METABOLOMICS2010

27 JUNE – 1 JULY, 2010
AMSTERDAM RAI
THE NETHERLANDS

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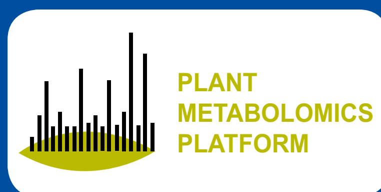

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SCHEDULE AT GLANCE

Time slot	Sunday 27th June	Monday 28th June	Tuesday 29th June	Wednesday 30th June	Thursday 1st July
Plenary session 09.00 - 10.30		Plenary session Applications Galore!	Plenary session Advanced Technologies	Plenary session Young Scientists	Nutrition & Health Sponsored by Unilever co-organized with NUGO
	Workshops 10.00 - 12.30			Sponsored by MPF	Metabolomics-assisted breeding
Coffee 10.30 -11.00		Coffee 10.30 -11.00	Coffee 10.30 -11.00	Coffee 10.30 -11.00	Coffee 10.30 -11.00
Parallel Session 11.00 - 12.30	1A Mass Spectrometry	Model systems for translational research	Databases, Bioinformatics & data analysis	Genome, metabolome and microbiome	Plenary session The future
Parallel Session 11.00 - 12.30	1B NMR	Technology Updates		Biotic interactions and plant stress	Society issues Awards Metabolomics2011
Lunch & Posters 12.30 - 14.00	Lunch & Posters	Lunch & Posters& Sponsor Workshops	Lunch & Posters& Sponsor Workshops	Lunch & Posters& Sponsor Workshops	Lunch & departure
Parallel session 14.00 - 15.30		Metabolomics and Biomarker discovery	Pharmacometabolomics, personalized medicine & future of health system	Pathways discovery and disease pathophysiology	NuGO Workshop (invitees only)
	Workshops 14.00 - 16.30				
Parallel Session 14.00 - 15.30		Environment and Ecology	Developments in plant metabolomics	Plant Systems biology	
	2A Biostatistics				
Tea 15.30 – 16.00	Chemometrics & Bioinformatics	Tea 15.30 – 16.00	Tea 15.30 – 16.00	Tea 15.30 – 16.00	
Parallel Session 16.00 - 17.30		Drug discovery & drug development	Systems biology of mammalian/microbial metabolism	Hot Topics	
	2B Plant Metabolomics				
Parallel Session 16.00 - 17.30		Plant Physiology	Volatiles & secondary metabolism	Food & Applied metabolomics	
Early evening activity 17.30	Opening Ceremony 17.30 – 18.45 Kick-off Plenary Prof. LEROY HOOD	Posters + wine 17.30 - 19.00	Posters + wine 17.30 - 19.00		
	Reception 18.45 - 20.00	Workshops 18.30 - 19.45	Workshops 18.30 - 19.45	Conference Dinner Okura Hotel 19.30	

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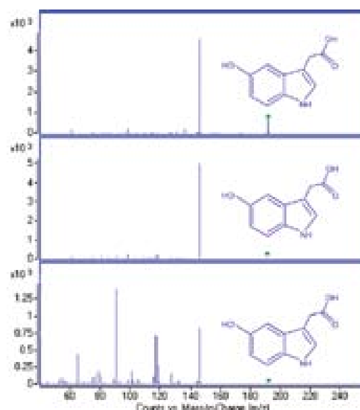

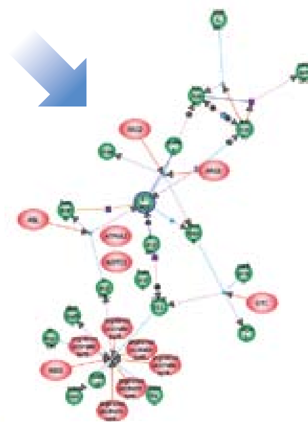

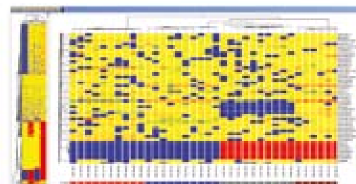

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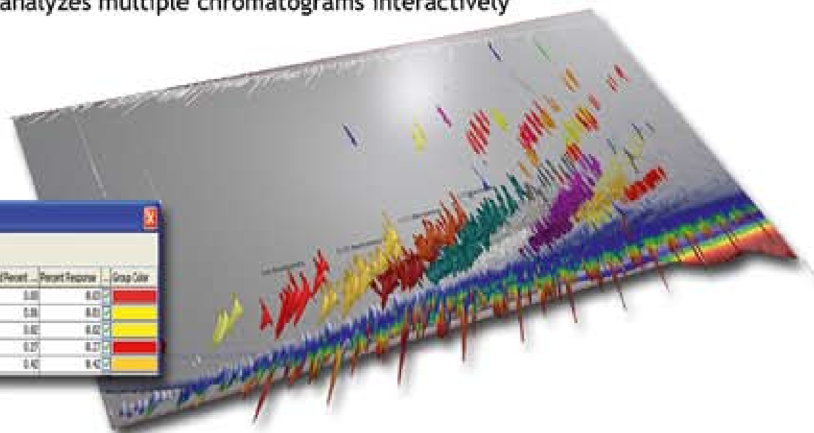

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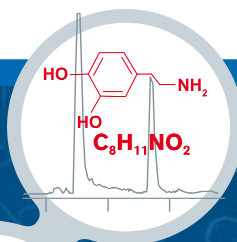

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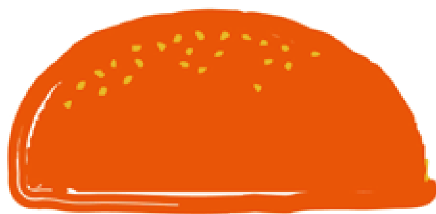

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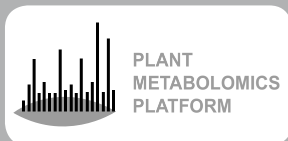

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LETTER OF WELCOME

DEAR PARTICIPANT, METABOLOMICS2010,

It is our very great pleasure to welcome you, on behalf of all those involved in organization of this meeting, to metabolomis2010 – the biggest meeting dedicated to metabolomics to date! For the first time we have been able to combine the activities of the International Metabolomics Society, the Plant Metabolomics Platform and the Metabolic Profiling Forum into one joint meeting. We therefore appreciate the trust these organizations have placed in us to organize metabolomics2010.

As local organizers we have done our best to coordinate the compilation of what we hope you agree is an excellent programme of state-of-the-art, innovative metabolomics presentations comprising both lectures and posters. This programme would of course never have been possible without your contributions by submitting a huge number of excellent abstracts. Special thanks must therefore firstly go out to our distinguished group of experts who made up the International Scientific Advisory Board (ISAB), whose job it has been to sift through all these abstracts and to vote on selections for the individual sessions. The quality of the submissions was excellent and the diversity of topics and approaches was impressive. Unfortunately, even by having parallel sessions, almost 200 hopeful speakers had to be disappointed. The result however is a set of top quality posters to complement the oral presentations.

The ISAB was initially asked to compile a programme of sessions and, later, to select lectures which together give a good overview of the main activities currently at the forefront of metabolomics research. We aimed to cover both the development and application of metabolomics technologies, giving more emphasis on the biological implication of metabolomics results. Metabolomics has (almost) come of age and the impact of the approaches is becoming more and more evident and relevant. Technology developments are still ongoing and special thanks must therefore also go out to the organizers of the complementary Workshops which are being held all day on Sunday and also on Monday and Tuesday evenings. Through these workshops we aim both to inform on progress as well as create forums for interactive discussion regarding how we can move forward, communicate and collaborate better, facilitate data exchange etc. We encourage everyone to attend and participate in these workshops.

No metabolomics meeting would be complete without the active participation of our commercial partners. Their continued commitment to support our efforts – in cash and in kind – is essential to our continued success. Without the financial support of our sponsors, be they instrument makers, software companies or local enterprises, this meeting would literally not have been possible. The field of metabolomics is blessed in many ways by having commercial entities strongly committed to future development and implementation of what is still a nascent technology. Consequently, many of our sponsors are also making active contributions to both scientific programmes and five of our Platinum sponsors have organized Lunchtime Showcases to bring you up to date on their most recent activities. In addition, 18 of our sponsors are also present as exhibitors and we encourage all of you to join their activities, talk with them and let them know what you want in the future. They are just as happy to hear what is going wrong as well as what is going right! Grab your chance to help them develop even better products and protocols for us.

Finally, we offer a final thanks to all the volunteers from both the Netherlands Metabolomics Centre and the Centre for BioSystems Genomics offices and all the PhD and postdoc volunteers who have offered their services during the prior organization of the meeting and to help with the smooth running of the conference on site.

Best wishes and hoping you have a great conference,
Your local organizers
Robert Hall, Centre for BioSystems Genomics, Plant Research International, Wageningen
Thomas Hankemeier, Netherlands Metabolomics Centre, Leiden University, Leiden

PS – While we have done our best to come up with a full scientific programme to keep you busy with science, information exchange and networking, we do recommend that if you have the chance, you take the opportunity to see some of the beautiful city of Amsterdam, the ‘Venice of the North’. There are several cultural highlights not to be missed (van Gogh Museum, Rembrandthuis, Rijksmuseum etc) and in a short boat trip (Rondvaart) you can get a great impression of the whole sphere of the city is just an hour! We and the people at the registration desk are happy to advise!

Netherlands Metabolomics Centre

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SPEAKERS

Opening Keynote speaker

Leroy Hood	Institute for Systems Biology, USA

Keynote speakers

Jack Newman	Amyris Biotechnologies, USA
Graham Cooks	Purdue University, USA
Hannelore Daniel	Munich Technical University, Germany

Invited Speakers

Henri Brunengraber	Case Western Reserve University, USA
Jan van der Greef	University of Leiden, The Netherlands
Rick Dunn	University of Manchester, UK
Nicole van Dam	Radbout University Nijmegen, The Netherlands
Lothar Willmitzer	MPI Potsdam, Germany
Joshua Rabinowitz	Princetown University, USA
Dan Jones	Michigan State University, USA
Uwe Sauer	ETH Zurich, Switzerland
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Annick Moing	INRA Bordeaux, France

METABOLOMICS2010 - GENERAL INFORMATION

Congress Venue

Amsterdam RAI
Congress Centre FORUM (Entrance E)
Europaplein 22
NL 1078 GZ
Amsterdam

The RAI is a large complex of buildings. The conference has its own signposting - signposted 'metabolomics2010' from both the tram stop 'de RAI'and the Amsterdam RAI train station. Please follow these signs or otherwise the signs bringing you to the entrance of our conference building – this is Entrance E.

Arrival

- If you wish to go straight to the RAI from the airport – the conference venue is just 12 minutes by train from Schiphol Airport Train Station (located directly underneath the Airport terminal building) to Amsterdam RAI station. Trains run every 15 minutes throughout most of the day. From RAI station it is an 8 minute (300 m) walk which is well signposted. Buy a single ticket at the counters with the blue lighting (on the ground floor)
- If you have a hotel in the centre of Amsterdam you can best take a train from the below ground Schiphol station to Amsterdam Central Station (CS). From there you can take a tram or taxi to your hotel.
- If you arrive by train at Amsterdam Central Station you can take a tram No 4 to the Conference site which takes about 25 minutes; exit at the stop 'de RAI'.
- If you are travelling by train to the Amstel station, you can take the Amstelveen express tram 51 (travelling time: 5 minutes) or the bus (route 15, 69 or 169), which will bring you to the RAI within 10 minutes. You should get off at 'de RAI.

Public transport

http://www.gvb.nl/english/travellers/tickets-and-fares/Pages/Ticketsandfares.aspx

The RAI is most easily accessed by tram line No 4. You can buy a 1 hour ticket in the tram (2.60 Euro). There are also 24h tickets (7.00 Euro) and a 4 day ticket (19.80 Euro) with unlimited use on all busses, metro and trams for the relevant period. These can be bought at all stations, tobacconists and post offices as well as the GVB office across the square at Amsterdam Central Station.

By car / Parking

From the motorway follow the signs to the RAI. Those coming by car are recommended to then follow the signs to and use, parking area P1.

Congress Secretariat

The secretariat / registration desk is located on the ground floor, on the right just inside the Lounge Forum, Entrance E. The desk will be manned from 08.00 – 20.00 each day.

Registration

Registering for the conference gives you 'Access to all areas' and the fee includes receptions, all lunches and the conference dinner. The registration desk is open from 08.30 on Sunday 27th June and from 08.00 on all other days.

Badges

All conference participants and exhibitors will be given a badge at registration. YOU NEED THIS BADGE TO ENTER THE BUILDING AND YOU MUST WEAR IT AT ALL TIMES WHEN INSIDE.

Floor plan / room allocations

All plenary sessions will be held in the main Auditorium. The entrance is directly across from the main entrance E past the exhibitor stands. In addition, half of the parallel sessions will be held in rooms E104-107 which is on the first floor. Please follow the signs. Posters are presented in the Forum Lounge on the ground floor and in the Ruby Lounge and in Room numbers E102, E103 and along the main corridor on the first floor.

Full floor plans are provided elsewhere in this book.

Catering arrangements

Coffee and tea during the breaks as well as the buffet lunches are available BOTH on the Lounge Forum (ground floor) and Ruby Lounge (First floor). The receptions are also divided between the two floors. To avoid congestion please make use of both areas.
FOOD AND BEVERAGES MAY NOT BE TAKEN INTO THE MAIN AUDITORIUM.

Dinner

Except for the conference dinner on Wednesday evening you are free to choose your own restaurant of which there are very many.

There are a few in the street close to the RAI (Scheldestaat). In the centre of town there are very many restaurants around the Leidseplein and the Rembrandtplein.

Sponsor booths

We recommend you visit all our sponsors! The sponsor exhibition stands are located on the ground floor (between the entrance E and the entrance to the main auditorium) and on the first floor at the top of the stairs. The stands will be manned at least during the breaks and during the receptions. Our sponsors also have access to a private meeting room should you to wish to talk in confidence. Please contact the sponsors for this service.

Speaker presentations

ALL SPEAKERS ARE REQUESTED TO HAND IN / UPLOAD THEIR PRESENTATIONS THE DAY BEFORE THEIR TALK HAS BEEN PLANNED. Please go to the registration desk to arrange this.

Lectures

- All the plenary sessions will be held in the main Auditorium on the ground floor.
- The biomedical parallel sessions are also in the Auditorium.
- The plant / environment sessions are held in Rooms E104-E107 which are on the first floor.

Internet

It is not possible to access internet in the main conference areas. Free WiFi and on line computer access has been made available on the first floor. Please ask at registration for directions.

Poster sessions

All posters should be hung up (using the pins provided) on Sunday or Monday morning. Please check your poster code and find the location at the registration desk. The poster session is completely full. Therefore, PLEASE DO NOT HANG UP ANY POSTERS WHICH HAVE NOT BEEN REGISTERED.
The posters can remain throughout the entire conference. There are two poster sessions on Monday 28th and Tuesday 29th from 17.30 – 19.00.

- Even number posters should be manned on Monday 28th
- Odd number posters should be manned on Tuesday 29th

Conference banquet

The conference dinner is always an excellent opportunity to talk at length and socialise with your friends and colleagues. The dinner is included in the registration fee and will be held on Wednesday evening 30th June in the Okura Hotel (http://www.okura.nl/) just a 15 min short walk from the conference site. The entrance to the Grand Ballroom is on the left just before the main entrance to the hotel. Look for the red carpet! We shall begin with a reception and drinks at 19.30 after which we shall have a buffet dinner in the Grand Ballroom at 20.00. PLEASE BRING THE DINNER INVITATION WITH YOU!

Luggage / Coats

There is a secure manned cloakroom for coats and luggage available throughout the conference at a cost of 1.50€ / item. For fire and safety reasons luggage may not be taken into the lecture theatres

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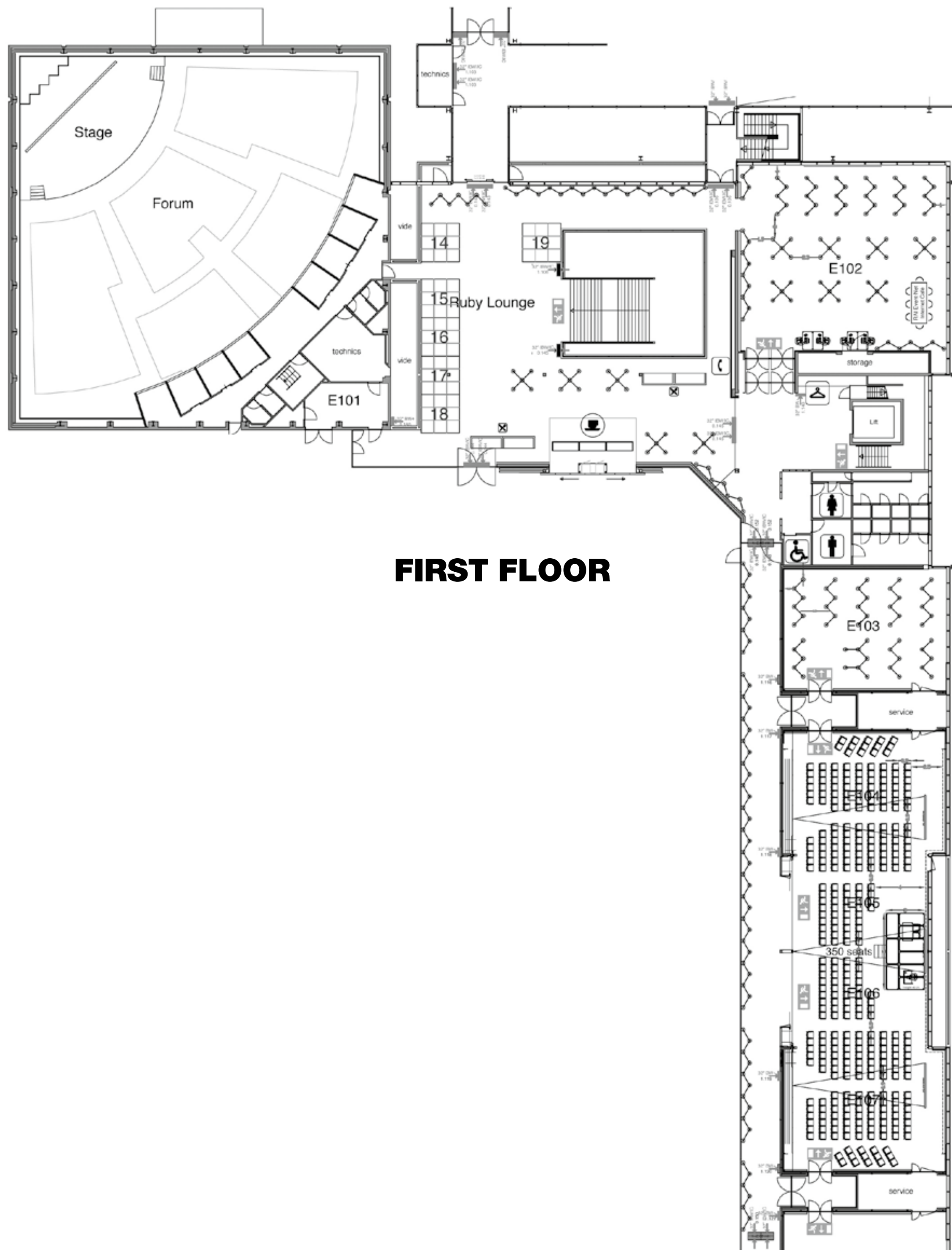

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Booth No	Company
1	Sigma Aldrich
2	Gene Data AG
3	Zoex
4	Shimadzu Benelux
5	Waters
6	Bruker Daltonik GmbH
7	Dionex
8	Thermo Fisher Scientific
9	AbSciex
10	LECO
11	Human Metabolome Technologies
12	Agilent Technologies
14	TNO
15	BIOCRATES Life Sciences AG
16	Chenomx Inc.
17	Netherlands Metabolomics Centre
18	Metabolomic Discoveries GmbH
19	Metanomics Health GmbH

PROGRAMME WORKSHOPS

Workshop Program Morning Sunday 27th June
Parallel session 1A
Mass Spectrometry: identification and quantification strategies
Room: Forum

	Topic	Coordinators
10.00 – 12.30	**Mass Spectrometry: identification and quantification strategies** This workshop aims to give an overview of MS-based techniques used in metabolomics research. Furthermore, we will discuss and review current trends in quantifying the right metabolites as well as strategies to identify (novel) metabolites using accepted protocols and novel methodologies. The workshop will address issues, like GC-MS and LC-MS, Quantification and ion suppression, various ionization techniques, why sample preparation?, multi-dimensional chromatography and mass spectrometry, metabolite identification from complex metabolomics data, etc. The workshop will supply you with the necessary knowledge to fully indulge in the programme of the conference and to get the best insight in the latest trends, techniques, challenges but also possibilities at hand nowadays. Rick Dunn (UK) & Rob Vreeken (Netherlands)	Rick Dunn (UK) & Rob Vreeken (Netherlands)
10.05 – 10.35	**Introduction to the application of Mass Spectrometry in Metabolomics** Ute Roessner (Australia)	
10.35 – 10.55	**Biomarker and profiling strategies for the diagnosis of Tubercolosis using GC and GC X GC-Tof-MS.** Erwin Kaal (Netherlands) (Sponsored by LECO)	
10.55 – 11.20	**Latest trends in getting accurate Metabolite concentrations in metabolomics studies** Rob Vreeken (Netherlands)	
11.20 – 11.45	**Identification of metabolites: Current Techniques and Novel Approaches** Warwick (Rick) Dunn (UK)	
11.45 – 12.05	**Technology Spotlight: Latest developments in High definition Mass Spectrometry(HDMS).** Presented by John Rontree (Sponsored by Waters)	
12.05 - 12.30	**Open discussion forum** Lead by Rob Vreeken and Rick Dunn.	

PROGRAMME WORKSHOPS

Workshop Program Morning Sunday 27th June
Parallel session 1B
NMR spectroscopy: What's new?
Room: E104 - 107

	Topic	**Coordinators**
10.00 – 12.30	**NMR spectroscopy: What's new?** To date these workshops have focused on teaching various aspects of NMR spectroscopy. This year, we have decided to break from this format and instead tried to show the full range of what NMR spectroscopy has to offer. While all talks will be geared to educate, new results and techniques will be presented to show how NMR spectroscopy is used in solution state and solid state, in vitro and in vivo, and in both steady state and in dynamic experiments.	Jules Griffin (UK) & Aalim Weljie (Canada)
10.00 – 10.30	**A journey from in vivo NMR to metabolomics: from MRS to OPLS.** Hans Vogel (Calgary, Canada)	
10.30 – 10.55	**HR-MAS proton NMR based metabolomic analysis of breast cancer tissues highlight the importance of choline metabolites in tumour grading.** Reza Salek (Cambridge, UK)	
10.55-11.20	**Metabolic Profiling Detects Systemic Effects of Environmental and Lifestyle Exposure to Cadmium in a Human Population.** James Ellis (London, UK)	
11.20-11.45	**Investigating the Metabolic Effects of Heart Failure Progression using Hyperpolarized Magnetic Resonance** Helen Atherton (Oxford, UK)	
11.45-12.10	**Novel Methods for Identifying and Quantifying Metabolites in Complex Biological Extracts by Multidimensional Nuclear Magnetic Resonance Spectroscopy** Ian Lewis (Princeton, USA)	
12.10 - 12.30	**Don't put my peaks in a bucket – alternative ways for data processing of NMR spectra + Discussion.** Julian Griffin (Cambridge, UK) & Aalim Weljie (Calgary, Canada)	

PROGRAMME WORKSHOPS

Workshop Program Afternoon Sunday 27th June
Parallel session 2A
Biostatistics, chemometrics and bioinformatics
Room: Forum

	Topic	Coordinators
14.00 – 16.30	**Biostatistics, chemometrics and bioinformatics** The goal of the biostatistics workshop is to review a large part of the data analysis pipeline. Study design as well as measurement design, thus which samples are taken when? and when are they measured? are important questions we aim to answer. Furthermore the whole data laundry process to clean up the "dirty data" will be reviewed. We will look into the use of databases during the data analysis process and finally the data analysis of designed studies and its validation receives sufficient attention.	Johan Westerhuis (Netherlands) & Roy Goodacre (UK)
14.00 – 14.25	**Measurement design and corrections in metabolomics**. Adrie D. Dane (Netherlands)	
14.25 – 14.50	**Use of web-based databases and applications for quantitative metabolomic studies.** Jianguo (Jeff) Xia (Canada)	
14.50 – 15.15	**Analyzing structured metabolomics data.** Age K. Smilde (Netherlands)	
15.15 – 15.40	**Processing of mass spectrometry based molecular profile data** Matej Orešič (Finland)	
15.40 – 16.05	**Validation and biomarker selection in metabolomics data analysis** Johan A. Westerhuis (Netherlands)	
16.05 – 16.30	**General Discussion on future initiatives** Lead by Roy Goodacre and Johan Westerhuis	

PROGRAMME WORKSHOPS

Workshop Program Afternoon Sunday 27th June
Parallel session 2B
Plant metabolomics
Room: E104 - 107

	Topic	Coordinators
14.00 – 16.30	**Plant metabolomics** In the plant metabolomics workshop the potentials and pitfalls of the main technologies available for wet lab analyses will be covered. Starting with the importance of experimental design and sampling approaches, aspects data acquisition and processing will be covered as will opportunities for automation of procedures for large sample numbers. This will be followed by a 'Research blast' where 6 scientists with the most innovative poster abstracts will each have 5 minutes to present their work. A general discussion of future developments will round off the workshop.	Robert Hall (Netherlands) & Lloyd Sumner (USA)
14.00 – 15.00	**Plant Metabolomics technology review: Separating the wheat from the chaff – getting the most out of plant metabolomics experiments** Mike Beale & Jane Ward (UK)	
15.00 – 15.45	**Research Blast 6 x 7 minute poster shots!** • **Metabolic distance, a novel feature in metabolomics.** Benyamin Houshyani (Netherlands) • **Metabolite Profiling of Volatile and Nonvolatile Compounds in 32 Pepper Accessions.** Yuni Wahyuni (Netherlands) • **Multifaceted Metabolomic Approaches Profiling of Soluble Byproducts Formed During Lignocellulosic Biomass Pretreatment.** Ramin Vismeh (USA) • **Analysis of photosynthetic carbon assimilation by a combination of in vivo 13C-labelling from 13CO2 and LC-MS/MS, GC-MS analytic platforms.** Marek Szecowka (Germany) • **Clarification of metabolite compartmentalization by metabolomics in a single cell of the alga Chara australis.** Akira Oikawa (Japan) • **Metabolomic Analysis of the Stem Holoparasite Plant Cuscuta (Cuscutaceae).** Takeshi Furuhashi (Austria)	
15.45 – 16.30	**Open discussion on future developments, opportunities for collaborative efforts, future meetings etc** Robert Hall (Netherlands) & Lloyd Sumner (USA)	

PROGRAMME WORKSHOPS

Additional Workshop Program Monday 28th June
Parallel Session 3A
Databases & Standards Discussion session
Room: Forum

	Topic	Coordinators
18.30 – 19.45	**Databases & Standards Discussion session** In this session a number of key points will be brought to the table for discussion and exchange of ideas and experiences. Important here are for example, topics related to ease of accessibility and reliability of (unpublished) datasets and how we can ensure that these are robust and usable to others. Robustness also relies upon correct unambiguous annotation – what are your experiences? The general aim therefore is to exchange ideas and if possible come to a consensus on at least some of these issues and / or to establish a means to continue discussions in order to solve some of these issues.	Oliver Fiehn (USA) & Christoph Steinbeck (UK)
18.30 – 19.30	(1) Publications: how can we improve the public accessibility of metabolomic data sets? (2) Existing databases: how can we improve usability of databases from MassBank to MetaCyc and ChEBI? What are the sets of data that users would like to get from databases but that are not yet available? (3) Metabolite queries: How do we make sure we talk about the same compounds when using queries? How do we correct wrong entries in databases? (4) Quantifications: How do we deal with the problem that absolute quantifications are inherently difficult but relative normalizations are not comparable between studies? Are they?	
19.30 – 19.45	(5) Round –up discussion: How and where can we collect ideas, links and comments in a Wiki- or Blog-style to continue discussions?	

PROGRAMME WORKSHOPS

Additional Workshop Program Monday 28th June
Parallel Session 3B
Nordic Interest Group
Room: E104 - 107

	Topic	Coordinators
18.30 – 19.45	**Nordic Interest Group**	Matej Oresic (Fi)
18.30 – 19.00	**Introduction of metabolomics research activities in Nordic countries** Group presentations from Denmark, Finland, Norway, Sweden	
19.00 – 19.45	**General discussion and Organization issues: Conferences, workshops, training etc** Lead by Matej Oresic	

PROGRAMME WORKSHOPS

Additional Workshop Program Tuesday 29th June
Parallel Session 4A:
Environmental metabolomics
Room: 104-107

	Topic	Coordinators
18.30 – 19.45	**Environmental metabolomics** The environmental metabolomics session has been organised to cover key issues of wide relevance to the community. The workshop has been divided into 4 main topics which will be introduced by leading scientists and followed by an open floor discussion for input from anyone involved. The main aim is to exchange information *(who is doing what? Using which organisms? Which systems? Which technical and biological limitations are being experienced? etc)* and discuss ideas and establish an interactive forum for future collaboration, organisation of workshops / meetings etc.	Mark Viant (UK) & Dan Baerden (USA)
18.30 – 18.45	**Discussion Topic 1 – International NMR intercomparison exercise** Lead: Dr Dan Bearden, (USA)	
18.45 – 19.00	**Discussion Topic 2 – Metabolomics in environmental/ecological risk assessment** Lead: Dr Mark Viant, (UK)	
19.00 – 19.15	**Discussion Topic 3 – Brainstorming on "what are the top 5 or 10 measurement problems in environmental metabolomics?"** Lead: Dr Dan Bearden (USA)	
19.15 – 19.30	**Discussion Topic 4 – What is the purpose of environmental metabolomics?** Lead: Dr Jake Bundy (UK)	
19.30 – 19.45	**Round up summary**	

PROGRAMME WORKSHOPS

Additional Workshop Program Tuesday 29th June
Parallel Session 4B:
Metabolomics & Future of Health System
Room: Forum

	Topic	**Coordinators**
18.30 – 19.45	**Metabolomics & Future of Health System** The aim of this session is to address key issues in the area of the application of metabolomics in health care, daily clinical decisions and in the pharmaceutical industry. The following topics will be addressed by leading scientists from academia, pharmaceutical industry, clinics and patient organizations followed by a discussion to exchange ideas and experiences. • What is necessary to make metabolomics a routine tool in clinical decision making? • How can metabolomics change the current practice in health care and in the pharmaceutical industry?	Thomas Hankemeier (NL) & Rima Kaddurah - Daouk (USA)
18.30 – 19.00	Short presentations of visions & bottlenecks by experts on future of health care	
19.00-19.30	Open discussion: • What are the lessons to be learned from other omics? • What will be impact of metabolomics on clinical care? What is expected horizon for this to happen? What will be the first clinical applications? When will this happen? • When will be metabolomics a standard tool in Pharma? What can be done now, what is required for a broader application in the pharmaceutical industry? • What is the role of the patient, what is about IP on biomarkers? Confidentiality of patient data?	
19.30-19.45	Summary of discussion: what are the next steps?	

PROGRAMME:

LECTURES & SPONSOR TECHNOLOGY SHOWCASES

Sunday, 27 June 2010

Opening ceremony	**Welcome message from** **Dr Colja Laane, Director, Netherlands Genomics Initiative**	**Forum**
Kick off Plenary speaker:		
17.30 -18.45	**Leroy Hood:** Systems Approaches to Medicine	**Forum**

LPlenary
Systems Approaches to Medicine

Leroy Hood, Institute for Systems Biology, Seattle, Washington, USA

One powerful way to attack biological complexity is to view biology (medicine) as an informational science requiring systems approaches. I will discuss this and the emerging technologies that will transform medicine over the next 10 years, including next generation DNA sequencing, protein assays, single-cell analyses, and the capacity to generate stem cells for each individual patient. It appears that systems medicine, together with pioneering technology changes , as well as the creation of powerful new computational and mathematical tools, will transform medicine over the next 5 to 20 years from its currently reactive state to a mode that is proactive (P4) medicine; predictive, personalized, preventive and participatory. P4 medicine will have striking implications for healthcare costs as well as leading to a transformation of the healthcare industry. I will also talk about ISB strategic partnerships that will permit us to bring P4 medicine to the patient.

Monday, 28 June 2010

Plenary session		
09.00 - 10.30	**PLENARY SESSION P1** **APPLICATIONS GALORE!** **Chair: Roy Goodacre (UK)**	**Room**
09.00 - 09.50 Keynote speaker	**Jack Newman (USA)** Microbial Fermentations for Diesel Fuel Production	Forum
09.50 - 10.10 Selected speaker	**John Draper (UK)** Common patterns of metabolic reprogramming are associated with cellular penetration in two very different plant-pathogen interactions	Forum
10.10 - 10.30 Selected speaker	**Steffen Neumann (Germany)** In silico fragmentation and chemical similarity for metabolite identification from tandem-MS	Forum

LP1-001

Microbial fermentations for Diesel fuel production: The role of metabolite analysis in strain improvement.

Jack Newman, Amyris, California

Conversion of biomass to fuels, chemicals and pharmaceuticals can be achieved cost-effectively by microbial metabolism. Understanding the flux of carbon through the cell is key to uncovering limitations in metabolism that reduce the effectiveness of the microbe. Amyris has built strains that produce a high level of flux through the isoprenoid pathway to produce a variety of isoprenoid products including Diesel fuel and Artemisinin, an anti-malarial drug. Production of all of these isoprenoid products relies on a common pathway to produce prenyl pyrophosphates as a precursor to the final product. Targeted metabolomic analysis is used extensively at Amyris for quality control of strain construction, pathway bottleneck identification and a better understanding of the physiological state of highly productive strains. These analyses, along with complementary approaches, are demonstrated technologies for bringing impactful, renewable products to the world. The speed and high-throughput application of the latest metabolite analysis technology dramatically accelerates the timeline from idea to product.

LP1-002

Common patterns of metabolic reprogramming are associated with cellular penetration in two very different plant-pathogen interactions

John Draper, Hassan Zubair, Stuart Snowden, Barbara Hauck, Kathleen Tailliart, Ifat Parveen, Kathleen Tailliart, Gordon Allison and Manfred Beckmann Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Ceredigion, UK

Metabolite fingerprinting using flow infusion electrospray MS in a linear ion trap provided a primary discovery tool to investigate the re-programming of plant defence metabolism in pre-symptomatic leaf tissue of two important host-pathogen interactions, namely *Phytophthora infestans (*potato blight) invasion of potato (*Solanum tuberosum*) and *Magnaporthe grisea* (rice blast) infection of the model host, *Brachypodium distachyon*. *Solanum tuberosum leaves* inoculated with a virulent strain of *Phytophthora infestans* showed a strong metabolic response to the pathogen as early as 12 hours post inoculation. These early stages of pathogen attack represent a phase several hours before penetration of the first host cell which occurs typically by 16-20hrs. Major metabolic changes (confirmed by LC-ICR-MS accurate mass analysis, MS/MS and use of standards) observed included a diversion of the TCA cycle likely to effect the energy metabolism of the host. Later metabolic reprogramming events (20-24hrs) just after successful host cell penetration were associated with disruption/modification of defensive phenylpropanoid and monolignol biosynthesis pathways. Analysis of metabolic re-programming in resistant interactions is

currently under investigation. Susceptible interactions of M. grisea with *Brachypodium distachyon* leaves have been described recently (Parker et al., Plant Journal 59, 723-737, 2009). Using a higher inoculation density, earlier phases of the infection process were studied and, similar to fungal-infected potato, a disruption of the TCA cycle and energy metabolism was evident just before cellular penetration. Later changes (72-96 h) concurrent with the first appearance of visible lesions were similarly dominated by a disruption in phenylpropanoid metabolism and lignin synthesis compared to a resistance response. Specific consequences on redox balance are postulated in both host-pathogen interactions. The similarity of metabolome alterations in pathogen-challenged leaf tissue plant prior to penetration of the first infected cells in these two very different hosts was striking and suggests an evolutionary conservation of pathogenicity strategies. Host enzyme activity potentially modulated by the invading pathogen will be discussed.

LP1-003

In *silico* fragmentation and chemical similarity for metabolite identification from tandem-MS

Sebastian Wolf(1), Michael Gerlich(1), Carsten Kuhl(1), Stephan Schmidt(1), Matthias Müller-Hannemann(2) and Steffen Neumann(1) (1) Leibniz Institute of Plant Biochemistry-Department of Stress- and Developmental Biology, Weinberg 3, 06120 Halle(Saale), Germany (2) Institut für Informatik, Martin-Luther-Universität, Halle-Wittenberg, Von-Seckendorffplatz 1, 06120 Halle (Saale), Germany

Mass spectrometry has become the analytical method of choice in metabolomics research, but the identification of unknown compounds remains the main bottleneck. In addition to the precursor mass, MS^2 (or MSn) spectra carry rich (sub-)structure information; but the coverage of spectral libraries of measured reference compounds is far from complete. Compound databases such as KEGG, PubChem or ChemSpider on the other hand describe a large number of compounds, but provide no spectral searches. We first present the MetFrag suite (http://msbi.ipb-halle.de/MetFrag/) to create a ranked list of structure proposals from compound libraries: candidates are retrieved based on an exact mass search with the precursor (neutral) mass. Then, all bonds are fragmented and fragment masses are compared to the measured spectrum. The scoring also incorporates bond dissociation energies. Especially for large compound libraries, the candidates with a good score usually show a high structural similarity (or just different stereochemistry). A subsequent clustering based on chemical distances reduces this redundancy. We evaluate MetFrag on a large set of QTOF MS^2 spectra of different instruments from MassBank, and present the results. In case of tied ranks, we report the worst-case position. The median of the correct solution is 14 (out of 2500 candidates retrieved from PubChem). Compared to a previously published study, MetFrag obtained better results than the commercial MassFrontier software. The in-silico fragmentation requires less than a second to process a molecule, and MetFrag finishes a KEGG or PubChem search on average within 30 to 300 seconds, respectively, on an average desktop PC. MetFrag is available through a web application, web services and as Java library. The web front end allows the end-user to analyse single spectra, whereas the latter are aimed at evaluation and batch searches. Second, we present a method to integrate MassBank and MetFrag results, to combine the respective strengths of both. On the same dataset as above, we obtain the correct solution on average at rank 4. The search function is available from our websites at http://msbi.ipb-halle.de/MetFlow/

Monday, 28 June 2010

Parallel Session 1A

11.00 - 12.30	**MODEL SYSTEMS FOR TRANSLATIONAL RESEARCH** **Chair: Mark Viant (UK)**	**Room**
11.00 - 11.30 Invited speaker	**Henri Brunengraber (USA)** Uncovering the metabolism of 4-hydroxyacids: drugs of abuse and products of lipid peroxidation	Forum
11.30 - 11.50 Selected speaker	**Jake Bundy (UK)** Metabolic changes of ageing and longevity in the nematode *C. elegans*	Forum
11.50 - 12.10 Selected speaker	**Aalim Weljie (Canada)** Hypoxia-induced metabolic shifts in cancer cells: beyond the 'Warburg effect'	Forum
12.10 - 12.30 Selected speaker	**Paul Lee (USA)** Application of Tracer-based Metabolomics and Phenotypic Phase Plane Analysis in Metabolic Studies	Forum

L1A-001

Uncovering the metabolism of 4-hydroxyacids: drugs of abuse and products of lipid peroxidation

Zhang, G-F. (1), Harris, S. (1), Sadhukhan, S. (1), Gibson, K.M. (2), Anderson, V.E. (1) Tochtrop, G.P. (1), Brunengraber, H. (1): Case Western Reserve University, Cleveland Ohio, USA; (2) Michigan Technical University, Houghton Michigan, USA.

4-Hydroxyacids are drugs of abuse (4-OH-butyrate, 4-OH-pentanoate) and products of lipid peroxidation (derived from 4-OH-nonenal and 4-OH-hexenal). We used a combination of metabolomics and mass isotopomer analysis to investigate the metabolism of these compounds in vivo and in isolated rat livers. We synthesized singly and multiply 13C-labeled substrates, and identified their metabolites by GC-MS and LC-MS/MS. The profile and mass isotopomer distribution of CoA esters provided a gold mine of information on the pathways of 4-OH-acid catabolism. All 4-OH-acids with 4 to 11 carbons form 4-phospho-acyl-CoAs, a new class of CoA esters. The metabolism of 4-OH-acids with 5 to 11 carbons proceeds via 2 new pathways: (i) isomerization of 4-OH-acyl-CoAs to 3-OH-acyl-CoAs via 4-phospho-acyl-CoAs, followed by regular beta-oxidation to acetyl-CoA-propionyl-CoA, and (ii) a sequence of beta-, alpha- and beta-oxidation steps with production of formate, acetyl-CoA-propionyl-CoA. 4-OH-butyrate is a physiological neurotransmitter derived from GABA. When ingested at high doses, it is a drug of abuse (date-rape drug, GHB). We showed that it is metabolized by 4 processes: (i) anaplerosis of the citric acid cycle via succinate, (ii) to 3-OH-propionate + formate via two parallel alpha-oxidation processes starting from each end of the molecule, (iii) to glyoxylate + acetyl-CoA by a variant beta-oxidation process, and (iv) to 4-phospho-butyryl-CoA. The latter also accumulates in the brain and liver of mice unable to dispose of physiological 4-OH-butyrate. The metabolism of the new drug of abuse 4-OH-pentanoate (GHV, more toxic analog of GHB) leads to the accumulation of very high concentrations of 4-phospho-pentanoyl-CoA + 4-OH-pentanoyl-CoA + 4-keto-pentanoyl-CoA. This trapping of CoA perturbs a number of reactions using CoA. It is likely that 4-phospho-acyl-CoAs are neuromodulators which contribute to the brain toxicity of 4-OH-butyrate and 4-OH-pentanoate. This work, supported by the NIH (NIDDK RoadMap Initiative and NIEHS), illustrates the potential of the association of metabolomics and mass isotopomer analysis for pathway discovery.

L1A-002

Metabolic changes of ageing and longevity in the nematode *C. elegans*

JG Bundy (1), SK Davies (2), FM Geier (1), AM Leroi (2): (1) Biomolecular Medicine, and (2) Biology, Imperial College London, London SW7 2AZ, UK.

The nematode Caenorhabditis elegans is widely used as a model organism. Because it has only a short lifespan and rapid generation time, it is frequently used for the study of ageing. Since the classic discovery in 1993 that mutations in the gene daf-2 (an orthologue of the human insulin receptor) can increase longevity, a large number of genes have been identified that increase C. elegans lifespan when deleted or mutated. These genes are from many different biological areas, including insulin and insulin-like signalling (IIS), protein translation, mitochondrial genes, autophagy, caloric restriction, and others. We used NMR spectroscopy and GC-MS to study worms associated with three separate longevity-increasing mechanisms: IIS, protein translation, and dauer larvae (a long-lived, non-ageing, and stress-resistant larval C. elegans stage). Even though these mutants have apparently very different underlying genetic bases for long life, a number of metabolites were increased in all the separate long-lived worms. In order to investigate these further, we also looked at the daf-16 and daf-2;daf-16 double mutants: daf-16 is a FOXO family transcription factor, and daf-16 null mutants do not undergo IIS-induced lifespan extension. In particular, the branched-chain amino acids (BCAAs) exhibited the classic phenomenon of being daf-16 repressible, suggesting they may be closely linked to mechanisms of lifespan extension – and generating some interesting hypotheses for future testing. We also present some data on the metabolic changes associated with the normal ageing process in wild-type C. elegans, together with a novel explanation for these changes based on C. elegans biology that does not rely on standard metabolic network explanations.

L1A-003

Hypoxia-induced metabolic shifts in cancer cells: beyond the 'Warburg effect'

Weljie, AM (1), Bondareva, A (2), Zhang, P (1), Jirk, FR (2) (1) Department of Biological Sciences, University of Calgary (2) Department of Biochemistry and Molecular Biology, University of Calgary, Canada

Hypoxia has been recognized to play a role in promoting the invasive and metastatic behavior of cancer cells. Largely via the transcription factor, hypoxia-induced factor 1 (HIF1), hypoxia exerts significant effects on cellular metabolism, with numerous downstream consequences. Energetically, for example, there is a significant shift away from oxidative phosphorylation in mitochondria towards glycolysis (the 'Warburg effect'). The proteins involved in mediating the metabolic pathways triggered in response to hypoxia thus represent prime targets for therapeutic intervention. Hypoxia has been associated with increasingly aggressive phenotypes in cancer cells, and some of these have been linked to changes in carbohydrate metabolism important for adhesion and angiogenesis (e.g. via effects on E-selectin and integrin mediated effects). Here, illustrating the ability of metabolomics approaches to furthering our understanding of hypoxia-mediated events, we initially examined the responses of a breast cancer cell line to 1% oxygen. Metabolomics technologies can simultaneously measure a wide range of metabolites in an untargeted manner, but to date this technology has been relatively under utilized in the study of hypoxia. Examining the effects of hypoxia in the MDA-MB-231 cell line by gas chromatography mass spectrometry (GC-MS), we found not only that intracellular metabolite profiles indicated a significant shift in energy metabolites and carbohydrates, as expected, but we also found changes in metabolites involved in the urea cycle, as well as the metabolism of arginine, proline, glutamate, aspartate, and asparagine. Intriguingly, there was a clear time-dependence in hypoxia-induced metabolic changes, indicating that the hypoxic effect may transition through several generations during reprogramming of cellular metabolism. We also searched for extracellular biomarkers by nuclear magnetic resonance spectroscopy (NMR) of cell supernatants ('footprinting '), and found evidence for oxidative stress and energy metabolites when cells were hypoxic. These changes correlated with metabolite profiling studies on the sera of mice with xenografted MDA-MB-231 tumors. Thus, in addition to the 'Warburg effect', there appears to be range pathways impacted by hypoxia. These pathways may represent targets for therapeutic intervention.

L1A-004

Application of Tracer-based Metabolomics and Phenotypic Phase Plane Analysis in Metabolic Studies

WN Paul Lee, Jun Xu, Paulin Wahjudi and Vay-Liang Go. Center of Excellence for Pancreatic Disease, UCLA, Los Angeles.

Tracer-based metabolomics is the experimental counterpart of in silico constrained based modeling. While metabolic phenotype can be solved in constrained based modeling as the space bounded by “extreme pathways”, it is precisely determined in tracer-based metabolomics, allowing a better characterization of metabolic phenotype. The utility of tracer-based metabolomics and PPP analysis is illustrated by an investigation of metformin (Met) and rosiglitazone (Rosi) in HepG2 cells. Human hepatoma HepG2 cells were grown in tissue cell culture in Dulbecco’s modified Eagle’s medium containing 50% [1, 2 13C2]-glucose in

the presence of metformin (1 mM) or rosiglitazone (1 micromolar). Cells were harvested in 48 hours and mass isotopomers of deoxyribose and long chain fatty acids were determined. Isotopomer fractions m1/Sm in deoxyribose were: 0.560 (control), 0.575 (Rosi) and 0.4971 (Met). Isotopomer fractions m2/Sm were: 0.328, 0.314 and 0.379 respectively. Plot of phenotypic phase plane (m1 vs m2) showed different metabolic phenotype changes due to action of Met vs Rosi. The respective isocline in PPP analysis showed that cells treated with Met increased contribution from non-oxidative pentose cycle to compensate for the loss of contribution from oxidative pentose cycle in deoxyribose synthesis. On the other hand, Rosi treatment resulted in a loss of contribution from oxidative pathways without compensation to deoxyribose synthesis. Plot of acetyl-CoA enrichment against fraction of new palmitate synthesis showed that effect of Rosi on fatty acid synthesis is orthogonal to that of deoxyribose synthesis. However, treatment with Met significantly increased conversion of glucose to acetyl-CoA associated with diminished fatty acid synthesis in a futile manner. Conclusion: Unlike metabolite profiling, tracer-based metabolomics is a quantitative approach in which metabolic phenotype is characterized by extreme pathways. Phenotypic phase plane analysis provides unambiguous interpretation of the metabolic effect of Met and Rosi on glucose utilization through pentose and fatty acid synthesis pathways. Tracer-based metabolomics and PPP analysis is a powerful quantitative systems biology approach to understanding of metabolic pathways in metabolic studies.

Monday, 28 June 2010

Parallel Session 1B

11.00 - 12.30	**TECHNOLOGY UPDATES**	
	Chair: Lloyd Sumner (USA)	**Room**
11.00 – 11.30 Selected speaker	**Volker Kruft (sponsored by AB Sciex)** A strategy for the quantitative analysis of all lipids in complex samples with a single acquisition method.	E104 - 107
11.30 - 11.50 Selected speaker	**Ed Ledford (sponsored by ZOEX)** Classification of Breast Cancer Grades by Pattern Recognition in GC x GC x HiResTOMFS Images	E104 - 107
11.50 - 12.10 Selected speaker	**Martin Hornshaw (sponsored by ThermoFisher)** Discovering Unexpected Products of CYP Catalysed Reactions in Brain using Charge-Tagging™ and LTQ Orbitrap MSn.	E104 - 107
12.10 - 12.30 Selected speaker	**Theodore Sana (sponsored by Agilent)** A QTOF generated LC-MS/MS library facilitates compound identification of non-targeted metabolomics data.	E104 - 107

L1B-001

A strategy for the quantitative analysis of all lipids in complex samples with a single acquisition method

Volker Kruft, AB SCIEX

The fast identification and quantification of lipid biomarkers without extensive fractionation or method development would be an advantage for laboratories worldwide. So far, the comprehensive study of lipids is done by accurate mass measurements for lipid identification or the use of precursor ion sans for the analysis of different functional groups. We describe novel workflow for the identification and quantification of hundreds of lipids in total lipid extracts on the AB SCIEX TripleTOF 5600 system in a single, fast acquisition. Direct nano-electrospray infusion of less than 10 l of lipid extract enabled the acquisition of a complete quantitative and qualitative dataset: After a high-resolution survey scan, MS/MS data are acquired for each mass between 200 and 1000 m/z. The resulting 3-dimensional datafile comprises dimensions for selection mass, fragment ion masses, and intensity. We will show the identification of more than 800 lipid species from 6 classes and 15-subclasses in crude rat brain lipid extract in a single polarity acquisition. The combined datasets from positive and negative mode provide a complete lipid profile that can be queried by specific parent or signature ions. Relative quantification of lipid species against their lipid class, or absolute quantification with the use of specific internal standards, can be achieved with a comprehensive dataset acquired in less than 10 minutes.

L1B-002

Classification of Breast Cancer Grades by Pattern Recognition in GC x GC x HiResTOMFS Images

Ledford, Edward B. (1), Wu, Zhanpin (1), Reichenbach, Stephen, E. (2), Tao, Qingping (3), Hutchinson, Dan (3), Tian, Xue (3) Tanner, Christain (4), Tanner, Martin (4), Gonin, Marc (4), and Furher, Katrin (4) (1) Zoex Corporation, Houston TX USA (2) University of Nebraska at Lincoln, NE, USA (3) GC Image, LLC., Lincoln, NE, USA (4) Tofwerk AG, Thun, Switzerland

This paper is a preliminary report on new software and hardware for GC x GC x MS analysis of metabolomics samples. The focus of the study was human breast cancer tissue, specifically, the assignment of breast cancer grades by means of pattern

recognition in complex GC x GC x MS images. New software has been developed for the purpose of "aligning" GC x GC x MS images for comparison. The method employs feature-based parsing and affine transformation of images to match a so-called "consensus template," as a means of locating regions of similarity and difference in GC x GC x MS images. The "features" on which pattern recognition is based may be derived from TIC patterns alone, a combination of TIC and Low Resolution MS patterns, or of TIC and High Resolution MS patterns. The latter provide elemental composition determinations on sufficiently intense mass spectral peaks. In preliminary studies, 13 of 18 samples were correctly graded on the basis of TIC data alone. The grading was statistically significant relative to the null hypotheses (even for this relatively small data set) with greater than 99.9% confidence. The effects of incorporating low and high resolution MS data into the feature sets will be discussed. A new high-speed (50 to 500 scans/sec) high resolution (7,000 at 500 Th) time-of-flight mass spectrometer will be briefly described, and preliminary data on exact mass measurements and elemental composition assignments presented. Future research directions will be indicated.

L1B-003
Discovering Unexpected Products of CYP Catalysed Reactions in Brain using 'Charge-Tagging' and LTQ Orbitrap MSn

Martin Hornshaw[1], Jenny Ho1, Gary Woffendin1, Yuqin Huang2 and William Griffiths2. 1Thermo Fisher Scientific, Hemel Hempstead, UK; 2Swansea University, Swansea, UK

Cytochrome P450s (CYPs) are key enzymes in the metabolism of brain lipids. They are particularly important in the metabolism of sterols in brain, where CYP46A1, 27A1 and 7B1 are all involved. However, the detection of endogenous reaction products can be difficult, owing to their low levels against a large lipid background. For example, cholesterol is present in adult brain and CSF at levels of 10-20 mg/g and ~3μg/mL, while 24S-hydroxycholesterol, a major cholesterol metabolite formed in a CYP46A1 catalysed reaction in brain, is present at 10-20 μg/g and ~3ng/mL in adults. Lipid metabolites are usually identified in biological systems by mass spectrometry, originally by GC-MS, and now more often by LC-MS. However, many metabolites may be transparent to MS analysis and require derivatisation to allow their detection. Here we report the use of a new charge-tagging LCMSn strategy utilizing LTQ Orbitrap technology to identify novel sterol metabolites in developing rodent brain and in CSF and plasma from adult human. Further we discuss the subsequent proteomic effects of sterol metabolites on cell culture systems. Our charge-tagging strategy has allowed the identification of low amounts of 24S-hydroxycholesterol (10-30 ng/g) and 24S,25-epoxycholesterol (100-200 ng/g) in developing rodent brain and high amounts of 3β-hydroxycholest-5-en-27-oic acid and others formed from cholesterol in reactions catalysed by CYP27A1 and 7B1. This leads to the question, what are the functions of these sterols in brain?

L1B-004
A Q-TOF generated LC-MS/MS Library Facilitates Compound Identification of Non-Targeted Metabolomics Data

Theodore R. Sana & Steven Fischer , Agilent Technologies, Santa Clara, CA, USA

The determination of compound identities is a crucial analytical problem for metabolomics scientists using LC/MS detection. Without compound identities it is impossible to make biological sense of study results. Many compounds observed to be differentially expressed in metabolomics studies are known primary metabolites. Easily identifying these known metabolites would facilitate metabolomics analysis. One accepted way to identify compounds is to compare the observed MS/MS spectra of the unknown to a library of MS/MS spectra of metabolite standards. Using a Q-TOF LC/MS system, we have constructed an accurate mass MS/MS spectral library of common metabolites using three different collision energies. In this presentation we show an un-targeted metabolomics workflow and how the utility of a metabolite LC/MS/MS spectral library was used for compound identification. We will present data demonstrating the library's utility by analyzing MS/MS spectra of metabolites in urine sample extracts.

Sponsor Lunch Session Monday, 28 June 2010
Parallel session 1A: Sponsored by LECO
Room: Forum

13.00 – 13.55	**Technology showcase: LECO**
	GC-TOFMS GC×GC LC-TOFMS GC×GC-TOFMS
	Real life research applying Metabolomic approaches using the LECO Pegasus GC-TOFMS and Pegasus 4D GC×GC-TOFMS will be presented during this session from users in the field.
13.00 – 13.15	Inter-laboratory repeatability of rapid GC-TOFMS plant metabolite profiling and GC-TOFMS spatial metabolite analysis during melon fruit development
Speaker	Will Allwood, University of Manchester, UK
13.15 – 13.30	Metabolomics of small samples: Miniaturized sample-preparation for GC×GC-TOFMS based metabolite analysis.
Speaker	Alexander Erban, MPIMP-Golm, Germany
13.30 – 13.45	Metabolomics of the hydrogen producing algae Chlamydomonas reinhardtii using GC×GC-TOFMS.
Speaker	Matthias Keck, University of Bielefeld, Germany
13.45 – 13.55	Discussion

Allwood Abstract: Within the EU Framework 6 META-PHOR (plant and food metabolomics) project (http://www.meta-phor.eu/), LECO, the University of Manchester and Max Plank Institute for Molecular Plant Physiology - Golm, have collaborated on a number of research projects focusing upon the strengths of GC-TOFMS based plant metabolomics. First the three groups wished to compare their three SOP's for GC-TOFMS analysis of polar metabolites on a common sample set generated by a single technician within one laboratory. An evaluation of the pros and cons of each laboratories SOP was made as was a comparison of data from between the three laboratories. In our hands we found the inter-laboratory repeatability of GC-TOFMS analysis of the common sample sets was extremely promising with the processed datasets of the three laboratories producing near identical results when compared by classical Principle Component Analysis. In result of the comparison of the three laboratories SOP's, a single analytical method was selected for all follow up GC-TOFMS experiments. One such experiment again performed between researchers from all three laboratories, studied spatial metabolite patterns in melon fruit throughout their development from immature fruit to commercial ripeness. Polar metabolite data from GC-TOFMS was combined with that from 1H-NMR and LC-MS, data based upon the analysis of VOC's by SPME-GC-TOFMS and data based upon micronutrient content were also integrated thus providing many insights as to how the fruit develops through changes in primary and secondary metabolites and how these related to further changes in the VOC profiles.

Erban Abstract: Within the last years robust GC-MS metabolite fingerprinting and profiling platforms and routines were established for fresh plant material in a range of 50mg to 150mg basic raw material. Since the scientific progress shows a tendency towards observations of more specific cell-types and organs from plants, miniaturization during sample-preparation became necessary. We present a range of miniaturization methods during sample-preparation, such as manual tissue micro dissections, liquid micro sampling and laser-microdissection coupled to laser pressure catapulting of cyrosections, in short laser-microdissections (LMD). An outlook into the potential of GC×GC-TOFMS will be given, with focus on plant cyrosamples prepared with LMD.

Keck Abstract: The microalgae *Chlamydomonas reinhardtii* is able to produce molecular hydrogen under specific conditions which is an important aspect with regard to renewable, CO2-free

energy supply. In this study, we analysed the metabolite profiles of the high hydrogen producing strain Stm6Glc4 and the wild type cc406 (WT) before and during the hydrogen production phase. We have established GC×GC analysis coupled to fast TOFMS (Leco Pegasus IV) to analyse hydrophilic extracts of *Chlamydomonas reinhardtii*. GC×GC-TOFMS results in a good separation of these complex samples, which expands the chromatographic plane for coeluting compounds. Using the GC×GC-TOFMS together with the statistical compare feature of the LECO ChromaTOF software we were able to obtain a detailed view of metabolomic changes during hydrogen production.

Sponsor Lunch Session Monday, 28 June 2010
Parallel session 1B: Sponsored by Waters
Room: E104 -107

13.00 – 13.55 Technology showcase: Waters

13.00 – 13.55 Metabolic profiling workshop

From the post genomics and proteomics era, metabolic profiling (metabolomics/metabonomics) has emerged as a vital new area of research. Metabolic profiles of biological fluids contain a vast array of endogenous low-molecular weight metabolites, the composition of which depends upon the sample type (plasma urine, bile etc) and factors such as the species, age, sex, diet of the organism from which the sample derives and indeed even the time of day at which the sample was taken. Disease, drugs (and other biologically active molecules) perturb concentrations and fluxes in intermediary metabolic pathways. The response to this perturbation involves adjustment of intracellular and extracellular environments in order to maintain homeostasis.

Both the perturbations and the adjustments are expressed as changes in the normal composition of the biofluids or tissues that can be characteristic of the nature or site of the disease process, toxic insult, pharmacological response or genetic modification.

This presentation guides the auditorium through an efficient and robust work-flow for profiling endogenous metabolites. Following an introduction to the principle work-flow, a set of data will be mined using a variety of multivariate statistical methods, facilitated by MarkerLynx XS software.

Speaker Richard Lock, Waters UK

Monday, 28 June 2010

Parallel session 2A

14.00 - 15.30	**METABOLOMICS & BIOMARKER DISCOVERY** **Chair: Rima Kaddurah-Daouk (USA)**	**Room**
14.00 – 14.30 Invited speaker	**Rick Dunn (UK)** Traversing the methodological gap - defining the role of metabolism in human function at the systems level with advances in large-scale mass spectrometry-based studies	Forum
14.30 - 14.50 Selected speaker	**David Broadhurst (Ireland)** Uncovering the Metabolomic Signature of Intrauterine Growth Restriction in Early Pregnancy: Search, Discovery and Validation	Forum
14.50 - 15.10 Selected speaker	**Guowang Xu (China)** Where and how to "fish" disease-related metabolite biomarkers by using LC-MS based metabonomics	Forum
15.10 - 15.30 Selected speaker	**Matej Oresic (Finland)** Metabolome in schizophrenia and related psychotic disorders: Findings from the general population cohort	Forum

L2A-001

Traversing the methodological gap - defining the role of metabolism in human function at the systems level with advances in large-scale mass spectrometry-based studies

Warwick B. Dunn (1), David Broadhurst (2), Wanchang Lin (3), Marie Brown (3), Mamas Mamas (3), Paul Begley (3), Sue McIntyre (3), Nadine Anderson (3), Ian Wilson (4), Andrew Nicholls (5), Ludwig Neyses (3), The Husermet consortium, Douglas B. Kell (3) and Roy Goodacre (1). 1 The Manchester Centre of Integrative Systems Biology and The Centre for Advanced Discovery and Experimental Therapeutics, The University of Manchester, UK 2 University College Cork, Ireland 3 The University of Manchester, UK 4 AstraZeneca, UK 5 GlaxoSmithKline, UK

Most metabolomic studies have been relatively small (n<100) and consist of single analytical experiments. However, the large variations in genotype, environment and lifestyle of humans produce a diverse range of phenotypes as detected in biofluid or tissue metabolomes. Chromatography-mass spectrometry and NMR platforms provide complementary data for systems-wide studies of metabolism. NMR can provide the study of 1000s of samples in epidemiological studies without drift in response and chemical shift. However, drift in response, retention time and mass accuracy is observed during the analysis of 10-100s of sample by chromatography-mass spectrometry. The HUSERMET project, a BBSRC/AZ/GSK-funded study, has the objective to define the human diversity in serum metabolomes of greater than 3000 subjects and relate this diversity to causal factors (age, gender, lifestyle, health and others). Developments in the project enabled chromatography-mass spectrometry platforms to be applied in this epidemiological-type study. The large biological experiment (n=1000s) was divided, with appropriate experimental design, in to smaller analytical experiments where n<120 samples. These apply Quality Control (QC) samples to provide (i) univariate quality assurance of data within a single analytical experiment, (ii) univariate normalisation of data to the QC within a single analytical experiment and (iii) integration of data from multiple analytical experiments. This presentation will discuss small and large-scale metabolomic studies; (i) the application of GC-MS and LC-MS in the

HUSERMET project including a discussion on biological discoveries and (ii) the application of discovery and validation studies in an investigation of metabolism during cardiac ischemia and how metabolism plays a major role in the development of cardiac arrhythmias and acute myocardial infarction.

L2A-002

Uncovering the Metabolomic Signature of Intrauterine Growth Restriction in Early Pregnancy: Search, Discovery and Validation.

Broadhurst, D. I. (1), Dunn, W. B. (2), Horgan, R. P. (1), Brown, M. (2), Kenny, L. C. (1) (1) The Anu Research Centre, Department of Obstetrics and Gynaecology, University College Cork, Cork University Maternity Hospital, Cork, Ireland. (2) The Manchester Centre for Integrative Systems Biology, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK.

Intrauterine growth restriction (IUGR) refers to a condition in which a fetus is unable to achieve its genetically determined potential size. IUGR affects 3-10% of nulliparous pregnancies and confers significantly increased risks of perinatal morbidity and mortality. Several biomarkers have been proposed for prediction of IUGR but there is currently no clinically useful early screening test. Accumulating evidence suggests that the aetiology of IUGR is associated with poor placental vascular development in early pregnancy such that the fetus does not receive the necessary nutrients and oxygen needed for growth and development. As trophoblast differentiation and invasion begins in early pregnancy, altered levels of circulating factors should be detectable throughout pregnancy. We examined metabolomic differences in three independent studies (a) venous cord blood plasma from normal babies and babies with IUGR, (b) plasma from a rat model of fetal growth restriction: reduced uterine perfusion pressure (RUPP) rat, (c) plasma samples obtained at 15±1 weeks gestation from women who subsequently delivered an IUGR baby and matched controls. All samples were analyzed using Ultra Performance Liquid Chromatography coupled to a LTQ-Orbitrap Mass Spectrometer. In both the cord blood and RUPP studies there was comprehensive disruption of plasma metabolism due to IUGR. Multivariate predictive models gave area under the Receiver Operator Characteristic (AuROC) curve of 1 in both cases. Disruption was specific to lipid and amino acid metabolism. When the time-of-disease biomarker signature of cord blood was validated using the pre-symptomatic 15-week maternal blood, a multivariate predictive model with AuROC of 0.96 was produced. This is the first time any clear biomarkers for IUGR have been discovered using any technology. A pre-symptomatic predictive test at 15 weeks gestation will have a significant impact on clinical care, allowing scarce resources to be concentrated on those at greatest risk.

L2A-003

Where and how to ‘fish’ disease-related metabolite biomarkers by using LC-MS based metabonomics

Xu,G.W.,Wang,W.Z.,Yin,P.Y.,Huang,Q.,Zhao,X.J.,Chen,S.L.,Chen ,J.,Zhou,L.N.,Kong,H.W., Lu,X. CAS Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R &A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

Complex diseases such as cancer, diabetes and obesity arise from an intricate interaction of inherited–'nature' and environmental–'nurture' factors. Their (early) diagnosis is very difficult, especially based on only singular biomarker. The move toward "multi biomarkers" is a necessity. Metabonomics is a technique based on analyzing as many endogenetic metabolites as possible. It has shown the great potential of finding biomarker group for disease diagnosis. Normally Blood and urine are the best samples for clinical assay or metabonomics. Unfortunately, they are similar to a reservoir, any physiological and pathological changes will be integrated into urine or blood, the discovery of biomarkers taking them as samples become very difficult. To resolve this disadvantage, we are using the strategy from tissue to body fluids, from bile to blood and from animal models to human to ‘fish’ the potential metabolite biomarkers. Based on it, it is possible to find the potential biomarkers from tissue, bile or animal, then to validate them in human body fluids, it can reduce the disturbance of other non-pathological factors, in the meantime find early diagnosis biomarkers which are usually difficult in human samples because of the difficult in early diagnosis. As the examples, we shall report our newest work on the metabolic biomarker discovery of liver cancer by using non-target metabonomics analysis to ‘fish’ the potential differential metabolites from the tissue, bile or animal model based on LC-MS/MS, and target metabolic analysis in human serum/plasma to confirm the found biomarker group based on selective ion LC-MS or MRM LC-MS.

L2A-004

Metabolome in schizophrenia and related psychotic disorders: Findings from the general population cohort

Orešič, M. (1), Tang, J. (1), Sysi-Aho, M. (1), Seppänen-Laakso, T. (1), Hyötyläinen, T. (1), Perälä, J. (2), Suvisaari, J. (2): (1) VTT, Espoo, Finland; (2) National Institute for Health and Welfare, Helsinki, Finland

Persons with schizophrenia and other psychotic disorders have high prevalence of obesity, impaired glucose tolerance, and lipid abnormalities, particularly hypertriglyceridemia and low HDL [1]. More detailed molecular information on the metabolic

abnormalities may reveal clues about the pathophysiology of these changes, as well as about the disease specificity. From a population-based study [2], we analyzed serum samples from all persons with DSM-IV primary psychotic disorder (schizophrenia n=45, other nonaffective psychosis (ONAP) n=57, affective psychosis n=37) and controls matched by age, sex, and region of residence. We applied lipidomics using UPLC/MS and metabolomics using GCxGC-TOFMS [3]. A total of 360 molecular lipids and 201 metabolites were measured. Bayesian model based clustering [4] was performed to reduce the data into a subset of 13 lipid and 8 metabolite clusters, respectively. We used linear mixed models to analyze the effect of diagnosis on metabolic cluster variables after adjusting for antipsychotic medication use, nutritional variables, smoking, obesity, waist circumference, and type 2 diabetes. The effect of schizophrenia was independently associated with 4 metabolite and 5 lipid clusters, which e.g. included insulinotropic metabolites and saturated triacylglycerols. These metabolic abnormalities were much less pronounced in persons with ONAP, and persons with affective psychosis did not differ from their matched controls. The schizophrenia-associated clusters strongly correlated with gamma-glutamyl transferase values, despite significantly lower alcohol consumption as compared to controls. Our findings suggest that specific lipid abnormalities related to saturated triglycerides are specifically associated with schizophrenia. These affected lipids are known to be enriched in VLDL particles [5], thus VLDL secretion and the amount of liver fat may play a role in schizophrenia. [1] Suvisaari JM, et al. J Clin Psychiatry 2007; 68:1045-55. [2] Perälä J, et al. Arch Gen Psychiatry 2007; 64:19-28. [3] Oresic M, et al. J Exp Med. 2008;205(13):2975-84. [4] Fraley C and Raftery AE. J. Classif. 2003; 20: 263-286. [5] Kotronen A, et al. Diabetologia. 2009 Apr;52(4):684-90.

Monday, 28 June 2010

Parallel Session 2B		
14.00 - 15.30	ENVIRONMENT & ECOLOGY	
	Chair: Jake Bundy (UK)	Room
14.00 – 14.30		
Invited speaker	**Nicole van Dam (Netherlands)**	
	Metabolomics for plant-herbivore interactions: Let's go wild!	E 104 - 107
14.30 - 14.50		
Selected speaker	**Dan Bearden (USA)**	
	Environmental Metabolomics with Marine Organisms	E 104 - 107
14.50 - 15.10		
Selected speaker	**Simone Rochfort (Australia)**	
	Metabolomics for Functional Metagenomics	E 104 - 107
15.10 - 15.30		
Selected speaker	**Andrew Southam (UK)**	
	A Multi-Platform Metabolomic Approach to Investigate Toxicant Induced Disruption of Sexual Development in Wild Male Fish	E 104 - 107

L2B-001
Metabolomics for plant-herbivore interactions: Let's go wild!

Nicole M. van Dam, Radboud University Nijmegen, PO Box 9010, 6500 GL Nijmegen, The Netherlands

Plant metabolomics has been mostly applied to analyse the metabolomes of crops and a hand-full model species. However, metabolomic analyses may also be extremely valuable for plant ecologists studying wild plants in their natural environment. Because of the untargeted nature of metabolomic platforms, the analyses provide a comprehensive overview of both the primary and secondary plant metabolome. This is especially valuable for chemical ecologists studying plant-herbivore interactions, as both primary and secondary metabolites together determine the performance and preference of herbivores. In the early days of chemical ecology various theories regarding optimal defence strategies for plants have been postulated. Depending on the severity of the damage, plants may employ different strategies. For example, when plants are attacked by only a few small insect herbivores, they may increase their chemical defences at the feeding site to deter or kill the herbivores. When larger herbivores are feeding or when there is a massive insect outbreak, plant tissue will be removed much faster. In those cases, plants more likely rely on tolerance strategies which involve the reallocation of primary metabolites to 'safe havens'. These stored resources are used to fuel plant regrowth after the herbivores have moved on. Originally, defence and tolerance were thought to be contrasting strategies. However, it is more likely that they are at the ends of a continuous spectrum of survival strategies that plants have evolved in the evolutionary arms-race against herbivores. So far, the chemistry underlying these strategies has mainly been assessed using targeted analyses. This limits our understanding of why and how plants have evolved these strategies to survive in a world full of herbivores. By using metabolomic approaches, optimal defence theories may be better substantiated and even expanded. In this presentation I aim to convey my enthusiasm about the chemical ecology of plant-herbivore interactions in wild plant species. I hope that this will elicit the scientific curiosity of chemists and bioinformaticians, and that I can convince them to join ecologists in resolving these classic ecological theories using modern metabolomic methodologies.

L2B-002
Environmental Metabolomics with Marine Organisms

Daniel W. Bearden (1), Arezue F. B. Boroujerdi (1), Tracey Schock (1) (1) National Institute of Standards and Technology, Analytical Chemistry Division, Hollings Marine Laboratory, 331 Ft. Johnson Rd., Charleston, SC 29412 USA

The metabolomic response of marine organisms in carefully planned experiments is key to understanding the effects of anthropogenic and natural stressors in coastal regions. In individual case studies, organisms such as fish, crabs and bacteria are candidates for investigation. In addition, efforts to improve data quality and improve comparability of interlaboratory results are important [1]. Several marine organisms have recently been assesed for suitability in metabolomics studies. For example, the metabolic trajectory for Atlantic blue crabs (Callinectes sapidus) subjected to injection with the microbe Vibrio campbelli, which causes nodules to form in the gill and impacts oxygen uptake, is initially different than treatment with 2, 4-Dinitrophenol, which is a known uncoupler of oxidative phosphorylation. [2] The microbe Vibrio coralliilyticus has been found in high concentrations in the bleached coral Pocillopora damicornis, but not in healthy corals. When inoculated into healthy corals at temperatures above 25 °C, V. coralliilyticus caused bleaching. A relationship between elevated temperature and virulence of V. coralliilyticus has been hypothesized. Recent metabolic studies indicate distinct metabolic responses of V. coralliilyticus at different temperatures, and the differential production of metabolites indicates a complex response to the elevated temperature. [3] 1. M. R. Viant, D. Bearden, J. G. Bundy, I. Burton, T. W. Collette, D. R. Ekman, V. Ezernieks, T. Karakach, C. Y. Lin, S. Rochfort, J. S. de Ropp, Q. Teng, R. S. Tjeerdema, J. Walter and H. Wu, "International NMR-based Environmental Metabolomics Intercomparison Exercise", Environmental Science and Technlogy. 43(1), 219-225 (2009). 2. T. B. Schock, K. G. Burnett, L. E. Burnett, L. Thibodeaux, D. A. Stancyk, D. W. Bearden: "Metabolomic Analysis of Atlantic Blue Crab, Callinectes sapidus, Hemolymph Following Oxidative Stress", Metabolomics, published on-line 20 Jan 2010, doi:10.1007/s11306-009-0194-y. 3. A. F. B. Boroujerdi, A. Meyers, E. C. Pollock, S. L. Huynh, T. Schock, M. Vizcaino, P. J. Morris, and D. W. Bearden: "NMR-Based Microbial Metabolomics and the temperature-dependent coral pathogen Vibrio coralliilyticus", Environmental Science & Technology, 43(20), 7658–7664 (2009).

L2B-003
Metabolomics for Functional Metagenomics

Rochfort, S (1), Ezernieks, V (1), Sawbridge, T (1), Cocks, B (1), Kitching, M (2), Prell, M (3), Hayden, H (4), Mele, P (4), Methe, B (5), Lewis, M (5): (1) Biosciences Research, Biosciences Research Division, Department of Primary Industries, 1 Park Drive, Bundoora, Victoria, Australia, 3083. (2) Future Farming Systems Research, Department of Primary Industries, 621 Sneydes Rd, Werribee, Victoria, Australia, 3030. (3) Hochshule Niederrhein, 47799 Krefeld, Germany. (4) Bioprotection, Biosciences Research Division, Department of Primary Industries, 1 Park Drive, Bundoora, Victoria, Australia, 3083. (5) J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, United States of America.

Metagenomic sequencing is currently being employed to assess microbial diversity and function at the genetic level for systems including, soils, seawater, the human microbiome and the bovine rumen. Two Australian soils (Calcarosol remnant and managed) have recently been sequenced to explore genetic and functional diversity. The meta-metabolomics analysis of these, and a larger set of paired site remnant and managed soil samples, was simultaneously investigated. Organic extracts of soils were examined by LCMS and NMR to explore the 'meta-metabolomics' of the soil system. The soil samples were also analysed by traditional inorganic chemistry methods as well as by MIR. Interestingly, metabolomics analysis more accurately reflected broad land use categories (native remnant or agriculturally managed) while the inorganic measures and MIR analysis better reflected soil locale. The metabolomic analysis also provided a snapshot into the functional state of the soil community, with organic nutrients (e.g. free sugars) being key differentiators associated with land use. These metabolomics findings correlated with differences in carbon and nitrogen cycling genes discovered through whole shotgun sequencing of the soil metagenome. A number of the soils also demonstrated antibiotic activity, a key factor in soil health and important for disease suppression in cropping systems. Metabolomics analysis along with isolation and structure elucidation of one bioactive soil constituent will be described. Correlation with metagenomic sequencing and the opportunities for data integration from meta-omic approaches will be discussed along with the potential pitfalls.

L2B-004
A Multi-Platform Metabolomic Approach to Investigate Toxicant Induced Disruption of Sexual Development in Wild Male Fish

Southam,A.D.(1),Hines,A.(1),Lange,A.(2),Hill,E.M.(3),Tyler,C.R.(2) and Viant,M.R.(1) (1)School of Biosciences,Birmingham University,B152TT,UK (2)School of Biosciences,Exeter University,EX44PS,UK. (3)School of Life Sciences,Sussex University,BN19QG,UK.

Endocrine disruption in wild fish populations is of major international concern. There is a critical need to discover biomarkers that can be measured rapidly to determine the causative class(es) of pollutants and inform directly on reproductive fitness. This presentation will describe a metabolomic study investigating the effects of an endocrine disrupting chemical, the anti-androgen fenitrothion, on a freshwater fish species, the roach (Rutilus rutilus). Sexually mature male fish were exposed to 2, 20 and 200 μg/L of fenitrothion for 28 days and their tissues harvested for histology

and metabolomics. Two non-targeted metabolomic techniques, NMR spectroscopy and direct infusion Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), were used to identify changes to the polar metabolome in liver and gonad tissue. Additionally, ultra-high pressure liquid chromatography time-of-flight (UPLC-TOF) MS was utilised as a targeted metabolic analysis to investigate perturbations of sex steroids in gonad tissue. Univariate statistical tests of each variable within the non-targeted datasets (with false discovery correction, FDR<10%) revealed a much greater change to the metabolome of liver tissue (9.9% of NMR bins and 5.8% of MS peaks were significant) compared to gonad tissue (0% of NMR bins and 1.6% of MS peaks were significant). This was mirrored by multivariate PCA results and indicated that fenitrothion exerted a greater metabolic effect in the liver compared to the gonad. Specific metabolic effects included highly significant (FDR<10%) changes to the levels of creatine (NMR:p=4.5x10-7;MS:p=1.9x10-5) and phosphocreatine (NMR:p=4.3x10-4;MS:p=1.3x10-3) in liver tissue only. The MS data showed significant increases of N-acetyl-phenylalanine as fenitrothion dose was increased in both liver (p=1.3x10-3) and gonad (p=3.8x10-4). Additionally, FT-ICR-MS identified highly significant changes in a fenitrothion-associated metabolite, desmethylfenitrothion, and a compound specifically linked to its detoxification, S-methyl-glutathione. UPLC-TOF revealed a significant decrease in cortisone (p=0.004) and a near significant increase in 11-hydroxyandrostenedione (p=0.057) as the fenitrothion dose was increased, confirming an endocrine disruptive effect. To conclude, these results highlight how a combination of NMR- and MS-based metabolomics can give a comprehensive insight into the potential mode of action of fenitrothion.

Monday, 28 June 2010

Parallel Session 3A

16.00 - 17.30	**DRUG DISCOVERY & DRUG DEVELOPMENT** **Chair: Bruce Kristal (USA)**	**Room**
16.00 - 16.30 Selected speaker	**Michael Reiley (USA)** Metabolomics in Drug Discovery: From Screening to Mechanistic Insights	Forum
16.30 - 16.50 Selected speaker	**Catherine Winder (UK)** Global metabolic profiling as a tool to determine the multiple intervention sites for targeted chemical effectors	Forum
16.50 - 17.10 Selected speaker	**Alain van Gool (Singapore)** Integrated Molecular Profiling Approaches towards Biomarker Discovery in Neuroscience	Forum
17.10 - 17.30 Selected speaker	**Johan Lindberg (Sweden)** Safety Biomarker discovery/qualification for drug induced liver injury	Forum

L3A-001

Metabolomics in Drug Discovery: From Screening to Mechanistic Insights

Michael Reily, Bristol-Myers Squibb

Metabolomics technology has been employed by the Pharmaceutical industry for well over a decade. As with other “omics” approaches, metabonomics is under increased scrutiny due to shrinking budgets and shortened timelines. Many companies who helped pioneer the technology have reduced or eliminated active involvement. However, metabolomics still delivers value in the quest for effective and safe therapeutics. We will present four case studies where a standardized metabolomics platform has proven useful and decisive for project teams. Three of these derived from the application of the technology in early drug discovery efforts as a safety screening paradigm and one from an investigational toxicity study aimed at a significant preclinical toxicological problem, phospholipidosis. In all four of these cases, metabolomics delivered information that altered the course of a project or influenced decisions which would not have been possible had the technology not been applied.

L3A-002

Global metabolic profiling as a tool to determine the multiple intervention sites for targeted chemical effectors.

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The complexity of biological systems severely limits the ability to predict the role of novel pharmacological agents on a host organism. In an ideal scenario the effect of a chemical inhibitor would have a specific point of interaction in a biological network. However, it is likely that other changes in the network may be observed either as a result of knock-on effects to the primary mode of action or secondary effects unrelated to the original target site. The reconstruction of metabolic networks (for example, yeast(1)) and the application of global metabolic profiling provide a powerful tool to investigate the effects of drug intervention on biological systems. It is well-known that cellular effects are amplified in the metabolome and as such will provide valuable information on subtle changes occurring in the biological network as a result of drug intervention. The effect on

the exometabolome or overflow metabolism also provides a sensitive measure of changes on the biological network. Therefore measurements on both the intracellular and exometabolome are preferential to obtain a holistic understanding of the system changes. In order to demonstrate the applicability of these tools to drug mode of action studies, we have exposed cultures of Saccharomyces cerevisiae to a number of chemical inhibitors (methotrexate, mizorbine, methionine sulphoxime and fluconazole) with known modes of action in metabolism. Measurements of both the intracellular and exometabolome were performed with complementary analytical platforms (GC-ToF-MS and UPLC-LTQ/Orbitrap-MS) to maximise the coverage of the metabolic network. A combination of univariate and multivariate statistical analyses were employed to identify changes in the metabolome at the primary mode of action, and to identify the widespread responses in the biological network to the chemical inhibitors. Further work will expand to the human metabolic reconstruction. (1)Herrgard et al., 2008, Nature Biotech 26, 1155-1160

L3A-003

Integrated Molecular Profiling Approaches towards Biomarker Discovery in Neuroscience

Gool, van AJ(1), Ingelse, B(2), Sollewijn Gelpke, M(2), Heisterkamp, S(2), Ruigt, G(2): Merck Research Labs, MSD, (1) Singapore,(2)The Netherlands.

To successfully develop innovative drugs in neuroscience, pharmaceutical researchers cannot limit themselves to classical methods to classify patients and to assess clinical efficacy following drug administration. In many cases, a black box exists that leaves many questions unanswered, including disease diagnosis, prognosis, underlying mechanism, extent of drug exposure and target engagement, adverse events and efficacy of the drug. To reveal this black box, drug developers need to follow a rational, data-driven approach linking basic Research to clinical Proof of Concept in man. This strategy will enable early selection of both the best candidate medicines and the patient population that is most likely to respond to this drug(1). In neuroscience, approaches like non-invasive imaging and molecular profiling are increasingly being applied. By combining genetics, transcriptomics, proteomics and metabolomics, project teams can better understand the mechanism underlying the clinical symptoms leading to better patient selection and more personalized treatment regimes. This approach benefits greatly from external collaborations, whereby the collaboration partners join forces to work on mutually interesting translational research projects. Examples from various neuroscience biomarker projects will be discussed. (1) AJ van Gool, B Henry, ED Sprengers. From Biomarker Strategies to Biomarker Activities, and back. Drug Discovery Today, 2010 Feb;15(3-4):121-6

L3A-004

Safety Biomarker discovery/qualification for drug induced liver injury

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Drug Induced Liver toxicity represents a major challenge for drug development and patient healthcare. There is a need for more informative biomarkers to predict, prevent and monitor drug-induced liver injury. Biliary toxicities such as Bile Duct Hyperplasia(BDH), a pre-stage to the more overt cholestasis, can be monitored preclinically using histopathology. Safety Biomarkers are necessary in order to progress compounds with inadequate margins for BDH to the clinic. Bile acids have important roles in liver physiology / pathology and levels of total bile acids and bile flow are commonly used as general liver function markers. We have used a non targeted analysis approach based on LC/MS and NMR technology to provide extensive bile acid and bile profiling. Depending on species and selection of matrix (bile, plasma or urine) approximately 60 bile acids were detected, some which were non expected or unknown. The LC/MS analysis of bile identified several bile acids, conjugated and unconjugated, as biomarker candidates for BDH. Further mechanistic understanding and biomarker qualification were achieved by analysis of the plasma and urine samples, and the influence of different species and drug treatments on the bile acid pattern. The proton NMR platform was primarily used to profile bile. This profile is complimentary to the LC/MS data and the ratio of conjugated bile acids (glycin and taurine) vs. unconjugated is determined. Glucose and the concentration of the bile was found to be highly correlated to the BDH toxicity in rat. Use of this untargeted metabolomics approach has led to new possibilities for understanding unexpected toxicities and elucidation of toxicological mechanism.

Monday, 28 June 2010

Parallel Session 3B

16.00 - 17.30	**PLANT PHYSIOLOGY** **Chair: Kazuki Saito (Japan)**	**Room**
16.00 - 16.30 Invited speaker	**Lothar Willmitzer (Germany)** Changing environments lead to extensive rewiring of metabolic networks in Arabidopsis thaliana	E 104 - 107
16.30 - 16.50 Selected speaker	**Ric de Vos (Netherlands)** Untargeted comparative plant metabolomics to determine gene function in vivo	E 104 - 107
16.50 - 17.10 Selected speaker	**Charles Warren (Australia)** What does it take to tolerate water and nutrient deficiencies? Insights from stress-tolerant plant species	E 104 - 107
17.10 - 17.30 Selected speaker	**Mathew Davey (UK)** Cold acclimation duration and freezing temperatures - distinct intra-specific metabolic phenotypes of two geographically isolated plant populations	E 104 - 107

L3B-001

Changing environments lead to extensive rewiring of metabolic networks in Arabidopsis thaliana

Lothar Willmitzer, Camila Caldana, Thomas Degenkolbe.
Max-Planck-Institut für Molekulare Pflanzenphyiologie Potsdam-Golm, Germany

In response to changed environmental conditions plants like other organisms react by extensive reprogramming at the cellular and organismal level, as exemplified by multiple changes which occur at all levels of the realization of genomic information. A graph-based analysis of metabolite data in which the changes of *Arabidopsis thaliana* following the shift from ambient conditions to seven different conditions over a period of six hours was monitored using a dense-sampling approach in which material was harvested every 20 minutes will be presented. Extensive re-wiring of networks is observed as a function of these environmental perturbations. Transformation of condition specific networks into one another follows both temperature and light gradients. The combined network reflects known biochemical pathways. Correlation with changes in gene expression of corresponding biochemical pathways is observed in a condition dependent manner.

L3B-002

Untargeted comparative plant metabolomics to determine gene function in vivo.

Ric de Vos1,2,3, Ana-Rosa Ballester1,2, Arnaud Bovy1,2, Jules Beekwilder1, Wessel van Leeuwen4, Nicole van Dam5, Ralph Stracke6, Bernd Weisshaar6, Avital Adato7, Ilana Rogachev7, Asaph Aharoni7, and Robert Hall1,2,3 1 Plant Research International, PO Box 16, 6700 AA Wageningen 2 Centre for BioSystems Genomics, PO Box 98, 6700 AA Wageningen, The Netherlands 3 Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC Leiden, The Netherlands 4 Nickerson-Zwaan/Limagrain. PO Box 4, 1749 ZG Warmenhuizen, The Netherlands 5 Netherlands Institute of Ecology, PO Box 40, 6666 ZG Heteren, The Netherlands 6 Genome Research, Bielefeld University, 33594 Bielefeld, Germany 7 Department of Plant Sciences, Weizmann Institute of Science, PO Box 26, Rehovot 76100, Israel

Metabolomics is rapidly growing as key technique to identify differences and similarities between plant samples. By using comprehensive comparative metabolomics approaches in natural or specifically induced plant mutants, detailed insight into the alterations in the metabolite composition, and hence in the in vivo gene functioning, can be obtained. Such detailed

information is essential in breeding programs directed towards new plant varieties with improved or specific quality characteristics. Several examples of determining in vivo gene functioning in plants using comprehensive untargeted metabolomics techniques will be highlighted. For instance, we determined the effect of a natural, single nucleotide mutation, resulting in light-hypersensitivity, on the metabolite composition of ripe tomato fruit. Also, while a specific mutation resulting in pink tomatoes has been known for 50 years, by using comparative metabolomics approaches, combined with transcriptomics, we have recently been able to identify the mutated gene and its in vivo effect on fruit metabolite composition. Likewise, comparative metabolomics has been applied to the model plant Arabidopsis thaliana, for instance to characterize a gene family related to flavonol biosynthesis, as well as genes involved in the production and transport of glucosinolates, a group of anti-herbivore compounds. The examples presented indicate how powerful comparative metabolomics can be as a tool to study in detail the in vivo functioning of genes in plants. Such information is essential in breeding activities directed towards new crop varieties with improved or specific quality characteristics.

L3B-003

What does it take to tolerate water and nutrient deficiencies? Insights from stress-tolerant plant species

Charles Warren, University of Sydney

Metabolomics is providing major insights into what it is that makes plants tolerant of abiotic stresses. The field is growing rapidly, but to date has primarily focused on model plant species. Unfortunately the special traits that make model species easy to work with also makes them non-representative of many plants (e.g. perennials and anything stress tolerant). It is axiomatic that to uncover what it is that makes plants stress tolerant we ought to examine stress tolerant species, rather than solely focusing on species that are easy to study. To investigate what it takes to be tolerant of water and nutrient deficiencies seedlings of Eucalyptus and Acacia were exposed to prolonged drought stress (3+ months) or a wide range in N and P availability. Polar metabolites in leaves and roots of stress-tolerant plants species were quantified by GC-MS of TMS derivatives (for most primary metabolites) and tBDMS derivatives (for amines). Mass spectra were deconvoluted and metabolites identified based on retention index and comparison with purified standards, commercial libraries (Fiehn, NIST), public libraries (Golm metabolome database, GMD) and in-house EI and methane-CI libraries. Methane CI was particularly useful for confirming identification and differentiating among isomers and other closely related metabolites Five of the ten most abundant metabolites in stress-tolerant *Eucalyptus* and *Acacia* species were either absent or at low concentrations in the model species *Arabidopsis*. These metabolites included cyclohexanepentols, methylated inositols, proline homologues, and shikimic acid. In most species, exposure to soil water stress for 3-4 months significantly affected concentrations of 20-30% of metabolites. Responses of the metabolome to N and/or P were shown to be complex and not restricted to any particular class of compounds. The significance and functional interpretation of the metabolic responses to soil water stress and limitation by N and/or P will be discussed.

L3B-004

Cold acclimation duration and freezing temperatures – distinct intra-specific metabolic phenotypes of two geographically isolated plant populations

Matthew P. Davey(1,2), FI Woodward(2) and WP Quick(2) : (1) Department of Plant Sciences, Downing Street, Cambridge, CB2 3EA, UK (2)Animal and Plant Sciences, Western Bank, University of Sheffield, Sheffield, UK

Plant populations growing at the margin of their range may exhibit traits that indicate genetic differentiation and adaptation to their local abiotic environment. *Arabidopsis lyrata* subspecies petraea, is sparsely distributed across Europe. Our goal is to measure the similarities and differences between geographically isolated populations of this species to provide a clearer understanding of the mechanisms involved in limiting plant distribution. We have found that the survival of young *A. petraea* after exposure to sub-zero temperatures is dependent on the duration of pre-shock cold acclimation time. Therefore, we investigated whether such differences in cold acclimation duration and survival can be detected at the metabolic level. Seeds of European *A. l. petraea* were obtained from populations along a latitudinal gradient (High altitude Norwegian and Lowland Irish) followed by germination and growth in a controlled cabinet environment. These plants were subjected to either control, cold (2 ºC) acclimation (2 or 14 days) and sub-zero shocks. Metabolite fingerprints were obtained for populations of *A. l. petraea* by direct-injection mass spectrometry. Metabolite fingerprints of each population were assessed using principal component analysis (PCA). PCA of metabolite fingerprints revealed metabolic phenotypes for each population, cold acclimation duration and sub-zero temperatures. Cold acclimation had a clear effect on the metabolic fingerprints of the Norwegian samples, with clear clustering of control and cold acclimation samples. However, the difference between control and acclimation times in the metabolic fingerprints of the Irish samples was less distinct. As the acclimated Irish plants were metabolically similar to control plants, this may help explain why after a 2 day acclimation period prior to sub-zero shocks, the Irish plants perform better in terms of percent survival after exposure to -9 ºC than Norwegian plants. These results suggest that there is significant natural variation in metabolism among these populations of *A. l. petraea.*

Tuesday, 29 June 2010

Plenary session		
09.00 - 10.30	**PLENARY SESSION P2** **ADVANCED TECHNOLOGIES** **Chair: Masura Tomita (Japan)**	**Room**
09.00 - 09.50 Keynote speaker	**Graham Cooks (USA)** Mass Spectrometry and Metabolomics: Miniaturization, Ambient Ionization, Microorganisms and Disease Diagnosis	Forum
09.50 - 10.10 Selected speaker	**Justin van der Hooft (Netherlands)** Systematic metabolite identification using HPLC-MSn fragmentation trees and LC-MS-SPE-NMR	Forum
10.10 - 10.30 Selected speaker	**Clare Daykin (UK)** Interactive Metabolomics: A Powerful New Technique	Forum

LP2-001

Mass Spectrometry and Metabolomics: Miniaturization, Ambient Ionization, Microorganisms and Disease Diagnosis

Cooks, R. Graham (1), Ouyang, Zheng (2) Zhang, Isabella (1) W. Andy Tao (3) (1) Department of Chemistry, (2) Weldon School of Biomedical Engineering, (3) Department of Biochemistry, Purdue University, West Lafayette, IN 47907. USA

The rapid evolution of mass spectrometry (MS) continues unabated. This presentation covers recent progress in two ares (i) ambient ionization which is characterized by minimal sample preparation, high throughput and in situ chemical analysis and (ii) miniature mass spectrometers, especially fully autonomous handheld instruments fitted with ambient ionization sources and capable of tandem mass spectrometry experiments to allow complex mixture analysis in situ. This combination of capabilities is particularly appealing for the characterization of small molecules including fatty acids, phospoholipids and other compounds in biological samples in situ. Examples are given of the application to microoganism typing using two ambient ionization methods, low temperature plasma (LTP) ionization and desorption electrospray ionization (DESI). The former uses a low power rf discharge in air, the latter employs charged microdroplets as ambient projectiles. In both cases identificaion of microorangism species is rapidly and directly made without sample preparation and in some cases distinction extends to sub-species. The same ambient ionization methods can be used to identify disease states in intact untreated tissue sections and examples of human tissue analysis allow recognition of several types of human cancers as well as recognition of the stage of the disease.

LP2-002

Systematic Metabolite Identification Using Hplc-Msn Fragmentation Trees And Lc-Ms-Spe-Nmr

Justin J.J. van der Hooft(1,2,3), Piotr Kasper(2,3), Miguel Rojas(2,3), Jacques Vervoort(1,2),and Ric de Vos(2,4,5) Justin.vanderHooft@wur.nl (1)Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands (2)Netherlands Metabolomics Centre, Leiden, The Netherlands (3)LACDR, Leiden University, Leiden, The Netherlands (4)Plant Research International, Wageningen, The Netherlands (5)Centre for Biosystems Genomics, Wageningen, The Netherlands

The exact, unbiased and complete analysis of the metabolite content of biological extracts becomes increasingly important. The wide spectrum of structurally diverse metabolites asks for the use of state-of-the-art analytical technologies, which enable rapid annotation of both known and unknown metabolites. New developments in both mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, aimed towards systematic metabolite identification, will be presented. Firstly, a reproducible high resolution MSn spectral tree method that systematically fragments metabolites, using a NanoMate (Advion)

– Ion Trap-Orbitrap FT-MS (Thermo) device, has been developed. The NanoMate robot facilitates small volume sample injection combined with chip-based nano-electrospray ionization, whereas the Ion Trap – Orbitrap FT-MS combination ensures robust MSn fragmentation with accurate mass determination. Using this approach, we obtained structure-specific fragmentation trees for a large series of phenolic compounds, including positional isomers and stereoisomers which so far were difficult to elucidate using MS. For instance, glucose and galactose moieties attached to the same carbon position on a phenolic core could be reproducibly discriminated. Examples of this MSn spectral tree approach as a potent tool in the identification of metabolites in biological extracts will be shown. Secondly, a rapid identification strategy for yet completely unknown compounds, based on a LC-MS-solid phase extraction (SPE)-NMR platform (Bruker), will be presented. With this platform, compounds separated by LC are on-line trapped on SPE cartridges, triggered by the TOF-MS signal, after which the compounds are transferred to the cryogenic-NMR-detection probe (600 MHz). In this manner, NMR spectra of lower abundant metabolites in a small sample volume can be generated. Examples of using this LC-MS-SPE-NMR platform in the unambiguous identification of novel metabolites in crude sample extracts, such as tomato fruit, will be provided. The examples presented will show that MSn fragmentation trees and LC-MS-SPE-NMR are powerful tools in the systematic identification of compounds in metabolomics approaches.

LP2-003

Interactive Metabolomics: A Powerful New Technique

Clare Daykin, University of Nottingham

All published metabolomics studies investigate changes in either absolute or relative quantities of metabolites. However, blood plasma, one of the most commonly studied biofluids for metabolomics applications, is a complex, heterogeneous mixture of lipoproteins, proteins, small organic molecules and ions which together undergo a variety of possible molecular interactions including metal complexation, chemical exchange processes, micellular compartmentation of metabolites, enzyme-mediated biotransformations and small-molecule-macromolecule binding. In particular, many low molecular weight (MW) compounds (including drugs) can exist both ‘free’ in solution and bound to proteins or within organised aggregates of macromolecules. To study the effects of e.g. disease on these interactions we have developed a technique termed ‘interactive metabolomics’ or i-metabolomics. i-metabolomics can be defined as: “The study of interactions between low MW biochemials and macromolecules in heterogenous biosamples such as blood plasma, without pre-selection of the components of interest”. Standard 1D NMR experiments commonly used in metabolomics allow metabolite concentration differences between samples to be investigated because the intensity of each peak depends on the concentration of the compound in question. On the other hand, the instrument can be set-up to measure molecular interactions by monitoring the diffusion coefficients of molecules. According to the Stokes-Einstein equation, the diffusion coefficient of a molecule is inversely proportional to its effective size, as represented by the hydrodynamic radius. Therefore, when low MW compounds are non-covalently bound to proteins, the observed diffusion coefficient for the compound will be intermediate between those of its free and bound forms. By measuring diffusion by NMR, the degree of protein binding can be estimated for either low MW endogenous biochemicals or xenobiotics. This type of experiment is referred to as either Diffusion-Ordered Spectroscopy (DOSY) or Diffusion-Edited Spectroscopy, depending on the type of post-acquisition data processing applied to the spectra. Results will be presented which demonstrate the non-selective modelling of metabolite-macromolecule interactions. These studies show that individuals differ not only in metabolite profile (“classical” metabolomics), but also in how these metabolites interact with their environment (i-metabolomics). Our approach is powerful, novel and calls into question the interpretation of “classical” metabolomics results obtained from NMR spectroscopy of whole blood plasma.

Tuesday, 29 June 2010

Plenary session		
11.00 - 12.30	**DATABASES, BIOINFORMATICS & DATA ANALYSIS P3**	
	Chair: Oliver Fiehn (USA)	**Room**
11.00 - 11.30		
Invited speaker	**Ben van Ommen (Netherlands)**	
	How to deal with metabolomics data and databases?	Forum
11.30 - 11.50		
Selected speaker	**Amine Ghozlane (France)**	
	Metaboflux : a method to analyse flux distributions in metabolic networks.	Forum
11.50 - 12.10		
Selected speaker	**Jeroen Jansen (Netherlands)**	
	Between-Metabolite Relationships: metabolomics with new glasses	Forum
12.10 - 12.30		
Selected speaker	**Miguel Rojas-Cheto (Netherlands)**	
	The metabolite identification pipeline based on MS fragmentation	Forum

LP3-001
How to deal with metabolomics data and databases?

Ben van Ommen, TNO, Zeist, The Netherlands

Like all '' omics' technologies, metabolomics needs standards, ontologies, information on the (bio)chemical background of its parameters, knowledge bases, embedding in biological information of its parameters, etc. Many of these have been constructed or are under construction. And like all 'omics' technologies, all of these activities are under development and range from utter chaos till complete and global agreement on structuring.This are processes that need funds, attention and coordination. These topics are usually not on the agenda of metabolomics biology researchers but the deliverables make their life very easy. Yet, the danger exists that metabolomics technology is viewed as the endpoint of biological research, which it is not. Metabolomics is a technology that conveniently analyzed the low molecular weight parameters of the phenotype. Researchers ask questions, design studies, and measure parameters (among which metabolomics), and draw conclusions based on elaboration of the results. In other words, from a biological point of view, metabolomics data and databasing needs to be integrated in study data and study databasing. Study databasing consists (in a minimal setting) of a study capture tool, databasing and pipelining of all involved analytical technologies and a part that integrated and queries all study data. As an example, the nutritional phenotype database will be discussed. Finally, data standardization allows data sharing and integrated analysis. Yet, many reasons for not sharing our data exists, at least until we have obtained all possible scientific credits. We need tools to optimize data sharing with adequate IP-protection where needed. Various options and models will be discussed.

LP3-002
Metaboflux : a method to analyse flux distributions in metabolic networks

Amine Ghozlane, Université Bordeaux, Bordeaux, France

Trypanosoma brucei is a parasitic protist of vertebrates that causes sleeping sickness in Africa. A part of its energetic metabolism, including the 6 or 7 first glycolytic step, occurs in an organelle called glycosome. A metabolic pathway for the glycosome had been built by exploiting genomic, reverse genetic and metabolomic data [1]. Some known biological constraints, such as the maintenance of the glycosomal ATP/ADP and NADH/NAD+ balances, have not been carefully addressed in the current model. We propose a modelling approach including structural pathway and metabolic flux analysis to help in the understanding of the system's structure and its semi-quantitative behaviour. We model known biological information with a stochastic Petri net (where transitions are given for the reaction

and places for metabolites) where delays can be assigned to transitions given a probability distribution. From a given set of probability distribution representing the flux amount of reactions (the input set of parameters), the simulation of the Petri net allows the exploration of the possible behaviours of the system. At the end of a run, if all input metabolites are consumed, we get concentration for intermediate and output metabolites. We integrate expected metabolites concentrations revealed by biological experiments within an objective function, and use simulated annealing and simplex minimization approach for its global optimization. Therefore, simulations are carried out by fitting the set of input parameters until the system reach the best optimization of the objective function. To explore a large set of possible behaviour of the system, several run of simulations combined with the simulated annealing approach are made. A set of solutions is given by different groups of fluxes distributions (that best fit expected metabolites concentrations), and are helpful to make some assumptions and analysis for a given metabolic system. “Metaboflux” was developed to this purpose and applied to *T. brucei*. Resulting scenarios strongly argue in favour of an unrealistic NADH/NAD+ imbalance and suggest adding to the model new metabolic pathways. A realistic solution may be to integrate the pentose phosphates to the previous model. The resulting new model was tested with Metaboflux and shows relevant fluxes scenarios. References [1] Bringaud F., Rivière L., Coustou V. (2006) Energy metabolism of trypanosomatids : adaptation to available carbon sources. Molecular and biochemical parasitology. 149: 1-9

LP3-003

Between-Metabolite Relationships: metabolomics with new glasses

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The ‘Vitruvian Man’ of Leonardo da Vinci shows that different anatomical measures are highly related in humans. These relationships are crucial to the functioning of the system. Such dependencies may also be present in the organism biochemistry: relationships between different metabolite levels might indicate the metabolic state. Usually metabolomic studies focus on metabolite levels, but studying Between-Metabolite Relationships (BMRs) may provide additional information on the metabolism. The behavior of BMRs, e.g. in time or induced by a treatment, may show how the metabolic system reacts within the experiment. Because most metabolomics studies focus on changes in metabolite levels, data analysis methods that characterize BMRs are not available yet. These BMRs can be expressed as a covariance matrix, relating all metabolites to each other. Comparing such covariance matrices of different experimental groups (e.g. differing in time or treatment) may reveal changes in the BMRs. Because covariance matrices contain only information about the relations between metabolites summarized for all biological replicates in an experiment. This structure is different from the conventional ‘sample by variable’ table used in e.g. Principal Component Analysis. Therefore data analysis methods dedicated to the analysis of covariance matrices are essential. Such methods, referred to as ‘indirect fitting’, are available for psychological research where relations between personality traits are of prime interest. We will focus on the ‘INdividual Differences SCALing’ (INDSCAL) method. This method provides models that express the emergence and disappearance of BMRs as components, analogous to Principal Component Analysis. These models are therefore relatively easy to interpret, while greatly extending the insight into the metabolic system of interest. We illustrate the different steps of the method by the analysis of several metabolomics studies. The INDSCAL results clearly reveal how experimental manipulations and dynamics may lead to changes in BMRs. This novel viewpoint on metabolic responses provides additional information that may lead to an increased understanding of metabolic systems.

LP3-004

The metabolite identification pipeline based on MS fragmentation.

Miguel Rojas-Chertó, Julio E. Peironcely, Piotr T. Kasper, Andreas Bender, Jean-Loup Faulon, Theo Reijmers, Leon Coulier, Rob Vreeken, Thomas Hankemeier. Netherlands Metabolomics Centre, Leiden, The Netherlands

Structural characterization and identification of components of complex biological mixtures is one of the central and challenging aspects within metabolomics reserach. Because of its high sensitivity and specificity, mass spectrometry is widely and successfully applied in the analysis of biological samples. Especially, high resolution multistage mass spectrometry (MSn) is used for the identification of metabolites. However, neither general methodology for the identification nor extensive databases of metabolites with multistage mass spectrometric data are available yet. Here we show different parts of the metabolite identification pipeline based on MS fragmentation data. MSn spectral trees, acquired on the LTQ-Orbitrap (Thermo) equipped with a Triversa nanoMate (Advion) nanoelectrospray ion source, form the basis of the pipeline. By using in-house developed software, the Chemistry Development Kit (CDK) and XCMS libraries and the spectral data was processed. Application of a Multi-stage Elemental Formula (MEF) tool resolved the

elemental composition of the parent compound and its fragment ions. Repeatability, reproducibility and robustness of fragmentation tree acquisitions were tested by changing experimental conditions (fragmentation energy, isolation width, etc.) and varying the concentration of the metabolite of interest. An acquisition protocol is established for reliable and reproducible acquisition of mass spectral trees. Currently, a database containing fragmentation trees of metabolite standards is in-place that will facilitate the task of assigning the identity of a metabolite by comparing the acquired topology with the topologies of fragmentation trees already in the database. Besides, it enables the characterization of fragments from an unknown compound by querying and matching subtrees from known compounds. The 'Metabolite Space' is the total chemical universe of metabolites present in all compartments and in all states from any organism. We build models based on discriminative features that predict 'metabolite likeness'. Here, we focus on the human metabolite space. Metabolites are compared with other molecules representative from different areas of the whole chemical space. The integration of all developed computational tools with the analytical platform makes the identification of metabolites based on MSn data feasible.

Sponsor Lunch Session Tuesday, 29 June 2010
Parallel session 2A: Sponsored by ThermoFisher
Room: E104 - 107

13.00 – 13.55	**Technology showcase: ThermoFisher**	
13.00 – 13.25	New automated software for metabolome profiling and biomarker discovery with high resolution LC-MS data,	Mark Sanders Thermo Fisher Scientific
13.25 – 13.55	Mitochondrial lipid profiling and identification using high resolution LC-MS and MS/MS	Susan Schiavo, Brigham and Women's Hospital, Boston, USA

Mark Sanders Abstract: Many stages of drug discovery require robust biomarkers and analytical capabilities to quantify them in various biological samples. High resolution LC/MS provides the sensitivity, accuracy and the wide dynamic range required for quantitation and is suitable for high throughput automation making it a widely used tool for discovery and utilization of biomarkers. A typical 10 minute high resolution LC/MS profile of a biological sample may contain over a million data points. Reduction of the data to accurately represent the endogenous metabolites under study, their proper identification and statistical comparison across samples remains a major challenge for LC/MS metabolomic analyses. Blood samples were taken from 4 different groups of rats (male fully satiated, female fully satiated, male food deprived and female food deprived) and analyzed using LC/MS. The data was then analyzed with Component Elucidator software to determine the metabolic effects of food deprivation on the rats. A typical LC/MS metabolomics data file, urine, plasma or tissue extract, could easily yield over one million signals. It has previously been shown that a single component - hippuric acid, an endogenous metabolite found in urine, generates more than 20 related peaks, including isotope clusters, various adducts, multimers and fragments. Overall, plasma and urine are estimated to each contain between 500-1500 unique quantifiable metabolites and, therefore, it is not surprising that the LC/MS data obtained from these samples are extremely complex. Proper removal of chemical noise and identification of sample related peaks is essential. A variety of noise filtering approaches, including blank subtraction were used to reduce the complexity of the sample data. In addition, grouping related signals, i.e. isotope peaks, adduct, dimers, fragments, etc. significantly simplified the data set, reducing the number of components by a factor of 10. An approach encompassing all of the features above represents a comprehensive, integrated solution to processing LC/MS metabolomics data. A data set consisting of 24 samples can be processed in less than 1/2 hour. Easily accessible visualization tools showed substantial differences in endogenous metabolite levels between groups of animals. Automated annotation of components was accomplished using a search of the ChemSpider database or a local, private database.

Susan Schiavo Abstract: An LC-MS method was developed for optimal lipid class separation and detection in rat liver mitochondria, both qualitative and quantitatively, using a Thermo Scientific Exactive mass spectrometer. The method was validated using biological and internal standards spanning all 7 lipid classes and a quality control (QC) sample, created by pooling mitochondria from each rat in the study. In addition, this QC pool sample was used in profiling studies to correct for any chromatographic or MS variations observed over time and for lipid identification in HCD studies. In profiling experiments, lipids were ionized in both positive and negative mode using the same LC conditions and buffer system and detected using full scan MS acquisition. Lipid identifications were done by alternating between full scan MS and HCD fragmentation at 3 different fragmentation energies, facilitating the detection of lipids via class specific fragmentations, as well as lipid specific fragmentations. Linearity and limit of detection in mitochondria was assessed for 5 different lipid class standards spiked into the pool and extracted under normal conditions. Triacylglycerol, glycerophosphocholine and phosphatidylglycerol standards all showed linearity over 5 orders of magnitude, with R-squared values of 0.99 and coefficient of variance (CV) less than 12%. Lysoglycerophosphocholine and fatty acid standards were linear over 4 orders of magnitude also with R-squared values of 0.99 and CVs less than 12%. Extraction efficiencies for the above compound classes were between 70 and 105%. Using this method, developed to separate and detect multiple lipid classes in a single analysis, we identified 19 unique cardiolipin species, which are notoriously of lower abundance, usually requiring class fractionation prior to LC-MS analysis and 2 monolysocardiolipin species which are often associated with mitochondria stress and dysfunction. We will present this method and its application to study the linkages between diet, mitochondria function and disease.

Sponsor Lunch Session Tuesday, 29 June 2010
Parallel session 2B: Sponsored by AB Sciex
Room: E104 - 107

13.00 – 13.55	**Technology showcase: AB Sciex**	**Speakers**
13.00 – 13.15	Lipidomics: advantages of enhanced sensitivity, selectivity and speed of LC/MS/MS analysis	Volker Kruft
13.15-13.30	Metabolomics: advances towards simultaneous qualitative and quantitative analysis	Axel Besa
13.30- 13.45	Introducing LipidView™ software	Christof Lenz
13.45-13.55	Discussion	

Lipidomics: advantages of enhanced sensitivity, selectivity and speed of LC/MS/MS analysis
Mass spectrometry systems with fast scanning capabilities are pivotal for quantitative profiling of a vast variety of lipid molecules that are structurally very closely related. There are hundreds of molecules in each of the 8 lipid classes with different biological functions or activities depending on specific structure or concentration. The enhanced sensitivity, selectivity and speed of the hybrid triple quadrupole/linear-ion trap mass spectrometer AB SCIEX QTRAP® 5500 enables the simultaneous acquisition of both quantitative and qualitative information for a large number of lipids within a single run. Suitability and advantages of the approach and technology will be demonstrated for monitoring inflammatory response in LPS treated monocytes and for evaluating lipid profiles in sets of human blood plasma.

Metabolomics: advances towards simultaneous qualitative and quantitative analysis
In metabolomic studies, the simultaneous analysis of the concentration profiles of a drug, its metabolites and changes in endogenous metabolites is of great interest. However, constraints on the LC as well as technical limitations on the MS side have made two analyses necessary so far: part of the sample is analyzed by SRM/MRM, another on a high resolution instrument.
The recent introduction of the AB SCIEX TripleTOF™ 5600 system does allow this data analysis in a single run without splitting of the sample. The TripleTOF operates at acquisition speeds of up to 100 Hz while maintaining a resolution of 30,000 or higher in MS and MS/MS. This short duty cycle at high resolution allows an extremely high rate of full scan and dependent scan acquisitions. Post-acquisition analysis tools like PeakView and MarkerView™ software packages do extract and process relevant information. This allows post-processing of the experiment for targeted and non-targeted quantification and identification.

Introducing LipidView™ software
We will give a short introduction and demonstration of the LipidView™ software. LipidView is a data processing software for complex lipid profiling datasets. It is supported by a lipid fragment database with more than 40 lipid classes and 23,000 lipid species. A simple 4-step workflow allows easy sample comparison in terms of lipid class, fatty acid, or molecular species profiles. Experimental data from AB SCIEX Triple Quad™, QTRAP®, QSTAR®, and AB SCIEX TripleTOF™ 5600 systems can be processed.

Tuesday, 29 June 2010

Parallel session 4A		
14.00 - 15.30	**PHARMACOMETABOLOMICS, PERSONALIZED HEALTH & THE FUTURE OF THE HEALTH SYSTEM**	
	Chair: Guowang Xu (China)	**Room**
14.00 – 14.30		
Invited speaker	**Jan van der Greef (Netherlands)**	
	One size fits all? : a systems perspective on Personalized Health	Forum
14.30 - 14.50		
Selected speaker	**Rima Kaddurah-Daouk (USA)**	
	Pharmacometabolomics Research Network: Approach for Personalized Medicine	Forum
14.50 - 15.10		
Selected speaker	**Rachel Cavill (UK)**	
	Integrating transcriptomic and metabolomic data to enhance the detection of pathways associated with drug response	Forum
15.10 - 15.30		
Selected speaker	**Jildau Bouwman (Netherlands)**	
	Let's visualize personalized health	Forum

L4A-001

One size fits all? : a systems perspective on Personalized Health

Jan van der Greef, TNO Quality of Life, Leiden University and Sino-Dutch centre for Preventive and Personalized Medicine, The Netherlands

In recent decades various important scientific developments have been based on a systems-based view. The key research topic being the interconnectivity of systems and the study of the organizing principles, realizing that new properties emerge at different levels of complexity. System thinking is gaining a boost globally due to recent crisis's in the financial, social and environmental domains, which all underpin the need for a systems approach. In Life sciences this resulted in Systems Biology research, which has developed in recent years from a technology driven enterprise to a new strategic tool in Life Sciences, particularly for innovative drug discovery and drug development. The One size fits all approach seems to be at its end and new personalized strategies are getting shaped. The latter will be discussed from a systems perspectives from both the diagnostic systems view as well as the systems intervention needs. Metabolomics-based systems biology plays a key role in this new strategy while integration of Chinese and Western Medicine opens the way to design combinatorial interventions opportunities including life style and psychology. 1. Van der Greef J, Martin S, Juhasz P, Adourian A, Plasterer T, Verheij ER, McBurney RN. The art and practice of systems biology in medicine: mapping patterns of relationships. J Proteome Res. 2007 Apr;6(4):1540-59. 2. Van der Greef J, Hankemeier T, McBurney RN Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials? Pharmacogenomics. 2006 Oct;7(7): 1087-94 3. Van der Greef, J and McBurney, R.N. Rescuing Drug Discovery and Drug Development: In Vivo Systems Pathology and Systems Pharmacology, Nature Reviews Drug Discovery, 4, 1-7 December, 2005

L4A-002

Metabolic signatures of response to sertraline and placebo

Kaddurah-Daouk, R.(1), Matson, W. (2), Sharma, S. (2), Boyle, S.H. (1), Matson, S. (2), Krishnan, R.R. (1), Rush, A.J (1): Duke University Medical Center, Department of Psychiatry and Behavioral Sciences, Box 3903, Durham, NC 27710. (2)Bedford VAMC Building 70, Room 262, Mail Stop 152, 200 Springs Road, Bedford, MA 01730, USA

Objective: The metabolome defines the complete repertoire of small molecules present in an individual and captures a metabolic state "metabotype" that is regulated by net interactions between genome and total cellular environment. We put forward a hypothesis that a metabolic profile of a patient with major depression at baseline prior to treatment would define how an individual responds to treatment and that response to drug and response to placebo have common and also unique metabolic underpinnings. Method: Patients meeting DSM-IV criteria for major depressive disorder were randomly assigned to receive placebo (N = 40) or Sertraline (N=36) therapy. Serum blood samples collected at baseline were profiled using an electrochemistry based metabolomics platform (LCECA) that quantifies redox active compounds. Digitizing the output of the LCECA platform creates a "digital map" of the entire response of the platform for a particular sample. A positive response to therapy was defined as >50% reduction in HAMD-17 score after four weeks of treatment. Results: Using the digital maps partial least squares discriminant models were built with samples taken from ends or middle of the response curve (training set) with the assignment of the rest of samples (replication set) to responder or non-responder groups. The correct classification rate (CCR) of these models were 69-85% for Sertraline and 100% for placebo. Metabolic profiles at baseline seem to define a group of responders to drug who would have responded to placebo; a group who would respond to drug but not placebo, and a group of patients who seem resistant to treatment. Conclusions: The metabotype of patients with major depression seems to define how an individual responds to treatment with Sertraline or placebo. Metabolomics provides tools that can help subclassify depressed patients and to define the biochemical basis for variation in response to treatment.

L4A-003

Integrating transcriptomic and metabolomic data to enhance the detection of pathways associated with drug response.

Rachel Cavill, Dr Hector C Keun, Dr Timothy MD Ebbels, Imperial College London, UK

Many technologies such as transcriptomics, metabolomics and proteomics are now commonly used by biologists as hypothesis generating tools to aid the understanding of behaviour in their systems. Each of these technologies gives only part of the overall picture, therefore integrating the data from multiple sources is a crucial, yet complex task we currently face. Here we take publicly available baseline metabolomic and transcriptomic measurements on the NCI60 cell line panel and using a novel integration method, alongside the sensitivity of each cell line to a range of drugs, find pathways which are significantly associated with drug sensitivity for each drug. The initial work focuses on four platinum-based chemotherapeutics. We find sets of pathways associated with sensitivity to platinum drugs are highly coincident and consistent with previously reported molecular determinants of platinum resistance. Using our approach to combine data from multiple sources we improve the sensitivity for the detection of pathways by ~82% compared to using a single data source. We then extend our approach to a panel of 82 drugs, enabling us to reveal details of the complex relationships between biological pathways and drug response. Clustering drugs according to the pathways with which they are associated shows many clusters of drugs with similar modes of action or similar structure. In summary, we present a straightforward approach to data-integration which has been demonstrated to deliver biologically relevant pathways.

L4A-004

Let's visualize personalized health

Bouwman J(1,2,3), Wopereis S(1,2), Vogels JTWE(1), Rubingh CM(1), van Ommen B(1,2) (1)TNO, The Netherlands, (2)Eurreca, (3)NMC, The Netherlands

Good health begins with good nutrition, and good nutrition starts with a balanced diet that provides necessary levels of essential nutrients. For micronutrients Dietary Reference Intakes are defined, which are based on average population needs in a particular life stage and gender group. However, the dietary requirements depend on many factors such as genotype, lifestyle, stress, disease state etc. Therefore, we should find a way to define personalized needs. We have developed a visualization method, called the 'health space' method that separates subjects according to the underlying biological processes. In this method the measured nutrigenomics parameters are grouped in the three biological processes: the processes chosen will depend on you research question. A PLS-DA model is build for each of these processes. This model is scaled between 0 (the healthy/treated group) and 1 (the unhealthy/untreated group). A 3-dimensional space is built on the combination of processes with on every axis one of the three processes. In this health space every person will have his own score that shows to what extend the treatment or disease is affecting the related processes. This information can be used for further treatment strategies. We have tested this 'health space' concept on a recently published study (Bakker et al, 2010). In this study non-diseased subjects are treated with an anti-inflammatory dietary mix containing n-3 fatty acids, EGCG, Vitamin-E, Vitamin-C, resveratrol and tomato-extract. The plasma concentrations of proteins and metabolites before and after a five week treatment were analyzed. Central processes that are regulated by this dietary intervention are stress in oxidation, inflammation, and metabolism. Therefore, these

processes were at the axes of the health space in this example. The treated and untreated groups were clearly separated in space. In the earlier paper it has been shown that the health status of these people improves. Some people mainly modulate their metabolic stress profile, while others show a specific inflammatory or oxidative response to the anti-inflammatory dietary mix. We show that different response subgroups can be distinguished and may be treated accordingly.

Tuesday, 29 June 2010

Parallel Session 4B

14.00 - 15.30	**DEVELOPMENTS IN PLANT METABOLOMICS** **Chair: Ute Roessner (Australia)**	**Room**
14.00 – 14.30 Invited speaker	**Dan Jones (USA)** Deep profiling and localization of specialized plant metabolites	E104 -107
14.30 - 14.50 Selected speaker	**Miyako Kusano (Japan)** Multi-platform metabolomics approach for an objective substantial equivalence assessment of transgenic tomato	E104 -107
14.50 - 15.10 Selected speaker	**Stephanie Moon (USA)** Metabolomics based annotation of novel genes in Arabidopsis thaliana	E104 -107
15.10 - 15.30 Selected speaker	**David Portwood (UK)** Plant metabolomics: Tomato Metabolite Profiling and Identification Employing High Resolution MS Strategies	E104 -107

L4B-001

Deep profiling and localization of specialized plant metabolites

Jones, A. D. (1) Department of Biochemistry and Molecular Biology and Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA

Plants synthesize an incredibly diverse suite of specialized metabolites long valued for their medicinal properties. While some plant tissues are prolific chemical factories, accumulation of desirable metabolites often fails to reach desirable levels. Some phytochemicals been purported to play important roles in plant defenses and as signaling molecules that regulate plant responses to changing environments, but our understanding of the pathways responsible for metabolite accumulation is often limited. Our current efforts have focused on establishing the chemical diversity of specialized metabolism across different cell and tissue types using both model and medicinal plants. To establish chemical diversity across plant tissues, hundreds to thousands of separate chemical analyses must be performed, often on tiny samples. Analytical technologies are further stretched by our desire to investigate numerous plant species including genetic variation within individual species. Our recent efforts have focused on two complementary approaches: (1) acceleration of LC/TOF MS-based deep profiling of specialized metabolites with minimal loss of chemical information, and (2) localization of metabolites in specific cell types using laser desorption ionization and mass spectrometry imaging. Findings to date suggest remarkable chemical complexity, unexpected connections between metabolic pathways, and important quantitative spatial diversity in levels of specialized metabolites across plant tissues.

L4B-002

Multi-platform metabolomics approach for an objective substantial equivalence assessment of transgenic tomato

Kusano, M. (1)#, Redestig, H. (1)#, Hirai, T. (2), Oikawa, A. (1), Matsuda, F. (1), Fukushima, A.(1), Arita, M. (1), (3), Watanabe, S. (2), Yano, M. (2), Hiwasa-Tanase, K. (2), Ezura, H. (2), Saito K. (1), (4) (1) RIKEN, PSC, Yokohama, Kanagawa 230-0045, Japan (2) Grad. Sch. Life Env. Sci., Univ. Tsukuba, T Tsukuba Ibaraki 305-8572, Japan (3) Dept. Info. Sci. Grad. School Sci., Univ. Tokyo, Tokyo 113-0033, Japan. (4) Grad. Sch. Pharm. Sci., Chiba Univ., Chiba 263-8522, Japan #equal contributors to this work.

Metabolomics offers a unique opportunity to perform a detailed study of an organism's phenotype and is therefore a promising approach for a substantial equivalence (SE) assessment of genetically modified crops. Here we propose the use of gas chromatography- (GC), liquid chromatography- (LC) and capillary

electrophoresis (CE)-time-of-flight (TOF)/mass spectrometry (MS) in parallel with the advantages that (1) multiple platforms increase the chemical coverage; (2) the consensus data obtained by our novel data summarization approach is annotated and directly interpretable and; (3) can be evaluated in terms of actually achieved coverage. We used our multi-platform approach to perform a SE assessment of tomatoes that over-express the taste-modifying protein miraculin. The identified metabolites by using three platforms were found to be representative of the tomato metabolome as they covered 86% of the chemical diversity of the public database TomatoCyc. We show that 95% of all metabolite abundances were within an acceptable range of variation but at the same time indicate a reproducible transformation related metabolic signature. We conclude that multi-platform metabolomics is a both sensitive and robust approach that constitute a good starting point for characterizing novel organisms.

L4B-003

Metabolomics based annotation of novel genes in Arabidopsis thaliana

Stephanie M. Moon, Preeti Bais, Julie Dickerson, Philip Dixon, Oliver Fiehn, Kun He, B. Markus Lange, Seung Rhee, Mary Roth, Vladimir Shulaev, Lloyd Sumner, Ruth Welti, Eve Wurtele, and Basil J. Nikolau NSF2010-funded Arabidopsis Metabolomics Consortium (www.plantmetabolomics.org)

A plant metabolomics consortium has been established to generate and evaluate metabolomics data as a tool for generating hypotheses concerning the metabolic and physiological function of genes of unknown function. This consortium integrates 11 analytical platforms, which have the combined ability to generate relative abundance data of nearly 3100 Arabidopsis metabolites/analytes. The strategy combines the power of reverse genetics (T-DNA tagged Arabidopsis lines) and metabolomics to evaluate the consequence of the loss-of-gene function on the metabolome of the organism. The project database at www.plantmetabolomics.org, is publicly available and contains metabolomics data along with detailed information about mutant selection, material processing, analytical platform protocols, tools to aid in data visualization and more. Initially the consortium established pipelines for large-scale analyses, including mutant gene selection, tissue production and harvest, data collection and data processing. These experiments (termed EIE2 and fatB in the database) showed that the pipelines could clearly distinguish between a mutant metabolome from a wild-type metabolome, even we as experimenters introduced large environmental pressures during the growth of the organisms (experiment EIE2). In addition, the fatB experiment provided a degree of validation of the platforms based on prior characterization of the mutant allele. Based on the initial experiments (termed ME1 to ME5 in the database), the platform has been modified to enhance the reliability and robustness of the metabolomics data. These modifications include increasing the number of mutants for analysis within a single growth experiment, increasing the number of replications per mutant line, and distributing samples of pooled biological materials for analysis. These modifications have been incorporated in the extension of the project, in which the metabolomes of 200 different Arabidopsis mutants are being assessed.

L4B-004

Plant metabolomics: Tomato Metabolite Profiling and Identification Employing High Resolution MS Strategies

Helen Welchman (1), David Portwood(2), Mark Earll(2), Mark Seymour(2), Madalina Oppermann(3) (1) Thermo Fisher Scientific, UK (2) Syngenta, UK (3) Thermo Fisher Scientific, Sweden

Food nutritional value, quality, resistance to pathogens, flavor are among the traits monitored by the food industry, in an attempt to promote the creation of robust, healthy, nutrition-rich cultivars that contribute to a sustained agro development. Metabolomics has been identified as a key mass spectrometry-based approach in the analysis of such characteristics. Syngenta is a world-leading agribusiness with a particular interest in seeds and crop protection. Herewith, results from metabolite profiling and identification experiments on tomato samples will be presented. Tomato samples were extracted as follows: triplicate biological replicates of two tomato cultivars were analyzed at four time points of fruit development stages using fast reversed-phase chromatography prior to mass spectrometric analysis, carried out on a hybrid high resolution mass spectrometer instrument. Strategies for metabolite profiling and identification were successfully applied and encompassed sample measurement in positive and negative ion mode electrospray ionization in conjunction with multiple dissociation techniques and extensive data mining. Preliminary results indicate that the high sample complexity in survey scans in the mass range 85-900Da benefits from highly-resolving, profile mode analysis. Hundreds of components were profiled at resolutions up to 100,000 useful for accurate and sensitive relative quantification experiments. Using external instrument calibration analyte masses were measured with high, sub-ppm to max 2ppm accuracy, leading to strongly suggestive identifications based on elemental composition analysis. Unambiguous identification of analytes was used to corroborate the performance of the different MS/MS fragmentation regimes, carried out either via resonance excitation CID or higher energy collisional activation (HCD) experiments. Profiling experiments followed by extensive statistical analysis reveal biologically interesting putative markers which should be identified during supplementary experiments. We report on a plant metabolomic study where high resolution data generated on a novel type of hybrid Fourier-based accurate mass measurement system is used to link plant development stage-specific metabolomic characteristics to cultivar phenotype.

Tuesday, 29 June 2010

Parallel Session 5A

16.00 - 17.30	**SYSTEMS BIOLOGY OF MAMMALIAN/MICROBIAL METABOLISM** **Chair: Marta Cascante (Spain)**	**Room**
16.00 - 16.30 Invited speaker	**Uwe Sauer (Switzerland)** High throughout intracellular metabolomics and what concentration data tell us	Forum
16.30 - 16.50 Selected speaker	**Dirk Walther (Germany)** Metabolic pathway relationships revealed by an integrative analysis of the metabolic and transcriptional temperature stress response dynamics in yeast.	Forum
16.50 - 17.10 Selected speaker	**Paula Gaspar (Portugal)** In vivo metabolite profiling of Lactococcus lactis mutants towards the optimal production of reduced compounds	Forum
17.10 - 17.30 Selected speaker	**Marjan de Mey (Belgium)** Catching prompt metabolite dynamics of E. coli with the BioScope at oxygen rich conditions.	Forum

L5A-001
High throughout intracellular metabolomics and what concentration data tell us

Jörg Büscher, Sarah-Maria Fendt, Stephanie Heux, Nicola Zamboni and Uwe Sauer. Institute of Molecular Systems Biology, ETH Zurich, Switzerland

Metabolomics offers promising insights into how complex networks respond to genetic or environmental perturbations. To make full use of this potential, we are currently faced with two challenges: analytics and data interpretation. To address the former challenge, we will present a high-throughput work-flow for targeted (MS/MS) and untargeted (TOF) intracellular metabolomics based on mini-scale cultivation and flow injection. This method is then applied to a drug-metabolism interaction screen of S. cerevisiae, and the metabolomics results are contrasted to 13C-flux data from the same experiments. In particular we will discuss the relevance of our findings for early on detection of off-target drug effects. The second challenge, data interpretation is particularly difficult for metabolomics because i) there is no direct link to the genome and ii) metabolite concentrations are not a direct functional measurement of flux through metabolic networks (1, 2). To provide a general interpretation concept for metabolomics data, we postulate three hypotheses on the relationship between enzyme capacity and metabolite concentrations that were subsequently tested by correlation of metabolite concentrations to transcript and protein data in yeast. From these analyses, we conclude that substrate metabolite concentrations, at least in central metabolism, can be used to conclude on the in vivo capacity of their enzymes (3). 1) U. Sauer, M. Heinemann & N. Zamboni (2007) Getting closer to the whole picture. Science 316: 550. 2) Zamboni N & Sauer U (2009) Novel biological insights through metabolomics and 13C-flux analysis. Curr. Opin. Microbiol. 12: 553-558. 3) Fendt SM, Büscher JM, Rudroff F, Picotti P, Zamboni N & Sauer U (2010) Tradeoff between enzyme and metabolite efficiency maintains metabolic homeostasis upon perturbations in enzyme capacity. Mol. Sys. Biol. 6: 356

L5A-002
Metabolic pathway relationships revealed by an integrative analysis of the metabolic and transcriptional temperature stress response dynamics in yeast.

Walther D, Strassburg K, Kopka J Max Planck Institute for Molecular Plant Physiology, Potsdam-Golm, Germany, Present address KS: Netherlands Metabolomics Centre, LACDR/ Leiden University, The Netherlands

The parallel and integrated analysis of metabololite data with datasets covering other levels of molecular organization has become a central task of metabolomics research. We investigated the metabolomic and transcriptional response of yeast exposed to increased and lowered temperatures relative to optimal reference conditions in the context of known metabolic pathways. Pairwise metabolite correlation levels were found to carry more pathway-related information and to extend to farther distances within the metabolic pathway network than associated transcript level correlations. Metabolites were detected to correlate stronger to their cognate transcripts (metabolite is reactant of the enzyme encoded by the transcript) than to more remote transcripts reflecting their close metabolic relationship. We observed a pronounced temporal hierarchy between metabolic and transcriptional molecular responses under heat and cold stress. Changes of metabolites were most significantly correlated to transcripts encoding metabolic enzymes, when metabolites were considered leading in time-lagged correlation analyses. By applying the concept of Granger causality, we detected directed relationships between metabolites and their cognate transcripts. When interpreted as substrate-to-product directions, most of these directed Granger causality pairs agreed with the KEGG-annotated preferred reaction direction. Thus, the introduced Granger causality approach may prove useful for determining the preferred direction of metabolic reactions in cellular systems.

L5A-003

In vivo metabolite profiling of Lactococcus lactis mutants towards the optimal production of reduced compounds

Gaspar, P. (1), Neves, A.R. (1), Gasson, M.J. (2), Shearman, C.A. (2) and Santos, H. (1): (1) Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apt. 127, 2780-156 Oeiras, Portugal, (2) Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom

Manipulation of NADH-dependent steps in Lactococcus lactis is common to many strategies envisaging the accumulation of compounds other than lactate. In particular, disruption of the major lactate dehydrogenase (ldh gene) is frequently considered. In this work, we pursued an engineering approach based on the combined inactivation of enzymes involved in NAD+ regeneration downstream of pyruvate. Based on the end-product and transcript profiles in LDH-deficient strains, we selected as targets for further manipulation in an LDH/MTLF-negative strain (FI10089, Gaspar et al. 2004) the two additional lactate dehydrogenases genes ldhB and ldhX, as well as adhE (acetaldehyde/ethanol dehydrogenase). Subsequently, a series of triple and quadruple knockout mutants was obtained. Likewise the parental strain, FI10089-ldhB and FI10089-ldhB-ldhX showed a mixed-acid fermentation profile. Lactate production was not completely abolished, but it decreased considerably with the combined deletion of ldh genes. Surprisingly, FI10089\ adhE was fully homolactic, which indicated activation of alternative ldh genes in this strain. Combination of ldh, ldhB and adhE deletions (FI1089-adhE-ldhB) affected drastically glucose metabolism and impaired growth under anaerobic conditions. The metabolism of [1- 13C]glucose in resting cell suspensions of the mutant strains was characterized by in vivo 13C-NMR to follow the dynamics of intracellular metabolite pools in a non-invasive way. Fructose 1,6 bisphosphate, mannitol 1-phosphate, 3-phosphoglycerate, and phosphoenolpyruvate were accumulated to different extents by the mutant strains. Moreover, data on the qualitative expression of ldh genes in the different mutants was obtained by RT-PCR. The levels of key glycolytic enzymes (6-phosphofructokinase, glyceraldehyde 3 phosphate dehydrogenase and pyruvate kinase) as well as lactate, ethanol and mannitol 1-phosphate dehydrogenases were measured in the different strains and compared with those of the wild-type strain MG1363. Data demonstrating the usefulness of these constructs to direct the metabolic flux to the production of mannitol or 2,3-butanediol will be presented. P. Gaspar, et al. (2004) Appl. Environ. Microbiol. 70, 1466-74

L5A-004

Catching prompt metabolite dynamics of E. coli with the BioScope at oxygen rich conditions

De Mey, M. (1,2), Taymaz-Nikerel, H. (2), Baart, G. (3), Waegeman, H. (1), Maertens, J. (3), Soetaert, W.K. (1), Heijnen, J.J. (2), van Gulik, W.M. (2) (1): Department of Biochemical and Microbial Technology, Ghent University, Coupure links 653, 9000 Ghent, Belgium (2): Department of Biotechnology, Delft University of Technology, Kluyver Centre for Genomics of Industrial Fermentation, Julianalaan 67, 2628 BC Delft, The Netherlands (3): Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Previously, enzyme kinetics derived from in vitro studies have been used in kinetic models of the central metabolism. However, these studies have typically been performed under optimized conditions, which rarely resemble the natural environment of enzymes inside the cell. Applying these in vitro obtained enzyme kinetic properties for kinetic modeling of the in vivo behavior of metabolic pathways might lead to erroneous predictions. Therefore, there is an urgent need for accurate data of enzyme kinetics which are valid under in vivo conditions. These can be obtained from perturbations of well defined steady-state conditions of whole cells and requires to measure enzyme levels, fluxes and metabolite levels. If perturbation experiments are carried out in a short time frame (seconds to minutes), the enzyme levels can be assumed not to change and hence only intra- and extracellular metabolite concentrations as a function of time are required to obtain the rates from the mass balances. This contribution presents the

design and application of a BioScope, a mini plug-flow reactor, specifically designed for Escherichia coli. The dynamics of a large range of central metabolites were monitored in the first 40 seconds after perturbations applied directly in the reactor as well as in the BioScope. The obtained dynamic metabolite profiles appeared highly reproducible. Moreover, the metabolite profiles obtained in the BioScope were almost identical with the profiles obtained from the perturbation experiments carried out directly in the reactor. Furthermore, it was observed that it is imperative to maintain oxygen rich conditions during such experiments. This is also true for *E. coli* which shows a fast response (< 10 s) to environmental perturbations.

Tuesday, 29 June 2010

Parallel Session 5B

16.00 - 17.30	**VOLATILES & SECONDARY METABOLISM**	
	Chair: Dan Jones (USA)	**Room**
16.00 - 16.30		
Invited speaker	**Joe Chappell (USA)**	
	Understanding the molecular wizardry of terpene metabolism in plants	E104 -107
16.30 - 16.50		
Selected speaker	**Roland Mumm (Netherlands)**	
	A metabolomic approach to decipher fragrance in rice	E104 -107
16.50 - 17.10		
Selected speaker	**Hong Soon Rhee (S. Korea)**	
	Comparative profiling of metabolites, gene transcripts and proteins involved in benzylisoquinoline alkaloid biosynthesis of Papaver somniferum for targeted metabolic engineering	E104 -107
17.10 - 17.30		
Selected speaker	**John Hugh Snyder (USA)**	
	Exploiting Medicago Germplasm Diversity for Triterpene Saponin Biosynthetic Gene Discovery Using an Integrated Metabolomics and Transcriptomics Approach	E104 -107

L5B-001
Understanding The Molecular Wizardry Of Terpene Metabolism In Plants

Shuiqin Wu, Jeanne Rasbery and Joe Chappell

Plant Biology Program University of Kentucky Lexington, KY, USA

My laboratory was initiated with a goal to better understand the mechanisms plants use to defend themselves against microbial pathogens. For many years, and like many laboratories, we focused our attention on how plants regulate the biosynthesis of anti-microbial phytoalexins. In particular, our studies have been dedicated to understanding the biosynthesis of sesquiterpene phytoalexins in solanaceous plant species, and has utilized a wide range of experimental strategies including genetic engineering, structure-function comparisons of genes and proteins, as well as very simple physiological experiments to uncover putative signal molecules. Our studies have, however, yielded several unexpected results that have been interpreted with novel models for the organization of this biosynthetic machinery (Plant Physiol. (1995) 109:1337), the structure-function relationships of terpene synthase genes/enzymes (Proc. Natl. Acad. Sci. (2006) 103:9826), and the development of new strategies to engineer these biochemical traits into plants (Nat. Biotech. (2006) 24:1441) and into microbial host systems (J.B.C. (2007) 282:1744). In more recent work, we have been examining the relationship between sesquiterpene metabolism and flowering in lettuce. Flowering in lettuce is a light-regulated process associated with the accumulation of bitter and noxious sesquiterpenoids, and results in reduced yields and quality of leaf lettuce produce. Delaying the time to flowering is thus a major goal to improving overall yield and quality of this crop. Our preliminary evidence has demonstrated that RNAi suppression of a select sesquiterpene biosynthetic gene alters the profile of sesquiterpene lactones in leaves, delays flowering very significantly, and extends the vegetative growth period. One inference from these experiments is that select sesquiterpenoids might represent novel cues inducing the flowering program in plants within the Asteraceae.

L5B-002
A metabolomic approach to decipher fragrance in rice

Mumm, R. (1,2), Calingacion, M. (3), de Vos, R.C.H. (1,2), Stoopen, G.M.(1,2), Jonker, H.H. (1,2), Fitzgerald, M.A. (3), and Hall, R.D. (1,2) (1) Plant Research International, Business Unit

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Rice is the most important food crop in the world. It is the staple of almost half of the world's population and it contributes around 75% of the calorific intake of Asian people. Fragrance is considered one of the most important quality traits in rice, determining market price and which has a link to a clear local and national identity. Important rice flavours are often associated with the Basmati and Jasmine style rices. The flavour and aroma of these two rice types can be discriminated easily by consumers, even though the major aromatic component in both types is 2-acetyl-1-pyrroline (2-AP). Within the EU project META-PHOR, the detailed composition of the volatile components of fragrant and non-fragrant rice varieties was studied using solid-phase micro extraction (SPME) and gas chromatography mass spectrometry (GC-MS). A unique selection of 33 pure fragrant Basmati, Jasmine type rices, and non-fragrant (no 2-AP) varieties from 10 countries were analysed. Many of the varieties were traditional lines and from all varieties the storage history was known. Results show that key metabolite groups differ significantly between rice types and that it is within these differentiating groups that we must search for those characteristics defining the typical Jasmine / Basmati sensory attributes as well as key off-flavours as experienced by the consumer.

L5B-003

Comparative profiling of metabolites, gene transcripts and proteins involved in benzylisoquinoline alkaloid biosynthesis of Papaver somniferum for targeted metabolic engineering

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Papaver somniferum produces a variety of benzylisoquinoline alkaloids (BIAs) and is the major plant source of morphinan alkaloids including thebaine, codeine and morphine. Plant cell cultures has attracted interest as bioreactors for in vitro production of phytocompounds, however, undifferentiated cells of *P. somniferum* have been reported to lose their ability to synthesize morphine with unknown reasons. To elucidate the differences of secondary metabolism between plant cells and intact plants of P. somniferum and to determine potential targets of metabolic engineering for in vitro morphine production, we compared expression profiles of gene transcripts and proteins involved in the BIA biosynthesis using plant tissues showing different BIA profiles. Additionally, as it has been reported that morphine biosynthesis in *P. somniferum* is under developmental regulation, correlation of morphine production to differentiation of laticifer where morphine accumulates in plants was investigated through somatic embryogenesis from opium poppy callus. The indirect somatic embryogenesis contributed to overall accumulation of gene transcripts and proteins involved in the BIA biosynthesis accompanying morphine production in somatic embryos. Among genes on the morphinan alkaloids biosynthetic pathway, salutaridine reductase (SR) transforming salutaridine into salutaridinol before thebaine, accumulated not in undifferentiated callus but in morphine-producing plant tissues and somatic embryos. From the integrated –omics studies, SR was selected as a potential target to modify for in vitro morphine production using *P. somniferum* suspension cultures; because its transcript and protein expression was closely correlated to both morphine production in plant tissues and differentiation of laticifer in early stage of development. We established genetically engineered *P. somniferum* callus where SR was over-expressed via Agrobacterium-mediated transformation and the SR-activated transgenic callus synthesized morphinan alkaloids even in undifferentiated callus state. This study represents that comparative metabolic profiling integrated with transcripts and proteins expression profiles enables to understand the developmental regulation of morphinan alkaloid biosynthesis in opium poppy and to determine a proper target for metabolic engineering for in vitro morphine production in P. somniferum suspension cultures.

L5B-004

Exploiting Medicago Germplasm Diversity for Triterpene Saponin Biosynthetic Gene Discovery Using an Integrated Metabolomics and Transcriptomics Approach

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Triterpene saponins are a class of structurally diverse plant natural products with a wide range of demonstrated bioactivities. Individual triterpene saponins have been demonstrated to possess allelopathic, anti-fungal, anti-bacterial, anti-insect, anti-feedant, and anti-cancer activities. The biosynthesis of triterpene saponins is poorly characterized. The model legume Medicago truncatula is known to accumulate a large variety of triterpene saponin compounds, resulting from the differential glycosylation of at least seven triterpene aglycone structures. In

this project, UPLC-ESI-qTOF-MS was used to profile the accumulation of triterpene saponin metabolites in a collection of 100 M. truncatula ecotypes (germplasm accessions). Analyses of both aerial and root organs was performed. These metabolomic analyses revealed interesting trends in differential spatial and structural accumulation patterns between the various ecotypes, and between the organs. For example, zanhic acid saponins were detected exclusively in aerial organs, while soyasapogenol B saponins were detected exclusively in root organs. The high-resolution biochemical phenotyping data for the whole ecotype collection enabled an informed selection of hypo- and hyper accumulating ecotypes for subsequent transcriptomic analyses via Affymetrix Medicago GeneChips®. Correlation analyses of saponin accumulation phenotypes with transcript expression data led to the identification of several biosynthetic gene candidates. A cytochrome P450 gene candidate was cloned and introduced to Wat11 yeast cells, enabling microsomal isolation and detailed in vitro characterization of enzyme function. This cytochrome P450 showed sequential oxidase activity for carbon 23 of oleanolic acid and several structurally related compounds in the triterpene sapogenin biosynthesis pathway. Genetic confirmation of in planta function for this gene is under way via mutant analysis.

Wednesday, 30 June 2010

Plenary session		
09.00 - 10.30	**PLENARY SESSION P4**	
	Chair: Jules Griffin (UK)	**Room**
09.00 - 09.30		
Invited speaker	**Thomas Hankemeier (Netherlands)**	
	Metabolomics tools & technologies: now & tomorrow?	Forum
	YOUNG SCIENTISTS: SPONSORED BY MPF	
09.30 - 09.45		
Selected speaker	**Cheryl Strelko (USA)**	
	NMR metabolic analyses with 13C-glutamine identify altered TCA and gamma-glutamyl cycles in the metastatic VM-M3 tumorigenic cell line	Forum
09.45 - 10.00		
Selected speaker	**Lynsey MacIntyre (UK)**	
	Mapping Biomarkers in the Brain using Mass Spectrometry-Based Metabolomic Profiling on Several Platforms MS	Forum
10.00 - 10.15		
Selected speaker	**Peter Wurtz (Finland)**	
	Quantitative metabolic profiling of early risk for atherosclerosis by serum NMR metabonomics	Forum
10.15 - 10.30		
Selected speaker	**Jeongwoon Kim (USA)**	
	A tomato EMS mutant with altered trichome flavonoid methylation identified by LC-MS screening	Forum

LP4-001
Metabolomics tools & technologies: now & tomorrow?

Hankemeier, T. Metabolomics Centre & Leiden University, 2333 CC Leiden, The Netherlands.

The application of metabolomics to answer biological questions is significantly increasing in recent years. Metabolomics is applied to find biomarkers for disease diagnostics in clinical and epidemiological studies. For this, large sample series have to be analyzed. In this presentation the focus will be on what kind of data for metabolomics are required, and what on the other hand is currently possible. The validation of analytical methods and the challenges in obtaining reproducible data will be addressed. Obviously, the best output of metabolomics methods is a list of metabolites and their concentrations. Options to achieve this will be discussed. In addition, various metabolite classes have to be analyzed at low concentrations and sometimes also in very small sample volumes. Examples for analyzing ultra-small samples will be discussed. Actually, many of this goals are addressed within the research program of the Netherlands Metabolomics Centre, and examples are given on how new tools are developed and might help to get better metabolomics data in the future.

LP4-002
NMR metabolic analyses with 13C-glutamine identify altered TCA and γ-glutamyl cycles in the metastatic VM-M3 tumorigenic cell line

Strelko, C.L. (1) Shelton, L.M. (2) Seyfried, T.N. (2) Roberts, M.F. (1): (1)Boston College Department of Chemistry (2)Boston College Department of Biology, Boston, USA

The VM-M3 macrophage-like cell line, unlike the overwhelming majority of tumorigenic cell lines, is highly metastatic in vivo. It

therefore may serve as a more accurate in vitro representation of the metabolism of the deadliest cancers. While typical cancer cell lines get their energy primarily from glucose, these unique cells can survive on glutamine alone and cannot maintain viability without it. Clearly these cells display an altered metabolism, which may involve energy derived from substrate level phosphorylation through the TCA cycle rather than using glycolysis which has historically been linked to tumorigenicity. Various single and multidimensional NMR techniques were used to determine the steady state metabolite pools in cell extracts and media samples when the cells were incubated with 4 mM glutamine alone, with 25 mM glucose alone, or with both glucose and glutamine. The metabolic fingerprint is similar to that of macrophages, the cell type from which the VM-M3 cells seem to be derived. In order to monitor metabolism more accurately and determine how and why the cells use glutamine to maintain viability, the cells were incubated with uniformly 13C labeled glutamine under the same conditions. Several labeled metabolites related to the TCA cycle were identified in both the cell and media extracts. Some very unique metabolites were found to be 13C labeled including itaconate (in both media and cell extracts) and pyroglutamate (in the media alone). These metabolites (at low levels in unlabeled extracts, but easily detected in 13C-labeled samples) have rarely (if ever) been reported in mammalian cells. This combination of labeled metabolites strongly suggests that the TCA and gamma-glutamyl cycles have alterations that enable these cells to survive by using glutamine as their primary energy source. Given the metastatic characteristic of the VM-M3 cell line, either of these metabolites (itaconate in particular) may serve as a biomarker for metastatic capability in cancer cells.

LP4-003

Mapping Biomarkers in the Brain using Mass Spectrometry-Based Metabolomic Profiling on Several Platforms

L. MacIntyre (1), D.G. Watson (1), R.J.A. Goodwin (2), A.R. Pitt (2), P. Scullion (3), B. Pickard (1) and S.J. Clapcote (4) (1) Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK. (2) Division of Integrative and Systems Biology, University of Glasgow, UK. (3) Schering-Plough Research Institute, Newhouse, UK. (4) Institute of Membrane and Systems Biology, University of Leeds, UK.

Methods based on matrix-assisted laser desorption ionisation imaging mass spectrometry (MALDI-IMS), liquid chromatography coupled to an LTQ-Orbitrap mass spectrometer and gas chromatography-mass spectrometry (GC-MS) were used to carry out comprehensive metabolite analyses to characterise alterations in metabolites in brain tissue in response to either drug treatment or disease. A dry matrix coating was used to map the distribution of CNS-targeted drugs in situ using MALDI MSI. The technique was used to profile the distribution of 4-bromophenyl-1,4-diazabicyclo(3.2.2)nonane-4-carboxylate, monochloride (SSR180771), in rat brain sections. To confirm the semi quantitative data obtained from the signal intensities in MALDI IMS, quantification of the drug was carried out by LC-MS analysis of laser micro-dissected tissue regions. Metabolomic profiling of the microdissected tissue was then carried out to identify biomarkers collocated with the drug using hydrophilic interaction chromatography in combination with FT-MS. Metabolomic profiling of brain tissue taken from an animal model of schizophrenia was also carried out. The neuronal PAS domain protein 3 (NPAS3) is a candidate gene for schizophrenia that is principally expressed in the CNS. Brain tissue was taken from transgenic Npas3 knockout mice to identify biomarkers relating to the underlying pathology of the disease. It was found that a dry matrix coating allowed successful mapping of pharmaceuticals in situ. MALDI IMS revealed that the drug was found in high intensity in the white matter of the cerebellum, which was confirmed through LC-MS analysis of laser microdissected tissue regions. It was also possible to identify biomarkers correlating to regions of drug accumulation, for example increased levels of o-acetylcarnitine. It was also found that there were numerous changes in small molecules in Npas3 KO mice. One marker appears to be NAD+ which was highly elevated in the animal model and may be indicative of oxidative stress.

LP4-004

Quantitative metabolic profiling of early risk for atherosclerosis by serum NMR metabonomics

Würtz, P.(1,2,3), Soininen, P.(1,4), Kangas, A.J.(1), Magnussen, C.G.(2,5), Raiko, J.(2), Thomson, R.(5), Mäkinen, V.P.(6), Groop, P.H.(6), Savolainen, M.J.(1), Viikari, J.(2), Kähönen, M.(2), Lehtimäki, T.(2), Juonala, M.(2), Raitakari, O.T.(2), Ala-Korpela, M. (1) 1 Computational Medicine, Internal Medicine, University of Oulu, Finland 2 Cardiovascular Risk in Young Finns Study Group, Turku & Tampere University Hospitals, Finland 3 Epidemiology and Biostatistics, Imperial College London, UK 4 NMR Metabonomcis Laboratory, Department of Biosciences, University of Eastern Finland, Finland 5 Menzies Research Institute, University of Tasmania, Australia 6 Folkhälsan Research Center, Biomedicum Helsinki, Finland.

Background: Atherosclerosis is the primary cause of cardiovascular disease. The disease is characterized by a long incubation period before heart attack and stroke occur. We aimed to determine associations of systemic metabolites with preclinical atherosclerosis, in particular whether the metabolite data would suggest different phenotypes conveying similar cardiometabolic risk. Methods: 1H NMR spectroscopy was applied to 4,309 serum samples from the population-based

Cardiovascular Risk in Young Finns Study. Two spectra were measured at 500 MHz from each sample; a standard 1H spectrum and a CPMG spectrum for quantification of lipoprotein subclasses and low-molecular-weight metabolites, respectively. The extent of preclinical atherosclerosis, in terms of carotid intima-media thickness (IMT), was assessed by ultrasound. Numerous lipoprotein subclasses as well as low-molecular-weight metabolites were quantified from the spectral data by regression models. Results: In these young adults (aged 24-45 years) data-driven analysis using self-organizing maps on the spectral data revealed quantitatively different metabolic phenotypes associated with elevated carotid IMT. The phenotypes were characterized by varying combinations of metabolic disturbances including elevated VLDL and LDL subclasses, but also several low-molecular-weight metabolites. Results for prediction of 6-year incidence of high carotid IMT in terms of discrimination and reclassification will also be discussed. Conclusion: The study revealed different metabolic phenotypes inherently associated with preclinical atherosclerosis. Prediction of subclinical atherosclerosis was improved by comprehensive metabolic profiling. The findings give insight into the pathophysiology of early stage atherosclerosis and substantiate developments toward the use of multi-metabolic risk phenotypes in cardiovascular risk assessment.

LP4-005

A tomato EMS mutant with altered trichome flavonoid methylation identified by LC-MS screening

Jeongwoon Kim1,2,A.Daniel Jones3,4,Robert L.Last1,3
1Department of Plant Biology 2MSU-DOE Plant Research Laboratory 3Department of Biochemistry and Molecular Biology 4Department of Chemistry Michigan State University, USA

Trichomes are specialized epidermal cells that protrude from the surface of various plant tissues. Trichome metabolites from a variety of plants are contributed to flavor and taste or are medically important. We and others have demonstrated that secretory and glandular trichomes (SGTs) in tomato produce diverse secondary metabolites, which are presumably involved in plant defense. Tomato (*Solanum lycopersicum*) is our system of choice for studying SGT metabolism because it makes SGTs and is genetically tractable. We employed 5 minute LC-ToF MS screen (Gu et al. 2009; Schilmiller, Shi et al. 2010) to identify randomly generated EMS mutants with altered trichome non-volatile chemistry. Approximately 1,400 tomato EMS mutants were screened to identify genetic variants with altered secondary metabolites. LC-MS analyses of single leaf dip extract have generated profiles of metabolites including acylsugars, alkaloids, flavonoids and others yet to be identified. Eleven groups of mutants were identified for various chemical phenotypes. For example, in contrast to wild type plants, mutant JP117 accumulates more monomethylated myricetin (mono-) than dimethylated myricetin (di-). This phenotype was consistent in the progeny, indicating that altered chemical phenotype is heritable. Further chemical analysis of backcross F1 plants revealed that the mutant allele is recessive and likely loss of function. The wild type allele showed gene dosage effect in the F1, suggesting the mutated gene could be a structural gene for an O-methyltransferase. In backcross F2, the phenotype segregated to 3:1, supporting that a single gene is mutated. The JP117 mutant was outcrossed to the S. pennellii 0716 wild tomato and chemical phenotypes of progenies were tested. Of 75 outcross F2 plants, 16 showed the mutant phenotype. The locus responsible for the mutant phenotype was mapped to chromosome 6. The identification and characterization of the mutant gene is ongoing. As shown in this study, the combination of chemical and genetic analysis provides a foundation for discovery of biosynthetic pathways leading to the production of secondary metabolites in tomato trichomes.

Wednesday, 30 June 2010

Parallel Session 6A

11.00 - 12.30	**GENOME, METABOLOME, MICROBIOME**	
	Chair: Uwe Sauer (Switzerland)	**Room**
11.00 - 11.30		
Selected speaker	**Marc-Emmanuel Dumas (UK)**	
	Genomics and network biology of metabolic profiles	Forum
11.30 - 11.50		
Selected speaker	**Jerzy Adamski (Germany)**	
	Metabolites profiled by targeted metabolomics associate with lipid levels	Forum
11.50 - 12.10		
Selected speaker	**Thomas Binsl (Netherlands)**	
	Metabolic flux determination under non-steady state conditions in an in vitro model of the large intestine containing human faecal microbiota	Forum
12.10 - 12.30		
Selected speaker	**Nicola Zamboni (Switzerland)**	
	Deep phenotyping of genomes by high-throughput metabolomics	Forum

L6A-001

Genomics and network biology of metabolic profiles

Marc-Emmanuel Dumas, Imperial College London / Ecole Normale Superieure de Lyon

The study of human multifactorial diseases like insulin resistance represents a real healthcare challenge for the western and developing world. Integration of metabolic phenotyping with other –Omics provides a systems biology approach to identify biomarkers and susceptibility genes related to the cardio-metabolic syndrome (glucose intolerance, insulin resistance, dyslipidemia, hypertension, obesity). In particular, approaches such as metabolomic Quantitative Trait Locus (mQTL) mapping1,2, or Metabolomic Genome-Wide Association Studies3 consist of the robust and accurate statistical integration of genome-wide genotyping (single nucleotide polymorphisms, microsatellites) and metabolome-wide profiling by NMR spectroscopy and mass spectrometry. New signal processing and statistical developments were performed to enhance signal recovery, locus detection and biomarker identification. Integration of mQTL studies with expression and physiological QTL studies (eQTL and pQTL) provide a powerful validation, thanks to co-localisation (cis-QTL). However, very few systems-wide cis-QTL have been identified. Alternatively, trans-QTLs can also be explained through protein interactions. From a network biology angle, candidate genes and metabolites are mapped onto biological networks, allowing an efficient visualisation of metabolomic pattern formation. Mechanistic insights derived from this systems biology approach clarify the influence of gene variants on metabolic profiles and results in a better understanding disease phenotypes and identification of potential drug targets. 1. Keurentjes, JJ. et al. The genetics of plant metabolism. Nat. Genet. 2006; 38, 842–849. 2. Dumas ME. et al. Direct quantitative trait locus mapping of mammalian metabolic phenotypes in diabetic and normoglycemic rat models. Nat Genet. 2007; 39, 666-672. 3. Illig, T. et al. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. Nat Genet. 2010; 42:137-41.

L6A-002

Metabolites profiled by targeted metabolomics associate with lipid levels

Cornelia Prehn1, Anke Nissen2 und Dominik Achten2, Christian Gieger3, Florian Kronenberg4, H.-Erich Wichmann3, Klaus M. Weinberger5, Thomas Illig3, Karsten Suhre2, Jerzy Adamski1 1 Institute of Experimental Genetics, Genome Analysis Center,

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In several diseases total cholesterol (Chol) and triglyceride plasma concentrations are considered as a risk factor. The associations between lipids concentrations and metabolic pathways in humans are still not fully understood, especially how the plasma lipid levels are modulated by disease (e.g. diabetes or cardiovascular) or drug (e.g. statin) treatment. To address these questions we used targeted metabolomics to analyze Chol-associated lipidome and further metabolic pathways in human individuals. We quantified 363 metabolites in 283 serum samples from the human cohort KORA with profiling by electrospray ionization on API 4000 tandem mass spectrometer (1). High-throughput analyses were assisted by robotized liquid handling, quality assurance and multivariate data analyses. We targeted selected analytes from the following classes: amino acids, hexoses, biogenic amines, oligosaccharides prostaglandins, acylcarnitines, sphingomyelins, and glycerophospholipids. When analyzing the concentrations of total Chol, HDL, and LDL and triglycerides we discovered novel significant associations with several analytes pointing to so far unknown cross-talks in metabolic pathways. Some phosphatidylethanolamines correlated with HDL concentrations (C36:2 at p=1.0E-09, C40:6 at p=2.2E-09), further phosphatidylcholines with that of triglycerides (C38:4 at p=3.2E-31) or total Chol (C38:1 at p=1.4E-26, C38:2 at p=4.4E-25) and sphingomyelines with total Chol (C16:0 at p=2.3E-20, C18:0 at p=2.2E-17). We discovered a significant correlation of amino acid concentrations with triglyceride concentrations (e.g. Glu at p=4.7E-10, Phe at p=2.1E-08, Trp at p=1.4E-07) with that of triglycerides. These new associations and potential links with endpoints such as cardiovascular disease will have to be investigated in the future.

(1) Th. Illig, et al. (2010) A genomewide perspective of genetic variation in human metabolism. Nature Genetics, 42(2):137-41

L6A-003

Metabolic flux determination under non-steady state conditions in an in vitro model of the large intestine containing human faecal microbiota

Binsl, T.W. (1), de Graaf, A.A. (2), Venema, K. (2), Maathuis, A. (2), de Waard, P. (3), van Beek, J.H.G.M. (1): (1) VU University (Medical Center), Amsterdam (2) TNO Quality of Life, Zeist (3) Wageningen NMR Centre, The Netherlands

Metabolism is often not in steady state under physiological conditions and the determination of transient metabolic fluxes is challenging. To determine metabolic fluxes in human faecal microbiota after food enters the intestine, isotopic labeling experiments were performed in the TNO in vitro model of the human colon (TIM-2, de Graaf et al., NMR in Biomedicine 2010,23:2-12). The TIM-2 model was inoculated with faecal microbiota and allowed to adapt to the model conditions (37°C and pH 5.8) for 16h. After 2h starvation, 1g of [U-13C]starch was added and samples were taken at t=0,30,60,120,240 minutes after starch addition. For each time point 13C mass isotopomers and 13C NMR multiplets were measured for acetate, propionate and butyrate by MS and NMR. By decomposing a metabolic model of the microbiota, pathway-discriminating labeling patterns in the 13C labeled metabolites could be inferred. Matching these labeling patterns with measured isotope incorporation enabled us to establish the metabolic flux distribution in acetate, butyrate and propionate production pathways. After deriving equations for the time-resolved dynamic mass balances, including concentration changes, we also calculated the absolute fluxes into the product pools. We found that solely 10% of the total carbon flux from starch reaches propionate. The main contribution to that flux (83%) is initially via succinate. Over time this contribution decreases, shifting towards production via acrylate (51%). The remainder of the carbon flux from starch went to acetate (79%) and butyrate (11%). The absolute flux into acetate was estimated to be 0.16 micromol/min between 0-30 min and 0.14 micromol/min between 120-240 min. We demonstrate that deriving pathway-discriminating labeling patterns by decomposition of a metabolic model, combined with dynamic mass balance analysis, is suitable to calculate flux distributions in transient metabolic systems. This method makes it possible to time-resolve the effects of nutrients on the metabolic fluxes in human faecal microbiota and will help to discover the regulation of such fluxes.

L6A-004

Deep phenotyping of genomes by high-throughput metabolomics

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Institute of Molecular Systems Biology, ETH Zurich, Switzerland

In all questions of biology that aim at - or rely on - gathering a holistic picture of cellular life, there is a pressing demand for large scale studies with thousands of strains or conditions. This applies for example to functional genomics, causal elucidation of regulatory networks, mapping of gene-environment interactions in quantitative traits, inhibitor screens, etc. To meet the need of analysing tens of thousands of sample, we established a metabolomics pipeline capable of handling > 1000 samples/day and yet provide a broad coverage of metabolism. The effort included scale-down to microscale cultivation, parallelization of

sample preparation, development of high-throughput metabolomics by flow injection – time-of-flight mass spectrometry, and novel data processing workflow. The analytical platform was thoroughly optimized for sensitivity, linearity, robustness, accuracy to ultimately attain reproducible detection of typically 2000-4000 ions in a single sample in less than a minute. Our ad-hoc processing software exploits the size of the dataset to filter background signal, remove noise, convolute multimers, adducts and isotopomers, and merge thousands of samples without sacrificing rare markers. To date the platform delivers accurate monitoring of ca. 1000-2000 biological compounds with good coverage of primary metabolism. The routine throughput is of >1400 samples/day and thus far beyond any other existing -omics workflow. With this platform, we recently completed a genome-wide analysis of the metabolome in ca. 8500 single-knockout mutants of Escherichia coli (Keio collection). For this purpose, we performed ca. 35000 analyses in 6 weeks on a single instrument obtaining excellent reproducibility. Exemplary cases for the information content of such screens and the unprecedented potential of this platform will be presented.

Wednesday, 30 June 2010

Parallel Session 6B

11.00 - 12.30	**BIOTIC INTERACTIONS AND PLANT STRESS** **Chair: Simone Rochfort (Australia)**	**Room**
11.00 – 11.30 Invited speaker	**Lloyd Sumner (USA)** Metabolomics Reveals that the Devastating Phymatotrichopsis omnivora (root rot) Pathogen Circumvents Traditional Medicago truncatula Defense Responses and Suggests Strategies for Metabolic Engineering of Resistance	E104 -107
11.30 - 11.50 Selected speaker	**Joachim Kopka (Germany)** The GC-MS metabolite profiling platform for Legume analysis (2003-2010): A case study from metabolite atlas to the testing of robustness for translational genomics	E104 -107
11.50 - 12.10 Selected speaker	**Kirsten Leiss (Netherlands)** An eco-metabolomic approach to study host plant resistance	E104 -107
12.10 - 12.30 Selected speaker	**Susanne Rasmussen (New Zealand)** Metabolomics analysis of plant-fungal associations: New insights from the Lolium perenne - Neotyphodium lolii symbiosis	E104 -107

L6B-001

Metabolomics Reveals that the Devastating *Phymatotrichopsis omnivora* (root rot) Pathogen Circumvents Traditional Medicago truncatula Defense Responses and Suggests Strategies for Metabolic Engineering of Resistance

Li, W.(1,2), Shen, W.(1), Snyder, J.H.(1,3), Uppalapati, S.R.(1), Mysore, K.E.(1), Dixon, R.A.(1), Sumner, L.W.(1) (1)The Samuel Roberts Noble Foundation, Ardmore OK; (2)Monsanto Company, St. Louis, MO. (3)Cornell University, Ithaca, NY, USA

Phymatotrichopsis omnivora is a devastating pathogen that causes substantial economic losses in more than 2000 dicotyledonous plant species including alfalfa. Currently, no cost effective chemical control methods nor sources of genetic resistance have been identified for P. omnivora. Here, metabolomics was used to study the complex biochemical interactions between the model legume *Medicago truncatula* and *P. omnivora*. Analyses of the interactions between 0 and 9 days post-inoculation revealed massive metabolic changes. Contrary to many Medicago-fungal interactions, secondary metabolite profiling by UPLC-MS revealed no significant increase in medicarpin; which is the typical and predominant isoflavonoid induced during Medicago interactions with fungal pathogens. However, increased flavone levels were observed and particularly increased 7,4-dihydroxyflavone. We conclude that *P. omnivora* circumvents traditional *Medicago* defense responses by suppressing isoflavonoids/medicarpin biosynthesis, while simultaneously inducing flavonoid biosynthesis. Quantitative RT-PCR suggests that the suppression of the isoflavonoid pathway was at the transcript level and less likely due to catabolic detoxification of isoflavonoids by the fungus. In vitro growth inhibition assays revealed that medicarpin and 7,4-dihydroxy flavone possess significant anti-microbial activity against *P. omnivora* and suggest that increased constitutive levels of these compounds represents a strategy for future metabolic engineering of alfalfa resistant to *P. omnivora*. In a parallel study, activity based fractionation of *Maclura pomifera* (Osage orange or horse apple) fruit was used to purify and identify wighteone as a potent antimicrobial compound against *P. omnivora*. Wighteone, a.k.a 6-prenyl genistein, is also present in white lupin (*Lupinus albus*) and lupin cDNA libraries were queried to identify potential prenyltransferases. One target was cloned and heterologously expressed. In vitro enzymatic assays with the

recombinant protein yielded successful prenylation of genistein to form wighteone. These results provide another mechanism for metabolic engineering of resistance in alfalfa by the introduction of the prenyltransferase and accumulation of wighteone and other prenylated (iso)flavnonoids.

L6B-002

The GC-MS metabolite profiling platform for Legume analysis (2003-2010): A case study from metabolite atlas to the testing of robustness for translational genomics

Joachim Kopka (1), Diego H. Sanchez (2), Michael K. Udvardi (3) (1) Max Planck Institute for Molecular Plant Physiology, Wissenschaftspark Golm, Am Mühlenberg 1, Potsdam-Golm, 14476, Germany. (2)Present address: Division of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA. (3) Samuel Roberts Noble Foundation, 2510 Sam Noble Pky., Ardmore, OK 73401, USA.

Translational genomics, namely the use of model species to generate knowledge about biological processes and the functions of genes, offers great promise to biotechnologists. Metabolome information contributes to this approach. For example, the metabolite targeted and also non-targeted GC-MS based profiling promises new and potentially transferable insights into the metabolic aspects of plant acclimation responses to environmental stresses, perhaps even more so, when combined with profiling results from other systems levels. With this vision in mind a GC-MS metabolomics platform was established for the Legume analysis. The project started out in 2003 as an atlas of metabolites observed in diverse legume tissues and is now in 2010 used to seek for robust responses of model legumes to environmental stresses. Salinity stress responses were tackled by altering the stress dosage and more importantly by repeating experiments independently and in consecutive years. Aspects from the data mining of such legume salt acclimation experiments are presented and discussed. A special focus is set on the mining of robust system features at the ionomic, transcriptomic and metabolomic levels. We applied best possible controlled greenhouse conditions and asked two main questions: How reproducible are results obtained from physiologically meaningful salinity experiments, and what degree of bias may be expected if conclusions are drawn from less well-repeated sampling? A surprisingly large fraction of the transcriptional and metabolic responses to salt stress were not reproducible between experiments. But a core set of robust changes was found which was clearly shared between experiments. Many of these robust responses were qualitatively and quantitatively conserved between different accessions of the same species, indicating that the robust responses may be a sound starting point for translational genomics.

L6B-003

An eco-metabolomic approach to study host plant resistance An eco-metabolomic approach to study host plant resistance

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Due to a massive increase in international movement of plant material crop pests have spread worldwide. To control these integrated pest management (IPM) is necessary. An important strategy of IPM is the use of chemical host plant resistance. Up to now the study of chemical host plant resistance has, for technical reasons, been restricted to the identification of single compounds applying specific chemical analyses adapted to the compound in question. In biological processes however, different compounds, which are 'a priori' unknown, are involved. A way to solve this problem is to use metabolomics, which allows the simultaneous detection of a wide range of metabolites. We have developed an eco-metabolomic approach, using NMR (Nuclear Magnetic Resonance Spectroscopy), to identify candidate compounds involved in host plant resistance. We classify resistant and susceptible plants using 'in-vivo' bioassays. Subsequently, we compare their metabolomic profiles by applying multivariate statistical analysis to identify metabolites involved in host plant resistance. The negative effect of the candidate compounds is validated with 'in-vitro' bioassays. As a proof of principle we used western flower thrips ('Frankliniella occidentalis') in different host systems including Senecio as a wild plant, chrysanthemum as an ornamental and tomato as a crop. In all three host systems the metabolomic profiles of thrips-resistant and –susceptible plants were significantly different leading to a range of different metabolites involved in thrips resistance. Thrips resistant Senecio contained higher amounts of the pyrrolizidine alkaloids (PA) jacobine and jaconine and the flavanoid, kaempferol glucoside. Chrysanthemums resistant to thrips contained higher amounts of the phenylpropanoids chlorogenic acid and feruloyl quinic acid. Tomatoes with little thrips damage contained high amounts of acylsugars. Besides their negative effect on herbivores kaempferol and the phenylpropanoids are investigated for their positive effect on human health preventing cancer development. This unique combination makes them the candidates of choice for development of host plant resistance. Our results show that NMR- metabolomics constitutes a significant advance in the study of plant-insect relationships providing key information for the implementation of herbivore resistance breeding programmes in plants.

L6B-004

Metabolomics analysis of plant-fungal associations: New insights from the *Lolium perenne – Neotyphodium lolii* symbiosis

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The association of plants with endosymbiotic microorganisms poses a particular challenge to metabolomics studies. The presence of endosymbionts can alter metabolic profiles of plants by introducing non-plant metabolites like e.g. fungal specific alkaloids, and by metabolic interactions between the two organisms. Here, we provide a synthesis of some of our recently published studies on metabolic profiles of *Lolium perenne* plants infected with endophytic *Neotyphodium lolii* fungi. The vast majority of literature has focussed on a limited number of alkaloids produced by N. lolii which have been shown to negatively affect insect pests and vertebrate herbivores. Much less is known about the effects on other metabolites, or effects of resource supply on the symbiotic metabolism; and how metabolic consequences of the symbiosis depend on the amounts (concentrations) of endophyte present. Symbiotic tissues were analysed using targeted and untargeted metabolic profiling methods as well as quantitative PCR to estimate abundances of the fungal endosymbiont. Based on these analyses we present and discuss a hypothetical schematic representation of possible links between plant and fungal metabolic networks. A multiple regression analysis of insect responses to endophyte infected plants and their metabolic profiles revealed an intriguing differential pattern of insect population sizes to non-alkaloid metabolites.

Sponsor Lunch Session Wednesday, 30 June 2010
Session 3A: Sponsored by Agilent
Room: Forum

13.00 – 13.55	**Technology showcase: Agilent**	**Speakers**
13.00 – 13.25	Advances In Data Processing Software For Metabolomics (1)	Steven Fischer
13.25 – 13.50	Advances In Data Processing Software For Metabolomics (2)	Theodore Sana

Advances In Data Processing Software For Metabolomics

Abstract:
Features finding and compound identification are critical for successful discovery metabolomics. Agilent's advanced software suit enables analysis by an un-targeted data mining approach and / or by a user defined list of compounds. Each of these approaches has advantages that result in more thoroughly mined data for subsequent statistical analysis, compound identification and data visualization. Confirmation of any potential biomarkers or testing a discovery generated hypothesis can then be accomplished using a targeted metabolomic study.

We demonstrate the advantages of our Metabolomics workflow in the analysis of a host/parasite model of Malaria infection. Molecular feature data sets were generated using MassHunter Qual and subsequently evaluated for differences in Mass Profiler Professional (MPP). Statistically significant features were matched to compounds in our METLIN database using an ID browser for feature annotation. A Pathway Analysis module within MPP was then used to map the annotated compounds to specific pathways. The research driven hypothesis was then tested using a targeted QQQ analysis.

Wednesday, 30 June 2010

Parallel session 7A

14.00 - 15.30	**PATHWAYS DISCOVERY AND DISEASE PATHOPHYSIOLOGY** **Chair: Matej Oresic (Finland)**	**Room**
14.00 – 14.30 Invited speaker	**Joshua Rabinowitz** Viral Hijacking of Host Cell Metabolism	Forum
14.30 - 14.50 Selected speaker	**Kenjiro Kami (Japan)** CE-MS-based Metabolomics Identified a Novel Anaerobic Energy Metabolism of Cancer Cells	Forum
14.50 - 15.10 Selected speaker	**Jean-Charles Portais (France)** Complementarity of NMR and MS for pathway discovery using 13C-labelling strategies: the example of the ethylmalonyl-CoA pathway in bacteria	Forum
15.10 - 15.30 Selected speaker	**Dinesh Barupal (USA)** Impact of hormonal receptors expression on breast cancer metabolic phenotypes	Forum

L7A-001

Viral Hijacking of Host Cell Metabolism

Joshua D. Rabinowitz, Princeton University, Princeton, USA

Viruses rely on the metabolic network of their cellular hosts to provide energy and building blocks for viral replication. We used liquid chromatography-mass spectrometry to quantitate metabolite concentration and flux changes induced by viral infections. The flux measurement approach relies on monitoring metabolome labeling kinetics after feeding cells (13)C-labeled forms of glucose and glutamine. For influenza A and herpes simplex virus (HSV), the most profound metabolome alterations occur in pathways that are the targets of current antiviral therapies: neuraminidase for influenza A and thymidine biosynthesis for HSV. In contrast, for human cytomegalovirus (HCMV), flux increases are broad based and include a particularly notable increase in flux through the tricarboxylic acid cycle and its efflux to the fatty acid biosynthesis pathway. Pharmacological inhibition of fatty acid biosynthesis suppresses the replication of HCMV. Intriguingly, it also blocks influenza A replication. These results show that systems-level metabolic flux profiling can identify metabolic targets for antiviral therapy.

L7A-002

CE-MS-based Metabolomics Identified a Novel Anaerobic Energy Metabolism of Cancer Cells

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Chronically hypoxic and glucose-deprived microenvironment of tumors, as typically observed in pancreatic cancer, raises a paradox; cancer cells perpetually proliferate while exhausting glucose and oxygen from the surrounding tissue. Intrinsically, cancer cells actively consume glucose even under aerobic condition (Warburg effect) and their adaptive responses to hypoxia further enhance glucose consumption. With a limited supply of glucose, upregulation of glycolysis alone cannot fully explain energy production of hypoxic cancers. Here, we used

state-of-the-art metabolomics technology based on capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and analyzed energy metabolism of 9 cancer and 4 normal cell lines exposed to hypoxia and glucose deprivation by measuring more than 130 compounds with time. The results disclosed unexpected cancer-specific metabolic profiles, which sparked an idea that cancer cells exposed to a severe nutrient deprivation rely on an atypical anaerobic energy metabolism that resembles so-called NADH-fumarate reductase system identified in parasitic helminthes. Intriguingly, pyrvinium pamoate, a commercially available anthelmintic, is known to be cytotoxic against not only parasites but also cancer cells exclusively under nutrient deprived condition, supporting an idea that cancer cells and parasitic helminthes share unique anaerobic energy production machinery in common. Accordingly, we demonstrated a potential of CE-MS-based metabolomics, realizing comprehensive and temporal monitoring of the energy metabolism of cancer cells. This unique metabolic machinery may constitute a novel therapeutic target to eradicate not only parasites but also tumors.

L7A-003
Complementarity of NMR and MS for pathway discovery using 13C-labelling strategies: the example of the ethylmalonyl-CoA pathway in bacteria.

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Mass spectrometry and NMR are powerful tools for resolving the topology of complex metabolic networks from 13C-labeling experiments. The two techniques are most often applied separately but their combination is highly valuable, since the increasing sensitivity of mass spectrometers allows short term labeling experiments to be carried out, resulting in dynamic information on a given metabolic network, while NMR is unique in providing detailed positional labeling information from which metabolic pathways can be directly identified and quantified. We have combined both mass spectrometry and NMR to demonstrate the operation of a novel metabolic pathway, namely the ethylmalonyl-CoA (EMC) pathway (Erb et al. PNAS), in the isocitrate lyase-negative methylotroph Methylobacterium extorquens AM1 (Peyraud et al. PNAS 2009). This pathway involves a series of 11 reactions where intermediates are all CoA esters, and represents an alternative of the classical glyoxylate cycle for the biosynthesis of glyoxylate. The operation of this pathway was first demonstrated by very short-term labeling experiments carried out with 13C-acetate, which allowed determination of the sequence of reactions from the order of label incorporation into the different CoA derivatives. The analysis of 13C positional enrichments by NMR during steady-state labeling experiments with 13C-methanol proved not only the operation of the EMC pathway during growth on methanol, but also revealed that 2 molecules of glyoxylate are regenerated in this process. A complete set of mass and positional isotopomer data (165-170 isotopomer data per experiment) was collected on proteinogenic amino-acids to establish the distribution of metabolic fluxes during methylotrophic growth. Together with the reconstruction of the genome-scale metabolic network of this organism, this work provides the complete topology of the central metabolic network actually operating during methylotrophic growth in M. extorquens AM1. The operation of the ethylmalonyl-CoA pathway has major implications for the physiology of ICL- methylotrophs and provides a common ground for C1 and C2 compound assimilation in isocitrate lyase-negative bacteria.

L7A-004
Impact of hormonal receptors expression on breast cancer metabolic phenotypes

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Breast cancer is a leading cause of deaths worldwide. The expression of hormonal receptors (ER, PR, and HER2) influences cellular signaling and metabolic pathways. In this study, we aimed to identify distinct metabolic phenotypes for mammary tumors, specifically for patients with grade 3 tumors and triple-negative hormone receptor status tumors (estrogen ER, herceptin HER2 and progesterone PR). Using a cohort of 261 clinically well-characterized breast cancer patients in independent training and validation series, over 400 unique metabolites were detected by GC-TOF MS profiling and BinBase data processing of which a total of 154 non-redundant metabolites were structurally identified by a multi-tiered retention index and mass spectral scoring algorithm. Subsequently, a range of statistical comparisons were undertaken to distinguish specific metabolic phenotypes in grade 1, 2 and 3 tumors and subcategories of receptor status. 50% of all patients had ER+,PR+,HER- receptor status , 12% triple positive status and 14% triple negative receptor status (ER-,PR-,HER-). The latter group has worse prognosis and fewer therapy options and thus, a better understanding of cancer cell metabolism may help finding novel target pathways. We found that the most important metabolic phenotypes distinguishing different tumors were the morphological differentiation status (grade 1-3) and expression of the estrogen receptor (ER-/ ER+), specifically for alterations in nucleotide metabolism and the arachidonate pathway . Triple negative tumors (ER-,PR-,HER-) were mostly of the poorly differentiated

grade 3-subtype with dysregulation of amino acids, nucleotide and energy metabolism in comparison to grade 3 tumors of the double-positive ER+,PR+,HER- category. Subsequently, the metabolic impact of the presence of the herceptin receptor HER2 was investigated by comparing triple negative to double-negative tumors (ER-,PR-,HER+). In this comparison, a clear influence of lipid metabolism was found by significantly higher levels of free fatty acids and glycerol phosphates pointing towards biosynthesis of membrane lipids that are a hallmark of cancer cell metabolism and cell division. Together, these studies may lead to novel hypotheses on cancer cell metabolism with potential high impact for therapeutic options.

Wednesday, 30 June 2010

Parallel Session 7B		
14.00 - 15.30	**PLANT SYSTEMS BIOLOGY**	
	Chair: Mike Beale (UK)	**Room**
14.00 – 14.30		
Invited speaker	**Wolfram Weckwerth**	
	Green Systems Biology – from genomes, metabolomes and proteomes to ecosystems	E104 -107
14.30 - 14.50		
Selected speaker	**Annemie Geeraerd (Belgium)**	
	Systems biology investigation of the ethylene metabolism of tomato fruit	E104 -107
14.50 - 15.10		
Selected speaker	**Atsushi Fukushima (Japan)**	
	Comparative metabolomics characterizes the impact of genotype-dependent methionine accumulation in Arabidopsis thaliana	E104 -107
15.10 - 15.30		
Selected speaker	**Animesh Acharjee (Netherlands)**	
	Integrating genetic markers with ~omics data using genetical genomics and modern regression methods	E104 -107

L7B-001

Green Systems Biology: from genomes, metabolomes and proteomes to ecosystems

Wolfram Weckwerth, Department of Molecular Systems Biology (MOSYS; http://www.univie.ac.at/mosys/), University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Plants have shaped human life forms since their rising. With emerging recognition of world population feeding, global climate change and limited energy resources with fossil fuels, the relevance of plant biology and biotechnology becomes dramatically important. Consequently, it can be anticipated that plant biology and applications will have even more indispensable future roles in all socio-economic aspects of our life. In parallel the last 10 years have recognized a revolution in biology basically as a result of three main developments: (i) shotgun and next-generation genome sequencing, gene reconstruction and annotation, (ii) genome-scale molecular analysis using omics-technologies and (iii) computer-assisted analysis, modelling and interpretation of biological data. Systems biology is the approach to combine these molecular data, genetic evolution, environmental cues and species-interaction with the understanding, modelling and prediction of active biochemical networks. The idea relies strongly on the development of new technologies for the analysis of molecular data, especially metabolomics, proteomics and transcriptomics based on genome sequencing and gene annotation. The ambitious aim of these non-targeted 'omic' technologies is to extend our understanding beyond the analysis of only parts of the system. However, before data reveal their interrelation, extended statistical and mathematical concepts are required for the integrative analysis of multifactorial phenomena. Most of these data mining tools are closely related - based on covariance and/or correlations within a data matrix and will reveal the connectivity of a networking system and a novel understanding of the system by integration of metabolite-, protein-, transcript-levels and environmental data using biostatistics and mathematical modeling 1-4. References 1 Morgenthal, K., et al. Metabolomics 1, 109-121 (2005). 2 Wienkoop, S. et al. Mol Biosyst 6, 1018-1031, doi:10.1039/b920913a (2010). 3 Wienkoop, S. et al. Mol Cell Proteomics 7, 1725-1736 (2008). 4 Weckwerth, W. Annu Rev Plant Biol 54, 669-689 (2003).

L7B-002

Systems biology investigation of the ethylene metabolism of tomato fruit

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Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium (3) Laboratory of Protein Phosphorylation and Proteomics, Department of Molecular Cell Biology, Katholieke Universiteit Leuven, Herestraat 49, 3000 Leuven, Belgium (4) Laboratory of Tropical Crop Improvement, Department of Biosystems, Katholieke Universiteit Leuven, Kasteelpark Arenberg 13, 3001 Leuven, Belgium (5) Plant Physiology Laboratory, Department of Biosystems, Katholieke Universiteit Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium

A systems biology approach is used to investigate the ethylene metabolism of tomato during fruit development, fruit ripening and postharvest storage. Ethylene plays an important role in the ripening of climacteric fruit and is biosynthesized autocatalytically during ripening. Our results show that fruit ethylene production showed a normal climacteric behavior. Besides ethylene other pathway intermediates (1-aminocylopropane-1-carboxylic acid (ACC), N-malonyl-ACC (MACC), S-adenosyl-L-methionine (SAM) & Methionine (Met)) were fully profiled by different analytical techniques (GC, GC-MS and CE). ACC-oxidase (ACO) in vitro enzyme activity was found to be strongly correlated with the whole fruit ethylene production. ACO protein levels were studied by western blotting and did not completely correlate with its activity. After the climacteric rise, fruit ethylene production and ACO in vitro enzyme activity decreased, while ACO protein levels remained highly abundant. This observation was confirmed at the genetic level by means of RT-qPCR. Overall ACO expression levels stayed high after the climacteric peak. A shift between two ACO isoforms during the decline in ethylene production was observed. These combined results indicate that different isoforms have different enzyme activities and are regulating different stages of fruit development. Further potential post-translational modification or enzyme inactivation are being investigated. Our work shows that a systems biology approach, covering three hierarchical biological domains, might reveal multiple pathway regulation mechanisms.

L7B-003
Comparative metabolomics characterizes the impact of genotype-dependent methionine accumulation in Arabidopsis thaliana

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Methionine (Met), an essential amino acid for all organisms, has many important functions in plant cellular metabolism such as a precursor of plant hormones, polyamines, and glucosinolate. The regulatory mechanism of Met biosynthesis is highly complex and, despite its great importance, remains largely uncharacterized. To answer the key question of how accumulation of Met influences metabolism in Arabidopsis, we examined three methionine over-accumulation (mto) mutants using a established gas chromatography (GC)-time-of flight (TOF)/mass spectrometry (MS) protocol for metabolite profiling. Principal component analysis showed distinct metabotypes of the three mto mutants, mto1, mto2, and mto3. A more sophisticated approach based on orthogonal projection to latent structures–discriminant analysis highlighted genotype-related differences. Though Met accumulation in mto1 had no dramatic effect on other metabolic pathways except for the aspartate family, metabolite profiles of mto2 and mto3 indicated that several extensive pathways were affected in addition to over-accumulation of Met. The marked changes in metabolic pathways in both mto2 and mto3 were associated with polyamines. We suggest that comparative metabolomics can not only reveal the impact of Met over-accumulation on metabolism, but also may provide clues to identify crucial pathways for regulation of metabolism in plants.

L7B-004
Integrating genetic markers with ~omics data using genetical genomics and modern regression methods

Animesh Acharjee, Wageningen University Laboratory of Plant Breeding

Utilization of the natural genetic variation in traditional breeding programs remains a major challenge in crop plants. In the post genomic era, high throughput technologies give rise to data collection in fields like transcriptomics, metabolomics and proteomics and as a result, large amounts of data have become available. We have screened a diploid potato population for gene-expression and obtained LC-MS data resulting in the identification of many expression and metabolite QTL's across the genome. However, the integration of these data sets with phenotypic and marker data is still problematic. Here we present novel approaches to study the various ~omic datasets to allow the construction of networks integrating gene expression, metabolites and markers. We used univariate regression and modern regression methods like lasso, elastic net, sparse partial least squares regression to select subset of the metabolites and transcripts which shows association with potato tuber flesh colour. Selected subset of metabolites and transcripts shows high significant (p value <2.2e-16)to the flesh colour trait and variance explained by regression model is about seventy one percent.

Wednesday, 30 June 2010

Parallel Session 8A

16.00 - 17.30	**HOT TOPICS** **Chair: Joshua Rabinowitz (USA)**	**Room**
16.00 - 16.30 Selected speaker	**Roland Wohlgemuth (Switzerland)** Bioinspired Synthesis of Chiral and Non-Chiral Metabolite Standards	Forum
16.30 - 16.50 Selected speaker	**Michael Lassman (USA)** Polar metabolite profiling in biological tissues and fluids for qualitative and quantitative analysis	Forum
16.50 - 17.10 Selected speaker	**Huiru Tang (China)** Combined NMR and LC-DAD-MS Analysis Revealed Comprehensive Metabonomic Variations for Three Phenotypic Cultivars of Salvia Miltiorrhiza Bunge	Forum
17.10 - 17.30 Selected speaker	**Roldán Cortés (Spain)** Determination of metabolic Volatile Organic Compounds in exhaled breath of patients with Chronic Obstructive Pulmonary Disease by Gas Chromatography Mass Spectrometry.	Forum

L8A-001

Bioinspired Synthesis of Chiral and Non-Chiral Metabolite Standards

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For the many metabolites typically present in biological systems, authentic material for unambiguous assignment is useful. Therefore, the extension of the range of avai-lable well-defined and pure metabolite standards is key for a variety of experiments. The chemical synthesis of densely and differentially functionalized small molecules is not a small endeavour, but requires combinations of the best selective methods known. The revitalization of this classical biochemistry area has been started with the focus on the central metabolites at pathway intersections of healthy biological systems and with the synthesis of the metabolite of interest in racemic form. As the chirality of many important metabolites has biochemical relevance, the synthesis of key meta-bolites in both racemic and chiral form is of interest. In our metabolite initiative, this has been achieved in a three-phase process by first synthesizing the racemic form, then developing the analytical methods for the separation of the enantiomers and in the third phase synthesizing the chiral metabolite. The natural metabolic pathways have not only inspired both our classical chemical and biocatalytic syntheses, but have also been an starting point for assembling a number of biocatalytic steps by preparing the required enzymes and auxiliary reagents [1-3]. New results from the synthesis of metabolites in glycolysis, pentose phosphate, mevalonate and non-mevalonate, steroid and vitamin B6 pathways has been achieved with new tools and ingredients. These tools are also utilized for the synthesis of stable-isotope-labelled central metabolites. Remote metabolite synthesis can build on the central metabolites as hubs and selected examples will be presented. References: [1] Wohlgemuth R. Biotechnol. J. 2009, 9:1253-1265. [2] Richter N, Neumann M, Liese A, Wohlgemuth R, Eggert T, Hummel W. ChemBioChem 2009, 10:1888-1896. [3] Richter N, Neumann M, Liese A, Wohlgemuth R, Weckbecker A, Eggert T,Hummel W. Biotech. Bioeng. 2010, DOI 10.1002/bit.22714 (in press). [4] Wohlgemuth R. J. Mol. Catal. B: Enzymatic 2009, 61:23-29. [5] Schell U, Wohlgemuth R, Ward JM. J.Mol.Catal.B: Enzymatic 2009, 59: 279-285.

L8A-002
Polar metabolite profiling in biological tissues and fluids for qualitative and quantitative analysis

1Michael Lassman, 1Rory Rohm, 2Margaret Wu, 1Dan Xie, 1Stephen Previs, 1Jose Castro-Perez, 2Robert Myers, 2Joel Berger, 2James Mu, 1Thomas Roddy 1Atherosclerosis Exploratory Biomarkers, 2Diabetes Biology. Merck & Co Inc. Rahway, USA

In order to advance a potential pharmacological target, pharmaceutical research groups are faced with multiple analytical challenges. Included is the challenge of not only confirming target engagement for a given molecule, but often of determining mechanism of action for a molecule that has demonstrated pharmacodynamic effects but whose target and or mechanism of action is yet unclear. For targets affecting metabolic function such as those for the treatment of diabetes, obesity or atherosclerosis, the target may frequently be an enzyme responsible for shuttling carbon between key metabolic nodes. We apply a general strategy for simultaneous qualitative and quantitative analysis of key polar metabolites involved in energy metabolism. A dual HILIC chromatography strategy combined with high resolution full scan mass spectrometry allows for detection and quantitation of metabolites from a single sample preparation. The sample preparation is limited and the analysis is optimized for throughput. The use of multiple stable labeled internal standards allows for absolute quantitation and better analytical precision. When applicable, we use stable isotope labeled substrates to increase the analytical window between vehicle and pharmacological intervention. Preliminary Data: Biological samples including plasma, urine, liver, muscle, adipose, pancreas and heart were homogenized in 75% acetonitrile, which precipitates proteins and inactivates metabolite degrading enzymes. The supernate is collected and can be injected directly onto the LC/MS system. We employ two different HILIC methods for the analysis of polar metabolites: one for the analysis of anions and another for the analysis of cations. Here we present data collected from multiple mouse animal models as well as multiple species, demonstrating the translational approach of metabolite profiling. Mice treated with insulin show clear increases in glucose uptake as expected, as well as corresponding increased production of downstream amino acids and TCA cycle metabolites as monitored in plasma. Analysis of individual tissues reveals the target tissues responsible for these effects.

L8A-003
Combined NMR and LC-DAD-MS Analysis Revealed Comprehensive Metabonomic Variations for Three Phenotypic Cultivars of Salvia Miltiorrhiza Bunge

Hui Dai, Chaoni Xiao, Hongbing Liu, Fuhua Hao, Huiru Tang
State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, P.R.China

Metabonomic analysis is an important molecular phenotyping method for understanding plant ecotypic variations and gene functions. Here, we systematically characterized the metabonomic variations associated with three *Salvia miltiorrhiza* Bunge (SMB) cultivars using the combined NMR and LC-DAD-MS detections in conjunction with multivariate data analysis. Our results indicated that NMR methods were effective to quantitatively detect the abundant plant metabolites including both the primary and secondary metabolites whereas the LC-DAD-MS methods were excellent for selectively detecting the secondary metabolites. We found that the SMB metabonome was dominated by 28 primary metabolites including sugars, amino acids and carboxylic acids and 4 polyphenolic secondary metabolites, amongst which N-acetylglutamate, asparate, fumurate and yunnaneic acid D were reported for the first time in this plant. We also found that three SMB cultivars growing at the same location had significant metabonomic differences in terms of metabolisms of carbohydrates, amino acids and choline, TCA cycle and the shikimate-mediated secondary metabolisms. We further found that the same SMB cultivar growing at different locations differed in their metabonome. These results provided important information on the ecotypic dependence of SMB metabonome on the growing environment and demonstrated that the combination of NMR and LC-MS methods was effective for plant metabonomic phenotype analysis.

L8A-004
Determination of metabolic Volatile Organic Compounds in exhaled breath of patients with Chronic Obstructive Pulmonary Disease by Gas Chromatography Mass Spectrometry.

Roldán Cortés (1), Ms. Ana Guaman (2), Ms. Idoya Agudo (2), Dr. Daniel Calvo (3), Dr. Antonio Pardo (3), Dr. Santiago Marco (2,3), Dr. Joan Albert Barberà (4), Dr. Federico P. Gómez (4) and Prof. Dr Marta Cascante (1): (1) Department of Biochemistry and Molecular Biology, IBUB, University of Barcelona and IDIBAPS, Barcelona, Spain, 08028; (2) Artificial Olfaction Group, Inst. for Bioengineering of Catalonia (IBEC), Barcelona, Spain, 08028; (3) Intelligent Signal Processing (ISP), Department of Electronics, Universitat de Barcelona, Barcelona, Spain, 08028 and (4) Department of Respiratory Medicine, Hospital Clínic, CIBERES, IDIBAPS, Universitat de Barcelona, Barcelona, Spain, 08036.

Metabolism generates Organic Volatile Compounds (VOCs) detectable in exhaled air. Due to an alteration in normal metabolism, altered patterns of exhaled VOCs can be found in several diseases. In lung disorders, it is expected that abnormal

pulmonary and systemic metabolism result in altered exhaled VOCs pattern, with the potential for diagnosis and pathological understanding. Chronic Obstructive Pulmonary Disease (COPD) is characterized by increased inflammation and oxidative stress, and there are indications that the pattern of exhaled VOCs in COPD can be different from that in healthy patients. Aims: We present a method to identify VOCs profile in breath samples to separate COPD patients from healthy volunteers. Methods: In a pilot study, 12 COPD patients (FEV1, 48 9% pred) and 17 controls were evaluated. End-exhaled breath samples (5x80mL, in duplicate) were obtained using a breath sampler (BioVOC) and transferred to a Tedlar Bag. Solid Phase Microextraction (SPME) was used to preconcentrate the samples. Analysis was carried out with a Gas Chromatographer coupled to a Mass Spectrometer. MZmine software was used to process the spectra obtained. Results: Analysis of the spectra showed differences between COPD and healthy VOCs profiles, even though variability between subjects is still high. The pattern of exhaled VOCs differs when considering environmental VOCs for the analysis. Conclusions: Pre-concentrated end-exhaled breath VOCs analysis using GC/MS is a non-invasive method with promising potential in distinguishing VOCs patterns from COPD and healthy breath samples. Supported by: SEPAR 06, ISCIII-RTICC (RD06/0020/0046) and PI080283-FIS.

Wednesday, 30 June 2010

Parallel Session 8B

16.00 - 17.30	**FOOD & APPLIED METABOLOMICS** **Chair: Robert Hall (Netherlands)**	**Room**
16.00 - 16.30 Invited speaker	**Annick Moing (France)** Metabolic, enzymatic and mineral element profiling issued from multiple analytical platforms renews the study of melon fruit quality and physiology	E104 -107
16.30 - 16.50 Selected speaker	**Ilka Abreu (UK)** Metabolomics as a potential selection tool for novel traits in soft fruit breeding programs	E104 -107
16.50 - 17.10 Selected speaker	**Yury Tikunov (Netherlands)** An integrated x-omics approach to uncover tomato fruit quality traits	E104 -107
17.10 - 17.30 Selected speaker	**Federico Marini (Italy)** The influence of a sports drink on the post-exercise metabolism of elite athletes as investigated by NMR-based metabolomics.	E104 -107

L8B-001

Metabolic, enzymatic and mineral element profiling issued from multiple analytical platforms renews the study of melon fruit quality and physiology

Annick Moing (1,2) and about 30 scientists from 10 research groups within the EU META-PHOR project (http://www.meta-phor.eu/) (1) INRA - UMR619 Fruit Biology (2) Metabolome-Fluxome Facility of Bordeaux Functional Genomics Center, IBVM, Centre INRA de Bordeaux, F-33140 Villenave d'Ornon, France

Variations in fruit flesh development and composition, along fruit growth and maturation, have a major impact on the taste, flavor and nutritional quality of ripe melon fruit (*Cucumis melo L.*). In order to characterize melon fruit quality and physiology, we studied the metabolic and mineral element changes in melon fruit, using an unprecedented range of analytical platforms: targeted LC-DAD of isoprenoids and ICP-MS of mineral elements, untargeted proton NMR, GC-MS and LC-MS of non-volatile and volatile primary and secondary metabolites, as well as enzymatic measurements. First, we used metabolite and element profiling of commercial varieties in order to characterize the effects of year, culture conditions and genotypes on fruit flesh quality and highlight discriminant compounds. Second, after a preliminary study on mature fruit (Biais et al. 2009, Anal. Chem. 81:2884), we dissected the spatial and temporal variability of metabolic, enzymatic and mineral element profiles in the flesh of one cultivar in order to improve our knowledge of fruit metabolism and physiology. The changes in absolute or relative quantity of about 2000 metabolites, 15 mineral elements and 10 enzymes were determined from the outside to the inside of fruit mesocarp at three stages of development. We took advantage of the metabolite and element variability, to study co-regulated compounds using k-means clustering and correlation networks. This analysis revealed hub metabolites in the networks, and highlighted the crosstalk between primary and secondary metabolites and between metabolites and mineral elements. In addition, the relationships between primary metabolite contents and activities of several enzymes of central metabolism were studied. Proton NMR and GC-MS spectra and data, as well as metadata, were deposited into MeRy-B database (http://bit.ly/meryb) and will be open to the community. The present results demonstrate the potential of combined metabolomics approaches for diagnostic studies of fruit quality as well as new physiology studies corroborating previously known metabolic links and revealing unexpected metabolic relationships.

L8B-002
Metabolomics as a potential selection tool for novel traits in soft fruit breeding programs.

Ilka N. Abreu, Eapen N Kanichukattu, Sandra Gordon, Rex Brennan, Derek Stewart Scottish Crop Research Institute, Invergowrie, DD2 5DA, Dundee – UK

In traditional fruit breeding programmes, the selection of new varieties depends on targeted analysis of flavour, resistance, texture, etc. Such analyses are expensive and difficult when analysing large populations of progenies. Metabolomics can be used as a non-targeted analysis approach to guide in breeding programmes for trait selection. We tested commercial blackcurrant varieties as a model to characterize important traits to be considered in breeding programmes. As part of our blackcurrant breeding programme, wherein selection encompasses improved pest resistance, fruit quality and nutritional value, a segregating population (~200 progeny) has been established. These were genotyped for a marker for gall mite resistance and samples (from two years) were analysed for productivity (juice yield and berry size) and metabolite profile. Different liquid chromatographic methods coupled to different detectors (PDA, Amperometric and Orbitrap/MS) were carried out for measurements of primary and secondary metabolites. The correlation between the metabolite profiles of commercial varieties with sensory data showed that different classes of phenolic compounds, positively correlated with aroma and flavour. Negative correlations were found for sugars and organic acids. A correlation network helped to understand the balance between specific polyphenols and primary metabolites. With the help of multivariate statistics we could identify potential metabolite markers for gall mite susceptibility and resistance. This approach allowed us to select candidates for resistance with superior nutritional traits. Hence, large populations can be reduced to a few candidates, which later can be subjected to sensory analysis. Furthermore, we found that about 20% of the progeny displayed metabolic plasticity year-on-year for most of the metabolites analysed. Those genotypes could potentially be used in breeding programmes to target positive adaptation to climate change or reduced inputs.

L8B-003
An integrated x-omics approach to uncover tomato fruit quality traits

Tikunov, Y.M., Ballester Frutos, A.R., de Vos, R.C.H., Molthoff, J., Bovy, A.G. Plant Research International, 6708 PB, Wageningen

The past decade has been marked by a significant breakthrough in development of x-omix methods. These methods enable comprehensive quantitative and qualitative analyses of plants at different levels of organization: genome, transcriptome, proteome and metabolome. Molecular and biochemical interactions at these levels form a crop's quality characteristics. Unraveling these interactions is a key to control crop quality. Sensory characteristics, such as flavour and taste, are important attributes of quality of vegetables and fruit. The typical flavour of fresh tomato fruit and products produced thereof are appreciated worldwide. Volatile organic compounds (VOCs) have a significant impact on tomato fruit flavour. The qualitative and quantitative composition of VOCs in tomato fruit is the result of a balance in a chain of internal cellular processes: biosynthesis – degradation – emission – conversion – storage. This balance is affected by both internal genetic and external environmental factors. The scope of this study is to identify environmentally consistent metabolite QTLs (mQTL) in tomato, to unravel their interactions and to discover the factors underlying these mQTLs, by integration of different x-omics data. Fruit of 33 S. lycopersicum x S. chmielewskii introgression lines (ILs) grown in two different climatic regions in Europe, France (INRA, Avignon) and the Netherlands (WUR, Wageningen) were profiled for VOC and non-VOC metabolites using GC-MS and LC-MS approaches, respectively. Environmentally consistent mQTLs were determined by comparative analysis of both geographic data sets. Putative identification followed by multivariate statistical analyses showed that structurally or biochemically related metabolites seem to be regulated in a coordinate manner by certain genomic loci. This suggests that these loci may carry structural or regulatory genes affecting metabolite levels. A high-throughput qRT-PCR platform for expression analysis of transcription factors (TF) has been developed and applied to profile the IL population for transcription factors expressed in ripening tomato fruits. Several transcription factors showed expression patterns correlating to the VOC and non-VOC metabolite profiles. Analysis of tomato genomic data showed co-location of some of candidate TFs and mQTLs determined. Functional analyses of candidate TFs are currently underway.

L8B-004
The influence of a sports drink on the post-exercise metabolism of elite athletes as investigated by NMR-based metabolomics.

Federico Marini, Sapienza University of Rome

The aim of this study is to evaluate the systemic effects of an isotonic sports drink on the metabolic status of athletes of the Italian Olympic rowing team during recovery after strenuous and prolonged physical exercise by NMR-based metabolomics analysis on plasma and urine. Forty-four male athletes of the Italian Olympic rowing team have been enrolled in a double-blind, crossover study. All of the subjects were subjected to two evaluations, spaced out by a week interval. The evaluation was performed on a rowing ergometer after strenous physical

exercise in order to produce a state of dehydration. Afterwards the athletes were rehydrated either with a green tea-based carbohydrate-hydroelectrolyte drink or with oligomineral water. Three blood samples were drawn for each subject: at rest, after the exercise and following rehydratation, while two urine samples were collected: at rest and after the rehydratation period. Biofluid samples were analyzed by high resolution H-1 NMR metabolic profiling combined with Multilevel Simultaneous data-Analysis (MSCA) and Partial-Least Squares-Discriminant Analysis (PLS-DA). The between-subject variations, as evaluated by MSCA, agreed with metabolic variations depending on individual response by lactate level changes to physical exercise. The analysis of within-subject by multilevel PLS-DA models of plasma and urine metabolic profiles showed an effect of the green tea-based sports drink on glucose, citrate, lactate levels in plasma and acetone, 3-OH-butyrate and lactate levels in urine. The increase of caffeine and hippuric acid levels in urine showed the absortion of green tea extract components. NMR-based metabolomics allowed to evaluate the complex effects of a green tea extract-based carbohydrate/hydroelectrolyte beverage on the energy metabolism of athletes during recovery by post-exercise re-hydration.

Thursday, 1 July 2010

Parallel Session 9A		
09.00 - 10.30	**NUTRITION & HEALTH** **SPONSORED BY UNILEVER** **Co-organized with NUGO** **Chair: Ben van Ommen (Netherlands)**	**Room**
09.00 - 09.30 Invited speaker	**Lorraine Brennan (Ireland)** Identification of nutritional responsive phenotypes: the role of metabolomics	Forum
09.30 - 09.50 Selected speaker	**Ali Moazzami (Sweden)** Metabolomics study of the effect of rye bran intervention on the progression of prostate cancer	Forum
09.50 - 10.10 Selected speaker	**Amanda Lloyd (UK)** Development of metabolomics as novel approach to biological indicators which characterise and quantify dietary exposure	Forum
10.10 - 10.30 Selected speaker	**Ewa Szymanska (Netherlands)** Tracking nutritional effects: a biostatistics view on applied metabolomics studies	Forum

L9A-001
Identification of nutritional responsive phenotypes: the role of metabolomics

Lorraine Brennan UCD School of Agriculture, Food Science and Veterinary Medicine, UCD Conway Institute, Belfield, UCD, Dublin 4, Ireland.

The metabolic phenotype (metabotype or nutritype) describes the metabolic state of an individual and is a product of genetic and environmental influences. Among the environmental influences habitual diet is one of the most important influences. If progress is to be made in this field the influence of habitual diet on the phenotype needs to be fully understood. Metabolomics offers great potential for the studying of metabolic phenotypes and there is an expectation that assigning individuals to a particular metabotype / nutritype will help in evaluating individual health status, in establishing metabolic targets for preventing and treating diseases, and in monitoring response to intervention. The current presentation will look at the link between habitual diet and the metabolic profile. Analysis of dietary intake patterns and the relationship to the metabolic profile will be presented. In a group of 160 Irish adults, three dietary patterns were identified: “Traditional Irish”, “wholefoods” and “light-meal foods”. These dietary clusters were reflected in the urinary metabolomic profiles. Following on from this a metabolic phenotyping strategy will be presented and the application of this approach to the identification of nutritional responsive phenotypes will be discussed.

L9A-002
Metabolomics study of the effect of rye bran intervention on the progression of prostate cancer

Ali A. Moazzami (1), Jie-Xian Zhang (2), Afaf Kamal-Eldin (1), Per Åman (1), Göran Hallmans (2), Jan Erik Johansson (3), Sven Olof Andersson (3) (1) Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, 756 51 Uppsala, Sweden. (2) Department of Public Health and Clinical Medicine, Umeå University, 90187, Umeå, Sweden. (3) Department of Urology, Örebro University Hospital, Örebro University, 70110 Örebro, Sweden.

Prostate cancer is the most common cancer in the western world and the second cause of death, after lung cancer, in United States and Britain. However, many men die with prostate cancer

rather than because of it. Active surveillance, together with lifestyle and diary changes to control the development of cancer, are the main routine measures in many of the prostate cancer cases. With regard to the dietary changes, it has been shown that prostate specific antigen is reduced after intervention with products containing whole grain rye/rye bran indicating reduced tumor activity. In this study, metabolomics was used to elucidate the possible mechanism by which rye/rye bran reduce prostate cancer activity. In a cross-over design 17 prostate cancer patients received either a diet containing 485 g whole grain rye/rye bran per day or a control diet containing refined grain products adjusted for fiber and energy intake. 1D & 2D NMR-based metabolomic (400 MHz) analysis of plasma and urine samples collected after intervention periods showed that intervention with rye/rye bran causes an increase in urinary excretion of short chain fatty acids, glutamine, and alkylresorcinol metabolites. Short chain fatty acids are known to be associated with activation of AMP-activated protein kinase, whose role is shown in energy expenditure and incident of cancers associated with metabolic syndrome e.g. prostate, colon and breast cancer. The increase in glutamine also indicates a shift in energy metabolism. Our findings suggest a change in energy metabolism as a possible mechanism by which rye/rye bran intervention can reduce prostate cancer activity and consequently progression.

L9A-003

Development of metabolomics as novel approach to biological indicators which characterise and quantify dietary exposure

Amanda J. Lloyd(1), Manfred Beckmann(1), Gaëlle Favé(2), Long Xie(2), Wanchang Lin(1), Kathleen Tailliart(1), John C. Mathers(2) and John Draper(1) (1)Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, SY233DA, UK (2)Human Nutrition Research Centre, Institute for Ageing and Health, University of Newcastle, NE24HH, UK

Discovery of chemical biomarkers indicative of specific food consumption is difficult due to the complexity of metabolite content of foods, metabolism of these metabolites, and the lack of databases and standards for possible biomarkers. The MEDE Study (MEtabolomics to characterise Dietary Exposure) aimed to provide ‘Proof of Principle’ that a non-targeted metabolomics approach could identify chemical signals typical of individual foods in human biofluids. The first challenge was to design and validate protocols for i) subject recruitment and management and ii) biological sample collection, processing and storage. A standardised breakfast (orange juice, croissant, tea and cornflakes) was employed as an initial food ‘challenge’ to fasting volunteers. FIE-MS fingerprinting coupled with PC-LDA and Random Forest provided evidence of chemical differences in post-prandial urines. Feature-ranking methods highlighted nominal mass bins which were ‘explanatory’ of exposure to the breakfast, which were further targeted by ultra FT-ICR-MS and MS/MS. Markers indicative of orange juice consumption were revealed, as reported in previous literature, thus validating the utility of non-targeted metabolomics. Following this initial success to calibrate methodology, we sought to determine whether metabolite fingerprints indicative of different foods, substituted for Cornflakes, could be discriminated from the standard breakfast. FIE-MS data subjected to PC-LDA revealed that it was possible to separate raspberries, smoked-salmon and broccoli from the standard breakfast. Following FT-ICR-MS and MS/MS, several specific metabolites were identified as possible biomarkers for future targeted, quantitative analysis. It was not possible to discriminate Weetabix from Cornflakes for the study volunteers as a whole. However, analysis showed distinct ‘metabotype’ sub-groups within the study volunteers and when analysed within each sub-group, discriminatory metabolites could be identified. Overall this study demonstrated the potential of metabolomics to identify and develop novel and robust biomarkers of dietary exposure. There is clear potential of using metabolomics to identify candidate chemistries that can be explored using targeted, quantitative methods to confirm biomarker status.

L9A-004

Tracking nutritional effects: a biostatistics view on applied metabolomics studies

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Nowadays, the number of successful applications of the metabolomic approach in large-scale studies of nutritional effects is still limited. Amongst others this is due to the high complexity of the studies and the generated data where treatment effects cannot easily be explored by standard tools such as uni-variate tests, PCA or regular PLS-DA. The aim of this study is to find reliable nutritional effects in metabolomic projects. A nutritional study concerning the effects of two different food products on serum lipid profiles will be discussed with the main focus on comparison of outcomes (significant metabolites) found by different data analysis tools. A randomized, double-blinded and placebo-controlled study comprised of 100 mildly hypercholesterolaemic subjects who were divided into three treatment groups (including two groups on different cholesterol lowering foods). Serum samples were collected before and after treatment together with standard lipoprotein profiles. Lipidomics

analysis was performed using a UPLC-ESI-QTOF method. The two tested food products were found to significantly lower serum total cholesterol and low-density cholesterol to the same extent. Thus, an effect of these products on the lipidomics profiles would be expected and this could help to further understand diet-induced health benefits. A first issue concerns the high biological variation between study subjects vs. the relatively small nutritional effect. Multilevel analysis was applied including a comparison of differences in levels of metabolites after the treatment period for each studied food product and placebo product (paired uni-variate tests, multilevel PLS-DA). Secondly, a statistical validation of results of the different methods and of the different groups of subjects was evaluated by false discovery rates and permutation tests. Finally, study design information was included by a tailor-made linear model per metabolite to deliver a group of serum metabolites which is reliably affected by the test product intervention.

Thursday, 1 July 2010

Parallel Session 9B

09.00 - 10.30	**METABOLOMICS-ASSISTED BREEDING**	
	Chair: Joachim Kopka (Germany)	**Room**
09.00 - 09.30 Invited speaker	**Arnaud Bovy (Netherlands)** Metabolomics-assisted breeding to improve the consumer quality of tomato fruit fruit	E104 -107
09.30 - 09.50 Selected speaker	**Henning Redestig (Japan)** Covering the chemical diversity of rice kernels to investigate correlations between metabolite levels and phenotypical traits	E104 -107
09.50 - 10.10 Selected speaker	**Takayuki Tohge (Germany)** Metabolomics-assisted breeding: a viable option for crop improvement?	E104 -107
10.10 - 10.30 Selected speaker	**Yaakov Tadmor (Israel)** Comparative intraspecific metabolomic and genomic variation analyses in Cucumis melo	E104 -107

L9B-001

Metabolomics-assisted breeding to improve the consumer quality of tomato fruit fruit

Yury M. Tikunov1,2,3, Ric C.H. de Vos1,2, Ana-Rosa Ballester1,2, Jos Molthoff1,2, Robert D. Hall1,2, Arnaud G. Bovy1,2 1 Centre for BioSystems Genomics, POB 98, 6700 AB Wageningen, the Netherlands, 2 Plant Research International, POB 16, 6700 AA Wageningen, the Netherlands, 3 Laboratory for Plant Physiology, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, the Netherlands

Tomato is one of the most important vegetable crops worldwide. Producer traits, such as yield and disease resistance traditionally have been the most important for tomato breeders. Recently, however, the importance of consumer-related traits, such as flavour and nutritional value is increasingly recognised. The tomato program of the Centre for Biosystems Genomics aims at elucidating the genetic and biochemical basis of tomato flavour, by screening a diverse collection of tomato cultivars and breeding populations for variation in taste attributes and metabolite content. Our analyses revealed that, in addition to sugars and organic acids, volatiles play an important role in determining the flavour of tomato fruit. By analysing non-targeted GC- and LC-MS metabolomics datasets we found that many volatiles are present in tomato fruit as glycoconjugates, supporting the hypothesis that glycosylation plays an important role in the storage and emission of volatiles. The phenylpropanoid-derived volatiles methyl salicylate, guaiacol and eugenol appeared to be one of the most discriminatory among our germplasm collection. Emission of these volatiles took place upon disruption of fruit tissue through cleavage of the corresponding disacharide glycoconjugates. However, in certain genotypes, phenylpropanoid volatile emission was arrested due to conversion of the corresponding disacharide precursors into glycoconjugate species of a higher complexity: trisacharides and malonyl-trisacharides. This glycoside conversion was established to occur in tomato fruit during the later phases of fruit ripening and has consequently led to the inability of red fruits of these genotypes to emit key phenylpropanoid volatiles upon fruit tissue disruption. Our current efforts are geared towards the isolation and characterisation of the genes underlying this novel principle of volatile emission regulation. This research was supported by the Dutch NWO-genomics initiative Centre for Biosystems Genomics

L9B-002
Covering the chemical diversity of rice kernels to investigate correlations between metabolite levels and phenotypical traits

Redestig, H.(1), Kusano, M.(1), Ebana, K.(2), Fukushima, A.(1), Oikawa, A.(1), Okazaki, Y.(1), Matsuda, F.(1) and Saito, K.(1) (1) RIKEN, PSC, Yokohama, Kanagawa 230-0045, Japan (2) NIAS, Tsukuba, Ibaraki 305-8602 Japan

These authors contributed equally The ongoing demand for improved and tailored crops makes it essential to develop improved breeding methods. Current breeding programs are geared towards optimization of high-level phenotypical traits but such traits are the sum of multiple variables making direct marker identification difficult. Metabolite profiling can give a more high-resolution picture of the phenotype and decompose visible traits into its molecular factors. A major obstacle for this application is that no single analytical platform can detect all types of molecules and therefore result in biased data sets. We have developed a multi-platform metabolomics approach based on a combination of gas chromatography-, liquid chromatography-, and capillary electrophoresis-time-of-flight/ mass spectrometry to address this problem. Using a novel data summarization approach which is supported by our automatic metabolite identifier linking program MetMask, we obtain an consensus data set with strongly reduced chemical bias compared to the single platforms. Here we describe our strategy for data analysis and evaluation in an application where we profiled rice kernels from the 70 cultivars of the World Rice Core-Collection. The resulting data was used to mine for correlations with phenotypical traits using multivariate regression. Taken together, our results show that multi-platform metabolomics can be an efficient tool for identifying the molecular background of phenotypical traits in a major crop species and for investigating links between genotype and phenotype.

L9B-003
Metabolomics-assisted breeding: a viable option for crop improvement?

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The increase in productivity and quality on crop cultivation was offset by the narrowing of the crops genetic base which has lead to greater susceptibility to environment stress. However complete genome information for several plant species facilitates another approach for designing crops. In order to perform the strategy of metabolomics-assisted breeding, we have to know not only metabolites displaying natural variation but also their underlying biosynthetic pathways and to identify the key genes related to target productivity. Since S. lycopersicum can easily be crossed with many of its wild species relatives, such exotic germplasm represents a valuable source for the improvement of agriculturally important traits. In higher plants, phenolic compounds play important roles in many biological processes such as pigmentation of fruits and vegetables, plant-pathogen interactions, protection against high light, salt stress and chilling. On the other hand, these polyphenolic compounds are an integral part of the diet and there is increasing report that dietary polyphenols are likely candidates for the observed beneficial effects of a diet rich in fruits and vegetables on the prevention of cardiovascular diseases and some other chronic diseases. Even within a single plant species, there is a large variance in the levels and composition of phenolic compounds. An LC-MS based phenolic compound analysis was performed to allow comparisons between the relative metabolic levels of leaves and fruits of S. lycopersicum and seven wild species tomato that can be crossed with it. Flavonols and chlorogenic acid accumulated to different levels in the various Solanum species. The significance of these changes is discussed with respect to the use of the various wild species for metabolic engineering within wide breeding strategies. We will discuss similarities of differences between the levels of variance observed between the different metabolite classes for the purpose of metabolomics-assisted breeding.

L9B-004
Comparative intraspecific metabolomic and genomic variation analyses in Cucumis melo

Yaakov Tadmor1, Nurit Katzir1, Burger Josef1, Vitaly Portnoy1, Tamar Lahav1, Galil Tzuri1, Uzi Sa-ar1, Efraim Lewinsohn1, Ric DeVos2, Roland Mumm2, Joachim Kopka3, Alexander Erban3, Jane Ward4, Mike Beale4, Roy Goodacre5, William J. Allwood5, Benoit Biais6, Mickael Maucourt6, Catherine Deborde6, Stéphane Bernillon6, Isabelle Quintana6, Dominique Rolin6,Thomas H. Hansen7, Jan K. Schjoerring7, Asaph Aharoni8, Ilana Rogachev8, Sagit Meir8, Leonid Brodsky8, Arthur Schaffer9, Annick Moing6, and Robert Hall2 1 - Newe Ya-ar Research Center, Agricultural Research Organization, Israel 2 - Plant Research International and Centre for BioSystems Genomics, The Netherlands 3 - MPI Max-Planck-Institute of Molecular Plant Physiology, Germany 4 - The National Centre for Plant and Microbial Metabolomics, Rothamsted Research, UK 5 - School of Chemistry, Manchester Interdisciplinary Biocentre, UK 6 - Metabolome Facility of Bordeaux Functional Genomics Centre, Centre INRA de Bordeaux, France 7 - Plant and Soil Science Laboratory, University of Copenhagen, Denmark 8 - Department of Plant Sciences, Weizmann Institute of Science, Israel 9 - Volcani Center, Agricultural Research Organization, Israel.

We characterized a selection of 52 genotypes representing two subspecies and nine taxonomic groups of Cucumis melo using biochemical phenotyping and molecular genotyping. Metabolomic and mineral element analyses of mature melon fruit utilized an unprecedented range of approaches, including LC/MS, GC/MS, NMR, IR/MS and ICP/MS. Metabolomic analyses of melon fruit is challenging due to the high fruit sugar content therefore specific SOPs were developed when needed. In parallel, the 52 genotypes were analyzed for genetic relatedness utilizing different genetic markers, including internal transcribed spacer (ITS), short sequence repeats (SSR), amplified length polymorphism (AFLP) and the sequences of 22 genes. The objectives of this study were: 1. to compare the genetic distances revealed by DNA analysis to those determined by the different metabolomic strategies; 2. to identify metabolites that are more informative for taxonomic classification. The results of our analyses indicate that the taxonomic clustering of cultivated melon varieties is complex, utilizing either DNA markers or metabolomic data. Still, one can find metabolites that distinguish between the C. melo subspecies and even between different C. melo taxonomic groups.

Thursday, 1 July 2010

Plenary Session

11.00 - 12.30	**PLENARY SESSION P5** **THE FUTURE** **Chair: Thomas Hankemeier**	**Room**
11.00 - 11.50 Keynote speaker	**Hannelore Daniel (Germany)** Metabolomics between powerful technologies and lack of knowledge	Forum

LP5-001
Metabolomics between powerful technologies and lack of knowledge

Hannelore Daniel, Technische Universität München Nutrition and Food Science Center

Currently the metabolomics field is driven by the breathtaking advancements of the NMR- or mass-based techniques providing constantly improved sensitivity and larger metabolite panels. In addition, new targeted data-bases for annotation and identification of so far unknown metabolites are created. Genetic and molecular tools in combination with metabolite profiling help to dissect the metabolic pathways from which the metabolites are derived. Seemingly unlimited when applied to cells in culture or model organisms, metabolomics in humans however relies mainly on the profiling of plasma and urine samples. When used as a diagnostic tool, changes in the concentrations in metabolites or metabolite ratios are used to discriminate healthy from disease conditions. What is essentially not known is what determines the plasma and urinary metabolome and its dynamics as each organ and cell type has a distinct metabolite pattern with intracellular concentrations exceeding those in plasma up to 200-fold. Urinary metabolites do in most cases also not reflect plasma levels and the contribution of the intestinal microbiota to the body fluid metabolome remains as well to be defined. Although classical physiological chemistry defined the basis of the human metabolism, the metabolites identified today by far exceed the knowledge on their origin and regulation under various conditions. What we need to determine is the variability and the dynamics of changes of the human metabolome based on well defined human studies. In addition, stable isotopes need to be applied to determine the fate and kinetics of the metabolites in health and disease states. Metabolomics therefore needs a push - I call it Physiology 2.0 - to advance from a descriptive to an explanatory science.

ABSTRACTS POSTERS

Code	Session
P1A	Model systems for translational research
P2A	Metabolomics and Biomarker discovery
P2B	Environment and Ecology
P3A	Drug discovery & drug development
P3B	Plant Physiology
P4A	Pharmacometablomics, personalized medicine & future of health system
P4B	Developments in plant metabolomics
P5A	Systems biology of mammalian/microbial metabolism
P5B	Volatiles & Secondary metabolism
P6A	Genome, metabolome and microbiome
P6B	Biotic interactions and plant stress
P7A	Pathways discovery and disease pathophysiology
P7B	Plant systems biology
P8A	Hot Topics
P8B	Food & Applied metabolomics
P9A	Nutrition & Health
P9B	Metabolomics-assisted breeding
P10A	Bioactive Compounds and Pharmacognosy
PP2	Advanced Technologies
PP3	Databases, bioinformatics & data analysis
PP4	Young Scientists

P1A-001

Anatomically resolved metabolomics of mutant Arabidopsis pollen for the functional analysis of pollen exine biosynthesis

Bennie Bench, Samuel Roberts Noble Foundation

Exine is a complex polymeric material that forms the outer wall of pollen grains and serves as an adhesive that binds to the stigma during pollination. Unfortunately, exine is a strong chemically inert polymer that is impervious to direct chemical analysis. Thus, a genetic approach is being pursued to identify the genes and gene networks necessary for exine biosynthesis. During the screening of a broad spectrum of mutants for pollen exine deficient phenotypes, several mutants were identified and we are now using metabolomics to define and better understand the functional role of the tDNA mutants. This approach is technically challenging due to the limit quantities and tedious nature of the high resolution anatomically sampling. Arabidopsis flowers were collected and the anthers dissected from wild-type and 30 different pollen mutant lines including three different lap (for less adherent pollen) mutants. The metabolomes of these mutants were then analyzed using an optimized ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC/TOFMS) and gas chromatography/mass spectrometry (GC/MS). UPLC/TOFMS has the substantial benefits of higher chromatographic resolution, efficiency, and peak capacity which enable a greater visualization of the metabolome, especially secondary metabolism. Extraction and analysis methods were based upon our previous reports but significantly scaled-down significantly to accommodate smaller quantities of materials such as those encountered with dissected floral organs. Currently, we are able to identify approximately 150 different components in anther extracts collected from 50 Arabidopsis inflorescences with a cumulative weight less than 500 µg. Multivariate statistical analyses and ANOVA testing were utilized to identify differential compounds of interest related to exine production amongst the various mutants. Differential levels of several secondary metabolites including phenolics, fatty acids, and as yet unidentified compounds were observed and used to characterize multiple mutants to date (Dobrista et al, 2009, 2010). The characterization of these mutants will be presented.

P1A-002

Comprehensive Quantitative Characterization of the Human CSF, Serum, and Urine Metabolome

Frolkis, A. (1), Brons, C. (2), Mandal, R. (1), Psychogios, N. (1), Bouatra, S. (1), Sinelnikov, I. (1), Knox, C. (2), Guo, A. (2), Gautam, B. (2), Eisner, R. (2), Dong, Y.W. (1), Huang, X.P. (1), Krishnamurthy, R. (1), Wishart, D.S. (1,2) (1) Department of Biological Sciences (2) Department of Computing Science

Metabolomics is an emerging field that is gaining wide-spread interest in disease diagnostics, agriculture food and safety, and pharmaceuticals. Developments in metabolomics have led to biomarker discovery and improved screening methods, and the possibilities do not end there. In order to utilize all that metabolomics has to offer, there needs to be concerted efforts in helping with its development. In an effort to contribute to metabolomics, the Pan-Alberta Metabolomics Platform (PANAMP) initiated a project to characterize cerebrospinal fluid (CSF), serum, and urine using holistic quantitative metabolomics. In order to characterize the aforementioned human biofluids, PANAMP utilized multiple platforms including NMR, GC-MS, LC-MS, LC-FTMS, LC-ESI-MS/MS, TLC/FAMES/GC-FID, and DI-MS/MS. PANAMP was able to identify and quantify metabolites within each biofluid and determine the degree of identification overlap that exists amongst the platforms. The results from these platforms were combined with those found with an in-house text mining program as well as manual literature searches to expand the list of identifiable and quantifiable metabolites. For CSF, the computer-aided and manual literature survey allowed PANAMP to identify 308 metabolites that constitute the detectable human CSF. We utilized NMR, GC-MS, DI-MS/MS and LC-FTMS technologies and identified and quantified 159 unique metabolites. In a similar literature analysis of human serum, we identified 868 metabolites that make up the human serume metabolome. PANAMP employed NMR, GC-MS, LC-ESI-MS/MS, TLC/FAMES/GC-FID, and DI-MS/MS and identified and quantified 3687 metabolites. In an ongoing study, the text-mining and manual literature search has helped identify 865 compounds that comprise the human urine metabolome. PANAMP has been using NMR, GC-MS, LC-MS, and DI-MS/MS and has identified and quantified 232 unique metabolites. While these results represent significant progress in mapping the human metabolome, work still needs to be done to realize the potential that metabolomics holds in contributing to the understanding of health and other areas.

P1A-003

Uncovering the metabolism of 4-hydroxyacids: drugs of abuse and products of lipid peroxidation

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4-Hydroxyacids are drugs of abuse (4-OH-butyrate, 4-OH-pentanoate) and products of lipid peroxidation (derived from 4-OH-nonenal and 4-OH-hexenal). We used a combination of metabolomics and mass isotopomer analysis to investigate the metabolism of these compounds in vivo and in isolated rat livers. We synthesized singly and multiply 13C-labeled substrates, and identified their metabolites by GC-MS and LC-MS/MS. The profile and mass isotopomer distribution of CoA esters provided a gold mine of information on the pathways of 4-OH-acid catabolism. All 4-OH-acids with 4 to 11 carbons form 4-phospho-acyl-CoAs, a new class of CoA esters. The metabolism of 4-OH-acids with 5 to 11 carbons proceeds via 2 new pathways: (i) isomerization of 4-OH-acyl-CoAs to 3-OH-acyl-CoAs via 4-phospho-acyl-CoAs, followed by regular beta-oxidation to acetyl-CoA / propionyl-CoA, and (ii) a sequence of beta-, alpha- and beta-oxidation steps with production of formate, acetyl-CoA / propionyl-CoA. 4-OH-butyrate is a physiological neurotransmitter derived from GABA. When ingested at high doses, it is a drug of abuse (date-rape drug, GHB). We showed that it is metabolized by 4 processes: (i) anaplerosis of the citric acid cycle via succinate, (ii) to 3-OH-propionate + formate via two parallel alpha-oxidation processes starting from each end of the molecule, (iii) to glyoxylate + acetyl-CoA by a variant beta-oxidation process, and (iv) to 4-phospho-butyryl-CoA. The latter also accumulates in the brain and liver of mice unable to dispose of physiological 4-OH-butyrate. The metabolism of the new drug of abuse 4-OH-pentanoate (GHV, more toxic analog of GHB) leads to the accumulation of very high concentrations of 4-phospho-pentanoyl-CoA + 4-OH-pentanoyl-CoA + 4-keto-pentanoyl-CoA. This trapping of CoA perturbs a number of reactions using CoA. It is likely that 4-phospho-acyl-CoAs are neuromodulators which contribute to the brain toxicity of 4-OH-butyrate and 4-OH-pentanoate. This work, supported by the NIH (NIDDK RoadMap Initiative and NIEHS), illustrates the potential of the association of metabolomics and mass isotopomer analysis for pathway discovery.

P1A-004

Investigation of Cytotoxic Effects of Au(III), Pt(II) complexes of 5-chloro-1,10-phenanthroline on HL-60(Acute Promyelocytic leukemia cells) Cell Line

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Au(III) complexes, the last isostructural and isoelectronic with platinum(II) complexes, are potentially attractive as anticancer agents. The purpose of this study was to investigate the cytotoxic effect of Au(III) and Pt(II) metal complexes on human leukemic cell lines. In this study Au(III) and Pt(II) metal complexes of 5-chloro-1,10-phenanthroline (5-Cl-phen) were synthesized and elucidated of their structure was performed by IR, 1H-NMR and MASS spectroscopic data and elemental analyses results. Then, we studied the antiproliferative effects of Au(III) and Pt(II) metal complexes of 5-chloro-1,10-phenanthroline, and cisplatin on HL-60 cell line. The antiproliferative effects of these compounds were evaluated by MTT cell viability assay by following the 24 and 48 hours incubation of leukemic cells in 5,10,25,50,100 ul concentration of Au(III) and Pt(II) complexes and cisplatin. When HL-60 cells exposered to 50 and 100 ul [Au(5-Cl-phen)Cl2]Cl for 24 h, the cell viabilites decreased to 30% and 43% respectively compared to control. After 48 h 50-100 ul [Au(5-Cl-phen)Cl2]Cl concentrations significantly decreased the cell viabilty ($p<0.001$). The concentrations of 50, and 100 ul [Au(5-Cl-phen)Cl2]Cl for 48 h, the cell viability percenties were determined to 44%, 55% respectively. HL-60 cells exposered to 50 and 100 ul [Pt(5-Cl-phen)Cl2]Cl and cisplatin for 24 h, the cell viabilites decreased to 24% and 42% ; 16% and 26%. The concentrations of 50, and 100 ul [Pt(5-Cl-phen)Cl2]Cl and cisplatin for 48 h, the cell viability percenties were determined to 31%, 44% and 16% , 39% respectively Especially, [Au(5-Cl-phen)Cl2]Cl observed an inhibition of cancer cell proliferation higher than [Pt(5-Cl-phen)Cl2]. In conclusion, the present study demonstrates a powerful in vitro antitumor action of Au(III) complexes of 5-chloro-1,10-phenanthroline ([Au(5-Cl-phen)Cl2]Cl) on HL-60 cell lines.

P1A-005

A comprehensive functional genomic study of Δ9 desaturase deletion in Caenorhabditis Elegans by metabolomics.

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Obesity is a complex trait influenced by many factors. However, many studies have demonstrated that genetic predisposition is a key contributing factor (estimated between 40% and 70%). Furthermore, genes causing low- or high-fat phenotypes in nematodes, rodents and humans have been identified. Therefore, it is important to understand how the network of genes involved in fat metabolism exerts regulation across the whole system to produce the complex phenotype associated with obesity. The nematode Caenorhabditis elegans has become a popular model for exploring the genetic basis of fatty acid synthesis and regulation of fat storage. Although worm and mammalian physiologies differ greatly, many of the proteins involved in making, metabolising and transporting fats, as well as many of the fat-regulatory components are highly conserved between C. elegans and mammals. C. elegans synthesize a wide variety of fatty acids using the full range of desaturases activities found in plants and animals. Among these, the most important is the Δ9 desaturases expressed by three genes (fat-5, fat-6 and fat-7): it is the rate limiting enzyme in the biosynthesis of monounsaturated fatty acid, used as major substrates for the synthesis of various kinds of lipids including phospholipids, triglycerides and cholesteryl esters. The aim of this work is to characterize the metabolic impact of knocking-out all the possible Δ9 desaturase genes in C. elegans, making use of a range of approaches including liquid chromatography mass spectrometry, gas chromatography mass spectroscopy and nuclear magnetic resonance spectroscopy. Despite the genes having similar functions, excellent discrimination was achievable for all single and viable double mutants according to both total fatty acids as measured by GC-FID and intact lipids as measured by LC-MS. Furthermore, the distinctive roles of fat-6 and fat-7, genes both expressing steroyl-CoA desaturases, are highlighted by the profiles. The metabolomic changes also extend to the aqueous fraction of metabolites demonstrating the profound influence the Δ9 desaturases have on regulating global metabolism.

P1A-006

Advances in Data Processing and Compound Identification Software for Metabolomics

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Agilent's advanced software suite enables metabolomics analysis by an un-targeted data mining approach and / or by a user defined list of compounds. Each of these approaches has advantages that result in more thoroughly mined data for subsequent statistical analysis, compound identification and data visualization. Agilent Mass Profiler Professional (MPP) is a chemometric software package designed specifically for processing mass spectrometric data (GC/MS, LC/MS, CE/MS and ICP-MS), such as that resulting from metabolomics experiments. Advanced visualization tools enable the inspection and annotation of results. With functionality for unsupervised (classification without prior group assignments) or supervised (using pre-classified groups) analysis, the software allows: - Quick and easy discovery of differences between sample groups - Plot changing patterns of compound abundances over time - Development of useful multivariate models for class prediction Mass Profiler Professional includes an integrated ID Browser that allows compound identification using databases, libraries or empirical formula calculations. For LC/MS metabolomics, the new enhanced Agilent METLIN Personal Compound Database and Library contains more than 27000 compounds with approximately 3000 compounds having MS/MS Spectra. The spectra are acquired at 4 different collision energies (0, 10, 20 and 40 eV) both in negative and positive mode in ESI mode using an Agilent Q-TOF LC/MS system. The use of the MS/MS spectral search assists in compound identification where the accurate precursor mass is not sufficient for a unique identification. The ID Browser annotation capability enables the use of integrated pathway software to mine interactive databases.

P1A-007

New insight in metabolic routes: the difference between blood samples obtained from the portal vein, the hepatic vein, and the jugular vein

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Prevention and treatment of metabolic disorders or gut-related diseases by nutritional means strongly depend on understanding the mechanisms involved in the complex processes of digestion. Some samples (blood, faeces, and urine) are easily accessible in humans as well as animals, however, these samples represent endpoints of the digestion meaning that important information on e.g. digestive processes, uptake and partitioning of nutrients, and microbial fermentation is lost. In model animal studies it is possible to obtain samples that are not accessible in humans under normal conditions. In the present study, postprandial blood samples from the portal vein, the hepatic vein, and the jugular vein were taken 3h after the morning feeding from anaesthetized pigs. The plasma samples were precipitated with methanol and resuspended in H2O/acetonitril/formic acid (95:5:0.1). The samples (5 µl) are injected into a reversed-phase C18 column and detection is performed with a MicrO-TOF Q II mass spectrometer. Data will be processed using XCMS, a nonlinear alignment software. A principal components analysis (PCA) that allows a visualisation of the similarities/dissimilarities of the data with respect to their biochemical composition will be performed. Furthermore, XCMS will be used to directly compare the relative metabolite ion intensities to identify changes in specific endogenous metabolites such as potential biomarkers. The approach used in the present study allows studies on the net flux of metabolites, including digested dietary compounds absorbed by the gut as well as non-nutrient compounds produced by the microbiota, from the gastrointestinal tract to the liver as well as insight to the metabolism of these metabolites taking place in the liver. Metabolomics provides a systems approach to advance our understanding of the metabolic processes. Combining the use of animal model with metabolomic studies may prove to be a valuable tool for further investigations and may provide insight to metabolic routes that would otherwise be impossible to get.

P1A-008

Targeted Lipidomics – high-throughput analysis of lipid metabolites in biological samples via flow injection ESI-MS/MS

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Since many years it is without doubt that the diverse classes of endogenous lipids play important roles in living organisms. Their functions go far beyond the storage of energy and the assembly of cell membranes to complex signalling pathways of e.g. apoptosis, cell differentiation and inflammation. As a consequence, several states of diseases are associated with changes of the lipid composition. Therefore, it is of great interest to provide methods for the analytical determination of the lipidom as a prerequisite to the discovery of lipid biomarkers. We present a targeted lipidomics method for the high-throughput analysis of the biologically most abundant members of the 7 lipid classes glycerophosphocholines (PC), glycerophosphoethanolamines (PE), glycerophosphoserines (PS), glycerophosphoglycerols (PG), sphingomyelins (SM), ceramides and dihydroceramides. This method requires only 20µL of biological sample for a methanol/chloroform extraction procedure and is based on flow-injection ESI-MRM technology. Besides five internal standards to compensate for matrix effects, 43 external standards are used for a multi point calibration. The data analysis is performed with our inhouse software MetIQ offering the possibility for isotopic correction. To check quality parameters of the method like accuracy, precision, selectivity, recovery and stability, validation experiments for human plasma and calf brain homogenate were performed. During this validation process it became clear that the most severe problem to solve was the carry-over effect. In this poster we will also present the strategies we used to get this problem under control.

P1A-009

Metabolomics based integrative analysis of selection for resistance to environmental stress in Drosophila melanogaster.

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Evolution has provided an impressive multitude of biological variance, which has largely been shaped by the temporal or special variability in environmental conditions. Thus species performances and tolerances are formed by the constant selection for genotypes that prevail/prosper in the face of adverse/favorable conditions. Here we investigate the metabolomic signal in D. melanogaster left by artificial selection for tolerance to stressful conditions such as cold, heat, starvation and dehydration, and for longevity. Indeed we see metabolomic effects of selection for environmental stress resistance, a large fraction of which occurs in the same direction independent of the stress selected for. There is thus a general stress selection response at the metabolite level. Interestingly, the metabolite composition of the selection lines shows an almost continuous distribution, and there does not seem to be one unique metabolomic response to each selection regime. The effects of selection for temperature stress on the temperature stress response at the metabolite level were also studied, and the main effect was found to be a change in the timing of the response. When the findings at the metabolite level are compared with gene expression and phenotypic data (such as resistance for the stresses selected for), there is very little agreement between the similarities between the different selection regimes on the three levels. However, various features can be correlated to the metabolite data using self-organizing maps, including features that show no clear correlation to selection regime. The correlations between the different levels were further explored using O2PLS-based methods.

P1A-010

Development of CSF metabolomics for biomarker discovery in neurological research

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Until recently, the application of metabolomics research in neurological studies was limited and lately published results cover very small number of identified and validated compounds. A survey of the Central Nervous System (CNS) metabolism is possible through tissue and fluid samples. Most commonly undertaken is the analysis of cerebrospinal fluid (CSF), which is generated continuously in CNS and its contents manifest the current state of CNS. However, due to the invasive sampling procedure, only sparse amounts of CSF are available for investigations, imposing strong constrains on the required sensitivity of analytical methods. This is especially true for CSF of mice or rat. Moreover, typical concentrations of many metabolites in CSF are significantly lower than in plasma or urine. We show the development and optimization of novel analytical platforms that provide reliable quantitative information of a wide range of metabolites and their respective concentrations, present in CSF. We present profiling of amine containing compounds using LC-MS/MS, based on derivatization of amino groups with 6-aminoquinoly-N-hydroxysuccinimidyl (AccQ-Tag). We show example application in search for CSF biomarkers in an animal model of Multiple Sclerosis - Experimental Autoimmune Encephalomyelitis (EAE). Multiple Sclerosis is a chronic demyelinating neurodegenerative Central Nervous System (CNS) disorder of autoimmune origin. However, molecular processes associated with onset and progression of MScl and its etiology are still unknown. Application of metabolomics gives opportunity to discover novel biomarkers related to onset and progression of the disease. We show application of two targeted metabolomics platforms, LC-MS and GC-MS based, covering 39 and 114 fully identified and validated compounds, respectively.

P1A-011

Functional Genomics of Caenorhabditis elegans by Whole organism NMR Spectroscopy in Applications to Ageing and Toxicology

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Caenorhabditis elegans is used to monitor a wide range of biological processes and to characterize genetic mutations responsible for human diseases. We have developed a robust protocol based on 1H HR-MAS NMR spectroscopy of intact C. elegans worms to investigate the metabolic signature induced by genetic mutations, and demonstrated its use as a molecular phenotyping device for functional genomics at the system level (1). Here we focus on the metabonomic characterization of two different C. elegans mutants to obtain insight into the metabolic mechanisms ageing and toxicological processes. We derived the metabolic signature of ageing in a C. elegans mutant that mimics caloric restriction (CR), a well-known process responsible for increase in lifespan in various organisms. We compared metabolic profiles obtained by 1H HR-MAS NMR spectroscopy for wild type (N2) nematodes and CR mutants, young adults and 7-day old adult worms. We used supervised statistical analyses, such as OPLS, coupled to statistical recoupling of variables to derive significant metabolic discriminations (2). The metabolic signature of both ageing and CR in intact C. elegans is found to share similarities with signatures previously described from the plasma of non-human primates (3). Furthermore, we find that the difference between the metabolic profiles of wild-type worms and CR mutants increases with age. 7-day old CR mutants appear metabolically younger than their wild type counterparts. We also illustrate the characterization of the metabolic signature of mutants of the aryl hydrocarbon receptor (AhR) which plays a central role in xenobiotic-induced toxicity and carcinogenesis. 1. Blaise B. J. et al. Proc. Natl. Ac. Sci. USA 104, 19808 (2007). 2. Blaise, B. J. et al. Anal. Chem. 81(15), 6242-6251 (2009). 3. S. Rezzi et al. Exp. Gerontol. 44(5), 356 (2009).

P1A-012

Mutations in the Saccharomyces cerevisiae succinate dehydrogenase result in distinct metabolic phenotypes revealed through 1H-NMR based metabolic footprinting.

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Combining genomic studies with metabolomic analysis has proven to be a powerful method of understanding the connections between mutation, metabolism and disease. Metabolic footprinting is an innovative method for functional analysis. It examines the extracellular metabolome or exometabolome and has been used successfully in bacteria, yeast and cultured human cells. We utilized metabolic footprinting, in conjunction with multivariate statistical analysis, to examine a yeast model of mitochondrial dysfunction. We examined the effects of single amino acid substitutions in succinate dehydrogenase (SDH), a key enzyme in both the mitochondrial respiratory chain and in the tricarboxylic acid cycle. SDH mutations can cause neurodegenerative disorders or tumours. We identified and quantified 36 metabolites in the exometabolome. Our results indicate that SDH mutations cause significant alterations to yeast metabolism. Multivariate statistical analysis allowed us to discriminate between the different metabotypes of individual mutants. Metabotypes were linearly and highly correlated to mutant growth rates. Our study provides considerable insight into the metabolic effects of SDH dysfunction. It also highlights the effectiveness of metabolic footprinting for examining mitochondrial diseases.

P1A-013

You are what you eat: a critical evaluation of the effects of diet on the nematode Caenorhabditis elegans.

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Diet has a profound effect on health. Metabolomic studies offer a unique opportunity to correlate diet with health and disease phenotype. Using model systems for metabolomic studies provides the additional advantage of optimal experimental control, limiting genetic and external factors affecting metabolism. We used the free-living soil nematode, Caenorhabditis elegans, in effort to understand the role that metabolism plays in mitochondrial disease. Two E. coli strains, OP50 and HT115, are commonly used as food sources when culturing C. elegans. OP50 is the standard food strain; HT115 is used for RNA interference (RNAi) mediated gene suppression studies. We found that when placed on the different diets, the worms exhibited significant metabolic and phenotypic differences. Multivariate statistical analyses of the metabolic data revealed that dietary effects on metabolism were similar in magnitude to those produced by gene mutations or RNAi-mediated gene suppression. Genetic and phenotypic studies revealed that diet could cause significant changes to mitochondrial DNA (mtDNA) copy number, brood size and lifespan. This is the first critical evaluation of these two commonly used food sources for the nematode C. elegans. Our study highlights the effect that diet can have on health and phenotype and the need for controlled conditions in nutritional and metabolic studies.

P1A-014

A metabolomics study on the toxic effects of TCDD on HepG2 cells

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Among the various "omics" techniques, metabolomics is a developing field which is driven by advances in separation and detection technologies. It can have a high potential in complementing other genomics based technologies. The main goal of this work is to study the usefulness of metabolomics in providing information on the toxicity of a well known toxic model compound, TCDD, in an in vitro test system such as HepG2 cells. Key issues among others are robustness and avoiding contaminations when looking at intracellular responses. In order to determine robustness and reproducibility, exposures (5 biological replicas) were done using 5 different cell passage numbers. After a 48 hr exposure, the metabolism was quenched and two fractions were extracted from the cells: a polar fraction for 1H-NMR and UPLC-TOF\MS analysis and an apolar fraction for 1H-NMR and GC\MS analysis. All obtained data were preprocessed and aligned with in house developed software. Resulting spreadsheets were analyzed in a non-targeted manner by subjection to multivariate analysis. Several statistically relevant and passage-number independent consistent effects have been detected and identified in both polar and apolar fractions as a result of the TCDD exposure. Interestingly, differences between different passage numbers have also been detected as well as differences in TCDD response of the cells as a function of passage number or culture age. The metabolomics results will be discussed with regard to the biological effects (other omics studies and classical studies) already described in literature.
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P1A-015

A Stress Response Comparison: LC/MS metabolomics analysis of Saccharomyces cerevisiae exposed to the immunosuppressant drugs FK506 and Cyclosporin A

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Baker's yeast, Saccharomyces cerevisiae, is extensively used as a model organism for all eukaryotic cells. We have developed an optimized, robust and controlled metabolite extraction technique to study the stress response of yeast exposed to different immunosuppressant drugs. Culturing of the yeast in different conditions was performed in parallel, being exposed to either vehicle control or to immunosuppressant drug, followed by Ca2+ exposure, with the goal of perturbing calcineurin and any other Ca2+/immunosuppresant responsive pathways. Strain BJ5459 was cultured to target OD600, split and exposed to vehicle control, 4µg/mL FK506, or 4µg/mL cyclosporin A for 1 hour. Cultures were then exposed to 100mM Ca2+, allowed to reach target OD600, centrifuged and washed with phosphate buffered saline. Quenching was done with 1mL of cold methanol at -40°C, followed by lyophilization. 30mg of dry sample was wet milled in 2mL Eppendorf tubes using a Retsch MM301 5mm ball mill mixer concomitant with 1.2mL of 6:3:3 chloroform:methanol:water extraction solvent. Liquid-liquid extraction resulted in polar and non-polar phases. Sample analysis was done using a liquid chromatograph coupled to a quadrupole/time-of-flight (Q-TOF) mass spectrometer in both positive and negative modes from the polar phase samples. These samples were analyzed using electrospray ionization by reverse phase and Aqueous Normal-Phase chromatography. The non-polar samples were dried and analyzed by reverse phase chromatography using atmospheric pressure chemical ionization. All data was analyzed using naïve feature finding and multivariate statistical software. This allowed for a non-targeted determination of which compounds were present in each culture treatment sample set. PCA results revealed tight grouping of sample replicates for both immunosuppressant drugs, and was well separated from the calcium control treatment. This indicated that the drug treatment culture conditions were having a significant effect. Fold expression analysis was used to develop a list of potential biomarkers from each set of data. This list of compounds was then extracted from the original raw data for confirmation. This resulted in a short list of samples shown to be reproducible in each culture condition, with variation between sample sets. In addition, all data was analyzed for compounds that were present across all conditions.

P1A-017

Metabonomic fingerprint of the isolated and perfused liver.

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Metabonomics studies based on spectroscopic analysis of biofluids are a useful tool to explore the biological and metabolic profiles of an organism. However, biofluids are composed of metabolites from various tissues origins, making it very difficult to determine which organ/tissue a particular metabolite is arising from. In order to overcome this issue and validate the origin of potential biomakers, we applied the metabonomic approach to isolated and perfused organs, including the rat liver for which we have characterized the metabonomic fingerprint from perfusion fluid sampling. Materials and methods Livers isolated from male Wistar rats were perfused through the portal vein with a recycling Krebs-Henseleit solution using a peristaltic pump. Perfusion fluid was sampled every 10 minutes for two hours. Samples were prepared for 1H-NMR spectroscopy by adding 200µl of phosphate buffer (with D2O) to 400µl of fluid. TSP was used an NMR reference. At the end of the experiment, the entire volume of recirculating perfusion liquid was lyophilised for NMR analysis. During all the experiment, liver viability was followed by 31P NMR. A liver lobe was prepared for histology and another lobe was used for acid extraction. Results The metabonomic fingerprint of viable perfused livers was characterized from 1H-NMR and correlated with high ATP levels as measured by 31P-NMR. Then livers were submitted to various alterations, such as hypoxia or addition of a hepatotoxicant to the perfusion. For each treatment, specific changes in the normal metabonomic fingerprint were observed and correlated with decreases in ATP levels. . Perspectives The discovery of new biomarkers of drug-induced toxicity is a promising application for metabonomics. However, urinary or plasma components may originate from many different tissue/organs which makes it very difficult to validate such markers. The isolated and perfused organ approach could be very useful tool to confirm the tissue origin of proposed biomarkers before their validation.

P1A-018

An LC-MS/MS Investigation of Amino Acid Profiles in Chronic Obstructive Pulmonary Disease (COPD) patients.

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One of the aims of the ECLIPSE (Evaluation of Chronic obstructive pulmonary disease to Longitudinally Identify Predictive Surrogate Endpoints) study is to investigate and identify biomarkers that correlate with clinically relevant COPD subtypes, and to assess how these may predict disease progression. 1H NMR spectroscopy identified changes in serum branched chain amino acids, which correlated with disease markers. A quantitative LC-MS/MS assay was developed to measure 36 amino acids and dipeptides as an initial step in the analytical and biological variation of these findings. Serum samples from pancreatic cancer patients exhibiting cachexia (muscle wasting) were also studied to evaluate whether markers were correlated with COPD per se or cachexia, a common consequence of COPD. Methods A sub-group of 60 ECLIPSE (n=30 smoker controls; n=30 GOLD IV) and 12 pancreatic cancer (n=6 cachexic; n=6 non-cachexic) serum samples were derivatised and extracted using the EZ:Faast kit (Phenomenex Inc). Amino acid serum profiles were measured using a Waters Ultra Performance Liquid Chromatograph (UPLC) coupled to a Waters Quattro Premier XE mass spectrometer. All serum amino acid concentration were normalised to the concentration of creatinine, as determined by automated biochemical assay. PCA and PLS-DA was used to visualise the data. Unpaired Student's t tests were calculated to determine statistical significance, assuming unequal variance, with a Benjamini-Hochberg correction, = 0.1 for controlling the false discovery rate. Results PCA analysis of control versus GOLD IV sera showed distinct clustering between the two patient groups. The main differences being increased concentrations of 1- and 3-methylhistidines, aminoisobutyrate, gamma-aminobutyrate, aspartate and glutamate and decreased concentrations of aminobutyrate, proline, 4-hydroxyproline, aminoadipate, proline, leucine, isoleucine and valine in GOLD IV samples relative to controls. Results from the pancreatic cancer study suggest that aminoisobutyrate, 1-methylhistidine, thiaproline, alpha and gamma-aminobutyrate distinguish between cachexic and non-cachexic patients. Conclusions Targeted metabolomics distinguished COPD GOLD IV samples from smoker controls. Altered serum aminoisobutyrate, 1-methylhistidine, thiaproline, alpha and gamma-aminobutyrae levels were also increased in cancer cachexic patients, suggesting that these particular changes in COPD patients are related to cachexia.

P1A-019

Hypoxia-induced metabolic shifts in cancer cells: beyond the 'Warburg effect'

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Hypoxia has been recognized to play a role in promoting the invasive and metastatic behavior of cancer cells. Largely via the transcription factor, hypoxia-induced factor 1 (HIF1), hypoxia exerts significant effects on cellular metabolism, with numerous downstream consequences. Energetically, for example, there is a significant shift away from oxidative phosphorylation in mitochondria towards glycolysis (the 'Warburg effect'). The proteins involved in mediating the metabolic pathways triggered in response to hypoxia thus represent prime targets for therapeutic intervention. Hypoxia has been associated with increasingly aggressive phenotypes in cancer cells, and some of these have been linked to changes in carbohydrate metabolism important for adhesion and angiogenesis (e.g. via effects on E-selectin and integrin mediated effects). Here, illustrating the ability of metabolomics approaches to furthering our understanding of hypoxia-mediated events, we initially examined the responses of a breast cancer cell line to 1% oxygen. Metabolomics technologies can simultaneously measure a wide range of metabolites in an untargeted manner, but to date this technology has been relatively under utilized in the study of hypoxia. Examining the effects of hypoxia in the MDA-MB-231 cell line by gas chromatography mass spectrometry (GC-MS), we found not only that intracellular metabolite profiles indicated a significant shift in energy metabolites and carbohydrates, as expected, but we also found changes in metabolites involved in the urea cycle, as well as the metabolism of arginine, proline, glutamate, aspartate, and asparagine. Intriguingly, there was a clear time-dependence in hypoxia-induced metabolic changes, indicating that the hypoxic effect may transition through several generations during reprogramming of cellular metabolism. We also searched for extracellular biomarkers by nuclear magnetic resonance spectroscopy (NMR) of cell supernatants ('footprinting'), and found evidence for oxidative stress and energy metabolites when cells were hypoxic. These changes correlated with metabolite profiling studies on the sera of mice with xenografted MDA-MB-231 tumors. Thus, in addition to the \'Warburg effect\', there appears to be range pathways impacted by hypoxia. These pathways may represent targets for therapeutic intervention.

P2A-001

The use of Solid Phase Extraction in a Lipidomic Study of Non-genotoxic Hepatocarcinogenesis

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Non-genotoxic carcinogens (NGCs) are compounds that promote tumour growth through a variety of biological mechanisms, affecting processes such as apoptosis and cellular proliferation. As these compounds do not directly damage DNA they are difficult to detect via conventional screening methods. There is a growing need to reduce the cost of developing new pesticides and drugs and the identification of biomarkers that can predict, anticipate and detect NGCs would be a significant advance towards this. Due to the proliferation of sub-cellular organelles by some NGCs we can hypothesise that these carcinogens will remodel the lipidome. Determination of the lipid composition in tissue could give insight into the functional role of organelles and has the potential to explain novel biology with high relevance to toxicology. The field of lipidomics has been largely driven by the developments in mass spectrometry and liquid chromatography mass spectrometry (LC-MS) is at present the only analytical technique that, alone, can be used to separate, identify and quantify the vast number of closely related compounds that comprise lipids. We have developed a solid phase extraction (SPE) LC-MS method to profile a wide range of lipid species. SPE was used in order to pre-fractionate the total lipid extract into cholesterol esters, triacyl glicerols and phospolipids. These fractions were then analyzed using a Shimadzu LCMS-IT-TOF (Ion Trap - Time of Flight) system. Both SPE and mass spectrometry were able to provide consistent separation and recovery. With the fractionation step we also achieved an increased number of detected analytes and an enhancement of individual signals Data will also be presented to elucidate the actions of non-genotoxic hepatocarcinogen exposure in the rat liver. This project is founded by the MRC ITTP and Syngenta.

P2A-002

Comprehensive metabonomic study of malaria parasite *Plasmodium falciparum* by nuclear magnetic resonance spectroscopy

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The malarial parasite is a rapidly growing organism that exhibits a high metabolic rate and has a large demand for small molecular metabolites that will serve as precursors for the synthesis of nucleic acids, proteins and peptides. This necessitates that the parasite like all other organisms, acquire nutrients and metabolize these various biological molecules in order to survive and reproduce. The rapidly growing parasite requires large amounts of lipids for this increase in parasite surface area and volume of internal membranes. This huge demand for lipids makes lipid metabolism an attractive target for anti-malarial drugs and several potential drugs targeting lipid metabolism have been identified. Metabonomics or 1H NMR spectroscopy is a novel post genomics science in that all the molecules with low molecular weight (metabolome) are studied in an organism at a same unit of time. Although metabolites in the parasite has been studied by 1H NMR, but these studies were confined to trophozoite stage of parasites and did not focus on the different stages separately. So, in this survey polar and non-polar metabolome profile of ring and trophozaite's parasite stages were studied separately, and the result was correlated with a copper, calcium, zinc and selenium level in parasites. Data were normalized by help of Chenomx software. Multivariate data analysis techniques such as Principal component analysis (PCA) and Partial least squares (PLS) were performed for calibration of related metabolites.

P2A-003

GC-MS-Based Metabolomics Reveals Mechanism of Action for Hydrazine-Induced Hepatotoxicity in Rats

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GC-MS has great advantages for analyzing organic/amino acids, which are often targets in efficacy and/or toxicity studies. Although GC-MS has been used for the detection of many metabolic disorders, the application of GC-MS based metabolomics for pharmacology/ toxicology is relatively underdeveloped. We intended to investigate applicability of GC-MS-based metabolomics approach for toxicological evaluation, and tried to elucidate the mechanism of hydrazine-induced hepatotoxicity. Hydrazine has been widely used as an intermediate in industrial synthetic chemistry and also been found as a metabolite of some important drugs. It has been reported that hydrazine induces hepatotoxicty, carcinogenicity, mutagenicity, teratogenicity, and neurotoxicity, but to date, the definitive mechanism of toxicity has not been understood. Rats were administered hydrazine chloride orally (120, 240 mg/kg), and urine, plasma and liver samples were collected at 24 or 48 hours post-dosing. Conventional clinical chemistry and liver histopathology were performed, urine and plasma were analyzed by GC-MS, and metabolic profiles were assessed using chemometric techniques. As a result, principal component analysis score plots showed that each group was clearly separated, indicating dose-dependent toxicity and recovery. Furthermore, the mechanism of toxicity was investigated based on semi-quantification data of identified metabolites. Amino acid precursors of glutathione (cystein, glutamate and glycine), and a product of glutathione metabolism (5-oxoproline) were elevated in a dose-dependent manner, accompanied with elevation of ascorbate levels. In addition, intermediates of the TCA cycle were decreased, whereas participants of the urea cycle and other amino acids were increased. These alterations were well associated with histopathological changes. In conclusion, we found that oxidative stress and GSH consumption play an important role in the etiology of hydrazine-induced hepatotoxicity by application of GC-MS-based metabolomics, and the present study demonstrated that this approach is a useful tool in pharmacology and toxicology; screening, elucidating mode of action and biomarker discovery.

P2A-004

Metabolic characterization of human gastric cancer using HR-MAS 1H NMR spectroscopy

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Metabolic characterization of human gastric cancer was performed by NMR-based metabolomics and multivariate analysis that is a powerful tool in clinical application including cancer. The 1H-NMR spectra for gastric cancer and normal tissue were measured without extraction using high resolution magic angle spinning (HR-MAS). The metabolic differences were observed between gastric cancer and normal group from the PLS-DA model using multivariate analysis. This study provided the valuable metabolic information of gastric cancer and could be applied for clinical diagnosis in gastric cancer.

P2A-005

Fingerprinting metabolomic approach following fractionated sample preparation to identify new markers of selenium status

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Recommended Dietary Allowances (RDA) for micronutrients fluctuate noticeably within European Union countries. The Network of Excellence Eurreca (EURopean micronutrient RECommendations Aligned) aims at harmonising micronutrient intake recommendations through population groups. The lack of proper markers of status for some micronutrients limits progress in this area: metabolomics could help identifying such new markers. An original metabolomics strategy is developed here. A list of 270 metabolites known to be influenced by the micronutrient of interest has been established [1]. In order to monitor the largest fraction of these metabolites in plasma, a protocol based on plasma fractionation has been set up. It starts with a cold methanol precipitation that is followed by an extraction with a chloroform/methanol mixture. The aqueous fraction is analysed by UPLC QTOF. The organic layer is fractionated using Solid Phase Extraction on an aminopropyl cartridge. The 3 resulting fractions are analysed either by UPLC QTOF or GCMS. It has been applied to samples collected in a human intervention study investigating the effect of selenium intake on status and immune function [2]. Four groups of individuals were administered either selenium enriched yeast tablets, selenium enriched onion meals, unenriched onions meals or placebo. Data has been processed using Markerlynx or XCMS, and samples were statistically analysed using supervised and unsupervised multivariate methods. We present here the results obtained for two of the four fractions. This research was undertaken as an activity of the EURRECA Network of Excellence (www.eurreca.org), funded by the European Commission Contract Number FP6 036196-2 (FOOD) [1] Van Ommen, B. et al., Brit. J. Nutr., 2008, 99: S72-S80 [2] Hurst R. et al., Am. J. Clin. Nutr., 2010, 91: 923-31

P2A-006

Metabolomics for discovery of known and novel biomarkers for whole grain intake in 'behavioural phase' urine samples

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Assessment of wholegrain (WG) food intake is difficult and the level at which health benefits can be achieved is unknown, presenting a major obstacle to the generation of sound public health targets. The levels of alkylresorcinols (AR) and mammalian lignans (ML) in plasma have been suggested as potential biomarker of WG intake, but such targeted analyses are not trivial. We investigated whether non-targeted metabolomics approaches could identify novel characteristics of plasma and various \'behavioural phase\' urine samples linked to WG intake in a small cohort of 68 volunteers exposed 4 weeks to 3 servings and then 6 servings of WG food per day after a washout period of 4 weeks. Metabolite fingerprints and profiles were generated by FIE-MS and GC-TOFMS, respectively. In targeted analyses plasma AR were strongly predictive of WG-intake; the pattern of AR homologues has potential to identify biological sources of WG-intake. Urine proved to be the more informative biofluid for non-targeted, high throughput metabolomics profiling using GC-TOFMS. Data mining revealed a high number of metabolites strongly discriminating the three intervention phases. Two alkyl resorcinol (AR) metabolites, 3,5-dihydroxy-benzoic acid and 3-(3,5-dihydroxyphenyl)-1-propanoic acid were highly explanatory of WG-intake. Other currently structurally uncharacterised metabolites of bran components strengthen the discriminatory power for identifying WG-intake; tentatively identified novel metabolites discriminate groups of participants by WG wheat or WG rye consumption. Discriminatory metabolites, and therefore potential new biomarkers, are not only accessible in pooled day and night voids or 24 h urine samples, but also in spot fasting and post-prandial urine samples of volunteers with habitual WG intake. Non-targeted high-throughput metabolite profiling using GC-TOFMS of spot urine samples might be therefore an alternative methodology to assess WG intake in epidemiological studies as both, extensive sample clean-up and collection of 24 h urine samples is not required.

P2A-007

Automated Workflows for Putative Metabolite Identification in UPLC/MS-derived Metabolomic Datasets

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Studies in Manchester have assessed the level of complexity of electrospray UPLC-MS data derived from biological extracts in a metabolic profiling strategy [1]. A multitude of different ion types are observed including commonly described ions (protonated, deprotonated, sodium or potassium adducts, 13C isotope) and many other unexpected types of ions including adducts (e.g. sodium chloride and formate), fragment, multiply charged and instrument specific ions are also detected. A single metabolite is typically detected as many different ion types each with a unique mass and identical retention time. This data complexity negatively influences the accuracy of current methodologies for metabolite identification and increases the probability of false negatives. The non-existence of software for automated and high-throughput metabolite identification was also observed. Automated workflows have been developed in the Taverna environment (www.taverna.org.uk) to provide putative metabolite identification based on accurate mass. These are currently being tested on data acquired on ThermoFisher (LTQ-Orbitrap) and Waters (LCT Premier) platforms. The two workflows (1 and 2) incorporate the following steps (1a) Annotation and grouping of features derived from the same metabolite (based on mass differences, retention times (+/-3s) and correlation between peak responses) (1b) Matching of m/z to unique molecular formula (MF) with specified tolerance (typically 3ppm for Orbitrap acquired data) (2) Matching of MF to metabolites listed in the Manchester Metabolomics Database (MMD, [1]) The workflows are rapid (<5 min for 5000 features) and reduce the number of false positives by eliminating the inaccurate matching of many artifact, isotope and complex adduct peaks. Subsequent definitive identification can be performed. Additional information based on similarity measures (e.g. metabolite class or metabolite pathway) is being incorporated into the Manchester Metabolomics Database for future application On completion the two Taverna workflows will be available on myExperiment (www.myexperiment.org/) [1] Brown M, Dunn W.B. et al. Analyst, 2009, 134, 1322-1332.

P2A-008

LC-ESI-TOF MS method for profiling polyphenols from olive oil and their metabolites in human urine: new findings about metabolism of polyphenols

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The regular consumption of olive oil, an essential component of Mediterranean diet, has been associated with a lower incidence of coronary heart disease and certain cancers. Those beneficial effects can be partially ascribed to the high content of polyphenols and their effective antioxidant properties. Biological properties of polyphenols depend on their bioavailability; consequently, understanding of the polyphenols absorption, metabolism and the clearance is essential for the correct assessment of olive oil healing effects. To address this question, we challenged a group of ten healthy volunteers with single intake of 50 ml extra-virgin olive oil and collected urine samples at baseline (0 h), 2, 4 and 6 hours after the intake. To evaluate the metabolic composition of urine we have established an analytical methodology based on rapid resolution liquid chromatography-mass spectrometry (RRLC-ESI-TOF MS) in combination with chemometrics methods. To the best of our knowledge, this is the first systematic study of the metabolic composition of human urine after olive oil intake. Using high mass accuracy and resolving power of TOF mass analyzer, together with a priory chemical knowledge and rules of metabolic transformation, we have identified about 60 metabolites related to olive oil. Moreover, we report that the bioavailability of the different polyphenol categories appears to be different. For example, the most abundant metabolites came from those phenolic compounds containing a catechol group, such as hydroxytyrosol and their secoiridoid derivatives. Phenolic compounds were subjected to different Phase I and Phase II reactions, but the most common metabolic reactions were methylation and glucuronidation. The developed method was also successfully applied to monitor the levels of the identified metabolites in human urine at different times (2, 4 and 6 h) after the intake of olive oil. Finally, we discuss our findings in the context of available literature on phenolic compounds metabolism.

P2A-009
Towards the identification biomarkers for multiple sclerosis in human urine using 1H-NMR Spectroscopy

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Multiple sclerosis (MS) is an autoimmune disorder resulting in inflammatory events, formation of sclerotic plaques and demyelinated lesions within the white matter of the central nervous system. Interpretation of experimental data from human MS cases presents a number of problems due to differences among patients life style, disease phenotype, medications and diet. Hence a multidisciplinary approach is necessary to obtain a clear understanding of the molecular changes associated with the disease process. We are using such a strategy to obtain a better understanding of the changes in biochemical composition of urine from 12 MS patients [1] and 39 healthy volunteers from Sicily (Italy). In addition to urine, hair and toenails have been collected from the study population for biomarker identification. Urine samples were studied using 1H-NMR for analysis of metabolites, ICP-MS for trace element analysis and MALDI-TOF for protein analysis. Furthermore, a questionnaire was used to obtain demographic information, dietary habits, medications and general life-style habits of the healthy and MS patients. 1H-NMR spectra (Bruker, 400 MHz)of the urine samples were analysed to identify different metabolites (NMR suite, Chemonomx 6.1) and principal component analysis (using R) was used to determine differences between the control and MS groups. Interpretation of the complex spectral data was aided by information derived from the questionnaire. Preliminary results show differences in urinary metabolomic profiles between the MS cases and controls. The results of the findings will be discussed. Reference: 1. Nicoletti, A., Lo Fermo, S., Reggio, E., Tarantello, R., Liberto, A., Le Pira, F., Patti, F., Reggio, A. (2005) Journal of Neurology, 252 (8), pp. 921-925.

P2A-010
Plasma metabonomic study of Guillain-Barre syndrome by ultra-performance Liquid chromatography coupled with Q-TOF mass spectrometry

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Guillain-Barre syndrome (GBS) is an autoimmune inflammatory disease of peripheral nervous system that characterized by demyelination. Acute inflammatory demyelinating polyneuropathy (AIDP) is the most common form of GBS, usually triggered by acute infectious process, however, more than 60% of cases do not have known cause. In order to find potential disease biomarkers and reveal its pathophysiological changes, a metabonomic technique based on ultra-performance liquid chromatography (UPLC) coupled with Q-TOF mass spectrometry was employed to investigate the plasma from 12 patients with GBS and 12 healthy volunteers. Based on this approach, more than fifty metabolites were significantly changes between two groups. These metabolites all were compared with database and further identified by MS/MS. Several potential biomarkers, acylcarnitines, lysophosphatidylethanolamine and lysophosphatidylcholines, were identified. These metabolites were reduced in GBS patients. The unknown metabolites, the m/z values of 602.4465 and 704.5176 were significantly increased in GBS patients, will be identified by further study. These disturbances in plasma metabolites are likely due to metabolic changes in GBS disease. These metabolites may be potential biomarkers for the evaluation of GBS disease.

P2A-011
UPLC quadrupole oa-TOF MS and MarkerLynx XS software for metabolomic profiling of creatine supplementation in human serum samples

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Creatine is present in muscular tissue of many vertebrates and is a widely used dietary supplement. Since it represents an endogenous substance, determining its absolute effect in individuals is very challenging. Serum from sportsmen during seven weeks training with/without creatine in a double blinded trail was analyzed using accurate mass UPLC® quadrupole oa-TOF MS in MSE mode. The data was processed with MarkerLynx extended statistics software and a comprehensive metabolomic profile was created using principle component analysis (PCA). Results of the creatine group (CC) were compared with a placebo group (PP) in an OPLS-DA approach which showed clear differences in the detected markers. Multiple marker candidates could be identified in CC using MassFragment™ software. One candidate is sarcosine, which is part of the metabolic degradation pathway of choline. The increase of sarcosine can be interpreted in terms of a decreased somatic production of creatine in supplemented patients. Further, anthranilate has been detected in CC samples only. Since it was used as artificial flavor in the creatine supplement, it can be used as a compliance marker throughout the study. The combination of UPLC-quadrupole-oaTOF-MS and embedded Software package led to an efficient detection of marker candidates in serum samples.

P2A-012
Targeted Urinary and Fecal Amino Acid Profiling Using Gas Chromatography Mass Spectrometry: It's Role in Risk-Factor Epidemiology and Chemoprevention

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Epidemiological studies have identified diet as a major environmental risk factor for a number of chronic diseases, including colorectal cancer, the United Kingdom's third most prevalent carcinoma (2006 mortality rate ~16,000). Nutritional epidemiological studies have attempted to further define the food types responsible for the association between food consumption and colorectal carcinogenesis. There is a substantial body of evidence suggesting a positive association between red/processed meat consumption and increased colorectal cancer risk and an inverse association between vegetable/fibre consumption and colorectal cancer risk. However, these findings are inconsistent. It is proposed that the origin of this inconsistency is attributable to the nature of dietary assessment methods currently employed in nutritional epidemiology. Individual volunteers are required to "self-report" their own food consumption by a variety of dietary assessment instruments and this is the primary source of data subsequently used to test diet/disease associations. However, this data source is inherently biased as individuals have a propensity to over-report food types perceived as healthy, whilst under-report those viewed less healthy. Hence it is difficult to attribute putative protective/cytotoxic effects to particular dietary components. Biomarkers of nutritional exposure circumvent the uncertainty of traditional dietary assessment methods, facilitating a more accurate, unbiased assessment of an individual's dietary consumption. Hence, they permit the association between diet and gastrointestinal carcinogenesis to be unequivocally addressed. However, the complex nature of diet, both at the level of an individual meal and especially over an extended time span, result in a miscellany of low concentration metabolites in urine, rendering the process of nutritional biomarker discovery a formidable task. Here, we describe the use of GC-MS and pattern recognition techniques in the "predictive" biomarker discovery process. Volunteers were recruited and subsequently required to live in a metabolic suite where they were exposed to a number of different dietary regimens. GC-MS and multivariate analysis of urine and fecal samples was used to discriminate between diets on the basis of their metabolic profiles and identify a number of potential biomarkers indicative of the particular food-types consumed within the intervention study. Robust discrimination was achieved for diets associated with high meat intake and vegetarianism.

P2A-013

"Hormesis : Myth or reality? A temptative metabonomic answer"

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Two models of dose-response relationships are currently accepted in risk assessment: the "linear threshold model" involving a dose below which exposure to the chemical is safe, and the "linear no-threshold model" in which exposure to a unique molecule of the chemical can be deleterious. Besides, a "hormesis concept" is proposed but is still controversial. It suggests that a primary exposure to a low non toxic level of a chemical protects against a subsequent toxic dose. For example, it is well documented that a low dose of CCl4 protects against the hepatotoxicity of a higher dose administered later. However, cellular events underlying this phenomenon are still misunderstood. In order to unveil some of those cellular mechanisms, we have used a metabonomic approach to investigate the presumed hormetic effect of CCl4. Material & Methods 12 male rats divided in 3 groups were individually housed in metabolism cages. Urine samples were collected over time and analyzed by 1H-NMR spectroscopy at 400 MHz. CCl4 was given i.p. in corn oil. Group 1 ("hormesis group") received a low non-toxic dose (100 µl/kg) of CCl4, followed by a high toxic dose (1ml/kg) injected 24 hours later. Group 2 ("unstimulated group"), received corn oil on day 1 followed by a high toxic dose of CCl4 on day 2. Group 3 ("toxic group") received 2 subsequent toxic doses. Results & Discussion Urinary excretion of hippurate was severely decreased in the unstimulated and toxic groups. Taurine was higher in hormesis group than in pretest urine, but decreased in the unstimulated and toxic groups. A high creatine level was only observed in the toxic group. Finally, Kreb's cycle intermediates fell down more severely in toxic group than in unstimulated group. In hormesis group, this change was also noticed, but negligible when compared to pretest urine. Those preliminary metabonomic results confirm the protective effect of a low dose of CCl4. Mechanisms involved in this protective process include: improved osmoprotection (taurine), unaltered liver synthesis ability (hippurate), a stronger resistance of energy production pathways. Taking together, those results suggest that hormesis is more a reality than a myth.

P2A-014

Quantitative analysis of trehalose-6-phosphate in *Arabidopsis thaliana* seedlings by solid-phase extraction-based sample pretreatment and anion-exchange chromatography–electrospray ionization mass spectrometry

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Plant synthesized starch is an essential raw material for industry and a crucial component of human diet and livestock feed. Therefore, understanding its synthesis and regulation mechanisms is essential. The first committed step in starch synthesis involves the conversion of glucose-1-phosphate by ADP-glucose pyrophosphorylase (AGPase) into ADP-glucose. AGPAse has been shown to be post-transcriptionaly redox regulated and trehalose-6-phosphate (T6P) affects this regulation. But the exact mechanism by which T6P acts in this regulation has yet to be elucidated. Study of plants disrupted in their response to T6P would represent a key step in understanding the regulatory role of T6P in carbon utilization. In such studies the ability to readily quantify levels of T6P in plant tissues is of crucial importance. However, the low quantity of T6P present in plants and the high complexity of plant matrices provide an analytical challenge. Here we present a method for the selective detection of T6P in tissue of the model plant *Arabidopsis thaliana*. Liquid/liquid extraction (LLE) and solid phase extraction (SPE) were used as sample pretreatment followed by anion-exchange chromatography (AEC) coupled with electrospray ionization mass spectrometry (ESI-MS) for highly specific quantitative analysis. LLE of plant material was performed with chlorophorm/acetonitrile/water (3/7/16, v/v/v) followed by SPE with OasisMax material, which significantly reduced the complexity of the sample. On-line coupling of MS with gradient AEC using a sodium hydroxide eluent was accomplished with a post-column ion suppressor which exchanged sodium for hydrogen ions. The method is shown to allow specific quantification of T6P with good linearity ($R^2>0.98$) in the 8 nM to 8 µM range. The recovery of the method was above 80% for relevant T6P levels. The method was applied to the quantification of T6P in seedlings from Arabidopsis thaliana mutant lines that resist growth arrest caused by external supply of trehalose.

P2A-015

Characterization of the human adenocarcinoma HT29 cell line lipidome during cell cycle progression.

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Phosphatidylcholine (PC) is the major membrane phospholipid in mammalian cells and with sphingomyelin (SM) comprise approximately 50% of the glycerophospholipids (GPL) in the outer leaflet of the mammalian plasma membrane. Phosphatidylcholine:ceramide phosphocholine transferase, also known as sphingomielyn synthase (SMS), catalyzes the transfer of the phosphorylcholine headgroup from PC to ceramide (CER), forming SM and releasing diacylglycerol. This activity permits SMS to switch from an anti-mitogenic signal, as ceramide, to a mitogenic signal, as DG. As such, SMS may play a key role in cell cycle regulation pathways. Furthermore, the sphingomyelin signal transduction pathway are important in cell cycle regulation due to enzymatic degradation of SM to generate different products with pro-apoptotic or pro-mitotic function. Activation of acidic sphingomyelinase (aSMase) often produces an increase in ceramide concentration that leads to cell apoptosis, however simultaneous activation of Mg2+-dependent neutral sphingomyelinase (nSMase) and ceramidase (CDase) produces sphingosine (Sph) and sphingosine-1-phosphate (S1P) acting as pro-mitotic signal. The aim of the current study is to determine the lipid species most directly involved in the regulation of tumor cell proliferation and correlate it with SMase and/or CDase activities. Human adenocarcinoma HT29 cells were synchronized and G1, S and G2 rich populations were obtained. Membrane lipid composition of these populations was analyzed by Fourier Transform-Ion Cyclotron Ressonance-Mass Spectrum (FT-ICR-MS) and assigned using Precalculated Exact Mass Isotopologue Search Engine (PREMISE). SMase activities were evaluated by non-radioactive fluorimetric methods. Our results implicated role(s) of certain species of PC and SM in cell cycle progression in HT29 cell line. Length and degree of saturation of acyl chains in GPL may be important in cell cycle regulation. This work was supported by the Catalan (2009 SGR 1308) and Spanish (SAF2008-00164) Governments and the ISCIII-RTICC (RD06_0020_0046).

P2A-016

Large-scale genetical lipidomics: analytical method development and bioinformatic strategies

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In epidemiological research, large-scale genome wide association studies have been carried out in the recent years and let to a wide range of novel insights with regard to common diseases like obesity, type 2 diabetes or cardiovascular disease. However, these approaches often only link clinical outcomes or relatively blunt phenotypical attributes like the Body Mass Index (BMI) to genetical information. Lacking detailed information on functional endpoints like metabolites, little can be inferred about the mechanisms causing the disease. Recent advances in mass spectrometry now enable us to match and complement genome wide association studies encompassing several ten thousand participants with unprecedented throughput and detailed metabolic information. We suggest that our approach should enable us to unravel novel associations on a mechanistic level and facilitate a deeper understanding of distinctive features in the person-to-person variability of the lipidome. To facilitate this approach, we developed new analytical and computational strategies. On the analytical side, we exclusively focussed on the lipophilic fraction of human plasma samples. Chip based direct-infusion nanospray coupled to an orbitrap mass analyser was used to ensure both high-throughput and high-resolution mass spectra in an unbiased profiling approach. To enhance the reproducibility of our method, we used a 96 well plate layout with quality control samples and blanks, as well as a range of internal standards and automated sample preparation. The high-throughput capabilities and the complex and feature-rich character of the spectra, demonstrate the need for efficient and scalable bioinformatics routines. To tackle this, we chose a workflow-based approach utilising the Taverna framework. Much attention was paid to develop automated quality control mechanisms; furthermore computationally intensive tasks like peak detection were carried in a distributed environment harnessing several high-end machines. In addition, compound databases like LIPIDMAPS, HMDB, ChEBI and Chemspider were linked to the analysis workflow and queried automatically. An overview of the complete workflow and future developments will be demonstrated.

P2A-017

Normalization to account for dilution in a hemorrhagic shock model

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Hemorrhagic shock changes intravascular volume and renal blood flow. These changes alter urine output and concentration after hemorrhage and throughout resuscitation. Metabolomic analysis of urine is attractive since urine contains filtered metabolites, is reflective of kidney function, and can be obtained noninvasively. In hemorrhagic shock, dilution effects confound the absolute abundances of the metabolites present in each sample. We seek to understand different urinary normalization methods in the context of hemorrhagic shock and resuscitation to identify a normalization technique that is useful in our experiments and for human studies. Methods: Twelve Yorkshire pigs were subjected to a standardized hemorrhagic shock and resuscitation protocol. Urine samples were collected at set timepoints throughout. All samples were processed with 1H NMR using a NOESY pulse sequence on a Bruker 700 MHz spectrometer. Seven normalization methods were applied to raw urine concentrations: constant sum (CS), both with and without lactate, glucose, and urea signals, total spectral intensity (TSI), probabilistic quotient (PQ) with two different reference spectra, osmolality (OSM), and urine output (UO). The normalization constants were correlated using Spearman Rank correlation. These constants, blood gas lactate, and urinary lactate concentrations were analyzed using repeated measures analysis of variance (rmANOVA). Results: Correlation indicated that CS, TSI, OSM, and UO are correlated ($r \geq 0.47$). The PQ methods are correlated with each other, but not with CS and TSI ($r<|0.18|$) and less correlated with OSM and UO ($r>0.17$). RmANOVA indicated a decrease in urine concentration after resuscitation as indicated by the normalization constants across normalization methods ($p<0.0149$ in all but CS and TSI). Blood gas lactate increased by 45 minutes after shock but did not increase in urine until the next measured timepoint. Conclusions: Normalization constants reflect the relative concentration of urine samples and each suggest a delayed increase in lactate concentration as measured in urine compared to blood gas lactate.

P2A-018

Advances in Data Processing and Compound Identification Software for Metabolomics

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Agilent's advanced software suite enables metabolomics analysis by an un-targeted data mining approach and / or by a user defined list of compounds. Each of these approaches has advantages that result in more thoroughly mined data for subsequent statistical analysis, compound identification and data visualization. Agilent Mass Profiler Professional (MPP) is a chemometric software package designed specifically for processing mass spectrometric data (GC/MS, LC/MS, CE/MS and ICP-MS), such as that resulting from metabolomics experiments. Advanced visualization tools enable the inspection and annotation of results. With functionality for unsupervised (classification without prior group assignments) or supervised (using pre-classified groups) analysis, the software allows: - Quick and easy discovery of differences between sample groups - Plot changing patterns of compound abundances over time - Development of useful multivariate models for class prediction. Mass Profiler Professional includes an integrated ID Browser that allows compound identification using databases, libraries or empirical formula calculations. For LC/MS metabolomics, the new enhanced Agilent METLIN Personal Compound Database and Library contains more than 27000 compounds with approximately 3000 compounds having MS/MS Spectra. The spectra are acquired at 4 different collision energies (0, 10, 20 and 40 eV) both in negative and positive mode in ESI mode using an Agilent Q-TOF LC/MS system. The use of the MS/MS spectral search assists in compound identification where the accurate precursor mass is not sufficient for a unique identification. The ID Browser annotation capability enables the use of integrated pathway software to mine interactive databases.

P2A-019

Finding robust bio-markers in small samples

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Bio-marker identification is a hot topic in many of the -omics sciences: knowing which compounds (metabolites, genes, proteins, ...) are indicative of class differences is important for a better understanding of the underlying biological processes. Two main approaches exist: the first is to fit a multivariate statistical model with good predictive properties, and to assess the absolute size of the coefficients. The other is to rely on (uni-variate) statistical tests to determine whether class averages for specific variables are significantly different. Both approaches become "risky" as the number of biological replicas decreases. Unfortunately, in many cases it is practically impossible to gather more data. In animal testing in particular, due to ethical and legal reasons as well as public concerns, it is indispensable to develop strategies which use the smallest possible number of animals necessary to assure the statistical validity and usefulness of research results. We highlight strategies to identify potential bio-markers with small numbers of objects, and illustrate the key ideas using both simulated and real data sets.

P2A-020

UDP-GlcNAc as a metabolic marker in neuronal cells – facile detection by NMR

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Between 2 and 5% of the intracellular glucose is shuttled into the hexosamine biosynthetic pathway (HBP) [1]. One major intermediate along this pathway is UDP-GlcNAc that serves as a donor substrate for e.g. O-GlcNAc transferase that mediates O-GlcNAcylation of cytosolic proteins. O-GlcNAcylation is a stress and nutrient-sensitive dynamical posttranslational modification analogous to phosphorylation and plays an important role in Alzheimers's disease and diabetes [2]. Prior NMR studies have focused on monitoring the fate of 13C labelled metabolites occurring during the citric acid cycle or glycolysis. Metabolic products from the HBP have attracted little attention so far. We are currently exploring the potential of UDP-GlcNAc as a NMR detectable biomarker in neuronal cells within the framework of the so called "Selfish Brain" theory that provides a new model of energy regulation within the human body. It states that the brain satisfies its own energy requirements first and dysregulation of these mechanisms can lead to obesity, type II diabetes or other metabolic diseases [3]. On a cellular basis energy production of neurons is described by the astrocyte-neuron lactate shuttle hypothesis [4]. Here, NMR spectroscopy in combination with selective isotope labelling in a neuronal cell line is used to map the functional and energy state of neurons, i.e. we follow the fate of metabolic species in cell extracts and supernatants [5,6]. Furthermore, we follow the specific labelling pattern of UDP-GlcNAc under different physiological and manipulating conditions. Additional studies on quantification of UDP-GlcNAc and other metabolites to compare the energy status of the cell with its metabolic state are planned. [1] McClain D. and Crook E (1996) Diabetes 45(8), 1003 [2] Dias W. B. and Hart G. W. (2007) Mol. BioSyst. 3, 766 [3] Peters A. et al. (2004) Neurosci. Biobehav. Rev. 28(2), 143 [4] Pellerin L. (2005) Mol. Neurobiol. 32, 59 [5] Zwingmann C. et al. (2003) J. Cereb. Blood Flow Metab. 23, 756 [6] Bouzier-Sore A.-K. et al. (2006) Eur. J. Neurosci. 24, 1687

P2A-021
Caenorhabditis elegans metabolite extraction method benchmarking for GC-MS, LC-MS and NMR profiling

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C. elegans is a widely used model organism in biomedical research. Despite being the first eukaryote organism to have its genome sequenced and having given rise to important methods (i.e. RNAi), it is still not widely used in metabolomics research. As already well characterised for toxicology, ageing studies, functional genomics or environmental sciences, metabolomics could provide new and exciting insights. A first and crucial step to ensure good results is an optimised metabolite extraction strategy – especially because the nematode has a small body size and tough cuticle, and so extraction methods developed for soft tissue samples are not necessarily applicable. In this study six extraction methods and two solvent systems (methanol/ water and methanol/chloroform) were evaluated for use with GC-MS, UPLC-MS, and NMR to achieve the widest possible metabolome coverage. The extraction methods were (1) mortar and pestle, (2) homogeniser, (3) bead-beater, and a 'Tissuelyser' ball mill with either (4) metal spheroids, (5) glass beads, or (6) metal balls at cryogenic temperatures. NMR: All methods and solvents were roughly equivalent in terms of numbers of observed metabolites and reproducibility, except that use of the homogeniser was gave very outlying results in one solvent system. GC-MS: Again, most methods showed a coefficient of variation for each metabolite similar to the derivatization and runtime QC samples, indicating a good overall reproducibility. Differences in overall yield were also observed. LC-MS: As expected, LC-MS showed the clearest distinction between MeOH and MC extracts. Conclusions: This cross-platform study provides data on choosing an appropriate metabolite extraction strategy for C. elegans, together with useful baseline data on the observed metabolome. Certain methods can be seen as a cross-platform compromise: for example bead-beating and manual grinding both gave highly reproducible data and high numbers of visible metabolites.

P2A-022
Fatty acid composition of serum glycerophospholipids in children

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Aims: Polyunsaturated fatty acids (PUFA) have important biochemical and physiological functions. An adequate PUFA availability is important for growth and development. Monitoring and therapeutic interventions are applied in children with impaired fatty acid (FA) intake, absorption or metabolism, e.g. with gastrointestinal, hepatic or metabolic diseases, which requires reliable assessment of FA status. FA composition of serum glycerophospholipids (GP) is a sensitive and reliable biomarker of the organism\'s FA status. We aimed to establish reference values for children using a new and precise high-throughput methodology. Method: We analyzed the GP FA composition of 1326 serum samples obtained from a cohort of 951 children at 2 and 6 years participating in a prospective birth cohort study in Germany, the LISA study. FAs were determined from 100 µl serum with a method as previously described (Glaser et al., 2010). Results: We categorized the distribution of FAs in GPs by gender for both age groups (2 years: 412 boys, 325 girls; 6 years: 330 boys, 259 girls). Medians and interquartile ranges were similar for both genders. The two-sided Mann-Whitney rank test revealed no significant differences between boys and girls. The FA distribution in GPs was similar to values in phospholipids published by Decsi and Koletzko, 1994: total saturated FA 43.95% ± 1.59% (vs. 45.17% ± 3.49%), total cis monounsaturated FA 14.11% ± 1.88% (15.37% ± 2.43%), total n-6 LC-PUFA 13.3% ± 2.14% (12.34% ± 2.68%), total n-3 LC-PUFA 4.44% ± 1.08% (3.73% ± 1.45%). Conclusion: The obtained FA values may serve as reference values for children and can be applied to assess results of analyses from patient samples, as well as clinical and epidemiological studies. FA values in GPs are in very good agreement with FA values in phospholipids for most of the analyzed FAs. GP FA composition is considered a valuable new biomarker that is very sensitive for PUFA and LC-PUFA status.

P2A-023
MarVis: Metabolite-based clustering and visualization of LC-MS data for marker discovery and pathway recognition

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Metabolic fingerprinting using mass spectrometric measurements provides large volumes of metabolite intensity profiles. For marker discovery similar intensity profiles of these metabolites need to be grouped by means of intensity-based clustering. One-dimensional self-organizing maps (1D-SOMs) realize a linear array of prototypes that correspond to local averages of the data ordered according to similarity of intensity profiles. The MarVis tool provides a graphical user interface for exploratory data analysis, which implements the concept of 1D-SOM clustering, data base search and pathway recognition. The corresponding visualization supports data identification even if the number of biologically meaningful groups of intensity profiles is unknown. Relevant groups of markers can be identified based on their position in the prototype array and are attached to the corresponding pathways. To demonstrate the functionality of MarVis, metabolic fingerprinting was applied to plant wound response. Oxidized fatty acid derivatives (oxylipins) play a crucial regulatory role in mediating this response. To extend our knowledge on global metabolic changes after wounding, wild type and respective mutant plants of Arabidopsis thaliana were analyzed. Data resulting from both an untargeted UPLC-TOF-MS analysis and a targeted HPLC-Iontrap-MS analysis were explored using MarVis. Using the untargeted approach, not only a significant number of well-known oxylipins was identified as wounding markers but also so far unknown markers independently from the oxylipin pathway. Moreover, MarVis can be used to find a meaningful biological context of comprehensive quantitative profiling data resulting from targeted analyses. Leaf wounding is accompanied by the formation of oxygenated galactolipids (Arabidopsides) which may be a storage-form of signaling molecules to be liberated upon stress. To identify so far unknown metabolic pathways involved in Arabidopside biosynthesis, quantitative profiles of more than 100 different galactolipid species were inspected with MarVis. The 1D-SOM arrays indicate that Arabidopsides may be synthesized via an enzymatic pathway which acts on lipid-bound fatty acids.

P2A-024
Plasma lipidomic profiles and familial longevity: The Leiden Longevity Study

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Offspring of long-lived individuals have lower prevalence of cardiovascular disease, hypertension, and type-2 diabetes. Interestingly, variations in specific lipid classes are present in these age-related diseases, but its relevance to human longevity has not been investigated. In this study, we aim to depict the plasma lipidome of the offspring of nonagenarian siblings and to assess potential differences between their lipid profiles and that of controls. A previously developed UPLC-MS(QToF) method was validated to determine 180 lipids from different lipid classes in human plasma (lysophosphatidylcholines, phosphatidylcholines, cholesterylesters, sphyngomyelins, phosphatidyethanolamines, diacylglyerols, and triglycerides). A total of 112 lipids fulfilled the quality control and replicate criteria (RSD < 15%) and were selected for further statistical analysis. Here, we present preliminary results of 25% of the cohort. Using a generalized linear model we compared relative lipid levels between controls (n=221, 34-78 years) and the offspring of nonagenarian siblings (n=477, 38-80 years) from The Leiden Longevity Study. Sixteen lipids from the lysophosphatidylcholine, phosphatidylcholine, and sphyngomyelin classes showed slight but significant higher levels in the offspring of nonagenarians compared to the control group. In the offspring of nonagenarian siblings, LPC O-16:0 and SM 18:1/17:0 showed the highest increase (9.8% and 9.3%; $p<0.05$), while phosphatidylcholines showed a modest increase (4%). Other lipid classes such as triglycerides and cholesteryl esters did not present differences between groups. This study depicts the plasma lipidome of a population genetically enriched for longevity. Several lipids of the LPC, PC, and SM classes showed minor but statistically significant higher relative concentrations in the offspring of a long-lived population. In long-living individuals the onset of aging diseases such as cancer, metabolic syndrome, and cardiovascular ailments tends to be postponed compared to the general population. It will be of interest to investigate whether these lipid findings have an effect on the healthy aging of long-living individuals.

P2A-025

Determination of urinary 6β-hydroxycortisol-to-cortisol ratio for metabolic phenotyping of human CYP 3A4 activity using LC-QTOF MS

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CYP 3A4, an isoform of cytochrome P450, metabolizes numerous substances including half of marketed drugs in human liver. The ratio of urinary 6β-hydroxycortisol (6β-HC) to cortisol is currently considered as the only noninvasive indicator of human CYP 3A4 activity. Often, determination of two steroids in body fluids was carried out by LC-UV, GC-MS or LC-MS/MS. We herein proposed a new approach by use of UPLC-QTOF MS for reliable quantitation of urinary 6β-HC and cortisol in premenstrual women. Urine was extracted with ethyl acetate and the extracts were analyzed by reversed-phase UPLC-QTOF MS using gradient elution and (+) ESI detection. The LC-MS conditions were optimized for the highest analytical performance and minimum matrix effects. Quantitation was performed using internal standard calibration. The method was validated for dynamic range, linearity, specificity, sensitivity, precision and accuracy to ensure reliability of quantitation. The use of isopropanol and 0.01% formic acid as mobile phase significantly increased analytical sensitivitiesm, >3 and 5 folds for 6β-HC and cortisol as compared to a standard LC condition. 6β-HC (m/z 379.2115) and cortisol (m/z 363.2166) achieved good separations from their isomeric components. Method validation results demonstrated reasonable dynamic response ranges (ca. 750) and good linearities (R>0.9995) for both compounds. The LODs were 0.3 and 0.1 ng. mL(-1), and the LOQs were 1.5 and 0.3 ng.mL(-1) for 6β-HC and cortisol, respectively. The sample recoveries at three spiking levels and the inter- and intra-day analysis variations were shown to be satisfactory, indicating good quantitative accuracy and precision. As a proof-of-principle study, this method was used for analysis of the urinary 6β-hydroxycortisol-to-cortisol ratio as an endogenous indicator of CYP 3A4 activities in a cohort of premenopausal women in relation to their ovulatory status during menstrual cycle. Result from correlation of the CYP 3A4 enzyme activities with the urine metabolic profiles will be presented.

P2A-026

Sensitive Quantification of 22 Amino Acids from 10 µL Plasma Combining Derivatization and Ion-Pair LC-MS/MS

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Time efficient and comprehensive quantification of amino acids continues to be a challenge. We developed a sensitive and precise method for analyzing amino acids from very small plasma and serum volumes. Ion-pair chromatography of amino acid butylesters proved to provide an optimal combination of selectivity, sensitivity and robustness. 10 µL of plasma or serum are added to precipitation reagent containing stable isotope standards. After protein precipitation, the supernatant is dried and incubated with 3N butanolic HCl for improving chromatographic separation and ionization efficiency. Amino acid butylesters are separated using ion-pair (heptafluorobutyric acid) reversed-phase chromatography coupled to triple quadrupole mass spectrometry. The established method enables quantitative analysis of 22 amino acids, all 20 proteinogenic amino acids, ornithine and citrulline. Cysteine is measured as cystine. The combination of precipitation, derivatization and chromatographic separation effectively avoids ion suppression and coelution. Simultaneous with quantification, the identity of the analytes is verified in each sample using qualifier ions. The micro-method is very sensitive and accurate. The intra-assay precision for the analysis of plasma was 2.6 - 10.1%. Absolute accuracy as determined by comparison of external reference samples was 81.5 - 108.6%. Excellent linearity of detection response was demonstrated for all compounds in the range representative for clinical samples from infants and adults (from aspartic acid = 15 µmol/L to glutamine = 1000 µmol/L). Lower limits of quantification were in the range of 30 nM - 300 nM for all analytes. In conclusion, the method is ideally suited for cost-effective high-throughput analysis of large numbers of samples in clinical studies and metabolomics research.

P2A-027

CE-MS-based metabolome profiles differentiate stages of diabetic nephropathy

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Diabetic nephropathy (DN) is one of the major complications of diabetes mellitus (DM) and is the most prevalent cause of end-stage renal disease (ESRD). Recent studies showed that several medical interventions at early stage in the development of nephropathy can significantly slow down the progression of DN and thus have great impact on disease management outcome. Therefore, the identification of novel biomarkers for early prediction of DN is an important task. Here, we applied capillary electrophoresis-mass spectrometry (CE-MS) to identify serum metabolite biomarkers that may facilitate early diagnosis of DN. Serum samples (n=77) were obtained from type 2 diabetic patients with successive development of DN [stage I (normoalbuminuria; n=20), stage II (microalbuminuria; n=19), stage III (macroalbuminuria with/without renal dysfunction; n=20) and stage IV (renal failure; n=18)]. We conducted non-target analysis and obtained 289 metabolite peaks after removal of redundant peaks. From these, we identified 24 biomarker candidate metabolites showing significant differences between at least two groups (P<0.0001; Kruskal-Wallis test). Furthermore, we constructed the decision tree classification models, making use of the markers. The tree with selected seven metabolites yielded high area under the receiver operating characteristic curves. In conclusion, global metabolite profiling coupled with decision tree classification techniques can help to discriminate the progression of DN.

P2A-028

Lipidomics of liver tissue of ApoE*3 Leiden CETP transgenic mice: comparing intervention of Rimonabant and a multi-component Chinese herbal formula

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We applied here a sophisticated liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometry (LC-FTMS) lipidomics method to study an animal model of ApoE*3 Leiden (E3L) transgenic mouse, expressing human-like cholesterol ester transfer protein (CETP), relevant to hyperlipidemia. A total of 30 female E3L CETP mice were fed a semi-synthetic modified Western-type diet 4 weeks prior to the treatment in order to get mildly elevated lipid levels and a mild increase in body weight. Then 24 out of 30 female mice were divided into 3 groups and were studied (1) without treatment, (2) after treatment with Rimonabant, and (3) after treatment with a multi-herbal Chinese formula SUB885C for a 4-week intervention period. Liver samples were collected from the sacrificed mice after the treatment. Liver lipid extraction was achieved by liquid-liquid extraction and subjected to LC-FTMS system. Score plots from Principal Component Analysis (PCA) of the LC-FTMS lipidomics data showed distinct patterns among the groups. SUB885C treatment was found to notably change the hepatic lipid metabolism toward a more healthy direction than the Rimonabant treatment compared to the control. Significantly decreased cholesterol ester lipid species (ChoEs) such as ChoE (16:0) (28.5%, P = 0.0299), ChoE (18:1) (21.2%, P = 0.0292), ChoE (18:2) (43.7%, P = 0.0046) and ChoE (20:4) (52.7%, P = 0.0057) caused by SUB885C suggested the multi-component Chinese herbal formula can effectively influence the CETP pathway, leading to decelerated atherosclerosis. This LC-FTMS lipidomics method holds promises for lipid biomarker screens in relation to, for example, the metabolic syndrome, disease prevention and health promotion. Its applicability has been demonstrated in revealing treatment effects and differences of a Western and a complex Chinese herbal formula.

P2A-029

Targeted metabolomics of clinically well characterised serum samples of patients with prostate cancer

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Prostate cancer (PCa) is the most commonly diagnosed cancer among men and the second leading cause of cancer-related death. The etiology of prostate cancer still remains controversial, with environmental, hormonal and hereditary factors implicated as key players. The current methods of classifying prostate cancer stages or outcomes clinically, such as Gleason score, Epstein criteria and TMPRSS2: ERG-gene fusion status are largely inconsistent and non quantitative. Herein, we have employed a LC-MS/MS based targeted metabolomic approach, as opposed to metabolomic profiling, to quantitatively analyse metabolites in sera of 105 patients with prostate cancer previously characterized by Gleason scores, Epstein and TMPRSS2: ERG-fusion classification schemes. In a panel of 238 metabolites analysed in this study, some associations between metabolites and the different classification schemes were established. Several metabolites were able to distinguish control sera from PCa sera irrespective of the clinical classification, whereas only few metabolites could stratify prostate cancer within each classification scheme. Out of the 238 metabolites analysed, 11 metabolites differentiated prostate cancer characterized as low Gleason scores from high Gleason scores, 11 metabolites differentiated prostate cancer characterized as Epstein insignificant from Epstein significant, and 21 metabolites differentiated prostate cancer with TMPRSS2:ERG-gene fusion to those without TMPRSS2:ERG-gene fusion. Interestingly, most metabolites capable of differentiating PCa within a classification scheme were restricted to one classification scheme, with only octadecadienoylcarnitines (saturated and unsaturated) and hydroxykynurenine being able to differentiate PCa outcome in more than one classification scheme. These findings underscore the inherent differences in the currently used schemes to classify prostate cancer, and reveal the potential of using metabolites for prostate cancer diagnosis and for the stratification of the disease.

P2A-030

NMR-based metabolic profiling of serum and urine in stroke patients

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Stroke is the leading cause of adult disability and death in develop country. Nevertheless, early diagnosis of stroke is too difficult and no reliable biomarker is currently available for the diagnosis of stroke. So, we applied 1H NMR metabolomics approach to investigate the perturbed metabolic pattern in serum and urine from stroke patients and identify metabolic marker associated with stoke. Metabolic profiles of serum and urine from stroke patients were investigated using NMR spectroscopy coupled with pattern recognition method such as the principle component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA). The accurate concentrations of metabolites in serum and urine were rapidly measured using the target-profiling procedure. The results showed a statistically significant separation between stroke patients and healthy individuals. The serum of stroke patient was characterized by increased excretion of lactate, pyruvate, glycolate, and formate, and decreased excretion of glutamine and methanol. The urine of stroke patient was characterized by decreased levels of citrate, hippurate, and glycine. These metabolites detected from serum and urine of stroke patients associated with anaerobic glycolysis, folic acid deficiency, and hyperhomocysteinemia. Furthermore, the presence of stroke in the external validation model was predicted with high accuracy. These data demonstrate that metabolomics approach may be useful for the effective diagnosis of stroke.

P2A-031

Risperidone and Aripiprazole Induced Lipid Changes in Patients with Schizophrenia

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Risperidone and Aripiprazole Induce Lipid Changes in Patients with Schizophrenia Schizophrenia is a debilitating mental disorder characterized by psychosis, negative symptoms and neurocognitive deficits. Theories of the pathophysiology underlying schizophrenia have centered on neurotransmitters and their receptors and therapeutic drug development has largely targeted dopamine, serotonin and glutamate systems. Increasing evidence suggests that phospholipids which play a critical role in the structure and function of membranes seems to be impaired in schizophrenia. Neuronal cell membranes form the vesicles in which neurotransmitters are stored and through which neurotransmitters are released and hence membrane lipid changes could have a direct effect on proper neurotransmission. We used a targeted lipidomic approach to evaluate changes in lipids and lipid metabolism prior to and post short-term treatment (2 week average) with Aripiprazole or Risperidone in forty patients with schizophrenia. All patients were kept in hospital setting with standardized diet and exercise regimen. Outcome measurements of body mass index (BMI), weight and waist circumference were all significantly increased, while BPRS a clinical measure of disease severity was significantly decreased by both medications. Major changes in lipids were identified in patients with recurrent disease as well as in first episode patients suggesting that these changes happen early in the course of the disease and prior to treatment. A set of n6 lipid metabolites were significantly increased by treatment with either Aripiprazole or Risperidone. Monounsaturated lipid metabolites were selectively decreased with Aripiprazole treatment, while Risperidone treatment resulted in increases in phosphatidylcholine and phosphatidylethanolamine metabolites. Changes in multiple lipid metabolites were correlated with drug response and with changes in BMI and weight. Metabolites known to be involved in lipid oxidation were correlated with changes in BPRS scores suggesting a role of inflammation in the pathology of schizophrenia and its treatment.

P2A-032

Loss of expression of Mlh1 and its association with mitochondrial dysfunction and platinum resistance in cancer

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The mechanisms by which platinum drug resistance develops and, most importantly, its reversal to a more sensitive phenotype are of major interest in modern medical oncology. Alterations in MMR proteins such as Mlh1 have been shown to play an important role in the development of resistance to platinum drugs. Additionally, loss of Mlh1 has been speculated to be associated with mitochondrial dysfunction and the effects can be apparent even in cell lines of different genetic origin. In this study, in vitro cell models of platinum resistance involving cell lines either positive or negative for Mlh1, were examined within a metabolic context using \$^{1}$H NMR spectroscopy. More specifically, focus was given on the comparative metabolic analysis of the following cell models: a model of transient Mlh1 expression, involving kidney-derived cell lines (HEK-293T) of common genetic background using selective mitochondrial depletion, and a platinum resistance model including ovarian cancer cell lines (A2780 & CP70) of well-established Mlh1 status. The analysis and comparison of these models resulted in the isolation and identification of a metabolic signature unique for Mlh1, which included metabolites such as glutathione, alanine, phosphocholine, myo-inositol, lactate, and branched-chain amino acids. Overall, our findings offer a range of possible explanations on the role of specific metabolites in cancer development and their association with Mlh1, mitochondria and platinum resistance.

P2A-033

Metabolomic approach for classification of medicinal plants

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Artemisia genus is one of the most important herbs in the traditional medicinal herb market, and they have great variety of species all over the world. In addition, their enormous utility to cure and prevent various disease led many well-known bioactive compounds such as artemisinin, eupatilin and etc. from specific *Artemisia* species. In fact, the selection of specific medicinal sources as well as bioactive compounds is important before making good quality of medicine and products. It is necessary to pay close attention for choosing correct medicinal sources, particularly in case of medicinal plants, because of their diversity, which can affect the quality and efficacy of medicine. However, identification and classification of plants is difficult with no established method. Discrimination of plants based on morphological or genetic characteristics determined by specialists has been used as a conventional classification method of pharmaceutical sources so far; however, more need demands more general methods for accurate quality assessment of medicinal plants. We tried to classify ten different species of *Artemisia* using gas chromatography coupled with mass spectrometry (GC/MS) and showed a result on the relationship of *Artemisia* sp. with other species. Moreover, we suggested that GC/MS-based metabolite profiling has a potential as a new approach for classifying medicinal herbs.

P2A-034

LC/MS-based metabolomic analysis for age discrimination of Panax ginseng

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Panax ginseng C. A. Mayer is a highly important and widely used medicinal herb in the world. Various biological and pharmacological activities of *P. ginseng* are well-known, and the activities differ from ages, origins, and cultivars. In the case of ages, types and composition of bioactive components in ginseng samples are different according to ages, and quality and value are the best when the ginseng reaches an age of six years. Since it is hardly possible to determine ginseng ages with conventional methods based on physical appearance, illegal distribution of ginseng ages are prevalent in the market to meet consumers\' demand. In this study, an ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-Tof MS) analytical method has been developed for metabolite profiling and pattern analysis of *P. ginseng* to identify ginseng ages from 4 to 6 years, which are the most demanding ages in the market. Total 30 *P. ginseng* extracts were analyzed by UPLC/Q-Tof MS with the optimized method for *P. ginseng* extracts, and total profiled metabolites were followed by multivariate statistical analysis such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) to compare the patterns among tested samples. The dataset was subsequently applied to various feature selection methods to select optimal numbers of metabolites which are influential to discriminate each sample group. The PCA and HCA results with the selected metabolites show that each sample was clearly clustered according to its age compared to those with total metabolites. Therefore, the application of UPLC/Q-Tof MS–based metabolomics technique, which is accurate, reliable, and effective, for identification and classification of the real ages of *P. ginseng* samples presents a blueprint for establishment of a distribution system of P. ginseng using advanced technology.

P2A-035

UPLC-TOF/MS based metabolic profiling on human cervical cancer cell lines exposed to Indinavir and Lopinavir

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Human papilloma virus (HPV) can cause cervical cancer, where the pathogenesis of high-risk HPV arises from expression of E6 oncoproteins which induce improper activity of the 26S proteasome, thus leading to the degradation of the tumour suppressor p53 and other cellular proteins. Recently, it has been reported that HIV protease inhibitors, such as indinavir and lopinavir, could inhibit E6-mediated proteasomal degradation of mutant p53 in E6-transfected C33A (E6) cells. LC-MS is a powerful technique for not only the quantification of metabolites in biological samples but also for their identification, with LC giving metabolite separation, followed by electrospray ionisation (ESI) to generate ions followed by high resolution Time-of-Flight (TOF) MS for high precision mass and intensity measurements. In order to contribute to an understanding of the mechanism of these drugs against HPV on human cervical cell lines, we investigate the level and compositional changes in intracellular components of control and HPV16 E6 expressing cervical carcinoma cells upon exposure to a series of physiological relevant indinavir and lopinavir concentrations. C33A parent and E6-transfected cells exposed to indinavir and lopinavir at physiologically relevant concentrations were analysed using UPLC (Waters, Acquity) - MS (Waters, Synapt HDMS Qtof) for metabolite profiling. Using Bayesian networks allowed key mass ions to be determined from the MS data and these key metabolites were identified by LC-MS/MS analysis. Finally, drug quantification using UPLC-MS was carried out to estimate the drug levels in drug exposed parent and E6-transfected cells. We shall report that this approach can reveal distinct and common effects that the two drugs have on the metabolome of these two different cell lines.

P2A-036

Metabolomics: How to Validate Mass Spectrometry based Assays for endogenous metabolites?

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There is a big need of high quality assay for endogenous metabolites today e.g. for biomarker discovery, metabolomics and optimization of fermentation. The method validation is one of the most critical analytical issues in the mass spectrometry based quantification of assays for these metabolites. A standardization of the method validation procedure for endogenous metabolites is not given so far. The current method validation guidance for industry/bioanalytical method validation may be used as general basis for the validation procedure, however, do not cover all aspects for endogenous metabolites. An overview of the open and critical points in method validation for endogenous metabolites will be presented and discussed with main focus on biomarker discovery. Lit.: (1)Koal T., Deigner H-.P. Challenges in Mass Spectrometry Based Targeted Metabolomics. Curr Molecul Med 2010; 10 (2): 216-226. (2)Griffiths W., Koal, T., Wang Y., Kohl M., Enot D.P., Deigner H.-P. Targeted Metabolomics for Biomarker Discovery, Angewandte Chemie 2010, in press

P2A-037
Validated method for the analysis of free and bonded fatty acids in biofluids without fractionation using GC-MS after methylation

Therese Koal, BIOCRATES Life Sciences AG

Fatty acids are basic components in living organisms. They are either the building blocks or biosynthetic precursors in all the lipid compound classes. Fatty acids can be bound to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. Up till now standardized methods for fatty acid analysis can only determine the total fatty acid content. For the determination of free acid content, a pre-fractionation of free fatty acid from other lipid classes is often necessary. We have developed a highly sensitive and selective method for the determination of individual fatty acids, both in free form and bonded in glycerides and/or phospholipids without the need of (pre) fractionation. A 0.3 M methanolic HCl solution can selectively methylate free fatty acids at 25°C while leaving the bonded fatty acids largely intact. A higher concentration of HCl of 0.6 M, together with a higher reaction temperature of 90°C and longer reaction time, on the other hand, can completely convert the total fatty acid content into their respective methyl esters (FAMEs). Individual FAMEs are then separated on a DB-23 column and quantified by the GC-MS in the SIM modes. Characteristic ions are used for each of the FAME classes: m/z 74 for saturated and monounsaturated FAMEs, m/z 81 for di-unsaturated and m/z 79 for polyunsaturated FAMEs. The selectivity of methylation reactions has been proved using model compounds from different lipid classes. Some 35 FAMEs in the range from C10 to C24 have been quantified against external standards. Further 33 FAMEs have been identified based on the retention time and ratios of characteristic ions. These FAMEs can be semi-quantified using response factors deducted from the closely eluted FAME standards which are in the same class. With 25 µL sample, depending on the FAME class, LLOQ in the range of 0.3 – 5.0 µM can be achieved. The precision of analysis is generally between 5-8%, while the accuracy falls in the 80-115% range. The method has been validated and subjected to accreditation for human plasma, serum and cell culture media samples.

P2A-038
A simple and fast method for the analysis of water and fat soluble vitamins in cell culture media using HPLC-ESI-MS/MS

Therese Koal, BIOCRATES Life Sciences AG

We have developed a simple and fast method to measure quantitatively 12 water soluble vitamins/vitaminoids: Cholin, Betaine (a metabolite from Cholin), B1 (Thiamine), B2 (Roboflavin), B3-Nacid (Nicotinic acid), B3-Namide (Nicotinamide), B5 (Pantothenic acid), B6-PxalPO4 (Pyridoxal 5´-phosphate), B6-Pxine (Pyridoxine), B7 (Biotin), B9 (Folic acid) and B12 (Cyanocobalamine) and 4 fat soluble vitamins: A (Retinol) and E (alfa-, gamma- and delta-tocopherol). Target vitamins in 20 µL cell culture media samples are fractionated using a filter plate. Water soluble vitamins are eluted with the water in the 1st washing step. In this step, fat soluble vitamins stay on the filter and are eluted in the next washing step, when hexane-ethylacetate mixture is used as elution solvent. The solvent is then changed to MeOH after drying under N2. A 100% water compatible column, ZORBAX-Aq column (Agilent), is used to separate water soluble vitamins with a total runtime of 10 min. Fat soluble vitamins, were separated on the same column using mobile phase with almost 100% MeOH. The total runtime in this case is 9 min. Different types of filter plate have been tested for their selectivity during vitamin fractionation. Different columns has been tried to achieve the best separation for the complex mixture of vitamins. The best candidate was the ZORBAX-Aq column. Due to the very hydrophilic nature of water soluble vitamins, a high ionic strength mobile phase (200 mM ammonium formate) was necessary to maintain the separation and peak shape of target compounds. Standard mixtures of vitamins have been stabilized with Vitamin C and fumaric acid. For most compounds corresponding isotope labeled internal standards were used for absolute quantification. LLOQs in the range of 50 nM – 1 µM have been achieved. The calibration curves have shown at least three orders of magnitude of linearity. The imprecision is generally under 10%, while accuracy of spiked samples always falls in between 85-115%.

P2A-039
On-line electrochemistry/MS - a novel tool in predicting drug metabolism

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Oxidation of target compounds in an electrochemical cell is a complementary approach to traditional methods and delivers the oxidative metabolic fingerprint of the molecule in a very short time. The acquired mass spectra are presented in three-dimensional plots, so-called mass voltammograms. A mass voltammogram visualizes the ion abundance versus m/z as a function of applied potential to the electrochemical cell. With a mass voltammogram the optimal potential can be determined for electrochemical generation of the desired metabolite for further research e.g. a phase II metabolism study. It is a quick method to identify reactive pathways of the compound of interest. Additionally, electrochemistry allows to trace the reactive metabolite conjugates with targets (e.g. proteins, glutathione) without matrix interactions in contrary to classical methods. A dedicated software program has been developed to automate and simplify the mass voltammogram acquisition. The program controls the syringe pump, the potentiostat and triggers the acquisition of mass spectra at the designated cell potentials. The total acquisition time needed for recording of a full mass voltammogram can be as short as 5 minutes. Acetaminophen was chosen as one of the model drugs to investigate oxidative metabolism using the on-line EC/MS system with automated mass voltammogram acquisition. Electrochemical conversion of the acetaminophen into the reactive phase I metabolite (N-acetyl-p-benzoquinoneimine, NAPQI) and the NAPQI – glutathione (GSH) phase II conjugate was successfully achieved and identified with MS. Additionally, mass voltammograms of other drugs and xenobiotics (e.g. amiodarone; amodiaquine) are presented. The data demonstrate that hyphenation of electrochemistry with electrospray mass spectrometry provides a versatile and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) in phase I and phase II metabolomics studies.

P2A-040
Annotation of unknown metabolites in breast cancer samples using accurate mass GC-TOF MS and substructure features

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The structural annotation of unknowns poses a formidable challenge to analytical and computational chemistry. While a single analytical method (in mixtures) is inadequate for full structural elucidation, we here follow the route of structure dereplication, i.e. using all physicochemical data from metabolomic profiles to result in hit lists of candidate structures that are subsequently constrained by matching experimental and predicted features of chemicals such as accurate mass product ion spectra, retention indices and proton donor capacity. A Waters Micromass GCT Premier (Milford, MA, USA) orthogonal time-of-flight mass spectrometer coupled to an Agilent 6890N GC was used for accurate mass analysis of derivatized quality control mixture, blood plasma and breast cancer tissue sample from the European MetaCancer biomarker discovery consortium. All samples were subjected to electron ionization (EI) and chemical ionization (CI) analysis with three reagent gases i.e methane, ammonia and isobutane. We have first analyzed the quality control samples in three chemical ionization conditions. The overall mass error was determined at 5.7±5.3 ppm. The errors for isotopic abundance ratios for the A+1/A and A+2/A ion ratio were found as 2.1±2.6 % and 1.6±2.0 %, respectively. Using these data, the Seven Golden Rules algorithm was applied to constraint and scores all chemically possible formulas. For unknowns that were found as statistically significantly altered in breast cancer samples, results were constrained by query in chemical and biochemical databases, fit to the number of acidic protons, substructure analysis and retention index prediction. The top hits were further constrained by matching fragmentation pattern to predicted product ions using the Mass Frontier software, including matching predicted and experimental accurate product ion masses. Current prospects and pitfalls using this strategy will be presented. A range of novel compounds have been annotated using this approach, which eventually need to be validated as ‘identified compounds’ by comparison to authentic standards.

P2A-041
Investigation of the effects of PPAR activation on brown adipose tissue metabolism

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The Peroxisome Proliferator-Activated Receptors (PPARs) are members of the nuclear receptor superfamily. They are ligand-modulated transcription factors and regulate a large number of metabolic processes. There are three PPARs: alpha, delta/ beta and gamma. PPAR-alpha is a target for the fibrate class and is mainly expressed in the liver. PPAR-gamma is activated by Thiazolidinediones and is highly present in adipose tissue. Finally, PPAR-delta is almost ubiquitous and is known to regulate numerous pathways including glucose and lipid metabolism [1]. In this study, the metabolic effects of PPAR activation have been investigated in brown adipose tissue of rats using a PPAR-pan agonist that is known to target all three PPARs. The role of brown adipose tissue is to produce heat by oxidizing fatty acids in mitochondria [2]. Brown adipose tissue is well represented in rodents and also in cervical-supraclavicular depots in humans. In this study, rats received either 1000 mg/kg/day of GW625019X, a PPAR-pan agonist, over the course of 13 weeks (n=18) or corn oil vehicle control (n=20).1H-Nuclear Magnetic Resonance (NMR) and GC-MS analysis were used to profile the aqueous extract of brown adipose tissue and GC-FID and UPLC-MS were used on the organic extract. All of these analyses have been followed by multivariate statistics. The PPAR agonist affects fatty acid metabolism in multiple ways, including increasing fatty acid oxidation and the stearoyl-CoA desaturase activity, as well as decreasing total glucose concentration. The agonist appears to largely act through PPAR-delta activation and hence PPAR-delta's role in regulating fatty acid oxidation does not seem to be limited to "highly metabolic" tissues such as liver, white adipose tissue and skeletal muscle. 1: Kleiner, S., Nguyen-Tran, V., Baré, O., Huang, X., Spiegelman, B., Wu, Z. (2009) Journal of Biological Chemistry 284, 18624-18633 2: Himms-Hagen, J. (1985) Ann. Rev. Nutr. 5, 69-94

P2A-042
Metabolomic biomarkers of rat testes treated with di-butyl phthalate (DBP)

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Dibutyl phthalate (DBP), one of suspected endocrine disrupting chemicals (EDCs), has been reported to be a developmental toxicant especially in the testes.. Metabolomic biomarkers were therefore investigated in Sprague Dawley rats (10 heads/ dose) treated orally with DBP (0, 0.5, 5 mg/kg) for 1 month and testes samples were collected at the end of experiment. Sperm count was significantly decreased in the testes of animals treated with DBP treatment. In addition, analysis by NMR spectroscopy showed that levels of 2-aminoadipate, 2-hydroxybutyrate, glycine, isoleucine, malate, O-phosphocholine, and threonine were elevated, whereas 1,6-anhydro- -D-glucose, alanine, choline, citrulline, creatinine, cysteine, glycerol, N-acetylaspartate, trigonelline, tyrosine, and sn-glycero-3-phosphocholine were decreased in a dose-dependent manner in DBP treated animal testes compared to control (corn oil treatment). Of metabolites elevated, threonine (3-fold)and glycine (2.5-fold) were shown to be most affected in animal testes after 1 month treatment of 5 mg DBP/kg. Of metabolites decreased, trigonelline (6.4-fold) and cysteine (5-fold) were most affected. These data suggest that change in metabolites in the testes might be involved in the mechanism of testicular toxicity of DBP.

P2A-043
Metabolomic profiling of melamine-induced renal toxicity in Sprague-Dawley rats

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Melamine-induced renal toxicity is associated with crystal formation in the kidney by the combination of melamine and cyanuric acid. The metabolic response of intact kidney tissue to chronic intake of melamine and cyanuric acid mixtures has rarely been studied. The present study utilized (1)H-Nuclear Magnetic Resonance (NMR)-based metabolomics to investigate melamine-induced renal toxicity. Melamine (63 mg/kg) and cyanuric acid (6.3 mg/kg) were coadministered to rats via oral gavage for 30 days. The mixture of melamine and cyanuric acid (63/6.3 mg/kg) induced nephrotoxicity, as determined by increased blood urea nitrogen (BUN), uric acid, and creatinine levels as well as reduced total protein. Kidney weights were significantly increased in animals treated with the combination of melamine and cyanuric acid (63/6.3 mg/kg). Histological analysis revealed epithelial degeneration and necrotic cell death in proximal or distal tubules. Furthermore, a number of metabolites were changed in both renal medulla and cortical tissues. Among these, choline, lactate, taurine, valine, leucine and threonine levels were elevated, but creatine, glutamine, and inositol levels were reduced in cortical and medullar tissues, suggesting melamine-induced renal cell injury. These data suggest that melamine-induced metabolites may be useful biomarkers for the detection of kidney injury.

P2A-044
Functional metabolomics – Identification and characterization of biomarkers detected under moderate intensity exercise conditions

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Physical activity is an extreme physiological challenge for the human body. Performed regularly, it has notable health benefits. In our study we aimed to identify and characterize metabolite components possibly mediating the beneficial metabolic adaptation to exercise. Healthy human subjects completed two independent running studies under moderate, predominantly aerobic conditions. Plasma samples obtained prior to and immediately after running and then 3 and 24 h into the recovery phase, were analyzed by a non-targeted metabolomics approach applying liquid chromatography-qTOF-mass spectrometry. Dominant biomarkers were further characterized in a cell culture model and mouse tissue. Acylcarnitines were found to be the most discriminant plasma biomarkers of moderately intense exercise. Immediately after a 60 min (at 93 % velocity of the individual anaerobic threshold; VIAT) or a 120 min run (at 70% VIAT) a pronounced, transient increase was observed. Further investigations in primary human myotubes as well as in mouse tissue revealed an unexpected, novel functional role of acylcarnitines in lipid metabolism under physiological conditions.

P2A-045

The Metabolomics of -hydroxybutyrate and Melatonin Treatment in a Porcine Model of Hemorrhagic Shock

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We have previously demonstrated improved survival rates in animals (rats and pigs) receiving \β-hydroxybutyrate/Melatonin (BHB/M) as an alternative fuel source in models of hemorrhagic shock. While this treatment improved outcomes, the metabolic basis of this effect is poorly understood. This study utilized metabolomics to evaluate the effects of BHB/M on the metabolome associated with improved survival. Methods: Pigs (n=17) were sedated, intubated, and instrumented. Hemorrhagic shock was induced by withdrawing blood to systolic blood pressure <60mmHg for 60 minutes. Nine pigs were treated with BHB/M using a 4 hour infusion (after an initial weight-based bolus over 10 min). Eight pigs were treated with 4M NaCl. All animals were then resuscitated for 20 hours. Serum samples were collected from the animals at baseline, after 60 minutes of shock, and at subsequent intervals throughout the experiment. Serum metabolomic profiles were determined by 1H-NMR spectroscopy with a CPMG pulse sequence. Results: BHB/M treatment resulted in changes in metabolite levels compared to NaCl treated animals. The most dramatic changes were seen in the following metabolites; 3-hydroxybutyrate (BHB), 2-oxovalerate, adipate, hippurate, succinate and urea. We have previously shown that increases in cytidine, s-adenosylhomocysteine, and serine are markers of mortality. In our current study, lower levels of these markers were observed in the BHB/M group during the early time points after shock. Perturbations in metabolites led to a two component PLS-DA model that showed statistically significant separation between the BHB/M and NaCl treated animals. Conclusion: Treatment with BHB/M in hemorrhagic shock resulted in very distinct profiles of metabolomic changes when compared to NaCl treated controls. These metabolic shifts are associated with markers of increased survivability.

P2A-046

A GC-MS Metabolomics Approach Exploring ESX-3 Gene Functionality in Mycobacterium Smegmatis.

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The ESAT-6 or ESX gene cluster is thought to be involved in mechanisms relating to the pathogenicity and viability of various *Mycobacterium* species, including *M. tuberculosis*. The ESX-3 gene cluster in particular is thought to be specifically involved in bacterial cell viability. Novel metabolites/metabolite profiles associated with ESX-3, were identified using a metabolomics comparison of ESX-3 knock-out *M. smegmatis* (a non-infectious model for M. tuberculosis) and an isogenic wild type group. This was done in order to determine if a possible metabolic link exists, bridging the genotypic and phenotypic characteristics associated with ESX-3 functionality. The principal component analysis (PCA), shows a clear separation between the two sample groups, due to the constant variation in the GC-MS analysed metabolite profiles of the individual samples of each group, respectively. A partial least squares discriminate analysis (PLS-DA) validation indicated 100% correct group membership prediction for all new, "unknown" samples analysed in the same manner. Subsequently, those metabolites showing comparatively the largest influence on the separation seen in both the PCA and PLS-DA analyses, were identified. On the basis of these metabolites/metabolite profiles, four distinct energy-related metabolic pathways, namely; the glyoxolate shunt, TCA cycle, urea cycle and a glutamine pathway, appeared altered in the ESX-3 knockouts, similarly to that seen during hypoxia or energy limiting studies reported previously. These observations, in addition to the reduced viability observed in ESX-3 knockout cultures, indicate that energy metabolism may be influenced by the absence of this gene cluster. This is not only the first study giving clues into ESX-3 gene functionality on a metabolic level, but is also indicative of possible changes that occurs enabling mycobacteria to survive energy limiting conditions. Additionally, this study proves the capacity of using a metabolomics approach for identifying the underlying metabolic process behind the phenotypic characteristics, due to genotypic alterations or abnormalities in mycobacteria.

P2A-047

Characterisation of hESC conditioning media by 1H-NMR-based metabonomics

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Human foreskin fibroblasts (HFFs) excrete a complex mixture of protein and metabolic factors into defined culture media providing it with the capacity to support pluripotent hESC culture in vitro. Recent studies have investigated the protein component of conditioned culture media (CM), yet detailed information regarding the metabolic component of this media is lacking. Moreover, there is currently no way of determining prior to culture, the functionality of CM batches. Using a 1H-Nuclear Magnetic Resonance (1H-NMR) metabonomics approach, 32 metabolites were identified and quantified in CM representing excretion and utilisation of metabolic factors. Higher concentrations of lactate, alanine, and formate were detected in CM whilst levels of tryptophan, folate and niacinamide were depleted in CM. Principal Components Analysis of the 1H-NMR data revealed marked age-related differences in the metabolic profile of CM collected from HFFs every 24 h over 5 days. Also, differences in the metabolic profile of CM could be observed following freeze storage at -20°C for 2 weeks. Conditioned media derived from passage 18 HFFs (CMp18) was incapable of maintaining hESCs in an undifferentiated state beyond 5 days of culture. CMp18 contained lower concentrations of lactate and ethanol and a more basic pH as indicated by a consistent shift in the 1H-NMR spectra of HEPES. In conclusion, 1H-NMR-based metabonomics assessment of CM enables the rapid and accurate characterisation of metabolic components present in the media and the selection of preparations most suitable for maintaining and preserving hESCs in vitro. We envisage this approach being utilised broadly for monitoring and controlling culture media preparation and in turn, preventing the loss of valuable cells, reagents and time.

P2A-048

Method Development Strategies for the Analysis of Hydrophilic Metabolites using a Silica Hydride-Based Stationary Phase.

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A limiting factor in many metabolomics analyses is the ability to robustly chromatograph mixed classes of hydrophilic compounds. Using mass spectrometry for detection, metabolites can easily be divided into two categories: compounds analyzed in the positive ion mode and those which are analyzed in the negative ion. Amino acids and carbohydrates are analyzed in the positive ion mode with a mobile phase containing formic or acetic acid. In the negative ion mode nucleotides and organic acids are retained on the chromatographic column using ammonium acetate and ammonium formate. The single stationary phase that can provide the retention properties and selectivity to analyze a broad range of polar metabolites is silica hydride that has been modified to contain a small amount (2-3%) of carbon on the surface. Silica hydride is a fundamentally different material than ordinary silica because it is somewhat hydrophobic so it has a relatively small amount of water on the surface. This property results in good efficiency using high amounts of organic constituent in the mobile phase, controlled selectivity for many types of metabolite analyses and rapid equilibration when using gradient elution methods. Because the mechanism of retention and other features of the hydride material are different than hydrophilic interaction liquid chromatography (HILIC), this approach to the analysis of polar compounds is referred to as aqueous normal phase (ANP) chromatography. Various examples of ANP chromatography will be presented to illustrate the versatile nature of the silica hydride stationary phase. This chromatographic information will also demonstrate how method development strategies are formulated for various classes of metabolites. In addition to methods utilizing mass spectrometry for detection, a number of challenging metabolic problems can be solved with UV detection. Many methods are applicable to either MS or UV detection because of the mobile phases developed for use on silica hydride stationary phases.

P2A-049
Comparative Metabolomics of Medicago truncatula roots and shoots under salt stress and recovery

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Medicago truncatula is a very important model plant for the analysis of plant-microbe symbiosis, since it forms nodules with nitrogen fixing bacteria. Experimentally obtained metabolite data lead to differential stress response effects in the metabolism of the plants strongly related to nitrogen nutrition. The results indicated a significant difference on the Metabolites of symbiotically N fixing compared to N fertilized plants. Medicago is known to be relatively salt tolerant. A comparative Metabolomics analysis has been carried out using GC-MS in order to test whether a similar stress alleviation effect may be also found under high salt concentrations. Here, first results of a time dependent analysis of roots and shoots will be presented. Multivariate data analysis revealed differences in N metabolism between control and plants treated with salt for about one week; between N-fixating and N-assimilating plants and also after two days of recovery. Comparison between salt and drought treatment is still in progress.

P2A-050
The application of metabolomics to an animal model of type 1 diabetes to identify biomarkers of pre-symptomatic disease and treatment efficacy

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Type 1 diabetes arises as a result of autoimmune destruction of the pancreatic beta cells. It is known that the autoreactive destruction of the beta cells may have been occurring for a number of years before any clinical symptoms become obvious [1]. It would therefore be advantageous to be able to fully identify susceptible individuals and intervene before irrevocable beta cell destruction occurs. We are using the established non-obese diabetic (NOD) mouse model of type 1 diabetes [2] to identify biomarkers of underlying pathological processes at the pre-symptomatic stage of the disease. The aim of which is to determine whether the biomarkers we identify provide accurate markers of predisposition to develop diabetes and whether they can be used to monitor the success of therapeutic intervention. This would represent a considerable advance in the potential treatment of type 1 diabetes. Pancreas, kidney, liver and plasma samples were taken from female NOD mice at 4-6, 11-12, and 20 weeks of age. These age ranges, respectively, represent animals which have not developed diabetes, those in a pre-diabetic state, and those which have the full symptoms of glucosuria. Samples were also taken from NODE mice of similar age which, despite being genetically similar, do not go on to develop type I diabetes. We report our initial findings from the analysis of aqueous metabolites by high resolution 1H Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography – Mass Spectrometry (GC-MS) and in particular perturbations in amino acid metabolism in the NOD mice prior to overt diabetes. 1. Atkinson, M.A., Maclaren, N.K. (1994) N. Engl. J. Med. 331:1428-1436 2. Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K., Tochino, Y. (1980) Jikken Dobutsu 29 (1): 1–13

P2A-051
Quantitative metabolomic profiling of serum and urine in DSS-treated mice by proton NMR spectroscopy

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Metabolic profiling of a large number of metabolites whose concentration and composition are influenced by genetic, immunologic and environmental factors may be a promising method to detect biomarkers for inflammatory bowel diseases (IBD). In order to distinguish between mice suffering from an experimental form of IBD and healthy animals, we measured and characterized serum vs. urine metabolites by 1H-NMR spectroscopy and targeted profiling analysis. Experimental IBD was induced in CD1 mice by 4% dextran sulfate sodium (DSS) supplied in the drinking water for 8 days. The body weight was checked daily and fresh void 24h-urine was sampled on day 1, 4, 6 and 8. In addition, serum was collected at the end of experiment. 69 compounds in urine and 53 compounds in serum were quantitatively identified using Chenomx software. DSS treatment resulted in severe colitis, as determined by weight loss together with a significant increase in the macroscopic damage score and myeloperoxidase activity. Multivariate Orthogonal Partial Least Squares analysis (OPLS) was employed to detect and predict separation of the treatment groups. Based on the loss of their body weights, OPLS data from both urine and serum demonstrated a metabolic pattern that clearly separated DSS-treated from control mice with a slightly higher predictive power (Q2) for serum (Q2=0.73) than urine (Q2=0.71). Combined assessment of serum and urine vs. urine or serum alone provided for a slightly higher predictive power (Q2=0.76). In serum from DSS-treated mice, strongest increases were measured for acetoacetate, hypoxanthine and tryptophan while glucose, Krebs cycle intermediates, and several amino acids were decreased. In urine samples from DSS-treated mice, creatine, allantoine and trimethylamine N-oxide significantly increased whereas concentrations for nicotinamide, methionine and urea were lower. Metabolomic profiling of serum and urine therefore clearly discriminates between mice with active experimental colitis and healthy controls. In addition, analysis of serum or urine seems to be equally powerful to detect and distinguish colon inflammation.

P2A-052
Clustering Analyses and Metabolomics in the Diagnosis of Diseases.

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Nuclear magnetic resonance (NMR) spectroscopy has emerged as a technology that can provide metabolite information within organ systems in vivo. Conventional statistical tools are not designed to handle the complexity and volume of data generated by today's high resolution NMR spectroscopy, and new approaches that can accomplish that task with speed and accuracy are required. In this study, we introduced the novel concept of employing a clustering method to render a differential diagnosis in a given disease. We developed a mathematical approach that rendered the aforementioned novel concept a reality, and we devised three tests to assess the suitability and the accuracy required for diagnostic purposes of the four clustering methods we investigated (K-means, Fuzzy, Hierarchical, and Medoid Partitioning). To accomplish this goal, we studied the striatal metabolomic profile of R6/2 Huntington disease (HD) transgenic mice, as well as that of wild type (WT) mice, using high field in vivo proton NMR spectroscopy (9.4 Tesla). We tested all four clustering methods 1) with the original R6/2 HD mice and WT mice, 2) with unknown mice, whose status had been determined via genotyping, and 3) with the ability to separate the original R6/2 mice into the two age subgroups (8 and 12 wks old). Unsupervised Fuzzy and ROC-supervised Fuzzy and K-means clustering passed all three stringent tests with a total accuracy of 100% [Positive Likelihood Ratio approximating infinity ($1/0 \longrightarrow \infty$) and Negative Likelihood Ratio equal to zero ($0/1 = 0$)], proving that they may be used for diagnostic purposes.

P2A-053

Discovery and validation of plasma markers of liver fat content: Metabolomics approach

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Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in affluent societies, representing the hepatic metabolic consequence. It is in many cases is linked to obesity or overweight. At present, there is a lack of accurate and sensitive diagnostic tests for NALFD that do not involve invasive procedures. For the development of diagnostic tools for NALFD, information on the metabolic profiles associated with the NALFD is important. In this study, potential biomarkers for the prediction of liver fat content in NAFLD were studied using two analytical platforms, covering large range of lipids and polar metabolites. Sample set included over 900 plasma samples from ~800 individuals, which were divided into discovery and validation groups. Liver fat content was measured using proton magnetic resonance spectroscopy or histology. In addition, detailed clinical information was available for all subjects. For determination of molecular lipids, ultra high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-QTOFMS) was applied. For further identification of unknown lipids, fractions collected from UPLC run were infused to a LTQ-Orbitrap mass spectrometer by a TriVersa Nanomate using chip-based nanoelectrospray in positive and negative ionisation mode. Identifications were based on the exact mass and MS2 and MS3 spectra. For the determination of polar metabolites, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS) was used. Statistical analyses were performed using a freely available R package. In the discovery series of samples several lipids, including many triacylglycerols, and several amino acids were found to significantly correlate with liver fat content. On the contrary a strong negative correlation was observed with certain phospholipids. Using these marker lipids in addition to the routinely available clinical and laboratory data (Liver Fat Score developed by Kotronen et al.) may increase the accuracy of the prediction of liver fat content in NAFLD. The validation series of samples will be used to confirm these results.

P2A-054

Evaluation and identification of markers of damage in mushrooms (Agaricus bisporus) using a GC/MS metabolomics approach

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The major cause of quality loss that accounts for the reduction in market value of mushrooms is browning. Mushrooms are very susceptible to tissue damage and bruising during handling, storage and transport due to their soft pericarp and lack of protective tissues. Damage triggers the browning process within mushroom tissues changing the metabolic state of the mushroom. GC/MS is a sensitive analytical technique that allows simultaneous analysis of metabolites in complex extracts. This technique was introduced into plant research for metabolic profiling purposes in the nineties in, for example apricots and potato tubers . The objective of this study was to use GC/MS and chemometric tools to profile undamaged and damaged mushrooms and to identify metabolic markers of damage. Mushrooms were grown in controlled conditions and transported to the laboratory in specially designed trays to avoid contact between (a) mushrooms and (b) between the top of mushroom caps and the tray. A set of 120 closed cap, defect-free mushrooms were selected for this study. Three levels of damage were chosen: undamaged (UD), damaged for 20 minutes (D20) @ 300 rpm on a shaker table, damaged for 40 minutes (D40) @ 300 rpm and analysed on day zero (D0) and day one (D1). Twenty mushrooms were selected for each day and damage level. Mushrooms were divided into their tissues; cap, gills and stalk and frozen at -70ºC in a cryogenic fridge. Once frozen an extraction, fractionation and derivatisation was carried out and samples were injected into the GC/MS. A mass spectral library was built with over 100 metabolites including sugar and polyalcohols, fatty acid metabolites, phenolic compounds and amino acids for spectral comparison and metabolite identification. Random forest modelling was used to predict damage with an out-of-bag (OOB) error rate of 8.3%. Metabolites identified by the model as important variables for predicting damage (markers) were 7,10-octadecadienoic acid, Inositol, pentadecanoic acid, benzoic acid and nonanoic acid.

P2A-055

Metabolome in schizophrenia and related psychotic disorders: Findings from the general population cohort

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Persons with schizophrenia and other psychotic disorders have high prevalence of obesity, impaired glucose tolerance, and lipid abnormalities, particularly hypertriglyceridemia and low HDL [1]. More detailed molecular information on the metabolic abnormalities may reveal clues about the pathophysiology of these changes, as well as about the disease specificity. From a population-based study [2], we analyzed serum samples from all persons with DSM-IV primary psychotic disorder (schizophrenia n=45, other nonaffective psychosis (ONAP) n=57, affective psychosis n=37) and controls matched by age, sex, and region of residence. We applied lipidomics using UPLC/MS and metabolomics using GCxGC-TOFMS [3]. A total of 360 molecular lipids and 201 metabolites were measured. Bayesian model based clustering [4] was performed to reduce the data into a subset of 13 lipid and 8 metabolite clusters, respectively. We used linear mixed models to analyze the effect of diagnosis on metabolic cluster variables after adjusting for antipsychotic medication use, nutritional variables, smoking, obesity, waist circumference, and type 2 diabetes. The effect of schizophrenia was independently associated with 4 metabolite and 5 lipid clusters, which e.g. included insulinotropic metabolites and saturated triacylglycerols. These metabolic abnormalities were much less pronounced in persons with ONAP, and persons with affective psychosis did not differ from their matched controls. The schizophrenia-associated clusters strongly correlated with gamma-glutamyl transferase values, despite significantly lower alcohol consumption as compared to controls. Our findings suggest that specific lipid abnormalities related to saturated triglycerides are specifically associated with schizophrenia. These affected lipids are known to be enriched in VLDL particles [5], thus VLDL secretion and the amount of liver fat may play a role in schizophrenia.
[1] Suvisaari JM, et al. J Clin Psychiatry 2007; 68:1045-55. [2] Perälä J, et al. Arch Gen Psychiatry 2007; 64:19-28. [3] Oresic M, et al. J Exp Med. 2008;205(13): 2975-84. [4] Fraley C and Raftery AE. J. Classif. 2003; 20: 263-286. [5] Kotronen A, et al. Diabetologia. 2009 Apr;52(4):684-90.

P2A-056

Development of UHPLC- Ultra High Resolution TOF MS based workflow for evaluation of the treatment effectiveness in Urinary Tract Infection.

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Detected in primary care as a mild illness Urinary Tract Infection (UTI) may rapidly develop into a life-threatening condition of septic shock and multiple organ failure. Given the spectrum of clinical presentation, disease severity and outcome, this clinically well-recognizable disease represents a good model for development of an analytically based scoring system of disease severity or/and evaluation of a treatment effectiveness. Here, we present an analytical workflow for evaluation of a treatment strategy based on combination of high performance liquid chromatography, mass spectrometry and multivariate data analysis. This project is a part of a large multidisciplinary clinical study designed to evaluate the efficacy of novel antibiotic treatment strategy and to develop an analytical scoring system for the prediction of a treatment outcome. For the workflow development, we randomly selected 40 patients with urine culture confirmed E. Coli febrile UTI from a study cohort of 642 consecutive patients presenting with febrile UTI at primary care or emergency department. Baseline urine sample (day of enrolment) and sample collected 30 days after the initiation (T30) of antibiotic treatment were used for this project. In addition, a carefully matched group of controls was included. The analytical core of the workflow is a combination of ultra high performance liquid chromatography (Dionex, Ultimate 3000 RS tandem LC system) and time of flight mass spectrometry (UHR-TOF maXis, Bruker Daltonics). Using a randomized acquisition scheme with build-in series of quality controls, we demonstrate the robustness of our workflow and ability to provide data quality required for such a study. In addition, evaluation of data consistency using Principal Component Analysis (PCA) has shown that analytical variance was significantly lower than variance associated with biological/ clinical nature of the samples. Consequently, using a class based modelling we have demonstrated that two-class model based on patients at baseline and matched control group can be used for an evaluation of treatment response of patients at T30. A practical implications and possibility to improve the quality of prediction using a cross-platform analysis (including NMR or/and GC-MS data) and more advanced ways of data analysis are discussed.

P2A-057

Predicting idiopathic toxicity of the anticancer agent cisplatin with a pharmacometabolomic approach

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Cisplatin has been one of the most widely-used anticancer agents for decades, but its nephrotoxicity remains a dose-limiting complication. Here, we studied the idiopathic nature and the pre-dose prediction of cisplatin-induced nephrotoxicity with an NMR-based pharmacometabolomic approach. Cisplatin (10 mg/kg) produced serious toxic responses to some animals (T-group), as judged by hematologic and histologic results, but had little effect in others (NT-group). The individual metabolic profiles, assessed by urine NMR spectra, showed large differences between the post-administration profiles of the two groups, indicating the relevance of the NMR approach. Importantly, multivariate analysis of the NMR data showed that the N- and NT- groups can be differentiated based on the pre-administration metabolite profiles. By NMR structural analysis, we found that allantoin, succinate, creatinine, and oxoglutarate had a biased distribution between the two groups. Leave-one-out analysis, performed to evaluate the practical performance of our approach, gave 70% sensitivity and 60% specificity in predicting the toxic responses with pre-administration metabolite profiles. We also present a working model that can explain the idiopathic toxic responses based on metabolic differences. This pharmacometabolomic approach using pre-administration metabolite profiles may help expedite personalized chemotherapy of anticancer drugs.

P2A-058

Analysis of Hydrophilic Metabolites in Physiological Fluids by LCMS using a Silica Hydride-Based Stationary Phase

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Aqueous normal-phase chromatography is used for the analysis of metabolites in human saliva, urine and red blood cell extracts. The HPLC column contains a separation material composed of silica having a hydride surface further modified with a minimal amount of a hydrophobic moiety. Gradients are evaluated with different mobile phase additives to produce retention for amino acids, small organic acids, carbohydrates and nucleotides. A single mobile phase composition suitable for all hydrophilic metabolites was also investigated. The premise is that a mobile phase containing an additive with a pH above the pKa of acidic metabolites is needed to retain the compounds in their ionized form. This additive must be removed and the mobile phase switched to a more acidic pH to elute amino acids and carbohydrates. One option uses ammonium acetate as the additive to produce a pH sufficiently high to retain the acidic metabolites. A pH gradient is created with a lower concentration of ammonium acetate in acetonitrile followed by DI water to remove the additive. The final solvent consists of either DI water with formic acid or methanol/water with formic acid to lower the pH further to elute strongly retained compounds. Detection is done by time-of-flight (TOF) mass spectrometry. Among the examples to be presented are: the relative concentration levels of various metabolites in human saliva for normal patients and patients with pancreatic cancer or pancreatitis; and a clinical method for detecting the metabolic disease glycosemia based on the analysis of UDP-glucose and UDP galactose in red blood cell extracts. The reproducibility of retention of individual metabolites in these complex matrices for a broad range of compounds is usually 0.5% or less. Column re-equilibration after gradients occurs in less than five minutes. Column lifetimes are at least several hundred injections and can often be regenerated when contaminated by components from physiological fluids.

P2A-059

Fission yeast metabolites implicated in stress response, nutrient starvation and longevity

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The fission yeast *Schizosaccharomyces pombe* is an excellent model organism for the study of important cell biological problems, resembling higher eukaryotes in many aspects. While transcriptomic analyses have been extensively conducted in this organism, knowledge on metabolic compounds is limited. To understand cell division, quiescence and longevity through the changes in metabolic regulations, we recently performed the first global semi-quantitative analysis of *S. pombe* metabolome using liquid chromatography high-resolution mass spectrometry (Pluskal et al., 2010). The procedures to obtain metabolic compounds (MW 100-1000 Da) from *S. pombe* extracts were established and one hundred and seventeen metabolites were identified. In order to perform detailed analyses of temperature sensitive mutants, the effect of temperature stress was thoroughly investigated. We demonstrated the great benefit of combined genetic and metabolomic approach. Good reproducibility of metabolome data was exemplified by the fact that the deletion mutant of ferrichrome synthetase sib1 showed no significant change except the disappearance of ferrichrome. However, secondary effects prevailed in other mutants. Results using hcs1 mutant defective in the HMG-CoA synthase confirmed the expected decrease of HMG-CoA. In addition, complex metabolic changes that included urea cycle intermediates and acetylated compounds were observed. Proteasome mutant mts3, which suffered from oxidative stress generated by mitochondria, strongly accumulated two prominent antioxidative metabolites, glutathione and ergothioneine, in order to support the longevity of quiescent cells (Takeda et al., 2010). Cells cultured in standard laboratory media are usually supplied with abundant nutrients, however such excess nutrient conditions rarely occur in nature. We are studying the starvation effect of nitrogen source and glucose (carbon and energy source) regarding the longevity of cells. We attempt to identify biomarkers for starving and fasting conditions, which may be critical for cellular viability and longevity.
Pluskal T., et al. Molecular BioSystems 2010, 6(1):182-98
Takeda K., et al. PNAS 2010, 107(8):3540-5

P2A-060

Introducing a High Throughput Metabolomics Platform at the Helmholtz Zentrum München

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Metabolomics is a very fast expanding research field for phenotyping of biological samples with an unbiased approach of characterisation. Especially, either not manifested phenotypes or subsidiary phenotypes could be determined if many different parameters are correlated. At present, two main approaches in metabolomics are performed: targeted (quantification of a chosen set of metabolites) and non targeted (profiling or search for biomarkers). The Metabolomic Platform (metaP) of the Helmholtz Zentrum München is designed to mediate progress in science through development of new metabolomic methods and provision of measurement services applicable to man, animal models, plants, environmental samples and ex vivo systems. Part of our activities is related to targeted metabolomics. The quantification is based on the Biocrates Kit AbsoluteIDQ and own developments for mass spectrometry and covers more than 150 endogenous metabolites like lipids, amino acids, acylcarnitines, carbohydrates and steroids. For this, minimal amounts of plasma are needed. The measurements perform very well with high reproducibility. We successfully performed studies in the population based human KORA (Coop. Health Research in the Region of Augsburg) cohort and in animal models in elucidating metabolomic effects in complex diseases or drug development, respectively. To facilitate high quality standards and sample tracking we build up a tailor made LIMS. We recently established MassTRIX service (http://masstrix.org) identifying chemical compounds from mass spectrometry analyses in their genomic context on KEGG pathway maps. The evaluation of data is done on metaP-server which provides automated and standardized data analysis for quantitative metabolomics data freely accessible at http://metabolomics.helmholtz-muenchen.de/metap2/. The processivity of the Metabolomic Platform is reached by integration of different expertise at the campus of the Helmholtz Zentrum München. At present, in metaP cooperate the following groups: robotics for sample preparation and analyte quantification (LC-MS/MS 4000 QTrap; J. Adamski), high resolution analysis and profiling (FT-ICR-MS; Ph. Schmitt-Kopplin), biobanking (Th. Illig) and bioinformatics/project integration (K. Suhre).

P2A-061

A High Throughput Protocol for Metabolomic Analysis of Tissue Samples

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While biological processes in higher organisms mainly take place in specialized cells and whole tissues, the established matrices for metabolomic studies, like blood, urine, or saliva, are typically of extracellular type. Metabolite concentrations from body fluids therefore reflect the result of various simultaneously occurring processes over different places and cell types in a given organism. Hence, accurate and reproducible quantification methods for metabolites in tissue samples are of high importance to identify metabolic changes that occur in different tissue types. Especially, animal models for specific diseases could be characterized more intrinsically, yet the preparation of metabolite extracts from tissue samples is often a critical and very labour intensive step. We developed a high-throughput method for parallel extraction of metabolites from multiple tissue samples. The method utilizes a homogenizer with ceramic beads in individual disposable tubes in combination with a simple extraction protocol and the AbsoluteIDQ™ Kit. The AbsoluteIDQ™ kit was originally validated for human plasma and can simultaneously quantify 163 endogenous metabolites by flow injection analysis mass spectrometry (FIA-MS). The metabolite spectrum of the kit covers molecules with significant different lipophilic and hydrophilic properties, including acylcarnitines, amino acids, hexose, glycerophospholipids, and sphingolipids. Thus, different extraction solvents were tested, and reproducibility as well as suppression effects were evaluated for several different animal tissue types including liver, kidney, muscle, brain, and fat tissue.

P2A-062

A new approach to metabolite profiling using tandem liquid chromatography.

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A tandem liquid chromatography (LC) setup has been developed to analyze a larger subset of molecules in a complex sample from a single analysis by combining multiple chromatographic chemistries. We have coupled reverse phase (RP) chromatography (Zorbax Eclipse XDB-C18, Agilent) with stationary phases that resolve hydrophilic compounds, including aqueous normal phase (ANP) chromatography (Cogent Diamond Hydride, MicroSolv Technology). The setup allows sequential separation of hydrophobic and hydrophilic compounds on respective, complementary stationary and mobile phases. In a metabolite profiling analysis of a urine extract, approximately 900 and 600 molecular features can be detected on RP and ANP columns, respectively. The Tandem-LC setup presented here is a unique configuration that allows detection of approximately 1500 resolved molecular features with both hydrophilic and hydrophobic properties from a single injection. The Tandem-LC setup can be used to resolve many complex samples, including molecules extracted from plant material, animal tissue, culture fluid and single cellular organisms. The Tandem-LC configuration is robust, relatively simple to setup and greatly reduces instrument and analyst time, even more so when coupled with a fast-polarity switching MS.

P2A-063

Metabolomics defines a bio-signature for respiratory chain disorders

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The respiratory chain (RC) comprises of a system for electron transport from reducing cofactors (NADH and FADH2) to molecular oxygen to generate ATP. RC defects (RCD) do not induce the accumulation of specific metabolites and thus does not generate metabolites with a diagnostic significance. Clinical procedures are consequently mainly used for diagnosis of RCD. Insight into the metabolic consequences of the RCD is thus important for a better understanding of the pathogenesis and for further development of guidelines for diagnosis of RCD's. We here report on an untargeted GC-MS metabolomics study of urinary organic acids, regarded as secondary indicators of RCD, from samples coming from a cohort of patients with confirmed enzyme defects in the RC (Smuts et al 2010). The organic acids were isolated, separated and identified (AMDIS) using conventional analytic techniques. Our main findings were: (1) The original data matrix consisted of 420 variables (mmol organic acid/mol creatinine) from 78 controls and 54 cases. We introduced the concept of a bio-filter and some statistical methods to reduce the applicable variables to 65. (2) Using the Mahalanobis distance measure, we eliminated 14 outliers from the control group, and the enzyme profile and a defined cut-off point to reduce the cases to 26. (3) Using the standard multivariate techniques (PCA and PLS-DA) and univariate analysis (effect size, t-tests and Mann Whitney), a list of 12 biomarkers could be defined for RCD. (4) The biomarkers originated from the Krebs cycle, β-oxidation and some minor metabolic pathways, apparently due to increased NADH and FADH2. (5) The combined group of 12 biomarkers, designated as a bio-signature, were validated as a possible predictive instrument for RC deficiencies. The significance of this investigation is that this metabolomics analysis defined a bio-signature that may pave the way for an alternative approach for screening for the complex RCD's encountered in paediatric medicine. Smuts et al, Journal of Inherited Metabolic Diseases, DOI 10.1007/s10545-009-9031-8 (2010)

P2A-064

Characterizing the Metabolic Changes in Response to Radiation by UPLC-QTOF

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We utilized the UPLC-QTOF to understand the metabolic level differential response to radiation exposure in two isogenic cell lines (AT5BIVA and ATCL8). The AT5BIVA cells were derived from a patient with ataxia telangiectasia (A-T) exhibiting extreme radiosensitivity. ATCL8 contains an introduced A-T mutated (ATM) gene. We investigated the metabolic changes in these cell lines in response to radiation exposure, owing to the differential expression status of ATM. Shrinkage t statistic and orthogonal partial least squares-discriminant analysis (OPLS-DA) methods were utilized to select ions whose signal intensities were significantly altered by radiation. This is accomplished through pair-wise comparison of radiation treated cell lines (at 30min, 1hr, 2hrs, 3hrs, 6hrs, and 24hrs following radiation) against the untreated cell lines (time zero). Each time point was represented by five replicates, thus a total of 35 metabolomic profiles were acquired by UPLC-QTOF. The metabolites represented by the ions selected through the Shrinkage t statistic and OPLS-DA methods were identified using databases such as Metlin, MMCD, and HMDB. We verified some of the metabolites by comparing their MS/MS fragmentation pattern with standard compounds. The results of our pathway analysis indicate an ATM regulated induction of major pathways in response to radiation treatment. A number of metabolites involved in purine metabolism were changed due to radiation. This is consistent with our previous proteomics analysis where we found enzymes involved with purine metabolism. We observed that ATCL8 showed more enrichment of metabolites involved in purine metabolism than AT5BIVA. While ATCL8 showed significant enrichment of metabolites involved in purine metabolism, linoleic acid metabolism, pentose and glucurronate interconversions, fructose and mannose metabolism, etc., AT5BIVA showed a predominance of glycerophospholipd metabolism and phospholipid degradation. Taken together, these results show that the presence of ATM in the ATCL8 cells elicits a normal radiation response leading to inhibition of cell growth and proliferation and increased DNA repair. Future mechanistic studies will be needed in order to correlate the role of these pathways with respect to ATM functionality.

P2A-065

Multi-Stage Elemental Formula Tool for Metabolite Identification

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Because of its high sensitivity and specificity, mass spectrometry is widely and successfully applied in metabolomics studies for the analysis of all types of different biological samples. For biological interpretation, the identity of the metabolites have to be known. However, still there are many metabolites detected are not identified, which is one major bottleneck in metabolomics. For metabolites detected with LC-MS, high resolution multi-stage mass spectrometry (MSn) is used for the identification of metabolites because it provides a feature rich fragmentation fingerprint of the precursor ion. However, neither general methodology for the identification nor extensive databases of metabolites with MSn data are available at the moment. To obtain proper matches for non-identified compounds a database containing robust, high quality and standardized MSn data from known metabolites is needed. For this reason, we created a database composed of fragmentation trees from most metabolite standards listed in the HMDB (Human Metabolites DataBase). To be able to process the MSn data and to store the relevant mass spectral information in the database, the in-house developed Multi-stage Elemental Formula (MEF) tool is used. Additionally the MEF tool assigns unique elemental compositions to the parent ion and its fragment ions, and removes artefacts. The MSn database model that stores this information is making use of the HDF (Hierarchical Data Format) format. Here we will show the viability of using MSn data to identify metabolites stored in the MSn database.

P2A-066

Discriminating inflammation-specific urinary biomarkers from other transgenic metabolic differences in a gene-knockout mouse model of Crohn's disease

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The interleukin-10-gene-deficient (IL10-/-) mouse develops intestinal inflammation in the presence of gut microflora and is useful as a model for Crohn's disease, an inflammatory bowel disease. After a microbial challenge, urine from IL10-/- and wildtype mice was analyzed by GCMS and Fast LCMS. GCMS identified a number of metabolites that were consistently different between IL10 and wildtype mice, including xanthurenic acid, fucose, and glutaric, 2-hydroxyglutaric acid and 2-hydroxyadipic acids. Fast-LCMS identified a number of other biomarker candidate including the glucuronides of xanthurenic acid and alpha-CEHC (2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman). The significance of these metabolic differences was evaluated in an experiment which compared urinary metabolites in IL10-/- and wildtype mice housed either under conventional conditions and dosed with intestinal microflora (control), or maintained under specific pathogen free (SPF) conditions without microbial dosing. Our rationale was that inflammation, and inflammation specific biomarkers, should be reduced in IL10-/- mice under SPF conditions while metabolic differences arising from other genetic differences between the mouse strains or as a consequence of microbial dosing should not be similarly affected. The use of SPF conditions as a treatment to distinguish between inflammation-specific and other types of metabolic differences between IL10-/- and wildtype mice will be described.

P2A-067

HR-MAS proton NMR based metabolomic analysis of breast cancer tissues highlight the importance of choline metabolites in tumour grading

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Breast cancer is the most common cancer in women where approximately one in eight women in the Western world will develop an invasive breast carcinoma in their lifetime. While the disease is curable in the early stages, about 50% of the patients have stage II or III tumours requiring potential systemic therapy and are in need of better biomarkers for diagnosis. The aim of the METAcancer project (www.metacancer-fp7.eu - an EU FP7 funded project) is to investigate metabolites that can be used as prognostic and predictive biomarkers by applying different metabolic profiling technologies (i.e. NMR, GC-MS and LC-MS) to maximize the coverage of the breast cancer metabolome. We have used high resolution solution 1H NMR and solid state Magic Angle Spinning (HRMAS) NMR spectroscopy to analyse blood plasma (~350) and tumour tissue (~450 samples). Spectra were acquired using a Bruker ADVANCE III spectrometer interfaced with 11.7 Tesla/500 MHz superconducting magnet using a 4 mm HRMAS 1H-13C NMR probe with a z-gradient at room temperature. For each tumour spectra were acquired using the NOESYPR1D, a solvent suppression pulse sequence and CPMG pulse sequences. Multivariate pattern recognition techniques using SIMCA-P+ 12.0 (Umetrics AB) and statistical total correlation spectroscopy (STOCSY) were used to analyse the NMR data set. Proton HRMAS NMR spectroscopy as a tool for investigating tumour biology has the advantage of measuring both aqueous and lipophilic metabolites simultaneously as both components are affected by tumour aggressiveness, malignancy and cell death. HRMAS 1H NMR spectroscopy proved to be highly discriminatory between tumours and healthy breast tissue as well as to discriminate between grade II and grade III tumours. Lipids, choline, phosphocholine and glycerophosphocholine were among the metabolites shown to be highly discriminatory. The analysis of blood plasma from cancer patients is currently under investigation.

P2A-068

A metabonomics evaluation of breast cancer patients with and without tamoxifen therapy

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The heterogeneity of breast cancer and responses to tamoxifen achieved long term metastasis-free survival in only 1/3 of patients. Parameters which accurately predict clinical manifestation in individual patients are required in personalising therapy. Objective: Profiling different metabolite expression levels in patients treated with tamoxifen and identification of potential markers for therapeutics monitoring. Methods: Plasma samples were obtained from breast cancer patients and controls. Technical triplicates of patients and controls were analysed using LCMS. Samples were injected after protein precipitation with acetonitrile and centrifuged for 10 min at 10,000×rpm at 4°C. The chromatography was performed on an Agilent 1200 Series using an XDB C18 4.6 μM, 150×1.8 mm. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The flow rate was 0.25 mL/min and the injection volume was 5 μL. A linear gradient with the following proportions (v/v) of phase B (5% to 95% for 18 min; hold 95% for 5 minutes and then equilibration to 5% again for 7 minutes) was used. The HPLC system was coupled to Q-TOF 6520 (Agilent Technologies). The MS acquisition was performed in positive ionization mode. Statistical analysis was performed using Mass Profiler Professional (MPP) and Metlin Personal. Combined partial least square discriminant analysis (PLSDA) and hierarchical clustering with Bonferroni correction was used to extract differential expression. Results/ Discussion: The PLS-DA analysis showed patterns of metabolites increased in patients treated with tamoxifen compared to patients before therapy and healthy controls. The initial results provide cues of the effect of tamoxifen on the endogenous biological system. Some of these metabolites identified could be useful markers to monitor patient's response to therapy. Conclusion: This report shows that MPP and Metlin Personal Database provide an excellent platform for scientists to unravel the complex relations between drugs application and their expected effects on biological systems. Keyword: Tamoxifen, PLS-DA analysis, breast cancer patients

P2A-069

Metabolic response of intracellular protein production in microorganisms

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Metabolite profiling via GC-TOF-MS was used to identify the metabolic response of protein production across model species (Aspergillus niger, Bacillus megaterium and Escherichia coli). A model protein production system based on the green fluorescent protein (Gfp) was established for B. megaterium and E. coli with an inducible promoter. The promotor construct was systematically optimised and results in different expressed protein levels. Complementary in A. niger a constitutively overexpressed intracellular sucrase strain was compared against control strain without enhanced protein production. The analysis of the three biological systems results in individual metabolic fingerprints related to an increased protein production, however equal metabolic pattern are determinable. Basically, changes in the metabolic profile of the overexpressing strains involve mainly intermediates from TCA cycle and amino acid biosynthesis. Several amino acids (glutamine, alanine, proline, ornithine) are up regulated and directly linked to TCA intermediates (fumarat, malat, succinat and 2-Ketoglutarat). In opposite some amino acids show no changes in response to the enhanced intermediate flow through the TCA cycle, whereby individual specific amino acids are down regulated and indicate a bottle neck in the protein production system. The same metabolic response for all investigated microorganisms was manifest for ornithine and malat. Increased ornithine concentrations indicate enhanced amino acid turn over in the urea cycle, which is directly associated to fumarat. Decreased Malat concentrations indicate changed redox and energy status. In summary, our study with three different biological systems determines that protein production induces a ubiquitous metabolic pattern in microorganisms, whereby individual adaptations to specific expressed proteins are realised.

P2A-070

Combined LC-MS and GC-MS approach to exploring hydrophilic metabolites in Mycobacterium tuberculosis infected guinea pig lung reveals substantial up regulation of kynurenine pathway metabolites

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The World Health Organization (WHO) estimates that one third of the human population is infected with Mycobacterium tuberculosis; in 2007, 1.7 million deaths and more than 9 million new cases were attributed to the disease. Very little information is available on metabolome wide changes that occur in infectious disease, and virtually no information is available regarding metabolome changes in the host upon M. tuberculosis infection. In the present study we explored changes in small hydrophilic metabolites in the guinea pig lung upon infection. Methods: For this study we examined several tissue types including granuloma (host immune response to M. tuberculosis), uninvolved (non-granuloma tissue), and uninfected controls. After tissue pulverization a two phase liquid extraction technique was used to obtain lipid and water soluble fractions. Non-targeted metabolomic investigations on the water soluble fractions were done sequentially; first using a QTOF coupled HILIC mode LC-MS/MS system operated in the ESI+ mode then after methoximation and silylation, samples were analyzed by GC-MS with electron impact ionization. Data files were then converted from either Agilent (.d) or Varian (.XMS) to .mzData format and XCMS was used to identify and align features between samples, and generate CSV tables with statistical, fold change, retention time, and mass information for each feature. Relative abundance of features between tissue types was assessed, and fold changes of \>2 with P\≤0.05 were considered significant. Preliminary data: LC-MS: Fold change analysis for granuloma vs. uninfected revealed 163 significant features, with melatonin, aniline, 2-aminomuconic acid, 3-hydroxykynurenine, and kynurenine having large fold changes. For GC-MS fold change analysis revealed massive accumulation of quinolinic acid in infected tissues. The increased tissue concentrations of these kynurenine pathway metabolites in infected tissue point to the induction of the tryptophan degrading enzyme indoleamine-2,3-dioxygenase (IDO) which has been implicated in creating a "tolerogenic state" where pathogens and cancerous tissues can seemingly be ignored by the immune system . The current study illustrates how untargeted analysis techniques can be very valuable in implicating potentially important but previously unsuspected metabolic pathways in infectious disease progression.

P2A-071

Biomarkers of Levofloxacin response by metabolic profiling of human urine with Liquid Chromatography-Mass Spectrometry

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Levofloxacin are most commonly used drug for treatment of various bacterial infections, and it was well known to ECG abnormalities, gastrointestinal and CNS toxicity adverse effect in clinical therapy. But, levofloxacin's effect on endogenous metabolism that related to drug response did not study in human urine. So, we here for effect of levofloxacin dose on urine metabolome and its potential to predict the pharmacokinetic response. When it using clinical therapy, has not information about drug response and assumed that high drug concentration in plasma was induced heighten the frequency of adverse effect, so, it need that AUC correlated endogenous metabolite biomarker. In 24 health Korean male volunteer, collected pre-dose urine sample as spot-urine and post-dose urine was collected continuously for 12-hr period after levofloxacin (200mg) administration. We analyzed all urine samples using LC-MS full scan and then subsequent data analysis using XCMS software. We have detected features representing respective m/z and retention time for metabolites. After quantile normalization, we performed principal component analysis (PCA). The principal component of the scored plot allowed separation between pre-dose urine and post-dose urine. The loading plot of metabolite variables used to find endogenous metabolite markers that change after dose. These metabolite markers after identification can be used to understand biochemical pathways that were affected by levofloxacin. Furthermore, we will investigate related drug response metabolite identification and predicted biomarker. Then we are study that urine metabolite with AUC correlation and find to potential key metabolite for PK prediction.

P2A-072

NMR metabolomics studies reveal that placental lactogens modulate glucose homeostasis during pregnancy in mice

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In mice, placental lactogens are produced by endocrine cells in the placenta. These hormones are detectable in the maternal sera of pregnant mothers from mid-gestation onwards. They act through the prolactin receptor, which is ubiquitously expressed, and they are thought to maintain the signaling potential of prolactin when pituitary expression of prolactin is depressed during the second half of pregnancy. However, placental lactogen mutant mice have not been generated and is complicated by the fact that there are 4 separate genes encoding placental lactogens. As such, it has not been clearly demonstrated that placental lactogens are necessary or sufficient to maintain rodent pregnancy to term. Our objective was to use serum NMR metabolomics to study the function(s) of placental lactogens during murine pregnancy. Pituitary prolactin and four placental lactogens act through the prolactin receptor, thus a comparison between pregnant prolactin ligand null (Prl-\/-) and prolactin receptor null (Prlr-\/-) female mice was used to reveal the function(s) of placental lactogens. Progesterone was administered to null mice in order to rescue known implantation defects in these mutants. No differences in litter size were observed near term between Prl-\/- and Prlr-\/- females. However, serum blood glucose levels were elevated in pregnant Prlr-\/- mice, but not Prl-\/- pregnant mice. Serum metabolomic studies of pregnant mice near term revealed that trimethylamine-N-oxide (TMAO) was elevated in Prlr-\/- but not Prl-\/- mice. Unexpectedly, Prl+\/-, Prl-\/- and Prlr-\/- animals that were administered progesterone were found to have reduced litter sizes and leaner pups near term, as well as significant changes to maternal serum metabolites compared to wildtype mothers. We conclude that the major function of placental lactogens in post-implantation murine pregnancy is to modulate maternal glucose levels and prevent gestational diabetes. Additionally, we found that administering subcutaneous progesterone had unexpected adverse effects on pregnancy.

P2A-073
Metabolic profiles in various neurologic disorders in children - PCA study of 1H MRS in vivo spectra

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Principal component analysis (PCA) was used to explore the dataset of 1HMRS in vivo spectra acquired from children suffering from neurologic disorders. The studied group consisted of 64 children diagnosed with epileptic encephalopathy, cerebral palsy, developmental delay and neurometabolic disorders (metachromatic leukodystrophy (ML), Canavan disease (CD), megalencephalic leukoencephalopathy with subcortical cysts (MLC), mitochondrial cytopathy (MC), Menkes disease (M), non-ketotic hyperglycinemia (NKH), globoid leukodystrophy (GL), congenital disorders of glycosylation (CDG) and ethylmalonic encephalopathy (EE)). MRI and 1HMRS were performed using 1.5T GE scanner (the MRS acquisition parameters: TE 35 ms, TR 1500 ms, voxel volume 8 ml). Absolute metabolite quantification was performed by means of a water scaling technique. LCModel software was exploited in the analysis of the spectra and N-acetylaspartate (NAA), N-acetylaspartate+N-acetylaspartylglutamate (NAA+NAAG), creatine (Cr), choline containing compounds (Cho), myo-inositol (Ins), glutamate+glutamine (Glu+Gln) were analyzed. The metabolite levels and ratios were adjusted for age-related variation and subjected to PCA. MLC is separated along PC1 and characterized by a low level of all the metabolites. CD is separated along PC2 from ML, GL and M. The higher levels of NAA and NAA+NAAG, and lower levels of GPC+PCH and Cr in CD as compared to ML, GL and M are responsible for the separation. In the projection plane formed by PC1 and PC3, CD, GL, ML and NKH are clustered due to the highest contribution of Ins(Gly?) to PC3. PCA analysis of the metabolite levels and ratios to creatine revealed the separation of CD along PC1 (high NAA, NAA+NAAG, NAA/Cr, NAA+NAAG/Cr and low GPC+PCH and GPC+PCH/Cr). MLC is separated along PC2 due to low Cr, Glu+Gln, GPC+PCH and high Ins(Gly?)/Cr and GPC+PCH/Cr. The separation of ML, GL and NKH from the main bulk of data is seen in the PC1 and PC3 scores plot(low NAA, NAA+NAAG, NAA/Cr,NAA+NAAG/Cr,high Ins(Gly?),Ins(Gly?)/Cr and GPC+PCH). M is characterized by high GPC+PCH, GPC+PCH/Cr and low NAA, NAA+NAAG, NAA/Cr, NAA+NAAG/Cr. Combination of 1H MRS in vivo with multivariate projection techniques of data analysis enables efficient visualization of inter-spectra relationships.

P2A-074
Salivary metabolome profiling for cancer diagnosis

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Saliva is a readily accessible and informative biofluid providing various functions for oral cavity. Various omics analyses have proven the salivary diagnostic potential for wide range of diseases [1, 2]. In this study, we conducted non-target metabolome analysis of saliva samples from totally 215 individuals (69 oral, 18 pancreatic and 30 breast cancer patients, 11 periodontal disease patients and 87 healthy controls) using capillary electrophoresis-mass spectrometry (CE-MS) [3]. In the obtained thousands of peaks, 57 metabolites were identified to show statistically significant differences between at least two disease groups. Multiple logistic regression (MLR) models consisting of multiple metabolites yielded high area under the receiver operating characteristic curves (AUCs) for discriminating healthy controls and each disease; the AUCs were 0.865 for oral cancer, 0.973 for breast cancer, 0.993 for pancreatic cancer, and 0.969 for periodontal diseases. These models also produced high AUC values in cross-validation analysis. The differences in metabolomic profiles among diseases were more distinct than clinical features, such as age, gender, race and ethnicity. In conclusion, cancer-specific signatures in salivary metabolomic profiles showed a high potential for cancer detection. (1)Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, Wong DT. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res, 2009, 15, 5473-5477 (2)Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, Chia D, Wong DT, Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology, 2010, 138, 949-957 (3)Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M, Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles, Metabolomics, 2010, 6, 78-95

P2A-075
Differential Profiling of Endogenous Metabolites in Colorectal Cancer Patients Treated with 5-fluorouracil

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5-fluorouracils (5-FU) and leucovorin is the first line therapy for colorectal cancer CRC)patients in Malaysia. Objective: Profiling endogenous metabolites to detect potential markers for the monitoring of patients' responses to 5-FU. Methods: Serum was obtained from CRC patients treated with 5-FU and healthy volunteers. Three biological and technical triplicates of patients and controls were analysed using LC/MS. Samples were injected after protein precipitation with acetonitrile and centrifuged for 10 min at 8,500×rpm at 4°C. The chromatography was performed on an Agilent 1200 Series using an XDB C18 4.6 μM, 150×1.8 mm. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The flow rate was 0.25 mL/min and the injection volume was 2 μL. A linear gradient with the following proportions (v/v) of phase B (5% to 95% for 18 min; hold 95% for 5 minutes and then equilibration to 5% again for 7 minutes) was employed. The HPLC system was coupled to MS-QTOF 6520 (Agilent Technologies). The MS acquisition was performed in positive mode. Statistical analysis was performed using Mass Profiler Professional (MPP). Combined partial least square discriminant analysis (PLS-DA) and hierarchical clustering with Bonferroni correction was used to extract differential expression. Results/ Discussion: The PLS-DA analysis showed a pattern related to the differences of metabolites between the CRC patients and healthy controls. The initial results unravel the patterns of the complex biological relations between the consumption of 5-FU and its effects on patients. Some of these metabolites identified may prove to be useful markers to monitor patient's response to therapy. Conclusion: This report shows that MPP and Metlin Personal Database provide an excellent platform for scientists to unravel the complex relations between drugs application and their expected effects on biological systems. Keyword: 5-fluorouracils (5-FU), PLS-DA analysis, colorectal cancer patients

P2A-076
Capillary electrophoresis-mass spectrometry as tool in early-stage biomarker discovery for diabetic nephropathy

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Diabetic nephropathy (DN) is the most common complication of diabetes mellitus and often leads to end-stage renal disease. Typical characteristics of DN are amongst others persistent proteinuria, elevated arterial blood pressure and decline in renal function. There is no cure once the disease is diagnosed, but early treatment at a sub-clinical stage can prevent or at least halt the progression. Today, the commonly used biomarker for the development of DN is albumin excreted via the urine, although nephropathy has already developed significantly when these markers are present in urine. Therefore a biomarker or biomarker profile able to allow early prediction of the development DN would be very valuable to allow proper treatment at an early stage. In this project we hypothesize that subtle changes in the urine metabolome precede the clinically significant rise in albumin excretion rate (AER). To test this, 109 type-1 diabetic patients were recruited by the FinnDiane study: at baseline, 52 patients had normal AER, while other patients already progressed from normal AER to micro- or macroalbuminuria. Metabolite profiles of baseline 24h urine samples were obtained using different analytical platforms: NMR, GC-MS and LC-MS. In addition, a CE-qTOF-MS method was extensively explored and evaluated, and applied to the baseline samples. Data analysis such as logistic regression modeling was applied to the metabolomics data to differentiate between those patients who progressed to microalbuminuria and those who remained normal. The accuracy and precision of the predictive models obtained were better than 70%. These results demonstrated the potential of metabolomics for the prediction of the development of diabetic complications.

P2A-077
NPxRP-ESI-MS of phospholipids

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The aim of this work is to achieve better chromatographic separation of complex mixtures resulting in less co-elution of compounds. As a result, less ion-suppression and improved quantification will be observed. This can be achieved in various ways. In this poster we focus on a real on-line two-dimensional coupling of LC, increasing peak capacity, and as a result reduce matrix interferences. Phospholipids are chosen as model compounds in this study, they are composed of glycerol, two fatty acids, and a phosphate group with a (polar) head group. The hydrocarbon tails of the fatty acids are hydrophobic, but the phosphate group end of the molecule is hydrophilic. These different functionalities are used in this comprehensive separation- the first step is a normal phase (NP) separation which separates the phospholipids according to polarity of the head groups as the second step is a reversed phase (RP) separation enabling separation of the individual lipids on the basis of their fatty acid chain lengths. This is achieved by transferring the effluent from the NP column to the RP column using a electronically controlled 10-port 2-positions valve, equipped with 2 sample loops. Rapid switching of this latter valve ensures full 2-D coverage. As mixing of different solvent/polarity-types is a core problem of this approach, several measures were taken: 1)'2nd' Column ID was chosen to be 4.6 mm ID 2)Flow-rate of 2nd dimension was optimized still allowing proper mixing of solvents 3)Column temperature was optimized at '60 °C' without jeopardizing resolution 4)Solvent gradient composition was studied extensively. 5)The 2nd column was operated in specific group gradient conditions Further details and discussions on these measures as well as the application of this LCXLC system to both academic and biological samples will be shown.

P2A-078
Metabolomic and Transcriptomic Analysis of the Pichia pastoris Unfolded Protein Response

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Pichia pastoris is a methylotrophic yeast commonly used for recombinant protein production. However, obtaining good recombinant protein yields is largely an empirical process and little is understood about the constraints. Evidence suggests that inefficient trafficking of recombinant protein through the export pathway induces a stress response known as the unfolded protein response (UPR).[1-2] While the initial UPR expression of chaperones and protein disulphide isomerase may be beneficial, it ultimately leads to reduced protein production and secretion, and proteolysis. We have constructed P. pastoris clones containing 1 and 3 copies of the human Trypsinogen-1 (TRY1) expression cassette to investigate gene dosage effects on the UPR. Protein expression of TRY1 was decreased in the 3 copy strain compared with the 1 copy strain, and RT-qPCR results confirmed the upregulation of UPR responsive transcripts. Chemostat cultures of the two TRY1 clones were sampled for RNA extraction and also directly into a quenching solution at -50°C to rapidly halt metabolism. Variations in levels of transcripts were measured by microarray analysis, while intracellular and extracellular metabolites were analysed by NMR spectroscopy and GC-MS. The data indicates significant metabolic differences between the TRY1 x1 and x3 P. pastoris strains. Multivariate Principal component analysis (PCA) of the NMR and GC-MS metabolite data show a clear separation of the two TRY1 strains, and we have identified a number of metabolites that are significantly different. With the information from this investigation we aim to develop reporter systems for the P. pastoris UPR, which could then be used as a tool for early stage small scale screening to indicate problems in secretion and also as a process tool to determine the optimal level of expression. Furthermore, integration of the metabolomic and transcriptomic data will provide a better understanding of the cellular pathways involved in recombinant protein production and secretion. 1. Macauley-Patrick et al., 2005, Yeast, 22, 249-270; 2.Hohenblum et al., 2004, Biotech Bioeng, 85, 367-375;

P2A-079
Nuclear Magnetic Resonance (NMR) Analysis of Urine Establishes Metabolomic Fingerprints that Distinguish IBD from Non IBD and the Bacterial Small Molecules Present at the Initiation of Disease

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Metabolomic “fingerprints” on urine from animals with IBD have implicated bacteria, or bacterial products, as a driving element that permits metabolomics to distinguish IBD from non-IBD. Confirmation of bacteria in this role requires metabolomic testing, in a luminally sterile environment, before and after bacteria are present. Aim: The purpose of this project was to determine if bacterial inoculated mice, genetically predisposed to IBD and raised in an axenic environment, would lead to a similar metabolomic “fingerprint” as mice raised in a conventional environment. Methods: Urine was collected at week 20 from IL-10 knockout and wild type mice raised in conventional or axenic environments, all other aspects of care were identical. 20 week old mice were then removed from the axenic environment and inoculated with fecal material from conventional mice of similar age. Urine was collected on days 1, 4 and 7 and a 600 MHz 1 H NMR spectrum was obtained. The spectra were analyzed with Chenomx software to develop the metabolomic fingerprint. Metabolite concentrations were subjected to multivariate statistical methods. Results: PLS-DA analysis demonstrated that mice from the axenic colony had a completely different metabolomic fingerprint relative to mice from the conventional colony, implying that bacteria or bacterial products were driving the difference. Axenic mice, then fecally inoculated, demonstrated: on day 1, a metabolomic fingerprint that was intermediate between the axenic and conventional baseline, and on day 4 & 7 a metabolomic fingerprint that was similar to that seen with mice raised in the conventional environment. This metabolomic shift occurred in conjunction with the development of florid histologic IBD, confirming the necessary role of bacteria in both the metabolomic fingerprint and in the development of IBD. Analysis demonstrated that bacterial metabolites were dominant in the IBD metabolomic fingerprint. Conclusion: This study is the first to confirm the role of bacteria in determining the metabolomic fingerprint associated with the identification of IBD. Using fecal transfer into the axenic model will allow us, in the future, to identify specific bacterial groups and/or bacterial products that play a role in the very earliest initiation of IBD in genetically predisposed hosts.

P2A-080
A metabolomic approach to predictive liver toxicology

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Before approval of a new drug it will be tested intensively for safety in preclinical studies and clinical trials. Many drugs are found to be hepatotoxic in animal studies, but sometimes the hepatotoxicity is only noticed in phase III clinical studies or later. We aim to develop new tests that shall improve preclinical testing and diminish the number of hepatotoxic compounds going into animal experiments and clinical trials, based on metabolomic profiling of in vitro liver models. First focus will be on fatty acid and bile acid metabolism, which most likely play a major role in two important hepatotoxic mechanisms, namely steatosis and cholestasis. We have set up a Fluorescent Activated Cell Sorting (FACS) method for the measurement of steatosis in vitro in HepG2 cells. By optimizing this method we will have a high throughput test to screen compounds for steatotic properties. We will optimize this assay for primary mouse hepatocytes and we want to set up a FACS method for cholestasis using fluorescent bile acid conjugates. Next step will be a metabolomic investigation of hepatotoxicity using 1H-Nuclear Magnetic Resonance spectroscopy and Liquid Chromatography coupled to Mass Spectrometry. The ultimate goal would be to find one or several biomarkers which are specific for hepatotoxicity and which can be used to develop a sensitive in vitro assay for early discovery of hepatotoxicity. A systems wide approach combining metabolomics, proteomics and transcriptomics with bioinformatics will lead to a better understanding of the underlying mechanisms of drug-induced hepatotoxicity. Keywords: Hepatotoxicity, steatosis, cholestasis, metabolomics, in vitro

P2A-081

Using LC with Parallel Electrochemical Array – MS (LC/ECArray-MS) to Discover Metabolic Biomarkers in the Zucker Diabetic Fatty Rat Model

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LC/ECArray-MS was used for profiling endogenous metabolites in urine from Lean Zucker (LZ) and Zucker Diabetic Fatty (ZDF) rats, to search for potential biomarkers associated with genotype and phenotype. Since ZDF rats are known to acquire a diabetic phenotype at different rates, a particular focus was on identification of predictive markers of disease progression. Analyses were performed with gradient HPLC and a quadrupole-linear ion trap MS in parallel with a serial array of 16 electrochemical cells. Separate methods of positive and negative ion electrospray MS were used with a series of exploratory (enhanced MS) and targeted [neutral loss (NL) and precursor ion (PI)] scans while simultaneously gathering parallel ECArray data. The use of ECArray in combination with MS resulted in complementary data based on orthogonal detection of redox active and ionizable metabolites, respectively, with pg sensitivity and over a wide dynamic range. Principal components analyses (PCA) of ECArray data allowed differentiation according to genotype and disease progression and loadings plots were used to identify regions of variability. This allowed targeted interrogation of corresponding MS data and several ions were identified that exhibited differences in intensities associated with disease progression and genotype. Structural elucidation studies of potential early onset biomarkers are ongoing and include MS for targeted substructural analyses (e.g. glucuronide, sulfate) and comparison of EC voltammetric data to that of metabolite library standards.

P2A-082

1H NMR-based metabolomics for the diagnosis of Huntington Disease in serum

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Huntington's Disease (HD) is a hereditary brain disease. The exact mechanism of its pathogenesis is still unknown. [1,2] Metabolites in biofluids are in dynamic equilibrium with those in cells and tissues. A healthy individual attempts to retain the concentration of metabolites in cells and tissues constant by homeostasis. Abnormal cellular processes due to sickness, toxins, etc. will result in altered biofluid compositions. [3] In this study, proton nuclear magnetic resonance spectroscopy (NMR) was used to analyse serum taken from presymptomatic rats. Spectra of rats transgenic for HD are compared with those of healthy littermates. Visual inspection of these spectra only revealed a small percentage of the available information. However, spectral interpretation was simplified by applying an automatic data reduction method. Subsequently, these reduced datasets were analyzed with univariate (Student\'s t-test) and multivariate (PLS) statistical methods. It is anticipated that these investigations lead to a gain in knowledge about the pathogenesis of HD and make it possible to identify some potential biomarkers. [1] S.E. Browne, M.F. Beal, Neurochemical research (2004), 29, 531-546 [2] I. Tkac, J.M. Dubinsky, et.al., Journal of Neurochemistry (2007), 100, 1397-1406 [3] J.K. Nicholson, J.C. Lindon, Nature (2008), 455, 1054-1056

P2A-083

An integrated 1H NMR, HPLC-(ESI/TOF) and GC-MS based metabolomics approach to the study of acute exercise in human serum metabolome

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This study is aimed to explore the serum metabolic changes induced by an acute physical exercise. Eleven healthy young men (32.7±2.8 years old) were enrolled in the study. Fasting serum samples were withdrawn prior and after 30 min of acute exercise (bicycle, 70% VO2max). A combined GC-MS, HPLC-MS (ESI/TOF) and NMR-based metabolomics approach was used to the assessment of the global metabolic rearrangement produced by the impact of acute short-term exercise. Regardless of the analytical platform, metabolomics derived data presented a multilevel structure since it contains either NMR spectra regions, HPLC-MS or GC-MS m/zRT features taken at two different time-points (prior and post-acute physical exercise) for several subjects simultaneously. Thus, a multilevel two-component PCA model fitted either on 1D-CPMG spectra or in GC-MS m/zRT features showed two clearly separated clusters corresponding to individuals prior and post-acute exercise. Their corresponding loadings revealed significant raised levels of lactate, pyruvate and glycerol after acute exercise. In the NMR spectra, these changes were paralleled by significantly increased levels of alanine and succinate. GC-MS data showed that acute exercise induced significantly higher levels of -ketoisocaproic acid (KIC) together with raised levels of some fatty acids such as stearic or oleic. Preliminary studies performed on HPLC-MS data points to the same direction as those encountered using either NMR or GC-MS. Since serum closely reflects muscle cellular changes in lactate and pyruvate, their increased levels might be indicative of an accumulation of such glycolisis metabolites in muscular cells after short-term intensive exercise. Serum alanine levels appeared to be increased since it is transported to the liver where it acts as a gluconeogenesis substrate. Raised levels of glycerol together with elevated levels of some fatty acids suggest mobilization of triglycerides with acute exercise. Increased levels of KIC might be indicating leucine catabolism.

P2A-084

Pathological mechanisms of bacterial infections

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In earlier attempts to develop new methods for early diagnosis of bacterial infections the metabolomics approach has already been applied in growing cultures and for the differentiation between bacterial and viral meningitis as well as for different bacterial lung infections. In this work we demonstrate in mouse-models that were intraperitoneally infected with Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa that NMR-based metabolomics can be a powerful tool to distinguish between mice infected with different bacterial strains. We obtained significantly different 1H NMR spectra and the comparison of 43 metabolite concentrations, which were quantitatively determined using Chenomx software, led to complete class distinction using multivariate statistical techniques. Similarities in a wide range of energy metabolites and amino acids were identified among bacteria with the same Gram property and Gram-positive and Gram-negative infections were clearly separated by orthogonal partial least squares discriminant analysis (OPLS). The bacteria-specific metabolic profiles represent both metabolites activated by the innate defense system in mice and bacterial metabolites that are released into biological fluids. To differentiate the metabolites according to their pathological mechanism we compared the in vivo study with culture experiments and could identify some of the bacteria-specific metabolites as bacterial footprints. The immune cascade that is activated in response to the lipopolysaccharides (LPS) from the cell walls of Gram-negative bacteria mainly acts through the Toll-like receptor 4 (TLR4) and causes many of the clinical features. However, there are a number of critical differences between LPS- and bacteria-induced inflammatory responses which supports the notion that other bacterial molecular elements are involved as well. Therefore we also compared serum metabolite changes resulting from LPS treatment and from E. coli infection in both wildtype and TLR4 deficient mice.

P2A-085

Metabolomic Profiling of Neurospora Cultures Using Orthogonal Chromatographic Selectivity

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The general aim of metabolomic profiling is to document the set of metabolites from a defined sample for the determination of physiological changes. The specific sample can included a variety descriptors or parameters, such as cell type, organelle, age, tissue, and treatment. In this study, Neurospora cultures grown over a specified time period were compared and contrasted for a set of identified analytes. The metabolomic profiling was conducted by a high efficiency HPLC polar embedded phase utilizing accurate mass Time of Flight (TOF) mass spectrometry. Fused Core™ phases in two different chemistries HILIC and RP Amide provided orthogonal separation solutions to the complex profiling; HILIC generated 672 features, while RP Amide provided a further 312 features. Samples were evaluated for changes in metabolite composition and concentration using Molecular Feature Extraction. By performing batch processing, samples were compared for common components within all samples from the batch. This batch processing can also identify components that are common in only one set of samples or attributed to a subset with in the batch. This study demonstrates the power of orthogonal chromatography for metabolomic profiling.

P2A-086

Metabolomics and Detection of Colorectal Cancer in Humans – A Systematic Review

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Colorectal cancer (CRC) is a major public health concern. Current screening methods are not optimal. Metabolomics is being investigated as a novel screening method. Objectives: This systematic review summarizes existing literature on the use of metabolomics in CRC screening and diagnosis. Data Sources: A comprehensive literature search of established electronic databases (MEDLINE, EMBASE, PubMed, Cochrane Library, Scopus, and Web of Science), as well as of the pertinent grey literature was performed. Study Selection: Randomized, quasi-randomized, non-randomized, retrospective and prospective cohort studies and case-series involving human adult subjects with primary CRC were included. The index tests made use of serum, urine or tissue metabolomics. The reference standard was histopathological analysis of resected CRC specimens. Studies were included if the sensitivity and specificity of the index tests were either available or derivable from the primary data, or if they reported on specific metabolites that distinguished CRC from controls. Data Extraction: Screening of titles and abstracts was performed by the author; all subsequent steps were performed by two independent reviewers, to minimize bias. Results: Five studies are included in this review: 4 on tissue metabolomics and 1 on urine metabolomics. The data was insufficient for a meta-analysis. All five studies reported on metabolites that effectively distinguished CRC from controls. Ten unique metabolites (taurine, lactate, choline, glycine, phosphocholine, proline, alanine, threonine, valine and leucine) were found to be more prevalent in CRC; additionally, glucose was found in higher proportions for control specimens. Conclusions: This review highlights the emerging field of metabolomics as a diagnostic and screening tool for colorectal oncology. Individual metabolites are providing insight into mechanisms of action and pathogenesis of colorectal cancer.

P2A-087

Metabolite Profiling in Tomato Fruit Employing Automated Software for Biomarker Discovery and Identification

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Food crop characteristics such as nutritional value, quality, resistance to pathogens and flavor are important traits for the food industry and consumers. Metabolomics has been identified as a key mass spectrometry-based approach in the analysis of such characteristics. Herewith, results from metabolite profiling and identification experiments on tomato samples will be presented. Triplicate biological replicates of two tomato fruit cultivars were analyzed at four time points of fruit development stages using fast reversed-phase chromatography prior to mass spectrometric analysis, carried out on a hybrid high resolution mass spectrometer instrument. Strategies for metabolite profiling and identification were successfully applied and encompassed sample measurement in positive and negative ion mode electrospray ionization in conjunction with multiple dissociation techniques and extensive data mining. Preliminary results indicate that the high sample complexity in survey scans in the mass range 90-900Da benefits from highly-resolving, profile mode analysis. Hundreds of components were profiled at resolutions up to 100,000 useful for accurate and sensitive relative quantification experiments. Proper reduction of the number of detected signals through identification of sample related peaks and filtering out signals related to system background is essential. A GUI (Graphical User Interface) was developed to ensure simple fast operation of the software by novice users. In addition, grouping related signals, i.e. isotope peaks, adduct, dimers, fragments, etc. significantly simplified processing the results reducing the number of components by a factor of 10. This approach encompassing all of the features above presents a comprehensive, integrated solution to processing LCMS metabolomics data. Using external instrument calibration analyte masses were measured with high accuracy, leading to strongly suggestive identifications based on elemental composition analysis. Unambiguous identification of analytes in Mass Frontier software was used to corroborate the performance of the different MS/MS fragmentation regimes, carried out either via resonance excitation CID or higher energy collisional activation (HCD) experiments.

P2A-088

Predictive and Diagnostic Markers for High Fat Diet Induced Insulin Resistance in ApoE3Leiden Transgenic Mice

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Insulin resistance (IR) is a characteristic feature of the pre-diabetic stage and a critical element in the pathogenesis towards overt diabetes mellitus type 2 (DM2). The prevalence of DM2 could be significantly reduced by early identification of persons at risk allowing better prevention and earlier treatment. As IR has multifactorial causes, biomarkers are needed which detect IR predispositions in an early phase or already in healthy subjects. Individual transgenic ApoE3Leiden mice susceptible to diet-induced IR/DM2 and exhibiting a humanized lipid metabolism reacted differently to high fat diet conditions, as reflected by a strong variation in the degree of resulting IR. This natural variation was used to identify potential biomarkers for IR in plasma lipidome and white blood cell (WBC) gene expression profiles. Specific plasma free fatty acid ratios determined at the start (t=0) of the study were predictive markers for the degree of IR developed after 12 weeks of high fat feeding. The expression of 10 specific genes in WBC accurately reflected the individual degree of whole body IR over time and may discriminate between IR in critical tissues. To our knowledge it is for the first time that predictive markers for IR and easy accessible early markers indicating whole body IR have been identified. Interestingly, RNA transcription levels of the white blood cell gene most reflective for whole body IR was found to correlate with hepatic concentrations of metabolites that are related to the enzymatic function of this gene. Our results suggest that individual differences in adipose fatty acid desaturase capacity are critical for the degree of induced IR, in contrast to obesity itself. These differences can be exploited as predisposition marker for IR. RNA levels of a specific set of WBC transcripts may serve as easy accessible diagnostic marker for the stage of insulin resistance. Furthermore, we show new concepts for biomarker discovery that can be applied in many scientific disciplines.

P2A-089

Metabolomics as a tool for studying amyotrophic lateral sclerosis (ALS) in cerebrospinal fluid (CSF) of human subjects

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Amyotrophic lateral sclerosis (ALS) is a deadly, adult-onset neurodegenerative disorder characterized by progressive loss of upper and lower motor neurons, resulting in evolving paresis of the linked muscles. ALS is defined by classical features of the disease, but may present as a wide spectrum of phenotypes. Research has advanced the understanding of ALS, but the cause is still unknown, no reliable diagnostic test exists, no cure has been found and the current therapies are unsatisfactory. About 10% of all ALS cases have been reported as familial (FALS), of which about 20% have been associated with mutations in the gene encoding for CuZn superoxide dismutase (SOD1). The remaining cases are regarded as sporadic (SALS). Metabolomics, in combination with chemometrics, have been used as a tool to study the disease in cerebrospinal fluid from human subjects with ALS and matched controls suffering from neurological conditions. A panel of potential biomarkers was found for ALS where FALS specifically showed an overall decrease in metabolite concentration for subjects with ALS compared to matched controls. Glutamic acid was one of the metabolites found to be decreased in patients with ALS in controversy of previous research findings reported in literature. A larger metabolic heterogeneity was also detected among SALS cases compared to FALS in the metabolic pattern. Looking at SALS and FALS respectively against their respective matched controls, no significant difference from control was found for SALS while the FALS samples significantly differed from their matched controls. Significant deviating metabolic patterns were also found between ALS subjects carrying different mutations in the gene encoding SOD1.

P2A-090

MSEA: A web-based tool to identify biologically meaningful patterns in quantitative metabolomics data

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Gene set enrichment analysis (GSEA) is a widely used technique in transcriptomic data analysis that uses a database of predefined gene sets to rank lists of genes from microarray studies to identify significant and coordinated changes in gene expression data. While GSEA has been playing a significant role in understanding transcriptomic data, no similar tools are currently available for understanding metabolomic data. Here we introduce a web-based tool, called MSEA (Metabolite Set Enrichment Analysis), to help researchers identify and interpret patterns of human or mammalian metabolite concentration changes in a biologically meaningful context. Key to the development of MSEA has been the creation of a library of ~1000 predefined metabolite sets covering various metabolic pathways, disease states, biofluids and tissues. MSEA also supports user-defined or custom metabolite sets for more specialized analysis. MSEA offers three different enrichment analyses for metabolomic studies including over representation analysis (ORA), single sample profiling (SSP) and quantitative enrichment analysis (QEA). MSEA generates easily understood graphs and tables embedded with hyperlinks to relevant pathway images and disease descriptors. The MSEA server also supports conversion between metabolite common names, synonyms, and major database identifiers. MSEA has the potential to help users identify obvious as well as \'subtle but coordinated\' changes among a group of related metabolites that may go undetected with conventional approaches. MSEA is freely available at http://www.msea.ca.

P2A-091

Different patterns of serum metabolites by dialysis modalities: accumulation of inosine and hypoxanthine in patients on hemodialysis

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The pattern of abnormal metabolites may be different by the dialysis modality. However, there was no data showing the screening of comprehensive metabonomic patterns in patients on dialysis. We applied a novel method of 1H-Nuclear Magnetic Resonance (NMR)-based metabonomics to screen the comprehensive profiles of metabolites and identify the differences of metabonomic patterns by the dialysis modalities. The metabolic profiles of serum in hemodialysis (HD) and peritoneal dialysis (PD) were investigated using high-resolution NMR spectroscopy coupled with pattern recognition method. The patients on HD and PD who were anuric and non-diabetic were matched with age, sex and dialysis duration. The accurate concentrations of metabolites in serum were rapidly measured using the target-profiling procedure, and thereafter the differences in the levels of metabolites were compared using multivariate analysis such as Principal Components Analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA). PCA revealed that the general pattern is apparently different by dialysis modalities. Hypoxanthine and inosine were presented only in HD. Serum xanthine oxidase activity and uric acid level was not different by dialysis modality. Acetoacetate increased in HD. In PD, lactate, glucose and maltose were dominant and pyruvate, succinate, alanine and glutamate linked to glucose metabolism and tri-carboxylic acid (TCA) cycle were higher. Maltose appeared in patients using icodextrin solution. Known uremic retention solutes such as urea, creatinine, myoinositol and trimethylamine-N-oxide (TMAO) were increased in both dialysis groups. Citrate and phenylalanine are higher in both dialysis groups. In conclusion, metabolomics of serum based on 1H-NMR spectroscopy shows apparent difference of metabonomic patterns by dialysis modalities in anuric non-diabetic patients, which is influenced by dialysis-related process including solution. Inosine and hypoxanthine were detected only in HD patients, which may be representative of hypoxia and oxidative stress generated by HD.

P2A-092

The Metabolic Signature of Hypoxic cells by Hilic-FTMS

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Hypoxia is one of the features of poorly vascularised areas of solid cancers. Cancer cells can survive and proliferate in these areas despite the low oxygen tension reached. The adaptation to hypoxia requires both biochemical and genetic responses that culminate with a metabolic rearrangement to balance the decreased energy supply from mitochondrial respiration. However, the characterization of the metabolic adaptation to hypoxia is still poorly understood. In this study a biochemical and metabolomic analysis is carried to assess the effects of hypoxia on cellular metabolism. Two different cancer cell lines, A375 and HCT116 were kept under normoxia or at 1% O2 oxygen for 36 hours and different aspects of cellular and mitochondrial metabolism were analyzed. First, by using a phosphorescent oxygen sensor, it was demonstrated that hypoxic cells decreased their respiratory rate. To better define the metabolic signature of the hypoxic cells, a comprehensive metabolomic analysis using Hilic-Fourier Transform Mass Spectrometry (Hilic-FTMS) were performed. A375 and HCT116 cells presented a distinct normoxic and hypoxic signatures as showed by Principal Component Analysis indicating that these cells metabolically differ from each other. In the meantime, a conserved metabolomic signature in response to hypoxia was also observed. Biochemical analyses further confirmed a similar increase in glucose uptake, glucose consumption and lactate production under hypoxia. In conclusion, we show here that different cancer cell lines react to hypoxia maintain a distinct metabolic signature.

P2A-093

Phospholipidomic identification of potential biomarkers for diabetic mellitus and diabetic nephropathy

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Diabetic nephropathy (DN) is one of the serious complications of diabetic mellitus (DM).The prevalence of DN and the related treatment cost have increased dramatically during recent decades. The researches on discovering biomarkers are of major importance to prevent the progression from DM to DN, as well to the end stage renal disease (ESRD). Chinese Medicine (CM) focuses on health preservation and maintenance guided by a holistic and personalized approach for thousands of years. Its treatment for DM and DN reached remarkable effects and improved the quality of life of patients. Here we aim to combine Western Medicine (WM) stage classifications and CM subtypes to find novel potential phospholipids biomarkers in patients with DM and DN. In this poster, the combination of multivariate statistical analysis and targeted quantification based on an external calibration were applied to discover and validate DM or DN-specific potential biomarkers. Plasma phospholipids profiles were established by using NPLC-TOF/MS. The identification of the molecular structure of these biomarkers was obtained by Ion Trap-MS/MS. The plasma samples of 30 healthy individuals, 30 DM patients and 52 DN patients were collected and analyzed. PLS-DA and ANOVA were successfully utilized to screen out biomarker candidates and an external standard quantitative method was applied to evaluate potential biomarkers. Totally, 18 compounds were validated as potential biomarkers of DM and DN, including 3 DM-specific biomarkers, 8 DN-specific biomarkers and 7 common biomarkers for the two diseases. Among these potential biomarkers, 2 members can be used to distinguish DM and DN cases from healthy individuals. Correlation networks based on these potential biomarkers and some known clinical diagnostic biomarkers were also built to systematically understand phospholipids metabolism disorder happened to DM and DN patients. Further study will focus on discovering potential biomarkers of different subtypes of DM and DN diagnosed by CM and WM and investigating the correlation between CM and WM. (Note: Author affiliates will be detailed on poster.)

P2A-094

Metabolomics Of Amp-Activated Kinase Activation In Acute Lymphoblastic Leukemia Cell Lines

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Metabolism plays an important role in the physiology of human diseases. The complex and intricate nature of human metabolism however makes it difficult to evaluate the disease specific changes and requires a holistic approach. For instance, cancer is showing a clear metabolic link and metabolic enzymes have been directly implicated in cancer phenotypes. AMP activated kinase (AMPK) is a major sensor of cellular energy balance and regulates various pathways resulting in inhibition of energy consuming pathways and activation of energy producing processes. AMPK activators have been suggested as cancer drugs and the effect of AMPK activation on several metabolic pathways has been elucidated. However, its effect on the cellular metabolome has not been studied. To gain insight into the metabolic changes occurring upon AMPK activation, two acute lymphoblastic leukemia cell lines were treated with direct (A-769662) and indirect (AICAR) AMPK activators. Extracellular metabolites were analyzed by mass spectrometry and the effect on growth and apoptosis was evaluated. The growth of the cell lines was reduced upon AMPK activation while apoptosis increased. The extracellular metabolic profile showed cell-specific and activator-specific changes, including decrease in amino acid and glucose uptake and an increase in purine metabolism intermediates. Only A-769662 resulted in a decrease of lactate secretion. We are currently combining the metabolic measurements with a genome-scale reconstruction of human metabolism (Recon 1) to assess systemic metabolic changes caused by the drugs. This computational modeling effort will result in further insight into the complex interaction between AMPK and metabolism.

P2A-095

NMR-based profiling of fat content and detection of metabolic variations in ATGL mouse models

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Lipotoxicity is a well-established mechanism in the development of insulin resistance in association with hepatic steatosis, diabetic cardiomyopathy, and altered oxidative capacity of the skeletal muscle. Currently, patients will only be diagnosed with advanced stages of liver disease. Milder stages of hepatic lipotoxicity, and lipotoxicity in other organs, play only a minor role in diagnosis and patient stratification. Yet non-invasive imaging would allow monitoring of lipids within the organs, and lipid profiles can also be readily made available from serum. Recently, it has become clear that lipotoxicity does not simply develop in response to fatty acid overload of the target organ. Within the Predicct project (CTMM), researchers from Maastricht University, UMC St Radboud and Spinnovation Analytical are addressing the different aspects of lipotoxicity in order to generate the basis for the identification of biomarkers that would enable to monitor early onset of diabetes complications. ATGL mice have been initially chosen as a model to study fat accumulation resulting from an unbalanced muscular fatty acid uptake and oxidation, and leading to lipotoxicity. Using NMR-based methods, we are investigating the ATGL-mice fatty acid profile in tissue (heart, liver, skeletal muscles) and plasma, and compare them to wild-type mice in order to identify differences, eventually other than overall fat content. Secondly, in depth analysis of the NMR profile of ATGL plasma reveals differences in metabolite content and distribution as compared to wild-type. We are currently working on the identification of these differences and interpret them in order to evaluate whether they could be of value for the establishment of biomarkers for lipotoxicity.

P2A-096

The Demonstration & Competence Lab A high quality lab using State-of-the-Art technology in metabolomics studies

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The Demonstration & Competence Lab (DCL) is the central facility of the Netherlands Metabolomics Centre (NMC), a consortium of academia and (semi)industrial partners aiming to create a world-class metabolomics knowledge infrastructure for the improvement of personal health and quality of life. The research program focuses on technology development and application in biology driven projects in application areas like: nutritional-, clinical-, plant- and pharmaceutical science. The DCL focuses at the complete metabolomics work-flow but mostly concentrates on sample preparation, data acquisition and data preprocessing. Furthermore, the DCL combines innovative analytical platforms mainly using high resolution, state-of-the-art, mass spectrometry with a high standard of quality. The DCL provides support for method-validation and -demonstration, feasibility- and (pre)clinical-studies. Next, it offers a metabolite identification pipeline and subsequently several quantitative platforms to quantify this/these metabolite(s). These quantitative platforms operational in the DCL cover a wide range of metabolites; from lipids to amino acids and peptides to neurotransmitters as well as e.g. citric acid cycle intermediates using GC-, LC-, CE-, or HILIC-, MS platforms.. Furthermore, global profiling methods (non-targeted) are being setup/available for a variety of biological fluids (plasma, urine, serum etc) and tissue samples. Prior to application, the available platforms are extensively validated in order to determine the performance characteristics, resulting in an error model per metabolite. Continuous quality control during application of these methods offers highly reliable data to be used for advance data analysis. By this the DCL helps to answer (biological) questions in e.g.; energy metabolism, inflammation, oxidative stress, signaling and immune response. Various examples of novel tools applied to real-life questions resulting in preliminary biomarkers will be reported. The DCL also gives training courses and workshops to transfer the knowledge to the scientific community!

P2B-001

Environmental Metabolomics with Marine Organisms

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The metabolomic response of marine organisms in carefully planned experiments is key to understanding the effects of anthropogenic and natural stressors in coastal regions. In individual case studies, organisms such as fish, crabs and bacteria are candidates for investigation. In addition, efforts to improve data quality and improve comparability of interlaboratory results are important [1]. Several marine organisms have recently been assesed for suitability in metabolomics studies. For example, the metabolic trajectory for Atlantic blue crabs (*Callinectes sapidus*) subjected to injection with the microbe *Vibrio campbelli*, which causes nodules to form in the gill and impacts oxygen uptake, is initially different than treatment with 2, 4-Dinitrophenol, which is a known uncoupler of oxidative phosphorylation. [2] The microbe *Vibrio coralliilyticus* has been found in high concentrations in the bleached coral Pocillopora damicornis, but not in healthy corals. When inoculated into healthy corals at temperatures above 25 °C, *V. coralliilyticus* caused bleaching. A relationship between elevated temperature and virulence of *V. coralliilyticus* has been hypothesized. Recent metabolic studies indicate distinct metabolic responses of V. coralliilyticus at different temperatures, and the differential production of metabolites indicates a complex response to the elevated temperature. [3] 1. M. R. Viant, D. Bearden, J. G. Bundy, I. Burton, T. W. Collette, D. R. Ekman, V. Ezernieks, T. Karakach, C. Y. Lin, S. Rochfort, J. S. de Ropp, Q. Teng, R. S. Tjeerdema, J. Walter and H. Wu, "International NMR-based Environmental Metabolomics Intercomparison Exercise", Environmental Science and Technlogy. 43(1), 219-225 (2009). 2. T. B. Schock, K. G. Burnett, L. E. Burnett, L. Thibodeaux, D. A. Stancyk, D. W. Bearden: "Metabolomic Analysis of Atlantic Blue Crab, Callinectes sapidus, Hemolymph Following Oxidative Stress", Metabolomics, published on-line 20 Jan 2010, doi:10.1007/s11306-009-0194-y. 3. A. F. B. Boroujerdi, A. Meyers, E. C. Pollock, S. L. Huynh, T. Schock, M. Vizcaino, P. J. Morris, and D. W. Bearden: "NMR-Based Microbial Metabolomics and the temperature-dependent coral pathogen Vibrio coralliilyticus", Environmental Science & Technology, 43(20), 7658–7664 (2009).

P2B-002

Environmental Metabolomics Intercomparison Exercise

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Recent results of an international intercomparison exercise [1] for NMR-based environmental metabolomics show that NMR metabolome analysis yields robust results with consistent trends in metabolite-based biomarker identification among laboratories. Seven laboratories on three continents participated in the exercise. This type of demonstrated comparability is necessary as the technique is considered for regulatory environmental studies. Based on the lessons learned from this first effort, a second exercise is starting, and updated data collection and processing protocols are being developed to further investigate the comparability and suitability of NMR-based metabolomics data for environmental research, assessment and regulatory roles. The design for the exercise leverages the experience gained previously, so the participants will be asked to process and analyze two classes of materials with a set of robust NMR experiments and data analysis protocols. The exercise design emphasizes the steps that occur after sample collection and initial sample preparation. The samples to be used for the exercise are a set of simplified artificial metabolite mixtures and a set of fish muscle extracts from a marine aquaculture feeding study. Factors to be evaluated in the exercise include sample preparation, NMR data collection at multiple NMR-field strengths, quantitative evaluation and multivariate data analysis (principal component analysis). Several data quality evaluation protocols for multivariate data sets will be utilized to assess participants' success with the exercise. This poster will summarise the plans of the second intercomparison and provide an opportunity for interested groups to consider participating. 1. M. R. Viant, D. Bearden, J. G. Bundy, I. Burton, T. W. Collette, D. R. Ekman, V. Ezernieks, T. Karakach, C. Y. Lin, S. Rochfort, J. S. de Ropp, Q. Teng, R. S. Tjeerdema, J. Walter and H. Wu, "International NMR-based Environmental Metabolomics Intercomparison Exercise", Environmental Science and Technlogy. 43(1), 219-225 (2009).

P2B-003

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Desulfovibrio vulgaris Hildenborough (*D. vulgaris*) is a sulfate reducing deltaproteobacterium that has the potential to be widely used in the bio-containment of toxic heavy metal and radioactive waste. However, the effective implementation of remediation strategies and the use of natural attenuation for the cleanup of waste sites are dependent upon understanding critical chemical, physical, and biological processes. Thus, an understanding of regulatory mechanisms and cellular responses to different environmental factors affecting the metal remediation activity, in situ, is of great importance. The comprehensive analysis of metabolites from D. vulgaris can illuminate gene regulatory pathways responding to various environmental stimuli. However, the quantification of a large number of metabolites is impractical and time consuming. For this reason, our laboratory has designed an isotope dilution mass spectrometry (IDMS) strategy that improves upon precision, identification, and simplifies quantification. This strategy entails the mixing of unlabeled and 13C labeled lactate grown *D. vulgaris* cultures. To strengthen the efficacy of this strategy, an algorithm was developed that successfully facilitates the identification of unlabeled and labeled metabolites.

P2B-004

Variation in the concentrations of phenolic compounds of *Olea europaea* L. leaves with different water and nitrogen applications

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Abiotic stresses, especially in the context of global climate change, are important factors affecting crop productivity and quality. The effects of irrigation and nitrogen applications on the concentrations of selected phytochemicals of olive (*Olea europaea* L., cv. Cobrançosa) leaves were investigated. These studies were performed in a commercial orchard in Northeast Portugal. Trees were subjected to a rain-fed control and a drip irrigation treatment equivalent to 100% of the estimated local evaporative demand in combination with annual applications of 0 or 1.0 kg N tree-1. The analyses showed that olive leaves are a significant source of phytochemicals and that all samples presented a similar qualitative profile, although the levels varied among treatments. Seventeen individual compounds of four groups of phenolics were identified: simple phenolics, flavonoid aglycones and the more abundant flavonoid glycosides and iridoids. Oleuropein and ligstroside were the major compounds identified while, in general, luteolin 4´-O-glucoside, apigenin 7-O-rutinoside and luteolin 7-O-glucoside were the three main flavonoids. The concentration of some phenolics was changed by water and mainly by nitrogen application. In fact, a decrease of tyrosol, luteolin 4´-7-Di-O-glucoside, rutin, luteolin 7-O-glucoside, apigenin 7-O-rutinoside, apigenin 7-O-glucoside and luteolin 4´-O-glucoside were observed in nitrogen fertilized plants submitted to irrigation. Conversely, these trees had a higher concentration of diosmetin than rain-fed fertilized plants. Meanwhile, rutin and apigenin 7-O-rutinoside levels decreased with irrigation, but only in the presence of nitrogen application. Therefore, abiotic factors can influence the composition of leaves and there is clearly great potential to use olive leaves, which are otherwise considered as agricultural waste, into functional food ingredients (tea and as a source of natural bioactives and antioxidants). Our results suggest that controlled manipulation of water and nitrogen supplies in olive orchards can be used to modulate the levels of bioactive components in olive leaves.

P2B-005

Metabolomics and histopathology approaches to characterize naphthalene toxicity in mouse respiratory system

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Pulmonary diseases pose their significant impact to human. Clara cell, a susceptible site of tracheobronchial epithelium within the respiratory system, is in charge of xenobiotic metabolism. Clara cell may be injured due to exposure to environmental chemicals such as naphthalene (NA). NA, a major PAH in ambient polluted air, has resulted in a transboundary health problem. Naphthalene is demonstrated carcinogenic from animal investigations; moreover, possibly associated with human cancer risk. Metabolomics and histopathology approaches are applied to examine NA toxicity in a susceptible species, mouse, using ip administration. Dose response and time course experiments were carried out in male ICR mice whose bronchoalveolar lavage fluid (BALF) and lung were then taken for metabolic analysis. BALF and the lung metabolomes were analyzed by using 1D 1H and 2D JRES NMR following principal component analysis (PCA). For morphological observation, mouse lung was embedded and examined by light microscopy (LM). The PCA results of BALF and lung metabolomes demonstrated a clear trend of naphthalene dose effect. Numerous metabolites such as glycerophosphocholine (GPC), acetate, and succinate were decreased ($p<0.05$) when NA dose increases. GPC was found drastically decreased when treated with 100 and 200 mg/kg of naphthalene. The pathological data demonstrated Clara cell injuries as doses increase. The LM photographs of mouse Clara cell were swollen and vacuolated after 75 mg/kg and 100 mg/kg of naphthalene treatment. GPC is a pro-phospholipid source for cell membranes and an osmoregulator. From our BALF investigations, the decrease of GPC which results in unbalance osmolarity is likely associated with morphologically swollen and vacuolated Clara cells. The metabolic results may be used in biomarker discovery and mechanistic studies for investigating lung injury. The method can also be applied in studying other lung toxicants and diseases.

P2B-006

Health impact assessment based environmental metabolomics after oil spill exposure

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Environmental metabolomics is an emerging technology for examining metabolic profiles in biological systems exposed to environmental stress using mass spectrometry, which provides a blend of sensitive, rapid, and selective qualitative and quantitative analyses with the ability to metabolites. It involves the application of advanced analytical and statistical tools to profile changes in levels of endo- and exo-genous metabolites in tissues and biofluids result from disease onset, stress, or chemical exposure. In this study, we performed a pattern analysis using UPLC-QTOF/MS in urine of residents who exposed to toxicant chemicals, and then we investigated a steroids profile in urine using the GC-MS. To visualize differences between the metabolite signatures between low- and high-exposure to chemical substances, both PLS-DA and supervised hierarchical clustering analysis (based on Pearson correlation coefficients) were the good discrimination between groups. We were searched histidine metabolism for up-regulated, and glutathione and steroid metabolism for down-regulated metabolites from library. For targeted metabolic profiling, androgen steroids metabolites were decreased in high-exposure group, while estrogen steroids were increased. The alterations in circulating steroids levels usually lead to compensatory adaptation of the production rate and the rate of degradation and excretion. Our results mean toxicant induced estrogenic and anti-androgenic effect. The present metabolomic approaches will provide the most comprehensive molecular description of organisms in the environment and could be a useful tool in investigation of health effect and biomonitoring.

P2B-007

Effects of concentrated ambient fine particles on plasma phospholipids in a myocardial rat model

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Background: Many epidemiologic studies have demonstrated that fine particles (PM2.5) will induce cardiovascular diseases. There have been two hypotheses about the toxic mechanisms of ambient particulate. The first one is through direct effect. PM or its' soluble materials activate the neural reflexes and disarray the heart rate rhythm, which sequentially conducting the cardiac arrhythmias. The other one is the indirect effect, which caused by increased oxidative stress and inflammation, then inducing the thrombosis and the atherosclerosis. Either direct effects or indirect effects will disturb the lipids' balance during the course of cardiovascular disease. Glycerophosphocholine (PC) and glycerophosphoethanolamine (PE), which accounted the most ratios in phospholipids, are likely to be affected by PM2.5 exposure and cause cardiovascular abnormal. Understanding the fluctuations of PC and PE in plasma can help us to elucidate the mechanism of PM2.5 toxicity and to mine the possible biomarkers. Objective: In our study, we compare the differences of PC and PE in rat plasma between PM2.5 exposure and control group. We intend to understand the cardiovascular toxicity of concentrated ambient particles in a susceptible population. Methods: Male SD rats were exposed to PM2.5 for 65 days after they became myocardial damaging rats by isoproterenol(150 mg/kg) injection. After animals were scarified, plasmas were taken and snap frozen. The samples were extracted by Folch's extraction method and the lower phase was removed for farther analysis. Both NMR and MS were used to analyze the hydrophobic metabolite extracts of the rat plasma. The spectra were then analyzed by principal components analysis (PCA) and analysis of variance (ANOVA). Lipids were identified by the Chenomx and MassLynx for NMR and MS respectively. Results and discussion: The statistic results demonstrated lipids' fluctuations in rat plasma after PM2.5 exposure. We conclude that plasma lipids provide useful information for developing biomarkers to investigate the PM2.5 toxicity during cardiovascular disease progress. Other classes' lipids, which take important roles in cardiovascular disease, will also be examined in the future.

P2B-008

Mass Spectrometry-based Metabolome Analysis for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) toxicity

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In this study, liquid chromatography/quadrupole time-of-flight mass spectrometry was employed to investigate the toxic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in blood, liver and skeletal muscle tissues of C57BL/6J (C6) and DBA/2J (D2) mice. By using non-targeted metabolomic approach in conjunction with multivariate data analysis, e.g. principal conponents analysis and support vector machines, hundreds of ions were aligned and extrated with P-value and fold-change in mice exposed with TCDD compared to control group. Through high resolution MS and MS/MS analyses as well as database searching and comparison with reference standards, differentiating metabolites were tentatively identified for the interpretation of metabolic pathways in sensitive or less-sensitive mouse model. As a result, in sensitive mouse model-C6, fatty acid beta-oxidation was markedly attracted. Meanwhile, perturbation of other metabolic pathways was also observed, namely the accumulation of fatty acids and lysophospholipids, the reduced amino acid biosynthesis, and the decreased purine metabolism. The intermediate metabolites in citric acid cycle was also affected to reveal energy metabolism. The metabolic signature of TCDD toxicity in C6 mice was delineated by mass spectrometry and data mining, indicating oxidative stress, reduced energy production and perturbation of immune system. In contract, in the less-sensitive mouse model-D2, as expected, much less metabolites were detected as the differentiating effects of TCDD toxicity. In conclusion, mass spectrometry-based metabolomics measures the different abundant levels of the large number of metabolites produced as intermediates and end-products in different mouse models. The multivariate data analysis offered highly efficient classification and improved visualization, which facilitates metabolomic approach to be a promising tool in the interaction of environment and health.
References 1. Ishida, T.; Kan-o, S.; Mutoh, J.; Takeda, S.; Ishii, Y.; Hashiguchi, I.; Akamine, A.; Yamada, H. Toxicol. Appl. Pharmacol. 2005, 205, 89-97.

P2B-009

Identification of Distinct Susceptibility Metabotypes with Trichlorethylene Exposure Using an Inbred Mouse Panel

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Trichloroethylene (TCE) is a widely used industrial chemical and a common environmental contaminant. Evidence of its toxicity has been shown in rodent models but its mode of action and dose response are known to differ both within and across species (Lash, L.H., Envrion Health Perspec, 108, Suppl 2, p177, 2000). Studies utilizing panels of mouse inbred strains afford a unique opportunity to understand both metabolic and genetic basis for differences in responses to TCE. We tested the hypothesis that individual and liver-specific toxic effects of TCE are genetically controlled and that the mechanisms of toxicity and susceptibility can be uncovered exploring responses to TCE using a diverse panel of inbred mouse strains. TCE (2100 mg/kg) or corn oil vehicle were administered by gavage to 6-8 wk old male mice of 15 inbred strains. NMR-based metabolite profiling was performed on liver extracts at 2, 8 and 24 hours. The levels of four of the major TCE metabolites were also determined by a targeted mass spectrometry assay. At each time point a subset of mouse strains showed distinct metabolic perturbations due to the TCE treatment. The largest effect was observed at 8 hours wherein two distinct responder metabotypes were observed. These two metabotypes correlated with distinctly different levels of the P450 mediated metabolites, trichloroacetic acid and dichloroacetic acid. Significant perturbations a number of metabolites suggested that these metabotypes are characterized by differences in the degree of oxidative stress along with perturbations to branched chain amino acid catabolism, the glucose-alanine cycle, and tyrosine metabolism. This study demonstrates the potential of metabolomics analyses with diverse mouse panels to reveal distinct susceptibility metabotypes. These metabotypes will be integrated with genomics analyses to provide a mechanistic understanding of susceptibility to TCE toxicity.

P2B-010

Metabolomics using aquatic macrophytes in ecotoxicology

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Macrophytes are important model organisms in aquatic stress ecology and are more and more implemented in ecotoxicology. Their stress responses may be quantified by observing growth or photosynthesis. These established observation parameters have their limitations e.g. regarding their sensitivity in detecting effects in aquatic plants exposed to contaminants. Further, observation of chronic effects at environmental concentrations is a challenge. Molecular approaches such as metabolomics are considered to be promising to increase detection sensitivity of organisms exposed to chemicals under environmental conditions. To this end, we have established metabolic profiling of field-grown *Myriophyllum spicatum* and moreover, mesocosm studies for chemical assessment have been carried out to investigate the applicability of the metabolic profiling as an early-warning approach for alerting about toxic effects in macrophytes. A proof-of-principle study for low-level chronic fungicide exposure of *M. spicatum* in a mesocosm experiment was carried out at Alterra, Wageningen in summer 2009. Azoxystrobine was applied in mesocosms at six treatment levels. Plants were harvested at day 17 and day 45 after fungicide treatment. For the metabolomics approach hydrophilic extracts of leaves were analyzed using GC-MS. Data were pre-processed using XCMS and evaluated employing PCA. In the model ecosystem no effect of fungicide exposure on *M.spicatum* was observed for biomass increase and shoot growth of the plants. First analysis of molecular responses of exposed macrophytes also showed no effect on metabolite level using unsupervised statistics. Distinguishable metabolic profiles of M.spicatum leaves have, however, been found on the second PC axis. We attribute this to a time effect corresponding with the day of harvest. It seems that the metabolic pattern of *M.spicatum* exposed to azoxystrobine under realistic exposure is not determined by a stress response but merely mirrors biological variability. Additionally, supervised multivariate statistics will be used to discuss the suitability of metabolic profiling for environmental risk assessment.

P2B-011

Stress responses of the metabolome and proteome of the chlorophyte S. vacuolatus under exposure to N-phenyl-2-naphthylamine

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Toxicity evaluation of chemicals mainly relies on non-specific, population-based observations. To address current challenges in ecotoxicology, integrated approaches including toxicogenomics may improve our causal understanding of effects of toxicants like the elucidation of modes-of-action. N-phenyl-2-naphthylamine (PNA) can be naturally produced by plants as an allelopathic substance but was also characterized as an environmental contaminant at former industrial megasites. PNA shows a high anti-algal activity, however its mode-of-actions still not fully understood. We aimed to analyse the stress response in the proteome and the metabolome of a chlorophyte after exposure to PNA in order to (1) identify concentration-dependent changes in the biochemical pattern and to (2) assess the sensitivity of stress responses in biomolecules in comparison to established observation parameters, used in effect assessment of toxicants. Synchronized cultures of S. vacuolatus were exposed for 14 hours to a concentration series of PNA ranging from no to full growth inhibition (0.00143 (environmental concentration) -1.82 µmol L-1). 2D-gel electrophoresis-based proteomics was performed using DIGE. Metabolites were analysed by GC-MS after extraction in water/methanol/chloroform, followed by derivatization and silylation. To be able to relate changes in biomolecules to established phenotypic responses, changes in cell volume and selected physiological parameters were evaluated. Multivariate data were analysed using PCA. Metabolites clustered in three groups, indicating two different response patterns of the metabolites. The first group clearly separated from controls at levels above 0.00713 µmol L-1 and captured variation of metabolites like few amino acids and several sugars and fatty acids. The second group of metabolites, e.g. amino acids showed concentration-dependent changes comparable to the proteins or to growth inhibition at concentrations above 0.228 µmol L-1. 115 proteins altered in expression at high concentrations including proteins of the Calvin cycle or stress proteins like heat shock proteins. In conclusion, the combined approach revealed the high sensitivity of metabolomics for identifying effects of toxicants and a concentration-dependent pattern in metabolites.

P2B-012

Simultaneous GC-MS Profiling of Biomarkers of Occupational Exposure and Natural Acidic Metabolites in Urine Simultaneous GC-MS Profiling of Biomarkers of Occupational Exposure and Natural Acidic Metabolites in Urine

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People in their living and working environments are exposed to large quantities of chemicals that may be potentially harmful. The important industrial organic pollutants such as aromates, alkoxyalcohols, monomers and common organic solvents are absorbed, metabolized and excreted from an organism mostly as carboxylic acids, which are determined as biomarkers of exposure. For several tens of the chemical pollutants, biological limits (levels of biomarkers in biological material) have been introduced in most industrial states. Compliance with these limits should guarantee health of persons employed in daily 8-hour exposure. Till now, the biomarkers of occupational exposure have been analysed more or less separately in body fluids by chromatographic techniques coupled with mass spectrometric detection. Here we report a comprehensive approach to rapid profiling of the biomarkers together with natural acidic metabolites present in human urine. The metabolic products of the most common pollutants, including those derived from benzene, toluene, styrene, xylenes and alkoxyalcohols, carbon disulfide, fural and N, N-dimethylformamide are determined in the form of e.g hippuric or mercapturic acids simultaneously after in situ extractive alkylation with alkyl chloroformates followed by capillary gas chromatography with mass spectrometric detection. The developed method was validated for the quantitative determination of fifteen, the most common biomarkers occurring on the 0.1-500 µmol/L level in exposed human urines. The methodology enables rapid analysis of xenobiotics and natural acidic metabolites like amino and organic acids simultaneously and opens way for comprehensive studies of metabolic profiles in urine of workers exposed to the harmful industrial chemicals. Financial support: Fund for Research Support, EEA/Norway grant No. A/CZ0046/1/0018 and Grant Agency of the Czech Republic project No. 213/09/2014.

P2B-013

A Metabolomic Approach to Study Toxicity, Compensation, and Recovery in Small Fish Exposed to EDCs

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Determining the impact(s) of exposure on aquatic organisms by endocrine disrupting chemicals (EDCs) is essential for determining the risks that these chemicals pose. However, to accurately evaluate these risks, beyond simply measuring a “before and after exposure” snapshot, researchers must assess the ability of the exposed organisms to adapt or compensate for the presence of these compounds. The extent of true harm from sub-lethal exposure is often a complex relationship of both time and chemical concentration. Due to the large number of samples required to map this complex response profile, a robust molecular technique with low per-sample cost of analysis is desirable. Therefore, we have employed a metabolomics approach for studying these responses in small fish toxicity models (e.g., fathead minnow) using nuclear magnetic resonance (NMR) spectroscopy. This approach provides the ability to measure molecular responses in different tissue and biofluid types, both rapidly and inexpensively, making it ideal for this application. Using this approach, we have been able to observe apparent compensatory responses to the presence of EDCs over the duration of an exposure. Furthermore, it appears that after the chemical has been removed from the water (i.e. during a depuration phase) that fish are able in some cases to return to a near “pre-exposure” state, providing evidence of partial recovery. These results demonstrate the potential of this approach for improving the assessment of risk(s) that various EDCs pose to sentinel small fish species.

P3A-001
Direct Surface Sampling of Planar Tissues combined with nESI-MS in early studies of drug candidates

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Metabolite distributions directly in tissues require atmospheric pressure surface sampling/ionization of small sample volumes, where analytes can be directly analyzed from a variety of surface types without sample preparation. A direct analyte extraction from a surface in combination with nano-ESI-MS was recently developed,and is a complementary analytical approach to other surface-oriented MS methods such as DESI, LDI, DART or MALDI imaging. Extraction is performed by placing a small solvent droplet on the tissue area of interest and then aspirated into a conductive pipette tip for automated chip based nano electrospray infusion using a modified Advion NanoMate TriVersa system. Thin mouse tissue slices pre dosed with sulforafane or propranolol, were investigated by this method for analysis of drug and drug metabolite distribution of whole body and compared with control tissue. The parent drug (SFN / Propranolol),and the phase II metabolites (SFN-GSH,SFN-NAC / Hydroxypropranolol-glucuronide) were screened in MRM mode in different thin tissue sections(lung, liver, kidney, brain) from the dose and control mouse. Excellent signal to noise ratio could be achieved to visualize the drug distribution.

P3A-002
Aptamer-Conjugated Gold Nanoparticles for the Improved Inhibition of HIV-1 Reverse Transcriptase

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We had developed the aptamer - RT1t49 (Aptpol), and ODN 93 (AptRH) - conjugated gold nanoparticles (AptRH-Au NPs and Aptpol-Au NPs) as the highly effective inhibitors for HIV-1 reverse transcriptase (HIV-1 RT) polymerization. Based on the multivalent effect, the inhibition efficiency of Aptpol and AptRH were further improved after their bioconjugations with Au NPs. Since Aptpol direct reacted with the polymerase site of HIV-1 RT with the smaller Kd, the decreasing order of the inhibition efficiency for HIV-1 RT was Aptpol-Au NPs > AptRH-Au NPs   Aptpol > AptRH >> TBA27 (27-base-long thrombin binding aptamer). The surface aptamer density on the Au NPs was also important in determining their enzymatic inhibition of HIV-1 RT. Our results suggested that the use of 40Aptpol-Au NPs (40 Aptpol molecules per Au NP) provided the highest activity (IC50 = 27 ± 1 nM) for inhibiting HIV-1 RT. To test the potential of this enzyme assay in biological media, we found that there were just 2% decreases in the inhibition efficiency of 40Aptpol-Au NP in the present of BSA (100 μM). Furthermore, the increased resistance of the Apt-Au NPs against the digestion of nucleases (DNase I) was examined. Free Aptpol lost its inhibition efficiency about 34% within 2 h, while 40Aptpol¬-Au NPs only slightly lost its efficiency (3%). To further improve the inhibition efficiency, the Aptpol with more flexible conformation were added into the AptRH-Au NPs which led to the synergistic effect to get the best inhibition (IC50 =17 ± 1 nM). From our results, the novel Apt and Au NPs based reverse transcriptase inhibitors held greater potential of developing alternative therapeutics in clinical therapy.

P3A-003
Apoptotic and Cytotoxic Effects of Methanol Extract of Camelia sinensis Leaves on Osteosarcoma Cells

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Tea polyphenols has received much attention over the last few years as cancer chemopreventive and chemotherapeutic agent. These dietary polyphenols have shown to possess antitumor effects in several malignant cell lines including breast, skin, liver, pancreas, lung, prostate and bladder. In this study, It was investigated apoptotic and cytotoxic effects of methanol extract of Camelia sinensis leaves (CsM) on human osteosarcoma (U2OS) cells. Cells were cultured and incubated with 5, 10, 20, 40, and 60 μg/mL CsM concentrations. Cytotoxic and apoptotic effects were determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and FITC Annexin V flow cytometric analysis, respectively. As a result, U2OS cell proliferations were significantly decreased at the 40 and 60 μg/mL concentrations of extract for 24h, and at all the concentrations (5-60 μg/mL) of extract for 48 h. When cells were treated with concentrations (5-60 μg/mL) of CsM for 24 h, apoptotic rates were determined 0.3, 1.4, 5.2, 4.2 and 18.9 %, respectively. The extract showed significant cytotoxic effect and inhibited the proliferation of U2OS osteosarcoma cells. Espicially, the most important apoptotic effect was determined at the 60 μg/mL extract concentration. As a result, It was shown that CsM had significant anticancer activities on U2OS cells. This study suggested that CsM may have a potentially therapeutic role for osteosarcoma cells. It that a significantly higher

P3A-004
Metabolomics Identifies the Building Blocks of Pharmacologically Active Metabolites in Marine Invertebrates and its Microbial Symbionts

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Marine invertebrates harbour microorganisms that include bacteria, cyanobacteria and fungi within their tissues and in some cases these associated microorganisms may constitute up to 40% of their biomass. A number of pharmacologically active sponge natural products have been found to be structurally related to microbial metabolites. One example is ecteinascidin (ET-743) which was first isolated from the tunicate *Ecteinascidia turbinata*. The structure of ET-743 reveals striking similarities to safracin B, a metabolite of *Pseudomonas fluorescens*. ET-743 is commercially available as Yondelis® or under the generic name trabectedin and is used for the treatment of undifferentiated uterine sarcoma in women. To date, Yondelis® is made feasibly available through biotechnological methods and partial synthesis. Renieramycin is an analogue of ET-743 which was obtained from sponges *Reniera and Xestospongia*. Building blocks have also been isolated from these sponge genera. Sponges become fermenter vessels for the microorganism to produce these interesting metabolites. Through tools of metabolomics and genomics, the production of other novel drugs can be optimised to solve and come up with a sustainable solution to address the supply problem. Recently, we have applied metabolomics to screen for potential new antibiotics from sponge-derived microorganisms collected from under-investigated and under-exploited marine habitats of a geographic distance of more than 10,000 km coastline of Scotland.

P3A-005
Metabolomic analysis reveals metabolic conditionality of antibiotic resistance in Staphylococcus aureus

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Staphylococcus aureus (S. aureus) is an antibiotic-resistant pathogen second only to HIV in scope and significance. Existing knowledge indicates that antibiotic resistance incurs a fitness cost to which the bacterial host must adapt. The identities of such adaptations, however, remain lacking. Our work focuses on identifying the adaptations that accompany hetero-intermediate vancomycin resistance in *S. aureus* (hVISA). Knowledge of such adaptations may provide novel means of controlling the growing medical threat of antimicrobial resistance. We previously demonstrated that vancomycin heteroresistance is accompanied by a systematic shift in the *S. aureus* metabolome. Untargeted profiling of the metabolomes of vancomycin -susceptible and -heteroresistant *S. aureus* isolates revealed changes in pathways of anaerobic metabolism. To further evaluate the role of these pathways in supporting heteroresistance, we performed metabolomic and microbiologic analyses of hVISA isolates under aerobic and anaerobic conditions. Sample preparation methods using a combination of thermal cooling, mechanical lysis and organic extraction were used to ensure efficient extraction of intracellular metabolites. Hydrophobic and hydrophilic metabolites were resolved using a combination of C18-reverse phase and aqueous normal phase chromatography, followed by ESI-TOF. Metabolites were identified using retention time-accurate mass pair qualifiers and analyzed using unsupervised statistical methods. Global metabolite profiles of unrelated clinical *S. aureus* isolates were obtained and analyzed using two unsupervised statistical methods. These studies showed that the phenotypic expression of vancomycin heteroresistance was accompanied by conserved alterations in the S. aureus metabolome. Analysis of these changes revealed that the expression of resistance was accompanied by changes in metabolite pools related to anaerobic metabolism. Microbiological analyses of these, and other, hVISA isolates demonstrated that growth in either minimal media or anaerobic conditions was sufficient to suppress the expression of vancomycin heteroresistance. Moreover, this suppression in heteroresistance was accompanied by a specific reversion of the metabolic alterations associated with expression of vancomycin heteroresistance. Based on our recent findings, we hypothesize that S. aureus redirects enzyme intermediates of anaerobic metabolism to express vancomycin heteroresistance. Future studies examining the role of intermediates of these pathways in supporting vancomycin heteroresistance may identify novel chemotherapeutic targets that could specifically suppress the emergence of resistance itself.

P3A-006
High-throughput screening for novel prostate cancer drug targets identifies PLA2G7 as a putative target for ERG oncogene positive cancers

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There is an urgent need for more efficient and more targeted methods of prostate cancer treatment. Here, we combined information from gene expression studies of prostate cancer tissues in vivo and RNA interference studies of prostate cancer cell lines to nominate potential targets and pathways for therapeutic exploration. We selected 300 prostate and prostate cancer-specific genes and performed high-throughput siRNA screening with cell viability and apoptosis as end-points. The results highlighted a significant role for enzymes associated with the production of bioactive lipids and signalling molecules, especially the arachidonic acid pathway, in prostate cancer cell proliferation. Many of the arachidonic acid pathway targets identified here, such as PLA2G2A and FAAH, have been previously linked to prostate cancer. However, there were also novel genes along this pathway, suggesting strong dependency of tumor cells on arachidonic acid metabolism. One of these novel genes was PLA2G7 (phospholipase A2 group VII). Clinical validation confirmed high PLA2G7 expression especially in ERG oncogene-positive prostate cancers and its silencing sensitized ERG-positive prostate cancer cells to oxidative stress. Silencing of ERG reduced PLA2G7 mRNA expression in ERG positive prostate cancer cells, supporting a functional link between these two genes. Global lipidomic analysis indicated that PLA2G7 silencing reduces the amount of lysophosphatidyl choline, a precursor of lysophosphatidic acid in ERG positive prostate cancer cells. Taken together, our results illustrate the power of high-throughput RNAi coupled with systems biological data analysis in the exploration of potential new target genes for prostate cancer.

P3A-007
Metabolomics Study for Hepatotoxicity Evaluation Using In Vitro Systems

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The purpose of this study was to profile endogenous metabolites of 25 hepatotoxic drugs and to develope metabolic biomarker candidates in rat primary hepatocytes. Hepatocytes were treated with the drugs at inhibition concentration 20 (IC20) for 24h. The 25 drugs were classified into hepatocellular, cholestatic and mixed type depending on their pattern of liver injury. After Q-TOF LC/MS analysis of cell extract, global profiling using PLS-DA was performed and endogenous metabolites were identified using Metlin DB. As results, 25 targeted metabolites was selected for further analysis. Expression of targeted metabolites was classified into 4 groups, which was not closely correlated with 3 patterns of liver injury. However, aminoacyl-tRNA biosynthesis and glutathione metabolism showed significant correlation. A targeted profiling analysis was further applied for identification of metabolic biomarkers. As results, oleamide, palmitic acid, arginine, oleic acid, leucine/isoleucine, glycine were identified as biomarker candidates for hepatotoxicity and oleamide, palmitic acid and leucine/isoleucine for classification of liver injury pattern. These results might provide better understanding of the biological metabolic changes for drug-induced hepatotoxicity and closer safety evaluation tool to the phenotype.

P3A-008
Discovery of novel antimicrobials in filamentous fungi

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The purpose of this project is the production, structural and functional characterisation of novel antibiotics produced by *Penicillium chrysogenum*. Therefore, three PhD students from different universities and fields are working on this project. The group in Groningen led by Prof. Dr. Arnold J.M. Driessen will focus on the awakening of sleeping gene clusters. The group in Erasmus University headed by Prof. Dr. Alex van Belkum concentrates on the biological activity of products generated by *P. chrysogenum* whereas our group will focus on the structural characterisation of the produced metabolites. The full sequenced genome of P. chrysogenum revealed the presence of 50 potential gene clusters which mostly remain non-expressed under typical fermentation conditions as used in the laboratory. By using a P. chrysogenum host strain in which all expressed antimicrobial gene clusters have been genetically inactivated, we will revive these sleeping gene clusters. This provides a low background and will facilitate compound analytics, isolation and screening. Based on the identified structural information of new produced compounds we will elucidate the biosynthetic pathway and predict the functions of the individual genes. Both in-vitro and in-vivo screens are used to elucidate the mechanisms of action. In addition, allergic testing of the compounds, the assessment of possible toxicity and eventually complicating immunogenicity will be performed. Due to the complex variety of antibiotic active compounds several analytical platforms like Reversed Phase Liquid Chromatography (RP-HPLC), Hydrophilic Interaction Chromatography (HILIC) as well as Gas-Chromatography (GC), all combined with Mass Spectrometry (MS) are necessary for a comprehensive screening and subsequent identification. In combination with new algorithms, which help to predict structures of unknown metabolites and chemical synthesis of promising leads, the exact structure will be determined. Within the first phase of this project a GC-MS method for the identification and quantification of primary and secondary metabolites will be developed. First results reveal this GC-MS platform as an promising approach for metabolomic profiling of secondary metabolites produced by P. chrysogenum.

P3A-009

Targeted metabolomics of oxylipins for the investigation of the mode of action of xenobiotics

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Oxylipins are a group of oxidized metabolites of unsaturated fatty acids produced by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes. These compounds as well as their metabolites are highly potent biological mediators. The metabolites of LOX and COX mediated conversion of arachidonic acid (AA) are well characterized and the attenuation of these lipid mediators is an important component of the non-steroidal anti-inflammatory drugs (NSAIDs). Many other lipids are also potent bioactive molecules yet their biological roles remain poorly understood. Specifically, epoxy-fatty acids, produced by CYP450s are implicated in numerous biological processes. Inhibition of the soluble epoxide hydrolase (sEH), the major enzyme which degrades the metabolites to inactive or less active molecules, has a variety of effects in vivo models, including anti-inflammatory and pain blocking activities, which render sEH inhibitors (sEHi) as promising drug candidates. However, the three diverging pathways of oxylipin formation seem to be involved in an elaborate and dynamic cross-talk. The arachidonic acid epoxide 14,15-EET has been shown to suppress the induction of vascular COX-2 expression and to competitively inhibit prostaglandin E2 synthesis. In order to understand the multiple modes of action of a drug on the formation of these bioactive lipids, we monitor all three pathways simultaneously. Therefore we developed an LC-MS/MS based targeted metabolomics approach, allowing the quantification of 88 oxylipins in plasma after solid phase extraction (SPE) in only 21 minutes. Our panel not only includes the metabolites of AA (eicosanoids), but also allows the quantification of the lipoxygenase products and epoxides and diols of the unsaturated fatty acids gamma-linoleic, alpha linoleic, eicosapentaenoic and docosahexaenoic acid. This approach enables the quantitative monitoring of a targeted panel of bioactive materials, thus providing numerous advantages over focusing on only one or several analytes and reducing bias in understanding the pathophysiology of disease states. In the presentation the performance of our approach will be demonstrated based on the investigation of inhibitors of the soluble epoxide hydrolase as the antibacterial compound triclocarban, and the investigation of their interaction with other drug compounds.

P3A-010

Multipole Platform Mass Spectrometric Profiling of the Effects of an LXR Agonist on Metabolism in Mouse Liver

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The effects of an LXR agonist on the metabolic profile in mouse liver was assessed with a variety of mass spectrometric methods. The methods used included on matrix assisted laser desorption mass spectrometry (MALDIMS), two modes of hydrophilic interaction liquid chromatography coupled to an LTQ-Orbitrap mass spectrometer and two gas chromatography-mass spectrometry (GC-MS) methods. Small molecule profiles were obtained for mouse liver samples obtained from mice which were treated with either saline (control) or 0.3 mg/kg, 1 mg/kg, 10mg/kg, 30mg/kg or 60mg/kg of an LXR test compound once daily over a 5 day period. It was possible to detect an increase in triglyceride (TG) accumulation in the livers of animals treated with the drug, even at the lowest dosage levels, using in the first instance MALDI mass spectrometry. There was both accumulation of the triglycerides and an increase in relative degree of TG saturation in the treated samples. In addition changes in the profiles of phosphatidyl choline lipids were also observed. Using a lipidomics LC-MS method, it was possible to determine that these changes were due to an increase in the levels of oleic acid containing lipids. The changes in lipid profiles were also observed by GC-MS which also demonstrated a large increase in the level of oleic acid in the samples. GC-MS analysis revealed that there was a fall in cholesterol levels although there was no marked increase in any of the minor steroid intermediates involved in cholesterol biosynthesis. Polar metabolites in the samples were analysed by hydrophilic interaction chromatography in combination an LTQ Orbitrap. There were many changes in the metabolite profiles some of which might simply related to generalised toxicity. The clearest marker compounds which showed very marked changes with dose were methylglutaryl carnitine and hydroxymethylglutaryl carnitine (HMGC). Another marker of some interest was uridine diphosphate N-acetylglucosamine.

P3A-011

Extracellular Metabolite Biomarkers of Bortezomib Resistance in Multiple Myeloma Indicate Involvement of Unexpected Metabolic Pathways

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Bortezomib (BZ) is a chemotherapeutic agent approved for the treatment of multiple myeloma (MM). BZ acts through proteasome inhibition, inducing significant ER stress and cell death. Unfortunately, nearly 20% of MM patients are primarily resistant to BZ treatment and responses to BZ are difficult to predict based on the currently available clinical, cytogenetic and genomic biomarkers. Our functional hypothesis is that extracellular metabolites have a greater potential to be found in circulating biofluids as biomarkers. As a result we used a metabolite \'footprinting\' approach in cell growth media to examine the metabolic consequences of BZ treatment using eight human MM cell lines, three of which have been determined to be less sensitive to BZ treatment than the others with a 10 fold difference in their IC50 at 24 hours (5 nM vs 50 nM). Our aims were to: 1) establish whether analysis of growth media was suitable for monitoring metabolic changes and 2) determine specific biopatterns of BZ resistance. Methods: Media from eight MM cell lines were sampled at 6 and 24 hours. Metabolite profiling was accomplished using gas chromatography mass spectrometry (GC-MS), and significant metabolites were identified using multivariate regression analysis using SIMCA-P (Umetrics). Results: An initial O2PLS-DA model was successfully built from the GC-MS feature set using both growth time (p=0.03) and BZ status (p=6.9e-13). The remarkable metabolic difference between BZ-treated and untreated cells resulted from reduced energy-related metabolites such as citric acid cycle intermediates and sugars, with a concomitant increase in selected amino acids. To further probe the phenomenon of BZ resistance, the treated group was analyzed independently, with the 37 most influential variables providing discriminating ability between the BZ-insensitive and BZ-sensitive cells (p= 0.04) in an OPLS-DA model. Conclusions: Our results suggests that BZ function remains largely intact in both sensitive and insensitive cell lines, and resistance is conferred through alternate mechanisms with measureable metabolic endpoints. Success in measuring extracellular metabolites also supports the notion of serum-accessible biomarkers or biopatterns of BZ resistance.

P3A-012

Chemical genomics and metabolic suppression to characterize probes of biosynthetic pathways in Escherichia coli

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Microorganisms have a remarkable capacity to rapidly adapt to various changes in their environment. They can efficiently modulate their physiological responses to adapt to various stresses in their environment. Of these stresses, we focus in this work on nutritional limitation with special emphasis on the Gram-negative model bacterium, *Escherichia coli*. Growth of E. coli on glucose minimal media results in a general up-regulation of genes involved in the synthesis of amino acids, vitamins, cofactors, prosthetic groups and carriers. In the goal of targeting these biosynthetic pathways, we have completed a high-quality high-throughput screen against a library of ~ 30,000 structurally diverse small molecules and identified those with growth inhibitory activity against E. coli when grown in glucose minimal media. From this screen, we have identified ~ 500 small molecules of which about 300 constitute a set of novel chemically synthetic compounds with previously uncharted biological activity. In secondary screens, we use metabolite chemical suppression as a systematic approach to identify the potential cellular pathways targeted by these bioactives. Furthermore, we make use of genome-scale, ordered high expression and deletion clone sets in E. coli to further investigate the effect of these compounds on biosynthetic pathways. Our ultimate goal is to uncover the mechanism of action and describe the chemical genetic interactions of this set of small molecules on a genome-wide level. Among these characterized bioactives there is a potential for identifying new classes of antibiotics with novel mechanisms of drug action.

P3B-001
Light interacts with salt stress in regulating superoxide dismutase gene expression in Arabidopsis

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Arabidopsis thaliana plants (Col and N1438) were grown for 15 d under two light regimes providing different growth rates. The medium contained 0 to 85 mM NaCl. Shoot biomass and ion accumulation were measured. Superoxide dismutase (SOD) activity was assayed on gels, and the expression of six SOD genes was studied using real-time PCR. Mean growth rate was increased in high light (HL) regime as compared to low light (LL) regime. Parallely, most of SOD genes were overexpressed in Col and underexpressed in N1438 in response to HL. Plant growth was inhibited when 50 mM NaCl was present in the medium, differently according to the light regime and the accessions, and a complex pattern of SOD gene response was recorded. This pattern, including the differences between the accessions, could be interpreted as a consequence of interaction between light and salt, hypothesizing that oxidative stress occurred when light energy input exceeded energy utilization when salt inhibited growth, and that oxidative stress induced overexpression of some SOD genes. Salt-induced excess Na+ accumulation in leaves and limitation of K+ provision to these organs might also participate in eliciting SOD genes' response. Variability was observed between the two accessions for all these traits.

P3B-002
Down regulating an epigenetic-related protein, SlEZ2, in tomato induces metabolome modifications

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The term epigenetics refers to changes in gene transcription, which often leads to changes of gene expression and functionality. These changes remain through cell divisions and can last for multiple generations. However, there is no change in the underlying genomic DNA sequence of the organism. Epigenetic mechanisms include DNA cytosine methylation, covalent modifications of histones, chromatin structure, pathways recognizing aberrant transcripts and small RNAs (Grant-Downton and Dickinson, 2005). In this context, the Enhancer of zeste (E(z)) proteins, which belongs to the Polycomb Goup, are known to repress gene expression. This protein belongs to the Polycomb Repressive Complex 2 (PRC2), which sets up the trimethylation of the Lysine 27 on histone H3 (H3 K27me3) therefore repressing gene expression (Köhler and Villar, 2008). The aim of the present work was to obtain biochemical information to initiate the functional analysis of the tomato E(z) protein, SlEZ2, by analyzing the metabolic profiles of plants with a reduced SlEZ2 gene expression. Constitutive RNAi tomato plants were generated to down regulate expression of SlEZ2 in all plant organs. On one hand, plants from three independent transformation events were characterized using several morphological, anatomical and molecular traits. On the other hand, extracts of tomato fruits at two stages of development (orange and red-ripe) were analyzed using 1H NMR and LC-MS profiling. These data were processed using multivariate analysis. On a morphological basis, transformed plants showed a slightly different phenotype than wild-type plants. Though, some characteristic traits were not consistent for all plants. For fruits, the quantification of 21 polar metabolites by NMR including organic acids and sugars revealed several differences between fruits of RNAi transformed and wild-type plants. Besides, the analysis of LC-MS profiles highlighted other differences between transformed and wild-type fruits.

P3B-003
Metabolic characterization of systemic acquired resistance in BTH-treated *Arabidopsis thaliana leaves*

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In response to local infection many plants develop enhanced broad-spectrum resistance which protects the whole plant against subsequent pathogen attack. This type of immune response is referred to as systemic aquired resistance (SAR). Its onset requires the accumulation of salicylic acid and the coordinated expression of PATHOGENESIS-RELATED genes, which encode small secreted or vacuole-targeted proteins with antimicrobial activities or fungal cell wall degrading enzymes. Besides triggering by infection exogenous application of salicylic acid or one of its functional analogs such as 2,6-dichloroisonicotinic acid or benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) results in chemical induction of SAR. Although SAR has been known for a long time and extensively studied at transcriptome and proteome levels, characterization of this phenomenon at metabolite level is incomplete. In order to elucidate changes in the secondary metabolism of the model plant Arabidopsis thaliana during the onset of SAR, we applied non-targeted UPLC/ESI-QTOF-MS-based metabolite profiling on BTH-treated leaf material. To dissect SAR-related metabolic changes in wildtype lines within the performed time course experiment, an npr1 mutant line being strictly impaired in the formation of SAR was included in the experimental design. First results on the metabolism of BTH, salicylic acid and several indolic compounds, as well as technical details corresponding to the experimental design, explorative data analysis and structure elucidation will be highlighted on the poster.

P3B-004
Evaluation of the development and ripening processes of Hayward kiwifruits by NMR metabolic profiling

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Aim of this study is the metabolic characterization of the processes occurring during the development and ripening of the kiwifruit (*Actinidia deliciosa*, Hayward cultivar). The metabolic profiles of aqueous extracts were monitored over the season (June-December) using high field NMR Nuclear Magnetic Resonance (NMR) spectroscopy. A large number of water soluble metabolites was assigned by means of 1D and 2D NMR experiments. The change in the metabolic profiles monitored over the season allowed the kiwifruit development to be investigated. Specific temporal trends of aminoacids, sugars, organic acids and other metabolites were observed. PLS analysis of up to 29 metabolites who are present in the metabolic profile all over the observation periods shows that there are two main distinct processes characterizing the development of the Hayward kiwifruit: the first one occurs at an early stage and involves mainly the aminoacids metabolism, after which a second process involving the carbohydrate metabolism takes place. Further analyses over the two separate periods, including more metabolites that are detectable only in those stages, shed light over the nature of the metabolic relations that take place in the early and late profiles. The knowledge of the different metabolic routes occurring during the development of kiwifruits is a further step to answer questions about the treatment, harvest and storage times.

P3B-005

Progress in Chemometrics and Biostatistics for Plant Application: A Good Red Wine is a Bad White Wine

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Gas-chromatography coupled to mass spectrometry is one of the most widespread technologies applied for large scale screenings and discovery of new metabolite markers. We used this technique to analyze a dataset that aims to understand the contributions that the metabolome can make to the prediction of the enological quality of white and red wines. For that purpose we used the power of "simple" statistical analysis such as principal component analysis (PCA), hierarchical cluster analysis (HCA) and decision tree analysis (DT). The set of samples discussed in this poster comprises commercial wines,that have been quality graded using a numerical scale. The fundamental question addressed by this study of taste was the demonstration of volatile and soluble compounds from consumer wines which are associated with enological quality. The investigation was aimed towards the discovery of lead compounds for white wine quality, and is also seen as a prerequisite for future attempts to discover metabolic markers in the early processing steps of white wine production. In conclusion, we formally demonstrated using HCA and PCA that red wines tend to have metabolite patterns similar to inferior white wines. As red and white wine production is known to be aimed towards different taste optima we find the general saying that "A Good Red Wine is a Bad White Wine" is supported by our data set, hence the extended title. Acknowledgement This study was funded in part by the EU META-PHOR (FOOD-CT-2006-036220) and EU GRASP (ERA-Net Plant Genomics 0313996B) projects. Dr. J. William Allwood, Prof. Royston Goodacre (School of Chemistry, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK) Dr. S. Hilz, Landwirtschaftskammer Rheinland-Pfalz (D-67433 Neustadt/Weinstraße, Germany) Dr. E. Zyprian and Dr. R. Töpfer (Julius Kühn-Institute, JKI, Bundesforschungsinstitut für Kulturpflanzen Institut für Rebenzüchtung Geilweilerhof, D-76833 Siebeldingen, Germany)

P3B-006

Early response mechanisms of perennial ryegrass (Lolium perenne) to phosphorus deficiency.

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Improving phosphorus (P) nutrient efficiency in *Lolium perenne L.* (perennial ryegrass) is likely to result in considerable economic and ecological benefits. To date, there has been limited research into the global transcriptomic and metabolomic responses of perennial ryegrass to P deficiency and in particular to early response mechanisms to P-deficit. This study aimed to identify molecular mechanisms activated in response to the initial stages of P deficiency. A barley microarray was successfully used to study gene expression in perennial ryegrass and was complemented with a gas chromatography-mass spectrometry (GC-MS) based metabolomic approach to obtain an overview of the global response to early stages of P deficiency. After 24 hrs P deficiency, internal phosphate concentrations were reduced and significant alterations were detected in the metabolome and transcriptome of two perennial ryegrass genotypes. Results indicate a replacement of phospholipids with sulfolipids in response to P deficiency and that this occurs at the very early stages of P deficiency in perennial ryegrass. Additionally, the results suggested an important role of glycolytic bypasses and the re-allocation of carbohydrates in response to P deficiency

P3B-007

Metabolomic Analysis of a Stem Holoparasite Plant (Cuscutaceae)

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Cuscuta is a well-known stem holoparasitic plant without leaf and root that causes crucial problems for crops. Although several aspects of this plant have been studied, no detailed profile of its metabolites is available. Here, we applied metabolomic approaches with GC/MS metabolites profiling. We compared early-stage *Cuscuta japonica* seedlings, using a far red light (FR) cue and contact signal, with haustorium-induced seedlings as well as with adult plant parasites on host plants. Sugars, amino acids, nucleic acids, and polyols were identified from the polar phase fraction. Neutral sugars (e.g., fructose and glucose) were dominant in all specimens including host plants. Early-stage, seedlings contained a larger proportion of nucleic acids, and stimulating the far red light (FR) or contact signal decreased this. Metabolic profiling was generally similar between haustorium-induced seedlings and seedlings with touch stimulation only. This indicates that the metabolic change of the developing haustorium was provoked by touch stimulation. After attachment to host plants, the proportion of disaccharides (e.g., sucrose) was dominant. *Cuscuta japonica* absorbs other metabolites such as pinitol and quinic acid from host plants. At the same time, host plants increased certain metabolites after infestation, although these changes differed between host plants. The first metabolite profiling for plant-plant interactions showed that the sieve of *Cuscuta japonica* for absorbing metabolites has a certain selectivity.

P3B-008

Integrated transcriptomics and metabolomics reveals the involvement of the Arabidopsis thaliana ubiquitin ligase, NLA, in maintaining carbon and nitrogen metabolic homeostasis required for adaptation to nitrogen-limiting environments

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Plants growing under environmental conditions where nitrogen (N) is limiting undergo an adaptive reorganization of carbon (C) and N metabolism. However, the mechanism responsible for achieving C/N metabolic homeostasis during growth in these environments is poorly understood. Recently, we showed that the Arabidopsis thaliana nitrogen limitation adaptation (nla) mutant is incapable of undergoing adaptive responses that are crucial for survival during prolonged exposure to soils with insufficient N levels. The nla mutant displays a low N-induced early senescence phenotype compared to wild-type (WT) plants as a result of a deletion mutation in the RING domain of the RING-type ubiquitin ligase NLA protein. In order to gain insights into the metabolic processes regulated by the NLA, transcriptome and metabolome analyses of WT and nla mutant plants subjected to N-limitation were performed using a whole genome GeneChip array and GC/MS, respectively. The absence of a functional NLA protein in the nla mutant severely altered transcriptional responses to N limitation, resulting in differential expression of 3-fold more transcripts (>1700) compared to WT plants subjected to identical N-limiting conditions. Similarly, principal component analysis of GC/MS-based metabolic profiles of WT and nla mutant plants subjected to N-limitation showed that WT and nla mutant responses to N-limitation were distinct, as clear separation between their metabolic profiles occurred in PC plots that accounted for 90% of the variance. Discriminatory metabolites included several amino acids, and glycolysis and TCA cycle intermediates, a finding that was substantiated at the level of the transcriptome where an alteration in expression of transcripts encoding enzymes involved in these pathways was also observed. Our results suggest that an important component for Arabidopsis adaptation to N-limiting environments involves a fine balance between C/N metabolism. The involvement of the NLA in achieving C/N metabolic homeostasis during plant exposure to N-limiting environments will be discussed.

P3B-009

Ultra-performance LC/TOF MS analysis of the fruits of Ligustrum Lucidum for metabolomic research

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Objective: To establish an ultra-performance LC-quadrupole TOF MS (UPLC-Q-TOF-MS) method of the crude and processed fruits of Ligustrum Lucidum for metabolomic research, comparative study in their metabolite profiling was performed to explore the mechanism of processing the fruits of *Ligustrum lucidum*. Methods: Metabolite profiling of crude and processed fruits of *Ligustrum lucidum* were performed using UPLC-Q-TOF-MS and multivariate statistical analysis technique. Results: There is significant difference of metabolite profiling among the crude and different processed fruits of *Ligustrum lucidum*. The chemical markers such as Ligustaloside B for such variations was identified. And its contents in the crude *Ligustrum lucidum* was significantly higher than the one in processed one. Conclusion: This study indicated that UPLC-Q-TOF-MS coupled with multivariate statistics is able to provide quality control of the crude and processed fruits of *Ligustrum lucidum*. While the results provided the basis for the mechanism of processing.

P3B-010

Metabolic Alteration in Mung Bean Seedlings under Salinity Stress and Regulatory Role of Exogenous Proline and Glycinebetaine in Antioxidant Defense

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The purpose of this study was to assess the antioxidant protection offered by exogenous proline and glycinebetaine (betaine) against salt stress (150 and 300 mM NaCl, 48 h) by investigating the metabolites and the ascorbate-glutathione (AsA–GSH) cycle and catalase (CAT) enzyme activity in mung bean seedlings. Ascorbate (AsA) and glutathione (GSH) content increased dramatically with 150 mM NaCl stress, while their levels were only slightly increased with 300 mM NaCl stress with a sharp increase in the oxidized glutathione (GSSG) content, hydrogen perxoide, and lipid peroxidation level. Ascorbate peroxidase (APX) activity increased significantly with increased salt stress. Monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) activities were increased with 300 mM NaCl stress, but strong increases were observed with 150 mM NaCl stress. Dehydroascorbate reductase (DHAR) activity increased significantly with 150 mM NaCl stress whereas the activity declined with 300 mM NaCl stress. CAT activity declined upon the introduction of salt stress. Exogenous application of 15 mM proline or 15 mM betaine lead to an increase in AsA and GSH content, maintenance of a high glutathione redox state, and increased the activity of APX, DHAR, MDHAR, GR and CAT enzymes involved in the reactive oxygen species (ROS) detoxification, even under severe salt stress (300 mM NaCl), compared to the control and most other salt stressed plants, with a simultaneous decrease in GSSG content, hydrogen peroxide, and lipid peroxidation level. These findings suggest that both proline and betaine provide protective effects against salt-induced oxidative stress by reducing hydrogen perxoide and lipid peroxidation levels and by increasing the non-enzymatic antioxidants and AsA-GSH cycle and CAT enzyme activities. However, betaine was more effective than proline. *Corresponding author Email: fujita@ag.kagawa-u.ac.jp Telephone: +81-87-891-3133 Fax: +81-87-891-3021

P3B-011

Metabolic Flux Phenotyping of Excess and Limited Inorganic Carbon Supply in Cells of the Wild Type and Photorespiratory Mutants of the *Cyanobacterium Synechocystis* sp. Strain PCC 6803

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Gas chromatography-mass spectrometry (GC-MS) based metabolic flux phenotyping of primary metabolic pathways was performed in order to monitor the 13C-dilution after a very high 13CO2 (VHC) pulse in photoautotrophically grown cultures of Synechocystis sp. PCC 6803. The pulse was followed by a chase using a high 5% CO2 (HC) or a limited 0.035% CO2 (LC) regime of ambient isotope composition corresponding to the pre-acclimation conditions of the cells. We introduce the initial rate of 13C-accumulation 0.5-10 min after the pulse and maximum observed 13C-enrichment at 20-60 min after the pulse in metabolite pools and respective ratios over 3-phosphoglycerate (3PGA) or phosphoenolpyruvate (PEP) as flux phenotyping parameters. With these simplified tools we formally demonstrate 13C-flux into the glycolate pool under conditions thought to suppress photorespiration. Using the glycolate accumulating glcD1 mutant we demonstrate enhanced 13C-flux into the glycolate pool under conditions favouring photorespiration and enhanced 13C-flux into the glycine pool of the glycine accumulating gcvT mutant. We demonstrate two major paths of CO2 assimilation in Synechocystis under VHC conditions, namely from 3PGA via glucose-6-phosphate to sucrose and from 3PGA via PEP to aspartate, malate and citrate. The flux phenotypes of 3PGA and sucrose were consistent with previous knowledge on altered rates of photosynthesis under HC compared to LC and in the above mutants. Furthermore, the results reveal evidence of carbon channelling from 3PGA to the PEP pool and of enhanced flux through the PEP carboxylase path in the glcD1 and gcvT mutants under conditions favouring photorespiration.

P3B-012

Metabolome and lipidome analyses under nitrogen-deficient conditions in oil-rich algae, *Pseudochoricystis ellipsoidea*

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Oil-rich algae have promising potential for a source of next-generation biofuels. *Pseudochoricystis ellipsoidea*, a novel unicellular green alga, accumulates a large amount of lipids (oil droplets) under nitrogen-deficient conditions. Although these oil droplets are easily visualized by nile red fluorescence staining in the cells, little is known how oil droplets are synthesized in the metabolisms. Clarification of the metabolic changes under nitrogen-deficient conditions is important to understand the physiological mechanism of lipid accumulation, and it will be useful to optimize culture conditions for efficient lipid accumulation. In this study, *P. ellipsoidea* was grown in flat flasks under continuous illumination (200 µmol m-2 s-1) and aeration with 1% CO_2 at 25°C. Metabolome and lipidome profiles were obtained from *P. ellipsoidea* under both nitrogen-rich (rapidly growth) and nitrogen-deficient (lipid accumulating) conditions using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and liquid chromatography TOFMS (LC-TOFMS), respectively. Relative quantities of approximately 300 metabolites including central metabolites, free fatty acids, glycerolipids, glycerophospholipids and glycolipids are systematically compared between these two conditions by using our proprietary software. As a result, the levels of more than twenty central metabolites were found to differ by more than two-fold. We found that most of these metabolites are involved in arginine metabolism which is closely relate to de novo synthesis of amino acids. In lipid metabolisms, the quantities of most triglycerides (TGs) were greatly increased in nitrogen-deficient conditions; however, those of nearly all the other lipids were either decreased or only slightly altered. We also report the morphological changes under nitrogen-deficient (lipid accumulation) conditions, and discuss the relationship between the metabolic changes and morphological changes.

P3B-013
Metabolomics of Arabidopsis lap5 and lap6 Pollen Mutants

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Arabidopsis less adhesive pollen mutants, lap5 and lap6, have abnormal anther and pollen exine morphology. Anthers of these mutants appear glossy and the pollen lacks the characteristic reticulate structure. Molecular mapping and sequence analyses revealed that lap5 and lap6 genes encode anther-specific proteins with homology to chalcone synthase (CHS), a key flavonoid biosynthetic enzyme that catalyzes the condensation of coumaroyl-CoA and malonyl-CoA into naringenin. To further investigate the functions of lap5 and lap6 genes, metabolomics was used to profile anthers of lap5 and lap6 mutants. UPLC-qTOF MS and GC-MS analyses of anther methanolic extracts revealed a dramatic reduction in flavonoids and carbohydrates in the mutant anthers. Naringenin chalcone and naringenin, the first two compounds in the flavonoid pathway from which all flavonids are derived, were significantly reduced in the lap5 and lap6 mutants, and completely absent in the lap5/ lap6 double mutants. Other flavonoids were also found to decrease in the mutant anthers, suggesting the roles for the Lap5 and Lap6 proteins in flavonoid biosynthesis in anthers. However, lap5 and lap6 mutants could not be complemented with the well characterized Arabidopsis CHS gene, tt4. In vitro enzymatic assays with the recombinant Lap5 and Lap6 proteins showed that they were capable of catalyzing the condensation of coumaroyl-CoA and malonyl-CoA into bisnoryangonin (BNY) but not naringenin, indicating that Lap5 and Lap6 proteins are different from CHS (tt4). They were also found to catalyze the formation of n-alkyl pyrones from different fatty acyl-CoAs and malonyl-CoA in vitro. However, whether these fatty acyl-CoAs are the physiological substrates of Lap5 and Lap6 proteins remains to be determined as CHS also showed similar activity in vitro and no n-alkyl pyrones were observed in the wildtype. In addition to flavonoids, some carbohydrates including sucrose and glucose also decreased significantly while several non-aromatic amino acids increased dramatically. These findings demonstrate that a single gene mutation can result in the alternation of a wide spectrum of metabolites ranging from primary to secondary metabolites, revealing the complexity of interactions among metabolic pathways.

P3B-014
Metabolomic reprogramming mediated by the transcription factor AtbZIP11 in Arabidopsis: a connection to trehalose metabolism

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AtbZIP11 is one of the transcription factors priming the global transcriptomic reprogramming down stream of SnRK1 (plant homolog of animal AMPK and yeast SNF1 complex) in response to energy depletion. ASN1 and ProDH2 have been identified as its direct targets, which are key genes involved in asparagine and proline metabolism, respectively. Here, we report that AtbZIP11 mediates metabolomic reprogramming in seedlings as the consequence of its function on the transcriptome. The metabolic changes largely occur in primary metabolism. In detail, the levels of sugars and sugar phosphates are elevated together with increased level of several aromatic amino acids. Whereas the proline level is severely decreased, which is the consequence of the induced expression of ProDH2 by AtbZIP11. Furthermore, we find AtbZIP11 induces the expression levels of several trehalose metabolism associated genes which results in increased enzymatic activity thereby altering the level of associated metabolite. The metabolite from trehalose metabolism, trehalose 6-phosphate (T6P) is an essential signaling molecule that controls plant growth, while the quantification of T6P is difficult. Trehalase is transcriptionally induced by AtbZIP11 and this leads to increased trehalase activity as assayed. T6P level is altered in the seedlings over expressing AtbZIP11 as determined by HPAEC-MS. Recently, it has been reported that T6P inhibits SnRK1 activity at physiological concentrations. Thus our findings shed light on understanding the regulatory circuit of SnRK1/bZIP11/T6P in response to energy depletion.

P3B-015
LC-MS Based Untargeted Plant Metabolomics Approach : the study of salicylic acid response in Arabidopsis thaliana

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Plant can display a remarkable reduction in its susceptibility to future infection after its recovery from disease. This systemic acquired response (SAR) results from infection with necrotizing pathogens and lead to whole plant systemic resistance not only to inducing agent but also to a broad spectrum of other pathogens such as virus, fungus, and bacteria. Though SAR is the best-studied salicylic acid (SA)-requiring systemic immune response, many things remained to be elucidated. Metabolic profiling is of growing importance in plant functional genomics field offering a direct link between a gene and function of metabolic network in plants. Following up the dynamic behavior of metabolome can offer a clue to decipher the biological roles of gene and metabolites at the conditional perturbation. In this study, we applied ultra performance liquid chromatography/quadrupole time of flight mass spectrometry (UPLC/Q-TOF MS) based metabolite profiling and multivariate data analysis in SA response defective mutant or transgenic Arabidopsis plants - npr1, dir1, nahG. Multivariate analysis using chromatography retention time and mass spectrum discriminated the control and SA treated group and three SA-response defective mutants and wild type plants. It also discriminate local and systemic leaf groups. Using this approach, we got list of potential salicylic acid responsive ions.

P3B-016
Clarification of metabolite compartmentalization by metabolomics in a single cell of the alga *Chara australis*

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The process of metabolite compartmentalization in the cell is not thoroughly understood. The analysis of metabolites in single organelles has consequently presented a significant challenge. We have developed a single cell metabolomic analysis system by applying a single vacuole and cytoplasm isolated from a single giant internodal cell of the alga Chara australis to the CE-MS (capillary electrophoresis / mass spectrometer) –based metabolomic technique. We determined that compartmentalization of 125 known metabolites found in vacuole and cytoplasm of C. australis was different under changing light conditions. Under various stress conditions such as high temperature, localization and dynamics of metabolites fluctuated asynchronously between vacuole and cytoplasm, suggesting a spatial regulation of metabolites in a cell. By using a microinjection technique, metabolite transportation across the vacuolar membrane can be directly detected, suggesting a previously unknown function of the vacuole. Our metabolomics approach provides novel insights on metabolic dynamics in a single organelle derived from a single cell. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN).

P3B-017
Lipid Profiling by LC-MS Revealed a Unique Glycerolipid Class Related to Phosphate-Limiting Growth of Arabidopsis thaliana

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Phosphorus limitation leads to a drastic change in membrane lipid composition in plants. Lipidome analysis by liquid chromatography-ion trap mass spectrometry confirmed compositional changes of membrane lipid in *Arabidopsis thaliana* by phosphorus limitation, i.e., decreases of phospholipids (PC, PE, PG, and PI) and increases of two glycoglycerolipids (DGDG and SQDG). In addition to these well-known changes of lipid profiles, an unknown lipid group was found to accumulate in the leaves of A. thaliana by phosphorus limitation. Based on the MS/MS analyses, this inducible lipid group was elucidated as diacylglycerol bound to hexuronosyl moiety. During the course of the investigation, an Arabidopsis mutant, which does not accumulated this hexuronosyldiacyglycerol under phosphate-limiting condition, was isolated. Since a glycosyltransferase gene was disrupted in this mutant, this glycosyltransferase is postulated to be involved in the final condensation reaction of diacylglycerol and hexuronic acid. Under phosphate-depleted condition, the mutant showed an enhanced senescence compared with the wild-type plant, suggesting that the hexuronosyldiacyglycerol plays a role to mitigate the depletion stress of phosphorus in A. thaliana. This inducible lipid group was also found in leaves of rice, and the level of this lipid increased by phosphorus limitation, suggesting a general physiological significance of this lipid class across plant species.

P3B-018
An efficient antioxidant defense system enables *Pennisetum typhoides* to tolerate As-induced oxidative stress

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Arsenic contamination in food chain is a serious environmental problem especially in south East Asia. Although As is not a redox active metal but reports has been suggested that As causes oxidative stress by production of reactive oxygen species during conversion of arsenate to arsenite. However, few attempts have already been made to understand the uptake and accumulation of As in plants. But, no serious effort has been made so far to evaluate As induced oxidative stress in crop plants. Therefore, Present study is aimed to determine role of antioxidant defense system against As induced oxidative stress in *Pennisetum typhoides*. Seedlings of test plant were grown hydroponically in Hoagland nutrient solution enriched with different concentrations of As (0, 10, 25, 50, 100, 200 μM) for 10 days. Time course study was carried out at sublethal concentration of As (50 μM). High antioxidant potential was exhibited by enhanced level of thiol content and activities of antioxidant enzymes viz. SOD, CAT and APX in *Pennisetium typhoides* exposed to higher tested concentrations of As (100, 200 μM). This finding was further supported by lesser growth inhibition, lipid peroxidation, with subsequent reduction in chlorophyll and carotenoid content in test plants exposed to elevated concentrations of As. Based on available evidences the present study suggests that antioxidant defense system plays an important role in protection from As induced oxidative damage in *Pennisetum typhoides*.

P3B-019
Analysis of photosynthetic carbon assimilation by a combination of in vivo 13C-labelling from 13CO2 and LC-MS/MS, GC-MS analytic platforms

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Over the years labelling of plants with CO2 using radioactive or stable carbon isotope enabled to discover the main entry point of CO2 into plant metabolism and to characterize photosynthetic carbon assimilation. Recently availability of stable isotopes and improvement of analysis methods (e.g. mass spectrometric analysis) opens new possibilities for plant metabolism research. To further understand the control of metabolic pathways in plants, we needed sensitive and robust analytical methods to measure metabolic turnover rate. For this purpose, additional LC-MS/MS and GC-MS methods were established allowing the quantification of all isotopomers of a total of 40 metabolites. These include metabolites from the Calvin cycle, sucrose and starch synthesis, TCA cycle, glycolysis and organic acid metabolism. By combining these analytical platforms with an in vivo 13C-labelling system from 13CO2 , the time course of the 13C-labelling ratio in each metabolite were determined in A. thaliana rosette leaves. The results showed that metabolites from the Calvin-Benson cycle were almost completely labelled within a minute, whereas amino acids and metabolites from the TCA cycle presented a 13C-labelling occurring after several minutes and representing only a fraction of these metabolite pools. In future described approach will be applied to study metabolic turnover with a special emphasis on the photorespiration process under different photorespiratory conditions. In addition, metabolic fluxes analysis will be determined on the sub-cellular level by using non-aqueous fractionation coupled with in silico modelling. Literature: Arrivault et al., 2009, Plant Journal, 59(5): 826-839"

P4A-001

Automated GC x GC-TOF MS urine analysis - application to diagnosing of inherited metabolic diseases

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Background: Urine represents an extremely complex matrix; hundreds of structurally different compounds with great concentration variability have been identified so far. Frequently occurring coelutions make comprehensive analysis a challenging task. GC/MS has been traditionally used for human urine analysis, e. g. in diagnosing so-called organic acidurias. We reported here an automated GC x GC-TOF MS approach for urine analysis. Methods: Urine samples with suboptimal average analytical results reported in the quality control schemes in diagnosing of inherited metabolic disorders were selected to evaluate the performance of our approach. Samples were ethoxymated, extracted into ethyl acetate and derivatized to the trimethylsilylderivates. Analyses were performed on GC x GC (Agilent 6890 with LECO thermal modulator) coupled with TOF MS (LECO Pegasus 4D). The nonpolar/polar (RXi-5 MS, 30 m x 0.25 mm/BPX-50, 2.5 m x 0.1 mm, both Supleco) column arrangement was chosen. Splitless injection volume was 0.2 uL at 250 OC. MS acquisition rate was 125 Hz at the range m/z 35 - 550. An automated data processing was performed by the ChromaTOF software (LECO). Results: Urinary profiles of healthy children and samples from patients with inherited disorder were analyzed. The list of normal and pathological metabolites (so called reference, which includes retention characteristics and mass spectra) was created. After applying the reference automated identification and quantification of pathological metabolites was performed. Diagnosing of even very difficult samples that were missed by GC/MS was possible by our approach. Conclusions: Using this approach all pathological compounds were correctly assigned and quantitative data allowed establishing proper diagnosis in all samples. Supported by grants from Iceland, Liechtenstein and Norway through the EEA Financial Mechanism and the Norwegian Financial Mechanism (A/CZ0046/2/0011), MSM6198959205 and Internal grant agency of Palacky University grant No. LF_2010_013.

P4A-002

Assessing the use of aminopterin as internal standard for methotrexate measurement using LC-MS/MS

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BACKGROUND: The folate antagonist methotrexate (MTX) is the most widely used drug in the treatment of rheumatoid arthritis. The therapeutic effects of MTX can be attributed to the intracellular levels of MTX, which is present in the cell as polyglutamates (MTXPGn). Assessment of intracellular MTXPGn levels might predict how well patients respond to treatment. We developed an LC-MS/MS based assay to determine MTXPGn in red blood cells (RBC). To be able to quantify MTXPGn an internal standard displaying similar behaviour as MTXPGn has to be used. Because of the high cost of a stable isotope-labelled internal standard, we have looked for a suitable alternative. Based on literature and structure we decided to use aminopterin (AMP) for that purpose. The aim of this study was to determine whether AMP is indeed a suitable internal standard for our assay. METHOD: RBC were isolated from fresh whole blood by centrifugation. The RBC pellets were spiked with several concentrations of MTXPGn (2-1000nM) using 400nM AMP per sample. RBC pellets were lysed by 1:1 reconstitution in H2O and protein removal was done with 7% perchloric acid and subsequent centrifugation. Concentration was measured by liquid chromatography with tandem MS. LC separation was performed using a 5-100% organic gradient of 10mM Ammonium Bicarbonate (pH10) and Methanol. Due to the properties of our method, AMP co-elutes with MTXPG3 at 2.7 min. RESULTS: AMP had considerably more variation (CV 15-20%) in signal than any of the MTXPGn (CV <10%) at a comparable concentration. Response of AMP was six-fold weaker than MTXPG3 and five-fold weaker than MTXPG1 despite similar elution (MTXPG3) or structure (MTXPG1). Also, the concentration of AMP decreased 25% over 12 hours. CONCLUSION: Within the specifications of our method aminopterin is not a suitable internal standard for the measurement of MTXPGn.

P4A-003

Screening of Endogeneous Substrate of Organic Cation Transporter 2 by GC-TOF Based Metabolomic Analyses

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Our previous study revealed that the genetic variants of SLC22A2 identified in a Korean population appear to have a significant impact on the disposition of metformin, a substrate of OCT2. As expected from the primary distribution of OCT2 in the kidney, the tubular excretion was influenced mainly by the c.808G>T variants of SLC22A2, leading to an increase in plasma metformin concentrations in subjects with this variant. In this study, we investigated the potential endogeneous marker related on OCT2 activity through global metabolomic analyses. The untargeted metabolite profiling was performed on urine samples obtained from healthy subjects with different OCT2 genotype using GC-TOF mass spectrometry. Multivariate analysis separated three groups (WT, 808GT, and 808TT) by PLS-DA vector 1+3+4. The results demonstrated OCT2 genotype effects on the level of several primary metabolites such as tryptophan, oxoproline, glycine, alanine, uridine, and taurine. The substrate specificity of these metabolites for OCT2 protein is under investigation.

P4A-004

Targeted Metabolomics: Qualitative and Quantitative Analysis Using GC-QQQ Mass Spectrometry

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Triple quadruple (QQQ) mass spectrometers coupled with liquid chromatographs (LC) are well established techniques. In recent years, the benefits of this detection technique motivated the development of gas chromatographs (GC) coupled with QQQ, which are well suited for targeted analysis of small molecules in complex matrices due to their excellent selectivity and high sensitivity and signal-to-noise ratios. In this presentation, the human standard plasma from NIST and standard human serum supplied by European serum was screened for fifty common metabolites simultaneously, setting multiple reaction monitoring (MRM) transitions for each metabolite. Absolute concentration results from NIST plasma and European standardized serum samples are compared using univariate statistical techniques to discriminate between two different sample groups. Drug screening: Propofol was found unsuitable for quantification in LC-QQQ mass spectrometry due to the lack of suitable product ions. However, after derivatization for GC-QQQ MS, both precursor and product ions were detectable and optimized for selectivity. A linear range of quantification was obtained from 0.05 µg/ml to 1 µg/ml for propofol and 0.25 µg/ml to 25 ug/ml for guaifenesin. Method of detection and limit of quantitation were 0.23 µM and 0.56 µM for propofol, and 0.25 \µM and 0.50 µM for guaifenesin. Metabolomics: Chromatograms of replicate standard human plasma were processed for identification of detectable metabolites. Fifty compounds were unambiguously assigned by the Mass Hunter software using the Fiehn/Agilent Metabolomics Library. Subsequently, product and precursor ions were selected for each metabolite. The MS acquisition method was divided into time segments to enhance the sensitivity and selectivity. For each compound, selectivity was validated by three different parameters, i.e. the retention time and the two m/z values for MRM transitions. Semi-quantitative concentration results are calculated using Mass Hunter QQQ/Quantitation software.

P4A-005

Pharmacometabolic investigation can predict cardiovascular adverse effects of fluoroquinolone drugs

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Some new drugs were forced out from the market due to their fatal cardiovascular side effects. The possibilities of inducing the cardiovascular toxicity cannot be easily determined by traditional safety pharmacology experiments; therefore, it is necessary to establish predictable indices for securing safety from the cardiovascular side effects with rapid and accurate detecting techniques. So we tried to apply the pharmacometabolomics approach for developing new biomarkers to evaluate cardiovascular toxicity of drugs It is considered that QT prolongation at the initial stage of developing drugs should be estimated with a special method for the preclinical trial to evaluate the cardiac toxicity. In this study we used guinea pigs which are suitable to measure the proarrhythmic effect since they have a specific ion channel fairly similar to that of human, especially the hERG channel that is known to cause QT prolongation. Guinea pigs underwent intravenous medication of Sparfloxacin and Levofloxacin known to have potential QT prolongation effect. Plasma samples were collected before and after medication. QT intervals normalized by RR interval were measured. Plasma metabolomics profiling was performed using liquid chromatography - mass spectroscopy (LC-MS). Percent change of QT intervals had correlation with drug dose. Score plot of PCA carried out with metabolomic data showed distinct two groups; control and drug dosed groups. PLS was carried out using metabolomics data as X variables and percent change of QT intervals as Y variables. The metabolites which show the highest VIP values(>1.5) in the finally selected model were selected and identified, and then used to determine the metabolic network. As a result, the cardiotoxicity of sparfloxacin was revealed through the actions of steroids, phospholipids, and sphingolipids in plasma; Whereas the cardiotoxicity of levofloxacin was appeared intensively through the actions of steroids in plasma. As a conclusion, the cardiotoxicity of fluoroquinolones is considered to be caused mainly by the actions of steroids in plasma. Especially lanosterol, pregnenolone, and progesterone involved in C21-steroid hormone metabolism, a common network of plasma, were found to be the most useful intrinsic biomarkers for the prediction of cardiotoxicity.

P4A-006

High-throughput screening of inhibitory effects of BHT (Bo-yang- hwan-o-tang) on human cytochrome P450 isoforms in vitro using liquid chromatography/tandem mass spectrometry.

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The detection of potential drug-herb interactions is an important issue of drug industry in korea, commonly used herbal medicine. We developed the economically feasible and time-saving high-throughput screening method for potential interactions with inhibitory drugs for nine human P450 enzymes and BHT(Bo-yang-hwan-o-tang), an oriental herbal medicine for treatment of brain disorder. The inhibitory effects of BHT on catalytic activity of major CYP isoenzymes(CYP1A2, CYP 2A6, CYP2C8, CYP2C19, CYP2D6, CYP3A4, CYP 2B6, CYP2C9, CYP2E1) were assessed by using cocktail incubation and tandem mass spectrometry in vitro. Two cocktail set were used for minimize the solvent effects and drug interactions each other. Cocktail A was consists of phenacetin for CYP1A2, coumarin for CYP2A6, paclitaxel for CYP2C8, omeprazole for CYP2C19, dextromethorphan for CYP2D6, and midazolam for CYP3A4. Cocktail B was consists of bupropion for CYP2AB6, tolbutamide for CYP2C9, chlorzoxazone for CYP2E1. Each two substrate cocktail sets were incubated with human liver microsomes. The metabolic reactions were terminated with ACN containing chlorpropamide as an internal standard. After centrifugation, the supernatant of reaction mixture was pooled and analyzed at the same time by using UPLC/MS/MS with fast gradient. The concentrations of the substrate metabolites – acetaminophen, hydroxycoumarin, hydroxypaclitaxel, hydroxyomeprazole, dextrorphan, hydroxymidazolam, hydroxybupropion, hydroxytolbutamide, hydroxychlorzoxazone – were represented by substrate metabolite / IS ratio. This study suggests that BHT extract has no inhibitory effects on human CYP isoforms. So We concluded that BHT might be free of drug-herb interactions when co-administrated with other western medicine. The high-throughput screening method used in this study can be useful tools in the drug discovery and for understanding drug interactions.

P4A-007

Treatment effects of rimonabant and a multi-targets approach on plasma lipidomics in ApoE*3 Leiden CETP transgenic mice

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Obesity and its related diseases such as diabetes, cardiovascular diseases, dyslipidemia, are becoming a public health concern globally. Using lifestyle modification, diet and exercises intervention to control obesity have obtained sub-optimal results and limited long-term efficacy. Therefore, safe and effective treatments for obesity are in high demand. Rimonabant, a selective cannabinoid-1 receptor antagonist for obesity treatment, targets on the endocannabinoid system, which regulates food intake, lipid and glucose metabolism in the central nervous system and peripheral tissues. Both pre-clinical and clinical studies showed that rimonabant intervention result in long-term maintained weight loss and optimizing cardio-metabolic risk factors including insulin resistance and lipid profiles. But its psychiatric adverse events including depression and suicidal attempts withdrew rimonabant's general application. A Chinese multi-herbal formula (namely SUB885C in this poster) using multiple -targets approach to regulate body weight and dyslipidemia has long been used in China for treatment of metabolic syndrome, in particular early stage of diabetes type 2 in combination with obesity. SUB885C contains eight natural herbs: Fructus Crataegi, Folium Nelumbinis, Folium Apocyni, Flos Rosae rugosae, Radix et Rhizoma Rhei, Depuratum mirabilitum, Thallus Sargassi, Honey fried Radix Glycyrrhizae. One SUB885C intervention study on male ApoE*3Leiden transgenic mice with pre-diabetes has already shown its control effect on insulin resistance. The aim of the present study is to further evaluate the treatment effects of SUB885C on weight and dyslipidemia regulation. ApoE*3 Leiden CETP female mouse model was used to compare treatment effects of SUB885C, rimonabant and the placebo control on body weight and plasma lipidomics. After 2-week intervention, three treatments showed a separation trend in lipidomics pattern. Only rimonabant showed a significant weight reduction while SUB885C showed a wide range regulation on cholesterol esters, triglycerides, sphingomyelin and fatty acids and result in a prominent optimization in lipid profile.

P4B-001

Metabolomics of tree root exudates

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The root exudates are compounds continuously synthesized and secreted by plants in their immediate environment. With up to twenty percent of all photosynthetically fixed carbon transferred by roots to the rhizosphere via exudation, the root exudates represent a major natural source of plant metabolites. To avoid biological and chemical interference caused by the interactions characterizing the rhizosphere environment, root exudates are commonly investigated in hydroponics system, where herbaceous plants are grown or sampled under axenic conditions. Less is known about the role and composition of root exudates secreted by arboreal species cultivated in their natural habitat conditions. In this work, we developed an original procedure for in-vivo sampling of tree exudates, consisting in carefully introducing root tips into differently filled sampling tubes, while leaving the plant in place. The collected samples were filtered, dried, derivatized to produce \tert-butyldimethylsilyl or trimethylsilyl-methoxime derivatives and subjected to gas chromatography-mass spectrometry metabolomic analysis. Statistical comparison between blank and sample runs allowed significant qualitative and quantitative identification of about three hundred metabolites, including amino, organic, keto and fatty acids, poly-alcohols, phenolics, mono and polysaccharides, in exudates released, under various conditions, by *Euonymus spp., Pinus sylvestris* and other arboreal species. Also, we recorded changes in exudate composition, at the single tip level, due to the presence of *Pseudomonas putida* IsoF/gfp. During these investigations, the following observations emerged. When a root tip was exposed to water, the exudation process reached its peak concentration in thirty to sixty minutes, after which the exudation rate decreased abruptly. One-hour exudation patterns in physiological solution of single tips, sampled when the rest of the root system was immersed in water, clustered separately from their counterparts sampled when the plant was left in soil. This highlighted that, despite exudation is commonly under peripheral control, metabolites may be released under central control, in response to events involving the tree as a whole, like flooding of the root system. At night, compared to the day, the accumulation rate of half compounds identified from tip exudation was increased by two to six times.

P4B-002

Applications and Tools in Plant Metabolomics at Bordeaux Metabolome-Fluxome Facility (PMFB; http://www.bordeaux.inra.fr/umr619/NMR.htm)

Catherine Deborde, Stéphane Bernillon, Mickaël Maucourt, Cécile Cabasson, Patricia Ballias, Guillaume Ménard, Hélène Ferry-Dumazet, Laurent Gil, Daniel Jacob, Yves Gibon, Dominique Rolin and Annick Moing. Main collaborations: in Japan (Univ. of Tsukuba - C. Matsukura), in Israel (Volcani center, A. Schaffer), in UK (IFR Norwich - I. Colquhoun and M. Defernez; Univ. Manchester -R. Goodacre and W. Allwood), in Ireland (Teagasc - J. Valverde) in France (Avignon, M. Génard, JL. Poëssel; Bordeaux, D. Thiéry,Montpellier, F. Tardieu).

The Bordeaux Metabolome-Fluxome Facility (PMFB) develops and applies plant metabolomics for local, national and international projects. Applications range from the characterization of plant derived extracts to systems biology: 1- Quantitative metabolic profiling of plant organs or tissues by 1H-NMR [1,2,3, J. Valverde Teagasc)] 2- Plant metabolomics by LC-HRMS. 2- Robotised high throughput measurements of metabolite concentrations and enzyme activities and -kinetics [4]. 3- Storage of metadata and raw data and biostatistical analysis. A web-based application, “Mery-B” (Metabolomics Repository of Bordeaux) is being developed in collaboration with the Bordeaux Bioinformatics Centre (http://bit.ly/meryb) 4- Identification of metabolic markers of environmental changes [5] or agricultural practice [6] 5- Characterization of plant extracts having bioactive properties (JL. Poëssel Avignon and D. Thiéry Bordeaux). 6- Characterization of mutants [7] and transformants ([8], France and Japan) for candidate genes for grain or fruit quality. 7- Screening of genetic resources for fruit composition (A. Schaffer, Israel and META-PHOR consortium http://www.meta-phor.eu/; ISAFRUIT consortium http://www.isafruit.org) and resistance to water stress (F. Tardieu, Montpellier, FP7 DROPS) 8- Integrative modelling of tomato fruit metabolism (ERASysBio+ FRIM) 9- Integration of metabolomics data with other ‘omics data for the study of fleshy fruit development and metabolism [8,9]. In this poster, we will provide an overview of the major features of some of these metabolomics studies and tools developed at Bordeaux Metabolome-Fluxome Facility. References: 1 Moing et al. (2004) Funct. Plant Biol. 31:889–902. 2 Mounet et al. (2007) Metabolomics 3:273-88. 3 Biais et al. (2009) Anal. Chem. 15:2884-94 4 Gibon et al. (2004) Plant Cell. 16:3304-25 5 Pereira et al. (2006) Anal. Chim. Acta 563:346-52 6 Deborde et al. (2009) Metabolomics 5:183-98 7 Cossegal et al. (2008) Plant Physiol. 146:1553-70 8 Garcia et al. (2009) C. R. Biologies 332:1007-21 9 Mounet et al. (2009) Plant Physiol. 149: 1505-28

P4B-003

Applications and Tools in Plant Metabolomics at Bordeaux Metabolome-Fluxome Facility (PMFB; http://www.bordeaux.inra.fr/umr619/NMR.htm)

Catherine Deborde, Stéphane Bernillon, Mickaël Maucourt, Cécile Cabasson, Patricia Ballias, Guillaume Ménard, Hélène Ferry-Dumazet, Laurent Gil, Daniel Jacob, Yves Gibon, Dominique Rolin and Annick Moing. Main collaborations in: Japan (Univ. Tsukuba- C. Matsukura), Israel (Volcani center, A. Schaffer), UK (IFR Norwich - I. Colquhoun and M. Defernez; Univ. Manchester-R. Goodacre and W. Allwood), Ireland (Teagasc- J. Valverde); France (Avignon- M. Génard, JL. Poëssel; Bordeaux- D. Thiéry; Montpellier- F. Tardieu).

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P4B-004

NMR Discrimination of Ginseng Landraces

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Ginseng cultivation in Ontario began over 100 years ago when seed was cultivated from wild ginseng plants. Today Ontario ginseng *(Panax quinquefolius)* comprises several unimproved landraces, which are farmer selected populations that have likely diverged significantly from wild populations, developing separately for several decades. In a previous study, using HPLC-DAD ginsenoside content within and between Ontario ginseng landraces was examined, using 6 major ginsenosides (Rg1, Re, Rb1, Rc, Rb2, Rd) as markers. Using this method, it was clear that significant variation in ginsenoside content does occur between landraces though unique characteristics based on these markers could not be identified which would rapidly identify each landrace. There is great interest in identifying unique characteristics of landraces as differences in the phytochemical characteristics could lead to the development of unique cultivars with unique activities. For this purpose, NMR has been used, examining the metabolic profile of Ontario ginseng, with the potential to identify a unique fingerprint for each landrace. This has important quality control and standardization implications along with the possibility that distinctive landrace characteristics may be correlated to unique activities. This current work evaluates the use of NMR spectroscopy for providing information on the amount of desired components and the ‘fingerprinting’ of landrace. In this work we present our progress towards developing a NMR based quality and fingerprinting screen to provide information such as: 1. discrimination analysis of different landraces 2. relative or absolute quantity of key components present in the crude extract 3. quantitative distribution of ginsenosides

P4B-005

Metabolic distance, a novel feature in metabolomics

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Since the boom of metabolomics in 2000, a few approaches have been introduced to study the metabolome. Metabolite fingerprinting approach, the unbiased global screening of metabolome, and multivariate data analysis has been extensively used to classify or group samples by metabolites patterns or “fingerprints”. These groupings result from observational evidences on a paper or screen that due to limitations in our imaginary power and visualization techniques involve just two or three components of variation. They do not incorporate other components of variation, although some of those components contain relevant biological variation and are important for understanding the metabolic relationships between a priori group of samples. These problems become more complex by an increased number of a priori groups in the experiment. In this research, we fingerprinted the natural metabolic variation of nine *Arabidopsis thaliana* accessions grown in four different environments. High through-put untargeted metabolomics by three type of analytical techniques was exploited to produce fingerprints of a wide range of metabolites. We applied current statistical methods on the precious fingerprints data and elaborated on the metabolic relationship of accessions. For the first time, a metabolic distance between *A. thaliana* accessions was calculated. The magnitude of sources effects and their interaction on the metabolic variation in different environments and in different analytical methods were compared. Moreover, we quantified the correlation between the metabolic and the genetic diversity between the set of analyzed accessions.

P4B-006

Metabolic engineering of astaxanthin biosynthesis in Capsicum annuum

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Astaxanthin (3,3’-dihydroxy-β, β-carotene-4,4’-dione) is a high-value ketocarotenoid, having powerful antioxidative activity and protection against a broad range of human diseases. In nature only some organisms synthesize astaxanthin. The health benefits and high cost of natural astaxanthin (~US$7000/kg) have attracted considerable interest in engineering astaxanthin pathways in potential hosts. β-Carotene ketolase (BKT) and hydroxylase (CHYb) catalyze the rate-limiting steps for astaxanthin formation from β-carotene. Plants generally lack BKT activity and synthesize only significant amounts of carotenes and their hydroxyl derivatives. Plants are therefore promising hosts to be engineered as astaxanthin producers by expressing a microbial BKT gene. One major challenge of engineering astaxanthin pathways in plants is to achieve optimal biosynthetic capacity of astaxanthin. We hypothesize that the specific function of BKT enzyme and the limited supply of accessible β-carotene may be the rate-limiting factors and bottleneck for astaxanthin formation in plants. Capsicum annuum has the ability to accumulate high amounts of the keto-carotenoids capsanthin and capsorubin. Pepper accessions with deletion of capsanthin-capsorubin synthase (CCS) gene generate yellow fruits enriching zeaxanthin. Our study focused on addressing our hypothesis by introducing a novel BKT gene into yellow fruit pepper. Seven BKT genes were isolated from 5 astaxanthin-producing green algae, which were functional characterized and further modified for high catalytic activity in ketolating zeaxanthin to astaxanthin. An efficient regeneration system of the pepper accession was established, by which the modified BKT gene was transferred into the plant. A number of transgenic peppers have been developed and analyzed for the biosynthesis of astaxanthin in the plants.

P4B-007

1H-NMR based metabolic profiling of cytokinin-receptor mutants of Arabidopsis plant

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Arabidopsis has been used as a model plant in plant biotechnology research due to its relatively small genome size, short life cycle, and easiness of cultivation. Cytokinin is a plant hormone that plays various regulatory roles in many aspects of plant growth and development. In this study, metabolic profiling of wild-type (control) and cytokinin-receptor mutants (ore3, ore12) of Arabidopsis plant was performed using 1H-NMR spectrometry coupled with multivariate statistical analysis and the metabolic changes were investigated by the acceleration of aging by cultivating the Arabidopsis plants under darkness for 0, 2, and 4 days. Leaf senescence was significantly delayed in the cytokinin-receptor mutants. Various metabolites, such as isoleucine, leucine, valine, threonine, alanine, acetate, glutamate, malate, asparagine, histamine, malonate, glucose, fumarate, gallic acid, tryptophan, phenylalanine, and formate, were assigned in the 1H-NMR spectra of aqueous extracts of Arabidopsis leaves. In addition, there was a clear separation between samples in the score plots derived from principal partial least squares discriminant analysis (PLS-DA) of 1H-NMR data sets. By loading plot analysis derived from PLS-DA, the compounds contributing to the separation of each sample in the score plots was elucidated, and the major difference of metabolic profiles during senescence process of each sample was compared and discussed in the view point of aging of Arabidopsis plants.

P4B-08

Technology developments in metabolite profiling, large-scale annotations and correlation analysis, and their applications toward biomass engineering

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Since plants can fix carbon dioxide into useful biomass, studies of their metabolic system may be an important field in the era of bio-refinery as an alternative to oil-refinery. The NMR-based metabolomics approach has much potential for not only basic science but also for applied science in plant systems. First, I will introduce techniques for the correlation of 1H-NMR based metabolic profiling with genetic SNPs makers [1], as well as plant hormone responses [2] in plant systems. Furthermore, stable isotope labeling with 13C-carbon dioxide [3], 13C-glucose [4-5] or specific substrates [6] allows for the elucidation of metabolic pathways and movements by 1H-13C correlation NMR. In order to annotate a large number of metabolites from metabolite mixtures by 2D-NMR spectra, we have established a standard metabolite signal database and semi-automatic signal assignment software written in Java[7-8]. In particular, we have recently developed new statistical indices for large-scale annotations from a single 2D-NMR spectrum, enabling 211 plant metabolite annotations [9]. In addition to these metabonomics platform technologies, we have also introduced magic angle spinning (MAS) methods for characterization of low-solubility metabolites using intact plant tissues [10]. The potential for tackling low-solubility plant biomass such as lignin and hemicellulose will be discussed in this presentation. References: [1] Mochida et al. BMC Genomics. 10, e563 (2009). [2] Okamoto et al. Plant Biotechnol. 26, 551-560 (2009). [3] Kikuchi & Hirayama, Method Mol. Biol. 358, 273-286 (2007). [4] Tian et al. J. Biol. Chem. 282, 18532-18541 (2007). [5] Sekiyama & Kikuchi, Phytochemistry 68, 2320-2329 (2007). [6] Ohyama et al. Proc. Natl. Acad. Sci. USA 106, 725-730 (2009). [7] Akiyama et al. In Silico Biology 8, e27 (2008). [8] Chikayama et al. PLoS ONE 3, e3805 (2008). [9] Chikayama et al. Anal. Chem. 82, 1653-1658 (2010). [10] Sekiyama et al. Anal. Chem. 82, 1643-1652 (2010).

P4B-009

Multi-platform metabolomics approach for an objective substantial equivalence assessment of transgenic tomato

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Metabolomics offers a unique opportunity to perform a detailed study of an organism's phenotype and is therefore a promising approach for a substantial equivalence (SE) assessment of genetically modified crops. Here we propose the use of gas chromatography- (GC), liquid chromatography- (LC) and capillary electrophoresis (CE)-time-of-flight (TOF)/mass spectrometry (MS) in parallel with the advantages that (1) multiple platforms increase the chemical coverage; (2) the consensus data obtained by our novel data summarization approach is annotated and directly interpretable and; (3) can be evaluated in terms of actually achieved coverage. We used our multi-platform approach to perform a SE assessment of tomatoes that over-express the taste-modifying protein miraculin. The identified metabolites by using three platforms were found to be representative of the tomato metabolome as they covered 86% of the chemical diversity of the public database TomatoCyc. We show that 95% of all metabolite abundances were within an acceptable range of variation but at the same time indicate a reproducible transformation related metabolic signature. We conclude that multi-platform metabolomics is a both sensitive and robust approach that constitute a good starting point for characterizing novel organisms.

P4B-010

1H NMR metabolic profiling of A. *thaliana* expressing Vitreoscilla hemoglobin (VHb) under oxygen deprivation and NO treatment

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Aerobic bacterium *Vitreoscilla stercoraria* is known to synthesize elevated quantities of homodimeric hemoglobin (VHb) under hypoxic growth conditions. Expression of VHb in heterologous hosts, such as plants, when expressed in various hosts in oxygen-limited conditions, has been shown to improve growth, metabolite productivity and stress resistance of host plants. This makes VHb promising candidate for metabolic engineering, especially in plant metabolism optimization. Mechanism of Vhb is believed to rise from enhanced respiration and energy metabolism by promoting oxygen delivery. The ability of haemoglobins to bind NO can also be cause of beneficial effects of VHb expression in plants. Two model organisms, an herbaceous plant, Arabidopsis (*A. thaliana*) and a tree, Populus (*P. tremula x tremuloides*) as well as VHb-expressing lines of both species has been set up as an experimental system to study further function and physiological role of both endogenous Hbs and heterologous VHb. 1H NMR metabolomics is a well established method for studying plant metabolomics in general level, especially when the metabolome itself or the effect of a transgene or a treatment to metabolome is unknown. This approach is fast and extremely reproducible, giving excellent viewpoint on plant's metabolism for plant biotechnological applications such as this. We have now conducted an experiment, where two transgenic (mitochondrial and cytosolic versions of the transgene VHb) plant lines of A. thaliana and their metabolism were compared to wild type plants during time series of 24 hours in different treatment conditions, using 1H NMR metabolic profiling. The treatments chosen for this experiment were anoxic conditions, NO donor treatment with DETA(diethylenetriamine) and NO2, all done in parallel to each other. To establish as comprehensive view of the metabolism related to VHb as possible, plant shoots and roots samples were analyzed separately in metabolite extraction. Our results show a viewpoint on the metabolism of A. thaliana during these treatments and the distinct metabolic signatures of the VHb expressing plants.

P4B-011

Metabolomics based annotation of novel genes in *Arabidopsis thaliana*

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A plant metabolomics consortium has been established to generate and evaluate metabolomics data as a tool for generating hypotheses concerning the metabolic and physiological function of genes of unknown function. This consortium integrates 11 analytical platforms, which have the combined ability to generate relative abundance data of nearly 3100 Arabidopsis metabolites/analytes. The strategy combines the power of reverse genetics (T-DNA tagged Arabidopsis lines) and metabolomics to evaluate the consequence of the loss-of-gene function on the metabolome of the organism. The project database at www.plantmetabolomics.org, is publicly available and contains metabolomics data along with detailed information about mutant selection, material processing, analytical platform protocols, tools to aid in data visualization and more. Initially the consortium established pipelines for large-scale analyses, including mutant gene selection, tissue production and harvest, data collection and data processing. These experiments (termed EIE2 and fatB in the database) showed that the pipelines could clearly distinguish between a mutant metabolome from a wild-type metabolome, even we as experimenters introduced large environmental pressures during the growth of the organisms (experiment EIE2). In addition, the fatB experiment provided a degree of validation of the platforms based on prior characterization of the mutant allele. Based on the initial experiments (termed ME1 to ME5 in the database), the platform has been modified to enhance the reliability and robustness of the metabolomics data. These modifications include increasing the number of mutants for analysis within a single growth experiment, increasing the number of replications per mutant line, and distributing samples of pooled biological materials for analysis. These modifications have been incorporated in the extension of the project, in which the metabolomes of 200 different Arabidopsis mutants are being assessed.

P4B-012

Covering the chemical diversity of rice kernels to investigate correlations between metabolite levels and phenotypical traits

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The ongoing demand for improved and tailored crops makes it essential to develop improved breeding methods. Current breeding programs are geared towards optimization of high-level phenotypical traits but such traits are the sum of multiple variables making direct marker identification difficult. Metabolite profiling can give a more high-resolution picture of the phenotype and decompose visible traits into its molecular factors. A major obstacle for this application is that no single analytical platform can detect all types of molecules and therefore result in biased data sets. We have developed a multi-platform metabolomics approach based on a combination of gas chromatography-, liquid chromatography-, and capillary electrophoresis-time-of-flight/mass spectrometry to address this problem. Using a novel data summarization approach which is supported by our automatic metabolite identifier linking program MetMask, we obtain an consensus data set with strongly reduced chemical bias compared to the single platforms. Here we describe our strategy for data analysis and evaluation in an application where we profiled rice kernels from the 70 cultivars of the World Rice Core-Collection. The resulting data was used to mine for correlations with phenotypical traits using multivariate regression. Taken together, our results show that multi-platform metabolomics can be an efficient tool for identifying the molecular background of phenotypical traits in a major crop species and for investigating links between genotype and phenotype.

P4B-013

LC/MS/MS profiling of flavonoid conjugates in *Lupinus reflexus*

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Flavonoids and their derivatives constitute an interesting group of secondary metabolites because of their important role in plant physiology and biochemistry. Application of two systems, LC/IT-MS and LC/qToF-MS, allowed for profiling of flavonoid conjugates in extracts obtained from roots and leaves of plants from the Mexican lupine species *L. reflexus*. Over sixty flavonoid and isoflavonoid glycoconjugates were detected, few recognized compounds were not reported earlier in plant tissue. Many of these natural products were isomeric or isobaric compounds. Glycoconjugates of genistein, 2'-hydroxygenistein, biochanin and prenylated isoflavones were found in the analyzed extracts. CID/MS/MS spectra registered in positive and negative ion modes permitted structural characterization of these compounds. Both LC/MS systems allowed to obtain complementary data from the registered mass spectra. MSn experiments on IT permitted identification of flavonoid aglycones after comparison with spectra of aglycone standards. High resolution of qToF analyzer enabled the determination of the elemental composition of protonated or deprotonated molecules $[M+H]^+$ / $[M-H]^-$ of the studied compounds. Careful control of collision energy and ionization potential at the entrance to the analyzer of hybrid qToF spectrometer permitted the registration of mass spectra of the product ions with highly repeatable relative intensities of fragments. The observed differences of the product ion relative intensities could be used as a tool for the distinguishing compounds with different substitution pattern of sugar moieties or acyl groups. However, placement of the substituents is not possible without standard compounds. The glycosides identified in L. reflexus were acylated with malonic acid or their derivatives. Presence of the latter substitution of flavonoid glucoconjugates was not reported until now. Neither positions of sugars on the aglycone moieties nor positions of glycosidic bonds and configuration on sugar C-1 atoms could be established on the basis of registered mass spectra. Application of mass spectrometric techniques allowed for tentative identification of compounds.

P5A-001
Metabolite Profiling of Deinococcus radiodurans to Discover the Basis for Radioresistance Conferred by Endogenous Nitric Oxide

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Deinococcus radiodurans (Drad) is a 2.5 billion year old eubacteria that is the most radioresistant organism known. This radioresistance of Drad owes to an extremely efficient system for repairing double-strand DNA breaks and other mechanisms that await definition. Drad is also unusual in that it is one of a small group of bacteria known to possess a NO synthase (bNOS) gene. Recently, it was shown that a bNOS knockout strain of Drad (ΔNOS) is sensitized to killing by UV irradiation, establishing the first function for Drad-derived NO. UV-irradiation was shown to rapidly trigger bNOS gene expression and NO synthesis in wildtype Drad (WT), where NO functions as a transcriptional regulator that promotes recovery after UV insult. Nonetheless, the specific actions of endogenously-produced NO on Drad metabolism is unknown and molecular mechanisms that underlie NO-promoted survival of UV-irradiated Drad is largely unexplored. This investigation sought to use untargeted profiling to broadly discover actions of NO on metabolism in control (non-irradiated) and UV-irradiated Drad. Using an LC-TOF platform with aqueous normal phase chromatography and positive/negative ion monitoring MS, we could quantify relative expression levels of >1000 molecules in all samples from at least one treatment group. Treatment groups comprised WT and ΔNOS Drad strains, with and without UV-irradiation (200–500 nm, 30 mW/cm2; n = 5 cultures/group). We found >2-fold UV-induced changes in levels of 81 and 170 features (50 -1000 Da) in WT and ΔNOS Drad, respectively; – 26 features were induced in both groups. Molecular identification of differentially-expressed features by MS/MS revealed UV-induced upregulation of bacterial membrane constituents, including species previously recognized to resist phototoxicity, levels of some were profoundly altered by NOS gene deletion. Results suggest that endogenous and UV-induced upregulation of bacterial NO regulates activity and/or expression of enzymes involved in the metabolism of carotenoids and glycerolipids. Further experiments will be needed to detail biochemical pathways, enzymes and molecular mechanisms by which NO specifically confers radioresistance in Drad. Untargeted metabolite profiling offers the potential to uncover these details and may additionally shed light on the enigmatic basis for radiosensitization by NOS inhibitors of some mammalian tumors.

P5A-002
A metabolomic view on the pathogenic bacterium *Staphylococcus aureus*

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Staphylococcus aureus as a facultative anaerobic bacterium is part of the mammalian commensal flora. Nevertheless under specific conditions *S. aureus* causes strong infections and is able to invade tissues and cells. With regard to its role as a leading nosocomial pathogen and its increasing multidrug resistance, investigations on *S. aureus* are of great interest. During host infection the bacterium has to cope with changing supply of carbon sources and varying oxygen availability up to anaerobic conditions. For a better understanding of its adaptive mechanisms and its regulatory processes, *S. aureus* COL were cultivated under different growth conditions. By using 1H-NMR, GC-MS and LC-MS we investigated the extra- and intracellular metabolome and observed distinct changes between aerobically and anaerobically grown *S. aureus* COL cells.

P5A-003
In vivo metabolite profiling of Lactococcus lactis mutants towards the optimal production of reduced compounds

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Manipulation of NADH-dependent steps in *Lactococcus lactis* is common to many strategies envisaging the accumulation of compounds other than lactate. In particular, disruption of the major lactate dehydrogenase (ldh gene) is frequently considered. In this work, we pursued an engineering approach based on the combined inactivation of enzymes involved in NAD\+ regeneration downstream of pyruvate. Based on the end-product and transcript profiles in LDH-deficient strains, we selected as targets for further manipulation in an LDH/MTLF-negative strain (FI10089, Gaspar et al. 2004) the two additional lactate dehydrogenases genes ldhB and ldhX, as well as adhE (acetaldehyde/ethanol dehydrogenase). Subsequently, a series of triple and quadruple knockout mutants was obtained. Likewise the parental strain, FI10089-ldhB and FI10089-ldhB-ldhX showed a mixed-acid fermentation profile. Lactate production was not completely abolished, but it decreased considerably with the combined deletion of ldh genes. Surprisingly, FI10089-adhE was fully homolactic, which indicated activation of alternative ldh genes in this strain. Combination of ldh, ldhB and adhE deletions (FI1089-adhE-ldhB) affected drastically glucose metabolism and impaired growth under anaerobic conditions. The metabolism of [1- ^{13}C]glucose in resting cell suspensions of the mutant strains was characterized by in vivo ^{13}C-NMR to follow the dynamics of intracellular metabolite pools in a non-invasive way. Fructose 1,6 bisphosphate, mannitol 1-phosphate, 3-phosphoglycerate, and phosphoenolpyruvate were accumulated to different extents by the mutant strains. Moreover, data on the qualitative expression of ldh genes in the different mutants was obtained by RT-PCR. The levels of key glycolytic enzymes (6-phospho-fructokinase, glyceraldehyde 3 phosphate dehydrogenase and pyruvate kinase) as well as lactate, ethanol and mannitol 1-phosphate dehydrogenases were measured in the different strains and compared with those of the wild-type strain MG1363. Data demonstrating the usefulness of these constructs to direct the metabolic flux to the production of mannitol or 2,3-butanediol will be presented. P. Gaspar, et al. (2004) Appl. Environ. Microbiol. 70, 1466-74

P5A-004
Untargeted Plasma Metabolite Profiling to Discover Inborn Errors of Metabolism in Babies

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Inborn Errors of Metabolism (IEMs) are a large group of rare and potentially lethal conditions that arise from single gene mutations. If discovered within days of an afflicted baby's birth, dietary modifications and supplements can often be instituted to prevent lethality or permanent brain damage that may result with severe IEMs. Given the critical importance for rapid diagnosis of life-threatening IEMs, many governments have mandated disease screening. However, nowhere does this screening exceed 10% of the > 440 recognized IEMs. Challenges to more comprehensive screening include prohibitive costs, limited availability of neonatal plasma, inadequate assay procedures and a need to complete all analyses within 2-3 days of birth for optimal benefit to the neonate. Notwithstanding, untargeted metabolite profiling can overcome these limitations and offers enormous potential for the diagnosis of conditions that arise from rare defects in metabolism. Examples will be presented to demonstrate the efficacy of plasma metabolite profiling for discovering rare inborn errors of metabolism in plasma from human babies.

P5A-005

Metabolomics of parasite differentiation: metabolomic profiling of the human enteric protozoan parasite *Entamoeba histolytica* revealed activation of unpredicted pathways during differentiation of the proliferative into dormant stage

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The intestinal parasite *Entamoeba histolytica*, the causative agent of amoebiasis, is estimated to infect 50 million people annually, mainly in developing countries. Humans are infected by the amoeba through ingestion of water or food containing dormant cysts, which are differentiated from the motile proliferative trophozoites (encystation). Once in the human host, the cysts become the active trophozoites (excystation). These trophozoites live in the mucosal layer of the colon, and occasionally invade other organs. Therefore, encystation and excystation are the two major differentiation events essential for completion of *E. histolytica* life cycle in the human intestine. As *E. histolytica* does not efficiently encyst in vitro, encystation and excystation has been investigated using *Entamoeba invadens*, the reptilian sibling of *E. histolytica*. In order to understand molecular events in encystation at the metabolomic level, we applied capillary electrophoresis-tandem mass spectrometry to comprehensive and quantitative analysis of charged metabolites during encystation. Our data revealed that the concentrations of the majority of metabolites drastically decreased during encystation, whereas the concentrations of the metabolites involved in cyst wall biosynthesis increased. We also found remarkable changes in biogenic amine metabolites such as cadaverine and isoamylamine, which increased during the early period of encystation, when the trophozoites form large multicellular aggregates (precyst), and then decreased when the precyst differentiates to the cyst form suggest that these metabolites play an important role in inducing the encystation process. L-cystine, N-acetylputrescine, and -aminobutyric acid (GABA) increased in the late period of encystation. Significance of these metabolomic changes in encystation will be discussed.

P5A-006

Integrative metabolomics strategy to analyse cellular metabolism

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The cellular metabolism is for us of major interest, because it integrates all regulatory levels and environmental influences in a most comprehensive way. The metabolome represents on one side an endpoint of cellular regulation but its turnover is orders of magnitudes higher compared to the turnover proteins or transcripts. The aim of our group is to apply ‘cutting edge’ metabolomics and proteomics techniques for quantification and analysis of turnover rates of metabolites and proteins. Metabolomic analyses of Arabidopsis thaliana revealed that the transcriptional priming of the raffinose oligosaccharide (RFO) pathway by abcisic acid (ABA) did not induce metabolic flux in RFO pathway. More detailed studies provided evidence that ABA, as well as a plastid localized GSK3ß-like protein kinase, could regulate the starch breakdown pathway. These examples clearly demonstrated that metabolic activity could not be predicted by transcript expression data. Furthermore, the use of comprehensive GC-MS (GCXGC-MS) for metabolomic characterization of Chlamydomonas reinhardtii suggested the presence of further metabolic enzymes encoded in the genome. High throughput techniques are the tool for such large scale “-omics” studies allowing the obtainment of a nearly complete picture of a determinate cell state, concerning its metabolites, transcripts and proteins. However, single level study of a living organism cannot give a complete understanding of the mechanism regulating biological functions. The integration of transcriptomics, proteomics and metabolomics data in the newly emerging field of Systems Biology, combined with existing knowledge, allows connecting biological processes which were treated as independent so far. The systems biology unit of the MDC Berlin-Buch, the Berlin Institute for Medical Systems Biology (BIMSB/MDC) is a unique place to conduct such integrative studies at a systems level because it hosts cutting edge technologies for genome, proteome and metabolome analyses. Using an approach called ‘integrative metabolomics’ we study cellular dynamics during development, stress response and metabolic dysfunction in model organisms. Current projects focus on a comprehensive molecular analysis of oncogene-depended metabolic regulation.

P5A-007

Gaussian graphical modeling reveals molecular signatures of underlying pathway mechanisms in metabolic profiles

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The metabolite profile of a biological system is considered as the ultimate response to genetic and environmental factors, and thus represents the integrated phenotypic endpoint of cellular processes. The underlying metabolic network is driven by a set of catalytic enzymes, leading to specific inter-metabolite dependencies in metabolomics datasets. In this work, we extend correlation-based statistics by the application of Gaussian graphical models (GGMs). GGMs are weighted networks where nodes represent the measured entities, metabolites in our case, and each edge is annotated with the partial correlation coefficient between the two incident metabolites. This methodology allows for the detection of conditional dependencies between metabolite concentrations, i.e. direct relationships corrected for unspecific high correlations usually present in metabolomics datasets. Direct relationships in this context refer to metabolites that are linked through a single enzymatic step in the underlying metabolic pathway. First, we demonstrate the capability of GGMs to discriminate between direct and indirect interactions in computer-simulated toy models with a small set of metabolites. We illustrate the effects of kinetic constants and noise strength on GGMs and point out possible pitfalls of the procedure. After careful preprocessing, our method is evaluated on a real metabolomics dataset by projecting partial correlation coefficients onto a literature-derived model of fatty acid biosynthesis. In this analysis we find that metabolite pairs that are directly linked in the metabolic pathway indeed show a significantly higher partial correlation than metabolite pairs with a pathway distance of 2 or higher. Finally, we present predictions of novel pathway interactions in the fatty acid biosynthesis and phospholipid pathways. We identify several pairs of metabolites that show a high partial correlation, but cannot be attributed to known metabolic pathway steps. These pairs represent candidates of yet unknown enzymatic reactions suitable to be verified in experimental studies.

P5A-008

Staphylococcus aureus - A metabolomics view on an emerging pathogen

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Staphylococcus aureus is a versatile pathogenic bacterium responsible for a wide range of nosocomial infections found in humans and animals. As a commensally microorganism S. aureus is resting on mucosa and skin. Most severe forms of staphylococcal infections are endocarditis, osteomyelitis, sepsis and forms of the toxic shock syndrome. Many *S. aureus* strains are able to express a large number of virulence factors like cell-surface exposed proteins, enzymes and toxins supporting invasion into tissues and cells. Little is known about pathogens like *S. aureus* in terms of intracellular metabolite pools. For survival within the host, several regulatory strategies, defined structural and functional features of virulence factors and the interaction with the core cell metabolism are responsible. These interactions between eukaryotic and bacterial cells caused e.g. by nutritional limitation, anaerobic life or antibiotic stresses result in an adaption of the microbial virulence factor expression and metabolism to survive within the host environment. There is also an urgent need for new antimicrobial drugs especially against *S. aureus* and its Methicillin and Vancomycin resistant strains (MRSA & VRSA). To find new antibiotic targets or to evaluate the connection between virulence and metabolism in *S. aureus*, we have to understand the physiology of this versatile pathogen and it is therefore of crucial importance to decipher its metabolome. Approaches to understand the metabolic adaption of S. aureus towards environmental stresses represent a main focus of our research. In combination with proteomics, the metabolomics approach allows a global view and a better understanding of regulatory systems, dynamic ranges and the control of metabolic pathways of pathogens like *Staphylococcus aureus*. The talk will give an introduction into the life of *S. aureus* and presents results of the investigation of its metabolism.

P5A-009

The way to a metabolic profile of Staphylococcus aureus cells after uptake by macrophages

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Infections caused by the gram positive bacterium *Staphylococcus aureus* are a current harassment, since a third of the population worldwide are carriers of these bacteria. Indeed most strains do live as commensals without leading to any infection, but once natural barriers have been breached, furuncles and carbuncles may occur. Harmful *S. aureus* strains produce a huge diversity of exotoxins, which can cause different diseases like toxic shock syndrome or scalded skin syndrome. Recently, an intracellular lifestyle of *S. aureus* was observed by in vitro infection assays with different post-infectional fates depending on parameters like host-system, *S. aureus* strain and environmental conditions. Macrophages, as a part of the innate immune system take up staphylococci very efficiently. Trapped in the phagolysosome the bacteria have to face a very low pH and a high concentration of reactive oxygen- and nitrogen species. Despite this rough treatment, *S. aureus* is still able to avoid cell lysis, to persist in the phagolysosome and to replicate inside the host. This suggests an active metabolism is going on in *S. aureus* dealing with oxidative burst in phagolysosomes and replicating in the environment inside the host-cell. To investigate metabolomic adaptation of *S. aureus* to intracellular life in macrophages, we developed a protocol for fast sampling of intracellular living staphylococci and subsequently analyzed the metabolic profile. Furthermore, we determined the intracellular environment, the macrophage metabolome which is the nutrition source for the bacteria. Investigations were performed mostly by GC-MS and in addition 1H-NMR and LC-MS analytics to cover the most abundant metabolites.

P5A-010

A metabolomic view of *Staphylococcus aureus* and its eukaryotic-like serine/ threonine kinase and phosphatase deletion mutants: Involvement in cell wall biosynthesis

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Little is known about intracellular metabolite pools in pathogens such as *Staphylococcus aureus*. We have studied a particular metabolome by means of the presented LC-MS method. By investigating the central carbon metabolism which includes most of the energy transfer molecules like nucleotides, sugar mono- and biphosphates and cofactors, a conclusion about phenotypes and stress answers in microorganisms is possible. Quantitative metabolite levels of *S. aureus* grown in complex LB-Broth and in minimal medium were compared in the wild-type S. aureus strain 8325 and the isogenic eukaryotic-like protein serine/threonine kinase (ΔpknB) and phosphatase (Δstp) deletion mutants. Detection of several remarkable differences, e.g. in nucleotide metabolism and especially cell wall precursor metabolites, indicates a previously unreported importance of serine/ threonine kinase/phosphatase on peptidoglycan and wall teichoic acid biosynthesis. These findings may lead to new insights into the regulation of staphylococcal cell wall metabolism.

P5A-011

Effects of Exercise Intensity and Hypoxia on Urinary Metabolomics in Humans

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Physical exercise is advised as the “wonder drug” to cut the risk of heart disease, make a dramatic improvement in the delivery of oxygen to our body muscles, decrease the mental anxiety and depression, and lowers the blood pressure and cholesterol levels. Further research indicated that increased physical activity is associated with decrease incidence and mortality rates for various cancers. However, reports also emphasized the injuries of top athletes, the potential for heat exhaustion and collapse by runners during races, which may cause from elevating the level of oxidative stress and promoting platelet activation and coagulation cascades. On the other hand, hypoxic conditions occurring at high altitude lead to acclimatization processes in responses to minimize tissue damage, such as gradually increase pulmonary ventilation, enhance oxygen transportation efficiency in circulating system, and subsequently improve physical fitness. However, hypoxic states of human tissue belong to the most frequent and dangerous diseases of modern times. They results from disturbed oxygen supply to cells, which is insufficient to meet their metabolic demands. The beneficial or detrimental effects of systematic hypoxia may vary substantially with the concentration of O2 exposure. Apparently no work has explored how hypoxia/exercise intervention affects metabolic profiles of human biofluids. Liquid chromatography-mass spectrometry-based metabolomics was applied to investigate the effects of urinary metabolic profiles by hypoxia/exercise intervention and identify and quantify the changes of metabolites into the biological meaningful metabolic networking correlate to hypoxia/exercise intervention. The results will develop suitable exercise combined environmental regimens for health-related sciences, further may present a framework for assessing studies in exercise physiology. In addition, they will provide a better understanding of exercise physiology to guide individuals wishing to attain their objectives of optimal fitness and health with minimal risk, and update the knowledge base of exercise physiology.

P5A-012

Is vitamin C involved in carnitine biosynthesis? An LCMS based analysis.

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Carnitine is a metabolite necessary for transport of fatty acids from the cytosol to the site of β-oxidation in the mitochondrial matrix. In vitro evidence advocates ascorbic acid (vitamin C) as a critical cofactor for carnitine biosynthesis; yet a more recent in vivo study containing an ascorbic acid biosynthesis enzyme knockout did not confirm this view. However, the previously studied knockout was an intermediate step in ascorbic acid biosynthesis. Other reported biosynthetic mechanisms can potentially bypass this step retaining some ascorbic acid production. Overall, the role of ascorbic acid in carnitine biosynthesis remains unclear. We applied metabolomics to explore the relationship between ascorbic acid and carnitine levels in a mouse model containing a knockout corresponding to the final catalyzed step of ascorbic acid biosynthesis. We present combinations of numerous columns and solvents for liquid chromatography ion trap mass spectrometry analysis of carnitine and acyl carnitine derivatives. The Acquity UPLC BEH HILIC column (Waters Corp., Milford, MA, USA) provided the best peak shape and enabled resolution of all tested carnitine species, including carnitine and acyl carnitines of varying acyl chain lengths. This LC method was coupled with a MS/MS detection method for quantitation. Ascorbic acid concentrations were evaluated using gas chromatography TOF mass spectrometry. Liver samples from wild type mice and knockout mice, both supplemented with ascorbic acid, and knockout mice without supplemented ascorbic acid were selected for analysis. Ascorbic acid was identified in supplemented wild type mice and knockout mice, but remained below the detection limit in the unsupplemented knockout. Analysis of carnitine and selected acyl carnitines did not reveal significant variance in concentration between tested conditions. This result correlates with the previously published knockout study and clarifies that ascorbic acid is not a critical cofactor necessary for carnitine biosynthesis *in vivo*.

P5A-013
A metabolomics view on the lithoautotrophic bacterium Ralstonia eutropha

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Ralstonia eutropha is used for the industrial production of stable isotopic labeled metabolites. The ability of this organism to grow autotrophically is of great advantage for the production of such compounds. This feature of *R. eutropha* allows e.g. for the use of 13CO2 as a cheap carbon source for the production of 13C-labeled molecules. Furthermore, *R. eutropha* grows to high cell densities yielding large amounts of biomass in a single fermentation. By use of analytical techniques like NMR spectroscopy, LC-MS and GC-MS for the identification and quantification of primary metabolites in cells and in the medium, metabolomics is an essential part of the "omics"-technologies to understand special physiological problems. Monitoring the phenotype at the level of metabolites under different environmental conditions allows a differentiated insight into the bacterial physiology. A major benefit from a combination of proteome and metabolome studies is the potential to connect changing concentrations of metabolites to the amount of key enzymes of metabolic pathways. To provide a comprehensive insight into the metabolism of cells grown autotrophically for the production of labeled peptides and amino acids proteome studies will be complemented by a study of the extracellular and intracellular metabolites formed during lithoautotrophic growth. Results on the development of a metabolome sampling protocol for R. *eutropha* and initial studies on its heterotrophic and autotrophic growth will be presented.

P5A-014
Approaches to decipher the metabolome of Bacillus subtilis

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The Gram-positive bacterium *Bacillus subtilis* has been studied for over 40 year and become widely adopted as a model organism. To amplify the biological knowledge of B. subtilis a qualified sampling protocol for metabolome studies were developed. During the protocol establishment one of the most important criteria is, that the sampling procedure does not introduce artifacts, so that the sample reflects the biological status of interest. For this reason sampling, quenching, extraction of the metabolites, cell disruption as well as the leakage of metabolites during quenching were tested and optimized for Bacillus subtilis metabolome analysis. As main indicator for an optimal sampling protocol the energy charge was determinded for each method. By the use of complementary analytical methods (GC-MS, LC-MS and 1H-NMR) and the established sampling protocol, we could gain a global insight into the metabolome of Bacillus subtilis under different physiological conditions. Moreover the metabolomic profile of different Bacilli species can be compared.

P5A-015
Glycine metabolism in T. denticola when co-cultured with P. gingivalis.

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Porphyromonas gingivalis and *Treponema denticola* are bacteria associated to chronic periodontitis. Previous studies have suggested that interactions between these bacteria enhance the progression of the disease. We have used a transcriptomics and proteomics approaches to analyze the effect of polymicrobial growth on gene expression in *T. denticola* and *P. gingivalis* grown in continuous culture either separately or in co-culture. Some of the most up-regulated *T. denticola* genes were those encoding enzymes of the glycine cleavage system, and glycine-specific enzymes of the glycine reductase complex and a gene encoding a glycine/Na+ symporter. Corresponding gene products that were detected have also been confirmed to be more abundant using a stable isotope labeling strategy, identified and quantified by LC-MALDI-TOF/TOF. GC-MS analysis of media metabolites confirmed that glycine is rapidly depleted from a glycine supplemented complex media when *T. denticola* is grown in batch culture. NMR results indicate that 13C uniformly labeled glycine is metabolized to acetic acid. Identification of communication and symbiotic processes between these bacteria are thought to be important for the development and progression of periodontitis. Investigating the cooperative processes of pathogenic bacteria that cause chronic periodontitis, may identify therapeutic targets within these organisms.

P5A-016
Improving metabolite annotation in untargeted MS-based metabolomics datasets

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Metabolomics, most often associated with metabolite profiling, generates valuable qualitative and quantitative information about the small molecule complement of biological samples. However, among all signals that can be detected using MS instruments, only a fraction can be readily assigned to specific metabolites. There is thus an important need to integrate different experimental and computational approaches to facilitate metabolite identification. Toward this goal we used isotopic labeling of metabolites with both 13C and 15N in E. coli to confirm or rule-out possible candidate metabolites or molecular formulas generated from a high mass accuracy capillary electrophoresis time-of-flight instrument. The presence of labeled isotopes allows to \"count\" unambiguously the number of carbon and nitrogen atoms in a metabolite thus confirming/ruling-out candidates while at the same time eliminating background signals whose origin is not from the actual biological sample. In combination with additional data filters derived from information such as migration time prediction, the predicted metabolome based on whole genome metabolic model reconstruction and other databases, we provide an exhaustive and high confidence depiction of the CE-MS-observable metabolome of E. coli. In addition, we generate high confidence candidates for compounds for which no chemical standards were available. In total we assigned identity to hundreds of signals observed in E. coli using a single analytical method, and extend the list with multiple likely candidate metabolites. We expect this work and similar endeavors to increase the value of information collected during untargeted metabolite profiling.

P5A-017

Small molecule-mediated metabolic switching in Escherichia coli

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Small molecules, such as external or internally accumulated metabolites, affect cellular metabolic activities via activation or inhibition of transcription and enzyme activities. In bacteria, nucleotide-based signal molecules transmit signals responding to changes in the environment or in intracellular conditions and pleiotropically control biological processes. Here we present the extensive effects of a guanosine penta- and tetra-phosphate ((p)ppGpp)-mediated stringent response on *Escherichia coli* metabolism. The time series metabolome data was obtained using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). The accumulation of ppGpp responding to amino acid starvation caused changes in the dynamics of intracellular metabolites in the central carbon metabolism, amino acids and nucleotides biosynthetic pathway. The metabolic responses in the ppGpp synthetase gene (relA) knockout E. *coli strains* showed great difference with that in the wild-type. These metabolite profiles suggested the strict regulation by ppGpp to the nucleotides biosynthetic pathway under the amino acid starvation. Time series metabolome analysis consequent to the induction of intracellular ppGpp, with inducible relA gene, and the subsequent biochemical analyses revealed that the activities of several metabolic enzymes in the nucleotides biosynthetic pathway were directly controlled by ppGpp. These results suggested that ppGpp regulates extensive metabolism than previously recognized and plays a role in the immediate metabolic switching under the environmental perturbation.

P5A-018

Metabolomic analysis of the human enteric parasite Entamoeba histolytica: Discovery of unique pathways and potential targets for chemotherapeutics.

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Amoebiasis, which is caused by the infection with the enteric protozoon *Entamoeba histolytica*, is responsible for the second leading cause of death from parasitic diseases after malaria. Although the obvious clinical resistance has not yet been demonstrated, the paucity of effective drug and potential resistance, similar to that acquired by other pathogens, has been major threat worldwide. Therefore, the development of a novel anti-amoebic drug is urgently needed. Previous biochemical studies and whole genome transcriptomics demonstrated that E. histolytica has reductive metabolic pathways, possibly as a consequence of parasitic adaptation. For example, *E. histolytica* lacks TCA cycle and electron transport chain using cytochromes, and relies on the glycolysis and subsequent acetate/ethanol fermentaton for energy production. The major substrates in the latter process are likely α-keto acids derived from amino acids. Despite its reduced biosynthetic pathways, *E. histolytica* retains several unique pathways for sulfur-containing amino acid (SAA) metabolism; it possesses a series of enzymes of cysteine de novo biosynthesis from inorganic sulfur. To understand the role of glycolysis and SAA metabolism, the metabolomic profile of *E. histolytica* was analyzed by CE-TOFMS. Flux of labeled-glucose indicated that glucose is utilized for the production of acetyl-CoA and glycerol-3-phosphate, the latter of which suggests that glycolysis is linked to glycerophospholipid metabolism, which may be reflected by the active turnover of membrane. Comparison of the metabolites under cysteine deprivation showed that *E. histolytica* accumulates S-methylcysteine via an alternative sulfur salvage pathway partly using the de novo cysteine biosynthetic pathway. Moreover, cysteine deprivation led to the synthesis of unconventional phospholipid, phosophatidyl-isopropanolamine, and a decrease in the phosphatidylethanolamine level. These results may help us to identify new targets to develop novel chemotherapeutics against amebiasis.

P5A-019

Identification of growth phase associated metabolite markers in the pathogen Yersinia pseudotuberculosis

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Yersinia pseudotuberculosis, a gram-negative pathogen, was investigated by metabolite profiling via GC-MS in different growth phases and different media. It is known that the expression of virulence factors in *Y. pseudotuberculosis* is growth phase dependent (Heroven et al. 2004). In order to understand the interlinkage to the metabolism we utilized untargeted metabolite profiling of *Y. pseudotuberculosis* under different growth conditions and found corresponding patterns in the profiles. We investigated the metabolic profile of *Y. pseudotuberculosis* during growth in exponential phase and stationary phase on LB medium and minimal medium (MM) with glucose as single carbon source. As could be expected, glycolysis intermediates like phosphorylated sugars are present in higher concentrations after cultivation on MM than on LB, while most amino acid pools are increased in LB medium. Interestingly some amino compounds like lysine, glutamate and putrescine show unusual high abundance in the metabolic profile of Y. pseudotuberculosis in both media. It occurs also that specific amino acids like lysine, valine and alanine accumulate in the stationary phase independent of the culture media while TCA intermediate pools are reduced. The results show that metabolite profiling is a powerful diagnostic tool to reveal metabolic responses to environmental influences.

P5A-020

Determination of intracellular metabolites and mathematical modeling of E.coli MG1655 metabolism

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In systems biology, one of the main tasks is to understand the global regulation of metabolic networks. The microorganism *Escherichia coli* is a favourable model organism for any kind of systems biological approach, because its network structure is largely known and its experimental handling is in many aspects very simple. With in the SysMo-SUMO consortium we like to fully investigate the regulation of the central metabolism of Escherichia coli by analysing the influence of oxygen on three different cellular levels e.g. the transcriptome-, the proteome- and on the metabolome-level. Here we present an integrative systems biology approach of quantitative metabolic data and mathematical modelling of the central metabolism. First: intracellular metabolite concentrations of *Escherichia coli* MG1655 for metabolites of central metabolic pathways (e.g. TCA and glycolysis) have been quantified applying LC/MS analysis. Therefore, *Escherichia coli* MG1655 was grown in glucose-limited chemostat cultures, with different oxygen concentrations ranging from 0\% to 120\% of Aerobiosis (acc. Alexeeva et al. 2002). Cells were quenched with cold methanol-glycerol-solution (acc. Link et al. 2008). Extraction of metabolites was done with methanol-chloroform and a later addition of methanol-tricine for separation of cell debris from the extracts (acc. Ritter et al. 2008). Second: we built a mathematical model of the central metabolism and its enzymatic and genetic regulation. Model parameters were adjusted in order to explain the metabolite data and mRNA data (PCR and microarray). The model explains the steady state oxygen response of Escherichia coli in a coherent and comprehensive way. As metabolite pools are difficult to interpret as stand alone information this model is used to understand the kinetics and regulative structures behind them. In the beginning the presented approach was done for the wildtype strain of Escherichia coli MG1655 and later the model will be used to characterise isogenic mutant strains e.g. knock-out of TCA enzymes and others.

P5A-021
Metabolic pathway relationships revealed by an integrative analysis of the metabolic and transcriptional temperature stress response dynamics in yeast.

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The parallel and integrated analysis of metabololite data with datasets covering other levels of molecular organization has become a central task of metabolomics research. We investigated the metabolomic and transcriptional response of yeast exposed to increased and lowered temperatures relative to optimal reference conditions in the context of known metabolic pathways. Pairwise metabolite correlation levels were found to carry more pathway-related information and to extend to farther distances within the metabolic pathway network than associated transcript level correlations. Metabolites were detected to correlate stronger to their cognate transcripts (metabolite is reactant of the enzyme encoded by the transcript) than to more remote transcripts reflecting their close metabolic relationship. We observed a pronounced temporal hierarchy between metabolic and transcriptional molecular responses under heat and cold stress. Changes of metabolites were most significantly correlated to transcripts encoding metabolic enzymes, when metabolites were considered leading in time-lagged correlation analyses. By applying the concept of Granger causality, we detected directed relationships between metabolites and their cognate transcripts. When interpreted as substrate-to-product directions, most of these directed Granger causality pairs agreed with the KEGG-annotated preferred reaction direction. Thus, the introduced Granger causality approach may prove useful for determining the preferred direction of metabolic reactions in cellular systems.

P5A-022
Cobalt Chloride: A Hypoxic Mimicker; but is it a Suitable Substitute?

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The transcription factor Hypoxia Inducible Factor 1 (HIF-1) plays a pivotal role in the onset and development of many cancers. Under ordinary circumstances, the [alpha] subunit of HIF-1 is degraded in the presence of oxygen, but under hypoxic conditions, has the ability to regulate a number of genes which are profoundly associated with cancer initiation/progression. Furthermore, tumorous cells have been found to maintain high levels of HIF-1[alpha] even under normoxic conditions. Due to experimental restraints, it is a common feature that scientists use the hypoxic impersonator, cobalt chloride ($CoCl_2$), which is known to inhibit the degradation of HIF-1[alpha] in the presence of oxygen via interfering with the pVHL:HIF-1[alpha] interaction and/or poly-ubiquitination of the HIF-1[alpha] protein, thereby increasing its concentration. $CoCl_2$ is a potent substance, even at low concentrations, and as a result is likely to have off target effects on the metabolic network of the cell. The aim of this experiment was to investigate these off target effects of $CoCl_2$ on human telomerase reverse transcriptase (hTERT) cells utilising Fourier Transform Infrared (FT-IR) spectroscopy and Gas Chromatography Mass Spectrometry (GC-MS). A $CoCl_2$ concentration of 100[micro]M is frequently utilised in order to inhibit HIF-1[alpha] degradation and so this concentration was adopted and the off target effects analysed. hTERT cells were subjected to normoxia (21% oxygen), hypoxia (1% oxygen) or 100[micro]M $CoCl_2$ (21% oxygen) for 4 hours prior to analysis. The FT-IR spectra and metabolites from GC-MS were analysed using multivariate statistical analyses. Although 100[micro]M $CoCl_2$ may be sufficient to induce HIF-1[alpha] concentrations, a number of off target effects were observed. Therefore, if $CoCl_2$ usage is obligatory for a hypoxia experiment, then these off target effects should be taken into consideration.

P5B-001

A new high-throughput approach to volatile analysis by Thermal Desorption - Gas Chromatography - Mass Spectrometry - applied to the characterisation of various melon cultivars by aroma

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The fruit of *Cucumis melo* is highly valued globally for its sweet and refreshing flesh, in fact sweetness is one of the fruits most valued traits, although the flavour and thus commercial value are also highly influenced by aroma. The aroma of a melon fruit is dictated by the concentration and balance of specific volatile organic compounds (VOC's). The VOC profiles of three types of C. melo Var. cantalupensis group Charentais (Cézanne, Escrito, and Dalton) known to exhibit differences in ripening behaviour and shelf life, as well as one type of C. melo Var. cantalupensis group Ha'Ogan (Noy Yisre'el) and one non-climacteric type of C. melo Var. Indorous (Tam Dew) were investigated. A simple and yet extremely robust method of passively sampling melon VOC's upon Polydimethylsiloxane (PDMS) membrane was first developed. Fifty eight VOC's were successfully sampled and detected, forty seven of which were identified (including 19 esters, 3 sulphur compounds, 2 aldehydes, 6 alcohols, and a range of other VOC classes) by means of Thermal Desorption - Gas Chromatography - Mass Spectrometry (TD-GC-MS). Multivariate data analysis via principal components analysis (PCA) and univariate data analysis via the non-parametric Kruskal Wallis significance test indicated which VOC's were significantly altered between the melon varieties. A reduction in VOC's was observed for the non-climacteric Tam Dew variety compared to the four *C. melo* cantalupensis varieties. Many differences were also noted between the *C. melo* cantalupensis group Ha'Ogan (Noy Yisre'el) variety and the three *C. melo* cantalupensis group Charentais varieties. The differences between the short-, mid- and long-shelf life C. melo cantalupensis group Charentais varieties were also considerable and of significance in terms of the melon fruit ripening behaviour, commercial shelf life and value.

P5B-002

Determination of metabolic Volatile Organic Compounds in exhaled breath of patients with Chronic Obstructive Pulmonary Disease by Gas Chromatography Mass Spectrometry.

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Introduction: Metabolism generates Organic Volatile Compounds (VOCs) detectable in exhaled air. Due to an alteration in normal metabolism, altered patterns of exhaled VOCs can be found in several diseases. In lung disorders, it is expected that abnormal pulmonary and systemic metabolism result in altered exhaled VOCs pattern, with the potential for diagnosis and pathological understanding. Chronic Obstructive Pulmonary Disease (COPD) is characterized by increased inflammation and oxidative stress, and there are indications that the pattern of exhaled VOCs in COPD can be different from that in healthy patients. Aims: We present a method to identify VOCs profile in breath samples to separate COPD patients from healthy volunteers. Methods: In a pilot study, 12 COPD patients (FEV1, 48 9% pred) and 17 controls were evaluated. End-exhaled breath samples (5x80mL, in duplicate) were obtained using a breath sampler (BioVOC) and transferred to a Tedlar Bag. Solid Phase Microextraction (SPME) was used to preconcentrate the samples. Analysis was carried out with a Gas Chromatographer coupled to a Mass Spectrometer. MZmine software was used to process the spectra obtained. Results: Analysis of the spectra showed differences between COPD and healthy VOCs profiles, even though variability between subjects is still high. The pattern of exhaled VOCs differs when considering environmental VOCs for the analysis. Conclusions: Pre-concentrated end-exhaled breath VOCs analysis using GC/MS is a non-invasive method with promising potential in distinguishing VOCs patterns from COPD and healthy breath samples. Supported by: SEPAR 06, ISCIII-RTICC (RD06/0020/0046) and PI080283-FIS.

P5B-003

Targeted Metabolomic Analysis of Volatiles Contributing to the Unique Aromas of Cantaloupe Melon (Cucumis melo L.) varieties

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Aroma is one of the most important factors in fruit quality affecting consumer's preference of melon varieties (*Cucumis melo* L.). We investigated the volatile compositions of 60 melon cultivars, known to exhibit an extent of genetic and phenotypic variation. The aim was to find key aroma compounds determining differences in melon aromas and infer the metabolic pathways involved in their biosynthesis. The concentration of 110 volatile compounds was determined by targeted metabolimics using solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry (GC-MS). A hierarchical cluster analysis using the Pearson correlation coefficient revealed the presence of a few major structurally related compound clusters derived from the same biochemical origin. Sesquiterpenes, norisoprenes and esters grouped in a distinctive pattern along the diagonal line. In addition, a few interesting associations with no obvious biosynthetic relations were found. The approach chosen can further enhance our understanding of the complex interactions that ultimately contribute to the unique flavor of melon, and in the longer term, coupled to genotyping and transcriptomic analyses will provide important tools to discover novel metabolic pathways affecting the unique aromas of melons.

P5B-004

A tomato EMS mutant with altered trichome flavonoid methylation identified by LC-MS screening

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Trichomes are specialized epidermal cells that protrude from the surface of various plant tissues. Trichome metabolites from a variety of plants are contributed to flavor and taste or are medically important. We and others have demonstrated that secretory and glandular trichomes (SGTs) in tomato produce diverse secondary metabolites, which are presumably involved in plant defense. Tomato (*Solanum lycopersicum*) is our system of choice for studying SGT metabolism because it makes SGTs and is genetically tractable. We employed 5 minute LC-ToF MS screen (Gu et al. 2009; Schilmiller, Shi et al. 2010) to identify randomly generated EMS mutants with altered trichome non-volatile chemistry. Approximately 1,400 tomato EMS mutants were screened to identify genetic variants with altered secondary metabolites. LC-MS analyses of single leaf dip extract have generated profiles of metabolites including acylsugars, alkaloids, flavonoids and others yet to be identified. Eleven groups of mutants were identified for various chemical phenotypes. For example, in contrast to wild type plants, mutant JP117 accumulates more monomethylated myricetin (mono-) than dimethylated myricetin (di-). This phenotype was consistent in the progeny, indicating that altered chemical phenotype is heritable. Further chemical analysis of backcross F1 plants revealed that the mutant allele is recessive and likely loss of function. The wild type allele showed gene dosage effect in the F1, suggesting the mutated gene could be a structural gene for an O-methyltransferase. In backcross F2, the phenotype segregated to 3:1, supporting that a single gene is mutated. The JP117 mutant was outcrossed to the S. pennellii 0716 wild tomato and chemical phenotypes of progenies were tested. Of 75 outcross F2 plants, 16 showed the mutant phenotype. The locus responsible for the mutant phenotype was mapped to chromosome 6. The identification and characterization of the mutant gene is ongoing. As shown in this study, the combination of chemical and genetic analysis provides a foundation for discovery of biosynthetic pathways leading to the production of secondary metabolites in tomato trichomes.

P5B-005

Exploiting *Medicago* Germplasm Diversity for Triterpene Saponin Biosynthetic Gene Discovery Using an Integrated Metabolomics and Transcriptomics Approach

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Triterpene saponins are a class of structurally diverse plant natural products with a wide range of demonstrated bioactivities. Individual triterpene saponins have been demonstrated to possess allelopathic, anti-fungal, anti-bacterial, anti-insect, anti-feedant, and anti-cancer activities. The biosynthesis of triterpene saponins is poorly characterized. The model legume *Medicago truncatula* is known to accumulate a large variety of triterpene saponin compounds, resulting from the differential glycosylation of at least seven triterpene aglycone structures. In this project, UPLC-ESI-qTOF-MS was used to profile the accumulation of triterpene saponin metabolites in a collection of 100 M. *truncatula* ecotypes (germplasm accessions). Analyses of both aerial and root organs was performed. These metabolomic analyses revealed interesting trends in differential spatial and structural accumulation patterns between the various ecotypes, and between the organs. For example, zanhic acid saponins were detected exclusively in aerial organs, while soyasapogenol B saponins were detected exclusively in root organs. The high-resolution biochemical phenotyping data for the whole ecotype collection enabled an informed selection of hypo- and hyper accumulating ecotypes for subsequent transcriptomic analyses via Affymetrix Medicago GeneChips®. Correlation analyses of saponin accumulation phenotypes with transcript expression data led to the identification of several biosynthetic gene candidates. A cytochrome P450 gene candidate was cloned and introduced to Wat11 yeast cells, enabling microsomal isolation and detailed in vitro characterization of enzyme function. This cytochrome P450 showed sequential oxidase activity for carbon 23 of oleanolic acid and several structurally related compounds in the triterpene sapogenin biosynthesis pathway. Genetic confirmation of in planta function for this gene is under way via mutant analysis.

P5B-006

Metabolite Profiling of Volatile and Nonvolatile Compounds in 32 Pepper Accessions

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The genus *Capsicum* spp. (pepper) comprises up to 25 wild and domesticated species and forms a rich source of health-related metabolites, such as carotenoids, flavonoids and vitamins C and E. These metabolics content in pepper has been studied intensively using targeted metabolic approaches. However, such approaches cover a limited number of metabolites, often measured in a restricted number of genotypes. We used non-targeted MS-based approaches to study biochemical variation of 32 pepper accessions, selected from the Centre for Genetic Resources of the Netherlands (CGN) based on variation in fruit morphology (size, shape and colour), pungency level and country of origin. Accessions of four intercrossable Capsicum species, C. annuum, C. chinense, C. frutescens and C. baccatum, were selected, including commercial cultivars, landraces and wild accessions. They were grown under controlled conditions in a greenhouse located in Wageningen (The Netherlands). The pericarp of ripe fruits was used to determine semi polar and volatile metabolites, using LC-PDA-QTOF-MS and headspace SPME-GC-MS, respectively. Analysis of both data sets using several multivariate statistical approaches showed a large variation in both semi polar and volatile metabolome. The different of Capsicum species could be clearly separated based on their semi-polar or volatile metabolite profile. The most discriminative contrast in the volatile dataset was related to pungency, suggesting that pungent accessions, in addition to capsaicin production, have a very different aroma compared to sweet accessions. We are currently exploring several hypotheses which may explain these results: (i) pungent accessions contain volatiles derived from capsaicinoid pathway, (ii) volatile QTLs are closely linked to loci regulating pungency or (iii) divergent evolution of pungent and sweet capsicum germplasm as a result of domestication and breeding. Metabolite identification in combination with genetic analysis will lead to identification of novel mQTLs which can be used in future breeding programs for pepper fruit quality.

P5B-007

Metabolomic fingerprinting of plant resins collected by the European honey bee, Apis mellifera

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'Propolis' (pro – "at the entrance to", polis – "city") is an apicultural term for plant resins used by honey bees (*Apis meliffera*) in the construction of their nests. These resins are by bees to seal their nest architecture, embalm intruders too large to carry out of the nest, and to reduce the size of the nest entrance. In temperate regions of Europe and the US, honey bees collect resin primarily from the young leaves and buds of trees in the *Populus* genus (poplar, cottonwood, and aspen). Honey bees in tropical regions seem to collect resin from a variety of plants including *Clusia* genus flowers (where resin may be a reward for pollination) and from alecrim plants like *Baccharis dracunculifolia*. Propolis collected from managed colonies of honey bees from different geographic regions is chemically diverse and display varying levels of antimicrobial properties that may be important to colony level disease resistance. We have used two different platforms, (1) LC-TOF and (2) GC-MS equipped with a thermal desorption unit (TDU) to analyze the chemical diversity of propolis samples from different temperate and tropical locations. 2450 spectral features were identified in LC-TOF profiles of acetonitrile extracts from four propolis samples collected from Northern Minnesota (USA), Mariana (Brazil), Muzambinho (Brazil), and a pooled sample from China. TDU-GC-MS profiles were less rich in the total number of peaks captured compared to LC-TOF profiles, but showed significant differences between temperate (Northern US) and tropical (Brazil) samples. Using these methods we hope to identify patterns of spectral features as well a individual compounds that (1) may be related to antimicrobial activity against Paenibacillus larvae, a bee brood pathogenic bacterium, (2) can be used to identify the botanical sources of propolis samples, and (3) could serve as foraging cues for resin collecting workers.

P5B-008

Comparative metabolic profiling of alfalfa trichomes to better understand potato leafhopper resistance

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Plant trichomes synthesize, accumulate and secrete large amounts and various specialized metabolites including volatiles to protect themselves against pathogens and herbivores. To better understand the role of trichomes and metabolites in alfalfa and potato leafhopper (PLH) interactions, the metabolite profiles of a resistant line with a high density of glandular trichomes on its stem was compared to a susceptible alfalfa line with a high density of glandular and non-glandular trichomes on its stem. The trichome density in the susceptible line was significantly higher (1.6 fold) than that in the resistant line indicating that alfalfa biochemical factors likely provide greater contribution to PLH resistance than its physical factor. To more thoroughly interrogate the biochemical factors, volatile and nonvolatile primary and secondary metabolites were analyzed using SPME-GC-MS, GC-MS, and UPLC- qTOF-MS. Numerous volatile and nonvolatile primary metabolites and secondary metabolites in the resistant line accumulated to significantly higher levels than in the susceptible line. Former reports suggested that fatty acid amides could form the basis for potato leaf hopper resistance. However, 9 fatty acid amides which were previously reported for their contribution to PLH resistance, did not show higher levels in resistant alfalfa line relative to the susceptible line. The volatile metabolic profiles obtained from an optimized SPME-GC-MS method revealed clear metabolic differences between tissues and between the trichomes from resistant and susceptible lines. Thirteen volatile metabolites were significantly over 2 times higher in resistant alfalfa trichomes, and two unknown volatile metabolites showed the highest different ratio (14 and 8 folds) of resistant line to susceptible line and specifically accumulated in trichomes. A triterpenoid saponin, tentatively identified as 3-Glu-Glu-Glu-28-Ara-Rha-Xyl-medicagenic acid on the basis of tandem spectrometry, was 14 times higher in the resistant line. This metabolite was also observed at higher levels in the stem and leaf than in trichome, suggesting the medicagenic acid glycoside is not specific to the trichome. Therefore, the two unknown volatile metabolites and one medicagenic acid glycoside are currently prioritized candidate metabolites contributing to PLH resistance.

P5B-009

Volatile Metabolites in Human Breath and Classification Models for Lung Diseases

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Since about 2000 several types of ion mobility spectrometers coupled to multi-capillary columns are used in clinical trials for investigations of metabolic profiles in human breath. Results of clinical applications within the Lung Hospital Hemer[3], the Ruhrlandklinik Essen[4-6] and the University of Göttingen[7] will be presented, using instrumentation and data partly obtained also at ISAS in Dortmund[8]. The examples consider chronic obstructive pulmonary disease (COPD), bronchial carcinoma (BC), lung fibrosis and others.[2-3,8] including non-small-cell lung cancer and COPD stage III and IV [2,4-6], different VOCs in bronchi close to the tumor[2,4] and at Hunter disease treated with idursulfase[2-3].The concentrations of Propofol in exhaled air and in serum showed satisfying agreement [2,7-8] Volatile metabolites are relatable directly to the healthy condition and could be used for bedside applications with respect to personalized medicine. The long-term objective at the Department of Clinical Diagnostics of KIST Europe is the combination of transcriptomic and metabolomic signal analysis by direct and parallel investigation of volatile and non-volatile metabolites in breath of humans and animals using a palm-size real-time PCR system combined to a µIMS. Acknowledgements: The author wants to acknowledge thankfully the cooperation with the following scientists: B. Bödeker2, M. Westhoff3, P. Litterst3, K. Darwiche4, St. Welter5, Th. Gauler6, L. Freitag4, V. Bessa4, U. Sommerwerck4, O. Anhenn4, M. Jünger7, Th. Perl7, M. Quintel7, W. Vautz8, J. Nolte8 - located at 3 Lung Clinic Hemer, Theo-Funccius-Str. 1, 58675 Hemer, Germany, 4 Department of Pneumology, Ruhrlandklinik - University of Essen, 5 Department of Thoracic Surgery, Ruhrlandklinik - University of Essen 6 Department of Thoracic Oncology, Ruhrlandklinik - University of Essen, Tüschener Weg 40, 45239 Essen, Germany, 7 Department of Anaesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Robert-Koch-Str. 40, 37099 Göttingen, Germany and 8 Leibniz-Institute for Analytical Sciences - ISAS - e.V., Bunsen-Kirchhoff-Str. 11, 44139 Dortmund, Germany. * former address: Leibniz-Institute for Analytical Sciences - ISAS - e.V., Bunsen-Kirchhoff-Str. 11, 44139 Dortmund, Germany

P6A-001

Metabolomic analysis of secondary metabolites from the medicinal plant, Prunella vulgaris.

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Prunella vulgaris is a perennial plant of the Lamiaceae family that is widely distributed over the world. *Prunella* is classified as a medicinal plant and has a long history in folk medicine. For example scientific publications have reported it's antioxidative, antimicrobial, antiviral, anti-inflammatory and immunomodulatory properties. However, little is known concerning the small molecule metabolites composition in these plants, and the geographic and genetic variation that may occur among this genus. In our study we conducted fingerprint metabolomic analyses and chemically identified the phytochemicals that occur in *Prunella*. In these studies we surveyed relative metabolite differences among different *Prunella* accessions that are maintained by the USDA-ARS North Central Regional Plant Introduction Station. Using non-targeted and targeted profiling platforms we analyzed 4 accessions collected form different USA wild populations and one collected from a site in South Ossetia, Georgia (Eastern Europe). There are no visual phenotypic differences between the five accessions, however metabolite profiling revealed clear differences among the five accessions, and as may be expected from the geographic distance, the South Ossetia accession is much more distinguishable from the 4 USA accessions.

P6A-002

Time Resolved Metabolic Foot-printing of Δpqs mutants of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a major cause of morbidity and mortality in cystic fibrosis patients. Virulence in Pseudomonad infection is regulated by a hierarchical quorum sensing system which includes N-acylhomoserine lactones (AHLs) and the 4-quinolones (4Qs). The pseudomonas quinolone signal ((PQS) 2-heptyl-3-hydroxy-4-quinolone) is an inter-cellular signalling molecule that controls multiple virulence factors and adaptive mechanisms in pseudomonas infection. Under stressful conditions PQS induces the entry of undamaged bacteria into a less metabolically active and less susceptible state, increasing pseudomonas tolerance to stress. Although these have been studied widely using genetic methods, little is known about their effects on general bacterial metabolism. The bacterial metabolome is dynamic and is a direct reflection of the cells physiological state, and as such any effects from changes in gene expression or in the environment will be integrated into the organism's metabolic profile. We sampled supernatants (metabolic footprinting) over the course of growth for wild-type and ΔpqsA and ΔpqsH knockouts, and analysed them by NMR spectroscopy. Fitting equations to individual metabolite changes (time-resolved footprinting) can summarize the complex biological data in a principled fashion. Here we present the metabolic effects of PQS mutations on P. aeruginosa metabolism.

P6A-003

NMR and MS-based profiling of gut microbial fermentation of tea and wine polyphenols in the Simulator of the Human Intestinal Ecosystem (SHIME)

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Dietary intake of polyphenols has been associated with beneficial health effects. A major fraction of dietary polyphenols is degraded by gut microbiota, resulting in smaller phenolic acids that may be more readily absorbed and potentially contribute to the health benefits. Dietary intake of polyphenols may also affect the gut microbial population or activity. This study describes the microbial degradation of two polyphenol-rich ingredients, i.e. black tea extract and a red wine/grape juice mix, in the in vitro Simulator of the Human Intestinal Ecosystem (SHIME). The tea and wine extracts, containing equivalent amounts of polyphenols, were studied in parallel in a TWIN-SHIME model inoculated with the same faecal sample. The effect of the tea or wine extracts on microbial population and on polyphenol degradation was studied in two subsequent experiments, i.e. a single dose experiment with 7-day follow-up, and a 2-week continuous feeding experiment followed by a 2-week washout. Culture media from SHIME vessels representing ascending, transverse and descending parts of the colon were sampled at regular time intervals during these experiments. PCR-DGGE and real time qPCR showed that the wine extract exhibited a transient antimicrobial effect, which was stronger than for the tea extract and also more prominent for some bacterial groups. GC-MS profiling was used to identify microbial polyphenol degradation products and monitor their formation in time at different colon locations. Global NMR metabolite profiling was used to monitor microbial production of short-chain fatty acids and several more abundant phenolic acids. An important finding was that the tea and wine polyphenols displayed some marked differences in the kinetics and colon site of microbial phenolic acid production. Thus, polyphenol source and gut microbial population may be important determinants of bioavailability and potential health benefits of dietary polyphenols.

P6B-001
Simultaneous quantification of salicylic, absisic and jasmonic acids in coffee leaf extracts by HPLC-DAD-MS/MS

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Salicylic acid (SA), absisic acid (ABA) and jasmonic acid (JA) are known to be plant hormones that play a crucial role in controlling plant growth, development, and response to biotic and abiotic cues. It has been suggested that crosstalk occur among JA, SA and ABA in plant response to biological threats. The use of a simple and sensitive method to simultaneously quantify multiple classes of phytohormones will facilitate the research of hormone networks and functions. The reduced concentrations of these compounds in real samples corresponding to complex matrixes, as it is the case of coffee leaves, requires the optimization of sample preparation techniques and the use of sensitive and selective techniques for their quantification. In this work different sample preparation methods were used to obtain leaf extracts of Coffea arabica and the results of the various methodologies were compared considering SA, ABA and JA content. The best results were achieved when coffee leaves were grounded with liquid nitrogen and extracted with a methanol-water mixture. Analytical conditions were also optimized for the analysis of the extracts by HPLC-DAD-MS/MS. For a better sensitivity and selectivity a MRM mode was used for the quantification, using the best transitions obtained for each compound. Validation parameters as specificity, linearity, accuracy, precision and LOD (Limit of detection) and LOQ (limit of quantification) were determined using both detection modes. The optimized method seems adequate for the quantification of SA, ABA and JA in coffee leaf extracts. Studies are being conducted to understand the role of these phytohormones in the resistance response of coffee to leaf rust (Hemileia vastatrix) Acknowledgments: This work was funded by Fundação para a Ciência e Tecnologia (PTDC/AGR-AAM/71866/2006 and REDE/1518/REM/2005 for the LC-MS/MS equipment).

P6B-002
Effect of fungal elicitors on podophyllotoxin production in *Linum album* cell culture

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Podophyllotoxin (PTOX) is a lignan compound which occurs in a few plant species and has pharmacological significance for its anticancer activities. Linum album, one of endemic species in Iran, has PTOX and other lignans. Lignans are potentially involved in plant defence against pathogens. Fungal elicitors can be used for triggering of secondary metabolite synthesis in plant cell culture. In this study, we investigated the effect of 4 fungal elicitors on cell growth and PTOX production. Cell suspension cultures of Linum album were treated with elicitor preparations made from mycelium extracts of *Fusarium graminearum, Rhizoctonia solani, Rhizopus stolonifer and Sclerontinia sclerotiarum*. Cell cultures were inoculated at day 7 by fungal elicitors at rate of 5 and 10 % (v/v). Cells were collected for analysing at 48h after elicitation. The highest yield of PTOX, 5µg/gr (FW), and cell growth was achieved from cells were treated by 5% F. graminearum exteract. Key words: fungal elicitors, podophyllotoxin, Linum album and cell culture.

P6B-003
Metabolomics in Poplar Research

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Populus is a widely used bioenergy crop cultivated in short rotation plantations, since it is a fast-growing and robust tree, which can be cultivated on low quality soils, including nutrient-poor, saline and contaminated soils as well as floodplain habitats. The importance of understanding underlying mechanisms of nutrient uptake, growth promotion and stress tolerance is evident in order to improve efficiency of energy production. Thus, poplar as a well studied perennial tree with a completely sequenced genome is an excellent model system to apply metabolomics studies and correlate them to morphological and growth data. Colonization of endophytic bacteria can promote plant growth, stress resistance and enhance control of pathogens. In vitro-grown explants of the Chinese hybrid poplar clone 741 (♀*[Populus alba x (P.davidiana + P. simonii) x P. tomentosa]*) free from culturable bacteria show significant differences in growth, root development (adventitious roots) and metabolic signature compared to inoculated shoots with *Paenibacillus* sp. strain P22 (Scherling et. al, 2009). On nitrogen free medium inoculated plants are capable to survive and even grow whereas endophyte free plants die. Presented data point to a mutualistic interaction between poplar plants and endophytic bacteria resulting in better adaption to environmental pressure. The observed phenotypic plasticity is investigated by metabolic profiling. References: Scherling C, Ulrich K, Ewald D, Weckwerth W. 2009. A Metabolic Signature of the Beneficial Interaction of the Endophyte Paenibacillus sp. Isolate and In Vitro-Grown Poplar Plants Revealed by Metabolomics. Molecular Plant-Microbe Interactions 22(8): 1032-1037.

P6B-004
Differential expression of metabolites in barley genotypes varying in resistance to Fusarium head blight

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Resistance in barley (*Hordeum vulgare* L.) to fusarium head blight (FHB) caused by Fusarium graminearum is quantitative, involving several resistance mechanisms. Metabolomics approach was used to phenotype resistance in six barley genotypes. The genotypes in the order of resistant to most susceptible were: CI-4196, Zhedar-1, Zhedar-2, Fredrickson, Harbin-2r, and CH 9520-30. The plants were inoculated with macroconidia or mock at anthesis and spikelets were sampled at 96 hours post-inoculation. The lemma and palea were extracted with methanol+water, and analyzed using liquid chromatography and mass spectrometry (LC-ESI-LTQ-Orbitrap) in negative mode. Univariate analysis identified 150 treatment significant metabolites, and those in higher abundance in resistant than in susceptible genotype were designated as resistance related metabolites. These were assigned with putative names based on the accurate masses and fragmentation pattern. Phenylpropanoid, flavonoid and jasmonate pathways were found active against FHB. The genotype CI 4196 was associated with several significant metabolites in jasmonate and flavonoid pathway, while Fredrickson and Harbin 2r with phenylpropanoid pathway, and Zhedar-2 with flavonoid pathway. Genotype Zhedar-1 was able to degrade DON to DON-O-glucoside, and as compared to others it had less number of resistance related metabolites. Most common resistance related metabolites detected were: coumaric acid, phenylalanine, caffeoyl alcohol and few lignans from phenylpropanoid pathway, and conjugates of kaempferol from flavonoid pathway.

P6B-005

The GC-MS metabolite profiling platform for Legume analysis (2003-2010): A case study from metabolite atlas to the testing of robustness for translational genomics

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Translational genomics, namely the use of model species to generate knowledge about biological processes and the functions of genes, offers great promise to biotechnologists. Metabolome information contributes to this approach. For example, the metabolite targeted and also non-targeted GC-MS based profiling promises new and potentially transferable insights into the metabolic aspects of plant acclimation responses to environmental stresses, perhaps even more so, when combined with profiling results from other systems levels. With this vision in mind a GC-MS metabolomics platform was established for the Legume analysis. The project started out in 2003 as an atlas of metabolites observed in diverse legume tissues and is now in 2010 used to seek for robust responses of model legumes to environmental stresses. Salinity stress responses were tackled by altering the stress dosage and more importantly by repeating experiments independently and in consecutive years. Aspects from the data mining of such legume salt acclimation experiments are presented and discussed. A special focus is set on the mining of robust system features at the ionomic, transcriptomic and metabolomic levels. We applied best possible controlled greenhouse conditions and asked two main questions: How reproducible are results obtained from physiologically meaningful salinity experiments, and what degree of bias may be expected if conclusions are drawn from less well-repeated sampling? A surprisingly large fraction of the transcriptional and metabolic responses to salt stress were not reproducible between experiments. But a core set of robust changes was found which was clearly shared between experiments. Many of these robust responses were qualitatively and quantitatively conserved between different accessions of the same species, indicating that the robust responses may be a sound starting point for translational genomics.

P6B-006

Whiteflies interfere with indirect plant defense against spider mites in Lima bean

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Plants under herbivore attack are able to initiate indirect defense by synthesizing and releasing complex blends of volatiles that attract natural enemies of the herbivore. However, until now little is known about how plants respond to infestation by multiple herbivores, particularly if these belong to different feeding guilds. Here, we report the interference by a phloem-feeding insect, the whitefly Bemisia tabaci, with indirect plant defense induced by spider mites (*Tetranychus urticae*) in Lima bean (*Phaseolus lunatus*) plants. Additional whitefly infestation of spider-mite infested plants resulted in a reduced attraction of predatory mites (*Phytoseiulus persimilis*) compared to attraction to plants infested by spider-mites only. This interference is shown to result from the reduction in (*E*)-β-ocimene emission from plants infested by both spider mites and whiteflies. When using exogenous salicylic acid (SA) application to mimic B. tabaci infestation, we observed similar results in behavioral and chemical analyses. Phytohormone and gene-expression analyses revealed that B. tabaci infestation, as well as SA application, inhibited spider mite-induced jasmonic acid (JA) production and reduced the expression of two JA-regulated genes, one of which encodes for the P. lunatus enzyme β-ocimene synthase that catalyzes the synthesis of (*E*)-β-ocimene. Remarkably, B. *tabaci* infestation concurrently inhibited SA production induced by spider mites. We therefore conclude that in dual-infested Lima bean plants the suppression of the JA signaling pathway by whitefly feeding is not due to enhanced SA levels. Zhang et al. (2009) Proceedings of the National Academy of Sciences 106: 21202

P6B-007

A novel function of flavonoids for abiotic stress tolerance

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Taking abiotic stresses, plants exercise flavonoid biosynthetic mechanism which involves signal perception, transcriptional network, expression of genes encoding structural enzyme, and biosynthesis and accumulation of flavonoids with anti-oxidant activity. Under the stresses, plants also exercise stress response mechanism which involves signal perception, abscisic acid (ABA) biosynthesis, transcriptional network, and expression of stress-related genes to obtain stress tolerances. Although induction of both mechanisms under stress conditions implies relationship between them, we have minimal information on the relationship. In *Arabidopsis thaliana* (Arabidopsis), MYB12 and MYB75/PAP1 has been promptly characterized as regulator of flavonol and anthocyanin biosynthtesis, respectively. Expression of MYB12 gene (At2g47460) and PAP1 (At1g56650) which are abiotic-stress-inducible suggests that flavonoid biosynthetic mechanism has network to stress response mechanism. Here, we report a novel function of flavonoids as stress mitigation against abiotic stresses revealed by comparative integrated analysis with stress tolerance evaluation in Columbia-0 as control, single flavonoid-related TF over-expressing plants (MYB12OX and pap1-D), and double flavonoid-related TF over-expressing plants (WOX1-1 and WOX1-2). Ectopic over-expression of MYB12 and MYB75/PAP1, regulating flavonoid biosynthesis, in *Arabidopsis thaliana* resulted in elevated production of both flavonols and anthocyanins. Tolerance to abiotic stresses of drought, salt, and oxidation was conferred in these transgenic Arabidopsis plants in which the expression of a set of stress-related genes was up-regulated. Our knowledge will provide new insights of plant survival strategy and evolution on secondary metabolites. Biotechnological application from the insights could lead to the development of plant breading for improved secondary metabolites accumulation and stress tolerance in Arabidopsis and other plants, crops.

P6B-008

Application of metabolomics tool to study inducible biochemical responses of mountain birch trees to herbivorous insect damage

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Mountain birch (*Betula pubescens ssp. czerepanovii*) is the main host plant for autumnal moth, Epirrita autumnata, in northern parts of Fennoscandia. Populations of *E. autumnata* have been shown to display cyclic fluctuations in density (with 9–10-year periodicity), which may culminate in outbreak density for 1–3 successive years and severely damage or even kill birch trees on vast areas. Monitoring of body size variables of E. autumnata larvae from the early increase phase of the cycle and through the outbreak to the end of the density decline showed that increasing of larval density was associated with decreasing body size and fecundity of the moth without a time lag. It was supposed that one of the most plausible reasons for these changes could be a rapid inducible biochemical response of birch trees to insect damage that strongly lowered the quality of leaves as a food. To characterize biochemical quality of birch leaves in the years with different larval density, we applied metabolomics tool that is based on the GC-MS and HPLC-DAD-MS platforms. Samples of leaves were taken from the same 15 trees during of three successive years with low, moderate and outbreak densities of *E. autumnata* larvae. PCA of metabolome database clearly discriminated three groups of trees that belong to years with low, moderate and outbreak densities of *E. autumnata* larvae. Among the 289 metabolites from the initial database, 93 metabolites had the best discriminating efficiency. In the year with outbreak density of *E. autumnata* larvae, the leaves of damaged (induced) trees were characterized by relatively low level of nutritive metabolites such as carbohydrates, amino acids, and lipids, etc., and accumulation of hydrolysable tannins; contents of individual galloylglucoses and ellagitannins were increased from 3 to 11 folds. At the same time total content of phenylpropanoids (p-coumaroylquinic acids, chlorogenic acids, flavonoid-glycosides and proanthocyanidins) did not change. Role of hydrolysable tannin pathway in the mechanism of inducible resistance of mountain birch trees to herbivorous insects will be discussed.

P6B-009

Unravelling mechanism of resistance against aphids in lettuce

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Aphids are an important pest of lettuce(*Lactuca* L.) in many parts of the world, like the USA, Europe and New-Zealand making lettuce unmarketable as a fresh market product. Aphids belong to the Aphididae family and have a complicated life cycle. They cause damage in several ways. Building up to high density populations, they can cause leave deformation, gall formation, withering or even plant death as a consequence of removing too much phloem sap. They also play a role as vector transmitting viruses such as cucumber mosaic virus and lettuce mosaic virus. Resistance to aphids was introgressed in 1980s from Lactuca virosa (wild relative of lettuce) into *Lactuca sativa* (cultivated lettuce). Even though it was hence possible to breed resistant lettuce, the resistance mechanism has never been unraveled. We analyse wild lettuce species and resistant and susceptible cultivars of lettuce, using untargeted metabolic profiling on GC-TOF-MS, GC-MS and LC-QTOF-MS followed by statistical analysis, to investigate and understand this resistance mechanism.

P6B-010

Re-programming of the pre-lignin pathway and lignin synthesis in susceptible interactions during *M. grisea* infection of B. *distachyon* leaves

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Induction of the phenylpropanoid pathway is an important component of plant defence against pathogens, which often includes cell wall strengthening by lignification during a resistant response. In susceptible interactions of *M. grisea* with its model host, *Brachypodium distachyon* the phenylpropanoid pathway is certainly activated but any specific effect of the pathogen on lignin biosynthesis was unclear. Phenolic extracts of healthy and infected leaves of susceptible (ABR1) and resistant (ABR5) *B. distachyon* ecotypes were therefore compared to investigate whether defensive lignification was altered during cellular penetration by a virulent pathogen. HPLC-PDA analysis of phenolic extracts at different stages of infection revealed flavonoid levels to be almost identical in healthy and infected plants of both ecotypes. In contrast, HPLC peaks with absorption spectra typical of compounds containing hydroxycinnamic acid moieties (HCA) were found elevated in infected plants. For example, the monolignol precursor and strong antioxidant, chlorogenic acid (caffeoylquinate) was only found to be present in ABR5 plants. At 72-96h after infection in ABR1 the levels of several previously reported HCA conjugates along with two novel conjugates had increased significantly and were maintained at high levels. HPLC-MS/MS analysis, accurate mass determinations by LTQ-FT FIE-MS and analysis of chemical standard are currently being used to confirm the identity of these compounds. In a resistant response (ABR5) the levels of most of these compounds had dropped significantly by the time lesions had appeared at 96hr. The reason for the continued accumulation of HCA conjugates in a susceptible response might reflect a perturbation of lignin polymerisation as our previous studies revealed the modification of reactive oxygen species (ROS) induction in ABR1. This hypothesis is currently being investigated by GC-MS analysis of cell wall bound lignin in both ABR1 and ABR5 plants. We conclude that successful fungal pathogens do not simply down-regulate the phenylpropanoid pathway but rely on subtle manipulation of the lignin synthesis machinery as they invade plant tissue.

P7A-001

Apoptotic effect of the Brazilian berry Bacaba (Oenocarpus bacaba Martius) on MCF-7 breast cancer cells.

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Bacaba berry (*Oenocarpus bacaba Martius*) is a native Brazilian fruit, found either in Amazon or Cerrado (Brazilian Savannah) biomes. Bacaba berry is one of the native fruits used from local rural communities either as food or as an agricultural product. In this context, Bacaba has an important role not only to the diet but also as a source of income generation to poor people. However, until recently no information about its biofunctionality is available in scientific the literature. In addition, previous studies have shown apoptotic effects of another Brazilian berry on cancer cells. The aim of this study is to investigate the apoptotic effects of Bacaba berry on MCF-7 human breast adenocarcinoma cells. The acetone Bacaba extract was assessed for apoptotic effects at the concentration range of 0-1000µg/mL. Antiproliferative effect was evaluated by mitochondrial activity (MTT assay). Apoptotic effect was assessed by Ethidium Bromide/Acridine Orange staining in a fluorescent microscope using the Axioplan microscope with 20x, 40x and 100x objectives, DNA Laddering and immunoblotting of PARP cleavage and caspases cleavage after 24 and 48h of Bacaba extract incubation. Staurosporine was used as a positive control. Bacaba extract had antiproliferative and apoptotic effects in a dose dependent manner on MCF-7 cell line. The IC50 of Antiproliferative effect was 252.10µg/mL with a confidence interval ranged at 242.8-261.7 µg/mL. After 48h of incubation the cleavage of PARP was observed. As due a genetic defect MCF-7 cell line doesn’t express Caspase 3, other caspases were investigated. Bacaba extract didn’t have any effect on Caspase 8 and Caspase 7, but Caspase 9 was cleaved after 48h of incubation. Results indicate that Bacaba extracts could trigger the intrinsic pathway of apoptosis on MCF-7 breast cancer cell line. Based on this, authors are engaged to investigate the biochemical mechanisms involved on it.

P7A-002

Metabolites profiled by targeted metabolomics associate with lipid levels

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In several diseases total cholesterol (Chol) and triglyceride plasma concentrations are considered as a risk factor. The associations between lipids concentrations and metabolic pathways in humans are still not fully understood, especially how the plasma lipid levels are modulated by disease (e.g. diabetes or cardiovascular) or drug (e.g. statin) treatment. To address these questions we used targeted metabolomics to analyze Chol-associated lipidome and further metabolic pathways in human individuals. We quantified 363 metabolites in 283 serum samples from the human cohort KORA with profiling by electrospray ionization on API 4000 tandem mass spectrometer (1). High-throughput analyses were assisted by robotized liquid handling, quality assurance and multivariate data analyses. We targeted selected analytes from the following classes: amino acids, hexoses, biogenic amines, oligosaccharides prostaglandins, acylcarnitines, sphingomyelins, and glycerophospholipids. When analyzing the concentrations of total Chol, HDL, and LDL and triglycerides we discovered novel significant associations with several analytes pointing to so far unknown cross-talks in metabolic pathways. Some phosphatidylethanolamines correlated with HDL concentrations (C36:2 at p=1.0E-09, C40:6 at p=2.2E-09), further phosphatidylcholines with that of triglycerides (C38:4 at p=3.2E-31) or total Chol (C38:1 at p=1.4E-26, C38:2 at p=4.4E-25) and sphingomyelines with total Chol (C16:0 at p=2.3E-20, C18:0 at p=2.2E-17). We discovered a significant correlation of amino acid concentrations with triglyceride concentrations (e.g. Glu at p=4.7E-10, Phe at p=2.1E-08, Trp at p=1.4E-07) with that of triglycerides. These new associations and potential links with endpoints such as cardiovascular disease will have to be investigated in the future. (1) Th. Illig, et al. (2010) A genomewide perspective of genetic variation in human metabolism. Nature Genetics, 42(2):137-41

P7A-003

Impact of hormonal receptors expression on breast cancer metabolic phenotypes

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Breast cancer is a leading cause of deaths worldwide. The expression of hormonal receptors (ER, PR, and HER2) influences cellular signaling and metabolic pathways. In this study, we aimed to identify distinct metabolic phenotypes for mammary tumors, specifically for patients with grade 3 tumors and triple-negative hormone receptor status tumors (estrogen ER, herceptin HER2 and progesterone PR). Using a cohort of 261 clinically well-characterized breast cancer patients in independent training and validation series, over 400 unique metabolites were detected by GC-TOF MS profiling and BinBase data processing of which a total of 154 non-redundant metabolites were structurally identified by a multi-tiered retention index and mass spectral scoring algorithm. Subsequently, a range of statistical comparisons were undertaken to distinguish specific metabolic phenotypes in grade 1, 2 and 3 tumors and subcategories of receptor status. 50% of all patients had ER+,PR+,HER- receptor status , 12% triple positive status and 14% triple negative receptor status (ER-,PR-,HER-). The latter group has worse prognosis and fewer therapy options and thus, a better understanding of cancer cell metabolism may help finding novel target pathways. We found that the most important metabolic phenotypes distinguishing different tumors were the morphological differentiation status (grade 1-3) and expression of the estrogen receptor (ER-/ ER+), specifically for alterations in nucleotide metabolism and the arachidonate pathway . Triple negative tumors (ER-,PR-,HER-) were mostly of the poorly differentiated grade 3-subtype with dysregulation of amino acids, nucleotide and energy metabolism in comparison to grade 3 tumors of the double-positive ER+,PR+,HER- category. Subsequently, the metabolic impact of the presence of the herceptin receptor HER2 was investigated by comparing triple negative to double-negative tumors (ER-,PR-,HER+). In this comparison, a clear influence of lipid metabolism was found by significantly higher levels of free fatty acids and glycerol phosphates pointing towards biosynthesis of membrane lipids that are a hallmark of cancer cell metabolism and cell division. Together, these studies may lead to novel hypotheses on cancer cell metabolism with potential high impact for therapeutic options.

P7A-004

Metabolite set enrichment analysis: A tool to analyze metabolomics data

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Gene set enrichment analysis (GSEA) is now an established method in RNA expression analysis. This statistical method makes it possible to calculate the enrichment based on gene sets. Biological interpretation of -omics data in general and metabolomics data specifically is difficult. Firstly, because presently a limited amount of bioinformatics tools is available and secondly because it is currently impossible to analyze the complete metabolome, which is not taken into account by the bioinformatics tools available that calculate pathway enrichment. One of the questions often raised in metabolomics studies is whether there is a relation between the significantly affected metabolites in the study. Finding these relations can be done by hand, but for large dataset this will be laborious. Moreover, such an analysis will be biased to certain pathways and processes (generally to the well known pathways). We show how metabolite set enrichment analysis (MSEA) can help and accelerate the interpretation of metabolomics data.

P7A-005
Metabolic profile of HDAC inhibitors in human colon adenocarcinoma cell line HT29

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Cell differentiation is an orderly process that begins with modifications in gene expression. This process is regulated by the acetylation state of histones. Removal of the acetyl groups of histones by specific enzymes (histone deacetylases, HDAC) usually downregulates expression of genes that can cause cells to differentiate, and pharmacological inhibitors of these enzymes have been shown to induce differentiation in several colon cancer cell lines. Butyrate at high concentration is both a precursor for acetyl-CoA and a known HDAC inhibitor that induces cell differentiation in colon cells. The dual role of butyrate raises the question whether its effects on HT29 cell differentiation are due to butyrate metabolism or to its HDAC inhibitor activity. To distinguish between these two possibilities, we used a tracer-based metabolomics approach to compare the metabolic changes induced by two different types of HDAC inhibitors (butyrate and the non-metabolic agent trichostatin A) and those induced by other acetyl-CoA precursors that do not inhibit HDAC (caprylic and capric acids). [1,2-$^{13}C_2$]-D-glucose was used as a tracer and its redistribution among metabolic intermediates was measured to estimate the contribution of glycolysis, the pentose phosphate pathway and the Krebs cycle to the metabolic profile of HT29 cells under the different treatments. The results demonstrate that both HDAC inhibitors (trichostatin A and butyrate) induce a common metabolic profile that is associated with histone deacetylase inhibition and differentiation of HT29 cells whereas the metabolic effects of acetyl-CoA precursors are different from those of butyrate. The experimental findings support the concept of crosstalk between metabolic and cell signalling events, and provide an experimental approach for the rational design of new combined therapies that exploit the potential synergism between metabolic adaptation and cell differentiation processes through modification of HDAC activity. This work was supported by the Catalan (2009-SGR1308) and Spanish (SAF2008-00164) Governments and the ISCIII-RTICC (RD06_0020_0046), the European Union FEDER funds and the European Commission (FP7) Etherpaths KBBE(#222639).

P7A-006
Identification of a novel gamma-glutamyltranspeptidase pathway by metabolic profiling studies in *Trypanosoma brucei*

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Trypanosoma brucei is the causative agent for Human African trypanosomiasis (HAT), a potentially fatal parasitic disease of sub-Saharan Africa. Existing HAT treatment options are unsatisfactory, primarily due to toxicity and resistance, and discovery of new trypanocidal drugs is urgently required. Emerging metabolomics technologies offer new opportunities to explore parasitic metabolism, and discover novel pathways and enzymes that may provide potential drug targets. The complex lifecycle of T. brucei requires transmission between mammalian hosts by the tsetse fly vector, and energy metabolism differs as the predominant carbon source changes from glucose-rich blood, to the proline-rich tsetse fly midgut. In this study, an untargeted metabolomics approach was used to characterise the metabolic differences between procyclic forms of T. brucei cultured in vitro with either glucose-rich, or proline-rich, media. Cell extracts were analysed by accurate mass LCMS, with hydrophilic (HILIC) chromatography coupled to an Exactive Orbitrap mass spectrometer. Metabolites were identified based on accurate mass matching to metabolite databases, after removing common adducts and fragments, followed by evaluation of retention times to improve the accuracy of metabolite identification. The expected differences in energy metabolism were observed between glucose and proline grown cells, however additional metabolic differences were also observed that do not appear to be directly related to energy metabolism. Interestingly, the presence of pyroglutamic acid, and glutamyl dipeptides, suggested the presence of a gamma-glutamyltranspeptidase pathway, which has not been previously reported in trypanosomes. Gamma-glutamyltranspeptidase activity was validated by classical biochemical techniques, however the presence this enzyme was not predicted by database mining within the published T. brucei genome, indicating that the trypanosome has enzymes that are not orthologous with those in species where the transpeptidase has been characterised, thus highlighting the usefulness of an untargeted metabolic profiling approach for the identification of novel metabolic pathways.

P7A-007
REGULATION OF LIPID METABOLISM IN BREAST CANCER

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Previous studies have shown that specific changes in lipid metabolism at the gene expression level are a prominent feature in many tumors. However, very few studies so far have investigated the lipid molecular composition in tumor cells and tissues. Here we applied the lipidomic approach to characterize the lipidome in breast cancer tissues, as part of the METAcancer EU project. Global lipidomics using UPLC/MS was performed on a series of 267 breast cancer samples, divided into discovery and validation series. The data were processed with MZmine 2 software and the peaks identified by tandem MS. Large differences between tumors and normal breast tissue were observed for specific classes of membrane lipids. Generally, phospholipid concentrations were elevated in malignant tissue, with the highest concentrations found especially in grade 3 and estrogen receptor negative tumors. The specific enrichment of palmitate in phospholipids, indicative of increased de novo synthesis of fatty acids, was a prominent feature of these aggressive tumors. Several related lipids were also associated with poorer survival of the patients. Motivated by the observed phospholipid changes, we performed comprehensive mining of published cancer gene expression data for selected genes of potential relevance to the findings. The lipid metabolism genes found specifically overexpressed in breast cancer tissues or cells were then silenced using RNAi in multiple breast cancer cell lines. In agreement with the tumor lipidomics results, for most genes the silencing affected cell viability as well as lipidomic profiles of the cancer cells. Together, our results are consistent with earlier studies highlighting the increased de novo fatty acid synthesis in tumors. Our study may also provide a basis for better understanding of upstream regulation of lipid metabolism in cancer cells.

P7A-008
Metabolic Flux Phenotyping of Excess and Limited Inorganic Carbon Supply in Cells of the Wild Type and Photorespiratory Mutants of the Cyanobacterium Synechocystis sp. Strain PCC 6803

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Gas chromatography-mass spectrometry based metabolic flux phenotyping of primary metabolic pathways was performed in order to monitor the ^{13}C-dilution after a very high $^{13}CO_2$ pulse in photoautotrophically grown cultures of *Synechocystis* sp. PCC 6803. The pulse was followed by a chase using a high 5% CO_2 or a limited 0.035% CO_2 regime of ambient isotope composition corresponding to the pre-acclimation conditions of the cells. We introduce the initial rate of ^{13}C-accumulation 0.5-10 min after the pulse and maximum observed ^{13}C-enrichment at 20-60 min after the pulse in metabolite pools and respective ratios over 3-phosphoglycerate or phosphoenolpyruvate as flux phenotyping parameters. With these simplified tools we formally demonstrate ^{13}C-flux into the glycolate pool under conditions thought to suppress photorespiration. Using the glycolate accumulating glcD1 mutant we demonstrate enhanced ^{13}C-flux into the glycolate pool under conditions favouring photorespiration and enhanced ^{13}C-flux into the glycine pool of the glycine accumulating gcvT mutant. We demonstrate two major paths of CO_2 assimilation in *Synechocystis* under VHC conditions, namely from 3PGA via glucose-6-phosphate to sucrose and from 3PGA via PEP to aspartate, malate and citrate. The flux phenotypes of 3PGA and sucrose were consistent with previous knowledge on altered rates of photosynthesis under HC compared to LC and in the above mutants. Furthermore, the results reveal evidence of carbon channelling from 3PGA to the PEP pool and of enhanced flux through the PEP carboxylase path in the glcD1 and gcvT mutants under conditions favouring photorespiration.

P7A-009

Early metabolomic changes in cancer and normal cells following H_2O_2 exposure

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Most cancer cells have been widely known to enhance glycolysis even under normoxia, yet this still remains a subject of controversy. A recent hypothesis proposes that cancer cells enhance glycolysis to protect themselves from oxidative stress. Since cancer cells show increased steady-state levels of reactive oxygen species (ROS) in comparison to normal cells, the cells would have different strategies to eliminate ROS. Energy production metabolism is an important system to remove ROS. NADPH is mainly produced through pentose phosphate pathway (PPP) and increases reduced form of glutathione (GSH) to eliminate ROS. To reveal a possible correlation between glycolysis and ROS deletion system in normal and cancer cells, we investigated the metabolomic changes of pancreatic normal and cancer cells responding to hydrogen peroxide by using capillary electrophoresis time-of-flight mass spectrometer (CE-TOFMS). Interestingly, the metabolites involved in glycolysis and PPP significantly changed within 1 hr from the initiation of oxidative stress in both normal and cancer cells and nearly returned to the initial levels within 3 hrs. Moreover, cancer cells, relative to normal cells, showed more significant increases in PPP metabolites within 10 min. The amount of GSH in a cancer cell was approximately 3 to 10 times higher than a normal cell. These results support the hypothesis that cancer cells increase glucose metabolism in order to relieve oxidative stress. On the other hand, TCA intermediates showed cancer-cell-specific metabolic changes though the underlying mechanisms are still unknown. This study compared the difference in metabolic strategies against oxidative stress between cancer and normal cells and may lead to identify a crucial biochemical component that would be targeted to selectively kill cancer cell.

P7A-010

Unravelling starch metabolism in Arabidopsis using metabolite profiling

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Metabolomics is emerging as an important tool for the understanding of plant systems. We show here the application of a supervised multivariate technique, partial least squares discriminant analysis (PLS-DA), as a method for the classification of mutant Arabidopsis plants based on metabolite levels and we present the results of two distinct applications. In both cases, the technology used to profile the low molecular chemicals was chromatography, followed by mass spectroscopy (LC-MS and GC-MS). The first and preliminary application was to discover whether PLS-DA, which has been examined before (Scott et al, 2010; Westerhuis et al 2008), could be used here to assess the metabolic role of particular genes and their contribution to the overall metabolic functioning of plant cells and organs. We classified well-characterized Arabidopsis mutants with the aim of making predictions on a selection of transposon insertion lines. A clear discrimination of the lipid mutants act 1 and fad2-1 was one of the most informative observations. For the second application, the metabolite fingerprint was used as a complementary tool for the chararacterization of starch metabolism in Arabidopsis leaves. We focus on two particular features of starch degradation pathway, the phosphorylation and dephosphorylation of the surface of the starch granule involving the enzymes SEX1 (GWD1), and the export of maltose from the chloroplast and its subsequent metabolism in the cytosol involving the maltose transporter protein MEX1 and the transglucosidase DPE2. We will present the outcomes of this analysis.

P7A-011

CE-MS-based Metabolomics Identified a Novel Anaerobic Energy Metabolism of Cancer Cells

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Chronically hypoxic and glucose-deprived microenvironment of tumors, as typically observed in pancreatic cancer, raises a paradox; cancer cells perpetually proliferate while exhausting glucose and oxygen from the surrounding tissue. Intrinsically, cancer cells actively consume glucose even under aerobic condition (Warburg effect) and their adaptive responses to hypoxia further enhance glucose consumption. With a limited supply of glucose, upregulation of glycolysis alone cannot fully explain energy production of hypoxic cancers. Here, we used state-of-the-art metabolomics technology based on capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and analyzed energy metabolism of 9 cancer and 4 normal cell lines exposed to hypoxia and glucose deprivation by measuring more than 130 compounds with time. The results disclosed unexpected cancer-specific metabolic profiles, which sparked an idea that cancer cells exposed to a severe nutrient deprivation rely on an atypical anaerobic energy metabolism that resembles so-called NADH-fumarate reductase system identified in parasitic helminthes. Intriguingly, pyrvinium pamoate, a commercially available anthelmintic, is known to be cytotoxic against not only parasites but also cancer cells exclusively under nutrient deprived condition, supporting an idea that cancer cells and parasitic helminthes share unique anaerobic energy production machinery in common. Accordingly, we demonstrated a potential of CE-MS-based metabolomics, realizing comprehensive and temporal monitoring of the energy metabolism of cancer cells. This unique metabolic machinery may constitute a novel therapeutic target to eradicate not only parasites but also tumors.

P7A-012

In Vitro Elucidiation of Troglitazone-Induced Hepatotoxicity Using a Metabolomics Approach

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Troglitazone is a member of the thiazolidinedione (TZD) class of drug, and was the first TZD to be marketed for the treatment of type 2 diabetes. Troglitazone is an agonist for the peroxisome proliferator-activated receptor gamma, and reduces blood glucose levels by enhancing insulin sensitivity, primarily in the adipose and skeletal muscle tissues. Troglitazone was withdrawn from the market following concerns of idiosyncratic hepatotoxicity although other TZDs are still safely prescribed. A considerable effort has been made to elucidate the mechanisms of troglitazone-induced hepatotoxicity and proposed mechanisms include the formation and accumulation of toxic metabolites, oxidative stress, mitochondrial dysfunction, steatosis, apoptosis, and inhibition of bile salt excretory protein and cholestasis. This study aimed to use an in vitro cell culture system (HuH7 hepatic-type cell-line) and a metabolomics approach (liquid chromatography coupled to mass spectrometry, LC-MS) to identify metabolites correlated with troglitazone hepatotoxicity and subsequently differentiate affected metabolic pathways. Following incubation of the cells either with troglitazone or the non-hepatotoxic TZDs (rosiglitazone and pioglitazone), the cell culture medium and cells were subjected to analysis by LC-MS. Data analysis by projection to latent structures discriminant analysis (PLS-DA) identified several differentiating metabolites that have been linked to glutathione utilisation (GSH), nicotinamide metabolism (nicotinuric acid), glycolysis (pyruvate), and the tricarboxylic acid cycle (malate). Work currently in progress will verify the identity of these metabolites, and assess the impact of troglitazone on the proposed metabolic pathways.

P7A-013

Metabolomic analysis of mouse tissues deficient for Agpat4, a putative acyltransferase involved in glycerophospholipid metabolism

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Sequence homologies indicate that Agpat4 (1-acyl-sn-glycerol-3-phosphate acyltransferase 4) belongs to a family of acyltransferases participating in glycerophospholipid metabolism. However its function in vivo has not yet been established. Members of this family have been reported to exhibit different activities and expression patterns, which may reflect tissue-specific functions. Our RNA expression data show that Agpat4 transcripts are present in most catecholaminergic tissues including the dopaminergic cell groups of the midbrain, the superior cervical sympathetic ganglia and the adrenal medulla. These observations suggest that Agpat4 function might be related to catecholamine metabolism. To explore the endogenous pathways in which Agpat4 is involved, we engineered knockout mice and decided to compare the metabolome of Agpat4-deficient and wild-type mice. Using liquid chromatography coupled to an electrospray LTQ-Orbitrap mass spectrometer, we examined tissues in which Agpat4 gene expression was established beforehand - in particular the ventral midbrain and the adrenal gland. We also analyzed the striatum, which contains dopaminergic projections from the midbrain. The results in negative ionization mode highlight ~ 80 signals whose intensities differ between the knockout and wild-type mouse tissues. Several of them can be attributed to different classes of glycerophospholipids. MS\² experiments are currently being carried out to identify these signals. The analysis of the data acquired in positive ionization mode is still in progress. First results reveal that dopamine levels are lower in the striatum of knockout mice than in wild-type striata. This work shows that Agpat4 knockout affects multiple metabolic pathways, notably dopamine metabolism. It should allow the identification of the reactions catalyzed by Agpat4 in vivo and may uncover unexpected relationships between pathways.

P7A-014

Comparative Analyses of Metabolomics and Phospho-Proteomics Reveal Discriminatory Metabolic Profiles in Lung, Prostate, and Pancreas Tumors

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MOTIVATION. Environment of cancer cells is often severe in oxygen and nutrient supplies, because blood flow in most tumors are very poor, resulting in hypoxia and nutritional starvation of cancer cells. However, cancer cells in internal organs with poor blood flow, e.g., pancreas, are extremely malignant. On the other hands, because metabolite levels are reflection of change of metabolisms, combinatorial analysis of phosphorylation pattern of enzymes and metabolome is the hopeful approach to understand cancer metabolisms. Here, we present far different profiles of metabolome and protein phosphorylation in pancreas (poor blood flow), prostate (middle blood flow), and lung (rich blood flow) tumors relative to that of normal tissues. METHODS. Tumor and surrounding normal tissue samples were collected surgically from identical patient; thirteen, eight, and six for lung, prostate, and pancreas cancers, respectively. Most of the cancer cells are pathologically diagnosed as adenocarcinoma. Metabolites were extracted by a methanol/chloroform/water method and quantified by CE-TOFMS. Phosphorylated peptides were extracted and separated by phospho-affinity column. A nano-LC-Orbitrap MS was used. FINDINGS. In the CE-TOFMS-based metabolome analyses, we obtained the data of more than 250 metabolites from the samples, and performed multivariate analysis. For the lung and pancreas cancer, the metabolome profiles were well-distinguished between tumor and normal tissues, but not for prostate cancer. Interestingly, inverted accumulation trends of metabolites were observed between lung and pancreas cancers, indicating reciprocal change of metabolite levels in major metabolic pathways including the glycolysis, the tricarboxylic acid (TCA) cycle, amino acids, and energy-carrier metabolites. Furthermore, profiles of protein phosphorylation were varied between the tumors in metabolic enzymes, signal transduction-associated proteins, and transcription factors. Our findings suggest that the difference of oxygen and nutrient supplies in each internal organ affects the metabolic states of tumors, and the cancer types can be characterised by the combinatorial approach of phospho-proteomics and metabolomics.

P7A-015

Remodeling of Adipose Tissue Lipidome as Adaptation to Acquired Obesity: Benefits and Costs

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Identification of early mechanisms that may tilt obesity towards the complications such as metabolic syndrome is of great interest. We studied 26 healthy monozygotic twins discordant for obesity and show that adipose tissue of obese co-twins is characterized by specific lipid remodeling. Despite lower dietary polyunsaturated fatty acid (PUFA) intake, the obese co-twins had increased proportions of palmitoleic and arachidonic acids (AA) in the adipose tissue, including increased levels of AA containing ethanolamine plasmalogens. Based on the observed differences in phospholipid composition, we performed molecular dynamics simulations of different phospholipid membrane systems. Surprisingly, the study revealed that the observed lipid remodeling maintains the biophysical properties of lipid membranes. However, such remodeling comes at a price since AA-containing plasmalogens are precursors of potent inflammatory mediators. We then reconstituted a dependency network by combining clinical, lipidomics and gene expression data from the adipose tissue. We found that the lipid remodeling is controlled by a complex network involving fatty acid desaturation and elongation, and that the elongase Elovl6 is an important network hub sensitive to decrease of dietary PUFA content in obese co-twins. Knock-down of Elovl6 in 3T3-L1 adipocyte cell line revealed a mirroring lipid pattern as observed in obesity and thus confirmed the importance of Elovl6 in observed lipid remodeling. In another study, adipose tissue lipidome was analyzed from 8 morbidly obese subjects. In these subjects, the proportion of AA containing plasmalogens in the adipose tissue was markedly decreased, suggesting a breakdown of the adaptive mechanism observed in healthy obese individuals. Together, our study identified a new adaptive mechanism amenable to modulation which maintains the adipocyte membrane properties in acquired obesity with a price of increased vulnerability to inflammation. Our study may also open new opportunities for the prevention or treatment of obesity-related metabolic complications.

P7A-016

EFFECTS OF NITRIC OXIDE-INDUCED ALDOLASE NITRATION ON THE GLYCOLYTIC METABOLITES OF MAST CELL

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Introduction Mast cells (MC) are primary effector cells of IgE-mediated allergic inflammation. Nitric oxide (NO) is a short-lived free radical that regulates MC activities including inhibition of MC degranulation. To elucidate the molecular mechanisms underlying the effects of NO in MC, we investigated protein tyrosine nitration in human mast cell lines treated with the NO donor S-Nitrosoglutathione (SNOG). Methods Two dimensional electrophoresis and western blot analysis with an anti-nitrotyrosine antibody together with mass spectroscopy was used to identify the targets for nitration in human MC lines HMC-1 and LAD-2. The aldolase activity and the intracellular fructose 1,6 bisphosphate (FBP), the substrate for aldolase were measured using a standard aldolase assay. Nuclear Magnetic Resonance (NMR) was employed to define the metabolic changes associated with NO treatment. MC degranulation was measured using \-hexosaminidase assay. Results Aldolase A, an enzyme of the glycolytic pathway was identified as a target for tyrosine nitration in MC. Aldolase A nitration was associated with reduction in the Michaelis constant (Km) and maximum velocity (Vmax) of aldolase in HMC-1 and LAD-2. NMR analysis revealed no significant changes in total cellular ATP content, although the AMP/ATP ratio was altered. Elevated levels of lactate and pyruvate suggested that NO treatment enhanced glycolysis in MC. Moreover, reduction in MC aldolase activity was associated with increased intracellular levels of its substrate, fructose 1,6 bisphosphate (FBP). Interestingly, FBP inhibited IgE-mediated MC degranulation in LAD-2 cells. Conclusions Inhibition of MC degranulation by FBP has the potential to regulate MC function through multiple signaling pathways including phospholipase C (PLC). We are currently dissecting the precise signaling pathways underlying the effects of FBP. Analyses of the possible links between aldolase nitration, altered glycolytic metabolites including FBP levels and the regulation of MC function will evaluate the potential immunoregulatory role of FBP in allergic and immune diseases.

P7A-017

Interaction between primary and secondary metabolism of the aromatic amino acids in Arabidopsis thaliana

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The aromatic amino acids; phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp), are central molecules in plant metabolism, functioning both as primary building blocks of proteins and also as precursors for a variety of wide range of aromatic secondary metabolites with multiple biological functions and biotechnological values. Yet, the interplay and regulation of their synthesis is still far from being understood. To address this question, we expressed in transgenic Arabidopsis plants a bacterial bifunctional PheA* (Chorismate Mutase /Prephenate Dehydretase) gene that converts chorismate via prephenate into phenylpyruvate. These plants displayed a significant overproduction of Phe as well as a number of Phe-derived metabolites. This implied that plants can convert phenylpyruvate into Phe and also that the level of Phe influences the pattern of its catabolism into various classes of secondary metabolites. Notably, the levels of homogentisate and tocochromanol (vitamin E) that are derived from Tyr catabolism were enhanced in these plants. Furthermore, these plants were more sensitive to the Trp inhibitor, 5-mehtyl-Trp, in the growth medium and the levels of several Trp catabolic products, including derivatives of the hormone IAA, were reduced. This study engenders novel insights into the regulation of plant Phe metabolism, its cross-regulation with the metabolic networks of Trp and Tyr and the regulatory interface between primary and secondary metabolism.

P7A-018

A metabolomic investigation of diabetic cardiomyopathy in mouse models of diabetes

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Patients with diabetes (both type I and II) are at increased risk of developing cardiovascular disease and heart failure. Among these cardiovascular complications, diabetic cardiomyopathy, first described by Rubler et al(1), is characterized by ventricular dysfunction independent of coronary artery disease (CAD) or hypertension. Although the pathogenesis is not fully understood, altered myocardium substrate utilization and energy metabolism have become important contributors to the development of diabetic cardiomyopathy in both human and animal studies, where the diabetic heart shows enhanced fatty acid uptake with a coordinate decrease in glucose uptake(2). As the existence of diabetic cardiomyopathy is increasingly recognized, a better understanding of mechanisms responsible for the development of diabetic cardiomyopathy is required for early diagnosis and therapeutic treatment. The purpose of this study is to build a snapshot of altered metabolism in hearts from a wide range of diabetic mouse models, both type 1 and type 2 diabetes, using metabolomic approach to gain mechanistic insights in the progression of diabetic cardiomyopathy. Heart and skeletal muscle tissues were obtained from 3 different age groups (4-6 weeks, 11-12 weeks, and 20 weeks) from type 1 diabetes non-obese diabetic (NOD) mice. NOD-E mice were used as controls as this strain is almost congenic with the NOD mice but they are completely protected from diabetes onset. Analytical platforms utilized in this study include high resolution 1H NMR spectroscopy, Gas-Chromatography Mass spectrometry (GC-MS) and Liquid-Chromatography Mass spectrometry (LC-MS). In addition, targeted analysis of carnitine derivatives, which are implicated in disease progression, was performed using a triple quadrupole LC-MS. Our primary results show a distinct metabolic profiling of NOD mice from NOD-E mice across all the age groups for both organic and aqueous metabolite analysis. Reference 1. Rubler S et al. The American journal of cardiology. 1972;30(6):595-602. 2. Carley AN et al. Biochimica et biophysica acta. 2005;1734(2):112-26.

P7A-019

Application of High-Throughput Metabolomics to Inherited Cardiomyopathies

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Inherited dilated cardiomyopathies (DCMs) are monogenic disorders that are caused by mutations in more than 30 genes. DCM is characterised by substantial phenotype heterogeneity thus making it difficult to diagnose the condition by traditional genetic testing. While DCM can be caused by many external factors, in about 50% of cases is familial. This work will be carried out as part of the EU Framework 7 project INHERITANCE (Integrated Heart Research In TrANSlational genetics of dilated Cardiomyopathies in Europe) which encompasses 11 research centres in Europe. Heart tissue samples of 2000 human cohorts, 1000 with known disease mutation and 1000 with unknown disease mutation, will be metabolically profiled and cross referenced with data from analyses of plasma samples from these patients with the hope of finding biomarkers for the condition from the peripheral fluid. Heart tissue for a mouse model of hypertrophy cardiomyopathy (HCM) will also be profiled. The mouse model will be analyzed by an open profiling strategy to provide possible directions for the analysis of the human samples using a targeted high-throughput approach.. With this strategy in mind, assays have been developed in tandem with traditional extraction methods, such as the methanol/chloroform extraction, for the MS analysis of a range of metabolite classes. Total fatty acids will be analysed via GC-FID, amino acids via GC-MS (utilising the EZ:faast SPE extraction kit by Phenomonex) and intact lipids using LC-MS on a QToF Ultima. The bulk of the analysis, however, is conducted on a Quattro Premier triple quadrupolar MS where analyses of acyl CoA derivatives, carnitines, Krebs cycle intermediates, sugar phosphates and sugar nucleotides have been developed. The emphasis on triple quadrupolar MS is intended to provide the assays with greater sensitivity and selectivity by making use of tandem MS-MS capabilities.

P7A-020

Absence of Cross-pathway Associations of Reduced Tyrosine with Tryptophan Metabolites in First-Episode Neuroleptic-Naïve Patients with Schizophrenia

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Schizophrenia is a biologically complex disorder with perturbations in multiple neurochemical systems whose dynamic interrelationships, until recently, have been difficult to examine. Rather, evidence for these alterations has been collected piecemeal, limiting our understanding of the interactions amongst relevant biological systems. Using high-pressure liquid chromatography coupled with a coulometric multi-electrode array system, we evaluated both tyrosine and tryptophan pathways by comparing 25 metabolites simultaneously in the plasma between first-episode neuroleptic-naive patients with schizophrenia (FENNS, n=25) and healthy controls (HC, n=30) as well as between FENNS at baseline (BL) and 4 weeks (4w) after antipsychotic treatment. The monoamine variables were found to have significant mutual dependencies, but these did not show the same pattern in the HC and the FENNS groups. Wilcoxon rank-sum tests found significant differences between HC and each of the FENNS groups; specifically, tyrosine was lower and N-acetylserotonin was higher in the FENNS groups. The tyrosine levels were significantly correlated with tryptophan, kynurenine, 3-hydroxykynurenine and melatonin in all three groups. By contrast, correlations of tyrosine with each of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid were all significant for the HC but not for the FENNS. Our data suggest significant correlations between tyrosine and tryptophan metabolites; although some of these correlations persist across disease or medication status, others appear to be lost among FENNS. Specifically, reduced tyrosine appears to be associated with the up-regulation of tryptophan hydroxylase pathway leading to the production of N-acetylserotonin in FENNS patients. Together, both reduced tyrosine and elevated N-acetylserotonin may in part result from excessive sympathetic tone in acutely psychotic patients. Considering N-acetylserotonin as a potent antioxidant, such increases in N-acetylserotonin might also be a compensatory response to increased oxidative stress in schizophrenia.

P7B-001

Integrating genetic markers with ~omics data using genetical genomics and modern regression methods

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Utilization of the natural genetic variation in traditional breeding programs remains a major challenge in crop plants. In the post genomic era, high throughput technologies give rise to data collection in fields like transcriptomics, metabolomics and proteomics and as a result, large amounts of data have become available. We have screened a diploid potato population for gene-expression and obtained LC-MS data resulting in the identification of many expression and metabolite QTL's across the genome. However, the integration of these data sets with phenotypic and marker data is still problematic. Here we present novel approaches to study the various ~omic datasets to allow the construction of networks integrating gene expression, metabolites and markers. We used univariate regression and modern regression methods like lasso, elastic net, sparse partial least squares regression to select subset of the metabolites and transcripts which shows association with potato tuber flesh colour. Selected subset of metabolites and transcripts shows high significant (p value<2.2e-16) to the flesh colour trait and variance explained by regression model is about seventy one percent.

P7B-002

Omics Assisted Identification of Genes Involved in the Cladosporium-Tomato Interaction

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The Cladosporium-Tomato interaction is one of the most extensively studied and best characterized pathosystems that is employed in gene for gene interaction researches. The so-called "dying seedling model" is one of the valuable tools used to study this pathosystem. These plants are generated by crossing tomato plants expressing fungal avirulence gene (Avr-4) to resistant Cf-4 expressing tomato lines. When such plants are grown at 33oC and high RH, the hypersensitive response (HR) is suppressed. A synchronized defense response is subsequently induced by transferring the plants to 20oC. This results in a massive HR which represents the response of a resistant plant to Cladosporium fulvum. Hence, such a pathosystem can be used to amplify the localized HR response that is observed during the infection of tomato leaves by C. *fulvum*. We have used the dying seedlings and a number of the currently available cutting-edge, high throughput technologies to study defense associated transcriptome and metabolome reprogramming. The integrated approach revealed a strong reprogramming of gene expression and corresponding change in the metabolome during the defense response. Many genes that are associated with the biosynthesis of primary and secondary metabolites showed up-regulation in the dying seedlings. Inline with this, the GC-TOF-MS and LC-QTOF-MS analyses showed a strong accumulation of a number of polar primary metabolites and secondary metabolites in the dying seedlings. Using multivariate statistics and bioinformatics tools, associations of known and unknown genes that are or may be involved in the biosynthesis of primary and secondary defense metabolites could be pinpointed.

P7B-003

Determination of metabolic fluxes in developing crop seeds using steady-state ^{13}C metabolic flux analysis

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One of the main sources for food, feed and industrial raw material are plants. In order to optimize the yield of plant products metabolic engineering becomes more and more important. For this reason it is essential to understand the control and the dynamics of plant metabolism. A powerful tool for determination of plant metabolism is Metabolic Flux Analysis (MFA). It has become well-established especially in microorganisms. The objectives of research are to understand how to manipulate the metabolism in a rational way leading to an increase of the yield of favoured products. Due to the higher complexity of plants compared to microorganisms it is more difficult to manipulate plants beyond the scope of secondary metabolites and composition of storage compounds. Attempts to redirect major carbon flows were much less successful. Hence a system wide analysis and a more general understanding of metabolic processes in plants are necessary. Metabolic flux is a direct measure of a cell\'s metabolic activity thus representing a very specific phenotype. It includes extremely important information for targeted improvement of crop metabolism. MFA can be deduced from a combination of stable isotope labeling experiments and computer modeling. In contrast to the well established Flux Balance Analysis, this method allows the generation of detailed flux maps, including parallel, bidirectional, and cyclic fluxes. With the main focus on crop seeds we are developing experimental and computational pipelines for performing MFA including development suitable cultivation method for embryos, adaption of existing protocols for GCMS analysis to the new material, setup of methods for data extraction and correction and establishment of a computational model of central metabolism. First results on intracellular metabolic fluxes will be presented.

P7B-004

Comparative metabolomics characterizes the impact of genotype-dependent methionine accumulation in Arabidopsis thaliana

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Methionine (Met), an essential amino acid for all organisms, has many important functions in plant cellular metabolism such as a precursor of plant hormones, polyamines, and glucosinolate. The regulatory mechanism of Met biosynthesis is highly complex and, despite its great importance, remains largely uncharacterized. To answer the key question of how accumulation of Met influences metabolism in Arabidopsis, we examined three methionine over-accumulation (mto) mutants using a established gas chromatography (GC)-time-of flight (TOF)/mass spectrometry (MS) protocol for metabolite profiling. Principal component analysis showed distinct metabotypes of the three mto mutants, mto1, mto2, and mto3. A more sophisticated approach based on orthogonal projection to latent structures–discriminant analysis highlighted genotype-related differences. Though Met accumulation in mto1 had no dramatic effect on other metabolic pathways except for the aspartate family, metabolite profiles of mto2 and mto3 indicated that several extensive pathways were affected in addition to over-accumulation of Met. The marked changes in metabolic pathways in both mto2 and mto3 were associated with polyamines. We suggest that comparative metabolomics can not only reveal the impact of Met over-accumulation on metabolism, but also may provide clues to identify crucial pathways for regulation of metabolism in plants.

P7B-005

Modeling the network of genes responsible for polyphenol biosynthesis in Tomato

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In order to elucidate the structure of the network of genes responsible for phenylpropanoid biosynthesis a new experimental system has been performed using tomato seedlings as a model. Extensive metabolomic datasets have been generated from seedlings growing under fully climate controlled conditions. Seedlings from five to nine days after sowing were harvested, extracted and their metabolic profile evaluated using an accurate mass spectrometer (LC-QTOF). The existing database for tomato fruit has been used for the putative annotation of the metabolites present in the tomato seedling model. Some of the compounds already detected in the tomato fruit have been detected in the tomato hypocotyls. Anthocyanins are clearly present in tomato hypocotyls but are generally absent in fruit. By comparing the previously reported anthocyanins in transgenic tomato fruits and seedlings, anthocyanins present in tomato hypocotyls have now been identified. Additionally, a group of unknown (polyphenolic) compounds having a strong correlation with the already annotated metabolites has been selected and subjected to further chemical identification using an Orbitrap-FTMS. Datasets placing the tomato seedlings under different stress conditions (e.g. light, nutrients) have also been produced to generate contrasting metabolite profiles. Significant differences have been observed on the annotated polyphenol compounds between the tomato seedlings subjected to standard or modified conditions. Gene expression patterns of these perturbed seedlings have been analyzed and correlations with the metabolomic data are being studied. Using these metabolomic and gene-expression data, new statistical methods and mathematical models are being generated and applied to find the structure of the gene network underlying the polyphenol biosynthesis in tomato. We expect that this model will help us to predict and expand our knowledge to improve agronomic traits in tomato.

P7B-006

Metabolite Profiling in Tomato Fruit Employing Automated Software for Biomarker Discovery and Identification

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Food crop characteristics such as nutritional value, quality, resistance to pathogens and flavor are important traits for the food industry and consumers. Metabolomics has been identified as a key mass spectrometry-based approach in the analysis of such characteristics. Herewith, results from metabolite profiling and identification experiments on tomato samples will be presented. Triplicate biological replicates of two tomato fruit cultivars were analyzed at four time points of fruit development stages using fast reversed-phase chromatography prior to mass spectrometric analysis, carried out on a hybrid high resolution mass spectrometer instrument. Strategies for metabolite profiling and identification were successfully applied and encompassed sample measurement in positive and negative ion mode electrospray ionization in conjunction with multiple dissociation techniques and extensive data mining. Preliminary results indicate that the high sample complexity in survey scans in the mass range 90-900Da benefits from highly-resolving, profile mode analysis. Hundreds of components were profiled at resolutions up to 100,000 useful for accurate and sensitive relative quantification experiments. Proper reduction of the number of detected signals through identification of sample related peaks and filtering out signals related to system background is essential. A GUI (Graphical User Interface) was developed to ensure simple fast operation of the software by novice users. In addition, grouping related signals, i.e. isotope peaks, adduct, dimers, fragments, etc. significantly simplified processing the results reducing the number of components by a factor of 10. This approach encompassing all of the features above presents a comprehensive, integrated solution to processing LCMS metabolomics data. Using external instrument calibration analyte masses were measured with high accuracy, leading to strongly suggestive identifications based on elemental composition analysis. Unambiguous identification of analytes in Mass Frontier software was used to corroborate the performance of the different MS/MS fragmentation regimes, carried out either via resonance excitation CID or higher energy collisional activation (HCD) experiments.

P7B-007

A metabolomic approach to the identification of health based consumer traits in tomato

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The presence of antioxidants in human diets has been attributed to the prevention of chronic diseases such as certain cancers and cardiovascular disease. When consumed in a plant based matrix, the beneficial effects of antioxidants on human health are greater than via dietary supplements. Tomato contains many health-promoting phytochemicals such as the antioxidant lycopene, which is known to reduce the incidence and progression of prostate cancer. Production of tomato fruit with increased levels of these antioxidants is therefore likely to be more attractive to the consumer and of significant benefit to health. The objectives of this project are to apply a metabolomic approach to characterise the introgression line (IL) populations of Solanum pennellii and Solanum habrochaites, over two seasonal crops, using both targeted (HPLC profiling of carotenoids, flavonoids and phenylpropanoids) and non-targeted (NMR and MS chemical fingerprinting) approaches to characterise each IL. Two seasonal crops have been grown and harvested and the colour index has been determined for each IL. Both crops have been profiled by NMR and MS and the targeted carotenoid and flavonoid/phenylpropanoid analyses have been completed. A number of ILs show significant changes in important metabolites including lycopene, beta- and delta - carotene and rutin. Over-lapping ILs that share common genomic regions have also shown changes in the same metabolites, which has allowed us to focus on the underlying genomic-transcriptional-metabolomic interactions."

P8A-001
Exchange of pooled human serum for method evaluation and comparison in nutrigenomics.

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Reports of the plasma metabolome composition are widely variable but range from 10 - 30 thousand small molecules. The ultimate goal of metabolomics is the quantitative analysis of all these metabolites. However, the chemical diversity of metabolites exceeds the span of any analytical method. Moreover, the concentration range to be covered in metabolomics is also extremely large. This is also reflected in the literature where many methods are described for analyzing metabolites, depending on the application of interest. Most of the published methods report semi-quantitavive concentrations rather than absolute concentrations of the metabolites detected. One drawback of so many methods is that even though they are locally validated, by the laboratory that developed them, their quantitative performance can not be compared with other methods, designed to analyse (partly) the same metabolites. Reference samples are lacking, even though recently, NIST has characterized a large pool of plasma for the use as a reference sample in metabolomics. Within NuGo, we started a similar initiative, where we purchased a larger volume of a pooled serum sample (healthy individuals, pooled genders) for the purpose of creating a reference sample for exchange of data and comparison of methods. The sample was distributed between 10 participants and data acquired at the different labs are collected, exchanged and evaluated on different levels. The poster will show examples of the shared quantitative results e.g. bile acids, amino acids and others, as well as examples of shared qualitative results

P8A-002
Analytical error reduction for accurate and precise metabolomics phenotyping

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Analytical errors caused by suboptimal performance of the chosen platform for a number of metabolites and instrumental drift are a major issue in large-scale metabolomics studies. Especially for MS-based methods, which are gaining common ground within metabolomics, it is difficult to control the analytical data quality without the availability of suitable labeled internal standards and calibration standards even within laboratory. In this paper, we suggest a workflow for significant reduction of the analytical error using pooled study samples and multiple internal standard strategies. In this paper we demonstrate that results from metabolomics studies can be improved using single point calibration based upon results obtained from pooled study samples (QC samples) that are repeatedly measured in between study samples. Two types of QC samples are required whereby the first type, is used to perform a one-point calibration and the second type is used to assess how well the calibration procedure improved the data quality. We will show that it is feasible to increase the number of metabolites with a relative standard deviation for replicated measurements below 10% significantly. The methodology presented is applied to GC-MS data but is applicable also to other data sets obtained with other analytical techniques.

P8A-003
The French Metabolomics and Fluxomics network (RFMF Réseau Français de Métabolomique et Fluxomique)

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The French Metabolomics and Fluxomics network (RFMF Réseau Français de Métabolomique et Fluxomique) was created in 2005. Its purposes are : - to make an inventory and promote French skills in the field of Metabolomics and Fluxomics - to assess strengths, weaknesses and to position the French community in that field - to promote the growth and development of Metabolomics - to provide opportunity for collaborations between labs or platforms in that field (and beyond that field, i.e. collaborations with biologists, physiologists, etc.) - to provide and support scientific meetings or workshops in Metabolomics and Fluxomics and create environments that will catalyze collaborations among researchers within that field (and beyond) - to provide knowledge transfer to students and new starters in the field and help students advertise their work - to promote recognition of French activities in Metabolomics and Fluxomics domain in France and Worldwide. The French Metabolomics and Fluxomics network is an independent, non-profit organization, governed by a Board of Governors. The RFMF activities belong to the field of systems biology and are organized around 4 topics : 1 Metabolomics / Metabolite analysis; 2 Fluxomics / Metabolic flux analysis ; 3 In situ analysis of metabolism ; 4 Modelling of metabolic systems. RFMF is one of the Technological Networks of IBiSA (French national scientific consortium for Biology, Health & Agronomy Facilities) since 2010 and is supported financially by IBiSA. Four scientific meetings were organized by the RFMF in France (1-2 december 2005 in Toulouse ; 13-15 December 2006 near Clermont-Ferrand, 7-8 February 2008 in Bordeaux, 4-6 May 2010 in Marseilles) and one summer school on Practical aspects of Metabolomics near Montpellier in 2009. Nowadays 101 people from 57 laboratories/companies are members of RFMF. http://www.bordeaux.inra.fr/ifr103/reseau_metabolome/accueil.htm contact : rfmf@bordeaux.inra.fr

P8A-004
New automated software for metabolome profiling and biomarker discovery with high resolution LC-MS data

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High resolution LC-MS provides the sensitivity, accuracy and the wide dynamic range required for metabolite quantitation and is suitable for high throughput automation making it a widely used tool for biomarker research. A typical high resolution LC-MS profile of biological sample may contain over a million signal peaks which may correspond to several thousand endogenous metabolites. Reduction of the number of acquired signals, their proper identification and statistical comparison across samples remains a major challenge for LC-MS metabolomic analyses. A typical metabolomics data file, urine, plasma or tissue extract, could easily yield over one million signals. It has previously been shown that single mixture component - hippuric acid, an endogenous metabolite found in urine, generates more than 20 related peaks, including isotope clusters, various adducts, multimers and fragments. Overall, plasma and urine are estimated to each contain between 500-1500 unique quantifiable metabolites and, therefore, it is not surprising that the obtained data files are extraordinarily complexity. Proper reduction of the number of detected signals through identification of sample related peaks and filtering out signals related to system background is essential. A variety of noises filtering approaches including blank subtraction were used to reduce the number of irrelevant components. A GUI (Graphical User Interface) was developed to ensure simple fast operation of the software by novice users.. In addition, grouping related signals, i.e. isotope peaks, adduct, dimmers, fragments, etc. significantly simplified processing the results reducing the number of components by a factor of 10. This approach encompassing all of the features above presents a comprehensive, integrated solution to processing LCMS metabonomics data. The software achieved data analysis time for the 24 samples in this set of less than 1/2 hour. Easily accessible visualization tools such as PCA (Principle Component Analysis) showed substantial differences in endogenous metabolites levels between groups of animals. Annotation of components was accomplished using a search of the ChemSpider database. Obtained tentative metabolite assignments were subjects for verification with synthetic standards.

P8A-005
Bioinspired Synthesis of Chiral and Non-Chiral Metabolite Standards

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For the many metabolites typically present in biological systems, authentic material for unambiguous assignment is useful. Therefore, the extension of the range of avai-lable well-defined and pure metabolite standards is key for a variety of experiments. The chemical synthesis of densely and differentially functionalized small molecules is not a small endeavour, but requires combinations of the best selective methods known. The revitalization of this classical biochemistry area has been started with the focus on the central metabolites at pathway intersections of healthy biological systems and with the synthesis of the metabolite of interest in racemic form. As the chirality of many important metabolites has biochemical relevance, the synthesis of key meta-bolites in both racemic and chiral form is of interest. In our metabolite initiative, this has been achieved in a three-phase process by first synthesizing the racemic form, then developing the analytical methods for the separation of the enantiomers and in the third phase synthesizing the chiral metabolite. The natural metabolic pathways have not only inspired both our classical chemical and biocatalytic syntheses, but have also been an starting point for assembling a number of biocatalytic steps by preparing the required enzymes and auxiliary reagents [1-3]. New results from the synthesis of metabolites in glycolysis, pentose phosphate, mevalonate and non-mevalonate, steroid and vitamin B6 pathways has been achieved with new tools and ingredients. These tools are also utilized for the synthesis of stable-isotope-labelled central metabolites. Remote metabolite synthesis can build on the central metabolites as hubs and selected examples will be presented. References: [1] Wohlgemuth R. Biotechnol. J. 2009, 9:1253-1265. [2] Richter N, Neumann M, Liese A, Wohlgemuth R, Eggert T, Hummel W. ChemBioChem 2009, 10:1888-1896. [3] Richter N, Neumann M, Liese A, Wohlgemuth R, Weckbecker A, Eggert T,Hummel W. Biotech. Bioeng. 2010, DOI 10.1002/ bit.22714 (in press). [4] Wohlgemuth R. J. Mol. Catal. B: Enzymatic 2009, 61:23-29. [5] Schell U, Wohlgemuth R, Ward JM. J.Mol.Catal.B: Enzymatic 2009, 59: 279-285.

P8B-001

Metabolite Profiling of the Polypholic Compounds in *Rubus coreanus* Miquel (Bokbunja) by UPLC-qTOF-MS/MS

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Rubus coreanus Miquel (Bokbunja) has been used in oriental medicine traditionally based on its unique antioxidative activity; however, the differences of their efficacy among various cultivars were not clearly elucidated scientifically to date. In this study, metabolite profiling was attempted using UPLC-qTOF-MS/MS according to ripening stages & cultivation regions for the development of *Rubus coreanus* Miquel as a functional ingredient in nutraceuticals. *Rubus coreanus* Miquel cultivated in two different regions of southern area of South Korea were collected depending on their ripening stages (immature, middle, and mature). Whole metabolites were extracted with 80% of ethanol and the analyses were carried out with UPLC-qTOF-MS/MS for metabolite profiling. Accurate mass chromatographic fingerprinting of negative ion modes successfully classified based on their polyphenolic metabolites. Reliable classification models by principal component analysis (PCA) and partial least square discrimination analysis (PLS-DA) gave good capability in categorizing the tested samples. PCA analysis demonstrated four significantly different categories along with component 1 (32.9%) and component 2 (29.1%). Within each cultivars, the metabolites in matures showed clear distinction compared the others indicating a significant changes in the polyphenolics during ripening. A variety of polyphenolic compounds were tentatively identified in Rubus coreanus Miquel with different cultivars & ripening stages and most dramatic diversity were investigated according to ripening stages. Polyphenolics such as coumaric acid hexose, galloyl-HHDP-glucose, galloyl-bis-HHDP-glucose, digalloyl-HHDP-glucose, triterpenoid hexose, ellagic acid, ellagic acid pentose, quercetin glucuronide, kaempferol-3-o-glucoside, catechin were found in the immature. A score plot by PCA analysis revealed remarkable increase of anthocyanin derivatives such as cyanidin-3-o-glucoside, cyanidin-3-o-rutinoside as ripening, while the anthocyanins were rarely found in the immature. The results can be used as a useful basement for application of *Rubus coreanus* Miquel as netraceuticals.

P8B-002

Pre- and postnatal exposure to low doses of pesticides alone or in mixture: metabolic fingerprint and Impact on haematopoiesis in the offspring

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Epidemiological studies indicate that occupational exposure to pesticides is often associated with various pathologies in specific individuals and their offspring. On the other hand, few data dealing with impact of exposure to low doses of residual pesticides in food are available. In this study we have investigated, in vivo, the effect of selected pesticides or mixtures administrated to mice during pre- and postnatal period on haematopoiesis and general metabolism. Atrazine, endosulfan and chlorpyrifos alone or in mixture were added in food at low concentrations derived from Admissible Daily Intake values. Female mice were fed during gestation and lactation, and then pups were fed during an additional period of 11 weeks. Peripheral haematopoiesis was assessed both in weaning and in 14 weeks-old animals by the count of each blood cell type and the quantification of myeloid progenitors in blood by flow cytometry; plasma and liver extracts were also analyzed by 1H NMR spectroscopy for metabonomic study. Central haematopoiesis was evaluated by culturing bone marrow cells. Our results were different according to the animal sex, the effects being more pronounced in female. NMR-based metabonomic analysis of plasma and liver aqueous extracts revealed metabolic perturbations in the mouse offspring upon maternal and post-natal exposure to low dose of pesticides. Identification of metabolites that permits the distinction between treated and control is in progress. Our results showed a perturbation of the capacity of bone marrow progenitors to differentiate. Moreover, we observed that some treatment with single pesticide even at low doses were associated with an increased number of stem cells in peripheral blood and a significant change in white cells number. We observed that the effect of the mixture was not predictable from the effect of each pesticide alone (nor synergic no additive effects were observed in our parameters) and was sometimes at the opposite. Effects were more pronounced in 14 weeks-old animal than in weaning animal suggesting a role of the duration or the window of exposure.

P8B-003

Quality assessment of broccoli florets by 500 MHz-1H NMR profiling.

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In order to determine the effect of cultivar on organoleptic quality in purple broccoli floret tissue, 8 varieties were cultivated on the same plot using a Randomised Complete Block Design in 2008-2009 in Ireland. Florets were harvested at commercial maturity (Februar to March 2009). Each sample is constituted by a composite sample of 50-80g of secondary florets from 3 plants within each replicate. In purple broccoli the secondary florets are interspersed with leaves – large leaves were removed from the florets for sampling (as would be done with preparing them for cooking). 4 biological replicates were collected by broccoli varieties. Hydro-methanolic extraction was performed with Dionex ASE 200. Proton NMR analysis (1H-NMR) of polar extracts of secondary florets samples was performed on a 500 MHz Bruker spectrometer equipped with a QNP probe to provide an overview of some major determinants of the broccoli floret organoleptic quality. Each 1H-NMR spectra was acquired in less than 8 min. The 1H-NMR spectra were processed to identify the major metabolites including soluble sugars, organic acids and amino acids. The NMR data were visualized with Principal Component Analysis using AMIX software (v 3.7.10) to reveal sample dissimilarities and to highlight discriminant metabolites. Acknowledgements: The Irish Department of Agriculture, Fisheries and Food (FIRM 06/NITARFC6) and the Ambassade de France en Irlande are gratefully acknowledged for financial support of this work.

P8B-004

LC/MS-based metabolic profiling of Japanese green tea leaf extracts improving vascular endothelial dysfunction

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Green tea (*Camellia sinensis* L.) is a popular beverage worldwide, and its possible health effects have been received considerable attention. Prevention and reduction of endothelial inflammation and arteriosclerosis have been reported as potential physiological activities of green tea. However, the effect of green tea on vascular endothelial dysfunction, one of the causal factors of arteriosclerosis, still remains unclear. At the inflamed vascular endothelium, thrombin induces phosphorylation of myosin regulatory light chain (MRLC). This leads to the cytoskeletal rearrangement and the subsequent enhancement of permeability of endothelial cells, and finally causes atherosclerosis. Therefore, here we evaluated the inhibitory activity of green tea infusions from various Japanese tea cultivars on thrombin-induced phosphorylation of MRLC in human unbilical vain endothelial cells (HUVEC). Among tea infusions from forty-five kinds of Japanese tea cultivars, Okumusashi, Cha Chuukanbohon Nou-6, and Sunrouge showed a potent inhibition of MRLC phosphorylation. With the recent developments in plant metabolomic techniques, it is now possible to detect several hundred metabolites simultaneously and to compare samples reliably to identify differences and similarities in an untargeted manner. To explore potential active components, we measured comprehensive low molecular weight metabolites in their tea infusions by liquid chromatography equipped with time-of-flight mass spectrometry (LC/MS)-based metabolic profiling method. Principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) indicated various known or unknown tea components contributed to the inhibitory effect on thrombin-induced phosphorylation of MRLC.

P8B-005
A metabolomic view of wine micro-oxygenation

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To date is widely accepted that a well controlled, limited exposure to oxygen, is indispensable for the optimal aging of premium red wines. When oxygen is in contact with wine, the metal catalysed Fenton reaction have been shown to be a major route to ethanol oxidation. Strong oxidant species are produced, which have been suggested to react directly with the wine constituents, bypassing the pool of polyphenols. We report here the first results of an experience of micro-oxygenation of a Sangiovese wine of the vintage 2009 performed at the Tuscania experimental winery. The micro-oxygenation was carried on during the early phases of winemaking, just after the alcoholic fermentation and prior to the malo-lactic fermentation. The wine was divided in 24 stainless-steel tanks and subjected to 7-weeks of controlled micro-oxygenation under 8 different conditions, in triplicate. The variables were the amount of oxygen (0-5-10-15 mg/L/month) and the iron concentration (1.5 and 2.0 mg/L), while all other conditions were strictly standardized and monitored. During and after the micro-oxygenation trials, the wine composition, antioxidant and sensorial properties were measured. A data-driven experiment, aimed at measuring the effect of the treatments on all measurable low-molecular weight organic compounds in wine, was performed by Synapt UPLC-Q-TOF (Waters), with ESI interface operating under both positive and negative conditions. A number of >1000 and >3000 features (i.e. unique couple of retention time and exact mass) were measured in the wines. After data alignment and processing with the s/w MarkerLynx (Waters), a preliminary list of the biomarkers of wine micro-oxygenation in presence of variable levels of iron and oxygen were extracted. This experiment confirmed that both factors influence in a complex manner the wine composition. The effects induced from both iron and oxygen during the treatments involved a very large number of wine constituents. This experiment highlights the feasibility of unbiased, non-target metabolomic experiments, for improving our understanding of wine chemistry.

P8B-006
New Insights in NMR-based Mixture Analysis

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Traditionally, NMR has been perceived as a tool for structure verification, elucidation and purity analysis. However, driven by the needs of the emerging field of Metabonomics, NMR has rapidly expanded in recent years into the areas of mixture analysis and screening applications. In such studies, hundreds of samples may have to be screened per day with regard to the identity and concentration of selected compounds as well as for between-sample comparison of spectral patterns using multivariate statistics to obtain classification and discrimination information. NMR is a particularly well-suited detector for screening applications, deriving truly quantitative and structural information while featuring high throughput and excellent reproducibility. The high stability of the instruments and the reproducibility of the measurements inter-instruments allow to build statistical models for long-term studies. Under typical conditions in NMR screening of intact mixtures, where sample preparation just means addition of defined amounts of buffer, compounds can usually be detected down to the lower micromolar range directly from the mixture spectrum. At the same time the spectra provide information on high concentrated compounds like sugars in fruit juices (typical concentrations up to 100g/l). Complementary information about lower-concentrated compounds in mixtures (e.g. polyphenols in fruit juices) can be obtained by applying solid-phase extraction (SPE) prior to NMR. Although SPE pre-treated NMR samples are still complex mixtures, separation properties of different SPE phases can be used to optimize spectroscopic accessibility and detection limit with respect to certain pre-selected classes of chemicals. The proof of concept of solid-phase extraction prior to NMR-based mixture analysis has been demonstrated. The ability of SPE to concentrate the sample and to open new spectral regions, together with a good reproducibility while working in automation, supports SPE-NMR potential as a new method for screening purposes. Thanks to the use of appropriate SPE-phases, SPE-NMR has been tested and has shown good results on other matrices like wine, honey and biofluids, thus opening a new world in NMR screening.

P8B-007
Application of metabolite profiling to adherent growing cell lines

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Sampling is known to potentially introduce considerable analytical variance to compound quantitation. Numerous reports state that proper sampling within metabolite profiling is particularly difficult since non-selective determination of compounds is envisaged including those that undergo rapid modification in vivo. Every biological matrix might impact analysis differently and needs to be validated for each analytical method. We set out to investigate the handling of the adherent-growing model cell line MCF-7 using GCMS metabolite profiling. Adherent-growing cells are challenging because cell sampling is difficult and routine protocols are not readily applicable. Analytical parameters such as working range, variance introduced by different automated evaluation procedures, injection, derivatisation, and different extraction protocols in comparison to biological variance were evaluated. Sample preparation details were assessed such as the use of phosphate-buffered saline (PBS) and normalisation to dry weight instead of cell number. Exemplarily, accuracy of lactate analysis was tested by an enzymatic reference method because quantitation within GCMS profiling was difficult due to coelution and contamination. 124 metabolites identified with the Golm Metabolome Library and 57 abundant unknown peaks were included for further evaluation. Obtained average analytical variances were within the known tolerances, namely between 20-30% relative standard deviation. Among the tested commonly used extraction protocols, methanolic extraction was found to be most useful with respect to number of detected compounds and handling of the protocol. Replacement of PBS with isotonic NaCl for trypsinization did not result in different phosphate response. Linear working range of lactate analysis was found to be limited due to contamination of silylating reagents used for GCMS derivatisation with lactate-bis-tms. However, lactate response by different methods of calibration was found to be linear with respect to further matrix effects. Results of enzymatic analysis confirmed accuracy of results obtained with GCMS.

P8B-008
Metabolome in human hepatic vein and artery: liver metabolite fluxes in a hepatic venous catheterization study in subjects with NAFLD

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Non-alcoholic fatty liver (NAFLD) disease describes a range of conditions caused by a build-up of fat within liver cells and a wide spectrum of liver diseases are categorized in this group. Currently, the pathogenic mechanisms in fatty liver remain poorly understood and are difficult to study in humans. In this study, the specific goal was to study metabolites associated with the NAFLD, and the general aim was to gain a global insight about metabolite fluxes into and out of the liver. For this, a hepatic venous catheterization was performed in nine subjects with various degrees of hepatic steatosis as determined using liver biopsy. In combination with the catheterization, [2H2]palmitate infusion in the fasting state and during low-dose insulin infusion was also performed. In addition, arterialized venous blood was obtained from the patients. The metabolite profiles were obtained by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. The metabolic profile covered over 1000 metabolites. Statistical evaluation of the metabolite profiles together with the clinical data revealed significant differences in levels of several metabolites in between the artery and hepatic vein (40 metabolites with $P<0.05$), including multiple amino acids. After insulin infusion the changes between these vessels became even more prominent. Specifically, the levels of branched-chain amino acids were decreased in the artery but not in the vein. On the other hand, the levels of several short-chain fatty acids were increased both in the artery and the vein while the levels of several saturated fatty acids were decreased in the artery but increased in the vein. In addition to new insights into the liver metabolism in NAFLD, the results obtained by hepatic venous catheterization also provide valuable insights into the liver metabolic fluxes in humans and will be of great help in interpretation of serum metabolomics data.

P8B-009

Metabolic profiling of processed fruits and vegetables

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There is ample epidemiological evidence that consumption of fruits and vegetables reduces long-term cardiovascular disease risks. Few people however manage to consume recommended daily intake levels. It is a considerable challenge to design food manufacturing processes that provide the consumer with an acceptable balance between nutritional and sensorial quality and convenience. A bottleneck in the design of such processes is the lack of insight in the impact of processing on the overall nutritional profile of fruits and vegetables. In this work we explored the use of metabolic profiling as a tool to obtain such an overall view. Blending (B) and heat/high-pressure treatment (H) are known to have impact on sensory and nutritional quality. We investigated how order of treatment (B, H vs. H, B) and different temperatures affected phyto-chemical profiles of broccoli, tomato and carrot. Vitamins C/E (HPLC-PDA), and carotenoids (HPLC-PDA-FL) were assessed by target analysis as markers for antioxidants and lipophilic phytochemicals. Crude aqueous-methanol extracts were profiled by LC-PDA-QTOF MS. B, H treatment had a most profound effect on vitamins C/E, in particular in broccoli. Carotene levels were hardly affected by the different treatments. The processing parameter with most pronounced impact on the profiles was the order of the B,H/H,B treatments, most clearly observed in broccoli. Within broccoli, applying different temperatures during heating, in both B,H/H,B treatments, resulted in differential metabolite profiles. In order to validate these findings, broccoli, carrot and tomato were B, H/H, B treated in alternate order in triplicate. Broccoli again showed most and largest effects, in particular on glucosinolates, flavonoids and lipid breakdown products. Effects on tomato and carrots were generally smaller. In conclusion, metabolomics was able to provide a comprehensive view into those metabolites and enzymatic reactions that are most influenced by specific food processing treatments. This insight will help us in defining the best strategy for achieving optimal nutritional profiles of vegetable products.

P8B-010

Optimal Fungi and Fermentation Time of Rice Koji Revealed by Metabolomics

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Inoculation of Koji on wheat, rice or barley with zymotic fungi is the key ingredient for the alcoholic fermentation of traditional beverages in Korea and Japan. The inoculants, *Aspergillus kawachii, Aspergillus oryzae*, and *Rhizopus* sp., were utilized for rice Koji fermentation and the fermented metabolites were analyzed in time dependent manner using gas chromatography- electron impact ion trap mass spectrometry (GC-EI-MS) and ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS). In the Principal Component Analysis (PCA) score plots showed that the metabolite patterns were clearly distinguished between the Koji fermentation. In particular, rice Koji fermented with *A. kawachii* produced highly significant metabolites than the others. The quantity of sugars and sugar alcohols (glucose, galactose and glycerol) were gradually increased according to fermentation time. These compounds were correlated with the enzyme activities including glucoamylase, α-amylase and saccharogenic power. As a result of comparison of each Koji metabolites, fatty acids (linoleic acid, stearic acid and hexadecanoic acid), sugar alcohol and sugars (xylofuranose, glucopyranose, turanose and arabitol) and other metabolites (malic acid, butane, citric acid and phospholic acid) increased depends on fermentation of *A. kawachii*. In the case of *Rhizopus* sp., citric acid, linoleic acid and turanose increased in particular and sugars (xylofuranose, turanose) and fatty acids (linoleic acid, stearic acid) were increased in *A. oryzae* fermentation. This study showed that the GC/LC-MS based metabolomic approaches which revealed the time dependent metabolite production could be used as a valuable tool in selecting inoculants fungi and the optimal fermentation time for rice Koji.

P8B-011

Metabolomics based determination of blanching dependent anti-oxidative biomarkers of *Chrysanthemum coronarium* L.

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Chrysanthemum coronarium L. is an annual herbaceous plant and its blanched or fresh one has been regarded as a health food in East Asia. LC-MS/MS based metabolomic characterization was applied to elucidate the correlation between secondary metabolites and antioxidant activity changes during blanching process. By principal component analysis (PCA), a significant separation was observed between blanched and flesh samples. The major metabolites of flesh samples were selected by PCA and PLS-DA loading plots and those structures were determined as dicaffeolyquinic acid, succinyl dicaffeoylquinic acids and mycosinol series by LC-MS/MS analysis. The other clustering with blanched one was observed with higher levels of glyceroglycolipids derivatives than fresh one. By partial least square (PLS) regression analysis about the correlation between contributed components and antioxidant effects, the candidate antioxidative metabolites of the flesh sample could be predicted by a strong positive correlation with phenolic constituents which are mainly dicaffeolyquinic acid derivatives and by a week positive correlation with mycosinol series. The radical scavenging effects of blanched sample decreased about 3.1%~26.0% and correlated antioxidant effects of water extracts increased after blanching process. These findings indicated that a metabolite profiling approach is a useful tool for analyzing blanching process dependent metabolite changes with antioxidant activities of C. *coronarium* L.

P8B-012

Metabolic footprinting by MS-based analyses for characterization of fermentations of dietary fibres with human gut flora

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Dietary fibres may act as prebiotics, i.e. stimulation of growth of health-promoting bacteria such as bifidobacteria and lactobacilli. The fermentation of carbohydrates by the gut microflora generates short-chain fatty acids (SCFA), of which particularly butyric acid is considered as beneficial for the health. In vitro fermentations of fibres with human faeces have frequently been used for comparison of fibres, with analyses of SCFA by GC or HPLC. In contrast to techniques directed against analyses of specific compounds, the use of MS-based methods will provide an overall metabolite profile – a "metabolic footprint". Such data may provide useful information about the function of the microflora, as a supplement to information gained from DNA-analyses about the composition of the flora. In this study, a range of dietary fibres, including pure polysaccharides such as -glucans, arabinoxylan and poly-uronic acids, and selected crude fibre fractions, have been screened by in vitro batch fermentations with faeces from human infants. The commercial prebiotic inulin was included for comparison. SCFA were monitored by HPLC-analyses, while a complete metabolite profile was achieved by use of GC-MS. The GC-MS analyses revealed that consumption of amino acids provided by the inoculum (faeces) had a significant contribution to the production of organic acids. Production rates of individual SCFAs varied depending on the substrate (fibre or amino acids) being consumed. The present results demonstrate interesting differences between the dietary fibres, as well as the usefulness of more extensive analyses of substrate consumption and product formation. A future aim will be to correlate the metabolite analyses with the changes in microflora.

P8B-013

The quality evaluation of Angelice Radix by means of GC-based metabolomics technique

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Angelicae Radix is one of the most essential gynecological herbal medicines with the tremendous expansion in use of traditional medicines worldwide. It has been used for thousands of years in China, Korea and Japan. In the market of herbal medicines, the quality control of Angelicae Radix depends a great deal on a sensory evaluation. The standards for Angelicae Radix quality including Japanese pharmacopeia are obscure and nonnumerical. In addition, training and maintenance of professionals for sensory analysis are time-consuming and expensive. There is no discussion how accuracy professionals can evaluate by and what sensory attributes and compounds affect quality of Angelicae Radix. Fingerprinting has been accepted as one of the efficient methods for quality control.The aim of this research is to disclose the components which contribute to the quality assessment and gain the feedback on the practical evaluation by using metabolomics technology. Angelicae Radix was evaluated by 5 professional panels on herbal medicines. In total 8 attributes based on Japanese pharmacopeia , shape/size, leaf sheath, density, moisture, taste/sweetness, odor, external and fractured surface color were evaluated. All attributes were ranked on a scale from 0 to 5. By applying the evaluated scores to correlation analysis and principal component analysis (PCA), the effect of sensory attributes and the difference of sensory panel occupation were revealed. PCA was used to explore the gas chromatography-flame ionization detection (GC/FID) data structure and revealed the classification based on production areas; China and Japan. When comparing GC/FID fingerprints and sensory scores, poor correlations between the area value of sugars (glucose, fructose and sucrose) known as markers for quality control of Angelicae Radix and the sweetness scores were observed. Calibration models with respect to the averaged, producer's and manufacture's scores of sweetness were calculated for each attribute separately using orthogonal partial least squares (OPLS) regression methods and validated using test sets. The result will be used to carry on the feedbacks of the information to improve the accuracy of quality control for herbal medicines.

P8B-014

Characterization of black and green tea using ESI-Q-TOF-MS and data evaluation by principle component analysis

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Black and green tea account for more than 95% of the total tea consumption around the world. In order to improve the quality and taste, characterization of food and beverages as well as quality control is a topic of interest in academia and a large market in industry. In our study, we used high resolution electrospray time-of-flight mass spectrometry to study different tea's using statistical methods. Several black tea's, among them one decaffeinated tea, and one green tea have been analyzed. Infusions were prepared with 100ml hot water for 5 minutes and analyzed using a reversed phase gradient separation on a UHPLC system interfaced to a high resolution ESI-TOF-MS. Full scan data were acquired in ESI positive mode (scan range m/z 75-1000). A mathematical algorithm was applied to detect all compounds in the analyses. This "Find Molecular Features" algorithm extracts all relevant information and differentiates between real signals and background noise. The processed data were submitted to principal component analysis (PCA) in order to differentiate the samples and to identify differences between the tea types. As expected, the decaffeinated black tea was distinct from the other teas by the absence of caffeine. Excluding caffeine from the PCA calculation, other differences between the teas were revealed, mainly originating from the different "flavonoid profiles" of the teas. In order to identify those differences, sum formulae were calculated, taking both the accurate mass and the isotopic pattern of the compounds into account. With increasing molecular mass, the number of possible sum formulae in a certain mass window increases exponentially. Therefore, an autoMS/MS run was performed focusing on the largest differences between the teas as precursor ions, so that also the accurate mass and isotope pattern of the fragments could be used for sum formula generation to reduce the number of sum formula suggestions. In this study, high resolution ESI-Q-TOF mass spectrometry, coupled to UHPLC, and data evaluation with PCA followed by sum formula generation of the detected differences proved to be a valuable tool for the characterization and quality control of tea.

P8B-015

Metabolomics and transcriptomic analysis of brassicaceae for the evaluation of the effects of manure amendment

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Given the rising demand for 'organic' agricultural commodities, and cropping systems that efficiently recycle nutrients and mitigate the emissions of carbon dioxide, organic soil amendments such as manure and organic fertilizers have attracted considerable attention. Although amendments using organic compounds such as manure have been recommended for improving soil physical properties and nutrient availability, there has been no critical evaluation of quality characteristics of products grown with these materials. We introduce GC/MS-based metabolic profiling on *Brassica rapa* L., and transcripotome analysis on *Arabidopsis thaliana* as a holistic view on the plants grown with organic manures. A simple factorial design with applied N and dairy manure levels served to investigate whether any compound(s) specifically fluctuated with levels of manure amendments. *Brassica rapa* L. was grown in the experimental field, located at the National Agricultural Research Center for the Hokkaido Region. Metabolite analysis was carried out using a GC-sector-MS system (JMS GCmate II; JEOL, Tokyo, JAPAN). Each metabolite was identified by mass spectral and retention index using AMDIS software, referencing a private library of 183 self-purchased standards. Transcriptome analysis was carried out using microarray on *Arabidopsis thaliana*. In growth chamber, *Arabidopsis thaliana* grown in plastic pot with or without 20 g L-1 manure. For leaf metabolites, principal component analysis was applied to 56 metabolite peaks revealed by GC/MS analysis. The first principal component accounted for 48.0 % of total variance and indicated a close relationship between metabolite profiles and inorganic-N application rates, whereas the second principal component, accounting for 8.1 % of total variance, pointed to a close relationship between metabolite profiles and manure application rates. Transcriptome analysis using *Arabidopsis thaliana* represented the candidate genes which responded to manure application. The transcripts relating super-oxide dismutase and cell wall metabolism were up-regulated and the transcripts relating myb family and phosphorus metabolism were down-regulated. These compounds and transcripts responses will be useful in the further characterization of the effects of organic amendments on crop metabolisms.

P8B-016

Studying the seed metabolome of ricinus communis for cultivar and provenance determination

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The seeds of the castor bean plant, *Ricinus communis*, contain the protein toxin ricin. Ricin is declared by the Chemical Weapons Convention as a Schedule 1 agent.[1] Ricin has an intravenous LD50 of 2 μg/kg in standard mouse models,[2] and thought to have a human LD50 of 3 – 30 μg/kg.[3] Ricin is a heterodimeric type II ribosome-inactivating protein,[4] which selectively depurinates adenine within a highly conserved fourteen nucleotide region of the 28S rRNA subunit of the large 60S ribosome.[5] This results in the inhibition of protein manufacture, preventing chain elongation of polypeptides, leading to cell death. The plant is also a common environmental weed in many localities within Australia, and importation of the seed is banned. Therefore, the only available source of the seed, and ricin, is from specimens growing in the wild. This presents a unique problem to Australian law enforcement and forensic agencies, making techniques that can determine both cultivar and provenance essential. DSTO is applying metabolomic strategies to generated extracts R. communis seeds from different specimens collected around Australia. Extracts of local and overseas specimens were analysed using 1H NMR. Collected data was manually phased and baseline corrected, and analysed using multivariate statistical analysis, and in particular PLS-DA and OPLS-DA. This presentation will discuss recently generated results from the study of the metabolome of both Australian and overseas specimens. [1] Department of Foreign Affairs and Trade, Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction (Chemical Weapons Convention) - Annex on Chemicals. [2] T. Fu, C. Burbage, E. P. Tagge, T. Brothers, M. C. Willingham, A. E. Frankel, Int. J. Immunopharmaco. 1996, 18, 685. [3] L. G. Doan, Clin. Toxicol. 2005, 42, 201. [4] Y. Endo, K. Tsurugi, T. Yutsudo, Y. Takeda, T. Ogasawara, K. Igarashi, Eur. J. Biochem. 1988, 171, 45. [5] C. Chen, L. Jiang, R. Michalczyk, I. M. Russu, Biochem. 2007, 46, 1116.

P8B-017

Metabolomic Evaluation of Bacillus species Conferring Nutritional Properties

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A metabolomic platform that facilitates the rapid screening of diverse bacterial isolates has been established. Extraction efficiency of polar and non-polar metabolites was assessed using a range of aqueous/organic solvent mixtures in the presence and absence of acid. Extractions performed with acidified methanol (50%) yielded the highest number of extractable metabolites, as well as the highest recoveries. Introducing a sequential chloroform extraction enabled the extraction and separation of polar and non-polar compounds, creating a procedure capable of delivering an improved representation of the metabolome. Bacterial metabolism is rapid and therefore arresting the metabolism in a quick manner which prevents cell disruption or intracellular metabolite leakage is an important aspect of microbial metabolomics. Numerous methodologies utilised to quench bacterial metabolism involve rapid filtering, temperature or pH shock and the use of organic solvents as quenching agents. After evaluating these procedures it was concluded that the use of 60% methanol in an isotonic solution minimised intracellular leakage, while enabling rapid sampling. Several analytical platforms have been employed to cover the widest range of chemical classes found in the extracts. Metabolite libraries have been created and semi-automated data analysis achieved. The use of GC-MS and HPLC-PDA allows the development of reliable analytical methods with good peak resolution and simultaneous unambiguous identification of metabolites. The protocols developed have been validated by analysing different species from the Bacillus genus with probiotic activity and the presence of novel isoprenoids. Multivariate principal component analysis performed on the datasets generated show that Bacillus indicus HU36 strain clusters away from the rest of the Bacillus species analysed. Loading values suggest that this variance is due to pentose phosphate pathway metabolites. The presence of secondary metabolites such as apocarotenoids does not affect the clustering. However, the occurrence of these and other triterpenoids like squalene account for the clustering of the red pigmented *Bacillus firmus* GB1. The metabolomic approach developed will be utilised further in the analysis of G+ve bacteria used in the food industry and those of medical significance.

P8B-018

Comparative qualitative and quantitative evaluation of two metabolic biomarkers (carotenoids and phenolics) of four berries (Seabuckthorn, Aronia, Black Currant and Bilberries) using HPLC and UV-Vis analysis

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Berries are known to be rich sources of phytochemicals, especially phenolics and carotenoids with antioxidant and health beneficial properties. Last years, the evaluation of these phytochemicals as metabolic biomarkers is increasingly reported. We aimed to evaluate comparatively four types of the berries mostly used in food and nutraceuticals , e.g. Seabuckthorn (*Hippophae rhamnoides*), aronia (*Aronia melanocarpa*), black currant (*Ribes nigrum*) and bilberries (*Vaccinum myrtilis*), by means of metabolite fingerprinting. Among many techniques employed for the separation and identification of these metabolites, HPLC coupled with UV-VIS spectrometry is widely applicable in metabolomics. To investigate accurately these biomarkers, two types of extraction procedures were employed, ethyl acetate and petroleum ether - specific for lipophilic carotenoids, vs methanol for phenolic compounds. The UV-Vis analysis showed the quantitative ratios of these markers, while HPLC revealed their fingeprint using two different reversed-phase C-18 columns and gradient elution protocols. Distinct fingerprints and compositions among these four berries were obtained. The highest carotenoid content was found in seabuckthorn berries (121.47 mg/100 g dry matter, DM) while bilberries, aronia and black currant had only 6.45, 6.42 and 2.79 mg/100 g DM, respectively. Total phenolic content ranged from 2723.83 (aronia) to 4668.28 mg/100 g DM (bilberries), expressed as gallic acid equivalents. Seabuckthorn and black currant had similar results: 3627.20 and 3875.02 mg/100 g DM. The HPLC profiles showed specific fingerprints for each type of berries. Zeaxanthin and carotene were common to all types of berries, free lutein was present in black currant and bilberries whereas its esterified form was present in seabuckthorn and aronia. The profile of phenolic compounds was mainly represented by phenolic acids and anthocyanins glucosides. Chemometric analysis (PCA) was applied to make appropriate interpretations of significant differences between their fingerprints (1-3). 1. Määttä-Riihinen K.R., et al., J Agr.Food Chem. 52:4477-4486 (2004). 2. Scholz M., et al., Bioinformatics 20:2447-2454 (2004). 3. P.D. Fraser and P.M. Bramley, Metabolic Profiling and Quantification of Carotenoids in Plant Metabolomics, K. Saito, R.A. Dixon, and L. Willmitzer Eds., Springer-Verlag Berlin, Berlin, Germany, 2006, pp 229-240.

P8B-019

Metabolomic Grading of Red Grape Wine; Characterisaion of Maturation and Maloractic Fermentation by Metabolite.

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Chemical components of wine are important as the determining factors for the quality of wine. Wine contains many metabolites from raw grape materials or those generated during alcoholic fermentation by wine yeast and malolactic fermentation (MLF) by lactic acid bacteria or maturation. In this study, to aim to grade wine by metabolite, we investigated the changes in metabolites in wine to characterise MLF and maturation. METHODS. Some kinds of wine, which were yielded by different fermentation processes and production year, have been analysed by metabolome techniques using a capillary electrophoresis time-of-flight mass spectrometer (CE-TOFMS) and a liquid chromatography time-of-flight mass spectrometer (LC-TOFMS). FINDINGS. The level of malic acid was significantly decreased in induced-MLF wine relative to non-MLF wine, and that of lactic acid is increased. Metabolites that displayed a good correlation with malic acid or lactic acid included mevalonic acid and citric acid; the former is the nutrition of lactic acid bacteria, and the latter is consumed by the minor reaction of MLF. These results suggest that the metabolites are involved in the sub-reaction in MLF. In addition, metabolites that displayed a high correlation with the production year contained anthocyanin pigments, e.g. petunidin 3-glucoside, which showed a tendency to decrease in accordance with the past-years of production. Decrease of the anthocyanin pigments by maturation is consistent with change in colour tone of wine, indicating that they are the indicators of quality changes of wine by maturation. We suggest that the MLF and maturation can be characterised by metabolome analysis of the product wine using CE-TOFMS and LC-TOFMS.

P8B-020

Applied Metabolomics in the Agricultural Industry and Industrial Biotechnology

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Here, we present examples on the improvement of taste and aroma of agricultural products and the optimization of production conditions in industrial-scale production. Furthermore, we present ways to discover biomarker for metabolomics-assisted breeding and bioprocess applications. Metabolomic Discoveries provides mass spectrometry based metabolite profiling and fingerprinting of biological material. We have extensive experience in analyzing microbes, plant and animal tissues, body fluids, complex fluids, food and beverages.

P8B-021
Sustainable production of platform chemicals by crops: lysine and itaconic acid

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In many industrialized countries there is a growing consciousness about the diminishing fossil resources, and that it is preferable for economical, environmental en social reasons to make use of bio fuel and chemical building blocks from more durable resources, such as plants. This research is focusing on the production of high value base chemicals by plants. Plants present a good production platform for compounds that contain in combination with C en H (the typical petrochemical compounds) also N and O. This functionalisation is for petrochemical carbon hydrates a chemically and energetically very costly proces. A disadvantage of plants, however, is that not all chemicals can be produced, and that the production level of natural occurring base chemicals can be very low. Still there are several examples of compounds from plant origin that could form a link between agriculture and chemistry. Examples of such linking compounds, called platform chemicals, are organic acids and amino acids. When these chemicals can be produced in crops to a relatively high extent, without hindering the production of the main product (e.g. sugar or starch), and without interfering with the extraction process of the main product, these chemicals can be produced much cheaper compared to production by fermentation. Several platform chemicals can be produced in crops such as starch potato and sugar beet. These crops have a high level of water content, remaining after the extraction of the main product (starch, sugar). We produced via metabolic engineering potato plants that accumulate a high level of lysine and itaconic acid in their tubers. These chemical building blocks for chemical industry give the crop added value, because they are produced in combination with starch for which these starch potatoes are grown and harvested. Lysine is a precursor of Nylon-6 and itaconic acid is a green replacement of acrylic and metacrylic acid in petro chemistry.

P8B-022
Using tomato as a cell factory for astaxanthin production

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Carotenoids are important pigments for all photosynthetic organisms as well as essential components of human diets. Ketocarotenoid astaxanthin is one of the most important carotenoids due to its health benefits by boosting immune function and preventing tumor formation. Biosynthesis of astaxanthin is limited to a few organisms. The burgeoning demand for natural astaxanthin in nutraceutical and pharmaceutical industries has attracted much recent interest in engineering astaxanthin pathways in higher plants. Beta-carotene ketolase (BKT) and hydroxylase (CHY) catalyze the conversion of beta-carotene to astaxanthin via several alternative intermediates. Plants usually exhibit CHY activity but lack BKT activity. Therefore, overexpressing a microbial BKT gene in plants is expected to enable the biosynthesis of astaxanthin. One major challenge of engineering astaxanthin pathways in plants is no or low astaxanthin content achieved. As hydroxyl carotenoid intermediates are accumulated, we hypothesize that the microbial BKT gene introduced is the limiting step in astaxanthin biosynthesis. In this study, we used a modified BKT gene from green alga to transform crop plant tomato which is the major dietary source of lycopene and beta-carotene. Transgenic tomato expressing the function-enhanced BKT gene accumulated astaxanthin up to 1030 \µg/g dry weight together with 5250 µg/g of its direct precursor canthaxanthin in the leaves, turning the green color to brown. The poor enzymatic activity of endogenous hydroxylase (CrtR-b1) toward canthaxanthin was proved to be the reason of high accumulation of canthaxanthin. In contrast, astaxanthin was the predominant ketocarotenoid in transgenic tomato fruit, indicating that unlike CrtR-b1, the fruit specific CrtR-b2 exhibits efficient conversion from canthaxanthhin to astaxanthin which induced obvious color change. The total carotenoid contents in all transformants are much higher than untransformed plants. Our study supports the hypothesis that BKT activity is the limiting step of astaxanthin biosynthesis in transgenic plants. Furthermore, we showed that the cooperation of BKT and CHY was also critical for efficient biosynthesis of astaxanthin. We proposed that tomato fruit and probably other plant fruits may be served as cell factories for astaxanthin production by metabolic engineering.

P8B-023
Analysis of whisky by electrospray ft-icr mass spectrometry: proof of origin by statistical methods

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Whisky is a high-class consumed alcoholic beverage with a several billion dollar market. Due to the high value of this liquor counterfeiting and manipulation have been observed. Therefore, the proof of the origin of this luxury alcoholic drink is of major interest of distillers and beverage importers. Whisky consists beside water and alcohol of a variety of volatile and non-volatile chemical components, e.g. organic acids and esters, aldehydes, phenols, polyphenols and lactones. Recently the proof of origin and authenticity of whisky has been studied by electrospray mass spectrometry [1]. However, in our study we used ultra-high resolved mass spectrometry to study whiskies from different origins using statistical methods and fingerprinting. The whiskies have been diluted 1:20 in 50% MeOH for direct infusion measurements using electrospay FT-ICR (Bruker solariX 12T) in negative ion mode. Several Scottish whiskies from two different origins as well as several whiskies from the japanese distillery Suntory have been analyzed. Using electrospray ionization the most polar components of whisky are detected. Beside dominant species like ellagic acid and gluconic acid, the mass spectra of whisky show a complex pattern with several peaks at one nominal mass resulting in several thousand peaks in a mass spectrum. The molecular formulas of more than thousand compounds have been identified. Principal component analysis (PCA) as well as cluster analysis have been performed of the full and of a part of the mass spectra with and without using the isotopic fine structure to validate the origin of the studied whiskies and to proof the relevance of the highly resolved mass spectra for the characterization of whisky. The importance of the isotopic fine structure for fingerprinting of whisky will be shown. In this study electrospray FT-ICR-mass spectrometry has been proven as a powerful tool for the characterization of extremely complex mixtures such as whisky.
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P9A-001

Targeted metabolomics of conjugated and microbial-derived phenolic metabolites after consumption of an almond skin extract

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Almond (*Prunus dulcis* (Mill.) D.A. Webb) skins comprise diverse kind of bioactive polyphenols, in particular flavan-3-ols, flavonols and flavanone. The health effects of these polyphenols depend on their bioavailability implying the presence of metabolites formed in tissues and in the colon by the microbiota. A placebo-controlled study was carried out with 16 healthy volunteers in order to perform a targeted analysis of conjugated and microbial-derived polyphenols metabolites in urine. Urine samples were collected and 0-2, 2-6, 6-10 and 10-24-h after consumption and analysed by HPLC-MS/MS. Maximum urinary excretion of (epi) catechin and naringenin conjugates derived from phase II metabolism, was attained at 2-6 h after consumption of the almond skin extract and resulted significantly different from the control group. Concerning microbial derived metabolites the hydroxyphenylvalerolactones (derived from flavan-3-ols), reached the maximum urinary levels at 6-10 h after the intake of almond polyphenols. Microbial phenolic acids formed in subsequent stages of the biotransformation process, showed significant differences in function of the time period for most of them. Their levels were also higher for the intake group than for the control group although not statistically significant, due in part to the large inter-individual variation and to their incomplete formation up to 24h. PCA analysis revealed that changes in the urinary metabolome during the first 6 h after the intake of almond polyphenols were attributed to conjugates of (epi)catechin whereas changes observed from 6 to 24 h were mainly due to conjugated forms of hydroxyphenylvalerolactones and other microbial-derived phenolic metabolites. The application of targeted metabolomics approach allows to obtain a new quantitative picture of specific portions of the urinary metabolome.

P9A-002

Vitamin Levels in Plasma from Children and Adults in the Arkansas Delta Region

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Lack of micronutrients in utero and early childhood has been linked to childhood and adult onset obesity and related diseases. 24-hour dietary recall surveys suggested that people in rural Delta region of the U.S. may be at risk for vitamin deficiencies. The average weight and BMI of children in this population is higher than the recommended for their ages suggesting they may have sub-optimal levels of some vitamins. Blood specimens were collected from participants in a community based participatory research designed summer day camp at two sites in 2008 and 2009. Two nutritious meals per day plus a healthy snack were provided to children attending the camp. Blood was collected at the start of camp, after 5 weeks of camp and at one-month after the camp ended. Vitamins in plasma samples from children and adults were analyzed by a LC/MS/MS targeted profiling method. Levels of S-adenosyl-l-methionine (SAMe) and S-adenosyl-l-homocysteine (SAH) were evaluated by HPLC. In the 2008 samples, plasma levels of B2 (riboflavin) were lower than the published normal range of 4-24 µg/dL. Levels of B6 were within the normal range of 5-30 ng/mL. Folic acid levels were within the normal range of 3-16 ng/mL. The five-week nutritional intervention in 2008 did not appear to change the average serum levels of B2, B6 or folate in the study participants. In the second year, plasma levels of thiamine, folic acid, B2 (riboflavin) and 25-OH Vitamin D3 increased during the study in the second and third months while niacin and vitamin B6 dramatically increased in the second month but were closer to the first month levels during the third month. The mean of the SAMe in the RBC for this group was 1.15±0.55 nmol/ml (2008, n=15), 1.19±0.40 nmol (2009, n=25). The SAH for this group was 0.93 ±0.11 (2008), 0.95 ±0.15 (2009). The SAMe/SAH ratio was 1.27±0.66 (2008) and 1.27±0.46(2009). The levels for most of the participants in both years were below those of previously published ranges from studies with healthy European-American adults (SAMe/SAH ratios in earlier studies were 4.86 ± 0.32).

P9A-003

The RESMENA-S study: A non-targeted metabolic profiling approach in the Reduction of the Metabolic Syndrome in Navarra-Spain through a strategy based on personalized tailoring-diets with psychological control

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The RESMENA-S project aims to reduce body weight and to improve the oxidative and inflammatory status of Spanish obese adults with metabolic syndrome by means of a 8-week controlled parallel nutritional intervention based on personalized diets and psychological control. The subjects have been randomized either to a hyperproteic weight-loss tailoring-diet group (group A, n = 50) or to a weight-loss tailoring-diet based on the American Heart Association recommendations (Group B, n = 50). Diets in both groups have been designed on a daily caloric restriction of 30% of the subjects total energy baseline needs. Group A diets are characterized by a macronutrient content of 40/30/30 (carbohydrate/lipid/protein), 5-7 meals/day, a higher distribution of protein at the end of the day, a weekly intake of at least 3 portions of wholegrain pasta, 3-4 portions of legumes, 3 portions of fatty fish and 6 fruits/vegetables portions. Group B diets are characterized by a macronutrient content of 55/30/15 (CH/L/P) and 4-5 meals/day. Validated psychological tests, appetite questionnaires and weight dietary records have been filled in by the volunteers every 15 days to assess the acceptance and adherence of each subject to their personalized diet. After the intervention period, subjects have followed their ruled dietary patterns, but without any dietary or psychological control. Fasting plasma and 24-h urine samples have been collected at the baseline and endpoint of each period. Next step is to apply HPLC-MS and NMR- metabolomic platforms to evaluate the metabolic effects of both nutritional interventions in the oxidative and inflammatory status of the patients. Identification of biomarkers of intake of food groups will be planned as an objective tool for dietary intake assessment. Finally, it will be tested whether there are any different behaviour changes that allow an easier adaptation to personalized diets in obese subjects that can be reflected in different metabolic profiles.

P9A-004

Plasma free fatty acids and free/total carnitine in ESRD patients on hemodialysis

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Background: The high prevalence of hyperlipidemia is a critical issue for patients with end stage renal disease on hemodialysis (HD). Levels of plasma free fatty acids (FAAs), as well as free/total carnitine are significant indicators of lipid metabolism and nutritional status assessment. We measured plasma FAAs and F/T carnitine in Taiwanese patients on maintenance dialysis, and thus provide valuable information in monitoring the therapeutic strategy. Material and Method: Thirty patients with ESRD on long-term HD were investigated, including 17 males and 13 females, aged from 48–68 years old. The subjects have been on dialysis for an average of 22 months. Plasma samples obtained from the ESRD patients in pre-HD and in post-HD, as well as the healthy controls (n=30) were first esterified. The methyl esters of FAs were then analyzed by gas chromatography/mass spectrometry method. Plasma F/T carnitines were detected with enzymatic assay by automatic centrifugal analyzer. Results: The total plasma FFAs found in pre-HD (6802 µmol/l) and in post-HD (8284 µmol/l) groups were significantly higher than that of the control group (6033µmol/l)(p <0.05). When evaluating the individual mean of plasma FFAs in each group by Percentile Ranking, 3 saturated fatty acids (C14, C16, and C18) and 2 monounsaturated omega-9 fatty acid (C16:1 and C18:1) appeared in the 5th Percentile in pre-HD group, and all FFAs except C18:3, C22:6, C22, and C24 showed in the 5th Percentile in post-HD group when comparing with the normal values. Both plasma free and total carnitine levels were significantly reduced after maintenance dialysis, about 56.1% and 57.2%, respectively. Conclusion: Llipid metabolism in ESRD patient received HD therapy is defect, and this may result in high incidence of cardiovascular complications. Maintenance dialysis can not effectively eliminate excess plasma FFAs, instead of plasma carnitine. The abnormalities in FA metabolism found in patients with ESRD necessitate careful consideration of dialysis and dietary measures.

P9A-005
Resistant starch improves insulin sensitivity in rats fed a high-fat diet: a metabolomic study

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Fibre-rich food consumption is linked to reduced risk of chronic diseases. Among dietary fibres, resistant starch (RS) is widely consumed and of interest in human nutrition. The RS effects on intestinal function, lipid and carbohydrate metabolisms have been extensively studied but its role in insulin resistance (IR) and type 2 diabetes prevention is still unclear. Metabolomics, combining high-throughput analytical methods and multivariate statistical analyses, allows characterizing diet or nutrient metabolic effects in a more integrative way as compared to classical approaches. Our objective was to use metabolomics for better understanding the RS mechanisms of action in a nutritionally induced IR. Male Wistar rats were fed for 9 weeks with: low-fat (C, 5% w/w fat); high-fat (HF, 30.4% fat); RS-supplemented HF (HF-RS, Hi-Maize260®, 41.6%) diets (n=14/group). Oral Glucose Tolerance tests were performed at 0, 6, 8 weeks of feeding. At 9-wk, fasted plasma metabolic parameters were measured and insulin sensitivity was evaluated with glucose transport in the epitrochlearis muscle. Urine and fasted plasma metabolic fingerprints were obtained with UPLC-QToF MS. RS prevented the HF induced weight gain ($p<0.05$) and was associated with decreased plasma lipid concentrations ($p<0.05$). Glucose tolerance was impaired and insulin sensitivity decreased by HF; both were restored with RS (HF vs HF-RS; HF vs C: $p<0.05$). PCA analyses of urine and plasma metabolomic data showed clear diet distinctions (HF vs HF-RS). So far, among the 45 plasma metabolites discriminant for HF vs HF-RS, lysophosphatilylcholines, leucine/isoleucine, and prostaglandins have been identified, consistently to changes reported with IR and/or nutrients/drugs having protecting effects on this development. More than 20 urine metabolites were found discriminant (HF vs HF-RS). Identification of metabolites is still in progress as well as metabolomic analyses of faecal water samples. Preliminary metabolomic results show that RS induces difference in metabolome patterns which may shed new lights on its effects on metabolism with potential identification of new biomarkers.

P9A-006
Food plant derived 13C-internal standards for improved biofluids analysis

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Metabolite and metabolome analysis by mass spectrometry can be significantly improved by stable isotope labelled internal standards (isotope dilution MS), both for quantification and identification, in both targeted and profiling metabolomics, and in metabolic flux analysis [e.g. 1,2,3]. Stable isotope labelled plant metabolites offer innovative solutions as internal standards and tracers in life science research. The production of uniformly labelled 13C-plants requires advanced life support technology in closed atmosphere systems [4]. Although uniformly 13C-labeled (U-13C, >98 atom%) food plant materials have only recently become available [5], a novel U-13C based method for quantification of quinic acid in foods has already been developed [6]. Other recent applications include improved identification of metabolites in tomato [7] and the physiology of de novo sulforaphane biosynthesis in broccoli [8]. The production and characteristics of U-13C labeled internal standards are described, including nutritional compounds, like flavonoids (U-13C quercetin and kaempferol), phenolic acids (e.g. U-13C quinic acid, chlorogenic acid), and fructo-oligosaccharides (U-13C FOS). Other standards being developed include U-13C carotenoids, tocopherols, antioxidants and mitochondrial metabolites. Mass spectra demonstrate a carbon isotope enrichment of >98 atom% 13C, thus making these U-13C compounds suitable for use as internal standard for quantitative analyses of compounds up to 60 C atoms, in –for instance- blood plasma, urine, plant extracts and other biofluids. [1] Dettmer K., et al. (2007) Mass Spec Rev 26:51–78; [2] Scalbert et al. 2009. Metabolomics 5, 435-458; [3] Hall, R.D. (2006). New Phytologist, 169(3), 453-468; [4] Dueck T.A. et al. 2007. New Phytologist 175: 29-35; [5] http://www.isolife.nl/about%20us.php?letter= ; [6] Erk T. et al. (2009). J. AOAC Int. 92: 730-733. [7] De Vos C.H., H. Verhoeven & F. Verstappen, personal commun.; [8] Kraut, N.U. et al. (2010) Metabolomics 2010, Amsterdam. [6] Stringham JM et al. (2010) J. Food Sci. 75: R24-R29.

P9A-007
Discrimination of acute exposure to different test foods using metabolomics

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Conventional tools for measuring dietary exposure, an essential component of much health-related research, have significant limitations. The MEDE Study (MEtabolomics to characterise Dietary Exposure) was designed to provide proof of principle that metabolomics could be an alternative non-subjective approach to characterise dietary exposure without using diaries or questionnaires [Favé, 2009]. Under carefully controlled conditions, 24 healthy adults consumed a test breakfast, providing 5 fixed items (orange juice, croissant, tea with milk and sugar) and 1 changing item viz. corn flakes with milk (SB), salmon (Salm), broccoli (Broc), raspberries (Rasp) or Weetabix with milk (Weet). Each volunteer attended 6 visits and consumed the SB (twice) and each of the test foods (once) in a Latin Square design. Urine and blood samples were collected fasting and at 1.5, 3, and 4.5 hours after meal consumption. Metabolites in urine samples were analysed by FIE-MS and GC-MS followed by multivariate data analysis. PC-LDA score plots showed that, except for Weet, all breakfasts were well discriminated in 3h post-prandial urine samples analysed by FIE-MS (Eigenvalues: 2.55 and 1.3 in DF1 and DF2 dimensions, respectively). PC-LDA on GC-MS data showed even stronger discriminations between SB, Salm, Broc and Rasp exposures (Eigenvalues: 19.46 and 6.14 in DF1 and DF2 dimensions, respectively), but still none between SB and Weet. However, PCA of the 3h samples collected after Weet consumption revealed a subset of "high responders", and re-analysis of the data from this sub-set lead to a good discrimination between the two cereal breakfasts. Also, the data from the two SB exposures showed a nice grouping of both visits' samples, demonstrating the repeatability of our protocols. Our metabolomics approach succeeded in discriminating acute exposure to foods of high public health importance. Determination of which combinations of metabolome signals are responsible for these discriminations will help to develop novel exposure biomarkers (FSA Project N05073).

P9A-008
Non-targeted metabolite profiling of the phytochemicals of rye and effects of microbiota on their composition in in vitro colon model

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Diets rich in whole grain products are convincingly associated with reduced incidence of chronic diseases such as cardiovascular disease and diabetes. One of the most frequently consumed whole grain products in Northern Europe is rye (*Secale cereale*, L.) bread. Especially the bran layer of rye grain is known to be a very rich source of phytochemicals like phenolic compounds, which are suggested to contribute to the health protective effects of whole grain rye. The human distal gut hosts diverse microbial communities living in a mutualistic relationship with the human body. The metabolic activity of colonic microbiota results in major changes on the dietary phytochemical spectrum and also alters their absorbability and bioactivity. The impact of gut-modified phytochemicals is increasingly pointed out in research related to diet and health, and thus it is of major interest to elucidate the metabolic process occurring in the colon between the microbes and the food components. In this study non-targeted metabolite profiling by UPLC-qTOF-MS was conducted on whole grain rye as such, and on rye bran incubated in an in vitro colon model. Parallel profiling was conducted in order to gain information on the phytochemical repertoire of rye in detail, as well as to monitor how the composition of the rye bran semi-polar metabolite fraction alters as time course in the fermentation process. In the whole grain rye crude extracts several novel lignan metabolites, phenolic acids in different polymerized forms, as well as metabolites from other chemical classes previously not found in rye grain were identified. The results from the analysis of the microbiota converted metabolite fractions indicate a major change e.g. in the composition of lignan metabolites, and show the accumulation of several small phenolic compound –derived metabolites during the fermentation. This work is part of the Nordforsk Nordic Centre of Excellence project HELGA- Whole Grains and Health.

P9A-010

Impact of short-term intake of wine and grape polyphenols on human metabolism

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Red wine and grape polyphenols are considered to promote cardiovascular health and have been reported to be involved in multiple biological functions by acting as antioxidant, anti-carcinogen, anti-microbial, anti-inflammatory, insulin sensitizing and neuron-protective agents. However, the overall impact of polyphenols on the human metabolism remains largely undefined. In a placebo-controlled, randomized, cross-over study we investigated the metabolic impact of a four-day intake of red wine and grape polyphenols in healthy male adults by performing untargeted GC-MS- and LC-MS-based metabolite profiling as well as targeted profiling of catecholamines and steroids. Overall, the wine and grape polyphenols only had a mild impact on the metabolism in urine and plasma. Using the method of local false discovery rates, a limited set of metabolites was identified that were clearly affected by the polyphenol intake. These included mainly exogenous metabolites from gut microbial fermentation of polyphenols such as 3-hydroxyhippuric acid, pyrogallol, 3-hydroxyphenylacetic acid, hippuric acid, catechol, 4-hydroxyhippuric acid, 3,4-dihydroxyphenylacetic acid, vanillic acid and trans-ferulic acid in urine. Furthermore, a reduction in urinary p-cresol sulfate and 3-indoxylsulfuric acid was observed suggesting altered microbial protein fermentation. In addition, increased levels of urinary indole-3-lactic acid, nicotinic acid and 1-methylhistidine and reduced levels of tyrosine and taurine in plasma were found indicating a modified amino acid metabolism. These results provide a rationale for further research probing the microbial-host metabolic cross-talk in relation to dietary polyphenols and protein consumption.

P9A-011

Metabolite profiles in human urine after a high vs. low dietary fiber intake

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Objectives: The health effects of dietary fiber in healthy subjects might be subtle and looking at several early biomarkers simultaneously, rather than measuring just a few endpoints, might better explain these effects. The aims of the study are to find unique biomarkers of dietary fiber exposures and to search for early biochemical changes in urine metabolites related to this exposure. Method: In a randomized cross-over five week intervention, 25 subjects were given high fiber diet (HF) and low fiber diet (LF). At the end of each intervention period, 24-h urine samples were collected and stored at -80°C. Samples were separated by an UPLC system equipped with a 1.7μm C18 BEH column using a 6 min gradient from 0.1% aqueous formic acid to 0.1% formic acid in 20% acetone: 80% acetonitrile followed by high mass resolution and accuracy QTOF-MS detection in positive and negative electrospray ionization modes. Raw QTOF-MS data are aligned and normalised in MarkerLynx (Waters, Milford, MA, USA) and exported to Excel to identify markers that differ between the diets by using algorithms and restrictions in mean and CV%. Multivariate data analysis will also be performed to investigate any discrimination between groups (e.g. between diets, gender) to identify variables (metabolites) that contribute to any discrimination. Results: In positive mode, the peak response of 30 metabolites was higher after HF compared to LF ($p<0.0001$). In negative mode, peak response of 42 metabolites were increased after HF ($p<0.0001$). Identification of these metabolites is ongoing using Human Metabolome DataBase and ChemSpider with following verification using standards and fragmentation pattern analysis.

P9A-012

Effects of mutations on its structure and functional properties of the sweet-tasting protein, brazzein

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The demand for non-calorigenic protein-based sweetners with favorable taste properties is high. Brazzein is an intensely sweet-tasting plant protein with good stability at high temperature and pH. Brazzein is 500~2,000 times sweeter than sucrose. It is a single-chain protein and its molecular mass is 6.5 kDa. To elucidate relationship between structure and sweetness of brazzein, a 159-bp synthetic brazzein-encoding gene was constructed for expression in *Escherichia coli.* We designed a gene for a minor component of brazzein (des-pGlu1) which has twice the sweetness of the major component that incorporates codons that are optimal for protein production in Escherichia coli. We also constructed pET26b(+)-brazzein expression plasmid containing the periplasm signal sequence. Based on the constructed pET26b(+)-brazzein, 18 brazzein mutants were constructed by site-directed mutagenesis to gain further insight on the relationship between sweetness and sweetness of brazzein. The mutant enzymes were expressed in Escherichia coli and purified the same methods as pET26b(+)-brazzein. Some of brazzein mutants were not sweeter than the wild-type brazzein. On the other hands, substitutions of His31, Ala32, Glu36 and Glu41 residues increased sweetness. These mutants result in approximately 2.5 to 5.0-fold the sweetness of the wild-type brazzein, and approximately 2,000 to 4,000-fold sweeter than sucrose.

P9A-013

Metabolic profiling of human breast milk samples through the DART-FTMS and LESA-FTMS analysis of dried paper spots

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Human milk is considered the most important diet for human development. The dietary composition of infants is thought to program many aspects of the metabolism that can have health effects in the rest of the life-span. However we currently lack a detailed understanding of human breast milk composition. The detailed analysis of human breast milk is difficult due to the rapid changes in breast milk composition during a feeding and during the whole period of lactation. This is further hampered by the long and tedious methodology to determine for instance lipid composition of breast milk (using either HPLC or (FAME)GC analysis). We developed a completely new strategy based on dried milk spots, which are small milk samples (5-25 μl) spotted on filter paper. This preserves the milk samples over several days and requires minimal technical skill for collection. The dried milk spot can then be analysed by ambient desorption ionisation (DART) or surface sampling using the Triversa Nanomate (LESA), both coupled to high-resolution FTMS. Human milk samples were spotted on Whatman filter paper and air-dried. The paper disks with the milk spots we placed for up to 5 min in the helium stream of the DART source, or the surface of the milk spot extracted with 1 μl of MeOH that was then directly infused with chip-based nanospray. The total lipid profile corresponded to literature data, although the amount of detail of the presented method is much a higher. Samples spotted on paper could be stored at room temperature over several days without any qualitative changes. In addition to the triglyceride profile also information could be obtained on sugars (lactose) and other lipids (sterol lipids and free fatty acids) Data will be shown on the changing lipid levels at 2, 13 and 52 weeks lactation. The method proofs to be extremely quick and easy, requiring no additional sample preparation, and resulting unprecedented detail of the milk composition.

P9A-014
Biomarkers of caloric intake -- from rats to humans

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Caloric restriction (CR) is the most potent and reproducible known means of increasing longevity and reducing morbidity in mammals. Risk of breast cancer, for example, is generally decreased by more than 90% in CR rodents, and the CR-mediated effects are usually dominant to those induced by genetic risk factors, carcinogens, or co-carcinogens. The robust observations of reduced morbidity in CR animals is directly analogous to studies in humans that link obesity with poor health outcomes, including increased risk of neoplastic disease. We therefore proposed to test the general concept that biomarkers of diet in rats will predict risk of future disease in humans. Metabolomics measurements in sera/plasma were conducted by HPLC coupled with coulometric detector arrays (N~600 rats, ~1700 humans). Classification and predictive power were tested, optimized, and validated using megavariate data analysis in sequential blinded cohorts. Exploratory studies identified 93 redox-active small molecules from sera with potential to distinguish dietary groups in both male and female rats. PLS-DA built models with >95% accuracy in distinguishing groups. Data processing choices of transformation, scaling, and winsorizing each affected strength of the models, and, in some cases, revealed distinct metabolites to be of importance in building these models, often in gender-specific ways. Diets varying in extent and duration of CR were used to develop models for intermediate caloric intakes, which are more relevant for human studies (total N=180 females). Markers were adapted for human study, analytically validated at both the instrumentation and at the sample collection levels, then biologically validated (N~200, metabolites and profiles had intraclass correlation coefficients from ~0.65-0.85). We will present these modelling approaches, the models, and their ability to distinguish sera based on caloric intake, as well as data from the initial application of these markers to address risk of breast cancer in case-control studies nested within the Nurses' Health Study.

P9A-015
Effects of low insulin response grain products, fish and bilberries on plasma lipidomics profile in individuals with the metabolic syndrome

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Background. Low insulin response grain products, fatty fish and berries are known to have beneficial effects on glucose and lipid metabolism. In this study, we aimed to study the synergistic effects of these foods on lipid metabolism in subjects at risk for type 2 diabetes. Methods. This study included altogether 105 subjects with age of 40-70 y, BMI 26-39 kg/m2, and at least three of the features of the metabolic syndrome (NCEP Adult Treatment Panel III, 2001) in a 12-week parallel dietary intervention with 3 different groups. The subjects were randomized into a group consuming cereal products with low postprandial glucose and insulin response, fatty fish at least 3 times a week, and bilberries 3 portions per day (“Sysdimet”), a group consuming the same cereal products, but no change in fish and berry consumption (HealthGrain), and a group consuming refined wheat breads as cereal products (Control). Fasting blood samples were taken before and after the intervention. The analytical platforms were ultra performance liquid chromatography coupled to electrospray ionization mass spectrometry (UPLC/MS) with time of flight (TOF) detection for lipidomics and gas chromatography for fatty acids. Results. Altogether 369 lipids and 22 fatty acids were identified and quantified. Statistical analyses are ongoing. Based on mixed model analyses 87 lipids changed significantly ($p \leq 0.05$, corrected for multiple comparisons) during the dietary intervention. The most considerable changes were found in the “Sysdimet” group. Specifically, multiple triacylglycerols with long chain polyunsaturated fatty acids, lysophosphatidylcholine 20:5, phosphatidylserines 36:1 and 38:1, and phosphatidylethanolamine 38:7e increased in the “Sysdimet” group. Conclusion. Preliminary results suggest that the dietary modification causes significant changes in lipidomics profile. Further analysis is needed to reveal the meaning and the clinical relevance of these results.

P9A-016
Metabolomics of faecal extracts: ulcerative colitis and irritable bowel syndrome

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The balance of bacterial species in the gut has been suggested to play a role in some gut disorders. Ulcerative colitis (UC) and Crohn’s disease (CD) are inflammatory bowel diseases suspected to be caused by a reduction in immune tolerance and an overreaction of the host’s immune system to the bacterial community in the gut. Irritable bowel syndrome (IBS) has also been linked to the composition of the gut microbiota. Low molecular weight metabolites in the faecal water are produced by bacterial fermentation of undigested polysaccharides and protein in the colon. The metabolites present are of interest because of their potential influence on gut health and because they reflect the make-up of the microbiota. Metabolomics studies of faecal extracts have been carried out previously by NMR and LC/MS with the emphasis on CD. We now report results of NMR studies in which the composition of faecal extracts from subjects with UC or IBS was compared with healthy controls. We have found that UC extracts are more easily separated from controls than IBS. Compounds responsible for the separation show some overlap with the earlier studies on CD although new disrupted pathways are highlighted (choline-related, polyamines and branched short chain fatty acids). We also illustrate a strong correlation between NMR and PCR-Denaturing gel Gradient Electrophoresis (DGGE) data which emphasises a direct link between the composition of the gut microbial communities and metabolites from faecal waters.

P9A-017
Plasma monitoring of laparoscopic adjustable gastric banding (LAGB) patients by proteomics and metabolomics

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Morbid obesity is associated with increased prevalence of inflammatory related diseases and cancer. The risk of these co-morbidities has been shown to decline with weight-loss after bariatric surgery. High-throughput “-omic” technologies provide a unique opportunity to gain knowledge about the complexity of the metabolic adaptations occurring after such surgery. We conducted a prospective controlled study of morbidly obese patients undergoing LAGB. Fasted blood tests were taken pre- and 3, 6, 9 and 12 months post- surgery. Proteomic analysis was conducted by 2-D gel electrophoresis of albumin/ IgG depleted plasma. Further analysis was performed using liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. Average BMI was significantly reduced at 12 months after surgery ($p=0.0015$). Proteomics showed a complex spectrum of protein changes. CRP levels were significantly reduced at 12 months ($p=0.04$), whilst haptoglobin, adiponectin, alpha-2 macroglobulin, alpha-1 antitrypsin, fibrinogen and complement factor B were all higher at 12 months ($p<0.05$). Metabolomics demonstrated progressive changes in HDL, branched amino acids and markers of oxidative stress (lysyl-albumin) and inflammation (N-acetyl-glycoproteins) with a trend towards control levels with increasing time post-op Weight-loss was associated with changes in acute-phase proteins and metabolite changes generally consistent with those detected by other methods. These results show the value of proteomic and metabolomic techniques for broad-spectrum metabolic assessment in this group. Interestingly, the relatively modest changes in several key metabolite levels post LAGB surgery suggest that the patient metabolism adjusts progressively to a healthier status in relation to the weight loss observed. Acknowledgments, Lynda Olivier for technical assistance and Mike Naldrett JIC for the proteomics multiTOF

P9A-018

Human urinary metabolome modifications after almond polyphenols consumption. A metabolomics approach to identify new nutritional biomarkers.

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Almond-skin is a source of polyphenols, mainly procyanidins. The health effects of these polyphenols depend on their bioavailability implying tissue metabolites and gut microbial metabolites. An important challenge of the nutri-metabolomics is to identify new biomarkers that allow monitoring the intake of dietary phytochemicals (food-metabolome) and finally relate them with the expected biological effects (endogenous-metabolome). The modifications occurred in urine metabolome during the 24-h after consumption of almond-skin extract were studied. Twenty-four healthy subjects were randomized into two group (n=12, placebo or dosed). Placebo-group ingested microcrystalline cellulose (4-g,10-capsules) whereas Dosed-group ingested almond-skin extract (3.5-g,10-capsules). Urine samples were collected and 0-2, 2-6, 6-10 and 10-24-h after consumption. Samples were analyzed by HPLC-q-ToF (Applied-Biosystems) followed by multivariate data analysis. To evaluate possible analytical artefacts several QCs were carried-out. Identification was performed using public databases (KEGG, HMDB) and in-house-database focused on polyphenols metabolites and MS/MS experiments. Urinary metabolic profiling showed a clear discrimination between both test-meals at the four assayed times. Kinetic patterns for different biomarkers related with almond-skin consumption were detected. Host metabolites were excreted within the first 10-h and others such as microbial-polyphenol metabolites were present at 10-24-h period. Several metabolites have been identified including polyphenol host-metabolites as well as colonic-microbiota metabolites such as phase-II conjugates of hydrophenylvalerolactones and hydroxyphenylvaleric acid. The results showed that LC-MS based metabolomics contributes to characterize the urine metabolome modifications associated to almond polyphenols intake. These modifications were related to almond polyphenols intake and mainly with the complex profile of colonic microbiota metabolites. Several of these metabolites have been related for the first time as markers of procyanidins intake. These results confirm that metabolomics is a powerful tool that allows to characterize the food metabolome and to explore the metabolism impact of dietary components.

P9A-019

Development of metabolomics as novel approach to biological indicators which characterise and quantify dietary exposure

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Discovery of chemical biomarkers indicative of specific food consumption is difficult due to the complexity of metabolite content of foods, metabolism of these metabolites, and the lack of databases and standards for possible biomarkers. The MEDE Study (MEtabolomics to characterise Dietary Exposure) aimed to provide 'Proof of Principle' that a non-targeted metabolomics approach could identify chemical signals typical of individual foods in human biofluids. The first challenge was to design and validate protocols for i) subject recruitment and management and ii) biological sample collection, processing and storage. A standardised breakfast (orange juice, croissant, tea and cornflakes) was employed as an initial food 'challenge' to fasting volunteers. FIE-MS fingerprinting coupled with PC-LDA and Random Forest provided evidence of chemical differences in post-prandial urines. Feature-ranking methods highlighted nominal mass bins which were 'explanatory' of exposure to the breakfast, which were further targeted by ultra FT-ICR-MS and MS/MS. Markers indicative of orange juice consumption were revealed, as reported in previous literature, thus validating the utility of non-targeted metabolomics. Following this initial success to calibrate methodology, we sought to determine whether metabolite fingerprints indicative of different foods, substituted for Cornflakes, could be discriminated from the standard breakfast. FIE-MS data subjected to PC-LDA revealed that it was possible to separate raspberries, smoked-salmon and broccoli from the standard breakfast. Following FT-ICR-MS and MS/MS, several specific metabolites were identified as possible biomarkers for future targeted, quantitative analysis. It was not possible to discriminate Weetabix from Cornflakes for the study volunteers as a whole. However, analysis showed distinct 'metabotype' sub-groups within the study volunteers and when analysed within each sub-group, discriminatory metabolites could be identified. Overall this study demonstrated the potential of metabolomics to identify and develop novel and robust biomarkers of dietary exposure. There is clear potential of using metabolomics to identify candidate chemistries that can be explored using targeted, quantitative methods to confirm biomarker status.

P9A-020

Long-Term Metabolic Effects of a Glucose Challenge in C57BL6 Mice

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Although the molecular events occurring following a glucose tolerance test have been well studied, the longer term effects of this model of metabolic stress are less well known. In the present study forty mice were fed a standard low-fat (10%) diet for twelve weeks before being administered a bolus of glucose (2g/Kg body weight). Eight animals were then sacrificed at each of five time points (0, 0.6, 2, 18 and 48 hours) and serum, liver and muscle tissue samples were collected for analysis using GC-MS, LC-MS and NMR spectroscopy. The temporal analysis allowed for changes in different processes and tissues to be followed as they occurred over the 48 hour period. Serum glucose levels were mirrored in the liver throughout the experiment with a rapid increase at 0.6 hours and a gradual decrease over the remaining time-course. Lactate levels in the liver peaked at 0.6 hours and returned to normal levels at 2 hours. Serum levels of LDLs and VLDLs were both increased at 2 hours and 48 hours while HDLs were decreased at the same time points. Glutamate and glutamine levels in the liver increased rapidly from 2 hours, whilst serum levels of branched chain amino acids were decreased. Overall, the results demonstrate a rapid increase in glycolysis (lactate), an exportation of lipids from the liver and a decrease in circulating branched chain amino acids. More surprisingly, glutamate and glutamine levels increased over the time-course, possibly reflecting an increase in TCA cycle turnover. Additionally, a trend towards normalisation to basal metabolite levels was not apparent, even after forty-eight hours. Our results imply that the level of metabolic stress induced in mammals by high-sugar consumption may be more significant than was previously assumed and that this would warrant further analysis.

P9A-021

Characterization of the peculiar obese urinary metabotype by metabolomic analysis of the gut microbiome

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Obesity is a complex multifactorial disease involving genetic and environmental factors and influencing several different metabolic pathways. In this regard, metabonomics may provide a systems approach to understand the global metabolic regulation of the organism in relation to this peculiar pathology. Here we present the results of a pilot study conducted by NMR-based metabolomics on urinary samples of morbidly obese subjects. Urine samples of morbidly obese insulin resistant male patients and of age-matched control subjects were collected, frozen and analyzed by high resolution 1H-NMR spectroscopy combined with Partial-Least Squares-Discriminant Analysis (PLS-DA). Furthermore, two obese patients who underwent two different bariatric surgery interventcions were monitored during the first 3 months after surgery and their urinary metabolic profiles were characterized. NMR-based metabolomic analysis allowed us to identify an obesity-associated metabolic phenotype (metabotype) that differs from that of lean controls. Mainly gut flora-derived metabolites contributed most to the classification model and were responsible for the discrimination. This preliminary results confirmed that in humans gut microflora metabolism is strongly linked to obesity phenotype. Moreover, the typical obese metabotype is lost after weight loss induced by bariatric surgery.

P9A-022

Omega-3 fatty acid ethyl ester supplementation reveals a tight regulation of plasma and blood cell membranes HUFA and oxy-HUFA pools

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Omega-3 fatty acid (n3-FAs) consumption is associated with health benefits with respect to cardiovascular risk, inflammation, and hypertriglyceridemia. While the n3-FAs compete with arachidonic acid, an omega-6 fatty acids (n6-FAs), for placement in membrane lipids and for the production of oxygenated lipid mediators, the magnitude and breadth of these effects are poorly described. Using targeted procedures quantifying an array of eighteen to twenty-two carbon fatty acid metabolites and their parent lipids in esterified lipid pools, we investigated the influence of four weeks of 4g/day n3 FA ethyl ester supplementation on plasma oxylipin profiles, as well as plasma, erythrocyte, and platelet fatty acid profiles in 30 healthy volunteers. While the majority of plasma fatty acids, including plasma arachidonate concentrations were unchanged, concentrations of other long chain n6 HUFAs were decreased 20 - 50% ($p<0.001$) while n3-HUFAs increased 70 – 550% ($p<0.001$). Changes in both red blood cell and platelet lipids were consistent with these changes. Similarly, a variety of plasma long chain oxylipins classes including fatty acid alcohols, epoxides and diols also showed reduced n6-FA species and increased n3-FA oxylipins ($p<0.001$). The magnitude of change in each lipid pool composition induced by the 4 week supplementation was dependent on the initial pool concentration, and despite high compliance and equivalent intake, some individuals showed minimal shifts in lipid pools. Therefore, this study suggests the existence of a maximum concentration for n3-FA enrichment in multiple lipid pools.

P9A-023

Targeted Metabolomic Profiling of Lipid Signaling Domains with Semi-Automated QA and Data Mining for Health and Nutrition Research

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Targeted metabolic profiling provides a means to quantify low abundance metabolites with known physiological and biological significance. Lipid signaling domains are nutritionally sensitive and play critical roles in the regulation of cell growth, inflammation, and energy balance, making them excellent analytical targets for health and nutrition research. While state of the art instrumentation allows for fast and selective acquisition of many targeted molecules down to femtomole levels, the production of high quality data requires laborious QA/QC regimens resulting in bottlenecks. Here we present an efficient Flow of Work that alleviates some of these bottlenecks. Components include single extraction procedures for multiple profiles, identical chromatographic conditions for multiple profile acquisitions, static QA/QC regimens and first-pass data interpretation approaches using the public domain interface between Microsoft Excel and the Gnu project statistical package R, RExcel. Implemented analysis routines include novel supervised PCA and PLS procedures, directed and undirected biosynthetic network construction, and predictive modeling algorithms. The accuracy and efficiency of the developed system to evaluate QA/QC paradigms are compared to the manually curated data set of BMI and age matched human plasma.

P9A-024

Barley arabinoxylan and mixed linked beta-glucan exhibit low responses in selected in vitro test systems

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High intake of dietary fibre has been claimed to protect against the development of colo-rectal cancer (CRC), but to date this is not well documented. Barley is a rich source of dietary fibre, and possible immunomodulatory effects of barley carbohydrate polymers might explain a protective effect. Dietary fibre was isolated from barley (*Hordeum vulgare* var. Tyra) by extraction and treatment with specific enzymes giving two types of high molecular weight carbohydrate polymers; a mixed-linked beta-glucan and an arabinoxylan. Fractions containing purified beta-glucan (WSM-TPX), arabinoxylan (WUM-BS-LA), a beta-glucan rich fraction containing 10% arabinoxylan (WSM-TP) and an arabinoxylan rich fraction containing 30% beta-glucan (WUM-BS) were subjected to immunological test systems. The fractions had no significant effect on secretion of IL-8 from two different colon cancer cell lines, Caco-2 and HT-29, and they had no significant effect on NF-kappaB activity on U937-3kappaB-LUC which is a monocytic cell line. Fractions isolated from four different barley varieties (Tyra, NK96300, SB94897 and CDC Gainer) were subjected to the complement-fixing test. The beta-glucan rich fraction from CDC Gainer showed the same level of activity as the positive control while the beta-glucan rich fractions from Tyra, NK96300 and SB94897 were less active. Fractions rich in arabinoxylan exhibited lower complement-fixing activity than the beta-glucan rich fractions.

P9A-025

Factors that influence human urine 1H NMR metabolomics

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It is well known that a large inter- and intra-subject variability affects nutritional metabolomic studies (nutri-metabolomics) and therefore, in order to achieve meaningful and reliable results, it becomes essential to limit the variance introduced by external factors. This research work describes the findings of a controlled study, conducted with full provision of all food for a standardized intervention, in which human urinary profiles have been analyzed using 1H NMR and chemometrics. Different factors, such as diet standardization time-compliance, sample cooling, gender, food culture and cohabitation have been investigated. In the first part of the study (A), eight healthy subjects (4 men; 4 women) collected 24-h urine, splitting each void into two specimens stored either at 4 C or at room temperature. In the second part (B), sixteen healthy subjects (7 men; 9 women) collected 24-h urine for three days while being on a standardized diet. The collected NMR data have been processed and analyzed using classic and advanced chemometric tools. On the basis of the analysis of duplicate samples (part A), it can be state that no significant changes were found in the NMR profiles of the urine samples stored at 4 C for 24h. On the contrary, metabolites assigned to bacterial contamination were observed in three out of 16 samples stored at room temperature. The study (part B) also showed that, during three days of diet standardization, a trend towards reduced intra- and inter-individual variation was achieved. Furthermore, the urine metabolome showed a clear grouping effect of the samples on the basis of both food culture and cohabitation. However, these effects almost completely vanished after diet standardization. Finally, gender-specific differences were found in both studies. Using iECVA, a new classification chemometric tool, the NMR signals of the biomarkers responsible for these discrimination could be found and, subsequently, assigned. Our results confirm that, prior to a dietary intervention, careful sample practices and diet standardization are indispensable in order to minimize intra- and inter-subject variability

P9A-026
Metabolic disorders associated to weight gain in humans: a metabolomic approach

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Obesity has become a major public health concern worldwide and epidemiological studies have established strong association of obesity with cardiovascular diseases and type 2 diabetes. Obesity is associated with increase risk of metabolic syndrome which is a complex disease involving dysregulation of several metabolic pathways. The major objective of the METAPROFILE project is using an integrated metabolomic approach to identify new biomarkers of early metabolic dysfunctions associated to overfeeding, predisposing for the development of obesity and associated diseases. For this purpose a metabolic exploration was carried out on humans. Forty men (18-55 years old) were recruited according to their body mass index (BMI) and waist circumference. Twenty slim men with no family history of obesity (20≤BMI≤ 25) and twenty overweight men with family history of obesity (25≤BMI≤ 30) were submitted to a hypercaloric and hyperlipidic diet for 8 weeks. Samples of blood, urine and feces were collected before, during and after the feeding intervention. Plasma (after deproteinization) and urine samples were analyzed on a Waters Acquity UPLC module coupled with an ESI-QToF micro mass spectrometer, in positive and negative ion modes. Fecal water prepared by centrifugation of the feces was analyzed by GC/MS using ethyl chloroformate and trimethylsilylation derivatizations. Both analysis of variance and PLS discriminant analysis were carried out to determine the potential biomarkers. For urine and plasma samples, some discriminant metabolites were identified by comparison with existing databases and further confirmed using high resolution mass spectrometry. Preliminary metabolomic data showed to which extend overfeeding induced metabolic dysfunctions.

P9A-027
Metabolic Profiling of Oxylipids at the Netherlands Metabolomics Centre

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Oxylipids, including eicosanoids, act as lipid mediators involved in inflammation and cellular growth processes. They are generated by oxidation of polyunsaturated fatty acids (PUFAs) like arachidonic acid and linoleic acid (both known as omega-6-fatty acids). Prostaglandines (PG), leukotriens (LT) and thromboxanes (TX) are the most broadly investigated representatives of this compound class, however oxylipids encompass other important chemical classes including hydroperoxides, alcohols, epoxides, and diols. The involvements of these compounds in conditions with inflammatory components including cardiovascular disease, hypertension, atherosclerosis, type 2 diabetes mellitus and obesity make their profiling an interesting challenge for nutritional studies carried out within the framework of the Netherlands Metabolomics Centre. These studies aim at resolving the relations between diet and cardiometabolic risk, weight management and gut health. Inflammation is overarching in these processes hence a comprehensive oxylipid profiling platform is developed. Here we describe the implementation and analytical validation of a robust LC-MS/MS targeting method for approximately 120 analytes. Current efforts are aimed at demonstrating the applicability of this analytical platform in biological processes.

P9A-028
Towards identification of polyphenol metabolites in biofluids by LC-SPE-cryoNMR-MS

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Polyphenols are an important class of functional ingredients that are currently being investigated for their potential health benefits. Upon consumption, polyphenols undergo conjugation in the human host and/or bioconversion by the colonic microbiota. Many of the resulting metabolites are not know and their identification is a considerable challenge. In many cases one can take recourse to spectral databases (NMR or MS), but often one needs to embark on a de-novo molecular identification. This requires isolation and enrichment of the metabolite(s) of interest and subsequent structural elucidation by a combination of both NMR and MS. An efficient metabolite identification platform is provided by combining high-performance LC with NMR and MS. The increased automation and the incorporation of on-line solid-phase extraction (SPE) into an integrated system recently improved the detection limits. A further gain in sensitivity is provided by the use of cryogenic NMR probeheads. LC-SPE-cryoNMR-MS platforms have already been deployed for identification of metabolites from single drug compounds dosed at high levels. Nutritional polyphenol formulations are much more complex and dosages are mostly low. Hence we needed to design a strategy for identification of low-abundance metabolites against the highly complex metabolic background of biofluids. This strategy has successfully been applied to identify metabolites that are being produced upon microbial fermentation of green tea in a colonic in-vitro model. A range of low-abundance metabolites has been identified which could be used to further expand the known colon microbial degradation pathway of epicatechines in green tea.

P9A-029
Semi-automated non-target processing in GC×GC-MS metabolomics analysis: applicability for biomedical studies

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Due to the complexity of typical metabolomics samples and the many steps required to obtain quantitative data in GC×GC-MS (deconvolution, peak picking, peak merging, and integration), the unbiased non-target quantification of GC×GC-MS data still poses a major challenge in metabolomics analysis. The feasibility of using commercially available software for automated non-target processing of GC×GC-MS data was assessed. For this purpose a set of mouse liver samples (24 study samples and 5 QC samples prepared from the study samples) were measured with GC×GC-MS and GC-MS to study the development and progression of insulin resistance, a primary characteristic of diabetes type 2. A total of 170 and 691 peaks were quantified in, respectively, the GC-MS and GC×GC-MS data for all study and QC samples. The quantitative results for the quality control samples were compared to assess the quality of automated GC×GC-MS processing compared to the targeted GC-MS processing that involved manual correction of all wrongly integrated metabolites. Although the RSDs obtained with GC×GC-MS were somewhat higher than with GC-MS, still the biological information in the study samples was preserved and the added value of GC×GC-MS was demonstrated; many additional candidate biomarkers were found compared to GC-MS.

P9A-030

An unbiased metabolic profiling approach to lipidomics using high resolution mass spectrometry

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The study of lipids (lipidomics) involves the identification and quantitation of thousands of cellular lipid species especially during perturbations of biological systems. Using high resolution mass spectrometers with sufficient scan speed, capable of measuring m/z ratios with relative mass measurement uncertainties of 1 ppm or less, it is possible to obtain excellent qual/quan data in full scan mode. This is an important step forward in comprehensive analysis for lipidomics using an unbiased profiling approach. Plasma samples were collected from healthy volunteers and prepared by protein precipitation for HPLC analysis. The total analysis duration was 10 min using an Accela UPLC and Exactive (Thermo, Hemel Hempstead, UK) mass spectrometer. The MS was operated in positive mode, using full scan from m/z 150-1000 at 50,000 resolving power (2 Hz). The stability of mass accuracy was very high and combined with a 2 Hz scan speed this opens up the possibility to extract ion chromatograms with a bandwith of 2 ppm using the theoretical m/z of compounds of interest. The resulting chromatograms are highly specific for the chosen molecular formula. In the presentation we show how the data can be analysed using the Sieve metabolomics software which may be customized for lipidomics by using lipid specific databases in ChemSpider to identify differentially expressed compounds.

P9A-031

Postprandial challenge test to demonstrate subtle dietary effects on human health

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The aim of healthy diets is to keep us healthy for longer, but it is difficult to quantify ‘health optimization’ as biomarkers for this purpose are essentially absent. One approach to quantify health is to apply a homeostatic perturbation on processes that are relevant in maintaining health. Measuring classical and nutrigenomics markers after these challenges provide information about the resilience of the subject against different types of stressors and how this is affected by diet. The classical example is the oral glucose tolerance test versus fasting glucose concentration to demonstrate insulin resistance. Here, we introduce the postprandial challenge test (PPCT), perturbing specific areas of metabolism, oxidation and inflammation control. After a 5-week intervention with a diet rich in antioxidants and anti-inflammatory compounds in a cross over design with 36 overweight subjects, a PPCT was applied as a 500ml high fat dairy shake. The effect on metabolism, oxidation and inflammation control was determined by quantification of 79 relevant plasma proteins by multiplex assays, 145 plasma metabolites by GCMS and 7 clinical chemistry parameters during a 6 hour time curve. The majority of these parameters (106 of 145 metabolites, 5 of 7 clinical chemistry parameters and 31 of 79 proteins) reacted significantly to the postprandial challenge test irrespective of the diet. The effect of the dietary intervention on the metabolism, oxidation and inflammation control could be quantified in the fasting state (Bakker et al. 2010), but was more significantly demonstrated by the response to the PPCT. Specifically, 35 of 231 quantified parameters had significantly different time courses in response to the PPCT, while only 16 of these parameters were found to be changed at the fasting state. Specifically, these plasma dynamic changes indicated differences in dealing with oxidative stress and endocrine metabolism after dietary intervention. This nutrigenomics based PPCT provides a firm proof for the relevance of homeostatic perturbation in quantification of subtle phenotypic changes. In nutrition research, applying challenge tests and measuring the integrative response is a new and promising strategy to quantify and understand maintenance of health.

P9A-032

Mass spectrometry-based analysis of eicosanoids and related compounds in an in vivo and ex vivo human LPS challenge study

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There is a growing interest in the diagnosis and classification of inflammatory processes. Acute inflammation is normally easy to diagnose, but a more subtle , often chronic, inflammatory response is more difficult to categorize and understand. However, increasing evidence suggests that subclinical inflammation plays an important role in diseases like diabetes type 2 and atherosclerosis and for that reason it is often called ‘the secret killer’. Inflammation is a natural reaction of the body to pathogens (e.g. bacteria, viruses, and chemicals). Its goal is to clear the body of pathogens and dead cell material, and to repair damaged tissue. White blood cells play an important role in these processes. It is still not completely understood how a chronic inflammatory process starts. However, it is clear that a disturbed balance in the regulation of inflammatory cascades is involved. To investigate different processes that are involved in inflammation a comprehensive analytical platform was set-up to analyze 41 eicosanoids simultaneously in plasma and culture media. The eicosanoids are analyzed using a relative straightforward reversed phase UPLC-MS/MS method after sample clean-up and concentration using C18 solid phase extraction. An important issue is that eicosanoids are present at very low concentration in plasma and for hat reason, are difficult to detect. Moreover the half life of many eicosanoids is short and they are susceptible to oxidation. This necessitates the use of special inhibitor cocktails. Furthermore there was a need to develop a biological test system to measure the subtle effects of chronic inflammation and the even more subtle effects of dietary supplements on the degree of chronic inflammation. Using lipopolysaccharide (LPS), a compound from Gram negative bacteria it is possible to induce an inflammatory response. In our study, LPS was administered under standardized conditions to healthy volunteers (in vivo) or to their blood cells using TruCulture tubes (ex vivo). The eicosanoids UPLC-MS/MS platform was used to detect the subtle modulations in the inflammatory process and to compare the in vivo and ex vivo results.

P9A-033

The analysis of the effect of fish oil capsules intake on endocannabinoid levels using Liquid Chromatography - tandem mass spectrometry

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Omega-3 polyunsaturated fatty acids (n-3 PUFAs) such as docosahexaenoic acid (DHA; C22:6; n-3) and eicosapentaenoic acid (EPA; C20:5; n-3) have been linked to several positive health effects, such as a reduced risk for cardiovascular diseases, potentially cancer and certain mental illnesses. Moreover, consumption of DHA, EPA or fatty fish containing high levels of n-3 PUFAs have been shown to reduce the inflammatory component and improve other characteristics of the metabolic syndrome. The exact mechanisms behind these effects are unknown, but evidence suggests that DHA and EPA can be converted to anti-inflammatory eicosanoids known as resolvins. Another possibility is that their anti-inflammatory properties are due to an interaction with the endocannabinoid system. Fatty acids are precursors for N-acyl ethanolamines (NAEs), a class which includes several endocannabinoids including anandamide (arachidonoyl ethanolamine, AEA) with known anti-inflammatory properties. NAEs can be rapidly synthesized from their fatty acid precursors in membranes, released on demand and broken down again by fatty acid amide hydrolase (FAAH). Therefore, the local availability of a specific type of fatty acid precursor may determine product formation and hence bio-activity. This suggests a link with dietary intake, since it is well known that incorporation of fatty acids into membranes can be modulated by their proportional abundance in the diet. To investigate the effect of the daily intake of supplementary fish oil capsules on the level of endocannabinoids in plasma, a comprehensive analytical platform was set-up and validated to analyze simultaneously, for plasma relevant endocannabinoids and related fatty acid acylamides. The endocannabinoids are analyzed using a reversed phase LC-MS/MS method after sample clean-up and concentration using C8 solid phase extraction. The results show that the short term daily fish oil intake has a minimal effect on the formation of docosahexaenoyl ethanolamine (DHEA; ethanolamine from DHA) and no effect on the formation of eicosapentaenoyl ethanolamine (EPEA; ethanolamine from EPA). However, when daily use of fish oil capsules by frequent users was terminated, a clear reduction in plasma DHEA levels was observed. Again no effect on EPEA was detected.

P9B-001

Heredity of fruit aroma volatiles in Solanum *lycopersicum x Solanum pimpinellifolium* interspecific crosses

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Much of the research in fruit and vegetable breeding that was carried out during the last decades focused on obtaining desirable agronomical characteristics that often resulted in the loss of unselected traits such as aroma and flavor. The unique flavor of tomato is mainly determined by sugar content, free acids and their ratio as well as more than 400 volatile compounds. Of those, only a few have positive log odor units and are likely to contribute to the tomato full flavor. *Solanum pimpinellifolium* is an "aromatic" wild tomato, that contains relatively high levels of volatiles, such as carotenoid derivatives (especially 6-methyl-5-hepten-2-one, geranylacetone and geranial), methyl salicylate, 2-pentylfuran, hexanal and trans-2-hexenal. In addition, volatile nitrogen containing compounds that are important to the "tomato-like flavor" such as 1-nitro-2-phenylethane and 2-isobutylthiazole are also prominent in S. pimpinellifolium. To study the genetics of the key volatiles contributing to the flavor of domesticated tomato and to assess if *S. pimpinellifolium* would be a good source for genes imparting desirable aromas, an *S. lycopersicum* line was crossed with *S. pimpinellifolium* (LA1589) and 250 F5 recombinant inbred lines (RILs) developed. Volatiles composition in fruits harvested from selected RILs were evaluated by SPME GC-MS. We found marked differences in the volatile compositions associated with different overall flavors of the introgression lines as evaluated by taste panelists. These results suggest that *S. pimpinellifolium* can be a good source for the introgression of volatile compounds into the cultivated tomato.

P9B-002

Potato Genetical Genomics: Investigating QTLs controlling tuber metabolites

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The advent of large scale and high throughput analytical technologies alongside the availability of genome sequences has permitted to explore different organisms from a more systematic view. A more comprehensive understanding of the regulation of different biological processes facilitates the implementation of molecular breeding strategies to improve agriculturally important traits. In this study GC/MS profiling will be applied to explore metabolite composition and variation of a segregating potato mapping population. Subsequently these data will be used to investigate associations with genomic regions involved in the regulation of primary metabolites. Furthermore, metabolite QTLs (mQTLs) will be analyzed and interesting co-localizations with physiological QTLs will be investigated. Additionally, in previous studies transcriptomics and LC/MS metabolic profiling data were produced for the population under study and are available to be used. Here, different sets of data will be integrated in attempt to identify gene-to-metabolite functions and candidate genes coordinating primary and secondary biosynthesis pathways. This approach will ultimately lead to the identification of genes responsible for the observed metabolite variation and their impact on potato tuber physiology.

P9B-003

Combining targeted and untargeted metabolomics to support the development of high-yielding artemisinin lines of the anti-malarial plant, Artemisia annua.

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The sesquiterpene lactone artemisinin is synthesized in leaf trichomes of the plant *Artemisia annua* and is the essential ingredient in the manufacture of artemisinin combination therapies (ACTs) – currently the most effective drugs for treating malaria. Artemisinin is sourced from an immature supply chain where small-scale farmers in Africa and Asia may use commercial seed, locally bred varieties or even wild seed to produce crops with variable performance, with commercial yields averaging around 0.8% DW. In order to improve the supply of artemisinin for use in ACTs we embarked on a fast-track plant breeding program in 2006 funded by the Bill & Melinda Gates Foundation to supply new, robust *A.annua* varieties by 2012, with a target to provide commercial lines with artemisinin yields 50-100% above those currently grown. The core of this work involved performing a phenotypic forward screen of over 23,000 individuals exploiting the natural variation in this essentially wild species. In parallel, we profiled mapping populations to develop the first genetic map of this species to enable ongoing marker assisted breeding (Graham et al., Science (2010) 327:328). The phenotypic and genotypic data were then combined with the goal to identify suitable parental lines for commercial hybrid seed production. Here we describe the LCMS and GCMS platforms and the software tools we developed for the large-scale targeted and untargeted phenotypic screening of *A. annua* extracts from glasshouse-grown populations and field trials. These procedures were developed with two main goals in mind. First, a UPLC-MS platform was developed to meet the challenge of providing accurate, rapid, targeted measurements of artemisinin in high-throughput screening. Secondly, data extraction procedures were developed to mine untargeted metabolite information from the mass-accurate UPLC-MS data and additional methods were developed for complementary terpene metabolite analysis by GC-MS. This larger body of data was combined and is being used to provide insight into biosynthetic pathway elucidation and to select lines with high artemisinin yields but also diverse metabolite profiles to take forward in more advanced field trials.

P9B-004

Biochemical phenotyping of Vitis by 1H-NMR profiling and targeted enzymatic analysis.

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We characterized a selection of 61 genotypes representing 19 species of *Vitis* genera including *Vitis vinifera*, cultivated near Bordeaux, using biochemical phenotyping of the flesh juice of berries. 1H-NMR profiling on two millesimes revealed large variations in the metabolic composition of the must across genotypes and indicated that the genotype effect was higher than the millesime effect. The grouping of the different genotypes was visualized using PCA analysis of the 1H-NMR spectral signatures. These findings were confirmed by a targeted enzymatic analysis of key metabolites, which was performed on three millesimes. Further objectives of this study will be to compare the genetic distances revealed by molecular genetics to those determined by the different metabolomic strategies.

P10A-001

Changes of Flavonoid Content and Tyrosinase Inhibitory Activity in Kenaf Extract after Far Infrared Irradiation

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The tyrosinase inhibitory activity of kenaf (*Hibiscus cannabinus* L.) extract was evaluated after far-infrared (FIR) irradiation. The ethanol extract of kenaf was prepared and its main component was analyzed as a kaempferitrin (kaempferol-3,7-O- -dirhamnoside). Inhibitory activity of kenaf extract was not detected in tyrosinase assay. However, tyrosinase inhibitory activity was observed in kenaf extract treated with FIR irradiation. After 60 min of FIR irradiation onto kenaf extract at 60 oC, a ethanolic extract was prepared and it showed significant tyrosinase inhibitory activity (IC50 = 3500 ppm). According to HPLC analysis, kaempferol, afzelin and minor product were detected. The inhibitory activity may be due to the existence of kaemperol, afzelin and minor product. This study showed that FIR irradiation method can be used as a convenient tool for deglycosylation of flavonoid glycoside.

P10A-002

Metabolite Profiling based Novel Antibiotics Screening from Phomopsis longicolla

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Bacterial leaf blight caused by *Xanthomonas oryzae pv. oryzae* (Xoo) is a major catastrophic disease in rice. Limited success in chemical usage and development of resistance to the available antibiotics are major problems in the disease control. The aims of this study was to isolate a suitable compound from a fermentation time optimized endophytic fungus, Phomopsis longicolla, using mass spectrometry based metabolomic approaches for the effective management of bacterial blight in rice production. The metabolome changes of P. longicolla according to the fermentation time were investigated by liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) analysis coupled with a multivariate dataset. The principal component analysis (PCA) and projection to latent structures discriminate analysis (PLS-DA) of *P. longicolla* fermentations were clearly divided into two phase, phase 1 (2-4 days) and phase 2 (6-20 days). The highest antibacterial activity against Xoo was observed in the phase 2, fermented for 14 days, and 12 metabolites were selected as biomarkers to determine the antibacterial activity. Among them, monodeacetylphomo-xanthone B (a novel compound) and four known antibiotics (dicerandrols A, B, C and deacetlyphomoxanthone B) were identified and significantly correlated with antibacterial activity change of *P. longicolla*. This metabolomic approaches provides valuable information in regards to optimizing the fermentation process for antibacterial activity against Xoo and describes an efficient way to screen for novel bioactive compounds from *P. longicolla*.

P10A-003

Metabolic profiling and a search for bioactive constituents in rice

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The antioxidant properties and glycosidase inhibitory properties of methanolic extracts of a number of white varieties and purple/brown varieties of Indian rice were analyzed. The antioxidant properties of the rice extracts were determined by their DPPH radical scavenging activity, total antioxidant capacity, superoxide radical scavenging activity, reducing power and ferrous ion chelating properties. -Amylase and -glucosidase inhibitory properties of the rice extracts were also measured. The extracts were analyzed for determination of total phenol content, anthocyanin content. Phenolic acids were determined by HPLC. Both white varieties and purple/brown varieties of rice showed antioxidant activities. Some white varieties of rice have very good antioxidant activity. Free phenolic acids e.g. protocatechuic acid, caffeic acid, vanillic acid, p-coumaric acid, ferulic acid and sinapic acid and the phenolic aldehyde vanillin could be detected in all the rice samples analyzed. Principal component analysis and classification shows that superoxide radical scavenging activity, total phenolic acid, protocatechuic acid and ferulic acid are the components to differentiate the varieties from each other. It was also observed that the purple/brown varieties of rice have good -amylase and -glucosidase inhibitory properties. But such properties are weak in white varieties. An anthocyanin has been identified to have -glucosidase inhibitory property.

P10A-004

LC-MS analysis of sulforaphane, a potential anti-cancer agent in Broccoli sprouts.

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Sulforaphane (SFN) (CH3SO(CH2)4NCS) is an isothiocyanate, found in various cruciferous vegetables. It has potential anti-cancer activity and inhibits Heliobacter pylori growth, related to stomach cancer. Broccoli sprouts are particularly rich in glucoraphanin, which is transformed into sulforaphane by the enzyme myrosinase upon damage to the plant. BroccoCress™, a micro-vegetable owned by Brassica Protection Product LLC, is known for its relatively high SFN content (250 mg/100g fresh weight). However, for health studies and development of health claims, bioavailability of SFN is important. This requires a marker that differentiates the compound of interest from compounds from other sources present in the human body. Labeling plants with non-radioactive 13C is a state-of-the-art approach for bioavailability studies using liquid chromatography hyphenated electrospray ionisation high resolution mass spectrometry (LC-ESI-HRMS). BroccoCress™ was grown by IsoLife BV in an uniformly 13CO2-labeled atmosphere to incorporate 13C into all plant components. 13C-seeds (~3 mg) were homogenized in 600 µL water, 25-fold diluted with 0.1% formic acid and 2 µL injected into the LC-ESI-HRMS. 13C-Leaves (~30 mg) were homogenized in 500 µL water, incubated for 30 minutes at 37 °C to activate the plant's myrosinase, acidified and further incubated for 4 hours at 42 °C. After centrifugation, 10 µL of supernatant was diluted to 100 µL and 1 µL was injected. Mass spectra revealed the presence of 89% 13C6H12ONS2, and 11% 13C512CH12ONS2, demonstrating an almost complete labeling (~98,2 atom %) of carbon atoms in SFN resulting in a 6 Dalton mass shift from 177.29 to 183.29. We present evidence that sulforaphane in broccoli sprouts fully originates from the seed and not from de novo synthesis during germination or sprout development. Uniform labeling of SFN makes it a perfect tracer for bioavailability studies. Preliminary data on 13C-sulforaphane analysis in plasma indicate promising perspectives for oral administration of 13C-enriched foods in bioavailability and health claim studies.

P10A-005

Quantitative Estimation of Azadirachin and Nimbin in Azadirachta inidca A. Juss Grown in Nepal

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The leaf and bark fraction extracts of *Azadirachta indica* A. Juss. (neem) grown in the foothills (subtropical region) of Nepal were evaluated for their antioxidant activity, total phenolic (TP) and total flavonid (TF) contents. HPLC method was employed to quantify the amount of azadirachtin and nimbin present in the seed, leaf and the bark extracts of neem. The result showed that the highest azadirachtin content was found in the methanolic extract of the seed (3300 µg/g dw). Similarly, the hexane fraction of bark showed the highest nimbin content (271 µg/g dw) followed by the methanolic extract (260 µg/g dw). Antioxidant activity was determined by measuring 1,1-diphenyl-2 picrylhydrazyl free radical scavenging activity, hydroxyl radical scavenging activity, DNA protection assay, metal chelating and the inhibition of peroxidation using linoleic acid system and their results were found at different magnitudes of potency. The results of TP content expressed in tannic acid equivalents ranged from 66.63 to 629.04 µg/mg in the bark extracts and 23.85 to 237.00 µg/mg in the leaf extracts. Likewise, the content of TF expressed in quercetine equivalents ranged from 12.87 to 17.07 µg/mg in the bark and 13.72 to 93.17 µg/mg in the leaf extracts.

P10A-006

Extra Virgin Olive Oil Phenolic Extract (EVOO PE) prevents human colon adenocarcinoma growth. A metabolomic approach

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Ever-growing epidemiological and laboratory-based studies have begun to accumulate strong evidence that the so-called “Mediterranean diet” significantly reduces the risk for several types of human carcinomas. It has been repeatedly suggested that this protective anti-carcinogenic effect is largely due to the relatively safe and even protective dietary habits of this area. Phenolic compounds could play a major role in the healthy effects of Extra Virgin Olive Oil (EVOO), including the prevention of chronic diseases such as cancer, obesity, diabetes, or coronary diseases, has gradually been increasing and several studies have attempted to elucidate the ultimate mechanisms through which EVOO-derived phenols might contribute to these healthy properties. EVOO-derived complex phenols have been shown to suppress cell growth of human colon carcinoma efficiently. We have obtained crude phenolic extracts (PE) naturally bearing Spanish Arbequina EVOO. The EVOO-PE presents different polyphenols families such as secoiridoids, phenolic alcohols, lignans, flavones and other compounds yet unknown. The effects of crude EVOO PE have been demonstrated on the growth of SW480 and HT-29 cells, two human colon adenocarcinoma cell lines, showing statistically significant effects in SW480 at a concentration of 0.01% in 24 h. The analysis of metabolized compounds from EVOO PE in culture media and citoplasms were carried out using reverse phase high-performance liquid chromatography (RP-HPLC) and nano-liquid chromatography (nLC) coupled with electrospray ionization-time-of-flight-mass spectrometry (ESI-TOF-MS). The results shown many compounds metabolized in both culture media and citoplasms. The most important compound found was hydroxylated luteolin, as well as sulphated, metilated and glucuronidated compounds from EVOO PE.

P10A-007

Evaluation of Antioxidant Compounds Contents and Biological Activities in Chrysanthemum indicum L. Flower Extract Fractions

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This research we evaluated the antioxidant, anti-inflamatory and anticancer activity of different fraction of wild *Chrysanthemum indicum* L. The research data revealed that the total flavonoid contents were highest in ethyl acetate fraction of Chrysanthemum. However, other fraction also showed high flavonoid content. The antioxidant activity evaluated on DPPH free radicals revealed that ethylacetate fraction showed highest percent of radical scavenging activity compare to other fraction. Similarly, the effect of C. indicum extracts on LPS induced NO production in RAW264.7 cells showed that all the fractions effect in a does dependent manner. However, water and butanol fraction showed highest percent of NO scavenging activity compare to other fractions. Likewise, the ethylacetate fraction also showed moderate inhibiting effect on TNF- . Further, the *Chrysanthemum indicum* fractions also inhibit the PGE2 and COX-2 production in a dose dependent manner. Hexane fraction showed highest inhibition on PGE2 compare to other fractions. The western blot analysis showed significant effect to inhibit COX-2 protein by all the fractions of *Chrysanthemum indicum*.

P10A-008

Astragalosides: cytotoxic cycloartane saponins from hairy roots of Astragalus membranaceus Bge.

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One of the goals of cancer chemotherapy and prevention is the discovery of compounds that are relatively selective to tumor cells and, therefore, have reduced effects on normal cell growth. In the current study, three different cycloartan saponins, isolated from transformed hairy roots of *A. membranaceus*, cultivated in air-lift bioreactor were tested for their cytotoxic potential and apoptosis induction in a panel of human tumor cell lines, which is representative for some common human malignancies. The structural identification of saponins was performed using detailed analysis of NI-FAB, MS/MS, EI-MS, 1H- and 13C-NMR (1D and 2D). Root cultures, cultivated in bioreactor gave 18.5 g.l-1 dry wt roots with the highest astragalosides production in vitro up to now - 1.64% (astragaloside I), 1.12 % (astragaloside II) and 1.08% (astragaloside III). The isolated saponins exhibited strong cytotoxic effects against the used tumor cell lines, in a concentration dependent-manner. Highest chemosensitivity was established with the leukemic cell lines HL-60, HL-60/Dox and SKW-3, as well as with the breast cancer-derived cell line MDA-MB-231, whereas the colorectal carcinoma -29 proved to be far less responsive to the cytotoxicity of the saponin compounds. The tested saponins were evaluated for their ability to trigger apoptosis. The results prove that the hairy root cultures of A. membranaceus can be a valuable alternative approach for the production of cycloartane saponins. Using a selected high productive clone, inducing by *Agrobacterium rhizogenes* LBA 9402, a relatively high saponin production can be achieved. This now provides a system for both productions of active compounds, but also a means to correlate structure of individual triterpene cycloartane glycosides with specific cellular target activity in human tumor cell targets. In this manner the production in airlift bioreactor can be used as means of reliable supply of cycloartane saponins to extend the research to human clinical studies. Acknowledgements: Financial support from Ministry of Education and Science, Sofia, Bulgaria (D002-128/2008) is acknowledged.

P10A-009

Development of an accurate LC-MS/MS method for analysis of bioactive compounds in *Scutellaria* species.

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Scutellaria species are widely used in herbal remedies for inflammation, allergy, diarrhea, bronchitis and hepatitis. We have developed a rapid and accurate reverse-phase liquid chromatography, tandem mass spectrometry (LC-MS/MS) method for quantitative determination of bioactive phenolics and flavonoids in different species of *Scutellaria (S. baicalensis, S. lateriflora, S. racemosa, S. tomentosa*, and S. wrightii) following extraction with 70% methanol. Chromatographic separation was performed on a C18 column with a mobile phase consisting of 0.1% formic acid and acetonitrile at a flow rate of 0.3 mL/min. The compounds were detected by positive electrospray ionization with multiple-reaction monitoring using a triple-quadrupole mass spectrometer. Quantitative results indicated that scutellarin, baicalin and wogonoside are the major compounds whereas acteoside, scutellarein, apigenin, chrysin and oroxylin A are minor components in all *Scutellaria* species analyzed. Significant variations in the levels of the various phenolics and flavonoids were observed in the different species tested.

P10A-010

In silico prediction of the metabolism of food phytochemicals facilitates the identification of markers in MS-based metabolic fingerprints in rat urine

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Identification of phytochemical metabolites in metabolic fingerprints is essential for several reasons. First, considering the importance of plant foods in the human diet, phytochemical metabolites are expected to be significant features of the plasma and urine metabolomes, especially when acquired using sensitive mass spectrometry approaches. Second, phytochemicals, especially polyphenols, today arouse considerable interest in both the scientific community and in the food industry because of their various suspected or proven health effects. Identifying the phytochemical metabolites in metabolomes will allow searching associations between their presence and diverse health outcomes in clinical and population studies, in order to identify the most active compounds and better assess their actual impact on health. However, annotation of phytochemical metabolites is still very difficult. This is due to limited coverage of the currently available metabolite databases, lack of commercially available standards, and too limited knowledge on the metabolism of phytochemicals in humans. For many of the 20,000 known phytochemicals in foods, their metabolism is still entirely unknown. However the main biotransformations of xenobiotics and drugs are now well documented and common mechanisms also apply to food phytochemicals. In the intestine and the liver, they are metabolized by Phase I and Phase II enzymes, e.g. methylated, hydroxylated, and/or conjugated with glucuronic acid, sulphate, glycine, or glutathione with further metabolism through the mercapturic acid pathway. This knowledge has been the basis of some rule-based expert systems able to predict the metabolic fate of chemicals from their chemical structure. Meteor is one of the most commonly used tool for drug discovery. The present work is an evaluation of the reliability of Meteor to predict phytochemical metabolism and facilitate annotation of the urine metabolome of rats fed a diet supplemented with a mixture of common dietary polyphenols.

P10A-011

Bioactive metabolites variation in different parts of Punica granatum L. in two provinces , North of Iran

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Punica granatum L. belongs to *Punicaceae* family with locally name "Anar" is one of the most important trees with wilde distribution in sea level to 1600 m temperate region, North of Iran. Different parts of plant has been used for its delicious edible fruit and traditional medicine by the rural people as a heart tonic, anti diabetic , antibacterial , antitumour, expel worms and cardiovascular diseases especially with combination of *Crataegus* species and *Mespilus germanica* and for the treatment of various diseases, such as ulcer, hepatic damage, snake bite. Leaves and flowers were collected in Mazandaran province(sea level) and mountainous Region of Golestan province (1600m) respectively. Methanolic extracts were obtained and their bioactive metabolites were determined. Total phenolic content (TPC) in leaves (249.8-178.8 mg) and flowers (298.9-283.5) mg gallic acids equivalents per gram and total anthocyanin content (TAC) in flowers(0.75-14.11mg) cyanidine-3-glucoside equivalents per gram of dried methanolic extracts respectively. Results in this research showed total phenolic content (TPC) of flowers were more than leaves and increased especially in Mazandaran province(sea level) but total antocyanine content of flower were increased in 1600m (Golestan province). These results that confirmed ,its usage by rural people believed the plant parts that collected in higher region have more secondary metabolites than sea level region to treat various ailments. Key Words : *Punica granatum* L. , leaves and flowers, total phenol and total anthocyanin content, two provinces , North of Iran

P10A-012

Homoisoflavonoid Derivatives from the Roots of *Ophiopogon japonicus* and Their In vitro Anti-inflammation Activity

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Introduction: *Ophiopogon japonicus* (Liliaceae) is an evergreen perennial. Its tuber is sweet with a slightly bitter aftertaste and has been employed in traditional Chinese medicine as an expectorant, antitussive, and tonic agent as well as showing pharmacological effects on the cardiovascular system. In the folk medicine of Vietnam, it serves as expectorant, anti-cough and tonic agent. Recently, the anti-inflammatory effect of the aqueous extract from radix O. japonicus was examined in mouse and rat models, and results demonstrate that the aqueous extract presents remarkable anti-inflammatory activity. Methods: Repeated chromatography of the EtOAc-soluble fraction of the 70% EtOH extract of O. japonicus on silica gel, YMC gel, Sephadex LH-20, and C18 columns led to the isolation of three new compounds (1 3). The anti-inflammatory activities of isolated compounds were investigated by their effects on the release of the inflammatory chemokine eotaxin, stimulated by IL-4 and the combination of IL-4 and TNF- in BEAS-2B cells, which mimics the in vivo conditions in bronchial allergic asthma. Results: Three new homoisoflavonoids, ophiopogonanone G (1), ophiopogoside A (2), and ophiopogoside B (3) were isolated from the roots. The structures of new metabolites were determined on the basis of spectroscopic analyses including 2D NMR.physicochemical data and in the comparison with published references. Compounds (1–3) significant downregulated IL-4-induced eotaxin production in a dose-dependent manner. At the concentration of 25 μM, compounds 1–3 reduced eotaxin production to 30.8, 28.5 and 25.5 pg/mL, respectively. References 1. Adinolfi, M.; Parilli, M.; Zhu, Y. Phytochemistry 1990, 29, 1696. 2. Kou, J.; Sun, Y.; Lin, Y.; Cheng, Z.; Zheng, W.; Yu, B.; Xu, Q. Biol. Pharm. Bull. 2005, 28, 1234.

P10A-013
Marthasterias glacialis: cytotoxic carotenoids metabolite profiling by HPLC-PAD-APCI-MS

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Nature constitutes one of the most important sources of bioactive compounds. Nowadays, the number of bioactive molecules from marine sources is rising, a trend that is thought to continue given the tremendous biodiversity of marine environment. As a result of the many challenges that these ecosystems present, including low temperatures, high pressures and low light availability, marine organisms respond by synthesizing a number of secondary metabolites, some of them with remarkable pharmacological properties. In this work, the echinoderm Marthasterias glacialis (spiny sea-star) was studied from a metabolomic point of view. Analysis using liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry (LC-MS/APCI) led to the finding of several carotenoids. After a deeper chemical insight, we evaluated its cytotoxic activity against the cancer cell line RBL-2H3 (rat basophilic leukemia), with an IC25 of 268 µg/ml being found. Adding to the interest of these carotenoids as cytotoxic agents, the same compounds revealed lower toxicity against healthy cells (V79 cell line, rat fibroblasts), thus being more specific for cancer cells. This work shows how marine organisms can constitute an interesting source of bioactive compounds, due to their diversified chemistry.

P10A-014
In vitro pharmacokinetic study of phenolic compounds from olive leaves in breast cancer cell lines

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In recent years, a rising interest has been focused on phenolic compounds from olive oil due to their antioxidant, antiinflammatory, antimicrobial, cardioprotective and anticancer activities. Olive leaves contain a high amount of phenolic compounds present in olive oil and therefore, they could be considered as an easily available and low cost natural source of these phytochemicals. We have demonstrated in a previous study the ability of an olive leaf extract to decrease breast cancer cell viability in SKBR3 and JIMT-1 cell lines . As a continuation of this study, a pharmacokinetic assay of the main compounds from this extract has been carried out. In order to perform this assay, culture media were spiked with 200 µg/ml of the olive leaf extract dissolved in DMSO and both tumoral cell lines were incubated independently. The control group consisted in culture media spiked with the extract but without cellular growth. Quantification of the main compounds was carried out by HPLC-ESI-TOF in culture medium at different incubation times (0, 15 min, 1 h, 2 h, 24 h and 48 h) in order to monitor their cellular absorption. Cytoplasms from lisated cells were analyzed too by HPLC/nanoLC-ESI-TOF to determine which compounds from the extract had entered through the cellular wall as well as the formed metabolites.

P10A-015
Bioactive fatty acids and cerebrosides from the TCM drug Arisaema sp.

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In this study active compounds from the TCM drug Arisaema sp. [1] were characterized by bioassay-guided isolation. Extracts and fractions of Arisaema sp. were tested for an agonistic activity towards peroxisome proliferator-activated receptor-α and -γ (PPAR) and for activation of the AMP-activated protein kinase (AMPK). These proteins are therapeutical targets in the treatment of metabolic disorders [2,3]. An apolar fraction strongly activated PPAR-α and -γ and had positive effects on AMPK activity in vitro. Among the main compounds were n-hexadecanoic acid, 9,12-octadecadienoic acid, 9-octadecenoic acid, octadecanoic acid, 13-phenyltridecanoic acid, pentadecanoic acid and 8-octadecenoic acid. Since cerebrosides from Arisaema with antihepatotoxic activity reported by Jung et al [4], were found to bind PPAR-α and -γ in silico, isolation and activity studies on these glycosphingolipids were continued. From a polar fraction, with moderate agonistic effect on PPAR-α and -γ in vitro, cerebrosides were isolated. Their structures were elucidated by NMR, ESI-MS-MS and matrix free LDI-TOF-MS-MS. In conclusion, in the present activity and analytical studies chemical constituents of Arisaema sp. that showed in vitro activity on important anti-diabetic targets were revealed.
Acknowledgements: Sino-Austria Project (Austrian Federal Ministry of Science and Research and Federal Ministry of Health, Women and Youth) and partly by the Austrian Science Fund [NFN S10704-B037] and the Austrian Federal Ministry for Science and Research [ACM-2009-01206]. References: 1. Bensky D. et al (2004) Chinese Herbal Medicine Materia Medica. Eastland Press. Seattle. 2. Kersten, S. et al (2000) Nature 405:421-424 3. Winder W.W. et al (1999) Am. J. Physiol. Endocrinol. Metab. 277:1-10 4. Jung, J.H. et al. (1996) J. Nat. Prod. 59:319-322

P10A-016
Combined NMR and LC-DAD-MS Analysis Revealed Comprehensive Metabonomic Variations for Three Phenotypic Cultivars of Salvia Miltiorrhiza Bunge

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Metabonomic analysis is an important molecular phenotyping method for understanding plant ecotypic variations and gene functions. Here, we systematically characterized the metabonomic variations associated with three *Salvia miltiorrhiza* Bunge (SMB) cultivars using the combined NMR and LC-DAD-MS detections in conjunction with multivariate data analysis. Our results indicated that NMR methods were effective to quantitatively detect the abundant plant metabolites including both the primary and secondary metabolites whereas the LC-DAD-MS methods were excellent for selectively detecting the secondary metabolites. We found that the SMB metabonome was dominated by 28 primary metabolites including sugars, amino acids and carboxylic acids and 4 polyphenolic secondary metabolites, amongst which N-acetylglutamate, asparate, fumurate and yunnaneic acid D were reported for the first time in this plant. We also found that three SMB cultivars growing at the same location had significant metabonomic differences in terms of metabolisms of carbohydrates, amino acids and choline, TCA cycle and the shikimate-mediated secondary metabolisms. We further found that the same SMB cultivar growing at different locations differed in their metabonome. These results provided important information on the ecotypic dependence of SMB metabonome on the growing environment and demonstrated that the combination of NMR and LC-MS methods was effective for plant metabonomic phenotype analysis.

PP2-001
Profiling cationic polar metabolites using SCX, SCX-HILIC-MS

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Liquid chromatography mass spectrometry (LC-MS) is a commonly used technique for metabolic profiling. For LC-MS there is a wide choice of stationary phases. To separate polar compounds, hydrophilic interaction liquid chromatography (HILIC) can be used. Here we present a profiling approach using an amine-based HILIC column in combination with a strong cation exchange (SCX) trap column. With this approach cationic polar compounds in plasma can be profiled. The compounds are trapped by Coulombic interactions. By switching the solvent composition the trap column retains the compounds under HILIC conditions after which gradient elution is performed. The sample preparation consists of the removal of proteins and (inorganic) cations, using SCX.

PP2-002
Challenges in Metabolomics addressed by targeted and untargeted UHR-Q-TOF analysis

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Myxobacteria are promising producers of natural products exhibiting potent biological activities, and several myxobacterial metabolites are currently under investigation as potential leads for novel drugs. However, the myxobacteria are also a striking example for the divergence between the genetic capacity for the production of secondary metabolites and the number of compounds that could be characterised to date. The number of identified metabolites is usually significantly lower than expected from genome sequence information. Here, we present an ESI-UHR-Q-TOF based analysis of myxobacterial secondary metabolites, which permits to solve several challenges frequently encountered in metabolite profiling studies. Wildtype and mutant strains are analyzed concerning the production patterns of known metabolites and with regard to the discovery of new metabolites. Sample throughput: Since mass accuracy and resolution of TOF instruments are independent of the acquisition rate, they are perfectly suited for a coupling to UHPLC separations. These hyphenations enable a reduction of analysis time in combination with a high chromatographic resolution and therefore permit an increased sample throughput. The UHR-TOF analysis revealed that an acquisition rate of up to 20Hz did not compromise the achieved mass accuracy or resolution. Targeted and untargeted metabolite profiling: Acquisition of full scan accurate mass spectra enable the targeted screening for known compounds e.g. from the class of DKxanthenes based on very selective high resolution EIC (hrEIC) traces with small mass windows of 1.0 - 0.5 mDa. A comparison of several datasets following a “comprehensive feature extraction” combined with a statistical analysis permits an untargeted discovery of novel biomarkers using the same data files as for the targeted analysis. Identification of unknowns: With increasing molecular mass of a compound, the number of possible molecular formulae increases exponentially. Even a mass accuracy of 0.1 ppm is not sufficient for an unambiguous formula identification for m/z values above 500. A combination of accurate mass data and isotopic pattern information in MS and MS/MS spectra can extend this m/z range for reliable formula suggestions. Examples for novel metabolites from Myxobacteria will be shown.

PP2-003
Structure Elucidation with an Ultra-High Resolving TOF Instrument by Alternating MS and Broad-Band CID Analyses

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Typical metabolite profiling applications are faced with complex samples – partly overlapping chromatographic peaks of co-eluting compounds resulting in many potentially relevant precursors to be isolated and fragmented to create a product ion spectrum. Alternating full-scan-MS and broad-band-CID or in-source-CID-MS is an alternative acquisition mode for generating fragment data of complex samples. A mixture of 6 pharmaceutical compounds with a MW from 350 up to 1450 g/Mol has been separated using fast liquid chromatography. First the clean mixture of compounds and second the compounds spiked into matrix were analysed. The UHPLC was combined with acquisition of alternating full scan precursor mass spectra and broad-band collision-induced fragment mass spectra (bbCID) using an ultra-high resolving quadrupole-Time-Of-Flight (Q-TOF) instrument with electrospray ionization (ESI). Broad-band fragmentation in the collision cell is achieved by setting a higher collision energy (e.g. 25 eV) without precursor isolation, whereas the precursor spectrum is taken at a low collision energy (e.g. 8 eV) with basically no fragmentation. A mathematical algorithm unequivocally assigns the observed product ion peaks to its precursor ion peak. The algorithm analyses the complete LC-MS dataset at once and produces a list of deconvoluted, chromatographic compounds, each with precursor and product spectrum annotated with a chemical formula. These compound spectra are fully background subtracted and deconvoluted from overlapping chromatographic peaks. Also, the isotope pattern of each ion species is fully preserved. The accurate mass and correct isotopic pattern information of parent and fragment ions is the key for clearly assigning the correct elemental composition to fragment and precursor ions enabling the correct identification of potential biomarkers.

PP2-004
Derivatization for lipidomics: analysis of free fatty acids, neutral and polar lipids by UHPLC-FTMS without prior fractionation

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Changing concentrations of the different lipid classes known in plasma and tissues are important markers of life style associated diseases. Lipid analysis is therefore often included in nutritional studies with genetically modified rodent models. In these types of studies the mice are sampled several times during the course of the study, with only tens of microliters of plasma or serum available for each time point. In such cases it is very important that the methods are very sensitive and use a very low volume (or amount of tissue) of sample and provide as much information as possible. With our method, lipids are extracted from very low volume of plasma (5 microliters), part of the extract is derivatised with a novel derivatisation method and analysed by UHPLC-Orbitrap. In ESI positive mode FFA, LPC, PC and SPM are detected, in APCI positive mode neutral lipids such as CER, MG, DG,TG, ChE are detected. This method combines quantitative analysis of free fatty acids with a comprehensive lipidomics approach. The introduction of a new polar group into the molecule greatly improves ionization in ESI+ and also separation to such extent that for unsaturated fatty acids the isomers with different position of the double bonds can be separated on reversed phase UHPLC column. The method enables high throughput. The derivatization method for the fatty acids was optimized and fully validated. The sensitivity of the method for free fatty acids is 10 pmol per ml, which is much more sensitive than conventional GC methods. As an example in human plasma, 28 FFA were detected, identified and quantified, 87 neutral lipids and 68 polar lipids were detected, and identified. The quality of the data is monitored in each study by using pooled study samples.

PP2-005
Analytical challenges in Zebrafish metabolomics.

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The zebrafish model organism is increasingly used for assessing drug and chemical toxicity and safety. Numerous studies confirm that mammalian and zebrafish toxicity profiles are strikingly similar. Zebrafish can be used to eliminate potentially unsafe compounds rapidly in the early stages of drug development and to prioritize compounds for further preclinical and clinical studies. In addition to toxicity testing, zebrafish models are used to study human diseases like cancer and cardiovascular -, muscular -, neurological- and metabolic diseases. Needless to say, the use of zebrafish as a model in different studies is a new analytical challenge. Very low amounts of sample are available (different tissues) and extremely low amounts of plasma. In these low sample amounts, determination of dosed chemicals or drugs needs to be performed as well as concentrations of endogenous metabolites, if mechanistic insight into the studies processes is required. For this reason, adaptation of conventional methods combined with new technology developments are required in order to expand use of zebrafish for chemical and drug screening or as a model for studying different disease states. As a preparation step for further studies, we tested our metabolomics platforms for suitability in zebrafish applications. The GC-MS method was downsized in order to be able to handle lower volumes of plasma or tissue extracts. Our LC-MS lipid platform and amino acid platforms showed to be sufficiently sensitive to observe lipid and amino acid profiles comparable to those obtained with larger sample quantities. Results of this assessment will be presented.

PP2-006
Retrospective quantification of endogenous metabolites in GC-MS metabolomics data.

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Analytical data obtained within metabolomics studies are often not quantitative and at the most semi quantitative which means that measured intensities in different samples are more or less comparable to each other, but absolute concentrations are unknown and can not be compared to the literature. While hundreds of metabolites are measured per sample, it becomes impossible to setup calibration lines for each metabolite even if the identities of all metabolites would be known. However, quantification is of the utmost importance to enable biological interpretation of assessed results by means of known reaction mechanisms. Recently, we described the possibilities on the analytical error reduction using a pooled study sample analysed regularly during the study [1]. The sample can be used to assess the performance of the method during long studies, but also as a single calibration point to remove i) offsets between batches or ii) instrumental drift. In this poster we will show an example of a third use of the pooled study sample, that is a single point calibration to obtain absolute concentrations of selected metabolites in a GC-MS study without the need of analyzing standards in each batch, only after the completion of the study.

[1] Analytical error reduction using single point calibration for accurate and precise metabolomic phenotyping, F.M. van der Kloet, I. Bobeldijk, E.R. Verheij, and R.H. Jellema Journal of proteome research 2009;8(11):5132-41.

PP2-007
Effect of Harvest Time and Extraction Procedure on Ingredients of Ginkgo Leaves – A Comprehensive Study Using UPLC/TOF MSE/Multi-Variant Statistical Analysis

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Traditional Chinese Medicine (TCM) is a medicinal system that utilizes naturally occurring resources such as plants and animals for treatment of diseases. Most TCM plants have to go through some specific processing procedures prior to their medicinal usage; hence the actual effective ingredients may be different from the freshly harvested plant extracts. In this work, we present a fast and generic approach using the UPLC/TOF MSE coupled with Multi-Variant Statistical Data Analysis to systematically profiling the ingredient changes between the fresh and processed samples. A bird\'s eye view of the major differences was easily obtained with most significant ingredient changes identified. We believe that this approach will ultimately facilitate the progress of TCM migrating into Modern Chinese Medicine (MCM).

PP2-008
In-situ pretreatment of urine with fluoroalkyl chloroformates as a novel GC-MS metabolite profiling strategy

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The chloroformate-mediated derivatization-extraction became mature and attractive method of sample preparation and compound derivatization during the last two decades [1,2]. Unlike silylation, which requires strictly anhydrous conditions and sample heating, protic functional groups of polar metabolites can be treated directly with an alkyl chloroformate in aqueous media and transferred into an immiscible organic layer in seconds. This approach has been examined in simple urine pretreatment with ethyl chloroformate (ECF) and subsequent metabolite profiling [3,4]. Here we report direct treatment of urine with novel fluoroalkyl chloroformates, heptafluoroalkyl chloroformate (HFBCF) and pentafluoroalkyl chlororoformate (PFPCF) synthesized in our laboratory [5,6]. The novel reactive reagents convert smoothly polar metabolites into more volatile species, less retentive in GC capillary columns; analogously to perfluorinated anhydrides established earlier in GC derivatization techniques. Moreover, the sample preparation protocol is simpler because there is no necessity to dilute urine with the analogous alcohol and a mere addition of organic phase with the reagent provides for both derivatization and extraction. Performance of the novel methodology is documented on metabolite profiling in urine of patients with different pathological stages. Financial support: Ministry of Health of the Czech Republic, NS9755-3/2008 and Grant Agency of the Czech Republic, P206/10/2401. [1] Husek P., Simek P., LC-GC North America 2001, 19: 986-999. [2] Husek P., Simek P. Current Pharmaceut Analysis 2006, 2(1): 23-43 [3] Husek P. Clin Chem 1997, 43(10): 1999-2001. [4] Hušek P., Simek P., Matucha P.: Chromatographia 2003, 58: 623-630. [5] Hušek P., Simek P., Hartvich P., Zahradnickova H.. J.Chromatogr. A 2008, 1186, 391–400. [6] Simek P., Husek P., Zahradnickova H. Anal Chem. 2008, 80: 5776–82

PP2-009
Biomarker and profiling strategies for the diagnosis of Tubercolosis using GC and GC X GC-Tof-MS

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There is an increased demand for fast detection and identification of bacteria causing diseases. Of particular interest to human health is the diagnosis of mycobacteria tuberculosis (M. Tuberculosis). The present methods, mainly X-ray and microscopy, suffer from important limitations because they are not specific and sensitive, but slow, labor intensive and/or expensive in terms of running costs. A promising approach is the use of GC hyphenated to thermochemolysis (THM). In previous work, an in-liner THM GC-MS method was developed for rapid diagnosis of M. Tuberculosis in sputum. This new in-liner THM-method was based on direct liquid injections of dispersed bacteria into the GC. Drying of the sample, addition of the reagent, incubation, and derivatization were performed inside the liner of a programmable temperature vaporizer (PTV) - injector. Because of the complex sample matrix, the obtained profiles were rather complex. Fortunately, specific biomarkers could be used for rapid and reliable identification of the mycobacteria. Although the use of biomarkers results in a fast and reliable method, finding the markers is difficult and time consuming. Moreover, the markers might not be 100% specific. Therefore, using complete compound profiles instead of a limited set of biomarkers is preferable. To improve the quality of the profiles more resolution is needed. This can be obtained with two dimensional GC preferably in combination with ToF mass spectrometric detection. In the present study, we studied the potentials of THM GC X GC-TOF-MS for profiling bacteria. A set of (cultured) bacteria samples was measured and evaluated by chemometric analysis of the THM-profiles. It was investigated whether the profiles obtained with two-dimensional GC resulted in more specific information for the identification of bacteria than one dimensional GC. Additionally, the presence of new, or recently proposed biomarkers was investigated.

PP2-010
Optimization of the ionization efficiency of anionic metabolites in capillary electrophoresis-electrospray ionization-mass spectrometry

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Capillary electrophoresis (CE) coupled with time-of-flight mass spectrometry (TOF-MS) using a sheath-liquid interface is a powerful technique for profiling of highly polar and charged metabolites. CE-MS of anionic metabolites is commonly carried out using electrospray ionization (ESI) and ammonium acetate as background electrolyte (BGE). However, with this BGE analyte responses in negative ionization mode are often relatively low, thereby limiting sensitivity in metabolomics applications. In this study, possibilities to improve the ionization efficiency of anions in CE-TOF-MS are investigated. Pressure-induced and voltage-induced infusion experiments have been performed to assess responses of anionic test metabolites using BGEs and sheath liquids of various compositions. BGEs and sheath liquids containing triethylamine showed optimum ionization efficiencies. BGEs with triethylamine also showed good separation for anionic test metabolites. With CE-MS, signal intensities were significantly higher in 10 mM triethylamine (pH 11.7) compared to 25 mM ammonium acetate (pH 9) and total analysis times were decreased. Results of the use of triethylamine BGEs for the analysis of anionic metabolites in urine will be shown. Comparisons will be made with the currently used CE-MS systems for metabolic profiling in negative ionization mode.

PP2-011
Electrochemistry(LC)/MS a Powerful Analytical Technique to Simulate Oxidative Metabolism Processes

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Traditional methods to investigate oxidative drug metabolism are based on in-vitro (e.g. microsomes) or in-vivo (urine, plasma, etc.) methods, which are time-consuming. Therefore, mimicking of oxidative metabolic processes using electrochemistry is of great interest as a fast screening tool. Recently, the use of EC(LC)/MS has been extended towards new applications such as: fast synthesis of metabolites, rapid risk assessments of drug-protein binding, signal enhancement in MS, and oxidative damage of DNA. In this presentation we will show the application of on-line EC/ESI-MS as a powerful tool to simulate various oxidative processes in life sciences. A specially designed μ-preparative electrochemical flow cell will be presented. The cell allows the generation of sufficient amounts of metabolites, which can be used as reference material (e.g. NMR). Moreover, EC/MS approach can be used to collect the oxidative metabolism profiles of the compound of interest in a very short time. Oxidative metabolism of the different drugs mimicked in the electrochemical cell will be shown (e.g. amiodarone, amodiaquine, boscalid). Electrochemistry can be applied to study the oxidative damage of DNA. On-line EC/ESI-MS is a novel tool to study oxidative processes of nucleic acids, as well as to create covalent drug adducts with nucleic acids. Furthermore, we will demonstrate a novel and flexible EC/LC/MS approach, based on the integration of an amperometric thin layer cell into the autosampler flow path of an EC/LC system, which allows fully automated oxidation, conjugation (i.e., adduct formation), separation and MS analysis of multiple samples. The principle of operation is based on the modified flow path and flexible user-defined injection programs. All these applications illustrate the tremendous power and broad applicability of electrochemistry as a tool to mimic nature's Redox reactions, including oxidative damage of DNA, protein stress, lipid oxidation, etc.

PP2-012
Automated Electrochemistry/LC/MS system for oxidative metabolism studies

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Oxidation reactions occurring in drug metabolism are regulated by enzymes (e.g. cytochrome P450). Traditional research involves time-consuming in-vitro or in-vivo methods. Therefore, mimicking of oxidative metabolic processes using electrochemistry is of great interest as a fast screening tool. In this poster a novel electrochemistry (EC)/LC system (upfront MS) is presented for automated screening of samples (drugs, xenobiotics) in oxidative metabolism studies. Current EC/LC/MS approaches used in oxidative metabolism studies are either based on the generation of metabolites on-line using an electrochemical cell integrated in the LC flow path (pre or post-column) or off-line with an EC cell connected to a sampling valve. We demonstrate a novel and flexible EC/LC/MS approach, based on the integration of an amperometric thin layer cell (reactor cell) into the autosampler flow path of an EC/LC system, which allows fully automated oxidation, conjugation (i.e., adduct formation), separation and MS analysis of multiple samples. The principle of operation is based on the modified flow path and flexible user-defined injection programs. The system performance was evaluated for the model drug compound acetaminophen (phase I) and its conjugation with glutathione (phase II). The EC/LC platform shows good reproducibility (conversion, retention times and peak area) and no carry over. Control experiment conducted with the EC cell with no potential applied, confirmed that conjugation reactions occurs only when acetaminophen is oxidized into its reactive NAPQI metabolite. The conjugation product was additionally identified by means of high resolution MS in combination with MS/MS fragmentation. With this new concept it is possible to screen automatically for Phase I and II metabolites of multiple samples.

PP2-013
A strategy for the quantitative analysis of all lipids in complex samples with a single acquisition method

Volker Kruft, AB SCIEX

The fast identification and quantification of lipid biomarkers without extensive fractionation or method development would be an advantage for laboratories worldwide. So far, the comprehensive study of lipids is done by accurate mass measurements for lipid identification or the use of precursor ion sans for the analysis of different functional groups. We describe novel workflow for the identification and quantification of hundreds of lipids in total lipid extracts on the AB SCIEX TripleTOF™ 5600 system in a single, fast acquisition. Direct nano-electrospray infusion of less than 10 µl of lipid extract enabled the acquisition of a complete quantitative and qualitative dataset: After a high-resolution survey scan, MS/MS data are acquired for each mass between 200 and 1000 m/z. The resulting 3-dimensional datafile comprises dimensions for selection mass, fragment ion masses, and intensity. We will show the identification of more than 800 lipid species from 6 classes and 15-subclasses in crude rat brain lipid extract in a single polarity acquisition. The combined datasets from positive and negative mode provide a complete lipid profile that can be queried by specific parent or signature ions. Relative quantification of lipid species against their lipid class, or absolute quantification with the use of specific internal standards, can be achieved with a comprehensive dataset acquired in less than 10 minutes.

PP2-014
Metabolic Profiling Workshop

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From the post genomics and proteomics era, metabolic profiling (metabolomics/ metabonomics) has emerged as a vital new area of research. Metabolic profiles of biological fluids contain a vast array of endogenous low-molecular weight metabolites, the composition of which depends upon the sample type (plasma urine, bile etc) and factors such as the species, age, sex, diet of the organism from which the sample derives and indeed even the time of day at which the sample was taken. Disease, drugs (and other biologically active molecules) perturb concentrations and fluxes in intermediary metabolic pathways. The response to this perturbation involves adjustment of intracellular and extracellular environments in order to maintain homeostasis. Both the perturbations and the adjustments are expressed as changes in the normal composition of the biofluids or tissues that can be characteristic of the nature or site of the disease process, toxic insult, pharmacological response or genetic modification. This presentation guides the auditorium through an efficient and robust work-flow for profiling endogenous metabolites. Following an introduction to the principle work-flow, a set of data will be mined using a variety of multivariate statistical methods, facilitated by MarkerLynx XS software.

PP2-015
Sum Formula Calculation and Identification of a Bacterial Metabolite with m/z >1100

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In the course of identification and structural characterization of compounds the unambiguous sum formula calculation is an essential step. With increasing molecular mass the number of possible sum formulae is increasing exponentially. In order to significantly extend the mass range for reliable sum formula generation the mass accuracy information can be combined with additional information such as the isotope pattern in MS and MS/MS. In order to show the efficiency of this approach, a bacterial metabolite was analyzed using a reversed phase gradient separation on an UHPLC system interfaced to either an ESI-TOF-MS (HR-TOF) or an ultrahigh-resolution ESI-TOF-MS (UHR-TOF) system. Full scan and MS/MS data were acquired in ESI positive mode (scan range m/z 50-1300). Sum formula suggestions were calculated and the results combining different levels of information were compared. On the HR-TOF system, full scan mass spectra gave an exact mass value of m/z 1170.7112 for the bacterial metabolite. The observed ion was identified as ammonium adduct. Allowing only C, H, N, O as elements 30 sum formula suggestions were calculated in a 5 ppm mass accuracy window. If additional elements were allowed, the number of theoretical suggestions increased rapidly, even if additional filters were applied. The results were rated according to the matching of experimental and theoretical isotope patterns, so that the number of meaningful suggestions was reduced to 12. The sum formula suggestions were verified by combining full scan MS and MS/MS data, taking also the fragment\'s accurate mass and isotope pattern information into account. This reduced the sum formula candidate list to 6, of which only one could be found in online available databases. This query suggested the bacterial metabolite to be cereulide. A similar analysis using the UHR-TOF-MS system showed the benefit of enhanced mass accuracy, since this approach only left one sum formula in addition to the cereulide formula for combined mass, isotope and fragment information.

PP2-016
Identification of phenolic compounds using a database of experimental and predicted 1H-NMR spectra

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Polyphenolic compounds are abundantly found in nature and are very diverse in structure due to a very large number of possible substitution patterns. Therefore, the identification of these compounds cannot be done on the sole basis of MS data. The identification process can be accelerated when MS and high quality 1H-NMR data are combined. Although 1H-NMR spectra are relatively easy to obtain, the existing databases contain only a limited number of polyphenolics. Here we present a database of experimental and predicted 1H-NMR spectra of polyphenolics based on high quality NMR data. The predicted proton spectra were generated from 3D structures using the PERCH NMR Software trained on a large set of experimental spectra. By incorporating the stereochemistry, intra-molecular interactions, and solvent effects into the mathematical model, 1H chemical shifts and 1H-1H couplings were predicted with great accuracy. The predictive model was used to extend the database with 1H-NMR spectra of about 3000 polyphenolics available from public resources. The 3D structures were generated using fragments with the correct stereochemistry, which is important for compounds containing sugar moieties. The 1H-NMR spectra were automatically annotated confirming the atom labels accepted in the literature. The spectrum querying was done in combination with the mass using a list of chemical shifts. When the correct compound is present in the database it is easily distinguished from false positives. For example, there are 15 compounds with chemical formula of $C_{15}H_{10}O_7$. When querying the list of experimental chemical shifts of quercetin against the database, only one hit gave a good match. The 3D structure of quercetin was confirmed by an automated fit of the experimental and theoretical 1H-NMR data carried out using the PERCH NMR Software. The remaining 14 hits failed to produce a satisfactory fit. The 3D Mol files and the predictions in a binary format are available for download.

PP2-017

MALDI-MS-based Metabolite Analysis for High-throughput Metabolic Dynamics and *in situ* Metabolomics Imaging

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Metabolomic studies can lead to the enhanced understanding of disease mechanisms and the discovery of new diagnostic biomarkers as well as the enhanced understanding of mechanisms for drug or xenobiotic effects and the increased ability to predict individual variation in drug response phenotypes. Thus, this rapidly developing discipline has important potential implications for the pharmaceutical research field. To date, mass spectrometry (MS) coupled with pre-separation techniques such as liquid chromatography (LC-MS) or gas chromatography (GC-MS) has been known to be a conventional strategy for metabolomics, but these methods have several disadvantages for pharmaceutical metabolomics. First, sample preparation of such methods includes complicated steps including sample extraction, derivatization, desalting, and/or concentration before further MS analysis. Second, a large sample volume is required because of the lower sensitivity. Third, total LC analysis time is still on the order of several minutes. In contrast to the methods mentioned above, direct MS analysis, especially matrix-assisted laser desorption ionization (MALDI)-MS has advantages for metabolite analysis because it is a highly sensitive, high-throughput, and low sample-consuming (~ 1 µL) technique compared with other conventional analytical platforms based on LC- or GC-MS. In addition, MALDI has recently been reported to be suitable not only for high-molecular-weight polymers or peptides, but for low-molecular-weight metabolite analysis. In addition to above-mentioned advantages, MALDI system, mass spectrometry imaging, enables us to analyze tissue sample directly. In the present study, a high-throughput and non-targeted metabolomic technique using MALDI-MS was developed for the rapid analysis of cellular metabolites. Furthermore, this technology was applied to tissue metabolite imaging. Our developed technique showed ultra-high sensitive (single cell level detection limit) and high-throughput (within 1 minute per sample) performances, suggesting that this high-throughput MALDI-MS-based metabolomic technique can be utilized for drug screening, validation of drug efficacy and safety, and will facilitate biological discovery in both preclinical and clinical settings.

PP2-018

Comparative metabolomics by direct infusion isotope-coded metabolite profiling (DICMET) using fourier transform ion cyclotron mass spectrometry.

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Direct infusion fourier transform ion cyclotron mass spectrometry has been put forward as a high throughput metabolic fingerprinting method yet allowing to gain information from individual metabolites based on their accurate mass. However, neglecting the presence of multiple isomers, the quantification of these accurate masses is biased due to matrix effects that are presumed to occur in the condensed phase rather than in the gas phase during the electrospray ionization of the metabolites. Therefore it was suggested that matrix effects would be less problematic using nano-electrospray ionization in which droplets are expected to completely evaporate. Using standards mixtures, we investigated to what extent matrix effects are still occurring in the condensed as well as in the gas phase during nano-electrospray, and to what extent these are removed by derivatizing with a permanently charged moiety. By simultaneously infusing two differentially isotope-labeled sample mixtures, we show that the variation due to matrix effects can be controlled by considering the ratio between both isotopes of the derivatized standards. Finally, when comparing candidate metabolites in mannitol-stressed and control Arabidopsis seedlings, results of this direct infusion isotope-coded metabolite profiling method were in agreement with those obtained by GC- and LC-MS.

PP2-019

Metabolite discovery through trend detection in multi-dose kinetic studies

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Metabolic profiling is rapidly gaining importance in pharmaceutical and nutritional intervention studies. Chromatography in combination with mass spectrometry is an excellent tool to analyse sample sets from such studies as it provides a fingerprint of the whole sample as well as specific information on target compounds. Therefore, untargeted biomarker discovery studies can be performed simultaneously with targeted kinetic studies, i.e. metabolomics studies in which the time response of specific metabolites is monitored after an intervention. Clearly, complex data sets containing a wealth of information are obtained. This complexity and information-richness may be increased further when the intervention includes several doses of the compound(s) of interest. Obviously, only the combination of advanced analytical techniques and sophisticated data analytical tools will allow the extraction of useful information from the huge quantity of data in a timely manner. In this work, we present a new strategy to discover potential biomarkers in kinetic dose-response studies that are usually only used for target-compound analysis. The strategy makes use of the fact that an interesting metabolite will exhibit certain behaviour versus sampling time following the intervention. Additionally, an interesting metabolite will respond similarly for all doses applied. This type of information can be used to distinguish the more relevant metabolites from those not following the expected trends. The method is based on principal component analysis that is applied locally, i.e. on one metabolite at a time. Thus, not the correlation between metabolites is of interest, but each metabolite is investigated independently, allowing varying trends between them. With this method, the common extensive manual evaluation of the data sets can be simplified and reduced to only investigate pre-defined interesting compounds. The new strategy will be demonstrated on a sample set obtained from a polyphenols gut fermentation study with the response studied versus time at different levels.

PP2-020

Micro/nanofluidic preconcentrator for single cell metabolomics

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The development of tools for single-cell analysis is receiving increasing attention. The analysis of low-abundant analytes in ultrasmall volumes of complex biological samples is an important challenge in today's science and technology. The goal of our research is to develop a micro/nanofluidic platform for the analysis of metabolites in samples from single cells. This platform will couple electrokinetic techniques for cell positioning, sampling, preconcentration, separation and interfacing with mass spectrometry. It has been reported that a micro/nanofluidic preconcentrator can be used to achieve more than a million-fold preconcentration of ionic species (1). In our lab, such a preconcentrator is being characterized to obtain optimal preconcentration regimes for biological metabolite samples. We aim to integrate the preconcentrator in the micro/nanofluidic platform for single-cell analysis. 1) Wang et al, Million-fold preconcentration of proteins and peptides by nanofluidic filter, Anal Chem. 2005; 77 (14): 4293-4299

PP2-021

Technology Spotlight: Latest Developments in High Definition Mass Spectrometry (HDMS), Demonstrating the Powerful Analytical Capabilities of Ion Mobility and High Resolution Mass Spectrometry

John Rontree Waters Corporation, Atlas Park, Wythenshawe, Manchester M22 5PP

This presentation begins by providing an introduction to a novel folded geometry high res ToF coupled to a novel high dynamic range ADC based detection system (combining the benefits of TDC and averaging ADC detection systems) which provides un-compromized levels of performance (resolution >40,000FWHM, dynamic range <105, mass measurement accuracy <1ppm and precision) for the characterisation of the most analytically challenging samples at the fast acquisition rates (20 spectra/sec) demanded by Ultra Performance LC or Atmospheric Pressure GC. One of the more recent developments in ion mobility technology has been the travelling wave-based approach used in a commercially available hybrid quadrupole/ion mobility/oa-ToF MS instrument. Whilst this technology has provided greater access to the potential of ion mobility spectrometry in combination with mass spectrometry for many analysts, the mobility resolution afforded by the travelling wave separator is relatively low in comparison with current, albeit significantly larger, instruments in some research laboratories. What is presented here is a novel approach towards increasing the mobility resolving power (<4 times) of the travelling wave device together with a description of an enhanced detection system for acquiring mobility data. The novel ADC-based detection system has been developed which can process data on the mobility timeframe and provides exact mass accuracy and an increase in dynamic range. These enhancements, combined with novel software tools enable the separation of small molecule isomers, facile generation of collision cross section (CCS) information for conformational studies, comprehensive separation and peak detection of complex mixtures, and enhanced routine structural characterization of compounds with the use of exactly mass measured first and second generation fragment ions. In summary, the new Mass Spectrometry research system described here provides significantly enhanced, accessible performance levels and new research capabilities not possible with other MS technologies or analytical platforms. This will be demonstrated with examples from the analysis of complex mixtures and compounds encountered in proteomics, metabolite profiling and biomarker discovery applications.

PP2-022

Optimal measurement designs: a neglected topic in metabolomics studies

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It is a matter of fact that the majority of metabolomics papers do not make any reference to the measurement design (MD) underlying the generation of the data analyzed. We see this as a major concern because a misconceived measurement design can lead to biased and misleading experimental results. Using the DiOGenes study as a benchmark to devise an optimal MD for large-scale metabolomics studies, we here define a three step operation pipeline. Usually, a metabolomics study stems from the interaction among biologists and/or physicians, experimentalists and data analysts, the former phrasing the problem being studied as a series of biological questions. The first step is then to translate these questions, which are encoded in the study design, in terms of sources of variation by identifying the factors which can affect the results of the experiments. The MD must be realized in such a way that the effects of uncontrolled factors are minimized. An MD is then a trade-off between the study design which is usually given, the biological information one needs to retrieve and the experimental constraints imposed by the measurement technique chosen. Once all the factors and experimental constraints have been identified, the second step is to frame the MD under one of the many theoretical MD's available. Due to its special design and to experimental limitations (namely, a limited number of measurement batches (<30) and of samples per batch (<100)), DiOGenes could not be framed under an Incomplete Block Design. We then developed an optimal MD tailored for these constraints. The third step deals with the operative realization of the measurement scheme. For DiOGenes, having > E+245 possible sample dispositions across the batches, a computer assisted procedure had to be devised to select the optimal samples placement. These results are important in all fields of large-scale functional genomics analysis.

PP2-023

A Q-TOF generated LC-MS/MS Library Facilitates Compound Identification of Non-Targeted Metabolomics Data

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The determination of compound identities is a crucial analytical problem for metabolomics scientists using LC/MS detection. Without compound identities it is impossible to make biological sense of study results. Many compounds observed to be differentially expressed in metabolomics studies are known primary metabolites. Easily identifying these known metabolites would facilitate metabolomics analysis. One accepted way to identify compounds is to compare the observed MS/MS spectra of the unknown to a library of MS/MS spectra of metabolite standards. Using a Q-TOF LC/MS system, we have constructed an accurate mass MS/MS spectral library of common metabolites using three different collision energies. In this presentation we show an un-targeted metabolomics workflow and how the utility of a metabolite LC/MS/MS spectral library was used for compound identification. We will present data demonstrating the library's utility by analyzing MS/MS spectra of metabolites in urine sample extracts.

PP2-024

AB SCIEX TripleTOF™ 5600 System; High Performance for Qualitative and Quantitative LC/MS/MS

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The AB SCIEX TripleTOF™ 5600 System is the first accurate-mass, high-resolution LC/MS/MS system for qualitative analysis, with the speed and sensitivity to deliver high-performance triple quad-like quantitation. The system enables enhanced workflows for comprehensive exploring, profiling, and quantifying of low abundance analytes in very complex matrices. The combination of high mass accuracy and resolution with high sensitivity allows very high acquisition speeds without compromising performance. The system enables advanced workflows that allow conclusive identification and quantification of low abundance compounds in complex samples, all in a single run, making it the ideal platform for transitioning from qualitative workflows such as ID, confirmation or screening to early quantitation experiments.

PP2-025

Re-optimization of the SIM-stitching method for routine use of direct infusion mass spectrometry in a metabolomics facility

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The use of high-resolution mass spectrometers and direct infusion MS (shot-gun metabolomics) is an important approach for the analysis of large metabolomics sample sets. Data acquisition in selected ion monitoring (SIM) windows followed by application of the "SIM stitching" algorithm [1,2] reduces ion suppression and significantly increases sensitivity in mass spectrometry driven metabolomics. The previously published method was developed and utilised for high-throughput analyses on a Thermo LTQ-FT mass spectrometer, using a mass resolution of 100,000. Following the upgrade of this instrument to a LTQ-FT Ultra, which comprises a larger ion cyclotron resonance detector cell with greater ion capacity, re-optimization of the SIM-stitching method was required. In particular the following parameters were re-optimised: the automatic gain control (AGC) value, the m/z range of the SIM windows, and the acquisition time (i.e. number of scans) per window. Our re-optimised methods on the LTQ FT Ultra (in both the positive and the negative ion mode) allows measurements of the same sensitivity and mass accuracy (typically < 1 ppm before internal calibration) to be performed in half the time (3 min per analysis), enabling a higher sample throughput of a whole 384-well plate in one day. We also observed fewer "failed" samples, due to the shorter time that stable spray is required, while the relative standard deviation values (as a measure of spectral repeatability) are similar to those for the original LTQ FT method [3]. The re-optimised methodology comprises of 7 SIM windows of each 100 Da, and overlapping by 15 Da, covering the range of m/z 70-590. It has been tested on several biological datasets in our NERC Biomolecular Analysis Facility (NBAF) Metabolomics facility at Birmingham, including for newly introduced QC standards. (1) A.D. Southam et al. (2007). Anal. Chem. 79, 4595-4602. (2) T.G. Payne et al. (2009), J. Am. Soc. Mass Spectrom. 20, 1087–1095. (3) H.M. Parsons et al. (2009). Analyst 134, 478–485.

PP2-026

Spatial metabolomics of wheat seeds using ToF SIMS imaging

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Spatial metabolomics aims to capture metabolic changes with respect to the spatial distribution of metabolites, ideally in situ (i.e. where it occurs in the cellular context). A capability to image and map the spatial distribution of metabolites and the associated metabolic changes enables a powerful tool to monitor and understand several biological processes at the molecular level. Imaging mass spectrometry offers the potential to screen for spatial metabolic distribution. We are developing the application of time-of-flight secondary ion mass spectrometry (ToF-SIMS) as an imaging tool for spatial metabolomics. ToF-SIMS is a surface technique that uses pulsed primary ions to desorb and ionise molecules from a sample surface so the emitted secondary ions can be analysed (using a ToF mass spectrometer). The advent of polyatomic primary ions promises 3D spatial biochemical imaging of cell surfaces, sub-surfaces and tissue sections, at submicron resolutions. In this investigation we have applied the technique to map spatial metabolic changes in developing wheat seeds. Cryosectioned seeds from different stages of development were examined using bismuth cluster ions as the primary ion source. Metabolic changes associated with different stages of development could be discerned and these will be discussed.

PP2-027

SYSTEMATIC METABOLITE IDENTIFICATION USING HPLC-MSn FRAGMENTATION TREES AND LC-MS-SPE-NMR

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The exact, unbiased and complete analysis of the metabolite content of biological extracts becomes increasingly important. The wide spectrum of structurally diverse metabolites asks for the use of state-of-the-art analytical technologies, which enable rapid annotation of both known and unknown metabolites. New developments in both mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, aimed towards systematic metabolite identification, will be presented. Firstly, a reproducible high resolution MS\n spectral tree method that systematically fragments metabolites, using a NanoMate (Advion) – Ion Trap-Orbitrap FT-MS (Thermo) device, has been developed. The NanoMate robot facilitates small volume sample injection combined with chip-based nano-electrospray ionization, whereas the Ion Trap – Orbitrap FT-MS combination ensures robust MS\n fragmentation with accurate mass determination. Using this approach, we obtained structure-specific fragmentation trees for a large series of phenolic compounds, including positional isomers and stereoisomers which so far were difficult to elucidate using MS. For instance, glucose and galactose moieties attached to the same carbon position on a phenolic core could be reproducibly discriminated. Examples of this MS\n spectral tree approach as a potent tool in the identification of metabolites in biological extracts will be shown. Secondly, a rapid identification strategy for yet completely unknown compounds, based on a LC-MS-solid phase extraction (SPE)-NMR platform (Bruker), will be presented. With this platform, compounds separated by LC are on-line trapped on SPE cartridges, triggered by the TOF-MS signal, after which the compounds are transferred to the cryogenic-NMR-detection probe (600 MHz). In this manner, NMR spectra of lower abundant metabolites in a small sample volume can be generated. Examples of using this LC-MS-SPE-NMR platform in the unambiguous identification of novel metabolites in crude sample extracts, such as tomato fruit, will be provided. The examples presented will show that MS\n fragmentation trees and LC-MS-SPE-NMR are powerful tools in the systematic identification of compounds in metabolomics approaches.

PP2-028

Two pH optimized LC-MS methods for metabolomics analysis of hydrophilic compounds on silica hydride stationary phase

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The determination of hydrophilic metabolites is a crucial analytical problem for metabolomics scientists, where coverage needs to be both comprehensive and broad. Silica hydride columns have demonstrated good retention reproducibility, ruggedness and metabolite coverage. Examples of classes that can be separated in complex biological matrixes using the aqueous normal phase (ANP) technique on a silica hydride surface are sugars (neutral), amino acids (basic) and organic acids (acidic). The retention mechanism of acidic and basic compounds on the silica hydride column is highly pH dependent, whereas neutrals are not. Therefore, two general purpose ANP chromatographic methods were developed to achieve retention and separation of (1) neutral and basic compounds by (+) ESI-MS, and (2) neutral and acidic compounds by (-) ESI-MS We discovered that ammonia permanently alters the physio-chemical properties of the silica hydride surface which results in shorter RTs for basic molecules; one Diamond Hydride (150mm×2.1mm) column was dedicated for each method. High acetonitrile to water gradients were used for both methods. Method (1) used a buffered ammonium acetate mobile phase, at neutral pH; with a pH gradient generated by the addition of increasing formic acid. Method (2) used a simple pH system with a constant amount of formic acid. The total analysis time for each method was approximately 20 minutes. We changed the strong solvent to include 50% methanol to improve solubility of metabolite extracts from different biological matrices containing both polar and hydrophobic molecules such as phospholipids. Organic acids and nucleotide phosphates presented major development challenges for method 1 optimization. To achieve good retention of organic acids, mobile phase must be above pH 5. Gradual column degradation occurs above pH7. For citric acid , these pH conditions lead to poor peak shape. Development of a pH gradient was resolved this issue. Surprisingly, initial attempts to chromatograph ATP were unsuccessful due to excessive tailing but it was dramatically less for AMP. By switching from glass solvent bottles to HDPE we eliminated trace sodium leaching, which resulted in dramatic improvement of ATP peak shape. The robustness of these methods was subsequently tested in extracts of plasma, urine and Yeast.

PP2-029

MALDI-MS-based Analysis of Intracellular Metabolic Dynamics in Human Acute Lymphoblastic Leukemia Cells under Drug Treatments

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Herein we demonstrate a high-throughput analysis on metabolic dynamics of human acute lymphoblastic leukemia Jurkat cells against drug treatment based on matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS). The development of MALDI-MS-based method has received considerable attention as high-throughput techniques for metabolic profiling on mammalian cells or for metabolic dynamics analysis on bacteria [1, 2]. This technique enables a highly sensitive, quantitative and nontargeted metabolomic analysis with minimal operations for sample preparation, which contributes effectively toward minimizing errors observed during a series of experimental processes. Applications of the method include a large-scaled data acquisition of metabolite level fluctuations consequent upon an exogenous perturbation. In the present study, Jurkat cells collected from the culture medium were mixed with the matrix and dropped onto a stainless MALDI sample plate by 2,500 cells/well. As the result, approximately 100 metabolite peaks were detected from a single analysis completed within two minutes. To investigate influences on the metabolism of Jurkat cells against the perturbation caused by anticancer drug, the cells were continually collected and subjected to MALDI-MS analysis to illustrate time-dependent transitions of intracellular metabolite levels. Time-courses of the metabolism indicated behaviors characteristic to the dosed drug within three hours. Studies on such metabolic responses against drug treatment in early stage can be facilitated by the high-throughput method developed here. Insights into the metabolic dynamics responding to perturbations provide a novel aspect for the mechanism of drug effectiveness. [1] Miura, D.; Fujimura, Y.; Tachibana, H.; Wariishi, H. Anal. Chem. 2010, 82, 498–504. [2] Yukihira, D.; Miura, D.; Saito, K.; Takahashi, K.; Wariishi, H. Anal. Chem. 2010, 82, 4278–4282.

PP3-001
Computational Analysis of Halotolerance Genes from Halophilic Prokaryotes to Infer their Signature Sequences

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Halophiles represent organisms which live, grow, and multiply in highly saline environments. It has been found that many proteins and enzymes do not withstand industrial reaction conditions. Thus, the characterization of gene\/enzymes from the microorganisms that are able to thrive in hypresaline environments can be valuable information to infer what makes halotolerance proteins to work under halophilic environments. Computational analysis of eukaryotic and prokaryotic organisms resulted in identification of forty two genes involved in halotolerance. Comparative gene structure and protein properties were studied in extreme halophiles (EH), moderate halophiles (MH), mesophiles (MS) and non-halophiles (NH). Forty two genes involved in halotolerance were identified in 18 completely sequenced archaea and bacteria using blastP. Homologs of genes EctB, GbuA, URA5 and CysK were found in all the selected archaeal and bacterial halophiles, whereas genes EctA, EctC, Hal4, Hal5, Trk1, CcpA, Gpd1, Gsk1, GroES and Hkt1 were found only in bacterial halophiles, which have been reported to be involved in ectoine synthesis, halotolerance and protein translation. Gene eIF-1A, involved in translation, was present only in archaeal halophiles. The most predominant family of proteins involved in halotolerance among halophiles was of various types of transporters. The halotolerance proteins of EH were found to be more acidic in comparison to proteins of MH, MS and NH. The frequency of acidic AA (amino acid) Asp was higher in EH, whereas, in case of MH and MS the frequency was lesser than that of NH. The frequency of Glu was found to be greatest in MH\'s. The frequency of basic AA lysine in EH\'s was much lower than the other three groups. There was an increase in frequency of Pro and Gly in case of EH. There was a marked reduction in the frequency of basic AA\'s at the protein surface. There was an enormous increase of non-polar AA's at the protein surface of EH\'s . The occurrence of non-polar AA\'s at the surface of EH's was one of the distinctive observation.

PP3-002
Development and application of an integrated software package for high-throughput FTMS-based metabolomics

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Introduction The state-of-the-art ultrahigh-resolution FTICR mass spectrometry enables simultaneous detection of thousands of compounds in a complex sample without sacrificing ultimate mass accuracy, providing a robust platform for high-throughput metabolomics via metabolic fingerprinting or footprinting. The large volume of information-rich FTMS datasets necessitates software tools for equally high-throughput data processing and analysis. We herein developed a new integrated software package for streamlined processing of chromatography-free and chromatography-coupled FTMS metabolomic datasets. Methods Metabolites were extracted from different biological samples and analyzed via direct infusion (DI)-, MALDI- or LC-MS on a Bruker 12T FTICR instrument. Two VBA scripts (1D- and 2D-MIPP), have been written for use within the vender's processing software to automate batched data extraction. Two additional programs (1D- and 2D-PAMD) were developed within National Instruments LabVIEW for mining and aligning the peak lists extracted from individual MS and LC-MS profiles, respectively. Another program (GMF & DB Search) was further written for automatically identifying the known metabolites, typically within 2 ppm, by searching the metabolome databases (METLIN, KNApSAcK and/or LIPID MAPS) using the measured accurate masses and/or the molecular formula generated from statistical analyses of the isotope intensity profiles. Results The MIPP scripts incorporated automatic post-acquisition internal mass calibration, mass exclusion, metabolite feature detection (via mono-isotopic peak picking) and charge state deconvolution. The PAMD programs provided multiple options for sample normalization, adduct ion discrimination, correlation analysis, and biological/technical replicate alignments. The resultant metabolite features across all the samples, in a format of either m/z's vs. peak intensities or retention time_m/z pairs vs. peak responses, were saved for subsequent statistical analysis and hierarchical clustering. GMF & DB Search enables real-time update of the metabolome databases, and, for example, >350 metabolites in mouse liver can be assigned from 'one-click' search of the HMDB database. Application of this software package to processing of several datasets from DI- or MALDI-MS of body fluids, tissues and plants, and from LC-MS of a set of mimic-spiked urine samples has been achieved with minimal manual intervention. Typically, more than 2,000 metabolite features were dissected and measured from each sample type. The details about these results will be presented.

PP3-003
Let's visualize personalized health

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Good health begins with good nutrition, and good nutrition starts with a balanced diet that provides necessary levels of essential nutrients. For micronutrients Dietary Reference Intakes are defined, which are based on average population needs in a particular life stage and gender group. However, the dietary requirements depend on many factors such as genotype, lifestyle, stress, disease state etc. Therefore, we should find a way to define personalized needs. We have developed a visualization method, called the 'health space' method that separates subjects according to the underlying biological processes. In this method the measured nutrigenomics parameters are grouped in the three biological processes: the processes chosen will depend on you research question. A PLS-DA model is build for each of these processes. This model is scaled between 0 (the healthy/treated group) and 1 (the unhealthy/untreated group). A 3-dimensional space is built on the combination of processes with on every axis one of the three processes. In this health space every person will have his own score that shows to what extend the treatment or disease is affecting the related processes. This information can be used for further treatment strategies. We have tested this 'health space' concept on a recently published study (Bakker et al, 2010). In this study non-diseased subjects are treated with an anti-inflammatory dietary mix containing n-3 fatty acids, EGCG, Vitamin-E, Vitamin-C, resveratrol and tomato-extract. The plasma concentrations of proteins and metabolites before and after a five week treatment were analyzed. Central processes that are regulated by this dietary intervention are stress in oxidation, inflammation, and metabolism. Therefore, these processes were at the axes of the health space in this example. The treated and untreated groups were clearly separated in space. In the earlier paper it has been shown that the health status of these people improves. Some people mainly modulate their metabolic stress profile, while others show a specific inflammatory or oxidative response to the anti-inflammatory dietary mix. We show that different response subgroups can be distinguished and may be treated accordingly.

PP3-004
Detailed study capturing: Give your data more impact!

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The challenge of modern biology is studying systems in a holistic way to find properties and functions that emerge for the interaction of individual components (systems biology). This research profits enormously from the revolution in 'omics' technologies and requires many analytical and bioinformatics methods. In addition to measurements of the parameters of interest, extensive description of the study, subjects and environment is central for describing the phenotype and answering systems related questions. We are developing a systems biology study evaluation platform that facilitates both multi-omics research and collaborations between researchers at various locations. This application is a combined effort of NuGO (Nutritional Phenotype Database; van Ommen et al, 2010, Genes and Nutrition) and NMC (Data Support Platform) and several other consortia. This application is study-orientated instead of technology orientated and can therefore be used to answer biological questions that exceed the usage of a single technology. A key feature of the application is the detailed capturing of the study description. This module can be easily linked to technology specific modules. New technologies can easily be included in the system. Studies are stored with a high degree of detail in design, intervention and sampling strategies. Complex designs (multiple doses, cross-over designs, multiple groups, sampling time points and challenge tests, etc) can be stored in the system. Tailoring of the tool to specific applications (e.g. human, mouse, plant) is possible. The import part of the study capturing application consists of a web-based step-by-step data importer and a spreadsheet importer. The output of the system allows searches for studies with a specific treatment, species or organs and thus allows easy retrieval and comparison of studies. In addition, a free text search option is available that searches all fields in the database. Moreover, studies can be viewed and compared to other studies via a table structured web interface. See www.dbnp.org for all technical information and to download the code.

PP3-005
Automated Identification and Quantification of metabolites using J-Resolved NMR spectroscopy.

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NMR-based metabolomic studies typically use 1D NMR methods to minimize spectral acquisition times and hence maximize sample throughput. These advantages, however, are offset by high spectral congestion that limits the number of metabolites that can be uniquely identified and quantified. Furthermore, spectral congestion hinders the interpretation of multivariate pattern recognition analyses, since resonances from several metabolites are often averaged together into single NMR variables (i.e. bins). 2D NMR techniques enable deconvolution of multiplets in congested spectra, and one such method is J-resolved NMR spectroscopy (JRES). JRES spectra display the chemical shift and spin–spin coupling data on orthogonal axes, and a skyline projection of the 2D spectrum along the chemical shift axis provides a proton-decoupled 1D 1H NMR spectrum. Assignment of spectral peaks is necessary for biochemical interpretation of metabolomics studies, but this is a time-consuming process when done manually. Automated assignment is therefore desirable. We present here a spectral library and accompanying software package for the automatic identification and quantification of metabolites in JRES NMR spectra. The spectral library consists of 2D JRES and 1D pJRES spectra of 210 metabolites measured at pH 6.6, 7.0 and 7.4. The metabolites were selected to provide as wide a coverage as possible of metabolites typically measured in metabolomics studies. The software uses a peak-picking approach to produce a “hit-ratio” for each metabolite in the library, which indicates the likelihood that the metabolite is present in a mixture NMR spectrum (e.g., of a biological sample). Quantification is achieved using a non-negative constrained least squares linear algebra approach. The software was tested using simulated mixtures of library spectra and also chemically defined mixtures of metabolites which are representative of real biological samples. The results of these validation studies are reported and discussed.

PP3-006
Redundancy analysis of sensory directed metabolomics data

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Metabolomics data can be measured with several different analytical techniques such as GC-MS, LC-MS or NMR. The question arises to what extent the data from these platforms are overlapping. Metabolomics data are frequently used as predictors for response variables such as sensory traits of crops. It is expected that the predictive parts of these data sets for the response variable contains information about the underlying metabolic networks. The actual interest of the redundancy analysis therefore lies in the predictive parts of the data sets. A method is proposed that quantifies the redundancy between two data sets with respect to a response variable. For each data set a partial least squares model is built. The redundancy of the predictive parts of the data blocks is determined by performing a canonical correlation analysis on the relevant score vectors of the two data blocks. Inspection of the loadings and coefficients of the two models further reveals the active metabolites. An additional partial least squares model is built with the joint data blocks. In this case, the data blocks have common score vectors and the loadings give an indication of the active metabolites. A randomization test is used to see whether the joint data improves the predictive power which is also an indication additive information between the data blocks. A synthetic data set is used to demonstrate the procedure. In addition, a real life data set is used consisting of 94 tomato genotypes containing three groups of metabolic compounds: targeted volatiles (GC-MS), untargeted volatiles (GC-MS) and Non-Volatile (LC-MS), and sensory attributes, i.e. taste sweet, that are used as response variable. The targeted volatiles are a subset of the untargeted volatiles but are measured in a different manner.

PP3-007
Non-negative Matrix Factorization Based Scaling: Application to Biomarker Identification in NMR Spectroscopic Metabolomics

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Scaling is an important data preprocessing step prior to multivariate statistical analysis for nuclear magnetic resonance (NMR) spectroscopic metabolomics. The commonly used methods, such as unit variance (UV) scaling and variable stability (VAST) scaling, scale each variable of the data independently, which ignores the chemical meaning of the spectra (and hence the natural correlates) and may make the subsequent analysis uninterpretable. A new scaling method based on non-negative matrix factorization (NMF) is proposed in this paper. The new method aims to perform scaling on the concentration of the metabolites rather than on the variables of the data. By using the NMF algorithm, the data matrix is firstly factorized into the product of a base (metabolites spectra) matrix and a coefficient (concentration) matrix. Secondly, a proper scaling algorithm is performed on the concentration matrix. At last, the scaled data matrix is reconstructed with the scaled concentration matrix and the metabolites spectral matrix. Both simulated data and real-world data are used to demonstrate the utility of the new method in enhancing multivariate models for biomarker identification. Results show that NMF-based scaling method is more suitable than UV and VAST scaling for biomarkers identification in NMR spectroscopic metabolomics.

PP3-008
Group Differences in the Distribution of Metabolomic Data

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If multivariate data are approximately normal, one may have comfort that all information resides in the mean vector, variances and correlation matrix, and the error is due to many small additive processes. However, often metabolomic data have large rightward tails proscribing univariate normality, and nonlinear variable relationships and perhaps higher order dependencies can rule out multivariate normality. Concave, monotonic transformations to normality are commonly used, but this approach has limitations. A chosen transformation is part of one\'s predictive model and should apply to all groups and repeated measurements of a given variable. In some cases it may be difficult to find a transformation that brings a variable to approximate normality for all groups studied, at all time points. Therefore we sought to define features of the natural distributions of metabolite data. In this study, we examined metabolite distributions using a dataset of 38 metabolites measured in plasma of 30 healthy controls and 25 patients with first episode schizophrenia, at drug naïve state and again after 4 weeks of neuroleptic treatment. Differences of univariate distribution between 2 groups was examined by Kolmogorov-Smirnov tests. We found 8 variables were different at p\<.01 and 2 of these at p\<.0004. However, more often, group distributions found to be not different were still not able to be transformed to approximate normality by the same power transformation. This finding suggests that we are not capturing the proper division of random variation and parameter variation by the transformation-to-normality approach. The group distributions found to be significantly different for several metabolites, showed the same pattern of difference between controls and drug-naïve patients, with more broad central tendency for the former and high left peak and long tail for the latter. Thus, direct testing and modeling of the natural non-normal distribution of the data are felt to be superior to transformation-to-normality.
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PP3-009
MetAssimulo: Realistic Simulation of NMR Metabolic Profiles

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Statistical and computational tools are vital ingredients of metabolomic research. The development of these tools relies on the availability of representative training data for which the true answers are known. This is hard to obtain with real analytical data and thus simulation is a productive route towards algorithm development. MetAssimulo is a MATLAB-based package that has been developed to simulate 1-dimensional 1H-NMR spectra of complex mixtures such as metabolic profiles. Drawing data from a metabolite standard spectral database in conjunction with concentration information input by the user or constructed automatically from the Human Metabolome Database, MetAssimulo is able to create realistic metabolic profiles with a range of user-defined properties. Current features include the simulation of two groups (case and control) specified by means and standard deviations of concentrations for up to several hundred metabolites. The case group may also be parameterised more simply by altering the levels of a few metabolites with respect to the control group. A crucial feature of the algorithm in providing realistic test data for new methods is its ability to simulate inter-metabolite correlations. Further, MetAssimulo is able to simulate shifts in NMR peak positions that result from matrix effects such as pH differences. These are often observed in metabolic NMR spectra and pose serious challenges for statistical algorithms. Along with accurate simulation of the autocorrelation structure of the spectral noise and an intuitive graphical interface, the features of MetAssimulo combine to allow creation of realistic 1-d NMR metabolic profile data sets which can be hard to distinguish from real spectra. This talk will describe the algorithm behind MetAssimulo and demonstrate how it can be used to simulate realistic NMR metabolic profiles with which to develop and test new data analysis techniques.

PP3-010
MeRy-B: a web knowledgebase for the storage, visualization, analysis and annotation of plant metabolomics profiles obtained from NMR

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Thanks to the improvement of metabolomics analytical techniques, and the growing interest in metabolomics approaches, more and more metabolic profiles are generated. This high quantity of high throughput data needs to be saved and structured according to accepted standards (MSI). To exploit these data, scientists need tools to store data, identify metabolites and disseminate results. To meet this need, different tools already exist which are specific of a species, an analytical technique or a given usage: reference spectra databases (BMRB), profiles management databases (GMD, PlantMetabolomics.org), metabolites databases (KEGG), or knowledge base (HMDB). Each of these tools addresses one or more of the above needs. However, management of NMR plant metabolomics profiles remains poorly addressed. To fill this lack, we have developed MeRy-B (http://www.cbib.u-bordeaux2.fr/MERYB/index.php), a plant metabolomics platform allowing the storage and display of NMR plant metabolomics profiles. MeRy-B is a web-based application with either public or private access. Currently, MeRy-B contains more than one hundred different plant metabolites and unknown compounds with information about experimental conditions and metabolite concentrations from several plant species compiled from more than one thousand of annotated NMR profiles on various organs or tissues. We will describe the MeRy-B knowledge base functionalities: -Data capture: metadata, spectra data, peak lists, and detected analytes. MeRy-B uses MSI requirements for metadata description as well as suitable OBO ontologies. The application supports pdf format for protocols and Jcamp-DX for NMR spectra outputs. -Data visualization: spectrum viewer, spectra overlay and statistical tools for synthetic representation. -Analyte identification support, thanks to MeRy-B knowledge base, fed with new identifications. -Export for data exploitation by other statistical analysis tools that may contribute to biomarker discovery.

PP3-011
Metaboflux : a method to analyse flux distributions in metabolic networks

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Trypanosoma brucei is a parasitic protist of vertebrates that causes sleeping sickness in Africa. A part of its energetic metabolism, including the 6 or 7 first glycolytic step, occurs in an organelle called glycosome. A metabolic pathway for the glycosome had been built by exploiting genomic, reverse genetic and metabolomic data [1]. Some known biological constraints, such as the maintenance of the glycosomal ATP/ADP and NADH/NAD+ balances, have not been carefully addressed in the current model. We propose a modelling approach including structural pathway and metabolic flux analysis to help in the understanding of the system's structure and its semi-quantitative behaviour. We model known biological information with a stochastic Petri net (where transitions are given for the reaction and places for metabolites) where delays can be assigned to transitions given a probability distribution. From a given set of probability distribution representing the flux amount of reactions (the input set of parameters), the simulation of the Petri net allows the exploration of the possible behaviours of the system. At the end of a run, if all input metabolites are consumed, we get concentration for intermediate and output metabolites. We integrate expected metabolites concentrations revealed by biological experiments within an objective function, and use simulated annealing and simplex minimization approach for its global optimization. Therefore, simulations are carried out by fitting the set of input parameters until the system reach the best optimization of the objective function. To explore a large set of possible behaviour of the system, several run of simulations combined with the simulated annealing approach are made. A set of solutions is given by different groups of fluxes distributions (that best fit expected metabolites concentrations), and are helpful to make some assumptions and analysis for a given metabolic system. "Metaboflux" was developed to this purpose and applied to T. brucei. Resulting scenarios strongly argue in favour of an unrealistic NADH/NAD+ imbalance and suggest adding to the model new metabolic pathways. A realistic solution may be to integrate the pentose phosphates to the previous model. The resulting new model was tested with Metaboflux and shows relevant fluxes scenarios. References [1] Bringaud F., Rivière L., Coustou V. (2006) Energy metabolism of trypanosomatids : adaptation to available carbon sources. Molecular and biochemical parasitology. 149: 1-9

PP3-012
Reverse engineering of metabolic networks, a critical assessment.

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Reverse engineering of metabolic networks from metabolite concentration data is one of the challenging topics in systems biology since it can improve our understanding of the functioning of cellular systems. Many mathematical approaches have been proposed in the literature to extract information about the network from the data. For most of these techniques, there exist examples where they perform well. However, these examples are limited to small networks of 4 to 6 metabolites or linear networks. In this study, we give a critical assessment on reverse engineering methods applied to larger networks, where the inference task becomes more complicated. Problems occurring when applying network inference methods on larger networks are illustrated with four methods. We will do so only from simulated data and extract the conditions the data should fulfill in order to predict the network completely with these methods. These conditions are confronted with the state-of-the-art of current measurement techniques. Our results show that if full inference of a large metabolic network is the goal then the requirements for the sampling frequency and noise levels are not consistent with contemporary practice. Integration of reverse engineering methods with a priori biological knowledge deposited in databases, could be an option for further research.

PP3-013

ChromA4D – An integrated OpenSource Application Framework for comprehensive GCxGC-MS based Metabolomics

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Two-dimensional gas-chromatography mass-spectrometry (GCxGC-MS) has developed into an important technique for the analysis of complex biological samples. It is applied by a growing number of researchers in the field of metabolomics due to its increased peak capacity compared to one-dimensional GC-MS. Yet, this has lead to larger amounts of data, which are even harder to inspect and analyze manually than one-dimensional GC-MS data. Currently, only a few commercial software solutions exist, which cover most parts of the workflow from raw data preprocessing to statistical data analysis, such as ChromaTOF (LECO Corp.) and GC Image (GC Image, LLC). However, there are no OpenSource solutions available yet, which integrate all steps of processing, analysis, and visualization of GCxGC-MS data into a complete solution. We present such a solution: ChromA4D, which combines visual data analysis and inspection with a semi-automatic processing toolkit in order to perform the tasks of denoising and baseline removal, peak finding and integration by adaptive seeded region growing, library identification of mass spectra on individual chromatograms, as well as automated peak matching and grouping by determining cliques of bidirectional best-hits, and finally alignment together with differential visualization of multiple GCxGC-MS chromatograms. All processing steps generate data formats compatible with OpenSource statistics software such as R or general spreadsheet programs such as OpenOffice, so that they can be integrated easily into existing workflows. Additionally, we provide a graphical user interface, which integrates the setup of experiments, file import, processing definition, editing, visualization and analysis of GCxGC-MS data. Finally, we demonstrate the applicability of our software on a biological experiment with the hydrogen producing algae Chlamydomonas rheinhardtii.

PP3-014

An evaluation of missing values in direct infusion FT-ICR mass spectrometry based metabolomics

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Data pre-processing is a crucial step in metabolomics experiments. It influences the outcome of data analysis, thus affecting biochemical interpretation. One aspect of pre-processing – the handling of missing data - has not yet been adequately addressed in metabolomics, despite missing data being a common problem. Here we focus on direct infusion FT-ICR mass spectrometry datasets (although these studies could be applicable to both DIMS and LC-MS) to address the questions: a) what is the nature of missing data?; b) to what extent does their treatment influence the outcome of univariate and multivariate data analyses?; and c) what would be the best approach (disregarding, imputation methods) prior to data analysis to, for example, retain the power of biomarker discovery. Three datasets with different degrees of biological variation (K562 leukaemia cell line extracts < water flea (Daphnia magna) extracts < human liver biopsy extracts) were considered. We analyzed the characteristics of the missing data and examined their influence on finding discriminatory markers between biological groups (parametric univariate testing and multivariate principal component analysis). The following approaches to replace the missing data were compared: imputation algorithms such as small value-, mean- and median-substitution, k-nearest neighbour imputation, Bayesian principal component estimation and multiple imputation. We show that missing data arise from both technical and biological causes (intensity-, measurement procedure- and sample biological variation-dependent). Replacement of missing values greatly influences the outcome of data analysis. For univariate testing the mean percentage of significantly changed peaks between treatment groups was 8.33\±5.97 (cell line), 9.78\±2.38 (water flea) and 5.28\±4.64 (liver), across the six tested methods. For PCA the mean variations captured by PC1 were 66.03\±14.96, 83.48\±13.43 and 39\±2.15 and by PC2 14.69\±12.65, 4.32\±3.59 and 17.98\±1.42, for the three biological datasets respectively. The considerable differences between the results from the six imputation approaches illustrates that choosing the optimal approach should involve the analysis of the nature of missing data as well as the type of dataset.

PP3-015

Decision tree supported substructure prediction of metabolites from GC-MS profiles

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Gas chromatography coupled to mass spectrometry (GC-MS) is one of the most widespread routine technologies applied to the large scale screening and discovery of novel metabolic biomarkers. However, currently the majority of mass spectral tags (MSTs) remains unidenti-fied due to the lack of authenticated pure reference substances required for compound identi-fication by GC-MS. Here, we accessed the information on reference compounds stored in the Golm Metabolome Database (GMD) to apply supervised machine learning approaches to the classification and identification of unidentified MSTs without relying on library searches. Non-annotated MSTs with mass spectral and retention index (RI) information together with data of already identified metabolites and reference substances have been archived in the Golm Metabolome Database (GMD). Structural feature extraction was applied to sub-divide the metabolite space contained in the GMD and to define the prediction target classes. Decision Tree (DT)-based prediction of the most frequent substructures based on mass spectral features and RI information is demonstrated to result in highly sensitive and specific detections of sub-structures contained in the compounds. The underlying set of DTs can be inspected by the user and are made available for batch processing via SOAP (Simple Object Access Protocol) -based web services. The GMD mass spectral library with the integrated DTs is freely accessible for non-commercial use at http://gmd.mpimp-golm.mpg.de/. All matching and structure search functionalities are available as SOAP-based web services. A XML+HTTP interface, which follows Representational State Transfer (REST) principles, facilitates read-only access to data base entities.

PP3-016

Between-Metabolite Relationships: metabolomics with new glasses

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The 'Vitruvian Man' of Leonardo da Vinci shows that different anatomical measures are highly related in humans. These relationships are crucial to the functioning of the system. Such dependencies may also be present in the organism biochemistry: relationships between different metabolite levels might indicate the metabolic state. Usually metabolomic studies focus on metabolite levels, but studying Between-Metabolite Relationships (BMRs) may provide additional information on the metabolism. The behavior of BMRs, e.g. in time or induced by a treatment, may show how the metabolic system reacts within the experiment. Because most metabolomics studies focus on changes in metabolite levels, data analysis methods that characterize BMRs are not available yet. These BMRs can be expressed as a covariance matrix, relating all metabolites to each other. Comparing such covariance matrices of different experimental groups (e.g. differing in time or treatment) may reveal changes in the BMRs. Because covariance matrices contain only information about the relations between metabolites summarized for all biological replicates in an experiment. This structure is different from the conventional 'sample by variable' table used in e.g. Principal Component Analysis. Therefore data analysis methods dedicated to the analysis of covariance matrices are essential. Such methods, referred to as 'indirect fitting', are available for psychological research where relations between personality traits are of prime interest. We will focus on the 'INdividual Differences SCALing' (INDSCAL) method. This method provides models that express the emergence and disappearance of BMRs as components, analogous to Principal Component Analysis. These models are therefore relatively easy to interpret, while greatly extending the insight into the metabolic system of interest. We illustrate the different steps of the method by the analysis of several metabolomics studies. The INDSCAL results clearly reveal how experimental manipulations and dynamics may lead to changes in BMRs. This novel viewpoint on metabolic responses provides additional information that may lead to an increased understanding of metabolic systems.

PP3-017
Towards standardised MSn databases: evaluation of between-laboratory reproducibility of high resolution MSn acquisition.

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Metabolite identification is one of the central aspects of metabolomics and one of the major bottlenecks prohibiting rapid biological interpretation of the results obtained from quantitative studies. Identification of metabolites can, in principle, be achieved using high resolution multistage mass spectrometry (MSn) because it provides a feature-rich fingerprint of the structure of the precursor ion. However, neither general methodology for the identification nor extensive databases of metabolites with MSn data are available at the moment. In strive for a generic and efficient mass spectrometry centric identification platform a database of metabolite fragmentation data is constructed. In this database the MSn fragmentation data of metabolites are represented as fragmentation trees - hierarchical representations of relations between observed fragment ions. The fragmentation trees are generated from high resolution MSn experiments performed on a LTQ-Orbitrap (Thermo) equipped with a Triversa NanoMate (Advion) nanoelectrospray ion source. An in-house developed software tools are used for spectral data processing. The resulting fragmentation trees are highly characteristic for structure of the precursor ion and therefore can be used for metabolite identification when acquired under defined conditions. In order to assess and demonstrate the reproducibility of the acquisition of fragmentation trees, the MSn spectra for various human and plant metabolites were collected in both ionisation modes in several NMC laboratories. Although the analysis of obtained fragmentation trees exposed multiple differences in the fragmentation spectra and performance of the compared instruments the fragmentation trees were highly reproducible. The instrument dependent artefacts were removed during the data analysis leaving highly reproducible fragmentation trees containing only data relevant for the metabolite structure. These results demonstrate that the reproducibility is sufficient to allow comparison of the fragmentation trees obtained on different instruments, providing firm basis for developing a generic, multistage mass spectrometry based platform for efficient identification of metabolites.

PP3-018
Knowledge Based LC-MS Deconvolution

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Traditional methods for quantifying and identifying metabolites from Liquid Chromatogram Mass Chromatography (LC-MS) datasets are target-analysis, peak-peaking and deconvolution. Target-analysis is a manual procedure to identify specific metabolites signatures from raw datasets, while peak-picking and deconvolution are either partially or fully automated non-targeted methods. Non-targeted methods generate peak-tables bearing the mass-spectral information, retention-times and peak areas corresponding to every metabolite discovered from the raw-dataset. Given the peak-table information, library search methods are implemented to identify the metabolites. The number of metabolites accurately identified and quantified depends on the efficiency and the robustness of the non-targeted method. Conventional application of non-targeted methods like peak-picking and deconvolution have not been very successful in identifying every metabolite from LC-MS datasets. The peak-picking methods usually suffer from the problem of unique peak identification and hence needs an additional integration step to mass all those peaks belonging to a certain metabolite. A deconvolution method although is an elegant spectral decomposition method for LC-MS datasets, in some situations, fails to identify certain metabolite classes. This presentation will first discuss those metabolite classes ceased to be identified by a deconvolution method and then followed by a knowledge-based deconvolution procedural flow capable addressing this problem.

PP3-019
Development and evaluation of an accurate mass LC/MS/MS spectral library for metabolomics

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The determination of compound identities is a crucial analytical problem for metabolomics scientists using LC/MS detection. Without compound identities it is impossible to make biological sense of study results. Many compounds observed to be differentially expressed in metabolomics studies are known primary metabolites. Easily identifying these known metabolites would facilitate metabolomics analysis. One accepted way to identify compounds is to compare observed MS/MS spectra of the unknown to a library of MS/MS spectra of metabolite standards. In this poster we present the creation of an accurate mass MS/MS library of common metabolites using three different collision energies. The MS/MS spectral library was created by analyzing metabolite standards on a Q-TOF by either flow injection analysis or chromatography. An electrospray source was used and data was collected on all molecules in positive and negative ion mode. Only the $[M+H]^+$ or $[M-H]^-$ ion was used to produce MS/MS spectra. Targeted MS/MS analysis was performed on each ion at three collision energies; 10, 20 and 40 eV. Collected spectra were filtered; only ions in the spectrum that meet both a minimum required absolute count threshold and a minimum percentage of the strongest ion signal threshold were included in the spectral library entry. Ion values entered into the library were set to the calculated accurate mass value based on empirical formula. An initial MS/MS library has been built following the above described method. Data for the library was acquired with the quadrupole filter set to transmit a peak width of 1 amu; only the isotope selected is transmitted and not the adjacent naturally occurring isotopes. As one would expect not all compounds ionize in positive and negative ion mode. Only spectra acquired from ESI are included in this library. The companion MS/MS library search routine is capable of forward and reverse searches. Setting proper spectral filter thresholds can help improve match scores. Background ion interference is a problem when MS/MS spectra from complex matrices are searched. We will show this effect by searching data from plasma, urine and yeast sample extracts and compare forward and reverse search results.

PP3-020
Open-access software and Databases to help metabolites recognition in Metabonomics: the user's point of view

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Nowadays, metabolomics is underpinned by a number of freely and commercially available databases and automated approaches for metabolite identification and chemical structure elucidation. We have built a CCPN metabolomics project containing around 80 standard compounds(1). Standards have been chosen between metabolites present in the common biofluids' and common urine's library of the open-access software MetaboMiner(2). 2D 1H-13C HSQC, 1H-1H TOCSY and 1D 1H spectra of standard compounds have been free obtained from public databases(3). With the presented CCPN metabolomics project, spectra acquired on an unknown mixture can be easily compared, superimposed and assigned on the basis of the standard compounds spectra available in the project. Moreover, any other standard compounds required by users can be easily implemented in the same project and used for assignment purposes. The project enables (a) to have an overview of the similarity and differences between spectra coming from different samples; (b) to have an overview of the number of unassigned peaks; (c) to have detailed lists of the unambiguous and ambiguous assigned peaks. These peak lists could also be quality checked, exported or screened against other public databases, or could be the starting point for a de novo metabolite identification. In conclusion, here we present a protocol based on open-access databases and software, which we believe could help in the management of complex mixtures of spectra, and in the identification of metabolites on the basis of 2D and 1D -NMR spectra. References: 1. http://www.ccpn.ac.uk/ccpn 2. http://wishart.biology.ualberta.ca/metabominer/index.html 3. BMRB: www.bmrb.wisc.edu; HMDB: www.hmdb.ca; MMCD:mmcd.nmrfam.wisc.edu/

PP3-021
Plants, Pipes and Ancient Dreams: A Chemotaxonomy Study

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Archaeological and anthropological sciences are augmented by results obtained using mass spectrometry of organic residues. Often, chemical analyses of archaeological specimens are hard to reproduce due to exposure to different environmental conditions, diversity of specimens and use of target compound analytics instead of profiling methods. In this study, we aimed at discovering which plant species were used for ceremonial procedures by Native American hunter-gatherer tribes of Northern California. Even though most archaeological pipes are assumed to be associated with tobacco smoking, a variety of smoked plants were used for ritual and medicinal purposes in ethnographic North America. Tobacco was supposedly not introduced to the region at the time for which the pipes were associated. We compared residue analysis of ancient pipe specimens to leaf and seed extracts of native Northern California plants and to experimental clay pipes that were smoked using these potential medicinal plants. We used GC-TOF MS-based mass spectrometry with BinBase data processing for data acquisition. In order to test the hypothesis that tobacco was available at the time, chromatograms were specifically investigated for nicotine and nicotine-derivatives. Multivariate statistics was employed to provide a statistical model to establish which of the plant chemotaxonomic signatures and residue analyses would best predict the source material present in the ancient pipes.

PP3-022
Exploring different GC/MS-based methods for urine metabolomics

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Public health studies often include the collection, shipping and long term storage of urine samples, but conventional methods used to handle these specimens are not efficient. We proposed using a metabolomics approach to examine how different treatments of elastomeric matrices, used for the stabilization of urine in the air-dried state, would affect its metabolic profile. Aliquots of pooled human urine sample were applied and dried onto elastomers which had been treated with five different formulations. After a one-month storage period at room temperature, samples were reconstituted in water and submitted to GC/MS analysis to compare metabolic profiles across the different formulations. Urine extracts pose challenges to GC/MS analysis due to the abundance of urea which causes overload on the column and the detector, limiting the identification of compounds eluting nearby. Eliminating urea by urease also alters the metabolomic profile of urine, so it cannot be used for this analysis. Alternatively, we used the Prosep (APEX) large volume temperature programmable injector with GC-quadrupole MS (Agilent) to eliminate some of the matrix effects caused by urea. Compared to our standard analysis method of cold injection of 0.5 \µl derivatized urine extracts (CIS, Gerstel) into a GC-TOF MS instrument (Leco), large volume injections clearly show an advantage in detecting low abundant metabolites if front-cutting of the large urea peak is achieved. We explored the Mass Profiler Professional data processing software (Agilent) and compared results to freely available programs for data alignment and data binning (Binbase, AMDIS/SpectConnect). For statistical assessments discriminating different formulations used for urine metabolomics, we tested MetaboAnalyst, a recent and free web-based software, which offers options for data normalization, multivariate statistical analysis, graphing and pathway mapping. Unfortunately, MetaboAnalyst can only handle two different treatment classes. Mass Profiler Professional, as demonstrated in this study, facilitates the handling and visualization of various classes and any number of experimental samples and provides further data analysis tools.

PP3-023
Understanding Metabolite Space

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In metabolomics the identity and role of low mass molecules called metabolites that are produced in cell metabolic processes are investigated. These make them valuable indicators of the phenotype of a biological system. The ‘Metabolite Space’ is the total chemical universe of metabolites present in all compartments and in all states from any organism. These molecules exhibit common features that form what can be called ‘metabolite likeness’. Here, we focus on the human metabolite space, including both endogenous and exogenous (such as drug) metabolites. In order to analyze the ‘Metabolite Space’, we collected data from the Human Metabolome Database (HMDB) which is a comprehensive database for human metabolites containing over 7000 compounds that were identified in several human biofluids and tissues. As there still remain many compounds to be identified that lay outside the boundaries of this known space, exploring this unknown region is crucial to evaluate ‘metabolite likeness’. In order to expand ‘Metabolite Space’ in our approach we employed the Retrosynthetic Combinatorial Analysis Procedure (RECAP) to generate new molecules that possess features similar to those present in metabolites, however in other (but still likely) rearrangements. We studied how discernible these new molecules are from real metabolites and, hence, whether synthetic organic chemistry reactions are indeed able to expand the known universe of metabolites. We further studied the new chemistry present in the expanded metabolite space by looking at Murcko assemblies, ring systems and other chemical properties. The new metabolite space is compared to other small molecules, such as those obtained from the ZINC database, that are not metabolites. By combining all the above analyses we expect to characterize better the metabolite space, and furthermore, to predict the metabolite-likeness of a molecule and to understand its immanent properties.

PP3-024
Intelligent LC-MS signal annotation resources which take into account ionisation behaviour in biological matrices.

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There are numerous web-accessible databases containing information on metabolites. As an aid to data interpretation it is of great importance to link these physical descriptions of metabolites to their representation in real metabolomics data. An informatics resource cognisant both of expected ionisation behaviour and presence/absence of specific metabolite signals in different matrices can guide signal annotation in metabolite fingerprinting/profiling by LC-MS under circumstances where either mass resolution is not quite adequate and/or when many isobaric alternatives are possible for a signal. Flow infusion ESI-MS data was generated (100,000) for a number of matrices using a Thermo LTQ FT-ICR-MS instrument. Mass peaks were then manually annotated from 50–620 m/z and only metabolites matching at 1ppm or better were recorded after using the MZedDB1 database to predict common adducts, clusters and neutral losses. As a rule-of-thumb the most reproducible whole matrix annotation was achieved using a mass signal intensity cut off of 400 and a mass difference from other signals of at least 10ppm. The frequencies of all classes of ionisation products were then enumerated overall for each matrix. The two most predominant ions were [M-H]- (~75%) and [M+K]+ (~60%), but their relative frequency compared to other possible products varied depending on the matrix. When abundance ratios were examined in different sample types some metabolites showed little matrix specific effects whereas others were matrix sensitive, reinforcing the need for bespoke information on individual metabolites. The ARMeC2 database captures information on metabolite representation in specific biological matrices (e.g. leaves, starchy tubers, urine and plasma) linked to typical physical descriptions (e.g. Tryptophan will typically form predominantly [M+Cl]- and [M-H]- ions at a ratio of 1:0.4 in potato tubers). In the future, new implementations of MZedDB will utilise the ionisation behaviour patterns of chemical classes in combination with ARMeC2 information on adduct formation likelihood in a particular matrix to sort and rank annotation solutions in automated queries. 1. BMC Bioinformatics, July 21 2009

PP3-025
LC-MS according to the Systems Theory

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Liquid chromatography with mass spectrometry (LC-MS) detection is one of the major tools in proteomics and metabolomics. Metabolite transformation by protein enzymes and protein- and lipid-mediated signal transduction are elements of the pathways responsible for the non-linear dynamics of living cells. The goal of experiments in metabolomics and proteomics is to identify the molecule (or its fragment) and quantify its amount, at the best inside the cell or in a representative sample of the culture. Mass spectrometers are sophisticated, fine instruments which are essential in many applications. However, their results are usually interpreted in a rather primitive way, without knowing the errors of the results we get. We divide the output of the LC-MS into three parts: (a) useful output, (b) random noise (c) systemic noise of the instrument related to the particular experiment. The characteristics of the systematic noise change in time and depend on the analyzed substance. This allows us to quantify the probability of error and, at the same time, retrieve some peaks which get lost in the noise when using the existing methods. There are no user-defined parameters. Our software tool, Expertomica Metabolomics Profiling, automatically evaluates the given instrument, detects compounds and calculates the probability of individual peaks.

PP3-026
Towards sub-typing of rheumatoid arthritis patients using a questionnaire based on a fusion of Chinese and Western diagnosis

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Arthritis like diseases affect billions of people worldwide and are a common cause of disability. The current trial and error approach of choosing medication for these diseases leads to a large group of non-responders. Therefore there is a great need to develop tools to get the right drug to the right patient. In this endeavor towards personalized medicine the key is diagnosis. Chinese medicine (CM) developed a personalized approach to treat rheumatic diseases in which several sub-types of patients are recognized. Different gene expression and metabolomics profiles of Cold and Heat rheumatoid arthritis patients have recently been characterized (van Wietmarschen 2009). A large text mining study has shown relationships between Cold diseases and hormone disorders and between Heat diseases and immune systems disorders (Li 2007). Our hypothesis is that Cold arthritis patients might therefore respond better to steroids and Heat arthritis patients more to biologicals. In the present study a questionnaire is developed and tested based on symptoms used in Chinese medicine diagnosis. Scores from 47 arthritis patients on the questionnaire were explored. Network theory and mapping algorithms were used to visualize the connections between the symptoms and the relationship with Chinese syndromes. Personal symptom patterns were created to visualize differences between patients. The patients do not clearly separate in a Cold and Heat group using hierarchical cluster analysis. Therefore the analysis is continued with a semi-supervised approach in which two Chinese medicine experts ranked the Cold and Heat status of all the patients. These rankings were used in a categorical principal component analysis to reveal important questions for the Heat and the Cold variables. After validating this model further the questionnaire can be optimized for use in a clinical setting to predict the Cold and Heat status of arthritis patients.

PP3-027
NMR Deconvolutor: A Robust Deconvolution Algorithm for Quantitative Metabolomics

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A fundamental problem in quantitative metabolomics is to identify components in the mixtures of body fluids. 2D NMR methods (including DOSY, COSY, and TOCSY, etc) and pseudo-2D methods (such as STOCSY) have been applied to facilitate identification with its own limitation. Chemometrics software allows users to manually identify and quantify the single component in a mixture spectrum utilizing small number of reference spectra. However, it is still time-consuming and user-dependent to identify and quantify the single components. Automatic curve fitting with matrix factorization was also proposed but often generates negative quantity assignments when applied to large database. To resolve the issue stated above, we proposed a robust deconvolution algorithm, NMR deconvolutor, for quantitative metabolomics spectrum analysis. We screened and selected 529 proton NMR spectra from HMDB as reference spectra. Spectra were phase and baseline corrected. A fuzzy segmented peak alignment was also developed to align the reference spectra to the metabolomics spectrum. The mixture spectrum is then deconvoluted utilizing a least square matrix factorization algorithm with an upper bound and the non-negative constraint. The algorithm determines the number of components in the metabolomics spectrum and their concentrations. We tested this algorithm on 1) simulated mixed spectra of 100 pure compounds from HMDB assigned with random peak shifts, 2) spectra of 12 fresh prepared samples with various concentrations, and 3) a set of metabolomics NMR spectra from human samples. The results demonstrate that NMR deconvolutor produces reliable quantitative prediction on single components from metabolomics NMR spectra and therefore can potentially facilitate the biomarker discovery process greatly.

PP3-028
Characterisation of carbon isotope patterns in FT-ICR mass spectra for improved confidence of metabolite identification

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Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is a powerful tool for measuring metabolites. The FT-ICR analysis yields a complicated mass spectrum with typically 3000 or more peaks that arise from the metabolites, including adducts as well as naturally occurring isotopes. The excellent specifications (i.e. high mass accuracy and mass resolution) of the FT-ICR mass spectrometer are ideal for identifying such isotopes based on peak differences (e.g. m/z 1.00336; 13C-12C spacing). Isotope intensity ratios are widely used in MS for estimating the number of carbon atoms in a compound. This is particularly useful for reducing the number of possible empirical formulae that can be assigned to a peak. Here we investigate the accuracy with which FT-ICR MS (Thermo LTQ FT Ultra) can determine the number of carbon atoms in a metabolite, and discuss the implications for metabolite identification in metabolomics. First, using a mixture of known chemicals (polyethylene glycols), we measured carbon isotope intensities, calculated the empirical number of carbon atoms, and then deduced the associated carbon errors. Several filtering rules were defined based on this dataset (e.g. signal-to-noise ratio (SNR)). Next we recorded mass spectra of biological samples, and isotope intensities were measured and filtered by the defined rules. Finally, a statistical approach was used to select the most likely elemental composition for a particular all-12C-containing peak. This statistical approach was validated using known contaminant peaks in the biological mass spectra. The range of errors in the number of carbon atoms decreases with higher SNR (e.g. mean and standard deviation for SNR 13C peak >10: -0.32 ±1.30; >100: -0.48 ±0.63). These values indicate that the number of carbon atoms predicted was on average lower than the theoretical number of carbons. We show that although FT-ICR MS cannot determine the number of carbon atoms with high accuracy, by using an extensive training set, strict filters and a statistical approach, it is capable of yielding information for reducing the uncertainty in metabolite identification.

PP3-029
Maltcms 1.0 - a Modular Application Toolkit for Chromatography-Mass Spectrometry in Metabolomics

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We present the current stable release 1.0 of our modular application toolkit for chromatography-mass spectrometry (Maltcms). Maltcms provides a flexible, pipeline based infrastructure for processing data acquired from gas-chromatographs (GC) and liquid- chromatographs (LC) with single- (FID, FL) or multidimensional (MS) detectors. It provides methods for signal denoising, peak-finding, retention time alignment and annotation of signal peaks found within the data, as well as visualizations of unaligned and aligned data and differential reports. Furthermore, processing results are exported to csv formats for downstream statistical analysis with other packages. Maltcms supports multiple open formats for analytical data as input, such as AIA/ANDIMS (netcdf), mzXML, mzData, mzML and csv-compatible formats. MSP compatible data files can be used to set up a custom database for MS-based peak identification. Our current work is focused on the development of a user-friendly, plugin based extensible GUI which provides tools for the creation and configuration of processing pipelines, as well as for the visual inspection and interactive annotation and exploration of datasets from metabolomics and the processed results. Future work is aimed at the parallelization of processing tasks on arbitrary grid infrastructures. Maltcms is freely available at http://maltcms.sourceforge.net under the L-GPL v3 license. It runs under all personal computer operating systems, for which a JAVA Runtime Environment is available.

PP3-030
Chemical Translation Service – a database approach to standardize conversion of chemical identifiers

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Metabolomics urgently needs standardized databases to compare data across studies, laboratories and different mass spectrometry platforms. The optimal way to annotate small molecules (0-1500 u) is by the corresponding structure in a machine-readable format. Open-source structure codes were introduced by the IUPAC five years ago (InChI codes) but have not yet been widely adopted in literature. One problem is that there are no easy translator tools for analytical chemists who lack time or expertise in computational chemistry to transform lists of identified metabolites into structure codes or to batch convert metabolite database identifiers (like CAS, KEGG, HMDB or PubChem) into unambiguous and public InChI keys. The database content was obtained from publicly available databases, such as KEGG, LipidMaps, HMDB, Pubchem, BioCyc and Chebi. To avoid entry replication, we merged the shared content by the use of the unique InChI Code, which was generated from the Mol files in the obtained downloads. For storage reasons we have only included certain chemical information, such as structures, chemical names, chemical synonyms, database identifiers, molecular masses, XlogP and proton-donor/acceptor information. If available, additional properties can be easily accessed through direct links to the original databases. A key feature of this program is its ability to extract chemical compounds out of any given text with very high precision. For this feature we employ existing techniques from regular expressions to the use of sophisticated tools. To reduce the risk of false positives we integrated manually generated wordlist filters and dictionaries into the algorithm. This database can be accessed by the use of a Grails based Web interface or by a REST-based Web service. This web service is required primarily for automatic access from other programs and databases. For the storage and querying the data we use a PostgresSQL database system. Further optimization was obtained by generating an index over the database with the use of the compass framework. This minimizes the actual database access for most queries and allows you to perform 'Google' like queries.

PP3-031
GCxGC-TOFMS Data Interpretation of Metabolic Biomarkers from Diabetic and Non-diabetic Urine Utilizing Fisher Ratios Prior to Multivariate Analysis

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Metabolomics presents challenges for both the analytical methods used and the data reduction required to interpret the results. Comprehensive multi-dimensional gas chromatography time-of flight mass spectrometry has emerged as an excellent instrumental option for the characterization of small metabolite profiles. GCxGC-TOFMS provides increased peak capacity and resolution for the chromatographic separation while fast TOFMS acquires the mass spectral data density necessary to characterize complex biological samples. This poster presents a data mining strategy from results obtained from a diabetic and non-diabetic Trimethylsilyl (TMS) derivatized urine study analyzed by GCxGC-TOFMS. A Fisher Ratio plot was also generated from grouped sample comparison results. A compound table based on the Fisher Ratio results was then applied to principal component analysis (PCA) whereby possible differences between non-diseased and diseased state groups were graphically represented. The Initial experimentation focused on optimized method development for sample extraction, BSTFA derivatization, and GCxGC-TOFMS method optimization. Following method development, GCxGCTOFMS analysis was conducted on six derivatized samples from each of four subjects, two diseased, and two non-diseased. The GCxGC-TOFMS data was refined through background elimination of erroneous peaks produced by column bleed and derivatization reagents before processing by statistical comparison and Fisher Ratio calculations. GCxGC-TOFMS analysis was conducted on diabetic and non-diabetic urine samples that were first extracted with Methylene Chloride and then derivatized with BSTFA. A total of twenty-four samples were analyzed and data processed before applying a statistical comparison for the diseased and non-diseased state classes. Data alignment was carried out by the Statistical Compare feature contained in LECO's ChromaTOF software. A Fisher Ratio plot was then calculated for each analyte of the Compound Table and utilized to identify metabolites with the highest variance. The high variance data was subsequently exported as a spreadsheet and applied to multivariate principal component and clustering analysis. Graphical representations of the multivariate analysis show significant analyte differences between diseased and non-diseased state sample classes.

PP4-001
Investigating the Metabolic Effects of Heart Failure Progression using Hyperpolarized Magnetic Resonance

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Heart failure (HF) is a progressive disease with multiple causes including myocardial infarction (MI), hypertension and cardiomyopathy. Regardless of cause, HF is accompanied by changes in cardiac energy metabolism. The rat ischaemia-reperfusion model of MI provides a powerful tool for investigating HF progression. The aims of this study were to use hyperpolarized MRS to investigate in vivo temporal metabolic effects associated with HF progression up to 22wks post MI, and to use ex vivo 1H-NMR spectroscopy and spectrophotometric assays to further define metabolic defects at 22wks. The left anterior descending coronary artery of female Wistar rats (n=16) was occluded ~2mm from the origin for 50mins, followed by reperfusion. Cardiac metabolism was non-invasively assessed using hyperpolarized MRS at 2days, and 2, 6, 12, and 22wks post MI. At each time point ejection fraction (EF), a measure of heart function, was assessed using echocardiography. At 22wks, hearts were removed for metabolomic and biochemical analyses. Echocardiography revealed EFs ranging from ~20-80%. In vivo pyruvate dehydrogenase (PDH) flux was unchanged in all animals throughout the study, indicating acetyl CoA production from pyruvate was maintained regardless of cardiac function. This was confirmed using a spectrophotometric assay which revealed no correlation between PDH activity and EF at 22wks. Levels of 13C-labelled citrate and glutamate correlated with EF ($P<0.05$), suggesting a reduction in intracellular pool size and/or a defect in Krebs cycle flux. 1H-NMR spectroscopy revealed a reduction in glutamate, consistent with decreased Krebs cycle flux. Using a spectrophotometric assay, citrate synthase activity was also found to be reduced. Together, this data provided evidence of defective Krebs cycle metabolism from 6wks in HF, which could contribute to the well characterised perturbation in energy homeostasis in the failing heart. The results also highlight the importance of being able to non-invasively assess metabolism at multiple time points and demonstrates the potential of hyperpolarized MRS for investigating the metabolic effects of progressive diseases such as HF.

PP4-002
On the outside looking in – Applications of Time-Resolved Metabolic Footprinting

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To understand bacterial physiology and/or virulence, the ideal scenario is to monitor the cells' complete metabolic state constantly to see which events trigger virulence factor synthesis. However, due to various experimental constraints, large-scale omic-data sets are often acquired at a single-time point, thus potentially losing vital information. Here, we present Time-Resolved metabolic Footprinting (TReF), an integrated sampling/data analysis approach, which provides a systematic description of bacterial metabolism over the course of growth. TReF combines NMR-based analysis of bacterial culture supernatants with non-linear modelling of compound concentration changes and multivariate statistical analysis. This improves the interpretability of the data by expressing the time dimension as more biologically meaningful parameters. We successfully applied TReF to different biological systems and were able to show that the method can be used for biomarker detection as well as for the generation of testable biological hypotheses. In the well-studied Gram-negative model organism Escherichia coli, TReF led to the detection and identification of keto-leucine as an extra-cellular biomarker for nitrogen stress in bacteria. For the opportunistic human pathogen Pseudomonas aeruginosa, we tested the effects of mucA mutation, as this is a common hallmark of chronic cystic fibrosis infections. The TReF data showed changes in metabolites potentially related to osmotic tolerance, and we confirmed that the mucA mutants did indeed have increased sensitivity to osmotic stress – which may be directly relevant to a common clinical treatment. Finally, combining non-linear fitting parameters with hierarchical principle component analysis we were able to recreate taxonomical separation for a subset of the very closely related Burkholderia cepacia complex species based on phenotypic data alone. In summary, these examples demonstrate the successful application of TReF to three principal areas of microbiology: physiology, functional genomics and phylogeny. We therefore believe time-resolved metabolic footprinting could be a valuable tool for many questions in bacteriology, including isolate comparisons, phenotyping deletion mutants, and as a functional complement to taxonomic classifications.

PP4-003
Metabolomics: Isolation of Putative Endophytes from Selected Peninsular Malaysia's Plants and Their Screening for Cytokinin-Like Compounds

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Endophytic microorganisms reside inter or intra cellularly in most of the plant tissues without causing any disease symptoms. Plant-growth-promoting endophytic bacteria stimulate plant growth by producing and/or inducing the plant to release secondary metabolites facilitating the uptake of nutrients and/or inhibiting plant pathogenic organisms. Cytokinin is a group of plant growth regulators (PGRs) which can be used in increasing or maintaining leafy vegetables, fruits and cut flowers crop yield. Cytokinins are known to stimulate chlorophyll synthesis and to increase shelf-life of leafy vegetables, cut flowers and fruits. Hence, isolation of endophytes and screening them may lead to identification of cytokinin-like compound producing endophytes which could be used in agriculture for various applications. Therefore the objective of this study is to isolate endophytic bacteria from various plants collected from peninsular Malaysia and to screen them using cucumber cotyledon bioassay. Plant samples were collected from various states of peninsular Malaysia and endophytes were isolated from different tissues using the standard isolation method. In this study, so far 1099 endophytic bacterial strains are isolated and being screened for cytokinin-like compound using cucumber cotyledon bioassay. Pure cultures of putative bacterial endophytes were grown separately in Luria Bertani (LB) medium and cell-free broth was used in the cucumber cotyledon bioassay. The amount of total chlorophyll content in cucumber cotyledon samples was estimated by spectrophotometry and compared with positive and negative controls. To identify the putative endophytic bacterial strains 16S rDNA was amplified using PCR and sequencing was carried out. The isolation, screening results for isolated putative endophytes using metabolomics approach and 16S rDNA based identification of isolates will be discussed in this paper. This work is fully supported by a grant from the Ministry of Agriculture and Agro-Based Industry of Malaysian Government (05-02-16-SF1001).

PP4-004
Molecular characterization of beta-ketoacyl-ACP synthase II (KAS-II) gene *in silico* to understand the metabolomics differences in American and African oil palm fruit tissues

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Oil palm (*E. guineensis* Jacq. Tenera) is a valuable source of cooking-oil and ranks first for its oil yield (per hectare) among the oil producing crops. The beta-ketoacyl-acyl carrier protein (ACP) synthase (KAS) II catalyzes the elongation of 16:0-ACP to 18:0-ACP in plastids. The objective of this study is to understand the differences and similarities in American and African oil palm KAS-II gene (cDNA) structure to understand the metabolomics differences in their fruit tissues. Elaeis oleifera 17 weeks old mesocarp tissue cDNA library was constructed using CloneMiner cDNA library construction kit. By random method of gene isolation, E. oleifera KAS-II cDNA clone was isolated and compared in silico with sequence of E. guineensis Jacq. Tenera KAS-II cDNA clone isolated previously. Conserved domain search analysis revealed the presence of beta-ketoacyl-ACP synthase, polyketide synthases, and elongation condensing enzymes domains responsible for the elongation steps in fatty acid biosynthesis. Comparative analysis showed that E. guineensis and E. oleifera KAS-II is 98 % and 95% identical with each other at nucleotide at amino acid level respectively. The E. oleifera KAS-II phyllogenetic analyses showed its close phyllogenetic relation with E. guineensis KAS-II. In silico molecular characterization of oil palm KAS-II gene will be discussed in this paper. This work is fully supported by a grant from the Ministry of Science, Technology and Innovation (MOSTI) of Malaysian Government [Grant Code: IRPA: 01-02-02-0014P (extended work)].

PP4-005
Quantitation of Biogenic Amines using LC-Triple Quadrupole-ESI-MS

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Previous methods developed for amino acids quantitation can also be used to measure biogenic amines sourced from a variety of biological matrices.1 Modification of primary and secondary nucleophilic amines by use of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC, Waters) reagent leads to production of a tethered amino-quinoline moiety via a carbamide linkage. Complex mixtures of modified amines are separated by liquid chromatography then analysed by electrospray ionization mass spectrometry (ESI-MS). Upon collision induced decay (CID) the modified molecule fragments into discrete product ions, with one corresponding to a characteristic m/z of 171.0550+ that identifies the amino-quinoline moiety. By using the mass selective capabilities of a triple-quadrupole ESI-MS and comparison against a standard concentration curve, the concentration of a variety of biogenic amines can be determined. We have performed tagging and quantitation experiments for many of the pungent biogenic amines including ethylamine, cadaverine, putrescine, spermine and spermidine. We have found each of these to be present in samples of both red and white wine. Further, mining for the characteristic AQC fragment has uncovered a rich variety of molecules that have been modified, these remain to be identified. Further development has included exploring quantitation of other biological nucleophiles including glutathione, the simple neurotransmitters and their biological metabolites: serotonin, dopamine, epinephrine, metanephrine, norepinephrine, normetanephrine and octopamine. Each possesses at least one nucleophilic amine which is modified upon incubation with the AQC reagent. 1. Callahan, D.L.; Kolev, S.D.; O'Hair, R.A.J.; Salt, D.E.; Baker, A.J.M., New Phytologist (2007), 176(4), 836-848.

PP4-006
Consequences of the exposure of human adipocytes to Mono-2-ethylhexyl phthalate

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Phthalates are widely used plasticizers that can leak from plastics and lead human populations to high levels of exposure. Di-2-ethylhexyl phthalate (DEHP) is the most wildly used of the phthalate esters and mono-2-ethylhexyl phthalate (MEHP) is its main metabolite. It has been proposed that such pollutants, by modulating the differentiation or the metabolic functions of adipocytes, could lead to the development of obesity related disorders. MEHP urinary concentrations have thus been positively correlated to waist circumference in non diabetic adults. Our study aimed at describing the global consequences of human adipocytes exposure to MEHP, using an integrative, untargeted approach that combined both transcriptomics and 1H-NMR based metabonomics. Transcriptomic analysis indicated activation of the PPARg signaling pathway in cells treated for 24h with MEHP. Consistent with PPARg activation, metabolic profiling revealed increased glycerol-triglyceride signals in MEHP treated cells after 48h of treatment. This result was confirmed using an enzymatic assay that revealed an increased triglyceride content in 48h-MEHP treated adipocytes. Metabolic profiling also showed a decreased lactate and amino acids contents after 24h and 48h of treatment. Statistical integration of the metabonomic and transcriptomic data highlighted a high correlation between transcriptomic data at 24h and metabonomic data at 48h, and the perturbations observed on both data sets were coherent with increased triglyceride synthesis in MEHP treated cells. However, poor correlation between transcriptomic data at 24h and metabonomic data at 24h led us to the hypothesis that other early metabolic pathways were activated and we found that neoglycerogenesis was also activated early after MEHP treatment. In conclusion, metabonomic analysis of in-vitro human adipocytes culture, combined with transcriptomics, enabled here a global insight of the metabolic consequences of the exposure of human adipocytes to MEHP. We showed that MEHP activated the PPARg receptor, which led to increased adipogenesis and neoglycerogenesis. Those two mechanisms, that both lead to increased intracellular triglyceride content, could partially explain the potential pro-obesogenic effect of this compound in humans.

PP4-007
Metabolomic Approach to study *Rosmarinus officinalis* extract effect on diabetic rats

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Antioxidant therapy has been proposed to improve the oxidative stress status of diabetic patients. Natural products are a source of substances with known antioxidant properties with possible benefits on diabetes. Among them, carnosol, carnosic acid, and rosmarinic acid are active constituents of rosemary that can be extracted via an environmentally clean process such as supercritical fluid extraction with CO2. Five doses of rosemary extract with in vitro antioxidant properties were intragastrically administrated to adult male streptozotocin (STZ) diabetic rats and the corresponding controls. Urine fingerprints of control and diabetic rats, both with and without treatment, were obtained by LC-MS. Data were collected in positive ESI mode in separate runs on a QTOF (Agilent 6520) operated in full scan mode from 50 to 1,000 m/z. When the profiles were submitted together to pattern recognition techniques they showed the effects of rosemary on this acute and short term treatment animal model. After checking the analytical quality of the process with the corresponding QCs, a PLS-DA model was built with variables found in diabetic and control, non-treated groups. Afterwards, the model was used to predict treated diabetic animals and they appeared clustering between both, which could prove an improvement in the general status. In order to have further biochemical knowledge of the effect, after treatment, groups were studied by pairs and metabolites identified will be described to explain the effect.

PP4-008
MSeasy: an R package for the pre-processing of GC/LC-MS data adapted to chemical ecology

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Motivation: The democratization of metabolic analyses has extended the scope of metabolomics to ecological questions. Chemical ecology interprets the variation and diversity of chemical signals of non-model organisms in the light of species interactions. Elucidating the biological information within such complex signals, using robust statistical analyses, requires a large number of replicates. Results: We developed an unsupervised pre-processing method, for the treatment of large GC/LC-MS dataset, that efficiently detects individual compounds within complex mixtures. The method is based on the clustering of mass spectra and does not require any profile correction, retention time alignment or normalization. It is robust to the use of different types of columns and to shifts in retention times particularly common for large/long-term experiments. We validated our method, and compared it to other pre-processing approaches by carrying out analyses on two different experimental datasets. This method is based on the clustering of mass spectra and does not require any profile correction, migration time alignment and normalization. On those datasets, we found that the best clustering method for grouping similar mass spectra was the hierarchical clustering analysis with the Euclidean distance and the Ward linkage. However, it is not excluded that other clustering algorithms could be more adapted for other datasets. For that reason, we\'ve developed the function MS.test.clust to identify the best clustering algorithm on any new dataset. Availability and implementation: an R package "MSeasy" implementing our pre-processing method is freely on demand. Contact: yann.guitton@univ-st-etienne.fr

PP4-009

Comparative analyses of the metabolome and proteome of G-protein signaling mutants of *Stagonospora nodorum*

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The necrotrophic fungal pathogen *Stagonospora nodorum* is the causal agent of leaf and glume blotch on wheat. In Australia alone, the disease causes in excess of 100 million dollars (AUD) in yield losses each year, making it one of the most significant diseases to wheat in this country. G-protein signalling has long been implicated in the pathogenicity of phytopathogenic fungi such as *S. nodorum*. Strains of this fungus have been created lacking the G-alpha, G-beta and G-gamma proteins, with all mutants exhibiting abnormal development and defects in pathogenicity. Advanced analytical techniques are being used to explore changes in the metabolome of *S. nodorum*, occurring as a result of the deletion of the G-alpha, G-beta and G-gamma proteins. The metabolomic data highlights various changes to both primary and secondary metabolism that have occurred as a result of the mutations. A redirection of carbohydrate metabolism is also evident through an altered abundance of some sugars, including trehalose, in mutants failing to accumulate to the levels detected in wild-type. Additionally, a mycotoxin previously identified in metabolite extracts of *S. nodorum* (Tan et al., 2009) has shown to accumulate in the tissue of the mutant strains. Complementary proteomic studies have also been performed on the *S. nodorum* wild-type and the G-alpha-lacking strain using iTRAQ (Casey et al., 2010). This data and the metabolomic data generated by gas chromatography, mass spectrometry (GC-MS) and comprehensive two-dimensional gas chromatography, time-of-flight mass spectrometry (GC×GC-TOF) will be presented.

PP4-010

Genomics meets secondary metabolomics: towards a comprehensive view of myxobacterial natural product diversity

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Myxobacteria represent an important source of biologically active natural products with considerable promise for human therapy. Several studies have recently highlighted the enormous and hardly tapped potential of many myxobacterial species for secondary metabolite biosynthesis.[1,2] Although more than 100 basic structures from myxobacteria have been characterized to date, the number of compound classes reported from individual strains clearly falls short of the genetic capabilities. Thus, the discovery of novel secondary metabolites which are presumably only produced in small quantities from genetically proficient bacteria currently constitutes a substantial bottleneck in the discovery process of novel natural product lead structures. Furthermore, whole-genome sequence analysis of various myxobacterial strains has revealed that myxobacteria devote an unexpectedly high proportion of their genetic resources to encode regulatory systems. Deciphering the complex regulatory networks that seem to govern secondary metabolite production in myxobacteria is of outstanding interest with regard to their biotechnological potential as suppliers of bioactive compounds. Metabolomics-style experiments, based on the combined use of LC-coupled high-resolution mass spectrometry and statistical data evaluation, are now beginning to pave the way towards an enhanced view of myxobacterial natural product diversity. These techniques can significantly enable the mining of myxobacterial secondary metabolomes for the presence of previously undiscovered metabolites.[3,4] Aiming at the definition of a "comprehensive molecular phenotype", metabolomics-based investigation of myxobacterial secondary metabolism is also a promising approach for studying the regulatory mechanisms controlling natural product biosynthesis. Recent examples from research on the myxobacterial model species *Myxococcus xanthus* will be presented and the impact of improved analytical methods on our view of the myxobacteria as proficient producers of novel natural products will be discussed. [1] Wenzel SC,Müller R (2009). Curr.Opin.Drug Disc.Dev. 12(2), 220-230 [2] Garcia RO, Krug D, Müller R (2009). Methods in Enzymology 458, 59-91 [3] Krug D, Zurek G, Schneider B, Garcia R, Müller R (2008). Anal.Chim.Acta, 624, 97-106 [4] Krug D, Zurek G, Revermann O, Vos M, Velicer GJ, Müller R (2008). Appl.Environ.Microbiol. 74, 3058-2068

PP4-011

Mass Spectrometry-based on Metabolome Analysis for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) toxicity

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In this study, liquid chromatography/quadrupole time-of-flight mass spectrometry was employed to investigate the toxic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in blood, liver and skeletal muscle tissues of C57BL/6J (C6) and DBA/2J (D2) mice. By using non-targeted metabolomic approach in conjunction with multivariate data analysis, e.g. principal conponents analysis and support vector machines, hundreds of ions were aligned and extrated with P-value and fold-change in mice exposed with TCDD compared to control group. Through high resolution MS and MS/MS analyses as well as database searching and comparison with reference standards, differentiating metabolites were tentatively identified for the interpretation of metabolic pathways in sensitive or less-sensitive mouse model. As a result, in sensitive mouse model-C6, fatty acid beta-oxidation was markedly attracted. Meanwhile, perturbation of other metabolic pathways was also observed, namely the accumulation of fatty acids and lysophospholipids, the reduced amino acid biosynthesis, and the decreased purine metabolism. The intermediate metabolites in citric acid cycle was also affected to reveal energy metabolism. The metabolic signature of TCDD toxicity in C6 mice was delineated by mass spectrometry and data mining, indicating oxidative stress, reduced energy production and perturbation of immune system. In contract, in the less-sensitive mouse model-D2, as expected, much less metabolites were detected as the differentiating effects of TCDD toxicity. In conclusion, mass spectrometry-based metabolomics measures the different abundant levels of the large number of metabolites produced as intermediates and end-products in different mouse models. The multivariate data analysis offered highly efficient classification and improved visualization, which facilitates metabolomic approach to be a promising tool in the interaction of environment and health.
References 1. Ishida, T.; Kan-o, S.; Mutoh, J.; Takeda, S.; Ishii, Y.; Hashiguchi, I.; Akamine, A.; Yamada, H. Toxicol. Appl. Pharmacol. 2005, 205, 89-97.

PP4-012

Surface fitting of 2D diffusion-1H NMR spectra of human plasma lipoproteins

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The characterization of the lipoprotein profile of human plasma is clinically relevant. The atherogeneity of lipoproteins depends not only on the amount of carried lipids, but also on its concentration and size, so it is of great importance to have a measure of these parameters. The traditional techniques used for this purpose (ultracentrifugation, gel electrophoresis, etc.) are manual and time consuming. Several new approaches are currently attempted. For instance, the analysis of the methyl peak of 1H-NMR spectra performed by Liposciences Inc. allows the quantification of both, concentration and size, of up to 11 lipoprotein subclasses in human blood samples. This methodology is based on the lineshape fitting of a full serum spectrum using a previously characterized spectra library of isolated lipoprotein subclasses. Other approaches are mainly based on statistical regression of the plasma 1H-NMR spectra against lipid concentrations measured by classical biochemistry. This work is based on the surface fitting of both methyl and methylene peaks of a 2D diffusion-based 1H NMR spectra, considering as a second dimension the gradient strength. A set of lorentzian functions characterized by their position, amplitude, width and average diffusion coefficient was optimized. Using 8 functions both the amplitude and the averaged diffusion coefficient were found to be significantly correlated with biochemically assayed concentrations of triglycerides, cholesterol, ApoA and ApoB measured in each of the isolated fractions (VLDL, IDL, LDL1, LDL2, HDL2, HDL3) from the same plasma samples. Our experiment demonstrates that the averaged diffusion coefficient of each fitting function can be considered as a direct estimation of the size of its related lipoprotein. The presented approach based on surface fitting leads to a reliable quantification and characterization of lipoprotein particles. Additionally, these data can also be used as further input information for multivariate algorithms used in metabolomics leading to more parsimonious models.

PP4-013
Explorative analysis of urinary metabolome by 1H NMR: a new insight into progression of osteoarthritis

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Osteoarthritis (OA) is a disease characterized by joint degradation, involving both articular cartilage and subchondral bone. Up until now there are no reliable biomarkers for diagnosis or prognosis which is an impediment to the management of OA, the targeted development and monitoring of disease modifying drugs and increases the cost of therapeutic trials. Thus there is a strong need for alternative characteristics of the diagnosis and prognosis as well as progression for osteoarthritis. In the current work, we present an explorative 1H NMR analysis of GARP (Genetics, osteoARthritis and Progression) cohort. This cohort consists of genetically predisposed sibling pairs affected with OA at multiple joint sites. 1H NMR data was obtained for urine samples collected from 4 timepoints in the same subjects within 2 years span. Clinical follow-up and progression data also includes a 5-year time point. The unique cohort design gives us a possibility to monitor urinary metabolic signatures along the time in the studied group of people and investigate if the observed changes can be associated with OA and its progression, other clinical features or established biochemical markers. Our hypothesis is that using previously described person recognition approach [1] we can discover subjects with reduced rate of recognition, which indicates significant changes in NMR profiles of those individuals. These changes can be related to either the clinical course of OA or changes in lifestyle, eating habits etc. The aim of the present study is to explore to which extent personal recognition rates can be used to evaluate progression of OA and what are the causes for their disturbance. [1] Bernini, P.; Bertini, I.; Luchinat, C.; Nepi, S.; Saccenti, E.; Schäfer, H.; Schütz, B.; Spraul, M.; Tenori, L. Individual Human Phenotypes in Metabolic Space and Time. J. Proteome Res. 2009.

PP4-014
Functional characterization of fungal cytochromes P450 involved in metabolism of antifungal substances

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The complex biochemistry and metabolism of filamentous fungi aids in successful resistance to adverse environmental phenomena. Cytochrome P450 enzymes (CYPs) constitute a large family of enzymes that are remarkably diverse oxygenation catalysts found throughout nature, from archaea to humans. Fungal CYPs are found in primary and secondary metabolism. They are involved in biosynthetic pathways of natural compounds and in detoxification of other antifungal substances (xenobiotics). A novel cytochrome P450, classified into the CYP53 family, CYP53A15, was identified in the pathogenic filamentous ascomycete Cochliobolus lunatus. The protein CYP53A15 (BPH, benzoate parahydroxylase) is capable of para hydroxylation of benzoate which is a key intermediate in the metabolism of aromatic compounds in fungi and yet basically toxic to the organism. Phenolic compounds such as benzoic acid, derivatives and other phenolic compounds (e.g., eugenol, isoeugenol, vanillin, thymol) play a pivotal role in the plant resistance processes during the phase of infection. Some of these compounds are inhibitors of CYP53 enzymes and could serve as alternative antifungal drug targets. Cytochrome P450 reductase (CPR) is a membrane-bound flavoprotein and redox partner in eukaryotic microsomal P450 monooxygenase systems. We found that the filamentous ascomycete Cochliobolus lunatus possesses two reductases, CPR1 and CPR2. They were functionally characterizated in the reconstituted monooxygenase complexes with CYP53A15. It was determined that both redox partners support CYP53A15 activity to different extent. Kinetic parameters of the systems were determined by following product formation by RP-HPLC analyses.

PP4-015
Metabolomics and Infant Nutrition: Inference of Differential Metabolic Regulation in a Randomized Clinical Intervention Trial.

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High protein intake in infancy has been associated with adverse outcomes in later life regarding obesity and metabolic disease risk. A European multicenter trial, funded under the 6th Framework Programme (EARNEST, www.metabolic-programming.org), randomly assigned 1138 healthy formula-fed infants to either receive high- or low-protein infant formula during the first year. Additionally, 619 breastfed infants were observed as a reference population. At the age of six months, serum samples were drawn (726 valid cases in total) that were analyzed by Targeted Metabolomics based on BIOCRATES AbsoluteIDQ direct infusion-MS/MS technology combined with HPLC-MS/MS profiling, covering exact quantitative assessment of amino acids, acylcarnitines, hexoses, (lyso-)phosphocholines und sphingomyelins (180 metabolites in total). Extensive quality control and data normalization strategies were applied to ensure data consistency across the large number of samples studied. Statistical comparison of formula intervention and breastfed groups revealed distinct changes in metabolic network units as defined by consensual movement of biochemically related metabolite groups. Between group differences reached high statistical significance in conventional univariate testing. The functional relevance of these findings was underscored by showing that similarity measures between metabolites learned directly from data compared very well with prior knowledge documented in biochemical textbooks and databases like KEGG. Functional data interpretation was further aided by using Bayesian network analysis to explore the conditional dependence structure of metabolite concentrations. Bayesian networks excelled in providing visual models of metabolic interactions within and between highly connected subunits. Together with clustering techniques, probabilistic network models were shown to provide a valuable tool for explicitly taking advantage of the informative correlation \/ dependence structure of Metabolomics datasets to generate data-driven hypotheses from clinical trials on metabolism and nutrition.

PP4-016
Nmr based metabolomics study of ptp1b deficiency influence on hepatic regeneration in mice.

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Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of tyrosine kinase growth factor signaling. Lack of PTP1B receptor gene leads to increased insulin sensitivity, energy expenditure and decreased adiposity. Levels of PTB1B may exert a pivotal role in maintaining the balance between survival and death in hepatocytes1. Recently, it has been proposed that PTP1B deficiency accelerates hepatic regeneration in mice. The present work is aimed to analyze the metabolic events produced upon partial hepatectomy (PH) and liver regeneration in PTP1B-/- versus WT mice. After a HR-MAS-NMR metabolic profiling pilot study, using directly fresh liver tissue, we have continued the study with the lipid and aqueous liver extracts2 obtained from PTP1B-/- and WT mice at different time points (12h, 24h, 36h, 48h) after hepatectomy. Also the effect of a high fat diet (HFD) during 48 hours of hepatic regeneration has been assessed. In general PTP1B-/- and WT mice regenerating livers revealed significant increase of triglycerides and cholesterol, being more prominent in WT animals. These changes were paralleled by rising levels of oleic, linolenic and monounsaturated fatty acyls (MUFA) and depleted levels of phospholipids. In the aqueous extract, after PH, glucose and glycogen were decreased and methionine were increased in regenerating livers, with a less pronounced effect in PTP1B-/- mice. In conclusion, NMR is able to provide a biochemical snapshot at different time and conditions of changes occurring after and with PH allowing for a rapid assessment of the regeneration status in the liver. 1.A. M. Valverde, et al., Journal of Cellular Physiology 2007, 212, 76–88. 2.M. Vinaixa et al., Journal of Proteome Research 2010, 9, 2537-2528.

PP4-017

13C-Flux analysis and stoichiometric network modeling of the carbohydrate metabolism from *Xanthomonas campestris pv. campestris*

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The Gram-negative γ-Proteobacteria *Xanthomonas campestris* pv. campestris is a plant pathogen for Brassicacea (e.g. the model plant *Arabidopsis thaliana*) and produces the polysaccharide xanthan gum used in various industrial fields. To analyse the carbohydrate metabolism, which is relevant for the xanthan gum production and the pathogenicity, stoichiometric network modelling and 13C-flux analysis were applied. The carbohydrate metabolism of *Xanthomonas campestris pv. campestris* was reconstructed and the established network was stoichiometrically analyzed. Stoichiometric network modelling predicts an optimal efficiency for glucose utilisation using the Entner-Doudoroff pathway. The stoichiometically predicted optimal flux for Xcc through the Entner-Doudoroff pathway is confirmed using [1-13C]- and [U-13C]- flux measurements.

PP4-018

NMR metabolic analyses with 13C-glutamine identify altered TCA and γ-glutamyl cycles in the metastatic VM-M3 tumorigenic cell line

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The VM-M3 macrophage-like cell line, unlike the overwhelming majority of tumorigenic cell lines, is highly metastatic in vivo. It therefore may serve as a more accurate in vitro representation of the metabolism of the deadliest cancers. While typical cancer cell lines get their energy primarily from glucose, these unique cells can survive on glutamine alone and cannot maintain viability without it. Clearly these cells display an altered metabolism, which may involve energy derived from substrate level phosphorylation through the TCA cycle rather than using glycolysis which has historically been linked to tumorigenicity. Various single and multidimensional NMR techniques were used to determine the steady state metabolite pools in cell extracts and media samples when the cells were incubated with 4 mM glutamine alone, with 25 mM glucose alone, or with both glucose and glutamine. The metabolic fingerprint is similar to that of macrophages, the cell type from which the VM-M3 cells seem to be derived. In order to monitor metabolism more accurately and determine how and why the cells use glutamine to maintain viability, the cells were incubated with uniformly 13C labeled glutamine under the same conditions. Several labeled metabolites related to the TCA cycle were identified in both the cell and media extracts. Some very unique metabolites were found to be 13C labeled including itaconate (in both media and cell extracts) and pyroglutamate (in the media alone). These metabolites (at low levels in unlabeled extracts, but easily detected in 13C-labeled samples) have rarely (if ever) been reported in mammalian cells. This combination of labeled metabolites strongly suggests that the TCA and gamma-glutamyl cycles have alterations that enable these cells to survive by using glutamine as their primary energy source. Given the metastatic characteristic of the VM-M3 cell line, either of these metabolites (itaconate in particular) may serve as a biomarker for metastatic capability in cancer cells.

PP4-019

Comparative metabolomics: reconstructing the evolution of central carbon metabolism in 14 yeast species

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The basic topology of metabolic networks (and particularly of central carbon metabolism) is conserved across a wide range of species. However, the macroscopic metabolic phenotypes of species with otherwise similar metabolic networks are surprisingly diverse, even across comparatively small evolutionary distances. For instance, the Ascomycota yeasts (which include Saccharomyces cerevisiae) exhibit significant variations in growth rate, carbon source utilization, ability to ferment, and preference for fermentation under aerobic conditions. However, much remains unclear about the origins and mechanics of these differences in metabolism. How did so many closely related species evolve such different behaviors with nearly identical metabolic networks? What are the evolutionary processes that drive these changes? What are the regulatory mechanisms that allow for such flexible rewiring of metabolism on evolutionary (and shorter) time scales? How do these changes relate to similar metabolic phenotypes in higher eukaryotes, like the Warburg effect in cancer? In our work, we have used metabolomics and comparative functional genomics to study the dynamics and evolution of metabolism in 14 Ascomycota yeasts spanning over 300 million years of evolution. We studied the response of cells to different nutritional conditions and the transitions between these conditions. We used metabolomic measurements, with parallel microarray analysis of the same samples, to measure the dynamic responses of the species in corresponding physiological conditions. Using advanced computational tools, we have begun to unravel the differences in dynamic metabolomic responses and their potential transcriptional causes. We have identified dynamic metabolic behaviors that are consistent across all species, conserved within phenotypically similar or closely related species, and unique to certain species. We have also identified evolutionary trends that suggest selective pressures unique to certain metabolites, even within pathways with otherwise poorly conserved metabolic dynamics. Currently, we are using our data to reconstruct the evolutionary history of modules of co-regulated metabolites and genes, and to infer the metabolic phenotypes of non-extant yeast ancestors.

PP4-020

Early detection of celiac disease by metabolomics

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Celiac disease is an autoimmune disorder triggered by modification of the glycoprotein gliadin and certain other prolamins found in wheat by the transglutaminase enzyme [1]. The modified proteins lead the immune system to react with the small-bowel tissue causing an inflammatory reaction. This results in erosion of the villa of the small intestine responsible of nutrient absorption and thus alterations to metabolite levels are expected. The only known treatment is gluten free diet. Early detection of the disease would help start the gluten free diet as early as possible and prevent destruction of the villa and its reflected symptoms. We analyzed longitudinally collected serum samples between birth and 10 years of age from 15 subjects who progressed to celiac disease, and 28 controls matched by age, sex and HLA genotype. A total of 614 samples were analyzed by lipidomics using UPLC/MS and metabolomics using GCxGC-TOFMS [2], measuring a total of 333 molecular lipids and 130 metabolites. At the age of 4 ± 2 months, before antibody tests alert, 54 of the lipids and 24 of the metabolites were statistically significantly different at $p<0.05$ (Wilcoxon's test). When samples were collected shortly before the clinical diagnosis the diet was not restricted and 69 of the 333 lipids were statistically significantly different. When gluten free diet started following the diagnosis, only 22 of these 69 lipids still differed. The consistency of the metabolic markers at different ages was also evaluated using logistic regression [3] by explaining the disease status with selected lipid profiles at early ages and then testing whether the same model discriminates cases from controls at later ages. Together, our findings suggest that metabolomics approach may help detect and possibly prevent celiac disease early.

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PP4-021
Metabolome analysis of oil-rich alga, *Pseudochoricystis ellipsoidea*, using nitrogen stable isotope labeling.

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Pseudochoricystis ellipsoidea, an undescribed genus and species of green alga accumulates a large amount of lipids (oil) intracellularly, including diesel class hydrocarbons, and is thus expected to be a promising source of bioenergy. *P. ellipsoidea* grows rapidly under nitrogen-rich conditions, but lipid accumulation is limited. However, under nitrogen-deficient conditions, the growth rate of *P. ellipsoidea* is considerably reduced and the cell accumulates large amounts of lipids. To optimize culture conditions for efficient lipid accumulation, it is important to understand the metabolic changes associated with growth under nitrogen-rich and nitrogen-deficient environmental conditions. Using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), we obtained metabolomic profiles of *P. ellipsoidea* grown under nitrogen-rich and -deficient conditions. Under nitrogen-deficient condition, essential amino acids except for tryptophan and histidine, and total protein were decreased. This is because amino acids are not biosynthesized without nitrogen. On the other hand, synthesis of de novo protein synthesis will be necessary to accumulate lipids. We, therefore, presumed that protein degradation might be occurred to produce amino acids for the source of other metabolism and de *novo* protein synthesis under nitrogen-deficient conditions In this study, the protein turnover was detected under nitrogen-deficient conditions in *P. ellipsoidea* by metabolome analysis using nitrogen stable isotope ^{15}N labeling. We also discuss the several nitrogen flows in central metabolism under nitrogen-deficient conditions.

PP4-022
Using targeted metabolomics to identify new substrates of the efflux transporter MRP2 in vivo.

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Multidrug resistance protein 2 (MRP2, ABCC2) is an ATP-dependent efflux transporter. Absence of functional MRP2 in humans results in the Dubin-Johnson syndrome, a hallmark of which is an increase in plasma bilirubin-glucuronide levels. In vitro studies have shown that MRP2 is a typical organic anion efflux transporter and substrates include many compounds conjugated to glucuronic acid. High levels of MRP2 are found in liver, kidney and gut, organs with a high capacity for glucuronidation. MRP2 is present in the apical membrane of polarized cells and transports its substrates towards bile, gut lumen and urine. In the absence of MRP2 a larger part of its substrates will be transported over the basolateral membrane towards the circulation, leading to their increased presence in plasma. It is presently unclear which substrates are transported by MRP2 in vivo under normal conditions. We have previously used Mrp3-/- mice to identify new MRP3 substrates in vivo using metabolomics and now used a similar approach to identify the preferred in vivo substrates of MRP2. We used LC/MS to screen in plasma for compounds containing a glucuronic acid moiety by scanning for a neutral loss of 176 Da. We found that many glucuronides were more abundant in plasma of mice lacking MRP2. Several of these compounds were identified as phytoestrogen-conjugates containing a glucuronic acid as well as a sulphate moiety. In vitro vesicular transport experiments showed that these phytoestrogen glucuronosyl-sulpho-conjugates were high-affinity/ high-capacity substrates of MRP2, in agreement with published in vitro data showing that MRP2 has a preference for substrates containing two negative charges. The identification of several other compounds that were highly abundant in plasma of Mrp2-/- mice is still ongoing. In conclusion, by applying an unbiased metabolomics-like approach to biological samples of wild type and Mrp2-/- mice we have identified several new MRP2 substrates and expect to find many more, notably sulphate and GSH conjugates.

PP4-023
Multifaceted Metabolomic Approaches Profiling of Soluble Byproducts Formed During Lignocellulosic Biomass Pretreatment

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One of the main hurdles in the process of “Biomass to Ethanol” conversion is providing hydrolytic enzymes with access to cell wall glycopolymers. In lignocellulosic biomass, cellulose and hemicellulose are rigidified by a diverse array of polyphenols called lignin which presents a barrier inhibiting digestion of cell walls to fermentable sugars. Acid or alkali pretreatments of cellulosic biomass can enhance enzymatic digestion. Ammonia Fiber Expansion (AFEX) provides a promising pretreatment that improves digestion yields; however, degradation products produced using all of these pretreatments inhibit downstream fermentation, and there is a need for comprehensive profiling of these products for process optimization. In addition, profiling of soluble oligosaccharides released by biomass pretreatments is essential for rational optimization of conversion of cell walls to bioethanol. Profiling of such a complex oligosaccharide mixture presents one of the great challenges to modern analytical chemistry owing to the vast structural diversity among these compounds. Oligosaccharide characterization has relied on degradation of oligomers to monomers that are more easily characterized, but understanding of oligosaccharide diversity is elusive when such “bottom-up” approaches are employed. Integration of several analytical improvements offers a promising “top-down” alternative based upon: oligosaccharide enrichment, HPLC separations of oligosaccharides, electrospray ionization mass spectrometry, and collision induced dissociation of oligosaccharide ions. We have developed fast LC/MS/MS screening using a hybrid-linear ion trap mass analyzer to profile byproducts from biomass pretreatment. To differentiate effects of process conditions on formation of potential fermentation inhibitors, LC/MS data were processed using Principal Component Analysis (PCA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA). Short GC/MS methods were also developed to identify and quantify small (MW<140) nitrogen containing compounds formed during the pretreatment process. For profiling oligosaccharides, we have developed methods for enrichment of oligosaccharides using porous graphitized carbon followed by analysis using LC/TOF MS and multiplexed collision-induced dissociation. This approach successfully profiled glucans with molecular masses up to 3500 Da in extracts of AFEX-treated corn stover. Analyses of enzymatic digests of treated biomass results also revealed presence of water-soluble arabinoxylans.

PP4-024
Metabolomics as a tool in fertility research

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Assisted reproductive techniques have become widespread and figures show they account for the birth of more than 3 million babies worldwide. A serious complication of invitro fertilisation (IVF) is a high multiple pregnancy rate, circa 30%, which leads to medical complications. Single embryo transfer is an effective way of minimising these risks but its acceptance is limited as there lacks an accurate method of assessing embryo reproductive viability. We hypothesise that metabolomic analysis of follicular fluid could prove a useful non-invasive technique for the prediction of oocyte quality and thus embryo viability. 67 patients undergoing IVF at the Merrion Fertility Clinic were recruited. At time of oocyte retrieval, the follicular fluid from two follicles were retained per patient. 1H NMR spectra of the follicular fluid were acquired and analysed using multivariate data analysis. The concentration of lactate and glutamine in the samples was determined using a YSI analyser. Of the 67 patients, 40 patients had follicular fluid retained from an oocyte that resulted in an embryo transferred during the IVF cycle. Of these 40 patients, 14 had a successful pregnancy outcome. Multivariate data analysis of the 1H NMR spectra resulted in the formation of a PLS-DA model which showed separation between successful and unsuccessful IVF outcomes. Preliminary analysis of the loadings and VIP plots identified a series of metabolites as being discriminatory. Lactate and glutamine were among these and independent confirmation of their levels showed that a successful pregnancy was associated with reduced levels of glutamine (0.38 ± 0.01 and 0.41 ± 0.07, $p = 0.034$) and an increased lactate/glutamine ratio (12.02 ± 3.33 and 10.39 ± 2.13, $p = 0.002$). Our results, demonstrate for the first time, the potential use of metabolomic profiling of follicular fluid as a determinant of the reproductive potential of oocytes. This novel non-invasive technique may improve gamete/embryo selection and therefore assist in the establishment of single embryo transfer as a method to reduce the occurrence of multiple pregnancies.

PP4-025

NMR Based Metabolomic Studies of Arterial Disease

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Atherosclerosis is a disease of large and medium-sized arteries and contributes to the increasing worldwide burden of myocardial infarction (heart attack) and stroke. Plasma has been studied from three groups of patients, two suffering from arterial disease, either coronary or peripheral, and one without arterial disease. Peripheral arterial disease can cause intermittent claudication, which is aching or pain in the legs reproducibly brought on by walking and relieved by rest. For this preliminary study, sample data from 80 patients, with similar numbers in each group, has been acquired using 1H-NMR spectroscopy and various bin widths investigated, initially using principal component analysis (PCA) then partial least squares discriminant analysis (PLS-DA). Firstly, PCA showed diet was strongly influencing results, principally glucose and lactate, but other interesting trends have been shown. These will be investigated through further analysis and the acquisition of more samples from new patients in all groups.

Author	Abstract ids
Aardema	PP2-001
Aasen	P8B-012
Abadio Finco	P7A-001
Abdullah	P2A-075
Abete	P9A-003
Abreu	L8B-002
Acharjee	L7B-004
Achten	L6A-002
Acworth	P2A-081
Adam	P4A-001
Adamski	L6A-002, P2A-060, P2A-061
Adato	L3B-002
Agudo	L8A-004
Aharoni	L3B-002, L9B-004, P7A-017, P9A-008
Ahn	P2A-042, P2A-043, P4A-003
Akamatsu	P8B-019
Akbari	P2A-002
Ala-Korpela	LP4-004
Alanen	P3A-006
Alcarraz-Vizán	P2A-015, P7A-005
Alexandre	P9A-028
Aligner	P9A-026
Allen	L5B-004
Allison	LP1-002, P6B-010
Allwood	L9B-004, P2A-035, P5A-022, P5B-001
Almeida	P3A-001, P6B-001
Altenburger	P2B-010, P2B-011
Amelia	PP4-004
Ament	P2A-001, P2A-087, P7B-006
Anders	P5A-006
Andersen	P2A-089
Anderson	L1A-001, L2A-001
Andersson	L9A-002
Andres-Lacueva	P9A-001, P9A-018
Angulo	PP4-007
Ankley	P2B-013
Ano	P3B-012, PP4-021
Antonova	P10A-008
Antti	P2A-089
Anwar	PP3-001
Arbustini	P7A-019
Architecture	P9B-001
Arita	L4B-002
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