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METABOLOMICS SOCIETY

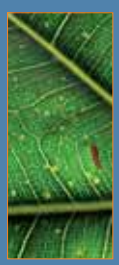
Metabolomics Society's 3rd Annual International Conference

11-14 June, 2007

Manchester, UK

International leaders in Metabolomics Field At 2007 event

Delegate Book



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Metabolomics Society's 3rd Annual International Conference

11-14 June, 2007 - Manchester, UK

1 Security Lodge

2 Renold Building (Main Conference)

3 Staff House (Biorad Technology Showcase)

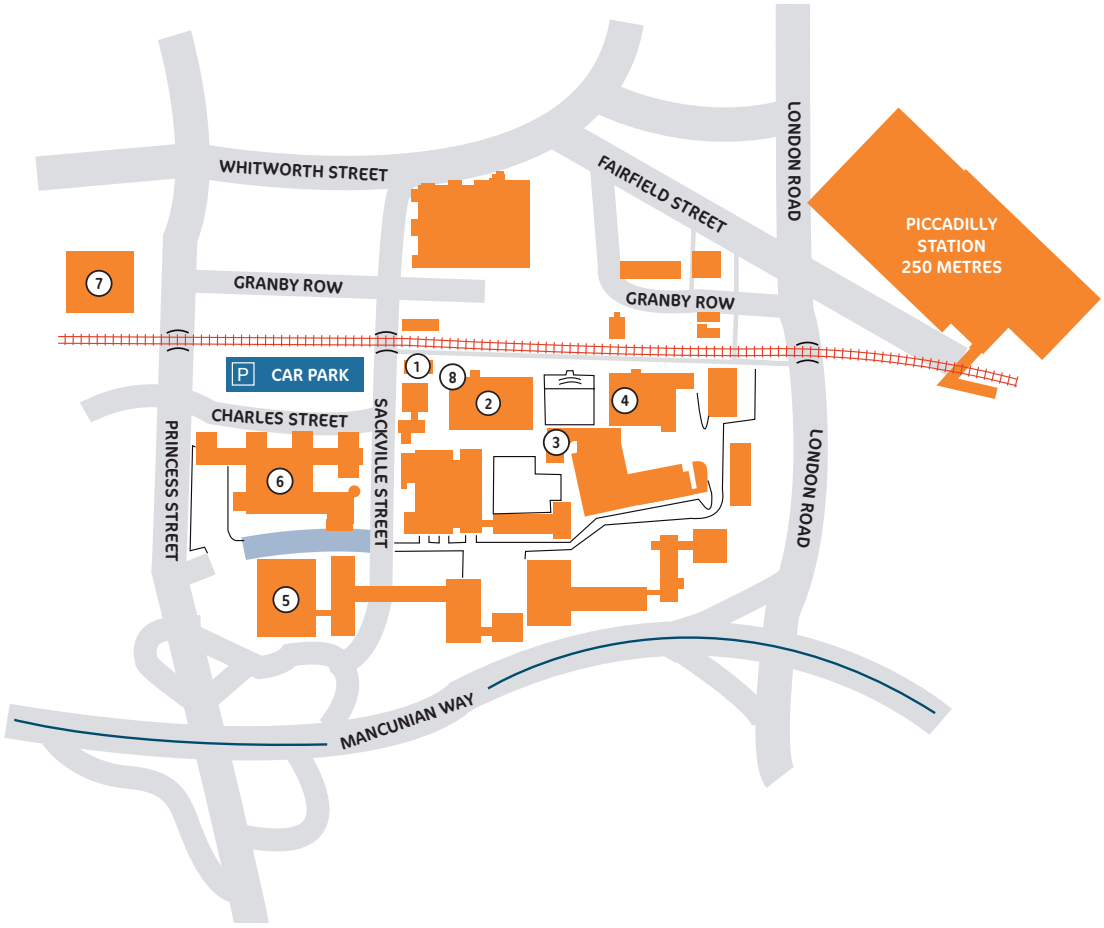
4 Barnes Wallis Restaurant (Bruker and Waters Technology Showcase)

5 MIB (Workshop Sessions)

6 Days Inn Hotel

7 Ibis Hotel

8 Bus to / from Midland Hotel and Radisson Hotel



life inspiring ideas

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AstraZeneca

Metabolomics Society's
3rd Annual International Conference

Welcome To Metabolomics 2007

Dear Friends and Colleagues,

It gives me great pleasure to welcome you to the 3rd annual scientific meeting of the Metabolomics Society in our beautiful host city Manchester, UK.

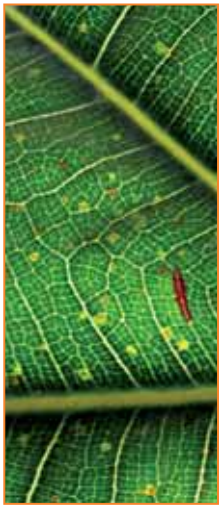
Three years ago, we collectively put forward a vision and mission for the society. We aspired to promote the growth and development of the field of metabolomics internationally; to create opportunities for collaboration and association among researchers in metabolomics and its related sciences and foster connections between academia, government and industry in the field of metabolomics. Critical for us was to provide opportunities for presentation of research achievements, creation of workshops, and to promote the publication of meritorious research in the field.

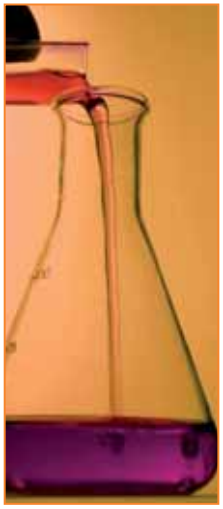
In reviewing the first three years of the life of our society I must say we have gone a long way towards achieving some of our goals. Metabolomics 2005 our first scientific meeting held in Japan was a wonderful podium to launch the society. With over 300 in attendance we hit the ground running. Metabolomics 2006 held in Boston at the Harvard Medical Conference Center attracted over 500 participants and was another home run for the society. The many workshops that we held in collaboration with the National Institute of Health provided great means to educate scientists outside this field as well as policy makers on the promise of this new field of metabolomics. The Metabolomics Standards Initiative was an important project to launch under the umbrella of the society. The Metabolomics Journal is thriving and continues to grow at a steady rate. All these achievements are due to the collective effort of all of you, and for all that we are most grateful.

This year Drs. Roy Goodacre and Douglas Kell co chairs of Metabolomics 2007 along with our program committee have crafted a meeting program that we believe is outstanding in its scope and content. We are delighted to see breadth of topics covered and inclusion of the pharmaceutical sector. Based on great success we had last year we are glad to bring again workshops that will precede the official meeting where experts will share knowledge around metabolomics technologies and informatics tools.

On behalf of the Board of the Metabolomics Society and on behalf of the 2007 Program Committee, we welcome you to Metabolomics 2007 and we hope you will enjoy the meeting.

Rima Kaddurah-Daouk, Ph.D.
President
Metabolomics Society





A letter from the Organizers

On behalf of the Organising Committee, we would like to welcome you all to the 3rd International Meeting of the Metabolomics Society, held here in Manchester UK at the University of Manchester.

We are enormously grateful for the generously given and expert assistance of the Session and Workshop Chairs, the Programme Committee and the Board of Directors of the Metabolomics Society. With their help we have been able to put together a really exciting programme of lectures and workshops. The oral scientific programme, split into 10 sessions and based on a strategy of equal numbers of pre-invited lectures and open submissions, was designed to bring together presentations by established researchers with those from colleagues who are newer to metabolomics, so as to ensure a wide and deep coverage of the latest and most exciting advances in metabolomics.

In addition, we are fortunate enough to have sufficient poster space to enable all posters to be available for viewing throughout the meeting. With four poster sessions there should be ample time for their scientific discussion

Even at the time of writing, just a week before the Conference, this meeting is set to be the largest metabolomics conference ever, with more than 500 delegates from 24 countries, and including 44 lectures, 283 abstract submissions and some 266 poster presentations. This shows both the importance and timeliness of the topic and the benefits of having a Society to help bring all interested parties together. It is particularly gratifying for the health and vigour of metabolomics that 79 of the registrations are from students.

As well as those from further afield, we have received considerable assistance from many colleagues in Manchester during the preparations for this Conference. We would particularly like to thank Andy Tseng and Roger Jarvis for their help with the website and mailing lists.

Finally, we are indebted to Anita Howard of Ya-Ya for taking care of all the non-scientific organisational arrangements. Anita, you are a conference organiser's dream!

We trust that everyone has a wonderful time here in Manchester, both intellectually and socially, meeting old friends and making new ones.

Roy Goodacre
Conference Chair

Douglas Kell
Conference co-Chair

School of Chemistry and the Manchester Interdisciplinary Biocentre, University of Manchester



Workshop and Conference Programmes

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think forward

NMR / LC-MS

WORKSHOP PROGRAMME

Monday 11th June

NMR Workshop 9:00-12:30
Organized by Jake Bundy (Imperial College, London) and Andrew Lane, U. Louisville, USA

Sponsored by: Chenomx, Bruker, Varian Inc

I Invited Presentations

Chair: **Jake Bundy**

8:55 Introduction

9:00 **Kevin Brindle,** *University of Cambridge.* Detecting the early responses of tumours to therapy using magnetic resonance imaging and (hyperpolarised) spectroscopy

9:30 **John Markley,** *U. Wisconsin, USA* Fast and accurate method for determining millimolar concentrations of metabolites in complex solutions from two-dimensional ^{1}H-^{13}C NMR spectra

10:00 **Andrew Lane,** *U. Louisville, USA* Atom-resolved isotopomer analysis and metabolic pathways

10:30 **Ulrich Günther** *U. Birmingham, UK* Probing DNP enhancements for biological applications

11:00 Coffee Break

II Sponsored Presentations

Chair: **Andrew Lane**

11:10 Introduction

11:15 **Manfred Spraul** *Bruker Biospin*, **Germany** New tools for NMR-based Metabonomics

11:35 **Jack Newton,** *Chenomx, USA* Combining targeted and global profiling techniques.

11:55 **Daina Avizonis,** *Varian Inc, USA*

12:15 Conclusion and Discussion-future trends

12.30 Lunch

MS Workshop 13.30-17:10
Organized by Professor Richard Higashi, University of Louisville, USA

14.00 **Joachim Kopka** *Max Planck Institute of Molecular Plant Molecular Physiology, Potsdam-Golm, Germany*
GCxGC-TOF-MS: Discussion of potential and limitations for metabolite profiling

14.35 **Thomas Hankemeier** *Division Analytical Biosciences, LACDR, Leiden University, Netherlands*
LC-MS and CE-MS-based metabolite profiling

15.10 Coffee Break

Chair : **Warwick Dunn,** *University of Manchester, UK*

15.30 **Lorraine Kay** *LECO Instruments, UK* Metabolomics using GC- and GCxGC-TOF/MS

15.50 **John P. Shockcor** *Waters Corp., USA* Application of ion mobility mass spectrometry and advanced statistical methods to metabolomic studies

16.10 **Anne Ferguson** *Thermo Fisher Scientific, USA* High speed, high mass accuracy, automated differential analysis and MSn- a combined quantitative and qualitative approach to metabolomics

16.30 **Steve Fischer** *Agilent Technologies, USA*
Novel approaches coupled with powerful tools to address today's metabolomic challenges

16.50 **Ron Bonner & Dr Julie Wingate** *Applied Biosystems/MDS SCIEX, Canada*
Practical considerations in MS-based metabolomics

17.10 Open discusion session involving all speakers

Tuesday 12th June

Data Analysis Workshop 9:00-12:00
Organized by David Broadhurst and Bruce Kristal

9.00 **Bruce S. Kristal,** *Brigham and Women's Hospital, Dept of Neurosurgery, USA* A Practical View of Basic Workflows (and Pitfalls...) in a Metabolomics-based Classification Problem

9.35 **Johan Westerhuis** *Biosystems Data Analysis Swammerdam Institute for Life Sciences Universiteit van Amsterdam, Netherlands* Validation of metabolic differences and variable importance.

10.10 **John Haselden,** *GlaxoSmithKline, UK* Design of large scale clinical metabolomics studies.

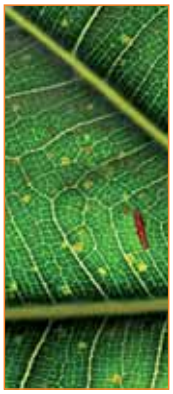
11:45 Coffee break

10.55 **Mark R. Viant** *School of Biosciences,University of Birmingham, UK*
From peaks to knowledge: Interpreting multivariate metabolic models

11-35 **Christian Spieth,** *Genedata, Switzerland* Challenges of MS analysis software in Metabolomics research.

11.50 Open discussion and debate

12.00 Lunch served in Barnes Wallis Building, Sackville Street



CONFERENCE PROGRAMME

Day 1: Tuesday 12 June 2007

09:00 Registration desk opens in Renold Building, Sackville Street. Posters to be hung.

12.00 Finger lunch commence in Barnes Wallis Building

13.00 Opening Comments from
Rima Kaddurah-Daouk, Roy Goodacre *and* **Simon Gaskell**
Location: C16

13.15 Plenary speaker 1: **Gary Siuzdak,** *Scripps Center for Mass Spectrometry, La Jolla, CA, USA.* Novel approaches for small molecule detection
Location: C16

14.15 Coffee Break
Location: Main exhibition room

14.45 Session 1: Metabolomics in health and disease
Location: C16
Chairs: **Rima Kaddurah-Daouk,** *Duke University, USA.*
Ian Wilson, *AstraZeneca, Alderely Park, UK.*

14.45 **David Wishart,** *University of Alberta, Canada.* The human metabolome project: An update for 2007

15.15 **Sabine Bahn,** *The Cambridge Centre for Neuropsychiatric Research, UK* Disease Biomarkers in first-onset Schizophrenia

15.45 **Laura Schnackenberg,** *FDA, USA* Characterization of multi-age rodent pediatric models of toxicity

16.15 **Carolyn Slupsky,** *University of Alberta, Canada.* Can the etiology of pneumonia be determined using metabolomics?

Session 2: Fluxomics and pathway discovery; informatics meets isotopomer analysis
Location: C2
Chairs: **Jacky Snoep,** *University of Manchester, UK.*
Masaru Tomita, *Keio University, Japan.*

14.45 **Uwe Sauer,** *ETH Zurich, Switzerland.* Molecular traffic through metabolic networks: data, principles and their prediction

15.15 **Jörg Schwender,** *Brookhaven National Laboratory, Upton, NY, USA.* Probing seed development by metabolic flux analysis

15.45 **Masaru Tomita,** *Keio University, Japan.* Large-scale multi-omics analyses for E. coli systems biology

16.15 **Fionnuala Morrish (to be confirmed),** *Fred Hutchinson Cancer Center, Seattle, WA, USA* ^{13}C NMR isotopomer analysis of metabolic network regulation by the oncogene c-MYC during cell cycle entry

16.45 Poster session including Tea and Coffee
Technology Showcase – **Bruker BioSpin** *and* **Bruker Daltonics**
Fully Integrated and Dedicated Solution for Metabolic Profiling
Location: Harwood Room

17.45 Coaches leave for drinks reception

18.00 Welcome drinks reception
Manchester Town Hall, Albert Square, Manchester M60 2LA

19.00 End of Day One

Day 2: Wednesday 13 June 2007

08:30 Exhibit opens

09:00 Plenary speaker 2: **Steve Oliver,** *University of Manchester, UK.* Metabolomics as a tool for functional genomics: studies in yeast
Location C16

10.00 Coffee Break

10.30 Session 3: Integrating metabolomics data with systems biology models
Location C16
Chairs: **Jan van der Greef,** *Leiden/TNO, Netherlands*
Douglas Kell, *University of Manchester, UK*

10.30 **Matthias Heinemann,** *ETH Zurich, Switzerland.* Insight from quantitative metabolome data via network-embedded thermodynamic analysis

11.00 **Age Smilde,** *University of Amsterdam, The Netherlands.* Metabolomics as an essential module in systems biology models

11.30 **Domenico Bellomo,** *University of Delft, The Netherlands* Reconstructing metabolic networks from perturbation experiments

12.00 **Marc-Emmanuel Dumas,** *Ecole Normale Supérieure de Lyon, France.* Metabolic profiling and statistical genomics of insulin resistance

Session 4: Environmental metabolomics
Location C2
Chairs: **Mark Viant,** *University of Birmingham, UK.*
Dan Bearden, *Center for Coastal Environmental Health and Biomolecular Research, USA.*

10.30 **Tim Collette,** *Environmental Protection Agency, USA.* Metabolomics in small fish toxicology and ecological risk assessments

11.00 **Jake Bundy,** *Imperial College London, UK.* Metabolomics and 'non-model' organisms – earthworms as a case study

11.30 **Mark Viant,** *University of Birmingham, UK.* Can metabolomics be used for environmental monitoring in free living aquatic wildlife? - Building the weight of evidence

12.00 **Simone Rochfort,** *Primary Industry Research Victoria, Australia.* Wine, soils and complex community interactions

12.30 Exhibition and poster session.
Finger buffet lunch

13.00 Technology Showcases
Bio-Rad Laboratories Inc Data processing and analysis – an integrated informatics approach for metabolomics research
Location: Staff Room 4 and 5
Waters Corporation New developments in system solutions for metabolic profiling
Location: Harwood Room

CONFERENCE PROGRAMME

14.00 Session 5: Drug Discovery
Location: C2

Chairs: **John Haselden,** *GlaxoSmithKline, UK.*
Don Robertson, *Pfizer, USA*

14.00 **Ina Schuppe-Koistinen,** *AstraZeneca, Sweden* The application of metabolic profiling in preclinical and clinical drug development

14.30 **Susan Connor,** *GlaxoSmithKline, UK* Metabolomics in pharmaceutical R&D: Potential for biomarker discovery

15.00 **Yutai Li,** *Merck US.* Metabolomic analysis of bile acids as biomarkers of hepatobiliary toxicity

15.30 **Aalim Weljie,** *University of Calgary, Canada.* Metabolite biomarkers of scleroderma elucidated using 1H NMR metabolomics

Session 6: Plant Metabolomics
Location: C16

Chairs: **Mike Beale,** *Rothamsted Research, UK*
George Harrigan, *Monsanto, USA*

14.00 **Søren Bak,** *Danish Technical University, Lyngby, Denmark* Lessons to be learned from metabolic engineering of cyanogenic glucosides

14.30 **Rik de Vos,** *Plant Research International, Wageningen, The Netherlands* QTL analysis of Arabidopsis RI lines by metabolomics

15.00 **Joachim Kopka,** *Max Planck Institute of Molecular Plant Physiology, Germany.* GC-EI-TOF-MS analysis of in-vivo carbon-partitioning into soluble metabolite pools of higher plants by monitoring isotope dilution after $^{13}CO_2$ labelling

15.30 **Kazuki Saito,** *RIKEN Plant Science Center/ Chiba University, Japan.* High-throughput gene discovery through integration of metabolomics and transcriptomics in *Arabidopsis thaliana*

16.00 Society elections/other

16.30-18.00 Exhibition and poster session.

19.30-0.00 Conference dinner/party
Location: Whitworth Hall, Oxford Road, Manchester

Day 3: Thursday 14 June 2007

09.00 Plenary speaker 3: **Robert Hall,** *Centre for BioSystems Genomics, Wageningen, The Netherlands.*
Metabolomics assisted plant breeding
Location : C16

10.00 Coffee Break

10.30 Session 7: Nutrigenomics and metabolomics
Location C16

Chairs: **Ben van Ommen,** *TNO Quality of Life, Netherlands*
Bruce German, *Nestle, Switzerland*

10.30 **Augustin Scalbert,** *INRA Centre de Rechereé de Clermont Ferrand/Theix, France* Phytochemicals as prominent constituents of the human metabolome - from dietary exposure to health effects

11.00 **John Newman,** *US Department of Agriculture, University of California, Davis, USA* Metabolomics studies in the context of human nutrition and health

11.30 **Sunil Kochhar,** *Nestlé Research Center, Lausanne, Switzerland* Nutri-metabonomics approach in understanding gut microbiome-mammalian metabolic interactions

12.00 **Bruce Kristal,** *Brigham & Women's Hospital, Boston MA.* Validation of a metabolic profile for caloric intake in rats

Session 8: Novel technologies for metabolomics
Location: C2

Chairs: **Jules Griffin,** *University of Cambridge, UK*
Teresa Fan, *University of Louisville, USA*

10.30 **Rolf Gruetter,** *EPFL, Lausanne, Switzerland.* ^{13}C MRS-based metabolomics

10.30 **Justin Wiseman,** *Prosolia, Inc. Indianapolis, USA.* Desorption electrospray ionization (DESI) MS: a new tool for metabolomics

10.30 **Matej Oresic,** *VTT Technical Research Centre of Finland.*
Comprehensive metabolomic characterisation of lipoprotein fractions reveals differential lipoprotein-specific regulation of xenobiotic and pro-inflammatory metabolites in patients with metabolic syndrome

10.30 **Seetharaman Vaidyanathan,** *Manchester Univ, UK.* Biomolecular imaging using ToF-SIMS and buckminsterfullerene (C_{60}) primary ions to study spatial metabolite distribution in cells

12.30 Exhibition and poster session
Finger buffet lunch

14.00 Session 9: Microbial metabolomics
Location C16

Chairs: **Jørn Smedsgaard,** *Technical University of Denmark*
Pedro Mendes, *Virginia Bioinformatics Institute, USA.*

10.30 **Vladimir Shulaev,** *Virginia Bioinformatics Institute, USA.* Metabolic responses of Plasmodium falciparum to anti-malarial drugs

10.30 **Gianni Panagiotou,** *Technical University of Denmark* Metabolomics for genotyping aspergilli

10.30 **Mariët J. van der Werf,** *TNO, The Netherlands.*
Strain improvement using metabolomics information

10.30 **Judy Hwang,** *UC Berkeley, USA.* Mapping metabolic pathways in the metal reducer, *Shewanella oneidensis* MR-1

Session 10: Bioinformatic strategies for metabolomics
Location: C2

Chairs: **Susanna-Assunta Sansone,** *EBI, UK*
Thomas Moritz, *University of Umeå, Sweden*

Nigel Hardy, *University of Wales, Aberystwyth, UK.* Turning Metabolomics Reporting Requirements into useful software and biological output

Johan Trygg, *University of Umeå, Sweden.* The OPLS and O2PLS approach in metabolomics and metabonomics

Margriet Hendriks, *Univ Medical Centre, Utrecht, NL.*
Simplivariate methods: creating interpretable metabolomics models

Lily Tong, *MIT, MA, USA* Investigation of processing and imputation in metabolomics datasets

16.00 Coffee Break

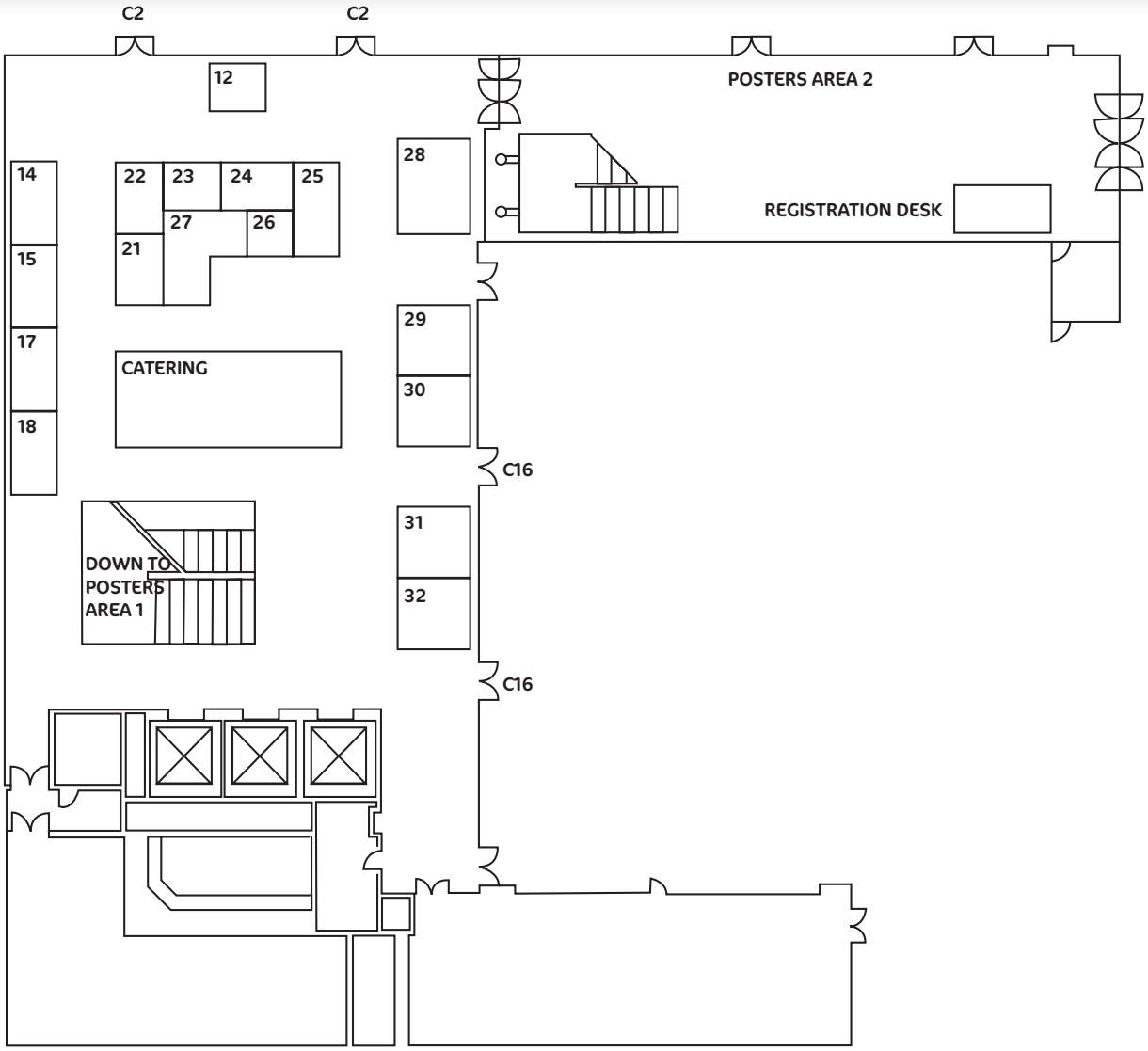
16.30 Plenary speaker 4: **Bernhard Palsson,** *University of California at San Diego, La Jolla, USA.* Systems biology and metabolomics: mapping the human metabolome
Location C16

17.30 Closing Remarks

17.45 End of Conference

EXHIBITORS & FLOORPLAN

Renold Building - 2nd Floor



Exhibitors

29 Agilent Technologies UK Ltd - www.home.agilent.com/

27 Applied Biosystems Ltd - www.eu.appliedbiosystem.com

17 Bruker Biospin Ltd - www.bruker.co.uk

32 Chenomx - www.chenomx.com

15 CK Gas Products - www.ckgas.com/contact.html
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28 Leco UK - www.lecouk.com

25 Metanomics Health GmbH - www.metanomics.de

21 Shimadzu Europa - www.shimadzu.co.uk

18 Thermo Fisher Scientific - www.thermofisher.com

12 Umetrics UK - www.umetrics.co.uk
Umetrics are a leading supplier of software, training and consultancy in “Experimental Design” and “Multivariate Analysis”, complementary methods for handling vast amounts of research data. Our flexible SIMCA-P software is widely used for analysis of Metabonomic datasets and incorporates Orthogonal PLS for enhanced interpretation of results for biomarker discovery.

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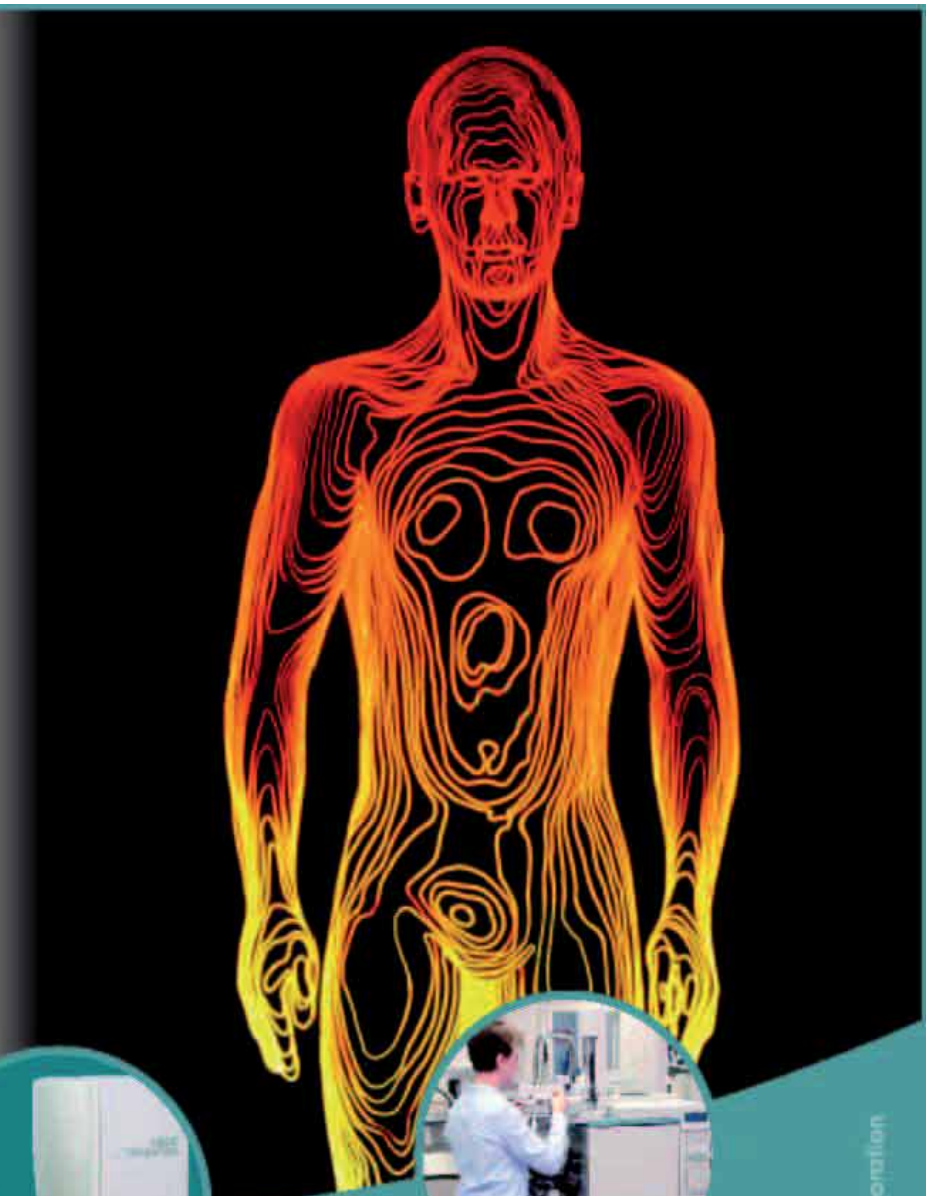

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TECHNOLOGY SHOWCASES

Tuesday 12 June

16.30hrs - 17.45hrs

Bruker BioSpin and Bruker Daltonics

Dr Manfred Spraul, Dr Gabriela Zurek and Special Guest

Don't miss the unique Bruker Technology Showcase on Day One of the Conference. Join both Bruker BioSpin and Bruker Daltonics on Tuesday 12th June at 16:30 and discover the world's only, fully integrated and dedicated solution for Metabolic Profiling. Our unique combination of NMR & LC-MS is the ideal platform for conducting metabonomics studies, traditional metabolism studies, and analyses of complex mixtures.

Join us for a beer and a snack in the Harwood Room. More details will appear inside your conference bag!

Wednesday 13 June

13.00hrs – 14.00hrs

Bio-Rad Laboratories, Inc

Data Processing and Analysis – An Integrated Informatics Approach for Metabolomics Research

Michelle D' Souza, Regis Grenier and Gregory Banik

The ever-increasing demand for improved data analysis tools within the metabolomics research community initiated Bio-Rad Laboratories' development of an integrated analytical approach. This approach encompasses all the steps involved in such analyses from raw data processing to biomarker identification and integrates market-leading analytical, cheminformatics, and chemometrics tools from Bio-Rad and its partners into a single system. At the 3rd Metabolomics Society Meeting, a technical talk and a poster presentation introduced this new approach and integrated system.

We invite you to join us for a brief discussion of Bio-Rad's overall analytical data management environment and the advances that have occurred since the last meeting. Areas of focus will include streamlined multivariate analysis, metabolite projection analysis (projecting a database of pure metabolite spectra onto a metabolomics analysis to highlight the metabolites that appear to change most in the study), and automated pathway identification (based upon the metabolites implicated in the metabolite projection analysis). Finally, we will share recent initiatives to provide easy and comprehensive access to spectral resources for educational institutions.

Water Corporation

New Developments in System Solutions for Metabolic Profiling

John Shockcor, Business Development Manager, Metabolic Profiling, Waters Corporation

Our new developments seminar has been designed to introduce you to several ground breaking advances in hyphenated MS techniques and data analysis for small molecules.

Workshop Abstracts

NMR Workshop Abstracts

Detecting the early responses of tumours to therapy using magnetic resonance imaging and (hyperpolarised) spectroscopy

Kevin M. Brindle
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge UK and Cancer Research UK Cambridge Research Institute, UK

A better understanding of the molecular signature of cancer has allowed the development of a new generation of anti-cancer drugs that target specific molecular entities, such as receptors, genes, or signalling pathways. However, DNA microarray-based disease profiling, together with the results of recent clinical trials using targeted therapies, have clearly demonstrated the intrinsic heterogeneity of human tumours, both genetically and phenotypically. Patients with similar tumour types frequently have markedly different responses to the same therapy. The development of these novel targeted cancer therapies could benefit significantly, therefore, from the introduction imaging methods that allow an early assessment of treatment response in individual patients. These would allow an oncologist to rapidly assess the effectiveness of a new therapy. Ineffective treatments could be abandoned at an early stage and more effective treatments selected, with attendant welfare benefits for the patient and cost benefits for the health care system.
We have been developing non-invasive and clinically applicable magnetic resonance-based methods for detecting the early responses of tumours to therapy. A primary focus has been on the development of methods for detecting tumour cell apoptosis, or programmed cell death, since the level of tumour apoptosis after drug treatment has been shown, in preclinical and clinical studies, to be a good prognostic indicator for treatment outcome. Thus by monitoring tumour cell death an oncologist may get an indication of whether a particular drug is working very early during treatment, possibly within 24-48 hours, and long before there is any evidence of tumour shrinkage. In this talk I will describe the different approaches that we have taken in detecting and imaging cell death in tumours using NMR, including imaging measurements of a targeted contrast agent that binds to dying cells and spectroscopic measurements of metabolic changes. The latter includes recent measurements using hyperpolarised 13C-labelled pyruvate.

Fast and accurate method for determining millimolar concentrations of metabolites in complex solutions from two-dimensional ^{1}H-^{13}C NMR spectra

Ian A. Lewis, Brendan Hodis, Kate A. Robb, Marco Tonelli, Seth C. Schommer, William M. Westler, Michael R. Sussman and John L. Markley
Department of Biochemistry, University of Wisconsin, Madisson, USA

One-dimensional 1H Nuclear Magnetic Resonance (1D 1H NMR) has been used extensively as a high-throughput analytical tool for investigating metabolites in unfractionated biological fluids and tissue extracts. In this role, NMR spectra are usually treated as multivariate statistical objects rather than as collections of quantifiable metabolites. As a result, NMR-based analyses have been largely restricted to generating spectral "fingerprints" for the purpose of classifying samples. We have recently developed a practical two-dimensional (2D) 1H-13C NMR strategy for identifying and quantifying approximately 80% of the NMR-observable metabolites present in biological samples. To validate this technique, we prepared mixtures of synthetic compounds and extracts from Arabidopsis thaliana, Saccharomyces cerevisiae and Medicago sativa. We show that accurate (technical error < 3 %) molar concentrations of more than 40 metabolites per cell extract can be determined in 12 minutes using our quantitative 2D 1H-13C NMR strategy. In contrast, traditional 1D 1H NMR analysis under nearly ideal conditions results in technical error > 16 %. We will present the details of our quantitative approach and comment on the metabolic composition of the cell extracts analyzed for this study. We propose our quantitative 2D strategy as a practical alternative to the established 1D 1H NMR metabolomics approach.
Supported by NIH grant R21 DK070297; I.A.L. was the recipient of a fellowship from the NHGRI 1T32HG002760; NMR data were collected at the National Magnetic Resonance Facility at Madison (NMRFAM) funded by NIH grants (P41 RR02301 and P41 GM GM66326).

Atom-resolved isotopomer analysis and metabolic pathways

Andrew N. Lane, Teresa W-M. Fan, Mariusz Z. Ratajczak, Lynn Deleeuw
JG Brown Cancer Center, U. Louisville, USA

The ability to trace the flow of individual atoms through metabolic pathways is immensely powerful for understanding the control of metabolism and dysregulation in disease states. NMR offers the potential of following the fate of individual atoms from a precursor molecule to end products using stable isotopes as tracers, under a wide variety of conditions.
Recent developments in stable isotope editing of NMR spectra of mixtures of metabolites in crude extracts by 1D and 2D methods will be presented. Isotopomer distributions in metabolites in the extract mixtures can be determined using a variety of isotope-edited experiments (e.g. HSQC, HSQC-TOCSY and HCCH-TOCSY) and quantified by TOCSY. The advantages of the 2D H-1 detection approach include sensitivity and resolution with simultaneous identification of isotope distributions of all protiated carbon sites. The dependence of quantitative precision and accuracy on sample integrity, experimental design and instrumental considerations will be treated with concrete.
The information content of these various experiments will be discussed, along with techniques for determining isotopomer distributions and quantification, and the relationship to pathway delineation and relative flux determination. The choice of labeled precursor compounds for specific and general metabolomics questions will be introduced. The principles will be illustrated with examples from nucleotide biosynthesis, pentose phosphate pathway, glycolysis, citric acid cycle and phospholipid biosynthesis.

Probing DNP enhancements for biological applications

Ulrich Günther, Martin Saunders, Christian Ludwig
HWB-NMR, CR UK Institute of Cancer Studies, University of Birmingham, Vincent Drive, Edgbaston, Birmingham B15 2TT

Dynamic Nuclear Polarisation (DNP) is used to transfer the high spin polarization of unpaired electrons to coupled nuclear spins. Stable radicals are added to a solution of the analyte and irradiating with microwaves is applied at the EPR lines of the radical. At temperatures of 1.5K polarisation times of 1-3h are required for optimal polarisation of 13C and 15N. In such experiments enhancements of >10,000 were achieved [1] by rapidly warming up samples to approx. 300K where spectra are recorded after transfer to a high field magnet. Since the temperature factor of Tobs/Tmwave contributes a factor of 200 to the enhancement various experiments were designed to polarize at low temperature and observe the signal at higher temperatures [1,2].
This implementation of DNP requires efficient transfer of polarisation from stable radicals to the analytes which is facilitated by an optimal contact between the radical and the analyte in a glass state formed at low temperature.
We have studied enhancements for different radicals to optimise polarisation for different substances, including typical metabolites found in body fluids. The life time of the polarisation after the transfer depends primarily on the longitudinal relaxation time of the polarised molecules. Here we have analysed the determinants of optimal polarisation transfer and we have studied the possibility of polarisation transfer from long-lived 13C-labelled carbonyl groups. Carbonyl groups substantially improve the contact to the radical and allow a transfer of polarisation via a 13CC-nuclear Overhauser effect when the carbonyl is 13C isotope enriched. This principle can be used to enhance the life-time of polarisation and to further enhance the observed polarisation at room temperature. Here we probe the applicability of long-lived carbonyl groups to enhance the life-time of the polarisation for various biological applications. Using this method we have evaluated the suitability of this implementation of DNP to study various metabolic pathways.

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NMR Workshop Abstracts

Application of ion mobility mass spectrometry and advanced statistical methods to metabolomic studies

John P. Shockcor
Waters Corp. USA

From the post genomics and proteomics era, metabolomics has emerged as a vital area of research. Metabolic profiles of biological fluids contain a vast array of endogenous low-molecular weight metabolites Changes in these profiles resulting from perturbations of the system can be observed using information rich analytical techniques, such as mass spectrometry. Due to the complexity of the samples new separation techniques like ultra performance liquid chromatography (UPLC) is an appropriate tool to employ. A recent development, ion mobility mass spectrometry (IMS) is now also being employed to aid in extracting even more critical information from these sample sets. The additional information obtained from these approaches has increased the complexity of the data, rendering them even more difficult to mine. Traditional profiling techniques which involve scan by scan comparison of the data have been used to compare small datasets; however, these approaches are not well suited to studies involving large numbers of samples with complex spectral information. Metabolomic approaches have been employed to mine large complex data sets with great success. These approaches typically use multivariate statistical methods, such as principal component analysis (PCA), to highlight differences between samples based on observed spectral patterns. However, these methods are often not well suited to identifying subtle changes and can be biased by large variations within a sample class. New multivariate statistical methods, like orthogonal partial least squares (OPLS), have been developed, which can overcome many of the problems observed when using PCA.

We will illustrate these statistical and analytical methods with several examples obtained on a variety of sample types.

New tools for NMR-based Metabonomics

M.Spraul, H.Schäfer, P.Neidig
Bruker BioSpin GmbH, Rheinstetten Germany

NMR is a very important analytical Tool for metabolic fingerprinting and profiling. Its main advantages are quantitative results, robustness, high throughput under full automation and low cost per sample as well as structural information. Being used in statistical applications creates the need for lowest system internal variance. This can be achieved by extensive control of temperature, creating flat baseline and error-free phase correction to just name a few parameters. Besides the NMR experiment itself, the sample preparation and transfer to the instrument has to be integrated using barcodes all through the process to avoid sample mixup.

Integrating LC-MS into the fully automatic NMR scheme allows to record time synchronized NMR and LC-MS measurements, which are the prerequisite for integrating both types of data. Routines are shown that make use of both data types to secure analytical findings. Also the use of homo- and hetero-analytical covariance methods is demonstrated on example metabonomics datasets. On the NMR side, a reference compound database is an absolute necessity for successful identification and quantification. This cannot be achieved in automation without the use of 2D-experiments, that can be integrated in the screening process.

Therefore a reference compound database is explained, that contains 1D and 2D spectra of metabolites from biofluids, tissues and cell extracts. This database is fully integrated into automatic data evaluation using statistics and matching technology. Before a successful quantification can be done, safe identification of compound signals is needed, knowing the pH-shift pH dependence. All functions mentioned are demonstrated on metabolic sample sets.

Combining Targeted and Global Profiling Techniques

Jack Newton
Chenomx Inc., Edmonton, Alberta;CA

Targeted profiling is a technique for directly recovering quantitative compound information from complex mixtures acquired via 1D NMR experiments. Targeted profiling uses a predefined library of individual compound signatures that are scaled quantitatively and summed together using a linear combination model to create a "simulated" NMR spectrum. In an ideal scenario, this "simulated" spectrum would precisely match the experimental spectrum. However, in all but the most contrived examples, there will be unknown compounds present in the mixture that are not captured using the targeted profiling methodology. Global, compound-agnostic techniques, such as spectral binning or full spectrum analysis, are commonly used to model NMR spectra when the compounds present in a mixture are not known a priori. However, these global profiling techniques can be confounded by a variety of factors, such as the "usual suspect" metabolites. Here we present a method for fully integrating targeted and global profiling techniques. By combining both approaches, we gain the benefits of targeted profiling for known compounds, and retain the extensive spectral coverage of global profiling techniques.

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Daina Avizonis,
VarianInc, Palo Alto, CA

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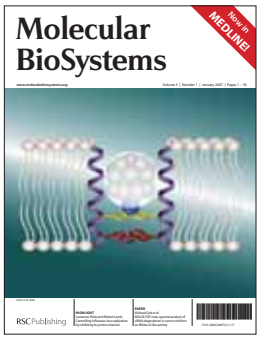

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MS Workshop Abstracts

GCxGC-TOF-MS: Discussion of potential and limitations for metabolite profiling

Joachim Kopka
Max Planck Institute of Molecular Plant Molecular Physiology, Potsdam-Golm, Germany

GC-MS technology has been used for decades in studies, which aim at the exact quantification of metabolite pool size and metabolite flux. Exact quantification has traditionally been focused on a single or small set of predefined target metabolites. Today GC-MS is one of the most widely applied technology platforms in modern metabolomic studies. This presentation will use GC-MS technology as an example and touchstone for the discussion of the work flow comprising metabolomic studies. Specifically the potential and possible limitations of GCxGC-TOF-MS based metabolite profiling will be highlighted, as conventional GC-TOF-MS and the novel 4D extension of this analytical technology are useful tools for computer-assisted and automated analyses of complex separations. Instead of peak lists comprising single values for each constituent compound, each component is represented by a mass spectrum consisting of an array of mass and intensity pairs. These mass spectral tags including the chromatographic retention behaviour can be used for compound identification utilizing a public library such as provided by the Golm Metabolome Database (GMD, http://csbdb.mpimp-golm.mpg.de/gmd.html) [1,2]. In addition the intensity of single mass fragments provide quantitative information on each respective compound and mass isotopomer distribution analysis opens the path towards enhanced quantitative accuracy and flux analysis [3].

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LC-MS and CE-MS-based metabolite profiling

Thomas Hankemeier
Division Analytical Biosciences, LACDR, Leiden University, Netherlands

Metabolic profiling of blood, urine, and cerebrospinal fluid is likely to become instrumental in predicting disease-risks and treatment responses. The potential of metabolomics has already been demonstrated, but, as a technology, metabolomics needs further improvement of the sensitivity, specificity, and capacity of the analytical and biostatistical methods.
In this presentation, various approaches in the comprehensive analysis of metabolites using LC-MS and CE-MS will be discussed. Attention will be paid to the requirements of an LC-MS and CE-MS method for metabolite profiling. Important is a reliable, repeatable and preferably reproducible quantification of metabolites in different types of samples. For this, it is important to understand the role of ion suppression using electrospray MS. Another important issue is the proper validation of this kind of methods, as metabolites cover a broad range of physicochemical properties, and optimization has to aim at a good performance of wide range of metabolites. Various methods and applications will be covered in the presentation. Finally, recent and future trends in CE-MS and LC-MS for metabolite profiling will be discussed.

Metabolomics using GC- and GCxGC-TOF/MS

Lorraine Kay
LECO Instruments, UK

Samples analysed in metabolomic studies are by their very nature extremely complex. The ability to detect biomarkers of disease from samples such as human breath, blood and urine or uncover metabolites in plants that have potential medicinal properties is an exciting concept. However, the complete metabolite characterization of a biological system is an enormous challenge for any analyst irrespective of the starting material.
Gas Chromatography coupled to Mass Spectrometry (GCMS) is an ideal tool that can assist in the huge task of analysing the metabolites in these various matrices. Nonetheless, following chromatographic separation of hundreds or thousands of metabolites and dozens of different chemical families, identification and quantification is also necessary. Although GC-MS is widely used in this field many methods are limited in their capacity to give anything more than target analysis in a single chromatographic run. Modern methods that use Time of Flight mass spectrometers (TOFMS) such as GC- & GC × GC-TOFMS vastly increase the information gleaned from a single sample injection and help to fill this gap [1]. Dallüge et al. [2] reported that only detectors able to acquire fifty or more spectra per second enable effective reconstruction of the two dimensional chromatogram and subsequent quantification. Currently, the only compatible MS is time-of-flight mass spectrometry (TOFMS). Additionally, O'Hagan et al have reported the optimisation, automation and high throughput essential for metabolomic studies using GC-TOFMS [3] & GC × GC-TOFMS [4]. Furthermore, Dallüge et al. [5] and Shellie et al. [6] concluded that GC × GC–TOFMS provides a reliable basis for the automated analysis of complex samples. It is possible to achieve both non-target and target analysis in one sample run by acquiring all the peak information initially. Once data are acquired they are processed accordingly, whether this be looking for specific biomarkers or alternatively to provide metabolic profiles/footprints in order to search for 'unexpected' or 'unknown' metabolites. Both GC- & GCxGC-TOFMS as tools for metabolomic analyses are discussed here.

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Challenges of MS analysis software in Metabolomics research.

Christian Spieth
Genedata

Modern software solutions have to accommodate the increasing demand of MS data analyses in both Metabolomics and Proteomics. The challenges for software providers as well as researchers are manifold. Large scale experimental setups lead to extensive data volumes to be stored and processed, resulting either in expensive hardware, or very inert programs, or in the worst case in both. Moreover, the data often presents a challenge in itself. Chemical noise and variable background signals as well as shifts in m/z or retention time between single experiments complicate the analysis process or simply render it impossible.
This talk will focus on strategies to overcome current problems in Metabolomics analysis, covering

- Challenge of large data volumes
- Visualization of MS data
- Issues with noise and background signals
- Alignment of multiple experiments
- Problems in peak detection
- Compound identification

It will be aimed towards researchers and software developers, pointing out potential bottlenecks and sketching possible solutions for the mentioned problems.

High speed, high mass accuracy, automated differential analysis and MS^n- a combined quantitative and qualitative approach to metabolomics

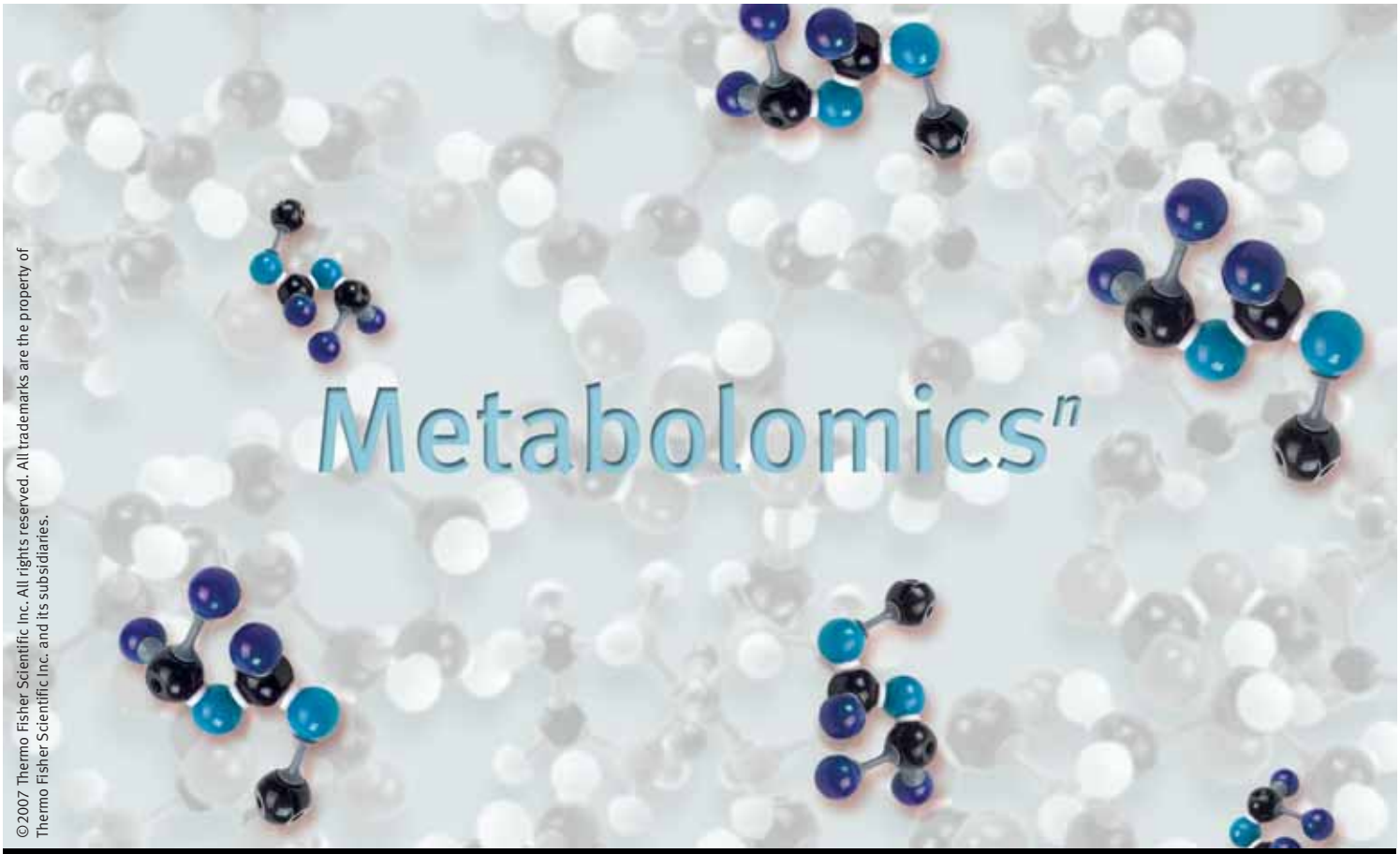
Anne Ferguson
Thermo Fisher Scientific, USA

Recent advances in liquid chromatography and mass spectrometry combined with bioinformatics can provide an integrated approach to mammalian metabolomic studies. This presentation highlights the synergistic value of high speed and high resolution chromatography and high mass resolution and mass accuracy mass spectrometry coupled with powerful data analysis software for sensitive detection of differentially abundant metabolites in human body fluids. The benefits of each of these technologies to metabolomics studies will be described with relevant examples.

Novel approaches coupled with powerful tools to address today's metabolomic challenges

Steve Fischer
Agilent Technologies, USA

Metabolomics is a logical compliment to large scale expression profiling and proteomics studies, offering valuable insight in to the biochemistry of organisms. But metabolomics also presents analytical challenges. Endogenous metabolites exhibit vast chemical diversity, with tremendous variation in structures, functional groups, and physicochemical properties. Thus no single instrument or technology is suitable for all metabolomic analysis.
This presentation will highlight a multi-step mass-profiling based approach that involves finding metabolites with significant variation in abundance between experiment and control groups. In addition, as metabolomic studies generally involve large numbers of samples and complex data processing, powerful software tools for metabolite identification, quantitation, statistical analysis and validation will be discussed.



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MS and Data Analysis Workshop Abstracts

Practical considerations in MS-based metabolomics

Ron Bonner & Dr Julie Wingate
Applied Biosystems/MDS SCIEX, Canada

The ability of mass spectrometry to rapidly provide detailed peak information for many samples has made it a popular tool in metabolomics. This ability is, however, accompanied by a number of potential issues that must be considered when designing and conducting metabolomics experiments. Here we briefly review some of these issues and suggest techniques that can be used to detect and eliminate them.
In general two main areas must be considered:

- Generating appropriate high quality data that can be analyzed to identify compounds that are causing observed sample differences
- Avoiding variation (systematic or random) that will confound the analysis, mask real effects and potentially lead to false conclusions.

Point 1) mainly affects the choice of experiment (for example targeted vs. non-targeted) and the equipment to be used, while point 2) affects how the samples are handled and how the data is acquired and processed. In most cases it is impossible to control all sources of unwanted variation and it is necessary to analyze the data to determine and remove experimental artefacts.
Using LCMS data from real samples acquired on AppliedBiosystems/MDS Sciex QSTAR® (QqTOF) and QTRAP (QqLIT) instruments, and processed using MarkerView™ Software to find and analyze peaks, we will illustrate many of these issues but with particular focus on:

- Targeted vs. non-targeted workflows
- The effects of data acquisition speed and resolution
- Data pre-processing such as peak finding, normalization, alignment and scaling
- Artefact detection and removal (contamination and carryover for example)
- The identification of sample related effects such as diurnal variation and aging

We will also discuss ways to identify compounds.

A practical view of basic workflows (and pitfalls...) in a metabolomics-based classification problem

Bruce S. Kristal
Brigham and Women's Hospital, Dept of Neurosurgery, Boston, USA

Metabolomics greatest advantages – such as its immediacy and its sensitivity -can also conspire to confound seemingly straightforward analysis such as defining a single metabolic profile reflective of nutritive intake. This talk will focus on practical observations on the strengths – and weaknesses -- of clustering and projection methods (supervised and unsupervised, descriptive and discriminant) in a progressive, unified approach to a representative, but unexpectedly complex problem in metabolomics-based classification.

Validation of metabolic differences and variable importance.

Johan Westerhuis
Biosystems Data Analysis, Swammerdam Institute for Life Sciences, Universiteit van Amsterdam, The Netherlands

Classification of individuals based on their metabolic profile is an important goal in metabolomics research. The classification serves two goals, being the prediction of class labels of new individuals and the selection of important metabolites in blood or urine that show a difference between the groups being treated vs nontreated, healthy vs diseased etc.
Unfortunately, classification of groups of individuals based on their metabolic profile is a difficult task. The large number of variables compared to the small number of individuals makes the use of standard discrimination techniques impossible. Therefore latent variable methods are being used to compress the data. Validation of these latent variable classification models is of major importance because of the chance of overfit. Cross validation is often used because of the small number of individuals. This can lead to overoptimistic results when not performed properly.
In this presentation a permutation approach will be used to validate the whole procedure of class prediction and metabolite selection. Permuting the class labels over the individuals provides a H0 distribution of no effect as well as a H0 distribution of peak importance in case of no effect. These H0 distributions can be used to test the validity of the discrimination as well as the peak importance. This permutation approach clearly shows the validity of the cross validation procedure as well as the selection of relevant metabolites.

Design of large scale clinical metabolomics studies.

John Haselden
GlaxoSmithKline, UK

Although metabolomics has been used in a number of disciplines, the major area that remains to be proven is in the application to the clinical situation. Notable examples exist of the use of metabolomics for the study of kidney transplantation, pre-eclampsia, cardiovascular and vascular disease. These all represent a powerful application of metabolomics, but from the pharmaceutical viewpoint, although compelling, they provide a frustration due the extreme (i.e. disease-related) modifications to the metabolome. Indeed measurement of the 'extreme' has been the basis of much of the work published in the discipline of metabolomics, which in turn has lead to questions about the biological sensitivity of the method in less extreme situations. From the pharmaceutical perspective, the use of metabolomics falls into broad camps – the application to the discovery of novel mechanisms (hence potential drug targets), application to Phase I volunteer trials, and Phase II to IV patient studies. In the former situation extreme cases are used, but patients with minimal disease development are usually focused on since they offer the best understanding from a treatment development perspective and from a longitudinal viewpoint, allow for the potential to offer novel treatment as a well as to monitor any amelioration of the mechanistic dysfunction. The latter situation represents the greatest complexity due to the lack of a genuine appreciation of the extent of human variability from the metabolomics perspective.
Complications include: a definition of the precise composition of the human metabolome, the concentration variations that exist for each metabolite, the localisation and global interplay of numerous metabolites, the stability of the metabolome under various sources of normal physiological variation, the innate variation resulting from genetic differences within the human population, the metabolic consequences of one's lifestyle, the effects of the wider environment, and the effects from parasitic/symbiotic/commensal relationships. This presentation aims to exemplify areas that need to be considered when designing large scale clinical studies, show some data from an in-house volunteer study, whilst highlighting ongoing projects that look to define 'normal' human populations.

From peaks to knowledge: Interpreting multivariate metabolic models

Mark R. Viant
School of Biosciences, University of Birmingham, UK

Having designed a good metabolomics experiment, collected the metabolic data, and constructed a validated multivariate model, what should you do next? This presentation will introduce some of the important next steps required to move from spectral peaks to biological knowledge, including pitfalls to avoid. Specifically, the following issues will be introduced and discussed:

- Introduction to interpreting scores plots and loadings plots from a principal components analysis.
- Metabolite identification: the initial (critical) step for relating spectral data back to metabolic pathways and biological mechanism.
- Importance of anchoring the metabolic measurements to organism phenotype and genotype, in order to maximise the interpretation of multivariate models and to reduce "metabolic noise".
- The "so what?" of molecular biomarker research: relating the molecular measurements to whole organism responses.

The talk will draw upon examples from the speaker's laboratory as well as information being produced by the Metabolomics Standards Initiative. It will be aimed towards researchers with relatively minimal previous experience in the interpretation of multivariate metabolic models.

Invited Speaker Abstracts

Invited Speaker Abstracts

Disease biomarkers in first-onset schizophrenia

Sabine Bahn and colleagues

Institute of Biotechnology, University of Cambridge, UK

At present, little is known about the basic mechanisms that underlie the schizophrenia disease process. This lack of knowledge is most likely due to the fact that until recently large-scale expression profiling studies were technologically impossible. Thus, most researchers employed a "candidate gene/protein" approach. With recent technological advances in genomics, proteomics and metabolomics techniques, it is now possible to globally investigate the molecular underpinnings of psychiatric conditions which should result in improved knowledge and hopefully new (pre-symptomatic) diagnostic, therapeutic and preventative regimes.

Our laboratory combines advanced computing and bioscience technologies with functional genomics studies. Using this powerful approach we explore the molecular "fingerprints" of psychotic disorders from early onset through their progressive stages, exploring alterations at the gene, protein, lipid and metabolite level.

A further aim is to establish whether disease related changes can be traced in peripheral tissues. Amongst other tissues, we have investigated T-cell function in schizophrenia patients and have identified significant alteration in signaling mechanisms. The key aim of this study is to establish a suitable surrogate 'disease model', allowing for dynamic functional investigations of disease-associated pathophysiological mechanisms as well as the identification of schizophrenia biomarkers, whilst limiting problems such as drug and post mortem effects.

Thank you to the Stanley Medical Research Institute for Centre support.

Lessons to be learned from metabolic engineering of cyanogenic glucosides

Søren Bak

Danish Technical University, Lyngby, Denmark

Plants produce a plethora of secondary metabolites which presents a wealth of potential pharmaceuticals, pro-vitamins, flavours, aromas and colours as well as a source of natural pesticides. Metabolic engineering can be used to alter and improve the secondary metabolite composition in crop plants for improved traits. The genetic simplicity of the cyanogenic glycoside pathway has given cyanogenic glucosides a pioneering status in metabolic engineering of plant secondary metabolism. We report the lessons learned from metabolic engineering of cyanogenic glucosides in plants, and demonstrate that metabolic engineering is a powerful tool to elucidate the impact of secondary metabolism in plant-insect interactions.

Metabolomics and 'non-model' organisms – earthworms as a case study

Jake Bundy

Imperial College London, UK

Earthworms have been widely used as sentinel organisms for monitoring soil contamination. As a result, there is a substantial amount of existing information about their suitability as environmental indicators, including ecological, cellular, and molecular endpoints. A metabolomic approach adds a great deal of potential advantages, including the ability to screen for novel and/or unexpected biomarkers of toxicity, and to use multivariate profiles to distinguish confounding variables from 'real' toxic responses. (There are many other additional potential applications, e.g. physiological/ecological.) Some results will be presented from both laboratory and field experiments, identifying metabolites that are potential biomarkers of toxic response across very different field sites and from different sampling years. Finally, because of the untargeted nature of metabolomics, the experiments have led to the detection of some unusual and unexpected secondary metabolites.

Metabolomics in small fish toxicology and ecological risk assessments

Timothy Collette[1], Drew Ekman[1], Quincy Teng[1], Dan Villeneuve[2], and Gerald Ankley[2]

US Environmental Protection Agency
1 National Exposure Research Laboratory, Athens, GA
2 National Health and Environmental Effects Research Laboratory, Duluth, MN

The US EPA is tasked with protecting not only humans, but also ecosystems from potentially harmful effects of chemical pollutants. Although lagging behind applications targeted to human endpoints, metabolomics offers great potential in ecotoxicology. Indeed, the advantages of metabolomics (relative to other 'omic techniques) may be more tangible in ecotoxicology because there is often not a sequenced genome available for ecologically relevant species. We are conducting metabolomics studies on small fish, such as the fathead minnow, that are used both as model organisms in ecotoxicology research, and in regulatory testing programs. As part of a project involving a large, interdisciplinary team of scientists from US government, academia, and industry, we are integrating transcriptomic, proteomic, and metabolomic data to describe endocrine disruption in the fathead minnow. Our long-term goal is to understand how chemical exposures are linked through early molecular changes to whole-organism adverse outcomes and, ultimately, to changes in population status. To achieve this goal, a systems-based approach is being used to define toxicity pathways for model chemicals with well defined modes of action within the hypothalamic-pituitary-gonadal (HPG) axis of the fathead minnow. We will describe the unique role that metabolomics plays in this ecotoxicological application, and also the role that we envision for metabolomics in regulatory decision making.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

Metabolomics in Pharmaceutical R&D: Potential for biomarker discovery

Metabolomics (metabolic profiling/metabonomics) continues to thrive and is beginning to provide more than mere promises. The scope of metabolomics applications within GSK is briefly summarised and a clinical diabetes example is discussed. Key results are put in context of both of known aspects of the disease and in terms of their usefulness in assessing drug development in the preclinical and clinical areas. A key message will be that metabolomics, using whatever platform technology, offers huge potential to highlight candidate (putative) small molecule biomarkers in support of all stages of pharmaceutical R&D - from drug discovery to patient stratification/disease classification.

New insights into cerebral carbohydrate and neurotransmitter metabolism

Rolf Gruetter

Centre d'Imagerie BioMedicale (CIBM), Laboratory for functional and metabolic imaging (LIFMET), EPFL, Lausanne, Switzerland.

The imaging methods most ubiquitously used for measuring brain activation are based on either determining the glucose metabolic rate, CMRglc, using e.g. FDG PET or on studying the hemodynamic correlates of brain activation by measuring CBF changes or changes in tissue deoxyhemoglobin, using e.g. BOLD fMRI. Of these, the last has taken center stage in its ability to depict cortical networks in humans. However, the link between the rather indirect hemodynamic events with metabolic events linked to cellular activation is currently not as well understood.

Traditionally, brain function is attributed solely to the neuronal networks, however, to the pre- and postsynaptic neuron, astrocytes play an important role in synaptic functionality, exemplified by e.g. the glutamate-glutamine cycle, now recognized as the main metabolic pathway of the excitatory neurotransmitter glutamate following release and receptor interaction. In the last two decades experimental evidence has been mounting to support the notion that astrocytes are vital and important contributors to brain energy metabolism and in recent years the consensus has been increasing that their energy metabolism is substantial.

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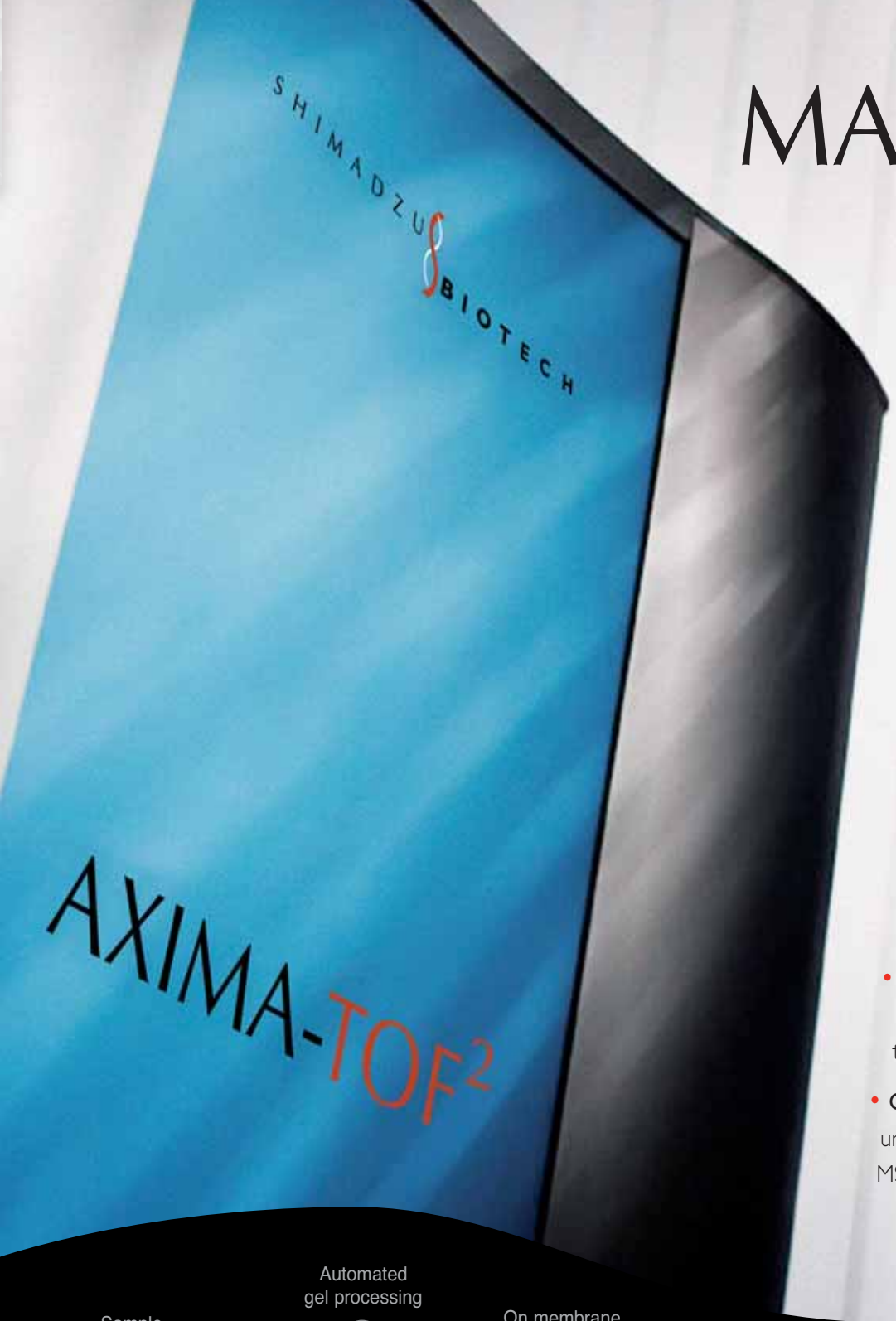

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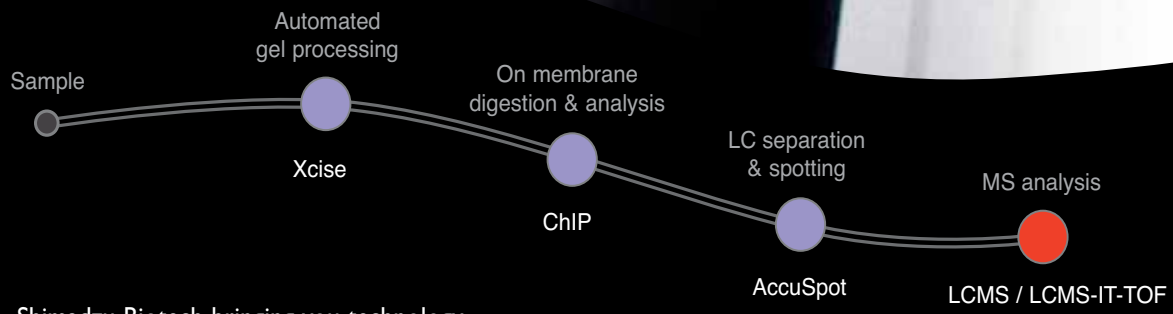

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Invited Speaker Abstracts

The activity of many processes in the brain is highly compartmentalized, with CO2 fixation and glutamine synthesis occurring primarily in the astrocytes, whereas most but not all of the oxygen consumption occurs in the neuronal compartment. In vivo the measurement of energy metabolism in the two major compartments is difficult to achieve, primarily because the established imaging methods (see above) generally lack the ability to separate individual compartments.

This limitation can potentially be overcome by using the stable isotope 13C in conjunction with NMR spectroscopy, as the fate of 13C label can in vivo not only be followed into different molecules, such as glutamate and glutamine, but also into different positions in these molecules. Because of the primary location of glutamine in astrocytes, the labeling of glutamine relative to glutamate provides insight into relative metabolic rates between astrocytes and neurons. Research exploiting these unique labeling patterns has shown that glial energy metabolism is a substantial fraction of overall energy metabolism in brain, underscoring the important role of astrocytes. Furthermore, recent NMR evidence overwhelmingly suggests that the majority of energy produced during activation is by synthesis of ATP produced by oxidative combustion of glucose. Oxidative metabolism of glucose is under important control of the malate-aspartate shuttle. While a significant fraction of energy metabolism has been linked to electrical activity, substantial energy metabolism remains under iso-electric conditions, yielding a significant glucose concentration gradient across the blood-brain barrier. Glucose, being the primary substrate for brain energy metabolism, is stored also in the brain in the form of glycogen. Recent evidence by 13C NMR suggests that brain glycogen is under resting conditions and mild physiological activation highly stable and only activated with strong adrenergic stimuli or when free cellular glucose becomes rate-limiting for metabolism, such as during hypoglycemia.

In conclusion, the complementary nature of insights provided by NMR spectroscopy in vivo has the potential to allow the measurement of astrocytic and neuronal energy metabolism, as well as the rate of glutamatergic action. These experimental capabilities have already lead to important advances in our understanding of the regulation of energy metabolism, hypoglycemia and are expected to yield further insights into the metabolic effects of many brain diseases.

Metabolomics assisted plant breeding

Robert D. Hall

BU Bioscience, Plant Research International, Wageningen, The Netherlands and Centre for BioSystems Genomics, Wageningen, The Netherlands.

The field of plant metabolomics is developing rapidly and applications for the technologies being developed are very broad. Fundamental research is delivering much information on e.g. the relationship between phenotype, genotype and metabolic profile. Changes in metabolite profile in relation to environment, treatment or biotic stress also help us to better understand the flexibility of plant metabolism and how we might develop strategies to direct plant metabolism and metabolic profiles in a specific manner. This is of particular importance in the food production and processing industries.

Much of the published work in the area of applied plant metabolomics is focused upon aspects of comparative metabolomics where different genotypes, varieties, tissues etc are compared and, using multivariate statistics approaches, significant metabolite differences are identified. Metabolomics is very much seen as an hypothesis generator where detected metabolic differences, found to correlate with a specific quality or phenotypic trait, may be further tested for potential causality. In this way metabolomics is being used to generate data and information which can be used to understand how plant metabolism is endogenously controlled and is under the influence of external factors. We already have some excellent examples where the genetics behind these changes are also being characterized and, so doing, we can not only identify potentially useful biomarkers but also we can determine how e.g. breeding strategies might be designed to directly lead to improved crop varieties with e.g. enhanced nutritional value or disease resistance. Metabolomics is also being applied in a post harvest context – concerning aspects of transport, storage and processing in order to, once again, help us understand the broader biochemical changes taking place in time, identify where there are opportunities for improvement, and decide upon the best strategy for improved food quality.

Bioinformatic strategies for metabolomics

Nigel Hardy

Dept. Computer Sci, University of Wales, Aberystwyth, UK

Progress by the Metabolomics Standards Initiative (MSI) of the Metabolomics Society will bereviewed and the next steps outlined. The minimum reporting standards produced by MSIwork groups provide a basis for development of a range of tools and facilities. These areexplored. They include data collection tools, specifically configured LIMS and public databasesof comparable data in addition to depositories for regulatory and publication purposes. Thesedevelopments will benefit from input by potential users, not least to ensure principledcomparability. Extraction of comparable biological output from data collections is a harderchallenge than ensuring meaningful archival but offers significant benefits.

Insight from quantitative metabolome data via network-embedded thermodynamic analysis

Matthias Heinemann, Anne Kümmel

ETH Zurich, Institute of Molecular Systems Biology, Zurich, Switzerland

The concept of network-embedded thermodynamic analysis (NET analysis) is presented as a framework for mechanistic and model-based analysis of quantitative metabolome data: Metabolite data and intracellular fluxes are computationally integrated via the second law of thermodynamics and the metabolites' Gibbs formation energies. We demonstrate that the developed optimization framework is a valuable tool to systematically investigate experimental data sets for consistency and to identify metabolic reactions that most likely are subject to active allosteric or genetic regulation. Using cell-averaged metabolite data from Saccharomyces cerevisiae, we will demonstrate that NET analysis is even capable of resolving intracompartmental metabolite concentrations.

Nutri-metabonomics approach in understanding gut microbiome-mammalian metabolic interactions

François-Pierre J. Martin, Serge Rezzi, and Sunil Kochhar

Metabonomics & Biomarkers, BioAnalytical Science Department, Nestlé Research Center, Lausanne-26, Switzerland

The human gastrointestinal tract is a remarkable organ with prodigious length, surface area, cellular diversity, signalling capacity and metabolic activity. Adult humans are composed of an estimated 10 times less human cells than microbial symbionts, which communicate with each other and disperse their metabolic functions thus behaving like a multi-cellular organism. The intestinal surface is an important host organism-environment boundary and interactions of gut microbes in the gastrointestinal tract very likely influence the mammalian metabolic phenotype. The mammalian-microbiome symbiotic relationship lends extraordinary capacity to human gut to physically

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propel, mechanically manipulate, enzymatically digest and actively absorb nutrients even from the most intractable biological matrices. Gut microbiota was recently shown to vary significantly in obese animals and humans, and its essential role in energy recovery may be crucial in several metabolic variations commonly encountered in toxicology or diseases.

Manipulation of the microbiome either by probiotics, prebiotics and/or by diet shows promise in improvement of human health, e.g. improved immune response, lowering of inflammation, etc. Supplementation with specific probiotics appeared to offer considerable promise in the treatment of post-infective Irritable Bowel Syndrome (IBS) by modulating host metabolism and host-microbiota interactions. In addition, microbial-induced modifications of the intestinal metabolic profile were shown to be region-dependent and correlated with the intestinal functions as manifested by the effects on digestion, absorption, amino acid homeostasis, lipid metabolism and protection of oxidative stress. The presentation will cover results from metabonomic investigation of post-infective murine models and germ-free mice re-colonized with a variety of microbiomes to understand complex metabolic interactions and their impact on the host health and nutritional status.

Metabolomics studies in the context of human nutrition and health

John W. Newman USDA- ARS,

Western Human Nutrition Research Center and UC Davis Dept. of Nutrition, USA

Nutritional status has both subtle and overt effects on the physiological state of an individual. Moreover, inter-individual variability can dramatically influence the response of individuals to dietary components and nutritional supplements. The USDA Western Human Nutrition Research Center is integrating metabolomics into the basic experimental design of a variety of investigations on the biological impact of nutrients. Selected results will be shown from studies of marginal vitamin B12 deficiency, plasma biomarker discovery in whole vs. refined grain consumption, the influence obesity on circulating inflammatory mediators, and explorations of the mitochondrial metabolome to complement studies of metabolic disease. We have found that by using clinical chemistry measurements to select subjects for metabolomic screening, meaningful metabolomic responses can be produced in small human cohorts. In concert with a growing collaborative metabolomics research effort at the University of California, and our ability to perform long-term live in feeding studies on human subjects, the Western Human Nutrition Research Center is poised to capitalize on the variability in human metabolism to enhance our understanding of the impact of nutrition on health and health risks.

Metabolomics approaches to the systems biology of yeast.

Steve Oliver

Manchester Centre for Integrative Systems Biology and Centre for the Analysis of Biological Complexity, The University of Manchester, Michael Smith Building, UK

Metabolic Control Analysis (MCA) is a conceptual and mathematical formalism that models the relative contributions of individual effectors in a pathway to both the flux through the pathway and the concentrations of individual intermediates within it. Detailed modelling requires that 'natural' biological systems be identified. The combination of flux balance analysis with both genetics and metabolomics in the definition of metabolic systems will be discussed.

Defining metabolic systems through flux coupling analysis.

Genome-scale metabolic models promise important insights into cell function. However, the definition of pathways or functional network modules within these models, and in the biochemical literature in general, is often based on intuitive reasoning. Although mathematical methods have been proposed to identify these modules in an unbiased way, there is a need for experimental verification. We have used metabolite profiling of intra- and extra-cellular metabolites in single-gene deletion mutants of the yeast Saccharomyces cerevisiae and have shown that such data can, when combined with multivariate statistical methods, be used to validate functional modules within a metabolic network that have been identified by theoretical analysis. More importantly, we have demonstrated that this method will also identify those enzymes or proteins that are not part of a functional module to which they may have been assigned. The approach does not require any knowledge of how a gene deletion might perturb the metabolic network and provides an empirical method for validating and ultimately refining the predicted network structure.

Defining metabolic systems through flux balance analysis of genetic interactions.

We have analysed the context dependency of synthetic genetic interactions that define redundant functions and alternative pathways. We performed systems-level flux balance analysis of the yeast metabolic network to identify genetic interactions and then tested the model's predictions with in vivo gene deletion studies. We found that the majority of synthetic genetic interactions are restricted to certain environmental conditions. Moreover, the phylogenetic co-occurrence of synthetically interacting pairs is not significantly different from random expectation. These findings suggest that compensation is only a side effect, and these gene pairs have at least partially independent functions. Experimental analyses, using multiple gene deletion strains, not only confirmed predictions of the model, but also showed that investigation of false predictions may both improve functional annotation within the model and also lead to the discovery of higher order genetic interactions.

Systems biology and metabolomics: mapping the human metabolome

Bernhard Palsson

Dept of Bioengineering, University of California—San Diego, USA

We have manually reconstructed the global human metabolic network based on Build 35 of the genome annotation and a comprehensive evaluation of >50 years of legacy data (i.e., bibliomic data). Herein we describe the reconstruction process, the content of the reconstruction, and demonstrate how the resulting genome-scale (or global) network can be used for basic and applied purposes. Some of the results discussed are: comparison of the metabolites that are included in the reconstruction with recently published metabolomic data sets, the assessment of the dimensionality of the steady state flux space and the reaction sets that create its principal directions, and the use of metabolomic data to create genome-scale kinetic models through he use of the k-cone

Metabolomics for genotyping aspergilli

Gianni Panagiotou

Center for Microbial Biotechnology, BioCentrum-DTU, Denmark

The protein 6-methylsalicylic acid synthase (6-MSAS) is among the simplest and most-well characterized members of the polyketide synthase (PKS) family of multienzyme systems. Clearly, heterologous expression of functional PKSs in organisms such as A. nidulans could be advantageous because it would enable use of the advanced knowledge and technology available with this microorganism. Aiming to develop a robust platform for 6-MSA production, 6-MSAS from P. griseofulvum was functionally expressed in A. nidulans

Physiological studies on different carbon sources accompanied by the analysis of metabolome data of three recombinant A. nidulans strains were proven extremely useful in the evaluation of the function of the metabolic network after genetic perturbations and the optimization of 6-MSA production. A gas-chromatography-mass spectrometry (GCMS) profiling protocol was used to identify more than 300 metabolites in the samples. Neural networks have then been applied to discriminate between the genotypes and the different environmental conditions.

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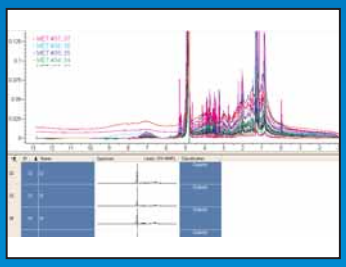
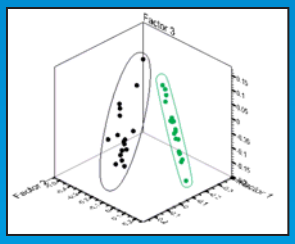
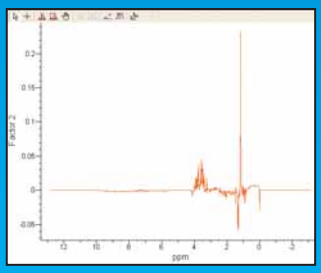


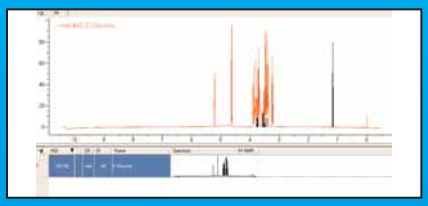
Input data of NMR FIDs



PCA yields class differentiation

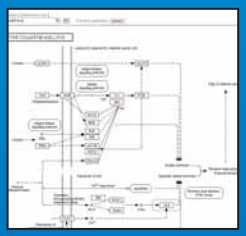


Loadings plot used as search query against metabolite DB



Results retrieved from database search indicate putative biomarkers

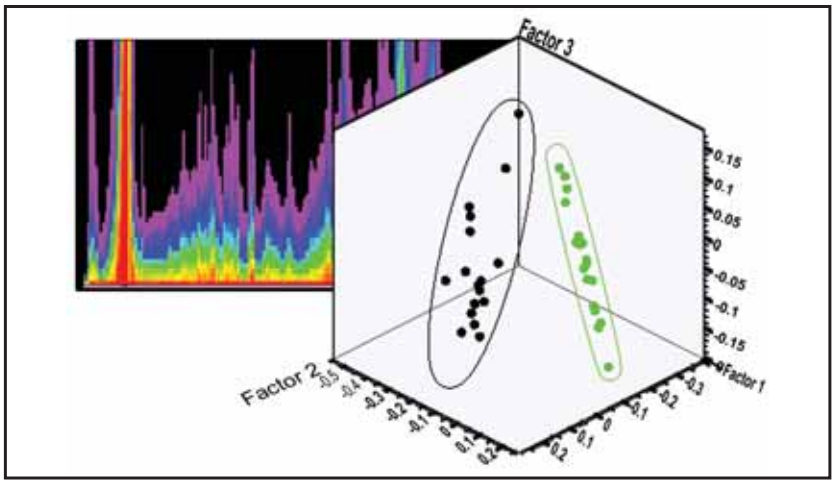
Link to Kyoto Encyclopedia of Genes and Genomes (KEGG*)



Biochemical pathway of identified biomarker

BIO-RAD

From NMR FIDs to Biomarker Identification



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*Kyoto Encyclopedia of Genes and Genomes:
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Invited Speaker Abstracts

QTL analysis of metabolomics data using *Arabidopsis* RI lines

Ric de Vos[1,5], Joost Keurentjes[2,3], Jingyuan Fu[4], Arjen Lommen[1,6], Harro Bouwmeester[1,5], Robert Hall[1,5], Raoul Bino[1,3,5], Ritsert Jansen[4], Dick Vreugdenhil[3] & Maarten Koornneef[2,7]

1 Centre for Biosystems Genomics, Wageningen, The Netherlands.
2 Laboratory of Genetics, Wageningen University, The Netherlands.
3 Laboratory of Plant Physiology, Wageningen University, The Netherlands.
4 Department of Bioinformatics, Groningen University, The Netherlands.
5 Plant Research International, Wageningen, The Netherlands.
6 Institute for food safety, RIKILT, Wageningen, The Netherlands.
7 Max Planck Institute for Plant Breeding Research, Cologne, Germany.

Plants are known to contain a huge array of metabolites present at highly variable levels, including the large and often economically important group of secondary metabolites such as terpenoids, alkaloids, saponins, flavonoids, glucosinolates, polyamines, and all kinds of derivatives thereof. However, it is still poorly understood to what extent this variation in plants has a genetic basis, which might be valuable in plant breeding. To address this issue, we apply genetical metabolomics approaches integrating large-scale metabolite analyses and genetics. Instead of focusing on specific metabolites (targeted metabolomics), essentially untargeted metabolomics approaches are used consisting of LCMS- or GCMS-based profiling of crude extracts followed by automated mass peak extraction and alignment, and statistical analyses of the mass signal intensities obtained.

In this presentation we will give recent examples obtained by reversed-phase LC-QTOF MS profiling, using Arabidopsis thaliana as model. This method uncovered many qualitative and quantitative differences in (mainly secondary) metabolites between different ecotypes, grown under identical conditions, with only 13% of the mass signals being present in all genotypes. Profiling of a Recombinant Inbred Line (RIL) population, derived from the two most divergent ecotypes, and subsequent Quantitative Trait Locus (QTL) analysis identified at least one QTL for about 75% of the mass signals. More than one-third of the signals were not detected in either parent, demonstrating the large potential for modification of metabolic composition through classical breeding. Subsequent identification of statistically relevant signals, using MS/MS and exact mass analyses, not only confirmed some known metabolite QTLs, thus validating our untargeted approach, but also identified novel metabolite QTLs and biosynthetic steps. These data show the power of untargeted metabolomics combined with genetic analyses in the identification of loci controlling metabolic profiles. This can lead to the identification of the underlying genes and the construction of biochemical networks in relation to other phenotypic traits.

Molecular traffic through metabolic networks: data, principles and prediction

Robert Schütz, Annik Nanchen, Eliane Fischer and Uwe Sauer

Institute of Molecular Systems Biology, ETH Zurich, Switzerland

Direction and rate of molecular fluxes through metabolic networks depend on thermodynamic principles, kinetic properties of the participating enzyme(s), and a complicated regulatory network that includes transcriptional and allosteric regulation. Hence, intracellular fluxes are the functional output of integrated biochemical and genetic interactions within complex metabolic networks that are pivotal for understanding of network operation (1). In contrast to the directly measurable concentrations of metabolites and proteins, however, fluxes are per se non measurable and must be inferred from measurable quantities. For this reason, quantification of intracellular fluxes has long lagged behind our capability to track global metabolite, mRNA or protein concentration changes.

With recent advances in 13C-labeling experiments, large-scale experimental analysis of intracellular fluxes is now feasible (2). After a brief introduction of the principles of flux analysis, general principles of carbon traffic that include network rigidity and robustness will be discussed for model microbes. The control of flux distribution will be addressed by focusing on the transcriptional regulation network that overlays metabolism. How such knowledge can be used to model the behavior of large metabolic networks will then be addressed for the example of predicting flux responses to gene deletions and environmental conditions.

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Phytochemicals as prominent constituents of the human metabolome - from dietary exposure to health effects

Augustin Scalbert[1], Rafael Llorach[1], Claudine Manach[1], Estelle Pujos[1], Jean-François Martin[1], Marie Agier[1], Johanna Lampe[2]

1 UMR 1019, Unité de Nutrition Humaine, INRA, Centre de Recherche de Clermont-Ferrand/Theix, Saint-Genes-Champanelle, France.
2 Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

Hundreds of phytochemicals are regularly ingested with our food. Among them, polyphenols, carotenoids, phytosterols or glucosinolates participate to the prevention of diseases such as cardiovascular diseases, cancers, osteoporosis or diabetes. Metabolomics offers considerable promise to better characterize both their intake and effects on the metabolism and should contribute to better understand their effects on health. We have characterized the urinary metabolome in human subjects and rats submitted to various diets containing either isolated phytochemicals or fruit and vegetables rich in phytochemicals. Urine samples were analyzed by LC-QTof and the markers identified by comparison with freely accessible databases such as HMDB or KEGG for endogenous metabolites or for phytochemical metabolites with a proprietary database currently developed in our laboratory. These studies show that phytochemical metabolites are major sources of variability in urine metabolome fingerprints. The phytochemical metabolome provides unique markers to compare exposure to a large variety of phytochemicals or foods in different individuals and populations and thus search for correlations with disease risk factors. Analysis of the phytochemical metabolome also provides novel insights on the metabolism of phytochemicals in the body. Its in-depth characterization should allow better delineating the endogenous metabolome in cohort studies.

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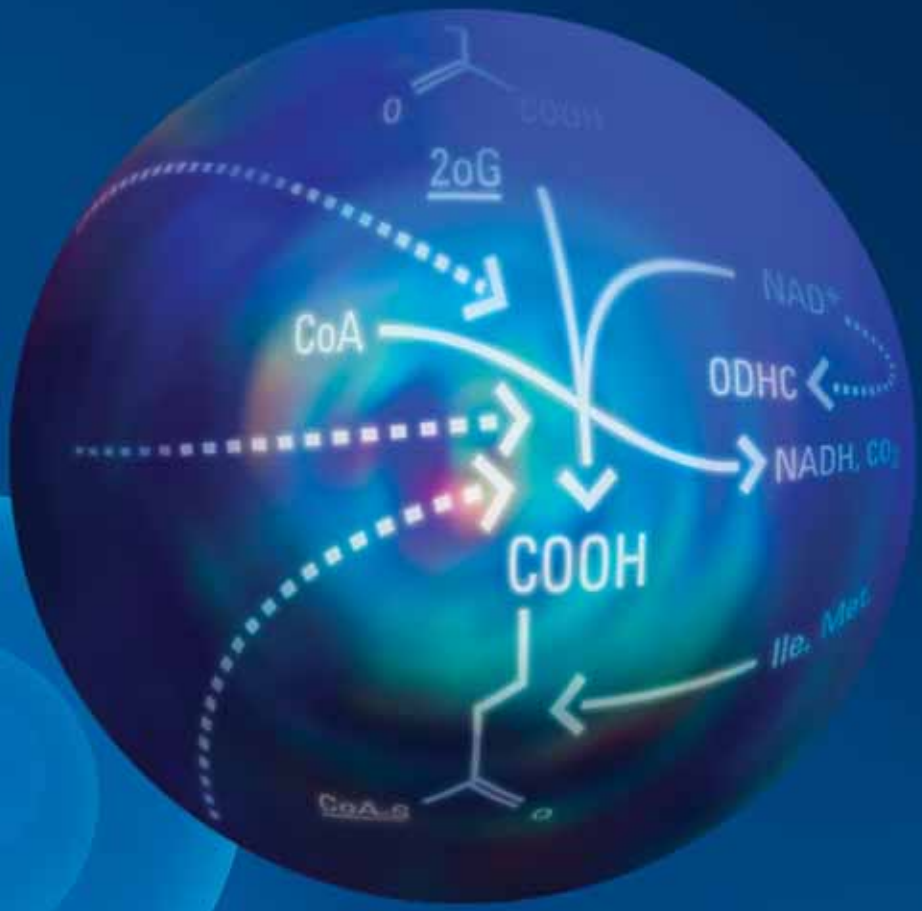

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Invited Speaker Abstracts

The application of metabolic profiling in preclinical and clinical drug development

Ina Schuppe-Koistinen

AstraZeneca, Sweden

Monitoring the metabolome allows the study of normal homeostasis as well as the effects of drugs and toxicity. An important potential of metabolic profiling is the possibility to develop "fingerprints" of diseases or cellular responses to classes of compounds with known common biological effect. Such fingerprints have the potential; to allow classification of disease states or compounds, to provide mechanistic information on cellular perturbations and pathways, and to identify biomarkers specific for disease severity, drug efficacy and toxicity. Potentially, this will lead to the development of tools for the prediction of mechanisms based on fingerprints of biomarker and pathway responses and should facilitate selection of compounds for drug development and increase the efficiency of toxicological testing strategies.

Probing seed development by metabolic flux analysis

Jörg Schwender,

Biology Department, Brookhaven National Laboratory, Upton, USA

Seeds are the major economic value of agricultural crops. In particular oilseeds may become a major renewable source of reduced carbon for the chemical industry in the future. To characterize partitioning of maternal carbon resources into different seed storage products, central metabolism in developing seeds of rapeseed (Brassica napus L) was studied in cultured embryos isolated from developing seeds. Using different 13C-labeled tracers in steady state labeling experiments, we could determine metabolic flux during seed filling in part with resolution of sub-cellular compartmentation. The capabilities and limitations of different approaches of flux analysis for the analysis of plant primary metabolism will be exemplified on basis of the unconventional metabolic function that we recently could assign to RuBisCO (Ribulose 1,5-bisphosphate carboxylase/oxygenase). During B. napus seed oil synthesis with the conversion of sugars to fatty acids, RuBisCO acts in a novel metabolic context, bypassing steps of glycolysis and recycling CO2. In addition, due to the observed use of amino acids as nitrogen sources in developing embryos, PEP carboxylase and pyruvate kinase appear not to constitute the key branch point for the control of amino acid synthesis - as described for autotrophic leaves. It turns out that the function of well known metabolic pathways of central metabolism cannot be understood independently but must be analyzed quantitatively in the context of the metabolic network.

Metabolic responses of *Plasmodium falciparum* to anti-malarial drugs

Vladimir Shulaev[1], Sunil Bajad[1], Joel Shuman[1], John M. Pisciotta[2], Wei Sha[2], Dominique Rasoloson[2], Lirong Shi[2], Oluwatosin Ginsanrin[2], and David Sullivan[2]

1 Virginia Bioinformatics Institute, Virginia Polytechnic Institute & State University, Blacksburg, USA; 2 John Hopkins Bloomberg School of Public Health, John Hopkins University, Baltimore, USA.

Malaria is a major threat in the developing world, with more than 1 million clinical episodes and 3000 deaths every day. Current antimalarial drugs affect specific developmental stages of Plasmodium falciparum, although the precise mode of action for the widely used quinolines and artemisinins is still controversial. The continued progression of resistance to antimalarials in P. falciparum requires the development of new drugs with original modes of action that target a specific developmental stage of the parasite.

Global transcriptional analysis of the P. falciparum response to diverse drugs has shown very limited or no variation in nearly all of the functional genes. Metabolomics, therefore, can provide an alternative platform to study stage-specific metabolite profiles that can potentially lead to validation of quinoline and artemisinin targets and to development of novel antimalarial drugs.

We have studied the metabolic phenotypes of the intra-erythrocytic Plasmodium faciparum (clone 3D7) ring and trophozoite developmental stages. Data was obtained through a combination of a non-targeted GC-MS and a targeted and non-targeted LC-MS based metabolomics approach.

P. falciparum invades a metabolically defined mature erythrocyte lacking nucleus and ribosomes with a limited number of active pathways. The early 10-12 hour ring stage-infected erythrocytes have a distinct profile from uninfected erythrocytes. The trophozoite infected erythrocytes are markedly transformed with many novel metabolites. One group of compounds that are differentially expressed at different developmental stages includes several amino acids like L-threonine, L-alanine and L-lysine. A simple interpretation relates the amino acid differences to hemoglobin catabolism as threonine represents 6% of the amino acid in hemoglobin, while alanine is 13%. These amino acids may be an independent marker of hemoglobin degradation and differential export, as many amino acids in hemoglobin are exported.

We will present data on the metabolic differences between the un-treated trophozoite developmental stage of P. falciparum, and the same stage following treatment with several antimalarial drugs, including chloroquine and artemisinin.

Novel approaches for small molecule detection

Gary Siuzdak

Scripps Center for Mass Spectrometry, La Jolla, USA

Quantitative global analysis of endogenous metabolites from cells, tissues, fluids or whole organisms - metabolomics, is becoming an integral part of functional genomics efforts as well as a tool for finding diagnostic biomarkers. Where proteomics is largely enabled by the predictable fragmentation pattern of peptides, metabolomics is complicated by the tremendous chemical diversity of metabolites. The experimental aim in our global metabolomics studies is to obtain a comprehensive quantitative with an unbiased view of the metabolome, a key aspect to this goal is the mass spectrometry ionization event. We have explored multiple ionization methods and novel mass spectrometry platforms including both solution-based approaches as well as novel surface-based mass spectrometry, such as nanostructure-initiator mass spectrometry (NIMS). These platforms will also be presented in the context of specific biochemical applications such as neonate screening, gut microbes, and tissue imaging.

Metabolomics as an essential module in Systems Biology models

Age K. Smilde

Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands and TNO Systems Biology, Zeist, The Netherlands

Systems biology is the study of biology as an integrated system of genetic-, protein-, metabolite-, cellular- and pathway events that are in flux and interdependent. Due to the availability of advanced instrumentation it is possible to generate very complex data sets and a systems biology approach becomes a possibility.

Metabolomics can and should play a role in systems biology models. This role can be fulfilled in several ways, depending on the type of systems biology model and the level at which such a model is made. Although the field of systems biology is rapidly evolving, some general trends on combining metabolomics with systems biology models can be seen, e.g. by incorporating metabolic networks in systems biology models. Some of these trends will be highlighted and an attempt will be made to categorize those. This will be illustrated with some real-life examples and also some ideas will be given for future


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Invited Speaker Abstracts

The OPLS and O2PLS approach in metabolomics and metabonomics

Johan Trygg

Research group for Chemometrics, Umeå University, Sweden

The most common chemometrical tool used in the evaluation of complex biological data is principal component analysis (PCA). PCA is always recommended as a starting point for analyzing multivariate data and will rapidly provide an overview of the information hidden in the data. Unfortunately, often the PCA method is the only tool applied as opposed to using modified and better suited multivariate methods when a priori information is available.

A novel extension of PLS, called OPLS1 can be used for modeling two classes of data to increase the class separation, simplify interpretation, and find potential biomarkers as well as provide an understanding of the intraclass variation. The S-plot is a visualization tool for multivariate classification models having two or more classes. The S-plot helps identifying putative biomarkers based both on contributions to the model as well as their reliability. An extension of the S-plot, the SUS-plot (Shared and Unique Structure), is applied to compare the outcome of multiple classification models with a common reference.

The O2PLS method is used for the huge task of combining and integrating multiple data tables obtained either from an array of profiling technologies, or different compartments. In this context, traditional chemometric methods (e.g. Ridge regression and PLS regression) do not have the proper model structure to describe these data structures. The O2PLS2 model has that proper model structure and fits very nicely into the multi-block framework required for any systems biology approach. The benefits of the OPLS method and its extensions O2PLS and OPLS-DA4 are improved model diagnostics, interpretation3 and quality control. Examples from human and plant biology will be presented and discussed.

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Desorption electrospray ionization (DESI) MS: a new tool for metabolomics

Justin M. Wiseman[1], Nari N. Talaty[2], Demian R. Ifa‡, Ayanna Jackson‡, R. Graham Cooks‡

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Desorption electrospray ionization (DESI) mass spectrometry enables rapid analysis of biological fluids and intact tissue preparations at atmospheric pressure without chromatographic separation. In DESI, pneumatically directed charged solvent droplets impact the surface generating secondary droplets containing the analyte. In this presentation, two key developments are emphasized which relate to metabolomics studies. The first is the ability to obtain rich spectral information on serum and urine samples without prior chromatographic separation. Preliminary data is shown in a study of serum samples collected from 60 patients (30 control and 30 cancer) with breast cancer. Distinct lipid and metabolite profiles were recorded using DESI-MS that distinguish the diseased versus non-diseased states of the individuals. Previously data was obtained on mouse urine samples to distinguish lung cancer from control samples successfully. Advanced statistics were applied to both data sets to further discriminate the diseased and non-diseased samples. The second development is the ability to record two-dimensional images of surfaces. Recording spatial and molecular information, simultaneously, of surfaces is a particularly powerful approach, especially when limited preparation of the surface is required and the sample is maintained under ambient conditions. This allows faster throughput and the ability to record information on samples in situ. Here, two-dimensional imaging of brain tissues is presented and were recorded based on the presence of specific lipids with a spatial resolution of less than 400 µm. Furthermore, studies showing the distribution of clozapine, an atypical antpsychotic, in rat brain and lung tissue throughout the course of a typical pharmacokinetic experiment will also be discussed. In these studies, the detection of clozapine in the tissue is confirmed by tandem mass spectrometry (MS/MS), as compared to the MS/MS of the authentic compound, and HPLC MS/MS. The results discussed here demonstrate the capacity of DESI-MS for rapid analysis of biological fluids without chromatographic separation and the ability to record two-dimensional images of tissue samples.

The human metabolome project: an update for 2007

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For the past 3 years the Human Metabolome Project Consortium (HMP) has been working on trying to obtain a complete or nearly complete characterization of the human metabolome. This project has had two main phases. The first phase was called "backfilling", and the second phase was called experimental validation. The backfilling phase, which is now largely complete, involved the compilation of information about what was previously known about human metabolites. This required the development of a number of novel software tools and considerable hand curation. This data is now available in several freely available electronic databases including

1) The Human Metabolome Database (HMDB)
2) DrugBank and
3) FooDB.

I will briefly describe these databases and provide and update on their current status, their content and potential applications. The second phase, which involves validation (and potential discovery of novel compounds), is now well underway.

We are in the process of completing a full characterization of the human CSF metabolome, urine metabolome and blood metabolome using a combination of LC-MS, NMR and GC-MS. An update on the status on these efforts will also be provided.

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Reconstructing metabolic networks from perturbation experiments

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The advances recently achieved in experimental techniques like gas/liquid chromatography and mass spectrometry allow us to separate and identify an increasing number of chemical compounds within the cell. Moreover, the identification of these metabolites is not restricted, as in the past, to steady-state measurements, but it can also take advantage of dynamic measurements in perturbation experiments (e.g. pulse experiments). The availability of such time-resolved metabolic data offers the opportunity to reconstruct on large-scale the functional relations between metabolites, by means of data-driven approaches, previously developed for the discovery of genetic networks (and based on micro-array data). Discovering the structure of a metabolic network means to determine how metabolites (indirectly) interact. Metabolite interactions are, in fact, always mediated by enzymes, so effects of one metabolite level on the other is either caused (i) by mass action, when the two metabolites are involved in the same chemical reaction, or (ii) by allosteric enhancement/inhibition of enzyme activity, when the metabolites participate in different reactions. In this work, we first show how such an identification problem, can be formulated as a (linear) regression problem. Then we discuss how it can be negatively affected by the dependencies in the time-series data (introduced by an improper excitation of the system, by moiety conservations, and by under-sampling of fast dynamic modes). Finally, as a possible solution to the above challenges, we investigate the least absolute shrinkage and selection operator (LASSO) algorithm. The choice of LASSO is primarily motivated by the beneficial effects introduced by the regularization term of LASSO: it can (at least partially) alleviate the problem of the dependencies in the data. The key idea of LASSO is to minimize a quadratic function of the prediction error (difference between the measured metabolite levels and the output estimated by a linear model of the metabolic network), plus an additive term penalizing solutions with many nonzero interactions. As a consequence, the algorithm performs a feature selection and the solutions provided are sparse metabolite-metabolite interaction matrices (many interactions are set to zero), in agreement with the evidence that, in general, the metabolites in a metabolic network only influence the concentrations of a limited number of other metabolites. This work has been entirely carried out by in silico experiments with artificial metabolic pathways, with the goal of providing useful tools for the evaluation of existing and future experimental metabolic data. Since LASSO in combination with standard pulse-experiments data, does not perform satisfactorily in reconstructing the benchmark metabolic networks, we have investigated possible pre-processing steps on the data to (partly) remove the dependencies, we have considered the issue of designing better perturbation experiments, in order to generate more informative data-sets, and, finally, we have considered the issue of incorporating prior knowledge in the form of constraints dictated by physical and chemical laws (e.g. elemental balance, thermodynamics constraints, etc).

Metabolic profiling and statistical genomics of insulin resistance

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The study of human multifactorial diseases like insulin resistance is a real healthcare challenge for the western and developing world (1). Animal models of human disease can be used to decipher genetic from environmental variation. In that regard, high-throughput "-omics" biotechnologies like genomics, transcriptomics and metabonomics are invaluable tools for investigating insulin resistance-related pathologies (type 2 diabetes, obesity, non-alcoholic fatty liver disease). In this lecture, I will show how metabolic profiling can be used as a structuring tool to understand the effect of microbial metabolism in insulin-resistance (2). I will then show the integration of metabolic profiles with genome-wide genotyping (3) and expression profiling data to extend the spectrum of rodent genetics to the study of transgenomic interactions between the symbiotic gut flora and the mammalian host.

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Simplivariate methods: Creating interpretable metabolomics models

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A typical metabolomics data set contains hundreds of metabolites and a (relatively) small number of experiments. Much used exploratory data analysis methods for these types of data are mainly projection and clustering methods. Projection methods aim at representing the complete dataset in a lower dimensional subspace, while clustering methods aim finding groups in the data based on some similarity measure. However, none of these methods takes into account the interpretability of the resulting model. Metabolomic profiling data comprise informative as well as non-informative parts and these should ideally be separated. From a biological point of view, an appropriate method should be able to identify groups of biochemical compounds that are functionally related and relevant for the problem under study. Most of the explorative data analysis methods do not fulfill these requirements. In this presentation we demonstrate new data analysis tools that can improve the interpretation of large scale metabolomics data. We refer to models that describe the data in an interpretable way and use only the informative part of the data as simplivariate models. The biological basis as well as the data analytical consequences and statistical framework of simplivariate models will be addressed. Applications and limitations of two existing methods, Interpretable Dimension Reduction (IDR), and PLAID, will be discussed, based on results for real metabolomics datasets. Furthermore, results of steps taken in the development of new simplivariate methods are presented.

Mapping metabolic pathways in the metal reducer, shewanella oneidensis MR-1

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Our research focuses on understanding the general metabolism of Shewanella oneidensis MR-1. This microorganism is an attractive candidate for bioremediation because of its metabolic versatility. It has the capacity of utilizing a wide range of terminal electron acceptors in respiration, including toxic metals, such as uranium and chromium. Based on previous biochemical studies and recent complete genome sequence, several unusual features of S. oneidensis metabolism have been investigated but not rigorously verified by 13C tracer experiments. In our study, 13C isotopomer analysis is applied to determine the actual balances of intracellular metabolic fluxes under

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various oxygen conditions. By feeding 13C labeled lactate to S. onedensis MR-1, Nuclear Magnetic Resonance (NMR) and gas chromatography mass spectrometry (GC-MS) are coupled to analyze labeling patterns of proteinogenic amino acids, and further to quantify metabolic fluxes in the key central metabolic pathways. It is important to gain insights into intracellular metabolism of S. oneidensis because it might help us improve its metal reduction ability through rational metabolic engineering. The metabolic flux distributions under aerobic and microaerobic conditions were determined and several active pathways were identified. The tricarboxylic acid (TCA) cycle was the main carbon metabolism route. Although MR-1 is genetically fairly closed to Escherichia coli, it has higher fluxes through the serine oxidation metabolism and lower fluxes through the pentose phosphate (PP) pathway. It also substitutes the Entner-Doudoroff (ED) pathway for the common glycolysis pathway. There is no clear explanation for the presence of the detected futile cycles involving the reactions, pyruvate Â® malate Â® oxaloacetate Â® phosphoenolpyruvate. These cycles might help to increase the flexibility in central carbon metabolism to allow MR-1 to utilize various electron acceptors, or to maintain stability in central carbon metabolism for survival under environmental stresses. These findings provided us the first insights into the general metabolism in MR-1 under aerobic conditions. Ability to use amino acids as carbon sources is also widespread in MR-1. Therefore, we are interested in probing specific metabolic utilization in MR-1 by using 13C labeled amino acids. It is relevant to understand the amino acids transport mechanisms before choosing a particular labeled amino acid to target the specific metabolic pathway. Different growth profiles were observed when supplementing various amino acids to MR-1 grown on lactate as the primary carbon source. From the one-dimensional (1D) 1H presaturation NMR spectra of the cell extra-cellular supernatant, utilization rates of lactate and amino acids were measured. Four different utilization categories with respect to lactate usage were found when supplementing with a single amino acid. When a mixture of sixteen amino acids was fed to the cells, several amino acids shared common transport mechanisms while others used specific transport mechanisms. We are currently trying to correlate the observed amino acid transport behavior with existing bioinformatics information.

GC-EI-TOF-MS analysis of in-vivo carbon-partitioning into soluble metabolite pools of higher plants by monitoring isotope dilution after $^{13}CO_2$ labelling

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The established GC-EI-TOF-MS method for the profiling of soluble polar metabolites from plant tissue was employed for the kinetic metabolic phenotyping of higher plants. Approximately 100 typical GC-EI-MS mass fragments of trimethylsilylated and methoxyaminated metabolite derivatives were structurally interpreted for mass isotopomer analysis, thus enabling the kinetic study of identified metabolites as well as so-called functional group monitoring of yet non-identified metabolites. The monitoring of isotope dilution after $^{13}CO_2$ labelling was optimized using Arabidopsis thaliana Col-0 or Oryza sativa IR57111 plants, which were maximally labelled with 13C. Carbon isotope dilution was evaluated for short (2 h) and long term (3 days) kinetic measurements of metabolite pools in root and shoots. Both approaches were shown to enable the characterization of metabolite specific partitioning processes and kinetics. Simplifying data reduction schemes comprising calculation of 13C-enrichment from mass isotopomer distributions and of initial 13C-dilution rates were employed. Metabolites exhibited a highly diverse range of metabolite and organ specific half-life of 13C-label in their respective pools (13C-half-life). This observation implied the setting of metabolite specific periods for optimal kinetic monitoring. A current experimental design for the kinetic metabolic phenotyping of higher plants is proposed.

Validation of a metabolic profile for caloric intake in rats

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Over-nutrition and obesity are associated with increased risks of cancer at many sites. At the other end of the spectrum, laboratory research spread over 70 years has clearly established dietary or caloric restriction (CR) as the most potent and reproducible known means of increasing longevity and decreasing morbidity in mammals. While the direct extrapolation of the lessons of CR to humans remains unclear, it is does appear clear that nutritive intake is a major factor in human health. We have therefore developed serum metabolomic profiles that can identify ad libitum fed and caloric-restricted rats with a high degree of accuracy. These profiles are being adapted for use in human epidemiology studies, with several long-term goals, including objective analysis of diet in humans and individualized risk prediction for diseases involving metabolic components, such as breast cancer. Exploratory studies previously identified 93 redox-active small molecules from sera (measured by HPLC coupled with coulometric detector arrays) with potential to distinguish dietary groups in both male and female rats. Classification and predictive power were addressed using a series of megavariate data analysis approaches in both open and blinded analyses (Clustering, PCA, Knn, SIMCA, PLS-DA, O-PLS). Notably, we found that the use of appropriate algorithms (eg, PLS-DA) allowed us to distinguish such diets across several years, even when the signal due to caloric intake was apparently swamped by the cohort-cohort differences observed. We have now shown that profiles built using O-PLS are predictive in blinded studies across multiple cohorts spread out over several years.

Metabolomic analysis of bile acids as biomarkers of hepatobiliary toxicity

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The aim of this study was to profile endogenous metabolites that could serve as biomarkers of hepatobiliary toxicity. The liver plays a major role in metabolism and serves a number of functions in the body including glycogen storage, plasma protein synthesis, drug detoxification, and bile acid synthesis. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids. About 90% of the excreted bile acids are reabsorbed and recycled through enterohepatic circulation. Therefore, trace amounts of bile acids are circulating in blood and are excreted in urine. The increased levels of bile acids that are excreted in urine appear to correlate with hepatobiliary toxicity.

A generic metabolite profiling method was developed to analyze urine and serum components from toxicity studies using UPLC-LTQ-FTMS. Urine and serum samples were processed by simple protein precipitation with methanol. After removal of proteins by centrifugation at 14,000 RPM for 15 min, the supernatants were further diluted with water. Aliquots of these samples and bile acid standards were then injected into the LC-MS system for metabolite profiling. LC/MS analysis was performed on a Waters Acquity UPLC coupled to a Finnigan LTQ-FTMS using an Acquity UPLC BEH C18 column (1.0 x 100 mm, 1.7 µ particle

size) with a flow rate of 100 µL/min. Negative ion ESI-MS full scan data were acquired using FTMS with a mass range of 95- 950 m/z.

A single dose of genipin (75 mg/kg) was administrated intraperitoneally to male rats. Urine samples from the control and 75 mg/kg dose groups were collected overnight (over dry-ice) in metabolism cages for metabolomic analysis. LC-MS profiling of rat urine samples showed a significant increases in specific bile acids (>10-fold) in the 75 mg/kg dose group compared to the control group. The increase in bile acids in urine correlated with increases in AST, ALT, and total bilirubin in serum, as well as with the hepatocellular degeneration score. Urine and blood samples were collected from a second study using a proprietary Merck compound that caused liver toxicity in dogs. LC-MS profiling of the dog urine and serum samples from this study also showed a significant increase in bile acids. Similar to the genipin study, the increase in bile acids in urine and serum correlated with increases in AST, ALT, ALP, and bilirubin in serum. In conclusion, specific urinary bile acids may represent useful, non-invasive and accessible biomarkers for hepatobiliary toxicity.

^{13}C NMR isotopomer analysis of metabolic network regulation by the oncogene C-MYC during cell cycle entry

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Analysis of signaling events during cell cycle entry has allowed the discovery of many cell cycle regulators and aided the development of drugs for cancer therapy. Proliferative growth requires high levels of metabolites and regulation of energy generating metabolic pathways. However, detailed analysis of how oncogenes coordinate the regulation of the metabolome to meet this metabolic demand have not yet been conducted. One of the few genes capable of independently inducing cell cycle entry in the absence of growth stimulating mitogens is the oncogene c-Myc (Myc). This transcription factor is involved in the pathogenesis of many human cancers, and extensive ChIP and array analysis has demonstrated Myc's ability to regulate many genes including cyclins, CDKs and metabolic genes. To begin to investigate the metabolic events underlying Myc's ability to regulate cell cycle entry, we have used Myc knockout and wild type Rat1A fibroblasts to study the fate of [U-13C] glucose during serum induced cell cycle entry. This model is ideal for studies of metabolic events required for cell cycle entry because the myc-null cells have a pronounced delay in S phase entry on serum induction, and the confounding effects inherent in the over-expression of an introduced gene are avoided. Furthermore, extensive efforts to reconstitute the Myc phenotype using cyclins and other regulators of the cell cycle have been unsuccessful, suggesting a requirement for the increased expression of multiple genes such as those in a metabolic network. Prior to performing 13C NMR, we characterized the metabolic phenotype of the myc-null cells, which we found had severe mitochondrial dysfunction with low oxygen consumption, low mitochondrial membrane potential and high ROS production. We also demonstrated that the coupling of mitochondrial function and glycolysis are required for Myc-induced cell cycle entry, as treatment of myc-positive cells with small molecule inhibitors of oxidative phosphorylation reduced cell cycle entry and recapitulated the myc-null phenotype. Our NMR analysis, utilizing 1D 13C direct observe for isotopomer analysis and 2D 1H - 13C HSQC, 1H - 13C HMBC, 1H - 13C HCCH-TOSCY and 1H TOSCY analysis for metabolite identification, revealed that Myc induced the activation of multiple metabolic pathways, leading to increases in production of purines, pyrimidines, and amino acids required for synthesis of DNA, RNA and protein. Furthermore, our isotopomer analysis demonstrated that the expression of Myc increased flux through the TCA cycle and pentose phosphate pathway, and is thus linked to an overall increase in the flow of metabolites through these metabolic networks. These studies, combined with our small molecule inhibitor analysis, demonstrate that Myc-induced cell cycle entry involves coordinate regulation of the cell metabolome to meet the energy and metabolite requirements of cells entering cell cycle. Further work is required to characterize how these metabolic events are linked to Myc's dysregulation of cell cycle entry during neoplasia and these studies may reveal unique targets for cancer drug development or uncover biomarkers for detection of the diverse array of human cancers known to involve deregulated Myc expression.

Comprehensive metabolomic characterisation of lipoprotein fractions reveals differential lipoprotein-specific regulation of xenobiotic and pro-inflammatory metabolites in patients with metabolic syndrome

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The composition of lipoprotein particles at the molecular level is not well understood. New mass spectrometry technologies afford characterization of human metabolome at the level of detail that was not possible before. Therefore, it is expected metabolomic characterization of lipoproteins may provide novel insights into their roles in cardiovascular diseases. In order to characterise the lipid composition of lipoprotein fractions, we studied the metabolite composition of different lipoprotein fractions. Serum samples were collected from healthy individuals and patients with metabolic syndrome (MS), total 17 subjects. HDL (both HDL2 and HDL3), IDL, LDL, VLDL fractions and residue were separated from the total serum by ultra-centrifugation. Lipidomic profiling was performed using UPLC/MS platform separately for each fraction in both positive (ESI+) and negative (ESI-) ion modes, followed by a comprehensive identification of lipid species using tandem mass spectrometry. To optimize coverage of small molecules, GCxGC-ToF based metabolomic analysis was performed all fractions. For the purpose of validation, NMR analysis was performed on total serum samples. We detected significantly altered molecular composition in multiple lipoprotein fractions in patients with metabolic syndrome. LDL fraction of MS patients was characterized by increase of ceramides and long chain fatty acids. Several specific xenobiotic metabolites were increased in HDL. In IDL the pro-inflammatory urea cycle metabolites and amino acids were up regulated in metabolic syndrome subjects. The overall change in total serum metabolome was detectable, but less pronounced. In order to facilitate future interpretation of serum lipidomics data in context of lipoprotein composition, we have been also developing a novel method based on Bayesian regression model which estimates the amounts of metabolites in each of the lipoprotein classes given amounts of metabolites in serum. As a background data used for model building, we utilized the available data from the present lipoprotein study. Our metabolomic strategy provides for the first time evidence about the lipoprotein distribution of multiple pro-inflammatory and potentially toxic metabolites, as well as suggests a link between the lipoprotein and xenobiotic metabolisms.

Wine, soils and complex community interactions.

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Environmental metabolomics techniques can be applied to complex, multifactorial, agricultural issues. Wine making and appropriate land use for agriculture are two such issues. 1. Wine making

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is seen as an art as much as a science with quality affected by grape cultivar, environmental factors, harvest, crushing, fermentation, storage, aging and transport. Certain wine quality factors have now been investigated by metabolomics techniques. Bunch shading is an environmental variable that has a direct effect on wine quality, e.g. mouth feel. These parameters have been measured by a trained tasting panel and the results compared with investigation of the same wines by NMR, LC and LCMS metabolomics techniques. Metabolomics analysis correlated well with the variables measured by the tasting panel and demonstrated that the bunch shading effect can have more influence on certain factors than grape variety. The correlation between the tasting panel analysis and metabolomics has implications for the monitoring of wine quality aspects throughout the grape to wine value chain and has the potential to offer wine makers new, less expensive tools to measure certain quality attributes. 2. Appropriate and environmentally sustainable land use depends on understanding soil function and how this can be manipulated. Despite the importance of organic content in soil (which can affect water use, the need for fertilisers and pesticides) little is understood about carbon form and how this may change with land use practice. Metabolomics analysis (NMR, NIR, LCMS) has allowed the identification of plant and microbial metabolites in certain soils. Change in land use has had a clear effect on soil structure, as indicated by metabolomics analysis of remnant (native state) and managed (farmed) land. Initial results are discussed with emphasis on some of the biological qualities of the soils.

Characterization of multi-age rodent pediatric models of toxicity

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The search for early biomarkers of pediatric drug toxicity should be initiated at each major stage of growth because of the rapidity of developmental changes. Gentamicin, and cisplatin were evaluated in a multi-age rodent toxicity model. Sprague-Dawley (SD) rats (10, 25, 40, 80 days old) thought to be developmentally equivalent to human infants, toddlers, young and mature adults were treated daily with gentamicin (50 or 100 mg/kg, s.c., for 6 or 14 days) or cisplatin (1, 3, or 6 mg/kg, s.c., 1 day) in separate studies to identify potential differences in pediatric vs. adult susceptibility to liver and kidney toxicity. Urine samples were collected for metabonomic analysis in gentamicin and cisplatin studies at 0, 8, 24, 48 and 72 hrs after initial dosing. Blood and tissues were collected at sacrifice for clinical chemistry, histopathological and omic analyses. Histopathology findings indicated that the patterns of liver or renal toxicity induced by these agents in the different age rats were quite dissimilar. The order of age-dependent sensitivity to gentamicin was 10>80>40>25 and to cisplatin was 80>40>25>10 days. The magnitude of change in gene expression level of a set of nephrotoxicity biomarkers in animals treated with gentamicin and cisplatin correlated with the extent and severity of renal pathology. The levels of urinary metabolic biomarkers of nephrotoxicity and evaluation of metabonomic data indicated a distinctly separate and unique pattern for each drug and different age groups. These findings indicate that multi-age animal models could be used as a means for predicting pediatric drug safety in preclinical and clinical studies.

Can the etiology of pneumonia be determined using metabolomics?

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Pneumonia is a catch-all phrase for any disease of the alveoli and respiratory bronchioles caused by an infectious agent. There are more than 100 different microbial species that can cause Community Acquired Pneumonia (CAP) including bacteria, viruses, parasites and fungi. CAP is the number one infectious cause of death and the sixth leading cause of death overall. However, the pathogen responsible for CAP can only be determined in approximately 30% of patients due to the difficulty of current testing procedures. Since there are many different causes of pneumonia, it is important to be able to quickly and accurately diagnose the source so that appropriate medical, pharmaceutical, and/or public health interventions may be employed. We have collected urine samples from over 1000 CAP patients from different geographic areas, as well as an extensive control set. Using urinary metabolomic profiles, we have discovered biomarkers for several of the pathogens that are not masked by patient co-morbidities. We have found that CAP etiology can be predicted with a sensitivity of over 80% and specificity close to 100%. Based on these profiles, as well as animal and cell culture models, we have been able to define specific metabolic fingerprints for several CAP pathogens that provide some mechanistic insights into the disease process.

Investigation of processing and imputation in metabolomics datasets

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Metabolomic data contain missing values for a number of reasons including instrumental limits of detection and improper data alignment between samples. While many supervised and unsupervised data analysis techniques have been used to analyze metabolomic data, the topic of how to process data and handle missing data has largely been ignored. These are well-studied issues in the field of microarrays that may have an important parallel to metabolomics datasets. We surveyed a number of techniques from the microarray literature and determined their applicability to metabolomics data. We used a publicly available GC-MS dataset of Medicago truncatula (ten replicates for each sample type of roots, stems, and leaves). We first ran the data through SpectConnect, a metabolomic data analysis tool, with 100% metabolite presence in all replicates in order to obtain a complete dataset with no missing values. We then processed the data in three different ways: no data pre-processing, log2 data transformation and log2 transformation with data whitening (zero mean and unit standard deviation). For each processed data version, we then deleted values at random 1) with a uniform prior distribution and total missingness of 1% to 20% and 2) with a nonuniform prior distribution such that 95% of the missing values are expected to come from the 10% smallest GC peaks, up to 5% total missingness. 100 such deletion datasets were created for each processed dataset. We imputed new values for the missing values of each deletion dataset using six different techniques found in the literature: imputation of a value of zero, imputation of the row average value, imputation via k-nearest neighbors analysis (KNN), imputation via linear least squares (LLS) analysis, imputation via iterated local least square imputation (ILLSimpute), and imputation via Bayesian principal component analysis (BPCA). Error with respect to the complete datasets was calculated based on a normalized root mean square error, and error values for each processing method/imputation method combination were averaged for the 100 iterations at each missingness value. For the uniform prior distribution, imputation with zeros yielded the highest error for every processing method. Imputation with no data transformation was comparable to imputation with log2 data transformation at very low missingness (1-5%). However, it yielded much higher errors at higher missingness. For those datasets with no data transformation, KNN yielded the lowest error values. Generally speaking, log2 transformation is

preferred at all levels of missingness and there is little difference between whitened log2-transformed data and log2-transformed data. In addition, the lowest overall error values were obtained with the BPCA and row-average methods. As expected, errors under the nonuniform prior distribution were much higher than those under the uniform prior distribution as the missing data was targeted towards those peaks with the lowest abundance. For these datasets, omitting log2 transformation was preferable to transformed data, and again both BPCA and row-average methods produced the lowest overall error values. At high missingness (4-5% of the total), all methods except imputation via row-average and zero produced extremely high errors. This work has shown that parallels can be drawn between the natures of microarray data and metabolomics data and those techniques used to process and impute missing microarray data can – and perhaps should- be applied to metabolomics data.

Large-scale multi-omics analyses for e.Coli systems biology

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We performed multiple high-throughput measurements to study the response of Escherichia coli cells to genetic and environmental perturbations. Making use of a set of nearly 4000 single gene mutants in E. coli we previously developed (Keio collection), we analyzed mutants associated with central energy metabolism. In addition, the non-mutated or wild type cells were also grown at different rates to observe the response to such changes. For each strain, an exhaustive global survey of intracellular components was performed using DNA microarrays, two-dimensional gel electrophoresis, and capillary electrophoresis mass spectrometry (CE-MS) to quantify messenger RNAs, proteins, and metabolites. We also performed a more detailed and precise analysis of 85 different intracellular RNAs, 57 proteins, and about 130 metabolites representing most components of energy metabolism, and simultaneously derived the metabolic fluxes through most reactions by combining quantitative measurements with a computational model of energy metabolism. Deletion of energy metabolism genes, in most cases, did not result in large compensatory changes in the level of RNAs, proteins, or metabolites. On the other hand, while significant changes in RNA and protein levels were seen upon changes in growth rate, the overall metabolite levels remained stable. The results thus demonstrate that E. coli can use different and complementary strategies to maintain a stable metabolic state, according to the circumstances, and also compensate for mutations through functional redundancy in its metabolic network.

Metabolite biomarkers of scleroderma elucidated using 1H NMR metabolomics

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Objective: A metabolic profiling approach used to investigate systemic sclerosis (SSc) compared with normal controls, as well as rheumatoid arthritis with the goal of identifying specific SSc metabolite biomarkers and metabolic pathways.

Methods: Sera from 25 SSc patients were analyzed and compared to samples from 15 healthy adults and nine rheumatoid arthritis (RA) patients. The sera were analyzed using 1H NMR spectroscopy, and concentrations of metabolites determined using a targeted profiling approach.

Results: A metabolite bioprofile of SSc was identified which included metabolites from a diverse set of pathways including amino acids, short chain fatty acids, purines, and energy-related metabolites. Each metabolite was found to be individually statistically significant relative to control levels using ANOVA testing ($p < 0.05$). MANOVA testing of the bioprofile indicates that the overall profile was highly discriminatory ($p = 2.8x10-7$). This result was corroborated by multivariate analysis using orthogonal partial least squares discriminant analysis (OPLS-DA) and preliminary validation conducted using permutation testing.

Conclusions: The scleroderma metabolite "bioprofile" identified in this study suggests alterations in several significant areas of metabolism.

- Elevation of the short chain fatty acids indicates elevated omega-oxidation and impaired mitochondrial beta-oxidation, and are unique to SSc.
- Decreased levels of oxidative stress related metabolites are indicative of damage by reactive oxygen species. There are some similarities to RA in the identified oxidative stress metabolites.
- Bioprofiling may have diagnostic and/or prognostic value in the clinical setting. Moreover identifiable patterns may assist towards individualized medicine, including monitoring disease activity and response to treatment. Our studies are ongoing and current data will be discussed.

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High-throughput gene discovery through integration of metabolomics and transcriptomics in arabidopsis thaliana

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The completion of the whole genome sequence of Arabidopsis thaliana has made it possible to discover the genes involved in metabolism in a high throughput manner by determining gene-to-metabolite correlation through the comprehensive analysis of metabolite accumulation and gene expression. In silico co-expression analysis of genes involved in flavonoid metabolism in Arabidopsis was performed using a publicly available transcriptome database of DNA microarrays. We inferred a co-expression framework model of the genes involved in the pathways of flavonol, anthocyanin, and proanthocyanidin synthesis, suggesting specific functions and co-regulation of the genes of pathway enzymes and transcription factors. Changes in flavonoid profiles of wild-type plants and T-DNA insertion mutants of the delimited genes led to the confirmation of gene function (Yonekura-Sakakibara et al., J Biol Chem, doi:10.1074/jbc.M611498200 (2007)). We also applied this strategy to glucosinolate biosynthetic pathway for identification of MYB transcription factors crucial for aliphatic glucosinolate production (Hirai et al., PNAS, 104, 6478 (2007)). These results suggest that the functional genomics approach by integration of metabolome with transcriptome co-expression analysis provides an efficient way of identifying novel gene functions involved in plant metabolism.

Strain improvement using metabolomics information

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Metabolomics is an emerging powerful functional genomics technology that involves the comparative, non-targeted analysis of the complete set of metabolites in an organism. By linking differences in the metabolomes to phenotypic properties, such are yield or productivity, using multivariate statistical approaches metabolomics targets or bottlenecks for strain improvement can be identified in an unbiased manner. Recently, we have set-up a quantitative metabolomics platform that allows the analysis of 'snapshot' metabolomes [1-4].

To demonstrate the merits of the combined metabolomics/multivariate statistics approach, we are studying phenylalanine production by

Escherichia coli. The strain used in this study was previously optimized and already produced 2.5 g/l phenylalanine. An experimental design was defined that resulted in large differences in the phenylalanine titer when E. coli was cultivated under different defined environmental conditions. Samples were taken from these fermentations, quenched to halt cellular metabolism and subsequently analyzed by comprehensive GC-MS and LC-MS techniques. The metabolomics data was analyzed with the multivariate data analysis tool PLS, in order to rank the relevant metabolites based on the strength of their correlation with the phenylalanine titer. Subsequently, from the biological interpretation of the function of the highest ranking metabolites, a number of genetic targets were identified. Mutants were constructed by overexpression or deletion of these target-genes and tested on their phenylalanine production under defined fermentation conditions. A marked increase in phenylalanine production was observed after a single genetic adaptation solely based on the metabolomics leads, showing that the selection of targets via the metabolomics-methodology is a valid approach.

Moreover, we have extended this 'static' metabolomics approach to a chronobiotechnology approach in which also time lag's in the induction of biological processes are taken into account, thus allowing the discrimination between cause and effect. When analyzing the longitudinal metabolomics data collected with the multiway multivariate statistical tool nPLS, compounds could be identified that correlated, all or not shifted in time, with the phenylalanine titer.

Reference List:

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Biomolecular imaging using tof-sims and buckminsterfullerene (C_{60}) primary ions to study spatial metabolite distribution in cells

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Cellular processes are often mediated by differential distribution of metabolites within the cell and between intracellular compartments. Consequently, contextual (co-)localization of metabolites within the cell defines their role in cellular processes. Investigating intracellular spatial distribution of metabolites is therefore a key component of metabolome analyses, and one that is likely to provide valuable information on the associated biochemical processes and in turn on biological systems behaviour. This aspect is often lost in global metabolomic approaches where the metabolites are extracted to be analysed. Imaging mass spectrometry offers a potential route to studying intracellular metabolite distributions. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) using buckminsterfullerene (C60) as the primary ion source has the ability to generate chemical images of surfaces with high sensitivities and minimal chemical damage. We are developing this technique for biomolecular imaging of biological cells and tissues. Here we present the application of C60+ to image cell surfaces and discuss its utility as a potential tool for studying spatial metabolite distributions, briefly highlighting the associated challenges with respect to sample preparation, ion yield enhancements and matrix influences. We will also present the application of C60+ to etch (erode) biological cell surfaces in a controlled manner and to subsequently image the revealed subsurfaces, in order to assess not only lateral but also depth wise distribution of molecular species. The application of multivariate analysis as an exploratory tool in such analyses will also be discussed. Studies on the distribution of secondary metabolites in Streptomyces coelicolor, a mycelial bacterium and of three dimensional distributions of molecular species in Xenopus laevis oocytes will be presented.

Can metabolomics be used for environmental monitoring in free living aquatic wildlife? - Building the weight of evidence

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Metabolomics has been applied successfully to fields such as drug discovery and toxicology, where the animals studied are genetically similar and housed in pristine conditions with a highly controlled environment and diet. Clinical metabolomics is considerably more challenging from the perspective of metabolic variability, but human subjects can at least give a medical history and can control their diet prior to sampling. The application of metabolomics to free living wildlife poses some significant challenges since the metabolomes of these organisms can be affected by a plethora of environmental factors that are typically not controllable by the researcher. Furthermore, even the most fundamental of genotypic and phenotypic (e.g., sex, age) traits of wildlife studied using metabolomics may not be known. Here we report the first large metabolomics study of free-living wildlife from the aquatic environment, and demonstrate that with appropriate rigorous characterisation of the study organism as well as optimised spectral processing, meaningful and interpretable metabolomics data can be obtained. Specifically, we report NMR based metabolomic studies on the marine mussel, the most widely utilised sentinel species in biomonitoring and aquatic ecotoxicology, and initially address the following questions: can metabolomics identify stress-induced phenotypes in animals experiencing a highly variable environment or must animals be stabilized in a laboratory prior to sampling? Is knowledge of genotype and phenotype required to interpret metabolic data from free-living wildlife? Contrary to expectation, laboratory stabilization increased metabolic variability in adductor muscle, masking the response to hypoxia, an environmental stress. From this we concluded that direct field sampling of mussels is the optimal strategy. The principal source of metabolic variability in mantle was gender-based, highlighting the importance of anchoring the metabolic measurements to known life history traits. Further studies identified metabolic differences between mussel species. Finally, we have characterised temporal changes in the metabolomes of three species of mussel, every month for one year, from two different field sites (one clean, one polluted). In addition to species and gender-specific metabolic fingerprints, our analyses have revealed seasonal changes in the mussel metabolome that are correlated with reproductive status. We therefore conclude that with rigorous characterisation of the sentinel organism, environmental metabolomics can identify metabolic changes associated with reproductive condition, which begins to build support for the use of this high throughput approach in environmental monitoring.

Notes

Notes

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