metabomeeting 2012

Manchester Conference Centre, September 25-27th 2012,

Manchester, UK

Organised by the Metabolic Profiling Forum and the University of Manchester

Metabolic Profiling Forum

MANCHESTER 1824
The University of Manchester

Contents

Welcome

Welcome to Manchester and to Metabomeeting 2012, the seventh in this series of meetings dedicated to the further development of metabolic profiling (metabolomics and metabonomics) and its applications. These areas of application cover a variety of topics including plants, microbes, and preclinical modelling as well as human health and nutrition. Sessions will also cover the latest developments in sample analysis within the whole metabolic profiling pipeline along with data analysis and integration with Systems Biology approaches and including metabolite annotation and identification. A student and postdoctoral session is also included to promote and encourage the younger generation of researchers.

The lectures for this year's meeting will all be held in the Weston Theatre of the Manchester Conference Centre. In Weston One there will be exhibition displays from the sponsors for this meeting and we encourage you to meet with the sponsors to discover the latest developments on offer for metabolic profiling studies. Five of the sponsors are also hosting seminars to emphasise in more detail the instrumentation and software available to apply in metabolic profiling (see the programme and speaker abstracts for further information.

We are pleased to welcome as our key-note speaker this year Dr Robert Hall from Plant Research International in The Netherlands, who will provide an address entitled "**Metabolomics and the art of deception".**

We hope that Metabomeeting 2012 provides a rewarding and stimulating meeting, and that you enjoy your time in the city of Manchester. The local organisers will be happy to answer any questions regarding the Manchester locale and attractions. Information can also be found at http://www.visitmanchester.com/

Metabomeeting 2012 and the Metabolic Profiling Forum are organised on a purely voluntary basis.

LOCAL ORGANISERS:

- Dr Warwick Dunn, Institute of Human Development, University of Manchester, UK
- Dr Will Allwood, School of Chemistry, University of Manchester, UK

METABOLIC PROFILING ORGANISERS:

- Dr Andy Nicholls, MPF and GlaxoSmithKline, UK
- Dr Julian Griffin, MPF and MRC Human Nutrition Research, UK
- Dr John Haselden, MPF and GlaxoSmithKline, UK
- Professor Roy Goodacre, MPF and University of Manchester, UK

Location

The Manchester Conference Centre is located in central Manchester, close to excellent transport links. Piccadilly railway station is a 5-10 minute walk away where there are direct links to Manchester International Airport, the Metrolink tram system operates from the station and next to the station is a large taxi rank.

Arriving by Air

Manchester Airport is 15 miles from Manchester City Centre and the conference centre. From the airport, trains to the city centre run every 15 minutes into Piccadilly train station; the average journey time is 20 minutes. Alternatively you can jump into a taxi to the city centre. For further information visit www.manchesterairport.co.uk.

Arriving by Train

There are four key train stations in Manchester city centre - Deansgate, Piccadilly, Oxford Road and Victoria. Piccadilly and Oxford Road railway stations are both a 5-10 minute walk from The Manchester Conference Centre. See the following websites for further information (Piccadilly - http://www.nationalrail.co.uk/stations/MAN.html and Oxford Road - http://www.nationalrail.co.uk/stations/mco.html).

Arriving by Coach

National Express run coaches from major cities in the UK to Manchester. Visit the National Express website for full details (http://www.nationalexpress.com/home.aspx). The coach station is a 5-10 minute walk away (for further information see http://www.nationalexpress.com/coach/destinations/england/north-west/manchester/Manchester-coach-station.aspx).

Arriving by Car

Manchester's ring road (M60) connects Manchester to motorways north, south, east and west. The M6 offers easy access from the north and south. The M62 offers easy access from east and west.

Once you have arrived in Manchester there are two car parks close to the conference centre on Charles Street; one is east of the Charles Street/Princess Street junction (MCP) and one is west of the Charles Street/Princess Street junction (mult-storey car park).

Travelling by Bus, Tram and Train while in Manchester

A comprehensive network of buses, trams and trains offer frequent services to many destinations in Greater Manchester and the surrounding area. For further information see http://www.gmpte.gov.uk/

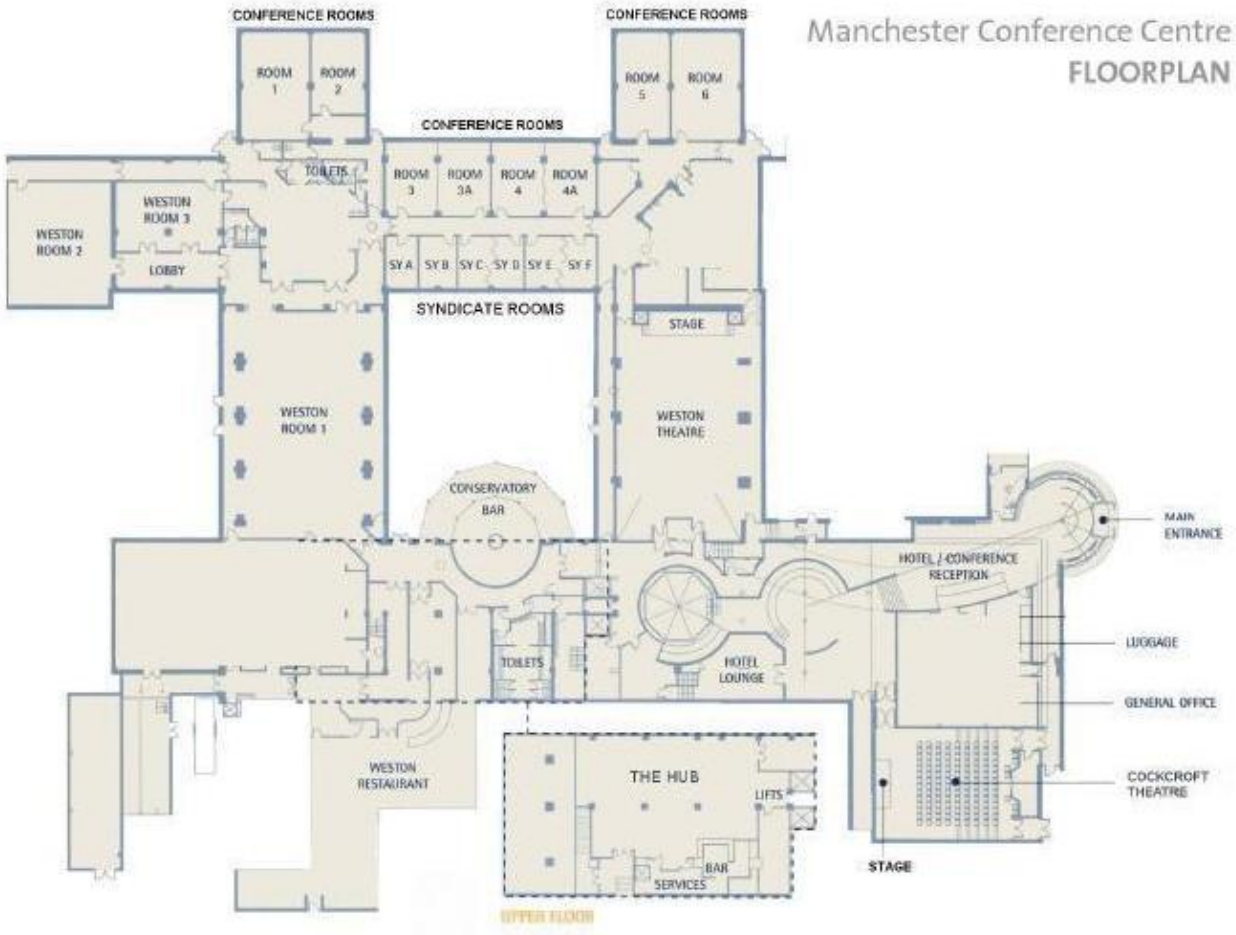
Exploring Manchester On foot

Walking is the easiest way to get around. Manchester is easily explored on foot, with plenty of pedestrian-only zones. It is possible to traverse the main central locations within around 20-minutes,

end-to-end. We recommend walking in groups for safety after dark. For further information visit http://www.visitmanchester.com/.

Manchester Conference centre

All lectures, poster sessions, lunches and refreshments (with the exception of the conference meal) will be held in the Manchester Conference Centre. A floorplan is shown below.



Presentations

All of the lectures for the meeting will be held in the Weston Theatre.

Lunches and Refreshments

All lunches and refreshments will be served in Weston Room 1.

Exhibitions

Sponsor's exhibits are located in Weston Room 1. We encourage you to go and chat with the sponsors regarding their services and products.

Posters

The boards for posters are located in Weston Room 1, Weston Room 2 and Weston Room 3. Posters can be displayed from Tuesday September 25th, but must be removed by 5pm on Thursday September 27th. Poster presentation will be split with presenters for odd numbered posters on the Tuesday evening (poster session A) and for even numbered posters on the Wednesday afternoon (poster session B).

Poster Session Mixer (Sponsored by Waters)

A Poster session mixer will be held on Tuesday evening.

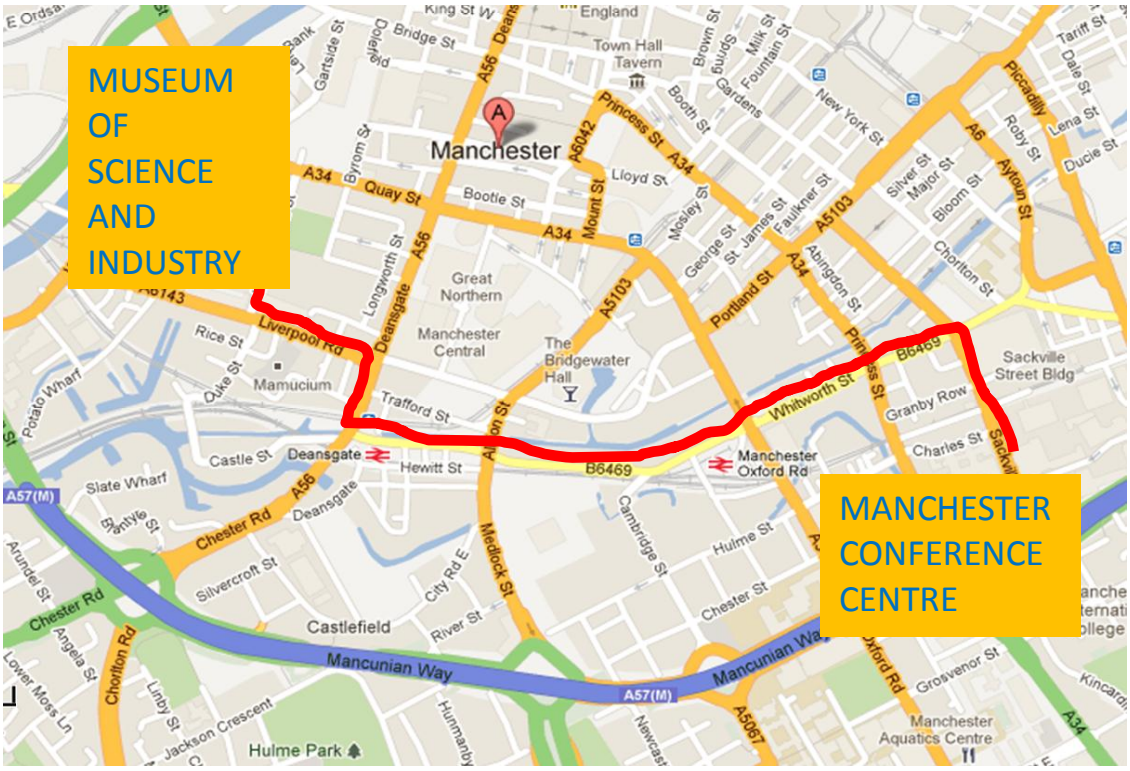
Wireless Internet Facilities

Open access wireless (WiFi) internet is available at the conference. Details of how to connect are provided separately in your conference materials and can be obtained from the registration desk.

Conference Meal Location/Logistics

The Conference meal will be held at The Museum of Science and Industry (http://www.mosi.org.uk/) on the evening of Wednesday 26th September. A drinks reception in The Engine Room will commence at 6pm and the meal will commence in the Revolution Manchester Hall from around 7pm.

Buses will be provided to transport attendees from outside the conference centre to the museum; 100 seats will be available at 17.30 and 100 seats will be available at 18.15. Local Organisers will also walk to MOSI while highlighting some of the history of the area; further information is available from the registration desk. You may also walk to MOSI yourself (25-40 minutes), a map is provided below.



METABOMEETING 2012 PROGRAMME

TUESDAY SEPTEMBER 25TH

08.15 – 09.15	*Registration*
09.15 – 09.30	***Introduction and Welcome – Dr Andy Nicholls and Dr Warwick Dunn***
09.30 – 10.30	**Plenary Presentation** ***Chair: Dr Warwick Dunn***
	Metabolomics and the art of deception ***Dr Robert Hall, Plant Research International, The Netherlands***
10.30 – 11.00	***Coffee Break***
11.00 – 12.30	**Session 1. Applied Metabolomics - Plants** ***Chair: Dr Robert Hall***
11.00 – 11.30	*Compositional and metabolomics analyses of crops derived from modern agricultural biotechnology* ***Dr George Harrigan, Monsanto, USA***
11.30 – 12.00	*Profiling of spatial metabolite distributions in wheat leaves under normal and nitrate limiting conditions* ***Dr Will Allwood, University of Manchester, UK***
12.00 – 12.30	*Early metabolic re-programming and diversion of lignin biosynthesis in rice blast infection of Brachypodium distachyon plants* ***Dr Hassan Zubair, University of Aberystwyth, UK***
12.30 – 14.00	***Lunch***

	Thermo Fisher Scientific seminar (Conference Room One) ***Bruker seminar (Weston Theatre)***
14.00 – 15.30	**Session 2. Applied Metabolomics – Human Health and Nutrition** ***Chair: Dr Julian Griffin***
14.00 – 14.30	*Metabolite profiling reveals a coherent signature of a dietary ω-3 LC-PUFA supplementation in mice and humans* ***Prof. Hannelore Daniel, Technical University of Munich, Germany***
14.30 – 15.00	*A data driven strategy to discover dietary biomarkers and validate new diet recording tools suitable for epidemiological studies* ***Prof. John Draper, Aberystwyth University, UK***
15.00 – 15.30	*Mountains out of molehills? The role of experimental design, quality assurance and parsimonious regression models, in effective clinical metabolomics biomarker discovery* ***Dr David Broadhurst, University of Alberta, Canada***
15.30 – 16.00	***Coffee/Tea Break***
16.00 – 18.00	**Session 3. Student and Postdoctoral Presentations** ***Chair: Dr Will Allwood***
16.00 – 16.20	*Metabolomics approach for the differentiation between organic and conventional grown carrots* ***Dr Elena Cubero, Directorate General Joint Research Centre Institute, Belgium***
16.20 – 16.40	*Targeted metabolic profiling of umbilical cord blood reveals promising prognostic markers, and insight into the pathogenesis, of hypoxic-ischaemic encephalpathy* ***Dr Stacey Reinke, University of Alberta, Canada***
16.40 – 17.00	*Liquid chromatography-mass spectrometry calibration transfer and metabolomics data fusion*

	Dr Andrew Vaughan, University of Manchester, UK
17.00 – 17.20	*Rheumatoid Arthritis: Targeted and global metabolic profiling to study disease progression*
	Mr Fillippos Michopoulos, University of Thessaloniki, Greece
17.20 – 17.40	*Variation in the metabolite contents of bulbs Narcissus pseudonarcissus from different locations and of different age measured by GC-FID and GC-MS*
	Mr Muhammad Nadeem Akram, Leiden University, The Netherlands
17.40 – 18.00	*A Bayesian approach to metabolite peak identification in liquid chromatography-mass spectrometry experiments*
	Dr Rónán Daly, University of Glasgow, UK
18.00 – 20.00	**Poster Session A** **Poster Session Mixer (sponsored by Waters)**

WEDNESDAY SEPTEMBER 26TH

08.30 – 09.00	*Registration continues*
09.00 – 10.30	**Session 4. Applied Metabolomics - Drug Discovery and Pharma** ***Chair: Dr Andy Nicholls***
09.00 – 9.30	*Metabolites to Systems - Understanding the effects of drugs* ***Prof. Ian Wilson, Imperial College London, UK***
09.30 – 10.00	*Metabolic profiles of urine and serum from children with Acetaminophen overdose* ***Dr Rick Beger, US Food and Drug Administration, USA***
10.00 – 10.30	*Determining mechanisms of anti-parasitic drug action by untargeted metabolomics* ***Dr Darren Creek, University of Melbourne, Australia***
10.30 – 11.00	***Coffee Break***
11.00 – 12.30	**Session 5. Analytical Advances and Imaging** ***Chair: Dr Darren Creek***
11.00 – 11.30	*New prospects for metabolic profiling and imaging using Secondary Ion Mass Spectrometry* ***Dr Nick Lockyer, University of Manchester, UK***
11.30 – 12.00	*Drug and metabolites study in whole animal body by high definition MALDI imaging* ***Dr Emmanuelle Claude, Waters Corp., UK***

12.00 – 12.30	*High resolution MAS-NMR detection of nano-litre tissue biopsies and living organisms* ***Dr Alan Wong, CEA Saclay, France***
12.30 – 14.00	***Lunch*** ***LECO seminar (Weston Theatre)***
14.00 – 15.30	**Session 6. Applied Metabolomics – Cells to Organisms** ***Chair: Prof. Roy Goodacre***
14.00 – 14.30	*Elaborating the use of metabolomic technologies to cell layer resolution and chemical genetics screens* ***Dr Asaph Aharoni, Weizmann Institute of Science, Israel***
14.30 – 15.00	*Metabolic phenotyping of Caenorhabditis elegans by whole organism NMR spectroscopy; applications for functional genomics in aging and toxicology* ***Dr Clement Pontoizeau, University of Lyon, France***
15.00 – 15.30	*NMR faecal metabolic profiling of mice fed with high fat diet and two different fermentable carbohydrates* ***Dr Ruey Leng Loo, Universities of Kent and Greenwich, UK***
15.30 – 16.00	***Coffee/Tea Break***
16.00 – 18.00	**Poster Session B**
18.00 - 23.00	**Conference meal at The Museum of Science and Industry, including a drinks reception in the Power Hall**

THURSDAY SEPTEMBER 27TH

08.30 – 09.00	*Registration continues*
9.00 – 10.30	**Session 7. Data Analysis and Integration with Systems Biology** ***Chair: Dr David Broadhurst***
9.00 – 9.30	*TA Framework for Convergence: Closing the Metabolomics Pipeline into a Knowledge Cycle* ***Dr Jeroen Jansen, Raboud University, The Netherlands***
9.30 – 10.00	*MetaboLights: a database for collecting metabolomics experimental data using ISA tools* ***Dr Reza Salek, European Bioinformatics Institute, UK***
10.00 – 10.30	*FaST metabolome- and genome-wide association* ***Dr Rene Pool, VU University Amsterdam, The Netherlands***
10.30 – 11.00	***Coffee Break***
11.00 – 12.30	**Session 8. Applied Metabolomics - Human Disease** ***Chair: Prof. Ian Wilson***
11.00 – 11.30	*Going to the extremes: using lipidomics to understand the causes of insulin resistance* ***Dr Julian Griffin, University of Cambridge, UK***
11.30 – 12.00	*Understanding pregnancy complications using a metabolomics approach* ***Dr Alexander Heazell, University of Manchester, UK***
12.00 – 12.30	*Lipidomic analysis of acute myeloid leukaemia cell responses to bezafibrate and medroxyprogesterone acetate reveals potential insight into their anti-leukemic action* ***Dr Andrew Southam, University of Birmingham, UK***

12.30 – 14.00	***Lunch***
	Waters seminar (Conference Room One) ***Agilent Technologies seminar (Weston lecture Theatre)***
14.00 – 15.30	**Session 9. Metabolite Annotation and Identification** ***Chair: Dr Warwick Dunn***
14.00 – 14.30	*The mzMatch pipeline: Recent developments* ***Prof. Rainer Breitling, Univesity of Glasgow, UK***
14.30 – 15.00	*Advanced analysis of LC/MS metabolite profiling data with the Bioconductor package CAMERA* ***Mr Carsten Kuhl, Leibniz Institute of Plant Biochemistry, Germany***
15.00 – 15.30	*Combination of MS-based metabolomics and microNMR for studying fungal interactions and induction of bioactive natural products* ***Dr Jean-Luc Wolfender, University of Geneva, Switzerland***
15.30 – 16.00	***Coffee/Tea Break***
16.00 – 17.30	**Session 10. Applied Metabolomics - Microbial** ***Chair: Dr Cate Winder***
16.00 - 16.30	*Microbial Metabolomics in the Service of Quantitative Food Microbiology* ***Prof. George Nychas, Agricultural University of Athens, Greece***
16.30 - 17.00	*Discovery of metabolic patterns of gut microbiome in C57BL/6J and C57BL/6N mice using high resolution mass spectrometry* ***Dr Alesia Walker, HelmholtzZentrum Munchen, Germany***
17.00 - 17.30	*Urinary Tract Infection (UTI):through the looking-glass of metabolomics* ***Dr Ekaterina Nevedomskaya, Leiden University Medical Centre, The Netherlands***

17.30 - 18.00	**Conference Close** **including awards ceremony for oral and poster presentations**

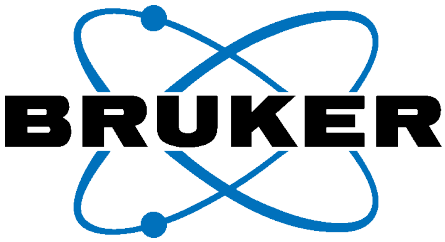
Sponsors

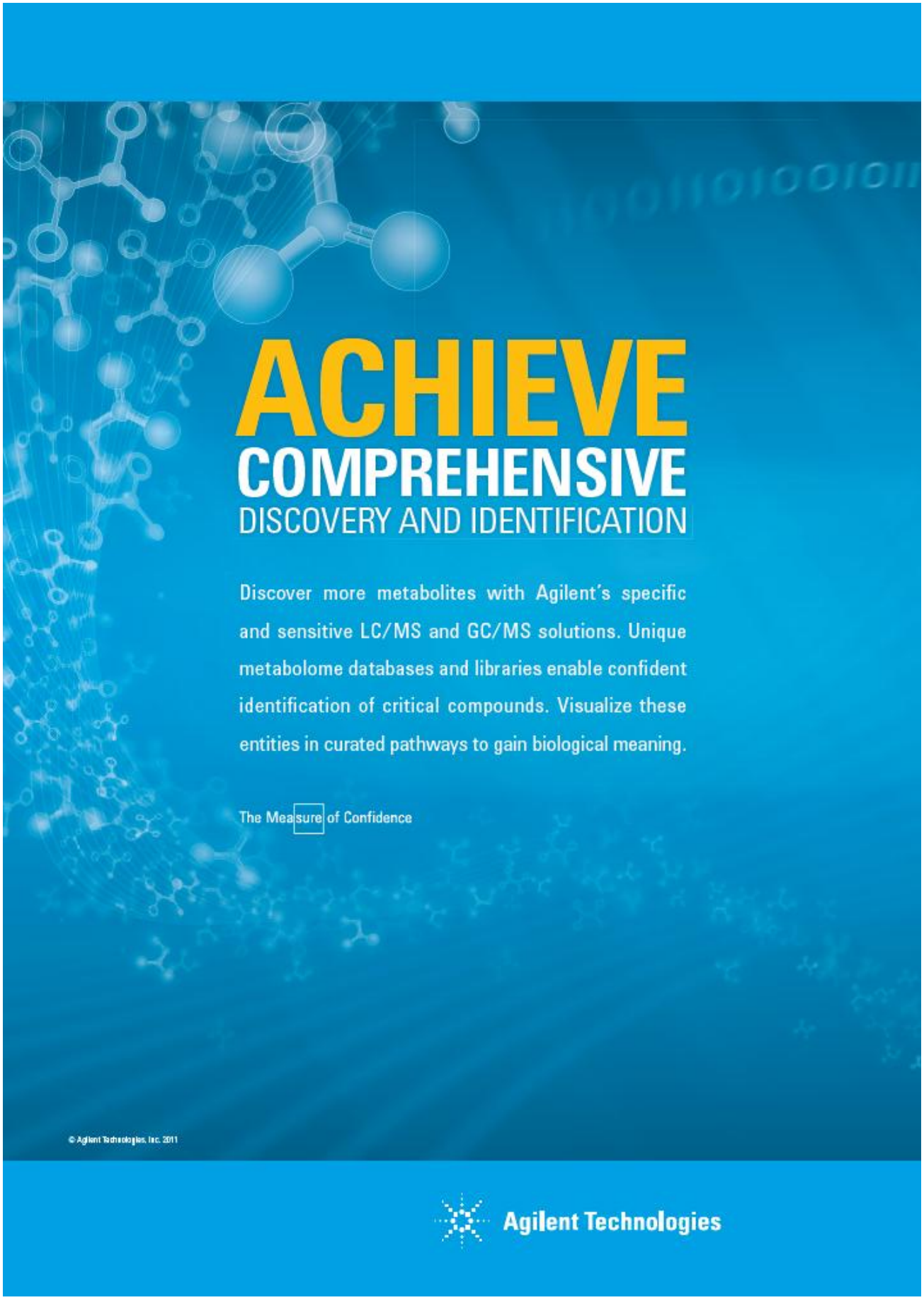
The organisers are grateful for the generous support from the following sponsors:

Gold Sponsors

Agilent Technologies

Silver Sponsors




ACHIEVE
COMPREHENSIVE
DISCOVERY AND IDENTIFICATION
Discover more metabolites with Agilent's specific and sensitive LC/MS and GC/MS solutions. Unique metabolome databases and libraries enable confident identification of critical compounds. Visualize these entities in curated pathways to gain biological meaning.
The Measure of Confidence
© Agilent Technologies, Inc. 2011
Agilent Technologies

The Biotechnology and Biological Sciences Research Council (BBSRC) has a unique and central place in supporting the UK's world-leading position in bioscience. We are an investor in research and training, with the aim of furthering scientific knowledge, to promote economic growth, wealth and job creation and to improve quality of life in the UK and beyond. With a budget of around £445M (2012-13) our vision for world-class 21st century bioscience is structured around curiosity-led research, key strategic research priorities and enabling themes.

BBSRC has identified **Industrial Biotechnology and Bioenergy** (IBBE) as a key strategic research priority in its Strategic Plan[1]. It is anticipated that innovative research programmes will lead to the development of new or improved biological processes and technologies that will contribute towards reducing our dependency on petrochemicals, thereby helping the UK to achieve its ambitious targets for reductions in greenhouse gas emissions, as set out in the Climate Change Act 2008. Moreover, increased adoption of bioenergy and industrial biotechnology will also contribute to the growth of the green economy[2]. BBSRC envisages the enabling technologies of systems and synthetic biology as being crucial for this.

BBSRC is developing mechanisms to promote, encourage and develop the UK IBBE community further, including via our Doctoral Training Partnerships (DTPs), Strategic Longer and Larger (sLoLa) grant scheme, International funding activities and other directed funding mechanisms[3] including highlight schemes. BBSRC also welcomes the submission of proposals relating to the Industrial Biotechnology[4] and Bioenergy[5] strategic priorities to our Responsive Mode closing dates.

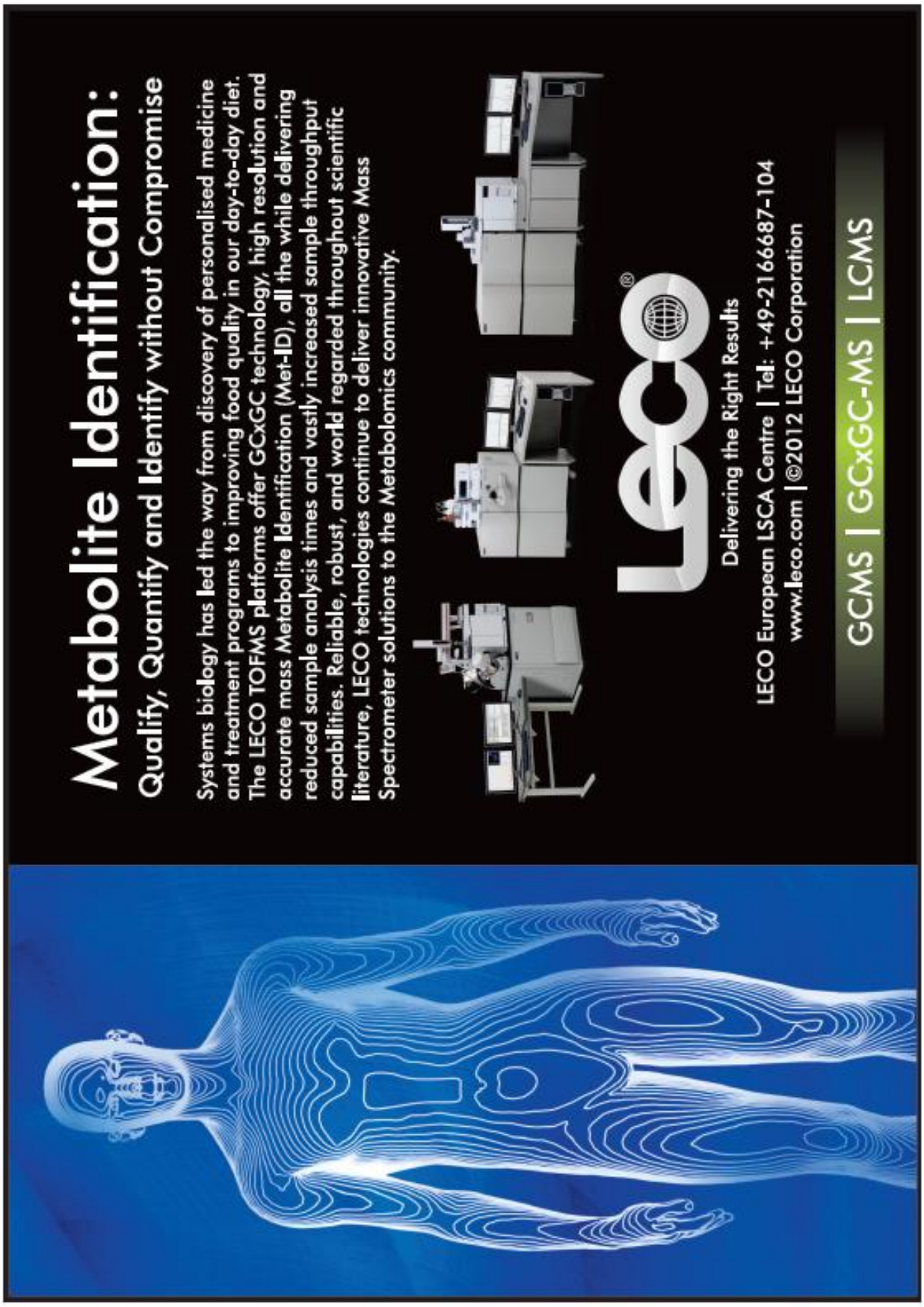
[1] http://www.bbsrc.ac.uk/web/FILES/Publications/strategic_plan_2010-2015.pdf

[2] https://online.businesslink.gov.uk/Horizontal_Services_files/Enabling_the_transition_to_a_Green_Economy__Main_D.pdf

[3] http://www.bbsrc.ac.uk/funding/opportunities/opportunities-index.aspx

[4] http://www.bbsrc.ac.uk/funding/priorities/ibb-industrial-biotechnology.aspx

[5] http://www.bbsrc.ac.uk/funding/priorities/ibb-bioenergy.aspx


Metabolite Identification:
Qualify, Quantify and Identify without Compromise
Systems biology has led the way from discovery of personalised medicine and treatment programs to improving food quality in our day-to-day diet. The LECO TOFMS platforms offer GCxGC technology, high resolution and accurate mass Metabolite Identification (Met-ID), all the while delivering reduced sample analysis times and vastly increased sample throughput capabilities. Reliable, robust, and world regarded throughout scientific literature, LECO technologies continue to deliver innovative Mass Spectrometer solutions to the Metabolomics community.
leco
Delivering the Right Results
LECO European LSCA Centre | Tel: +49-2166687-104
www.leco.com | ©2012 LECO Corporation
GCMS | GCxGC-MS | LCMS

The Organising Committee would like to express their appreciation of the following organisations in providing support to allow us to provide awards to outstanding scientific contributions and in providing information and support for your visit to Manchester.

Plenary Lecture

Metabolomics and the art of deception

Robert Hall

Dept. of Bioscience, Plant Research International, The Netherlands

While the first true metabolomics papers only really started to emerge ca. 10 years ago we have since progressed leaps and bounds with the technologies and their application. No more so than in the field of plant and crop science. Plants are nature's cleverest biochemists and their metabolites are a continual inspiration to the agrochemical and pharmaceutical industries. Furthermore, most of the reasons why we need, use and enjoy plants are centred upon their metabolic composition – from nutritional value to fragrance and from personal hygiene to murderous intention. 80% of what we eat comes from plants and the other 20% wouldn't be available without them. But yet still we know too little about which compounds are made by plants or when and why. Metabolomics and systems-levels approaches are now providing valuable inroads to helping us decipher plant metabolism, understand better its control and determine the mechanisms underlying environmental and genetic perturbation. Only with this knowledge will we be in a position to exploit more efficiently what plants have to offer and design targeted strategies to produce improved crop varieties with enhanced value to help solve, for example, serious future food security issues. Metabolomics still has much more to offer but nevertheless has already come of age – but is what you see what you get?

Speaker Abstracts

Session 1

Applied Metabolomics – Plants

COMPOSITIONAL AND METABOLOMICS ANALYSES OF CROPS DERIVED FROM MODERN AGRICULTURAL BIOTECHNOLOGY

Dr George Harrigan

Monsanto, USA.

Compositional analyses represent a key aspect of the comparative assessment process for new genetically modified (GM) crops. These analyses have consistently shown that GM crops are compositionally equivalent to conventional counterparts. The increasing number of comparative assessments based on metabolomics technologies that are being reported in the scientific literature have further substantiated these findings. Advantages and limitations in the application of 'omics technologies in Regulatory assessments are reviewed.

PROFILING OF SPATIAL METABOLITE DISTRIBUTIONS IN WHEAT LEAVES UNDER NORMAL AND NITRATE LIMITING CONDITIONS

William Allwood (1), Surya Chandra (2), Elon Correa (1), Warwick B. Dunn (4), Yun Xu (1), Laura Hopkins (5), Alyson Tobin (5), Royston Goodacre (1,3), Caroline Bowsher (2)

1. School of Chemistry, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester, M1 7DN, UK.
2. Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK.
3. Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester, M1 7DN, UK.
4. Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, York Place, Oxford Road, Manchester, M13 9WL, UK.
5. School of Biology, Biomolecular Sciences Building, University of St Andrews, St Andrews, Fife KY16 9ST, Scotland UK.

Nitrogen and carbon assimilatory pathways must be co-ordinated in higher plants in photosynthetic as well as in non-photosynthetic tissue. Physiological differences between the basal to mid section and on to the mature tip region of wheat primary leaves confirmed that there was a change from heterotrophic to autotrophic metabolism. This difference between the basal, mid and terminal 20 mm sections of the developing wheat leaf (*Triticum aestivum*) was further studied using metabolic fingerprinting and profiling in order to produce a detailed description of the metabolic response of the wheat leaf as it develops photosynthetic capacity while growing in the presence or absence of nitrate in the nutrient solution. Fourier Transform Infrared (FT-IR) spectroscopy confirmed the suitability and phenotypic reproducibility of the leaf growth conditions used. Principal Component-Discriminant Function Analysis (PC-DFA) revealed distinct clustering between base or tip leaf sections and from plants grown in the presence or absence of nitrate. More detailed analysis of the metabolite profile using Gas Chromatography-Time of Flight / Mass Spectrometry (GC-TOF/MS) combined with multivariate chemometric approaches, univariate data interpretation, and an extremely novel approach within the metabolomics field known as Bayesian Network (BN) based correlation analyses confirmed that the metabolite composition in the base and tip of the primary wheat leaf was distinct. Furthermore this study indicates that such changes represent the different metabolic processes operating in young and mature wheat leaf cells. The operation of nitrogen metabolism also impacted on the levels of amino acids, organic acids and carbohydrates within the wheat leaf. This research represents both a well validated approach to plant metabolic profiling from the technical aspect as well as revealing significant changes in metabolite distributions with respect to leaf position, nitrate supplementation, and the development of photosynthetic capacity.

EARLY METABOLIC RE-PROGRAMMING AND DIVERSION OF LIGNIN BIOSYNTHESIS IN RICE BLAST INFECTION OF *BRACHYPODIUM DISTACHYON* PLANTS

Hassan Zubair, Manfred Beckmann, Kathleen Tailliart and John Draper

Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Ceredigion, UK

Non-targeted metabolite fingerprinting using flow infusion electrospray (FIE) MS in a linear ion trap provided a primary discovery tool to investigate the re-programming of plant's early defence metabolism in pre-symptomatic leaf tissues of two *Brachypodium distachyon* (*B. distachyon*) ecotypes, ABR1 (susceptible) and ABR5 (resistant), during infection with *Magnaporthe grisea* (*M. grisea*). Fourier-transform ion cyclotron resonance (FT-ICR) MS was used for obtaining accurate masses for the explanatory (*m/z*) bins and the signals were identified and confirmed by an accurate mass database MZedDB search at less than 1ppm mass accuracy and by comparing MS/MS fragmentation patterns with available standards.

In susceptible host-pathogen interactions, the metabolite fingerprinting showed an early metabolic shift between 24-28 h after inoculation (i.e. during the penetration of leaf epidermal cells by the fungal pathogen). A disruption of the TCA cycle (energy metabolism) and amino acid biosynthesis was evident during this phase along with effects on the levels of trisaccharide and some flavonoids. In resistant host-pathogen interactions, however, the metabolic response to *M. grisea* infection was much robust and was prominent as early as 8 h after inoculation. By 24 h (before the start of fungal penetration) infected ABR5 plants had already accumulated specific antifungal compounds (e.g. sphingofungin E) at significantly higher levels. A range of further compounds were accumulated at significantly higher levels in ABR5 plants by 24 h, including phospholipids such as PC(18:2), PC(18:3) and 2-16:1-lysoPG, along with pantothenate, melibionate and 2-aminoadipate.

The changes associated with phenylpropanoid metabolism and lignin synthesis were characterised by targeted LC-MS/MS PDA analysis of the phenolics extracts after an initial screening with non-targeted FIE-MS fingerprinting, which suggest alteration in levels of phenylpropanoid metabolites at later (>48 h) stages of the *M. grisea* infection, when visible symptoms started to appear. In infected ABR1 plants, these changes were dominated by a disruption in phenylpropanoid metabolism and lignin synthesis compared to ABR5 plants. A range of hydroxycinnamic acid conjugates (-quinate, -threonate and -glucoside conjugates) were found to have accumulated in infected ABR1 plants, suggesting modulation in three of the key phenylpropanoid pathway enzymes, including cinnamoyl-CoA reductase (CCR), cinnamoyl alcohol dehydrogenase (CAD) and hydroxycinnamoyl-CoA quinate: shikimate hydroxycinnamoyl transferase (HCT). Transcriptional and proteomic analysis including blast-n search, phylogenetic analysis, protein sequence alignment and active site determination of these enzymes have identified important candidate genes for these enzymes. Host gene expression analyses of CCR enzyme by q-RT-PCR and CCR enzyme activity potentially modulated by the invading pathogen are currently under investigating.

We conclude that although successful fungal pathogen down-regulate the primary (energy and amino acid) metabolism at a very early phase of the infection process in susceptible plants, but they rely on subtle manipulation of the plant's secondary phenylpropanoid metabolism as well as they invade plant tissue.

Speaker Abstracts

Session 2

Applied Metabolomics - Human Health and Nutrition

METABOLITE PROFILING REVEALS A COHERENT SIGNATURE OF A DIETARY Ω-3 LC-PUFA SUPPLEMENTATION IN MICE AND HUMANS

Tobias Ludwig (1), Manuela Sailer (2), Kurt Gedrich (2), Alexander Haag (2) and **Hannelore Daniel (2)**

1. Clinical Nutritional Medicine, ZIEL – Research Center for Nutrition and Food Sciences, Technische Universität München (TUM), Gregor-Mendel-Strasse 2, 85350 Freising-Weihenstephan, Germany
2. Molecular Nutrition Unit, ZIEL – Research Center for Nutrition and Food Sciences, Technische Universität München (TUM),Gregor-Mendel-Strasse 2, 85350 Freising-Weihenstephan, Germany

Dietary ω-3 long-chain polyunsaturated fatty acids (ω-3-LC-PUFA) have been demonstrated to affect a multitude of biological processes. As constituents of the cell membrane phospholipids they serve as substrates of COX and LOX pathways which lead to highly active lipid-mediators. We employed a LC-MS/MS method to analyze the changes in phospholipids, lysophospholipids (LP) and sphingomyelins (SM) that occur in C57BL/6 mice during a 12 week feeding trial when a control (13 kJ% fat) or high fat diets (48 kJ% fat) without or with ω-3-LC-PUFA (EPAX, 7.5% w/w) were provided. At the end of the trial, plasma, liver as well as mesenteric and epididymal fat depots were collected, extracted and submitted to profiling. In addition, a human trial with 16 volunteers undergoing a supplementation for 24 days with 600 mg n-3-LC-PUFA (360 mg EPA + 240 mg DHA + 24 mg Vit. E per day) was performed with metabolite profiling from dry blood spots before, during and after the trial. The ω-3 LC-PUFA supplementation caused most impressive changes in numerous of the lipid entities in all mouse tissue samples as well as in plasma. For some sub-classes of metabolites, the ω-3 fatty acids reversed the signature of the HF diet similar to that observed in control mice – mainly in the fat depots. Although each compartment provided a tissue-specific signature of changes in the lipid entities, there were coherent alterations that span over all organs (liver, fat depots and plasma). Amongst others, PC aa 32:1 decreased in concentration around 3-fold in all compartments during supplementation while PC aa 36:5 increased around 3-fold in plasma as well as tissues. In humans, PC aa 36:5 was as well the most responsive entity that increased during the supplementation followed by a decline in the wash-out period. In summary, our metabolite profiling approach demonstrates coherent changes in subclasses of complex lipids upon a dietary supplementation of ω-3 LC-PUFA that occur in liver, different fat depots but also in blood and that may serve as marker metabolites reflecting the supply status. Moreover, dry blood spot analysis (finger prick) as a minimal invasive sampling proved to work reasonably well for metabolite profiling in humans.

A DATA DRIVEN STRATEGY TO DISCOVER DIETARY BIOMARKERS AND VALIDATE NEW DIET RECORDING TOOLS SUITABLE FOR EPIDEMIOLOGICAL STUDIES

John Draper, Amanda Lloyd, Manfred Beckmann, Sumanto Haldar, Chris Seal and John Mathers

IBERS, Aberystwyth University, UK

Diet has a major effect on human health but even after decades of research it has proved difficult to demonstrate in epidemiological studies causative relationships between consumption of specific foods and a reduction in the risk of major chronic disease. To strengthen the evidence base for dietary recommendations it is essential to have robust methodologies both for measuring dietary exposure and to understand the impact of individual 'nutritional metabotype' variation in humans. Conventional methods of measuring habitual dietary exposure such as Food Frequency Questionnaires (FFQs) depend upon food intake estimates and are subject to errors, which can confound interpretation of subsequent data. Several reports have described the targeted analysis, in blood and/or urine samples, of specific metabolites known from previous detailed analytical work to be present in individual foods. Such chemicals could potentially provide direct biomarkers of dietary exposure, however, the biotransformation of many dietary metabolites is often complex and to date putative biochemical markers are available for only a relatively small number of specific foods and food components. Our recent studies [1-3] have demonstrated the use of non-targeted metabolite fingerprinting and machine learning [4] to identify putative dietary biomarkers in post-prandial urines after exposure to specific foods. We describe our current studies evaluating how this technology can be translated into an epidemiological context in which habitual food exposure in human populations is determined by analysing randomly sampled overnight urines.

Most comprehensive FFQs and diet diary methods require participants to record the consumption of a large number (often several hundred) of different foods components which places considerable burden on volunteers and researchers trying to interpret the information. Descriptors of individual FFQ food components vary in degree of distinctiveness and consumption patterns generally typical of each food component display great variability. We describe a data-driven procedure to determine first of all which major food components are appropriate for biomarker development and in the future will collaborate with nutritionists to validate new quantitative dietary assessment methods suitable for large scale population surveys.

The habitual diet of 68 participants was assessed using 4 Food Frequency Questionnaires over 3 months and individuals assigned to different consumption frequency classes for 58 dietary components. Flow Infusion Electrospray-Ionization Mass Spectrometry followed by supervised multivariate data analysis was used to determine whether urine chemical composition was related to specific differences in the consumption levels of each food. Our data indicate that foods are eaten habitually in one of 5 basic patterns differing in range and distribution of consumption frequency. 24hr, overnight and fasted-state urines proved useful for biomarker lead discovery using habitual citrus exposure as a paradigm. For all foods their distinctiveness and consumption frequency range had an influence on whether differential dietary exposure could be detected. Analysis of model output statistics indicated foods for which biomarker lead-discovery was feasible. Metabolites proposed previously as acute intake biomarkers of citrus fruits, oily fish, wholegrain foods, red meat and coffee were shown to be explanatory of habitual exposure. A significance threshold in modelling output statistics was determined to guide discovery of potential biomarkers in other foods. We conclude that this data-driven strategy can identify urinary metabolites associated with habitual exposure to specific foods.

[1] Favé G *et al.,* (2011) Metabolomics 7:469-484; [2] Lloyd *et al.,* (2011) Br. J. Nutr.106:812-824; [3] Lloyd *et al.,* (2011) Am. J. Clinical Nutrition 94: 981-991; [4] Beckmann *et al.,* (2008) Nature Protocols 3:486-504.

MOUNTAINS OUT OF MOLEHILLS? THE ROLE OF EXPERIMENT DESIGN, QUALITY ASSURANCE, AND PARSIMONIOUS REGRESSION MODELS, IN EFFECTIVE CLINICAL METABOLOMICS BIOMARKER DISCOVERY

David Broadhurst

Department of Medicine, University of Alberta, Canada

Many clinical metabolomics, and other high-content or high-throughput, 'omic experiments are set up such that the primary aim is the discovery of biomarker metabolites that can discriminate, with a certain level of certainty, between nominally matched 'case' and 'control' samples. However, it is unfortunately very easy to find markers that are apparently persuasive but that are in fact entirely spurious. The main types of danger, which are not entirely independent of each other, include: bias in patient selection; inconsistent biobanking; poor choice in clinical endpoint; inadequate sample size; inappropriate choice of machine learning methods; inadequate model validation; poor experiment design; minimal and ineffective lab based quality assurance protocols. Many studies fail to take these issues into account, and thereby fail to discover anything of true significance, or more seriously report spurious findings which prove impossible to validate. Here I summarise these problems, and provide pointers to assist in the improved design and evaluation of metabolomics experiments, thereby allowing robust scientific conclusions to be drawn from the available data.

The statistical methodologies presented will focus mainly on the use of pooled QC samples, integrated into the experimental design, enabling the researcher to perform post-hoc assessment of metabolite reproducibility (experimental/biological signal-to-noise ratios), and thus remove metabolites with low information content before subsequent statistical modelling. I will also explain the utility of projecting of these QC data through any subsequent statistical models in order to assess the precision of predicted outcome. In addition a robust non-linear method of correcting for unavoidable peak area attenuation within and between analytical batches will be presented. This is version 2.0 of the QC-RLSC algorithm described in Dunn *et al.* [1].

[1] Dunn, W.B. *et al.* (2011) Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols, 6(7): 1060-1083.

Speaker Abstracts

Session 3

Student and Postdoctoral Presentations

METABOLOMICS APPROACH FOR THE DIFFERENTIATION BETWEEN ORGANIC AND CONVENTIONAL GROWN CARROTS

E. Cubero and A. Maquet

Directorate General Joint Research Centre Institute for Reference Materials and Measurements, Belgium

A growing market share and a premium price of organically grown products are the background for the need to develop analytical methods which aim to discriminate conventionally and organically grown agricultural products. Mass spectrometry-based profiling techniques are increasingly being used to study how the metabolome of an organism changes as a result of in tissue differentiation, disease or in response to environmental stressors. In this study, different metabolite extraction methods were investigated and their suitability to profile the metabolome of carrots assessed. The best performing method was used to profile the metabolome of organic and conventional carrots using ultraperformance liquid chromatography-time-of-flight-mass spectrometry (UPLC-TOFMS). Using this method, a comparison of the metabolome of organic and conventional carrots (*Daucus carota L.*) harvested from 2005 revealed significant differences in a number of metabolites. Our studies show that the plant metabolism may be affected by the agricultural system employed and that UPLC-TOF-MS profiling methodology could be a promising approach to investigate specific differences in conventional and organically grown carrots.

TARGETED METABOLIC PROFILING OF UMBILICAL CORD BLOOD REVEALS PROMISING PROGNOSTIC MARKERS, AND INSIGHT INTO THE PATHOGENESIS, OF HYPOXIC-ISCHAEMIC ENCEPHALPATHY.

Stacey N. Reinke (1), B.H. Walsh (2), B.D. Sykes (3), G.B. Boylan (2), L.C. Kenny (4), D.M. Murray (2), and D.I. Broadhurst (1)

1. Department of Medicine, University of Alberta, Canada
2. Neonatal Brain Research Group, Department of Paediatrics and Child Health, Cork University Maternity Hospital, Ireland
3. Department of Biochemistry, University of Alberta, Canada
4. Department of Obstetrics and Gynaecology, University College Cork, Ireland

Perinatal asphyxia is a medical condition resulting from interruption of the oxygen supply to an infant around the time of birth. Approximately one quarter of asphyxiated infants will develop hypoxic-ischaemic encephalopathy (HIE), with metabolic acidosis, abnormal neurology, multi-organ dysfunction and a risk of long-term neurological sequelae. HIE occurs in approximately 2 per 1000 live births in the developed world, and causes 1 million neonatal deaths globally each year. The long term outcome for HIE-affected infants varies considerably depending on severity of encephalopathy, but includes cerebral palsy, severe cognitive delay, and hearing or visual loss. Currently, the only effective therapeutic intervention is induced hypothermia. Hypothermia must be initiated within six hours of birth to be effective; for this reason, a quick, cost-effective, non-user dependent, reproducible method for defining severity of HIE, and likely prognosis, is an urgent clinical need. In addition, the biochemical consequences, and thus pathophysiological mechanisms of brain injury in HIE are not clearly understood hampering the development of new therapeutic strategies. We report the results of a nested case/control targeted metabolomic study of umbilical cord blood derived from full-term infants with signs of perinatal asphyxia (with and without HIE) and matched healthy controls. Samples were analyzed using Direct Flow Infusion MS/MS (Biocrates AbsoluteIDQ kit) and ^{1}H-NMR spectroscopy (Chenomx workflow), providing quantitative results for acylcarnitines, glycerophospholipds, sphingolipids, amino acids, biogenic amines, and metabolites of central energy pathways. These data expose wide-spread disruption to not only energy pathways, but also nitrogen and lipid metabolism in both asphyxia and HIE. In addition, a logistic regression model using 5 metabolites clearly delineates severity of asphyxia and classifies HIE infants with an area under Receiver Operator Characteristic curve of 0.93. Our results show that a robust highly reproducible multi-platform targeted approach to metabolomic analyses using accurately phenotyped and meticulously biobanked blood samples provides an effective springboard for the development of a clinically effective prognostic blood test and new therapeutic strategies.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY CALIBRATION TRANSFER AND METABOLOMICS DATA FUSION

Andrew Vaughan (1), Warwick B. Dunn (2,3), J. William Allwood (1), David C. Wedge (1), Fiona H. Blackhall (4), Anthony D. Whetton (5), Caroline Dive (4) and Royston Goodacre (1,2)

1. School of Chemistry, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester, M1 7DN, U.K.
2. Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester, M1 7DN, U.K.
3. Centre for Advanced Discovery & Experimental Therapeutics (CADET), Central Manchester NHS Foundation Trust and School of Biomedicine, University of Manchester, Manchester Academic Health Science Centre (MAHSC); York Place, Oxford Road, Manchester, M13 9WL, U.K.
4. Clinical and Experimental Pharmacology Group, Paterson Institute for Cancer Research and Manchester Cancer Research Centre (MCRC), Manchester Academic Health Science Centre, University of Manchester, Wilmslow Road, Withington, Manchester, M20 4BX, U.K.
5. School of Cancer and Enabling Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, M20 3LJ, U.K.

Metabolic profiling is routinely performed on multiple analytical platforms in order to increase the coverage of detected metabolites. Biological samples from a study can also be distributed between instruments of the same type in order to share the workload between different laboratories. The ability to combine metabolomics data arising from different sources is therefore of great interest, particularly for large-scale or long-term studies, where samples must be analysed in separate blocks, often months or years apart. This is not a trivial task, however, due to differing data structures, temporal variability and instrumental drift. Data fusion is the process of combining data from multiple sources in order to achieve improved inferences than could be gained by the use of a single data source alone, and calibration transfer methods can be used to eliminate or reduce the variation in response observed between these sources.

In this study, we collected blood serum and plasma samples from 29 subjects suffering from small cell lung cancer, and analysed each sample on two separate liquid chromatography-mass spectrometry (LC-MS) instruments. The response matrices from the two instruments were compared and then transformed so that they could be combined. We describe a method for mapping retention times and matching features between instruments, and a low-level fusion approach for combining the raw data for metabolite feature grouping and identification. A mid-level approach is described for fusing subsets of matched features for subsequent use in calibration transfer models. We show that these methods are successful at mapping the response of one LC-MS instrument to another, and allow us to merge the data from different samples analysed on different instruments. This data fusion is assessed by multivariate cluster analysis methods, and also in a clinical context.

RHEUMATOID ARTHRITIS: TARGETED AND GLOBAL METABOLITE PROFILING TO STUDY DISEASE PROGRESSION

Filippos Michopoulos (1), Niki Karagianni (2), Georgios Theodoridis (1), Georgios Mosialos (3), Ian D Wilson (4) and Georgios Kollias (5)

1. Department of Chemistry, Aristotle University of Thessaloniki, 54124 Greece
2. Biomedcode Hellas, Vari, Athens, 16672 Greeece
3. School of Biology, Aristotle University of Thessaloniki, 54124 Greece
4. Drug Metabolism and Pharmacokinetics IM, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK
5. Institude of Immunology, BSRC Alexander Fleming, Vari, Athens, 16672 Greeece

Autoimmunity is the biological condition where an organism's immune system does not recognize its own constituents resulting to a series of immune responses against its own cells and tissues. Rheumatoid arthritis (RA) is one of the most common autoimmune diseases that principally attacks synovial joints. The disease starts with an inflammation around the joint cartilage, which can lead to destruction of articular cartilage and ankylosis, ultimately leaving patients experiencing disabling conditions as well as severe pain. The female population in western countries is among the most commonly affected with a disease prevalence of 1% of the population worldwide. There is no early and specific sign, symptom or diagnostic feature (biomarker) to distinguish RA from other forms of inflammatory arthritis. The disease diagnosis of RA is still mainly based on clinical assessment in combination with x-ray imaging to monitor joint damage. Laboratory tests using the rheumatoid factor have been central to the diagnostic exploration but have very low sensitivity in early disease. The need for a reliable diagnostic tool based on an early disease biomarker is still unmet.

Here we used mass spectrometry-based targeted and untargeted metabolic profiling to study the progress of RA in a rodent (mouse) model. Global metabolite profiles of urine, obtained from samples collected on weekly basis for six weeks, from humanized TNF transgenic mice (Tg197), transgenic mice following treatment of Remicade at 10mg/kg as well as from their wild-type counterparts revealed clear differences between Tg and control animals. Similarities between the urinary metabolic profiles of control and drug-treated animals demonstrated a response to drug treatment. Similar results were obtained when plasma samples from this study, was analysed with reversed and normal phase chromatography. Lipid profiles obtained from whole joints after extraction with dicloromethane/methanol revealed a clear separation between control and transgenic animals following multivariate statistical analysis (PCA). Targeted metabolite analysis using a reversed-phase ion-pairing separation of hydrophilic intracellular metabolites obtained from the whole mouse joint (after extraction with acetonitrile/water 50/50) showed metabolic changes associated mainly to central carbon metabolism, nucleotides and aminoacids metabolism.

These preliminary results clearly show the potential for UHPLC-MS-based techniques to illuminate the disease process for RA in mice and their response to therapy.

VARIATION IN THE METABOLITE CONTENTS OF BULBS *NARCISSUS PSEUDONARCISSUS* FROM DIFFERENT LOCATIONS AND OF DIFFERENT AGE MEASURED BY GC-FID AND GC-MS.

Muhammad Nadeem Akram, Robert Verpoorte and Barbora Pomahacova

Natural Products Laboratory, Institute of Biology, Leiden University, 2300 RA, Leiden, The Netherlands.

Narcissus is a spring flowering plant which belongs to family *Amaryllidaceae*. *Narcissus* is mostly grown for decorative purposes but research has shown that its alkaloids possess a lot of useful biological activities. These alkaloids with activities include galanthamine lycorine, hamanthamine, tazitine, homolycorine and many others. The aim was to investigate whether there are differences in the alkaloid content and the total metabolic profiles of bulbs of the same species and cultivar grown in different locations, and if so, to identify the metabolites responsible for the variation.

In this study bulbs of *Narcissus pseudonarcissus cv Carlton* from different locations i.e. The Netherlands and U.K. are checked for variations. An acid-base method was used for the extraction and samples were analysed by GC-FID and GC-MS. Results have shown that the alkaloids can be quantified by using a single standard i.e. Galanthamine hydrobromide which is easily available in the market with the help of a correction factor used. The results have also shown that the alkaloid contents from the same variety at different locations vary from each other. The results have also showed that the alkaloid contents of bulbs also vary on the basis of age of the bulb. Some alkaloids are found to be more in quantity in younger bulbs than the older bulbs.

A BAYESIAN APPROACH TO METABOLITE PEAK IDENTIFICATION IN LIQUID CHROMATOGRAPHY MASS SPECTROMETRY EXPERIMENTS

Rónán Daly (1), Simon Rogers (1), Rainer Breitling (2)

1. University of Glasgow
2. University of Groningen

In recent years, the use of liquid chromatography coupled to mass spectrometry (LC-MS) has enabled the high-throughput profiling of the metabolic composition of biological samples. However, the resulting surfeit of data poses problems, with extensive processing needed in most cases to make sense of the mass of information. Recent efforts have provided much relief in this regard [1, 2], but much work remains in improving the accuracy and reliability of these automated techniques.

Here, we present a novel computational technique that is designed to deal with a problem inherent in MS data, that of detecting (and potentially removing) derivatives of a substance of interest, which are created as artefacts of the technology and tend to make up roughly 80% of detected signals. Our technique uses a Bayesian statistical model, that not only identifies derivatives, but automatically clusters them in order to increase the detection power for the peak of interest. The arbitrary thresholds and unstable maximum operators of currently used greedy algorithms are eschewed in favour of a statistical clustering treatment that inherently tolerates noise in both the peak intensities and peak shapes. Also, because of the use of the Bayesian paradigm, a measure of confidence in the derivative detection can be given. This technique can be easily incorporated into existing open processing pipelines, such as mzMatch, in order to increase the accuracy of the analysis by eliminating spurious base-peak detections. It can also be easily joined to previous work on Bayesian metabolite identification [3].

[1] Andris Jankevics, *et al.* Metabolomics, 2011; [2] Richard A. Scheltema, *et al.* Analytical Chemistry, 83(7):2786-2793, 2011; [3] Simon Rogers, *et al.* Probabilistic assignment of formulas to mass peaks in metabolomics experiments. Bioinformatics, 25(4):512-518, 2009.

Speaker Abstracts

Session 4

Applied Metabolomics - Drug Discovery and Pharma

METABOLITES TO SYSTEMS – UNDERSTANDING THE EFFECTS OF DRUGS

Ian D. Wilson

Imperial College London, UK

Untargeted global metabolic profiling (metabonomics/metabolomics) provides one methodology for the detection of potential biomarkers using a hypothesis free approach. An example relating to the long term effects of the administration of ethanol to rodents will be provided examining the effects of ethanol treatment on liver, plasma and urinary, UPLC-MS-derived, metabolome profiles, and the dependency of those profiles on the type of animal model used. From this type of study potential metabolites that could serve as biomarkers of ethanol exposure can be derived that might be useful for monitoring the onset of toxicity etc., but without necessarily illuminating mechanisms. However, whilst hypothesis free, such approaches should be hypothesis generating. This will be illustrated by in vitro and in vivo studies on the hepatotoxin paracetamol which highlighted metabolites involved in the glutathione pathway, such as 5-oxoproline and ophthalmic acid, as potential biomarkers of liver toxicity. The hypothesis that resulted from the detection of these compounds did however, require both validation and a better understanding of why these compounds might reflect paracetamol toxicity. Experimental validation of these metabolites as biomarkers was pursued via specific, targeted, UPLC-MS-based methods. In vitro experiments, undertaken using a hepatocyte-derived cell line were used to enable a deeper mechanistic understanding of the linkage between the biomarkers and the underlying biology to be derived via the construction of a systems biology model that linked these biomarkers to the underlying biochemistry. This systems model also allowed predications to be made about biomarker behaviour under various conditions and provided an insight into why these particular metabolites could indeed represent biomarkers of the reactive metabolite-based toxicity of the drug. The application of this approach to understand the effects of a glutathione-depleting drug such as paracetamol will be used to illustrate the process of going from biomarker detection, through biomarker validation and then the development of a systems biology model of hepatotoxicity.

METABOLIC PROFILES OF URINE AND SERUM FROM CHILDREN WITH ACETAMINOPHEN OVERDOSE

Laura K. Schnackenberg (1), Jinchun Sun (1), Ando, Yosuke Ando (2), Xi Yang (1), Qiang Shi (1), James Greenhaw (1), Lisa Pence (1), Sudeepa Bhattacharyya (3), Ke Yan (4), Pippa Simpson, (4), William F. Salminen (1), Donna L. Mendrick (1), James, Laura James (3) and **Richard D. Beger (1)**

1. National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079;
2. Medicinal Safety Research Laboratories, Daiichi Sankyo Co. Ltd., Tokyo, Japan;
3. Arkansas Children's Hospital Research Institute, Little Rock, AR 72202
4. Medical College of Wisconsin, Milwaukee, WI 53226

Drug-induced hepatotoxicity is one of the major reasons for drug recall and hence it is of major concern to the FDA and to consumers. Overdose of acetaminophen (APAP), a widely used over-the counter analgesic and antipyretic drug, can cause acute liver failure. The results of our and other preclinical studies indicated that serum acylcarnitines and urinary amino acids related to oxidative stress are potential biomarkers of APAP-induced hepatotoxicity. Therefore, the early metabolic profiling results of an ongoing study with children (aged 2-18 years) that were hospitalized following toxic APAP ingestion were quantitatively profiled by LCMS and compared to values in normal healthy subjects are provided. Serum and urine samples were collected daily from each patient during the hospitalization for treatment of APAP overdose. Indicators of hepatotoxicity (serum alanine aminotransferase [ALT]) and APAP-protein adducts, quantified by HPLC-EC) were compared between groups. Acylcarnitine and amino acid values were stratified by the toxic APAP protein adduct concentration of 1 nmol/mL, where > or = to 1.0 nmol/mL adduct was deemed "high" and < 1.0 nmol/mL adduct was considered "low" in the APAP overdose patients. Non-parametric tests were used to compare metabolic profiles between the APAP overdose patients and the normal healthy subjects and between high and low adduct groups within the APAP overdose group. Palmitoylcarnitine was higher in the APAP overdose patients than the healthy control subjects and the maximum peak of palmitoylcarnitine ($p<0.05$) and ALT ($p<0.001$) were each higher in the high adduct group compared to the low adduct group. In addition, cysteine, methionine, taurine and 5-oxo-proline were higher in the APAP overdose group than the normal healthy controls. No differences were observed in the urine amino acids within the APAP overdose patients when the results were stratified by alanine aminotransferase levels (< or > 1000 IU/L) or acetaminophen protein adduct levels (< or > 1.0 nmol/mL serum). Urine amino acids are increased in children with acetaminophen overdose, compared to normal healthy controls, but did not associate with accepted measures of toxicity severity such as serum ALT or acetaminophen protein adducts. Additional factors, such as timing of antidotal treatment with N-acetyl cysteine, may contribute to metabolic profiles in the clinical setting of APAP overdose

DETERMINING MECHANISMS OF ANTI-PARASITIC DRUG ACTION BY UNTARGETED METABOLOMICS

Darren Creek (1,2) and Michael Barrett (1)

1. Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, United Kingdom
2. Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Flemington Rd, Parkville, Victoria 3010, Australia.

The discovery, development and optimal utilisation of pharmaceuticals can be greatly enhanced by knowledge of the modes of action, yet many drugs currently on the market act by unknown mechanisms. Untargeted metabolomics offers the potential to discover modes of action for drugs that perturb cellular metabolism. We studied the metabolomic profile of in vitro cultures of Trypanosoma brucei, the causative parasite of human African trypanosomiasis, following treatment with clinically used drugs or experimental trypanocidal compounds. Development of the IDEOM software allowed rapid interpretation of high resolution LC-MS data for the detection of drug-induced changes to cellular metabolism in an untargeted manner. In a proof-of-concept study, eflornithine, a known inhibitor of ornithine decarboxylase, induced significant accumulation of the enzyme substrate, ornithine, and depletion of product, putrescine. The metabolic profiles induced by other clinically used trypanocides showed remarkably few changes, indicating that the modes of action for these drugs are not due to inhibition of metabolic enzyme targets. Nevertheless, detection of drug-derived metabolites for nifurtimox and melarsen oxide provide important mechanistic information for these drugs. We have also applied this method to experimental anti-parasitic compounds, with detected metabolic changes demonstrating modes of action for compounds with previously unknown targets. Untargeted metabolomic analysis of drug action allows detection and semi-quantitative analysis of endogenous, and drug-derived, metabolites, providing unbiased target discovery and validation to assist with optimisation of new drug candidates.

Speaker Abstracts

Session 5

Analytical Advances and Imaging

NEW PROSPECTS FOR METABOLIC PROFILING AND IMAGING USING SECONDARY ION MASS SPECTROMETRY

Helen L Kotze (1), Emily G Armitage (1), John S Fletcher (1), Alex Henderson (1), Kaye J Williams (2), John C Vickerman (1) and **Nicholas P Lockyer (1)**

1. Surface Analysis Research Centre, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester M1 7DN, UK
2. School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK

Mass spectrometry imaging (MSI) has in recent years seen a very rapid growth, particularly in biological applications e.g. the localization of drugs and metabolites in tissue sections [1,2]. Amongst the range of techniques available, Secondary Ion Mass Spectrometry (SIMS) is particularly well-suited to elemental and small molecule analysis and imaging [3]. The advantages of imaging SIMS over other chemical imaging techniques such as fluorescence microscopy, autoradiography, or the range of microspectroscopy techniques is the combination of high spatial resolution, sensitivity and chemical selectivity, and that the sample requires minimal or no preparation for analysis. Recent technological advances in the 'primary' ion beam systems used to probe the sample have delivered new capabilities for chemical imaging in 2D and 3D [4]. This presentation will review the current state-of-the-art regarding Time-of-Flight SIMS analysis of biological systems including tissue sections and single cells. Examples will include the metabolic profiling and imaging of hypoxic vs normoxic regions in multicellular tumour models, illustrating the potential use of this technology in cancer studies.

[1] Chughtai K and Heeren RMA. Chem. Rev. 110, 3237–3277 (2010); [2] Sugiura Y and Setou M J. Neuroimmune Pharmacol. 5, 31-43 (2010); [3] Lockyer NP. Bioanalysis 3, 1047-1051 (2011); [4] Fletcher JS, et al. Mass Spectrom. Rev. 30, 142-174 (2011).

DRUG AND METABOLITES (ENDOGENOUS AND EXOGENOUS) STUDY IN WHOLE BODY ANIMAL BY HIGH DEFINITION MALDI IMAGING

Emmanuelle Claude (1), M. Towers (1), L. Bendahl (2), L. Edwards (1) and L.Fernandes (1)

1. Waters Corporation, Manchester, UK
2. Lunbeck A/S, Denmark

Introduction: Mass spectrometry Imaging (MSI) is increasingly used in pharmacokinetic studies during preclinical studies. It has been recognized as a complementary technique to Whole-Body Autoradiography (WBA), which is traditionally used for approval of a drug by the Food and Drug Administration (FDA) agency. The two main advantages of MSI are cost savings compared to radio-labeling of the drug and the absolute confirmation that the drug and its possibly produced metabolites are indeed visualized. Here, we present the results from a study where two MS based imaging approaches were used to illustrate the spatial distributions of a drug and its metabolites, along with untargeted analysis of endogenous molecules in a whole body section.

Methods: Olanzapine (OLZ) was administrated to rats at 8 mg/kg. Animals were euthanized at 2 or 6 hours post-dose. 30-µm-thick whole-body sagittal tissue sections were mounted on invisible mending tape. A single sagittal section of a whole rat spanned three MALDI plates. CHCA matrix was applied evenly to the sample in several coats using a SunCollect nebulising spray device. Data were acquired using a MALDI SYNAPT G2 mass spectrometer in both MS and MS/MS mode with tri-wave ion guide optics to separate ions according to their ionic mobility in the gas phase. The obtained data sets were subsequently processed using High Definition Imaging (HDI) MALDI software for detailed image analysis.

Preliminary data: OLZ is a drug of which its pharmacokinetics1 have been intensively studied in different animal systems. However, spatial distribution analysis solely by MSI lacks certain, specific information. The two main metabolites of OLZ (m/z 313.15) that have been identified in rat are N-desmethyl OLZ (m/z 299.13) and 2-hydroxymethyl OLZ (m/z 329.14). Typically, drug distribution analysis by MSI is conducted by using a targeted analysis method for the drug of interest, aiming to achieve better sensitivity and specificity, where the molecular ion is selected by a quadrupole, fragmented in a collision cell and finally the product ions m/z separated before detection. In this study, traditional targeted and untargeted MSI analysis experiments have been explored and contrasted as detailed below.

A first experiment was carried out on the 2 h post-dose tissue section in a multiplex targeted MS/MS, approach where the drug and the two known metabolites were MALDI imaged from a single tissue section. A second experiment was performed on the 6 h post-dose tissue section in an untargeted MS approach. Also in this instance, both the drug and the two main metabolites were imaged. However, here a vast amount of information is also generated by the ionisation of the endogenous species present in the whole body tissue section. The final part of the study involved the performance evaluation of isobaric species separation in the gas phase by means of ion mobility separation, which is integrated in the MALDI SYNAPT G2 HDMS instrument, for untargeted MALDI-MS imaging experiments of OLZ and its two main metabolites.

HR-MAS NMR DETECTIONS OF NANO-LITER TISSUE BIOPSIES AND LIVING ORGANISMS

Alan Wong, Xiaonan Li and Dimitris Sakellariou

CEA Saclay, UMR3299 – SIS2M, LSDRM, F-91191, Gif-sur-Yvette Cedex, France

High-Resolution Magic-Angle sample Spinning (HR-MAS) NMR spectroscopy of tissue biopsies combined with chemometric techniques has now emerged as a powerful methodology for metabolonmics NMR, and has led to many important disease diagnosis and environmental assessments [1]. However, the required tissue mass quantity is of the order of 10 mg. Such large mass quantity can compromise the metabolic evaluation because of the high degree of tissue heterogeneity. The tissue availability, such as tumor, is also often a limitation and prevent from the possibility of multi-clinical diagnostic analyses. Moreover, the large tissue mass also renders great challenges for localization analysis.

Here, we present the use of a spinning nano-liter volume NMR detector that optimizes the signal sensitivity for mass-limited tissue analysis [2]. This miniature NMR detector is wirelessly coupled to any standard commercial HR-MAS probe without modifications, making it easy to adapt in any laboratory. Using this spinning nano-liter NMR detector, we show the capability of obtaining high mass-sensitivity and high spectral-resolution 1D and 2D NMR results for nano-gram tissue biopsies (3) and small quantity of nano-size living organisms such as *Caenorhabditis elegans* worms. We also present the results from slow sample spinning (<500 Hz) nano-liter NMR detection [4]. The combine miniature detection with slow spinning greatly minimizes the centrifuge force that is known to be destructive to tissue biopsy and living organism. Lastly, we also present a new and cost-effective methodology for multidimensional microscopy simply using the NMR stray-field with the spinning nano-liter detector. The high sensitivity and resolution demonstrated here from this nano-liter NMR detection offers the possibility of studying few cells and few living organisms by metabolomics NMR.

[1] Beckonert O, *et al.* Nature Protocols 2010;5:1019-1032; [2] Sakellariou D, *et al*. Nature 2007;447:694-697; [3] Wong A, *et al.* Anal Chem 2012;84:3843-3848; [4] Wong A, *et al.* Magn. Reson. Med. 2010;63:269-274.

Speaker Abstracts

Session 6

Applied Metabolomics - Cells to Organisms

ELABORATING THE USE OF METABOLOMICS TECHNOLOGIES TO CELL LAYER RESOLUTION AND CHEMICAL GENETICS SCREENS

Aharoni Asaph

Department of Plant Sciences, Weizmann Institute of Science, Rehovot, Israel.

Metabolite composition analysis through metabolomics approaches offers a powerful tool for decoding biological processes. However, most studies including those applying metabolomics in multicellular organisms have thus far been performed primarily on whole organisms, organs or cell-lines, losing information about individual cell-types within a tissue. In this study we aimed at increasing the cellular resolution of metabolomics assays. Fluorescent Activated Cell Sorting (FACS) was used to dissect fluorescent reporter (GFP)-marked cells from Arabidopsis roots for metabolomics analysis. Here, we present the metabolic profiles obtained from five GFP-tagged lines representing core cell-types in the root. Fifty metabolites were putatively identified, the most prominent groups being glucosinolates, phenylpropanoids, and dipeptides, the latter yet unexplored in roots. A workflow for metabolomics analyses of cell-type populations will be presented. In a different study we elaborated the use of metabolomics to combine with a chemical genetics/genomics approach that uses small molecules to temporarily modulate protein function. Joining metabolomics with chemical genetics/genomics to what we term “ChemoMetabolomics” is a promising future application of both technologies that is expected to facilitate the detection of metabolic modulators, the dissection of the crosstalk in the metabolic network, and the development of hypotheses on how changes in metabolism affect developmental or cellular responses. In the project presented, extracts of Arabidopsis seedlings treated with 120 different cell trafficking-related chemicals were subjected to metabolomics assays. This screen revealed 2 chemicals that reproducibly alter the levels of a specific branch of the phenylpropanoid pathway. We are currently investigating the cellular targets of these 2 molecules in order to identify a yet unknown mode of phenylpropanoids trafficking in the plant cell.

METABOLIC PHENOTYPING OF *CAENORHABDITIS ELEGANS* BY WHOLE ORGANISM NMR SPECTROSCOPY, APPLICATIONS FOR FUNCTIONAL GENOMICS IN AGING AND TOXICOLOGY

Clément Pontoizeau (1), Laurent Mouchiroud (2), Linh-Chi Bui (3), Laurent Molin (2), Nicolas Dallière (2), Pierre Toulhoat (1), Lyndon Emsley (1), Robert Barouki (3), Xavier Coumoul (3), Florence Solari (2) and Bénédicte Elena-Herrmann (1)

1. Université de Lyon, UMR 5280 CNRS/ENS-Lyon/UCBL 1 Centre de RMN à Très Hauts Champs, Villeurbanne, France.
2. Université de Lyon, Centre de Génétique et de Physiologie Moléculaire et Cellulaire,, UMR CNRS 5534, Villeurbanne, France
3. INSERM UMR-S 747, Université Paris Descartes, Paris, France

The model organism Caenorhabditis elegans (*C. elegans*) is widely used to investigate biological processes and disease mechanisms. We developed a robust protocol based on ^{1}H High Resolution Magic Angle Spinning (HR-MAS) Nuclear Magnetic Resonance (NMR) spectroscopy for studying the metabolism of intact *C. elegans* worms. We demonstrated its potential for the characterization of metabolic signatures induced by genetic mutations allowing functional genomics at the system level [1]. Here, we present metabolic signatures of a range of *C. elegans* mutants that provide insight into molecular mechanisms of aging and toxicology.

We investigate aging processes in *C. elegans* by monitoring the metabolic perturbations linked to caloric restriction (CR), a well-known process responsible for lifespan increase in various organisms. We compared during ageing (1-day old and 7-day old) the metabolic profiles obtained by ^{1}H HR-MAS NMR spectroscopy for wild type nematodes and CR mutants eat-2, an established model of CR, or slcf-1 mutants, which mimic caloric restriction when fed ad libitum (2). Metabolic signatures of both ageing and CR in intact *C. elegans* are found to share similarities with signatures previously described from the plasma of non-human primates. Furthermore, we found 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 analyse the metabolic profiles of short-lived *C. elegans* daf-18 mutants and slcf-1;daf-18 double mutants for which lifespan increase is inhibited, to derive metabolic markers of ageing.

Finally, we characterize metabolic fingerprints of *C.elegans* mutants lacking 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] Mouchiroud L. et al. Aging Cell 10, 39 (2011).

NMR FAECAL METABOLIC PROFILING OF MICE FED WITH HIGH FAT DIET AND TWO DIFFERENT FERMENTABLE CARBOHYDRATES.

Ruey Leng Loo (1), Tulika Arora (2), Jelena Anastasovska (3), Glenn R. Gibson (4), Kieran M. Tuohy (4), Raj Kumar Sharma (5), Jonathan R. Swann (4), Eddie R. Deaville (3), Michele L. Sleeth (2), E. Louise Thomas (3), Elaine Holmes (6), Jimmy D. Bell (3) and Gary Frost (2)

1. Medway School of Pharmacy, Universities of Kent and Greenwich, Central Avenue, Chatham Maritime, Kent, ME4 4TB, United Kingdom
2. Nutrition and Dietetic Research Group Department of Investigative Medicine, Imperial College London, Hammersmith Hospital, London W12 0NN, United Kingdom.
3. Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, London W12 0NN, United Kingdom
4. Department of Food and Nutritional Sciences, University of Reading, Reading, RG16 6AP, United Kingdom
5. Division of Animal Biochemistry National Dairy Research Institute, Karnal-132 001 (Haryana), India
6. Department of Surgery & Cancer, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom

Background: Worldwide obesity has doubled since 1980. It increases the risk of developing diabetes, cardiovascular disease and some cancers, as well as increasing the burden of the health bill. Approaches that may reduce obesity are therefore highly sought after.

Aim: The aim of this study was to investigate how inulin and ß-glucan can attenuate weight gain in mice fed with a high fat diet.

Methodology: Male C57BL/6 mice were maintained on high fat diet supplemented with 10% (w/w) cellulose as control, or with either 10% (w/w) inulin or 10% (w/w) ß-glucan for 8 weeks (N = 6 per group). Faecal and caecal gut microbiota changes were evaluated by fluorescent in situ hybridisation. In addition, faecal metabolic profiling was obtained by proton nuclear magnetic resonance (^{1}H NMR). Pair-wise orthogonal projection to latent structure discriminating analysis (OPLS-DA) models between different types of diet intervention and at different time points were calculated separately using faecal ^{1}H NMR spectral data.

Results: No significant difference in cumulative food intake was observed between control and inulin groups. The ß-glucan group showed a significantly lower cumulative food intake from week 4 as compared to control (pt gain).

Speaker Abstracts

Session 7

Data Analysis and Integration with Systems Biology

INDIVIDUAL DIFFERENCES OPEN UP THE ROAD TOWARDS PERSONALIZED HEALTH

Jeroen J. Jansen

Department of Analytical Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

The traditional paradigm to find systematic responses in biological systems is to look for features (*i.e.* metabolites) that either in- or decrease for all comparable biological replicates. However, this may be too limited and too strict: novel data analysis methods may give a dedicated and information-rich view on the heterogeneity in the response of different individuals, essential to answer the questions relevant to biology and medicine in the 21st century—the aeon of Personalized Health.

Metabolomics opens up the potential to observe relations between metabolites, which may change considerably upon treatment—in addition to, or even in absence of changes that follow the traditional paradigm. The INdividual Differences SCALing (INDSCAL) method focuses on such Between Metabolite Relationships (BMR) and their systematic change upon experimental perturbation [1]. Extending the metabolomics data analysis toolbox that consists of 'standard methods' like Principal Component Analysis and (Orthogonal) Partial Least Squares, with INDSCAL will greatly extend the view on the biochemistry of system response.

The variation that underlies these changes is that between the biological replicates that is completely disregarded in the traditional paradigm. However, researchers in all branches of biology—from ecology to personalized health—will be able to elaborate on chemically diverse, alternative responses to the same treatment, the presence of non-responders to a treatment and large variation in response dynamics in many different biological systems. The standard methods—specifically Discriminant Analysis—completely disregard such information and are therefore far too strict to observe such response heterogeneity. As it is encapsulated by the BMRs, this heterogeneity can be highlighted by an extension of INDSCAL, called Simultaneous Component Analysis with INDSCAL Constraints (SCA-IND) [2].

The novel Individual Differences tools will be demonstrated on a dataset from chemical ecology, in which also many medically relevant processes occur.

[1] Jansen, J., et al., Metabolomics, 2012. **8**(3): p. 422-432; [2] Jansen, J., et al., Metabolomics, 2012. **8**(1): p. 94-104.

METABOLIGHTS: A DATABASE FOR COLLECTING METABOLOMICS EXPERIMENTAL DATA USING ISA TOOLS

Reza Salek (1), K. Haug (1), P. Conesa (1), P. de Matos (1), J.L. Griffin (2), C. Steinbeck (1) , P. Rocca-Serra (3), E. Maguire (3) and S.A. Sansone (3)

1. European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD.
2. Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, CB1 9NL, UK/University of Cambridge, Department of Biochemistry, Cambridge CB2 1QW.
3. Oxford e-Research Centre, University of Oxford, 7 Keble Road, Oxford OX1 3QG.

MetaboLights is a resource developed to archive metabolomics experimental evidence, covering raw, processed data and ancillary metadata. It is the first comprehensive, cross-species, cross-platform database that combines curated reference data of metabolites, information about their occurrence and concentration in species, organs, tissues and cell types under various conditions. Protocols documenting how metabolomics experiments were conducted will also be captured and made available.

One of the main submission channels for MetaboLights is the ISA Tools Suite [1, 2]. The ISA software suite comprises several platform-independent components, which run as 'desktop' applications built around ISA-Tab based format. The 'Investigation/Study/Assay' ISA-Tab format, developed to represent experimental metadata independently from the assay technology used, is utilised here to capture the raw file and associated metadata. In order to support the MetaboLights mission, dedicated MIBBI (The Minimum Information for Biological and Biomedical Investigations) and MSI (Metabolomics Standard Initiative) standards compliant, 'ISAconfiguration' files have been produced. Two general settings exist: one for NMR spectroscopy and a second for mass spectrometry based experiments. Furthermore, courtesy of the plugin functionality of ISA Tools, specialized components have been created to support documenting metabolite identifications with the format based on mzTAB (tab-delimited file format standard for proteomics data). This allows pairing of generic components describing experimental set-up with community centric components as this one. Future plans are the development and integration of autoloaders, working with instrument vendors for export format support and addition of metabolomics specific ontologies that currently are needed.

MetaboLights is funded by the BBSRC as a joint project between The Chemoinformatics and Metabolism team at The European Molecular Biology Laboratory- European Bioinformatics Institute (EMBL-EBI) (Christoph Steinbeck) and The MRC Human Nutrition Research (MRC-HNR) (Jules Griffin).

[1] http://www.isa-tools.org/; [2] ISA software suite: supporting standards-compliant experimental annotation and enabling curation at the community level. Bioinformatics (2010) 26 (18): 2354-2356.

FAST METABOLOME- AND GENOME-WIDE ASSOCIATION

René Pool, Harmen, H.M. Draisma and Dorret I. Boomsma

Department of Biological Psychology, Faculty of Psychology and Education, VU University Amsterdam, Van der Boechorststraat 1, 1081BT AMSTERDAM, the Netherlands

Using metabolomics data as phenotypes for genome-wide analysis (GWA) studies, the number of metabolites reported in all study participants determines the number of predicted variables that need to be evaluated for their association with single nucleotide polymorphisms (SNPs). For many metabolomics platforms, the number of metabolites NM reported is large. If single metabolites are related to each other as the substrates and products of enzymatic conversions, including their ratios as phenotypes in GWA can yield associations with genetic polymorphisms that are much stronger compared to the associations with the single metabolites. However, this dramatically increases the number of statistical tests to be performed, e.g. if NM = 100, the amount of ratios NR = NM (NM-1)/2 ≈ 5000. Such amounts of tests can become prohibitive with respect to the computation times needed.

In our new GWA pipeline, we employ the GWA tool FaSTLMM [1]. This application results in an 11-fold computation time reduction compared to conventional tools (e.g. plink [2] or snptest [3]) and hence contributes to the practical feasibility of GWA studies using metabolomics data (single metabolites and their ratios). The computing time reduction is accomplished by a reformulation of the linear mixed model framework that FaSTLMM utilizes. In addition, FaSTLMM links to advanced third-party math libraries that enable multi-threading and that are freely available to academic users.

A GWA run of a single phenotype over ~ 4 million SNPs requires roughly 22 hours of computing time on a single CPU using plink or snptest. Even if the NR analyses are distributed across multiple nodes, the computation time remains considerable. We applied FaSTLMM to our metabolomics data that were generated in human serum using the Biocrates kit that reports concentrations of NM = 163 metabolites, measured in 1372 participants. As a benchmark, the FaSTLMM association p-values compare well to those produced by plink and snptest. By choosing an appropriate distribution of the analyses over the available nodes, we show that we can perform NR ≈ 13000 GWA runs for the ratios within 5 days of wall-clock time. We provide intuitive visualizations of the typically enormous amounts of data resulting from the GWA analyses of all metabolic traits data and to post process them. Furthermore, we will show the preliminary results that come from this study.

We conclude that using FaSTLMM in our GWA pipe line, enables processing large amounts of metabolomics data due the relatively low amount of computation time involved. The outcomes of FaSTLMM are in agreement with those from plink and snptest, using the same data sets.

[1] C. Lippert, *et al.,* Nat Meth 8, 833 (2011); [2] S. Purcell, *et al.,* The American Journal of Human Genetics 81, 559 (2007); [3] J. Marchini, *et al.,* Nat Genet 39, 906 (2007).

Speaker Abstracts

Session 8

Applied Metabolomics - Human Disease

GOING TO THE EXTREMES: USING LIPIDOMICS TO UNDERSTAND THE CAUSES OF INSULIN RESISTANCE

Michael Eiden (1), Albert Koulman (1), Michael Osei (1), Mojgan Masoodi (1), Steven Murfitt (3), James A. West (3), David B. Savage (2), L. Bluck (1) & **Julian L. Griffin (1,3)**

1. Medical Research Council Human Nutrition Research, Cambridge, CB1 9NL, United Kingdom.
2. Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, Cambridge, CB2 0QQ, United Kingdom
3. Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, Cambridge, CB2 1GA, United Kingdom

It is clear that at the epidemiological level obesity is associated with insulin resistance, but a fundamental question is why some individuals develop type 2 diabetes with relatively modest obesity while others retain adequate glycaemic control despite higher degrees of body fat. Despite significant advances in the genetics of type 2 diabetes with the identification of about 20 loci with contributions to type 2 diabetes risk, these loci have relatively small effects and only represent 5-10% of the inherited component of type 2 diabetes. This suggests that a significant proportion of the underlying mechanistic causes of type 2 diabetes are not described by genetic approaches. There is growing evidence that there are clear associations between insulin resistance and the handling of dietary lipids, in particular the uptake, synthesis and storage of lipids, and thus a lipidomic study of type 2 diabetes may be more profitable in defining the underlying pathologies associated with type 2 diabetes.

One of the key aims of the Lipid Profiling and Signalling (LPS) group at MRC Human Nutrition Research (HNR) is to use lipidomic biomarkers to stratify patients and people at risk of developing diabetes into groups that reflect the underling mechanistic causes of their metabolic dysfunction. To achieve this we have developed a range of mass spectrometry assays to address the different aspects of type 2 diabetes. This includes a high throughput screening assay of intact lipids using direct infusion high resolution mass spectrometry which measures a range of intact lipids in both positive and negative mode including triglycerides, diglycerides, phospholipids, free fatty acids, cholesterol esters and sphingolipids in approximately three minutes, oxidized amino acids and nucleotides to reflect reactive oxygen species damage, carnitines to monitor beta-oxidation and mitochondrial function, and eicosanoids to follow tissue inflammation. In addition MRC HNR have developed a bioinformatics suite of tools in R for performing lipidomics called CALDERA (CAmbridge Lipidomics Data Exploration and Reproducible Analysis Framework). We will present recent results from examining rare inherited forms of type 2 diabetes, including those with lipodystrophy, insulin receptoropathies and individuals who fail to produce leptin. This has allowed us to demonstrate that triglycerides remodelling towards species that contain more palmitate and sterate, and in general shorter chain and saturated fatty acids, are not associated with insulin resistance per se as previously suggested but de novo lipogenesis.

UNDERSTANDING PREGNANCY COMPLICATIONS USING A METABOLOMIC APPROACH

Alexander Heazell (1), Marie Brown (1), Stephanie Worton (1), Priya Pantham (2), Kyle Davies (1), Rebecca Jones (1), Larry Chamley (2), Douglas Kell (1) and Warwick Dunn (1)

1. The University of Manchester, UK
2. The University of Auckland, New Zealand

Pregnancy provides unique biochemical and physical challenges to mother and fetus. Failure to adapt to these challenges can lead to complications during pregnancy including preterm labour, intrauterine growth restriction, pre-eclampsia or stillbirth. The placenta plays a crucial role during pregnancy; it is the interface between mother and fetus supplying nutrients and removing waste products. The placenta produces a range of hormones which maintain and promote pregnancy by manipulation of maternal physiology. In the majority of pregnancies the placenta performs these functions well, giving a healthy baby with minimal maternal stress. However, in some pregnancies the placenta has a significant role in the development of complications although the precise mechanisms underlying these diseases have not been elucidated.

Placental tissue can be obtained from early pregnancy (terminations) and after birth. In Manchester metabolomics strategies have been applied to placental tissue. Untargeted metabolomics has been applied to characterize metabolic changes related to normal placental development, to investigate the response of normal placental tissue to noxious insults and to characterize pregnancy complications. These have included the direct analysis of tissue homogenates [1] and the analysis of spent culture media (metabolic footprint; represents the maternal blood) from tissue cultures [2] and maternal serum [3] applying GC-MS and UPLC-MS.

Here we will present work describing:

1. The development of placental tissue culture systems in Manchester and elsewhere and their emergence as a tool for research into pregnancy complications including the study of placental hypoxia, oxidative stress and the effects of anti-phospholipid antibodies [4]
2. Methods applied for analysis of metabolic footprints of cultured placental tissue explants and for preparation and analysis of placental tissue. This will include discussions on appropriate experimental design in the study of placental tissue [2].
3. The study of biochemical changes in first trimester tissue associated with changes in oxygenation and a change from histiotrophic to haemotrophic nutrition [1]
4. The study of hypoxia and oxidative stress and its relationship to pre-eclampsia [2,6].

A metabolomic approach has confirmed some previously held hypotheses while challenging others; it has enabled a more accurate understanding of disease phenotype in pregnancy. Future research includes application of these techniques to enhance current in vitro models and to broaden the range of conditions under investigation.

[1] Dunn, W.B., *et al.* Metabolomics, 2011, in press, DOI 10.1007/s11306-011-0348-6; [2] Heazell, A.E., *et al.* Placenta, 2008, 29, 691-698; [3] Heazell A.E. *et al.* Reproductive Sciences 2012, in press, DOI 10.1177/1933719112438446; [4] Miller, R.K., *et al* Placenta, 2005;26, 439-448; [5] Heazell, A.E., *et al.* Placenta, 2008, 29, 175-186; [6] Dunn, W. B., *et al.* Placenta, 2009, 30, 974-980.

LIPIDOMIC ANALYSIS OF ACUTE MYELOID LEUKAEMIA CELL RESPONSES TO BEZAFIBRATE AND MEDROXYPROGESTERONE ACETATE REVEALS POTENTIAL INSIGHT INTO THEIR ANTI-LEUKEMIC ACTION

Andrew Southam, F.L. Khanim, R.E. Hayden, R.H. Michell, A.J. Lilly, U.L. Gunther, M.T. Drayson, M.R. Viant and C.M. Bunce

School of Biosciences, The University of Birmingham, Egdbaston, Birmingham, B15 2TT

We have recently shown that a combination of bezafibrate (Bez) and medroxyprogesterone acetate (MPA) (combination denoted BaP) kills acute myeloid leukaemia (AML) cell lines in vitro, but is not toxic to non-leukaemic myeloid cells [1]. In addition, a phase II trial of low dose BaP in elderly and relapsed AML showed clinical activity in the absence of toxicity [2]. We are about to embark on a Phase II trial of BaP at higher doses that reflect those at which the drugs are maximally active in vitro. In this study, we built on our existing polar NMR metabolomic analysis of BaP activity in AML cells [3], by utilising high-resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to investigate BaP-induced changes to the AML cellular lipidome. The aims were to increase understanding of the molecular mechanism of BaP and to identify specific lipid signatures of BaP exposure that might be used to monitor the effects of BaP in patients. AML cell lines (HL60 and K562) were exposed to 0.5 mM Bez and 5µM MPA for 24 hours, then immediately quenched at -40°C to halt metabolism. Lipids were extracted and analysed by direct-infusion FT-ICR MS. Significant changes were seen in cellular phospholipid profiles, including a widespread increase in the unsaturation of phospholipid fatty acyl residues. A significant decrease of the carbon:double-bond ratio (i.e. the degree of saturation) within the fatty acyl tails of diacyl-glycerophosphatidylcholine (GPC; HL60; 1.10-fold reduction, $p = 5x10^{-7}$, K562; 1.08-fold reduction, $p = 4x10^{-14}$) and diacyl-glycerophosphatidylinositol (HL60; 1.09-fold reduction, $p = 4x10^{-11}$, K562; 1.21-fold reduction, $p = 2x10^{-14}$) was observed after BaP treatment. There was also an alteration to the composition of ether-phospholipids. Most notably, BaP exposure caused a depletion of arachidonic acid-containing alkylacyl-GPC in both cell lines (HL60; 1.11-fold reduction, $p = 1x10^{-4}$; K562; 1.19-fold reduction, $p = 5x10^{-8}$). Alkylacyl-GPCs are involved in the release of arachidonic acid and the biosynthesis of platelet activating factor, which are key molecules in cellular signalling processes. The similarity of phospholipid disruption in two cell lines after BaP treatment suggests a common action of the drugs, which may contribute to the selective killing of AML cells.

[1] PLoS One. 2009 4(12):e8147; [2] B.r J. Haematol. 2010 149(1):65-9; [3] PLoS One. 2009;4(1):e4251

Speaker Abstracts

Session 9

Metabolite Annotation and Identification

THE MZMATCH PIPELINE: RECENT DEVELOPMENTS

Rainer Breitling

University of Glasgow, UK.

The freely available mzMatch pipeline is a flexible command-line tool for the processing, filtering, and annotation of mass spectrometry data in metabolomics [1,2].

In this talk, the focus will be on recent improvements and applications of the mzMatch software, for example for the processing and visualization of data from stable-isotope labeling experiments.

The features of mzMatch will be illustrated using metabolomics data sets from a variety of microorganisms [3,4].

[1] Scheltema RA, *et al.* (2011). Anal. Chem. 83:2786–2793; [2] Jankevics A, *et al.* (2012), Metabolomics in press; [3] Jankevics A, *et al.* (2011) Proteomics 11:4622–4631; [4] t'Kindt R, *et al.* (2010) PLoS Negl. Trop. Dis. 4:e904.

ADVANCED ANALYSIS OF LC/MS METABOLITE PROFILING DATA WITH THE BIOCONDUCTOR PACKAGE CAMERA

Carsten Kuhl and Steffen Neumann

Research Group Bioinformatics & Mass Spectrometry, Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale)

Liquid Chromatography coupled to Mass spectrometry (LC/MS) is one of the major analytical platforms for metabolite profiling. The data processing workflow for resulting data sets can be highly automated, for example using the R package xcms [1] for feature detection and -alignment. Depending on the analytical setup, a single metabolite may give rise to several or even several dozens features. These include typically isotopes, adducts and fragments, and the subsequent feature grouping and annotation are often done manually. Depending on the experimental design, more targeted and complex analysis reveals features-of-interest, but still requires a lot of manual inspection.

We present CAMERA [2], a Bioconductor package recently published, which supports high-throughput analysis of LC/MS data in direct combination with xcms. CAMERA provides different algorithms for grouping related features into compound spectra and annotation of ion species.

In addition, CAMERA is an integrated toolbox for scripting (semi-)targeted screening approaches. We demonstrate this for two plant metabolomics case studies.

1) We performed a neutral loss screen for phenolic choline esters based on untargeted LC/MS profiles of methanolic extracts of Brassicaceae seeds, measured on a quadrupole time-of-flight (QTOF) instrument. We obtained 90 putative choline esters, of which 22 were previously known. The high resolution and mass accuracy of the QTOF instrument improve the specificity of the candidate selection compared to neutral loss scan on a triple quadrupole instrument with nominal mass accuracy.

2) We used isotope-labeled tryptophan (Trp) as tracer to identify Trp-derived metabolites in Arabidopsis thaliana. CAMERA detected pairs of features with corresponding mass shift, in a narrow retention time window between the control and label-fed sample. We verified 23 metabolites with tandem-MS, of which 20 were already known in literature, and three new ones.

The workflow combining xcms and CAMERA allows fast analysis of LC/MS metabolomics experiments. As demonstrated in the two case studies, advanced analysis benefit from the scriptable interface to CAMERA.

[1] C.A. Smith, *et al.* Anal Chem, 78(3):779-787, 2006; [2] Carsten Kuhl, *et al.* Anal Chem, 84(1):283-289, 2012

COMBINATION OF MS-BASED METABOLOMICS AND MICRONMR FOR STUDYING FUNGAL INTERACTIONS AND INDUCTION OF BIOACTIVE NATURAL PRODUCTS

Jean-Luc Wolfender (1), Samuel Bertrand (1), Nadine Bohni (1), Olivier Schumpp (2) and Katia Gindro (2)

1. School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, quai Ernest-Ansermet, CH-1211 Geneva, Switzerland
2. Swiss Federal Research Station Agroscope Changins-WÃ¤denswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland

Classical pharmacognosy investigations have always been related to the bioactivity guided fractionation of constitutive secondary metabolites of plants for their isolation and use as possible leads for the development of new drugs. Plants and fungi possess inducible pathways that are activated in responses to stress that can produce highly bioactive defense compounds upon elicitation. Natural products produced by these pathways need to be identified and investigated further for an evaluation of their biological properties.

In this respect metabolomics represent an ideal approach to highlight biomarker induction in complex plant or microorganism crude extracts. In this context we have developed MS-based metabolomics methods that take advantages of UHPLC-TOF-MS fingerprinting and profiling for an efficient localization of stress-induced biomarkers. Their de novo structure identification is assured by subsequent LC-MS targeted microisolation and microflowNMR analysis. The same microfractionation enabled an early assessment of their bioactive potential by the development of adapted sensitive bioassays at the microgram scale.

We have use this type of strategy to identify biomarkers in various biological issues such as the wound response, the induction of resistance in vineyard or herbivory-maize interactions below and above-ground. In this context a new project aim at the study of natural product induction in co-culture of fungal and microbial strains will be discussed. In this case interaction zone between combative fungal strains in Petri dishes were analyzed differentially from their corresponding pure culture strains and the antifungal activity of the extracts was evaluated after stress elicitation. The approach revealed a strong induction of new metabolites at the confrontation zone.

The combination of both metabolomics with adapted bioassay has a great potential to identify new bioactive compounds produced dynamically in various stress responses. Furthermore a comprehensive interpretation of all the data generated may bring key information for a better understanding important stress response phenomena.

Speaker Abstracts

Session 10

Applied Metabolomics - Microbial

MICROBIAL METABOLOMICS IN THE SERVICE OF QUANTITATIVE FOOD MICROBIOLOGY

George. J.E. Nychas, E. Z. Panagou and A. Malouchos

Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.

The use of microbial metabolites as a consequence of microbial growth in food has been continuously recognized as a potential means for assessing food quality and/or safety. The various attempts that have been made over the last three decades to associate given metabolites with the microbial spoilage of food have not been appreciated very much, mainly because this effort was focused on single metabolites and the low understanding of the phenomena [1]. The potential of using the biochemical changes occurring during food storage instead of a single metabolite has been considered as an alternative approach to evaluate food quality and safety [2, 3]. These changes are mainly attributed to microbial activities, e.g. they utilize nutrients and produce by-products. The quantification of these activities could provide information about the type as well as the rate of spoilage, and can be used in parallel or even in conjunction with the predictive or alternatively quantitative microbiology, which involves knowledge almost exclusively of microbial growth responses to environmental factors expressed in quantitative terms by mathematical equations. Data treatment and application of advanced statistical methods (discriminant function analysis, clustering algorithms, chemometrics) and intelligent methodologies (neural networks, fuzzy logic, evolutionary algorithms and genetic programming) in collaboration with machine learning procedures, such as supervised learning algorithms, can be stored in databases and used to interpret the effect of processing, distribution and storage conditions on microbial growth. The last mentioned approach together with the development of artificial neural networks (ANN) could be shortly used to implement the evaluation of food spoilage. In this communication, an effort to combine different approaches for the optimization of food quality and safety at the time of consumption is reported.

[1] Dainty (1996) Int. J. Food Micr. 33,19-34; [2] Ellis & Goodacre (2001) Trends Food. Sci. & Techn. 12, 414–424; [3] Nychas *et al.* (2008) Meat Sci. 78, 77- 89.

DISCOVERY OF METABOLIC PATTERNS OF GUT MICROBIOME IN C57BL/6J AND C57BL/6N MICE USING HIGH RESOLUTION MASS SPECTROMETRY

Alesia Walker (1), M. Kahle (2), B. Pfitzner (3), J. Rozman 2 4, M. Klingenspor (4), V. Gailus-Durner (2), H. Fuchs (2), S. Neschen (2), M. Hrabé de Angelis (2) , A. Hartmann (3), P. Schmitt-Kopplin (1)

1. Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH),
2. Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH),
3. Research Unit Microbe-Plant Interactions, Research Group Molecular Microbial Ecology, Ingolstaedter Landstrasse 1 85764 Neuherberg, Germany,
4. Molecular Nutritional Medicine, Else Kröner-Fresenius Center, TUM, 85350 Freising-Weihenstephan, Germany

Non-targeted metabolomics (metabonomics) enables a hypothesis free insight into the global status of complex metabolic networks, reflecting a particular biological or pathophysiological state of an organism depending on the nutritional status, drug treatment and environmental or genetic background.

The aim of this study was to investigate the metabolic patterns in two C57BL/6 mouse substrains, the C57BL/6J and C57BL/6N, fed with different diets. Ongoing studies indicate that C57BL/6J males, gain less weight compared to C57BL/6N when fed the same high-fat diet, despite displaying increased food intake and energy assimilation from the diet as measured by bomb calorimetry. Several studies revealed that gut microbiota might be involved in many biological processes, e.g. energy homeostasis and also in metabolic diseases.

Therefore, the cecal content of mice was collected and the metabolome analysis of mouse gut microbiome was performed by high resolution power techniques: Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and Ultra Performance Mass Spectrometry (UPLC-MS) to provide deeper insight into the metabolome of gut. By means of unsupervised technique Principal Component Analysis (PCA) used for data reduction of multivariate data we could discriminate patterns in cecal content of C57BL/6J and C57BL/6N substrains, but also differences related to the dietary composition, measured with FT-ICR-MS. The annotation of the measured data was performed with MassTRIX, using different databases KEGG, HMDB and LIPID MAPS for mass assignment. Prospectively, the application of discriminant analyses (PLS and O-PLS) enables the extraction of most important variables that are responsible for the clustering of the groups. The information of the annotated masses plus the assignment on KEGG pathway enables to explore of how the gut microbiome is involved in different biological processes of its host. Correlating the metabolome and metagenome as well as generating high resolution datasets from 454 pyrosequencing will enable us to better understand the contribution of the gut microbiome to alterations in energy homeostasis and obesity and its adverse effects on health.

URINARY TRACT INFECTION (UTI): THROUGH THE LOOKING-GLASS OF METABOLOMICS

Ekaterina Nevedomskaya (1), Tiziana Pacchiarotta (1), Axel Meissner (1), Willize van der Starre (2), Cees van Nieuwkoop (2), Jaap J. van Dissel (2), André M. Deelder (1) and Oleg A. Mayboroda (1)

1. Biomolecular Mass Spectrometry Unit, Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
2. Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Urinary tract infection (UTI) is a common bacterial infection leading to substantial morbidity, mortality and health care expenditures across all ages. In fact, the term 'Urinary Tract Infection' encompasses a variety of clinical syndromes with one common denominator, namely a positive urine culture (i.e., bacteriuria >103 CFU/ml). However, the presence of bacteria in urine may be essential but is not sufficient for diagnosis of UTI and the correct assessment of disease relies on a coordinated effort of clinicians and microbiologists.

After a decade of an exponential development of metabolomics, with this discipline having unequivocally proven its clinical feasibility, it is only logical to apply the approach to such complex clinical entity as UTI. To this end, we have selected a group of patients with culture-confirmed febrile Escherichia coli UTI from a prospective multicenter cohort study. The study design included urine samples of UTI patients collected at baseline (t=0) as well as samples collected after antibiotic treatment (t=30). Moreover, urine samples were collected in controls without UTI.

Thus, we had the opportunity to not only compare UTI patients with symptom-free controls, but also to follow up on recovery of the patients after treatment. To achieve this we subjected the samples to metabolic profiling by NMR and UPLC-MS, followed by statistical analysis. Using the cross-platform dataset and taking advantage of the multi-level study design, which opens possibilities for case-control and longitudinal modeling, we were able to not only identify molecular discriminators that characterize UTI patients but also dissect those, which are specific for bacterial contamination, from those, which are possible markers of morbidity.

Speaker Abstracts

Lunchtime Vendor Seminars

Tuesday September 25th

Thermo Fisher Scientific - Seminar room 1

NOVEL TYPE OF SPECTRAL LIBRARY FOR THE IDENTIFICATION OF "UNKNOWN UNKNOWNS"

Robert Mistrik (1), Juraj Lutisan (1); Mark Sanders (2); Yingying Huang (2); Rose Herbold (2) and Tim Stratton (2)

1. HighChem, Ltd., Bratislava, SLOVAKIA
2. Thermo Fisher Scientific, San Jose, CA)

Novel Aspect: New type of spectral library for identification of "unknown unknowns" in forensic, metabolomics, or environmental applications.

Introduction: Identification of unknown compounds was traditionally limited to library search techniques and manual spectral interpretation. A few years ago, Precursor Ion Fingerprinting (PIF) was developed. This innovative approach identifies substructural information through the comparison of product ion spectra of structurally related compounds. Structural information is derived by utilizing previously characterized ion structures stored in reference libraries of tandem mass spectral data and matching them with unknown product ion spectra. PIF is a very powerful technique that heavily depends upon libraries containing spectra of precursor ions of various chemical classes acquired at various experimental conditions. Here we present a new type of spectral library providing the functionality required for elucidation of unknowns even if compounds are not present in the library.

Methods: For the purpose of library creation, precursor ions (isolated, activated, and dissociated ions) can be selected using various criteria; however, the more product ion spectra that are generated, the more comprehensive a library can be built. Spectra and associated experimental data are organized into a logical structure called spectral ion trees, where branches represent precursor ions and nodes the corresponding product ion spectra. The library is implemented in a relational database that will be accessible through a public domain web site of an emerging consortium named "m/z Cloud". Since the consortium is predominantly oriented towards high-resolution, accurate mass spectra, the database design, spectral management, and library search algorithms require a completely new architecture compared to traditional spectral databases.

Preliminary Data: Even if the acquisition process follows a standardized experimental protocol, reproducible product ion spectra are difficult to achieve since the probabilities of various possible ion decomposition products depend on its internal energy that can differ for identical ions if derived from different parent compounds. Since the standardized criteria cannot be met completely, several product ion spectra for the same precursor ion should be acquired using various experimental conditions (collision energies, isolation widths, etc.) to compensate for possible differences between reference and investigated spectra. Various mathematical methods have been developed to harness the spectral dissimilarities to allow correct identification of fragment structures. The idea of this project is to create a library of comprehensive spectral ion trees based on structurally characterized product ion spectra to enable the identification of substructures in unknowns. Each structurally characterized product ion spectrum will contain the precursor ion m/z value, a list of product ion m/z values with mass accuracies, corresponding absolute and relative intensities, ion polarity, charge state, the structure of the precursor ion, and the structure of the parent

molecule. For the assignment of fragment structures to a precursor ion in the process of creating structurally characterized product ion spectra, it is extremely beneficial to have high-resolution spectra since the accurate m/z values of precursor and product ions greatly reduce the number of possible molecular formulas for fragment structures. Also, the determination of the structural arrangement for the elucidated molecule benefits from exact mass measurements by constraining the elemental composition of the elucidated molecule and consistently validating the calculated mass of recognized fragment structures and accurate m/z values of precursor and product ions. The m/z Cloud public domain database aims to provide complete library technology based on spectral ion trees to enable elucidation of unknowns using the precursor ion fingerprinting method.

THE ROLE OF ORBITRAP TECHNOLOGIES IN METABOLOMICS RESEARCH: CONSIDERATIONS, METABOLITE IDENTIFICATION AND APPLICATIONS

Warwick Dunn

University of Manchester, UK

Mass spectrometry (MS) plays an important role in metabolomics research and provides the capabilities to perform both untargeted and targeted studies when coupled with chromatographic systems including Ultra High Performance Liquid Chromatography (UHPLC).

Since the introduction of the Orbitrap technologies in 2005 (initially hybrid ion trap-Orbitrap systems followed by Exactive systems) their development and application in the metabolomics community has been rapidly increasing in diverse application fields from bioproduction of chemicals in microbial systems to defining mechanisms related to human ageing and disease. Orbitrap technologies offer many advantages for untargeted metabolomics studies including the ability to (i) acquire full-scan/profile data with high mass resolution (up to 240,000FWHM) and mass accuracy (sub-ppm) in full-scan profiling, (ii) acquire in parallel on-line MS/MS data for metabolite annotation and (iii) acquire off-line MSn data to aid in metabolite identification of structurally similar metabolites. In combination with software tools (e.g. SIEVE 2.0 and mzCloud) a powerful metabolomics platform can be applied to complex biological problems.

In this presentation the capabilities of Orbitrap technologies will be discussed from a perspective of performing untargeted metabolomics applying UHPLC-MS including considerations related to experimental design and metabolite annotation/identification. Finally, applications from the study of mammalian systems will be discussed to highlight current capabilities.

Tuesday September 25th

Bruker – Weston Lecture Theatre

FAST DE-REPLICATION AND UNKNOWN IDENTIFICATION IN METABOLOMICS RESEARCH

Dr. Verena Tellström and Dr. Aiko Barsch

Bruker Daltonik GmbH, Bremen, Germany

In Metabolomics research the identification of unknown metabolites is often limited by the amount of sample available, the lack of reference material and comprehensive databases, as well as the presence of compounds in complex mixtures. High resolution LC-MS/MS systems in combination with orthogonal analytical techniques like NMR and dedicated software for data evaluation can be used to address these challenges. Here we present workflows for fast de-replication and de-novo identification exemplified on bacterial and plant metabolomics experiments.

NMR-BASED SCREENING OF NEONATE URINES

Claire Cannet

Bruker Biospin GmbH, Rheinstetten, Germany

With the high throughput capabilities of modern NMR spectrometers, NMR can deliver targeted and nontargeted results from one measurement of neonate urine in short time, thus combining quantification of a large set of compounds indicative of inborn errors with the statistical analysis of deviations from normality. In this case all kinds of deviations are accessible, be the reason known or unknown. This NMR analysis allows to deliver a general health assessment of the neonate by first of all building a model for healthy neonates and subsequently comparing new samples against this model. Results obtained on 2 studies in Germany and Turkey will be introduced and the procedures of analysis will be explained.

Wednesday September 26th

LECO – Weston Lecture Theatre

HIGH PERFORMANCE ANALYTICAL TOOLS FOR LIPIDOMICS AND METABOLOMICS

Jeffrey S. Patrick

Director of Marketed Technologies - Separation Sciences, LECO

Metabolomics provides information on diseases, differential physiology in organisms and information on stressed systems. The intent of these studies is to obtain differential analysis between two states (diseased/non; treated/non) and identify compounds (a.k.a. features) which will ultimately help to better understand the metabolic pathways implicated. The number and scope of metabolomic analyses are ever growing and the information content obtained from such studies can be enhanced by use of both GC and LC, dimensionality (GCxGC), and increasing spectral resolving power. Additional value comes from accurate mass measurements which reduces uncertainty in analyte identification and provides further selectivity in comparative analysis.

During this presentation analyses in complex systems will be discussed using each of these technologies – LC with high resolution MS; GC with high resolution MS; GCxGC with MS and even GCxGC with high resolution MS – all demonstrating their benefit to expanding metabolomic studies. The application and benefit of each technology is discussed with statistical analysis reviewed. Applications presented will be studies of plasma metabolites, tissue extracts, and plant metabolites with a focus on changes between states and strains of organisms. Interestingly the examples demonstrate differences in lipids in the plasma from diabetic and non diabetic, as well as trained and untrained rodents, differences among extracts from leaves from different rice strains, comparisons of botanical extracts and lipids from liver extracts from diseased states. Inference is made as to the biology behind the observed changes. The utility and complementary nature of each technology is discussed as is the benefit of exact mass analysis in precursor and fragment ions are discussed as is the utility of relative isotope abundance and some comparative concerns among different MS instrumentation technologies.

THE APPLICATION OF GC-HIGH RESOLUTION TIME-OF-FLIGHT MS TO THE PROFILING OF TOBACCO SMOKE

J. Frosina (1), J. van Heemst (1), A. Kettle (2) and C. Wright (1)

1. GR&D BAT
2. LECO UK

Tobacco smoke is a complex aerosol containing many chemical constituents, with recent research indicating the presence of over 5600 identified components. The total number of tobacco smoke constituents may exceed 100 000. Each type of cigarette produces a unique smoke profile that arises from the chemical composition of the tobacco blend. As part of our research we seek to understand the sources of formation of harmful and potentially harmful constituents of smoke, and to evaluate the changes that result from the

application of toxicant reduction technologies. There is thus a requirement to characterise the chemical profiles of tobacco smoke samples and to identify statistically significant differences between control and test samples.

In conjunction with exploring GC×GC-TOF approaches for the separation and identification of components in tobacco smoke, we are applying gas chromatography coupled to high-resolution Time-of-Flight Mass Spectrometry to the deconvolution of chromatographic data from tobacco smoke samples. Non-targeted screening offers the capability to compare the chemical profiles of smoke from conventional and modified products and to identify significant chemical changes.

The presentation will illustrate our technical approach and demonstrate how this has been applied to the comparison of conventional and modified combustible products.

Thursday September 27th

Waters – Conference Room One

WATERS TRANS-OMICS SOFTWARE: NOVEL TOOLS FOR "OMICS-BASED" ANALYSIS OF COMPLEX MIXTURES. APPLICATION TO A STUDY OF WHITE ADIPOSE TISSUE FROM RATS AFTER DOSING WITH A PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR)-PAN AGONIST

John P Shockcor (1,2,3) and Jules Griffin (2,3)

1. Waters. Corp. Milford, MA, USA;
2. University of Cambridge, Dept. of Biochemistry, UK;
3. Imperial College, Dept. of Surgery and Cancer, London, UK

The PPARs are a group of ligand activated transcription factors known to control the expression of a number of genes involved in lipid metabolism and energy homeostasis. The aim of this study was to characterize the metabolic effects of the Peroxisome Proliferator Activated Receptor (PPAR)-pan drug on white adipose tissue (WAT) using a mass spectrometry based lipidomic approach. The drug targets all 3 PPAR isoforms (PPAR-α, γ, and δ), although the specificity of the drug for each receptor isoform is as yet unknown. Therefore, a secondary aim was to determine whether the observed effects of drug administration were largely PPAR- δ mediated, resulting in up-regulated fatty acid oxidation, or PPAR- γ mediated, resulting in increased fatty acid sequestration and elongation.

The drug was administered at a range of dose levels (0, 30, 100, 300, and 1000 mg/kg/day; n =12 per group) to male Sprague Dawley rats for 13 weeks by oral gavage. Lipids were extracted using water: methanol: chloroform and 1/40 of the total lipid pool was reconstituted in methanol: chloroform (1:1; 1 in 20 dilution) prior to LC-MS analysis. The LC/MS analysis was performed on a Synapt G2 quadrupole time-of flight (QTof) mass spectrometer coupled to an ultra-performance liquid chromatograph (UPLC). The separation method was reverse-phase. Samples were analyzed in both positive and negative ion modes using elevated energy mass spectrometry (MSE), which provides both precursor and product in data from the same experiment.

The data obtained from LC/MS analysis was analyzed using Waters TransOmics software. From this analysis we identified 13 components (m/z, retention time pairs), which contributed to the variance between the control and high dose groups. The elemental composition of these components were calculated and used in database searches to obtained candidate structures. The product ion data, obtained using MS^E was then employed to confirm the structure of the component. The 13 components were all identified as triacylglycerides that are lower in concentration in the high dose group. These preliminary results suggest that the effect of the drug was largely PPAR- γ mediated.

Thursday September 27th

Agilent Technologies – Weston lecture Theatre

SOFTWARE FOR PATHWAY INFORMED METABOLOMICS

Steven M. Fischer (1) and Gordon A. Ross (2)

1. Agilent Technologies, Santa Clara, CA 95051, US
2. Agilent Technologies GmbH, Waldbronn, Germany

The historical approach to metabolomics research is to analyze samples in an un-targeted fashion, use software to find compounds in the data and then attempt to identify the found compounds. This approach is good for discovering new compounds but in practice it has yielded many unknown compounds that are difficult and slow to identify. If the primary purpose of the researcher is to perform translational biology, a more direct approach to the problem can be taken. In this case the data is still collected in an un-targeted fashion but now the data is mined using known biological pathways, the results are statistically analyzed and the results are projected onto biological pathways to better understand the biological system under study.

Poster Abstracts

POSTER 1.

THE HUSERMET PROJECT – DEVELOPMENTS TO ALLOW LARGE-SCALE EPIDEMIOLOGICAL AND METABOLOMIC STUDIES OF THE HUMAN POPULATION

Warwick Dunn (1), David Broadhurst (2), Paul Begley (1), Eva Zelena (1), Sue Francis-McIntyre (1), Nadine Anderson (1), Marie Brown (1), Andrew Vaughan (1), Wanchang Lin (1), Joshua D Knowles (1), Antony Halsall (1), John N Haselden (3), Andrew W Nicholls (3), Ian D Wilson (4), Douglas B Kell (1), Royston Goodacre (1) & The Human Serm metabolome (HUSERMET) consortium

1. University of Manchester, UK; 2. University of Alberta, Canada; 3. GlaxoSmithKline, UK; 4. AstraZeneca, UK

The Husermet project (Human Serum Metabolome; http://husermet.org/index.php) was funded to fulfil multiple objectives related to human health, ageing and disease risk factors. One objective was to characterise the human serum metabolome through a large-scale epidemiological investigation applying untargeted metabolomics. To fulfil this objective significant development and validation of experimental design and standard operating procedures (SOPs) for sample collection, sample preparation, data acquisition, data pre-processing and quality assurance was required and was successfully performed applying innovative developments.

Here, we will discuss the developments and innovative advances required to enable for the first time large-scale epidemiological studies of the human population applying untargeted metabolomics and chromatography-mass spectrometry platforms. We will discuss in detail (i) appropriate experimental design including the separation of a single biological study in to multiple analytical experiments, (ii) development of protocols for sample collection and preparation, (iii) development of chromatography-mass spectrometry methods to optimise metabolome coverage and (iv) the inclusion of pooled quality control (QC) samples for quality assurance and data integration [1].

These methods have, for the first time, been applied in the large-scale epidemiological study of the UK population applying untargeted chromatography-mass spectrometry metabolomics. Discussions on their development will be included along with their importance to allow valid, robust and biologically applicable data to be acquired.

[1] Dunn, W.B. et al., Nature Protocols, 2011, 6(7):1060-83

POSTER 2.

THE HUSERMET PROJECT – ADVANCES IN BIOLOGICAL KNOWLEDGE AND THE NEXT STEPS

Andrew Vaughan (1), Wanchang Lin (1), Warwick B Dunn (1), David Broadhurst (2), Paul Begley(1), Eva Zelena (1), Sue Francis-McIntyre (1), Nadine Anderson (1), Marie Brown (1), Joshau D Knowles (1), Antony Halsall (1), John N Haselden (3), Andrew W Nicholls (3), Ian D Wilson (4), Douglas B Kell (1), Royston Goodacre (1) & The Human Serum Metabolome (HUSERMET) Consortium

1. University of Manchester, UK; 2. University of Alberta, Canada; 3. GlaxoSmithKline, UK; 4. AstraZeneca, UK

The Husermet project (Human Serum Metabolome; http://husermet.org/index.php) was funded to fulfil objectives to characterise the human serum metabolome and to define metabolic biomarkers to be applied in diagnosis and therapeutic monitoring of ovarian cancer and Alzheimer's disease. The project has provided the collection and

biobanking of many thousands of serum samples from individuals in the UK which were applied for serum characterisation, studies associated with lifestyle and disease risk factors and to provide a resource for future research. This was the first large-scale epidemiological study applying mass spectrometry platforms and untargeted metabolomics to investigate the human population. Of particular biological and medical interest is the application of chromatography-mass spectrometry platforms to investigate human serum from a large UK population which has provided a mechanism to define changes in human metabolism related to healthy ageing and differences related to disease risk factors including body mass index (BMI), blood pressure (BP) and smoking status.

We will present data highlighting the importance of large-scale metabolomic studies with examples of how biological hypotheses have been constructed in relation to healthy ageing and disease risk factors. We will also discuss the open availability of the metabolomics and related data and future plans for the resources collected.

POSTER 3.

HIGH-THROUGHPUT MASS SPECTROMETRY BASED METHOD FOR QUANTITATIVE TARGETED METABOLOMICS

Vasudev Kantae, Jean-Christophe Yorke, Vidya R. Velagapudi and Matej Oresic

Institute for Molecular Medicine Finland FIMM, Helsinki, Finland.

Background: Metabolomics is a field of science, which aims at the comprehensive quantitative analysis of all the metabolites in any biological system or a specific physiological state. Quantifying broad range of targeted metabolites in a sample provides the 'functional readout' of a cellular phenotype.

Objective: To develop a high throughput quantitative method for targeted metabolite profiling across different classes of metabolites in a single run with as much coverage of metabolites as possible.

Methods: Liquid chromatography was performed on an Acquity UPLC system using HILIC (Hydrophilic Interaction Chromatography) column. MS/MS analysis was performed on Waters XEVO-TQ S triple quadrupole mass spectrometry with electro spray ionization (ESI) using multiple reaction monitoring (MRM) method. Metabolite extraction method by protein precipitation was automated using Hamilton Star liquid handling system to increase the throughput and decrease the inter-assay variations.

Results: We have developed and optimised a method that:

- is fast with a single chromatographic condition (15 min including equilibration),
- requires minimal sample amount (50 µL) and offers high throughput analysis,
- offers high sensitivity for trace level applications (pmol) and high degree of selectivity provided by MRM strategy,
- (semi) quantifies 94 metabolites from 15 different classes,
- provides an accuracy and precision of 10-15% RSD.

Conclusion: Our developed method has the ability to detect large number of polar metabolites in low and high concentrations in a single analysis. This targeted metabolite profiling method has high potential for the discovery of biomarkers for disease risk, to meet clinical demands.

POSTER 4.

ANALYSIS OF LONGITUDINAL METABOLOMIC DATA FROM ENDOCRINE DISRUPTION STUDIES: THE A-SCA METHOD

Marie Tremblay-Franco (1), Cécile Canlet (1), Nicolas J. Cabaton (1), Roselyne Gautier (1), Jean-Pierre Cravedi (1), Ana M. Soto (2) and Daniel Zalko (1)

1. INRA, UMR 1331, ToxAlim, Research Centre in Food Toxicology, F-31027 Toulouse; 2. Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.

Metabolomics techniques are increasingly being used in the field of toxicology. Experimental designs involving the study of dynamic changes in the metabolome (for instance in long term and perinatal in vivo exposure studies) raise new methodological challenges in the field of data analysis.

Multivariate analysis of variance (MANOVA), often used to analyze experimental data, is not always appropriate for metabolomics, especially when sample size is much smaller than the total number of variables. For this reason, normality and homoscedasticity hypotheses can neither be performed nor tested. In addition, multivariate methods, such as Principal Component Analysis (PCA) or Partial Least Squares-Discriminant Analysis (PLS-DA), which are often used to analyze metabolomic data, do not take into account data's temporal structure. Thus, a major part of the information is lost when either only ANOVA or PCA are used to analyze temporal changes in the metabolome. In this study, we applied a method which combines ANOVA and PCA: A-SCA (Anova-Simultaneous Component Analysis; SCA is a generalization of PCA.) taking into account the experimental design, as well as the relationship between variables (multivariate data), to allow data modelisation. Data were first separated into blocks corresponding to the various sources of variation (experimental design factors). Then SCA was independently applied on each block. Permutations test was then used to evaluate the significance of model parameters.

This method was applied to the study of the effects of low doses of BPA on global metabolism in Sprague-Dawley rats perinatally exposed to BPA, within the frame of the INRA-TUFTS medical school collaboration within the frame of NIEHS project #5RC2ES018822. Pregnant rats (n= 46 to 50 observations per group) were exposed to DMSO (vehicle-control), 0.25, 2.5, 25 or 250 ng BPA/kg BW/day. Serum samples of the F1 generation were collected at 5 time points, on days 21, 50, 90, 140 and 200 of the experiment, and submitted to 1H NMR spectroscopy (Bruker DRX-600 Avance spectrometer operating at 600.13 MHz).

Using this method, combining the analysis of variance and multivariate analysis of metabolomic data, time and time-dose interaction effects were observed. The ASCA results were compared to the results obtained by PLS-DA.

POSTER 5.

EFFECTS OF BISPHENOL-A (BPA) ON HEPG2 CELLS: AN UNTARGETED NMR-BASED METABONOMICS STUDY

Cécile Canlet, Marie Tremblay-Franco, Anne RIU, Nicolas Cabaton, Claire Lapeyre, Marc Audebert and Daniel Zalko

INRA, UMR 1331 TOXALIM (Research Center in Food Toxicology); 180 Chemin de Tournefeuille, F-31027 Toulouse, France

Humans are exposed daily to a variety of man-made chemicals entering the food-web

and/or present in the environment. Many are now known to behave as endocrine disruptors, which currently raises new challenges in the field of toxicology. Modern toxicology paradigms (low doses issues), the entering in force of specific regulations such as the REACH directive, but also the need to reduce the number of animals used for toxicity studies, now require the use of reliable in vitro tools, enabling to examine the potential effects of a variety of chemicals.

In vitro cellular systems, together with 'omics' techniques, represent a promising addition to classical toxicology tests. So far, only little data has been published regarding the potential use of metabonomics in in vitro studies investigating the toxicity of chemicals. The main goal of this work was to study the usefulness of a NMR-based metabonomics approach in providing information on the toxicity of Bisphenol-A (BPA), a model endocrine disruptor, based on the use of a human hepatic cell line (HepG2 cells).

A crucial step in NMR-based in vitro metabonomics is the extraction procedure. Conventional methods require 10 millions cells in order to provide suitable concentrations of intracellular metabolites that allow NMR measurements. In this study, we developed a method for quenching and extracting cell cultures for in vitro NMR-based metabonomics, based on only one million cells.

Metabonomic approaches were investigated on HepG2 cell extracts, after a 24-h exposure to low concentrations of BPA (10-6, 10-9 and 10-12 M). Sample extracts were submitted to 1H NMR spectroscopy and NMR data were analyzed by multivariate statistical methods. First, data were filtered using OSC (Orthogonal Signal Correction) method, to get rid of the variability not correlated with the treatment. PLS-DA (Partial Least Square Discriminant Analysis) was then applied to attempt to discriminate groups according to the BPA concentration. We were able to discriminate between control cells and all BPA-exposed cells.

This study hence confirms that the metabonomic approach using ^{1}H NMR spectroscopy is a promising tool to identify shifts in the global metabolism after an exposure to very low concentrations of xenobiotics.

POSTER 6.

THE IMPACT OF HIGH-RESOLUTION MS AND NMR TECHNIQUES FOR THE DISCOVERY OF NOVEL NATURAL PRODUCTS FROM MYXOBACTERIA

Aiko Barsch (3), Daniel Krug (1,2), Thomas Hoffmann (1,2), Alberto Plaza (1), Kirsten Harmrolfs (1), Gabriela Zurek (3) and Rolf Müller (1,2)

1. Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, Germany; 2. Saarland University, Saarbrücken, Germany; 3. Bruker Daltonik GmbH, Bremen, Germany

Myxobacteria represent an important source of novel natural products exhibiting a wide range of biological activities. Some of these so-called secondary metabolites are investigated as potential leads for novel drugs. Traditional approaches to discovering natural products mainly employ bioassays and activity-guided isolation, but genomics-based strategies become increasingly successful to reveal additional compounds. These "metabolome-mining" approaches hold great promise for uncovering novel secondary metabolites from myxobacterial strains, as the number of known compounds identified to date is often significantly lower than expected from genome sequence information. Their discovery enables characterization of the

underlying biosynthetic pathways and at the same time chances are good to find compounds with potent biological activity.

The most important key for the rapid identification of known compound classes is the ability to generate a partial (or even complete) structure based on MS and NMR information as early as possible in the course of the discovery workflow. LC-SPE-NMR/MS is an important tool to achieve this objective. After initial separation of a myxobacterial extract obtained from small scale fermentation into crude fractions by size-exclusion chromatography, analytical HPLC with MS monitoring was used to track fractions of interest. Candidate peaks were then trapped on SPE cartridges and transferred via a Cryofit flowprobe to the NMR system using deuterated solvent. The acquisition of 1D and 2D NMR spectra sets from small scales (~20µg) was achieved by applying multiple solvent suppression pulse programs. Combining NMR data with elemental composition information for precursor and fragment ions obtained from accurate mass MS measurements enabled the rapid identification of known and novel structural scaffolds. Thus, a well-founded decision whether to include a candidate compound into the labor-intensive up scaling, fermentation and purification pipeline can be rapidly made.

Here we show that UHPLC-coupled UHR-TOF mass spectrometry and LC-NMR represent complementary analytical tools for the discovery of novel natural products, efficient dereplication, comprehensive mining of metaboloms and rapid structure elucidation of novel metabolites.

POSTER 7.

LC-Q-TOF BASED METABOLIC PROFILING OF DANSYLATED METABOLITES TO STUDY YEAST ARGININE BIOSYNTHESIS DELETION MUTANTS

Aiko Barsch (1), Sandy Yates (1), Gabriela Zurek (2), Manhong Wu (3); Bob St. Onge (3), Sundari Suresh (3), Ron Davis (3) and Gary Peltz (3)

1. Bruker Daltonics, Fremont, CA, USA; 2. Bruker Daltonik GmbH, Bremen, Germany; 3. Stanford University, CA, USA

Yeast has been an import model system for biological studies because of its simplicity, easy of manipulation and the availability of strains with individual gene deletions. In this work, a non-targeted LC-Q-TOF based approach was used to profile the metabolism of two deletion mutants in the arginine biosynthesis pathway to identify metabolic changes.

To enable this metabolic profiling work using standard C18 chromatographic separation, the extracted amino acids metabolites were dansylated. Additionally, dansylation often leads to an enhancement of the electrospray ionization signal intensities.

Statistical analysis of wild type versus the gene deletion mutants using PCA and t-test approaches revealed an accumulation of several metabolites. Elemental formulae for these compounds were determined using SmartFormula3D by evaluating the MS and associated MS/MS data. Molecular formulae were queried against public access databases to derive candidate chemical structures. Correlating these database matches with information contained in MS/MS spectra enabled the confirmation of particular structural hypotheses using the FragmentExplorer. The characteristic reporter

fragment ion (*m/z* 170; $C_{12}H_{12}N$) for the dansylated compounds facilitated this work by limiting the candidate structures to classes of compounds which can be derivatised by dansylation.

Using this approach an accumulation of argininosuccinic acid and N-acetylornithine could be observed in arg4 mutant yeast cells accompanied with a decrease in relative amounts of aspartate. N-acetylornithine had a higher abundance in the arg7 deletion mutant compared to yeast wild type cells. These metabolic profiling data agreed with the hypothesis that there would be an accumulation of a precursor upstream of the blocked enzyme.

Further, unexpected, but highly statistically significant changes in the metabolic profile were determined which were not associated with arginine biosynthetic pathway. This represents the power of untargeted metabolic profiling as such changes might not be detected if a targeted approach were to be used.

POSTER 8.

IMPROVED METHODS FOR CHARACTERISATION OF PHENOLIC COMPOUNDS FROM ROSEMARY EXTRACTS USING ESI-QQ-TOF MS

Verena Tellström (2), Isabel Borrás-Linares (1), Aiko Barsch (2), Gabriela Zurek (2), David Arráez-Román (1), Antonio Segura-Carretero (1) and Alberto Fernández-Gutiérrez (1)

1. CIDAF, Granada, Spain; 2. BDAL, Bremen, Germany

Rosemary (*Rosmarinus officinalis L.*) is a shrub traditionally used as culinary spice, as well as in folk medicine, being a greatly valued medicinal herb. In fact, this plant exerts a great number of pharmacological activities. Most of these observed effects are linked to its phenolic content. Recently there has been a growing interest in the use of natural antioxidants in the food industry as a preservation method. Due to its high antioxidant capacity, rosemary turned out being one of the most used natural antioxidant because of its value for preservation and its beneficial effects mentioned above.

This work comprehensively characterizes the bioactive secondary metabolites present in two rosemary extracts obtained by SFE (CO_2 at 150 bar) and PLE (H_20 at 200°C).

A sensitive analytical method for phenolic compounds has been developed using high resolution chromatography and ESI-Qq-TOF mass spectrometry. The extracts were analysed by both positive and negative ionization modes for comprehensive characterization. Modern ESI-Qq-TOF-MS instrumentation provides high mass resolution and mass accuracy in combination with isotopic pattern information, for both MS full scan and MS/MS spectra thus providing an established method for molecular formula determination and confirmation.

The presented methodology proved to be an effective tool for the separation of a wide range of phenolic compounds in rosemary extracts: phenolic diterpenes (carnosol, carnosic acid, rosmadial, rosmanol and its isomers epirosmanol/epiisorosmanol), flavonoids (apigenin, genkwanin, salvigenin, ladanein, cirsiliol), phenolic acids (artepillin C), among other classes of phenolic compounds such as rosmarinic acid. The results of this study were in good agreement with prior characterization of rosemary or other species of the Lamiaceae family [1]. In this work, the analysis time and the flow rate were optimized without affecting the chromatographic resolution. Using the

methodology that will be presented, we were able to find new compounds which were not detected previously in these extracts such as cirsiliol, 5-methoxy-6,7-methylenedioxyflavone and 12-O-methylcarnosol.

[1] Borrás-Linares *et al,* J. Chromatography A, 1218 (2011) 7682-7690

POSTER 9.

COMPLEMENTARY GC-EI-MS AND GC-APCI-TOF-MS ANALYSIS OF A SHORT-LIVED MITOCHONDRIAL KNOCKDOWN OF CAENORHABDITIS ELEGANS TO STUDY METABOLIC CHANGES DURING AGING

Verena Tellström (1), Aiko Barsch (1), Gabriela Zurek (1), Marta Artal Sanz (2), Carsten Jaeger (2) and Bernd Kammerer (2)

1. Bruker Daltonik GmbH, Bremen, Germany; 2. Center for Biological Systems Analysis (ZBSA), University Freiburg, Germany

Aging is a highly complex biological process involving a multitude of alterations at the metabolic, cellular and organismal level. According to the free radical theory of aging, mitochondria are the major source of oxidative stress within the cell and a main cause of aging. Mitochondria may become increasingly dysfunctional with age and fundamental impacts on cellular energy metabolism may occur. The simple nematode worm *Caenorhabditis elegans* is a well-established model system for aging; several mitochondrial mutations significantly reduce life span. However, metabolic alterations associated with life span reduction are largely unknown.

In this study we compared whole-organism methanolic extracts of short-lived *C. elegans* depleted in mitochondrial prohibition to those of non-treated *C. elegans* with normal life span by non-targeted GC-MS based metabolite profiling.

Initial profiling with GC-EI-MS yielded 212 reconstructed mass spectra present in at least 40 of 48 chromatograms. Of these 212 mass spectra, 134 could be assigned structures at a match factor of >800 by database-assisted mass spectral/retention index matching (AMDIS, GMD/NIST05), while the rest remained unidentified. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) both allowed clear differentiation between metabolic phenotypes of short-lived and normal-lived worms. PLS-DA indicated that a set of 17 compounds was particularly relevant for phenotype differentiation; however, five of these relevant metabolites could not be identified by database search.

In contrast to GC-EI-MS, GC-APCI-TOF-MS analysis readily yields pseudomolecular ions facilitating the identification of unknowns. Feature extraction from the GC-APCI-TOF-MS analyses for statistical analysis resulted in approx. 650 features/chromatogram. Differentiation using pattern recognition methods between RNAi-treated and non-treated animals was similarly possible based on the APCI data. Molecular formula generation from accurate mass and isotopic pattern successfully confirmed identities of primary metabolites from GC-EI-MS analysis such as glycine or alanine. The identification of the unknown 5 phenotype related metabolites is currently ongoing.

POSTER 10.

METABOLOMICS REVEALS THE METABOLIC EFFECTS OF WHOLE GRAIN RYE PRODUCTS ON HUMANS

Ali A. Moazzami (1), Isabel Bondia-Pons (2), Jie-Xian Zhang (3), Afaf Kamal-Eldin (1), Per

Åman (1), Kati Hanhineva (2), Katri Juntunen (2), Nadja Antl (1), Göran Hallmans (3) Kaisa Poutanen (2,4), Hannu Mykkänen (2), Jan-Erik Johansson (5) and Sven -Olof Andersson (5)

1. Department of Food Science, Swedish University of Agricultural Sciences; 2. Department of Public Health and Clinical Nutrition, Clinical Nutrition, Food and Health Research Centre, University of Eastern Finland, Kuopio Campus; 3. Department of Public Health and Clinical Medicine, Umeå University, SE 90187 Umeå, Sweden4. VTT Technical Research Centre of Finland; 5. Department of Urology, Örebro University Hospital, SE 70110 Örebro, Sweden.

Epidemiological studies have consistently shown that whole grain cereals can protect against the development of chronic disease, i.e. type 2 diabetes (T2D) and cardiovascular disease (CVD). However, the underlying mechanism is not fully understood. Among WG products, WG rye is considered even more potent because of discrepancies in postprandial insulin and glucose responses known as rye factor. Here, we are reporting the results from NMR-based metabolomics analysis of two interventions (n=24, prostate cancer patients & n=33, Finish postmenopausal women) and one postprandial study (n=20, Finish postmenopausal women) in which subjects received whole grain rye (RP) or refined wheat (WP) products using a cross over design. The experiments were performed within Nordic Whole grain for Health Centre of Excellence (HELGA) on men and women in the risk of developing chronic diseases. Our aim was to detect short term and long term metabolic effects of whole grain rye in order to understand the health beneficial effects of RP. NMR-based metabolomics analysis revealed that the consumption of RP products compared with WP causes an increase in circulating ketone bodies and reduction in branch amino acids and homocysteine. These findings indicate favourable shifts in energy metabolism, branch amino acid metabolism, and single carbon metabolism. The deregulations of these three metabolic pathways have been associated with the development of T2D and CVD.

POSTER 11.

DATA INDEPENDENT MALDI IMAGING ION MOBILITY ACQUISITION FOR THE VISUALISATION AND IDENTIFICATION OF LIPIDS DIRECTLY FROM A SINGLE TISSUE SECTION

Emmanuelle Claude, M. Towers, K. Neeson, L.Fernandes and J.P.C. Vissers

Waters Corporation, Manchester, UK

Introduction: Advances in MS enabled the simultaneous analysis of a wide range of lipids and the rise of lipidomics. However, the spatial localization of lipids within tissue microstructures is often lost during the process of lipid extraction. Recently, MS imaging allowed visualizing lipid species in entire tissue section. Structural identification is traditionally performed after processing the untargeted MS imaging data, followed by further manual acquisitions either on the same or consecutive sections. A data independent MALDI imaging acquisition method is presented, where MS and MS/MS information are acquired within the same experiment, without any precursor selection. Post-acquisition, precursors and fragments are correlated on the basis of ionic drift in the gas phase, which is further refined utilizing their spatial distributions.

Methods: Data were acquired using a MALDI SYNAPT G2 instrument with tri-wave ion guide optics to separate ions according to their gas phase mobility. Within the same MALDI imaging experiment, the mass spectrometer was set to apply alternate collision energies to

the transfer cell between low energy and an elevated collision energy ramp, with the latter inducing lipid fragmentation. As fragmentation occurs post-ion mobility separation, the precursors at low energy share drift time with their associated fragments from the elevated energy scan. The dataset was subsequently processed using High Definition Imaging (HDI) MALDI software for enhanced image analysis. Drift time alignment and spatial correlation of the data from both low and elevated energy functions were carried out with the same platform.

Preliminary data: Proof-of-principal experiments have been carried out using a thin section of rat brain, produced using a cryotome and deposited on standard microscope slides. CHCA matrix was applied evenly to the sample in several coats using a SunCollect nebulising spray device. The MALDI imaging experiment was designed such that adjacent pixels had low and elevated CE applied. Using new HDI MALDI software, both elevated and low energy functions of the MALDI imaging data were independently processed using the Apex 3D detection algorithm. The two types of ion images were then visualised within the Analysis tab of the HDI software, with the low energy function displaying lipid precursor ion images and the elevated energy function lipid fragmentation profiles. During image and data processing, the low and elevated data streams were drift time aligned and further correlated based on their spatial distribution for supplementary refinement. Further work will be conducted with HDI generated output (i.e. pkl file) for the structural identification of multiple lipids from MALDI imaging experiment using LipidMaps and/or SimLipid softwares.

POSTER 12.

GC-MS PROFILING IN INDUSTRIAL FERMENTATIONS – A STRATEGY TO IMPROVE MEDIUM DESIGN

Stefanie Wiese (1), Dirk Bergmaier (2), Asger Geppel (1) and Morten Danielsena (1)

1. Chr. Hansen, Boege Allé 10-12, DK-2970 Hoersholm, Denmark; 2. Chr. Hansen GmbH, Große Drakenburger Straße 93-97, D-31582 Nienburg, Germany

Main goals in the industrial production of bacteria, e.g. in human or animal health, are the high yield of cells, robustness as well as stability of the final product. Controversially medium composition influences all outcomes but is due to cost reasons influenced by the composition of its complex ingredients. An approach to overcome the black box of complex medium components in improving fermentation performance is the monitoring of fermentation progresses by use of metabolic profiling techniques [1,2].

A GC-MS method was used to profile low molecular nutrients and metabolites in fermentation media. In a simple preparation step samples were centrifuged for 15 min at 3000 g and 4Â°C to remove particles. Supernatants were diluted with 66% ethanol. An internal standard was added, samples were oximized, silylated, and analyzed by GC-MS in full scan mode. Total ion chromatograms were aligned by the icoshift function (available from models.life.ku.dk) using MATLAB R2010b (The Mathworks Inc.) and corrected for the signal of the internal standard. For principal component analysis PLS Toolbox Version 6.7 (Eigenvector Inc.) was used.

This approach was used to investigate performance difference of *Bacillus subtilis* using two different medium formulations. It

could be shown, that a better growing performance might be caused by a higher concentration of amino acids in the medium. In contrast the presence of specific organic acids is correlated to a more efficient sporulation in the later progress.

Summarizing, GC-MS profiling combined with multivariate analysis could be shown to be a relative easy method to gain a more comprehensive insight into complex fermentation processes.

[1] M. Kennedy and D.Krouse J. Ind. Microbiol. Biot. 1999 456-475; [2] J. M. Cevallos-Cevallos *et al.* Trends Food Sci. Tech. 2009 557-566.

POSTER 13.

THE METABOLIC RESPONSE OF ARABIDOPSIS TO CO-CULTIVATION WITH WHITE CLOVER (TRIFOLIUM REPENS L.) SUGGESTS A HERBICIDE-LIKE MODE OF ACTION

Inge S. Fomsgaard (1), **Hans Albert Pedersen (1)**, Per Kudsk (1) and Oliver Fiehn (2)

1. Aarhus University, Department of Agroecology, Slagelse, 4200, Denmark; 2. UC Davis, Department of Molecular and Cellular Biology, College of Biological Sciences, Davis, California, 95616, United States

Allelopathy contributes to competition between plants and has potential use for weed suppression in agriculture. A co-cultivation system for *Arabidopsis thaliana* and *Trifolium repens* was used to study allelopathic effects. Plants were cultivated for up to two weeks to provide a timeline. Growth was modeled using Gompertz curves, and including the co-cultivation factor significantly affected the model (for Arabidopsis, P ed metabolomics. The decreases in *A. thaliana* of aromatic and branched-chain amino acids suggest a herbicide-like suppressant effect by *T. repens* because of the similarity with effects expected from glyphosate or sulfonylurea herbicide treatment.

POSTER 14.

SOFTWARE FOR PATHWAY INFORMED METABOLOMICS

Steven Fischer and Theodore Sana

Agilent Technologies, USA

Introduction: The historical approach to metabolomics research is to analyze samples in an un-targeted fashion, use software to find compounds in the data and then attempt to identify the found compounds. This approach is good for discovering new compounds but in practice it has yielded many unknown compounds that are difficult and slow to identify. If the primary purpose of the researcher is to perform translational biology, a more direct approach to the problem can be taken. In this case the data is still collected in an un-targeted fashion but now the data is mined using known biological pathways, the results are statistically analyzed and the results are projected onto biological pathways to better understand the biological system under study.

Methodology: We have developed three software tools to analyze data using pathways as the basis. The first tool is a software program that can read publicly available pathway databases, such as KEGG, and allow the user to select pathway(s) of interest based on name, common compound or related reaction. When extracting the pathway database, the compound information such as name, empirical formula, identifier and structure are captured. Duplicate entries and non-metabolites are stripped from the pathway database and added to a compound database. This yields a database of compounds of a set of pathways that are of

interest to the researcher. The second piece of software developed for this analysis is a mass spectrometer data feature extraction routine. This software works by taking an entry in the previously created database and calculates the ion to extract from the mass spectral data. It does this by taking user settable parameters such as charge state and adduct ion and calculates all the permitted combinations of ions to extract by combining it with the empirical formula in the database. For example; for glucose with an empirical formula of C6H12O6, charge state of one and adduct ion of H+ a mass of 181.0707 is extracted from the data with a user settable extraction window. Since the software is designed for an accurate mass TOF instrument the extraction window is typically +/- 20 ppm. The software then integrates the extracted ion chromatogram and detects all peaks found. The next step in the algorithm is to examine the spectra from each to determine if the isotopes for the compound are present. The software calculates the expected isotope pattern and accurate mass values based on the empirical formula. These are compared to the actual data and a score is calculated. The user can set the pass / fail score threshold. In addition, if the user has retention time information in the database it can be used as an additional test criteria. This process is repeated for all the entries in the compound database and a list of results is created. Accurate mass alone is not a positive identification but in a biological context it is often enough for a preliminary compound assignment. The last piece of the software then takes the result of the list of found compounds and searches a pathway database for matches. A number of steps must be performed to do this. The pathway database and the compound database can have different identifiers if the sources were not the same. A mapping tool aligns the two databases. This is done by mapping the chemical identifier in the compound database to an identifier in the pathway database by use of a look up file. The next step is to score each pathway based on the number of metabolites present in the compound list. A pathway list is created with the number of entities found in each pathway. The user can then filter the pathway list to look at only the most interesting pathways. By selecting a pathway in the pathway list, the user can visualize the acquired data projected onto that pathway. The software allows for zooming in or out for closer inspection of the pathway as well as the ability to select part or all of the pathway for export as a list of metabolites, genes or proteins. The utility of this approach is demonstrated using data acquired on a study of malaria infected erythrocytes. The data is acquired on a QTOF in MS-only mode. The data is then processed using the above approach.

Conclusion: By using this approach, we were able to identify many metabolites that vary in malaria infected erythrocytes. In this presentation I will focus on three of those metabolites and the ease with which these where then subsequently identified.

POSTER 15.

NMR BASED METABOLOMICS STUDY OF A CEREBRAL ISCHEMIA MODEL – A PRACTICAL LOOK IN TO THE METABOLIC PROFILING PIPELINE OF SMALL TISSUE AMOUNTS

Edoardo Gaude (1), Silvia Mari (1,5), Marco Cambiaghi (2), Luca Peruzzotti-Jametti (3), David Chang (4), Leocani Letizia (2), Martino Gianvito (3) and Giovanna Musco (1)

1. Dulbecco Telethon Institute c/o S. Raffaele Scientific Institute, Milan (Italy); 2. Experimental Neurophysiology, San Raffaele Scientific Institute, Milan (Italy); 3. Neuroimmunology, San Raffaele

Scientific Institute, Milan (Italy); 4. Chenomx Inc, Edmonton, Alberta (Canada); 5. R4R, Rodano (Italy) www.research4rent.com

Focal cerebral ischemia is caused by the occlusion of a cerebral vessel resulting in a significant reduction of cerebral blood flow into a discrete region of the brain. The lack of energetic substrates (mainly oxygen and glucose) induces a shift to anaerobic metabolism with a consequent decrease in the mitochondrial respiratory activity. The striking imbalance between energy use and production, irremediably leads to complex biological maladaptive responses (including release of excitatory neurotransmitters and activation of apoptotic pathways) that perpetuate the extensive ischemic damage. As it has been already pointed out, further experimental research is still needed to analyze this complex response to ischemia, and to identify early biochemical markers of injury as well as potential targets for future therapies [1]. With the aim to investigate the metabolic signature of cerebral ischemia we have performed a metabolomics study in a mouse-model of proximal middle cerebral artery occlusion (pMCAO). We have collected tissues from cerebral cortex and striatum on both ipsilateral and contralateral hemispheres of five animals. Despite the limited amount of tissue collected (weights 10-20mg) we were able to extract metabolites according to Viant protocol (2) and acquire NMR spectra of the polar phase extracts by means of a Bruker 600MHz equipped with cryo-probe. About 30 different metabolites were recognized and quantified using Chenomx NMR Suite software. Finally metabolite concentration matrix was the input for an in-house developed R-package, the so-called MUMA (Multivariate Univariate Metabolomic Analysis). MUMA package will be freely available and downloadable with its easy-to-way correlated tutorial. Briefly, once the matrix has been read MUMA performs sample normalization on the sum of all variables of each sample (total spectrum normalization) and different possible scalings (auto-, pareto-, vast- or range-scaling). Once data are scaled MUMA can perform both univariate (Shapiro Wilk's test for normality, Welch's T-test or Mann Whitney-Wilcoxon test between all possible class combinations and finally outputs Volcano plots) or multivariate analysis (such as PCA, PLSDA or O-PLSDA). MUMA also includes further correlation analysis such as STOCSY and RANSY. Here we present an integrated application based on the R-package MUMA and Chenomx software for the analysis, and classification of different brain regions in a pMCAO mouse model.

[1] Schaller B., Graf R., Journal of Cerebral Blood Flow & Metabolism, 2004, 24:351-371

POSTER 16.

METABOLOMICS OF INTACT TISSUES – DISCRIMINATION BETWEEN DIFFERENT REGIONS OF OSTEOLYTIC LESIONS IN A MULTIPLE MYELOMA PATIENT USING 1H HRMAS NMR SPECTRA

Silvia Mari (1, 6), Francesca Fontana (2), Jose Garcia Manteiga (2), Edoardo Gaude (1), Simone Cenci (2), Enrico Caneva (3), Giovanna Musco (1), Stanislav Sykora (4), Juan Carlos Cobas Gomez (5)

1. Dulbecco Telethon Institute c/o S. Raffaele Scientific Institute, Milan (Italy); 2. Protein Transport and Secretion Unit c/o S. Raffaele Scientific Institute, Milan (Italy); 3. Centro Interdipartimentale Grandi Apparecchiature c/o UniversitÃ degli Studi di Milano, Milan (Italy); 4. Extra Byte, Castano Primo, Italy, http://www.ebyte.it/; 5. Mestrelab Research, Santiago de Compostela, Spain, http://www.mestrelab.com/; 6. R4R, Rodano, Italy, http://www.research4rent.com/

Multiple myeloma (MM) is a malignancy of plasma cells characterized by multifocal bone lesions and systemic complications due to distant end-organ damage. The bone marrow is a natural niche for long-lived plasma cells, but upon malignant transformation an expanding clone diffusely infiltrates the bone marrow, altering its composition and functions. At a number of sites a dramatic alteration of the balance between bone resorption and deposition, locally induced by neoplastic cells, leads to the complete destruction of the bone, which becomes substituted by a mass of cancer cells. Such osteolytic lesions can grow to the point of jeopardizing the structural stability of bones and give rise to dangerous pathological fractures or spinal cord compression. Understanding the biology of such process, which is particularly severe in MM, could help design better diagnostic strategies to prevent pathologic fracture, but also shed light on the peculiar properties that differentiate the local bone-destroying from bone-infiltrating myeloma cells.

The metabolic profiling or metabolomics of disease has proven useful to identify diagnostic and prognostic markers. Its use in the clinic is beginning to increase exponentially; however, it is still largely underexploited. Although the potential of metabolomics has been established in solid tumors (prostate, breast cancer and colon cancer), much less is known about its use in hematological malignancies. Moreover, to our knowledge, metabolomics has not been applied so far to the evaluation of bone lesions. We thus set out to develop the metabolomic study of myeloma-induced bone disease. To this aim, bone tissue biopsies have been collected from MM patients undergoing orthopedic surgery and analyzed by High Resolution-Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR).

Since the actual nature of all the metabolites is rarely known in advance, metabolomics often uses alternative statistical evaluation methods, such as multivariate factor analysis. Such approaches require integration over predefined intervals (bins) and a meaningful integration of such intricate and artefact-burdened spectra may often be just as arduous as peaks fitting. Recently, a new algorithm called GSD (Global Spectrum Deconvolution) has been developed and made available in the Mnova software package (Mestrelab Research). GSD is capable of identifying even poorly resolved spectral signals and of fitting all recognizable peaks in even very complex 1D spectra. GSD produces a table of all detectable spectral peaks and their parameters. Such a table can be then used for various purposes like generation of artifact-free synthetic spectra, stick spectra, artifact-free integrals, as well as accurate binning void of any bin-crossover problems due to the overlapping wings of spectral peaks. Because of these attractive features, GSD is likely to become a very important pre-processing tool for all metabolomic approaches to the evaluation of NMR spectra of whole bio-samples. Finally, a GSD-based binning matrix was used as input to the in-house developed R-package MUMA (Multivariate & Univariate Metabolomic Analysis). MUMA is available online for a free download. It performs total spectra normalization and scaling (auto-, pareto-, vast- or range-scaling) as well as both univariate (Shapiro Wilk's test for normality, Welch's T- test or Mann Whitney-Wilcoxon test amongst all possible class combinations and outputs Volcano plots) and multivariate analysis (PCA, PLSDA or O-PLSDA). It can also carry out further correlation analysis such as STOCSY and RANSY.

Here we present an integrated application based on the R-package MUMA and Mnova

software for the processing, analysis and classification of different regions of osteolytic lesions in a MM patient's bone tissue biopsies.

POSTER 17.

NMR SPECTROMETERS AS 'MAGNETIC TONGUES' – PREDICTION OF SENSORY DESCRIPTORS IN CANNED TOMATOES

Anders Malmendal (1,2), Claudia Amoresano (3), Roberta Trotta (4), Ilaria Lauri (5), Stefano De Tito (5), Ettore Novellino (5) and Antonio Randazzo (4)

1. Center for Insoluble Protein Structures, Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, University of Aarhus, Langelandsgade 140, DK-8000 Aarhus C, Denmark; 2. Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen N, Denmark; 3. Loghìa Market Research, Piazza Immacolata 12, 80129, Napoli, Italy; 4. Dip. Chimica delle Sostanze Naturali and Universita degli Studi di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy; 5. Dip. Chimica Farmaceutica e Tossicologica, Universita degli Studi di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy

The perception of odor and flavor of food is a complicated physiological and psychological process that cannot be explained by simple models. Quantitative descriptive analysis by a team of trained assessors is a usually used to describe sensory features. Nevertheless, the availability of a number of instrumental techniques has opened up the possibility to calibrate the sensory perception. In this frame, we have tested the possibility to use NMR spectroscopy to predict sensory features. We were able to correlate the chemical composition of these canned tomato samples to the sensory descriptors bitterness, sweetness, sourness, saltiness, tomato and metal taste, redness and density, providing glimpses of information about the complex chemistry behind taste and suggesting NMR to be a useful tool in the characterization of sensory features.

POSTER 18.

SPANNING THE BRIDGE FROM SYSTEMS BIOLOGY TO CLASSICAL SCIENCE – NMR METABONOMIC INVESTIGATION OF PHOSPHINE RESISTANCE IN CAENORHABDITIS ELEGANS

Horst Joachim Schirra (1), Anita Goldinger (1), Li Ma (2), Cameron G. Anderson (1), Colin Cheng (2), Paul Ebert (2)

1. The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Qld 4072, Australia; 2. The University of Queensland, School of Biological Science, Brisbane, Qld 4072, Australia.

Currently, there is some perceived tension between systems biology ('data first') and classical biology ('hypothesis first'). Using the example of phosphine toxicity, we will show that both strategies are valid approaches and that it is not only possible to build a bridge between them, but that a combination of both methods in an iterative cycle can advance science faster than each in isolation. Phosphine is the most widely used fumigant in the world for controlling insect pests in grain stores without compromising the grain or leaving toxic residues. However, the increasing frequency of phosphine-resistant insects is threatening the useful life of phosphine as a fumigant. Despite its importance, little is known about the toxic action of phosphine or the resistance mechanisms in insect species.

In this study, we used a genetically, developmentally and metabolically well characterised model organism, *Caenorhabditis elegans*, to investigate and characterise the mechanisms of phosphine toxicity and

resistance with NMR-based metabonomics. Both phosphine-resistant and susceptible *C. elegans* strains were exposed to different concentrations of phosphine, and the changes in their metabolic profiles were analysed with NMR-based metabonomics of extracts of the whole nematodes. In addition, we investigated the response of wild-type C. elegans and the phosphine-resistant mutants against hydrogen sulphide, a toxic gas that modulates metabolism in a fashion similar to phosphine. The responses to both gases will be compared.

A combination of 1D NMR spectroscopy and multivariate statistical analysis allowed sources of metabolic variation between resistant and susceptible strains to be determined. Further NMR techniques, including 2D TOCSY and HSQC spectra, allowed characterisation of the compounds that caused metabolic variation, shedding some light on the metabolic pathways affected by phosphine poisoning and phosphine resistance. Subsequent combination of the metabonomic data with detailed genetic characterisation of the phosphine resistant mutants allowed identification of the affected metabolic pathways, which will facilitate identification of the likely target enzyme affected by phosphine.

This study demonstrates the usefulness and versatility of NMR spectroscopy in investigating the metabolic response to external stimuli and in identifying the responsible individual metabolites and/or metabolic pathways. It is one of the few cases in which a combination of systems biology methods has led to the identification of a single cause of phenotypic change that can subsequently be studied in detail with classical methods.

POSTER 19.

MASS SPECTROMETRY AND SHOTGUN METABOLOMICS IN ALZHEIMER'S DISEASE DIAGNOSIS

Jose´ Luis Gómez-Ariza (1), Raul González-Domínguez (2) and Tamara García-Barrera (3)

1. Departamento de Química y CC MM (Área de Bioquímica), Facultad de Ciencias Experimentales, Universidad de Huelva, Campus de El Carmen, 21007 Huelva, Spain; 2. Campus of Excellence International ceiA3, University of Huelva, Spain; 3. Research Center of Health and Environment (CYSMA), University of Huelva, Campus de El Carmen, 21007 Huelva, Spain

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by a progressive decline of cognitive functions (memory, reason, and orientation). The high complexity of this disease makes the etiology is not known completely, which means that diagnosis is actually based on clinical criteria that require the presence of significant cognitive deficits. Thus, the discovery of biomarkers that allow early diagnosis is extremely important. In biomarker discovery highlight 'omics' sciences, including metabolomics, which involves both gene expression and external factors (diet, exercise) and lifestyle habits, providing a more realistic view of the pathology.

High resolution mass spectrometry (hybrid systems QqQ-TOF, triple quadrupole - time of flight) has great potential in metabolomic studies, being possible to obtain comprehensive metabolomic profiles. Furthermore, the high resolution of the technique allows direct infusion analysis of the sample without prior chromatographic separation, which means a very rapid and reproducible methodology.

In the present study was developed a procedure for metabolomic analysis of blood serum by direct infusion into a mass spectrometer with electrospray ionization (DI-ESI-QQQ-TOF-MS). The application of this technique to samples of AD patients and healthy controls allowed obtaining distinct metabolomic profiles. In addition, using multivariate analysis techniques, discriminant models were constructed that allowed us to distinguish between sick and healthy cases, and the identification of metabolites that could serve as potential biomarkers of Alzheimer's disease. These markers can be related to different metabolic pathways altered in neurodegenerative processes, so it can be concluded that the methodology developed represents an interesting approach to understanding the pathogenesis of Alzheimer's disease.

POSTER 20.

PHASE II SULPHATED AND GLUCURONIDATED BIOACTIVE PHENOLICS ARE POTENTIAL URINARY BIOMARKERS FOR HABITUAL EXPOSURE TO WHOLEGRAIN RYE BREAD

Amanda Lloyd (1), John Draper (1), Manfred Beckmann (1), Chris Seal (2), Sumanto Haldar (2) and Kirsten Brandt (2)

1. Institute of Biological Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, SY23 3DA, United Kingdom; 2. Human Nutrition Research Centre, School of Agriculture, Food & Rural Development, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

There is strong observational evidence supporting the link between increased consumption of wholegrain foods and reduced risk of several diseases including cardiovascular diseases, type 2 diabetes and some cancers. However, understanding of causal relationships between dietary wholegrain and health is hindered by incomplete knowledge of the range of potentially bioactive metabolites derived from cereal bran. We aimed to discover urine biomarkers indicative of habitual exposure to rye bread to explore the fate of plant secondary metabolites found in wholegrain foods.

We examined 24 hour urine samples from free-living individuals (n=15) after they switched from a diet avoiding wholegrain to a new diet in which they elected to consume habitually initially 3 portions and then 6 portions of rye bread per day, each for a period of 4 weeks. We analyzed these samples using Flow Infusion Electrospray-Ionization Mass Spectrometry (FIE-MS) followed by supervised multivariate data analysis to identify chemicals related to increased rye bread consumption.

Urines from participants reporting high levels of rye bread consumption were discriminated robustly (AUC = 0.89) from urine samples taken during the washout period. Around 20 mass-to-charge ratio (m/z) signals provided the majority of the discriminatory power in 24 hr urine. A Hierarchical cluster analysis based on the correlation coefficient (Pearson correlation method) of 24 hr urine samples indicated several of the top ranked signals were correlated. Further investigation of these top ranked correlated mass bins was performed by targeted accurate mass analysis using Fourier Transform-Ion Cyclotron Resonance Ultra-Mass-Spectrometry (FT-ICR-MS) followed by FIE-MSn fragmentation. Several phase II sulphated and glucuronidated bioactive phenolics were identified as well as a cluster of ferulate derivatives. To summarise, a wider than previously thought range of potentially bioactive phenolics are potential urinary biomarkers for exposure to wholegrain rye bread.

POSTER 21.

HR-MAS NMR METABONOMICS FOR THE CLASSIFICATION OF MEN1 VARIANTS

Claire Lopez (1), Cécile Vercherat (2,3), Benjamin J. Blaise (1), Annie Lacheretz-Bernigaud (2,3), Mathilde Bayet-Robert (1), Lamya Rezig (1,2,3), Jean-Yves Scoazec (2,3), Alain Calender (2,3), Lyndon Emsley (1), Bénédicte Elena (1) and Martine Cordier-Bussat (2,3)

1. Université de Lyon, CNRS / ENS-Lyon / UCB Lyon 1, Centre de RMN à Très Hauts Champs, 5 rue de la Doua, 69100 Villeurbanne, France; 2. Inserm, U1052, CRCL team4, Faculté de Médecine Laennec, 7-11 rue Guillaume Paradin, Lyon, F-69372 France; 3. Univ Claude Bernard, Lyon1, Lyon, F-69372 France

Multiple endocrine neoplasia syndrome type 1 (MEN1) is a rare genetic disorder, characterized by the association of endocrine tumours in tissues. The MEN1 syndrome is caused by mutations of the MEN1 gene, which encodes a protein known as menin. Menin has multiple potential partners, making it difficult to predict the functional consequences of its invalidation and its role in tumorigenesis. The disease is associated with a large diversity of mutations, where missense mutations resulting in amino acid substitution account for ~20% of mutations identified in patients with MEN1 syndrome. As a consequence, functional tests allowing their classification as 'pathogenic mutations' remains to be developed.

Here, we determine the metabolic perturbations associated with different variants or disease-related mutations in the MEN1 gene. Several independent germline mutations distributed throughout the MEN1 gene coding region have been identified from patients with MEN1 syndrome [1,2]. A murine cell model was developed allowing overexpression of human wild type (WT) menin or mutated menin after a doxycycline treatment. High-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR) at 700 MHz is applied on cell lines to evaluate the metabolic consequences of pathogenic mutations and to classify several missense variants. Metabolic fingerprints are obtained from ^{1}H HRMAS NMR of whole murine cells overexpressing either the human wild type or mutant menin. We focus on menin variants with amino acid substitution in their N-terminal, central or C-terminal parts, such as pathogenic mutants corresponding to disease-related missense mutations in MEN1 patients, suspect SNP (single-nucleotide polymorphism) representing non pathogenic or benign polymorphism, as well as silent SNPs corresponding to simple DNA mutations (change of a nucleotide, without change in amino acid), or artificial mutants for which amino acids are not conserved. Negative controls that do not overexpress menin (no effect of doxycycline) and wild type menin were also analysed.

We show that a robust metabolic signature discriminating cell samples with the WT or pathogenic menin can be obtained from multivariate statistical modeling of the NMR profiles. Moreover, we observed that controversial menin variants display a metabolic behavior close to the disease-related proteins. We identified significant metabolites, as phosphocholine or taurine, for the discrimination of pathological mutations versus WT, some of them being identified in the literature as metabolic biomarkers for tumors.

[1] Chandrasekharappa SC, *et al.* J Intern Med 253:606 (2003); [2] Wautot V, *et al.* Hum Mutat 20:35 (2002).

POSTER 22.

METABOLIC CHANGES IN INDOLENT AND AGGRESSIVE PROSTATE CANCER – CORRELATIONS WITH THE GLEASON GRADING SYSTEM

Guro F. Giskeødegård (1,2), Helena Bertilsson (3,4), Kirsten M. Selnæs (1,2), Alan Wright (5), Tone F. Bathen (1,2), Trond Viset (6), Jostein Halgunset (3,6), Anders Angelsen (4), Ingrid S. Gribbestad (1,2), May-Britt Tessem (1,2)

1. MI Lab, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; 2. St. Olavs Hospital, Trondheim University Hospital, Norway; 3. Department of Laboratory Medicine and Children's and Women's Health, NTNU, Trondheim, Norway; 4. Department of Urology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway; 5. Department of Radiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 6. Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Introduction: Currently there are no accurate diagnostic tools for discriminating aggressive from harmless types of prostate cancer. In this study, high resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS) was used to provide the metabolite profiles of human prostate cancer and normal adjacent tissues. The purpose was to identify metabolic biomarkers for prostate cancer aggressiveness.

Materials and methods: Using a new harvesting method [1], high quality prostate tissue samples (n=162 samples, 48 patients) were obtained from normal tissue and cancer tissue with different Gleason score (score 6-9, where 9 is the most aggressive) and analyzed by HR MAS MRS (Bruker avance DRX600). Multivariate analysis (PLS, PLS-DA) and absolute quantification using LCModel were used to examine the metabolic changes and predict cancer aggressiveness by comparing normal, low grade (Gleason score = 6) and high grade (Gleason score > 7) cancers. The multivariate models were validated by double cross-validation and permutation testing, and differences in metabolite concentrations were examined using linear mixed models.

Results and discussion: Based on the metabolite profiles, normal tissue, low grade and high grade tissue were discriminated with a classification accuracy of 85%, 66% and 77%, respectively, using PLS-DA. Out of 23 quantified metabolites, 17 metabolites were significantly changed in cancer samples compared to normal adjacent tissue ($p < 0.05$). The metabolite profiles could be related to Gleason score ($r = 0.71$) by PLS analysis. High grade cancer tissues were distinguished from low grade cancer tissues by decreased concentrations of spermine ($p = 0.0044$) and citrate ($p = 7.73 \bullet 10\text{-}4$), suggesting spermine and citrate as metabolic biomarkers for prostate cancer aggressiveness.

Conclusion: HR-MAS MRS provides detailed metabolite profiles distinguishing cancer and normal adjacent tissues. The metabolite profiles are related to prostate cancer aggressiveness, and spermine and citrate are promising biomarkers for separating indolent from aggressive prostate cancers. HR-MAS MRS can be used as an additional diagnostic tool, and the results illustrate the benefit of MRS in future in vivo investigations of prostate cancer patients.

[1] Bertilsson *et al* (2011) The Prostate 71: 461-469.

POSTER 23.

EFFECT OF MILLING AND COOKING PROCESSES ON BENZOXAZINOIDS IN WHEAT AND RYE

Fariha Tanwir (1), Per L. Gregersen (1) and Inge S. Fomsgaard (2)

1. Dept. of Molecular Genetics and Biotechnology, Aarhus University, Forsogsvej 1, 4200 slagelse, Denmark; 2. Dept. of Agroecology, Aarhus University, Forsogsvej 1, 4200 slagelse, Denmark

Benzoxazinoids belong to an important group of phytochemicals, potentially conferring various health promoting effects, i.e. anti-allergic, anti-inflammatory and anti-cancer effects. Rye contains significant amounts of these benzoxazinoids compared to wheat and is an important part of staple food in northern Europe and America. The aim of this study was to quantify levels of different benzoxazinoids in seed fractions during the milling process as well as to understand the effect of various cooking processes on these compounds and their biotransformation in cereals.

Our results on different seed fractions indicated that significantly high concentrations of these compounds are present in germ that constitutes only 3 to 5% of whole seed compared to other fractions, i.e. bran or endosperm. HBOA, HBOA-gly and HBOA-gly-hexose are the predominant compounds found in different seed fractions.

During cooking, polished rye seeds and rye flakes were either soaked or boiled. After soaking or boiling, samples were freeze dried and analysed on LC-MS/MS. Water left after soaking or boiling was also analysed for excreted benzoxazinoids. LC-MS/MS analysis of benzoxazinoids in soaked cereals showed a significant increase of DIBOA-gly, DIBOA and HBOA-gly as compared to either boiled or raw polished rye seeds, although an increase of these compounds was observed during boiling, but the level of these compounds was low as compared to level in soaked cereals.

In rye flakes the pattern of these benzoxazinoids was different to that in polished rye seeds. High levels of DIBOA-gly, HBOA-gly-hexose and HBOA-gly were observed during boiling compared to soaking. A considerable amount of these benzoxazinoids was also present in the water left after soaking or boiling. This study will give the consumers an understanding of the reasons for choosing potentially healthier foods in daily life.

POSTER 24.

METABOLIC RESPONSE TO GLYCEROPHOSPHOCHOLINE PHOSPHODIESTERASE GDPD5 SILENCING IN HUMAN BREAST CANCER CELLS

Maria D. Cao (1,2), Lu Jiang (1), Tiffany R. Greenwood (1), Balaji Krishnamachary (1), Zaver M. Bhujwalla (1), Ingrid S. Gribbestad (2) and Kristine Glunde (1)

1. Johns Hopkins University In Vivo Cellular and Molecular Imaging Center, Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; 2. Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), 7489 Trondheim, Norway

INTRODUCTION: We recently demonstrated that the glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) gene is associated with choline phospholipid metabolite levels and breast cancer malignancy [1]. In this study, we investigated the metabolic effects of GDPD5 silencing using short interfering RNA (siRNA) in human breast cancer cells.

EXPERIMENTAL: We tested three different siRNA targeting sequences for silencing GDPD5 (siRNA-GDPD5) in weakly malignant human MCF-7 (n=8) and highly malignant human MDA-MB-231 (n=9) breast cancer cells. The siRNA-GDPD5 treated cells were compared with respective controls that were treated with scrambled siRNA. The knockdown efficiency of GDPD5 was assessed by quantitative real time-polymerase chain reaction (qRT-PCR). The metabolite levels were measured using fully relaxed high-resolution proton magnetic resonance spectroscopy (HR 1H MRS) on a Bruker Avance 500 MR Spectrometer as previously described [2].

RESULTS: GDPD5 silencing resulted in a significant reduction of GDPD5 mRNA levels in MCF-7 as well as MDA-MB-231 cells to about 10% of the respective scrambled siRNA controls. Cellular glycerophosphocholine (GPC, p=0.041) and choline containing metabolites (tCho, p=0.042) levels increased following siRNA-GDPD5 treatment in MCF-7 and MDA-MB-231 breast cancer cells, respectively.

DISCUSSION and CONCLUSION: Here we demonstrated for the first time that silencing GDPD5 increased the choline phospholipid metabolite levels in breast cancer cells, suggesting GDPD5 as a potential anticancer target in regulating choline phospholipid metabolism in breast cancer. tCho levels increased in highly malignant MDA-MB-231 cells, but not in weakly malignant MCF-7 cells upon GDPD5 silencing, proposing different roles for GDPD5 in these two breast cancer cell lines. We are currently further investigating the molecular and metabolic effects of GDPD5 silencing in breast cancer cells and animal models to uncover the role of GDPD5 in choline phospholipid metabolism and malignancy of breast cancer.

[1] Cao M.D. *et al,* NMR in Biomed 2012; [2] Glunde et al, Cancer Res 2004.

POSTER 25.

METABOLIC PROFILING OF STEROIDOGENESIS IN THE HUMAN H295R ADRENOCORTICAL CELL LINE FOR DETECTION OF ENDOCRINE DISRUPTORS

Arjen Lommen (1,2), Jeroen C.W. Rijk(1,2), Toine F.H. Bovee (1,2), Ron L.A.P. Hoogenboom (1) and Ad A.C.M. Peijnenburg (1,2)

1. RIKILT – Institute of Food Safety, Wageningen UR, The Netherlands; 2. Netherlands Toxicogenomics Centre (NTC), The Netherlands.

Endocrine disruptors can cause non-receptor mediated effects either indirectly by altering common signal-transduction pathways or directly via (non-)competitive inhibition of enzymes involved in the steroidogenic pathway. The H295R steroidogenesis assay provides an in vitro cell-based assay to evaluate the potential interference of compounds with steroid hormone production. Current endpoints of this assay are limited to measuring a selected set of hormones using targeted analytical methods such as LC- and GC-MS or EIAs. Recent developments in LC-MS and bioinformatics however, allow more comprehensive approaches to evaluate changes in steroid profiles. In the current work, a metabolomics approach was developed that monitors changes in metabolite profiles in both a targeted and untargeted way.

Therefore, H295R cells were exposed for 48h to forskolin, aminoglutethimide, prochloraz, trilostane and atrazine, which are compounds known to affect steroidogenesis. After exposure, the culture medium was subjected to a solid phase extraction clean-up procedure and analyzed by Ultra Performance Liquid

Chromatography Time-Of-Flight Mass Spectrometry (UPLC-TOF/MS). Generated profiles were compared to profiles obtained from DMSO blanks using sophisticated preprocessing and alignment software (MetAlignTM). Differential mass signals (p-value <0.05 and fold change >2) were selected and profiles typical for the mode of action of the selected reference compounds were constructed. The observed differences in metabolite profiles were mainly caused by alterations in levels of free and sulfated steroids. These alterations were considered to be very relevant as the identity of the differential metabolites could be related to the expected effect of the compound under investigation. In conclusion, it can be stated that application of a comprehensive metabolite profiling methodology provides a promising analytical approach to screen compounds for steroidogenic modulating properties as well as chemical class prediction.

POSTER 26.

FROM TARGETED ANALYSIS TO STEROIDOMICS

Serge Rudaz (1,2), Julien Boccard (1,2), Flavia Badoud (1,2,3), N. Baume (2,3) and Martial Saugy (2,3)

1. School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland; 2. Swiss Centre for Applied Human Toxicology, University of Geneva, CMU, Geneva, Switzerland; 3. Swiss Laboratory for Doping Analyses, University Center of Legal Medicine, Epalinges, Switzerland

Analytical procedures regularly applied for steroids control in doping analysis rely on the detection of unexpected variations of the steroid profile, which include a restricted number of endogenous compounds. This monitoring is related to the quantification of levels and concentrations ratios of testosterone, epitestosterone, androsterone, etiocholanolone, dehydroepiandrosterone, 5α- or 5β-androstane-3α,17β-diol and dihydrotestosterone in urine.

A targeted analysis was performed with these reference compounds thanks to an original method based on UHPLC-QTOF-MS. The latter was developed as a promising alternative to assess the steroid metabolism by simultaneously measuring compounds in glucuronidated and sulfated forms. It was applied to sample coming from a clinical study related to testosterone oral intake. Time kinetics was assessed and the classical detection window was confirmed. The results were in accordance with well-established anti-doping screening protocols. Thanks to TOF full mass range acquisition, a new data evaluation was further engaged to provide a broader coverage of urinary steroid metabolites. Among the 5,750 detected mass features, automatic peak detection was applied by using reference m/z values corresponding to putative steroids. This filtering procedure ensured the selection of an information rich variable subset of 234 steroid-related peaks. Chemometric tools, including N-PLS, O-PLS, SUS-plot and ROC curves were used for data mining to provide a insight into the urinary excretion pattern. The filtered temporal monitoring of urine samples provided useful information about kinetics of steroid excretion.

Both known metabolites and new biomarkers could be highlighted by these means and allowed the extension of the urinary detection window after testosterone intake. Further investigations of promising candidates by targeted analysis need to be performed to ensure proper identification.

POSTER 27.

METABOLOMIC PROFILING OF WATER SUPPLY NETWORK BIOFILMS TO INFORM ASSET MANAGEMENT DECISIONS

David Beale (1), Michael S. Dunn (2), Paul D. Morrison (2), Enzo A. Palombo (3) and David R. Marlow (1).

1. Land and Water, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Highett VIC, Australia; 2. School of Applied Sciences, RMIT University, Melbourne VIC, Australia; 3. Environment and Biotechnology Centre, Swinburne University of Technology, Hawthorn VIC, Australia.

Pipe biofilms are known to contribute to waterborne disease outbreaks, accelerate corrosion of pipes and cause aesthetic water quality issues within the potable water supply network. One way to better understand and manage this problem is to develop a strategy for identifying if corrosion or water quality failure is microbially influenced prior to implementing strategies to ameliorate the problem. Metabolomics is one approach to achieve this goal. It was found experimentally in a lab scale biofilm rig that a profile of the extracellular metabolites from a microbial biofilm attached to the inside of a pipe could be achieved by analysing the passing water using a suite of gas chromatography techniques, in particular GC-MS, GCxGC-FID and GC-TOFMS. Chemometric analysis of the one and two dimensional chromatograms in conjunction with the mass spectrometric data of the biofilm/water samples (bacterium consortium), along with seven isolated biofilm bacteria, enabled differentiation of those believed to cause microbial corrosion or water quality failure and those that did not. The suitability and practical implications of this technique to inform water practitioner asset management decisions is also discussed via the presentation of a pilot study, where samples were collected and analysed for metabolomic biomarkers from a water mains cleaning program.

POSTER 28.

SERUM METABOLOMIC PROFILING FOR EARLY DETECTION OF COLORECTAL CANCER

Masaru Yoshida (1,2,3), Shin Nishiumi (1), Yoshihiro Izumi (1), Atsuki Matsubara (1), Takeshi Azuma (1)

1. Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; 2. The Integrated Center for Mass Spectrometry, Kobe University Graduate School of Medicine, Kobe, Japan; 3. Division of Metabolomics Research, Kobe University Graduate School of Medicine, Kobe, Japan

To improve the quality of life of colorectal cancer patients, it is important to establish new screening methods for early diagnosis of colorectal cancer. We performed serum metabolome analysis using gas-chromatography/mass-spectrometry (GC/MS). First, the accuracy of our GC/MS-based serum metabolomic analytical method was evaluated by calculating the RSD% values of serum levels of various metabolites. Second, the intra-day (morning, daytime, and night) and inter-day (among 3 days) variances of serum metabolite levels were examined. Then, serum metabolite levels were compared between colorectal cancer patients (N=60; N=12 for each stage from 0 to 4) and age- and sex-matched healthy volunteers (N=60) as a training set. The metabolites whose levels displayed significant changes were subjected to multiple logistic regression analysis with the stepwise variable selection method, and a colorectal cancer prediction model was established. The validity of the prediction model was confirmed using colorectal cancer

patients (N=59) and healthy volunteers (N=63) as a validation set.

The prediction model was composed of the 4 metabolites selected, and its AUC, sensitivity, specificity, and accuracy were 0.9097, 85.0%, 85.0%, and 85.0%, respectively, according to the training set data. In contrast, the sensitivity, specificity, and accuracy of CEA were 35.0%, 96.7%, and 65.8%, respectively, and those of CA19-9 were 16.7%, 100%, and 58.3%, respectively. At the validation set, the sensitivity, specificity, and accuracy of the prediction model were 83.1%, 81.0%, and 82.0%, respectively, and these values were almost the same as those obtained with the training set. In addition, the model displayed high sensitivity for detecting stage 0-2 colorectal cancer (82.8%).

Our prediction model established via GC/MS-based serum metabolome analysis is valuable for early detection of colorectal cancer and has the potential to become a novel screening test for colorectal cancer.

POSTER 29.

ALLELOPATHIC EFFECTS OF BIOCHANIN A ON SELECTED WEED SPECIES

Hans Albert Pedersen, Tariqul Islam Shajib, Anne G. Mortensen, Per Kudsk, Inge S. Fomsgaard

Aarhus University, Department of Agroecology, Slagelse, 4200, Denmark

The uptake and allelopathic effects of biochanin A on monocot and dicot weed species were evaluated in agar medium bioassays with concentrations up to 400 µmol/L. The monocot *Echinochloa crus-galli*, the dicot *Geranium molle*, and the dicot *Silene noctiflora* were non-susceptible, susceptible, and very susceptible, respectively. Biochanin A and its known transformation products genistein, dihydrobiochanin A, pratensein, and coumaric acid were found and quantified in all tested weed species using LC-MS and pure reference standards. Concentrations of all compounds were higher in roots than in shoots. The uptake of biochanin A was highest in *G. molle*. For identification of unknown compounds principal component analysis was performed using the peaks observed during LC-MS. An unknown compound displaced along the first principal component was identified as biochanin A 7-O-D-glucopyranoside (sissotrin). Glucosylation thus appears to be a mechanism for detoxification of biochanin A.

POSTER 30.

USE OF MULTIVARIATE ANALYSIS FOR SELECTION OF DIAGNOSTIC MARKERS IN URINE IN NEONATES WITH 21-HYDROXYLASE DEFICIENCY

Sofia Christakoudi (1), Georgios Christakudis (3), David Cowan (2), Norman Taylor (1)

1. King's College Hospital, London, UK; 2. King's College London, UK; 3. University of Sofia, Bulgaria

Urine from neonates with 21-hydroxylase deficiency, unlike that from affected older children and adults, contains a multitude of 17-hydroxyprogesterone, 21-deoxycortisol and androgen metabolites, most with incompletely defined structure. The classical markers of the disorder are at low levels in the first days of life. Treatment given prior to sample collection is a further diagnostic confounder. This drives the search for alternative markers.

Urine from affected neonates (111 untreated and 42 treated) aged between birth and 60 days and 7 unaffected controls (at ages around 1, 4, 10 and 29 days) was studied. Steroids were analysed, after enzymatic conjugate hydrolysis, as methyloxime-

trimethylsilyl ether derivatives. Structural characterisation was based on GC-MS and ion trap GC-MS/MS spectra and was aided by methods of microchemistry. Some 300 endogenous metabolites, most characterised for the first time, were quantified relative to creatinine, using a limited number of available standards as calibrants. Metabolite ratios representative of different enzyme activities were also calculated.

Distinction between affected and unaffected neonates was examined by the method of orthogonal projections to latent structures-discriminant analysis (OPLS-DA). Two approaches to variable selection were compared; based solely on the regression coefficients b1 of the PLS model fitted to the OPLS pre-treated matrix and based on a combination of b1 and the regression coefficients bO1 of the first orthogonal (unrelated to the group separation) component. Regression coefficients of comparisons between untreated patients and controls and between treated patients with high level of adrenal suppression and controls were simultaneously considered. The untreated patients were used as a training set and the complete group of treated patients as a test set. A very successful model based on b1 alone comprised six 11-oxo metabolites derived from 21-deoxycortisol, characteristic of the disorder and one metabolite ratio. These had very high F-ratios when one-way ANOVA comparison between different age groups of untreated patients and controls was performed. A model based on a combination of b1 and bO1, however, had worse performance.

In conclusion, OPLS-DA provides an excellent mathematical platform for discrimination between neonates with 21-hydroxylase deficiency (with and without treatment) and controls. Use of regression coefficients as selection criteria results in a biologically sound choice of variables, while targeting low orthogonal variation is not beneficial.

POSTER 31.

METABOLOMICS AND DEREPLICATION STUDIES OF ENDOPHYTIC METABOLITES FROM SOME EGYPTIAN MEDICINAL PLANTS IN THE SEARCH FOR NEW POTENTIAL ANTI-CANCER DRUGS

Ahmed F. Tawfike (1,2), Muhammad Romli (1), Roth Tate (1), Marc Diederich (3), Marc Schumacher (3) and RuAngelie Edrada-Ebel (1)

1. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, The John Arbuthnott Building , 161 Cathedral Street, Glasgow G4 0NR, Scotland; 2. Faculty of Pharmacy, Helwan University, Cairo, Egypt; 3. Fondation Recherche sur le Cancer et les Maladies du Sang, Laboratoire de Biologie Moleculaire, et Cellulaire du Cancer, Hopital Kirchberg 9, rue Edward Steichen, L-2540 Luxembourg.

Endophytic fungi associated with medicinal plants represented a potential source of novel chemistry and biology. This study involved isolation of five endophytic fungal strains from different Egyptian medicinal plants. Identification of the strain has been achieved through molecular biological methods. Metabolomic profiling, using 2D-NMR and HR-ESIFTMS were done at different stages of the growth phase for both solid and liquid culture media. Dereplication studies were accomplished by utilizing the Mzmine software with the Antimarin database. The optimised method in terms of media, incubation time, and maximum production bioactive compounds were taken into account for the scale-up. The thirty-day rice culture extract of Aspergillus flocculosus were fractionated using different high-throughput chromatographic techniques and subjected to selected bioactivity-guided isolation approaches. This led to the identification of

compounds 1a and 1b which are both novel and possess moderate activity against NFk-b.

POSTER 32.

ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION OF DIETARY BENZOXAZINOIDS IN RATS

Khem Adhikari (1), Helle N. Lærke (2), Anne G. Mortensen (1) and Inge S. Fomsgaard (1)

1. Department of Agroecology, Faculty of Science and Technology, Aarhus University, Slagelse, Denmark; 2. Department of Animal Science, Faculty of Science and Technology, Aarhus University, Tjele, Denmark.

Benzoxazinoids are a group of bioactive phytochemicals mostly found in cereal plants including grains. Several potentially beneficial healthful and pharmacological effects of benzoxazinoids have been described previously in some epidemiological and in vitro studies. Concerning the daily consumption of 50 g of rye bread (the major wholegrain product in Scandinavian region), the daily total benzoxazinoids intake through a specific brand of wholegrain rye bread can be estimated to be higher than 5 mg/d. However, there is a paucity of information regarding the absorption, distribution, metabolism, and elimination of these dietary compounds in mammals. A rye bread-based diet containing a daily dose of 4780 ± 68 µmol benzoxazinoids, principally 2-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-glc; 2243 ± 32 nmol) was fed for 2 wk to 12 rats which were housed in metabolic cages for last 7 d followed by euthanasia. In parallel, 12 other rats were fed AIN-93G as control. The benzoxazinoid compounds and their conjugated derivatives in diets, plasma, urine, and faeces were identified and quantified using high-performance liquid chromatography coupled to electrospray ionization triple quadrupole mass spectrometry. Three benzoxazinoid compounds: 2-hydroxy-1,4-benzoxazin-3-one (HBOA), its glucosidic analogue HBOA-glc, and DIBOA-glc (17 ± 4, 74 ± 27, and 17 ± 8 nmol/L, respectively) were detected in rat plasma collected at 3 h or more after the cessation of feed intake. The total urinary excretion of benzoxazinoids was 1176 ± 66 nmol/d corresponding to approximately 25% of the total intake. The urinary benzoxazinoids profile was noticeably different from that of plasma with major urinary components; HBOA-glc and DIBOA-glc (647 ± 31 and 466 ± 33 nmol/d, respectively). The glucuronide conjugates of HBOA and DIBOA were detected in plasma and urine, high intensities of peaks in the latter, indicating substantial phase II metabolism. The N-dehydroxylation of hydroxamic acids (DIBOA, DIBOA-glc, etc.) into lactams (HBOA, HBOA-glc, etc.) is a critical mechanism for the gastrointestinal absorption and metabolism. This study revealed for the first time that bioactive benzoxazinoids in rye bread are highly bioavailable in rats. The findings of the present study might be helpful to understand the healthful and pharmacological effects of benzoxazinoid compounds in vivo. Moreover, benzoxazinoids could be an important component to contribute the overall healthful effects of wholegrain rye products.

POSTER 33.

COMPARATIVE METABOLIC PROFILING OF STAPHYLOCOCCUS AUREUS USING LC-QTOF-MS AND GCXGC-TOF-MS FOR POTENTIAL BIOMARKER IDENTIFICATION

Konstantinos Kouremenos (1), Maria Fallman (1), Anders Johansson (2), Anna Fahlgren (1), Henrik Antti (3), Peter Haglund (3),Thomas Mortiz (4), Philip Marriott (5), Hans Wolf-Watz (1)

1. Department of Molecular Biology, Umeå University, SE-901 87, Umeå, Sweden; 2. Department of Clinical Microbiology, Umeå University, SE-901 85, Umeå, Sweden; 3. Department of Chemistry, Umeå University, SE-901 87, Umeå, Sweden; 4. Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 87, Umeå, Sweden; 5. Centre for Green Chemistry, School of Chemistry, Monash University, Clayton, 3800, Victoria, Australia

Staphylococcus aureus (*S.aureus*) is a major pathogen that highlights the serious antibiotic resistance problem. A comparative metabolomic study of 'normal' MSSA (methicillin-sensitive *staphylococcus aureus*) and MRSA (methicillin-resistant *staphylococcus aureus*) was conducted on both in-vitro and human serum samples. The aim was to identify potential biomarkers using LC-QToFMS (in both +ESI and -ESI modes), as well as LLE (liquid-liquid extraction) and SPME (solid-phase microextraction) with GCxGC-ToFMS, in order to obtain a comprehensive coverage of the metabolome. Data processing steps (baseline correction, alignment, normalization) and multivariate data analysis techniques - PCA (principal component analysis) and OPLS-DA (orthogonal partial least squares-discriminant analysis) - were used to discriminate normal, MSSA and MRSA samples. Differentially expressed metabolites were obtained which could be used as possible biomarkers to distinguish normal and diseased states. Overall, the results of this study demonstrate that the two sensitive and robust metabolic profiling approaches can be used as promising screening tools for the comprehensive, fast diagnosis of infections caused by *S.aureus*, complementary to existing clinical procedures.

POSTER 34.

METABOLOMICS: A POWERFUL TOOL FOR INVESTIGATING THE RELATIONSHIP BETWEEN FOOD AND HEALTH?

Frédérique Courant (1), Sylvain Chéreau (1), Gaud Dervilly-Pinel (1), Jean-Philippe Antignac (1,2), Emmanuelle Bichon (1), Sébastien Anizan (1), Fabrice Monteau (1) and Bruno Le Bizec (1)

1. LUNAM Université, Oniris, Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France; 2. INRA, Nantes, F-44307, France

LABERCA, the Laboratoire d'Étude des Résidus et Contaminants dans les Aliments, is a research unit of the Nantes Atlantique College of Veterinary Medicine, Food Science and Engineering (Oniris). The general field of activity of LABERCA fits within a global and integrated approach of risk assessment, from agricultural supply to human and its mankind. LABERCA generates knowledge and data related to the sources, transport and metabolism of chemical substances, in order to simultaneously characterise both exposure of consumers to chemical pollutants (occurrence of these parameters in foodstuffs) and their internal dose (occurrence of the same parameters, their metabolites or their degradation products in biological fluids and human tissues). Besides conventional and historical targeted approaches based on liquid/gas chromatography/mass spectrometry developed by the unit to monitor and quantify these compounds, LABERCA has focused its research efforts toward the development and implementation of new and more open-ended methodological strategies, notably metabolic profiling or metabolomic. Overall, the unit aims at characterising the biological changes that occur following exposure to a chemical and/or

revealing new biomarkers of exposure and/or effects associated with these pollutants. A general framework has been designed and implemented to conduct such studies, with respect to sample preparation [1,2], generation of metabolic profiles by GC [3,4] or LC [5,6] coupled with low [7,8] or high [9] resolution mass spectrometry and raw data processing to statistical analysis by multivariate techniques [10,11]. This led to the to the definition and validation of an analytical strategy that today represents the laboratory's master plan with regard to this kind of studies [12]. It has demonstrated the benefit of untargeted approaches to get new insights into various research fields such as health [13], environment [14] and food safety [15,16] issues.

[1] Dervilly-Pinel G., et al., Drug Testing and Analysis 2012, in press; [2] Kieken F., et al., Metabolomics, 2011, 7:84-93; [3] Anizan S., et al., J Chrom A, 2010,1217:6652-6660; [4] Anizan S., et al., J Mass Spec 2012;47:131-140; [5] Boyard-Kieken F., et al., J Separation Science 2011,34:3493-3501; [6] Courant F., et al., Analyst, 2009, 134:1637-1646; [7] Anizan S., et al., Talanta 2011,86:186-194; [8] Anizan S., et al., Anal. Chim. Acta, 2011, 700:105-113; [9] Pinel G., et al., Anal. Chim. Acta, 2011, 700:144-154; [10] Qannari El M et al., Curent Anal Chem 2012, in press; [11] Moyon T., et al., Metabolomics 2012, in press; [12] Antignac J.P., et al., TrAC, 2011, 30:292-301; [13] Alexandre-Gouabau M.C., et al., J Prot Res 2011,10:3292-3302; [14] Gervais G. et al., Chemosphere 2011,83:1553-1559; [15] Pinel G., et al., TrAC, 2010, 29:1269-1280; [16] Kieken F., et al., Anal Bioanal Chem, 2009, 394:2119-2128.

POSTER 35

MZMATCH/MZMATCH.R – A PRIORITISATION PIPELINE FOR THE ANALYSIS OF METABOLOMICS DATASETS

Andris Jankevics (1,2), Achuthanunni Chokkathukalam (2)and Rainer Breitling (1,2)

1. University of Groningen, Groningen, The Netherlands; 2. University of Glasgow, Glasgow, United Kingdom

Liquid Chromatography Mass Spectrometry (LC-MS) is a powerful and widely applied method for the study of biological systems, biomarker discovery and pharmacological studies. LC-MS measurements are, however, significantly biased by several factors, including: (1) ionisation suppression/enhancement, interfering with the correct quantification of analytes; (2) detection of large amounts of derivative ions, increasing the complexity but not the information content; and (3) machine drift during extensive sample sequences, altering mass and quantification accuracy.

Here we present our efforts to produce a customisable pipeline for mass spectra calibration [1], processing [2], filtering [3-5], and annotation [3]. The benefits of such a processing pipeline include an easy "rewind" option to roll back to intermediate steps in the data analysis and increased verifiability of the performance of the analytical methods. The developed approaches have provided us with a fuller understanding of the information content of our observations and a better assessment of the metabolites detected in the analyzed data sets [6]. This pipeline can be extended to include advanced techniques such as Bayesian statistical models and stable isotope labelling studies.

We illustrate the application of this pipeline in a variety of large metabolomics studies, ranging from work on bacterial cell extracts to human biofluids.

[1] Scheltema RA, et al. (2008) Proteomics 8 (22):4647-4656; [2] Scheltema RA, et al. (2011) Anal. Chem. 83 (7):2786-2793; [3] Scheltema RA, et al. (2009) Bioanalysis 1 (9):1551-1557; [4] Jankevics A, et al. (2011) Metabolomics In Press, DOI:10.1007/s11306-011-0341-0; [5] Creek DJ, et al. (2011) Anal. Chem. 82 (22): 8703-8710; [6] Jankevics A, et al. (2011) Proteomics 11 (24):4622:4631

POSTER 36.

MZMATCH-ISO: AN EXTENSION OF MZMATCH FOR THE IDENTIFICATION AND RELATIVE QUANTIFICATION OF ^{13}C LABELLED MASS SPECTROMETRY DATA

Achuthanunni Chokkathukalam, Andris Jankevics and Rainer Breitling

Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK

Stable isotope labelling experiments have recently gained increasing popularity in global metabolomics studies providing unique insight into the dynamics of metabolic fluxes, beyond the steady-state information gathered by routine mass spectrometry. Here we describe mzMatch-ISO, a new extension to mzMatch.R [1] that can be used for the rapid and reliable identification and relative quantification of isotope peaks in ^{13}C-labelled mass spectrometry (MS) data. mzMatch.R was introduced recently as an open-source software toolbox for the MS data processing, and is the underlying platform for user-friendly software such as IDEOM [2].

mzMatch-ISO can perform both targeted and untargeted profiling for labelled metabolites. In both these approaches, missing monoisotopic and related isotope peaks of individual samples are gap-filled from the raw data, while stringent filters exclude peak sets that are not reproducibly labelled. The output of mzMatch-ISO are plots that highlight trends in labelling and a table containing the area under each isotope peak in each sample for each metabolite. While the latter can be used for further statistical analysis, the former provide a convenient visual representation of the quality and quantity of labelling. For each metabolite identified four different types of plot are provided that show; a) the isotope peaks of each monoisotopic peak in each sample group; b) the ratio of monoisotopic and labelled peaks; c) the average peak area of monoisotopic and labelled peaks in each sample group; and d) the trend in the relative amount of labelling in a predetermined isotope. The power of mzMatch-ISO is demonstrated on tissue resolved 13C labelled metabolome data from cancer cells.

[1] Scheltema RA*, et al.* (2011) Anal. Chem. 83 (7):2786-2793; [2] Creek DJ, *et al.* Bioinformatics. 2012 28(7):1048-9

POSTER 37.

IN VITRO AND IN VIVO METABOLITE IDENTIFICATION OF *ARGEMONE MEXICANA L.*

Amina Haouala (1), C. Simoes-Pires (1), J. Deglon (2), K. Ndjoko (1), M. Lakovlev (1), J. Houriet (1), J. Falquet (3), B. Graz (3), C. Staub (2), M. Cuendet (1) and P. Christen (1)

1. School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland; 2. Unit of Toxicology, University Center of Legal Medicine, rue Michel-Servet 1, 1211 Geneva 4, Switzerland; 3. University of Lausanne, 1000 Lausanne, Switzerland

Malaria is responsible for the death of over one million people every year, mostly children under the age of 15. Several factors contribute to the malaria burden: the lack of access to a treatment in poor regions, as well as inappropriate treatments with counterfeit drugs, contributing to the emergence of resistance [1]. An interesesting strategy is to encourage the use of local resources to provide early treatment for malaria in regions where conventional therapy is not available. Many plants used in traditional medicine have shown antiplasmodial activity [2], but often little is known about their mode of action and

the compounds responsible for the activity [3]. A decoction of *Argemone mexicana* (AM), a pantropical weed, is traditionally used to treat malaria in Mali and in other African countries. In a clinical study [4], the efficacy of the decoction was found non-inferior to the standard drug artesunate-amodiaquine in the case of non complicated malaria. Subsequent studies showed that three alkaloids (protopine, berberine and allocryptopine) present in the AM decoction had in vitro activity against Plasmodium falciparum [5]. At this point, the study of the poorly understood metabolization of the decoction and the three active alkaloids is important [6]. The aim of this work was to evaluate the in vitro CYP450 activity by using microsomes and supersomes and to confirm the results in vivo in healthy rats.

The analyses were performed using an LC system coupled with a QTRAP triple quadrupole linear ion trap (HPLC/ESI-QqQLIT) for quantitative measurements (MRM mode), using a fully validated method according to FDA recommendations, as well as enhanced product ion (EPI) scans for metabolites identification. Moreover, a UHPLC system hyphenated with a Time-of-Flight (UHPLC/ESI-TOF) mass spectrometer was used for adding high resolution information. The in vitro and in vivo studies showed the formation of the same metabolites, with the major pathways of phase I metabolism for the three alkaloids being demethylation and demethylenation. CYP2D6 was the main enzyme involved in the metabolism of berberine and allocryptopine, whereas protopine was very poorly metabolized by the five cytochromes (1A2, 2C9, 2C19, 2D6 and 3A4). Phase II metabolites (glucuronides) were observed in rats with protopine and berberine only. Moreover, pharmacokinetic curves were determined during the in vivo study, showing a better absorption of the alkaloids when the decoction was administered, compared to the administration of single compounds. Interestingly, a very low concentration of unmetabolized alkaloids was observed in the intestine and the liver after gavage with the decoction.

[1] Phyo, A. P.; *et al.* Lancet 2012; [2] Wells, T. N. Malar. J. 2011, 10 Suppl 1, S3; [3] Diallo, D.; *et al.* Trans. R. Soc. Trop. Med. Hyg. 2006, 100 (6), 515-520; [4] Graz, B.; *et al.* Trans. R. Soc. Trop. Med. Hyg. 2010, 104 (1), 33-41; [5] Simoes-Pires C; *et al.* Planta Med. 2009, 75 (PD22); [6] Bao L.; *et al.* Pharmacol. Bull. 1997, 13, 95.

POSTER 38.

EXPLORING THE METABOLISM OF STREPTOMYCES COELICOLOR USING MASS SPECTROMETRY-BASED METABOLOMICS

Maria Elena Merlo (1,2), Andris Jankevics (2,3), Rainer Breitling (2,3) and Eriko Takano (1)

1. Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands; 2. Groningen Bioinformatics Centre, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands; 3. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Joseph Black Building B3.10, G11 8QQ Glasgow, United Kingdom.

Microorganisms depend on their ability to modulate their metabolic composition according to specific circumstances, such as different phases of the growth cycle and circadian rhythms, fluctuations in environmental conditions, as well as experimental perturbations. A thorough understanding of these metabolic adaptations requires the ability to comprehensively identify and quantify the metabolome of bacterial cells in different states.

Here we present an overview of two diverse metabolomics approaches in which a combination of advanced analytical equipment and novel computational analysis was used to explore the metabolism of the antibiotic producing bacterium *Streptomyces coelicolor*. Time series metabolite extracts were analyzed by liquid chromatography coupled to the high accuracy LTQ Orbitrap mass spectrometer. The LC-MS data were analyzed using the dedicated preprocessing software MzMatch developed for LTQ Orbitrap MS data, which exploits the high mass accuracy for reliable alignment of the mass spectra.

In the first metabolomics investigation, we have studied the adaptation of *S. coelicolor* to osmotic stress and shown that the bacterium accumulates specific compounds in response to salt stress. In the second more general analysis, a metabolomics approach was used to assess the response of S. coelicolor to the overexpression of a small non coding RNA targeting glutamine synthethase I, an important player in the nitrogen assimilation. The metabolite analysis showed that the metabolite response was rapid and dynamic and affected different areas of the metabolism.

POSTER 39.

APPLYING Q EXACTIVE BENCHTOP ORBITRAP LC-MS/MS AND SIEVE 2.0 SOFTWARE FOR CUTTING-EDGE METABOLOMICS AND LIPIDOMICS RESEARCH

Madalina Oppermann, David A Peake, Jessica Wang, Mark Sanders, Yingying Huang

Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA

Application of metabolomics to disease phenotype analysis and identification of unique biomarkers that distinguish healthy individuals from those with a disease has renewed the promise of personalized medicine. We present here the application of a benchtop quadrupole-Orbitrap mass spectrometer coupled to a UHPLC and advanced label-free differential analysis software to several metabolomics and lipidomics studies. The performance of the entire platform is illustrated with relevant examples including the metabolomic analysis of lean and obese Zucker diabetic fatty (ZDF) rat plasma, California wine and mitochondrial lipids in yeast.

Metabolites were extracted from ZDF rat plasma with cold methanol. The supernatant was removed, dried under nitrogen, and reconstituted with 80:20 water/methanol for LC/MS. Isopropanol was used to extract mitochondrial lipids from wild-type (WT) yeast (S. cerevisiae) and a knockout (KO) strain that does not produce coenzyme Q (CoQ) [1]. High-resolution, accurate-mass full-scan LC/MS and LC-MS/MS analyses were performed with a short UHPLC gradient at 70,000 resolving power on the Thermo Scientific Q Exactive mass spectrometer in both positive and negative ion modes with an electrospray ionization source. All of the datasets were analyzed with Thermo Scientific SIEVE software version 2.0, employing the Component Elucidation algorithm designed specifically for optimal data analysis in untargeted metabolomics experiments. Components that showed significantly different abundance between the sample groups were detected and identified via local or online database search. MS/MS data were then acquired and used to confirm the structure of these components. Thermo Scientific Mass Frontier software version 7.0 RS1 provided spectral interpretation tools including MS/MS library search and assigning structures to the fragment ions automatically

in the MS/MS spectrum. Thermo Scientific TraceFinder software version 2.1 was then used for streamlined, targeted quantitative analysis.

Analysis of the ZDF fatty rat plasma compared to normal rat plasma served as a benchmarking study to determine the merits of the technology platform. We observed an increase in acylcarnitines, branched-chain amino acids [2], phospholipids, fatty acids and conjugated bile acids ($P < 0.05$) in fatty ZDF rat plasma relative to normal ZDF rats.

Preliminary analysis of yeast mitochondrial lipid extracts demonstrated the ability to obtain statistically significant results with a single injection of each biological replicate. The expected change in CoQ6 levels was accompanied by changes in over 60 different components including amino acids, acylglycerols, sterols, phospholipids and sphingolipids.

[1] D. A. Peake, *et al.* Lipid Maps Annual Meeting, May 7-8, 2012, La Jolla, California, USA; [2] J. R. Bain, *et al.* Diabetes, 2009, 58, 2429-2443.

POSTER 40.

RELATED SEASONAL AND GEOGRAPHICAL DIFFERENCES IN WINE FROM CALIFORNIA'S CENTRAL COAST

Madalina Oppermann (1), Michael Athanas (1), Mark Dreyer (1), Paul Tarr (2), Michaela Scigelova (3)

1. ThermoFisher Scientific, San Jose, USA; 2. California Technical Institute, Pasadena, USA; 3. ThermoFisher Scientific, Bremen, Germany

Several factors may influence the complexity of the organoleptic properties of wine, including micro-climate, soil composition, and seasonal variations of these to name a few. Vintage must and wine samples were collected from vineyards in the Ballard Canyon and Santa Ynez Valley AVA during the 2007 through 2011 growing seasons in Santa Barbara county. Samples were analyzed using LC-MS differential analysis to determine the relative levels of various expected flavonoids as well as unexpected constituents. The data samples were compared both seasonally, geographically (terrior influences among certain clonal varietals), and pre- and post-fermentation to identify relationships among identified compounds.

Instrumentation were chosen and configured to deliver high fidelity and robust measurements over the multi-day acquisition. 42 samples were acquired in duplicate and were analyzed in a two pass workflow. In the first pass, instrumentation was optimized for full mass scan for best quantitation. The measurements were chromatographically aligned based upon correlating overall shapes of full scan spectra. A novel component detection algorithm was applied to the aligned data to discover families of related ions (isotopes and adducts) to reduce the data set to fundamental components. In addition, components discovered in solvent blanks (10% methanol) were removed. Differential and multivariate analysis uncovered components of potential interest. These components were selected and reacquired from a subset of the wine samples in a second acquisition pass where fragmentation spectra were acquired for identification and structural elucidation.

The results from this study provide a catalogue of the compounds present in the samples and a statistical analysis reflecting the changes in the compound levels observed across the samples. This data set may serve as a first approximation of how certain influences during the winemaking process from vineyard to winery are potentially reflected in the organoleptic properties of the final wine product. Efficient verification of

known constituents as well as detection of unexpected compounds is an important area of research for the agricultural industry.

POSTER 41.

METABOLOMIC CHARACTERIZATION OF SOD1 NULL MUTANT FLIES USING LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

Aiko Barsch (3), Jose M. Knee (1), Teresa Z. Rzezniczak (1), Kevin Kun Guo (2) and Thomas J. S. Merritt (1)

1. Department of Chemistry & Biochemistry,Laurentian University Sudbury, Ontario, Canada; 2. Bruker Daltonics Inc., Billerica, MA; 3. Bruker Daltonics GmbH, Bremen, Germany

Mass spectrometry is a powerful and fast developing tool in metabolomics, providing researchers with a highly sensitive and accurate method for quantification of a broad array of small molecule metabolites. The field is, however, still in its infancy and well-established methods and protocols are not broadly available. We are developing such protocols for liquid chromatography/mass spectrometry (LC/MS) metabolomics using oxidative stress and the cytosolic Superoxide Dismutase gene (Sod1) in *Drosophila melanogaster* as a model system. The SOD1 protein is involved in reactive oxygen species (ROS) scavenging and Sod1 mutants accumulate both ROS and products of ROS damage. Our initial work is targeting these 'known' metabolites to allow us to develop and optimize sample preparation and chromatography protocols. Using known standards, fly homogenates, and spiked-homogenates, we are investigating different homogenization buffers, filtering and sample clean-up techniques and the use of diamylammonium as an ion pairing reagent to improve separation of small polar metabolites. Later work will use these protocols to expand our investigation to a broader, currently unknown, suite of metabolites. This combination of targeted and discovery metabolomics is allowing us to develop methods and protocols for investigating SOD1 function, oxidative stress in general, and biological networks and their response to genetic alterations.

POSTER 42.

A ^{1}H NMR-BASED METABOLOMIC APPROACH REVEALS SIGNIFICANT CHANGES IN BREAST MILK COMPOSITION INDUCED BY ORAL PROBIOTIC SUPPLEMENTATION

Giulia Pratico'(1), Giorgio Capuani (1), Alberta Tomassini (1), Paola Mastromarino (2), Maurizio Delfini (1), Maria E. Baldassarre (3), Alfredo Miccheli (1)

1. Department of Chemistry, Sapienza University of Rome, Italy; 2. Public Health and Infectious Diseases, Sapienza University of Rome, Italy; 3. Department of Gynecology, Obstetrics and Neonatology, Section of Neonatology and NICU, University of Bari, Italy.

Background: Pregnancy and the first two years of life are the most critical time periods for interventions to improve child growth and development. In this scenario, both the microbiota and breast milk which are 'transmitted' from mother to infant seem to play a crucial role for the promotion of a healthy growth in newborn. Perinatal supplementation with probiotics has been recently proposed as a possible strategy to manipulate maternal physiology, from gut microbiota to breast milk composition, in order to orchestrate the infant development. In this study, we applied a ^{1}H NMR-based metabolomic approach to characterize the breast milk metabolome and possible changes in breast milk composition induced by oral

supplementation with VSL#3 probiotic mixture.

Methodology: Pregnant women were given either oral VSL#3 or placebo. Breast milk was collected one month after delivery, extracted into polar and organic phases and analysed by ^{1}H NMR spectroscopy. Breast milk metabolomic profiles were defined by multivariate statistical methods on the collected spectra.

Principal findings: NMR-based metabolic profiling allowed the classification of breast milk samples on the basis of fucosyl-oligosaccharide signals, highlighting the relation between the mother's Lewis blood group and breast milk composition. Moreover, VSL#3 supplementation induced reliable changes in milk metabolome, including a decrease in fucosyl-oligosaccharides such as Fucosyl-Lactose (2-FL) and Lacto-N-fucopentaose I (LNFP1) and citrate levels, and an increase in Monounsaturated Fatty Acids (MUFA), phosphatidylglycerols, and succinate levels.

Conclusions: 1H-NMR-based global metabolic profiling represents a powerful analytical tool to characterize human milk composition and investigate the changes induced by dietary interventions. Our metabolomic approach revealed a systemic effect of the maternal probiotic supplementation on the production or secretion of human milk oligosaccharides (HMOs) and lipids in the mammary gland. Such an approach may become increasingly useful as a tool to design and evaluate dietary and therapeutic strategies aimed at improving the health status of the mother-infant dyad.

POSTER 43.

EFEFCTS OF AGE AND PROBIOTIC SUPPLEMENTATION ON THE MOUSE METABOLOME – A LOOK INTO THE HOMEOSTENOSIS WINDOW

Riccardo Calvani (1), Elisa Brasili (2), Giorgio Capuani (3), Alberta Tomassini (3), Fabio Sciubba (3), Elena Mengheri (2) and Alfredo Miccheli (3)

1. Institute of Crystallography, National Research Council (CNR), Bari, Italy; 2. INRAN, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy; 3. Department of Chemistry, Sapienza University of Rome, Rome, Italy

Background. Aging is characterized by a general decline in cellular functions, which ultimately affect whole body homeostasis. The study of the complex biomolecular networks involved in the aging process is still in its infancy. Recently, particular interest has been placed into the age-related shift in the composition and function of the gut microbiota, which may contribute to the homeostenosis phenomenon, that is the progressive constriction of the homeostatic reserve that occurs with aging. The metabolic signature of a deranged human-gut microbiota relationship can be retrieved through the metabolomic profiling of biological fluids. It is proposed that the administration of probiotics, prebiotics, or symbiotics could counteract age-related gut microbial changes in experimental animals and humans.

Objectives. In this study we characterized the age-related changes in urinary and fecal metabolic profiles of adult and old BALB/c mice through a ^{1}H-NMR-based metabolomic approach. We also assessed the effects of a 4-week supplementation of a mixture of two probiotics [*Lactobacillus acidophilus* (La5) and *Bifidobacterium lactis* (BB12)] on metabolic

phenotypes of adult and old mice. Urine and feces were collected at the beginning and the end of treatment, and analyzed by high-resolution ^{1}H-NMR spectroscopy combined with multivariate statistical analysis.

Results. An age-related metabolic phenotype was detected both in urine and feces. The metabolic signature of aging consisted in changes in the levels of metabolites associated with amino acid metabolism, citric acid cycle, and host-microbiota metabolic axis. The probiotic supplementation induced changes in urinary and fecal metabolic profiles mainly affecting transmethylation pathways, NAD metabolism, and host/microbiota co-metabolism. These effects were only marginally affected by age.

Conclusions. Our ^{1}H-NMR-based metabolomic approach was able to characterize the effects of age and probiotic supplementation on urinary and fecal metabotypes. This analytical strategy will provide new metrics to design and evaluate interventions aimed at driving the host-microbiota relationship into healthier directions.

POSTER 44.

NETHERLANDS METABOLOMICS CENTRE – DATA SUPPORT PLATFORM

Theo Reijmers (1,2,6), Micheal van Vliet (2,6), Kees van Bochove (1), Jildau Bouwman (3,6), Jeroen Wesbeek (3,6), Gooitzen Zwanenburg (4,6), Siemen Sikkema (4,6), Isthiaq Ahmad (4,6), Tim Dorscheidt (4,6), Margriet Hendriks (2,5,6), Tjeerd Abma (5,6) and Thomas Hankemeier (2,6)

1. BioAssist, Netherlands BioInformatics Centre; 2. Analytical BioSciences, Leiden University, The Netherlands; 3. TNO Quality of Life, Zeist, The Netherlands; 4. Biosystems Data Analysis, University of Amsterdam, The Netherlands; 5. Department of Metabolic Diseases, University Medical Center Utrecht, The Netherlands; 6. Netherlands Metabolomics Centre

Large amounts of data are being generated in metabolomics studies. The need for tools and applications to support the data handling and biological interpretation is huge, but online availability of metabolomics data and tools is poor. The process of extracting biological information can be seen as an integrated workflow. It is recognized that this workflow can benefit highly from coordinated (and automated) handling and processing of the data. The Netherlands Metabolomics Centre (NMC), in collaboration with the Netherlands BioInformatics Centre (NBIC), has a dedicated project that supports the development of an infrastructure to share metabolomics data and tools: the NMC Data Support Platform. This project addresses two major bottlenecks for metabolomics research. The first is sharing of metabolomics studies and data. The second addresses the accessibility of dedicated processing and biostatistics tools.

Sharing and storing of metabolomics studies and data is provided for by a web-based, open source, study capture and experimental data framework, where study information can be stored together with metabolomics mass spectrometric or NMR data. Statistical analysis tools are made available using the Galaxy interface which provides a toolkit dedicated to metabolomics data analysis (next to basic data manipulation tools, also different sorts of univariate and multivariate statistical analysis tools are accessible).

POSTER 45.

AN AUTOMATED METABOLITE IDENTIFICATION PIPELINE USING MASS SPECTRAL TREES

Theo Reijmers (1,3), Julio Peironcely (1,2,3), Miquel Rojas-Chertó (1,3), Piotr Kasper (1,3),

Leon Coulier (2,3), Albert Tas (2,3), Rob Vreeken (1,3), Thomas Hankemeier (1,3)

1. Analytical BioSciences, Leiden University, The Netherlands; 2. TNO Quality of Life, Zeist, The Netherlands; 3. Netherlands Metabolomics Centre

Identifying metabolites has been reported as one of the major bottlenecks in metabolomics. In part, this is due to the absence of good computational tools to automate metabolite identification. To address this issue, we have developed computational tools to process and compare multi-stage mass spectrometry data (MS^n), in order to extract as much information as possible from the fragmentation trees. In addition, candidate structures are generated computationally and filters are used to reject improbable chemical structures. In this work, we present the integrated use of these tools in a pipeline fashion to identify metabolites in human urine. MS^n data was obtained using an HPLC coupled to an Orbitrap MS^n instrument.

Metabolite Identification Pipeline:

- MS^n data were processed into fragmentation trees using the MEF [1] tool. This tool annotates all the nodes of the trees with unique elemental compositions and neutral losses.

- The way to identify substructures in the fragmentation trees was based on comparing the processed trees with an in-house MS^n reference database, as described by Rojas-Chertó et al. [2]. This approach finds for an unknown fragmentation tree, the most similar trees in the reference database, and from their known structures the maximum common substructure (MCS). MCS information was used as additional input criterion for a structure generator.

- The Open Structure Generator (OMG) [3] has been implemented and used to generate all the possible chemical structures for an unknown metabolite. It takes as an input the elemental composition and multiple prescribed substructures (e.g. MCS) and outputs an exhaustive list of all possible molecules fitting these criteria.

- The list of candidates produced by OMG was reduced by first establishing an internal energy threshold that rejected unstable molecules. Next a Metabolite-Likeness [4] filter was used to retain only molecules resembling human metabolites. Finally the fragmentation prediction tool MetFrag [5] was applied to rank the remaining molecules using similarity between predicted and observed fragmentation spectra.

The results of using such a pipeline to identify 31 urine metabolites will be presented. Furthermore, it is discussed how this approach improves on existing metabolite identification methods and what needs to be improved to achieve the metabolomics dream, a fully automated metabolite identification pipeline. Most of the tools used are open-source and part of the pipeline can already be accessed via www.metitree.nl [6].

[1] Rojas-Chertó, M.; *et al.* T.H. Bioinformatics 2011, 27, 2376-83; [2] Rojas-Chertó, M.; *et al.* Analytical Chemistry 2012, 84, 5524-34; [3] Peironcely, J.E. *et al.* submitted; [4] Peironcely, J.E. *et al.* PLoS ONE 2011, 6, e28966; [5] Wolf, S. *et al.* BMC Bioinformatics 2010, 11, 148; [6] Rojas-Chertó, M. Bioinformatics, in press.

POSTER 46.

METABOLOMIC INVESTIGATION OF A SIMULATED COMBAT TRAUMA INJURY IN A PORCINE MODEL USING ^{1}H NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Anna Karen Carrasco Laserna (1), Fang Guihua (1), Wu Jian (2), Lai Yiyang (2), Rajaseger Ganapathy (2), Shabbir Moochhala (2) and Sam Li Fong Yau (1)

1. Department of Chemistry, National University of Singapore, Singapore; 2. Defence Medical & Environmental Research Institute, DSO National Laboratories, Singapore

Trauma injury has been identified as one of the leading causes of morbidity and mortality worldwide [1], more so in combat scenarios wherein the nature of the wounding agents are far more lethal and the resources to administer treatment are usually limited as compared to civilian settings. Furthermore, even after survival of the initial insult, the patient may also succumb to multiple organ failure (MOF) and die. The identification of biomarkers that can serve as indicators of the extent and progression of damage, the onset of organ failure, and the effects of the treatment administered, can help in improving patient outcome. Here, we investigate on the metabolic perturbations occurring after a simulated combat trauma injury using ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy. Based on a complex combat trauma injury model [2], pigs were subjected to femur fracture and soft-tissue injury with 60% blood loss, followed by a 30-minute shock period, and induced hypothermia. Plasma samples before and after injury, as well as sham samples, were analyzed to evaluate the changes in the metabolic profiles of the pigs. The web server, Metaboanalyst [3], was used for the multivariate and univariate analyses of the data. Principal Component Analysis (PCA) revealed distinct separation of the after trauma injury samples from the baseline and sham samples. Significance Analysis of Microarrays (SAM) was used to identify compounds that have significant changes after injury. The differentiation of the after trauma injury samples from the baseline and sham samples was found to be highly influenced by signals associated with lipids, low density lipoproteins (LDL and VLDL), albumin and N-acetyl glycoproteins. Other metabolites that were also found to be significantly differentiating after injury include creatine, creatinine, betaine, 3-hydroxybutyrate, isopropyl alcohol, and some amino acids.

[1] Global burden of disease: 2004 update. 16 June 2011; Available from: http://www.who.int.libproxy1.nus.edu.sg/healthinfo/global_burden_disease/2004_report_update/en/index.html; [2] Cho, S.D., et al., 2009. 31(1): p. 87-96; [3] Xia, J., *et al.,* Nucleic Acids Research, 2012. 40(W1): p. W127-W133.

POSTER 47.

DISSECTING THE MECHANISM OF ANTIFUNGAL DRUG ACTION BY GC/Q-TOF-MS

Ashley Sage (1), Sofia Aronova (2), Stephan Baumann(2), Manhong Wu (3), Bob St. Onge (3), Sundari Suresh (3), Ronald Davis (3) and Gary Peltz (3)

1. Agilent Technologies, Stockport, UK; 2. Agilent Technologies, Santa Clara, CA; 3. Stanford University, Stanford, CA

Introduction: Ergosterol is a key component of yeast and fungal membranes. It performs a similar function as cholesterol in animal cells. However, many of the enzymes involved in yeast sterol biosynthesis are quite different from their counterpart in mammalian cells. Therefore, agents that inhibit ergosterol biosynthetic pathways often have therapeutic potential. Drugs that inhibit a step in the ergosterol biosynthesis pathway can be identified by Haploinsufficiency Profiling (HIP) screening. In this process gene deleted yeast is grown against a drug at a concentration that inhibits the yeast pool growth rate to 80% of wild type. This could provide a robust and high-throughput approach for evaluating new antifungal agents. The approach was validated with two novel and one drug of known inhibition.

Methods: Wild type yeast cultures were incubated with drug concentrations that inhibited growth by 10%. Yeast sterols were extracted by the Folch method. The lower chloroform aliquots were dried by speed vacuum, and the active functional groups were derivatized. The carbonyl groups were first protected by methoximation and the samples were then derivatized with MSTFA + 1 % TMCS, prior to analysis by Agilent 7200 series GC/Q-TOF. The resulting data were deconvoluted, metabolites were identified by comparison with a mass spectral metabolomic library, and finally processed using Mass Profiler Professional (MPP), a multivariate software package.

Results: We evaluated several antifungal drugs including Fluconazole, Totarol, as well as a new chemical entity (NCE) 1181-0519. Fluconazole is an azole antifungal agent, which is one of a commercially important group of drugs that inhibits the ERG11 gene product, the cytochrome P450 14-demethylase. This drug prevents the conversion of lanosterol to its subsequent intermediate, and results in accumulation of 14-methyl sterols. We evaluated the abundance differences of extracted metabolites in drug treated and non-treated samples. As expected, lanosterol accumulated in Fluconazole-treated samples. Changes in several 14-methyl sterols were also detected, and their empirical structures determined. Metabolic profiling of yeast sterols followed by untargeted analysis and MS/MS experiments to provide additional structural information clearly indicated NCE 1181-0519 and Totarol targets as being Erg25 and Erg26 respectively. We used the exact mass of the ergosterol biosynthetic intermediates to identify the point of inhibition. In this manner the GC/Q-TOF played a crucial role in elucidating mechanisms of poorly characterized antifungal drugs.

POSTER 48.

RATIONAL ISOLATION STRATEGY OF PLANT BIOMARKERS AT THE MG SCALE BASED ON CHROMATOGRAPHY MODELLING, MPLC-ELSD AND FAST UHPLC-TOF-MS MONITORING

S. Challal, E.F. Queiroz, B. Debrus, D. Guillarme and J.L Wolfender

School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, Quai Ansermet 30, 1211 Geneva, Switzerland

In natural product (NP) research, the isolation of biomarkers or bioactive compounds from complex natural extracts, for identification or bioactivity assessment, is generally an essential but laborious and time consuming step. This procedure usually requires multiple chromatographic steps. While de novo structure identification of biomarkers can already be achieved at the microgram level with state-of-the art microNMR techniques [1], the determination of the bioactivity profile of a natural product of interest requires mg amounts. To obtain in one step mg amounts of pure NPs directly from crude plant extracts (tens of grams), the up-scale transfer of a generic gradient method that provide efficient separation from analytical HPLC to large preparative MPLC has been investigated [2]. The proposed method takes the advantage of HPLC modelling based on generic linear gradients at the analytical level to maximize the separation of the biomarkers of interest in an extract. This step was performed with an HPLC column packed with the same C-18 material than in MPLC. The gradient was geometrically transferred to MPLC by system characterization and chromatographic calculation. For the monitoring of the largest possible number of constituents, UV and ELSD detections were used simultaneously at the analytical and

preparative scale. MS monitoring was performed by post-chromatographic high throughput UHPLC-TOF-MS profiling of the aliquots of all the fractions in the 96 well microtiter plate format. A rapid evaluation of the performance of the preparative separation was obtained by combining all UHPLC-MS profiles in a LC-LC 2D plot which also provides an efficient localization of the biomarkers. Examples of separations of crude plant extracts yielding in one step pure biomarkers will be presented and the possibilities and limitations of the approach are discussed.

[1] Wolfender JL, et al., Chimia 2011, 65, 400; [2] Guillarme D, *et al.,* Eur. J. Pharm. Biopharm. 2008, 68, 430.

POSTER 49.

NON TARGETED LC-MS METABOLITE PROFILING HIGHLIGHTS THE BETWEEN-TISSUE DIFFERENCES CAUSED BY A HIGH-FAT DIET IN A PIG MODEL

Kati Hanhineva (1), Thaer Barri (2), Marjukka Kolehmainen (1), Jenna Pekkinen (1), Jussi Pihlajamäki (1), Gloria Solano-Aguilar (3), Hannu Mykkänen (1), Lars Dragsted (2), Joseph Urban (3), Kaisa Poutanen (1,4)

1. University of Eastern Finland, Institute of Public Health and Clinical Nutrition, PO Box 1627, FIN-70211 Kuopio, Finland; 2. Institute of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, DK-1958 Frederiksberg, Copenhagen, Denmark; 3. United States Department of Agriculture, Beltsville Human Nutrition Research Center, Diet, Genomics, and Immunology Laboratory, Beltsville, Maryland, USA; 4. VTT Technical Research Centre of Finland, PO Box 1000, Tietotie 2, FI-02044 VTT, Finland

Obesity and obesity related diseases such as metabolic syndrome and type 2 diabetes are among the most significant health problems in the Western world. The molecular changes behind these disorders have been investigated extensively in both humans and experimental models, but so far mostly concentrating on metabolic changes in the most accessible samples, such as plasma and urine. Animal trials assist in gaining a more comprehensive view of the tissue level metabolic alterations caused by diet- induced obesity and subsequent metabolic disorders.

The Ossabaw miniature swine is an isolated breed evolved at the Ossabaw Island at the coast of Georgia. It has the capability to store large amounts of fat for survival during famine, and without physical activity this leads to obesity. The Ossabaw miniature swine develops metabolic syndrome under high caloric diets showing characteristic symptoms such as obesity, insulin resistance and glucose intolerance more clearly than other large animal models.

Non-targeted LC-MS metabolite profiling was used for analysis of biofluids (plasma, urine, bile) and organs (liver, pancreas, cortex, jejunum, and proximal colon) collected from the Ossabaw pigs after feeding with either normal or high-fat diet. Significant changes in the biofluid metabolomes were observed, and the impact of the high-fat diet was clearly pronounced in the organs. Moreover, several metabolic changes were shown to occur mutually in different organs and biofluids, but metabolite differences were also identified that were predominant in only some of the organs and not visible in any of the biofluids. We could confirm many of the metabolite level changes shown by earlier studies, like the decrease of hippuric acid and betaine after the high fat diet, as well as observed various metabolite alterations not previously reported. These findings suggest the importance of tissue level analyses to find out mechanisms of dietary signaling in prevention of obesity-related pathologies.

POSTER 50.

^{1}H NMR-BASED METABOLOC PROFILING OF BLOOD PLASMA AND MILK IN DIARY COWS REVEALS PHYSIOLOGICAL MECHANISMS UNDERLYING ECONOMICALLY RELEVANT TRAITS

Anthony Maher (1,2,3), Benjamin Hayes (1,2,3), Benjamin Cocks (1,2,3), Leah Marett (4), Bill Wales (4) and Simone J Rochfort (1,2,3)

1. Biosciences Research Division, Department of Primary Industries, Bundoora Victoria 3083, Australia; 2. Dairy Futures Cooperative Research Centre, Victoria 3083, Australia; 3. La Trobe University, Bundoora Victoria 3083, Australia; 4. Department of Primary Industries, Ellinbank Centre, Ellinbank, Victoria 3820, Australia.

We have adapted 1D and relaxation-edited NMR methods to measure total and small-molecule metabolic profiles from blood serum and milk samples from cows selected for extremes of residual feed intake (RFI). We have applied projections to latent structures (PLS) to build quantitative relationships between metabolic profiles and economically important traits. We have further developed Statistical Heteroscpectroscopy (SHY), a powerful approach to recovering latent biological information in NMR spectroscopic datasets from multiple complementary samples, to understand the metabolic relationships between blood and milk from these animals.

The results from PLS regression indicated several economically important traits could be predicted from metabolic profiles of milk, such as milk yield, and the metabolites responsible give insight into the biological mechanisms underlying these traits. SHY revealed a number of metabolites were significantly statistically correlated across these two metabolic compartments including trimethylamine (TMA) and dimethyl sufone (DMSO2).

The findings have potential for development of diagnostics for economically relevant parameters in dairy cows. The methods developed here will be applied to cows with extreme FCE phenotypes to assess statistically significant correlations for the existence of predictable relationships between metabolic parameters and important economic traits such as milk yield.

POSTER 51.

FROM WHOLE-ORGANISM TO SUB-CELLULAR LOCALIZATION OF METABOLITES BY MALDI FTICR MS AND TOF-SIMS IMAGING APPROACHES IN AN INVERTEBRATE

Manuel Liebeke (1), Jens Fuchser (2), Sarah Fearn (3), David McPhail (3), Aiko Barsch (2) and Jacob Bundy (1)

1. Department of Surgery and Cancer, Imperial College London, London, UK; 2. Bruker Daltonik GmbH, Bremen, Germany; 3. Department of Materials, Imperial College London, London, UK

In order to fully understand metabolic problems, it is important to be able to map metabolites in tissues or even within single cells. Advances in mass spectrometry imaging (MSI) allow the mapping and identification of individual molecules across a spatial distribution. We report here the complementary use of matrix assisted laser desorption ionization (MALDI) FTICR-MS to image tissue cross-sections (0.5 cm^2, 20µm resolution) and TOF-SIMS to further elucidate the cellular distribution of selected small molecules (125-500μm^2, 250 nm resolution). Both MSI techniques gained reproducible tissue specific metabolite distributions with unique pattern for classification of different cell types. The use of MALDI in conjunction

with an ultra-high mass resolution mass spectrometer and MS/MS capabilities makes in-situ identification of metabolites feasible. In addition, the application of TOF-SIMS with much higher spatial resolution, and lower mass range detection gives information on detailed cellular distribution, thus giving complementary data. We present results from an exemplar project: the localization and identification of selected metabolites in an invertebrate (the 'super-sentinel' Lumbricus rubellus), demonstrating the power of metabolite imaging from the whole-organism to sub-cellular levels, and which enables hypothesis generation about novel biological functions of small molecules.

POSTER 52.

METABOLOMICS APPROACHES: A POWERFUL TOOL FOR THE SCREENING OF TRENBOLONE ACETATE/ESTRADIOL COMBINATIONS IN CATTLE

Cristina da Costa Jacob (1), Gaud Dervilly-Pinel (1), Giancarlo Biancotto (2), Roberto Stella (2), Fabrice Monteau (1), Roberto Angeletti (2) and Bruno Le Bizec (1)

1. LUNAM Université, Oniris, École nationale vétérinaire, agroalimentaire et de l'alimentation Nantes-Atlantique, Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France; 2. Istituto Zooprofilattico Sperimentale delle Venezie, Department of Chemistry, 35020 Legrano (PD), Italy.

Cattle producers in USA have had the option of using implants containing the combination of androgenic and estrogenic steroids as growth promoters since 1992. Although these implants are registered in USA and in many other countries, within European Union they are forbidden as they are considered as a danger for public health like all growth promoters [1,2]. Detection of illegal practices classically relies on residue monitoring in a targeted approach. However, this strategy fails when faced with new xenobiotic growth-promoting agents or new ways of application, such as administration of low-dose cocktails, therefore new analytical tools to detect such abuse are today mandatory.

As hormonal therapy influences endocrine and/or metabolism, strategies based on the detection of physiological actions of anabolic practices are promising approaches to screen for their misuse. In this context, metabolomics strategies may offer a reliable solution for screening hormonal abuse in livestock production animals [3-7]. The purpose of the present study was to set up and assess a mass-spectrometry metabolomics strategy as a new tool to screen for combined trenbolone acetate/estradiol implant abuse in cattle and demonstrate the feasibility of such approach. Therefore, an untargeted metabolomics approach, based on liquid chromatography coupled to high resolution mass spectrometry, was developed and applied to characterize and compare cattle urinary metabolic profiles from untreated and treated animals. After minimal sample preparation, urine samples were analyzed by LC-ESI(+)-HRMS. Data processing were performed by XCMS software and multivariate data analysis applied to the generated data set allowed building of OPLS models to discriminate anabolic treated from untreated animals. Moreover, OPLS models permitted to highlight the candidate biomarkers appearing as the ions which contribute the most in the observed discrimination between the two groups. The metabolic modifications in urinary profiles upon combined trenbolone acetate/estradiol exposure have been evaluated in the scope of real application for the screening of anabolic steroid abuse.

[1] Council Directive 96/22/EC, Official Journal of European Communities, 1996, L125: 3-9; [2] Council Directive 2008/97/EC, Official Journal of European Communities, 2008, L318: 9-11; [3] Courant, F., *et al.,* Analyst, 2009, 134: 1637-1646; [4] Rijk, J. C. W., *et al.,* Analytical Chemistry, 2009, 81 (16): 6879-6888; [5] Anizan, S., *et al.,* Journal of Chromatography A, 2010, 1217: 6652-6660; [6] Pinel, G., *et al.,* Trends in Analytical Chemistry, 2010, 29: 1269-1280; [7] Pinel, G., *et al.,* Analytica Chimica Acta, 2011, 700: 144-154.

POSTER 53.

SEMIQUANTITATIVE ANALYSIS OF NONRIBOSOMAL SYNTHESIZED LIPOPEPTIDES AND POLYKETIDES BY UHPLC-TOF-MS TO UNDERSTAND THE MECHANISM CAUSING ANTIFUNGAL EFFECTS OF BACILLUS AMYLOLIQUEFACIENS FZB42

Jenny Westphal (1), Rainer Borriss (2), Soumitra Paul Chowdhury (3), Kristin Dietel (2), Anton Hartmann (3), Magdalena Hennig (4), Andreas Schlüter (4), Philippe Schmitt-Kopplin (1)

1. Helmholtz Zentrum München, Research Unit Analytical BioGeoChemistry, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany; 2. ABiTEP GmbH, Glienicker Weg 185, 12489 Berlin, Germany; 3. Helmholtz Zentrum München, Research Unit Microbe-Plant Interactions, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany; 4. Universität Bielefeld, Centrum für Biotechnolgie - CeBiTec, Universitätsstraße 27, 33615 Bielefeld, Germany

Rhizoctonia solani is a world-wide distributed phytopathogen, which affects important agricultural and horticultural crops. Diseases caused by isolates of *R. solani* have been yet suppressed using fungicides. To limit chemical pesticides from industrial agriculture it is important to explore new fungicide compositions in order to replace chemopesticides. FZB42, a bioformulation that reduces lettuce bottom rot caused by *R. solani*, presents a plant-associated strain of the genus Bacillus amyloliquefaciens which promotes growth and immune defense of crops.

To understand the mechanism causing these antifungal effects, different mutants of *Bacillus amyloliquefaciens* were cultivated in minimal medium and the metabolic composition was analyzed by reversed phase liquid chromatography coupled with an ultra-high resolution time of flight mass spectrometer (maXis UHR-Qq-ToF MS, Bruker Daltonik). Nonribosomal-synthesized lipopeptides and polyketides were identified and could be allocated to *Bacillus amyloliquefaciens* strains. Afterwards, selected strains were co-cultivated with R. solani to detect changes in molecular compositions and to identify relevant secondary metabolites involved in an interaction of *Bacillus amyloliquefaciens* and *Rhizoctonia solani.*

POSTER 54.

PREDICTABLE PROTEIN PRODUCTION IN SACCHAROMYCES CEREVISIAE APPLYING METABOLIC FLUX MEASUREMENTS

Catherine Winder (1), Ettore Murabito (1), Helen Bryant (1), Alan Dickson (2), Roy Goodacre (1) and Hans V. Westerhoff (1)

1. Manchester Centre for Integrative Systems Biology, Manchester Institute of Biotechnology, University of Manchester; 2. Faculty of Life Sciences, University of Manchester.

Recombinant proteins are important industrial products, with a vast number of existing and potential applications. The synthesis of recombinant proteins requires complex systems such that biological cells are routinely applied in protein production. The complexity of these 'cell factories' is far beyond that of man-made production systems and far less understood. Molecule-based

biology has long been unable to understand the complex self-sustaining networks of living cells. As a consequence protein production is unpredictable. In this project we are applying a systems biology approach integrating experimental and mathematical approaches to enable predictable protein production to increase product yields.

Saccharomyces cerevisiae was used as the cell factory for recombinant protein production. The microorganism was cultured at the maximum growth rate in permittistat cultures. Quantitative analysis of the exometabolome by gas chromatography- mass spectrometry was performed to facilitate the calculation of the exchange fluxes (consumption and production of metabolites and biomass) in the biological system. A genome-scale reconstruction of yeast metabolism was enriched with a pseudo-reaction representing the production of the recombinant protein and experimentally determined exchange fluxes were used to constrain flux balance analysis (FBA) models. The results of the FBA were used to provide prediction of the protein production and facilitate the optimisation of feed design to improve protein production.

POSTER 55.

MULTIVARIATE METHODS IN METABOLOMICS - FROM PRE-PROCESSING TO DIMENSION REDUCTION AND STATISTICAL ANALYSIS

Kristian Hovde Liland

Nofima, Postboks 210, 1431 Ås and Norway and Norwegian University of Life Sciences, Postboks 5003, 1432 Ås, Norway

This poster presents some of the multivariate methods used in metabolomics, and addresses many of the data types and associated analyses of current instrumentation and applications seen from the point of view of data analysis.

I will cover most of the statistical pipeline - from pre-processing to the final results of statistical analysis (i.e. pre-processing of the data, regression, classification, clustering, validation and related subjects). Most emphasis is on descriptions of the methods, their advantages and weaknesses, and their usefulness in metabolomics. Of course, the selection of methods presented is not exhaustive, but should shed some light on some of the more popular and relevant methods.

POSTER 56.

DYNAMIC FLUX IN CENTRAL CARBON METABOLISM

Catherine Winder (1), Natalie J. Stanford (1), Warwick B. Dunn (1,2), Kieran Smallbone (1) and Pedro Mendes (1)

1. Manchester Centre for Integrative Systems Biology, Manchester Institute of Biotechnology, University of Manchester; 2. Centre for Advanced Discovery & Experimental Therapeutics, Institute of Human Development, University of Manchester

The quantitative understanding of the dynamics of metabolism requires a dual approach of quantification and flux analysis. The application of stable isotopes (^{13}C and ^{15}N) of labeled metabolites to 'visualize' metabolic flux is applied in both static and dynamic flux analysis [1]. In static flux analysis the steady state distribution of the isotope label is determined in the proteinogenic amino acids. This is then used to identify the pathways the flux is predominantly proceeding through. In dynamic flux analysis the isotope label is added to a steady state system in a time dependent approach such that the incorporation of the isotope may be traced in associated metabolites. The changing ratio of

isotopically unlabeled to labeled molecules along with the pool size (metabolite concentration) is used to calculate the dynamic flux through each reaction of the system.

Recent developments in the metabolomics field have led to an increase in the number of metabolites that can be accurately identified and quantified, increasing the coverage of the metabolic network. This coupled with enhanced knowledge of the biochemical interaction networks, gained from genome-scale reconstructions [2], has provided a viable platform for dynamic flux calculations to be undertaken.

In this investigation U-$^{13}C_6$ glucose was added to steady-state cultures of *Saccharomyces cerevisiae*, maintaining the macro steady state, whilst introducing a dynamic isotopic state to the system. Steady-state cultures were performed using a permittistat such that the growth was conducted without the limitation of nutrients and at the maximum specific growth rate. Time dependent measurements were applied to track the distribution of the ^{13}C label in the metabolic network. The $^{13}C/^{12}C$ distribution and concentration of metabolites involved in central metabolism was performed by gas chromatography mass spectrometry. Computational methods were applied to calculate the metabolic fluxes from the metabolite specific $^{13}C/^{12}C$ ratio data, and metabolite quantification.

Here we show the dynamic flux results attained, focusing on central carbon metabolism of S. cerevisiae.

[1] Winder, C.L., *et al.* (2011). Trends in Microbiology. 19 (7) 315-322; [2] Herrgard, M.J., *et al.* (2008) Nature Biotechnology, 26, 1155-1160.

POSTER 57.

DEVELOPMENT OF STRATEGIES FOR INTEGRATED FULL-SCAN PROFILING AND DATA DEPENDENT MS/MS APPLYING CID AND HCD ON HYBRID ORBITRAP MASS SPECTROMETERS

Warwick Dunn, Graham Mullard, Paul Begley, Katherine A. Hollywood, Marie Brown, Richard D. Unwin, Paul N. Bishop, Garth J. S. Cooper

Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust & Institute of Human Development, The University of Manchester, UK.

The identification and characterisation of metabolites is a current bottleneck in untargeted metabolomics. Although accurate measurement of mass-to-charge ratio is applied routinely in UPLC-MS applications to putatively annotate metabolites, the acquisition of MS/MS or MSn data is required to further reduce the chemical search-space sufficiently and to provide greater confidence in metabolite identity [1].

We have assessed, and now provide strategies, for integrated full scan metabolic profiling and MS/MS data acquisition (CID and HCD) on LTQ-Orbitrap platforms. This research has shown that (i) CID and HCD mass spectra of a wide diversity of endogenous metabolites can provide complementary information, (ii) different activation energies are required for acquisition of appropriate MS/MS spectra to enable identification of metabolites with diverse structural and physicochemical properties and (iii) narrow pre-cursor ion m/z ranges provide greater MS/MS coverage of the metabolome. The application of different and unique MS/MS experiments can increase the coverage of

metabolites for which MS/MS data are acquired. The strategies described provide for a suitable data acquisition first pass to improve metabolite identification capabilities. Further targeted identification studies will potentially be required in any given study to focus on identification of specific metabolites.

[1] Dunn, W.B. *et al.* Metabolomics, 2012, DOI: 10.1007/s11306-012-0434-4.

POSTER 58.

FUNCTIONAL CHARACTERIZATION OF COMMON OBESITY AND DIABETES RISK VARIANTS BY METABOLIC CHALLENGE TESTS – THE USE OF TARGETED METABOLOMICS

Simone Wahl (1), Cornelia Then (2), Susanne Krug (3), Tina Brand (3), Kerstin Ehlers (3), Melina Claussnitzer (3), Thomas Skurk (3), Andreas Lechner (2), H.-Erich Wichmann (4), Margit Heier (5), Cornelia Huth (5), Barbara Thorand (5), Christa Meisinger (5), Cornelia Prehn (6), Ferdinand Stückler (7), Gabi Kastenmüller (7), Jerzy Adamski (6), Karsten Suhre (7,8), Thomas Illig (1,9), Harald Grallert (1), Helmut Laumen (3,10), Hans Hauner (3,10) and Jochen Seissler (2)

1. Research Unit of Molecular Epidemiology, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 2. Diabetes Center, Medical Clinic Innenstadt, Ludwig Maximilians University, Munich, Germany; 3. EKFZ, Chair for Nutritional Medicine, Technische Universität München, Freising-Weihenstephan, Germany; 4. Institute of Epidemiology I, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 5. Institute of Epidemiology II, Helmholtz Zentrum Muenchen, Neuherberg, Germany ; 6. Institute of Experimental Genetics, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 7. Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Muenchen, Neuherberg, Germany; 8. Department of Physiology and Biophysics, Weill Cornell Medical College, Education City, Qatar; 9. Hanover Unified Biobank, Hanover Medical School, Hannover, Germany; 10 ZIEL, Research Center for Nutrition and Food Sciences, Technische Universität München, Freising-Weihenstephan, Germany

Objective: To date, common variants at more than 50 genetic loci have been consistently associated with obesity or type 2 diabetes mellitus. The mechanisms underlying these associations remain poorly understood. In this study, we focused on the functional characterization of variants of the fat mass- and obesity-associated (FTO) and the transcription factor 7-like 2 (TCF7L2) genes. We present an approach comprising measurement of plasma metabolite concentrations during nutritional and intravenous challenge tests to investigate early metabolic alterations in healthy risk allele carriers.

Methods: 78 healthy male participants of the KORA S4/F4 cohort, including homozygous carriers of the FTO locus risk allele rs9939609, carriers of the TCF7L2 locus risk allele rs7903146 and non-carriers of both alleles, were recruited. Nutritional challenges comprised an oral glucose tolerance test after overnight fasting, a standardized fast food meal, and a lipid tolerance test within a two-day study period. The intravenous challenge consisted of an intravenous glucose tolerance test and a subsequent euglycemic hyperinsulinemic glucose-clamp test. During the intervention, blood was sampled for biochemical and metabolomics measurement. Plasma samples were analyzed using a mass spectrometry-based metabolomics approach targeting 163 metabolites (AbsoluteIDQTM p150 kit, Biocrates Life Sciences AG).

Results: Challenges induced marked metabolic changes independent of genotype. FTO risk allele carriers did not differ from control subjects in their metabolic challenge response. However, we observed significant differences

in the ivGTT response comparing TCF7L2 risk allele carriers and control subjects, including a reduced first-phase insulin secretion and a stronger decline of a range of sphingomyelin and lysophosphatidylcholine metabolites in the carriers.

Conclusion: Our study confirms a role of TCF7L2 in insulin secretion. Furthermore, a connection of TCF7L2 to phospholipid metabolism was shown. The presented design provides a framework for further analysis of additional risk variants.

POSTER 59.

FAMILIAL RESEMBLANCE FOR 120 SERUM METABOLITES

Harmen Draisma (1,2,4), René Pool (1,3), Gonneke Willemsen (1,2), Jouke Jan Hottenga (1,2) and Dorret I. Boomsma (1,2,3,4)

1. Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands; 2. The EMGO+ Institute for Health and Care Research, Amsterdam, The Netherlands; 3. BBMRI-NL: Infrastructure for the Application of Metabolomics Technology in Epidemiology (RP4), The Netherlands; 4. ENGAGE: European Network of Genomic And Genetic Epidemiology

Background: Shared genetic background and shared environmental influences contribute to phenotypic similarity among family members. In this study, we sought to highlight two aspects of serum metabolite level familiality: heritability and spouse correlations.

Methods: Serum levels for 163 serum metabolites were assessed using the Biocrates targeted metabolite profiling platform in N=151 spouse pairs (mean age 59.3 years; SD, 5.8) and N=85 of their offspring (64% female; mean age 31.0 years [SD, 4.7]), who are enrolled in the Netherlands Twin Register. After quality control, log-transformed values for 120 metabolites were retained for analysis: eight acylcarnitines, fourteen amino acids, eighty-three glycerophospholipids, fourteen sphingolipids, and a compound measure for hexose. All analyses were carried out for single metabolites as well as for the log-transformed ratios for each possible pair of metabolites. Spouse correlations were computed as product-moment correlations after regressing out age effects from the metabolite scores for each sex. Potential associations of absolute within-spouse pair differences with self-reported relationship duration were investigated by simple linear regression analysis. Heritability was estimated by midparent-offspring regression analysis. We used "p-gain" to assess significance of associations for metabolite ratios; multiple testing correction was attained for all results using appropriate Bonferroni-based critical values.

Results: Spouse correlations were statistically significant for 553 metabolic traits; 535 of these were metabolite ratios. Heritability was significant for eighty-three metabolic traits (eighty ratios). The mean of the significant spouse correlations was equal to 0.46 (range, 0.36-0.62); the mean of the significant midparent-offspring regression coefficients equaled 0.74 (range, 0.56-0.97). Thirty-nine traits (37 ratios) were significant in both spouse correlations and in midparent-offspring regression. None of the metabolic traits displayed significant association between relationship duration and within-spouse pair differences. Conclusions: These results suggest that family members have significantly more similar metabolic trait values than expected by chance, in particular for log-transformed metabolite ratios. Our data do not provide evidence that these similarities are linked to the relationship duration of the parents, which argues against a significant influence of the shared ("familial") environment.

POSTER 60.

EXPOSURE OF MICE TO IONIZATION RADIATION INDUCES SPECIFIC METABOLOMIC BIOSIGNATURES

Marc Breit (2), Evagelia C. Laiakis (1), Ralf Bogumil (2), Steven Lai (3), James Langridge (3), Albert J. Fornace Jr. (1) and Giuseppe Astarita (1,3)

1. Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Washington DC, USA; 2. BIOCRATES Life Sciences AG, Innsbruck, AUSTRIA; 3. Waters Corp., Milford, MA, USA.

Biomarkers of acute exposure to ionizing radiation are highly needed, particularly in the case of accidental exposures and terrorist acts. The classic cytogenetic methods available for biodosimetry are laborious and time consuming. Here, we screened mouse sera for alterations in major metabolic pathways following exposure to gamma radiation. Male C57Bl/6 mice were exposed to high doses of gamma radiation associated with the hematopoietic syndrome (8 Gy). Blood was obtained by cardiac puncture twenty-four hours post irradiation.

Metabolites were extracted from mouse sera using multiple steps involving solid-phase extraction and derivatization (AbsoluteIDQ(tm) p180 Kit, Biocrates). Metabolites were analyzed by mass spectrometric methods using flow injection (FIA-MS/MS) and UPLC-TOF and UPLC-MS/MS. Verification of the molecular identity was conducted using retention times and tandem mass spectrometry (MS/MS); quantification of the metabolites was achieved by multiple reaction monitoring (MRM) detection in combination with the use of isotope-labelled and chemically homologous internal standards. Calibration curves and quality control samples were simultaneously analyzed. The data analysis was performed with the MetIDQ software, allowing an automation of the assay workflow, from sample registration to data processing.

We detected and quantified over 180 metabolites from different metabolite classes belonging to major metabolic pathways, including amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingomyelins and hexose. Specific biomarkers of radiation exposure were identified, with significant changes in lipid and amino acid metabolism. Multivariate data analysis, such as principal component analysis (PCA), was applied to interpret the large datasets that arose.

Our results indicate that ionizing radiation induces specific metabolomic signature in mouse blood, which could be utilized as biomarkers of exposure. Furthermore, our results might indicate novel targets of intervention to ameliorate the devastating effects of radiation.

POSTER 61.

INVESTIGATING THE SYSTEMIC AND GUT-SPECIFIC MOUSE METABOLOME BY NON-TARGETED METABOLITE PROFILING

Silke Heinzmann and Philippe Schmitt-Kopplin

Helmholtz Zentrum Muenchen

Many analyses have recently been performed to investigate the influence of the composition of the gut microbiome on health and disease status. However, information about the metabolic functionality of the whole gut microbiome and species-specific metabolic activity and interaction with the host is sparse.

Metabolite profiling provides the opportunity to characterise and quantify metabolites from

different chemical classes. The non-targeted manner of metabolic profiling allows discovery of novel metabolites and a combination of complementary analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) and ultra-high resolution MS (FT-ICR-MS) provides a holistic picture of a broad range of metabolites present in each sample.

To systematically characterize the metabolic composition of the gastro-intestinal tract and bodily tissues we collected gastro-intestinal contents, bodyfluids and tissues from n=3 C57BL/6 mice. Different tissues, biofluids and the gut content was analysed; the gut content was divided into 7 sections to allow an overview of the progression of nutrient breakdown and microbial activity in the gut. Metabolites were extracted using a step-wise extraction with aqueous (ACN:H2O) and methanol solvents and submitted to NMR spectroscopic analysis and MS analysis to obtain a comprehensive overview of the metabolites. Pattern recognition analysis was employed to illustrate similarities and differences among the tissue and gut compartments.

The chosen method strategy allowed an overview of a wealth of metabolic information within one analytical experiment and the non-targeted approach opens the possibility to discover novel or unexpected metabolic information.

This preliminary analysis unveils promising insights in the metabolic functionality of the symbiotic activity of host digestion and microbial activity and provides the opportunity to link details about the gut microbiome composition to its direct effects on the host.

POSTER 62.

MS-BASED METABOLOMIC STRATEGY FOR THE SCREENING OF NEW INDUCED METABOLITES IN MINIATURIZED FUNGAL CO-CULTURES

Antonio Azzollini (1), Samuel Bertrand (1), Olivier Schumpp (2), Nadine Bohni (1), Michel Monod (3), Katia Gindro (2) and Jean-Luc Wolfender (1)

1. School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; 2. Swiss Federal Research Station Agroscope Changins-Wädenswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland; 3. Department of Dermatology and Venereology, Laboratory of Mycology, CHUV, CH-1011 Lausanne, Switzerland

In natural product research, the search of new sources of compounds is a key element. In this respect microorganisms have provided a large number of biologically active molecules [1]. Recently, the use of fungal co-culture for the induction of new natural products has emerged as a new field in drug discovery [2].

A key point for the success of such studies is the development of co-culture experiments that provide high reproducibility of metabolite induction pattern and that are compatible with high throughput analytical procedure for the screening of a large number of single and co-culture samples and further data mining.

To tackle this issue, a method based on 12-well plate miniaturized Petri dishes compatible with high throughput UHPLC-TOF-MS metabolomics [3] has been developed. Various culture condition parameters were optimized for fungal growth such as culture medium volume and culture duration. This strategy was used to screen for metabolite induction and study their dynamics in the co-

cultures of a plant pathogenic fungi Aspergillus Clavatus and a systemic human pathogenic fungi Fusarium sp.

This approach provided a satisfactory reproducibility and was used for the identification of induced biomarkers. This study demonstrates the consistent induction of new metabolites through co-culture. Moreover upscaling of the co-cultures conditions to large Petri dishes show that the main induced metabolites were also produced allowing their purification for further de novo identification and evaluation of their bioactivity.

[1] Berdy J, J. Antibiot. 2005, 58, 1; [2] Glauser G *et al.,* J. Agr. Food. Chem., 2009, 57, 1127; [3] Bertrand S. *et al.,* J. Chromatography A, submitted manuscript

POSTER 63.

QUANTIFICATION OF URINARY BETAINE AND CHOLINE EXCRETION IN RATS FED FRACTIONS OF RYE AND WHEAT

Mette Skou Hedemann and Knud Erik Bach Knudsen

Department of Animal Science, Aarhus University, 8830 Tjele, Denmark

Life style related diseases has become of more and more concern in the Western world. Due to our sedentary life style and the consumption of diets with high energy and fat density and low in dietary fibre (DF) the prevalence of overweight, obesity and type 2 diabetes is alarming. Development of healthy food products that increase the intake of components from whole cereals or cereal fractions is of major interest. Betaine has been suggested to one of the components of wholegrain with health benefits [1]. Betaine is an osmolyte and it plays an important role in remethylating homocysteine, a risk factor for cardiovascular disease. Furthermore it is a lipotropic compound preventing excess fat deposition in the liver. The betaine content is high in wheat but it is appreciable in rye bran as well. The present study aimed to investigate the betaine and choline content in 20 fractions of rye and wheat and to study the bioavailability of betaine and choline in a rat model.

In the present study 20 fractions of rye and wheat were incorporated in experimental diets for rats. The diets were composed to contain the same amount of DF (10 %), protein (15 %) and fat (5 %). A semi-synthetic diet with Vitacel (purified cellulose from wheat) as the only fibre source was used as control. Each diet was fed to 8 rats for 16 days. During the last three days of the experiment urine was collected quantitatively. The urine was collected in bottles containing ascorbic acid. Urine was collected and frozen (-20°C) daily. Betaine and choline was quantified using a modification of the method of Bruce et al. [2]. To 100µl of urine was added 300µl methanol with D9-betaine and D9-choline (10µM), the samples was incubated for 10 min (4°C) and centrifuged (13.200 x g, 10 min). The supernatant was transferred to vials. The samples were injected to a hydrophilic interaction liquid chromatography column (HILIC) and data was acquired using a MicrO-TOF Q II mass spectrometer in positive electrospray ionization mode. A standard curve of betaine and choline ranging from 3.9-1000 µM and 0.39-100µM for betaine and choline, respectively, was made and the concentration of betaine and choline in the samples was calculated using QuantAnalysis 2.0 (Bruker daltonics GmbH, Bremen, Germany). The calibration curves were linear with the mean r2 value of 0.999 for nine betaine and choline standards.

The content of betaine was found to be highest in aleurone fractions of wheat which was expected as the aleurone layer is part of the bran where high concentrations of

betaine are usually found [1]. The high concentration of betaine in aleurone fractions resulted in a high excretion of betaine from rats fed these diet. Generally, rats fed fractions of wheat bran had a high excretion of betaine as well. Rat fed diets containing wheat whole grain showed variation in the excretion on betaine depending of the wheat variety. Rats fed white wheat had the highest excretion of betaine. The excretion of choline showed a smaller variation. In conclusion, the present study shows that it is possible to produce mill fractions of cereals with a high content of betaine and that betaine is absorbed and excreted in the urine.

[1] Fardet, A. (2010) Nutr. Res. Rev. 23, 65-134; [2] Bruce, S.J., *et al.* (2010) J. Agric. Food Chem. 58, 2055-2061.

POSTER 64.

ABSOLUTE METABOLITE CONCENTRATIONS AND METABOLIC CHANGES ASSOCIATED WITH OXIDATIVE STRESS AND DIFFERENT STAGES OF CELL GROWTH IN *TRYPANOSOMA BRUCEI*

Dong-Hyun Kim (1), Darren J. Creek (1), Rainer Breitling (1) and Michael Barrett (1)

College of Medical, Veterinary and Life Sciences, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow, G12 8TA

Human African trypanosomiasis (HAT) is a neglected tropical parasitic disease in sub-Saharan Africa caused by the protozoan parasite, *Trypanosoma brucei* (*T. brucei*) and transmitted by tsetse flies. It is fatal if untreated so effective drug treatment is necessary. New drugs are urgently required to eliminate the disease and so discovery of new targets for chemotherapy is of great importance.

LC-MS based metabolomic studies enable simultaneous measurement of a number of the small molecule metabolites in biological systems and can give information on how drugs perturb metabolism. However, although the relative quantification of metabolites gives us information regarding cell responses, absolute metabolite concentrations is critical to perform metabolic flux analysis and ultimately construct a quantitative mathematical model of metabolic flux. Quantitative modelling will play a critical role in developing optimised anti-parasite drugs. In order to obtain absolute concentrations, uniformly (U)-^{13}C-labelled *E. coli* extract can be used as an internal standard to overcome non-linear responses in MS with labelled metabolites behaving identically to their unlabelled forms in sample extract. Therefore, absolute intracellular concentrations of metabolites can be calculated by adding known amounts of U-^{13}C-labelled cell extract prior to the extraction procedure.

T. brucei bloodstream forms at different stages of cell growth in culture were analysed using LC-MS for metabolite profiling and absolute quantitative analysis. A total of 58 metabolites were absolutely quantified and key mass ions were determined by using univariate and multivariate analyses from the MS data. We then use this approach to reveal not only metabolic changes caused by different cell densities but also metabolic responses to oxidative stress.

POSTER 65.

METABONOMIC INVESTIGATION OF THE EFFECT OF ALLOPURINOL ADMINISTRATION IN PLASMA OF EXERCISING RATS

Alexandros Pechlivanis (1), Chrysovalantou-Anastasia Chatziioannou (2), Vassilis Mougios (3) and, Georgios A. Theodoridis (2)

1. Department of Surgery and Cancer, Imperial College London, SW7 2AZ; 2. Department of Chemistry, Aristotle University of Thessaloniki, 54124 Greece; 3. Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, 54124 Greece

Exhaustive exercise is a generator of free radicals and reactive species in human and rats. Allopurinol is a known inhibitor of xanthine oxidase, one of the most important sources of free radicals during exercise. In this study the influence of allopurinol in the metabolic profile of rats' plasma that had undergone exhaustive swimming is investigated by GC-MS method. Rats used in this study were divided in four groups: Rats that have been administered with placebo and were sacrificed without exercise (control), rats that have been administered with placebo, exercised until exhaustion and sacrificed just after that (exercise control), rats that were administered with allopurinol and sacrificed 2,5 hours after the administration (allopurinol control), and rats that were administered with allopurinol exercised until exhaustion and were sacrificed just after that (allopurinol exercise).

Samples were analysed after derivatization on GC-MS and the data were analyzed using multivariate statistical analysis methods. Safe prediction/classification of the samples was accomplished according to exercise and administration of allopurinol. Separation of the samples was mainly due to allopurinol and compounds in its catabolic pathway such as alloxanthine, hypoxanthine and uric acid, along with the 'usual suspects' after exercise, lactate and pyruvate.

POSTER 66.

METABOLOMICS IN DIABETES RESEARCH

Marianna Lucio (1), Kilian Wörmann (1), Sara Forcisi (1), Dimitrios Tziotis (1), Rainer Lehmann (2), Hans-Ulrich Häring (2) and Philippe Schmitt-Kopplin (1)

1. Helmholtz Zentrum München - Research Center for Environment and Health, Research Unit Analytical BioGeoChemistry, Neuherberg, Germany; 2. Dept. of Internal Medicine 4, University of Tübingen, Germany

This project involves the establishment and the proof of a non-targeted metabolomics platform within the BMBF Diabetes Mellitus Network for the metabolic screening and biomarker identification in human plasma and urine in order to enable the diagnosis of the onset of pre- Type 2 Diabetes in non-diabetic individuals of the TULIP (Tübingen LifeStyle Intervention Program) program, presenting a pre-diabetes phenotype. The combination of high resolution and high sensitivity techniques is the strong point of this platform development. The employment of UPLC-MS, FT-ICR-MS, NMR and 2D-chromatography leads to a multidimensional separation and detection of metabolites distinctive for a pre-illness stage as well as metabolic disorders and other kinds of illnesses.

Here we report as example a non-target metabolomics approach by ultrahigh resolution mass spectrometry (ICR-FT/MS) was developed to evaluate 46 plasma samples of subjects exhibiting high to low insulin sensitivities. The aim of our study was to investigate for the first time metabolic patterns with a 'metabolomics data modelling approach' to elucidate the conversion from physiological to pathophysiological insulin sensitivity by an individual metabolic fingerprint. Furthermore, this let us to detect pathways and to discover putative metabolite

biomarkers altered in the pathogenesis of insulin resistance. The mass spectra measured by Ion Cyclotron Resonance Fourier Transform Mass Spectrometer (ICR-FT/MS, 12 Tesla) were evaluated with multivariate approaches. All the information was achieved through multivariate display and statistical analysis, visualization and classification tools through the use of different databases like KEGG, Lipid databases, METLIN.

Our results suggest that altered metabolite patterns that reflect changes in insulin sensitivity respectively the ISIMatsuda are dominated by lipid-related pathways. Furthermore, a metabolic transition state reflected by heterogeneous metabolite fingerprints may precede severe alterations of metabolism. Our findings offer future prospects for deeper insights in the pathogenesis of the pre-diabetic phase.

[1] K. Wörmann, M. *et al.* (2012) "Metabolomics" in der Diabetesforschung. Der Diabetologe, Springer-Verlag, DOI: 10.1007/s11428-011-0778-9; [2] M. Lucio, A. *et al.* (2010): PLoS One. 5(10):e13317.

POSTER 67.

A COMPREHENSIVE METABOLOMIC CHARACTERIZATION OF TYPHOID FEVER IN HUMAN SERUM USING A MULTI-PLATFORM APPROACH

Elin Näsström (1), Stephen Baker (2), Henrik Antti (1)

1. Department of Chemistry, Computational Life Science Cluster, CLiC, Umeå University, SE-901 87 Umeå, Sweden; 2. Oxford University Clinical Research Unit, The Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Typhoid fever is an infectious disease caused by the bacteria *Salmonella enterica serovars Typhi* (*S. Typhi*) and *Paratyphi A* (*S. Paratyphi A*). Current diagnostic methods suffer from major limitations; therefore, there is a need for a more rapid, sensitive and specific diagnostic method for typhoid fever. Here, 75 human serum samples including: control samples (no detected infection), *S. Typhi* samples and *S. Paratyphi A* samples have been analysed with a mass spectrometry-based metabolomics approach including three analytical techniques: GC/TOFMS, LC/QTOFMS and GCxGC/TOFMS. Data obtained from these analyses have been compared to evaluate the different analytical platforms when it comes to detection and identification of as many relevant metabolites connected to infectious disease as possible. Metabolic fingerprints of the three sample types have been investigated by multivariate analyses to find differences between control and infectious samples as well as between the two bacterial species. The results show that all techniques gave a clear separation between the control and infectious samples, while the two bacteria could be separated only by the LC/QTOFMS and GCxGC/TOFMS data. The detected metabolites cover a wide range of the metabolome of interest. The discovered metabolic differences provide useful information for the continuing work in searching for biomarkers for diagnosis of typhoid fever, something that ultimately could be helpful in obtaining a regional and eventual global elimination of the disease.

POSTER 68.

THE EFFECT OF FOOD PULSES ON METABOLITES THAT EXHIBIT SIGNIFICANT TIME-OF-DAY VARIATION

Sarah Davies (1), Victoria Revell (1), Joo Ern Ang (2), Anuska Mann (1), Katrin Ackermann (3), Manfred Kayser (3), Alfred Thumser (1), Florence I. Raynaud (2) and Debra J. Skene (1)

1. Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK; 2. Cancer Research UK Cancer Therapeutics

Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK; 3. Department of Forensic Molecular, Erasmus MC University Medical Centre Rotterdam, Rotterdam, The Netherlands

Background: Determining which metabolites vary significantly with time of day may impact greatly on the interpretation of results from metabolomics studies. This baseline knowledge will be vital in the identification of disease biomarkers and in drug design. Here, we have used liquid chromatography-mass spectrometry (LC-MS) to examine the effect of food pulses on metabolite rhythms already known to vary significantly with time of day in human plasma [1].

Methods: Fifteen, healthy male subjects aged 18-35 years with regular sleep wake cycles were recruited. Subjects maintained a regular sleep/wake schedule (23:00-07:00 h) for one week prior to an in-laboratory session during which subjects remained in highly controlled conditions with respect to light, sleep, meals and posture (18:00 - 23:00 h and 07:00-09:00 h: semi-recumbent, < 5 lux; 23:00 - 07:00 h: recumbent (sleep), 0 lux; 09:00 - 18:00 h: freely moving, 100 lux). Regular blood samples were taken and standardised meals were given at 07:00, 13:00, and 19:00 h with a snack at 22:00 h; water was available ad libitum. Two-hourly plasma samples were prepared using a methanol/ethanol liquid phase extraction prior to analysis using reverse phase LC coupled to a Waters Micromass UPLC-QTOF Premier MS system. Thirty-two metabolites, previously identified as varying significantly with time of day (Ang et al., 2012), were quantified for 12 subjects over a 24 hour period (12.00-12.00 h) using MassLynx v4.1 (Waters Corporation, Milford, USA). Each individual's metabolite data were z-scored prior to cosinor analysis to determine amplitude, acrophase (peak) time and mesor of the rhythm of each metabolite.

Results: The 24 h rhythms in the quantified amino acids (phenylalanine, methionine, tyrosine, proline and leucine) were affected by the food pulses (peaks occurring 1-3 h post meal). By contrast, 14 carnitine, 6 lysoPC and 5 lysoPE compounds, along with cortisol and bilirubin, all exhibited daily rhythms that were not observably affected by food pulses.

Conclusions: The impact of food pulses on human plasma metabolites that exhibit significant time-of-day variation under strictly controlled laboratory conditions is dependent upon the type of metabolite, with amino acids being considerably affected. This study highlights the importance of strict control of external conditions in metabolomics studies and the need to further investigate the interaction between these exogenous factors and endogenous circadian time-of-day variation.

[1] Ang JE, *et al.* (2012). Chronobiology International 29: 868-881.

POSTER 69.

TIME-OF-DAY VARIATION IN HUMAN PLASMA METABOLITES USING AN UNTARGETED LIQUID CHROMATOGRAPHY MASS SPECTROMETRY METABOLOMIC APPROACH

Joo Ern Ang (1), Victoria Revell (2), Anuska Mann (2), Simone Mäntele (2), Daniella T. Otway (2), Jonathan D. Johnston (2), Alfred E. Thumser (2), Debra J. Skene (2) and Florence Raynaud (1)

1. Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, UK; 2. Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Background: Daily rhythms regulate multiple aspects of human physiology but rhythmic control of the metabolome remains poorly understood. The primary objective of this proof-of-concept study was identification of metabolites in human plasma that exhibit significant 24-h variation.

Methods: Metabolite rhythms were assessed via an untargeted metabolomic approach using liquid chromatography-mass spectrometry (LC-MS). Eight lean, healthy, and unmedicated men, mean age 53.6 (SD±6.0) yrs, maintained a fixed sleep/wake schedule and dietary regime for 1 wk at home prior to an adaptation night followed by a 25-h experimental session in the laboratory where the light/dark cycle, sleep/wake, posture, and calorific intake were strictly controlled. Plasma samples from each individual at selected time points were prepared using liquid-phase extraction followed by reverse-phase LC coupled to quadrupole time-of-flight MS analysis in positive ionisation mode. Time-of-day variation in the metabolites was screened for using orthogonal partial least square discrimination between selected time points of 10:00 vs. 22:00 h, 16:00 vs. 04:00 h, and 07:00 (d 1) vs. 16:00 h, as well as repeated-measures analysis of variance with time as an independent variable. Subsequently, cosinor analysis was performed on all the sampled time points across the 24-h day to assess for significant daily variation.

Results: Analytical variability, assessed using known internal standards, was low with coefficients of variation <10%. A total of 1069 metabolite features were detected and 203 (19%) showed significant time-of-day variation. Of these, 34 metabolites were identified using a combination of accurate mass, tandem MS, and online database searches. These metabolites include corticosteroids, bilirubin, amino acids, acylcarnitines, and phospholipids. The magnitude of the 24-h variation of these identified metabolites was large, with the mean ratio of oscillation range over MESOR (24-h time series mean) of 65% (95% confidence interval: 49-81%). Importantly, several of these human plasma metabolites, including specific acylcarnitines and phospholipids, were hitherto not known to be 24-h variant.

Conclusions: Identification of metabolites that vary with time of day will be useful in guiding the design and interpretation of future metabolite-based studies.

POSTER 70.

METABOLIC EFFECTS OF MILK PROTEIN INTAKE

Morten Rahr Clausen (1), Xumin Zhang (1), Haldis Lillefosse (3,2), Bjørn Liaset (2), Karsten Kristiansen (3), Hanne Bertram (1)

1. Department of Food Science, Aarhus University, Denmark; 2. National Institute of Nutrition and Seafood Research, Norway; 3. Department of Biology, University of Copenhagen, Denmark

Obesity is a wordl-wide problem, and nutrition research is focused on pinpointing diets reducing the risk of obesity development. Diets rich in proteins are generally recognized to have a beneficial effect on adiposity and body weight. However, the metabolic effects of proteins from different sources may vary, and the reasons for this are still far from fully understood. In the present study mice were fed a high fat diet with different preparations of milk proteins as main protein sources. Mice fed whey proteins gained significantly less adipose tissue than mice fed casein proteins. In order to shed light on underlying mechanisms, LC-MS and NMR metabolic profiling were used to map the metabolic phenotypes associated with this difference in adiposity. Several body fluids and organs were

analysed. While no pronounced effects could be identified on the serum and liver metabolomes, we observed some striking effects on the urinary metabolome. These effects indicate that whey proteins induce secretion of energy-rich citric acid cycle intermediates, which may explain the whey protein-induced reduction in adiposity.

POSTER 71.

A NEW APPROACH TO ASSESS REPEATaBILITY AND REPRODUCIBILITY OF A GC-MS BASED METABOLOMICS DATA MATRIX

Carolus Reinecke (1), Shayne Mason (1), Marceline van Furth (2), Regan Solomons (3), Mari van Reenen (4) and Gerhard Koekemoer (4)

1. Centre for Human Metabonomics, North-West University (NWU), South Africa; 2. Department of Paediatric Infectious Diseases, Vrije Universiteit, Amsterdam; 3. Department of Paediatrics, University of Stellenbosch; 4. Statistical Consultation Services, NWU, South Africa

Introduction: The analytical procedures to generate global metabolomics data for metabolite profiling or to detect markers require a high degree of reliability and repeatability to exclude the identification of false biomarkers due to experimental procedures used (see [1]) . Here we present results and a model to evaluate repeatability and reproducibility of a GC-MS analysis of a complex CSF sample obtained from a treated patient suffering from tuberculosis meningitis (TB-M).

Methods: Organic acids were isolated from 5 aliquots from a CSF sample of a treated patient with TB-M (see [1]). The trimethylsilyl (TMS) derivatives from each extraction was analyzed on two Agilent 7890A gas-chromatographs, used for research and routine diagnostics services, respectively . They were coupled to an Agilent 5975B XL MSD and Agilent 5975C VL MSD mass-spectrometer. Features from the GC-MS were deconvoluted, identified and quantified using a custom created library on AMDIS. Bioinformatics data processing included normalization, data reduction and the development of a kernel density estimate model to qualitatively assess the repeatability of the comprehensive metabolomics data set (abbreviated as the KEDREP method).

Data and results: Analysis of 5 samples each on the GC-MS-1 and GC-MS-2 yielded 398 and 239 features respectively. Using a filter based on retention time CV of less than 10%, reduced them to 175 and 112. The average concentration and standard deviation were calculated for each feature and a coefficient of variation (CV) distribution was plotted for each GC-MS. From a plot of the cumulative log-scaled concentration of the features against retention time, corresponding density plots were generated, using kernel density estimation. Overlays of these plots was used for the qualitative and quantitative assessment of (1) the complete data set derived from the 10 samples and two GC-MSs; (2) the profiles obtained from the individual GC-MSs; (3) the outcome of the data reduction steps and (4) for the assessment of 95 variables found to be common between the two GC-MS apparatuses.

Conclusion: We have developed a novel and practical model for the qualitative assessment of repeatability and reproducinility for comprehensive metabolomics data sets.

[1] Del Boccio *et al,* Ann Neurology 2007, 62, 201-204; [2] Reinecke *et al,* Metabolomics, 2012, 8, 264-28

POSTER 72.

WHAT THE SMALL MOLECULES IN SENILE FLIES CAN TELL US ABOUT ALZHEIMER'S DISEASE – NMR METABOLOMICS OF Aß-EXPRESSING DROSOPHILA HEADS

Anders Malmendal, Stanislav Ott and Damian Crowther

Department of Biomedical Sciences, University of Copenhagen

As we age the ability of our cells and tissues to retain proteins in their proper, native conformation is impaired. The consequences of the proteostatic collapse in old age are varied and include a number of very common neurodegenerative disorders, the most prevalent of which is Alzheimer's disease (AD). AD is characterised microscopically by the accumulation of two distinct protein amyloid deposits, neuritic plaques that are extracellular deposits of the amyloid peptide (Aß), and the intracellular tangles composed of the tau protein.

The current gold standard for measuring ageing and associated physiological fragility is the determination of mortality trajectories. While mortality trajectory data is robust as a population-based measurement, it cannot be applied to small groups or individual organisms and so there is a need to find biomarkers for the "physiological age" of individuals.

Here we use NMR metabolomics of Drosophila melanogaster heads to see how the expression of different variants of Aß, mimicking AD, affects the normal metabolomic changes that occur with healthy ageing. These results are used asses whether metabolomics can provide a measure of physiological age, and to identify specific AD-related responses.

POSTER 73.

LIPIDOMIC PROFILING OF DRIED SERUM SPOTS (DSS)

Rebeca Mayo (1), Ibon Martínez-Arranz (1), Ziortza Ispizua (1), Cristina Alonso (1), Yudelsy Tandrón (2) and Miriam Pérez-Cormenzana (1)

1. OWL, Derio, Bizkaia, Spain; 2. MD Renal, Derio, Bizkaia, Spain

DSS offers a number of advantages such as easy and cheap sample transportation at room temperature, sample conservation and storage, facilitating for instance multicenter studies. An ultra-performance liquid chromatography/time-of-flight tandem mass spectrometry (UPLC/TOF-MS/MS)-based metabolomics approach was employed for the simultaneous determination of different classes of lipids, including diacylglycerides, triacylglycerides, phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, cholesterol esters and sphingolipids from human dried serum spots (DSS). The assay employed simple solvent extraction of a punch taken from the DSS sample, followed by reversed phase separation using a Waters BEH C18 column in combination with a tandem Xevo G2 QTof (Waters Corp.) mass spectrometer. Serum extracts were spotted on the cards and once dried they were stored at room temperature in the absence of light until sample extraction. Dried serum spots were punched out of a collection paper with a 3 or 8 mm diameter punch and then extracted with different solvents. Card types, volume of serum, punch sizes, stability of the metabolites along time and different extraction procedures were some of the aspects taken into account in order to obtain the most complete serum lipidomic profile. Results confirm that lipid species have different behavior along time. However, a common issue for most of

metabolites was that their intensities increased 48 hours after serum was spotted in the cards.

POSTER 74.

THE DEVELOPMENT AND VALIDATION OF A TARGETED LC-MS/MS METHOD FOR THE DETECTION AND QUANTIFICATION OF HEPARAN SULPHATE SACCHARIDES

Katherine Hollywood, Nina Kureishy, Graham Mullard, Paul Begley, Garth JS. Cooper and Warwick B. Dunn.

Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust & Institute of Human Development, The University of Manchester, UK.

Heparan sulphate (HS) is a member of the glycosaminoglycan (GAG) family; highly sulphated, charged and linear polysaccharides composed of a repeating disaccharide structure of 1,4 hexuronic acid and glucosamine residues. Their biological importance has been noted in a number of diseases including diabetes, Alzheimers disease, Parkinsons disease and carbohydrate storage disorders (for example, mucopolysaccharidosis). For investigations of their changes in disease and potentially therapeutic monitoring, a targeted assay to quantify structural components of heparan sulphate in human biofluids is required.

A liquid chromatography-mass spectrometry-based assay for the absolute quantification of heparan sulphate disaccharides and monosaccharides has successfully been developed. The assay utilises a Thermo Scientific Accela UHPLC system coupled to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer. Chromatographic separation of the analytes was achieved employing Hydrophilic Interaction Chromatography (HILIC), specifically a Sequant ZIC HILIC column.

The analytical method allows for the absolute quantification of heparan sulphate monosaccharides and disaccharides to be performed with high sensitivity and specificity with use of a non-natural heparan disaccharide analogue (I-P) as an internal standard. Limits of detection were observed in the pM range. Results from the digestion of heparan sulphate purified from urine will be presented to highlight applicability.

POSTER 75.

STATISTICAL ANALYSIS OF COMBINED METABOLOMICS DATASETS FROM THE POTATO TUBER LIFE CYCLE

Colin Alexander (1), LVT Shepherd (2), JA Sungurtas (2), JW McNicol (1), D Stewart (2) and HV Davies (2)

1. Biomathematics & Statistics Scotland; 2. The James Hutton Institute

An approach is presented for the combined statistical analysis of multiple datasets derived from a range of metabolomics technologies. Using a study on potatoes, several stages of the tuber life cycle were sampled and analysed using various mass-spectrometry based approaches. Each individual dataset is subjected to Principal Components Analysis, Analysis of Variance and Hierarchical Cluster Analysis to assess the potential for separating the life cycle stages, to define the major profiles for metabolite changes during the life cycle stages examined, and to inform on which metabolites underpinned these profiles. For each technology's dataset, Analysis of Variance (ANOVA) is applied to each individual metabolite to identify those which show a significant differential response among the life cycle stages. We define a development profile as the set of estimated

mean intensities (from the ANOVA) at each of tuber stages analysed. Further to this, partitioning of the subset of metabolites with significant responses is performed using the standardised differences among the development stages. This standardisation is based on dividing the difference in means by their 5% Least Significant Differences (LSD). Since these standardised differences are scaled by their variability, this allows metabolites to be compared both within and across technologies. Hierarchical Cluster Analysis on the standardised differences pooled from all the technologies generates clusters which share the same patterns; these are defined by significant changes rather than absolute values. Each cluster represents different profile types of metabolites pooled across all of the technologies. Graphical representation is also achieved by standardising the development profiles, allowing each metabolite in a cluster to be plotted on the same scale. This is accomplished for each metabolite by dividing its estimated mean values by its average standard error of difference. This methodology provides a procedure for combining data from multiple metabolomics technologies. It produces clusters of metabolites from this pooled dataset and appropriate graphical representation.

POSTER 76.

METABOLOMICS OF INTACT TISSUES: DISCRIMINATION BETWEEN DIFFERENT REGIONS OF OSTEOLYTIC LESIONS IN A MULTIPLE MYELOMA PATIENT USING 1H HRMAS NMR SPECTRA

Silvia Mari (1,6), Francesca Fontana (2), Jose Garcia Manteiga (2), Edoardo Gaude (1), Simone Cenci (2), Enrico Caneva (3), Stanislav Sykora (4), Juan Carlos Cobas Gomez (5), Giovanna Musco (1)

1. Dulbecco Telethon Institute c/o S. Raffaele Scientific Institute, Milan (Italy); 2. Protein Transport and Secretion Unit c/o S. Raffaele Scientific Institute, Milan (Italy); 3. Centro Interdipartimentale Grandi Apparecchiature c/o UniversitÃ degli Studi di Milano, Milan (Italy); 4. Extra Byte, Castano Primo, Italy, http://www.ebyte.it/; 5. Mestrelab Research, Santiago de Compostela, Spain, http://www.mestrelab.com/; 6. R4R, Rodano, Italy, http://www.research4rent.com/

Multiple myeloma (MM) is a malignancy of plasma cells characterized by multifocal bone lesions and systemic complications due to distant end-organ damage. The bone marrow is a natural niche for long-lived plasma cells, but upon malignant transformation an expanding clone diffusely infiltrates the bone marrow, altering its composition and functions. At a number of sites a dramatic alteration of the balance between bone resorption and deposition, locally induced by neoplastic cells, leads to the complete destruction of the bone, which becomes substituted by a mass of cancer cells. Such osteolytic lesions can grow to the point of jeopardizing the structural stability of bones and give rise to dangerous pathological fractures or spinal cord compression. Understanding the biology of such process, which is particularly severe in MM, could help design better diagnostic strategies to prevent pathologic fracture, but also shed light on the peculiar properties that differentiate the local bone-destroying from bone-infiltrating myeloma cells.

The metabolic profiling or metabolomics of disease has proven useful to identify diagnostic and prognostic markers. Its use in the clinic is beginning to increase exponentially; however, it is still largely underexploited. Although the potential of metabolomics has been established in solid tumors (prostate, breast cancer and colon cancer), much less is known about its use in

hematological malignancies. Moreover, to our knowledge, metabolomics has been not applied so far to the evaluation of bone lesions. We thus set out to develop the metabolomic study of myeloma-induced bone disease. To this aim, bone tissue biopsies have been collected from MM patients undergoing orthopedic surgery and analyzed by High Resolution-Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR). Since the actual nature of all the metabolites is rarely known in advance, metabolomics often uses alternative statistical evaluation methods, such as multivariate factor analysis. Such approaches require integration over predefined intervals (bins) and a meaningful integration of such intricate and artefact-burdened spectra may often be just as arduous as peaks fitting. Recently, a new algorithm called GSD (Global Spectrum Deconvolution) has been developed and made available in the Mnova software package (Mestrelab Research). GSD is capable of identifying even poorly resolved spectral signals and of fitting all recognizable peaks in even very complex 1D spectra. GSD produces a table of all detectable spectral peaks and their parameters. Such a table can be then used for various purposes like generation of artifact-free synthetic spectra, stick spectra, artifact-free integrals, as well as accurate binning void of any bin-crossover problems due to the overlapping wings of spectral peaks. Because of these attractive features, GSD is likely to become a very important pre-processing tool for all metabolomic approaches to the evaluation of NMR spectra of whole bio-samples. Finally, a GSD-based binning matrix was used as input to the in-house developed R-package MUMA (Multivariate & Univariate Metabolomic Analysis). MUMA is available online for a free download http://cran.r-project.org/web/packages/muma. It performs total spectra normalization and scaling (auto-, pareto-, vast- or range-scaling) as well as both univariate (Shapiro Wilk's test for normality, Welch's T- test or Mann Whitney-Wilcoxon test amongst all possible class combinations and outputs, Volcano plots) and multivariate analysis (PCA, PLSDA or O-PLSDA). It can also perform correlation analysis such as STOCSY and RANSY.

Here we present an integrated application based on the R-package MUMA and Mnova software for the processing, analysis and classification of different regions of osteolytic lesions in a MM patient's bone tissue biopsies for which also histopathological analysis could be performed. Importantly histopathology perfectly correlates with HR-MAS NMR data. From the translational point of view, metabolomics analysis of bioptical samples may be used in the future to improve prognostic stratification or differential diagnosis.

POSTER 77.

^{1}H NMR SPECTROSCOPY AND GC/MS ANALYSIS FOR A METABOLOMIC STUDY OF PRETERM HUMAN AND FORMULA MILK

Luigi Atzori (1), F. Cesare Marincola (1), A. Noto (2), PL Caboni (3), A. Reali (2), L. Barberini (6), M. Lussu (5), F. Murgia (5), ML Santoru (5) and V. Fanos (2)

1. Department of Chemical and GeoScience, University of Cagliari,Italy; 2. Department of Surgery, University of Cagliari,Italy; 3. Department of Life and Environmental Sciences, University of Cagliari,Italy; 4. Department of Public health and clinical and molecular medicine, University of Cagliari,Italy; 5. Department of Biomedical Sciences, University of Cagliari,Italy; 6. Department of Public Health and clinical and molecular medicine, University of Cagliari, Cagliari, Italy

Preterm infants (less than 37 completed weeks of gestation) of low birth weight (LBW; less than 2500 g) due to their metabolic immaturity are especially predisposed to develop diseases both in early and later in life. While the pathways responsible of these mechanisms are not completely understood, it is clear that an optimal nutritional support for this special class of patients plays an important role not only for short effects, in achieving an appropriate growth and nutritional accretion, but also for long-term effects on health and well-being.

In this study we tested the potential of the metabolomic approach to investigate the composition of human breast milk from mothers delivering a preterm-LBW newborn during the first month of lactation. For the sake of comparison, some commercially available formula milk (FM) samples were also analyzed. Our attention was focused on the carbohydrate and fatty acid moieties that were analyzed by NMR and GC-MS techniques, respectively. At specific week intervals from 1 to 4 weeks postpartum, complete 24-hr human breast milk (HBM) collections were obtained from 30 mothers giving birth at 26 to 42 weeks of gestation. At the completion of each 24-hr period, the total daily volume of milk was well mixed and an aliquot was removed and stored at -20°C for analysis. ^{1}H NMR experiments on the aqueous extracts of milk were carried out on a Varian Unity 500. For the GC/MS experiments, 1µL of derivatised sample was injected splitless into a HP 5971A GC/MS chromatography system (Agilent, USA). Principal components analysis (PCA) of NMR and GC/MS data was performed using SIMCA software package (version 13.0, Umetrics, Umea, Sweden).

An explorative PCA analysis of the middle field spectral region was performed with all milk samples. For the sake of comparison, milk from full-term mother was included. The score plot of the first two PCs, which expressed almost 80% of the total variability, showed a clear differentiation between HBM and FM, reflected by a high goodness of fit and predictability as indicated by an R^2 value of 0.97 and by a Q^2 value of 0.91, respectively. The analysis of the corresponding loading plot revealed variables of importance for the clustering, thus allowing the identification of metabolites responsible of the observed variance. The PC1 loadings corresponding to lactose (5.25, 4.45, and 3.30 ppm) showed negative values, indicating that HBM contains relatively higher contents of this sugar with respect to FM samples. By contrary, the commercial products were suggested to be richer in maltose. Examination of the score plot for PC2 showed that milk samples of term infant were located at the opposite side with respect those expressed by mothers at the lowest gestational age under investigation (i.e. 26 weeks). PCA using only preterm HBM provided by the same mothers at different lactation weeks postpartum evidenced the occurrence of a progressive change of the metabolic profile of milk suggesting a temporal variation in the carbohydrate composition. The analysis of the corresponding loading plot indicated an increase of the lactose level during the lactation period, in good agreement with the literature data. Other relevant metabolites were choline, myo-inositol, and maltose. Chromatograms from preterm HBM and FM extracted sample showed significant qualitative and quantitative differences between the two groups in the fatty acid profile, the HBM chromatogram being characterized by peaks higher in number and intensity with respected to FM. The PCA analysis of the data evidenced a fairly clear separation between the groups. Among the metabolic differences observed, the level of oleic and linoleic acids appeared to be higher in the artificial formulas than in HBM.

In summary, the preliminary results of the present study suggests that metabolomics may become a promising tool in the future for nutrition and health in preterm infants. Indeed, this approach may be a powerful instrument of knowledge about the factors responsible of the metabolic modifications of HBM and a promising approach for maximally improving the nutritional quality of artificial milk.

POSTER 78.

THE USE OF MULTILEVEL MODELS TO OVERCOME HIGH BETWEEN-SUBJECT VARIABILITY AND DISCOVER THE HIDDEN PATTERN WITH APPLICATIONS TO CLINICAL METABOLIC PROFILING STUDIES

Yun Xu (1), Stephen J Fowler (2) and Royston Goodacre (1)

1. Manchester Institute of Biotechnology (MIB) & School of Chemistry, University of Manchester; 2. University of Manchester and Lancashire Teaching Hospitals NHS Foundation Trust.

In clinical related metabolic profiling studies, the between-subjects variability was often the most dominating source of variation and it could mask the potential separation caused by other reasons and one of which could be the one the experiment was aimed to discover. Conventional models such as PCA, PLS-DA were not effective in such situations and find a suitable model which is able to discover the underlying pattern hidden behind the high between-subjects variability is highly desirable. In this study we employed two clinical metabolic profiling data sets as the testing ground and demonstrated that a proper choice of multilevel model can help overcoming such difficulty. One data set was obtained from a small scaled study which involved with analysing the volatile organic compounds (VoCs) collected from the skins of chronic wounds by using a skin patch device and GC-MS. Five patients were recruited and for each patients 3 sites were sampled in triplicates: healthy skin, boundary of the lesion and the top of the lesion. The aim was to see whether it was possible to discriminate these 3 types of samples based on their VoCs profile. The other data set was coming from a much larger study which had involved with a total number of 115 subjects. Among them 35 were healthy subjects, 47 were chronic obstructive pulmonary disease (COPD) patients and 33 were asthma patients. The VoCs in the breath of each subject were collected by using a mask device and analysed by using GC-MS and the aim of the study is to discriminate the three types of subjects based on the VoC profiles in their breath. Multilevel simultaneous component analysis (MSCA), ANOVA-PCA and a simplified ANOVA-PCA model which we name it ANOVA-Mean centre (ANOVA-MC) were applied on these two data sets and results were compared with the ones obtained by PCA. Significantly improved results were obtained by using the multilevel models. We also presented a validation procedure to validate the results obtained from the multilevel models. The results of validation confirmed that the patterns showed by the multilevel models were the genuine ones hidden behind the high between-subject variability.

POSTER 79.

SYSTEMS BIOLOGY ANALYSIS OF THE EFFECTS OF ACETAMINOPHEN TREATMENT ON ENERGY METABOLISM PATHWAYS

Lisette Leonhardt (5), Jinchun Sun (1), Yosuke Ando (1,2), Dörthe Ahlbory-Dieker (5), Laura K. Schnackenberg (1), Xi Yang (1), James Greenhaw (1), Lisa Pence (1), Feng Qian (3), William Salminen (1), Donna L. Mendrick (1),

Richard D. Beger (1), Oliver Schmitz (5) and Christopher Macri (4)

1. Division of Systems Biology, NCTR, FDA, Jefferson, AR USA; 2. Medicinal Safety Research Labs, Daiichi Sankyo Co., Ltd., Tokyo, Japan; 3. Z-Tech, Jefferson, AR, USA; 4. Metanomics Health GmbH, Tegeler Weg 33, 10589 Berlin; 5. Metanomics GmbH, Tegeler Weg 33, 10589 Berlin

Drug-induced hepatotoxicity is one of the major reasons for drug recall and hence it is of major concern to the FDA and consumers. An overdose of the analgesic and antipyretic drug acetaminophen (APAP) can lead to liver failure due to saturation of the normal metabolic pathway. In this case, N-acetyl-p-benzosemiquinone (NAPQI), generated by metabolic oxidation of APAP, can bind irreversibly and covalently to macromolecules. As a consequence, disruption of cell function and necrosis of hepatocytes take place. Although a lot of effort has been put into research over the last 45 years and a lot is known about the toxicity and metabolism of APAP, the community still lacks in a complete mechanistic understanding of APAP-induced hepatic necrosis. Our goal therefore was to comprehensively understand APAP-induced hepatotoxicity regarding its influence on energy metabolism by applying an integrated transcriptomic and multi analytical platform-based metabolomics approach.

Sprague Dawley rats were orally gavaged with different concentrations of APAP. Blood, liver tissue and urine samples were analyzed to evaluate the hepatotoxic effects and impact on energy metabolism at different time points. Open metabolic profiling (by NMR and LC/MS) and broad metabolic profiling (by LC/MS/MS and GC/MS) were applied to examine metabolic changes; 270 metabolites were detected in total from serum/plasma and urine. The metabolic data combined with gene expression profiles of the liver served as a basis for this systems biology investigation of APAP-induced hepatotoxicity. The findings indicate that hepatocyte necrosis is a multistage process that was initiated by GSH-depletion and results from a lack of energy or impaired energy production by mitochondria.

POSTER 80.

^{1}H NMR SPECTROSCOPY AS A NEW TOOL FOR BODY FLUID IDENTIFICATION IN FORENSIC MEDICINE

Luigi Atzori (2), P. Scano (1), E. Locci E (1), M. Lussu (2), F. Murgia (2), A. Noto (3), L. Barberini (4), G. Navarra (1), F. De Giorgio (4), E. d'Aloja (5)

1. Department of Chemical Sciences, University of Cagliari, Cagliari, Italy; 2. Department of Biomedical Sciences, University of Cagliari, Cagliari,Italy; 3. Department of Surgery, University of Cagliari,Cagliari, Italy; 4. Institute of Legal Medicine, Catholic University, Rome, Italy; 5. Forensic Science, Department of Public Health, University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Cagliari, Italy

Identification of body fluid (BF) traces in a crime scene plays a pivotal role in forensic medicine, helping investigators in figuring out the events which occurred or in directing the course of further inquires, as well as in contributing to the evidence in a case. High Resolution 1H Nuclear Magnetic Resonance (1H NMR) spectroscopy coupled with mathematical strategies is proposed as a valid tool for body fluid trace identification in forensic science. A total of 9 healthy subjects were recruited for this study. Samples of blood, urine, saliva, and semen were collected from different donors, and, in order to assess the feasibility of this approach also for the identification of traces composed by different body fluids, mixtures were prepared. Samples were divided in a training set and in a test set.

1H NMR analyses (Varian UNITY INOVA 500) were carried out for all the samples. Principal component analysis (SIMCA-P+ program ,Version 13.0, Umetrics, Umeå, Sweden) was applied to the spectral data to ascertain whether samples of the same body fluid from different donors have common characteristics that make them clustering in a multivariate space. Furthermore, an average spectral profile, weighted on different donors, was obtained and validated for each body fluid class, showing that the inter-body fluid variability is much greater than the inter-individual variability. The 1H NMR average spectral profile for each BF was obtained computing the average values of the spectral data of the 9 samples.

The distance of each sample with respect to the 4 average spectral profiles has been computed and results reported in terms of root square difference (RSD). The mathematical strategy for BF identification in the test set samples was based on fitting procedure as implemented in MATLAB software. A system of linear equation has been solved, where the coefficients of each average spectral profile are the unknowns. Goodness of fitting is given by the RSD based on the residual analysis, i.e. how the average spectral profiles, each weighted by its c-value, match the test set spectral data. The coefficient c obtained for each class of BF gives an estimation of the possibility that the sample is composed by that specific BF.

A fitting procedure was applied to the test set samples, and it showed a great potential for unambiguous identification of body fluids also in mixtures. The overall strategy is based on the intrinsic properties of 1H NMR spectroscopy of giving an unique metabolite profile of each body fluid. Furthermore, in this feasibility study, we demonstrated that the spectra of mixtures retain the features of the original body fluids, thus electing this technique as a valid, fast and no destructive tool for composite traces identification in forensic medicine.

POSTER 81.

EVALUATION OF A FOLCH-STYLE EXTRACTION METHOD FOR UNTARGETED METABOLOMIC STUDIES OF MAMMALIAN TISSUES

Paul Begley, Graham Mullard, Katherine A. Hollywood, Elizabeth J. Pawson, Garth J.S. Cooper, and Warwick B. Dunn

Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust & Institute of Human Development, The University of Manchester, UK

Mammalian tissues are increasingly being studied in untargeted and targeted metabolomics studies, to investigate tissue-specific changes related to physical or biological perturbations including in the study of disease onset and progression and in drug discovery and development. However, evaluation of sample extraction methods for a wide variety of mammalian tissues studied in untargeted metabolomics studies has not been previously reported.

Here we will report the evaluation of a Folch-style extraction method for the preparation of mammalian tissue samples prior to untargeted metabolomics. Seven different tissues were collected from healthy mice (liver, brain, pancreas, adipose fat, kidney, heart and muscle) for the evaluation. Tissue samples were homogenised in 1:1 MeOH:CHCl3 using a bead homogeniser (Qiagen Tissuelyser). Following extraction, water was added to a final composition of 1:1:1 H2O:MeOH:CHCl3, causing separation into two phases. Polar and non-polar phases were separately collected, dried and analysed by GC-MS (Leco Pegasus

HT MS coupled to an Agilent 7890 GC) and reversed phase UHPLC-MS (Thermo Scientific Accela UHPLC coupled to a LTQ-Orbitrap Velos hybrid MS), respectively.

Method reproducibility, intra-subject and inter-tissue variability will be reported and the method shown to be suitable for comparisons within a single tissue type. However, systematic variations were observed in the internal standards (GC-MS) between tissue types, which suggest that tissue-specific matrix effects were present. This complicates the interpretation of inter-tissue comparisons, which should be approached with caution if the presented method is used. Furthermore, it emphasises the utility of employing internal standards as a form of internal QA, regardless of subsequent approaches to data normalisation and/or drift correction.

POSTER 82.

CHANGES IN GRAPE PHENYLPROPANOID COMPOSITION INDUCED BY LEAF REMOVAL IN TWO 'PINOT NOIR' VINEYARDS

Melita Sternad Lemut (1), Paolo Sivilotti (1), Domenico Masuero (2), Roberto Zorer (3), Luca Zulini (4) and Urska Vrhovsek (2)

1. University of Nova Gorica, Wine Research Centre, Ajdovscina, Slovenia; 2. Food Quality and Nutrition Department, IASMA Research and Innovation Centre, Edmund Mach Foundation, San Michele all'Adige, Italy; 3. Biodiversity and Molecular Ecology Department, IASMA Research and Innovation Centre, Edmund Mach Foundation, San Michele all'Adige, Italy; 4. Genomics and Biology of Fruit Crop Department, IASMA Research and Innovation Centre, Edmund Mach Foundation, San Michele all'Adige, Italy

Beyond the biological significance, secondary metabolites are crucial in the determination of grape and wine quality attributes, and their composition, can be significantly modulated by exogenous (site, climate, soil properties) and endogenous factors (variety, clone, rootstock) and finally by agricultural practices. The grape composition is undoubtedly crucial for Pinot noir production and any improvements in polyphenol complexity are welcomed. Leaf removal is an important canopy management practice, leading either to an improvement of fruit-zone microclimate and grape quality [1]. Different results can be achieved depending on the timing of performing, and pre-flowering leaf removal seems to be more promising even if further results are needed [2].

In this study, leaf removal was performed in two 'Pinot noir' vineyards (located in Vipava Valley, Slovenia and in San Michele all'Adige, Italy) at different phenological stages: 10 days before flowering (pre-flowering leaf removal) and at veraison, while untreated vines were used as a control. For each shoot 4-to-6 leaves were removed manually. At harvest, the grapes from all the treatments were collected separately, processed, and a comprehensive LC-MS/MS metabolic profiling approach [3] was adopted in order to highlight the induced quantitative changes of several dozens of phenolic compounds including a number of up to date very poorly studied ones.

The results revealed a number of changes related both to vineyard location and to the timing of leaf removal. As related to vineyard location, the occurrence of secondary metabolites was 40%-higher in Italian samples, probably due to the different ripening status of the grapes (25 Brix in Italy vs 22 Brix in Slovenia). On the other hand, comparing leaf removal treatments, the most significant changes were observed in the group of 18 flavonols. The amount of total flavonols was increased by 216% and 109% in Slovenia and by 56% and 20% in Italy, in the pre-flowering and in the veraison treatments, respectively,

as compared with the controls. Pre-flowering leaf removal has also triggered significant changes in total hydroxycinnamic acids (both vineyards) and anthocyanins (in Slovenia), while other phenolic groups showed some changes in occurrences within individual group members, but were not significant in total amount. Despite location differences, grape polyphenols included in the study were positively affected by both leaf removal treatments, particularly when performed at early phenological stages. Metabolic profiling technique represents a powerful tool, but a lot of efforts and data are still required towards a comprehensive understanding of the mechanisms behind it.

[1] Haselgrove L., *et al.,* Australian Journal of Grape and Wine Research 6, (2000) 141-149; [2] Sternad Lemut M., *et al.,* Journal of Food Composition and Analyses 24, 6 (2011) 777-784; [3] Vrhovsek U., Journal of Agricultural and Food Chemistry, DOI: 10.1021/jf2051569.

POSTER 83.

AN NMR-BASED METABONOMICS INVESTIGATION OF PLASMA USING AN OVINE MODEL OF PERICONCEPTIONAL OVERNUTRITION

Horst Joachim Schirra (1), Juan Li (2), Lisa Nicholas (3), Song Zhang (3), Janna Morrison (3), Caroline McMillen (3) and Ross L. Tellam 92)

1. The University of Queensland, Centre for Advanced Imaging, Brisbane, Qld 4072, Australia; 2. CSIRO Animal, Food and Health Sciences, 306 Carmody Road, St Lucia, Brisbane, QLD 4067, Australia; 3. University of South Australia, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, City East Campus, Level 5 Playford Building P5-06, Frome Road, Adelaide, SA 5000, Australia.

The growing prevalence of obesity is a major health concern because it is a risk factor for several chronic diseases including hypertension, coronary heart disease, some cancers and type two diabetes. There is increasing evidence indicating that factors such as maternal obesity or overnutrition during pregnancy can influence long-term energy balance in offspring. Maternal obesity has been associated with offspring weight at birth as well as childhood and adolescence obesity. The mechanisms underlying these observations are unclear. To investigate these effects we have used an ovine model of periconception overnutrition and employed NMR-based metabonomics profiling of plasma from fetuses at 140 days of development - the latter were transferred as embryos from donor ewes into control recipient ewes. NMR-based metabonomics technology can now provide system-wide information on the major metabolites in plasma. Metabonomics has been successfully applied in diagnostic biomarker discovery, toxicology and pharmaceutical research. One-dimensional 1H NMR spectra of plasma samples from the donor ewes during the period of periconceptual overnutrition and fetuses at 140 days of development were recorded and exported for multivariate statistical analyses. The resulting data indicated that several metabolic pathways were involved in responses of donor ewes and fetuses to periconceptional overnutrition.

POSTER 84.

EVALUATION OF EXTRACTION METHODS FOR WHOLE BLOOD SAMPLES

Anna Kauppi, Alicia Edin, Åsa Gylfe, Anders Johansson

Department of Clinical Microbiology, Umeå University, SE-901 85 Umeå, Sweden

Choosing the right extraction method for metabolomic analyses is critical for getting desired results. Various methods and extraction solvents have been evaluated

previously for serum, plasma and erythrocytes [1,2,3]. Common criteria for a good extraction method for global metabolome analysis are reproducibility and sensitivity [2]. Most studies have found that extractions using methanol or methanol/chloroform mixes have the highest reproducibility and number of extracted compounds [1]. Since there is little data available on extraction of whole blood samples for metabolomic analysis, we wanted to evaluate two standard methods and compare their performance. Methanol/water was compared to Methanol/Chloroform/water with regards to reproducibility and sensitivity for extraction of whole blood samples. The variance in quantity of internal standards and the number of extracted and identified metabolites were evaluated after GC/MS and LC/MS analysis.

[1] Zhang et al. Journal of Chromatography B, 877 (2009) 1751-1757; [2] Want et al. Anal. Chem. 2006, 78, 743-752; [3] Jiye et al. Anal. Chem. 2005, 77, 8086-8094

POSTER 85.

BIOMARKERS OF DIOXIN EXPOSURE IN HUMAN URINE – A METABOLOMIC STUDY OF THE POISONING OF VICTOR YUSHCHENKO

Fabienne Jeanneret (1,2), Julien Boccard (3), Flavia Badoud (4), Olivier Sorg (1, 2), Jean-Hilaire Saurat (1, 2), Denis Hochstrasser (5) and Serge Rudaz (1, 2).

1. Swiss Centre for Applied Human Toxicology, Geneva, Switzerland; 2. University of Geneva, Geneva, Switzerland; 3. AgroParisTech, Paris, France; 4. Swiss Laboratory for Doping Analyses, Lausanne, Switzerland; 5. Geneva University Hospitals, Geneva, Switzerland.

Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) is a by-product of industrial processes involving chlorine, such as waste incineration and chemical manufacturing. Human populations are principally exposed to low levels of dioxin through animal foods and the effects of chronic exposure to dioxin are unknown. However in case of acute intoxication like for the poisoning of Victor Yushchenko in 2004, when he was candidate for the presidency of Ukraine, hepatitis, neuropathy and skin damages are just a few examples of pathologies that were observed.

Urine samples of Victor Yushchenko (TCDD 'group'), collected at different time points after the intoxication and urine of healthy volunteers (control group) were analyzed by ultra high-pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (Acquity UPLC & Xevo G2 QTOF, Waters). Multivariate analysis was carried out to distinguish the two groups (Comet, Nonlinear Dynamics & SIMCA-P, Umetrics).

First identifications highlight the drugs that were administered to treat the clinical complications of this poisoning. Previous blood and urine clinical analyses of Victor Yushchenko pointed to some abnormal steroid levels; therefore we applied a filtering strategy to identify metabolites alteration within this class of compounds. A 'steroid' filter was established to select steroid mass-related features: m/z values of known conjugated steroids were retrieved from The LIPID MAPS Lipidomics Gateway.

The resulting models revealed modified metabolic pattern of some putative steroids and bile acids after acute TCDD intoxication. Further investigations of promising biomarkers will be performed to ensure proper identification and the method will be applied to other cases of dioxin exposure for a better understanding of the acute and chronic effects of this toxic.

POSTER 86.

DOES ANTIBIOTIC IONISATION STATUS EFFECT THE MICROBIAL METABOLOME – AN INVESTIGATION IN TO *E. COLI* K12 CHALLENGED WITH TRIMETHROPRIM AT VARYING PH

Haitham AlRabiah, J. William Allwood, Elon Correa, Yun Xu and Royston Goodacre

School of Chemistry, Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester, M1 7DN

Antibiotics are among the most frequently prescribed therapeutics. They are chemical agents that can selectively kill or inhibit the growth of bacteria. In this investigation, metabolomics-based approaches were applied in order to understand the interaction of Trimethoprim, a basic dihydrofolate reductase inhibitor, with *Escherichia coli* K12 at two pH levels (5 and 7). Such an investigation will provide a greater level of insight and understanding of the antibiotic mode(s) of action and takes into consideration the ionisation status of this antimicrobial.

In preliminary investigations *E. coli* K12 was cultured in Lysogeny broth at different pH levels, and then challenged with a range of concentrations of trimethoprim to determine the most appropriate conditions for further analysis.

Fourier transform infrared (FT-IR) spectroscopy was used for preliminary metabolic fingerprinting under the different conditions (i.e., pH levels and drug concentrations). The data produced were interrogated with multivariate analysis approaches, namely, principal component analysis (PCA) as an unsupervised method and principal component-discriminant function analysis (PC-DFA) as a supervised method. Some variations were found between the conditions which were thought to reflect phenotypic effects, however further investigations applying more advanced analytical techniques were required. Samples were prepared for gas chromatography mass spectrometry (GC-MS) using 60% methanol for quenching and 80% methanol for extraction. The samples were methoxyaminated and siliyated with MSTFA prior to GC-TOF/MS profiling. The GC-MS profiles again indicated the much greater effect of the antibiotic at pH7 compared to pH5, and also showed an escalating effect with increasing concentration. The differential metabolites were mapped onto metabolic pathways and these results will be presented in this poster.

POSTER 87.

C-SOURCE UTILIZATION AND OVERFLOW METABOLISM OF *STAPHYLOCOCCUS AUREUS*

Hanna Meyer and Michael Lalk

Ernst-Moritz-Arndt-University Greifswald, Germany

Staphylococcus aureus is a wide spread facultative anaerobic Gram-positive bacterium. It is frequently found as part of the skin flora and the mucous membranes. While *S. aureus* can cause a wide range of diseases in mammalians, it is as well known to colonize healthy people. 30 % of the population is estimated to be carrier, whereby mostly the nasal passages are colonized without causing a disease. Especially if the immune system is weakened, *Staphylococcus aureus* infections can provoke a number of illnesses like minor skin infections and abscesses, pneumonia, meningitis, mastitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. During infection

Staphylococcus aureus has the possibility to infiltrate the host cell.

Hence, dependent on the current niche or life state (dormant, growing, invasive) of *S. aureus*, different nutrients are available in the environment. Nevertheless for scientific research in the laboratory, mostly complex medium like LB or chemical defined medium with glucose as predominant C-source is used, which not necessarily reflect the natural lifestyle of *S. aureus*. Accordingly in this work seven different compounded media (glucose, glucose-6-P, glycerol, lactose, lactic acid, pyruvic acid, a complete mix of all mentioned) were used to study the growth behavior and the exo-metabolome of *S. aureus* depending on the available nutrients. Furthermore three different *Staphylococcus aureus* strains (HG001, EN493, RF122) were used to consider that different strains are known to colonize different niches. Thereby this work aims to investigate the adjustment of *S. aureus* to different available C-sources by an exo-metabolomic point of view realized by ^{1}H-NMR measurements.

POSTER 88.

METABONOMIC UNVELILING OF COMPREHENSIVE METABOLIC MECHANISM AND MULTIPLE BIOMARKERS OF INFLAMMATION IN A RAT MODEL OF EXPERIMENTAL AUTOIMMUNE MYOCARDITIS

Hong Young-Shick (4), Hyeyoung Moon (1,2), Jongeun Kang (1,3), Eun-Hee Kim (1), Chaejoon Cheong (1), Eunjung Bang (4), Kiyuk Chang (5) and Kwan Soo Hong (1,2,3)

1. Division of MR Research, Korea Basic Science Institute, Cheongwon, Republic of Korea; 2. Department of Bio-analytical Science, University of Science and Technology, Daejeon, Republic of Korea; 3. Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon, Republic of Korea; 4. Seoul Branch, Korea Basic Science Institute, Seoul, Republic of Korea; 5. Department of Cardiovascular Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Myocarditis is an inflammatory heart disease with characteristic inflammatory cellular infiltration into myocardium, causing many events in myocardium from cardiac inflammation to cellular dysfunction. However, comprehensive metabolic characterization of host systemic response against myocarditis has not been established. Myosin immunization was used to induce experimental autoimmune myocarditis (EAM) in rats. We have characterized holistic response of plasma and urinary metabolome in EAM rats to progression of myocarditis through metabonomics approach. Large elevation of fatty acid -oxidation were found at acute phase of EAM. Cellular anaerobic glycolysis at subacute phase was noted by marked depletion of plasma glucose and large accumulation of plasma lactate. Chronic phase was characterized mainly by accumulation of plasma glucose. In particular, depletions of fatty acid and citrate and accumulations of acetyl glycoproteins and albumin lysyl group in plasma or urine of EAM rats demonstrate still large energy demand and involvement of myocardial inflammation until the chronic phase, which are distinct from cardiac ischemia. This study highlights simultaneous observations of global and distinct metabolic perturbations at different stages of myocarditis by using metabonomics.

POSTER 89.

A METABOLIC PROFILING APPROACH TO INVESTIGATE AQUATIC RHIZOSPHERE RESPONSES TO MICROPOLLUTANTS IN URBAN FRESH WATER SYSTEMS

Kalyan Mynampati (1), A. Wijdeveld (2), S. Reuben (3), S. Swarup (4) and L. Samavedham (5)

1. Singapore-Delft Water Alliance, National University of Singapore, 117577, Singapore; 2. Deltares, P.O. Box 177, 2600 MH Delft, The Netherlands; 3. Singapore-Delft Water Alliance, National University of Singapore, 117577, Singapore; 4. Department of Biological Sciences, National University of Singapore, 117543, Singapore; 5. Department of Chemical and Biomolecular Engineering, National University of Singapore, 117576, Singapore

Reservoirs in urban areas receive rainwater runoff from different land use types, including construction sites, industrial warehouses, parks/gardens, commercial, and residential areas. The runoff contains organic compounds such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals such as Lead. These micropollutants induce chemical stress on the biota, including plants and microbial organisms.

We developed a metabolic profiling approach to investigate the chemical stress experienced by plants and microbes around the root zone (rhizosphere) in fresh water systems. A novel bioreactor was designed for direct and non-destructive extraction of metabolites released in the rhizosphere. This device, called RhizoFlowCell, facilitates in situ filtration and extraction of root exudates with minimal recovery loss for metabolomics studies. The resultant metabolic profiles, from high throughput UPLC-TOF system, were analyzed using MetDAT, a Metabolomics Data Analysis Tool [1,2].

The metabolic profiles from the dose-time experiments indicate that when plant rhizosphere was exposed to high concentrations of toxicant for a short interval, the stress response was immediate and it took more than 24hrs to adapt to a different metabolic state. In the early time points, the difference in the metabolomic response between stressed and non-stressed rhizosphere was approximately 80% in terms of metabolites detected. After 24hrs, only 10% of the metabolites were found to be dissimilar between the two root zones. Similar metabolomic response was observed in the rhizosphere to both organic as well as heavy metal stress. Multivariate regression and clustering were applied to discern the collinear salient features that account for similarities and differences in the metabolomic response between the dose-time groups. The results were used to develop a pattern of root exudation responses in aquatic plants undergoing toxicant exposure.

[1] Biswas et.al., Bioinformatics, 26(20):2639-2640; [2] Biswas et. al. Metabomeeting 2009.

POSTER 90.

GLOBAL URINE METABOLOME ANALYSIS – A COMPARISON OF PREPARATION TECHNIQUES

Andrew Chetwynd, A. Abdul-Sada and E. M Hill

School of Life Sciences, University of Sussex, Falmer, Brighton UK BN1 9QG

Non-targeted urine metabolomic analysis is a quick, non-invasive technique for detection of biomarkers of disease, toxic exposure and nutritional status. To analyse as much of the urinary metabolome as possible, a reliable and sensitive analytical method needs to be implemented. This method must overcome the inherent problem of ion suppression due

to the high salt level of urine, while allowing for the detection and quantification of the least abundant metabolites. For this study we used composite urine samples prepared from the first void collected over ten days from two healthy volunteers (1 male, 1 female). Urine samples were prepared as neat, or diluted (with water or methanol) preparation, and also as SPE concentrates using polymeric (HLB) and ion exchange cartridges. The different preparations were analysed using a UPLC-QTOF platform, with both traditional C18 and HILIC columns in positive and negative ESI modes. The number of metabolites was quantified using appropriate blanks to remove any background ions. The results indicated that HLB SPE concentration of urine extracts resulted in detection of significantly more metabolites compare with diluted and neat urine preparations. Principal component analysis of the datasets revealed that SPE treatment was necessary to detect low abundant compounds including many sex specific metabolites such as steroidal conjugates which were not detected in neat and diluted urine extracts. These results indicate that the incorporation of an SPE step significantly increases the number of metabolites detected in urine samples and is necessary to obtain the most complete profile of the urinary metabolome, thus increasing the efficiency of screening for biomarkers of disease, toxic exposure or nutrition.

POSTER 91.

A COMPARISON OF DIFFERENT APPROACHES FOR THE IDENTIFICATION AND CLASSIFICATION OF *BACILLUS* SPORES AND SPECIES BASED ON METABOLOMICS AND SPECTROSCOPIC DATA

Piotr Gromski (1), Elon Correa (1), Andrew A. Vaughan (1), Michael L. Turner (2) and Royston Goodacre (1)

1. Manchester Institute of Biotechnology, School of Chemistry, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK; 2. School of Chemistry, University of Manchester, Manchester, M13 9PL, UK.

In this study we used different approaches for the identification of *Bacillus* spores and classification of *Bacillus* species as alternatives to the genetic algorithm-Bayesian network (GA-BN) approach previously used for the analysis of metabolomics data [1]. The comparison is based upon on the classification accuracy of several methods. We used random forest (RF) to establish variable importance and reduce dimensionality, then for selected variables we applied several methods to compare the prediction accuracy of partial least square discriminant analysis (PLS-DA), linear discriminant analysis (LDA) and support vector machines (SVM) with different kernels, which we proposed as alternatives to the GA- BN algorithm. We show that the classification accuracy for physiological states (vegetative/spores) for all used techniques is >95 % for selected variables, whereas for all variables the PLS-DA accuracy prediction is only 50 %. For the classification of species we observed significant results with the exception of PLS-DA and SVM with polynomial and sigmoid kernels. We show that by applying a combination of random forest for variable

selection with other methods for prediction we achieve fast and accurate classification results.

[1] Correa, E. and Goodacre, R. (2011). BMC Bioinformatics, 12.

POSTER 92.

A SNAPSHOT OF CORRELATIONS BETWEEN METABOLITES AND THE PREDICTION OF SAMPLE IDENTITY VIA BAYESIAN NETWORKS

Elon Correa and Royston Goodacre

Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

Biochemical correlation between metabolites is an intrinsic phenomenon of metabolic networks. Most of the traditional analytical techniques used in metabolomics, such as Gas chromatography-mass spectrometry (GC-MS) and Liquid chromatography-mass spectrometry (LC-MS), produce data that quantify metabolites but do not explicitly preserve any information about how those metabolites correlate to each other. An accurate mapping of such correlations and the strength at which they occur can provide valuable information for both a clear interpretation of the network and the prediction of expected metabolite levels. Retrieving such information directly from the data is difficult and requires the application of specialized statistical and data mining techniques.

This work proposes the use of Bayesian networks (BN) to statistically represent the relationships between measured metabolites and to quantify the strength of these correlations. Based on the data provided, the BN model builds a graphical network of the relationships between metabolites. This BN framework offers not only a simple way to visualize correlations present in the metabolic network under study, but also a classification model capable of grouping similar samples and predicting expected metabolite levels based on the observed data and the network only. Using concrete metabolomics datasets we demonstrate the capabilities of a BN model to produce a snapshot of correlations between metabolites and the ability of the resulting model to correctly identify unknown samples.

POSTER 93.

GAMMA-AMINOBUTYRIC ACID (GABA) IN PLANTS – A SIGNALING MOLECULE OR 'JUST' A METABOLITE

Albert Batushansky (1), Menny Kirma (2), David Toubiana (1), Nicole Grillich (3), Ilse Balbo (3), Alisdair R. Fernie (3), Gad Galili (2) and Aaron Fait (1)

1. Ben-Gurion University of the Negev, Jacob Blaustein Institute for Desert Research, French Associates Institute for Agriculture & Biotechnology of Drylands; 2. Department of Plant Science, Weizmann Institute of Science; 3. Max Planck Institute of Molecular Plant Physiology

Gamma-Aminobutyric acid (GABA) has been isolated from potato more than half a century ago, but its role in plants remains unclear. Numerous observations of GABA accumulation in plants under various types of stress and similarity in metabolic pathways between plant and animal cell where major function of GABA is inhibition of signal transduction led to hypothesis hypothesize about signaling role of GABA in plants.

To investigate this hypothesis we used Arabidopsis thaliana (ws) wild type and GABA transporter-1 (gat1) knockout mutant, which was suggested as regulating GABA influx to the cell. GC-MS based metabolic profiling and microarray analysis of seedlings grown

without exogenous GABA and on 1mM exogenous GABA under full nutrient medium and under C and N starvation. Next, the results of metabolome and transcriptome profiling have been integrated according to the biological question.

The results of the analysis showed large differences in metabolism and gene expression across different media in both control and mutant plants. Differences in GABA concentrations mostly affected ws plants suggesting that knockout mutation in GAT1 protein partially blocks a signal transduction in respect to C-N metabolism. Under C starvation in wild type a significant decrease in the relative amount of TCA cycle and shikimate pathway components caused by external GABA was observed. Under N starvation the effect of exogenous GABA was minor compared to that under C starvation. The analysis of transcriptome of ws plants under C starvation revealed a significant effect of exogenous GABA on the gene expression of specific catabolic processes of Phe and phenylpropanoids biosynthesis, while effect of GABA under C starvation on gat1 genotype was wider and general.

POSTER 94.

APPLICATION OF METABOLOMICS TO THE SU.VI.MAX2 COHORT FOR DISCOVERY OF BIOMARKERS OF COFFEE INTAKE

Yohann Fillâtre (1), J.A. Rothwell (1), M. Touvier (2), B. Lyan (1), J-F. Martin (1), S. Gueho (2), L. Fezeu (2), N. Arnault (2), J-L. Sébédio (1), B. Compte (1), S. Hercberg (2), E. Pujos-Guillot (1), P. Galan (2) and C. Manach (1)

1. Human Nutrition Unit, UMR 1019 INRA / University of Auvergne, Clermont-Ferrand, France; 2. Unité Recherche Epidémiologie Nutritionnelle, INSERM U557 / INRA / CNAM / PARIS 13, Bobigny, France

The 'food metabolome' is the subset of the human metabolome originating from the digestion of food components. As part of the ANR PhenoMeNEp project, non-targeted profiling is currently being used to identify and validate potential biomarkers of plant food consumption. Using 24 hour dietary recall and food frequency questionnaire data, 66 high (median 974 grams/day) and 144 low (median 305 grams/day) consumers of fruit and vegetables were selected from the French SU.VI.MAX2 cohort. Morning spot urine samples from each subject were analyzed by QTOF and LTQ-orbitrap mass spectrometry. The consumption data available from the SUVI.MAX.2 cohort allows the comparison of low and high consumers of specific foods of interest. Coffee, for instance, is one of the most widely consumed beverages in the West and contains various bioactives implicated with health and the prevention of disease. From the SU.VI.MAX pool, the profiles of 25 high coffee consumers (median 180 ml coffee/day) and 20 low coffee consumers (median 0 ml coffee/day) were compared. Metabolomic profiling and partial least squares discriminant analysis (PLS-DA) after orthogonal signal correction filtration (OSC) clearly distinguished low and high coffee consumption and revealed a number of discriminant ions. Some of the most significant discriminants, both by PLS-DA and ANOVA, were found to be metabolites of caffeine, although the non-caffeine derived discriminants observed may be more specific to coffee consumption and are thus may better reflect coffee consumption. The use of samples from cohort study subjects should lead to the discovery of more reliable biomarkers of food consumption than those from intervention study subjects, since biomarkers found this way are not only valid

for acute consumption of the food shortly before biofluids are taken. Robust biomarkers of food consumption are potential replacements for self-reported traditional dietary assessment methods, which are subject to considerable bias.

POSTER 95.

THE PHYTOMETABOBANK DATABASE – A NEW RESOURCE FOR THE IDENTIFICATION OF PHYTOCHEMICAL METABOLITES IN FOOD METABOLOMICS

Joseph Rothwell (1), Y. Fillatre (1), D. Cesaire (1), M. Quintana (1), C. Knox (2), J-L Sébédio (1), B. Comte (1), E. Pujos-Guillot (1), C. Manach (1) and F. Giacomoni (1)

1. Human Nutrition Unit, UMR 1019 INRA / University of Auvergne, Clermont-Ferrand, France; 2. In Siliflo Inc, Edmonton, AB T5M 1K2

The 'food metabolome' comprises all metabolites present in biological fluids that are directly derived from the digestion of food. A large proportion of the food metabolome consists of phytochemical metabolites, which are products of intestinal and hepatic or microbial metabolism of molecules such as polyphenols, terpenoids and alkaloids. Identification of unknowns in metabolome fingerprints is a laborious step-by-step process and often a bottleneck in biomarker discovery. One major limitation for the interpretation of the food metabolome fingerprints is the incompleteness of existing databases with regard to phytochemical metabolites. As part of the ANR PhenoMeNep project, we aim to construct a new database tailored to the study of the phytochemical component of the food metabolome. Provisionally named PhytoMetaboBank, the database will be an inventory of known metabolites described in the literature for all dietary phytochemicals. Built with MySQL and Perl processing chains, an efficient relational design will underpin a powerful and intuitive web interface. For a queried monoisotopic mass or elemental formula, the database will return a list of possible metabolites, with their physicochemical properties, spectral data and possible dietary precursors linked to food sources. PhytoMetaboBank will be the first database to collate information on phytochemical metabolites from a metabolomics standpoint, and should improve the identification of discriminant ions in non-targeted profiling.

POSTER 96.

METABOLIC STUDIES OF LEAD TOXICITY AND TOLERANCE IN GREEN MICROALGAE

Wenlin Zhang and Li Fong Yau Sam

Department of Chemistry, National University of Singapore, Science Drive 3, Singapore 117543

Lead (Pb) is a biologically non-essential element for plant metabolism and exhibit varied degrees of phytotoxicity including adverse effects to photosynthesis [1]. Although Pb is toxic to most plant species even at low concentration, several metal-tolerant plants have the capability to accumulate large amount of Pb in contaminated soil and water [2]. Algae have been widely reported for its incredible metal biosorption capability, with most studies focusing on the elucidation of mechanisms active in metal biosorption using dried algal biomass [3]. In an attempt to understand the complete metal detoxification mechanism of algae, we are investigating the metabolic response of green microalgae exposed to various concentrations of Pb. This poster presents results on the uptake and accumulation of Pb in *Chlorella vulgaris* by inductively coupled plasma mass spectroscopy (ICP-MS), and gives an overview

of the Pb-detoxification mechanism, Pb-induced oxidative damage and antioxidative defense in *C. vulgaris* by nuclear magnetic resonance (NMR)-based metabolomics. Elemental analysis of algal medium and biomass showed that *C. vulgaris* displays high Pb-removal efficiency (> 70%), and concentration-dependent Pb-accumulation capability (BCF > 1000). The high bioconcentration factor (BCF) can be interpreted as Pb being favourably taken up by *C. vulgaris* from the growth medium. This observation coincided with current knowledge of Pb-detoxification mechanism by phytochelatins, where *C. vulgaris* responds to Pb exposure through the chelation of metals ions by this key class of chelators, and thus improved the Pb-accumulation capability in *C. vulgaris*. NMR-based metabolomics of *C. vulgaris* exposed to various concentration of Pb revealed the net changes in biochemical response between sample groups. Based on principal component analysis (PCA) of ^{1}H spectra from C. vulgaris biomass, the Pb-dosed and control groups were significantly differentiated. The changes that influence the discrimination between the sample groups were in the concentrations of lipids, surcose, betaine and several amino acids such as glutamate, lysine and arginine, which were substantially reduced in the Pb-dosed group as compared to the control group. We infer from the metabolic changes that excess Pb in *C. vulgaris* led to the formation of reactive oxygen species (ROS), which initiated lipid peroxidation and altered cell biochemical activities. In response to the oxidative stress, antioxidants (glutathione) and phytochelatins were upregulated to remove ROS and metal ions in the plant cells respectively. Significant reduction of glutathione and phytochelatin precursor (glutamate) demonstrated the Pb-detoxification and antioxidative defense mechanism of *C. vulgaris* in the presence of Pb.

[1] Tang, Y.T. *et al.* Franch. Enviro. Exp. Bot. 66, 126-134, 2009; [2] Rascio, N. & Navari-Izzo, F. Plant Science, 180, 160-181, 2011; [3] Davis, T.A., *et al.* Water Research, 37, 4311-4330, 2003.

POSTER 97.

INVESTIGATING THE GROWTH PATTERN OF GEOBACTER SULFURREDUCENS DURING BATCH CULTURE SCALE-UP USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

Howbeer Muhamadali, J William Allwood, Royston Goodacre, Jonathan R. Lloyd

The University of Manchester, Manchester, M13 9PL

The aim of this study was to investigate the effects of medium scale-up on the growth pattern of *G.sulfurreducens* and distinguish the different stages of growth using Fourier transform infrared (FT-IR) spectroscopy. FT-IR can be said to provide several advantages including minimal sample preparation, rapid high-throughput analysis, relatively simple to operate, generation of qualitative and quantitative data, and is able to analyse a range of samples in different formats (live cells, powder, liquid/gels, and bacterial suspensions). FT-IR has an established record of microbiological applications such as identification, discrimination, detection, and classification of a wide-range of bacteria and fungi. During the past decade, FT-IR has also been applied as a rapid identification and diagnostic tool for different diseases and clinical states. For this study, *G. sulfurreducens* cells were grown in 100 mL flasks and a 5 L bioreactor and multiple samples analysed at different time points by turbidimetric, FT-IR, and mass spectrometry techniques. In order to determine growth phase and potentially identify the critical biochemical differences between cells. Turbidimetric results of Geobacter cells grown

in the bioreactor showed a prolonged lag phase compared to the cells grown in flasks. FT-IR spectra were analysed using principal component analysis (PCA) and principal component-discriminant function analysis (PC-DFA). Results were in agreement with the turbidimetric findings and revealed the same prolonged lag phase for cells grown in the bioreactor. In addition, it was observed that FT-IR was able to distinguish between different stages of growth and demonstrate the growth trend for both sets of samples (flasks and bioreactor). The findings of this study further demonstrate the potential applications of FT-IR as a rapid, robust and reliable high-throughput screening tool for increasing our understanding of the biochemical composition of the bacterial cells, and their physiology and behaviour, in different batch culture systems during all stages of growth.

POSTER 98.

A NEW APPROACH TO UNTARGETED ANALYSIS OF HIGH RESOLUTION LC-MS DATA

Frans van der Kloet

Division of Analytical Biosciences, Leiden Amsterdam Center for Drug Research/Netherlands Metabolomics Centre, Leiden University, P.O. Box 9502, Einsteinweg 55, 2300 RA Leiden, NL

Because of its high sensitivity and specificity hyphenated mass spectrometry has become the predominant method to detect and quantify the metabolites present in bio-samples relevant for all sorts of life science studies being executed. Global profiling acquisition methods allow new metabolites to be analyzed at the expense of sensitivity. In case of these untargeted approaches, i.e. without a list of target metabolites, the vendor integration software cannot be used. We propose a new algorithm that enables untargeted integration of samples that are measured with high resolution liquid chromatography mass spectrometry (LC-MS). In addition a strategy is proposed that reduces the large amount of features extracted from each sample to a smaller list of feature-sets representative for all samples. Furthermore, these feature-sets allow for easier interpretation and identification. We show that the automatic obtained integration results for a set of known target metabolites match those generated with vendor software but that many more feature-sets are extracted as well. We demonstrate our approach using high resolution LC-MS data acquired for 151 samples on a lipidomics platform. The data was also integrated (with a combination of automatic and manual integration) using vendor software for a set of over 150 targets. The untargeted extraction procedure is run per sample and per mass trace which makes the implementation of it scalable. Because of the generic approach, we envision this data extraction method to be used in a targeted as well as untargeted analysis of LC-MS data.

POSTER 99.

ACUTE CONSUMPTION OF FLAVAN-3-OL-ENRICHED DARK CHOCOLATE AFFECTS HUMAN ENDOGENOUS METABOLISM

Gwen Le Gall (2), Luisa M. Ostertag (1), Mark Philo (2), Henri Tapp (2), Ian J. Colquhoun (2), Shikha Saha (2), Garry G. Duthie (1), Kate Kemsley (1), Baukje de Roos (1) and Paul A. Kroon (2)

1. University of Aberdeen, Rowett Institute of Nutrition & Health, Aberdeen, UK; 2. Institute of Food Research, Norwich, UK

Cardiovascular disease (CVD) is a primary cause of premature deaths worldwide, with incidence rates in the United Kingdom being

amongst the highest in the world. Consumption of dietary polyphenols, secondary plant metabolites that are ubiquitously present in plant-derived foodstuffs and beverages, has been linked to improved cardiovascular health in humans. Plant-derived substances such as flavan-3-ols may beneficially affect atherosclerosis and impact on cardiovascular risk, but information on their bioavailability is limited.

We performed a randomised controlled cross-over intervention trial to assess the acute effects of consumption of dark chocolate enriched in flavan-3-ols and procyanidins, compared with standard dark chocolate and white chocolate. NMR and MS-based metabolomics were used to profile urine and blood plasma samples collected at time 0, 2 and 6 hours post intake for each of 42 healthy volunteers.

Multivariate (MV) statistics could readily separate the different time points. MV and univariate statistics showed that the largest differences between pre and post intake urines were due to exogenous metabolites originating from the chocolate intake (epicatechin derivatives, methylxanthines and microbial breakdown products). Interestingly a proportion of the variance was also associated with changes in the levels of endogenous compounds such as N1-methylnicotinamide, creatinine, lactate, and some amino acids. Urinary levels of these metabolites decreased 6 h after intake of both dark chocolates compared to white chocolate and all followed the same trend: mean level in flavan-3-ol-enriched dark chocolate < dark chocolate < white chocolate.

These results demonstrate the power of untargeted NMR-based metabolite profiling to reveal the modulation of human metabolism following a controlled dietary challenge.

POSTER 100.

MS^N SPECTRAL TREE DATABASES: ANNOTATION AND IDENTIFICATION OF PLANT METABOLITES

Justin J.J. van der Hooft (1,2,3), Miguel Rojas-Cherto (3), Thomas Hankemeier (3), Michael van Vliet (3), Theo Reijmers (3), Robert D. Hall (1,2,3) and Ric C.H. de Vos (1,2,3)

1. Plant Research International, Wageningen-UR, Wageningen, The Netherlands; 2. Centre for BioSystems Genomics, Wageningen, The Netherlands; 3. Netherlands Metabolomics Centre, Leiden, The Netherlands

Annotation and identification of metabolites detected in untargeted liquid chromatography coupled to mass spectrometry (LC-MS) profiling techniques is still a major challenge that needs to be overcome in order to interpret the results obtained in a biological context. Protocols for generating MS^n and LC-MS^n data from metabolites detectable in plants, including Arabidopsis leaves and tomato fruit, have recently been developed, resulting in robust spectral trees [1,2]. Meanwhile, a software tool called MEF has been developed to process the MS^n raw data and assign elemental formulas to the detected metabolite MS^n signals [3]. Very recently, a web-based program called MetiTree, embedding the MEF tool and algorithms to visualize and compare spectral trees [4] has become available [5].

Here we introduce the use of high mass resolution MSn data, generated from plant metabolites through Ion trap-Orbitrap Fourier transformed MS fragmentation, as input for a plant spectral tree database that can be populated, visualized, and queried for spectral matching. Spectral trees were obtained from a series of 121 reference flavonoids, thereby using a NanoMate robot for direct infusion of small sample volumes from 96 wells plates

into the MS, i.e., offline fragmentation [2]. In order to generate spectral trees of metabolites from plant extracts, LC-MS fractionation into 384 wells plates was performed with the NanoMate, after which in-depth MS^n fragmentation data was recorded offline [1]. As proof of principle, a spectral tree database was created from (phenolic) diterpenes and diterpene-like structures from *Rosmarinus officinalis trichomes*. Based on spectral tree similarities with the known phenolic diterpenes rosmanol and carnosol yet unknown diterpene compounds could be annotated.

These preliminary results indicate the potential for MS^n spectral trees in combination with the MetiTree software in spectral tree database generation, visualization, and comparison. Future work includes both optimization of the tree processing parameters and fragment structure annotation. Furthermore, we will expand the spectral tree database by populating with compounds from plant species like Arabidopsis thaliana and tomato, to facilitate metabolite annotation and structural elucidation of secondary metabolites.

[1] Van der Hooft, J. J. J., *et al.* (2012). Metabolomics 8(4): 691-703; [2] Van der Hooft, J. J. J., *et al.* (2011). Anal. Chem. 83(1): 409-416; [3] Rojas-Chertó, M., *et al.* (2011). Bioinformatics 27(17): 2376-2383; [4] Rojas-Cherto, M., *et al.* (2012). Anal. Chem. Article ASAP. DOI:10.1021/ac2034216; [5] Rojas-Chertó, M., *et al.* (2012). Bioinformatics.

List Of Posters By Presenting Author

Poster	Poster Title	First name	Last Name
32	Absorption, distribution, metabolism, and elimination of dietary benzoxazinoids in rats	Khem	Adhikari
75	Statistical Analysis of Combined Metabolomics Datasets from the Potato Tuber Life Cycle	Colin	Alexander
86	Does antibiotic ionisation status effect the microbial metabolome: an investigation into *E. coli K12* challenged with Trimethroprim at varying pH.	Haitham	AlRabiah
69	Time-of-day variation in human plasma metabolites using an untargeted liquid chromatography-mass spectrometry metabolomic approach	Joo Ern	Ang
77	^{1}H NMR Spectroscopy and GC/MS analysis for a metabolomic study of preterm human and formula milk	Luigi	Atzori
80	^{1}H NMR Spectroscopy as a New Tool for Body Fluid Identification in Forensic Medicine	Luigi	Atzori
62	MS-based metabolomic strategy for the screening of new induced metabolites in miniaturized fungal co-cultures	Antonio	Azzollini
6	The impact of high-resolution MS and NMR techniques on the discovery of novel natural products from myxobacteria	Aiko	Barsch
7	LC-Q-TOF based metabolic profiling of dansylated metabolite to study yeast arginine biosynthesis deletion mutants	Aiko	Barsch
41	Metabolomic characterization of SOD1 null mutant flies using liquid chromatography/mass spectrometry	Aiko	Barsch
93	Gamma-Aminobutyric Acid (GABA) in plants: a signaling molecule or 'just' a metabolite?	Albert	Batushansky
27	Metabolomic Profiling of Water Supply Network Biofilms to Inform Asset Management Decisions	David	Beale
81	Evaluation of a Folch-style extraction method for untargeted metabolomic studies of mammalian tissues	Paul	Begley
60	Exposure of mice to ionization radiation induces specific metabolomic biosignatures	Marc	Breit
43	Effects of age and probiotic supplementation on the mouse metabolome: a look into the homeostenosis window	Riccardo	Calvani
5	Effects of Bisphenol-A (BPA) on HepG2 cells: an automated untargeted NMR-based metabonomics study	Cécile	Canlet
24	Metabolic response to glycerophosphocholine phosphodiesterase GDPD5 silencing in human breast cancer cells	Maria	Cao

48	Rational isolation strategy of plant biomarkers at the mg scale based on chromatography modelling, MPLC-ELSD and fast UHPLC-TOF-MS monitoring	S.	Challal
90	Global urine metabolome analysis: A comparison of preparation techniques	Andrew	Chetwynd
36	mzMatch-ISO: an extension of mzMatch for the identification and relative quantification of ^{13}C labelled mass spectrometry data	Achuthanunni	Chokkathukalam,
30	Use of multivariate analysis for selection of diagnostic markers in urine in neonates with 21-hydroxylase deficiency	Sofia	Christakoudi
11	Data independent MALDI Imaging ion mobility acquisition for the visualisation and identification of lipids directly from a single tissue section	Emmanuelle	Claude
70	Metabolic effects of milk protein intake	Morten Rahr	Clausen
92	A snapshot of correlations between metabolites and the prediction of sample identity via Bayesian networks	Elon	Correa
34	Metabolomics: a powerful tool for investigating the relationship between food and health?	Frédérique	Courant,
52	Metabolomics approachs: a powerful tool for the screening of trenbolone acetate/estradiol combinations in cattle	Cristina	da Costa Jacob
68	The effect of food pulses on metabolites that exhibit significant time-of-day variation	Sarah	Davies
59	Familial resemblance for 120 serum metabolites	Harmen	Draisma
1	The Husermet Project: developments to allow large-scale epidemiological and metabolomics studies of the human population	Warwick	Dunn
57	Development of strategies for integrated full-scan profiling and data dependent MS/MS applying CID and HCD on hybrid Orbitrap mass spectrometers	Warwick	Dunn
94	Application of metabolomics to the SU.VI.MAX2 cohort for discovery of biomarkers of coffee intake	Yohann	Fillâtre
14	Software for Pathway Informed Metabolomics	Steven	Fischer
15	NMR based metabolomics study of a cerebral ischemia model: a practical look into the metabolic profiling pipeline of small tissue amounts	Edoardo	Gaude
22	Metabolic changes in indolent and aggressive prostate cancer- correlations with the Gleason grading system	Guro	Giskeødegård
19	Mass spectrometry and shotgun metabolomics in Alzheimer's disease diagnosis	Jose´ Luis	Gómez-Ariza

91	A comparison of different approaches for the identification and classification of Bacillus spores and species based on metabolomics and spectroscopic data.	Piotr	Gromski
49	Non targeted LC-MS metabolite profiling highlights the between- tissue differences caused by high-fat diet in pig model	Kati	Hanhineva,
37	In vitro and in vivo metabolite identification of *Argemone mexicana L.*	Amina	Haouala
63	Quantification of urinary betaine and choline excretion in rats fed fractions of rye and wheat	Mette Skou	Hedemann
61	Investigating the systemic and gut-specific mouse metabolome by non-targeted metabolite profiling	Silke	Heinzmann
74	The development and validation of a targeted LC-MS/MS method for the detection and quantification of Heparan Sulphate saccharides.	Katherine	Hollywood
35	MzMatch/mzMatch.R: a prioritisation pipeline for the analysis of metabolomics datasets	Andris	Jankevics
85	Biomarkers of Dioxin Exposure in Human Urine: a Metabolomic Study of the Poisoning of Victor Yushchenko	Fabienne	Jeanneret
3	High-throughput Mass Spectrometry based Method for the Quantitative Targeted Metabolomics	Vasudev	Kantae
84	Evaluation of Extraction Methods for Whole Blood Samples	Anna	Kauppi
64	Absolute metabolite concentrations and metabolic changes associated with oxidative stress and different stages of cell growth in *Trypanosoma brucei*	Dony-Hyun	Kim
33	Comparative metabolic profiling of staphylococcus aureus using LC-QToFMS and GCxGC-ToFMS for potential biomarker identification	Konstantinos	Kouremenos
46	Metabolomic Investigation of a Simulated Combat Trauma Injury in a Porcine Model using ^{1}H Nuclear Magnetic Resonance Spectroscopy	Anna	Laserna
99	Acute consumption of flavan-3-ol-enriched dark chocolate affects human endogenous metabolism	Gwen	Le Gall
82	Changes in grape phenylpropanoid composition induced by leaf removal in two 'Pinot noir' vineyards	Melita Sternad	Lemut
79	Systems Biology Analysis of the Effects of Acetaminophen Treatment on Energy Metabolism Pathways	Lisette	Leonhardt
51	From whole-organism to sub-cellular localization of metabolites by MALDI FTICR MS and TOF-SIMS imaging, approaches in an invertebrate	Manuel	Liebeke
55	Multivariate methods in metabolomics: from pre-processing to dimension reduction and statistical analysis	Kristian Hovde	Liland

20	Phase II sulphated and glucuronidated bioactive phenolics are potential urinary biomarkers for habitual exposure to wholegrain rye bread	Amanda	Lloyd
25	Metabolic profiling of steroidogenesis in the human H295R adrenocortical cell line for detection of endocrine disruptors	Arjen	Lommen
21	HR-MAS NMR metabonomics for the classification of MEN1 variants	Claire	Lopez
66	Metabolomics in the diabetes research	Marianna	Lucio
50	^{1}H NMR-based Metabolic Profiling of Blood Plasma and Milk in Dairy Cows Reveals Physiological Mechanisms Underlying Economically Relevant Traits	Anthony	Maher
17	NMR spectrometers as 'magnetic tongues': prediction of sensory descriptors in canned tomatoes.	Anders	Malmendal
72	What the small molecules in senile flies can tell us about Alzheimer's disease-NMR metabolomics of Aß-expressing Drosophila heads.	Anders	Malmendal
16	Metabolomics of Intact Tissues: Discrimination Between Different Regions of Osteolytic Lesions in a Multiple Myeloma Patient using ^{1}H HRMAS NMR spectra	Silvia	Mari
73	Lipidomic profiling of dried serum spots (DSS)	Rebeca	Mayo
38	Exploring the metabolism of *Streptomyces coelicolor* using mass spectrometry-based metabolomics	Maria Elena	Merlo
87	C-source utilization and overflow metabolism of *Staphylococcus aureus*	Hanna	Meyer
10	Metabolomics Reveals the Metabolic Effects of Whole Grain Rye Products on Humans	Ali	Moazzami
97	Investigating the growth pattern of *Geobacter sulfurreducens* during batch culture scale-up using Fourier transform infrared spectroscopy	Howbeer	Muhamadali
89	A Metabolic Profiling approach to investigate Aquatic Rhizosphere responses to Micropollutants in Urban fresh water systems	Kalyan	Mynampati
67	A comprehensive metabolomic characterization of typhoid fever in human serum using a multi-platform approach	Elin	Näsström
39	Applying Q Exactive Benchtop Orbitrap LC-MS/MS and SIEVE 2.0 Software for Cutting-Edge Metabolomics and Lipidomics Research	Madalina	Oppermann
40	Related Seasonal and Geographical Differences in Wine from California's Central Coast	Madalina	Oppermann
65	Metabonomic investigation of the effect of allopurinol administration in plasma of exercising rats.	Alexandros	Pechlivanis

13	The metabolic response of Arabidopsis to co-cultivation with white clover (*Trifolium repens L.*) suggests a herbicide-like mode of action	Hans Albert	Pedersen
29	Allelopathic effects of biochanin A on selected weed species	Hans Albert	Pedersen
42	An 1H NMR-based metabolomic approach reveals significant changes in breast milk composition induced by oral probiotic supplementation.	Giulia	Pratico'
44	Netherlands Metabolomics Centre - Data Support Platform	Theo	Reijmers
45	An Automated Metabolite Identification Pipeline Using Mass Spectral Trees	Theo	Reijmers
71	A new approach to assess repeatability and reproducibility of a GC-MS based metabolomics data matrix	Carolus	Reinecke
95	The PhytoMetaboBank database: a new resource for the identification of phytochemical metabolites in food metabolomics	Joseph	Rothwell
26	From targeted analysis to steroidomics	Serge	Rudaz
47	Dissecting the Mechanism of Antifungal Drug Action by GC/Q-TOF	Ashley	Sage
18	Spanning the bridge from systems biology to classical science: NMR metabonomic investigation of phosphine resistance in *Caenorhabditis elegans*	Horst Joachim	Schirra
83	An NMR-based metabonomics investigation of plasma using an ovine model of periconceptional overnutrition.	Horst	Schirra,
23	Effect of milling and cooking processes on benzoxazinoids in wheat and rye	Fariha	Tanwir
31	Metabolomics and dereplication studies of endophytic metabolites from some Egyptian medicinal plants in the search for new potential anti-cancer drugs	Ahmed	Tawfike
8	Improved method for characterization of phenolic compounds from rosemary extracts using ESI-Qq-TOF MS	Verena	Tellström
9	Complementary GC-EI-MS and GC-APCI-TOF-MS analysis of a short-lived mitochondrial knockdown of *Caenorhabditis elegans* to study Metabolic changes during aging	Verena	Tellström
4	Analysis of longitudinal metabolomic data from endocrine disruption studies: the A-SCA method	Marie	Tremblay-Franco
100	MS^n spectral tree databases: annotation and identification of plant metabolites	Justin	van der Hooft
98	A new approach to untargeted analysis of high resolution LC-MS data	Frans	van der Kloet
2	The Husermet Project: advances in biological knowledge and the next steps	Andrew	Vaughan

58	Functional characterization of common obesity and diabetes risk variants by metabolic challenge tests? The use of targeted metabolomics	Simone	Wahl
53	Semiquantitative analysis of non-ribosomal synthesized lipopeptides and polyketides by UHPLC-TOF-MS to understand the mechanism causing antifungal effects of *Bacillus Amyloliquefaciens* FZB42	Jenny	Westphal
12	GC-MS profiling in industrial fermentations ? a strategy to improve medium design	Stefanie	Wiese
54	Predictable Protein Production in *Saccharomyces cerevisiae* applying metabolic flux measurements.	Catherine	Winder
56	Dynamic Flux in Central Carbon Metabolism	Catherine	Winder
78	The use of multilevel models to overcome high between-subject variability and discover the hidden pattern with applications to clinical metabolic profiling studies	Yun	Xu
28	Serum metabolomic profiling for early detection of colorectal cancer	Masaru	Yoshida
88	Metabonomic unveiling of comprehensive metabolic mechanism and multiple biomarkers of inflammation in a rat model of experimental autoimmune myocarditis	Hong	Young-Shick
96	Metabolic studies of lead toxicity and tolerance in green microalgae	Wenlin	Zhang

Delegate List

FirstName	LastName	Affiliation	Country
Khem	Adhikari	Aarhus University	Denmark
Asaph	Aharoni	Weizmann Institute of Science	Israel
Muhammad Nadeem	Akram	Institute of Biology Leiden University	Netherlands
Colin	Alexander	BioSS	United Kingdom
William	Allwood	University of Manchester	United Kingdom
Haitham	Alrabiah	University of Manchester	United Kingdom
Joo Ern	Ang	The Institute of Cancer Research	United Kingdom
Christopher	Armstrong	University of Melbourne	Australia
Luigi	Atzori	Department of Biomedical Sciences	Italy
Antonio	Azzollini	University of Geneva	Switzerland
Aiko	Barsch	Bruker Daltonik GmbH	Germany
Albert	Batushansky	Ben-Gurion University of the Negev	Israel
David	Beale	CSIRO	Australia
Richard	Beger	National Center for Toxicological Research/FDA	United States
Paul	Begley	University of Manchester	United Kingdom
Celia	Berkers	The Beatson Institute for Cancer Research	United Kingdom
Rainer	Breitling	University of Glasgow	United Kingdom
David	Broadhurst	University of Alberta	Canada
Riccardo	Calvani	Sapienza University	Italy
Cecile	Canlet	UMR 1331 TOXALIM	France
Maria Dung	Cao	Norwegian University of Science and Technology (NTNU)	Norway
Mynampati Kalyan	Chakravarthy	National University of Singapore - Singapore Delft Water Alliance	Singapore
Soura	Challal	University of Geneva	Switzerland
David	Chang	Chenomx Inc.	Canada
Andrew	Chetwynd	University of Sussex	United Kingdom
Achuthanunni	Chokkathukalam	University of Glasgow	United Kingdom
Sofia	Christakoudi	King's College London	United Kingdom
Emmanuelle	Claude	Waters Corp.	United Kingdom
Morten	Clausen	Aarhus University, Department of Food Science	Denmark
Cristian	Cojocariu	Waters	United Kingdom
Kenneth	Cook	Thermo Fisher Scientific	United Kingdom
Elon	Correa	University of Manchester	United Kingdom
Frédérique	Courant	Oniris - LABERCA	France
Darren	Creek	University of Melbourne	Australia
Elena	Cubero-Leon	IRMM	United Kingdom
Felicity	Currie	Liverpool School of Tropical Medicine	United Kingdom
Cristina	Da Costa Jacob	Oniris - LABERCA	France
Rónán	Daly	University of Glasgow	United Kingdom

FirstName	LastName	Affiliation	Country
Hannelore	Daniel	Technical University of Munich	Germany
Matthew	Davey	University of Cambridge	United Kingdom
Sarah	Davies	University of Surrey	United Kingdom
Clare	Daykin	The University of Nottingham	United Kingdom
Joanna	Denbigh	University of Manchester	United Kingdom
Harmen	Draisma	VU University Amsterdam	Netherlands
John	Draper	Aberystwyth University	United Kingdom
Warwick	Dunn	University of Manchester	United Kingdom
Alicia	Edin	Umeå University	Sweden
Ebony	Escalona	Imperial College London	United Kingdom
Lucy	Fernandes	Waters	United Kingdom
Steven	Fischer	Agilent Technologies	United States
Raul	Gonzalez-Dominguez	University of Huelva	Spain
Roy	Goodacre	University of Manchester	United Kingdom
Julian	Griffin	Medical Research Council Human Nutrition Research	United Kingdom
Piotr	Gromski	University of Manchester	United Kingdom
Robert	Hall	Plant Research International	Netherlands
Kati	Hanhineva	University of Eastern Finland	Finland
Amina	Haouala	Geneva University	Switzerland
George	Harrigan	Monsanto	United States
Kenneth	Haug	EMBL-EBI	United Kingdom
Alexander	Heazell	Maternal and Fetal Health Research Centre	United Kingdom
Mette	Hedemann	Aarhus University	Denmark
Silke	Heinzmann	Helmholtz Zentrum München	Germany
Maria	Hodges	Genome Medicine - BioMed Central	United Kingdom
Katherine	Hollywood	University of Manchester	United Kingdom
Young-Shick	Hong	Korea Basic Science Institute (KBSI)	South Korea
Yingying	Huang	Thermo Fisher Scientific	USA
Charlotte	Hulme	University of Manchester	United Kingdom
Andris	Jankevics	University of Groningen	Netherlands
Jeroen	Jansen	Raboud University	Netherlands
Vasudev	Kantae	Institute for Molecular Medicine Finland (FIMM)	Finland
Anna	Kauppi	Umeå University	Sweden
Lorraine	Kay	LECO	United Kingdom
John	Kelly	Bruker UK Limited	United Kingdom
Alec	Kettle	LECO	United Kingdom
Dong-Hyun	Kim	University of Glasgow	United Kingdom
Konstantinos	Kouremenos	Umeå University	Sweden
Rebecca	Krisher	National Foundation for Fertility Research	United States
Carsten	Kuhl	Leibniz Institute of Plant Biochemistry	Germany
Gavin	Laing	Liverpool School of Tropical Medicine	United Kingdom
Anna Karen	Laserna	National University of Singapore	Singapore
Gwenaelle	Le Gall	Institute of Food Research	United Kingdom

FirstName	LastName	Affiliation	Country
Lisette	Leonhardt	Scientist Data Interpretation Health	Germany
Kristian	Liland	Nofima	Norway
Amanda	Lloyd	Aberystwyth University	United Kingdom
Nicholas	Lockyer	Manchester Institute of Biotechnology	United Kingdom
Arjen	Lommen	RIKILT	Netherlands
Ruey-Leng	Loo	Medway School of Pharmacy	United Kingdom
Claire	Lopez	CRMN	France
Marianna	Lucio	Helmholtz Zentrum Muenchen	Germany
Anders	Malmendal	University of Copenhagen	Denmark
Silvia	Mari	S. Raffaele Scientific Institute	Italy
Rebeca	Mayo	OWL	Spain
Maria Elena	Merlo	Microbial Physiology group, University of Groningen	Netherlands
Hanna	Meyer	Uni-Greifswald	Germany
Filippos	Michopoulos	Aristotle University of Thessaloniki	Greece
Murad	Mubaraki	Liverpool School of Tropical Medicine	United Kingdom
Howbeer	Muhamadali	University of Manchester	United Kingdom
Giovanna	Musco	Dulbecco Telethon Institute/S. Raffaele Hospital	Italy
Elin	Näsström	Umeå University	Sweden
Ekaterina	Nevedomskaya	Leiden University Medical Center	Netherlands
Andy	Nicholls	MPF	United Kingdom
George	Nychas	Agricultural University of Athens	Greece
Madalina	Oppermann	Thermo Fisher Scientific	Germany
Debora	Paris	Istituto di Chimica Biomolecolare - CNR	Italy
Jeff	Patrick	LECO	USA
Alexandros	Pechlivanis	Imperial College London	United Kingdom
Hans	Pedersen	Aarhus University	Denmark
Andrew	Peters	Waters	United Kingdom
Clément	Pontoizeau	Université de Lyon	France
René	Pool	VU University Amsterdam	Netherlands
Giulia	Pratico'	Sapienza University	Italy
Ganapathy	Rajaseger	DSO National Laboratories (Kent Ridge)	Singapore
Antonio	Randazzo	University of Naples	Italy
Nicholas	Rattray	University of Manchester	United Kingdom
Theo	Reijmers	Leiden University	Netherlands
Carolus	Reinecke	North-West University	South Africa
Stacey	Reinke	University of Alberta	Canada
Joseph	Rothwell	French National Institute for Agricultural Research	France
Serge	Rudaz	School of Pharmaceutical Sciences	Switzerland
Reza	Salek	MRC-HNR / EMBL-EBI	United Kingdom
Ali	Sayqal	University of Manchester	United Kingdom
Horst Joachim	Schirra	The University of Queensland	Australia

FirstName	LastName	Affiliation	Country
Gesine	Schmidt	Nofima - Norwegian Institute for Food, Fisheries and Aquaculture Research	Norway
John	Shockcor	University of Cambridge/Imperial College London	United States
Andrew	Southam	The University of Birmingham	United Kingdom
Melita	Sternad Lemut	University of Nova Gorica	Slovenia
Eriko	Takano	University of Groningen	Netherlands
Fariha	Tanwir	Aarhus University	Denmark
Ahmed	Tawfike	Strathclyde Institute Of Pharmacy And Biomedical Science	United Kingdom
Verena	Tellström	Bruker Daltonik GmbH	Germany
Marie	Tremblay-Franco	UMR 1331 Toxalim	France
Justin	van der Hooft	Plant Research International	Netherlands
Frans	van der Kloet	Leiden University	Netherlands
Andrew	Vaughan	University of Manchester	United Kingdom
Simone	Wahl	Helmholtz Zentrum München	Germany
Alesia	Walker	Helmholtz Zentrum München	Germany
David	Waterhouse	Liverpool School of Tropical Medicine	United Kingdom
Stefan	Weidt	University of Glasgow	United Kingdom
Erwan	Werner	Technologie Servier	France
Johan	Westerhuis	University of Amsterdam	Netherlands
Jenny	Westphal	Helmholtz Zentrum München	Germany
Stefanie	Wiese	Chr. Hansen A/S	Denmark
Paul	Willcock	University of Manchester	United Kingdom
Ian	Wilson	Imperial College London	United Kingdom
Catherine	Winder	University of Manchester	United Kingdom
Jean-Luc	Wolfender	University of Geneva	Select Country
Alan	Wong	CEA Saclay CNRS UMR3299	France
Yun	Xu	University of Manchester	United Kingdom
Masaru	Yoshida	Kobe University Graduate School of Medicine	Japan
Noha	Yousri	Weill Cornell Medical College Qatar	Qatar
Guangju	Zhai	Memorial University of Newfoundland	Canada
Wenlin	Zhang	National University of Singapore	Singapore
Hassan	Zubair	Aberystwyth University	United Kingdom