METABOLOMICS SOCIETY'S

5TH ANNUAL INTERNATIONAL CONFERENCE

AUG 30 - SEPT 2, 2009
EDMONTON, CANADA

Metabolomics Society
2009 Conference
Final Program

Day 1 – Sunday, August 30, 2009

Workshop 1 – NMR Workshop
Workshop 2 - Sample Preparation Workshop
Workshop 3 - Mass Spectrometry Workshop
Workshop 4 - Pathways and Statistics Workshop
Opening Reception with Posters

Day 2 – Monday, August 31, 2009

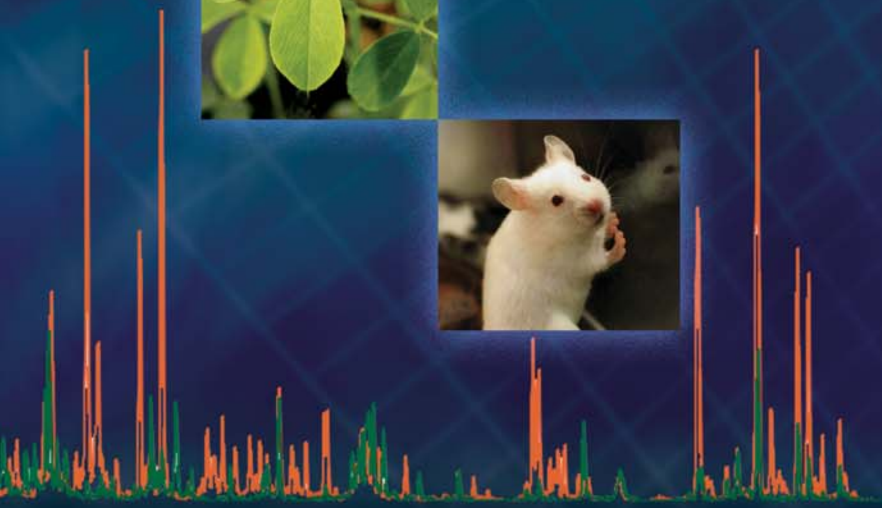
Keynote Plenary 1 - Kazuki Saito
Session 1 - Metabolomics and Nutrition
Session 2 - Biomedical Biomarkers
Session 3 - Pharmacometabolomics

Day 3 – Tuesday, September 1, 2009

Keynote Plenary 2 - Hans Westerhoff
Session 4 - Plant/Phytochemical Metabolomics
Session 5 - Fluxomics and Metabolic Engineering
Session 6 - Microbial/Environmental Metabolomics
Conference Dinner

Day 4 – Wednesday, September 2, 2009

Session 7 - Metabolomics and Informatics
Keynote Plenary 3 - Philip N. Baker
Session 8 - Novel Technologies and Imaging
Session 9 - National Metabolomics Initiatives



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The Fifth International Conference of The Metabolomics Society
August 30 – September 2, 2009
Crowne Plaza Hotel
Edmonton, Alberta, Canada

Schedule at a Glance

Sunday, August 30

Start	End	Event	Room
7:30 a.m.	7:00 p. m.	Registration	Promenade
8:30 a.m.	12:00 p.m.	**Concurrent Workshops 1& 2**	Ballroom A, Lacombe
8:30 a.m.	12:00 p.m.	**Workshop 1 – NMR** **8:30 a.m. – 9:15 a.m.** Jules Griffin, Chair, Cambridge University **9:15 a.m. – 10:00 a.m.** Aalim Welijie, University of Calgary **10:00 a.m. – 10:30 a.m. Break Room: Ballroom C** **10:30 a.m. – 11:15 a.m.** Risto Kauppinen, Dartmouth Medical School **11:15 a.m. – 12:00** Jules Griffin, Chair, Cambridge University	Ballroom A
8:30 a.m.	12:00 p.m.	**Workshop 2 - Sample Preparation** **8:30 a.m. – 9:15 a.m.** Ute Roessner, Chair, University of Melbourne **9:15 a.m. – 10:00 a.m.** Rick Dunn, University of Manchester **10:00 a.m. – 10:30 a.m. Break Room: Ballroom C** **10:30 a.m. – 11:15 a.m.** David deSouza, University of Melbourne **11:15 a.m. – 12:00 (Noon)** Daniel Jones, Michigan State University	Lacombe

12:00 (Noon)	1:00 p.m.	Lunch – Delegates on own	

1:00 p.m.	4:30 p.m.	Exhibit and Poster Set-up	Promenade Foyer & Ballroom B

1:00 pm	4:30 p.m.	**Concurrent Workshops 3& 4**	Ballroom A, Lacombe
1:00 p.m.	4:30 p.m.	**Workshop 3 – Mass Spectrometry** **1:00 p.m. – 1:40 p.m.** Thomas Hankemeier, Chair, Netherlands Metabolomics Centre **1:40 p.m. – 2:20 p.m.** Liang Li, University of Alberta **2:20 p.m. – 2:50 p.m. Break Room: Ballroom C** **2:50 p.m. – 3:30 p.m.** Mark Viant, University of Birmingham **3:30 p.m. – 4:10 p.m.** Robert Trengrove, Murdoch University **4:10 p.m. – 4:30 p.m.** David Heywood, Waters	Ballroom A
1:00 p.m.	4:30 p.m.	**Workshop 4 – Pathways and Statistic Room: Lacombe** **1:00 p.m. – 1:40 p.m.** David Wishart, Chair, University of Alberta **1:40 p.m. – 2:20 p.m.** Chris Steinbeck, European Bioinformatics Institute **2:20 p.m. – 2:50 p.m. Break Room: Ballroom C** **2:50 p.m. – 3:30 p.m.** Robert Haw, University of Toronto **3:30 p.m. – 4:10 p.m.** Jeff Xia, University of Alberta **4:10 p.m. – 4:30 p.m.** Theodore R. Sana, Agilent	Lacombe

5:00 p.m.	8:00 p.m.	Opening Reception with Exhibitors and Odd Numbered Poster Authors Present	Promenade Foyer & Ballroom B

Monday, August 31

Start	End	Event	Room
7:30 a.m.	9:00 a.m.	Registration	Promenade

Start	End	Event	Room
9:00 a.m.	9:45 a.m.	**Keynote Plenary**: Kazuki Saito, Riken Plant Science Center	Ballroom A

Start	End	Event	Room
9:45 a.m.	10:00 a.m.	Break	Promenade

Start	End	Event	Room
10:00 a.m.	12:00 (Noon)	**Session 1: Metabolomics and Nutrition** David Wishart, Chair	Ballroom A
10:00 a.m.	10:40 a.m.	Invited Speaker: Bruce German, UC Davis	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 1: Philip Britz-McKibbin, McMaster University	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 2: Wei Jia, University of North Carolina	Ballroom A
11:20 a.m.	11:40 a.m.	Podium Speaker 3: Richard Beger, US Food & Drug Administration	Ballroom A
11:40 a.m.	12:00 (Noon)	Podium Speaker 4: Bruce Kristal, Harvard Medical School	Ballroom A

Start	End	Event	Room
12:00 (Noon)	2:00 p.m.	**Lunch with Poster Authors and Exhibitors**	Promenade Foyer & Ballroom B

Start	End	Event	Room
12:30 p.m.	1:30 p.m.	**Luncheon Workshop – Agilent Technologies**	Lacombe

Monday, August 31

2:00 p.m.	4:00 p.m.	**Session 2: Biomedical Biomarkers** Matej Oresic, Chair	Ballroom A
2:00 p.m.	2:40 p.m.	Invited Speaker: John Ryals, Metabolon, Inc.	Ballroom A
2:40 p.m.	3:00 p.m.	Podium Speaker 1: Marko Sysi-Aho, VTT Technical Research Centre of Finland	Ballroom A
3:00 p.m.	3:20 p.m.	Podium Speaker 2: Robert Weiss, UC Davis	Ballroom A
3:20 p.m.	3:40 p.m.	Podium Speaker 3: Michael Milburn, Metabolon	Ballroom A
3:40 p.m.	4:00 p.m.	Podium Speaker 4: Takushi Ooga, Human Metabolom Technologies, Inc.	Ballroom A

4:00 p.m.	4:15 p.m.	Break	Promenade

4:15 p.m.	6:15 p.m.	**Session 3: Pharmacometabolomics** Rima Kadurrah Daouk, Chair	Ballroom A
4:15 p.m.	4:55 p.m.	Invited Speaker: Jules Griffin, Cambridge University	Ballroom A
4:55 p.m.	5:15 p.m.	Podium Speaker 1: Jerzy Adamski, German Research Center f Environmental Health	Ballroom A
5:15 p.m.	5:35 p.m.	Podium Speaker 2: Yuan Ji, Mayo Clinic	Ballroom A
5:35 p.m.	5:55 p.m.	Podium Speaker 3: Steven Gross, Weill Cornell Medical College	Ballroom A
5:55 p.m.	6:15 p.m.	Podium Speaker 4: Ewoud Van Velzen, University of Amsterdam	Ballroom A

6:15 p.m.	8:30 p.m.	**Reception with Exhibitors** and Even Numbered Poster Authors	Promenade Foyer & Ballroom B

Tuesday, September 1

Start	End	Event	Location
7:30 a.m.	9:00 a.m.	Registration	Promenade

Start	End	Event	Location
9:00 a.m.	9:45 a.m.	**Keynote Plenary:** Hans Westerhoff, Free University	Ballroom A
9:45 a.m.	10:00 a.m.	Break	Promenade
10:00 a.m.	12:00 (Noon)	**Session 4: Plant / Phytochemical Metabolomics** Robert Hall, Chair	Ballroom A
10:00 a.m.	10:40 a.m.	Invited Speaker: John Draper, Aberystwyth University	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 1: Peter Facchini, University of Calgary	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 2: Jane Ward, Rothamsted Research	Ballroom A
11:20 a.m.	11:40 a.m.	Podium Speaker 3: Lloyd W. Sumner, The Samuel Roberts Nobel Foundation	Ballroom A
11:40 a.m.	12:00 (Noon)	Podium Speaker 4: Ajjamada Kushalappa, McGill University	Ballroom A

Start	End	Event	Location
12:00 (Noon)	2:00 p.m.	**Lunch with Poster Authors and Exhibitors**	Promenade Foyer & Ballroom B

Start	End	Event	Location
12:30 p.m.	1:30 p.m.	**Luncheon Workshop – Thermo Scientific**	Lacombe

Tuesday, September 1

2:00 p.m.	4:00 p.m.	**Session 5: Fluxomics & Metabolic Engineering** Marta Cascante, Chair	Ballroom A
2:00 p.m.	2:40 p.m.	Invited Speaker: Teresa Fan, University of Louisville	Ballroom A
2:40 p.m.	3:00 p.m.	Podium Speaker 1: Thomas W. Binsl, VU University of Amsterdam	Ballroom A
3:00 p.m.	3:20 p.m.	Podium Speaker 2: Deyu Xie, North Carolina State University	Ballroom A
3:20 p.m.	3:40 p.m.	Podium Speaker 3: Fionnuala Morrish, Fred Hutchinson Cancer Research Center	Ballroom A
3:40 p.m.	4:00 p.m.	Podium Speaker 4: Hunter Moseley, University of Louisville	Ballroom A

4:00 p.m.	4:15 p.m.	Break	Promenade

4:15 p.m.	6:15 p.m.	**Session 6: Microbial/Environmental Metabolomics** Mark Viant, Chair	Ballroom A
4:15 p.m.	4:55 p.m.	Invited Speaker: Steven Oliver, Cambridge University	Ballroom A
4:55 p.m.	5:15 p.m.	Podium Speaker 1: Sara Cooper, University of Washington	Ballroom A
5:15 p.m.	5:35 p.m.	Podium Speaker 2: Manuel Liebeke, Ernst-Moritz-Arndt University Greifswald	Ballroom A
5:35 p.m.	5:55 p.m.	Podium Speaker 3: Myrna Simpson, University of Toronto	Ballroom A
5:55 p.m.	6:15 p.m.	Invited Speaker 4: Mark Viant, University of Birmingham	Ballroom A

7:00 p.m.	8:00 p.m.	Reception with Exhibitors Featuring the Dave Babcock Trio	Promenade

8:00 p.m.	11:00 p.m.	**Conference Dinner, Awards and Entertainment**	Ballroom A

Wednesday, September 2

Start	End	Event	Room
7:30 a.m.	9:00 a.m.	**Registration**	Promenade

Start	End	Event	Room
9:00 a.m.	11:00 a.m.	**Session 7: Metabolomics and Informatics** Roy Goodacre & Bruce Kristal, Chairs	Ballroom A
9:00 a.m.	9:40 a.m.	Invited Speaker: Oliver Fiehn, UC Davis	Ballroom A
9:40 a.m.	10:00 a.m.	Break	Promenade
10:00 a.m.	10:20 a.m	Podium Speaker 1: Karen W. Phinney, National Institute of Standards and Technology	Ballroom A
10:20 a.m.	10:40 a.m.	Podium Speaker 2: Piotr T. Kasper, Netherlands Metabolomics Centre	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 3: David Enot, Biocrates AG	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 4: Ajjamada Kushalappa, McGill University	Ballroom A

Start	End	Event	Room
11:20 a.m.	12:00 (Noon)	**Keynote Plenary:** Philip N. Baker (Manchester University and University of Alberta)	Ballroom A

Start	End	Event	Room
12:00 (Noon)	1:00 p.m.	**Lunch with Poster Authors and Exhibitors**	

Start	End	Event	Room
1:00 p.m.	3:00 p.m.	**Session 8: Novel Technologies & Imaging** Oliver Fiehn, Chair	Ballroom A
1:00 p.m.	1:40 p.m.	Invited Speaker: Richard D. Smith, PNL	Ballroom A
1:40 p.m.	2:00 p.m.	Podium Speaker 1: Wei Zou, UC Davis	Ballroom A
2:00 p.m.	2:20 p.m.	Podium Speaker 2: Steven Fischer, Agilent Technologies	Ballroom A
2:20 p.m.	2:40 p.m.	Podium Speaker 3: Kazuki Saito, Riken Plant Science Center	Ballroom A

Wednesday, September 2

3:15 p.m.	5:15 p.m	**Session 9: National Metabolomics Initiatives** Thomas Hankemeier, Chair	Ballroom A
3:15 p.m.	3:45 p.m	Invited Speaker 1: Thomas Hankemeier, Netherlands Metabolomics Centre	Ballroom A
3:45 p.m.	4:05 p.m.	Invited Speaker 2: Tony Bacic, University of Melbourne	Ballroom A
4:05 p.m.	4:25 p.m.	Invited Speaker 3: Kazuki Saito, Riken Plant Science Center	Ballroom A
4:25 p.m.	4:50 p.m.	Invited Speaker 4: Hans Vogel, University of Calgary	Ballroom A
4:50 p.m.	5:15 p.m.	Invited Speaker 5: Rima Kaddurah-Daouk, Duke University Medical Center	Ballroom A

5:15 p.m.	5:30 p.m.	Conference Closing	Ballroom A

Letter of Welcome

Dear Participants,

On behalf of the organizers of the 5th Annual Metabolomics Society Conference in Edmonton, we are delighted to welcome you to the meeting. We truly hope that you will enjoy the program that the Scientific Program Committee has put together.

We are particularly grateful for the efforts of the Scientific Program Committee members: Drs. Oliver Fiehn (USA), Jules Griffin (UK), Thomas Hankemeier (Netherlands), Choonghwan Lee (Korea), Matej Oresic (Finland), Ute Roessner (Australia), Kazuki Saito (Japan), and Hans Vogel (Canada). This international collection of distinguished scientists has put in many hours designing the program, mulling over speaker invitations, poring over abstracts, editing the conference booklet and trying their best to make this the most enjoyable and enlightening conference possible. Their many hours of volunteer effort are greatly appreciated and we are indeed fortunate to have worked with such an excellent team. We are also very grateful to the session and workshop chairs, many of whom volunteered their time in addition to their duties as either Program Committee members or Society Board Members. Certainly without their efforts and their time commitment, much of this could not have happened.

As with past Metabolomics Society Conferences, this meeting brings together both students and scientists from a wide range of disciplines and organizations, all of whom share a passion for understanding metabolism, metabolites and metabolic regulation. In trying to reach out to such a broad community we have tried to offer a little something for everyone. Indeed, the 2009 conference program has been designed to be an enlightening combination of basic and applied science with 3 plenary speakers, 4 workshops and 9 thematic sessions spanning such diverse areas as technology development, metabolic engineering, environment, nutrition, plant biology, biomedicine and bioinformatics.

For the first time ever we have partnered with an off-site, hands-on NMR metabolomics workshop offered through NANUC (Canada's National NMR Centre). We are hopeful that this will lead to other hands-on workshops in mass spectrometry, bioinformatics, separation technology or sample preparation in upcoming Metabolomics Society conferences. In addition, we hope you will be able to attend as many workshops and oral sessions as possible and that you will take the time to view the posters and visit the exhibitor booths. Of course conferences are also about meeting people, developing collaborations, sharing information, learning about new ideas and seeing parts of the world that you haven't seen before.

Please make sure to take some time off to explore the city of Edmonton and its surroundings. Edmonton is a real 'summer' city with long sunny days, pleasant daytime temperatures, plenty of outdoor cultural festivals and 1000s of acres of river valley parks to explore. It is also only a few hours away from the Rocky Mountains (to the west) and the fossil rich badlands (to the south). Hopefully you will be able to arrive before the conference or stay a few days after the conference to enjoy some Canada's most beautiful scenery and one of its most culturally diverse cities.

Best Wishes,

David Wishart, Program Committee Chair
Lloyd Sumner, President, Metabolomics Society

The Fifth International Conference of The Metabolomics Society August 30 – September 2, 2009

Organizing Committee

David Wishart (Conference Chair), University of Alberta, Edmonton, Canada

Oliver Fiehn, University of California, Davis, USA

Jules Griffin, University Of Cambridge, Oxford, UK

Thomas Hankemeier, Netherlands Metabolomics Centre, The Netherlands

Choong Hwan Lee, Korea

Matej Oresic, VTT Technical Research Centre of Finland, Espoo, Finland

Ute Roessner, Australian Centre for Plant Functional Genomics, University of Melbourne, Victoria, Australia

Kazuki Saito, Riken Plant Science Center, Japan

Hans Vogel, University of Calgary, Alberta, Canada

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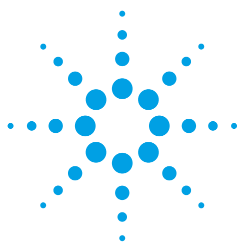

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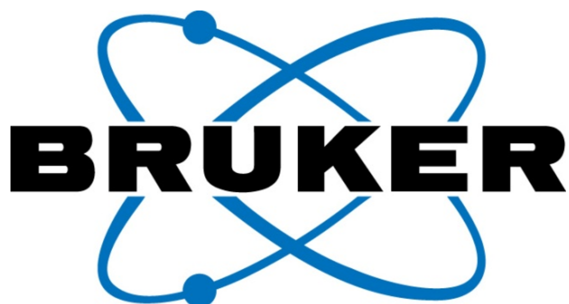

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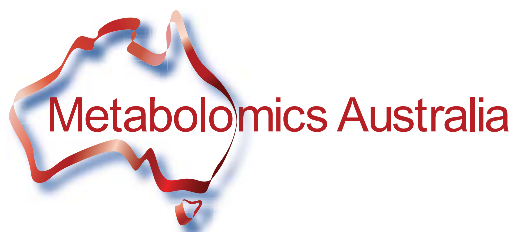

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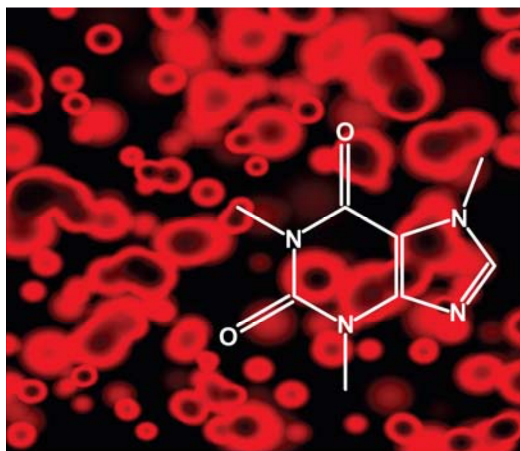

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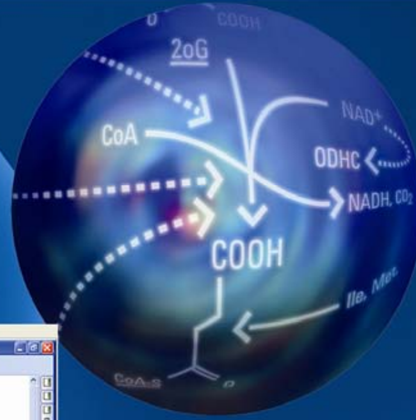

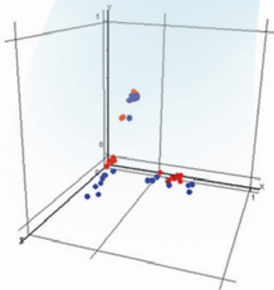

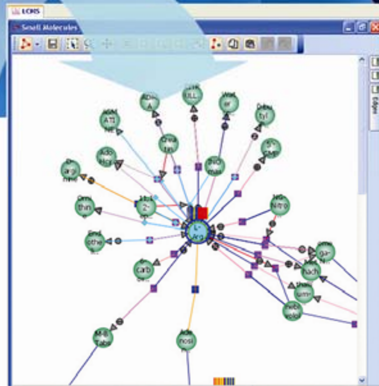

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General Information

Delegate Meals

Delegates are responsible for the purchase of breakfasts, lunches and dinners (except Conference Dinner September 1, which is included as part of the conference registration). Please confirm your attendance of the Conference Dinner at the registration desk and receive your banquet dinner ticket no later than 9:00 a.m. Tuesday, September 1. Dinner tickets must be presented upon entrance.

Delegate will find five (5) $5.00 meal coupons in their registration envelope. These can be used for meals at the conference hotel for food and non-alcholoic beverages in the hotel restaurants and/or lounge. They have no cash value and cannot be redeemed for cash. Lost coupons will not be replaced.

In addition, delegates receive four (4) drink tickets which can be redeemed during the receptions at the cash bars for a complimentary conference glass of beer or wine.

All conference sessions are held at the:
Crowne Plaza Edmonton-Chateau Lacombe
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Exhibition Hours:

Sunday, August 30
1:00 p.m. – 5:00 p.m. – Exhibition Set-up
5:00 p.m. – 8:00 p.m. – Reception with Posters and Exhibitors

Monday, August 31
9:00 a.m. – 6:15 p.m. – Exhibits Open
6:15 p.m. – 8:00 p.m. – Reception with Posters and Exhibitors

Tuesday, September 1
9:00 a.m. – 7:00 p.m. – Exhibits Open
7:00 p.m. – 8:00 p.m. – Pre-Dinner Reception in Exhibition

Wednesday, September 2
9:00 a.m. – 5:15 p.m. – Exhibits Open
5:30 p.m. – Exhibits Tear Down

Poster Presentations:

Authors of accepted posters should hang their poster according to their assigned poster number. Poster numbers are identified in this book. Posters are displayed for the duration of the conference. However, authors are expected to be with their posters, as shown in the schedule below, in order to present their poster research and findings to conference attendees.

Poster Hours:

Set-up: Sunday, August 30 – Posters placed between 1:00 p.m. – 5:00 p.m.

Authors should be present at their posters at the following times:

Presenters for odd numbered posters should be present Sunday, August 30, 5:00 p.m. – 8:00 p.m.

Presenters for even numbered posters should be present Monday, August 31, 6:15 p.m. – 8:30 p.m.

Exhibitors Listing

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Exhibitors Listing

Chenomx Inc.
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http://www.chenomx.com

> *Chenomx is a life sciences company offering world leading solutions for metabolomics research. Chenomx's patented technology platform combines state-of-the-art spectroscopic technology for the identification of metabolic markers with advanced algorithms to provide quantitative analysis of biological samples. Chenomx offers access to its technology platform via both software and services. Come visit us at our booth to find out more.*

Genome Alberta
#115, 3553-31st St. N.W.
Calgary, AB T2L 2K7
ph: 403-503-5222
fax: 403-503-5225
www.genomealberta.ca (look for Genomics on Facebook and on Twitter for mikesgene)

> *Genome Alberta is a not for profit organization that initiates, funds, and manages genomics research and partnerships. We were established in 2005 as part of Alberta's Life Sciences Strategy through an initiative between the Alberta Government and federally funded Genome Canada. Genome Alberta is based in Calgary but leads projects at institutions around the province and participates in a variety of other projects based across the country. We are one of Canada's 6 Genome Centres and work closely with these centres to advance the science and application of genomics, metabolomics and many of the related 'omics'.*

Human Metabolome Technologies, Inc.
246-2 Mizukami Kakuganji,
Tsuruoka, Yamagata
997-0052, Japan
www.humanmetabolome.com

> *Human Metabolome Technologies, Inc. (HMT) is a Japan-based bio-venture company utilizing CE-MS technologies to develop and commercialize metabolomic testing for drug discovery, diagnostic products development and fermention process optimization. HMT's Basic Plan provides primary metabolome analysis, which covers the major metabolic pathways, at an affordable price. We offer Basic Plan from just one sample.*

Exhibitors Listing

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www.metanomics-health.de

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University of Alberta
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Scientific Luncheon Workshops

*Please note participation is based on first come, first served and limited to the room capacity. A box lunch is provided by the sponsoring organization.

Monday, August 31

Agilent Technologies Luncheon Workshop

12:30 – 1:30 p.m. Room: Lacombe

Mass Profiler Professional statistical analysis and visualization software: a new chemometric package for mass spectrometry data

Theodore R. Sana
Senior Scientist
Agilent Technologies
Santa Clara, CA, USA

Agilent Mass Profiler Professional (MPP) is a chemometric software package designed specifically for processing mass spectrometric data, typically resulting from metabolomics, proteomics, food safety, environmental, forensics, and toxicology experiments. Using a combination of filtering steps, statistical analyses and model building, it manages and analyzes trends in complex MS data sets, enabling the analyst to classify, compare and analyze results from different platform technologies: GC/MS, LC/MS, CE/MS and ICP-MS, all in one project. In addition, an optional mzXML package enables processing of data from other vendor instruments. Navigation is easy and intuitive and includes a guided workflow for the newcomer, with pre-defined data import and analysis steps, so one can quickly perform a basic analysis. There is also an advanced workflow that includes tools such as PCA, PLS-DA, ANOVA, Clustering, ID browser and pathway analysis. All tools are wizard-based, with easily customizable settings. In addition, one can execute R scripts to further extend and customize statistical analysis and visualization capabilities. We have also developed a recursive feature extraction workflow, a unique feature of MPP, that lets you easily re-mine across data sets, resulting in an iterative improvement in the quality and accuracy of mass, retention time and abundance values for each candidate feature. It’s also easy to export an inclusion list for Q-TOF MS/MS analysis, and re-import the results back into MPP

Tuesday, September 1

Thermo Scientific Luncheon Workshop

12:30 – 1:30 p.m. Room: Lacombe

Accurate and Sensitive All-Ions Quantitation Using Ultra High Resolution LCMS and its Application to Endogenous Metabolite Profiling

Mark Sanders
Thermo Scientific

Metabolomics is an extreme example of multi-component quantitation. Here we demonstrate the use of ultra high resolution LCMS to provide sensitive, high quality quantitative data. This provides a simple, relatively unbiased and highly quantitative assessment of metabolomics samples and negates the need for multiple MS platforms. Typically screening is performed on a high resolution instrument and once potential biomarkers have been found more rigorous, absolute quantitative assessments are made to validate the initial observations using triple quadrupole SRM techniques. However, this approach requires MS/MS methods to be developed for each analyte, and as the number of analytes increases the advantages of using a triple quadrupole diminish. The move to smaller SRM dwell times to accommodate more components across narrow uHPLC peaks compromises both sensitivity and precision. We have observed that the use of high resolution LCMS provides high quality quantitative results comparable to that of a triple quadrupole when profiling multiple endogenous metabolites in urine or plasma. The instrument set up is very simple, with a full-scan over the mass range of interest and no prior knowledge of the analytes needed. Selectivity for the quantitative measurement is provided by high resolution MS and the use of narrow mass windows.

Workshop Speakers Program & Abstracts

Sunday, August 30

Start	End	Event	Room
8:30 a.m.	12:00 p.m.	**Concurrent Workshop 1 - NMR**	Ballroom A
8:30 a.m.	12:00 p.m.	**8:30 a.m. – 9:15 a.m.** Jules Griffin, Chair, Cambridge University **9:15 a.m. – 10:00 a.m.** Aalim Welijie, University of Calgary **10:00 a.m. – 10:30 a.m. Break Room: Ballroom C** **10:30 a.m. – 11:15 a.m.** Risto Kauppinen, Dartmouth Medical School **11:15 a.m. – 12:00** Jules Griffin, Chair, Cambridge University	Ballroom A

Structural Identification by NMR spectroscopy

Julian Griffin[1] and Aalim Weljie[2]
1. Dept of Biochemistry, University of Cambridge, Cambridge, CB2 1QW, UK.
2. University of Calgary, Calgary, Alberta, Canada

NMR spectroscopy is a powerful tool for structural identification of unknown compounds. This presentation will focus on approaches that can be used to define the structure of unknown compounds when the use of databases and literature values have drawn a blank. Through a series of examples we will discuss common multidimensional pulse sequences for the identification of unknowns including homonuclear and heteronuclear approaches, as well as how these pulse sequences can be used in conjunction with diffusion and relaxation editing, and HPLC to isolate individual components from complex mixtures.

Carbon-13 NMR applications for metabolite profiling

Aalim Weljie, University of Calgary. Calgary, Alberta, Canada

Typical NMR metabolomics experiments are conducted using 1H NMR due to the sensitivity, speed, and resolution with which information can be obtained. In a number of situations, significant additional information can be derived through 13C NMR approaches, including both one and two dimensional experiments. 1D direct detection13C NMR can be advantageous in resolving highly overlapped resonances from entities with similar proton chemical shifts (such as sugars or lipids). Additionally, targeted metabolic flux can be readily followed using labeled substrates using proton-detected NMR experiments. Furthermore, two-dimensional 1H-13C

NMR experiments allow for significant resolution enhancement and identification certainty as compared with 1D. These advantages, as well as current developments to overcome sensitivity limitations, such as 13C hyperpolarisation, will be discussed along with specific examples in plants, cell lines, and human urine analysis.

In Vivo Nuclear Magnetic Resonance Spectroscopy: A Metabolomics Perspective

Risto A. Kauppinen, Biomedical NMR Research Center, Department of Radiology, Dartmouth College, Hanover, NH, USA

In vivo NMR spectroscopy, often abbreviated as MRS, has a longer history than the commonplace medical counterpart, MRI. Virtually all modern MR scanners provide a truly 'hybrid' imaging platform with non-invasive access to anatomy, physiology and biochemistry *in vivo*. MRS offers a large arsenal of techniques to 'assay' metabolites and metabolic activity in selected tissue volumes using multi-nuclear readout. The most commonly used nucleus ^{1}H possesses a good sensitivity in NMR terms and typical tissue volumes from which metabolite data are acquired for evaluation amount to few ml and 1 ml in preclinical and clinical settings, respectively. Modern preclinical MR scanners operate typically at 9.4T, but *in vivo* systems up to 16.4T are now commercially available for rodent MRS. Clinical field strengths are typically 3T, but scanners operating at 9.4T have been installed for human use. In this presentation, principles of common *in vivo* MRS techniques are covered. Emphasis is placed on (bio)medical value of MRS data in the context of 'imaging biomarker search' using both conventional and advanced spectral analysis routines, such as neural networks, independent and principal component analyses. Cancer will be used as an example of a clinical condition with promising role in patient management.

Location, location, location – using NMR spectroscopy to probe metabolic compartmentation

Julian Griffin, Dept of Biochemistry, University of Cambridge, Cambridge, UK.

The exact role of a metabolite often varies depending on its subcellular localisation and this can be probed using NMR spectroscopy. This presentation will examine how spectral editing techniques such as T1 and T2 relaxation editing and diffusion weighting can be used to probe the subcellular organisation of metabolites within intact cells, tissue and even organisms. In addition, approaches using stable isotopes to probe subcellular and inter-cellular compartmentation of metabolism will also be discussed, and in particular how such approaches have been used to examine neuronal/glial cell compartmentation in the brain.

Sunday, August 30

Start	End	Event	Room
8:30 a.m.	12:00 p.m.	**Concurrent Workshops 2 – Sample Preparation**	Lacombe
8:30 a.m.	12:00 p.m.	**8:30 a.m. – 9:15 a.m.** Ute Roessner, Chair, University of Melbourne **9:15 a.m. – 10:00 a.m.** Rick Dunn, University of Manchester **10:00 a.m. – 10:30 a.m. Break Room: Ballroom C** **10:30 a.m. – 11:15 a.m.** David deSouza, University of Melbourne **11:15 a.m. – 12:00 (Noon)** Daniel Jones, Michigan State University	Lacombe

How to prepare samples for metabolomics analyses from different plant tissues?

Ute Roessner, Australian Centre for Plant Functional Genomics, The University of Melbourne Melbourne, Australia

Sample preparation is the most crucial step in metabolomics applications to ensure sufficient metabolite extraction out of the tissue of interest and therefore representative analysis of actual metabolite levels in the system under investigation. Special care has to be taken for the extraction of metabolites from different plant tissue. Plant cells have unique structures compared to cells of other organisms; in addition they contain a central vacuole, plastids and a thick, plasma-membrane surrounding cell wall. In this workshop we explore where to take care when aiming the analysis of metabolites from plant tissues. Crucial steps in the process of harvesting, quenching of metabolism, homogenisation of harvested tissue, extraction of metabolites for different metabolomics approaches (e.g. targeted versus untargeted), storage of tissue and extracts, purification and enrichment of metabolites if required leading to a successful analysis and quantification of metabolites will be discussed.

Investigating human phenotypes – discussions regarding sample collection and preparation for blood, urine and mammalian tissue

Warwick B. Dunn, Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK

The metabolomics workflow is composed of many separate but inter-related processes from sample collection and sample preparation, through data acquisition and data analysis

to biological knowledge. Importantly, the quality of data produced at the end of the workflow is impacted upon by each step in the workflow.

Mammals are complex biological systems with biofluids and tissues being composed of many different chemical species (proteins, cells, complexes) including many hundreds to thousands of metabolites of a wide diversity of chemical reactivity, hydrophobicity/hydrophilicity and concentration. Specific steps are required to collect and prepare samples depending on the sample type and the objective of the experiment to be performed.

This presentation will discuss methodologies for collection and preparation of a range of mammalian sample types (serum, plasma, urine and tissue) for subsequent mass spectrometric analysis. The difficulties to overcome in sample preparation, the extraction of metabolites from complex matrices and the selectivity in methodologies applied will all be discussed in relation to both targeted/quantitative methods and semi-quantitative metabolic profiling methods. The impact of the types of extractions on instrument stability will also be described. Examples will be given for specific processes and the requirement for method validation, reproducibility and quality assurance will also be discussed.

Preparation techniques for microbial metabolite profiling

David De Souza, Metabolomics Australia, Institute for Molecular Science and Biotechnology, University of Melbourne, Parkville, Victoria, Australia,

Abstract not available at time of printing

Preparation of tissues and specialized cell types for metabolite profiling using LC/TOF MS and laser desorption ionization imaging

A. Daniel Jones, Chao Li, Department of Biochemistry and Molecular Biology and Department of Chemistry, Michigan State University, East Lansing, MI, USA

Metabolite synthesis and transport is organized at cellular and subcellular levels, and improved understanding of the roles of specific cell types requires reliable methods for microsampling and imaging of metabolites. To develop suitable methodologies, we have investigated several approaches for sampling specialized epidermal cell types from leaf and stem tissues from plants in the genus *Solanum*. These include removal of cells using freezing and mechanical depilation, sampling using micropipettes, and laser desorption ionization (LDI) imaging. Metabolite analyses in individual cell types using LC/TOF MS will be evaluated in the context of assessing the need for pooling individual cells to achieve adequate metabolite pools for analysis. Several different approaches for metabolite imaging will be compared including LDI analysis of individual cells and tissues and contact transfer of cells to substrates for matrix-free LDI imaging. The merits and pitfalls of these sampling and analysis methods will be compared. In

the process, the challenges, merits, and pitfalls of performing spatially-resolved metabolome analysis will be explored.

Sunday, August 30

1:00 pm	5:00 p.m.	**Concurrent Workshops 3 – Mass Spectrometry**	Ballroom A
1:00 p.m.	5:00 p.m.	**1:00 p.m. – 1:40 p.m.** Thomas Hankemeier, Chair, Netherlands Metabolon Centre **1:40 p.m. – 2:20 p.m.** Liang Li, University of Alberta **2:20 p.m. – 2:50 p.m. Break Room: Ballroom C** **2:50 p.m. – 3:30 p.m.** Mark Viant, University of Birmingham **3:30 p.m. – 4:10 p.m.** Robert Trengrove, Murdoch University **4:10 p.m. – 4:30 p.m.** David Heywood, Waters	Ballroom A

The role of LC-MS in metabolomics: basics & perspectives

Thomas Hankemeier, Netherlands Metabolomics Centre, The Netherlands

This lecture provides an introduction of the role of LC-MS in metabolomics. After a short introduction of the basics of LC-MS, the various LC modes used for metabolomics will be discussed, as different metabolite classes require different separation approaches. Examples are reversed phase LC, hydrophilic liquid interaction chromatography, the use of an ion pair reagent, normal phase LC, or derivatization of metabolites prior to the separation. Next, the coupling of LC with MS will be discussed, mostly for electrospray ionization; aspects such as ion suppression and challenges in the quantification will be covered in the presentation. Different MS detector options will be introduced and the pro's and con's of the different MS options for quantitative metabolite profiling discussed. Different approaches for data processing will be introduced, i.e. how to come from raw data to a list of features or, if possible, a list of identified metabolites. Several metabolite profiling methods will be discussed. A discussion of possible bottlenecks of LC-MS and the validation of an LC-MS method for metabolomics will be given. Finally, future trends will be discussed. The presentation will include some examples of the application of LC-MS methods to biological samples.

Tandem mass spectrometry for metabolite identification

Liang Li, Avalyn Lewis, and Azeret Zuniga, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

Tandem mass spectrometry or MS/MS is a power tool for metabolite identification, particularly when it is combined with liquid chromatography (LC). In this presentation, we will first provide a brief summary of several tandem MS instruments commonly used for metabolite identification, followed by an introduction to several different modes of tandem MS operations. A strategy

based on MS/MS spectral library search for metabolite identification will be presented. Methods and software used to unknown metabolite identification will be discussed.

High mass accuracy and resolution: key specifications in mass spectrometry based metabolomics

Mark Viant, NERC Environmental Metabolomics, Facility School of Biosciences, The University of Birmingham, Birmingham UK

Mass spectrometry based metabolomics is increasingly exploiting instruments with high mass accuracy and resolution. These include for example the Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer and Orbitrap mass spectrometer. This workshop presentation will introduce these two types of mass spectrometer, briefly describe how they work, and then explain why high mass resolution and accuracy are important in metabolomics studies. Particularly attention will be paid to the challenges and pitfalls of metabolite identification (e.g. using accurate mass measurements, isotope patterns etc.). Examples will be provided throughout, with a particular emphasis on direct infusion mass spectrometry.

Calibration of GCMS based Metabolomics methods across multiple mass analyser geometries.

Garth Maker[1,2,3], Joel Gummer[4], Catherine Rawlinson[2], Bruce Peebles[3], Richard Oliver[4], Peter Solomon[5] and **Robert Trengove**[2,3]
[1] School of Pharmacy, Murdoch University, Perth, WA 6150, Australia
[2] Metabolomics Australia, Murdoch University Node, Perth, WA 6150, Australia
[3] Separation Science and Metabolomics Laboratory, Murdoch University, Perth, WA 6150, Australia
[4] Australian Centre for Necrotrophic Fungal Pathogens, Murdoch University, Perth, WA 6150, Australia
[5] Plant Cell Biology, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia

GCMS systems with quadrupole, time of flight, ion trap and triple quadrupole mass analysers have been “tuned” to allow comparable untargeted Metabolomics analysis to be undertaken independent of instrument used, thus gaining the benefits of each mass analyser geometry. The GCMS linear quadrupole system was used to calibrate the other instruments using extracts of the “wild type” wheat pathogen *Stagonospora nodorum*. For the ion trap instrument the threshold was used as the primary calibration parameter and the impact is shown in figure 1 below, whilst for the TOF based instrument the EMV for the detector was the parameter used. The triple quadrupole instrument was operated in full scan mode and the EMV

again was the key parameter used in the calibration process. The comparability of the "calibrated" instruments for Metabolomics analysis was validated using plasma samples.

As a precursor to the cross platform calibration the inlet liner systems have been optimised to maximise sensitivity and minimise mass discrimination. Artefacts of different inlet systems and column configurations will be discussed and illustrated using extracts of the "wild type" wheat pathogen *Stagonospora nodorum.*

Advances in Hybrid Quadrupole IMS oa-Tof Mass Spectrometry

David Heywood, Waters Limitée, Québec, Canada

In this presentation we will describe several new innovations in hybrid quadrupole IMS oa-Tof mass spectrometry. These include, high extraction fields, dual stage reflectrons and a novel approach towards increasing the mobility resolving power of the travelling wave device together with a description of an enhanced detection system for acquiring mobility data.

High extraction fields help to reduce the spread of the arrival time distributions (ATD) for ions of a given mass to charge ratio (m/z) at the detector. Dual stage reflections together with folded instrument geometries extend the time of flight for the narrow arrival time distributions. The combination of the two effects is to increase the resolving power by more than a factor of 2 over similar instrumentation configurations.

We will also describe the ion -mobility separation of flavanoid glycoside structural isomers and how the collisional cross-sectional values determined by a travelling wave ion-mobility device agree with theoretically derived CCSs of a variety of small molecules.

Sunday, August 30

1:00 pm	5:00 p.m.	**Concurrent Workshops 4**	Lacombe
1:00 p.m.	5:00 p.m.	**Workshop 4 – Pathways and Statistic Room: Lacombe** **1:00 p.m. – 1:40 p.m.** David Wishart, Chair, University of Alberta **1:40 p.m. – 2:20 p.m.** Chris Steinbeck, European Bioinformatics Institute **2:20 p.m. – 2:50 p.m. Break Room: Ballroom C** **2:50 p.m. – 3:30 p.m.** Robint Haw, University of Toronto **3:30 p.m. – 4:10 p.m.** Jeff Xia, University of Alberta **4:10 p.m. – 4:30 p.m.** Theodore R. Sana, Agilent	Lacombe

Statistics, Informatics and Pathway Databases in Metabolomics: An Introduction

David Wishart, Departments of Biological Sciences and Computing Sciences, University of Alberta
Edmonton, Alberta, Canada

This workshop will give attendees and introduction to some of the statistical approaches to interpreting metabolomic data. Simple examples of how PCA, PLS-DA and other techniques can be used or misused will be provided. In addition attendees will be introduced to various software tools for metabolite annotation (identification) and for pathway interpretation, with a particular emphasis on freely accessible web-based resources. This lecture is intended to help lay the groundwork for the other three presentations in this workshop session.

Cheminformatics and Metabolism at the European Bioinformatics Institute

Christoph Steinbeck, EMBL Outstation – Hinxton, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

The Cheminformatics and Metabolism team at the European Bioinformatics Institute is interested in the elucidation of metabolomes by means of computer assisted structure elucidation (CASE) and other prediction methods. To this end we have developed algorithms for screening large molecular spaces for candidates with desired properties and have worked on methods for the prediction of NMR spectra as well as the semantic encoding of spectral and assigned molecular information as part of the chemical markup-language (CML). We implement our methods based on a number of open source software packages in Chemo- and Bioinformatics originating from our lab, including the Chemistry Development Kit (CDK), a Java library for chemo- and bioinformatics, Bioclipse, an Eclipse-based Rich Client for everything and nothing in particular, and OrChem, a chemical database cartridge for the Oracle database system. In this

talk we will outline our research so far and describe our future plans for metabolomics databases at the EBI.

Reactome knowledgebase of human biological pathways and processes.

Robin Haw, Ontario Institute for Cancer Research, Toronto, Ontario, Canada

The Reactome project is a collaboration between Ontario Institute for Cancer Research, Cold Spring Harbor Laboratory, The European Bioinformatics Institute, and The Gene Ontology Consortium to develop a curated, peer-reviewed resource of human biological processes. Given the genetic makeup of an organism, the complete set of possible reactions constitutes its reactome. The basic unit of the Reactome database is a reaction; reactions are then grouped into causal chains to form pathways. The Reactome data model allows us to represent many diverse processes in the human system, including the pathways of intermediary metabolism, regulatory pathways, and signal transduction, and high-level processes, such as the cell cycle. Reactome provides a qualitative framework, on which quantitative data can be superimposed. Tools have been developed to facilitate custom data entry and annotation by expert biologists, and to allow visualization and exploration of the finished dataset as an interactive process map. Although our primary curation domain is pathways from Homo sapiens, we regularly create electronic projections of human pathways onto other organisms via putative orthologs, thus making Reactome relevant to model organism research communities. Collaborations to create manually curated Reactome pathway datasets for species including Arabidopsis, Drosophila and Gallus gallus (chicken) are currently underway. The database, located at http://www.reactome.org, is publicly available under open source terms, which allows both its content and its software infrastructure to be freely used and redistributed.

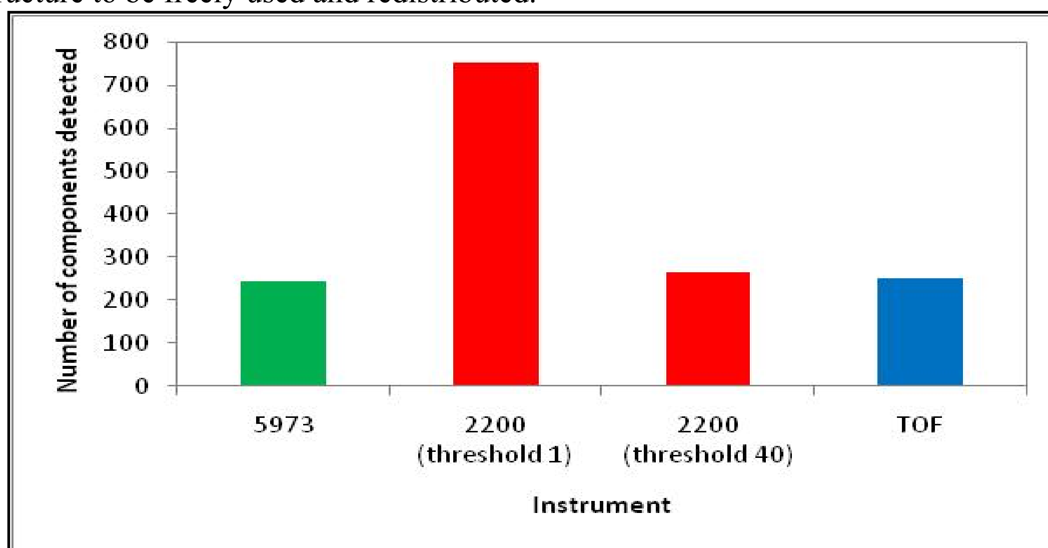

Figure 1. Comparison of the number of metabolites detected for GCMS systems with different mass analyser geometries (5973 - linear quadrupole; 2200 – ion trap; TOF – time of flight)

Metabolomic Data Analysis using MetaboAnalyst

Jeff (Jianguo) Xia, Departments of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

Workshop attendees will be introduced to a new, on-line tool for web-based metabolomic data analysis – called MetaboAnalyst. In this presentation I will provide a short description of this analysis pipeline and highlight some of its unique features. I will also provide a number of example cases demonstrating how MetaboAnalyst can be used to analyze GC-MS, LC-MS and NMR data either via metabolic profiling or via quantitative (targeted) metabolomics.

Metabolomic Factors in Red Blood Cell Malaria Infections: From LC/MS to Pathway Analysis

Theodore R. Sana, Agilent Technologies, Santa Clara, CA, USA

The metabolomic situation was examined in the malaria parasitic infection of red blood cells. Streptolysin O cell membrane permeabilizing agent is thought to enrich the infected red blood cell population. By using LC/MS analysis in both ESI and APCI modes, a molecular feature data set was generated which was evaluated for differences, and then subsequently identified and linked directly with pathway analysis software. In this example arginine, ornithine and citrulline were identified as being either up or down regulated in the infected cells. This work illustrates the usefulness of being able to link the LC/MS work through to the pathway analysis without having to merge or apply different software packages.

Invited Speakers program

Monday, August 31

Start	End	Event	Room
9:00 a.m.	9:45 a.m.	**Keynote Plenary**: Kazuki Saito, Riken Plant Science Center	Ballroom A

Metabolomics-based plant functional genomics – From Arabidopsis and beyond

Dr. Kazuki Saito, RIKEN Plant Science Center, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Metabolomics is one of the major components of omics research. It holds a fundamental position in functional genomics and biotechnology. Metabolomics research is particularly important in the plant field, because plants collectively produce a huge variety of chemical compounds, far more than animals and even microorganisms. In addition, most of the human-beneficial properties of plants – foods, medicinal resources, or industrial raw materials – are ascribed to the metabolites produced by plants.

Using *Arabidopsis thaliana* as a model system, an integrated analysis of metabolome and transcriptome led to prediction of gene-to-metabolite relations. Co-regulation framework models of genes and metabolites in the pathways of flavonoids, sulfur-containing metabolites and lipids, suggested the specific involvement of co-expressed genes in the synthesis and accumulation of the metabolites in the pathways. Metabolomic analysis of Arabidopsis natural variants indicated the diversity of metabolite accumulation pattern among those variants, which is presumably caused by genetic polymorphism. Metabolomics was further applicable to crops and medicinal plants to decipher the genes' functions and to predict the agronomical and food-chemical traits. In this presentation, the crucial roles of metabolomics in plant functional genomics and plant biotechnology will be discussed.

Start	End	Event	Room
9:45 a.m.	10:00 a.m.	Break	Promenade

Monday, August 31

10:00 a.m.	12:00 (Noon)	**Session 1: Metabolomics and Nutrition** David Wishart, Chair	Ballroom A
10:00 a.m.	10:40 a.m.	Invited Speaker: Bruce German, UC Davis	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 1: Philip Britz-McKibbin, McMaster University	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 2: Wei Jia, University of North Carolina	Ballroom A
11:20 a.m.	11:40 a.m.	Podium Speaker 3: Richard Beger, US Food & Drug Administration	Ballroom A
11:40 a.m.	12:00 (Noon)	Podium Speaker 4: Bruce Kristal, Harvard Medical School	Ballroom A

Metabolomics and Nutrition

Bruce German, Department of Food Science and Technology, University of California, Davis, CA,USA

The global epidemic of diet-related metabolic diseases, due to inadequate and or unbalanced nutrient intakes, is a vivid indictment of the failure of scientific research to understand the links between diet and metabolism and of health care to improve them. Obesity, type 2 diabetes, atherosclerosis, hypertension and osteoporosis are largely preventable. Preventing them however will require a more detailed knowledge of human metabolism and a more personal approach to its management. Metabolomics, as the accurate and comprehensive measurement of a significant fraction of important metabolites in accessible biological fluids, is a promising field of science to improve our understanding of diet-related metabolism and to apply this understanding to assessing individual metabolic status. What unique demands will nutrition make on the field of metabolomics? Analytically, nutritional metabolomics needs to be quantitatively accurate. Alterations in metabolism due to diet are small and analytical platforms of metabolomics must capture these variations. Biologically, nutritional metabolomics must be able to assign metabolites to pathways and physiologies. The disregulation of metabolism within the principal metabolic organs (e.g. intestine, adipose, skeletal muscle, liver) is at the center of the diet-disease paradigm that includes metabolic syndrome, type 2 diabetes, and obesity. Experimentally, nutritional metabolomics must capture the temporal variations in metabolism that correspond to diet. Metabolomics must understand the dynamics of the fasted through fed transitions and nutrition studies must standardize protocols to render metabolite data comparable. Mathematically, nutritional metabolomics must capture the diversity of the human condition. The variations in humans that lead to metabolic diseases are subtle and failures to maintain optimal metabolic regulation are highly personal. In summary, the challenges are substantial, however so too are the rewards. Metabolomics could be the leading field in resolving the 21st

centuries most critical health problems and guiding health care to a prevention rather than curative model.

Differential Metabolomics for Quantitative Assessment of Cellular Oxidative Stress and Antioxidant Efficacy with Strenuous Aerobic Exercise Abstract: D02

Philip Britz-McKibbin, McMaster University, Canada
Richard Lee, Department of Chemistry, McMaster University
Daniel West, Department of Kinesiolog, McMaster University
Stuart Phillips, Department of Kinesiology, McMaster University

A fundamental understanding of the mechanism(s) of oxidative stress remains elusive due to inconsistent results from large-scale clinical trials using antioxidants, as well as the seemingly contradictory and synergistic functions of reactive oxygen/nitrogen species in-vivo. Herein, we describe a time-resolved differential metabolomics strategy for quantitative assessment of oxidative stress and antioxidant efficacy with strenuous aerobic exercise using capillary electrophoresis-electrospray ionization-mass spectrometry (CE-ESI-MS). A healthy untrained volunteer was recruited to perform a high intensity ergometer cycling routine until exhaustion with frequent blood collection over a 6 hour time interval, which included pre-, during and post-exercise periods while at rest. A follow-up study was subsequently performed by the subject after a high dose oral supplementation of the antioxidant N-acetyl-L-cysteine (NAC) prior to performing the same exercise protocol under standardized conditions. Differential metabolomic analyses of filtered red blood cell lysates by CE-ESI-MS in conjunction with multivariate analysis revealed significant attenuation of oxidative after NAC supplementation as reflected by a lower change in half-cell reduction potential for glutathione, a shorter recovery time for attaining homeostasis, as well as unchanged levels of specific biomarkers reflective of residual fatigue relative to control. This work demonstrates the proof-of-concept that timely nutritional intervention can effectively attenuate acute periods of oxidative stress by global perturbations in metabolism that has relevant implications ranging from enhanced athletic performance to improved post-surgical recovery.

Metabolomics study of traditional Chinese medicine Abstract: D13

Wei Jia, UNCG Center for Research Excellence in Bioactive Food Components, University of North Carolina

The treatment of common multi-factorial, system-wide diseases using traditional Chinese medicine (TCM) can be regarded as a ‚Äúsystem to system‚Äù approach. It is difficult to evaluate the systemic pharmacological effect of the multi-component TCM agents in the context of single-target based pharmacological models. Metabonomics/metabolomics, defined as the measurement of multiparametric metabolic responses of a biological compartment or a living system to pathophysiological stimuli or genetic modification, is a newly thriving systems biology

which has been highly favored in botanical science, environmental research, and toxicological study in preclinical and clinical fields.

The Chinese ginseng extracts typically contain more than 50 known compounds, mainly, ginsenosides. We demonstrated, in an animal model, that ginseng extracts were able to attenuate alterations in several metabolic pathways in response to acute cold stress and chronic unpredictable mild stress, using a combined chemical profiling and metabolic profiling approach. We also conducted metabolomics study on a traditional Chinese tea ‚Äì Pu-erh, regarding its chemical constituents prepared in different locations and different storage times, and human metabolic profiles of tea drinkers, using a high-performance liquid chromatography coupled with quardrupole-time of flight mass spectrometry. The results indicate that comprehensive molecular descriptions of a pathophysiological state and the response to TCM and dietary intervention can be achieved, so that the global biochemical changes contributing to a drug response can be taken into account, leading to much improved understanding of the efficacy, toxicity and mechanisms of action.

Metabolomics Analysis of Urine from Rats Chronically Dosed with Acrylamide

Abstract: C04

Richard Beger, National Center for Toxicological Research, US Food and Drug Administration United States
Laura Schnackenberg, National Center for Toxicological Research, US Food and Drug Administration
John Bowyer, National Center for Toxicological Research, US Food and Drug Administration
Daniel Doerge, National Center for Toxicological Research, US Food and Drug Administration

Acrylamide (AA) is a chemical formed during cooking of food. AA is neurotoxic, toxic to the testis, mutagenic, and carcinogenic in rodents. This study compares the NMR-based metabolomics of AA in urine and serum of male F344 rats during subchronic exposure to AA. Five male F344 rats were administered control water or dosed with AA (2.5, 10 or 50 mg/kg bw) in the drinking water for two weeks. There were two groups of rats treated with the high dose, one was sacrificed after the last dose while the other group was sacrificed 1 week after the end of AA exposure. Urine was collected daily and blood was collected on days 0, 1, 3, 7, 10, 14, 17, and 21. Metabolomics analysis showed that the creatine concentration increased by two orders of magnitude at the high AA dose, which could be related to decreased food intake or an oxidative stress response caused by the thiol reactivity of AA and glycidamide, the epoxide metabolite. All Kreb's Cycle intermediates (2-oxogluturate, succinate, and citrate) detected in urine were decreased by ~19-44% during AA dosing, while formate was up by approximately 35%. Urinary levels of N-acetylaspartate were increased by ~16% at the high dose, which might be related to the neurotoxicity of AA. Higher urinary levels of lactate and alanine could signal an increase in glycolysis. Changes in these urinary metabolites caused by the high dose of AA may be due to reduced weight gain (possibly due to changes in dietary intake or intestinal absorption) and physiological changes associated with altered energy metabolism, particularly in neural and testicular tissues.

Dietary macronutrients modify serum metabolomics in rats Abstract: D16

Bruce S. Kristal, Department of Neurosurgery, Brigham and Womens Hospital, and Department of Surgery, Harvard Medical School USA
Sergei Baranov, Department of Neurosurgery, Brigham and Womens Hospital, and Department Of Surgery, Harvard Medical School
Irina Stavrovskaya, Department of Neurosurgery, Brigham and Womens Hospital, and Department of Surgery, Harvard Medical School
Heather Greenberg, Department of Neurosurgery, Brigham and Womens Hospital
Diane Sheldon, Department of Neurosurgery, Brigham and Womens Hospital
Vasant Marur, Department of Neurosurgery, Brigham and Womens Hospital
Neil Russell, Department of Neurosurgery, Brigham and Womens Hospital
Matt Sniatynski, Department of Neurosurgery, Brigham and Womens Hospital
Caryn Porter, Department of Neurosurgery, Brigham and Womens Hospital

Suboptimal dietary macronutrient choices are arguably the major environmental stressor in individuals living in Western societies. Trans and saturated fats contribute to cardio- and cerebrovascular disease, as do diets high in easily digested carbohydrates, which themselves may also contribute to metabolic syndrome and overt diabetes. A massive literature links diet and both neoplastic and non-neoplastic diseases, and a causal role for mitochondria in this relationship is strongly supported by broad areas of inquiry represented in the literature. We are funded as a part of the NIH-sponsored Genes and Environment Initiative, Exposure Biology Program (Biological Response Indicators component) to conduct an interdisciplinary, approach to discover and confirm plasma metabolomic biomarkers for dietary intake of subclasses of fats and carbohydrates and their effects on mitochondrial function. We will present the initial results of these diets on the sera metabolome.

12:00 (Noon)	2:00 p.m.	Lunch with Poster Authors and Exhibitors	

12:30 p.m.	1:30 p.m.	Luncheon Workshop – Agilent Technologies	Lacombe

Monday, August 31

2:00 p.m.	4:00 p.m.	**Session 2: Biomedical Biomarkers** Matej Oresic, Chair	Ballroom A
2:00 p.m.	2:40 p.m.	Invited Speaker: John Ryals, Metabolon, Inc.	Ballroom A
2:40 p.m.	3:00 p.m.	Podium Speaker 1: Marko Sysi-Aho, VTT Technical Research Centre of Finland	Ballroom A
3:00 p.m.	3:20 p.m.	Podium Speaker 2: Robert Weiss, UC Davis	Ballroom A
3:20 p.m.	3:40 p.m.	Podium Speaker 3: Michael Milburn, Metabolon	Ballroom A
3:40 p.m.	4:00 p.m.	Podium Speaker 4: Takushi Ooga, Human Metabolom Technologies, Inc.	Ballroom A

The Use of Biochemical Profiling for Biomarker Discovery

John A. Ryals, Metabolon, Inc., Durham, NC, United States

Metabolomics is defined as “the non-biased quantification and identification of all metabolites present in a biological system” but in practice the term metabolomics is used in a rather broad sense and covers many different analytical methodologies. To address the challenges associated with metabolomics, a comprehensive, integrated analytical and data handling platform was developed that provides a chemo-centric global metabolomics analyses of biological systems. Using this platform, prostate cancer aggressiveness was investigated. Diagnostic tests that distinguish indolent from aggressive tumors have the potential to augment prostate health management programs and reduce the number of unnecessary biopsies and prostatectomies. Toward this goal, tissue specimens with different degrees of clinically ascertained disease (i.e. localized prostate cancer, metastatic disease, or control benign adjacent tissue) were analyzed. One of the significantly altered compounds in prostate cancer was sarcosine (N-methyl glycine). This finding was confirmed by a number of approaches and sarcosine was shown to be causally related to the aggressiveness of prostate tumors.

In a separate study, we have used a metabolomic approach to discover the mode of action for a potent antitumor drug GMX1778. Since its discovery the antitumor activity of GMX1778 has been widely studied but the identification of the molecular target and the mechanism of action of GMX1778 have been elusive. Based on our results we have shown that GMX1778 is a potent and specific inhibitor of nicotinamide phosphoribosyl transferase (NAMPRT), a NAD+ biosynthetic enzyme.

Combining MR imaging and metabolomics ‚Äì towards early markers of dilated cardiomyopathy Abstract: A31

Marko Sysi-Aho, VTT Technical Research Centre of Finland, Finland
Juha Koikkalainen, VTT Technical Research Centre of Finland
Tuulikki Seppänen-Laakso, VTT Technical Research Centre of Finland
Kirsi Lauerma, Helsinki Medical Imaging Center, Helsinki University Hospital
Jurki Lötjönen, VTT Technical Research Centre of Finland
Tiina Heliö, HYKS Hospital District of Helsinkin and Uusimaa
Matej Oresic, VTT Technical Research Centre of Finland

In order to form a holistic picture of human physiology it is necessary to put empirical data obtained at different scales into physiologic context and to fill the gaps that remain unobserved between the organismal levels. We present an approach that can be used to bridge the gap between tissue level magnetic resonance (MR) images and the molecular level of metabolism. We studied two human sample sets in the context of dilated cardiomyopathy (DCM): (1) lamin positive set consisting of 11 asymptomatic lamin A/C gene mutation (LMNA) carriers who are known to be at risk for developing DCM, and 11 controls, and (2) lamin negative set consisting of 8 non-LMNA DCM progressors and 8 controls. Lipids were analyzed by UPLC/MS platform. One of our main aims was to search for early predictive DCM biomarkers from the lamin positive set and then test their performance in the lamin negative set. We developed a predictive model for DCM by combining the most dysregulated lipids and some of their ratios. In addition, we identified and visualized correlations among the heart MR images and the lipid profiles. The heart images were decomposed into 86 physiologically relevant parameters [1] and regression models were applied to explain these parameters with the lipid profiles. Regression models were also applied to reconstruct the heart MR images by lipids and to study the effects of lipid concentration changes on these images. As a result we identified associations between heart images and the lipid profiles. We also visualized the effect of lipid concentration changes on the left reconstructed image of the ventricle of the heart. Our approach may, in addition to its potential relevance to early diagnostics, inspire development of methods for integrating physiological data across different organismal levels.
[1] J.R. Koikkalainen et al., Radiology 2008;249:88-96.

A metabolomics approach to identifying biomarkers and therapeutic targets for polycystic kidney disease Abstract: A38

Robert Weiss, Univ. of Calif., Davis USA
Sheila Ganti, UC Davis
Oliver Fiehn, UC Davis
Kyoungmi Kim, UC Davis
Sandra Taylor, UC Davis

Polycystic kidney disease is one of the most common of all life-threatening human genetic disorders. It is an incurable genetic disorder affecting 12.5 million people worldwide. It is characterized by the formation of fluid-filled cysts in the kidneys of affected individuals and currently has no biomarkers or effective treatment. Although requiring a second mutational ‚Äúhit‚Äù, PKD arises from germline mutations, and because of this, we propose that metabolic changes can be detected in the urine before changes in kidney size and filtration function are noted. Our project seeks to identify novel biomarkers which can be used to develop an early diagnostic test as well as to reveal new therapeutic targets for disease treatment. In the jck mouse model (which has a mutation in the Nek8 gene), cysts appear starting as early as 26 days, but renal failure is not seen until 45 days. Urine was collected from mice at these two ages. Metabolomic analysis was performed using GC-MS, and the resulting data were baseline corrected, log transformed, body weight adjusted, and normalized to total mass the account for variations in urine concentration. These data were then analyzed by partial-least squares and other statistical analyses to identify the metabolites that are significantly different between diseased and control individuals. Complete separation of the two groups of mice can be seen as early as 26 days when renal function is normal and is maintained at the 45 day time point. Metabolites which contribute the most to the segregation between groups are currently being identified and validated. Once confirmed, these metabolites may be used to identify patients with PKD before the onset of kidney failure, and, once metabolic pathways are elucidated, novel targets for therapy will be identified.

Identification of novel insulin resistance metabolites in a non-diabetic population by global biochemical profiling Abstract: A09

Walter Gall, Metabolon USA
E. Ferrannini, University of Pisa School of Medicine
M. Nannipieri, University of Pisa School of Medicine
M. Anselmino, University of Pisa School of Medicine
Mauro Rossi, University of Pisa School of Medicine
Kirk Beebe, Metabolon
Costel Chirila, Metabolon
Klaus-Peter Adam, Metabolon
John Ryals, Metabolon
Michael Milburn, Metabolon

An unmet medical need is the early identification of insulin resistance (IR) with high accuracy. Our goal was to carry out a global, non-targeted biochemical profiling analysis on human plasma samples to identify novel small molecule metabolites that can distinguish insulin-sensitive from IR subjects in a non-diabetic population. Fasting baseline plasma samples from the EGIR-RISC cohort that were representative of the spectrum of insulin sensitivity and glycemic status, based on hyperinsulinemic euglycemic clamp and oral glucose tolerance testing, were analyzed. Several hundred metabolites were detected and quantified in each sample using UHPLC and GC mass spectrometry-based platforms and proprietary cheminformatics software. Analysis showed that 2-hydroxybutyrate (2-HB), creatine, certain fatty acids and other lipid metabolites such as lysoglycerophosphocholines and acylcarnitines, were the most highly significant metabolites separating insulin-sensitive subjects from IR subjects. Quantitative, targeted mass spectrometric measurements were carried out to confirm the screening results for the IR/IS metabolites. To test their clinical significance, these IR metabolites were further measured in 11 morbidly obese subjects (aged 44¬±8 years, mean¬±SD) undergoing bariatric surgery. One year after surgery, BMI had declined from 52¬±7 to 35¬±6 kg/m2 ($p<0.001$) and the M value had risen from 22¬±11 to 39¬±10 ¬µmol.min-1.kgFFM-1, ($p<0.01$). Consistent with 2-HB being a sensitive marker of IR, we observed a 1.7-fold reduction in plasma 2-HB concentrations post-surgery for this obese cohort. The significance of these IR metabolites is discussed in the context of their production in IR states, and their observed changes when insulin sensitivity and energy balance are improved.

The anatomical metabolomics of hyperlipidemia Abstract: A27

Takushi Ooga, Human Metabolome Technologies, Inc. Japan
Hajime Sato, Human Metabolome Technologies, Inc.
Atushi Nagashima, Human Metabolome Technologies, Inc.
Tmoyoshi Soga, Institute for Advanced Biosciences, Keio University and Human Metabolome Technologies, Inc.
Masaru Tomita, Institute for Advanced Biosciences, Keio University and Human Metabolome Technologies, Inc.
Yoshiaki Ohashi, Institute for Advanced Biosciences, Keio University and Human Metabolome Technologies, Inc.

The marked imbalances of metabolites in body fluids, such as blood and urine, represent ensemble of metabolic dysfunctions in several organs and tissues, therefore are regarded as available markers for the diagnoses or assessment of medical practices. On the other hand, understanding of metabolic imbalances in each tissue, that is anatomic metabolomics, further provide the clues to the pathologic mechanism, which renders biological supports for the metabolic markers found in body fluids. Hyperlipidemia is known to be one of the serious MetSs and characterized by abnormal elevation of blood lipids and/or lipoproteins. For familial hypercholesterolemia (FH), mutation in the LDLR gene, which encodes a low-density lipoprotein (LDL) receptor expressed in the liver, causes juvenile hyperlipidemia, and resulting in severe cardiovascular event. Recently, adding to the elevation of blood lipid level, other

possible pathogeneses, such as oxidative stress, are regarded to be essential for severe cardiovascular events as second hit, whereas the diagnostic assessment of those factors has not yet been established. For the evaluation of the systemic metabolic imbalances including the second hit, we performed LC/CE-TOFMS-based systemic metabolic profiling for Homozygous Watanabe heritable hyperlipidemic (WHHL) rabbit as model for FH. The changes of metabolic pool were investigated for blood, liver, aorta, cardiac muscle, and brain between WHHL rabbit and healthy controls. For the pathologic rabbit, prominent changes were observed for lipid catabolism, purine metabolism, and glutathione biosynthesis, indicating advanced oxidative stress resulting in folic acid depletion for whole body, especially for liver. We also assessed the pharmaceutical effect of statin, which is broadly used for treating patients by its strong effect to control plasma lipids. After the 10-days administration, metabolic pool of tissues exhibited extensive changes suggesting both of the restoration to the healthy state and possible side effect, which are inconsistent with the restoration.

4:00 p.m.	4:15 p.m.	Break	Promenade

Monday, August 31

4:15 p.m.	6:15 p.m.	**Session 3: Pharmacometabolomics** Rima Kadurrah Daouk, Chair	Ballroom A
4:15 p.m.	4:55 p.m.	Invited Speaker: Jules Griffin, Cambridge University	Ballroom A
4:55 p.m.	5:15 p.m.	Podium Speaker 1: Jerzy Adamski, German Research Center f Environmental Health	Ballroom A
5:15 p.m.	5:35 p.m.	Podium Speaker 2: Yuan Ji, Mayo Clinic	Ballroom A
5:35 p.m.	5:55 p.m.	Podium Speaker 3: Steven Gross, Weill Cornell Medical College	Ballroom A
5:55 p.m.	6:15 p.m.	Podium Speaker 4: Ewoud Van Velzen, University of Amsterdam	Ballroom A

Hearts and Minds in Pharmacometabolomics

Julian L. Griffin, Department of Biochemistry, University of Cambridge, Cambridge, UK

This presentation will focus on the use of a combination of global metabolomics and targeted stable isotope analysis to elucidate drug mechanisms of action in two contrasting systems. The first application involves the examination of peroxisomal proliferation activated receptor (PPAR)-delta agonists as treatments of type II diabetes and obesity. We have examined the action of a highly specific PPAR-delta agonist in the obese ob/ob mouse and contrasted it with the action of a specific PPAR-gamma agonist. Physiological responses identified in the whole animal by metabolomics of key tissues and biofluids were then explored in cell culture. The latter approach also allowed the use of 13C labelled substrates to confirm modes of action, including PPAR-delta induced increases in both beta-oxidation and the citric acid cycle rates in white adipose tissue, contrasting with PPAR-gamma where there was an increase in fatty acid synthesis. In addition this approach also confirmed an increase in alpha-oxidation of fatty acids. In the second example, an approach based on perfused brain slices will be presented as a versatile tool for studying neurochemical changes induced by stimulation and inhibition of the GABA receptors in the cortex [1, 2]. Using a combination of total pool measurements and 13C labelling following perfusion with [2-13C]pyruvate, a variety of different drugs could be classified according to their metabolic responses in intact brain slices. This metabolomic profiling demonstrated that the reported pharmacological classes of the different agonists and antagonists may not always be appropriate, with many being promiscuous for the different GABA receptors.
References:

1. Nasrallah FA, Griffin JL, Balcar VJ, Rae C. Understanding your inhibitions: effects of GABA and GABAA receptor modulation on brain cortical metabolism. J Neurochem. 2009 Jan;108(1):57-71.
2. Rae C, Nasrallah FA, Griffin JL, Balcar VJ. Now I know my ABC. A systems neurochemistry and functional metabolomic approach to understanding the GABAergic system. J Neurochem. 2009;109 Suppl 1:109-16.

Genotypes contribute to human metabolic phentotypes Abstract: A02

Jerzy Adamski, Helmholtz Zentrum München, German Research Center for Environmental Health Germany
Cornelia Prehn, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Elisabeth Altmaier, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Gabriele Kastenmueller, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Christian Gieger, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Florian Kronenberg, Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria
H.-Erich Wichmann, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Klaus M. Weinberger, Biocrates Life Sciences AG, Innsbruck, Austria
Thomas Illig, Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
Karsten Suhre, Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

Cholesterol (Chol) is an important molecule controlling the fluidity of membranes and a precursor for many signaling and regulatory molecules. Total Chol blood concentrations are considered as a risk factor for cardiovascular and other diseases. The associations between Chol concentrations and metabolic pathways in humans are still not fully understood, especially how the lipids are modulated by diabetes or statin treatment. To address these questions we used targeted metabolomics to analyze Chol-associated lipidome and further metabolic pathways in human individuals.

We quantified and analyzed up to 363 metabolites in 283 serum samples from the human cohort KORA (Cooperative Health Research in the Region of Augsburg) with profiling by electrospray ionization (ESI) on API 4000 tandem mass spectrometer (1). High-throughput analyses were assisted by robotized liquid handling, quality assurance and multivariate data analyses. We targeted selected analytes from the following classes: amino acids, biogenic

amines, oligosaccharides prostaglandins, acylcarnitines, sphingomyelins, and glycerophospholipids.
When analyzing the concentrations of total Chol, HDL, and LDL and triglycerides we discovered novel significant associations with several analytes pointing to so far unknown cross-talks in metabolic pathways. Some phosphatidylethanolamines correlated with HDL concentrations (C36:2 at p=1.0E-09, C40:6 at p=2.2E-09), further phosphatidylcholines with that of triglycerides (C38:4 at p=3.2E-31) or total Chol (C38:1 at p=1.4E-26, C38:2 at p=4.4E-25) and sphingomyelines with total Chol (C16:0 at p=2.3E-20, C18:0 at p=2.2E-17). We discovered a significant correlation of amino acid concentrations (e.g. Glu at p=4.7E-10, Phe at p=2.1E-08, Trp at p=1.4E-07) with that of triglycerides. These new associations and potential links with endpoints such as cardiovascular disease may now be investigated.

1. Gieger, C., Geistlinger, L., Altmaier, E., Hrab√© de Angelis, M., Kronenberg, M., Meitinger, T., Mewes, H. W., Wichmann, H. E., Weinberger, K. M., Adamski, J., Illig, T., and Suhre, K. (2008) PLoS Genet 4, e1000282

Metabolomics of SSRI Response in Depression: Pathway and Functional Genomics

Abstract: H11

Hongjie Zhu, North Carolina State University USA
Yuan Ji, Mayo Clinic
Scott Hebbring, Mayo Clinic
Oliver Fiehn, UC Davis
Zhao-Bang Zeng, North Carolina State University
Richard Weinshilboum, Mayo Clinic
Rima Kaddurah-Daouk, Duke University Medical Center

Treatment of major depressive disorder (MDD) has changed dramatically over the years, beginning with the introduction of monoamine oxidase inhibitors and the “tricyclic” antidepressants and, later, the selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs). However, the detailed molecular mechanisms responsible for depression remain unknown despite of numerous studies implicating the norepinephrine (NE) and serotonin (5-HT) systems. Response to current therapies varies considerably, with approximately 40% of MDD patient nonresponders and 60% “nonremitters” after an initial trial of therapy. In this study members of the “Metabolomics Network for Drug Response Phenotype” and in partnership with members of the Pharmacogenomics Research Network embarked on a metabolomics -pharmacogenomic analysis of response to the SSRI drug escitalopram. Specifically, studies were performed with groups of 20 depressed “responders” and 20 “nonresponders” to SSRI escitalopram therapy to define metabolites that correlate with response. A series of compounds that included glycine showed significant associations with response. We then mapped these metabolomic signatures to biochemical/biological pathways, followed by the identification of genes encoding components of the pathway. For genes encoding key pathway regulators, we also initiated in-depth gene resequencing to identify common genetic

polymorphisms that might be associated with variation in metabolomic signatures, thus, moving from response to SSRI treatment to metabolomic signatures to pathways to pharmacogenomics.

Proof-of-principle for the Effectiveness of Untargeted Plasma Metabolite Profiling to Discover Molecular Aberrations That Arise From Gene Defects and Drug Treatments Abstract: H4

Steven Gross, Weill Cornell Medical College USA
Qiuying Chen, Weill Cornell Medical College
Steven Fischer, Agilent Technologies
Brian Ratliff, New York Medical College
Michael Goligorsky, New York Medical College

Untargeted plasma metabolite profiling has enormous potential for the diagnosis of conditions that arise from defects in metabolism. Here, we evaluated the capability of an LC/MS-based metabolite profiling platform to provide a broad survey of the plasma metabolome and to discover the defect in purine metabolism that predictably results in mice when xanthine oxidoreductase (XOR) is inactivated, either by gene ablation or pharmacological inhibition with allopurinol. Since XOR catalyzes the conversion of xanthine and hypoxanthine to uric acid and then allantoin, we wondered whether this anticipated metabolic disturbance would be identified using untargeted metabolite profiling. Applying two distinct chromatographic modes, positive/negative ion monitoring TOF MS and statistical analysis of the resulting data with Profiler and GeneSpring MS software, we were able to confidently survey >3,700 distinct molecular features in 1 ¬µl of murine plasma and found that 23 and 26 of these features were altered in expression by >8-fold (up or down) vs. control in xor-knockout and allopurinol-treated mice. Searching of these most differentially-expressed features against an in-house modified METLIN database revealed profound changes in purine metabolism; as expected, knockdown of XOR activity (both genetically and pharmacologically) was associated with a marked increases in xanthine and hypoxanthine levels as well as the disappearance of plasma urate and allantoin relative to control. Associated with the predicted changes in purine metabolism, numerous unanticipated changes in metabolism were observed, some readily reconciled and others not. These unanticipated changes cast new light on the functions of XOR and its unappreciated metabolic linkages. Taken together, the present studies confirm the emerging power of untargeted metabolite profiling as an unprecedented tool for biomedical discovery.

Phenotyping consumers of black tea and red grape flavonoids by nutrikinetic analysis of gut microbial metabolites Abstract: D29

Ewoud van Velzen, Universiteit van Amsterdam, (Unilever R&D Vlaardingen), The Netherlands
Johan Westerhuis, Universiteit van Amsterdam, The Netherlands
John van Duynhoven, Unilever R&D Vlaardingen The Netherlands

Age Smilde, Universiteit van Amsterdam Amsterdam The Netherlands

The bacterial conversion in the gut to smaller phenolic acids (catabolism) involves the successive formation of a large number of phenolic metabolites and may include bioactive molecules with beneficial effects on the human (cardiovascular) health. The extent to which flavonoids are being metabolized are depended on the composition and the activity of the resident microflora and can largely vary among individuals. To asses the bioavailability, metabolism and the gastrointestinal transport of flavonoid metabolites through the human system (human metabolome & gut microbiome), the time course of the microbial conversions has to be addressed. In this current work we will present a new integrated method of metabolomics and nutrikinetics that is particularly designed to examine the metabolic flows through the gastrointestinal tract without the need for any prior knowledge about the underlying metabolic pathways [1]. The method furthermore exploits the cross-over design in the data using multilevel analysis and permits a separate analysis of the between-individual and the within-individual variation in the data [2]. We applied this integrated method for the analysis of flavonoid metabolites that derived from a single-dose oral intervention of black tea solids and a red grape extract. 1H NMR and chromatographic profiles from blood and plasma were collected to provide information about their existence, identity, absorption, elimination and (changing) output levels over a 48 hours period. This information allowed us to assess the time response of the gut-mediated flavonoid metabolites, and furthermore allowed us to distinguish gut-microbial phenotypes within a human test population. Knowledge about the time response of flavonoid catabolism is particularly important to assess the activity of the microbial community, transport mechanisms and metabolic routes underlying the inter-individual differences in effects on human health.

[1] van Velzen, E.J.J. et.al, (2008), J. Prot. Res 7(10), 4483-4491.
[2] van Velzen, E.J.J. et.al. (2009), J. Prot. Res. DOI: 10.1021/pr801071p.

6:15 p.m.	8:30 p.m.	**Reception with Exhibitors** and Even Numbered Poster Authors	Promenade Foyer & Ballroom B

Tuesday, September 1

9:00 a.m.	9:45 a.m.	**Keynote Plenary:** Hans Westerhoff, Free University	Ballroom A

Hans Westerhoff
Department of Molecular Cell Physiology
BioCentrum Amsterdam
VU University
Amsterdam, The Netherlands

Abstract not available at time of printing

9:45 a.m.	10:00 a.m.	Break	Promenade

Tuesday, September 1

10:00 a.m.	12:00 (Noon)	**Session 4: Plant / Phytochemical Metabolomics** Robert Hall, Chair	Ballroom A
10:00 a.m.	10:40 a.m.	Invited Speaker: John Draper, Aberystwyth University	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 1: Peter Facchini, University of Calgary	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 2: Jane Ward, Rothamsted Research	Ballroom A
11:20 a.m.	11:40 a.m.	Podium Speaker 3: Lloyd W. Sumner, The Samuel Roberts Nobel Foundation	Ballroom A
11:40 a.m.	12:00 (Noon)	Podium Speaker 4: Ajjamada Kushalappa, McGill University	Ballroom A

Metabolome Modeling in Plant-Pathogen Interactions

John Draper, University of Wales Aberystwyth, Aberystwyth, Ceredigion, UK

Authors: David Parker[1], Manfred Beckmann[1], Hassan Zubair[1], David P. Enot[1], Zaira Caracuel-Rios[2], David P. Overy[1], Stuart Snowdon[1], Nicholas J. Talbot[2], and John Draper[1]
[1]Institute of Biological Environmental and Rural Sciences, Aberystwyth University, Penglais Campus, Aberystwyth, SY23 3DA, United Kingdom.
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The mechanism(s) by which biotrophic and hemibiotrophic fungal pathogens simultaneously subdue plant defence and sequester host nutrients are poorly understood. Using metabolite fingerprinting, we show that *Magnaporthe grisea,* the causal agent of rice blast disease, dynamically re-programmes host metabolism during plant colonisation. Identical patterns of metabolic change occurred during *M. grisea* infections of three different grass species; barley, rice and *Brachypodium distachyon*. Targeted metabolite profiling by GC-MS confirmed the modulation of a conserved set of metabolites. In pre-symptomatic tissues, malate and polyamines accumulated, rather than being utilised to generate defensive reactive oxygen species (ROS) and the levels of metabolites associated with amelioration of redox stress in different cellular compartments increased dramatically. Early accumulation of quinate, hydroxycinnamic acids and un-polymerised lignin precursors were consistent with the manipulation of defensive phenylpropanoid metabolism by *M. grisea* and the inability of susceptible hosts to mount a hypersensitive reaction or produce lignified papillae (both requiring ROS) to restrict pathogen invasion. Rapid proliferation of *M. grisea* hyphae in plant tissue after 3 days was associated with accelerated nutrient acquisition and utilisation by the pathogen. Conversion of photoassimilate into mannitol and glycerol for carbon sequestration and osmolyte production appear to drive hyphal growth. Taken together, our results indicate that fungal pathogens deploy common metabolic re-programming events in diverse host species to suppress plant defense and colonize plant tissue.

References: (1) Parker, D., Beckmann, M., Enot, D., Overy, D., Rios, Z.C., Gilbert, M., Talbot, N. & Draper, J. (2008). Rice blast infection of *Brachypodium distachyon* as a model system to study dynamic host/pathogen interactions. *Nature Protocols* **3**, 435-445.
(2) David Parker, Manfred Beckmann, Hassan Zubair, David P. Enot, Zaira Caracuel-Rios, David P. Overy, Stuart Snowdon, Nicholas J. Talbot, and John Draper (2009) Metabolomic analysis reveals a common pattern of metabolic re-programming during invasion of 3 host plant species by *Magnaporthe grisea.* Plant Journal 59 , In Press.

Gene and Metabolite Prospecting as a Platform to Identify Novel Benzylisoquinoline Alkaloid Biosynthesis Abstract: I03

Peter Facchini, University of Calgary Canada
Jörg Ziegler, Department of Biological Sciences, University of Calgary
Jürgen Schmidt, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany
Jake Stout, Department of Biological Sciences, University of Calgary
Jobran Chebib, Department of Biological Sciences, University of Calgary
Dustin Cram, National Research Council of Canada - Plant Biotechnology Institute
Jacek Nowak, National Research Council of Canada - Plant Biotechnology Institute

Benzylisoquinoline alkaloids (BIAs) are a structurally diverse group of ~2,500 specialized metabolites found mostly in a limited number of related plant families. Many BIAs exhibit potent pharmacological activities including morphine and codeine (narcotic analgesics), berberine and sanguinarine (antimicrobials), (+)-tubocurarine (muscle relaxant), papaverine (vasodilator) and noscapine (cough-suppressant). The chemical diversity of BIAs results from (1) the reconfiguration of the original benzylisoquinoline moiety via the formation of novel C-C or C-O bonds, with each new structural backbone leading to one of several branch pathways, and (2) the decoration of various BIA structural variants with a variety of functional group and covalent bond modifications (e.g. oxidation, reduction, hydroxylation, methylation, acetylation and glycosylation) catalyzed by a suite of categorically conserved enzymes. We have established a platform based on targeted metabolite profiling, modest expressed sequence tag (EST) libraries and relevant bioinformatics tools to tap into the rich diversity of BIA biosynthetic pathways in plants. Cell cultures of 18 BIA-producing plant species, representing four related plant families (i.e. the Papaveraceae, Ranunculaceae, Berberidaceae and Menispermaceae) were analyzed by Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS) and liquid chromatography electrospray ionization-tandem mass spectrometry (ESI-MS/MS) to establish accurate alkaloid profiles for each cell culture. Approximately 6,000 clones from a cDNA library prepared for each of the 18 cell cultures were subjected to random dye-termination sequencing. Assembly of the empirical metabolite profiles from each species into putative biosynthetic networks inferred the occurrence of myriad pathway intermediates and products, and revealed the active BIA branch pathways and modifications in each system. Integration of known and candidate biosynthetic genes in each EST database into the corresponding biosynthetic network has facilitated the identification of new enzymes involved in the elaboration of BIA diversity. The utility of this comparative and integrative approach to the discovery of novel specialized metabolism genes will be discussed.

Quality vs Quantity: Metabolomics in cereal crop improvement Abstract: I18

Jane Ward, Rothamsted Research UK
Claudia Harflett, Rothamsted Research
Sonia J. Miller, Rothamsted Research

Melissa A. Fitzgerald, International Rice Research Institute
Robert D. Hall, Plant Research International
Peter R. Shewry, Rothamsted Research
Michael H. Beale, Rothamsted Research

Cereals are major sources of human nutrition either directly or indirectly through animal food, and breeding of new varieties with improved nutritional and other quality parameters, as well as stress and pest resistance is a major research activity. We are applying metabolite profiling and metabolomics to assist in understanding biochemical processes leading to quality enhancement , biomarker discovery and QTL localisation. We are involved in a number of projects involving both field- and CE-grown cereals. These range from studies of the development of Fusarium sp fungal infections of wheat ears to studies of the effects of different S and N fertiliser regimes. The talk will mainly draw on examples of the use of, high-throughput, NMR-ESI-MS screening and targeted phytochemical analysis in two major EU-funded projects . HEALTHGRAIN is a project concerning health promoting compounds including phenolics , folates, methyl-donors and phytosterols and involves a diversity screen of grains of over 200 different cereal cultivars as well as G X E assessment of metabolome variability. A component of the EU META-PHOR project involves metabolomic analysis of rice (leaf and grain tissue) in the context of the production of fragrance, an important metabolite-based quality that provides an economic premium in basmati and jasmine varieties. In both projects statistical correlation of metabolomic data against quality parameters, including other phytochemical data, has proven very informative for breeders in ‚Äúbest‚Äù variety selection.

<u>Spatially Resolved, Integrated Transcriptomics and Metabolomics Reveal Enhanced Secondary Metabolism in Medicago truncatula Root Border Cells</u>

Bonnie Watson Plant Biology Division, Samuel Roberts Noble Foundation
Ewa Urbanczyk-Wochniak Plant Biology Division, Samuel Roberts Noble Foundation, Monsanto Company
Mohamed Bedair Plant Biology Division, Samuel Roberts Noble Foundation
Yuhong Tang Plant Biology Division, Samuel Roberts Noble Foundation
David V. Huhman Plant Biology Division, Samuel Roberts Noble Foundation
Stacy Allen Plant Biology Division, Samuel Roberts Noble Foundation
Lloyd W. Sumner, Plant Biology Division, Samuel Roberts Noble Foundation

The root tips of many plant species, including legumes, produce thousands of differentiated cells which remain appressed to the root until separated from the root by exposure to water. These cells, termed border cells, provide a biotic boundary fundamental in ecological interactions, rhizosphere modifications, and plant defense. Unfortunately, little is known about the molecular or biochemical roles of these cells in plant-microbe interactions. Here, we report a systematic evaluation of the integrated molecular and biochemical differences between border cells, root tips, and whole roots of the model legume Medicago truncatula.

Border cells begin their life as root cap initial cells. They then evolve as columella and peripheral root cells, which ultimately transition to border cells. Starch deposition is increased in the root cap cells as determined by staining and serves as a carbon reserve for developing root cap and border cells. The comparative microarray data revealed dramatic increases in border cell β-amylase resulting in increased starch hydrolysis. Increased glycolysis intermediates such as glucose-6-P, fructose-6-P, fructose and increased TCA cycle components support an increase in central carbon metabolism. Increases were also observed in branched chain amino acids and polyamines such as β-alanine which we associate with increased CoA biosynthesis and carbon shuttling into secondary metabolic pathways. Both flavonoid and triterpene pathways were also increased in border cells with specific increases in compounds associated with defense and signaling observed.

We conclude from the cumulative constitutive data that border cells have an enhanced metabolic capacity and content relative to root cap and whole root. Root tips have increased starch deposition and as these cells transition into border cells, the border cells expend their starch reserves for fortification of primary and secondary metabolism better enabling border cells as front-line defenders in plant-pathogen interactions and important ambassadors in mutualistic signaling.

Metabolomics of plant and necrotrophic pathogen interaction Abstract: I12

Ajjamada Kushalappa, McGill University Canada
Kenchappa Kumaraswamy McGill University
Venkatesh Bollina McGill University
Thin Choo AAFC
Yves Dion CEROM
Sylvie Rioux CEROM

Necrotrophic pathogens produce toxins and enzymes to kill plant cells at the site of invasion so that they can obtain food for continued growth. The plants defend pathogen attack based on constitutive and induced biochemicals, in addition to structural and morphological resistance to invasion. The biochemicals can be either metabolites or proteins. Metabolomics was applied to better understand the mechanisms of resistance in barley against a necrotrophic pathogen, Fusarium graminearum. The spikelets of barley genotypes varying in resistance were spray inoculated with pathogen and the disease severity, as proportion of spikelets diseased in a spike, was assessed. The mock or pathogen inoculated spikelets were harvested, metabolites extracted and analyzed using a hybrid mass spectrometer (LC-nESI-LTQ OrbiTrap), in both negative and positive ionization modes. The raw data was aligned using XCMS and sieved for adducts using esi/camera. Depending on the study, about 5000 peaks/accurate masses were consistently detected over replicates, of which about 600 metabolites had significant treatment effects. A t-test identified about 200 resistance related (RR) metabolites, of which more than150 were assigned putative names of identity based on accurate masses, isotope ratios (IntelliXtract), and fragmentation patterns using several databases (METLIN). These metabolites were searched in the metabolic pathway databases to identify their pathways of synthesis. The RR metabolites

mainly belonged to four metabolic pathways: lipid/fatty acid, phenylpropanoid, flavonoid and terpenoid. JA signaling is one of the major ways to resist F. graminearum and instead of SA more phenylpropanoids and flavonoids, known antimicrobials and cell wall enforcing compounds, were detected. Some terpenes detected are known phytoallexins. The potential application of these RR metabolites to identify functions of FHB resistance QTLs, and screening advanced breeding lines for resistance to FHB will be discussed.

12:00 (Noon)	2:00 p.m.	**Lunch with Poster Authors and Exhibitors**	

12:30 p.m.	1:30 p.m.	**Luncheon Workshop – Thermo Scientific**	Lacombe

Tuesday, September 1

2:00 p.m.	4:00 p.m.	**Session 5: Fluxomics & Metabolic Engineering** Marta Cascante, Chair	Ballroom A
2:00 p.m.	2:40 p.m.	Invited Speaker: Teresa Fan, University of Louisville	Ballroom A
2:40 p.m.	3:00 p.m.	Podium Speaker 1: Thomas W. Binsl, VU University of Amsterdam	Ballroom A
3:00 p.m.	3:20 p.m.	Podium Speaker 2: Deyu Xie, North Carolina State University	Ballroom A
3:20 p.m.	3:40 p.m.	Podium Speaker 3: Fionnuala Morrish, Fred Hutchinson Cancer Research Center	Ballroom A
3:40 p.m.	4:00 p.m.	Podium Speaker 4: Hunter Moseley, University of Louisville	Ballroom A

Integrating Stable Isotope-Resolved Metabolomics (SIRM) with Functional Genomics

Dr. Teresa W.-M. Fan, Center for Regulatory & Environmental Analytical Metabolomics (CREAM), Department of Chemistry, University of Louisville, Louisville, KY, United States

Authors: Teresa W-M Fan[1,2,3], Richard M. Higashi[1,2,3], Hunter Moseley[1,2], and Andrew N. Lane[1,3]
[1] Center for Regulatory and Environmental Analytical Metabolomics (CREAM), [2] Dept of Chemistry and [3] J.G. Brown Cancer Center, University of Louisville, Louisville, KY, USA

Transcriptomics provides information on gene expression networks while proteomics link this network to protein expression. Metabolomics is the 3rd essential partner that defines metabolic network(s). All three are required to achieve systems biochemical understanding. Both NMR and MS enable broad perspective analysis of the metabolome, particularly the transformation pathways. We combined these tools with stable isotope tracers to explore the anticancer mechanisms of different selenium forms in human lung adenocarcinoma A549 cells. Using [U-C-13]-glucose as tracer, we demonstrated that the toxic action of redox-sensitive selenite [1] and methylseleninic acid (MSA) on A549 cells involves glycolytic and mitochondrial dysfunctions, while redox-insensitive selenomethionine (SeM) was much less toxic with a different mode of action. SeM also showed no chemopreventive efficacy in the A/J mouse lung cancer model. We have modeled changes in metabolic fluxes using ^{13}C-isotopomers of metabolites in Se-treated A549 cells grown in [U-C-13]-glucose or [U-C-13/N-15]-glutamine. The time course changes in intracellular metabolites and C-13-isotopomers indicate early oxidative damage that disrupts the Krebs cycle activity (e.g. anaplerotic pyruvate carboxylation), protein synthesis machinery, lipid/nucleotide biosynthesis, and/or amino acid transporters, leading to inhibition of cell

proliferation and adhesion. Using the metabolomics-edited transcriptomic approach (META) [1], we found the expression patterns of key genes and proteins to be consistent with key metabolic dysfunctions. More importantly, we have successfully applied this approach clinically to human lung cancer patients [2].

1 Fan, T. et al. (2005) Metabolomics-edited transcriptomics analysis of Se anticancer action in human lung cancer cells. Metabolomics 1, 325-339
2. Fan, T. WM et al. (2009) Altered regulation of metabolic pathways in human lung cancer discerned by ^{13}C stable isotope-resolved metabolomics (SIRM). Molec. Cancer, In press.

TCA Cycle Flux Estimation Using Stable Isotope Snapshots in a Single Animal Biopsy

Abstract: B03

Thomas W. Binsl, VU University Amsterdam The Netherlands
David J.C. Alders, VU University Medical Centre Amsterdam
Jaap Heringa, VU University Amsterdam
A.B. Johan Groeneveld ,VU University Medical Centre Amsterdam
Johannes H.G.M. van Beek, VU University Amsterdam, VU University Medical Centre Amsterdam

It is difficult to determine metabolic fluxes quantitatively in single tissue biopsies from experimental animals. Here we report a novel approach for in vivo flux quantification using stable isotope labeling. Our approach overcomes the difficulty that it is impossible to obtain a time series of metabolite levels from the same tissue area at high resolution.
We developed a protocol based on timed (5.5 min) infusion of 13C isotope enriched substrates for the TCA cycle followed by quick freezing of tissue biopsies. NMR measurements of the extract were followed by flux estimation based on a computational model of carbon transitions between TCA cycle metabolites and connected amino acids. To this end we developed a computational framework in which metabolic systems can be flexibly assembled, simulated and analyzed. Implementing a model of the TCA cycle we showed by extensive simulations that the timed infusion protocol reliably quantitates multiple fluxes.
An experimental validation of the method was done in vivo on hearts of anesthetized pigs. The experiments were done under two different conditions: basal state (n = 7) and cardiac stress caused by infusion of dobutamine (n = 7). About 8-10 tissue samples, 50-160 mg dry mass, were taken per heart. Using the isotope snapshot method the TCA cycle flux was 6.11 ¬± 0.28 (SEM) micromol/g dry/min for control vs 9.39 ¬± 1.03 micromol/g dry/min for dobutamine stress. Oxygen consumption calculated from the TCA cycle flux and from 'gold standard' blood-gas-based measurements were nearly identical, correlating with r = 0.87 (p < 5e-5). Given good NMR spectra, further flux parameters (e.g. carbon substrate flux) could also be quantified. We propose that our novel isotope snapshot methodology is suitable for flux measurements in biopsies in vivo.

Gas chromatography-mass spectrometry based metabolomics of transgenic tobacco plants (Nicotiana tabacum var. Xanthi) reveals differential effects of complete and incomplete flavonoid pathways on the metabolome Abstract: B12

De-Yu Xie, North Carolina State University USA
Corey Broeckling, Department of Horticulture and Landscape Architecture, Colorado State University

Gas chromatography-mass spectrometry (GC-MS) based metabolomics was used to comparatively examine the metabolic consequences of introduction of a complete anthocyanin pathway by expressing PAP1 gene, an incomplete proanthocyanidin pathway by expressing anthocyanidin reductase (ANR) gene, and a complete proanthocyanidin pathway by expressing both PAP1 and ANR genes in tobacco(Nicotiana tobacum) plants. Eighty-seven identified metabolites including primary and secondary metabolites were clearly detected from young leaf, old leaf and stems of wild type tobacco plants, among which the abundance of 71 metabolites was tissue specific and developmentally dependent. Our data clearly show that constitutive over-expression of a complete anthocyanin(PAP1) or proanthocyanidin (PAP1 and ANR) pathway in tobacco resulted in altered pools of numerous primary metabolites; for example, the levels of shikimic acid and malonic acid, which are the early metabolic precursors of flavonoids pathway, were significantly diminished. Introduction of an incomplete proanthocyanidin pathway by only expressing the ANR gene resulted in altered accumulation of nineteen identified primary and secondary metabolites, many of which are metabolically unrelated to flavonoids biosynthesis. Included in this list are several terpenoid cembranoids, which are aphid-defensive metabolites that are significantly increased by the expression of ANR transgene. In addition, coupled expression of the PAP1 and ANR genes led to clear separation of the accumulation patterns of certain metabolites from the PAP1 or ANR alone transgenic plants. Our results support the value of using comparative metabolomics approaches for revealing and measuring the off-target effects of transgenes on the metabolomes in metabolic engineering efforts.

Oncogene-induced cell cycle entry and cellular re-programming for induction of neoplasia engages pathways for de novo lipid synthesis Abstract: B08

Fionnuala Morrish, Fred Hutchinson Cancer Research Center USA
Jhoanna Noonan, Fred Hutchinson Cancer Research Center
Felix Nau, Fred Hutchinson Cancer Research Center
Carissa Perez, Fred Hutchinson Cancer Research Center
Ben Reed Endocrinology and Nutrition, University of Washington
Gerard Evan, University of California, San Francisco, Cancer Research Institute
Ian Sweet, Endocrinology and Nutrition, University of Washington
Marc Van Gilst, Fred Hutchinson Cancer Research Center
Joanna Kelleher, Laboratory for Metabolic Engineering and Bioinformatics, Massachusetts Institute of Technology
David Hockenbery, Fred Hutchinson Cancer Research Center

The generation of lipids for cell division and neoplasia requires the coordination of substrate uptake and carbon flux into metabolic pathways to initiate de novo lipogenesis. However, molecular factors involved in the regulation of these events have not been fully elucidated. The Myc oncogene is a serum induced cell cycle regulator and reprogramming factor involved in both induction of pluripotency and neoplasia. De novo lipid metabolism is important for cell cycle entry and Myc regulates carbon flux through the TCA cycle but Myc,Äôs influence on lipid metabolism, during cell cycle entry or cellular reprogramming to a neoplastic state, have not been investigated. In this study we confirm Myc,Äôs ability to alter carbon flux into lipids using stable isotope analysis. Labeling of myc-/- and myc+/+ fibroblasts with multiple metabolic substrates, including [U-13C] glucose, glutamate, acetate and acetoacetate reveal that Myc increases the production of fatty acids from each of these substrates during cell cycle entry. In addition, metabolic inhibitor studies confirm Myc,Äôs dependence on de novo fatty acid synthesis for continued proliferation. Temporal studies on lipid synthesis in isolated pancreatic islets, from the pInsMycERTAM/Bcl-XL model of Myc inducible pancreatic beta cell neoplasia, demonstrate that Myc increases de novo lipid metabolism during induction of tumorigenesis. Since this model displays an embryonic stem cell-like gene expression profile these data suggest lipid metabolism may be associated with reprogramming of cells to embryonic stem cell-like states. To our knowledge, this is the first study of temporal analysis of glucose carbon flux into lipids during Myc-induced neoplasia. When combined with our prior work on Myc,Äôs regulation of central carbon metabolism, these data suggest a significant role for this oncogene in integrating cell cycle signaling networks with metabolic processes required to drive bioenergetic and synthetic pathways for cell proliferation and tumorogenesis.

Stable Isotope Resolved Metabolomics (SIRM) of UDP-GlcNAc and UDP-GalNAc Metabolism in Prostate Cancer Abstract: B09

Hunter Moseley, University of Louisville USA
Alex Belshoff, University of Louisville
Richard Higashi, University of Louisville
Teresa Fan University of Louisville
Andrew Lane, University of Louisville

Supranutritional dietary selenium supplement reduces the risk of prostate cancer and shows promise as a chemoprevention agent for several other prominent cancers. Previous in vitro studies demonstrated methyl seleninic acid's (MSA) effectiveness at inhibiting prostate cancer cell growth. We found that MSA has numerous inhibitory effects on central metabolism, including a striking effect on sugar nucleotides, which we identified as uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) and uridine diphospho-N-acetylgalactosamine (UDP-GalNAc) by NMR and FT-ICR-MS. In particular, UDPGlcNAc is an important sugar donor for regulating intracellular proteins by O-glycosylation, including c-Myc, p53 and VDAC. We used FT-ICR-MS for rapid, high-confidence measurement of the time course of C-13 incorporation into both UDP-GlcNAc and UDP-GalNAc molecules from [U-C-13]-Glucose, in LNCaP and LN3 prostate cancer cells. This incorporation involves the flow of different C-13 labeled

functional moieties via parallel, converging metabolic pathways. The ultra high mass resolution and high precision of the isotopomer intensities is critical for reliable quantification of all 17 combined UDP-GlcNAc/UDP-GalNAc carbon mass isotopomers. We developed a combined simulated annealing and genetic algorithms (SAGA) method to parse the observed mass isotopomer intensities into individual species, that are comparable with positional isotopomer information from NMR. This SAGA method also provides information about the relative importance of specific metabolic pathways to C-13 incorporation. Replacement of initial unlabeled UDP-GlcNAc and UDP-GalNAc by labeled species is consistent with a regulated steady state synthesis and consumption of this important intermediate. The rate of C-13 enrichment of both UDP-GalNAc and UDP-GlcNAc was substantially decreased by MSA along with a similar decrease in the rate of unlabeled UDP-GlcNAc/UDP-GalNAc disappearance. This suggests an effect of MSA on the enzymatic synthesis and utilization of both UDP-GlcNAc and UDP-GalNAc.

4:00 p.m.	4:15 p.m.	Break	Promenade

Tuesday, September 1

4:15 p.m.	6:15 p.m.	**Session 6: Microbial/Environmental Metabolomics** Mark Viant, Chair	Ballroom A
4:15 p.m.	4:55 p.m.	Invited Speaker: Steven Oliver, Cambridge University	Ballroom A
4:55 p.m.	5:15 p.m.	Podium Speaker 1: Sara Cooper, University of Washington	Ballroom A
5:15 p.m.	5:35 p.m.	Podium Speaker 2: Manuel Liebeke, Ernst-Moritz-Arndt University Greifswald	Ballroom A
5:35 p.m.	5:55 p.m.	Podium Speaker 3: Myrna Simpson, University of Toronto	Ballroom A
5:55 p.m.	6:15 p.m.	Invited Speaker 4: Mark Viant, University of Birmingham	Ballroom A

Context and content of the yeast metabolic network

Steve Oliver, Cambridge Systems Biology Centre & Department of Biochemistry, University of Cambridge, Cambridge, UK

Abstract: The complete genome sequence of the bread and ale yeast Saccharomyces cerevisiae has been available for over 13 years now and, together with the facility with which that genome can be manipulated genetically, this has meant that yeast has been an experimental organism of choice for both Functional Genomics and Systems Biology. In the area of metabolism, this has led to global studies that exploit either logical or stoichiometric models of the metabolic network and has enabled the prediction of the phenotypes of both single- and double-deletion mutants. From this, it is very clear both that our current picture of the metabolic network of even this simple eukaryote is incomplete. My talk will address both these issues. I shall give an account of impact of different nutrient limitations on gene expression, redundancy, and the metabolic network. I shall then explain how we have employed a Robot Scientist to discover the genes encoding enzymes for 'orphan' chemical reactions. (Orphan reactions are ones that we know must occur in yeast metabolism but for which no gene has been assigned to the necessary enzyme.) Finally, I shall consider how the joint efforts of both human and robot scientists will be required to fully elucidate the genetic, physiological, and biochemical control of metabolism in this simple and rather specialised eukaryote.

Genome-wide profiling of small molecule metabolites in Saccharomyces cerevisiae
Abstract: E06

Sara Cooper University of Washington USA
Greg Finney University of Washington
Shauna Brown University of Washington
Sven Nelson University of Washington
Jay Hesselberth University of Washington
Michael MacCoss University of Washington
Stanley Fields University of Washington

Quantification of metabolite levels is an important aspect of biology that is often neglected in genome-wide studies. We have developed a method for high throughput screening of metabolites in the yeast Saccharomyces cerevisiae, which uses capillary electrophoresis in combination with fluorescent derivatization of yeast cell extracts. Using this approach, we quantified primary amine-containing metabolites in nearly 6000 yeast strains, each lacking a single gene. This large dataset provides a resource for understanding the role of individual genes in metabolism. We identified several strains with altered metabolic profiles. For example, many strains lacking one of the ribosomal protein genes, such as RPS19, show accumulation of lysine and significant accumulation of a second unique peak. These strains are being characterized under alternative growth conditions and through the use of HPLC and GCxGC-TOFMS for more detailed profiles. Many of the genes identified in my analyses have not been previously characterized; others encode components of well-known complexes. To identify additional genes that may be involved in metabolic pathways, we clustered the strains based on the similarities among profiles. Using this method, we demonstrate that strains deleted for genes involved in the biosynthesis of arginine (ARG1, ARG3, ARG4, ARG5,6) cluster together based on lack of arginine and accumulation of arginine precursors such as citrulline and ornithine. Similarly, other clusters likely represent groups of genes with related functions. In addition to characterizing variation resulting from specific genetic changes, we have profiled thirty-seven wild yeast strains whose genomes have been resequenced. Together the metabolite and genetic data reveal genetic changes underlying metabolic differences among wild yeasts, providing insight into the natural variation among individuals of a species. Ongoing studies include metabolomic profiles of these strains using GCxGC-TOFMS. Together these data provide significant insight into metabolism in Saccharomyces cerevisiae.

Staphylococcus aureus under oxygen and nutritional limitations, stress responses by a metabolomic and proteomic view Abstract: E14

Manuel Liebeke, Ernst-Moritz-Arndt-University Greifswald Germany
Kirsten Doerries Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald
Daniela Zuehlke, Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald
Stephan Fuchs, Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald
Hanna Meyer, Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald
Michael Hecker, Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald
Michael Lalk, Competence Center Functional Genomics, Ernst-Moritz-Arndt-University Greifswald

Mimic important stress conditions to Staphylococcus aureus, a human colonizing pathogen bacterium and measuring the response of the metabolome and proteome will lead to a more detailed view into the physiology and regulation of this microorganism. By choosing oxygen depletion and nutrient starvation, we try to address wound conditions or growing in other niches of our body.

Along a cultivation time of 24 hours under aerobic/glucose limitation and anaerobic/oxygen limitation conditions we take multiple samples for comprehensive time resolved proteome and metabolome analysis of the methicillin resistant strain S. aureus COL. We used 1H-NMR for extracellular metabolome investigations and GC-MS as well as LC-MS(TOF) for intracellular metabolite quantifications. The results were compared with the 2D-gel proteome data and predictions were made for regulation of distinct stress responses.

By glucose deprivation the ATP level decreased and a stringent response mediated by (p)ppGpp starts. A complex system of up- and down-shifts begins to adopt this nutrition limitation stress. Somehow different is the answer of S. aureus to oxygen limitation, proteins for mixed acid fermentation increase, as a consequence lactate, formate and alcohols followed on the metabolome level. A vast number of proteins and metabolites are altered in their level to protect the bacterium against a redox imbalance.

Surviving under these limited conditions required a perfect adaption of the regulatory system of the bacterium, we try to decipher this interplay between metabolites and proteins with the new data for S. aureus.

1H NMR Metabolomics of Earthworm Responses to Phenanthrene Exposure in Soil
Abstract: E26

Myrna Simpson, University of Toronto Canada
Andre Simpson, University of Toronto
Jennifer McKelvie, University of Toronto
Sarah Brown, University of Toronto

1H NMR metabolomics of earthworm tissues may provide insight into any metabolic changes that may occur during exposure to sub-lethal and bioavailable concentrations of environmental contaminants. We have examined the response of Eisenia Fetida, the recommended earthworm species for ecotoxicity tests, to phenanthrene (a model polycyclic aromatic hydrocarbon) exposure in soil. We studied both short-term and long-term responses to varying concentrations of phenanthrene. Earthworm tissues were extracted into a D2O-based buffer and analyzed by 1H NMR (using PURGE for water suppression) and data were analyzed with multivariate statistics (AMIX). Our first study showed that PCA separation and clustering of exposed earthworms was observed for the higher phenanthrene concentrations when compared to control earthworms. PC1 loadings plots showed that alanine, leucine, lysine and valine were responsible for the majority of the observed variation. Furthermore, amino acid concentrations were observed to increase with increasing phenanthrene concentration suggesting a strong link between metabolomic responses and soil phenanthrene concentrations after only 48 hours of exposure. Maltose was also identified in loadings plots but was also found to be variable amongst the control group. PLS regression of earthworm responses as compared to both total and bioavailable phenanthrene revealed strong, positive correlations suggesting that 1H NMR metabolomics may be a potential tool for measuring bioavailability directly. E. fetida responses to phenanthene exposure over a 30 day period showed a correlation between amino acid levels and the concentration of phenanthrene in soil. Earthworm responses were still detectible even when the bioavailable phenanthrene concentration diminished. These studies demonstrate the potential for 1H NMR metabolomics to further the understanding of E. fetida ecotoxicity to sub-lethal concentrations of organic chemicals in soil. Future research will focus on testing the inherent variability of observable 1H NMR metabolites in E. fetida and the response of E. fetida in different soil types.

Toxicity screening in *Daphnia magna* for ecological risk assessment: Application of FT-ICR mass spectrometry based metabolomics

Nadine S. Taylor[1], Ralf J. M. Weber[1], Andrew D. Southam[1], Tristan G. Payne[2], Olga Hrydziuszko[1] **and Mark R. Viant[1]**

[1] School of Biosciences, University of Birmingham, Edgbaston, Birmingham, U.K.
[2] School of Engineering, University of Birmingham, Edgbaston, Birmingham, U.K.

Abstract: The water flea (*Daphnia magna*) is one of the most widely used aquatic organisms for toxicity testing as part of the ecological risk assessment of new chemicals. With many gene expression studies of chemical toxicity in *D. magna* published, it is surprising that essentially no metabolomics research has been reported. There is currently widespread interest in exploiting toxicogenomic approaches to screen the toxicity of chemicals, enabling their rapid categorisation into classes of defined mode-of-action (MOA) and prioritising them for further testing. Here we present the first metabolomics studies of chemical toxicity screening in *D. magna* using direct infusion (DI) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. First we optimised the analytical methods and confirmed that mass spectra comprising of thousands of reproducible features can be recorded from whole-body homogenates of 30 neonates or single adult daphnids. Validation of these methods using copper as a model toxicant found significant changes to the metabolome consistent with the MOA of copper, including markers of oxidative stress. Subsequently we confirmed that spectra can be obtained from the haemolymph of individual adults (1µl sample). A series of 24-hr acute toxicity studies in adult daphnids were then conducted using four chemicals with differing MOAs: cadmium (oxidative stress), dinitrophenol (uncoupler of oxidative phosphorylation), fenvalerate (neurotoxicant) and propranolol (beta-blocker). Comparing the biochemical responses in whole organism homogenates versus haemolymph, we have shown that whole-organism metabolic fingerprints provide the greatest discrimination between control and toxicant-exposed animals. Further analyses are on-going, with the goal to determine the ability of FT-ICR to differentiate the MOAs. The ability to measure metabolic fingerprints from individual daphnids raised the potential of correlating molecular toxicity to whole organism reproductive fitness. Such studies are now underway. In conclusion, these experiments highlight the potential of DI FT-ICR metabolomics for high-throughput screening of chemical toxicity.

7:00 p.m.	8:00 p.m.	Reception with Exhibitors Featuring the Dave Babcock Trio	Promenade

8:00 p.m.	11:00 p.m.	**Conference Dinner Awards and Entertainment**	Ballroom A

Wednesday, September 2

Start	End	Event	Room
7:30 a.m.	9:00 a.m.	**Registration**	Promenade

Start	End	Event	Room
9:00 a.m.	11:00 a.m.	**Session 7: Metabolomics and Informatics** Roy Goodacre & Bruce Kristal, Chairs	Ballroom A
9:00 a.m.	9:40 a.m.	Invited Speaker: Oliver Fiehn, UC Davis	Ballroom A
9:40 a.m.	10:00 a.m.	Break	Promenade
10:00 a.m.	10:20 a.m	Podium Speaker 1: Karen W. Phinney, National Institute of Standards and Technology	Ballroom A
10:20 a.m.	10:40 a.m.	Podium Speaker 2: Piotr T. Kasper, Netherlands Metabolomics Centre	Ballroom A
10:40 a.m.	11:00 a.m.	Podium Speaker 3: David Enot, Biocrates AG	Ballroom A
11:00 a.m.	11:20 a.m.	Podium Speaker 4: Henning Redestig RIKEN Plant Science Center Japan	Ballroom A

Towards a Standardized Metabolomics Repository

Prof. Oliver Fiehn, UC Davis Genome Center, 451 Health Sci. Dr. Davis CA 95616
U.S.A.

Co-Authors: Oliver Fiehn, Tobias Kind, Gert Wohlgemuth, Martin Scholz, Dinesh Barupal, Kirsten Skogerson, Kwang-Hyeon Liu

Today's metabolomic approaches by LC- or GC-based mass spectrometry enables detection of up to 500 metabolites in complex biological matrices. To understand systemic changes in metabolism, these metabolites need to be quantified and referenced in a standardized manner to allow comparisons across studies, and especially across organs and species. Current steps towards a standardized metabolomic repository are presented which are aimed to evolve to a platform-independent public database, in analogy to transcriptomic DBs such as NCBI's Gene Expression Omnibus.

Metabolomic databases need to be supported by unambiguous metabolite identification using at least two independent parameters, such as 'retention index' and 'mass spectrum', in order to be compliant to Minimum Reporting Standards that have been recommended by the Metabolomics Society. For primary metabolites up to 550 Da, the FiehnLib libraries comprise over 2,000 retention-index based spectra for over 1,000 different metabolites using quadrupole and time-of-flight GC-MS platforms. The complementary LipidBLAST library annotates complex lipids based on presence and relative abundance of parent ions and MS/MS fragmentations for use in QTOF, ion trap or OrbiTrap mass spectrometry. The volatile BinBase is used to profile and disseminate data for volatile emissions, e.g. in plant pathogen defense or in human breath condensates. The major database SetupX/BinBase currently hosts data for over 17,000 samples in more than 250 studies covering microbes, plants and animals. Data are available for downloads and queries including cross-references to PubChem and KEGG. However, many identified metabolites are not yet referenced in biochemical pathway databases. We present a three-tiered approach combining biochemical, chemical and mass spectral similarity distances to provide network graphs for both identified and novel, structurally unknown signals.

A Reference Material to Support Metabolomics Measurements Abstract: C19

Karen Phinney, National Institute of Standards and Technology United States
Nathan Dodder, National Institute of Standards and Technology
Daniel Bearden, National Institute of Standards and Technology
Stephen Long, National Institute of Standards and Technology
Lane Sander, National Institute of Standards and Technology
Michele Schantz,National Institute of Standards and Technology
Katherine Sharpless, National Institute of Standards and Technology
Stephen Stein, National Institute of Standards and Technology
Gregory Turk, National Institute of Standards and Technology
Stephen Wise, National Institute of Standards and Technology

Metabolomics can provide a unique perspective on the biochemical status of an organism through systematic analysis of the metabolites in relevant samples such as plasma, urine, cells, or tissues. Interpretation and understanding of the results of metabolomics experiments is aided by identification and, when possible, quantification of the relevant metabolites in the samples. No single technique can provide a full catalog of all the metabolites in a sample, and sample handling and preparation techniques can affect both metabolite identification and quantification. For these reasons, comparison of data sets from different metabolomics experiments can be quite difficult. NIST has collaborated with the National Institutes of Health (NIH) to develop a Standard Reference Material (SRM) for metabolomics, with the goal of providing a stable, well-characterized reference material for metabolomics research. SRM 1950 Metabolites in Human Plasma is a human plasma pool collected from 100 individuals. The SRM was prepared from both male and female donors and has an ethnic makeup that is representative of the U.S. population. The concentrations of more than 50 metabolites, including electrolytes, hormones, glucose, creatinine, vitamins, amino acids, and fatty acids have been determined by various methods. Quantification was based upon isotope-dilution methodology when possible. In addition to the quantitative aspects of this SRM, qualitative information may be valuable for those interested in metabolite profiling and identification. Therefore we have also pursued identification of additional metabolites through a number of different techniques, including LC-MS, GC-MS, GCxGC-TOF-MS, and NMR. Although this qualitative information is anticipated to broaden the utility of the SRM, assigning a level of confidence or uncertainty to a compound identification poses some unique challenges.

The analysis of MS fragmentation trees as an integral part of the metabolite identification pipeline Abstract: C12

Piotr T. Kasper, Netherlands Metabolomics Centre, The Netherlands
Miguel Rojas-Cherto, Netherlands Metabolomics Centre, Leiden University
Theo Reijmers, Netherlands Metabolomics Centre, Leiden University
Rob J. Vreeken, Netherlands Metabolomics Centre, Leiden University
Thomas Hankemeier, Netherlands Metabolomics Centre, Leiden University

Metabolite identification constitutes one of the central aspects of metabolomics. Identification of metabolites can, in principle, be achieved using high resolution multistage mass spectrometry (MSn) because it provides a feature rich fingerprint of the structure of the precursor ion. However, neither general methodology for the identification nor extensive databases of metabolites with multistage mass spectrometric data are available at the moment. We demonstrate in this presentation the feasibility of the strategy for metabolite identification based on analysis of fragmentation trees.

High resolution MSn experiments were performed on LTQ-Orbitrap equipped with TriVersa NanoMate (Advion) nanoelectrospray ion source. A software tool, integrating among others Chemistry Development Kit (CDK) and XCMS libraries, was used for spectral data processing

Multistage Molecular Formula software tool has been developed for resolving an elemental composition of a compound and fragment ions derived from MSn data. The process of elemental formula assignment and fitting within elemental formula paths removes unwanted artefacts of the spectra.

Reproducibility and robustness of fragmentation tree acquisitions were tested by varying experimental conditions and a concentration of a metabolite and an optimised acquisition protocol was established. It was investigated to which extent the variation of conditions such as fragmentation energy, isolation width etc. did change the fragmentation pattern or topology of hierarchical relations between fragments.

A library of fragmentation trees of metabolite standards is being created. The database will facilitate the challenging task of metabolite identification by comparing the topology of fragmentation trees.

We demonstrate how the developed analytical strategy based on analysis of fragmentation trees can be used to discriminate between metabolite isomers with the same elemental composition and only a slightly different structure, but with a significantly different biological function.

Our results provide firm basis for developing a generic, multistage mass spectrometry based platform for efficient identification of metabolites.

Housekeeping metabolites: a new normalization concept in metabolomics Abstract: C07

David Enot, Biocrates AG Austria
Matthias Kohl, University Bayreuth, Bayreuth, Germany
Matthias Keller, University Hospital Essen, Germany
Therese Koal, Biocrates AG
Klaus Weinberger, Biocrates AG
Hans-Peter Deigner, Biocrates AG

Comparability of quantitative data generated in different laboratories, on different platforms and obtained by varying sample work-up conditions remains a challenge in omics data analysis. Present concepts in metabolomics require further evaluation, a gold standard for normalization of metabolomics data is lacking. To date, normalisation in metabolomics relates to a large panel of more or less sophisticated data manipulation ranging from correcting for samplewise bias (e.g. total ion count), using context specific parameter (e.g. creatinine), calibrating to internal/external standards or applying numerical transformations (e.g. logarithm).

These methods, usually as a combination, are routinely employed to rub sample homogeneity discrepancies off and to improve data characteristics such as variance stabilisation. Nevertheless, these cannot guarantee that inherent additional experimental error is completely corrected, hindering both data modelling and followed up interpretation.

This problem (the reduction of the technical error) is known also in the context of transcriptomics and proteomics. We here present very promising results for the use of housekeeping/reference metabolites in quantitative metabolomics. Our data show that the use of housekeeping/reference metabolites increases the power of statistical analyses, i.e., reduces technical errors. In addition, we have identified several metabolites which qualify as candidates for ‚Äûuniversal‚Äú housekeeping metabolites as they show a constant concentration level independent of experimental conditions.

Interestingly, these ‚Äûuniversal‚Äú housekeeper or reference metabolite candidates were found stable among various species and may facilitate animal model comparison and transfer of data to man. In fact, this may be a unique feature of metabolomics, unlike the situation e.g. in transcriptomics.

Cross-contribution Robust Normalization of Mass Spectrocopy Based Metabolomics Data
Abstract: C21

Henning Redestig, RIKEN Plant Science Center Japan
Atsushi Fukushima, RIKEN Plant Science Center
Thomas Moritz, Umea Plant Science Center
Arita Masanori, RIKEN Plant Science Center
Kazuki Saito, RIKEN Plant Science Center
Miyako Kusano, RIKEN Plant Science Center

Most mass-spectroscopy (MS) based metabolomics studies are only semi-quantitative and therefore depend on efficient normalization techniques to suppress systematic error arising from e.g. chromatography and derivatization efficiency. A commonly used approach is to include isotope labeled internal standards (ISs) and then express the estimated metabolite abundances as relative to the recovered standard. Due to problems such as insufficient chromatographic resolution, the analytes may however directly influence the estimates of the IS - a phenomenon known as cross-contribution (CC). Confounding between analytes and internal standards is a well-studied problem in other fields of MS coupled chromatography but has not been given much attention in the metabolomics application. Normalization using ISs that suffer from CC-effects will cause significant loss of information if the biological analyte is affected by the experimental design. We examined several GC-MS based metabolomics datasets and found that correlation between analytes and the utilized ISs was quite common and that standard normalization approaches therefore suppressed the variance of interest. Here we present a novel normalization algorithm dubbed Cross-contribution Robust Multiple standard Normalization (CRMN) which solves this problem by correcting the ISs for any CC effects before using them for normalization. We tested our method on two biological datasets and a multi-component dilution mixture and found that it was superior at purifying the relevant signal compared to current normalization methods. We therefore believe that the inclusion of the CRMN method may significantly increase the precision of mass spectroscopy based metabolomics protocols. The CRMN method was implemented as an open source software package for the statistical programming environment R and is distributed via http://www.metabolome.jp/download/r-package.

11:20 a.m.	12:00 (Noon)	**Keynote Plenary:** Philip N. Baker (Manchester University and University of Alberta)	

Application of metabolomic technologies for patient benefit.

Philip N Baker, Maternal and Fetal Health Research Centre, St Mary's Hospital, University of Manchester, UK, and Dean-Elect, Faculty of Medicine and Dentistry, University of Alberta, Canada

Identification of discriminatory metabolites offers the potential to generate novel biomarkers and new screening tests for a range of clinical conditions, and may generate original hypotheses regarding disease pathogenesis. Clinical studies must use extremely well curated, well matched samples, accompanied by comprehensive metadata. These principles will be illustrated using two examples.

The pregnancy complication preeclampsia remains a leading cause of maternal death and significant baby morbidity/mortality. A maternal predisposition may be evident in the early pregnancy plasma metabolome. We performed a nested case control study within the Screening fOr Pregnancy Endpoints (SCOPE) Study. Plasma obtained at 15 ± 1 weeks from 60 women who subsequently developed preeclampsia and 60 matched controls was analyzed using GCMS and UP-LCMS. 45 metabolites were significant indicators of subsequent preeclampsia ($P<0.05$). The majority were chemically identified as fatty acids, keto- or hydroxy- fatty acids, carnitines, lipids or phospholipids. A model combining metabolites gave an odds ratio for developing preeclampsia of 19.5 (95%CI 7.5-50), with area under ROC 0.91. This metabolic derangement may be incorporated into future screening tests.

There is intense interest in novel biomarkers which improve the diagnosis of heart failure. Serum samples from 52 patients with systolic heart failure and 57 controls were analyzed by GCMS and the raw data reduced to 272 statistically robust metabolite peaks. 20 peaks showed a significant difference between case and controls. Two such metabolites were pseudouridine, a nucleotide present in t- and rRNA and a marker of cell turnover, as well as tricarboxylic acid cycle intermediate 2-oxoglutarate. 3 new compounds were also excellent discriminators: 2-hydroxy,2-methylpropanoic acid, erythritol and 2,4,6-trihydroxypyrimidine.

12:00 (Noon)	1:00 p.m.	**Lunch with Poster Authors and Exhibitors**	

Wednesday, September 2

1:00 p.m.	3:00 p.m.	**Session 8: Novel Technologies & Imaging** Oliver Fiehn, Chair	
1:00 p.m.	1:40 p.m.	Invited Speaker: Richard D. Smith, PNL	
1:40 p.m.	2:00 p.m.	Podium Speaker 1: Wei Zou, UC Davis	
2:00 p.m.	2:20 p.m.	Podium Speaker 2: Steven Fischer, Agilent Technologies	
2:20 p.m.	2:40 p.m.	Podium Speaker 3: John Shockcor, Waters Corp & University of Cambridge	
3:45 p.m.	4:05 p.m.	Podium Speaker 4 Rawi Ramautar Utrecht University, The Netherlands	

Advances in LC-MS and LC-IMS-MS for High Throughput and Ultra-Sensitive Metabolomics Measurements

Richard D. Smith, Battelle Fellow and Chief Scientist, Environmental Molecular Sciences Laboratory
and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, United States

Co-Authors: Richard D. Smith, Thomas O. Metz, Qibin Zhang, Jie Ding, Mike Belov, Keqi Tang, Jason Page, and Christina M. Sorensen

Key challenges in metabolomics derive from the complexity of biological samples, the chemical diversity of constituent metabolites, the range of relative abundances of possible interest, as well as the growing desire for faster and more effective measurements to facilitate e.g. large population studies or systems biology approaches. To address these challenges, we have been developing improved analysis platforms that incorporate ion mobility separations (IMS) coupled with fast nanoscale liquid chromatography (LC) and mass spectrometry (MS). IMS separations take place on a millisecond time scale, enabling much faster analyses of complex chemical or biochemical mixtures in conjunction with the high speeds achievable for time-of-flight (TOF) mass spectrometer spectrum acquisition. A new nanoLC-IMS-MS platform developed at PNNL and has been characterized for its performance in metabolomics and proteomics applications. The initial evaluation has shown that the new platform provides significant gains in the sensitivity, throughput, and dynamic range of measurements. The gains achieved depend upon the implementation of a more intense and efficient ion source and interface. These improvements are particularly important for multiplexing in the IMS stage, which circumvents space charge limitations on the size of ion populations that can be separated by IMS. The IMS stage is particularly effective for distinguishing different chemical classes and, in conjunction

with high mass accuracy, provides useful information for metabolite identification. The performance achieved with the platform to date will be illustrated and compared with existing platforms in studies at our laboratory.

Predictive multiple reactions monitoring (MRM) as a functional test of hepatocyte-like human embryonic stem cells (ESC) Abstract: A41

Wei Zou, UC Davis USA
Vladimir Tolstikov, UC Davis Genome Center

Analysis of low abundant metabolites represents significant challenge in xenobiotic metabolism and in endogenous metabolomics. In several cases, we examined a promising approach detecting low abundant metabolites in complex biological matrixes. It was found that predictive multiple reactions monitoring (MRM) detection method offers the highest sensitivity among various acquisition modes used to detect secondary metabolites at trace levels in plant extracts, human embryonic stem cells lysate and media, or human urine. Utilizing high performance liquid chromatography (HPLC) coupled to 4000 Qtrap mass spectrometry (MS) allowed identification of specific molecular locations where biotransformation had altered the parent compounds. Further, accurate masses and isotopes ratios of the identified metabolites were obtained in the selected ion monitoring (SIM) mode using high-resolution high-accuracy ultra performance liquid chromatography (UPLC) LTQ Orbitrap MS. Spectral accuracy and isotopic ratio filters were considered for metabolite identification using MassWorks. Theoretical fragmentation patterns generated by Mass Frontier were compared to those acquired from LC-MS acquisitions for further validation. This platform of hybrid triple quadruple ion-trap MS together with software-supported intelligent data acquisition allowed identification of trace-level secondary metabolites that were not detectable in full scan mode.

A novel method for robust LC/MS-TOF Analysis of Hydrophilic Metabolite Classes by Aqueous Normal Phase on a Silica Hydride-Based Column Abstract: F2

Steven Fischer, Agilent Technologies USA
Theodore Sana, Agilent Technologies
William Ciccone, Microsolv Technology Corporation
Maria Matyska, San Jose State University
Joe Pesek, San Jose State University

A limiting factor in many metabolomics analyses is the ability to robustly chromatograph mixed classes of hydrophilic compounds. A generic LC/MS-TOF method has been developed that is capable of separating sugars (polar neutral), amino acids (polar basic) and organic acids (polar acidic) in complex mixtures using the aqueous normal phase (ANP) technique on a silica hydride surface. This material can be used over a wide range of mobile phase compositions from pure aqueous to pure non-polar and is compatible with analysis in both positive and negative ion

modes. This highly reproducible method for hydrophilic compounds now enables the use of retention time as an orthogonal physical parameter for identification with an Accurate Mass Retention Time (AMRT) database. In order to encompass a broad range of polar metabolites it was necessary to buffer the system at neutral pH. That was accomplished with a pyridine/acetic acid buffer in the acetonitrile solvent. The aqueous component of the mobile phase contains formic acid as the additive. A Cogent Diamond Hydride (150mm√ó2.1mm) column, using a gradient starting at high acetonitrile, and buffered at neutral pH, results in a relatively high initial pH (near 7). As the amount of water (containing formic acid) is increased the pH is lowered. Therefore, the gradient changes with respect to both polarity (increasing) and pH (decreasing). Under these conditions, virtually all polar metabolites are retained. Isobaric compounds or compounds differing by one amu can be separated. The method demonstrates the ability to retain and chromatograph a chemically diverse compound set with the potential for rapid positive / negative ion switching to allow single run analysis.

Development of a Lipidomic Platform Based on a Hybrid Quadrupole Time-Of-Flight (QTof) Ion-Mobility Abstract: H10

John Shockcor, Waters Corp & University of Cambridge USA
Jose Castro-Perez, Waters Corporation
Kate Yu, Waters Corporation
Emma Marsden-Edwards, Waters Corporation
Henry Shion, Waters Corporation
Robert Vreeken, The Netherlands Metabolomics Centre
Thomas Hankemeier, The Netherlands Metabolomics Centre

Mass spectrometry plays an important role in the study of lipid biochemistry. For example, the information it provides can be critical to understanding the mechanism of pathogenesis for diseases which are linked to abnormal physiological levels of certain lipids including atherosclerosis and diabetes.

Recent technological advances have yielded hybrid instruments such as the quadrupole time-of-flight (QTof) ion-mobility mass spectrometer which is an ideal platform for lipid analysis. This instrument possesses clear analytical advantages over conventional nominal mass instruments in full scan sensitivity, mass accuracy, spectral resolution and fragmentation. It can also provide an added dimension of separation to the analysis via ion-mobility. With the hybrid QTof it is possible to conduct class specific precursor and neutral loss acquisitions over a single experimental run using an instrument acquisition mode called elevated-energy mass spectrometry (MSE). MSE is a term which is used to describe a strategy which performs data-independent fragmentation experiments. The exact mass information obtained provides a more definitive descriptor of the molecule and is very important to removal false positives.

Applying this technology allowed the specific detection of intact molecular ions, precursor ions and neutral losses in either positive or negative ionization mode that upon collision-induced dissociation generated characteristic diagnostic fragment and neutral loss ions. However, ions yielding structural information about the fatty acid side-chains are of low

abundance and typically other solvents such as LiOH (post column or addition to the mobile phase) are used to obtain structural information about them. Utilizing the unique characteristics of the ion-mobility sector of the instrument we are able to perform time-aligned parallel fragmentation experiments which yield fragment ions that facilitate assignment of the fatty-acid side chains.

In this paper, a robust LC/MS platform for detection and characterization of multiple lipid classes is described and illustrated with data from extracted human plasma samples.

Stable and flexible metabolic profiling by comprehensive CE-ToF-MS Abstract: D21

Rawi Ramautar, Utrecht University The Netherlands
Oleg Mayboroda, Leiden University Medical Centre
André Deelder, Leiden University Medical Centre
Govert Somsen, Utrecht University
Gerhardus De Jong, Utrecht University

Combined capillary electrophoresis-time-of-flight mass spectrometry (CE-ToF-MS) is a powerful technique for the fast and efficient analysis of ionogenic compounds. The application of CE-ToF-MS to metabolic profiling of body fluids, however, may be hindered by reproducibility and coverage problems. In this presentation a novel approach for reproducible and comprehensive metabolic profiling by CE-ToF-MS will be outlined. Very stable CE performance is accomplished by the use of noncovalent capillary coatings comprised of double and triple layers of charged polymers. These easy-to-produce coatings provide high migration-time reproducibility and good tolerance against sample matrix compounds. Moreover, one capillary with different coatings can be used for both cationic and anionic compounds demonstrating the flexibility of this approach for providing an extended coverage of metabolites in only two CE runs. Incorporation of in-capillary preconcentration by pH-mediated stacking further aids the detection of low-level metabolites.

The performance of this novel CE-ToF-MS platform for metabolic profiling of body fluids has been studied for large groups of urine samples. Using multivariate techniques, it is demonstrated that the CE-ToF-MS platform allows high quality metabolomic data to be obtained. Accordingly, it can be applied for delineation of the urinary metabolome in a clinical setting. For example, the applicability and usefulness of the CE-ToF-MS platform will be outlined by the elucidation of metabolites involved in complex regional pain syndrome (CRPS) and urinary tract infection (UTI). Multivariate statistical analysis of the recorded profiles revealed biomarker candidates, which were subsequently identified by accurate mass and/or MS-MS using QToF-MS.

Wednesday, September 2

3:15 p.m.	5:15 p.m	**Session 9: National Metabolomics Initiatives** Thomas Hankemeier, Chair	
3:15 p.m.	3:45 p.m	Invited Speaker 1: Thomas Hankemeier, Netherlands Metabolomics Centre	
3:45 p.m.	4:05 p.m.	Invited Speaker 2: Tony Bacic, University of Melbourne	
4:05 p.m.	4:25 p.m.	Invited Speaker 3: Kazuki Saito, Riken Plant Science Center	
4:25 p.m.	4:50 p.m.	Invited Speaker 4: Hans Vogel, University of Calgary	
4:50 p.m.	5:15 p.m.	Invited Speaker 5: Rima Kaddurah-Daouk, Duke University Medical Center	

Netherlands Metabolomics Centre: what can you expect?

Thomas Hankemeier, Netherlands Metabolomics Centre, The Netherlands

This lecture gives an overview of the ambition and research program of the Netherlands Metabolomics Centre (NMC). NMC's ambition is to create a world-class metabolomics knowledge infrastructure to improve personal health and quality of life. Major challenges of metabolomics with regards to quantitative metabolomics, identification of metabolites and data analysis are addressed by a comprehensive and multidisciplinary research program. The focus is on developing tools which are not available yet, but are crucial to obtain biologically relevant answers. For example, new metabolomics platforms are being developed addressing relevant metabolite classes which are not covered yet sufficiently, new algorithms and modeling methods are developed to obtain biological interpretable in a better way. The developed tools are applied in biology-driven projects to find answers to important biological/biomedical questions. Projects are, for example, directed on finding biomarkers for better diagnostics and (personalized) interventions for multi-factorial diseases, to support the development of functional food promoting health and develop plants for food products with better taste and nutritional value.

The tools developed will de made available to the metabolomics community in several ways. A Demonstration & Competence Lab has been established to validate and implement the methods developed by the researchers and to apply them to (pre)clinical and other metabolomics studies in a medium/high throughput manner. Hands-on trainings and guest visits are to transfer the developed technology to interested researchers. A data support platform has been established to integrate all tools required for the metabolomics pipeline as a web-based platform, which will be made available for the worldwide metabolomics community. These include tools for experimental design, data processing, identification of metabolites, data analysis and biological interpretation, including a data warehouse to store all data including relevant metadata/clinical data.

In the lecture a perspective on the development of metabolomics will be given, the need for standardization and databases will be discussed, and the necessity to collaborate and exchange results between research groups and consortia in an open and worldwide manner

NMC has recently received a major fund by the Netherlands Genomics Initiative (www.genomics.nl), and has a research budget of 53M€ from 2008-2013. Core partners of the NMC are DSM, Leiden University, Leiden University Medical Centre, Plant Research International, TNO, Schering-Plough, Unilever, University of Amsterdam, Utrecht Medical Centre, Wageningen University. In addition, a larger number of associate partners are involved in the research projects, such as various academic hospitals, Dutch Genomics Centres of Excellence and instrument manufactures.

Metabolomics Australia: an integrated national biomolecular "omics" and informatics initiative of the Federal Government.

Antony Bacic[1], Steven Smith[2], Robert Trengove[3], Lars Nielsen[4], Sakkie Pretorius[5], Malcolm McConville[6], Ute Roessner[1]

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[6] Department of Biochemistry & Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, 30 Flemington Rd, The University of Melbourne, VIC 3010, Australia

Metabolomics comprises the combination of high-throughput analytical technologies for the detection and quantification of metabolites in biological systems with the application of sophisticated bioinformatic tools for data mining and analysis. The National Collaborative Research Infrastructure Initiative (NCRIS; http://ncris.innovation.gov.au/) established a Biomolecular Platforms and Informatics consortium, managed by Bioplatforms Australia Pty Ltd (BPA), to form national technology platforms to provide the analytical- and informatics-based capabilities to deliver "Systems Biology" to the Australian research community, including industry. This investment, totaling approx. A$80 million over 5 years, involves 4 platforms including Genomics Australia (GA), Proteomics Australia (PA), Metabolomics Australia (MA) and the Australian Bioinformatics Facility (ABF). MA provides "state-of-the-art" metabolomics capabilities including sophisticated analytical facilities, man-power and expertise as well as high-

throughput informatic solutions for metabolomics data analysis and interpretation. MA has 5 nodes including The University of Melbourne shared between the School of Botany and the Bio21 Institute, the Australian Wine Research Institute Ltd (AWRI) in Adelaide, Murdoch University and University of Western Australia in Perth and the University of Queensland in Brisbane. These nodes possess synergistic technologies and expertise enabling the consortium to offer a broad integrated range of services to the research community and industry. For more information, please visit www.metabolomics.net.au.

National Metabolomics Initiatives: Japan

Kazuki Saito, RIKEN Plant Science Center; Graduate School of Pharmaceutical Sciences, Chiba University, Japan

In Japan, several prominent metabolomics research centers are taking initiatives of national metabolomics research. These include RIKEN Plant Science Center in Yokoyama, Keio University in Tsuruoka, Kazusa DNA Research Institute in Kisarazu and other several universities. Strategic funding to fundamental metabolomics or metabolism-oriented research is programmed by Japan Science and Technology Agency (JST). More industry-oriented strategic grant comes from New Energy and Industrial Technology Development Organization (NEDO). Metabolomics meeting is held annually in autumn since 2006 to provide a forum for an interactive discussion of researchers in this field.

Metabolomics of Mice and Men, a bird's-eye view of the Alberta and Canadian perspectives

Hans Vogel, BioNMR Centre Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada

Canada has traditionally been strongly represented in the area of Biological NMR spectroscopy of proteins. This activity has over the years been based at many Canadian universities and numerous research groups are extremely active today and are internationally well recognized for their work. At the same time research activity in the use of NMR spectroscopy for the study of metabolism has been more localized at several National Research Council laboratories; this work also extended beyond the use of NMR spectroscopy and it included extensive use of infrared spectroscopy as well as mass spectrometry. Some of these earlier studies relied on methodologies that are similar to those used in what we now generally call 'metabolomics'. More recently funding provided by Genome Canada has allowed a group of Alberta-based university researchers to develop the Human Metabolome Database. In addition research into various disease biomarkers and plant and microbial metabolism has been initiated province-wide, where we have been able to benefit from some provincial funding streams, which allow us to carry out research that is not necessarily hypothesis-driven. Starting from a brief historic perspective, in

this talk I will discuss some examples of the successful application of (mostly) NMR-based metabolomics research in Alberta-based studies of infectious diseases, cancer and inflammatory bowel diseases. This work benefits from the current push towards translational research and close cooperation between clinicians, basic scientists and technology experts.

Metabolomics Network for Drug Response Phenotype

Rima Kaddurah-Daouk, Director of Pharmacometabolomics Center, Duke University Medical Center, Durham, NC, USA

The Metabolomics Network for Drug Response Phenotype was established two years ago with funding from NIH with the goal of integrating the rapidly evolving science of metabolomics with molecular pharmacology and pharmacogenomics to better define mechanisms involved in variation in response to therapies. Metabolomic "signatures" present in patients who do and do not respond to drug therapy, i.e., signatures that reflect the drug response phenotype, could lead to mechanistic hypotheses that would provide insight into the underlying basis for individual variation in drug response. This could lead a more personalized approach to therapy. The network has assembled under one virtual roof complimentary metabolomics platforms, informatics tools, pathway analysis and modeling capabilities for the broadest coverage of biochemical information and for defining pathways implicated in variation in response to therapy. Metabolic data enabled mapping of several pathways implicated in response to statins and SSRIs classes of therapies. The goals of the Network include developing standards for metabolomics research, a national metabolomics data repository and pathway database.

5:15 p.m.	5:30 p.m.	Conference Closing Remarks	

CORPORATE

POSTER

ABSTRACTS

Agilent Technologies

METLIN Personal Metabolite Database: Enhancements to include MS/MS Spectral Libraries for more confident identification

Steven Fischer
Senior Applications Chemist
Agilent Technologies
Santa Clara, CA, USA

The most common workflow for LC/MS metabolomics typically involves the use of an accurate mass LC/TOF or Q-TOF system. Identifying metabolites is a key step in untargeted metabolomic experiments. Metabolite database searches such as the Agilent METLIN Personal Metabolite Database using accurate-mass MS information can greatly improve compound identification by significantly narrowing the list of possible identities. The METLIN Personal Metabolite Database has not been enhanced to a version that includes an accurate mass MS/MS spectral library for over 1000 compounds. The spectra are acquired at 4 different collision energies (0, 10, 20 and 40 eV) both in negative and positive mode in ESI mode using an Agilent Q-TOF LC/MS system.

The use of the MS/MS spectral search assists in the identification of metabolites when the retention time is not known, or when isomers elute at similar retention times, that is, in cases where the accurate precursor mass is not sufficient for a unique identification.

Mass Profiler Professional Software: Statistical Analysis and Visualization Software - A New Chemometric Package for Mass Spectrometry Data

Agilent Technologies

Theodore R. Sana
Senior Scientist
Agilent Technologies
Santa Clara, CA, USA

Agilent Mass Profiler Professional (MPP) is a chemometric software package designed specifically for processing mass spectrometric data, typically resulting from metabolomics, proteomics, food safety, environmental, forensics, and toxicology experiments.

Using a combination of filtering steps, statistical analyses and model building, it manages and analyzes trends in complex MS data sets, enabling the classification, comparison and analysis of results from different platform technologies: GC/MS, LC/MS, CE/MS and ICP-MS, all in one project. In addition, an optional mzXML package enables the processing of data from other vendor instruments.

Navigation is easy, intuitive and includes a guided workflow for the newcomer with pre-defined data import and analysis steps, so one can quickly perform a basic analysis. There is also an advanced workflow that includes tools such as PCA, PLS-DA, ANOVA, Clustering, ID Browser and Pathway Analysis.

A novel and unique "recursive feature extraction" workflow has been developed to enable the re-mining of data sets, resulting in an iterative improvement in the quality of statistical analysis results. It's also easy to export an inclusion list for Q-TOF MS/MS analysis, and re-import the results back into Mass Profiler Professional.

Bruker BioSpin

Blueberry Extract: Towards the Development of a Quality and 'Fingerprinting' Identification Tool Using NMR

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Efforts are underway to study the potential of plant extracts as a complementary medicine for the treatment of Type II diabetes. 1,2 This disease, which may lead to serious complications, is often initially managed through diet and exercise. Traditional medicines, such as those from the *Vaccinium* genus (i.e. blueberry), are used for treatment by indigenous cultures and herbalists in Canada. Evaluation of the constituents in the extracts from various members of this genus show variation in the quantities of the potentially antidiabetic compounds.2

This current work evaluates the use of NMR spectroscopy for providing information on the amount of desired components and the 'fingerprinting' of plant species to assist in making assessments on quality and efficacy. In this work we present our progress towards developing a NMR based quality and fingerprinting screen to provide information such as:

- relative or absolute quantity of key components present in the crude extract
- geographic origin
- plant part
- information on possible adulterants.

Human Metabolome Technologies, Inc.

Human Metabolome Technologies, Inc. (HMT) is a Japan-based bio-venture company utilizing CE-MS technologies to develop and commercialize metabolomic testing for drug discovery, diagnostic products development and fermention process optimization.

The company was founded in July 2003 by Dr. Masaru Tomita and Dr. Tomoyoshi Soga, two preeminent leaders in metabolomics, and is led by President Ryuji Kanno, a former Agilent Technologies Japan, Ltd. Vice-president with over 25 years experience. HMT is actively establishing licensing and collaborative research agreements with leading companies and research institutions on the basis of our unparalleled CE-MS techniques.

HMT is the first company to specifically harness the incredible measurement capability of Capillary Electrophoresis Mass Spectrometry (CE-MS) for metabolomic testing. Intracellular metabolites are mostly small ionic compounds and thus can be comprehensively analyzed by CE-MS with high resolution and high sensitivity.

Utilizing the technological edge of CE-MS, HMT has developed and patented proprietary methods for the comprehensive detection and quantification of metabolites. By the magic of CE-MS, we can make your research problems disappear.

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For over 70 years, industries around the world have trusted LECO to deliver technologically advanced products and solutions for analytical science. Today's technologies for separation science resolve complex samples and pioneer high sample throughput using GCxGC, GCxGC-TOFMS, GC-TOFMS, and LC-TOFMS. This state-of-the-art instrumentation, combined with the industry's most powerful and intuitive software package, provides an innovative solution for today's most demanding applications, including food, flavor/fragrance, environmental, and metabolomics. Our new LECO/Fiehn Metabolomics Library features over 1,100 spectra of 700 unique metabolomics for metabolite identification in your most complex samples.

PANAMP

PANAMP is a contract metabolomic core facility offering clients one-stop compound characterization that uses NMR, GC-MS, and LC-MS to perform quantitative metabolomics and lipidomics. PANAMP is able to identify and quantify in excess of 3000 metabolites and has also developed a quantitative global metabolome profiling platform based on novel high performance isotope labeling.

There are several features that make PANAMP unique in its abilities in the metabolomic profiling. The first is experience. With 15 years of experience in "-omics" sciences, the scientists involved in PANAMP have not only had the opportunity to develop and refine methods for data collection with excellent sensitivity, but also to develop outstanding resources for data interpretation such as custom software and databases of metabolic compounds. The publically accessible databases developed include the Human Metabolome Database (HMDB), DrugBank, the Toxin and Toxin Target Database (T3DB) and FooDB, which together receive over 1.5 million visits per year. However, PANAMP also has exclusive access to a specially-developed proprietary database of theoretical compounds, allowing for identification and quantification of additional metabolites.

Another significant advantage of PANAMP analysis is the ability to quantify a continually-expanding list of metabolites in both relative and absolute terms. This capability has recently been enhanced by the addition of special techniques in LC-MS to our existing expertise in LC-MS, GC-MS and NMR. The recent PANAMP publication of a global metabolome profiling platform based on High Performance Isotope Labeling (HPIL) chemistry for LC-MS (patent pending) has proven an exceptionally effective strategy for identifying additional metabolites. At present, our list of quantifiable metabolites stands at 3000 compounds.

In addition to all of these assets, PANAMP also excels in the field of lipidomics and is recognized as one of the few metabolomics platforms with the proven ability to provide quantitative characterization across all lipid classes including human, plant, and microbial samples.

Waters Corporation

A Tool Kit for Mass Spectrometry Based Metabolomics

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In order to effectively use mass spectrometry for metabolomic studies it is necessary to have a tool kit of software aids that will allow the user to effectively mine the complex data. In this poster we will present a description of some of the tools needed to effectively "turn data into Information". Among the tools being described are a DataBase Manager, a fragment analysis tool, an integrated multivariate statistical analysis tool and an elemental composition tool with isotopic pattern fitting.

A Shotgun Approach for Profiling Traditional Chinese Medicine Samples Using UPLC/TOF MS^E and Multi-Variant Statistical Data Analysis

Waters Corporation

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Traditional Chinese Medicine (TCM) is a medicinal system that utilizes naturally occurring resources such as plants and animals for treatment of disease. Most TCM plants have to go through some specific processing procedures prior to their medicinal use; hence the actual composition may be different from that of the freshly harvested plant extracts.

In this work, we present a fast and generic approach using the UPLC/TOF MS^E coupled with Multi-Variant Statistical Data Analysis to systematically profile the differences between fresh and prepared samples. A bird's eye view of the major differences was easily obtained with most significant changes identified. We believe that this approach will ultimately facilitate the progress of TCM migrating into Modern Chinese Medicine (MCM).

ACADEMIC

POSTER

ABSTRACTS

A01: Biomedical Biomarkers
Large Volume Injection GC/MS Analysis of Cultured Human Skin Fibroblasts for Metabolomic Studies

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Introduction: Gas chromatography-mass spectrometry (GC/MS) is often used method for metabolomic studies to analyze organic acids, amino acids and sugars. Human cells in a culture provide an important research and diagnostic tool because influence of major part of the external factors is minimized in culture where defined extracellular environment takes place. We report here the method for analysis of cultured human skin fibroblasts by programmable temperature vaporizing injector.

Methods: Fibroblasts (one million cells) were cultured by standard protocol (DMEM, supplemented with 10% FBS and antibiotics ‚Äì streptomycin and penicilin). Cells were quenched by spraying with cold 60 % aqueous methanol (v/v) and immediately extracted into cold 80 % aqueous methanol (v/v). The cell extract was freeze-dried, derivatizated to trimethylsilylderivates, and analyzed with PTV-GC/MS. Conditions of the PTV injector (splitless mode) were optimized using Design of Experiment (DOE) method (model mixture of organic acids). A Plackett-Burman design was used to identify significant factors and a central composite design to choosing their best values. Final conditions were followed: injection volume 5 ¬µl, injection temperature 250 ¬∞C, splitless time 1 min. No backflush or surge was used.

Results: In intracellular content we determined more than 20 metabolites ‚Äì 13 amino acids, 5 organic acids, cholesterol, 7 peaks of not fully characterized sugars and 9 undefined peaks. The efficiency of separation was 200 000 theoretical plates.

Conclusion: Techniques of large volume injection belong to frequently used ones for decreasing a limit of their detection. We optimized the method of PTV-GC/MS analysis of intracellular metabolites from human skin fibroblasts.

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A02: Biomedical Biomarkers
Genotypes contribute to human metabolic phentotypes

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Cholesterol (Chol) is an important molecule controlling the fluidity of membranes and a precursor for many signaling and regulatory molecules. Total Chol blood concentrations are considered as a risk factor for cardiovascular and other diseases. The associations between Chol concentrations and metabolic pathways in humans are still not fully understood, especially how the lipids are modulated by diabetes or statin treatment. To address these questions we used targeted metabolomics to analyze Chol-associated lipidome and further metabolic pathways in human individuals.

We quantified and analyzed up to 363 metabolites in 283 serum samples from the human cohort KORA (Cooperative Health Research in the Region of Augsburg) with profiling by electrospray ionization (ESI) on API 4000 tandem mass spectrometer (1). High-throughput analyses were assisted by robotized liquid handling, quality assurance and multivariate data analyses. We targeted selected analytes from the following classes: amino acids, biogenic amines, oligosaccharides prostaglandins, acylcarnitines, sphingomyelins, and glycerophospholipids.

When analyzing the concentrations of total Chol, HDL, and LDL and triglycerides we discovered novel significant associations with several analytes pointing to so far unknown cross-talks in metabolic pathways. Some phosphatidylethanolamines correlated with HDL concentrations (C36:2 at p=1.0E-09, C40:6 at p=2.2E-09), further phosphatidylcholines with that of triglycerides (C38:4 at p=3.2E-31) or total Chol (C38:1 at p=1.4E-26, C38:2 at p=4.4E-25) and sphingomyelines with total Chol (C16:0 at p=2.3E-20, C18:0 at p=2.2E-17). We discovered a significant correlation of amino acid concentrations (e.g. Glu at p=4.7E-10, Phe at p=2.1E-08, Trp at p=1.4E-07) with that of triglycerides.

These new associations and potential links with endpoints such as cardiovascular disease may now be investigated.

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A03: Biomedical Biomarkers

Tear metabolome analysis in atopic keratoconjunctivitis

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In acute exacerbations of atopic keratoconjunctivitis (AKC) corneal complications that can sometimes lead to severe visual loss can occur. At the same time, atopic diseases are sometimes complicated by potentially blinding complications such as cataract and retinal detachment, however the precise etiology of such disease is still largely unknown. Metabolome analysis has recently become an important systems analysis method in research, however metabolome analysis of tear samples has not yet been reported. We analysed the metabolome profile of tears of patients with AKC to study the mechanism of AKC. 5 patients with AKC with corneal complications, 5 patients with AKC without corneal complications and 5 control subjects were enrolled in this study. Tear samples were collected (30micro litter) from each subject. Metabolome analysis was conducted by capillary electrophoresis time-of-flight mass spectrometry (CE-TOF/MS) and the profiles were compared between the groups. gamma-carboxy-L-glutamic acid was significantly lower in AKC with corneal complications compared to the other groups. Levels of gamma-carboxy-L-glutamic acid in tears may be associated with the pathogenesis of corneal lesions.

A04: Biomedical Biomarkers

Distinguishing Benign from Malignant Pancreatic Disease by Serum Metabolomic Profile

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Background. It is currently difficult to accurately distinguish between benign and malignant causes of pancreatic tumors and biliary strictures. As a result, some patients with benign disease mimicking pancreatic cancer are submitted to major surgeries associated with considerable morbidity. Others are not referred sufficiently early to surgery because a diagnosis of malignancy cannot be confidently achieved. We postulated that the abundance of various metabolites in blood would facilitate the diagnosis of pancreatic and biliary lesions.

Methods. Serum from patients with benign hepatobiliary disease (N=50) and from patients with pancreatic cancer (N=62) were collected under general anesthesia and submitted to 1H-NMR spectroscopy to quantify metabolites typically detectable in this modality. Metabolic profiling was done using Chenomx NMR Suite 4.6 software followed by supervised pattern recognition and orthogonal partial least squares-discriminant analysis (O-PLS-DA), which enables comparison of the whole sample spectrum between groups.

Results. 51 compounds were identifiable. O-PLS-DA was able to predict class separation between patients with benign and malignant disease with greater than 83% accuracy, on seven-fold cross validation. The metabolic profile of patients with pancreatic cancer was significantly different from that of patients with benign disease ($P < 0.001$ by MANOVA). To better determine the

usefulness of this test, a comparison was made to age- and gender-matched controls with benign pancreatic lesions, controlling also for presence or absence of jaundice and diabetes (N=12/group). The metabolic profile in patients with pancreatic cancer remained distinguishable from patients with benign pancreatic lesions.
Conclusions. The serum metabolic profile may be useful for distinguishing benign from malignant pancreatic lesions. Further studies will be required to study the effects of jaundice and diabetes. In addition, a more comprehensive metabolic profile will be evaluated using mass spectrometry.

A05: Biomedical Biomarkers

Label-Free Analysis of Thyroid Hormones by Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry

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L-Thyroxine (T4) and 3,3,Ä≤,5-Triiodo-L-thyronine (T3) represent the major circulating thyroid hormones for regulating basal metabolism and the growth and development of virtually every cell in the human body. Abnormal levels of T3 and T4 characterize a number of serious thyroid diseases. Thus, reliable quantitation of these two low abundance hormones is critical for accurate diagnosis and monitoring of thyroid disorders. Congenital hypothyroidism is one such disease. It affects approximately 1 in 3500 live births and causes neurodevelopmental retardation if not diagnosed and treated in early stages prior to clinical symptoms. Sensitive radioimmunoassays are currently used for routine clinical thyroid analysis but suffer from significant cross-reactivity and poor selectivity. In this work, a new strategy for the analysis of nanomolar levels of T3 and T4 in biological samples was developed using capillary electrophoresis-electrospray ionization-mass spectrometry with on-line sample preconcentration. Sensitivity was maximized using a rational experimental design to optimize analyte responses using on-line sample preconcentration and electrospray ionization conditions while minimizing ion suppression effects. Method validation and applicability to quantifying total T3 and T4 in human plasma and filtered dried blood spot samples will also be examined.

A06: Biomedical Biomarkers

Global quantitation of carbonyl metabolites in human urine and plasma using (14)N-/(15)N-dansylhydrazine labeling and nanoLC/FT-ICR-MS.

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The analyses of ketones and aldehydes in the metabolome have been of recent interest for biological functional studies and disease biomarker discovery. A differential (14)N-/(15)N-isotope dansylhydrazine derivatization strategy for the quantitative analysis of carbonyl compounds in the human metabolome has been developed. This is a universal technique specific for the identification and quantitation of ketones and aldehydes in human urine and plasma by the formation of the dansylhydrazone derivatives. The detection of ketones and aldehydes in biological fluids has been considered in the past to be a challenging task as these analytes are poorly ionized by ESI.

Dansylhydrazine labeling has shown to improve the ESI surface activity and the chromatographic properties of the analyte making it amenable for analysis by LC/MS. The enhanced analyte signal allows for significant reduction of sample size. To this end, nano flow LC methods appropriate for the analysis of derivatized biological samples have been developed which are suitable for the interface with nanoESI-MS in a 9.4 Tesla FT-ICR-MS. This method of derivatization can be used to generate a (14)N-stable isotope labeled internal standard for each corresponding (15)N-labeled analyte which allows for reliable, absolute quantitation of carbonyl metabolites. Derivatization of ketone and aldehyde standards has been carried out and tentative confirmation of thirty of these carbonyls in derivatized urine and plasma has been achieved thus far. A larger standard library for absolute quantification of carbonyls in biofluids is being established. This technique has been applied to the quantitative profiling of ketones and ketoacids in biological fluids as indicators of ketoacidosis, as well as in the analysis of short chain aldehydes as biomarkers of lipid peroxidation. The results of differential analysis of biofluids from healthy individuals and individuals with ketone/aldehyde abnormality, such as in diabetes patients, will be presented.

A07: Biomedical Biomarkers

High-throughput lipidomic phenotyping of human plasma samples using next generation Orbitrap direct-infusion mass spectrometry. A step closer to genetical lipidomics in the context of epidemiology

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Introduction: The success of genome wide association studies in epidemiology is highly dependent on the level of detail of phenotypic data used. The usage of high-throughput lipidomic phenotyping now opens up the possibility to exploit the lipid domain for phenotypic characterization. Orbitrap mass spectrometers equipped with nanospray direct-infusion technology enable high-resolution, stable mass accuracy as well as high-throughput capabilities with high reproducibility. However, tailored data handling and analysis strategies are essential to exploit the vast amount of information generated. In this context we designed a scalable data analysis pipeline, tailored for lipidomic phenotyping in context of large-scale epidemiological studies.

Methods: We used second generation benchtop Orbitrap instruments equipped with chip based direct-infusion technology to investigate human plasma samples. Data were acquired in positive ion mode using a full scan mode from m/z 150 to 1500 and a scan rate of 1 Hz and subsequently stored in a LIMS system. To facilitate analysis of the high-throughput data, an analysis pipeline was designed using the Taverna workbench framework. The analysis pipeline consists of several analysis modules which are organized in a consecutive manner. The pipeline starts with modules performing pre-processing of the raw data followed by modules performing peak-picking and compound identification and finally end up with modules performing multivariate statistical analysis. The modular and controlled architecture of the analysis pipeline not only enables us to

systematically test for optimal data pre-processing schemes but also to monitor and reproduce every single step in the analysis pipeline and evaluate its importance for the final outcome. Furthermore, specific meta-data can be automatically fed into the analysis pipeline. This can be structures and properties of compounds identified, known pathway interactions or literature references, which all can provide valuable information in subsequent analysis steps. First results of this approach, carried out in a large-scale obesity study, will be presented.

A08: Biomedical Biomarkers

Quenching and extraction of human skin fibroblasts in adherent culture for metabolomic studies

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Introduction: Metabolomics aiming to characterize of a phenotype through analysis of metabolome developed to an accepted and valuable tool in life sciences over the recent years. Human cells in a culture provide an important research and diagnostic tool.
Metabolomic analysis of adherent cell cultures present complex challenges mostly given by limited sample size. Rapid quenching of intracellular metabolism simultaneously with considerable removal of superabundant growing medium are the main prerequisites. The aim of our work was to develop efficient quenching and extraction procedure for human skin fibroblasts in culture for metabolomic studies.

Methods: Fibroblasts were cultured in DMEM using standard protocol. After optimization quenching of cells was performed by spraying with 60 % aqueous methanol (v/v; -50 ¬∞C). Leakage of cells during quenching was determined by radio-tracing (14C-glycine), stable isotope dilution (D7- glucose) and determination of ATP. Influence of quenching on intracellular metabolome was analyzed using GC/MS and capillary electrophoresis.

Results: Average leakage of the measured compounds - ATP, D7-glucose and labeled amino acids after incubation of 14C-glycine - was below 4%. Influence of harvesting method on metabolome (quenching vs. trypsinisation) were compared as the natural logarithms of ratios of means and variations. It is clearly visible that quenching technique substantially affects concentrations of number of metabolites. Several metabolites (e.g. citrate, lysine) differ approximately order of magnitude. From variations it is also evident that sample preparation by conventional trypsinisation provides substantially more variable data compared to quenching by spray.

Conclusion: We developed quenching technique for cultured adherent cells providing stable data sample-to-sample with minimal leakage.

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A09: Biomedical Biomarkers

Identification of novel insulin resistance metabolites in a non-diabetic population by global biochemical profiling

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An unmet medical need is the early identification of insulin resistance (IR) with high accuracy. Our goal was to carry out a global, non-targeted biochemical profiling analysis on human plasma samples to identify novel small molecule metabolites that can distinguish insulin-sensitive from IR subjects in a non-diabetic population. Fasting baseline plasma samples from the EGIR-RISC cohort that were representative of the spectrum of insulin sensitivity and glycemic status, based on hyperinsulinemic euglycemic clamp and oral glucose tolerance testing, were analyzed. Several hundred metabolites were detected and quantified in each sample using UHPLC and GC mass spectrometry-based platforms and proprietary cheminformatics software. Analysis showed that 2-hydroxybutyrate (2-HB), creatine, certain fatty acids and other lipid metabolites such as lysoglycerophosphocholines and acylcarnitines, were the most highly significant metabolites separating insulin-sensitive subjects from IR subjects. Quantitative, targeted mass spectrometric measurements were carried out to confirm the screening results for the IR/IS metabolites. To test their clinical significance, these IR metabolites were further measured in 11 morbidly obese subjects (aged 44¬±8 years, mean¬±SD) undergoing bariatric surgery. One year after surgery, BMI had declined from 52¬±7 to 35¬±6 kg/m2 (p<0.001) and the M value had risen from 22¬±11 to 39¬±10 ¬µmol.min-1.kgFFM-1, (p<0.01). Consistent with 2-HB being a sensitive marker of IR, we observed a 1.7-fold reduction in plasma 2-HB concentrations post-surgery for this obese cohort. The significance of these IR metabolites is discussed in the context of their production in IR states, and their observed changes when insulin sensitivity and energy balance are improved.

A10: Biomedical Biomarkers

Exploring the structural dynamics of the interaction of MUC1 and Src in the context of MUC1/ICAM-1 binding induced signaling in Breast Cancer Metastasis

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Breast cancer is the second most frequent cause of cancer deaths in North American women with death resulting from the spread of cancer cells to distant organs. Our lab was the first to show that MUC1, a mucin present in breast milk, may contribute to breast cancer metastasis by binding to the Intercellular adhesion molecule-1 (ICAM-1). We have recently demonstrated that MUC1/ICAM-1 binding initiate the non-receptor tyrosine kinase, Src dependent signaling cascade that promotes migration of breast cancer cells. However, the structural mechanism(s) of MUC1 that activate Src are still unknown. MUC1 consists of i) bulky Extracellular (ECD), ii) transmembrane (TM ,Äì 28 residues) and iii) cytoplasmic (CD ,Äì 72 residues) domains. We hypothesize that ICAM-1 binding triggers cleavage of MUC1-ECD, which releases MUC1-CD to allow competitive binding of Src-SH3 domain leading to Src activation.

Our objectives are; i) to determine the structural elements of MUC1-CD, ii) identify the Src interaction motif(s) on MUC1-CD and iii) to investigate the structural dynamics of the MUC1/Src interaction. We have obtained proton NMR spectra (2D TOCSY and NOESY) of a synthetic 18 residue peptide and a disulfide-linked dimer of the same peptide (for comparison), both encompass the SH2 and putative SH3 binding motifs of MUC1-CD. The monomeric peptide is largely unstructured and would be in keeping with a SH3 binding motif while the dimer shows a higher order structure. Further investigations with full length MUC1-CD and Src SH3-SH2 domains are underway to explore the MUC1/Src interaction using NMR spectroscopy and Surface Plasmon Resonance.

We are the first to investigate the structural mechanism of Src recruitment via SH3 domain by the cytoplasmic domain of MUC1. These findings may be crucial in developing anti-metastatic therapeutic targets such as small molecular inhibitors to reduce Src activation in MUC1 overexpressing breast cancers.

A11: Biomedical Biomarkers

BACTERIAL INFECTIONS AND THEIR METABOLOMIC RESPONSES STUDIED IN MOUSE-MODELS

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Severe sepsis is currently one of the most common causes of morbidity and mortality in intensive care units and its incidence has increased steadily over the past several decades. One of the main problems with sepsis is its diagnosis at an early stage. The conventionally applied culture methods, which involve recovering microorganisms in patients,Äô blood, urine or specimen are time-consuming, labour-intensive and show a high rate of failure, but are still routinely employed. Consequently, innovative new methods for early diagnosis are of utmost importance. Combining metabolomic profiling and multivariate data analysis, different bacterial species have already been automatically identified in growing cultures [1] and this metabolomics approach allowed for the differentiation between bacterial and viral meningitis from an analysis of cerebrospinal fluid samples [2].

Here we demonstrate in mouse-models of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa infections that NMR-based metabolomics can be a powerful tool to

distinguish between different bacterial strains. The bacteria-specific metabolomic profiles represent both metabolites activated by the innate defense system in mice and bacterial metabolites that are released into biological fluids. In our study, we investigated serum samples of infected mice as well as finger- and footprints of bacterial cultures. By combining the results of the in-vivo study with those of the culture experiments potential bacteria-specific biomarkers could be identified.
So far it is known that the immune cascade is activated in response to the lipopolysaccharides (LPS) from the cell wall of gram negative bacteria. However, the influence of further bacterial constituents is not yet disclosed. Therefore we also compared serum metabolite changes resulting from either LPS treatment alone or from E. coli infection in both wildtype and TLR4 deficient mice.

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[2] Coen M. et al. Clin. Infect. Dis., 41:1582 (2005).

A12: Biomedical Biomarkers

Metabolomic Markers of End-stage Heart Failure

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Heart failure is the leading cause of human morbidity and mortality worldwide. To begin the quest for predictive markers that would allow early detection of patients at risk of heart failure, the Biomarkers in Transplantation team within the Centre of Excellence for the Prevention of Organ Failure (PROOF) has evaluated blood metabolomic markers of end-stage heart failure.

Serum was collected from 21 patients with chronic heart failure (CHF) and 20 individuals with normal cardiac function (NCF). Serum samples were analyzed using nuclear magnetic resonance (NMR). In NMR data, there are many metabolites not identified in all samples and there is no consensus on how to best deal with missing values. We performed the statistical analysis using four ways of dealing with this issue. The methods are: 1) replacing missing values with half of the minimum concentration level of the metabolite, 2) replacing missing values with zero, 3) analyzing the data with the missing values and 4) analyzing only metabolites identified in all samples. Comparisons were made between the metabolite concentration levels in CHF versus NCF. Statistical analysis was performed using the robust limma method. A metabolite with an FDR <5% was considered statistically significant.

Using methods 1) and 2) we identified eight metabolites with differential concentration levels in serum between CHF and NCF. Method 3) yielded six statistically significant metabolites and method 4) only three. When we compared the results, we noticed that the metabolites identified by 3) were a superset of the ones from 4), and methods 1) and 2) found all except one of the metabolites from method 3). In addition, methods 1) and 2) identified the same eight statistically significant metabolites.

In summary, the PROOF Centre of Excellence team has identified possible metabolomic markers of late-stage heart failure and tested several methods for dealing with missing values.

A13: Biomedical Biomarkers

Quantification of Glycine- and Taurine-conjugated Bile acids, Total Bile acids, and Phospholipids in Human Bile using 1H MRS

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Bile acids, phospholipids, and cholesterol are the major lipid components in human bile. The composition of bile is altered in various chronic liver diseases, and determining the alteration in the levels of biliary lipids will be of great clinical importance in understanding the pathophysiology of these diseases. The bile acids in bile are generally conjugated to the amino acids glycine and/or taurine. As a result, distinct amide proton (NH) signals are seen in the downfield region of 1H MR spectrum of bile (7.75 ‚Äì 8.05 ppm). These signals are in dynamic exchange with biliary water, and do not represent 100% signal intensity. Previously, these signals have been utilized for the quantification of total glycine- and taurine-conjugated bile acids after changing the pH of bile to 6 ¬± 0.5, minimizing the exchange rate. Although the use of amide signals has specific advantages in in-vitro studies, it is not ideal for in-vivo applications. Thus, we propose an alternative approach more suitable for the in-vivo quantification of glycine- and taurine-conjugated bile acids using their characteristic methylene signals resonating at 3.73 and 3.07 ppm respectively. This method also allows the quantification of total bile acids and phospholipids which can be estimated from the peak areas of the bile acids methyl signal at 0.65 ppm and the phospholipids ‚ÄìN+(CH3)3 signal at 3.22 ppm. The peak areas of these lipid signals can be obtained simultaneously by deconvolution, making the method fast and robust. We compared the results of this method with an NMR-based literature method (which involves dissolution of bile in DMSO, limiting its in vivo utility). We obtained a good correlation between both methods with regression coefficients- 0.97, 0.99, 0.97 and 0.93 for glycine-conjugated bile acids, taurine-conjugated bile acids, total bile acids, and phospholipids respectively, showing good accuracy of the proposed method.

A14: Biomedical Biomarkers

Determining the Metabolic Profile of Cirrhosis and its Progression towards Minimal Hepatic Encephalopathy: A 1H NMR Study of Plasma Samples

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Hepatic encephalopathy is a neuropsychiatric complication of patients suffering cirrhosis or acute liver failure. With severe liver impairment, toxic substances normally removed by the liver accumulate in the blood and damage the function of brain cells. Signs can include impaired cognition, a flapping tremor (asterixis), and decreased levels of consciousness including coma and, ultimately, death. The lower degree of this disorder is called Minimal Hepatic Encephalopathy (MHE) and its diagnosis is based on psychometric tests like the Psychometric Hepatic Encephalopathy Score (PHES) and Critical Flicker Frequency (CFF). These tests are not widespread and distinction between cirrhotic and MHE patients is difficult. Thus, novel, reliable tests that can be easily implemented in routine clinical care are highly desirable.
Here we have analysed the blood plasma metabolome of a control group (n=70) as well as a group of cirrhotic (n=67) and MHE patients (n=39). The plasma metabolic pattern of the controls was markedly different from disease patients as identified using Principal Component Analysis. Increased levels of glucose and decreased levels of lipids, choline and acetyl signals from alpha1-acid glycoproteins were characteristic of disease samples. Partial Least Squares-Discriminatory Analysis together with Orthogonal Signal Correction were used for enhancing the detection of metabolic differences between cirrhotic and MHE samples. Levels of glucose were observed to increase with disease progression as lipids and choline levels decrease suggesting that MHE affects the energetic metabolic pathway. Additionally lactate, citrate and glycerol levels were found to be elevated in MHE patients. The metabolic profile of cirrhotic patients exhibited comparatively elevated levels of various aminoacids including valine, isoleucine, alanine, glycine and histidine. Collectively our study shows NMR based metabonomics is a useful tool for monitoring progression of cirrhosis to MHE and thus offers much potential for determining patient prognosis.

A15: Biomedical Biomarkers

Metabolome Analysis of Cancer Cells under Hypoxia and Glucose Starvation

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Hypovascular tumors are characterized by their chronically hypoxic and glucose-deprived microenvironment, as typically observed in pancreatic cancer. This raises a paradox, however, from a viewpoint of energy metabolism; cancer cells perpetually proliferate while exhausting glucose and oxygen from the surrounding tissue. Intrinsically, cancer cells actively consume glucose even under aerobic condition (Warburg effect) and their adaptive responses to hypoxia further enhance glucose consumption. With a limited supply of glucose, upregulation of glycolysis alone cannot fully explain energy production of hypoxic cancers. Here, applying state-of-the-art metabolomics technology based on capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), we analyzed energy metabolism of several human cancer and normal cell lines exposed to hypoxia and glucose deprivation by measuring more than 130 compounds with time. The results exhibit unexpected cancer-specific metabolic features, which sparked an idea that cancer cells exposed to a severe nutrient deprivation rely on an atypical anaerobic energy metabolism that resembles the one identified in parasitic helminthes. Intriguingly, pyrvinium pamoate, a commercially available

anthelmintic, is known to be cytotoxic against not only parasites but also cancer cells exclusively under nutrient deprived condition (Esumi, H., et al. (2004) Cancer Sci. 95(8): 685‚Äì90), supporting an idea that cancer cells and parasitic helminthes share a unique anaerobic energy production machinery in common. Accordingly, we demonstrated a potential of CE-MS-based metabolomics, realizing comprehensive and temporal monitoring of the energy metabolism of cancer cells. This unique metabolic machinery may constitute a novel therapeutic target to eradicate not only parasites but also tumors.

A16: Biomedical Biomarkers

ANALYSIS OF CARBONYL COMPOUNDS IN EXHALED BREATH CONDENSATE BY LC/MS

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The detection and monitoring of biomarkers for oxidative stress has received increasing attention in the medical literature. Oxidative stress is increasingly seen as a major upstream component in the signaling cascade involved in many cellular functions, such as cell proliferation, inflammatory responses, stimulating adhesion molecule, and chemoattractant production. A growing body of evidence suggests that many of the effects of cellular dysfunction under oxidative stress are mediated by products of non-enzymatic reactions, such as the peroxidative degradation of polyunsaturated fatty acids. Aldehydic molecules generated during lipid peroxidation have been implicated as causative agents in cytotoxic processes initiated by the exposure of biological systems to oxidizing agents.

Analysis of aldehydes in exhaled breath condensate (EBC) is of great importance for the diagnostics and monitoring of several lung diseases. The most widely used method for qualitative and quantitative analysis of carbonyl compounds is the 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). The new method for the simultaneous determination of several classes of aldehydes in EBC, comprising the most important oxidative stress biomarkers, malondialdehyde and 4-hydroxynonenal, was developed. The method combining derivatization with Girard T reagent with mass spectrometry detection was sensitive and easy-to-use for high throughput analysis of aldehydes in the EBC and enabled insight into the pathophysiology of diseases induced by oxidative stress.

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A17: Biomedical Biomarkers

Mass spectrometry-based urinary metabolomic profiling of ovariectomized rats

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A study has been focused on the clinical application to understand urinary metabolic alterations between ovariectomy and control in rats based on metabolomics. For non-targeted metabolic profiling, urine samples were purified and analyzed by ultraperformance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC-QTOF). The retention time and response data pair for each peak were detected, and the data of metabolites were fed to the principal component analysis as well as partial least square regression-discriminant analysis. The clustering between ovariectomy and control was well separated, and we found progesterone and its metabolites from variable importance plot analysis. A targeted approach was applied to quantify progesterone and its metabolites in urine samples under optimal procedure by gas chromatography-mass spectrometry (GC-MS). The level of urinary allo-pregnanolone was decreased in ovariectomy rat than normal control ($p<0.05$). This study indicates that metabolomics may play a key role in understanding the alteration of progesterone metabolism after ovariectomy.

A18: Biomedical Biomarkers

Serum Metabolomic Biomarkers in Hypothermic and Normothermic Models of Hemorrhagic Shock Associated with Increased Mortality

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Introduction: Hemorrhagic shock, a state of inadequate blood and oxygen perfusion in the tissues, is a leading cause of trauma related deaths. Although treatment for hemorrhagic shock has greatly improved, there is still a need to identify patients that are at risk for complications. Therefore, the development of a reliable prognostic indicator such as a serum biomarker becomes paramount to effective trauma treatment. In this study we use metabolomics as a tool to identify potential biomarkers associated with poor outcome as a result of shock.

Methods: 1H-NMR spectroscopy was used to analyze serum collected from our porcine model of controlled hemorrhagic shock. Animals were hemorrhaged, then resuscitated to a goal of 80 mm Hg systolic blood pressure after 45 minutes. Animals underwent a standard hemorrhagic shock protocol and resuscitation under either hypothermic (n=7) or normothermic (n=7) conditions, or were in one of two control groups (un-shocked control (n=2), minimal invasive control (n=2)). Serum samples were collected from the animals at baseline (prior to hemorrhage), shock45 (after 45 min of shock), and at 1, 4, 8, 23, and 48 h post resuscitation.

Results: Hypothermic animals had a significantly greater survival rate and outcome compared to the normothermic group (P=0.002). Several perturbations in serum were observed in the 1H-NMR spectra as a result of hemorrhagic shock. Hypoxanthine was identified as the most interesting candidate biomarker with a significant increase (P=0.01) at the shock 45 time point in the group of animals with lower survival. Other marked changes were identified in metabolites involved in glycolysis and the Krebs cycle including glucose, lactate, pyruvate and succinate.

Conclusions: Metabolomic profiling and biomarker discovery in hemorrhagic shock has significant potential to add to the current understanding of derangements in energy metabolism as well as to predict outcome.

A19: Biomedical Biomarkers

Metabolomic profiling of urine from rats dosed with carbon tetrachloride

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Carbon tetrachloride (CCl4) induces hepatotoxicity and also causes oxidative stress via free radical reactions that damage lipids, DNA, and proteins. The objectives of this study were to discover biomarkers associated with oxidative stress and liver toxicity using both targeted analysis and metabolomic profiling approaches. Daily doses of 0, 30, and 120 mg/kg of CCl4 were administered by oral gavage to male Sprague-Dawley rats for up to 14 days. Urine samples were collected overnight on dry-ice on days 4, 8, and 15. Targeted analysis of lipid oxidation products, isoprostanes, in rat urine samples was conducted using LC-MS/MS. Metabolomic profiling was performed on a Waters Acquity UPLC coupled to a Waters LCT premier using an Acquity UPLC 1 x 100 mm BEH C18 column with a flow rate of 100 ¬µL/min. Positive and negative ion ESI-MS full scan data were acquired using LCT with a mass range of 100-1000 m/z.

Urinary isoprostane levels with and without normalization to creatinine in rat urine showed significant increases with dose and time, and also correlated with clinical chemistry and histopathology findings. Metabolomic profiling data were processed using Elucidator software. PCA analysis of metabolites showed good separation based on dose. A list of markers that were associated with CCl4 administration was identified and consistent with liver damage.

A20: Biomedical Biomarkers

Lipoprotein subclass distribution measured by ^{1}H NMR as a potential oxidative stress biomarker in HIV-1 infection and antiretroviral treatment

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The application of body fluid proton nuclear magnetic resonance (1H NMR) spectroscopy is showing promise as a diagnostic tool for disease risk. HIV-1 infection is the cause of chronic

oxidative stress and often treatment with antiretrovirals is associated with dyslipidemia and increased risk of cardiovascular disease. 1H NMR metabonomics was used to measure lipoprotein profiles as a potential biomarker for increasing oxidative stress during HIV-1 infection and antiretroviral treatment (ART).

Significant changes in the lipoprotein subclass distribution was observed in the serum 1H NMR spectra of HIV-1 infected patients (n=60) compared to the uninfected subjects (n=30). Within the group of HIV-1 infected individuals, those not receiving ART (n=30) had a significant decline ($p<0.05$) in high density lipoprotein (HDL), whereas those receiving ART (n=30) showed only a marginal decline. Unique to the group of HIV-ART subjects, an atherogenic lipid profile was observed. Malonaldehyde levels (an established indicator of oxidative stress) in serum was used to confirm the presence of lipid peroxidation. Increased lipid peroxidation was positively correlated with HIV-1 infection, with a significant increase within the group receiving ART. Taken together, an atherogenic lipid profile was positively correlated with increasing lipid peroxidation. Our data suggests the production of a more atherogenic lipid profile with ART. In conclusion, data collected demonstrate that 1H NMR can provide diagnostic information highly relevant to a setting of an increasing population of HIV-infected patients on ART, who are known to be at risk for developing atherogenic diseases such as type 2 diabetes and dyslipidemia. 1H NMR metabonomics has a future role to play as a diagnostic and potentially prognostic tool applicable to clinical settings, through high-throughput data collection and analytics

A21: Biomedical Biomarkers

Lipidomic changes in hepatic lipase knockout mice revealed by chip-based direct infusion nanoelectrospray ion trap mass spectrometry

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We performed a comparison of five representative extraction protocols such as protein precipitation method and liquid-liquid extraction method for their ability to extract lipids from plasma samples. Extracted samples were analyzed by nanoelectrospray-ion trap tandem mass spectrometry. Spectra were aligned using the Genedata Expressionist Refiner MS software directly from raw files. Quantitative readouts were evaluated using unsupervised and supervised statistical analyses. While all extraction protocols provided moderate accuracy (positive ionization mode, <9.3%; negative ionization mode, < 51.7%) over all detected lipids, it was also evident that each extraction method resulted in distinct clusters in unsupervised PCA plots. These findings suggest that there is a clear influence of the solvent composition and method details on the recovery of lipid extractions. Though both Matyash and Folch method showed higher recovery of lipid ions and good precision, the Matyash method which uses tert-butyl methyl ether as extraction solvent allowed faster and easier lipid recovery and was well suited for automated lipid profiling by direct infusion nanospray tandem mass spectrometry.

This method was applied to study lipid profiles of hepatic lipase knockout mice and normal mice. Thirty seven phospholipids and sixteen triglycerides were identified in mice blood plasma through tandem mass spectrum analysis. Multivariate analysis revealed a clear separation of the two mice groups in the principal component plots by combining PC1 and PC2, which cumulatively accounted for 54.6% of the variance. Major compounds contributing to the separation of two mice groups were phosphatidylethanolamines, phosphatidylcholines, and lysophosphatidylcholines. HL knockout mice with decreased hepatic lipase activity showed higher levels of phospholipids and lower levels of lysophospholipids compared to normal mice. The present study suggests the usefulness of a direct infusion nanospray tandem mass spectrometry based lipidomics approach to elucidate the function of mice hepatic lipase.

A22: Biomedical Biomarkers

A metabolomic survey on Crohn's disease

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The metabolomics study applied to disease,Äôs evaluation has the main goal to diagnose health and identify factors that cause disease. These studies can lead to enhanced understanding of disease mechanisms Here it is presented a study of the Crohn,Äôs disease. The causes and etiology of this disease are currently unknown although both host genetics and environmental factors are thought to play a role. Examination of identical twins with Crohn,Äôs disease enabled us to focus on the contribution of metabolites produced by the gut microbiota towards disease status of the host. Pathways with differentiating metabolites included those involved in the metabolism and or synthesis of amino acids, fatty acids, bile acids and arachidonic acid. Several metabolites were positively or negatively correlated to the disease phenotype and to specific microbes previously characterized in the same samples. The statistical tools start from unsupervised method to lead to supervised methods; such as principal component analysis, partial least square regression, discriminate analysis and a variety of clustering techniques. Common to a great part of these methods is that they build up interdependencies between metabolites, relationships between the abundances of the metabolites as revealed by correlation, covariance or distance matrix.

A23: Biomedical Biomarkers

Urinary Nicotinamide and 1-Methylnicotinamide Levels are Elevated in Injury: a Metabolomic Analysis

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Introduction: Hemorrhagic shock, characterized by acute blood loss and tissue injury, results in tissue ischemia. Resuscitation and restoration of tissue oxygen may create what is known as ischemia/reperfusion (I/R) injury. We wished to evaluate metabolomic findings associated with this process in an established porcine model.

Methods: Animals underwent instrumentation for physiologic monitoring, then a standardized hemorrhage/resuscitation protocol meant to mimic battlefield trauma. Animals were hemorrhaged to a systolic blood pressure of 50 mmHg. Forty-five minutes later, they received limited resuscitation for 8 hours, at which point they were fully resuscitated and observed for another 48 hours (experimental group). Control groups of instrumented, non-hemorrhaged animals (sham group), and sedated animals (sedated group) were included for comparison. Urine and other samples were collected at set timepoints, flash-frozen, and stored at -80¬∞C until processed for NMR analysis. Urine was prepared with a phosphate buffer to control pH, with TSP added to serve as an internal standard. 1H NMR spectra with presaturation of the water signal and 128 scans were taken with a 600 MHz Varian NMR spectrometer. Spectra were processed and analyzed using Chenomx software. Constant sum normalization was applied to account for dilution.

Results: We identified thirty-six metabolites for all 11 animals (n=7 experimental, n=2 in each of the sham and sedated groups). Nicotinamide and 1-methylnicotinamide levels were elevated in both experimental and sham animals, but were not present in sedated control animals.

Conclusions: NMR analysis of urine in a porcine model of hemorrhagic shock and resuscitation identified metabolites that indicate a physiologic response to I/R injury. Nicotinamide and 1-methylnicotinamide were observed in the urine of animals undergoing hemorrhagic shock and those with tissue injury associated with instrumentation. These compounds may represent tissue injury or ischemia in the setting of trauma.

A24: Biomedical Biomarkers

Analysis of the serum metabolome by 1H-NMR reveals differences between Chronic Lymphocytic Leukaemia molecular subgroups

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Chronic lymphocytic leukaemia (CLL) is a heterogeneous disease exhibiting variable clinical course and survival rates ranging from months through to decades. Mutational status of the immunoglobulin heavy chain variable regions (IGHV) of CLL cells offers useful prognostic information for high risk patients but substantial time and economical costs have prevented it from being routinely employed in a clinical setting. Instead, alternative markers of IGHV status such as ZAP70 protein and mRNA levels are often clinically employed. Here we report a 1H-NMR-based metabolomics approach to examine serum metabolic profiles of early stage, untreated CLL patients (Binet stage A) classified by IGHV mutational status or ZAP70 protein and mRNA expression levels. Metabolic profiles of CLL patients (n=29) exhibited higher concentrations of pyruvate and glutamate and decreased concentrations of isoleucine compared to healthy controls (n=9). Differences in metabolic profiles between unmutated (UM-IGHV; n=10) and mutated IGHV (M-IGHV; n=19) patients were determined using partial least square discriminatory analysis (PLS-DA; R2Y=0.74, Q2Y=0.36). UM-IGHV patients had elevated cholesterol, lactate, uridine and fumurate and decreased pyridoxine, glycerol, 3-hydroxybutyrate and methionine serum concentrations. PLS-DA models derived from ZAP70 classifications showed comparatively poor goodness of fit values suggesting IGHV mutational status correlates better with disease-related metabolic profiles. Our results highlight 1H-NMR-based metabolomics as a potential non-invasive prognostic tool for identifying CLL disease state biomarkers.

A25: Biomedical Biomarkers

Urine based metabolic biomarkers as indicators of cognitive status: development of a non-invasive test for susceptibility to Alzheimers.

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This work seeks to develop a simple test for susceptibility to Alzheimer‚Äôs disease (AD) using urine based biomarkers. Specifically, we are conducting an exploratory investigation to correlate biomarkers indentified in urine by a Nuclear Magnetic Resonance Spectroscopy (NMR) based metabolic analysis, with cognitive assessments of seniors (>60 years old) stratified by three groups: (1) age-appropriate cognitive functioning, (2) at or below the MCI diagnostic level, (3) clinically diagnosed AD. NMR profiles, and specific biomarkers, are analyzed separately and statistically matched to pencil and paper assessments to determine the relationship of specific molecules (e.g., N-acetyl-aspartate, NAA, myo-inostol, choline, creatine, gamma amino butyric acid, GABA, amino acids, cholesterol and oxysterols) and NMR metabolic pathway profiling with cognitive performance utilizing both univariate and multivariate analyses. Past research with biomarkers and MCI/AD has largely relied on invasive samples, e.g., blood or cerebrospinal fluid (CSF). In contrast, the present proposal will utilize non-invasive urine samples. Our research outcome therefore will be a non-invasive portable public health early warning protocol for susceptibility to MCI/AD that is applicable to both minority and non-minority elders in both urban and rural locales.

A26: Biomedical Biomarkers

Serum metabolic profiles in DSS treated mice identifying potential biomarkers for inflammatory bowel disease

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Background: Novel biomarkers or biomarker patterns for the diagnosis, surveillance and direction of treatment in inflammatory bowel disease (IBD) are needed. Presently the only clear way to assess disease requires invasive endoscopy, thus medications are often modified without clear assessment of disease activity resulting in both under and over treatment. Metabolomic analysis of small molecule profiles has been applied to various biofluids for disease assessment.

Aim: Using the established dextran sulfate sodium (DSS) model of colitis, our aim was to identify whether metabolomic analysis of serum could differentiate those animals with colitis and to identify principal compounds of the metabolomic profile that could be further assessed in patients with inflammatory bowel disease.

Methods: Methods: 12 week old male mice were treated with either 2.5% DSS (n=29) in drinking water or drinking water alone (n= 30) and sacrificed on day 7. Body weight was observed daily and 0.5 ml blood serum was collected and NMR metabolomic analysis (Chenomx NMR Suite 5.1) was performed.

Results: DSS resulted in intestinal inflammation as indicated by tissue myeloperoxidase activity (14.5 ¬± 1.0 u/mg vs (0.4 ¬± 0.1 u/mg in controls) and significant weight loss. 76 compounds were identified from sera. Multivariate analysis by orthogonal partial least squares discriminant analysis (OPLS-DA) was employed to establish a model and was able to predict class separation between mice with colitis and controls with > 95% accuracy based on seven-fold cross-validation. Interpretation of OPLS-DA found tryptophan, 2-hydroxyisovalerate, and 2-oxoisocaproate were most elevated and citrate, ethanol and histidine were most decreased during active colitis.

Conclusion: Our results demonstrate that serum metabolomics can clearly discriminate mice with active colitis from control animals. Our study indicates that metabolic profiling is a powerful tool to identify intestinal inflammation and may be useful in the management of IBD and in studies exploring disease pathogenesis.

A27: Biomedical Biomarkers
The anatomical metabolomics of hyperlipidemia

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The marked imbalances of metabolites in body fluids, such as blood and urine, represent ensemble of metabolic dysfunctions in several organs and tissues, therefore are regarded as available markers for the diagnoses or assessment of medical practices. On the other hand, understanding of metabolic imbalances in each tissue, that is anatomic metabolomics, further provide the clues to the pathologic mechanism, which renders biological supports for the metabolic markers found in body fluids. Hyperlipidemia is known to be one of the serious MetSs and characterized by abnormal elevation of blood lipids and/or lipoproteins. For familial hypercholesterolemia (FH), mutation in the LDLR gene, which encodes a low-density lipoprotein (LDL) receptor expressed in the liver, causes juvenile hyperlipidemia, and resulting in severe cardiovascular event. Recently, adding to the elevation of blood lipid level, other possible pathogeneses, such as oxidative stress, are regarded to be essential for severe cardiovascular events as second hit, whereas the diagnostic assessment of those factors has not yet been established. For the evaluation of the systemic metabolic imbalances including the second hit, we performed LC/CE-TOFMS-based systemic metabolic profiling for Homozygous Watanabe heritable hyperlipidemic (WHHL) rabbit as model for FH. The changes of metabolic pool were investigated for blood, liver, aorta, cardiac muscle, and brain between WHHL rabbit and healthy controls. For the pathologic rabbit, prominent changes were observed for lipid catabolism, purine metabolism, and glutathione biosynthesis, indicating advanced oxidative stress resulting in folic acid depletion for whole body, especially for liver. We also assessed the pharmaceutical effect of statin, which is broadly used for treating patients by its strong effect to control plasma lipids. After the 10-days administration, metabolic pool of tissues exhibited extensive changes suggesting both of the restoration to the healthy state and possible side effect, which are inconsistent with the restoration.

A28: Biomedical Biomarkers
2D-LC Fractionation and Dansylation Labeling Combined with FT-MS and NMR for Human Urine Metabolome

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Introduction: There is much interest in comprehensively characterizing the human urine metabolome. However, urine metabolome analysis is a challenging task, as many of the metabolites

detected are unknown. To address this issue, we are developing efficient separation and compound identification techniques. In this report, we present a method of two dimensional LC fractionation followed by FT-MS and NMR analysis for unknown metabolite identification.

Methods The urine samples were firstly fractioned by semi-Pep HPLC (Agilent 1100 HPLC system equipped with a 9.4mm Rx-C18 column) using 12 mM HFBA as ion pair reagent. Individual fractions were subjected to dansylation which mainly labels the amino acids, amine and phenolic hydroxyl . The dansyl labelled metabolites were further fractioned by HPLC using the same column. The fractions were analysed using LC-FT-MS. In addition, MS/MS spectra were obtained using hybrid quadrupole/time-of-flight MS. A standard library including 250 dansylated metabolites was first used to confirm the identification of the known metabolites. The unknowns were subjected to chemical formula calculation, MS/MS analysis and NMR.

Results A novel two dimensional (2D) HPLC fractionation strategy was developed. 7 fractions were obtained from a high-resolution ion pair HPLC separation. A total of 6-10 fractions were obtained from the second dimensional reversed-phase LC. Each resulting fraction was dried and re-dissolved in deuterated solvent for NMR structural analysis. A dansylation metabolite standard library containing 250 compounds was constructed and the library consists of NMR and MS spectra of each labelled metabolite. This library was used to confirm the identity of known metabolites in the fractionated urine samples.

In a parallel experiment, a data processing strategy was developed for processing the dansylation labelled metabolites to facilitate peak identification in both MS and NMR. The integration of MS and NMR results may potentially be semi-automated for unknown metabolite identification.

A29: Biomedical Biomarkers

Identifying important variables in a time-dependent metabolomics dataset with disjoint PCA

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Time-dependent metabolic experiments are important in investigations of various biological treatments, like on the effect of any exogenous substance on the normal metabolism. The resulting data generated from such experiments forms a three dimensional data array in contrast with the two dimensional data arrays as mostly used in metabolomics experiments. In this presentation we show how a new approach of data analysis by a SIMCA method may be used for the analysis of time-dependent metabolomics data. We used the pharmacokinetic approach of the time-dependent formation of metabolites formed after the consumption of a single dose of alcohol to generate the metabolomics data. The metabolites were obtained according to the procedure used to detect organic acids, derivitized and separated and identified by our GC-MS-AMDIS procedure. All metabolites were expressed as mg metabolite per gm creatinine. Ten experimental subjects formed the cases of the protocol, and the time period covered 5 hours after the alcohol consumption.
Variable selection has been performed by eliminating variables that did not show mayor deviation over time. This was done to control the size of the data matrix used in the analysis. The resulting data formed the three dimensional data array with cases, metabolites and time as the three dimensions. PCA is however only applicable, to a 2-dimensional data matrix. Hence, before analyzing 3-dimensional data array using PCA, we first transform the data array into a more suitable structure. This is achieved using the method of unfolding, a technique that reduces the 3-dimensional

data array to one with only 2-dimensions. Using the 2-dimensional matrix, we then build a PCA model, illustrate the results of the time-dependence graphically and identify important variables related to the short-term consequence of acute alcohol consumption.

A30: Biomedical Biomarkers

Common metabolic phenotypes en route to autoimmunity and type 1 diabetes in man and mouse

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In a recent study we found that dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes (T1D) [1]. Individuals who developed diabetes had reduced levels of triglycerides and ether phospholipids throughout the follow up, and increased levels of lysoPCs, glutamate and leucine several months before seroconversion to autoantibody positivity. We conducted a separate study using non-obese diabetic (NOD) mouse model with the aim of comparing longitudinal human and NOD mouse lipid profiles. Altogether 39 mice, 26 females (12 progressors, 14 nonprogressors) and 13 males (7 progressors, 6 nonprogrerssors) were included in the analysis, comprising 1172 serum samples. Tail blood samples were collected weekly, starting at the age of 3 weeks. At 8 weeks of age the mice were also tested for antibody positivity. Lipids were analyzed by UPLC/MS. By using Hidden Markov Models [2] and a new algorithm for multi-view learning [3], we found that the metabolic states of progressors prior to T1D are similar between human and female mice. Using lipidomics and autoantibody profiles we developed a predictive model for T1D in NOD mice, by which in a separate study where the mice were sacrified at 8 weeks of age, we were also able to compare tissue metabolic profiles of T1D progressors. Comparison of lysoPCs on antibody positive and negative progressors to corresponding non-progressors supported our hypothesis that the initial autoimmune response in progressors to T1D normalizes abnormal metabolic profiles found prior to autoimmunity. By using metabolomics (GCxGC-TOF/MS) and gene expression analysis, we also identified major alterations of energy metabolism in mice who are likely progress to T1D, independent of autoimmune status.

[1] Oresic et al., J Exp Med 2008;205:2975-84.
[2] Nikkil√§ et al., Mol Syst Biol 2008;4:197.
[3] Tripathi et al., (2009, in preparation).

A31: Biomedical Biomarkers

Combining MR imaging and metabolomics - towards early markers of dilated cardiomyopathy

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In order to form a holistic picture of human physiology it is necessary to put empirical data obtained at different scales into physiologic context and to fill the gaps that remain unobserved between the organismal levels. We present an approach that can be used to bridge the gap between tissue level magnetic resonance (MR) images and the molecular level of metabolism. We studied two human sample sets in the context of dilated cardiomyopathy (DCM): (1) lamin positive set consisting of 11 asymptomatic lamin A/C gene mutation (LMNA) carriers who are known to be at risk for developing DCM, and 11 controls, and (2) lamin negative set consisting of 8 non-LMNA DCM progressors and 8 controls. Lipids were analyzed by UPLC/MS platform. One of our main aims was to search for early predictive DCM biomarkers from the lamin positive set and then test their performance in the lamin negative set. We developed a predictive model for DCM by combining the most dysregulated lipids and some of their ratios. In addition, we identified and visualized correlations among the heart MR images and the lipid profiles. The heart images were decomposed into 86 physiologically relevant parameters [1] and regression models were applied to explain these parameters with the lipid profiles. Regression models were also applied to reconstruct the heart MR images by lipids and to study the effects of lipid concentration changes on these images. As a result we identified associations between heart images and the lipid profiles. We also visualized the effect of lipid concentration changes on the left reconstructed image of the ventricle of the heart. Our approach may, in addition to its potential relevance to early diagnostics, inspire development of methods for integrating physiological data across different organismal levels.

[1] J.R. Koikkalainen et al., Radiology 2008;249:88-96.

A32: Biomedical Biomarkers

Melanotransferrin facilitate transferrin/transferrin receptor-independent iron transport in melanoma cells

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Iron is an essential element for cellular metabolism. However, high cellular iron content has been connected to the development of cancers in human. One way iron may affect cancer development and progression is by altering cell growth and proliferation. Iron has been proposed to promote progression from G1 to S phase of the cell cycle through activation of ribonucleotide reductase during DNA synthesis. Iron is normally absorbed by the enterocyte of the duodenum where it is transported throughout the body via serum transferrin. However, the existence of transferrin-transferrin receptor independent iron transport pathways has been shown. Such pathways are thought to play critical roles in non-transferrin bound iron overload in diseases such as hemochromatosis and Alzheimer‚Äôs disease, though the molecular details of these pathways remain obscure. Many genes are up-regulated to aid the abnormal proliferation of cancer cells and subsequent invasion of tissues. In the case of melanoma, the expression of melanotransferrin (p97), an iron binding molecule, is elevated. The cellular and functional role of glycosylphosphatidylinositol (GPI)-anchored p97 in melanoma was investigated. Using confocal immunofluorescence microscopy and quantitative immunoelectron microscopy, iron bound GPI-anchored p97 was shown to be internalized via a caveolae vesicles dependent endocytotic pathway. In addition, endosomal disruption studies demonstrated that the intracellular trafficking of the GPI-anchored p97 in melanoma cells is endosomal-dependent. The studies performed demonstrate that GPI-anchored p97 protein can mediate the iron uptake in melanoma cells. Furthermore, over-expression and down-regulation of GPI-anchored p97 in melanoma cells show that the GPI-anchored p97 can promote melanoma cell proliferation and melanoma tumor growth in vivo. Taken together, these studies sheds new light on the relationship between GPI-anchored p97, melanoma development and cellular iron uptake.

A33: Biomedical Biomarkers

Applying Metabolomics Platform towards Small Molecule Biomarkers Discovery

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Small Molecule Biomarkers have been utilized in different fields of medicine for years. We apply well established Metabolomics platform towards the detection, finding and identification of small molecule biomarkers. Recent achievements in mass spectrometry coupled with high resolution different separation techniques allow to detect and analyze novel potential biomarkers in highly complex biological samples. Recent efforts invested in development data processing programs and algorithms make available efficient handling very complex datasets generated during small molecule biomarkers discovery process. De novo characterization of putative small molecule biomarkers is extremely important, sometimes difficult, but vital procedure. We utilize Mass Spectrometry based approach which can be extended to and supported with NMR technique. Data acquisition is performed with the assistance of Time off Flight (TOF), Linear Ion Trap (LTQ), Triple Quadrupole (Qtrap4000) and hybrid Linear Ion Trap Fourier Transform Ion Cyclotron (LTQ-FT-ICR Ultra) mass spectrometers coupled with a variety of separation techniques, in particular GC and LC (HPLC/UPLC) chromatography and nano-scale infusion (Nanomate). Sophisticated data mining approach using different algorithms deals with electron impact (EI) generated data as well as data generated with the unit resolution, high resolution and ultrahigh resolution hybrid instruments having API sources. We illustrate Small Molecule Biomarkers Discovery with the next case studies: Kidney diseases: Targeting endogenous metabolites. Pancreatic Cancer: Targeting endogenous metabolites. Human Embryonic Stem Cells functional characterization: Targeting exogenous metabolites.

A34: Biomedical Biomarkers

A full quantitative NMR metabolomics liver profiling on genetically modified mice: assessing metabolic disarrangements in hepatic inflammation

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Dysfunctions in metabolism are a common finding in clinical medicine. We report the use of 1H-NMR-based full quantitative metabolomics liver profiling to assess the metabolic disarrangements in the progression of steatosis to Nonalcoholic Steatohepatitis (NASH).

This study is mainly aimed to establish a full quantitative NMR based metabolomic approach for liver phenotyping in genetically modified mice. Besides, using this approach, it was our goal i) to establish concentration ranges for different metabolite markers of the actual risk factors that drive hepatic inflammation during the progression to NASH (ii) to identify metabolic pathways which may explain this progression.

This metabolomic analysis proved to be fast, simple, reproducible and informative, revealing a suggestive relationship of the occurrence of SAMe depletion at the early stages of NASH development and a causal role of impairment of hepatic transsulfuration reactions. Furthermore our data suggests an intensive role of dietary cholesterol as a risk factor to the progression to hepatic inflammation in diet-induced NASH.

The full quantitave profiling approach we report allows for the quantification of up to 54 different metabolic markers and the simultaneous evaluation of water-soluble and lipid-soluble extract metabolites altogether in the same analysis. As far as we know, this is the first reported work where NMR derived quantitative data obtained is used for further multivariate modelling leading to a challenging opportunity in the assessment of the metabolic scenario of the progression of steatosis to NASH. We propose that the approach presented hereby should be considered in the evaluation and phenotyping of genetically modified mice when a metabolic alteration is suspected but also it could provide a high valuable tool in clinical application and important new insights in clinical research.

A35: Biomedical Biomarkers

THE ROLE OF NMR URINE METABOLOMICS AS A NEW METHOD FOR SCREENING COLORECTAL CANCER -- A PRELIMINARY ANALYSIS

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Aims: Colorectal cancer is a major public health concern since it is among the leading causes of death in North America, but it can be curable if identified early, even better if found at the adenomatous polyp stage. Current screening methods have limitations or potential risks associated with them. With the emerging field of metabolomics, we hypothesize that urine metabolomics could represent a more sensitive, non-invasive and accessible method of identifying asymptomatic subjects with colorectal cancers, and perhaps even precursor adenomatous polyps.

Methods: This is the preliminary analysis from a 1000 subject prospective, multi-centered trial to assess whether a simple urine test can play a role in the screening of colorectal cancer. Urine samples were collected from 52 subjects going through an established colorectal screening program (29 had no polyps, 10 adenomatous polyps, 12 hyperplastic polyps, and 1 malignant polyp). Using nuclear magnetic resonance (NMR) spectroscopy, the 1H NMR spectrum of each urine sample was analyzed to define a unique metabolomic signature, and it was compared to the recently established metabolomic signature of cancer (Sawyer and Slupsky et al.).

Results : Using the metabolomic signature, the cancer subject was correctly predicted as having cancer. Of the 10 adenomatous polyp subjects, and 12 hyperplastic polyp subjects, 8/10 and 4/12, respectively, were correctly predicted by the metabolomic signature. Of those patients with no polyps, only 14/29 were identified as 'normal'. Comparison of those with adenomatous and hyperplastic polyps revealed no clear distinction using multivariate statistical analysis.

Conclusions: These results are encouraging; as it appears that urine metabolomics has a high sensitivity for diagnosis of colorectal cancer and identifying colonic polyps. Additional patient analyses will likely increase specificity and decrease false-positives. Urine metabolomics has the potential to provide society with an accurate, non-invasive, and inexpensive screening tool for colorectal cancer.

A36: Biomedical Biomarkers

A metabolomics approach to identifying biomarkers and therapeutic targets for polycystic kidney disease

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Polycystic kidney disease is one of the most common of all life-threatening human genetic disorders. It is an incurable genetic disorder affecting 12.5 million people worldwide. It is characterized by the

formation of fluid-filled cysts in the kidneys of affected individuals and currently has no biomarkers or effective treatment. Although requiring a second mutational ‚Äúhit‚Äù, PKD arises from germline mutations, and because of this, we propose that metabolic changes can be detected in the urine before changes in kidney size and filtration function are noted. Our project seeks to identify novel biomarkers which can be used to develop an early diagnostic test as well as to reveal new therapeutic targets for disease treatment. In the jck mouse model (which has a mutation in the Nek8 gene), cysts appear starting as early as 26 days, but renal failure is not seen until 45 days. Urine was collected from mice at these two ages. Metabolomic analysis was performed using GC-MS, and the resulting data were baseline corrected, log transformed, body weight adjusted, and normalized to total mass the account for variations in urine concentration. These data were then analyzed by partial-least squares and other statistical analyses to identify the metabolites that are significantly different between diseased and control individuals. Complete separation of the two groups of mice can be seen as early as 26 days when renal function is normal and is maintained at the 45 day time point. Metabolites which contribute the most to the segregation between groups are currently being identified and validated. Once confirmed, these metabolites may be used to identify patients with PKD before the onset of kidney failure, and, once metabolic pathways are elucidated, novel targets for therapy will be identified.

A37: Biomedical Biomarkers

1H NMR-based metabolic profiling in a mouse model of tuberculosis

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Tuberculosis(TB) is a world-wide problem and the TB case has increased dramatically in the past decade. The TB infections has contributed to the multidrug-resistant(MDR) and extensively drug-resistant(XDR-TB) strains of Mycobacterium tuberculosis which are associated with high mortality. For TB treatment and elimination, this metabolomic approach could show the possibility to improve diagnosis, shorten treatment, improve outcomes in MDR and XDR-TB, and enhance protection afforded by vaccination. In this study, we investigated endogenous metabolic changes in the urines, sera, and tissue extracts of livers, lungs, and spleens between M. tuberculosis infected mice and healthy control, using 1H NMR spectroscopy coupled with multivariate statistical analysis. Aromatic amino acids and branched-chain amino acids were increased in tissue extracts of TB mice. Urinary TCA cycle intermediates, such as citrate, 2-oxoglutarate, and succinate, were decreased and the sera level of LDL and unsaturated lipids were also decreased in TB mouse model.

A38: Biomedical Biomarkers

Homeostatic Imbalance of Purine Catabolism and Its Clinical Relevance in Schizophrenia

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Dysregulation of the antioxidant defense system (AODS) has consistently been found in schizophrenia. The dynamics of purine pathway are critical to the AODS. Thus, seven breakdown products in purine pathway were determined by high-pressure liquid chromatography coupled with a coulometric multi-electrode array system from the plasma of healthy controls (HC, n=27) and first-episode neuroleptic-na√Øve patients with schizophrenia (FENNS, n=25) at baseline and 4-week after initiation of treatment with clinician‚Äôs-choice antipsychotics. Associations between these metabolites and clinical assessments using standard rating scales were evaluated. Wilcoxon rank-sum tests showed significantly higher levels of xanthosine and lower levels of guanine in FENNS group before and after antipsychotic treatment compared to HC subjects. Moreover, xanthosine/guanine ratio was higher, and guanine/guanosine and urate/xanthosine ratios were lower in both FENNS groups compared to HC subjects. On the other hand, all 3 groups had significant correlations between urate and guanine or between hypoxanthine and xanthine. Contrarily, in the HC but not either FENNS groups, urate was significantly correlated with xanthine or hypoxanthine, and xanthine was correlated with guanosine. In addition, a significant and positive correlation was also demonstrated between the ratio of urate to guanine at neuroleptic-na√Øve baseline and treatment improvement as measured by changes in the Global Assessment Scale scores. During purine catabolism, both conversions from guanosine to guanine and from xanthosine to xanthine are reversible. Decreased ratios of product to precursor suggested a shift favorable to the precursor in the FENNS. Taken together, the potential for steady formation of antioxidant urate from purine is altered early in the course of illness and independent of treatment effects. Thus, homeostatic imbalance in the purine pathway may be important for the pathophysiology of schizophrenia. Such product-precursor relationship may therefore be informative about the degree of clinical improvement that can be expected as a result of pharmacological treatment.

A39: Biomedical Biomarkers

Predictive multiple reactions monitoring (MRM) as a functional test of hepatocyte-like human embryonic stem cells (ESC)

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Analysis of low abundant metabolites represents significant challenge in xenobiotic metabolism and in endogenous metabolomics. In several cases, we examined a promising approach detecting low abundant metabolites in complex biological matrixes. It was found that predictive multiple reactions

monitoring (MRM) detection method offers the highest sensitivity among various acquisition modes used to detect secondary metabolites at trace levels in plant extracts, human embryonic stem cells lysate and media, or human urine. Utilizing high performance liquid chromatography (HPLC) coupled to 4000 Qtrap mass spectrometry (MS) allowed identification of specific molecular locations where biotransformation had altered the parent compounds. Further, accurate masses and isotopes ratios of the identified metabolites were obtained in the selected ion monitoring (SIM) mode using high-resolution high-accuracy ultra performance liquid chromatography (UPLC) LTQ Orbitrap MS. Spectral accuracy and isotopic ratio filters were considered for metabolite identification using MassWorks. Theoretical fragmentation patterns generated by Mass Frontier were compared to those acquired from LC-MS acquisitions for further validation. This platform of hybrid triple quadruple ion-trap MS together with software-supported intelligent data acquisition allowed identification of trace-level secondary metabolites that were not detectable in full scan mode.

A40: Biomedical Biomarkers

Sample preparation and extraction techniques for the analysis of acylcarnitines in human urine and blood by UPLC MS/MS

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Sample preparation and analyte extraction are key steps in the analysis of metabolites in biofluids. Acylcarnitine analysis has become important for the diagnosis of inherited metabolic disorders. However, acylcarnitines possess certain characteristics that make their extraction a challenge. They have a wide range of hydrophobicities and thus different extraction techniques are necessary for acylcarnitines of different chain lengths. Moreover, acylcarnitines are known to interact with various proteins in blood causing difficulty in applying conventional protein precipitation protocols for their extraction. In this work, an optimized sample preparation method for the extraction of acylcarnitines from urine and blood amenable for analysis by UPLC MS/MS is presented.

Short- and medium-chain acylcarnitines were extracted using solid phase extraction, whereas liquid-liquid extraction was employed for long-chain analogs. A Waters UPLC system using a C18 column was coupled to an AB 4000 QTRAP system using multiple reaction monitoring as a survey scan.

Acylcarnitines were identified based on their fragmentation pattern. Characteristic neutral losses of 59 and 161 Da corresponding to the loss of the trimethylamine moiety and the loss of the carnitine backbone respectively were searched for, as well as peaks at m/z 60, 85 and 144. Compounds were identified as acylcarnitines when at least 3 of these peaks were present in their MS/MS spectra. Structural assignments were done based on fragmentation pattern and retention time.

Using this method, a total of 329 acylcarnitines were detected in urine, only 40 of which have been previously reported in the urine of healthy individuals.

The analysis of acylcarnitines in blood is currently underway. By screening for a large number of acylcarnitines including isomeric species and phase I metabolites in different biofluids new biomarkers for inherited metabolic disorders may be discovered.

A41: Biomedical Biomarkers

Optimizing brain tissue sample preparation as a powerful tool in targeted metabolomics for biomarker discovery

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Animal models of brain injury are usually the prerequisite for the development of neuroprotective strategies. Targeted metabolomics in brain offers the unique opportunity for direct characterization of animal models on a functional level, thus gaining new knowledge of the pathophysiology as a fundament for drug development and direct translational biomarker discovery and validation. The probability of success in the pre-clinical research phase for biomarker discovery is intimately linked to the analytical platform efficiency to overcome several challenges: brain complex interactions, specificity of the metabolome and range of concentration that can dramatically differ from well studied biofluids. Current analytical methods assessing the effects of such strategies are limited by certain weaknesses on chemical identification, quantification of effects, duration of method (e.g. immunostaining) and selection of little number of outcome parameters analyzed.

In the present study, we report recent developments of a targeted and quantified strategy to approach metabolomics in animal brain models. Multiparametric, highly robust, sensitive and high-throughput targeted metabolomic FIA (Flow Injection Analysis)/MS/MS and LC/MS/MS methods are used for the simultaneous quantification of endogenous intermediates in brain samples. The target analyte list comprises highly relevant chemical classes including eicosanoids and oxysterols alongside a broader range of metabolites (amino acids, biogenic amines and polyamines, acylcarnitines, phospholipids and lysophospholipids, sphingo(phospho)lipids, intermediates of energy metabolism).

Adequate brain homogenization and sample preparation are discussed to optimize extraction, quenching and suppression effects with consideration to extracted chemical content, data quality and reproducibility and assay relevance in the context of brain studies. The benefits for our methodology are illustrated through several examples where it is proven efficient to better relate biochemical mechanisms and identify relevant markers in response to brain injury.

A42: Biomedical Biomarkers

Identifying important variables with disjoint PCA

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The aim of the Principal Components Analysis (PCA) is to reduce the dimensionality of a set of p variables while retaining the maximum variability in terms of the variance-covariance structure. This method is often used as an unsupervised pattern recognition technique. Variables that are identified as important are those that have a high communality. Hence, the variables that are modeled well by the PC model. Variables that cause groups to be disjoint (discrimination) are however not identified.

In this respect the SIMCA method improves the important variable identification. SIMCA builds independent PCA models for each class and uses a discrimination measure based on error matrixes to determine which variables are important in discrimination.

We here present results on the development of the SIMCA method to address the abovementioned limitations of the PCA approach. For this purpose we used the Iris dataset, and applied the new approach to a typical metabolomics dataset. For the latter we chose the quantified organic acid profile of metabolites isolated from urinary samples of controls and patients suffering from propionic acidaemia, using our standard GC-MS-AMDIS approach . The latter is a life-threatening disorder with autosomal recessive inheritance, caused by deficiency of propionyl CoA carboxylase. This perturbation, however, provides an ideal metabolomics dataset for comparative purposes in the development of statistical models in metabolomics investigations.

The results that we present here deals with the development of our alternative SIMCA model with the Iris dataset and the refinement of this model by using the more complex dataset from the propionic acidaemia cases. According to the results that we obtained, we propose a model which produces a informative set of variables important in the discrimination between groups.

A43: Biomedical Biomarkers

Fast-LCMS identification of urinary biomarkers of colon inflammation in interleukin-10-gene deficient mice

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A Fast-LCMS method was developed for the rapid screening of complex biological samples whilst retaining the versatility and robustness of LCMS. Fast-LCMS metabolite profiling of urine from interleukin-10-gene deficient (IL10-/-) and C57 mice was used to identify mass spectral ions associated with the development of colon inflammation in samples from two mouse trials where urine was collected over five weeks. Potential biomarkers of colon inflammation were selected from the non-targeted Fast-LCMS candidates and further characterized by LCMS/MS and correlated to indices for colon inflammation. Further candidate selection was based on the elimination of adduct ions, isotopologues, weak intensity peaks or difficult candidates (multiple charged and or high mass candidates). Possible metabolite structures were inferred from LCMS/MS ion trees using web-based metabolite databases, high resolution mass spectral data, and by comparison with authentic compounds and published mass spectral fragmentation pathways.

Xanthurenic acid (206 (M+H)+ and 204(M-H)-) and its glucuronide adduct (382 (M+H)+ and 380 (M-H)-) were clearly identified as differentiating metabolites in both positive and negative mode LCMS. Xanthurenic sulphate was also detected (m/z 284 (M+H)-) but was not significantly different between the IL10-/- and the C57 samples. These metabolites are implicated in the tryptophan pathway. In a cross-platform comparison of metabolomics methods, the candidate biomarkers detected by Fast-LCMS were compared with a number of putative biomarkers of

inflammation previously reported from GCMS analysis of the urine samples. The changes in metabolite levels indicated changes in biochemical pathways providing insights into the effects of disease progression. The multiple metabolomics platforms extended the range of detectable metabolites and possible perturbations in biochemical pathways that may be observed.

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A44: Biomedical Biomarkers

Eye lipidome as a reporter of metabolic stress over time: effect of gut microbiota on retinal and lens lipid composition

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The adult intestine contains ~100 trillion bacteria (gut microbiota), a number 10 times greater than the number of human cells in our bodies. The gut microbiota affects host lipid metabolism and is considered an environmental factor that contributes to development of obesity. Compared to metabolically active organs such as liver, lens tends to be deprived of the allostatic adaptive mechanisms which maintain lipid homeostasis. In this regard, we recently argued that the lens can become a reporter of integrated metabolic stress over time [1]. Probably partly due to its diminished allostatic control, the lifespan of many species has been found to positively correlate with the sphingolipid content in the lens and inversely correlate with the lens phosphatidylcholine, which is known to be more prone to oxidative damage. To investigate whether the gut microbiota affects the eye lipidome, we performed comprehensive lipidomic profiling of lens and retina from conventionally raised and germ-free and mice using UPLC/MS [2]. Conventionally raised mice had diminished phosphatidylcholines in the lens and elevated ethanolamine plasmalogens in the retina. Diminishment of lens phosphatidylcholines in the presence of gut microbiota suggests that the conventionally raised mice are exposed over time to more oxidative stress than germ-free mice. Consistent with this, their lifespan is also shorter. In conclusion, we provide for the first time evidence that the gut microbiota affects the lens and retinal lipid composition. Our findings may open a new area of investigation how modulation of gut micriobiota affects the eye health. Comparative lipidomic analysis of lens and other surrounding tissues could also be a novel approach able to identify early pathogenic insights or biomarkers related to conditions or diseases that are characterized by long prodromal periods.

[1] M. Oresic et al., Trends Biotechnol 2008;26:647-52.
[2] M. Oresic et al., Exp Eye Res 2009, in press.

A45: Biomedical Biomarkers

Changes in the metabolic footprint of cultured villous trophoblast identifies metabolic disturbances related to hypoxia and pre-eclampsia

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Pre-eclampsia (PE) is a multi-system disorder of pregnancy thought to be mediated by circulating factors released from damaged placental villous trophoblast, with release potentially a result of hypoxia [1]. PE has a prevalence of 3-8%, is a major cause of maternal and prenatal morbidity and provides an increased risk for infant of cardiovascular disease and type II diabetes in adult life.

Metabolic footprinting [2,3] offers a strategy to investigate factors released from placental villous trophoblast tissue in vitro during cultivation in a serum-based medium. The current research investigates differences in the metabolic footprints from villous trophoblast from uncomplicated pregnancies (n=6) and those with PE (n=6) cultured at either normoxic (6% O2) or hypoxic (1% O2) conditions. Metabolites consumed from and released into serum-conditioned culture medium were analysed by Ultra Performance Liquid Chromatography coupled to a hybrid LTQ-Orbitrap mass spectrometer. The relative concentration of 154 features of the metabolic footprint were observed to change when comparing culture medium from uncomplicated pregnancies cultured in normoxic and hypoxic conditions ($p<0.00005$). When comparing all 4 groups, 47 features showed a similar relative concentration in PE-derived tissue cultured in normoxic conditions to normal villous tissue cultured in hypoxic conditions. These data suggest that hypoxia has a role in the placental pathogenesis of PE. Three areas of metabolism were highlighted for further systems-biology investigation; glutamate and glutamine, tryptophan metabolism and leukotriene or prostaglandin metabolism.

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A46: Biomedical Biomarkers

Altered regulation of metabolic pathways in human lung cancer discerned by 13C stable isotope-resolved metabolomics (SIRM)

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Metabolic perturbations arising from malignant transformation have not been systematically characterized in human lung cancers in situ. Metabolomic analysis allows genome-wide functional analysis of the dysregulation of cancer-specific genes. We investigated metabolic changes by infusing uniformly labeled 13C-glucose into human lung cancer patients, followed by resection and processing of paired normal lung and tumor tissues. 13C-isotopomer-based metabolomic analysis was performed using NMR, GC-MS, and FT-ICR-MS. Many polar metabolites were consistently found at higher levels in tumor tissues than their normal counterparts. As expected, the 13C-isotopomer analysis indicated activated glycolysis in the tumor tissues. In addition, the Krebs cycle activity was altered, as evidenced by an enhanced buildup of 13C-succinate in tumor tissues, consistent with the pathway from glucose to succinate via glycolysis, anaplerotic pyruvate carboxylation (PC), and the reverse Krebs cycle sequence from oxaloacetate to succinate. PC activation in tumor tissues was also supported by the increased expression of pyruvate carboxylase transcripts. PC activation ,Äì revealed for the first time in human patients ,Äì is likely necessary to replenish the Krebs cycle intermediates to fulfill the anabolic demands for growth in lung tumors. We hypothesize that this is an important dysregulatory event in lung and possibly other tumor development. FT-ICR-MS analysis of lipid extracts of paired normal and cancerous lung tissues as well as plasma of lung cancer patients and normal volunteers was also performed. Thousands of automated assignment on phospholipids and their 13C-isotopomers were made by employing ,ÄúPREMISE,Äù (Precalculated Exact Mass Isotopomer Search Engine) software developed in-house. Preliminary comparison of the assigned lipids indicates a fast turnover of plasma phospholipids in lung cancer patients. Further comparison is being conducted to reveal potential lipid marker(s) for lung cancer.

Fan et al. (2009) Altered regulation of metabolic pathways in human lung cancer discerned by 13C stable isotope-resolved metabolomics (SIRM). Molec. Cancer, In press.

A47: Biomedical Biomarkers

Analysis of the human serum metabolome in health and disease: the HUSERMET project

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The Human Serum Metabolome project (HUSERMET: http://www.husermet.org/) is a four year multi-disciplinary collaboration between the University of Manchester, AstraZeneca and GlaxoSmithKline. Using a multi-platform analytical approach this project aims to assemble comprehensive serum metabolic profiles for several thousand individuals, recruited and analysed over a 3-5 year period.
The key aims of this project are:

- To develop and optimise reliable methodologies for characterising the serum metabolome
- To demonstrate the applicability of these methods through the characterization of the "healthy" human serum metabolome
- To monitor disease progression, and/or response to treatment, in disease-based studies
- To establish the disease-independent ranges of metabolites observed in 'healthy" human serum
- To make these data available in a web-accessible manner.

Over 3000 serum samples have currently been collected from healthy volunteers, with an additional 500 samples provided from patients diagnosed with Alzheimer's disease or Ovarian Cancer.

A range of analytical technologies including GC-MS, UPLC-MS, NMR, FT-IR and ICP-MS, are being employed to analyse these samples. Since data from each technique are complementary, broad coverage of the metabolites present in the human serum metabolome is ensured.

The size and scale of this project has required the development of robust standard operating procedures, and intelligent experimental design, to allow the acquisition of long-term, reproducible, analytical data. In addition, sample collection and experimental procedures have been standardized to ensure consistency over the life time of the project.

The raw and processed analytical data generated, together with relevant clinical and physiological metadata for all subjects, are currently being assembled into a database to aide future metabolomic and clinical research.

A48: Biomedical Biomarkers

Development and performance of a GC-TOF-MS analysis for the large-scale non-targeted metabolomic studies of human serum

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Many factors influence the reproducibility and robustness of data obtained from untargeted metabolomic analyses of mammalian biofluids, but in particular those concerned with sample complexity and analytical operations. Within the Bioanalytical Sciences Group at Manchester, GC-ToF-MS of trimethylsilyl derivatives is one of the techniques being employed to analyse human serum samples as part of the HUSERMET project (http://www.husermet.org).

A method for the preparation and GC-ToF-MS analysis of serum samples has been developed and evaluated for application in long-term untargeted metabolomic studies. This methodology involves an optimised metabolite extraction and derivatisation procedure, coupled with the extensive use of Quality Control (QC) samples to quantify process variability.

Here we present a summary of the extraction, derivatisation and instrumental parameters used and the rationale for their selection. We also describe a simple, generally applicable experiment design which we have found helpful in distinguishing metabolites derived from serum from contaminants and other artefacts arising from sample preparation and analysis. In addition, we demonstrate that our methodology allows the acquisition of consistent, reproducible, data over a period of 18 months.

A49: Biomedical Biomarkers

The response of HaCaT cells to dithranol anti-psoriatic treatment: A combined approach incorporating top down metabolic profiling and shotgun proteomics

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Psoriasis is a common inflammatory skin disorder characterized by the presence of areas of inflamed, thickened & discoloured skin. There is limited understanding regarding the exact pathogenesis of the disease although both genetic and environmental factors are believed to impact its occurrence and development. Whilst there have been a considerable number of studies conducted to enhance knowledge and understanding of the pathogenesis of psoriasis, here we present the first ‚Äòomics based investigation. The HaCaT cell line was chosen as a model system for psoriasis and the effect upon application of dithranol was investigated.

HaCaT cells were grown using standard culture protocols. Upon reaching approximately 85-90% confluency the drug solution was applied to the cells at concentrations in the range of 0.1-0.5¬µg/ml for 24h. Experimental sampling protocols were developed for the generation of samples for both metabolomic and proteomic based analyses. Metabolic profiling was conducted through GC-MS analysis of both the cell‚Äôs footprint (exometabolome) and fingerprint (internal metabolome) while proteomic analysis incorporated the use of LC-MS for fingerprint investigation.

GC-MS analysis yielded a total of 127 fingerprint and 107 footprint metabolite peaks. An ANOVA based analysis was performed and metabolite peaks were selected for further analysis at a significant value of $p < 0.05$. From these metabolite subsets spring embedded plots were constructed using a correlation threshold of 0.7 1. To relate the list of significant metabolites to central metabolism a schematic metabolite map with associated box and whisker plots was generated; to allow the cellular response to dithranol to be highlighted. In this cognate approach LC-MS analysis provided qualitative lists of proteins present at each drug treatment concentration. The presence or absence of a proportion of these recorded proteins has been correlated to the metabolite map mentioned above.

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A50: Biomedical Biomarkers

Early metabolic changes in preneoplastic nodules of the liver

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Hepatocellular carcinoma is, worldwide, the fifth most frequent neoplasm and the third most common cause of cancer related death. In spite of the identification of the main risk factors for this cancer (e.g. hepatitits B and C virus, alcohol abuse, non-alcoholic steatohepatitis, aflatoxin contaminated food) the prognosis of these patients is poor, mainly due to advanced stage of the lesion at the time of the diagnosis and the lack of effective therapy. Hyperplastic liver lesions developing after administration of carcinogens are early precursors for cancers. Hepatic hyperplastic nodules were induced in male Fischer rats (n= 12) by a single dose (150 mg/kg) of diethylnitrosamine (DENA), followed by a 2-week exposure of the animals to 2-acetylaminofluorene (diet containing 0.02%) and partial hepatectomy according to the Solt and Farber protocol. Controls (n=12) were sham treated without DENA. Animals were sacrificed seven weeks later. Liver was collected and analysed morphologically. Treatment induced a high number of placental glutathione-S-transferase (GSTP)-positive nodules (N¬∞ of GSTP+ nodules/cm2: 19.7-46.6; area persistent lesions in per cent: 0-35, arbitrary score 2-10)). Liver extracts and plasma were analysed using a combination of 1H-nuclear magnetic resonance (NMR) spectroscopy and gas-chromatography mass spectrometry (GC-MS) Multivariate statistics, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were used to identify clusters and metabolites responsible for differences. PCA identified two clusters corresponding to nodule density. PLS-DA of these samples indicated two well defined classes (R2Y=91%, Q2(cum)=82%). Principal metabolites responsible for the discrimination were identified as: decrease of glucose, phophocholine, glutathione and increase of glutamate in the DENA treated group . Analysis is ongoing to discover the metabolic changes in the liver preneoplastic nodules in order to identify early modifications responsible for the carcinogenic process in the liver.

A51: Biomedical Biomarkers

Topographical Variation in Metabonomic Signatures of the Rat Intestinal Content Revealed by High-Res

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The metabolite composition of gastrointestinal contents carries important information for the complex interactions between mammal hosts and their gut microbiome. Here, we characterized the metabolic profiles of rat gastrointestinal contents using high-resolution 1H NMR spectroscopy coupled with multivariate pattern recognition techniques. We found that significant metabonomic variations were present between the contents of jejunum, ileum, cecum, colon and feces although many metabolites were commonly found in these samples including lactate, creatine, choline and branched chain amino acids. The topographical variations reflect the unique and specific metabolic fingerprints for each intestinal region with the greatest differences found between the contents of small and large intestines. We further found that compared with the large intestinal content and feces, the jejunal and ileal contents contained higher levels of lactate, creatine, taurine and amino acids together with lower levels of short chain fatty acids. The feces had high levels of glucose but low levels of amino acids. Moreover, metabonomic changes of intestinal contents and feces were associated with growth development of animals which was featured with the alterations in the levels of lactate, choline, methanol, amino acids and short chain fatty acids. Such findings suggest that the symbiotic gut microbiota and activities not only modulate the adult host metabolism but also could play some important roles in the host development.

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A52: Biomedical Biomarkers

ACUTE TOXICOLOGICAL RESPONSES OF WISTAR RATS TO MEQUINDOX REVEALED BY NMR-BASED METABONOMICS

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Mequindox is used as a veterinary antibiotic drug in livestock and poultry. However, the toxicity of mequindox remains unknown. The aim of the investigation is to assess the toxicity of the mequindox using NMR-based metabonomics strategy which combines NMR metabolic profiles of biofluids and

pattern recognition data analysis. In this study, we investigated the dynamic NMR metabolic profiling of serum and urine in rats gavaged with mequindox. Clinical biochemistry analysis (AST, TP, ALB, AREA) showed liver and kidney toxicities both at moderate and high dosages; the PLS-DA plots disclosed significant rat metabonomic alterations induced by mequindox treatment. Even with the low dosage used practically at present, the clinical chemistry and metabonomic analyses showed some metabonomic differences between the controls and exposed rats. In order to fully understand the damage caused by mequindox and recoverability, further investigation is necessary to assess the chronic toxicology and the metabolic responses of different species (rats, mice, chicken and pigs) exposed to mequindox. Our work provides important information for mequindox usage and animal food safety.

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A53: Biomedical Biomarkers

Dynamic mice metabolic responses to Schistosoma japonicum infection

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About 600 million people are exposed to schistosomiasis in the world and 200 million are estimated to be infected. As the endemic species in China, S japonicum infection often leads to anemia, diarrhea, portal vein hypertension syndrome, etc [1]. Traditional diagnostic tests, including pathological and immunological approaches, are not suitable for early diagnosis and are insensitive. As a new powerful analytical tool, metabonomics has been used to study the urine metabolic profiling of mice infected with S mansoni, hamster infected with S. japoniucm. [2-3]. In order to understand the dynamic metabolic responses of mice to S. japonicum infection, in this study, we analyzed the metabonomic alterations of mice blood plasma induced by the infection with 80 S. japonicum cercariae using the combination of NMR spectroscopy and multivariate data analysis. The results showed that the glucose was elevated at early infection probably due to immune reactions [4] and alleviated in the heavy-infected group probably due to anemia resulting from the affection of the host red blood cells by the adult schistosomes. The levels of lipid component (mainly LDL, VLDL) were also reduced due to the lipid peroxidation damage in cell membranes caused by reactive oxygen species in the infection group [5]. The depletion of plasma amino acids may reflect a compensatory mechanism for maintaining host energy homeostasis and ketogenesis[6-7]. Elevated O-acetyl glycoprotein signals in plasma were considered to be associated with inflammatory conditions[8].

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B01: Fluxomics & Metabolic Engineering
Metabolomics: A Powerful Tool for Biopharmaceutical Production

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Metabolomics is the unbiased global profiling of biochemicals in a complex sample. This technology has been successfully applied to problems such as the discovery of biochemical markers of prostate cancer1 and identification of markers of early drug-induced kidney toxicity2. Recently, this approach has been used to better understand the production of recombinant proteins. Because of its ability to analyze hundreds of biochemicals from a single sample, it can be used to compare different lots of media, media from different vendors, or chemically-defined versus animal-derived media. In addition to analyzing media formulations, metabolomics can be used to profile biochemicals in a shake flask or bioreactor. This ‚Äúsnapshot‚Äù of cellular metabolism provides insight into how biochemicals are changing, not only in the cells but also in the media over time. This presentation will discuss several case studies from biopharmaceutical collaborations, describing how metabolomics was used to examine different media compositions and monitor of hundreds of biochemicals and metabolites, found both inside the cell and in the spent media.

1 Sreekumar, A, et al. Metabolomic Profiles Delineate Potential Role for Sarcosine in Prostate Cancer Progression. Nature 457:910-914 (2009)
2 Boudonck, K, et al. Discovery of Metabolomics Biomarkers for Early Detection of Nephrotoxicity. Toxicologic Pathology Volume 37, Number 3 (2009)

B02: Fluxomics & Metabolic Engineering
Metabolic labeling studies with the alga Chlamydomonas reinhardtii under heterotrophic and mixotrophic growing conditions

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The ability of the freshwater alga Chlamydomonas reinhardtii to grow heterotrophically is due to the presence of the acetate-inducible glyoxylate pathway, bypassing two decarboxylation steps in the citric acid cycle. The metabolic flux through the non-photosynthetic central metabolic pathways was determined by 13C-labeling experiments. After supplying continuous algal cultures, growing either in the light or the dark, with 13C-labeled substrates, the incorporation of the 13C-label in the proteinogenic amino acids was determined by NMR. The position and the relative intensity of the 13C-label in these amino acids is indicative of the pathways utilized to metabolize the labeled substrate molecules. Preliminary data confirms the occurrence of the glyoxylate pathway under heterotrophic and mixotrophic growing conditions.

B03: Fluxomics & Metabolic Engineering

TCA Cycle Flux Estimation Using Stable Isotope Snapshots in a Single Animal Biopsy

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It is difficult to determine metabolic fluxes quantitatively in single tissue biopsies from experimental animals. Here we report a novel approach for in vivo flux quantification using stable isotope labeling. Our approach overcomes the difficulty that it is impossible to obtain a time series of metabolite levels from the same tissue area at high resolution.
We developed a protocol based on timed (5.5 min) infusion of 13C isotope enriched substrates for the TCA cycle followed by quick freezing of tissue biopsies. NMR measurements of the extract were followed by flux estimation based on a computational model of carbon transitions between TCA cycle metabolites and connected amino acids. To this end we developed a computational framework in which metabolic systems can be flexibly assembled, simulated and analyzed. Implementing a model of the TCA cycle we showed by extensive simulations that the timed infusion protocol reliably quantitates multiple fluxes.
An experimental validation of the method was done in vivo on hearts of anesthetized pigs. The experiments were done under two different conditions: basal state (n = 7) and cardiac stress caused by infusion of dobutamine (n = 7). About 8-10 tissue samples, 50-160 mg dry mass, were taken per heart. Using the isotope snapshot method the TCA cycle flux was 6.11 ¬± 0.28 (SEM) micromol/g dry/min for control vs 9.39 ¬± 1.03 micromol/g dry/min for dobutamine stress. Oxygen consumption calculated from the TCA cycle flux and from 'gold standard' blood-gas-based measurements were nearly identical, correlating with r = 0.87 ($p < 5e-5$). Given good NMR spectra, further flux parameters (e.g. carbon substrate flux) could also be quantified. We propose that our novel isotope snapshot methodology is suitable for flux measurements in biopsies in vivo.

B04: Fluxomics & Metabolic Engineering

Characterization of the metabolic changes underlying growth factor angiogenic activation: identification of new potential therapeutic targets

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One of the critical stages in tumor growth is angiogenesis, which consists of recruiting endothelial cells toward an angiogenic stimulus. The cells subsequently proliferate and differentiate to form endothelial tubes and capillary-like structures. Little is known about the metabolic adaptation of

endothelial cells through such a transformation. We studied the metabolic changes of endothelial cell activation by growth factors using human umbilical vein endothelial cells (HUVEC), [1,2-13C2]-glucose and mass isotopomer distribution analysis (MIDA). The metabolism of [1,2-13C2]-glucose by HUVEC allows us to trace many of the main glucose metabolic pathways, including glycogen synthesis, the pentose cycle and the glycolytic pathways. So we established that these pathways were crucial to endothelial cell proliferation under vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) stimulation. A specific VEGF receptor 2 (VEGFR-2) inhibitor demonstrated the importance of glycogen metabolism and pentose cycle pathway. Furthermore, we showed that glycogen was depleted in a low glucose medium, but conserved under hypoxic conditions. Finally, we demonstrated that direct inhibition of key enzymes to glycogen metabolism and pentose phosphate pathways reduced HUVEC cell viability and migration. In this regard, inhibitors of these pathways have been shown to be effective antitumoral agents. To sum up, our data suggest that the inhibition of metabolic pathways offers a novel and powerful therapeutic approach, which simultaneously inhibits tumor cell proliferation and tumor-induced angiogenesis.

B05: Fluxomics & Metabolic Engineering

Using Metabolic Flux Analysis with Flux Balance Analysis To Understand Regulation in E. coli

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The development of rational metabolic engineering is limited by our understanding of metabolism. Methods such as genome-based Flux Balance Analysis (FBA) give insight to metabolism and allow predictions about the impact of genetic perturbations. By testing the predictions with steady state isotopic-labeling-based Metabolic Flux Analysis (MFA), we can gain insight to cellular regulation. Here, we have generated a 13C isotopic MFA network for Escherichia coli (E. coli) based on a FBA model in previous studies (Reed et al., Genome Biol, 2003). To investigate cellular regulation, our model includes all central carbon metabolism pathways, along with amino acid and DNA/RNA biosynthesis. We have established analytical protocols for analysis of microbial amino acid and intracellular metabolic intermediates using gas chromatography-mass spectrometry (GC-MS). Additionally, we have measured directly the substrate uptake and product secretion rates in cultured medium using NMR and enzyme assay. Labeling measurements and direct flux measurements have been performed with wild type E. coli (Mg1655) cultured in M9 medium under aerobic conditions and supplied to our isotopic network model to obtain a detailed map of carbon and energy flows through metabolism. The resulting flux map is being used to test predictions made using Flux Balance Analysis and will shed light on metabolic regulation in transgenic strains.

B06: Fluxomics & Metabolic Engineering

Integrating Various Levels of "Omics" Data into Mathematical Models - Mint Essential Oil Biosynthesis as an Example

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The essential oil distilled from peppermint (Mentha x piperita) leaves is used in numerous consumer products (e.g., chewing gum, toothpaste, and mouthwash), as a flavor in the confectionary and pharmaceutical industries, and as a source of active ingredients for aromatherapy (value of finished products > $ 6 billion per year). Because of the depth of data available for peppermint, it has been developed as an experimental model system for understanding essential oil biosynthesis in general. We developed a kinetic mathematical model of monoterpenoid essential oil formation by integrating numerous levels of experimental data: the kinetic properties of biosynthetic enzymes were incorporated as static parameters; developmental patterns of biosynthetic enzyme activities, enzyme concentrations in different subcellular compartments, metabolite levels, and the distribution of the specialized anatomical structures producing essential oils (glandular trichomes) were included as dynamic parameters. Using an iterative approach of mathematical modeling and experimental testing, we recently discovered previously unknown mechanisms of feedback control [1].

We have now developed a second generation mathematical model, once again based on extensive experimental data, to evaluate the effects of environmental perturbations and targeted transgenic modifications on essential oil yield and composition. In general, environmental and transgenic effects on oil composition correlated well with biosynthetic gene expression patterns, whereas impacts on oil yield correlated strongly with the distribution of glandular trichomes. Theimplications of these new findings for developing peppermint as a production host for high value terpenoids will be discussed.

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B07: Fluxomics & Metabolic Engineering

HIGH-ACCURACY LC-MS/MS QUANTIFICATION OF ISOTOPOMER DISTRIBUTION IN INTRACELULLAR METABOLITES FOR 13C-FLUXOMICS

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Metabolic flux determination is mostly based on the 13C labelling experiments and thus relies on an accurate quantification of the 13C atom distribution in intermediates or products of metabolism. These methods are well established for steady-state conditions where the isotopic information valuable for flux calculations can be collected from metabolic end-products, which accumulate at extents much larger than true intermediates. The current challenge is to extend these methods to the analysis of the dynamic response of metabolism to changes in the environment or in genome

expression. To achieve flux measurement in such conditions, instationary 13C-labelling experiments must be performed where the time-courses and rates of label incorporation in true metabolic intermediates are monitored in detail, which require specific sampling procedures, sensitive analytical tools and sophisticated mathematical models. Because intracellular metabolites are found at low quantities and have high turn-over rates, highly-sensitive LC-MS(/MS) strategies are increasingly used for the quantification of mass isotopomer distributions (MID) in these compounds. The accurate quantification of MIDs is critical for constraining metabolic fluxes and establishing reliable flux maps. Differents factors, ranging from the sampling procedure to MS parameters - affect the accuracy of this quantification. In addition, no commercial standard is available for measuring the isotopic accuracy of MS detectors, especially at high 13C enrichments. Here, we describe the design and validation of a biological standard of labelled intracellular metabolites that allow determining the accuracy of MID quantification by MS strategies. Methylotrophic yeast Pichia pastoris was grown on partially 13C labelled methanol as a sole carbon source so that the incorporation of the label in metabolites follows a predictable binomial law. MIDs were quantified by LC-MS/MS for a wide range of intracellular metabolites, and the comparison of the experimental results with the expected ones indicated that the isotopic accuracy of the method was within 1%.

B08: Fluxomics & Metabolic Engineering

Oncogene-induced cell cycle entry and cellular re-programming for induction of neoplasia engages pathways for de novo lipid synthesis

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The generation of lipids for cell division and neoplasia requires the coordination of substrate uptake and carbon flux into metabolic pathways to initiate de novo lipogenesis. However, molecular factors involved in the regulation of these events have not been fully elucidated. The Myc oncogene is a serum induced cell cycle regulator and reprogramming factor involved in both induction of pluripotency and neoplasia. De novo lipid metabolism is important for cell cycle entry and Myc regulates carbon flux through the TCA cycle but Myc‚Äôs influence on lipid metabolism, during cell cycle entry or cellular reprogramming to a neoplastic state, have not been investigated. In this study we confirm Myc‚Äôs ability to alter carbon flux into lipids using stable isotope analysis. Labeling of myc-/- and myc+/+ fibroblasts with multiple metabolic substrates, including [U-13C] glucose, glutamate, acetate and acetoacetate reveal that Myc increases the production of fatty acids from each of these substrates during cell cycle entry. In addition, metabolic inhibitor studies confirm Myc‚Äôs

dependence on de novo fatty acid synthesis for continued proliferation. Temporal studies on lipid synthesis in isolated pancreatic islets, from the pInsMycERTAM/Bcl-XL model of Myc inducible pancreatic beta cell neoplasia, demonstrate that Myc increases de novo lipid metabolism during induction of tumorigenesis. Since this model displays an embryonic stem cell-like gene expression profile these data suggest lipid metabolism may be associated with reprogramming of cells to embryonic stem cell-like states. To our knowledge, this is the first study of temporal analysis of glucose carbon flux into lipids during Myc-induced neoplasia. When combined with our prior work on Myc,Äôs regulation of central carbon metabolism, these data suggest a significant role for this oncogene in integrating cell cycle signaling networks with metabolic processes required to drive bioenergetic and synthetic pathways for cell proliferation and tumorogenesis.

B09: Fluxomics & Metabolic Engineering

Stable Isotope Resolved Metabolomics (SIRM) of UDP-GlcNAc and UDP-GalNAc Metabolism in Prostate Cancer

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Supranutritional dietary selenium supplement reduces the risk of prostate cancer and shows promise as a chemoprevention agent for several other prominent cancers. Previous in vitro studies demonstrated methyl seleninic acid's (MSA) effectiveness at inhibiting prostate cancer cell growth. We found that MSA has numerous inhibitory effects on central metabolism, including a striking effect on sugar nucleotides, which we identified as uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) and uridine diphospho-N-acetylgalactosamine (UDP-GalNAc) by NMR and FT-ICR-MS. In particular, UDPGlcNAc is an important sugar donor for regulating intracellular proteins by O-glycosylation, including c-Myc, p53 and VDAC. We used FT-ICR-MS for rapid, high-confidence measurement of the time course of C-13 incorporation into both UDP-GlcNAc and UDP-GalNAc molecules from [U-C-13]-Glucose, in LNCaP and LN3 prostate cancer cells. This incorporation involves the flow of different C-13 labeled functional moieties via parallel, converging metabolic pathways. The ultra high mass resolution and high precision of the isotopomer intensities is critical for reliable quantification of all 17 combined UDP-GlcNAc/UDP-GalNAc carbon mass isotopomers. We developed a combined simulated annealing and genetic algorithms (SAGA) method to parse the observed mass isotopomer intensities into individual species, that are comparable with positional isotopomer information from NMR. This SAGA method also provides information about the relative importance of specific metabolic pathways to C-13 incorporation. Replacement of initial unlabeled UDP-GlcNAc and UDP-GalNAc by labeled species is consistent with a regulated steady state synthesis and consumption of this important intermediate. The rate of C-13 enrichment of both UDP-GalNAc and UDP-GlcNAc was substantially decreased by MSA along with a similar decrease in the rate of unlabeled UDP-GlcNAc/UDP-GalNAc disappearance. This suggests an effect of MSA on the enzymatic synthesis and utilization of both UDP-GlcNAc and UDP-GalNAc.

B10: Fluxomics & Metabolic Engineering

Use of 13C-glucose to infer metabolic flux in respiration-disabled *Escherichia coli*

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A respiratory deficient Escherichia coli strain was generated for use as a platform strain for metabolic and evolutionary engineering. The E. coli Cytochrome Oxidase Mutant (ECOM3) strain described before (Portnoy et al., Appl. Environ. Microbiol. 74, 7561-9, 2008) was further mutated by removal of the ygiN gene, followed by adaptation to glucose minimal medium, producing a strain (ECOM4) that utilized no oxygen even when grown in air. This strain grew similarly in oxic and anoxic conditions and exhibited almost-stoichiometric conversion of glucose into D-lactate. To examine the metabolism of ECOM4 in more detail, this strain and its MG1655 wild-type parent were grown in minimal medium with [U-13C], [1-13C] or [6-13C] glucose. Cell pellets were digested in 6N hydrochloric acid and the 13C labeling of amino acids was determined by GC-MS. Labeling patterns were used to infer input of converging metabolic pathways to amino acids and related central metabolites. As expected from the lack of respiratory-chain function, the complete tricarboxylic acid cycle did not operate in ECOM4. Oxaloacetate (aspartate) was derived wholly from anaplerotic conversion of phospho-enol pyruvate. Flux through the pentose phosphate pathway relative to glycolysis was much reduced in ECOM4, but relative inputs to the pentose phosphate pool from the oxidative or non-oxidative branches of the pentose phosphate pathway were similar to wild-type. Otherwise, the ECOM4 strain was metabolically similar to WT: there was still some reverse flux of aspartate/ oxaloacetate back through the TCA cycle to symmetrical intermediates and there was no enhancement of the Entner-Doudoroff pathway or pyruvate-formate lyase activity. These results, based on analysis of local flux, were compared to a global constraint-based metabolic flux analysis. This procedure also indicated decreased TCA and pentose phosphate pathway flux and increased glycolytic activity in the ECOM4 cell line.

B11: Fluxomics & Metabolic Engineering

Targeted Metabolomics in Cell Culture Applications: From Clone Selection to Medium Optimization

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Cell cultivation is an established system for the production of biologicals in large-scale fermentation processes. Efficient production strains and optimal growth conditions are required to achieve a high yield of quality products. As metabolic processes play a key role in respect to cell vitality, productivity, and process economy, a targeted metabolomics approach to cell culture applications was taken to improve the outcome.

Utilizing a mass spectrometry platform (FIA-, LC-MS/MS, GC-MS), several hundred metabolites of different classes, e.g. amino acids, hexoses, intermediates of the energy metabolism, biogenic amines and polyamines, fatty acids, phospholipids and acylcarnitines were concurrently analyzed in small sample volumes of cell culture supernatants.

The obtained quantitative results enabled the metabolic characterization of diverse production cell lines originating from different species, which showed a highly distinctive metabolite pattern. It will be outlined that this metabolic discrimination can be easily applied to the selection of high producer clones within one production cell line, as high productivity needs to go along with an optimal metabolic capability of the cell.

Furthermore it will be shown that targeted metabolomics also comprises a rapid and comprehensive method to monitor changes in medium composition that are relevant for cell growth and vitality, i.e. consumption of supplied nutrients or accumulation of defined growth-inhibitory metabolites.

B12: Fluxomics & Metabolic Engineering

Gas chromatography-mass spectrometry based metabolomics of transgenic tobacco plants (*Nicotiana tabacum* var. Xanthi) reveals differential effects of complete and incomplete flavonoid pathways on the metabolome

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Gas chromatography-mass spectrometry (GC-MS) based metabolomics was used to comparatively examine the metabolic consequences of introduction of a complete anthocyanin pathway by expressing PAP1 gene, an incomplete proanthocyanidin pathway by expressing anthocyanidin reductase (ANR) gene, and a complete proanthocyanidin pathway by expressing both PAP1 and ANR genes in tobacco(Nicotiana tobacum) plants. Eighty-seven identified metabolites including primary and secondary metabolites were clearly detected from young leaf, old leaf and stems of wild type tobacco plants, among which the abundance of 71 metabolites was tissue specific and developmentally dependent. Our data clearly show that constitutive over-expression of a complete anthocyanin(PAP1) or proanthocyanidin (PAP1 and ANR) pathway in tobacco resulted in altered pools of numerous primary metabolites; for example, the levels of shikimic acid and malonic acid, which are the early metabolic precursors of flavonoids pathway, were significantly diminished. Introduction of an incomplete proanthocyanidin pathway by only expressing the ANR gene resulted in altered accumulation of nineteen identified primary and secondary metabolites, many of which are metabolically unrelated to flavonoids biosynthesis. Included in this list are several terpenoid cembranoids, which are aphid-defensive metabolites that are significantly increased by the expression of ANR transgene. In addition, coupled expression of the PAP1 and ANR genes led to clear separation of the accumulation patterns of certain metabolites from the PAP1 or ANR alone transgenic plants. Our results support the value of using comparative metabolomics approaches for revealing and measuring the off-target effects of transgenes on the metabolomes in metabolic engineering efforts.

C01: Metabolomics and Informatics

A strategy for structure elucidation on unknown compounds using gas chromatography - accurate mass time of flight mass spectrometry

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Advanced methods for the identification of novel metabolites are direly needed because unknown metabolites are detected as being statistically significant in almost all published metabolomic studies. GC/MS methods have the advantage of large mass spectral libraries of known compounds which therefore typically yield already around 150 identified structures per study, besides some 250 unidentified signals. Determination of elemental compositions is the first crucial step in structural annotations for these unknown compounds. We here report on use of accurate mass and isotope abundance ratio using the Waters GCT-premier time of flight mass spectrometer. To check performance of the instrument, 18 known reference metabolites were analyzed, applying the ‚ÄòSeven Golden Rules‚Äô algorithm to constrain chemical formulae hit lists. The average errors of the mass accuracy and of the isotope abundance ratio for A+1 and A+2 in chemical ionization mode or high-mass tune EI-mode were -6.3 ppm, 2.2% and 1.9%, respectively. As result, 14 out of 18 known reference metabolites were ranked among the top 3 hits. Investigation of the M-15 (methyl cleavage) fragment ions yielded average errors of -5.2 ppm mass accuracy and 3.7% and 2.7% isotope abundance ratio errors for A+1 and A+2 ions. Results were shown to be dependent on the ratio of metabolite concentration to the lock mass intensity. Applications of the instrument for metabolomic samples will be demonstrated.

C02: Metabolomics and Informatics

An Interactive Plant Metabolomics Web Portal for Exploring the Arabidopsis Metabolome

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PlantMetabolomics.org is a web portal and database to store, exchange and visualize plant metabolomics data. The NSF 2010 plant metabolomics consortium is developing metabolomics as a functional genomics tool by identifying metabolites of Arabidopsis and applying that knowledge to genes of known and unknown functions respectively to verify the functions of genes and generate hypotheses about the gene functions. The participating labs in this consortium use different

analytical platforms on the similar biological material. PlantMetabolomics.org provides a hypothesis building platform where data visualization tools such as ratio plots and error plots can be easily applied to the metabolomics data. PM is publicly available at http://www.plantmetabolomics.org.

The portal provides:

- Metabolomics abundance data for viewing and download.
- Full metadata of experiments based on MSI standards.
- Searches by metabolic pathway or individual metabolite.
- Comparative visualization tools allow mutants to be compared to each other and to the wild type with a click of a button.
- Annotation links between the measured metabolomics and small molecule data from TAIR, PubChem, MetNetDB, and other databases.
- Detailed protocols and video tutorials on conducting plant metabolomics experiments.
- Data quality plots to guide the user on replicate reliability.

C03: Metabolomics and Informatics

A Data Mining Process Model for Metabolomics

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Metabolomics experiments produce a substantial amount of data which include hundreds of variables. There is a general agreement that the development of metabolomics depends not only on advances in chemical analysis techniques, but also on advances in computing and data analysis methods.

Metabolomics data analysis and mining are often approached in an informal and unjustifiable manner. A systematic and manageable process is needed to promote the achievement of analytical objectives and to enhance the validity of metabolomics results. To achieve this, reporting standards as proposed by MSI is not enough, we also need a process model that governs the conduct, the procedures and the activities applied to assure their quality and justify their results.

Scientific applications in general and metabolomics in particular, require attention to issues including traceability, justification and reproducibility of outcomes. Several process models are currently available in data mining e.g. CRISP-DM, KDD. They are not directly applicable to scientific experiments and are not strong in quality control, reporting or management issues. Our data mining process is built around the specific requirements of metabolomics. It combines and utilises the best practices, techniques and procedures in software engineering with those in machine learning and statistics in addition to the fundamentals of scientific methodology.

The process is designed as an iterative process defining the flow and feedbacks between its phases as well as the inputs and outputs of each phase. A set of tasks is defined for each its phases including objective setting, planning, activities performing, reporting and validation. The process provides mechanisms for management, human interaction, standardisation, and quality assurance. It also supports justifying the selection of data analysis and mining techniques, results traceability validation and evaluation, not only from a data mining perspective, but also from a biological perspective through effective representation, interpretation of the discovered knowledge.

C04: Metabolomics and Informatics

Metabolomics Analysis of Urine from Rats Chronically Dosed with Acrylamide

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Acrylamide (AA) is a chemical formed during cooking of food. AA is neurotoxic, toxic to the testis, mutagenic, and carcinogenic in rodents. This study compares the NMR-based metabolomics of AA in urine and serum of male F344 rats during subchronic exposure to AA. Five male F344 rats were administered control water or dosed with AA (2.5, 10 or 50 mg/kg bw) in the drinking water for two weeks. There were two groups of rats treated with the high dose, one was sacrificed after the last dose while the other group was sacrificed 1 week after the end of AA exposure. Urine was collected daily and blood was collected on days 0, 1, 3, 7, 10, 14, 17, and 21. Metabolomics analysis showed that the creatine concentration increased by two orders of magnitude at the high AA dose, which could be related to decreased food intake or an oxidative stress response caused by the thiol reactivity of AA and glycidamide, the epoxide metabolite. All Kreb's Cycle intermediates (2-oxogluturate, succinate, and citrate) detected in urine were decreased by ~19-44% during AA dosing, while formate was up by approximately 35%. Urinary levels of N-acetylaspartate were increased by ~16% at the high dose, which might be related to the neurotoxicity of AA. Higher urinary levels of lactate and alanine could signal an increase in glycolysis. Changes in these urinary metabolites caused by the high dose of AA may be due to reduced weight gain (possibly due to changes in dietary intake or intestinal absorption) and physiological changes associated with altered energy metabolism, particularly in neural and testicular tissues.

C05: Metabolomics and Informatics

The Human Urine Metabolome

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Metabolomics is an emerging field that is complementary to the other omics and proving to have unique advantages. The field of metabolomics is gaining increasing interest across all disciplines, including functional genomics, integrative and systems biology, pharmacogenomics, and biomarker discovery for drug development and therapy monitoring. For the field of metabolomics to prosper, much effort must be expended to build a basic understanding of the metabolites that are found in

biology. In an effort to contribute to this understanding, we have chosen to focus on human urine, the most easily obtained biofluid sample. High-resolution NMR spectroscopy, GC-MS and LC-ESI-MS/MS together with computer-aided literature mining tools were combined to identify and quantify the metabolites that can be commonly detected (with today's technology) in the human urine metabolome. Samples from healthy subjects and patients were collected for the analysis. With NMR spectroscopy approximately 60 distinct metabolites were detected and quantified, whereas GC-MS methods could detect 90 metabolites. Data acquisition and analysis was performed according to SOPs and in house developed protocols. The Human Urine Metabolome Database consists more than 650 metabolites. Normal as well as abnormal concentration values for each metabolite are reported. A computational text mining tool (Polysearch) was used to generate a hyperlinked list of abstracts and papers from PubMed containing relevant information about urine metabolites and their concentration data.

C06: Metabolomics and Informatics

Study of Different Variable Selection Approaches on NMR Serum measurements for the Metabolomic Assessment of a Novel PCOS Treatment

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In this study we assessed the performance of different variable selection algorithms and pre-processing steps to minimise the high dimensionality problem associated to the metabolomic data generated by NMR instruments used to characterize complex bio fluid samples.

A total of 11 patients with polycystic ovary syndrome (PCOS) were followed from diagnosis (baseline state) up to 3 years during which they received combined low-dose therapy with Metformin, Flutamide and Pioglitazone plus an oral contraceptive. NMR measurements were performed on both pre-treatment serum samples and on their corresponding samples at the end of the treatment.

While regular PCA or PLS-DA analysis already proves that a direct NMR metabolomic approach can differentiate between basal and treated samples, both algorithms had to cope with NMR spectra consisting on 14001 data points. Our aim was to see if better results could be obtained with a reduced subset of the data points from the original NMR spectra while evaluating the best pre-processing approach needed for these type of measurements.

The pre-processing approaches applied to the 22 spectra were mean centring, auto scale and Pareto Scaling. The variable selection algorithms studied included Genetic Algorithms, Wavelets, Simulated Annealing, Simulated Annealing plus wavelets and ANOVA analysis.

We used ten serum samples from five patients (both before and after treatment) for variable selection and model calibration. Then, we validated the regions chosen and models built with the remaining six patients. Therefore, the results were validated with completely independent subjects not included before.

Results obtained show that using the Anova variable selection approach for an 1H NMR spectra reduces the Mean Square Error of Prediction from 0.36 to 0.19 (almost a 50% reduction in error) using only 524 variables from the entire spectrum, barely a 3.5% of the original data points. This result was obtained using a Pareto Scaling pre-processing algorithm.

C07: Metabolomics and Informatics

Housekeeping metabolites: a new normalization concept in metabolomics

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Comparability of quantitative data generated in different laboratories, on different platforms and obtained by varying sample work-up conditions remains a challenge in omics data analysis. Present concepts in metabolomics require further evaluation, a gold standard for normalization of metabolomics data is lacking. To date, normalisation in metabolomics relates to a large panel of more or less sophisticated data manipulation ranging from correcting for samplewise bias (e.g. total ion count), using context specific parameter (e.g. creatinine), calibrating to internal/external standards or applying numerical transformations (e.g. logarithm).

These methods, usually as a combination, are routinely employed to rub sample homogeneity discrepancies off and to improve data characteristics such as variance stabilisation. Nevertheless, these cannot guarantee that inherent additional experimental error is completely corrected, hindering both data modelling and followed up interpretation.

This problem (the reduction of the technical error) is known also in the context of transcriptomics and proteomics. We here present very promising results for the use of housekeeping/reference metabolites in quantitative metabolomics. Our data show that the use of housekeeping/reference metabolites increases the power of statistical analyses, i.e., reduces technical errors. In addition, we have identified several metabolites which qualify as candidates for ‚Äûuniversal‚Äú housekeeping metabolites as they show a constant concentration level independent of experimental conditions.

Interestingly, these ‚Äûuniversal‚Äú housekeeper or reference metabolite candidates were found stable among various species and may facilitate animal model comparison and transfer of data to man. In fact, this may be a unique feature of metabolomics, unlike the situation e.g. in transcriptomics.

C08: Metabolomics and Informatics

Identification and Quantification of Selected Metabolites in a Human Plasma Standard Reference Material by Comprehensive two Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry

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NIST is developing a standard reference material, Metabolites in Human Plasma (SRM1950), to serve as a common material to help researchers evaluate new techniques or platforms in this field. Both qualitative and quantitative information will be provided to the users of this material. Comprehensive two dimensional gas chromatography (GCxGC) connected with time-of-flight mass spectrometry detector (TOFMS) and peak deconvolution software offers a major tool for non-targeted analysis in metabolomics. GCxGC-TOFMS was first investigated to identify and characterize unknown polar and semi-polar low molecular weight compounds in SRM1950. Prior to GC analysis, a derivatization step is necessary to increase the volatility of the metabolites. The optimized GCxGC-TOFMS method was able to resolve more than 1000 peaks and therefore potential compounds. The data was first processed with ChromaTOF software for qualitative identification. Parameters included minimum similarity value with the NIST MS library (cut-off value of 600), peak width, S/N ratio of 10 and retention time. Using these rules, the software automatically generated a list of approximately 250 compounds that fulfilled the criteria. In addition, linear retention indices (RI) in the first dimension were calculated using SRM1494, containing 18 n-alkanes (C10 to C34). Currently, more than 100 metabolites were confirmed by triplicate analyses and authentic compounds standards. The metabolites list encompassed several classes of metabolites such as amino acids, hydroxyl/carboxylic acids, fatty acids, carbohydrates, alcohols, miscellaneous organics including some exogenous compounds. Among this list, seventeen amino acids were quantified by GC-TOFMS and GCxGC-TOFMS. Two different derivatization agents were investigated: tertbutyl silyl (MTBSTFA) and propyl chloroformate (PCF). In GC-TOFMS, terbutyl silyl and propyl chloroformate derivatives provided comparable concentrations. However, some amino acids are not able to be quantified under the conditions used with PCF. GCxGC TOFMS provided comparable data with GC-TOFMS. The added sensitivity of GCxGC did not seem to improve the precision.

C09: Metabolomics and Informatics

Filling the gap between so many ions and a really lower number of true metabolites

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Mass Spectrometry (MS) has gained large popularity in metabolomics and in metabolic profiling of complex biological samples, since nearly all analytical problems can be addressed by it. MS sources like ESI are almost universal and allow the ionization and the detection of different classes of molecules in a wide range of concentrations.
Unfortunately there is no one to one correspondence between the ionic signals and the neutral metabolites present in the sample. Ionization, indeed, is a highly variable phenomenon which results in (one or more) sequential events such as fragmentation, reaction and complexation of the analytes, both in-source and in the spectrometer. The overall result is hardly predictable even in presence of state-of-the-art chromatographic separation, optimal MS setup, and advanced software.
In these conditions the automatic analysis of the experimental results is practically impossible, thus hampering the setting up of a real 'high throughput' work-flow. The number of candidate metabolites to be identified is greatly over-estimated, making a realistic estimate of the dimension of the metabolome impossible.
A possible strategy able to reduce the effects of this phenomenon envisages the integration of MS experiments performed in different conditions (i.e. high and low collision energy) and with different ionization interfaces (ESI, APCI, MALDI, EI, CI) and separation modes (LC and GC).
In this contribution the effects of ionization dynamics on metabolome estimation will be discussed and preliminary results on data integration will be presented.

C10: Metabolomics and Informatics

Utilization of Statistical Compare Software and Fisher Ratios prior to Multivariate Analysis for Complex GCxGC-TOFMS Data in Order to Define Statistical Variation Between the Small Molecule Metabolite Profiles of Different Fish Species

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Metabolomics presents challenges for both the analytical methods used and the data reduction required to interpret the results. Comprehensive multi-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) has emerged as an excellent instrumental option for the characterization of complex metabolite profiles. Metabolomic samples demand analytical solutions and instrumental methods that will identify the small molecule metabolite profile completely as well as discover significant key components of interest. This study investigates tissue extraction techniques to optimize a simple and fast procedure which provides characterization of the small

metabolite profiles between different species. The experimental research explores the differences between the metabolite profiles of MTBSTFA derivatized tissue from different fish species analyzed by GCxGC-TOFMS. The combination of TOFMS data and deconvolution algorithms facilitate trace level analyte detection that would otherwise be hidden and coeluted with other compounds in the sample. This research presents metabolomic data illustrating the benefits of multidimensional chromatography coupled with time-of-flight mass spectrometry. Two dimensional chromatographic plots of biological samples showing increased peak capacity and structural orientation not possible in one dimensional chromatography will be highlighted. In addition, this research demonstrates a data mining strategy from GCxGC-TOFMS results which focus on locating the metabolite differences between species. Processed sample data was loaded as separate classes into statistical comparison software for peak table alignment, statistics generation, and Fisher Ratio calculations to define the unknown chemical variations between metabolites from different fish species. Results from the statistical comparison were subsequently exported to peripheral multivariate analysis software whereby analyte differences between the metabolite profiles of two fish species were examined. This research illustrates the capability of the Statisical Compare software feature to facilitate data reduction, and define metabolite variance thereby increasing the overall experimental results between complex biological sample classes.

C11: Metabolomics and Informatics

A Feature-Extraction and Data-Fusion Workflow for Metabolomic Analysis

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We have recast our previously published metabolomic analysis methodology as object-oriented software, extending it to implement data fusion of features extracted from multiple sources. Our process is geared for multivariate data such as nuclear magnetic resonance (NMR) spectra, mass spectra, and microarrays. The workflow proceeds with parallel preprocessing to a feature extraction step for each measurement technique. Illustrating feature extraction with NMR spectra, a graphical interactive process allows a user to select obvious (e.g., doublets and other multi-peak) structures, or complex regions (equivalent to ‚Äúintelligent binning‚Äù) as single features characterized by their mean amplitudes. Feature fusion requires 1) handling missing data, and 2) equalization of feature amplitudes among disparate measurement types. Data centering by mean subtraction handles missing data naturally, as zero values in missing samples do not alter the mean. Normalization to the sample variance commonly is used for equalization, but for typical metabolomic data, the

variance arises from biological diversity more than measurement error. Rather than normalize out the biological variation, we choose to equalize by the instrumental noise level determined from spectral regions with no signal. This expresses feature amplitudes in natural dimensionless signal-to-noise ratio units. The data matrix of fused features is then Principal-Component Analyzed, and a linear-kernel support vector machine determines a class-separating hyperplane in the space of principal component scores. The normal to the separating hyperplane is a vector of loadings that give the maximal variation of class score. We illustrate the workflow with 1H, 13C, and 31P NMR spectral analysis of aqueous and lipid liver extracts obtained from control and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treated rat and mouse models. Feature extraction has aided discovery of important features not originally included in targeted analysis, and data fusion provides a universal importance ranking of all features across the four spectral types. Supported by NIEHS R01-ES013927.

C12: Metabolomics and Informatics

The analysis of MS fragmentation trees as an integral part of the metabolite identification pipeline

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Metabolite identification constitutes one of the central aspects of metabolomics. Identification of metabolites can, in principle, be achieved using high resolution multistage mass spectrometry (MSn) because it provides a feature rich fingerprint of the structure of the precursor ion. However, neither general methodology for the identification nor extensive databases of metabolites with multistage mass spectrometric data are available at the moment. We demonstrate in this presentation the feasibility of the strategy for metabolite identification based on analysis of fragmentation trees.

High resolution MSn experiments were performed on LTQ-Orbitrap equipped with TriVersa NanoMate (Advion) nanoelectrospray ion source. A software tool, integrating among others Chemistry Development Kit (CDK) and XCMS libraries, was used for spectral data processing.

Multistage Molecular Formula software tool has been developed for resolving an elemental composition of a compound and fragment ions derived from MSn data. The process of elemental formula assignment and fitting within elemental formula paths removes unwanted artefacts of the spectra.

Reproducibility and robustness of fragmentation tree acquisitions were tested by varying experimental conditions and a concentration of a metabolite and an optimised acquisition protocol was established. It was investigated to which extent the variation of conditions such as fragmentation energy, isolation width etc. did change the fragmentation pattern or topology of hierarchical relations between fragments.

A library of fragmentation trees of metabolite standards is being created. The database will facilitate the challenging task of metabolite identification by comparing the topology of fragmentation trees.

We demonstrate how the developed analytical strategy based on analysis of fragmentation trees can be used to discriminate between metabolite isomers with the same elemental composition and only a slightly different structure, but with a significantly different biological function.

Our results provide firm basis for developing a generic, multistage mass spectrometry based platform for efficient identification of metabolites.

C13: Metabolomics and Informatics

Metabolic profiling using UPLC-MS on human cervical cancer cell lines exposed to Indinavir and Lopinavir

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Cervical cancer is a major gynaecological cancer among women diagnosed in the UK. Each year in the UK over 2,700 women are diagnosed and approximately 1,000 deaths are caused by this disease. (Cancer Research UK. www.cancerresearchuk.org).

It is known that the human papilloma virus (HPV) can cause cervical cancer, where the pathogenesis of high-risk HPV arises from expression of E6 oncoproteins which induce improper activity of the 26S proteasome thus leading to the degradation of the tumour suppressor p53 and other cellular proteins. Recently, it has been reported that anti-viral drug inhibitors, such as Indinavir and Lopinavir (originally targeted for HIV), could also inhibit E6-mediated proteasomal degradation of mutant p53 in E6-transfected C33A cells.

LC-MS is a powerful technique for not only the quantification of metabolites but also for their identification, with LC giving metabolite separation, followed by electrospray ionisation (ESI) to generate ions followed by high resolution Time-of-Flight (TOF) MS for precise mass and intensity measurements.

In order to understand more about these drugs‚Äô mode-of-action(s) we are investigating their effect on the metabolome of cervical cell lines. C33A parent, vector and E6-transfected cells were seeded and allowed to adhere overnight at 37¬∞C, 5% CO2. Indinavir and Lopinavir, were added to the relevant flasks and cells were incubated for 24h.

Cells were quenched in methanol (-48¬∞C) then freeze-thaw extracted and lyophilised. Samples were reconstituted in aqueous methanol just prior to UPLC (Waters, Acquity) - MS (Waters, Synapt HDMS) analysis. By using a combination of multivariate analyses, such as principal components analysis, with Spearman‚Äôs correlation analysis, MS data were investigated for analytical and biological reproducibility and to reveal key metabolite differences between the various sample groups. We shall report that this approach can reveal distinct and common effects that the two drugs have on the metabolome of these three different cell lines.

C14: Metabolomics and Informatics
Chromaligner: a web server for chromatogram alignment

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Peak shifts in chromatograms cause errors if chromatograms are used for further chemometrics analysis. Chromaligner resolves this issue by a constrained chromatogram alignment. For any given chromatograms C1, C2, …, Cn, and their pre-defined peaks S1, S2, …, Sn, the constrained chromatogram alignment is to find the correlation optimized warping (COW) of C1, C2, … Cn with the optional alignment on predefined peaks first. For a reasonable chemometrics analysis with chromatograms, for example, the fingerprint analysis on plant extracts, certain known peaks, as the key chromatogram features should be aligned together before statistical treatments. Current peak alignment methods such as COW and dynamic time warping only focus on comprehensive optimal alignment of full chromatogram instead of aligning the peaks of the same components first. They also failed to treat chromatograms when there are large peak shifts as commonly seen in capillary electrophoresis. Our goal is to provide a constrained chromatogram alignment to resolve above issues. Chromaligner aligns chromatograms based on COW with optional alignment on predefined peaks. The inputs of Chromaligner include a set of chromatograms comprising intensity, retention time and constraints if avialable. The constraints are the common user-defined peaks (or spike peaks) or automatically assigned by Chromaligner by mapping the full spectrum. The outputs files from Chromaligner are aligned chromatograms along with overlaid spectrum before and after alignment. The current Chromaligner service can be accessed by a straightforward web interface. Chromaligner has a time complexity of O(nm3/k2) where n, m and k are the number of chromatograms, the complete time of a chromatogram and the number of predefined peaks in a chromatogram, respectively. It is faster than the original COW by k2 times. With the optional constraints provided by Chromaligner, it can successful provide accurate alignment based on known component peaks to reach best results in chemometrics analysis.

C15: Metabolomics and Informatics
Small Molecule Pathway Database: illustrating human metabolism at a subcellular and organ level

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This project involves a detailed determination of human metabolic pathways at the (1) subcellular level and at the (2) organ/organ compartment level. A collection of pathways illustrating metabolism in human beings, have been drawn and compiled into a database known as Small Molecule Pathways Database (SMPDB). 77 pathways illustrating normal metabolic conditions, such as Glycolysis, and the Urea Cycle were drawn. Another 150 pathways indicating specific diseased states, such as Phenylketonuria, Hyperchoslestrolemia, and Argininosuccinic Aciduria were also created. Finally a series of about 150 drug pathways were drawn to indicate the metabolic action and fate of drugs that are consumed by human beings. The basic framework (metabolites, enzymes, and reactions) for normal metabolic pathways was obtained from the pathway maps in KEGG. From the generic pathways in KEGG, we only extracted pathway information that was relevant to human beings, and to that we added cellular and organ compartment annotation. The disease metabolic pathways were created based on the normal metabolic pathways, but include additional annotation retrieved from textbooks and literature describing which enzymes would be deficient, and which metabolites would be in excess. This comprehensive database is a unique resource that illustrates metabolism in an accurate, exhaustive, and exciting way. It also serves as an efficient means to indicate relationships between metabolites on the HMDB, drugs on DrugBank, and proteins on Expasy. All pathways were drawn in Microsoft Powerpoint, and the resulting images were image mapped with hyperlinks to HMDB, DrugBank and Expasy. It is also fully searchable by metabolite drug and protein names and accession numbers, and it even has a built-in chemical structure search. The pathways are in jpeg format and are available at 3 different zoom levels. SMPDB is available at http://www.smpdb.ca

C16: Metabolomics and Informatics

Rapid Information Extraction from 1H NMR Urine Profiles

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1H NMR analysis is a widely employed metabolic profiling technology due to several favorable characteristics, e.g. rapid data acquisition leads to high-throughput, fully quantitative and nondestructive, minimal sample preparation, excellent within and between laboratory reproducibility. Chemometrics methods applied to frequency-bucketed NMR traces yield variables whose differential regulation discerns between various treatment groups. However, their interpretation leads to many difficult identification and quantification problems due to co-resonant spectral signatures arising from hundreds of endogenous metabolites. Bucketed data variables may split peaks, have redundant information, and include contributions from multiple metabolites with varying differential regulation. These characteristics compromise the validity of the statistical analysis for selecting markers. Applying pure compound spectral libraries to fit components of mixture signals mitigates these problems, but there is a need to relate this method to the standard

bucketing method. Using rat urine data from a set of nephrotoxicity experiments (for D-serine, puromycin, and 2-bromoethanamine) as case studies, we will show that this goal may be realized by presenting a new data reduction method, called Reliable Targeted Binning (RTB) that is based on bucketing data by identifying the subset of frequency intervals that allow an annotation of each variable with single compounds (restricted to the set of 300 compounds from the Chenomx spectral library). We will also show how the development of RTB addresses solutions to the following issues: which endogenous metabolites may consistently be identified and quantified in rat urine, the precise limitations of binning with respect to metabolite identification, how RTB defines input needed from complementary methods (e.g. LC-MS), how to use RTB to improve the quality of statistical analyses, how RTB automates the rapid extraction of information from large data sets, and how to overcome some of the limitations of RTB with follow-up studies using direct peak fitting from a spectral library.

C17: Metabolomics and Informatics

Phenolic database of experimental and predicted 1H-NMR spectra

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NMR spectroscopy is the most selective technique for identification of unknown metabolites. Compared to MS, NMR suffers from relative low sensitivity especially of two-dimensional NMR-experiments needed for unique identification. This identification process can be accelerated when MS (MS/MS) and high quality 1H-NMR data are combined. 1H-NMR chemical shifts and 1H-1H couplings are very sensitive to changes in the 3D structure and the chemical environment. Here we present a database of experimental and predicted NMR spectra of phenolic compounds based on high quality NMR data acquired under very well controlled identical conditions. The predicted proton spectra were generated from 3D chemical structures using the PERCH NMR Software trained on a set of experimental spectra. The structures were first optimized in three-dimensional space, and then the conformational space was mapped using Monte-Carlo/Molecular Dynamic analysis. Solvent effects were also taken into account. Equivalent and nonequivalent protons were automatically differentiated based on the symmetry of the molecule. By incorporating the stereochemistry, intra-molecular interactions, and solvent effects into the mathematical model, chemical shifts and couplings can be predicted with much greater accuracy. The predictive model will be used to extend the database with predicted 1H-NMR spectra of a vast number of phenolic compounds available from public resources. The 1H-NMR spectra and 3D Mol files are automatically annotated confirming the accepted in the literature atom labels. The experimental NMR data were completely analyzed, which makes the prediction very reliable and the database so valuable. A molecule is considered as a match if the mass is within 1-2 ppm deviation from the obtained from MS (and/or MS/MS) accurate mass and the predicted 1H-NMR spectrum correctly matches the 1H-NMR

experimental spectrum. Automated fitting of experimental and theoretical 1H-NMR data is provided by PERCH NMR Software. Text searching, spectrum and structure querying, and data browsing tools are also provided.

C18: Metabolomics and Informatics

Enhancing Metabolomic Data Analysis with Progressive Consensus Alignment of NMR Spectra (PCANS)

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Nuclear magnetic resonance spectroscopy is one of the primary tools in metabolomics analyses, where it is used to track and quantify changes in metabolite concentrations in response to disease, toxicants or drugs. NMR spectra are often confounded by highly overlapping and often variable peak positions which can hinder downstream statistical analysis. Such issues are becoming increasingly significant as greater numbers of large-scale systems and/or longitudinal studies are being performed, in which many spectra from different conditions need to be compared simultaneously. Here we describe a novel approach, termed Progressive Consensus Alignment of NMR Spectra (PCANS), for the alignment of NMR spectra. Through the progressive integration of many pairwise spectrum comparisons, this approach generates a single consensus spectrum as an output. We characterize the performance of PCANS by aligning simulated NMR spectra which have been provided with user-defined amounts of chemical shift variation as well as inter-group differences as would be observed in control-treatment applications. Moreover, we demonstrate how our method provides better performance than either template-based alignment or binning. Finally, we further evaluate this approach in the alignment of real mouse urine spectra and demonstrate its ability to improve downstream PCA and OPLS analyses. By avoiding the use of a template or reference spectrum, PCANS allows for the creation of a consensus spectrum that enhances the signals within the spectra while maintaining sample-specific features. This approach is of greatest benefit when complex samples are being analyzed and where it is expected that there will be spectral features unique and/or strongly different between subgroups within the samples.

C19: Metabolomics and Informatics

A Reference Material to Support Metabolomics Measurements

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Metabolomics can provide a unique perspective on the biochemical status of an organism through systematic analysis of the metabolites in relevant samples such as plasma, urine, cells, or tissues. Interpretation and understanding of the results of metabolomics experiments is aided by identification and, when possible, quantification of the relevant metabolites in the samples. No single technique can provide a full catalog of all the metabolites in a sample, and sample handling and preparation techniques can affect both metabolite identification and quantification. For these reasons, comparison of data sets from different metabolomics experiments can be quite difficult. NIST has collaborated with the National Institutes of Health (NIH) to develop a Standard Reference Material (SRM) for metabolomics, with the goal of providing a stable, well-characterized reference material for metabolomics research. SRM 1950 Metabolites in Human Plasma is a human plasma pool collected from 100 individuals. The SRM was prepared from both male and female donors and has an ethnic makeup that is representative of the U.S. population. The concentrations of more than 50 metabolites, including electrolytes, hormones, glucose, creatinine, vitamins, amino acids, and fatty acids have been determined by various methods. Quantification was based upon isotope-dilution methodology when possible. In addition to the quantitative aspects of this SRM, qualitative information may be valuable for those interested in metabolite profiling and identification. Therefore we have also pursued identification of additional metabolites through a number of different techniques, including LC-MS, GC-MS, GCxGC-TOF-MS, and NMR. Although this qualitative information is anticipated to broaden the utility of the SRM, assigning a level of confidence or uncertainty to a compound identification poses some unique challenges.

C20: Metabolomics and Informatics

T3DB: The Toxin and Toxin Target Database

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Toxicogenomics is a field of study that uses the combined fields of toxicology and genomics in order to decipher the mechanisms of action by which toxic substances exert their effects. In order to facilitate this study, we have created the Toxin and Toxin Target Database (T3DB), a unique bioinformatics resource that combines detailed toxin data with comprehensive toxin target information. The database currently houses over 2 500 toxins, including pollutants, pesticides, drugs, and food toxins, with over 800 corresponding toxin target records. Each toxin record (ToxCard) contains over 30 data fields and holds information such as chemical properties and descriptors, toxicity values, molecular and cellular interactions, and medical information. This information has been extracted from numerous sources, including other databases, government documents, books, and scientific literature. The focus of the T3DB is on providing mechanisms of toxicity and target proteins for each toxin. This dual nature of the T3DB, in which toxin and toxin target records are interactively linked in both directions, makes it unique from existing databases. It is also fully searchable and supports extensive text, sequence, chemical structure, and relational query searches. The T3DB is housed on an Apple XServe in the Wishart Lab on the University of Alberta campus, and consists of a LIMS built using the Ruby On Rails framework along with a Perl-based HTML

public interface. It is both modelled after and closely linked to the Human Metabolome Database (HMDB) and DrugBank. Potential applications of T3DB include toxin metabolism prediction, toxin/drug interaction prediction, and general toxin hazard awareness by the public, making it applicable to various fields. Overall, the variety and accessibility of the T3DB make it a valuable resource for both the casual user and the advanced researcher. The T3DB is available online at: http://www.t3db.org.

C21: Metabolomics and Informatics

Cross-contribution Robust Normalization of Mass Spectrocopy Based Metabolomics Data

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Most mass-spectroscopy (MS) based metabolomics studies are only semi-quantitative and therefore depend on efficient normalization techniques to suppress systematic error arising from e.g. chromatography and derivatization efficiency. A commonly used approach is to include isotope labeled internal standards (ISs) and then express the estimated metabolite abundances as relative to the recovered standard. Due to problems such as insufficient chromatographic resolution, the analytes may however directly influence the estimates of the IS - a phenomenon known as cross-contribution (CC). Confounding between analytes and internal standards is a well-studied problem in other fields of MS coupled chromatography but has not been given much attention in the metabolomics application. Normalization using ISs that suffer from CC-effects will cause significant loss of information if the biological analyte is affected by the experimental design. We examined several GC-MS based metabolomics datasets and found that correlation between analytes and the utilized ISs was quite common and that standard normalization approaches therefore suppressed the variance of interest. Here we present a novel normalization algorithm dubbed Cross-contribution Robust Multiple standard Normalization (CRMN) which solves this problem by correcting the ISs for any CC effects before using them for normalization. We tested our method on two biological datasets and a multi-component dilution mixture and found that it was superior at purifying the relevant signal compared to current normalization methods. We therefore believe that the inclusion of the CRMN method may significantly increase the precision of mass spectroscopy based metabolomics protocols. The CRMN method was implemented as an open source software package for the statistical programming environment R and is distributed via http://www.metabolome.jp/download/r-package.

C22: Metabolomics and Informatics

Improving untargeted differential analysis of mass spectrometric data by recursive feature extraction

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Processing of mass spectrometric data for chemometric analysis typically involves a feature extraction step, followed by an alignment of features across multiple sample data sets. Feature extraction algorithms for LC/MS and GC/MS data often process single data files at a time, owing to the complexity and memory requirements of the raw data. Due to the high cost in processing time for large data sets, most feature extraction algorithms are optimized for speed, at the expense of sensitivity and quality, which can introduce both false positives and false negatives. Alignment of data containing false positives results in a sparse matrix of mass-retention time-abundance values. These deficiencies in feature extraction result in unreliable post-processing statistics and introduce noise into abundance profiling experiments. To improve the quality of feature extracted data and alignment matrix fill-in, we have developed a recursive feature extraction workflow to address the current shortcomings of mass spectrometric feature extraction on large data sets. This approach results in an iterative improvement in the quality and accuracy of mass, retention time and abundance values for each candidate feature, as well as validates the initial feature finding results, reducing false positives and false negatives. Eight high resolution Q-TOF LC/MS data files representing four technical replicates of two sample conditions were individually extracted using a feature finding and ion clustering algorithm. Between 1478 and 2389 features were found for each data file. Due to the presence of many false positives, the aligned feature list contained 4175 features. This candidate feature list was recursively re-extracted to obtain more accurate RT and signal abundance values. Following re-extraction, between 2321 and 2494 features were found per sample file, showing marked improvements in matrix fill-in (false negative reduction) and a two-fold loss of false positive noise.

C23: Metabolomics and Informatics

HMDB (version 2.5): Human Metabolome Database

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Metabolomics has emerged as a new field of ‚Äòomics' research and it has evolved from a little-known branch of analytical chemistry to a mainstream research discipline being practiced by hundreds of laboratories around the world. It can be defined as the complete complement of al metabolites (small molecules M <1500 Da) found in a specific cell, organ or organism. Combined

with genomic, transcriptomic and proteomic studies, metabolomics can help in the understanding and interpretation of complex biological processes.

Due to recent advances in analytical instrumentation such as NMR spectroscopy, Gas Chromatography (GC) and Mass Spectrometry (MS) and compound separation techniques, thousands of metabolites have been identified and quantified. Thus, within the metabolomics community, there is a significant demand for the availability of high quality electronic databases that can be searched for metabolite concentrations and their properties, disease-related associations, characteristic MS or NMR spectra, etc. To address these needs the Human Metabolome Database (HMDB) is being developed in our lab. To date, HMDB is the most complete and comprehensive collection of human metabolites. It focuses on quantitative, analytical information about metabolites at molecular-scale, their associated enzymes or transporters and their disease-related properties. Since its first release in 2007, the HMDB has been used to facilitate the research of more than 120 published studies.

Here, we report recent additions and improvements to the HMDB since its last release. The number of fully annotated metabolites has increased by more than 20%; the number of metabolites with biofluid or tissue concentrations data has grown by more than 10%; the number of compounds in Human Metabolome Library (HML) has increased by more than 15%; the number of ‚Äòhand-drawn‚Äô human metabolic pathways has increased to more than 70; a number of metabolite entries were modified based on feedback from HMDB users.

C24: Metabolomics and Informatics

Automatic processing of CE-TOFMS metabolome data

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Capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), a powerful platform to quantify numerous charged metabolites simultaneously, is becoming widely used in metabolomics research field. Since charged metabolites are the most common intermediates in central metabolic pathways, comprehensive CE-TOFMS profiling has great potential to facilitate the understanding of cellular metabolism at the systems level. However, large run-to-run variability in migration time and the variety of peak shapes observed during CE-TOFMS data hinders data processing and quantification. Here, we introduce software optimized for CE-TOFMS data analysis, named MasterHands. The data processing features include (1) highly sensitive and automatic peak picking and integration, (2) estimation of accurate mass of detected features, (3) alignment of multiple samples using dynamic programming with optimization algorithm, (4) interpretation and visualization of mass spectra, (5) assignment of metabolite ID for peaks derived from known metabolites, (6) exploration of potential biomarkers with statistical analysis such as principal component analysis, and (7) generation of structured reports including complete data matrix for

analysis with third party tools. Although each process works with minimum user intervention, a flexible graphical user interface allows users to interactively fine-tune parameters. MasterHands can considerably facilitate high-throughput CE-TOFMS-based metabolome data processing.

C25: Metabolomics and Informatics

Distribution-based Reconstruction Model for Baseline Correction in Metabolomic NMR Spectrum

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We here presented a new baseline correction method for complex metabolomic NMR spectra

NMR spectra for complex biofluids usually require further baseline correction. Current post-processing NMR baseline correction method, for example, used intensity range to detect the peaks generally resulted in smoothing the low intensity peaks to zero intensity after baseline correction. Penalized smoothing method advantaged in preserving the low signal peaks during the smoothing process without noise identification required, but failed to treat wide peak area with large overlapped peaks. Metabolomic NMR spectrum often complicated with multiple overlapped and low signal peaks. Therefore, a new method to handle the widely variant spectra highly is demanded.

Our method compared the standard deviation of peak intensity instead of intensity range in a 41 points window with the standard deviation of the noise to robust identify the noise region and then further interpolated sectional linearly to eliminate the peak segments for noise. After the peaks elimination, the reconstructed full spectrum is smoothed by penalized smoothing to preserve the undetected small peaks. We also established a statistic model to estimate the noise detection and error rates. Moreover, we successfully resolved the issues on wide peak region where large overlapped peaks existed.

We have tested this method at different metabolomic NMR measurement with different types of biofluid samples. The results showed this method as a fully automated, robust, reliable and more accurate baseline correction method.

C26: Metabolomics and Informatics

MetaboAnalyst-A Web Service For Metabolomic Data Analysis

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The data processing challenges in metabolomics are quite unique and often require specialized (or

expensive) data analysis software and a detailed knowledge of cheminformatics, bioinformatics and statistics. In an effort to simplify metabolomic data analysis while at the same time improving user accessibility, we have developed a freely accessible, easy-to-use web server for metabolomic data analysis called MetaboAnalyst. It accepts most commonly used data formats including NMR/MS peak lists, NMR/MS spectral bins, compound concentration data, as well as raw GC/LC-MS spectra. It also supports a variety of options for metabolomic data processing, data normalization, multivariate statistical analysis, visualization, metabolite identification and pathway mapping. In particular, MetaboAnalyst offers univariate analysis (fold change analysis, t-tests, volcano plot), chemometrics methods (PCA, PLS-DA), significant feature identification (SAM, EBAM), clustering (hierarchical clustering, SOM, K-means), as well as classification (Random Forest, SVM). MetaboAnalyst also employs a large library of reference spectra to facilitate compound identification from most kinds of input spectra. MetaboAnalyst guides users through a step-by-step analysis pipeline using a variety of menus, information hyperlinks and check boxes. Upon completion, the server generates a detailed report describing each method used, embedded with graphical and tabular outputs. MetaboAnalyst is capable of handling most kinds of metabolomic data and was designed to perform most of the common kinds of metabolomic data analyses. MetaboAnalyst is accessible at http://www.metaboanalyst.ca.

C27: Metabolomics and Informatics

Lipid identification by ion trap tandem mass spectrometry using an in-silico generated fragment database

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Shotgun lipidomics is performed using electrospray ionization either under direct infusion condition or with HPLC. In many research projects, triple quadrupole mass spectrometers are used. The use of unbiased lipid screening instruments such as ion trap or QTOF analyzers was limited due to the absence of a universal mass spectral library of lipids.

We present an in-silico tandem mass spectral library of 70,000 lipid mass spectra for glycerolipids generated from LipidMaps structures and confirmed by authentic standards with respect to accurate mass fragment ions and relative intensities. This feature allows scoring theoretical and actual experimental spectra instead of using simple fragment lookups in the LipidMaps DB. The library covers 35,000 lipid species including glycerophosphocholines, glycerophosphoethanolamines, glycerophosphoserines, glycerophosphoglycerols, glycerophosphoinositols, glycerophosphates, sphingomyelins and select triacylglycerols. For positive/negative ionization modes, tandem mass spectra with different adducts were created. Identification steps comprise the extraction of tandem mass spectra, precursor ion matching in specific m/z windows and a subsequent MS/MS matching using a dot-product algorithm to create the final hit-score.

The performance of the library was evaluated using external standard compounds and plasma and tissue samples. By scoring the experimental MS/MS fragment spectra we identified major phospholipid head groups together with the correct acyl and alkyl side chain modifications and their double bond position and degree of unsaturation. In one minute nanoelectrospray infusion experiments and ion trap analysis with data dependent MS/MS scans, up to 80 lipid species can be

assigned. Up to twenty compounds were identified at very high hit sores and 30-50 compounds with medium or low score, due to lower signal to noise ratio or interfering or overlapping fragment ions. The creation and application of the library as well as false positive and false negative identification rates and their implications on high throughput lipid profiling will be discussed.

C28: Metabolomics and Informatics

New Metabolite Identification Tools: Accurate Mass Retention Time and MS/MS Libraries for Reverse Phase LC/MS

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Steve Fischer, Agilent Technologies

The identification of small molecules is often the most challenging aspect of metabolomics analyses, as positive identifications require comprehensive libraries and databases that integrate chromatographic behavior, accurate mass data, MS/MS fragmentation pattern, and potentially independent spectral data (e.g, UV/VIS, IR and/or NMR). We have previously reported the development of an Accurate Mass and Retention Time (AMRT) database for LC/MS for metabolites commonly found in animals, microbes and plants. Identification is achieved using METLIN Personal database software to match observed high accuracy mass spectrometric data and retention time against a database built analyzing standards using a fixed chromatographic method. Chromatography was standardized by using a 50 mm reverse phase SB-Aq column on an Agilent 1200 SL Series Rapid Resolution LC system. The use of standardized chromatography facilitated the detection of compounds by ESI and APCI modes and in both ion polarities. A comprehensive list of ion species formed by each metabolite was compiled and then used to populate a METLIN Personal Metabolite Database.

We are currently expanding the AMRT content to include a database of phytochemicals of hundreds of plant-specific metabolites, including carotenoids, chlorophylls and various other terpenoids, plant growth hormones, flavonoids, anthocyanins, isoflavones, stilbenes, lignans and essential vitamins. In addition, we are accumulating MS/MS fragmentation patterns (using a Q-ToF) of these phytochemicals, thus providing multidimensional data for metabolite identification.
The development of such multidimensional libraries is a significant advance in LC/MS and will help with further metabolomics innovations in diverse application fields, including agricultural, diagnostic and pharmaceutical research.

C29: Metabolomics and Informatics

Automatic Organization of GC/MS and LC/MS Data into Chemical Libraries

Corey DeHaven, Metabolon

One of the fundamental challenges of metabolomics is to reduce a complex set of individual ions (m/z, RT, intensity values) into a smaller set of chemical library entries representing the distinct chemicals found in the samples being analyzed. This goal is made difficult by several factors such

as the unpredictable set of compounds, lack of complete chemical libraries for matching and co-elution issues. Conventional approaches to this problem are based on the detailed analysis of a single LC/MS or GC/MS injection. Ions are then grouped into sets by analysis of chromatographic peak attributes, such as highly similar peak RT and peak shape, and application of chemical knowledge, such as isotopic ratios, fragmentation tendencies or common adduct formation.

This presentation will discuss new developments in the approach to this problem which utilize data from multiple samples rather than one. This method exploits the fact that biological samples are diverse and that chemicals are largely non-covariant with each other when viewed over multiple samples. Thus when ions are partitioned by both RT and covariance they likely represent ions from a single underlying chemical. This approach also benefits from using the mass, RT and intensity data in aggregate over the sample set to reject outliers and noise thus producing higher quality chemical library entries. By applying software that functions in this way, researchers can greatly accelerate and automate organization of ions into chemically related sets and expedite creation of chemical library entries.

C30: Metabolomics and Informatics

Software Advancements for Performing Compound Identification QC on Large Metabolomic Datasets

Corey DeHaven, Metabolon

Traditional collection, sorting and analysis of metabolomic data has generally involved single data files each corresponding to a single biological sample. Data is then individually compared to spectral libraries of known metabolites in order to identify compounds contained in each biological sample. This labor-intensive approach does not lend itself to industrialization of the process; it also presents a problem correlating the analysis of multiple biological samples, including meta-data, with one another to determine trends and population differences.

The ability to analyze and perform QC on large-scale, multi-sample metabolomics data on an industrial scale is an important step in the evolution of metabolomics technology as a whole. This presentation will provide an overview of software advancements which provide options for quickly performing rapid quality-control of metabolomic data. Functions discussed include:

Plotting of library-matched hits and other components within an RI window that may fall below threshold versus the library entry‚Äôs retention index or time which enables the displaying of data from multiple biological samples dramatically reducing the time and potential error from the analysis of very large datasets

Direct links to raw, chromatographic data, the library spectra, and the display of raw response versus time from the entire set

Plotting in run order or sample name order and the ability to show data in terms of retention index or time

Summary statistics being displayed with each identified compound and being individually annotated allowing for individual data points to be zoomed in on, summarized and the RI or RT window can be changed so that a more granular view of the data is capable

C31: Metabolomics and Informatics

Metabolic responsiveness to short term exercise training in lean and diet-induced insulin resistant mice

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Hans Vogel, Bio-NMR center, Department of Biological Sciences, University of Calgary
Aalim Weljie, Department of Biological Sciences, University of Calgary
Jane Shearer, Department of Kinesiology, University of Calgary

Exercise training is a common therapeutic approach known to antagonize the metabolic consequences of obesity. Aims of the present study were to examine i) whether short-term, moderate intensity exercise training alters basal metabolite profiles and ii) if exercise training can correct obesity induced shifts in metabolic spectra. Following weaning, male C57BL/6J littermates were randomly divided into two dietary groups; low (LF) or high fat (HF). HF animals were obese and hyperglycaemic compared to LF. After 12wk of dietary manipulation, mice from each treatment were further divided into sedentary or exercise treatments. Exercise consisted of wheel running exercise (2h/d, 10d, 5.64m/min). Following exercise training, rested (30h) animals were fasted (6h) before serum was collected. Samples were analyzed by high resolution-1D proton NMR. Fifty high- and medium-concentration metabolites were identified. Pattern recognition algorithms were used to identify and isolate significant metabolites changing in response to HF and exercise training. Hierarchal metabolomic clustering of 1H spectral data provided distinct classification of animals in both treatments. Multivariate modelling demonstrated energy metabolites including glucose, glutamine, pyruvate and threonine to be lower in exercised animals. Conversely, the concentration of signalling metabolites associated with the onset of insulin resistance (branched-chain and other large non-charged amino acids such as leucine, isoleucine, valine and phenylalanine) are mostly unaffected by exercise but drop substantially in animals on a high fat diet. Results demonstrate metabolomics to effectively characterize obesity induced perturbations in metabolism and support the concept that short-term exercise training is beneficial therapeutic approach for obesity.

C32: Metabolomics and Informatics

Methodologies for putative and definitive metabolite identification in mass spectrometry-related metabolomic studies of yeast and humans

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Marie Brown, Department of Chemistry, University of Manchester
Paul Dobson, Department of Chemistry, University of Manchester
Yogendra Patel, Department of Chemistry, University of Manchester
Catherine Winder, Department of Chemistry, University of Manchester
Paul Begley, Department of Chemistry, University of Manchester
David Broadhurst, Department of Chemistry, University of Manchester
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Douglas Kell, Department of Chemistry, University of Manchester

Many hundreds or thousands of features are detected in holistic metabolomic studies of complex biological systems, including yeast and humans. This is particularly relevant when employing gas or

liquid chromatography coupled to mass spectrometric detection. A feature is defined as a retention time/index-mass spectrum pair (GC-MS) or a retention time-accurate mass pair (LC-MS). A common and substantial problem to overcome in these studies is the determination of relationships between features detected chemical identification of these metabolites. Putative or definitive chemical identification can be performed and specific guidelines have been reported by The Metabolomics Standards Initiative (MSI) [1]. The chemical identification of mass spectrometric signals is important to provide conversion of analytical data to biological knowledge.

A range of methods are applied and many are currently being further developed and validated for metabolomic applications. GC-MS typically apply searching of mass spectrometric libraries (in-house, commercially available, publicly available) and matching of mass spectra to that acquired for an authentic standard (putative identification) or matching of retention time/index and mass spectrum to that acquired for an authentic standard. LC-MS typically apply accurate mass data to putatively identify appropriate molecular formulae from which metabolites can be putatively assigned to.

The presentation will describe advances in metabolite identification for both GC-MS and LC-MS [2]. The complexity of electrospray mass spectrometric data will be specifically discussed along with methods combining correlation analysis and the complexity of electrospray data to assist in putative identification. The construction of mass spectral libraries and metabolite databases will also be discussed, including The Manchester Metabolomics Database.

[1] Sumner, L.W. et al. Proposed minimum reporting standards for chemical analysis. Metabolomics 2007 3 211-221
[2] Brown M, Dunn WB, Dobson P, et al. Mass spectrometry tools and metabolite-specific databases for molecular identification in metabolomics. The Analyst 2009 134 1322-1332.

C33: Metabolomics and Informatics

MetPath: Metabolomics pathway visualization

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Ching-Hua Kuo, School of Pharmacy, College of Medicine, National Taiwan University

MetPath is a visualizing system which can fast display metabolic pathways among identified metabolite markers in the global metabolism network as well as the local bionetworks between identified markers. The final steps of every Metabolomics studies involved reconstruction of identified metabolites. Currently, most manuscripts and researchers organized and produce metabolic pathways manually.

MetPath auto generates comprehensive maps of local related paths and global metabolic pathways with metabolite. MetPath takes common names and also KEGG IDs of metabolites and the ratio of fold change as the input. Local metabolic network can be constructed with shortest paths of two metabolites collected through metabolic network. Metabolites with shortest paths are then arranged by a grid layout algorithm and displayed as the local map. On the local map, large nodes represent higher fold changes. The distances between metabolites are proportional to the relative distance in the full metabolic network. Global metabolic map of involved pathways of the metabolites is based on KEGG global map. All involved pathways are colored in the pre-defined blocks on global map to allow fast visualization of all the sub-metabolisms involved.

With MetPath, auto global and local metabolic pathway maps are generated without manual editing and organization. The metabolic maps generated help to understand the relationship among metabolites and the involved pathways. MetPath is a convenient tool providing comprehensive maps for instant and clear visualization.

C34: Metabolomics and Informatics

Quantitative liquid-chromotography mass spectrometry in metabolomics: assessing ionization behaviour

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Liquid chromatography coupled to mass spectrometry is one of the primary platforms used in metabolomics,research which characterizes small-molecules in biological samples. Quantitative analysis of metabolomics MS data is critical for statistical processing, such as multivariate statistical analysis and integration with cross-platform ‚Äòomics‚Äô data. As absolute quantification of all metabolites, of which many are unknown, is not possible, we detail a mathematical method to determine the relative quantitative behavior of mass spectral features using orthogonal partial least squares analysis. The response of every retention time / mass pair is correlated to relative analyte concentrations. Relevant ions are further characterized to determine the linearity of detected response, with the aim of choosing a suitable injection volume that optimizes the number of features detected with linear ionization properties. The method is demonstrated by UPLC-TOF MS on lipid samples derived from liver extracts as part of a rodent model of fatty liver. We propose that experimental times may be held relatively constant for existing work flows by using different injection volumes as technical replicates, and application of the method effectively increases the dynamic range of reliable ions for interpretation.

C35: Metabolomics and Informatics

MetPath: Metabolomics pathway visualization

Y. Jane Tseng, National Taiwan University,
Tien-Chueh Kuo, Graduate Institute of Biomedical Electronic and Bioinformatics, National Taiwan University,
Ching-Hua Kuo, School of Pharmacy, College of Medicine, National Taiwan University

MetPath is a visualizing system which can fast display metabolic pathways among identified metabolite markers in the global metabolism network as well as the local bionetworks between identified markers. The final steps of every Metabolomics studies involved reconstruction of identified metabolites. Currently, most manuscripts and researchers organized and produce metabolic pathways manually.

MetPath auto generates comprehensive maps of local related paths and global metabolic pathways with metabolite. MetPath takes common names and also KEGG IDs of metabolites and the ratio of fold change as the input. Local metabolic network can be constructed with shortest paths of

two metabolites collected through metabolic network. Metabolites with shortest paths are then arranged by a grid layout algorithm and displayed as the local map. On the local map, large nodes represent higher fold changes. The distances between metabolites are proportional to the relative distance in the full metabolic network. Global metabolic map of involved pathways of the metabolites is based on KEGG global map. All involved pathways are colored in the pre-defined blocks on global map to allow fast visualization of all the sub-metabolisms involved.

With MetPath, auto global and local metabolic pathway maps are generated without manual editing and organization. The metabolic maps generated help to understand the relationship among metabolites and the involved pathways. MetPath is a convenient tool providing comprehensive maps for instant and clear visualization.

C36: Metabolomics and Informatics

GC/APCI-TOF MS: GC/APCI with ultra high resolution TOF-MS - analytical validation and applicability to metabolic profiling

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Introduction: Most of the commercial GC/MS systems use ionization under vacuum conditions: electron ionization (EI) and chemical ionization (CI). Atmospheric pressure ionization sources (API), which are probably the key of the ‚Äúovernight success‚Äù of MS-detectors in analytical sciences due to coupling with liquid chromatography, are rarely used with GC instruments. A recently introduced multipurpose AP source created the opportunity to reconsider the importance of AP ionization for GC. Here, we present an analytical evaluation of GC/APCI-MS showing the benefits of soft atmospheric pressure chemical ionization for GC in combination with an orthogonal-accelerated ultra high-resolution TOF mass spectrometer (UHR-TOF-MS MaXis).

Result: A very sensitive and accurate GC/APCI-TOF-MS method was developed for the automated analysis of metabolites in biological samples. At present, the analytical evaluation of the method was made using amino acids, organic acids, alcohols, xanthines, indoles, dipeptides, compounds with imidazole groups, amines and analytes with hydroxyl and amine groups, demonstrating ability of the analytical procedure to deal with nine different chemical families within one experiment (chromatogram). Excellent repeatability was obtained, with relative standard deviations (RSDs) of peak areas between 0.7% and 2.1% in the intra-day study, and between 3.8% and 6.4% in the inter-day study. Analysis of CSF has demonstrated a rich chromatographic pattern consisting of hundreds of features. We have detected more than 300 distinct features in CSF, even when using very strict peak detection criteria. This fact in combination with the presented analytical characteristics (LODs, repeatability and reproducibility) demonstrates the potential of GC/APCI-TOF MS for metabolic profiling. The high quality of spectra creates an opportunity to make structural assignments of metabolites based on accurate mass position and isotopic distribution. However, accurate MS/MS spectra can be efficiently in order to resolve more difficult cases and

support identification by fragmentation data.

C37: Metabolomics and Informatics
MZmine 2: Open-source toolbox for processing of metabolomics data

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MZmine 2 (http://mzmine.sourceforge.net/) is an open-source and multiplatform project delivering a software for metabolomics data processing, with the main focus on LC-MS data. MZmine 2 is constructed by modules which made it very flexible and extendable framework, useful for the entire data analysis workflow. The most important modules are:

Data import: MZmine 2 can read and process both unit mass resolution and exact mass resolution (e.g. FTMS) data in both continuous and centroided modes, including fragmentation (MSn) scans. Supported data formats are mzML (version 1.0), mzXML (versions 2.0, 2.1 and 3.0), mzData (versions 1.04 and 1.05), NetCDF (no MSn support) and Thermo RAW (only on Windows with Thermo Xcalibur installed).

Visualization: MZmine 2 can visualize your raw data together with peak picking and identification results, which is very useful for evaluating different peak detection methods.

Peak detection: Peak detection in MZmine 2 is performed in a three-step manner: first, mass values are detected within each spectrum (several methods are available, depending on the nature of the data). In the second step, a chromatogram is constructed for each of the mass values which span over certain time range. Finally, deconvolution algorithms are applied to each chromatogram to recognize the actual chromatographic peaks.

Peak list processing: There are several modules for further processing of peak detection results, including deisotoping, filtering and alignment. Identification of peaks can be performed by searching a custom database or by connecting to PubChem Compound database.

Statistical analysis: MZmine 2 contains basic methods for statistical analysis of processed data.

C38: Metabolomics and Informatics
Reactome, a curated knowledgebase of human biological pathways

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Reactome (http://www.reactome.org) is a manually curated database of core pathways and reactions in human biology that functions as a data mining resource and electronic textbook. Information is authored by expert biological researchers, maintained by the Reactome editorial staff and cross-referenced to publicly available web-based informatics resources. Its robust data model describes life processes ranging from metabolism to signal transduction and the cell cycle. As of Release 29, Reactome contains 4181 human proteins, 3335 reactions and 990 pathways. An entity-level pathway viewer and data mining tools facilitate searching and visualizing pathway data and the analysis of user-supplied high-throughput data sets. Reactome has increased its utility to the model organism communities with improved orthology prediction methods allowing pathway inference for 22 species and through collaborations to create manually curated Reactome pathways for species including Arabidopsis, Oryza sativa (rice), Drosophila and Gallus gallus (chicken). All data content and software can all be freely used and redistributed under open source terms.

D01: Metabolomics and Nutrition

A systems biology approach to examine gut microbial effects on host metabolism in mice

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The gut microbiota has coevolved with the mammalian host and acquired functions that complement the host's metabolism. To understand how the gut microbiota affects host physiology, we investigated how the gut microbiota regulates the metabolome and transcriptome in germ-free (GF) and conventionally raised (CONV-R) mice. To this end, we have applied global metabolomics and lipidomics profiling using mass spectrometric techniques, GCxGC-ToF-MS and UPLC-MS coupled to QToF respectively. Metabolomic analysis revealed that the gut microbiota affects several important metabolic processes including energy metabolism, amino acid, and lipid metabolism. The serum metabolome was associated with increased hepatic transcription of genes involved in proteolysis, energy, and xenometabolism. Surprisingly, we detected increased levels of neurotransmitters in serum of CONV-R animals which suggests that the gut microbiota may affect animal behavior. Lipidomic studies of serum disclosed that the presences of a gut microbiota increase serum phosphatidylcholine and cholesterol ester levels, while decreasing triglyceride levels. In contrast, livers from CONV-R mice contained increased triglyceride levels in fasted and fed state and produced VLDL particles with increased lipid content, which correlated with increased activity of the main lipidating enzyme in the liver, MTP. These results suggest that variations in an individual's gut microbiota may have profound effects on host metabolism and physiology and will be an important factor when considering personalized medicine.

D02: Metabolomics and Nutrition

Differential Metabolomics for Quantitative Assessment of Cellular Oxidative Stress and Antioxidant Efficacy with Strenuous Aerobic Exercise

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Richard Lee, Department of Chemistry, McMaster University
Daniel West, Department of Kinesiology, McMaster University
Stuart Phillips, Department of Kinesiology, McMaster University

A fundamental understanding of the mechanism(s) of oxidative stress remains elusive due to inconsistent results from large-scale clinical trials using antioxidants, as well as the seemingly contradictory and synergistic functions of reactive oxygen/nitrogen species in-vivo. Herein, we describe a time-resolved differential metabolomics strategy for quantitative assessment of oxidative stress and antioxidant efficacy with strenuous aerobic exercise using capillary electrophoresis-electrospray ionization-mass spectrometry (CE-ESI-MS). A healthy untrained volunteer was recruited to perform a high intensity ergometer cycling routine until exhaustion with frequent blood collection over a 6 hour time interval, which included pre-, during and post-exercise periods while at rest. A follow-up study was subsequently performed by the subject after a high dose oral supplementation of the antioxidant N-acetyl-L-cysteine (NAC) prior to performing the same exercise protocol under standardized conditions. Differential metabolomic analyses of filtered red blood cell lysates by CE-ESI-MS in conjunction with multivariate analysis revealed significant attenuation of

oxidative after NAC supplementation as reflected by a lower change in half-cell reduction potential for glutathione, a shorter recovery time for attaining homeostasis, as well as unchanged levels of specific biomarkers reflective of residual fatigue relative to control. This work demonstrates the proof-of-concept that timely nutritional intervention can effectively attenuate acute periods of oxidative stress by global perturbations in metabolism that has relevant implications ranging from enhanced athletic performance to improved post-surgical recovery.

D03: Metabolomics and Nutrition

LC-TOF-MS-based metabolomic approach for studying the cellular responses to dehydroepiandrosterone induced-bioenergetic dysfunction

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Daniel Tsun-yee Chiu, Chang Gung University

Dehydroepiandrosterone (DHEA) and its sulfate ester form (DHEAS) are marketed as dietary supplements in United States. DHEA is a multi-functional steroid that has been implicated in a broad range of biological effects. One of the effects of DHEA is known to have anti-proliferative effect on a number of cell lines in vivo and in vitro, but the mechanism is not completely understood. We investigated the mechanism underlying DHEA-induced growth arrest and depletion of intracellular ATP in hepatoma cells. It is likely that DHEA suppresses cell growth by altering mitochondrial functions. Nonetheless, little is known about how DHEA affects metabolism. Here, a metabolomic approach, using liquid chromatography coupled with electrospray time-of-flight mass spectrometry, was used to profile the hydrophilic metabolome of SK-Hep1 cells exposed to DHEA. Multivariate data analysis of the positive ions revealed the anomalous alteration of the levels of intermediates of urea cycle, carnitine or derivatives, and phospholipids in DHEA-treated cells. Depletion of carnitine and related derivatives may be associated with mitochondrial dysfunction in DHEA-treated SK-Hep1 cells.

D04: Metabolomics and Nutrition

Metabolite Profiling Based Classification and Novel Compound Identification of Cordyceps militaris Grown on Germinated Soybean by LC-ESI-IT-MS/MS and Multivariate Statistical Analysis

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Cordyceps militaris grown on germinated soybeans (CMGGS) might be a promising efficacious source of novel bioactive compounds. LC-ESI-IT-MS/MS based metabolomic approach was used to investigate the variation of secondary metabolites during CMGGS fermentation. It revealed clear time dependent metabolite differences during early stage of fermentation in the principal component plots by combining PC1 and PC2, which cumulatively accounted for 89.5% of the variance. PCA scores plots showed that major fermentation process and metabolite changes were completed within

3 weeks after the inoculation with Cordyceps militaris. The corresponding metabolites of those differences were identified as soyasaponins, daidzein, genistein, glycitein and their glycosides by comparing MS/MS spectral data with in-house MS/MS library. Several unknown peaks were also detected after one week fermentation of CMGGS and identified as novel isoflavone methyl-glucosides by 1D and 2D NMR spectroscopy. During the cultivation, the antioxidant activity and total flavonoid contents were showed a similar change with movement of PCA scores plot. Also, discriminatory compounds of the antioxidant activity were detected as soyasaponins (Soyasaponin Bd, Soyasaponin Bb and Soyasaponin Bc) and isoflavone aglycones (daidzein and genistein) by partial least-squares (PLS) biplots. The information of this study is valuable for the optimizing cultivation process for bioactive compound production of CMGGS.

D05: Metabolomics and Nutrition

Metabolism, N-glycan biosynthesis and adaptation at the cell surface

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Contextual regulation of signaling often involves shuttling of proteins between nuclei, nucleoli, Golgi, centrioles, and endoplasmic reticulum. In cell membranes, more transient structures including focal adhesions, cell junctions and lipid microdomains induce ‚Äúmolecular crowding" of receptors, and thereby threshold to ligand-induced activation. Most transmembrane receptors and solute transporters are glycoproteins, and the Asn (N)- and Ser/Thr (O) - linked oligosaccharides (glycans), bind animal lectins forming a multivalent lattice that regulate their mobility in the plane of membrane. The N-glycan number (sequence-encoded NXS/T) and Golgi N-glycan branching (context-dependent on tissue-type and metabolism) cooperate to regulate affinities for the galectin family of lectins. Galectins bind glycoproteins forming lattice microdomain that reduce glycoprotein trafficking into coated-pits and caveolae lipid rafts, decrease ligand-independent receptor activation, and promote Œ±5Œ≤1 integrin remodeling in focal adhesions. The extent of N-glycan branching in the medial Golgi is sensitive to UDP-GlcNAc, supplied by the hexosamine pathway, a nutrient sensor implicated in metabolic syndrome. Metabolite availability (fructose, glutamine and acetylCoA) and flux though the hexosamine pathway promotes N-glycan branching on glycoprotein receptors and solute transporters, increasing their affinities for galectins at the cell surface. Thus lattice avidity regulates glycoprotein trafficking and thereby cellular responsiveness to cues in an adaptive manner.

D06: Metabolomics and Nutrition

Diagnostic markers of cancer-associated skeletal muscle wasting using 1H-NMR profiling of urinary metabolites and machine learning approaches

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Jeff Xia, University of Alberta
Cynthia Stretch, University of Alberta
Thomas Eastman, University of Alberta
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Vickie Baracos, University of Alberta
David Wishart, University of Alberta
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Cachexia is a complex metabolic syndrome characterized by weight loss and skeletal muscle depletion, which bodes a poor prognosis in cancer patients. While image-based methods (dual energy x-ray, computed tomography (CT)) have the specificity and precision to detect early or slowly evolving wasting, or muscle wasting without fat loss, they are costly, inconvenient and expensive. NMR-based metabolomics and statistical approaches based machine-learning potentially offer new opportunities to simplify the detection and monitoring of muscle catabolism. We obtained high-resolution 1H-NMR spectra from the urine of 96 advanced cancer patients, from which we identified and quantified 71 common urinary metabolites. We evaluated lumbar skeletal muscle area (cm2) from clinical CT images, from which we estimated the patient‚Äôs muscle change (loss, maintenance, or gain) over a period of 100 days. We applied statistical and machine-learning techniques to this labeled data to identify urinary metabolite patterns discriminating the condition of muscle loss versus muscle gain, outside a minimal margin of +/-0.75% / 100days. Several metabolites correlated with muscle loss and this may provide some biological clues to the nature of the wasting process. We produced a classifier based Pathway-Informed Analysis of Markov Random Fields, which robustly achieved a prediction accuracy of 79.2% for muscle loss. This method outperformed other machine learning approaches (Naive Bayes, Support Vector Machines) and Partial Least Squares Discriminant Analysis. We thus generated a simple, single-time point diagnostic urine test for cancer-associated loss of skeletal muscle. Our methods may be used to develop similar classifiers that can predict other metabolic conditions.

D07: Metabolomics and Nutrition

Metabolites of the methionine cycle in autoimmune neurological disease

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The methionine cycle is a major contributor to oxidative injury and DNA damage, yet its role in autoimmune neurodegenerative diseases is largely unknown. Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), in which a relapsing phase is followed by neurodegenerative phase, which leads to permanent disability. Since the cause of MS is unknown, our lab studies human T-lymphotrophic virus type 1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neurologic disease that mimics the neurodegenerative phase of MS. HAM/TSP patients are infected with HTLV-1 and can be indistinguishable form progressive forms of MS. HTLV-1 may infect up to 10 million people worldwide, however only 1-5% of infected individuals develop HAM/TSP or acute T-cell leukemia, the remainder being asymptomatic carriers of the virus. Homocysteine (Hcy) is a sulfur-containing metabolite of the methionine cycle that can be elevated in the plasma as a result of genetic or nutrient-related disturbances. Total homocysteine (tHcy) levels are inconsistent in MS patients, and have not been reported in HAM/TSP patients. We hypothesized that tHcy levels would be different in HAM/TSP patients compared to controls. Therefore, we investigated tHcy levels in HAM/TSP patients from Peru. Three groups were

evaluated in this study. Group 1: HAM/TSP (n=19), 10 out of 19 patients had rapidly progressive HAM/TSP; group 2: asymptomatic carriers of HTLV-1 (n=11); and group 3: normal age-matched controls (n=10). The mean plasma tHcy levels were higher in HAM/TSP group (9.49¬±1.53¬µM/L) and in asymptomatic group (12¬±1.4¬µMol/L) in comparison to controls (6.56¬±0.98¬µMol/L). Thus, tHcy levels were significantly higher (P=0.0277, Kruskal-Wallis test) in HTLV-1 infected individuals compared to normal controls. These data suggest that high tHcy levels may be a trigger or marker for a neurotoxic effect and cause damage of neuronal cells by activation of oxidative injury and DNA damage pathways in autoimmune neurological disease.

D08: Metabolomics and Nutrition

Rapid Quantitative Metabolome Profiling by Differential 13C-/12C-Isotope Dansylation Labeling and Fast LC FT-ICR MS

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Large scale quantitative metabolome analysis remains to be a major analytical challenge. In this work, we report a rapid metabolome profiling technique based on differential 12C-/13C-isotope dansylation labeling combined with LC/MS for detection and quantification. It is shown that dansylation derivatization significantly enhanced the LC/MS signals and RP chromatographic properties.

A Bruker apex-Qe 9.4-T FT-ICR-MS was employed. A Waters BEH C18 column (2.1x50 mm, 1.7 Œºm) was used for fast RP separation. For labeling the metabolites, an equal volume of carbonate buffer (0.5M, pH9.4) was added into the sample. The solution were added with either 12C- or 13C-dansyl chloride and then incubated at 60oC. The 12C-/13C-labeled urines or standards were then combined and injected onto LC/MS. The ion pair was identified based on accurate mass difference of 2.006709 Da and quantified based on their peak ratio.

The heavy isotope reagent 13C-dansyl chloride along with commercially purchased 12C-dansyl chloride provides a simple and robust means of labeling metabolites containing primary amine, secondary amine or phenolic hydroxyl group(s) in aqueous reaction condition. It is shown that dansylation labeling offers one to three orders of magnitude ESI signal enhancement over the underivatized counterparts. Dansylation improves the chromatographic behaviors of polar and ionic metabolites on a reversed phase column. There is no isotopic effect on RPLC separation of 12C-/13C-labeled isoforms. 12C-/13C-labeled metabolites are co-eluted and detected by MS, thus leading to precise and accurate quantification and confident identification. A dansylation standard compound library consisting of 121 known amines and phenols has been created. As an example, the absolute concentrations of 93 metabolites can be determined from human urine labeled with 12C-dansylation and spiked with the 121 13C-dansylated standards. We are currently in the processing of applying this method for metabolome profiling of human CSF and blood and these results will be reported.

D09: Metabolomics and Nutrition

Non-targeted LC-MS profiling of rye bran metabolome processed by human microbiota in vitro

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The human distal gut hosts a diversity of microbial communities living in a mutualistic relationship with human body. It is widely known that the metabolism of the microbiota modifies the food-borne compounds ingested in our diet, and alters their bioactivity. These components are believed to add to the health related impact of plant derived foods like whole grain products. The impact of microbial conversion of the phytochemicals is increasingly pointed out in research related to diet and health, but very little is known so far about their metabolic effect on molecular level, thus it is of major interest to elucidate the metabolic process occurring in the colon between the microbes and the food components. In this study, the phytochemicals present in in vitro microbiota - treated rye bran are studied by non-targeted UPLC-qTOF-MS profiling in order to monitor the change in the semi-polar metabolite fraction as time course in the fermentation process. The results show, that the microbiota treatment has remarkable impact on the metabolite content of the rye bran, as shown by clear PCA separation of the metabolite markers from samples collected at different time points along the incubation. In addition to the microbiota processed rye we have applied the non-targeted profiling strategy also for the screening of metabolite content of different whole grain varieties. The preliminary results point out the differences in the various grain species (rye, wheat, barley and oat), as well as illustrate the impact of malting on the barley metabolites.
This work is part of the Nordic Centre of Excellence program in the area of nutrition, food and health, within the project HELGA- Whole Grains and Health.

D10: Metabolomics and Nutrition

Cancer Specific Metabolism revealed by CE-TOFMS

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Many types of cancer are exposed to severe and chronic hypoxia in contrast to their surrounding normal tissues. However, most cancer cells are known to obtain their energy primarily by glycolysis rather than oxidative phosphorylation even in the presence of adequate oxygen supply (Warburg

effect). This implies that tumor hypoxia is caused not by the excessive oxygen consumption by cancer cells, but rather the inadequate blood supply that results from structurally and functionally defective angiogenesis. Nutritional conditions are also unfavorable under the limited blood supply. From this perspective, tumor microenvironment in vivo might be considerably different from typical in vitro culture conditions. Here, we applied capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) to simultaneously quantify metabolites in tumor and grossly normal tissues obtained from 16 colon and 12 stomach cancer patients. Quantification of 94 and 95 metabolites in colon and in stomach, respectively, involved in glycolysis, the pentose phosphate pathway, the TCA and urea cycles, and amino acid and nucleotide metabolisms resulted in the identification of several cancer-specific metabolic traits. The glucose concentration in tumor tissues was only 1/10 (in colon) or 1/3 (in stomach) of that in the surrounding normal tissues. Moreover, tumor lactate and glycolytic intermediate concentrations were higher than their respective normal counterparts in both tissues, which indicated enhanced glycolysis and thus confirmed the Warburg effect. In addition, significantly high levels of most amino acids and hydroxyproline in the tumors implied autophagic degradation of proteins. The results uncovered unexpectedly poor nutritional conditions in the actual tumor microenvironment and showed that CE-TOFMS-based metabolomics could be a powerful tool for the development of novel anticancer agents that target cancer-specific metabolism.

D11: Metabolomics and Nutrition

Metabolome Analysis of Pancreatic Cancer and Normal Cells under H2O2-mediated Oxidative Stress

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Most cancer cells produce energy mainly by energy-inefficient glycolysis rather than mitochondrial respiration for yet unclear reasons. An intriguing hypothesis suggests that cancer cells,Äô active use of glycolysis is their preventive measure against reactive oxygen species (ROS) because they appear to be more sensitive to oxidative stress and exhibit lower antioxidant capacity than normal cells. Indeed, recently-developed potential anticancer drugs such as arsenic trioxide and 2-methoxyestradiol exert their selective toxicity against cancer cells by inducing oxidative stress. However, few studies examined the difference between antioxidative strategies used by cancer and normal cells from a viewpoint of metabolomics. Here, by using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), we investigated time-course changes of more than 120 metabolites in pancreatic cancer and normal cells in response to oxidative stress induced by administrating three different concentrations of hydrogen peroxide. In addition to reasonable alterations in redox-related metabolites, unexpected changes in several amino acids and TCA intermediates were observed in a cancer-cell-specific manner. Elucidations of these metabolic features in cancer and normal cells will be crucial for the development of clinically more effective and sustainable anticancer drugs targeting a susceptibility of cancer cells against oxidative stress.

D12: Metabolomics and Nutrition

In vitro Bioconversion of Polyphenols in Tea and Wine by Intestinal Microbiota

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Epidemiologic data as well as experimental studies suggest beneficial health effects of natural polyphenols that are abundantly present in foods such as tea and wine. However, the complex underlying mechanisms have not been identified completely yet. After ingestion, some phenolic compounds can be absorbed by the gut epithelium, whereas others pass to the large intestine and are metabolized by the colonic microbiota, followed by absorption of bacterial degradation products. Therefore, potential positive effects of polyphenol-rich foods might be dependent on the bioconversion by intestinal bacteria and would therefore vary between individuals due to differences in the inherent microbiota composition.
These complex interactions are addressed in the EU-Transfer of Knowledge project GUTSYSTEM, with the present study particularly focussing on in vitro fermentation models to simulate intestinal microbial bioconversion of polyphenols. In particular, in vitro batch fermentation models were carried out to assess the inter-individual variability of microbial polyphenol metabolism. The bioconversion of tea and wine polyphenols was monitored using NMR-based metabolite profiling and GC-MS-based profiling of phenolics. In contrast to targeted techniques focusing on isolated substances, the profiling methods allowed to detect a wide range of potentially active breakdown products from complex polyphenol extracts. A substantial inter-individual variation was observed depending on both the activity of the microbiota and the precursor polyphenol. It was characterized by different levels of intermediate and end products as well as by variable time responses. The results demonstrate the usefulness of combined analytical profiling techniques to shed light on the colonic metabolism of dietary polyphenols.

D13: Metabolomics and Nutrition

Metabolomics study of traditional Chinese medicine

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The treatment of common multi-factorial, system-wide diseases using traditional Chinese medicine (TCM) can be regarded as a ‚Äúsystem to system‚Äù approach. It is difficult to evaluate the systemic pharmacological effect of the multi-component TCM agents in the context of single-target based pharmacological models. Metabonomics/metabolomics, defined as the measurement of multiparametric metabolic responses of a biological compartment or a living system to pathophysiological stimuli or genetic modification, is a newly thriving systems biology which has been highly favored in botanical science, environmental research, and toxicological study in preclinical and clinical fields.

The Chinese ginseng extracts typically contain more than 50 known compounds, mainly, ginsenosides. We demonstrated, in an animal model, that ginseng extracts were able to attenuate alterations in several metabolic pathways in response to acute cold stress and chronic unpredictable mild stress, using a combined chemical profiling and metabolic profiling approach. We also conducted metabolomics study on a traditional Chinese tea ,Äì Pu-erh, regarding its chemical constituents prepared in different locations and different storage times, and human metabolic profiles of tea drinkers, using a high-performance liquid chromatography coupled with quardrupole-time of flight mass spectrometry. The results indicate that comprehensive molecular descriptions of a pathophysiological state and the response to TCM and dietary intervention can be achieved, so that the global biochemical changes contributing to a drug response can be taken into account, leading to much improved understanding of the efficacy, toxicity and mechanisms of action.

D14: Metabolomics and Nutrition

Identification of dietary fiber-related markers in human urine

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Objectives: The health effects of dietary fiber in healthy subjects might be subtle and looking at several early biomarkers simultaneously, rather than measuring just a few endpoints, might better explain these effects. The aims of the study are to find unique biomarkers of dietary fiber exposures and to search for early biochemical changes in urine metabolites related to this exposure. Additional aim will be to search for possible gender differences in metabolic response.

Method: In a randomized cross-over five week intervention, 25 subjects were given high fiber diet (HF) and low fiber diet (LF). At the end of each intervention period, 24-h urine samples were collected and stored at -80¬∞C. Samples were separated by an UPLC system equipped with a 1.7¬µm C18 BEH column using a 6 min gradient from 0.1% aqueous formic acid to 0.1% formic acid in 20% acetone: 80% acetonitrile followed by high mass resolution and accuracy QTOF-MS detection in positive and negative electrospray ionization modes. Raw QTOF-MS data were aligned in MarkerLynx (Waters, Milford, MA, USA) and exported to Excel to identify HF markers that differed significantly from LF by using statistical tests and restrictions in mean and CV%. Multivariate data analysis will be performed in MATLAB (MatWorks).

Results: In positive mode, the peak response of 30 metabolites was higher after HF compared to LF ($p<0.0001$). Additionally, peak response of 13 metabolites in women and 11 metabolites in men were increased after HF-diet ($p<0.001$). In negative mode, peak response of 42 metabolites were increased after HF ($p<0.0001$). Furthermore, the peak response of 13 metabolites in women and 5 metabolites in men were increased after HF-diet ($p<0.001$). Ongoing analysis will show any fiber marker overlap between the modes. Further analysis will include determination of down-regulated fiber markers, and marker identification by database search and fragmentation pattern analysis.

D15: Metabolomics and Nutrition

Metabolic Profiles of Urine from Patients with Cystic Fibrosis and Controls Employing Liquid Chromatography/Mass Spectrometry: a Pilot Study

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As part of an ongoing study of methyl and lipid metabolism in children with cystic fibrosis (CF) we conducted pilot experiments employing liquid chromatography combined with electrospray mass spectrometry to establish metabolite profiles for 40 urine samples from children with CF and 9 children without CF as controls. All of the children with CF were outpatients at the CF clinic of the British Columbia Children,Äôs Hospital and were seen during a routine outpatient clinic appointment. Subsequent comparison of the obtained spectra with multivariate statistical software, including principal component analysis, revealed significant differences in respect to specific phthalate metabolites which were associated with plasticizers contained in enteric-coated pancreatic enzyme supplements. Up to 90% of individuals with CF are pancreatic enzyme insufficient, and for these individuals, pancreatic enzyme replacement therapy is a critical part of clinical nutrition care. Furthermore, among other metabolite differences, hippurate levels were consistently lower in urine from children with CF compared to children without CF. Hippuric acid is the hepatic conjugation product of benzoic acid and glycine. After activation to its coenzyme A ester and conjugation to glycine, it is eliminated via renal excretion. Benzoic acid is a natural component of foods, such as berries and other fruits and, importantly, is widely used in high doses as a preservative in processed foods and soft drinks. Due to widespread use of benzoates in foods and no evidence of lower benzoate intakes among children with CF when compared to other children, metabolic limitation of conjugation due to the limiting factors glycine and/or coenzyme A appears likely. The limited ability to conjugate and excrete benzoic acid and related compounds, such as for example salicylic acid, might indicate potential reduced toxicity thresholds for individuals with CF. Supported by the CF Foundation (U.S.) and a Michael Smith Foundation for Health Research Unit Award.

D16: Metabolomics and Nutrition

Dietary macronutrients modify serum metabolomics in rats

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Suboptimal dietary macronutrient choices are arguably the major environmental stressor in individuals living in Western societies. Trans and saturated fats contribute to cardio- and cerebrovascular disease, as do diets high in easily digested carbohydrates, which themselves may also contribute to metabolic syndrome and overt diabetes. A massive literature links diet and both neoplastic and non-neoplastic diseases, and a causal role for mitochondria in this relationship is strongly supported by broad areas of inquiry represented in the literature. We are funded as a part of the NIH-sponsored Genes and Environment Initiative, Exposure Biology Program (Biological Response Indicators component) to conduct an interdisciplinary, approach to discover and confirm plasma metabolomic biomarkers for dietary intake of subclasses of fats and carbohydrates and their effects on mitochondrial function. We will present the initial results of these diets on the sera metabolome.

D17: Metabolomics and Nutrition

Application of Metabolomics to Indentify Manufacturing types and to Predict Preservation Year of Pu-erh Teas and Their Antioxidant Activity

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Liquid chromatography-mass and multivariate analyses were employed for measuring composition of Pu-erh teas and generalization of composition pattern change of Pu-erh teas during post ferment process. Partial least square analysis of Pu-erh teas indicated two large distinct clusters on score plot: ripened Pu-erh teas, raw Pu-erh teas. The raw Pu-erh teas contained more antioxidant compounds compared to ripened Pu-erh teas. As a result, the raw Pu-erh teas showed significantly higher antioxidant activity than the ripened Pu-erh teas in 1,1-diphenyl-2-picrylhydrazyl, Trolox equivalent antioxidant capacity, and ferric reducing antioxidant power assay. Significant correlations between compound-type and preservation year were observed in raw Pu-erh teas; (-)-gallocatechin, (-)-epigallocatechin, and quinic acid were decreased and gallic acid was increased in a year dependent manner. As decreased antioxidant compounds of raw Pu-erh tea, the antioxidant activity was also decreased. These findings indicate that manufacturing type, preservation year, and its antioxidant activity of Pu-erh tea can be predicted based on metabolomics approach.

D18: Metabolomics and Nutrition

Comprehensive Analysis of Urinary Acylglycines using UPLC MS/MS

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Analysis of acylglycines in urine is important for the diagnosis of inherited metabolic disorders. Conjugation of free fatty acids with glycine is effective in balancing the ratio between free and esterified CoA in the mitochondria of liver cells. In inherited metabolic disorders, increased

concentration of acylglycines in urine is directly related to the accumulation of acyl-CoA esters. Currently, urinary acylglycines are analyzed using derivatization followed by GC/MS and/or ESI-MS/MS. Although these methods offer some advantages, GC-MS requires specific derivatization reactions and direct infusion ESI-MS/MS lacks the ability to distinguish structural isomers and suffers from matrix and ion suppression effects. Herein, we describe a sensitive method that uses the resolving power of UPLC to separate isomers, followed by QTRAP MS analysis.

Urine samples were collected from six healthy volunteers, with no known diagnoses of metabolic disorders. Acylglycines were extracted from urine and microsome incubates using mixed-mode anion-exchange (MAX) solid phase extraction (SPE) cartridges. The collected fractions were analyzed by UPLC-MS/MS using a Waters UPLC system coupled to a MDS SCIEX hybrid linear ion trap mass spectrometer.

Comparison of positive and negative scans revealed that the overall intensities were similar in the two methods and positive scan yielded more structural information in the MS/MS mode. Due to the higher selectivity and sensitivity, MRM scans were used as survey scans, using the most intense transitions observed in the precursor or neutral loss scans. The higher sensitivity and resolution of the UPLC were used to separate isomeric and isobaric peaks and to enhance detection of the low abundance acylglycines. A total of 40 acylglycines were detected, only 15 of which are currently used in the diagnosis of inherited metabolic diseases. The current work demonstrates a highly selective and sensitive method for the detection of acylglycines in human urine.

D19: Metabolomics and Nutrition

Phenol-Explorer: a comprehensive database on the content of dietary polyphenols in foods

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Polyphenols are natural antioxidants present in plant foods. They may exert protective health effects and prevent cardiovascular diseases or cancers. Food composition tables for polyphenols are still incomplete and largely focused on a limited number of flavonoid aglycones. Comprehensive tables are needed by epidemiologists to estimate polyphenol intake, and by the agricultural and food industry to compare the polyphenol content in various food products. Composition data for polyphenols are scattered throughout a large number of literature sources, and therefore not easily exploited.

Polyphenol composition data for foods and beverages were collected from 878 peer-reviewed scientific papers and inserted in the Phenol-Explorer database using Access software. Phenol-Explorer contains content data for 498 polyphenols, either aglycone, glycosides or esters, linked to the reference of the publication, and details on the corresponding polyphenols and foods as well as on the analytical methods used for their estimation. 35,837 data points from 608 publications were selected after evaluation, out of a total of 63,291 original data collected, to produce mean

content values for all the different polyphenols in 429 foods and beverages.
An open-source user-friendly web interface has been developed (www.phenol-explorer.eu) to allow anyone to easily query the Phenol-Explorer data. Simple text searches as well as advanced searches are supported. Composition tables can be quickly obtained using the name of a given food or polyphenol. Different displaying options are available depending on the choice of the analytical method or selection of measurement units. Various calculations can also be performed such as the total amount of polyphenols in different classes or the contents in aglycone equivalents. A graph can be displayed for each average content value showing the distribution of the original values used for aggregation. All original values can also be viewed together with the corresponding literature sources.

D20: Metabolomics and Nutrition

Methods development for metabolic profiling of plasma samples for nutrition studies

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The study of small molecules defined as metabolome is an important challenge in the area of nutrition. The analytical technology for measuring the macronutriments already exists but the study of lower abundance metabolites requires some technological developments. We present, here, a top down approach using mass spectrometry coupled to liquid chromatography (LC/MS). Mass spectrometry (electrospray / Qtof micromass) is a suitable tool for this type of analysis because of its resolution, mass accuracy and sensitivity.
In order to develop an LC/MS profiling method, important parameters were tested i) the anticoagulant type for sample collection, ii) the protein precipitation method as these the mass spectrometry analytical conditions are a major problem. Special attention was given to the injection solvent and eluents. To improve methods profiling, metabolites identified in human plasma were monitored during the different step of the study. The method was validated using a large batch of 126 plasma samples, randomized in 4 analytical sequences, over a period of 4 days. The stability of the analytical system was monitored using Pool Quality Control (PQC) samples injected one time at the beginning of a sequence and then every 10 samples to monitor the stability of the analysis. We demonstrated that the main source of variability can be attributed to biological variation and not to variations in the analytical system. However, the variation of signal intensity which depends on the nature of metabolites and concentration, was found to be the main source of variability.

D21: Metabolomics and Nutrition

Stable and flexible metabolic profiling by comprehensive CE-ToF-MS

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Combined capillary electrophoresis-time-of-flight mass spectrometry (CE-ToF-MS) is a powerful technique for the fast and efficient analysis of ionogenic compounds. The application of CE-ToF-MS to metabolic profiling of body fluids, however, may be hindered by reproducibility and coverage problems. In this presentation a novel approach for reproducible and comprehensive metabolic profiling by CE-ToF-MS will be outlined. Very stable CE performance is accomplished by the use of noncovalent capillary coatings comprised of double and triple layers of charged polymers. These easy-to-produce coatings provide high migration-time reproducibility and good tolerance against sample matrix compounds. Moreover, one capillary with different coatings can be used for both cationic and anionic compounds demonstrating the flexibility of this approach for providing an extended coverage of metabolites in only two CE runs. Incorporation of in-capillary preconcentration by pH-mediated stacking further aids the detection of low-level metabolites.
The performance of this novel CE-ToF-MS platform for metabolic profiling of body fluids has been studied for large groups of urine samples. Using multivariate techniques, it is demonstrated that the CE-ToF-MS platform allows high quality metabolomic data to be obtained. Accordingly, it can be applied for delineation of the urinary metabolome in a clinical setting. For example, the applicability and usefulness of the CE-ToF-MS platform will be outlined by the elucidation of metabolites involved in complex regional pain syndrome (CRPS) and urinary tract infection (UTI). Multivariate statistical analysis of the recorded profiles revealed biomarker candidates, which were subsequently identified by accurate mass and/or MS-MS using QToF-MS.

D22: Metabolomics and Nutrition

Establishing population based cohorts with urinary metabolome profiles, diet and lifestyle variables for comprehensive analysis to define health and disease status

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Approximately one third of cancers in humans may be prevented by appropriate nutrition, being physically active and maintaining an appropriate body weight(1). However, despite years of research, there is a paucity of conclusive evidence linking intakes of specific foods, nutrients and other dietary components with cancer risk. This is not surprising, given that diet in free-living humans is difficult to measure accurately, and metabolism is controlled by nutrigenetic and nutrigenomic variables. In future, the science of metabolomics may help shed more light on the link

between diet and cancer risk, but currently there is a need to develop the framework of basic knowledge surrounding the influence of human phenotype and behaviour on metabolomic profiles obtained from analysis of human body fluids(2). In Alberta, Canada, we are establishing a long-term prospective study (the Tomorrow Project) to examine links between cancer risk and genotype, diet, physical activity, lifestyle and environmental variables in 50,000 adults aged 35-69y. As part of a larger national project (Canadian Partnership for Tomorrow Project), the Tomorrow Project in Alberta is currently banking samples of blood and urine, taking physical measurements (height, weight, body composition, grip strength, blood pressure, resting heart rate, heel bone ultrasound), and obtaining detailed information concerning health and lifestyle. One aim is to construct a database in which metabolomic data obtained from this cohort of randomly selected, free-living adults are linked with a wide range of phenotypic and behavioural variables, thereby facilitating greater understanding of how potential confounding factors may influence metabolomic profiles.

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D23: Metabolomics and Nutrition

Determination of the bovine metabolome

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Recent advancements in the omics technologies have enabled the use of novel approaches to investigate the etiology and pathogenesis of metabolic disorders in humans and animals. The evolving field of metabolomics has seen the development of several electronic resources and outstanding metabolomic/genomic databases for multiple-organisms (KEGG, Reactome, the Cyc databases, ArkDB) or organism-specific (EcoCyc, HMDB, SYSTONOMAS). Interestingly, the release of the sequence of the bovine genome earlier this year, revealed not only that cattle share 80 percent of their genes with humans but also that they are much more similar to humans than rodents. Therefore, we have launched an effort to characterize the bovine metabolome.

Completing the first step of this effort, we have created an ‚Äúin-silico‚Äù bovine metabolome database, modeled after the HMDB. Due to this high degree of similarity between the two genomes, our concept was to modify the HMDB by removing compounds unlikely to exist in cattle (such as drug metabolites, synthetic compounds together with food, drug and cosmetic

additives) and by adding bovine-specific metabolites that arise from unique genes or pathways in cattle (more than 100 so far).

In the second step we are confirming and extending this ‚Äúin silico‚Äù construction by analyzing real experimental data from a detailed set of samples from control cows and cows fed diets containing high proportions of grain. Grain-rich diets in cattle have been associated with a greater incidence of several metabolic diseases. This will allow us to establish normal and abnormal metabolite concentration ranges compiled from NMR, GC/MS and LC/MS spectra for the 4 major bovine biofluids: rumen, urine, serum and milk. Data mining and extensive literature surveys will compliment the effort of building a comprehensive metabolomics database. We are also developing a web-application (Bovine LIMS) for managing the data using Ruby on Rails with a MySQL back end.

D24: Metabolomics and Nutrition

Exploring the impact of hospital treatment of anorexia nervosa on the blood metabolome: A CE-TOFMS-based study

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Anorexia nervosa (AN) is characterized by a low body mass index (BMI), fear of gaining weight, denial of current low weight and its impact on health, and amenorrhea. AN restricting type (ANr) is a subtype that has strong inhibition of eating but no experiences of binge-eating and vomiting. There have been no evidence of organic brain defects and the conclusive results of specific genetic loci associated with development of ANr, so far. To identify potential targets for treatment and biomarker of nutrient and/or mental state of ANr patients, we collected serum samples from ANr patients (N=10) at the time of hospital admission and discharge as well as healthy control (N=9) and profiled them using highly sensitive and comprehensive capillary electrophoresis coupled with time-of-flight mass spectrometry (CE-TOFMS)-based platform (1). Interestingly, despite the fasting state of the patients, the level of majority of nonessential amino acid was not significantly altered in patients at the time of admission compared to control, apparently reflecting amino acid homeostasis. Changes in intermediates in choline metabolism were significant, reflecting nutrient state of liver. The hospital treatment was associated with a significant change in level of 80 metabolites ($P < 0.05$). Some of these metabolites included molecules belonging to pathways postulated to be responsible for intestinal bacterial metabolism. Although replication studies are warranted, these identified changes in the blood metabolome might provide the opportunity to identify clinically relevant biomarkers of state of an ANr patient.

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D25: Metabolomics and Nutrition

Metabolomics-derived Biochemical Markers of Prostate Cancer Aggressiveness

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The elucidation of the complex molecular and physiological events that characterize the differences between normal cells and cancer cells is under intense investigation both at the research level and in clinical practice. A large number of studies have been reported with DNA, RNA, and protein-based technologies, however, few studies have been performed to characterize cancer at the biochemical level.

It is by gaining this type of mechanistic understanding of a disease that researchers will unlock the keys to discovering new diagnostics. This presentation will provide an overview of a study undertaken to better understand and profile the biochemical changes associated with prostate cancer aggressiveness. Using metabolomics, a global biochemical profiling technology, tissue, urine and plasma samples were analyzed enabling researchers to identify a series of biochemicals (including sarcosine) that are key potential predictors of cancer aggressiveness. (Nature, 12 February 2009)

Attendees will learn about this study as well as how the underlying technology that fueled this discovery is being applied in hundreds of other areas ‚Äì like diabetes, drug safety research and even to gain a better of understanding of consumer goods products.

D26: Metabolomics and Nutrition

Metabolomics of human urine: variations in metabolic patterns are dominated by muscle metabolism with minimal effects due to factors such as age, diet or energy expenditure

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Background: Urine is the most accessible and expedient biofluid for human metabolomic studies. However, urine contains metabolites originating from different endogenous metabolic pathways and organs and exogenous sources (diet).

Objective: We investigated the urinary metabolome from a human population expressing variation in dietary intake, energy expenditure, respiratory quotient, age, and total muscle and fat mass. We hypothesized that any of these features could contribute to metabolite variation, but since muscle protein metabolism results in the production of tissue ‚Äì specific metabolites (i.e. creatinine, 3-methylhistidine) and the fate of these compounds is urinary excretion, muscle metabolism will be strongly represented in the urinary metabolome.

Design: Patients with Stage IIIb or IV cancer were selected for their known variation in the selected features. Participants were assessed by computed tomography, indirect calorimetry, provided a detailed diet record and fasting urine sample (n=48). Urine was analyzed by ¬πH NMR targeted-profiling method producing data that was analyzed using principal components analysis and partial least squares discriminant analysis (PLS-DA).

Results: The study population displayed a great range in every measured feature (P <0.001). PLS- DA indicated the bulk of the variation in urine metabolite profiles to be related to muscle mass

(R¬≤= 0.79, Q¬≤=0.63); when patients who were catabolic (losing muscle) were compared with those maintaining or gaining muscle this also generated a strong model (R¬≤= 0.72, Q¬≤=0.52). Gender also resulted in a relatively good model (R¬≤= 0.59, Q¬≤=0.49) but this separation relied heavily on muscle specific metabolites (creatine). By contrast other features resulted in weak models R¬≤ ~ 0.30 (age, total fat mass, respiratory quotient, resting energy expenditure) or very weak models with negative Q¬≤ values (diet).

Conclusions: The variation of the urine metabolome is a reflection of muscle mass and muscle catabolism. The effects due to differences in age, diet, and energy expenditure are minimal.

D27: Metabolomics and Nutrition

Serum lipidomics analysis to study the effect of cholesterol-lowering diets in mildly hypercholesterolaemic human volunteers

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Cholesterol-lowering diets play an important role in reducing the risk of cardiovascular disease by improving the plasma/serum lipoprotein profile. Typically, such diets are aimed at reducing serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). However, the impact of various cholesterol-lowering diets on individual serum lipids including e.g. glycerophospholipids is still unknown. A metabolomic approach including extensive lipid profiling and multivariate data analysis could give novel information about the effect of cholesterol-lowering diets on different components of the lipid profile and thus improve our mechanistic understanding of diet-induced health benefits.

The goal of our study was to assess the effect of two cholesterol-lowering diets on serum lipid profiles in mildly hypercholesterolaemic subjects. In addition, special attention was given to relate changes in specific lipid profiles to those in standard serum lipoproteins such as TC, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triacylglycerols (TAG).

A randomized, double-blinded and placebo-controlled study comprised of 100 mildly hypercholesterolaemic subjects who were divided into three similarly sized treatment groups (one placebo group and two groups on different cholesterol lowering diets). Serum samples were collected before and after four weeks of treatment and standard lipoprotein profiles were measured. Lipid profiling was performed by a liquid-liquid extraction procedure followed by UPLC-ESI-QTOF analysis(1). Collected profiles were composed of >100 metabolites from seven different lipid classes. Data analysis included univariate statistical tests, principal component analysis (PCA), partial least squares (PLS) regression analysis and partial least squares-discriminant analysis (PLS-DA) with appropriate model validation tools. These tools were used to establish diet-related changes of lipid metabolites profiles. Changes in lipid profiles were also correlated to changes of LDL-C and

other lipids parameters which will provide valuable new metabolic information which may support the health benefits of specific cholesterol-lowering diets.

(1) C. Hu et al. J. Proteome Res., 2008, 7 (11), pp 4982‚Äì4991.

D28: Metabolomics and Nutrition

Phenotyping consumers of black tea and red grape flavonoids by nutrikinetic analysis of gut microbial metabolites

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The bacterial conversion in the gut to smaller phenolic acids (catabolism) involves the successive formation of a large number of phenolic metabolites and may include bioactive molecules with beneficial effects on the human (cardiovascular) health. The extent to which flavonoids are being metabolized are depended on the composition and the activity of the resident microflora and can largely vary among individuals. To asses the bioavailability, metabolism and the gastrointestinal transport of flavonoid metabolites through the human system (human metabolome & gut microbiome), the time course of the microbial conversions has to be addressed. In this current work we will present a new integrated method of metabolomics and nutrikinetics that is particularly designed to examine the metabolic flows through the gastrointestinal tract without the need for any prior knowledge about the underlying metabolic pathways [1]. The method furthermore exploits the cross-over design in the data using multilevel analysis and permits a separate analysis of the between-individual and the within-individual variation in the data [2]. We applied this integrated method for the analysis of flavonoid metabolites that derived from a single-dose oral intervention of black tea solids and a red grape extract. 1H NMR and chromatographic profiles from blood and plasma were collected to provide information about their existence, identity, absorption, elimination and (changing) output levels over a 48 hours period. This information allowed us to assess the time response of the gut-mediated flavonoid metabolites, and furthermore allowed us to distinguish gut-microbial phenotypes within a human test population. Knowledge about the time response of flavonoid catabolism is particularly important to assess the activity of the microbial community, transport mechanisms and metabolic routes underlying the inter-individual differences in effects on human health.

[1] van Velzen, E.J.J. et.al, (2008), J. Prot. Res 7(10), 4483-4491.
[2] van Velzen, E.J.J. et.al. (2009), J. Prot. Res. DOI: 10.1021/pr801071p.

D29: Metabolomics and Nutrition

Target versus non-target analytical approach for describing metabolism of anthocyanins in rat

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Understanding biochemical pathways involved in metabolism of natural polyphenols in mammals might represent one of the major goals of future research in this field. Epidemiological, clinical and experimental studies have shown that the dietary intake of flavonoids confers protection against many chronic diseases. Anthocyanins are among the most common flavonoids in human diet. Following their oral ingestion they are rapidly detected in plasma and organs both as intact compounds and as metabolites of phase 1 and phase 2 biotransformation. However, at present, the detailed metabolic pathways of anthocyanins in mammalian organisms still remain largely unknown. In our study cyanidin 3-glucoside was administered at low dose intravenously in anaesthetised rats. The tissue distribution and metabolism of cyanidin-3-glucoside was investigated using both target and non-target UPLC-MS and UPLC-Q-TOF experiments. With the target approach, cyanidin 3-glucoside and its metabolites were investigated in plasma, liver, kidney and urine. It was found that the kidney was the organ accounting for most of cyanidin 3-glucoside uptake and metabolism. The further aim of this work was also to apply non-target experimental approach in order to investigate diverse tissues and organ-specific metabolites which might also be differentially expressed in blood, kidney, liver and urine.

D30: Metabolomics and Nutrition

Study on the effects of resistant starch on insulin resistance in rats fed a high fat diet using metabolomics

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Fibre-rich food consumption is linked to reduced risk of chronic diseases. Among dietary fibres, resistant starch (RS) is widely consumed and of interest in human nutrition. The RS effects on intestinal function, lipid and carbohydrate metabolisms have been extensively studied but its role in insulin resistance (IR) and type 2 diabetes prevention is still unclear. Metabolomics allows characterizing global metabolic effects of diets. Our objective was then to better understand the mechanisms of action of RS in high-fat fed, insulino-resistant rats using a metabolomic approach. Male Wistar rats (n=46) were fed for 9 weeks the following diets (n=14/group): control low-fat diet (C, 5% w/w fat); high-fat diet (HF, 30.4% fat); RS-supplemented HF diet (HF-RS, Hi-Maize260¬Æ substituting sucrose and starch, 41.6%). Oral Glucose Tolerance tests (OGTT) were performed at 0, 6, 8 weeks. At week 9, fasted plasma metabolic parameters were measured and peripheral insulin sensitivity was evaluated with glucose transport by the epitrochlearis muscle. Urine and fasted plasma metabolic fingerprints were obtained with a UPLC-QToF MS. RS (HF-RS) prevented the weight gain induced by the HF diet ($p<0.05$). RS diet was also associated with a decrease in plasma lipid profiles compared to HF diet ($p<0.05$). The OGTT showed that HF rats had an impaired glucose tolerance ($p<0.05$) which was restored with RS (HF vs HF-RS: $p<0.001$). Insulin sensitivity of muscle glucose transport was also normalized in the HF-RS group (HF vs HF-RS; HF vs C: $p<0.05$). PCA analysis of plasma metabolomic data showed clear diet distinctions (HF vs HF-RS). More than 4,000 detected ions are discriminating the diets and among those, 144 are significant in HF vs HF-RS diets. Identification of several of the characteristic metabolites will be reported. Preliminary metabolomic results show that RS induces difference in metabolome patterns which may shed new lights on its effects on metabolism.

D31: Metabolomics and Nutrition

A Novel Strategy for Structural Estimation of Metabolites

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Metabolic profiling (MP) is a methodology to discover novel biomarkers that are substances used as an indicator of a biological state. To utilize metabolites as a biomarker, the identification of a chemical structure is a key strategy for further functional and application studies in pharmaceutical research or diagnostics. Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) are often used as analytical platform for MP. However, determination of chemical structure of candidate biomarkers from independent data of NMR or MS is tough task because data shows only a partial structure or molecular weight information of metabolites, respectively. In the present study, the integration of NMR- and MS-based MP to develop a novel strategy for identifying the chemical structure of unknown metabolites was investigated.

Urine samples of spontaneously hypertensive (SHR/Izm) and normal (Wistar Kyoto, WKY) rat were used. The 1H NMR spectra were acquired using 800 MHz JEOL ECA spectrometer at 25oC. A pre-saturation sequence was used to suppress the water signal. MS analyses were performed with pre-separation on reverse-phase or HILIC (LCMS-IT-TOF; Shimadzu), and MALDI-FT-ICR-MS (Apex-Q94e FT-ICR-MS; Bruker Daltonics) methods. Acquired data were converted to ppm/area (the case of NMR) or m/z/intensity (the case of MS) matrices, and then performed the principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were carried out using SIMCA-P+ version 11.5 (Umetrics).

From integration of the data that acquired from different analytical platforms, we can successfully showed the novel strategy to identify the chemical structure of candidate marker compounds. These data are preliminary, but it thought to be a powerful strategy for standard-independent identification of metabolite structure. Further investigation toward identifying other metabolites is now underway.

D32: Metabolomics and Nutrition

Coffee Metabolomics: A New Approach to Defining Quality Markers

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Metabolomics provides sensitive and specific tools for the assessment of quality markers of coffee. Recently, a sensitive method has been published for the quantification of coffee chlorogenic acid metabolites from plasma samples by LC-ESI-MS/MS. Green coffee beans of the two main varieties, Coffea arabica (Arabica) and Coffea canephora (Robusta) have been characterized by mass spectrometric analyses. However, also coffee roasting and products obtained by further technological treatments, including the final beverage consumed, need to be profiled. Herewith, results from metabolite profiling and identification experiments in light and medium roasted coffee samples will be presented.

Samples of light and medium roasted varieties were analyzed by a minimum of three technical replicates each (5mg/mL). Methanol and acetonitrile were evaluated as mobile phases, with reverse-phase chromatographic separations performed on Phenomenex Phenyl-Hexyl packing columns and Thermo Hypersil Gold C18 columns. Metabolite profiles were acquired in high resolution (R = 60000), full scan mode, with positive and negative ionization. Alignment, peak detection and metabolite identification based on elemental composition were followed by statistical analysis. Multiple dissociation techniques such as HCD and CID were employed for metabolite identification, with accurate MS/MS complemented by Data Dependant Analysis experiments and inclusion list-driven MSn for metabolite structural determination.
Metabolites were measured with high mass accuracy, leading to strongly suggestive identifications made by elemental composition software. Statistical analyses were used to distinguished components of interest, which were identified employing a mix of accurate mass and MSn strategies, searches in spectral libraries and fragment ion searches (FISH).
Notably, among them chlorogenic acid components were identified, some of which can be used as quality markers. However, further analyses and systematic studies are needed to establish direct correlations between composition (fingerprint), individual compounds, and quality attributes such as taste, color, and health benefits.

D33: Metabolomics and Nutrition

Metabonomic Assessment of Probiotic Effects in a Colitis Mouse Model using 1H NMR Spectroscopy

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Metabolic profiling of the fecal extracts of male mice was carried out to assess the effects of probiotics on colonic inflammation using 1H NMR spectroscopic analysis coupled with multivariate statistical datasets. The control group (n=5) was administered phosphate buffered saline (PBS) for 14 days. Acute colitis was induced with dextran sulfate sodium (DSS) for 7 days following administration of PBS for 7 days (DSS-treated group, n=5). LAB + DSS-treated group (n=5) was administered lactic acid bacteria (LAB) daily for 14 days and treated with DSS simultaneously for the last 7 days to investigate protective effect of LAB against DSS-inducible colitis. Histological damage, myeloperoxidase (MPO) activity, and malondialdehyde (MDA) content of colon tissue were reduced whereas colon length increased in LAB + DSS-treated mice compared to those in DSS-treated mice. DSS treatment was associated with fecal excretion of amino acids, short chain fatty acids (SCFAs), and nucleotides, revealing significant decreases of threonine, alanine, glutamate, glutamine, aspartate, lysine, glycine, butyrate, uracil, and hypoxanthine together with increases of monosaccharides, glucose, and trimethylamine in the feces of mice with DSS-induced colitis. Increased levels of acetate, butyrate, and glutamine and decreased levels of trimethylamine were found in the feces of LAB + DSS-treated mice compared to DSS-treated mice alone. The increased SCFAs levels in the feces of mice fed with LAB indicate that the probiotics have

protective effects against DSS-induced colitis via modulation of the gut microbiota. This work highlights the alternative approach of metabonomics in feces for assessing the probiotic effect in an animal model of inflammatory bowel disease (IBD).

D34: Metabolomics and Nutrition

Development of assessment systems for gut homeostasis

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The biological evolutionary development of mammalian-microbial symbiosis leads to extensive ‚Äòtransgenomic‚Äô modulation of metabolism and physiology. The genomes of the microbial symbionts (the microbiome) provide traits that humans did not need evolve on their own, and they synthesize essential amino acids and vitamins, and also process components of otherwise indigestible contributions to our diet such as plant polysaccharides. The composition of the gut microbiome is highly variable, and its diversity can be significantly affected by alterations in diet. The ‚Äòtransgenomic‚Äô modulations of individuals reflected by variations in the microbial symbionts are likely to impact human health and disease.We report an approach to evaluate the intestinal variation and to predict metabolic pathways of major microbial symbionts affected by the variation of fiber intakes. The covariance of structural variation in gut microbiome and host metabolism was visualized based on the correlation with the denaturing gradient gel electrophoresis (DGGE) profiles and the urinary nuclear magnetic resonance (NMR) profiles. We improved the DGGE-NMR correlation analysis and the multiple sample collection of a single subject, and integrated the metabolic information, gene expression profiles and microbial community structure in intravital systems. Our approach provides a foundation for evaluation of systemic effects of drugs and diet that are of relevance to personal and public heath care solutions, and a step for opening up a new window that will clear up metabolic dynamics in the complex microbial community in the gut.

D35: Metabolomics and Nutrition

Reproducible metabolomics : Consistency of OPLS models across replicate mouse studies as validation of multivariate signficance

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The mathematical and spectral complexity intrinsic to metabolic studies represents a significant barrier to understanding and communication, especially when results are shared with clinicians and other non-specialists. Follow up studies using the same experimental structure help demonstrate

reliability and consistency, but direct comparison of the resulting models can be complex or misleading; metabolites often show a wide range of significance and variability. The resulting lack of understanding hampers acceptance of results, so a clear explaination of the methods used in multivariate model validation is of significant importance.

Models such as those generated by Orthogonal Projection to Latent Structures (OPLS) can simplify this interpretation. We demonstrate a meaningful and intuitive relationship between internal validation metrics (eg: Variable Influence on Projection/VIP) and the consistency across experimental replicates of model parameters (loadings), in real world biological study data. In each of two pre-clinical animal studies, SIMCA-P was used to generate OPLS models of replicate datasets which we compare using custom R software. Monte Carlo selection of variable subsets was used to maintain consistent model structure. The result was a sigmoidal, monotonic correlation between a variable's influence (VIP score) and its loadings' consistency across replicates (as measured by linear regression within subsets). In the first study (three equivalent experimental cohorts, n=20 per cohort), metabolites with increasing VIP scores above showed an increased linear regression coefficient (R2) as compared to metabolites at lower thresholds. A second study, using two cohorts of a metabolic knockout strain (n=20 per cohort), yielded similar results.

In essence, this relationship allows investigators to state with higher confidence that OPLS variables having high internal validation scores in a first study model are worthy of further consideration. A cutoff value for the VIP of value of "significant" metabolites is suggested based on the plateau of the sigmoidal curve.

D36: Metabolomics and Nutrition

Integrating Stable Isotope-Resolved Metabolomics (SIRM) with Functional Genomics

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Transcriptomics provides information on gene expression networks while proteomics link this network to protein expression. Metabolomics is the 3rd essential partner that defines metabolic network(s). All three are required to achieve systems biochemical understanding. Both NMR and MS enable broad perspective analysis of the metabolome, particularly the transformation pathways. We combined these tools combined with stable isotope tracers to investigate the anticancer mechanisms of different selenium forms in human lung adenocarcinoma A549 cells. Using [U-C-13]-glucose as tracer, we demonstrated that the toxic action of both redox-sensitive selenite [1] and methylseleninic acid (MSA) on A549 cells involves glycolytic and mitochondrial dysfunctions, while redox-insensitive selenomethionine (SeM) was much less toxic with a different mode of action. This could be related to the production of reactive oxygen species. We also modelled changes in metabolic fluxes from time courses of 13C-isotopomers of metabolites in Se-treated A549 cells grown in [U-C-13]--glucose or [U-C-13/N-15]-glutamine. The time course changes in intracellular metabolites and C-13-isotopomers are consistent with early oxidative damage that disrupts the Krebs cycle activity (e.g. anaplerotic pyruvate carboxylation), protein synthesis machinery, lipid/nucleotide biosynthesis, and/or amino acid transporters, leading to inhibition of cell proliferation and adhesion. Using the metabolomics-edited transcriptomic approach (META) [1], we found the expression patterns of key genes and proteins to be consistent with key metabolic dysfunctions. Most importantly, we have successfully applied this approach clinically to human lung cancer patients [2].

1 Fan, T., Bandura, L., Higashi, R. & Lane, A. (2005) Metabolomics-edited transcriptomics analysis of Se anticancer action in human lung cancer cells. Metabolomics 1, 325-339
2. Fan, T. WM, Lane, A.N., Higashi, R.M., Farag, M.A., Gao, H., Michael Bousamra, M. & Donald M. Miller, D.M. (2009)Altered regulation of metabolic pathways in human lung cancer discerned by 13C stable isotope-resolved metabolomics (SIRM). Molec. Cancer, In press.

D37: Metabolomics and Nutrition

Metabolomics: Its Role in Risk-Factor Epidemiology and Chemoprevention

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Epidemiological studies have identified diet as a major risk factor for a number of chronic diseases, including colorectal cancer (UK 2006 mortality rate ~16,000). There is a substantial body of evidence suggesting a positive association between red / processed meat consumption and increased colorectal cancer risk and an inverse association between vegetable / fibre consumption and colorectal cancer risk. However, these findings are inconsistent across studies. It is proposed that the origin of this inconsistency is attributable to the nature of dietary assessment methods currently employed in nutritional epidemiology, largely focussed on "self-reporting." This data source is inherently biased as individuals have a propensity to over-report food types perceived as healthy, whilst under-report those viewed less healthy.

Biomarkers of nutritional exposure circumvent the uncertainty of traditional dietary assessment methods, facilitating a more accurate, unbiased assessment of an individual,Äôs dietary consumption. However, the complex nature of diet, both at the level of an individual meal and especially over an extended time span, result in a miscellany of low concentration metabolites in urine, rendering the process of nutritional biomarker discovery a formidable task. Here, we describe the use of high resolution 1H NMR spectroscopy-based metabolomics and pattern recognition techniques in the ,Äúpredictive,Äù biomarker discovery process. Volunteers were recruited and subsequently required to live in a metabolic suite where they were exposed to a number of different dietary regimens. NMR spectroscopic and multivariate analysis of urine samples was used to discriminate between diets on the basis of their metabolic profiles and identify a number of potential biomarkers indicative of the particular food-types consumed within the intervention study. Robust discrimination was achieved for diets associated with high meat intake, vegetarianism and high fish intake.

D38: Metabolomics and Nutrition

Lipidomics: A Quantitative Approach for Analysis of Lipids in Biological and Plant Samples

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Lipidomics is a systems-based study to describe the complete lipid profile within a cell, tissue or organism and is a subset of metabolomics. It is a relatively recent and rapidly growing research field with an impressive array of applications. Rapid advances in technologies have greatly facilitated and allowed lipidomics to become an integral part of the large multidisciplinary effort supporting systems biology, biomarker discovery and drug development. It involves the identification and quantification of thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites.

We have recently developed a quantitative lipidomics approach for various sample types such as plant tissues (leaves, fruits, seeds), oils (vegetable oils, seed oils) and biofluids (serum, plasma). As a first part of this approach, the extraction of lipid components in different samples was performed by commonly used organic solvent extraction protocols such as the Folch method. The second step was the lipid class separation which was first done by using solid-phase extraction and then further separation via HPLC coupled with evaporative light scattering detector. After the lipid class separation was completed, the HPLC fractions were dried down with nitrogen and subjected to Fatty Acid Methyl Ester (FAME) derivatization followed by GC-MS analysis to characterize the fatty acid composition of each lipid class. FAMEs were identified using AMDIS and an in-house developed GC/MS processing software, by matching retention indices and EI spectra to known FAMEs. All FAMEs were quantified with a specially prepared FAME standard using a five-point calibration curve with two replicate injections. This GC-MS-FAME approach allows the identification and quantification of hundreds to thousands of lipids from a given sample. More than 500 lipids in oil samples, 5000 lipids in plasma samples and 4000 lipids in berry samples, were identified and quantified.

D39: Metabolomics and Nutrition

Human Cerebrospinal Fluid Targeted Metabolomics Analysis using the BIOCRATES AbsoluteIDQ Kit

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Metabolomics is an emerging area of -omics research that involves the global or near global analysis of metabolites (small molecules<1500 Da) found in living organisms. Here, a targeted metabolomics analysis of human cerebrospinal fluid (CSF) using AbsoluteIDQ Kit is presented. CSF is the secretion product of the central nervous system that fills the ventricles and the subarachnoid space of the brain and spinal column. It has many putative roles including mechanical protection of the brain, distribution of neuroendocrine factors and prevention of brain ischemia. The composition of CSF is directly dependent upon metabolite production rates in the brain, the metabolite concentrations in the blood and transport processes at the blood-CSF barrier. Therefore, metabolomic analysis of CSF can offer biochemical insights into central nervous system disorders such as brain injury, multiple

sclerosis, and also could result in the identification of biomarkers for these diseases. The AbsoluteIDQ kit is a commercially available assay from BIOCRATES Life Sciences, and it was originally validated for plasma samples. Recently, the kit has been optimized for the analysis of human CSF using pooled human CSF samples. This Kit is based on a targeted metabolomics approach and quantifies a large number of endogenous metabolites such as amino acids, acylcarnitines, glycerophospholipids and sphingolipids. We have analyzed different human CSF samples using this Kit with a 4000 QTrap mass spectrometer. The application of this Kit in analyzing CSF samples will be presented and discussed in detail.

D40: Metabolomics and Nutrition
The Human Serum Metabolome

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Continued advances in detection and separation analytical technologies in the field of metabolomics call for in-depth metabolic profiling of biofluids and underline the need for developing comprehensive reference resources. As part of our objective to systematically characterize the human metabolome and advance the fields of quantitative metabolomics, we currently present a global metabolic profiling of the human serum. For this holistic analysis, high-resolution NMR spectroscopy, GC–MS, LC-ESI-MS/MS for oxidized lipid mediators and LC/GC-FID (Flame-Ionization Detector) for lipidomics together with computer-aided literature mining were combined to identify and quantify the metabolites that can be commonly detected (with today's technology) in the human serum metabolome. Samples from healthy individuals and various patients were collected for the NMR, the LC-ESI-MS/MS analysis and the LC/GC-FID, whereas pooled blood serum samples were prepared for the GC–MS studies. Data acquisition and analysis was performed according to SOPs and in-house developed protocols. Our literature survey was facilitated by several computational tools developed for the Human Metabolome Database (http://www.hmdb.ca) and the in-house text-mining tool called PolySearch (http://wishart.biology.ualberta.ca/polysearch/). Our experimental results indicated that global metabolic profiling methods can routinely detect more than 4200 different compounds in serum. With NMR spectroscopy 44 compounds were detected and quantified, GC–MS methods could detect and quantify about 70 compounds, LC-ESI-MS/MS techniques were able to quantify 84 oxylipid mediators, whereas with LC/GC-FID more than 3100 lipids were quantified. Comprehensive, web-

accessible tables containing the compounds, concentrations, spectra, protocols and links to disease associations that were revealed or identified from these combined experimental and literature mining efforts are presented, which are also freely available at: http://www.serummetabolome.ca.

D41: Metabolomics and Nutrition

Metabolomic profiling of rumen fluid in dairy cows fed high concentrate/low forage diets

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Dairy cows fed diets containing high concentrate/low fiber diets (HC/LF) develop metabolic disorders such as, sub-acute rumen acidosis, displaced abomasum, fatty liver, and laminitis. The mechanism(s) underlying the development of such disorders are not fully understood at present; however, recent progress in metabolomics technologies has enabled the use of novel approaches to investigate the role of feeding HC/LF diets on development of those disorders.

The objectives of the current study were to identify and quantify metabolites present in the rumen fluid of cows fed different concentrate/forage ratios in the diet. Eight rumen-fistulated dairy cows were used in a replicated 4 x 4 Latin square design with four periods of 21 days each (11 days of adaptation and 10 days of measurements) to test the effects of diets containing 0, 15, 30, and 45% barley grain on rumen fluid metabolites. Rumen samples were collected on days 1, 3, 5, 7, and 10 of the measurements period. High-resolution NMR spectroscopy and GC-MS were used to identify and quantify rumen metabolites and changes occurring with diets. A detailed computer-aided literature survey also was performed to identify rumen metabolites reported in public domains.

The data-mining results revealed 50 metabolites identified/quantified in the bovine ruminal fluid. Our NMR analyses revealed 50 quantifiable compounds, whereas the GC‚ÄìMS detected another 25 measurable metabolites. Data also indicated that concentrations of amino acids, biogenic amines, and organic acids were at low whereas those of carbohydrates were at medium to high Œ°Œú scale. In addition, volatile fatty acids, including butyric, valeric, propionic, and acetic acids were at low mM level. Results also showed major differences in the concentrations of metabolites in relation with the diets used.

In conclusion, metabolomics technology is a reliable technology to study the etiology and pathogenesis of nutrition-related metabolic disorders in dairy cows.

D42: Metabolomics and Nutrition

Metabolomic profiling of Wistar rats reared on diets high in protein or fiber and then switched to a high fat, sucrose diet in adulthood

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Background: Large differences in the composition of diets between early development and adulthood can have detrimental effects on obesity risk. We examined the effects of re-matching rats to their developmental diets high in protein or fiber following a temporary high fat/sucrose diet challenge in adulthood.

Methods: At 21d Wistar rat pups were weaned onto control (C), high fiber (HF) or high protein (HP) diets. The rats consumed the diets until 4 mth of age (stage 1) when they were given a high fat/sugar (HFHS) diet for 6 wk (stage 2) and then reverted back to their respective C, HF, or HP diet for an additional 4 weeks (stage 3). Body weight and fat mass were determined and an oral glucose tolerance test (OGTT) performed. Serum samples taken at time 0 (fasted) and 30 minutes post-oral glucose (fed) were analyzed by proton nuclear magnetic resonance spectroscopy and a total of 50 known compounds identified and measured.

Results: The HF group had the lowest body weight (BW) at all 3 time periods examined ($p<0.05$). Body fat (%) was also lowest in the HF group. In the fasted state, glucose, citrate, leucine, isoleucine and valine were the most important metabolites discriminating between the HF and HP diets. In the fed state, serine, citrate, and glucose contributed most to this discrimination.

Conclusions: This work suggests that in the face of a high energy challenge in adulthood, rats reared on a HF diet had the most favorable body composition compared to C and HP. Metabolite profiles provide a biochemical signature of these diets and may help explain the protective versus harmful effects of these diets on obesity risk and/or identify biomarkers for evaluating the effectiveness of dietary interventions.

D43: Metabolomics and Nutrition

Combining a targeted and fingerprinting metabolomic approaches to identify new markers of health for micronutrients

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Recommended Dietary Allowances (RDA) for micronutrients fluctuate noticeably within European Union countries. The Network of Excellence Eurreca (EURopean micronutrient RECommendations Aligned) aims at harmonising micronutrient intake recommendations through population groups. The lack of proper markers of status for some micronutrients limits progress in this area. Metabolomics should help identifying such new markers as it allows a paradigm shift from public health to personalized health though individual phenotyping.

An original approach combining a targeted and fingerprinting approaches is developed here. A list of 270 metabolites known to be influenced by or dependent on the availability of the micronutrient of interest (eg selenium, folate,Ä¶) has been established (van Ommen et al., 2008) and UPLC-QTof analytical methods have been developed using both positive and negative electrospray ionisation modes to monitor the largest fraction of these metabolites in plasma. This list comprises molecules belonging to various chemical families such as fatty acids, steroids, vitamins, carotenoids or carbohydrates. A spectrum library comprising fragment ions has also been built. Two separated chromatographic stationary phases had to be used so that polar compounds and lipids could be monitored. Comparison between HILIC and ion pairing chromatography is presented for the retention of polar molecules.

Different methods for protein removal and sample preparation are also compared. Such a combined approach together with the multivariate statistical analysis of all mass signals collected will be applied to controlled intervention studies with varying levels of the micronutrient considered.

Acknowledgments: This work is supported by the European Network of Excellence Eurreca.

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D44: Metabolomics and Nutrition

Interactions between Symbiotic Microbiome and Host Metabolism

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The interactions between the symbiotic microbiota (microbiome) and host metabolism are of importance for host physiology and pathophysiology, drug efficacy and nutritional functions. As a systems approach, metabonomic analyses have a major role to play in understanding such interactions since metabonomics is the branch of science concern with the quantitative understandings of the metabolite complement of integrated living systems and its dynamic responses to the changes of both endogenous factors and exogenous factors. Here, we will briefly review the aforementioned importance of symbiosis and report some recent works dealing with the mechanistic

details of the microbiome-metabonome interactions with particular focus on the regulatory functions of gut microbiota in mammals. Examples related to the nutrition effects and drug interventions will be discussed to illustrate the rapid developments of this exciting field and its potentials in biomedical sciences.

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E01: Microbial/Environmental Metabolomics

1H HRMAS NMR Based Metabolomics of Mycobacterium tuberculosis Infected Guinea Pig Lung Tissues: A Novel approach to study the host response and therapeutic efficacy

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Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide. The effect of Mycobacterium tuberculosis (M.tb) on the host still remains an unexplored area. Lung lesions in guinea pigs (GP) infected with M.tb have remarkable similarities to natural infection in humans and are best models to study the host response. Presently, 1H HRMAS NMR has been used for the first time to study the host metabolism of intact M.tb infected GP lungs. GPs (n=12) were infected with M.tb (106 CFU) and 12 GPs were considered as controls. Following 30 and 60 days of infection, GPs were sacrificed (6 at each time point), lungs were harvested, snap frozen in liquid nitrogen and stored at -80oC. At similar time points control GPs were also sacrificed. The tissues from various sites of the infected and uninfected GP lungs were studied. Significantly elevated metabolites viz. lactate, alanine, glutamate, glutamine, glutathione, aspartate, creatine, phosphocholine, glycerophosphocholine, TMAO, betaine, scyllo-inositol, myo-inositol and glycine were observed in 30-day infected tissues compared to the control and these metabolites were further elevated in 60-day infected tissues. Some strong unidentified signals were also observed in 60-day infected tissues which were not detected in control. Metabolic pattern of some tissues in infected lungs appeared similar to uninfected tissues which were thought to be free of infection. PCA performed on NMR spectra showed clear grouping among infected and uninfected tissues, with the tissues which were free of infection being grouped among uninfected ones. Histopathology performed on the tissues after obtaining NMR correlates well with NMR observations. The dynamic variations in metabolite concentration levels have shown good correlation with the amount of necrosis in the tissues. The dramatic metabolic perturbations observed in the infected tissues have been used to establish the defects in host metabolism.

E02: Microbial/Environmental Metabolomics

Metabolomic analysis of the differentiation of *T brucei*

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African trypanosomes are devastating human and animal pathogens. Trypanosoma brucei rhodesiense and T. b. gambiense subspecies cause the fatal human disease known as African sleeping sickness. T. brucei is transmitted by the tsetse fly. The parasite is obliged to make significant biochemical changes to adapt its physiology to the divergent environments of mammalian

blood and the tsetse fly. In this ongoing project we are identifying and characterising changes in the metabolism of T. brucei that take place during the differentiation process, between long slender, intermediate and stumpy bloodstream forms, and procyclic forms that differentiate from stumpy forms in the tse-tse fly midgut. Metabolic profiling of T. brucei and its different life forms has been carried out using hydrophilic interaction chromatography (HILIC) coupled to an Orbitrap Fourier transform mass spectrometer. Changes in abundance of many metabolites have been detected, and several metabolites have been detected to change significantly, giving rise to hypotheses about their significance for the differentiation process. These hypotheses are important from the standpoint of understanding trypanosome biology and identifying novel drug targets. This project is part of the Systems Biology of trypanosomes project ('SysTryp') that aims for comprehensive analysis of trypanosome‚Äôs metabolome, using advance bioinformatics techniques based on metabolic connectivity and response correlation to build metabolic networks.

E03: Microbial/Environmental Metabolomics

Toward a protocol for untargeted metabolomic profiling of Brettanomyces bruxellensis

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Brettanomyces bruxellensis is a spoilage yeast in wine production, because its metabolic products can impart off-flavor to wine. The two main undesired products related to off-flavor occurrence are 4-ethylguaiacol and 4-ethylphenol, although the responsibility of other metabolites not yet characterized has been often hypothesized. Besides strain differences, the production of volatile phenols can probably be related to three parameters: i) grape and wine composition; 2) oenological practices; 3) environmental conditions. Compared to S. cerevisiae, B. bruxellensis is more resistant to ethanol, which can be also used as carbon source, and has lower nutritional demanding. These features are favoring its capability to proliferate when alcoholic fermentation is completed. B. bruxellensis strains can be gathered in several group characterized by similar ethyl phenols production capacities, and nutritional needs, therefore they have different development faculties and spoilage ability in wine. More fundamental studies on the physiological behavior of B. bruxellensis strains are needed to progress in the comprehension of this diversity, and provide important knowledge to control its development in wine.
In order to better understand the behavior of this oenological yeast, strains of B. bruxellensis were characterized by SPME/GC-TOF for their volatile metabolite production under defined growth conditions in the presence of different ethanol concentrations. Furthermore non-target metabolomic investigation was conducted trying to integrate NMR, UPLC-Q-TOF and GC-TOF data as a first step to develop a protocol for metabolome analysis in Brettanomyces yeast.

E04: Microbial/Environmental Metabolomics
Environmental NMR-based Metabolomics Intercomparison

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Recent results of an international intercomparison exercise for NMR-based environmental metabolomics show that NMR metabolome analysis yields robust results with consistent trends in metabolite-based biomarker identification among laboratories. Seven laboratories on three continents participated in the exercise. This type of demonstrated comparability is necessary as the technique is considered for regulatory environmental studies. The exercise design emphasized the steps that occur after sample collection and initial sample preparation. The samples used for the exercise were a set of simplified artificial metabolite mixtures and a set of fish liver extracts from two sites with different pollution profiles. Factors evaluated in the exercise included sample preparation, NMR data collection at multiple NMR-field strengths, quantitative evaluation and multivariate data analysis (principal component analysis). Several data quality evaluation protocols for multivariate data sets were utilized to assess participants‚Äô success with the exercise. A second exercise is being planned, and additional data collection and processing protocols are being developed to further investigate the comparability and suitability of NMR-based metabolomics data for environmental research, assessment and regulatory roles. The preliminary design for the exercise leverages the experience gained previously, so the participants will be asked to process and analyze two classes of materials with a set of robust NMR experiments and data analysis protocols. In an optional second phase, the participants will be asked to collect more varied experimental data and process the data using more advanced schemes in order to expand the breadth of the exercise.

E05: Microbial/Environmental Metabolomics
NMR-BASED MICROBIAL METABOLOMICS AND THE TEMPERATURE-DEPENDENT CORAL PATHOGEN VIBRIO CORALLIILYTICUS

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Coral bleaching occurs when the symbioses between coral animals and their zooxanthellae is disrupted, either as part of a natural cycle or as the result of unusual events. The bacteria Vibrio coralliilyticus has been shown to cause bleaching in the coral Pocillopora damicornis at temperatures higher than 27 ¬∞C.[1] This temperature-dependence of V. coralliilyticus in regard to its metabolome was addressed. One-dimensional nuclear magnetic resonance (NMR) proton spectra were obtained of methanol-water extracts of intracellular metabolites (the endo-metabolome) from multiple samples of the bacteria cultured into late stationary phase at both a high temperature (27 ¬∞C) and a low temperature (24 ¬∞C). The spectra were subjected to principal components analysis

(PCA), and significant temperature-based separations in PC1, PC2, and PC3 dimensions were observed. Betaine, succinate, and glutamate were identified as metabolites in the V. coralliilyticus samples that cause the greatest temperature-based separations in the PC scores plots. With increasing temperature, betaine was shown to be down regulated and glutamate up regulated. These metabolites function as compatible solutes by helping the cells cope with salt from their seawater environment.

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E06: Microbial/Environmental Metabolomics

Genome-wide profiling of small molecule metabolites in Saccharomyces cerevisiae

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Quantification of metabolite levels is an important aspect of biology that is often neglected in genome-wide studies. We have developed a method for high throughput screening of metabolites in the yeast Saccharomyces cerevisiae, which uses capillary electrophoresis in combination with fluorescent derivatization of yeast cell extracts. Using this approach, we quantified primary amine-containing metabolites in nearly 6000 yeast strains, each lacking a single gene. This large dataset provides a resource for understanding the role of individual genes in metabolism. We identified several strains with altered metabolic profiles. For example, many strains lacking one of the ribosomal protein genes, such as RPS19, show accumulation of lysine and significant accumulation of a second unique peak. These strains are being characterized under alternative growth conditions and through the use of HPLC and GCxGC-TOFMS for more detailed profiles. Many of the genes identified in my analyses have not been previously characterized; others encode components of well-known complexes. To identify additional genes that may be involved in metabolic pathways, we clustered the strains based on the similarities among profiles. Using this method, we demonstrate that strains deleted for genes involved in the biosynthesis of arginine (ARG1, ARG3, ARG4, ARG5,6) cluster together based on lack of arginine and accumulation of arginine precursors such as citrulline and ornithine. Similarly, other clusters likely represent groups of genes with related functions. In addition to characterizing variation resulting from specific genetic changes, we have profiled thirty-seven wild yeast strains whose genomes have been resequenced. Together the metabolite and genetic data reveal genetic changes underlying metabolic differences among wild yeasts, providing insight into the natural variation among individuals of a species. Ongoing studies include metabolomic profiles of these strains using GCxGC-TOFMS. Together these data provide significant insight into metabolism in Saccharomyces cerevisiae.

E07: Microbial/Environmental Metabolomics
Metabolic fingerprinting of Chlamydomonas reinhardtii during light stress.

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Chlamydomonas reinhardtii, a unicellular green alga, is a model organism for the study of various processes including photosynthesis. During photosynthesis, plants and green algae use sunlight to convert carbon dioxide into organic sugars. Although light is essential for photosynthesis, high light intensity causes photo-oxidative damage to photosynthetic cells. Photosynthetic organisms have evolved various photo-protective responses to cope with changes in incident light intensity. These responses have been studied extensively, but signaling pathways that trigger stress responses are not well understood. Our previous data suggests that metabolites may act as sensors of the energy status of cells. Changes in metabolite concentration or flux through pathways may thus initiate compensatory changes in gene expression during stressful conditions. Our current study will examine metabolic changes occurring in C. reinhardtii during light stress. Metabolic fingerprinting will be used to study short term and long term changes in intracellular metabolite concentrations due to high light exposure. Metabolic fingerprints will be obtained using nuclear magnetic resonance (NMR) and these data will be analyzed in detail to identify light stress related biomarkers. Metabolic fingerprints will also be correlated to changes in the transcriptional profiles of a variety of diverse genes involved in mitochondrial and plastid metabolism. Correlation of metabolic changes to observed physiological responses can aid the identification of key pathways affected by short-term light stress. Characterizing metabolic changes will contribute to providing a comprehensive view of high irradiance stress in C. reinhardtii and how the organism is able to sense and acclimate to light stress.

E08: Microbial/Environmental Metabolomics
A metabolomics approach to determine gene function in the yeast Saccharomyces cerevisiae.

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The yeast Saccharomyces cerevisiae has been used over the past century as a model organism for the study of eukaryotic biochemistry. Our laboratory uses yeast to study how metals interact with metabolism in relation to basic biology and disease. We use a GC-MS metabolomics platform in conjunction with a yeast gene knock-out library to screen for genetic mutations predicted to have a role in metal related metabolism.

To implement this combined metabolomics and genetics approach to gene function discovery an initial series of profiling experiments for the parent strain BY4741 was performed in batch culture using iron limited and normal media. From this data a metabolic model of iron limitation was developed. Using this model we wished to assign a phenotype to genes of unknown molecular function involved in iron limitation. One metabolite with a retention index of 1939 was increased in iron limited media and identified by the NIST database with 95% certainty as methyl citrate. A search of the Saccharomyces Genome Database suggested that the product of PDH1 may be responsible for the transformation of methyl citrate to methyl isocitrate. Using a yeast knock-out library we tested BY4741 null Pdh 1 using glucose or glycerol/ethanol as the carbon source. A peak with an RI of 1866 with a high NIST probability to methyl citrate was observed only in glycerol/ethanol media. Analysis of an authentic standard confirmed that it was indeed methyl

citrate and not the compound with a RI of 1939. A second round of analysis found that ACO2, a gene of unknown phenotype, is responsible for the metabolite at RI 1939 which is putatively identified as homo citrate. Thus, using batch analysis methodology in conjunction with a yeast knock out library we are able to assign phenotype to genes of unknown function.

E09: Microbial/Environmental Metabolomics

Characeterizing and correlating the plasma proteome and metabolome of *Danio rerio* (zebrafish)

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The zebrafish (Danio rerio) is a well-characterized vertebrate model widely used in a variety of disciplines including genetics, developmental biology and physiology. Fish are a valuable model system for investigating the impact of toxins on aquatic environments and the zebrafish is gaining popularity as a model in this field.

The genome of the zebrafish is mostly sequenced and partially annotated, which facilitates proteomic analyses by allowing for the identification and characterization of proteins using existing databases. The use of proteomics and metabolomics can potentially provide complementary information to gain an understanding of biochemical pathways.

Blood was collected from zebrafish in anticoagulant-containing capillary tubes and centrifuged to obtain plasma. Protein precipitation was used to separate the plasma into the two components for analysis: the proteins in the precipitate and the metabolites in the supernatant. We utilized two-dimensional liquid chromatography-electrospray ionization tandem mass spectrometry (2D LC-ESI MS/MS) shotgun methods to identify proteins in the zebrafish plasma proteome using a Waters QToF Premier,Ñ¢ equipped with a nanoACQUITY UPLC¬Æ system. The plasma metabolome was chromatographically separated on a Waters Xterra column using an Agilent capillary LC system interfaced to a Bruker 9.4 Tesla Apex-Qe Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR-MS). Our metabolomic approach involved using accurate mass, retention time, and standards to identify metabolites of interest. We employed a number of databases (NCBInr, Kyoto Encylcopedia of Genes and Genomes (KEGG), Human Metabolome Database (HMDB)) to search the results.

Preliminary analysis of the data shows correlation between proteins and metabolites in a number of biochemical pathways. We are currently analyzing the biological significance of our findings.

E10: Microbial/Environmental Metabolomics

Analysis of trichloroethylene metabolism in a genetically diverse panel of inbred mouse strains

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Trichloroethylene (TCE) is a widely used industrial chemical, and a common environmental contaminant. TCE is a well-known carcinogen in rodents and a probable carcinogen in humans. Although the metabolism of TCE is qualitatively similar in mice and man, the toxicity outcomes are thought to differ. Studies utilizing panels of mouse inbred strains afford a unique opportunity to understand both metabolic and genetic basis for differences in responses to TCE. In this project, we tested the hypothesis that apparent individual- and organ-specific toxic effects of TCE are genetically controlled and that the mechanisms of toxicity and susceptibility can be uncovered using a diverse panel of inbred mouse strains. TCE (2100 mg/kg) or corn oil vehicle were administered by gavage to 6-8 wk old male mice of 16 inbred strains. Serum, liver and kidney were collected 2, 8, and 24 hr post dosing. Serum samples were analyzed for markers of hepatocellular injury and the profiles of TCE metabolites. Both oxidative metabolites and glutathione conjugates of TCE were measured by liquid chromatography coupled with tandem mass spectrometry. NMR based metabolomics was run on liver extracts. At each time point a subset of mouse strains showed distinct metabolic perturbations from the TCE treatment. Both global and targeted metabolomics analysis have been carried out to determine the critical perturbations in the TCE susceptible mouse strains. These studies provide better understanding of the mechanisms of TCE-induced toxicity anchored on metabolism and genotype-phenotype correlations that may define susceptibility or resistance. (Supported by ES005948 and ES10126)

E11: Microbial/Environmental Metabolomics

The *E. coli* Metabolome

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Escherichia coli is perhaps the best studied microbe in biology. Not only is E. coli the "workhorse" for most of molecular biology, it was also one of the fist bacteria to have its genome fully sequenced. With its genome fully determined, E. coli is now the subject of intense structural genomics efforts to have all of its protein structures determined by X-ray and NMR techniques. While E. coli's genome and proteome are well-characterized, its metabolome is somewhat less well known. This is in part due to the fact that E. coli can grow on a wide variety of substrates under both aerobic and anaerobic conditions. It is also, in part, due to the fact that up to 1/4 of all of the E. coli genes have unknown or unclear functions. While several E. coli metabolite databases do exist (KEGG, EcoCyc), the data in these databases is highly discordant (<50% compound overlap) and poorly referenced. Furthermore, both of these databases include many non-metabolites and exclude many well known metabolites (i.e. lipids and glycans). In an effort to correct this situation we have used software and literature mining techniques originally developed for the Human Metabolome Project to re-define and correct the E. coli metabolome. We have also applied a number of experimental (NMR, GC-MS, lipidomis) methods to confirm, characterize and quantify many additional E. coli metabolites. The result is a comprehensive database (www.ecdb.ca) that contains detailed chemical, biological and biochemical data on more than 2900 E. coli metabolites and 1076 E. coli proteins. This new database is approximately 2X larger than existing E. coli metabolite databases and contains significant corrections and improvements over existing databases. With an improved picture of the

E. coli metabolome we are hopeful that this will open the door to more detailed systems biology studies of this important model organism.

E12: Microbial/Environmental Metabolomics

NMR based metabolomics to characterize naphthalene toxicity in mouse respiratory system

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Background: Lung diseases are one of important contributors to morbidity and mortality. The prevalence of lung diseases has been correlated with environment air pollutants such polycyclic aromatic hydrocarbon (PAH). Human are exposed to naphthalene, the most common PAH, via occupational exposure and living style chosen (ie. cigarette smoke). Previous studies have shown that naphthalene leads to airway epithelial cell injury in mice. A more complete and molecular-based approach to characterize the toxic events in target compartment is necessary to elucidate the mechanism of naphthalene toxicity and develop biomarkers.

Objective: We intend to examine the metabolic effects of naphthalene in mouse respiratory system to understand the toxic action of naphthalene.

Methods: Male ICR mice were treated with a series dose of naphthalene via ip. After animals were scarified, bronchoalveolar lavage fluid (BALF) and lung tissues were removed and snap frozen. Both BALF and the metabolite extracts of the lung tissues were analyzed by using 1D 1H NMR and 2D JRES NMR. NMR spectra were then analyzed by principal components analysis (PCA). Metabolites were identified by Chenomx software.

Results and discussion: The PCA results demonstrated a clear trend of naphthalene dose response from BALF samples. Numerous metabolites responding to naphthalene have also been identified. We conclude that BALF metabolome is a viable technique for investigating lung injury. The technique can also be applied in studying other lung toxicants and diseases.

E13: Microbial/Environmental Metabolomics

Concentration-dependent response of Eisenia fetida to sub-lethal concentrations of phenanthrene

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Earthworms are commonly used in ecotoxicology to assess contaminant risks in the environment. Metabolomics may be a novel method of detecting sub-lethal responses to problematic environmental contaminants. In this study, 1H NMR metabolomics was used to monitor Eisenia fetida responses after exposure to sub-lethal concentrations of phenanthrene, a persistent environmental contaminant. Earthworms were exposed to six concentrations of phenanthrene ranging from 0.8 to 0.025 mg/cm2 which are 1/2 to 1/ 64th of the LC50 (the concentration of a chemical that kills 50% of a species at a given time) using contact tests. Earthworms were exposed to phenanthrene treated filter papers for 48 hours. After exposure, earthworms were flash frozen, lyophilized and tissues were extracted using a D2O-based buffer with 10 mg/L DSS and 0.1% (w/v) NaN3. 1H NMR spectra of E.fetida extracts were analyzed using partial least squares (PLS) and

partial least squares discriminant analysis (PLS-DA). The PLS and PLS-DA scores plots indicated that higher phenanthrene-exposure led to greater differences in the overall metabolic profile of the exposed compared to the control earthworms. The PLS T/U scores plot showed that a correlation exists between the concentration of phenanthrene and the resulting metabolite composition. The intensity of the residual peaks in the 1H NMR difference spectra, (obtained by subtracting the spectrum of the control worms from the spectra of the exposed worms), increased in magnitude as the exposure concentration increased, further suggesting concentration-dependence. There was a statistically significant (=0.05) increase in alanine and decrease in maltose in phenanthrene-exposed worms. Betaine, an osmolytic stress indicator, was also identified in E.fetida extracts and was shown to generally decrease as phenanthrene exposure increased. This study shows a concentration-dependent response of E. fetida to sub-lethal concentrations of phenanthrene and demonstrates that 1H NMR metabolomics is a powerful tool for monitoring earthworm responses to environmental contaminants.

E14: Microbial/Environmental Metabolomics

Staphylococcus aureus under oxygen and nutritional limitations, stress responses by a metabolomic and proteomic view

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Mimic important stress conditions to Staphylococcus aureus, a human colonizing pathogen bacterium and measuring the response of the metabolome and proteome will lead to a more detailed view into the physiology and regulation of this microorganism. By choosing oxygen depletion and nutrient starvation, we try to address wound conditions or growing in other niches of our body. Along a cultivation time of 24 hours under aerobic/glucose limitation and anaerobic/oxygen limitation conditions we take multiple samples for comprehensive time resolved proteome and metabolome analysis of the methicillin resistant strain S. aureus COL. We used 1H-NMR for extracellular metabolome investigations and GC-MS as well as LC-MS(TOF) for intracellular metabolite quantifications. The results were compared with the 2D-gel proteome data and predictions were made for regulation of distinct stress responses.

By glucose deprivation the ATP level decreased and a stringent response mediated by (p)ppGpp starts. A complex system of up- and down-shifts begins to adopt this nutrition limitation stress. Somehow different is the answer of S. aureus to oxygen limitation, proteins for mixed acid fermentation increase, as a consequence lactate, formate and alcohols followed on the metabolome level. A vast number of proteins and metabolites are altered in their level to protect the bacterium against a redox imbalance.

Surviving under these limited conditions required a perfect adaption of the regulatory system of the bacterium, we try to decipher this interplay between metabolites and proteins with the new data for S. aureus.

E15: Microbial/Environmental Metabolomics

Effects of ultrafine and fine zinc oxide particles to rat respiratory system by analyzing metabolome of bronchoalverolar lavage fluids

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Zinc oxide particles can be found from combustion sources and industrial processes. Zinc oxide nanoparticles are also used commercially for paint, rubber, and cosmetics. While the nanotechnology has the potential to promote the quality of life, the adverse effects of the nano-products should be considered. Previous studies have linked ultrafine and fine particles to oxidative stress leading to inflammation and exacerbation of preexisting respiratory syndromes. Researchers also recognized that ultrafine particles may induce greater lung and systemic inflammatory than equal mass of larger particles. A systemic and mechanistic approach to describe the adverse effects of zinc oxide particles using a validated exposure system is necessary to study toxic effects of zinc oxide particles. We have applied nuclear magnetic resonances-based metabolomics to construct the toxic actions of zinc oxide particles in the respiratory system of Sprague Dawley rats exposed to the particles. A validated zinc oxide particle generation system and whole-body animal exposure systems were applied in this study. Rats were exposed to a series does of ultrafine (35 nm) or fine (250 nm) zinc oxide particles for 6 hours. Bronchoalverolar lavage fluids (BALF) were collected for metabolic analysis. We have found that the metabolic effects of zinc oxide particles are size dependent. A clear trend of dose-response has also been demonstrated. We conclude that the stimulation of inhaling zinc oxide particles in the respiratory system can be recorded by metabolic disturbances in the BALF. Moreover, metabolomics provide a more systemic molecular approach to investigate toxic actions of zinc oxide particles. The results of this study could provide important information for risk assessment of metal oxide particles.

E16: Microbial/Environmental Metabolomics

Metabolic flux analysis and 31P NMR spectroscopy: complementary techniques for proving the existence of dual metabolic pathways operating in *L. reuteri* ATCC 55730

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Lactic acid bacteria (LAB) in general are classified as either homofermentative or heterofermentative organisms. These strains are commonly used in the dairy industry for the production of fermented foods; they are also used as probiotics. Homofermentative strains use the Embden-Meyerhof pathway (EMP) to generate lactic acid whereas heterofermentative strains use the phosphoketolase pathway (PKP) to generate a mixed acid profile. Lactobacillus reuteri ATCC 55730 is considered to be a heterofermentative strain, however metabolic flux analysis (MFA) of bacterial fermentations in exponential growth showed (unexpectedly) that up to ~30% of the carbon flux went through the EMP when cells were grown on glucose or a mixture of glucose and fructose and ~15% when grown on sucrose, suggesting that both pathways were operating simultaneously in this particular organism. Bacterial extracts from log-phase cells grown under identical conditions and analyzed by phosphorus-31 NMR spectroscopy gave rise to a metabolic profile of all the phosphorylated metabolites from both pathways. Extracts were made by rapid filtration and extracted with perchloric acid, giving rise to better phosphorus signals than observed with cold methanol extracts. The combination of both techniques was thus useful for proving the existence of dual metabolic pathways in this organism. 31P NMR data also showed that extracts made of glucose-grown cells had relatively lower ATP levels than sucrose grown cells, in agreement with data that cells grew slower and had lower biomass yields on glucose. This failure to thrive in glucose media is attributed to a redox imbalance (causing energy starvation) as fructose released from sucrose is normally used to generate mannitol and NADP+ required for the first enzymatic step of the PK pathway, by which the majority of glucose is metabolized. Fructose and mannitol, on the other hand, are excreted from these cells.

E17: Microbial/Environmental Metabolomics

Effects of Systematic Hypoxia on Human Urine Metabolites Using LC-MS-based Metabolomics

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The human urinary metabolic variability and response to the systematic hypoxia have been studied by LC-TOF-MS. A clear separation was visualized by principle component analysis between nomoxia and hypoxia. About 10% of features observed showed significant changes in their relative signal intensity by ANOVA, whereas 10 features have been identified by MS/MS and their metabolic pathway and meaningful interpretation will be further discussed.

E18: Microbial/Environmental Metabolomics

Quantitative Analysis of the Intracellular Nucleotides Using Ion Pair Reversed Phase Liquid Chromatography-Electrospray Isotope Dilution Tandem Mass Spectrometry

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"Quantitative data on intracellular metabolite concentrations in microorganisms play an increasingly important role in metabolic engineering studies. These data have been used to elucidate the in vivo reaction kinetics [1, 2] for strain improvements aimed at increasing the productivity of fermentation processes.

Nucleotides are important group of metabolites in these type of studies. These molecules are not only the precursors of DNA and RNA but they also fulfill central roles in metabolism, such as storage and transport of metabolic energy and cellular signaling and are incorporated into cofactors of enzymatic reactions.

Here, we report a new sensitive and accurate analytical method which is developed for quantification of intracellular nucleotides in samples from different microorganisms such as *Saccharomyces cerevisiae, Escherichia coli* and *Penicillium chrysogenum*. This method is based on ion pair reversed phase liquid chromatography electrospray ionization isotope dilution tandem mass spectrometry; IP-LC-ESI-ID-MS/MS. A good separation and low detection limits were observed for these compounds using dibutylamine as volatile ion pair reagent in the mobile phase of the LC. Uniformly 13C-labeled isotopes of nucleotides were used as internal standards for both extraction and quantification of intracellular nucleotides. The method was validated by determining of the linearity, sensitivity and repeatability.

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E19: Microbial/Environmental Metabolomics

Identification of Putative Genes in Phospholipid Biosynthesis in Sinorhizobium meliloti using Shotgun Lipidomics

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Sinorhizobium meliloti is a soil bacterium that fixes nitrogen and establishes symbiotic relationships with some important crop plants. The performance of S. meliloti under abiotic stress conditions is an important issue in crop science. Phospholipid biosynthesis in S. meliloti is more complicated than in E. coli, with a number of alternate biosynthetic pathways and a variety of mechanisms for coping with abiotic stresses. We have used a shotgun lipidomics approach to investigate the function of several genes of unknown function in S. meliloti, genes proposed to be involved in phospholipid biosynthesis by sequence homologies with E. coli genes. This presentation will focus on two studies. Two S. meliloti genes, cfa1 and cfa2, were proposed to encode for two cyclopropane synthases involved in cyclopropane lipid biosynthesis, a key stress response while another S. meliloti gene, annotated as plsC, was proposed to function as the single lysophosphatidic acyl transferase in S. meliloti.

A shotgun lipidomics approach was used to compare the lipid profiles of S. meliloti wild type wild type and gene knockout mutants in the presence (2 mM) and absence (0 mM) of inorganic phosphate. Crude non-polar cell extracts were infused at 1 uL/min directly into a triple quadrupole

mass spectrometer under both negative ion and positive ion electrospray ionization conditions, the latter in the presence of LiCl. Distributions of fatty acids in eight lipid classes were obtained using a combination of precursor ion and neutral loss scans. Furthermore, the relative positions of fatty acids (i.e., sn-1 vs sn-2) were determined from from product ion scans. Fatty acid profiles were also obtained by GC-MS analyses of fatty acid methyl esters (FAMEs), prepared using using a new one-vial method. The outcomes of these experiments, data analysis using principal components methods and the advantages of using a shotgun lipidomics approach will be discussed.

E20: Microbial/Environmental Metabolomics

NMR Metabolomics and System Biology

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Metabolomics is routinely being used as a tool of system biology to monitor drug toxicity, to identify disease markers and to explore in vivo protein function and activity. We have developed an NMR-based method to monitor changes in the metabolome caused by changes in enzymatic activity. The technique is based on a differential comparison of the metabolome between wild-type and mutant cells under different environmental and therapeutic stress conditions. Principal component analysis (PCA) is used to identify major variations in the NMR spectra obtained from whole cell lysates. NMR spectra obtained for samples with similar metabolome compositions will cluster together in a 2D scores plot produced by PCA. Our differential NMR metabolomics methodology relies on the analysis of these clustering patterns. Specifically, the cellular activity of a deletion mutant and an environmental factor (drug) can be functionally correlated based on a similar clustering pattern in the 2D scores plot (i.e., essentially identical impact on the metabolome). The analysis of clustering patterns in PCA 2D scores plots are generally qualitative. To address this issue, we have developed a new method to visualize NMR metabolomics data and to quantify the relative separation in PC-space. Basically, we apply phylogenetic tree techniques to calculate an average position for each data set. Simply, each PC value (PC1, PC2, PC3, etc) is treated as an axis in a Cartesian coordinate system. An average position is calculated for each data set, which is then used to calculate distances between each data set to generate a distance matrix. The distance matrix are then used to generate a dendrogram. Our differential NMR metabolomics technique has been

applied to explore biofilm formation by Staphylococcus epidermidis, and antibiotic resistance in Mycobacterium smegmatis.

E21: Microbial/Environmental Metabolomics

Metabolic Profiles of field-populations of *Myriophyllum spicatum*

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Macrophytes are important model organisms in aquatic stress ecology and their stress responses are mainly quantified by observing growth or photosynthesis. These classical endpoints have their limitations and biochemical endpoints are requested which may increase response sensitivity for aquatic plants under stress, e.g. chemical exposure. Therefore, metabolomics as a comprehensive method for detecting low molecular weight compounds present in an organism may be appropriate to give new insights in stress physiology of macrophytes on primary metabolite level. Before applying the method in stress ecology we addressed the following questions: Can individual plants from one species grown under different abiotic environmental conditions be discriminated by their metabolom? How variable are biochemical phenotypes of field-grown individual plants from two populations and within population development?

Metabolic profiling was applied for submerse M. spicatum grown at two lakes near Leipzig, Germany and harvested weekly during three subsequent weeks of their flowering period. For each sampling condition four plants have been investigated individually. Hydrophilic and lipophilic extracts of leaves were analyzed using GC-MS. Data of polar compounds were pre-processed using XCMS and subsequently evaluated with principle component analysis.

The first and second principal component capture more than 50% of metabolom variability of polar metabolites of M.spicatum leaves from individual plants. Metabolic profiles of M.spicatum from two lakes could be clearly discriminated. Within one population a time-related trend over the three weeks of sampling was found, which may be related to developmental trajectories during growth. The variability of individual plants was the lowest within one population at one sampling date and the highest between the populations from different lakes.

Therefore, metabolic profiling seems to be a promising tool for stress ecology. Its potential for detecting specific stress responses of field-grown macrophytes exposed to a pesticide is currently under investigation.

E22: Microbial/Environmental Metabolomics

A generic mass spectrometry-based strategy to assign activity to orphan metabolic enzymes

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Numerous putative metabolic enzymes have no experimentally assigned function even in organisms where a complete genome sequence is available. Moreover, existing metabolomic datasets include metabolites that are not connected to known pathways. Together, these represent a pool of orphan targets for functional enzyme discovery. Targeted assays based on mass spectrometry can be used to assay any biochemical reaction, provided there is an associated change in mass. We introduce a screening platform based on simple in vitro biochemical assays, where purified E. coli recombinant proteins are incubated with a mixture of a single or only a few metabolites. Enzyme candidates are selected based on sequence information, gene structure and conservation, gene expression profile, protein-protein interactions, or other types of functional genomics information. The presence of reaction causes changes in the mixture composition that are monitored by mass spectrometry. We show that the platform can be used for different types of activities and also report test results on some E. coli targets. This generic approach can facilitate the assignment of specific metabolic activity to orphan enzymes and is broadly applicable.

E23: Microbial/Environmental Metabolomics

Plant pathogenic bacteria - Rapid identification of Xanthomonas campestris pathovars using metabolite profiling

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Plant pathogenic bacteria are a risk to food security and cause significant economic losses in agriculture. There is considerable biosecurity effort directed to prevent the establishment of exotic pathovars in Australia. Rapid identification of plant pest and pathogen incursions is essential to reduce the cost of eradication and impacts through rapid response. The ability to differentiate between endemic and exotic diseases is therefore critical. Diagnostic technology and data underpin the capacity for early identification and monitoring. Many bacterial pathovars are difficult to rapidly identify. By definition they are distinguished by their host specificity and in most cases, pathogenicity testing remains the only definitive means to differentiate between closely related pathovars. These tests can be complicated and time consuming; as such pathovar differentiation can potentially delay diagnosis and incursion management.

Here we report the use of metabolomics to differentiate pathovars of Xanthomonas campestris which cause disease in many hosts, including citrus, beans, grapes, cotton and rice. In this proof of concept study, NMR and LCMS metabolomics were used to investigate pathovars of Xanthomonas campestris. The bacteria were grown in liquid culture for 24 hours before analysis. Samples of both broth and cell extracts were analysed. The results of footprinting (broth) and fingerprinting (cell extract) analyses will be presented and the effects of different culture medium discussed. Of the six pathovars investigated (X. campestris pv. armoraciae, X. campestris pv. aberrans, X. campestris pv. barbarae, X. campestris pv. campestris, X. campestris pv. incanae and X. campestris pv. raphani) good discrimination of four pathovars was achieved. This suggests that metabolomic approaches will offer new tools to significantly improve pathovar identification and enhance incursion investigation efficacy. Selected pathovars were also grown in planta to determine if pathovars can be identified from environmental or horticultural samples and initial findings will be presented.

E24: Microbial/Environmental Metabolomics
Metabolite profiling reveals a novel enzyme involved in succinic semialdehyde metabolic pathway in *Escherichia coli*

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The search for novel enzymes is important to better our understanding of the cell‚Äôs metabolic systems. We previously developed a screening method, based on in vitro assays in combination with metabolite profiling by capillary electrophoresis-mass spectrometry (CE-MS), to discover novel enzymatic activities. Using this method, the enzymatic activity of any uncharacterized protein can be tested by monitoring changes in a complex metabolite mixture that are induced by the test protein. We hereby refer to this method as Metabolic Enzyme and Reaction discovery by Metabolite profile Analysis and reactant IDentification (MERMAID).

Here we screened the uncharacterized protein YihU in Escherichia coli using the MERMAID approach. A complex metabolite mixture was profiled in the presence or absence of YihU using CE-TOFMS, and succinic semialdehyde was identified as a substrate for YihU. YihU catalyzed in vitro the NADH-dependent reduction of succinic semialdehyde to gamma-hydroxybutyrate. Profiling of intracellular metabolites following treatment of E. coli with succinic semialdehyde supports the existence of an alternative succinic semialdehyde metabolic route leading to gamma-hydroxybutyrate formation via YihU in E. coli. These findings suggest that YihU is a novel gamma-hydroxybutyrate dehydrogenase involved in the metabolism of succinic semialdehyde that may accumulate under particular stress conditions.

E25: Microbial/Environmental Metabolomics
Metabolomic analysis of the Atlantic blue crab, *Callinectes sapidus*, following oxidative stress

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The Atlantic blue crab, Callinectes sapidus, is a key organism in estuarine ecosystems and the blue crab fishery is important economically and recreationally along the Atlantic and Gulf coasts of United States. Assessing the health of these crustaceans is also important for evaluating the health of the coastal environment. Blue crabs are subjected to many stressors including anthropogenic contaminants and viruses and bacteria. Bacterial infection results in the depression of oxygen uptake, and impairs normal metabolic function in a manner that has not yet been fully elucidated. Our laboratory is developing NMR-based metabolomic tools for environmental research based on the

ability to discover metabolomic biomarkers of stress and exposure in marine organisms. We are investigating the response of the crab metabolome to Vibrio campbellii injection, 2,4-dinitrophenol (DNP) injection (a known uncoupler of oxidative phosphorylation resulting in an increase of oxygen uptake), and saline (control) injection with NMR spectroscopy of crab hemolymph. The corresponding NMR spectral variations between individual crabs were investigated using chemometric tools for pattern recognition and biomarker identification, including principle components analysis (PCA) and partial least-squares (PLS) analysis. Significant metabolic changes were identified in crab hemolymph injected with V. campbellii at the 30 minute time point. Glucose, considered a reliable index for biological stress in crustaceans, provided the largest variation in the metabolome. Trajectory analysis revealed two different modes of oxidative stress, corresponding to the chemical and bacterial challenge. However, biological variability and/or tight regulation of the hemolymph may have masked subtle metabolic changes and evaluation of tissues collected during this study would provide a better assessment of changes to the metabolome.

E26: Microbial/Environmental Metabolomics

1H NMR Metabolomics of Earthworm Responses to Phenanthrene Exposure in Soil

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1H NMR metabolomics of earthworm tissues may provide insight into any metabolic changes that may occur during exposure to sub-lethal and bioavailable concentrations of environmental contaminants. We have examined the response of Eisenia Fetida, the recommended earthworm species for ecotoxicity tests, to phenanthrene (a model polycyclic aromatic hydrocarbon) exposure in soil. We studied both short-term and long-term responses to varying concentrations of phenanthrene. Earthworm tissues were extracted into a D2O-based buffer and analyzed by 1H NMR (using PURGE for water suppression) and data were analyzed with multivariate statistics (AMIX). Our first study showed that PCA separation and clustering of exposed earthworms was observed for the higher phenanthrene concentrations when compared to control earthworms. PC1 loadings plots showed that alanine, leucine, lysine and valine were responsible for the majority of the observed variation. Furthermore, amino acid concentrations were observed to increase with increasing phenanthrene concentration suggesting a strong link between metabolomic responses and soil phenanthrene concentrations after only 48 hours of exposure. Maltose was also identified in loadings plots but was also found to be variable amongst the control group. PLS regression of earthworm responses as compared to both total and bioavailable phenanthrene revealed strong, positive correlations suggesting that 1H NMR metabolomics may be a potential tool for measuring bioavailability directly. E. fetida responses to phenanthene exposure over a 30 day period showed a correlation between amino acid levels and the concentration of phenanthrene in soil. Earthworm responses were still detectible even when the bioavailable phenanthrene concentration diminished. These studies demonstrate the potential for 1H NMR metabolomics to further the understanding of E. fetida ecotoxicity to sub-lethal concentrations of organic chemicals in soil. Future research will focus on testing the inherent variability of observable 1H NMR metabolites in E. fetida and the response of E. fetida in different soil types.

E27: Microbial/Environmental Metabolomics

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROMETRY METABOLOMIC URINE FINGERPRINT SEPARATES IBD FROM NON-IBD

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Aims: The purpose of this project is to: (1) use NMR metabolomics to identify the urinary metabolomic fingerprint of IBD and non-IBD mice, and (2) examine the microbe-genotype-metabolite relationship over time and at the precise initiation of intestinal inflammation.

Methods: IL10 gene deficient (IL10KO) and control mice will be exposed to either a germ free (axenic) or conventional environment. Urine will be collected at 4, 6, 8, 12, 16, 20 wks of age and analyzed on a Varian 600 MHz NMR to obtain a one dimensional 1H metabolomic spectrum. The spectrum will be analyzed with Chenomx software to compare metabolites and their concentrations and thus develop the metobolomic ‚Äúfingerprint‚Äù for each sample. Metabolite concentrations will then be subjected to both standard and multivariate statistical methods of analysis.

Results: NMR spectrum analysis revealed discrete ‚Äúfingerprints‚Äù for different groups of mice. Specifically, separating IL10KO mice with IBD raised in a conventional environment from those without IBD raised in an axenic environment. In addition there was a distinct difference in metabolomic ‚Äúfingerprints‚Äù for males versus females. Ongoing experiments are exploring the relationships between different age groups and also the microbe-genotype-metabolite relationships.

Conclusion: This study illustrates that measurement of a urine metabolomic fingerprint may be effective in distinguishing disease from non-disease animals. Further studies should be able to use the identified metabolites to define initiating and perpetuating factors for IBD.

E28: Microbial/Environmental Metabolomics

Toxicity screening in Daphnia magna for ecological risk assessment: Application of FT-ICR mass spectrometry based metabolomics

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The water flea (Daphnia magna) is one of the most widely used aquatic organisms for toxicity testing as part of the ecological risk assessment of new chemicals. With many gene expression studies of chemical toxicity in D. magna published, it is surprising that essentially no metabolomics research has been reported. There is currently widespread interest in exploiting toxicogenomic approaches to screen the toxicity of chemicals, enabling their rapid categorisation into classes of defined mode-of-action (MOA) and prioritising them for further testing. Here we present the first metabolomics studies of chemical toxicity screening in D. magna using direct infusion (DI) Fourier

transform ion cyclotron resonance (FT-ICR) mass spectrometry. First we optimised the analytical methods and confirmed that mass spectra comprising of thousands of reproducible features can be recorded from whole-body homogenates of 30 neonates or single adult daphnids. Validation of these methods using copper as a model toxicant found significant changes to the metabolome consistent with the MOA of copper, including markers of oxidative stress. Subsequently we confirmed that spectra can be obtained from the haemolymph of individual adults (1Œ°L sample). A series of 24-hr acute toxicity studies in adult daphnids were then conducted using four chemicals with differing MOAs: cadmium (oxidative stress), dinitrophenol (uncoupler of oxidative phosphorylation), fenvalerate (neurotoxicant) and propranolol (beta-blocker). Comparing the biochemical responses in whole organism homogenates versus haemolymph, we have shown that whole-organism metabolic fingerprints provide the greatest discrimination between control and toxicant-exposed animals. Further analyses are on-going, with the goal to determine the ability of FT-ICR to differentiate the MOAs. The ability to measure metabolic fingerprints from individual daphnids raised the potential of correlating molecular toxicity to whole organism reproductive fitness. Such studies are now underway. In conclusion, these experiments highlight the potential of DI FT-ICR metabolomics for high-throughput screening of chemical toxicity.

E29: Microbial/Environmental Metabolomics

Metabolomics and in silico simulation as tools for studying the phosphate starvation response of Corynebacterium glutamicum

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Metabolomics has been developed for studying metabolism and its regulation. We applied this technology to study the phosphate starvation response of Corynebacterium glutamicum, a soil bacterium used for production of more than two million tons of amino acids per year. GC-EI/CI-TOF mass spectrometry in combination with 13C-labelled metabolites was used. For identification of authentic metabolites, the exact mass of small molecules was measured. Molecular formula was calculated and identified in public databases using Matlab. The mass fragment patterns were scanned against a metabolite library. The levels of many TCA intermediates and intracellular amino acids were found to be decreased under phosphate limitation except for L-glutamate, which showed a 1.5-fold increased level. Most interestingly, maltose levels were found to be elevated under Pi limitation. As maltose was not used as a substrate, it is probably formed within glycogen metabolism and consequently we analyzed glycogen formation. Whereas under Pi excess a glycogen pool is formed and then degraded again within the exponential phase, Pi starved cells form a glycogen pool in the exponential phase, but retain it also in the stationary phase. Interestingly, even acetate-grown cells, which do not form glycogen under Pi excess, do so under Pi limitation. The glycogen synthesis rate in glucose-grown cells was found to be 1.24-fold higher under phosphate limitation, which is consistent with in silico simulation. Thus, Pi starvation apparently leads to a redirection of carbon metabolism towards glycogen synthesis. Analysis of 31P-NMR spectrometry and luminescent assays

showed that phosphate-starved cells show polyphosphate is depleted and decreased ATP levels, as expected. Our data show for the first time a link between phosphate starvation and glycogen metabolism as well as a link between phosphate starvation and energy metabolites.

E30: Microbial/Environmental Metabolomics

NMR metabolomics of phenotypic variants derived from Pseudomonas fluorescens biofilms

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In the environment, bacteria live primarily attached to surfaces through the production of numerous extracellular polysaccharides, proteins, and DNA. This lifestyle, known as a biofilm, leads to a variety of physiological adaptations such as an increased resistance to antimicrobials and increased phenotypic diversity. Phenotypic variation is the phenomenon where by multiple phenotypes can arise from a single parental genotype and seems to be important for bacterial survival in rapidly changing and diverse environments. This phenomenon can be readily observed in laboratory grown biofilms of certain species of Pseudomonas, a broad and ubiquitous bacterial genus. How this process occurs is a subject of considerable research, and genetic analysis has revealed roles for signal transduction systems such as the Wsp pathway and the Gac/Rsm pathway. Our goal as part of the research presented here is to further understand the process of phenotypic variation by analyzing the metabolic diversity of isolated variants. Metabolomics methodologies are particularly suited for this type of analysis, especially on a global scale. 1H-NMR is one of several analytical platforms used for metabolomics and in combination with multivariate analysis is a powerful tool for the quantitative analysis of intracellular metabolite levels. We have previously isolated two distinct types of variants from biofilms of Pseudomonas fluorescens. Metabolites were extracted from these variants and relative concentrations, determined by 1H-NMR, were then subjected to multivariate analysis. OPLS-DA modeling revealed that the process of phenotypic variation induces a number of changes in core metabolic pathways. The variants showed differences in glutathione metabolism as well as metabolites important for osmoregulation. An involvement of pyruvate, as a precursor to gluconeogenesis, suggests that the variants may be rearranging their metabolism to accommodate exopolysaccharide biosynthesis. Our analysis demonstrates that quantitative NMR metabolomics is a powerful tool for studying important processes in the bacterial lifestyle.

E31: Microbial/Environmental Metabolomics

Investigation of metabolic impact of motorcycle exhausts in rats using 1H NMR spectroscopy

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Motorcycle exhausts (ME) contain numerous gas-phase and particulate-phase substances. In Taiwan, motorcycles represents 68% of all motor vehicles. Taiwan also has the highest motorcycle per capita density in the world. Previous studies have demonstrated toxic effects of ME in male reproductive sytem in rats. Moreover, vitamin E may reverse toxic effects of ME. The objective of this study is to apply metabolomic approach to investigate changes in endogenous metabolites in the testis and liver of rats after ME exposure.

Male Wistar rats (treated with or without treat vitamin E) were exposed to ME for 4 weeks. The liver and testis were excised and snap frozen for metabolite extraction. Hydrophilic tissue extracts were analyzed using both 1H NMR and 2D JRES NMR at 500.13 MHz spectrometer. Principal components analysis (PCA) and partial least square discriminate analysis (PLSDA) were used to examine metabolic variation in the spectra.

According to PLSDA model in liver samples, latent variable 1(LV1) separated control and exposure group. LV2 divided ME exposure and other two groups. In testis, the LV1 of PLSDA plot took apart vitamin E group from samples and distributed into three portions along LV2.

2D JRES NMR which generates flatter spectral baseline with numerous peaks is a better approach for metabolite identification and quantification. The present findings show that ME inhalation exposure caused metabolic changes in both liver and testis tissues. Besides, the antioxidant, vitamin E, adversed the metabolic effects of ME exposure in rats.

E32: Microbial/Environmental Metabolomics

Application of 1D and 2D NMR techniques to examine the metabolic response of *Eisenia Fetida* to endosulfan

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Nuclear Magnetic Resonance (NMR) spectroscopy is a key analytical tool in metabolomic studies. However, with the analysis of complex mixtures, such as those from biofluids or tissues, it is crucial to select optimized experiments and acquisition parameters that best identify metabolites and maximize the discrimination between the control and treatment groups. Here we describe the application of three 1D NMR techniques (J-resolved spectroscopy (J-RES) projections, Carr-Purcell-Meiboom-Gill (CPMG) and Presaturation Utilizing Relaxation Gradients and Echos (PURGE)) and two 2D NMR techniques (1H-H COrrelation SpectroscopY (COSY) and 1H-13C Heteronuclear Single Quantum Coherence (HSQC) spectroscopy to distinguish between Eisenia Fetida control earthworms and those exposed to the common organochlorine pesticide, endosulfan in contact tests. Tissue samples were flash frozen, lyophilized and extracted using a D2O buffer prior to NMR analyses. Separation between the two groups was observed in the partial least squares discriminant analysis (PLS-DA) scores plots obtained from the spectra for all three 1D NMR techniques. PLS-DA loadings plots showed alanine, leucine and maltose were the main contributors to the separation. PURGE demonstrated slightly improved water suppression and spectral detail in comparison to CPMG. J-RES projections simplified the complex and overlapping spectral regions spanning 3.0-4.5ppm. However, there was difficulty in identifying the metabolites in this region from the J-RES projections alone. The additional connectivity information provided by COSY helped confirm glucose as another potential biomarker. The HSQC analysis provided the best discrimination and

ability to identity potential biomarkers in the control and exposed specimens. HSQC further confirmed the metabolites identified by all other 1D and 2D NMR techniques but also identified two additional amino acids, lysine and glutamate. This study showed that PURGE had higher spectral detail in comparison to the other two 1D NMR methods while HSQC proved to be an excellent discriminatory tool for assessing earthworm responses to environmental contaminants.

E33: Microbial/Environmental Metabolomics

Phospholipid profiling by Liquid Chromatography Electrospray Ionisation Mass Spectrometry in *Leishmania donovani* and *mexicana*

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With the evidence showing that lipids and their metabolism affect cell signalling to accelerate or slow down the cell growth and apoptosis, the importance of lipid research has been widely recognized. Measurements of lipid species are straight to know the quantitative change involved in biochemical and pathological processes. Previous studies on the separation of phospholipids have been conducted mainly on normal phase or conventional C-18 reverse phase. It has either peak broadening for reverse phase or ESI-unsuitable eluents for normal phase. In this study, Several novel chromatographies like C-4, Zic-Hilic and CN column coupled with high resolution mass spectrometry were created to characterise phospholipids. Separations on C-4 and CN column were successful and then tested on biological samples from Leishmania mexicana to profile phospholipids. C-4 Separions can be used for lipid quantification based on the fatty acid tails and CN can be used based on the lipid class separations. Those two methods can also be applied in future lipidomics research.

E34: Microbial/Environmental Metabolomics

Exploration of Taxonomical and Bioactivity Diversity in Spectral Data of Natural Extracts

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An extensive work is required finding novel chemical entities for drug development, since only few hits survive dereplication studies and proceed into preclinical analysis. Therefore, efficient dereplication tools are required to avoid working with previouly known samples; in this step, some

taxonomical classification and evaluation will be helpful for selection of new biomedical significant hits, but this process can be tedious and arduous.(1-2)

Recent developments have enabled rapid processing of acquired spectral data and identification of metabolites from complex samples; at the same time, these approaches are used for discrimination of organisms by chemotaxonomic methods, as well as recognition of patterns related to genome function and toxic response.(3-9)

In this research we explore taxonomical and bioactivity patterns in three sample sets. The first set were 1H-NMR and MS spectral fingerprints from Fijian marine organisms with NF-kB inhibitory potential; the second one were 1H-NMR fingerprints from Costa Rican microbes with antimicrobial activity; and the third one were 1H-NMR fingerprints from Costa Rican endophytic fungi. We retrieved models correlating the taxonomy and bioactivity at genus level; however, retrieved partial information at phylum or general diversity levels, basically due to data overlaping when diverse complex groups were considered.

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E35: Microbial/Environmental Metabolomics

Comparative metabolite analysis of protein secreting and non-secreting Streptomyces lividans TK24

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Streptomycetes naturally synthesise and secrete a variety of secondary metabolites which are extensively used in the agricultural, environmental and pharmaceutical industries. Although physiological and genetic factors affecting protein expression have been well described in Streptomyces lividans, there are very limited metabolome studies to investigate heterologous protein biosynthesis and secretion.

In order to identify metabolites changes associated with protein expression in S. lividans TK24, a comparative global metabolite analysis was performed using hTNF, mTNF and xyloglucanase secreting strains against non-secreting empty pIJ486 and wild type.

The metabolite profiles revealed differences in the levels of several metabolites between protein secreting and non-secreting strains. In particular, a decrease in metabolites (succinate, malate, citrate and Œ±-ketoglutarate) associated with the TCA cycle was observed during protein secretion. In addition, relatively lower levels of sugars and sugar phosphates were detected in producing strains. On the other hand, the levels of many acidic metabolites were significantly higher in non-secreting strains.

E36: Microbial/Environmental Metabolomics

Exchange of nutrients in bacterial cross-talk identified by a novel metabolic monitoring system

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Microbial communities are important in industrial processes and environmental maintenance. Moreover, the human gut is colonized by many bacteria with roles in the maintenance of health and the pathogenesis of infection due to cross talk between commensal and pathogenic bacteria. Commensal-pathogenic bacterial cross talk involves antimicrobial peptides that inhibit pathogen growth, but it concerns only part of the microbial community and mostly does not include the effects of commensal bacteria on the growth of pathogens. Not only secondary metabolites, including quormon and antimicrobial peptides, but also primary metabolites, such as organic acids are important in the regulation of microbial communities because bacteria utilize these compounds as nutrients. Therefore, we developed a new two dimensional nuclear magnetic resonance(NMR)profiling method for real-time monitoring of extracellular metabolites. Using this method, we analyzed Escherichia coli O157:H7 (O157) and Bifidobacterium longum (BL) as a model of noncompetitive inhibition by pathogenic and commensal bacteria. As a result, serine and aspartate were dramatically consumed and acetate and succinate were accumulated in coculture compared to monoculture. From transcriptome and proteome analysis, we observed the change of metabolism associated with fermentation and aspartate utilization in O157. Integrating omics data, we suggested that BL produced serine and aspartate and O157 utilized these amino acids and produced acetate and succinate in coculture. These amino acids are not essential for O157 but O157 utilized them and changed metabolism, which indicated O157 adapted to environmental changes caused by coculture.

E37: Microbial/Environmental Metabolomics

Phenotypic effects and metabolic changes in the chlorophyte Scenedesmus vacuolatus under chemical exposure

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Ecotoxicology traditionally uses phenotypic observations to assess toxic effects in organisms. Today, metabolomics technology can support this approach by providing information at the biochemical level.

The aim of this study was to relate metabolic changes in the chlorophyte S. vacuolatus to conventional parameters of toxicity, in order to evaluate the sensitivity of metabolomics and to derive a causal understanding of toxic effects. Therefore, synchronized cultures of the alga were exposed for 14 h to the phytotoxicant N-phenyl-2-naphthylamine (PNA) in the range of 0.00089

µmol L-1 (environmental concentrations) up to 1.82 µmol L-1. Variable fluorescence (by PAM-fluorometry), oxygen evolution during photosynthesis and cytosol esterase activity (evaluated after FDA-staining by flow-cytometry) were observed for physiological changes, as well as developmental parameters such as growth and reproduction (after 24h). They indicated no effects below experimental concentrations of 0.456 µmol L-1. Changes in the biochemical composition of algae were measured by GC-MS in both lipophilics and hydrophilics extracts after extraction in water/methanol/chloroform, followed by derivatization and silylation.

Results, evaluated by PCA, revealed no effects on metabolites below exposure concentration of 0.00356 µmol L-1 of PNA. However, a clear change was detected at concentrations higher than 0.00713 µmol L-1. A combined visualization of PCA results from metabolic changes and concentration-response relationships from phenotypic parameters inhibition highlighted (I) a two-orders of magnitude higher sensitivity of the metabolomics approach to evaluate PNA-induced effects in µalgae and (II) two types of response patterns of metabolites. One group of metabolites, containing for instance sugars and sugar alcohols, was reflecting pharmacological effects at low exposure concentrations. A second group, mainly composed of amino acids, corresponded to adverse effects along with conventional observations of toxicity. The strategy employed for this study and the results will be discussed. This work was funded by the EU-RTN Keybioeffects MRTN-CT-2006-035695 and the EU IP MODELKEY (Contract number 511237-GOCE).

E38: Microbial/Environmental Metabolomics

Metabolic changes in periphyton after exposure to an herbicide

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Traditionally, the assessment of effects of contaminants is based on population-based observations in single-species investigations. However, first steps have been done to apply metabolomics on exposed organisms in order to identify biochemical changes. But in the environment, organisms are generally exposed within communities, i.e. a complex network of interacting species. Therefore, insights in stress-induced metabolic changes in communities may support a causal understanding of stress responses in the environment, e.g. species shifts. However, community-level metabolic profiling may be limited due to high variability of multispecies systems.

To approach this challenge, we performed a microcosm experiment using microalgal communities (biofilms). 16 aquaria of 15 Litres were grown for 10 weeks at semi-natural conditions. 8 controls and 4 solvent controls were run to evaluate the variation in the community-metabolome during algal growth over this period. 4 other aquaria were treated for the entire time of the experiment with 15 microg/L prometryn, a PSII ‚Äì inhibiting herbicide, which induced distinct metabolic changes in a single-species algal culture (Kluender et al., 2009) (long-term-experiment). Additionally, the 8 control aquaria were exposed for 24 hours to the same stressor to capture short-term changes in the metabolome. Low molecular weight compounds from hydrophilic and lipophilic extracts of the biofilms were analysed after liquid-liquid extraction in water/methanol/chloroform, subsequent derivatization and analysis using gas chromatography-mass spectrometry (GC-MS).

Data, inspected by PCA, revealed a clear variability induced by both long and short term exposures

to prometryn but also a high variability between replicates of the same group. Stress-induced changes in the metabolome will be discussed in relation to phenotypic observations. The suitability of metabolomics for community-level studies in an ecotoxicological context will be commented.
This work was funded by the EU-RTN Keybioeffects MRTN-CT-2006-035695 and the EU IP MODELKEY (Contract number 511237-GOCE).

E39: Microbial/Environmental Metabolomics

<u>Gender differences in the metabolic profiles of blood plasma from Polar bear (Ursus maritimus) analyzed by 1H-NMR metabolomics</u>

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Plasma samples from Ursus maritimus were collected in Spitsbergen in 2008 from individuals with ages ranging from 0-26 years. Samples from 70 individuals were analyzed by 1H-NMR spectroscopy on a Bruker DRX 500 MHz instrument. Data analysis was performed in MATLAB. CPMG-filtered spectra were imported and preprocessed with ProMetab v3.3 software from MR Viant(1) and subjected to principal component analysis (PCA) using the PLS Toolbox v5.2 from Eigenvector Research to study variations in the metabolic profiles.

PCA showed an increasing differentiation between genders with increasing age. Kennard-Stone sample selection(2) using the TOMCAT toolbox(3) was done on PC1 and PC2 from individuals at 5 years and older (n=47) to select spectra for model (n=30) and test (n=17) sets to perform partial least squares discriminant analysis (PLSDA). Subsets were also used for linear discriminant analysis(4) (LDA) using the toolbox of Kiefte(5) on score-values from PC1 and PC2 from PCA. PLSDA predicted gender with a sensitivity of 0.89 and a specificity of 1.0, using two latent variables accounting for 67.99% of the variation. LDA predicted one sample (male) erroneously of the 17 samples in the test set.

The loading profiles from PCA and PLS gave the variables responsible for the differences between gender clusters. Among these were lipid resonances which were more dominating in the profile of females, and glucose and amino acid resonances which were more dominant in males. A detailed assignment of the variables in the loading profiles and discussion on the results will be given. Further use of the NMR data together with other analyses performed will be discussed.

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E40: Microbial/Environmental Metabolomics

Exploring diethanolamine toxicity in Calanus finmarchicus

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Calanus finmarchicus is a key species in the marine eco-system in the North Atlantic with an annual production of 300 million tons per year, and is as such highly relevant as a species for assessing effects of pollutants. At BioTrix, NTNU and SINTEF in Trondheim we have unique possibilities for doing experiments on C. finmarchicus with a laboratory culture which is currently in its 23rd generation.

Diethanolamine (DEA) is an alkanolamine used in a wide range of industrial, agricultural and pharmaceutical applications. Owing to its chemical properties, DEA has also been employed as a solvent in CO2 capture processes and its usage is expected to increase in the future. Although DEA has low acute toxicity (EC50 Skeletonema: 356.9 mg L-1, LC50 C. finmarchicus 380 mg L-1, LC50 Acartia tonsa: 223 mg L-1) it is not readily biodegraded in cold marine environments, implying persistence. Despite reported carcinogenic effects on vertebrates, there is still very limited information regarding the potential eco-toxicity of DEA.

We exposed C. finmarchicus to a sublethal concentration of DEA for 48 hrs. Impacts of DEA exposure were studied using high resolution magic angle spinning nuclear magnetic resonance spectroscopy (HR MAS NMR) on a Bruker DRX 600 spectrometer to study differences in metabolic profiles using multivariate analysis In addition changes in gene expression were assessed using a suppression subtractive hybridization gene library. Together these two methods offer complementary screening tools for the assessment of molecular responses to DEA. Effects were observed on genes putatively involved in lipid metabolism, antioxidant systems, metal binding, and amino acid and protein catabolism and were accompanied by altered levels of fatty acids, amino acids. The presence of three potential, and currently unidentified, biomarkers for DEA toxicity in the HR MAS NMR spectra will be discussed along with plans for follow up experiments.

E41: Microbial/Environmental Metabolomics

Hormone sensitivity of goldfish tissue: a step towards endocrine disrupting chemical metabolomics

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Waterborne chemicals are serious sources of environmental toxicity, ultimately having impact both in aquatic ecosystems and human health. Endocrine disrupting chemicals (EDCs) are a major class of toxicants that have been shown to interact with sex steroid receptors in higher organisms. Aquatic ecosystems are particularly susceptible to EDCs due to constant streams of effluent (e.g. treated waste, pulp and paper, heavy industry, mining, oil and gas), as well as periodic surface runoff from agricultural activities. This worldwide problem has received increasing recognition in Canada and Alberta. Phenotypic effects on aquatic organisms include reduced testes size, and the so-called 'feminization' of fish populations, where exposure the EDCs results in a unnatural increase in the female/male ratio. Androgenic effects have also been noted, indicating that EDC impact is not limited to estrogen-sensitivity. In this study, we examined metabolic response of goldfish populations exposed to low levels of sex steroids, thyroid hormone as well as a number of environmental contaminants with hormone-like activity. Liver, muscle, and gonad tissue were extracted using chloroform/methanol and the aqueous fraction was analysed using NMR spectroscopy. The results were interpreted with respect to metabolic changes related to tissue type and gender (identified during tissue collection) using orthogonal partial least squares discriminant analysis (OPLS-DA) on the spectral data. The results demonstrate that tissue from goldfish exposed to sex steroids have significantly altered metabolic profiles. The specific metabolites varied by hormone as well as tissue type. Intriguingly, gender response was similar in liver and muscle tissue. Significant alterations were noted for males exposed to estrogenic compounds and contaminants with thyroid-like activity. The results are consistent with the hypotheses that EDCs with estrogen and thyroid-like activity cause dysregulation of energy and lipid metabolism in fish. These results provide a framework for further studies of EDC-related metabolic dysregulation in fish and other species.

E42: Microbial/Environmental Metabolomics

Metabolomic profiling reveals differences between aggregative and non-aggregative cells of Salmonella

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The future of Microbiology lies in the discovery and characterization of the many small molecules or "metabolites" produced by bacteria. This study was performed as a proof of principle to determine if we could differentiate Salmonella enterica serovar Typhimurium ATCC 14028s (wt) cells from an isogenic DeltaagfD strain based on their metabolite profiles. Metabolites were extracted after growth of the two strains for two and five days at 28°C on solid media containing 1% Tryptone. Under these

growth conditions, wt cells produce an aggregative, extracellular matrix comprised of protein and polysaccharide polymers, including curli fimbriae and cellulose, whereas DeltaagfD cells are devoid of an extracellular matrix. Analysis by GC-MS and 1-D and 2-D 1H-NMR was limited by existing databases, yet still led to the identification of over 80 metabolites. The levels of 35 compounds differed between the wt and DeltaagfD strains, as determined by statistical comparisons of the spectral profiles as well as concentration values determined by NMR. The 35 compounds fit primarily into defined pathways, leading to an overall model where wt metabolism was geared toward the production of glucose and precursor sugars (Gal, Man) for the production of extracellular polysaccharides and sugar-derived osmoprotectants (trehalose), while the DeltaagfD mutant accumulated TCA cycle intermediates (succinate, fumarate, malate) and polyamines (cadaverine, putrescine), which may be evidence of amino acid breakdown. We are currently in the process of verifying these metabolic changes using promoter-luciferase reporters based on a variety of target genes.

E43: Microbial/Environmental Metabolomics

Mining the myxobacterial secondary metabolome using high-resolution mass spectrometry: natural product discovery as an analytical challenge

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Introduction: Myxobacteria and other secondary metabolite producers represent an important source of biologically active natural products with considerable promise for human therapy. Several studies have recently highlighted the enormous and hardly tapped genetic potential of many myxobacterial species for secondary metabolite biosynthesis.[1,2] Although more than 100 basic structures from myxobacteria have been characterized to date, the number of compound classes reported from individual strains clearly falls behind genetic capabilities. Thus, the discovery of novel secondary metabolites from genetically proficient myxobacterial producers currently constitutes a substantial bottleneck. Improved analytical methods, based on the combined use of LC-coupled high-resolution mass spectrometry and statistical data evaluation, can significantly enable the process of uncovering these ‚Äúhidden‚Äù bacterial secondary metabolomes.[3,4]

Method: Extracts from cultivations of Myxococcus xanthus were analyzed by a separation of complex samples on RP-C18 column (1.7 ¬µm particle size) under UPLC conditions and coupling to a novel ultra-high resolution TOF mass spectrometer. Atmospheric pressure ionization was carried out using positive and negative ESI and APCI. The obtained data were pre-processed using a compound finding algorithm prior to statistical interpretation by principal component analysis (PCA)

Preliminary Data: Metabolomics-based experiments employing high-resolution LC-MS measurements are convenient because they provide the opportunity to apply both targeted queries and unbiased statistical treatment to the same dataset. The feasibility of the latter approach depends critically on the number of chemical ‚Äúfeatures‚Äù detected by the compound finding algorithm. In this study, varying combinations of ion source and ionization modes were rigorously put to the test in order to establish an analytical system for the optimal coverage of myxobacterial secondary

metabolomes. An analytical performance assessment was carried out including the evaluation of mass position stability, isotope pattern accuracy, dynamic range and sensitivity.

F1: Novel Technologies & Imaging

GC/APCI-TOF MS: GC/APCI with ultra high resolution TOF-MS - analytical validation and applicability to metabolic profiling

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Introduction: Most of the commercial GC/MS systems use ionization under vacuum conditions: electron ionization (EI) and chemical ionization (CI). Atmospheric pressure ionization sources (API), which are probably the key of the ‚Äúovernight success‚Äù of MS-detectors in analytical sciences due to coupling with liquid chromatography, are rarely used with GC instruments. A recently introduced multipurpose AP source created the opportunity to reconsider the importance of AP ionization for GC. Here, we present an analytical evaluation of GC/APCI-MS showing the benefits of soft atmospheric pressure chemical ionization for GC in combination with an orthogonal-accelerated ultra high-resolution TOF mass spectrometer (UHR-TOF-MS MaXis).

Result: A very sensitive and accurate GC/APCI-TOF-MS method was developed for the automated analysis of metabolites in biological samples. At present, the analytical evaluation of the method was made using amino acids, organic acids, alcohols, xanthines, indoles, dipeptides, compounds with imidazole groups, amines and analytes with hydroxyl and amine groups, demonstrating ability of the analytical procedure to deal with nine different chemical families within one experiment (chromatogram). Excellent repeatability was obtained, with relative standard deviations (RSDs) of peak areas between 0.7% and 2.1% in the intra-day study, and between 3.8% and 6.4% in the inter-day study. Analysis of CSF has demonstrated a rich chromatographic pattern consisting of hundreds of features. We have detected more than 300 distinct features in CSF, even when using very strict peak detection criteria. This fact in combination with the presented analytical characteristics (LODs, repeatability and reproducibility) demonstrates the potential of GC/APCI-TOF MS for metabolic profiling. The high quality of spectra creates an opportunity to make structural assignments of metabolites based on accurate mass position and isotopic distribution. However, accurate MS/MS spectra can be efficiently in order to resolve more difficult cases and support identification by fragmentation data.

F2: Novel Technologies & Imaging

A novel method for robust LC/MS-TOF Analysis of Hydrophilic Metabolite Classes by Aqueous Normal Phase on a Silica Hydride-Based Column

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A limiting factor in many metabolomics analyses is the ability to robustly chromatograph mixed classes of hydrophilic compounds. A generic LC/MS-TOF method has been developed that is capable of separating sugars (polar neutral), amino acids (polar basic) and organic acids (polar acidic) in complex mixtures using the aqueous normal phase (ANP) technique on a silica hydride surface. This material can be used over a wide range of mobile phase compositions from pure aqueous to pure non-polar and is compatible with analysis in both positive and negative ion modes. This highly reproducible method for hydrophilic compounds now enables the use of retention time as an orthogonal physical parameter for identification with an Accurate Mass Retention Time (AMRT) database. In order to encompass a broad range of polar metabolites it was necessary to buffer the system at neutral pH. That was accomplished with a pyridine/acetic acid buffer in the acetonitrile solvent. The aqueous component of the mobile phase contains formic acid as the additive. A Cogent Diamond Hydride (150mm√ó2.1mm) column, using a gradient starting at high acetonitrile, and buffered at neutral pH, results in a relatively high initial pH (near 7). As the amount of water (containing formic acid) is increased the pH is lowered. Therefore, the gradient changes with respect to both polarity (increasing) and pH (decreasing). Under these conditions, virtually all polar metabolites are retained. Isobaric compounds or compounds differing by one amu can be separated. The method demonstrates the ability to retain and chromatograph a chemically diverse compound set with the potential for rapid positive / negative ion switching to allow single run analysis.

F3: Novel Technologies & Imaging

Combining unbiased metabolic profiling with targeted analysis of specific metabolites using high resolution mass spectrometry: A step forward in metabolomics

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Most metabolomics methods allow either the analysis of limited numbers of known metabolites or compound classes or the analysis aims to identify as many features as possible in a given metabolome without prior knowledge. Using high resolution mass spectrometry it is possible to combine these two strategies.

Sixty fortified plasma samples from ten healthy volunteers (glucose, palmitate, N-octanoylsphingosine or a combination thereof), were analysed 4 times using two injection volumes all within the same LC-MS batch using a benchtop orbitrap mass spectrometer. The results showed that the mass accuracy was comparable to conventional FTICR-MS. The stability of mass accuracy allows the use of high mass accuracy extracted ion chromatograms (2ppm) which are specific for the chosen molecular formula and only likely affected by isomers.

The experimental data was analysed using standard data-mining tools allowing determination of qualitative sample differences. The fortified samples were readily identified. The same raw data was used for specific analysis of 75 different metabolites using high resolution extracted ion chromatograms based on the theoretical metabolite m/z. This method can be used for semi-quantitative analysis of many metabolites. We report the first metabolomics method allowing

both targeted analysis of specific metabolites and unbiased profiling without compromising results.

F4: Novel Technologies & Imaging

Ordered v.s. Random Peak Distributions in Comprehensive Multi-dimensional Analysis Systems

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Comprehensive two-dimensional chromatographic separators, such as GC x GC, LC x LC, and two dimensional gel electrophoresis, can separate complex chemical mixtures not amenable to single dimensional (linear) techniques. Two factors account for the separation power of comprehensive multi-dimensional systems: 1) peak capacity, and 2) ordered distributions of peaks. The former issue, peak capacity, is widely understood. The latter, ordered separations, is not. In 1995, Calvin Giddings advanced general criteria for achieving ordered separations in comprehensive multi-dimensional chromatographs, and discussed the strong effect of orderly peak distributions upon resolving power. Key ideas are "sample dimensionality", "separator dimensionality", and "dimensionality matching", i.e., tuning the dimensionality (design) of a comprehensive chromatograph to match the intrinsic chemical dimensionality (complexity) of a sample. Giddings demonstrated that a two-or higher-dimensional chromatograph will efficiently resolve a two- or higher dimensional sample if, and only if, the dimensionality of the chromatograph matches that of the sample. In particular, if the intrinsic chemical dimensionality of a complex sample is two or higher, no one dimensional chromatograph can efficiently separate it. These considerations bear fundamentally upon the analysis of complex mixtures, and will be considered vis-a-vis human breast cancer tissue extracts analyzed by a GC x GC instrument equipped with a fast scanning(100 Hz) high resolution time-of-flight mass spectrometer. The current state of the art suggests that, in most cases, we have not matched the dimensionalities of instruments to the dimensionalities of samples derived from natural products (with the exception of petroleum derived samples, in which two-dimensional matching is largely achieved). Results suggest that the peak capacities of higher-dimensional comprehensive separators are critical to metabolomics. Dimensionality matching, should it prove possible for metabolomic samples, would further improve resolving power.

F5: Novel Technologies & Imaging

in situ Metabolomics Imaging by MALDI mass spectrometry

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Metabolomic studies can lead to the enhanced understanding of disease mechanisms and the discovery of new diagnostic biomarkers as well as the enhanced understanding of mechanisms for drug or xenobiotic effects and the increased ability to predict individual variation in drug response phenotypes. Thus, this rapidly developing discipline has important potential implications for the pharmaceutical research field. To date, mass spectrometry (MS) coupled with pre-separation techniques such as liquid chromatography (LC-MS) or gas chromatography (GC-MS) has been known to be a conventional strategy for metabolomics. However, these methods have disadvantages for measuring tissue samples because these samples require the step of metabolite extraction and this process cause a loss of the information of both tissue morphology and metabolite distribution. In this study, we tried to develop in situ metabolomics imaging technique with matrix-assisted laser desorption ionization (MALDI) mass spectrometry.
C57BL/6N mouse brain was sagittally sectioned at 15 Œºm thickness with cryostat and then thaw-mounted onto a ITO-coated glass slide. A matrix solution (100% methanol) was sprayed using air brush. Mass imaging data were acquired in negative ionization mode using AXIMA Confidence (Shimadzu, Japan) with 50 Œºm spatial resolution.
In our previous study, we reported that MALDI mass spectrometry is highly sensitive and quantitative methodology for metabolite analysis. Applying this technique to in situ tissue metabolomics imaging, more than 70 metabolites including nucleotides, cofactors, phosphorylated sugers, and carboxylic acids were detected and visualized the unique distribution on the mouse brain tissue. These data suggest that in situ metabolomics imaging developed in the present study can be utilized for visualization of different distribution of metabolome and pharmacometabolomics directry on the tissue section.

F6: Novel Technologies & Imaging

Accurate and Sensitive All-Ions Quantitation Using Ultra High Resolution LCMS and its Application to Endogenous Metabolite Profiling

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Metabolomics is an extreme example of multi-component quantitation. Even though in most cases the comparisons are relative, the key to a successful study is high quality quantitative results. Once potential biomarkers have been found, more rigorous, absolute quantitative assessments are usually made to validate the initial observations. Here we demonstrate the use of ultra high resolution LCMS to provide sensitive, high quality quantitative data. This then provides a simple, relatively unbiased and highly quantitative assessment of metabolomics samples and negates the need for multiple MS platforms. However, the triple quadrupole mass spectrometer is considered the gold standard quantitation and so typically, the validation of potential biomarkers are performed with LCMS SRM techniques. This approach requires MS/MS methods to be developed for each analyte, and that as the number of analytes increases the advantages of using a triple quadrupole diminish. The move to smaller SRM dwell times to accommodate more components across narrow uHPLC peaks compromises both sensitivity and precision. In addition, the more analytes the more labor intensive the SRM method development becomes. In contrast, the instrument set up for high resolution quantitation is very simple, with a full-scan over the mass range of interest and no prior knowledge of the analytes needed. Selectivity for the quantitative measurement is provided by high resolution

MS and the use of narrow mass windows. Quantitative performance was comparable to that of a triple quadrupole when multiple analytes were monitored. One of the most significant obstacles for this type of non-targeted metabonomics is to identify components that change between the groups. Most components observed are not in any of the available endogenous metabolite databases. In many cases accurate mass and MS/MS are not sufficient to identify the components. Our approach uses MSn library trees that provide the ability to identify structurally related compounds.

F7: Novel Technologies & Imaging

A mass spectrometric approach to spatial metabolomics

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An important aspect of cellular and tissue biochemistry is to know if and (if so) how metabolic localizations mediate and influence cellular processes. Subcellular localization of biomolecules control several biological processes with localized activities occurring within the cells or in the extracellular space in cellular proximity. This key aspect is lost in traditional methods of biochemical analysis or metabolomics, where the components are extracted and analysed away from the cellular context. A capability to image metabolic changes in cells and population of cells in spatial dimensions enables a powerful tool to monitor and understand several biological and biomedical processes, at the molecular level. Spatial metabolomics aims to capture metabolic changes with respect to the spatial distribution of metaboites in situ (i.e. where it occurs in the cellular context).

Imaging mass spectrometry offers the potential to screen for spatial metabolic distributions. Time of flight secondary ion mass spectrometry (ToF-SIMS) enables intrinsic biochemical distributions to be monitored, at meaningful spatial resolutions. It is a surface technique that uses pulsed primary ions to desorb and ionize molecules from a sample surface so the emitted secondary ions can be analysed (using a ToF mass spectrometer). The advent of polyatomic primary ions promises 3D spatial biochemical imaging of cell surfaces, sub-surfaces and tissue sections, at submicron spatial resolutions. In reality, the application of the technique to derive spatial metabolomic information requires addressing several challenges. These include assessing the different chemistries amenable to direct detection and imaging, influences of ion suppression and preferential ionizations, maximizing secondary ion yields, etc. We are addressing some of these and are involved in the development of the technique for spatial metabolomics. Our experience with animal and plant tissue sections and microbial cell populations, in deriving spatial metabolomic information using the technique, will be discussed.

F8: Novel Technologies & Imaging

High-throughput and sensitive analysis of intracellular metabolites using MALDI-TOF-MS and MALDI-FT-ICR-MS

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Since the central metabolic pathway represents critical aspects of cellular metabolism, it provides indispensable data for metabolic engineering and metabolomics to quantify temporal changes of concentrations of intermediates in glycolysis and pentose phosphate pathway and their corresponding cofactors. As well as quantitative method, the demand for high-throughput and sensitive analytical method is increasing as quite a large number of biological samples inevitably need to be analyzed for investigating the cellular metabolism and intracellular metabolic intermediates normally exist at such low concentrations. Compared to other analytical methods used for metabolite quantification (GC-MS, LC-MS, etc.), matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) enables highly sensitive, high-throughput, and low sample-consuming analysis. Furthermore, negative-mode MALDI-MS analysis provides remarkable sensitivity of phosphorylated compounds including important metabolic intermediates and corresponding cofactors in the central metabolism. In the present study, we developed an exclusively high-throughput and sensitive method for quantification of metabolites in the central pathway using MALDI-MS.

The quantitative performance of MALDI-MS in negative ion mode was confirmed using metabolite standards. This technique then applied to investigate intracellular metabolism of Escherichia coli, analyzing its cell extracts taken consecutively before and after a perturbation of carbon source. MALDI-FT-ICR-MS was used to identify the elemental compositions of detected metabolites.

The correlation coefficients obtained from calibration curves of each metabolites showed a good linearity ($R2 > 0.99$). In the analysis of time-course extracts, observed temporal changes of concentrations of metabolic intermediates and their corresponding cofactors strongly correlated with the perturbation. These results suggested that the MALDI-MS-based analytical method developed in this study is suitable for high-throughput analysis of dynamic intracellular metabolism.

G1: National Metabolomics Initiatives

High-resolution fragmentation trees of phenolic compounds: Can we differentiate between isomers?

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Metabolomics needs efficient protocols for recognition and identification of small molecules. Mass spectrometry (MS) is a widely used, sensitive technique to detect metabolites and solve the molecular formula. Isomers do have the same molecular formula and it seems to be a hard task for MS to differentiate between them. However, fragmentation patterns of metabolites can give insight in the position of substituents on a core structure. In this study, fragmentation patterns of phenolic compounds were obtained in the format of spectral trees, based on a method developed in Leiden University. The spectral tree method systematically breaks down a molecule down to the smallest measurable fragments, resulting in a fragmentation tree. This is in contrast to tandem-MSn, a method where only the highest intensity fragment is taken for further fragmentation. Spectral tree data were obtained with a NanoMate-Orbitrap set up, which resulted in high-resolution mass chromatograms. The identification of molecules is based on the presence or absence of fragments and the difference in relative intensity of the fragments in the different MS levels from MS2 down to MS5. Due to the use of normalized collision energy, fragments posses a reproducible relative intensity. The mass accuracy of the Orbitrap (Fourier transformed mass spectrometry) enables easy assignment of molecular formulas to the fragments. As a result, the spectral tree method could differentiate between four methoxy-flavone and three quercetin-glucoside isomers, in a concentration range of 0.5 ,Äì 5 microgram/ml. In collaboration with Leiden University, within the Netherlands Metabolomics Centre, the spectral tree method will be further developed. More groups of phenolic compounds will be measured and analyzed to investigate the discriminative power of the spectral tree method and the fragmentation behavior of metabolites.

H1: Pharmacometabolomics

Metabolomics: A Novel Tool for Understanding the Early-Stage Mechanistic Underpinnings of Drug Action and Safety

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Traditionally, drug action and safety has involved monitoring gross physiological changes to animals dosed with drug. With increased regulatory scrutiny, understanding the mechanistic underpinnings of drug action and safety has become paramount. Furthermore, with increasing numbers of drug candidates and potential targets, earlier information on potential drug safety issues is required before deciding which compounds to take into the clinic.

Metabolomics, the global profiling of biochemicals, provides unparallel insight into the mechanistic action of drugs. The simultaneous analysis of hundreds of biochemicals and metabolites enables the identification of both on-target and off-target effects. Also, many of these biochemical changes are seen within hours of dosing, providing early-stage indication of drug safety issues.

This presentation will demonstrate the utility of metabolomics in drug safety research through a series of case studies done in collaboration with leading pharmaceutical companies.

Case Study: Fenofibrate and Phenobarbital are two known rodent carcinogens. To better understand their mechanism of toxicity, rats were dosed with fenofibrate (300 mg/kg/day), or phenobarbital (50 mg/kg/day) or vehicle. Plasma and 24-hour urine samples from rats were collected at day 2 and day 14. A metabolomics analysis yielded 496 biochemicals in plasma and 974 compounds in urine.

H2: Pharmacometabolomics

The comparison of sampling method for the analysis of endogenous metabolites in urine, serum and liver tissue

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In the field of metabolomics, the diverse sampling method and instrumental analysis method have been used to the analysis of endogenous metabolites such as amino acid and fatty acid in living organism. Endogenous metabolites affected by xenobiotics and diseases could demonstrate mechanism of interaction in biological systems. On that account, a quantitative and qualitative analysis of endogenous metabolites should be accurate to enhance our understanding of biological systems. In this study, the sampling method and condition of instrument, which is used for metabolomics, were compared. Also, metabolites profiling changed by various solvent extraction was compared using 1H nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS). The urine, serum and liver tissue used for analysis were collected by different conditions upon temperature or preservative addition to compare the metabolite change. For example, the urine and serum samples were collected at 4 degree and room temperature for comparison. In case of liver sample, perfused liver tissue and not treated liver tissue were exhibited. As the results, the spectral data of 1H NMR was changed when the analysis was performed without preservative such as sodium azide. Moreover, elimination of urea by urease treatment was important for GC/MS analysis

because some metabolites co-eluted with urea in chromatogram. In the results of diverse solvent extraction, the solvent which has a high recovery of endogenous metabolites was determined in each instrument, respectively.

H3: Pharmacometabolomics

Analysis of Hamster Urinary Metabolomic Profiles of NNK Modulated by Safrole Using Liquid Chromatography-Mass Spectrometry

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The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a documented human carcinogen. We have previously shown that safrole pretreatment induced hamster hepatic CYP2As activity and reduced urinary NNAL formation. This study evaluated the effect of safrole on NNK metabolism by metabolomic approach. Male hamsters received a single administration of safrole (1 mg/kg) or an equal volume of corn oil (vehicle control) by oral gavage nine days prior to buccal painting of NNK (1 mg/kg). Urinary metabolomic profiles were determined by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/QTOF-MS) in 24 h urine after NNK treatment. The acquired LC-MS raw data were extracted with the Agilent MassHunter Qualitative software. Multivariate data analysis was performed with GeneSpring MS software. The activation indices (hydroxy acid/total NNAL ratio and keto acid/total NNAL ratio) were significantly increased in safrole-pretreated group. The hydroxy acid /total NNAL ratios in safrole-pretreated and control groups were 3.27 and 2.74, respectively ($p=0.05$); the keto acid /total NNAL ratios in safrole-pretreated and control groups were 1.85 and 1.28, respectively ($p=0.004$). Besides, the detoxification index (NNAL-glucuronides/free NNAL ratio) were decreased in safrole-pretreated group (4.03, $p=0.06$). Our findings show the synergistic effect of safrole on metabolic activation of NNK and thus modulate the carcinogenic potential of NNK.

H4: Pharmacometabolomics

Proof-of-principle for the Effectiveness of Untargeted Plasma Metabolite Profiling to Discover Molecular Aberrations That Arise From Gene Defects and Drug Treatments

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Untargeted plasma metabolite profiling has enormous potential for the diagnosis of conditions that arise from defects in metabolism. Here, we evaluated the capability of an LC/MS-based metabolite profiling platform to provide a broad survey of the plasma metabolome and to discover the defect in purine metabolism that predictably results in mice when xanthine oxidoreductase (XOR) is inactivated, either by gene ablation or pharmacological inhibition with allopurinol. Since XOR catalyzes the conversion of xanthine and hypoxanthine to uric acid and then allantoin, we wondered whether this anticipated metabolic disturbance would be identified using untargeted metabolite profiling. Applying two distinct chromatographic modes, positive/negative ion monitoring TOF MS

and statistical analysis of the resulting data with Profiler and GeneSpring MS software, we were able to confidently survey >3,700 distinct molecular features in 1 ¬µl of murine plasma and found that 23 and 26 of these features were altered in expression by >8-fold (up or down) vs. control in xor-knockout and allopurinol-treated mice. Searching of these most differentially-expressed features against an in-house modified METLIN database revealed profound changes in purine metabolism; as expected, knockdown of XOR activity (both genetically and pharmacologically) was associated with a marked increases in xanthine and hypoxanthine levels as well as the disappearance of plasma urate and allantoin relative to control. Associated with the predicted changes in purine metabolism, numerous unanticipated changes in metabolism were observed, some readily reconciled and others not. These unanticipated changes cast new light on the functions of XOR and its unappreciated metabolic linkages. Taken together, the present studies confirm the emerging power of untargeted metabolite profiling as an unprecedented tool for biomedical discovery.

H5: Pharmacometabolomics

A new NMR-based metabolomics approach for the diagnosis of biliary tract cancer

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Abstract: Background/Aims: Biliary tract cancer is highly lethal at presentation, with increasing mortality worldwide. Current diagnostic measures employing multiple criteria such as imaging, cytology, and serum tumor markers are not satisfactory, and a new diagnostic tool is needed. Because bile is a cognate metabolite-rich bio-fluid in the biliary ductal system, we tested a new metabolomic approach to develop an effective diagnostic tool.

Methods: Biles were collected prospectively from patients with cancer (n=17) or benign biliary tract diseases (n=21) with percutaneous or endoscopic methods. Nuclear magnetic resonance spectra (NMR) of these biles were analyzed using orthogonal partial least square discriminant analysis (OPLS-DA).

Results: The metabolomic 2-D score plot showed good separation between cancer and benign groups. The contributing NMR signals were analyzed using a statistical TOCSY approach with verification. The diagnostic performance assessed by leave-one-out analysis exhibited 88% sensitivity and 81% specificity, better than the conventional markers (CEA, CA19-9, and bile cytology).

Conclusion: The NMR-based metabolomics approach provides good performance in discriminating cancer and benign biliary duct diseases. The excellent predictability of the method suggests that it can, at least, augment the currently available diagnostic approaches.

H6: Pharmacometabolomics

Endogenous Metabolites Profiling of Urine; Protective Effect of Morin on Dimethylnirtosamine (DMN) induced Hepatic fibrosis in Rats

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The morin [2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one], a kind of flavonoid found in almonds and mill, has various pharmacological properties. Recently, the protective effect of morin on dimethylnitrosamine(DMN)- induced liver fibrosis in rats was investigated through mRNA expression of TGF-Œ≤1 and Œ±-SMA. In this study, metabolomics approach, which could simultaneous systematic profiling of various metabolite concentrations, was performed using urine sample. The metabolic profiling, which is correlated with DMN induced fibrosis and the protective mechanism against hepatotoxin of morin, was determined by gas chromatography/mass spectrometry (GC/MS) and 1H NMR spectroscopy. In multivariate analysis, orthogonal-projection to latent structures discriminant analysis (O-PLS-DA) was used to maximize the class separation. Also, to identify the urinary metabolites, which could discriminate between control and treated animals, VIP (variable importance in the projection) plot obtained from O-PLS-DA, was applied the spectral data. Histopathological data and clinical chemistry measurements (AST, ALT) demonstrated the protective effect of morin. The body and liver weight was decreased concurrent with increasing severity of liver damage assessed by a histopathologic data. In GC analysis, creatine, 2-ketoglutarate, citric acid, glycine, uric acid, 4-hydroxyproline, arabitol, erythritol and D-glucose were increased in DMN treated sample. The increased level of urinary 4-hydroxyproline and glycine showed the collagen degradation mechanism during the liver fibrosis. After morin treatment to DMN treated sample, these metabolites were increased than control group, but they were significant decreased than DMN treated sample. In NMR analysis, threonine, acetate, 2-ketoglutarate, creatine, citric acid and taurine were increased and allatoin, hippuric acid and succinate were decreased. The elevated creatine concentration in DMN treated sample showed that hepatic fibrosis induced by DMN was involved in glutathione generation mechanism, which is important to protect cells from toxins such as free radicals. The level of creatine was down-regulated in morin treated sample, which demonstrated the protective effect of morin.

H7: Pharmacometabolomics

Normalization for mass spectrometry metabolomics data from urine and plasma sample of healthy human

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Data normalization is an important step for investigating metabolomics data to remove unwanted systemic error invoked from various experimental conditions. Several kinds of normalization methods are used for metabolomics data, but which one is the most appropriate method is not confirmed.

Quantile normalization is based on the assumption of underlying common distribution of intensity across samples. It is known that this is the simplest and quickest normalization method which is not required of an iterative process through microarray and proteomics data.
In this study we applied quantile normalization method to mass spectrometry (MS) metabolomics data from human urine and plasma sample and evaluated the effectiveness of this method to reduce the variation of non-biological origin.

Pre- and post-dose blood and urine samples obtained from 29 healthy male subjects after receiving tacrolimus were analyzed by MS-based non-targeted metabolomics profiling method. Partial least squares-discriminant analysis (PLS-DA) was carried out with metabolomics data as X variables and pharmacokinetic parameters as Y variables with discrimination by clearance extent. X variables with high variables important to projection(VIP) value represented significant correlation with Y variables after quantile normalization, but no significant correlation with metabolomics data before normalization.

And MS-based metabolomics data from urine samples collected from 8 healthy human volunteers before cyclosporine administration (high dose and low dose) represented more obvious grouping in principal component analysis (PCA) score plot according to the amount of administered drug after quantile normalization than before normalization.
In conclusion, quantile normalization is a simple and effective method to reduce non-biological variation from human MS-based metabolomics data and reveal the biological variance more obviously.

H8: Pharmacometabolomics

Metabolomics with LC/MS to detect sparfloxacin induced QT prolongation in guinea pig

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Several drugs induced QT prolongation were withdrawn from market. But detection of this potential toxicity is difficult with traditional toxicological experiments. We applied metabolomics approach to develop new biomarkers to detect drug induced QT prolongation. We used guinea pigs which are

suitable for measuring proarrhythmic effect due to their specific ion channel fairly similar to that of human - especially the hERG channel known to cause QT prolongation.
Guinea pigs underwent intravenous medication of sparfloxacin known to have potential QT prolongation effect. Blood and urine samples were collected before and after medication. QT intervals normalized by RR interval were measured. Plasma, urine metabolomics profiling was performed using liquid chromatography - mass spectroscopy (LC-MS).
Percent change of QT intervals had correlation with drug dose. Score plot of PCA carried out with metabolomic data showed distinct two groups; control and drug dosed groups. PLS was carried out using metabolomics data as X variables and percent change of QT intervals as Y variables. The key metabolites, essential for distinguishing between QT intervals were selected from the results of important metabolites (VIP>1.50). Theses metabolites have possibility of biomarkers to predict drug induced QT prolongation and can be applied to other drugs.

H9: Pharmacometabolomics

Pharmacokinetics can be predicted through predose Metabolomic profiling

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Background: Genetic heterogeneity alone cannot explain and predict individual‚Äôs response to drugs; as it doesn‚Äôt consider environmental and other external contributions to individual‚Äôs biological condition. So, possible alternative can be measuring and statistically modeling the metabolic phenotype of an individual before drug administration and use it to predict post-dose pharmacokinetic response. Drugs like tacrolimus where therapeutic index is narrow and inter-individual variation in pharmacokinetics is high it becomes much critical. In this study, we demonstrate ability of predose urinary metabolite profile through PLS multivariate modeling to predict pharmacokinetic parameters of tacrolimus in healthy human subjects.

Methods: Clinical trial has been conducted on 29 healthy Korean male volunteers. Predose urine samples were collected during 24hrs before tacrolimus drug dose (0.075mg/kg). The postdose tacrolimus blood concentration was measured up to 72hrs using UPLC-MS/MS and AUC (area under curve) was calculated. LC-MS based global metabolomic profiling was performed on this predose urine which measures more than 1,200 metabolite ions. This generated individual predose metabolic phenotype was then used as X (prediction) variables and AUC of tacrolimus as Y (response) variable in building PLS model.

Results: Two component PLS model shows statistically significant results as R2=0.9, Q2=0.466 and Eigen-value= 3.27. Using about 100 metabolite ions which significantly correlate with AUC having (VIP>1.5) we have built final PLS model for prediction of AUC. This model shows excellent cross-validation results and predictability. Identification of those metabolite ions (VIP>1.5) reveal that some amino acid, steroid, bile acid and nucleoside metabolic pathways are responsible for early drug intervention of tacrolimus.

Conclusion: This pharmacometbolomic approach can be used for the prediction of pharmacokinetic response (AUC) of tacrolimus using predose urine metabolite profile of an

individual. Biochemical knowledge of those metabolite markers can provide alternativecomplementary approach to the field of ‚ÄòPersonalized drug therapy‚Äô.

H10: Pharmacometabolomics

Development of a Lipidomic Platform Based on a Hybrid Quadrupole Time-Of-Flight (QTof) Ion-Mobility

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Mass spectrometry plays an important role in the study of lipid biochemistry. For example, the information it provides can be critical to understanding the mechanism of pathogenesis for diseases which are linked to abnormal physiological levels of certain lipids including atherosclerosis and diabetes.

Recent technological advances have yielded hybrid instruments such as the quadrupole time-of-flight (QTof) ion-mobility mass spectrometer which is an ideal platform for lipid analysis. This instrument possesses clear analytical advantages over conventional nominal mass instruments in full scan sensitivity, mass accuracy, spectral resolution and fragmentation. It can also provide an added dimension of separation to the analysis via ion-mobility. With the hybrid QTof it is possible to conduct class specific precursor and neutral loss acquisitions over a single experimental run using an instrument acquisition mode called elevated-energy mass spectrometry (MSE). MSE is a term which is used to describe a strategy which performs data-independent fragmentation experiments. The exact mass information obtained provides a more definitive descriptor of the molecule and is very important to removal false positives.

Applying this technology allowed the specific detection of intact molecular ions, precursor ions and neutral losses in either positive or negative ionization mode that upon collision-induced dissociation generated characteristic diagnostic fragment and neutral loss ions. However, ions yielding structural information about the fatty acid side-chains are of low abundance and typically other solvents such as LiOH (post column or addition to the mobile phase) are used to obtain structural information about them. Utilizing the unique characteristics of the ion-mobility sector of the instrument we are able to perform time-aligned parallel fragmentation experiments which yield fragment ions that facilitate assignment of the fatty-acid side chains.

In this paper, a robust LC/MS platform for detection and characterization of multiple lipid classes is described and illustrated with data from extracted human plasma samples.

H11: Pharmacometabolomics
Metabolomics of SSRI Response in Depression: Pathway and Functional Genomics

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Treatment of major depressive disorder (MDD) has changed dramatically over the years, beginning with the introduction of monoamine oxidase inhibitors and the tricyclic antidepressants and, later, the selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs). However, the detailed molecular mechanisms responsible for depression remain unknown despite of numerous studies implicating the norepinephrine (NE) and serotonin (5-HT) systems. Response to current therapies varies considerably, with approximately 40% of MDD patient nonresponders and 60% nonremitters after an initial trial of therapy. In this study members of the ‚ÄúMetabolomics Network for Drug Response Phenotype‚Äù and in partnership with members of the Pharmacogenomics Research Network embarked on a metabolomics -pharmacogenomic analysis of response to the SSRI drug escitalopram. Specifically, studies were performed with groups of 20 depressed responders and 20 nonresponders to SSRI escitalopram therapy to define metabolites that correlate with response. A series of compounds that included glycine showed significant associations with response. We then mapped these metabolomic signatures to biochemical/biological pathways, followed by the identification of genes encoding components of the pathway. For genes encoding key pathway regulators, we also initiated in-depth gene resequencing to identify common genetic polymorphisms that might be associated with variation in metabolomic signatures, thus, moving from response to SSRI treatment to metabolomic signatures to pathways to pharmacogenomics.

H12: Pharmacometabolomics
Using functional metabolomics and proteomics for the identification of novel anti-aging drugs and studying the molecular mechanisms underlying their ability to extend longevity

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The yeast Saccharomyces cerevisiae is a valuable model for unveiling the mechanisms of cellular aging in multicellular eukaryotes. Aging in yeast and other organisms can be delayed by a low-

calorie dietary regimen known as calorie restriction (CR). To define a specific pattern of metabolism that is responsible for the anti-aging effect of CR and to establish the mechanisms underlying such effect, we assessed the effect of CR and numerous mutations extending life span on the age-dependent dynamics of cellular and organellar metabolomes and proteomes, interorganellar metabolic flow, concentration of reactive oxygen species, frequencies of nuclear and mitochondrial DNA mutations, mitochondrial morphology, stress response, and apoptosis. We found that chronologically aging yeast merge a number of cellular processes, which we call modules, into a longevity network. Our findings imply that 1) yeast establish a diet- and genotype-specific configuration of the network by setting up the rates of the processes taking place within each of its modules; 2) the establishment of a network‚Äôs configuration occurs before yeast enter a non-proliferative state; and 3) different network‚Äôs configurations established prior to entry into a non-proliferative state define different rates of survival following such entry. Thus, by designing a specific configuration of the metabolic longevity network prior to reproductive maturation, yeast define their life span. We concluded that the chronological aging of yeast is a developmental program. Implementing our understanding of the longevity network, we developed a life-span assay that was used for a high-throughput screening of extensive compound libraries. We identified five groups of novel anti-aging small molecules that greatly extend yeast longevity by 1) remodelling lipid metabolism in the ER, peroxisomes and lipid bodies; 2) promoting ‚Äúmitohormesis‚Äù through the activation of a distinct set of stress response-related processes in mitochondria; and 3) modulating nutrient signaling pathways governed by the protein kinase Rim15p.

H13: Pharmacometabolomics

Distinct metabolic roles of Peroxisome Proliferator-Activated Receptor delta and gamma in murine skeletal muscle and adipose: Implications for the treatment of type II diabetes

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The Peroxisome proliferator-activated receptors (PPARs) family consists of three members: PPAR-alpha, PPAR-gamma, and PPAR-delta. PPAR-delta controls the transcription of genes involved in multiple physiological pathways, including cellular differentiation, lipid metabolism and energy homeostasis. The receptor is expressed almost ubiquitously, with high expression in adipose tissue, liver and skeletal muscle. PPAR-gamma expression is highest in adipose tissue where is has roles in the regulation of differentiation and control of lipid metabolism. Pharmacological agonists of PPAR-gamma in the form of thiazolodinediones are currently available in the clinic for the treatment of type-2 diabetes mellitus (T2DM). Although the physiological ligands of PPAR-delta remain undefined, a number of high affinity synthetic ligands have been developed for the receptor as a therapeutic target for T2DM, dyslipidaemia and the metabolic syndrome. In this study, the metabolic role of PPAR-delta activation has been investigated in skeletal muscle, liver, adipose tissue and blood serum from ob/ob mice using a high affinity synthetic ligand and contrasted with PPAR-gamma activation. In addition the action of agonists has been investigated in the 3T3-L1 murine cell line; a robust and widely used model for the study of adipogenesis and processes occurring in mature adipocytes. Proton-NMR spectroscopy, GC-MS, LC-MS and direct infusion MS were used to

examine metabolites from tissue and cell extracts in order to maximise the coverage of the metabolome. Analysis by multivariate statistics demonstrated that PPAR-delta activation profoundly affected glycolysis, gluconeogenesis, the TCA cycle and linoleic acid and alpha-linolenic acid essential fatty acid pathways and pentose sugar metabolism. Although activation of both PPAR-delta and PPAR-gamma lead to increased insulin sensitivity and glucose tolerance in the mice; PPAR-delta activation was functionally distinct from PPAR-gamma activation, and was characterised by increased fatty acid oxidative metabolism, demonstrating the distinctive physiological role of this receptor compared with PPAR-gamma

H14: Pharmacometabolomics

Metabolomics analysis on complex Chinese remedies - Examples from a series of Aristolochic Acid containing remedy induced Nephrotoxicity in Rodents

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Metabolomics study of urine using 1H NMR spectroscopy is a non-invasive approach to detect nephrotoxicity. Aristolochic acid (AA) is a potent nephrotoxicant in humans and rodents. Traditional renal function indicators such as serum creatinine and urea nitrogen are not sensitive to detect early acute renal injury. AA induced Nephrotoxicity detection has been successfully performed with metabolomics in literature. However, it was performed mostly on single AA standard compound.

We were interested in understanding the complex nature of Chinese herbal remedy not just with its single component but mixture at molecular systems biology level. We designed a series of AA, an AA containing herb Aristolochia contorta and one commonly used mixture A. contorta containing herb Bu-Fei-A-Jiao-Tang (BFAJT) in rodents to pinpoint the effect of AA nephrotoxicity in molecular level.

Rats were given with AA 0, 4 and 8 mg/kg, mice were given AA 0, 5 and 7.5 mg/kg, Fructus A. contorta 0, 559, 1118, and 2236 mg/kg (with equivalent AA-I 0, 15, 30 and 60 mg/kg) and BFAJT 0, 2 and 4 g/kg (with equivalent AA-I 0, 7.5 and 15 mg/kg) in 3 separate experiments. With chemometric approach including 1H NMR spectra preprocessing and principal component analysis (PCA), different groupings were found within several days (2-10 days) after treatment. Scoring plots of PCA showed the groupings were time, dose and AA source dependent. Renal pathology showed acute renal injuries at end of experiment in all high AA containing groups. Lactate, acetate, glucose hipurrate and creatinine were among urine metabolites significantly related with AA nephrotoxicity. With metabolomics, it is possible to detect AA nephrotoxicity at early stage and at molecular level.

I01: Plant/Phytochemical Metabolomics

Use of reverse phase liquid chromatography linked to tandem mass spectrometry to profile Calvin cycle and other metabolic intermediates in Arabidopsis rosette leaves

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Understanding the regulation and integration of primary metabolism is one of the major challenges in plant biochemistry. Within this perspective, quantitative determination of metabolic intermediates, particularly sugar phosphates, which are notoriously difficult to separate and quantify, is required.

For this purpose, we developed and validated a convenient, highly selective and sensitive reverse phase liquid chromatography‚Äìtandem triple quadrupole mass spectrometry (LC‚ÄìMS/MS) method. It allows identification and quantification of 28 negatively charged metabolites from the Calvin cycle, sucrose and starch synthesis, glycolysis and organic acid metabolism, in Arabidopsis leaves. Metabolites that could not be reliably measured, such as glycerate-3-phosphate, pyruvate and ATP, were determined enzymatically. To overcome ion suppression, eight isotope-labelled authentic standards, either commercially available or enzymatically synthesised and purified by paper chromatography, are used. Additional ten labelled compounds are being currently prepared.

The applicability and biological utility of this new approach was established by experiments on Arabidopsis leaves. We compared the levels of intermediates of the Calvin cycle and associated pathways during photosynthesis in ambient, limiting and compensation point CO2 concentrations. The levels of most Calvin cycle intermediates are unaltered at compensation point CO2, whereas the levels of metabolites in end-product synthesis pathways decrease strongly. This has important implications for the regulation of photosynthetic carbon metabolism.

Our main goal is to understand the regulation of photosynthetic metabolism at the subcellular level. The fractionation of cells under non-aqueous conditions has been optimized for Arabidopsis leaves, allowing the separation of chloroplasts, cytosol and vacuoles. The newly established LC-MS/MS method has been used to measure the subcellular metabolite levels in Arabidopsis leaves. In the near future, we will measure metabolites fluxes in and between compartments, under different environmental conditions and in various Arabidopsis mutants.

I02: Plant/Phytochemical Metabolomics

Metabolic profiling to discriminate six-row barley genotypes varying in resistance to fusarium head blight, based on LC-ESI-LTQ Orbitrap

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Fusarium head blight (FHB) is one of the most devastating diseases of barley and wheat. Occurrence of more than 100 resistance QTLs indicate the presence of several resistance mechanisms. The objectives of this study were to identify metabolites involved in resistance and to discriminate resistance phenotypes. Barley genotypes Chevron (Resistant) and Stander (Susceptible) were grown in greenhouse, spikelets were inoculated with mock (M) or pathogen (P) and samples were collected 48 h post inoculation. In addition, the disease severity was assessed as proportion of spikelets diseased. The genotype Chevron was more resistant than the Stander (PSD = 0.37 and 0.88; AUDPC = 3.34 and 8.83). Metabolites from spikelets were extracted in methanol and water, and analyzed in negative mode of ionization using LC-nESI-LTQ-Orbitrap. The output was imported to XCMS, peaks were deconvoluted and consistent peaks were retained. Out of 1826 consistent peaks, a student,Äôs t-test identified 497 metabolites with significant treatment effects. Among these, 194 were resistance related constitutive metabolites (RRC = abundance of RM>SM) and 82 were irregular RR induced metabolites (iRRI =RP>SP). A total of 61 metabolites were assigned with putative names based on accurate mass, isotope ratio and fragmentation pattern. The RR metabolites mainly belonged to phenylpropanoid (quinic acid, 7-O-(4-methoxycinnamoyl) tecomoside, seselinol isovalerate), flavanoid (naringenin 7-glucoside, 6-prenylnaringenin, kaempferide 3-glucoside-7-rhamnoside), lignan (dihydrocubebin), fatty acid (Omega-Hydroxydodecanoic acid, methyl-decanoic acid) and terpenoid (juanislamin, isofurocaespitane) metabolic pathways. The RR metabolites, coumarins and naringenin glycoside are known antimicrobials. Increased flavonoid biosynthesis pathway (kaempferol) indicates a possible defense against stress. Fatty acids identified here also are antimicrobials and in addition they reduce pathogen penetration by forming wax layers. The phenylpropanoid metabolites lead to the cell wall enforcement, through formation of lignins, thus reducing pathogen spread.

I03: Plant/Phytochemical Metabolomics

Gene and Metabolite Prospecting as a Platform to Identify Novel Benzylisoquinoline Alkaloid Biosynthesis

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Benzylisoquinoline alkaloids (BIAs) are a structurally diverse group of ~2,500 specialized metabolites found mostly in a limited number of related plant families. Many BIAs exhibit potent pharmacological activities including morphine and codeine (narcotic analgesics), berberine and sanguinarine (antimicrobials), (+)-tubocurarine (muscle relaxant), papaverine (vasodilator) and noscapine (cough-suppressant). The chemical diversity of BIAs results from (1) the reconfiguration of the original benzylisoquinoline moiety via the formation of novel C-C or C-O bonds, with each new structural backbone leading to one of several branch pathways, and (2) the decoration of various BIA structural variants with a variety of functional group and covalent bond modifications (e.g. oxidation, reduction, hydroxylation, methylation, acetylation and glycosylation) catalyzed by a suite

of categorically conserved enzymes. We have established a platform based on targeted metabolite profiling, modest expressed sequence tag (EST) libraries and relevant bioinformatics tools to tap into the rich diversity of BIA biosynthetic pathways in plants. Cell cultures of 18 BIA-producing plant species, representing four related plant families (i.e. the Papaveraceae, Ranunculaceae, Berberidaceae and Menispermaceae) were analyzed by Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS) and liquid chromatography electrospray ionization-tandem mass spectrometry (ESI-MS/MS) to establish accurate alkaloid profiles for each cell culture. Approximately 6,000 clones from a cDNA library prepared for each of the 18 cell cultures were subjected to random dye-termination sequencing. Assembly of the empirical metabolite profiles from each species into putative biosynthetic networks inferred the occurrence of myriad pathway intermediates and products, and revealed the active BIA branch pathways and modifications in each system. Integration of known and candidate biosynthetic genes in each EST database into the corresponding biosynthetic network has facilitated the identification of new enzymes involved in the elaboration of BIA diversity. The utility of this comparative and integrative approach to the discovery of novel specialized metabolism genes will be discussed.

I04: Plant/Phytochemical Metabolomics

Deciphering how metabolic networks re-program through metabolomics

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Metabolome data has significant number of correlations between metabolite concentrations when repeated samplings across individual plants are carried out. This is different from transcriptome and proteome data in that correlated metabolites are not always likely to be involved in a common biological function. However, the approach based on such metabolomic correlations is apparently widely used in metabolomics (e.g. principal component analysis; PCA). Little studies have been performed on the comprehensive and systematic comparison of the metabolomic correlations across different times, tissues, genotypes, and stress treatments. To elucidate the influence of sampling point in time [1] and global stress treatment (additional nitrogen source or not) [2], we highlighted comparative metabolite-metabolite correlation derived from gas chromatography-time of flight/mass spectrometry (GC-TOF/MS)-based metabolomics in Arabidopsis thaliana and Oryza sativa. Such meta-analysis with metabolomic data toward systems biology can accelerate the investigation to fill in the missing blank on our knowledge about how cellular processes cooperate.

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I05: Plant/Phytochemical Metabolomics

NMR Metabolome Analysis of Dang Gui Botanical Dietary Supplements

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According to pharmacopoeial and GMP guidelines, one key aspect of the quality control (QC) of botanical dietary supplements (BDSs) is the authentication of the plant material and identification of subsequent products such as extracts. Extracts of the roots of Angelica sinensis (syn. Dang Gui) represent an example of a phytochemically and therapeutically well-established BDS, and are among the most popular Traditional Chinese Medicines in the US market. Hydroalcoholic and hot water extracts have traditionally been used for gynecologic indications and as a general blood tonic, and recent research has focused on the evaluation of properties such as cardiovascular, hematopoietic, antioxidant, and immunomodulatory.

Characteristic, bioactive secondary metabolites contained in A. sinensis extracts are alkylphthalides, in particular Z-ligustilide and Z-butylidenephthalide, ferulic acid derivatives, polyynes, and fatty acids such as linoleic acid. For the unambiguous identification of authentic plant material, a variety of methods have been employed to detect the presence of the above mentioned compounds. QC measures typically employ chromatographic methods such as TLC or GC/LC-UV/MS. However, the qualitative and quantitative potential of 1D and 2D nuclear magnetic resonance (1D/2D NMR) has not been systematically studied for the QC of Dang Gui BDSs. This study aims at the establishment of a metabolomic NMR method that allows establishment of botanical identity and purity of Dang Gui (A. sinensis) preparations by means of a two-step analysis: (a) solid phase extraction to obtain a defined, lipophilic portion of the metabolites; (b) 1D and 2D NMR metabolomic analysis. The latter includes identification of marker signals that can be assigned to characteristic metabolites, which allow unambiguous botanical identification.

I06: Plant/Phytochemical Metabolomics

Metabolomics: A Powerful Tool for Wine Research

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Metabolomics is the study of the whole complement of small chemicals in a biological sample. Wine represents one the most complex chemical systems with contributions from several different biological organisms an enological practices. Metabolomics allows for the isolation and characterization of the relative contributions of each of these factors and different stages of fermentation. Our study compared Merlot (Vitis vinifera L. var Merlot)grapes, barrel samples and wines from the north and south Okanagan for total metabolite content and differences in the chemical composition. The number of compounds observed in the grape (3719 to 4016), barrel (6278 and 7696), and wine (6867 to 7645) samples did not vary greatly for the wineries sampled relative to the distance between the winery locations. Within the total number of compounds isolated in the grape tissue, 3665 compounds were shared between grapes grown in conditions typical of the northern part of the Okanagan Valley and those located in the south of the Valley. However, over 2000 additional compounds in the grape tissue were specific either to the north or to the south of the Okanagan Valley. The overall trend showed an increase in compounds from grape to barrel to wine and was attributed to yeast and bacteria metabolisms in both the primary and secondary malolactic fermentation. One of the principle components with an exact mass 231.1131m/z was found at high concentrations in the wines, moderate concentrations in barrels and trace amounts in grapes demonstrating how the chemical composition changes during the fermentation and processing. These data demonstrate the potential of metabolomics as a tool for characterization and ensuring quality in wines.

I07: Plant/Phytochemical Metabolomics

A streamlined FTMS platform for high throughput plant metabolomics

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Introduction: There are up to 200,000 primary and secondary metabolites in the plant kingdom. Given the complexity in molecular size and polarity of these metabolites and the magnitude of biological and technical replicates to be analyzed, plant metabolomics posts significant challenges in omics research. High throughput analytics and cutting-edge bioinformatics are necessary for successful metabolite detection, identification and quantification. In this work, we provide a streamlined direct infusion-FTMS platform for plant metabolomics.

Methods: Plant tissues or cells were pulverized in liquid nitrogen and extracted with water/methanol/chloroform or water/ethyl acetate/acetonitrile. The metabolite extract pools were either directly infused into a 12-T FTMS instrument or further partitioned into aqueous/organic phases. Full-scan MS datasets were acquired in both (+) and (-) ESI modes with or without mass spectral stitching acquisition. A comprehensive software package was developed for automation of mono-isotopic peak picking, background mass exclusion, post-acquisition internal mass calibration, adduct ion/charge states discrimination, intensity normalization and peak alignment. The software package also incorporated automatic molecular formula generation followed by preliminary metabolite identification through searching available metabolome databases. This pipeline was

tentatively used to profile the metabolites in chitosan-elicitated spruce cell culture and transgenic Brassica seeds as the proof-of-principle studies.

Results: We routinely detected thousands of metabolite features in each plant sample and successfully assigned many features to metabolite candidates in plant metabolome databases in a streamlined way. Our results have showed that liquid-liquid partition and mass spectral stitching efficiently enhanced the metabolite detection; the integrated software pipeline rendered reliable data processing and elucidation in a high-throughput manner. Dozens of primary and secondary metabolites were detected as up- or down-regulated in both cases in correlation to the specific treatment and the genetic modification. As a result, this study has demonstrated the usefulness of the platform for high throughput-compatible plant metabolite fingerprinting by chromatography-free FTMS.

I08: Plant/Phytochemical Metabolomics

1H NMR-based Metabolomic Profiling of Key Water Soluble Metabolites in *Arabidopsis thaliana* during Freezing and Thawing Affected by the Lipophilic Components of Brown Seaweed *Ascophyllum nodosum*

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Lipophilic components of the brown seaweed Ascophyllum nodosum improve freezing tolerance in Arabiodpsis thaliana. The physiological, biochemical and metabolic changes elicited by A. nodosum extracts that imparts stress tolerance, however, remain largely unknown. 1H NMR metabolite profile of A. nodosum extract treated Arabidopsis exposed to freezing stress revealed a spectrum dominated by chemical shifts (Œ¥) representing lipophilic components like fatty acids and accumulation of sugars, as compared to control plants. Additionally, 2D 1HNMR suggest an increase in the unsaturation of fatty acids in plant treated with lipophilic component under freezing stress.

For water soluble metabolites, a targeted metabolite profiling tool was used and several key polar metabolites such as glucose, sucrose, proline, choline, alanine, glutamine, pyroglutamate, asparagine, 3-phenyllactate, adenosine, and uridine, were identified and tentatively quantified. Based on multivariate analysis using PCA and PLS-DA, A. nodosum components had induced greater metabolite changes in Arabiodpsis thaliana during the thawing period than in the freezing stage, as compared with the control.

In summary, our results suggest that A. nodosum mediates freezing tolerance in Arabidopsis by priming the plant to accumulate higher concentration of proline and sugar and by increasing the unsaturation of fatty acid possibly altering the fluidity of cell membrane.

I09: Plant/Phytochemical Metabolomics

Metabolic profiling on flax varieties: a first step to develop selection-dedicated tools

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Because of its interest for human health, the use of flaxseed (Linum usitatissimum L.) is actually growing. Lignans (in the seedcoat) and high omega-3 fatty acid content (in the embryo) are two targets for the breeding of high quality varieties. The beneficial effects of these compounds on human health are now well recognised. Lignans - and other phenylpropanoid derivatives - appear to be anticarcinogenic compounds, whereas omega-3 fatty acids are known to reduce heart disease and would be helpful in the case of inflammatory diseases such as rheumatoid arthritis. Besides applications of flaxseed components reported in pharmaceutical, food and cosmetic products, it can be used to feed animals and poultry, once processed. It is therefore of interest to have a variety selection tool of flaxseed based on its metabolite content.

Here we report the development of such a method by using MS and NMR-based metabolomics after optimisation of the extraction process by microwave. 1H NMR spectra coupled with principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were applied to investigate the metabolite variations in different flaxseed varieties. It was notably observed a negative correlation between the lignan and the omega-3 fatty acid contents.

I10: Plant/Phytochemical Metabolomics

Quality control and discrimination of Scrophularia spp. based on HPLC-UVD and multivariate analysis

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A high-performance liquid chromatography coupled with diode array detector (HPLC-DAD) method for the simultaneous determination of angoroside C, harpagoside, 8-O-(E-p-methoxycinnamoyl)harpagide, E-cinnamic acid and E-p-methoxycinnamic acid of Scurophularia

spp. was developed. This method was validated in terms of specificity, linearity, precision and recoveries. The validated method was successfully applied to quantitatively analyze five components in Scurophularia spp. In addition, the samples were clustered according to their geographic origins by a principal component analysis (PCA). A partial least squares-linear discrimination analysis (PLS-DA) was subsequently developed for the effective classification of the samples. The proposed method shows an efficient strategy for quality control of Scurophularia spp.

I11: Plant/Phytochemical Metabolomics

Metabolomics reveals the crucial role of cytosolic glutamine synthetase 1;1 in maintaining the metabolic balance for normal growth of rice seedlings

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Rice plants grown in paddy fields preferentially use ammonium ions as a source of inorganic nitrogen. Glutamine synthetase (GS) catalyzes the conversion of ammonium ions to glutamine. Of the 3 genes encoding cytosolic GS in rice, Os-GS1;1 is a critical regulator of normal growth and grain filling. However, its physiological function in the regulatory relationships between nitrogen assimilation, growth, and metabolic networks remains unclear. To address this issue, metabolite profiling was performed using gas chromatography,Äîtime-of-flight mass spectrometry (GC-TOFMS). The metabolite profiles of mutants lacking Os-GS1;1 showed (1) an imbalance in the levels of sugars, amino acids, and metabolites belonging to the tricarboxylic acid (TCA) cycle in the third leaf blade (LB), and (2) overaccumulation of secondary metabolites containing nitrogen group in the roots under a continuous supply of ammonium. Metabolite-correlation analysis revealed the appearance of new metabolic networks between tryptamine,Äìother metabolites belonging to central metabolism in the mutant roots. Furthermore, the metabolite profiling together with quantitative real-time polymerase chain reaction (qRT-PCR) analysis reveals the non-redundant and indispensable role of Os-GS1;1 in maintaining metabolite levels required for normal growth in rice in the presence of ammonium ions.

I12: Plant/Phytochemical Metabolomics

Metabolomics of plant and necrotrophic pathogen interaction

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Necrotrophic pathogens produce toxins and enzymes to kill plant cells at the site of invasion so that they can obtain food for continued growth. The plants defend pathogen attack based on constitutive and induced biochemicals, in addition to structural and morphological resistance to invasion. The biochemicals can be either metabolites or proteins. Metabolomics was applied to better understand the mechanisms of resistance in barley against a necrotrophic pathogen, Fusarium graminearum. The spikelets of barley genotypes varying in resistance were spray inoculated with pathogen and the disease severity, as proportion of spikelets diseased in a spike, was assessed. The mock or pathogen inoculated spikelets were harvested, metabolites extracted and analyzed using a hybrid mass spectrometer (LC-nESI-LTQ OrbiTrap), in both negative and positive ionization modes. The raw data was aligned using XCMS and sieved for adducts using esi/camera. Depending on the study, about 5000 peaks/accurate masses were consistently detected over replicates, of which about 600 metabolites had significant treatment effects. A t-test identified about 200 resistance related (RR) metabolites, of which more than150 were assigned putative names of identity based on accurate masses, isotope ratios (IntelliXtract), and fragmentation patterns using several databases (METLIN). These metabolites were searched in the metabolic pathway databases to identify their pathways of synthesis. The RR metabolites mainly belonged to four metabolic pathways: lipid/fatty acid, phenylpropanoid, flavonoid and terpenoid. JA signaling is one of the major ways to resist F. graminearum and instead of SA more phenylpropanoids and flavonoids, known antimicrobials and cell wall enforcing compounds, were detected. Some terpenes detected are known phytoallexins. The potential application of these RR metabolites to identify functions of FHB resistance QTLs, and screening advanced breeding lines for resistance to FHB will be discussed.

I13: Plant/Phytochemical Metabolomics

GC-MS based Analysis of Essential Oil and Anatomical Characterization of Oil Cell from Flowers of Litsea euosma

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Litsea euosma was a deciduous woody species from the family Lauraceae. It is native to Southern and South-Western China. Its flower and fruit are very aromatic and traditionally used by local people to make spicy food and to treat different diseases, e.g. abdominal pain, edema and rheumatism associated with arthritis. In this study, a supercritical fluid extraction through carbon dioxide was used to extract essential oils from flowers collected at different flowering times. GC-MS analysis was used to identify metabolites of essential oils and estimate the levels of individual components. The contents of total essential oil from flowers obviously declined from flower buds (15.14%) through wilting flowers (0.26%). Thirty-four components of essential oils were identified throughout the whole flowering period, mainly belonging to alcohol, aldehyde, alkane, ester, ketone, lactone, and terpenoid. Eucalyptol, terpineol, and tetracosane were constitutively produced throughout the whole flowering period. Microscopical analysis was performed to characterize anatomical location of essential oil formation. Idioblast oil cells were identified in mesophyll of bracts and perianths. The structure of mature oil cells was characterized by an elliptical or spherical shape and a large oil sac but without a nucleus and an evident cytoplasm. The size and density of oil cells slightly changed from flower buds to wilting flowers. Oil cells are suggested to be the main cellular location involved in the accumulation of essential oils. The study suggests that flower buds and early opening flowers are appropriate materials to obtain a high production of total essential oils.

I14: Plant/Phytochemical Metabolomics

Oxidative stress responses in ozone-sensitive Arabidopsis RCD1 mutant: transcriptional and biochemical characterization

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The ozone insensitive rcd1-1 (radical-induced cell death1) mutant of Arabidopsis has been shown to be defective in the containment of programmed cell death and in the signalling of several plant hormones. The Radical-Induced Cell Death1 (RCD1) protein of Arabidopsis thaliana is thought to be an integrative node in plant stress and developmental signalling. Its biochemical function is unknown, but knocking this gene out causes a large number of phenotypes, including alterations in hormonal signalling, development and progression of cell death. RCD1 belongs to a novel gene family with 5 unknown genes encoding proteins distinctively similar to RCD1 (SRO1-SRO5; SIMILAR TO RCD-ONE 1-5). Interestingly, a conserved domain of ADP-ribosylation has been assigned to all the RCD1-SRO proteins. RCD1 appears to have partially overlapping functions with at least SRO1, because rcd1-sro1 double mutant plants have a severely stunted phenotype even in control conditions.

In addition to global gene expression with microarrays, we have accomplished metabolite profiling of the rcd1-1, and Col wild type, both after stress treatments and in control condition by HPLC-MSn and GC-MS. Recent HPLC-MSn and GC-MS analysis have revealed differences between Col and rcd1-1 in the diurnal rhythm of some amino acids and their TCA cycle intermediates, respectively. The observed metabolic changes are discussed in respect to the latest results obtained from microarrays.

I15: Plant/Phytochemical Metabolomics

Detection of Volatile Compounds Released in Vine Canopies for use in Field Measures of Fruit Maturity

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Plants interact with the environment through the emission of volatile compounds. Volatile compounds comprise an important part of plant secondary metabolism. Biotic and abiotic factors affect the identity, quantity and timing of their release. Despite our understanding of the strong correlation between plant status and volatile emissions, little work has been performed to investigate the identity of such compounds in Vitis vinifera. By measuring and identifying compounds present in vineyards and correlating them to vine or fruit states (healthy, diseased, under-ripe, mature, water stressed, etc.) we intend to discover key marker compounds that could inform viticulturalists about a vine or vineyard‚Äôs status, and more specifically, on the best time to harvest. Once volatile biomarkers are identified, tools (e.g. hand-held electronic noses) can be developed to detect them. These tools will allow for real-time measures in the field, and could change the way that agricultural management decisions are made.

Vineyard volatiles were monitored using Twister-based sampling techniques. Volatiles were thermo-desorbed for GC-TOF-MS analysis. To facilitate automated annotation and database construction, all samples were spiked with a mixture of retention index (RI) markers (fatty acid methyl esters C4-C24) prior to analysis. Database capabilities for storing, comparing and disseminating volatile compounds from GC-TOF-MS data have been established and implement similar algorithms employed in the Fiehn laboratory for liquid injections of TMS-derivatized metabolite profiles (BinBase version 3.3).

Vineyard volatiles were sampled in three Cabernet Sauvignon blocks during the 2008 growing season. Automated annotation of the 1120 sample chromatograms by the Fiehn Lab Volatile BinBase yielded more than 600 reliably detected compounds. Partial least squares regression analysis of data has revealed correlations between a subset of canopy volatiles and traditional measures of grape berry development including ¬∞Brix, pH and TA values. Identification of key compounds by retention index and mass spectral matches is on-going.

I16: Plant/Phytochemical Metabolomics

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

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Phymatotrichopsis omnivora is a devastating pathogen that causes substantial economic losses in more than 2000 dicotyledonous plant species including alfalfa. Currently, no cost effective chemical control methods or sources of genetic resistance have been identified for P. omnivora. Here, a metabolomics approach was used to study the complex biochemical interactions between the model legume Medicago truncatula and P. omnivora to enhance opportunities for developing resistance to this devastating pathogen. Temporal analyses between 0 and 11 days post-inoculation revealed massive metabolic changes. GC/MS revealed decreased sucrose and increased mannitol, arabitol, proline and trehalose in plant roots following fungal infection. Contrary to many Medicago-fungal interactions, secondary metabolite profiling by UPLC-MS revealed no significant increase in medicarpin; which is the typical and predominant isoflavonoid induced during Medicago-fungal interactions. Interestingly, increased flavone levels were observed and particularly increased 7,4,Äô-dihydroxyflavone. We conclude that P. omnivora circumvents traditional Medicago defense responses by suppressing isoflavonoids/medicarpin biosynthesis, while simultaneously inducing flavonoid biosynthesis. Quantitative RT-PCR of suggests that the suppression of the isoflavonoid pathway was at the transcript level and less likely fungal catabolic detoxification of isoflavonoids. In vitro growth inhibition assays revealed that medicarpin and 7,4,Äô-dihydroxy flavone possess significant anti-microbial activity against P. omnivora and suggest that increased constitutive levels of these compounds represents a strategy for future metabolic engineering of alfalfa resistant to P. omnivora.

I17: Plant/Phytochemical Metabolomics

Spatially Resolved, Integrated Transcriptomics and Metabolomics Reveal Enhanced Secondary Metabolism in *Medicago truncatula* Root Border Cells

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The root tips of many plant species, including legumes, produce thousands of differentiated cells which remain appressed to the root until separated from the root by exposure to water. These cells, termed border cells, provide a biotic boundary fundamental in ecological interactions, rhizosphere modifications, and plant defense. Unfortunately, little is known about the molecular or biochemical roles of these cells in plant-microbe interactions. Here, we report a systematic evaluation of the integrated molecular and biochemical differences between border cells, root tips, and whole roots of the model legume Medicago truncatula.

Border cells begin their life as root cap initial cells. They then evolve as columella and peripheral root cells, which ultimately transition to border cells. Starch deposition is increased in the root cap cells as determined by staining and serves as a carbon reserve for developing root cap and border cells. The comparative microarray data revealed dramatic increases in border cell Œ≤-amylase resulting in increased starch hydrolysis. Increased glycolysis intermediates such as glucose-6-P, fructose-6-P, fructose and increased TCA cycle components support an increase in central carbon metabolism. Increases were also observed in branched chain amino acids and polyamines such as Œ≤-alanine which were associated with increased CoA biosynthesis and carbon shuttling into secondary metabolic pathways. Both flavonoid and triterpene pathways were also increased in border cells with specific increases in compounds associated with defense and signaling observed.

We conclude from the cumulative constitutive data that border cells have an enhanced metabolic capacity and content relative to root cap and whole root. Root tips have increased starch deposition and as these cells transition into border cells, the border cells expend their starch reserves for fortification of primary and secondary metabolism better enabling border cells as front-line defenders in plant-pathogen interactions and important ambassadors in mutualistic signaling.

I18: Plant/Phytochemical Metabolomics

Quality vs Quantity: Metabolomics in cereal crop improvement

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Cereals are major sources of human nutrition either directly or indirectly through animal food, and breeding of new varieties with improved nutritional and other quality parameters, as well as stress and pest resistance is a major research activity. We are applying metabolite profiling and metabolomics to assist in understanding biochemical processes leading to quality enhancement , biomarker discovery and QTL localisation. We are involved in a number of projects involving both field- and CE-grown cereals. These range from studies of the development of Fusarium sp fungal infections of wheat ears to studies of the effects of different S and N fertiliser regimes. The talk will mainly draw on examples of the use of, high-throughput, NMR-ESI-MS screening and targeted phytochemical analysis in two major EU-funded projects . HEALTHGRAIN is a project concerning health promoting compounds including phenolics , folates, methyl-donors and phytosterols and involves a diversity screen of grains of over 200 different cereal cultivars as well as G X E assessment of metabolome variability. A component of the EU META-PHOR project involves metabolomic analysis of rice (leaf and grain tissue) in the context of the production of fragrance, an important metabolite-based quality that provides an economic premium in basmati and jasmine varieties. In both projects statistical correlation of metabolomic data against quality parameters, including other phytochemical data, has proven very informative for breeders in ‚Äúbest‚Äù variety selection.

I19: Plant/Phytochemical Metabolomics

Differentiation of cultivars of Ganoderma lucidum by NMR-based metabolomics approach

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Correct identification of the cultivars of oriental medicine is important for proper quality control. To develop an efficient way of discriminating the cultivars, we carried out metabolomic differentiation of the cultivars for a mushroom Ganoderma lucidum, a widely-used and high-value oriental medicine. We collected samples from various regions in two countries, Korea and China, and obtained NMR spectra of the extracts. Although conventional principle component analysis showed some overlaps, orthogonal projections to latent structure discriminant analysis provided clean distinction between samples from the two countries. Contributing signals were also identified using S-plot, and further verified with independent t-test. Final validation of the model was obtained using prediction test of the unknowns, where the model predicted all of the 14 test samples correctly. We also showed that the cultivars of the Chinese samples can be differentiated with similar methods. The easiness and transferability of our NMR-based approach should contribute to addressing an important aspect of quality control process of Ganoderma lucidum. We believe the method can be easily applied to other herbal medical products.

I20: Plant/Phytochemical Metabolomics

Rapid determination of S-Adenosylmethionine and S-Adenosylhomocysteine in plant tissues by UPLC-Tandem Mass Spectrometry

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S-Adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), the components of the methionine cycle, are the substrate and product of methyltransferase reactions. SAM is produced from an ATP-dependent transfer of adenosine to methionine and serves as the proximal methyl donor for methylation reactions of a host of compounds, including phospholipids, proteins, histones, neurotransmitters, DNA and RNA. SAH, the demethylated product of these reactions, is a competitive inhibitor for several SAM-dependent methyl transferases. Altered SAM and SAH levels are found in several human disease states. HPLC coupled with ultraviolet detection is frequently used for measuring their concentrations in red blood cells and lymphocytes. Recently, LC-tandem mass spectrometry was used to measure SAM and SAH in plasma and cerebrospinal fluid. In plants, SAM is the precursor of ethylene and polyamines, which are important signalling molecules in plant stress tolerance. Plants tissues are usually more complex than plasma and cerebrospinal fluid and thus pose a challenge for the determination of SAM and SAH. Here, we report a sensitive and rapid method for the measurement of SAM and SAH in plant tissues using solid-phase extraction and UltraPerformance LC-tandem mass spectrometry.

I21: Plant/Phytochemical Metabolomics

Metabolomic Applicaiton to Determine Blanching Effects on Metabolite Profile and Bioactivity of *Chrysanthemum coronarium* L.

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Chrysanthemum coronarium L. is an annual herbaceous plant widely distributed in the Mediterranean region and its blanched stem and leaves has been regarded as a health food in East Asia. In addition to these common nutrients some compounds responsible for the chemoprevention of cancer and other disease are through to be contained in it. Our approach has applied LC-MS/MS based metabolomic characterization of the secondary metabolites of C. coronarium L. based on blanching process from the samples grown in four different geographical origins in South Korea (Pocheon, Seoul, Yongin, and Yeoju). After LC-ESI MS analysis, XCMS software is used for automated baseline correction and alignment of all extracted mass peaks across all samples. The information in the data matrix was observed using principal component analysis (PCA) and partial least squared-discriminant analysis (PLS-DA). A significant separation among plants was reveled

according to blanching or not. The major metabolites of flash samples contributing to the separation were elucidated as quinic aicd, dicaffeolyquinic acid, chlorogenic acid, and succinyl dicaffeoylquinic acids by PCA and PLS-DA loading plots and its peaks were identified or tentatively annotated with in-house metabolite database, which has 5,000 MS/MS spectra of plant and microbial secondary metabolites. According to contributed component, it was deduced that the flash sample showed higher antioxidant activity than blanched one, and as decreased antioxidant compounds of blanched one, the antioxidant activity was also decreased in various antioxidant assay systems. The other clustering was presented according to the induction of NQO1 (NAD(P)H dehydrogenase) and blanched samples. Candidate metabolites for this separation could be predicted to mycosinol derivative and several unidentified peaks.

I22: Plant/Phytochemical Metabolomics

Evaluation of extraction solvents for comparative metabolome analysis and profiling plant metabolites throughout extraction processes using two-dimensional 1H-13C NMR spectra

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NMR techniques can provide information on the global pool of all measurable metabolites, including both soluble low molecular weight compounds and insoluble metabolites. At first, we describe the evaluation of extraction solvents that can be applicable for a wide range of organisms and their application to comparative metabolome analysis using 13C-labeled plants, animals and microorganisms. In metabolomic analyses, care should be exercised as to which metabolites are extracted from the sample and which remain in the residue; the remaining metabolites are typically discarded following the extraction process. The second, we tried to visualize plant metabolite profiles throughout a series of repeated extraction process. The metabolites remaining in the extraction residues of 13C-labeled Arabidopsis thaliana were recovered by repeated extraction with methanol-d4 (MeOD) and deuterium oxide (D2O). The soluble extracts and residual pellets from each step of the extraction process were analyzed by both solution-state and high-resolution magic angle spinning (HR–MAS) NMR. Metabolic profiling based on chemical shifts in two-dimensional 1H–13C heteronuclear single-quantum coherence (HSQC) spectra allowed elucidation of both structural and chemical proprieties. In addition to the metabolite profile, we will discuss a relationship between metabolite structure and behavior along the repeated extraction process.

I23: Plant/Phytochemical Metabolomics

Inter-laboratory reproducibility of fast gas chromatography - electron impact - time of flight mass spectrometry (GC-EI-TOF/MS) based plant metabolomics

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The application of gas chromatography-mass spectrometry (GC-MS) to the ‚Äòglobal‚Äô analysis of metabolites in complex samples (i.e. metabolomics) has now become routine. The generation of these data-rich profiles demands new strategies in data mining and the need for standardisation of experimental and reporting aspects across laboratories. In parallel, food policies demand ever stricter surveillance of food quality and safety. Quality traits such as fragrance, taste, appearance, shelf-life and nutritional value are determined and reflected by the biochemical composition of the food we consume. As part of the META-PHOR project‚Äôs (METAbolomics for Plants Health and OutReach: http://www.meta-phor.eu/) priorities towards robust technology development, a GC-MS ring experiment based upon three complex plant matrices (melon, broccoli and rice) was undertaken between three sites: Manchester, UK; Golm, DE; LECO, DE. All sample preparation, data processing, multivariate statistical analyses and comparisons of major metabolite features followed standardised protocols. As typical for GC-TOFMS analyses, minor method variants were employed between the laboratories, although identical models of GC (Agilent 6890N) and TOFMS (Leco Pegasus III) instrumentation were employed; in addition comprehensive GCxGC-TOFMS was also compared. Comparisons of the paired data from the various laboratories were made with a single data processing and analysis method (pre-selected from an assessment of seven different data analysis work flows). The procedure provided unbiased assessments of the different analytical method variants, the inter-laboratory reproducibility, and the almost equivalent suitability of a range of data processing and statistical analysis routines. By comparing cluster plots generated by principal components and independent component analysis (PCA and ICA) as well as directly comparing the levels of key metabolites reported by each of the laboratories, high inter-laboratory reproducibility of qualitative GC-TOFMS based metabolomics was revealed. Further investigations of long-term reproducibility are required, though the future generation of global and valid metabolomics databases offers much promise.

I24: Plant/Phytochemical Metabolomics

Direct correlation of 1H-NMR and GC-EI-TOF-MS profiling data reveals significant spatial localisations and metabolite gradients in Charentais melon fruit

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META-PHOR (METAbolomics for Plants Health and OutReach: http://www.meta-phor.eu/) aims to establish a European-based platform for the analysis of plant metabolites based on developing innovative cutting-edge metabolite profiling and identification technologies. Knowledge of the metabolites in our food which determine key characteristics such as nutritional value, quality and health needs to be enhanced and the tools required for their detection need to be improved. Focusing towards technology development a metabolomics approach using 1H-NMR and GC-EI-TOF/MS profiling was employed to characterise three cultivars (C√©zanne, Escrito and Hugo) of Charentais melon (Cucumis melo L.). In a first step, the major metabolites of melon flesh were analysed by quantitative 1H-NMR of polar extracts. Principal Component Analyses (PCA) of 1H-NMR data revealed the similarities and differences between the three cultivars in metabolite concentration, including sugars, organic acids and amino acids. In a second step, the spatial localisation of metabolites was investigated on one representative fruit for each cultivar. Direct 1H-NMR profiling of juice or GC-EI-TOF/MS profiling of tissue extracts collected from different locations in the fruit flesh provided information on advantages and drawbacks of each technique for the analysis of a sugar rich matrix such as fruit. 1H-NMR and GC-EI-TOF/MS datasets were compared using independently performed PCA and multiblock Hierarchical PCA (HPCA) respectively. In addition a correlation based multiblock HPCA was used for direct comparison of both analytical datasets. These data analyses firstly indicated the suitability of multiblock HPCA for correlation of data from two (or potentially more) metabolomics platforms, and secondly identified several gradients of metabolites in fruit flesh which can be related to differences in metabolism including some possibly related to a fermentation process due to hypoxia. Further quantification of adenylate nucleotides permitted the confirmation of the spatial changes in the melon flesh energetic status in relation to hypoxia.

I25: Plant/Phytochemical Metabolomics

Development of a Universal Metabolomics Platform for Assessing Health Promoting Phytochemicals in Fruits and Vegetables

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Increased consumer interest in the health benefits associated with the consumption of fresh produce have necessitated the development analytical techniques for the comprehensive profiling, discovery and monitoring of high value phytochemicals in commercial crops. Reduced risk of heart disease, cancer and stroke have been strongly associated with the consumption of fruits and vegetables that contain significant amounts of bioactive phytochemicals and may thus provide health benefits in addition to basic nutrition. Among the most prominent phytochemicals with demonstrated health-promoting effects are polyphenolic antioxidants (e.g., anthocyanins and flavonoids), phytosterols (having cholesterol lowering effects) and phytoestrogens (e.g., lignans and isoflavones), and essential vitamins.

We have developed custom extraction protocols for numerous fruits and vegetables and combined these with a universal metabolomics workflow for comprehensive phytochemical profiling utilizing GC-MS and LC-qTOF-MS technologies. As part of this effort we are also developing an Accurate Mass Retention Time (AMRT) library supplemented with MS/MS fragmentation spectra to assist in the identification of phytochemicals detected by LC/MS. Based on statistical analyses of our data we are able to differentiate between fruit varieties (e.g., Fuji, Gala, Red Delicious, Golden Delicious, Granny Smith, and other apples), agricultural management practices (e.g., organic vs. conventional), and content in potentially health-promoting phytochemicals. The opportunities of utilizing our metabolomics-based screening approach for discovering novel phytochemical leads are also discussed.

I26: Plant/Phytochemical Metabolomics

Challenges in the investigation of the metabolic changes in Nicotiana attenuata during insect herbivory using an improved HPLC-TOF-MS method

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Introduction: This study investigates metabolic changes in leaf extracts of Nicotiana attenuata after simulated insect herbivory by mechanically wounding and applying oral secretions of Manduca sexta (W+OS) using HPLC-ESI-TOF-MS. Responses of wild-type (WT) plants compared with genetically modified plants where hydroxyproline-rich glycopeptide systemin precursor (ppHypSys) is over-expressed (OVsys) or down-regulated (IRsys). The challenges inherent to this type of study and strategies for quality control are discussed.

Methods: Treated and untreated leaves (control) from WT, IRsys, and OVsys plants were harvested 1, 14, 86, and 120 h after treatment. Plant tissue was extracted with MeOH/acetic acid buffer 40:60 (v/v). Five biological replicates per time-point, analyzed by HPLC-MS, 45min binary acetonitrile-water gradient using an ESI-TOF in electrospray positive mode. Additional MS/MS information for structure elucidation was obtained by analysis with an ESI-Qq-TOF mass

spectrometer.
Statistical analysis was performed using ProfileAnalysis software (Bruker Daltonik, Germany) as well as the freely available XCMS and R software. Microsoft Office Excel was used for calculation of kinetic profiles.

Results: Preliminary results obtained from a first data set of the same sample origin (7 time-points with three biological replicates per time-point, treatment and genotype analyzed with a 17min gradient) were used to design the experiment presented here.

The data sets were analyzed using Principle Component Analysis in order to evaluate the overall variance and filter out changes induced by the treatment and genotype. Quantitative information about relevant ions was extracted independently and the time dependent changes were summarized. The most time consuming challenge in this process is the identification of the ions of interest. An important key for the identification is the unambiguous assignment of molecular formulae derived from accurate mass and isotopic pattern. The application of accurate and high resolution MS/MS data to support structure elucidation will be demonstrated.

Novel Aspects: Challenges in data interpretation of a large plant metabolomics data set

I27: Plant/Phytochemical Metabolomics

NMR Studies of the Metabolic Responses of *Salvia miltiorrhiza* Bunge to Drying

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The metabolite composition of the aqueous methanol extracts of the dry roots of Salvia miltiorrhiza Bunge (SMB), a phytomedicine used for treatments of cardiovascular diseases, were characterized using 1H NMR spectroscopy coupled with multivariate data analysis. The results showed that the SMB metabolites were dominated by amino acids, sugars and salvianolic acid B. The PCA scores plot illustrated clear clustering of SMB extracts from three different drying methods. OPLSDA results showed that compared with freeze-drying, air-drying led to significant level increase for proline, glutamine, malate and salvianolic acid B but decrease for sugars. Compared with air-drying, sun-drying induced significant level increase for proline, glucose and sucrose but decline for glutamine, malate, salvianolic acid B. Compared with air-drying, sun-drying caused significant elevation of glucose and sucrose and decline of proline, glutamine, malate, salvianolic acid B. This suggests that prolonged drought stress (e.g. air-drying) causes increase levels of proline, glutamine, malate, salvianolic acid and decrease levels of glucose and sucrose; more severe and rapid drought stress (e.g. sun-drying) induces the opposite results. The results also indicate that the NMR-based metabonomics technology is a powerful tool for composition-based quality control of traditional Chinese medicines and probably for plant metabolic response to stresses.

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I28: Plant/Phytochemical Metabolomics

HPLC-DAD-ESI-MS Analysis of the Rosemary Metabolites

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Rosemary (Rosmarinus officinalis L.) is used as natural antioxidant attributable to its secondary metabolites such as polyphenolic acids, flavonoids and phenolic diterpenens. Here, we systematically characterized 30 rosemary secondary metabolites using HPLC-DAD-MS. The water extracts were dominated by polyphenolic acids such as vanillic acid and caffeic acid, and flavonoids including diosmin, genioside, hesperidin, luteolin 3,Äô-O-b-D-glucuronide and 6-methoxylutelin-7-glycoside. The aqueous methanol extracts contained polyphenolic acids such as rosmarinic acid, sagerinic acid and salvianolic acid B, and flavonoids including 6-methoxylutelin-7-glycoside, luteolin 3,Äô-O-(4,Äù-O-acetyl)-b-D-glucuronide, luteolin 3,Äô-O-(3,Äù-O-acetyl)-b-D-glucuronide, 6-hydroxyluteolin-7-glycoside, criocitrin, and cirimaritin together with some terpenens such as rosmanol, epiisorosmanol, epirosmanol, carnosic acid and carvacrol-2-O-b-glucopyranosyl-b-glucopyranoside. The chloroform-methanol extracts contained mainly phenolic diterpenens such as rosmadial, rosmanol, epirosmanol, epiisorosmanol, epiisorosmanol methyl ether, carnosic acid, methylcarnosate, carnosol and 12-methyoxy carnosic acid. The results showed that HPLC-DAD-ESI-MS is a powerful tool for identification of plant secondary metabolites and the selection of appropriate solvents is crucial for such studies. chloroform-methanol is an excellent solvent system for selective extraction of phenolic diterpenes and that methanol-water is a better solvent for extraction of polyphenolic acids and flavonoids than water. Among all the metabolites detected, p-coumaric acid, syringic acid, carvacrol 2-O-b-glucopyranosyl-b-glucopyranoside, genioside, salvianolic acid B and quinic acid were found in rosemary for the first time.

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I29: Plant/Phytochemical Metabolomics

NMR analysis of the metabonomic variations for four Salvia species

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Salvia miltiorrhiza Bunge(SMB) is one of the most commonly used traditional Chinese medicines for cardiovascular disease treatments. Although a few other Salvia species have been used as danshen replacements in some areas of China, there were no comprehensive studies on the molecular basis for such replacements. Here, the aqueous methanol extracts from SMB, Salvia bulleyana (Sb),

Salvia yunnanensis (Sy) and Salvia flava (Sf) were characterized using 1H NMR spectroscopy coupled with multivariate data analysis (PCA and O-PLS-DA). The metabolites in these Salvia species are mainly composed of many amino acids, organic acids and polyphenolic acids such as rosmarinic acid and salvianolic acid B. The O-PLS-DA results showed that the metabolite compositions of the other three Salvia species were remarkably different from that of SMB. Compared to SMB, Sb had significant higher levels of valine, alanine, proline, Œ≥-aminobutyrate, glutamine, succinate, citrate, malate, choline, Œ±-glucose, rosmarinic acid but lower levels of N-acetylglutamate, pyroglutamate, Œ±-galactose, sucrose, danshensu; Sy contained significant higher levels of valine, alanine, proline, Œ≥-aminobutyrate, glutamine, malate, choline and danshensu but less N-acetylglutamate, pyroglutamate, succinate, Œ±-galactose and Sucrose; Sf contained significantly more malate, malonate, Œ±-glucose and galactose and less N-acetylglutamate, pyroglutamate, succinate, sucrose and danshensu. The results revealed that the metabolite compositions differed obviously amongst different salvia species. Although these results do not have any pharmcological inference, they started to provide basic information on the used SMB substitutes both in clinical and pharmacological investigations. The results also revealed that the NMR-based metabonomics technology is a useful tool for composition-based quality control and authenticity of traditional medicines.

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I30: Plant/Phytochemical Metabolomics

A three-faceted approach to reveal dynamic changes in metabolism

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Many plant species show induced responses that protect them against exogenous attacks. These responses involve the production of many different bioactive compounds. Each responding compound may have its own dynamic profile and metabolic relationships with other compounds. The chemical background of the induced response is therefore highly complex and may therefore not reveal all the properties of the response in any single model.

This study therefore aims to describe the dynamics of the induced response of a feral Brassica to above- and belowground herbivory. The chemistry of the response was measured at two time points after the attack, using a Liquid Chromatography coupled to Mass Spectrometry platform. A three-faceted approach based on Principal Component Analysis was used to analyse this response: first the large-scale aspects of the response are described in a 'global model' and then each time-point in the experiment is individually described in 'local models' that focus on phenomena that occur at specific moments in time. Although each local model describes the variation among the plants at one time-

point, the response dynamics are lost from them because they are not directly comparable. Therefore a novel method called the ‘Crossfit’ is described that links the local models of different time-points to each other.

Each element of the described analysis approach reveals different aspects of the response. The crossfit shows that smaller dynamic changes may occur in the response that are overlooked by global models, as illustrated by the analysis of a metabolic profiling dataset.

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