DELEGATE HANDBOOK

21st Annual Lorne Proteomics Symposium

Thursday 4th - Sunday 7th February, 2016

Mantra Lorne, Lorne, Victoria, Australia

www.australasianproteomics.org

INDEX

AUSTRALASIAN PROTEOMICS SOCIETY 3

SPONSORS 4

DELEGATE INFORMATION 4

- VENUE AND LOCATION 5
- ORGANISER'S OFFICE – ASN EVENTS 6
- WHAT YOUR REGISTRATION INCLUDES 6
- SPEAKER PREPARATION INSTRUCTIONS 6
- DISPLAYING YOUR POSTER 6
- INTERNET ACCESS 6
- SETTLING YOUR ROOM ACCOUNT WITH YOUR HOTEL 6
- USEFUL PHONE NUMBERS 7
- BUS TRANSFERS HOME 7
- PARENT VIEWING ROOM 7
- CONFERENCE APP 7
- SOCIAL PROGRAM 8

INVITED SPEAKERS 9

- INTERNATIONAL 9
- NATIONAL 12

PRE-LORNE WORKSHOPS 14

VENDOR WORKSHOPS 15

PROGRAM 18

ABSTRACTS 27

TRADE DIRECTORY 80

INDEX OF ABSTRACT AUTHORS 83

NOTES 86

EXECUTIVE COMMITTEE OF MANAGEMENT 2016

PRESIDENT
A/Prof. Stuart Cordwell
Charles Perkins Centre
School of Life and Environmental Sciences,
Discipline of Pathology, School of Medical Sciences
Director, Mass Spectrometry Core Facility
The University of Sydney

VICE PRESIDENT/CONFERENCE CHAIR
Prof. Peter Hoffmann
Director, Adelaide Proteomics Centre
School of Biological Sciences
School of Physical Sciences
University of Adelaide, SA, Australia

SECRETARY
A/Prof. Mark Molloy
Director,
Australian Proteome Analysis Facility
Macquarie University, NSW, Australia

TREASURER
Prof. Gavin E Reid
School of Chemistry, Bio21 Molecular Science & Biotechnology Institute
The University of Melbourne, VIC, Australia

MEMBERS

MEMBER
A/Prof Michelle Hill
The University of Queensland Diamantina Institute,
The University of Queensland, Translational Research Institute, QLD, Australia

MEMBER
Prof. Marc Wilkins
Director, Ramaciotti Centre
School of Biotechnology and Biomolecular Sciences
The University of NSW, NSW

MEMBER
A/Prof. Vera Ignjatovic
Murdoch Childrens Research Institute, VIC

MEMBER
Prof. Anthony Purcell
NHMRC Senior Research Fellow
Monash University, Vic

SPONSORS

The Australasian Proteomics Society would like to thank the following sponsors and supporters for Lorne APS 2016

PLATINUM SPONSOR

BRUKER

GOLD SPONSORS

SCIEX

ThermoFisher SCIENTIFIC

Agilent Technologies

SHIMADZU Excellence in science

SILVER SPONSOR

novachem superior standards

Waters THE SCIENCE OF WHAT'S POSSIBLE.®

EXHIBITORS

TRAJAN

GENESEARCH

PEAK SCIENTIFIC

SoAPS FUNCTION SPONSOR

PILL Proteomics International

SUPPORTER

FIAT LUX Walter+Eliza Hall Institute of Medical Research DISCOVERIES FOR HUMANITY

PROTEOMICS & METABOLOMICS VICTORIA

APAF australian proteome analysis facility

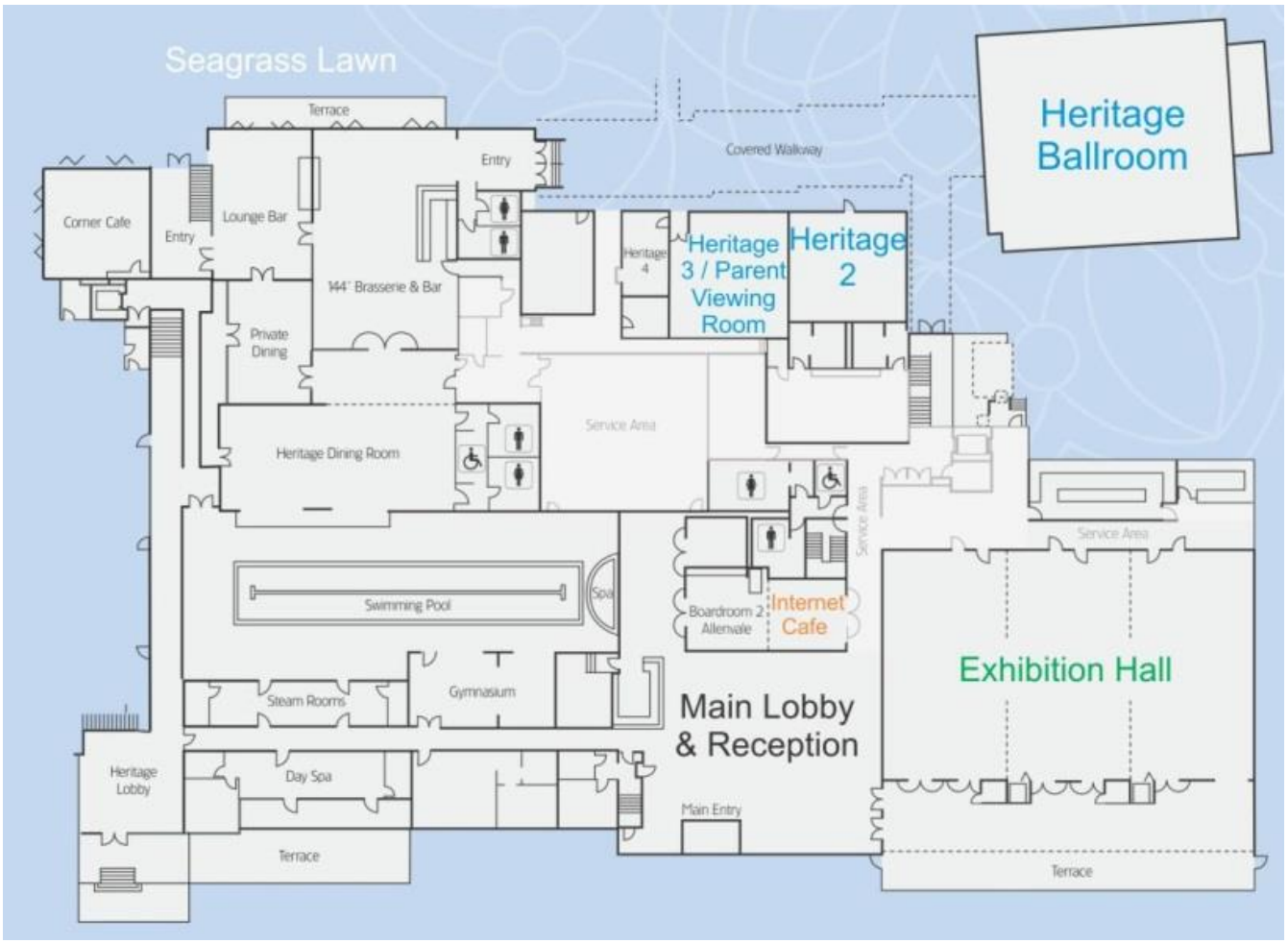
SIGMA-ALDRICH®

VENUE AND LOCATION

Mantra Lorne
Mountjoy Parade
Lorne, VIC, 3232
Phone: (03) 5228 9777
Fax: 03 5289 1185
www.mantralorne.com.au

Lorne is 140 kilometres south west of Melbourne and an approximately 2 hour drive. Travel is by car along the spectacular Great Ocean Road. Mantra Lorne is located on absolute beachfront and is nestled between the crystal clear water of Louttit Bay and the Otway Hinterland.

Please refer to this map for room locations



ORGANISER'S OFFICE – ASN EVENTS

The organiser's office is located in the Mantra Lorne Lobby. Any enquiries can be directed to ASN Events staff at the organiser's office, with the exception of enquiries regarding accommodation which should be directed to

The Conference office hours are:

Thursday 4th February: 12:00 pm –7:30 pm
Friday 5th February: 8:00 am – 7:00 pm
Saturday 6th February: 8:00 am – 6:00 pm
Sunday 7th February: 8:00 am – 1:00pm

WHAT YOUR REGISTRATION INCLUDES

Delegate and student registrations include:

- Access to the sessions of your choice
- Conference materials on USB
- Morning tea, and lunch for the days of nominated attendance (except Thursday and Sunday)
- Saturday Night APS Carnival

CONFERENCE SECRETARIAT – ASN EVENTS PTY LTD

Bianca Hanna-Atkinson
Level 1, 9/397 Smith Street, Fitzroy, Vic, 3065
Phone: 03 8658 9530 Fax: 03 5983 2223
Email: bha@asnevent.net.au

SPEAKER PREPARATION INSTRUCTIONS

All conference sessions take place in the Heritage Ballroom.
Audio-visual equipment will be supplied and there will be a technician on site to assist with any enquires.
It is the conference preference that ALL presentations be pre-loaded onto the laptop in the session room. Please bring your presentation to the meeting on a USB drive and give this to the technician well in advance of the session you are participating to allow for timely loading and testing.

A remote mouse/laser pointer will be provided at the lectern. Please use the pointer to ensure delegates in the overflow rooms are able to follow as you present.

You will be able to use your own MAC to present if preferred, however, please remember to bring necessary adapters.

DISPLAYING YOUR POSTER

Posters will be displayed throughout the Symposium on panels in the Exhibition Hall. Posters numbers 101 - 145 will be displayed from Friday morning and must be removed by Saturday morning tea. Posters starting at 201 – 246 will be displayed from Saturday morning tea and must be removed by Sunday morning tea. Please locate your abstract number for correct positioning. The maximum size provided is 1.0 m wide by 1.2 m high (portrait orientation). The approved method for attaching your poster is with Velcro. Please visit the organiser's office for additional supplies of Velcro.

INTERNET ACCESS

Free Wi-Fi is available to conference delegates. Simply select the username Mantra Lorne and enter the code **GU2G62ZZ** on your mobile device and connect. Please note: Access is restricted to one device per person. If you wish to use Wi-Fi on an additional device you must disconnect on the original device first before logging onto the second device.

ThermoFisher SCIENTIFIC

This conference acknowledges the sponsorship of Thermo Fisher Scientifc.

SETTLING YOUR ROOM ACCOUNT WITH YOUR HOTEL

Those individuals departing Lorne on Sunday 7th February will be required to settle their room accounts with Mantra Lorne that morning - you should check out before 10:00 am. Concierge has facilities to store baggage if required.

USEFUL PHONE NUMBERS

Mantra Lorne - 03 5228 9777
ASN Onsite – 0423 157 693
Gull Airport Service - 03 5222 4966
VLine Bus Service – 1800 800 007
Qantas – 13 13 13
Jetstar – 131 538
Virgin Blue – 136 789

BUS TRANSFERS HOME

Those who have pre-booked their bus transfer home from the conference will find the buses waiting to depart as they fill from the end of the last session. The buses will depart at 2:00 pm sharp and will travel first to the Melbourne airport then into the city, dropping passengers at Royal Parade outside Melbourne Brain Centre, Florey Institute (30 Royal Parade Parkville VIC). This location is opposite Royal Melbourne Hospital. Bus travellers will need to make their way promptly to the bus and should have already checked out from their accommodation earlier in the day.

PARENT VIEWING ROOM

The Walter and Eliza Hall Institute of Medical Research will again be supporting parents with young children attending the Lorne Conferences in 2016. They will make an overflow room (Heritage Room 3) available as a viewing room for parents with children. The room will have space for activities and play for children, while parents will be able to listen to the presentations with the same AV projection. ***This conference acknowledges the sponsorship of Walter + Eliza Hall Institue.***

CERTIFICATE OF ATTENDANCE

On conclusion of the Conference you will be able to download your certificate of attendance. Please login to your Currinda profile ***(members.asnevents.com.au/register/event/1419)*** by entering as a returning delegate and using your email address and password to login. Then click on the downloads section. From here you will be able to download your certificate of attendance. Please note that you will only be able to download your certificate of attendance if full registration payment has been received.

CONFERENCE APP

The official **Lorne Proteomics Symposium** mobile app will keep you organised during the meeting. You can view:

- an up-to-date daily program
- speaker abstracts
- speaker bios (where supplied)
- venue maps
- conference sponsors

You can update your profile information too!

Instructions for Apple devices

Step 1 Copy **proteomics-2016.m.asnevents.com.au** to your browser or use the above QR code.

Step 2 To install this web app;

Tap the **Share button** in Safari's menu bar

Tap the **Add to home screen** icon

This web app icon will now appear on a homescreen page for future reference.
For further benefits and instructions for Android devices please see staff at the registration desk.

EXHIBITOR PRIZE DRAW

After engaging with the staff at an exhibition booth they will present you with a draw ticket. Complete your details on the ticket and enter the draw at the Registration Desk. You could be taking home a new iPad mini. Prize/s will be drawn on Saturday 6th February, please to refer to the noticeboard for winners.
Remember, you've got to be in it to win it!

SOCIAL PROGRAM

WELCOME FUNCTION

The Welcome Function will be held amongst trade on Thursday 4th February, from 7:00pm – 10:00pm. This event will include food and beverages. If you have not selected a ticket in your registration and would like to attend please visit the registration desk for further information.

SoAPS DINNER

On Friday 5th February, the Students of APS (SoAPS) Pizza Dinner will be held at Mantra Lorne restaurant area on the Seagrass Lawn from 6:00pm – 7:30pm. A pizza dinner will be provided (drinks attendees expense). Visit the registration desk for further information if required.

THE SCIEX MUSIC AND PIZZA NIGHT

Join us on the Mantra lawn, Friday night 5th February, for food, drinks and live music from 7:30pm – 10:30pm. This is a great chance to catch up with your industry colleagues and the SCIEX team.

ERSKINE FALLS BUS/WALK TOUR

On Saturday 6th February at 2:30pm-3:30pm, there is an excursion to the local sights surrounding Lorne. This tour will be weather dependent. Notification about the tour will be provided in the Friday morning sessions. A bus will leave Mantra Lorne lobby at 2:30pm and take delegates to explore the area. Please visit the registration desk for further information.

APS CARNIVAL

On Saturday 6th February the APS Carnival will be held from 7:00pm until midnight in the Mantra Dining Area and the Seagrass Lawn Area. There will be live music, beverages, carnival themed food and entertainment. All are welcome and there is no cost to attend for delegates (tickets can be purchased for partners).

INTERNATIONAL

ASSOCIATE PROFESSOR EMMA LUNDBERG

SciLifeLab Stockholm and School of Biotechnology, KTH Karolinska Institutet Science Park, Sweden

Associate Professor Emma Lundberg is heading the Cell Profiling group at the Department of Proteomics and Nanobiotechnology at the Royal Institute of Technology (KTH) in Stockholm, Sweden. Her research is focused in the interface between affinity proteomics and bioimaging for cell biology applications. Emma's group is located to the Science for Life Laboratory in Stockholm, a newly started national research center focused on high-throughput molecular bioscience. Furthermore she is the Director of the Subcellular Protein Atlas, part of the Swedish Human Protein Atlas program, and elected member of the Executive Committee of both the Human Proteome Organization as well as the international Human Proteome Project. Dr. Lundberg received her Ph.D. in Biotechnology in 2008 and holds a M.Sc. in Biotechnological Engineering, both from KTH. Dr. Lundberg has co-authored 55 publications and two licensed patent application.

PROFESSOR MATTHIAS MANN

Max Planck Institute of Biochemistry, Martinsried, Germany

Matthias Mann studied physics and mathematics at Göttingen University in Germany and obtained his Ph.D. in chemical engineering at Yale University. Here he was decisively involved in the development of electrospray ionization, which has become a key technology of the life sciences. As a post-doctoral fellow and later as a professor for bioinformatics at the University of Southern Denmark in Odense, he developed, amongst others techniques, the first bioinformatic search algorithms for peptide fragmentation data and SILAC, a new method of quantitative proteomics and a breakthrough in the mapping of protein interactions. In 2005, Matthias Mann took up a director position at the Max-Planck Institute of Biochemistry in Munich. Here his group continues to address a wide range of biological questions using proteomic technology, as well as to develop this technology. The group is also heavily involved in providing proteomic methods and tools to the community. Most importantly in this regard, they have provided the MaxQuant suite of computational proteomics algorithms; this software promises to significantly advance the state of the field. More recently his group used the SILAC technology in conjunction with MaxQuant to described the first comprehensive identification and quantification of a proteome. (http://www.biochem.mpg.de/en/rd/mann) In 2009 Dr. Mann was additionally appointed director of the proteomics department of the Novo Nordisk Foundation Center for Protein Research in Copenhagen. Matthias Mann has authored and co-authored more than 580 publications with a total citation count of more than 100,000, making him one of the most highly cited researchers worldwide, has been elected to membership of the European Molecular Biology Organization, Royal Danish Academy of Arts and Sciences and the Leopoldina German National Academy of Sciences as well as to a visiting professorship at Harvard Medical School. He has received two honorary degrees from Utrecht University and the University of Dundee, respectively. In 2012 he was awarded the Leibniz Prize from the German Research Foundation, the Ernst Schering Prize, the Louis-Jeantet Foundation Prize for Medicine and the Körber European Science Prize.

ASSOCIATE PROFESSOR GANESH ANAND

Department of Biological Science, National University of Singapore, Singapore

Associate Professor Ganesh Anand received his Bachelor in Pharmacy and Master of Science in Biological Sciences from the Birla Institute of Technology and Science, Pilani, India and doctorate from Rutgers University, New Brunswick, NJ. He carried out postdoctoral research at the Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA. Since 2006, he has been a member of the faculty at the Department of Biological Sciences (DBS), National University of Singapore (NUS) and is also the Director of Protein and Proteomics Centre core facility. A/P Anand heads the Chemical Biology and Drug Discovery group at the Department of Biological Sciences, NUS. His research group investigates allostery, conformational dynamics of intrinsically disordered signaling enzymes, and transmembrane receptor signaling. Other projects include protein dynamics in viral maturation and fragment-based drug discovery. The lab uses amide hydrogen/deuterium exchange mass-spectrometry (HDXMS) as a primary tool for protein dynamics and has been recognized as a Waters World Centre of Innovation in HDXMS since 2011.

ASSOCIATE PROFESSOR MATTHEW CHANG

Department of Biochemistry, National University of Singapore, Singapore

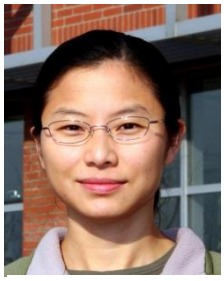
Matthew Chang is Associate Professor at the National University of Singapore and Program Leader of NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI). His research interests lie in synthetic biology of microbial systems, with particular emphasis on development of synthetic microbes that perform programmable functions for engineering applications. In particular, he has pioneered the development of synthetic microbes that show novel programmable therapeutic behaviors. His work has received international recognition and is featured in leading media agencies worldwide. He has been honored with the Scientific and Technological Achievement Award from U.S. Environmental Protection Agency, and serves as an editorial board member for ACS Synthetic Biology and Biotechnology Journal, and as an associate editor for Biotechnology for Biofuels.

PROFESSOR ANNE DELL

Department of Life Sciences, Imperial College, London

Anne Dell was a chemistry undergraduate at the University of Western Australia before undertaking a PhD at the University of Cambridge supported by an 1851 Exhibition Research Scholarship. She moved to Imperial College London for her postdoc where she rose through the ranks to a Personal Chair in 1991. She was Head of the Biochemistry Department from 1999-2001. She was elected to the Fellowship of the Royal Society in 2002 and was awarded a CBE in recognition of her services to science in 2009. She has received honorary DSC degrees from the University of Western Australia and the University of Waterloo in Canada. Anne was amongst the first investigators to successfully apply soft ionization mass spectrometry to carbohydrate containing biopolymers. Over the past thirty years she has led a major group that has been at the leading edge of the development of many mass spectrometric methods for glycomic and glycoproteomic analyses, especially in the area of biomedical research. Her laboratory provides structural underpinning for programmes of research seeking to define the biological roles that glycans play in health and disease. She is currently a Wellcome Trust Senior Investigator and heads the Glycobiology Training, Research and Infrastructure Centre at Imperial College.

ASSOCIATE INVESTIGATOR MENG-QIU DONG



National Institute of Biological Sciences, Beijing

Grown up in the southwestern region of China, Meng-Qiu Dong moved steadily eastwards for higher education until she obtained her Ph.D. degree at Yale University in 2001. Then, her migratory track reserved; after six years of postdoc training in UCSD and the Scripps Research Institute, she moved back to China in 2007 to start her independent research at NIBS, Beijing. In her laboratory, biology and mass spectrometry (MS) goes hand-in-hand. On the biology side, she is interested in understanding the secrets of aging using C. elegans as a model. On the mass spec front, her current focus is perfecting the technology of chemical cross-linking of proteins coupled with mass spectrometry (CXMS). She and collaborators have developed a complete CXMS workflow, which features the most inexpensive and readily available cross-linkers and the software program pLink for data analysis. CXMS is an effective tool for locating the interface between interacting proteins, and is gaining popularity in structural analysis of large protein complexes. Recently, she has extended the technology to mapping native disulfide bonds of proteins from simple or complex samples.

PROFESSOR ANDREA SINZ

Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle, Germany

Andrea Sinz received her degree in Pharmacy from the University of Tübingen (Germany) in 1993. She obtained her Ph.D. in Pharmaceutical Chemistry from the University of Marburg (Germany) in 1997. From 1998 to 2000 she was a post-doctoral fellow at the National Institutes of Health in Bethesda, MD (USA) where she got introduced into chemical cross-linking and protein mass spectrometry. From 2001-2006, she was head of the junior research group 'Protein-Ligand Interaction by Ion Cyclotron Resonance Mass Spectrometry' at the University of Leipzig (Germany). Since 2007, she is Full Professor and head of the Department of Pharmaceutical Chemistry and Bioanalytics at the Martin-Luther University Halle-Wittenberg (Germany).

Andrea Sinz is an expert in chemical cross-linking and mass spectrometry for studying protein 3D-structures and protein interactions. Her research interests are the development of novel analytical strategies and reagents to advance the cross-linking/MS approach. She has authored more than 100 papers and is member of the presidential board of the German Society for Mass Spectrometry (DGMS). She has received the Mattauch-Herzog award from the German Society for Mass Spectrometry and the Innovation Award in Medicinal/Pharmaceutical Chemistry from the German Society of Chemists (GDCh) and the German Pharmaceutical Society (DPhG).

DR TORSTEN KLEFFMANN

University of Otago, Dunedin, New Zealand

Torsten Kleffmann is director of the Otago Centre for Protein Research at the University of Otago, New Zealand, a facility for biological mass spectrometry with the main focus on proteomics. He is working in the area of proteomics and biological mass spectrometry for more than 15 years and his current interest and research is focused on the dynamics of the protein cargo associated with different lipoprotein particles in the context of cardiovascular disease. After completing his PhD, which he received from the Ruhr University in Bochum, Germany, he moved to Switzerland to work as a postdoctoral fellow with Prof. Wihelm Gruissem in the Group of Plant Biotechnology at the ETH in Zurich. Here he undertook some of the early research on proteome dynamics during plant plastid differentiation. He then moved to New Zealand in 2006 to take on his current position at the University of Otago where he has established the current capabilities for biological mass spectrometry in Centre for Protein Research. Besides his work on the protein composition of lipoproteins in general and lipoprotein(a) in particular, he is involved in various other research projects that focus on mass spectrometry-based proteomics on different biological and biomedical systems.

DR THOMAS KISLINGER

Princess Margaret Cancer Center, Ontario, Canada

Thomas Kislinger received his MSc in Analytical Chemistry from the University of Munich, Germany (1998). He completed his PhD in 2001, investigating the role of Advanced Glycation Endproducts in diabetic vascular complications at the University of Erlangen, Germany and Columbia University, New York. Between 2002 and 2006 he completed a post---doctoral fellowship at the University of Toronto using shotgun proteomics to investigate organelle dynamics in mouse models of human disease. In 2006 he joined the Princess Margaret Cancer Center as an independent investigator. Dr. Kislinger holds positions as Senior Scientist at the Princess Margaret Cancer Center and as Associate Professor at the University of Toronto in the Department of Medical Biophysics. He is a Tier 2 Canadian Research Chair in Proteomics in Cancer Research. The research interests in the Kislinger lab are focused on the application of proteomics and computational tools to cancer biology and biomarker discovery. We are particularly interested in combining in---depth proteomics with chemistry, biochemistry and cell & molecular biology to gain novel insights into the function of poorly studied membrane proteins.

DR GARY KRUPPA

MRM Proteomics Inc., United States

Gary Kruppa is the CEO of MRM Proteomics. Gary and his team at MRM Proteomics Inc. work with the staff at the University of Victoria/Genome BC Protein Centre led by Prof. Christoph Borchers to develop MS assays for the quantitative analysis of proteins using LC-MRM/MS (PeptiQuant™ assays) and iMALDI methods. MRM Proteomics Inc. sells PeptiQuant™ assays as a service and has developed PeptiQuant™ kits for use in standardization of LC-MRM/MS workflows across laboratories worldwide. PeptiQuant™ kits are available both for LC-MRM/MS platform quality control and validation and for biomarker discovery and validation via Cambridge Isotope Laborarories, Inc, an its distributors worldwide. Gary received his Ph.D. in chemical physics from the California Institute of Technology.

DR CHRISTIE HUNTER

SCIEX United States

Christie Hunter is the Director of Omics Applications at SCIEX. Christie and her team are focused on developing and testing innovative MS workflows to analyze biomolecules, and work collaboratively with the instrument, chemistry and software research groups. Her primary area of focus is the application of MS based tools for the quantitative analysis of proteins and using MRM and SWATH® based strategies.

Christie Hunter is the Director of Proteomic Applications at AB SCIEX. Christie and her team are focused on developing and testing innovative MS workflows for the quantitative analysis of proteins and peptides, and work collaboratively with the instrument, chemistry and software research groups. Her primary area of focus is targeted peptide quantification, mainly using MRM based strategies. Most recently, she has focused on new technologies for further improving quantitative proteomic applications using differential mobility separation and the new MS/MSALL with SWATH Acquisition workflow. Christie received her Ph.D. in protein biochemistry from the University of British Columbia (Canada).

DR YUE XUAN

Thermo Fisher Scientific, Germany

Dr. Yue Xuan is currently working in the global Product Marketing team for Q Exactive Series platforms at Thermo Fisher Scientific in Bremen, Germany. In her current role, she develops novel application workflows and collaborates with customers in the "omics" and biopharma researches for the existing and further developed Orbitrap based Mass Spectrometers. Since 2013 she has put focus on the Data Independent Acquisition (DIA) method development on Q Exactive MS platform. The lately activities also include DIA publications, webinars, collaborations as well as IS-PRM. She has more than 10 years experience in LC-MS related application development area, starting from the Finnigan LTQ FT till today's Thermo ScientificTM Q ExactiveTM HF mass spectrometer. She is also one of the pioneers in the development of the 1st Thermo ScientificTM Q Exactive MassTM Spectrometer. Dr. Xuan holds a Ph.D. in Analytical Chemistry from University of Dortmund and a M.S. in Chemistry from the Free University of Berlin, Germany.

DR ROBERTO CASTANGIA

Shimadzu United Kingdom

Roberto is a Pharmaceutical Chemist.
After his BSc in Italy, he worked at the CNRS in France and at the Max Planck Institute in Germany, before moving to the University of Manchester, UK, to pursue his Ph.D. in Chemical Biology. After a stay as a post-doc at the Manchester Institute of Biotechnology (MIB), he started at Kratos Analytical Ltd. in 2012 working as a Global MALDI Applications Scientist.Since 2015; he is managing the Global Sales Department for Shimadzu MALDI Division.

NATIONAL

PROFESSOR NICKI PACKER

Macquarie University, Sydney

Prof Nicki Packer has had an extensive and varied career in biomolecular research. She was part of the team that established the Australian Proteome Analysis Facility (APAF) and co-founded Proteome Systems Limited, an Australian biotechnology company. She is now Professor of Glycoproteomics, Director of MQ Biomolecular Frontiers Research Centre, Deputy Director of the ARC Industrial Transformation Training Centre for Molecular Technology in the Food Industry and Discovery Leader for the ARC Centre of Excellence in Nanoscale BioPhotonics. She has gained international profile by linking glycomics with proteomics and bioinformatics to determine biological function. Her research interests are now in the structure, function, informatics and application of glycans as molecular markers, particularly in their role in cancer, imaging and microbial infection.

DR TARA PUKALA

University of Adelaide, Adeleaide

Tara Pukala obtained a PhD from the University of Adelaide in 2006. This was followed by a postdoctoral position at the University of Cambridge, UK, working in the field of native mass spectrometry. Tara returned to Australia to her current role as lecturer in the Discipline of Chemistry at the University of Adelaide in 2008. Here she leads a multidisciplinary research group focused on developing new approaches, primarily utilising mass spectrometry, to investigate the higher order structure, function and interactions of macromolecules important in biology.

DR NICOLAS TAYLOR

University of Western Australia, Western Australia

Nic Taylor completed his undergraduate studies and MSc at Massey University, New Zealand and in 2000 moved to UWA to undertake his PhD. After his PhD he was awarded and European Molecular Biology Organization (EMBO) Long Term Fellowship to study at the Department of Plant Sciences at the University of Oxford. He was recruited back to UWA in 2006 to the newly established ARC Centre of Excellence in Plant Energy Biology (ARC CoE PEB). Here he has applied and developed a wide range of quantitative proteomics approaches in his research. He is particularly well known for his pioneering work in the development of peptide selective reaction monitoring (SRM) mass spectrometry approaches in plants and developed an SRM toolbox (APP, http://www.plantenergy.uwa.edu.au/APP/) for plant biologists wishing

to conduct SRM experiment in the model plant Arabidopsis.
His lab seeks gain a comprehensive understandings of how metabolites, proteins and lipids within plant cells respond to extremes of temperature and salt with the hope this knowledge can be applied to future breeding programs to allow the production of thermal and salinity tolerant crop plants. He is currently an ARC Future Fellow at the ARC CoE PEB and School of Chemistry & Biochemistry at UWA, a member of the Multinational Arabidopsis Steering Subcommittee for Proteomics (MASCP) and the Australian Representative of the International Plant Proteomics Organisation (INPPO). In 2015 he was awarded the Robson Medal for Research Excellence in Agriculture and Related Areas.

DR DARREN CREEK

Monash University, Melbourne

Dr Darren Creek is a Senior Lecturer and head of the metabolomics laboratory at the Monash Institute of Pharmaceutical Sciences. He completed his PhD at Monash University in 2007, which led to the discovery of a new antimalarial, OZ439, and conducted post-doctoral training on clinical pharmacokinetics with UCSF-Makerere research collaboration in Uganda. Dr Creek received a CJ Martin Fellowship (NHMRC) to study parasite metabolomics at the University of Glasgow and the University of Melbourne. He developed several novel analytical methods and software for the metabolomics field, and discovered novel pathways and drug mechanisms in protozoan parasites. His group continues to develop novel metabolomics techniques and apply systems approaches to understand the role of metabolism in drug action and resistance mechanisms for cancer and infectious diseases. He currently holds an RD Wright Career Development Fellowship (NHMRC) and is a Director of the international Metabolomics Society.

DR MELISSA DAVIS

Walter+Eliza Hall Institute for Medical Research, Melbounre

Dr Melissa Davis is a computational biologist, with a background in genetics and computational cell biology and expertise in the analysis of protein interaction networks, genome-scale regulatory networks, and knowledge-based modelling. Melissa did her post-doctoral training at the Institute for Molecular Bioscience in Brisbane, before taking up a position as Senior Research Fellow and Group Leader in Cancer Systems Biology at the University of Melbourne in 2014. In 2016 Melissa will relocate her group to the Walter and Eliza Hall Institute for Medical Research, where she will take up an appointment as a Group Leader in the Division of Bioinformatics. Melissa holds a four year NBCF Career Development Fellowship to study the systems biology of epithelial-mesenchymal plasticity in breast cancer as part of the national EMPathy Breast Cancer Network.

ASSOCIATE PROFESSOR UTE ROESSNER

The University of Melbourne, Melbourne

A/Prof. Roessner has obtained her Diploma in Biochemistry at the University of Potsdam and the John Innes Institute in Norwich, UK after which she pursued a PhD in Plant Biochemistry at the MPI for Molecular Plant Physiology in Germany, where she developed novel GC-MS methods to analyse metabolites in plants. Together with the application of sophisticated data mining the field of metabolomics was born and is today an important tool in biological sciences, systems biology and biomarker discovery. In 2003 she moved to Australia where she established a GC-MS and LC-MS based metabolomics platform as part of the Australian Centre for Plant Functional Genomics for which she led the node at the University of Melbourne. In addition, since 2007 A/Prof. Roessner has been involved in the setup of Metabolomics Australia (MA), a federal and state government funded national metabolomics service facility and now leads the MA node at The University of Melbourne. In 2013 A/Prof Roessner was successful being awarded an Australian Research Council Future Fellowship to establish her own research program applying Imaging Mass Spectrometry for spatial metabolite and lipid analyses to solve biological questions.

WEDNESDAY 3rd FEBRUARY 2016

3D STRUCTURE DETERMINATION BY MASS SPECTROMETRY

Time: 9:00am – 3:45pm
Location: The Bio21 Institute, The University of Melbourne, Victoria

Confirmed Speakers
Prof. Andrea Sinz, *Martin-Luther University Halle-Wittenberg, Germany*
– Cross-linking and mass spectrometry for structure determination
Dr. Tara Pukala, *The University of Adelaide, Australia*
- Ion mobility-mass spectrometry for structure determination
Assoc Prof. Ganesh Anand, *National University of Singapore, Singapore*
- HDX mass spectrometry for structure determination

Program:

9:00-9:15 am	Registration and Welcome
9:15-10:00 am	**Ion Mobility Mass Spectrometry: An Added Dimension for Structural Biology.** Dr Tara Pukala, The University of Adelaide, Australia
10:00-10:55 am	**Dynamics of Macromolecular Assemblies by Amide Hydrogen/Deuterium Exchange Mass Spectrometry: From protein-ligand interactions to signalling particles.** A/Prof Ganesh S. Anand, National University of Singapore, Singapore
10:55-11:15 am	Coffee Break
11:15- 12:00 pm	**Chances and Pitfalls of Chemical Cross-Linking and Mass Spectrometry.** Prof Andrea Sinz, Martin-Luther University Halle-Wittenberg, Germany
12:00-1:00 pm	Lunch break
1:00-1:45 pm	**Native mass spectrometry; practical consideration, experimental optimisation and current limitations.** Dr Tara Pukala, The University of Adelaide, Australia
1:50-2:30 pm	**HDXMS: Practical considerations and opportunities for complementarity with orthogonal biophysical and computational approaches** A/Prof Ganesh S. Anand, National University of Singapore, Singapore
2:30-2:45pm	Coffee Break
2:45-3:30pm	**Practical Aspects of Cross-linking and Introduction of StavroX/MeroX Software.** Prof Andrea Sinz, Martin-Luther University Halle-Wittenberg, Germany
3:30-3:45 pm	Concluding remarks

Proudly Sponsored by

Agilent Technologies

THURSDAY 4th FEBRUARY 2016

Mass Spectrometry '101' Tutorial Series

Presented by: **Dr. Nicholas Williamson**
Mass Spectrometry and Proteomics Facility, Bio21 Institute, The University of Melbourne

Time: 12:30pm - 3:30pm
Location: Heritage 2, Mantra Lorne
Lunch: Provided

This pre-conference lecture aims to provide a basic working knowledge of mass spectrometry, proteomics and simple proteomic bioinformatics. It is deliberately aimed at novice users with little or no prior experience in the field. Explanations are kept as simple as possible. Emphasis will be placed on understanding the broad concepts that will be assumed knowledge during the conference.

Mass Spectrometry '202' Tutorial Series

Presented by: **Prof. Gavin E. Reid**
School of Chemistry, Department of Biochemistry and Molecular Biology, Bio21 Institute, The University of Melbourne

Time: 12:30pm - 3:30pm
Location: Heritage 3, Mantra Lorne
Lunch: Provided

This pre-conference lecture will provide a more advanced understanding of the underlying principles of mass spectrometry and tandem mass spectrometry methods applied to proteome analysis, with an emphasis on the characterization of PTM's and quantitation. It is aimed at those people who have previously taken part in the Mass Spectrometry '101' Tutorial Series (or its equivalent), or users with some prior experience of the field.

VENDOR WORKSHOPS

FRIDAY 5th FEBRUARY 2016

Thermo Fisher Scientific Breakfast Workshop:
New Advances in Liquid Chromatography & Mass Spectrometry to Transform the way you work

Time: 7:30 - 9:00am | Breakfast served at 7:00am
Location: Heritage 2, Mantra Lorne

Proudly Sponosored by

ThermoFisher SCIENTIFIC

Confirmed Speakers

7:30 – 8:10AM
Prof. Matthias Mann, *Department of Proteomics and Signal Transduction on Max Planck Institute of Biochemistry, Germany*
"Technology development towards high-throughput proteomics"

Mass spectrometry (MS)-based proteomics has advanced tremendously during the last few years in terms of depth of proteome coverage that can routinely be obtained. Despite these advances, routine application of shotgun proteomics with large sample numbers is challenging because the workflows are not sufficiently scalable and robust. With the bioinformatic workflow increasingly streamlined and versatile [1], work in our laboratory has focused on reducing the number of steps in sample preparation [2] as well as the degree of fractionation needed to achieve good proteome coverage [3]. Here we will discuss developments in the entire workflow upfront of the mass spectrometer that are aimed at achieving high uptime and high throughput. In particular, we have investigated protein digestion in a 96-well format, streamlined fractionation and column designs with these goals in mind. Our results indicate that MS-based proteomics can indeed be performed with high reliability and reproducibility, a precondition to applying the technology in a systems biological and clinical context.

8:10 – 8:50AM
Prof Andrea Sinz, *Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin-Luther-Universität Halle-Wittenberg, D-06120 Halle (Saale), Germany*
"An Integrated Workflow for Structural Proteomics Studies based on Cross-linking/Mass Spectrometry with an Orbitrap Fusion Mass Spectrometer"

Cross-linking combined with MS has evolved as an alternative strategy in structural biology for characterizing three-dimensional structures of protein assemblies and for mapping protein-protein interactions. One of the most pressing questions to be addressed in the chemical cross-linking/MS approach is the development of automated workflows. In my talk, I will describe an integrated workflow for an automated identification of cross-linked products that is based on the use of an MS/MS cleavable cross-linker generating characteristic reporter ion patterns upon fragmentation. I will evaluate different fragmentation methods available on an Orbitrap Fusion mass spectrometer (CID, HCD, ETciD, and EThcD) in combination with a dedicated software tool, MeroX, for conducting fully automated analyses of cross-linked products. The confidence of cross-link assignment is validated by a decoy search strategy. Moreover, I will show ETD data of intact cross-linked protein/peptide complexes.

Bruker Afternoon Workshop:
Gain Deeper Biological Insight by Accelerating Your Omics Research

Time: 2:45pm - 4:15pm | *Afternoon drinks and snacks will be served between 4:15 pm – 4:45pm*
Location: Heritage Ballroom, Mantra Lorne

Proudly sponsored by

Confirmed Speakers
Prof. Matthias Mann, *Max Planck Institute of Biochemistry, Germany*
"In depth proteomics with the impact II and beyond"
Prof. Peter Hoffmann, *Adelaide Proteomics Centre, University of Adelaide*
"MALDI TOF Mass Spectrometry Imaging on Cancer Tissue and MALDI TOF Mass Spectrometry Profiling on Beer"
Dr. Andrew Webb, *Walter+Eliza Hall Institute of Medical Research*
"Dissecting disease biomarkers using intact protein profiling with UHR-QTOF MS"

Agilent Technologies Afternoon Workshop:
Technologies and Tools to Assist Disruptive and Orthogonal Approaches for Proteomics

Time: 2:45pm - 4:15pm | *Afternoon drinks and snacks will be served between 4:15 pm – 4:45pm*
Location: Heritage 2, Mantra Lorne - Refreshments provided

Proudly sponsored by

Confirmed Speakers
Dr. David Bradley & Tom Hennessy, *Agilent Technologies, Australia*
Dr. Hyun Joo An, *ChungNam National University, South Korea*
Christine Miller, *Agilent Technologies, Santa Clara, United States*

Program

2:45-2:50pm Welcome & Introduction & Review Agenda
Dr David Bradley

2:55-3:15pm Agilent Technologies Academia Team & Program
Dr David Bradley & Tom Hennessy

3:15-3:45pm Decoding Glycosyl Modification of Biotherapeutics by Intact Protein Analysis using Mass Spectrometry
Dr Hyun Joo AN

3:45-4:15pm Technologies and Tools to Assist Disruptive and Orthogonal Approaches for Proteomics
Christine Miller

Agilent Technologies' Academia Team & Programs
What you will learn:
- Meet the Academia & Collaborations Team
- Unique programs specifically designed to facilitate academic research & collaborations
- Mutual benefits by design: reflection on programs' focus, science, connections, relevance & outcomes

SATURDAY 6th FEBRUARY 2016

SCIEX Breakfast Workshop:
Next-Generation Sequencing meets Next-Generation Proteomics

Time: 7:30am - 9:00am
Location: Heritage Ballroom, Mantra Lorne

Proudly sponsored by

Confirmed Speakers
Dr. Christie Hunter - *SCIEX, United States*
Dr. James Broadbent - *SCIEX*

As a first step in enabling such research on a large scale, SCIEX has partnered with Illumina to enable processing of SWATH® next-generation proteomics data on BaseSpace®, Illumina's cloud computing environment. OMICs processing in the cloud promises; up to 10x faster processing of SWATH® data, a user friendly app environment for integrating new tools, easier collaboration and sharing with colleagues worldwide and the ability to perform data management and analysis from any web-equipped computer.
In this workshop, SCIEX Field Application Specialist James Broadbent will demonstrate the latest versions of the Protein Extractor and Workflow Manager applications that enable the conversion of raw SWATH data files to detailed protein expression data and visuals in fast, intuitive manner. The Workflow Manager also incorporates the Protein Expression Analytics application, which provides the mass spectrometrist a detailed review of the data quality, such as data completeness, precision and false discovery rates.
Following the software demonstration, SCIEX's Global Director of OMICs Applications, Christie Hunter, will outline the first multi-omics study to be conducted using BaseSpace®. In this study - a collaboration between SCIEX, Advaita and researchers from the University of California, San Francisco (UCSF) - cytotrophoblast (CTB) cells were studied to investigate their role in placental abnormalities. In this investigation, transcriptomics and SWATH™ proteomics data from CTB cell extracts were processed in BaseSpace and the results combined in Advaita's iPathway guide, providing a seamless processing pipeline from data to meaningful biological answers.

Novachem Breakfast Workshop:
The inside story on Peptiquant™ and how it will change your research forever

Time: 7:30 - 9:00am
Location: Heritage 2, Mantra Lorne

Proudly sponsored by

Confirmed Speaker
Dr. Gary Kruppa - *MRM Proteomics Inc, Canada*

Researchers in academia and life science industries continue to adopt a targeted, bottom-up MS-based proteomic workflow for biomarker discovery and validation. Biomarker validation requires absolute quantification of surrogate peptides in the sample matrix, a requirement that is best achieved using stable isotope enriched peptide internal standards (SIS). Although the use of SIS has increased the reliability of LC/MRM-MS based assays, optimal results require properly functioning equipment and a workflow with minimal human error and bias.
To help researchers establish a stable LC/MRM-MS platform for bottom-up quantitative proteomics, MRM Proteomics in conjunction with Cambridge Isotopes Limited now offer PeptiQuant™ products. These innovative products are used to assess and track LC/MS performance of the LC/MS platform in a proteomic workflow in order to highlight any possible issues affecting quantitation.
In addition to doing away with the less accurate and more expensive ELISA and Western Blot protein quantitation methods, PeptiQuant™ now provides researchers with a variety of customisable biomarker assessment assays which provide a huge advantage in biomarker identification for specific disease profiles and allow results to be accurately verified with high levels of certainty.
The workshop with Gary Kruppa, CEO of MRM Proteomics promises a wealth of insight into how the technology works, some real-life examples of the benefits of the methods and an opportunity to ask your questions about how PeptiQuant™ can benefit your research.

PROGRAM

Thursday 4th February		
12:00 PM	**Registration Opens**	Mantra Lobby
12:30 PM - 3:30 PM	**Mass Spec 101** Chair: Nick Williamson	Heritage 2
12:30 PM - 3:30 PM	**Mass Spec 202** Chair: Gavin Reid	Heritage 3
4:00 PM - 4:10 PM	**Welcome Address** Stuart Cordwell, President	Heritage Ballroom
4:10 PM – 5:00 PM	**The Simpson Lecture** *Session Sponsored by* BRUKER Chair: Stuart Cordwell	Heritage Ballroom
	Matthias Mann *Moving MS-based proteomics closer to the clinic* ***abs#001***	
5:00 PM - 5:40 PM	**Ken Mitchelhill Young Investigator Award Lecture** *Session Sponsored by* SHIMADZU Chair: Stuart Cordwell	Heritage Ballroom
	Rommel Mathias *SIRT4 is a host mitochondrial lipoamidase that restricts human cytomegalovirus* ***abs#002***	
5:40 PM - 6:45 PM	**Symposium One: Students of the APS (SoAPS)** *Session Sponsored by* PROTEOMICS & METABOLOMICS VICTORIA Chair: Tony Purcell	Heritage Ballroom
5:40 PM	**Zeynep Sumer Bayraktar** *Asn-347 Glycosylation of Corticosteroid Binding Globulin Finetunes the Host Immune Response by Modulating Proteolysis by P. aeruginosa and neutrophil elastase* ***abs#003***	
5:55 PM	**Iain Berry** *The N-terminome of Mycoplasma hyopneumoniae reveals functionally diverse proteins as targets of endoproteolysis* ***abs#004***	
6:10 PM	**Lauren Smith** *Large-scale Phopshorylation Study of the Type 2 Diabetic Heart subjected to Control and Infarct Conditions* ***abs#005***	
6:25 PM	**Tuo Zang** *The proteome of blister fluid from pediatric burn injuries* ***abs#006***	
6:40 PM	**Daniel Winter** *A combination of bottom-up and top-down mass spectrometry provides insights on the post-translational regulation of yeast protein methyltransferases* ***abs#007***	
7:00 PM - 10:00 PM	**Welcome Reception** *Sponsored by* PEAK SCIENTIFIC	Exhibition Hall

Friday 5th February		
7:00 AM	**Breakfast**	
7:30 AM - 9:00 AM	**Thermo Fisher Scientific: Breakfast Workshop** *"New Advances in Liquid Chromatography & Mass Spectrometry to Transform the way you work"* ThermoFisher SCIENTIFIC **Prof Matthias Mann** **Prof Andrea Sinz**	Heritage 2
9:00 AM - 10:30 AM	**Symposium Two: Signalling and Post-Translational Modifications** Chair: Vera Ignjatovic	Heritage Ballroom
9:00 AM	**Meng-Qiu Dong** *Mitochondrial Changes Contribute to the Longevity of the daf-2 Mutant* ***abs#008***	
9:30 AM	**Stuart Cordwell** *Proteomic-scale approaches for identifying reversible and irreversible cysteine redox post-translational modifications in myocardial ischemia / reperfusion* ***abs#009***	
9:50 AM	**Mark Larance** *Starvation regulates ubiquitin-like modifiers critical for stress responses* ***abs#010***	

10:10 AM	**Gary Kruppa** *Higher-Order Structural Characterization of Therapeutic Antibodies at Residue Level by Middle-Down HDX-MS* *Speaker sponsored by* novachem superior standards ***abs#011***	
10:30 AM - 11:00 AM	**Morning Tea**	Exhibition Hall
11:00 AM - 12:25PM	**Symposium Three: Plant, Earth and Environmental Proteomics** Chair: Paul Haynes	Heritage Ballroom
11:00 AM	**Nicolas Taylor** *Harnessing Targeted Proteomics to Enhance Yield, Salinity Tolerance and Thermal Tolerance of Wheat* *Speaker sponsored by* Agilent Technologies ***abs#012***	
11:25 AM	**Alison McAfee** *Proteome profiling of persistent honey bee (Apis mellifera) primary cells* ***abs#013***	
11:45 AM	**Vincent Bulone** *Quantitative Proteomics Reveals that Plant Plasma Membrane Microdomains are involved in Molecular Transport, Stress Responses and Callose Biosynthesis* ***abs#014***	
12:05 PM	**Eivind AB Undheim** *Limitations on a biochemical arsenal – do centipede venoms evolve under morphological constraint?* ***abs#015***	
12:30 PM - 1:00 PM	**Lightning Talks One** Chair: Angus Grey	Heritage Ballroom
1st	**Daniel Broszczak** *Proteome survey of wound fluid from non-healing wounds reveals key biological processes associated with poor healing outcomes* ***abs#101***	
2nd	**Soumya Mukherjee** *Identification of the Fragmentation Features of Dityrosine cross-linked Amyloid beta in ESI-MS/MS abs* ***abs#102***	
3rd	**Kate Scull** *A bioinformatic solution for identifying non-genomic peptides in the immunopeptidome* ***abs#103***	
4th	**Shivashankar H Nagaraj** *Multi-omic analysis of Epithelial-Mesenchymal Transition in pancreatic cancer* ***abs#104***	
5th	**Pouya Faridi** *Proteomics-based approach to elucidate the mechanism of anti-hypercholestrolemia activities of selected herbal medicines* ***abs#105***	
1:00 PM - 2:30 PM	**Lunch | Poster Session One**	Exhibition Hall
2:30 PM – 5:00 PM	**Free Afternoon**	
2:45 PM – 4:45PM	**Bruker: Afternoon Workshop** *"Gain Deeper Biological Insight by Accelerating Your Omics Research"* **Prof Matthias Mann, Max Planck Institute** **Prof Peter Hoffmann** **Dr Andrew Webb** BRUKER	Heritage Ballroom **Afternoon drinks and snacks will be served from 4:15pm*
2:45 PM – 4:45PM	**Agilent: Afternoon Workshop** *"Technologies and Tools to Assist Disruptive and Orthogonal Approaches for Proteomics"* **Dr David Bradley & Tom Hennessey** **Dr Hyun Joo An** **Christine Miller** Agilent Technologies	Heritage 2 **Afternoon drinks and snacks will be served from 4:15pm*
5:00 PM - 6:00 PM	**Symposium Four: Metabolomics** Chair: Gavin Reid	Heritage Ballroom
5:00 PM	**Ute Roessner** *Tissue specific metabolite and lipid analysis using MALDI-FT-MS – linking spatial distribution to metabolic function* *Speaker sponsored by* BRUKER ***abs#016***	
5:20 PM	**Darren Creek** *Discovery of mechanisms of action for antimalarial drugs by medium-throughput*	

	metabolomics screening ***abs#017***	
5:40 PM	**Angus Grey** *MALDI imaging analysis of the aging human lens metabolome* ***abs#018***	
6:00 PM – 7:30 PM	**Student of APS (SoAPS) Dinner with Invited Speakers** *Sponsored by* Proteomics International	Seagrass Lawn Area
7:30 PM – 10:30 PM	**The SCIEX Pizza and Music Night** *Enjoy live music, beverages & pizzas with the SCIEX Team* SCIEX	Seagrass Lawn Area
Saturday 6th February		
7:00 AM	**Breakfast**	
7:30 AM - 9:00 AM	**SCIEX: Breakfast Workshop** SCIEX *"Next-Generation Sequencing meets Next-Generation Proteomics"* **Dr Christie Hunter** **Dr James Broadbent**	Heritage Ballroom
7:30 AM - 9:00 AM	**Novachem: Breakfast Workshop** *"The inside story on Peptiquant™ and how it will change your research forever"* novachem superior standards **Dr Gary Kruppa**	Heritage 2
9:00 AM - 10:25 AM	**Symposium Five: Glycoproteomics and Glycomics** Chair: Benjamin Parker	Heritage Ballroom
9:00 AM	**Anne Dell** *Glycomics in Health and Disease* ***abs#019***	
9:30 AM	**Nicolle Packer** *Alterations in Glycosylation in Ovarian Cancer* ***abs#020***	
9:55 AM	**Matthew Briggs** *MALDI mass spectrometry imaging on formalin-fixed paraffin-embedded tissue: A spoonful of sugar of the N-glycome* ***abs#021***	
10:10 AM	**Ian Loke** *Probing human white blood cells for a novel type of protein N-glycosylation* ***abs#022***	
10:30 AM - 11:00 AM	**Morning Tea**	Exhibition Hall
11:00 AM - 12:40 PM	**Symposium Six: New Technology** Chair: Mark Molloy	Heritage Ballroom
11:00 AM	**Andrew Webb** *MSCypher: A high-throughput peptide identification strategy for complex mixtures* ***abs#023***	
11:20 AM	**Nichollas Scott** *Speaker sponsored by* SIGMA-ALDRICH *Characterization of the protein complex landscape of murine tissues* ***Winner of the International Early Career Award*** ***abs#024***	
11:40 AM	**Christie Hunter** *Speaker sponsored by* SCIEX *Extending the Reach of Data Independent Acquisition – Microflow SWATH® Acquisition* ***abs#025***	
12:00 PM	**Yue Xuan** *Speaker sponsored by* ThermoFisher SCIENTIFIC *IS-PRM: A Smarter MS Method for Reproducible and Sensitive Pathway Characterization* ***abs#026***	
12:20 PM	**Roberto Castangia** *Speaker sponsored by* SHIMADZU *MALDI mass spectrometric imaging in an Alzheimer disease mouse model* ***abs#027***	
12:40 PM - 1:00 PM	**Lightning Talks Two** Chair: Ben Crossett	Heritage Ballroom

1st	**Alok Shah** *Towards a blood test for oesophageal adenocarcinoma: Serum glycoprotein biomarker candidates for oesophageal adenocarcinoma* ***abs#201***	
2nd	**Parul Mittal** *MALDI Imaging of primary endometrial cancers reveals proteins associated with lymph node metastasis* ***abs#202***	
3rd	**Desmond Li** *Post translational modifications in the type 2 diabetic liver and their contribution to the diseased state* ***abs#203***	
4th	**Melanie White** *The myocardial degradome following ischemia/reperfusion injury* ***abs#204***	
5th	**Naomi Belic** *Neuronal differentiation and analysis of Multiple sclerosis patient adipose derived stem cells* ***abs#205***	
1:00 PM - 2:30 PM	**Lunch | Poster Session Two**	Exhibition Hall
2:30 PM – 3:30 PM	**Annual General Meeting**	Heritage Ballroom
2:30 PM – 4:15 PM	**Free Afternoon and Erskine Falls Tour**	
4:15 PM – 5:20 PM	**Symposium Seven: Systems Biology** Chair: Marc Wilkins	Heritage Ballroom
4:15 PM	**Melissa Davis** *Proteomics in the systems biology of breast cancer: Enhancing pathway analysis and simulation* ***abs#028***	
4:40 PM	**Brian Drew** *A Trans-omics approach to identify novel regulators of hepatic metabolism* ***abs#029***	
5:00 PM	**Esteban Marcellin** *Proteomic characterisation of Clostridium fermentation* ***abs#030***	
5:20 PM – 7:00 PM	**Symposium Eight: Disease Proteomics** Chair: Michelle Hill	Heritage Ballroom
5:20 PM	**Thomas Kislinger** *Membrane proteomics: surface markers and horizontal signaling* ***abs#031***	
5:45 PM	**Torsten Kleffmann** *Quantitative proteomics of lipoproteins: insights into individual differences of lipoprotein associated proteins in the context of cardiovascular disease* ***abs#032***	
6:10 PM	**Sri Ramarathinam** *A Distinct Proteomic Signature of Latently Infected Cells Reveals New Drug Targets and Immunotherapeutic Targets for HIV* ***abs#033***	
6:30 PM	**Christoph Krisp** *Data-independent mass spectrometry phenotyping of patient-derived melanoma cancer cell lines correlates with MEK inhibitor resistance independent of genotype and predicts poor patient outcome* ***abs#034***	
7:15 PM – 12:00 AM	**APS Carnival**	Mantra Dining & Seagrass Lawn Area
Sunday 7th February		
9:00 AM - 10:30 AM	**Symposium Nine: Microbial Proteomics** Chair: Steve Djordjevic	Heritage Ballroom
9:00 AM	**Matthew Chang** *Reprogramming biological functionalities for autonomous microbial factories and therapeutics* ***abs#035***	*Speaker sponsored by* Agilent Technologies
9:30 AM	**Karel Novy** *Proteotype-based decoding of the viral signalling network orchestrating the assembly of poxvirus* ***abs#036***	

9:50 AM	**Florian Weiland** *Rapid identification of beer spoilage microrganisms using Biotyper* ***abs#037***	
10:10 AM	**James Broadbent** *S. aureus strain functional diversity as observed through pan-proteomics: an emerging approach for proteome measurement within a background of genetic heterogeneity* ***abs#038***	
10:30 AM – 11:00 AM	**Morning Tea (Winners of Vendors Prizes and Passport Prize)**	Exhibition Hall
11:00 AM - 12:15 PM	**Symposium Ten: Structural Proteomics** Chair: Andrew Webb	Heritage Ballroom
11:00 AM	**Andrea Sinz** *The Advancement of Chemical Cross-Linking/Mass Spectrometry in Structural Proteomics* ***abs#039***	
11:25 AM	**Ganesh Anand** *Whole Dengue viral particle breathing dynamics unravels quaternary temperature-specific changes across capsid proteome* ***abs#040***	*Speaker sponsored by* Waters THE SCIENCE OF WHAT'S POSSIBLE.®
11:50 AM	**Tara Pukala** *Structural characterisation of biomolecular assemblies by ion mobility-mass spectrometry* ***abs#041***	
12:20 PM - 1:00 PM	**Closing Keynote Lecture** Chair: Peter Hoffmann	Heritage Ballroom
12:20 PM	**Emma Lundberg** *Characterizing human organelle proteomes - Towards a complete Cell Atlas* ***abs#042***	
1:00 PM - 1:30 PM	**Closing Ceremony / Award Presentation** Chair: Stuart Cordwell	Heritage Ballroom
2:00 PM	**Buses Depart**	Mantra Lobby

POSTER SESSION ONE

Daniel A Broszczak
Proteome survey of wound fluid from non-healing wounds reveals key biological processes associated with poor healing outcomes ***abs# 101***

Soumya Mukherjee
Identification of the Fragmentation Features of Dityrosine cross-linked Amyloid beta in ESI-MS/MS ***abs# 102***

Kate E Scull
A bioinformatic solution for identifying non-genomic peptides in the immunopeptidome ***abs# 103***

Shivashankar H Nagaraj
Multi-omic analysis of Epithelial-Mesenchymal Transition in pancreatic cancer ***abs# 104***

Pouya Faridi
Proteomics-based approach to elucidate the mechanism of anti-hypercholestrolemia activities of selected herbal medicines ***abs# 105***

Chi-Hung Lin
A novel MRM_HR based glycosyltransferase assay ***abs# 106***

Christopher Ashwood
Effect of carbon source on the glycosylation pathway of Trichoderma reesei RUT-C30 ***abs# 107***

Laura F Dagley
Quantitative proteomics reveals novel diagnostic and prognostic markers of acute rheumatic fever. ***abs# 108***

Jarrod J Sandow
Proteogenomic profiling of acute myeloid leukaemia reveals novel insights into leukaemogenesis. ***abs# 109***

Nathan Croft
Differential abundance, but not kinetics, of virus-derived epitopes presented on infected fibroblasts and dendritic cells ***abs# 110***

David C.L. Handler
A new method of validation using the protein quantitation false discovery rate from control/control analysis ***abs# 111***

Edmond M Linossi
Investigation of SOCS5 signalling complexes by mass spectrometry uncovers a role in breast cancer ***abs# 112***

Anup D Shah
Life on the Rafts ***abs# 113***

Kris Ford
N-linked glycopeptide analysis of plant glycoproteins ***abs# 114***

Vineet Vaibhav
Queensland Unknown (QX) and Winter Mortality (WM) disease of Sydney rock Oysters: A comparative proteomics study. ***abs# 115***

Amanda P Woon
A proteomic approach to characterising antigen processing and presentation in bats. ***abs# 116***

Daniel Bucio Noble
Antioxidant and anti-inflammatory properties of sugarcane dietary fibre ***abs# 117***

Nicolai Bache
A Systematic Investigation of CID Q-TOF Collision Energies for Complete Ddentification of Glycopeptides by Mass Spectrometry ***abs# 118***

Roberto Castangia
Investigating lipid changes with PI3-K inhibition in colorectal cancer liver metastases by MALDI-MS imaging ***abs# 119***

Matthew O'Rourke
The development and implementation of a new microbial pathovarience system. ***abs# 120***

Samira Aili
Peptidomic and proteomic comparison of electrically stimulated and manually dissected venom from the South American bullet ant Paraponera clavata ***abs# 121***

Kayla Madonis
Characterisation of lipoprotein MPN_284, a potential adhesin of Mycoplasma pneumoniae ***abs# 122***

Marcelo Moreno
Moonlighting proteins in Mycoplasma hyopneumoniae: Investigating a pathogenic role for glycolytic enzymes and their cleavage fragments ***abs# 123***
Yunqi Wu
Comparative Proteomic Analysis of Two Rice Genotypes Exposed to Drought Stress and Recovery ***abs# 124***
Ghizal Siddiqui
Combination of metabolomics, peptidomics and proteomics to elucidate mechanisms of drug resistance in the malaria parasite ***abs# 125***
Chao Zhang
Characterization of vulvar cancer progression and metastasis by imaging mass spectrometry from formalin-fixed paraffin-embedded tissues ***abs# 126***
Samantha J Emery
Quantitative proteomics of metronidazole drug resistance in Giardia duodenalis ***abs# 127***
Jess Tacchi
Using proteomics to decipher novel protein function in a genome-reduced pathogen ***abs# 128***
Joshua J Hamey
A novel protein methyltransferase catalyses conserved N-terminal and lysine methylation on translational elongation factor 1A **abs# 129**
Daniela-Lee Smith
The "SRGG" Protein Interaction Code ***abs# 130***
Gene Hart-Smith
Large-scale mass spectrometry-based identifications of enzyme-mediated protein methylation are subject to high false discovery rates ***abs# 131***
Michael Widjaja
Multiple roles of elongation factor Tu in Mycoplasma pneumoniae ***abs# 132***
Ira Cooke
Proteomics of Cephalopod Venoms ***abs# 133***
Morten Thaysen-Andersen
Automated N-glycopeptide identification in glycoproteomics ***abs# 134***
Karthik Kamath
Understanding adaptation dynamics of Pseudomonas aeruginosa in cystic fibrosis lungs ***abs# 135***
Rafea Naffa
Isolation and Characterization of Natural Crosslinks in Animal skins by Liquid Chromatography and Electrospray Ionization-Mass Spectrometry Detection ***abs# 136***
Steven Ramsay
LC/MS Method Optimization for TMT Quantification using Q-Exactive instruments ***abs# 137***
Christie Hunter
Automated Protein Digestion to Reduce the Sample Preparation Bottleneck ***abs# 138***
Heather Patsiouras
DESI-MS Optimisation for the application of proteomic Imaging ***abs# 139***
Heather Patsiouras
Characterization and Collision Cross Section Determination of Lipids from Metabolic Syndrome Disorders ***abs# 140***
Lucy A Woods
Improvements in accurate mass GC-MS based Metabolomics: A novel atmospheric pressure GC-APCI Source increases quantitative and qualitative performance for metabolic profiling ***abs# 141***
Sean J. Humphrey
EasyPhos – a high-performance, scalable and universal phosphoproteomics platform ***abs# 142***
Richard Wilson
New elements of the chondrocyte stress response identified in a model of cartilage degeneration ***abs# 143***
Jodie L Abrahams
The N-Glycosylation Profile of Metastatic Melanoma Lymph Node Tumours ***abs# 144***
Joel A Cain
Proteomic and Degradomic Analysis of a Virulence-Associated Serine Peptidase from Campylobacter Jejuni ***abs# 145***

POSTER SESSION TWO

Alok Shah
Towards a blood test for oesophageal adenocarcinoma: Serum glycoprotein biomarker candidates for oesophageal adenocarcinoma ***abs# 201***

Parul Mittal
MALDI Imaging of primary endometrial cancers reveals proteins associated with lymph node metastasis ***abs# 202***

Desmond K Li
Post translational modifications in the type 2 diabetic liver and their contribution to the diseased state ***abs# 203***

Melanie White
The myocardial degradome following ischemia/reperfusion injury ***abs# 204***

Naomi Koh Belic
Neuronal differentiation and analysis of Multiple sclerosis patient adipose derived stem cells ***abs# 205***

Alison McAfee
Towards improving the genome annotation of the honey bee (*Apis mellifera*) ***abs# 206***

Stuart J. Cordwell
The *N*-linked glycosylation system of *Campylobacter jejuni*: a functional proteomics approach ***abs# 207***

Nicolai Bache
Applying a Proteoform profiling method for neurological disorder biomarker discovery ***abs# 208***

Ali Azimi
Label-free Proteomic Analysis of Microdissected Formalin-fixed and Paraffin Embedded Cutaneous Squamous Cell Carcinoma Tissue ***abs# 209***

Geoff Birrell
Proteomic Analysis of an Artelinic Acid-Resistant P. falciparum strain ***abs# 210***

Shannon Cornett
High-Speed Imaging Mass Spectrometry of N-Linked Glycans Provides a new approach to Molecular Histology and Tissuetyping of Tumors ***abs# 211***

Michael Mariani
Quantitative peptide assay for optimized and reproducible sample preparations for mass spectrometry applications ***abs# 212***

Thomas Stoll
Look who's talking: UHPLC-MRM/MS assay for staphylococcal quorum-sensing and virulence peptides ***abs# 213***

Bronwyn J Barkla
Epidermal bladder cells as a single-cell-type system to further our understanding of the molecular mechanisms involved in plant salt tolerance and water use efficiency. ***abs# 214***

Benjamin L Parker
Terminal galactosylation and sialylation switching on membrane glycoproteins upon TNF-alpha-induced insulin resistance in adipocytes ***abs# 215***

Kasper Engholm-Keller
Modulation of neurotransmitter release by phospho-signalling: Major substrates and master regulators ***abs# 216***

Kate Harvey
Elongation factor Thermo unstable (Ef-Tu) is proteolytically processed on the extracellular surface of *Staphylococcus aureus* ***abs# 217***

Steven Ramsay
An Integrated Approach to Metabolomics Studies in Pancreatic Cancer Cells: Discovery to "Quanfirmation" on a Single Platform ***abs# 218***

Abdulrahman M Shathili
Insight into protein glycosylation in human capacitated sperm using PGC-LC-MS/MS ***abs# 219***

Lawrence Mok
A comparative transcriptomic and proteomic analysis of the infection of mammalian cells with Nelson Bay orthoreovirus ***abs# 220***

Christie Hunter
Multi-omics analysis of primary cytotrophoblasts from second trimester and term placentas ***abs# 221***

Thiri Zaw
Quantitative proteomic analysis of plasma proteins in healthy neonates, young children and adults using SWATH-MS data independent acquisition ***abs# 222***

Jemma Wu
SWATH mass spectrometry performance using extended ion assay libraries ***abs# 223***

Michelle M Hill
Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS/MS) Proteomic Analysis ***abs# 224***

Serene Gwee
Phosphoproteomics reveals Aurora kinase B (AurkB) as a regulator of neuronal development ***abs# 225***

Nikeisha Caruana
A slimy situation: Using proteomics to highlight mechanisms of slime secretion in the southern bottletail squid, *Sepiadarium austrinum.* ***abs# 226***

Scott Bringans
PromarkerD: Biomarkers for Diabetic Kidney Disease ***abs# 227***

Lok Man
Characterisation of a Potential Nutrient Transporter Protein (Cj0025) in *Campylobacter jejuni* ***abs# 228***

Liang Li
Defining IGF-II and IR-A signalling in Breast Cancer Cells ***abs# 229***

Melanie C Foell
Investigation of prostasin's role in placental development by proteomic and degradomic approaches ***abs# 230***

Patricia Illing
Haptens Happen: Searching for drug modified Human Leukocyte Antigen-Peptide Complexes ***abs# 231***

Hans-Juergen Wirth
A Simple and Convenient Way to Pack Capillary LC Columns ***abs# 232***

Heather Patsiouras
Systematic Comparison of Integrated Microfluidics/Nanoscale LC Platforms and High/Low Resolution Mass Spectrometers for Quantitative MRM Analysis of Candidate Peptide Biomarkers ***abs# 233***

Heather Patsiouras
Enhancing the Analytical Capabilities of DESI Imaging Using Ion Mobility Separation - Providing Superior Insights of Biological Samples ***abs# 234***

Umesh Gaikwad
Compartment specific PTM study of Endogenous Protein Kinase C Using Mass Spectrometry in Human Breast Cancer Cell line ***abs# 235***

Maja Christiansen
Why use Ultra-High Resolution Quadrupole Time of Flight Instruments for Proteomics Applications? ***abs# 236***

Stephanie Rayner
Investigating the role of a new E3 ubiquitin ligase implicated in ALS disease pathology ***abs# 237***

Lucy A Woods
Improved capacity trapped ion mobility spectrometry ***abs# 238***

Lucy A Woods
Megapixel tissue imaging at high-speed: Evaluation of a next generation MALDI-TOF instrument ***abs# 239***

Mehdi Mirzaei
TMT One Stop Shop: from reliable sample preparation to computational analysis platform ***abs# 240***

Michelle Colgrave
The development of barley-specific peptide markers for food testing applications ***abs# 241***

Arlene D'Silva
A top-down proteomic approach to the identification of early serum biomarkers predictive of spontaneous preterm birth ***abs# 242***

Sean J. Humphrey
A human stimulome – mapping signalling network intersection downstream of major cell surface receptors ***abs# 243***

Christopher Ashwood
Novel *O*-glycans containing acidic sugars on fungal secreted protein and their characterisation using ESI-PGC-LC-MS/M ***abs# 244***

Jeffrey Molendijk
Mass spectrometry-based lipidomics analysis of Barrett's oesophagus and oesophageal adenocarcinoma ***abs#245***

ORAL PRESENTATIONS

001

Moving MS-based proteomics closer to the clinic

Matthias Mann[1]

1. *Max-Planck Institute of Biochemistry, Munich and Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark*

Mass spectrometry based proteomics has advanced tremendously in the last few years. We describe a shotgun proteomics workflow that allows us to detect and quantify the large majority of the proteins expressed in a biological system such as cancer cell lines and even formalin-fixed, paraffin embedded material. This included streamlined and highly efficient sample preparation, analysis with very high sequencing speed using modern mass spectrometers and bioinformatic analysis using the MaxQuant and Perseus platforms. Efforts in our group have focused on 'single shot' analysis and we demonstrate very high coverage in this mode (Mann et al., Mol. Cell, 2013). We have also extended this concept to the analysis of interactomes (Hein et al. Cell 2015) and phosphorylation, where the 'EasyPhos' method now allows acquiring large numbers of phosphoproteomes, for instance for the analysis of in vivo signaling (Humphrey et al. Nat. Biotech, 2015). Finally, we are currently re-visiting body fluid proteomics with a view to obtain protein profiles of large numbers of individuals in health and disease. Together such developments make proteomics increasingly relevant to translational research as I will discuss in this talk.

002

SIRT4 is a host mitochondrial lipoamidase that restricts human cytomegalovirus

Rommel Mathias[1, 2], **Todd M. Greco**[1], **Adam Oberstein**[1], **Hanna G. Budayeva**[1], **Elizabeth A. Rowland**[1], **Thomas Shenk**[1], **Ileana M. Cristea**[1]

1. *Department of Molecular Biology, Princeton University, Princeton, New Jersey, United States*
2. *La Trobe Institute for Molecular Science, Bundoora, VIC, Australia*

Publish consent withheld

1. Koyuncu E, Budayeva HG, Miteva YV, Ricci DP, Silhavy TJ, Shenk T et al. Sirtuins are evolutionarily conserved viral restriction factors. MBio 2014; 5.
2. Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA et al. Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. Cell 2014; 159: 1615-1625.

003

Asn347-Glycosylation of Corticosteroid Binding Globulin Fine-tunes the Host Immune Response by Modulating Proteolysis by *P. aeruginosa* and Neutrophil Elastase

Zeynep Sumer Bayraktar[1], **Oliver Grant**[2], **Vignesh Venkatakrishnan**[1], **Robert J. Woods**[2], **Nicolle H. Packer**[1], **Morten Thaysen-Andersen**[2]

1. *Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*
2. *Complex Carbohydrate Research Center, The University of Georgia, Athens, Georgia, USA*

Corticosteroid binding globulin (CBG) delivers anti-inflammatory cortisol to inflamed tissues upon elastase-based proteolysis of an exposed reactive center loop (RCL). However, the molecular basis of the RCL proteolysis by co-existing host and bacterial elastases and mechanisms regulating these events in inflamed and infected tissues remain unknown. We document that the RCL-localized Asn347-glycosylation is ideally positioned to regulate the RCL cleavage by human neutrophil elastase (NE) and P. aeruginosa elastase (PAE) and that it does so by different mechanisms. NE- and PAE-generated fragments of native and exoglycosidase-treated blood-derived CBG of healthy individuals were monitored by gel electrophoresis and LC-MS/MS to determine the cleavage site(s) and N-glycosylation status as a function of NE/PAE incubation time. The site-specific (Val344-Thr345) and rapid (seconds-minutes) NE-based RCL proteolysis was significantly antagonized by several volume-enhancing Asn347-glycan features i.e. site occupancy, triantennary β1,4/6-GlcNAc branching, β-galactosylation and α1,6-fucosylation and strongly augmented by Asn347 α2,3-sialylation (all $P < 0.05$). In contrast, the inefficient (minutes-hours) PAE-based RCL cleavage occurring equally well at Thr345-Leu346 and Asn347-Leu348 was completely antagonized by the Asn347-glycan, but received beneficial electrostatic interactions from sialoglycans of other CBG glycosylation sites. In addition, virulent P. aeruginosa growth was augmented by the free cortisol, which may explain the weak PAE efficiency in cleaving glycosylated RCL during host infection. Finally, molecular dynamics simulations of relevant CBG Asn347-glycoforms, carried out using a homology model of uncleaved CBG, largely confirmed the experimental observations and suggested additional interesting relationships between Asn347-glycosylation and RCL cleavage. In conclusion, we are the first to show that site-specific CBG N-glycosylation regulates the bioavailability of cortisol in inflamed environments by fine-

tuning the RCL proteolysis by endogenous and exogenous elastases via different mechanisms. This study offers new molecular insight into host- and pathogen-based manipulation of the human immune system.

004

The N-terminome of *Mycoplasma hyopneumoniae* reveals functionally-diverse proteins as targets of endoproteolysis.

Iain J Berry[1, 2], Jess L Tacchi[1, 2], Benjamin B A Raymond[1, 2], Michael Widjaja[1, 2], Matt P Padula[1, 2], Steven P Djordjevic[1, 2]

1. *Proteomics Core Facility, University of Technology, Sydney*
2. *The ithree Institute, University of Technology, Sydney*

Mycoplasma hyopneumoniae is a primary colonising agent and economically-devastating porcine respiratory pathogen that is controlled by partially-effective bacterin vaccine formulations and widespread antibiotic therapy. Adherence and colonisation is in part mediated by the P97 and P102 adhesin families that directly interact with the host extracellular matrix, via glycosaminoglycans, fibronectin and the fibrinolytic protein, plasminogen. Post-translational proteolytic cleavage is essential to the production of functional, mature proteoforms on the surface of this ubiquitous pathogen. We aim to determine whether proteolytic cleavage is restricted to the adhesin families using a high-throughput, proteome-wide methodology.

Using protein dimethyl labelling, reversed-charge enrichment of N-termini and 'shotgun' mass spectrometry, the N-terminal sequences of mature *M. hyopneumoniae* proteins were characterised. Our data interrogated protein start sites and identified the precise location of protein cleavage in functionally diverse proteins. These data were combined with surfaceome studies, affinity chromatography using diverse host molecules as bait and mass mapping by SDS-PAGE to confirm the existence of proteoforms and to investigate their putative functions.

Our approach confirmed the existence of multiple cleavage events, previously characterised in the adhesin families, validating our approach. Additionally, we identified internal cleavage events in lipoprotein families and proteins with well-studied canonical functions in the cytosol. The majority of the proteins targeted by endoproteolysis were identified in our surfaceome studies. Furthermore, our chromatography studies suggest that these proteins are multifunctional, with implications for the function of moonlighting proteins on the surface of *M. hyopneumoniae*. Consistent with this hypothesis, homologues of these 'moonlighting' proteins have been identified as virulence factors in other pathogenic bacteria but the data reported here is the first description of them being targets of endoproteolysis. The sequence motifs associated with the characterised cleavage events in this study are similar to those already described in studies of the adhesin families suggesting that the same protease(s) process functionally-diverse proteins on the cell surface. These observations have widespread ramifications for microbial pathogenesis and vaccine design.

005

Large-scale Phosphorylation Study of the Type 2 Diabetic Heart subjected to Control and Infarct Conditions

Lauren Smith[1, 2], Stuart Cordwell[1, 3, 2], Melanie White[1, 3, 2]

1. *Discipline of Pathology, University of Sydney, Sydney, NSW, Australia*
2. *Charles Perkins Centre, University of Sydney, Sydney*
3. *School of Molecular Bioscience, University of Sydney, Sydney, NSW*

Type 2 Diabetes (T2D) significantly increases the risk of a cardiovascular event, with acute myocardial infarction (AMI) the leading cause of mortality in these patients. The impact of T2D on the myocardial molecular mechanisms is however, poorly understood. Previous studies have demonstrated the essential role of protein phosphorylation to the outcomes following AMI. The current study uses large-scale phosphoproteomics to offer insight into the global impact of T2D on the heart and aims to elucidate the altered signalling cascades mediated by phosphorylation in response to AMI in the T2D setting.

Rats were fed a CHOW (12% fat) or high fat (HF) (42% fat) diet for 8 weeks, with T2D induced in 50% of the animals after 4 weeks utilising a low dose of Streptozocin (STZ; 35mg/kg) a pancreatic β-cell toxin. At the cessation of the feeding protocol, hearts were excised and subject to Langendorff perfusion to produce either non-ischemic time control (NITC) or ischemia / reperfusion (I/R) injury (30I/30R). Peptides were labelled with iTRAQ prior to enrichment of phosphopeptides utilising the TiSH method for analysis by tandem MS.

HF T2D hearts showed a significant decrease in functional recovery post-I/R (6.91 ± 2.42% versus 32.68 ± 4.98% in CH controls). This study identified 16,845 and 11,803 unique phosphosites, originating from 3,586 and 2,786 unique proteins from hearts subjected to NITC and I/R respectively. Using z-score cutoff >+1 or <-1, we observed 9,086 phosphosites significantly regulated following NITC and 6,432 phosphosites regulated by I/R. These regulated phosphosites were mapped to functional pathways utilising Kyoto Encyclopedia of Genes and Genomes (KEGG), identifying components of vital regulatory pathways such as the PI3K/Akt signalling cascade. By elucidating potential mechanisms behind the severely reduced capacity of the T2D heart to recover from AMI, improved outcomes and novel therapeutic targets are possible for these patients.

006

The proteome of blister fluid from paediatric burn injuries

Tuo Zang[1, 2], Daniel Broszczak[1, 2], James Broadbent[1, 2], Leila Cuttle[3, 1], Tony Parker[1, 2]

1. *TRR Program, IHBI-QUT, Kelvin Grove, Queensland, Australia*
2. *School of Biomedical Sciences, Queensland University of Technology, Brisbane, Queensland, Australia*
3. *Children's Burns and Trauma Research, Centre for Children's Health Research, South Brisbane, Queensland, Australia*

Burn injury is highly traumatic for paediatric patients. Scar formation is a nature outcome in severe burn and dependent on the severity of the burn injury. The early and accurate diagnosis of medium to severe burn is different according to current clinical assessment which is mainly based on the clinician's experience. These wounds represent a grey-area in scar formation and skin grafting. Thus, a more objective and quantitative measure to distinguish between burn severities is urgently required. Burn blister fluid is a viable study matrix that reflects systemic responses as well as the local microenvironment. This project seeks to characterise the blister fluid from children with different degrees of burn severity and determine severity specific protein markers to potentially aid in future clinical practice.

Blister fluid (BF) was collected from superficial thickness (n=12), deep-partial thickness (n=14), and full-thickness burn wounds (n=4). In order to characterise and identify BF proteins, the samples within each depth classification were pooled and subjected a variety of fractionation methods, including: filter aided sample preparation (FASP), lithium dodecyl sulphate polyacrylamide gel electrophoresis (LDS-PAGE), and Isoelectric OFFGel. Another 11 individual samples were processed by Multiple Affinity Removal Spin (MARS) cartridge. Then the enzymolytic products were analysed using LC-MS/MS to profile protein composition. The underlying biological processes were discovered using a gene ontology analysis.

We have demonstrated the strength of fractionation approaches to facilitate a deeper characterisation of burn blister fluid proteome. We identified, 228 proteins using the FASP, 365 by LDS-PAGE, 460 by MARS and 551 by OFFGel. Importantly, there were 812 different proteins identified in total including 107, 84, and 146 unique proteins in different burn severities, respectively, and 317 common to all three severities. The differences in proteins and biological processes demonstrated that there are subtle, yet significant, differences in the biochemistry of burn wounds according to severity. Moreover, this study represents the most comprehensive study of paediatric burn wound microenvironment to date.

007

A combination of bottom-up and top-down mass spectrometry provides insights on the post-translational regulation of yeast protein methyltransferases

Daniel L Winter[1], Muhammad Bin Zenaidee[2], Huixin Wang[2], Gene Hart-Smith[1], William A Donald[2], Marc R Wilkins[1]

1. *Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, UNSW, Sydney, New South Wales, Australia*
2. *School of Chemistry, UNSW, Sydney, New South Wales, Australia*

In recent years, protein methylation has been established as a major intracellular post-translational modification (PTM). Over twenty protein methyltransferases have been identified in *Saccharomyces cerevisiae* that target histone and non-histone substrates such as RNA-binding proteins, elongation factors and ribosomal subunits. The question remains as to how these enzymes are regulated. We overexpressed and purified six *Saccharomyces cerevisiae* protein methyltransferases (Hmt1, Rkm1, Rkm3, Rkm4, Set5, Efm7) in their native host and subjected them to digestion by either trypsin or LysargiNase, followed by analysis with either LC-HCD-MS/MS or LC-ETD-MS/MS. Additionally, undigested samples were analysed via top-down mass spectrometry with FT-ICR MS to investigate the co-occurrence of PTMs and the modform distribution of protein methyltransferases. This multi-protease, multi-fragmentation approach revealed a range of PTMs on several protein methyltransferases including phosphorylation, acetylation and methylation. Of particular interest is a range of PTMs on Rkm1, Set5, and the N-terminal tail of Hmt1. The N-terminal tail of Hmt1 has been shown to be implicated in modulating its oligomerisation state, which in turn regulates its activity. The close proximity of certain PTMs on Hmt1 and Set5 also raises the possibility of crosstalk between PTM pairs. In summary, the identification of several PTMs on protein methyltransferases suggests at least some methyltransferases are regulated post-translationally and are end points of signalling pathways.

008

Mitochondrial Changes Contribute to the Longevity of the daf-2 Mutant

Meng-Qiu Dong[1], Chun-Qing Song[1], En-Zhi Shen[1], Chao Liu[1], Wen-Hong Zhang[1], Yu-Xin Li[1], Han-Qing Zhao[1], Mei-Jun Zhang[1], Liping Wei[1], Si-Min He[2]

1. *National Institute of Biological Sciences, Beijing, China*
2. *Institute of Computing Technology, Chinese Academy of Sciences, Beijing, China*

We analyzed the mitochondrion organelle in the long-lived daf-2 mutant to find out whether reduced insulin/IGF-1 signaling affects mitochondria and if so, whether these changes contributed to the longevity phenotype. Compared to wild-type C. elegans, the long-lived daf-2 mutant showed a better ability in maintaining a youthful mitochondrial morphology and respiratory function at an old age. We therefore asked whether a young daf-2 mutant worm is equipped with more young mitochondria (therefore, more healthy mitochondria) to withstand aging. However, on adult day 1 the total amount of mitochondrial proteins in a daf-2 mutant

worm was found to be about the same as that in a wild-type worm. From quantitative proteomic analyses of purified mitochondria of wild type, daf-2 and daf-2; daf-16 mutant worms, we identified 81 up-regulated and 44 down-regulated mitochondrial proteins in the daf-2 mutant, and most of these changes were dependent on daf-16. These differentially expressed proteins revealed a shift of metabolic activity, especially in the metabolism of reactive oxygen species, branched-chain amino acids, β-alanine, propionate, and fatty acids. Further experiments suggested that altered metabolism contributed to the longevity of the daf-2 mutant. No abundance changes were detected in the electron transport chain proteins. There was a slight (10-20%) decrease of mitochondrial ribosomal proteins in the daf-2 mutant; although these changes were not significant for individual proteins, they were as a group. Consistently, by ribosome profiling, we found that the amount of assembled 28S mitochondrial ribosomes decreased by about 20% in the daf-2 mutant, suggesting a decrease in protein synthesis inside mitochondria and echoing with previously reported decrease of protein synthesis in the cytoplasm. We did not find evidence of mitonuclear protein imbalance in our data. Collectively, our results suggest that insulin/IGF-1 signaling regulates mitochondria and mitochondrial changes contribute to the longevity of the daf-2 mutant.

009

Proteomic-scale approaches for identifying reversible and irreversible cysteine redox post-translational modifications in myocardial ischemia / reperfusion

Jana Paulech, Melanie Y. White, Stuart J. Cordwell[1]

1. *School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia*

Redox post-translational modifications (PTM) are emerging as important regulatory mechanisms in signaling and pathogenesis. Cysteine (Cys) is the most redox active amino acid and is a target for these PTM, some of which are biologically reversible (e.g. disulfides, sulfenic acid) while others (sulfinic [Cys-SO_2H] and sulfonic [Cys-SO_3H] acids) are considered irreversible. We have developed enrichment methods to examine these PTM on a proteome-wide scale. Rapid and specific alkylation of free Cys, followed by thiol-based reduction and resin capture by thiol-disulfide exchange chemistry was applied to isolate reversibly modified Cys-containing peptides. The method was applied to a complex protein lysate generated from rat myocardial tissue and 6559 unique Cys-containing peptides from 2694 proteins were identified by tandem mass spectrometry (MS/MS). We next developed an enrichment method to isolate Cys-SO_2H/SO_3H-containing peptides from complex tissue lysates. The method is based on electrostatic repulsion of Cys-SO_2H/SO_3H-containing peptides from cationic resins (i.e. 'negative' selection) followed by 'positive' selection using hydrophilic interaction liquid chromatography (HILIC). We identified 181 Cys-SO_2H/SO_3H sites from rat myocardial tissue subjected to physiologically relevant concentrations of H_2O_2 (<100 μM) or to ischemia / reperfusion (I/R) injury *via* Langendorff perfusion. I/R significantly increased Cys-SO_2H/SO_3H-modified peptides from proteins involved in energy utilization and contractility, as well as those involved in oxidative damage and repair. Finally, we have combined these methods to enable multiplexed quantitative analysis of reversible/irreversible Cys redox PTM in response to I/R and in the presence of a broad-spectrum antioxidant (*N*-2-mercaptopropionyl glycine, MPG). We quantified >1350 Cys sites that are reversibly and/or irreversibly oxidized by I/R, including many sites that are protected by MPG. This technique allows for the quantitative profiling of reversible/irreversible Cys PTMs in response to oxidant / antioxidant stimulus, and their delineation within the context of protein abundance, during I/R injury and cardioprotection.

010

Starvation Regulates Ubiquitin-like Modifiers Critical for Stress Responses

Mark Larance[1], Angus I Lamond[1]

1. *Centre for Gene Regulation and Expression, University of Dundee, Dundee, United Kingdom*

We have shown previously that nutrient deprivation in the model animal *C. elegans* will alter signalling pathways that control longevity[1]. These responses are known to be regulated by protein-protein interactions, but such changes are largely uncharacterised. To identify the pathways regulated during mammalian starvation responses, we have used proteome-wide analysis of protein abundance and interactions in mouse tissues. We analysed liver, white adipose tissue, spleen and skeletal muscle after starvation (16 hours), compared to animals fed *ad libitum*. This label-free analysis allowed quantitation of >6000 proteins, the most comprehensive thus far.

One of the most surprising findings was the decreased abundance of the ubiquitin-like protein UFM1. The total abundance of UFM1 (including conjugated and unconjugated forms) was significantly ($P<0.000015$) decreased more than 2-fold in starved mouse liver. Other proteins in the UFM1-conjugation pathway were not significantly changed in abundance. Quantitative immunoblotting pinpointed the UFM1-UFC1 (E2) conjugate as significantly decreased in abundance in starved mouse liver. In addition, we were able to identify novel direct substrates of UFM1 conjugation involved in liver metabolism, using high resolution tandem MS analysis. We hypothesise that UFM1-conjugation is detrimental to their activity.

Previously, we developed a workflow for the analysis of native protein-protein interactions using protein correlation profiling[2]. We have now applied this method to analyse the starvation response in liver. These data show, for the first time, protein complex regulation is significant among the ~4000 proteins analysed, even in the absence of protein abundance changes. We observed a significant decrease in the interaction of UFM1 and its corresponding E1 (UBA5) and E2 (UFC1) enzymes after starvation. Like the UFM1 immunoblotting, this suggests a significant reduction in UFM1 pathway activation in response to starvation stress. Interestingly, UFM1 has previously been shown to have a role in stress responses such as ER stress and oxidative stress. Our finding

that starvation regulates this pathway and UFM1-target proteins has provided new mechanistic insight into the nutrient deprivation response.

1. Larance M, Pourkarimi E, Wang B, Brenes Murillo A, Kent R, Lamond AI, Gartner A. (2015). Global Proteomics Analysis of the Response to Starvation in C. elegans. Mol. Cell. Proteomics. 14(7):1989-2001.
2. Kirkwood KJ, Ahmad Y, Larance M, Lamond AI. (2013). Characterization of native protein complexes and protein isoform variation using size-fractionation-based quantitative proteomics. Mol. Cell. Proteomics. 12(12):3851-3873.

011

Higher-Order Structural Characterization of Therapeutic Antibodies at Residue Level by Middle-Down HDX-MS

Gary Kruppa[1], Christoph H. Borchers[2, 3], Jingxi Pan[2], Suping Zhang[2], Albert Chou[2, 1]

1. *MRM Proteomics Inc., Victoria, British Columbia, Canada*
2. *University of Victoria-Genome BC Proteomics Centre, Victoria, British Columbia, Canada*
3. *Department of Biochemistry & Microbiology, University of Victoria, Victoria, British Columbia, Canada*

(HDX-MS) strategies currently being used for protein structure and dynamics measurements include bottom-up and top-down approaches. The peptide-based bottom-up approach has no limit in protein size, but the small size of peptic peptides can lead to significant back exchange and incomplete sequence coverage, and the spatial resolution is often limited to peptide level. The top-down approach tackles these issues by analyzing intact proteins, but its success decreases rapidly as the protein size increases. In this presentation, we will discuss our development of a new 'middle-down' approach and its application in antibody characterization with ca. 100% sequence coverage.

Disulfide linkages in the antibody limit the ETD cleavage in top-down experiments and thus need to be. By performing ECD on the light chain and heavy chain separately after reduction and HPLC separation, we achieved complete sequence coverage for the light chain, but only 50% for the heavy chain (45 kDa). To expand the applicability of top-down HDX to larger proteins, we attempted to digest antibodies under HDX quench conditions into a limited number of specific fragments, We found three very large protein fragments after a brief proteolysis (1 min) of HER with a molecular weight of 12-25 kDa, that were well separated by HPLC within a 12 min run. The specific fragments obtained this way covered 100% of the light chain, and 95.3% of the heavy chain, representing a total coverage of 96.8% for the whole antibody. ETD fragmentation on these reached a spatial resolution of less than two residues and complete coverage for every fragment. The effect of deglycosylation on the HER antibody structure as revealed by HDX with top-down ETD and the structural characterization of intact antibodies will be presented, and the advantages and disadvantages of different HDX-MS strategies will be discussed.

012

Harnessing Targeted Proteomics to Enhance Yield, Salinity Tolerance and Thermal Tolerance of Wheat

Nicolas Taylor[1]

1. *ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley, WA, Australia*

Publish consent withheld

013

Proteome profiling of persistent honey bee (*Apis mellifera*) primary cells

Alison McAfee[1], Danica Baines[2], Shelley Hoover[2], Leonard J Foster[1]

1. *UBC, Vancouver, BRITISH COLUMBIA, Canada*
2. *Lethbridge Research Center, Lethbridge, Canada*

Honey bee (*Apis mellifera*) research has been limited by a scarcity of resources for molecular biology. Cell lines in particular are important tools for studying, *e.g.*, signalling cascades or host-pathogen interactions; however, only one stable honey bee cell line has been reported in the literature[1] and it is not widely used. Here we provide proteomic characterization of honey bee primary cells which can be maintained in cell culture for up to one year. The cells are a mixed population derived from worker pupae, so we were first interested in determining what proteins are regularly expressed and what organ or tissue type has the most overlap in expression profile. In this analysis, we identified 2,498 protein groups and the expression profile unequivocally overlaps with the thoracic salivary gland. We are now developing a lipid nanoparticle transfection method and are exploring the utility of the T2A self-cleaving peptide system in these cells. The T2A sequence can be used to liberate protein concatamers, allowing for expression of multiple proteins from one promoter. This system has been shown to be effective in Sf21 cell extracts for *in vitro* translation,[2] but has never before been investigated in the honey bee. The work described here represents important developments in the biochemical tools available to honey bee researchers, and will facilitate further research on this important insect.

1. Goblirsch MJ, Spivak MS, Kurtti TJ (2013) A Cell Line Resource Derived from Honey Bee (Apis mellifera) Embryonic Tissues. PLoS ONE 8(7): e69831. doi:10.1371/journal.pone.0069831
2. Wang QS, Jan E. Switch from Cap- to Factorless IRES-Dependent 0 and +1 Frame Translation during Cellular Stress and Dicistrovirus Infection. Preiss T, ed. PLoS ONE. 2014;9(8):e103601. doi:10.1371/journal.pone.0103601.

014

Quantitative Proteomics Reveals that Plant Plasma Membrane Microdomains are involved in Molecular Transport, Stress Responses and Callose Biosynthesis

Vaibhav Srivastava[1] and Vincent Bulone[1,2]

1. *Division of Glycoscience, School of Biotechnology, Royal Institute of Technology (KTH), AlbaNova University Centre, 106 91 Stockholm, Sweden*

2. *ARC Centre of Excellence in Plant Cell Walls and School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae, South Australia 5064, Australia*

The plasma membrane (PM) is one of the most interactive and dynamic membrane structures of the cell. It represents an interface between the cell and the extracellular environment and, as such, it is involved in many biological processes, e.g., metabolite and ion transport, endocytosis, defense against pathogens, cell differentiation and proliferation. The PM contains microdomains enriched in sphingolipids and sterols that are resistant to certain concentrations of detergents. The aim of this work was to determine the main functions of such microdomains in poplar cells through quantitative proteomics, using gel-based and solution (iTRAQ) approaches. Compared to PM, 80 proteins related to cell wall biosynthesis, transport, stress responses and signaling processes were found to be significantly enriched in the microdomains. The majority of these proteins were predicted to contain up to 16 transmembrane domains and/or membrane-anchoring acylation sites. The occurrence of S-acylation sites in PM and microdomain proteins was experimentally determined using an acyl-biotin exchange method and mass spectrometry. Out of the 450 S-acylated proteins in PM, 24 were enriched in microdomains, suggesting S-acylation plays a key role in the localization of these proteins to specialised PM subdomains. In addition, the number of transmembrane domains and their length were higher in the proteins isolated from the microdomains than in the total PM proteins. An important proportion of the most enriched microdomain proteins corresponded to cell wall biosynthetic enzymes, namely (1→3)-β-glucan (callose) synthases and related proteins, indicating that the isolated microdomains are the site for callose biosynthesis and regulation. The proteins identified most likely reflect the biological specialisation of the isolated microdomains in cell surface specific responses that trigger callose formation, e.g. stress responses, as well as a potential role in plasmodesmata formation and structure. The data will be discussed in relation with these key biological processes in plant cells.

015

Limitations on a biochemical arsenal — do centipede venoms evolve under morphological constraint?

Eivind AB Undheim[1], Brett Hamilton[2], Nyoman Kurniawan[3], Greg Bowlay[2], Darren Brown[1], Carsten Mueller[4], Andy Sombke[4], Bronwen Cribb[5], Jennifer Stow[1], Bryan G Fry[5], Deon J Venter[2, 6], Glenn F King[1]

1. *Institute for Molecular Bioscience, UQ, St Lucia, QLD, Australia*
2. *Pathology, Mater Hospital, Brisbane, QLD, Australia*
3. *Centre for Advanced Imaging, University of Queensland, St Lucia, QLD, Australia*
4. *Zoologisches Institut und Museum, Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany*
5. *School of Biological Sciences, University of Queensland, St. Lucia, QLD, Australia*
6. *School of Medicine, University of Queensland, St Lucia, QLD, Australia*

Venoms are complex biochemical arsenals, often containing hundreds to thousands of unique proteins and peptides. Despite their utility for prey capture, venoms are energetically expensive commodities, and consequently it is hypothesized that venom complexity is inversely related to the capacity of a venomous animal to physically subdue prey[1]. Centipedes, one of the oldest yet least studied venomous lineages, appear to defy this rule[2]. We examined a taxonomically diverse range of centipedes using a multidisciplinary approachthat included proteomics, transcriptomics, MALDI imaging, histology, immunohistochemistry, MRI, mCT, and electron microscopy to provide the first comprehensive insight into the composition and evolution of these ancient and unique venoms. Our results reveal a rich set of both novel and convergently recruited toxin types, but also substantial variation in toxin diversity between higher-order taxa. Surprisingly, despite lacking the formidable physical weaponry of scolopendrid centipedes, scutigeromorph venoms appear relatively non-complex. We show that the observed disparity in venom complexity between scolopendrid and scutigeromorph centipedes likely stem from morphological limitations of the venom gland, and that scolopendrids may be unique among centipedes in having evolved venom glands that can accommodate the peptide diversity characteristic of many arthropod venoms[3,4]. Moreover, the same morphological adaptations that allow great venom complexity in scolopendrid centipedes also appears to enable exquisite control over the composition of secreted venom. Our results provide further support for the concept that toxins are not universally expressed throughout the glands of venomous animals, and that this heterogeneity of toxin production can enable modulation of the composition of secreted venom. Our finding of morphological

constraint on toxin diversity has substantial implications for centipede venoms as a biodiscovery resource, and our results are a clear example of an interdisciplinary approach to understanding the evolution of venom systems may guide such efforts.

1. Morgenstern, D., King, G.F. (2013) The venom optimization hypothesis revisited. Toxicon 63:120-128.
2. Undheim E.A.B., Jones A., Holland J.W., Clauser K., Pineda S.S., King G.F., Fry B.G. (2014) Clawing through evolution: toxin diversification and convergence in the ancient lineage Chilopoda (centipedes). Molecular Biology & Evolution 31, 2124–2148
3. Undheim E.A.B., Sunagar K., Hamilton B.R., Jones A., Venter D.J., Fry B.G., King G.F. (2014) Multifunctional warheads: diversification of the toxin arsenal of centipede venoms via novel multidomain transcripts. Journal of Proteomics 102: 1–10
4. Undheim E.A.B., Hamilton B.R., Kurniawan N., Bowlay G., Cribb B., Merritt D., Fry B.G., King G.F., and Venter D.J. (2015) Production and packaging of a biological arsenal: centipede venom evolution under morphological constraint. Proceedings of the National Academy of Sciences USA, 112: 4026–4031

016

Tissue specific metabolite and lipid analysis using MALDI-FT-MS – linking spatial distribution to metabolic function

Ute Roessner[1, 2], Berin A Boughton[1], Daniel L Sarabia[2]

1. *Metabolomics Australia, School of BioSciences, The University of Melbourne, Melbourne, VIC, Australia*
2. *School of BioSciences, The University of Melbourne, Melbourne, VIC, Australia*

Imaging Mass Spectrometry (IMS) is a novel technique allowing the measurement of the spatiotemporal distribution of many biomolecules, including lipids and metabolites, in thin sections of biological tissues. The growing field of spatial lipidomics and metabolomics is developing and adopting IMS as a complementary analytical tool to conventional 'omics analyses of crude tissue extracts. Here we present the advantages and disadvantages of Ultrahigh Mass Resolution Matrix Assisted Laser Desorption Ionisation Fourier Transform Ion Cyclotron Resonance IMS and describe potential applications of spatial lipid and metabolite analysis in different biological tissues such as from mammals or plants. We will discuss how IMS will contribute to our increasing knowledge base of biological systems under investigation by providing information on the spatial distribution of lipids and metabolites.

017

Discovery of mechanisms of action for antimalarial drugs by medium-throughput metabolomics screening

Darren Creek[1]

1. *Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia*

New drugs are urgently required for the treatment of malaria, due to the emergence of multi-drug resistant forms of the causative parasite, Plasmodium falciparum. Recent drug discovery programs utilising high-throughput phenotypic screens have identified thousands of small molecules that have antiparasitic activity against P. falciparum in vitro, and a representative subset of these, known as the 'Malaria Box', have been released openly to the research community . However, little is known about the mechanism of action of these compounds, restricting further development of these hit compounds.

Systems biology techniques offer an ideal platform to investigate mechanisms of drug action in a hypothesis-free manner. This approach is ideal for the de novo identification of drug mechanisms where no prior knowledge about the mode of action is available. Specifically, metabolomics provides a snapshot of the metabolic state of the parasite after drug treatment, and can reveal the direct impact of a test compound on metabolism.

LCMS-based untargeted metabolomics methods for P. falciparum cultures were optimised for 96-well plate format, allowing analysis of the metabolic phenotype induced by 100 known and novel antimalarials. Many of the novel compounds induced metabolic phenotypes that clustered with known antimalarials. In particular, inhibition of pyrimidine biosynthesis was a common mechanism of antimalarial action. Furthermore, depletion of unique parasite peptides and lipids indicated inhibition of parasite-specific pathways that may provide attractive new targets for drug discovery. This work demonstrates the suitability of metabolomics approaches for medium-throughput screening of the impact of drug candidates on cellular biochemistry, and revealed mechanisms of action associated with many of these novel antimalarial compounds.

018

MALDI imaging analysis of the aging human lens metabolome

Angus C Grey[1], Seung Seon Lee[1], Michael J-C Lin[1], Mitchell G Nye-Wood[1], Paul J Donaldson[1]

1. *University of Auckland, Auckland, New Zealand*

The transparent ocular lens focusses light onto the retina in order to form a sharp image. The lens is remarkable in that it is able to remain transparent over many decades of life despite the lack of a blood supply or *de novo* protein synthesis and turnover in the lens centre. Cells in the lens centre are formed *in utero* and maintained throughout life, and contain long-lived proteins whose function must also be maintained. To protect against oxidative-stress induced protein damage, the lens contains high levels of antioxidants, such as glutathione and vitamin C. The development of age-related cataract is thought to be related to changes in lens antioxidants and other metabolites in specific lens regions, and leads to the protein damage, insolubilisation and opacification that

characterises lens cataract. In this study, lens small molecules and metabolites have been mapped in the aging human lens to define lens metabolome changes associated with normal lens aging.

Frozen donor human lenses were sectioned at 20mm and collected on gold MALDI targets using methanol soft-landing. NEDC matrix (7mg/ml in 90% MeOH) was applied using a TM-Sprayer (HTX Technologies). MALDI imaging data sets of all lens ages were analysed simultaneously at 50-150um spatial resolution using a MALDI-TOF/TOF mass spectrometer (Bruker UltrafleXtreme) in negative ion mode. Data sets were normalised to total ion current, and MALDI images plotted using flexImaging v4.1with pixel interpolation on. Where possible, on-tissue tandem mass spectrometry was used to confirm metabolite identities.
Over one hundred signals for small molecules were detected in the m/z range 80-1000, including major lens antioxidants, amino acids and nucleotides. Generally, ion signal for lens antioxidants decreased with increasing lens age, while other unidentified ion signals increased in specific lens regions. This study highlights the metabolic changes that take place in the aging human lens, providing a baseline measure for age-related changes to be compared with metabolic changes associated with cataract formation.

019

Glycomics in Health and Disease

Anne Dell[1]

1. *Department of Life Sciences, Imperial College , London, United Kingdom*

Every cell has multiple types of glycan on its surface where they play vital roles in cell-cell and cell-matrix interactions. Structural determination is essential for understanding the roles that glycans play in biological systems. Mass spectrometry (MS), with its ultra-high sensitivity and ability to analyse complex mixtures of glycans, is the most powerful tool currently available for glycan structure analysis. Our laboratory is engaged in numerous world-wide collaborations in which we exploit high sensitivity glycomic and glycoproteomic methodologies for the structural characterization of glycans found in a diverse range of biological material. This presentation will describe MS strategies for defining the glycomes of cells, tissues and purified glycoconjugates, as well as establishing glycoprotein site-specific glycosylation. The broad range of our research activities will be illustrated by data from ongoing collaborative projects embracing host pathogen interactions and human health and disease.

This work is supported by the BBSRC and the Wellcome Trust.

020

Alterations in Glycosylation in Ovarian Cancer

Nicolle H. Packer[1, 2], Merrina Anugrahama[2], Francis Jacob[3], Viola Heinzelmann-Schwarz[3], Matthew Briggs[4], Peter Hoffmann[1, 4], aRUN Everest – Dassa[1, 2]

1. *ARC Centre of Excellence for Nanoscale BioPhotonics (CNBP), Macquarie University , Sydney, NSW, Australia*
2. *GLYCO group @ MQ, Department of Chemistry & Biomolecular Sciences, Biomolecular Frontiers Research Centre, Faculty of Science, Macquarie University, Sydney, NSW, Australia*
3. *Ovarian Cancer Research, Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland*
4. *Adelaide Proteomics Centre, School of Biological Sciences, University of Adelaide, Adelaide, SAq, Australia*

Altered glycans have long been associated with cancer and are displayed on such cancer protein markers as CA-125, CA19-9, a fetoprotein, MUC 1, PSA, etc but have never been fully exploited as indicators of cancer in their own right or as an adjunct to other biomarkers.

We now have the mass spectrometric technology to detect the glycosylation changes on proteins and lipids and to accurately assign glycan structures including sequence and linkage in both cell lines and tissue samples[1]. We can also now correlate these changes with the genes and epigenetic changes responsible for the aberrant glycans observed. Furthermore, we have shown that specific glycan masses can be used to precisely image the localisation of different tissue types in formalin fixed paraffin embedded tissue sections [2].

Ovarian cancer is a cancer which is not only difficult to detect at early stage but is also controversial in regard to its tissue origin and metastatic progression. We show that there are significant changes in glycosylation in proteins and lipids between ovarian cancer cell lines that are reflected by changes in expression of the glycosylation pathway genes. We also show that glycan structures can differentiate between patient tissues of ovarian, peritoneal and tubal origin subtypes. Furthermore we demonstrate that specific single glycan structures can localise tissue types in formalin fixed paraffin embedded tissue sections of ovarian cancer samples.

1. Everest-Dass AV, Kolarich D, Campbell MP, Packer NH (2013)Tandem mass spectra of glycan substructures enables the multistage mass spectrometric identification of determinants on oligosaccharides. Rapid Commun Mass Spectrom.27:931-9
2. Gustafsson OJ, Briggs MT, Condina MR, Winderbaum LJ, Pelzing M, McColl SR, Everest-Dass AV, Packer NH, Hoffmann P. (2015) MALDI imaging mass spectrometry of N-linked glycans on formalin-fixed paraffin-embedded murine kidney. Anal Bioanal Chem. 407:2127-39.

021

MALDI mass spectrometry imaging on formalin-fixed paraffin-embedded tissue: A spoonful of sugar of the *N*-glycome

Matthew Briggs[1, 2], Arun Dass[3], Julia Kuliwaba[4, 5], Dzenita Muratovic[4, 5], David Findlay[4], Martin Oehler[6], Nicki Packer[3], Peter Hoffmann[1, 2]

1. *University of Adelaide, Adelaide, S.A., Australia*
2. *Adelaide Proteomics Centre, School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA*
3. *Faculty of Science, Biomolecular Frontiers Research Centre, Macquarie University, Sydney, NSW*
4. *Discipline of Orthopaedics and Trauma, The University of Adelaide, Adelaide, SA, Australia*
5. *Bone and Joint Research Laboratory, SA Pathology, Adelaide, SA, Australia*
6. *Department of Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, SA, Australia*

In humans, it is estimated that 50-60% of proteins are glycosylated. *N*-linked glycans are of particular interest in diseases such as ovarian cancer and osteoarthritis because structural alterations have been observed. Further investigation of these alterations may unearth novel biomarkers for early stage diagnosis. A novel method for investigating tissue-specific *N*-linked glycans was recently developed by our group on formalin-fixed paraffin-embedded (FFPE) murine kidney. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) spatially profiles *N*-glycans in tissue-specific regions, while through liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) the corresponding glycol compositions are structurally characterized.

Regions of interest such as tumour, stroma, adipose and necrotic tissue were isolated from FFPE serous ovarian cancer tissue sections while cartilage and bone marrow tissue were isolated from FFPE osteoarthritic bone tissue sections. *N*- and *O*-linked glycans were structurally characterized through enzymatic peptide-N-glycosidase F (PNGase F) release of N-glycans, followed by β-elimination of*O*-glycans. The released glycans were analyzed through porous graphitized carbon liquid chromatography (PGC-LC) and collision induced negative mode fragmentation analysis. Following structural characterization, high resolution MALDI-IMS revealed the spatial distribution of these identified *N*-glycans and their tissue-specificity on consecutive FFPE tissue sections. For example, tumour and non-tumour tissue regions established clear demarcation based on their *N*-glycan distribution.

MALDI-IMS and LC-ESI-MS/MS were used as complementary techniques to generate high resolution images and structural information of tissue-specific *N*-glycans. Furthermore, *O*-glycoforms were characterized for the first time in FFPE ovarian tumour tissue sections. Application of this method enabled the first steps in the glycomic quest for the Holy Grail: an early stage diagnosis biomarker.

022

Probing human white blood cells for a novel type of protein *N*-glycosylation

Ian Loke[1], Nicki Packer[1], Morten Thaysen-Andersen[1]

1. *Macquarie University, North Ryde, NSW, Australia*

Mammalian asparagine (*N*)-glycosylation covers three well-established classes of high mannose, hybrid and complex type glycans expressed on membrane or secreted glycoproteins. We have recently proposed paucimannosylation ($Fuc_{0-1}Man_{1-3}GlcNAc_2$) as an unconventional *N*-glycan class in human neutrophils [1,2]. Despite their suggested immuno-functions, the presence of these truncated *N*-glycoproteins in other white blood cells has received little attention. We here explore the expression of paucimannosylation across multiple white blood cell types involved in the innate and adaptive immune response. Utilising PGC-LC-MS/MS, the *N*-glycomes of neutrophils, lymphocytes, monocytes and platelets isolated from whole blood of a healthy individual were profiled. Existing glycome data of white blood cells from multiple donors deposited in the Consortium for Functional Glycomics repository were also reinterpreted for the existence of these novel structures. In both approaches, paucimannosidic *N*-glycans were found to be highly enriched in the granulocytes i.e. eosinophils and neutrophils relative to the other immune cells in the myeloid and lymphoid lineages. Paucimannosidic glycoproteins were confirmed in isolated neutrophils with immunofluorescence using paucimannose-recognising antibodies. Co-localisation of paucimannosidic epitopes with β-hexosaminidase A and myeloperoxidase yielded clues to their subcellular localisation in the azurophilic granules of the neutrophil. Collectively, this suggests that protein paucimannosylation is a granulocyte-enriched glycosylation feature arising from cell-specific mechanisms during granulopoiesis. This map of protein paucimannosylation in white blood cells is important to understand the immunological role(s) of this novel class of human glycoproteins.

1. Thaysen-Andersen, M., Venkatakrishnan, V., Loke, I., Laurini, C., Diestel, S., Parker, B.L., Packer, N.H., 2015. Human Neutrophils Secrete Bioactive Paucimannosidic Proteins from Azurophilic Granules into Pathogen-Infected Sputum. Journal of Biological Chemistry 290 (14), 8789-8802.
2. Loke, I., Packer, N.H., Thaysen-Andersen, M., 2015. Complementary LC-MS/MS-Based N-Glycan, N-Glycopeptide, and Intact N-Glycoprotein Profiling Reveals Unconventional Asn71-Glycosylation of Human Neutrophil Cathepsin G. Biomolecules 5 (3), 1832-1854.

023

MSCypher: A High-Throughput Peptide Identification Strategy for Complex Mixtures

Eugene Kapp[1], Giuseppe Infusini[1], Yunshan Zhong[1], Laura Dagley[1], Terry Speed[1], Liam O'Connor[1], Andrew I Webb[1]

1. *Walter and Eliza Hall Institute, Parkville, VIC, Australia*

Shotgun proteomics based on high-resolution mass spectrometry (MS) is transforming biological research in the post-genomic era and offers an unbiased analytical profiling method that complements existing targeted MS and antibody-based approaches. However, using current technology only about 15-30% of the >100,000 observable individual peptide isotopic patterns[1] are ever identified. During traditional data-dependant MS acquisition (DDA) of complex samples, nearly all MS/MS spectra generated contain contaminating fragment ions that arise from the isolation and fragmentation of multiple co-eluting peptides. Identification of these chimeric spectra is limited by the 'one MS/MS spectrum–one peptide' strategy used in nearly all available MS/MS search algorithms.

Here we report MSCypher, a new proteomics workflow that combines an improved tandem MS acquisition strategy with a novel search algorithm. Our initial benchmarking of MSCypher shows significant improvements in speed and sensitivity of peptide detection and identification. This high-resolution MS approach takes advantage of co-eluting peptides and uses a dynamic isolation window to evenly distribute the number of co-fragmented species during acquisition. Processing these multiplexed spectra datasets has required a redesign of the current analysis workflow to include retention time prediction, dynamic probabilistic fragment ion matching and a supervised ensemble learning method for peptide feature identification.

Our initial results indicate that this multiplexed MS/MS approach provides both enhanced sensitivity and a gain in coverage. Each of these improvements was proportional to the number of peptides simultaneously analysed. We also maintain high confidence levels for peptides identified and our latest experiments indicate that identification of modified peptides will also be improved using this strategy.

1. Michalski, A., Cox, J., & Mann, M. (2011). More than 100,000 detectable peptide species elute in single shotgun proteomics runs but the majority is inaccessible to data-dependent LC– MS/MS. Journal of proteome research, 10(4), 1785-1793

024

Characterization of the protein complex landscape of murine tissues

Nichollas E Scott[1], Leonard J Foster[1]

1. *Centre for High-Throughput Biology, Foster Laboratory, Vancouver, BC, Canada*

The recent improvements in proteomics technologies have enabled the distribution and relative abundances of proteins to be characterized across tissues in unprecedented detail. These studies have shown that even tissues from unrelated developmental lineages are composed of extremely similar protein repertoires, suggesting that additional layers of co-ordination are responsible for tissue functionality. As the ability of proteins to form higher order associations, also known as protein complexes, allows for dramatic changes in functionality by the exchange of subunits global differences within tissues interactomes may account for tissue functionality. As traditional high-throughput protein-protein interaction (PPI) approaches are untenable for tissues, we have applied an alterative means to explore this hypothesis by use of size exclusion chromatography protein correlation profiling SILAC (SEC-PCP-SILAC). Using a super-SILAC based approach, we profiled the interactome landscape of seven tissues (Brain, Lung, Liver, Heart, Skeletal Muscle, Thymus and Kidney), and we generated quantitative profiles of protein distributions across both SEC fractions and between tissues. Protein chromatograms across the SEC gradients were assessed based on correlation and co-enrichment, to determine PPIs using the principle of guilt by association. Using our PCP-SILAC approach, 7054 protein groups were identified across the seven tissues, with 5231 enabling the mapping of protein profiles. From these profiles, 36554 protein interactions could be determined with a precision of ~68% based on comparison to the CORUM database., Over 80% of proteins were observed in all tissues, indicating that at the protein level these tissues were extremely similar, however of note only ~30% of interactions were shared between tissues. Between functionally related tissues, this overlap increased to >60% consistent with the interactome shaping function. In total, 3304 protein complexes were mapped across tissues with the majority showing high similarity in membership (>0.75), however tissue specific exchanges of subunits were common. Taken together, we observed that complex heterogeneity between tissues is extremely common, and may shapes the functionality of tissues.

025

Extending the Reach of Data Independent Acquisition – Microflow SWATH® Acquisition

Christie Hunter[1], Nick Morrice[2]

1. *SCIEX, Redwood City, CA, United States*
2. *SCIEX, Warrington, Cheshire, United Kingdom*

Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow variable width Q1 windows can improve peptide detection and increase sample

coverage. Many labs are now using DIA to perform larger scale quantitative proteomic experiments with solid reproducibility on 1000s of proteins in complex matrices. As this technique increasingly proves to be a solid tool for biomarker research, larger sample sets are being analyzed, driving the need for further investigation of workflow improvements for throughput and robustness.

Here microflow LC was investigated in combination with SWATH® acquisition on a number of complex matrices, to assess depth of coverage and robustness relative to current nanoflow strategies. The MS analysis was performed on a TripleTOF® 6600 System (SCIEX) equipped with an nanoLC™ 425 system (SCIEX) with microflow modules. A number of acquisition parameters were explored to understand the workflow options in this flow regime. Key workflow recommendations have been established to provide researchers additional strategies for performing large scale, higher throughput SWATH acquisition studies.

026

IS-PRM: A Smarter MS Method for Reproducible and Sensitive Pathway Characterization

Yue Xuan[1], Michael A. Blank[1], Daniel Ayoub[2], Sebastien Gallien[2], Antoine Lesur[2], Bruno Domon[2], Andreas Huhmer[1]

1. *Thermo Fisher Scientific , San Jose, CA, United States*
2. *Luxembourg Clinical Proteomics Center, Luxembourg Institute of Health, Strassen, Luxembourg*

Publish consent withheld

027

MALDI mass spectrometric imaging in an Alzheimer disease mouse model

Lyna Sellami[1], Marcia Roy [2], Roberto Castangia[1], Omar Belgacem [1]

1. *Shimadzu UK, Manchester, UK, United Kingdom*
2. *Centre for Clinical Brain Sciences/Centre for Neuroregeneration , University of Edinburgh, Edinburgh, UK*

Mass Spectrometry Imaging (MSI) is an emerging technology for biomarker discovery and for enabling comprehensive molecular imaging in complex biological matrices. It is a suitable technique for the identification of compounds without an *a priori* knowledge of the composition of the sample. Since the location of compounds on tissue sections is preserved, MSI allows the identification of local abundant species in cases where spatial distribution would be lost if the whole tissue is homogenised.

In our study, we aimed to visualize the molecular distribution of molecular entities in various brain regions during the progression of Alzheimer's disease using a well-known mouse model. This neurodegenerative disease was investigated by comparing the CRND8 mouse model to the wild type. A pilot study of nine mice was carried out. We performed in-situ digests on tissue sections which were subsequently analysed by MALDI TOF-TOF. High resolution images were acquired on various regions of interest including the hippocampus, cortex and cerebellum.

We examined molecular changes and mapped targeted entities according to their location. These data were investigated in parallel with relative quantification experiments performed using complementary techniques i.e. LC-MS.

Statistical analyses were performed on the mapped species and correlated to the brain regions. Spectra from the regions of interest, such as cerebellum, were investigated.

Our aim is to highlight specific candidates that may be linked to Alzheimer's disease.

028

Proteomics in the systems biology of breast cancer: Enhancing pathway analysis and simulation

Melissa Davis[1]

1. *Biochemistry and Molecular Biology, University of Melbourne, Parkville, VIC, Australia*

Much pathway analysis is conducted using transcriptomic data to identify statistically significant associations between genes that are grouped by pathway membership and the differentially expressed transcripts measured in a set of samples. While this has yielded valuable insights into the functional differences between normal and cancerous tissues, or between subtypes of cancer, it is well known that changes in transcript abundance are not necessarily predictive of protein function in signalling cascades. Here I will describe our work to generate a simulation framework for integrating transcriptomic and proteomic data using description logic models of signalling pathways to interpret the measured molecular abundances in breast cancer cell lines.

029

A Trans-omics Approach to Identify Novel Regulators of Hepatic Metabolism

Anna C Calkin[1], Benjamin L Parker[2], Sarah C Moody[1], Eser J Zerenturk[1], Yingying Liu[1], Ross Lazarus[1], Peter J Meikle[1], Elizabeth J Tarling[3], Aldons J Lusis[3], Thomas Q Vallim[3], David E James[2], Brian G Drew[1]

1. *BakerIDI Heart & Diabetes Institute, Prahran, VIC, Australia*
2. *Metabolic Systems Biology Group, Charles Perkins Centre, University of Sydney, Sydney, NSW, Australia*
3. *David Geffen School of Medicine, UCLA, Los Angeles, CA, USA*

Background: The liver controls numerous pathways central to the maintenance of whole body lipid and glucose metabolism. Accordingly, disruption of these pathways promotes diseases including hepatosteatosis, insulin resistance and cardiovascular disease. However, even though these ailments are amongst the leading causes of death in developed countries, their mechanistic underpinnings are still not well defined.

Aims & Approach: In this study we sought to use a trans-omics approach utilising genetics, metabolomics, phenomics, lipidomics and proteomics to identify novel pathways involved in regulating hepatic metabolism. To do this we took advantage of our exclusive access to a panel of >100 genetically diverse inbred mouse strains, which to our knowledge is the largest of its kind in the world, known as the hybrid mouse diversity panel (HMDP) at UCLA.

Methods: We collected livers (n=3) from male mice of 107 HMDP strains that were all housed, fed and sacrificed under the same conditions. We performed deep proteomic analysis on livers (~320) by performing 34 separate TMT-10plex multidimensional LC-MS/MS experiments with SPS-MS3 acquisition on an Orbitrap Fusion. On the same samples we performed quantitative lipidomics analysis using LC-MS/MS on an AB Sciex API4000 Q/TRAP and Analyst 1.5 data system.

Results: Proteomic analysis resulted in quantification of >5,000 proteins with excellent reproducibility within strains and significant variance in ~2500 proteins between the 107 strains. Lipidomics analysis resulted in quantification of 312 lipid species across 23 lipid classes in which significant variation was observed in >100 lipid species. Bioinformatics analysis has identified numerous protein-lipid associations. Once combined with pre-exisiting phenomics and metabolomics data from the HMDP, we will begin dissecting the interacting networks and their influence on disease phenotypes.

Conclusions: We have established a high-resolution trans-omics network for the identification of novel regulators of hepatic metabolism. When considering both the depth in quantitative analyses and the unique sample sets utilized, we believe this to be the most comprehensive of its kind in Australia, if not internationally.

030

Proteomic characterisation of Clostridium fermentation

Esteban Marcellin[1], Nicolas Zaragoza[1], Cuauhtemoc Licona-Cassani[1], Lars K Nielsen[1]

1. *The University of Queensland, St Lucia, QLD, Australia*

Clastridia offers numerous advantages for the production of sustainable fuels and chemicals without compromising food security or being dependent the availability of arable land. Advantages over the use of conventional (sugar, oil or algae) feedstocks also include minimal water and nutrient demand and the capability to capture greenhouse gases that would otherwise be emitted into the atmosphere. Practically all carbon-containing feedstocks, including industrial off-gases and gasified agricultural and municipal waste, can be readily utilised or converted to syngas for subsequent transformation into fuels and chemicals.

Acetogens such as *Clostridium ljungdahlii* or *Clostridium autoethanogenum* use the reductive acetyl-CoA (Wood-Ljungdahl) pathway as a terminal electron-accepting, energy-conserving, CO2-fixing process. This pathway is speculated to be the first biochemical pathway in existence on Earth and continues to play a key role in the global acetate cycle with annual acetogenesis in sediments and termite hindguts estimated to amount to several trillion kg of acetate. Energy metabolism in acetogens is complex and many aspects are only partly understood.

While all acetogens use the Wood-Ljungdahl pathway to fix CO2, they vary in terms of redox coupling (cytochromes, sodium translocating Rnf or proton translocating Rnf) and co-factor use in the bifurcating hydrogenase (NADPH or NADH). Understanding the complex energy metabolism is critical, since ATP availability is a fundamental limitation inengineering acetogen metabolism. *C. autoethanogenum* offers a robust engineering system and a flexible platform for syngas fermentation. Fermentation of *C. autoethanogenum*has high product selectivity, tolerates a broad range of gas compositions and accepts contaminants well, making it the preferred microorganism for industrial gas fermentation.

Using *C. autoethanogenum* as the system for this study, we used a multi-omics approach in order to gain a measure of enzyme capacity (transcriptomics and proteomics) and thermodynamic driving force (metabolomics). By comparing gene expression and transcription when cells are grown autotrophically and heterotrophically (CO, CO2 and H2 vs fructose fermentation) we found that, compared to *C. ljungdahlii* in *C. autoethanogenum* the Rnf complex is extremely efficient and is able to maintain high ATPase activity at the transcriptional (RNA-seq) and translational (iTRAQ) levels even when cells are fermented exclusively using gas. Furthermore, our RNA-sequencing data show that under autotrophic conditions, genes in the Rnf complex are highly transcribed, resulting in equi-molar quantities of ATP (metabolomics data). This high efficiency of the electron chain transfer, coupled to the recently discovered electron bifurcating hydorgenases is potentially what makes *C. autoethanogenum* so unique and a great industrial platform for the conversion of syngas into valuable fuels and chemicals.

031

Membrane Proteomics: Surface Markers & Horizontal Signaling

Thomas Kislinger[1]

1. *Princess Margaret Cancer Center, Toronto, ONTARIO, Canada*

Solid tumors are complex tissues composed of different cell types (i.e. cancer cells, fibroblasts, immune cells, endothelial cells, etc.) and noncellular components such as the extracellular matrix, soluble factors, pH, oxygen tension and interstitial pressure. While decades of research have identified key oncogenic signaling pathways and more recently through next-gen sequencing (epi)genetic alterations, current therapeutic strategies are still inefficient in curing most cancers. It has become evident that the highly complex crosstalk within the tumor microenvironment actively modulates tumor growth, metastasis and response to therapy. This intracellular crosstalk is mediated by cell-surface receptors, soluble ligands and extracellular vesicles. In-depth studies of membrane proteins have proven to be difficult because of their low abundance and hydrophobicity. However, recent advances in proteomic technologies make it possible to investigate proteins in this cellular compartment, to an impressive depth. Here, I will present our recent work using in-depth proteomics to obtain novel insights into head and neck cancer. Cell-surface labeling strategies, such as silica-bead coating, have enabled us to interrogate the head and neck cancer surfaceome to an unprecedented depth. Integration of these data with publicly available genomics data enabled us to discover novel, previously unstudied surface markers that could serve as novel drugable targets. Functional data on some surface markers will be presented. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type of the stroma and are key components involved in regulating carcinogenesis. Recently, CAF-secreted exosomes were shown to be important mediators of paracrine signals that promote motility, and metastasis in breast cancer. To investigate stromal heterogeneity in OSCCs we isolated matched pairs of human primary fibroblasts from resected tumors (CAFs) and adjacent tissue (AFs) and characterized them according to established CAF markers. Comprehensive proteomics identified a signature of CAF-enriched exosomal proteins potentially involved in pathways mediating tumor-stromal crosstalk. Functional experiments in the context of migration, metabolic coupling and radiation response are currently in progress.

032

Quantitative proteomics of lipoproteins: insights into individual differences of lipoprotein associated proteins in the context of cardiovascular disease.

Anne von Zychlinski[1]**, Michael Williams**[2]**, Sally McCormick**[1]**, Torsten Kleffmann**[3]

1. *Department of Biochemistry, University of Otago, Dunedin, New Zealand*
2. *Department of Medicine, University of Otago, Dunedin, New Zealand*
3. *Centre for Protein Research, Department of Biochemistry, University of Otago, Dunedin, New Zealand*

A disordered balance between plasma lipoproteins is a major risk factor for developing cardiovascular disease (CVD). High plasma levels of Lipoprotein(a) (Lp(a)) is another, independent risk factor for developing premature CVD. Various underlying disease mechanisms are not fully understood and details of Lp(a) physiology are still unclear.

We utilised different quantitative proteomics approaches to gain further insights into the biochemistry and pathophysiology of different lipoproteins with a main focus on Lp(a). Using absolute quantification (AQUA) of proteins by mass spectrometry we elucidated the stoichiometry of proteins per particle for the different lipoprotein classes. We then quantified the protein cargo of Lp(a) isolated from a cohort of 40 subjects using SWATH-MS in conjunction with AQUA.

The AQUA approach provided accurate numbers of lipoprotein-associated proteins per particle and showed significant individual differences in the apolipoprotein profiles of various particle classes such as strong variations in the numbers of apoC1, apoC2 and apoC3, which may indicate alterations in triglyceride metabolism. Proteins involved in immune response and complement activation which have been discussed in the context of lipoprotein-related CVD risk were only detected in very low numbers. Quantification of Lp(a)-associated proteins using AQUA and SWATH-MS identified a group of subjects with high levels of Lp(a)-associated histidine-rich glycoprotein (HRG). Those subjects also showed significantly higher levels of known HRG-interactors that are involved in coagulation and immune response. We hypothesise that Lp(a)-HRG interaction competes with HRG-plasminogen promoted regulation of fibrinolysis at sites of atherosclerotic plaques. However, in the low number of investigated subjects we could not detect any correlation of HRG-rich Lp(a) with disease associated factors such as high Lp(a) levels, high triglyceride levels or CVD events. More detailed information about the biochemistry of Lp(a)-HRG interactions is required and first insights into the influence of the apolipoprotein(a) isoforms on the HRG-interaction will be presented.

033

A Distinct Proteomic Signature of Latently Infected Cells Reveals New Drug Targets and Immunotherapeutic Targets for HIV

Sri H. Ramarathinam[1]**, Georges Khoury**[2]**, Leigh Harty**[2]**, Damian F.J. Purcell**[2]**, Anthony W. Purcell**[1]

1. *Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia*
2. *Department of Microbiology and Immunology, University of Melbourne, Parkville, VIC, Australia*

Combination antiretroviral therapy (cART) has vastly improved the quality of life of those suffering from HIV. However, interruption of therapy leads to re-emergence of the virus from the latent cellular reservoirs that include macrophages, dendritic cells and

particularly CD4+ T cells. J-Lat cells are a well-established CD4+ T cell line (derived from Jurkat cells) that harbour one integrated copy of the HIV provirus. These cells have been used to study both the factors that help maintain latency and to identify signals that can break latency and lead to viral reactivation. Previous studies have examined this at the transcript-level or attempted limited protein pull-down experiments. As a first step towards understanding the persistence of HIV and activation of latent provirus, analysis of the changes in host cell protein expression at the early stages of activation is crucial and may provide vital clues to develop new ways to control or eliminate viral reservoirs as well as defining the mechanisms that maintain HIV persistence. This study has provided a comprehensive quantitative protein expression map of latently infected J-Lat cells and follows changes in protein expression after viral reactivation of J-Lat clones by TNF-alpha. More than 9000 proteins and their modifications were identified and quantitated in latent and reactivated cells, making this study the largest of its kind. Unexpectedly, statistical analyses of the data revealed perturbation of several pathways in latently infected cells including proteins involved in cell signalling, energy generation and key transcription factors. This data highlights that latently infected cells are not "invisible" or indistinguishable from uninfected cells and that they have a discrete proteomic signature which has unveiled new drug targets and immunotherapeutic targets that could be used to eliminate HIV.

034

Data-independent mass spectrometry phenotyping of patient-derived melanoma cancer cell lines correlates with MEK inhibitor resistance independent of genotype and predicts poor patient outcome

Christoph Krisp[1], Robert Parker[1], Dana Pascovici[1], Nicholas Hayward[2], Mark Molloy[1]

1. *Australian Proteome Analysis Facility, Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, New South Wales, Australia*
2. *QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia*

Melanoma accounts for only 5% of all skin cancer variants, but is the most common cause of skin cancer related deaths, and is responsible for killing more young (<40yrs) people than any other cancer. Despite initial success of drugs that target specific enzyme mutations, melanomas rapidly acquire drug resistance. To understand baseline protein processes in melanomas with different genetic mutational status, mitogen activated protein kinase (MAPK) pathway mutant melanomas were compared to MAPK wild type (wt) melanomas using data-independent mass spectrometry (SWATH-MS) profiling.

Ten patient-derived melanomas with known MAPK mutational status (3x$BRAF^{mut}$, 3x$NRAS^{mut}$, 3x$MAPK^{wt}$, 1xMEK^{mut}) were cultured. Tryptic digests were submitted to LC-MS/MS on a TripleTOF 5600 MS (SCIEX) utilizing a multiphasic chip-based LC approach (reverse phase (RP), strong cation exchange, RP), for ion library generation to extract protein information from rapid 1h 1D RP SWATH-MS acquisitions per cell line. Presto Blue Assay was performed to assess ten-day cell viability in presence of the MEK inhibitor (MEKi) AZD6244.

This approach enabled comprehensive protein detection identifying 3200 proteins (10 cell lines; FDR<1%). Data extraction from SWATH-MS datasets revealed 2500 proteins quantifiable among these cell lines. Principal component analysis demonstrated segregation of melanomas based on sensitivity to MAPK inhibition (2 µM AZD6244), whereas genotype did not. In total, we show 57 proteins whose abundance correlate (r^2>0.75) with MEKi cell viability, revealing changes in cell pigmentation, lipid metabolism, and in adherence and inter-cell communication. Kaplan – Meier survival analysis using post-surgery patient survival data demonstrated untreated patients with MEKi sensitive melanoma cells showed significantly (p=0.01) lower mortality rates than patients with MEKi resistant tumours (mean survival >7.5 years MEKi sensitive versus 1.7 years MEKi resistant).

Therefore, data-independent MS phenotyping of MAPK pathway mutant and wt melanoma cell lines demonstrated explicitly segregation of MEKi sensitive and resistant cell lines and is associated with patient outcome.

035

Reprogramming biological functionalities for autonomous microbial factories and therapeutics

Matthew Chang[1]

1. *Department of Biochemistry, National University of Singapore, Singapore*

Synthetic biology aims to engineer genetically modified biological systems that perform novel functions that do not exist in nature, with reusable, standard interchangeable biological parts. The use of these standard biological parts enables the exploitation of common engineering principles such as standardization, decoupling, and abstraction for synthetic biology. With this engineering framework in place, synthetic biology has the potential to make the construction of novel biological systems a predictable, reliable, systematic process. While the development of most synthetic biological systems remains largely ad hoc, recent efforts to implement an engineering framework in synthetic biology have provided long-awaited evidences that engineering principles can facilitate the construction of novel biological systems. Synthetic biology has so far demonstrated that its framework can be applied to a wide range of areas such as energy, environment, and health care. In this talk, our recent efforts to develop synthetic microbes with programmable behaviors will be presented. In particular, an emphasis will be placed on our recent development of auto-regulatory genetic circuits for microbial chemical production and therapeutic applications. http://synbiolab.org/

036

Proteotype-based decoding of the viral signaling network orchestring the assembly of poxvirus

Karel Novy[1], Samuel Kilcher[2], Ulrich Omasits[1], Christopher KE Bleck[3], Mohammedyaseen Syedbasha[4], Alessio Maiolica[1], Jason Mercer[2], Bernd Wollscheid[1]

1. *Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland*
2. *MRC-Laboratory for Molecular Cell Biology, University College London, London, United Kingdom*
3. *Center for Cellular Imaging and NanoAnalytics (C-CINA), Biozentrum, University of Basel, Basel, Switzerland*
4. *Institute of Biochemistry, ETH Zurich, Zurich, Switzerland*

Poxviruses, the largest mammalian viruses, encode two dual-specificity enzymes, F10 kinase and H1 phosphatase. These enzymes are essential signaling mediators during poxvirus infection. Yet their precise functional role in reversible protein phosphorylation during the virus lifecycle remains enigmatic. Using a phosphoproteomic strategy in combination with F10 and H1 inducible viruses, we established the F10/H1 dependent viral signal network in HeLa infected cells by the prototypic poxvirus, vaccinia. In order to relate defined perturbations of this signaling network to a phenotypic outcome, we performed a detailed characterization of the progeny virions' proteotype. Our phosphoproteomic strategy allowed us to pinpoint relevant phosphorylation sites dependent on F10 and H1 which guided us towards focused functional analysis of two specific factors, the I7 protease required for proteolytic processing during virion maturation, and the viral early transcription factor, A7, showing that virion morphogenesis and the transcriptional competence of newly assembled virions are directly dependent on reversible phosphorylation of S134 in I7 and Y367 in A7. These results establish the molecular mode of action and a key role of F10/H1 in dynamic phospho-regulation during multiple stages of infectious poxvirus assembly.

037

Rapid identification of beer spoilage microorganisms using Biotyper

Florian Weiland[1, 2], Michelle E Turvey[1, 2], Jon Meneses[3], Nick Sterenberg[3], Peter Hoffmann[1, 2]

1. *The University of Adelaide, Adelaide, South Australia, Australia*
2. *Institute for Photonics and Advanced Sensing, Univesrity of Adelaide, Adelaide, South Australia, Australia*
3. *Coopers Brewery Ltd., Adelaide, South Australia, Australia*

Beer spoilage microorganisms present a major risk for the brewing industry and can lead to cost intensive recall of contaminated products, damage to brand reputation or in extreme cases, to the loss of business. Current microorganism detection procedures for bacterial and wild yeast contaminations involve cultivation-based enrichment and optical analyses, coupled with more recent molecular methods such as PCR, riboprinting, rRNA hybridization and antibody-based techniques. However, optical read-out methods are prone to misidentifications, whilst molecular methods like PCR are cost intensive. We investigated the applicability of molecular profiling using mass spectrometry in combination with the Biotyper software for the identification of beer spoilage microorganisms as a rapid and cost effective method for brewery quality control testing. Reference mass profiles for the five most common beer spoilage microorganisms (*Lactobacillus brevis*, *Lactobacillus lindneri*, *Pediococcus damnosus*, *Pectinatus frisingensis* and *Megasphaera cerevisiae*), four commercially-available brewing yeasts (top- and bottom-fermenting) and *Brettanomyces bruxellensis* wild yeast were established and incorporated into the Biotyper reference library. The method was validated by inoculation of these microorganisms into beer samples and identification using Biotyper. All tested bacteria, bottom- and top-fermenting and the wild yeast strain could be accurately identified and distinguished from one another, as well as from the other approx. 5,600 microorganisms present in the Biotyper database. The applicability of mass spectrometry profiling for the brewing industry was assessed by testing routine quality control samples from a local brewery, where contaminating microorganisms could be reliably identified. This renders the Biotyper platform a promising candidate for routine biological quality control within the brewing industry, especially considering the time-to-result is faster and analysis cost per sample is smaller relative to other molecular methods such as PCR.

038

***S. aureus* strain functional diversity as observed through pan-proteomics: an emerging approach for proteome measurement within a background of genetic heterogeneity**

James A Broadbent[1, 2], Imalka UK Tennakoon[1], Daniel A Broszczak[2], Flavia Huygens[1]

1. *Microbial Molecular Pathogenesis Group, Infection Disease Program, Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, QLD, Australia*
2. *Omics and Systems Biology Group, Tissue Repair and Regeneration Program, Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, QLD, Australia*

Background

Pan-proteome analysis is an emerging method that seeks to qualitatively and quantitatively compare the proteomes of genetically heterogeneous microorganisms. This approach has been reported in a single paper within the scientific literature;(1) however, there are major technical considerations that need to be addressed in order for this method to progress towards a valuable and accessible analytical technology.

Experimental Approach

FIve unique strains of *Staphylococcus aureus*, four of which are unsequenced, were grown under standard conditions and harvested at mid-exponential and stationary points on the growth curve. Cells were processed and comprehensive proteome samples subjected to data-independent acquisition LC-MS/MS as well as data-dependant acquisition LC-MS/MS, following deep proteome fractionation.

Qualitative mass spectrometry data were searched against all *S. aureus* sequences (~125,000) found within the UniProt database (Sept, 2014) using the Paragon algorithm. The resulting data were used to generate a spectral library of empirical *S. aureus* peptide mass spectra; the library was used to extract peptide ion areas from within the data-independent mass spectra. Peak area data were exported and processed in *R* in order to compare the summed protein abundance information under a set of unified pan-protein identifiers, based on protein sequence homology. Pan-protein abundances were normalized then compared across all strains using permutation statistics. Global protein abundance ratios between strains were compared to matched RNAseq quantification data.

Results and discussion

Pan-proteomics offers new opportunities to measure and interpret microorganisms' biochemistry within the context of genetic diversity. In this example, the contribution of protein abundance to unsequenced *S. aureus* strain types were measured and interpreted to reveal potential metabolic and virulence advantages that may underpin pathogenic observation. With robust development, pan-proteomics may prove valuable in the areas of drug development and/or industrial strain selection and, furthermore, could be applied to the proteome measurement of genetically-diverse but related plants and invertebrates.

1. 1. Zhang, L.; Xiao, D.; Pang, B.; Zhang, Q.; Zhou, H.; Zhang, L.; Zhang, J.; Kan, B., The core proteome and pan proteome of Salmonella Paratyphi A epidemic strains. PLoS One 2014, 9, (2), e89197.

039

The Advancement of Chemical Cross-Linking/Mass Spectrometry in Structural Proteomics

Andrea Sinz[1]

1. *Department of Pharmaceutical Chemistry & Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany*

During the last 15 years, chemical cross-linking combined with mass spectrometry (MS) and computational modeling has advanced from investigating 3D-structures of isolated proteins to deciphering protein interaction networks [1,2]. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-links, three-dimensional structural models of proteins and protein complexes can be constructed. Most commonly, homobifunctional amine-reactive cross-linkers, such as *N*-hydroxysuccinimide esters, are used for studying protein-protein interactions. One of our goals is to extend the arsenal of existing cross-linkers to obtain complementary 3D-structural information of proteins and protein complexes. To facilitate the identification of cross-linked products, we have designed novel MS/MS cleavable cross-linkers creating characteristic marker ions upon fragmentation [3].

In my talk, I will describe an integrated workflow for the automated identification of cross-linked products based on an MS/MS cleavable cross-linker using the different fragmentation methods available on an Orbitrap Fusion mass spectrometer (CID, HCD, ETciD, and EThcD). For conducting fully automated analyses, we employ our *in-house* developed software tool MeroX [4]. A direct way to probe protein-protein interactions in vivo is by site-specific incorporation of genetically encoded photo-reactive amino acids or by non-directed incorporation of photo-reactive amino acids. I will illustrate the different cross-linking strategies based on two protein systems: The tetrameric tumor suppressor protein p53 [5] and the complex between the basement membrane proteins laminin and nidogen-1 [6].

Literature References:

[1] Sinz, A., *Expert Rev. Proteomics* **2014**, 11, 733.[2] Herzog *et al., Science* **2012**,337, 1348. [3] Müller, M.Q. *et al.*, *Anal. Chem*. **2010**, *82*, 6958. [4] Götze, M. *et al*., *J. Am. Soc. Mass Spectrom*. **2015**, 26, 83. [5] Arlt *et al., Proteomics* **2015**,14, 3996. [6] Lössl *et al*., *PLoSOne* **2014**, 9:e112886.

040

Whole Dengue viral particle breathing dynamics unravels quaternary temperature-specific changes across capsid proteome

Ganesh S. Anand[1]

1. *Department of Biological Sciences, National University of Singapore, Singapore*

Temperature is one of the most important perturbations for initiating the infectious phase of a virus as it is transmitted from a vector to host. Dengue serotype 2 shows a large expansion in viral capsid size at a temperature of 37 °C corresponding to the environment within the human host. Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) is a powerful method to probe in parallel changes in dynamics across all constituent proteins in the viral capsid proteome at peptide resolution. Our results indicate that DENV2 viral particle shows high intrinsic and non-uniform dynamics across the C, E and M-proteins in the capsid and in alignment to the symmetry related contacts. These dynamic hotspots form the basis for where the largest changes are observed with temperature shift to 37 °C. These temperature-dependent changes in dynamics are largely irreversible and are specific to the

viral particle. The breathing dynamics observed solely in whole viral assemblies highlight the importance of protein quaternary contacts, packing of lipid bilayer and RNA genome for the coordinated changes to temperature. The increased dynamics and temperature-dependent expansion measured by HDXMS expose hidden linear epitopes and modulate interactions with neutralizing antibodies. These results have enormous implications for targeted antibody discovery. HDXMS has also been applied to map antibody-whole viral complexes and allows a molecular understanding of antibody-dependent expansion.

041

Structural characterisation of biomolecular assemblies by ion mobility-mass spectrometry

Tara Pukala[1], Henry Sanders[1], Michael Graetz[1]

1. *School of Physical Sciences, University of Adelaide, Adelaide, SA, Australia*

A precise network of interacting biomolecules, including proteins, DNA and lipids, allow for the function and control of all biological processes. Consequently, to understand these processes and offer potential for intervention, for example in the treatment of human disease, it is useful to have an understanding of the molecular components and their binding interactions. However, structural characterisation of such complex, heterogeneous and dynamic systems remains a challenge, in part due to analytical limitations of current structural biology approaches.

Ion-mobility mass-spectrometry (IM-MS) has emerged as a complementary tool for biomolecule structure determination, and in some cases a structural biology method in its own right. It is capable of defining identity, stoichiometry, size, structural arrangement and subunit interactions in a biomolecular assembly in a single experiment.

Here will be discussed recent advances in IM-MS methodology, and our application of native IM-MS and chemical cross-linking methods, in combination with complementary biophysical methods, to offer new insight into biological assemblies. We have applied these methods to assemblies of interest in human disease, for example; characterising the protein-protein and protein-lipid interactions mediating misfolding and toxicity in amyloid diseases such as Parkinson's and Alzheimer's Disease.

042

Characterizing human organelle proteomes - Towards a complete Cell Atlas

Emma Lundberg[1]

1. *SciLifeLab Stockholm and School of Biotechnology, Stockholm, Sweden*

Compartmentalization of biological processes is a fundamental principle of eukaryotic cells that gathers the relevant molecules and enables multiple processes to occur in parallel. Despite a great deal of research, basic questions about the spatial organization of many proteins and biological processes remain unanswered. The Cell Atlas aims to systematically localize the human proteome using an antibody-based approach as part of the Human Protein Atlas project.

Data generation include automated sample preparation, high-resolution confocal microscopy and computational image analysis. An integrative approach with strict validation criteria is used including gene silencing, paired antibodies and fluorescently tagged proteins. This allows identification of novel protein components of both known and unknown cellular structures.

In total, over 12000 human proteins have been localized to 25 organelles and cellular structures. As much as 50% of all proteins localize to multiple compartments and 20% show cell cycle dependent expression. The high spatial resolution allows pinpointing of protein localization to fine structures such as the cytokinetic bridge, microtubule ends, nuclear bodies, and rods and rings.

Here we discuss the importance of spatial proteomics for cell biology and present the content of the Cell Atlas as well as the path ahead to define the human organelle proteomes.

This research was supported by grants from Knut and Alice Wallenbergs foundation.

POSTER PRESENTATION

101

Proteome survey of wound fluid from non-healing wounds reveals key biological processes associated with poor healing outcomes

Daniel A Broszczak[1, 2], James A Broadbent[1, 2], Tony J Parker[1]

1. *Queensland University of Technology, Kelvin Grove, QLD, Australia*
2. *Wound Management Innovation Co-operative Research Centre, Brisbane, QLD, Australia*

Non-healing wounds are a significant problem for patients and healthcare systems worldwide. The underlying biochemistry, which drives non-healing outcomes in self-perpetuating leg wounds, is poorly understood. To address this knowledge deficit, a study of the proteins that compose the fluid, which exudates from these wounds, may provide important insight regarding treatment response and healing outcome for patients. In this respect, we have conducted a clinical study that included the collection of biological samples and clinical / psychosocial data over a 24 week period, during which time patients received best-practice care. Biological samples were analysed using mass spectrometry-based proteome measurements to detect and quantify the protein complement of the wound fluid. The resulting data were integrated with clinical measurements and contextualized by gene ontology annotations to enable deeper insight into the dynamic biological processes taking place within non-healing wounds. This identified key biological processes that may indicate specific underlying issues for a sub-set of wounds and their recalcitrant nature towards clinical care. A number of biological markers that are indicative of the wound healing outcome were also derived from these analyses. Unravelling the complex biology of non-healing wounds through proteome and clinical data integration provides some insight into the mechanisms associated with a patient's adverse or positive responses to clinical care. Such information can be developed further to inform clinical practices and enable the meaningful personalisation of wound management.

102

Identification of the Fragmentation Features of Dityrosine cross-linked Amyloid beta in ESI-MS/MS

Soumya Mukherjee[1], Berin Dr. Boughton[2], Somdatta Dr Ghosh Dey[3], Ashley Dr Bush[1], Colin Dr Masters[1], Blaine Dr Roberts[1]

1. *The Florey Institute of Neuroscience and Mental Health, Carlton, ACT, Australia*
2. *School of Botany, The University of Melbourne, Melbourne, Victoria, Australia*
3. *Department of Inorganic chemistry, Indian Association for the Cultivation of Science, Kolkata, West Bengal, India*

The use of mass spectrometry coupled with chemical cross-linking of proteins has become one of the most useful tools for proteins structure and interactions studies. One of the challenges in these studies is the identification of the cross-linked peptides. While lysine and cysteine cross-linked peptides have been investigated, identification of fragmentation patterns of dityrosine cross-linked peptides is yet to be understood. Oxidative stress is known to play a key role in the neurodegenerative disease like Alzheimer's disease (AD) and dityrosine in the amyloid plaques in AD brain is a potential biomarker of oxidative stress. Dityrosine cross-linked amyloid beta has been investigated in vitro to be toxic but whether its presence in the AD brain has any correlation with the disease progression and neurodegeneration is not well established. A quantitative analysis of covalently cross-linked dityrosine amyloid beta in AD brain using mass spectrometry requires a thorough understanding of the fragmentation pattern of these peptides. In this study we have investigated and characterized the fragmentation pattern of the LysC cleaved synthetically prepared amyloid beta(1-42) using ESI-MS/MS. In this study we report a detailed fragmentation study of the dityrosine containing peptide Aβ(1-16) generated by various techniques such as CID, HCD, ETD and ECD. The fragmentation features observed here can be helpful in the interpretation and identification of cross-linked dityrosine peptides present in nature and can be further implemented in search engine's algorithms.

103

A bioinformatic solution for identifying non-genomic peptides in the immunopeptidome

Kate E Scull[1], David N Perkins[2], Nadine L Dudek[3], Anthony W Purcell[3], Nicholas A Williamson[2]

1. *Biochemistry and Molecular Biology, University of Melbourne, Parkville, VIC, Australia*
2. *The Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Vic, Australia*
3. *Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia*

Human leukocyte antigen (HLA) molecules are cell-surface glycoproteins that present peptides for surveillance by T lymphocytes seeking signs of disease. It has been shown that the sequences of some class I HLA-bound peptides are not found in the genome, such as peptides arising from post-translational peptide splicing in the proteasome (Vigneron and Van den Eynde, 2012). Our group identifies large numbers of HLA-bound peptides by mass spectrometric analysis of purified immunopeptidome (i.e. HLA peptide) samples. However, non-genomic peptides such as spliced peptides are not susceptible to identification by conventional analysis software such as Mascot, since these search methods rely on matching tandem mass spectra to sequences in genome-based databases. This means that peptide sequences which are not present in the databases will be ignored or falsely identified. Here, we introduce software to address this problem by enabling searches to consider non-genomic sequences. Our program generates comprehensive 'artificial databases' which include all of the possible permutations of amino acids for peptides of a given length, then searches spectra using Mascot-based scoring. Our preliminary results, for which we analysed complex immunopeptidome samples by

LC-MS/MS and searched for peptides of 8-11 amino acids in length, shows that the program can identify many conventional sequences in agreement with Mascot, ProteinPilot and PEAKS DB searches. Despite statistical challenges necessitating more manual inspection than desired, it has also revealed a number of novel, non-genomic sequences for further investigation by MRM. This technique has potential to aid not only studies of the immunopeptidome but also analyses of other peptide samples for which genomic protein databases are incomplete or inapplicable.

Vigneron, N., Van den Eynde, B.J. (2012). Proteasome subtypes and the processing of tumor antigens: increasing antigenic diversity. Curr. Opin. Immunol. 24, 84-91.

105

Proteomics-based approach to elucidate the mechanism of anti-hypercholestrolemia activities of selected herbal medicines

Pouya Faridi[1], Peter Blattmann[2], Saman Sadraei[1], Anastasia Timofiiv[2], Ruedi Aebersold[2]

1. *Department of Phytopharmaceuticals, School of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Institute for Molecular Systems Biology, ETH Zurich, Zürich, Switzerland*

Introduction:

Hypercholesterolemia is a high incidence disease in developed and under developing societies. Natural products are becoming more popular for treatment of hyperlipidemia as alternatives or complementary medicines [1]. But the mechanisms of action of most of natural products are not clear yet. Recent studies shows that systems biology approaches and Omics techniques could better decipher mechanism of action of natural products. In this study we aimed to find mechanism of action of herbal remedies which used commonly for hyperlipidemia by using quantitative MS-based proteomics.

Materials and methods:

Hydroalcoholic extracts from 10 medicinal plant species were prepared and dried. The extracts were dissolved in DMSO and added to Hela and Huh7 cells (2ug/ml). The cells were harvested 48 hours after treatment and proteins digested into peptides. The samples were measured by selective reaction monitoring (SRM) [2] and 45 proteins involved in control of lipid metabolism were quantified. Analysis was performed with Skyline and MSstats and from three independent biological replicates.

Results:

Our results show 7 significant differential expression proteins by Glycyrrhiza glabra, 6 by Panax ginseng, 6 by Achillea wilhelmsii, 5 by Apium graveolens, 5 by Citrus aurantium, 4 by Trigonella foenum-graecum, 4 by Allium sativum, 4 by Zataria multiflora, 2 by Silybum marianum and one by Cynara scolymus.

Conclusion:

Herbal extracts are composed form multiple compounds and it is believed multi-ingredient drugs can interfere by multiple targeting in cells at same time. This study shows that each selected natural compounds can change the concentration of several proteins and control cholesterol metabolism by controlling of different pathways. By using proteomics techniques we will have a better holistic view on different mechanisms that are responsible for herbal remedies pharmacological activities.

106

A novel MRM_HR based glycosyltransferase assay

Chi-Hung Lin[1], Mark P Molloy[1], Nicki H Packer[1]

1. *Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*

Protein glycosylation is synthesized through concerted actions of glycosylation enzymes in the secretory pathway. Evidences have shown that functions of these enzymes are not only regulated at gene expression level but also post-transcriptionally at protein level. We have previously developed MRM_HR assays to quantitate differential expressions of endogenous glycosyltransferases. However, robust enzyme activity assays to detect endogenous glycosyltransferase activities is lacking in our proposed systems biology approach to study glycan biosynthesis.

Lectins are glycan binding proteins that could bind and distinguish structural isomers. We previously developed MRM_HR assays to analyze and quantitate degree of sialylation of glycopeptides from glycoproteins. These properties suggest lectin and MRM_HR analysis are useful tools to detect change of glycan structures before and after enzyme reaction.

In this study, we developed a novel glycosyltransferase assay that combines lectin detection and MRM_HR analysis. We demonstrated our method by detecting α2-6 sialyltransferase activity. We tested binding specificities of three sialic acid binding lectins, SNA, MALI and MALII using glycosidase modified fetuin and confirmed SNA binds specifically to α2-6 sialic acid. De-sialylated fetuin was immobilized on 96-well plate as substrate for sialyltransferase reaction. Probing by SNA showed only reaction with recombinant ST6Gal1 gave positive signal suggesting addition of sialic acid in α2-6 linkage on substrate. Trypsin digestion was then conducted in the same wells and peptide mixtures were analyzed by MRM_HR assays. The results confirmed the addition of sialic acid on fetuin and degree of sialylation could be calculated accordingly.

Our results showed this method is superior in that it is robust and compatible with cell lysate so that it could be used for high throughput screening. MRM_HR analysis provided quantitative results and confirmed the signal from SNA detection is truly from immobilized substrate. Thus, the method could be configured to fit different analysis requirements.

107

Effect of carbon source on the glycosylation pathway of *Trichoderma reesei* RUT-C30

Christopher Ashwood[1], Helena Nevalainen[1], Nicolle H Packer[1]

1. *Chemical and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*

Trichoderma reesei has been of industrial interest for several decades for the production of cellulases, resulting in the development of hypercellulolytic strains such as RUT-C30. High protein production and secretion, and eukaryotic glycosylation machinery, make *T. reesei* RUT-C30 a suitable expression host for recombinant proteins. The glycosylation of secreted proteins of RUT-C30 is known to vary depending on culture nutrients and this variability in glycosylation affects stability and activity of CBHI, the main secreted protein. However, the impact of media on the intracellular precursors of glycosylation and on the secreted protein glycosylation has received little attention, and techniques for their analysis in fungi are lacking.

This study investigated the effect of carbon source on nucleotide sugars of RUT-C30, important precursors of glycosylation. Methods were developed to extract and analyse these metabolites from mycelia grown on glucose or lactose. In parallel, the glycosylation profile of secreted protein was monitored. Differences were found in the abundance of nucleotide sugars depending on carbon source, not directly reflected in secreted protein glycosylation. In addition, previously unidentified O-glycan structures on the total secreted protein were identified. The work provides insight into the effect of carbon sources on the glycosylation and protein secretion pathways in *Trichoderma reesei* RUT-C30.

108

Quantitative proteomics reveals novel diagnostic and prognostic markers of acute rheumatic fever.

Laura F Dagley[2, 1], Giuseppe Infusini[2, 1], Willy-John Martin[2, 1], Liam O'Connor[2, 1], Gabriela Minigo[3], Jonathan Carapetis[4], Ian P Wicks[2, 1, 5], Andrew I Webb[2, 1]

1. *The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*
2. *Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia*
3. *Menzies School of Health Research, Casuarina, NT, Australia*
4. *Telethon Kids Institute, University of Western Australia, Subiaco, WA, Australia*
5. *Department of Rheumatology, The Royal Melbourne Hospital, Parkville, VIC, Australia*

Acute rheumatic fever (ARF), a condition which precedes the development of rheumatic heart disease (RHD), causes 275,000 deaths per year worldwide and has a global prevalence of 3.9 million. In Australia, ARF and RHD incidence remains high in Indigenous communities, where Aboriginal people are up to eight times more likely than other Australians to be hospitalised, and nearly 20 times more likely to die from RHD.

ARF is an autoimmune response that follows infection with group A streptococcus (GAS). RHD develops after repeated episodes of GAS infection and causes progressive heart valve damage. Accurate and timely diagnosis of an initial ARF episode and prophylaxis with antibiotics are the only current approaches for preventing RHD. At present, the diagnosis of ARF is made using the Jones criteria, which relies mainly on clinical features and has changed little in over 50 years. Misdiagnosis of ARF is common and a major contributor to the high rates of RHD seen in Aboriginal communities. Despite a clear need, there is no definitive method for diagnosing ARF.

In this study, we developed a novel peptide-based classification strategy using Random-forest based machine-learning algorithms in the R statistical computing environment. Data was generated using a label-free MS-based proteomics approach using Bruker's new Impact II UHR-QTOF and MaxQuant software. To detect novel diagnostic and prognostic markers of ARF and RHD, we compared affected individuals with healthy individuals and those with alternative diagnoses. A total of 53 plasma samples from patients in the Northern Territory were collected including those with ARF (21), RHD (8), healthy controls (7) and other diagnoses (17). In order to improve the dynamic range of low-abundance proteins, samples were immunodepleted with Agilent's Multi-Affinity Removal System (MARS-14) LC column. Utilising this approach we have identified statistically-relevant peptide signatures that distinguish patients with ARF from all other patient groups.

109

Proteogenomic profiling of acute myeloid leukaemia reveals novel insights into leukaemogenesis.

Jarrod J Sandow[1], Margherita Ghisi[2], Alexandra Garnham[1], Waruni Abeysekera[1], Gabriela Brumatti[1], Giuseppe Infusini[1], Ricky Johnstone[3], Gordon Smyth[1], Paul Ekert[4], Andrew Webb[1]

1. *Walter & Eliza Hall Institute, Parkville, VIC, Australia*
2. *Australian Centre for Blood Diseases, Monash University, Melbourne, VIC, Australia*
3. *Cancer Therapeutics Program, Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia*
4. *Murdoch Children's Research Institute, Parkville, VIC, Australia*

The Mixed Lineage Leukaemia (MLL) gene located on chromosome 11q23 is a frequent site of translocation that results in a number of oncogenic fusion proteins. MLL-translocations are present in a variety of paediatric and adult leukaemias and are associated with poor survival outcome. Despite intense research efforts the mechanisms underlying the leukaemogenic activity of MLL-translocations are poorly understood. Our aim was to identify proteins involved in leukaemogenesis that are regulated by MLL fusion protein expression

through quantitative proteome analysis using a murine leukaemia model. Our model of acute myeloid leukaemia (AML) involves the generation of leukaemic cells *in vivo* and is controlled by the regulated expression of a common MLL-translocation gene, MLL-AF9. Leukaemic cells were collected from mice before analysis of global protein expression using a SILAC quantitative proteomics workflow to generate a profile of proteins that are expressed in the presence or absence of MLL-AF9 expression. We extended this analysis by also profiling changes to the transcriptome using RNA-sequencing during MLL-AF9 regulation. These data underwent bioinformatic analysis to quantify and compare changes in protein and RNA expression before further comparison to human AML patient expression array and survival data to identify proteins and pathways of diagnostic and therapeutic interest. Our analysis has confirmed several known targets in AML and also highlighted novel pathways and targets that are regulated when MLL-AF9 is expressed. These pathways include epigenetic regulators, cell signaling and cell-cell or cell-extracellular matrix interactions. The results from this study not only identify mechanisms by which MLL-AF9 regulates leukaemogenesis, but also demonstrate the potential identification of clinical biomarkers and novel drug targets.

110

Differential abundance, but not kinetics, of virus-derived epitopes presented on infected fibroblasts and dendritic cells

Nathan Croft[1], Stewart Smith[2, 3], Erica Kellar[2], Inge Flesch[2, 3], David Tscharke[2, 3], Anthony Purcell[1]

1. *Monash University, Melbourne, VIC, Australia*
2. *Research School of Biology, The Australian National University, Canberra, ACT, Australia*
3. *John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia*

The presentation of peptide epitopes by major histocompatibility complex (MHC) class I molecules is a critical process governing anti-viral $CD8^+$ T cell-mediated immunity. Here we have used quantitative mass spectrometry to profile the presentation of 46 vaccinia virus-derived MHC-bound epitopes during infection of dendritic cells and fibroblasts, including a comparison between immortalized cell lines and *ex vivo* primary cells. Our data show that, in general, dendritic cells outperform in terms of absolute presentation capacity, with *ex vivo* fibroblasts the poorest presenters. However, several epitopes remained refractory to this fibroblast deficiency, highlighting the need for such wide-scale analyses. Conversely, despite such differential presentation across cell lines, the kinetic profile of each epitope remained markedly unchanged – subsequent analysis of vaccinia proteome kinetics strongly supports the notion that nascent protein synthesis drives MHC-peptide display, and that the similarities in infection kinetics across cell types is therefore providing a comparable rate of peptide supply.

In order to try to relate this dataset to the immunogenicity of each peptide, we measured in parallel the $CD8^+$ T cell response from infected mice, with the panel of peptides eliciting a robust immunodominance hierarchy. However, despite thorough analysis, the data so far show a complete disconnect between abundance and kinetics and the ensuing T cell response. These results highlight the complexities of epitope presentation during virus infection and the multifaceted processes that drive immunogenicity.

111

A new method of validation using the protein quantitation false discovery rate from control/control analysis

David C.L. Handler[1], Samantha J Emery[1], Iniga S George[1], Prathiba Ravishankar[1], Dana Pascovici[1], Paul A Haynes[1]

1. *Macquarie University, Epping, NSW, Australia*

Label-free protein quantitation by spectral counting has proved to be a useful method for accurate protein profiling. However, much work still remains to be done in applying useful and valid statistical methods for validation of shotgun results. Due to the large number of protein identifications involved, any t-test will invariably yield false positives; this is known as the multiple testing problem. My work focuses on the development of a novel form of statistical validation based on control-control experiments, where the statistical parameters for empirically minimising false discovery rates are determined from triplicate analyses of biologically identical material. An appropriate statistical measure (q value) is then carried forward into the analysis of control vs different samples. Through this additional statistical step, the resulting output comprises highly confident protein identifications with a minimised protein quantitation false discovery rate (PQ-FDR), which represents a work-around to solve the multiple testing problem.

112

Investigation of SOCS5 signalling complexes by mass spectrometry uncovers a role in breast cancer

Edmond M Linossi[2, 1], Laura F Dagley[2, 1], Tatiana B Kolesnik[2, 1], Jeffrey J Babon[2, 1], Jane E Visvader[2, 1], Marie-Liesse Asselin-Labat[2, 1], Nicos A Nicola[2, 1], Andrew I Webb[2, 1], Sandra E Nicholson[2, 1]

1. *University of Melbourne, Parkville, Vic, Australia*
2. *The Walter & Eliza Hall Institute, Parkville, VIC, Australia*

The Suppressor Of Cytokine Signalling (SOCS) family of proteins are critical negative regulators of cytokine and growth factor signalling. Whilst the roles of some SOCS family members have been clearly elucidated, the function of SOCS5 remains poorly characterised. SOCS5 has been postulated to act as a tumour suppressor in both EGFR and JAK/STAT-driven malignancies through expression analyses and functional studies of the Drosophila homologue Socs36E. However its precise physiological role and how it acts on these distinct pathways is yet to be elucidated. SOCS5 contains a C-terminal SOCS box motif that forms part of an E3 ubiquitin-ligase

complex, a central SH2-domain and large uncharacterised N-terminal region. In this study, we have used mass spectrometry to characterise the SOCS5 interactome and have identified a number of known and novel interacting proteins. These analyses have also revealed that the SOCS5 N-terminal region, which is predicted to be largely unstructured, to be heavily phosphorylated and we hypothesis that SOCS5 acts as a scaffold to support multiple signalling complexes. Additionally, many of the proteins enriched by SOCS5 are known drivers of tumorigenesis, particularly in the context of breast cancer. Expression analysis of SOCS5 in breast cancer revealed that is down regulated in a majority of patients with invasive ductal breast carcinoma (TCGA dataset, top 7% under-expressed genes, p=2.53E-23). Excitingly, SOCS5-deficient mice exhibited accelerated tumour onset and growth relative to wild-type mice in the Polyoma Middle T antigen model of breast cancer, thus providing the first *in vivo* evidence that mammalian SOCS5 can act to regulate tumorigenesis. Using our interactome data, we are now seeking to determine which specific protein targets are regulated by SOCS5 in breast cancer cells.

113

Life on the Rafts

Anup D Shah[1], Michelle M Hill[1], Melissa J Davis[2]

1. *University of Queensland Diamantina Institute, Brisbane, QLD, Australia*
2. *Systems Biology Laboratory , University of Melbourne, Melborne, VIC, Australia*

Lipid rafts are specialized membrane microdomains enriched in cholesterol and sphingolipids thought to act as dynamic signalling and sorting platforms. Given their fundamental roles in cellular regulation, there is a plethora of information on the size, composition and regulation of these membrane microdomains, including a large number of proteomics studies. To facilitate the mining and analysis of published lipid raft proteomics studies, first we have developed a searchable database RaftProt. Currently it hosts information about 117 proteomes derived from 69 different cell and tissue types across six mammalian species. In addition to variety of different search and browse features, we have captured the lipid raft preparation methods and implemented advanced search option for methodology and sample treatment conditions, such as cholesterol depletion. Furthermore, we have identified a list of high confidence proteins, and enabled searching only from this list of likely bona fide lipid raft proteins. Secondly, with the aim to identify of a common mechanism for lipid raft-mediated tumor progression, we integrated subcellular proteomics with computational systems biology approach. For this, we identified 'core cancer raft proteins' by analysing rafts from ovarian cancer, prostate cancer, breast cancer, renal cell carcinoma and melanoma which may offer novel pan-cancer therapeutic targets/strategies. Our analysis suggest that the proteins from cancer rafts have enhanced tendency to engage in protein-protein interactions, leading to much larger and more-dense interaction networks. Moreover, we observed strong enrichment of cytoskeletal assemblies in the membrane rafts during cancer progression which was abrogated by tumor suppressors OPCML and PTRF in epithelial ovarian cancer and prostate cancer respectively. Taken together, our results demonstrate the value of integrative computational analysis of subcellular proteomics data in biomedical research.

114

N-linked glycopeptide analysis of plant glycoproteins

Kris Ford[1], Wei Zeng[1], Ching-Seng Ang[2], Monika S Doblin[1], Joshua L Heazlewood[1], Antony Bacic[1]

1. *ARC Centre of Excellence in Plant Cell Walls, University of Melbourne, Parkville, Vic, Australia*
2. *Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Vic, Australia*

N-glycosylation of plant proteins has been shown to be important for protein folding, correct subcellular location and secretion, enzyme catalytic activity as well as plant pathogen interactions[1]. At the modification site there is (micro) heterogeneity of the glycans attached, making it difficult to detect in routine proteomics studies and the labile nature of the glycan hinders the analysis by mass spectrometry (MS). Plant *N*-glycosylation profiling studies performed have primarily relied on removal of the *N*-glycans to ease both the identification of the peptide and the glycan but at the cost of losing information on site-specific microheterogeneity[2].

Current MS-based ionisation and fragmentation techniques allow the comprehensive sequencing of intact glycopeptides revealing both the peptide and glycan sequences as well as the N-glycosylation site position. We describe a method for the enrichment of *N*-linked glycopeptides from the model plant *Arabidopsis*, followed by LC-ESI-MS/MS analysis using higher-energy collision dissociation product dependent electron transfer dissociation (HCD-pd-ETD) fragmentation for peptide identification, glycan site attachment and glycan sequence.

1. Song, W., Henquet M.G.L., et al. (2011) N-glycoproteomics in plants: Perspectives and challenges, Journal of Proteomics 74, 1463-1474
2. Ford KL, Zeng W, Heazlewood JL, Bacic A (2015) Characterization of protein N-glycosylation by tandem mass spectrometry using complementary fragmentation techniques, Frontiers in Plant Science 6: 674

115

Queensland Unknown (QX) and Winter Mortality (WM) disease of Sydney rock Oysters: A comparative proteomics study.

Vineet Vaibhav[2, 1], Maxime Lepretre[2], Emma Thompson[2, 3], David Raftos[2, 3], Wayne O'Connor[4], Haynes Paul[1]

1. *Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW, Australia*
2. *Sydney Institute of Marine Sciences, Sydney, NSW, Australia*
3. *Department of Biological Sciences, Macquarie University, North Ryde, NSW, Australia*
4. *NSW Department of Primary Industries (DPI), Port Stephens, NSW, Australia*

Sydney rock oysters (SRO) are indigenous to Australian waters and contribute to the economy greatly. In recent years, however, the SRO industry has witnessed a continuous decline in production, which can be attributed mainly to Queensland unknown (QX) and winter mortality (WM) diseases, in conjunction with environmental factors and anthropogenic pollution. The diseases together are responsible for > 80% of the mortality of oysters in the estuaries in NSW.

In 1990s DPI-NSW initiated a breeding program to tackle the loss by selection of broodstock that survived the disease outbreaks, without any knowledge of the molecular basis of resistance. This project is an attempt to connect the missing links between selection and the biological processes associated with it.

In this baseline study we aim at identifying the proteins differentially regulated in QX and winter mortality selected Sydney rock oysters when compared against non-selected oysters, employing both two dimensional protein gel electrophoresis and shotgun proteomics. We have identified proteins which are differentially regulated in winter mortality and QX. These proteins were found to be associated with different biological processes including stress response, cytoskeleton, cell communication, energy metabolism and protein synthesis. Identification of their biological significance has led to a better understanding of the underlying molecular processes associated with the disease selection. This ongoing study requires further substantiation, but results are already being used to help in breeding better oysters.

116

A proteomic approach to characterising antigen processing and presentation in bats.

Amanda P Woon[1], James W Wynne[2], Nadine L Dudek[1], Justin HJ Ng[3], Michelle L Baker[2], Linfa Wang[3], Anthony W Purcell[1]

1. *Department of Biochemistry & Molecular Biology, Monash University, Clayton, VIC, Australia*
2. *Australian Animal Health Laboratory, CSIRO, East Geelong, VIC, Australia*
3. *DUKE-NUS Graduate Medical School, Singapore*

Bats are a major reservoir of emerging infectious diseases and harbour deadly viruses such as Ebola, SARS, MERS and Hendra virus which pose serious threats to animal and human health. We hypothesise that the bat adaptive immune system contributes to their ability to co-exist asymptomatically with these viruses. One of the key adaptive immune responses is the presentation of antigens by major histocompatibility complex (MHC) class I molecules to cytotoxic $CD8^+$ T cells. Using a proteomic approach, we have characterised the proteins and peptides bound to three distinct bat MHC class I molecules. We determined that these bat MHC class I molecules, like their human and mouse homologues, associate with a peptide-loading complex to facilitate optimal ligand selection. Using a peptidomics approach, we also characterised the first repertoires of bat MHC class I bound peptides. These peptides ranged in length from 8-15 amino acid residues and motif analysis of the endogenous peptides revealed strong amino acid biases at various anchor positions. Furthermore, we were able to identify Hendra virus-derived peptides from infected bat cells which also display this binding motif, suggesting these motifs may be exploited to predict epitopes for vaccine development and immunological studies. In conclusion, this study provides fundamental insights into the antigen processing and presentation pathways of bats, which ultimately can be used to understand viral control.

117

Antioxidant and anti-inflammatory properties of sugarcane dietary fibre

Daniel Bucio Noble[2, 1], Liisa Kautto[1], Malcolm Ball[3], Mark Molloy[2, 1]

1. *CBMS, Macquarie University, Sydney, NSW, Australia*
2. *Australian Proteome Analysis Facility, Macquarie University, NSW, Australia*
3. *Gratuk Pty Ltd., Sydney, NSW, Australia*

Chronic inflammation involves the dysregulation in the synthesis of pro-inflammatory mediators which are associated with several diseases including autoimmune diseases, diabetes, cancer, etc. Some natural plant products are known to possess anti-inflammatory properties based on their high content of phytochemicals. In this study, we demonstrate that sugarcane dietary fibre (SCF) is a potent source of phytochemicals presenting more than two-fold polyphenols, flavonoids and antioxidants compared with raisins and cranberry juice. In vitro studies performed in a cellular model of intestinal inflammation using LPS-stimulated SW480 and HepG2 cells show that extracts from SCF supresses the phosphorylation of transcription factor NF-kB, and the protein kinase Akt as has been shown for the well-known polyphenol, resveratrol. *Mass spectrometry based phosphoproteomic analysis is being used to uncover*

other modes of action to explain the anti-inflammatory events associated with SCF extracts. Preliminary data shows profound changes in the phosphorylation of proteins involved in the inflammatory response. Our findings suggest that sugarcane fibre is a valuable source of antioxidants with potential to impart health benefits associated with inflammatory-related conditions.

118

A Systematic Investigation of CID Q-TOF Collision Energies for Complete Ddentification of Glycopeptides by Mass Spectrometry

Hannes Hinneburg[1], Kathrin Stavenhagen[2], Ulrike Schweiger-Hufnagel[3], Stuart Pengelley[3], Nicolai Bache[4], Peter H Seeberger[1], Daniel V Silva[1], Manfred Wuhrer[2], Daniel Kolarich[1]

1. *Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany*
2. *VU University Amsterdam, Amsterdam, Netherlands*
3. *Bruker Daltonik GmbH, Bremen, Germany*
4. *Bruker Daltonics Inc., Odense , Denmark*

In most naturally occurring glycoproteins, pools of glycans are attached to one or more glycosylation sites. The analysis of each individual site is challenging and requires the analysis of glycopeptides. For this, quadrupole time of flight (QTOF) mass spectrometers (MS) with high mass accuracy, fast duty cycles, and high m/z range are highly suited due to the usability of multiple collision energies. In this study we systematically investigated the optimum collision energies required for fragmentation of the glycan and the peptide parts of glycopeptides, and present a software-supported approach for data interpretation. For the initial studies of fragmentation energy, synthetic N-glycopeptides were analysed on a QTOF MS instrument (impact II) with CaptiveSpray nanoBooster^{tm} (Bruker Daltonics). For further testing, tryptically digested standard glycoproteins (fetuin, antibodies) were separated by nano LC before MS analysis using systematically varied collision energies. Glycopeptide spectra were detected and the peptide masses were determined automatically (ProteinScape 4.0, Bruker Daltonics). Glycan structures were identified using the integrated GlycoQuest search engine, and for peptide identification Mascot (Matrix Sciences) was used. The energies required for optimal glycan fragmentation were found to be clearly below the ones necessary for the peptide part. Collision energy stepping allowed identification of both peptide and glycan moieties from a single MSMS spectrum in ProteinScape 4.0. In summary, the optimized parameters were successfully applied on digested monoclonal antibodies and glycoprotein mixtures, which resulted in the identification of complete N- and O-glycopeptides. This improved methodology is particularly useful in the fields of glycoproteomics research as well as biopharmaceutical development and quality control.

119

Investigating lipid changes with PI3-K inhibition in colorectal cancer liver metastases by MALDI-MS imaging

Fiona Henderson[1, 2], Omar Belgacem[3], Roberto Castangia[3], Kaye Williams[2], Adam McMahon[1]

1. *Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK*
2. *Manchester Pharmacy School, University of Manchester, Manchester , UK*
3. *Shimadzu UK, Manchester, UK, United Kingdom*

Colorectal cancer (CRC) is one of the leading causes of cancer deaths worldwide, with metastases to the liver being responsible for very poor diagnoses. It is therefore crucial to develop a therapeutic approach to target these metastases. Phosphoinositide-3-kinase (PI3-K) is upregulated in many cancers, including CRC; and its cascades include various downstream lipid effectors. MALDI-MS imaging has been used to investigate the effect of a PI3-K inhibitor GDC-0941 on lipid profiles in CRC liver metastases.

An in-house mouse model was used to generate CRC tumours. Mice that underwent drug therapy were administered two doses of 50m/kg of GDC-0941. Vehicle and drug treated tumours were flash-frozen and sectioned for MALDI imaging .Adjacent sections were kept for immuno fluorescence. MALDI imaging and MS/MS experiments were carried out on the MALDI-7090 (Shimadzu, UK).

Clear lipid profile changes occur between non-treated and treated tumours, with certain phoshphatidylcholines showing a decrease in abundance after drug addition. Comparing MALDI images to H&E stains reveal which lipids are localised to necrotic, tumour, normal tissue; or those which are found heterogeneously in the tumour, and are hence significant for effective therapy. MALDI images have also been compared to immunofluorescence to show which lipids co-localise with immune cell infiltration. Future work will involve elucidating exactly what lipid mechanisms are occurring in these tumours to give the lipid changes seen as a result of PI3-K inhibition.

120

The development and implementation of a new microbial pathovarience system.

Matthew O'Rourke[1], Steven Djordjevic[1], Matthew Padula[1]

1. *University of Technology, Sydney, Broadway, NSW, Australia*

There is an ever increasing necessity for the rapid identification of microbiological pathogens in human clinical samples and agricultural samples. Generating information such as strain level identification, in a rapid, robust and automated way, is the way forward for microbiology. Traditional techniques rely on sometimes subjective morphological and biochemical information which requires a high level of technical expertise and expensive reagents. We aim to replace traditional microbiology identification

workflows with a single simple mass spectrometry-based protocol that allows walk up users to quickly and accurately identify a disease causing organism from multiple collected samples.
We have developed a new pathovarience technique which is an analysis technique that uses Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) to identify microorganisms from a range of clinical samples. In short, a small amount of cells are grown (or sampled) and prepared according to a proprietary protocol to extract molecules that can be analysed by the mass spectrometer. Currently this technology is limited, with several major pathogen groups, such as Staphylococcus spp., Streptococcus spp., and Escherischia coli/Shigella subgenus indistinguishable beyond the species level.
We have significantly improved on this current technology by developing a proprietary sample preparation protocol and computational package that allows the differentiation and identification of a range of organisms at strain and sub strain level with a high level of accuracy. We have termed this new technology the Karora Pathovarience System (patent pending).

121

Peptidomic and proteomic comparison of electrically stimulated and manually dissected venom from the South American bullet ant *Paraponera clavata*

Samira Aili[1], Axel Touchard[2], Alain Dejean[3], Jerome Orivel[3], Fréderic Petitclerc[3], Matt Padula[4], Graham Nicholson[1]

1. *University of Technology, Sydney, UTS, Broadway, NSW, Australia*
2. *Venoms and Biological Activities Laboratory, PRES-Université Toulouse, Albi, France*
3. *CNRS, UMR Ecologie des Forêts de Guyane (EcoFoG) , 97379 Kourou Cedex, France*
4. *Faculty of Science, university of techonology Sydney, Proteomics core facility, Sydney, NSW, Australia*

Venom peptides are currently being developed as novel drugs and bioinsecticides. A large number of ant species have evolved a venom rich in peptides and proteins used for predation, defence and communication and therefore represent an untapped source of potential lead compounds, with only 72 currently described peptides. One aspect of ant venoms that has not been investigated is the difference in venom composition obtained using differing venom collection methods and the potential variations in venom composition between ant colonies of the same species. The present study therefore aimed to determine any differences in the peptide and protein components of the bullet ant (*Paraponera clavata*)collected by either manual venom gland dissection or electrical stimulation, as well as difference between ant venoms collected from different colonies. Venom proteins were separated by 2D-PAGE and analysed by nanoESI-MS. Peptidomic analyses were carried out by separating venoms on C18 RP-HPLC then analysing by MALDI-MS. The proteomic experiments revealed numerous proteins that were assigned a biological function (96 in total) of which 70% were common to both collection methods. The remaining 30% of proteins were either structural or metabolic proteins. The peptidomic analysis revealed a large number of peptides (309 in total) of which only 30% were common to both collection methods. This was far lower than what was observed from protein matches. Therefore, we have shown that each method reveals a unique set of peptides and proteins, however, extracting venom via electrical stimulation, which is not destructive to the ants, is the preferred method of collection as it contains all the major components. There were also notable differences in peptide and protein expression between the venoms obtained from the two different *P. clavata* colonies. These findings further demonstrate the richness and diversity of ant venoms as potential sources of bioactive compounds.

122

Characterisation of lipoprotein MPN_284, a potential adhesin of *Mycoplasma pneumoniae*

Kayla Madonis[1, 2], Michael Widjaja[1, 2], Marcelo Moreno[1, 2], Iain Berry[1, 2], Matt Padula[1], Steven P Djordjevic[1, 2]

1. *UTS, Sydney, NSW, Australia*
2. *i3 Institute, Sydney, NSW, Australia*

Mycoplasma pneumoniae is the second most common cause of community acquired pneumonia. Infections are localised to the respiratory tract but can be systemic and affect multiple tissues and organs. *M. pneumoniae is a strict parasite and* must adhere to the respiratory epithelium to survive. Little is known of the mechanism of pathogenesis and attempts to develop efficacious vaccines have been unsuccessful.

M. pneumoniae possesses an unusually large repertoire of lipoproteins and their functions are largely unknown. Our proteome studies identified a number of lipoproteins in *M. pneumoniae*. One of these, MPN_284, was unusual in that it was highly expressed, proteolytically processed and surface accessible. N-terminomics of mature proteoforms and 2D SDS-PAGE followed by LC-MS/MS were used to characterize the cleavage sites of MPN_284. Two recombinant fragments were expressed that span the N-terminal (MPN_284 N-terminus) and C-terminal thirds (MPN_284 C-terminus) of MPN_284. Polyclonal antiserum to each fragment was generated to determine the cellular location of the lipoprotein fragments using 3D-Structured Illumination Microscopy and to confirm the identities of the cleavage fragments of MPN_284 by western blotting. Image analysis suggests that the C-terminal region of MPN_284 is accessible and distributed on the surface of *M. pneumoniae*, while the N-terminal region produced sporadic straining patterns. To investigate the adhesive characteristics of the N- and C-terminal cleavage fragments of MPN_284, inert latex beads were coated separately with MPN_284 fragments and incubated with respiratory epithelial cells (A549). Beads coated with MPN_284 fragments were found to bind to A549 cell monolayers while beads coated with bovine serum albumin did not.

Our data confirms that MPN_284 resides on the extracellular surface of *M. pneumoniae* and is proteolytically processed, generating multiple surface accessible fragments with putative binding functions for host molecules. The bead experiments were consistent with

these observations. This work provides new avenues to explore the identities of binding domains in MPN_284 and the development of an efficacious vaccine for *M. pneumoniae*.

123

Moonlighting proteins in *Mycoplasma hyopneumoniae*: Investigating a pathogenic role for glycolytic enzymes and their cleavage fragments.

Marcelo Moreno[1, 2], Benjamin B. A. Raymond[1, 2], Jessica L. Tacchi[1, 2], Iain J. Berry[1, 2], Michael Widjaja[1, 2], Matt P. Padula[1, 2], Steven P. Djordjevic[1, 2]

1. *UTS, Sydney, NSW, Australia*
2. *the ithree institute, Sydney, NSW, Australia*

Mycoplasma species are strictly parasitic bacteria from the Mollicutes class that are thought to have undergone extensive genomic reduction from a low G + C Firmicute ancestor. As such, Mycoplasma spp. lack genes for the TCA cycle and secretory mechanisms, and the biosynthesis of a cell wall, amino acids, nucleotides, and cholesterol. The pig pathogen *Mycoplasma hyopneumoniae* (*Mhp*) destroys mucociliary function in the respiratory tract and causes significant economic loss to pig production systems globally. Our surfaceome studies show that in addition to the P97 and P102 adhesin families, many lipoproteins and proteins with canonical cytosolic functions are surface-accessible targets of proteolytic processing events.

Proteins that do not contain known secretion motifs are often described on the surface of bacteria including metabolic enzymes, chaperones, and ribosomal proteins. The surface-expression of these proteins has been ignored for many years but has recently garnered widespread attention as their possible role in pathogenesis has developed. Many of these canonically cytosolic moonlighting proteins have since been shown to play important roles in binding to a functionally and structurally diverse array of host molecules and to activate host proteases such as plasminogen[1]. In *Mycoplasma pneumoniae*, subunits of the pyruvate dehydrogenase (PDH) complex are known to bind plasminogen and fibronectin[2,3].

Here we report processing of the PDH complex subunits that are located on the surface of *M. hyopneumoniae*. We argue that proteolytic processing may be a mechanism to release new, functional domains and expand the adhesive capacity of this pathogen as it colonises the host. To assist with the identification of cleavage fragments, a series of affinity chromatography assays using host molecules (plasminogen, fibronectin, and others) as bait were applied. Protein cleavage fragments were mapped by combining 1D and 2D-SDS-PAGE and LC-MS/MS data and precise cleavage sites within these molecules identified by dimethyl labelling of neo-N-termini. These enrichment strategies are useful for mapping functionally relevant cleavage fragments and assist with the identification of novel binding domains.

1. Kainulainen, V. and Korhonen, T. K., Dancing to another tune—adhesive moonlighting proteins in bacteria. Biology, 2014. 3(1): p. 178-204.
2. Dallo, S.F., Kannan, T. R., Blaylock, M. W., Baseman, J. B., Elongation factor Tu and E1 β subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in Mycoplasma pneumoniae. Molecular Microbiology, 2002. 46(4): p. 1041-1051.
3. Gründel, A., Friedrich, K., Pfeiffer, M., Jacobs, E., Dumke, R., Subunits of the Pyruvate Dehydrogenase Cluster of Mycoplasma pneumoniae Are Surface-Displayed Proteins that Bind and Activate Human Plasminogen. PLoS ONE, 2015. 10(5): e0126600

124

Comparative Proteomic Analysis of Two Rice Genotypes Exposed to Drought Stress and Recovery

Yunqi Wu[1], Mehdi Mirzaei[1], Brian Atwell[1], Paul Haynes[1]

1. *Macquarie University, North Ryde, NSW, Australia*

Rice is the major staple food for more than half of world's population. As global climate changes, many places have seen changes in rainfall, resulting in more floods, droughts and severe heat waves. Two rice varieties with contrasting genetic backgrounds, Nipponbare and IAC 1131, were used in this study. Nipponbare, the first fully sequenced agricultural plant, is a drought sensitive rice from Japan while IAC 1131, which is endemic to the hotter climate of upland rice fields in Java, is a drought tolerant upland tropical rice from the same sub-species as Nipponbare. Four-week-old seedlings of both cultivars were grown in large soil volumes and then exposed to moderate and extreme drought for 7 days by imposing two distinct watering regimes, followed by 3 days of re-watering. Mature leaves were harvested from plants from each treatment for protein extraction and subsequent quantitative proteomic analysis, with validation of selected proteins by Western blotting. Gene Ontology (GO) annotations of differentially expressed proteins provide insights into the metabolic pathways that are involved in drought stress resistance. Higher abundance of stress response proteins was found in IAC1131 than in Nipponbare. Additionally, six proteins involved in the porphyrin and chlorophyll metabolism were found to be significantly decreased in IAC1131 under extreme drought. Our data indicate that IAC 1131 appears to be better able to cope with stressful conditions by up regulating a suite of stress and defence response related proteins. Nipponbare, in contrast, lacks the range of stress response shown by the more stress tolerant variety, and responds to drought stress by initiating a partial shutdown of chlorophyll biosynthesis in an apparent attempt to preserve resources.

125

Combination of metabolomics, peptidomics and proteomics to elucidate mechanisms of drug resistance in the malaria parasite

Ghizal Siddiqui[1], Carlo R Giannangelo[1], Anubhav Srivastava[1], Darren J Creek[1]

1. *Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville Campus, Parkville, Victoria, Australia*

Drug resistance in the malaria parasite, *Plasmodium falciparum,* poses a major threat to global plans for the control and elimination of malaria. Resistance has been reported against all the currently approved antimalarial agents, although the underlying mechanisms associated with resistance are poorly understood. Most current drugs act by inhibiting cellular metabolism (e.g. atovaquone and antifolates) or act in the digestive vacuole (e.g. artemisinin and quinolines). Therefore, it is likely that a system-wide approach to monitor metabolism and protein turnover will reveal novel aspects of drug resistance for existing antimalarials. In order to identify key features associated with a delayed parasite clearance phenotype, we have developed a multi-omics platform based on high-resolution mass spectrometry combining proteomics, peptidomics and metabolomics to analyse global differences between drug-resistant and drug-sensitive isolates.

When applied to *P. falciparum* infected red blood cells, our multi-omics platform facilitated the identification of approximately 3000 proteins, 1000 metabolites and 10,915 naturally abundant peptides and integration of these data has provided a detailed view of protein expression and regulation associated with drug resistance. Preliminary findings for artemisinin-resistant isolates revealed that artemisinin resistance is associated with increased expression of proteins associated with the stress response and the unfolded protein response, as well as the accumulation of specific peptides and small molecule metabolites. These findings shed light on the biochemical pathways that are targeted by this critical antimalarial compound, and reveal the molecular adaptions that allow parasites to overcome the antimalarial effects of these front-line therapies.

126

Characterization of vulvar cancer progression and metastasis by imaging mass spectrometry from formalin-fixed paraffin-embedded tissues

Chao Zhang[1], Georgia Arentz[1], Lyron Winderbaum[1], Noor Alia Lokman[1], Manuela Klingler-Hoffmann[1], Parul Mittal[1], Martin Oehler[2], Peter Hoffmann[1]

1. *University of Adelaide, Adelaide, S.A., Australia*
2. *Obstetrics and Gynaecology , University of Adelaide, Adelaide, SA, Australia*

To date no tumour specific biomarkers for vulva cancer have been identified and the initiation and progression of the disease is poorly characterised and understood. Here we present an examination of vulva cancer progression in a cohort of 4 patients by the analysis of healthy tissue, vulvar intraepithelial neoplasia stage III (VINIII), vulvar squamous cell carcinoma (VSCC) and inguinal lymph node metastastases by MALDI mass spectrometry imaging (MALDI-MSI). 1821 peak groups were detected in the MALDI-MSI experiments, of which 567 were found to have different distributions of signal to noise ratios across the 4 tissue types according to a Kruskal-Wallis test ($P \leq 0.01$). Potential peptide identifications for the 567 peak groups were obtained by matching the MALDI-MSI data back to standard nanoLC-ESI-MS/MS data. Six of the highest intensity differentially expressed MALDI-MSI peak groups were targeted for identification by *in situ* MALDI MS/MS. The 6 *in situ* sequenced peptides matched back to 5 proteins, with one peptide identified from Histone H2A, Haemoglobin subunit β, Histone H4, and Cytoskeletal 10, and 2 unique peptides identified from β-Actin. In order to validate the MALDI-MSI results the spatial expression pattern and differential expression of β-Actin was further verified by immunohistochemistry and data independent nanoLC-ESI-MS/MS.

127

Quantitative proteomics of metronidazole drug resistance in *Giardia duodenalis*

Samantha J Emery[1], Brendan R Ansell[2], Mehdi Mirzaei[1], Dana Pascovici[3], Louise Baker[2], Paul A Haynes[1], Aaron R Jex[2]

1. *Macquarie University, North Ryde, NSW, Australia*
2. *Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Victoria, Australia*
3. *Australian Proteome Analysis Facility, Macquarie University, North Ryde, NSW, Australia*

Giardia duodenalis is a protozoan parasite of the gastrointestinal tract and the major global contributor to human diarrheal disease. Treatment of G*iardiasis* is predominately through nitroheterocyclics, specifically metronidazole, which are redox-active compounds believed to induce oxidative damage of DNA and proteins via activation by parasite oxioreductases. However, the effectiveness of metronidazole varies, and resistance is well-documented in both clinical and laboratory settings. Using three isolates of *G. duodenalis* from the human-infective Assemblage A taxonomic group, we have compared susceptible parent and metronidazole resistant strains of the same isolate using a TMT labelling approach. We identified over 1000 proteins in each isolate, and defined differentially expressed proteins in metronidazole-resistant strains. Though similarities in differential expression between isolates was observed at the level of protein function, there was limited overlap at the protein identification level. This may indicate multiple mechanisms are capable of producing metronidazole resistance in *G. duodenalis*. We observed differential expression in a wide range of oxidoreductases, some which have been previously implicated in metronidazole resistance and others in FAD and NADH dependent pathways, which are not as well-characterised. These oxidoreductases also showed variable combinations of up- and down-regulation

between isolates, further implicating alternate patterns of regulation may achieve similar outcomes for drug resistance. Our results also indicate several post-translational modifications may play a role in resistance, with multiple kinases up-regulated in drug-resistant strains. Furthermore, proteins associated with deacetylase activity were up-regulated, including histone deacetylase and a sirtuin homologue, as well as proteins associated with chromatin remodelling and silencing. This is a novel experimental observation which implicates epigenetic regulation in metronidazole resistance in *G. duodenalis*.

128

Using proteomics to decipher novel protein function in a genome-reduced pathogen

Jess Tacchi[1], Benjamin Raymond[1], Veronica Jarocki[1], Michael Widjaja[1], Iain Berry[1], Matt Padula[2], Steven Djordjevic[1]

1. *The ithree institute, University of Technology, Sydney, Broadway, NSW, Australia*
2. *Proteomics Core Facility, University of Technology, Sydney, Broadway, NSW, Australia*

Mycoplasma hyopneumoniae is a genome-reduced, cell wall-less, bacterial pathogen with a predicted coding capacity of less than 700 proteins and is one of the smallest self-replicating pathogens. The cell surface is extensively modified by processing events that target the P97 and P102 adhesin families. We analysed of the proteome of *M. hyopneumoniae* using protein-centric approaches (1D and 2D GeLC-MS/MS) that also enabled us to examine global processing events in this species. While these approaches only identified 52% of the predicted proteome (347 proteins) we identified 35 surface-associated proteins with widely divergent functions that were targets of unusual endoproteolytic processing events including cell adhesins, lipoproteins and proteins with canonical functions in the cytosol such as metabolic enzymes. Affinity chromatography assays that used key host substrates; heparin, fibronectin, actin, plasminogen and host epithelial cell surface proteins as bait, recovered cleavage products derived from these processed proteins, suggesting these fragments interact directly with the bait proteins and display previously unrecognised adhesive functions. Our combined methods of analysis were able to examine the interplay between structure, function and localisation of proteins. Previously well-characterised glycolytic enzymes were identified to be surface-exposed, bind key host substrates and displayed evidence of cleavage, and together these are strong indicators of protein moonlighting. We hypothesize that protein processing is underestimated as a post-translational modification in genome-reduced bacteria and prokaryotes more broadly and represents an important mechanism for creating cell surface protein diversity.

129

A novel protein methyltransferase catalyses conserved N-terminal and lysine methylation on translational elongation factor 1A

Joshua J Hamey[1], Daniel L Winter[1], Daniel Yagoub[1], Gene Hart-Smith[1], Marc R Wilkins[1]

1. *Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia*

Eukaryotic elongation factor 1A (eEF1A) is an essential protein that facilitates translational elongation by delivering aminoacyl-tRNAs to ribosomes. Protein N-terminal methylation is an under-characterised post-translational modification, with only three, highly homologous, N-terminal methyltransferases known in eukaryotes. Here we report a new eukaryotic protein N-terminal methyltransferase,*Saccharomyces cerevisiae* YLR285W, which methylates eEF1A at a previously undescribed high-stoichiometry N-terminal site and the adjacent lysine. Using parallel reaction monitoring (PRM) and MS/MS/MS (MS3), we unambiguously localised these modifications, showing that N-terminal trimethylation precedes dimethylation at the adjacent lysine. Deletion of YLR285W resulted in the loss of N-terminal and lysine methylation *in vivo*, whereas overexpression of YLR285W resulted in an increase of methylation at these sites. This was confirmed by *in vitro* methylation of eEF1A by recombinant YLR285W. Accordingly, we name YLR285W as elongation factor methyltransferase 7 (Efm7). This enzyme is a new type of eukaryotic N-terminal methyltransferase as, unlike the three other known eukaryotic N-terminal methyltransferases, its substrate does not have an N-terminal [A/P/S]-P-K motif. We show that the N-terminal methylation of eEF1A is also present in human; this conservation over a large evolutionary distance suggests it to be of functional importance. Indeed, proteomic analysis of the knockout of Efm7, with SILAC, reveals the role of this methylation in modulating the function of eEF1A.

130

The "SRGG" Protein Interaction Code

Daniela-Lee Smith[1], Melissa Erce[1], Gene Hart-Smith[1], Marc Wilkins[1]

1. *UNSW, Sydney, NSW, Australia*

Post-translational modifications (PTMs) can modulate protein-protein interactions. When used in combination, to modulate the specificity of interactions, PTMs can form “interaction codes”. To date, interaction codes have been characterised on a small number of proteins; histones, RNA polymerase II, FOXO transcription factors and chaperones. However, PTMs are known to be very widespread on eukaryotic proteins, raising the possibility that they may be a key means by which protein-protein interactions are regulated. We have termed this the “protein interaction code hypothesis”.

Finding new protein interaction codes is challenging, due to their complexity. Here we have simplified the study of interaction codes, by analysing paired PTMs, and through this defined a new interaction code based on an identical, recurring amino acid motif in yeast. The “SRGG” motif is found on 32 yeast proteins, many of which have functions in RNA maturation, transport or transcription.

Phosphorylation and methylation, of "SRGG" motifs on yeast protein Npl3p has been shown to modulate protein-protein interactions. Kinase Sky1p and methyltransferase Hmt1p are the modifying enzymes. Here we describe a second yeast protein, Nop1p, to also be phosphorylated by kinase Sky1p and methyltransferase Hmt1p on "SRGG" motifs. Via the use of our conditional two-hybrid system we also show these modifications to modulate the protein-protein interactions of Nop1p.

The proximity of phosphorylation and methylation on "SRGG" motifs suggests these modifications may be subject to PTM crosstalk. Using in vitro peptide assays, coupled to LC-ETD MS/MS, we found that phosphorylation of "SRGG" attenuates the methylation by Hmt1p. However, prior methylation has little effect on Sky1p phosphorylation. Together these results characterise an "SRGG" protein interaction code, which recurs across yeast proteins Nop1p and Npl3p. We discuss how this code is likely to be important for nucleo-cytoplasmic shuttling.

131

Large-scale mass spectrometry-based identifications of enzyme-mediated protein methylation are subject to high false discovery rates

Gene Hart-Smith[1], Daniel Yagoub[1], Aidan Tay[1], Russell Pickford[1], Marc Wilkins[1]

1. *University of New South Wales, Kensington, NSW, Australia*

Post-translational methylation is a protein modification that predominantly occurs on lysine and arginine residues, and a growing number of recent large-scale LC-MS/MS methylation site discovery experiments have indicated that these modifications are widespread and sometimes conserved in eukaryotes. In interpreting these LC-MS/MS data, methylpeptide spectrum matches (methyl-PSMs) must be identified at acceptably low false discovery rates (FDRs) following sequence database searching, and to estimate FDRs and determine methyl-PSM filtering criteria, the target-decoy approach is frequently employed. The efficacy of this methyl-PSM filtering approach has, however, yet to be thoroughly evaluated.

Here we conduct a systematic analysis of methyl-PSM FDRs across a range of sample preparation workflows and mass spectrometric instrument platforms (each employing a different mode of MS/MS dissociation). Through $^{13}CD_3$-methionine labeling (heavy-methyl SILAC) of *S. cerevisiae* cells and in-depth manual data inspection, accurate lists of true positive methyl-PSMs were determined, allowing methyl-PSM FDRs to be compared to target-decoy approach-derived methyl-PSM FDR estimates.

Our results show that global FDR estimates (i.e. FDR estimates obtained using all PSMs from target and decoy databases) produce extremely unreliable methyl-PSM filtering criteria; we demonstrate that this is an unavoidable consequence of the high number of amino acid combinations capable of producing peptide sequences that are isobaric to methylated peptides of a different sequence. Separate methyl-PSM FDR estimates (i.e. FDR estimates obtained using only methyl-PSMs from target and decoy databases) were also found to be unreliable due to prevalent sources of false positive methyl-PSMs that produce high peptide identity score distributions. Incorrect methylation site localizations, peptides containing cysteinyl-*S*-*β*-propionamide, and methylated glutamic or aspartic acid residues can partially, but not wholly, account for these false positive methyl-PSMs.

Together these results indicate that the target-decoy approach is an unreliable means of estimating methyl-PSM FDRs and methyl-PSM filtering criteria. We suggest that orthogonal methylpeptide validation (e.g. heavy-methyl SILAC) should be considered a prerequisite for obtaining high confidence methyl-PSMs in large-scale LC-MS/MS methylation site discovery experiments.

132

Multiple roles of elongation factor Tu in *Mycoplasma pneumoniae*

Michael Widjaja[1], Benjamin Raymond[1], Iain Berry[1], Matt Padula[1], Steven Djordjevic[1]

1. *ithree Institute, Sydney, New South Wales (NSW), Australia*

Mycoplasma pneumoniae (*Mpn*) is responsible for 15-20% of cases of community-acquired pneumonia. Up to 25% of infected patients develop extrapulmonary complications that can affect neurological, musculoskeletal, haematological and cardiovascular sites. An extracellular extension called the attachment organelle mediates attachment to the respiratory epithelium via the P1 and P30 adhesins. Vaccines incorporating both adhesins unfortunately exacerbate disease and a successful vaccine is yet to be developed. Our surfaceome studies identify about 160 proteins on the surface of *Mpn* including a number that lack signal secretion sequences. One of these, elongation factor Tu (Ef-Tu), is a protein that moonlights on the surface of Gram positive and Gram negative pathogens. Dallo *et al.*, (2002[1]) used immunogold EM microscopy to confirm Ef-Tu on the cell surface of *Mpn* and also described its ability to bind fibronectin (Fn). Subsequent studies identified two Fn binding motifs and six key amino acids in those regions that are needed to bind Fn in Ef-Tu (Balasubramanian *et al.*, 2008[2], 2009[3]). Notably, whole cell Labelling studies that target N-terminal amino acids identified nine neo-N-termini within Ef-Tu. LC-MS/MS analysis of protein spots separated by 2D SDS-PAGE and analysis of eluents from affinity chromatography columns loaded with fetuin, actin, plasminogen, heparin and Fn as bait has allowed us to map 15 putative cleavage fragments of Ef-Tu. Preliminary microscale thermophoresis experiments indicate that purified recombinant Ef-Tu binds heparin, plasminogen and actin and further experiments are underway to validate interactions between Ef-Tu and other host molecules implicated in our affinity studies. Our data indicates that Ef-Tu is a multifunctional adhesin that is processed into functional domains on the cell surface of *Mpn*.

1. 1. Dallo SF, Kannan TR, Blaylock MW, Baseman JB: Elongation factor Tu and E1 beta subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in Mycoplasma pneumoniae. Mol Microbiol 2002, 46(4):1041-1051.

2. 2. Balasubramanian S, Kannan TR, Blaylock MW, Baseman JB: The surface-exposed carboxyl region of Mycoplasma pneumoniae elongation factor Tu interacts with fibronectin. Infect Immun 2008, 76:3116-3123.
3. 3. Balasubramanian S, Kannan TR, Hart PJ, Baseman JB: Amino acid changes in elongation factor Tu of Mycoplasma pneumoniae and Mycoplasma genitalium influence fibronectin binding. Infect Immun 2009, 77, 3533–3541.

133

Proteomics of Cephalopod Venoms

Ira Cooke[1], Nikeisha Caruana[2], Brooke Whitelaw[2], Pierre Faou[3], Jan Strugnell[2]

1. *Latrobe University / VLSCI, BUNDOORA, VIC, Australia*
2. *Ecology, Environment and Evolution, La Trobe University, Bundoora, Vic, Australia*
3. *Biochemistry, Latrobe University, Melbourne, VIC, 3086*

Coleoid Cephalopods are a diverse molluscan sub-class that includes octopus, cuttlefish and squid. There is strong morphological, behavioural and transcriptomic evidence to suggest that many cephalopod species use a potent cocktail of proteinaceous toxins for predation and/or defense. Despite this, very few cephalopod proteins have been observed at the protein level and even fewer have been well characterised. As a consequence they are a conspicuously under-represented group in the Uniprot toxins database and potentially a rich source of novel toxic proteins.

We present our findings based on bottom-up proteomics on venom glands and toxic secretions from three different cephalopod species (two octopus and one squid). In order to perform this work we relied heavily on integrating information from transcriptomics and proteomics. For all organisms it was necessary to assemble high quality reference transcriptomes and transcriptome maps across multiple tissues. We used these to predict protein sequences and then further refined these predicted protein databases using proteogenomics. New bioinformatic tools were developed to facilitate these analyses. All these tools are open source and are available via the user-friendly Galaxy platform.

Toxins were identified based on well known general features of toxins, including small size, high cysteine richness, existence of a signal and/or pro peptides and sometimes homology to known toxins. Further evidence for toxicity was obtained from approximate quantitative protein expression estimates via intensity based absolute quantitation (iBAQ) in venomous secretions as well as relative expression in different tissues based on transcriptomic data.

Our work highlights the important role that mass spectrometry based proteomics can play in the discovery of novel toxin molecules from poorly studied taxa, and describes the non-standard bioinformatic techniques that enable such work.

134

Automated *N*-glycopeptide identification in glycoproteomics

Ling Yen Lee[1], Edward S.X. Moh[1], Benjamin L. Parker[2], Marshall Bern[3], Nicki H. Packer[1], Morten Thaysen-Andersen[1]

1. *Macquarie University, North Ryde, NSW, Australia*
2. *Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia*
3. *Protein Metrics Inc., San Carlos, CA*

Recent developments in LC-MS/MS-based *N*-glycoproteomics including advances in software-driven glycopeptide identification have facilitated biochemical studies reporting thousands of intact *N*-glycopeptides. In this early phase it is particularly important to scrutinize the automated glycopeptide identification to ensure confidence in the process. Herein, we explore the accuracy of software-assisted site-specific glycoprofiling using the PTM-centric search-engine Byonic (Protein Metrics) relative to manual expert annotation. To allow an appropriately deep comparison, the study utilised glycoproteomics-typic acquisition and data analysis strategies, but of a single glycoprotein, the previously uncharacterised triply (Asn160, Asn268 and Asn302) *N*-glycosylated human basigin. Initially, detailed site-specific reference *N*-glycoprofiles of purified basigin were established using manual annotation and relative quantitation of ion trap (CID) and high-resolution Q-Exactive Orbitrap (HCD) LC-MS/MS data of tryptic *N*-glycopeptides. Conventional *N*-glycome profiling supported the manual annotation. The *N*-glycosylation sites of basigin showed extensive and diverse micro- and macro-heterogeneity. Subsequently, the basigin peptide mixture was glycoprofiled using Byonic with or without a background of complex peptides using the same Q-Exactive Orbitrap LC-HCD-MS/MS data. By monitoring the software-assisted glycoprofiling accuracy and coverage relative to the reference profile, the influence of multiple search parameters and scoring thresholds in Byonic was investigated. In general, the search criteria and confidence thresholds suggested by the vendor provided highly accurate and sensitive automated glycopeptide detection. As expected, several search parameters i.e. search space (proteome and glycome size), mass tolerance and peptide modifications and the confidence thresholds influenced the glycoprofiling accuracy and coverage in particularly for Asn268 basigin peptides displaying extensive peptide heterogeneity formed by incomplete trypsinization and methionine oxidation and, unexpectedly, also carbamidomethylation. The latter produced abundant neutral losses in the HCD-MS/MS spectra that were left unassigned by Byonic reducing the identification scores. These are valuable lessons learned in our ambition to ensure high glycoprofiling accuracy and coverage as we transition rapidly into automated FDR-based *N*-glycopeptide identification in glycoproteomics.

135

Understanding adaptation dynamics of *Pseudomonas aeruginosa* in cystic fibrosis lungs

Karthik Kamath[1], Anahit Penesyan[1], Christoph Krisp[2, 1], Sheemal S Kumar[1], Dana Pascovici[2], Apurv Goel[2], Vignesh venkatakrishnan[1], Nicolle H Packer[1], Ian Paulsen[1], Mark P Molloy[2, 1]

1. *Faculty of Science and Engineering, Macquarie University, Sydney, NSW, Australia*
2. *Australian Proteome Analysis Facility, Sydney, NSW, Australia*

Pseudomonas aeruginosa (PA) is an opportunistic human pathogen and an etiological agent in cystic fibrosis (CF), leading to chronic infection and death. To understand how PA adapts to the harsh micro-environment of the CF host, we recently characterised three novel strains isolated from sputum of CF patients. We conducted genome sequencing, proteome profiling and phenotypic analysis and made comparisons to the laboratory strain PAO1. Genomic and phenotypic analyses revealed significant diversity in virulence, colonization and metabolism related traits in CF strains. 2D-LC-iTRAQ-MS analysis of carbonate-extracted membranes of all four strains of PA, grown in lung nutrient mimicking medium (SCFM) and M9 minimal medium revealed a total of 2,442 (SCFM) and 3,171 (M9) expressed proteins (global protein FDR <0.008%). Gene ontology and bioinformatics predictions assigned 994 (M9) and 765 (SCFM) proteins as membrane proteins. CF strains portrayed heterogeneity in membrane proteome expression which was reflected in phenotypes including biofilms, pigmentation, and virulence. We observed differential expression of key proteins involved in virulence, drug resistance, motility and adhesion. Interestingly, proteins involved in drug resistance (MexY, MexB, MexC, OprM) were up-regulated and chemotaxis, aerotaxsis (PA1561, PctA, PctB) motility and adhesion proteins (FliK, FlgE, FliD, PilJ) were downregulated in CF strains. Confirmatory phenotype assays including drug MIC, plate motility assay and sputum binding assay reinforced the observation by portraying increased antibiotic resistance and reduced motility and adhesion. In conclusion, our multi-omic analysis shows that the adaptation strategies of *P. aeruginosa* to CF lungs are both multifactorial and combinatorial and are tailored according to micro-environmental niches.

136

Isolation and Characterization of Natural Crosslinks in Animal skins by Liquid Chromatography and Electrospray Ionization-Mass Spectrometry Detection

Rafea Naffa[1], Gillian Norris[1], Richard Haverkamp[1], Meekyung Ahn[2]

1. *Massey University, Palmerston North, New Zealand*
2. *NZ leather and shoe research association, Palmerston North, New Zealand*

Natural crosslinks are important for stabilizing the collagen structure. However, because of their low concentration in collagen compared to amino acids, their isolation, characterization and quantitation are very challenging. To the best of our knowledge, no comparative study of the isolation and characterization of natural crosslinks in different animal skins has been reported. Herein, we describe an efficient and rapid method for the isolation of crosslinks from animal skins by liquid partition chromatography and preparative TLC followed by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and characterization using Electrospray Ionization-Mass Spectrometry (ESI-MS).

Skin is first treated with sodium borohydride to stabilize the crosslinks to prevent their destruction by acid hydrolysis. Reduced skin is then hydrolyzed using 6 M HCl at 105^{o}C for 24 hours. Crosslinks are separated from the bulk of the amino acid residues by liquid partition chromatography on fibrous cellulose using butanol:water:acetic acid and water as solvents. Water fractions were monitored by thin layer chromatography (TLC) and fractions containing crosslinks were pooled, concentrated, and purified using preparative TLC with ethylacetate:water:acetic acid as solvent. The purity of the crosslinks was analysed by RP-HPLC and the exact mass of each crosslink measured using ESI-MS. Three main crosslinks were identified in sheep, goat, deer and cow skins; hydroxylysinenorleucine (HLNL, 292.1736 m/z), histidinolysinonorleucine (HHL, 445.2207 m/z) and histidinohydroxymerdesmosine (HHMD, 574.2969 m/z). Non-reduced skin lacked HHMD crosslinks and cartilage showed a different crosslinking profile to skin. It contained dihydroxylysinonorleucine (DHLNL, 309.1040 m/z) and pyridinoline (Pyr, 429.1887 m/z) and lacked the ones in skin. This method allows direct analysis of crosslinks HLNL, HHL and HHMD without interfering compounds such as basic amino acids and salts which has been a drawback of previously reported methods. The crosslinks found in sheep, goat, deer and cow skins will be presented and discussed in terms of their contribution to skin strength.

137

LC/MS Method Optimization for TMT Quantification using Q-Exactive instruments

Steven Ramsay[1], Tabiwang Arrey

1. *ThermoFisher Scientific, Scoresby, VIC, Australia*

Isobaric mass tagging (e.g., TMT) has become a common technique in mass spectrometry for relative quantification of proteins. However, there are a couple of factors that impede accurate quantification of complex proteomic samples. One of these factors is co-isolation of peptides, which leads to systematic under/overestimation of quantitative ratios of the isobaric tags [2]. Co-isolation interference has been addressed using various kinds of approaches, such as using MS^3 experiments [3]. Due to the fast acquisition speed and efficient quadrupole isolation the Q-Exactive Plus/Q-Exactive HF can be used to address some of these challenges. The

effect of several parameters (isolation window, normalized collision energy (NCE), source fragmentation, and others) on the protein quantification accuracy and precision is discussed.

138

Automated Protein Digestion to Reduce the Sample Preparation Bottleneck

Christie Hunter[1], Qin Fu[2], Michael P. Kowalski[3], Jennifer E. Van Eyk[2]

1. *SCIEX, Redwood City, CA, United States*
2. *Cedars-Sinai Medical Center, Los Angeles, CA, United States*
3. *Beckman Coulter Life Sciences, Brea, CA, United States*

Today using targeted quantitative proteomics techniques such as MRM analysis or SWATH® Acquisition, protein panels can be quantified across a broad dynamic range with very high reproducibility. While these techniques have increased the reproducibility and scale of protein analysis, sample preparation remains a key bottleneck as the number of samples increases. Numerous processing steps are required, each susceptible to technical variation. Here, we have used automation to improve the throughput and day-to-day reproducibility of the protein digestion portion of the sample preparation workflow.

To increase throughput and reduce technical variation, we have implemented an automated peptide preparation protocol on a liquid handling workstation (Biomek NX^{P}) coupled with an MRM workflow using a QTRAP® 6500 system. Denaturation, reduction/alkylation and digestion were the steps included, total workflow time was ~5-6 hours, depending on # of samples processed. A large number of proteins/peptides were monitored to ensure that good general digestion was occurring. Two different automation workstations were used to confirm the method transferability and multi-day experiments were performed on each to confirm method reproducibility.

First the protocol was validated by performing a very careful manual digestion across 24 wells using MRMs to 150 peptides. Using this benchmark, the automation protocol was optimized to achieve similar or better performance. For off-deck digestion using separate incubators, on average, ~80% of peptides monitored had raw peak area CVs < 10% as monitored by LC/MRM analysis. Next steps will be to optimize the use of on-deck heating (incubators integrated on workstation) and assess variance. Use of internal standards could also be incorporated for specific workflows to further reduce variance and will be assessed.

The automation method provided excellent reproducibility for digestion across multiple days on multiple workstations.

139

DESI-MS Optimisation for the application of proteomic Imaging

M Towers[1], PJ Hart[1], E Jones[1], E Claude[1], Heather Patsiouras[2]

1. *Waters Organisation, Wilmslow*
2. *Waters Australia, Rydalmere, NSW, Australia*

Desorption electrospray ionisation (DESI) provides a useful alternative to matrix assisted laser desorption ionisation (MALDI) for mass spectrometry imaging (MSI) with the ambient nature of the technique increasing the scope of samples that can be analysed whilst reducing the sample preparation requirements. Furthermore DESI offers the possibility to generate multiply charged ions for biomolecules like peptides and proteins directly from a surface. Here we investigate sample analysis parameters and conditions to optimise the ionisation/desorption of biomolecules directly from glass surface and tissue sections.

Analyses were performed on a SYNAPT G2-S*i* Q-TOF-IMS-MS,positive MS mode with integrated tri-wave ion guide optics used to separate ions by ion mobility in the gas phase. The DESI (Prosolia) stage was mounted directly using an electrospray inlet.

Initial optimisation and experiments were performed on single peptides/proteins. Extracted proteins were also trypically digested spotted onto a Teflon coated glass slide. Various DESI solvent compositions were tested using methanol, Water, acetonitrile, formic acid. The MS spectra produced were in large comparable with those generated via a supporting ESI-MS analyses. However, it was noted that a number of peaks observed in the ESI spectrum were absent for the DESI data. In this case acetonitrile was found to be marginally better as the organic component than methanol. Furthermore the spray solution containing 0.2% formic acid was found to improve ionisation. In the case of the tryptic digest samples, peptide species with charge states of up to 4+ and 5+ were detected and were baseline separated using ion mobility.

Direct analysis of larger m/z molecules directly from tissue has shown to be more difficult because of the other endogenous molecules like lipids present within the tissue that ionise preferentially compare to larger endogenous molecules. Subsequent to these analyses, the focus turned to digested tissues. Trypsin , was reconstituted in 50 mM ammonium bicarbonate, 0.5% Octyl β-D-glucopyranoside with concentration of 20 µg/mL.

140

Characterization and Collision Cross Section Determination of Lipids from Metabolic Syndrome Disorders

Lee A Gethings[1], Gertjan Kramer[2], Nicholas Dekker[2], John P Shockhor[1], James I Langridge[1], Johannes P.C Vissers[1], Johannes M.F.G Aerts[2], Heather Patsiouras[3]

1. *Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands*
2. *Waters Corporation, Machester, United Kingdom*
3. *Waters Australia, Rydalmere, NSW, Australia*

Introduction

Obesity is a risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. This work provides additional characterization of the associated lipids using ion-mobility with collision cross section (CCS) databases.

Methods

Lipid were extracted from a variety of sources including plasma and liver tissue of control, obese and diabetic subjects. Extracts were separated over a 20 min reversed-phase LC gradient and data acquired using a data independent acquisition approach utilizing ion mobility. Data were processed and searched using Progenesis QI and dedicated lipid compound databases, providing normalized label-free quantitation results with additional specificity of CCS measurement.

Preliminary Data

Interrogation of the LC-IM-DIA-MS data revealed over 5000 potential features for further investigation as a result of positive and negative ion acquisitions combined. Data were further interrogated using multivariate statistical analyses, showing clear distinction between control and metabolic syndrome groups. OPLS discriminant analysis revealed 795 potential features that were of significant correlation and covariance. Database searching resulted in 163 candidates. Identifications were scored according to mass accuracy, isotoptic fit, CCS and MS/MS fragmentation. Additional filtering to curate the data was based on mass errors less than 2 ppm, fold change >2, 5% CCS tolerance and ANOVA p-value <6E-06. This resulted in 15 significant identifications including phosphatidylcholines, sphingomyelins, triglycerides and lysophosphatidylcholines. Ion mobility-derived CCS measurements allowed for improved specificity with the inclusion of drift time, providing additional confidence in the identifications returned. Pathway analysis revealed lipid metabolism as a significantly perturbed pathway with mapping highlighting example responses such as inflammation.

141

Improvements in accurate mass GC-MS based Metabolomics: A novel atmospheric pressure GC-APCI Source increases quantitative and qualitative performance for metabolic profiling

Lucy A Woods[1], Christian J Wachsmuth[2], Aiko Barsch[1, 3], Verena Tellström[3], Peter J Oefner[3], Katja Dettmer[3]

1. *Bruker Pty Ltd, Preston, VIC, Australia*
2. *Institute of Functional Genomics, University of Regensburg, Regensburg, Germany*
3. *Bruker Daltonics, Bremen, Germany*

Gas chromatography coupled to mass spectrometry (GC-MS) is one of the most widely used analytical techniques in metabolomics. While electron ionization (EI) is the common analytical standard for GC-MS, atmospheric pressure chemical ionization (APCI) became more prominent in recent years. The soft APCI ionization preserves molecular information and opens the doors to the world of unidentified metabolites which could not yet be annotated due to missing library data.

We report the application of a novel GC-APCI design coupled with high resolution oTOF-MS to analyse derivatized metabolite extracts and reference standards. Compared with earlier results [1], an improved analytical performance resulted in a higher number of compounds which could be identified in human cancer cell extracts.

Upon comparing previous APCI-I [1] and novel APCI-II ion sources, GC-APCI-II-TOFMS analysis resulted in improved peak shapes and much better peak area reproducibility for fatty acid methyl esters (FAMEs). Furthermore, overall decreased lower limits of quantification in the sub-micromolar range were found for twenty metabolites due to reduced background in the ion source. Meanwhile, the analytical linear working range was either maintained or slightly increased. The improved analytical performance enabled to approximately double the number of extracted peaks with signal-to-noise ratios >20 in cell culture supernatant samples of pancreatic cancer cells. Injecting PFTBA automatically into the source before each GC/MS run, lead to low ppm mass deviations for the standard compounds analyzed in this study. The higher number of peaks extracted and improved mass accuracy resulted in 36% more compounds which could be identified compared to the previous setup.

In summary, APCI-II has a number of notable improvements over APCI-I and holds great promise for further studies in metabolomics.

1. C. J. Wachsmuth, M. F. Almstetter, M. C. Waldhier, M. A. Gruber, N. Nürnberger, P. J. Oefner, K. Dettmer, Anal Chem 83 (2011) 7514-22.

142

EasyPhos – a high-performance, scalable and universal phosphoproteomics platform

Sean J. Humphrey[1, 2], S. Babak Azimifar[1], Matthias Mann[1]

1. *Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany*
2. *Cybernetics Lab, Charles Perkins Centre, School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia*

Tremendous advances in MS-based proteomics has revealed the pervasive nature of protein phosphorylation1. The widespread utility and application of existing approaches to quantifying signalling in vivo and en-masse however remains hampered by poor scalability of enrichment and labelling workflows, large input-material requirements, and inadequate coverage of key signaling networks, particularly in the context of tissue samples. Here, we set out to develop and refine a phosphoproteomics pipeline that was scalable, without compromising performance. Such a pipeline would enable processing of large sample numbers in parallel, and without using proprietary reagents to ensure its widespread applicability.

Here we describe EasyPhos2, a high-performance, scalable and extensible phosphoproteomics workflow that greatly streamlines the study of signalling networks across large sample numbers. We evaluated our platform in cells and tissues, measuring the phosphoproteomes of mouse liver cell lines, where replicate single-shot measurements quantified ~20,000 distinct phosphopeptides in one day. In liver, brain and kidney tissues, half-day measurements together quantified 24,000 phosphopeptides. Deep phosphoproteome coverage in the absence of fractionation was facilitated by high enrichment specificity (>95% of identified peptides phosphorylated). Compared with our previous studies in the same systems we achieved 3x the depth, despite using 10x less material and 1/3rd of the measurement time.

EasyPhos requires minimal input material and measurement time without compromising depth, making large-scale signaling studies much more practical. Just as cheap and rapid sequencing technologies have driven an explosion of data in the genomics fields, we envisage an acceleration of MS-driven signalling studies providing rich and complex insights about cellular network function in physiological and patho-physiological contexts.

1. Humphrey SJ, James DE, Mann M. Protein Phosphorylation: A Major Switch Mechanism for Metabolic Regulation. Trends in Endocrinology and Metabolism (2015). 26(12):676-87.
2. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. Nature Biotechnology (2015). 33(9):990-5.

143

New elements of the chondrocyte stress response identified in a model of cartilage degeneration

Richard Wilson[1], Sue B. Golub [2], Lynn Rowley [2], Constanza Angelucci [2], Yuliya Karpievitch [3], John F. Bateman[2], Amanda J. Fosang[2]

1. *Central Science Laboratory, University of Tasmani, Hobart, TAS, Australia*
2. *Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, VIC, Australia*
3. *Department of Mathematics and Physics, University of Tasmania, Hobart, TAS, Australia*

The destruction of articular cartilage in osteoarthritis involves chondrocyte dysfunction and imbalanced extracellular matrix (ECM) homeostasis. Pro-inflammatory cytokines such as interleukin-1a (IL-1a) contribute to osteoarthritis pathophysiology, but the effects of IL-1a on chondrocytes within their tissue microenvironment have not been fully evaluated. To redress this we used label-free quantitative proteomics to analyse the chondrocyte response to IL-1a within a native cartilage ECM. Mouse femoral heads were cultured with and without IL-1a and both the tissue proteome and proteins released into the media were analysed. Mass spectrometry identified 728 proteins and 368 proteins in the cartilage proteome and secretome, respectively. New elements of the chondrocyte response to IL-1a related to cellular stress included markers for protein misfolding (Armet, Creld2 and Hyou1), enzymes involved in glutathione biosynthesis and regeneration (Gstp1, Gsto1 and Gsr) and oxidative stress (Prdx2, Txn, Atox1, Hmox1 and Vnn1). Other proteins previously not associated with the IL-1a response in cartilage included ECM components (Smoc2, Kera and Crispld1) and cysteine proteases (cathepsin Z and legumain), while chondroadherin and cartilage-derived C-type lectin (Clec3a) were identified as novel products of IL-1a induced cartilage degradation. This comprehensive view of the cartilage IL-1a response identified candidate biomarkers of cartilage destruction and novel targets for therapeutic intervention in osteoarthritis.

144

The *N*-Glycosylation Profile of Metastatic Melanoma Lymph Node Tumours

Jodie L Abrahams[1], Matthew P Campbell[1], Nicolle H Packer[1]

1. *Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*

Metastasis accounts for the majority of mortality associated with melanoma, as limited treatment options exist for advanced stages of the disease. Alterations in cell surface glycosylation, in particular, increases in highly branched tetra-antennary *N*-glycan structures contribute to the invasive and metastatic potential of melanoma cells. Despite a growing understanding of the role of tetra-antennary N-linked oligosaccharides in melanoma biology, there has been little progress in using these glycans as a screening tool for the early diagnosis of metastasis and predictor of patient prognosis.

Here, we demonstrate a targeted method combining PGC-LC-MS with exoglycosidase digestion for the complete structural characterisation and relative quantitation of *N*-glycans released from metastatic melanoma lymph node tumours. Released glycans were treated with a full array of exoglycosidase enzymes to assign monosaccharide linkage and confirm terminal epitopes on pools of good and poor prognosis patient samples. The global membrane *N*-glycosylation profile of tumour tissue from individual patient samples have been compared and glycan structures quantitated before and after selected exoglycosidase combinations to confirm differences in structural features including the degree of branching, sialylation and fucosylation. Over 80 glycan structures were identified, including high mannose, pauci mannose, hybrid and complex type glycans.

This study contributes to our understanding of glycosylation alterations in melanoma metastasis towards using specific glycosylation changes as prognostic markers and specific targets for therapeutic intervention.

145

Proteomic and Degradomic Analysis of a Virulence-Associated Serine Peptidase from *Campylobacter Jejuni*

Joel A Cain[1, 2], Sherif Abouelhadid[3], Brendan W Wren[3], Stuart J Cordwell[1, 2, 4, 5]

1. *University of Sydney, Sydney, NSW, Australia*
2. *Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia*
3. *The London School of Hygiene & Tropical Medicine, London*
4. *Mass Spectrometry Core Facility , University of Sydney, Sydney, NSW, Australia*
5. *Discipline of Pathology, University of Sydney, Sydney, NSW, Australia*

Campylobacter jejuni is one of the leading causes of acute gastroenteritis in the developed world, and a major antecedent for a number of debilitating autoimmune disorders.Recently, the serine peptidase Cj0511 has been identified as being required for optimum virulence and is associated with number of virulence mechanisms including biofilm formation, stress tolerance and pancreatic amylase triggered α-dextran secretion. As a component of outer membrane vesicles, which are a key mechanism for delivery of a number of classical mediators of pathogenicity including components into host cells, there is also an implication that Cj0511 may also modify host proteins during infection.

Here, we employed iTRAQ-based labelling to determine the effect of loss of the peptidase on whole protein abundance in a hypermotile population of the original sequenced strain; *C. jejuni* strain 11168H. Of the 1306 *C. jejuni* proteins quantified, only 67 were deemed to have a significant change in abundance in the Δ*cj0511* strain relative to the wild-type isolate. Of these, 41 were reverted to WT levels in a complemented strain, Δ*cj0511*Ω*cj0046* including a number of proteins from the *dccRS* regulon, flagellar components and protein secretory systems all of which have known associations with virulence. *N*-terminal amine isotopic labelling of substrates (*N*-TAILS) was also employed for a pair wise comparison of the *N*-degradome of wild-type 11168H and the *cj0511* knock out strain to both identify biological targets of the protease as well as attempt to elucidate the sequence specificity of the protease. These proteomics-based approaches were complemented with various standard phenotypic tests to further establish the role of the protease towards *C. jejuni*'s physiology.

1. Karlyshev, A. V. et al.. 2014. Campylobacter jejuni gene cj0511 encodes a serine peptidase essential for colonisation. FEBS Open Bio 4:468-72
2. Jowiya, W. et al.. 2015. Pancreatic amylase ss an environmental signal for regulation of biofilm formation and host interaction in Campylobacter jejuni. Infect Immun 83(12):4884-95
3. Kleifeld, O. et al.. 2010. Isotopic labelling of terminal amines in complex samples identifies protein N-termini and protease cleavage products. Nat Biotechnol 12(3):281-288

201

Towards a blood test for oesophageal adenocarcinoma: Serum glycoprotein biomarker candidates for oesophageal adenocarcinoma

Alok Shah[1, 2], Kim-Anh Le Cao[1, 2], David Chen[3], Eunju Choi[1, 2], Benoit Gautier[1, 2], Derek Nancarrow[4], David Whiteman[4], Nicholas Saunders[1, 2], Andrew Barbour[5, 2], Virendra Joshi[6], Michelle Hill[1, 2]

1. *UQ Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia*
2. *Translational Research Institute, Brisbane, QLD, Australia*
3. *School of Information and Communication Technology, Griffith University, Brisbane, QLD, Australia*
4. *QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia*
5. *School of Medicine, The University of Queensland, Brisbane, QLD, Australia*
6. *Gastroenterology, Ochsner Health System, New Orleans, LA, United States*

While the incidence of most cancers are now steadying or declining, oesophageal adenocarcinoma (EAC) continues an upward trend. Rapid increase in EAC is not due to improved diagnosis, but attributed to the increased prevalence of risk factors gastro-esophageal reflux and obesity. Despite aggressive treatment, the survival rate for EAC is low at 9-24% five years post-diagnosis. The precursor condition, Barrett's oesophagus (BE), affects 0.2-2% of the adult population and increases EAC risk 30-100 fold. However, due to the low conversion rate of BE to EAC, studies indicate that current endoscopic screening programs may not be beneficial. Furthermore, a significant proportion of EAC patients do not have prior BE diagnosis, hence there is an urgent need for better detection of EAC. Our goal is to develop blood biomarker panels that can be used to screen at-risk patients, with positive or suspicious results triggering follow-on endoscopic screening.

We focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to enrich serum glycoproteins based on their glycan structures. Serum samples from healthy, BE and EAC patients (n=108) were analyzed by lectin magnetic bead array (LeMBA)[1, 2]-coupled biomarker discovery (LeMBA-QTOF-GlycoSelector http://glycoselector.di.uq.edu.au/index.php) and verification (LeMBA-MRM-MS-Shiny mixOmics http://mixomics-projects.di.uq.edu.au/Shiny/) pipeline.[3]

We have identified a list of putative serum glycoprotein biomarker that distinguish a) EAC from BE and b) EAC from healthy phenotypes. A multivariate panel achieved area under the receiver operating curve (AUROC) over 0.9, indicative of high diagnostic value. Selected biomarker candidates were further validated using an orthogonal technique, immunoblotting. The results indicate alteration of complement pathway in EAC pathogenesis. Currently we are evaluating the biomarker candidates in an independent patient cohort including early stage EAC patients. Continuing work will also evaluate performance of the biomarker panel to predict therapeutic response.

[1] Loo et al., J Proteome Res 2010

[2] Choi et al., Electrophoresis 2011

[3] Shah et al., Mol Cell Proteomics 2015

202

MALDI Imaging of primary endometrial cancers reveals proteins associated with lymph node metastasis

Parul Mittal[1], Manuela Klingler-Hoffmann[1], Georgia Arentz[1], Gurjeet Kaur[2], Lyron Winderbaum[1], Noor Alia Lokman[1], Chao Zhang[1], Martin Oehler[1, 3], Peter Hoffmann[1]

1. *University of Adelaide, Adelaide, SA, Australia*
2. *Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*
3. *Department of Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, South Australia, Australia*

Metastasis is a crucial step of malignant progression and remains the primary cause of death for patients with endometrial cancer (EC). However, clinicians presently face the challenge that conventional surgical-pathological variables, such as tumour size, depth of stromal invasion, histological grade, FIGO stage, lymphovascular space invasion or radiological imaging are unable to predict metastatic potential of the primary tumour with accuracy. In the current study, we have compared differential protein expression using primary tumour samples of EC patients diagnosed with (n=24) and without (n=34) lymph node metastasis (LNM). Using matrix assisted laser desorption and ionisation imaging mass spectrometry (MALDI-MSI), we have identified protein signatures from primary tumours to accurately predict LNM. By applying canonical correlation analysis (CCA) based variable ranking approach; we have generated a list of *m/z* values that could predict the status of LNM in EC. Protein identifications were achieved by *in situ* MALDI MS/MS in combination with LC-MS/MS. SCiLS lab software was used to visualize the spatial distribution of potential molecular discriminators and their AUC values. Using this approach, we could reliably identify two proteins, which can discriminate EC with and without LNM with an AUC score of 0.2-0.3. Further, the differential expression of proteins was validated by immunohistochemistry. In summary, MALDI-MSI has the potential to identify discriminators of metastasis using primary tumour samples.

203

Post translational modifications in the type 2 diabetic liver and their contribution to the diseased state

Desmond K Li[2, 1, 3], Lauren E Smith[1, 3], Joel A Cain[2, 3], Jana Paulech[2], Stuart J Cordwell[2, 1, 3, 4], Melanie Y White[2, 1, 3]

1. *Discipline of Pathology, School of Medical Sciences, The University of Sydney, Sydney, NSW, Australia*
2. *School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia*
3. *Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia*
4. *Mass Spectrometry Core Facility, The University of Sydney, Sydney, NSW, Australia*

Insulin resistance and oxidative stress are hallmarks of type 2 diabetes (T2D), commonly observed in insulin sensitive tissues including the liver. Insulin resistance has been attributed to impaired insulin signalling which normally promotes glucose uptake and utilisation through the IRS/PI3K pathway. Oxidative stress involves the increased generation/reduced clearance of reactive oxygen species (ROS), with high levels of ROS oxidising proteins producing redox modifications which may alter structure, functionality and signalling pathways. To investigate the changes in signalling mediated by phosphorylation and/or redox modifications with T2D, we investigated a rat model using global phosphoproteomics and redox proteomics. Rats were subjected to a high fat diet and streptozotocin to generate the T2D pathology. To quantify alterations in PTM status, samples were isobarically tagged and subjected to various enrichment strategies prior to mass spectrometry (MS). Phosphopeptides were enriched by a combination of TiO_2 and IMAC while redox modified peptides were enriched by thiol disulfide exchange for reversibly oxidised cysteines. Enriched and non-captured fractions were subjected to hydrophilic interaction chromatography prior to MS analysis. The insulin signalling pathway showed significant reduction in phosphorylation confirming impairment in T2D. Pathways regulating energy metabolism also showed significant perturbations in phosphorylation, possibly in response to or contributing to the energy imbalance. Oxidative modifications to the insulin receptor suggests contribution of redox modifications to insulin resistance, while modifications to proteins which maintain redox balance suggests dysregulation, contributing to the oxidative stress in T2D. The current study has identified changes in protein phosphorylation and redox state indicative of dysregulated signalling and energy utilisation while isolating species prone to oxidative damage, all able to contribute to the pathogenesis of T2D.

204

The myocardial degradome following ischemia/reperfusion injury

Melanie White[1], Nina Hartcher[1], Kiersten A. Liddy[1], Joel A. Cain[1], Stuart J. Cordwell[1]

1. *School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia*

Myocardial ischemia and the subsequent return of normal blood flow by reperfusion can both lead to significant injury in the heart. Ischemia / reperfusion (I/R) injury is characterized biochemically by an influx of reactive oxygen species (ROS) and calcium overload, which are both thought to induce proteases (e.g. matrix metalloproteinases and calpains) that target contractile proteins and other metabolic processes that are necessary for uninhibited function of the myocytes. Such proteases are therefore assumed to contribute to contractile dysfunction, apoptosis and necrosis during I/R injury. Here we describe a high-throughput approach to identifying and quantifying the targets of proteolysis in I/R. An *ex vivo* rat heart model of brief I/R was employed in the presence and absence of ROS scavengers. N-terminal peptides were enriched and quantified by N-terminal amine isotopic labelling of substrates (N-TAILS) and analysed by LC-MS/MS. Degradation products (neo-N-termini) were identified that corresponded to previously identified markers of proteolytic damage in I/R, including the troponins (TnI and TnT) and myosin light chains 1 and 2, as well as completely novel sarcomeric proteins such as desmin and myomesin. Cleaved proteins were also identified in key metabolic pathways including oxidative phosphorylation, the TCA cycle, and redox regulation. Finally, cysteine and glycine rich protein 3 (CSRP3) was confirmed as a target for degradation during I/R, which correlates with our previous work showing the release of CSRP3 peptides into the coronary perfusate post-I/R in both animal models and human STEMI patients. Collectively, our data confirm the increased activity of proteases during I/R injury and identify new molecular proteolytic targets that may contribute to the functional consequences of I/R in the myocardium.

205

Neuronal differentiation and analysis of Multiple sclerosis patient adipose derived stem cells

Naomi Koh Belic[1, 2], Innocent Macha[3], Matt Padula[2], Bruce Milthorpe[1, 3], Jerran Santos[1, 2]

1. *Advanced Tissue Engineering and Drug Delivery Group, School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney*
2. *Proteomics Core Facility, School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney*
3. *Department of Chemistry and Biomaterials, Faculty of Science, University of Technology Sydney, Sydney*

Multiple sclerosis (MS) is a chronic demyelinating disease that typically results in cognitive and physical disability. The high incidence, early onset and long-term morbidity presents an extensive problem for public health as well as decreasing the quality of life for MS patients, due to substantial psychological stress resulting from the impairment of daily function and unpredictable nature of the disease. Whilst there are treatments available to alleviate symptoms, treat attacks and prevent relapses, there are no treatments that have the capacity to recover neurological function.

This project has further explored the capacity of autologous non-MS and MS patient adipose derived stem cells (ADSCs) potential to differentiate into neuronal cells and identifies mechanisms with links to the disease progression. This proteomics and systems biology approach will assist in neuronal regenerative medicine by providing more targeted research with the hope of restoring neurological function, and overcoming the nervous systems limited capacity to regenerate.

ADSCs are an ideal target for neuronal regenerative therapy due to their ability to transdifferentiate into ectodermal lineages and consequently form neuronal cells, and they can be sourced ethically through liposuction of adipose tissue. Characterisation of the cells was achieved by analysis of cell morphology, secreted cytokines, the proteome and validation of known protein markers.

The proteomic analysis demonstrated that ADSCs from both non-MS and MS patients differ by a large number of proteins prior to differentiation. Non-MS neuronal differentiation was confirmed and the comparison to MS patient stem cells revealed that the latter has a reduced neurogenic potential. The explored function of unique and shared proteins at the variable time-points revealed an extensive difference between the two population's stem cells and their differentiated progenies. The identified proteins reveal mechanisms that play pivotal roles within neurogenesis and roles in the disease progression. Elucidating these proteins functionality is vital for understanding the disease and developing a targeted therapy which could utilise stem cells in a regenerative capacity.

206

Towards improving the genome annotation of the honey bee (*Apis mellifera*)

Alison McAfee[1], Sarah Michaud[2], Brock A Harpur[3], Amro Zayed[3], Leonard J Foster[1]

1. *UBC, Vancouver, BRITISH COLUMBIA, Canada*
2. *UVIC Genome BC Protein Research Center, Victoria, Canada*
3. *York University, Toronto, Canada*

Honey bees (*Apis mellifera*) are important pollinators in managed agriculture as well as natural ecosystems. Recently, the sequencing and annotation of the honey bee genome has allowed proteomics to become a powerful technique to probe aspects of honey bee biology; however, one troubling trend that emerged from these studies is that honey bee samples consistently result in lower peptide identification rates compared to other organisms. This suggests that either the genome annotation can be substantially improved, or some atypical biological process is interfering with the mass spectrometry (MS) workflow.

We used a publically available MS dataset (Peptide Atlas; 1,472 raw files) in a proteogenomic approach to search for missing genes, new exons, and to revive discarded annotations. To do this, we searched the data against a six-frame genome translation, a three-frame refSeq RNA translation and a database including sequences that were removed from previous annotations. We also considered unexpected post-translational modifications (PTMs), high genetic diversity and endogenous proteolysis as alternative explanations for low peptide identification rates.

While we found no significant effects of PTMs, sequence diversity or proteolysis in tissues other than the gut, we did discover 1,454 new coding regions matched by two or more peptides (1% FDR), including twelve sequences that were previously annotated as non-coding RNAs. In a separate search, we matched 748 previously annotated proteins that were not retained in the current official gene set (OGS). Importantly, when the sequences were added to the OGS protein database (increasing the database size by 13.5%), this improved MS identification rates across tissues.

Using this proteogenomic strategy, we have improved the completeness of the honey bee genome annotation. The information we present here can facilitate further research on this important insect and this workflow template can be used to aid the genome annotation of other under-studied species.

207

The *N*-linked glycosylation system of *Campylobacter jejuni*: a functional proteomics approach

Joel A. Cain[1], Nichollas E. Scott[1], Stuart J. Cordwell[1]

1. *School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia*

Campylobacter jejuni is the most common cause of food-borne illness worldwide, accounting for ~225,000 infections in Australia *per* year. Infection is zoonotic and is associated with consumption of under-cooked or poorly prepared poultry, in which *C. jejuni* is a commensal. A unique molecular feature of *C. jejuni* is the ability to post-translationally modify membrane-associated proteins by the *N*-linked addition of a heptasaccharide glycan. Glycan biosynthesis is performed by proteins encoded within the *pgl* (*p*rotein *gl*ycosylation) locus, and attachment is mediated by the PglB oligosaccharyltransferase. Disruption of *pgl* genes reduces chicken colonization and adhesion to human epithelial cells, however the proteins that mediate these phenotypes, and indeed the overall function of the *N*-glycan, remain to be determined. We developed mass spectrometric techniques that have allowed the identification of many novel *N*-linked glycoproteins in *C. jejuni*, and identified a non-canonical glycan that is itself modified by addition of phosphoethanolamine (pEtN) to terminal Gal*N*Ac. Quantitative proteomics and glycoproteomics have examined *C. jejuni* model strains NCTC11168 O (O), a clinical isolate, and NCTC11168 GS (GS), a laboratory-adapted avirulent strain derived from NCTC11168 O. Analysis of these strains demonstrated alterations in proteins responsible for the production of the *N*-linked glycan and its transfer. Increased Pgl proteins leads to elevated *N*-glycan biosynthesis and attachment, but this targets specific proteins rather than a general increase. Quantitative glycoproteomics also revealed glycopeptides that lack the typical *C. jejuni* glycosylation sequon (D/E-X-N-X-S/T). Recently, we have been working on the function of the *N*-glycosylation system, and hypothesize a role in protein stability, mediating protein complexes, and survival in diverse environments.

208

Applying a Proteoform profiling method for neurological disorder biomarker discovery

Jerome Vialaret[1], Sylvain Lehmann[1], Audrey Gable[1,2], Nicolai Bache[3], Pierre-Olivier Schmit[4], Christophe Hirtz[1]

1. *Laboratoire de Biochimie et Protéomique Clinique, Institut de Médecine Régénératrice et de Biothérapie, CHU de Montpellier , Hôpital St. Eloi, Montpellier, France*
2. *Centre Mémoire Ressources Recherche, CHU Montpellier, hôpital Gui de Chauliac, Montpellier, France*
3. *Bruker Daltonics Inc., Odense, Denmark*
4. *Bruker Daltonique, Wissembourg, France*

Measuring the intact masses of proteins from biological samples provides important insight into biological events which cannot be analyzed by standard bottom-up strategies. Despite technical limitations, mainly due to the large size of the biomolecules being measured, it has gained significant momentum over the last couple of years from improvements in both the general workflows and the associated technologies. Being able to analyze intact proteins rather than their proteolytic peptides enables direct measurements of the major and very important biological processes like alternative splicing, proteolytic processing and post translational modifications – including the complex patterns and stoichiometry of multi-modified proteins. The first step in this approach is to establish a set of quantitative proteoform profiles. The differentially expressed features are then targeted and subsequently identified through a separate top-down MS/MS experiment and if needed a more comprehensive characterization.

In this study we have used a quantitative proteoform profiling experiment to detect and identify several potential biomarkers for Alzheimer's disease based on cerebrospinal fluid (CSF). We were able to comfortably and reproducibly detect more than 1500 proteoforms in each sample leading to the detection and identification of 77 differentially regulated proteoforms (potential biomarker candidates) which are currently being identified and validated through a scheduled top-down MS/MS approach. Many of those correspond to proteoforms of proteins carrying a specific modification, a mutation or having undergone a proteolytic modification which would have made their characterization much more challenging, if not impossible, with a bottom-up workflow. One clear example of this is the diagnostic 1:2 distribution of the truncated and mutated versions of Secretogranin 2 which also clearly illustrates the big potential of the proteoform profiling approach. We are currently extending this study to larger cohorts of patients to both validate our current findings and potentially expand the list of new potential biomarkers.

209

Label-free Proteomic Analysis of Microdissected Formalin-fixed and Paraffin Embedded Cutaneous Squamous Cell Carcinoma Tissue

Ali Azimi[1], Kimberley Kaufman[2,3], Marina Ali[1], Steven Kossard[4], Pablo Fernandez-Penas[1]

1. *Dermatology, Westmead Hospital, The University of Sydney, Westmead, NSW 2145, Australia*
2. *School of Molecular Bioscience, Faculty of Science, The University of Sydney, Camperdown , NSW 2050, Australia*
3. *Brain and Mind Centre, The University of Sydney, Camperdown, NSW 2050, Australia*
4. *Dermatopathology, Skin and Cancer Foundation Australia, Darlinghurst, NSW 2010, Australia*

BACKGROUND: Cutaneous Squamous Cell Carcinoma (cSCC) is one of the most common types of non-melanoma skin cancer. Histopathologic variants of cSCC show significantly different clinical behavior and their correct classification is critical for diagnosis, prognosis and therapy.

METHOD: In the present study, we employed laser-capture microdissection coupled with liquid chromatography–tandem mass spectrometry (LC-MS) to test the feasibility of using archival formalin-fixed paraffin embedded (FFPE) cSCC material (five patients) for proteomic investigations that could provide reliable biomarkers for the cSCC clinical management. Pair-wise comparisons were performed between proteomes extracted from tumor and adjacent morphologically normal tissue for each patient. Ingenuity Pathway Analysis (IPA) bioinformatics suite was used to interpret the roles of altered proteins in cSCC pathophysiology. Oncomine database was used to assess the gene expression levels of proteins changed in our dataset.

RESULT AND DISCUSSION: In total, 1310 unique protein species were identified across all five patients. Of these, an average of 144 proteins were significantly increased and 88 proteins decreased in the tumor samples compared to their normal counterparts ($p<0.05$). IPA analysis revealed the disruptions are mainly in proteins and upstream regulators associated with the regulation and control of cell proliferation, apoptosis, and cell movement. Genes corresponding to 18 proteins were also differentially expressed at the transcript level in silico, supporting our initial proteomic measurements.

CONCLUSION: Our findings confirmed that label-free mass spectrometry-based quantitative proteomics is a useful platform for analyzing FFPE cSCC tissues.

210

Proteomic Analysis of an Artelinic Acid-Resistant P. falciparum strain

Geoff Birrell[1], Marcus Hastie[2], Nanhua Chen[1], Qin Cheng[1], Jeffrey Gorman[2], Mike Edstein[1]

1. *Australian Army Malaria Institute, ENOGGERA, QLD, Australia*
2. *Protein Discovery Centre, QIMR Berghofer Medical Research Institute, Brisbane, qld*

Resistance to artemisinin and its derivatives is becoming a serious threat to current antimalarial drug therapy. Understanding the molecular mechanisms underlying artemisinin resistance will aid drug development and malaria control. Recently, genetic screens have identified the involvement of the Kelch13 gene in screens of clinical samples with a slow parasite clearance time, and PfMDR1 gene amplification in laboratory-derived strains of P. falciparum resistant to 80 ng/ml artelinic acid, a semi-synthetic derivative of artemisinin. In these resistant strains, the resistance phenotype has been demonstrated to have (i) decreased sensitivity of mature-stage parasites, (ii) decreased sensitivity of the ring stage to the induction of dormancy, and (iii) a faster recovery from dormancy. In an effort to identify proteins and pathways involved in the resistance phenotype, we have used Orbitrap mass spectrometry-based proteomics to compare global protein expression levels in parallel cultures of resistant and wild-type strains. This has identified 11 proteins whose expression is significantly associated with artelinic acid resistance.These proteins and their potential involvement in artemisinin resistance will be discussed.

211

High-Speed Imaging Mass Spectrometry of N-Linked Glycans Provides a new approach to Molecular Histology and Tissuetyping of Tumors

Shannon Cornett[1], Peggi M Angel[2], Richard R Drake[2]

1. *Bruker Daltonics, Fairview, TN, United States*
2. *Medical University of South Carolina, Charleston, SC, USA*

Introduction: Imaging mass spectrometry (IMS) is a unique analytical tool that allows simultaneous label-free visualization of hundreds of compounds expressed in tissue. When applied to tumor samples, IMS provides a means of correlating many lipids/peptides/proteins to histological features thereby creating a multi-dimensional scale for molecular histology. N-linked glycosylation of proteins is an important subset of the molecular mechanisms important for cellular homeostasis, and disease state alters certain glycosylation mechanisms. The aim of this study was to use a new high-speed IMS tissuetyping platform to map and identify N-glycan markers from prostate and breast tumor samples to determine the suitability using the high-speed platform for N-glycan tissuetyping.

Experimental: Sections were cut at 5 micron from a tissue microarray of breast cancer, 48 HER2+/HER2- cores, and FFPE prostate biopsies. These were mounted onto conductive microscope slides. Each slide was processed with standard antigen retrieval methods, followed by aerosol applications of peptide N-glycanase to release N-linked glycans and matrix for matrix-assisted laser desorption (MALDI). Imaging mass spectrometry analysis of the released N-glycans was done on a Bruker rapifleX at image resolutions of 10-50 um/spot.

Results: Results from the high-speed imaging measurements were found to correlate with data generated from previous studies. A number of histopathology-specific glycan distributions were identified. The high-speed imaging system provided acquisition times similar to that of standard histology, ranging from seconds for each TMA core to a few minutes for the larger biopsies. Images of the most abundant N-glycans (15-20 species) distinguish tumor-related stroma vs normal stroma in the prostate biopsies. In the breast cancer TMA, images show that N-glycan abundances correlate with HER2 status of cancer regions in the cores.

Conclusion: High-speed images of N-glycosylation patterns are characteristic of tissue pathology, making it possible to create large-cohort databases.

212

Quantitative peptide assay for optimized and reproducible sample preparations for mass spectrometry applications

Michael Mariani[1], Xiaoyue Jiang[1]

1. *ThermoFisher Scientific, Scoresby, VIC, Australia*

New advances in mass spectrometry (MS) have pushed the limits of sensitivity, enabling comprehensive characterization and accurate quantitation of complete proteomes. Despite rapid advances in analytical instrumentation and software, data quality to a large extent still depends on upstream sample preparation techniques. A significant reduction in variability should be possible by standardizing sample preparation steps and analyzing accurate quantities of peptide material in each LC/MS analysis. In this study, we utilized a recently introduced peptide quantitation assay to monitor the protein and peptide concentration at each step of Thermo Scientific tandem mass tag™ (TMT™) reagents sample preparation workflow. Standardizing the digestion, labeling and fractionation steps using a peptide quantitation assay significantly improved reproducibility and quality of the MS results.

213

Look who's talking: UHPLC-MRM/MS assay for staphylococcal quorum-sensing and virulence peptides

Thomas Stoll[1], Richard Linedale[1], Mark Morrison[1], Ian H Frazer[1], Michelle M Hill[1]

1. *The University of Queensland Diamantina Institute, The University of Queensland, Woolloongabba, QUEENSLAND, Australia*

With the ongoing rise of antibiotic-resistant pathogens such as Methicillin-resistant *Staphylococcus aureus*, and the loss of their effective treatment, novel approaches to tackle this superbug crisis are urgently needed. The exploitation of bacterial quorum sensing (QS), i.e. the regulation of gene expression in response to fluctuations in cell-population density, has previously been discussed as a potential leverage point to combat pathogens. By interfering with the bacterial communication process, also known as quorum quenching, the goal is to shut down virulence production without developing resistance mechanisms. *S. aureus* employs oligopeptides, so-called autoinducer peptides (AIPs), as QS signaling molecules. AIPs consist of about 8 amino acids (AA) with a thiolester linkage between the central cysteine and the C-terminal carboxyl group. AIPs released by different species have divergent AA sequences but conserved thiolactone ring structure. After the detection of a minimal threshold concentration of AIPs, *S. aureus* expresses phenol-soluble modulins (PSMs), a toxin family usually composed of 21-26 AA arranged in an alpha-helix.

To facilitate monitoring of AIPs and PSMs in bacterial communities, the purpose of this project was to establish a high-throughput mass spec method for screening of staphylococcal isolates. To establish multiple reaction monitoring (MRM) methods, selected AIP and PSM peptides were custom-synthesized and used to experimentally compile a Q1/Q3 transition list and optimize collision energies. Finally, we have been able to squeeze the separation of the physico-chemically very different peptides in a 4.5 min gradient on an Agilent Poroshell column. Screening efforts are currently underway to sensitively detect and quantify AIP and PSM peptides in culture media filtrates from bacterial isolates. Furthermore, gathered knowledge about the fragmentation behavior of cyclic peptides will aid to generate transition lists *in silico* in the future.

214

Epidermal bladder cells as a single-cell-type system to further our understanding of the molecular mechanisms involved in plant salt tolerance and water use efficiency.

Bronwyn J Barkla[1]

1. *Southern Cross University, Lismore, NSW, Australia*

Crassulacean acid metabolism (CAM) is an alternative photosynthetic mode that achieves high water use efficiency through nocturnal CO_2 uptake and daytime stomatal closure. Crop plants that undergo CAM would be particularly attractive alternatives to C_3 crops for agricultural production in semi-arid, water-limited environments and there is increased interest in engineering CAM into C_3 crops (DePaoli et al., 2014). *Mesembryanthemum crystallinum* is a halophyte and facultative CAM plant, with the ability to switch its pathway of CO_2 assimilation from C_3 metabolism to CAM in response to environmental cues including water deficit, salinity or high light. This ability for photosynthetic plasticity makes it an extremely useful model experimental system for understanding the underlying molecular mechanisms of the CAM pathway and additionally, the mechanisms of environmental induction of complex traits in plants. Our lab has been using the specialized model trichomes called epidermal bladder cells that line the aerial parts of *M. crystallinum* as a single cell type system to gain further understanding of CAM and salt-tolerance mechanisms. Transcriptomics, proteomics, metabolomics and ionomics studies have allowed an integrated understanding of the role of these cells, with results contradicting earlier reports these cells were solely repositories for sodium and water (Oh et al., 2015; Barkla et al., 2012; Barkla et al., submitted; Barkla and Vera-Estrella, 2015). Our findings show that photosynthesis and primary metabolism in the cell supports cell growth, ion accumulation, compatible solute synthesis and CAM.

1. DePaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. 2014. Synthetic biology as it relates to CAM photosynthesis: challenges and opportunities. J. Exp. Bot. 65: 3381-3393.
2. Oh D-H, Barkla BJ, Vera-Estrella R, Pantoja O, Lee SY, Bohnert HJ, Dassanayake M. 2015. Cell type-specific responses to salinity-the epidermal bladder cell transcriptome of Mesembryanthemum crystallinum. New Phytologist 15: 207:627-644.
3. Barkla BJ, Vera-Estrella R, Pantoja O. 2012. Protein profiling of epidermal bladder cells from the halophyte Mesembryanthemum crystallinum. Proteomics 12: 2862-2865.
4. Barkla BJ, Vera-Estrella, Raymond CA. Single-cell-type quantitative proteomic and ionomic analysis of epidermal bladder cells from the halophyte model plant Mesembryanthemum crystallinum. Submitted manuscript
5. Barkla BJ, Vera-Estrella, Raymond CA. Single-cell-type quantitative proteomic and ionomic analysis of epidermal bladder cells from the halophyte model plant Mesembryanthemum crystallinum. Submitted manuscript
6. Barkla BJ, Vera-Estrella R. 2015. Single cell-type comparative metabolomics of epidermal bladder cells from the halophyte Mesembryanthemum crystallinum. Frontiers in Plant Science 6:435.

215

Terminal galactosylation and sialylation switching on membrane glycoproteins upon TNF-alpha-induced insulin resistance in adipocytes

Benjamin L Parker[1]**, Morten Thaysen-Andersen**[2]**, Daniel J Fazakerley**[1]**, Mira Holliday**[1]**, Nicolle H Packer**[2]**, David E James**[1]

1. *The University of Sydney, Sydney, NSW, Australia*
2. *Macquarie University, Sydney, NSW, Australia*

Insulin resistance (IR) is a complex pathophysiological state that arises from both environmental and genetic perturbations and leads to a variety of diseases including type-2 diabetes (T2D). Obesity is associated with enhanced adipose tissue inflammation, which may play a role in disease progression. Inflammation modulates protein glycosylation in a variety of cell types and this has been associated with biological dysregulation. Here we have examined the effects of an inflammatory insult on protein glycosylation in adipocytes. We

performed quantitative *N*-glycome profiling of membrane proteins derived from mouse 3T3-L1 adipocytes that had been incubated with or without the proinflammatory cytokine TNF-alpha to induce IR. We identified the regulation of specific terminal *N*-glycan epitopes, including an increase in terminal di-galactose- and a decrease in biantennary alpha-2,3-sialoglycans. The altered *N*-glycosylation of TNF-alpha treated adipocytes correlated with the regulation of specific glycosyltransferases including the up-regulation of B4GalT5 and Ggta1 galactosyltransferases and down-regulation of ST3Gal6 sialyltransferase. Knockdown of B4GalT5 down-regulated the terminal di-galactose *N*-glycans, confirming the involvement of this enzyme in the TNF-alpha regulated *N*-glycome. SILAC-based quantitative glycoproteomics of enriched *N*-glycopeptides with and without deglycosylation were used to identify the protein and glycosylation sites modified with these regulated *N*-glycans. The combined proteome and glycoproteome workflow provided a relative quantification of changes in protein abundance versus *N*-glycosylation occupancy versus site-specific *N*-glycans on a proteome-wide level. This revealed the modulation of *N*-glycosylation on specific proteins in IR, including those previously associated with insulin-stimulated GLUT4 trafficking to the plasma membrane.

216

Modulation of neurotransmitter release by phospho-signalling: Major substrates and master regulators

Kasper Engholm-Keller[1, 2], Ashley Waardenberg[1], Phillip Robinson[1], Mark Graham[1]

1. *Children's Medical Research Institute, Faculty of Medicine, University of Sydney, Westmead, NSW, Australia*
2. *University of Southern Denmark, Odense, Denmark*

To achieve understanding of how neural circuits function, we need to unravel the neuronal integration of incoming signals and modulation of synaptic transmission strength over time. This is a process known as synaptic plasticity and is an essential mechanism behind memory and learning. While focus has largely been on post-synaptic plasticity, pre-synaptic plasticity has been widely neglected. An important part of pre-synaptic plasticity is modulation of the synaptic vesicle cycle, which governs vesicle availability/localization – and thereby neurotransmitter release potential.

Protein phosphorylation modulates several aspects of the synaptic vesicle cycle and is thus a potential widespread mode of presynaptic plasticity. We have isolated presynaptic nerve terminals (synaptosomes) from rats and depolarized them with KCl to investigate the role of activity-dependent phospho-signaling in neurotransmission up to 15 min after depolarization. Performing enzymatic fluorescence-based assays of glutamate release, we show that this treatment induces synaptic depression. We have quantified >5000 phosphorylation sites in synaptosomes using dimethylation, phospho-peptide enrichment and fractionation via TiO2, Sequential elution from IMAC and HILIC (TiSH) followed by nanoLC-MS analysis. We observe prolonged reprogramming of the presynaptic phosphoproteome after depolarization. Changes in regulatory phosphosites on phosphatase regulators – key integrators of neuronal signaling networks – suggest a likely link between depolarisation and long-term global presynaptic phosphorylation changes. Importantly, the protein machinery of the active zone controlling the release of neurotransmitter-containing vesicles appears to be regulated by activity-dependent phosphorylation. Amongst these proteins, the key synaptic plasticity protein Rab3 interacting molecule (RIM1), which links calcium influx to neurotransmitter release, contained several regulated phosphorylation sites without known functions. This establishes RIM1 as a prime candidate for being the link between activity-dependent phospho-regulation and presynaptic plasticity.

MS-based protein pulldown experiments using phospho-deficient and -mimetic mutants of RIM1 are currently underway and will reveal the phospho-dependent mediators of presynaptic plasticity. Primary hippocampal neurons will be transfected with these constructs to validate the functional effect of the RIM1 phosphorylation sites using dye uptake/release assays.

217

Elongation factor Thermo unstable (Ef-Tu) is proteolytically processed on the extracellular surface of *Staphylococcus aureus*

Kate Harvey[1, 2], Joel R Steele[1], Jacqueline A Melvold[1, 2], Michael Widjaja[1, 2], Matt Padula[1, 3], Ian G Charles[4], Steven Djordjevic[1, 2]

1. *University of Technology Sydney , Broadway, NSW, Australia*
2. *The ithree institute, Faculty of Science, University of Technology, Sydney, Australia*
3. *Proteomics core facility, Faculty of Science, University of Technology, Sydney, Australia*
4. *Institute of Food Research, Norwich, England*

Staphylococcus aureus is a leading cause of nosocomial-acquired infections and has rapidly acquired resistance to multiple antibiotics. Pathogenic and commensal bacteria present multifunctional surface adhesins that bind to a wide range of host molecules, particularly those associated with the extracellular matrix. Glycosaminoglycans are components of the extracellular matrix (ECM) that are targeted by bacterial adhesins. Heparin is a glycosaminoglycan that mimics highly sulphated regions of proteoglycans and it has been used as a bait to identify bacterial virulence factors that facilitate adherence, colonisation and invasion of target host cells.

To characterise the surfacome of *S. aureus* we tagged surface proteins with biotin and identified them by streptavidin affinity chromatography and LC-MS/MS. A complimentary set of surface proteins were identified by shaving *S. aureus* with trypsin. Dimethyl labelling of neo-N-termini followed by LC-MS/MS was used to identify cleavage start sites. Unfractionated native WCL were used to identify heparin-binding proteins via heparin-agarose chromatography. We applied this technique to gain insights into the repertoire of *S. aureus* surface proteins that have evolved the ability to bind highly sulphated glycosaminoglycans and correlated the identities of these proteins with those from our surfacome studies.

318 proteins were identified on the surface of SH1000. Notably, Ef-Tu was identified as an intact protein and as cleaved fragments and both the intact molecule and the cleavage fragments bound heparin. Dimethyl-labelling studies of neo-N-termini enabled us to determine precise sites of cleavage within Ef-Tu. For the first time, these data suggest that Ef-Tu and processed fragments of Ef-Tu can perform alternate functions on the surface of *S. aureus*. Our studies indicate that cleavage events target a region of the molecule that separates the major subdomains within Ef-Tu, suggesting that processing may function to increase functional diversity.

218

An Integrated Approach to Metabolomics Studies in Pancreatic Cancer Cells: Discovery to "Quanfirmation" on a Single Platform

Gina Tan[1], Steven Ramsay[1]

1. *ThermoFisher Scientific, Scoresby, VIC, Australia*

Mass spectrometry (MS)-based metabolomics is one approach used to investigate the metabolic profiles of cancer cells. To gain a comprehensive interpretation of the metabolite variation profiles in biological systems, qualitative and quantitative information is required. This typically involves a discovery-driven untargeted profiling analysis using a high resolution accurate mass (HRAM) MS platform followed by a hypothesis-driven targeted analysis using a tandem triple quadrupole MS platform. Here, we propose a metabolomics research paradigm shift which utilizes a hybrid quadrupole-Orbitrap mass spectrometer to achieve an integrative approach of these two methodologies. The discovery and targeted workflows to study the metabolic profiles of *Panc-1* cancer cells will be discussed.

219

Insight into protein glycosylation in human capacitated sperm using PGC-LC-MS/MS

Abdulrahman M Shathili[1], Arun E Dass[1], Nicolle H Packer[1], Mark A Baker[2]

1. *Macquarie university, Wollstonecraft, NSW, Australia*
2. *Environmental and life sciences , University of Newcastle , Newcastle , NSW, Australia*

In order to achieve a successful pregnancy, sperm rely on surface glycans, which are constantly remodelled throughout their life cycle. Glycans within spermatozoa are essential for several processes, including passage through cervical mucus within the female reproductive track, protection from female immunity and Capacitation. Capacitation, which begins when a sperm is ejaculated, is the term used to describe the biochemical processes that modifies a sperm and enables it to fertilise an Oocyte. Interestingly, Glycans have shown to be highly modified during the capacitation (1) and as such, may play a major role in male infertility; something that affects 1 in 15 men of reproductive age. However, the role of specific glycans and their structures during sperm capacitation is still unknown.

In this work, *in vitro* capacitated sperm were enriched by Percoll density gradient centrifugation. Here we present glycan analysis of capacitated and non-capacitated sperm using porous graphitised carbon-liquid chromatography-MS/MS. Membrane proteins were extracted by different methods, immobilised on a PVDF membrane then treated with PNGase F to release *N*-linked glycans. The subsequent b-elimination allows the collection of *O*-linked glycans from the same proteins. Preliminary results suggested the presence of high mannose, paucimannose and hybrid-complex structures, and membrane enrichment by ultracentrifugation as the best method for high glycan yields from sperm proteins. The differences in glycosylation profiles between capacitated and non-capacitated samples will be presented. The identification of specific glycans associated with sperm capacitation will allow the selection of healthy sperm for *in vitro* fertilisation applications.

1. Tecle E, Gagneux P. 2015. Sugar coated sperm:unreviling the functions of the mammalian sperm glycocalyx. *Molecular Reproduction and Development*. 82:635–650.

220

A comparative transcriptomic and proteomic analysis of the infection of mammalian cells with Nelson Bay orthoreovirus

Lawrence Mok[1, 2], James W Wynne[3], Mary Tachedjian[3], Kris Ford[2], David Matthews[4], Tony Bacic[2], Wojtek P Michalski[1]

1. *CSIRO, Australian Animal Health Laboratory, East Geelong, Victoria, Australia*
2. *ARC Centre of Excellence in Plant Cell Walls School of BioSciences, The University of Melbourne, Melbourne, Victoria, Australia*
3. *CSIRO, Biosecurity Flagship, Australian Animal Health Laboratory, East Geelong, Victoria, Australia*
4. *Department of Cellular and Molecular Medicine, School of Medical Sciences, University of Bristol, Bristol, United Kingdom*

Nelson Bay orthoreovirus (NBV) is the prototypic member of the Pteropine othoreovirus family. Previous work in our laboratory has shown that mouse fibroblast cells are capable of restricting the replication of NBV, in vitro, compared to other mammalian cells. The mechanism involves suppressing viral induced cell fusion (termed syncytia). Here, we investigate this unique host-pathogen interaction further using an integrated transcriptomic and proteomic approach. Employing RNA sequencing and SILAC, the gene and protein expression profiles of mouse fibroblast and immortalised bat kidney cells following NBV infection was assessed.

The evaluation of the gene and protein expression data demonstrate that mouse fibroblasts respond to NBV through a robust type I interferon response. We show that knockdown of the type I interferon receptor (IFNAR1) results in an increase in NBV replication and

in syncytia formation in mouse fibroblasts. In contrast, stimulation of type I interferon signalling in bat cells with recombinant interferon decreases viral replication. Our results suggests type I interferon signalling plays a crucial role in mediating NBV replication and viral induced cell fusion. We are currently investigating which element of the broad type I interferon signalling pathway is involved in mediating this interaction between mouse fibroblast cells and NBV.

By understanding the molecular mechanisms that influence NBV replication, antiviral strategies may be applied to other fusogenic orthoreoviruses.

221

Multi-omics analysis of primary cytotrophoblasts from second trimester and term placentas

Christie Hunter[1], Katy Williams[2], Andrew Olson[3]

1. *SCIEX, Redwood City, CA, United States*
2. *University of California, San Francisco, CA, United States*
3. *Advaita Biosciences, Plymouth, MI, United States*

During human pregnancy, a subset of placental cytotrophoblasts (CTBs) differentiates into cells that aggressively invade the uterus and its vasculature, anchoring the progeny and rerouting maternal blood to the placenta. Defects in this process are the hallmark of the pregnancy complication preeclampsia. While disease-associated genes or transcripts may serve as useful biomarkers, they are not necessarily predictive of disease mechanisms. Thus, we performed global proteomic and transcriptional profiling to measure expression patterns of CTBs from second trimester and term normal placentas to gain further understanding of CTB differentiation in healthy pregnancy.

Primary CTBs were isolated using collagenase and trypsin digestion and Percoll gradient centrifugation. For proteomics analysis, cells were lysed and digested with trypsin. Variable window SWATH® Acquisition data were acquired using the TripleTOF 6600 System (SCIEX). A 180 min gradient using a nanoLC™ 425 with a cHiPLC® system (SCIEX) was performed for separation. SWATH data were processed using OneOmics™ applications in BaseSpace (Illumina). For transcriptomics, a second set of CTBs were analyzed using RNAseq (Kundaje, Nature 2015). iPathwayGuide (Advaita) was used to compare protein and RNA levels for pathway and gene ontology analyses.

Approximately 3000 proteins were quantified and ~400 showed differential expression in second trimester CTBs vs. term. Proteins known to function in CTB differentiation processes, e.g. angiogenesis and hypoxia response, as well as previously uncharacterized processes, e.g., NF-kappaB signaling, metal ion transport and muscle contraction, were altered. Integration with RNAseq data showed variations at the molecular level but concordance among biological processes. Multi-omics data sets comprised of SWATH MS protein quantification and RNAseq expression results showed gestation age differences in healthy CTB populations corresponding to known and novel processes. In future, these methods will be used to study CTBs from patients with preeclampsia is likely to identify aberrations that could contribute to disease and/or serve as diagnostic markers.

222

Quantitative proteomic analysis of plasma proteins in healthy neonates, young children and adults using SWATH-MS data independent acquisition

Thiri Zaw[1], Jemma Wu[1], Dana Pascovici[1], Xiaomin Song[1], Stefan Bjelosevic[2], Vasiliki Karlaftis[2], Mark Molloy[1], Paul Monagle[2], Vera Ignjatovic[2]

1. *Australian Proteome Analysis Facility, Macquarie University, NSW, Australia*
2. *The Royal Children's Hospital, Murdoch Childrens Research Institute, Parkville, Victoria, Australia*

SWATH-MS data independent acquisition is now commonly used to determine the abundance of proteins in complex biological samples. It is well suited for quantitative studies across many biological samples, but has not yet been fully exploited for applications of human plasma biomarker discovery.

In this paper, we used SWATH-MS to profile changes in plasma proteins associated with healthy human infant development. We profiled 40 individual plasma samples from four sample age groups (neonates, < 1-year olds, 1-5 year olds, adults, n=10 each). A local SWATH peptide library was built by combining 1D nanoLC MS/MS IDA runs from each pooled group, and an extended SWATH peptide library was also evaluated by merging the local library with publicly available MS spectral repository in SWATHAtlas.

Using undepleted plasma, 118 proteins with minimum of two peptides were quantified when the local library was used which contained 146 proteins while the extended library allowed for putative identification and quantification of more than 900 proteins. Overall, the changes in neonatal plasma proteins were the most significant when compared with the other three groups. Using both protein and peptide cut-off criteria to determine proteins changed in expression, the local library yielded 17 and 26 proteins up- and down-regulated respectively when compared with children under one year of age. Correspondingly, 8 and 35 proteins were found to be up- and down-regulated respectively when compared to 1-5 year old children; and 9 and 25 proteins with adults group. Interestingly, the number of differentially expressed proteins was only slightly higher with the extended library. In summary, this is the first description of using SWATH-MS to profile the expression of plasma proteins associated with human infant development.

223

SWATH mass spectrometry performance using extended ion assay libraries

Jemma Wu[1], Xiaomin Song[1], Dana Pascovici[1], Thiri Zaw[1], Natasha Care[1], Christoph Krisp[1], Mark Molloy[1]

1. *Australian Proteome Facility Centre, North Ryde, NSW, Australia*

The use of data-independent acquisition methods such as SWATH for mass spectrometry based proteomics is usually performed using peptide MS/MS reference ion assay libraries which enable identification and quantitation of peptide peak areas. Reference assay libraries can be generated locally through information dependent acquisition, or obtained from shared data repositories for commonly studied organisms. However, there have been no studies performed to systematically evaluate how locally-generated or repository-based assay libraries affect SWATH performance for proteomic studies. To undertake this analysis we developed a software workflow, *SwathXtend,* which generates extended peptide assay libraries using a local seed library and delivers statistical analysis of SWATH-based sample comparisons. We designed test samples using peptides from a yeast extract spiked into peptides from human K562 cell lysates at different ratios to simulate common protein abundance change comparisons. SWATH-MS data with 2, 5 and 10% of yeast peptides spiked into the human cell lysate were assessed using several local and repository-based assay libraries of different complexities and proteome compositions. We evaluated detection specificity and accuracy to detect differentially abundant proteins and reporting thresholds for statistical analyses. We demonstrate that extended assay libraries integrated with local seed libraries achieve better performance than local limited assay libraries alone from the aspects of the number of peptides and proteins identified and the specificity to detect differentially abundant proteins; the performance of extended assay libraries heavily depend on the similarity of the seed and add-on libraries; statistical analysis with multiple testing correction can improve the statistical rigor needed when using large, extended assay libraries.

224

Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS/MS) Proteomic Analysis

Peter Mollee[1, 2], Patricia Renaut[3], Samuel Boros[3], Dorothy Loo[1, 4], Michelle M Hill[1, 4]

1. *University of Queensland, Brisbane, QLD, Australia*
2. *Princess Alexandra Hospital, Brisbane, QLD, Australia*
3. *Pathology Queensland, Brisbane, QLD, Australia*
4. *Translational Research Institute, Brisbane, Australia*

Background & Aims

Correct identification of the protein that is causing amyloidosis is crucial for clinical management. Current standard laboratory methods have limited ability to detect the full range of amyloid forming proteins. We assessed the diagnostic value of LCM-MS/MS, which combines specific sampling of amyloid deposits by LCM with protein identification by MS/MS.

Methods

Biopsy specimens were referred to the Princess Alexandra Hospital Amyloidosis Centre. For all specimens, 10µm sections of formalin-fixed paraffin embedded tissue were stained with Congo Red using a standard technique. LCM was performed using an Arcturus XT instrument with an infrared capture laser. Proteins were extracted with FFPE Protein Extraction Solution, digested with trypsin and peptides were analysed by nano-liquid chromatography-coupled MS/MS using an Agilent Chip CUBE-QTOF.

Results

Biopsies were received for 136 patients from 35 different organ sites. There was insufficient tissue in the block for 7 cases, repeat LCM was required in 15 cases. An amyloid forming protein was identified in 121 out of 129 attempted cases (94%). Of the 121 successfully cases, the Mayo Clinic (at least two of SAP, ApoE and ApoA4) was detected in 92 (76%), and was associated with higher Amyloid protein score and % coverage. We found that vitronectin provided complementary information to the existing 3-protein amyloid signature. In terms of clinical impact, amyloid typing by immunohistochemical stains had been attempted in 87 cases and reported as diagnostic in 39. Five of these were subsequently revealed by proteomic analysis to be incorrect. Overall, the clinical diagnosis of amyloid subtype was altered by proteomic analysis in 24% of cases.

Conclusions

LCM with LC-MS/MS successfully identified an amyloid forming protein in 94% of clinical biopsy samples. Amyloid deposits often contain small amounts of other amyloid forming proteins. Because of this, results need to be interpreted in the context of full clinical information to enable correct diagnosis of amyloid subtype.

225

Phosphoproteomics reveals Aurora kinase B (AurkB) as a regulator of neuronal development

Serene Gwee[1], Albert Lee[1], Mark Malloy[1], Roger Chung[1]

1. *Macquarie University, NSW, Australia*

Protein kinases are utilized by cells during cell division and development, cytokinesis, and for the maintenance of metabolic homeostasis. Given the well-established role of Aurora kinase B (AurkB) in cellular division and cancer development, we have made a

surprising recent discovery – that AurkB has a critical role in axonal outgrowth of neurons (a non-dividing cell). We have recently identified AurkB in neuronal regeneration [1] but its substrates involved in neurite outgrowth remains to be investigated. Here, we aim to explore the potential phosphorylated substrates of AurkB and their involvement in neuritogenesis. Utilizing titanium dioxide enrichment and LC-MS/MS, differentiated RGC-5 cells grown in SILAC neurobasal media were treated with AurkB inhibitor, AZD1152-HQPA, and cell lysates were digested with trypsin and enriched for phosphopeptides. We identified >10,000 phosphopeptides, in which ~650 phosphopeptides were differentially down-regulated upon treatment. Preliminary analysis revealed that some key proteins involved in axonal transport are under the regulation of AurkB. The role of AurkB was further supported by in vivo studies of transgenic zebrafish, demonstrating that overexpression of AurkB regulates axonal elongation and neurite outgrowth. Collectively, this data suggests that apart from cellular division, AurkB could play a critical role in the development of neurons.

1. 1) Ng, J. M., Chen, M. J., Leung, J. Y., Peng, Z. F., Manikandan, J., Qi, R. Z., . . . Chung, R. S. (2012). Transcriptional insights on the regenerative mechanics of axotomized neurons in vitro. J Cell Mol Med, 16(4), 789-811. doi:10.1111/j.1582-4934.2011.01361.x

226

A slimy situation: Using proteomics to highlight mechanisms of slime secretion in the southern bottletail squid, *Sepiadarium austrinum.*

Nikeisha Caruana[1], Ira Cooke[1], Mark Norman[2], Pierre Faou[1], Jan Strugnell[1]

1. *La Trobe University, Bundoora, VICTORIA, Australia*
2. *Museum Victoria, Melbourne, VICTORIA, Australia*

Cephalopods comprise over 800 species, possess advanced nervous, cardiovascular and visual systems, and are masters of camouflage. They are also thought to use toxic secretions widely for defence and predation but very little is known about the proteomic composition of these secretions. One such species, *Sepiadarium austrinum*, the Southern bottletail squid, secretes viscous slime from its underside. This substance is thought to be toxic to predators such as crabs but the physical structure of slime itself is also of interest because it is remarkably voluminous and rapidly secreted.

Along with identifying the putative toxic proteins within the slime secretions of *S. austrinum,* this study compares its protein composition to the slime of sea stars and planarians, and speculates on potential similarities in slime mechanism to that of hagfish. Proteins involved in the hagfish slime secretion include calcium regulators as well as intermediate filament proteins, both of which are abundant within *S. austrinum* slime.

By combining a de novo assembled transcriptome from various tissues including the slime, with the proteomics of *S. austrinum* slime, our study was able to uncover this remarkable defence mechanism within a relatively unknown species. The method used for identifying proteins in non-model organisms has allowed a greater understanding of the proteins involved in the mechanisms that control the secretion of slime within *S. austrinum.* This method will also be used on other closely related species to create a comparative proteomic study on the secretions of cephalopods and highlight potentially novel proteins.

Identifying the proteins found within *S. austrinum* slime is important both from an ecological and evolutionary perspective as well as having the high potential to produce biomedical products, and biomimetics.

227

PromarkerD: Biomarkers for Diabetic Kidney Disease

Scott Bringans[1], Richard Lipscombe[1], Kaye Winfield[1], Jason Ito[1], Wendy Davis[2], Kirsten Peters[1, 2], Tim Davis[2]

1. *Proteomics International, Broadway, Nedlands, WA, Australia*
2. *Fremantle Hospital, Perth, WA, Australia*

Chronic kidney disease is a significant complication of diabetes that affects >35% of patients and is a major cause of renal transplantation globally with 10-20% of diabetics dying of kidney failure. The current diagnostic test for diabetic kidney disease uses a single urine biomarker that measures microalbumin levels (albumin/creatinine ratio, ACR) which has limited utility. Clinicians require better tools that deliver a more effective diagnosis, prognosis and monitor treatment to notably improve health outcomes.

A panel of **12 candidate biomarkers** were identified by mass spectrometry in a pilot study of 90 highly curated clinical samples. A further **diagnostic** study analysing 572 individuals has validated protein biomarkers at high stringency using mass spectrometry, including proteins involved in metabolism, inflammation and oxidative stress. Cross-validation of these findings against antibody-multiplexing approaches has yielded **highly significant correlations between biomarker levels and the ACR and eGFR** measurements of these patients. Specific ROC curves were constructed for combinations of biomarkers that show that the panel can discriminate different risk categories of diabetic kidney disease.

The final **prognostic** dataset was collected from 349 patients at three key time points (Years 0, 2, 4). The statistical analysis of this data revealed the predictive ability of the biomarker panel to **determine which patients were at risk of significant and rapid decline in kidney function** better than any other known measure. The preferred model of 3 biomarkers as a predictor of eGFR decline had an **AUC of 0.83 with 89% sensitivity, 68% specificity**. People who have altered levels of protein from the biomarker panel are up to 7 times more likely to be in the eGFR decliner trajectory group.

PromarkerD as a panel of biomarkers for diabetic kidney disease has the ability to both diagnose existing disease where the current measures are inadequate and predict future significant decline in kidney function. The target outcome is a **point-of-care test** or **companion diagnostic** for diabetic kidney disease.

228

Characterisation of a Potential Nutrient Transporter Protein (Cj0025) in *Campylobacter jejuni*

Lok Man[1]

1. *School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia*

Campylobacter jejuni is recognized as the most common pathogen responsible for bacterial gastroenteritis in the developed world, with the route of infection involving under-cooked or poorly prepared poultry as the organism resides as a commensal in the intestines of avian species. The ability of *C. jejuni* to survive in a diverse range of environments, colonise chickens and to adhere to and invade human gut epithelial cells has been strongly linked to factors including motility and the ability to uptake and utilise 'unusual' nutrients, especially organic and amino acids, rather than sugars. *C. jejuni* encodes a putative C_4-dicarboxylate transporter (gene designation *cj0025c*) that has been identified as the most up-regulated protein in proteomics analysis of *C. jejuni* grown under conditions of stress that mimic human hosts. The function of Cj0025c however, as well as the substrate it transports, remain unknown. Here, we use quantitative mass spectrometry-based proteomics using both iTRAQ and SWATH methods to compare proteomes of wild-type 11168H and *cj0025c* knockout strains with and without additional treatment conditions.

iTRAQ-based proteomic analysis of the *Δcj0025c* mutant revealed several functional clusters of proteins changing in abundance, including proteins involved in lipooligosaccharide modification and flagellar motility. SWATH-based proteomics successfully validated the *Δcj0025c* versus wild-type proteome comparison, and revealed clusters of proteins with altered abundance when cells were subjected to osmotic stress, including ribosomal and tryptophan biosynthesis proteins.

229

Defining IGF-II and IR-A signalling in Breast Cancer Cells

Liang Li[1], **Carlie Delaine**[2], **David Bersten**[1], **Peter Hoffmann**[1], **Briony Forbes**[2]

1. *University of Adelaide, Adelaide, SA, Australia*
2. *Medical Biochemistry, Flinders University of South Australia, Adelaide, SA, Australia*

Insulin like growth factor II (IGF-II) binds to Insulin Receptor isoform A (IR-A) to sustain cell growth and proliferation. This autocrine loop exists in many human carcinomas and provides an additional proliferation and survival pathway to the type 1 insulin-like growth factor (IGF-1R) signalling pathway. In addition, activation of the IGF-II/IR-A autocrine loop provides a mechanism of resistance to drugs targeting the type 1 insulin-like growth factor (IGF-1R). However, the mechanism of how IGF-II/IR-A differentiates itself from the IGF-II/IGF-1R pathway is still unclear. Here we use MDA MB 231 (Triple negative) breast cancer cells as study models. In this study, we aim to combine SILAC (Stable Isotope Labelling with Amino Acids in Culture) and MS/MS methods to define signalling molecules activated uniquely in the IGF-II/IR-A autocrine loop by determining the phospoproteomic profile. The MDA MB 231 cell line expresses components of both the IGF-II/IR-A and IGF-II/IGF-1R pathways. In order to study only the IGF-II/IR-A pathway, the IGF-1R was knocked down using shRNA targeting the IGF-1R gene delivered in the context of miR30 by a tightly regulated Doxycycline (Dox)-inducible lentiviral vector. Proliferation assays confirmed that signalling through the IGF-II/IR-A pathway enhances the cell growth in MDA MB 231 IGF-1R knock down cells. The IGF-1R knock down cells were SILAC labelled by growth in media containing three different labelled amino acid combinations (Lys^0Arg^0, Lys^4Arg^6 or Lys^8Arg^{10}). Data will be presented arising from MS analysis of phosphopeptides from insulin or IGF-II stimulated cells. Identification of specific proteins in IGF-II/IR-A pathway will provide new drug targets to help the treatment of cancers dependent on the IGF-II/IR-A autocrine loop.

230

Investigation of prostasin's role in placental development by proteomic and degradomic approaches

Melanie C Foell[1, 2], **Anna Keppner**[3], **Martin L Biniossek**[1], **Edith Hummler**[3], **Oliver Schilling**[1]

1. *Institute of Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany*
2. *Faculty of Biology, University of Freiburg, Freiburg, Germany*
3. *Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland*

Prostasin is a membrane-anchored serine protease, expressed in the epithelium of multiple organs and known to regulate the epithelial sodium channel. In several studies it has been linked to pre-eclampsia.

In mice, prostasin deficiency leads to placental malfunction and embryonal lethality from day 13.5 onwards. To understand the consequences of deleting prostasin in the placenta, we quantitatively compared placenta lysates of wildtypeand prostasin deficient mice by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in conjunction with stable isotope labeling. Furthermore we were interested in prostain's substrates and performed "terminal amine isotopic labeling of substrates" (TAILS), an N-terminomic technique for the identification and quantification of native and proteolytically generated protein N-termini.

Prostasin deficiency leads to alterations in the abundance of proteins involved in angiogenesis, placental development and several members of the carcinoembryonic antigen family. TAILS revealed that Prostasin affects a multitude of proteolytic processing sites. To underline these findings validations by immunohistochemistry and western blot analysis will be performed.

231

Haptens Happen: Searching for drug modified Human Leukocyte Antigen-Peptide Complexes

Patricia Illing[1], Nicole Mifsud[1], Nadine Dudek[1], Julian Vivian[1, 2], Jamie Rossjohn[1, 2], Anthony Purcell[1]

1. *Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia*
2. *Australian Research Council Centre of Excellence for Advanced Molecular Imaging, Monash University, Clayton, Victoria, Australia*

Adverse drug reactions (ADRs) inflict a significant toll on our health care system, preventing the use of key medications. A subset of ADRs involve the inappropriate activation of T cells resulting in immune responses targeted against healthy tissues. The Human Leukocyte antigen (HLA) class I and II molecules are cell surface proteins that present both self and pathogen derived peptides to CD8 and CD4 T cells, respectively. Under immunogenic conditions, peptides that are not part of the normal self-repertoire (for example pathogen derived peptides) stimulate T cell responses to help combat infection. Drugs such as beta-lactam antibiotics (including penicillins) have been shown to modify proteins and T cell responses to antigen presenting cells fed with drug modified human serum albumin have been observed. Here we explore modifications induced by penicillin-G in cell culture media containing either bovine or human serum. We use these data, in combination with the large amount of HLA-peptide ligand data amassed in the laboratory, to explore the potential for both direct haptenation of, and haptenated peptide presentation by, the HLA molecules. These data lay the groundwork for screening for drug-modified HLA-peptide complexes.

232

A Simple and Convenient Way to Pack Capillary LC Columns

Hans-Juergen Wirth[1], Mike Bailey[1], Harry Ritchie[2]

1. *Trajan Scientific and Medical, Ringwood, Vic, Australia*
2. *Trajan Scientific and Medical, Chester, UK*

Packing your own columns for capillary and nano LC is very common in the research community but has a number of disadvantages over commercially packed columns. In a lab it is harder to get the columns reproducible due the variation in bed height, the manufacture of the frits and the fact that fused silica capillaries are fragile. Packing your own columns also require a pressurised packing bomb and a high pressure gas supply.

We have developed a column packing kit where the fused silica capillaries are replaced by PEEKsil, a PEEK coated fused silica capillary. Columns with the desired ID and length are butt connected to connection capillaries with integrated low-volume screens and slurry packed using the HPLC pump as the packing solvent delivery system. The resulting columns have a precise bed height, are more robust than fused silica columns and can be reversed for column cleaning purposes. Independent test have also shown them to be equal in performance or superior to commercially available columns.

233

Systematic Comparison of Integrated Microfluidics/Nanoscale LC Platforms and High/Low Resolution Mass Spectrometers for Quantitative MRM Analysis of Candidate Peptide Biomarkers

Chris Hughes[1], Billy Joe Molloy[1], Johannes P.C Vissers[1], James I Langridge[1], Heather Patsiouras[2]

1. *Waters Corporation, Machester, United Kingdom*
2. *Waters Australia, Rydalmere, NSW, Australia*

Biomarker discovery and validation are the first steps in understanding disease and drug development. Validation is technologically challenged since it requires analyzing a large number of samples with high-throughput, but also requires high sensitivity, high resolution, large dynamic range and excellent selectivity. Targeted LC-MS based assays afford protein quantification with the reproducibility and throughput required in order to improve biomarker acceptance. MRM, using both low, high and ion mobility enabled high resolution mass spectrometry, are enabling technologies. Miniaturized LC systems offer improved mass-sensitivity but often lack throughput, robustness and reproducibility. Here, we compare the application of an integrated microfluidic device with nanoscale LC, using different MS platforms, for the quantification of marker peptides and proteins, considering speed, sensitivity and selectivity.

Important aspects when characterizing LC-MS systems for validation/translation experiments are retention time reproducibility, technical reproducibility of the monitored transitions, and consistent, quantitative measurement accuracy. MRM transitions were inspected ensuring that a minimum of two transitions per peptide were detected. Typical retention time reproducibility for the monitored peptides was shown to be acceptable at microfluidic and nanoscale LC system levels and equaled 0.02 min standard deviation. Ratio measurements experimentally determined for peptides present in the MS Qual/Quant mixture were in good agreement with the manufacturer specified values. For all peptides, the average variation from the expected values was on average

11.5%. The levels of various putative peptide biomarkers in tryptically digested human serum matrix were determined, for example, ITLYGR was present at 650 amol per 200 ng of plasma matrix using the microfluic LC system in combination with low resolution tandem mass spectrometry, and GYSIFSYATK present at 45 amol per 200 ng of matrix using the nanoscale LC system in combination with high resolution Q-Tof mass spectrometry, operating with and without ion mobility enhancements.

Comprehensive comparison of various LC-MS platforms for the quantitative analysis of potential peptide biomarkers, including ion mobility based MRM technology.

234

Enhancing the Analytical Capabilities of DESI Imaging Using Ion Mobility Separation - Providing Superior Insights of Biological Samples

Emanuelle Claude[1], Emrys A Jones[1], Mark Towers[1], Karolina Skraskova[2], Ron M.A Heeren[2], Jim Langridge[1], Heather Patsiouras[3]

1. *Waters Organisation, Wilmslow*
2. *Maastricht University,, Maastricht, NL*
3. *Waters Australia, Rydalmere, NSW, Australia*

Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) coupled with ion mobility separation has demonstrated significant utility over the last decade for the separation of matrix ion species from endogenous ion species in the gas phase. Since its introduction ten years ago, desorption electrospray ionization (DESI) has been gathering momentum as a complementary MSI technique to the more traditional SIMS and MALDI-MS approaches, proving especially beneficial for the analysis of metabolites/ lipids localization in tissue. In this study, we compare and contrast DESI imaging with MALDI imaging on the same ion mobility enabled mass spectrometer, with variety of samples. We demonstrate that additional classes of molecules are ionized by DESI which are clearly defined using ion mobility.

Different tissue samples including mouse brain sections and human tumor sections, have been analyzed using the same mass spectrometer by both MALDI and DESI. By keeping the parameters for the ion mobility and mass analyzer constant between the different techniques, the ion distribution overlap could be studied in detail. One advantage of MALDI imaging using an ion mobility enabled MS is ability to differentiate clustered matrix peaks from tissue derived analytes (e.g. lipids) as two distinct nested trendlines are observed in the m/z vs drift time plot. As DESI does not require a matrix compound for the ionization of molecules on the surface of tissue section, it could be expected that DESI MS spectra and 2D-plot m/z vs drift time would be cleaner. However, the DESI spectra show similar strong lipid peaks as observed in MALDI, but also intense fatty acid species were detected at the lower mass range. Closer inspection of the ion mobility dimension revealed further trendlines in the 2D-plot , corresponding to either different classes of molecules or different charge states of ions, present at much lower abundance.Additional information highlighted by the use of the ion mobility separation when DESI imaging was compared to MALDI imaging.

235

Compartment specific PTM study of Endogenous Protein Kinase C Using Mass Spectrometry in Human Breast Cancer Cell line

Umesh Gaikwad[1], Fei Dr Liu[1]

1. *Macquarie University, Marsfield, NSW, Australia*

Protein kinase C's are distributed in different compartment and found at the nexus of the many signalling pathways. PKCs regulate many vital pathways required for cell survival, proliferation and metabolism. Any aberrant alteration in the PKC structure/sequence/chemical modification leads to manifestation of many diseases ranging from inflammation to cancer. Recently, the compartment specific functions of these PKC isoforms are attributed to the post-translational modifications (PTM) at specific sites. PTM plays an important role in regulating their activation and translocation in different compartments of the cell. Most of the study of PKC PTM are done in non-endogenous conditions so necessarily not reflect the natural environment with in the cell. Here, we are trying to isolate endogenous PKC using membrane preparation and cell surface labelling methods.

Mass spectrometry has evolved dramatically, and is now considered a key method for site mapping, quantification of chemical modifications (PTM) and even for detecting protein–protein interactions. The right combination of MS method with maximum enrichment is crucial for mapping of proteins to study their PTMs in specific cell compartment.

Cell surface labelling helps to separate the cell surface protein by labelling the surface protein with biotin non-specifically followed by affinity purification using a neutravidin column. Membrane preparation will enrich the membrane proteins including membrane associated protein like protein kinase C's by using differential ultracentrifugation. By combining these two methods, selective enrichment of low abundant proteins like PKC's can be done. Selectively enriched membrane or membrane associated proteins can then be characterised for its PTM study by new MS method like SWATH.

236

Why use Ultra-High Resolution Quadrupole Time of Flight Instruments for Proteomics Applications?

Maja Christiansen[1], Stephanie Kaspar-Schoenefeld[2], Markus Lubeck[2], Annette Michalski[2], Oliver Raether[2], Christoph Gebhardt[2], Aiko Barsch[2]

1. *Bruker Pty Ltd, Alexandria, NSW, Australia*
2. *Bruker Daltonics, Bremen, Germany*

Introduction

In shotgun proteomics it is desirable to identify and quantify a large number of individual peptides from complex samples in the shortest possible time. Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. Several hardware modifications of a bench-top UHR-TOF instrument were carried out and evaluated addressing these particular performance aspects.

Methods

To test the impact of these modifications on proteomics performance, different complex tryptic digests were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II (Bruker Daltonik). For data processing the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)).

Results and Discussion

Proteomic capabilities were evaluated on a UHR-TOF instrument and results show that the high MS/MS acquisition speed is very suitable for large sample amounts (e.g. 5µg of tryptic HeLa digest) resulting in more than 4,000 protein identifications during a 90 min gradient. Preliminary quantitative data of 200 ng E.coli lysate spiked with iTRAQ labeled peptides were investigated with regard to the theoretical ratios of these proteins at 10:10:5:5:2:2:1:1. A reference amount of 4 ng was used providing a quantification accuracy of all four proteins at 10:10.2:5.5:5.4:3.3:3.5:2.1:2.8. Additionally, label-free quantitation results as well as identification numbers in very low sample amounts will be shown, further supporting UHR-TOF capabilities for proteomics applications.

Obtained data clearly reveal that hardware changes leading to higher sensitivity at fast acquisition speed and an increased resolution are beneficial for proteomics applications. The improvements result in higher identification rates and a very accurate quantification making the impact II a good choice for proteomics applications.

Conclusion

Improvements to several hardware components allow identification and quantification of complex proteomics samples with very high dynamic range.

237

Investigating the role of a new E3 ubiquitin ligase implicated in ALS disease pathology

Stephanie Rayner[1], Albert Lee[1], Kelly Williams[1], Ian Blair[1], Mark Molloy[2], Roger Chung[1]

1. *Biomedical Sciences, Macquarie University, Sydney, NSW, Australia*
2. *Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*

Amyotrophic Lateral Sclerosis (ALS) is a progressive and devastating neurodegenerative disease with poor prognosis. Our team has identified novel missense mutations in an *FBX* gene (manuscript submitted), some of which lead to aggressive ALS pathology. At the protein level, FBX is a component of an E3-ubiquitin ligase and is responsible for recognition of specific substrates for ubiquitination [1]. Our hypothesis is that disease-variants of FBX may have altered substrate recognition properties. To date, there are only three known FBX substrates. One of the main challenges of identifying substrates of E3 ligases is the transient and low binding affinity between the ligase and its substrates. Accordingly, one aim of this project is to identify novel substrates of FBX using proximity-dependant biotin identification (BioID) and LC-MS/MS. BioID is a new methodology that involves fusing mutant biotin ligase in frame with the enzyme of interest in order to biotinylate proteins that come into close proximity [2]. This approach has advantages in that it enables the selective isolation and identification of weak and transient protein-protein interactions using standard biotin-affinity capture. Furthermore, it provides a list of candidates for further interaction studies. In addition to identifying novel substrates of FBX, this project will also work toward producing recombinant FBX for structural studies. The structure will help us rationalise the changes in E3-ligase activity caused by mutations, and will provide a basis for structure-based drug design. Overall, this project will investigate the link between, and the degree to which, FBX affects ALS disease progression. It will also work toward identifying specific structural features of FBX that might be therapeutically targetable.

1. [1] Deshaies, R.J. and C.A. Joazeiro, RING domain E3 ubiquitin ligases. Annu Rev Biochem, 2009. 78: p. 399-434.
2. [2] Roux, K.J., et al., A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J Cell Biol, 2012. 196(6): p. 801-10.

238

Improved capacity trapped ion mobility spectrometry

Lucy A Woods[1], Karsten Michelmann[2], Jens Bossmeyer[2], Mark E Ridgeway[2], Joshua A Silveira[2], Matthias Mann[3], Melvin A Park[3]

1. *Bruker Pty Ltd, Preston, VIC, Australia*
2. *Bruker Daltonics, Billerica, US*
3. *Max Plank Institute, Martinsried, Germany*

In conventional drift tube ion mobility spectrometry, the overall duty cycle is principally limited by the injection of ions into the mobility analyzer. The initial ion pulse must be kept relatively small with respect to the drift time such that the resolving power is not significantly reduced. While multiplexed strategies can improve the sensitivity and duty cycle, peak aliasing still remains problematic.

Over the past few years, trapped ion mobility spectrometry (TIMS) has displayed several attractive features including: (1) rapid gas phase separations on a millisecond timescale, (2) resolving power that can approach ~300, (3) flexibility to tune the separation parameters (resolution vs. separation speed/duty cycle) in accordance with the analytical challenge, and (4) the ability to determine collision cross section values that closely match (within 1-2%) those obtained using drift tube IMS

Here, a new analyzer with an enhanced \ storage capacity is evaluated. This second generation TIMS analyzer features a tunnel region that is approximately double the length compared to the first generation prototype. An increase in the storage space of the new TIMS analyzer was observed by roughly the geometrical factor (2-fold) resulting in a duty cycle of ~60% at a resolving power of ~100.

239

Megapixel tissue imaging at high-speed: Evaluation of a next generation MALDI-TOF instrument

Lucy A Woods[1], Michael Becker[2], Ralf Schäfer[2], Janine Beckmann[2], Arne Fütterer[2], Armin Holle[2], Jens Höhndorf[2]

1. *Bruker Pty Ltd, Preston, VIC, Australia*
2. *Bruker Daltonics, Bremen, Germany*

MALDI Imaging (Mass Spectrometry Imaging, MSI) is a spatially resolved, label-free analytical technique for direct analysis of biological samples. Acquisition speed and sample throughput are a limiting factor in MSI experiments, in particular in clinical research where large cohorts of patients have to be analyzed to encompass the biological variance of human samples. Acquisition speed also limits MSI experiments at high spatial resolution, especially for larger tissue sections.

Here, we present data from a next generation MALDI-TOF instrument that enables acquisition speeds of up to 50 pixel (spectra) per second at pixel sizes of 10 µm and smaller. Data is shown from the most common application areas of MSI, including lipid, peptide and intact protein analysis.

240

TMT One Stop Shop: from reliable sample preparation to computational analysis platform

Mehdi Mirzaei[1], Dana Pascovici[2], Jemma Wu[2], Yunqi Wu[1], Joel M. Chick[3], Mark P. Molloy[1, 2], Paul A. Haynes[1]

1. *Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*
2. *Australian Proteome Analysis Facility, Macquarie University, North Ryde, NSW, Australia*
3. *Department of Cell Biology, Harvard Medical School, Boston, MA, United States*

Publish consent withheld

241

The development of barley-specific peptide markers for food testing applications

Michelle Colgrave[1], Keren Byrne[1], Hareshwar Goswami[1], Malcolm Blundell[1], Crispin Howitt[1]

1. *Agriculture Flagship, CSIRO, Brisbane, QLD, Australia*

Gluten is the collective name for a class of proteins found in wheat, rye, barley and oats. Coeliac disease (CD) is an immune-mediated inflammatory disease of the small intestine in a subset of genetically-susceptible individuals that is triggered by the ingestion of gluten, resulting in intestinal inflammation and damage. The only current treatment for CD- and gluten-intolerants (~70 million people globally) is lifelong avoidance of dietary gluten. Gluten-free (GF) foods are now commonplace, however, it is difficult to accurately determine the gluten content of GF-products using current methodologies as the antibodies are non-specific and show cross-reactivity. In processed products measurement is further confounded by protein modifications and/or hydrolysis. Global proteomic analysis of 14 economically important barley varieties utilising discovery LC-MS/MS was used to characterise the “gluteome” and select peptide markers specific to barley gluten (hordeins) and/or as general markers of barley for targeted quantitative MS assays. Barley-specific peptide markers were detected across 14 barley varieties with nine potential markers showing detection in all cultivars tested. The panel of barley peptide markers were then assessed against 16 commercial grains including those grown in crop rotation or typically processed in the same facility. Of these, seven of the peptide markers were unique to barley and can be used to develop

sensitive assays for the presence of barley as an unlabelled food ingredient and show promise for the quantification of gluten to protect consumers suffering from CD.

242

A top-down proteomic approach to the identification of early serum biomarkers predictive of spontaneous preterm birth

Arlene D'Silva[1], Jens R. Coorssen[1], Jon Hyett[2]

1. *Discipline of Molecular Physiology and the WSU Molecular Medicine Research Group, School of Medicine, Western Sydney University, Sydney, NSW, Australia*
2. *Department of Obstetrics, Gynaecology and Neonatology, University of Sydney, Maternal Fetal Medicine Unit, Royal Prince Alfred Hospital, Sydney, NSW, Australia*

Publish consent withheld

1. Wright, E.P., et al. (2014) Top-down proteomics: Enhancing 2D gel-electrophoresis from tissue processing to high sensitivity protein detection. Proteomics 14, 872-889.
2. Butt, R.H., Coorssen, J.R. (2005) Postfractionation for Enhanced Proteomic Analyses: Routine Electrophoretic Methods Increase the Resolution of Standard 2D-PAGE. J. Proteome Res. 4, 982-991.
3. Gauci, V.J., et al. (2013) Coomassie blue staining for high sensitivity gel-based proteomics. J. Proteomics 90, 96-106.
4. Wright, E.P., et al. (2014) Deep Imaging: How much of the Proteome Does Current Top-Down Technology Already Resolve? PLoS ONE 9(1): e86058. doi:10.1371/journal.pone.0086058.

243

A human stimulome – mapping signalling network intersection downstream of major cell surface receptors

Sean J. Humphrey[1, 2], Emily S. Humphrey[1], Matthias Mann[1]

1. *Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany*
2. *Cybernetics Lab, Charles Perkins Centre, School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia*

Reversible protein phosphorylation forms vast signalling networks that rapidly respond to intra- and extra-cellular cues to orchestrate molecular responses, linking the cell's genome with its environment. The misregulation of such signals cause or exacerbate the onset of complex diseases including cancer and ageing, and their rectification is therefore a major area of biomedical research. This is however complicated by the fact that major signaling nodes are functionally pleiotropic, being involved in several biological processes. Indeed, different cellular stimuli often signal through shared network branches. Designing effective therapies against dysregulated signalling therefore requires a more complete picture of the signalling landscapes downstream of cell surface receptors and the extent of network overlap.

Using EasyPhos1 – a new scalable, single-run phosphoproteomics platform – we quantified the response of signalling networks of diverse cell lines to a large panel of ligands, targeting pro-survival (e.g. EGF, IGF, VEGF, PDGF) and pro-inflammatory pathways (e.g. TNFα, TRAIL, IL1β, IL-6). We quantified over 20,000 phosphorylation sites, from which comparisons of ligand induced cellular signaling networks have been visualized, revealing highly specific network branches as well as large tracts of shared signalling components.

These systems-wide data provide an extensive view of ligand-induced signalling networks in different cell lines, greatly extending the current knowledge of the signalling occurring downstream of major cell surface receptors.

1. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. Nature Biotechnology (2015). 33(9):990-5.

244

Novel *O*-glycans containing acidic sugars on fungal secreted protein and their characterisation using ESI-PGC-LC-MS/M

Christopher Ashwood[1], Helena Nevalainen[2], Nicolle Packer[1]

1. *Centre for Nanoscale Biophotonics, Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*
2. *Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia*

Trichoderma reesei has been of industrial interest for the production of cellulases, resulting in the development of hypercellulolytic strains such as RUT-C30. High protein production and secretion, and eukaryotic glycosylation machinery, make *T. reesei* RUT-C30 a suitable expression host for recombinant proteins. The *N*-glycosylation of secreted proteins of RUT-C30 is known to vary depending on culture nutrients but *O*-glycosylation has been less extensively studied. Under glucose as a sole carbon source, secreted protein glycosylation featured novel *O*-glycan compositions which made up over a third of the total abundance of *O*-glycans released from these proteins.

This study characterised these novel compositions, confirming the presence of hexuronic acid in released *O*-glycans. Negative mode

ESI-PGC-LC-MS/MS using a ThermoFisher Velos Pro was used to identify and characterise these *O*-glycans through annotation of glycosidic bond and cross-ring fragments. Trap-HCD allowed targeted MS3 experiments to be performed on the hexuronic acid substituent of these structures which was not possible with CID, validating the novel *O*-glycan composition. One site of novel *O*-glycosylation was identified, revealing attachment to a peptide in the catalytic domain of CBHI. These are the first reported glycans to contain acidic sugars in fungi and could have significant implications for CBHI structure and activity.

245

Mass spectrometry-based lipidomics analysis of Barrett's oesophagus and oesophageal adenocarcinoma

Jeffrey Molendijk[1], Thomas Stoll[1], Federico Torta[2], Markus Wenk[2, 3], Tom Hennessy[1, 4], Michelle Hill[1]

1. *The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia*
2. *Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
3. *Department of Biological Sciences, National University of Singapore, Singapore*
4. *Life Sciences & Applied Markets Group, Agilent Technologies PTY LTD, Australia*

The incidence of oesophageal adenocarcinoma, which has a 5-year survival rate of 15%, has rapidly increased in the last years. The risk factors for oesophageal adenocarcinoma include chronic reflux, male gender, obesity and Barrett's oesophagus, a metaplastic change of the oesophageal squamous epidermal cells which are replaced by columnar cells. There is a need to understand the underlying mechanism of oesophageal adenocarcinoma development and find potential biomarkers. MALDI (Singhal et al. 2013) and spectroscopy (Dave et al. Eur J Gastroenterol Hepatol) studies report changes in the lipid profiles of Barrett's oesophagus and/or adenocarcinoma tissue compared to healthy squamous epithelium. Interestingly, cholesterol lowering drugs of the statin family have been reported to attenuate growth and malignant potential of oesophageal adenocarcinoma cells (Sadaria et al. 2011), and a more recent study report statin use as protective against Barrett's oesophagus and adenocarcinoma (Nguyen et al. 2014, 2015). To determine if lipid composition changes during the development of oesophageal adenocarcinoma, we conducted an untargeted mass spectrometry-based lipidomics experiment in five cell lines representing progression from Barrett's oesophagus, dysplasia to oesophageal adenocarcinoma. This poster will report the initial results.

1. Dave et al. 2004 Eur J Gastroenterol Hepatol 16:1199-205
2. Singhal et al. 2013 J Proteomics 80:207-15
3. Sadaria et al 2011 J Thorac Cardiovasc Surg 142:1152-60
4. Nguyen et al. 2014 Gastroenterology 147:314-23
5. Nguyen et al. 2015 Gastroenterology 149:1392-8

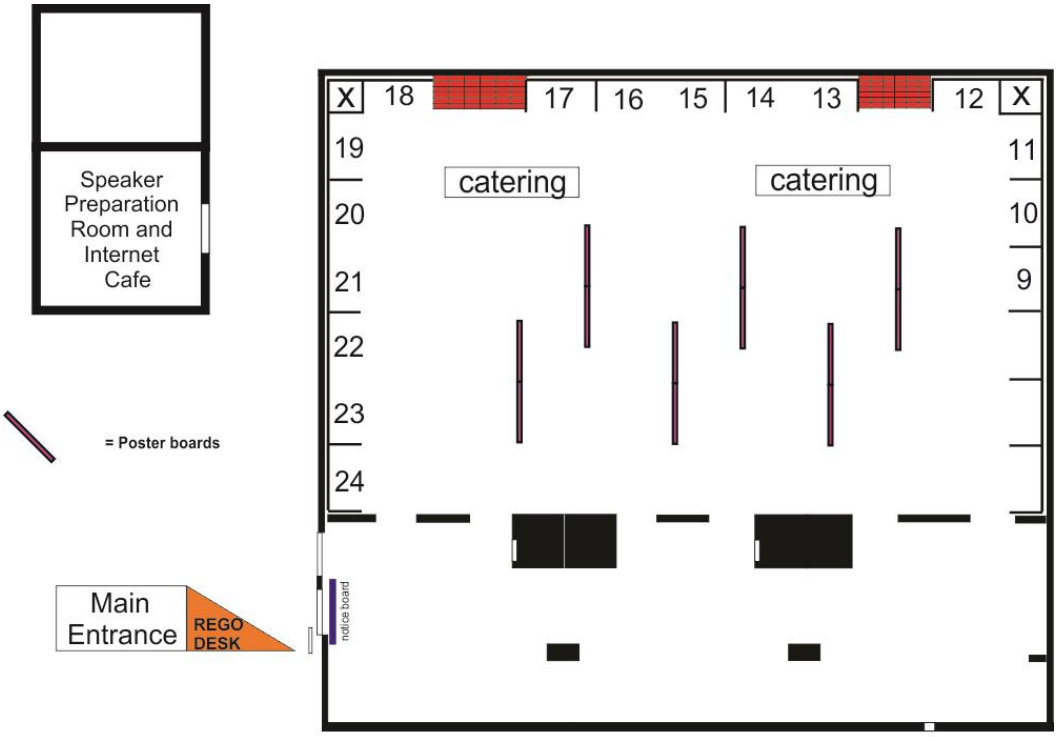
246

Identification of beer spoilage microorganisms using the MALDI Biotyper platform

Michelle Turvey[1, 2], Florian Weiland[1, 2], Jon Meneses[3], Nick Sterenberg[3], Peter Hoffmann[1, 2]

1. *Adelaide Proteomics Centre, The University of Adelaide, Adelaide, SA, Australia*
2. *Institute for Photonics and Advanced Sensing, The University of Adelaide, Adelaide, SA, Australia*
3. *Coopers Brewery Ltd, Adelaide, SA, Australia*

Beer spoilage microorganisms present a major risk for the brewing industry and can lead to cost intensive recall of contaminated products and damage to brand reputation. The applicability of molecular profiling using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in combination with Biotyper software was investigated for the identification of beer spoilage microorganisms from routine brewery quality control samples. Reference mass spectrum profiles for top- and bottom-fermenting brewing yeast strains, wild spoilage yeast and common bacterial spoilage organisms were established, incorporated into the Biotyper reference library and validated by successful identification after inoculation into beer. The applicability and integration of mass spectrometry profiling using the Biotyper platform into existing brewery quality assurance practices within industry was assessed by analysing routine microbiology control samples from Coopers Brewery Ltd., where contaminating microorganisms could be reliably identified and distinguished from over 5,600 microorganisms present in the database. This renders the Biotyper platform a promising candidate for biological quality control testing within the brewing industry as a more rapid, high-throughput and cost-effective technology that can be tailored for the detection of brewery-specific spoilage organisms from the local environment.



AGILENT TECHNOLOGY **SILVER SPONOSR & STAND 15-16**

Web: www.agilent.com.au

Agilent is a leader in life sciences, diagnostics and applied chemical markets. The company provides laboratories worldwide with instruments, services, consumables, applications and expertise, enabling customers to gain the insights they seek. Agilent's expertise and trusted collaboration give them the highest confidence in our solutions. Agilent focuses its expertise on six key markets, where we help our customers achieve their goals, namely: Food, Environmental and Forensics, Pharmaceutical, Clinical Diagnostics, Chemical and Energy and Life Science Research (Omics).

APAF **STUDENT POSTER AWARD SPONSOR & STAND 12**

Web: www.proteome.org.au

The Australian Proteome Analysis Facility, provides collaborative solutions for all your proteomic research needs. Review the testimonials on our website to discover how APAF has enabled scientists to progress their research. You will find links to numerous publications where APAF capabilities were used demonstrating the wide-ranging scientific activities available through working with us.

We offer proteomic services including advanced Mass spectrometry for biological discovery (SWATH, iTRAQ, SILAC, TMT), multiplexed ELISA, amino acid analysis, and N-Terminal sequencing. We also analyse post translational modifications and characterise recombinant proteins/antibodies.

BRUKER **PLATINUM SPONSOR & STAND 22-23**

Web: www.bruker.com

Bruker is a leading provider of Chromatography and Mass Spectrometry instruments and solutions for the Analytical Sciences. Our innovative and easy-to-use product families (ESI-QTOF, Ion Trap, FTMS, MALDI-TOF, LC-Triple Quads and GC-Triple Quads) provide the highest performance, ruggedness and value for a wide range of applications in the food, environmental, forensic, industrial, pharmaceutical and life science research markets.

GENESEARCH **STAND 11**

Web: www.genesearch.com.au

Genesearch is Australia's oldest continuously owned private biotechnology company, founded by 3 scientists as a partnership in 1980.

We became the Australian distributor for New England Biolabs in 1983 and took on Cell Signaling Technology in 1999. Our focus has been on enzymes and antibodies for molecular and cell biology research. Our distributorships also include: Cisbio for HTRF Immunoassays, Thomson high-aeration flasks for optimum recombinant protein expression and

GeneCodes for DNA sequence analysis.
Additionally, Genesearch is the developer of the e.Freezer, an innovative on-site ordering and dispensing system that saves researchers and their administrators time and money through its extensive automation and integration with stock control, ordering and invoicing systems.

NOVACHEM **SILVER SPONSOR & STAND 9**

Web: www.novachem.com.au

Novachem has been providing reagents and reference standards to the research and analytical community for over 35 years. You have come to expect quality, service and value from us and that is our commitment to you.
Novachem's key supply partners include Cambridge Isotopes Limited, MRM Proteomics, LGC Standards, Accustandard, Wako, Fluorochem, Acanthus, Bachem and many more from where we can source a range of over half a million different products.Our website provides access for you to search and get quotes for these products in a secure environment and above all ensures you get exactly what you want, when you want it.

PEAK SCIENTIFIC **WELCOME RECEPTION SPONSOR & STAND 13-14**

Web: www.peakscientific.com

With nearly two decades in pioneering reliable gas generator technology, Peak Scientific develops market-leading nitrogen, hydrogen and zero air systems mainly for the field of LC-MS and GC.

An on-site gas generator from Peak Scientific is the practical and cost-effective alternative to pressurized cylinders.

Our forte is in providing our customers with unrivalled peace of mind and hassle free gas generation solutions for their specific laboratory demands, backed up by our world-class technical support and ongoing service care throughout the generator's lifespan. With a rapid response and offices on every continent we deliver a local service on a global scale.

PROTEOMICS INTERNATIONAL **SoAPS FUNCTION SPONSOR & STAND 19**

Web: www.proteomics.com.au

Proteomics International is a specialist technology-driven life science company based in Perth, Western Australia. The Company's technology platform has been successfully applied to biomarker discovery in multiple project areas, in particular diabetic kidney disease, and the sensitivity and specificity of the approach is opening the door to personalised medicine.
Proteomics International operates in three synergistic business units: Diagnostics (biomarker discovery), Therapeutics (drug discovery of peptide medicines) and Analytics (biosimilars CRO). The Company, established in 2001, provides services to groups throughout the Indo-Asian region, and was the world's first provider of proteomics services to be accredited to ISO/IEC 17025 laboratory standard.

PROTEOMICS & METABELOMICS VICTORIA **STUDENT ORAL SPONSOR**

Web: www.pmv.org.au

Proteomics and metabolomics are new fields of science that are producing exciting advances in biology, biotechnology and medicine. Proteins and metabolites are two classes of biomolecules that are very diverse, so if we are to understand them properly we need to identify and classify thousands of different molecules. Increasingly, scientists are using mass spectrometry as a powerful tool to answer this problem. Proteomics and metabolomics aim to classify the full complement of proteins (proteomics) and metabolites (metabolomics) in living things and study how their patterns change with disease and changes of environment.
Like many fields of science, proteomics and metabolomics is driven by technological change. The methods for studying proteins and metabolites are becoming increasingly powerful but require access to specialised instruments and technical expertise. PMV aims to promote the use of these technologies by fostering a community of Victorian researchers and the scientific vendors that support them. This cross-sector collective encourages its members to share ideas, discuss problems, and develop the technical expertise required so that proteomics and metabolomics can provide productive outcomes for Victorian science.

SCIEX **GOLD SPONSOR & STAND 17**

Web: www.sciex.com

SCIEX is a global leader in the development of life science analytical technologies that help answer complex scientific challenges. The company provides scientific instrumentation, software and services used to discover new drugs, advance medical science and protect the food supply and the environment. The company has a more than 20-year history of innovation and market leadership as the former Applied Biosystems/MDS Analytical Technologies joint venture. For more information about SCIEX, go to www.sciex.com. Follow SCIEX on Twitter @SCIEXnews and on Facebook.

SHIMADZU **GOLD SPONSOR & STAND 20-21**

Web: www.shimadzu.com.au

Shimadzu Corporation's products include analytical instruments, medical X-ray imaging, aircraft equipment, and turbomolecular pumps. In particular Shimadzu Scientific Instruments is a global leader in analytical instruments, where it has earned a strong reputation in the pharmaceutical, chemistry, and environmental monitoring fields.

Shimadzu Scientific Instruments is committed to offering comprehensive support to customers in their business and R&D activities. To that end, we supply a wide range of products and services based on cutting-edge technologies for a multitude of industrial and academic applications.

SIGMA-ALDRICH (MERCK) **INTERNATIONAL EARLY CAREER AWARD SPONSOR**

Web: www.merck.com.au

Merck Millipore and Sigma-Aldrich have come together as the Life Science business of Merck. Lome brings us together for the first time as we unveil our new brand identify, as well as showcasing our products for the life-science industry.

THERMO FISHER SCIENTIFIC **GOLD SPONSOR & STAND 24**

Web: www.thermofisher.com.au

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Thermo Scientific and Invitrogen brands, we offer an unmatched combination of innovative technologies, purchasing convenience and support.

TRAJAN **STAND 10**

Web: www.thermofisher.com.au | Email: anz@trajanscimed.com | Australia Toll Free: 1800 257 213

Trajan's focus is on developing and commercializing technologies that enable analytical systems to be more selective, sensitive and specific, to improve biological, environmental or food related measurements. Serving the world with innovative scientific products, including GC Columns, SGE Analytical Syringes, and laboratory consumables for clinical applications.

Science that benefits people.

WALTER+ELIZA HALL INSTITUTE FOR MEDICAL RESEARCH **PARENT VIEWING ROOM SPONSOR**

Web: www.wehi.edu.au

The Walter and Eliza Hall Institute is Australia's oldest medical research institute, and for 100 years we have been making discoveries for humanity. We are committed to making fundamental scientific discoveries that can be translated to better treatments, benefiting the community on a global scale. We have 750 researchers who are working to understand, prevent and treat diseases including cancers, immune and inflammatory disorders, and infectious diseases. Our key programs include research into malaria, HIV, hepatitis, coeliac disease, multiple sclerosis, lupus, diabetes, allergy and asthma. Clinical trials based on institute discoveries include trials of vaccines for rheumatoid arthritis, coeliac disease, diabetes and malaria.

WATERS AUSTRALIA **SILVER SPONOSR & STAND 18**

Web: www.waters.com | Ph: 1800 222 133

Since 1958, Waters has been in the business of making innovative analytical instruments that assist scientists in reaching their scientific goals, increase productivity, and earn laboratory-based organisations a higher return on their investments in research, development, and quality control. Whether at work discovering new pharmaceuticals, inventing new and more effective ways to treat diseases, assuring the safety of the world's food and drinking water supplies, monitoring and controlling pollution, or conserving the world's greatest art treasures, scientists worldwide rely on Waters liquid chromatography and mass spectrometry products. Waters stands out among its peers and, year after year, is one of the best-performing companies in the industry.

INDEX OF ABSTRACT AUTHORS

Abeysekera, W	109
Abouelhadid, S	145
Abrahams, J.L	144
Aebersold, R	105
Aerts, J.M	140
Ahn, M	136
Aili, S	121
Ali, M	209
Anand, G.S	040
Ang, C	114
Angel, P.M	211
Angelucci , C	143
Ansell, B.R	127
Anugrahama, M	020
Arentz, G	126,202
Arrey, T	137
Ashwood, C	107,244
Asselin-Labat, M	112
Atwell, B	124
Ayoub, D	026
Azimi, A	209
Babak Azimifar, S	142
Babon, J.J	112
Bache, N	118,208
Bacic, A	114
Bacic, T	220
Bailey, M	232
Baines, D	013
Baker, L	127
Baker, M.A	219
Baker, M.L	116
Ball, M	117
Barbour, A	201
Barkla, B.J	214
Barsch, A	141,236
Bateman, J.F	143
Becker, M	239
Beckmann, J	239
Belgacem , O	027,119
Bern, M	134
Berry, I	122,128,132
Berry, I.J	004
Berry, I.J	123
Bersten, D	229
Bin Zenaidee, M	007
Biniossek, M.L	230
Birrell, G	210
Bjelosevic, S	222
Blair, I	237
Blank, M.A	026
Blattmann, P	105
Bleck, C.K	036
Blundell, M	241
Borchers, C.H	011
Boros, S	224
Bossmeyer, J	238
Boughton, B.A	016
Boughton, B.D	102
Bowlay, G	015
Briggs, M	020,021
Bringans, S	227
Broadbent, J	006
Broadbent, J.A	038,101
Broszczak, D	006
Broszczak, D.A	038,101
Brown, D	015
Brumatti, G	109
Bucio Noble, D	117
Budayeva, H.G	002
Bulone, V	12
Bush, A.D	102
Byrne, K	241
Cain, J.A	145,203
Cain, J.A	204,207
Calkin, A.C	029
Campbell, M.P	144
Carapetis, J	108
Care, N	223
Caruana, N	133,226
Castangia, R	027,119
Chang, M	035
Charles, I.G	217
Chen, D	201
Chen, N	210
Cheng, Q	210
Chick, J.M	240
Choi, E	201
Chou, A	011
Christiansen, M	236
Chung, R	225,237
Claude, E	139,234
Colgrave, M	241
Cooke, I	133,226
Coorssen, J.R	242
Cordwell, S	005
Cordwell, S.J	145,203
Cordwell, S.J	009,204,207
Cornett, S	211
Creek, D	017
Creek, D.J	125
Cribb, B	015
Cristea, I.M	002
Croft, N	110
Cuttle, L	006
D'Silva, A	242
Dagley, L	023
Dagley, L.F	108,112
Dass, A	021
Dass, A.E	219
Davis, M	028
Davis, M.J	113
Davis, T	227
Davis, W	227
Dejean, A	121
Dekker, N	140
Delaine, C	229
Dell, A	019
Dettmer, K	141
Djordjevic, S	120,128,132,217
Djordjevic, S.P	004,122
Djordjevic, S.P	123
Doblin, M.S	114
Domon, B	026
Donald, W.A	007
Donaldson, P.J	018
Dong, M	008
Drake, R.R	211
Drew, B.G	029
Dudek, N	231
Dudek, N.L	103,116
Edstein, M	210
Ekert, P	109
Emery, S.J	111,127
Engholm-Keller, K	216
Erce, M	130
Everest – Dassa, A	020
Faou, P	133,226
Faridi, P	105
Fazakerley, D.J	215
Fernandez-Penas, P	209
Findlay, D	021
Flesch, I	110
Foell, M.C	230
Forbes, B	229
Ford, K	114,220

Fosang, A.J 143
Foster, L.J 013,024,206
Frazer, I.H 213
Fry, B.G 015
Fu, Q 138
Fütterer, A 239
Gable, A 208
Gaikwad, U 235
Gallien, S 026
Garnham, A 109
Gautier, B 201
Gebhardt, C 236
George, I.S 111
Gethings, L.A 140
Ghisi, M 109
Ghosh Dey, S.D 102
Giannangelo, C.R 125
Goel, A 135
Golub , S.B 143
Gorman, J 210
Goswami, H 241
Graetz, M 041
Graham, M 216
Grant, O 003
Greco, T.M 002
Grey, A.C 018
Grimmond, S.M 104
Gwee, S 225
H Nagaraj, S 104
Hamey, J.J 129
Hamilton, B 015
Handler, D.C 111
Harpur, B.A 206
Hart, P 139
Hart-Smith, G 007,129,130,131
Hartcher, N 204
Harty, L 033
Harvey, K 217
Hastie, M 210
Haverkamp, R 136
Haynes, P 124
Haynes, P.A 111,127
Haynes, P.A 240
Hayward, N 034
He, S 008
Heazlewood, J.L 114
Heeren, R.M 234
Heinzelmann-Schwarz, V 020
Henderson, F 119
Hennessy, T 245
Hill, M 201,245
Hill, M.M 113,213,224
Hinneburg, H 118
Hirtz, C 208
Hoffmann, P 020,021,037,126,202,229,246
Höhndorf, J 239
Holle, 239
Holliday, M 215
Hoover, S 013
Howitt, C 241
Hughes, C 233
Huhmer, A 026
Hummler, E 230
Humphrey, E.S 243
Humphrey, S.J 142,243
Hunter, C 025,138,221
Huygens, F 038
Hyett, J 242
Ignjatovic, V 222
Illing, P 231
Infusini, G 023,108,109
Ito, J 227
Jacob, F 020
James, D.E 029,215
Jarocki, V 128
Jex, A.R 127
Jiang, X 212
Johnstone, R 109
Jones, A 104
Jones, E 139
Jones, E.A 234
Joshi, V 201
Kamath, K 135
Kapp, E 023
Karlaftis, V 222
Karpievitch , Y 143
Kaspar-Schoenefeld, S 236
Kaufman, K 209
Kaur, G 202
Kautto, L 117
Kellar, E 110
Keppner, A 230
Khoury, G 033
Kilcher, S 036
King, G.F 015
Kislinger, T 031
Kleffmann, T 032
Klingler-Hoffmann, M 126,202
Koh Belic, N 205
Kolarich, D 118
Kolesnik, T.B 112
Kossard, S 209
Kowalski, M.P 138
Kramer, G 140
Krisp, C 034,135,223
Kruppa, G 011
Kuliwaba, J 021
Kumar, S.S 135
Kurniawan, N 015
Lamond, A.I 010
Langridge, J.I 140,233
Langridge, J 234
Larance, M 010
Lazarus, R 029
Le Cao, K 201
Lee, A 225,237
Lee, L 134
Lee, S 018
Lehmann, S 208
Lepretre, M 115
Lesur, A 026
Li, D.K 203
Li, L 229
Li, Y 008
Licona-Cassani, C 030
Liddy, K.A 204
Lin, C 106
Lin, M.J 018
Linedale, R 213
Linossi, E.M 112
Lipscombe, R 227
Liu, C 008
Liu, F.D 235
Liu, Y 029
Loke, I 022
Lokman, N 126,202
Loo, D 224
Lubeck, M 236
Lundberg, E 042
Lusis, A.J 029
Macha, I 205
Madonis, K 122
Maiolica, A 036
Malloy, M 225
Man, L 228
Mann, M 001,142,238,243
Marcellin, E 030
Mariani, M 212
Martin, W 108
Masters, C.D 102

Mathias, R 002
Matthews, D 220
McAfee, A 013,206
McCormick, S 032
McMahon, A 119
Meikle, P.J 029
Melvold, J.A 217
Meneses, J 037,246
Mercer, J 036
Michael, M 104
Michalski, A 236
Michalski, W.P 220
Michaud, S 206
Michelmann, K 238
Mifsud, N 231
Milthorpe, B 205
Minigo, G 108
Mirzaei, M 124,127,240
Mittal, P 126,202
Moh, E.S 134
Mok, L 220
Molendijk, J 245
Mollee, P 224
Molloy, B 233
Molloy, M 034,117,222, 223,237
Molloy, M.P 106,135
Molloy, M.P 240
Monagle, P 222
Moody, S.C 029
Moreno, M 122,123
Morrice, N 025
Morrison, M 213
Mueller, C 015
Mukherjee, S 102
Muratovic, D 021
Naffa, R 136
Nancarrow, D 201
Nevalainen, H 107,244
Ng, J.H 116
Nicholson, G 121
Nicholson, S.E 112
Nicola, N.A 112
Nielsen, L.K 030
Norman, M 226
Norris, G 136
Novy, K 036
Nye-Wood, M.G 018
O'Connor, L 023,108
O'Connor, W 115
O'Rourke, M 120
Oberstein, A 002
Oefner, P.J 141
Oehler, M 021,126,202
Olson, A 221
Omasits, U 036
Orivel, J 121
Packer, N.H 106,107,135, 144,215,219
Packer, N.H 020,134
Packer, N 003,021,022, 244
Padula, M 120,121,122, 128,132,205, 217
Padula, M.P 004
Padula, M.P 123
Pan, J 011
Park, M.A 238
Parker, B.L 029,215
Parker, B.L 134
Parker, R 034
Parker, T 006
Parker, T.J 101
Pascovici, D 034,111,127, 135,222,223, 240
Patsiouras, H 139,140,233, 234
Paul, H 115
Paulech, J 009,203
Paulsen, I 135
Pearson, J.V 104
Penesyan, A 135
Pengelley, S 118
Perkins, D.N 103
Peters, K 227
Petitclerc, F 121
Pickford, R 131
Pukala, T 041
Purcell, A 110,231
Purcell, A.W 103,116
Purcell, A.W 033
Purcell, D.F 033
Raether, O 236
Raftos, D 115
Ramarathinam, S.H 033
Ramsay, S 137,218
Ravishankar, P 111
Raymond, B 128,132
Raymond, B.B 004
Raymond, B.B 123
Rayner, S 237
Renaut, P 224
Ridgeway, M.E 238
Ritchie, H 232
Roberts, B.D 102
Robinson, P 216
Roessner, U 016
Rossjohn, J 231
Rowland, E.A 002
Rowley , L 143
Roy , M 027
Sadraei, S 105
Sanders, H 041
Sandow, J.J 109
Santos, J 205
Sarabia, D.L 016
Saunders, N 201
Schäfer, R 239
Schilling, O 230
Schmit, P 208
Schweiger-Hufnagel, U 118
Scott, N.E 024
Scott, N.E 207
Scull, K.E 103
Seeberger, P.H 118
Sellami, L 027
Shah, A 201
Shah, A.D 113
Shathili, A.M 219
Shen, E 008
Shenk, T 002
Shockhor, J.P 140
Siddiqui, G 125
Silva, D.V 118
Silveira, J.A 238
Sinz, A 039
Skraskova, K 234
Smith, D 130
Smith, L 005
Smith, L.E 203
Smith, S 110
Smyth, G 109
Sombke, A 015
Song, C 008
Song, X 222,223
Speed, T 023
Srivastava, A 125
Srivastava, V 12
Stavenhagen, K 118
Steele, J.R 217
Sterenberg, N 037,246
Stoll, T 213,245
Stow, J 015

Strugnell, J	133,226
Sumer Bayraktar, Z	003
Syedbasha, M	036
Tacchi, J	128
Tacchi, J.L	004
Tacchi, J.L	123
Tachedjian, M	220
Tan, G	218
Tarling, E.J	029
Tay, A	131
Taylor, N	012
Tellström, V	141
Tennakoon, I.U	038
Thaysen-Andersen, M	003,022,134, 215
Thompson, E	104,115
Timofiiv, A	105
Torta, F	245
Touchard, A	121
Towers, M	139,234
Tscharke, D	110
Turvey, M.E	037,246
Undheim, E.A	015
Vaibhav, V	115
Vallim, T.Q	029
Van Eyk, J.E	138
Venkatakrishnan, V	003,135
Venter, D.J	015
Vialaret, J	208
Vissers, J.P	140,233
Visvader, J.E	112
Vivian, J	231
von Zychlinski, A	032
Waardenberg, A	216
Wachsmuth, C.J	141
Waddell, N	104
Wang, H	007
Wang, L	116
Webb, A	109
Webb, A.I	023,108,112
Wei, L	008
Weiland, F	037,246
Wenk, M	245
White, M	005,204
White, M.Y	203
White, M.Y	009
Whitelaw, B	133
Whiteman, D	201
Wicks, I.P	108
Widjaja, M	004,122,123, 128,132,217
Wilkins, M	130,131
Wilkins, M.R	007,129
Williams, K	119,221,237
Williams, M	032
Williamson, N.A	103
Wilson, R	143
Winderbaum, L	126,202
Winfield, K	227
Winter, D.L	007,129
Wirth, H	232
Wollscheid, B	036
Woods, L.A	141,238,239
Woods, R	003
Woon, A.P	116
Wren, B.W	145
Wu, J	222,223,240
Wu, Y	124,240
Wuhrer, M	118
Wynne, J.W	116,220
Xuan, Y	026
Yagoub, D	129,131
Yap, A.S	104
Zang, T	006
Zaragoza, N	030
Zaw, T	222,223
Zayed, A	206
Zeng, W	114
Zerenturk, E.J	029
Zhang, C	126,202
Zhang, M	008
Zhang, S	011
Zhang, W	008
Zhao, H	008
Zhong, Y	023

NOTES

NOTES