Index

The Metabolomics Society

The Metabolomics Society is dedicated to promoting the growth, use and understanding of metabolomics in the life sciences. Metabolomics is a rapidly growing field of "omics" research focused upon the comprehensive characterization of small molecule metabolites in biological systems. Metabolomics provides a high resolution biochemical phenotype of the metabolic status and global biochemical events associated with a cellular or biological system. As such, it can accurately and comprehensively depict both the steady-state physiological state of a cell or organism and their dynamic responses to genetic, abiotic and biotic environmental modulation.
The Metabolomics Society is an independent, non-profit organization, governed by a democratically-elected Board of Directors composed of dedicated members of the metabolomics community who are ultimately responsive to its members. The Metabolomics Society's vision is to become the premier organization devoted to the development of metabolism-based research. Constituted in 2004, the Metabolomics Society now has more than 500 members in more than 20 countries and publishes its own journal: Metabolomics.

Our Mission

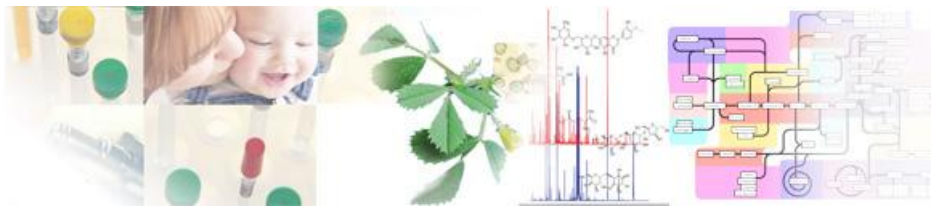
1. To promote the growth and development of the field of metabolomics internationally,
2. To provide the opportunity for collaboration and association among the workers in that science and in related sciences and connections between academia, government and industry in the field of metabolomics
3. To provide opportunities for presentation of research achievements and creation of workshops, and
4. To promote the publication of meritorious research in the field.

Welcome

Welcome to Australia, the Metabolomics Society, and our annual conference! We wish you a productive learning and social experience. By attending this conference you are now a member of the Metabolomics Society and will receive complementary access to our journal Metabolomics. Any suggestions you have on how to improve our Society and its activities are always welcome and we hope you will continue to be a supporter and active participant in our community.

Journal: http://www.springer.com/life+sci/biochemistry/journal/11306

Society: http://www.metabolomicssociety.org/



Letter of Welcome from the Metabolomics Society

Dear Participant,

On behalf of the Metabolomics Society, it is our very great pleasure to welcome you to metabolomics2011. The local organisers together with the International Advisory Board have done an excellent job in preparing a diverse and stimulating programme of workshops, lectures, poster sessions with plenty of opportunities for socialising and interacting. We hope you enjoy the experience and learn a lot during your time in Cairns.

As you are all aware, the original planned location for Metabolomics2011 was Tsuruoka, Japan. Prof Tomita and his local and national team had been working for more than a year on the preparations and had secured great support. They had invested a huge amount of work to prepare for an excellent meeting, following on from their successes in organising the first ever Metabolomics Society meeting in 2005. However, just days after the website registration opened, the devastating earthquake and tsunami struck the East coast of Japan. While Tsuruoka is on the West coast of the island and thankfully, suffered only minor damage, the consequences of the disaster affected the whole country. After many days of uncertainty and hopes that the meeting could still go ahead as planned, it was jointly agreed that the meeting would have to be relocated. In hindsight this was the best decision although we were greatly disappointed for our Japanese friends after all their hard work. We are therefore greatly appreciative that Prof Tomita and his colleagues are willing to organise a future Metabolomics Society meeting the next time the cycle comes to Asia. On behalf of the Society we thank him and his team wholeheartedly for their prolonged support for the Metabolomics Society and all its goals.

We were also thrilled and amazed to learn that Dr. Ute Roessner and her colleagues, supported by Prof Tomita's team, were willing to step up to the plate and take on the relocation metabolomics2011 to Australia. Their willingness and enthusiasm are inspiring. Therefore, a huge thank you must also go out to all our friends in Australia. Taking on such a difficult task of organising a large international meeting at short notice and with so little time is not to be envied. They have done an absolutely brilliant job and the Metabolomics Society will be eternally grateful that we have such trusted and loyal supporters across the world. The Australian and Japanese examples will be an inspiration for all future organisers!

A final thank you goes out to our trusted industrial sponsors, without whose continued support, meetings such as these would simply not be financially feasible. The Metabolomics Society regularly calls on you and especially this year, with the relocation, we have been able to rely on you for continued support – both financially and in contributing to the programme. Societies such as ours depend on you and we are truly grateful for your contributions in all forms.

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

Robert Hall, President,
Lloyd Sumner, Treasurer,
Jules Griffin, Secretary,
on behalf of the Metabolomics Society Board

Welcome Note from the Chairs

With the greatest pleasure we welcome you to the 7th Annual Meeting of the Metabolomics Society in Cairns, Australia. We believe that we have put together an exciting mix of speakers and topics relevant to metabolomics. The program is diverse, ranging from hot new technologies to applications of metabolomics in biomedical, microbial, environmental and plant sciences as well as fluxomics which promises new insights in the field. We especially look forward to providing a basis for gathering and networking of researchers and trade involved in metabolomics through which new connections can be made and collaborations initiated.

As you are all aware, the meeting was initially scheduled to be held in Tsuruoka, Japan. Due to the unforseen catastrophes our colleagues in Japan found themselves in circumstances too challenging to proceed with organising the meeting. We want to make sure you are all aware that Tsuruoka is located on the other side of the island of Japan and had been fortunate in that no damage was caused by the earth quakes and tsunami. Also, the city is far from the Fukushima nuclear plants and is considered safe and clean. However, due to severe nation-wide scheduled electrical black-outs the decision was made, together with the society board, to relocate to Cairns, Australia. At the same time the decision was made to organise the Metabolomics 2014 meeting in Tsuruoka and our Japanese colleagues are very much looking forward to hosting you all then. At this point we would like to express our sincere thanks for the financial and moral support from the local governments of Tsuruoka City and the Yamagata Prefecture.

We are aware that the change of venue has been partially confusing to our metabolomics friends. Therefore, special thanks to all participants of Metabolomics 2011 who have been able to change their travel arrangements and decided to visit Cairns, Australia. There has not been much time to organise the meeting and we hope that everyone will still enjoy the science and social events. At this point we would like to especially thank the team of ASN Events who have been able to provide us with tremendous and rapid support enabling us to pull the meeting off in this beautiful location. The event would also not have been possible without the generous support from all our sponsors, including trade, institutions, software and biotech companies. We also want to thank the society board, the international and regional organising committees for their involvement and support throughout the process of the meeting organisation. Special thanks to the very small, however very productive, local organising team including Dr Simone Rochfort and Associate Professor Rob Trengove. Simone has been tremendous in organising sponsorships and Rob has done an amazing job in bringing together the scientific program we are now able to enjoy in Cairns. Thanks guys for your continuous help, often at short notice!

We hope you all enjoy the next 4 days of metabolomics science and have the opportunity to take pleasure in your visit of one of the most beautiful destinations in Australia, Cairns.

Masaru Tomita and Ute Roessner

Sponsors

With thanks to our sponsors:

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Delegate Information

THE ORGANISER'S DESK – ASN Events

The Organiser's desk will be located in the lobby. Any enquiries about registration or the conference can be directed to ASN staff there. The registration desk hours are:

Sunday:	2:00pm – 4:00pm
Monday:	8:00am – 6:30pm
Tuesday:	8:00am – 6:00pm
Wednesday:	8:00am – 6:00pm
Thursday:	8:00am – 5:30pm

Note that the conference concludes at 5:30pm on Thursday.

WHAT YOUR REGISTRATION INCLUDES:

The Delegate registrations include:

- Access to the sessions of your choice;
- Conference program book;
- Welcome Reception Monday evening;
- Meal breaks

VENUE

- All scientific sessions will take place in either Hall A or Hall B
- All morning teas, lunches, afternoon teas, trade and poster sessions will be in Exhibition Hall

ACCOMMODATION

Accommodation has been offered at The Sebel Cairns, Pacific International Hotel and Park Regis City Quays. Check in time is from 2:00pm on the day of arrival. Individuals will be required to settle their room accounts with the hotel on the morning of checkout. Don't forget that those people who opted to only pay for a portion of their accommodation in advance can expect to have to settle the balance with the hotel, along with any other incidental expenses you have incurred upon checkout.

The Sebel Cairns – 17 Abbott Street, Cairns
Pacific International Hotel – 43 Esplanade, Cairns
Park Regis City Quays – 6 Lake Street, Cairns

SOCIAL PROGRAM

Welcome Reception – Monday 27th June, 5:30pm – 6:30pm, Exhibition Hall
Conference Dinner – Wednesday 29th June, 7:00pm – 11:00pm, in the Outdoor Plaza (weather permitting). Please note this function is not included in your registration and must have been selected as an add-on. Additional tickets may be available for purchase at the Registration Desk, subject to availability.

SPEAKER PREPARATION INSTRUCTIONS

The audio-visual equipment is being supplied and staffed by AV technicians. It is the conference preference to have **ALL** talks pre-loaded in the Speaker Preparation Room (Media Room). As per instructions already supplied, you should give your talk on a USB drive to the technicians well before the session you are participating in so it can be loaded and tested. A technician will always be attending the Media Room so it is always possible to find them.

DISPLAYING YOUR POSTER

Posters will be displayed for the entirety of the meeting, amongst the exhibition area. Please locate your abstract number for correct positioning. **ODD numbered posters will be presented during Poster Session I and EVEN numbered posters will be presented during Poster Session II**. The maximum size provided is 1m wide by 1.2m high. The approved way of attaching your poster is with Velcro which will already be at your poster location. Additional Velcro is available from the registration desk if required.

Local Facts and Tourism Information

From this stylish international city, the islands, rainforest and reef of tropical North Queensland are on your doorstep. Snorkel, dive or do a day trip to the Great Barrier Reef – a World Heritage-listed spectacular of coral islands and marine life. Enjoy the oceanfront energy of the Cairns Esplanade and trawl the restaurants, shops and bars. Go white water rafting in the nearby rivers and take the scenic railway to the tranquil, butterfly-fringed village of Kuranda. Don't miss a day trip to the magical Daintree Rainforest, thought to be the planet's oldest surviving tropical rainforest.

Population
Cairns has a population of over 150,000 making it the 16th largest city in Australia. More than 2 million people visit the area annually.

Location
Cairns is located in Australia's tropical north and is considered the capital of Far North Queensland (FNQ). Cairns, and the surrounding region, is one of the world's most desired destinations, as it is the only place on earth where two World Heritage listed sites live side by side. These World Heritage listed areas are the Great Barrier Reef and Australia's Tropical Rainforests, both are easily accessible from Cairns city.

Attractions
Although Cairns is situated a huge 1750km north of the state capital, Brisbane, it is conveniently situated close to the many attractions of FNQ. Some of the regions popular attractions, located within easy driving distance of Cairns, include:
Palm Cove - 26km north (approx. 20 mins driving)
Port Douglas - 67km north (approx. 1 hour driving)
Daintree - 110km north (approx. 2 hours driving)
Cape Tribulation - 140km north (approx. 2 and a half hours driving)

Climate
The Cairns region has a tropical climate ideal for outdoor enjoyment. June is a great time year to be in the area with monthly averages of:

Average daily max: 26.5°C
Average daily min: 18.6°
Average humidity: 34%

Transport around Cairns

Car Rentals - There are a number of car rental companies that operate within the airport terminals. They have staffed reception desks during all arrival times. Car rental companies can also be found in the CBD.

Taxis (Cabs) – Taxis are conveniently located outside both the domestic and international terminals at the Cairns airport. An approximate fair into the city is AUD$25 - $30. Taxis are also available from the main taxi rank in the City Place or along the Esplanade and in front of the reef Casino.
For more information or to make a booking call: Black & White Taxis Ph: 13 10 08

Public transport – Current bus timetables can be found on the Sunbus Cairns website – www.sunbus.com.au/sit_timetable_cairns.htm.

Restaurants
Cairns Dining provides a great service for casual diners offering reviews and ratings of local eateries. Please visit www.cairnsdining.com.

Invited Speakers

Plenary Speakers

Elaine Holmes, Imperial College, UK
Plenary 3 – Through the metabolic looking glass: A Window on health and disease
9:00am Wednesday 29th June

Nicholas Lockyer, University of Manchester, UK
Plenary 4 – Developments in biological analysis and imaging isomg Secondary Ion Mass Spectronomy
9:00am Thursday 30th June
Nicholas P. Lockyer performed post-graduate and post-doctoral work in the Vickerman group at UMIST, Manchester, developing imaging mass spectrometry instrumentation for biomoecular analysis. Following a Special Research Fellowship from the Leverhulme Trust, in 2002 he was appointed Lecturer in the Department of Chemistry at UMIST. In 2004 he joined the School of Chemical Engineering and Analytical Science at the University of Manchester where he currently holds a Senior Lectureship. His research interests include the development of ToF-SIMS applications in biology and medicine and probing fundamental aspects of the technique. He has co-authored over 70 papers on these subjects.

Peter Meikle, Baker IDI Heart & Diabetes Institute, Australia
Plenary 2 – Plasma Lipid Profiling in Diabetes and Cardiovascular Disease
9:00am Tuesday 28th June
A/Prof Peter Meikle is Head of the Metabolomics Laboratory at Baker IDI Heart and Diabetes Institute and a NHMRC Senior Research Fellow. He holds affiliate positions at Bio21, Melbourne University and the Department of Medicine, Monash Medical School, Monash University. The Metabolomics Laboratory has a focus on the dyslipidemia associated with obesity, diabetes and cardiovascular disease and its relationship to the pathogenesis of these disease states. The work is leading to new approaches to early diagnosis, risk assessment and therapeutic monitoring of these most prevalent diseases.

Masaru Tomita, Keio University, Japan
CE-TOFMS at work in medical, environmental and agricultural applications
4:15pm Monday 27th June
Masaru Tomita is a Professor and the Director of the Institute for Advanced•@Biosciences, Keio University, and a founder of Human Metabolome Technologies, Inc. He received Ph.D in Computer Science from Carnegie Mellon University (1985), Ph.D in Electrical Engineering from Kyoto University (1994) and Ph.D in Molecular Biology from Keio University (1998).
Research fields are Systems Biology, Bioinformatics, Metabolomics, Genome Informatics, and Biological simulation.

Invited Speakers

Burim Ametaj, University of Alberta, Canada
Nutrition – 4:00pm Tuesday 28th June

Daniel Bearden, National Institute of Standards and Technology, USA
Environmental Metabolomics – 2:00pm Tuesday 28th June

Richard Beger, United States Food and Drug Administration, USA
Pathways Discovery Disease & Disease Physiology – 11:00am Thursday 30th June

Steve Fischer, Agilent Technologies Life Sciences Group, USA
Biotic Interactions & Plant Stress – 11:00am Wednesday 29th June

Suha Jabaji, McGill University, Canada
Biotic Interactions & Plant Stress – 10:00am Wednesday 29th June

Wei Jia, University of North Carolina at Greensboro, USA
Pathways Discovery & Disease Physiology – 11:00am Thursday 30th June

Fabien Jourdan, French National Institute for Agricultural Research, France
Databases Bioinformatics & Data analysis & Medical Metabolomics – 2:00pm Wednesday 29th June
Systems Biology – 4:00pm Thursday 30th June

Joachim Kopka, Max Planck Institute of Molecular Plant Physiology, Germany
Technology Updates – 10:00am Wednesday 29th June

Irwin Kurland, Albert Einstein College of Medicine, USA
Pharmacometabolomics Personalised Medicine – Wednesday 29th June

Andrew Lane, University of Louisville, USA
Model Systems for Translational Research – Tuesday 28th June

Xinru Liu, Second Military Medical University, China
Development in Plant Metabolomics – 2:00pm Tuesday 28th June

Caroline Rae, University of New South Wales, Australia
Pathways Discovery & Disease Physiology – 10:00am Thursday 30th June

Reza Salek, University of Cambridge, UK
Databases Bioinformatics & Data Analysis – 4:00pm Wednesday 29th June

Kazuki Saito, Chiba University/RIKEN, Japan
Development in Plant Metabolomics – 2:00pm Tuesday 28th June

Age Smilde, University of Amsterdam, Netherlands
Databases Bioinformatics & Data Analysis – 4:00pm Wednesday 29th June

Hirofumi Uchimiya, University of Tokyo, Japan
Plant Metabolomics – 10:00am Thursday 30th June

Guowang Xu, Chinese Academy of Sciences, China
Lipidomics – 10:00am Tuesday 28th Jun

Organising Committee

International Committee
Choong Hwan Lee
Kazuki Saito
Ute Roessner
Thomas Hankemeier
Robert Hall
Jean-Charles Portais
Matej Oresic
Marta Cascante
Hannelore Daniel
Roy Goodacre
Rick Dunn
Joshua D. Rabinowitz
Oliver Fiehn
Rima Kaddurah-Daouk
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Weidong Zhang
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Amsha Nahid
Kazuki Saito
Masanori Arita
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Ryo Taguchi
Eichiro Fukusaki
Masaru Tomita

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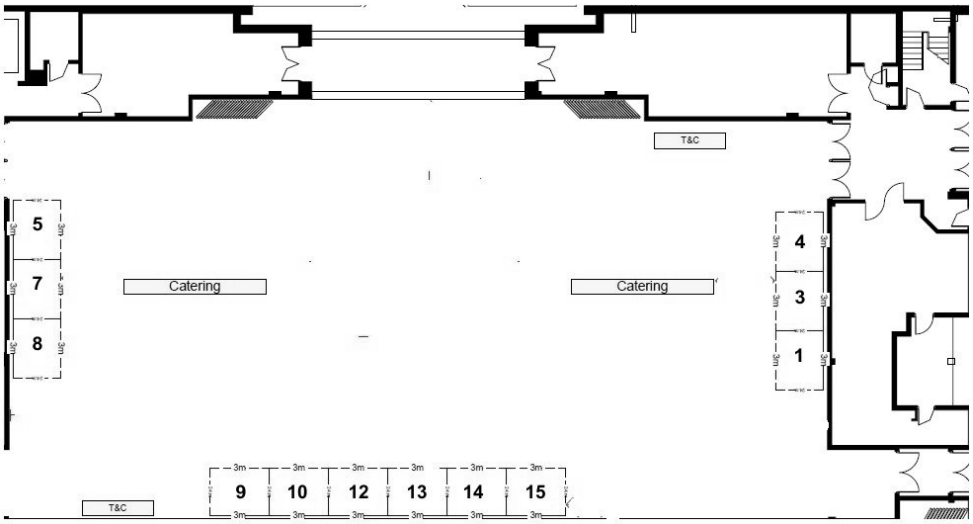
Ute Roessner
Robert Trengove

Sponsor and Exhibitor Listing



AB Sciex **Platinum Sponsor | Booth 3**

Website: www.absciex.com

AB SCIEX is a global leader in the development of best-in-class technologies that help answer complex scientific challenges and improve our world. We create scientific instrumentation, software and services for the life science, clinical research and industrial markets, empowering our customers with technologies that are used in drug discovery and manufacturing, food and environmental safety, and Metabolomics reasearch. With a more than 20-year history of innovation and market leadership under the Applied Biosystems/MDS Analytical Technologies joint venture, we now operate as AB SCIEX, a new company within the Danaher Corporation.

We have a long history of developing first-of-its-kind products, demonstrating our ability to continue innovating for the scientific community:

- First to introduce mass spectrometry/mass spectrometry on a triple quadrupole (MS/MS)
- First to introduce liquid chromatography/mass spectrometry (LC/MS)
- First to introduce time-of-flight/time-of-flight (TOF/TOF) mass spectrometry
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- Now Introduces the TripleTof ™ 5600 System

Agilent Technologies **Platinum Sponsor | Student Prize Sponsor | Booth 5**

Website: Metabolomics-lab.com

Agilent Technologies offers the industry's most complete range of products for metabolomics research, including GC/MS, LC/MS and NMR, as well as powerful software tools for metabolite identification, quantitation, and statistical analysis.

Agilent has taken the lead in metabolomics, and the newly designed virtual lab is where you'll find the knowledge, instruments, and data analysis tools you need to be successful. It is an information portal with peer-reviewed publications, presentations, posters and educational tools to help you learn more about metabolomics.

Metabolomics-lab.com

Agilent is proud to be the platinum sponsor at the Metabolomics Symposium so do drop by and visit us at the booth.

Bruker **Platinum Sponsor | Booth 4**

Website: www.bruker.com

Bruker remains the leader in integrated solutions for Metabolomics/ Metabonomics with its latest innovations for hyphenated NMR, LC-MS and GC-MS technologies.

Please come and join our lunch session on Monday 27th June where we will present the latest examples for targeted and untargeted Metabolomics/Metabonomics using Bruker's integrated NMR and MS solutions. The unique hyphenation of high resolution MS and NMR, the MetabolicProfilerTM, enables a rapid biomarker detection and identification by combined statistical evaluation of MS and NMR data.

Visit us at booth # 4 to see and discuss with us our latest innovations to solve the "Metabolomics Puzzle.

Chenomx Inc Silver Sponsor | Booth 7

Website: www.chenomx.com

Chenomx offers a proprietary platform for generating, classifying, and interpreting metabolic information obtained from biological fluids using nuclear magnetic resonance (NMR) spectroscopy. Chenomx combines state-of-the-art spectroscopic technology for identifying metabolic markers with advanced algorithms for analyzing biological samples.

Chenomx's technology platform is available through two channels: software licensing and contract services.

Dionex Australia **Booth 10**

Website: www.dionex.com

Genedata **Student Prize Sponsor**

Website: www.genedata.com

Human Metabolome Technologies Inc **Silver Sponsor | Booth 8**

Website: www. humanmetabolome.com

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

We provide several commissioned analysis services. Our main business line, Basic Plan, provides primary metabolome analysis, which covers the major metabolic pathways, at an affordable price. Furthermore, Basic Plan is priced for one exploratory sample.

Supported by our advanced technology, number of clients continuously grows both in and outside Japan. Our customer-satisfaction survey reveals that 99 % of our customers recommend our service.

Insiliflo **Gold Sponsor | 14**

Website: www.insiliflo.com

Welcome to the Next Generation of Data Management.

As an innovator in Laboratory Information Management Systems (LIMS), In Siliflo offers web-accessible complex scientific data management, sophisticated search technologies, and built-in analysis tools.

In addition to LIMS, In Siliflo offers custom programming, support, and scientific consultation. With 20 years of combined research experience, In Siliflo can save you time and money, building a custom solution that meets your unique needs.

Visit www.insiliflo.com for more information or see us at our booth!

Leco Australia **Gold Sponsor | Booth 12**

Website: www.leco.com.au

LECO Corporation has been a leader and innovator in analytical instrumentation since 1936. This technological drive has led to some of the industry's fastest, most accurate and cost-effective laboratory instrumentation in the world.

As the demands of life science research have changed throughout the years, so has the need for instrumentation that goes beyond normal expectations. LECO offers a number of solutions ideal for metabolomics, pharmaceutical, forensic science and flavour/fragrance analysis. These include the Pegasus® 4D GCxGC-TOFMS and winner of the Pittcon Editors' Gold Award for 2011, the CitiusTM LC-HRT High Resolution TOFMS.

Metabolomics Australia **Bronze Sponsor**

Website: www.metabolomics.net.au

Metabolomics Australia offers high throughput metabolomics services to all life science researchers. Services are offered through a consortium of Australian universities and research institutes with world class facilities and expertise in small molecule analysis. Metabolomics Australia can provide specific detection and quantification services and cater to complex investigations and systems wide analyses. Metabolomics Australia is committed to ensuring state-of-the-art services that keep up with emerging trends. We receive government funding for new technologies and constantly develop new techniques. We also employ advanced bioinformatics for data analysis and interpretation services and work closely with the Australian Bioinformatics Facility to continually enhance our capabilities.

SGE Analytical Science **Silver Sponsor | Booth 1**

Website: www.sge.com

SGE Analytical Science has five decades of expertise innovating, developing and manufacturing Chromatography, Mass Spectrometry and Liquid Handling products. View the SGE Product Selection Guide ebook at www.sge.com/selectionguide. We look forward to speaking with you at Metabolomics 2011 (booth #1) to show how our experience will enable your vision.

Technology Networks **Media Partner**

Website: www.technologynetworks.com/Metabolomics

Thermo Scientific **Platinum Sponsor | Booth 9**

Website: website

The Thermo Scientific brand represents Thermo Fisher Scientific's broad range of laboratory solutions for the life sciences industries. Our product offerings include chromatography, mass spectrometry, and spectroscopy products as well as guaranteed support to meet your analysis needs. Whether your sampling needs are routine, investigative, advanced research or process analysis, we offer comprehensive solutions for your applications. Our instruments are used throughout the chemical, pharmaceutical, proteomics, genomics, ADME/TOX, polymer, petrochemical, environmental, food, semiconductor, and energy industries, as well as in government and university laboratories. Visit booth #9 to learn about our cutting edge LC/MS technology, latest application developments in Metabolomics.

Waters Corporation **Platinum Sponsor | Booth 15**

Website: www.waters.com

Waters Corporation, the premium brand in the analytical instruments industry, creates business advantages for laboratory-dependent organizations by delivering practical and sustainable scientific innovation to enable significant advancements in healthcare delivery, environmental management, food safety, and water quality worldwide. Bringing keen understanding and deep experience to those responsible for laboratory infrastructure and performance, Waters helps customers make profound discoveries, optimize laboratory operations, deliver product performance, and ensure regulatory compliance. Pioneering a connected portfolio of separations and analytical science, laboratory informatics, mass spectrometry, as well as thermal analysis, Waters' technology breakthroughs and laboratory solutions provide an enduring platform for customer success.

Zoex Corporation **Bronze Sponsor**

Website: www.zoex.com

Zoex Corporation, based in Houston, Texas, USA has driven the commercial development of GC x GC (Comprehensive Two-dimensional Gas Chromatography) from its earliest days in the laboratory of its inventor, the late Professor John B. Phillips, to the present.

Today Zoex GC x GC systems are installed throughout the world, and are employed in such applications as petroleum, flavors and fragrances, environmental, forensics, and health related analysis.

The company has recently introduced a high resolution time of flight mass spectrometer detector specially designed for GC x GC applications. With this new product introduction, Zoex is able to continue with its history of supporting the analysis of most complex and demanding gas chromatographic problems.

Vendor Seminars

AB Sciex Vendor Seminar
Hall A | 1:00pm – 1:50pm
Wednesday 30th June

Agilent Vendor Seminar
Hall A | 1:00pm – 1:50pm
Tuesday 28th June

Bruker Vendor Seminar
Hall A | 1:00pm – 1:50pm
Monday 27th June

Thermo Scientific Vendor Seminar
Hall B | 1:00pm – 1:50pm
Tuesday 28th June

Waters Vendor Seminar
Hall B | 1:00pm – 1:50pm
Monday 27th June

Program

Sunday, 26 June 2011

Registration
2:00 PM - 4:00 PM — Entrance Foyer

Monday, 27 June 2011

Registration
8:00 AM - 6:30 PM — Entrance Foyer

GC-MS & LC-MS Compound ID
9:00 AM - 10:30 AM — Hall A

NMR Optimisation & Compound ID
9:00 AM - 10:30 AM — Hall B
Chair: Simone Rochfort

Morning Tea
10:30 AM - 11:00 AM — Exhibition Hall

Data Processing for Metabolomics
11:00 AM - 12:30 PM — Hall A
Chair: Amsha Nahid

Mass Spectral Imaging
11:00 AM - 12:30 PM — Hall B

Lunch
12:30 PM - 2:00 PM — Exhibition Hall

Bruker Vendor Seminar
1:00 PM - 1:50 PM — Hall A

Waters Vendor Seminar
1:00 PM - 1:50 PM — Hall B

Practical GC-MS & Cross Platform Analysis
2:00 PM - 3:30 PM Hall A
Chair: David De Souza

Biostatistics
2:00 PM - 3:30 PM Hall B

Coffee and Posters
3:30 PM - 4:00 PM Exhibition Hall

Welcome
4:00 PM - 4:15 PM Hall A
Chair: Robert Hall & Ute Roessner

Plenary 1
4:15 PM - 5:15 PM Hall A
Chair: Ute Roessner

Masaru Tomita
CE-TOFMS at work in medical, environmental and agricultural applications *abs#001*

Welcome Reception
5:30 PM - 6:30 PM Exhibition Hall

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** Winner of Pittcon Editors' Gold Award for 2011 **

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High performance MS capabilities including high acquisition speed, mass accuracy, isotopic abundance and wide dynamic range, all available simultaneously..... NO COMPROMISE.

- LECO's Folded Flight Path™ (FFPTM™) technology allows resolution of either 2,500, 50,000 or 100,000.
- Utilises KADAS® (Kinetic Algorithmic Data Acquisition System) to run in three separate modes of operation.
- Patented Dynamic Signal Tracking (DST) for accurate detection of the ion signal.
- ChromaTOF® software utilises True Peak Deconvolution® for accurate peak identification.

Pegasus® 4D GCxGC-TOFMS

Increases your Chromatograhic resolution of complex sample analysis allowing for detection of hundreds to thousands more compounds than previously seen using conventional GC.

- Comprehensive two-dimensional gas chromatography (GCxGC) using two orthogonal separation phases.
- Spectral collection rates up to 500 full range mass spectra/second (500 Hz).
- True Signal Deconvolution® - no other MS manufacturer in the world can match LECO's deconvolution experience and success.

Tuesday, 28 June 2011

Registration
8:00 AM - 6:00 PM Entrance Foyer

Plenary 2
9:00 AM - 10:00 AM Hall A
Chair: Tony Bacic

Peter Meikle
Plasma Lipid Profiling in Type 2 Diabetes and Cardiovascular Disease *abs#002*

Lipidomics
10:00 AM - 10:30 AM Hall A
Chair: Peter Meikle

Guowang Xu
Metabolic biomarker discovery and confirmation by using metabonomics *abs#003*

Metabolomics & Biomarker Discovery
10:00 AM - 10:30 AM Hall B
Chair: Rima Kaddurah-Daouk

Masaru Yoshida
Serum metabolomics by GC/MS as a novel diagnostic approach for diseases *abs#004*

Morning Tea
10:30 AM - 11:00 AM Exhibition Hall

Lipidomics - continued
11:00 AM - 12:30 PM Hall A
Chair: Peter Meikle

11:00am **Helen Atherton**
Investigating the roles of PPARδ and PPARγ in regulating the balance between lipid storage and oxidation in adipose tissue using a combined *ex vivo* and *in vitro* metabolomic approach *abs#005*

11:25am **Katrin Strassburg**
Quantitative Profiling of Oxylipins via comprehensive LC-MS/MS analysis: Application to patients undergoing cardiac surgery *abs#006*

11:50am **Anthony Don**
Mass and Relative Elution Time Profiling: Two-dimensional Analysis of Sphingolipids in Alzheimer's Disease Brains *abs#007*

12:10pm **Shane Ellis**
Ambient Air for Ambient Ionization Mass Spectrometry: A Method for Assigning Double Bond Positions in Unsaturated Lipids *abs#008*

Metabolomics & Biomarker Discovery - continued
11:00 AM - 12:30 PM Hall B
Chair: Rima Kaddurah-Daouk

11:00am **David Wishart**
MarkerDB - The Biomarker Database *abs#009*

11:25am **Claude Guillou**
Metabolomics analysis of exhaled breath condensate and urine of asthmatic children *abs#010*

11:50am **Thomas Hankemeier**
Metabolomics technologies: what can we improve? *abs#011*

12:10pm **Mark Allen**
Combining shotgun lipidomics and HPLC separation of lipids into a new workflow for total lipid analysis *abs#012*

Lunch
12:30 PM - 2:00 PM Exhibition Hall

Agilent Vendor Seminar
1:00 PM - 1:50 PM Hall A

Thermo Scientific Vendor Seminar
1:00 PM - 1:50 PM Hall B

Fluxomics & New Technology
2:00 PM - 3:30 PM Hall A
Chair: Dee Tull

2:00pm **Jens Krömer**
OpenFLUX: efficient modelling software for ^{13}C-based metabolic flux analysis *abs#013*

2:25pm **Raphael Aggio**
Comparing results obtained from the Pathway Activity Profiling (PAPi) algorithm with 13C-based metabolic flux analysis *abs#014*

2:50pm **Andei Bunescu**
The Impact of Bacterial Diet in *Caenorhabditis elegans* NMR Metabolic Profiling Studies *abs#015*

3:10pm **Jens Fuchser**
MALDI Imaging using Q-FTICR Mass Spectrometry: A Versatile Tool to Trace Small Molecules Directly in Tissue *abs#016*

Development in Plant Metabolomics

2:00 PM - 3:30 PM Hall A

Chair: Simone Rochfort

2:00pm **Kazuki Saito**
Development of plant metabolomics from Arabidopsis to crops *abs#017*

2:25pm **Xinru Liu**
Metabolomic application on the material basis and therapeutic mechanisms studies of TCM *abs#018*

2:50pm **Melissa Fitzgerald**
A metabolomics approach to unlocking the secrets of the eating quality of rice *abs#019*

3:10pm **Catherine Rawlinson**
Metabolomics study of the wheat defensome under pathogen challenge –Interlaboratory GC/MS method development *abs#020*

Coffee and Posters

3:30 PM - 4:00 PM Exhibition Hall

Nutrition

4:00 PM - 5:30 PM Hall A

Chair: Meagan Mercurio

4:00pm **Burim Ametaj**
Application of Metabolomics Technology in Dairy Cattle Research *abs#021*

4:25pm **John Draper**
Developing a data-driven framework for discovery and use of dietary exposure biomarkers in human epidemological studies *abs#022*

4:50pm **Federico Marini**
Probiotics and aging: evaluation of immunological and metabolic changes *abs#023*

5:10pm **James Lui**
The metabolomic characterization of changes in human milk metabolites under different storage temperature and time *abs#024*

Model Systems for Translational Research

4:00 PM - 5:30 PM Hall B

Chair: Malcolm McConville

4:00pm **Andrew Lane**
Analyzing breast cancer metabolism with multiple $^{13}C/^{15}N$-labeled substrates *abs#025*

4:25pm **Donald Robertson**
Routine metabolomic assessment in preclinical drug discovery and development *abs#026*

4:50pm **Warwick Dunn**
Mass Spectrometry and Mammalian-based Studies: Insights from the Husermet project *abs#027*

5:10pm **Roy Goodacre**
Meat, microbes and man: VOC-based metabolomics sniffs out pathogens and disease *abs#028*

Poster Session I

5:30 PM - 6:30 PM Exhibition Hall

ODD numbered abstracts will be presented in Poster Session I

EVEN numbered abstracts will be presented in Poster Session II

Wednesday, 29 June 2011

Registration
8:00 AM - 6:00 PM — Entrance Foyer

Plenary 3
9:00 AM - 10:00 AM — Hall A
Chair: Thomas Hankemeier

Elaine Holmes
Through the metabolic looking glass: A window on health and disease *abs#029*

Biotic Interactions and Plant Stress
10:00 AM - 10:30 AM — Hall A
Chair: Kazuki Saito

Suha Jabaji
Fungal metabolomics: challenges and applications *abs#030*

Technology Updates
10:00 AM - 10:30 AM — Hall B
Chair: Robert Trengove

Joachim Kopka
Tackling the unknown: Decision tree supported substructure prediction of metabolites monitored by GC-MS profiles *abs#031*

Morning Tea
10:30 AM - 11:00 AM — Exhibition Hall

Biotic interactions & Plant Stress - continued
11:00 AM - 12:30 PM — Hall A
Chair: Kazuki Saito

11:00am **Steven Fischer**
New software tools for Metabolomics facilitates multi-omics analysis of *the Rice response to Bacterial Leaf Blight disease*. *abs#032*

11:25am **Roland Mumm**
A metabolomics approach to detect an infection of white mushroom (*Agaricus bisporus*) compost by the green mould (*Trichoderma aggressivum*) *abs#033*

11:50am **Lauren Du Fall**
A metabolomics approach to elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors *abs#034*

12:10pm **Ute Roessner**
Investiating abiotic stress tolerance in plants using metabolomics *abs#035*

Technology Updates - continued
11:00 AM - 12:30 PM Hall B
Chair: Robert Trengove

11:00am **Jianguo Xia**
MetATT - a web-based Metabolomic tool for Analyzing Two-factor and Time-series data *abs#036*

11:25am **Jay Harrison**
Principal variance components analysis: A novel approach to quantify compositional and metabolomic responses *abs#037*

11:50am **Yeu-Chern Harn**
Structure Hunter: Prediction of novel chemical structures in a mixture *abs#038*

12:10pm **Arno Knorr**
Computer-Assisted Structure Identification (CASI)—A Mass Spectrometry-based Automated Platform for High-Throughput Identification of Small Molecules by Two-Dimensional Gas Chromatography − Mass Spectrometry *abs#039*

Lunch
12:30 PM - 2:00 PM Exhibition Hall

AB SCIEX Vendor Seminar
1:00 PM - 1:50 PM Hall A

Databases Bioinformatics & Data Analysis & Medical Metabolomics A
2:00 PM - 3:30 PM Hall A
Chair: Amsha Nahid & Don Robertson

2:00pm **Reza Salek**
Reza Selak - EBI Initiative: Metabolomics Database *abs#040*

2:25pm **David Broadhurst**
A longitudinal study of metabolomic changes in maternal plasma and urine during normal pregnancy using GC-MS and UPLC-MS metabolic profiling. *abs#041*

2:50pm **Juan Falcon-Perez**
Serum UPLC-MS/MS metabolic profiling in experimental animal models as a tool to reveal biomarkers for liver toxicity *abs#042*

3:10pm **Kosaku Shinoda**
Capillary Electrophoresis Mass Spectrometry-Based Metabolomics Reveals Ethanolamine Phosphate as a Major Depression Biomarker *abs#043*

Databases Bioinformatics & Data Analysis & Medical Metabolomics B
2:25 PM - 3:30 PM Hall B
Chair: Amsha Nahid & Don Robertson

2:25pm **Fabien Jourdan**
Use of reconstituted metabolic networks to assist in metabolomic data visualization and mining, the challenge of computing “metabolic stories” *abs#044*

2:50pm **Thomas Hankemeier**
Challenges for metabolomics study databases *abs#045*

3:10pm **Mingshu Cao**
"iontree"-- an R package for data management and analysis of ion trees from ion trap mass spectrometry *abs#046*

Coffee and Posters
3:30 PM - 4:00 PM Exhibition Hall

Pharmacometabolomics Personalised Medicine

4:00 PM - 5:30 PM Hall A

Chair: David Wishart

4:00pm **Irwin Kurland**
The Fasted/Fed Acetylome Coordinates Organ Specific Fuel Switching: Metabolomic and Fluxomic Validation *abs#047*

4:25pm **Anthony Maher**
Multivariate Paired Data Analysis (MVPDA) Reveals Unique Metabolic Fingerprints Underlying Surgically-induced Osteoarthritis in Sheep *abs#048*

4:50pm **Enzo Ranieri**
A non-targeted global metabolite profiling using high resolution accurate mass spectrometry, TripleTOF™ 5600 system, to identify novel makers in maternal serum screening for trisomy *abs#049*

5:10pm **Haripal Sonawat**
Metabonomics of Malaria: Alterations related to Transition of Non-cerebral to Cerebral Complications *abs#050*

Databases Bioinformatics and Data Analysis

4:00 PM - 5:30 PM Hall B

Chair: Age Smilde

4:00pm **Age Smilde**
Dynamic metabolomics data analysis: a tutorial review *abs#051*

4:25pm **Reza Salek**
APPLICATION OF MULTI-BLOCK DATA FUSION TECHNIQUES USING 1H HR-MAS NMR, GC-MS AND LC-MS METABOLOMIC PLATFORMS FOR THE STUDY OF BREAST CANCER *abs#052*

4:50pm **Marinus van Batenburg**
Analyzing multi-platform data using analytical repeatability: the metabolomics case *abs#053*

5:10pm **Amsha Nahid**
Analysis of Inter-laboratory Metabolomics Experiments *abs#054*

Poster Session II

5:30 PM - 6:30 PM Exhibition Hall

ODD numbered abstracts will be presented in Poster Session I

EVEN numbered abstracts will be presented in Poster Session II

Conference Dinner

7:00 PM - 11:00 PM Outdoor Plaza (weather permitting)

Thursday, 30 June 2011

Registration
8:00 AM - 5:30 PM — Entrance Foyer

Plenary 4
9:00 AM - 10:00 AM — Hall A
Chair: Roy Goodacre

Nicholas Lockyer
Developments in biological analysis and imaging using Secondary Ion Mass Spectrometry *abs#055*

Plant Metabolomics
10:00 AM - 10:30 AM — Hall A
Chair: Ute Roessner

Hirofumi Uchimiya
Plant metabolomics for the weed-biomass production under environmental constrains *abs#056*

Pathways Discovery & Disease Physiology
10:00 AM - 10:30 AM — Hall B
Chair: Rick Dunn

Caroline Rae
Neuropharmacology from a metabolomic perspective *abs#057*

Morning Tea
10:30 AM - 11:00 AM — Exhibition Hall

Plant Metabolomics - continued
11:00 AM - 12:30 PM — Hall A
Chair: Ute Roessner

11:00am **Aaron Fait**
From the desert to the cellar: response of grape berries metabolome to water deficit *abs#058*

11:25am **Huiru Tang**
Understanding the Systems Responses to Stresses with Integrated Metabonomic Analysis *abs#059*

11:50am **Damien Callahan**
LC-MS profiling of leaf tissue from *Thlaspi caerulescens* shows an involvement of lipids in Zn hyperaccumulation *abs#060*

12:10pm **Ric De Vos**
Metabolomics as powerful tool in seed quality research *abs#061*

Pathways Discovery & Disease Physiology - continued

11:00 AM - 12:30 PM Hall B

Chair: Rick Dunn

11:00am **Richard Beger**
Metabolomics analyses of biofluids from rats dosed with liver or kidney toxicants *abs#062*

11:25am **Wei Jia**
A metabolomic study of colorectal cancer *abs#063*

11:50am **Edoardo Saccenti**
A metabolomics investigation of weight loss in overweight and obese adults *abs#064*

12:10pm **Simon Ovenden**
Health monitoring biomarkers in elite soldiers *abs#065*

Lunch

12:30 PM - 2:00 PM Exhibition Hall

Microbial Metabolomics

2:00 PM - 3:30 PM Hall B

Chair: Silas Villas-Boas

2:00pm **Malcolm McConville**
Carbon metabolism of *Leishmania* parasites required for virulence in the mammalian host revealed by ^{13}C-stable isotopomer profiling *abs#066*

2:25pm **Kalesh Sasidharan**
Self-organisation of amino acid regulation in yeast *abs#067*

2:50pm **Kenji Nakahigashi**
Systematic phenome analysis of *E. coli* multiple-knockout mutants reveals hidden reactions in central carbon metabolism *abs#068*

3:10pm **Du Toit Loots**
A Metabolomics Approach to Exploring the Function of the ESX-3 Type VII Secretion System and *M. tuberculosis* viability *abs#069*

Environmental Metabolomics

2:00 PM - 3:30 PM Hall B

Chair: Choong Hwan Lee

2:00pm **Daniel Bearden**
Environmental Metabolomics at the Edge *abs#070*

2:25pm **Horst Schirra**
Spanning the bridge from systems biology to classical science – NMR metabonomic investigation of phosphine resistance in *Caenorhabditis elegans* *abs#071*

2:50pm **Gene Wijffels**
Altered Fatty Acid Metabolism in Long Duration Road Transport: An NMR-based Metabolomics Study in Sheep *abs#072*

3:10pm **Jun Kikuchi**
Energy security through next-generation metabolomics: a new avenue for polymerized metabolites, lignocellulose research *abs#073*

Coffee and Posters

3:30 PM - 4:00 PM Exhibition Hall

Systems Biology

4:00 PM - 5:30 PM Hall A

Chair: Lloyd Sumner

4:00pm **Fabien Jourdan**
Computing "metabolic stories" in genome scale metabolic networks based on metabolomics data *abs#074*

4:25pm **Tze-Feng Tian**
3Omics: a web based systems biology visualization tool by integrating transcriptomics, proteomics and metabolomics data in human *abs#075*

4:50pm **Craig Wheelock**
Systems-Based Approaches to Elucidating Gender-Specific Mechanisms in the Etiology of Atherosclerosis *abs#076*

5:10pm **Benjamin Blaise**
Statistical Recoupling of Variables for the Identification of Candidate Biomarkers and Perturbed Metabolic Networks. Application to whole organism NMR *abs#077*

Plant/Microbial Metabolomics

4:00 PM - 5:30 PM Hall B

Chair: Daniel Dias

4:00pm **Silas Villas-Boas**
Monitoring microbial contamination in fermentation processes using metabolic footprint analysis – an exometabolomics approach *abs#078*

4:25pm **Yuji Sawada**
Large-scale SRM assay system for un-targeted MS/MS of phytochemicals *abs#079*

4:50pm **Mohamed Bedair**
Quantification of plant hormones in *Medicago truncatula* roots using UHPLC-QqQ-MS/MS and multiple reaction monitoring *abs#080*

5:10pm **Albert Batushansky**
γ-Aminobutyric acid (GABA) in plants: a signaling molecule or "just" a metabolite? *abs#081*

Poster Listing

Konstantinos Aliferis

Metabolic systems biology for the study of plant-microbe interactions *abs#101*

Yongjin An

Non-invasive assessment of gastrointestinal toxicity of methotrexate using NMR based metabolomics *abs#102*

Cristina Andres-Lacueva

HPLC-q-ToF-MS Driven Untargeted Metabolomics Approach to Unveil Urinary Changes in MetS Subjects following 12-weeks Nuts Consumption *abs#103*

Masanori Arita

Chromatographic Comparison of Glycyrrhizae Radix from Different Regions *abs#104*

Mohammad Arjmand

Effect of *Artemisia annua* extract's on malaria parasite *Plasmodium falciparum* trophozoite metabolme profiling by ^{1}H NMR spectroscopy *abs#105*

Mohammad Arjmand

^{1}H Nuclear magnetic resonance based metabonomics approaches for evaluating rheumatoid arthritis metabolome finger printing *abs#106*

Mohammad Arjmand

Metabolomics profiling of Leishmania major in two phases of logarithmic and stationary by ^{1}H NMR abs#107

Aiko Barsch

Structure elucidation and confirmation for plant metabolomics: novel approaches *abs#108*

Aiko Barsch

Improving comprehensive analysis for myxobacterial metabolic profiling *abs#109*

Aiko Barsch

Metabolic profiling of a *Corynebacterium glutamicum* Δ*prpD* 2 by GC-APCI high Resolution Q-TOF Analysis *abs#110*

Devin Benheim

Protecting vines in stress environments: Early detection of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) infestation through identification of chemical biomarkers *abs#111*

Jairus Bowne

Scripting an Automated Lipid Analysis Workflow Using R *abs#112*

Arjan Brenkman

Mesenchymal Stem Cells induce resistance to chemotherapy through the release of platinum-induced fatty acids *abs#113*

Adam Carroll

The MetabolomeExpress Project: celebrating a year of rapid growth - new members, data and tools to enhance metabolomics research *abs#114*

Pui Hei Chan

1H-NMR metabonomics analysis of Danggui Buxue Tang effects in HEK293T kidney cell lines *abs#115*

David Chang

A Novel Highly Sensitive Test for Detecting Colon Cancer Using Spot Urine Metabolomics *abs#116*

David Chang

A New and Highly Sensitive Screening Tool for Colorectal Adenomatous Polyps Using a Spot Urine Metabolomics Test *abs#117*

Eisuke Chikayama

Theoretical Chemical Shift Database and Structural Investigation of Cellulose by NMR and Supercomputer *abs#118*

Jung Nam Choi

Novel Antibiotics and Optimized Fermentation Time of *Phomopsis longicolla* by Metabolomic Approaches *abs#119*

Hyung-Kyoon Choi

Biochemical monitoring of black raspberry (*Rubus coreanus* Miquel) fruits according to maturation stage by ^{1}H-NMR using multiple solvent systems *abs#120*

Hyung-Kyoon Choi

Geographical differentiation of black raspberry (*Rubus coreanus* Miquel) fruits of various origins by genomic and metabolic analysis *abs#121*

Edith Chow

Low-Cost, Portable Nanosensor Array for Identifying Low-Molecular Weight Molecules *abs#122*

Bong Chul Chung

Metabolomics approach for developing predictive biomarkers of colon cancer *abs#123*

Yu-Ting Chung

Metabolomic Approach on the Potential Role for Oxidative Stress in Reversible Cerebral Vasoconstriction Syndrome *abs#124*

Yasuhiro Date

NMR-based metabolomics approach in combination with ionomics for evaluation of compositional variations and diversities in intertidal seaweeds *abs#125*

Saravanan Dayalan

Web PyMS: An interactive web based gas chromatography - mass spectrometry data processing tool *abs#126*

David De Souza

Elucidation of Normal Human Epidermal Keratinocyte (NHEK) metabolic pathways using ^{13}C-stable isotope incorporation *abs#127*

Daniel Dias

Iron(III) Tartrate as a Potential Precursor of Light-Induced Oxidative Degradation of White Wine: Studies in a Wine-like Matrix *abs#128*

Eva Duchoslav

Information Independent MS/MS Data Collection of All Precursors Using High Resolution Accurate Mass Spectrometry *abs#129*

Lindsay Edwards

Exometabolomics reveals increased glycolysis in cultured neurons lacking alpha-synuclein *abs#130*

Jens Fuchser

A workflow for metabolic profiling and determination of the elemental compositions using MALDI-FT-ICR MS *abs#131*

Atsushi Fukushima

Global effects of the UV-B irradiation on metabolite-correlation networks in plants *abs#132*

Jennifer Gardner

Using Metabolomics to Probe the Impact of an Adaptively Evolved Wine Yeast on Oenological Fermentation *abs#133*

Anita Goldinger

Metabonomic investigation of phosphine toxicology and resistance in *Caenorhabditis elegans*: Method development improves analysis of how a modified metabolism contributes to fumigant tolerance and a novel longevity mechanism. *abs#134*

Ben Gordon

The Metabolome of *Symbiodinium* Phylotypes and their Coral Hosts: The Response to Environmental Stressors. *abs#135*

Na Guo

Qualitative and quantitative analysis of traditional Chinese medicine Gardenia jasminoides Ellis using high performance liquid chromatography and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry *abs#136*

Scott Harrison

Methods to increase our understanding of Fructans and their biosynthesis *abs#137*

Scott Harrison

A method for batch processing isobaric and isomeric compounds via Non-Targeted, relative retention time data processing *abs#138*

Angela Hendrickson Culler

Methodology for ^{13}C-Labeling of Entire Plant Metabolomes *abs#139*

Tom Hennessy

The Application of Mass Profiler Professional in Plasma Biomarker Discovery: A statistical analysis & visualization software tool in mental disorder metabolomics *abs#140*

Richard Higashi

Human non small cell lung cancer tissue has enhanced expression of pyruvate carboxylase, but not glutaminase *abs#141*

Camilla Hill

Characterization of ion contents and metabolic responses to salt stress of different *AtHKT1;1* genotypes and their parental strains in *Arabidopsis thaliana* *abs#142*

Akiyoshi Hirayama

Metabolomic profiling using capillary electrophoresis-mass spectrometry differentiates diabetic nephropathy *abs#143*

Tsung-Jung Ho

MetaPick, a denoising and peak picking algorithm for extracting single compound from complex mixtures in liquid chromatography/mass spectrometry (LC/MS) metabolomics data *abs#144*

Mark Hodson

The Application of GC-MS-based Metabolomic Profiling to the Optimisation of Polyhydroxybutyrate (PHB) Production in Transgenic Sugarcane *abs#145*

Ching-Shan Huang

Effect of genes on rosuvastatin therapy for hyperlipidemia *abs#146*

Yasuhiro Irino

GC-MS-based metabolomic study in mice with colitis induced by dextran sulfate sodium *abs#147*

Takamasa Ishikawa

Comparison of analysis platforms using mass spectrometry in metabolomics *abs#148*

Zihey Jang

Metabolomics study on alcoholic fatty liver in zebrafish using ^{1}H NMR spectroscopy coupled with multivariate statistical analysis *abs#149*

Renger Jellema

Comparative analysis between two different quenching methods for yeast metabolomics studies *abs#150*

JeeYoun Jung

^{1} H -NMR-Based Metabolomics Study of Asthma *abs#151*

Kenjiro Kami

Metabolomic Profiling of Dichloroacetate and Pyruvate Efficacy in Cybrid Cells Harboring MELAS Mitochondrial DNA Mutations *abs#152*

Sunmi Kang

An effective assessment of simvastatin-induced toxicity with NMR-based metabonomics approach *abs#153*

Masato Kawakami

Metabolomic profiling of pregnancy mice serum in preimplantation phase *abs#154*

Hyangyeon Kim

Secondary metabolites based classification of *Penicillium* sp. and relevance of its antioxidant activity *abs#155*

Suhkmann Kim

Characterization of the Effects of Silver Nanoparticles on Liver Cell Using HR-MAS NMR Spectroscopy *abs#156*

Sooah Kim

GC-MS based profiling of metabolic disruption and recovery of UV-irradiated lemon balm plant *abs#157*

Jinsup Kim

Metabolic profiling on peritoneal solute transport in peritoneal dialysis patients *abs#158*

Gemma Kirwan

Integration of large scale omic's datasets using O2PLS *abs#159*

Gemma Kirwan

OPLS as a variable selection tool: a comparative study *abs#160*

Craig Knox

Phenol-Explorer Version 2.0: A resource for polyphenol metabolism and pharmacokinetic profiles *abs#161*

Gerhard Koekemoer

Description of Concurrent Class Analysis and its Application in Defining a Biosignature for Mitochondrial Respiratory Chain Deficiencies *abs#162*

Christian Krill

The Secondary Metabolism of Stagonospora nodorum *abs#163*

Masae Kuboniwa

Metabolome profiles in *Porphyromonas gingivalis* stimulated with exogenous para-aminobenzoate *abs#164*

Tien-Chueh Kuo

HMO: Human Metabolome Ontology *abs#165*

Miyako Kusano

Evaluation of metabolomics data quality using artificial biological gradients *abs#166*

Andrew Lane

The gluconeogenic pathway is operative in lung cancer cells *abs#167*

Brian Lankadurai

^{1}H NMR-based metabolomic analysis of the toxic response of *Eisenia fetida* after exposure to sub-lethal phenanthrene concentrations *abs#168*

Jeongae Lee

Environmental metabolomics approach to estrogen metabolism based on bisphenol-A concentration *abs#169*

Joomi Lee

Targeted metabolite profiling to evaluate the therapeutic effects of metformin in the plasma of healthy volunteers *abs#170*

Wi Young Lee

Stem girdling enhanced strobilus production in *Larix kaempferi* via metabolic alterations *abs#171*

Vladimir Likic

The mzML vendor-neutral data format for gas chromatography-mass spectrometry (GC-MS) *abs#172*

Ching-yu Lin

NMR-based metabolomics to examine naphthalene toxicity in mouse urine *abs#173*

Hui-Ming Lin

Metabolomic analysis of urine from interleukin-10-deficient mice supplemented with kiwifruit extracts *abs#174*

Zhiqian Liu

A LC-MS based method for accurate quantification of plant primary metabolites *abs#175*

Amanda Lloyd

Developing a data-driven framework for discovery and use of dietary exposure biomarkers in human epidemiological studies *abs#176*

Bih-Show Lou

Effects of exercise intensity and hypoxia in Human Urine Metabolites by LC-MS-based Metabolomics Analysis *abs#177*

Ben Macleod

Utilization of GCxGC-TOFMS to Screen for Potential Metabolite Differences in Pooled Samples from Lean, Fat, and Obese Zucker Rat Plasma *abs#178*

Abstract Withdrawn

abs#179

Garth Maker

Automated library generation for untargeted metabolomics studies *abs#180*

Rupasri Mandal

An update on Canada's national metabolomics platform - TMIC *abs#181*

Ross Mann

Metabolomic profiling of endophytic fungi for bioprotection outcomes in agriculture *abs#182*

Brian McCarry

HILIC-LC-ESI-TOF-MS Analysis of the Polar Metabolome of *Sinorhizobium meliloti* following Exposure to Fluorene *abs#183*

Aurelien Monge

Computer-Assisted Structure Identification: Predictive Models and Automation *abs#184*

Cherie Motti

Qualitative variation in disease states of *Ianthella basta* (Porifera: Verongida) *abs#185*

Ian Mullaney

Development of a GC-MS metabonomic method for metabolite profiling of streptozotocin induced diabetes in rat liver *abs#186*

Douglas Murray

The Dynamics of the Yeast Metabolome *in vivo* *abs#187*

Aminata NACOULMA

Metabolic modifications of Nicotiana tabacum L. (Solanaceae) infected by Rhodococcus fascians *abs#188*

Ryo Nakabayashi

Flavonoid-targeted analysis using UPLC-PDA-QTOF-MS in rice *abs#189*

Klaus Neidig

Dietary Supplements: Towards the Development of a Quality and 'Fingerprinting' Identification Tool Using Nuclear Magnetic Resonance (NMR) *abs#190*

Jason Neo

High resolution LC/MS/MS metabolomic analysis of gastric cancer cell lines *abs#191*

Dung Nguyen

Micro-oxygenation impacts on the chromatic characteristics and off-odour sulfur containing volatiles in a Bordeaux blend *abs#192*

Mitra Nouri Koupaei

Towards a better understanding of uremic molecules: Development of a capillary electrophoresis-mass spectrometry procedure *abs#193*

Zain Nurazah

Investigation of Metabolites from Oil Palm (*Elaeis guineensis*) Tolerant and Susceptible to Basal Stem Rot *abs#194*

Sean O'Callaghan

Common Ion Approach for accurate quantitation of GC-MS metabolomics data *abs#195*

Tatsuki Ogura

Effects of rice straw pretreatment on changing cellulosic supramolecular structure and improving digestibility for paddy soil microbiota *abs#196*

Jeonghyeon Park

An integrative pharmacogenomic-pharmacometabolomic study to predict cyclosporine response using systems pharmacology in human *abs#197*

David Peake

Metabolomic Profiling in Drug Discovery: Understanding the factors that influence a metabolomics study and strategies to reduce biochemical and chemical noise *abs#198*

David Peake

Robust Metabolite Profiling and Identification Employing High Resolution MS Strategies and Dedicated Software *abs#199*

Matthias Pelzing

Monitoring the effect of exercise in human urine samples by ^{1}H-NMR and ultra-high resolution CZE-ESI-TOF-MS based metabolomics *abs#200*

Eloise Pigott

Studying the metabolome of Ricinus communis for cultivar and provenance determination *abs#201*

Farhana Pinu

Sauvignon blanc metabolomics: Correlation between juice metabolite profile and wine volatile thiols *abs#202*

Kannan Ponnusamy

Microbial community and metabolomiccomparison of irritable bowel syndrome feces *abs#203*

Catherine Rawlinson

Defining the metabolome using GC-MS: - Impact of temperature gradient and scan rate in quadrupole systems *abs#204*

Marlon Reis

Dairy polar lipids alter brain lipid metabolism of C57Bl/6J mice under diet-induced stress *abs#205*

Marlon Reis

Unsupervised classification of multi-way data by combining Tucker model, hierarchical cluster analysis and heat mapping data visualization *abs#206*

Daryl Rowan

Exploring the metabolome of the interlenkin-10-deficient mouse *abs#207*

Thusitha Rupasinghe

LCMS lipid profiling of intra-erythrocyte stages and intracellular organelles of *Plasmodium falciparum* *abs#208*

Daniel Sachse

NMR Metabolomics investigation of drastic hypoxia in a neonatal pig model *abs#209*

Natsumi Saito

Metabolic shifts under the stringent response in *Escherichia coli* *abs#210*

Martin Schaut

Accurate Mass HILIC with High Perfromance TOF-MS for Metabolomics Analysis of Blood Plasma *abs#211*

Martin Schaut

Differential Metabolomic Analysis of Corn Leaf Extracts by Liquid Chromatography - High Resolution Time of Flight Mass Spectrometry (LC-HRT) *abs#212*

Martin Schaut

Comprehensive metabolomic profiling of Zucker rat plasma using LC and GC ultra high resolution time-of-flight mass spectrometry and GCxGC-TOFMS *abs#213*

Horst Schirra

Of Mice and Men – NMR-based metabonomics from Animal Systems to Clinical Science *abs#214*

Horst Schirra

This won't hurt a bit – NMR-based metabolomics in medical diagnosis of prostate cancer *abs#215*

Petr Simek

Alkyl chloroformate-mediated metabolite labeling and its application in GC/MS and LC/MS metabolomics *abs#216*

Constance Sobsey

Identification of biomarkers associated with the onset and progression of major metabolic and infectious diseases of transition dairy cows *abs#217*

Constance Sobsey

The Bovine Milk Metabolome. *abs#218*

Mark Styczynski

Ovarian Cancer Metabolomic Dynamics Using GCxGC-MS *abs#219*

Karolina Sulek

Metabolic footprint of *Lactobacillus acidophilus* NCFM at different pH *abs#220*

Abstract Withdrawn

abs#221

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Abstracts

ORALS

001

CE-TOFMS AT WORK IN MEDICAL, ENVIRONMENTAL AND AGRICULTURAL APPLICATIONS

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Institute for Advanced Biosciences of Keio University and its spin-out company, Human Metabolome Technologies Inc., form Japan's largest metabolomics research center in Tsuruoka city. The center possess twenty one (21) sets of ESI-TOFMS, five (5) Q-TOFMS, twelve (12) Q-MS, two (2) Ion-trap-MS, three (3) Orbitrap MS, three (3) Triple Q-MS/MS, one (1) MALDI-TOFMS, one (1) NMR, as well as thirty five (35) sets of CE, fourteen (18) sets of LC and nano-LC, and two (2) sets of GC/MS.

The center's main force is capillary electrophoresis time-of-flight mass spectrometry (CE - TOFMS) developed by Keio [1] . The technology can simultaneously quantify a large number of cellular metabolites ranged from 70 to 1,000 molecular weights , and is being appli ed to various fields of biotechnology in the post-genomic era, such as medical diagnosis (blood, urine, saliva, tissue), food science , and systems biology of model organisms [2].

We have recently discovered serum biomarkers of hepatotoxicity [3 ,4] and saliva biomarkers of oral, breast and pancreatic cancer [5]. Furthermore, metabolome data have been used to successfully confirm simulation results of red blood cell metabolism [6,7] and to discover c ancer-specific energy metabolism [8].

In the area of food science, we conducted metabolome analysis of soybeans [9] and sake (rice wine) and its correlation with taste [10].

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002

PLASMA LIPID PROFILING IN TYPE 2 DIABETES AND CARDIOVASCULAR DISEASE

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Introduction: The metabolic syndrome incorporating obesity, hypertension, dyslipidemia and elevated plasma glucose has reached epidemic proportions in many countries leading to an increased prevalence of type 2 diabetes (T2D) and cardiovascular disease (CVD). D yslipidemia, as assessed by standard measures (raised plasma triglycerides and LDL-cholesterol, and decreased HDL-cholesterol) is an independent risk factor for T2D and CVD . However, current risk prediction algorithms have limited accuracy. Further to this, t he mechanistic links between dyslipidemia, T2D and CVD are complex and not well understood. Lipidomics presents a new set of tools to address these issues.

Methods: We have developed a targeted lipidomics platform using liquid chromatography electrospray ionization-tandem mass spectrometry to profile 300-400 lipids from 10 m L plasma. We have applied this technology to define the plasma lipid profiles associated with T2D and CVD and evaluate the potential application of these profiles to predict disease risk.

Results: Binary logistic regression analysis adjusting for covariates (age, sex, systolic blood pressure and obesity) identified multiple lipid species that were significantly associated with T2D or CVD. Many of these lipids also displayed an association with disease severity suggesting that they are altered prior to the onset of acute stage disease.

Multivariate analysis by either recursive feature elimination using support vector machine learning or reliefF feature selection was employed to create and test multivariate classification models incorporating different numbers of lipids and other risk factors. Relatively few lipids (8-32) were required to achieve maximum classification accuracy. Models based on lipids generally performed better than models based solely on traditional risk factors.

Conclusions: Plasma lipid profiling can provide insight into disease pathogenesis and may contribute to a new approach to risk stratification for T2D and CVD.

003

METABOLIC BIOMARKER DISCOVERY AND CONFIRMATION BY USING METABONOMICS

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Metabonomics is a part of systems biology, it has shown the great potential of finding biomarker group for disease diagnosis. Generally, NMR or chromatography-mass spectrometry is used to analyze as many metabolites with the molecular weights smaller than 1,000 daltons as possible. Multi-variable data analysis methods are used to classify different groups, and define the significantly changed metabolites to produce new biomarkers. Unfortunately, at this moment, many studies are only in the discovery stage, the confirmation with large scaled samples is very poor, leading to the over-use of the word 'biomarker'.

In this lecture we shall report a two-stage metabonomics method. In the discovery step the typical samples are selected to define the differential metabolites by using the non-target LC-MS metabolic profiling analysis. In the confirmation step, large amount of samples with different clinical backgrounds are investigated by using the target analysis based on MRM monitoring to test the usefulness of the above differential metabolites, further define the potential biomarkers. The liver cancer and ovarian cancer will be taken as the examples to show our method. It will be seen the confirmation is a very necessary step, the poor specificity is a main disadvantage for the metabolic markers in the discovery stage, many differential metabolites are found to be influenced by different life styles or other diseases.

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004

SERUM METABOLOMICS BY GC/MS AS A NOVEL DIAGNOSTIC APPROACH FOR DISEASES

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Abstract not available at time of print

005

INVESTIGATING THE ROLES OF PPARΑ AND PPARΓ IN REGULATING THE BALANCE BETWEEN LIPID STORAGE AND OXIDATION IN ADIPOSE TISSUE USING A COMBINED *EX VIVO* AND *IN VITRO* METABOLOMIC APPROACH

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Interest in targeting the Peroxisome Proliferator Activated Receptors (PPARs) pharmacologically to treat the various symptoms of the metabolic syndrome increased after the discovery that the fibrates and thiazolidinediones act specifically via these receptors. Despite the success of these pharmacological agents in alleviating symptoms of dyslipidaemia and type II diabetes, their usage dramatically decreased due to emerging evidence of severe side effects, with several compounds subsequently withdrawn from sale. Due to safety concerns, ongoing research is focussed on selective modulation of PPAR activity to produce highly efficacious PPAR agonists with minimal toxicity. In this study we used a combined *in vivo/ in vitro* approach to assess the effects of a PPARpan drug, which targets all three PPARs, on lipid metabolism in white adipose tissue (WAT). Male Sprague Dawley rats were dosed with PPARpan agonist for 13weeks at 0, 30, 100, 300 or 1000 mg/kg/day (n=12 per group). Metabolites were extracted from WAT and analysed using GC-MS and LC-MS. FAME analysis by GC-MS revealed an increase in mono-unsaturated fatty acids, most notably oleate, the formation of which is regulated by steroyl CoA-desaturase (SCD)-1, a known PPARγ target. Furthermore there were dramatic reductions in the concentrations of several polyunsaturated fats, including α-linoleate, docosahexaenoate and eicosapentaenoate, consistent with increased fatty acid oxidation, a known consequence of PPARδ activation. LC-MS revealed that the observed changes were likely associated with alterations in the triacylglyceride pool with significant reductions in several specific TAGs observed. Concomitant changes in TCA cycle intermediates were observed through increased succinate, fumarate and malate. Similar changes were observed when experiments were repeated using 3T3-L1 adipocytes. Our global pharmacometabolomic approach demonstrates the PPARpan compound upregulates a range of core metabolic processes associated with both PPARγ and PPARδ activity, with a number of these pathways having beneficial effects on insulin resistance and dyslipidaemia.

006

QUANTITATIVE PROFILING OF OXYLIPINS VIA COMPREHENSIVE LC-MS/MS ANALYSIS: APPLICATION TO PATIENTS UNDERGOING CARDIAC SURGERY.

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Oxylipins, including eicosanoids, act as lipid mediators involved in inflammation and cellular growth processes. These metabolites are generated by oxidation of polyunsaturated fatty acids such as arachidonic acid. Prostaglandins, leukotriens and thromboxanes are the most broadly investigated representatives of this class of compounds. Moreover oxylipins encompass other important chemical classes including hydroperoxides, alcohols, epoxides, and diols. All these lipid mediators belong to an important class of target compounds while investigating the inflammatory response of certain diseases such as cardiovascular diseases. For the detection of oxylipin compounds we performed high performance liquid chromatography coupled to triple quadruple mass spectrometry using the multiple reaction monitoring. Besides eicosanoids produced from the omega-6 polyunsaturated fatty acid arachidonic acid, we included oxylipins derived from the omega-6 polyunsaturated fatty acid linoleic acid, dihomo-γ-linolenic acid as well as from the omega-3 poly unsaturated fatty acids α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid into our target library. Our analytical approach allows the detection and quantification of more than 100 oxylipin compounds down to nanomolar level. We applied our methodology to human plasma samples from patients undergoing cardiac surgery. We have investigated samples taken the day before (baseline) and 24 hours after cardiac surgery. Compared to baseline levels, we monitored changes in the levels of oxylipins derived from arachidonic acid and eicosapentaenoic acid after cardiac surgery.

007

MASS AND RELATIVE ELUTION TIME PROFILING: TWO-DIMENSIONAL ANALYSIS OF SPHINGOLIPIDS IN ALZHEIMER'S DISEASE BRAINS

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The sphingolipids are a diverse family of lipids that are major constituents of myelin and play important roles in the organisation of cell membrane microdomains, cell-cell recognition, and signal transduction. Structural diversity occurs in the lipid headgroup and the two fatty acyl chains, which range in length from 14 to 28 carbons, carry different degrees of saturation, and may be hydroxylated. The diversity of acyl chains complicates the quantification of sphingolipids by mass spectrometry.

Current lipidomic profiling methods rely mainly on mass spectrometry to identify unknown lipids within a complex sample. We describe a new approach, involving LC×MS/MS analysis of sphingolipids based on both mass and hydrophobicity, and use this method to characterise the sphingomyelin, ceramide, and galactosylceramide (GalCer) content of hippocampus from Alzheimer's Disease (AD) and control subjects. Using a mathematical relationship we exclude the influence of sphingolipid mass on column elution time, generating three-dimensional plots that facilitate accurate visualization and characterization of the different lipid moieties within a given sphingolipid class. Major brain GalCer species that differ in mass by only 0.04 Da were easily differentiated on the basis of their hydrophobicity, and could be positively identified with a triple quadrupole instrument.

The importance of our method's capacity to define all of the major GalCer species in the brain samples is illustrated with the novel observation that the proportion of GalCer with hydroxylated fatty acids increased approximately two-fold in the hippocampus of AD patients, compared to age- and gender-matched controls. This suggests activation of fatty acid hydroxylase in AD. Overall GalCer content declined approximately three-fold in AD hippocampus, indicative of myelin pathology. Our method provides a practical approach for accurate analysis of lipid content in complex biological extracts, without the need for chemical derivatisation of lipids or high resolution mass spectrometers.

008

AMBIENT AIR FOR AMBIENT IONIZATION MASS SPECTROMETRY: A METHOD FOR ASSIGNING DOUBLE BOND POSITIONS IN UNSATURATED LIPIDS

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Determining double bond positions in unsaturated lipids presents an important challenge for lipidomics. Our group has previously reported the reaction of ozone with ionized lipids within the confines of an ion-trap mass spectrometer allows the assignment of lipid double bond positions. [1]. While this has proven to be a powerful analytical approach it requires an expensive high-concentration ozone generator and a specifically modified mass spectrometer. Interestingly, ambient ozone - present in the troposphere within urban environments - has previously been identified as a source of oxidation when samples are exposed to the ambient laboratory environment [2] . In this study the surface analysis technique Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) is used to investigate the reaction of ambient ozone with unsaturated phospholipids deposited onto Teflon and silica thin-layer chromatography (TLC) plates and allowed to dry in the ambient laboratory environment. Products originating from ozonolysis of the deposited lipids can be observed after only 5 minutes following deposition and allow the unambiguous assignment of the double bond positions within the parent lipid. The abundance of these ozonolysis products is observed to increase with longer exposure times to the ambient environment prior to analysis. This technique is applied to the analysis of a human lens lipid extract where the individual lipid classes are first separated by TLC and the distribution of lipids analysed by directly imaging the TLC plate by DESI-MS. Along with a number of individual lipid classes being detected, ions corresponding to n-5, n-7 and n-9 double bond isomers in unsaturated dihydrosphingomylein and lactosylcermaide and n-9 and n-7 isomers in unsaturated phosphatidylcholine are observed. This work describes the powerful combination of DESI-MS and TLC in allowing both the lipid composition and double bond positions within lipids to be investigated simply by exposing the sample to ambient laboratory air prior to analysis.

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009

MARKERDB - THE BIOMARKER DATABASE

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A significant portion of today's "omics" efforts is directed at the identification of biomarkers. Traditionally most biomarkers consisted of just single molecules, genes or proteins. However, with the development of improved techniques, biomarker "profiles" or multi component biomarkers are now becoming more prominent. A challenge with biomarker identification and validation is knowing whether these biomarkers have been previously identified or whether they appear to be common to other conditions or diseases. Some biomarkers that have been newly "discovered" have only turned out to be biomarkers that had previously been noted, but not widely known. Because there is no central repository for biomarkers (as there is for genes or proteins) there is no convenient method to check for previously known biomarkers or to find related biomarkers to a given condition or disease. In this presentation I will describe our efforts to develop a centralized electronic repository on biomarkers -- called MarkerDB. MarkerDB includes diagnositc, prognostic and predictive biomarkers as well as chemical (metabolite), genetic and protein biomarkers. It also includes single and multicomponent biomarkers and biomarker profiles. Information on their reliability, sensitivity and specificity is also provided. We believe the development of a comprehensive resources such as MarkerDB is essential for the identification, selection and validation of novel biomarkers

MarkerDB is still under development, but it is expected to be completed by Sept. 2011.

010

METABOLOMICS ANALYSIS OF EXHALED BREATH CONDENSATE AND URINE OF ASTHMATIC CHILDREN

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Asthma is a heterogeneous disorder and one of the most common chronic childhood diseases. Several asthma classifications have been proposed in the past, based mainly on the description of symptom triggers, lung function or clinical features. Most cases of pediatric asthma are mild because they are well controlled by low-to-moderate doses of inhaled corticosteroids (ICS), but some asthmatic children have a severe disease characterized by persistent symptoms and/or frequent exacerbations despite high-dose ICS treatment combined with other drugs. An improved characterization of asthma phenotypes would be invaluable for the understanding of the pathogenic mechanisms and the correct treatment of this disease. A few years ago we investigated 1H NMR Metabolomics approach applied to exhaled breath condensate (EBC) in childhood asthma (Carraro et al., 2007). Recently we explored the potential of metabolomics applied to urine samples in characterising asthma (Mattaruchi et al, 2011). Urine samples of 41 atopic asthmatic children (further subdivided in sub-groups according to the symptoms) and 12 age-matched controls were analyzed. Metabolic profiles were collected by LC-MS, and studied by multivariate statistical analysis. The group of the asthmatics was differentiated by a model that proved to be uncorrelated with the chronic assumption of controller drugs on the part of the patients. The distinct sub-groups were also appropriately modeled. A reduced excretion of urocanic acid, methyl-imidazoleacetic acid and a metabolite resembling the structure of an Ile–Pro fragment was found for the asthmatics. These findings seems mainly correlated with the modulation of immunity in asthma. In conclusion, the Metabolomic analysis of urine has revealed the potential to characterize asthma and enabled the identification of metabolites that may have a role in the underlying inflammation.

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011

METABOLOMICS TECHNOLOGIES: WHAT CAN WE IMPROVE?

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Metabolomics shows great promise for studying health and disease. However, still one may wish to have better tools available for biomarker discovery and to zoom into mechanisms.

In this presentation several recent technological innovations will be discussed. New platforms such as CE-MS have been recently added to our set of routine methods. Improvements in often applied lipid profiling have been achieved. One important recent improvement was the structure elucidation of lipids to reveal the position of fatty acids and double bonds. Next, miniaturization of analytical platforms offer new possibilities and the use of microfluidics for the analysis of small sample volumes will be discussed.

Examples of biomarker discovery will be discussed, and strategies and examples how to use tracers to zoom futher into biological mechanisms.

012

COMBINING SHOTGUN LIPIDOMICS AND HPLC SEPARATION OF LIPIDS INTO A NEW WORKFLOW FOR TOTAL LIPID ANALYSIS

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We compare the qualitative and quantitative aspects of direct chip-based infusion (shotgun) with online HPLC-MS analysis followed by combining the advantages of both strategies to design a new platform for total lipid analyses.

Shotgun lipidomic analyses of liver or yeast lipid extracts were performed on a LTQ-Orbitrap or QSTAR coupled to a TriVersa NanoMate operated in infusion mode. For HPLC separation, lipid extracts were loaded on a YMC(PVA-SIL) 150mmx1.0mm column and separated using a ternary gradient at a flow rate of 45uL/min: A: 0.1%TEA+FA in Hexane/IPA (98/2); B: 0.1%TEA+FA in MTBE/IPA (45/55); C: 0.1%TEA, 0.2%FA in MeOH. Online LC MS analysis was performed on an LTQ-Orbitrap using the NanoMate in LC-coupling mode. For combining shotgun analyses with HPLC, separation was performed as before, but using a post column split such that one part was used for online analyses and other for parallel fraction collection.

Lipid extracts were analysed by shotgun lipidomics and online normal phase HPLC MS. For shotgun lipidomics we used MPIS analysis on the QSTAR and DDA analyses on the LTQ Orbitrap. Online HPLC MS analysis was performed by Full scan MS and DDA analysis on the LTQ Orbitrap. Comparing the shotgun and LC MS lipidomic datasets showed a differential quantitative overlap. This is due to the changing solvent composition during the LC analysis whereas shotgun analysis are performed with a constant solvent composition. Detection of some lipid classes and species was enhanced during the LC analysis whereas the intensity of other species was reduced. This adverse property bias the quantitative analysis if appropriate lipid standards are not employed. To address these discrepancies we combined the shotgun approach with LC fractionation. We performed parallel online LC MS analysis and fraction collection followed by shotgun analysis of relevant lipid fractions. Importantly, we could enhanced lipid coverage, the sensitivity and normalise the quantitative response by utilizing the same solvent composition for analysis of all fractions.

013

OPENFLUX: EFFICIENT MODELLING SOFTWARE FOR ^{13}C-BASED METABOLIC FLUX ANALYSIS

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The quantitative analysis of metabolic fluxes, i.e., in vivo activities of intracellular enzymes and pathways, provides key information on biological systems in systems biology and metabolic engineering. It is based on a comprehensive approach combining (i) tracer cultivation on 13C substrates, (ii) 13C labelling analysis by mass spectrometry and (iii) mathematical modelling for experimental design, data processing, flux calculation and statistics. Whereas the cultivation and the analytical part is fairly advanced, a lack of appropriate modelling software solutions for all modelling aspects in flux studies is limiting the application of metabolic flux analysis.
We have developed OpenFLUX as a user friendly, yet flexible software application for small and large scale 13C metabolic flux analysis. The application is based on the new Elementary Metabolite Unit (EMU) framework significantly enhancing computation speed for flux calculation. From simple notation of metabolic reaction networks defined in a spreadsheet, the OpenFLUX parser automatically generates MATLAB-readable metabolite and isotopomer balances, thus strongly facilitating model creation. The model can be used to perform experimental design, parameter estimation and sensitivity analysis either using the built-in gradient-based search or Monte Carlo algorithms or in user-defined algorithms. Exemplified for a microbial flux study OpenFLUX allowed in a very user-friendly fashion to automatically compile the EMU-based model from an Excel file containing metabolic reactions and carbon transfer mechanisms. It reliably reproduced the published data and optimum flux distributions for the network under study were found quickly (<20 sec).
OpenFLUX is a fast, accurate application to perform steady-state 13C metabolic flux analysis. It will strongly facilitate and enhance the design, calculation and interpretation of metabolic flux studies. By providing the software open source, we hope it will evolve with the rapidly growing field of fluxomics.

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014

COMPARING RESULTS OBTAINED FROM THE PATHWAY ACTIVITY PROFILING (PAPI) ALGORITHM WITH 13C-BASED METABOLIC FLUX ANALYSIS.

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Metabolomics has gained increased popularity in the last 10 years. This popularity comes from its use as a functional genomics tool and its diverse range of potential applications. However, metabolomics data sets are usually complex, difficult to interpret and challenging to correlate. The Pathway Activity Profiling (PAPi) (1) is a method developed in our group to correlate metabolite levels with potential metabolic pathways likely to be active inside the cells. This method uses the number of metabolites detected from each metabolic pathway and their relative abundances in the samples to predict the activity of different pathways. PAPi has been recently published and became very popular. However, PAPi consider that pathways which intermediate are found at lower intracellular levels are down-regulated compared to pathways with intermediates accumulating inside the cells. This assumption has been controversial, and, therefore, we propose to compare the results generated by PAPi with metabolic flux analysis of two different microorganisms: *Escherichia coli* and *Enterococcus faecalis*. Based in the fraction of ^{13}C-labelling distribution in the biomass, we estimated the flux through different metabolic pathways from the central carbon metabolism of these bacteria when growing under different environmental conditions. Metabolic flux analysis is the ultimate measurement of metabolic pathway activity, and should answer the question of whether or not the intracellular metabolic intermediates of a pathway accumulate when down-regulated.

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015

THE IMPACT OF BACTERIAL DIET IN *CAENORHABDITIS ELEGANS* NMR METABOLIC PROFILING STUDIES

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The nematode *Caenorhabditis elegans* became in the last years one of the most investigated animal models in the context of studies on aging. Among various approaches, NMR metabolomics investigations have emerged to characterize aging nematodes or long-lived mutant stains. Under standard conditions worms were grown on Normal Growth Medium (NGM) agar plates seeded with bacteria, which serve as food for the worms. After seeding the plates were in general used for several days to weeks. The aging of the bacteria on the plates implies a potential evolution in the quality of the nematode food, including viability of the bacteria and/or changes in their metabolite composition that may finally impact on the metabolism of *C. elegans* . Here, we present the impact of aging bacteria on the metabolic fingerprints of *C. elegans* using high-resolution magic angle spinning (HR-MAS) NMR as a whole organism profiling technique. The study was performed on *C. elegans* N2 wild type and *dys-1(cx18)* mutant strains. As nematode food source we investigated the *Escherichia coli* OP50 strain. O-PLS models built from 1H HR-MAS NMR data revealed a significant discrimination between worms grown on fresh or aged OP50 plates. The differences are mostly explained by an altered composition of the *C. elegans* lipid profile. While worms grown on aged NGM agar plates contain less monounsaturated fatty acids than those bred on plates freshly seeded with bacteria, they are shown to integrate exogenous cyclopropane fatty acids (CFA) in their lipid profiles. The CFA level can exceed 30% of the total amount of fatty acids in the worm, and come as direct intake from the bacterial food. Such findings are of primary importance in the general design of metabolomic studies on *C. elegans*, where biais or alteration in the lipid profiles, as influenced by the bacterial diet, should carefully be monitored.

016

MALDI IMAGING USING Q-FTICR MASS SPECTROMETRY: A VERSATILE TOOL TO TRACE SMALL MOLECULES DIRECTLY IN TISSUE

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MALDI tissue imaging is already used extensively in the protein biomarker field [i] , [ii] . However, in the low mass range up to m/z 1000 matrix clusters and chemical background dominate MALDI TOF spectra rendering specific analysis of small molecules difficult. Ultra-high resolution MALDI-Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometers can overcome this lack of specificity which is mandatory for small molecule imaging and identification [iii] , [iv] . FT-ICR (resolving power > 250,000) can resolve matrix from analyte peaks and enables the identification of compounds directly based on high accuracy molecular weight determination. This technology provides a comprehensive analysis compared to the more restricted MS/MS analyses required using TOF systems. Therefore, the MS-mode acquisition yields localized information for thousands of ions simultaneously within a single imaging run.

To boost sensitivity in targeted experiments a mass selective quadrupol can be used to limit the observed mass range to a few hundred Daltons and hence increasing the dynamic range for detection. Therefore, FTMS based small molecule imaging allows for identification and localization of low abundant endogenous metabolites, such as acetylcholine directly in rat brain tissue.

For reproducible matrix application a piezo driven nebulizer was used to prepare different tissue slices. Mass spectra were acquired with a 12 T FT-ICR mass spectrometer equipped with a switchable ESI/MALDI ion source. Using a 1 KHz laser with modified beam pattern spot sizes of >20 µm are achieved. MALDI images were acquired in positive and negative ion mode depending on tissue type and analytes of interest. Different examples of MALDI images of tissue will be presented.

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017

DEVELOPMENT OF PLANT METABOLOMICS FROM ARABIDOPSIS TO CROPS

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Metabolomics plays a major role in plant functional genomics and biotechnology[1]. We have established an excellent analytical platform of plant metabolomics based on the combination of multiple mass spectrometry (http://prime.psc.riken.jp/). Using Arabidopsis thaliana, integrated analysis of metabolome and transcriptome led to the prediction of gene-to-metabolite relations[2-5]. The Arabidopsis tissue-specific metabolome accumulation database, AtMetExpress, was constructed[6]. AtMetExpress was designed to be compatible with AtGenExpress to allow efficient elucidation of metabolite-transcript networks during tissue development. An excellent coverage of chemical diversity of our analytical platform is suitably applied to the crops improvement, e.g., metabolomic assessment of GM tomato[7], elucidation of functionality of rice gene[8] and prediction of rice agronomical and food traits. In this presentation the crucial roles of metabolomics in plant functional genomics and crop biotechnology will be discussed.

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018

METABOLOMIC APPLICATION ON THE MATERIAL BASIS AND THERAPEUTIC MECHANISMS STUDIES OF TCM

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Myocardial ischemia (MI) is a worldwide epidemic. Compound Danshen Tablets (CDTs), an herbal compound preparation, are widely used to treat MI in China. T anshinone IIA (T), s alvianolic acid B (S) and g insenoside Rb1 (G) are the three major active ingredients of CDT for its protective effects on myocardial ischemia (MI).

In this study, we aimed to explore novel biomarkers to increase the understanding of MI, investigate therapeutic effects of CDT, and synergistic effects of TSG (combination of T, S and G) by using metabolomic approaches.

Plasma extracts from sham, MI model, CDT-,TSG- and western medicines(isosorbide dinitrate, verapamil, propranolol, captopril, and trimethazine)-treated rats were analyzed by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC–Q- TOF-MS). The orthogonal partial least square (OPLS) model was built to find metabolites expressed in significantly different amounts between MI and sham rats. Meanwhile, partial least squares discriminant analysis (PLS-DA) was used to investigate CDT and TSG's protective effects.

The results showed that CDT presented protective effects on MI by reversing potential biomarkers to sham levels, especially for the four metabolites in the pathway of purine metabolism (hypoxanthine, xanthine, inosine and allantoin). And TSG brings nearly equal therapeutic effects on MI as CDF and it plays more stable regulated action on those identified metabolites than single compound.

019

A METABOLOMICS APPROACH TO UNLOCKING THE SECRETS OF THE EATING QUALITY OF RICE.

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Rice is the staple food for about 50% of the world's population. In spite of significant investment over the decades to increase overall rice yields, traditional, low-yielding varieties continue to be grown for their excellent eating quality. The difficulty faced by rice improvement programs is that tools to measure eating quality are not able to discriminate sufficiently between traditional varieties and high-yielding breeding lines developed to replace them. The eating quality of rice is defined by taste, flavour and textural traits. Assays, such as amylose content, gelatinisation temperature and gel consistency have been developed to select for textural traits, but screening for taste and flavour is not part of any rice improvement program. The objective of this work was to use a range of metabolomics platforms to try and differentiate three varieties of rice from Lao PDR. Two of the varieties are traditional, and one is an improved high yielding variety. Current quality evaluation tools could not discriminate these three, but unique metabolite profiles, including volatile compounds, primary and secondary polar compounds and minerals, were found for the grains of each variety, several of which have low enough thresholds to contribute to aroma or flavour. Each profile agreed with anecdotal descriptions of taste by Lao consumers. Genome-wide single nucleotide polymorphism (SNP) genotyping at 1536 loci was also carried out for the three varieties, and genetic distance correlated very well with the metabolite distances from the first and second principle components for volatile compounds, polar compounds and minerals. The unique metabolite profiles of aroma and flavour compounds indicate that metabolomics can reveal the compounds of eating quality, and the association between the metabolomic and SNP profiles indicates that metabolomic profiling can be used in mapping populations to identify new genes for eating quality.

020

METABOLOMICS STUDY OF THE WHEAT DEFENSOME UNDER PATHOGEN CHALLENGE – INTERLABORATORY GC/MS METHOD DEVELOPMENT

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Pathogens are a major threat to Australian grain production, causing significant production losses in Australia every year. A 2008 review by Murray and Brennan estimated that pathogens cost the Australian wheat industry $913 million per annum, making up 19.5% of the current crop value. Without current management practices these losses would be far greater.

Bioplatforms Australia has facilitated the generation of reference data infrastructure for wheat and wheat pathogens by co-funding Australia's contribution to the International Wheat Genome Sequencing Consortium (IWGSC) and the generation of reference data sets from the measurement of the wheat defensome under pathogen attack using Metabolomics, Proteomics, Transcriptomics and Genomics.

The Metabolomics analysis is being undertaken at multiple nodes of Metabolomics Australia, using GC/MS, LC/MS, CE/MS and NMR platforms for analysis. The GC/MS extraction and instrument method has been validated using unchallenged Chinese Spring samples across nodes of Metabolomics Australia using quadrupole GC/MS and GCxGC TOF MS platforms.

Wheat pathology laboratories across the country are challenging wheat with the major pathogens causing production losses and these samples extracted in house for analysis by Metabolomics Australia nodes to determine the wheat defensome compounds activated by each pathogen type. Data for the wheat defensome challenged by a key pathogen from the Western region of Australia will be discussed.

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021

APPLICATION OF METABOLOMICS TECHNOLOGY IN DAIRY CATTLE RESEARCH

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Although metabolomics technology has been used intensively in human and plant's research, during the last decade, only recently cattle researchers are starting to use this new technology in their investigations. In this presentation a summary of data from several ongoing projects at the University of Alberta and elsewhere will be presented. Research activities of our team include: 1) Determining dairy cattle metabolome. This project intends to identify and measure metabolites present in the rumen fluid and in blood, urine, and milk of dairy cows. Up-to-date we have identified and measured ~120 metabolites in the plasma, ~230 metabolites in the rumen fluid, and ~600 metabolites in the milk of dairy cows. Identification and measurements of metabolites in various fluids continues; 2) Identifying biomarkers of disease in the plasma and urine of periparturient dairy cows. This projects aims at identifying biomarkers of disease in the plasma and urine of periparturient Holstein dairy cows between 8 weeks pre- and 8 weeks post-parturition. Preliminary data indicated that several metabolites, related to lipid metabolism and inflammatory states, are increased significantly in the plasma of dairy cows 4-8 weeks before dairy cows develop clinical disease; 3) Identifying metabolite perturbations in the rumen fluid and milk of dairy cows that have been fed graded amounts of cereal grains. Results of this investigation demonstrated that feeding 30 to 45% barley grain to dairy cows was associated with increased concentrations of several metabolites in the rumen fluid that might be involved in the etiopathology of various metabolic diseases. Research continues to evaluate whether similar changes occur in the milk of dairy cows that have been fed increasing amounts of grains; 4) Comparing metabolomes of raw milk, pasteurized milk, homogenized milk, organic milk, and various skimmed milks of dairy cows. Data from this project will be presented in future meetings of the metabolomics society.

022

DEVELOPING A DATA-DRIVEN FRAMEWORK FOR DISCOVERY AND USE OF DIETARY EXPOSURE BIOMARKERS IN HUMAN EPIDEMOLOGICAL STUDIES

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Western diets are generally complex and conventional methods of measuring habitual dietary exposure such as Food Frequency Questionnaires (FFQs) depend upon food intake estimates and are subject to errors, which can confound interpretation of subsequent data. Descriptors of individual FFQ food components vary in degree of distinctiveness and consumption patterns generally typical of each food component display great variability, including effects of seasonality.

Against this background we have been exploring the use of metabolomics to help validate FFQ dietary component descriptors without prior knowledge of biochemical markers potentially indicative of habitual exposure to specific foods. Initially we demonstrated that non-targeted metabolite fingerprinting using Flow Infusion ESI-MS (FIE-MS) in conjunction with machine learning data analysis can be used to explore relationships between the chemical content of overnight or fasting urine and reported levels of citrus exposure in 24 humans consuming a freely-chosen diet (1). Fourier-Transform Ion Cyclotron Resonance MS (FT-MS) and tandem MS, followed by signal annotation using MZedDB suggested that correlated explanatory signals indicative of high citrus consumption were ionisation adducts of proline betaine (stachydrine) and hydroxyproline betaine (2).

In an expansion of this preliminary study we describe a high throughput, data-driven approach to explore the food consumption habits (>130 standard food components) of a larger cohort of free-living humans. Using FFQ information and FIE-MS analysis of fasting and/or overnight ‘spot' urines we identify food components that are well discriminated between groups of individuals reporting either high or low habitual consumption. Ultra-high accurate mass analysis and tandem MS has revealed potential biomarkers for a range of foods of high public heath significance (including red and white meats, specific fruit/vegetables and dairy products). The likely role and impact of the use of biomarkers on future dietary exposure monitoring in human epidemiological studies will be discussed.

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023

PROBIOTICS AND AGING: EVALUATION OF IMMUNOLOGICAL AND METABOLIC CHANGES

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The aging process is characterized by changes in the gut microbiota community and in the immune and metabolic system. Recent studies have shown that specific lactic acid bacteria and bifidobacteria can ameliorate immune functions in the elderly. Up to now only few data are available on the regulation on metabolism by probiotics in elderly. This work was conducted to assess the effects of a mixture of two probiotics: Lactobacillus acidophilus (La5) e Bifidobacterium lactis (Bb12) on mucosal immune system and metabolic phenotype of old mice, with the aim to find a possible correlation of the probiotic-induced changes between the immune and the metabolic systems in old mice. Old and young BALB/c mice received orally the two probiotic strains (1.5×109 CFU/day of each strain) or PBS for 4 weeks. Urine and feces were analyzed by high-resolution H-NMR spectroscopy, and the resulting data were processed using partial least squares-discriminant analysis (PLS-DA). Feces NMR-based metabolomic analysis identified a gut flora-associated metabolic phenotype that differed from that of young controls. Metabolic profile of old mice after probiotic administration: showed altered metabolites such as urocanate, methanol, acetone, dimethylamine, histidine and butyrate. Urine NMR-based metabolomic analysis identified a metabolic phenotype (metabotype) in probiotic treated old mice different from that of control mice, demonstrating a systemic effect on trans-methylation and trans-sulfuration processes. These changes may have an important role in regulating the metabolic functions of different cell systems, including lymphocytes. This work was partly supported by an Italian grant from MiPAAF, project Qualifu

024

THE METABOLOMIC CHARACTERIZATION OF CHANGES IN HUMAN MILK METABOLITES UNDER DIFFERENT STORAGE TEMPERATURE AND TIME

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The metabolomic profile of human milk is not well characterized compared with other biological fluids like blood, saliva or urine. To date there is no extensive study to determine the "normal range" of the metabolite profile for human milk. In situations where infants are unable to breastfeed, expressed breastmilk is the recommended alternative. This is of significant importance for pre-term infants where breastmilk has been shown to increase their survival rate. Thus, optimal storage time and temperature are required to maintain the nutritional and functional benefits of expressed milk, while preventing the growth of pathogenic bacteria and break down of essential metabolites.

Through gas chromatography coupled with mass spectrometry (GC-MS), our study aims to unravel the "normal range" of the metabolomic profile for human milk. To test the suitability of GC-MS for this study, a pilot study was undertaken to determine the milk metabolite profile under different storage temperatures over a 24-hour period. Milk expressed from 5 individual mothers, between 3 to 7 months post-partum was collected, aliquoted, then stored immediately either at room temperature, 4^{o}C, -20^{o}C and -80^{o}C before processing for GC-MS analysis. Initial results shows metabolite levels were found to vary over time at higher in contrast to lower temperature stored milk samples. This variation over time could be either positive or negative depending on the metabolite.

Variation in the metabolomic profile of the milk when stored at room temperature suggests metabolic modification of the metabolomic profile of the milk occurred presumably due of the cytosolic enzymes escaping into milk during the secretory process. This pilot study shows that milk needs to be stored at -20°C to retain its metabolomic profile.

025

ANALYZING BREAST CANCER METABOLISM WITH MULTIPLE ^{13}C/^{15}N-LABELED SUBSTRATES

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Stable Isotope Resolved Metabolomics (SIRM) is a powerful means to map metabolic transformations in complex networks, in response to changes in external conditions. We have used several different labeled precursors to map different aspects of metabolism of several breast epithelial cell lines including MCF-7, LCC2, ZR-75-1 (estrogen sensitive), MDA-MB-231 (estrogen insensitive) and primary human mammary epithelial cells (HMEC) in response to varied nutrient conditions and hypoxia using high resolution NMR and accurate mass FT-ICR-MS techniques (1-3). [U-^{13}C]-glucose and [U-^{13}C/^{15}N]-glutamine were used to trace atom flow through glycolysis, the pentose phosphate pathways, Krebs cycle and anaplerosis, nucleotide, glutathione and phospholipid biosynthetic pathways. Lipid biosynthesis and turnover was further characterized using [U^{13}C]-glucose, glutamine, glycerol, serine and octanoic acid precursors to determine the major sources of fatty acid and headgroup carbon in the different classes of glycerophospholipids and triglycerides.

Under standard growth conditions (normoxia, 5 mM glucose and 2 mM glutamine, RPMI), glucose is preferentially used for hexose pathways and the pentose phosphate pathway including ribose synthesis, as well as fatty acid and GPL biosynthesis. Glutamine is preferentially utilized for pyrimidine biosynthesis, glutathione biosynthesis (from Glu) and as a nitrogen donor in nucleobase biosynthesis. Glycerol was primarily used for GPL synthesis, and serine entered the phosphatidylserine lipids. Octanoic acid was both incorporated into lipids, and also oxidized to acetylCoA. Hypoxia and glucose deprivation changed the balance of different biosynthetic pathways, as revealed by the quantitative analysis of the isotopomers and isotopologues. Significant quantitative differences in metabolic activities were observed among the cell types that correlated partly with growth rates, but also with other phenotypic properties.

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026

ROUTINE METABOLOMIC ASSESSMENT IN PRECLINICAL DRUG DISCOVERY AND DEVELOPMENT

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Metabolomics has been part of pharmaceutical drug discovery and development for over a decade. While academic interest in metabolomics has been growing exponentially in the past few years, metabolomics groups in industry have been struggling to demonstrate cost effectiveness and impact to warrant continued investment in the technology. Recent data suggest that metabolomics has had a significant impact in four key areas in preclinical drug discovery: 1) Model evaluation 2) Early assessment of off-target effects. 3) Biomarker identification 4) Elucidation of mechanisms of efficacy or toxicity. Specific examples for all these application areas can be provided. The fasting rat, though a simple model, has consequences at the metabolite and transcript level that are not completely understood. Over 1/3 of all measured metabolites and approximately 10% of transcripts change with fasting. These changes have the potential to unintentionally obscure or exacerbate meaningful drug-induced metabolic changes. Off-target effects identified in early discovery metabolomic studies include effects on plant sterol absorption (putting drug-induced cholesterol increases in perspective) and increased urinary nucleosides as an unanticipated effect of drug treatment. Biomarker work includes the identification of gulonic and ascorbic acids as biomarkers of CYP induction and the repurposing of an old biomarker, 3-methyhistidine, for the evaluation of skeletal muscle toxicity. Comprehensive evaluation of biofluids from rats treated with phospholipidosis-inducing compounds reveals extensive phospholipid disruption including many phospholipid ethers and bismonoacylglycerol phosphates (BMPs) providing novel mechanistic insights and potential biomarker identification for this troublesome toxicity. Finally, one clear advantage industrial metabolomics groups have over academic groups is the sheer volume of work that is done allowing the establishment of metabolic databases providing context around individual metabolomic changes (e.g how usual are the "usual suspects"). We conclude that metabolomics has and will continue to play an important role in preclinical drug discovery and beyond.

027

MASS SPECTROMETRY AND MAMMALIAN-BASED STUDIES: INSIGHTS FROM THE HUSERMET PROJECT

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The study of mammalian metabolomes (cells, tissues, biofluids) is hugely important to expand our knowledge of molecular processes related to health, ageing and the onset and progression of disease along with the development and testing of efficacy and toxicity of experimental therapeutics [1]. Mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy have been the most influential analytical platforms applied in holistic metabolic profiling studies. Separately or combined these platforms can provide good metabolome coverage, reproducible data in small and large-scale studies and the tools to perform identification of metabolites. However, further developments are required to enable mass spectrometry platforms to acquire reproducible data in large-scale studies and in their ability to perform holistic metabolite identification.

The presentation will focus on research performed in the Husermet project (www.husermet.org), a collaboration between The University of Manchester, AstraZeneca and GlaxoSmithKline with the objective to characterise the serum metabolome of a large healthy UK-based population (n>3000). The application of quality control (QC) samples for signal correction and quality assurance will be discussed for the robust acquisition of data related to 1200 subjects [2]. Changes in the serum metabolome related to age, gender, BMI and clinical biochemistry parameters (e.g. blood pressure and liver enzymes) will also be described. The current limitations to metabolite identification will be described including the development of PUTMEDID-LCMS for the holistic and automated putative identification of accurate mass LC-MS metabolic profiling data [3].

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028

MEAT, MICROBES AND MAN: VOC-BASED METABOLOMICS SNIFFS OUT PATHOGENS AND DISEASE

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We have been developing a novel volatile organic compounds (VOCs) metabolomics approach which we have applied to the analysis of microbial contamination on meat [1], infected lesions in man [2], and for breath analysis [3]. These techniques are based on the capture of VOCs using polydimethylsilicone (PDMS) patches suspended in the headspace of the sample to be analysed or via an active process using an adaptive breath sampler based on a full face mask of the sort commonly used for non-invasive ventilation which contains Markes' thermal desorption tubes packed with Tenax/Carbotrap.

Once the compounds are trapped they are analysed via thermal desorption into a gas chromatography mass spectrometer. Data analyses are performed using in house algorithms via a two stage process: (1) multivariate analysis is first based on the TIC chromatograms, (2) after which regions of interest undergo a modified hierarchical multivariate curve resolution (H-MCR) procedure for curve resolution to generate concentration profiles and the corresponding pure spectra.

The presentation will introduce the various analytical and data processing developments and highlight their application to the detection of *Salmonella typhimurium* contaminated pork and the differentiation of chronic obstructive pulmonary disease (COPD) from healthy controls and those individuals suffering from asthma.

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029

THROUGH THE METABOLIC LOOKING GLASS: A WINDOW ON HEALTH AND DISEASE

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Abstract not available at time of print.

030

FUNGAL METABOLOMICS: CHALLENGES AND APPLICATIONS

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The relevance of filamentous fungi and yeasts in various applications spanning crop protection, food chemistry to pharmaceutical biotechnology, makes them ideal model systems for research. However, complete exploitation of fungi can be achieved by detailed understanding of their metabolism. This presentation will focus on the utility of metabolite analysis and its application in the discovery of new biosynthetic targets for crop protection agents, chemotaxonomy of the economically important fungal pathogen Rhizoctonia solani, and the study of fungal metabolism. Sclerotia, are resting vegetative structures of the pathogen that are not only important for its survival and pathogenesis, but represent a rich and challenging source of extra- and intracellular metabolites of various functions. We applied 1H NMR and GC-EI/MS analyses in order to strengthen our capabilities in identifying metabolites, for the study of the physiology of sclerotia development, the mechanism of sclerotia formation and chemotaxonomy of the genetically-based anastomosis groups (AGs) of R. solani species complex. The disaccharide α-α-trehalose was identified as the key metabolite of sclerotia that progressively increases with maturation. Known as storage carbohydrate in eukaryotic cells, its key role in adaptation to desiccation tolerance of cell membranes renders its biosynthesis in R. solani a promising target for crop protection strategies or metabolic engineering. Additionally, α-α-trehalose was among the major metabolite biomarkers that attributed to the discrimination and classification of thirty R. solani isolates into chemotaxonomic groups that are consistent with the known AGs concept, based on GC/MS profiling and footprinting. The developed chemotaxonomic approach can potentially be used as a complimentary routine method to the already existing molecular methods for Rhizoctonia taxonomy and possibly to other filamentous fungi. Finally, application of metabolomics revealed functional changes between the metabolomes of R. solani isolates capable of producing sclerotia and those lacking such ability, providing insights into the metabolic mechanisms influencing sclerotia development.

031

TACKLING THE UNKNOWN: DECISION TREE SUPPORTED SUBSTRUCTURE PREDICTION OF METABOLITES MONITORED BY GC-MS PROFILES

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Gas chromatography coupled to mass spectrometry (GC-MS) is a widely applied routine technology for the large scale screening and discovery of novel metabolic biomarkers. However, the majority of mass spectral tags (MSTs) recorded by this and other mass spectrometry-based metabolomic methods remains unidentified largely due to the lack of authenticated pure reference substances, which are required for unambiguous compound identification. We accessed the information on identified reference compounds stored in the Golm Metabolome Database (GMD) and applied supervised machine learning approaches for the classification of yet unidentified MSTs. We thereby extended conventional mass spectral and retention index library searches towards a more refined assessment of yet unknown MSTs. Structural feature extraction was applied to sub-divide the metabolite space contained in the GMD and to define frequently occurring substructures of metabolites which were suitable for our cheminformatic approach. Decision tree (DT)-based prediction of the most frequent substructures was performed using mass spectral features and RI information. We established a highly sensitive and specific analysis of biologically relevant sub-structures contained within the GMD compendium which returns potential substructures for yet unknown MSTs from GC-MS profiles together with probability criteria and the rules that led to the respective prediction. The underlying set of DTs can be inspected by the user and are made available via a new user friendly web-based interface to GMD. In addition batch processing via SOAP (Simple Object Access Protocol)-based web services is enabled.

032

NEW SOFTWARE TOOLS FOR METABOLOMICS FACILITATES MULTI-OMICS ANALYSIS OF *THE RICE RESPONSE TO BACTERIAL LEAF BLIGHT DISEASE.*

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As part of our continued efforts to integrate different data types to reveal more biology, we have developed sample preparation, chromatography and data analysis workflows to facilitate the analysis of metabolomics, genomics and proteomics data in our new Integrated Biology software. Ultimately these tools will help lead to a more comprehensive biological understanding of the measurements we have made. We used Bacterial leaf blight (BLB) of rice as a model for this work. It is a pathogen that gives rise to devastating crop losses in rice. Disease resistant rice cultivars are the most economical way to combat the disease. Initially we used metabolomics and transcriptomics profiling workflows for discovery analysis to contrast the responses of two rice varieties to the Philippine bacterial Xoo strain PXO99, expressing Ax21 and TP309_Xa21, a transgenic variety of rice harboring the Xa21 PRR, which is resistant to Xoo. Accurate mass compound identification with molecular formula generation (MFG) ranking of 355 masses was achieved with the METLIN database. GC-MS analysis yielded an additional 441 compounds, of which 154 were structurally identified by retention index/MS library matching. Multivariate statistics revealed distinct profiles for the susceptible and resistant genotypes. Many differential changes in the transgenic BLB resistant line relative to mock infected controls were observed . Acetophenone, xanthophylls, fatty acids, alkaloids, glutathione, carbohydrate and lipid biosynthetic pathways were all affected. Significant transcriptional induction of several pathogenesis related genes, as well as differential changes to GAD, PAL, ICL1 and Glutathione-S transferase transcripts was observed. Fold change ratios between transgenic and control samples for several of the protein enzymes tracked well with the gene expression ratios. Q-TOF MS/MS confirmation of metabolite identities was performed and verified with our new Metabolomics PCDL , containing over 2,200 curated spectra. Finally, the results of the multiple technologies were superimposed onto biological pathways simultaneously using a connector to Cytoscape.

(1) Metabolomic and transcriptomic analysis of the rice response to the bacterial blight pathogen Xanthomonas oryzae pv. oryzae

033

A METABOLOMICS APPROACH TO DETECT AN INFECTION OF WHITE MUSHROOM (*AGARICUS BISPORUS*) COMPOST BY THE GREEN MOULD (*TRICHODERMA AGGRESSIVUM*)

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In the Netherlands, substrate colonisation by mushroom mycelium (spawn-run) is performed in bulk at so-called compost yards. This is referred to as phase 3 composting. Recently, infection of the compost by *T. aggressivum* has caused major problems in the Dutch mushroom industry. *T. aggressivum* infects mushroom compost at a very early stage. During this process, spawned compost is incubated in tunnels and ventilated with large volumes of air to control compost temperature. At this time the compost tunnels are inaccessible for monitoring for potential contamination. Therefore, there is a need for a non-invasive method that allows early detection of a *T. aggressivum* infection. We have used an untargeted metabolomics approach by comparing the complex volatile blends emitted by *T. aggressivum* infected and non-infected mushroom compost. We used a glass enclosure model system containing 300 g spawned, phase 2 compost. Dynamic headspace samples were taken at different time points during the colonisation of the compost. Headspace samples were desorbed thermally and analysed by gas chromatography mass spectrometry (TD-GC-MS). Multivariate data analysis techniques were applied to interpret the data. Within the 14-day colonisation period, mushroom mycelium developed in the non-infected compost. In the infected compost, the compost turned black producing occasional tufts of white mycelium and green spores. Volatile blends that are produced in non-infected compost significantly differ from those emitted from *T. aggressivum* infected compost. Some of the volatiles appear to be specific for *T. aggressivum* infected compost. Infections with *T. harzianum*, *T. atroviride* or Smokey mould (*Penicillium citreonigrum*) produced volatile blends that differed from those emitted by non-infected or *T. aggressivum* infected compost. Significant differences between the volatile blends of infected and non-infected compost are visible few days of compost colonisation. Through this approach we aim to identify early markers for infection in a non-destructive approach.

034

A METABOLOMICS APPROACH TO ELUCIDATING THE RESPONSE OF WHEAT TO THE EXPOSURE OF *STAGONOSPORA NODORUM* EFFECTORS

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Stagonospora nodorum is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB). This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of proteins (known as effectors) which are internalised into host cells of sensitive wheat cultivars. These effectors interact via an unknown mechanism with a dominant wheat sensitivity gene inducing tissue necrosis resulting in disease. The genes encoding three of these effectors, SnToxA, SnTox1 and SnTox3 have been fully characterised. Direct infiltration of these proteins into wheat leaves is sufficient for the development of disease-like symptoms providing an ideal system in which to study the plant response to these effectors. We have applied a metabolomics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. GC-MS analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To obtain further understanding of the host response to SnToxA at the secondary metabolite level, samples were also analysed using LC-Q-ToF-MS. These complementary approaches have provided a novel insight into how the SnToxA effector protein contributes to disease.

035

INVESTIATING ABIOTIC STRESS TOLERANCE IN PLANTS USING METABOLOMICS

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A plant's survival and reproduction is entirely dependent on its ability to adapt to an unpredictable and dynamic environment. We are using metabolomics as a functional genomics approach to increase our understanding of how plants develop mechanisms to adapt to or tolerate abiotic stresses. A clear understanding of these mechanisms will provide opportunities to generate new stress tolerant crops that will at least partly overcome the dramatic yield losses currently experienced in cereal crops. Examples presented will include in-depth investigations of the metabolite profiles of wheat, barley and *Arabidopsis* genotypes, characterised by different stress tolerance mechanisms, grown under conditions that simulate drought or salinity in the field compared with control plants. The global comparison of abiotic stress effects with changes in the metabolic networks allowed us to identify metabolites which were affected by drought or salinity in the different genotypes, as well as genotype-specific changes in metabolites. These changes are correlated with the relative tolerance levels to drought or salinity of each of the genotypes, allowing the identification of stress-tolerance related metabolite patterns.

036

METATT - A WEB-BASED METABOLOMIC TOOL FOR ANALYZING TWO-FACTOR AND TIME-SERIES DATA

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Time-series and multi-factor studies have become increasingly common in current metabolomics researches. The common tasks for analyzing data from these complex study designs include identification of major variations associated with each factor, comparison of temporal profiles across different biological conditions, as well as detection and validation of the presence of interactions. To address these challenges, we have implemented MetATT, a user-friendly, web-based tool dedicated to two-factor and time-series metabolomic data analysis. MetATT offers five different methods based on advanced data overview techniques, univariate and multivariate statistical algorithms. In particular, it provides interactive 3D PCA and two-way heatmap visualization, two-way ANOVA for between- and within-subjects analysis, ANOVA-simultaneous component analysis (ASCA), and multivariate empirical Bayes time-series analysis. The complex procedures are presented in a simple, stepwise manner through intuitive web interfaces. At the end of each session, a comprehensive analysis report will be generated which gives the background introduction of each method embedded with the corresponding graphical and tabular outputs. MetATT is freely available at http://metatt.metabolomics.ca.

037

PRINCIPAL VARIANCE COMPONENTS ANALYSIS: A NOVEL APPROACH TO QUANTIFY COMPOSITIONAL AND METABOLOMIC RESPONSES

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Advances in metabolomic technologies allow large volumes of data to be generated from designed experiments having multiple sources of variation. Improved approaches to assessing and visualizing sources of variability in metabolomic datasets will enhance the value of such experiments. The need for a robust, quantitative approach to analyze experimental effects on compositional and metabolomic variation has prompted the development of principal variance components analysis (PVCA, Scherer, 2009). This is a sequence of data analysis methods that summarizes the variation among analytes and measures the relative contributions of the effects in the experimental design to variation in the biological systems. First, principal components analysis is used to summarize the variation among many correlated analytes with a smaller number of variables. Next, factor analysis is applied to those principal components to identify the individual analytes that contribute to variation more effectively. Finally, variance components analysis estimates the relative contributions of the effects in the experimental design to the variation in each factor. The results are easy to interpret, since they are expressed with percentages of total variation explained by the factors and by the experimental effects. In a multilocation field trial for soybean composition, PVCA was used to show that the primary contributor to variation in composition was protein and amino acid-related metabolism, and this variation was primarily influenced by differences in germplasm. Additional steps needed to apply PVCA to more complex metabolomics data sets that contain large numbers of analytes and missing values will also be described.

(1) Scherer, A. (ed.). 2009. Batch Effects and Noise in Microarray Experiments: Sources and Solutions. John Wiley & Sons, West Sussex, United Kingdom.

038

STRUCTURE HUNTER: PREDICTION OF NOVEL CHEMICAL STRUCTURES IN A MIXTURE

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Identification of individual chemical constituents in a mixture solution, such as medicinal plants or bio-fluids, is a time-consuming task. In addition, the identification results are limited with available reference mass spectra. Here we present a new approach to efficiently and accurately predict individual components in a mixture by utilizing collections of natural products and a set of known reference compounds as "seeds". For an experiment, structure hunter accepts the peak table of the mixture sample from either GC-MS or LC-MS spectra and seeds information such as name or chemical structures of the presumable components in the mixture. While the seeds information may not be available, structure hunter automatically assigned seeds from the database. Aside from the user inputs, structure hunter includes a natural products scaffold relationship database containing scaffolds, the major chemical scaffold classifications and the parent and sibling relationship between each class of the natural products. A children scaffold contains chemical structure of its parent. The sibling scaffolds are scaffolds with same numbers of ring and similar size. The parent and sibling scaffolds of the seed along with seeds itself form the list of scaffolds for further comparison. Structure hunter computationally formulates possible chemical structures by extending the list of scaffolds with a weighted list of side chains from analyzing the collections of natural products. Therefore, the multiple components in a mixture spectrum will be proposed by matching to the most probably chemical structures of the natural products and its analogous. Compared to the previous developed method with heuristics rules or chemical structural search to generate structure, our method generates structures corresponding to their frequency in nature. Multiple natural products mixture samples were used to validate the efficacy of our approach. Scaffold Hunter predicts structures efficiently and outperforms other approaches in terms of accuracy.

039

COMPUTER-ASSISTED STRUCTURE IDENTIFICATION (CASI)—A MASS SPECTROMETRY-BASED AUTOMATED PLATFORM FOR HIGH-THROUGHPUT IDENTIFICATION OF SMALL MOLECULES BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY − MASS SPECTROMETRY

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Recent developments in non-targeted screening techniques, such as metabolomics and comprehensive small molecule screening in complex matrices using mass spectrometry techniques, result in huge amounts of data. While several software packages are available to automatize the data processing, the data evaluation and compound elucidation has to be done manually. However, manual interpretation is both time-consuming and subjective. Another drawback is that additional qualifiers, such as Kovats indices, are not always available in databases . Therefore, we designed and developed an automated platform which we call Computer-Assisted Structure Identification (CASI) to accelerate and standardize the identification of compound structures.

First, mass-spectra are searched for structural candidates and their associated match factors using an algorithm of NIST MS Search. Second, we developed quantitative structure − property relationship (QSPR) models that predict essential parameters to enhance the confidence in the compound identification. Kovats indices for 1st dimension (1D) separation as well as relative retention times for 2nd dimension (2D) separation are predicted. For the relative retention time model, we developed a new concept. Within each measured analysis, the 2D absolute retention time for each compound was referred to a hypothetical n-alkane, whose retention time was derived from the regression function of a deuterated n-alkanes reference system. In addition, boiling points were derived from 1D separation and matched to computationally predicted boiling points of the structural candidates. Finally, CASI combines the match results of NIST MS search and all parameters predicted in QSPR models and to generate a score of confidence. False positive identifications are minimized by ensuring that absolute score values exceed a threshold. In addition, CASI gives an estimate of discriminating the first hit structural proposal from alternative proposals.

In conclusion, we have developed a web interface which allows the scientist to consult the list of best-matched structural candidates for each analyte (highest CASI Scores).

040

REZA SELAK - EBI INITIATIVE: METABOLOMICS DATABASE

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The MetaboLights project aims to provide a comprehensive metabolomics based community resource. There are currently several metabolomics databases, which cater either to a specific analytical technique or focused on a certain species such as plant or yeast. The MetaboLights, will be cross-species, cross-platform and cross-application database, covering wide variety of experimental details, metabolite structures and their reference data with the drive to make data open access. Our secondary aim is to develop a curated reference layer with spectroscopic/spectrometry, chemical and biological information about metabolites. This work is collaboration between Cheminformatics and Metabolism team headed by Christoph Steinbeck at the EBI, in collaboration with Jules Griffin's lab at the University of Cambridge. This project is funded by Biotechnology and Biological Sciences Research Council (BBSRC) in the UK.

041

A LONGITUDINAL STUDY OF METABOLOMIC CHANGES IN MATERNAL PLASMA AND URINE DURING NORMAL PREGNANCY USING GC-MS AND UPLC-MS METABOLIC PROFILING.

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Pregnancy induces a unique progression of maternal metabolic changes. These changes have a single purpose - to enable a fetus to grow from a mass of cells into a complex living organism by the time of its birth. The main conduit for the exchange of metabolites between mother and fetus, is the placenta; which in itself independently engenders maternal metabolic change as it grows and adapts in harmony with the fetus. Controlled regulation of the metabolic interactions between mother, placenta and fetus is fundamental to the development of a healthy baby and equally important in maintaining a healthy state of homeostasis in the mother. It is logical to expect a continuous and systemic change in maternal metabolism throughout pregnancy; however, extreme perturbation of certain metabolites, whatever the cause, may result in adverse outcomes for both mother and/or fetus. A systems-wide understanding of metabolic changes during normal pregnancy will be essential when investigating molecular biomarkers for abnormal outcomes. The study presented here is a longitudinal study of 30 nulliparous women with a singleton pregnancy whom went on to have a normal healthy baby. Plasma and urine samples were collected at the first visit to the maternity hospital at 15 weeks, then further sampled at 20, 24, 28, 32, 36, 40 weeks and 6 weeks postnatally. Urine samples were analyzed using untargeted GC-MS and plasma samples were analyzed using untargeted UPLC-MS. Metabolic profiling exposed comprehensive disruption of both the urine and plasma metabolome throughout normal gestation, and described significantly different profiles between the antenatal and postnatal periods. In addition, correlation analysis, revealed how the dynamic nature of the metabolic change is dependent on an individual's unique physiology and personal exposure to environmental factors. The understanding of personalized metabolic changes during pregnancy may aid the development of personalized intervention strategies for pregnancy complications.

042

SERUM UPLC-MS/MS METABOLIC PROFILING IN EXPERIMENTAL ANIMAL MODELS AS A TOOL TO REVEAL BIOMARKERS FOR LIVER TOXICITY

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Drug-induced liver injury (DILI) remains a major cause of worldwide mortality and represents a serious clinical and financial problem because it is the single greatest cause of attrition in drug development and withdrawal of approved drugs from the market . Currently, besides liver biopsy the activity of a series of hepatic enzymes and proteins released into the blood by damaged liver cells are the most useful tools available in detecting liver damage in a non-invasive manner. The inherent deficiencies and limitations in sensitivity or specificity of current hepatic screening test preclude the use of any one marker as the "standard" for detecting liver damage or DILI. The development of novel diagnostic biomarkers with greater sensitivity and specificity can enhance the efficiency in the detection of low-degree liver damage, increasing the confidence of drug safety for release into the market. Metabolomics –a comprehensive study of global metabolites- has become a highly sensitive and powerful tool for biomarker discovery thanks to recent technological advances. We are currently applying ultra-performance liquid chromatography/time-of-flight tandem mass spectrometry (UPLC/TOF MS/MS)-based metabolomics to investigate sera from different animal models to find highly sensitive and specific biomarkers for liver injury. Our results indicate that serum levels of many membrane lipids including mono and diacylglycerolphospholipids, and sphingomyelins were significantly affected in liver injury conditions. Remarkably, a number of them were identified by using exact mass measurements, and found to be in high correlation with the degree of liver cell death as assessed by histological examination. Our results constitute a clear support to use UPLC-MS-based metabolomics in combination with animal models to identify non-invasive biomarkers for liver damage.

043

CAPILLARY ELECTROPHORESIS MASS SPECTROMETRY-BASED METABOLOMICS REVEALS ETHANOLAMINE PHOSPHATE AS A MAJOR DEPRESSION BIOMARKER

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Despite significant research efforts aimed at understanding the neurobiological underpinnings of psychiatric disorders, the diagnosis of these disorders are still based solely on subjective assessment of symptoms. Therefore, molecular markers which could improve the current classification of psychiatry disorders, and in perspective stratify patients on a biological basis into more homogeneous clinically distinct subgroups, are highly needed. In order to identify novel molecular markers for major depression, we have applied a capillary electrophoresis mass spectrometry (CE-MS)–based metabolomics using plasma samples. Patients were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV and a number of additional clinical variables were assessed. Plasma samples from 34 depressed patients and 38 controls were submitted to the metabolomic profiling allowing the evaluation of up to 538 metabolites, including a series of neurotransmitters and their metabolic intermediates. Statistical analysis highlighted ethanolamine phosphate (EAP) as high-sensitive biomarkers which ROC-area under curve were 0.87. The marker was validated by samples from another medical center and the level of the marker were above threshold level in patients in remission. The results illustrate the potential of plasma metabolomics for psychiatry research, and highlighted the metabolite as candidate biomarker for major depressive disorder, warranting further investigation in larger independent collections. All the participants included in this study were given written informed consent after the complete explanation of this study which is approved by ethical committee of National Center for Neurology and Psychiatry.

044

USE OF RECONSTITUTED METABOLIC NETWORKS TO ASSIST IN METABOLOMIC DATA VISUALIZATION AND MINING

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High-throughput metabolomic experiments aim at identifying and ultimately quantifying all metabolites present in biological systems. The metabolites are interconnected through metabolic reactions, generally grouped into metabolic pathways. Classical metabolic maps provide a relational context to help interpret metabolomics experiments and a wide range of tools have been developed to help place metabolites within metabolic pathways. However, the representation of metabolites within separate disconnected pathways overlooks most of the connectivity of the metabolome. By definition, reference pathways cannot integrate novel pathways nor show relationships between metabolites that may be linked by common neighbours without being considered as joint members of a classical biochemical pathway. MetExplore [1] is a web server that offers the possibility to link metabolites identified in untargeted metabolomics experiments within the context of genome scale reconstructed metabolic networks. The analysis pipeline comprises mapping metabolomics data onto the specific metabolic network of an organism, then applying graph-based methods and advanced visualization tools to enhance data analysis. The MetExplore web server is freely accessible at http://metexplore.toulouse.inra.fr.

(1) Cottret, L.; Wildridge, D.; Vinson, F.; Barrett, M. P.; Charles, H.; Sagot, M.-F. & Jourdan, F. MetExplore: a web server to link metabolomic experiments and genome-scale metabolic networks. *Nucleic Acids Res*, **2010**, 38, W132-W137

045

CHALLENGES FOR METABOLOMICS STUDY DATABASES

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As of 2011, metabolomics research can be facilitated by many online metabolomics-related databases. Numerous examples exist of metabolite/compound databases, consensus spectral libraries, and metabolic pathway databases. These resources have enabled the field of metabolomics to establish itself as a valuable emerging field of inquiry. However, relative to other *omics disciplines, the metabolomics field contains a paucity of online venues containing queriable study data and metadata. Likewise, only a few primitive tools exist for the exchange and comparison of spectral data for both known and novel compounds. Until these tools are developed, it is unlikely that the field as a whole can establish the precise metabolites that are the metabolic footprints of the pathways being investigated. Moreover, without standardized tools and repository schemata, it seems doubtful that research into the relative involvement and permutations of specific metabolic pathways in various health and disease phenotypes can progress beyond individual investigator-collaboration silos.

In order to advance to a point where knowledge stores of metabolic pathways can be interrogated for manipulation and intervention, the field of metabolomics needs standardized repositories of comparable results from metabolomic studies, methods of spectral data exchange that enable consensus metabolite resolution across multiple metabolomic platforms, and harmonization of the knowledge of pathways and their derived metabolites. Reflecting on recent workshops at NIH in December 2010 and EBI in March 2011, that identified the above concerns, we present here the challenges of designing and implementing such repositories and concomitant infrastructures. We also present MetaboKnow, a metabolomics community web portal aimed at organizing the worldwide metabolomics community around these issues.

046

"IONTREE"-- AN R PACKAGE FOR DATA MANAGEMENT AND ANALYSIS OF ION TREES FROM ION TRAP MASS SPECTROMETRY

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Metabolomics concerns identification and quantification of all the metabolites in a biological system and its association with other functional genomics. Coupled with chromatography mass spectrometry has become a dominant technical platform in metabolomics to achieve the strategic objectives. Despite recent advances in mass spectrometry structural elucidation of identified peaks, which are often described by mass-over-charge ratio (m/z) and its elution time, remains a challenging problem. Soft ionisation such as MALDI and ESI allows direct analysis of polar and thermally labile biomolecules in their intact form. Wider range of m/z can be measured in full scan and collision-induced dissociation (CID) fragmentation of selected precursor ions could assist structural inference on many classes of biomolecules. Although ESI MS/MS spectra are routinely used for peptide sequencing fragmentation spectra have not been systematically studied in metabolomics. There is no common computational framework to process, store, retrieve and analyse fragment spectra for metabolite identification in high throughput metabolomics experiments.

We developed a computational framework to capture, store and analyse MS^n spectral data in high throughput metabolomics. Utility functions are built as an R package "iontree". We present in this talk the overall framework of this system, functions to identify and comparatively investigate all the quality spectral trees collected from experiments, tools for annotating ions/peaks derived from DIMS and LCMS and integration to public (MassBank, HMDB) or commercial (NIST) MS/MS spectral databases.

047

THE FASTED/FED ACETYLOME COORDINATES ORGAN SPECIFIC FUEL SWITCHING: METABOLOMIC AND FLUXOMIC VALIDATION

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The elucidation, and functional importance, of extra-nuclear lysine acetylation in metabolic networks has been of growing interest, as the co-substrate for acetylation, acetyl CoA, is at a key metabolic intersection. Our hypothesis was that mitochondrial and cytoplasmic protein acetylation may be part of a fasted/re-fed feedback control system for the regulation of the metabolic network in fuel switching, where acetyl CoA could be provided by fatty acid oxidation, or glycolysis, for example. To test this we utilized a cross-omic approach, combining proteomic, metabolomic and fluxomic profiling. The mitochondrial and cytoplasmic acetylome in various organs that have a high metabolic rate relative to their mass, and/or switch fuels, was characterized under fasted and re-fed conditions (brain, kidney, liver, skeletal muscle, heart muscle, white and brown adipose tissues). Using immunoprecipitation, coupled with LC-MSMS label free quantification, we show there is a dramatic variation in global quantitative profiles of acetylated proteins from different organs. Fluxomic and metabolomic profiling, combined with structural visualization of the modified lysines in the proteins profiled, allows hypotheses to be made as to the functional impact of the modified lysines on the proteins affected, and on the metabolic network.

048

MULTIVARIATE PAIRED DATA ANALYSIS (MVPDA) REVEALS UNIQUE METABOLIC FINGERPRINTS UNDERLYING SURGICALLY-INDUCED OSTEOARTHRITIS IN SHEEP

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Characterising the metabolic response to an intervention can be challenging due to the large inter-subject variation. Because traditional chemometric approaches such as OPLS-DA do not take into account the paired data structure in these studies, we have adopted multivariate paired data analysis (MVPDA) to enhance the recovery of metabolic biomarkers from sheep subjected to surgically-induced osteoarthritis (OA). Sheep underwent one of three types of surgical procedure (sham (control), meniscal destabilisation, MD or anterior cruciate ligament transaction, ACLT), and for every animal a serum sample was collected prior to being operated on and at sacrifice. 1D 1H NMR spectra were acquired from each sample at 800 MHz. Results from traditional chemometric techniques (PCA, OPLS-DA) were compared and contrasted with MVPDA, which displayed enhanced classification and interpretability. MVPDA showed all types of surgical procedure were associated with elevated lactate and deceased TMAO/betaine. Serum from sheep that underwent ACLT was additionally characterised by elevated branched-chain amino acids (BCAAs) and decreased histidine, consistent with previous findings. There was no observable change in BCAAs for the MD cohort indicating that different OA subtypes were associated with unique metabolic fingerprints. This study reinforces the utility of MVPDA for datasets with a paired structure, and offers new insights into the metabolic consequences of OA.

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A NON-TARGETED GLOBAL METABOLITE PROFILING USING HIGH RESOLUTION ACCURATE MASS SPECTROMETRY, TRIPLETOF™ 5600 SYSTEM, TO IDENTIFY NOVEL MAKERS IN MATERNAL SERUM SCREENING FOR TRISOMY.

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Introduction:

Non targeted metabolite profiling has growing importance in metabolomics as it is a fully comprehensive survey of all endogenous metabolites for a given sample type. It is very important to have a systematic data processing strategy in order to fully benefit the huge amount of data collected using non-targeted approach on HRMS instrument. We demonstrate the use of global metabolite profiling to identify potential markers in first trimester maternal serum for trisomy. Maternal serum samples from normal (20) and those with a trisomy 21 (14) fetus were used to identify any potential endogenous metabolite differences between the two groups using a single injection non-targeted data acquisition with multiple data processing strategies.

Methods:

Briefly, serum samples were extracted using methanol and chloroform (2:1) ratio. Ultra high pressure liquid chromatography (UHPLC) coupled to high resolution accurate mass analysis was performed on a TripleTOF™ 5600 system in both positive mode and negative mode. Statistical data processing was performed using MarkerView™ software and targeted data analysis for endogenous metabolites was processed by PeakView™ and XIC manager software. From the same data file, lipid profiling was also achieved using LipidView™ software.

Preliminary data:

Principal component analysis (PCA) showed marked differences between individuals with a normal pregnancy and those with trisomy 21. Univariant analysis, t-test highlighted most significantly variable metabolites with log fold change value and p value. Since both TOF MS and MS/MS were acquired simultaneously in single injection, accurate mass parent ion is used to identify the biomarker and MS/MS was used for confirmation and structural elucidation of the biomarker.

Conclusion:

This is the first report of a metabolomics approach using high resolution accurate mass spectrometry to identify new set of potential markers to discriminate between a normal and trisomy pregnancy. Further details of the results will be discussed in the presentation.

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METABONOMICS OF MALARIA: ALTERATIONS RELATED TO TRANSITION OF NON-CEREBRAL TO CEREBRAL COMPLICATIONS

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Cerebral malaria is a life threatening disease caused by Plasmodium leading to death of several thousand per year. Not all individuals infected with the parasite come down with the cerebral complications of the disease. Murine malaria exhibits similar pattern and only a fraction of the infected animals show symptoms of cerebral stage. However, the fraction of animals transiting into cerebral malaria vary widely across labs suggesting that host parameters contribute significantly in the disease progression. The infection in the host triggers a cascade of events in different tissues as the host tries to maintain homeostatic condition. Here we have investigated the tissue level changes in metabolites using 1H NMR spectroscopy of brain, liver, serum and pleural effusion of mice with non-cerebral (NCM) and cerebral malaria (CM). Multivariate analyses such as OPLSDA and multiway PCA (MPCA) were used to investigate the interactions of these tissues/biofluids. Serum of mice with CM showed higher levels of triglyceride-cholesterol, VLDL-cholesterol, total cholesterol and lipoproteins in comparison with NCM. The mice with CM exhibited accumulation of pleural fluid. Furthermore, glucose in pleural effusion was negatively correlated with that in serum. This pleural effusion composed of significantly higher glucose concentration than the serum glucose level. These two body fluids had contributions to each other. In comparison to the uninfected controls the brains of mice with NCM showed decreased choline, glycerophosphorylcholine and phosphatidylcholine while those of CM had, in addition, less of GABA, myoinositol, taurine and leucine and enhanced levels of glutamine and pyruvate. A comparison of liver of mice with NCM and CM indicated that the latter had enhanced levels of histamine, betaine and myoinositol. The significance of these and other results will be presented.

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DYNAMIC METABOLOMICS

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In metabolomics, time-resolved, dynamic or temporal data is more and more collected. The number of methods to analyze such data, however, is very limited and in most cases the dynamic nature of the data is not even taken into account. This talk reviews current methods in use for analyzing dynamic metabolomic data. Moreover, some methods from other fields of science that may be of use to analyze such dynamic metabolomics data are described in some detail. The methods are put in a general framework after providing a formal definition on what constitutes a 'dynamic' method. Some of the methods are illustrated with real-life metabolomics examples.

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APPLICATION OF MULTI-BLOCK DATA FUSION TECHNIQUES USING 1H HR-MAS NMR, GC-MS AND LC-MS METABOLOMIC PLATFORMS FOR THE STUDY OF BREAST CANCER

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Breast cancer is a heterogeneous disease, consisting of several different subtypes with varying molecular and biological profiles. The aim of the METAcancer project (www.metacancer-fp7.eu) is to investigate metabolites that can be used as prognostic and predictive biomarkers by applying different metabolomic technologies including NMR, GC-MS and LC-MS. Here we have implemented a range of different data fusion techniques to elucidate the combined potential of the multiple profiling techniques for the diagnosis and classification of breast cancer. Compared to commonly used correlation methods, Multi-Block approaches not only allow fusion on the actual data level, but also are capable of analysing data from multiple platforms simultaneously whilst maintaining the underlying block-wise structure[1]. Multi-Block PCA and PLS models can be regarded as extensions of the traditional approaches, with the difference that the analysis is carried out on two levels of the individual "block" and on the so-called "super-level". Results generated from MB-PCA and MB–PLS can be inspected in the "super score" plots allowing a straightforward visual inspection of the "combined" results. We have systematically tested Multi-Block data fusion approaches on a cohort of 300 breast cancer patients with regard to various data pre-processing schemes and compared the outcomes. On the level of individual platforms the differentiation between healthy and diseased samples is often hampered by the heterogeneity of the tissue sample (fat content), with individual platforms being affected to different extents. Multi-Block PCA, as an unsupervised technique aiming to find the common structure inherent across the platforms, was able to differentiate between healthy and diseased subjects. Inspecting the individual block loadings, revealed that choline and phosphocholine measured by NMR spectroscopy demonstrated similar trends to the synthesis of phosphocholines as measured by LC-MS. These changes in fat metabolism contributed most to the observed separation of cancer from normal tissue, which is in accordance with current findings in the literature [2,3].

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(2) Katz-Brull, R., R. Margalit, et al. (1998). "Choline metabolism in breast cancer; 2H-, 13C- and 31P-NMR studies of cells and tumors." MAGMA 6(1): 44-52.

(3) M. Hilvo, et. al, "Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression", Cancer Res. (2011). doi: 10.1158/0008-5472.CAN-10-389

053

ANALYZING MULTI-PLATFORM DATA USING ANALYTICAL REPEATABILITY: THE METABOLOMICS CASE

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Analytical repeatability refers to the error associated with the repeated analysis of the same sample within the same assay, also called intra-assay accuracy or measurement error. Here we analyze bio-samples (e.g. blood plasma, urine) that contain many metabolites using a chromatography method in combination with a Mass Spectroscopy set up. Various error sources related to that complex analytical platform contribute to the measurement error of each metabolite analyzed. Moreover, different metabolites may have measurement errors that are correlated with each other (Ref. 1). Nowadays, different platforms are used to analyze as many as possible different metabolites belonging to the same sample. However, the integration of data from these different platforms (data fusion) does not account for platform-related measurement errors; as a consequence, no reliable quantification of the metabolites' responses in a sample may be obtained. To tackle this problem, we developed, based on Maximum-Likelihood Principal Component Analysis (MLPCA, Ref. 2), a novel exploratory multivariate data fusion approach that may account for both the platform-related measurement errors and the multivariate response levels of the metabolites under study. We validated this method by studying the extend to which the sample-specific response levels of metabolites from multi-platform metabolomics data (Ref. 3) with measurement errors of increasing complexity (ranging from i.i.d. measurement errors, to correlated analytical errors as (often) encountered in multi-platform metabolomics data) could be recovered. In this study it appeared that our novel data fusion approach recovers realistic metabolomics data to a larger extend than standard data fusion approaches, such as Maximum-Likelihood Simultaneous Component Analysis (MxL-SCA-P, Ref. 4-5) and Principal Component Analysis.

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ANALYSIS OF INTER-LABORATORY METABOLOMICS EXPERIMENTS

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Metabolomics Australia is developing standardised retention time locked GC-MS analyses methods that can be used in a laboratory independent manner and derive the same result. Achieving this level of reproducibility would allow for analysis of samples from large projects at multiple sites potentially increasing throughput substantially. To validate our methods we have prepared a standard mix containing ~40 known metabolites (mm). We have also prepared an additional version of this mix, where a selection of metabolites is spiked at a significantly higher abundance (mm+). Replicates (6-8) of the unspiked (mm) and spiked (mm+) metabolite mix were run on four different GC-MS machines at three laboratories using two GC-MS methods differing in temperature gradient. Target ion areas for the metabolites in the mix were extracted using Analyser Pro software (SpectralWorks) and presented as a matrix for statistical analysis. A linear model with an interaction term(s) is presented here to analyse and interpret these inter-laboratory metabolomics measurements. This model will give us a basis to build more complex models where different groups/types of samples from a single study are processed at different laboratories. This work is funded by Metabolomics Australia.

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DEVELOPMENTS IN BIOLOGICAL ANALYSIS AND IMAGING USING SECONDARY ION MASS SPECTROMETRY

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

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(2) Sugiura Y, Setou M, Imaging Mass Spectrometry for Visualization of Drug and Endogenous Metabolite Distribution: Toward In Situ Pharmacometabolomes, J. Neuroimmune Pharmacol. 5, 31-43 (2010).

(3) Lockyer NP, Bioimaging of elements and small molecules using Mass Spectrometry, Bioanalysis (2011) in press.

(4) Fletcher JS, Lockyer NP, Vickerman JC, Developments in molecular SIMS depth profiling and 3D imaging of biological systems using polyatomic primary ions. Mass Spectrom. Rev. 30, 142-174 (2011).

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PLANT METABOLOMICS FOR THE WEED-BIOMASS PRODUCTION UNDER ENVIRONMENTAL CONSTRAINS

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Metabolomics studies revealed that environmental changes trigger the change in composition of carbohydrates and a broad range of primary metabolites in plant species. Elevated biomass yield under high CO2 and rich nutrients were presented

by our work. While the effects of rising CO2 on global crop yield have received considerable attention, metabolomics information is currently lacking on typical weeds such as R. obtusifolius. Robustness of this plants against various environmental constrains suggest the application of weeds as raw materials for energy production. Furthermore, unique features of high oxalic acid contents in relation to metabolic pathway will be presented. Considering the future practice of agriculture, more attention to the weed managements in terms of atmospheric environment (high CO2) and residual nutrient levels in soil can be proposed.

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(3) Miyagi,A., Takahara,K., Kasajima,I., Takahashi,H., Kawai-Yamada,M. and Uchimiya,H., Fate of 13C in Metabolic Pathways and Effects of High CO2 on the Alteration of Metabolites in Rumex obtusifolius L., Metabolomics (in press) , 2011

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NEUROPHARMACOLOGY FROM A METABOLOMIC PERSPECTIVE

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A brain cortical tissue slice model, coupled with targeted neuropharmacology, $^{1}H/^{13}C$ NMR spectroscopy and multivariate statistics has been used to generate metabolic representations of brain activity to map the major neurotransmitter systems. Here, we present data from our work with the major inhibitory neurotransmitter, γ-amino butyric acid (GABA). We have used data from experiments with more than 40 different ligands targeting subcomponents of the GABAergic system to generate a GABAergic "footprint", identifying seven separate metabolic responses associated with receptor populations responding to different concentrations and locations of GABA. These responses are not described by classical GABAergic receptor classifications but include mainstream synaptic populations, peri- and extrasynaptic receptors and novel responses revealed by transmitter uptake blockade and release of non-synaptic (cytosolic) GABA (tonic inhibition) [1]. These data suggest that the classical two-compartment (neuron/glia) model for brain metabolism is under-describing the metabolic reality of brain compartmentation. We suggest a data-driven approach to describing brain compartmentation that may prove to be of more utility.

The GABAergic footprint has also been used to classify the sites of action of the party drug γ-hydroxybutyrate (GHB) [2] and to pinpoint possible antidotes for the sedative actions of this drug. It has also revealed evidence for activity of very low concentrations of alcohol, as well as satisfactorily verifying the site of action of alcohol in the GABAergic system.

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058

FROM THE DESERT TO THE CELLAR: RESPONSE OF GRAPE BERRIES METABOLOME TO WATER DEFICIT

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Water deficit (WD) reduces crop yields leading to economic losses worldwide. A large portion of the world wine production is located in regions suffering from WD. Whilst grapevine is relatively tolerant to WD, extended drought events accompanied by increased temperature can lead to decreased yield, alteration of fruit metabolism and hence corrupt product quality. Following these premises, we investigated the influence of WD on grape dark skinned berry developmental metabolism with particular emphasis on berry skin metabolome, harboring most aroma compounds important for wine quality and nutritionally valuable stilbenes. Field experiments were conducted on two *Vitis Venifera* cultivars, Shiraz and Cabernet Sauvignon. The vines were drip irrigated with 100% of estimated ETc (control) and two levels of deficit irrigation (70% and 50% of the control). Metabolic changes during berry maturation were assessed via GC-MS and UPLC-QTOF-MS/MS. Vines exposed to deficit irrigation showed clear gradual reduction in berry weight over the course of berry development compared to their control. WD induced changes in the metabolome, specific to harvest date and to variety. The two varieties differed significantly in the regulation of stilbene metabolism and in the accumulation of stress-associated compounds, during berry development and as affected by water deficit. Our data suggest that cropping and irrigation strategies of dark-skinned grapevines should be variety specific to maximize wine quality.

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UNDERSTANDING THE SYSTEMS RESPONSES TO STRESSES WITH INTEGRATED METABONOMIC ANALYSIS

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Metabolism represents all (bio)chemical changes in biological processes and is the fundamental feature of living systems. Analysis of the metabolite composition (metabonome) and its variations has, therefore, been an important way to understand the molecular aspects of biological activities ever for a long time. As a branch of science concerned with the metabolite compliment of biological systems and its dynamic responses to the changes of both endogenous and exogenous factors, metabonomics has shown rapid development in methodologies and found widespread applications in fundemental biological, environmental and biomedical sciences. Metabonomics involves comprehensive analysis of metabolite composition in biofluids, tissues and whole organisms with metabonomic complexity on one hand and demands for acquisition of quantitative information *in situ* on the other. It is thus obvious that the development and optimization of novel methods remain to be the essential requirements for further progress of metabonomics. The combined NMR-MS analysis and the integration of metabonome and other biological information (such as proteome, transcriptome and microbiome) have become the most effective ways to achieve holistic understandings of the molecular mechanistic aspects of biological systems and pathophysiology. In this report, we will report some of our recent progresses in the combined LC-MS/NMR metabonomic analytical methods and integrated metabonome-transcriptome analysis to understand the metabolic basis of the systems responses to various exposomic stresses in both plants and animal models. We will discuss the observation of common metabolic reprogramming upon oxidative stresses induced by environmental factors. The usefulness and effectiveness of integrated metabonomic analysis will be particularly reflected in this presentation.

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(2) 2. L Zhang, et al, ¡°Systems responses of rats to aflatoxin B1 exposure revealed with metabonomic changes in multiple biological matrices¡±, J Proteome Res, 10:614¨C23, 2011.

(3) 3. J Zhang, et al, ¡°Dynamic metabonomic responses of tobacco (Nicotiana tabacum) plants to salt stress¡±, J Proteome Res, 10:1904¨C14, 2011.

(4) 4. C Liu, et al, ¡°Revealing different systems responses to brown planthopper infestation for pest susceptible and resistant rice plants with the combined metabonomic and gene-expression analysis¡±, J P

(5) 5. H Dai, C Xiao, et al, ¡°Combined NMR and LC-MS analysis reveals the metabonomic changes in Salvia Miltiorrhiza Bunge induced by water depletion¡±, J Proteome Res, 9:1460¨C75, 2010.

(6) 6. L Ding, et al, ¡°Systems biological responses to chronic perfluorododecanoic acid exposure by integrated metabonomic and transcriptomic studies¡±, J Proteome Res, 8:2882-91, 2009.

(7) 7. M Li, et al£¬¡°Human symbiotic gut microbes specifically modulate metabolic phenotypes¡±, PNAS, 105:2117-22, 2008.

(8)

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LC-MS PROFILING OF LEAF TISSUE FROM *THLASPI CAERULESCENS* SHOWS AN INVOLVEMENT OF LIPIDS IN ZN HYPERACCUMULATION.

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Hyperaccumulators (extremophiles) are a group plants that have evolved the ability to not only survive in metal-rich soils but to sequester and store concentrations of metals which would be toxic to any other organism. These plants have developed unique molecular mechanisms which prevent them from succumbing to the toxic effects of high metal ion concentrations. These extremophiles essentially act as metal ion pumps. For example, it has been estimated, the total nickel content in one Ni-hyperaccumulating tree *Sebertia acuminata* was 37 kg, approximately 20,000 times the typical concentration[1]. A better understanding of how hyperaccumulators deal with these extremely toxic concentrations of metals could lead to an environmentally friendly means of restoring contaminated soils. The mechanisms behind the transportation of metal ions could also be used for improving the micronutrient value in crops, known as biofortification.

Thlaspi caerulescens (Brassicaceae) has been identified as a model species for studying hyperaccumulation2, 3. Reversed phase LC-QTOF-MS profiling of leaf extracts from T. caerulescens grown in increasing Zn2+ concentrations was carried out. From these extracts 1,688 metabolites were detected in at least 9 out of 10 biological replicates and 235 were found to have increased (fold change >2, $p<0.0005$, $n=10$) in the Zn-treated plants. Many of these were found to be Zn-complexes and membrane lipids such as phosphatidyl inostitol . The role of lipids in hyperaccumulation has not been explored and this is the first data showing their possible involvement in hyperaccumulation .

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(3) Kramer, U. Annual Review of Plant Biology 2010, 61, 517-534.

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METABOLOMICS AS POWERFUL TOOL IN SEED QUALITY RESEARCH

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Seed quality is an economically highly important trait, both in relation to seedling growth and plant production, as well as for seed-derived products such as coffee, rice and vegetable oils. In this presentation we introduce metabolomics as a powerful approach to study comprehensively the changes in metabolite composition during seed development, post-harvest treatments and germination. For instance, in field mustard and pepper seeds, metabolomics has enabled us to pinpoint those ripening stages mostly influencing the metabolite composition and germination quality of seeds. Experiments with lettuce and cabbage seeds indicated specific metabolite alterations upon seed ageing and priming, providing insights into the chemical and physiological processes taking place after harvest and enabling us to discover markers for seed quality. In coffee, we applied untargeted metabolomics to establish the effects of different post-harvest processing and drying methods on the metabolite profile of coffee beans, and to correlate metabolite profiles to the quality of the coffee drink.

The examples provided clearly indicates that metabolomics can give us novel insights into the physiology and quality of seeds. It will greatly enhance our possibilities to find novel markers for quality traits, to monitor and control changes occurring upon pre- and post-harvest treatments and to unravel seed biology.

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METABOLOMICS ANALYSES OF BIOFLUIDS FROM RATS DOSED WITH LIVER OR KIDNEY TOXICANTS

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Abstract not available at time of print.

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A METABOLOMIC STUDY OF COLORECTAL CANCER

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Tumor cells exhibit distinct metabolic phenotypes that are essential for them to sustain higher proliferative rates and resist some cell death signals, altering the flux along key metabolic pathways, such as glycolysis and glutaminolysis. Recent advances in metabolic alterations and adaptations of cancer cells are providing increasing support for the development of treatments that target tumor metabolic transformation. Colorectal cancer (CRC) is the third most common cancer in both men and women with an estimated 146,970 new cases and 49,920 estimated deaths, accounting for almost 9% of all cancer deaths in 2009 in the United States.

Metabolomics in clinical research aims at evaluating and predicting pathophysiological changes of an individual by investigating metabolic signatures in body fluids or tissues, which are influenced by genetics, epigenetics, environmental exposures, diet, and behavior. When used as a translational research tool, metabolomics enables the discrimination of distinct metabolic profiles and metabolite markers noninvasively *in vivo* that correlate to pathological stages and different responses to treatment modalities, providing a link between the laboratory and clinic. Powerful analytical techniques such as liquid or gas chromatography coupled to mass spectrometry (LC-MS or GC-MS) offer a rapid, effective and economical way to analyze significant alterations of global (unbiased approach) metabolome or pre-defined metabolites (targeted approach) in biological samples. A particular advantage of metabolomics over targeted metabolite measurement is in hypothesis generation: the discovery of changes in molecules that were not already associated with a biological phenomenon. We describe here our studies with mass spectrometry based metabolomic profiling of serum, urine and tissue samples from colorectal cancer patients. Our experimental results highlight the potential for the metabolomic approach to provide predictive and theranostic cancer markers and novel molecular targets, despite the lack of knowledge in the oncology community regarding its technology and methodologic process.

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A METABOLOMICS INVESTIGATION OF WEIGHT LOSS IN OVERWEIGHT AND OBESE ADULTS

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Overweight and obesity are increasing in both genders, and at all ages, across the majority of European countries. It is estimated that between 30% and 80% of adults and up to one-third of children in Europe are affected, with rates of obesity increasing. Body weight maintenance after weight reduction is a major challenge for obesity treatment and dietary interventions are the most popular method for weight maintenance. Understanding and highlighting the metabolic mechanism involved in weight loss, weight regain and/or maintenance is of the utmost importance for an effective treatment of obesity and overweight.

We present a broad spectrum metabolomics investigation of weight loss in adults in the frame of the DiOGenes project: DiOGenes (Diets, Obesity and Genes) is a Pan-European, randomized, controlled dietary intervention study investigating the effects of dietary protein and glycaemic index on weight (re)gain, metabolic and cardiovascular risk factors in obese and overweight families in eight European countries. A total of 891 families with at least one overweight/obese parent underwent screening. Serum and plasma samples collected from the participants matching the study inclusion criteria were analyzed through different measurement platforms (i.e. Nuclear magnetic resonance, LC-High Resolution Mass spectroscopy and NMR-based derivation of lipoproteins sub-fractions).

In this presentation we introduce and review one of the largest scale metabolomics studies up to date on obesity. The overall measurement strategy is illustrated with reference to the measurement design and to an integrated data analysis approach encompassing Quality control, standard chemometrics and statistical techniques as well modeling of lipids and lipoproteins metabolism. Preliminary results on gender dependent effects of weight loss on low molecular weight molecules, lipids and lipoproteins metabolism are also presented.

065

HEALTH MONITORING BIOMARKERS IN ELITE SOLDIERS

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The ability to interrogate human biofluids for insights into the health of military personnel could deliver significant improvements to Defence agencies by allowing them to monitor their occupational readiness for deployment/operations. "Predictive Diagnostics" could save these organisations a significant amount of compensation costs through identifying personnel that are pre-symptomatic of communicable disease, thereby allowing for their containment, and mitigating injury risk. It could also be used to monitor military personnel both pre- and post deployment for neurological stress (sleep disturbance, post traumatic shock etc). During a recent Australian Defence Force (ADF) training exercise, urine samples where taken throughout a 3 day endurance exercise, with the aim of monitoring the metabolome for putative biomarkers indicative of disease and physical/psychological stress associated with this type of activity. Following sample collection, specimens were subjected to LC-MS based metabolomic strategies. Subsequent database searches allowed for several compounds of potential interest to be identified. This presentation will discuss the outcomes of this research.

066

CARBON METABOLISM OF *LEISHMANIA* PARASITES REQUIRED FOR VIRULENCE IN THE MAMMALIAN HOST REVEALED BY ^{13}C-STABLE ISOTOPOMER PROFILING

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Parasitic protozoa cause a number of important diseases in humans. In particular, parasites belonging to the genus Leishmania, cause a spectrum of diseases that can be life threatening and afflict more than 12 million people worldwide. The genomes of these divergent eukaryotes have been sequenced providing information on the metabolic capacity of these pathogens. However, more than 60% of the encoded genes have no assigned function suggesting that many metabolic and regulatory processes in these organisms remain to be discovered. Here we describe the use of metabolite profiling and ^{13}C stable isotope labeling methods to investigate central carbon metabolism in the major insect (promastigote) and mammalian-infective (amastigote) stages of *Leishmania*. Promastigote stages were generally characterized by high metabolic activity, the preferential utilization of hexoses as major carbon source, and an active (and complete) TCA cycle. In contrast, intracellular amastigotes were characterized by low metabolic activity, the co-utilization of hexose and fatty acids as carbon sources, and the predominant use of a partial TCA cycle. These stage-specific differences in metabolism were induced by elevated temperature and reduced pH rather than changes in carbon source availability, suggesting that they are part of the hardwired differentiation response. We have also analysed carbon metabolism in Leishmania mutants with defects in carbon metabolism and virulence. These analyses indicate that hexose metabolism is essential for intracellular survival in the phagolysosomes of infected macrophages. The marked differences in carbon source utilization and metabolic capacity of different developmental stages detected using stable isotope labeling contrasts with the relative constancy of the transcriptome and proteome of these stages, highlighting the importance of post-translational processes in regulating the adaptive responses of these parasites to different host environments.

067

SELF-ORGANISATION OF AMINO ACID REGULATION IN YEAST.

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When *Saccharomyces cerevisiae* are grown continuously, cellular processes auto-synchronise resulting in stable oscillatory dynamics. Respiration is the most readily measured oscillatory parameter, however, transcriptome-wide and metabolome-wide studies indicate that the oscillation functions to temporally separate catabolic and anabolic processes. Consistent with this, the production of amino acids has distinct phase relationships with the oscillation cycle. Intracellular amino acids show different oscillation patterns during the respiration cycle, for example, the concentration of cysteine peaks prior to aspartate, glutamate, leucine and valine whereas threonine and isoleucine concentrations peak later during an oscillation cycle. Furthermore the oscillation is highly sensitive to amino acid and Rapamycin perturbation. Taken together these data indicate a role for the master amino acid regulator Gcn4p in the regulation of oscillatory dynamics, where the Gcn4p is activated by non-aminoacylated tRNAs. Here we show that the ratio between aminoacylated and non-aminoacylated tRNAs oscillates in phase with respiration cycle, indicating the observed Gcn4p dynamics result from translational activation by the cyclic amino acid synthesis and aminoacylation of tRNAs. Closer examination using Northern blotting of tRNAs representing single amino acids were used to explore the role of non-aminoacylated tRNAs in oscillatory regulation and the concentration response of individual amino acids thus revealing the dynamic activation of amino acid biosynthesis via the Gcn4 gene network (the immediate neighbors) during the respiration cycle.

068

SYSTEMATIC PHENOME ANALYSIS OF *E. COLI* MULTIPLE-KNOCKOUT MUTANTS REVEALS HIDDEN REACTIONS IN CENTRAL CARBON METABOLISM.

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Central carbon metabolism is a basic and exhaustively analyzed pathway. However, the intrinsic robustness of the pathway might still concealuncharacterized reactions. To test this hypothesis, we constructedsystematic multiple knockout mutants involved in central carboncatabolism in *E. coli* and tested their growth in 12 different nutrientconditions. Differences between in silico predictions and experimentalgrowth indicated that unreported reactions existed within thisextensively analyzed metabolic network. These putative reactions werethen confirmed by metabolome analysis and in vitro enzymatic assays.Novel reactions of sedoheptulose 7-phosphate to erythrose 4-phosphateand dihydroxyacetone phosphate were observed in transaldolase-deficientmutants, without any noticeable changes in gene expression. Thesereactions, triggered by an accumulation of sedoheptulose 7-phosphate,were catalyzed by the universally conserved glycolytic enzymesATP-dependent phosphofructokinase and aldolase. The emergence of analternative pathway that does not require any changes in gene expressionbut rather relies on the accumulation of an intermediate metabolite maybe a novel mechanism that mediates the robustness of these metabolicnetworks.

069

A METABOLOMICS APPROACH TO EXPLORING THE FUNCTION OF THE ESX-3 TYPE VII SECRETION SYSTEM AND *M. TUBERCULOSIS* VIABILITY.

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The genome of *Mycobacterium tuberculosis* contains five copies of a cluster of genes encoding a novel type VII secretion system, named the ESAT-6 or ESX gene cluster regions. The ESX-3 gene cluster has been implicated in iron and zinc homeostasis and is essential for *in vitro* growth of this organism. Although it does play a major role, it not essential for the *in vitro* viability of the fast growing, non-pathogenic mycobacterial species, *M. smegmatis*, and can thus be knocked out of the genome of this organism without totally inhibiting its growth. In order to gain a better understanding of ESX-3 functionality, we embarked on a study to determine the novel metabolite profiles associated with ESX-3, by comparison of an *M. smegmatis* ESX-3 knockout strain to an isogenic wild type parental strain. The results of the principal component analysis (PCA), of the gas chromatography mass spectrometry (GC-MS) analysed metabolite profiles, showed a clear separation between the wild type and knockout strain sample groups. A partial least squares discriminate analysis (PLS-DA) validation indicated 100% correct group membership prediction. Subsequently, those metabolites contributing most to the separation or showing the largest variation between the separated groups, in the PCA and PLS-DA analyses respectively, were identified as biomarkers, potentially explaining the functionality of ESX-3. Of all the metabolites identified, a number of amino acids in particular, in addition to other intermediate metabolites of other metabolic pathways related to these amino acids, appear to be most affected by the absence of the ESX-3 gene cluster. These observations are discussed in light of the hypothesized role of the ESX-3 gene region in iron and zinc homeostasis. This is not only the first study to provide clues into ESX secretion system functionality from a metabolomics perspective, but also demonstrates the potential of using a metabolomics research approach to identify metabolic processes underlying the phenotypic characteristics related to ESX-3 gene cluster expression.

070

ENVIRONMENTAL METABOLOMICS AT THE EDGE

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The use of metabolomics for understanding the complex interactions of biological systems in an environmental context is emerging as a powerful source of insight into these relationships. The condition of the coastal zone is key to human well-being and is subject to significant natural and anthropogenic stressors. Activities such as urbanization, recreation, commercial fishing, industrial development and natural events cause marine organisms to experience a wide range of stressors. The diversity of interacting organisms is fantastically wide and ranges from microbes to mammals. Many key species in the marine ecosystem are relatively uncharacterized, and their interactions are often confusing because of the difficulty of observing non-terrestrial effects. Metabolomics is an excellent platform for exploring these multi-species interactions and the effects of coastal zone stressors.

One initial approach to investigations in the marine environment involves case studies with partners who have utilized more traditional approaches to their investigations but who may have a system that can readily be sampled for metabolomics analysis. In our laboratory and others, several marine organisms have been assessed for suitability in metabolomics studies. For example, organisms such as fish, crabs and bacteria are candidates for investigation.

In one recent study, we observed that the metabolic trajectory for Atlantic blue crabs (*Callinectes sapidus*) subjected to injection with the microbe *Vibrio campbelli* and with 2,4-dinitrophenol, which is a known uncoupler of oxidative phosphorylation. [1] This study showed that it is feasible to use blue crabs as a model organism for determining different modes of action for different oxygenation-related stressors.

The microbe *Vibrio coralliilyticus* has been found in high concentrations in the bleached coral *Pocillopora damicornis*, but not in healthy corals. When inoculated into healthy corals at temperatures above 25°C, *V. coralliilyticus* caused bleaching. Recent metabolic studies indicate distinct metabolic responses of *V. coralliilyticus* at different temperatures, and the differential production of metabolites indicates a complex response to the elevated temperature. [2]

(1) Metabolomics, 6, 250-262 (2010)
(2) Environmental Science & Technology, 43(20), 7658–7664 (2009)

071

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

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Phosphine is the most widely used fumigant in the world for controlling insect pests in grain stores without compromising the grain or leaving toxic residues. However, the increasing frequency of phosphine-resistant insects is threatening the useful life of phosphine as a fumigant . Despite its importance, little is known about the toxic action of phosphine or the resistance mechanisms in insect species .

In this study, a genetically, developmentally and metabolically well characterised model organism, *Caenorhabditis elegans*, has been used to investigate and characterise the mechanisms of phosphine toxicity and resistance by application of metabonomic methods . Both phosphine-resistant and susceptible *C. elegans* strains were exposed to different concentrations of phosphine , and the changes in their metabolic profiles were analysed with NMR metabonomics of extracts of the whole nematodes.

A combination of 1D NMR spectroscopy and multivariate statistical analysis allowed sources of metabolic variation between resistant and susceptible strains to be determined. Further NMR techniques, including 2D TOCSY and HSQC spectra, allowed characterisation of the compounds that caused metabolic variation, shedding some light on the metabolic pathways affected by phosphine poisoning and phosphine resistance.

Subsequent combination of the metabonomic data with detailed genetic characterisation of the phosphine resistant mutants allowed the identification of the affected metabolic pathways, which opens up the avenue for identifying the likely target enzyme, affected by phosphine.

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

072

ALTERED FATTY ACID METABOLISM IN LONG DURATION ROAD TRANSPORT: AN NMR-BASED METABOLOMICS STUDY IN SHEEP

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Long distance/duration transport of livestock raises concern for animal welfare and production losses. Animals subjected to road transport longer than 12 hours are deprived for food and water before and during transport, and experience changed social and physical environments. The stress response to road transport has been studied under various conditions and on various livestock species for at least 20 years. However, the handful of traditional biochemical and haematological measures has not fully described the metabolic response of livestock subjected to long duration road transport.

To develop a system wide view of the metabolic responses to road transport, we applied NMR metabolomics both sera and urines obtained from sheep prior to transport, on arrival and on 1, 2 and 3 days after arrival. The subjects, 80 mature merino ewes were treated to 12 or 48 h road transport under normal industry conditions. NMR analyses of the samples and subsequent PCA indicated distinct metabolic changes due to transport and through recovery. As anticipated, the fast and water deprivation affected carbohydrate and lipid metabolism. The animals also experienced protein catabolism, and altered gut cometabolites indicated changed gut flora and metabolism. The animals transported for 48 hours were clearly discriminated from the 12 hour transported group as they exhibited deeper and more extensive metabolic responses.

The analyses also enabled detailed description of the recovery trajectory over the 3 days following the transport event. Recovery saw the two groups plot different but ultimately converging new metabolic states. An intriguing observation on arrival and post-transport was the excretion of acyl glycines and a dicarboxylic acid. These products of fatty acid oxidation implicate a role for the peroxisome in the metabolic response to transport induced stress.

(1) J. Proteome Res. 10, 1073-1087 (2011)

ENERGY SECURITY THROUGH NEXT-GENERATION METABOLOMICS: A NEW AVENUE FOR POLYMERIZED METABOLITES, LIGNOCELLULOSE RESEARCH

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A transition to biomass-based energy from nuclear and petrochemical sources has made the analysis of biomacromolecules in environmental samples a focus of current research. The chemical structure of such biomass products can affect differences in their degradability and hence suitability for energy needs. However, due to the difficulty of separating lignin-carbohydrate complexes into individual components (such as monosaccharides), information on the composition of biomass products such as lignocellulose are limited to particular plant species and for particular chemical components. Recently, advances in NMR spectral analysis have revealed the composition of lignocellulosic products from mixture biomacromolecular samples using simple ball-mill treatment. Furthermore, NMR has the potential to monitor insoluble components through both conventional 1D and 2D solution/solid-state measurements [1-3]. In order to improve the potential of such techniques, we developed the Bm-Char (*Bio*mass *Char*acterization) web tool to characterize the composition of lignocellulosic components in environmental samples. Here, we will present Bm-Char and related software as part of ECOMICS (https://database.riken.jp/ecomics/.)[4], a suite of web-based tools for ECosystem trans-OMICS investigation that targets meta-genomic[5], meta-transcriptomic, and meta-metabolomic systems[6], including biomacromolecular mixtures derived from biomass.

(1) Sekiyama, Y. et al. • gProfiling polar and semi-polar plant metabolites throughout extraction processes using a combined solution-state and HR-MAS NMR approach • h Anal. Chem. 82, 1643-1652 (2010)

(2) Ogura, T. et al. • gEffects of rice straw pretreatment on determining cellulosic supramolecular structure and improving digestibility for paddy soil microbiota • h in preparation.

(3) Mori, T. et al. • gSolid- and solution-state NMR analysis of the structural transition of cellulose upon dissolution with ionic liquids • h in preparation.

(4) Ogata, Y. et al. • gECOMICS: A web-based toolkit for investigating the biomolecular web in ecosystems using a trans-omics approach • h PLoS ONE, submitted.

(5) Ogata, Y. et al. • gE-class: a web tool for high-throughput metagenomic classification using modularized sequential databases • h Bioinformatics, submitted.

(6) Date, Y. et al. • gMetabonomics sequence of anaerobic fermentation upon sugar feedings by correlation analysis of microbial and metabolite profiling • h in preparation.

074

USE OF RECONSTITUTED METABOLIC NETWORKS TO ASSIST IN METABOLOMIC DATA VISUALIZATION AND MINING, THE CHALLENGE OF COMPUTING "METABOLIC STORIES"

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Metabolomic experiments generally focus on a sub-set of endogenous metabolites, in particular for biomarker identification. In order to decipher the metabolic processes involved by metabolic perturbations, it is necessary to identify the reaction cascades connecting monitored metabolites. To be efficient and exhaustive, this data mining has to be performed at the metabolic network scale [1,2]. But these genome scale metabolic networks often contain thousands of reactions and metabolites. In fact the improvement of *in silico* network reconstructions and the availability of high throughput data allow building larger and better models [3]. Manual inspection is thus becoming impossible, leading to the development of algorithms handling metabolic networks using the graph mathematical framework [1,4]. In order to automatically retrieve reactions connecting metabolites we first proposed an approach based on a gap filling strategy [5]. This approach was successfully used to improve raw data analysis and provided clues on metabolic processes involved in HepG2 cell metabolism. However, it was not sufficient to connect all the relevant metabolites identified in metabolomics. In order to span more globally the metabolic network, we are introducing the notion of *metabolic story*.

A metabolic story is a set of metabolic paths such that 1) their union doesn't create cycles 2) inputs and outputs of a metabolic story are metabolite identified in metabolomics (e.g. biomarkers). We developed a method allowing generating these stories given a network and a set of metabolites. Moreover we defined a way to rank these stories based on biological criteria.

This approach was successfully applied to metabolomics data (high resolution mass spectrometry) extracted from yeast exposed or not to cadmium [6]. The first metabolic story found corresponds to the biological interpretation made by experts in the article. Moreover this story raised new hypothesises on the metabolic process involved in the response to the cadmium exposure.

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3OMICS: A WEB BASED SYSTEMS BIOLOGY VISUALIZATION TOOL BY INTEGRATING TRANSCRIPTOMICS, PROTEOMICS AND METABOLOMICS DATA IN HUMAN

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3Omics is a one-click web tool for fast integration of multiple inter- or intra- transcriptomics, proteomics, and metabolomics data and visualization to cover and connect cascades from transcripts, proteins to metabolites in humans. It provides five commonly used analyses: correlation networking, co-expression analysis, phenotyping, KEGG pathway enrichment analysis, and GO enrichment analysis. By default, 3Omics generates inter-omic correlation networks to display relationships or common patterns in data over time or experimental conditions for all transcripts, proteins and metabolites. While users may only possess two of the three omics data, 3Omics supplements missing transcript, protein and metabolite information related to the user input data by text-mining the PubMed literature database. Co-expression analysis with heatmap visualization assists in revealing shared functions between different -omics data. Phenotypic analysis is performed by organizing Online Mendelian Inheritance in Man with available transcript or protein data. KEGG pathway enrichment analysis is designed for metabolomics data revealing enriched pathways in the KEGG Pathway database by ranking biological pathways commonly shared by metabolites. Gene Ontology-based functional enrichment analyses perform statistical analyses to display significantly overrepresented GO terms from transcriptomic experiments. While the main objective of 3Omics focuses on interpreting multiple -omics datasets, it is also capable of analyzing each omics data with the other available functions. All visualization and analysis results can be downloaded from the 3Omics website as plain text, SVG image or Cytoscape SIF files, allowing for user customization and further analysis. 3Omics is freely accessible at http://cmdd.csie.ntu.edu.tw/~3omics/

076

SYSTEMS-BASED APPROACHES TO ELUCIDATING GENDER-SPECIFIC MECHANISMS IN THE ETIOLOGY OF ATHEROSCLEROSIS

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Cardiovascular disease is the major cause of premature death in Europe, resulting in >4 million deaths annually. Cardiovascular disease is often regarded as a "men's" disease; however, it is the leading cause of mortality among women. In particular, since 1984, more US women than men have died annually from cardiovascular disease. Studies have suggested a key role for oxidized fatty acids (oxylipins) in inflammatory reactions of atherosclerosis; however, it is still unclear to what extent pro- and anti-inflammatory factors determine whether an atherosclerotic lesion develops into a stable plaque or ruptures, leading to stroke or myocardial infarction. To further probe the etiology of plaque development and subsequent rupture, we performed a combination of lipidomics and metabolomics as well as targeted oxylipin, free fatty acid and endocannabinoid profiling in human carotid atherosclerotic plaques and matching circulating plasma. These data were combined with sonographic gray-scale median plaque imaging and Affymetrix GeneChip® data as well as patient clinical parameters to develop a multivariate model of plaque gender-specificity. PCA analysis showed that plaque and plasma have unique composition, which was mainly driven by oxylipin levels, despite the fact that endocannabinoids represent >90% of the lipids measured. OPLS analysis based on gender resulted in a robust oxylipin-driven model with high predictive power, which was specific for plaque. The most important variables driving the separation between genders were primarily products of the 12/15 lipoxygenase pathway, suggesting gender-specific differences in this key pathway. These trends were supported by GSEA analyses showing enrichment in linoleic and eicosanoid specific pathways in plaque, but not plasma. Imaging data suggested that plaques in women had distinct morphological differences, with women having overall smoother plaques. Collectively, results point to gender-specific shifts in inflammatory lipid species as well as morphological differences that could potentially explain the higher incidence of cardiovascular disease in women.

077

STATISTICAL RECOUPLING OF VARIABLES FOR THE IDENTIFICATION OF CANDIDATE BIOMARKERS AND PERTURBED METABOLIC NETWORKS. APPLICATION TO WHOLE ORGANISM NMR

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We have developed an automated variable-sized bucketing procedure based on statistical relationships between neighboring variables, obtained by traditional 0.001ppm wide bucketing, to recouple into a cluster the points belonging to metabolic NMR signals. This Statistical Recoupling of Variables (SRV) procedure acts as an efficient noise-removing filter and identifies new variables, which have physical, chemical or biological meaning. This allows a large reduction of the number of variables and thus counters the "curse of dimensionality" observed in the "-omics" sciences.

SRV can be used in combination with multiple hypothesis testing corrections to evaluate the statistical significance of variations observed on the metabolic phenotypes discriminating the populations under study. It is thus possible after SRV to extract candidate biomarkers whose single variations are sufficient to sustain the discrimination. In a more comprehensive approach, SRV can be associated with Statistical Total Correlation Spectroscopy (STOCSY) to yield a recoupled form (R-STOCSY) and obtain a clear 2D pseudo-spectrum displaying spin and metabolic correlations. These correlations can then be identified as metabolic connectivities. By considering the shortest path length between the metabolites involved in these correlations, it is possible to visualize the perturbed metabolic network associated with a given pathophysiological condition, and to assess the robustness of the overall analysis. These approaches together provide a powerful way to secure the interpretation of metabonomics data. In the case of perturbation of minor intensity, we added an orthogonal filter to focus on the effect under scrutiny, which would otherwise be diluted in the complexity of the data set.

We illustrate this ability in whole organism NMR based studies. HRMAS NMR analysis of entire nematodes and cell pellets can lead to the functional genomics of *C. elegans* and a better understanding of the tumorogenesis pathophysiology in the *MEN1* cancer syndrome.

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MONITORING MICROBIAL CONTAMINATION IN FERMENTATION PROCESSES USING METABOLIC FOOTPRINT ANALYSIS – AN EXOMETABOLOMICS APPROACH

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Early detection of microbial contamination is crucial to avoid process failure and costly delays in fermentation industries. Traditional detection methods, such as plate counting and microscopy, are, however, labor-intensive, insensitive and time-consuming. Modern techniques are therefore sought that can detect microbial contamination rapidly and cost-effectively. In the present study, we propose gas chromatography-mass spectrometry (GC-MS) based metabolic footprint analysis as a rapid and reliable method for the detection of microbial contamination in fermentation processes. Our metabolic footprint analysis detected statistically significant differences in metabolite profile of axenic and contaminated batch cultures as early as 3 hours after contamination was introduced, whilst classical detection methods could only detect contamination after 24 hours. The data was analyzed by discriminant function analysis and was validated by leave-one-out cross validation. We obtained 96 per cent success rate in correctly classifying samples coming from contaminated or axenic cultures. Therefore, metabolic footprint analysis combined with discriminant function analysis presents a rapid and cost-effective approach to monitor microbial contamination in industrial fermentation processes.

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LARGE-SCALE SRM ASSAY SYSTEM FOR UN-TARGETED MS/MS OF PHYTOCHEMICALS

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In tandem mass spectrometry (MS/MS) using tandem quadruple mass spectrometer (TQMS), selected reaction monitoring (SRM) is most commonly used for high sensitive detection of targeted compounds. Recently, the high-performance TQMS, which has high sensitivity in short SRM dwell time, can be capable of a few hundred SRM conditions at a second. Previously, more than 700 standard compounds were successfully detected with the optimized SRM conditions by using liquid chromatography-TQMS (LC-TQMS). We named this methodology as "widely targeted metabolomics" [1-3]. In this study, we established a new SRM assay system of LC-TQMS for MS/MS tags (MS2Ts) obtained in un-targeted analysis of plant extracts [4]. We collected the MS2Ts by analyzing several model and non-model plants, and total 1,613,462 MS2Ts have been uploaded to our website (MS2T viewer, http://prime.psc.riken.jp/). As a case study to determine SRM condition for MS2T, approx. 500 MS2Ts in respective plant species were selected base on the intensity of fragment ions of each MS2T. To determine optimal collision energy for respective MS2Ts, collision-induced dissociation fragmentation analyses at 6 energy steps (10-60 eV) were conducted using plant extracts. As a result, more than three thousands of SRMs were analyzed using our SRM assay system for a plant species. The SRM condition which gave the highest signal intensity among 6 was defined as optimized SRM. The optimal SRM conditions were further selected based on the following criterion: the relative standard deviation of LC-TQMS peak area values is less than 10% (analytical replicates = 3). Finally, approx. 300 optimal SRM conditions were successfully assigned to each plant species (*e.g.*, *Arabidopsis thaliana*, *Lotus japonicus*, soybean and onion). In this presentation, we will introduce our case study for metabolite QTL analysis for new findings of phytochemicals and their metabolism.

(1) Sawada et al., PCP 2009, 50, 37-47
(2) Sawada et al., PCP 2009, 50, 1181-1190
(3) Sawada et al., PCP 2009, 50, 1579-1586
(4) Matsuda et al., Plant J 2009, 57, 555-577

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QUANTIFICATION OF PLANT HORMONES IN *MEDICAGO TRUNCATULA* ROOTS USING UHPLC-QQQ-MS/MS AND MULTIPLE REACTION MONITORING.

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Plant hormones regulate gene expression through complex signaling networks. Integrating hormone analysis with transcriptomic and metabolomics offers a global view of plant biological processes. However, the analysis of phytohormones is quit challenging due to their trace level concentrations in plant tissues, complex background matrix and small sample sizes. We have developed UHPLC-MS/MS method to quantify plant hormones, including auxins, abscisic, jasmonic and salicylic acid, in a single experiment using both positive and negative ion mode multiple reaction monitoring (MRM) on an Agilent 6430 triple quadrupole mass spectrometer. The MRM and chromatographic separation were optimized for hormone quantifications reaching a detection limit of low pg levels. Various sample preparation protocols, sample matrix effect of various plant tissues and internal standard recovery were also studied using stable isotope labeled standards.

This method was applied to the analysis of hormones in *Medicago truncatula* border cells, root tips and whole roots (elongation and mature root zones). Border cells develop from root cap initials and separate from the root during development, but remain appressed to it until contacting water. Detailed metabolic and transcriptional characterizations of *Medicago truncatula* border cells and root tips revealed substantial constitutive differences between these distinct cell types. Phytohormones were not detected using non-targeted metabolic profiling which was used to profile higher abundant metabolites. However, large changes in jasmonate biosynthesis and auxin-responsive transcript levels underscored the importance of hormones in border cell development. For example, the levels of three lipoxygenases, which function early in the JA pathway, were substantially higher in border cells than in root tips based upon microarray and qRT-PCR data. Also there were more auxin related transcripts elevated in border cells than any other class of hormone-related transcripts. Thus, quantitation of phytohormones in border cells and root tips was performed to enhance our understanding of biological functions and processes.

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Γ-AMINOBUTYRIC ACID (GABA) IN PLANTS: A SIGNALING MOLECULE OR "JUST" A METABOLITE?

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γ-Aminobutyric acid (GABA) has been isolated from potato more than 60 years ago, but its role in plants remains unclear. In plants, GABA was suggested as a signaling molecule in response to stress. To investigate this hypothesis we used Arabidopsis thaliana (ws) wild type and GABA transporter-1 (gat1) knockout mutant, which was suggested as regulating GABA influx to the cell. First we employed GC-MS based metabolic profiling on seedlings grown without exogenous GABA and on 1mM exogenous GABA under full nutrient medium, and under C and N starvation. Next, we performed microarray analysis of the seedlings under the same conditions. Last, the results of metabolome and transcriptome profiling have been integrated according to the biological question. Large differences were measured in metabolism and gene expression across different media in both control and mutant plants. Differences in GABA concentrations mostly affected ws plants suggesting that knockout mutation in GAT1 protein partly buffers perturbations across C-N metabolism. Under C starvation in wild type we observed a significant decrease in the relative amount of TCA cycle and shikimate pathway components caused by external GABA, while under N starvation the role of GABA was minor. The analysis of transcriptome of ws plants under C starvation revealed significant effect of exogenous GABA on the levels of expression of genes associated to the shikimate pathway.

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METABOLIC SYSTEMS BIOLOGY FOR THE STUDY OF PLANT-MICROBE INTERACTIONS

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In the last decade, plant metabolomics had provided critical insights into molecular and biochemical events that occur in very few examples of mutualistic and pathogenic plant-microbe interactions. Until now, perturbations of metabolomes of *Solanum* spp caused by fungal infections have been largely unexploited. Plant metabolism is a dynamic system and changes in the levels of any metabolite are expected to affect that of others. Thus, it is crucial in metabolomics studies to get the whole picture of all possible biosynthetic pathways. The lack of a robust pipeline for the integration of the vast amount of information of metabolomics analyses into the global metabolic networks of organisms under study has driven us to develop such approach, contributing to the achievement of the abovementioned goal. Here, we present an integrated metabolic systems biology approach combining FT-ICR/MS with GC/MS and bioinformatics for the study of potato-*Rhizoctonia solani* interaction. By means of integration of more than one analytical platform with bioinformatics, we reconstructed a network of all possible connected metabolic pathways that enabled the monitoring of perturbations of potato sprout's metabolomes in response to the pathogen. Mevalonic acid and deoxy-xylulose biosynthetic pathways leading to the biosynthesis of potato alkaloids were substantially up-regulated and significant fluctuations of the relative content of amino acid pool and carboxylic and lipid acids were recorded. Furthermore, components of the systemic acquired resistance (SAR) and hypersensitive reaction (HR) were detected and the pathogen's strategies to overcome plant defense were proposed. Our metabolic approach has not only greatly expanded the multitude of metabolites previously found in potato in response to pathogen exposure, but identified several antifungal plant-derived metabolites. Such molecules could be used as biomarkers in biomarker-assisted crop breeding or could be used *per se* or as lead structures for the development of new crop protection agents.

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NON-INVASIVE ASSESSMENT OF GASTROINTESTINAL TOXICITY OF METHOTREXATE USING NMR BASED METABOLOMICS

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Methotrexate (MTX) is a widely used anti-metabolite agent, with its mechanism lying in blocking folate synthesis. MTX also has anti-inflammatory and immune-modulating properties, leading to its various uses across multiple specialties. However, MTX has various dose-dependent side effects such as hepatotoxicity, nephrotoxicity, and gastrointestinal toxicity. Although there are toxicity markers for some of these toxicities, there is still much need for new non-invasive markers for assessing others. To this end, we performed metabolomics studies on the urine obtained from MTX-treated male Sprague-Dawley rats (n = 20) (20 mg/kg single dose). Hepatotoxicity and nephrotoxicity of the drug-treated animals were not definitive based on AST, ALT, BUN and Cr levels. However, we observed water-filled stomach and swelled intestinal lymphatic gland upon postmortem, indicating gastrointestinal toxicity. To see if we can correlate this invasive assessment with urine metabolomics profiles, we performed NMR-based metabolomics analysis combined with Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) for urine samples. Multivariable analysis of NMR data presented clear separation between treated and non-treated groups. We also identified 30 metabolites among which hippurate, taurine and creatinine were increased in the drug-treated group. To test the robustness of the approach, we built a prediction model for the toxicity and were able to predict all the unknown samples (n= 14) correctly. Our discovery in metabolic markers might have a clinical value in noninvasive detection of intestinal toxicity caused by this commonly used anti-cancer drug.

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HPLC-Q-TOF-MS DRIVEN UNTARGETED METABOLOMICS APPROACH TO UNVEIL URINARY CHANGES IN METS SUBJECTS FOLLOWING 12-WEEKS NUTS CONSUMPTION.

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In a prospective, randomized, parallel designed, interventional feeding trial [1], we used an HPLC-q-ToF driven metabolomics approach to explore urinary metabolome modifications in subjects with metabolic syndrome following 12-w of mixed nuts consumption (30g/day), compared to a control diet.

24-h urine samples were analyzed using an HPLC-ESI-q-ToF (QSTAR-Elite, AB/MDS-Sciex) (in positive and negative ion modes) followed by multivariate analysis (PCA and OSC-PLS-DA) . The PCA was used to have a rational overview on the data acquisition quality and OSC-PLS-DA models were carried-out for the exploration of the differences among the dietary interventions. Four classes of QC samples were included into the samples injections sequence, following a standardized protocol previously described [2]. A multiple-step procedure was followed to identify significant markers, consisting in the query of freely available and in-house databases, and the further MS^n fragmentation of the metabolites through a LC-ESI-LTQ-Orbitrap (Thermo Scientific) .

The urinary metabolome showed clear differences between dietary interventions. Significant markers of the nuts-enriched diet were obtained by using S-plots [3]. The metabolomics approach revealed 20 potential markers of nuts intake, belonging to distinct chemical families, including fatty acids conjugated metabolites, phase II and microbial-derived phenolic metabolites, and serotonin metabolites. Particularly, an increased excretion of serotonin metabolites was associated for the first time to nuts consumption, thus revealing new potential markers of mixed nuts intake. As well as, the detection of several urinary markers of microbial and host metabolism of nuts phytochemicals confirmed the understanding of their bioavailability and bioactivity as a priority area of research in the determination of health effects of nuts [4]. Summarizing, the results provided new information on the slight modifications of the body's metabolic homeostasis following nuts consumption, thereby giving prospects for new intervention targets.

(1) Casas-Agustench, Lopez-Uriarte et al. Nutr Metab Cardiovasc Dis 2009
(2) Llorach, Urpi-Sarda et al. J Proteome Res 2009
(3) Wiklund, Johansson et al. Anal Chem 2008
(4) Larrosa, García-Conesa et al. Mol Asp Med 2010

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CHROMATOGRAPHIC COMPARISON OF GLYCYRRHIZAE RADIX FROM DIFFERENT REGIONS

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Glycyrrhizae Radix is one of most popular medicinal plants used as crude drugs in Asian countries. The level of active compounds is known to vary widely depending on the plant species (G. uralensis or glabra), geographic source (China, Mongolia, or others), harvesting time (1, 2,3 or more years), and processing. As a first step investigation, we measured hot water extracts of 33 G. uralensis samples from China and Mongolia by Shimadzu LC-IT-TOF-MS (Waters Symmetry C18 column, 40 °C) and their lipase inhibiting activity. Although extracted from the identical species, they demonstrated high variance (almost 60 fold) in the level of biological activity. To find the reason for this variance, total ion chromatograms (TICs) were compared using our custom alignment procedure. TIC records were smoothed by the triangular moving average method, and significant peaks (around top 10 peaks) were selected as alignment landmarks. Major ion peaks were distributed in 16 regions, among which 9 regions shared characteristic mass spectra of saponins (glycyrrhizin and licoricesaponins). Variation in the remaining regions was separately realigned and subject to the support vector machine analysis. The biological activity roughly correlated with glycyrrhizin but other correlations were also found.

(1) Tanaka K, et al. "Multi-stage mass spectromtric analysis of saponins in glycyrrhizae radix" Natural Product Communications 6(1):7-10, 2011

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EFFECT OF *ARTEMISIA ANNUA* EXTRACT'S ON MALARIA PARASITE *PLASMODIUM FALCIPARUM* TROPHOZOITE METABOLME PROFILING BY ^{1}H NMR SPECTROSCOPY.

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Introduction: Artemisinin is a sesquiterpene lactone with C15H22O5 formula that has antiplasmodial and anticancer effects.Artemisinin is mostly extracted from Artemisia annua which is a common type of Artemisia genus from Asteraceae family and is native to temperate Asia. Artemisinin is effective against multidrug-resistant P. falciparum strains. 1H nuclear magnetic resonance has been used to study the metabolite composition of cell and tissue types in recent years. Metabolite profile might vary between different parasite strains and between different phases of the intraerythrocytic life cycle of parasites. In our present investigation, we study the metabolite profiling in trophozoite phases of malaria parasite Plasmodium falciparum.
Material and methods: Artemisinin, was obtained from dried leaves of Artemisia annua from Azerbaijan province (Iran) by continuous percolation method withnon-aqueous solvent selected from ethanol. Extracte was confirmed in1Hnuclear magnetic resonance Bruker 500MHZ with ZG30 protocol, Plasmodiumfalciparum strain 3D7 was cultured with 80% parasitemia and known amount of extract was incubated with trophozoiteculture for 24 hours. Trophozoite metabolites were extracted by percholoric acid methods and was lyophilized and spectra were obtained by same instrument with NOESY protocol with the addition of H2O2. Untreatedtrophozoite cell culture was used as control. Spectra was transferred to Chenomx suit (trail version) and ProMetab software for analysis. PCA and multivariate PLS were applied to our data for better classification of metabolites.
Result and discussion: PCA and PLS showed changes in nearly 40 metabolites, which will be discussed in detail.

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^{1}H NUCLEAR MAGNETIC RESONANCE BASED METABONOMICS APPROACHES FOR EVALUATING RHEUMATOID ARTHRITIS METABOLOME FINGER PRINTING

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Introduction[1] :H NMR spectroscopy was used to identify biomarkers in the sera of patients with rheumatoid arthritis (RA) and may be a potential approach for disease monitoring and personalized medication for RA therapy. Discriminant analysis provided evidence that the metabolic profiles predicted disease severity. Cholesterol, lactate , acetylated glycoprotein, and lipid signatures were found to be candidate biomarkers for disease severity. Metabonomics is the systematic study of the chemical finger print resulting from cell reactions. It is a useful method in the study of the disease process and toxic mechanisms by the help of1 H nuclear magnetic resonance technology .In the present investigation, we studied serum metabolome profile in rheumatoid arthritis (RA (in order to find out the metabolic finger print pattern
Material and methods: 20 sera sample were collected from an active patient and equal number of healthy subjects. They were evaluated during a one-year follow-up with assessments of disease activity and 1H NMR spectroscopy of sera samples. In all cases, the presence of active rheumatoid arthritis was shown by an increase in the T1 values of the synovium of the joints .Serum was collected and kept in -80 oC until assay for Elisa and NMR spectroscopy. We specified and also classified all metabolites using PCA and MLR methods .Chenomx (Trail Version) and ProMetab softwares were used for our data. Resulted data were compared with the NMR metabolite data bank (www.metabolomics.ca .(Antinuclear Antibodies (ANA ,(Uric Acid and Anti-CCP were also analyzed by Elisa's methods .
Result and discussion: Data showing the separation of specific metabolites in our experimental rheumatoid arthritis (RA) patients and findings indicate that NMR spectroscopy provides a sensitive method for the demonstration of inflammatory joint disease.

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METABOLOMICS PROFILING OF LEISHMANIA MAJOR IN TWO PHASES OF LOGARITHMIC AND STATIONARY BY ^{1}H NMR

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Introduction: Leishmania spp. cause a spectrum of diseases worldwide. Most research is now carried out on adaptation of this parasite to different nutrient environments, which were parasite encounters. *Leishmania major* is endemic in Iran. The genomes of three major leishmania parasite were analyzed and characterization of metabolic pathways was facilitated. We need full understanding of parasite metabolism and networks in order to know how these pathway effects on enzyme expression or for finding new drug targets. Metabolomics is the systematic study of chemical fingerprinting produce of cellular interactions, and a useful way to study mechanisms and metabolic cellular interactions. The use of ^{1}H NMR spectroscopy techniques help to determine the metabolome profiling of axenic amastigotes and promastigotes in different species of leishmania. In the present investigation, we studied the metabolome profiling pattern of this *Leishmania major* strain.
according to the method of *in vitro* was cultured (MRHO/IR/75/ER strain) *Leishmania major*:Materials and Methods carried out by samples were selected randomly for NMR spectroscopy. Metabolite extraction was Yehoshua (1988). 10 was analyzed with NOSEY techniques, and principle perchloric acid and was lyophilized before spectroscopy. Spectra .for classification of data component analysis was used

Results: Our classified data shows the pattern profiling of different class of carbohydrate specially TCA cycle in this strain of *Leishmania major*.

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STRUCTURE ELUCIDATION AND CONFIRMATION FOR PLANT METABOLOMICS: NOVEL APPROACHES

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Presently, the key bottleneck of (plant) metabolomics is structural confirmation and elucidation of secondary metabolites.

Nicotiana attenuata is a well-established model system to monitor plant-herbivore interactions with metabolomics being a novel approach to investigate the underlying biology [1]. 17-Hydroxygeranyllinallool diterpene glycosides (HGL-DTGs) are abundant direct defense compounds with their mode of action being largely unknown [1-3]. New acyclic HGL-DTGs were characterized using MS and NMR after extraction of several hundred grams of raw plant material [2, 3]. Such scale is not compatible to the analytical scope of metabolomics.

Here, we present novel solutions facilitating the identification and fast dereplication process of natural products when mass spectral libraries are not yet available and the sample amount is limited.

Plant samples were prepared as described previously [1]. Chromatographic separation was carried out using an UHPLC system combined with ultra high resolution (UHR) Q-TOF MS detection. Selected plant samples were fractionated. Peaks enriched in HGL-DTGs were subjected to detailed fragmentation studies by means of direct infusion measurements.

The dereplication of HGL-DTGs is rendered difficult by the large number of in-source fragments and adduct formation, and their molecular weight of 800-1000m/z. Novel algorithms were applied for deconvolution of LC-MS chromatograms by correlation analysis to safely determine the molecular ion in the presence of adducts and in-source CID fragments. Molecular formula determination was carried out by combined evaluation of mass accuracy, isotopic patterns, adduct and fragment information. The diagnostic fragments for the HGL-DTG backbone and successive sugar units, such as $[M+H]^+$ = 271.2420m/z = $C20H31^+$ and 417.2999m/z = $C26H41O4^+$ enabled the rapid identification of the entire compound family, which is subsequently characterized in more detail. For this, the fragmentation results have been combined with the structural information to visualize the interpretation. Simultaneously the necessary validation prior submission to a mass spectral library is achieved.

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(2) Jassbi, A., et al., Z. Naturforsch. 61b (2006), 1138-1142.
(3) Heiling, S., et al, Plant Cell 22 (2010), 273-292.

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IMPROVING COMPREHENSIVE ANALYSIS FOR MYXOBACTERIAL METABOLIC PROFILING

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As stated by many researchers, extracting relevant information from complex data sets is an important bottleneck in (microbial) metabolomics research. Compared to a focused targeted approach this is even more important in untargeted metabolomics, aiming for the identification of all compounds produced by a particular bacterial strain. Many of these compounds are part of primary metabolism and therefore out of scope when research concentrates on secondary metabolites. Other metabolites may be very common for a certain genus of bacteria and already well known but the large subset of compounds that is "really new" is hard to identify.

By combining targeted and untargeted metabolomics approaches, including searches in freely available data bases, it is possible to narrow down and identify numerous features derived from HPLC-high resolution MS/MS measurements. An automated feature finding algorithm extracted around 2000 – 5000 features within one HPLC-MS chromatogram. In a first step these features were subjected to a search against an in-house database using accurate mass, isotope pattern fit, and retention time in order to identify already known compounds. Untargeted metabolite profiling, using statistical methods such as ANOVA, t-test as well as PCA analysis, could identify features related to the growth of the bacteria by comparing myxobacterial extracts to blank samples (growth medium). These features were automatically added to a scheduled precursor list (SPL) to focus fragmentation experiments to the relevant subset of compounds within the complex mixture. This enabled a fragmentation of even low abundant features which might have been missed during an automatic precursor selection without predefined compounds of interest. High resolution full scan MS and MS/MS spectra were used to identify target metabolites by queries in open source libraries (e.g. METLIN). Remaining unidentified features were subsequently compared to an in-house database to identify putative derivatives of known compounds based on a similar fragmentation pattern.

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METABOLIC PROFILING OF A *CORYNEBACTERIUM GLUTAMICUM* Δ*PRPD* 2 BY GC-APCI HIGH RESOLUTION Q-TOF ANALYSIS

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Metabolomics studies based on Gas chromatography – Mass spectrometry (GC-MS) are well established and typically employ electron impact (EI) ionisation. Target compounds of interest can be identified by comparison to commercial or public databases. Unfortunately, many possible biomarkers detected in metabolic profiling experiments cannot be identified due to the lack of reference spectra for a majority of biologically relevant compounds. Therefore, many possible biomarkers remain "unknowns" up till now.

Hyphenating gas chromatography with high resolution TOF-MS technology with soft atmospheric pressure ionisation (APCI) can preserve the molecular ion information and delivers accurate mass and isotopic pattern information. This data enables a sum formula generation for known and unknown target compounds. Additionally, optionally acquired MS/MS data can extend the capabilities for structural elucidation. Mass accuracy, resolution and isotopic fidelity are independent of the TOF-MS acquisition rate. Therefore, these instruments can be coupled to gas chromatography, which typically delivers narrow peak width requiring fast MS scan speeds.

Corynebactrium glutamicum, a gram positive, non-toxic bacterium, is used in the industrial production of amino acids like lysine and glutamate. *C. glutamicum* can be grown on different carbon sources. Glucose is metabolised via glycolysis and the tricarboxylic acid (TCA) cycle, whereas propionate is catabolised through the methylcitric acid pathway. The *prpD*2 gene encodes a 2-methylcitrate dehydratase which is involved in the degradation of propionate.

Metabolic profiling of *Corynebacterium glutamicum ΔprpD*2 extracts of cells grown on glucose or glucose and propionate analyzed by GC-APCI-TOF-MS revealed several compounds elevated in cells grown on propionate. Identification of 2-methylcitric acid and alanine using accurate mass and isotopic pattern information in MS and MS/MS spectra provided a proof of concept for the identification of target compounds using high resolution MS technology.

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PROTECTING VINES IN STRESS ENVIRONMENTS: EARLY DETECTION OF GRAPE PHYLLOXERA (*DAKTULOSPHAIRA VITIFOLIAE* FITCH) INFESTATION THROUGH IDENTIFICATION OF CHEMICAL BIOMARKERS

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Grapevine phylloxera (*Daktulosphaira vitifoliae* Fitch) is a destructive, root-feeding insect that is difficult to detect and easily spread by the use of shared farm equipment. Early detection of this pest is essential to ensure the sustainability and profitability of the viticulture industry both in Australia and internationally. Late detections of the insect cause significant economic damage to affected vineyards, with replanting onto phylloxera resistant rootstocks costing AUS $20,000 - $25,000 per hectare. Metabolomics offers a new and exciting approach for the early diagnosis of phylloxera infestationon the host-plant root system. Initial studies of grapevine leaf material obtained from field studies in the Yarra Valley, Victoria, indicated that there were metabolic differences between non-infested and infested vines. Analysis suggested that certain flavonols, as well as other, as yet unidentified, metabolites could be useful biomarkers of phylloxera infestation. Validation of these putative biomarkers is crucial for the provision of useful diagnostic tools. Challenges in validation involve the accurate discrimination between biomarkers or metabolic profile changes caused by phylloxera infestation when compared to other environmental stressors. In order to address this challenge glasshouse trials are planned to test the metabolic response of vines to nutrient, water and phylloxera-induced stress. It is therefore critical that biomarkers of infestation can be detected under glasshouse conditions where the vines are relatively immature, under controlled conditions and have had less exposure to phylloxera compared to field conditions. This presentation will describe LCMS-based analysis of vine leaf material from initial glasshouse trials, the statistical analysis of the LCMS data that can differentiate between infested and non-infested vines and the molecules responsible for this differentiation.

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SCRIPTING AN AUTOMATED LIPID ANALYSIS WORKFLOW USING R

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The lipidome covers a diverse range of compounds, including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols, prenols, glycolipids and polyketides. These compounds form components of cell and organelle membranes, are a major part of energy storage and transport mechanisms, act as signalling molecules and include the vitamins A, D, E and K, making them an attractive target for study. Because of the number of possible combinations of chain lengths, bond types and modifications, there are currently over thirty thousand individual compounds in the LIPID MAPS database (http://www.lipidmaps.org) alone. Only recently has it become possible to measure a significant proportion of the lipidome simultaneously using hyphenated mass spectrometry (MS) methods. We have used liquid chromatography-mass spectrometry (LC-MS) with scheduled multiple reaction monitoring (MRM) on a triple quadrupole instrument to identify a large range of different lipid species in a single run (see poster Rupasinghe *et al*., also presented here). While the data output produced by Agilent's MassHunter program is informative, the format is cumbersome. We have created a set of R scripts to extract the data, calculate concentrations, create summaries for each class of compound and to produce graphs. This automation reduces processing time from weeks to minutes as well as reducing the potential for errors to accumulate during data processing. These scripts are available from http://code.google.com/p/ma-bioinformatics/

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MESENCHYMAL STEM CELLS INDUCE RESISTANCE TO CHEMOTHERAPY THROUGH THE RELEASE OF PLATINUM-INDUCED FATTY ACIDS

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The development of resistance to chemotherapy is a major obstacle for lasting effective treatment of cancer. Here, we demonstrate that endogenous mesenchymal stem cells (MSCs) become activated during treatment with platinum analogs and secrete factors that protect tumor cells against a range of chemotherapeutics. Through a metabolomics approach, we identified two distinct platinum-induced polyunsaturated fatty acids (PIFAs) that in minute quantities induce resistance to a broad spectrum of chemotherapeutic agents. Interestingly, blocking central enzymes involved in the production of these PIFA's (cyclooxygenase-1 and thromboxane synthase) prevents MSC-induced resistance. Our findings show that MSC are potent mediators of resistance to chemotherapy and reveal targets to enhance chemotherapy efficacy in patients.

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THE METABOLOMEEXPRESS PROJECT: CELEBRATING A YEAR OF RAPID GROWTH - NEW MEMBERS, DATA AND TOOLS TO ENHANCE METABOLOMICS RESEARCH

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MetabolomeExpress (https://www.metabolome-express.org) is a new web-based data processing pipeline and interactive data repository enabling the transparent online processing, systematic database storage and public mining of metabolomics data from all organisms. One year after its initial publication (Carroll et al., 2010), MetabolomeExpress is growing rapidly with a steady stream of new members from around the world, new datasets and importantly, new tools to enhance the transparency, preservation and utility of the ever-increasing torrents of new metabolomics data being generated by the community. This presentation will provide an overview of the current state of the MetabolomeExpress project, focusing on developments that have occurred in the year since publication and exciting new developments planned for the near future.

(1) Carroll, A.J., Badger, M.R., Millar, A.H. 2010, BMC Bioinformatics, 11:376

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1H-NMR METABONOMICS ANALYSIS OF DANGGUI BUXUE TANG EFFECTS IN HEK293T KIDNEY CELL LINES

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Danggui buxue tang (DBT), a Chinese medicinal decoction commonly used as hematopoietic medicine for treating menopausal irregularity, contains two herbs: radix Astragali (RA) and radix Angelicae Sinensis (RAS). Pharmacological results indicate that DBT can stimulate the production of erythropoietin (EPO), a specific hematopoietic growth factor, in cultured cells. Studies showed that the plasmid containing hypoxia response element (HRE) was highly responsive to the DBT treatment and this treatment would increase the mRNA and protein expressions of hypoxia-inducible factor-1¢G\ (HIF-1¢G\), which up-regulates EPO production. In addition, the activation of Raf/MEK/ERK signaling pathway by DBT could also enhance the translation of HIF-1¢G\, suggesting the dual actions of DBT in stimulating the EPO expression in kidney cells. In this experiment, we employed 1H-NMR metabonomics methods to investigate the metabolic changes of DBT treated kidney cells. We dosed HEK293T cells with 10mg/ml DBT (RA and RAS boiled together as a decoction), RA, RAS and RA+RAS (mixture of separate aqueous extracts of RA and RAS) for 24 and 48 hours. Data were then generated by using 400 MHz NMR. With the use of principle component analysis, we successfully discriminate 4 different dosed groups (RA, RAS, DBT, RA+RAS) and also the control based on their metabolic profiles, which suggests there are component-specific, as well as decoction-specific, effects on the cells. Glycero-phosphocholine is the main metabolite which discriminate DBT dosed group from all other groups. This result is consistent with our previous studies involving DBT, since it is known that choline-related metabolite would increase with HIF-1¢G\ expression. This experiment represents the first attempt to use NMR metabonomics to study the efficacy and action mechanism of traditional Chinese medicine, and the rationale behind the specific decoction preparation method mentioned in these ancient formulae.

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A NOVEL HIGHLY SENSITIVE TEST FOR DETECTING COLON CANCER USING SPOT URINE METABOLOMICS

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With 655,000 deaths worldwide per year, colorectal cancer (CRC) is the third leading cause of cancer-related death in the Western World. However, if identified early, CRC is curable. Current non-invasive fecal-based screening methods for CRC have suboptimal diagnostic accuracies of 30-40% sensitivity. Metabolomics is an emerging field of research that quantitatively identifies low molecular weight compounds generated by metabolism. Metabolomics could identify the biomarkers that predict health and disease states and in turn represent a highly sensitive, non-invasive, novel screening tool for detecting CRC. Urine samples were collected from 444 colonoscopy-negative normal subjects and 116 CRC patients and analyzed by nuclear magnetic resonance. The 1H NMR spectrum of each urine sample was analyzed using Chenomx NMRSuite v7.0 (Chenomx, Inc. Edmonton, Canada). The first 294 normal and 82 CRC samples were used to establish the diagnostic metabolomic model of normal vs. CRC using multivariate analysis with the aid of SIMCA-P+ v12.0.1 (Umetrics, Umea, Sweden) and STATA/SE 10.1 (TX, USA). The model was then validated with the remaining 150 normal and 34 CRC urine samples. Using 69 metabolites, the normal and cancer groups could be separated with a two-component orthogonal partial least squares model. A receiver operating characteristic curve was generated with an area under the curve of 0.8641. Sensitivity of 89.0% and specificity of 56.8% were achieved with this model. Validation with the testing set of 184 blinded samples resulted in sensitivity and specificity of 88.2% and 42.0%, respectively, confirming the robustness of the spot urine test. This study was able to distinguish normal healthy subjects from CRC patients with much superieor accuracy than that of current non-invasive fecal tests.

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A NEW AND HIGHLY SENSITIVE SCREENING TOOL FOR COLORECTAL ADENOMATOUS POLYPS USING A SPOT URINE METABOLOMICS TEST

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Adenomatous polyps are precursors of colorectal cancer (CRC) and their identification is the basis for population based CRC screening programs. Current non-invasive, fecal-based screening methods have poor diagnostic sensitivities (5 to 30%) for adenomatous polyps and limited patient uptake. Novel, patient-acceptable, highly sensitive CRC screening modalities are urgently required. Metabolomics identifies patterns of small molecule metabolites and has been shown to predict health and disease states. The aim of this study was to use urine metabolomics to distinguish healthy subjects from patients with colonic polyps and thereby develop a novel population-based CRC screening test. Urine samples were collected from 354 subjects with normal colonoscopies and 243 subjects with colonic adenomatous polyps (215 tubular, 28 villous). Nuclear magnetic resonance spectra were acquired for each urine sample. The 1H NMR spectrum of each urine sample was analyzed using Chenomx NMRSuite v7.0 (Chenomx, Inc. Edmonton, Canada). The first 294 normal and 200 adenoma urine specimens were used to establish the diagnostic metabolomic model using SIMCA-P+ v12.0.1 (Umetrics, Umea, Sweden). The model was validated with the remaining 60 normal and 43 adenoma samples. A two-component orthogonal partial least squares model for normal vs. adenoma was built; R2Y = 0.396, Q2 = 0.25. The model had a sensitivity and specificity of 89.5% and 71.8%, respectively. The area under the receiver operating characteristics curve was 0.891. Validation of the model with 103 blinded samples resulted in sensitivity and specificity of 72.1% and 40.0%, respectively. This is the first study to demonstrate that NMR urine metabolomics has the ability to distinguish healthy subjects from patients with adenomatous polyps with far-superior accuracy than that of current fecal-based screening tests.

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THEORETICAL CHEMICAL SHIFT DATABASE AND STRUCTURAL INVESTIGATION OF CELLULOSE BY NMR AND SUPERCOMPUTER

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NMR-based molecular detection relies on specific chemical shifts for each compounds. We have ever exemplified a metabolite chemical shift database is highly applicable for complex mixture of metabolites extracted from such as plants [1] in which more than 200 candidate metabolites were detected while half the detected peaks did not have no candidates in the experimental chemical shift database. We should thus extend the concept of the database to a theoretical chemical shift database. The theoretical database is constructed by using supercomputer and collecting ^{1}H and ^{13}C chemical shifts computed by quantum chemical calculation for compounds. While it has a deficiency in accuracy, the advantage easily results in a tremendous number of collected chemical shifts in a batch manner. Further application of quantum calculation of chemical shifts to the analysis of molecular mixture is structural investigation of polymers such as cellulose or lignin, which are important sources of green energy alternative to legacy atomic or fossil energy. Especially, cellulose or lignin is in the mixture of polymers inside cell wall and efficient analytical NMR-based methods for such complicated mixture have not been developed enough. Our database method prospects, also in polymers, similar approach to metabolites but addition of structural geometry of polymers. Our example of quantum calculation thus aimed at a structural investigation of amorphous states of cellulose; the structure of amorphous states have not been determined yet and important for degradation mechanisms of the crystal state. We replaced cellulose to a cellobiose molecule caused of calculation efficiency on the supercomputer. The result shows that a candidate local structure of amorphous states of cellulose was different in area of hydrophobic and hydrophilic surfaces from that of crystal structural state.

(1) Eisuke Chikayama et al., Analytical Chemistry, 82, 1653-1658 (2010)

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NOVEL ANTIBIOTICS AND OPTIMIZED FERMENTATION TIME OF *PHOMOPSIS LONGICOLLA* BY METABOLOMIC APPROACHES

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Bacterial blight is an important and potentially destructive bacterial disease in rice, caused by *Xanthomonas oryzae* (Xoo), recently it develops resistant to the available antibiotics. In this study, mass spectrometry (MS) and multivariate analyses were employed to investigate the correlation between fermentation time dependent metabolite changes of *Phomopsis longicolla* S1B4 and its antimicrobial activity changes during 20 days. The metabolite patterns were clearly distinguished based on the fermentation time, into phase 1 (4 - 8 days) and phase 2 (10 - 20 days) which appeared as distinct clusters in principal component analysis (PCA). The partial least-squares projections to latent structures-discrimination analysis (PLS-DA) showed that significantly contributed metabolites to phase 1 and 2 were deacetylphomoxanthone B, monodeacetylphomoxanthone B, fusaristatin A, dicerandrol A, B and C in LC-MS, and dimethylglycine, isobutyric acid, pyruvic acid, ribofuranose, galactofuranose, fructose, arabinose, hexitol, myristic acid and propylstearic acid in GC-MS based non-targeted metabolites profiling, respectively. The structure of monodeacetylphomoxanthone B was analyzed by mass and NMR spectroscopy and were determined to be novel secondary metabolite. Compared with the strong positive correlations of deacetylphomoxanthone B, monodeacetylphomoxanthone B, dicerandrol A, B and C with antimicrobial activity during fermentation, the pyruvic aicd and galactofuranos displayed strong negative correlations with the activity against Xoo during fermentation.

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BIOCHEMICAL MONITORING OF BLACK RASPBERRY (*RUBUS COREANUS* MIQUEL) FRUITS ACCORDING TO MATURATION STAGE BY ^{1}H-NMR USING MULTIPLE SOLVENT SYSTEMS

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Nuclear magnetic resonance (NMR) techniques coupled with multivariate data analysis were used to conduct monitoring of biochemical changes of black raspberry fruits at different stages of maturation and under various extraction and NMR dissolution solvent conditions: extraction with 50% methanol and D_2O as an NMR dissolution solvent, extraction with 50% methanol and 50% methanol-d_4 as an NMR dissolution solvent, and extraction with 100% ethyl acetate and 100% methanol-d_4 as an NMR dissolution solvent. Partial least-squares discriminant analysis reliably distinguished black raspberry fruits according to the maturation stage, whereby the relative levels of various compounds such as amino acids, organic acids, sugars and phenolic compounds were compared using analysis of variance. Sucrose and most of the amino acids, and organic acids decreased, whereas fructose, glucose, and cyanidins increased in relative concentration according to maturation of black raspberry fruits. The total number and kinds of assigned compounds of the three solvent systems were also compared. This research demonstrates that the metabolic profile of black raspberry fruits changes during maturation, and provides objective criteria for determining the stage of black raspberry fruit maturation via a ^{1}H-NMR-based metabolomics technique using multiple solvent systems.

(1) HS Kim, SJ Shin, SH Hyun, SO Yang, J Lee, JH Auh, JH Kim, SM Cho, PJ Marriott, HK Choi (2011) Food Res. Int. in press

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GEOGRAPHICAL DIFFERENTIATION OF BLACK RASPBERRY (*RUBUS COREANUS* MIQUEL) FRUITS OF VARIOUS ORIGINS BY GENOMIC AND METABOLIC ANALYSIS

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In this study, RAPD was applied to genetically differentiate the black raspberry samples, and nuclear magnetic resonance spectroscopy (NMR) techniques coupled with multivariate data analysis were used to conduct metabolic differentiation of black raspberry fruits of various origins. In addition, relative levels of total phenolic compounds, flavanols, flavonoids and anthocyanins were further analyzed. Hierarchical cluster analysis (HCA) based on genetic and metabolic data sets were separately performed. The HCA dendrogram derived from the NMR data was more matched well with genetic fingerprinting results than that from polyphenols, flavonoids, flavonols, and anthocyanins. This research demonstrates that the genetic and metabolic fingerprinting of black raspberry fruits provides objective criteria for determining the origin of black raspberry fruits.

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LOW-COST, PORTABLE NANOSENSOR ARRAY FOR IDENTIFYING LOW-MOLECULAR WEIGHT MOLECULES

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Chemiresistor sensors are devices that could potentially revolutionise the way low-molecular weight biological molecules (metabolites) are detected. In a recent advance, our Nanoscience team at CSIRO has demonstrated that gold nanoparticle chemiresistors can also be operated in a liquid environment rather than just in the gas/vapour phase.[1] These nanosensors rely on a change in electrical resistance when exposed to an analyte and can be made highly sensitive and selective by modifying the chemical properties of the gold nanoparticle/organic hybrid film. It is anticipated that by incorporating these gold nanoparticle/organic hybrid materials into a sensor array, one can increase the range and type of analytes that can be detected, thus leading to a myriad of potential applications.

In our early work, we used an array of chemiresistor sensors consisting of seven thiol-functionalised gold nanoparticle films for the detection of hydrocarbons in seawater.[2] Coupled with pattern recognition techniques we were able to distinguish crude oil, diesel and three different types of gasoline. We are currently extending the range of potential applications of our sensor by exploring different surface functionalities of the gold nanoparticles for identifying metabolic biomarkers in urine. The combination of sensors, and the information thus obtained could produce a paradigm shift in the diagnosis of disease states.

(1) B. Raguse, E. Chow, C. S. Barton and L. Wieczorek, Anal. Chem., 2007, 79, 7333.
(2) J. S. Cooper, B. Raguse, E. Chow, L. Hubble, K.-H. Müller and L. Wieczorek, Anal. Chem., 2010, 82, 3788.

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METABOLOMICS APPROACH FOR DEVELOPING PREDICTIVE BIOMARKERS OF COLON CANCER

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Metabolomics is a comprehensive quantitative measurement of low molecular weight compounds covering systematically the key metabolites, which represent the whole range of pathways of intermediary metabolism. It bridges information gap by depicting in particular such functional information since metabolite differences in biological fluids and tissues provide the closest link to the various phenotypic responses. The goal of this study was to classify differential expression of metabolites using metabolomics regarding various responses of preoperative chemoradiotherapy in patients with locally advanced rectal cancer (NeoA, 51.8+/-12.5 years and NeoB, 57.8+/-9.6 years) and of hepatic arterial infusion chemotherapy in patients with inoperable liver metastases of colorectal cancer (HAI, 55.4+/-102). The metabolic patterns of patients group were toward controls (27.7+/-5.1 years) from the nontargeted metabolic profiling in PCA. And also the quantitative results were showed similar patterns with the controls in PLS-DA from the steroids analysis, which deduced 16.7% (NeoA), 13.0% (NeoB), and 37.5% (HAI) of non-efficient. We may suggest candidate efficient biomarkers which are dehydroepiandrosterone and androsterone, and non-efficient biomarkers which are cholesterol, enterolactone, 16-keto-estradiol and 11-keto-estrone. It is not clear which part of the metabolome caused the different patterns in urine although it included all the mass ion information. However, the pattern analysis results indicate that the metabolome in urine can be represented the phenotype of disease state and predicted efficacy biomarkers of personalized medicine using metabolomics.

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METABOLOMIC APPROACH ON THE POTENTIAL ROLE FOR OXIDATIVE STRESS IN REVERSIBLE CEREBRAL VASOCONSTRICTION SYNDROME

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Reversible cerebral vasoconstriction syndrome (RCVS) is characterized by recurrent thunderclap headaches and reversible cerebral vasoconstrictions using transcranial color-coded Doppler sonography and magnetic resonance angiography. Excessive sympathetic activities were reasonable with the observations of blood pressure surge and triggers with elevated sympathetic tone in a great proportion of spontaneous RCVS patients. Endothelial dysfunction is caused by reduced activity of endothelium dependent vasodilators such as nitric oxide (NO) and increased activity of vasoconstrictors. Although the clinical presentation of RCVS is being elucidated, little is known about the global metabolomic alterations in its pathophysiology. In the present study, urinary metabolomic differences were profiled by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) across ten RCVS patients and ten matched controls. Pathway analysis using principal component analysis (PCA)-based approaches revealed that the metabolomic alterations between RCVS patients and controls were highly related to lipid peroxidation pathway. We further determined the non-enzymatically and enzymatically metabolites by liquid chromatography triple-quadrupole mass spectrometry (QqQMS) across 71 RCVS patients and 35 matched controls. The non-enzymatically metabolite, 8-isoprostane, was significantly higher in RCVS patients (0.30 ± 0.19 ng/mg creatinine) than controls (0.21 ± 0.11 ng/mg creatinine); the enzymatically metabolite, prostaglandin F2 alpha, was also significantly higher in RCVS patients (1.01 ± 0.15 ng/mg creatinine) than controls (0.65 ± 0.10 ng/mg creatinine). Regarding to the vasoconstrictory effect on cerebral arteries, our findings provided clear evidence that effects of 8-isoprostane and prostaglandin F2 alpha on endothelium might contribute to prolonged vascular changes, and were thus potentially important metabolites of the prolonged vasoconstrictions in RCVS.

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NMR-BASED METABOLOMICS APPROACH IN COMBINATION WITH IONOMICS FOR EVALUATION OF COMPOSITIONAL VARIATIONS AND DIVERSITIES IN INTERTIDAL SEAWEEDS

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NMR-based metabolomics approach has been used extensively to study metabolites in a wide range of biological systems such as plants, animals, and microbial ecosystems (1-4). This approach has flexibility and applicability for combination with other methods, thus we previously reported to develop an approach in combination of the metabolomics with microbial community analysis for monitoring the metabolic dynamics in microbial ecosystems and linking the relationships between microbial community and their metabolic information (1). Although these approaches are powerful tools for evaluation of low-molecular compounds such as short-chain fatty acids and amino acids, some scientists in next step face a significant challenge for evaluation and characterization of the complex biomacromolecules (e.g. plant biomass and polysaccharide in seaweed). To address this challenge, we are now attempting to develop an approach to characterize the compositional variations and diversities of polysaccharides in intertidal seaweeds for evaluation of the complex biomacromolecules in natural ecosystems. In this study, the relationships between compositional variations and elemental profiles in intertidal seaweeds were evaluated by the NMR-based metabolomics approach in combination with ionomics characterized by ICP-OES analysis. By multivariate statistical analysis of metabolome and ionome data matrices, the metabolic- and ion-profiles in intertidal seaweeds were likely to cluster according to the differences of the taxonomic groups (i.e. green, red, and brown algae). In addition, the relationships between metabolic compositions and elemental profiles in intertidal seaweeds were found by the metabolome-ionome correlation analysis. This approach will provide a platform technology for evaluating the compositional variations and diversities of polysaccharides in intertidal seaweeds and opening a new window that will clarify the complex biomacromolecules in natural ecosystems.

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(2) Fukuda S. et al. Nature (2011) 469, 543-547
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(4) Sekiyama Y. et al. Anal. Chem. (2011) 83, 719-726

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WEB PYMS: AN INTERACTIVE WEB BASED GAS CHROMATOGRAPHY - MASS SPECTROMETRY DATA PROCESSING TOOL

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Web PyMS is a web based interactive tool for processing gas chromatography – mass spectrometry (GC-MS) data. Web PyMS allows the processing of complete pipeline of GC-MS data, starting from reading of instrument generated files in standard data formats to creating data matrices ready to be analysed through statistical methods. Web PyMS provides access to reading ANDI-MS/NetCDF and JCAMP-DX files, noise smoothing, baseline correction, peak detection, peak deconvolution, peak integration and peak alignment through dynamic programming. Web PyMS is composed of two technologies - YABI for user interface, workflow construction, and compute pipelining, and the PyMS library for data processing. YABI is a web-facing workflow and high-performance computing interface developed by the Centre for Comparative Genomics, with Web PyMS customisations by the Australian Bioinformatics Facility. PyMS is a suite of data processing tools, and is implemented in Python. Future work on Web PyMS will include (a) the ability of users to interactively query data at the various stages of the pipeline to assist the user to make better informed decisions in setting values to the different parameters of the various modules and (b) the ability to perform targeted analysis of GC-MS data. PyMS is built as an open-source solution under the GPL licence and is available at http://code.google.com/p/pyms

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ELUCIDATION OF NORMAL HUMAN EPIDERMAL KERATINOCYTE (NHEK) METABOLIC PATHWAYS USING ^{13}C-STABLE ISOTOPE INCORPORATION.

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Normal Human Epidermal Keratinocytes (NHEK) are a model cell line for the study of dermal toxicology, and have been used in numerous applications, including investigations of human epidermal development, differentiation and cellular aspects of skin diseases. Comparatively little is known about the carbon metabolism of human epidermal cells, with existing research giving conflicting opinions as to whether epidermal metabolism is aerobic or anaerobic (Freinkel 1960; Decker 1971). Here, we investigate the metabolism of cultured NHEK cells using ^{13}C-stable isotope tracer approaches and analysis of intracellular metabolites by gas chromatography-mass spectrometry. Label derived from U-^{13}C-glucose was incorporated into intermediates in the glycolytic pathway and lactic acid, as well as into citric acid cycle intermediates. Glutamate and glutathione were also labelled, indicating that the TCA cycle may have an important cataplerotic function in these cells. While glucose was the major carbon source utilised by NHEK cells, significant levels of glutaminolysis was evident when NHEK cells were incubated in a serum-free medium supplemented wiht U-^{13}C-glutamine, with incorporation into glutamate, glutathione and TCA cycle intermediates. Label from U-^{13}C-glucose was also strongly incorporated into fatty acids and cholesterol. Collectively, these analyses show that NHEK have an active aerobic metabolism and can utilise both glucose and glutamine. Apart from energy generation, the mitochondrial catabolism of glucose appears to be required to sustain high rates of glutathione synthesis and lipid synthesis that may confer resistance to oxidative stress.

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IRON(III) TARTRATE AS A POTENTIAL PRECURSOR OF LIGHT-INDUCED OXIDATIVE DEGRADATION OF WHITE WINE: STUDIES IN A WINE-LIKE MATRIX

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Environmental pressure on the wine industry is increasing, with demands to reduce the mass of bottles (lower transport carbon costs) and to move away from darker coloured bottles to reduce recycling costs. While arguments relating to altering the colour and weight of bottles are often based on aesthetics and consumer appeal, there is a lack of information regarding the stability of wine in bottles that are thinner and lighter coloured. The wine industry has always assumed that UV radiation is critical in causing photodegradation of wine, but there is increasing evidence that low wavelength visible radiation can initiate oxidative breakdown. The potential of iron(III) tartrate to act as a photoactivator in light-induced oxidative degradation of white wine was identified, that is the formation of xanthylium type pigments. Using a tartaric-acid-based wine-like matrix containing 5 mg/L iron, exposure to light using the small wine irradiation setup led to the oxidative degradation of tartaric acid and the production of glyoxylic acid, a known pigment precursor. The critical wavelength of light for the degradation process was found to be below 520 nm. No glyoxylic acid was formed in the absence of iron and/or light. Flint glass offered little protection from the light-induced photodegradation of tartaric acid and Antique Green glass offered more protection but did not stop the photodegradation process. The impact of this light-induced production of a phenolic pigment precursor will be described. Small and large scale wine irradiation setups were designed to allow wine to be irradiated under controlled light, temperature and oxygen concentration conditions, conducive to enhanced oxidation. Bottles of different colours and weights were used as ‘filters' in the irradiation studies. Irradiated wine samples were profiled using a metabolomics approach utilising UV-Vis absorption spectroscopy and LC-MS which identified xanthylium type pigments characteristic of enhanced pigmentation in wine.

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INFORMATION INDEPENDENT MS/MS DATA COLLECTION OF ALL PRECURSORS USING HIGH RESOLUTION ACCURATE MASS SPECTROMETRY

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As lipidomics emerges as a necessary biomarker track for research in health and medicine, high resolution accurate tandem mass spectrometry strategies can offer the throughput and the molecular species characterization such studies require. A simple technique of stepping through a set mass range fragmenting everything, without applying criteria or any prioritization, may offer advantages when profiling lipids in complex biological extracts in an untargeted fashion. In this MS/MS^{ALL} workflow, a unit-resolved window defined in Q1 steps through a given mass range, with all product ions stored enabling the recall of any precursor ion spectrum and lipid-class specific profiling post-acquisition. This technique is similar to multiple precursor ion or neutral loss scanning on a QqQ-type instrument but is carried out with high resolution and accurate MS/MS mass information at high speeds. Novel data visualization tools provide the capabilities of filtering and profiling of any precursor characterized by a particular fragment or neutral loss in quantitative applications.

Through the nanoelectrospray infusion high resolution mass spectrometry analysis of very small lipid extract volumes, global lipid profiling experiments of yeast shows the identification of lipid species from 15 lipid classes encompassing a total of 179 lipids in positive mode and 337 lipids confirmed in negative mode in a total of two 1.8 min acquisitions. Data distilled by LipidView^{TM} software provided complex lipid array with 43, fully characterized, fatty acid moieties identified with high confidence. Linear dynamic range of the acquisition method was studied using a 6-point dilution series of liver tissue extracts with 12 internal standards added. Quantitatively, the lipid class internal standards showed excellent response and reproducible measurements with CVs < 10%.
This study presents a simple quantitative workflow for the identification of hundreds of lipids in total lipid extracts from biological samples using a new acquisition mode.

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EXOMETABOLOMICS REVEALS INCREASED GLYCOLYSIS IN CULTURED NEURONS LACKING ALPHA-SYNUCLEIN

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Alpha-synuclein is a molecular chaperone that is believed to play a pivotal role in the pathogenesis of Parkinson's Disease and dementia. We applied 1H-NMR metabolomics to media from cortical neuron cultures derived from C57Bl/6 (WT, n = 9) and alpha-synuclein null (sKO, n = 8) mice. Media was sampled at 15 days, deproteinized with acetonitrile, evaporated to dryness in an evacuated centrifuge and resuspended in NMR buffer (100% D2O + 2 mM DSS). One-dimensional 1H NOESY spectra were acquired using a Varian Inova 400 MHz MR spectrometer; the water signal was set to the centre of the transmitter offset and a presaturation pulse of 2 sec was applied. Individual free induction decays were Fourier transformed and baseline-corrected in NMR Manager 12.0 (Advanced Chemistry Development, Toronto, Canada). The spectra were normalized and bucketed using probabilistic-quotient normalization [1] and adaptive intelligent binning [2] respectively; both routines were custom-written in Matlab. The bucketed, normalized spectra were mean-centered, Pareto-scaled and orthogonal signal-corrected (for ease of interpretation) before being analyzed using PLSDA, all in PLS Toolbox 6.2 (Eigenvector Research Inc., Wenatchee, US). A PLSDA model comprising 2 latent variables was sufficient to correctly classify media from the two classes of cell (Figure 1). Latent variable 1 (LV1) was dominated by a variable that corresponded to a doublet at 1.31 ppm (Figure 2); this peak was confirmed as lactate by correlation spectroscopy. As fresh culture media (Neurobasal) did not contain lactate, our data indicate that sKO neurons produce approximately twice as much lactate as WT neurons. This is consistent with previous reports that alpha-synuclein knockout mice have impaired respiratory chain function [3] and indicate a critical role for alpha-synuclein in maintaining healthy neuronal mitochondrial function.

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A WORKFLOW FOR METABOLIC PROFILING AND DETERMINATION OF THE ELEMENTAL COMPOSITIONS USING MALDI-FT-ICR MS

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Mass spectrometry (MS) is a standard technique used in metabolomics applications. Although liquid chromatography or capillary electrophoresis coupled to MS have been widely used for this purpose, this methodology often depends on spectral databases for compounds identification. This approach is hampered by the fact that only ~20% of biologically relevant metabolites are commercially available. Here we present an MS-based metabolomics workflow which employs matrix assisted laser desorption ionization (MALDI)-Fourier transform ion cyclotron resonance (FT-ICR) MS.

HepG2 human liver carcinoma cells were administered with different concentrations of the anti cancer drug 5-fluorouracil (5FU). Intracellular metabolites of treated cells and a control group (five biological replicates each) were extracted by a biphasic extraction with methanol/water/chloroform=2/2/1. The extracts were analyzed by MALDI-FT-ICR MS using 9-aminoacridine as matrix in negative ion mode. Spectral data was acquired within 30 seconds per spectrum and the obtained spectral resolution was typically >400,000 (FWHM). Acquired data was evaluated using multivariate statistical analysis. Principal component analysis (PCA) showed a clear separation between dosed and control cell groups. Additionally, orthogonal partial least-squares discriminant analysis (OPLS-DA) could discriminate these groups and indicated several loadings contributing to this separation. Subsequently, elemental compositions were calculated for these compounds. The ultrahigh resolution data enabled to enhance the confidence for the formula assignment by comparing measured isotope fine structures with theoretical ones. A query of these sum formulae in public databases indicated that those compounds correspond to nucleotides and amino acids. Two of these analytes showed a clear dose-dependent reduction in peak intensities.

The present study shows that 5FU seems to inhibit the biosynthesis of several nucleotides and amino acids in HepG2 cells. Acquired ultrahigh resolution could separate both monoisotopic peaks close to each other as well as isotope fine structures. This methodology enables non-targeted metabolites profiling and biomarker discovery with high throughput capabilities.

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GLOBAL EFFECTS OF THE UV-B IRRADIATION ON METABOLITE-CORRELATION NETWORKS IN PLANTS

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One of the great challenges in plant systems biology is to understand genotype-phenotype associations [1]. Metabolomic correlation approaches have contributed to characterize biochemical phenotypes in plants [2-3]. Small studies have been done on the systematic and comprehensive evaluation of the metabolomic correlations across different genotypes, tissues, times, and stress treatments. To elucidate global effect of ultraviolet-B (UV-B) irradiation [4], which inhibits plant growth and causes serious damage to plants, we compared metabolite-to-metabolite correlations in metabolomic dataset of *Arabidopsis thaliana* obtained by using gas chromatography-time of flight/mass spectrometry (GC-TOF/MS) and liquid chromatography quadrupole-TOF/MS (LC-q-TOF/MS) under three light conditions (long day, continuous light and UV-B). We found that UV-B and continuous light treatments highly affect the rewiring of metabolite-correlation networks in wild-type Col-0 plants. Furthermore, mutants lacking flavonoids and anthocyanins (*transparent testa 4*, *tt4*; *transparent testa 5*, *tt5*) and sinapoyl-malate biosynthesis (sinapoylglucose accumulator *1*, *sng1*) also exhibited similar trends of metabolic reconfiguration in the correlation networks by UV-B irradiation. Such approaches with metabolomic data can facilitate the investigation to fill in the missing blank on our knowledge about how metabolic pathways cooperate in response to environmental stresses.

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USING METABOLOMICS TO PROBE THE IMPACT OF AN ADAPTIVELY EVOLVED WINE YEAST ON OENOLOGICAL FERMENTATION

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Adaptive evolution is the process by which a biological system evolves and therefore adapts under various environmental conditions and offers a versatile model for the development of optimised wine yeast. The robust and efficient fermentation of sugars under oenological conditions by the adaptively evolved wine yeast strain, FM16-C7, isolated by this laboratory, has been previously reported. Genomic changes in such strains are often complex and this is an area currently under investigation by whole genome sequencing. However, even with a known genome, relation of any genomic changes to a phenotypic outcome is challenging.

Examination of the metabolomic profiles of such strains potentially offers another way to unravel the improvements observed in an oenologically relevant fermentation. Furthermore, direct determination of those compounds relevant to winemaking will help to define the commercial potential of this strain. By using multiple methods (GC-MS and LC-MS) we measured both the intracellular and extracellular metabolites (i.e. the potential contributors to wine flavour and aroma) of FM16-C7 present after the completion of fermentation. A subset of discriminatory metabolites has been determined and the metabolic basis for the observed changes in their production and will then be related to the known genomic changes.

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METABONOMIC INVESTIGATION OF PHOSPHINE TOXICOLOGY AND RESISTANCE IN *CAENORHABDITIS ELEGANS*: METHOD DEVELOPMENT IMPROVES ANALYSIS OF HOW A MODIFIED METABOLISM CONTRIBUTES TO FUMIGANT TOLERANCE AND A NOVEL LONGEVITY MECHANISM.

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Phosphine is the most extensively employed fumigant in grain agriculture as it obviates the negative environmental and toxic effects of other pesticides. However, the rapid development of pest resistance has jeopardized the continued use of the fumigant. In spite of extensive research done on phosphine toxicology, a conclusive mechanism of action has not yet been fully elucidated. Phenotypic observations of resistant insect strains have further revealed a novel longevity mechanism that shares similar characteristics to other known long-lived mutations and lifespan increasing conditions such as caloric restriction.

In this study, the well-characterised model organism *Caenorhabditis elegans* was employed to investigate the physiological foundations behind phosphine resistance. Resistant *C. elegans* strains were exposed to several concentrations of phosphine, and whole-nematode aqueous extracts were analysed.

^{1}H NMR Spectra were analysed with both, bucketing methods (0.01 and 0.04ppm) and using the full resolution spectra. To minimize some of the effects of chemical shift variability due to pH, a specific R script was developed to align data to a reference ppm value. The data were then analysed using the multivariate statistical approaches PCA, PLS and orthogonally-filtered two-way PLS (O2PLS). Analysis and metabolite identification in the high resolution spectra was aided by 1D bivariate loading plots with univariate scaling, whilst Pareto scaling provided a clearer method of identification in 2D loadings plots of bucketed spectra. The characteristic metabolites identified were further confirmed from 2D TOCSY and HSCQ/HMBC spectra. The metabolites identified belong to common metabolic pathways associated with oxidative energy production. This area has long been implicated in life-span increasing effects.

These results demonstrate how sophisticated analysis methods greatly improve metabolite identification. In addition, combination of the metabolomic results with a genetic analysis has helped to elucidate the target enzyme involved, its effects on metabolic pathways and how they may contribute to the phenotypes observed.

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THE METABOLOME OF *SYMBIODINIUM* PHYLOTYPES AND THEIR CORAL HOSTS: THE RESPONSE TO ENVIRONMENTAL STRESSORS.

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Symbiodinium spp. lives freely in the water column and are in symbiosis with a number of marine invertebrates, such as coral, giant clams and anemones that are predominantly found in waters with low nutrient concentration and plankton densities [1, 2]. Some invertebrates have formed this symbiotic relationship with *Symbiodinium* in order to gain a competitive advantage through increased fitness [3], allowing the bilateral exchange of metabolites, including the production of metabolites that are not formed by either organism separately [4]. Of particular interest to researchers is the relationship of Symbiodinium with scleractinian (hard) corals. The importance of this relationship cannot be understated due to the fundamental role that scleractinian corals have played in the formation and maintenance of coral reefs. These habitats provide a livelihood for local communities with tourism and fishing industries relying heavily upon them. Unfortunately, due to anthropogenic pollution and global climate change this ecosystem is under increasing threat [5, 6]. The value of metabolomics to this project lies in its ability to analyse relevant elements of the symbiont/holobiont metabolism in response to selective pressures and provide novel data sets of the holobiont and symbiont metabolome. To date, no research has analysed the complex metabolome of the coral holobiont yet it is a vital element of understanding it's response to environmental stress. This poster illustrates the fundamental concepts of this research and presents the results of a newly developed sample handling and extraction method for metabolomics analysis of hard corals.

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QUALITATIVE AND QUANTITATIVE ANALYSIS OF TRADITIONAL CHINESE MEDICINE GARDENIA JASMINOIDES ELLIS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND RAPID RESOLUTION LIQUID CHROMATOGRAPHY COUPLED WITH TIME-OF-FLIGHT TANDEM MASS SPECTROMETRY

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A high performance liquid chromatography (HPLC) and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry (RRLC-Q-TOF) method was developed for the quality assessment of Gardenia jasminoides Ellis (Chinese name, Zhizi), a commonly used traditional Chinese medicine (TCM). The metabolite profiling of 4 batches of Gardenia jasminoides Ellis from Jiangxi province including the reference standard of crude drug from the National Institute for Control of Pharmaceutical and Biological Products , cultivated crude drugs from two different pharmaceutical companies and uncultivated crude drugs collected has been performed using RRLC-Q-TOF and multivariate statistical analysis techniques. The PCA scores plot could be readily divided into three different groups and successfully discriminated cultivated and uncultivated as well as the quality Gardenia jasminoides Ellis in accordance to the reference standard of crude drugs. In addition, gardenosid, which is biologically active and also the predominant compound in this crude drug, was quantified by HPLC method according to Phamacopoeia of the Peoples Republic of China (2010). This proposed HPLC method was successfully utilized to analyze gardenosid in 4 batches of Gardenia jasminoides Ellis extrats. The results demonstrate that this analytical method is simple and suitable for the original discrimination and quality control of this TCM.

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METHODS TO INCREASE OUR UNDERSTANDING OF FRUCTANS AND THEIR BIOSYNTHESIS

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Fructans are water soluble fructose polymers that are produced by a wide variety of both dicots and monocots. As well as being energy stores, they have been linked with tolerance of abiotic stresses, such as cold, drought and salinity. The model for fructan biosynthesis known as the SST/FFT model is commonly accepted, and applies in dicots, which produce only fructans with beta 1-2 bonding, known as inulins. Its applicability in monocots tends to be more complex and this is especially true in perennial ryegrass (Lolium perenne).

Ryegrass has been shown to produce Inulins, levans, neo-levans and possibly other fructans. The basic knowledge of fructan biosynthesis in ryegrass has generally been inferred from the biosynthesis in dicots. The fact that no confirmed FFT enzyme has been reported in ryegrass illustrates the fact that key pieces of information are still missing which are required for a fundamental understanding of the processes involved.

In this paper we show the steps we have taken to develop new analytical techniques to analyse fructan extracts from ryegrass and how these techniques will be applied in the future to elucidate the processes involved in fructan biosynthesis in ryegrass and the benefits on high resolution to expanding the size range that can be analysed

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A METHOD FOR BATCH PROCESSING ISOBARIC AND ISOMERIC COMPOUNDS VIA NON-TARGETED, RELATIVE RETENTION TIME DATA PROCESSING

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The use of chemical data to understand complex biochemical and biological problems requires the analysis of large quantities of data. The sample that are use to generate the data are usually extremely complex containing a large number of isobaric/ isomer compounds each of which can be involved in numerous different biochemical pathways. It is therefore critical that data reduction strategies that are robust and can be used to accurately assign identity are developed. The unambiguous identification of metabolites in chromatography depends on their reliable discrimination by retention time as well as qualitative properties such as *m/z* ratio.Retention time drifts can significantly complicate comparison of LC-MS datasets The subtlety of the interactions with the column or other analytes could result in variability in retention time, due to the fact that this is difficult to control either physically or chemically, and a computational approach may provide a solution. Reliable peak picking and assignment of identity are essential for subsequent data analysis which such as the inclusion of genomic and transcriptomic data in an attempt to understand the mechanisms and control of fructan biosynthesis. In this paper we explain the approach taken in our lab to analyse data showing significant temporal moments

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METHODOLOGY FOR ^{13}C-LABELING OF ENTIRE PLANT METABOLOMES

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Isotope dilution analysis using stable isotope-labeled standards is a well-established approach to metabolite quantitation by mass-spectrometry (MS). Cost-effective steps to acquiring labeled standards for all metabolites identified in MS-based metabolomics experiments, however, represent a key challenge. One innovative yet practical option is *in planta* ^{13}C-metabolome labeling. We have now developed a system to grow soybean plants, from seed, in a 100% $^{13}CO_2$-enriched environment. This system includes a transparent air-tight chamber with computer controlled $^{13}CO_2$ flow; nutrient application, pressure and humidity regulation, and ethylene and other volatile organic compound removal. Light intensity and temperature are measured continuously and controlled by placing the chamber in a larger environmental chamber. The system is equipped with other key monitoring features and incorporates a modular approach that allows assembly/disassembly of system components without compromising the integrity of ongoing experiments. All computer controls can be accessed remotely. Electronic records of conditions in the chamber are archived continuously throughout experiments, for future reference. Results have shown that over 90% enrichment of selected metabolites can be achieved in leaf at the R2 developmental stage. Use of ^{13}C-labeled metabolome standards generated through this technology will not only enable quantitation, but also improve the accuracy and reproducibility of metabolomics data. Key design features and plans for fully characterizing and utilizing the enriched metabolomes will be presented.

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THE APPLICATION OF MASS PROFILER PROFESSIONAL IN PLASMA BIOMARKER DISCOVERY: A STATISTICAL ANALYSIS & VISUALIZATION SOFTWARE TOOL IN MENTAL DISORDER METABOLOMICS

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Biomarker discovery can be performed at all levels of information flow in biological systems. Integrated Biology then is the discipline compiling and extracting meaningful data to comprehensibly describe any such system under scrutiny. However, in the quest for biomarkers it is desirable to obtain and retain only information which shows variance in expression levels at the expense of all other information.

Metabolite Biomarker discovery is the pursuit to display differentially expressed metabolite levels. MS based differential metabolite profiling is one way of monitoring up- and down-regulation between samples. Provided any perturbation causes a response in the system and here in particular at the metabolite level this variation may be monitored over time. Mass Profiler Professional (MPP) software is used to cluster biological and technical replicates and differentiate them in terms of the time domain of any variance occurring between samples where time course experiments are concerned or likewise expression levels where differential expression is the result of a treatment or a disease state. Only the molecular entities that show defined significant differences between samples are selected and retained by the MPP statistical analysis and visualization platform and ultimately identified. The resulting identified metabolites are candidate biomarkers for the response of the system to the perturbation.

The profiling strategy is, hence, ideally suitable to investigate experimental designs to study lifecycle stages and disease states in particular and any differential expression scenario in general. The analysis strategy on the statistical analysis level (MPP) will be exemplified via software data (profiling, molecular feature extraction, filtering, principle component analysis (PCA), condition- and mass-tree generation aka hierarchical clustering analysis (HCA), fold-change analysis, volcano plot representation) detailing the workflow to answer questions dedicated to plasma metabolomics in response to mental disorder.

HUMAN NON SMALL CELL LUNG CANCER TISSUE HAS ENHANCED EXPRESSION OF PYRUVATE CARBOXYLASE, BUT NOT GLUTAMINASE

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Altered cellular metabolism in tumors is well established, particularly an increased rate of aerobic glycolysis [1]. However, increased glycolysis alone is insufficient to sustain the enhanced cell growth and anabolic metabolism characteristic of cancer. Another key source of these precursors is the citric acid cycle (CAC). Removal of CAC intermediates for anabolism requires replenishment by other sources; these anaplerotic reactions are vital to cancer development. The two major pathways of anaplerosis are glutaminolyis (deamidation and transamination of glutamine to 2-oxoglutarate) [2] and carboxylation of pyruvate to oxaloacetate (OAA), catalyzed by pyruvate carboxylase (PCB) [2].

We have coupled ^{13}C-labeled glucose tracer with stable isotope resolved metabolomics (SIRM), gene microarray, and western blotting to track the anaplerotic PCB pathway in human lung cancer patients. The resected cancer tissue showed increased flux through the PCB pathway, along with increased transcription of the *PCB* gene and PCB protein expression compared with the adjacent benign lung tissue [3]. We have extended these findings to 50 paired tissue samples, and have correlated them with histological and clinical data. The protein expression of PCB was on average ten-fold higher in the tumor than in the benign lung tissue ($p=0.00001$, paired t-test). The normalized protein expression correlated with the fraction of the tumor that was identified as cancer cells, indicating that the increased expression was from the cancer cells rather than other lung cell types. Glutaminase was active in the lung tissue, but its expression was not different from, or lower in the tumors than in the benign lung tissue.

These data indicate that in NSCLC, PCB is important in anaplerosis and tumor metabolism. The balance between glycolysis and the choice of different anaplerotic pathways may depend on the tissue types.

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CHARACTERIZATION OF ION CONTENTS AND METABOLIC RESPONSES TO SALT STRESS OF DIFFERENT *ATHKT1;1* GENOTYPES AND THEIR PARENTAL STRAINS IN *ARABIDOPSIS THALIANA*

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Increasing soil salinity is one of the main environmental constraints on crop productivity worldwide. Plant strategies to maintain ion homoeostasis within the cell under salinity stress include the mediation of ion fluxes by transmembrane transport proteins and osmotic adjustment by accumulation of osmolytes. The HKT (high-affinity potassium transporter) gene family comprises of Na+ and Na+/K+ transporters in diverse plant species, with HKT1;1 as the only member of this gene family in Arabidopsis. AtHKT1;1 is expressed solely in root stelar cells, where it is hypothesized to remove Na+ from the transpiration stream, and has been shown to prevent overaccumulation of Na+ in the shoot under salinity stress [1]. Møller et al. [2] employed a enhancer trap expression system to express HKT1;1 specifically in the root stele of Arabidopsis, which led to a decreased Na+ accumulation in the shoot, and increasing salinity tolerance of the plants. Although several studies have successfully applied metabolomics to associate certain metabolites to abiotic stress responses, only few studies of metabolite profiling have been applied to dissect salt stress tolerance response pathways or have even combined metabolite data with data from elemental analysis. Here we describe the profiling of ten elements including calcium, sodium, and potassium in shoots and roots of different AtHKT1;1 genotypes (both cell-type specific overexpression and loss-of function mutants) and its parental strains before and after salinity stress using ICP-MS. Additionally, the effect of salt on the primary metabolism was investigated using gas chromatography-mass spectrometry (GC-MS), showing distinct metabolite responses to salinity stress of both cell-type specific overexpression mutants, and a stronger impact on the root than on the shoot metabolism. This approach has the potential to reveal currently unknown interactions and interdependent relationships between genes, metabolites, and ions in plants in response to salinity stress.

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METABOLOMIC PROFILING USING CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY DIFFERENTIATES DIABETIC NEPHROPATHY

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Diabetic nephropathy (DN) is the most common complication of diabetes and a major cause of end-stage renal disease around the world. Microalbuminuria, a currently available non-invasive marker for DN risk assessment, remains relatively inaccurate. Thus, the exploration of new biomarkers bearing high sensitivity and specificity for DN diagnosis is of great importance. In this study, we utilized capillary electrophoresis coupled with time-of-flight mass spectrometry (CE-TOFMS) for comprehensive analysis of serum metabolites from a total of 78 diabetic patients including 20 without microalbuminuria, 32 with microalbuminuria and 26 with macroalbuminuria. Multivariate analyses were used for the identification of biomarker candidates and the development of discriminative models. Among the 289 profiled metabolites, orthogonal partial least squares-discrimination analysis (OPLS-DA) identified 19 candidate metabolites, including creatinine, aspartic acid, ƒÁ-butyrobetaine, citrulline, symmetric dimethylalginine (SDMA), kynurenine, azelaic acid and galactaric acid, whole level were found to vary with albuminuria status. Correlations between all metabolites and urinary albumin creatinine ratio (UACR, $p < 0.009$; Spearman • fs rank test) were significant. Fifteen metabolites also showed significant correlation with estimated glomerular filtration rate (eGFR, $p < 0.035$). A multiple linear regression (MLR) model achieved high area under the receiver operating characteristic curves value (AUC) of 0.927 and 0.880 using whole datasets and cross validation (CV), respectively. Overall, the combination of serum metabolomics and multivariate analyses as demonstrated here enabled accurate discrimination of DN stages, suggesting a possible novel diagnostic approach for DN.

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METAPICK, A DENOISING AND PEAK PICKING ALGORITHM FOR EXTRACTING SINGLE COMPOUND FROM COMPLEX MIXTURES IN LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS) METABOLOMICS DATA

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Peak picking from a spectrum of mixtures is a challenging task in Metabolomics and often requires a denoising process. Current denoising methods can be a simple background subtraction procedure that checks all ions in the control scans within a specified time window around the analyte scan for potential subtraction of ions found in the analyte scan. Denoising can also combine with peak picking to generate peaks by first determining the noise by eliminating ions of interest from a given spectrum and then filtering all the spectra with the noise determined. In this study, we developed a denoising and peak picking algorithm, MetaPick, for extracting a single compound -- from complex liquid chromatography/mass spectrometry (LC/MS) mixtures -- to construct the NTU MetaCore reference LC/MS library. MetaPick contains three major steps; background subtraction, random noise reduction, and peak picking. MetaPick is able to effectively determine a single compound extracted ion chromatogram from complex mixtures effectively and to detect both low-intensity peaks and split peaks that may arise from either saturation or a general background subtraction and noise reduction algorithm.

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THE APPLICATION OF GC-MS-BASED METABOLOMIC PROFILING TO THE OPTIMISATION OF POLYHYDROXYBUTYRATE (PHB) PRODUCTION IN TRANSGENIC SUGARCANE

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Polyhydroxybutyrate (PHB) is a biopolymer that is produced from acetyl coenzyme A as a carbon and energy reserve by bacterial species such as *Ralstonia eutropha*. The polymer is of commercial interest due to its potential use as a biodegradable plastic. Bacterial-based production of PHB is considered to be commercially non-viable due to substrate, energy input and waste disposal costs and thus genetically-modified plants offer an alternative production platform. Sugarcane (*Saccharum* spp. hybrids) is an attractive candidate due to its high biomass yield, vigorous growth and low viable seed production.

We generated transgenic sugarcane lines containing expression cassettes with the *phaA*, *phaB* and *phaC* genes targeted to plastids. These lines produce up to 4.8% dry weight PHB in mature leaves. An apparent comparative reduction in vigour and leaf chlorosis in the higher producing lines was observed in a randomised, replicated glasshouse trial. Therefore metabolomic profiling of sugarcane leaves of the same physiological age from these lines was performed to determine whether changes in endogenous metabolite profiles could be correlated to the production of PHB, thus revealing pathway perturbations and possibly providing further bioengineering targets.

Similar growth-state leaves were harvested from each plant, processed and extracted for polar and non-polar metabolites and subsequently analysed using gas chromatography-mass spectrometry (GC-MS). The data was processed using AMDIS and Mass Profiler Professional, normalising data to the relevant internal standard(s) and filtering data based on group frequency. An HPLC method was used to quantify the PHB content of the leaves, and this data was used to anchor the GC-MS data and correlate it to the desired production endpoint using PLS/OPLS. PCA and PLS-DA were employed to define differences between the wild-type and transgenic lines and, along with PLS, to infer possible engineering targets to refine and increase the production output of the plants.

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EFFECT OF GENES ON ROSUVASTATIN THERAPY FOR HYPERLIPIDEMIA

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Rosuvastatin (RAV) is one of the powerful lipid-lowering statins. The metabolism of RAV is principally mediated by the cytochrome P450 2C9, UDP- glucuronosyltransferase (UGT) 1A1, UGT1A3 and organic anion transporter polypeptide 2 (OATP2). This study enrolled 107 hyperlipidemic patients who treated with 5 mg RAV daily. Before and 3 months after RAV treatment, lipid profile including total cholesterol (TC); low-density lipoprotein cholesterol (LDL-C); high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were measured. Single nucleotide polymorphisms (SNPs) at nucleotides (nts) -53/211 of UGT1A1 gene and SNPs at nts 388/521 of OATP2 gene were detected with the methods of PCR-restriction fragment length polymorphism, while SNPs at nts 31/140 of UGT1A3 gene were determined with DNA sequencing method. The 107 study subjects were divided into 4 groups according to their variation status of UGTs and OATP2 genes, respectively. Then the 4 groups were divided into 2 subgroups according to therapeutic effectiveness: ≥40% or <40% for LDL-C reduction; ≥30% or <30% for TC reduction; ≥15% or <15% for TG reduction and ≥6% or <6% for HDL-C increasing, respectively. Odds ratio (OR) and its 95% confidence interval (CI) of each genetic group were calculated. The results showed that only the 95% CI (1.37~54.48) of OR (8.63) in the subjects possessing variations at both nt 388 and nt 521 of OATP2 gene and with effective LDL-C reduction was statistically significant. Variations at nts 388/521 in OATP2 gene my cause decreased elimination rate of RAV and increase the plasma concentration of RAV. This is the reason that effective lipid reduction is observed in the subjects carrying such a genetic variation when they receive RAV treatment.

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GC-MS-BASED METABOLOMIC STUDY IN MICE WITH COLITIS INDUCED BY DEXTRAN SULFATE SODIUM

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Metabolomics provides data about all the metabolic processes of a cell or organism. We examined the changes of metabolite levels in the serum and colon tissue of colitis mice using gas chromatography mass spectrometry (GC/MS) with the aim of achieving a detailed understanding of the pathogenesis of inflammatory bowel disease (IBD). A total of 77 and 92 metabolites were detected in serum and colon tissue, respectively, and among the metabolites the compositions of TCA cycle intermediates and amino acids changed depending on the degree of colitis. Then, partial least square discriminant analysis (PLS-DA), a multiple classification analysis, showed distinct clustering and clear separation of the groups according to the degree of colitis. Furthermore, PLS-DA loadings plots revealed that succinic acid, indole-3-acetic acid, glutamic acid, and glutamine were the main contributors to the separation of each stage of colitis. In addition, it was revealed that supplementation with glutamine, the level of which was significantly decreased in the acute phase of colonic inflammation, attenuated colitis induced by DSS. Our results suggest that metabolomics is capable of representing the various degrees of colitis, and our findings will aid in the discovery of therapeutic agents for IBD and other inflammatory disorders by metabolomic approaches.

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COMPARISON OF ANALYSIS PLATFORMS USING MASS SPECTROMETRY IN METABOLOMICS.

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Although analysis platform using mass spectrometry (MS) is prevalent in metabolomics, the detection of molecular species is depend on the separation methods combined with MS, even more than the kind of MS itself. In current metabolomics, it is difficult to cover all metabolite in a single analysis platform, because varieties of chemical properties of metabolites. For instance, using analysis platform combining capillary electrophoresis (CE) and MS, ionic metabolites are effectively detected. By contrast, the quantitative capability for neutral metabolites are susceptible due to the effect of ion suppression resulted from the detection of these compounds in cluster as one inseparable peak. On the other hands, metabolites separation using liquid chromatography mass spectrometry (LC-MS) depends a great deal on column selection. For instance, using reverse phase column, hydrophobic metabolites are separable well, but hydrophilic metabolites are rarely retained and are susceptible to the effect of ion suppression. In either platform, unseparable metabolites are susceptible to matrix effect as typified by ion suppression. Additionally, stability of detection profiles in long-term operation is also important for the selection of analysis system, because of a large number of samples and target compounds in advanced metabolomics studies. In this presentation, we propose the best analysis method for each metabolite in according to the evaluation of reproducibility and linearity for standard sample, and influence of ion suppression and durability both of capillary and column for real samples, in CE-MS and LC-MS using octadecylsilyl column and pentafluorophenylpropinyl column.

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METABOLOMICS STUDY ON ALCOHOLIC FATTY LIVER IN ZEBRAFISH USING ^{1}H NMR SPECTROSCOPY COUPLED WITH MULTIVARIATE STATISTICAL ANALYSIS

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Zebrafish(*Danio rerio*) has been a prominent model in developmental biology and is becoming a popular model for disease study and drug discovery. Recently, new human disease models were generated to analyze the formation and functions of cell population within organs. In this study, we investigated the characteristic metabolic pattern of alcohol fatty liver disease model in zebrafish using 1H NMR and GC/MS based metabolite profiling to reveal the regulation metabolism of alcohol fatty liver disease .

Multivariate statistical analysis showed a significant discrimination between control and alcohol fatty liver disease groups . Alcohol fatty liver disease in zebrafish was characterized by the increased excretion of acetate, succinate, lactate/pyruvate ratio, creatine and ketosis , and by the decreased excretion of glucose and alanine . The metabolite alterations in alcohol fatty liver disease model were associated with liver metabolism, which causes not only oxidation-reduction (redox) changes but also oxidative stress.

This study demonstrate that NMR and GC/MS based metabolomic approach, in new disease models of zebrafish is provides new insights into the biological mechanism, potential biomarkers, and new therapeutic targets for disease.

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COMPARATIVE ANALYSIS BETWEEN TWO DIFFERENT QUENCHING METHODS FOR YEAST METABOLOMICS STUDIES

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Development of a generic workflow for intracellular metabolomics which can handle a high stream of samples is strongly required for rapid phenotyping as tools for improved strain desing and faster construction. One of the key steps of sample preparation is quenching of the biological samples. Many methods have been developed before using cold (-20 to -40°C) methanol[1,2]. However, processing of a high number of samples could be impractical when one uses -40°C methanol in view of maintaining samples at such a low temperature during treatment and workers' safety and health issues. Therefore we studied the impact of quenching using ice water (0°C), on yeast metabolomics, compared to a dedicated method based on cold methanol. We studied the quantitative recovery of intermediates of glycolysis, PPP, TCA cycle, nucleotides and free amino acids. We measured the metabolites in total broth, and separated supernatant and cell pellets and calculated the mass balances for each intermediate. Results showed that the quenching impact is depending on metabolite properties and pool sizes. We found that the ice water method may be used for quantitative and qualitative metabolomics of free amino acids in steady state cultures. In addition only the methanol quenching can be used for the quantitative analysis of the intermediates of the central metabolic pathways and the nucleotides. The variation among replicate samples within the same quenching method was within the acceptable range. Changes in the metabolite levels caused by experimental variations were of the same magnitudes. If it is realized that the metabolic state in other cultivation conditions and in other strains can lead to different metabolite level changes during sample treatment, then, the ice water quenching method may be suitable for qualitative purposes of some compounds after a dedicated validation procedure.

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^{1}H -NMR-BASED METABOLOMICS STUDY OF ASTHMA

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Asthma is a chronic inflammatory disease caused by complex interactions of genetic, epigenetic, and environmental factors. Metabolomics can be used to investigate the perturbed metabolic patterns in a complete set of metabolites in a body fluid or serum to clarify the pathogenesis of many diseases.

We applied a ^{1}H-nuclear magnetic resonance (NMR) metabolomics approach to investigate the altered metabolic pattern in sera from patients with asthma and sought to understand the mechanism underlying asthma and the potential therapeutic targets for asthma. The serum metabolic pattern in patients with asthma (n = 22) and controls (n = 17) was investigated using global profiling by ^{1}H-NMR spectroscopy, coupled with a pattern recognition method. Characteristic endogenous metabolites in serum of patients with asthma were identified using a target-profiling procedure. A principal components analysis showed a clear separation between patients with asthma and healthy subjects. Serum from patients with asthma was characterized by increased excretion of methionine, succinate, *N*-acetylneuraminic acid, glutamine, and lactate and by decreased excretion of formate, methanol, acetate, choline, O-phosphocholine, arginine, and glucose.

We demonstrated that ^{1}H-NMR-based metabolomics approach may be useful for understanding the biological pathways that are perturbed by asthma and for providing new therapeutic targets for asthma.

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METABOLOMIC PROFILING OF DICHLOROACETATE AND PYRUVATE EFFICACY IN CYBRID CELLS HARBORING MELAS MITOCHONDRIAL DNA MUTATIONS

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Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the mitochondrial cytopathies caused by a nucleotide A→G mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene. This mutation induces impaired oxidative phosphorylation and free radical generation, associated with diverse diseases including diabetes mellitus, deafness, hyperthyroidism and cardiomyopathy. Dichloroacetate (DCA), a lactate-lowering agent, has been used as a typical treatment agent for MELAS, but its clinical efficacy is considered limited or doubtful. In contrast, pyruvate treatment was recently found to alleviate clinical symptoms of MELAS and thus is considered promising as a highly effective and side-effect-free therapeutic. Here, we conducted comparative metabolome analysis to elucidate metabolomic effects of DCA and ^{13}C-labeled pyruvate treatment on human 143B osteosarcoma cells (2SA) and their MELAS-mutant cytoplasmic hybrids (2SD), or "cybrids," which contain a patient's mitochondria with MELAS mutant genomes that replaced the mtDNA of 2SA cells, by capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). Quantifications of the time-change in 161 intracellular and 85 medium metabolites and flux interpretations of ^{13}C-pyruvate in 2SA and 2SD cells clarified consistently low ATP levels and poor energy status in 2SD cells likely due to the impaired oxidative phosphorylation. The treatment of pyruvate, but not DCA, improved [NADH]/[NAD], thereby enhancing anaerobic glycolysis and partial TCA cycle for maintaining the energy status as high as that in 2SA cells. The results demonstrated a dramatic and sustainable effect of pyruvate administration on the energy metabolism of 2SD cells, supporting an idea that balancing the [NADH]/[NAD] is crucial for facilitating ATP production and improving energy status of MELAS mutant cells. Pyruvate was thus identified as a more effective and metabolically rational treatment regimen than DCA for improving symptoms associated with MELAS and even other mitochondrial diseases.

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AN EFFECTIVE ASSESSMENT OF SIMVASTATIN-INDUCED TOXICITY WITH NMR-BASED METABONOMICS APPROACH

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Simvastatin, which is used to control elevated cholesterol levels, is one of the most widely prescribed drugs. However, a daily excessive dose can induce drug-toxicity, especially in muscle and liver. Current markers for toxicity reflect mostly the late stages of tissue damage; thus, more efficient methods of toxicity evaluation are desired.

As a new way to evaluate toxicity, we performed NMR-based metabonomics analysis of urine samples collected from Female Wistar rats . Compared to conventional markers, such as AST, ALT, and CK, the urine metabolic profile provided clearer distinction between the pre- and post-treatment groups treated with toxic levels of simvastatin. Through multivariate statistical analysis, we identified marker metabolites associated with the toxicity. Importantly, we observed that the treatment group could be further categorized into two subgroups based on the NMR profiles: weak toxicity (WT) and high toxicity (HT). The distinction between these two groups was confirmed by the enzyme values and histopathological exams. Time-dependent studies showed that the toxicity at 10 days could be reliably predicted from the metabolic profiles at 6 days.

This metabonomics approach may provide a non-invasive and effective way to evaluate the simvastatin-induced toxicity in a manner that can complement current measures. The approach is expected to find broader application in other drug-induced toxicity assessments.

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METABOLOMIC PROFILING OF PREGNANCY MICE SERUM IN PREIMPLANTATION PHASE

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Maternal metabolism during pregnancy is important for physical condition of both maternal and child. Currently, pregnancy diagnosis is based on human chorionic gonadotropin (hCG), a glycoprotein hormone in embryos after implantation. Recently, hCG is also used for the diagnosis of maternal preeclampsia and fetal Down syndrome. However, diagnosis of fertilization failure and implantation failure in fertility treatment is difficult since pregnant marker in preimplantation is not available. To explore new markers, we conducted comprehensive metabolomic analysis using capillary electrophoresis - mass spectrometry (CE-MS) of pregnancy mice serum (after mating 24h ~ 120h) and false pregnancy mice in vitro fertilization. Overall, CE-MS detected and identified is 347 ± 140 metabolites. In this profile, 19 metabolites showed discriminating potential screened by both $p < 0.05$ (Mann-Whitney test) and area under the receiver operating characteristic curve (AUC)> 0.5 through the obtained time-course. Of these, N,N-dimethyglycine ($p = 0.028$, AUC = 0.87), providing an antioxidant function, and nicotinamide (p-value = 0.025, AUC = 0.90), protecting the embryo from teratogenic effect, were consistent with other reports. This study revealed dynamic metabolic alternation in immediately after fertilization and identified several pregnancy diagnostic markers.

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SECONDARY METABOLITES BASED CLASSIFICATION OF *PENICILLIUM* SP. AND RELEVANCE OF ITS ANTIOXIDANT ACTIVITY

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Penicillium is a large, ubiquitous group that has a wide geographical distribution with phenotypic and genotypic variation among the species. A complete classification of such species is important in terms of effective antibiotic production from penicillium. Traditional taxonomic classification faces lot of difficulties in terms of identification and characterization of such species because, the information about teleomorphic states and the morphological criteria similarities were not clear. Metabolites based classification (Chemotaxonomy) found to be one of the effective tools to differentiate among Ascomycota. So, the present study was made to classify four *Penicillium* species (seventeen strains) by secondary metabolite content using liquid chromatography electrospray ionization iontrap mass spectrometry (LC-ESI-IT-MS) and multivariate statistical analysis. Results revealed that ITS based dendrogram and secondary metabolite based dendrogram was found to be similar. *P. oxalicum* was separated from other three species based on its bioavailability of secondary metabolites. Simultaneously, *P. expansum* was discriminated from other two species. Distinct clustering was observed with *P. echinulatum* and *P. solitum*. To categorize the metabolite responsible for the clustering pattern was studied among the *Penicillium* strains using partial least squares discriminant analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA) analysis. The metabolites discriminated between *P. oxalicum* and three species were vermiculidiol, meleagrin, oxaline, glandicolin A, B and five unidentified metabolites. Gluconic acid, andrastin A, B, C and five unidentified metabolites were observed between *P. expansum* and two species (*P. solitum, P. oxalicum*). *P. echinulatum* was differed from *P. solitum* based on the availability of cyclopenol, Brefeldin B and six unidentified metabolites. It was observed that the antioxidant activity of four *Penicillium* species exhibits variation in terms of secondary metabolite based chemotaxonomy. ABTS radical-scavenging activity of *P. expansum* showed higher scavenging activity than that of *P. echinulatum* and *P. solitum*. Results of this study demonstrated that the secondary metabolite-based chemotaxonomy was not only used as a classification method but also for species-specific activity analysis.

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CHARACTERIZATION OF THE EFFECTS OF SILVER NANOPARTICLES ON LIVER CELL USING HR-MAS NMR SPECTROSCOPY

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AgNPs (silver nanoparticles) has been widely used for the commercial products, which have antimicrobial agent, medical devices, food industry and cosmetics. Despite, AgNPs have been reported as toxic to the mammalian cell, lung, liver, brain and other organs and many researchers have investigated the toxicity of AgNPs. In this study, we investigated toxicity of the AgNPs to the liver cell using metabolomics based on HRMAS NMR (High Resolution Magic Angle Spinning Nuclear Magnetic Resonance) technics, which could apply to the intact tissues or cells, to avoid the sample destruction. Target profiling and multivariative statistical analysis were performed to analyze the 1D ^{1}H spectrum. The results show that the concentrations of many metabolites were affected by the AgNPs in the liver cell. The concentrations of glutathione (GSH), lactate, taurine, and glycine were decreased and most of amino acids, choline analogues, and pyruvate were increased by the AgNPs. Moreover, the levels of the metabolites were recovered upto similar level of metabolites in the normal cell by the pre-treatment of NAC, external antioxidant. The results suggest that the depletion of the GSH by the AgNPs might induce the conversion of lactate and taurine to the pyruvate.

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GC-MS BASED PROFILING OF METABOLIC DISRUPTION AND RECOVERY OF UV-IRRADIATED LEMON BALM PLANT

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Lemon balm (*Melissa officinalis*) is a perennial herb has lately attracted increased attention due to its health-benefiting effect that is mainly caused by its secondary metabolites. The levels of plant metabolites are considered to possess the information of ultimate response of the plant to various environmental or genetic perturbations. In that context, metabolomic data could provide invaluable clues for the physiological state and reflect specific biochemical processes in the plant. For example, the ultra violet radiation (UV-B, 280-320 nm) is known to broad influence on plants including damages in DNA, RNA, protein and membrane, change of growth, productivity and morphology. Also, plants develop the defense and recovery mechanism against such UV damage. In this study, *M. officinalis* was irradiated with UV for 1 and 3 h and left for 0, 3 and 12 h for possible recovery, and the metabolites from the plant at each condition were analyzed by gas chromatography-mass spectrometry (GC-MS) and statistically analyzed by principal component analysis (PCA). A total 43 metabolites including alcohols, amines, amino acids, inorganic acids, organic acids and sugars were identified as the metabolites of *M. officinalis* by GC-MS. Depending on the UV irradiation time, three different groups with different UV irradiation times (0, 1 and 2 h) were clearly separated each other by PCA. With regard to recovery time, four different groups that were created with UV irradiation for 1 h and recovery for 0, 3 and 12 h were distinctively separated by PCA. These results indicated that metabolite profile of *M. officinalis* was altered by UV irradiation time and recovery time.

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METABOLIC PROFILING ON PERITONEAL SOLUTE TRANSPORT IN PERITONEAL DIALYSIS PATIENTS

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The body metabolism of patients with end stage renal disease may be altered in response to long term peritoneal dialysis (PD) treatment. The peritoneal solute transport could influence the metabolic pattern of PD patients, but metabolic changes associated withPD solution are poorly understood. In this study, we performed a comparative study on metabolite pattern changes which presumably occurred due to either PD treatment time or solutions.

The patients participated in this study were separated into two groups according to type of the prescribed PD solution: the conventional solution (CS) group and the biocompatible solution (BCS) group. We performed a prospective observational study with 97 incident PD patients who were treated with either BCS or CS. ^{1}H-NMR metabolomics approach was applied to investigate the metabolic pattern in peritoneal dialysate effluent (PDE) and serum from patients who had been 1, 6, 12 months after commencing PD.

Changes in serum and PDE metabolites were investigated using the target ed- profiling procedure, and differences in metabolic pattern according to PD solution type were compared using multivariate analysis. Moreover, the time-dependent effects for the one, six, and twelve month treatment groups were investigated. Partial least squares discriminant analysis(PLS-DA) showed a separation between CS group and BCS group. The major metabolites responsible for differentiation in PLS-DA were alanine, pyruvate, citrate, acetone, 3-hydrobutyrate, lipid CH_2CH_2CO and VLDL/LDL $(CH_2)n$. These findings demonstrate that ^{1}H-NMR-based metabolic profiling of serum and PDE metabolites is suitable for observing the change in peritoneal solute transport between CS and BCS in PD patients.

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INTEGRATION OF LARGE SCALE OMIC'S DATASETS USING O2PLS

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Data integration refers to the amalgamation of data from disparate parts of a system to produce an understanding of the system as a whole. In a systems biology-based research paradigm, these efforts often involve combining data from multiple analytical platforms (*i.e.,* metabolomics, proteomics, transcriptomics, genomics) into a single concatenated data set to identify biologically relevant shifts.

O2PLS is an extension of Orthogonal Projection to Latent Structures (OPLS), which is in turn an extension of Projection to Latent Structures (PLS). PLS finds linear regression models by projecting the predicted variables and the observable variables to a new space. While OPLS and PLS have identical predictive performance, the orthogonal components in OPLS provide a powerful tool for model interpretation. O2PLS defines the joint variations that exist between data from differing analytical platforms, separating predictive variations unique to each analytical platform, as well as residual variation for each platform.

We present a preliminary example workflow of how O2PLS can be used to integrate and reduce omics datasets from two different technology platforms. This study employed datasets derived from a study of ApoE*3Leiden mice fed an atherogenic diet in combination with 3 different cardiovascular therapeutic interventions, fenofibrate (FF), rosuvastatin (RO) and the liver X receptor agonist T0901317 (LXR). Individually, RO reduced plasma cholesterol and very low density lipid levels (VLDL), FF reduced triglyceride levels and dramatically reduced plasma VLDL, while the LXR increased plasma cholesterol and triglyceride levels. Transcriptomics (from liver) and lipidomics (from liver and plasma) datasets were integrated and analysed using the O2PLS method, giving a greater holistic understanding of the drug effects, from the levels of gene expression to individual metabolites. Concepts and implementation strategies of O2PLS are discussed along with the interpretation of model components.

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OPLS AS A VARIABLE SELECTION TOOL: A COMPARATIVE STUDY

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'Omics' data sets generally comprise of thousands of variable measurements performed on a limited number of samples or patients, of which only a relatively small percentage are altered between control and experimental samples. Herein lays the challenge of variable selection; to correctly identify the variables that are relevant to a mechanism of disease.

Univariate statistical methods (e.g. Student's t-test) in conjunction with a defined significance level (p value) are often used as a means of variable selection. To correct for the high false positive rate associated with multiple testing, False Discovery Rate (FDR) can be applied as an extension of the t-test to control for the high proportion of false positives during hypothesis testing. FDR uses characteristics of the p-value distribution to produce a list of q-values designed to limit the FP rate, but with the penalty of loss in statistical power.

Here we explore the use of multivariate statistics as an alternative variable selection approach. Orthogonal Projection to Latent Structures (OPLS) is an extension of PLS, where systemic variation not correlated between the input and response datasets is removed, aiding in interpretability. Correlation coefficients derived from the loadings of the predictive OPLS component, combined with a null distribution, highlight important variables between control and experimental groups. In this study, synthetic (simulated) data was engineered using both normal and Laplacian distributions with a pre-determined number of significant changes or true positives (TP). Gaussian noise was added incrementally to the data and the performance of three variable selection methods, the t-test, FDR, and OPLS, were evaluated by examining the number of correctly identified TP and true negatives (TN). Preliminary results suggest that OPLS may provide a viable alternative approach to variable selection and offer greater control between type I or type II error than traditional univariate procedures.

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PHENOL-EXPLORER VERSION 2.0: A RESOURCE FOR POLYPHENOL METABOLISM AND PHARMACOKINETIC PROFILES

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Phenol-Explorer (www.phenol-explorer.eu) is the first comprehensive and freely available electronic database on polyphenol content in foods. When initially released in 2009, it contained more than 35,000 content values for 500 different polyphenols in over 400 foods. These data were derived from the systematic collection of more than 60,000 original content values in more than 1,300 scientific publications. The original data were manually curated and hand-checked for accuracy. In the second major release of Phenol-Explorer, data on metabolism, pharmacokinetics, and intervention studies have been gathered on polyphenol sources and metabolites. These data are visible using intuitive searching tools and tables. Additionally, graphs comparing the data from intervention studies provide a visual overview of pharmacokinetic data following the consumption of various polyphenol sources. The aggregation of this key information about what happens to dietary polyphenols as they are consumed is vital to fully understanding their effects on health. The compilation of this data into an easy to understand format makes it possible to compare data across studies and should prove to be a priceless resource for metabolomics researchers. All data is open and free to download. Here, we explore some of the search tools and browsing capabilities present in the new Phenol-Explorer web application, developed by In Siliflo Data Management & Analysis.

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DESCRIPTION OF CONCURRENT CLASS ANALYSIS AND ITS APPLICATION IN DEFINING A BIOSIGNATURE FOR MITOCHONDRIAL RESPIRATORY CHAIN DEFICIENCIES

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This is a presentation on the description and application of concurrent class analysis (CONCA), which is a new multivariate approach for the analysis of complex and high dimensional metabolomics data. Standard parametric statistical methods such as regression is not easy applicable for identifying information from the data as there are insufficient data for parameter estimation. Principal component analysis (PCA) developed into the most generally used multivariate dimension reducing technique to disclose underlying relationships encapsulated in metabolomics data. Despite its widespread use, PCA has shortcomings that limit its applicability. Several approaches have been made to overcome these limitations and we here describe CONCA, an advanced disjoint PCA model. It is unique in linking disjoint PCA models to a traditional PCA model. This is accomplished by restructuring the input data matrix, applying disjoint PCA group models to the restructured data, and to combine the disjoint PCA models in order to replicate a traditional PCA. We applied the CONCA model to a data set on mitochondrial deficiencies related to oxidative phosphorylation, a group of inherited metabolic disorders linked to deficiencies in complexes I to V of the respiratory chain (RCDs). We have extended the GC-MS generated organic acid profiles (Reinecke, et al, 2011) to include those from the amino acids and acyl-carnitines of controls and patients with complex I, III and multiple complex (CM) deficiencies in the respiratory chain. The outcome of these analyses identified variables with high discrimination value (abbreviated as VID's) through the CONCA analysis, which could produce a putative biosignature for RCDs, using CONCA in combination with other multivariate analyses. The benefit of the CONCA model is to disclose information concerning individual groups and an ability to identify which variables are responsible for group separation.

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THE SECONDARY METABOLISM OF STAGONOSPORA NODORUM

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Secondary metabolites are small molecules that are not directly essential for growth under laboratory conditions. Many of those compounds, however, have a crucial role in the overall fitness of the producing organism in its natural environment[1]. Fungi are renowned for producing a vast number of secondary metabolites, both beneficial (pharmaceuticals such as penicillins or lovastatin) and detrimental (mycotoxins such as the aflatoxins) to human health. The fungal necrotroph Stagonospora nodorum is an important wheat pathogen in Western Australia, accounting for $108M (AUD) of crop loss in Australia each year[2]. The interaction of this fungus with its host plant has been studied extensively at the genome and proteome level, yet very little is known about a possible role of secondary metabolites during its pathogenic life cycle[3,4]. The ability of the fungus to produce the mycotoxin alternariol has been shown previously[3]. In this study, Liquid Chromatography – Mass Spectrometry is used to gain a comprehensive picture of the secondary metabolites profile of Stagonospora nodorum. Genetics and transcriptomics techniques are used to further elucidate the role of secondary metabolism during pathogenicity. To this date, more than 40 potentially biologically relevant candidate secondary metabolite genes have been identified. Experiments are underway to identify the metabolites associated with these genes. Targeted gene disruption experiments already yielded a minimum number of 20 compounds to differ significantly between the wild type fungus and the knockout mutants.

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METABOLOME PROFILES IN *PORPHYROMONAS GINGIVALIS* STIMULATED WITH EXOGENOUS PARA-AMINOBENZOATE

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Background: Pioneer organisms, such as *Streptococcus gordonii*, facilitate the colonization of later arrivals including a periodontal pathogen *Porphyromonas gingivalis* in the process of dental plaque development. We previously reported that a *S. gordonii* mutant deficient in a chorismate binding enzyme (Cbe), which is involved in the production of para-aminobenzoate (pABA), failed to develop a heterotypic biofilm with *P. gingivalis*. Objectives: To investigate a facilitatory effect of exogenous pABA on biofilm development, metabolome profiles in *P. gingivalis* cells treated with pABA were analyzed. Methods: *P. gingivalis* cells were anaerobically grown at 37□‹C in trypticase soy broth supplemented with yeast extract, menadione and hemin. At stationary phase, the cells were harvested and re-suspended with PBS with or without 1 mg/ml of pABA. After 6 hours incubation, ionic metabolites were extracted from 5 x 10^9 cfu of *P. gingivalis* cells. CE-TOFMS was used to simultaneously measure metabolite levels. Results: Metabolites in the glycolysis pathway (glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, 3-phosphoglyceric acid, 2-phosphoglyceric acid, and phosphoenolpyruvic acid) and pyrimidine (dCMP, dTMP, dTDP and UDP) were significantly increased when the exogenous pABA was available. Furthermore, pABA treated *P. gingivalis* cells formed GDP-mannose and N-acetylglucosamine 6-phosphate, constituent molecules of extracellular polymeric substances, at 8.8 and 2.1 times higher concentrations than control, respectively. Meanwhile, polyamines such as spermidine and N^8-acetylspermidine were barely detectable in *P. gingivalis* treated with pABA although they were present in substantial levels in control cells. With the exception of lysine, threonine and glutamine, production of amino acids was suppressed by pABA. Conclusion: The results suggest that exogenous pABA activates energy production, extracellular matrix development and nucleic acid synthesis, however, pABA suppresses protein turnover and remodeling in *P. gingivalis* cells. This phenomenon might reflect the accelerated production of folate and its derivatives, which lie down stream of pABA.

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HMO: HUMAN METABOLOME ONTOLOGY

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Final step in current metabolomics studies involve assessment and biological interpretation of metabolome and often require manual collections of literature or linking information scattered in Gene Ontology, BRENDA, KEGG Brite, KEGG Pathway, Human Metabolome Database, OMIM and etc. We have developed the Human Metabolome Ontology (HMO) to facilitate integration of biological functions, and chemical classification of metabolome and comprehensive understanding of metabolome and its target interactions as the common language and knowledge framework allowing further computational analysis. HMO provides three independent ontologies: biological functions, chemical taxonomies and metabolome targets. The biological function refers to the metabolic pathways involved by the metabolome. The chemical taxonomy specifies the chemical source such as endogenous or exogenous, and chemical classifications, for example carbohydrate or lipid, of the metabolome. The metabolome target provides information regarding to the target and its component interacting with metabolome of interest, favorable and unfavorable metabolome - target interactions, and the location of the target in tissue and cellular level. HMO was constructed in the Open Biomedical Ontology format (OBO) format with a user-friendly web interface. Currently version of HMO contains 16120 metabolome, 1840 metabolome targets and 161 diseases related to metabolome and covers 408 terms in biological function, 129 terms in chemical taxonomy and 22837 terms in metabolome targets. HMO provides a comprehensive metabolome centered resource that enables the sharing and reuse of the knowledge across domains of ontologies. HMO is free accessible at http://cmdd.csie.ntu.edu.tw/~hmo

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EVALUATION OF METABOLOMICS DATA QUALITY USING ARTIfiCIAL BIOLOGICAL GRADIENTS

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Metabolomics has become an integral part of many life-science applications but is technically still very difficult, because the ultimate aim of metabolomics is to measure the metabolome quantatively. Effective ways to evaluate how well acquired data capture actual concentration differences are a prerequisite for accelerated method development. Unfortunately, there are no widely applicable methods for realistic performance evaluation. Here we introduce a powerful but simple approach that provides calibration curves over a biologically defined concentration range for all detected compounds. By performing metabolomics on a stepwise gradient between two biological specimen, we obtain a dataset where each peak ideally would show a linear dependency on the mixture ratio. An example gradient between extracts of tomato leaf and fruit demonstrate good calibration statistics for a large proportion of the peaks but also highlights cases with strong background dependent signal interference. Analysis of artificial biological gradients is a general and inexpensive tool for calibration that greatly facilitates data interpretation, quality control and method comparisons.

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THE GLUCONEOGENIC PATHWAY IS OPERATIVE IN LUNG CANCER CELLS

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Accelerated aerobic glycolysis has been a hallmark of cancer metabolism since Otto Warburg's seminal discovery [1]. Glucose oxidation via glycolysis and the pentose phosphate pathway (PPP) not only produces energy and reducing equivalents but also anabolic precursors to meet the demands of proliferation and many essential functions of cancer cells. Although many of the anabolic precursors can be obtained from alternative fuel substrates, the de novo synthesis of ribose and therefore nucleotides normally requires glucose metabolism. Thus, under glucose deficiency, cancer cells will cease proliferation due to a lack of new synthesis of nucleic acids. This can occur in poorly vascularized areas of a tumor. Using Stable Isotope Resolved Metabolomics (SIRM) [2], we now have direct evidence that lung cancer cells can circumvent this problem by making ribose from Gln using part of the gluconeogenic pathway in different cancer cell lines. By using U-$^{13}C_5$,$^{15}N_2$-Gln as tracer, we tracked the fate of the ^{13}C and ^{15}N atoms simultaneously into various metabolites of A549, H1299 and ZR-75-1 cells using FT-ICR-MS and NMR [3]. We found that both labels were incorporated into ATP other nucleotides and lipids. Although the expected ^{15}N labeling patterns were found based on the purine biosynthetic pathway, the ^{13}C labeling patterns show that the adenine ring and/or ribosyl unit of ATP were also labeled. This can only occur through the metabolic sequence of glutaminolysis, Krebs cycle, pyruvate carboxylation, PEP carboxykinase (PEPCK) reaction, gluconeogenesis, and PPP. We believe this is the first time that gluconeogenesis is demonstrated for cancer cell lines not known to be gluconeogenic, which could have important implications for how cancer cells adapt to a glucose-limited microenvironment and the role of Gln in such adaptations. Support: NSF EPS-0447479; NIH NCRR 5P20RR018733, 1R01CA118434-01A2, NIH R21CA133668-02, the Kentucky Challenge for Excellence, and KY CTSPGP.

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^{1}H NMR-BASED METABOLOMIC ANALYSIS OF THE TOXIC RESPONSE OF *EISENIA FETIDA* AFTER EXPOSURE TO SUB-LETHAL PHENANTHRENE CONCENTRATIONS

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Earthworms are commonly used as indicators of soil toxicity. ^{1}H NMR-based metabolomics has shown promise in elucidating the toxic mode of action (MOA) of contaminants in organisms after sub-lethal chemical exposure. In this study, ^{1}H NMR-based metabolomics was used to delineate the MOA of phenanthrene, an ubiquitous environmental pollutant, in the earthworm, *Eisenia fetida*, after exposure to sub-lethal concentrations over time. Earthworms were exposed to 0.05, 0.2 and 0.4 mg/cm^2 of phenanthrene (which corresponds to 1/32nd to 1/4th of the LC_{50} respectively) via contact tests over one, two and three days. Earthworm tissues were extracted using a mixture of chloroform, methanol and water, resulting in polar and non-polar fractions that were analyzed by ^{1}H NMR. Metabolomic analysis revealed heightened *E. fetida* toxic responses with longer exposure times and higher phenanthrene concentrations. The principal component analysis (PCA) scores plots of the polar fraction showed significant separations between control and exposed earthworms. The PCA scores plot of the non-polar fraction showed significant separation between the controls and exposed earthworms for only the first day of exposure. Alanine, glutamate, maltose and fatty acids emerged as potential indicators of phenanthrene exposure. The deactivation of the succinate dehydrogenase enzyme of the Krebs cycle was also found in exposed earthworms. Partial least squares (PLS)-regression models showed that the polar metabolic profile of *E. fetida* was weakly but significantly correlated to phenanthrene exposure concentrations after both day one (cross-validated PLS-regression with 2 components, R^2X=0.57, R^2Y=0.64, Q^2Y=0.48, P=0.00004) and day two (cross-validated PLS-regression with 6 components, R^2X=0.85, R^2Y= 0.76, Q^2Y=0.48, P=0.0001) of exposure. This study shows that NMR-based metabolomics is capable of elucidating time and concentration-dependent relationships to understand the mode of toxicity of earthworms after phenanthrene exposure. Therefore, ^{1}H NMR-metabolomics shows potential as a powerful ecotoxicity tool for monitoring earthworm responses to environmental contaminants.

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ENVIRONMENTAL METABOLOMICS APPROACH TO ESTROGEN METABOLISM BASED ON BISPHENOL-A CONCENTRATION

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Bisphenol-A (BPA), one of endocrine disrupting chemicals, is widely used in the manufacture of plastic and acts as an estrogen agonist by binding to estrogen receptor both *in vitro* and *in vivo*. Moreover, low level of BPA may adverse effect on our body by binding to estrogen receptor mimic the body's own hormones and lead to negative health effects. The present study was designed to analyzed urinary estrogens association with high BPA group (n=100, 11.05+/-20.47 μg/g creatinine) and low BPA group (n=100, 0.70+/-0.22 μg/g creatinine) using gas chromatography-mass spectrometry and then investigated whole metabolic changes using ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. To visualize differences the metabolic signatures between low-and high-group of BPA, both PLS-DA and supervised hierarchical clustering analysis (based on Pearson correlation coefficients) were the good discrimination between groups. In estrogen profiling, estrogens were increased higher in the high BPA group than in the low BPA group. Interestingly, the level of 4-hydroxyestradiol (4-OH-E2) resulting from 4-hydroxylase was increased more than the level of 2-hydroxyestradiol in the high BPA group. The level of BPA depend on concentration may be induced activity of 4-hydroxylase and increased level of 4-OH-E2. It consequently can produce quinone and lead to oxidative damage to lipids, proteins and DNA as a result of production of reactive oxygen species. So BPA may be induced change of estrogen metabolism in human body. The present environmental metabolomics approache will provide the most comprehensive molecular description of organisms in the environment and could be a useful tool in investigation of health effect and biomonitoring.

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TARGETED METABOLITE PROFILING TO EVALUATE THE THERAPEUTIC EFFECTS OF METFORMIN IN THE PLASMA OF HEALTHY VOLUNTEERS

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Metformin is an effective oral agent for type 2 diabetes mellitus, with a variety of metabolic effects. In addition to controlling blood glucose level, it has been appeared to decrease the long-period complications of diabetes, including macrovascular disease. Few reports have addressed the metabolite profiling of metformin. The aim of this study was to evaluate if targeted metabolic profiling approach is sensitive enough to predict the therapeutic effects of metformin after a single oral dose. An ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS)-based metabolic profiling was performed on 28 healthy volunteers, by measuring the levels of endogenous metabolites in their pre-dose and post-dose plasma samples. After a single oral dose of 500 mg metformin, blood samples were collected at 0, 2 and 6 hours to determine the concentrations of endogenous metabolites. We quantified lactic acid, 7 amino acids (lysine, glutamic acid, alanine, valine, leucine, phenylalanine, tryptophan), and lysophosphatidylcholines(14:0, 16:0, 17:0, 18:0, and 18:1) using UPLC/MS/MS, and determined that the metabolic levels of lysine, valine, leucine, phenylalanine, tryptophan, and lysoPC(18:1) were slightly decreased with no significance. Accordingly, in order to explore the potential endogenous metabolites associated with the therapeutic effects of metformin, further study including non-targeted (global) metabolite profiling is needed.

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STEM GIRDLING ENHANCED STROBILUS PRODUCTION IN *LARIX KAEMPFERI* VIA METABOLIC ALTERATIONS

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Japanese larch (*Larix kaempferi*) is one of major economical planting tree species in Korea. The demand for the larch seeds has increased in Korea but their supply has been limited due to sporadic natural seed production. To enhance seed production, stem girdling was applied to Japanese larch seed orchard established from 1974 to 1978, resulting in remarkable enhancement of strobilus production in terms of the rate of strobilus-bearing tree and the number of strobilus per tree. Metabolic alterations in the cambium and phloem tissues of both girdled and the control trees were interrogated through GC/MS and HPLC analysis. Stem girdling affected $\delta^{13}C_{PDB,}$ $\delta^{15}N_{AIR,}$ total nitrogen content, and C/N ratio while total carbon content was not changed by girdling. Based on time-wise observation after girdling, the content of amino acids was significantly enhanced from August. Particularly the levels of Arginine (Arg) in the girdled trees were significantly higher (1.8 ~ 3.4-fold) than those in the control from June to September. When stem girdling was conducted at four different times such as March, April, May, and June, the significant metabolic alterations were found in the levels of amino acids in between the upper and the lower cambium and phloem tissues where the girdling was applied. The contents of amino acids were significantly higher in the upper than in the lower sections only when girdling was applied before April and later they became similar to the levels in the control trees. In conclusion, the stem girdling enhanced strobilus production through the quantitative increase of nitrogen sources, such as amino acids, and the girdling practice should be applied before the end of April. Although the role of amino acids on enhanced strobilus production remains unclear, the results presented in this study might provide useful information in elucidating metabolic network modulation induced by girdling.

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THE MZML VENDOR-NEUTRAL DATA FORMAT FOR GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Metabolomics community currently lacks a widely accepted and supported standard data format for the storage and exchange of mass spectrometry data. mzML (Mass Spectrometry Markup Language) is an XML-based vendor-neutral data format for storage and exchange of mass spectrometry data developed by an international proteomics consortium, including Human Proteome Organisation (HUPO) and Proteomics Standards Initiative (PSI). The mzML format has replaced two previous formats with the same purpose developed by the proteomics community (mzXML and mzData), and combines the best features of mzXML and mzData with the specific aim to provide a unified standard data format for mass spectrometry based proteomics. The metabolomic data in the mzML format obtained from liquid chromatography-mass spectrometry platforms (LC-MS) can be used through popular software packages such as XCMS [1] and mzMine [2]. However, no readily available software packages with the capability to read/write GC-MS data in mzML format currently exist. ANDI-MS (also known as netCDF) and JCAMP-DX, the predominant open file formats for GC-MS data, lack the ontology-backed metadata found in mzML. The objective of this work is to develop example GC-MS and GCxGC data sets in mzML format, augment the controlled vocabulary if required, and develop software tools that will allow conversion of ANDI-MS and JCAMP-DX data to mzML format. One specific platform aiming to achieve this is PyMS, the open source Python toolkit for the processing of GC-MS data (http://code.google.com/p/pyms/). We have recently succeeded in converting a data set from Thermo ISQ Single Quadrupole GC-MS instrument to mzML with the ProteoWizard libraries and software tools (http://proteowizard.sourceforge.net/).

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NMR-BASED METABOLOMICS TO EXAMINE NAPHTHALENE TOXICITY IN MOUSE URINE

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Naphthalene, the most common polyaromatic hydrocarbons, exists in the environment widely with significant human exposure. Previous studies have shown that naphthalene causes site and species selective cell injury. A comprehensive study examining the changes of small molecular metabolites is essential to understand the mechanisms of naphthalene toxicity and develop biomarkers. We hypothesize that naphthalene disrupts intracellular homeostasis and causes metabolic turbulences which can be recorded in urine and associated with cell injury.

Nuclear magnetic resonance (NMR)- followed by multivariate analysis were applied to characterize metabolic effects of naphthalene in leading to cell injury in a susceptible species, mouse. Both naphthalene metabolites and endogenous metabolites were examined in urine and correlated to the progress of cell injury. Time course and dose response of naphthalene-induced metabolic effects were identified.

Metabolic techniques provide a powerful tool to describe biological processes in a more complete manner. We hope that this study will suggest the best naphthalene biomarkers in noninvasive samples with potential to be applied in the human populations.

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METABOLOMIC ANALYSIS OF URINE FROM INTERLEUKIN-10-DEFICIENT MICE SUPPLEMENTED WITH KIWIFRUIT EXTRACTS

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Inflammatory bowel diseases (IBD) are characterised by recurring intestinal inflammation, which is attributed to a dysregulated immune response towards intestinal microbiota. Studies on IBD animal models such as the interleukin-10-deficient (IL10-/-) mouse indicate that dietary modulation may influence disease activity. We demonstrate using IL10-/- mice fed with kiwifruit fruit extracts, the potential application of metabolomic analysis as a non-invasive method to identify functional foods for the treatment of IBD. A metabolite profile associated with inflammation identified previously by GCMS metabolomic analysis, was used to determine the efficacy of the kiwifruit extracts in modulating inflammation. Non-targeted GCMS metabolomic analysis was performed to determine other metabolic effects of the kiwifruit extracts. We discovered IL10-/--wildtype genotypic differences in the metabolism of kiwifruit extracts, demonstrating the usefulness of metabolomic analysis in identifying metabolic differences that may have implications for disease treatment.

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A LC-MS BASED METHOD FOR ACCURATE QUANTIFICATION OF PLANT PRIMARY METABOLITES

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Plant primary metabolites include small, polar compounds with a wide range of chemistries. GC-MS is a powerful technique, able to determine simultaneously most polar metabolites, including sugars, amino acids and organic acids. However, there are drawbacks with the technique. Not all amino acids can be analysed and the method introduces an additional level of complexity in data analysis by the formation of two isomers from each sugar. Finally there is the requirement for a relatively complex derivatization process, which introduces additional error in the analysis. This has led to considerable effort in the development of alternative HPLC or LC-MS based methods for polar compound analysis. A failure of published methods is they generally deal with only one metabolite class and use columns and eluting conditions suitable for only a particular type of metabolite. Here we present a LC-MS method that enables quantification of all major amino acids, organic acids and sugars in plant tissue within a single run.

In a 30 minute run separation is achieved on a Synergi Hydro-RP column (Phenomenex) with metabolites detected by a LTQ Velos Orbitrap scanning in positive (FT) mode and negative (IT) mode. Structural isomers Leu and Ile are chromatographically resolved and accurate mass eliminates the interference of ^{13}C isotopes (Asn over Asp and Gln over Glu). Standard curves for 18 out of 19 amino acids and all four organic acids show excellent linearity ($R^2 > 0.99$). This method is also able to accurately determine the level of sucrose, although the two major hexoses present in plants are not fully resolved.

Applicability is demonstrated on ryegrass where quantification of 19 amino acids, four organic acids and three sugars in six different ryegrass lines was achieved. Importantly, for 18 amino acids and four organic acids the levels were determined with a RSD below 10%.

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DEVELOPING A DATA-DRIVEN FRAMEWORK FOR DISCOVERY AND USE OF DIETARY EXPOSURE BIOMARKERS IN HUMAN EPIDEMIOLOGICAL STUDIES

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Western diets are generally complex and conventional methods of measuring habitual dietary exposure such as Food Frequency Questionnaires (FFQs) depend upon food intake estimates and are subject to errors, which can confound interpretation of subsequent data. Descriptors of individual FFQ food components vary in degree of distinctiveness and consumption patterns of each food component generally display great variability, including effects of seasonality. Against this background we have been exploring the use of metabolomics to help validate FFQ dietary component descriptors without prior knowledge of biochemical markers potentially indicative of habitual exposure to specific foods. Initially we demonstrated that non-targeted metabolite fingerprinting using Flow Infusion ESI-MS (FIE-MS) in conjunction with machine learning data analysis can be used to explore relationships between the chemical content of overnight or fasting urine and reported levels of citrus exposure in 24 humans consuming a freely-chosen diet. F ourier-Transform Ion Cyclotron Resonance MS (FT-MS) and tandem MS, followed by signal annotation using MZedDB suggested that correlated explanatory signals indicative of high citrus consumption were ionisation adducts of proline betaine (stachydrine) and hydroxyproline betaine. In an expansion of this preliminary study we describe a high throughput, data-driven approach to explore the food consumption habits (>130 standard food components) of a larger cohort of free-living humans. Using FFQ information and FIE-MS analysis of fasting and/or overnight urines we identify food components that are well discriminated between groups of individuals reporting either high or low habitual consumption. Ultra-high accurate mass analysis and tandem MS has revealed potential biomarkers for a range of foods of high public heath significance (including red and white meats, specific fruit/vegetables and dairy products). The likely role and impact of the use of biomarkers on future dietary exposure monitoring in human epidemiological studies will be discussed.

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EFFECTS OF EXERCISE INTENSITY AND HYPOXIA IN HUMAN URINE METABOLITES BY LC-MS-BASED METABOLOMICS ANALYSIS

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Exercise is link with intensity-dependent metabolite response. We compare the effect of urinary metabolite profiling in eight untrained males cycled with moderate (60% VO_{2max}) vs. severe (VO_{2max}) exercise intensity for 30 min under hypoxic conditions (15% O_2) or under normoxic (20% O_2) conditions. The metabolic variability and response to exercise intensity with different oxygen level has been characterized by the high resolution of LC-TOF-MS in this study. A clear separation of dose dependent response was visualized by principle component analysis (PCA) between moderate and severe exercise. About 10% of a couple of thousand features were observed in both sample sets and showed significant changes in their relative signal intensity by ANOVA using a threshold of $p < 0.05$; however, there is a great diversity of concentrations of metabolites among the 8 subjects, which reflects the diversity between individuals. By comparing their personal tendencies, several metabolites have been identified and found significantly changed in their concentrations, such as N-trimethyllysine (a precursor of carnitine) and methylimidazoleacetic acid are down regulated. The increase in purine metabolic products (uric acid, xanthine, and hypoxathine) results from exercise and can be used as a marker. The increase of free carnitine and acetyl carnitines, on the other hand, signals a change in the pathway of energy, or lipid, metabolism.

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UTILIZATION OF GCXGC-TOFMS TO SCREEN FOR POTENTIAL METABOLITE DIFFERENCES IN POOLED SAMPLES FROM LEAN, FAT, AND OBESE ZUCKER RAT PLASMA

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This GCxGC-TOFMS research presents the comparison of pooled derivatized plasma samples from lean, fat, and diabetic obese Zucker rats. The small molecule metabolite profiles from the different sample pools were compared for similarity in mass spectral identification, retention time, and analyte variation by peak area. The enhanced chromatographic resolution and peak capacity available with comprehensive two-dimensional chromatography allows the fast acquisition of TOFMS, up to 500Hz, that will successfully acquire the data density needed to fully characterize low levels of metabolites common in complex sample matrices such as blood plasma. These complex and data rich files were processed with deconvolution algorithms which deliver qualitative identification as well as multiple component quantification in a single run. GCxGC-TOFMS was used to evaluate pooled trimethylsilyl-derivatized rat plasma samples in various states of health. Deproteinated rat plasma from each of the strains of rat (Zucker Lean, Zucker Fatty and Zucker Diabetic Obese) was derivatized with BSTFA after the proteins were removed. Sample analysis was conducted on the lean, fat, and obese pooled samples utilizing a GCxGC separation followed by TOFMS detection at an acquisition rate of 150 spectra per second. The raw data was processed and mass spectral searches conducted using Max Planck, Fiehn Rtx5, and NIST libraries. The data mining strategy compared the combined peak tables, mass spectral similarities, and overlaid chromatograms to discover significant metabolite variations between the lean, fat, and obese sample pools. The GCxGC-TOFMS analysis was performed on the pooled samples from various states of health, lean, fat and diabetic obese. This exploratory research was conducted as a screening tool to characterize and measure semi-quantitative variations in metabolites between the sample pools. A total of twelve metabolites between the different sample pools were found to have significant differences measured by their chromatographic peak areas. This exploratory research demonstrates the favorable and practical applicability of GCxGC-TOFMS as a screening tool to discover potential metabolic biomarkers.

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ABSTRACT WITHDRAWN

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AUTOMATED LIBRARY GENERATION FOR UNTARGETED METABOLOMICS STUDIES

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A major limitation for untargeted metabolomics studies is the generation of a library or target component list from the deconvoluted mass spectral data files. In order to achieve this, a script was written to compile a library from the results output of SpectralWorks AnalyzerPro. A set of 24 plasma samples from pigs were extracted and analysed using an Agilent 5973 GC-MS single quadrupole instrument. Generation of a library from this data set initially yielded a library with 2146 components, which reduced to 660 components after manual quality control. he same samples using an in-house target component list of 350 compounds generates an average of only 102 matches that can be used for subsequent analysis. This represents an increase of nearly 650% using the automated library generation. The same samples were analysed by GCxGC-TOF-MS using a LECO Pegasus 4D. Automated library generation was performed using the LECO ChromaTOF software. Generation of a library from these samples yielded a library with 3689 components, which reduced to 963 components after manual quality control. The key limitation of this technique is the requirement to manually quality control the data set. To overcome this, the process has now been integrated into AnalyzerPro. Automated library generation is a relatively simple option for strengthening metabolomics data sets and taking better advantage of the data generated by mass spectral analysis.

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AN UPDATE ON CANADA'S NATIONAL METABOLOMICS PLATFORM - TMIC

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TMIC (The Metabolomics Innovation Centre), which is serving as Canada's National Metabolomics Platform, was officially launched on April 1, 2011. This centre builds on the expertise and infrastructure developed by a number of Canadian researchers during their involvement with the Human Metabolome Project (2005-2009). TMIC has several mandates including the provision of low-cost metabolomics services to academic and industrial researchers, the maintenance of freely available metabolomics databases and web servers (HMDB, DrugBank, T3DB, MetaboAnalyst), the training/dissemination of metabolomics techniques/technologies to other researchers and the development of improved or more comprehensive metabolomics assays. TMIC specializes in performing quantitative metabolomic assays on human, animal, plant and microbial samples using a wide range of technologies including NMR, GC-MS, LC-MS/MS, LC-FTMS, HPLC-UV and TLC/FAMES/GC-FID. This allows for the identification and quantification of >3000 compounds for certain samples. In order to keep pace with the rapid technology developments in metabolomics, TMIC is constantly working towards developing, acquiring, testing and implementing new metabolomic technologies. Most recently, TMIC developed several quantitative assays to expand its list of detectable metabolites. These metabolites include polyphenols (phenolics, flavonoids, isoflavones), carotenoids, vitamins (B, C, D, E, A), trace metals, lipids (MAGs, DAGs, TAGs, PCs, PEs, PSs, SMs, CEs), nucleotides, thiols and bile acids. TMIC is also working towards the development of improved metabolite imaging technologies and the expansion of selective isotopic labeling for quantitative MS-based metabolomics. Descriptions of these new assays and technologies along with a brief discussion of their applications in medicine, nutrition, agriculture and other fields will be presented. TMIC, like Metabolomics Australia and the Netherlands Metabolomics Centre, is intended to be an enabling resource for all researchers in metabolomics.

METABOLOMIC PROFILING OF ENDOPHYTIC FUNGI FOR BIOPROTECTION OUTCOMES IN AGRICULTURE

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Agriculture industries worldwide are rapidly moving toward more environmentally sustainable practices, particularly in the area of disease management. This change has been driven by a shift in government and consumer sentiment, including tighter government regulations on pesticide usage (e.g. fumigants) and consumer demands for environmentally-friendly produce (e.g. reduced pesticide inputs).[1] Therefore, new products must meet strict environmental and commercial requirements, such as reduced application rates, low toxicity and minimal residues, while exhibiting new modes of action and targeted biocidal activity.[2]

Bioprotectant endophytes are an emerging tool in disease management that potentially offer a wide array of benefits (environmental and efficacy) over synthetically derived pesticides. Endophytes are microbes that reside in the tissues of living plants.[3] Many form mutualistic symbiotic relationships with their plant hosts, with the endophyte conferring increased fitness to the host, often through the production of bioprotectant defence metabolites. In return, the host provides the benefits of a protected environment and nutriment to the endophyte.

Promising bioprotectant endophytes include the fungus, *Muscodor albus*, which was isolated from *Cinnamomum zeylanicum* (cinnamon tree) in Hondurus and found to produce a suite of volatile biocidal metabolites that act synergistically to control a wide range of phytopathogens and pests.[4] A system was devised called mycofumigation, whereby the fungus was incorporated into soil, where it released its volatile biocidal metabolites, disinfesting the soil.[5] Mycofumigation offers a promising, environmentally-friendly and safer alternative to soil fumigation than toxic chemicals such as the ozone depleting fumigant, methyl bromide.

Our preliminary study identified an endophytic fungus (Family: Xylariaceae) from Australian native temperate rainforest plants, which also produces volatile metabolites with biocidal activity against soil-borne pathogens. This poster will report on: (a) the bioactivity of the isolate, (b) the identification of biocidal volatile metabolites and (c) delivery mechanisms for introducing the endophyte into soil (i.e. mycofumigation).

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HILIC-LC-ESI-TOF-MS ANALYSIS OF THE POLAR METABOLOME OF *SINORHIZOBIUM MELILOTI* FOLLOWING EXPOSURE TO FLUORENE

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The polar and non-polar metabolomes of the soil microorganism, *Sinorhizobium meliloti*, have been analyzed by gradient elution HILIC-LC-TOF-MS and by infusion tandem MS ('shotgun lipidomics') methods, respectively. A number of HILIC columns were evaluated for use in the LC-MS methodology; the column which gave the best performance overall was a zwitterionic column. This LC-MS methodology allowed us to detect over 230 unique peaks with k' values > 0.7 in both +ve ion and –ve ion MS modes. These 230 ions do not include adduct ions, in-source decay ions or isotopic peaks. We have shown that essentially no ion suppression was observed for peaks with k' >0.7. This methodology was applied to the metabolomic analyses of S. meliloti grown on M9 glucose minimal medium in the absence and presence of the PAH contaminant, fluorene, at two concentrations (0.14 and 1.4 mg per litre of growth medium). Analyses of biological replicates were performed in pentuplicate for each sample. Retentions times across the 25 min. chromatograms were aligned using xcms and Camera and showed retention time reproducibilities less than ± 2 seconds. The peak areas were determined and data analysis was performed using PCA and OPLS-DA methods. The reproducibilities within each biological treatment were in the 25%-35% range. The metabolic impacts of the fluorene exposures on *S. meliloti* will be discussed.

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COMPUTER-ASSISTED STRUCTURE IDENTIFICATION: PREDICTIVE MODELS AND AUTOMATION

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The CASI (Computer-Assisted Structure Identification) approach is used at Philip Morris International R&D to identify small molecules in complex matrices analyzed with GCxGC-TOF-MS. In the CASI approach, structure candidates and associated match factors for a mass spectrum are obtained using NIST MS Search. In order to refine the results of NIST MS Search, we developed quantitative structure−property relationship models to predict values of the two retention times of a GCxGC-TOF-MS instrument. A Kovats Indices model was built for the first dimension and a model developed for the second dimension using relative retention times that are specific for the GCxGC-TOF-MS instrument: non-polar (1st dimension) x polar (2nd dimension). The models can be adapted for different column combinations. Results obtained by k-nearest neighbors, multiple linear regression, and support vector machines for each type of model were compared. For each algorithm, the best sets of descriptors were chosen using genetic algorithms.

The process is fully automated using Java and several other standard tools, such as NIST MS Search for searches in a mass spectral database, Dragon for computing molecular descriptors, RapidMiner to apply predictive retention models, Pipeline Pilot to normalize chemical structures, and ACD/Labs PhysChem Batch to compute boiling points. CASI Web interface proposes a list of the best matched structure candidates allowing users to easily check and correct structure assignments. CASI also enables the user to easily add new instruments, analytical columns, and retention models to the platform.

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QUALITATIVE VARIATION IN DISEASE STATES OF *IANTHELLA BASTA* (PORIFERA: VERONGIDA)

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Marine sponges are critical to benthic-pelagic coupling across a wide range of habitats and have been shown to harbour dense and diverse populations of microorganisms. Sponges are also known to produce a large variety of structurally, highly diverse, secondary metabolites, many of which possess interesting biological activities, but whose ecological roles are not yet understood.

Reports of sponge disease have increased dramatically in recent years with several epidemics reportedly affecting *Ianthella* (order Verongida) on the Great Barrier Reef (GBR) (Webster, 2007). In an effort to better understand sponge disease we present here an investigation of healthy and diseased specimens of both yellow and purple colour morphs of *Ianthella* sp. In a previous study (Freckelton et al.) analysis of genetic markers confirmed the taxonomy of specimens collected as *I. basta*. It was also shown that the two colour morphs corresponded to two distinct metabolic profiles and two corresponding distinct bacterial community profiles. In the current study several untargeted metabolomic analytical platforms (FTMS, LCMS and ^{1}H NMR) and informatics tools (R, AMIX) will be used to start to define initial metabolic signatures for disease.

Preliminary results presented here indicate that the metabolic profile differs not only between colour morphs but also between healthy and diseased specimens of *I. basta*.

These results have potentially important implications to ecological investigations of sponge health where environmental factors including elevated seawater temperatures, anthropogenic pollution, nutrient enrichment and introduced species have been linked to marine diseases.

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DEVELOPMENT OF A GC-MS METABONOMIC METHOD FOR METABOLITE PROFILING OF STREPTOZOTOCIN INDUCED DIABETES IN RAT LIVER.

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Diabetes results in hyperglycaemia, due to decreased/absent insulin production or decreased insulin sensitivity. The lack of cellular glucose absorption induces compensatory mechanisms involving carbohydrate and nitrogen metabolism and oxidative stress mechanisms. Metabolomics can provide comprehensive characterisation of molecular pathways and may contribute to understanding metabolic changes in diabetes. The present study aims to combine experimental design optimisation with multivariate statistical analysis to validate a liver metabolite extraction protocol for the assessment of biomarkers of streptozotocin (STZ)-induced diabetes. Metabolic profiles of livers from STZ-induced diabetic rats and controls (n=8) were investigated with gas chromatography mass spectrometry (GC-MS). The data was further analysed by Principal Component Analysis to compare the metabolite profiles of diabetic and control rats. The analysis revealed an average of 143 ± 12.5 metabolites from the analysed livers. Of these 78 metabolites were positively identified. The two groups showed significant differences in metabolite profiles, including carbohydrates, amino acids, and organic acids. We have identified several relevant metabolites significantly altered in STZ-induced diabetes. These represent metabolites from key metabolic pathways, including gluconeogenesis and the tricarboxylic acid cycle. Metabolites that display significant and specific up or down regulation correspond well to existing data on diabetic metabolism. We have shown that GC-MS is a useful tool for characterising metabolic changes in diabetes. Understanding the biochemical changes occurring in diabetes will aid in the discovery and evaluation of possible treatments and provide a mechanism for further study of the disease itself.

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THE DYNAMICS OF THE YEAST METABOLOME *IN VIVO*

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Previous studies on the yeast reactome have produced a detailed functional map of its organisation. However, much of our understanding is derived from observations utilising static approaches, which change the network structure (such as mutation), measurements that are limited to one time domain or a few chemical species. Here we highlight that understanding the dynamics of the metabolome *in vivo* requires the understanding temporal dynamics on many time-scales (milliseconds to hours), by utilising synchronous (40 min oscillation in respiratory activity) continuously grown cultures of *Saccharomyces cerevisiae*. In these cultures, respiration cycles between phases of increased respiration (oxidative phase) and decreased respiration (reductive phase). We sampled from the culture (sampling frequency 0.004 Hz), rapidly quenched reactions, then extracted and measured metabolites using CE-MS. These data show clear phase relationships between all the calibrated metabolites and respiratory activity. A small but significant number of calibrated metabolites showed a "noisy" signal. Further investigation using more frequently sampled data, i.e., heat production (0.1 Hz) and online fluorimetry measurements (flavin and NAD(P)H; 10 Hz), revealed that these signals were multi-oscillatory and self-similar, i.e., showed the properties of a statistical fractal. Our data strongly suggests that a large proportion of the apparent "noise" in our CE-MS measurements was derived from this multi-oscillatory behaviour, and that perturbing one time-scale influences the dynamics of other time-scales.

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METABOLIC MODIFICATIONS OF NICOTIANA TABACUM L. (SOLANACEAE) INFECTED BY RHODOCOCCUS FASCIANS.

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Leafy gall is a severe symptom of the interaction between the phytobacteria Rhodococcus fascians and Nicotiana tabacum. In this structure, local proliferations of meristematic tissue are covered with many shoots which are inhibited in further growth by the presence of bacteria (1-3). The present project aims to identify metabolic changes induced by the microbe-plant interaction. The metabolomic analysis of tobacco plants extracts, infected or non infected by Rhodococcus fascians, was carried out by 1H NMR spectrometry and multivariate analysis techniques. A principal components analysis (PCA) was used as statistical tool to reduce the numerous NMR signals of the plant extracts to principal components that are characteristic for the metabolic changes after Rhodococcus fascians infection. PCA, an unsupervised clustering method, acts to reduce the dimensionality of multivariate data while preserving most of the variance within it (4). PCA of the 1H NMR spectra showed a clear discrimination between chloroform extracts on the first two components. A comparison of 1H NMR major peaks contributing to the discrimination with those of known tobacco constituents indicates that diterpene metabolites (probably of the cembrenoid family) considerably increase in response to R. fascians infection. Unfortunately, the great variability between aqueous extract samples does not allow the PCA to show a clear difference between tobacco plants samples. Thus, to allow an efficient interpretation of data, orthogonal projections to latent structures discriminant analysis (OPLS-DA), a supervised multivariate projection method was applied to highlight differences between aqueous tobacco extracts and to locate the class-distinguished variables contributing to the class separation (5). Our results are consistent with those obtained previously in the study of the interaction between Arabidopsis thalianna and Rhodococcus fascians (6).The elucidation of the major components contributing to the measured discrimination is being performed. This work should lead to the identification of metabolic pathways involved in the defence response against Rhodococcus fascians infection in Nicotiana tabacum.

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FLAVONOID-TARGETED ANALYSIS USING UPLC-PDA-QTOF-MS IN RICE

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Rice (*Oryza sativa*, Nipponbare) is one of the most important food crops and is a powerful model for functional genomics study of secondary metabolism in cereal crops. Flavone *C*-glycosides are common secondary metabolites among cereals with a wide range of biological activities. For examples, they function as UV-B protectants, antioxidants and allelochemicals for self-protection from biotic and abiotic stresses. The perceived human benefits of flavonoids have made them attractive targets for metabolic engineering of rice. However, rice flavonoids have been largely unknown due to lack of comprehensive rice flavonoid profiling method. Here, we demonstrate the method using ultra performance liquid chromatography (UPLC)-photodiode array (PDA)-quadorupole time-of-flight (QTOF)-mass spectrometry (MS). UV-based LC-MS profiling allows us to get useful UV spectra for suggestion of detected peaks as flavonoids (e.g. 340 nm). In this rice flavonoid-targeted profiling, we could show more than 30 flavonoids including flavone (apigenin, luteolin, crysoeriol and tricin) *C*- and *O*-glycosides, and flavonol (kaempferol, quercetin and isorhamnetin) *O*-glycosides. This analysis shows that rice has three types of flavonoid biosynthetic pathways for flavone *O*-glycosides, flavone *C*-glycosides and flavonol *O*-glycosides. Elucidation of the flavonoid biosynthetic pathways in rice will provide useful understandings to biotechnologically improve flavonoid amounts which may enhance various tolerances against biotic and abiotic stresses.

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DIETARY SUPPLEMENTS: TOWARDS THE DEVELOPMENT OF A QUALITY AND 'FINGERPRINTING' IDENTIFICATION TOOL USING NUCLEAR MAGNETIC RESONANCE (NMR)

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We describe our efforts towards a NMR based quality control screen for plant extracts used as dietary supplements. Raw material from plants vary widely due to agricultural, harvesting, or processing methods and adulteration may occur in the commercial product. The large variation in potential components, composition and adulterants suggests that a non-targeted 'fingerprinting' method is needed to characterize a dietary supplement. This allows the ability to quantify key components in the crude extract, to determine origin and plant part and get information on possible adulterants. Examples of pine bark, grape seed, blueberry leaves, skullcap and germander data are presented. PCA analysis to characterize spectra and identification and quantification of individual components was carried out with the AMIX software (Bruker BioSpin). The proposed screening method will improve the safety and efficacy to the consumer and meet the 2007 FDA ruling requiring cGMP compliance to ensure the quality of dietary supplements.

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HIGH RESOLUTION LC/MS/MS METABOLOMIC ANALYSIS OF GASTRIC CANCER CELL LINES

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Recent developments in mass spectrometry technology allow high resolution data to be collected on both MS and MS/MS analysis at fast speed at the same time. This enables the use of high resolution high mass accuracy MS data for more confident chemical composition prediction of chemical metabolites and the same high quality MS/MS data for structure elucidation, confirmation and identification of the predicted compounds. Using XIC Manager™ plug-in in PeakView™ software, a list of possible metabolites can be used to screened and confirmed based on their mass accuracy, isotope distribution and MS/MS spectra. PCA statistical analysis can also be used to differentiate potential targets based on similarities and differences of the metabolites. Here we are able to differentiate 4 different human gastric cancer cell lines based on their metabolites profiles and targeted metabolites were able to be identified. The results demonstrated the potential of this platform for untargeted metabolomic studies of gastric cancer.

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MICRO-OXYGENATION IMPACTS ON THE CHROMATIC CHARACTERISTICS AND OFF-ODOUR SULFUR CONTAINING VOLATILES IN A BORDEAUX BLEND

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Modern winemaking technologies provide winemakers with more powerful tools for oxygen management. Micro-oxygenation (MOX) is commonly applied to red wines, and is a technique that continuously delivers a small metered amount of oxygen into a wine by means of micro-bubbling using a porous micro-diffuser. While several beneficial effects of MOX on wine quality have been well examined in scientific research, information regarding the influence of MOX on the reductive sulfur containing off-odours is very limited. This study examined the influence of MOX during post malolactic fermentation on the chromatic characteristics and sulfur containing off-flavours, analysed by Gas Chromarography - Mass Spectrometry, in a fully replicate trial conducted on a *Vitis vinifera* var. Cabernet Sauvignon, Merlot, Cabernet franc and Malbec , vintage 2009, grown at a number of Hawkes Bay vineyards , New Zealand. MOX was conducted at 16 °C for 16 weeks in triplicate using 9 x 300 L stainless steel tank and 3 x 300 L polyethylene Flextanks.

Positive effects of oxygenation were seen in colour development, and the wine that received a high oxygen rate (2.5 mg/L/month) showed significantly higher colour intensity and improved SO2 resistant pigments, compared to the other wines. The values for the wines stored in Flextanks tracked between the low (0.5 mg/L/month) and high oxygen treated wines. While MOX lowered the concentrations of the off-odour sulfur containing volatiles found in the Bordeaux blend, of note is that the low oxygen treated wine had the most effects, which was also reflected in the sensory evaluation. Interestingly, the decrease in the concentration of the common off-odour methanethiol did not lead to any concurrent formation of disulfides, indicating that it is not oxidised directly to form disulfides but may react with other wine constituents such as polyphenols.

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TOWARDS A BETTER UNDERSTANDING OF UREMIC MOLECULES: DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY PROCEDURE

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Chronic kidney disease (CKD) affects one tenth of the Australian population causing premature death. The treatment for end stage kidney failure (ESKF) is around $70,000 per patient each year on dialysis and unfortunately, the number of patients is increasing each year.

Although improvements in dialysis procedures can improve the quality and quantity of life among the patients, still half of the patients die within 3 years while they are under modern dialysis treatment. The main reason which causes premature mortality is cardiovascular disease. It is hypothesised that there are some toxins which can't be removed using dialysis and those molecules contribute to or cause cardiovascular disease.

Among all separation methods developed for the recognition of uremic molecules, capillary electrophoresis (CE) isvery suitable for the analysis of highly polar and charged compounds (i.e. most of metabolites). As metabolites often don't have UV absorbance, hyphenation of CE with MS has provided a strong tool for their analysis and provides higher sensitivity. In addition,MS can provide structural information of unknown metabolites. Other advantages of CE are the small sample and organic solvent requirement and the running cost is relatively low.

In this work, some of our efforts towards developing a robust and reproducible CE-MS method for monitoring low-molecular weight metabolites in serum samples of patients with chronic kidney disease will be presented.

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INVESTIGATION OF METABOLITES FROM OIL PALM (*ELAEIS GUINEENSIS*) TOLERANT AND SUSCEPTIBLE TO BASAL STEM ROT

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Basal Stem Rot (BSR) in oil palm is a destructive disease caused by a group of wood decaying fungi called *Ganoderma.* It is affecting many oil palm plantations in Malaysia . Information about the response of oil palm to BSR is scarce , particularly concerning changes in plant metabolism. In order to understand and gain information on oil palm root metabolome, root tissue from parental palms that were tolerant (TP) and susceptible (SP) to *Ganoderma* were analysed. The metabolite composition of roots from tolerant and susceptible parental palms was evaluated by liquid chromatography- electrospray ionization mass spectrometry (LC-ESI-MS). Preliminary LC-ESI-MS analysis allowed the identification of specific compounds characterizing each group, and the compounds ID were presented. Altogether, the study revealed a significant increase of phenolic compounds in TP, compared with SP. Other metabolite alteration detected comprised increased level of an antioxidant Procyanidin B, which might be involved in defence mechanisms.

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COMMON ION APPROACH FOR ACCURATE QUANTITATION OF GC-MS METABOLOMICS DATA

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Accurate data analysis for gas chromatography-mass spectrometry (GC-MS) metabolomics experiments is dependent upon accurate peak area quantitation. Typically the most successful methods for comparing peaks across a number of samples depend on the operator choosing a target ion on which to calculate relative area. This approach works well for targeted experiments, but naturally excludes untargeted analyses, where target ions for unidentified peaks are not known in advance. Even in targeted analyses the process is manual, time consuming and depends on the knowledge of the operator. We have developed an automated, untargeted quantitation method for GC-MS data analysis based on the selection of common ions in the optimally aligned peak table of multiple experiments (this may include both within-state and between-state alignment). The algorithm based on dynamic programming (1) is used to align peaks between samples, and a highly abundant ion, shared between all aligned signal peaks in a specific position is chosen automatically for quantitation. This realises the benefits of the target ion approach without requiring the operator to manually select the target ion for each position. The algorithm is implemented within the open source Python-based toolkit for the processing of GC-MS data (PyMS, http://code.google.com/p/pyms). We demonstrate the power of the common ion quantitation on a highly controlled data set, based on a complex biological matrix dosed with increasing volumes of a known mix of metabolites. The areas of these known metabolites were calculated for each sample using both the common ion approach and a total area (sum of all ions in the peak) approach. For comparison, quantitation of the dataset using a commercial software package is also presented. The common ion approach offers many advantages in terms of reduced time, accuracy, and significantly increases the number of metabolites that can be accurately quantitated.

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EFFECTS OF RICE STRAW PRETREATMENT ON CHANGING CELLULOSIC SUPRAMOLECULAR STRUCTURE AND IMPROVING DIGESTIBILITY FOR PADDY SOIL MICROBIOTA

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Macromolecular complexe such as lignocellulose, major component of plant cell walls has not been targeted in metabolomics/metabonomics field. This cellulosic supramolecular structure plays many roles of providing physical and biological strength of plants, namely against microbial attack. However, little information about the cellulosic supramolecular structure and its degradation process by soil microbiota in natural ecosystems is available. In this study, we focused on the effects of rice straw pretreatment to cellulosic supramolecular structure and its digestibility by paddy soil microbiota. The rice straw samples were freeze-dried and milled by different mechanical pretreatment processes using two kinds of grinders with different milling time. Each milled sample was analyzed to obtain the structural, compositional, and physicochemical information by using various analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and thermogravimetric/differential thermal analysis (TG/DTA). By processing of different physical pretreatment, cellulosic chemical structures and linkages broken from crystalline to amorphous were observed by solid-state ^{13}C cross polarization magic angle spinning (CP/MAS) and ^{13}C-^{1}H heteronuclear correlation (HETCOR). In addition, thermodynamic properties of biomass samples processed by different milling conditions were characterized by TG/DTA. Furthermore, differential analysis of ^{1}H-^{13}C correlation NMR for 1 and 6 hour ball-milled samples exhibited xylopyranose moieties solubilized earlier than other lignin and hemicelluloses components. In order to evaluate the digestibility by the microbiota, milled samples were incubated with paddy soil for a month. The metabolic and microbial community profiles in the incubated soil samples were evaluated by NMR and denaturing gradient gel electrophoresis (DGGE) analysis, respectively. Surprisingly, the microbial community profiles and their metabolites production processes were found to be characteristically formed by the impact of feeding biomass pretreated with or without ball milling. Therefore, physical pretreatment of plant biomass was changed cellulosic structure and improved digestibility for microbiota.

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AN INTEGRATIVE PHARMACOGENOMIC-PHARMACOMETABOLOMIC STUDY TO PREDICT CYCLOSPORINE RESPONSE USING SYSTEMS PHARMACOLOGY IN HUMAN

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Although pharmacogenomic approach has been useful in personalized medicine, it cannot predict the individual variation in drug response that is influenced by environmental factors. We provide a new ' PharmacoGenomics (PG) – PharmacoMetabolomics (PM) ' approach, which is sensitive to both genetic and environmental contributions to drug response, with a study od cyclosporine administered to human. The aim of this study was to evaluate whether the combination of PM and PG can increase the predictability of individualized pharmacokinetics. This was single-center, randomized, open-label, parallel study in which 15 healthy male subjects selected according to the CYP3A5 genotypes, *3/*3 to group A (n=7) and *1/*3 to group B (n=8), were administered 100-mg cyclosporine orally. All subjects were hospitalized at the Clinical Trial Center of Kyungpook National University Hospital for 24 hours and given the controlled diet. We analyzed all pre- and post-dose plasma samples using liquid chromatography mass spectroscopy (LC-MS)-based non-targeted metabolomic profiling, with detection of the levels of 1,057 metabolite ions. The LC/MS spectra were analyzed using principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA), to identify metabolites predictive of individual variation in drug response including information related with both genetic and environmental factors. The use of CYP3A5 genotypes for variable selection methods was found to enhance the classification performance of the PLS-DA models. The loading plots obtained by PCA and PLS-DA were compared and various metabolites were selected that are responsible for the observed separations. The selection of these metabolites allowed us to understand their functional role and generate a clinically applicable index to predict individualized response of cyclosporine.

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METABOLOMIC PROFILING IN DRUG DISCOVERY: UNDERSTANDING THE FACTORS THAT INFLUENCE A METABOLOMICS STUDY AND STRATEGIES TO REDUCE BIOCHEMICAL AND CHEMICAL NOISE

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Introduction
Metabolomics is used within the pharmaceutical industry to investigate biochemical changes resulting from pharmacological responses to potential drug candidates. The ability to identify markers of toxicity/efficacy can significantly accelerate drug discovery and helps define the appropriate clinical plan. Data from LC-MS metabolomic profiling experiments contains large amounts of chemical background which often confounds biomarker discovery. New mass spectrometer technology and data processing software were utilized here to reduce chemical background; animal experiments were designed to investigate the influence of animal age and nutrition in relation to drug-induced changes.
Methods
Blood samples were taken from groups of male rats (fully satiated, acute and chronic fasting, different ages). LC-MS analyses were performed in positive and negative modes using a hybrid Orbitrap mass spectrometer capable of fast scanning at ultra high resolution (>50K), and a 12-minute UHPLC separation. Study data was analyzed using new component detection algorithms in beta SIEVE 2.0 software to determine metabolic effects of food deprivation and aging rats.
Preliminary data
In typical LC-MS metabolomics studies much of the data is redundant (multiple ions per component) and irrelevant (chemical noise). External factors that influence metabolic profiles (age, nutrition) increase biological variation. Since many of the chemical entities are unknowns, it is especially important to filter false positives before implementing structure elucidation. Ultra-high resolution instruments combined with UHPLC separations address the issues of chemical noise and redundancy by providing sufficient resolution to distinguish metabolites from chemical background. Accurate mass data allows sophisticated processing needed to recognize related signals leading to significant reduction in data size and providing improved quantitation of targeted metabolites. Biological factors have profound impact on metabolic profiles and even modest metabolic changes can obscure drug-induced metabolic effects. Understanding normal metabolic changes in rats helps to minimize "biological noise" and provides more confidence in assigning drug-related metabolic changes.

ROBUST METABOLITE PROFILING AND IDENTIFICATION EMPLOYING HIGH RESOLUTION MS STRATEGIES AND DEDICATED SOFTWARE

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Mass spectrometry-based metabolomics is a key approach to analysis of cultivars that contribute to sustained agro development by detecting plant varieties which are robust, healthy and nutrition-rich. High resolution/accurate mass (HRAM) LCMS analysis provides outstanding sensitivity, accuracy and wide dynamic range with high throughput. However, huge amounts of information are generated thus automated reliable extraction of relevant information is essential. Results from HRAM tomato metabolite profiling experiments followed by intelligent, automated data reduction are presented. Data processing employing novel algorithms is used to link development stage-specific metabolomic characteristics to cultivar phenotype.

Triplicate biological replicates of two tomato cultivars were analyzed at four times of development using fast reversed-phase LCMS analysis, using a hybrid high resolution mass spectrometer instrument. Strategies for metabolite profiling, data mining and metabolite identification were successfully applied and encompassed sample measurement in positive and negative ion mode electrospray in conjunction with multiple dissociation techniques and data analysis.

Hundreds of components were profiled at resolutions up to 100,000 useful for accurate and sensitive relative quantification experiments. Analytes were measured with high (<2ppm) mass accuracy, leading to putative identifications based on elemental composition. Resonance excitation CID or higher energy collisional activation (HCD) experiments were employed to further confirm the identity of metabolites of interest.

Data processing includes reduction of millions of data points to hundreds of actual components by eliminating noise, performing peak filtering and combining isotopic peak profiles, adducts and dimers into a single accurate mass and retention time corresponding to a unique analyte. Data processing continues with a sophisticated alignment procedure to generate a table of annotated components along with their relative abundance measured in each sample. Based on HRAM data components are identified in proprietary or public databases. Finally, univariate/multivariate statistics are applied across the samples to reveal changes and trends are correlated to biochemical events.

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MONITORING THE EFFECT OF EXERCISE IN HUMAN URINE SAMPLES BY ^{1}H-NMR AND ULTRA-HIGH RESOLUTION CZE-ESI-TOF-MS BASED METABOLOMICS.

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Proton nuclear magnetic resonance (^{1}H-NMR) and ultra-high resolution mass spectrometry coupled to capillary zone electrophoresis (CZE-ESI-TOF-MS) analysis has been applied to urine samples collected from patients (male, 61.0 ± 7.4 years; mean ± SD) before and after an 18 month exercise intervention, to determine significant molecular features and metabolites indicative of the effect of exercise. The exercise intervention consisted of high intensity progressive resistance training and moderate impact weight-bearing exercise 3 days/week. ^{1}H-NMR and CZE-ESI-TOF-MS profiling of the urine samples were used to accurately quantify and identify a number of high and low abundant metabolites. The ^{1}H-NMR experiments were performed on a Bruker Avance US^{2} spectrometer equipped with a 5 mm TCI probe, and a 1-dimensional noesy pulse sequence with presaturation (recycle delay = 1.5 s) was used. Each sample was spiked with a known concentration of 2,2-diemthyl-2-silapentane-5-sulfonic acid (DSS) for calibration and quantitation of proton resonances, and ^{1}H-NMR data analysis was performed using Chenomx 5.1 NMR Software, The Unscrambler and the R statistical software package. CZE-ESI-TOF-MS analysis was performed using the Bruker "maXis" high resolution mass spectrometer (resolution of 50,000 at 1522 m/z, sensitivity to 0.8 ppb), and MS data was analysed using Bruker's Compass 1.3 Software equipped with the SmartFormula algorithm for metabolite identification. Multivariate statistical analysis (including PCA, PLS-DA and HDA) revealed differences in overall urinary metabolite profiles between the before exercise and after exercise groups, and univariate data analysis depicted the significant changes amongst these metabolites. The approach of using ^{1}H-NMR and UHR-MS in this study highlights the importance of using complementary analytical techniques for a comprehensive metabolomics analysis.

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STUDYING THE METABOLOME OF RICINUS COMMUNIS FOR CULTIVAR AND PROVENANCE DETERMINATION

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Seeds of the castor bean plant, Ricinus communis, are the source of castor oil, an important commercial product that has wide applications in many and varied industries. In addition to the oil, the seed also contains the plant protein ricin. Ricin is declared by the Chemical Weapons Convention as a Schedule 1 agent,[1] which is a highly toxic substance with no legitimate use. Ricin is a heterodimeric type II ribosome-inactivating protein (RIP),[2] which selectively depurinates adenine within a highly conserved fourteen nucleotide region of the 28S rRNA subunit of the large 60S ribosome.[3] This results in protein manufacture inhibition within the cell, preventing chain elongation of polypeptides. The combination of the toxicity of ricin and its Schedule 1 status, have created an interest within Australian law enforcement agencies to establish a method that allows for cultivar and provenance determination of seed extracts.[4] To solve this problem, DSTO is applying metabolomic strategies to generated extracts of R. communis seeds from different specimens. The metabolome of plants is strongly influenced by the type of soil the plant is grown in, and the type of climate that the plant is exposed to. Extracts of plant specimens from overseas have been analysed using noesypresat solvent suppression 1H NMR. Collected data was manually phased and baseline corrected, and subsequently analysed using multivariate statistical analysis, and in particular PLS-DA and OPLS-DA. This presentation will discuss recently generated results from the study of the metabolome of overseas specimens.

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SAUVIGNON BLANC METABOLOMICS: CORRELATION BETWEEN JUICE METABOLITE PROFILE AND WINE VOLATILE THIOLS

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Wine is one of the most important export products in New Zealand that earns more than one billion dollars a year. More than 70% of NZ wine is Sauvignon Blanc, which is considered as the world's best due to its unique and distinctive aroma. Volatile thiols are key aroma compounds and among them, 3-mercaptohexyl acetate (3MHA) , 3 -mercaptohexan- 1-ol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP) are responsible for the passionfruit, grapefruit and box tree (cats pee) aromas respectively. The compounds are absent in grape juice and develop during fermentation by the action of yeasts from odourless precursors. There is ongoing debate about which compounds are precursors for volatile thiols. The goal of the metabolomics research in this project is to identify metabolites in grape juice that predict the level of thiol compounds in the wine. A total of 63 grape juices have been collected from 2006 to 2010 from different regions and vineyards. Metabolite profiling of all these juices was performed by GC-MS. About 1800 compounds were detected and over 100 compounds were identified by our in-house MS library. All 63 juices were also analyzed by FTIR WineScan and will undertake NMR. Quantification of putative thiol precursor compounds in a subset of 12 juices was performed by LC-MS but no correlation was found with thiols. All the juices were fermented by yeast strain EC1118 under controlled laboratory conditions. Thiol production in the final wine was correlated with initial metabolite profiles. No correlation of compounds was found with 4MMP. But alanine, valine, leucine, glycine, gamma-amino butyric acid, glutamic acid, glutamine, citric acid and succinic acid all showed positive correlation (range of r^2 0.35-0.48) with both 3MH and 3MHA. Juice addition experiments will be carried out in the 2011 season by adding subsets of these compounds to see whether thiol levels can be increased by direct manipulation and to address the issue of whether any of the correlations are causal.

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MICROBIAL COMMUNITY AND METABOLOMICCOMPARISON OF IRRITABLE BOWEL SYNDROME FECES

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Irritable Bowel Syndrome (IBS) is a chronic disorder of the large intestine and it is more prevalent in adult and adolescent without sex barrier. IBS is a functional disorder; it results in abnormal muscle contraction in the lower part of the colon and delays bowel movement which causes constipation. Sometimes, it may leads to more rapid passage of bowel movements, causing diarrhea. Symptoms may also be triggered by emotional stress or depression because muscles in the bowel are controlled by the nervous system. IBS is prevalent in worldwide; Western countries (10-15%) are more affected than Eastern countries and 6.6% was reported in Korean population. Microbiota plays an important role in gastrointestinal (GI) tract of human such as complex food digestion, metabolize drugs, detoxify toxic compounds, produce essential vitamins, and prevent colonization of pathogens. The most predominant probiotics are believed to prevent the GI tract and imbalance of microbial community causes various diseases. Information on the fecal microbiome among the IBS patients in Korean population is scarce. Microbial and metabolomics of gut will provide the complete information of indigenous microorganisms and its contribution in the IBS patients.

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DEFINING THE METABOLOME USING GC-MS: - IMPACT OF TEMPERATURE GRADIENT AND SCAN RATE IN QUADRUPOLE SYSTEMS

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Quadrupole GC-MS is often used as the initial tool of analysis for metabolomic studies due to its relative ease of use and the availability of commercial mass spectral libraries. In addition, the relativley low cost of running the instrument makes the technique highly desirable.

A key driver in Metabolomics is to produce a method that is high throughput without negatively impacting the integrity of the data obtained. High throughput methods are achieved through the use of faster temperautre ramps and an increased reliance on spectral deconvolution. However, the integrity of the data can be diminished if the combination of scan rate and temperature gradient are not fully optimised.

Using a Shimadzu QP2010 Ultra GC-MS, the effect of scan rate and temperature gradient was examined using urine samples. Temperature gradients ranged from 5.6ºC/min to 35ºC/min and scan rates varied from 2.5 to 20 scans per second scanning the range m/z 40-600.

This approach defines the necessary mass spectral scan rate required to maximise the number of metabolites in multifunctional samples with wide dynamic ranges for high throughput metabolomics analysis methods using single quadrupole GC-MS instruments. In particular, this work defines the compromise in the metabolome coverage resulting from unoptimised GC-MS methods. Significant decreases in the deconvolution capacity of the analysis occurred when the scan rate was not sufficiently fast to pull apart similar mass spectral detail. Specifically, metabolites belonging to the same chemical group (e.g. fatty acids or sugars) suffered greatly at the expense of higher throughput.

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DAIRY POLAR LIPIDS ALTER BRAIN LIPID METABOLISM OF C57BL/6J MICE UNDER DIET-INDUCED STRESS

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Diets rich in saturated fatty acids can lead to brain inflammation, reduce neurogenesis, and increase apoptosis. Studies have shown that some exogenous phospholipids are able to prevent apoptosis in cell culture. The present study evaluated whether a dietary supplementation of polar lipids from milk containing phospholipids and sphingolipids could ameliorate effects of high-fat diet in the brain. Twenty six C57Bl/6J female mice (4 weeks old) were fed a high-fat diet *ad libitum* for 5 weeks. Subsequently, mice were split into two groups and fed either the same diet (control) or the diet supplemented with the dairy polar lipids for further 5 weeks. During this period the mice also had free access to drinking water enriched with 4% of $^{2}H_2O$. The impact of dairy polar lipids supplementation on cell proliferation was evaluated by estimating the rate of incorporation of deuterium into the DNA of brain tissue. The deoxyribose derivative, from the hydrolysis of extracted DNA, was analyzed using NCI-GC/MS. The fatty acid lipid profile of the brain was analyzed by GC-MS and lipid profile by direct infusion mass spectrometry. There was no significant effect of supplementation of polar lipids on cell proliferation in the brain. Control and treatment group showed distinct correlation among levels of five fatty acids. Docosahexaenoic acid (C22:6) composition in the brain was negatively correlated with tetracosanoic acid (C24:0) for the treatment group (-0.71, P=0.02) but not for control (0.26, P=0.44), whereas arachidonic acid (C20:4) was negatively correlated with 24:0 for the treatment group (-0.77, P=0.008) and control (-0.81, P=0.003). Control and treatment group also showed a distinct correlation between lysophospholipids and phospholipids in the brain. These results suggest that the dietary administration of polar lipids from milk affected the metabolism of phospholipids and docosahexanoic acid in the brain when high fat diets are fed. To understand these effects further investigation is required.

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UNSUPERVISED CLASSIFICATION OF MULTI-WAY DATA BY COMBINING TUCKER MODEL, HIERARCHICAL CLUSTER ANALYSIS AND HEAT MAPPING DATA VISUALIZATION.

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The increased throughput and production of comprehensive data sets describing biological samples allow the identification of correlation between metabolites across samples, its dependence on treatment groups and on environmental factors, such as time variation. Although, it leads to an increase in the data complexity. Thus data mining methods have been applied to metabolomics to reduce data complexity and facilitate extraction of information. Tucker models accommodate different levels of complexity in the data set, allow identification of factors affecting the classification of biological samples and are useful for mining data produced in hyphenated instruments (e.g. GC/MS). However, there is a major challenge when using Tucker models that is the interpretation of the interaction among loadings and the core array. This work describes a novel approach for unsupervised classification of multi-way data, based on a Tucker model combined with hierarchical cluster analysis and data visualization. The developed method facilitates data mining and its application is illustrated in a GC/MS data set obtained from the fatty acid profile of milk samples aiming to provide unsupervised identification of groups of animals correlated significantly with age and breed. This data set includes different levels of complexity due to rank overlap in the mass spectra mode (due to high similarity in the mass spectra among some of fatty acid methyl esters) and also in samples mode since fatty acids produced by *de novo* biosynthesis might be highly correlated across groups of animals. The method allowed the identification of key grouping factors that can link milk components to genetic variation.

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EXPLORING THE METABOLOME OF THE INTERLENKIN-10-DEFICIENT MOUSE

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The interleukin-10-deficient ($IL10^{-/-}$) mouse is being used as a model for inflammatory bowel disease (IBD) with the expectation that foods that can reduce intestinal inflammation in the mouse model may provide relief to sufferers of IBD. Metabolite-based biomarkers predictive of intestinal inflammation in $IL10^{-/-}$ mice are being sought to non-invasively measure the efficacy of such functional food products.

We compare and contrast biomarker candidates generated by GC-MS and infusion-LC-MS/MS metabolomic analysis of urine from $IL10^{-/-}$ mice. We also distinguish between metabolic differences associated with intestinal inflammation and those resulting from residual differences between mouse strains or differences in intestinal microflora.

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LCMS LIPID PROFILING OF INTRA-ERYTHROCYTE STAGES AND INTRACELLULAR ORGANELLES OF *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum is the major cause of malaria, a disease that afflicts more than 500 million people world-wide. The intraerythrocytic stages of *P. falciparum* exhibit a highly orchestrated developmental cycle within red blood cells (RBC) that takes approximately 48 hr. During this time, intracellular stages synthesize massive amounts of new membrane. There is accumulating evidence that intraerythrocytic stages are dependent on both the salvage of lipids from the host, as well as *de novo* synthesis of phospholipids and other lipid classes for intraerythrocytic proliferation. In this study we have used a LC-MS lipidomic approach to identify changes in the lipid composition of *P. falciparum*-infected RBC during this cycle, and to probe the lipid composition of the unique *P. falciparum* apicoplast organelle that is thought to harbor a number of prokaryotic lipid biosynthetic pathways. Lipids were extracted from synchronized *P. falciparum*-infected RBC and targeted lipid analyses undertaken using LC-MS/MS (Agilent triple quadrupole). LC was performed using RP-amide columns and lipid species detected in precursor scan and neutral loss scan modes and quantified by multiple reaction monitoring (MRM). These analyses highlighted significant changes in both the overall abundance and fatty acid composition of major phospholipid classes (PC, SM, PG, PE, PS, PI), ceramides and cholesterol esters in infected and uninfected RBC. Most striking was the accumulation of PI following the differentiation of ring stage to trophozoite stages. The *P. falciparum* apicoplast was purified using a new immunoaffinity protocol and subjected to comparable analyses. Lipid profiling revealed the differences in PI levels between the apicoplast and unfractionated parasite membranes. Unlike the plastids of photosynthetic apicomplexans, the P. falciparum apicoplast lacked detectable galactolipids. Overall, these analyses suggest that intraerythrocytic stages are likely to be dependent on a number of pathways involved in lipid salvage from the host and remodeling/resynthesis of complex lipids.

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NMR METABOLOMICS INVESTIGATION OF DRASTIC HYPOXIA IN A NEONATAL PIG MODEL

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OBJECTIVE: To explore metabolic changes due to asphyxial cardiac arrest and cardiopulmonary resuscitation in a neonatal pig model; to find blood or urinary biomarkers for subject characterization and intervention effects.

METHODS AND RESULTS: A cohort of n=22 newborn pigs was subjected to progressive asphyxia until asystole.[1] Animals were randomised to receive compression to ventilation (C:V) ratios of 3:1 (n=11) or 15:2 (n=11). Plasma and urine were sampled at baseline and after two and four hours of post-resuscitational observation, and stored at -80°C. Samples were buffered, then profiled using ^{1}H-NMR (600 MHz, 1d-noesy). From the plasma spectra, cross-validated pairwise PLS-DA analysis could clearly distinguish between baseline and two-hour samples, identifying consistently elevated levels of metabolites such as lactate, alanine, branched-chain amino acids, pyruvate, glutamine, fumarate and hypoxanthine. While changes between the two-hour and four-hour samples were less systematic and could not be validated, the four-hour sample still exhibited elevated lactate and reduced levels of a lipid signal consistent with LDL as compared to baseline. This indicates that the pigs' metabolism had not yet returned to normal at the end of the experiment. Urine spectra were not able to consistently distinguish between either of the sampling time points. In accordance with previous studies[1], no difference between the intervention groups was observed in either fluid. However, both baseline urine and plasma were able to predict tendencies in the duration of hypoxia until asystole occurred, tying earlier cardiac arrest to elevated plasma lipid levels and to several currently unidentified metabolites of low concentration.

CONCLUSIONS: Tracing the effects of asphyxia, NMR Metabolomics detected significantly altered metabolite patterns in plasma, while urine proved less valuable. Neither fluid could distinguish the intervention groups, but both predicted tendencies in the time until cardiac arrest. Particularly the identity and role of lipids must be examined further.

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METABOLIC SHIFTS UNDER THE STRINGENT RESPONSE IN *ESCHERICHIA COLI*

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A guanosine tetra-phosphate (ppGpp) plays a key role in the bacterial stringent response that is induced by amino acid starvation. The stringent response provokes inhibition of stable RNA synthesis, and then induces stationary growth of bacterial cells. The regulatory effect of this response on global gene expression has become clear, however, its machinery for metabolic regulation such as the global metabolic shifts in response to ppGpp molecule remains incompletely understood.

We examined the metabolic effects of ppGpp mediated stringent response in *Escherichia coli*. The time series metabolome data during stringent response were obtained under overproducing a ppGpp and amino acid starvation in *E. coli* using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). The metabolic responses to ppGpp accumulation were analyzed by metabolites clustering. The levels of purine nucleotides, intermediates of *de novo* pyrimidine nucleotides biosynthesis, and intermediates of glycolysis pathway were dropped with the accumulation of ppGpp. In contrast, the metabolite levels of pyrimidine nucleotides and intermediates of purine nucleotides salvage pathway were rapidly accumulated in response to ppGpp. We confirmed that these rapid changes in metabolite levels are not correlated with changes in the amount of corresponding enzymes by parallel proteome analysis. *In vitro* approach to confirm the mechanism of rapid metabolite responses to ppGpp accumulation suggested that these are caused by direct action of ppGpp as an enzyme inhibitor. These results provide that possible insight into the new molecular target of ppGpp and mechanisms of metabolic regulation under stringent response.

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ACCURATE MASS HILIC WITH HIGH PERFROMANCE TOF-MS FOR METABOLOMICS ANALYSIS OF BLOOD PLASMA

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Accurate mass information is critical for analyzing the wealth of metabolomics data. Besides mass accuracy, isotope ratio accuracy, MS and fragment ion spectral acquisition rate, metabolomics data require powerful software for peak finding, mass spectral deconvolution, and calculating elemental formulas. The utility of these tools in general sample survey is explored and evaluated. Specifically, the ability to obtain correct elemental formulas and quality fragment ion spectra are utilized as tools to evaluate acylcarnitines as potential biomarkers of physical training and indicators of lipid metabolic cnages in unbiased metabolomics comparisons of mouse plasma. Analysis of biological replicates in each group enabled detection of hundreds of accurate mass/retention time features at or below 1-2 ppm mass error in full scan MS mode. Using the accurate mass matching and fragment ion evaluation in Metlin and other similar databases numerous complex lipids (triglycerides, phosphatidylethanolamines, phosphatidylcholines, and sphingomyelins), acylcarnitines, and various other small molecules were identified such as free carnitine and choline. Identified structures covered three to four log units of signal intensity. Trained and untrained mice differ in the ability to perform complete lipid oxidation. Statistically significant ($p < 0.05$) decreases in relative levels of lipids and fatty acid associated molecules of >30% in the trained population when compared to the untrained control, suggest changes relating to mitochondrial β-oxidation.

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DIFFERENTIAL METABOLOMIC ANALYSIS OF CORN LEAF EXTRACTS BY LIQUID CHROMATOGRAPHY - HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROMETRY (LC-HRT)

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The preferred tool in metabolomics studies has become mass spectrometry. Metabolomics benefits from using a very sensitive and high resolution mass spectrometer capable of rapid acquisition across a wide mass range, owing to the very complex samples may be biologically relevant. Differential metabolomic analysis of plant tissue is important for the development of crops with desirable traits such as increased yield. High performance Fourier-transformation type instruments have traditionally filled this role but have their limitations. Recent advances have enabled time-of-flight instruments to perform this type of analysis with equal facility. The economic importance of corn as a world-wide makes it a relevant example for testing a high performance time-of-flight mass spectrometer for differential metabolomic analysis. The chromatographic method appeared appropriate for analysis of a large number of metabolites. Although the early portion of the chromatogram exhibited peaks that were apparently distorted by the high fraction of methanol in the extraction solvent, about 75% of the chromatogram contained a multitude of peaks that could be repeatably integrated across samples. The final eluent was strong enough to elute chlorophyll as one of the last major peaks visible in the base peak chromatogram. About three hundred and four hundred analytically relevant signals were deconvolved per positive ion chromatogram and negative ion chromatogram, respectively. The spectrometer exhibited mass resolving power of approximately 35,000 at m/z of about 100, and consistently over 40,000 resolving power above m/z 250. A taurocholate internal standard present in each sample was used to verify accuracy of the m/z measurement and relative isotopic abundance accuracy, as well as to control for systematic sample variability. Throughout the run, m/z measurements for the internal standard were generally within about 1.5 ppm of the expected value, and relative abundances of the M+1 and M+2 isotopes were generally within about 10% of expected values. The data demonstrate the capability of the LC-TOF to provide a robust platform for accurate, high resolution evaluation of plant metabolomics.

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COMPREHENSIVE METABOLOMIC PROFILING OF ZUCKER RAT PLASMA USING LC AND GC ULTRA HIGH RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY AND GCXGC-TOFMS.

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Rodents represent a common model animal used in studying disease. UHPLC and GC are combined with high resolution time-of-flight mass spectrometry and utilized in the comparative analysis of metabolomic profiles of plasma from three strains of Zucker rats. Specifically, metabolite profiles for lean, obese, and fatty animals are differentially compared and statistically-significant features are identified. Differences in profiles for amino acid, ketone bodies, lipids and carbohydrates were observed. UPLC with ESI was utilized for non-volatile analyte identification. GC with EI ionization provided data on volatile and semi-volatile analytes after analyte derivatization. Protein was removed by acetonitrile precipitation or size-based filtration. Samples were analyzed at acquisition rates up to 40 spectra/s. The high speed acquisition capability facilitates faster analysis times for serum metabolomics with little compromise to coverage. The benefit of the complementary nature of the GC and LC data from high resolution MS are clearly demonstrated. Metabolites in the plasma from three phenotypes of Zucker rat were analyzed by a battery of complementary techniques which included GC and LC with high resolution (HRT) MS detection, GCxGC with TOFMS detection. LC-HRT identified several compounds and compound classes which changed in correlation with the phenotype. These included acyl carnitines and amino acids, among others. GC-HRT and GCxGC-TOFMS provided the identification of additional compounds from the same and related compound classes. These include short chain fatty acids, fatty acids, glycerol, amino acids and monosaccharides. Each of these showed a unidirectional change from Lean to Fatty to Obese. Each of these analytes also has a physiological relationship to fat and lipid processing. The high perfromace mass spectrometry provided typical mass accuracies of less than 1 ppm and resolving power of approximately 50,000 above 600 m/z. The combination of these complementary techniques provided a set of related but complementary metabolites and indicate the increased information content offered by this approach.

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OF MICE AND MEN – NMR-BASED METABONOMICS FROM ANIMAL SYSTEMS TO CLINICAL SCIENCE.

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We use NMR-based metabonomics to study systems from plants/animals to humans, with the aim to elucidate how disease, genetic and environmental factors influence metabolic profiles.

Mice: Growth hormone (GH) is the key factor regulating postnatal growth and an important regulator of metabolism. Excess or deficiency of GH result in abnormal growth and a number of metabolic changes. These changes can be monitored using NMR-based metabonomics. As a model system for obesity we have analysed the metabolic profiles of GH receptor (GHR) transgenic mice with truncations in the intracellular GHR domain at residues 569 and 391, respectively. These mice develop late-onset obesity as characteristic phenotype. NMR profiling identified several metabolites, including taurine, trimethylamine, and creatine/creatinine. These metabolites were correlated with genetic data obtained from microarray profiling and indicate significant changes in choline and lipid metabolism leading to the observed phenotype. They support a potentially important role for taurine in developing obesity.

Men: In preparation for clinical metabonomic studies we have extensively analysed the stability of human urine samples at room temperature. We show that the standard method of sample preservation with 0.06% NaN_3 is insufficient to prevent metabolite changes/bacterial growth over 24h. This can be a source of error in human clinical studies if subjects fail to store urine samples at 4°C before delivery to the clinic. We show that this problem can be solved by preserving urine samples in a minimum of 100 mM sodium borate upon collection. Afterwards, urine samples should be stored immediately at -20°C until analysed and NMR spectra be recorded within 24h of samples being thawed. Following this newly established standard procedure, samples will remain stable and an accurate metabolic fingerprint can be recorded, even if subjects do not comply with sample handling instructions.

These examples highlight the versatility of NMR-metabonomics in investigating complex biological problems.

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THIS WON'T HURT A BIT – NMR-BASED METABOLOMICS IN MEDICAL DIAGNOSIS OF PROSTATE CANCER

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Prostate cancer (PCa) is the most common internal male cancer, involving one in 9 Australian men. Early detection including a reliable prognosis of cancer aggressiveness is instrumental in facilitating early treatment. However, the current diagnosis of PCa, involving serum prostate-specific antigen (PSA) testing followed by trans-rectal ultrasound guided (TRUS) biopsy, are highly invasive and not very reliable. The combination of these two methods permits early detection but the PSA test lacks sensitivity and specificity with most patients undergoing invasive biopsies not having cancer detected. Thus, we are trying to develop a non-invasive test for prostate cancer detection that involves reliable, age-independent markers to identify the presence of PCa, indicate tumour aggressiveness and its metastatic risk.

We have used NMR-based metabolomics to investigate the metabolic profiles of post-ejaculate urine, collected from > 100 male patients attending the Urology Outpatients Department of the Royal Brisbane and Women's Hospital. The metabolic profiles were correlated via multivariate analysis with standard clinical data (serum PSA/biopsy) to determine metabolites correlated with cancer status and aggressiveness.

In addition, the metabolomic results were combined with those from the mRNA markers PCA3 and Hepsin from prostatic cells disaggregated into prostatic fluid and collected following ejaculation.

The combination of these genetic markers with metabolomic data was able to discriminate cancer from non-cancer samples, which correlated with an axis of cancer risk and status. Investigation of this clustering revealed currently unknown metabolites of interest. Further investigation of these promising results may identify potential foundations of a non-invasive diagnostic test for the early detection of PCa.

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ALKYL CHLOROFORMATE-MEDIATED METABOLITE LABELING AND ITS APPLICATION IN GC/MS AND LC/MS METABOLOMICS

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Alkyl chloroformates (RCFs) are highly reactive reagents that smoothly convert small molecule metabolites to much less polar species which are simultaneously extractable into immiscible organic solvents. The coupled RCF - mediated derivatization - extraction process has been an efficient sample preparation strategy for rapid analysis of polar metabolites by GC/MS and LC/MS techniques in complex aqueous biological matrices. The RCF - based derivatization reactions are briefly reviewed and demonstrated to be highly useful not only for the sample preparation but also are a versatile metabolite labeling strategy in both targeted and nontargeted GC/MS and LC/MS - based metabolomic applications.

The potential of the metabolite labeling with RCFs is illustrated on:

(i) Simultaneous nonchiral and chiral GC/MS profiling of acidic metabolites in human plasma (heptafluorobutyl RCF labeling) [1, 2];

(ii) Targeted profiling of 16 xenometabolites (of aromates, alkoxyalcohols, monomers and common organic solvents absorbed, metabolized and excreted mostly as carboxylates) together with the nontargeted profiling of native acidic metabolites in human urine (ethyl, d3-ethyl RCF labeling) [3];

(iii) Parallel LC/MS and GC/MS metabolite profiling in larvae extracts of the drosophilid fly, Chymomyza costata, (comprising ethyl, d3-ethyl RCF labeling) resulting in the targeted profiling of 61 metabolites and the discovery of the essential role of proline in the capability of the larvae to survive submergence in liquid nitrogen in a fully hydrated stage [4].

The research grant support: CSF 203/09/2014, CSF P206/10/2401, Ministry of Health of the C.R. NT11513-5/2010.

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IDENTIFICATION OF BIOMARKERS ASSOCIATED WITH THE ONSET AND PROGRESSION OF MAJOR METABOLIC AND INFECTIOUS DISEASES OF TRANSITION DAIRY COWS.

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Dairy cows are commonly affected by a variety of metabolic and infectious diseases during the transition period (3 wks before and 3 wks after calving). Four conditions in particular (mastistis, metritis, laminitis, and infertility) accounted for almost 60% of the 160,000 cows culled in Canada in 2008. This early culling costs the Canadian dairy industry more than $200 million per year. If these diseases or conditions could be detected early enough, effective and inexpensive treatments could be started and many dairy cattle could be saved. Currently there are no simple tests to predict or diagnose these conditions at their earliest stages. The overall goal of this investigation was to identify molecular biomarkers that are associated with the early prediction, diagnosis, and monitoring of major metabolic and infectious diseases of dairy cattle. Here, we used a multi-pronged quantitative metabolomics approach incorporating direct injection (DI)-MS/MS, NMR, and GC-MS to detect potentially predictive or diagnostic metabolites in dairy cows. We conducted a preliminary trial looking at plasma samples from 12 transition dairy cows that were sampled -4 and -1 wks before and +1 and +4 wks after calving. Six cows were free of diseases during the entire experimental period, whereas six other cows developed one or more conditions including mastitis, metritis, laminitis, and/or retained placenta 1-4 wks after calving. Using NMR and GC-MS approaches, 40-50 distinct plasma metabolites were measured. DI-MS/MS quantified another 120 metabolites. Statistical analysis of the combined data showed that a number of chemical biomarkers were able to clearly distinguish diseased cows from healthy ones and that these distinctions were detectable more than 4 wks before clinical symptoms appeared. Several phosphatidylcholines and acylcarnitines appeared to be the most important in separating the groups. More work is planned to monitor a larger number of dairy cows for an extended period of time (8 wks before and after calving).

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THE BOVINE MILK METABOLOME.

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With the recent determination of the bovine genome and given the close similarity (80% sequence identity) between the cattle genome and the human genome, we have undertaken the task of characterizing the bovine metabolome. As part of our effort to systematically characterize the bovine metabolome, we recently completed a comprehensive metabolomic characterization of bovine rumen. We are now focusing on characterizing the chemical composition of bovine milk. Characterization of the milk metabolome is important because: 1) milk composition is related to the growth of dairy cattle offspring and the health of human consumers; 2) milk composition is related to the structure, color, and odor of dairy products; 3) it can potentially identify candidate metabolites that promote health or cause allergies in humans; and 4) milk fat composition is important for the prevention of lipid related diseases. NMR spectroscopy, GC-MS, HPLC-UV and DI-MS/MS were combined with computer-aided literature mining tools to identify and quantify metabolites that can be commonly detected (with today's technology) in bovine milk. Samples of organic and conventional raw milk were collected from 3 different organic dairy farms around Edmonton, Alberta, Canada and at the Dairy Research and Training Centre at University of Alberta. Commercially available pasteurized organic and conventional milk for human consumption was purchased from a local retail store in Edmonton. The use of multiple metabolomics platforms allowed us to substantially enhance the level of metabolite coverage (up to 1,000 compounds) while critically assessing the relative strengths and weaknesses of these platforms or technologies. The results will be discussed in detail. The application of cutting-edge metabolomics technologies to characterize the dairy cow's milk metabolome should provide a far more complete perspective on milk's metabolite composition and also provide important insights into how health, breeds and diets of dairy cattle alter the nutritional content, utility and quality of milk.

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OVARIAN CANCER METABOLOMIC DYNAMICS USING GCXGC-MS

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The goal of this work is to characterize the metabolic dynamics (but not flux) of ovarian cancer cells, with the ultimate aim of identifying differences in metabolic behaviors between bulk tumor cells and the cancer-initiating cells whose chemoresistance is hypothesized to be a major cause of cancer recurrence.

The contribution of dysfunctional metabolism to the progression and phenotypes of cancer is increasingly being recognized, despite a decades-long stretch when genetics drove the vast majority of cancer research. While it is now well-known that cancers are usually characterized by an increase in glycolytic flux and fermentation, the extent of the metabolic changes induced in cancer cells and the impact of these changes on cancer's phenotypes and progression is still uncertain. For example, systems-level intracellular metabolic dynamics of ovarian cancer cells remain comparatively poorly understood, as many previous cancer metabolomics studies have focused on tumor or circulating biomarkers rather than the dynamic metabolic behaviors of cells. A deeper understanding of these metabolic behaviors could profoundly affect the way that cancer is understood, and potentially even how it is treated.

Here, we use two-dimensional gas chromatography coupled to mass spectrometry (GCxGC-MS) to measure intracellular and extracellular metabolites of ovarian cancer cell lines. We present some preliminary analyses confirming our ability to capture metabolic changes in response to simple environmental perturbations, including nutrient depletion, both on an individual metabolite level as well as through analysis of the entire dataset via dimensional reduction approaches. Future work will include comparative analysis of bulk tumor cells and cancer-initiating cells to understand the metabolic differences between these cell types, and to determine whether metabolism may serve as an appropriate therapeutic target for either class of cells.

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METABOLIC FOOTPRINT OF *LACTOBACILLUS ACIDOPHILUS* NCFM AT DIFFERENT PH

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The human organism lives in a symbiotic relationship with a very complex bacterial ecosystem. Around 10^{14} bacterial cells inhabit the gastrointestinal tract (GIT) (1), and are prone to communicate with each other and with the host in order to keep the balance of the vast ecosystem that they constitute. This type of bacterial community exhibits several levels of interaction, e.g. through protein production, enzymatic activity, DNA and RNA exchange as well through excretion of small molecules, metabolites (2), on which this work was focused.

The aim of this study was to analyze the potential role of extracellular metabolite production by microorganisms in the gut. The homofermentative *Lactobacillus acidophilus* NCFM has been chosen as an example of a well-described probiotic microorganism (3). In order to analyze the potential interactions of NCFM with the surrounding environment, *in vitro* tests with the metabolic footprinting approach were performed. It was found that NCFM increased the concentration of lactic acid, succinic acid, adenine and arginine in the medium. Assuming similar activity *in vivo*, NCFM shows metabolic possibilities of influencing the GIT, but this still needs to be addressed in the *in vivo* tests. The metabolism of NCFM did not change significantly between pH 5 and 7, suggesting that other environmental factors than pH might have bigger impact on its colonization throughout the gastrointestinal tract (4).

The metabolomic approach has opened new possibilities to address how NCFM interacts in the GIT ecosystem through external metabolite production.

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(2) Ben et al. (2004) Bacterial linguistic communication and social intelligence. Trends Microbiol 12: 366-372.

(3) Altermann et al. (2005) Complete genome sequence of the probiotic lactic acid bacterium Lactobacillus acidophilus NCFM. Proc Natl Acad Sci U S A 102: 3906-3912.

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METABOLIC FOOTPRINT OF *LACTOBACILLUS ACIDOPHILUS* NCFM AT DIFFERENT PH

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The human organism lives in a symbiotic relationship with a very complex bacterial ecosystem. Around 10^{14} bacterial cells inhabit the gastrointestinal tract (GIT) (1), and are prone to communicate with each other and with the host in order to keep the balance of the vast ecosystem that they constitute. This type of bacterial community exhibits several levels of interaction, e.g. through protein production, enzymatic activity, DNA and RNA exchange as well through excretion of small molecules, metabolites (2), on which this work was focused.

The aim of this study was to analyze the potential role of extracellular metabolite production by microorganisms in the gut. The homofermentative *Lactobacillus acidophilus* NCFM has been chosen as an example of a well-described probiotic microorganism (3). In order to analyze the potential interactions of NCFM with the surrounding environment, *in vitro* tests with the metabolic footprinting approach were performed. It was found that NCFM increased the concentration of lactic acid, succinic acid, adenine and arginine in the medium. Assuming similar activity *in vivo*, NCFM shows metabolic possibilities of influencing the GIT, but this still needs to be addressed in the *in vivo* tests. The metabolism of NCFM did not change significantly between pH 5 and 7, suggesting that other environmental factors than pH might have bigger impact on its colonization throughout the gastrointestinal tract (4).

The metabolomic approach has opened new possibilities to address how NCFM interacts in the GIT ecosystem through external metabolite production.

(1) Lebeer et al., 2008

(2) Ben et al., 2004

(3) Altermann et al., 2005

(4) Sulek et al., 2011

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INTEGRATED METABOLOMICS AND TRANSCRIPTOMICS REVEAL ENHANCED SECONDARY METABOLISM IN *MEDICAGO TRUNCATULA* ROOT BORDER CELLS

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Roots of many plants produce unique and often overlooked border cells with numerous specialized functions. Border cells develop from root cap initials and separate from the root during development, but remain appressed to it until contacting water. Detailed metabolic and transcriptional characterizations of *Medicago truncatula* seedling border cells and root tips revealed substantial constitutive differences between these distinct cell types. Large changes in jasmonate biosynthesis and auxin-responsive transcript levels underscored the importance of hormones in border cell development. Significant starch deposits served as a critical energy source and carbon reserve, documented through increased β-amylase transcript levels and associated starch hydrolysis. Many primary metabolism transcripts were decreased in border cells, but increases were observed in branched chain amino acids and other compounds associated with elevated CoA biosynthesis and carbon shuttling into secondary metabolic pathways. Flavonoid and triterpenoid-related metabolite and transcript levels were dramatically increased in border cells. The data provide compounding evidence that primary and secondary metabolism are differentially programmed in border cells relative to whole roots and root tips. Metabolic resources normally destined for growth and development are redirected towards accumulation of secondary metabolites in border cells. Border cells contain elevated defense related and signaling compounds needed to protect the delicate root cap and recruit motile rhizobia important for symbiotic nitrogen fixation to the roots. The cumulative and pathway specific data provide key insights into the metabolic programming of border cells that strongly implicate a more prominent mechanistic role in plant-microbe signaling and interactions than previously envisioned.

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LC/MS/MS INVESTIGATION OF OIL PALM (ELAEIS GUINEENSIS) LEAF METABOLOME

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Malaysia is one of the major producers of palm oil in the world and contributing around half of the world's palm oil production. In addition to its oil seed, other organs such as leaf is of use. Metabolomic studies are being undertaken to improve knowledge on oil palm leaf chemicals to allow efficient utilization of plant resources such as for livestock feed, biomass, fertilizer and pharmaceuticals. Information on chemical composition of oil palm leaf is a platform of discovery of phytochemicals of high value. It is also useful towards understanding metabolic pathways and the interactions of plants towards exogenous and endogenous factors. We describe the methods used to profile and characterize the phytochemicals of oil palm leaf using liquid chromatography/electrospray-mass spectrometry (LC/ESI-MS) and tandem MS/MS. The results are part of substancial equivalence study for provision of comparative evaluation of selected traits and clonal cultivars.

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DETECTION OF URINE, BLOOD, AND BRAIN BIOMARKERS FOR ALZHEIMER'S DISEASE BY CAPILLARY ELECTROPHORESIS/MASS SPECTROMETRY (CE-MS)-BASED METABOLOMIC ANALYSIS

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Alzheimer's disease (AD) is a progressive neurodegenerative disease, and represents the most prevalent cause of dementia in humans. The characteristic pathology of the AD brain is the deposition of amyloid plaques. Specific biomarkers to non-invasively diagnose the early stage of Alzheimer's disease (AD) and to monitor the progression of AD are essential to prevent and treat the disease. To find such biomarkers, we analyzed a wide range of ionic metabolites in urine, plasma, and brain tissue of a mouse model of AD (Tg2576 mice) and control mice by CE-MS. The AD mice overexpress the Swedish mutant of human amyloid precursor protein in their brains, thus leading to an age-dependent increase in amyloid plaque. The AD mice are known to exhibit defects in memory/learning even at a younger age (4 months) before the formation of amyloid plaques. Metabolomic profiling revealed that 4 urinary and 7 plasma metabolites were significantly increased in the AD mice at the age of 4 months, corresponding to the early stage of human AD. The analysis also showed an elevation of 2 plasma metabolites in the AD mice at the ages of 10 and 20 months, corresponding to the progressive stage of human AD. The analysis of brain tissues of AD mice indicated that a significant decrease in the brain ATP levels occurred at the age of 4 months, but that it recovered to the same level as the controls at 7 months, and that during the progressive stage (10 and 20 months), the ATP levels actually exceeded those of the controls. In conclusion, our metabolomic analysis provided possible novel urine and plasma biomarkers for the early diagnosis and monitoring of the progression of AD. We also demonstrated that a non-invasive measurement of brain ATP by fMRI could be relevant for the diagnosis of AD.

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GLYCEROPHOSPHOCHOLINES PROFILING IN THE BIOLOGICAL TISSUE USING UPLC/MS/MS

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The usefulness of ultra performance liquid chromatography coupled with a triple quadrupole mass spectrometer was demonstrated for glycerophosphocholines (GPCs) profiling of biological tissue. A strategy consisting of a two-phase analytical procedure was used to obtain a detailed GPCs molecular composition. In the first phase, the precursor ion scan was conducted to obtain the preliminary lipid profile that revealed the composition of the molecular species possessing phosphocholine structure in the sample. In the second phase, each product ion spectrum obtained for the GPC components in such profile was sequentially acquired for the molecular structure determination. A simple guide with high differentiability was proposed for the diacyl-, alkyl-acyl- and alk-1-enyl-acyl- GPCs, and related lyso-GPCs molecular structure decision. Also, the optimized chromatographic condition was proposed to handle serious GPCs peak broadening and tailing, and obtain satisfactory separation efficiency when using an octadecylsilica column. The performance analysis of this method was described and the practical usability was validated using a study of chemically induced early lung maturation in the premature mouse model. Total 93 GPCs molecular species were identified in the fetal mouse lung with the relative amounts from 14.39% to less than 0.01%. The metabolic difference between treated and control group fetal mouse lung was clearly distinguished by the composition of GPCs with several characteristics of molecular structure. The overall results showed that the analytical procedure proposed here is reliable for comprehensive GPCs profiling and should be useful for lipidomic research.

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STRATEGY FOR CHOOSING EXTRACTION PROCEDURES FOR NMR-BASED METABOLOMIC ANALYSIS FOR MAMMALIAN CELL

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Metabolomic analysis of mammalian cells can be applied across multiple field including medecine and toxicology. It requires the acquisition of reproducible, robust, reliable, and homogenous biological data sets. Therefore, we have developed a strategy for choosing extraction procedures from adherent mammalian cells for global analysis of the metabolome. After quenching of cells, intracellular metabolites are extracted from cells using one of the following solvent systems of varying polarities: perchloric acid, acetonitrile/water, methanol, methanol/water and methanol/chloroform/water. The hydrophobic metabolite profiles are analysed using 1H nuclear magnetic resonance (NMR) spectroscopy. We observe that extraction using perchloric acid produces the greatest variation between replicates due to peak shifts in the spectra, while other extraction methods lead to higher reproducibility. By an original geometric representation of metabolites, reflecting the efficiency of extraction methods, we demonstrate that higher portion of intracellular metabolites are extracted by using methanol or methanol/chloroform/water. The preferred method is evaluated in terms of biological variability for studying of metabolic changes caused by the phenotype of four different human breast cancer cell lines, showing that the selected extraction procedure is a promising tool to metabolomic and metabonomic studies of mammalian cells.

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VIRAL-VECTOR MEDIATED GENE THERAPY FOR CANAVAN DISEASE RESTORES NORMAL BRAIN METABOLISM IN A NOVEL MOUSE MODEL

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Canavan Disease (CD) is a recessive leukodystrophy generally characterized by gross white matter degeneration of the brain, macrocephaly, psychomotor retardation, seizures and premature death (Matalon et al., 1995). This inborn error of metabolism is ideally suited for a gene replacement therapy since CD pathology is limited to the brain and the genetic defect is a null mutation. The loss-of-function of the gene encoding the degrading enzyme ASPA results in pathological N-aspartyl acetate (NAA) enrichment in the brain and urine of CD patients. Seizures, disturbed neural osmoregulation and glia-neuronal signaling have been attributed to elevated NAA levels in the CD brain (Baslow, 2000). Loss of ASPA activity results in decreased levels of lipids and acetate in this fatal leukodystrophy and its mouse model (Madhavarao et al., 2005), however other metabolic changes have not yet been described or quantified. The aim of this study is to assess the full metabolic effects of ASPA knock-out (KO) in a novel CD mouse model (Mersmann, 2011) and to determine the effectiveness of viral vector mediated gene therapy in restoring normal brain metabolism in this model of CD. Metabolic phenotypes from wild type, APSA KO mice and the treated ASPA KO mice were determined using HR NMR of brain extracts and biofluids. Alterations in lipid and choline metabolism where among the changes detected by 1H and 13C NMR. Successful delivery of functional ASPA gene into the APSA KO mouse brain resulted in restoration of normal metabolism characterized by reduction of NAA and myo-inositol levels in the brain to that observed in wild type mice. This is the first study to demonstrate the effectiveness of targeted viral vector mediated gene delivery in rescuing CD brain metabolism. This work was supported by a European Leukodystrophy Association project grant and a NSW OSMR Life Science Research Award to M.K.

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IN-DEPTH METABOLIC PROFILING ANALYSIS OF ISOGENIC ENDOPHYTE-PERENNIAL RYEGRASS HOST ASSOCIATIONS

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Perennial ryegrass (*Lolium perenne* L.), the most important temperate forage grass on a global basis, can form a mutualistic association with the endophytic fungus *Neotyphodium lolii*. The presence of this fungus can protect the host plant from a variety of environmental stresses. Both beneficial and detrimental agronomic properties result from the association. Invertebrate resistance is provided by specific metabolites produced by the endophyte, in particular peramine. Other metabolites produced by the endophyte, such as lolitrems and ergot alkaloids, are toxic to grazing animals and reduce herbivore feeding. Novel endophyte strains that lack either or both of the toxins detrimental to animal welfare have been identified. An isogenic inoculation process was used to study host genotype–endophyte associations between novel endophytes and elite pasture grass germplasm. Five endophytes including standard toxic and four novel strains were successfully inoculated into five host genotypes representing a wide range of perennial ryegrass cultivars. Metabolic profiling of each of the associations was assessed using Liquid Chromatography-Mass Spectrometry (LC-MS). The results revealed significant biochemical differences between the symbionts. Lolitrem B, ergovaline, peramine and janthitrem were analysed semi-quantitatively based on LC-MS data. The results showed that alkaloids production varies between different endophytes inoculated into the same isogenic host and between different host plants harbouring the same endophyte.

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EFFICACY OF GREEN LIPPED MUSSEL EXTRACT FOR OSTEOARTHRITIS OF THE KNEE

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Osteoarthritis [OA], one of the most common forms of arthritis, affects approximately 7% of Australians and is associated with increasing age, female gender, trauma or overuse of the joint, obesity and genetics. OA can occur in any joint, but is most common in the knee, resulting in joint pain, reduced mobility and ultimately a reduced quality of life. Complementary and alternative medicines have become a popular treatment option for OA patients with the aim to limit the side effects that result from first-line pharmacological treatments, i.e. anti-inflammatory drugs and paracetamol . Green-Lipped Mussel (*Perna canaliculus*; GLM) products are marketed for the treatment of arthritic symptoms and numerous *in vitro* and animal studies have suggested an immunomodulatory role of GLM in regulating inflammation, with stabilised mussel powder extracts showing much greater anti-inflammatory effects than unstabilised extracts. The aim of this open-label, single group allocation trial was to investigate the therapeutic efficacy of a commercially available GLM extract in improving pain and joint mobility. Outcomes were evaluated through participant questionnaires, but also by metabonomics, since metabolic markers and marker ratios for OA have recently been identified.

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A NOVEL PROCOAGULANT THAT QUICKLY CLOTS THE MOST CHALLENGING SAMPLES

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Serum is often considered the 'cleanest' and therefore most suitable sample both for metabolomics investigations and for chemical pathology tests, due to the considerably lower levels of protein as compared to plasma. Conventionally, blood samples have to be left at room temperature for up to 30 minutes for clotting to be completed, even when collection tubes with clot activator are used, which can lead to changes in metabolite levels typically determined by metabolomics. Furthermore, blood samples of patients on anticoagulant therapy (warfarin, heparin) will often not clot at all in currently available serum tubes. For pathology applications, latent clotting and associated system downtimes when autoanalyzers are blocked by microclots pose a considerable problem.

We present here a novel procogulant that clots a large range of blood samples quickly and reliably and does not cause interference with metabolomics techniques and chemical pathology tests. Even fully heparinised blood samples (as would be found in patients prepared for bypass surgery) were clotted in less than 15 minutes, while in commercial serum tubes, they were not fully clotted even after hours. This extreme example indicates that this novel procoagulant allows rapid preparation of clean serum samples in virtually all situations.

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QUANTITATIVE FUNGAL METABOLOMICS USING GC/MS/MS - QUANTIFICATION OF THE MAJOR SOURCES OF VARIANCE FROM GC/MS STUDIES

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Stagonospora nodorum is a serious fungal pathogen of wheat and results in annual production losses of $108 million in Australia.

Comparative Metabolomics studies have been undertaken between the wild type *Stagonospora nodorum* and mutant strains that are defective in cyclic AMP signalling, exhibit abnormal development and reduction in pathogenicity, using single quadrupole GC/MS with a split/splitless inlet [1]. These comparative studies have identified metabolites that were differentially abundant between the wild type and mutants and studies are now in progress to correlate these differences with the compromised phenotype of the mutant.

We have developed a targeted tandem mass spectrometry method using triple quadrupole GC/MS to quantify the abundances of the fungal specific metabolites for the wild type and the mutants. The triple quadrupole instrument has been modified to include a PTV injection system and has collected both MS/MS and full scan data to correlate with the existing single quadrupole GC/MS results. In this initial methodology MRMs for almost 50 metabolites have been added to the method. .

The initial quantitative studies were undertaken on *in vitro* cultures of the strains and the next step to assess the biological significance of these changes is to determine the abundance of these metabolites during infection of the plant.

(1) Tan, K.-C., Trengove, R.D., Maker, G.L., Oliver, R.P., Solomon, P.S. 2009 Metabolomics 5 (3), pp. 330-335 Metabolite profiling identifies the mycotoxin alternariol in the pathogen Stagonospora nodorum

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GC-MS METHOD FOR ABSOLUTE QUANTIFICATION OF POLAR METABOLITES IN BIOLOGICAL SAMPLES USING DEUTERATED METHYL CHLOROFORMATE AS DERIVATIZATION AGENT

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Metabolite profiling methods are the most common analytical approach in metabolomics. However, most metabolite profiling methods require internal standardisation to correct technical variability imposed mainly by variations in matrix composition. Moreover, for absolute quantification of metabolites it is necessary to build up calibration curves for metabolites to be quantified. Recently, Kvitvang et al. [1] described a novel approach for quantitative analysis of amino and non-amino organic acids using isotopicaly labelled methyl chloroformate coupled to GC-MS/MS analysis. Since GC-Quadrupole-MS is a more widely available and affordable mass spectrometer, we report here a modified protocol for chemical synthesis of the deuterated-labelled methyl chloroformate (D-MCF), which present higher yields followed by global quantification of polar metabolites (amino acids, nonamino organic acids and amines) using a GC-Quadrupole-MS. As a result this method is a significant improvement of modern protocols for accurate quantitation of amino and non-amino organic acids and amines.

(1) Kvitvang H-F-N, Andreassen T, Adam T, Villas-Boas S-G, Bruheim P (2011) Highly sensitive GC/MS/MS method for quantitation of amino and nonamino organic acids. Anal. Chem. 2011, 83, 2705–2711

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NETHERLANDS METABOLOMICS CENTRE - DATA SUPPORT PLATFORM

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

This project addresses two major bottlenecks for metabolomics research. The first is sharing of metabolomics studies and data. The second addresses the accessibility of dedicated processing and biostatistics tools. Sharing and storing of metabolomics studies and data is provided for by a web-based, open source, study capture and experimental data storage framework, where study information can be stored together with metabolomics mass spectrometric or NMR data. See http://trac.nbic.nl/gscf. The processing of data will be supported by an online computing environment where sets of preprocessing, biostatistics and quality control tools are made accessible for the international community of biologists and biostatisticians working in metabolomics.

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THE EFFECT OF INSECT-VIRUS INTERACTIONS ON THE WHEAT METABOLOME UNDER INCREASING ATMOSPHERIC CARBON DIOXIDE.

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Rising CO_2 associated with climate change may affect the interactions between plants, pathogens and their vectors. Although plant response to elevated CO_2 (eCO_2) has been well studied, little is understood about the interactions between plants, insects and pathogens under simulated future climates. Subsequently, there is a lack of knowledge on how the plant metabolome will respond to future atmospheric changes.

The effect of eCO_2 on wheat production in Australia is being investigated at the Australian Grains Free-Air CO_2 Enrichment (AG FACE) research facility in Horsham, Victoria. Additionally, controlled environment growth chambers are being used to study the effect of eCO_2 on the feeding behaviour of the aphid *Rhopalosiphum padi* and its ability to acquire and transmit Barley yellow dwarf virus (BYDV). A significant decrease in the fecundity of *R. padi* as well as changes to its feeding patterns have been observed when the aphids are reared on wheat grown under eCO_2, potentially indicating changes in the nutritional quality of the wheat or the production of molecules that are inhibitory to aphid reproduction.

Understanding the link between wheat chemistry and aphid infestation under future climate scenarios will ensure better preparedness for pest and diseases occurrences. Further to this, chemical changes identified to have a negative association with the insect's fecundity and development may be exploited in future breeding lines. An initial metabolomics study has investigated the effect of virus infection on the wheat plant metabolome under both eCO_2 (550ppm) and ambient atmospheric levels 385ppm). LCMS profiling will be detailed as will statistical analysis highlighting metabolites changing to infected and uninfected wheat, under elevated and ambient CO_2.

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THE BIOLOGICAL INTERPRETATION OF METABOLOMIC DATA CAN BE MISLED BY THE EXTRACTION METHOD USED

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The field of metabolomics is getting more and more popular and a wide range of different sample preparation procedures are in use by different laboratories. Chemical extraction methods using one or more organic solvents as the extraction agent are the most commonly used approach to extract intracellular metabolites and generate metabolite profiles. Metabolite profiles are the scaffold supporting the biological interpretation in metabolomics. Therefore, we aimed to address the following fundamental question: can we obtain similar metabolomic results and, consequently, reach the same biological interpretation by using different protocols for extraction of intracellular metabolites? We have used four different methods for extraction of intracellular metabolites using four different microbial cell types (Gram negative bacterium, Gram positive bacterium, yeast, and a filamentous fungus). All the samples were identical. After extraction and GC-MS analysis of metabolites, we did not only detect different numbers of compounds depending on the extraction method used and regardless of the cell type tested, but we also obtained distinct metabolite levels for the compounds commonly detected by all methods (p-value < 0.001). These differences between methods resulted in contradictory biological interpretation regarding the activity of different metabolic pathways. Therefore, our results show that different solvent-based extraction methods can yield significantly different metabolite profiles, which impact substantially in the biological interpretation of metabolomics data. Thus, development of alternative extraction protocols and, most importantly, standardization of sample preparation methods for metabolomics should be seriously pursued by the scientific community.

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METABOLIC PROFILING OF B-CRYPTOXANTHIN AND ITS FATTY ACID ESTERS BY SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED WITH TRIPLE QUADRUPOLE MASS SPECTROMETRY

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A high-throughput and high-sensitive profiling system for β-cryptoxanthin (βCX) and β-cryptoxanthin fatty acid ester (βCXFA) was constructed by supercritical fluid chromatography (SFC) coupled with triple quadrupole mass spectrometry (QqQMS). βCX and nine βCXFAs, such as laurate (C12:0) ester, myristate (C14:0) ester, palmitate (C16:0) ester, stearate (C18:0) ester, oleate (C18:1) ester, linoleate (C18:2) ester, linolenate (C18:3) ester, eicosapentaenoic acid (C20:5) ester and docosahexaenoic acid (C22:6) ester, were separated successfully within 20 min using a column packed with octadecylsilyl-bonded silica particles. The limit of detection was 540 fmol for the free form and 32-130 fmol for the esterified forms. These results demonstrated that both throughput and sensitivity of this SFC-QqQMS system were significantly higher than those of conventional methods. When this system was applied to the analysis of *Citrus unshiu*, βCX and five βCXFAs were directly detected with simple pre-preparation of sample. Comparison among various citrus fruits revealed that the βCXFA profiles varied with their breed varieties. We also tried to quantify other fatty acid derivatives, of which standards are not available, by estimating the analytical parameters theoretically. As a result, it was suggested that citrus fruits contained βCX butyrate (C4:0) ester, caproate (C6:0) ester, caprylate (C8:0) ester, and caprate (C10:0) ester. Furthermore, gas chromatography/mass spectrometry was used to analyze total fatty acid profiles in *Citrus unshiu* in order to investigate the properties of the fatty acid used for βCX esterification. The results revealed that the profiles of fatty acids located in βCXFA were distinct. This is the first report on the analysis of βCX and its fatty acid derivatives by SFC-QqQMS. This profiling system will be a powerful tool for investigating xanthophyll fatty acid esters.

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A FAST ROBUST TOTAL ABUNDANCE REGRESSION CALIBRATION TO ADJUST SEVER BATCH EFFECT

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The liquid chromatography-mass spectrometry is widely used in metabolomics studies to identify and quantify metabolites in complex biofuilds. However, systematic biases due to instrumental calibration, imperfect sample preparations, and sample run orders can cause batch effects and often requires proper calibrations prior to quantitative analysis. In this work, we compared and applied current calibration methods derived from microarray analysis, proteomics, and metabolomics studies including centering, scaling, quantile-based, ratio-based, linear normalizer and linear regression algorithms on a metabolomics study containing 231 plasma and 25 pooled quality control samples. We also developed a fast robust total abundance regression calibration (RTARC) algorithm to adjust sever batch effect. We evaluated the performance by examining the distribution of relative standard deviation for all peaks detected in the pooled quality control samples and the distribution of quality control samples in the principal component analysis. We demonstrated that RTARC algorithm can produce better liquid chromatography/mass spectroscopy data calibration among all the method compared. Quality control samples can be calibrated in the range of 15% relative standard deviation and can be readily clustered in the principal component analysis scores plot.

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THE METABOLIC FOOTPRINT OF PLANTS & PLANT-SOIL INTERACTIONS

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Planet Earth bears the metabolic footprint of plants. This is because plants use the Earth as a substrate for chemical reactions (e.g. photosynthesis, N fixation) and as a repository for waste products (e.g. oxygen, organic carbon). In contrast to the well-known metabolic effects of plants on the atmosphere, the below-ground effects are poorly known. This is surprising given that the belowground action of plants controls interactions with other organisms (parasitism, mutualism, allelopathy), may help plants capture "unavailable" nutrients and avoid toxicities, and has a huge effect on soil respiration and ecosystem CO_2 exchange.

One of the ways plants interact with soil and soil organisms is by exudation of organic compounds from living roots ("rhizodeposition"). Knowledge of the chemical profile of rhizodeposits is in its infancy, and research in this area has been dominated by idiosyncratic analytical methods focussing on particular metabolite classes. In this study I am using "unbiased" GC-MS methods to profile the metabolites released from living plant roots. GC-MS chromatograms routinely contain around 300 peaks, of which 150 have been positively identified.

The movement of organic compounds across the root surface is in fact bi-directional. However, studies to date provide little information on net fluxes because studies have generally examined either uptake or efflux, rather than both fluxes. To quantify both fluxes simultaneously is challenging, but we have had considerable success in pilot studies focussing on amino acids. To determine uptake and efflux, roots were placed in a hydroponic solution containing uniformly ^{13}C, ^{15}N labelled amino acids. Uptake was determined by the appearance of isotope labelled amino acid in roots. Efflux was estimated from the appearance of ^{12}C,^{14}N amino acids in the hydroponic solution. On-going studies with isotope labelled plants and plant-derived metabolites are examining uptake and efflux of a broad suite of metabolites.

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COMBINED GENOMIC-METABOLOMIC APPROACH FOR THE DIFFERENTIATION OF GEOGRAPHICAL ORIGINS OF NATURAL PRODUCTS: DEER ANTLERS AS AN EXAMPLE

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Abstract

The correct identification of the geographical origin of deer antlers is essential to quality control, as its positive physiological effects correlate with chemical components. In this study, we applied both genomics and metabolomics to the origin-identification of 101 samples from Canada, New Zealand, and Korea. The genomics identified deer species in each country but failed to categorize all the samples, due to the presence of identical species in different countries. For identical species, NMR-based metabolomics gave clean separations, compounds specific to each country were identified, and the validity was confirmed by prediction analysis. As the genomics provided unambiguous read-outs for different species, and the metabolomics cleanly distinguished among identical species from different countries, their combined use could be a robust method for origin-identification even in difficult cases. We believe the method to be generally applicable to many herbal medicinal products for which various species are grown internationally

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THE METABOLIC RESPONSES TO INFECTION BY THE GASTROINTESTINAL PARASITE, *HAEMONCHUS CONTORTUS*, IN GENETICALLY RESISTANT AND SUSCEPTIBLE SHEEP: A STUDY BY GC-MS METABOLOMICS.

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Morbidity and mortality of sheep from infection with gastrointestinal parasites continues to be a burden on animal health and farm productivity. Vaccine development and genetic approaches have met limited success. Nutritional interventions have also been attempted, but in the absence of knowledge of the metabolic changes induced by gut worm infections.

H. contortus, one of the major gastrointestinal parasites of sheep and goats globally, is a blood sucking nematode which resides in the abomasum. We have investigated the metabolic responses to low level infection by this parasitic worm in two lines of sheep. The lines of sheep have been selected for Resistance or Susceptibility to infection by *H. contortus* over 20 generations. Seven sheep from each line were infected with 5,000 larvae for 6 weeks. As controls, seven age and weight matched sheep from each line were housed and fed under the same conditions as the infected groups. Sera were collected at days 0, 4, 14, 28 and 42 days. An acetonitrile extract of each sera were analysed by GC-MS.

The study has revealed a clear metabolic signal of infection, and clear differences in the responses to infection between the Resistant and Susceptible lines of sheep. The infected animals from the Resistant line were most different to their controls on day 42 of infection, with depressed serum levels of glucose and some TCA cycle intermediates. Otherwise, these animals appeared remarkably resilient to infection. In contrast, infection induced marked changes in the Susceptible line on day 28. Increased levels of several metabolites including small hydroxyl acids, uneven short chained dicarboxylic acids and some TCA cycle intermediates were observed. These changes coincide with the sexual maturation of the parasite which from day 21 of infection are producing large numbers of eggs and voraciously feeding on host blood to supply energy and protein.

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DAIRY CONSUMPTION AND THE REDUCED RISK OF T2D

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Recent observational studies have shown that circulating levels of trans-palmitoleic acid, a fatty acid derived from dairy food sources, are inversely associated with the risk of incident diabetes and metabolic risk factors (1). These findings suggest the possibility that certain dairy foods (e.g. yogurt and milk) might lower the risk of insulin resistance and incident diabetes.

To determine diabetes risk, we have developed a computational classification model based on an individual's plasma lipid profile. We trained our model with the plasma lipid profiles taken from 287 subjects of the AusDIAB (2) cross-sectional study cohort (117 incident diabetes cases and 170 matched healthy controls). A logistic regression-based classifier was applied to define the boundaries of separation between diabetes and control groups and diabetes risk was computed as the probability of case (diabetes) group membership. We applied our classification model to a dairy meal randomised crossover study where 13 subjects were given 4 distinct dairy meals and one low fat meal at 3 day intervals. The subjects' plasma was extracted for analysis at baseline, 3hrs and 6hrs postprandial for each meal and the lipid concentration for various lipid sub-species was measured in each sample. Applying our model, we computed a diabetes risk score for each sample corresponding to its lipid concentration composition. The computed diabetes risk scores revealed a trend of decline in postprandial diabetes risk associated with meals containing butter, cream and yogurt. The change in postprandial diabetes risk was statistically significant ($p < 0.05$) between baseline and the 6hr mark for these meals while the risks scores associated with a low fat and cheese meal did not show any significant change over the postprandial period. Our preliminary results corroborate recent findings that suggest diets rich in certain dairy foods may be protective against incident diabetes and insulin resistance.

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MULTIVARIATE ANALYSIS OF FULL-RESOLUTION ^{1}H-NMR SPECTRA OF URINE FROM GROWTH HORMONE RECEPTOR MUTANT MICE

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To reduce the amount of data for ^{1}H NMR-based metabonomic studies as well as alleviate the effects of peak position variation, data reduction by integrating over small spectral regions ('buckets') is widely used. However, the resulting loss of resolution is likely to have an impact on sample classification and the identification of metabolic biomarkers in chemometric models. In this study, we investigated differences in urinary samples from wild-type mice and growth hormone receptor (GHR) mutant mice who had truncations in the intracellular domain of the GHR at position 569 or 391[1]. For this analysis both, integrated (0.05ppm, 0.01ppm) and full-resolution ^{1}H NMR data (~64k variables) were employed. The results indicated that higher spectral resolution improves separation between different groups. When comparing different chemometric models for group separation, orthogonally corrected partial least squares discriminant analysis (O-PLS-DA) with unit variance scaling exhibited the best performance. Based on these models, bivariate loadings plots, which combine back-scaled O-PLS coefficients and variable weights were generated [2]. These plots provided more precise information about variables responsible for data clustering and the possibility of intra-molecular statistical correlations between different resonances. They are a useful tool in identifying the ^{1}H NMR resonances corresponding to the most influential metabolites without the need to re-consult the initial spectra. Combining this information with Statistical Total-Correlation Spectroscopy (STOCSY)[3], several significant metabolites contributing to classification were confirmed. Our analysis of full resolution spectra has been shown both to recover the previously deduced major metabolic effects of GHR mutation and to generate new hypotheses even on this well-studied model system.

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METABOLITE PROFILING OF SOY SAUCE USING GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRY AND CORRELATION ANALYSIS WITH QUANTITATIVE DESCRIPTIVE ANALYSIS

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Background and objective • z Different ingredients and brewing processes of soy sauces result in variations in components and quality. Therefore, it is extremely important to comprehend the metabolite profiles of components and their relationship to the sensory attributes of soy sauces. The current study sought to perform metabolite profiling in order to devise a method of assessing the tastes of soy sauces. • yMethod and Result • z Twenty four soy sauce samples were used in this study. Quantitative descriptive analysis (QDA) scores for soy sauce samples were obtained from skilled sensory panelists. Metabolite profiles primarily concerning low-molecular-weight hydrophilic compounds were based on GC/TOFMS. QDA scores for soy sauces were accurately predicted by projection to latent structure (PLS), with metabolite profiles serving as explanatory variables and QDA scores serving as a response variable. Moreover, correlation analysis of both matrixes of metabolite profiles and QDA scores indicated important components that were highly correlated with QDA scores. Especially, it is indicated that sugars are important components of the basic tastes and aftertastes of soy sauces. This study has indicated that GC/TOFMS based metabolite profiles primarily concerning low-molecular-weight hydrophilic compounds can evaluate the tastes of soy sauces. This new approach, which combines metabolite profiles and QDA, is applicable to analysis of sensory attributes of food as a result of the complex interaction between its components. This approach is effective to search important components that contribute to tastes.

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METABOLIC PROFILING FOR THE CONTENTS OF MURINE INTESTINAL COMPARTMENTS

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Intestinal microflora plays a key role in nutrition, metabolism, and immune responses of the host. Especially, microbial composition among different intestinal compartments influences on host • fs intestinal biochemistry. Unraveling the relationship between host and microorganisms, we utilized capillary electrophoresis-mass spectrometry (CE-MS) for metabolomics profiling of murine intestinal compartments (stomach, upper small intestine, lower small intestine, cecum, proximal colon and distal colon) in two specific pathogen-free (SPF) mice and CIEA flora mouse colonized with a few gut bacterial genera. We identified site-specific metabolites variance of each intestinal compartment; e.g. in lower small intestine, N-Acetyl-beta-alanine, component of the beta-alanine metabolism, was significantly higher than those in other parts of intestines in AC stock mouse (p=4.15 • ~10^{-9}, Kruskal-Wallis test). Cholate (p=3.88 • ~10^{-4}) and taurocholate (p=5.46 • ~10-^{6}) of small intestine were significantly higher than those of other parts in intestinal contents. Interestingly, pyridoxine, a kind of vitamin B6 produced from Bacteroides in SPF intestine. These site-specific features in metabolic profiles might reflect the biological systems of the host and intestinal microflora.

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METABONOMIC STUDY ON THE PROGRESSION OF HEPATITIS TO HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy and reported as the third most common cause of cancer-related deaths worldwide. It has been reported that chronic hepatitis can progress to cirrhosis and HCC. There is a high incidence for viral hepatitis infection and HCC in China. It is therefore of significance to explore the molecular biomarkers of the progression of hepatitis to HCC for the early diagnosis of HCC and the risk warning.

Metabolomics reflects the comprehensive changes of endogenous metabolites under certain pathophysiological condition. It can help us understand the disease process and the affected metabolic pathways, and to discover the biomarkers. In this study, nuclear magnetic resonance (NMR)-based metabolomics coupling with multivariate statistical methods was used to the study of serum and urine samples from HBV, cirrhosis, HCC patients and healthy controls. Partial least squares (PLS) analysis following orthogonal signal correction (OSC) of serum samples indicated systematic changes of lactate, valine, alanine, glutamine, glutamate, methionine, glucose, glycine, betaine and lipids were observed among groups. Pair-wised comparison provided more detailed changes of metabolites in the procession. An increase in N-acetyl glycoprotein was observed only in HCC group . Analysis of urine samples also demonstrated some systematic changeson metabolites such as creatinine, citrate, hippuric acid, tyrosine and some short chain fatty acids. This preliminary results suggested a potential of metabolomics in differentiation of different stages of liver diseases and identification of biomarkers of early diagnosis of HCC.

FISH METABOLIC PROFILING FOR ENVIRONMENTAL MONITORING IN THE ESTUARINE ECOSYSTEM

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Ecosystem services are precious and irreplaceable for human life, especially the estuarine environment is known as highest value among various natural ecosystems. In order to evaluate the estuarine ecosystem as bacteria-algae interaction level, we have reported the combination approach of environmental metabolomics with microbial community analysis (1). In estuarine ecosystems, however, relationships between variations of chemical compositions in fishes and environmental changes in their habitats are not extensively studied by -omics approach. Therefore, the aim of this study is to develop a NMR-based metabolomics approach for mining environmental information such as differences of metabolic profiling in fishes sampled from natural or artificial environments. In this study, we focused on the yellowfin goby for environmental monitoring in the estuarine ecosystem. The yellowfin goby is a primary consumer in the estuarine ecosystem of Keihin region in Japan, and is one of the suitable species to use for our experiments because of limited localization for their habits and behaviors. Extraction conditions and procedures for muscle and internal organs of sampled fishes were optimized from ^{1}H-NMR spectral patterns. Then, we processed the NMR spectra to data matrices, and evaluated the metabolic profiling of fishes from natural and artificial environments. In the results of principal component analysis, the metabolic profiles of fish muscles and organs were likely to be clustered according to the differences of their habitats, environments, or feeding substrates. The clustering of metabolic profiles of fishes were contributed by some amino acids, thus these metabolites may be used as a biomarker for environmental assessment. Therefore, fish metabolic profiling could be able to mine the environmental information in estuarine ecosystems.

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