Metabolomics 2015

Metabolism and its role in biology and medicine

11th Annual International Conference
of the Metabolomics Society

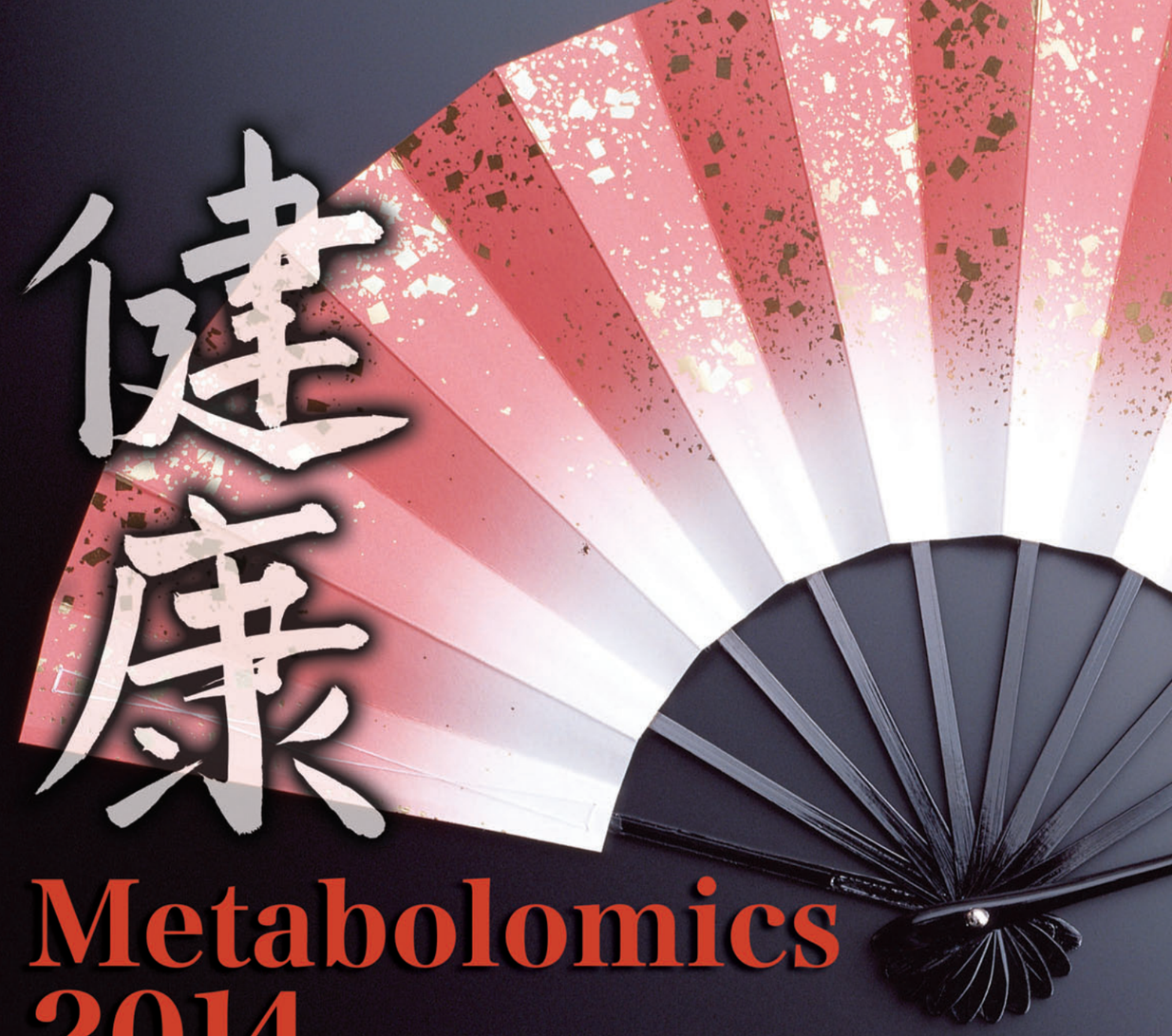
June 29-July 2, 2015
San Francisco Bay, California
Hyatt Regency, Burlingame

metabolomics2015.org
abstract deadline: March 27, 2015

METABOLOMICS
SOCIETY

Tenth Annual International Conference of the Metabolomics Society, 2014, Tsuruoka Japan

METABOLOMICS SOCIETY



Metabolomics 2014

23-26 June 2014 Tsuruoka, Japan

Program and Abstracts

METABOLOMICS
SOCIETY

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Metabolomics 2014

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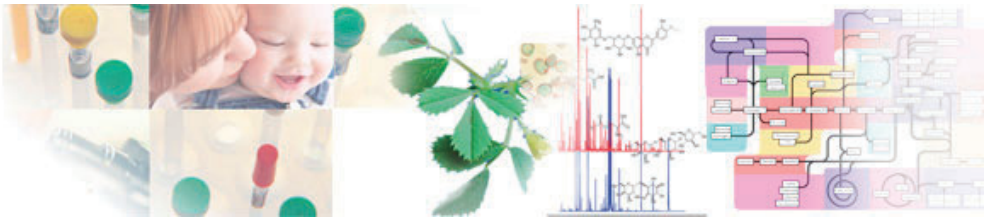
Letter of Welcome from the Metabolomics Society



Dear Metabolomics-2014 Participant,

On behalf of the Board of Directors of the Metabolomics Society it is our very great pleasure to welcome you to Tsuruoka and to Metabolomics 2014, the 10th Annual Conference of the Metabolomics Society held jointly with the Plant Metabolomics Forum. We also wish to welcome you as members of the international Metabolomics Society.

The Society's annual conference has a proven reputation for providing a stimulating array of opportunities to learn about cutting edge metabolomics, including both fundamental and applied sciences, as well as to bring together our metabolomics community to network. What is particularly special about this year is that we have reached our tenth international conference, and in part to recognise this achievement, we have returned to Tsuruoka where our 1st international conference was held in 2005. This year we have a series of workshops and scientific sessions across a diverse range of topics from methodology development through to applied research involving microbes, plants and animals, including humans. Our thanks are expressed in advance to our keynote and session speakers who will discuss their cutting-edge research in metabolomics.

It is with great pleasure this year to announce the launch of the Metabolomics Society's Early-career Members Network (EMN), to recognise the value and importance of our early-career members, to ensure that their views are heard, listened to, and acted upon, with the goal of ultimately improving their experience of metabolomics science and our community. The EMN have led the organisation of one of the workshops and I encourage you to support them there and also during the 'Young Researchers' platform sessions. This year there are 15 travel awards and prizes available, many to support the attendance of our early-career members, including ten travel awards provided by the Metabolomics Society.

The Metabolomics Society Board wish to show a huge appreciation to the local organising committee of Metabolomics 2014, with special thanks to the Chairs, Professors Masaru Tomita and Masanori Arita, for their dedication and significant contributions. The Board also wishes to thank our loyal and committed sponsors; the annual conferences would not be financially feasible without their continued financial support. Societies such as ours depend on you, and we are truly grateful for your contributions throughout the year. Metabolomics is still a young science and your support enables us to keep the registration costs – especially for the young scientists we need to support – to a minimum.

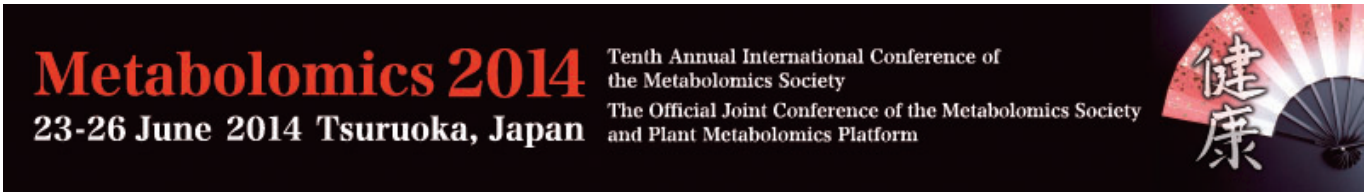
Finally, as a Society with ambitions to grow and serve our community, we want to highlight the activities and expanding portfolio of benefits to individual members, corporate members and international organisations. During the past two years we have witnessed record membership of the Society and attendance at the annual international conference, the launch of a second series of Metabolomics Society conferences comprising of smaller science-focused meetings, creation of the early-career members network, new international partnerships between the Society and national and regional metabolomics networks, an expansion of our publications and of several new member awards schemes. Thank you for renewing your membership and thereby supporting these activities.

Best wishes to everyone, and we hope you have a great conference!

Mark Viant, President,
Dan Bearden, Treasurer,
Ute Roessner, Secretary,

On behalf of the Board Members of the Metabolomics Society

Letter of Welcome from the Organizers



We are delighted to host the 10th Anniversary of the International Conference of the Metabolomics Society (Metabolomics2014) in Tsuruoka City, where the very first meeting of the society was held in 2005. Since then, Tsuruoka has grown to "a city of metabolomics"; various additional research buildings have been built, and two spin-out companies established. This year the event will also act as joint event with the Plant Metabolomics Platform.

Tsuruoka is a pretty city located 500 km north of Tokyo (about 1 hour flight), and surrounded by beautiful Japanese nature, historic spots, and exotic culture. You will also enjoy the best authentic Japanese food and sake (rice wine), as well as hot springs. So, come celebrate the 10th anniversary of the society, and enjoy high-quality scientific presentations by top-notch researchers around the world.

Masaru Tomita

General Chair, Metabolomics 2014
Director, Institute for Advanced Biosciences, Keio University

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Metabolomics 2014
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Metabolomics 2014
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Website: www.chenomx.com

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There are currently no article processing charges until 2015, due to generous support of the BGI; and time to first decision is 3 weeks.

Program

Mon 23 June 2014

Dai-ichi

1F Hoh-oh

10:00-18:30
Registration

11:00-14:40
Poster Viewing

14:40-15:25
Coffee Break

15:30-18:00
Poster Viewing

19:30-21:40
Excursion
1. Happosushi
2. Beniya

2F Tsuru

10:50-12:10
Workshop
Effective Metabolomics Software: From Design to Practice

12:30-13:20
LECO
Luncheon Seminar

13:40-14:35
Workshop
Early-career Member Network of Metabolomics Society

15:30-16:40
Workshop
Early-career Member Network of Metabolomics Society

18:40-19:30
Welcome Reception

19:30-21:40
Tsuruoka Noren

Marica

Marica Hall

10:50-12:10
Workshop
Metabolomics Measurement Technology and Application

12:30-13:20
Bruker Luncheon Seminar

13:40-15:00
Workshop
Metabolomics data standards, data capture and exchange

15:20-16:40
Workshop
Metabolomics data standards, data capture and exchange

17:00-17:40
Opening Ceremony

17:40-18:20
Keynote Lecture
Kazuki Saito

East

11:00-15:00
Poster Viewing
(Tea Ceremony)

15:00-15:20
Coffee Break

15:30-18:00
Poster Viewing

Tue 24 June 2014

Dai-ichi

1F Hoh-oh

08:20-17:00
Registration

08:40-10:10
Poster Viewing

10:10-10:30
Coffee Break

10:30-15:00
Poster Viewing

15:00-17:00
Poster Session
Crops
Flux, Pathways
Data Analysis, Networks
Nutrition
Cancer
Omics Integration

16:30-21:30
Excursions
16:30-19:30: Haguro (w/o dinner) or Kamo Aquarium
19:30-21:30: Happosushi or Beniya
16:30-21:30: Haguro (dinner) or Atsumi Onsen or Yunohama Onsen

2F Tsuru

08:40-09:20
Keynote Lecture
Markus Wenk

09:30-10:10
Keynote Lecture
Bas Teusink

10:30-12:10
Flux, Microbiology & Parasitology

12:30-13:20
Agilent Luncheon Seminar

13:40-15:00
Cancer

17:00-18:20
Evening Session
Thermo Fisher

Marica

Marica Hall

08:40-09:20
Keynote Lecture
Markus Wenk
(Live connection)

09:30-10:10
Keynote Lecture
Jules Griffin

10:30-12:10
Drug & Medicine

12:30-13:20
Shimadzu Luncheon Seminar

13:40-15:00
CVD, Diabetes & Neuroscience

19:00-21:30
Tsuruoka Noren

East

08:40-10:10
Poster Viewing

10:10-10:30
Coffee Break

10:30-15:00
Poster Viewing
(Tea Ceremony)

15:00-17:00
Poster Session
Room (1)
Environment
Microbiology
Room (2)
CVD, Diabetes
Neuroscience
Plant Physiology
Parasitology, Pathology
New Technology
Drugs, Medicine

16:30-18:45
IAB Tour with Agilent Seminar

Wed 25 June 2014

Dai-ichi

1F Hoh-oh

08:20-17:00
Registration

08:40-10:10
Poster Viewing

10:10-10:30
Coffee Break

10:30-15:00
Poster Viewing

15:00-17:00
Poster Session
Crops
Flux, Pathways
Data Analysis, Networks
Nutrition
Cancer
Omics Integration

16:30-18:45
IAB Tour with Agilent Seminar

2F Tsuru

08:40-09:20
Keynote Lecture
David Wishart
(Live connection)

09:30-10:10
Keynote Lecture
Mitsutoshi Setou

10:30-12:10
Data Analysis, Networks

12:30-13:20
AB-SCIEX Luncheon Seminar

13:40-15:00
Young Researchers

19:00-21:00
Conference Banquet

Marica

Marica Hall

08:40-09:20
Keynote Lecture
David Wishart

09:30-10:10
Keynote Lecture
Augustin Scalbert

10:30-12:10
Nutrition, Environment & Model Organism

12:30-13:20
HMT Luncheon Seminar

13:40-15:00
Young Researchers

17:00-18:20
Evening Session
AB SCIEX

East

08:40-10:10
Poster Viewing

10:10-10:30
Coffee Break

10:30-15:00
Poster Viewing
(Tea Ceremony)

15:00-17:00
Poster Session
Room (1)
Environment
Microbiology
Model Organisms
Room (2)
CVD, Diabetes
Neuroscience
Plant Physiology
Parasitology, Pathology
New Technology
Drugs, Medicine

Thu 26 June 2014

Dai-ichi

1F Hoh-oh

08:20-12:00
Registration

08:40-10:30
Poster Viewing

10:30-10:50
Coffee Break

10:50-12:00
Poster Viewing

19:00-21:30
Excursion
1. Happosushi
2. Beniya
3. Al-che-cciano

2F Tsuru

08:40-09:20
Keynote Lecture
Insuk Lee

09:30-10:30
New Technology

10:50-12:10
Omics Integration

12:30-13:20
Thermo Luncheon Seminar

13:40-14:20
Keynote Lecture
Pieter Dorrestein
(Live connection)

14:20-14:50
Closing Ceremony
(Live connection)

Marica

Marica Hall

08:40-09:20
Keynote Lecture
Ute Roessner

09:30-10:30
Plant Physiology

10:50-12:10
Crops

12:30-13:20
Waters Luncheon Seminar

13:40-14:20
Keynote Lecture
Pieter Dorrestein

14:20-14:50
Closing Ceremony

East

08:40-10:30
Poster Viewing

10:30-10:50
Coffee Break

10:50-12:00
Poster Viewing

15:15-16:45
IAB Tour

Scientific Program

Monday 23rd June Dai-ichi Hotel		
Time	**Session**	**Location**
10:00-18:30	**Registration**	1F
10:50-12:10	**Workshop: Effective Metabolomics Software: From Design to Practice**	2F Tsuru
10:50-11:05	**1A-D1** **Saravanan Dayalan** (Metabolomics Australia, Australia)	
11:05-11:20	**1A-D2** **Reza Salek** (EMBL-EBI, Cambridge UK)	
11:20-11:35	**1A-D3** **Atsushi Fukushima** (RIKEN-CSRS, Japan)	
11:35-11:50	**1A-D4** **Jianguo Xia** (University of Alberta, Canada)	
11:50-12:10	**1A-D5** **General Discussion**	
12:30-13:20	**LECO Corporation Luncheon Seminar**	2F Tsuru
13:40-14:35	**Workshop: Early-career Member Network of Metabolomics Society** **Session 1. Scientific writing and Grant writing: how to get published and funded in metabolomics**	2F Tsuru
13:40-13:55	**1P-D1** **Royston Goodacre** (University of Manchester, UK)	
13:55-14:05	**1P-D2** **Q&A**	
14:05-14:15	**1P-D3** **Krista Zanetti** (NIH, USA)	
14:15-14:25	**1P-D4** **Eiichiro Fukusaki** (Osaka University, Japan)	
14:25-14:35	**1P-D5** **Q&A**	
14:40-15:25	**1P-D6** **Extended Coffee Break** (1F Hoh-oh, exhibition room)	
14:40-15:25	**Coffee Break**	1F Hoh-oh
15:30-16:40	**Workshop: Early-career Member Network of Metabolomics Society** **Session 2. Identification of unknown metabolites**	2F Tsuru
15:30-15:45	**1P-D7** **Oliver Fiehn** (UC Davis, USA)	
15:45-16:00	**1P-D8** **David Watson** (University of Strathclyde, UK)	
16:00-16:15	**1P-D9** **Ralf Weber** (University of Birmingham, UK)	
16:15-16:40	**1P-D10** **Interactive Discussion**	
17:40-18:20	**Keynote Lecture: Kazuki Saito** (Live Connection) **Metabolomics revolutionizes phytochemical genomics and crop improvement**	1F Hoh-oh
18:40-19:30	**Welcome Reception**	2F Tsuru

Marica		
Time	**Session**	**Location**
10:50-12:10	**Workshop: Metabolomics Measurement Technology and Application**	3F Marica Hall
10:50-11:05	**1A-M1** **Ryo Nakabayashi** (RIKEN-CSRS, Japan)	
11:05-11:20	**1A-M2** **Hiroshi Tsugawa** (RIKEN-CSRS, Japan)	
11:20-11:35	**1A-M3** **Sastia Putri** (Osaka University, Japan)	
11:35-11:50	**1A-M4** **Ryu Nakata** (Kyoto University, Japan)	
11:50-12:00	**1A-M5** **Lloyd Sumner** (Noble Foundation, USA)	
12:00-12:10	**1A-M6** **Oliver Fiehn** (UC Davis, USA)	
12:30-13:20	**Bruker Corporation Luncheon Seminar**	3F Marica Hall
13:40-15:00	**Workshop: Metabolomics data standards, data capture and exchange** **Session 1. Metabolomics Data Standardization**	3F Marica Hall
13:40-13:55	**1P-M1** **Reza Salek** (EMBL-EBI, UK)	
13:55-14:10	**1P-M2** **Steffen Neumann** (Leibniz Institute of Plant Biochemistry, Halle Germany)	
14:10-14:25	**1P-M3** **Kenny Billiau** (MPI-MP Golm, Germany)	
14:25-14:40	**1P-M4** **Philippe Rocca-Serra and Alejandra Gonzalez-Beltran** (Oxford e-Research Centre, UK)	
14:40-15:00	**1P-M5** **Discussion and Questions**	
15:00-15:20	**Coffee Break**	3F East
15:20-16:40	**Workshop: Metabolomics data standards, data capture and exchange** **Session 2. Data Dissemination, Standardization and Exchange**	3F Marica Hall
15:20-15:50	**1P-M6** **Scott Edmunds and Rob Davidson** (Gigascience), **Susanna-Assunta Sansone** (Nature Scientific Data)	
15:50-16:10	**1P-M7** **Reza Salek** (EMBL-EBI, UK), **Philippe Rocca-Serra** (Oxford e-Research Centre, UK)	
16:10-16:25	**1P-M8** **Padma Maruvada** (NIH/NCRR, USA)	
16:25-16:40	**1P-M9** **Discussion and Questions**	
17:00-17:40	**Opening Ceremony**	3F Marica Hall
17:40-18:20	**Keynote Lecture: Kazuki Saito** **Metabolomics revolutionizes phytochemical genomics and crop improvement**	3F Marica Hall

Tuesday 24th June Dai-ichi Hotel		
Time	**Session**	**Location**
08:20-17:00	**Registration**	1F
8:40-9:20	**Keynote Lecture: Markus Wenk** **Natural variation of a signalling lipid**	2F Tsuru
09:30-10:10	**Keynote Lecture: Bas Teusink** **Use of metabolomics in systems biology: from data to understanding**	2F Tsuru
10:10-10:30	**Coffee Break**	1F Hoh-oh
10:30-12:10	**2A-D: Flux, Microbiology & Parasitology** Chairs: Choong Hwan Lee, David Watson	2F Tsuru
10:30-10:45	**2A-D1** **Identification of the metabolic reprogramming underlying metastasis in prostate cancer by combining multi-omic approach and model-driven data analysis** Marta Cascante, University of Barcelona	
10:45-11:00	**2A-D2** **OpenMebius: An Open source software for metabolic flux analysis** Hiroshi Shimizu, Osaka University	
11:00-11:15	**2A-D3** **Quantitative metabolic modeling for biofuel production at the Joint BioEnergy Institute.** Hector Garcia Martin, Lawrence Berkeley National Lab	
11:15-11:30	**2A-D4** **Stable-isotope labelled metabolomics for network-wide discovery of stage-specific pathways in Trypanosoma brucei** Darren J Creek, Monash University	
11:30-11:45	**2A-D5** **An altered M. tuberculosis metabolome induced by katG mutations resulting in drug resistance** Du Toit Loots, North-West University	
11:45-12:00	**2A-D6** **LC-HRMS fingerprinting: an innovative strategy to investigate bacterial metabolome during cheese ripening.** Le Boucher Clementine, INRA Agrocampus-Ouest UMR 1253 STLO and LUNAM Universite	
12:30-13:20	**Agilent Technologies Luncheon Seminar**	2F Tsuru
13:40-15:00	**24th: afternoon** **2P-D: Cancer** Chairs: Tomoyoshi Soga, Marta Cascante	2F Tsuru
13:40-14:00	**2P-D1** **Oncometabolites: Linking Altered Metabolism With Cancer?** Patrick John Pollard, University of Edinburgh	
14:00-14:20	**2P-D2** **Finding the right path: Constructing a temporal network of RAS activity in cancer** Emily Grace Armitage, Universidad San Pablo CEU	
14:20-14:40	**2P-D3** **Lipidomic profiling reveals a reconfiguration of sphingolipid metabolism that drives glioma angiogenesis and malignancy** Anthony Don, University of New South Wales	
14:40-15:00	**2P-D4** **Enhanced pyruvate carboxylation is crucial to non-small cell lung cancer proliferation and anabolism** Teresa Whei-Mei Fan, University of Kentucky	
15:00-17:00	**Poster Session** Crops / Flux, Pathways / Data Analysis, Networks Nutrition / Cancer / Omics Integration	1F Hoh-oh
17:00-18:20	**Evening Session** **Thermo Fisher Scientific**	2F Tsuru

Marica		
Time	**Session**	**Location**
8:40-9:20	**Keynote Lecture: Markus Wenk** (Live Connection) **Natural variation of a signalling lipid**	3F Marica Hall
09:30-10:10	**Keynote Lecture: Jules Griffin** **From mice to man and back again: applications of metabolomics to understand type 2 diabetes and obesity**	3F Marica Hall
10:10-10:30	**Coffee Break**	3F East
10:30-12:10	**2A-M: Drug & Medicine** Chairs: Rima Kaddurah-Daouk, Martin Robert	3F Marica Hall
10:30-10:45	**2A-M1** **Ceramide metabolism in the liver and its role in two lysosomal lipidoses - drug-induced phospholipidosis (DIPL) and Sandhoff disease (SD)** Emmanuelle Lecommandeur, University of Cambridge	
10:45-11:00	**2A-M2** **In Search of Metabolic Somnogens: A Unique Signature of Sleep Restriction from Blood in Rats** Arjun Sengupta, University of Pennsylvania	
11:00-11:15	**2A-M3** **Activation of Fatigue Metabolic Pathway Induces Hepatic Inflammation in a Rat Model of Fatigue** Satoshi Kume, RIKEN Center for Life Science Technologies	
11:15-11:30	**2A-M4** **Metabolomics biomarkers of doxorubicin-induced cardiac injury in B6C3F1 mice** Laura K Schnackenberg, NCTR, US FDA	
11:30-11:45	**2A-M5** **Metabolomics used as a tool to investigate the signature of etanercept treatment response in a psoriasis patient cohort.** Nicholas John William Rattray, The University of Manchester	
11:45-12:00	**2A-M6** **Using metabolomics to determine the modes of action of new antibiotics** Isabel M Vincent, Glasgow University	
12:30-13:20	**SHIMADZU CORPORATION Luncheon Seminar**	3F Marica Hall
13:40-15:00	**2P-M: CVD, Diabetes & Neuroscience** Chairs: Matej Oresic, Jules Griffin	3F Marica Hall
13:40-13:55	**2P-M1** **Longitudinal metabolome en route to type 1 diabetes: potential role of gut microbiota and metabolic vulnerability in the early disease pathogenesis** Matej Oresic, Steno Diabetes Center	
13:55-14:10	**2P-M2** **Large scale metabolomics in clinical epidemiology** Oliver Fiehn, UC Davis	
14:10-14:25	**2P-M3** **Examining the effects of metformin in a large clinical cohort and HepG2 and 3T3-L1 treated cells using untargeted LC-MS metabolomics** Emma Louise Boulton, University of Glasgow	
14:25-14:40	**2P-M4** **Metabolome and lipidome features of human brain development** Kasia Bozek, CAS-MPG Partner Institute for Computational Biology	
14:40-14:55	**2P-M6** **Accumulation of metabolic syndrome risk factors and plasma amino acids and their related metabolites: Tsuruoka Metabolomic Cohort Study** Toru Takebayashi, Keio University School of Medicine	
15:00-17:00	**Poster Session** Room (1) Environment / Microbiology Room (2) CVD, Diabetes / Neuroscience / Plant Physiology / Parasitology, Pathology / New Technology / Drugs, Medicine	3F East

Wednesday 25th June Dai-ichi Hotel		
Time	**Session**	**Location**
08:20-17:00	**Registration**	1F
08:40-09:20	**Keynote Lecture: David Wishart** (Live Connection) **Making metabolomics matter**	1F Hoh-oh 2F Tsuru
09:30-10:10	**Keynote Lecture: Mitsutoshi Setou** **Metabolomics imaging mass spectrometry in clinical research**	2F Tsuru
10:10-10:30	**Coffee Break**	1F Hoh-oh
10:30-12:10	**3A-D: Data Analysis, Networks** Chairs: Eiichiro Fukusaki, Masahiro Sugimoto	2F Tsuru
10:30-10:44	**3A-D1** **Complimentary LC- and GC-Mass Spectrometry Techniques Provide Broader Coverage of the Metabolome** Baljit Kaur Ubhi, AB Sciex	
10:44-10:58	**3A-D2** **An advanced computational and database framework for the compound identification in untargeted metabolomics** Robert Mistrik, HighChem	
10:58-11:12	**3A-D3** **MS-DIAL: Untargeted metabolomics software for data independent LC-MS/MS and mass spectral deconvolution: application to algae metabolomics** Hiroshi Tsugawa, RIKEN Center for Sustainable Resource Science	
11:12-11:26	**3A-D4** **MASTR-MS: A web-based collaborative Laboratory Information Management System (LIMS) for Metabolomics** Saravanan Dayalan, University of Melbourne	
11:26-11:40	**3A-D5** **Data-driven sample size determination for human metabolic phenotyping studies** Benjamin Blaise, Hospices Civils de Lyon	
11:40-11:54	**3A-D6** **Searching PubChem with tandem mass spectrometry data: Teaming molecular fingerprint prediction and fragmentation trees** Sebastian Boecker, Friedrich Schiller University Jena	
11:54-12:08	**3A-D8** **Systematic approaches for identification and removal of non-biological sources of variation in metabolomics data** Rohan Benjamin Hugh Williams, Singapore Centre for Environmental Life Sciences Engineering (SCELSE), National University of Singapore	
12:30-13:20	**AB SCIEX Luncheon Seminar**	2F Tsuru
13:40-15:00	**25th: afternoon** **3P-D: Young Researchers 1** Chairs: Tim Ebbels, Darren Creek	2F Tsuru
13:40-13:55	**3P-D1** **Construction of a metabolome library for transcription factor-related single gene mutants of Saccharomyces cerevisiae** Zanariah Hashim, Osaka University	
13:55-14:10	**3P-D2** **Metabolomics-based semi-rational identification of gene targets conferring 1-butanol tolerance in *Saccharomyces cerevisiae*** Shao Thing Teoh, Osaka University	
14:10-14:25	**3P-D3** **Quantitative Analysis of Stressed Caenorhabditis elegans using Isotopic Ratio Outlier Analysis** Gregory S Stupp, University of Florida	
14:25-14:40	**3P-D4** **Global Metabolomic Analysis of 13C-enriched Mixtures using INADEQUATE** Chaevien S Clendinen, University of Florida	
14:40-14:55	**3P-D5** **Classification of reliable MetFrag candidate identifications for tandem mass spectra from selected lipid samples** Christoph Ruttkies, Leibniz Institute of Plant Biochemistry	
15:00-17:00	**Poster Session** Crops / Flux, Pathways / Data Analysis, Networks / Nutrition / Cancer / Omics Integration	1F Hoh-oh
19:00-21:00	**Conference Banquet**	2F Tsuru

Marica		
Time	**Session**	**Location**
08:40-09:20	**Keynote Lecture: David Wishart** **Making metabolomics matter**	3F Marica Hall
09:30-10:10	**Keynote Lecture: Augustin Scalbert** **Metabolomics and the measurement of the exposome in epidemiological studies on cancer risk**	3F Marica Hall
10:10-10:30	**Coffee Break**	3F East
10:30-12:10	**3A-M: Nutrition, Environment & Model Organism** Chairs: Mark Viant, Roy Goodacre	3F Marica Hall
10:30-10:42	**3A-M1** **Characterization of food products by GCxGC with TOFMS and GC with high resolution TOFMS: a food-omics approach** Jeff Patrick, LECO Corporation	
10:42-10:54	**3A-M2** **A metabolomics approach shows that green tea extract attenuates UVB-induced skin metabolite alterations in mice** Eun Sung Jung, Konkuk University	
10:54-11:06	**3A-M3** **Non-targeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish and bilberries** Kati Hanhineva, University of Eastern Finland	
11:06-11:18	**3A-M4** **The secret life of the Mediterranean Lake Medee, the largest deep sea salt saturated formation in the world** David Rojo, University CEU San Pablo	
11:18-11:30	**3A-M5** **Differential lipid and metabolite levels in response to spawning-induced inappetence in Atlantic salmon Salmo salar** McKenzie Smith, Georgia Institute of Technology	
11:30-11:42	**3A-M6** **A metabolomic approach to assess neurotoxic effects of imidacloprid on the freshwater snail lymnaea stagnalis** Sara Tufi, VU Amsterdam	
11:42-11:54	**3A-M7** **Using omics for identification of the mechanism of the toxic action for perflourononanoic acid** Kasper Skov, Technical University of Denmark	
11:54-12:06	**3A-M8** **Metabolome analysis of Drosophila melanogaster during embryogenesis** Thuy An Phan Nguyen, Osaka University	
12:30-13:20	**Human Metabolome Technologies Luncheon Seminar**	3F Marica Hall
13:40-15:00	**3P-M: Young Researchers 2** Chairs: Dan Bearden, Robert Hall	3F Marica Hall
13:40-13:55	**3P-M1** **Characterization of the Sweat Metabolome in Screen-Positive Cystic Fibrosis Patients** Adriana Nori de Macedo, McMaster University	
13:55-14:10	**3P-M2** **Fatty acid profiling in breast cancer biomarker discovery by GC-MS** Huai-Hsuan Chiu, National Taiwan University	
14:10-14:25	**3P-M3** **Metabolome and transcriptome changes in human, macaque and mouse brain development** Hindrike Bammann, CAS-MPG Partner Institute for Computational Biology	
14:25-14:40	**3P-M4** **Metabolomics analysis reveals large effects of uremic toxin in heart failure outcome** Cheng-Cheng Lin, Chang Gung University	
14:40-14:55	**3P-M5** **Metabolic changes in stress-tolerant transgenic rice overexpressing calmodulin** Surachat Tangpranomkorn, Chulalongkorn University	
15:00-17:00	**Poster Session** Room (1) Environment / Microbiology / Model Organisms Room (2) CVD, Diabetes / Neuroscience / Plant Physiology / Parasitology, Pathology / New Technology / Drugs, Medicine	3F East
17:00-18:20	**Evening Session** **AB SCIEX session**	3F Marica Hall

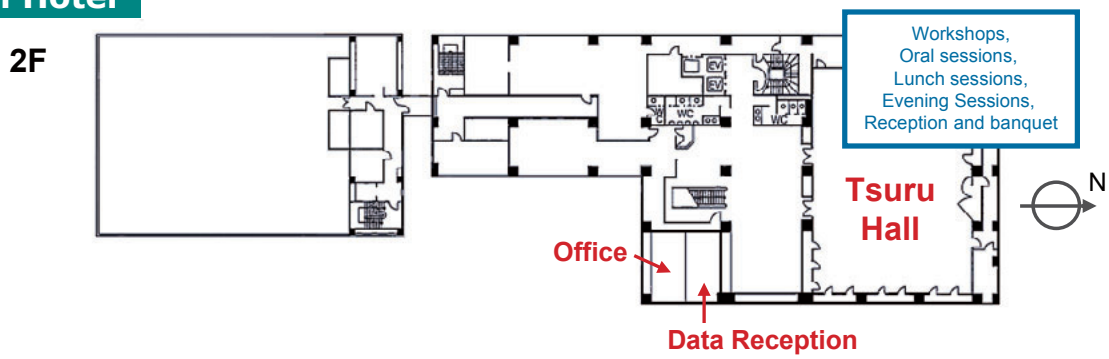
Thursday 26th June Dai-ichi Hotel		
Time	**Session**	**Location**
08:20-12:00	**Registration**	1F
08:40-09:20	**Keynote Lecture: Insuk Lee** **Googling biology using gene networks**	2F Tsuru
09:30-10:30	**4A-D: New Technology** Chairs: Lloyd Sumner, Robert Mistrik	2F Tsuru
9:30-9:50	**4A-D1** **A novel multi-platform software tool (MUSCLE) for the robust, objective and automated optimisation of targeted LC-MS/MS analyses** Mark R Viant, University of Birmingham	
9:50-10:10	**4A-D2** **CSPP networks aid the detection of in vivo substrates and products of enzymes** Kris Morreel, VIB	
10:10-10:30	**4A-D3** **Comprehensive two-dimensional liquid chromatography: a new technique for for high-resolution metabolomics?** Oliver Jones, RMIT Univeristy	
10:30-10:50	**Coffee Break**	1F Hoh-oh
10:50-12:10	**4P-D: Omics Integration** Chairs: Kenji Nakahigashi, Natsumi Saito	2F Tsuru
10:50-11:06	**4P-D1** **A unique brain lipidome and metabolome biosignature in alzheimer disease** Giuseppe Astarita, Waters Corporation	
11:06-11:22	**4P-D2** **13C-based Metabolomics: Integrating LC-MS IROA with NMR** Arthur S Edison, Southeast Center for Integrated Metabolomics	
11:22-11:38	**4P-D3** **MetaboLights: Open access metabolomics repository and reference data** Reza Salek, EMBL-EBI	
11:38-11:54	**4P-D4** **Reconstruction of global signal flow of insulin from phosphoproteome and metabolome data** Katsuyuki Yugi, The University of Tokyo	
11:54-12:10	**4P-D5** **A catalog of human metabolic individuality** Gabi Kastenmuller, Helmholtz Center Munich	
12:30-13:20	**Thermo Fisher Scientific Luncheon Seminar**	2F Tsuru
13:40-14:20	**Keynote Lecture: Pieter Dorrestein** (Live Connection) **A "GoogleMAP"-type view of specialized molecules from microbes, their communities and hosts including people**	2F Tsuru
14:20-14:50	**Closing Ceremony** (Live Connection)	2F Tsuru

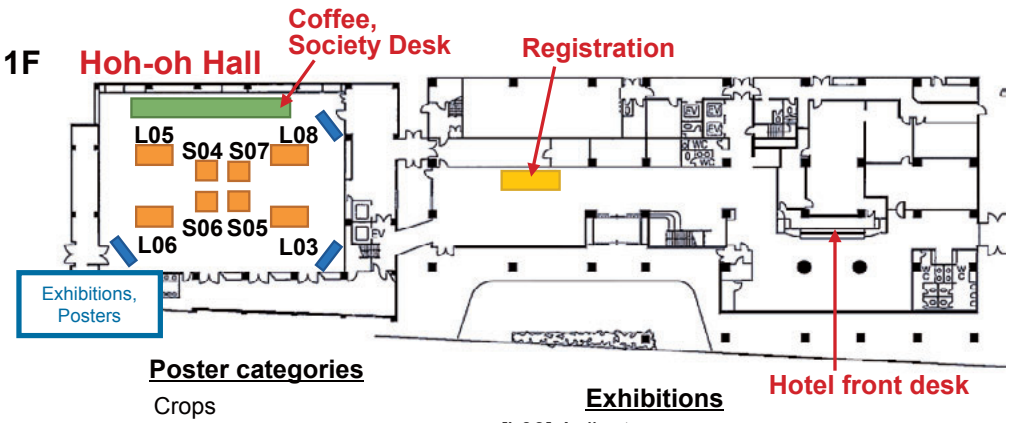
Marica		
Time	**Session**	**Location**
08:40-09:20	**Keynote Lecture: Ute Roessner** **Identifying novel salinity tolerance mechanisms by spatial analysis of lipids in barley roots**	3F Marica Hall
09:30-10:30	**4A-M: Plant Physiology** Chairs: Kazuki Saito, Oliver Fiehn	3F Marica Hall
9:30-9:45	**4A-M1** **Integrated Metabolomics, Gene Expression, and GWAS Identify New Saponin Biosynthetic Genes in Medicago truncatula** Lloyd W. Sumner, The Samuel Roberts Noble Foundation	
9:45-10:00	**4A-M2** **Metabolomic exploration toward understanding of key cellular events leading to wax ester fermentation in Euglena gracilis** Takumi Ogawa, Osaka Prefecture University	
10:00-10:15	**4A-M3** **Monitoring primary carbon metabolism in mature Arabidopsis thaliana leaves** Pernilla Linden, Swedish University of Agricultural Sciences	
10:15-10:30	**4A-M4** **Untargeted metabolomics of Habanero pepper (*Capsicum chinense* Jacq. var. Orange) fruits subject to Nitrogen and Phosphorus deficiency.** Carlos Rodriguez-Lopez, Tecnologico de Monterrey	
10:30-10:50	**Coffee Break**	3F East
10:50-12:10	**4P-M : Crops** Chairs: Ute Roessner, Akira Oikawa	3F Marica Hall
10:50-11:06	**4P-M1** **Composition and metabolite differences between near-isogenic gm and conventional maize are associated more with back-crossing than with the gm traits** Mark Leibman, Monsanto Company	
11:06-11:22	**4P-M2** **The application of metabolomics to the biochemical phenotyping of staple crops from developing countries** Elisabete Barros Carvalho, Royal Holloway University of London	
11:22-11:38	**4P-M3** **GC-MS based metabolite profiling of barley kernels - impact of induced drought-stress** Alexandra Wenzel, General Food Technology	
11:38-11:54	**4P-M4** **Combining metabolomic profiling and sensory evaluation to understand consumer perception of rice flavour** Venea Dara Daygon, University of Queensland	
11:54-12:10	**4P-M5** **Allelopathy in canola** Md Asaduzzaman, Charles Sturt University	
12:30-13:20	**Waters Corporation Luncheon Seminar**	3F Marica Hall
13:40-14:20	**Keynote Lecture: Pieter Dorrestein** **A "GoogleMAP"-type view of specialized molecules from microbes, their communities and hosts including people**	3F Marica Hall
14:20-14:50	**Closing Ceremony**	3F Marica Hall

Floor Map

Oral session streaming
Coffee
Sponsor booths

Dai-ichi Hotel





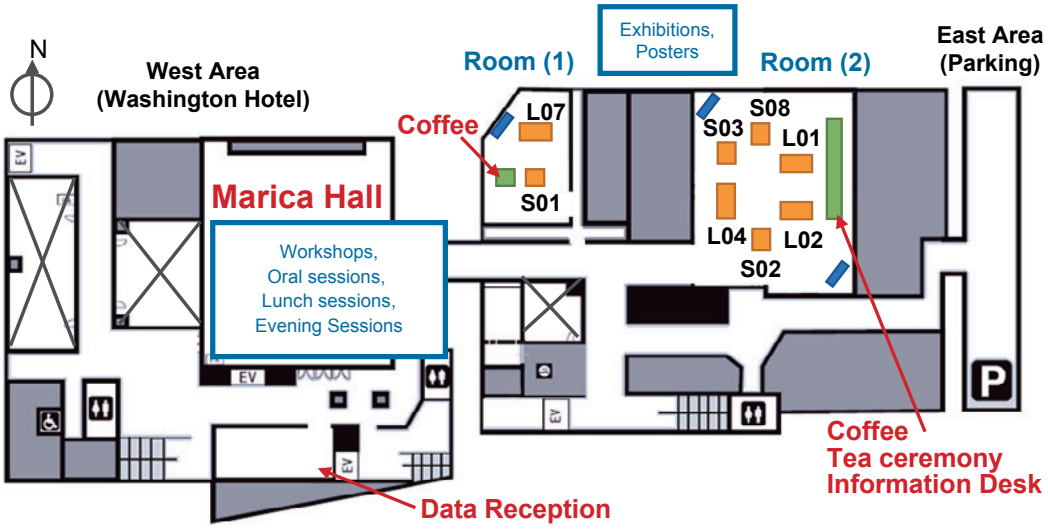
Poster categories

Crops
Flux, Pathways
Data Analysis, Networks
Nutrition
Cancer
Omics Integration

Exhibitions

[L03] Agilent
[L05] AB SCIEX
[L06] HMT
[L08] Waters
[S04] Chenomx
[S05] Monsanto
[S06] GL Sciences
[S07] Nonlinear

Marica 3F

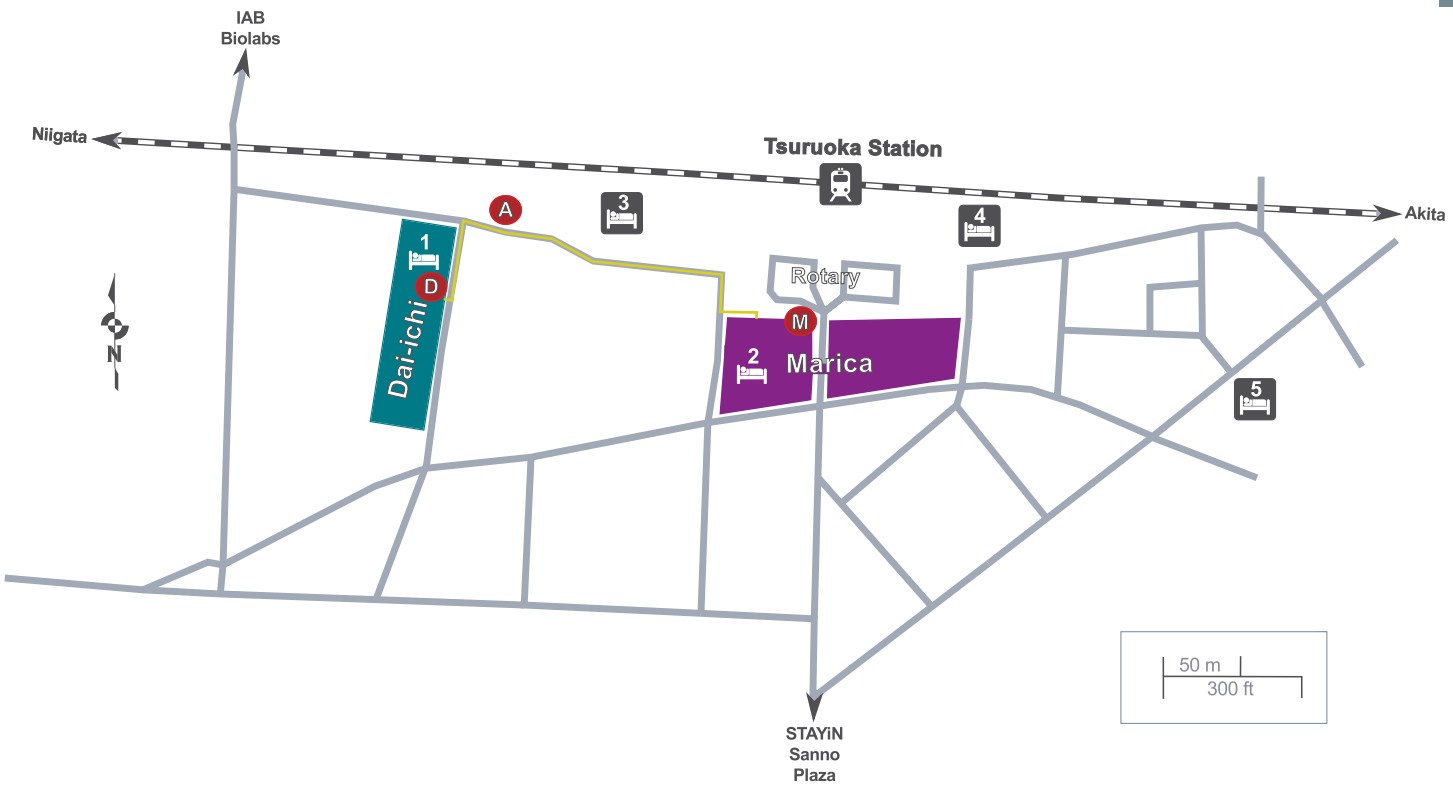


Poster categories

Room (1)	Room (2)
Environment	CVD, Diabetes
Microbiology	Neuroscience
Model Organisms	Plant Physiology
	Parasitology, Pathology
	New Technology
	Drugs, Medicine

Exhibitions

Room (1)	Room (2)
[L07] Thermo	[L01] LECO
[S01] SpectralWorks	[L02] Bruker
	[L04] SHIMADZU
	[S03] Metanomics Health
	[S08] Scrum
	[S02] AMR



1. Dai-ichi Hotel Tsuruoka
2. Marica/Washington Tsuruoka Hotel
3. Hotel Route Inn Tsuruoka Ekimae
4. STAYiN Sanno Plaza Premium Annex
5. Hotel α-1 Tsuruoka

A. Shuttle Bus Stop (Airport, Noren and Excursions)
D. Venue shuttle stop (Dai-ichi)
M. Venue shuttle stop (Marica)
Walking route between venues

General Information

General Information

Conference Venue

Dai-ichi Hotel Tsuruoka	Marica/Washington Hotel Tsuruoka
Address: 2-10 Nishikimachi, Tsuruoka, Yamagata, 997-0031, Japan TEL: +81-235-24-7611 FAX: +81-235-24-7621	Address: 5-20 Suehiromachi, Tsuruoka, Yamagata, 997-0015, Japan TEL: +81-235-25-0111 FAX: +81-235-25-0110
1F Poster & exhibitions (tea/coffee) Registration Desk **2F** Oral sessions & workshops Welcome reception and conference banquet	**3F West** Oral sessions & workshops Opening and closing events **3F East** Poster & exhibitions (tea/coffee) Information Desk, Japanese tea ceremony

Parking

Parking information and rules are available at Registration Desk. Numbers of parking spaces are limited.

Shuttle Bus Information

Free shuttle buses are provided for transportations to the Shonai airport, hotels, IAB lab tours, Tsuruoka Noren and excursions. Detailed information is available at Information Desk.

Registration Desk

Dai-ichi Hotel 1F

Standard delegates will receive their name badge, Program and Abstract, ordered tickets and all relevant conference information upon arrival.

Opening Hours

Monday, June 23:	10:00 – 18:30
Tuesday, June 24:	08:20 – 17:00
Wednesday, June 25:	08:20 – 17:00
Thursday, June 26:	08:20 – 12:00

Information Desk

Marica 3F East

Information about excursions, Tsuruoka Noren, Tsuruoka culture and sightseeing is available.

Opening Hours

Monday, June 23:	10:00 – 18:30
Tuesday, June 24:	08:40 – 17:00
Wednesday, June 25:	08:40 – 17:00
Thursday, June 26:	08:40 – 12:00

Badges

Your name badge must be worn at all times otherwise you will not be allowed to enter the conference venues.

Cloakroom

You may leave your luggage or poster tubes at the cloakroom located at Dai-ichi Hotel 1F. The desk will be open throughout the normal opening hours of the conference.

Certificate of Attendance

A certificate of attendance will be issued to delegates upon request at the conference.

Message Board

There will be a notice board at poster & exhibitions hall in Dai-ichi Hotel 1F “Hoh-oh Hall” for those wishing to leave messages or notifications during the conference.

WiFi Access

Free WiFi access is available to delegates. Please see the registration notice board for the password and log in details.

Conference Etiquette

Delegates are advised that no photographs of any posters or presentations are allowed without the consent of the author/presenter. Delegates should also obtain consent from presenters before quoting or citing any of their presentations at the conference. This applies to all 'blog' or 'tweet' activities.
Mobile phones should be switched off or placed on 'silent' during all sessions.

Notice of videotaping directed to Conference participants

The 2014 Conference is being videotaped by the Metabolomics Society, including interactions between and among presenters, panelists and participants. By attending the Conference, you consent to your image and voice being broadcast to our two conference venues, recorded, and possibly posted (in whole or in part) on the Society's website and/or used in educational materials that the Society may produce.

Please note that local media groups may also be filming in various Conference venues. They are unaffiliated with the Metabolomics Society. We have been informed that their focus is not the scientific content of the Conference, but rather the general proceedings and the international activity of the city of Tsuruoka.

Disclaimers

For possible use in the Conference program book:

The scientific data, analyses and conclusions presented at this Conference by lecturers, panelists and posters are solely those of the presenters. The Metabolomics Society and local organizers assume no legal liability or responsibility for their accuracy, completeness or non-infringement. Reference to any specific product, process or service does not necessarily constitute endorsement by the Society, and the opinions of presenters do not necessarily reflect those of the Society.

For possible use on the Society's website where Conference videos are accessed:

The scientific data, analyses and conclusions presented in these recordings of the 2014 Conference are solely those of the presenters. The Metabolomics Society and local organizers assume no legal liability or responsibility for their accuracy, completeness or non-infringement. Reference to any specific product, process or service does not necessarily constitute endorsement by the Society, and the opinions of presenters do not necessarily reflect those of the Society. The Society is not responsible for information that is referenced by or linked to this site.

Insurance

The Conference Organizers cannot accept any liability for personal injuries or for loss or damage to property belonging to delegates, either during, or as a result of the meeting. Please check the validity of your own personal insurance before travelling.

Catering Arrangements

Tea/Coffee Breaks

Refreshments during tea/coffee breaks will be served in the same rooms as posters and sponsor exhibitions: Dai-ichi Hotel 1F "Hoh-oh Hall" and Marica 3F East Area.

Lunch Arrangements

Lunches will be provided only for delegates who registered to attend lunchtime symposia by respective sponsors. Lunches will be distributed near the entrance of main halls. Please follow the directions of the conference staff.

Dinner

Except for the conference dinner on Wednesday, June 25 at Dai-ich Hotel 2F "Tsuru Hall", you are free to choose your own restaurant. Please see "Social Event" section for various outing options.

Speaker Preparation

All presenters are required to hand in their presentations 4 hours prior to their talk time. The data reception desk is located at both Dai-ichi Hotel 2F and Marica 3F West, from 9:00 to 17:00 each day. Please visit the desk of your talk place.

Poster Information (Dai-ichi Hotel 1F "Hoh-oh", Marica 3F East "Room1,2")

There are two dedicated poster sessions taking place at the following times.

Poster Session I
– Tuesday June 24, 15:00 - 17:00

15:00 - 15:45: ODD numbers
15:45 - 16:30: EVEN numbers
16:30 - 17:00: General discussion

Posters must be placed from 10:00 on Monday June 23 and must be removed by 18:00 on Tuesday June 24.

Poster Session II
– Wednesday June 25, 15:00 - 17:00

15:00 - 15:45: ODD numbers
15:45 - 16:30: EVEN numbers
16:30 - 17:00: General discussion

Posters must be placed from 10:00 on Wednesday June 25 and must be removed by 12:00 on Thursday June 26.

Authors are asked to stand by their boards during this time to discuss their work with delegates. The poster code and location are provided at the Registration Desk.
Please note that posters that are not removed by the designated time will be disposed by the organizers.

Sponsor Exhibitions (Dai-ichi Hotel 1F "Hoh-oh", Marica 3F East "Room1,2")

Visit our sponsor booths and get wonderful gifts!
Provide your business card at each sponsor booth and get a stamp on your activity sheet (provided at the registration desk).
By collecting stamps, you can win small gifts at Information Desk in Marica 3F East.

The exhibition will be open during the following times:

Monday, June 23:	11:00 – 18:00
Tuesday, June 24:	08:40 – 18:00
Wednesday, June 25:	08:40 – 18:00
Thursday, June 26:	08:40 – 12:00

Social Event

Welcome Reception - Monday June 23, 18:40 - 19:30 at Dai-ichi Hotel 2F "Tsuru Hall" (included in the registration fee)

The Welcome Reception is free for all delegates (except single-day participants). Starter beverage and small snacks will be served.

Conference Banquet - Wednesday June 25, 19:00 to 21:00 at Dai-ichi Hotel 2F "Tsuru Hall" (included in the registration fee)

You can enjoy delicacies of Japanese cuisine at the Conference Banquet. Tsuruoka is a city of gastronomy, home of top quality *sake* (rice wine), vegetables, fish and pork. The event is sponsored by Agilent Technologies and free for all delegates (except single-day participants).

There will be a Sake tasting booth and live performance of Taiko (Japanese drums) by a local group.

Tsuruoka Noren Culinary Experience - Monday June 23 & Tuesday June 24

The Local Organizer have teamed up with the city of Tsuruoka and local restaurateurs to offer the "Noren" Cuisine to all delegates. This scheme will allow delegates to experience fine dining at a reasonable price with the least stress of finding the right restaurant for you. Please visit our Information Desk at Marica 3F East for more details.

Tea Ceremony - Monday June 23 to Wednesday June 25, 11:00 - 15:30 at Marica 3F East

Japanese tea ceremony and demonstration by Enshu Sado will be provided from Monday June 23 to Wednesday June 25 at Marica 3F East between 11:00 to 15:30.

June 23
Monday

Effective Metabolomics Software: From Design to Practice

Dai-ichi Hotel 2F "Tsuru Hall"

Aim and Scope: Good design of scientific software is important. The care and awareness in using such software programs were highlighted in 2006, when a group at Scripps had to retract five papers, including three from *Science* and one from *PNAS* due to a problem in their software program (*Science* **314**, 1875;2006). As in other fields of life sciences, software is an intertwined part of Metabolomics. It plays a crucial role in all aspects of the workflow from data generation, management, processing, analysis, and to interpretation.

This workshop is aimed at both software users and developers, with the goal of teaching the fundamentals of efficient software design and development, and best practices in software usage. The speakers provide insights from developing a wide-range of metabolomics software programs.

List of speakers: (titles to be announced)

10:50-11:05 Saravanan Dayalan (Metabolomics Australia, Australia)

11:05-11:20 Reza Salek (EMBL-EBI, Cambridge UK)

11:20-11:35 Atsushi Fukushima (RIKEN-CSRS, Japan)

11:35-11:50 Jianguo Xia (University of Alberta, Canada)

11:50-12:10 General Discussion

Metabolomics Measurement Technology and Application

Marica 3F "Marica Hall"

Aim and Scope: Metabolomics covers wide range of topics and is effective not only in biomedical fields but also in energy or environmental sciences. This workshop is organized by the four research projects under the program title "Metabolomics for a Low Carbon Society," jointly coordinated by the U.S. National Science Foundation (NSF) and Japan Science and Technology Agency (JST) since 2011. Each team will introduce the recent progress including the measurement methods for metabolite identification, software development, and metabolic signalling among plants, insects, and disease.

Introduction to JST-NSF Program

Takaaki Nishioka (NAIST, Japan)

List of speakers: (titles to be announced)

10:50-11:05 Ryo Nakabayashi (RIKEN-CSRS, Japan)

11:05-11:20 Hiroshi Tsugawa (RIKEN-CSRS, Japan)

11:20-11:35 Sastia Putri (Osaka University, Japan)

11:35-11:50 Ryu Nakata (Kyoto University, Japan)

Introduction to Worldwide Networks

11:50-12:00 PAMM-NET RCN
Lloyd Sumner (Noble Foundation, USA)

12:00-12:10 Ring Trial Initiative and NIH common funds
Oliver Fiehn (UC Davis, USA)

Luncheon Seminars

LECO Japan Corporation

Dai-ichi Hotel 2F "Tsuru Hall" 12:30-13:20

GC-TOF-MS-based metabolomics for plant science – from starting up of the analytical pipeline to carrying out metabolite profiling

Miyako Kusano[1,2]

[1]RIKEN Center for Sustainable Resource Science (CSRS), Yokohama, Kanagawa 230-0045, Japan; [2]Kihara Institute for Biological Research, Yokohama City University, Yokohama, Kanagawa 244–0813, Japan.
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Metabolite-profiling methods using gas chromatography–mass spectrometry (GC-MS) has been used since the late 1970s. GC-MS-based metabolite profiling has been applied to detect volatilizable components because of its characteristics, while hydrophilic compounds have to be derivatized before analysis. The choice of mass analyzer is an important point when we conduct non-targeted metabolite profiling. Gas chromatography–time-of-flight–mass spectrometry (GC-TOF-MS) has high spectral acquisition rate, which allows a high throughput analysis with short analytical time. As plants can produce various types of metabolites including sugars, amino acids, organic acids, fatty acids and secondary metabolites, GC-TOF-MS has been regarded as the gold standard to conduct metabolite profiling to analyze metabolite composition in plants. In the seminar, I would like to introduce how we have developed the analytical pipeline for GC-TOF-MS-based metabolite profiling and application examples to analyze metabolites produced by plants at RIKEN in the decade. We used Pegasus GC-TOF-MS and Pegasus 4D GCxGC-TOF-MS to conduct comprehensive analysis for plant metabolites. We applied the established method to investigate metabolite changes toward model plant *Arabidopsis thaliana* (1, 2, 3), rice (4, 5) and vegetables (6). Metabolite profiling of volatiles (7) and wax components (8) in plants will be presented. I will also show current challenging to perform ultra-high resolution profiling of plant metabolites using GC coupled with high resolution TOF-MS (GC-HRT-MS).

References:

(1) **Kusano, M., Fukushima, A., et al.** (2007). BMC Syst Biol **1,** 53.
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(3) **Kusano, M., Tohge, T., Fukushima, A., et al.** (2011). Plant J **67,** 354-369.
(4) **Kusano, M.,et al.** (2007). J Chromatogr B **855,** 71-79.
(5) **Kusano, M., Tabuchi, M., et al.** (2011). Plant J **66,** 456-466.
(6) **Kusano, M., Redestig, H., et al.** (2011). PLoS One **6,** e16989.
(7) **Kusano, M., et al.** (2013). Metabolites **3,** 223-242.
(8) **Kimbara, J., et al.** (2012). Planta **236,** 1559-1570.

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Luncheon Seminars

Bruker Corporation

Marica 3F "Marica Hall" 12:30-13:20

Large-scale Computational and Empirical Annotation of the Medicago Metabolome using UHPLC-MS-SPE-NMR

Lloyd W. Sumner (The Samuel Noble Foundation, USA)

Metabolite identification is the number one challenge in metabolomics and provides the critical biological context of the experiments. We are developing a program that uses multiple methods for metabolite identification through the combination of computational and empirical methods. More specifically, we are developing custom special libraries, custom identification software and exploiting public/commercial software for the large-scale computational prediction of metabolite identification. Recent Grants from NSF MRI, NSF Metabolomics for a Low Carbon Society and Bruker Daltonics & BioSpin have also enabled us to assemble a sophisticated instrumental ensemble consisting of UHPLC-MS-SPE-NMR for higher throughput metabolite identification. This system enables the automated purification and concentration of metabolites for NMR analyses. Quality NMR can be reasonably obtained for plant metabolites in the 1-5 μg range. This presentation will provide a detailed overview of the computational and empirical technologies being used, and the application of these technologies in the systematic and biologically driven annotation of the metabolome of the model plant; *Medicago truncatula.*
(Lloyd W. Sumner, Dennis Fine, Feng Qiu and Daniel Wherritt, The Samuel Roberts Noble Foundation, Ardmore, OK.)

Assure-RMS: A suite of tools for targeted and non-targeted NMR-based analysis in automation

Michelle Markus (Bruker Biospin, Billerica, USA)

For a typical metabolomics study, many samples are required. It is extremely useful to lock down the experimental procedures to acquire consistent and comparable data. Even better would be to analyze the data as it is acquired. Assure-RMS provides tools for automated data acquisition and report generation for NMR analysis of complex mixtures. The analysis can draw on targeted methods, including matching individual components against a reference data base and quantification based on peak intensity or area, and non-targeted methods, including testing against the SIMCA model for classification and quantification based on PLS regression. These comprehensive tools will be presented in more detail, using blueberry leaf extracts as an example. Blueberry (genus Vaccinium) leaf extracts are widely regarded by indigenous peoples of the circumboreal floristic region as medicinal.
(Markus, Michelle A.[1]; Jimmy Yuk[1], Mark Garvey[2], Christian Fischer[2], Colson, Kimberly L.[1])

Bodyfluid-NMR with IVDr systems

Claire Cannet (Bruker Biospin, Rheinstetten, Germany)

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Early-career Member Network of Metabolomics Society

Dai-ichi Hotel 2F "Tsuru Hall"

Aim and Scope: The Early-career Members Network (EMN) committee presents their first workshop this year, consisting of a session on scientific writing and a session on the identification of unknown metabolites.

Organizers: The EMN committee of the Metabolomics Society (Sastia Putri, David Liesenfeld, Thomas Payne, Ralf Weber, Justin van der Hooft, Nicholas Rattray, Evangelia Daskalaki, Gabriel Valbuena, Vincent Asiago)

Session I. Scientific writing and Grant writing: how to get published and funded in metabolomics

This session will deal with something all scientists often face during their career: writing. Three excellent speakers will share their view on how to effectively communicate your science using written language. The different roles these scientists have, *i.e.*, journal editor, part of funding body, part of reviewing committee, promises a vivid and diverse setting of this first session of the workshop. The speakers will concentrate on technical aspects of writing and give some tips focused on early-career scientists. This session aims to provide early-career scientist with tools and resources to work on their writing skills.

Scientific writing

13:40-13:55	"Scientific inspiration *versus* literary perspiration: What makes a good *Metabolomics* paper? ", *Royston Goodacre (University of Manchester, UK)*
13:55-14:05	Q&A

Grant writing

14:05-14:15	"Navigating the Road Ahead: NIH Mechanisms to Obtain Funding for Metabolomics Research", *Krista Zanetti (NIH, USA)*
14:15-14:25	"How to be a good hunter of research grant", *Eiichiro Fukusaki (Osaka University, Japan)*
14:25-14:35	Q&A
14:40-15:25	**Extended Coffee Break (1st floor, exhibition room)** A chance to for the early career members to connect with each other and mingle with top scientists in metabolomics field. Please move to the exhibition area of Dai-ichi Hotel (poster area).

Session II. Identification of unknown metabolites

This session is focused on one of the biggest hurdles facing metabolomics research nowadays. The annotation and identification of unknown metabolites many of you will be confronted with during your metabolomics experiments, is a challenging part of the metabolomics pipeline. In this session, three speakers will explain their approaches and views on this topic and show useful metabolomics databases. The first two speakers will focus on data analysis of gas chromatography coupled to mass spectrometry (GC-MS) spectra and liquid chromatography coupled to high-resolution MS (LC-MS), and the role of NMR in *de novo* identification will be briefly touched upon. The last speaker will highlight the different metabolomics repositories that are available to the community nowadays.
This session aims to create an awareness of the different approaches that are out there and the current metabolomics databases that you can use to annotate and identify your metabolites of interest.

15:30-15:45 "Metabolite identification: mass spectral libraries, accurate mass MS/MS and structure ambiguities" *Oliver Fiehn (UC Davis, USA)*
15:45-16:00 "Identification of Metabolites Using High Resolution Mass Spectrometry MS", *David Watson (University of Strathclyde, UK)*
16:00-16:15 "Where would we be without metabolomic databases and repositories?", *Ralf Weber (University of Birmingham, UK)*
16:15-16:40 Interactive discussion

Metabolomics data standards, data capture and exchange

Marica 3F "Marica Hall"

Aim and Scope: The metabolomics community has seen a first round of standardization efforts culminating in a set of publications in 2006 and 2007. We are now witnessing the emergence of open, general purpose repositories such as MetaboLights (http://www.ebi.ac.uk/metabolights) at the European Bioinformatics Institute and Metabolomics Work bench by the NIH (http://www.metabolomicsworkbench.org/), which make use of those standards and which open up the opportunity for a worldwide network of metabolomics data deposition and exchange.
In this workshop, participants will be able to inform themselves about the current status in the development of this worldwide network of databases arising and current activities such as the European FP7 coordination action COSMOS (http://www.cosmosfp7.eu), the NIH Common Fund's Metabolomics Program and the Metabolite Standards Synthesis Core (MSSC) which aims to provide metabolomics researchers with high quality metabolite standards. Those initiatives aim to build on the earlier developments and continue the work on standardization of data and meta data and to establish best practices and workflows for metabolomics data capture, deposition, and dissemination.

Organisers and Speakers: Reza Salek, Christoph Steinbeck, Susanna-Assunta Sansone, Alejandra Gonzalez-Beltran, Philippe Rocca-Serra, and the COSMOS consortium

Session I. Metabolomics Data Standardization

13:40-13:55 Metabolomics standards Role of COSMOS (COordination Of Standards In MetabOlomicS) Initiative
Reza Salek (*EMBL-EBI, UK*)
13:55-14:10 "Data exchange formats - Why, how and a plea to the MS instrument vendors"
Steffen Neumann, Leibniz Institute of Plant Biochemistry (*IPB Halle, Germany*)
14:10-14:25 XEML metadata and COSMOS
Kenny Billiau, MPI-MP Golm, Germany
14:25-14:40 ISA in the context of multi-omics datasets and looking forward to linked data
Philippe Rocca-Serra and Alejandra Gonzalez-Beltran (*Oxford e-Research Centre*)
14:40-15:00 Discussion and questions

Session II. Data Dissemination, Standardization and Exchange

15:20-15:50 Scott Edmunds and Rob Davidson (Gigascience), Susanna-Assunta Sansone (Nature Scientific Data)
15:50-16:10 Reza Salek (EMBL-EBI, UK), Philippe Rocca-Serra (Oxford e-Research Centre, UK)
16:10-16:25 Padma Maruvada (NIH/NCRR, USA)
16:25-16:40 Discussion and questions

Opening Ceremony and Lecture

Marica 3F "Marica Hall" 17:00-17:40

17:00-17:10 Welcome to Tsuruoka and the 10th Annual Conference (Masaru Tomita, Keio University)
17:10-17:20 Welcome to the Metabolomics Society (Mark Viant, President of the Society)
17:20-17:30 Welcome to EMN (Sastia Putri, Chair of the Early-career Members Network)
17:30-17:40 Award Ceremony (Honorary Fellowships, Best Publication Awards)

Marica 3F "Marica Hall" 17:40-18:20

Metabolomics revolutionizes phytochemical genomics and crop improvement

Kazuki Saito[1,2]

[1] RIKEN Center for Sustainable Resource Science, Yokohama, Japan
[2] Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Plants as sessile organisms produce a huge array of metabolites (metabolome), which play multiple roles in the life of plants for interaction and adaptation to the environment. Mankind owes immense benefits to these plant metabolites for food, feed, fuel, medicines, flavors and cosmetics. The size of metabolome in plant kingdom is estimated to be 200,000-1,000,000, which far exceeds those estimated in animals and microorganisms. An important issue to be addressed is how this metabolomic diversity is originated at the levels of genome (phytochemical genomics) and how we could apply this knowledge to crop improvement. In this presentation, I would like to discuss how plant metabolomics revolutionizes functional genomics and crop improvement with the following points:

- Metabolomic analysis for understanding chemical diversity of plants
- From metabolomics-based functional genomics to systems biology in a model plant *Arabidopsis thaliana*
- Application to functional genomics and biotechnology of crops and medicinal plants

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Tuesday

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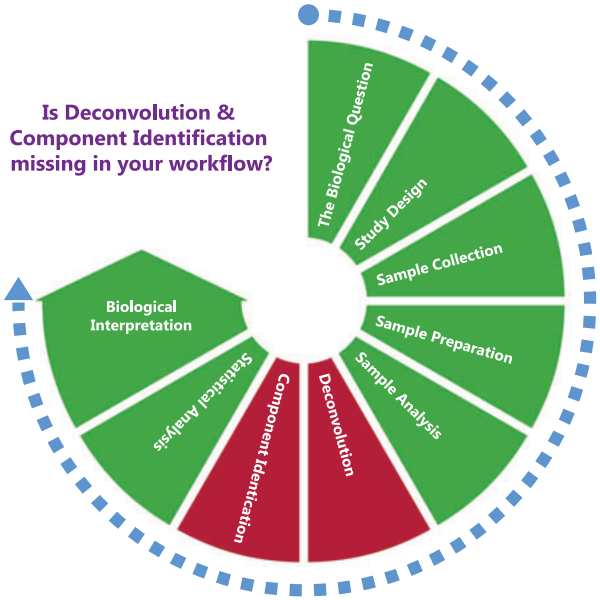

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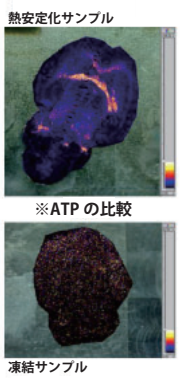

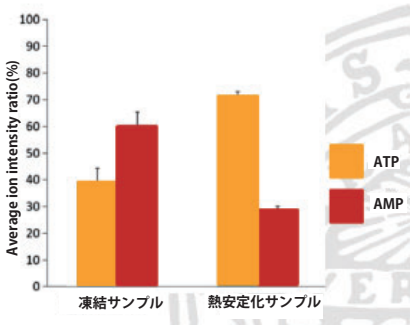

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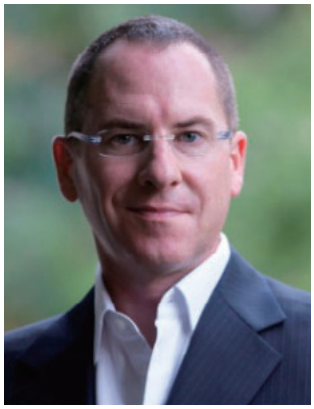
Keynote Lectures

Dai-ichi Hotel 2F "Tsuru Hall" 8:40-9:20

Natural variation of a signalling lipid



Markus Wenk

National University of Singapore
Associate Professor of Biochemistry & Biological Sciences
Director, Singapore Lipidomics Incubator
http://www.lipidprofiles.com

Once viewed simply as a reservoir for carbon storage, lipids are no longer cast as by-standers in the drama of biological systems. The emerging field of lipidomics is driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells (Wenk 2010 *Cell* 143(6):888-95).

Our recent results show extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans (Chua et al 2013 *Proc Natl Acad Sci U.S.A.* 110(35):14468-73). I will also introduce a strategy for capture of phospho-monoester lipids. Using this enhanced workflow we identified novel forms of sphingosine-1-phosphates, in tissue from human, mouse and fruit fly, respectively. (Narayanaswamy et al 2014, Anal Chem. 2014 Mar 18;86(6):3043-7).

Understanding better the fundamentals of natural variation in lipidomes as well as specific recognition of individual lipid species are the scientific aims of SLING, the Singapore Lipidomics Incubator. This centre is a global magnet for collaborating parties in lipidomics – from academia and industry – delivering new technologies and intellectual capital. SLING organizes the international Singapore Lipid Symposium (ISLS), a major symposium in lipidomic research in Asia Pacific and 'i c lipid', an intensive immersion course in mass spectrometry based lipidomics (http://www.lipidprofiles.com/index.php?id=139)

Keynote Lectures

Dai-ichi Hotel 2F "Tsuru Hall" 9:30-10:10

Use of metabolomics in systems biology: from data to understanding

Bas Teusink

Centre for Integrative Bioinformatics, Vrije Universiteit Amsterdam (IBIVU)
Netherlands Institute for Systems Biology (NISB), University of Amsterdam
Kluyver Centre for Genomics of Industrial Fermentation, Netherlands

Metabolomics data are used in the study of metabolic networks through largely two approaches, known as top-down and bottom-up systems biology. In the top-down approach, comprehensive data sets are generated to infer network or regulatory structures and to identify biomarkers. It uses inductive reasoning with statistics as its main computational method. In bottom-up systems biology, metabolomics data are used to develop and validate mechanistic models of metabolism. I will illustrate the use of metabolomics in systems biology approach to metabolism, through two studies.

In the first study, we used genome-scale metabolic models to develop a better understanding of the metabolic potential of a human pathogen. Model-directed comprehensive metabolomics analysis of fermentation broth showed the excretion of metabolites suspected to be invloved in host-pathogen interactions. Applying network-based medium optimisation algorithms led to a substantial simplification of growth media with over twofold higher productivity.

In the second study, a detailed kinetic model of glycolysis in yeast pointed to the co-existence of two metabolic states, one functional state and a state in which the reactions in glycolysis are unbalanced and intermediates accumulate. The co-existence of two metabolic states was reflected in two metabolic subpopulations within a clonal population. Dynamic fluxes within the first five minutes after glucose addition –estimated from time-dependent metabolomics data – determine which state is reached by individual cells (Van Heerden, *Science* 2014). This study shows the individuality of cells within a population, and the need for single-cell metabolomics technologies to understand the biology of cellular life.

Marica 3F "Marica Hall" 9:30-10:10

From mice to man and back again: applications of metabolomics to understand type 2 diabetes and obesity

Julian L. Griffin

Medical Research Council Human Nutrition Research, Cambridge & the Department of Biochemistry, University of Cambridge

There is a global epidemic in terms of the rise in the number of sufferers with type 2 diabetes (T2DM) with the world health organisation estimating currently 347 million people worldwide with diabetes and the disease representing the 7th largest cause of death globally. While a number of genes have been identified that play a role in the development of T2DM, most are associated with rare monozygotic forms of the disease, and do not model the complex interactions between genotype and the environment, and in particular diet and exercise, that is responsible for the development of T2DM in many human cases. We have been using a combination of high resolution mass spectrometry and NMR spectroscopy as tools to model the interactions between diet, age and genetic susceptibility in mouse models of T2DM. Both diet and age accelerate the development of T2DM in the obob mouse, recapitulating many of the features occurring in human disease. To translate the metabolomics results into clinical applications we have also cross compared our mouse studies with human patients possessing different forms of T2DM. In terms of both metabolomics and lipidomic perturbations both mouse models and human sufferers of T2DM showed a high degree of similarity. This has allowed us to return to the animal models and investigate tissue specific mechanisms using metabolomics, transcriptomics and proteomics that are responsible for specific metabolite changes. This translational systems medicine approach should allow us to define new biomarkers of various aspects of T2DM and identify the underlying mechanisms that they represent.

Dai-ichi Hotel 2F "Tsuru Hall"

Flux, Microbiology & Parasitology

2A-D1

Identification of the metabolic reprogramming underlying metastasis in prostate cancer by combining multi-omic approach and model-driven data analysis

Marta Cascante University of Barcelona; Esther Aguilar University of Barcelona; Igor Marin de Mas Biological Research Centre of the Hungarian Academy of Sciences; Silvia Marin University of Barcelona; Vitaly Selivanov University of Barcelona; Erika Zodda University of Barcelona; Pedro de Atauri University of Barcelona; Josep J. Centelles University of Barcelona; Oscar Meca-Cortes Molecular Biology Institute of Barcelona, National Research Council; Balazs Papp Biological Research Centre of the Hungarian Academy of Sciences; Timothy Thomson Molecular Biology Institute of Barcelona, National Research Council;

The metastatic potential of tumors resides in a minority of malignant cells, known as tumour-initiating cells (TICs), and it uses at several critical steps the process known as epithelial-mesenchymal transition (EMT). Using a well-characterized clonal neoplastic cell model and combining physiologic and multi-omic measurements with a novel model-driven genome scale metabolic network reconstruction analysis, we have identified key metabolic features that distinguish cells with either TIC or EMT properties, producing a metabolic landscape in which TICs engage redundant mechanisms for energy production and protection from cell death. Our observations have potential clinical implications.

2A-D2

OpenMebius: An Open source software for metabolic flux analysis

Syuichi Kajihata Osaka University; Chikara Furusawa QBiC, RIKEN; Fumio Matsuda Osaka University; Hiroshi Shimizu Osaka University;

Metabolic flux analysis is a method for *in vivo* measurement of metabolic activities of organisms. We develop a bioinformatics tool, OpenMebius, to determine metabolic flux from the ^{13}C-enrichment data of isotopically labeling experiments. OpenMebius provides the function of auto-generating metabolic models for simulating isotopic labeling enrichment from a user-defined configuration worksheet. Analysis using artificial data demonstrated the availability of OpenMebius for stationary and non-stationary ^{13}C-metabolic flux analysis. This software provides valuable information of metabolic states to create microbial cell factories to produce bio-based chemicals and fuels for low carbon society.

2A-D3

Quantitative metabolic modeling for biofuel production at the Joint BioEnergy Institute.

Hector Garcia Martin Lawrence Berkeley National Lab; Jorge Alonso-Gutierrez Lawrence Berkeley National Lab; Garrett Birkel Lawrence Berkeley National Lab; Amit Ghosh Lawrence Berkeley National Lab; Paul D Adams Lawrence Berkeley National Lab; Jay Keasling Lawrence Berkeley National Lab; Taek-Soon Lee Lawrence Berkeley National Lab; Christopher Petzold Lawrence Berkeley National Lab;

The goal of the Joint BioEnergy Institute (JBEI) is to produce fundamental scientific discoveries to enable large-scale conversion of lignocellulosic biomass into fuels. We will describe how the Quantitative Metabolic Modeling (QMM) directorate at JBEI uses experimental, computational and mathematical tools to achieve this goal. We will first introduce a new method that uses metabolomics data from 13C labeling experiments to measure fluxes for genome-scale models. Secondly, we will show how we used quantitative proteomic data to increase the production titers of limonene, a biofuel molecule. Finally, we will present new web-based tools for data storage and flux visualization.

2A-D4

Stable-isotope labelled metabolomics for network-wide discovery of stage-specific pathways in Trypanosoma brucei

Darren J Creek Monash University; Dong Hyun Kim Nottingham University; Jana Anderson University of Glasgow; Achuthanunni Chokkathukalam University of Glasgow; Andris Jankevics University of Manchester; Rainer Breitling University of Manchester; Michael P Barrett University of Glasgow;

Trypanosoma brucei is a protozoan parasite that causes fatal infections of humans and livestock. Metabolism differs significantly between the pathogenic bloodstream-form parasites and the procyclic form found in the Tsetse fly vector. A new metabolome-wide stable-isotope tracing approach was developed to characterise the active pathways in each stage. Advances in the IDEOM and mzMatch software enabled extraction of all isotopologue signals from high resolution LCMS data. Evaluation of relative isotopologue abundances across the metabolome revealed novel metabolites and unexpected pathways for sugar phosphate, organic acid and pyrimidine synthesis in pathogenic bloodstream-form parasites.

2A-D5

An altered M. tuberculosis metabolome induced by katG mutations resulting in drug resistance

Du Toit Loots North-West University

The most common form of drug resistance found in TB-positive clinical samples, is mono-resistance to the drug isoniazid. Despite the genomics and proteomics research done to date, this phenomenon is still only partly understood. Consequently, we used a GCxGC-TOFMS metabolomics approach, and indicated an increased susceptibility of these drug resistant strains to oxidative stress, and an up-regulation in the synthesis of a number of compounds involved in: 1) the uptake and use of alkanes and fatty acids as a source for carbon and energy, and 2) directly reducing oxidative stress, including an ascorbic acid degradation pathway, as a means to compensate for this.

2A-D6

LC-HRMS fingerprinting: an innovative strategy to investigate bacterial metabolome during cheese ripening.

Le Boucher Clementine INRA Agrocampus-Ouest UMR 1253 STLO and LUNAM Universite, Oniris, Laberca; Frederique Courant LUNAM Universite, Oniris, LABERCA; Anne-Lise Royer LUNAM Universite, Oniris, LABERCA; Jeanson Sophie INRA Agrocampus-Ouest UMR 1253 STLO; Lortal Sylvie INRA Agrocampus-Ouest UMR 1253 STLO; Gaud Dervilly-Pinel LUNAM Universite, Oniris, LABERCA; Anne Thierry INRA Agrocampus-Ouest UMR 1253 STLO; Bruno Le Bizec LUNAM Universite, Oniris, LABERCA;

Cheese ripening mechanisms has always been investigated with targeted approaches. We assessed the relevance of untargeted metabolomics to study the influence of a ripening parameter, known to generate fine differences on bacterial metabolism within cheese. Metabolic fingerprints were acquired by LC(ESI+/-)-HRMS throughout 1 month of ripening, based on two different extractions. Data processing (XCMS) and data analysis highlighted significant differences in bacterial metabolome profiles. 27 metabolites were further identified thanks to an in-house database. From now on, untargeted metabolomics can be considered as a new approach for the understanding of cheese ripening mechanisms.

Drug & Medicine

2A-M1

Ceramide metabolism in the liver and its role in two lysosomal lipidoses - drug-induced phospholipidosis (DIPL) and Sandhoff disease (SD)

Emmanuelle Lecommandeur University of Cambridge; Chloe Day GlaxoSmithKline; David Baker GlaxoSmithKline; Maria Begona Cachon-Gonzalez University of Cambridge; Timothy M. Cox University of Cambridge; Andrew W. Nicholls GlaxoSmithKline; Julian L. Griffin University of Cambridge;

Open-profiling of lipids and targeted analysis of six ceramide species were performed by LC-MS. Chloroquine DIPL was studied in Hep G2 cells and liver from rats, whilst SD was assessed in the liver from a mouse model. Total ceramide content was not affected by the lipidoses, but the proportions of the different fatty acyl chain lengths in ceramides were changed, including a common increase in C16:0-ceramide. In DIPL, these changes were accompanied by an alteration to the expression of the ceramide synthase genes. Given the important role of ceramides in cellular signalling and apoptosis, this may provide mechanistic insight to the common processes associated with lysosomal dysfunction.

2A-M2

In Search of Metabolic Somnogens: A Unique Signature of Sleep Restriction from Blood in Rats

Arjun Sengupta University of Pennsylvania; Peeter Meerlo University of Groningen; Namni Goel University of Pennsylvania; Matt Kayser University of Pennsylvania; David Dinges University of Pennsylvania; Morris Birnbaum University of Pennsylvania; Ted Abel University of Pennsylvania; Amita Sehgal University of Pennsylvania; Aalim M Weljie University of Pennsylvania;

Sleep deprivation is commonly experienced and produces significant clinical implications. We identified a unique molecular signature of sleep restriction (SR) from serum using a rat model. Ten rats were subjected 20 hours of SR over five days, alongside 10 control animals subject to 10 hours forced activity to normalize metabolic outputs. In a separate validation study, animals were subjected to SR followed by a three-day recovery period. Metabolites and lipids unique to SR were identified and validated. Branched chain amino acid, fatty acid and central carbon metabolism pathways were perturbed by SR while sphingomyelins and oxidized phosphatidylcholines were also significantly altered.

2A-M3

Activation of Fatigue Metabolic Pathway Induces Hepatic Inflammation in a Rat Model of Fatigue

Satoshi Kume Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Masanori Yamato Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Yukiharu Miyashige Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Masayuki Nakano Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Asami Eguchi Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Yasuhisa Tamura Cellular Function Imaging Team, RIKEN Center for Life Science Technologies; Yosky Kataoka Cellular Function Imaging Team, RIKEN Center for Life Science Technologies;

In metabolome analysis in the fatigued animals, we found a distinctive metabolic pathway in the fatigue condition; the metabolic flow from ornithine and glutamine to succinate of TCA cycle via GABA. In this study, we investigated the relationship between the fatigue metabolic pathway and hepatic inflammation using the fatigued model. The animals showed up-regulated gene expression of enzymes on the pathway and of proinflammatory cytokines including IL-1β. The oral administration of valproate suppressed such a metabolic flow into succinate, and attenuated the up-regulation of IL-1β. The results suggested that the activation of fatigue metabolic pathway induces inflammation in the liver.

2A-M4

Metabolomics biomarkers of doxorubicin-induced cardiac injury in B6C3F1 mice

Richard D Beger NCTR, US FDA; Varsa G Desai NCTR, US FDA; Lisa Pence NCTR, US FDA; Laura K Schnackenberg NCTR, US FDA; Josh C Kwekel NCTR, US FDA; Vikrant Vijay NCTR, US FDA; Carrie L Moland NCTR, US FDA; Tao Han

NCTR US FDA; **Taewon Lee** Korea University; **Sherry M Lewis** NCTR, US FDA; **James C Fuscoe** NCTR, US FDA;

Male B6C3F1 mice were given a weekly dose of 3 mg/kg doxorubicin (an anti-cancer drug) or saline via tail vein for 2, 3, 4, 6, and 8 weeks. Mice were euthanized a week after the last dose. Plasma levels of cardiac troponin T indicated cell injury at week 6 while lesions were observed at week 8. Metabolites in plasma and cardiac tissue were evaluated by MS and NMR. Several metabolites were increased in plasma and tissue at week 2. Levels of acetylornithine and arginine were significantly altered at all times in the heart while glycine and hexadecadienylcarnitine were altered in plasma at weeks 2-4. Pathway analysis of omics data suggested apoptosis and altered oxidative phosphorylation.

2A-M5

Metabolomics used as a tool to investigate the signature of etanercept treatment response in a psoriasis patient cohort.

Nicholas John William Rattray The University of Manchester; **Amy Foulkes** The University of Manchester; **Drupad Trivedi** The University of Manchester; **Richard B Warren** The University of Manchester; **Christopher E. M. Griffiths** The University of Manchester; **Royston Goodacre** The University of Manchester;

Psoriasis is a chronic inflammatory skin disease affecting 2-4% of the population in developed countries, with a high cost to patients and healthcare systems alike. To our knowledge, we present the first metabolomic assessment in a psoriasis cohort. Participants with severe disease were evaluated at 3 time points; pre-, during and post- TNF-inhibitor therapy with the agent etanercept. Subject serum and urine were analysed using GC-MS, RPLC-MS as well as HILIC-MS and subsequent statistical analysis, including PCA and DFA, were used to create a metabolomic signature of treatment response, representing an exciting pharmacogenomic advance in the treatment of this important inflammatory disease.

2A-M6

Using metabolomics to determine the modes of action of new antibiotics

Isabel M Vincent Glasgow University; **David Ehmann** Astrazeneca; **Manos Perros** Astrazeneca; **Scott Mills** Astrazeneca; **Karl Burgess** Glasgow University; **Michael Barrett** Glasgow University;

The metabolomes of *Escherichia coli* and *Pseudomonas aeruginosa* were perturbed with investigational antibacterials. pHILIC chromatography was coupled to a Thermo Exactive mass spectrometer and peaks were analysed using mzMatch and Xcalibur. An untargeted analysis of changes to the metabolomes was conducted along with targeted analyses of the five main pathways hit by antibiotics. These were compared to ceftazidime, a known PBPi. Putative modes of action were ascribed to the inhibitors and rates of drug uptake and processing were recorded. Peptidoglycan recycling was found to be one of the targets, and some inhibitors were modified by a resistant strain of *P. aeruginosa*.

Luncheon Seminars

Agilent Technologies

Dai-ichi Hotel 2F "Tsuru Hall" 12:30-13:20

Advances in Instrumentation and Software For Pathway Informed Metabolomics and Integrated Biology Analysis

Theodore Sana

PhD, Integrated Metabolomics Program Manager

Global metabolomics pathway profiling, mining and visualization workflows are increasingly being used to complement knowledge gleaned from traditional hypothesis driven experiments. I will describe Agilent's broad metabolomics program that includes a diverse portfolio of instrumentation and software tools for comprehensive analysis, metabolite identification and biological interpretation.

Untargeted Metabolomics Study of the Plant-Pathogenic Fungus Magnaporthe oryzae by GC x GC x QTOFMS

Sofia Aronova

Senior GC/ Q-TOF Applications Specialist

The rice blast fungus *Magnaporthe oryzae* causes significant losses of rice production. The precise regulatory mechanisms that control efficient growth of the fungus within its host remain unknown. Characterization of the metabolomes of non-pathogenic mutants could help better understand metabolic strategies employed by *M. oryzae* to colonize rice cells. We compared the metabolomes of a wild type strain to non-pathogenic mutant strains of *M. oryzae*. GC x GC/ Q-TOF was employed to ensure precise identification of the metabolites. This analysis was performed in order to take the first steps towards identifying and characterizing biochemical pathways essential for rice blast disease.

Using KEGG pathways to visualize meaningful biological correlations with LC/MS datasets

Dan Cuthbertson

PhD, Software Applications Engineer

As greater volumes of metabolomics data is generated, increasingly advanced and sophisticated software tools are required for feature extraction, mining, visualization and interpretation. As part of a large clinical study, patient plasma samples have been collected over several generations in order to find surrogate markers that can differentiate populations at risk for heart disease and diabetes. Data for a subset of this large cohort of samples was acquired in untargeted mode, using Agilent 1290 Infinity uHPLC and an Agilent 6550 QTOF. The feature extracted data was imported into Agilent Mass Profiler Professional (MPP) for statistical analysis and correlation between metabolites and metadata, and other metabolites. The strongly correlated compounds were isolated from the correlation matrix and searched against KEGG pathways for pathway visualization. We will demonstrate how this advanced software workflow can rapidly uncover meaningful biological relationships. Moreover, by incorporating RapidFire into the workflow, thousands of samples can be analyzed in a fast, robust, cost-effective manner.

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in Tsuruno ma at Daiichi Hotel Tsuruoka
IAB Lab Tour on Wednesday June 25, 17:00–18:15
Conference Banquet on June 25, sponsored by Agilent

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Luncheon Seminars

SHIMADZU CORPORATION

Marica 3F "Marica Hall" 12:30-13:20

Metabolome analysis for discovering biomarkers of cancers

Chief, Masaru Yoshida

M.D., Ph.D.
Division of Metabolomics Research & Gastroenterology, Kobe University
Graduate School of Medicine

Improvements in analytical technologies have made it possible to rapidly determine the concentrations of thousands of metabolites in any biological sample, which has resulted in metabolome analysis being applied to various types of research, such as clinical, cell biology, and plant/food science studies. The metabolome represents all of the end products and by-products of the numerous complex metabolic pathways operating in a biological system. Thus, metabolome analysis allows one to survey the global changes in an organism's metabolic profile and gain a holistic understanding of the changes that occur in organisms during various biological processes, e.g., during disease development. In clinical metabolomic studies, there is a strong possibility that differences in the metabolic profiles of human specimens reflect disease-specific states. Recently, metabolome analysis of biofluids, e.g., blood, urine, or saliva, has been increasingly used for biomarker discovery and disease diagnosis. Mass spectrometry-based techniques have been extensively used for metabolome analysis because they exhibit high selectivity and sensitivity during the identification and quantification of metabolites. Here, we show metabolome analysis using gas chromatography-mass spectrometry. Furthermore, we discuss metabolome analysis-based disease diagnosis.

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Metabolomics 2014

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Dai-ichi Hotel 2F "Tsuru Hall"

Cancer

2P-D1

Oncometabolites: Linking Altered Metabolism With Cancer?

Patrick John Pollard University of Edinburgh; Ming Yang University of Edinburgh; Tomoyoshi Soga Keio University;

The discovery of cancer-associated mutations in genes encoding key metabolic enzymes has provided a direct link between altered metabolism and cancer. Advances in mass spectrometry and nuclear magnetic resonance technologies have facilitated high-resolution metabolite profiling of cells and tumors, and identified the accumulation of metabolites associated with specific gene defects. Here, we review the potential roles of such oncometabolites in tumor evolution and as clinical biomarkers for the detection of cancers characterized by metabolic dysregulation.

2P-D2

Finding the right path: Constructing a temporal network of RAS activity in cancer

Emily Grace Armitage Centre for Metabolomics and Bioanalysis, Universidad San Pablo CEU, Madrid; David Rojo Centre for Metabolomics and Bioanalysis, Universidad San Pablo CEU, Madrid; Marta Isabel Barradas CNIO-Lilly Cell signalling Therapies section, Experimental Therapeutics Program, Centro Nacional de Investigaciones Oncologicas, Madrid; Laura Diezma CNIO-Lilly Cell signalling Therapies section, Experimental Therapeutics Program, Centro Nacional de Investigaciones Oncologicas, Madrid; Veronica Garcia Carpicio CNIO-Lilly Cell signalling Therapies section, Experimental Therapeutics Program, Centro Nacional de Investigaciones Oncologicas, Madrid; Susana Velasco-Miguel CNIO-Lilly Cell signalling Therapies section, Experimental Therapeutics Program, Centro Nacional de Investigaciones Oncologicas, Madrid; Coral Barbas Centre for Metabolomics and Bioanalysis, Universidad San Pablo CEU, Madrid;

Oncogene-induced senescence (OIS) is an anti-tumour mechanism of protection against cancer. To study this, we have designed a fibroblast cell line with induced RAS activation and analysed cell extracts over time by GC-MS, LC-QTOF-MS and CE-TOF-MS. The transition of cells towards RAS-OIS was marked by significant alterations in acyl carnitine, fatty acid and glycerophospholipid levels as well as alterations in central carbon metabolism and arginine and proline metabolism. A sequence of metabolic maps has been constructed that portrays the metabolic change over time, opening up new opportunities for fluxomics to highlight the relative importance of the pathways highlighted in the network.

2P-D3

Lipidomic profiling reveals a reconfiguration of sphingolipid metabolism that drives glioma angiogenesis and malignancy

Anthony Don University of New South Wales; Hazem Abuhusain University of New South Wales; Azadeh Matin University of New South Wales; Kerrie McDonald University of New South Wales;

Lipidomics coupled with transcript profiling for relevant metabolic enzymes was applied to investigate how the sphingolipid metabolic pathway is re-configured in malignant human gliomas, compared to normal brain. Two key enzymatic changes appear to direct a shift in sphingolipid metabolism that favours synthesis of the pro-survival, pro-angiogenic signalling lipid sphingosine 1-phosphate (S1P), at the expense of its pro-apoptotic precursor ceramide. This metabolic shift increases with increasing clinical grade and is essential for maintenance of cancer cell viability. S1P secreted by tumour cells promotes tumour angiogenesis and could potentially be targeted for therapeutic benefit.

2P-D4

Enhanced pyruvate carboxylation is crucial to non-small cell lung cancer proliferation and anabolism

Teresa Whei-Mei Fan University of Kentucky; Katherine E. Sellers National Institute for Medical Research, London; Matthew P. Fox Massachusetts General Hospital; Michael Bousamra II University of Louisville; Jun Yan University of Louisville; Mariia Yuneva

National Institute for Medical Research, London; **Richard M Higashi** University of Kentucky; **Andrew N Lane** University of Kentucky;
Anaplerosis to the Krebs cycle is vital to growing cancer cells. Major anaplerotic pathways involve glutaminase (GLS) and pyruvate carboxylase (PC). We found that PC was overexpressed (ca. 10 fold) in tumor tissues. With 13C6-glucose (Glc) as tracer and stable isotope-resolved metabolomics (SIRM), we saw enhanced PC activity in vivo in human NSCLC patients. PC knockdown (KD) in 5 NSCLC cell lines showed reduced growth. Using 13C6-Glc or 13C5-Gln and SIRM, we verified reduced PC activity and blocked entry of Glc and Gln carbon into the Krebs cycle, fatty acids, and nucleotides. PC KD reduced A549 cell growth in vivo. The data show that PC is indispensible for growth and anabolism of NSCLC.

Marica 3F "Marica Hall"

CVD, Diabetes & Neuroscience

2P-M1

Longitudinal metabolome en route to type 1 diabetes: potential role of gut microbiota and metabolic vulnerability in the early disease pathogenesis

Matej Oresic Steno Diabetes Center, Gentofte, Denmark; **Tuulia Hyotylainen** Steno Diabetes Center, Gentofte, Denmark; **Thomas Greiner** The Wallenberg Laboratory and Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Gothenburg, Gothenburg, Sweden; **Aleksandar Kostic** The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; **Heli Siljander** University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland; **Vallo Tillmann** Department of Pediatrics, University of Tartu, Estonia and Tartu University Hospital, Tartu, Estonia; **Curtis Huttenhower** The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; **Ramnik Xavier** The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; **Fredrik Backhed** The Wallenberg Laboratory and Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Gothenburg, Gothenburg, Sweden; **Mikael Knip** University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland;
We found earlier that specific metabolic derangements precede islet autoimmunity in children who later develop type 1 diabetes (T1D). Here we studied metabolomes in germ-free (GF) and conventionally raised NOD mice and longitudinal metabolomic and metagenomic profiles in children at risk for T1D. We observed decreased microbial gene richness preceding T1D in children and found that shifts in microbiota prior to seroconversion are correlated with altered serum levels of triglycerides and BCAAs. Similar associations were found in GF NOD mice, which were also characterized by elevated variation in blood glucose levels. Altered microbial metabolism may thus contribute to the pathogenesis of T1D.

2P-M2

Large scale metabolomics in clinical epidemiology

Oliver Fiehn UC Davis; **Carlos Leon** UC Davis; **Brian Defelice** UC Davis; **Tomas Cajka** UC Davis; **Bill Wikoff** UC Davis;
We present metabolome data for over 7,000 plasma samples from two clinical studies: (a) the TEDDY study on the onset and progression of type 1 diabetes in children and (b) on the SATURN study on cardiovascular health in adults. For all samples, over 150 identified primary metabolites and over 400 identified complex lipids were analyzed by GCTOF MS and by CSH-QTOF MS, in addition to over 300 unknowns. HILIC-QTOF MS for cationic and amino-compounds was used in the SATURN study. Use of BinBase and MPP/recursive analysis ensured that the data set did not have missing values. We present the overall workflow for quality controls, internal standards, data processing and statistical evaluations.

2P-M3

Examining the effects of metformin in a large clinical cohort and HepG2 and 3T3-L1 treated cells using untargeted LC-MS metabolomics

Emma Louise Boulton University of Glasgow
Metformin is the first line treatment for the majority of type 2 diabetics, however very little is known about its met-

abolic mode of action. We have used an untargeted liquid chromatography-mass spectrometry metabolomics approach to analyze 1) plasma samples from a large clinical cohort (CAMERA study) where coronary artery disease patients were treated with either metformin or placebo and 2) hepatocyte (HepG2) and adipocyte (3T3-L1) cells cultured and treated with metformin to explore mode of action under controlled conditions. The data were analyzed using an in-house bioinformatics pipeline to highlight significant differences between treatment groups and correlations with cohort data.

2P-M4

Metabolome and lipidome features of human brain development

Kasia Bozek CAS-MPG Partner Institute for Computational Biology, Shanghai, China; Patrick Giavalisco Max Planck Institute for Molecular Plant Physiology, Golm, Germany; Philipp Khaitovich CAS-MPG Partner Institute for Computational Biology, Shanghai, China;

How do metabolome and lipidome of human brain change during postnatal development? To assess this we measured lipid and hydrophilic metabolite composition of developing prefrontal cortex (PFC) in 40 humans, 40 chimpanzees and 40 macaques. Strikingly, 50% of lipids, but less than 10% of hydrophilic metabolites showed age-dependent concentration changes. The predominant patterns of age-dependent lipidome changes conserved among species include previously identified lipid markers of aging. By contrast, human-specific lipidome features include known markers of neurodegenerative diseases, as well as novel lipidome features potentially associated with cognitive functions unique to the human brain.

2P-M6

Accumulation of metabolic syndrome risk factors and plasma amino acids and their related metabolites: Tsuruoka Metabolomic Cohort Study

Toru Takebayashi Keio University School of Medicine; Sei Harada Keio University School of Medicine; Ayako Kurihara Keio University School of Medicine; Miki Akiyama Keio University Faculty of Environment and Information Studies; Tomonori Okamura Keio University School of Medicine; Yuji Nishiwaki Toho University School of Medicine; Taichiro Tanaka Toho University School of Medicine; Akiyoshi Hirayama Keio University Institute for Advanced Biosciences; Masahiro Sugimoto Keio University Institute for Advanced Biosciences; Tomoyoshi Soga Keio University Institute for Advanced Biosciences; Masaru Tomita Keio University Institute for Advanced Biosciences;

To elucidate the association between amino acids and their related (AA) metabolites and metabolic syndrome (MetS), we analyzed CE-based plasma matabolome of 1016 male and 1108 female Tsuruoka-city residents aged 35-74. MetS was defined according to IDF 2009 joint interim statement. Among 66 AA metabolites, 18 such as those from Gly, Ser, Thr metabolism, Ala, Asp, Glu metabolism, or BCAA catabolism were significantly increased or decreased while MetS risk factors accumulated by BH-FDR adjusted trend p-value of ANOVA in men aged below 65. A follow-up survey is planned to determine if they could be biomarkers predicting MetS-related health outcome such as cardio- and cerebro-vascular diseases.

Evening Session

Thermo Fisher Scientific

Dai-ichi Hotel 2F "Tsuru Hall" 17:00-18:20

Thermo Fisher Scientific Session: starting at 17:00 in Tsuru Room (Dai-ichi Hotel 2F)
Connect with your peers; share what you know, and learn from others through short topic talks at our Evening event. There are two parts: a short talk session with customer research topics followed by a mixer session. Join us for refreshments- beer, wine, "sake" and snacks, ending with "Kagami Biraki", a Japanese traditional ceremony.

Short Talk Abstract

Advantages of Capillary Ion Chromatography Coupled with High Resolution Accurate Mass MS to Profile Anionic Polar Metabolites

Terri Christison, Thermo Fisher Scientific, Ion Chromatography Marketing Specialist

We will discuss the application of ion chromatography, inline continuous desalting, and the IC-MS interface with the Q Exactive high resolution accurate mass (HR/AM) MS system.. This method, Cap IC HR/AM MS, was applied to the metabolic profiling of human oral squamous cell carcinoma (HOSCC) cells using 3-paired OSCC cell lines (UM1, UM5, CSC) and wild-type controls. The results demonstrated clear advantages over RPLC and HILIC methods.

Advances in Current Metabolomics Software Offerings: SIEVE, m/z Cloud, and Thermo Scientific™ Compound Discoverer™ software

Ralf Tautenhahn, Thermo Fisher Scientific, Life Sciences Mass Spectrometry Software Product Manager

This presentation will give an overview of the software solutions from Thermo Scientific for the common workflows in metabolomics, focusing on untargeted (discovery) metabolomics and metabolite/compound identification.

Fingerprinting Triacylglycerols in Seed Oils using Orbitrap Mass Spectrometry and Thermo Scientific™ LipidSearch™ Software

David Peake, Thermo Fisher Scientific, Life Sciences Mass Spectrometry Senior Marketing Specialist

This talk will illustrate the fingerprinting and comparison of different seed oils and the relationships of polyunsaturated omega-3 fatty acid containing species from canola to sesame seed oil.

June 25
Wednesday

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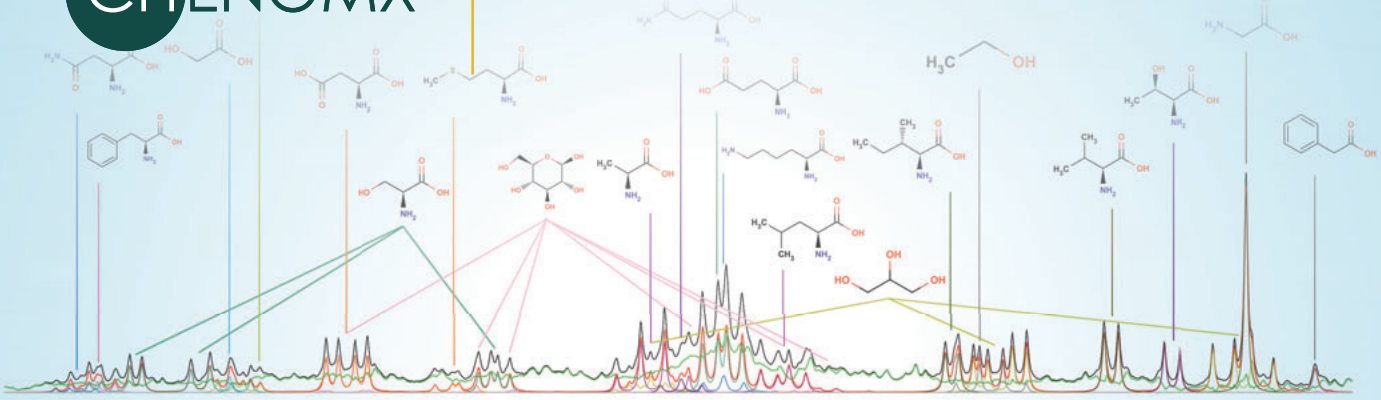

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Keynote Lectures

Marica 3F "Marica Hall" 8:40-9:20

Making metabolomics matter

David S. Wishart

Departments of Computing Science, Biological Sciences and Pathology/Laboratory Medicine, University of Alberta, Edmonton, Alberta, Canada

Metabolomics has long been the "poor cousin" to genomics and proteomics. It has struggled more than other "omics" sciences for visibility, acceptance and funding. In this presentation I will explore some of the reasons why metabolomics has struggled (historically) and what it needs to do to become more of a going concern within the life science community. In particular, I will outline a number of areas where metabolomics research, methodologies and technologies needs to be strengthened. I will also highlight a number of unrealized opportunities that could go a long way towards making metabolomics matter – to the public, to industry and to the funding agencies. Some of these opportunities lie in the little-known fact that more than 90% of plant, animal and human diseases have a non-genetic cause. This fact suggests that the development of environmentally, clinically and nutritionally useful chemical biomarkers needs to become a priority for the metabolomics research community. It also suggests that a shift to a far more "quantitative" and "democratized" approach to metabolomics needs to take place. This includes the development of "Next-Gen" metabolomics technologies such as higher throughput, fully automated metabolomic systems and the creation of low-cost, portable metabolomic devices.

Keynote Lectures

Dai-ichi Hotel 2F "Tsuru Hall" 9:30-10:10

Metabolomics imaging mass spectrometry in clinical research

Mitsutoshi Setou

Hamamatsu University School of Medicine, Department of Cell Biology and Anatomy, 1-20-1 Handayama, Hamamatsu, Shizuoka 431-3192, Japan

Metabolomics is the science applied to biological systems aiming to find the metabolite profiles produced by cellular processes at different hierarchical levels. An imaging mass microscope, iMScope, was developed in our laboratory in collaboration with Shimadzu, combining an optical microscope and a state-of-the-art imaging mass spectrometer (IMS) using MALDI (matrix-assisted laser desorption/ionization). The system allows high resolution IMS measurements for targeted sample regions and corroboration of results with histological diagnostic and other findings. An overview of recently obtained results for metabolomics IMS applied to clinical research will be presented.

Marica 3F "Marica Hall" 9:30-10:10

Metabolomics and the measurement of the exposome in epidemiological studies on cancer risk

Augustin Scalbert

International Agency for Research on Cancer (IARC)
Nutrition and Metabolism Section (NME)
Biomarkers Group (BMA)
150 cours Albert Thomas, F-69372 Lyon Cedex 08, France

Much effort has been made to identify the role of the genome in the development of cancer. However cancer aetiology appears to be largely governed by lifestyle and environmental factors. Various risk factors have already been identified but the causes of many cancers still remain largely unknown. Epidemiological studies conducted so far have been largely hypothesis-driven and focused on limited numbers of risk factors. These approaches were unable to take into account the considerable number of chemicals and metabolites to which individuals are commonly exposed along lifetime and that constitute altogether the exposome. The systematic comparison of metabolic profiles of individuals at varying risk of cancer using highly sensitive mass spectrometry techniques and fully agnostic metabolomic approaches opens exciting avenues to better understand the aetiology of these diseases (Wild et al., 2013). Not only endogenous metabolites can be measured in biospecimens like urine or plasma but also a considerable number of metabolites of exogenous origin, derived from dietary compounds, contaminants, pollutants or drugs. Dietary polyphenols, a class of over 500 compounds with diverse chemical structures and biological properties, scattered in a large diversity of foods, will be taken as an example to illustrate the possibilities of metabolomics for measuring complex environmental exposures in large-scale epidemiological studies. Limitations and challenges in the application of metabolomics to nutritional and cancer epidemiology will be emphasized.

Reference*:*

Wild, C. P., A. Scalbert and Z. Herceg (2013). "Measuring the Exposome: A Powerful Basis for Evaluating Environmental Exposures and Cancer Risk." Environmental and Molecular Mutagenesis **54**(7): 480-499.

Dai-ichi Hotel 2F "Tsuru Hall"

Data Analysis, Networks

3A-D1

Complimentary LC- and GC-Mass Spectrometry Techniques Provide Broader Coverage of the Metabolome

Baljit Kaur Ubhi AB Sciex; Jeff Patrick LECO Corporation; Joe Shambaugh Genedata Inc;

Metabolomics researchers often require the use of both GC/MS and LC/MS technologies to provide comprehensive coverage of the analytes in biological systems. Here we highlight the value added in using both GC/MS and LC/MS analyses for untargeted metabolomics as an integrated workflow by interrogating a well-established rat model for diabetes, obesity and cardiovascular disease effects. Combining the data meant we were able to generate PCA models with clear differences between the samples groups by non-discriminant analysis. We also observed that whilst GC/MS addresses primary metabolites, LC/MS most readily addressed secondary metabolites providing deeper coverage of the metabolome.

3A-D2

An advanced computational and database framework for the compound identification in untargeted metabolomics

Robert Mistrik HighChem; Tim Stratton Thermo Fisher Scientific; Milos Suchy HighChem; Alena Bednarikova HighChem; Yingying Huang Thermo Fisher Scientific; Mark Sanders Thermo Fisher Scientific;

Despite the increasing availability of modern high resolution mass spectrometers, untargeted metabolomics is hindered by an inability to identify thousands of observed components effectively. We will present an advanced computational and database framework leading to the much anticipated increase in mass spectral coverage of the metabolome, taking into account all the important experimental and calculated information necessary for efficient and reliable identifications. The resulting spectral space serves as a unique resource for the identification of unknowns even if reference spectra are not available, providing a major benefit for the metabolomics' community.

3A-D3

MS DIAL: Untargeted metabolomics software for data independent LC-MS/MS and mass spectral deconvolution: application to algae metabolomics

Hiroshi Tsugawa RIKEN Center for Sustainable Resource Science; Tobias Kind University of California, Davis; Tomas Cajka University of California, Davis; Yan Ma University of California, Davis; Kazutaka Ikeda Keio University; Mitsuhiro Kanazawa Reifycs Inc.; Atsushi Ogiwara Reifycs Inc.; Oliver Fiehn University of California, Davis; Masanori Arita National Institute of Genetics;

Data-independent MS/MS approaches such as SWATH, MS^E, or all ion fragmentation are much richer in information content compared to classic data-dependent MS/MS experiments. We have developed novel GUI-based software to identify far more metabolites using adduct speciation, spectral de-convolution and library comparisons. The software exploits two axes of de-convolution on MS/MS: "time-axis" across one chromatogram and "sample-axis" over different chromatograms. Using lipidomic extracts from the green microalga *Chlamydomonas reinhardtii*, we demonstrate far improved results from this data-independent LC-MS/MS approach with respect to accuracy of identification using the LipidBlast library.

3A-D4

MASTR-MS: A web-based collaborative Laboratory Information Management System (LIMS) for Metabolomics

Saravanan Dayalan Metabolomics Australia, University of Melbourne, VIC, Australia; David P De Souza Metabolomics Australia, University of Melbourne, VIC, Australia; Rodney Lorrimar Australian Bioinformatics Facility, Centre for Comparative Genomics, Murdoch

University, WA, Australia; **Adam Hunter** Australian Bioinformatics Facility, Centre for Comparative Genomics, Murdoch University, WA, Australia; **Jeremy Hack** Metabolomics Australia, The Australian Wine Research Institute, SA, Australia; **Robert Trengove** Metabolomics Australia, Murdoch University, WA, Australia; **Ute Roessner** Metabolomics Australia, University of Melbourne, VIC, Australia; **Dedreia Tull** Metabolomics Australia, University of Melbourne, VIC, Australia; **Antony Bacic** Metabolomics Australia, University of Melbourne, VIC, Australia; **Matthew Bellgard** Australian Bioinformatics Facility, Centre for Comparative Genomics, Murdoch University, WA, Australia; **Malcolm J McConville** Metabolomics Australia, University of Melbourne, VIC, Australia;

We present MASTR-MS, one of the first wholly functional, free, open-source LIMS solutions specifically designed for metabolomics laboratories. MASTR-MS has three modules:1. The User Management System manages user accounts, privileges, quotes and client communication. 2. The Sample Management System manages the setting up of projects, designing experiments, defining samples and the creation of sample lists to be used to run samples in the instruments and 3.The Data Management System manages the automatic capture of raw data from the instruments and the systematic storage of processed data and support files related to experiments and projects. MASTR-MS: http://mastr-ms.readthedocs.org

3A-D5

Data-driven sample size determination for human metabolic phenotyping studies

Benjamin Blaise Hospices Civils de Lyon; **Aurelie Gouel** Hospices Civils de Lyon; **Bernard Floccard** Hospices Civils de Lyon; **Thomas Rimmele** Hospices Civils de Lyon; **Guillaume Monneret** Hospices Civils de Lyon;

Sample size determination is a key question in medical studies. It is difficult in metabolic phenotyping studies considering the absence of a priori metabolic target. Starting from spectra acquired from a small cohort, we generate a larger data set based on kernel density estimation of variable distributions to determine the sample size. This method is exemplified on a human cohort dealing with sepsis risk in traumatized patients. Orthogonal partial least square analysis allowed the recovery of a predictive metabolic phenotype identifying patients with a later development of sepsis. A sample size of 200 patients was determined by our method and an extended cohort is being recruited.

3A-D6

Searching PubChem with tandem mass spectrometry data: Teaming molecular fingerprint prediction and fragmentation trees

Kai Duehrkop Friedrich Schiller University Jena; **Huibin Shen** Aalto University, Helsinki; **Juho Rousu** Aalto University, Helsinki; Sebastian Boecker Friedrich Schiller University Jena;

Searching tandem mass spectra in molecular structure databases can be a powerful tool for identifying a compound. We use fragmentation tree computation to boost the performance of molecular fingerprint prediction, predicting 528 molecular fingerprints using Machine Learning. We reach an accuracy of 85.0%, and an F1 score of 69.8%. This almost doubles the F1 score compared to the previously best method for this task. We then use the predicted fingerprint of a compound to search PubChem for similar molecular fingerprint. We were able to identify the correct 2D structure in about 20% of the cases. This outperforms all other methods that have been proposed for this task to date.

3A-D8

Systematic approaches for identification and removal of non-biological sources of variation in metabolomics data

Shivshankar Umashankar Department of Biological Sciences & NUS Environmental Research Institute (NERI), National University of Singapore; **Rasmus Kirkegaard** Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University & Singapore Centre for Environmental Life Sciences Engineering (SCELSE), National University of Singapore; **Sanjay Swarup** Department of Biological Sciences & NUS Environmental Research Institute (NERI), National University of Singapore, Singapore Centre for Environmental Life Sciences Engineering (SCELSE); Rohan Benjamin Hugh Williams Singapore Centre for Environmental Life Sciences Engineering (SCELSE), National University of Singapore;

High-throughput mass spectrometry based experiments generate large and complex data. Combining data from such experiments performed over time (weeks/months) or in different batches present numerous challenges. These are often overlooked in current pipelines leading to multiple sources of variation, such as batch effects. Using untargeted metabolite profile data from 22 Chlorella strains, compared over two growth stages and run in four batches, we show removal of run-day effect, using a filtering procedure based on the singular value decomposition, with demonstrable

preservation of biological signal (inter-strain variation). Our approach will be broadly applicable in metabolomics analysis.

Marica 3F "Marica Hall"

Nutrition, Environment, Model Organism

3A-M1

Characterization of food products by GCxGC with TOFMS and GC with high resolution TOFMS: a food-omics approach

Elizabeth Humston-Fulmer LECO Corporation; **Joe Binkley** LECO Corporation; **Jeff Patrick** LECO Corporation; **David Alonso** LECO Corporation;

Gas chromatography with mass spectrometry is an effective tool for characterizing food products. As sample complexity increases analytical space is enhanced with 2DGC-TOFMS or with HR-TOFMS. These tools are applied to food-omics. Specific samples analyzed here include hops, beer, and edible oils using HS-SPME. These methods allowed for comparing food products using chromatographic fingerprints, differential analysis with unique analyte identification, differentiating samples with PCA, and analyte identification using accurate mass data. This approach applies to quality control, process optimization, and nutritional evaluation, among others.

3A-M2

A metabolomics approach shows that green tea extract attenuates UVB-induced skin metabolite alterations in mice

Eun Sung Jung Konkuk University; **Hey Min Park** Konkuk University; **Kyung-Eun Lee** Yonsei University; **Jung-Hoon Shin** Kyungpook National University; **Sukyeong Mun** Yonsei University; **Jeong Kee Kim** AmorePacific R&D Center; **Sang Jun Lee** AmorePacific R&D Center; **Kwang-Hyeon Liu** Kyungpook National University; **Jae-Kwan Hwang** Yonsei University; **Choong Hwan Lee** Konkuk University;

We revealed green tea extract (GTE) administration significantly attenuated UVB-induced mouse skin damages by assessing physiological markers. The metabolites in the mouse skin after UVB irradiation and GTE administration were profiled using comprehensive MS-based metabolomics approach, and revealed that GTE administration attenuated UVB-induced alteration of skin metabolites such as lysophospholipids, fatty acids, ceramides, amino acids, organic compounds, and nucleobases. Among them, inosine, hypoxanthine, ascorbic acid, and lactose were remarkably influenced by GTE administration, which indicated that these metabolites could be biomarkers to explain GTE effects on UVB-irradiated skin.

3A-M3

Non-targeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish and bilberries

Kati Hanhineva University of Eastern Finland; **Maria Lankinen** University of Eastern Finland; **Anna Pedret** Universitat Rovira i Virgili; **Ursula Schwab** University of Eastern Finland; **Marjukka Kolehmainen** University of Eastern Finland; **Jussi Paananen** University of Eastern Finland; **Rosa Sola** Universitat Rovira i Virgili; **Marko Lehtonen** University of Eastern Finland; **Kaisa Poutanen** University of Eastern Finland; **Matti Uusitupa** University of Eastern Finland; **Hannu Mykkanen** University of Eastern Finland;

The metabolic effects of dietary modifications were investigated in the Sysdimet-HealthGrain trial containing three parallel diet groups: whole grain products, fatty fish, and bilberries (HD); whole grains (WG); and control. LC-MS metabolite profiling revealed marked differences in fasting plasma after the intervention diets as compared to control. In both intervention groups significant increase was observed for glucuronidated alk(en)ylresorcinols correlating strongly with intake of whole grains. Additionally, HD intervention increased signals for CMPF, hippuric acid and various lipid species. In particular, plasma CMPF correlated strongly with intake of fish, but not with any other foods.

3A-M4

The secret life of the Mediterranean Lake Medee, the largest deep sea salt saturated formation in the world

David Rojo Center for Metabolomics and Bioanalysis, University CEU San Pablo; Michail Yakimov Institute for Coastal Marine Environment, CNR; Violetta La Cono Institute for Coastal Marine Environment, CNR; Vladlen Slepak Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine; Coral Barbas Center for Metabolomics and Bioanalysis, University CEU San Pablo; Olga Golyshina School of Biological Sciences, Bangor University; Manuel Ferrer Institute of Catalysis, CSIC; Peter Golyshin School of Biological Sciences, Bangor University;

Hypersaline environments are relatively widespread on Earth. Their main characteristic is relative isolation, which makes them a perfect subject to study the biogeochemical cycles at the thermodynamic limits to life. In this study, the microbial metabolism in Lake Medee has been explored through a multi-omics approach. With particular reference to the contribution from targeted-metabolomics, we have measured ecologically relevant molecules including betaine, amines, amino acids and acetate by LC-Q-TOF-MS and CE-UV. As a result, it was found that osmolite betaine is reduced in order to produce trimetylamine, which is consequently used in the biosynthesis of methane.

3A-M5

Differential lipid and metabolite levels in response to spawning-induced inappetence in Atlantic salmon Salmo salar

Rocco Cipriano USGS; McKenzie Smith Georgia Institute of Technology; Kathleen Vermeersch Georgia Institute of Technology; Alistair Dove Georgia Institute of Technology; Mark Styczynski Georgia Institute of Technology;

Months-long inappetence can have pathological negative impacts on many species, but it is unclear whether this would be true for salmon, for which this behavior is part of their natural life cycle during spawning. Here, we used GCxGC-MS to measure serum analyte profiles in inappetent spawning Atlantic salmon and captively reared animals fed through sampling. The two groups were easily distinguished by their metabolite profiles. While most metabolites were depleted in inappetent salmon, the few that were not (including specific classes of lipids) indicate previously unknown metabolic behavior. Compounds expected to indicate pathological effects were not more abundant in inappetent salmon.

3A-M6

A metabolomic approach to assess neurotoxic effects of imidacloprid on the freshwater snail lymnaea stagnalis

Sara Tufi VU Amsterdam; Marja Lamoree VU Amsterdam; Pim Leonards VU Amsterdam;

Imidacloprid (IMI) is a neonicotinoid insecticide acting as agonist on nicotinic acetylcholine receptors of neuronal cells, leading to impaired nerve pulses. Due to the large quantities of IMI used in agriculture major concerns have been raised about its adverse effects on bees and aquatic species. Therefore, we investigated the effect of IMI exposure on the metabolome of the central nervous system of the pond snail Lymnaea Stagnalis by performing cross platform metabolomics and targeted neurotransmitter analysis. This study sheds light on the mode of action of IMI, highlighting the involvement of different metabolic and neuronal pathways and potential biomarkers of exposure.

3A-M7

Using omics for identification of the mechanism of the toxic action for perflourononanoic acid

Kasper Skov Technical University of Denmark; Kristine Groenning Kongsbak Technical University of Denmark; Niels Hadrup Technical University of Denmark; Anne Marie Vinggaard Technical University of Denmark; Joern Smedsgaard Technical University of Denmark; Henrik Lauritz Frandsen Technical University of Denmark;

In Toxicological studies, the analytical goal of metabolomics is to obtain a metabolic fingerprint and to study how this fingerprint is changed in response to doses of xenobiotics. Male rats were dosed with three concentrations of perflourononanoic acid (PFNA), 0.0125, 0.25 and 5 mg/kg/day. The two highest doses resulted in lowered phospholipid, and mono- and diglyceride levels. These observations were supported by transcriptomics where the major changes in liver mRNA levels corresponded to genes involved in steatosis, which is generally known to decrease plasma lipids. Based on these results we suggest a possible mechanism of PFNA-induced hepatotoxicity.

3A-M8

Metabolome analysis of Drosophila melanogaster during embryogenesis

Thuy An Phan Nguyen Osaka University; **Masamitsu Yamaguchi** Kyoto Institute of Technology; **Takeshi Bamba** Osaka University; **Eiichiro Fukusaki** Osaka University;

Drosophila embryo is an ideal model for developmental biology and the lack of metabolome information is an obstacle on understanding the mechanisms of *Drosophila* embryogenesis. Thus, the aim of this study is to assess the changes in metabolome during *Drosophila* embryogenesis by using GC/LC-MS-based metabolome analysis. Results showed that there was a strong correlation between the metabolome and actual developmental stages of *Drosophila* embryo. Moreover, we were able to construct a robust and accurate prediction model as well as identify the important metabolites for *Drosophila* embryogenesis. This is the first report of a high-resolution quantitative metabolome analysis of *Drosophila* embryo.

Luncheon Seminars

AB SCIEX

Dai-ichi Hotel 2F "Tsuru Hall" 12:30-13:20

Using SWATH mode analysis in metabolomics: the advantage of mass spectral deconvolution of high-resolution MS data

Oliver Fiehn

Director, West Coast Metabolomics Center, UC Davis Genome Center, CA, USA

The use of LC-MS with data dependent MS/MS spectra acquisition faces several severe problems: data acquisition is often not fast enough to acquire MS/MS on all parent ions, especially for low abundant compounds. Hence, untargeted metabolomics studies may end up with statistically significant biomarkers that do not comprise MS/MS data for structural investigations. Secondly, often up to 3 Da isolation widths for the precursor ions are used for sensitivity reasons, causing fragmentation of co-eluting peaks and hampering purity of MS/MS. In collaboration with Dr. Masanori Arita's research group, we have utilized an AB Sciex TripleTOF mass spectrometer using sequential window acquisition of all theoretical fragment-ion spectra (SWATH) with 25 Da isolation widths for algae and wine studies and performed mass spectrometry-data independent analysis (MS-DIAL) using newly developed software. MS-DIAL software enables true MS/MS data deconvolutions and analyzing MS/MS chromatograms while automatically associating all molecular ion adducts. Results will show comparisons between data-dependent and data-independent MS/MS fragmentation in algae and wine studies.

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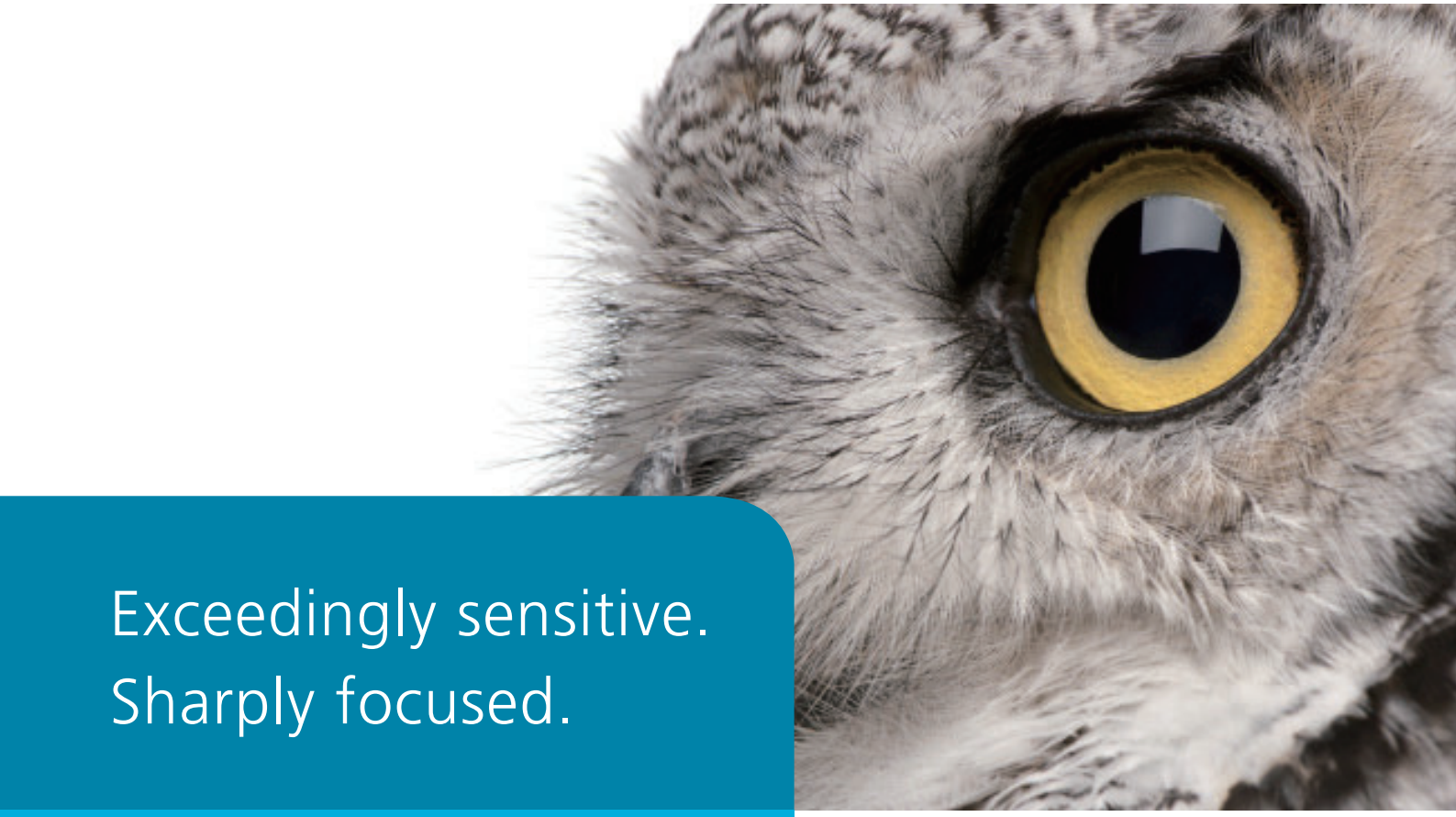

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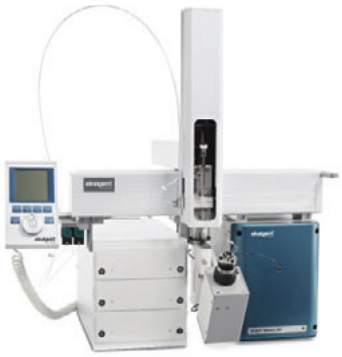

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Luncheon Seminars

Human Metabolome Technologies Inc.

Marica 3F "Marica Hall" 12:30-13:20

C-SCOPE: A Quantitative Energy Metabolism Analysis Package for Cells and Tissues

Kenjiro Kami

Ph.D., Human Metabolome Technologies, Inc., Tsuruoka, Yamagata, Japan

Concentrations of most primary metabolites in energy metabolism are often too low to be measured accurately by conventional metabolomics platforms. In addition, relative quantification makes is difficult to compare levels of different metabolites obtained in different projects. Further, data interpretation is often an inevitable bottleneck in metabolomics research. To resolve these issues in metabolomics, we developed C-SCOPE, a quantitative energy metabolism analysis package. Applying a capillary electrophoresis-triple quadrupole mass spectrometry (CE-QqQMS)-based metabolomics technique, we dramatically improved detection sensitivity of 63 anions up to 291-folds compared to a conventional TOF-MS-based analysis. Each of 116 target metabolites such as glycolytic, pentose phosphate pathway, citric acid cycle, and urea cycle intermediates, amino acids, and purines is absolutely quantified based on 3-point calibration, which thus realizes reliable quantitative comparisons among different metabolites in various projects. Absolutely quantified data can also be used to evaluate metabolic parameters such as energy charge, glutathione ratio, NAD(P)H/NAD(P)$^+$, and amino acid balances, which facilitate data interpretations and provide further insights into a more physiological state of cells and tissues. Applications of C-SCOPE in cancer research are also introduced: Dramatic and specific changes were observed in cell lines treated by molecular targeted drugs, providing clues for elucidating drug toxicity mechanism. Quantitative assessment of tissue metabolome data uncovers not only tumor-specific differences but also subtype-specific metabolic trends even within the tumor profile. Supported by highly sophisticated quantitative capacity and detection sensitivity of CE-QqQMS technology, C-SCOPE provides reliable and interpretable quantitative data for advancing a wide variety of research disciplines focusing on energy metabolism.

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Young Researchers

Dai-ichi Hotel 2F "Tsuru Hall"

3P-D1

Construction of a metabolome library for transcription factor-related single gene mutants of Saccharomyces cerevisiae

Zanariah Hashim Osaka University; Shao Thing Teoh Osaka University; Takeshi Bamba Osaka University; Eiichiro Fukusaki Osaka University;

Transcription factors play an important role in gene regulation, providing control for cells to adapt to different environments and physiological states. We performed metabolic profiling of 154 *S.cerevisiae*'s single gene knockouts each defective in a gene encoding transcription factor and built a metabolome library and conducted comparative analysis based on metabolic profile similarity. Using the metabolome dataset, we obtained significant correlations and identified differential strains that exhibit altered metabolism. This work presents a novel metabolome dataset which will be invaluable for researchers working on transcriptional regulation and yeast biology in general.

3P-D2

Metabolomics-based semi-rational identification of gene targets conferring 1-butanol tolerance in *Saccharomyces cerevisiae*

Shao Thing Teoh Osaka University; Sastia Prama Putri Osaka University; Yukio Mukai Nagahama Institute of Bio-Science and Technology; Takeshi Bamba Osaka University; Eiichiro Fukusaki Osaka University;

The metabolome is closely associated with phenotype, and comparison of metabolomes may reveal gene targets for strain improvement. Our objective was to demonstrate the usefulness of metabolomics in microbial strain improvement, by applying to 1-butanol tolerance of *Saccharomyces cerevisiae*. Mutant strains were subjected to metabolome analysis, regression modeling was used to identify metabolites positively correlated with tolerance, and new deletion mutants with higher levels of those metabolites were selected. These new strains were found to have higher tolerance over the parental strain, and the metabolome data also fit the regression model, proving the validity of this strategy.

3P-D3

Quantitative Analysis of Stressed Caenorhabditis elegans using Isotopic Ratio Outlier Analysis

Gregory S Stupp Biochemistry & Molecular Biology, University of Florida, Gainesville, FL; Chaevien Clendinen Biochemistry & Molecular Biology, University of Florida, Gainesville, FL; Ramadan Ajredini Biochemistry & Molecular Biology, University of Florida, Gainesville, FL; Francesca Ponce Biochemistry & Molecular Biology, University of Florida, Gainesville, FL; Chris Beecher IROA Technologies, Ann Arbor, MI; Timothy J Garrett Department of Pathology, Immunology and Laboratory Medicine & Southeast Center for Integrated Metabolomics, University of Florida; Lauren McIntyre Department of Molecular Genetics & Microbiology & Southeast Center for Integrated Metabolomics, University of Florida; Arthur S Edison Biochemistry & Molecular Biology & Southeast Center for Integrated Metabolomics, University of Florida, Gainesville, FL;

Isotopic Ratio Outlier Analysis identifies molecules of biological origin via LC-MS by isotopically labeling samples with 5% or 95% 13C and mixing for simultaneous analysis (1). In contrast to our previous experiments, we controlled for isotope effects by using as an internal reference the isotopically labelled 95% 13C worms; both control and experimental groups were labeled with 5% 13C. We analyzed 12 biological replicates; the larger number of samples and modified experimental design allows for a more rigorous statistical analysis whereby significant differences between features can be assessed while accounting for sources of variability. (1) Anal. Chem. 2013, 85, 11858-11865

3P-D4

Global Metabolomic Analysis of 13C-enriched Mixtures using INADEQUATE

Chaevien S Clendinen University of Florida; Christian Pasqual University of Florida; Ramadan Ajredini University of Florida;

Vijaykumar Ramaswamy University of Florida; **Gregory Stupp** University of Florida; **Christoph Turck** Max Planck Institute of Psychiatry; **Arthur Edison** University of Florida; Southeast Center for Integrated Metabolomics;

We present an approach to metabolomics using 2D 13C-detected NMR. We developed and validated this approach using an INADEQUATE of a 13C-labeled synthetic mixture. An INADEQUATE database was constructed using compounds in the BMRB and HMDB and we developed scripts to automatically find networks of spin systems in our spectra; these networks are matched to our database and a list of possible compounds is provided for each network. We analyzed INADEQUATEs of 13C-labeled C. elegans and 13C-labeled mouse tissues from labeled mice using our database and script to fully annotate, perform multivariate statistical analysis, and develop a metabolic profile of various tissue types and perturbations.

3P-D5

Classification of reliable MetFrag candidate identifications for tandem mass spectra from selected lipid samples

Christoph Ruttkies Leibniz Institute of Plant Biochemistry, IPB Halle, Department of Stress- and Developmental Biology; **Michael Witting** Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum Munich, German Research Center for Environmental Health; **Steffen Neumann** Leibniz Institute of Plant Biochemistry, IPB Halle, Department of Stress- and Developmental Biology; **Philippe Schmitt-Kopplin** Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum Munich, German Research Center for Environmental Health;

Lipids play an essential role in animal and plant metabolism, but their identification in high-throughput MS experiments still represents a major bottleneck. The use of data dependent acquisition allows to collect tandem mass spectra online during chromatographic separation, yielding large numbers of MS/MS spectra. MetFrag uses *in silico* fragmentation for metabolite identification, and was applied to tandem mass spectra of lipid extracts of the nematode *C. elegans*. The results show that detected fragments fit known fragmentation pathways. Lipid standard materials were used to build classifiers which are able to select reliable identifications out of a set of hundreds of spectra annotations.

Marica 3F "Marica Hall"

3P-M1

Characterization of the Sweat Metabolome in Screen-Positive Cystic Fibrosis Patients

Adriana Nori de Macedo Department of Chemistry and Chemical Biology / McMaster University; **Stephen Hill** Department of Pathology and Molecular Medicine / McMaster University; **Linda Pedder** Department of Pediatrics / McMaster University; **Philip Britz-McKibbin** Department of Chemistry and Chemical Biology / McMaster University;

The sweat test for chloride remains the gold standard in the diagnosis of cystic fibrosis (CF). However, disease severity is highly variable due to a complex phenotypic expression that results in ambiguous chloride test results. Multi-segment injection capillary electrophoresis-mass spectrometry (MSI-CE-MS) is used as a high-throughput screening approach for characterization of the sweat metabolome. An accelerated metabolomics workflow for biomarker discovery is introduced to identify and quantify sweat-derived metabolites differentially expressed in screen-positive CF patients. This work aims to improve stratification of CF patients to enable better treatment decisions in the clinic.

3P-M2

Fatty acid profiling in breast cancer biomarker discovery by GC-MS

Huai-Hsuan Chiu National Taiwan University; **Ching-Hua Kuo** National Taiwan University; **Yufeng Jane Tseng** National Taiwan University; **Chiun-Sheng Huang** National Taiwan University Hospital; **Sung-Jeng Tsai** National Taiwan University;

Fatty acids (FAs) have been shown to be differentially expressed in breast cancer patients. We used GC-MS to discover potential FA biomarkers in breast cancer patients. Several derivatization reagents were compared and acetyl chloride was selected considering method robustness and analytical cost. Plasma samples obtained from 50 breast cancer patients and 50 normal controls were derivatized and analyzed through GC-MS analysis. Among the detected 26 FAs, three FAs were significantly different between breast cancer patients and normal controls. These FAs were used to build a receiver operating characteristic curve to predict breast cancer with an area under the curve of 0.85.

3P-M3

Metabolome and transcriptome changes in human, macaque and mouse brain development

Hindrike Bammann CAS-MPG Partner Institute for Computational Biology; Masahiro Sugimoto Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan; Masaru Tomita Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan; Xi Jiang CAS-MPG Partner Institute for Computational Biology; Philipp Khaitovich CAS-MPG Partner Institute for Computational Biology;

Human brain evolution is characterized by a dramatic increase of brain/body size ratio and emergence of novel cognitive traits. Were these anatomical and functional changes accompanied by molecular adaptations? To answer this, we surveyed metabolome and transcriptome composition of developing brain in humans, macaque monkeys and mice using CE-MS and RNA-seq, respectively. The analysis revealed both conserved and rapidly evolving developmental features of brain metabolome and transcriptome. Notably, metabolome evolution proceeds at a more rapid pace and displays a larger number of human-specific features.

3P-M4

Metabolomics analysis reveals large effects of uremic toxin in heart failure outcome

Cheng-Cheng Lin Chang Gung University; Jui-Fen LIN Chang Gung University; Cheng-Yu Huang Chang Gung University; Chao-Hung Wang Chang Gung Memorial Hospital, Keelung; Mei-Ling Cheng Chang Gung University;

Heart failure (HF) is a complex clinical syndrome that represents the end stage of various cardiac diseases. Albeit with improved diagnosis and therapy, the end stage of HF contributes to a high mortality rate. Identification of any early biomarkers and/or risk factors for HF progression could lead to earlier intervention and potentially improve clinical outcomes. Through the present study, a thorough understanding of the perturbed metabolism in HF, we found two uremic toxins indoxyl sulfate (IS) and p-cresol sulfate (PCS) were high correlation with different severities of HF.

3P-M5

Metabolic changes in stress-tolerant transgenic rice overexpressing calmodulin

Surachat Tangpranomkorn Chulalongkorn University; Miyako Kusano RIKEN Center for Sustainable Resource Science; Ryo Nakabayashi RIKEN Center for Sustainable Resource Science; Apidet Rakpenthai Chulalongkorn University; Teerapong Buaboocha Chulalongkorn University; Kazuki Saito RIKEN Center for Sustainable Resource Science; Supaart Sirikantaramas Chulalongkorn University;

Stress-induced rice calmodulin 1-1 (*Os*CaM1-1) was reported to be an effective stress-counteracting signaling protein. In this study, metabolic changes in the transgenic rice overexpressing *OsCaM1-1* were investigated. We found the up-accumulation of many intermediates in energy metabolisms, namely glycolysis and citric acid cycle together with amino acid biosynthesis suggesting that the transgenic rice was induced to a proactive state. The accumulation of certain metabolites, such as flavonol glycosides which are known to have protective effects, was also found. These results elaborated on the supportive roles of *Os*CaM1-1 in rice acclimation to environmental stress.

Evening Session

AB SCIEX

Marica 3F "Marica Hall" 17:00-18:20

17:00-17:20 **Opening and Introduction**

17:20-17:50 **CESI-MS, an exciting new tool for the analysis of small charged and polar molecules from the cationic metabolome**
John C. Hudson (Sciex Separations, University of Regina, Regina, SK, S4V 0R4, Canada)

The integration of capillary electrophoresis and electrospray ionization into a single dynamic process (CESI) combined with mass spectrometry (MS) is an exciting new development in the area of metabolomics study. This presentation will discuss the advantages of low flow CESI-MS for the advancement of metabolomics investigations.
The promise of CESI-MS will be demonstrated using an amino acid subset of the metabolome as a performance standard and in a quantitative evaluation of various bio-fluids including plasma, urine and oral fluid. CESI-MS has the potential to be an effective tool to study endogenous polar compounds in targeted and untargeted analyses of the cationic metabolome.

17:50-18:20 **MRM-DIAL: software solution for quantitative metabolomics by means of SWATH technology**
Hiroshi Tsugawa (RIKEN Center for Sustainable Resource Science)

MRM-DIAL is a freely available software platform for targeted, SWATH-based metabolomics- and lipidomics research. It is ideal for targeted analysis and is intended for highly reproducible quantification and experimental validation from SWATH data. In combination with MS-DIAL for non-target analysis (see the talk 3A-D3 in Data Analysis session), the software environment offers a total solution for qualitative and quantitative metabolomics.
In MRM-DIAL, multiple reaction monitoring (MRM)-like chromatograms are extracted based on a user-defined transition library obtained by a non-targeted approach in advance. The user-interface facilitates speedy check of peak-picking results and the curation of peak-left and peak-right edges. The talk includes software demonstration of mouse liver lipidomics and introduces quantitative results of key fatty acids and glycerides.

Note: Snacks will be offered in this session.

June 26
Thursday

Keynote Lectures

Dai-ichi Hotel 2F "Tsuru Hall" 8:40-9:20

Googling biology using gene networks

Insuk Lee

Department of Biotechnology, Yonsei University, South Korea

Data-driven discovery is the new paradigm in science and technology, and one started to realize that utilizing data is much more challenging than generating data in various research fields. Biological science is not an exception. Therefore, unless we make dramatic improvement in data analysis technology, the massive amount of biological data will be burdens rather than new opportunities in the future. In this presentation, I want to introduce the network-assisted discovery pipeline in biological research, mimicking Google that effectively retrieves desired information from the internet based on building a network of documents and PageRank algorithm for document prioritization. We have constructed genome-scale gene networks for various organisms, from microbe to animal and plants. The gene networks could be utilized in many different ways to generate various types of biological hypotheses, for examples, gene function, domain function, gene-to-phenotype association, genetic interactions, gene set association, pathway-to-phenotype association.

Marica 3F "Marica Hall" 8:40-9:20

Identifying novel salinity tolerance mechanisms by spatial analysis of lipids in barley roots

Camilla Hill[1], Siria Natera[1], Berin Boughton[2], Stuart Roy[3], Mark Tester[4], Ute Roessner[1,2]

[1] Australian Centre for Plant Functional Genomics, School of Botany, The University of Melbourne, 3010 Victoria, Australia
[2] Metabolomics Australia, School of Botany, The University of Melbourne, 3010 Victoria, Australia
[3] Australian Centre for Plant Functional Genomics, School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, 5064, South Australia, Australia
[4] King Abdullah University of Science and Technology, Saudi Arabia

We are facing the challenging task to meet the growing demand for food which must occur in an environment of a changing climate with increasing environmental stresses such as drought, extreme temperatures, nutrient deficiencies and mineral toxicities. Less land available to cultivate crops, declining water quality and prioritization of biofuel production at the expense of food production further exacerbates the situation. A combination of climate change and poor agricultural practices signifies that 50% of current arable land is at high risk of increased salinity and hence unusable by 2050. Here we aim to develop and apply new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signaling is an integral part of the complex regulatory networks in the responses of plants to salinity. Modifications of membrane lipids occur through the activity of phospholipases, lipid kinases and phosphatases such as phospholipase D and diacylglycerol kinase that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and differentiation and for an appropriate response of the plant to environmental stimuli. We are using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. Our aim is to investigate the link between PM composition and functionality in aspects of salinity response by examining whether observed changes in lipids are involved in either the alteration of fluidity, or in lipid-based downstream signaling. We are also using MALDI-FT-MS based imaging technologies to monitor spatial distributions of lipids across root sections of salt treated tolerant and intolerant barley genotypes. These novel findings will lead to a better understanding of the role of lipids, lipid composition and signaling for plant salt tolerance.

Dai-ichi Hotel 2F "Tsuru Hall"

New Technology

4A-D1

A novel multi-platform software tool (MUSCLE) for the robust, objective and automated optimisation of targeted LC-MS/MS analyses

Mark R Viant University of Birmingham; James Bradbury University of Birmingham; Gregory Genta-Jouve University of Birmingham; James Allwood University of Birmingham; Shan He University of Birmingham; Warwick Dunn University of Birmingham; Royston Goodacre University of Manchester; Joshua Knowles University of Manchester;

The development of novel LC.MS.MS methods for the targeted analysis of metabolites can be time consuming even for expert analysts. We report the development of a multi platform, user friendly software tool (MUSCLE) for the robust and automated optimisation of LC.MS.MS methods. MUSCLE is designed to work across any manufacturers instrument and software. It optimises LC and MS parameters using an evolutionary algorithm against a set of predefined objective functions. We have validated MUSCLE on Thermo Scientific and Waters LC.MS.MS for the targeted analysis of steroids. This fully automated approach discovered faster and higher sensitivity methods than could be achieved manually.

4A-D2

CSPP networks aid the detection of in vivo substrates and products of enzymes

Kris Morreel VIB; Yvan Saeys VIB; Claudiu Niculaes VIB; Fachuang Lu University of Wisconsin-Madison; Hoon Kim University of Wisconsin-Madison; John Ralph University of Wisconsin-Madison; Wout Boerjan VIB;

The main bottleneck in metabolomics is the inability to annotate the large number of unknown metabolites. Here, an algorithm is presented that allowed structurally annotating 145 compounds of the LC-MS-profiled fraction of the Arabidopsis thaliana leaf metabolome by concatenating pairs of m/z features. The latter, called Candidate Substrate Product Pairs (CSPP), have mass and retention time differences corresponding with those of substrates and products from well-known enzymatic reactions. This method also allows searching in vivo substrates and products of enzymes. This is demonstrated for the poplar phenylcoumaran benzylic ether reductase, the most abundant protein in wood.

4A-D3

Comprehensive two-dimensional liquid chromatography: a new technique for for high-resolution metabolomics?

Oliver Jones RMIT Univeristy; Jessica Pandohee RMIT University;

Multi-dimensional chromatography employs more than one mechanism of separation, with each being considered an independent separation dimension. Multi dimensional gas chromatography (GC) has a notable history in metabolomics but, as with all forms of GC, samples are limited to those compounds that are, or can be made, volatile. In contrast 2D liquid chromatography (LC-LC) is a relative newcomer in which volatility is not required. In this study we demonstrate that the enhanced resolution and peak capacity of LC-LC allow peaks to be detected that could not be separated using a one-dimensional LC. We propose that 2D LC-LC has the potential to open new areas of exploration in metabolomics.

Marica 3F "Marica Hall"

Plant Physiology

4A-M1

Integrated Metabolomics, Gene Expression, and GWAS Identify New Saponin Biosynthetic Genes in Medicago truncatula

Lloyd W. Sumner The Samuel Roberts Noble Foundation; Bonnie S. Watson The Samuel Roberts Noble Foundation; Zhentian Lei The Samuel Roberts Noble Foundation; DongSik Yang The Samuel Roberts Noble Foundation; Yuhong Tang The Samuel Roberts Noble Foundation; Derek Nedveck University of Minnesota; Peter Tiffin University of Minnesota; Nevin Young University of Minnesota;

Triterpene saponins are structurally diverse secondary metabolites found in many plant families, including the Leguminosae, and saponins possess a broad spectrum of bioactivities. However, the biosynthetic pathways for saponin biosynthesis remain largely uncharacterized. Metabolic profiling of several germplasm collections, correlated gene expression and genome wide association studies (GWAS) have been performed. The cumulative data were used to identify and prioritize putative genes associated with saponin biosynthesis and several have now been characterized. This presentation will outline the overall strategy and the identification of novel genes.

4A-M2

Metabolomic exploration toward understanding of key cellular events leading to wax ester fermentation in Euglena gracilis

Adchara Padermshoke Osaka Prefecture University; Kazuki Nishio Osaka Prefecture University; Takumi Ogawa Osaka Prefecture University; Atsushi Okazawa Osaka Prefecture University; Takeshi Furuhashi RIKEN Center for Sustainable Resource Science; Masami Yokota Hirai RIKEN Center for Sustainable Resource Science; Masanori Arita National Institute of Genetics; Daisaku Ohta Osaka Prefecture University;

Aerobically grown Euglena gracilis accumulates wax esters upon exposure to anaerobic conditions. The wax ester fermentation is accompanied by a breakdown of reserve paramylon (β-1, 3-glucan) and a net synthesis of ATP. We employed GC-MS analyses to clarify accumulation profiles of wax ester species under a variety of environmental conditions and demonstrated that the presence of CO2 is essential for the fermentation. Here, we report a 13C-labelling experiment and discuss possible roles of CO2 in the fermentation.

4A-M3

Monitoring primary carbon metabolism in mature Arabidopsis thaliana leaves

Pernilla Linden Swedish University of Agricultural Sciences; Olivier Keech Umea University; Per Gardestrom Umea University; Thomas Moritz Swedish University of Agricultural Sciences;

We present a fast and reproducible method for monitoring metabolites in plant primary carbon metabolism. Mature leaves from Arabidopsis thaliana were labelled with $^{13}CO_2$ during two hours and analysed by gas- and liquid- chromatography coupled to mass spectrometry, resulting in both ^{13}C-labelling information and change in metabolite abundance. This method was used to investigate a mutant lacking a growth phenotype compared to wild type, but which proved to have a distinct metabolic phenotype. This difference was strengthened under low CO_2 conditions and weakened under high CO_2, proving that the mutant has a higher photorespiratory turnover than wild type.

4A-M4

Untargeted metabolomics of Habanero pepper (*Capsicum chinense* Jacq. var. Orange) fruits subject to Nitrogen and Phosphorus deficiency.

Carlos Rodriguez-Lopez Tecnologico de Monterrey; Rafael Urrea-Lopez Tecnologico de Monterrey; Victor Trevino Tecnologico de Monterrey; Juan I. Valiente-Banuet Tecnologico de Monterrey; Rocio I. Diaz de la Garza Tecnologico de Monterrey;

Habanero peppers are high-value fruits appreciated for their organoleptic properties. Although stress due to Nitrogen and Phosphorus deficiency (P-) showed no effect on fruit weight and quality metabolites (related to pungency, flavor and antioxidants), P- negatively affected on yield. In this work, an untargeted metabolomics approach (HPLC-TOF) was used to explore the mechanisms employed to maintain quality of the fruit. A Linear Mixed-Effect model revealed

that 832, 2.048, and 4.884 features significantly changed (FDR<0,1) under macronutrients deficiency in green, breaker and ripe fruits. Tandem MS from selected features revealed the involvement of glycosylated compounds in response to P-.

Oral Abstracts 26 June Thursday 10:50–12:10

Dai-ichi Hotel 2F "Tsuru Hall"

Omics Integration

4P-D1

A unique brain lipidome and metabolome biosignature in alzheimer disease

Giuseppe Astarita Waters Corporation, Milford, MA, USA.; Steven Lai Waters Corporation, Milford, MA, USA.; Giuseppe Paglia Waters Corporation, Milford, MA, USA.; James Langridge Waters Corporation, Milford, MA, USA.; Robert Plumb Center for Systems Biology, University of Iceland, Reykjavik, Iceland;

Alzheimer's disease (AD) is the most common cause of adult dementia, but the cause of this inexorable neurodegenerative disease remains still elusive. Here we used an integrated lipidomics and metabolomics approach to survey frozen brain tissue samples from clinically characterized AD patients and age-matched controls. Lipidome and metabolome data were fused and mined using multivariate statistics, pattern-recognition tools and pathway analysis. Our results reveal novel molecular alterations in AD and a unique molecular biosignature that differentiates the brains from individuals with AD compared from those from control subjects.

4P-D2

13C-based Metabolomics: Integrating LC-MS IROA with NMR

Arthur S Edison Southeast Center for Integrated Metabolomics; Gregory S Stupp University of Florida; Chaevien Clendinen University of Florida; Ramadan Ajredini University of Florida; Timothy J Garrett University of Florida; Richard A Yost University of Florida; Chris Beecher IROA Technologies;

We are developing two complementary approaches to 13C-based metabolomics, IROA and NMR. IROA compares samples that have been labeled with 5 and 95% 13C by combining them prior to extraction and simultaneously analyzing them by LC-MS (Anal Chem 2013, 85, 11858-11865). We have also developed a sensitive 13C NMR probe with a volume of 35 uL, allowing many previously impossible experiments to be conducted with NMR (J Magn Reson 2013, 235C, 58-65). I will describe the integration of IROA with 13C NMR into a single metabolomics workflow. These complementary analytical techniques can provide much deeper information on the same samples than either can provide alone.

4P-D3

MetaboLights: Open access metabolomics repository and reference data

Reza Salek EMBL-EBI; Kenneth Haug EMBL-EBI; Pablo Conesa EMBL-EBI; Mark Williams EMBL-EBI; Jules Griffin MRC-HNR; Christoph Steinbeck EMBL-EBI;

The MetaboLights database has now successfully established itself within the metabolomics community as the first open-access, general-purpose repository. In addition to a comprehensive metabolomics data archiving and curated metadata collection, we are now expanding its resources by adding a metabolite Reference Layer. This knowledge based resource, integrating with the deposition layer, holds over 9500 metabolites with chemical and biological information, pathway reactions, literature integration and NMR and MS reference spectra. Over the next few years MetaboLights will explore integrating open source online data analysis tools as part of its services.

4P-D4

Reconstruction of global signal flow of insulin from phosphoproteome and metabolome data

Katsuyuki Yugi Department of Biological Sciences, Graduate School of Science; Hiroyuki Kubota Department of Biological Sciences, Graduate School of Science; Yu Toyoshima Department of Biological Sciences, Graduate School of Science; Rei Noguchi Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo; Kentaro Kawata Department of Biological Sciences, Graduate School of Science; Yasunori Komori Department of Biological Sciences, Graduate School of Science; Shinsuke Uda Department of Biological Sciences, Graduate School of Science; Katsuyuki Kunida Department of Biological Sciences, Graduate School of Science; Yoko Tomizawa

Department of Biological Sciences, Graduate School of Science; **Yosuke Funato** Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University; **Hiroaki Miki** Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University; **Masaki Matsumoto** Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University; **Keiichi I. Nakayama** Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University; **Kasumi Kashikura** Institute for Advanced Biosciences, Keio University; **Keiko Endo** Institute for Advanced Biosciences, Keio University; **Kazutaka Ikeda** Institute for Advanced Biosciences, Keio University; **Tomoyoshi Soga** Institute for Advanced Biosciences, Keio University; **Shinya Kuroda** Department of Biological Sciences, Graduate School of Science;

We here developed a unbiased method to reconstruct the intracellular global network based on time-course phosphoproteome and metabolome data together with multiple databases and applied it to acute insulin action. We found that the insulin signal flowed through the global network that involved 13 protein kinases, 26 phosphorylated metabolic enzymes, and 35 allosteric effectors, resulting in quantitative changes in 44 metabolites. By use of experiments with phosphomimetic mutants of liver-type phosphofructokinase 1 (PFKL), and the kinetic modeling of glycolytic pathway, we found that insulin induces phosphorylation and activation of PFKL and controls a key reaction in glycolysis.

4P-D5

A catalog of human metabolic individuality

Matthias Arnold Helmholtz Center Munich; **Johannes Raffler** Helmholtz Center Munich; **Jan Krumsiek** Helmholtz Center Munich; **So-Youn Shin** Wellcome Trust Sanger Institute; **Christian Gieger** Helmholtz Center Munich; **Tim D Spector** King's College London; **Nicole Soranzo** Wellcome Trust Sanger Institute; **Karsten Suhre** Weill Cornell Medical College in Qatar; **Gabi Kastenmuller** Helmholtz Center Munich;

Combining genetics and metabolomics in genome-wide and metabolome-wide association studies identified genetic loci that influence metabolite levels in blood and urine in healthy populations and thereby marked the range of human metabolic individuality. Various studies including our recent study based on 7800 individuals uncovered more than 150 such loci in total and allowed reconstructing a first in vivo blueprint of the human metabolism in blood. To use their large overlap with pharmacogenetically and disease relevant loci and to facilitate exploration of these associations for hypothesis generation, we now built a comprehensive web-based catalog for published metabolite-SNP associations.

Marica 3F "Marica Hall"

Crops

4P-M1

Composition and metabolite differences between near-isogenic gm and conventional maize are associated more with back-crossing than with the gm traits

Mark Leibman Monsanto Company;

We hypothesized that metabolic differences between GM and non-GM comparators are associated with minor genomic differences between near-isogenic lines, and not specifically the GM trait. We confirmed this hypothesis in a study that contrasted the effect of three distinct GM traits (drought-tolerance, herbicide resistance, and insect-protection) on maize grain composition to the effects of residual genetic variation from backcrossing. The study further confirmed that conventional breeding is a major source of variation. Interpretation of hypothesis-free profiling of GM crops must therefore take into account possible influences of residual genetic variation in near-isogenic comparators

4P-M2

The application of metabolomics to the biochemical phenotyping of staple crops from developing countries

Elisabete Barros Carvalho Royal Holloway University of London; **Luis Augusto Becerra Lopez-Lavalle** International Center for

Tropical Agriculture; **Paul D Fraser** Royal Holloway University of London;

Cassava (Manihot esculenta) is an important staple food in tropical and sub-tropical countries, primarily due to the relatively low resource input required and tolerance to drought and abiotic stress. Breeding has focussed on improved traits such as productivity, pest and disease resistance and nutritional quality. With the advent of modern omic technologies the opportunity now exists for more strategic approaches towards modern breeding. In order to capture the biochemical diversity existing in cassava the metabolomic profile of 20 diverse varieties has been obtained using a multi-platform approach. Integration of these datasets with genomic information will assist future breeding studies.

4P-M3

GC-MS based metabolite profiling of barley kernels - impact of induced drought-stress

Alexandra Wenzel Chair of General Food Technology; **Thomas Frank** Technische Universitaet Muenchen; **Gabriela Reichenberger** Bavarian State Research Center for Agriculture; **Markus Herz** Bavarian State Research Center for Agriculture; **Karl-Heinz Engel** Technische Universitaet Muenchen;

The aim of the study was to investigate the impact of drought stress on the metabolite profiles of barley grains against the background of natural variability. A representative set of barley genotypes was grown under natural weather conditions (different sites and seasons) and under induced drought stress using a rain-out-shelter. The comparative assessment of the profiling data via multi- and univariate analysis revealed that drought stress is strongly reflected in quantitative changes of polar metabolites, irrespective of natural variability. In addition, the impact of KI-treatment, as alternative strategy to induce drought stress-like symptoms, on the metabolites was investigated.

4P-M4

Combining metabolomic profiling and sensory evaluation to understand consumer perception of rice flavour

Venea Dara Daygon University of Queensland; **Melissa Fitzgerald** University of Queensland; **Sangeeta Prakash** University of Queensland; **Arthur Riedel** University of Queensland; **Jennifer Waanders** University of Queensland;

The key to breeding fragrant rices is by understanding the essential compounds that contribute to pleasant and unpleasant aroma. Human perception of flavour is brought by a complex combination of odour active compounds. However, in the past years, breeding programs for fragrant rice have been focused on 2AP alone. This study aims to dissect rice aroma beyond 2AP on elite rice varieties. Metabolic profiles of rice volatile compounds are analysed using GCxGC TOF-MS and are associated with sensory evaluation data from trained panels. Results show that build-up of lipid degradation products negatively affects the aroma of rice and masks flavour of naturally occurring pleasant smelling compounds.

4P-M5

Allelopathy in canola

Md Asaduzzaman Charles Sturt University; **Min An** Charles Sturt University; **Jim Pratley** Charles Sturt University; **Deirdre Lemerle** Charles Sturt University; **David Luckett** NSW Department of Primary Industry;

Allelopathy is a process whereby plants provide themselves with a competitive advantage by releasing compounds into the adjacent environment. Allelopathy in canola (Brassica napus) is likely, given its reputation for soil biofumigation and its weak association with mycorhizae. This has been shown to contribute to the competitive ability of canola genotypes. Research is ongoing to identification and quantification of the allelopathic compounds involved and the associated gene expression in response to challenges by weeds. Development of a strategy based on allelopathy will help to reduce the dependence on herbicides and thus be an additional tool for integrated weeds management.

Luncheon Seminars

Thermo Fisher Scientific

Dai-ichi Hotel 2F "Tsuru Hall" 12:30-13:20

Transforming Metabolomics Research Using Orbitrap Based LCMS Platforms

This workshop is intended for scientists who are interested in applying high-resolution, accurate mass LC-MS platforms for Metabolomics (including Lipidomics) research. The featured speakers will demonstrate how endogenous metabolites are identified and quantified from various studies, as well as illustrate how the newly introduced hardware and software tools enable and transform cutting-edge Metabolomics research. Software and databases essential to Metabolomics and Lipidomics data analysis and compound identification will be also discussed.

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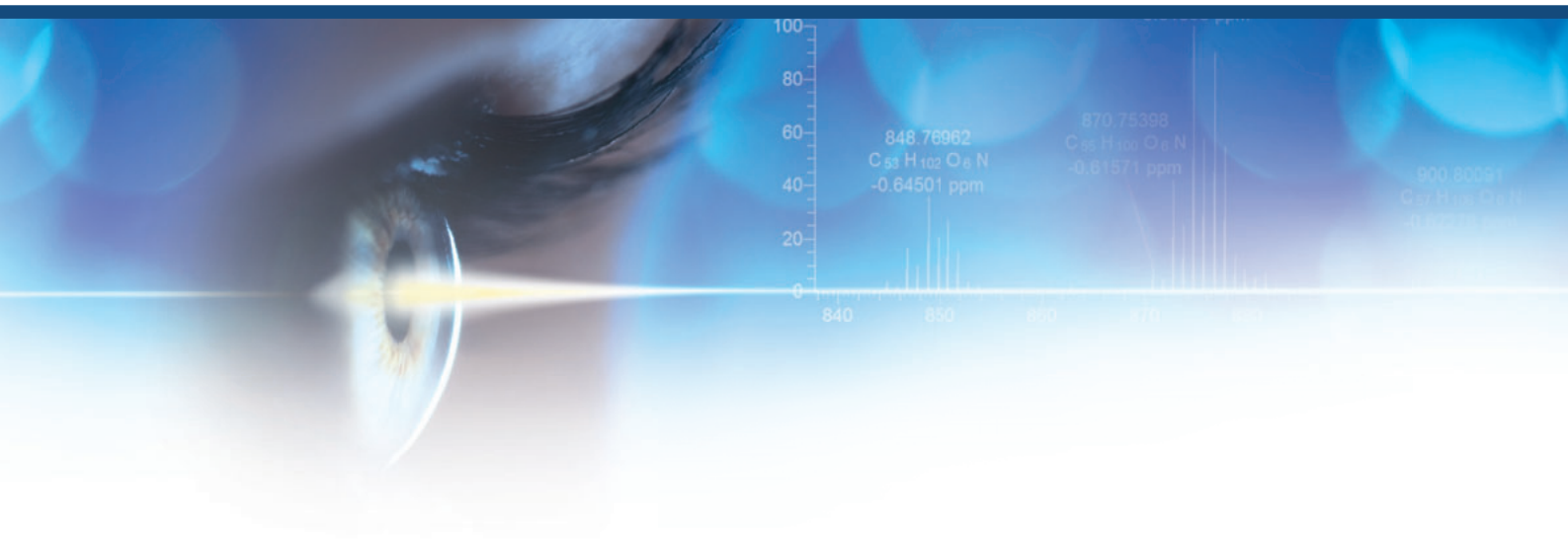

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Luncheon Seminars

Waters Corporation

Marica 3F "Marica Hall" 12:30-13:20

Metabolomics: from Biomarker Discovery to Molecular Epidemiology

Session Chair: Motoji Oshikata, Waters Corporation, Japan

UPLC-TOF/MS Based Metabolic Profiling for Biomarker Research in Human Plasma

Daisuke Saigusa

Research Associate, Tohoku Medical Megabank Organization, Department of Integrative Genomics, Tohoku University

Metabolomics is one of "Omics" approaches for researching a clinical biomarker of disease prediction and progression. Recently growth of metabolomics has greatly been depended on the improvement of mass spectrometry (MS). Especially, the method for "untargeted" metabolic profiling using a time of flight (TOF)/MS (Synapt-G2Si, Waters) coupled with ultra high-performance liquid chromatography (UPLC) has been widely used to research a clinical biomarker. Some of the papers reported the protocol of metabolic profiling in biological samples. However, the methodology is still developing. Therefore, we have developed a protocol of "untargeted" metabolic profiling in human plasma referenced from previous methods. In addition, we have tested the protocol for researching the predictive biomarkers for pregnancy hypertension. Attend our seminar to learn more.

Achieving large scale analysis of complex biofluids.

Matthew R. Lewis

Mass Spectrometry Manager, MRC-NIHR Phenome Center, Department of Surgery and Cancer, Imperial College, London, UK

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Closing Ceremony and Lecture

Marica 3F "Marica Hall" 13:40-14:20

A "GoogleMAP"-type view of specialized molecules from microbes, their communities and hosts including people.

Pieter C Dorrestein

Department of Pharmacology, Department of Chemistry and Biochemistry, University of California - San Diego, USA

Chemical crosstalk between cell populations is a universal phenomena. The chemistry involved in this crosstalk is very diverse and therefore capturing this information represents an analytical challenge especially since there are no amplification methods such as PCR used in sequencing. In this talk we will highlight the mass spectrometry based workflows that the lab has developed to increase our ability to tease apart the molecular components of interacting microbes and microbial communities in 2D and 3D. In our lab we are referring to this as our "Google-Map streetview" for molecular space and are developing a crowd source approach to characterize the signals detected by mass spectrometry of biological samples. The examples highlighted in this talk will range from interacting microbes such as P. aeruginosa with C. albicans, microbial communities such as lichen and sputa obtained from cystic fibrosis patients as well as molecular maps of molecular-microbial communities of people.

Marica 3F "Marica Hall" 14:20-14:50

14:20-14:30 Conference Awards Ceremony (Tim Ebbels, Chair of the Conference & Training committee)
14:30-14:40 San Francisco 2015 Conference (Oliver Fiehn, Chair of the 2015 meeting)
14:40-14:50 Closing remark (Mark Viant, President of the Society)

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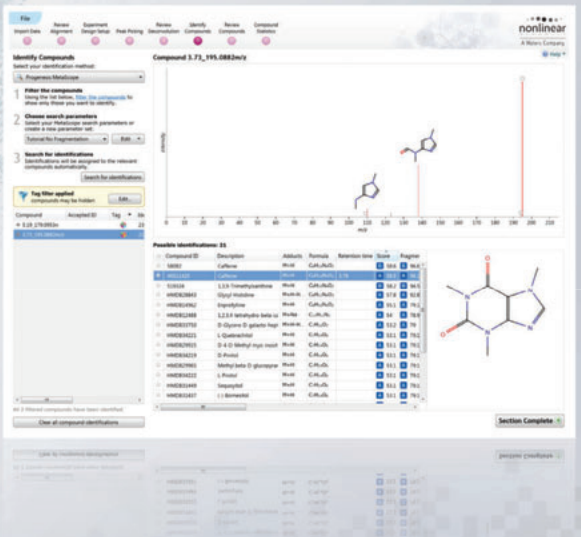

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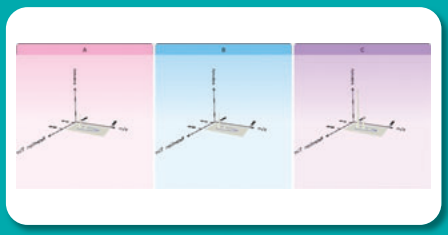

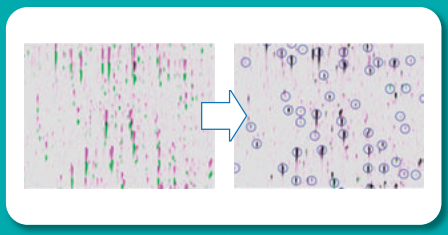

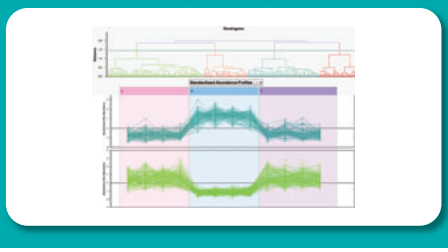

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Poster Abstracts

CVD, Diabetes

P1

Metabolic profiling of Wolfram syndrome 1 gene deficient mouse

Rando Porosk Institute of Biomedicine and Translational Medicine; Kalle Kilk Institute of Biomedicine and Translational Medicine; Riina Mahlapuu Institute of Biomedicine and Translational Medicine; Ursel Soomets Institute of Biomedicine and Translational Medicine;

The aim of the study was to analyse different metabolite concentrations of Wolfram syndrome 1 deficient mice (Wfs1-/-)and compare to heterozygous (Wfs1+/-) and wild-type mice (Wfs1+/+). Untargeted metabolomics of mice urine, trunk blood and liver, kidney, heart and brain (stiatum, hippocampus and hypothalamus) homogenates were analysed by LC-MS/MS. Principal component analysis showed significant separation of different Wfs1 genotypes in almost every tissue or biofluid. Metabolic profiling of Wfs1-/- mice showed remarkable changes in metabolite concentrations, which refer to higher oxidative stress and disruption of different metabolic pathways in Wfs1-/- mice.

P2

Metabolic profiling of T2DM model mouse response to glucose challenge identifies abnormal metabolic changes in the liver and muscle

Toshiya Kokaji Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo; Hiroyuki Kubota Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; Yuki Ito Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo; Yohei Sumitomo Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; Katsuyuki Yugi Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; Masashi Fujii Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; Shinsuke Uda Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; Tomoyoshi Soga Institute for Advanced Biosciences, Keio University; Shinya Kuroda Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo;

We performed an oral glucose tolerance test (OGTT) in wild-type and ob/ob mice, a model of type 2 diabetes, to identify abnormal regulation of glucose homeostasis in type 2 diabetes. A metabolomic measurement by means of CE-MS provided quantitative profiling of 493 metabolites in the liver and muscle homogenates taken from the wild type and ob/ob mice. Measured metabolomic data set was evaluated to identify differences in metabolite distributions before and after an OGTT in each type of mouse. This comparison identified changes in diverse metabolites specifically observed in ob/ob mice that indicate dysregulation of glucose metabolism in type 2 diabetes.

P3

Metabolic effects of glucocorticoid block in diabetes and non-alcoholic fatty liver disease

David George Watson University of Strathclyde; Brian R Walker University of Edinburgh; Ruth Andrew University of Edinburgh; Roland H Stimson University of Edinburgh; Mohammed Alwashih University of Strathclyde;

Glucocorticoid block (GC) was carried out with mifepristone in combination with metyrapone followed by an insulin infusion in 6 men with type 2 diabetes ± non-alcoholic fatty liver disease (NAFLD) in a double-blinded, placebo controlled crossover study. Marked differences in metabolite profiles were observed between diabetes and diabetes + NAFLD and in the response of the two groups to GC block. Marked effects of GC block were on biogenic amine metabolism, metabolism of various lipids and also on bile acid levels. Elevated amines could be explained by the fact that Glucocorticoids are known stimulate the oxidation of the monoamines predominantly via MAO-A and GCB could down regulate MAO-A.

P4

Metabolomic profiling reveals novel biomarkers of alcohol-induced hypertension in community-dwelling men: Tsuruoka metabolomic study

Sei Harada Keio University School of Medicine; Toru Takebayashi Keio University School of Medicine; Ayako Kurihara Keio University School of Medicine; Miki Akiyama Keio University Faculty of Environment and Information Studies; Daisuke Sugiyama Keio University School of Medicine; Kazuyo Kuwabara Keio University School of Medicine; Ayano Takeuchi National Institute for Environmental Studies;

Akiyoshi Hirayama Keio University Institute for Advanced Biosciences; **Masahiro Sugimoto** Keio University Institute for Advanced Biosciences; **Tomoyoshi Soga** Keio University Institute for Advanced Biosciences; **Masaru Tomita** Keio University Institute for Advanced Biosciences;

To reveal novel biomarkers of alcohol-induced hypertension (HT), we performed plasma metabolomic profiling by CE-MS in 1016 male Tsuruoka-city residents aged 35-74. We previously reported 33 polar metabolites significantly related to daily alcohol intake. Among these metabolites, 11 metabolites from choline metabolism ,BCAA catabolism, TCA cycle and urea cycle were significantly higher or lower in hypertensive, defined as SBP>=140 or DBP>=90 or on medication, as results of t-tests adjusted for BH method (α=0.05). Choline, 2-oxoglutarate, Tyr and Thr were specifically higher in hypertensive with high alcohol intake, thus those were considered as possible biomarkers of alcohol-induced HT.

P6

Mass Spectrometry Metabolomic Approaches Lead to a New Paradigm for Fabry Disease Patient Investigation

Christiane Auray-Blais Universite de Sherbrooke; **Pamela Lavoie** Universite de Sherbrooke; **Victoria Manwaring** University College London; **Michel Boutin** Universite de Sherbrooke;

Fabry disease (FD) is a panethnic lysosomal storage disorder characterized by accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Metabolomic mass spectrometric approaches targeting mass ranges from 50-1500 for structural variants of Gb3 and lyso-Gb3 have led to the discovery of 51 novel biomarkers in biological fluids. Patients with late-onset cardiac variant mutations showed abnormal analogs of lyso-Gb3, but normal Gb3 and lyso-Gb3 excretion levels. The identification of these novel biomarkers has paved the way for a new investigation paradigm for FD patients, where a global biochemical profile evaluation is strongly recommended.

P7

Discovery of Galabiosylceramide-Related Fabry Disease Urinary Biomarkers Using a Mass Spectrometry-Based Metabolomic Approach.

Michel Boutin Universite de Sherbrooke; **Christiane Auray-Blais** Universite de Sherbrooke;

Fabry disease is an X-linked lysosomal storage disorder characterized by the accumulation of globotriaosylceramide (Gb3), globotriaosylsphingosine (Lyso-Gb3), and of the less studied galabiosylceramide (Ga2) in biological fluids/tissues. Cardiac and nephrology complications lead to premature death for Fabry patients. Urine samples from untreated Fabry and control males were analyzed by UPLC-Tof/MS for detection of Ga2 structural variants (820-1020Da) and statistically compared. A total of 19 novel Ga2 isoforms/analogs were identified as Fabry disease biomarkers, and structurally elucidated by tandem mass spectrometry. These biomarkers offer a new tool for diagnosis/monitoring Fabry patients.

P201

Association of estimated absolute risk for coronary heart disease and metabolites in a Japanese general population: Tsuruoka Metabolomic Cohort Study

Daisuke Sugiyama Keio University School of Medicine; **Sei Harada** Keio University School of Medicine; **Toru Takebayashi** Keio University School of Medicine; **Ayako Kurihara** Keio University School of Medicine; **Tomonori Okamura** Keio University School of Medicine; **Yuji Nishiwaki** Toho University School of Medicine; **Kazuyo Kuwabara** Keio University School of Medicine; **Akiyoshi Hirayama** Keio University Institute for Advanced Biosciences; **Masahiro Sugimoto** Keio University Institute for Advanced Biosciences; **Tomoyoshi Soga** Keio University Institute for Advanced Biosciences; **Masaru Tomita** Keio University Institute for Advanced Biosciences;

We investigated association of absolute risk for coronary heart diseases (CHD) and metabolites to seek out new biomarkers predicting CHD. Subjects were 1050 Japanese participated in our cohort without past history of cardiovascular disease, cancer and medications for traditional risk factors such as hypertension. Amino acid and related metabolites were measured by CE/MS. Absolute risk for 10-year CHD mortality was estimated, which were categorized into 3 groups based on Japan Atherosclerosis Society Guideline. Some metabolites such as malate showed significant association with absolute risk of CHD both men and women. Further study should be needed to determine usefulness

of these biomarkers.

P202

Prospective clinical validation of a translational, metabolomics-based biomarker for asymptomatic, early stage heart failure

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Established biomarkers for heart failure are limited by their lack of sensitivity for the early detection of HF. We performed metabolite profiling of human plasma samples of HF patients by untargeted and targeted GC-MS and LC-MS/MS. In the single center identification phase metabolites were nominated for multimarker panels and their performance was clinically validated in a separate multi-centre validation cohort (in total >800 HF patients and >330 controls). The new marker panel demonstrated a significantly added diagnostic value for asymptomatic when combined with NT-proBNP. Compared to controls, numerous pathways, including lipid and amino acid metabolism, were altered in HF patients.

P203

Metabolic features of skeletal muscle in lipoprotein lipase transgenic rabbits protected against high-fat-diet-induced obesity and diabetes

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To identify metabolites that potentially play important roles in the anti-obesity/diabetes feature of LPL transgenic rabbits, we investigated metabolic profiles of skeletal muscle in lipoprotein lipase (LPL) transgenic and wild-type (control) rabbits. After fasting, gastrocnemius muscle (red portion) of high-fat diet-fed LPL transgenic (n=9) and control (n=9) rabbits were harvested and used for metabolic profiling using capillary electrophoresis mass spectrometry. Comparison of 165 metabolites revealed that concentrations of 38 metabolites were up- or down-regulated in the transgenic rabbits. Many of these metabolites were involved in amino acid metabolism, glucose metabolism, and TCA cycle.

P204

Plasma liped studies in atrial fibrillation patient after direct current cardioversion (DCC) treatment using UPLC/Q-TOF MS

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Atrial fibrillation is the most common arrhythmia, and recurrent AF was incurred in half of the patients after direct current cardioversion in a month. In this study, we applied lipidomic profiling based on UPLC/Q-TOF MS to investigate lipid changes in incidence of recurrent AF after one month from success of DCC treatment. Although the multivariate analysis from MS spectral data of plasma samples in AF patients didn't show the clear separation, significant difference in a few lipids were observed between recurrence and non-recurrence groups. This study demonstrates that plasma lipid profiles based on UPLC/Q-TOF MS could be used to predict recurrence possibility of AF after DCC.

P205

Metabolite profiling study in a rat model induced myocardial infarction

Miso Nam Korea Basic Science Institute; **Youngae Jung** Korea Basic Science Institute; **Jueun Lee** Korea Basic Science Institute; **Do Hyun Ryu** Sungkyunkwan Univ; **Geun-Sook Hawng** Korea Basic Science Institute;

Myocardial infarction (MI) is one of the leading causes of death, and carnitine levels in biofluids such as tissue or serum play a crucial role in the transport of the fatty acids. We applied UPLC/Q-TOF MS to perform metabolic profiling of heart tissue and serum in a rat model of myocardial infarction. Our results showed that long-chain acyl-

carnitine levels were up-regulated and short-chain acylcarnitine levels were down-regulated in the heart tissue over time. In contrast, acylcarnitine levels of serum were changed differently than heart tissue. This study suggests that metabolic profiling based on UPLC/Q-TOF MS can be a useful tool to investigate the metabolic changes of MI.

P206

Metabolomic profiling for the hypocholesterolaemic effects of Astragali Complanati Semen by GC-MS and multivariate data analysis

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This work aims to study the hypocholesterolaemic effect of Astragali Complanati Semen (SYZ) by metabolomics approach and the results indicate that administration of SYZ ethanolic extract for 28 days had significant effects, in term of reducing the serum total cholesterol and LDL, in hypercholesterolaemic rats. Serum fatty acid profiles further analyzed by PLS-DA and the results suggesting the mechanism of SYZ may be different from that of simvastatin. Further mechanistic analysis proved that SYZ, but not simvastatin, could provide its cholesterol lowering effect by up-regulating PEPCK and LDLR protein expressions in the livers of rats fed with hypercholesterolaemic diet.

P207

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) quantitation of dimethylarginines (DMA) in urine

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Asymmetric and symmetric dimethylarginines (ADMA and SDMA) are metabolites implicated in cardiovascular diseases. In this study, the isomers were reproducibly identified and quantified based on their unique ions with MALDI-TOF MS from urine samples. Sub-micromolar limits of detection (LOD) and quantitation (LOQ) with good calibration statistics (R2 &> 0.9), excellent recoveries, acceptable inter and intra-assay variation (within 15%) was achieved for the DMAs. ADMA and SDMA were in the range of 1.6-8.0 μM and 2.9-9.1 μM in normal urine from 11 healthy volunteers. The developed method offers rapid, sensitive and unambiguous detection and quantification of the isomers.

Neuroscience

P9

Multiple Metabolic Abnormalities in a Cohort of Idiopathic Parkinson's Disease Patients: Discovery via Urinary Metabolomics

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Abnormal metabolic phenotypes in urine reflect the pathogenesis of Parkinson's disease (PD). We employed metabolomics strategy using liquid chromatography coupled with orbitrap mass spectrometry to investigate urinary metabolic profiles and metabolic abnormalities in idiopathic PD patients. Our study revealed significant correlation between clinical phenotype and metabolite profile. Metabolic pathway variation in the idiopathic PD patients was clearly distinct from matched non-PD controls. A panel of metabolites that occurred at significantly altered levels in idiopathic PD patients might represent evidence for progression of PD and might be used as therapeutic targets for

drug development.

P10

A CE-MS based metabolome analysis of mice whole brain in light/dark phase

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In order to better understand sleep, we have comprehensively analyzed water-soluble metabolites of the whole brain in sleep/wake states, using a metabolomics approach by capillary electrophoresis mass spectrometry (CE-MS). In this study, we compared metabolite profiles between the light and dark phase in C57BL/6NCr mice. Of the 171 metabolites detected, 22, including those related to metabolism of neurotransmitters, such as histamine, acetylcholine and GABA, exhibited significant differences ($p<0.05$). This result revealed that there are significant changes in the metabolic pathway of neurotransmitters, between sleep and wake states. We will also report other metabolite changes.

P11

Global metabolomics reveals urinary biomarkers of alzheimers disease in the crnd8 transgenic mouse model

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Mass spectrometry-based metabolomics for characterizing the metabolic signatures in the CRND8 mice of Alzheimers disease was investigated, with the purpose of discovering metabolic markers for Alzheimers disease with diagnostic potential. A clear difference in the metabolic phenotypes between Tg and non-Tg mice was revealed by multivariate data analysis. Key changes included perturbation of aromatic amino acid metabolism, purine metabolism and oxidative stress.

P12

Blood metabolic patterns associate with the severity of traumatic brain injury and predict patient outcomes

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Traumatic brain injury (TBI) is a major cause of neurological damage and disability. We applied GCxGC-TOFMS to study longitudinal metabolic profiles in blood from (mild, moderate and severe) TBI patients and controls directly after the accident (n=122 for discovery, n=90 for independent validation). Several sugar derivatives were strongly associated with severity of TBI and patient outcomes, and most of them were also detected at high concentrations in patient-matched brain microdialysate and CSF samples. The elevated levels of these blood metabolites may be indicative of disrupted blood-brain barrier and may underline their potential as screening markers for TBI in healthcare setting.

P208

Targeted metabolomics appoach for evaluation of oxidative stress status in postmortem human brain

Stepan Melnyk UAMS; Oleksandra Pavliv UAMS; Teresa Evans UAMS; Jill S James UAMS;

Autism spectrum disorder (ASD) is affecting 1 in 110 children. Targeted metabolomics approach to evaluate levels of oxidative stress in postmortem human brain tissue utilizing LC-MS and HPLC-ECD techniques presented. It includes not only glutathione cycle metabolites, but also stable metabolites modified by oxidative stress. Comparing ASD cerebellum tissue with controls, we noticed significantly lower levels of total glutathione, cysteinyl-glycine, glutamine-cysteine and significantly higher levels of 3N-tyrosine, cysteine and 8-OH-guanosine in DNA. In sum-

mary, our targeted metabolomics pathways analyses provide a reliable algorithm in evaluating postmortem oxidative damage in the brain.

P209

Metabolomic analysis of the toxicity of acute and chronic pesticides exposure in B50 cultured neuroblastoma cells.

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Cultured rat neuroblastoma B50 cells were exposed to the neurotoxic insecticides permethrin and malathion, in order to determine both acute and chronic effects. Cell damage was assessed by morphological and biochemical analysis and resultant metabolite analysis was performed using gas chromatography-mass spectrometry. Metabolites that most contributed to the variance between treated and control cells were identified using PCA. This analysis indicated that cells damaged by pesticide could be grouped according to their metabolite profile. The metabolites detected were further investigated for changes in trend following exposure, and a proposed pathway of neurotoxicity determined.

P210

Untargeted metabolomics approach for the urine analysis of autistic patients using LC-HRMS, 1H-NMR and 1H-13C-HSQC NMR

Binta Bieme Tours University; **Sylvie Mavel** Tours University; **Helene Blasco** Tours University, Tours Hospital; **Cinza Bocca** Tours University; **Frederic Montigny** Tours University; **Frederique Bonnet-Brilhault** Tours University, Tours Hospital; **Catherine Barthelemy** Tours University, Tours Hospital; **Gabriele Tripi** Tours University, Tours Hospital; **Christian Andres** Tours University, Tours Hospital; **Lydie Nadal-Desbarats** Tours University, Tours Hospital; **Patrick Emond** Tours University, Tours Hospital;

Objectives are to compare urine metabolic signatures of autistic patients to normal controls using analytical multimodality platforms. Urine samples were collected from 29 autistic and 33 controls children. Based on data from LC-HRMS, 1H-NMR and 2D1H-13C-NMR, we performed a multivariate data analysis. OPLS-DA model using data from all platforms showed enhanced performance compared to single models with the following internal validation step parameters: R2Y=0.94, Q2=0.77, CVanova=1,24.10-14. This model used 71 features and 28 compounds are currently under identification. GABA, threonine, serine, glutamine, dopamine, tyrosine were found significantly altered between the two groups

P211

Blood biomarkers for Alzheimer's disease revealed by capillary electrophoresis mass spectrometry (CE/MS)-based metabolomics

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Alzheimer's disease (AD) is a progressive neurodegenerative disease, and represents the most prevalent cause of dementia. Specific biomarkers for diagnosis of AD at the early stage of AD are essential to prevent and treat the disease. To find such biomarkers, we analyzed 579 plasma metabolites of age-matched healthy controls, mild cognitive impairment (MCI) (an early stage of AD) patients, and AD patients by CE/MS. Our results revealed that plasma levels of some metabolites significantly changes in MCI and AD compared with the controls. ROC results of these metabolome analyses enable us to diagnose the early stage of AD with high accuracy (AUC>0.94).

Plant Physiology

P13

Wax ester profiling of Euglena gracilis by gas chromatography-mass spectrometry: toward understanding of anoxia wax ester fermentation

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Wax ester fermentation in Euglena is strongly increased by anoxia, but key events underlying the metabolic shift toward wax ester biosynthesis are poorly understood. We established a profiling method for wax esters in Euglena by GC-MS. Fermentation initiated 4 hours after the cessation of oxygen supply by halting the culture agitation resulting linear increase and proportional changes of wax ester amounts during 24 h. Complete anoxia by nitrogen gas aeration inhibited wax ester production and addition of bicarbonates reversed the inhibition, suggesting that there is a need for an additional carbon source. The proportion of wax esters was different between these two anoxic conditions.

P14

Modulation by temperature of purified phosphoenolpyruvate carboxylase from Amaranthus hypochondriacus L. (a C4 plant): protection by PEG-6000

Bhaskarrao Chinthapalli Arba Minch University; Agepati S Raghavendra University of Hyderabad;

<Temperature caused modulation of purified Phosphoenolpyruvate Carboxylase (EC: 4.1.1.31) in Amaranthus Hypochondriacus (a NAD-ME type C4 species). PEPC was purified from leaves of A. hypochondriacus with a specific activity of 34 U mg-1 protein. The activity of purified PEPC was maximal at 40oC and was quite dramatic when plotted as % of maximum activity. The decrease in the activity of PEPC at 15oC was much greater than that at 50oC upon incubation. As the temperature was raised from 15oC to 50oC, there was an increase in Vmax upto 40oC, while there was a marked decrease in malate sensitivity (from 68 to 33%). The inclusion of PEG-6000 during preincubation, decreased slightly the response of PEPC activity to temperature, but dramatically desensitized PEPC to temperature induced changes in the malate sensitivity. The extent of malate inhibition stayed in a narrow range of 51 to 48%, as the temperature was raised from 15oC to 50oC. The changes in the activity as well as malate inhibition of PEPC at either suboptimal or supraoptimal temperature, were significantly reversed. Temperature caused marked and reversible changes in the properties of PEPC protein purified from A. hypochondriacus leaves, implicating the physiological significance of temperature effects and PEPC could be one of the biochemical basis of temperature responses of C4 plants. The reversibility was partial when the PEPC protein was exposed to supraoptimal temperatures 50oC. Since these experiments were all done in vitro with purified PEPC, the changes are obviously independent of phosphorylation. The presence of PEG-6000 was effective in protecting PEPC against cold inactivation, indicating the importance of the conformation of PEPC. Nondenaturing PAGE of purified PEPC showed the existence of three different forms with proportionally increasing molecular weight: monomer, dimer and tetramer. ? Cold temperature tends to shift the equilibrium of PEPC protein towards tetramer. The presence of PEG and/or glycerol resulted in predominance of tetramer>

P15

A facile means for the identification of indolic compounds

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Indole-3-acetic acid (IAA) is a plant growth hormone present in very low amounts making its metabolism difficult to study using conventional approaches. Advances in analytical methods to deal with compounds at such low level are thus critical to future advances. We developed a simple yet powerful LC-MS method for fast identification of IAA related metabolites by using the quinolinium ion (m/z = 130.0651 m/z) with accurate mass. This signature ion is produced from substituted indoles in the CID process and is highly diagnostic for the later. With the method, we discovered new indolic metabolites including an IAA-tryptophan conjugate in soybean, and potential IAA precursors in Arabidopsis.

P16

Chemical regulation of photorespiratory hydrogen peroxide-dependent cell death

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Photorespiration relies heavily on peroxisomal catalase activity which removes photorespiratory hydrogen peroxide. By performing a chemical screen, we identified 34 small molecules that alleviate the negative effects of photorespiration in Arabidopsis thaliana mutants lacking peroxisomal catalase. We further characterized one of the hit compounds as an inhibitor of sirtuin activity. Sirtuin-dependent protein deacetylation is coupled to the energy status of the cell and is emerging as a crucial regulator of stress responses. This novel sirtuin inhibitor was used to explore the role of Arabidopsis sirtuins in metabolite reprogramming during adverse environmental conditions.

P17

Changes in membrane glycerolipids under heat stress in Arabidopsis leaf

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Environmental stress causes membrane damage in plants. Lipid studies are required to understand plant adaptation to climate changes. Here, an LC-MS-based lipidomics approach was carried out to elucidate the effect of short-term heat stress on Arabidopsis leaf membrane. Vegetative plants were subjected to high temperatures for a day, then continued to be grown under normal condition. The membrane glycerolipid composition was dynamic. TAG, 36:4/36:5 MGDG, 34:1/36:1/36:6 PC and 34:1 PE were increased by the stresses and decreased during recovery. The changes resulted in a decrease in membrane fluidity and an increase in bilayer stability, suggesting an acclimation response.

P18

Non-targeted metabolite profiling of different cultivars of rice seeds (Oryza sativa L.) by GC-TOF-MS and UPLC-Q-TOF-MS

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Rice (Oryza sativa L.) is a staple cereal food, particularly in Asia. In this study, we used both gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF-MS) and ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry (UPLC-Q-TOF-MS) followed by multivariate analysis to compare overall metabolite differences between different cultivars of rice seeds. Based on these analysis, sugars, sugar alcohols, amino acids, organic acid and flavonoids compounds were detected as significantly different metabolites in rice seeds. Our results imply an association between rice seed metabolites and their nutritional value.

P19

Metabolome analysis of rosaceous fruits

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Rosaceous plants include various edible fruits such as apples, pears, cherries, peaches, and strawberries. To understand metabolic dynamics in developing fruits, multiple metabolomics techniques including CE-TOF MS for ionic metabolites, LC-TOF MS for neutral metabolites, LC-tripleQ_MS for plant hormones, and HPLC for sugars and starch were applied to rosaceous fruits under developing. We found temporal accumulation of sulfur-containing amino acids in developing pear and apple fruits. In addition, several plant hormones such as abscisic acid and castasterone showed specific accumulation in mature fruits. These metabolomic data will be released on our database.

P20

Lipidome analysis of glucuronosyldiacylglycerol-deficient mutants of Arabidopsis

Yozo Okazaki RIKEN; Kazuki Saito Chiba University;

Phosphorus (P) is a major factor responsible for reduced crop yields. As a result, plants utilize various adaptive mech-

anisms against P depletion, including lipid remodeling. Glucuronosyldiacylglycerol (GlcADG) is synthesized by sulfolipid synthase, and deficiency of this lipid leads to an enhanced leaf senescence under P-limited conditions. To understand the effect of GlcADG deficiency in lipid metabolism in details, we analyzed wild type and GlcADG-deficient mutants of Arabidopsis grown under P-depleted conditions by untargeted lipidomics, and found several unknown metabolites decreased in GlcADG-deficient mutants. This result suggests pleiotropic effects of GlcADG deficiency in plants.

P21

Long-chain base desaturases modulate metabolic flow of complex glycosphingolipids in rice

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Plant sphingolipids are regarded as components of diverse biological processes but very little is known about molecular functions and metabolic regulation based on their structural variety. We developed LC-MS/MS-based sphingolipidomic analysis for comprehensive quantitative profiling of plant sphingolipids, which was applied to characterization of transgenic rice plants. Overexpression of long-chain base desaturases caused position-specific effects on metabolic flow of complex glycosphingolipids. The potential of desaturase-mediated metabolic engineering of plant sphingolipidome is discussed.

P22

Characterization of flavor in apple Fuji with a focus on pre-harvest conditions

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It has been in dispute that organic and conventional cultivation systems can affect contents of agricultural products or not. The level of primary metabolites as amino acids and sugars have been reported to be dependent on the N absorption history rather than on the origin. While, volatiles have not been investigated systematically in relation to cultivation systems. We attempted to apply metabolite profiling in study of organic products quality with quantitative description analysis (QDA). The feature of metabolic pathway in relation to cultivation method was also discussed.

P23

Analysis of the metabolite profiles of Arabidopsis thaliana expressing the enzymes from quinolizidine alkaloids producing plant: Lupinus angustifolius

Hajime Tomatsu Chiba University; Akira Oikawa RIKEN Center for Sustainable Resource Science; Yamagata University; Ryo Nakabayashi RIKEN Center for Sustainable Resource Science; Kazuki Saito RIKEN Center for Sustainable Resource Science; Chiba University; Mami Yamazaki Chiba University;

Heterologous genes encoding the candidate enzymes of quinolizidine alkaloids production in *Lupinus angustifolius*, including the gene encoding lysine decarboxylase (LDC) and candidates of amine oxidase (AO) and acyltransferase (AT), were introduced into *Arabidopsis thaliana*. The metabolite changes of transgenic *Arabidopsis* were analyzed. The transgenic plants expressing *La*-L/ODC accumulated cadaverine, the product of LDC activity. On the other hand, transgenic plants expressing *La*-L/ODC with *La*-AO or *La*-AT did not accumulate cadaverine. These results suggest that coexpressed AO and AT converted cadaverine and/or the cadaverine-derived metabolites.

P212

Mechanisms for soluble oxalate accumulation in plants

Atsuko Miyagi Saitama University;

Oxalate is useful in plants to detoxify metal ions, defense from predators, and so on. On the other hand, soluble oxalate is harmful for vertebrates because it leads to heavy syndrome such as kidney stone. However, mechanisms of oxalate accumulation in plants had been unknown. Thus, we analyzed the soluble oxalate-rich plant, *Rumex obtusifolius* L. (Polygonaceae), using CE-MS. Metabolome analysis with itaconate treatment showed that the isocitrate pathway contributed to oxalate synthesis. 13C tracer experiment revealed that citrate stored in stems was used for oxalate synthesis in leaves. These results would be available to lowering oxalate contents in crops such as spinach.

P213

Impact of drought stress on the secondary metabolite profile of sugarcane stalk

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Sugarcane is an important crop cultivated in tropical and sub-tropical regions mainly for its sucrose content. Several environmental factors affect its growth, metabolism and yield. In Brazil drought is the strongest and most frequent negative factor on sugarcane yield. A LC-MS-based approach was performed to investigate changes in the metabolite profile of a sugarcane drought tolerant variety under three irrigation regimes (control, moderate and severe stress). By PLS-DA analyses we were able to discriminate samples and identify differentially abundant metabolites among treatments

P214

Metabolic profile of sugarcane internodes during development

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Sugarcane is one of the most important cultivated grasses of the world mainly for its sucrose content, and Brazil is the largest producer. In order to elucidate the mechanisms of sucrose production and accumulation in metabolic level, we used the internodes 1, 5 and 9 of the sugarcane at 11 and 13 months after planting. The samples were analyzed by UPLC-ESI-QTOF-MS and multivariate statistical analysis. Analysis highlighted the biggest difference in metabolic profile between the samples from internode 9 with the other samples. The next steps aim to identify these metabolites and analyze their role in sucrose production and accumulation.

P215

Effects of illumination on leaf metabolomes of wild-type and energy-rich Arabidopsis thaliana

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Photosynthesis converts light energy into ATP and reducing power for carbon fixation. Overexpression (OE) lines of AtPAP2 grow faster and their leaves contain significantly higher ATP, sucrose, fructose and glucose levels than the wild-type (WT) in the middle-of-day. Even after 8 hours of darkness, the concentrations of ATP and sucrose were still higher in the OE line. Upon illumination, the level of NADPH increased significantly in both lines quickly (t = 1hr), whereas malate showed significant increase only at t = 8hr. The effects of illumination on the levels of TCA metabolites and amino acids in the lines were compared and their correlations to the levels of ATP and NADPH were examined.

P216

The metabolomics of a plant destroyer: using mass spectrometry to study tree disease

Ilena Isak Scion; Murray Robinson Scion; Armin Wagner Scion; Nari M Williams Scion;

Phytophthora are microscopic organisms which live up to their Greek name of plant destroyers. Sadly this is true with New Zealand's iconic indigenous trees and vast forest plantations increasingly threatened by Phytophthora diseases. Understanding the mechanisms of these diseases is critical for breeding, management and plant biosecurity. This calls for systems biology based approaches to investigate pathogen-host interactions for which metabolomic studies are an integral component. We are employing NMR and mass spectrometric technologies to characterize infection states in parallel to transcriptome and histological analyses. This approach will provide phenotypic data for disease research.

P217

Metabolomic analysis of Portulaca oleracea L. by using CE-TOF MS

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Portulaca oleracea is generally known as a weed, however, this plant is locally used as a herbal medicine and an edible plant. The purpose of our study is to find the potential of *P. oleracea* as an edible plant. Capillary electrophoresis time

of flight mass spectrometry (CE-TOF MS)-based metabolome analysis revealed specific accumulation of catecholamines such as dopamine in *P. oleracea* leaves.

P218

Metabolite profiling in barley mapping population Maresi x Cam/B1/CI subjected to drought

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Drought stress is one of the most important abiotic factors that has a negative impact on plant growth, development and survival massively limiting crop yield. The aim of conducted research was the analysis of the metabolome changes in spring type barley subjected to drought stress. The mapping population (100 recombinant inbred lines) used in the present study was developed through single seed descent from a cross between varieties Maresi CamB1CI. Analyses of barley metabolite extract samples were performed using gas chromatography mass spectrometer from three biological and two technical repetitions. In leaves and roots were identified 93 metabolites.

P219

Distinctive sugar metabolism in root parasitic weeds as a novel target for their selective control

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Because root parasitic weeds in Orobanchaceae cause serious problems in arid and semi-arid agriculture, an effective strategy for their control is required. The life cycle of the root parasitic weeds is significantly different from that of their host plants. For example, they require host-derived germination stimulants for seed germination. We focused on the unique germination process to find biological events specific to the root parasitic weeds. Hence, metabolic profiling was conducted on germinating seeds of a root parasitic species, *Orobanche minor*. As a result, we revealed that planteose, a galactosyl-sucrose trisaccharide, metabolism can be a target for the selective control.

P220

Visualization of the distribution of metabolites related to ripening in tomato fruit using MALDI-MS imaging

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Tomato fruit is one of the most important horticultural crops. In the present study, matrix-assisted laser desorption/ionization (MALDI)-MS imaging (MSI) technique was applied to visualize the distribution of metabolites during ripening in tomato fruit. Among metabolites detected, we successfully visualized the distribution of several metabolites between mature green and red tomato fruits. Especially, it was clearly shown that the quantities of primary metabolites related to umami compounds in mesocarp and locule were increased during mature process. In addition, we successfully visualized the distribution of secondary metabolites such as naringenin in epicarp in mature red tomato fruit.

P221

Detailed analyses of sphingolipids and their metabolite in Arabidopsis by LC-MS/MS

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LC-ESI-MS/MS approaches have enabled high selectivity and sensitivity for the identification and quantification of sphingolipids and their metabolite in Arabidopsis: (1) detailed molecular species analysis by reversed-phase HPLC coupled to ESI-MS/MS was performed for characterizing the cis-8 and trans-8 isomers of sphingoid bases among nine components of C18 sphingoid bases in glucosylceramides, (2) free sphingolid bases were analyzed by precolumn derivatization of the amino group of sphingoid bases with 4-fluoro-7-nitrobenzofurazan (NBD-F), and (3) reliable detection of several fmol of the derivatives of sphingolid base 1-phosphates were performed by LC-MS/MS.

Parasitology, Pathology

P24

Tryptophan metabolism in mice infected with Plasmodium berghei ANKA: 1H NMR-based metabolomic investigation

Rustam Singh Tata Institute of Fundamental Research; **Arjun Sengupta** Tata Institute of Fundamental Research; **Soumita Ghosh** Tata Institute of Fundamental Research; **Shobhona Sharma** Tata Institute of Fundamental Research; **Haripal Sonawat** Tata Institute of Fundamental Research;

Host response to Plasmodium infection is multimodal, involves several organs & metabolic lesions. Urine, serum, liver, kidney and brain metabolite profiles in infected mice exhibit malaria stage-specific changes. Levels of kynurenate & quinolinate, tryptophan metabolism intermediates, are specifically involved in cerebral malaria. The 1H NMR profiles of tryptophan challenged control & infected mice cluster distinctly. The metabolite profiles of un-infected (tryptophan challenged or unchallenged) are different from the parasite infected mice. The latter exhibited alterations in taurine/betaine, Glu, Gln, glutathione, glycerol, Leu, Ile, UDP-glucose & ADP/AMP in comparison to control animals.

P25

Transketolase in Leishmania mexicana: regulation of subcellular localisation and metabolic roles

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Transketolase is an enzyme of the non-oxidative branch of the pentose phosphate pathway, localised to both the glycosomes and cytosol in Leishmania mexicana. When deprived of transketolase, promastigote cells adopt a "stringent" metabolism, consuming two times less glucose and reducing the secretion of end products. Furthermore, we show that localisation of transketolase is regulated by altering the length of the C-terminal tail of the enzyme. Manipulating the sub-cellular localisation, we could determine the enzyme's contribution to metabolism in the respective compartments and impact on infectivity or oxidative stress response.

P26

Metabolome analysis for identifying biomarkers of Chronic Fatigue Syndrome

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Chronic fatigue syndrome (CFS) is a persistent and unexplained pathological state characterized by severely debilitating fatigue lasting for 6 consecutive months. We performed metabolome analysis in plasma from CFS patients and healthy volunteers, and identified a metabolic profile involved in the pathophysiology of CFS. CFS patients showed characteristic changes in metabolites levels, such as decreased citrate, cis-aconitate and isocitrate in TCA cycle and increased ornithine in Urea cycle, compared with healthy controls. We could discriminate CFS patients from healthy controls with over 80% accuracy using such metabolites. Our findings will contribute to the objective diagnosis of CFS.

P223

Assigning functions to unknown enzymes in trypanosomes using metabolite profiling

Katharina Johnston University of Glasgow; **Richard Burchmore** University of Glasgow, Glasgow Polyomics; **Karl Burgess** University of Glasgow, Glasgow Polyomics; **Michael Barrett** University of Glasgow, Glasgow Polyomics;

The parasitic protozoan, Trypanosoma brucei, is responsible for the disease sleeping sickness in Africa. An estimated 60% of its identified genes are annotated with unknown or putative function only. Here, we investigate the use of a method to detect enzyme function through interactions with metabolite mixtures, followed by mass spectrometry. Seven putative identified enzymes were randomly selected, heterologously expressed in E. coli, purified, and added to

yeast extract. Metabolite composition was assessed before and after enzyme-exposure. At least one of the enzymes confirmed putative function, although limitations with the system mean the approach may only be useful for some proteins.

P224

Global metabolomics for the determination of the role for AMP deaminase (Ampd3) mutation in malaria resistance

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Host genetic polymorphisms can confer resistance to malaria. Genomics and metabolomics were applied to identify a novel host factor involved in malaria resistance. Whole exome sequencing of a novel mutant mouse line, generated by ENU mutagenesis, identified a mutation in RBC *Ampd3*. LCMS-based metabolomics of Ampd3 mutant RBCs revealed significantly elevated GTP levels, 70-fold higher in homozygotes compared to wildtype, and depletion of adenosine nucleotides. Metabolomics provides the first evidence that the malaria-resistance mutation in Ampd3 leads to elevation of enzyme activity, and shows that the balance in the host purine metabolism is important for malaria infection.

P225

Lipidomics approach to study the effect of different chain length free fatty acids on HepG2 cells

Lu-Min Shih Chang Gung University; **Mei-Ling Cheng** Chang Gung University;

Non-alcoholic fatty liver disease (NAFLD) is an important healthy problem over the world. It is characterized by abnormal fatty acid (FA) accumulation in liver without excessively consuming alcohol. Hepatic lipotoxicity is implicated in dysfunction of hepatocytes and pathogenesis of NAFLD. Little is known about the effect of chain length of FA on lipotoxicity-induced metabolic changes and injury of hepatocytes. In this study, HepG2 cells detected by NMR and LC-TOFMS were treated with different free FAs. This data revealed the different lipid profiles in different chain length FAs treated cells.

P226

Fatty acid biosynthesis in apicomplexan parasites - a route for drug therapy?

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Apicomplexan parasites are responsible for high impact human diseases such as malaria and toxoplasmosis. These obligate intracellular pathogens are dependent on *de novo* lipid biosynthesis and lipid scavenging for biogenesis of parasite and protective vacuolar membranes. Here we combine novel metabolomics techniques with genetic and biochemical approaches to delineate fatty acid biosynthetic pathways in *Toxoplasma gondii*. We have employed metabolic labelling studies to elucidate the function of eight enzymes involved in core fatty acid biosynthesis, elongation, and phospholipid production, revealing that these pathways represent a putative drug target in these devastating organisms.

New Technology

P27

In vivo metabolite visualization using standardized imaging mass spectrometry

Shuichi Shimma National Cancer Center Research Institute; **Teruaki Fujishita** Aichi Cancer Center Research Instutute; **Masahiro Aoki** Aichi Cancer Center Research Instutute; **Tomoyoshi Soga** Keio University;

Conventional mass spectrometric analysis provides quantitative information on metabolites in normal and diseased tissues such as cancer. However, tissue extraction precludes precise information on their spatial distribution. To bring new viewpoints in metabolomics, Mass Microscope based on imaging mass spectrometry (IMS) was developed. In this presentation, we will provide an update on the metabolite visualization on the tissue surface using Mass Microscope, and offer a standardized methodology including sample preparations. Application of the method enabled visualization of cystathionine accumulation in intestinal tumors of *Apc* mice (colon cancer model).

P28

A novel high resolution human metabolite MS/MS spectral library enables rapid and accurate metabolite identification of human biofluids

Zhendong Li University of Alberta; **Mingguo Xu** University of Alberta; **Yiman Wu** University of Alberta; **Chiao-Li Tseng** University of Alberta; **Tao Huan** University of Alberta; **Wei Han** University of Alberta; **Jaspaul Tatlay** University of Alberta; **Tran Tran** University of Alberta; **Aiko Barsch** Bruker Daltonics; **Carsten Baessmann** Bruker Daltonics; **Liang Li** University of Alberta;

Metabolite identification remains a major analytical challenge in metabolic profiling of human biofluids. Here, we describe a workflow for rapid and accurate metabolite identification based on the use of a high resolution MS/MS spectral library of about 800 metabolites from HMDB created using a high performance QTOF mass spectrometer. For each metabolite, at least 5 different collision energies were used to generate MS/MS spectra. Manually annotated fragment ion structures and/or formula for each spectrum were found to be particularly useful for structural confirmation. We demonstrate the analytical performance of this workflow using profiles of the metabolomes of human urine and serum.

P29

A novel atmospheric pressure GC Source coupled to high resolution TOF-MS Analysis improves quantitative and qualitative metabolic profiling studies

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High resolution GC-APCI-MS generated interest in recent years because it enables the identification of unknown analytes. Soft APCI ionization preserves the molecular ion information, so the exact mass in a high resolution TOF enables the identification of still unknown metabolites. Here we present a novel GC-APCI design coupled with QTOF-MS. Compared with earlier results, an improved GC peak resolution, increased linear dynamic range and improved lower limits of quantification will be presented. The improved analytical performance enabled detection of approximately double the number of peaks in cell culture supernatant samples of pancreatic cancer cells.

P30

In vivo exhaled breath analysis: an alternative approach to address lung disease diagnosis, chronobiology and drug monitoring.

Pablo M-L Sinues ETH Zurich; **Xue Li** ETH Zurich; **Robert Dallmann** University of Zurich; **Lukas Bregy** ETH Zurich; **Esther Schwarz** University Hospital Zurich; **Yvonne Nussbaumer** University Hospital Zurich; **Steven Brown** University of Zurich; **Malcolm Kohler** University Hospital Zurich; **Renato Zenobi** ETH Zurich;

Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject. However, in contrast with other biofluids (e.g. plasma), the analysis of breath remains far less explored. We present here some recent examples of how new techniques developed in our laboratory to analyze breath in real time may contribute to the metabolomics field. We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant topics in translational medicine, including disease diagnosis, chronobiology and drug monitoring.

P31

Evaluating the integration of CE, ESI and mass pectrometry for the quantitative analysis of amino acids in the cationic metabolome.

John C. Hudson University of Regina;

The integration of capillary electrophoresis and electrospray ionization into a single dynamic process (CESI) combined with mass spectrometry (MS) has been previously demonstrated. The aims of the study were to investigate the quantitative capability of CESI-MS in metabolomics using commonly available sample preparation methods for bio-fluids including plasma, urine and saliva. The results of the study demonstrate that CESI-MS may be used for the quantitative analysis of amino acids from bio-fluids. This has the potential to be an effective tool to study endogenous polar compounds in untargeted and targeted analyses of the cationic metabolome.

P32

Multi-segment Injection-Capillary Electrophoresis-Mass Spectrometry: Multiplexed Separations for Biomarker Discovery in Metabolomics

Philip Britz-McKibbin McMaster University; Alicia DiBattista McMaster University; Naomi Kuehnbaum McMaster University; Adriana Nori de Macedo McMaster University; Karen Lam McMaster University;

Separation science plays a vital role in mass spectrometry (MS)-based metabolomic studies. However, sample throughput is limited when using conventional separation platforms. Multi-segment injection (MSI)-CE-MS offers a multiplexed separation format to enhance sample throughput and data fidelity that takes advantage of an accelerated metabolomics workflow for biomarker discovery. Customized serial injection configurations are used to encode information temporally to allow for unambiguous diagnostic testing of genetic diseases. Also, time-resolved metabolomic studies elucidate the putative health benefits of exercise training to improve glucose tolerance in obese women at risk for diabetes.

P33

Development of rapid whole cell metabolomic screening for volatile compounds

Ameerah Mohammed Bokhari KAUST; Zeyad AlTalla KAUST; Najeh Kharabatia KAUST; Vladimir Bajic KAUST; John Archer KAUST;

KSA is the leading petroleum exporting country in the Middle East; consequently it generates over 400 million tons of CO2 per year from industrial sites. Photosynthetic microalgae can fix CO2 to synthesis renewable and sustainable feedstock chemicals that offer a viable route to reducing carbon emissions and providing a sustainable source of chemical feedstocks. Volatile hydrocarbon (VHC) production by photosynthetic microalgae has been identified for their promising applications as a renewable source for many industrially desired compounds. Here we use Red Sea microalgae to develop a robust GC/MS/FID assay to establish the culture conditions related to particular metabolic profiles.

P36

Using PCI-IS for targeted metabolomics analysis of amino acids and its application on breast cancer research

Hsiao-Wei Liao National Taiwan University; Ching-Hua Kuo National Taiwan University; Yufeng Jane Tseng National Taiwan University; Chiun-Sheng Huang National Taiwan University Hospital;

Recent metabolomic studies revealed the potential key roles of amino acids (AAs) in various diseases including diabetes, cancers, and kidney diseases. In this study, we utilized the postcolumn infused-internal standard (PCI-IS) strategy to correct the matrix effects (MEs) caused errors in LC-MS for AAs research. Through PCI-IS correction, the accuracy of AAs comparison can be largely improved. Besides, accurate quantification can also be achieved by the combination of PCI-IS with matrix normalization factor (MNF). Finally, we applied this PCI-IS with MNF approach for the breast cancer research.

P37

A comparative metabolomic method for time series investigation of fatty acid expressions

Guan-Yuan Chen National Taiwan University; Ching-Hua Kuo National Taiwan University; Yufeng Jane Tseng National Taiwan

University; **Chiun-Sheng Huang** National Taiwan University Hospital;

Fatty acids (FA) are vital to living organisms. Time series experiment is frequently employed in biomedical researches. We proposed a comparative metabolomic approach for time series investigation of FA expressions. FAs extracted in samples obtained at different time points were differentially labeled by distinct isotope labeled ethanol and isopropanol and pooled for GC-MS analysis. Total sample size can be reduced and comparative information of FA expression between different time points could be obtained through this approach. The proposed method was validated in terms of accuracy and precision, and applied to investigate FA alternations in breast cancer patients after chemotherapy.

P38

Higher resolution LC-MS and MS-MS analysis of lipid extracts using benchtop Orbitrap-based mass spectrometers and LipidSearch software

David A Peake Thermo Fisher Scientific; **Reiko Kiyonami** Thermo Fisher Scientific; **Yasuto Yokoi** Mitsui Knowledge Industry; **Yingying Huang** Thermo Fisher Scientific;

LC/MS and MS-MS analysis of lipid extracts was performed using a novel benchtop quadrupole high-field Orbitrap mass spectrometer operating at low (15-30K) and high (120-240K) mass resolution. Bovine brain, heart, liver and yeast lipid extracts were analyzed to determine mass and chromatographic resolution required for separating overlapping isomeric/isobaric lipid species. The number of lipid species in each experiment were identified by LipidSearch software. At higher mass resolution the number of identified lipid species were at least 20% more than at the lower resolution. Thus, adequate mass resolution and sufficient speed are very important factors in LC-MS based lipidomics experiments.

P39

Novel metabolic assay for pre-analytical quality control of human plasma samples

Beate Kamlage Meatnomics GmbH; **Bianca Bethan** Metanomics GmbH; **Oliver Schmitz** Metanomics GmbH; **Philipp Schatz** Metanomics Health GmbH; **Tim Boelke** Metanomics Health GmbH; **Arndt Schmitz** Bayer Pharma AG;

Successful research in the healthcare area depends on high quality samples generated by standard sample collection methodologies to minimize the effects of pre-analytical variations that confound the analytical results and decrease the credibility of the research outcomes. MxP Quality Control Plasma is a novel metabolomics-based profiling assay which provides a holistic control of EDTA human plasma sample quality. In a blinded beta-test samples of a poor pre-analytical quality due to prolonged storage of blood or plasma were detected with a high sensitivity and specificity. This enables Pharma R&D and biobanks to upgrade their quality management from quality assurance to quality control.

P40

Strategic utilization of gas chromatography with both nominal and high resolution time-of-flight mass spectrometers for metabolomic studies

Jeffrey Patrick LECO Corporation; **Joe Binkley** LECO Corporation; **David Alonso** LECO Corporation;

Challenges in metabolomics include the number, diversity and concentrations of metabolites. This study defines an effective workflow for the structural identification of modulated metabolites based on advanced GCMS technologies. The bottleneck is identification and structural characterization of compounds not detected in available databases. The workflow utilizes nominal and high resolution time-of-flight (HRT) instrumentation for identification of metabolites. Both EI and CI, as well as, statistical analysis aid identification of modulated metabolites. The workflow leverages the 1ppm mass accuracy observed and the molecular and fragment ions detected in the two ionization techniques.

P41

Characterization of metabolites from M. truncatula using gas chromatography mass spectrometry for characterization of knowns and unknowns

David Alonso LECO Corporation; **Jeff Patrick** LECO Corporation; **Lloyd W. Sumner** The Samuel Roberts Noble Foundation, Ardmore,

OK; **Joe Binkley** LECO Corporation; **David V. Huhman** The Samuel Roberts Noble Foundation; **Feng Qiu** The Samuel Roberts Noble Foundation; **Dennis Fine** The Samuel Roberts Noble Foundation;

Identification of unknown analytes is a principle challenge to metabolomic analyses. Here extracts from M. truncatula are characterized using GC with HRTOF-MS detection. Accurate mass with either EI (library matching) or CI (molecular formula assignment) was used to identify metabolites. Analyses of the M. truncatula samples were performed with a focus on identification. The combination of EI and CI provided additional interpretative power with many unknowns detected as both their molecular species and fragments. Molecular formulae and structures can be proposed for the unknowns. Potential isomeric forms of pinnitol serve as one example with numerous others detected and interpreted.

P227

Novel unbiased derivatization methodology for broad-spectrum GC-MS metabolomic analysis of complex mixtures

Bekzod Bahromovich Khakimov University of Copenhagen; **Mohammed Saddik Motawia** University of Copenhagen; **Soren Bak** University of Copenhagen; **Soren Balling Engelsen** University of Copenhagen;

Quantitative GC-MS metabolomics of complex biological mixtures requires robust and broad-spectrum derivatization methods. We have developed a TMSCN based method that can outperform MSTFA, one of the most commonly used trimethylsilylation reagents. Two complex samples: a mixture of 35 metabolites, including amino acids, carbohydrates, small organic and phenolic acids, flavonoids, triterpenoids, and a phenolic extract of blueberry fruits were derivatized using either TMSCN or MSTFA and analyzed by GC-MS. Independently of prior methoximation, the TMSCN based method was more rapid, sensitive and reproducible than MSTFA. The TMSCN method thus has great potential for untargeted metabolomics.

P228

Automated NMR, GC-MS and HPLC-based kits for quantitative metabolomics

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A series of metabolomic kits consisting of reagents, protocols and/or software that can inexpensively and quantitatively measure approximately 30 selected metabolites in blood and urine is described. These kits are compatible with commonly available analytical platforms including NMR, GC-MS and HPLC. Fully automated software to identify and quantify the target metabolites have also been developed for each of these kits. Based on extensive testing with defined mixtures and real biological samples, these automated software systems consistently perform with sensitivity and specificity greater than 98% for compound identification and and a CV of less than 10% for compound quantification.

P229

Adductomic analysis of human serum albumin by mass spectrometry

Rupa Mandal University of Alberta, The Metabolomics Innovation Centre; **Lu Deng** University of Alberta, The Metabolomics Innovation Centre; **David S Wishart** University of Alberta, The Metabolomics Innovation Centre;

Adductomic analysis of Human Serum Albumin (HSA) represents a promising route to identify reactive electrophiles in blood. By comparing the concentrations of electrophilic HSA adducts across different populations, it may be possible to gain new insights into the environmental causes of many diseases. Here, we report an improved method that employs fixed-step multiple reaction monitoring (FS-MRM)-MS to characterize HSA adducts. This MS-based method has been used to investigate smoke exposure adducts in blood samples from smokers. Our improved FS-MRM-MS method is easier and faster than current approaches and offers the potential to identify the molecular composition of HSA adducts in serum.

P230

Accurate determination of metabolites in serum using MRM mode in a triple quadrupole GC/MS/MS and MRM metabolites database

Shuichi Kawana Shimadzu Corporation; Yukihiko Kudo Shimadzu Corporation; Kenichi Obayashi Shimadzu Corporation; Haruhiko Miyagawa Shimadzu Corporation;

Biological samples contain many metabolites and diverse matrices (contaminants). To reduce the effects of these matrices and achieve more sensitive and accurate measurement of the target metabolites, Multiple Reaction Monitoring (MRM) mode in GC/MS/MS is used. This poster will describe MRM analysis mode in GC/MS/MS, with an MRM Metabolites Database (186 metabolites) as it applies to analysis of metabolites in serum. These results demonstrate this system can identify and determine more than 100 metabolites in serum accurately compared to Scan mode. The wide-reaching utility of GC/MS/MS and MRM mode for accurate detection of metabolites in serum is discussed.

P231

Method development of GC/MS-based metabolome analyses for sweet fruits

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Agricultural products are including variety metabolites in broad range of concentration. For example, apples contain much amount sugars. Their concentrations are more than four orders of amino acids'. If the splitless injection would be applied to GCMS analysis in order to measure minute trimethyl-silylated metabolites, overloaded amount sugars will affect quantitative analysis of minute metabolites. As a possible solution for this problem, a combination of alkylation and silylation was evaluated with apple extract. As a result, influence of sugars was reduced and measurement of minute metabolites was achieved. It was proved to be a suitable method for GCMS analysis of sweet fruits.

P232

Novel rapid pretreatment method for biological samples using monolithic silica fixed spin column.

Shigenori Ota GL Science Inc.; Yuko Yui GL Science Inc.; Hiroshi Oikawa GL Science Inc.; Masayoshi Ohira GL Science Inc;

Ion suppression due to the presence of impurity has become a concern in LC/MS when analyzing biological samples, metabolites. The solid phase extraction is wildly used sample preparation technique for elimination of ion suppression, but at a method complexity and not suitable for small sample volume (<100 uL). We developed the Monospin for SPE column, is useful and effective for sample pretreatment when the biological sample volume is small. In this report, we developed a new pretreatment method for removal of lipids and hydrophobic compounds using several functional group modified Monospin.

P234

Profiling of anionic polar metabolites using capillary ion chromatography and high resolution accurate mass spectrometry

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Capillary ion chromatography (Cap IC) resolving power of anionic polar isobaric compounds coupled with high resolution accurate mass spectrometry (HR/AM) of Q Exactive MS is a highly sensitive platform needed to profile polar metabolites. Here Cap IC HR/AM was applied to the metabolic profiling of Human Oral Squamous Cell Carcinoma (HOSCC) cells using 3-paired OSCC cell lines (UM1, UM5, CSC) and wild-type controls. Cap IC demonstrated outstanding separation and peak shape with 100-fold increased sensitivities as compared to RPLC and HILIC methods. Additionally, HR/AM demonstrated accurate mass within 1 ppm. Significant changes in the energy cycle pathways were found by differential analysis.

P235

High throughput NMR spectroscopy of urine and serum or plasma for large scale metabolic phenotyping

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NMR instrumentation and technology has progressed to allow the acquisition of data in an effective, reproducible and high throughput approach that allows the study of general population samples from epidemiological collections for biomarkers of disease risk. The challenge remains to develop highly reproducible methods and standardised protocols that minimise technical or experimental bias, allowing realistic interlaboratory comparisons of subtle biomarker information. Here we present a platform that facilitates NMR spectroscopy usage across different large cohorts of biofluid samples, enabling integration of global metabolic profiling that is a prerequisite for personalised healthcare.

P236

Unambiguous determination of metabolite elemental compositions using isotopic fine structure: in silico evaluation and metabolomic application

Daisuke Miura Innovation Center for Medical Redox Navigation; Tatsuhiko Nagao Kyushu University; Daichi Yukihira Kyushu University; Yoshinori Fujimura Kyushu University; Kazunori Saito Bruker Daltonics K.K.; Katsutoshi Takahashi National Institute of Advanced Industrial Science and Technology; Hiroyuki Wariishi Kyushu University;

For metabolite identification, libraries or database search strategies using the exact MS and MS/MS pattern of mass spectra of known and available compounds are well-established chemical annotations with MS. However, there are still a tremendous number of unknown metabolites other than commercially available compounds. Therefore, reference-free MS-based metabolite identification is a key requirement in metabolomic studies. In the present study, we evaluated the potential effectiveness of the relative isotopic area as a constraint for determining elemental composition in metabolomic research. Some challenges regarding further use of the approach are also discussed.

P237

Coupling capillary ion chromatography with s new orbitrap mass spectrometer for targeted metabolomic analysis in oral cancer cells

Junhua Wang Thermo Fisher Scientific; Terri Christison Thermo Fisher Scientific; Krista Backiel Cambridge Isotope Laboratories, Inc.; Kaori Misuno UCLA, School of Dentistry; Shen Hu UCLA, School of Dentistry; Kevin Millis Cambridge Isotope Laboratories, Inc.; Linda Lopez Thermo Fisher Scientific; Yingying Huang Thermo Fisher Scientific;

A study using Capillary ion chromatography-Orbitrap MS for targeted metabolomic quantitative analysis of cell lysates. A new Thermo Scientific Q_ExactiveTM series mass spectrometer was operated using a method that consisted of full scan and targeted multiplexed SIM scans. Isotopically labeled standards were spiked into sample for relative quantitation and to generate standard curve for quantitation. Two methods were cross-validated and showed good correlation. Ten metabolites in citric acid cycle were detected and their amounts in two oral cancer cell samples were quantified. Isomeric metabolites were identified by accurate mass measurement and high resolution MS/MS fragmentation.

P238

Metabolomics: analytics methods driving progress in microbial cell factories

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We present here several high-throughout analytical methods that we have developed in-house and apply routinely for detection of metabolites. The platform is currently being served as a key technology for the development of yeast cell factories for the production of biofuels and biochemical products. Our analytical platform covers from sampling through the analysis based on several techniques. Up to date, we are able to set up methods/protocols for quantifying and profiling of several metabolites that we are highly interested e.g. lipids, fatty acids, amino acids and organic acids.

We are currently developing a method for global analysis of yeast metabolome based on high resolution LC-MS.

P239

Integration of computational and empirical methods in LCMS-based metabolomics for systematic annotation of plant metabolomes

Dennis Fine The Noble Foundation; Feng Qiu The Noble Foundation; Daniel Wherritt The Noble Foundation; Lloyd W. Sumner The Noble Foundation;

Computational and empirical methods based upon UHPLC-MS-SPE-NMR metabolomics were used for the systematic annotation and increased metabolome coverage of the model plant species Medicago truncatula. A Plant Metabolite Annotation Toolbox (PlantMAT) was developed that utilizes in silico software algorithms to predict secondary metabolite structures, mainly triterpenes and flavonoids, from empirically measured UHPLC-QToFMS accurate m/z values, elemental formulas, retention times, and MS/MS spectra. Further, metabolite identifications were confirmed by automated isolation and purification of targeted compounds using UHPLC-MS-SPE followed by structural determination by NMR.

P240

Poly-unsaturated fatty acids metabolome in sinomenine treated RBL-2H3 cell line using 2D nano LC-NSI-Q-TOF/MS

Jian-Lin Wu Macau University of Science and Technology; Na Li Macau University of Science and Technology; Wan Yi Gu Macau University of Science and Technology; Lu-Fen Huang Macau University of Science and Technology; Hua Zhou Macau University of Science and Technology; Liang Liu Macau University of Science and Technology;

This work described here employed a 2D nano liquid chromatography-nanospray ionization-time of flight-mass spectrometry (2D nano LC-NSI-Q-TOF/MS) method to monitor 57 poly-unsaturated fatty acids (PUFAs) were quantified along with 7 corresponding isotope dilution internal standards within 35min. The limits of quantification were between 0.008 and 20 pg. The method validations for linearity, accuracy, precision, recovery were satisfied. This approach was used to evaluate PUFAs metabolome in sinomenine treated RBL-2H3 cell line, an established allergic and inflammatory cell model.

Drugs, Medicine

P42

Saponarin isolated from barley sprouts attenuates LPS-induced inflammation by suppressing NF-kB activation in RAW 264.7 macrophages

Woo Duck Seo Rural Development Administration; Kyung Hye Seo Rural Development Administration; Sang Ik Han Rural Development Administration; Ji Eun Ra Rural Development Administration; Ji Young Park Rural Development Administration; Mi Jin Park Rural Development Administration; Min Hee Nam Rural Development Administration; Jung Hwa Cha Rural Development Administration;

Saponarin (SN), a flavone glucoside found in barley sprouts. We investigated that SN was inhibited lipopolysaccharide (LPS)-induced inflammatory response in RAW264.7 macrophage cells. The data results indicated that LPS-induced activation of nuclear factor kappa B (NF-kB) was suppressed significantly by saponarin in RAW264.7 macrophage cells without cytotoxicity. SN also decreased LPS-induced interleukin (IL) - 6 releases at mRNA level in RAW 264.7 cell. LPS-induced the expression of gene products involved in antiapoptosis (c-FLIP etc) and proliferation (e.g., Cox-2) that SN suppressed their expression. Moreover, SN inhibited the phosphorylation of IkBa and phosphorylation of p65 NF-kB.

P43

Metabonomic responses of MnIO-NPs as potential dual-modal contrast agents

Jianghua Feng Xiamen University; Jinquan Li Xiamen University;

MnO-embedded iron oxide nanoparticles (MnIO-NPs) could serve as promising dual-modal contrast agents. However, their overall bioeffects and potential toxicity remain unclear. In this study, their metabolic effects on rats were

investigated by using NMR-based metabonomic strategy. The analyses from body fluids (plasma and urine) and tissues (live, kidney and spleen) indicated that MnIO-NPs induced metabolic perturbation including energy, nucleotides, amino acids and phospholipid metabolisms. Besides, some supportive nutrients and conjugation substrates were involved in detoxification reaction. The obtained information would provide identifiable ground for candidate selection and optimization.

P44

Determination of lipoic acid and its metabolites in human urine by mass spectrometry

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Lipoic acid (LA) eliminates ROS and is an effective therapeutic agent for many oxidative stress-related disorders such as diabetes and cardiovascular diseases. This study explored dispersive liquid-liquid microextraction (DLLME) for extraction and concentration of LA in human urine. To improve the detection of LA by MALDI-TOF MS, microwave-assisted derivatization (MAD) was performed to render LA chromophores for high ionization efficiency in MALDI. The major and minor metabolites of LA were also extracted by DLLME and detected by MALDI-TOF MS and LTQ Orbitrap, respectively. After DLLME and MAD, all LA derivatizations and metabolites were structurally confirmed by LTQ Orbitrap.

P45

Determination of androgenic steroids and metabolites in urine by full-capillary sample injection combined with a sweeping CE method

Chun-Chi Wang Kaohsiung Medical University; Yen-Ling Chen Kaohsiung Medical University;

This study describes an on-line stacking CE approach by sweeping with whole capillary sample filling for analyzing five anabolic androgenic steroids and their metabolites in urine samples. The five analytes for detection were androgenic steroids and their metabolites including androstenedione, testosterone, epitestosterone, boldenone, and clostebol. Therefore, a sensitive detection method is imperatively required for monitoring the urine samples of athletes. After extraction by n-hexane, the simple and sensitive stacking capillary electrophoresis with full capillary injection could be used as a powerful tool for monitoring the illegal use of doping and their metabolic proportion.

P46

Determination of Saikosaponins and metabolites by chemometric experimental design and cyclodextrin modified micellar electrokinetic chromatography

Kuan-Ling Chen Kaohsiung Medical University; Yen-Ling Chen Kaohsiung Medical University;

The pharmacological components in Bupleuri Radix is saikosaponins, separated into saikosaponin a-d, which have inhibitory action against hepatitis. To assure the saikosaponins and their metabolites responsible for activities, a cyclodextrin modified micellar electrokinetic chromatography method with chemometric experimental design to identify the metabolic profile of saikosaponins is necessary. Compared to the traditional investigation of multiple parameters, chemometric experimental design was used for enhancing separation capability and exploring the interaction of parameters with less time consuming and low cost.

P47

Application of experimental design for the determination of ultraviolet absorbents and their metabolites by capillary electrophoresis

Wen-Yao Hsiao Kaohsiung Medical University; Yen-Ling Chen Kaohsiung Medical University;

In order to prevent UV damage to the skin, many sunscreen products that can absorb UV radiation and attenuate the negative effects of sunlight exposure were developed. However, ultraviolet absorbents have some adverse reactions such as skin irritation. On- line sample concentration technique, analyte focusing by micelle collapse-micellar electrokinetic capillary chromatography (AFMC-MEKC), was introduced to improve detection sensitivity. The aim of this study is to establish an on- line preconcentration method which can simultaneously determine these ultraviolet absorbents and their metabolites in plasma and urine samples.

P48

Gas chromatography mass spectrometry-based serum metabolomics for diagnosis and assessment of ulcerative colitis

Masaru Yoshida Kobe University; Shin Nishiumi Kobe University; Michitaka Kohashi Kobe University; Takeshi Azuma Kobe University;

To improve the clinical course of ulcerative colitis (UC), more accurate diagnostic and assessment methods is required. Therefore, we used serum metabolomics to develop diagnostic and assessment methods. Sera from UC patients and healthy volunteers (HV) were collected from multiple institutions. Their serum metabolites were analyzed by gas chromatography mass spectrometry. The diagnostic and assessment models for UC were established by multiple logistic regression analysis. The diagnostic model for discriminating UC from HV demonstrated 96.67% accuracy. The model for assessing UC showed 88.33% accuracy. Our models displayed high performance and expected to be clinical application.

P49

Metabolomic investigations of the effect of simvastatin on lipid synthesis by using UPLC coupled with TOF mass spectrometry

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Simvastatin is a cholesterol lowering drug, which is primarily used to regulate cholesterol levels for the management of dyslipidemia and the prevention of cardiovascular disease. However, its mechanisms of action are not yet fully understood. In this study, a metabolomic method was developed to investigate the effect of simvastatin on metabolites in mice liver by using UPLC/Q-TOF MS as well as multivariate statistical analysis. Results showed that simvastatin could inhibit the synthesis of lipids. The results were further confirmed with isotope tracer and Western-Blot assay. The inhibition of simvastatin may be related to the expressions of ACCa and FAS.

P50

Metabolomics application for searching adenosine a1 receptor ligands from indonesian medicinal plants

Nancy Dewi Yuliana Bogor Agricultural University; Slamet Budijanto Bogor Agricultural University; Young Hae Choi Leiden University; Robert Verpoorte Leiden University;

NMR metabolomics and orthogonal projection to the least square analysis were used to identify adenosine A1 receptor ligand from Indonesian medicinal plants;Orthosiphon stamineus(OS), Boesenbergia rotunda (BR),and Morus alba (MA). Seven methoxy flavonoids were identified as potential ligand from OS and indeed they were active when isolated and tested.Pinocembrine and hydroxy-panduratin were potential ligands from BR and need to be validated further. Aromatic compounds without prenyl or methoxy units were suggested as ligands from MA while betulinic acid, morusin, alkaloids and prenylated aromatic compounds abundant in MA did not correlate to the adenosine A1 binding activity of MA extract.

P51

Ftir metabolomics to study sorghum phytochemicals changes and its antiproliferative activity against hct 116 during analogue rice production

Slamet Budijanto Bogor Agricultural University; Nancy Dewi Yuliana Bogor Agricultural University;

The study aimed to identify the effect of processing step involved in sorghum based analogue rice (AR) production to sorghum phytochemicals composition and antiproliferative activity against HCT 116 in-vitro using FTIR metabolomics and orthogonal projection to the least square analysis. The antiproliferative activity tended to increase during the process. Typical FTIR patterns for functional groups present in ferulic acids, coumaric acid, procyanidins, and phytosterols were correlated with the activity. These compounds were reported elsewhere to correlate with various sorghum bioactivities. The signals were dominant in AR samples taken from mixing, extrusion, and oven drying steps.

P52

Metabolic imbalances due to oxidative stress in endometriosis: ^{1}H NMR based metabonomics

Saikat K Jana Indian Institute of Technology; **Mainak Dutta** Indian Institute of Technology; **Mamata Joshi** Tata Institute of Fundamental Research; **Sudha Srivastava** Tata Institute of Fundamental Research; **Baidyanath Chakravarty** Institute of Reproductive Medicine; **Koel Chaudhury** Indian Institute of Technology;

We have used 1H NMR based targeted metabolite profiling approach to explore dysregulation in metabolites expression in women with endometriosis. PLS-DA model could classify endometriosis with sensitivity and specificity of 92.83% and 100%, respectively. Glucose metabolism, citrate and succinate at elevated level, higher levels of ROS, lipid peroxidation, and advanced oxidation protein products and lower levels of total antioxidant capacity were observed. Oxidative protection, injury, recovery and possible sources and mechanism of ROS generation has been hypothesized.

P53

Integration of metabolomics with pharmacokinetic drug interaction: CYP3A-mediated metabomarkers in male and female

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Endogenous metabolic markers of hepatic CYP3A activity will be useful for early prediction for drug interaction of CYP3A substrate drugs. This study aimed to evaluate the endogenous markers for CYP3A activity in healthy male and female subjects. Midazolam CL was highly correlated with the urinary ratios of 6β-OH-cortisol:cortisol, 6β-OH-cortisone:cortisone, 16α-OH-DHEA:DHEA, and 7β-OH-DHEA:DHEA, as well as the plasma level of 4β-hydroxycholesterol. We demonstrated several endogenous metabolites that can be use as a reliable predictive marker of hepatic CYP3A activity in both male and female, which could be useful for assessing the drug interaction potential in the drug development.

P55

Application of metabolomics for quality evaluation of licorice (Glycyrrhiza uralensis Fisher)

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Herbal medicines, including Chinese herbal medicines, contribute complementary therapy and primary health care. However, quality evaluation and control of them are difficult issues. Pharmacological effects of herbal medicines are thought to be expressed by interaction of many components included in extract. So correlation analysis between chemical composition and biological activities would be useful for quality evaluation. In this study, we obtained LC/MS fingerprintings and biological activities of various samples of licorice, *Glycyrrhiza uralensis* Fisher, which is one of the most important herb, and analyzed correlation of these data.

P56

Noninvasive and real-time monitoring of ketamine pharmacokinetics by mouse breath analysis using secondary electrospray ionization mass spectrometry

Xue Li Jinan University; **Pablo Martinez-Lozano Sinues** ETH Zurich; **Maija Hollmen** ETH Zurich; **Robert Dallmann** University of Zurich; **Malcolm Kohler** University Hospital Zurich; **Michael Detmar** ETH Zurich; **Renato Zenobi** ETH Zurich;

Noninvasive and real-time pharmacokinetic monitoring of antidepressant drug ketamine (Ket) has been successfully achieved. A sub-anesthetic dose of Ket was injected to a mouse, whose breath was directly analyzed by secondary electrospray ionization high resolution mass spectrometry (SESI-HRMS). After injection, breath concentrations of Ket and its metabolite norketamine increased in 7 min and peaked in 20 min. This trend agrees with that reported in blood. Another three metabolites of Ket were also observed when a higher dose was applied. These results indicate SESI-HRMS breath analysis of small animal models is promising for noninvasive and real-time pharmacokinetic

monitoring of drugs.

P57

Rapid identification and comparative analysis of the chemical constituents and metabolites of Antrodia cinnamomea fruiting body extracts by UPLC-MS

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Antrodia cinnamomea (AC) is a precious edible fungus endemic to Taiwan that has long been used as a folk remedy for treating various disease. In the present study, an integrative pattern recognition approach including PCA and OPLS-DA analysis based UPLC-MS was successfully applied for the rapid discovery of natural compounds from Antrodia cinnamomea fruiting body crude extracts (ACE). In a 12-min analysis, 32 peaks were identified or tentatively characterized from ACE based on their fragmentation behaviors. A total of 5 metabolites in serum were identified from ACE, Among which, 4 prototypes and 1 metabolite of the compounds existed in ACE.

P58

Screening of kukoamine allies in Lycii Cortex using a multiple ion monitoring-dependent enhanced MS/MS acquisition method on Q-TRAP MS

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Kukoamines (Kuk) are major constitutes responsible for the anti-diabetic function of a Chinese herb, Lycii Cortex (LyC). A sensitive multiple ion monitoring (MIM)-dependent enhanced MS/MS acquisition method (MIM-EPI) based on Q-TRAP was established to qualitatively analyze Kuk allies in the herb. Based on the CID fragmentation behaviors, the typical fragment ions for diagnostic identification were found. The MIM-EPI highly enhanced the metabolite identification capability of the Q-TRAP MS. Fifteen Kuk allies were identified from LyC. Except for Kuk A and B, other species were first reported in the LyC. Our method can also be used for Kuk metabolites discovery in other biological samples.

P59

Serum ^{1}H-NMR Metabolomic Fingerprints of Acute-On-Chronic Liver Failure in Intensive Care Unit Patients with Alcoholic Cirrhosis.

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Acute-on-chronic liver failure is characterized by acute deterioration of liver function in patients with compensated or decompensated, but stable, cirrhosis. Using OPLS-DA, our study assessed the metabolomic profile of serum using 1H-NMR spectroscopy to identify metabolic changes related to acute-on-chronic liver failure. Metabolomic profiling may aid clinical evaluation of patients with cirrhosis admitted into intensive care units with acute-on-chronic liver failure, and provide new insights into the metabolic processes involved in acute impairment of hepatic function.

P241

A liquid chromatography tandem mass spectrometry based metabolic profiling to evaluate the biomarkers of imatinib response in human urine

Yong Chul Shin Kyungpook National University Graduate School; Mi-Ri Gwon Kyungpook National University Graduate School; Ji Yeon

Hyun Kyungpook National University Graduate School; **Eun Young Do** Kyungpook National University Graduate School; **Sung Min Park** Kyungpook National University Graduate School; **Moon-Young JeGal** Clinical Trial Center, Kyungpook National University Hospital; **Hae Won Lee** Clinical Trial Center, Kyungpook National University Hospital; **Young-Ran Yoon** Kyungpook National University Graduate School;

Imatinib is a used in chronic myeloid leukemia. The aim of this study was to apply the metabolomic approach using LC-MS in the urine samples of healthy volunteers for investigating the drug effect on endogenous metabolism after imatinib administration. Pre-dose spot urine and post-dose urine samples following oral administration of imatinib were collected. All the urine samples were analyzed by LC-MS full scan with XCMS software. In the corresponding loadings plot, the metabolites responsible for the separation observed in the scores plot were identified. After identification, these metabolite markers can be used to understand biochemical pathways that were affected by imatinib.

P242

Sensitive determination of fimasartan in human plasma by liquid chromatography-tandem mass spectrometry

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Fimasartan is an angiotensin II receptor blocker with selective AT1-receptor-blocking effect, approved for the treatment of essential hypertension. The aim of the study is to evaluate linearity, accuracy, precision, recovery and stability of the analytical method to determine the fimasartan levels in human plasma by rapid and sensitive ultra-performance liquid chromatography-tandem mass spectrometry. In this assay, protein precipitation procedure with acetonitrile was used. This UPLC-MS/MS method was validated for recovery, and stability that were found to be acceptable for bioanalytical applications, according to the guidelines by the Ministry of Food and Drug Safety, Republic of Korea.

P243

HerbsMed: Herbal medicine apps using integrated Jamu and Kampo formulas

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Nowadays, there is an increasing trend globally in the use of medicinal plants as blended herbal medicines. Unfortunately, the information about herbal medicine is currently still dispersed in an unorganized manner. We collected Indonesian Jamu and Japanese Kampo formulas and also integrated those databases from KNApSAcK Family database system based on their ingredients using extraction, cleaning, transformation, loading, and refreshing processes. HerbsMed Apps was then developed using Waterfall Model in the Android platform. HerbsMed Apps gives useful information about herbal medicines, their ingredients and efficacies for disease treatment and maintaining healthy life.

P244

Isolation, purification and structure elucidation of novel saponins from viscera of sea cucumber Stichopus vastus using HPCPC and mass spectrometry

Yadollah Bahrami Flinders University; **Chris Franco** Flinders University; **Wei Zhang** Flinders University; **Tim Chataway** Flinders university;

This study aims to purify and characterize novel bioactive saponins from the viscera of the Australian sea cucumber Stichopus vastus Sluiter, 1887. The 70% ethanolic extract of the viscera were purified by a liquid-liquid partition process and column chromatography. The saponin-enriched mixture was further purified by high performance centrifugal

partition chromatography (HPCPC) and analyzed via mass spectrometry. Twenty-two novel saponin congeners and sixteen reported ones have been identified with a high structural diversity, including acetylated, sulfated and non-sulfated triterpene glycosides. These novel saponins have a broad range of potential applications in the health industry.

P245

The metabolomics of frog skin secretions

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A pilot study was performed in order to investigate the hypothesis that the metabolome of frog skin secretions may have potential use in drug discovery and environmental management. Six frogs (xenopus) that were selected from a common source were divided into two groups and were exposed to cold and warm temperatures respectively. Frogs were then injected with 0.5 mg epinephrine to induce skin secretions which were collected for analysis. Samples were analysed with a LECO GCxGC-TOFMS. One hundred and twenty compounds were detected including 28 unknowns and a natural separation was achieved using principle component analysis. The preliminary data support the hypothesis.

P246

The metabolomics studies of drug candidate on diabetic-hypertensive rat model

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The metabolomics studies of drug candidate on diabetic-hypertensive rat model
GC-TOF MS was used to study the metabolic profile of a diabetic-hypertensive rat model treated by a drug candidate compared with ALT7-11. The metabolic phenotype showed differences with the control group and diabetic-hypertensive models. After the treatment of ALT-711 and drug candidate C36, both metabolic profile moved in the same trajectory indicating that the C36 played quite similar with ALT-711 in the metabolomics profile in the diabetic-hypertensive rat model.

P247

Metabolomic signatures in peripheral blood associated with alzheimer's amyloid-beta-induced neuroinflammation

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We aimed to explore changes in peripheral blood metabolome in response to amyloid-beta-induced neuroinflammation and to anti-inflammatory treatment using NMR-based metabolic profiling. We constructed Control, alzheimer-disease-like (ADL) and gallate treated ADL model mice, and profiled metabolites of whole blood, plasma, and intact hippocampal tissues. Overall, metabolomic profiles for plasmas well differentiated ADL mice with the other groups, and revealed that levels of several metabolites were significantly altered in plasma of untreated ADL mice relative to Controls. Moreover, notably, gallate treatment normalized the levels of most of affected plasma metabolite.

P248

Metabolomics discloses donor liver biomarkers associated with early allograft dysfunction

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Early allograft dysfunction (EAD) dramatically influences graft and patient outcome after orthotopic liver transplantation and its incidence is strongly determined by donor liver quality. Nevertheless, objective biomarkers, which can assess graft quality and anticipate organ function, are still lacking. A metabolomic biosignature that accurately differentiates donor livers, which later showed EAD or no-EAD, has been deciphered. The remarkable metabolomic differences among between donor livers before transplant can be related to their different quality. The proposed metabolomic approach may become a clinical tool for donor liver quality assessment

P249

Analysis for ingredients of crude drug with LC-(SPE)-NMR/MS system

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Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, which provide complementary information, are the two major methods used to address metabolomics research. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy provide complementary information each other. The LC-NMR/MS system is the hyphenation system that directly couples high performance liquid chromatography (HPLC) with MS and NMR spectrometers. we performed a chemical profiling on the extracts of licorice (Glycyrrhiza uralensis), a plant widely used for crude drug basis, by using the LC-NMR/MS equipped with ESI-QqTOF MS and NMR (600MHz) with the highly sensitive cryogenic probe (cryoprobe).

P250

Identifying species and quantifying components of Vaccinium leaf extracts by NMR

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The genus Vaccinium L. includes many familiar plants such as blueberry, bilberry, huckleberry, and cranberry. These plants have been used traditionally by indigenous cultures not only as food but also as treatment for a variety of diseases. Once the leaf is harvested from the plant and extracted, it becomes difficult to identify. We are developing statistical models based on NMR to identify V. leaf extracts. Principal component analysis separates the species angustifolium, macrocarpon, and ovalifolium. Components such as chlorogenic acid can be quantified in the extracts. With models for the different species, NMR will be a powerful tool to characterize extracts for clinical trials.

P251

Metabolomics and dereplication studies of endophytic metabolites from some Egyptian medicinal plants in the search for new potential anti-cancer drugs

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This study involved bioassay guided chromatographic isolation of three new natural compounds with 40 known bioactive metabolites from three endophytic fungi from different Egyptian medicinal plants. Identification of the strain has been achieved through molecular biological methods. Metabolomic profiling, using 2D-NMR and HR-ESIFTMS were done at different stages of the growth phase for both solid and liquid culture media. Dereplication studies were accomplished by utilizing the Mzmine software with the AntiBase database. The chemometric algorithms using SIMCA-P+ software were used to compare the active and inactive extracts and/or fractions in order to early predict the bioactive metabolites

P252

Oxylipin metabolome and signalling in endotoxin-induced sepsis in mice and the underlying mechanism of plant galactolipid and simvastatin treatment

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Oxylipins, a type of lipid mediators, play crucial roles in inflammatory responses and cascades in normal or pathological conditions. Comparative metabolomics approach was employed in this study to analyze systemic and organ-specific oxylipin mediator dynamics in LPS-induced sepsis in mice, with or without plant galactolipid or simvastatin treatment. A MAPK pathway-mediated oxylipins synthesis/activation was observed to be the drug target to reverse LPS-induced organ damages, inflammatory cytokines levels, and skew oxylipins profile toward homeostasis. This

study may offer new preventive and therapeutic strategy in attenuating acute or chronic inflammatory disorders, such as sepsis.

P253

Characterization of neural differention in mouse embryonic stem cells

Amy Su Georgia Institute of Technology; Mark Styczynski Georgia Institute of Technology;

The pluripotency of embryonic stem cells offers myriad applications in regenerative medicine, which has fueled extensive research in unraveling the effectors and markers of stem cell differentiation. However, the systems-level dynamics of metabolic remodeling during differentiation is thus far largely unexplored. We have characterized the metabolic profiles of mouse embryonic stem cells differentiating down a neural lineage, noting distinct separation between the daily time points which is largely driven by amino acid metabolism. We also describe the metabolic effects of different culture formats, which has implications for scale-up in biomanufacturing.

P255

Defining the training effect of prolonged exercise in combination with recombinant human erythropoietin (r-HuEPO) treatment in healthy trained males.

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The aim is to identify the metabolic variability and response of prolonged training as well as its combined effect with r-HuEPO treatment (50 IU.kg^{-1} body mass every two days for 4wk) via an untargeted LC-MS method in 18 healthy males. Blood and urine LC-MS data were processed via mzMatch and subjected to multivariate analysis. Raw data highlights variability of free carnitine and acetyl carnitine (normalizing factor), that signals a change in the pathway of energy/lipid metabolism. PCA/OPLSDA analysis of individual responses of normalized plasma data reveals a clear differentiation between phases compared to raw data. RBC S-plot analysis reveals a possible dietary effect on metabolome.

P256

The effect of haemolysis on umbilical cord blood metabolites

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There is increasing interest in the effect of early life status on life long health. Metabolomics in umbilical cord blood (UCB) may offer insight into many perinatal disease processes. Due to the relatively high haematocrit, haemolysis is a common technical problem affecting UCB sampling. We have examined both haemolysed and non-haemolysed UCB serum samples from 69 healthy term infants using H-NMR and DI-MS. We have identified 42 metabolites that are significantly and consistently altered in haemolysed UCB samples. This information will be useful for researchers in the field of neonatal metabolomics to avoid false positive findings in the face of haemolysis.

P257

Pharmacokinetics of Chlorpromazine and its metabolomic analysis using imaging mass microscope, iMScope

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Imaging Mass Microscope “iMScope”, visualizing the distribution of bio molecules in organ, is the new instrument for MALDI imaging MS (IMS) with high special resolution than ever. We performed imaging mass analysis using iMScope to mice liver tissues from the Chlorpromazine dosed mouse and untreated one. iMScope and its software succeeded to determine the drug and its metabolites at the same time. We confirmed not only well known biomarkers of hepatotoxicity, but also candidate Lipid biomarker molecules, which is the metabolites moved inside the body by drug medication.

P258

The application of global metabolomics to predict busulfan pharmacokinetics in pediatric cancer patients

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Busulfan, antineoplastic agent, has a narrow therapeutic range and considerable interpatient pharmacokinetic variability. In this study, we identified markers to predict busulfan pharmacokinetics using UPLC-TOF-MS. The 6-hours interval urine samples were collected before busulfan administration in 59 pediatric patients undergoing hematopoietic stem cell transplantation (HSCT). The urinary metabolites associated with high level of busulfan were identified to deferoxamine metabolites. Deferoxamine, iron chelating agent, is used to the iron overloaded patients before HSCT for preventing organ damage. From this result, hepatic damage from high iron level may cause busulfan hypometabolism.

P259

Metabolic fingerprinting of plasma samples in study of treatment-resistant hypertension

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It is accounted that about 15-20% of hypertensive patients are resistant to pharmacotherapy. Plasma samples (n=180) derived from RH patients and nonresistant ones were determined by LC/TOF/MS and GC/QqQ/MS. The obtained data sets were analyzed using data pretreatment procedures and univariate (U-Mann-Whitney), multivariate (PCA, PLS-DA) statistical analyzes. Such approach allowed for selection of the most relevant metabolites (n=72) which levels varied between two groups. The list of metabolites putatively identified included e.g.: prostaglandin, oxo-proline, HETE and Tri-HOME. These metabolites are known to be involved in endothelium dysfunction, oxidative stress and inflammation processes.

Environment

P61

Smoking of mothers causes mirrored changes in the endogenous metabolome of mothers at 34th of gestation and in unborn children

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To this day, the formation of detrimental effects of smoking during pregnancy remain unclear. During a pilot study, 40 mother-child pairs with different smoking habits were investigated. Information from a questionnaire was confirmed by measuring the smoking related exogenous metabolites in maternal urine. Subsidiary the anti-oxidative capacity was analyzed in the serum of smoking mothers. Additionalyl the impact of smoking was determined by assess-

ing 163 endogenous metabolites from different substance classes in serum of the mothers and from cord blood by children. The data indicates that smoking has an influence on fetal metabolism which could be responsible for later occurring diseases

P62

Metabolomic profiling of mice serum associated with trans-trans 2, 4-decadienal, a component of cooking oil fumes induced lung lesions by LC-MS

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Metabolomics has become an important tool in clinical research and the diagnosis of human disease. Intratracheal instillation of trans-trans 2,4-decadienal (tt-DDE), a major component in cooking oil fumes, has been demonstrated to cause lung lesions in mice at 8 weeks after treatment. This study was to identify any changes in metabolite profiles associated with the development of tt-DDE-induced lung lesions. Using a metabolomics strategy involving LC-MS and principal component analysis, we have demonstrated that ten amino acids were significantly reduced in serum. Our results suggest that amino acid profiles may be useful as an early indicator of the presence of tt-DDE-induced lung lesions

P64

Metabolomics approach to understand the resource partitioning in Chlorella during growth for enhanced biofuel production

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Cellular metabolism of organisms is tightly regulated in response to environmental pressures. Algal growth and lipid production are interlinked, and play a critical role during biofuel production processes. Here, we use untargeted high-resolution mass spectrometry to understand the metabolic differences at exponential and stationary growth stages using 22 Chlorella strains collected from S.E. Asia. This analysis revealed strain specific signatures of metabolic reprogramming strategies, and associations between physiochemical measures and metabolic pathways. These findings will aid the development of efficient bioprocess strategies to improve algal bio-products.

P65

MS-based lipidomics to explore the naphthalene toxicity in a tolerant mouse model

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Naphthalene causes respiratory toxicity in mice. Repeated exposure of naphthalene leads to less cell injury. Tolerant mice are adequate to square up the reduction in susceptibility. We profiled phosphorylcholine-containing lipids including Phosphatidylcholine and Sphingomyelin in injuried, tolerant, and the control mice by using UPLC-MS/MS. PLS-DA model from the analysis of these lipids showed the changes of plasmalogen lipids are related with naphthalene-induced injury.

P66

Lipidomics to study the biological effects of radiation exposure on two human lymphoblast cell lines

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Environmental Health; **Mong-Hsun Tsai** Institute of Biotechnology; **Ching-yu Lin** Institute of Environmental Health;
Irradiation might cause DNA damage, cancer and other serious problems. Lipids acted as the major targets when exposed to irradiation. This study intended to investigate the lipid effects when human lymphoblast cells exposed to different doses of gamma radiation. We used a validated UPLC-MS/MS method to analyze glycerophosphocholines and sphingomyelins. Results shows that the lipid profiling between cell lines and doses can be separated by PCA or PLS-DA model. Glycerophosphocholines and sphingomyelins may serve as potential markers for irradiation-induce toxicity.

P67

NMR-based Metabolomics to Determine the Acute Inhalation Effect of Nano- and Fine-Sized ZnO Particles in Rat Lung

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Although zinc oxide (ZnO) particles are extensively used, their toxic mechanism remains unclear. We intend to evaluate the acute inhalation effect of ZnO particles by NMR-based metabolomics. The metabolic signatures in the lung of rats exposed to series doses of 35 nm or 250 nm of ZnO particles via inhalation were analyzed by 1H and J-resolved NMR followed by multivariate analysis. PLS-DA model from the analysis of hydrophilic lung extracts showed a dose response trends in 250 nm ZnO particle group. Metabolic alterations are related with energy metabolism, membrane stability and antioxidant mechanism which may partially explain ZnO particles-induced lung inflammation.

P68

Intra-colonial distinction of cellular membrane accommodation to copper-induced oxidative stress in a pocilloporid coral Seriatopora caliendrum

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Functional separation/aging of polyp in the scleractinian corals has been related to the difference in resistance to environmental stress intra-colony. To gain insight into the molecular account, intra-colonial distinction of glycerophosphocholine profiling responded to copper-induced oxidative stress in a pocilloporid coral, Seriatopora caliendrum, was examined in this study. The results indicate that the lipid metabolism is programmed to accommodate cellular membrane to the oxidative conditions. Compared with old polyp, furthermore, young polyp has shown a dramatic turning in lipid metabolism to inform some particular faculties of the cell for conducting copper-induced oxidative stress.

P69

Long-term exposure to bisphenol-A affacts steroid hormone profile in mother and baby pairs based on envorinmental metabolomics

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People are exposed to bisphenol-A (BPA) as an endocrine disrupting chemicals from foods or drinks packaged or prepared in containers. The present study was designed to analyze urinary BPA in mother and baby pairs in Korea. And we performed a pattern analysis and investigated a steroids profile in urine. 4-Hydroxyestradiol was decreased in low concentration BPA mother group as negative effects of long-term exposure to BPA. And cortisol and cortisone were increased in low concentration BPA baby group. The alterations in circulating steroids levels usually lead to compensatory adaptation of the production rate and the rate of degradation and excretion.

P70

Identification of IDH3 as a novel target of tributyltin cytotoxicity by a metabolomic approach

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Organotin compounds, such as tributyltin (TBT), are known to cause various toxicities as endocrine disruptive chem-

icals (EDCs). We have previously reported that TBT at nM level inhibited growth in human embryonic carcinoma cells. To examine a target of TBT cytotoxicity, we performed comprehensive metabolomic analyses. We found that TBT reduced α-ketoglutarate (α-KG), succinate and malate. TBT inhibited isocitrate dehydrogenase (IDH3), which catalyzes the conversion of isocitrate to α-KG in TCA cycle. In addition, shRNA against IDH3 inhibited cell growth. These data suggest that IDH3 is a novel target of TBT. Thus, metabolomic approach may provide new insights into EDC action.

P260

Effects of environmental stress on sturgeon by NMR-based metabolomics

Lu-Hsueh Huang National Taiwan University; Ching-Yu Lin National Taiwan University; Silas S O Hung University of California;

Starvation is one of common environment stresses for environment organisms. It happens in nature because human activities or natural events. The purpose of this study is using NMR-based metabolomics to study effects of starvation in different tissues and life stage of green sturgeon. Green sturgeon fingerling and juvenile were fed four food restrictions (100%, 50%, 25%, 12.5%) for 2 and 4 weeks, respectively. Both hydrophilic and hydrophobic metabolites from the muscle, liver, and kidney were extracted and analyzed by NMR following multivariate statistical analysis. Results show numerous energy metabolites were altered after starvation. The metabolic effects are tissue-specific.

P261

Metabolomics on Honeybee hemolymph - first insight on multicausal colony losses

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The honeybee (Apis mellifera) colony losses recorded during the last decades represent a major issue and are thought to be multicausal. The microsporidian Nosema ceranae is the etiologic agent of the Nosemosis disease in A. mellifera. Such disturbances may have major consequences on bee metabolic pathways. For that purpose, we decided to perform metabolomic studies using the hemolymph. At first, emerging bees were infected by individual feeding. Then, hemolymph was collected at 2 and 10 days post-infection and analysed trough LC/MS-NMR. After data treatments, putative metabolites were highlighted as potential stress biomarkers. Validation was conducted using 2D-NMR and orbitrap HRMS.

P262

Metabolomic investigation of effect of Xenobiotics on human and model organisms

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Xenobiotics, especially pesticides and nanoparticles are encountered by humans on a daily basis due to their vast production, application in agriculture, industries. These chemicals undergo metabolism and have the potential to cause unintended toxic effects. Metabolomics approach was utilized to reveal the biological relevant perturbations that result from the nanoparticles, pesticides exposure on human population and various different model organisms like C. elegans, earthworms and mice. The study describes the application of metabolomics to reveal the impact of genetic, environmental variation has on the metabolome, and how these variables may interact with Xenobiotic metabolism.

P263

Metabolomics in the assessment of exposure and health outcomes

Susan Jenkins Sumner RTI International; Suraj Dhungana RTI International; Susan McRitchie RTI International; Wimal Pathmasiri RTI International; Darya Cheng RTI International; Aastha Ghimire RTI International; Rondey Snyder RTI International; James Carlson RTI International; Timothy Fennell RTI International;

Results from two investigations will be presented. We used LC-MS to profile the low molecular weight complement

of rice types purchased from general or wholefoods stores. Major differences in metabolite profiles were observed for brown rice purchased from the two vendors, but less differentiation was seen for jasmine rice. We used NMR, GC-TOF-MS, and UPLC-QTOF-MS to determine the impact of exposure to the anti-tubercular drug, isoniazid, on the urinary and serum profiles of exposed rats. Metabolites were identified that correlate with the presence of microvesicular lipid accumulation, and could serve as biomarkers for early detection of this reversible histopathology. NIH Grant 1U24DK097193.

P264

Role of co-factors in alleviating metabolic bottlenecks for mixotrophic production of algal biofuels

Brendan Thomas Higgins UC Davis; Jean VanderGheynst UC Davis; Tobias Kind UC Davis; Oliver Fiehn UC Davis;

Algal biofuels have the potential to displace petroleum but high lipid productivity is needed for cost-effective fuels. Mixotrophic algae growth can increase productivity but metabolomics analysis revealed bottlenecks (e.g. PDH complex) that contribute to inefficient substrate use. Exogenous thiamine was found to alleviate the PDH bottleneck and increase algae production 60x vs. autotrophic cultures. Co-culture of algae with E. coli resulted in enhanced algae growth and we hypothesize that E. coli secrete relevant co-factors. Our results show how metabolomics can identify bottlenecks and suggest industrially relevant strategies such as bacterial co-factor provision, to increase productivity.

P265

Metabolomic response of Spot Prawn (Pandalus platyceros Brandt, 1851) following exposure to the Aquaculture Chemotherapeutant Emamectin Benzoate

Jonathan P Benskin AXYS; Michael G. Ikonomou Institute of Ocean Sciences - Fisheries and Oceans Canada; Cory Dubetz Institute of Ocean Sciences - Fisheries and Oceans Canada; John R. Cosgrove AXYS;

Use of the commercial chemotherapeutant formulation SLICE (active ingredient emamectin benzoate; EB) to control parasitic sea lice in fin fish aquaculture is of concern due to its potential effects on non-target organisms. In the present work, a quantitative metabolomics platform was used to probe biological effects of EB in a non-target crustacean, the spot prawn. Exposures were carried out for up to 96 hrs at 0.1 - 1.2 mg EB/kg sediment (5 tanks/dose, 10 prawns/tank). Metabolites (>200) quantified in muscle tissue included amino acids, biogenic amines, sugars, fatty acids, phospholipids, and carnitines. The results shed new light on the mechanism of action of EB on non-target organisms.

P266

Barcoding contaminant responses in Zebrafish Embryos using a targeted metabolomics approach

John R. Cosgrove AXYS; Jonathan P. Benskin AXYS;

The application of metabolomics to effects-directed analyses involves linking perturbations in an organism's metabolome to contaminant exposure. In the present work, a targeted quantitative metabolomics platform was applied to zebrafish embryos exposed for 24 hrs to 4 stressors: bisphenol A (BPA), ethinyl estradiol (EE2), perfluorooctane sulfonate (PFOS) and triiodothyronine (T3), over a range of concentrations and developmental stages. These data form the basis of a library linking contaminant exposures to metabolomic responses in zebrafish with applications to chemical toxicity testing, mode-of-action studies, and effects-directed analysis in complex environmental samples.

P267

Serum metabolomics of lobomycosis infection in bottlenose dolphins indicates a catabolic state in infected animals

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Dolphins are apex predators in the marine food chain and thus potential sentinels of environmental health. Metabolomics presents a potentially high-impact way to monitor dolphin population health, but to date there has been fairly little use of this approach. Here we present a study of bottlenose dolphins from the Indian River Lagoon in Florida, some of which have the fungal infection lobomycosis. Serum metabolomics clearly distinguishes between diseased and asymptomatic dolphins. More than a dozen metabolites exhibit statistically significant differences between the

two groups of animals; taken together, these analytes suggest a previously unknown catabolic state in infected animals.

P268

Understanding cellular uptake of ceria nanoparticles into human lung-derived cells using microscopy and mass spectrometry-based metabolomics

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Ceria nanoparticles are increasingly being used to increase fuel efficiency in internal combustion engines. The resulting environmental exposure has raised concerns about the potential impact of ceria nanoparticles on human health via inhalation. We used microscopy and untargeted mass spectrometry to study the uptake and metabolic effects of ceria in lung-derived A549 cells. Protein corona formation around ceria, in a serum containing environment, was found to promote cellular internalisation. However, no effect on cell growth, viability and metabolic profile was observed after ceria internalisation, even at the relatively high concentration of 500 ug/ml.

P269

Investigations on the upper thermal acclimation response in the cyprinid Puntius sophore under multi-omics platform

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High temperature stress is a challenging environmental problem of this century and understanding mechanism of thermal acclimation could help in developing mitigation plans. Fishes inhabiting hot-spring runoffs could provide insights on such aspect. We investigated changes in Hsp transcriptome, whole body fatty acid lipidome and amino acid metabolome in Puntius sophore, a cyprinid like the zebrafish Danio rerio, collected from hot spring runoff (36-38 degreeC) to investigate the upper thermal acclimation response. The interesting biochemical changes observed could be part of the metabolomic response under adaptation pressure for survival under perennial thermal stress and will be discussed.

P270

A GC-MS based environmental metabolomics approach to detect oil hydrocarbon stress response of marine polychaetes

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We investigated the potential of metabolic fingerprints of marine polychaetes as pollution indicators linking the biological response to environmental stressors and assess organism function and health at a molecular level. The experimental set-up comprises: a) crude oil exposure of polychaetes in microcosms; b) metabolite extraction, c) GC-MS analysis; d) data pre-processing by XCMS, e) variable selection and f) multivariate statistics analysis. To improve the selectivity of the potential oil pollution indicator(s), it was required that the indicator metabolite pattern not be affected by other environmental stressors, such as salinity.

Microbiology

P71

Genetic and metabolomic dissection of the ergothioneine biosynthetic pathway in the fission yeast, S. pombe

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Ergothioneine is a thiol metabolite of mostly bacterial origin that accumulates in human body through diet. Despite showing antioxidative properties, its function *in vivo* is unclear. The fission yeast *S. pombe* is among only few eukaryotes that synthesize this compound. We characterized the genes forming the biosynthetic pathway in *S. pombe* and applied metabolomic analysis to measure the levels of ergothioneine and its intermediates in gene deletion, as well as over-expression, strains. Ergothioneine was nonessential for proliferation, but curiously accumulated in quiescent cells. In addition, we found *S. pombe* could produce selenoneine, a selenium-containing derivative of ergothioneine.

P72

Effect of arginine metabolic pathways on periodontopathic biofilms formation

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Periodontal disease is an infectious disease caused by a complex microbiome including *Porphyromonas gingivalis* (Pg) and *Filifactor alocis* (Fa). Pg posseses 2 distinct arginine (Arg) metabolic pathways to citrulline (Cit) and agmatine (Agm), respectively. CE-TOFMS metabolome analysis revealed that Arg and Agm production was increased but Cit when Pg was cocultured with Fa, with enhanced biofilm formation. In addition, Arg deiminase converting Arg to Cit was suppressed. While these effects were not observed by Pg coculture with *Streptococcus gordonii*, oral normal inhabitant. These results suggest that the Arg metabolic pathways play an important role in periodontopathic biofilm formation.

P73

Metabolomics perspective of acid stress response within Saccharomyces cerevisiae

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Acid stress often inhibits cell growth and productivity during bio-productions. In this study, metabolomics approach was conducted to understand the response of the acid stress by lactic acid within Saccharomyces cerevisiae. Cells were cultured with 0, 10, 14 g/L of lactic acid addition at initial pH of 6 and no pH control (around pH 2.4 in 10 or 14 g/L lactic acid-added conditions). Lactic acid addition decreased the cell growth under uncontrolled pH conditions. Metabolome analyses using capillary electrophoresis mass spectrometry identified increased concentrations of proline, ATP, glutathione and glutathione disulfide during the acid stress of 14 g/L lactic acid at no pH control.

P74

Metabolic and molecular dissection of bulk RNA degradation via autophagy in yeast

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Autophagy is a catabolic process conserved among eukaryotes. Upon induction, a portion of the cytoplasm is enclosed into autophagosomes, along which large amounts of ribosomes are delivered to the vacuole/lysosome for destruction. However, the precise mechanism of RNA degradation and its physiological implications remain unknown. We characterized autophagy-dependent RNA catabolism by a combination of metabolome and molecular biological analyses in yeast. RNA was first cleaved by Rny1, generating 3'-NMPs that were converted to nucleosides by Pho8. Being transported to the cytoplasm, the nucleosides were further broken down into bases by Pnp1/Urh1, most of which were excreted from the cell.

P75

Investigation of component changes in soy sauce resulted from microbial activities during fermentation using metabolome analysis

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Soy sauce is one of the most popular Japanese traditional seasoning that are produces by microbial fermentation. Compounds forming taste, aroma and color in soy sauce are so various and many that comprehensive investigations were difficult. In this study, we employed metabolome analysis in order to investigate low molecular weight hydrophilic and volatile compounds changes using GC/MS analysis during soy sauce fermentation. We also tried fermentation with or without specific microorganisms to clarify the relation of each components and microbes. In the results, we found components which characteristically change in each fermentation phase such as saccharides and amino acids.

P76

Metabolic characterization of three cyanobacterial strains by molar-based metabolic profiling

Yudai Dempo Osaka University; **Erika Ohta** Osaka University; **Yasumune Nakayama** Osaka University; **Takeshi Bamba** Osaka University; **Eiichiro Fukusaki** Osaka University;

Cyanobacteria have become the attractive candidates for bioproduction due to their inherent properties. For bioproduction, to understand and control their metabolism using metabolic profiling techniques are desirable. Here, absolute concentrations of 84 metabolites were successfully determined by QqQ-based target metabolic profiling in the three cyanobacteria which are commonly used for metabolic engineering. By comparing the differences in metabolic potentials, we found a relationship between intracellular energy state and growth in cyanobacteria. Lastly, we summarized the metabolic pathways in previous bioproductive studies and suggested the possibility for productive improvement.

P77

Adaptive mutations improve carbon flow and metabolic efficiency for fast growth of Escherichia coli on glycerol

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Comparative genome sequencing previously identified mutations in glpK and rpoC genes in an E. coli strain adapted to glycerol. However, the underlying growth-promoting mechanisms are not well characterized. Here, we used a multi-omics approach to show that an evolved strain uses two different metabolic strategies for faster growth. The rpoC mutation promotes efficient conversion of glycerol to biomass whereas the glpK gene mutation increases glycerol uptake but leads to unexpected carbon wasting. These contrasting mechanisms together confer a 89% growth rate increase in a strain carrying both mutations. These results provide clues about metabolic reprogramming during adaptive evolution.

P78

Profiling of stress-induced polar lipids in *Euglena gracilis*

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Identification of polar lipids by LC-MS/MS is hampered by the limited availability of their reference library. For the lipid annotation in *Euglena gracilis* we exploited the LipidBlast library, collection of 212,516 theoretical spectra for 26 lipid classes (Kind 2013). In *E. gracilis*, synthesis of wax ester under anoxia has been well documented, and we report generation of lysophosphatidylethanolamines and short-chain fatty acids within 24 hours of anoxia. The numbers of other polar species were unchanged. This indicates a significant remodeling of particular membrane lipids under anoxia, whose biological interpretation is under investigation.

P79

Effect of quenching method on the metabolic fingerprint of lactobacillus paracasei

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Three methods were evaluated for quenching of *L. paracasei*: fast filtration; buffered methanol (0.85 % (w/v) ammonium bicarbonate); and glycerol saline solution (0.9 % (w/v) sodium chloride). Fast filtration resulted in sampling times of minutes which is far from suitable for microbial metabolomics. Assessment of cell membrane integrity by propidium iodide assay demonstrated cell membrane damaged during methanol quenching. The loss of membrane integrity resulted in a decrease in metabolite levels measured by GC-MS metabolic fingerprinting. Cold glycerol quenching showed limited cell membrane damage and better recovery of intracellular metabolites as indicated by GC-MS fingerprint.

P272

A metabolomic evaluation of the effects of raffinose on murine gut microbial ecology

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Raffinose is a non-digestive prebiotic oligosaccharide that has been reported to significantly increase the number of beneficial gut microbiota. The purpose of this study is to evaluate the effects of raffinose dietary supplementation on murine gut microbial ecology using capillary electrophoresis-mass spectrometry-based metabolome analysis. We analyzed fecal metabolites of male 8-weeks-old BALB/c mice that were fed a diet containing 5% raffinose for a week. Murine fecal metabolites were dramatically changed during after intake of raffinose supplemented diet, indicating that murine gut microbiota plays a role in raffinose degradation and fermentation.

P273

Comparative metabolomic profiling of megasphaera elsdenii SU1 for hexanoic acid production under different temperature conditions

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Hexanoic acid, used as a platform chemical, can be produced by Megasphaera elsdenii SU1 from sucrose. In corn steep powder (CSP) medium, the composition of metabolites of M. elsdenii SU1 was different with that in peptone-yeast extract (PY) medium. In both media, total amounts of produced carboxylic acids were similar, but only in CSP medium, pentanoic acid was produced up to about 3 g/L. When cultivation temperature was changed, the produced amounts of carboxylic acids were also changed. To understand the metabolic response of microbe to the conditions of culture broth and temperature, the comparative metabolomics was performed. Metabolites were analyzed with GC TOF-MS and LC MS-MS.

P274

Study of the plasticity of secondary metabolites in the black Aspergili using UHPLC-qTOF molecular networking

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Among the filamentous fungi, the black *Aspergili* are regarded as some of the most diverse with respect to habitat as well as their secondary metabolites production. The *Aspergili* are some of the best studied fungi, making dereplication, the tentative identification of the metabolites, an essential first step to determine whether a sample contains metabolites of interest.

In this study extracts from around 100 different strains have been analyzed using a UHPLC-DAD-qTOF with auto MS/MS. Using accurate mass, an in-house MS/MS library for absolute identification, and molecular networking, we have been able to reveal the distribution of both known and unknown metabolites.

P275

Glucose metabolism in Bacillus subtilis coordinates with neotrehalosadiamine production

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Neotrehalosadiamine (NTD) is a secondary metabolite produced by *B. subtilis*. We previously reported the transcription of NTD biosynthesis operon is repressed in response to GlcP-mediated glucose transport. We also found that the GlcP disruption can restore the growth of a mutant lacking glucose-6-phosphate dehydrogenase by activating NTD synthesis. In this study, we performed genetic and metabolic analyses to investigate the coordination of NTD biosynthesis pathway with glucose transporter GlcP and glucose-6-phosphate dehydrogenase. We concluded that activation of the NTD biosynthesis resulted in the alternative production of NADPH through malate-to-pyruvate conversion.

P276

Complete genome sequence of Lactobacillus hokkaidonensis LOOC260 and its prospective usage as a starter culture for silage fermentation

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Lactobacillus hokkaidonensis, isolated from timothy grass silage in a subarctic region of Japan, shows remarkable psychrotolerance growing at as low as 4 degree C. Its whole genome sequencing by a PacBio sequencer, followed by the hierarchical genome-assembly process, produced 7 contigs, which were further error-corrected and finished as one circular chromosome and two plasmids. The standard genome features are not much different from closely related species in the *L. reuteri group*. Its lipid profile such as dominance of lactobacillic acid is also nonremarkable. The molecular reason for psychrotolerance and the prospect for an effective fermentation starter in cold regions are investigated.

P277

The Apicomplexan parasite Toxoplasma gondii is dependent on two fructose-1,6-bisphosphatases for replication and virulence during glucose metabolism

Martin Blume The University of Melbourne; **Richard Nitzsche** Humboldt University Berlin; **Ulrich Sternberg** Humboldt University Berlin; **Mordechay Gerlic** Walter and Eliza Hall Institute of Medical Research; **Seth Masters** Walter and Eliza Hall Institute of Medical Research; **Nishith Gupta** Humboldt University Berlin; **Malcolm McConville** The University of Melbourne;

Toxoplasma gondii is an obligate intracellular parasite that replicates in virtually all nucleated cells of warm-blooded animals. While intracellular stages utilize both glucose and glutamine as major carbon sources, they appear to derive most of their energy from glycolysis and oxidative phosphorylation of glucose-derived pyruvate. Paradoxically, we now report that *T. gondii* tachyzoites constitutively express two gluconeogenic fructose-1,6-bisphosphatases (TgFBP1 and 2), and that the enzyme activity of TgFBP2 is critical for replication and virulence. Extensive genetic and metabolomic analyses suggest that both TgFBPs regulate glucose metabolism and integrate it with cell-cycle progression.

P278

Evaluation of the gut ecosystem in mice fed with a western diet using metabolome and microbiome analyses

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Gut ecosystem is comprised of host intestinal cells, gut microbiota and microbiota-produced metabolites, which are modulated by host dietary habit. To uncover the effect of dietary habit especially western diet (WD) on the gut eco-

system, we performed fecal microbiome and metabolome analyses using a next generation sequencer and CE-TOF/MS, respectively. Our omics approaches showed that the fecal vitamin B6 concentration and the ratio of Bacteroides were decreased, whereas the butyrate concentration and the ratio of Prevotella and Lachnospiraceae were increased in WD group as compared to normal diet group, suggesting that WD habit largely modulates gut ecosystem via host-microbial crosstalk.

Model Organisms

P279

LC-MS based metabolomic platform for oxidative stress study

Cheng-Yu Huang Chang Gung University; Mei-Ling Cheng Chang Gung University; Jui-Fen Lin Chang Gung University;

Oxidative stress (OS) occurs as consequence of imbalance between oxidant generation and antioxidants. It is conceivable that OS affect cellular metabolism and physiological processes. Nonetheless, the relationship between redox homeostasis, metabolism and physiological changes is complicated, and remains incompletely understood. We have established a simple and effective LC-TOFMS platform to validate the relevance of OS and global metabolites. Five TOFMS data sets, after transomics software analysis, were integrated to generate oxidant-induced metabolic networks and to point out the pathways disturbances.

Crops

P101

Pseudomonas QuoA expression in Arabidopsis increases phenolamides channelling into lignin pathway

Amit Rai National University of Singapore; Sheela Reuben National University of Singapore; Sanjay Swarup National University of Singapore, Singapore Center for Environmental Life Sciences Engineering, Nanyang Technological University;

We studied phenylpropanoid network responses to perturbations caused by gain- and loss-of-function approaches affecting naringenin accumulation. Targeted metabolomics and gene expression studies using transgenic lines expressing a Pseudomonas quercetin oxidoreductase (QuoA) and flavonoid tt6 mutant revealed (i) increased phenolamides channelling into lignin pathways, which resulted in increased lignification and stem stiffness and (ii) coordinated metabolite-to-gene expression at four key branch points in the phenylpropanoid network. This biotechnology approach can provide duel benefit of imparting stem strength through lignification and stress tolerance by up regulation of flavonoids

P102

Metabolite profiling of shoot extracts, root extracts, and root exudates of rice under phosphorus deficiency

Keitaro Tawaraya Yamagata University; Ryota Horie Yamagata University; Weiguo Cheng Yamagata University; Tadao Wagatsuma Yamagata University; Kazuki Saito RIKEN Center for Sustainable Resource Science; Akira Oikawa RIKEN Center for Sustainable Resource Science;

Composition of root exudates is affected by plant nutrient status. *Oryza sativa* was grown in culture solution containing 0 (P0) and 8 (P8) mg P L^{-1}. Shoot and root extracts and root exudates were obtained from 5, 10 and 15 DAS and their metabolites were determined by CE-MS. One hundred and eighty-eight, 191, and 127 metabolites were identified in shoot extracts, root extracts, and root exudates, respectively. Sixty-one to 71 % of the metabolites were exuded to rhizosphere. Four to 17 % of metabolites in the root exudates showed higher concentration at P0 than at P8. These results suggest that rice actively release metabolites in response to P deficiency.

P104

Carotenoids and free amino acids in rapeseed (Brassica napus L.) flowers

Si-Chang Yang Chungnam National University; **Young-Seok Jang** Rural Development Administration; **Ki-Taek Lee** Chungnam National University; **Soon-Taek Hong** Chungnam National University; **Sun-Ju Kim** Chungnam National University;

Carotenoids and free amino acids were investigated in the flowers of rapeseed (Brassica napus L.) to increase value of local food products. Total carotenoids were the highest level in Naehan (64.46), whereas Hanla (12.32 mg/kg DW) was the lowest level. Lutein (ranging from 11.16 to 54.94 mg/kg DW) as the most abundant carotenoids was occupied by mean 87% of total carotenoid contents. The order of lutein levels were Naehan (58.24) > Mokpo 68 (54.94) > Tammi (32.46) > Youngsan (28.11) > Mokpo 111 (27.87) > Tamla (15.80) > Hanla (11.16). Total free amino acid content was the highest level in Naehan (ranging from 113.76 to 654.06 mg/100g FW).

P105

NMR-based metabolic profiling for the identification of potential metabolite markers to characterize apples from different origins

Satoru Tomita NARO National Food Research Institute; **Tadashi Nemoto** National Institute of Advanced Industrial Science and Technology; **Toshihiko Shoji** NARO Institute of Fruit Tree Science; **Fukuyo Tanaka** NARO Agricultural Research Center; **Hiroshi Ono** NARO National Food Research Institute; **Mayumi Ohnishi-Kameyama** NARO National Food Research Institute; **Jun Kikuchi** RIKEN Center for Sustainable Resource Science; **Yasuyo Sekiyama** NARO National Food Research Institute;

^{1}H NMR-based metabolic profiling was used to characterize apples from five cultivars produced in Japan and New Zealand. PCA showed a clear separation between Japanese (Fuji, Orin, Jonagold) and New Zealand cultivars (Jazz, Envy). The differences among the cultivars were primarily in sucrose, glucose, and fructose contents. Stepwise metabolite annotation and variable selection allowed the separation of the different geographic origins in Fuji by the levels of citramalate, quinate, and an unidentified metabolite. The structure of the unidentified metabolite was determined to be an alditol derivative by 2D NMR and MS analyses, followed by partial purification with a charcoal column.

P106

Metabolites profiling of rice leaf, brown rice, polished rice and rice bran using liquid chromatography quadrupole time-of-flight mass spectrometry

Zhigang Yang RIKEN Center for Sustainable Resource Science; **Ryo Nakabayashi** RIKEN Center for Sustainable Resource Science; **Yozo Okazaki** RIKEN Center for Sustainable Resource Science; **Tetsuya Mori** RIKEN Center for Sustainable Resource Science; **Kazuki Saito** RIKEN Center for Sustainable Resource Science; Graduate School of Pharmaceutical Sciences, Chiba University;

Oryza sativa (rice) is one of the most important staple crops in the world. We have enriched reference MS/MS spectra for specialized metabolites of rice leaf. Moreover, metabolite profiling of rice leaf, brown rice, polished rice and rice bran have been carried out using liquid chromatography quadrupole time-of-flight mass spectrometry. Principal component analysis score plot derived from MS data showed distinct three groups (rice leaf; rice bran; brown and polished rice). Tricin, tricin 7-*O*-rutinoside and tricin 7-*O*-neohesperidoside mainly presented in leaf and bran, not presented in polished rice. Further detail results will be presented on the conference.

P107

Prediction of the coffee blend mixing ratio by GC-MS-based metabolomics and its application to the authentication protocol for Kopi Luwak

Udi Jumhawan Department of Biotechnology, Graduate School of Engineering, Osaka University; **Sastia Prama Putri** Department of Biotechnology, Graduate School of Engineering, Osaka University; **Yusianto Yusianto** Indonesian Coffee and Cocoa Research Institute; **Takeshi Bamba** Department of Biotechnology, Graduate School of Engineering, Osaka University; **Eiichiro Fukusaki** Department of Biotechnology, Graduate School of Engineering, Osaka University;

Recently, incident of adulteration authentic Kopi Luwak, the world's most expensive coffee, has been reported in the coffee market. This poses serious concern among consumers over the authenticity of Kopi Luwak. To develop a prediction model for the adulteration ratio of coffee blend, GC-MS-based metabolite profiling using 2 sets of coffee blend from certified benchmark and commercial samples was employed. A robust prediction model was constructed and validated via orthogonal projection to latent structures (OPLS) regression. This report presents a proof of princi-

ple for the application of metabolomics to counteract adulteration in food and agricultural products.

P108

Metabolite differences between near-isogenic GM and conventional maize are associated more with back-crossing than with the gm traits

Minsang Lee Monsanto Company;

Here we tested the hypothesis that the effect of GM on maize grain composition is negligible relative to the effects of residual genetic variation from conventional backcrossing and other sources of variation. We confirmed this hypothesis, through metabolomic (NMR, GC-MS) analysis of maize grain. This study demonstrates that differences between GM and non-GM near-isogenic comparators cannot be associated solely with the GM trait but arise primarily from near-isogenic effects. Differences in growing location and germplasm were major sources of variation. The data presented here demonstrate that there is no basis for prioritizing GM as a source of metabolomic variation.

P109

Metabolomic diversity in the maize nested association mapping founders

Mutsu Takahashi Monsanto Company;

The present study tests the hypothesis that conventional plant breeding is associated with extensive metabolomic variability. It provides an assessment, through GC-TOF-MS and NMR analysis, of metabolomic diversity in maize B73 hybrids derived from the Nested Association Mapping (NAM) founders. The NAM founders represent a key population used extensively by the maize genetics research community. Overall, this study provides significant information on the contributions of conventional breeding to metabolomic variability as well as context to proposed applications of metabolomics in food and feed safety assessments of new GM crops.

P301

Assessing metabolomics and chemical diversity of a soybean lineage representing 35 years of breeding

George Harrigan Monsanto Company;

The objective of this study was to demonstrate that conventional plant breeding, more so than GM, is associated with extensive metabolomic variability. We assessed (CE, GC- and LC-MS) the metabolomes of seed from soybean varieties representing 35 years of crop development. Varieties included 6 conventional and 3 GM lines. OPLS-DA of the data differentiated newer higher-yielding soybean from earlier lines providing novel information on the impact of varietal development on metabolomic diversity. Results further confirmed that are no clear metabolic differences between conventional and GM comparators and that there is no basis for prioritizing GM as a source of unintended effects.

P303

Using metabolomics to capture high quality rice grain on a drought-tolerant plant

Mariafe Navarro Calingacion Wageningen University; **Roland Mumm** Plant Research International; **Arthur Riedell** University of Queensland; **Rosa Paula Cuevas** International Rice Research Institute; **Harro Bouwmeester** Wageningen University; **Robert Hall** Plant Research International; **Melissa Fitzgerald** University of Queensland;

Breeding programs now focus on developing new climate-ready germplasm. Adoption of these is due both to field performance and consumer acceptance. Apo is moderately drought-tolerant yet is not grown widely because of poor consumer acceptance, while IR64 is drought-susceptible and grown on millions of hectares of land. To determine what drives consumer acceptability, Apo and IR64 grains were extensively analysed. Using the current quality evaluation tools, no difference between varieties was found. However, sensory analysis of cooked grains by trained panel as well as metabolite profiling of volatiles showed significant differences between the samples, enabling new selection tools.

P304

The MS-based metabolite profiling analysis captures the extent of metabolite changes from phenotypic and genetic alternations in GM tomato

Miyako Kusano RIKEN CSRS; Atsushi Fukushima RIKEN CSRS; Tadayoshi Hirai University of Tsukuba; Masashi Suzuki Yokohama City University; Keiko Kobayashi Osaka University; Akira Oikawa Yamagata University; Yozo Okazaki RIKEN CSRS; Ryo Nakabayashi RIKEN CSRS; Tomoko Nishizawa RIKEN CSRS; Makoto Kobayashi RIKEN CSRS; Shoko Shinoda RIKEN CSRS;

Metabolomics is widely applied to investigate how much extent of metabolite changes caused by mutation(s) in a plant genome. We conducted metabolite profiling to evaluate metabolite changes of two genetically-modified tomatoes (GMT), overexpressing melon *3-hydroxy-3-methylglutaryl-coenzyme A reductase* (*HMGR*) and *miraculin* to the same genetic background cultivar (MM). Comprehensive metabolite profiling revealed that slight but clear difference was observed among the two GMTs and MM samples, respectively. Metabolite profiles of the GMTs with or without the genetic marker were also compared to know how much the inserted marker gene make effects toward metabolite changes in the GMTs.

P305

A metabolomics approach to aquaculture

Dan Bearden NIST; Miki Watanabe NIST;

The farming of fish and shellfish is a very large, worldwide industry that has tremendous potential for providing high-quality nutrition around the world while displacing less sustainable practices such as commercial fishing. For the growth in this industry to be economically and environmentally sustainable, improved practices and efficiencies must be realized. We are applying NMR-based metabolomics to understanding the metabolic effects of various feeds on marine species with aquaculture potential. Because of the complex interplay of physical factors and species-specific biology, metabolomics is an excellent tool for gaining novel insights for feed optimization at the systems level.

P306

Comparative profiling of volitile compounds in leaves of aroma and non-aroma rice

Young-Sang Lee Soonchunhyang University; Yoo-Hyun Cho Kongju National University; Soon-Wook Kwon Pusan National University; Yong-Jin Park Kongju National University;

As a part of rapid breeding program to select aroma rice lines, the leaves of aroma-flavoring Butan landrace rice (ABL) were collected at vegetative stage and their volatile compounds were compared to non-aroma Japonica rice (control). Total 205 and 214 volatiles could be identified in ABL and control, respectively. Among those, 82 volatiles were commonly found and 123 volatiles found unique to ABL inculded [2-acetyl-1-pyrroline] and [2-butyl-1-octanol] well known as major volatiles of aroma rice, and [3-Hexen-1-ol, formate, (Z)-], [Tridecane, 6-methyl-], [3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-] as major compounds which could be used in selection of aroma-flavoring rice lines.

P307

Metabolite profiling of Komatsuna (Brassica rapa L.) revealed factorial effects under field experiment.

Keiki Okazaki NARO Hokkaido Agricultural Research Center; Takuji Nakamura NARO Hokkaido Agricultural Research Center; Norikuni Oka NARO Hokkaido Agricultural Research Center; Takuro Shinano NARO Tohoku Agricultural Research Center; Masako Takebe NARO Agricultural Research Center;

The present study investigates which factor(s), particularly with regards to fertilization levels, most influence the changes in metabolitesthat result from different levels of soil amendment. Combining field experiments set up in a fractional factorial design and metabolite profiling of the Komatsuna crop revealed significant factorial effects of N absorption and manure application. The obtained results raise new questions about what other mechanism is involved to explain the changes brought by manure amendment which were not explained by N, P or K inputs.

P308

Evaluation of metabolites in soil solution extracted with methanol under soybean cultivation.

Takuji Nakamura NARO Hokkaido Agricultural Research Center; Keiki Okazaki NARO Hokkaido Agricultural Research Center; Takuro Shinano NARO Hokkaido Agricultural Research Center;

The rhizosphere is commonly defined as the zone where root activity significantly influences soil biological properties. The chemical and biological characteristics of this zone can very affected plant growth and nutrient uptake and so on. However, the nature of these chemicals in the rhizosphere soils in plant cultivation remain largely unidentified. To make clear the relation between plant growth and the chemical profiles in rhizosphere, we examined to develop the methods of chemical extraction in rhizosphere soils for adaptation the metabolomics technics.

Flux, Pathways

P110

Metabolic profiling and turnover analysis reveal the importance of synergy between two pathways for 1-propanol production in *Escherichia coli*

Sastia Prama Putri Osaka University; Yasumune Nakayama Osaka University; Claire Shen National Tsing Hua University; James C Liao University of California Los Angeles; Takeshi Bamba Osaka University; Eiichiro Fukusaki Osaka University;

The incorporation of the synergy concept as a design principle for strain engineering has been demonstrated in *E. coli* 1-propanol production. 1-Propanol production involves the synthesis of 2-ketobutyrate from either threonine pathway or citramalate pathway, or a combination of both pathways. The dual pathway strain achieved 3-fold higher titer compared to the strain with single pathway. Here, we provide further insight on the synergy concept using metabolic profiling and metabolic turnover analysis. The use of dual pathway reduced TCA cycle dependency of threonine pathway, minimized byproduct production, and resulted in faster turnover of citramalate compared to the single pathway.

P111

Reductive glutamine metabolism in p53-null soft tissue sarcoma cells

Nobuyuki Okahashi Osaka University; Fumio Matsuda Osaka University; Susumu Kohno Kanazawa University; Chiaki Takahashi Kanazawa University; Hiroshi Shimizu Osaka University;

The metabolic engineering tools for ^{13}C-metabolic flux analysis was employed to investigate reductive glutamine metabolism (RGM) via reverse reaction of isocitrate dehydrogenase in p53-null soft tissue sarcoma cells. A computer simulation of ^{13}C tracer experiment revealed that [1-^{13}C] glutamine was an appropriate substrate to quantify RGM. The specific consumption rate of glutamate and glucose as well as the specific production rate of lactate were determined from an analysis of the culture medium. The analysis of ^{13}C enrichment of intracellular metabolites by GC-MS with a correction of naturally occurring isotope showed that 10% of citrate in the cells was derived from RGM.

P112

Broadly conserved metabolic responses and the economics of cell growth

Martin Robert Institute for International Education, Tohoku University;

Metabolism connects all activities in living cells. Close similarities in high-level metabolic phenotypes are therefore expected to exist across a wide variety of cell types. Here, we describe underappreciated metabolic states shared by rapidly growing microbial cells, cancer cells, and other metabolically active cell types. As revealed by metabolomics, such cells rely on conserved energy spilling reactions that optimize metabolic function according to growth and activity rate. Fundamental trade-offs between biosynthetic costs and actual energy/biomass requirements thus shape highly conserved constraints that characterize the economics of cellular metabolism and growth.

P113

Metabolome analysis of the poly (gamma-glutamic acid) producing bacteria, Bacillus licheniformis

Hitoshi Mitsunaga Osaka University; Lena Meissner RWTH Aachen University; Thomas Palmen RWTH Aachen University; Takeshi Bamba Osaka University; Lars M. Blank RWTH Aachen University; Jochen Buechs RWTH Aachen University; Eiichiro

Fukusaki Osaka University;

Poly (γ-glutamic acid)(PGA) is a polymer composed of L- and/or D-glutamic acid and has attracted attention in many fields because of its various characteristics. PGA is produced by *Bacillus* sp. Although the effects of extra-cellular environmental conditions for PGA production have been studied since 1950's, there are few reports about the intracellular metabolism of the PGA production. In this study, PGA producing *Bacillus* sp. is subjected to metabolome analysis by GC/MS and LC/MS to identify the crucial metabolic steps. The information would be useful for optimization of the cultivation conditions and/or genetic manipulation driven strain improvement for PGA hyper-production.

P114

^{13}C-metabolic flux analysis of Escherichia coli wild-type and its mutant for co-consumption of glucose and glycerol

Ruilian Yao Shanghai Jiao Tong Univeristy; **Dewang Xiong** Shanghai Jiao Tong Univeristy; **Kazuyuki Shimizu** Keio University; **Hongbo Hu** Shanghai Jiao Tong Univeristy; **Xuehong Zhang** Shanghai Jiao Tong Univeristy;

Most bacteria can use various carbon sources. These carbon sources can be either co-metabolized or sequential metabolized, where the latter phenomenon typically occurs as carbon catabolite repression. It is of practical interest to investigate the carbon catabolite repression mechanism of *Escherichia coli*, such as co-consumption of glucose and glycerol by ^{13}C-metabolic flux analysis. In the present study, we calculated metabolic fluxes using the 13C-labeling technique for wild-type E. coli and ptsGglpK+ mutant at different dilution rates of 0.1 h-1 and 0.35 h-1. As compared to the wild-type, TCA cycle flux and glyoxylate flux were increased, and glycolytic flux was decreased.

P115

Metabolic flux analysis of Saccharomyces cerevisiae with a reduced Crabtree effect

Shuichi Kajihata Osaka University; **Chikara Furusawa** QBiC, RIKEN; **Fumio Matsuda** Osaka University; **Hiroshi Shimizu** Osaka University;

^{13}C-metabolic flux analysis was performed to investigate a metabolic state of *Saccharomyces cerevisiae* with a reduced Crabtree effect. ^{13}C-labeled glucose was fed to a glucose-limited continuous culture of *S.cerevisiae*. Proteinogenic amino acids were obtained from the *S.cerevisiae* cells whose isotopic labeling patterns were determined by GC-MS. A metabolic flux distribution was estimated by a non-liner fitting of metabolic model to the labeling data. The result showed that a flux through pentose phosphate pathway was larger than that of Embden-Meyerhof-Parnas pathway, suggesting an active supply of cytosolic NADPH under a reduced Crabtree effect condition.

P309

Targeted metabolic profiling of acyl-CoA compounds of 1-butanol-producing cyanobacteria Synechococcus elongatus

Shingo Noguchi Osaka University; **Sastia Prama Putri** Osaka University; **Ethan I Lan** University of California Los Angeles; **James C Liao** University of California Los Angeles; **Takeshi Bamba** Osaka University; **Eiichiro Fukusaki** Osaka University;

Direct photosynthetic production of 1-butanol from cyanobacteria *Synechococcus elongatus* is an attractive approach to a sustainable future. In order to gain insight to the metabolic state of cyanobacteria, targeted metabolic profiling of acyl-CoA compounds in 1-butanol production pathway was performed. Here, ion pair LC/MS-based metabolic profiling enabled strain characterization depending on the difference of genetic background in each strain. Moreover, a combination with a GC/MS-based non targeted metabolic profiling yielded several candidates for strain improvement.

P310

Toward manipulating biochemical reactions on Octave/Matlab

Yuki Hyodo Okayama University; Jun Ohta Okayama University;

Recent development of metabolomics technique has enabled us to get detailed information of the entire metabolism. Discovery of new metabolic pathways may be possible through these data. In this situation, informatics techniques for prediction of metabolic pathways would be valuable. We have proposed 3 approaches to generate hypothetical meta-

bolic networks for this purpose. Investigation of these approaches lead us to develop a methodology to manipulate chemical structure as a matrix. We present an attempt to generate biochemical reaction computationally using this methodology on Octave/Matlab environment.

P311

Dynamic simulation of metabolic network in Arabidopsis thaliana using parameters estimated by a genetic algorithm with modularity

Tetsuo Katsuragi Nara Institute of Sciences and Technology; **Naoaki Ono** Nara Institute of Sciences and Technology; **Tetsuo Sato** Nara Institute of Sciences and Technology; **Shigehiko Kanaya** Nara Institute of Sciences and Technology;

Metabolic pathway network in organisms have been reported to be organized into many topological modules in a hierarchical manner. In this study, we have developed dynamic simulation technique based on Genetic Algorithm (Modularity GA; MGA) taking topological modules of metabolic pathways into consideration. In experimental data concerning to metabolites concentrations of *Arabidopsis thaliana*, MGA has better performance than the conventional GA with a single population, since multiple subpopulations can keep optimized parameters for each modular. We demonstrate computation simulation of metabolome dynamics based on MGA along with the conventional GA.

P312

Construction of a model for flux balance analysis of Candida glabrata

Chisato Baba Keio University; **Douglas Murray** Keio University; **Ken Haynes** Exeter University; **Masaru Tomita** Keio University;

C. glabrata is an opportunistic pathogen associated with increased mortality rates in hospital patients. It has a high resistance to commonly used azole fungicides. Our goal is to have a deeper understanding of how *C. glabrata* and the closely related Bakers' yeast (*Saccharomyces cerevisiae*) have diverged. Using the community model of the *S. cerevisiae* we created a stoichiometric model of *C. glabrata* and integrated dynamic metabolic changes observed during the growth of *C. glabrata*.

P313

Prediction of PUFA pathways in Euglena gracilis

Shiho Mukaida National Institute of Genetics; **Takumi Ogawa** Osaka Prefecture University; **Atsushi Okazawa** Osaka Prefecture University; **Daisaku Ohta** Osaka Prefecture University; **Masanori Arita** National Institute of Genetics;

Euglena gracilis can synthesize even- and odd-numbered fatty acids (FAs) and perform wax ester fermentation. The main products are C12:0 to C16:0 saturated FAs but poly unsaturated (PU) FAs such as C20:3 and C20:4 are also observed. Although *E. gracilis* lacks Δ6-desaturase for synthesizing PUFAs but Δ8-desaturase complements their synthesis. We clarify its Δ8 pathways in relation to other algal species and estimates the lipid profiles under inhibition of related genes to overexpress PUFAs. This information is essential in enabling metabolic engineering in the future.

P314

Comparison of Separation Modes for UPLC-QQQ Approaches to High Throughput Targeted Analysis of Hydrophilic components of central metabolism.

James A Apffel Agilent Technologies;

There is a growing need to rapidly and quantitatively characterize the state of key metabolic pathways in assessing impact of biological perturbations on a system. The goal of the current research is to develop a quantitative targeted method for pathway specific metabolites based on UPLC-QQQ-MS. The target performance is >1000 Samples/Day for 50-100 central metabolism components. In initial studies, three different chromatographic modes (Reversed Phase Ion Pairing Chromatography (RP-IPC), Aqueous Normal Phase (ANP), Hydrophilic Interaction Chromatography (HILIC)) approaches have been compared. Results will be demonstrated for resolution, sensitivity, separation speed and robustness.

Data Analysis, Networks

P116

Steroid profiles considered as potential biomarkers in clinical diagnostics measured by CE and LC-MS methods supported by chemometrics analysis

Tomasz Baczek Medical University of Gdansk; Lucyna Konieczna Medical University of Gdansk; Alina Plenis Medical University of Gdansk; Ilona Oledzka Medical University of Gdansk; Piotr Kowalski Medical University of Gdansk;

Several determinations of steroids in view of biomedical and pharmacokinetics studies were first performed. Next, quantification of steroids in parachutists and depressed patients was done. Those analyses were a base to treat steroids as potential biomarkers in variable disease stages. CE and LC-MS analysis of steroids along with their metabolites considered as potential biomarkers of urogenital tract cancer were designed. Usefulness of developed analytical tools was further confirmed in patients with neuroendocrine tumors and cardiovascular diseases. Advanced chemometric analysis enabled to use steroid profiles to search variations between considered groups in view of clinical diagnosis.

P117

Model adaptive scaling for NMR-based metabolomic data preprocessing

Jiyang Dong Xiamen University; Lingli Deng Xiamen University; Kiankai Cheng Universiti Teknologi Malaysia;

Conventional variable scaling methods (e.g. unit variance scaling) assume all variables to be independent to each other. These methods scale data arbitrarily by unifying certain statistics of the data (e.g. standard deviation), without considering its impact on subsequent multivariate model,. In this study, a new scaling method called Model Adaptive Scaling is proposed. The current method updates the variables' weights iteratively based on the performance of the multivariable model. Using NMR-based metabolomic data, our study showed that the new scaling method facilitates biological interpretation of the resulting multivariate model. Hence it can be a useful scaling method for metabolomics.

P118

A mathematical approach to analyze dynamic structural changes of gene regulatory networks

Daisuke Tominaga National Institute of Advanced Industrial Science and Technology;

Gene expression levels are changing in time by mutual regulations. Although knowledge about these regulation schemes is growing rapidly, large parts of mechanisms are still unclear. In addition it is not unclear that whether fixed-value parameter models can represent reliably kinetics of these schemes even by complex non-linear models, such as ordinary differential equations (ODEs). We introduce decoupling and log-transformation to the S-system (canonical ODE model) to depict dynamic changes of gene-to-gene regulation strength. We applied this method to a time series dataset of selected circadian genes of mouse. The result shows that regulation schemes themselves dynamically change.

P119

A data sharing system for multiple metabolomics data sources

Ramon Francisco Mejia RIKEN CSRS; Masanori Arita RIKEN CSRS;

In this poster, we present a data sharing system for the metabolomics community called **MNN Node Network**. An MNN node functions as a local data hub, and cooperates with other nodes to index and query data at scale using a standard network API. We present a resource classification scheme centered on media type information, and protocols for node organization and communication to optimize network-wide operations. Also we propose a basic platform for running simple analysis scripts across multiple nodes, with tools for integrating and streaming results. Finally, we introduce a reference implementation of MNN nodes with an extensible architecture for quickly deploying new features.

P120

An integrated, web enabled tool for liquid chromatography mass spectrometry data analysis and visualization

Yoann Gloaguen Glasgow Polyomics, University of Glasgow; Fraser R Morton Glasgow Polyomics, University of Glasgow; Achuthanunni Chokkathukalam Glasgow Polyomics, University of Glasgow; David Wilson Glasgow Polyomics, University of Glasgow; Michael P Barrett Glasgow Polyomics, University of Glasgow; Karl Burgess Glasgow Polyomics, University of Glasgow;

We present a Web-based application to support biological specialists in analysing metabolomics data for LC-MS platforms. Sharing of experimental design and results enables collaboration between groups. A robust statistical analysis built into our pipeline enables differentiation and reporting of identified/annotated metabolites according to the MSI. Novel multi-scale visualization allows scientists to navigate and inspect sample data and to examine this information in a biological context. Our goal is to standardize and automate metabolomics analysis by integrating all workflow -- from planning to analysis to reporting -- into a comprehensive tool spanning raw data to biological insight.

P121

Metabolic networks of human, chimpanzee and macaque brain: mixed effect model analysis

Yuning Wei CAS-MPG Partner Institute for Computational Biology; Patrick Giavalisco Max Planck Institute for Molecular Plant Physiology; Lothar Willmitzer Max Planck Institute for Molecular Plant Physiology; Philipp Khaitovich CAS-MPG Partner Institute for Computational Biology; Zoran Nikoloski Max Planck Institute for Molecular Plant Physiology;

Concentrations of metabolites, and the conversion rates from substrate to product, reflect the physiological states of tissues. Here, we present a comprehensive study of metabolite levels in three brain regions - prefrontal cortex, primary visual cortex and cerebellar cortex - of humans, chimpanzees and macaque macaques. We applied mixed effect model analysis to all substrate-product metabolite pairs to determine (1) species-specificity of substrate concentrations and (2) species-specificity of conversion rate. Our result showed that metabolite concentrations and conversion rates diverge rapidly among species in a brain region dependent manner.

P122

Product Ion Analysis of Alkaloids in Plants by Liquid Chromatography Mass Spectrometry to Reveal Key Ion Regarding Pharmaceutical Activity

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Exploring alkaloids, which have pharmaceutical activities, in metabolomics develops phytochemical genomics, metabolic engineering, and/or synthetic biology to increase the production of such alkaloids. Although tandem mass spectrometry (MS/MS) is powerful for chemical assignment, available MS/MS spectra of alkaloids are limited unfortunately. Thus, high performance MS/MS spectra of 161 alkaloids were acquired by liquid chromatography-quadrupole time-of-flight-mass spectrometry. Using the spectra, we investigated key product ions regarding analgesic and anti-inflammatory actions on aconitine-type alkaloids.

P124

nmrML: an XML-based open standard for NMR data storage and exchange

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Trust Genome Campus; **Reza Salek** European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus; **Steffen Neumann** Leibniz Institute of Plant Biochemistry, Dept. of Stress and Developmental Biology;

Persistent NMR data storage and integration is hindered by incompatible vendor formats. A robust vendor-neutral NMR data standard is needed for NMR metabolomics data exchange, archival and re-use. The COSMOS EU consortium creates an open XML-based standard for metabolomics NMR data (http://nmrml.org), which can be automatically validated for completeness using rules and controlled vocabularies. We provide converters for all main vendor formats. The nmrML standard is sanctioned by the metabolomics standards initiative and accepted by major open source NMR data processing tools. It will serve the MetaboLights repository, and other metabolomics data repositories, with a stable storage format.

P125

Predicting total actinomycete chemical space through molecular networking

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What is the molecular diversity of Streptomyces, one of the genera of organisms that have provided many of our molecular therapies used in the clinic today? We are using mass spectral molecular networking, a tool that enables us to efficiently organize MS/MS data based on chemical similarity, to address this fundamental but important question. In this study, we utilized this approach to predict chemical space of actinomycetes. We are making correlative analysis of 1000 marine actinomycete strains at the molecular level. Both the discovery of old and new molecules will be highlighted.

P126

Pyrolysis GCxGC-TOFMS and multivariate statistical analysis for discrimination of coffee beans in different origins and brands

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Coffee beans obtained from different regions and brands were profiled using Pyrolysis GCxGC-TOFMS and multivariate data analysis. The relative peak areas of the different compounds identified on the pyrograms were used for multivariate analysis using PCA and HCA techniques. PCA of pyrolysis data from samples was able to represent 64% of the total variability within the first three principal components and led to the correct classification of samples. The results suggest that comprehensive profiling using Pyrolysis GCxGC-TOFMS combined with multivariate analysis can be used to discriminate minor differences and to provide characterization of complicate samples.

P127

Advanced chemometrics models for detailed factor analysis with application to crop composition data

Yun Xu University of Manchester; **Royston Goodacre** University of Manchester; **George G. Harrigan** Monsanto Company;

PCA is a predominant method for factor analysis. When there are multiple interacting factors in the data, the results of the PCA can be confusing as each of the extracted mathematical factors (PCs) describes a mixture of several known and/or unknown real factors and thus it is difficult to evaluate the effect of each of the real relevant factors. In this study, we analysed the influence of growing location, irrigation, and genotype on plant biochemical composition. Newly developed PCA variants, multiblock PCA and ANOVA simultaneous component analysis, which actively incorporate experimental design information into the model offered much better interpretability than traditional PCA.

P128

Novel recursive batch feature extraction algorithms for high resolution mass spectrometry data improves the accuracy of differential analysis results

Yuqin Dai Agilent Technologies; Steven M. Fischer Agilent Technologies; Norton Kitagawa Agilent Technologies; Theodore R. Sana Agilent Technologies;

The accuracy and robustness of feature finding algorithms can have a critical impact on the feature extraction results from accurate mass spectrometry data, and affects subsequent statistical analysis. Fully automated data processing workflows are described, that incorporate novel recursive feature extraction algorithms for larger LC/MS data sets from Yeast. Untargeted batch recursive feature extraction of Yeast data demonstrated an improvement in reducing the number of false positives and false negatives, as judged by 20% increase in the cumulative percentage of compound groups with low CV % values. This workflow improved the statistical results and biological interpretation.

P129

National institutes of health common fund- metabolomics funding opportunities

Padma Maruvada NIH Common Funds Metabolomics Program Working Group, National Institutes of Health (NIH); Keren Witkin National Institutes of Health; Charles Burant University of Michigan; Susan Sumner RTI; Oliver Fiehn University of California at Davis; Arthur Edison University of Florida; Sreekumar Nair Mayo Instittute; Richard Higashi University of Kentucky; Arthur Castle National Institutes of Health; Shankar Sunbramaniam National Institutes of Health; Barbara Spalholz National Institutes of Health;

The NIH Common Fund Program funded several awards for increasing the capacity in metabolomics. The current consortium includes researchers from 6 Regional Comprehensive Metabolomics Resource Cores (RCMRCs), 6 technology development grants, a Data Repository and Coordination Center (DRCC), training and education projects, and multiple collaborative projects. The RCMRCs provide metabolomics technology services. Two Metabolite Standards Synthesis Cores synthesize standards for the research community. The DRCC launched its data repository at http://www.metabolomicsworkbench.org/. This presentation will describe resources supported by the consortium for the international metabolomics community.

P130

Development of large-scale metabolite identification methods for metabolomics

Joshua M. Mitchell University of Kentucky; Teresa W-M. Fan University of Kentucky; Andrew N. Lane University of Kentucky; Hunter N.B. Moseley University of Kentucky;

A significant barrier to meaningful metabolomics data interpretation is the identification of a wide range of metabolites, especially unknowns. Our recent development of chemoselective (CS) probes to tag metabolite functional groups provides additional structural constraints for identification. We have developed a novel algorithm that efficiently detects functional groups within existing metabolite databases such as KEGG Ligand and the Human Metabolome Database, allowing for combined molecular formula (from FT-MS) and functional group (from CS tagging) queries to aid in metabolite identification. With this algorithm, we evaluated an exhaustive range of chemoselection strategies in silico.

P131

Strategy of integration between rna-seq and metabolome in curcuminoid biosynthesis pathways in cultivars of curcuma longa

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A rhizome of turmeric (Curcuma longa) has been used as a spice and herbal medicine in many Asian countries. Curcumin and its analogs are secondary metabolites that are known as the primary active constituent of turmeric. In this study, we compared 4 species of Curcuma family in order to understand the difference in their curcuminoids contents by analyzing their metabolome using mass spectrometry, and transcriptome using a next generation sequencer. We found that the difference of curcuminoids among the breeds can be explained by the expression changes of the genes

DCS and CURS1,2 at the very branch point of the curcuminoids biosynthesis.

P132

Extraction of quantitative information from mouse plasma using Complete Reduction to Amplitude Frequency Table (CRAFT)

Junichi Kurita Agilent Technologies; Katsuhiko Kushida Agilent Technologies; Noriko M Tsuji Advanced Industrial Science and Technology; Tadashi Nemoto Advanced Industrial Science and Technology;

Recently, NMR is increasingly considered a critical quantitative tool. However, complex NMR spectra are more common than not. So, to extract quantitative information of small molecules, large molecular filtering observation methods have been developed. However, NMR spectra are still complex even if these methods are used. And the quantitative information involves systematic error. If more quantitative methods are used, operator has to interact to more difficulty deconvolute peaks of interest. We will present an algorithm that achieves a Complete Reduction to Amplitude Frequency Table (CRAFT) in an automated and its application for NOESY 1st increment and T1rho filter spectra of mouse plasma.

P315

Exploratory analysis about metabolomic profile in community-dwelling men in Japan (Tsuruoka Metabolomics Cohort Study): Focused on smoking status

Ayano Takeuchi National Institute for Environmental Studies; Sei Harada National Institute for Environmental Studies; Toru Takebayashi Keio University School of Medicine; Ayako Kurihara Keio University School of Medicine; Taichiro Tanaka Toho University School of Medicine; Kota Fukai Keio University School of Medicine; Akiyoshi Hirayama Keio University Institute for Advanced Biosciences; Masahiro Sugimoto Keio University Institute for Advanced Biosciences; Tomoyoshi Soga Keio University Institute for Advanced Biosciences; Masaru Tomita Keio University Institute for Advanced Biosciences;

In cohort study, many important factors are measured as categorical variable. Either to evaluate its health effect or to adjust as confounder, we need the assumption that population within same level of categorical variable is homogeneous. We focused on smoking status (nonsmoker/ex-smoker/current smoker), and performed exploratory metabolomics profiling using principal component analysis method in 947 male Tsuruoka residents aged 35-74. 115 metabolites were measured routinely by CE/MS. Profile of metabolites was quite different and easy to distinguish between smoker and nonsmoker, but ex-smoker had diverse and wide ranging profile. We explore other factors to explain ex-smoker metabolomics.

P316

A new standalone Java tool - ShiftedIonsFinder - that helps find candidate peaks based on specified mass differences

Kota Kera Kazusa DNA Research Institute; Yoshiyuki Ogata Osaka Prefecture University; Takeshi Ara Kazusa DNA Research Institute; Yoshiki Nagashima Kazusa DNA Research Institute; Norimoto Shimada Kazusa DNA Research Institute; Nozomu Sakurai Kazusa DNA Research Institute; Daisuke Shibata Kazusa DNA Research Institute; Hideyuki Suzuki Kazusa DNA Research Institute;

Recently, data obtained using mass spectrometry are getting larger. Here we present a new standalone Java graphical user interface (GUI) tool - ShiftedIonsFinder - that increases the efficiency of the narrowing-down process of interesting metabolites. This tool helps find peaks with specified mass differences by comparing the mass spectra in two data sets and making a result list automatically. It enables you to find fully and partially labeled peaks by comparing a sample that is labeled with a stable isotope with an unlabeled sample. Further, it is also easy to find peaks that may have been modified, such as glycosylation and acylation. Now this tool is available free of charge at KOMICS.

P317

Prediction of the dynamic behavior of metabolite concentrations in a large-scale pathway system

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Hirai RIKEN Center for Sustainable Resource Science;

Although metabolomics has great potential for the generation of large-scale metabolic profiles, not all metabolites of interest can be measured simultaneously. We propose the "U-system" approach for constructing coarse mathematical models that predict dynamic behaviors of metabolite concentrations based on the structure of a metabolic pathway system. The approach was implemented to model a relatively large-scale metabolic reaction network of *Arabidopsis*. The predictive simulations from the model qualitatively agreed with measured metabolomic time-series data and identified biological variations. The model can also predict metabolic responses of a network in which gene expression is modified.

P318

Mass accuracy improvement of ESI-RPLC-MS based urinary metabolomic analysis by post-run calibration using sodium formate cluster ions

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The mass accuracy has influence on the metabolite identification. In the untargeted urinary metabolomics analysis, the signals of sodium formate cluster ions were detected at the step of column washing. The cluster ions were used as the internal standard for post-run mass calibration. In positive mode ESI, the average errors of sodium formate cluster ions were ± 0.48 ppm and in negative mode ESI, ± 0.94 ppm after calibration. The error window for metabolite identification was suggested to be 4 ppm after calibration. The results showed that the sodium formate cluster ions could be utilized for the calibration.

P319

An ion trace detection algorithm to extract pure ion chromatogram to improve untargeted peak detection quality for LC/TOF-MS-based metabolomics data

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Untargeted metabolomics studies using LC/TOF-MS is a powerful tool to detect and quantify a broad range of small molecules in the complex biological fluids. However, to detect the metabolites with LC/TOF-MS is still a major challenge. We present PITracker, a novel algorithm that accurately and sensitively detects the ions produced by metabolites contained in complex biological samples to generate pure ion chromatograms (PICs) for peak detection algorithms according to the distribution of the m/z values of ions. As a result, PICs can be generated for more precise chromatographic peak detection when compared to current LC/TOF-MS-based untargeted metabolomics studies.

P320

Computational analysis of gene expression involved in metabolic reprogramming associated to CDK4/CDK6 inhibition in HCT116 colon tumour-derived cells

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Looking for the metabolic reprogramming associated to the inhibition of cyclin-dependent kinases CDK4 and CDK6 in HCT116 colon tumour-derived cells, we performed a gene expression profiling by Affymetrix GeneChip arrays. We employed methods, such as the Gene Set Enrichment Analysis (GSEA), to test for sets of related genes that might be altered. These analyses focussed on potential links between cell cycle and metabolism, and suggested for vulnerabilities associated to cell viability in CDK4/CDK6-inhibited cells.

P321

Development of the metabolite profile database in Arabidopsis: AtMetExpress

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Although sharing metabolome data is important, mining public information of such data remains largely unexploited. The aims of this study are (i) to establish a new web-based platform to conduct meta-analysis of metabolite profile datasets and (ii) to explore the diversity of complex metabolic networks using multiple profiling experiments in the model plant Arabidopsis. We developed a database, AtMetExpress (http://prime.psc.riken.jp/AtMetExpress/), which is freely available and includes detailed information about small molecule metabolites detected in Arabidopsis and a small and simple GUI tool for performing meta-analyses, allowing easy metabolome meta-analysis for plant biologists.

P322

Robust high-throughput analysis: Identity, purity, strength and composition. Application of NMR spectroscopy in nutraceuticals

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There has been a significant increase in robust high-throughput analytical methods automating classification, identification and (absolute) quantification of compounds in mixtures by NMR spectroscopy. A simultaneous targeted and non-targeted NMR approach will be shown, it involves 1) Chemometric modeling for classification, 2) Identification of each component through a spectral comparison, 3) Screening check for any potential unknowns and 4) Absolute quantification using a calibrated NMR spectrometer Setup, validation and application of this analytical can be applied to many kinds of mixtures. We show the quality control of nutraceuticals as an example.

P323

Automatic lipid characterization based on charge remote and fatty acid fragmentation in MALDI MS/MS

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We have demonstrated structural analysis of a TAG mixture using MS/MS data generated by AB SCIEX TOF/TOF 5800 which supplies highly detailed fragmentation information for the structure elucidation of lipids. The MS/MS data of TAGs were subjected to batch analysis in SimLipid software which is a comprehensive high throughput informatics tool for characterizing lipids using precursors and product ions data from MS and MS/MS data. SimLipid identified unknown lipids from fats, e.g. m/z 907.742, which was identified as TAG 54:3 within olive oil. SimLipid facilitates automatic identification of structures of complex lipids mixture based on charge remote and fatty acid fragmentation in MALDI MS/MS.

P324

Effects of maternal separation stress model in rats as evaluated by NMR-based urinary metabolomics

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^{1}H-NMR-based metabolomic approach on urines was applied to evaluate the systemic metabolic consequences of the maternal separation stress in female rats after the beginning of weaning (postnatal day 26) and four weeks later, when

the rats were reaching the adulthood. The application of ANOVA-simultaneous component analysis (ASCA) allowed us to identify and separate the contributions of physiological adaptations to the development from the metabolic consequences attributable to maternal separation postnatal stress. Systemic metabolic differences in the maternal separated pups were mainly ascribable to the tryptophan/NAD pathway and to the urinary excretion of modified nucleoside compounds.

P325

SITAquant: A web-based resource for stable isotope tracer analysis from mass spectrometry data

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In order to quantify the metabolic fates of labeled substrates, several excellent programs exist to deconvolute mass spectrometry data via complex mathematical models. However, the paradigm of metabolic flux analysis (MFA) does not easily allow scientists with limited programming background to monitor, analyze, and understand metabolomics data. We have developed a simpler open-source program to remove the contribution of natural isotopic abundances and calculate proportional and absolute ion amounts for stable isotope tracer analysis (SITA). The outputs of this program can be further used to estimate changes in metabolite concentrations at steady-state or over time.

P326

Metabolonote: A semantic mediaWiki-based database for metadata of metabolomics experiments

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A set of metabolomics data accompanies with a vast amount of detailed information (metadata) for each step of experimental procedure. Management of metadata is a serious bottleneck for publication and re-utilization of metabolome data. We developed a metadata specific database Metabolonote (http://metabolonote.kazusa.or.jp/) based on Semantic MediaWiki. The metadata described by the users can be shared among multiple databases such as raw data repositories, metabolic profile databases, reference libraries of MS/MS, and research papers. Metabolonote functions as a hub of web resources related to users' work and is expected to increase citation of the work, thereby promoting data publication.

P327

Influence of mass spectrometry resolution on metabolite coverage in plasma

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The aim of this work was to describe influence of mass resolution on exact MS metabolomic experiments. In silico calculations based on mzMine database determined 15 711 unique masses without isobars (m/z range 70 - 2000 Da). Number of distinguishable masses was calculated for up to 3840k resolution. Plasma samples were run LC-MS using Orbitrap Elite at up to 480k resolution. In silico, the number of unique masses plateaued at the inflection point of 240k resolution. Real plasma experiments showed - same curve shape with inflection point at 120k resolution and number of observed features above 4k. Results suggest that resolution of 120k is needed for the analyses in metabolite rich biofluids.

P328

Highly sensitive identification of endogenous peptides using a combination of electron transfer dissociation and collision-induced dissociation

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Identification of endogenous peptides is still a challenge due to the structural characteristics, such as unpredictable

cleavages and various PTMs. We developed a method for highly sensitive identification by using different fragmentation methods. Peptide-to-spectrum matching is performed for collision-induced dissociation and electron transfer dissociation in parallel and the final score is made as a combination of the two matching scores. This method effectively discriminates true peptide from false hits. We adopted a GPGPU technology greatly enhancing the processing speed. This method enables the identification of difficult peptides such as numerous PTMs and genome-wide identification.

P329

Pharmacometabolomics and drug response phenotyping

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Pharmacometabolomics has the potential to transform clinical pharmacology. Patient's metabotypes and metabolic signatures of drug exposure inform about mechanisms of drug action and variation in response. We will share experience gained from the study of drugs that include statins, antidepressants, antipsychotics, antihypertensive and antiplatelet therapies. We will highlight pathways implicated in variation of response to antidepressants comparing within members of SSRI class; pathways implicated in response to aspirin and clopidogril and ethnic differences in response to antihypertensive therapies. Gut microbiome role in metabolic side effects of drugs is highlighted.

P330

A novel approach for refinement of structure candidate of known-unknown metabolites using CE-TOFMS

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Elucidating the chemical structure of unidentified peak is a time-consuming process. We propose here a novel approach which refines the number of structural candidates. It is distinguished by adoption of 1) computation of the consistent probability of the unidentified candidates based on the structural similarity of the candidates with relatives predicted from known metabolites, and 2) further refinement by migration-time prediction. In 1), we assume that the unidentified peak is synthesized by addition or desorption of specific functional group from known metabolites in same sample. We successfully applied the method to urine metabolome, resulting in prompt identification of glycocyamidine.

P331

Predicting preterm birth

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In the case of heterogenous diseases such as spontaneous preterm birth (SPTB) where it is assumed that a variety of metabolomic states could possibly exist across the entire range of cases a lower level analysis is required to find biomarkers . Here we examine the dataset assuming the possibility of the existence of multiple as yet unidentified subgroups among the cases. This method examines each and every data point in every case in the metabolomic dataset and compares it against the measurements across all controls for that particular metabolite. In this way potentially informative metabolites for predicting SPTB were identified that warrant further investigation.

Nutrition

P134

Study on the metabolic profilings of Chinese Oolong tea using ^{1}H NMR spectroscopy

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Metabolomics based on ^{1}H nuclear magnetic resonance spectroscopy is applied to obtain the metabolic profilings of Chinese Oolong tea. The metabolic composition in teas was found to vary with brewing time, and metabolic difference was detected between two varieties of Chinese Oolong tea (i.e. light or intense fragrance). Overall metabolic concentration decreases with increased number of times the tea was brewed. However the rate of decrease varies for each metabolite. Compare to the light fragrance tea, metabolites such as quinic acid, caffeine, fructose, EGCG are significant higher in intense fragrance variety, but theanine, alanine, sucrose and chlorogenic acid were found significant lower.

P135

^{1}H NMR-based metabonomic study on the physiological variations during the pregnancy process

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In this study, 1H NMR-based metabonomic approaches were used to analyze the physiological variations with pregnant Wistar Rats plasma samples during the late of pregnant period in order to visualize the metabolic trajectory and reveal any possible mechanism of physiological effects. The results show that the metabolic changes during the pregnancy process involved abnormal glucose, lipid and amino acid metabolism, which may exert negative influence on the intellectual development of newborns. Such information suggests that diet control and nutritional prevention can be served to improve the physiological conditions during pregnancy, and further, it can promote fetal health.

P136

Characterization of the muscle metabolome: is bicarbonate an ergogenic aide for high-intensity exercise?

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Metabolomics offers a unique approach for elucidating the underlying mechanisms associated with lifestyle modifications that promote human health. Strenuous exercise without training contributes to oxidative stress, metabolic acidosis and muscle fatigue that may be overcome by nutritional intervention. Bicarbonate pretreatment is evaluated as a putative ergogenic aide for subjects performing high intensity exercise when using a placebo-controlled cross-over design. Multi-segment injection-capillary electrophoresis-mass spectrometry is used as a high throughput screening approach for characterization of the muscle metabolome and treatment effects associated with bicarbonate intervention.

P137

Effects of manure amendment on sensory quality: metabolite profiling of mizuna (brassica rapa l. var. nipponsinica) in relation to sensory evaluation

Tomohiko Fukuda HOKUREN Agricultural Research Institute; Keiki Okazaki National Agricultural and Food Research Organization/Hokkaido Agricultural Research Center; Ayano Watanabe Food and Agricultural Materials Inspection Center; Takuro Shinano National Agricultural and Food Research Organization/Tohoku Agricultural Research Center for Fukushima Research Station; Norikuni Oka National Agricultural and Food Research Organization/Hokkaido Agricultural Research Center;

Plants change their physiological mechanisms through adjusting to environmental factors in an appropriate manner; therefore phenotype alterations including nutritional quality caused by fertilizer application could be detected. In the present study, we attempted to characterize the sensory quality of Mizuna based on metabolite composition affected by manure application. PLS analysis in relevance of metabolite profiling and sensory attributes such as “sharpness” and

"bitterness" showed that amino acids and isothiocyanates strongly associate to these sensory quality. These findings will provide a noble information for controlling sensory quality based on metabolite alteration.

P138

Association of postprandial high pyruvate level and fullness after natto and viscose vegetable intake

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Glucose metabolism and metabolites in obesity after natto and viscous vegetables as a traditional Japanese breakfast were assessed. A randomized crossover study was conducted in 10 obese men. Test meals were viscous meal, non-viscous meal and glucose. Plasma glucose (PG), insulin and fullness were corrected until 240 min. Serum sample was measured by CE-TOFMS. PG and insulin after the viscous meal were significantly lower than after non-viscous meal and glucose, but no significant differences at 120 and 240 min. Pyruvate and fullness after viscous meal were higher than non-viscous meal and glucose at 240 min. These data suggest the association between postprandial pyruvate level and fullness.

P139

Characterization of the seasonal variations on Momordica charantia metabolome using UPLC/Q-TOF MS

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The fruit of *Momordica charantia* has been a popular traditional medicine and functional food to prevent and treat diabetes for hundreds of years in Asia, India, Africa and South America. In the research, we utilize global metabolic profiling to establish LC-MS fingerprint of *M. charantia* harvested in the different seasons in Taiwan. The PCA scores present distinguished separation of seasonal classification. The result also displays the major alterations in metabolome including amino acids and glycerophospholipids based on the VIP analysis. The research benefits the construction of phytonutrient database and the determination of phytochemical diversity of popular vegetables in AVRDC.

P333

Metabolomics application for evaluation of effect of cooking on vegetables.

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Vegetables are well known as most important foods for us to keep healthy life, because they are rich in vitamins, minerals, dietary fibers and phytochemicals. We usually consumed vegetables not only as fresh but also after cooked. However, in many cases, the differences in composed metabolites between fresh and cooked vegetable are not known in detail. In this research, we purposed to evaluate the effect of cooking on vegetables by the non-targeted metabolomics approach. We chose tomato, onion and cabbage, which are popular to be consumed in both cases, and prepared each cooked sample. Non-targeted metabolic profiles were obtained by LC/FTICR-MS, and different metabolites were screened.

P334

Tryptophan in coffea arabica green beans is responsible for the quality of coffee flavors

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The quality of coffee green beans has been widely evaluated by cupping-test but not with chemical compounds objectively, as done with sugars for many fruits. The relation of the metabolites in green beans to the flavor quality remains unclear. Since the maturity is the most significant factor of quality, we set arabica green beans of four maturity stages

of nine cultivars, for the samples with distinct difference in quality. By the non-targeted metabolic profiling, we identified Trp (Tryptophan) as an immature-related marker, and revealed that the thermal decomposition of Trp gave indoles, providing off-flavor in beverages. Thus Trp is the key metabolite which has influence on the quality.

P335

A trial of MALDI-MS in metabolic profiling of green tea cultivars with antioxidant activity

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Here we explored the applicability of matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS)-based metabolic profiling to understand the relationship between composition profiles of green tea cultivars and their antioxidant activity. We measured the ability of hot water extracts from diverse Japanese green tea leaves to exhibit the oxygen radical absorbance capacity (ORAC), one of the antioxidant assays. Composition profiles of all tea extracts were determined by MALDI-MS. Multivariate analysis revealed differences among tea extracts with respect to their ORAC. This result suggests that MALDI-MS is a useful approach for metabolic profiling of green tea cultivars with ORAC.

P336

The identification of urinary markers of almonds, pecans and walnuts by nmr-based metabonomics

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Metabonomics has been successfully applied to identify biomarkers of different food groups e.g. cruciferous vegetables, citrus fruits and fish. This pilot study aims to identify urinary markers for nuts using a nutritional intervention study by NMR spectroscopy. Participants (n=4 for each nut) consumed 50 grams of almonds, pecans or walnuts after ingesting a standardised diet for a day. Urine was collected immediately before, 5-7 and 10-12 hours after nut intake. Visual examination of urine spectral data showed increased peaks in both aliphatic and aromatic regions following nut consumption. Future work aims to identify these compounds.

P337

Effect of beer intake on LC-MS plasma and urine profiles

Gozde Gurdeniz University of Copenhagen; **Morten Georg Jensen** Carlsberg; **Lars Ove Dragsted** University of Copenhagen;

Moderate beer consumption has been associated with beneficial health effects, potentially related to specific beer constituents: hop-related substances and malt. A meal study was conducted with three different beers varying in hop and alcohol content, and a soft drink. Plasma and urine samples were collected at various time points after the intervention and were analyzed with LC-MS. Initially ASCA has been applied to isolate the beer effect and later beer markers were extracted using PLSDA. The metabolites related with beer intake in plasma were mainly linked to effects on porphyrin and steroid metabolism whereas in urine hops alpha acids such as adhumulone and cohumulone were observed.

P338

The interaction of diet and age influences the metabolic phenotype of non-alcoholic fatty liver disease associated with obesity

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Non-alcoholic fatty liver disease (NAFLD) is a progressive liver disease associated with an increased risk of serious cardiometabolic abnormalities. To elucidate the multiple pathogenic mechanisms of NAFLD and its link with diet-induced obesity, a comprehensive metabolomic approach was performed to investigate the phenotype of the liver tissue in obese (ob/ob) mice fed with either a regular chow or a high-fat diet. The liver tissue of the ob/ob mice, compared with the matching wild-type controls, was characterised by increased de novo lipogenesis, changes in specific diacylglycerol species and a more significant ageing process.

Cancer

P140

Rapid quantitation of spermidine and spermine in human biological fluids by mass spectrometry

Huai-Hsin Su Kaohsiung Medical University;

Spermidine and spermine are bioactive amines, they participate in regulation of cellular function. In polyamine metabolism, spermine is degraded to spermidine by spermine oxidase. Spermidine is converted to putrescine by two enzymes. Catabolism of spermidine and spermine can potentially affect disease etiology or treatment. High polyamine intake increases the risk of colorectal adenoma, the spermidine/spermine ratio may correlate with tumor stage. This study developed a simple and fast method to detec spermidine and spermine in human urine and blood. The proposed method is suitable for high-throughput monitoring of these biogenetic amines in biological samples.

P141

Metabolomic investigation of ovarian cancer progression in a high-grade serous ovarian cancer DKO mouse model

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Ovarian cancer is the fifth deadliest cancer among women and the leading cause of death among gynecologic cancers. Asymptomatic early stages combined with a lack of high specificity biomarkers contribute to late diagnosis. A Dicer/Pten double knockout mouse model of high-grade serous ovarian cancer, the subtype causing 70% of ovarian cancer deaths, is here studied to better understand disease progression. Metabolic profiles of healthy (H) and early stage (ES) cancerous blood sera from Dicer-Pten DKO mice were acquired using UPLC-MS. With only eighteen features down-selected from these profiles, oPLS-DA models discriminated H from ES with 100% sensitivity and 100% specificity, respectively.

P142

Metabolomics investigation of liver cancer: delineating complex metabolic disorders under a systemic view

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Metabolomics is believed to be a promising technique for the discovery of biomarkers or therapeutic targets for Hepatocellular carcinoma (HCC). However, it is still challenging up to now. We study the metabolic disorders of HCC under a systemic view. Firstly, several analytical platforms were employed in combination to increase the coverage of metabolome. And we also developed a pseudotargeted strategy to get high quality data in large sample set. Secondly, important metabolic species were analyzed to focus on detail of the metabolic pathways and constructed systemic metabolic networks. Then, key compounds were discovered by data analysis and their diagnostic potential were validated further.

P143

Development and application of a LC-MS method with chemical derivatization for the determination of cis-diol metabolites in urine

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Metabolites containing cis-diol groups, such as modified nucleosides, have been extensively evaluated as cancer-related biomarkers. However, the determination of these metabolites is still a challenge due to the low abundance, high

polarity and serious matrix interferences of these compounds in urine samples. In this study, a chemical derivatization method was developed to analyze urinary nucleosides and other metabolites with cis-diol structure by using LC-MS based metabolomics. The derivatization conditions were optimized and validated. And then the method was applied to investigate urinary profiling of metabolites containing cis-diol structure from liver cancer urine samples.

P144

Changes in urinary metabolic profiles of colorectal cancer patients enrolled in a prospective cohort study (ColoCare)

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We profiled urinary metabolomes of patients enrolled in a colorectal cancer (CRC) cohort (ColoCare), aiming to describe changes during a longer clinical follow-up. Urine samples from patients pre-surgery (n=97), 1-8 days post-surgery (n=12) and after 6/12 months (n=52/38 respectively) were analyzed using GC-MS and 1H-NMR. Many gut microbial metabolites showed lower concentrations after surgery, whereas metabolites arising from anaerobic bacteria tended to increase. Associations of microbial metabolites with disease stage indicate an important role of the gut microbiome in CRC. Pre-surgery patients were differentiated from those at post-surgery timepoints using a multivariate model.

P145

Analysis of volatile human urinary metabolome by HS-SPME/GC-MS: application in a pilot study to discriminate patients with Renal Cell Carcinoma (RCC).

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An HS-SPME/GC-MS method was developed to study the volatile human urinary metabolome. Central composite design was used in the optimization of the extraction. The optimal conditions in terms of total response were achieved by performing analysis with at pH 2, with a DVB/PDMS fiber, addition of 0.59 g of NaCl, allowing the equilibration for 9 min and extracting at 68 ° for 24 min. The applicability was tested in a non-target analysis of urine samples from patients with RCC and healthy individuals. Chemometrics analysis carried on the volatile pattern of the samples showed the potential of volatile urinary profile to discriminate RCC and controls.

P146

Search of cancer markers in exhaled breath

Tsutomu Fujimura Juntendo University; Sei Matsumori Juntendo University; Naoko Kaga Juntendo University; Hikari Taka Juntendo University; Yoshiaki Kajiyama Juntendo University; Takashi Ueno Juntendo University;

Esophageal cancer has been increasing on the number of patients by the change of lifestyle. New cases of about 18000 people by the year have been reported in Japan. Esophageal cancer is an increasingly common cancer with a poor prognosis. An early non-invasive diagnosis of esophageal cancer would improve prognosis and enlarge treatment options. Analysis of exhaled breath would be an ideal diagnostic method, since it is non-invasive and totally painless. There were differences in the breath volatile organic compounds in patients with esophageal squamous cell carcinoma and healthy controls. It was possible to distinguish between the two groups the results of principal component analysis plots.

P148

HPLC-QTOF characterization of DNA deamination products induced by reactive oxygen species

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Nanyang Technological University; **Fangwei Shao** Division of Chemistry & Biological Chemistry, Nanyang Technological University;

Methylcytosine (mC) and hydroxymethylcytosine (hmC) are major epigenetic modifications of the genome and prone to pathological mutation by spontaneous deamination. We analyzed the deamination products of cytosines induced by OH-. Cytosine, mC and hmC were added to NaOH solutions (0.1M to 1M) and incubated at 37 oC for 8, 12, 24, 48, 96, 144 h. After the reaction, adenosine was added to the solution as internal standard, and the deaminaiton products were separated by HPLC and characterized with QTOF in both full-scan and MS/MS modes. Semi-quantitation of the products was conducted. The study could improve the understandings of reactive oxygen species (ROS) mediated DNA deaminaiton pathway.

P149

The role for glutamine as a key factor of cisplatin resistance in ovarian cancer cell line

Jing Guo Keio University; **Masaru Mori** Keio University; **Kiyotoshi Satoh** Keio University; **Tomoyoshi Soga** Keio University; **Masaru Tomita** Keio University;

Cisplatin is used for first-line chemotherapy of ovarian cancer. However, most patients become resistant to cisplatin during the treatment, therefore resistance of tumor cell to drug is a major obstacle to effective cancer chemotherapy. In this work, we found that consumption of glutamine in cisplatin-resistant cell line is lower than that of sensitive cell line. Furthermore, in glutamine-deprived medium, cisplatin-sensitivity of the resistant cell line was increased, and the metabolomic profile of resistant cell line was similar to that of the sensitive cell line. An inhibitor of the glutaminolysis may provide a new concept and method to overcome cisplatin-resistance.

P150

Untargeted mass spectrometry-based metabolomic profiling of pleural effusions: Fatty acids as novel tumor markers for malignant pleural effusions

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We exploit metabolomic profiling for tumor marker discovery for diagnosis of malignant pleural effusions (PE). The metabolomes of malignant and benign PE were analyzed by metabolomic profiling using high-resolution tandem mass spectrometry. Characteristic signature of cancer metabolism was detected in malignant PE. The biomarker with the largest area-under-ROC (AUC) was free fatty acid (FFA) 18:1. Using a ratio of FFA 18:1-to-ceramide (d18:1/16:0), the AUC was 0.98 (95% CI: 0.90 - 1.00) with sensitivity 94% at specificity 94%. The excellent diagnostic performance of FFA 18:1-to-ceramide (d18:1/16:0) ratio supports its use for diagnosis of malignant pleural effusions in clinical laboratories.

P344

Specific amino acid and phospholipid metabolism perturbations in elderly frail cancer patients identify by serum targeted metabolomics

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In this study we attempted to characterize the frailty clinical phenotype of elderly cancer patients. The serum metabolic profile of 89 elderly women with breast cancer clinically classified as Fit, Unfit and Frail was determined by LC-MS/MS targeted on amino acids, acylcarnitines, sphingo- and glycerol-phospolipids. The frail phenotype showed perturbations involving serine, Trp, Pro-OH, His, its derivate 3-methyl-His, cystine and aminoisobutyric acid and wide depletion of glycerol- and sphingo-phospholipid metabolites. These metabolic biomarkers may give new insight into the development of frailty phenotype useful to refine the geriatric assessment.

P345

Metabolic signatures of ovarian and colon cancer cell lines

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Qatar; **Karsten Suhre** Weill Cornell Medical College in Qatar;

Since ovarian and colon cancers are hardly distinguishable histologically, implementation of metabolomics may result in identification of novel diagnostic biomarkers. We applied non-targeted metabolomics to study metabolic features of cancer cell lines from colon and ovary carcinomas. The principle component analysis indicated separation among all cell lines. Metabolic pathways altered in ovarian cancer cells include increased TCA cycle and glycerophospholipid metabolism whereas the increased urea cycle and biogenic amine metabolism were characteristic of colon cancer cells.Our study provides new insights in the biology of cancer and can be explored in the context of improved diagnosis.

P346

Metabolomics of faecal extracts in human bowel diseases

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Metabolomics of faecal samples has the potential to characterise inflammatory bowel diseases like Ulcerative colitis (UC) and Crohn's disease (CD) and to develop possible biomarkers for early detection of other diseases such as bowel cancer. We have reported results of NMR studies comparing faecal extracts from subjects with UC and IBS with those of healthy controls (Le Gall et al, 2011). This time we have determined the absolute concontration of over 50 metabolites in faecal extracts from bowel patients and controls. Results will be presented. Le Gall et al, J. Prot. Res. 2011, 10, 4208-18.

P347

NMR metabolomics of human colon cancer cell lines: testing the stimuli of DNA G-quadruplex ligands as novel anticancer drugs

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Cell culture metabolomics has, in the recent years, gained an increasing attention because of its many potential applications, e.g. the understanding of *in-vitro* and *in-vivo* actions of drugs. In this study, the endo- and exo-metabolome of human colon cancer lines (HTC-116) was investigated *in-vitro* including its response to 3 anticancer drugs, 1 commercial and 2 novel. A standard operating procedure (SOP) was developed from cell growth to extraction of both hydrophilic and lipophilic fractions. Multivariate data analysis was able to cluster the acquired NMR spectra according to the different drug treatments and, in turn, address the specific chemical variations induced to the cell metabolome

P348

Urinary metabolome analysis from bladder cancer patients and its potential applicability as biomarker

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Bladder cancer (BCa) biomarkers research for detection and monitoring is evolving given the invasiveness of cystoscopy and the low sensitivity of cytology. The objective was to identify the urinary metabolome of BCa by LC-MS and study its value as a potential biomarker. The analysis strategy was to select discriminant metabolites and evaluate its performance as diagnosis and monitoring biomarkers by identifying their presence before and after transurethral resection. The positive and negative predictive values were 94 and 48%. A yield of 100% was obtained as monitoring biomarker. The urinary metabolomic profile of BCa patients identifies useful metabolites with discriminating capacity.

P349

Steroid metabolism and cancer

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Although steroids play a broad and vital role in human physiology, they are also implicated in the development and/or progression of many cancers viz. breast, ovarian, prostate, endometrial, liver, colon, etc. as well as in neurodegenerative diseases, cardiovascular disease and obesity. Present evidence shows the involvement of steroids; such as estrogens, testosterone, bile acids, oxysterols, etc. and their metabolites in carcinogenic processes. Given this we have developed the mass spectrometry based metabolomic platform to profile the entire fate of steroid metabolism. Recent studies involving steroid metabolic pathways and their association with various cancers will be discussed.

P350

Metabolome analysis of rasH2 mouse in the oncogenic process.

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To develop a prediction platform for the carcinogenic status, we performed time-course analysis of plasma metabolites of rasH2 mice carrying a human prototype c-Ha-ras gene. CE-TOF/MS-based metabolome analysis of murine plasma showed that eight amino acids were significantly decreased in 19 week-old rasH2 mice treated with N-methyl-N-nitrosourea (MNU) as compared to control mice, whereas the reduction was not observed in 13 week-old mice. In addition, changes of the plasma metabolome profiles in the MNU-treated rasH2 mice were positively correlated with the carcinogenic status. Taken together, the plasma metabolome profile might be useful for prediction of carcinogenic status in rasH2 mice.

P351

NMR Metabonomics Reveals Molecular Markers of Hepatocellular Carcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC)

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A NMR metabonomic investigation was undertaken in a case-control study nested within EPIC, a large prospective cohort from 10 Western European countries. We analyzed 336 serum samples collected from participants healthy at the time of enrollment that correspond to 112 cases of primary incident hepatocellular carcionoma (HCC), a highly malignant form of liver cancer, diagnosed after an average of 7.6 years follow-up and their matched controls. Multivariate untargeted analysis identifies biomarkers relevant to early HCC diagnosis, or indicative of etiologically relevant exposures. Conditional logistic regression models also assess associations of individual metabolic markers to HCC risk.

P352

Prostate cancer metabolome profiling by using proton nuclear magnetic resonance spectroscopy

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Prostate cancer is the second leading causes of death among men. In this investigation, we examined the chance of neural networking as tools in diagnosing this disease. Serum from 15 men with prostate cancer, and 15 healthy male with same range of age were used. H NMR spectroscopy with CPMG protocol were recorded by Bruker 400 MHZ and data were analyzed. Feed forward neural networking was run with seventy percent of samples. The model was tested with other thirty percent of samples. ROC test were also used and showed 85% of sensitivity in differentiation of two groups with 100 metabolites and 0.2875-error rate by using NNW. 31 metabolic pathways were showed changes.

P354

Fumarate induces redox-dependent senescence by modifying glutathione metabolism.

Leon Zheng Cancer Research UK, Beatson Institute; Christian Frezza University of Cambridge; Eyal Gottlieb Cancer Research UK, Beatson Institute;

The biallelic inactivation of Fumarate Hydratase leads to Hereditary Leiomyomatosis and Renal Cell Cancer. The reactive double bond of fumarate can covalently bind to cysteine residues of proteins which induces Nrf2. Through the computational modelling with transcriptomics and metabolomics, the protein posttranslational modification undergo metabolic reprogramming to support NADPH-production and glutathione biosynthesis.We also discovered that the oxidative stress is necessary and sufficient to elicit cellular senescence in non-transformed epithelial kidney cells. As a result, this will provide a therapeutic window for HLRCC.

P355

Metabolomics Approach for Predicting Response to Neoadjuvant Chemotherapy in Cervical Cancer Patients

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In clinical practice, only some patients could benefit from chemotherapy treatment. Identifying patients who will be response and nonresponse to chemotherapy has important implications to personalized treatment and outcomes. Plasma metabolite profiling was used cervical cancer patients. Metabolic profiles of plasma from cervical cancer patients with complete, partial and non-response to NACT were studies using a combination of LC-MS and multivariate analysis methods. L-valine and L-Tryptophan were finally identified. A prediction model with two markers correctly identified 80% nonresponse and 87% complete response patients, and has an excellent discriminant performance with AUC of 0.9407.

Omics Integration

P151

Integration of GC, LC and CE-MS: Metabolomics studies into the regulation of flower color properties of Primula species.

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Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) are common instrument for metabolite annotation using high-resolution MS spectral database which contains fragment patterns from tandem mass spectrometry and retention time. Here we show an example of sample preparation for integrated metabolomics using GC, LC and CE-MS. Six colors of Primula flowers were collected and extracted with methanol. After addition of half volume of water, aqueous phase were washed with equal volume of chloroform. Aqueous phase were concentrated, derivertized for GC-MS or reconstituted with water for CE- and LC- MS.

P152

GC-MS metabolomics approach to elucidate flower color properties of Primula species

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Six different cultivars were analyzed using gas chromatography-mass spectrometry equipped with capillary column

after two step derivatization of methoxyamine and N-methyl-N-trimethylsilyl trifluoroacetamide. Obtained chromatograms were deconvoluted and further analyzed with Fiehn metabolomics database which contains 1400 compounds information. As a result of database analysis, around 100 compounds were identified with matching of both retention index and mass spectra. Some sugar-like compounds with specific mass spectra were also detected. As a result, correlation between contrasting density of flower color and primary metabolites was indicated in statistical analysis

P153

GC-Q/Tof metabolomics approach to elucidate flower color properties of Primula species.

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The gas chromatography-quadropule/time of flight mass spectrometry (GC-Q/TOF) with soft ionization technique allows peak separation, molecular formula determination and molecular structure speculation for peaks that were not identified in previously established database. In this study, six different cultivars were analyzed by GC-Q/TOF using methylamine/methane as chemical ionization reagent gas after two step derivatisation of methoxyamine and N-methyl-N-trimethylsilyl trifluoroacetamide. Some unknown peaks that were critical in statistical analysis were determined their molecular formula and also speculated their molecular structure in sub 10 ppm level.

P154

CE-MS metabolomics approach to elucidate flower color properties of Primula species

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Capillary electrophoresis-electrospray ionization-quadrupole time of flight mass spectrometry was applied to the analysis of polar metabolites of six different cultivars of Primula x Juliana. The data were processed with metabolites database containing formula, migration time and MS/MS spectra. Statistical analysis suggested that the concentrations of certain amino acids in flower petal were reflecting flower color difference. Under a cationic analytical condition, some characteristic compounds were found in red/blue-color flower and they were estimated as anthocyanin glucosides, based on their electrophoretic behavior and compound structure estimation from their MS/MS spectra.

P155

LC-MS metabolomics approach to elucidate flower color properties of Primula species.

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Six different cultivars of Purimula species were analyzed using liquid chromatography-electrospray ionization-quadropule/time-of flight mass spectrometer. Obtained mass chromatograms were deconvoluted and further analyzed with database having information of accurate mass, MS/MS spectrum and retention time. As a result of database analysis, 4000-5000 compounds were determined in positive ion mode and approximately 2000 compounds were identified. On the other hand, 600-800 compounds were determined in negative ion mode and approximately 400 compounds were determined. PCA analysis revealed that 6 different colors were distinguished in three principle components at 70% of coverage.

P156

Comprehensive pathway analysis of GC-MS, LC-MS and CE-MS: Metabolomics studies into the regulation of flower color properties of Primula species.

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Six different cultivars of Purimula species were analyzed using GC-MS, LC-MS and CE-MS. The identified results via Fiehn library and METLIN database with RT information were integrated to the metabolism pathway. In this study we will show an expanded coverage of metabolites and the corresponding result of variation of each compounds by multiple instruments analysis for the consistent understanding of the biological regulations.

P157

High-throughput and high-resolution lipidomics platform using supercritical fluid chromatography coupled to Orbitrap mass spectrometry

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We developed a comprehensive lipid profiling method using supercritical fluid chromatography (SFC) system, quadrupole-Orbitrap Fourier transform mass spectrometer (Q-Orbitrap FT MS), and automated lipid identification software. Using this lipidomics platform, several hundreds of molecular species including phospholipids, sphingolipids, acylglycerols and cholesterol esters were detected from the total lipid extract of rat plasma within 15 minutes. Notably, the present study is the first report demonstrating a chromatographic separation based on polar head groups and hydrophobic fatty acyl moieties of lipid molecular species using a single octadecylsilyl (ODS) column.

P158

A new integration method for metabolome analysis

Teruko Matsuo Osaka University; **Yumiko Nagasawa** Osaka University; **Takeshi Bamba** Osaka University; **Eiichiro Fukusaki** Osaka University;

In metabolome analysis, metabolite concentrations are represented in relative intensities. As the intensities are affected by various factors, it is difficult to integrate data from different experimental batches. In this study, we propose a new integration method. The dilution series of sample mixture, used as reference samples are analyzed and the intensities are plotted against the reference sample weights. Then, the metabolite intensities of each sample analyzed parallelly are converted to the corresponding weight values. We analyzed 6 types of Senkyu by GC/MS on separate days and integrated the data from the two batches. The samples were successfully clustered according to their types.

P159

Insights into the central carbon and energy metabolism of a metabolic cell factory: The plant glandular trichomes.

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Metabolomics, proteomics, transcriptomics, and 13C-labeling were applied to compare the central carbon and energy metabolism of glandular trichomes (GT) and leaves of two tomato lines. Ion pairing UPLC-MS2-QToF revealed much lower levels of Calvin cycle intermediates in trichomes as compared to the leaves which was accompanied by very limited formation of transitory starch in GT. Despite the presence of plastids in GT, their contribution to photosynthetic carbon fixation from CO2 plays a minor role, whereas labelling studies using U13C-glucose and high expression levels of genes and proteins involved in sucrose metabolism imply that GT largely import their carbon from the plant leaves.

P161

Identifying functions of GLUD2 in the human brain by integration of metabolome and transcriptome approaches

Qian Li CAS-MPG Partner Institute for Computational Biology; **Song Guo** CAS-MPG Partner Institute for Computational Biology; **Masahiro Sugimoto** Keio University; **Masaru Tomita** Keio University; **Xi Jiang** CAS-MPG Partner Institute for Computational Biology; **Svante Paabo** Max Planck Institute for Evolutionary Anthropology; **Philipp Khaitovich** CAS-MPG Partner Institute for Computational

Biology;

GLUD2, a modified version of glutamate dehydrogenase GLUD1 gene, first appeared in the common ancestor of humans and apes. Previous work indicated roles of this protein in human and ape brain metabolism, although no direct studies of metabolic changes were conducted. Here, we present study of GLUD2 function conducted in transgenic mice, humans and macaque monkeys using combination of CE-MS and RNA-seq approaches. Our results reveal role of GLUD2 in early postnatal brain development and single out specific metabolic pathways affected by this recently evolved enzyme.

P162

Pathway driven targeted metabolomics of the arginine biosynthesis of corynebacterium glutamicum

Heiko Neuweger Bruker Daltonik GmbH; Frederik Walter Bielefeld University; Marcus Persicke Bielefeld University; Klaus Meyer Bruker Daltonik GmbH; Aiko Barsch Bruker Daltonik GmbH;

In a recent study by Petri et al. (2013) a novel synthase of the arginine biosynthesis of *C. glutamicum* was investigated applying targeted LC-MS analyses of knockout mutant metabolite profiles. Here we demonstrate a pathway driven workflow that re-analyzes the dataset in a wider metabolic context. Therefore a method was derived from associated KEGG pathways with the novel Compass PathwayScreener. The software tool streamlines method setup, batch screening and review of the generated datasets. In addition to the effects of the knock-outs on metabolites described in the original publication, significant changes on further metabolites in the proximal networks could be highlighted.

P164

Application of quantitative metabolomics in human disease biomarker studies

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TMIC specializes in developing quantitative metabolomics assays for many applications, including robust metabolite biomarker tests for first trimester prediction of early-/late-onset preeclampsia. Biomarkers have been identified for early-stage prenatal detection of Down syndrome, fetal chromosomal abnormalities and fetal congenital heart defects via maternal serum. Novel biomarkers have also been identified to more accurately diagnose adult heart failure, pediatric kidney transplant rejection and early/late-onset osteoarthritis. We will demonstrate the potential of quantitative metabolomics for improving medical diagnoses and contributing to an improved understanding of human health.

P165

The human saliva metabolome

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A comprehensive characterization of the human saliva metabolome is presented. Multiple analytical platforms including NMR, GC-MS, DI/LC-MS/MS, HPLC-UV/FLD and ICP-MS together with computer-aided literature mining tools were combined to identify and quantify human saliva metabolites. Our experimental methods yielded a total of 312 salivary metabolites that could be identified and quantified. More than 240 of these salivary metabolites are reported for the first time. Our literature survey identified another 420 salivary compounds, yielding a total of 732 different compounds. While this study is not the "final" word, it should be viewed as a starting point for future studies

in this field.

P166

Gas chromatography/mass spectrometry analysis of metabolites of urine on unpolished rice diet in healthy individuals

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An unpolished rice diet has been demonstrated to have a considerable influence on human metabolism and to impact on the prevention and improvement for metabolic diseases. In this study, gas chromatography/mass spectrometry (GC/MS) with comparison statistical analysis was applied to explore the variability in the metabolic urinary profiles of healthy individuals. The metabolites that showed differences in relation to diet were ferulic acid, fumaric acid, glycine, hydrocinnamic acid, stearic acid, succinic acid and tartaric acid, which are known to have anti-oxidant response, anti-tumorigenic properties and lowering LDL cholesterol.

P361

Quantitative proteomic and metabolomic analysis of Leishmania mexicana

Snezhana Nikolova Akpunarlieva University of Glasgow; Richard Burchmore University of Glasgow; Karl Burgess University of Glasgow;

Glucose transporter deficient mutants of the human parasite Leishmania mexicana exhibit highly reduced ability to take up and utilise sugars as primary sources of energy. To investigate carbon and energy metabolism of the mutant parasites, we have applied LC-MS-, GC-MS- and NMR-based proteomic, metabolomic and glycomic approaches. The data showed significant changes in amino acid, carbohydrate, nucleotide and lipid metabolism.

P362

Dietary effects influence on Gilthead seabream winter syndrome; an integrated metabolomics and proteomics analysis on plasma profiling.

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Gilthead seabream is a Mediterranean fish that can develop a winter syndrome, when water temperatures are low, affecting growth. Different diets were produced to help fish overcome this period. Plasma samples were analysed by DIGE+MS and Apolipoprotein A-I was identified as an overexpressed protein in fish fed a fortified winter feed (WF), demonstrating a relation of lipid metabolism with health and growth. Cholesterol ($p<0.05$), cortisol, glucose and lactate ($p>0.05$) slightly increased in fish fed WF, while triglycerides displayed lower levels ($p<0.05$) in fish fed WF, showing that diet can affect gilthead seabreams response to seasonal thermal stress.

P363

A new metabolomic information search using the KNApSAcK Metabolite Activity database

Yukiko Nakamura Nara Institute of Science and Technology; Aziza Kawsar Parvin Nara Institute of Science and Technology; Naoaki Ono Nara Institute of Science and Technology; Aki Hirai Morita Nara Institute of Science and Technology; Tetsuo Sato Nara Institute of

Science and Technology; **Tadao Sugiura** Nara Institute of Science and Technology; **Md. Altaf-Ul-Amin** Nara Institute of Science and Technology; **Shigehiko Kanaya** Nara Institute of Science and Technology;

To facilitate the understanding of interactions between the metabolites of organisms and their chemical-level contribution to human health, we constructed the KNApSAcK Metabolite Activity database (http://kanaya.naist.jp/KNApSAcK_Family/) which comprises 9,584 triplet relations (metabolite-biological activity-target species). Approximately 46% of the activities described in the database are related to chemical ecology; more than half the records are concerned with metabolites used in medicine. Our database is integrated within the KNApSAcK Family databases to facilitate further systematized research in various omics fields, especially nutrigenomics, foodomics and metabolomics.

P364

Integrated targeted metabolome and proteome analysis for dissecting energy metabolism in yeast

Fumio Matsuda Osaka University; **Syunsuke Nishino** Osaka University; **Tairo Ogura** Shimadzu Corporation; **Nobuyuki Okahashi** Osaka University; **Atsumi Tomita** Osaka University; **Ichiro Hirano** Shimadzu Corporation; **Hiroshi Shimizu** Osaka University;

An integrated platform for a targeted proteomics and an absolute quantification of central metabolic intermediates was developed for a system-level analysis of *Saccharomyces cerevisiae* metabolism. A MRM assay method for UFLC-MS/MS (Shimadzu nanoLC+LCMS-8040) to analyze 97 enzymes was constructed from the literature data. Levels of glycolytic intermediates were determined by using an internal standard mixture of ^{13}C labeled metabolites prepared from *S. cerevisiae* cells grown in [U-^{13}C]glucose. The integrated analysis of BY4742 pfk1Δstrain suggested that levels of Gnd1p and Tdh2p mainly control a metabolic redirection in respond to the reduced PFK activity.

P365

The metabolomical comparison of psoriatic skin with healthy controls

Aigar Ottas University of Tartu; **Ursel Soomets** University of Tartu; **Kylli Kingo** Tartu Ylikooli Kliinikumi Nahahaiguste Kliinik;

In this study 20 punch biopsy skin samples (from inflammatory and healthy skin) were taken from patients with plaque psoriasis and compared to biopsies taken from age and sex matched healthy volunteers. The samples were measured on an LC-MS system with a QTRAP 3200 mass-spectrometer. The data was analyzed with mzMatch in R Studio. Principal component analysis clearly shows a separation between all three groups. T-test reveals the most intense m/z ratios separating inflammatory skin from healthy controls: 256.08, 225.14 and 311.16. Between inflammatory and non-inflammatory skin m/z: 267.05, 242.08 and 225.14.

P366

Ion-pair uhplc-qtofms for intracellular metabolomics of various microorganisms

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Ion-pair chromatography-MS/MS is often used for intracellular metabolic profiling. However this does not detect the unexpected and the sensitivity is often low, due to the low ion yields for many metabolites, especially the phosphorylated. This point towards full scan accurate mass MS, which detect the unexpected and aids method development by identifying impurities. Here ion-pair UHPLC-QTOF is used for metabolomics of Saccharomyces and filamentous bacteria: Streptomyces and Microbispora. The methodology, including optimized quenching and extraction procedure, was used for targeted and untargeted analysis of organic acids, sugar phosphates, redox co-factors, coenzymes and nucleotides.

P367

Synthesis of stable isotope labelled lipid standards using Saccharomyces cerevisiae for quantitative lipidomic studies

Nyasha Munjoma Cambridge University; **Julian Griffin** Cambridge University; **Nianshu Zhang** Cambridge University; **Albert Koulman** HNR MRC Cambridge; **Clive Cornell** Selcia Ltd; **Mike Jones** Selcia Ltd;

One of the key factors that limits the use of lipidomics in functional genomic studies is the lack of appropriate internal standards for absolute quantification of lipid species in order to compare across different analytical platforms. A common approach for the quantification of lipids by mass spectrometry is to use stable isotope labelled (13C, 15N or 2D) internal standards. These are often chemically synthesized, making such standards expensive and limited in terms of specific species availability. This project is developing isotope labelled standards by culturing yeast on 13C labelled substrates to synthesis 13C labelled lipids. Our validated workflow will be presented.

P368

A strategy for UPLC/MS-based targeted metabolomics

Dong-Ming Tsai National Taiwan University; Ching-Hua Kuo National Taiwan University; Yufeng J. Tseng National Taiwan University;

HMDB affords MS/MS spectrometry for metabolite but no LC-MS data. While UPLC/MS is applied in preliminary metabolite identification, retention time is not available as the type of UPLC are widely different among labs. An UPLC/MS oriented database can facilitate metabolite identification. More than 500 human metabolites were divided into 30 mixtures. Spectrometry data with elective extraction, Hilic column combined with ESI-qTOF was collected. Same set of metabolites for each mixture was spiked to ensure quantitative features. An algorithm including data processing, chromatographic parameters was applied to XIC. It showed favorable in identification of targeted metabolites of low ion-counts.

P369

ChEBI as a knowledge resource for metabolomics

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ChEBI is a manually annotated database and ontology of chemical entities of biological interest, such as natural products. It contains around 40,000 chemical entities classified according to shared structural features and biological activities. It includes a web-based enrichment analysis tool which exploits the ontology classification to derive meaning from large sets of metabolites, such as measured in a biological sample. We are currently expanding our coverage of metabolites, aiming to fully annotate the known metabolome from several key model organisms in the next two years. Each metabolite is explicitly linked to all the species it is found in, with citations to the primary literature.

P370

Metabolomics-based integrated omics analysis of Wolfberry (Lyciumbarbarum) as a new dietary intervention on inflammatory bowel disease (IBD)

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We aimed to elucidate anti-inflammatory mechanisms of Wolfberry (WOL), an immune-boosting traditional Chinese food, in dextran sodium sulphate (DSS) induced colitis model by feeding 7-week-old male C57BL/6J mice either 2% WOL or control diet for 1 week thereafter 1.5% (w/v) DSS drinking water was administered for 9 days. WOL significantly suppressed colon length decrease and Disease Activity Index. Clues from the following analyses: colon transcriptome (Affymetrix), quantitative iTRAQ hepatic proteome and capillary electrophoresis-mass spectrometry (CE-MS)-based analysis of serum and hepatic metabolome complement each other, suggesting WOL as an effective nutraceutical in IBD management.

P371

An integrated omics extraction protocol for maximum recovery of biological information from a single sample

Tracey B Schock National Institute of Standards and Technology; Miki Watanabe University of Maryland; Satomi Kohno Medical University of South Carolina; John Bowden National Institute of Standards and Technology;

Samples collected for metabolomics are excellent specimens to gain cohesive insight into systematic responses at the metabolite, lipid, and macromolecular levels. Merging omics platforms with a single extraction technique will capitalize on time, resources and knowledge. To test the data quality and reproducibility of this multi-omics extraction, we employed a NIST- produced Standard Reference Material and considered requirements for each omic-specific extraction method, variability due to personnel, and sample stability. The phases of this modified Bligh and Dyer extraction were analyzed by NMR, electrophoresis and UV spectrophotometry, SDS-PAGE, and by both automated shotgun and LC-MS/MS.

P372

Increased throughput and analytical precision for targeted lipid quantification using HR/AM on an Orbitrap-based mass spectrometer

Reiko Kiyonami Thermo Fisher Scientific; David Peake Thermo Fisher Scientific; Yingying Huang Thermo Fisher Scientific;

Lipid species from complex biological samples are now routinely identified using the shotgun lipidomics approach. However, accurate quantification of the identified lipids remains a challenge because of the large heterogeneity of lipids and interferences from isobaric lipid species. Here we report that a targeted quantitative assay for a variety of phospholipid classes in total lipid extracts from bovine heart and liver can be rapidly developed using several higher mass resolution (up to 240K resolution) accurate (HR/AM) mass approaches on Orbitrap-based instruments. The LOD, LOQ and linear dynamic range were evaluated by spiking internal standards into lipid extracts in biological samples.

P373

Population-scale metabolic profiling in the MRC-NIHR national phenome centre

Jake TM Pearce Imperial College London; Matthew R Lewis Imperial College London; Anthony Dona Imperial College London; Rachel J Shaw Imperial College London; Elaine C Holmes Imperial College London; Jeremy K Nicholson Imperial College London;

The MRC-NIHR National Phenome Centre aims to generate untargeted metabolite-profiles from samples of human urine and blood-products, from a range of epidemiological and stratified-medicine cohorts. Studies are profiled by NMR and a range of UPLC-MS assays. Throughput will ramp up to over 50,000 assays / year. The Centre works to address these samples with a set of carefully characterised and validated methods, and comprehensive data-analysis. The Centre will facilitate UK medical science, providing an open-access resource for large-scale, qualitative assessment of metabolite-profiles, generation of hypotheses from the resulting data sets, and development of targeted assays to validate them.

P374

Structured metadata capture made easy

Kenny Billiau Max Planck Institute Molecular Plant Physiology; Jan Hummel Max Planck Institute Molecular Plant Physiology; Benjamin Dartigues Bioinformatics Center, University of Bordeaux; Macha Nikolski Bioinformatics Center, University of Bordeaux; Alexander Erban Max Planck Institute Molecular Plant Physiology; Joachim Kopka Max Planck Institute Molecular Plant Physiology; Dirk Walther Max Planck Institute Molecular Plant Physiology;

Tapping into the enormous potential of conducting meta-analyses on the ever increasing number of metabolomics studies requires structured metadata annotation to allow for a meaningful dataset integration and prepare data-mining analysis. We established a GUI-assisted metadata capture workflow based on the XEML-designer software system enabling the intuitive design of experiments. The XEML-designer has been developed further to run on all standard computing platforms. Successful integration into the Golm Metabolome Database has been achieved allowing to describe consistently experimental designs, conditions and export datasets to external databases.

P375

The metabolic response of high vs. moderate-intensity exercise

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Different metabolic pathways are activated in response to varying exercise intensity. This pilot study aims to use UP-LC-MS-based metabonomics to assess the acute and chronic metabolic responses to exercise. Participants completed a 4-week training programme at either high (n=3) or moderate (n=5) intensity. Spot urine was collected immediately before and for 24-hours post exercise on both the first and last training session. Preliminary results show differences in the acute metabolic response between exercise groups, as well as a chronic metabolic adaptation at rest in the high-intensity group after 4 weeks training. Future work aims to characterize trends and identify distinctive compounds.

P376

Comparison of the Orbitrap and qTOF configurations for the global metabolomic profiling of the Pseudomonas Aeruginosa biofilm and planktonic cells

Victor J Nesatyy NERI; **Peter Imre Benke** NERI; **Wei Ling Ng** NUS; **Sanjay Swarup** NUS;

Metabolic extracts from the P. aeruginosa samples were used as a model system. Metabolites were separated using identical chromatographic conditions and detected by Agilent 6550, Waters Xevo G2-S qTOF and Orbitrap Velos Pro in both positive and negative mode in a serially diluted samples spanning 4 orders of magnitude. The resulting data were analyzed by the XCMSonline, mzMine2, Sieve, and Genedata MSX software packages. Our data showed distinct differences in the LC-MS profiles between qTOF and Orbitrap for metabolite cell extracts. In particular, in all cases Orbitrap data revealed significantly less number of detected deisotoped and declustered features in comparison to both qTOFs.

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※Presenters are underlined.