20th Lorne Proteomics Symposium
5th - 8th February 2015 · Mantra Lorne, Lorne, Victoria, Australia

DELEGATE HANDBOOK
australasianproteomics.org
INDEX

AUSTRALASIAN PROTEOMICS SOCIETY EXECUTIVE COMMITTEE ........................................ 3

SPONSORS .......................................................................................................................... 5

DELEGATE INFORMATION

The Organiser’s Office - ASN Events ................................................................. 6
What Your Registration Includes ................................................................. 6
Conference Secretariat ............................................................................... 6
Social Program ................................................................................................. 6
Coupon Booklet ............................................................................................... 6
Speaker Preparation Instructions ................................................................. 7
Displaying Your Poster .................................................................................. 7
Email and Internet Access ........................................................................... 7
Mobile App ........................................................................................................ 7
Settling Your Room Account ....................................................................... 7
Useful Phone Numbers ................................................................................ 8
Bus Transfers Home ..................................................................................... 8
Parent Viewing Room ................................................................................... 8

SPEAKERS ...................................................................................................................... 10

TRADE WORKSHOPS

Friday ....................................................................................................................... 17
Saturday ............................................................................................................... 17

PROGRAM

Pre Lorne Workshop ......................................................................................... 18
Thursday ........................................................................................................... 19
Friday ................................................................................................................... 20
Saturday ............................................................................................................. 22
Sunday .................................................................................................................. 24

POSTER LISTING

Poster Session One ......................................................................................... 26
Poster Session Two ........................................................................................ 28

ABSTRACTS

Orals ....................................................................................................................... 31
Posters ............................................................................................................... 51

TRADE DIRECTORY .............................................................................................. 94

ATTENDEE LIST ..................................................................................................... 97

INDEX OF ABSTRACT AUTHORS ................................................................. 101
AUSTRALASIAN PROTEOMICS SOCIETY

EXECUTIVE COMMITTEE OF MANAGEMENT 2015

PRESIDENT
Prof Richard J Simpson
La Trobe University Institute for
Molecular Science (LIMS)
La Trobe University, VIC, Australia

SENIOR ADVISORY COMMITTEE

SECRETARY
Dr David W Greening
La Trobe University Institute for
Molecular Science
La Trobe University, VIC, Australia

VICE PRESIDENT/INDUSTRY LIAISON OFFICER
Dr Lou Fabri
CSL Limited, VIC, Australia
Bio21 Institute, VIC, Australia

VICE PRESIDENT/CONFERENCE CHAIR
Prof Peter Hoffmann
School of Biological Sciences,
University of Adelaide
SA, Australia

VICE PRESIDENT
A/Prof Stuart J Cordwell
School of Molecular & Microbial
Biosciences, Building GO8
The University of Sydney, Australia

STATE REPRESENTATIVES

New Zealand
A/Prof Bill Jordan
Director, Centre of Biodiscovery
Victoria University of Wellington
Wellington, New Zealand

Queensland
Dr Michelle Hill
University of Queensland, Diamantina
Institute
Princess Alexandra Hospital, QLD,
Australia

South Australia
Prof Peter Hoffmann
School of Biological Sciences,
University of Adelaide
SA, Australia

New South Wales
A/Prof Mark P Molloy
Director, APAF
Macquarie University, NSW, Australia

Western Australia
Dr Richard Lipscombe
Proteomics International
Lotterywest State Biomedical Facility
Centre for food and genomic medicine
Western Australia

Victoria
Prof Anthony Purcell
Dept. of Biochemistry & Molecular
Biology
Monash University, VIC, Australia
CO-OPTED MEMBERS

Education and Training Chair
Dr Michelle L Colgrave
Senior Proteomics Research Scientist
CSIRO Livestock Industries
St Lucia, QLD, Australia

International Representative
A/Prof Robert Moritz
Proteomics Research Laboratory
Institute for Systems Biology, Seattle, USA

Assistant Treasurer
Dr Lindsay Sparrow
CSIRO, Health Science and Nutrition, VIC, Australia

International Representative
A/Prof Gavin Reid
Michigan State University, Michigan, USA

Web Page Coordinator
Dr Ben Crossett
The University of Sydney, NSW, Australia

Director/Business Development
Mr Lindsay Woods
Australian Proteome Analysis Facility Ltd, NSW, Australia

A/Prof Hubert Hondermarck
School of Biomedical Science & Pharmacy
The University of Newcastle
NSW, Australia

APS SUB-COMMITTEES

Website / Advertising Committee
Co-ordinator: Ben Crossett
Stuart Cordwell, Peter Hoffmann

Trade Committee
Co-ordinator: Lou Fabri
Nicholas Williamson, Lindsay Woods, Peter Hoffman

Program Committee
Co-ordinator: Michelle Colgrave
Richard Simpson, Rob Moritz, Peter Hoffmann, Stuart Cordwell, David Greening, Nicholas Williamson, Michelle Hill, Mark Molloy

Finance Committee
Co-ordinator: Nicholas Williamson
Richard Simpson, Lou Fabri, David Greening, Lindsay Sparrow, Stuart Cordwell, Rob Moritz

Education Committee (Students)
Co-ordinator: Michelle Colgrave
Tony Purcell, Stuart Cordwell, Mark Molloy, Richard Simpson, Nicholas Williamson, Peter Hoffmann, Michelle Hill

SoAPS
President: Rong Xu
NSW Representative: Erin Sykes
VIC Representative: Rong Xu
SA Representative: Karina Martin
QLD Representative: Dayle Sampson
The Australasian Proteomics Society would like to thank the following sponsors and supporters for Lorne APS 2015:

**PLATINUM SPONSOR**

Thermo Fisher Scientific

The world leader in serving science

**GOLD PLUS SPONSOR**

AB SCIEX

**GOLD SPONSORS**

Shimadzu

Excellence in science

**EXHIBITORS**

Agilent Technologies

BSi Bioinformatics Solutions Inc.

BRUKER

GENESEARCH

MIMOTOPES

novachem

PEAK

PILL

Silantes

Waters

**SoAPS AWARD**

SIGMA-ALDRICH

**SUPPORTER**

APAF

CSL

Proteomics & Metabolomics Victoria

20th Lorne Proteomics Symposium 2015 – Page 5
DELEGATE INFORMATION

THE 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 Mantra Lorne.

The Conference office hours are:

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

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

Level 1, 9/397 Smith Street, Fitzroy, Vic, 3065
Phone: 03 8658 9530 Fax: 03 5983 2223
Email: dw@asnevents.net.au

SOCIAL PROGRAM

Welcome Reception: On Thursday 5th February, the Welcome Reception will be held from 6:30 pm - 9:30pm in the Exhibition Hall. Tickets can be selected when registering online. If you have not selected a ticket and would like to attend please visit the registration desk for availability.

Erskine Falls Bus/Walk Tour: On Saturday 7th 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 @ 2:30pm and take delegates to explore the area. Please visit the registration desk for further information.

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

APS Carnival: On Saturday 7th 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). If you have not selected a ticket in your registration and would like to attend please visit the registration desk for further information.

COUPON BOOKLET

You will find in your satchel a coupon booklet which includes offers from some of the exhibiting companies. Present the coupon to the appropriate exhibitor to go in the draw or receive your offer. The conference will also be offering the chance for a delegate to win a prize. Simply go to each exhibiting stand and collect a letter. When put together, all letters can be rearranged into a word which needs to be written into the appropriate page in the coupon booklet and handed into the registration desk by lunchtime on Saturday 7th February.
SPEAKER PREPARATION INSTRUCTIONS

All speakers are using the same lecture hall (Heritage Ballroom). The audio-visual equipment is being supplied and manned by operations staff. It is the conference preference to have ALL talks pre-loaded to the common laptop which is a PC (you will be able to use your own MAC if preferred, but please remember to bring necessary adapters if you wish to use your MAC). As per instructions already supplied, you should give your talk on a CD or USB stick to the technician well before the session you are participating in so it can be loaded and tested. There will be a mouse pointer also provided at the lectern, please use this as a pointer so delegates in the overflow rooms can follow your presentation.

DISPLAYING YOUR POSTER

Posters will be displayed throughout the Symposium on panels in the Exhibition Hall. Posters numbers 100 - 143 will be displayed from Friday morning and must be removed by Saturday morning tea. Posters starting at 200 - 243 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. The approved method for attaching your poster is with Velcro. Please visit the organiser’s office for additional supplies of Velcro.

EMAIL AND INTERNET ACCESS

The Internet Café is in the meeting room in the lobby foyer and will be open during conference hours. Wireless access will be available and is free for all delegates as well as in the Convention Centre. Delegates using the internet café service must restrict their access time to 15 minutes whenever there is a queue. Mantra Lorne has installed a new system and all guest rooms now have wireless internet access available — please visit the Mantra Lorne Reception Desk for rates and information.

*Internet Café proudly sponsored by Shimadzu*

CONFERENCE APP

The official Lorne Proteomics 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!

*Downloading the Lorne Proteomics Mobile App is easy!*

**Instructions for Apple devices**

**Step 1** Copy [http://proteomics-2015.m.asnevents.com.au](http://proteomics-2015.m.asnevents.com.au) to your browser or use this QR code.

**Step 2** You will be asked to install this web app onto your Iphone - tap the screen and the icon will appear on your home screen for future use.

For further benefits and instructions for Android devices please see staff at the registration desk

SETTLING YOUR ROOM ACCOUNT WITH YOUR HOTEL

Those individuals departing Lorne on Sunday 8th February will be required to settle their room accounts with their accommodation provider 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 - 0413 932 387
Gull Airport Service - 03 5222 4966
VLine Bus Service - 1800 800 007
Avalon Airport Shuttle - 03 5278 8788
Budget Care Hire - 1300 362 848
Qantas - 13 13 13
Jetstar - 131 538
Virgin Blue - 136 78

BUS TRANSFFERS 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 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

An overflow room (Heritage Room 3) is 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.

Biology is complex and understanding it is a big challenge. Identify and quantify more proteins and complexities such as PTMs faster and more accurately with our new portfolio of LC-MS instruments, sample prep solutions and software. HRAM solutions using Thermo Scientific™ Orbitrap™ MS quantifies all detectable proteins and peptides with high specificity and fewer false positives, while triple quadrupole MS delivers SRM sensitivity and speed to detect targeted proteins more quickly. Join us in meeting today’s challenges. Together we’ll transform proteomics.

Quantitation transformed.

• Discover more at thermoscientific.com/quan-transformed
Visit booth 16-18 to sign-up for our seminar on 7 Oct.
Prof. Juergen Cox, Max Planck Institute, Germany

Juergen Cox is an Independent Research Group Leader at the Max Planck Institute for Biochemistry in Martinsried, Germany where he heads the Lab for Computational Systems Biochemistry. He received his PhD in physics at the Massachusetts Institute of Technology and has since developed MaxQuant, a quantitative proteomics software package designed for the analysis of large mass spectrometric data sets. Dr. Cox is the author of numerous peer-reviewed publications in the field of data analysis in mass spectrometry and quantitative proteomics.

Prof. Albert Heck, Utrecht University, The Netherlands

Albert J.R. Heck (1964) is Professor of Biomolecular Mass Spectrometry and Proteomics at the Faculty of Sciences of Utrecht University and scientific director of the Netherlands Proteomics Centre. He received his Ph.D. in mass spectrometry from the University of Amsterdam. He was trained as postdoctoral fellow in the group of Dick Zare at Stanford and Lecturer in the Chemistry Department of Warwick (UK). He (co-)authored more than 400 publications and has received a number of awards. He is elected member of EMBO and the Royal Dutch Academy of Sciences (KNAW).

In general the work in the Heck-lab focuses on I. The development of enabling technologies to probe macromolecular protein complexes by mass spectrometry and II. The development of mass spectrometry-based enabling technologies for proteomics, with applications into the biomedical sciences, especially into stem cell biology, cancer and immunology. Heck’s lab is very active in quantitative proteomics and the targeted analysis of protein post-translational modifications. Heck introduced in 2004 the enrichment of phosphopeptides using TiO2 material, and later Ti4+-IMAC based enrichment. More recently we introduced for large-scale proteomics a simple and cost-effective stable isotope labeling by using reductive dimethylation, and an effective combination of the peptide fragmentation methods HCD and ETD termed ETnCD. Heck’s proteomics research focuses for a large part on (embryonic and adult) stem cells and immunology. Most of these studies are aimed at the understanding of embryonic, adult an induced pluripotent stem cell differentiation, which may eventually lead to these cells being used for regenerative purposes.

Dr. Pedro Cutillas, Imperial College London, UK

In 2004 Pedro graduated with a PhD from the laboratory of Mike Waterfield at UCL. He was also supervised by Robert Unwin, AL Burlingame and Rainer Cramer. After a postdoctoral position in Bart Vanhaesebroeck’s laboratory at the Ludwig Institute for Cancer Research, in 2007 Pedro obtained a lectureship at Barts Cancer Institute (part of Queen Mary University of London). There, he developed a programme of research in cell signalling and cancer biochemistry and in 2010 he was promoted to Senior Lecturer. After a ‘sabbatical’ year at MRC Clinical Sciences Centre in 2012, Pedro returned to Barts Cancer Institute in October 2013 where he now leads the Integrative Cell Signalling Group part of the Centre for Haemato-Oncology. Pedro’s latest research has involved the application of MS-based technology to rationalize responses of cancer cells to kinase inhibitors (MCP 2012, Genome Biology 2013, Science Signaling 2013). These proof-of-concept studies have shown that MS-based technology can be used to identify markers of responses of cancer cells to targeted inhibitors and can therefore contribute to advancing personalized cancer medicine.
Dr. Daryl Fernandes, Ludger Ltd, Oxford, UK

I am the founder and Chief Executive of Ludger - a company based near Oxford in the UK which helps biopharmaceutical companies design, measure and control their drug’s glycosylation patterns to improve clinical performance, increase profitability and satisfy the drug regulators. I gained my doctorate in glycoengineering from the Department of Biochemistry at the University of Oxford and in 1989 helped spin out a company Oxford GlycoSciences (OGS) to commercialise the glycoprofiling tools that my colleagues and I had developed at the University. In 1999, I left OGS to set up my own company, Ludger Ltd, focussing on systems to improve the optimisation, measurement and control of drug glycosylation.

I’ve spent the last fourteen years steadily developing my team of scientists and support staff at Ludger. Today, Ludger’s clients include drug developers and manufacturers across the world including over half the world’s top twenty biopharma companies. Although I gave up wearing my sandals, beard and lab coat a long time ago I still play a very active role in Ludger’s research programmes. At the moment, my main glyco-related interest is the application of medical glycomics to study inflammatory conditions - particularly those that arise from having a fat belly (I’m trying to get rid of mine now).

Dr Christie Hunter, AB SCIEX, USA

Christie Hunter is the Director of Omics 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. Most recently, she has focused on quantitative proteomic applications using differential mobility separation and the new SWATH Acquisition workflow. Christie received her Ph.D. in protein biochemistry from the University of British Columbia (Canada) in 1997 and did her post-doctoral research at a small biotechnology company, Gryphon Sciences, from 1997 to 2000.

Dr Helen Montgomery, SHIMADZU, UK

Dr Helen Montgomery, is the MALDI Marketing Manager in Shimadzu / Kratos Analytical (Manchester, UK). She obtained a PhD in Chemistry at The Michael Barber Centre for Mass Spectrometry (UMIST: University of Manchester Institute of Science and Technology, UK). Having spent nearly 10 years as a senior researcher in Shimadzu Koichi Tanaka Mass Spectrometry Research Laboratory (Manchester, UK), she has extensive experience in the applications of MALDI-TOF mass spectrometry and is specialized in proteomics and post translational modification identification and characterization (e.g. glycosylation, glycation and oxidation). Initially a research scientist for Oxford Glycosciences where she worked in the field of mass spectrometric analysis and high-throughput proteomics, she was then product manager at MWG biotech, where she gained valuable sales and marketing experience. In 2003, she obtained a position of senior researcher under the direct supervision of Nobel prize winner Koichi Tanaka. Helen Montgomery has also been a visiting lecturer for the MSc in analytical science at Manchester Metropolitan University.
A/Prof Robert Moritz, Institute for Systems Biology, Seattle, USA
Dr. Robert Moritz, a native of Australia, joined the Institute for Systems Biology faculty in 2008 as Associate Professor and Director of Proteomics Research. He began his work in 1983 in the Joint Protein Structure laboratory at the Ludwig Institute for Cancer Research, and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia where he designed and implemented a number of technologies currently used in many proteomics laboratories across the globe. His current research interests include proteomics software development for statistically valid proteome identification, protein crosslinking results and online resources for quantitative proteomics. His applications of proteomics include the discovery of normal and disease markers which constitute a molecular fingerprint using targeted quantitative mass spectrometry. Recently, these include the development of biomarker candidates for *Mycobacterium tuberculosis*, glioblastoma, prostate cancer, and the identification of previously unidentified proteins on the surface of Malaria sporozoites as potential vaccine targets. He has recently developed the complete Human Peptide- and SRM-Atlas, a quantitative atlas and community driven repository of mass spectrometric assays to all human proteins. Dr. Moritz has authored more than 200 peer-reviewed papers, and is a holder of several patents on his research interests in discovery protein science and the relationship of aberrant protein expression and its function in human disease.

Prof. Christopher M Overall, Vancouver, Canada
Dr. Overall is a Professor and Tier 1 Canada Research Chair in Proteinase Proteomics and Systems Biology at the University of British Columbia, Centre for Blood Research. He completed his undergraduate, Honors Science and Masters degrees at the University of Adelaide, South Australia; his Ph.D. in Biochemistry at the University of Toronto; and was a MRC Centennial Fellow in his post-doctoral work with Dr. Michael Smith, Nobel Laureate, Biotechnology Laboratory, University of British Columbia (UBC). On Sabbatical in 1997-1998 he was a Visiting Senior Scientist at British Biotech Pharmaceuticals, Oxford, UK and in 2004/2008 he was a Visiting Senior Scientist at the Expert Protease Platform, Novartis Pharmaceuticals, Basel, Switzerland. He was an External Senior Fellow, Freiburg Institute for Advanced Studies, Albert-Ludwigs Universität Freiburg, Germany and in November 2014 was appointed as an Honorary Professor, Albert-Ludwigs Universität Freiburg. Dr. Overall was awarded the Institute of Musculoskeletal Health and Arthritis CIHR Award as 2002 CIHR Scientist of the Year, the UBC Killam Senior Researcher Award (Science) 2005, and was the Chair of the 2003 Matrix Metalloproteinase Gordon Research Conference and the 2010 Protease Gordon Research Conference. He was elected to the HUPO Council and to the Executive Committee of the Chromosome-Centric Human proteome Project (CHPP) in 2014. With over 12,626 citations for his 208 papers (since 2000 his papers have been cited >8,813 times) and with an *h* factor of 62 he is a highly influential scientist in the field. Professor Overall is also the pioneer of degradomics, a term he coined. With 21 Nature Review, Nature Journal, Cell Journal, Science and Science Signaling papers he is a leader in the field, which was recently recognized by the International Society of Proteolysis with a Lifetime Achievement Award in 2011; by the Matrix Biology Society of Australia and New Zealand with the 2012 Barry Preston Award; in 2013 by the IADR Distinguished Scientist Award for Research in Oral Biology; and in 2014 by the Tony Pawson Canadian National Proteomics Network Award for Outstanding Contribution and Leadership to the Canadian Proteomics Community.

Dr. Andreas Roempp, University of Giessen, Germany
Dr. Andreas Römpp is a group leader / lecturer at the Institute of Inorganic and Analytical Chemistry of Justus Liebig University Giessen, Germany. In recent years mass spectrometry imaging has become his main area of interest. Dr. Römppp is the coordinator of the common data format for mass spectrometry imaging - imzML. He is actively involved in the COST action (BM1104) as management committee member and work group co-chair.
Dr Daniel Kolarich, Max Planck Institute of Colloids & Interfaces, Germany

Daniel Kolarich, born in Vienna, Austria became fascinated with glycbiology, mass spectrometry and the possibilities that a combination of both has to offer during his undergraduate studies of Food Science and Biotechnology at the University of Natural Resources and Applied Life Sciences, Vienna. During his PhD at the same University and his subsequent post doctoral work with Prof. Nicki Packer at Macquarie University in Sydney, NSW, he worked intensively on glycopeptide oriented glycoproteomics of major plasma and secreted glycoproteins as well as on plant and insect allergens. In autumn 2010 he joined the Department of Biomolecular Systems at the Max Planck Institute of Colloids and Interfaces in Potsdam, Germany, as a group leader establishing quantitative glycomics & glycosylation signatures.

NATIONAL

Dr Berin Boughton, Bio 21, Metabolomics Australia, University of Melbourne

Berin Boughton completed a PhD (2010) in Organic Chemistry from the University of Melbourne. After completing his PhD he took up a number of synthetic research roles focused on organometallic cross-coupling reactions and mass spectrometric analysis of organometallic complexes; in 2009 he significantly changed research focus taking up a post-doctoral position with Metabolomics Australia where he was awarded an Early Career Researcher Award and Vanderbilt-University of Melbourne Partnership Grants. Since 2013 he has lead the development of Imaging Mass Spectrometry (IMS) at Metabolomics Australia using Matrix Assisted Laser Desorption Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (MALDI-FTICR-MS) for the spatial analysis of metabolites in a variety of systems. His research is focused on developing new IMS methods to explore the interactions between parasite and host, mapping the distribution of plant secondary metabolites and the development of novel chemical matrices for MALDI-MS.

Dr. Mark Baker, University of Newcastle

I completed my Bachelor of Science Degree with Honours under the supervision of Dr. Alfons Lawen, Monash University at the Department of Biochemistry and Cell Biology. Shortly after, I took up a post-doctoral position with L/Prof. John Aitken and the pharmaceutical giant, Bayer, to work on male contraception. After five very successful years, in 2007, I was awarded the University of Newcastle research Fellowship, which allowed me to start an independent laboratory, whilst still being connected with John. Together we established the University of Newcastle’s Mass Spectrometry Core Facility, which now services not only the University, the greater Hunter area in general. In 2011 I was then awarded and NHMRC Career Development Fellowship for my work in proteomic analysis of human spermatozoa. Although my work now branches outward’s into stroke research, understanding male infertility is still an important priority for my work.

Prof. Mark S. Baker, Macquarie University

Born 1956 Maroubra, Sydney. Completed PhD 1985 at Macquarie University and subsequent research focused on biochemistry of proteins in human health and disease (e.g., arthritis, breast, ovarian, prostate and colorectal cancer). Mark made contributions to the growth of the science of proteomics and to the organisation of scientific societies at national, regional and global levels, as well as having a successful stint in the US biotech sector. Mark returned to serve as APAF CEO, supporting Australia’s national proteomics effort and securing NCRIS for national “omics” infrastructure. Mark has sat on the HUPO Board and Executive and is the current HUPO President. Mark co-chaired the 9th HUPO World Congress in Sydney where the Human Proteome Project was launched and drives many HUPO activities, sits on journal’ Editorial Boards and industry Advisory Boards. Mark was recognised in 2012
for his service to the community with the HUPO Distinguished Service Award. He has published >130 peer-reviewed papers, supervised more than 35 students and is the inventor of IgYultradepletion patents. He remains an advocate for industry:academic ventures, media engagement and promotion of career paths for young researchers.

**Prof. Steve Blanksby, Queensland University of Technology**

Stephen Blanksby holds a PhD (1999) in gas phase ion chemistry from the University of Adelaide (with Prof J.H. Bowie) and undertook postdoctoral research at the Technical University of Berlin (1999, with Prof H. Schwarz) and at the University of Colorado (with Prof G.B. Ellison). Blanksby was appointed to the School of Chemistry at the University of Wollongong in 2002 and was promoted to Professor in 2012. He was appointed Director of the Queensland University of Technology’s Central Analytical Research Facility in 2014. His research interests are at the interface between fundamental ion chemistry, spectroscopy and analytical mass spectrometry.

**Prof. Steven Djordjevic, University of Technology, Sydney**

Steven Djordjevic is Professor of Infectious Disease and Group Leader in the ithree Institute at the University of Technology Sydney. His group focuses on the molecular, structural and biochemical characterisation of bacterial molecules that function in adherence, colonisation and invasion of eukaryote cells and has a strong interest in the roles of protein processing and protein multifunctionality in microbial pathogenesis. Professor Djordjevic is a member of the governing board and the scientific management committee of Ausgem, the Australian Centre for Genomic Epidemiological Microbiology. As a part of Ausgem, we generate and analyse genomic sequence data to understand how pathogenic bacteria and the complex antibiotic resistance gene loci (CRL) they carry evolve in microbes inhabiting clinical, aquatic, food animal and agricultural environments.

**Dr. Mark Graham, CMRI, University of Sydney**

Dr Graham uses proteomics to understand the molecular mechanisms of neurotransmission. His Bachelor of Science Degree was in chemistry. During his PhD, he was an early user of quantitative proteomics approaches for the analysis of brain protein function regulated by protein phosphorylation. At the Children’s Medical Research Institute he progressed from Post-Doctoral Fellow to Group Leader and has a conjoint appointment with the University of Sydney. Dr Graham was the 2008 recipient of the ASBMB Applied Biosystems Edman Award and was awarded a Biomedical Career Development Award in 2009 from the National Health and Medical Research Council. Dr Graham has published work in a range of fields but his main focus is cellular signalling and protein interactions, including the assembly mechanisms and phospho-regulation of synaptic vesicle biogenesis.
**Dr Angus Grey, University of Auckland, NZ**

Dr Angus (Gus) Grey gained his PhD from the University of Auckland. He conducted post-doctoral research at the Medical University of South Carolina and Vanderbilt University with Prof Kevin Schey before returning to Auckland as a Research Fellow in the Department of Physiology. Gus is interested in investigating the molecular basis of ocular diseases using spatially resolved mass spectrometric techniques such as MALDI imaging. His current research aims to understand how long-lived lens proteins are modified over time to contribute to normal lens function and cataract formation, and the changes in the lens metabolome that lead to these proteomic changes. The long term goal is to develop effective therapeutic strategies to delay or prevent age-related nuclear cataract.

---

**Prof. Paul Haynes, Macquarie University**

Paul Haynes is a Professor in the Department of chemistry and biomolecular sciences at Macquarie University. His research group focuses on planned and environmental proteomics. He has published a large number of papers in proteomics and biochemistry journals, he teaches a senior undergraduate course in proteomics each year, and he is currently supervising and mentoring six Ph.D. students. He has been working in biological mass spectrometry and proteomics for more than 20 years, and retains a strong interest in using chemical methods to answer biological questions.

---

**Prof. David James, The University of Sydney**

David James is currently Professor of Systems Biology and Associate Academic Director (Research) at the Charles Perkins Centre, The University of Sydney. David received his PhD from the Garvan Institute in 1985 and since then has made major contributions to our understanding of insulin action. In the late 1980s he published a series of Nature papers describing the identification and characterization of the insulin responsive glucose transporter GLUT4. He then focused his efforts on unveiling the cellular and molecular control of insulin-stimulated glucose transport. He has also made contributions in the area of SNARE proteins, signal transduction and more recently has established new interests in systems biology. David has held positions at the Washington University School of Medicine in St Louis, at the University of Queensland, and at the Garvan Institute of Medical Research, where he was Director of the Diabetes & Obesity Research Program from 2002 until early 2014. David was the winner of the prestigious Glaxo Wellcome research medal in 1999, and was elected as a Fellow of the Australian Academy of Science in 2007.

---

**Prof. Malcolm McConville, University of Melbourne, Victoria**

Professor McConville is a NHMRC Principal Research Fellow and Acting Director of the Bio21 Institute of Molecular Science and Biotechnology at The University of Melbourne. His research group in the Department of Biochemistry and Molecular Biology utilizes multidisciplinary approaches to develop new therapies for malaria, tuberculosis and a number of other neglected tropical diseases that affect more than one billion people worldwide. Prof McConville was involved in the establishment of Metabolomics Australia, a national network of research centres that support the development of metabolomics and systems biology approaches in Australia and currently oversees the major biomedical node of Metabolomics Australia at The University of Melbourne.
**A/Prof Peter Meikle, Baker IDI Heart and Diabetes Institute, Melbourne**

A/Prof Peter Meikle is Head of the Metabolomics Laboratory at Baker IDI Heart and Diabetes Institute and a NHMRC Senior Research Fellow. He is Editor in Chief of Metabolites and holds affiliate positions at Bio21, Melbourne University and the Department of Medicine, Monash University. The Metabolomics Laboratory has a focus on the dyslipidemia associated with obesity, diabetes and cardiovascular disease and its relationship to the pathogenesis of these disease states. The work is leading to new approaches to early diagnosis, risk assessment and therapeutic monitoring of these most prevalent diseases.

---

**Prof. Harvey Millar, The University of Western Australia**

Harvey Millar is Director of the ARC Centre of Excellence in Plant Energy Biology, headquartered in Perth at The University of Western Australia. His field is plant biochemistry with a special interest in plant mitochondria, primary metabolism and proteomics. He had held four research fellowships offered by the ARC over time and had led and co-led successful discovery grants, linkage grants, equipment grants and centre grants. He has been the recipient of national and international awards in plant science and biology from governments, the Australian Academy of Science and the Australian and American Societies of Plant Scientists.

---

**Prof. Marc Wilkins, University of New South Wales**

Professor Wilkins is the Director the NSW System Biology Initiative and the Ramaciotti Centre for Genomics, at the University of New South Wales. His research group currently focuses on how protein post-translational modifications, or combinations of these, form an ‘interaction code’ that modulate the intracellular interactions of proteins. His group is also actively involved in the bioinformatic analysis of next-generation sequence data in genomics and transcriptomics projects, and the systems-level interpretation of these data in pathways and networks.
TRADE WORKSHOPS

FRIDAY 6TH FEBRUARY

THERMO FISHER SCIENTIFIC BREAKFAST WORKSHOP

Breakfast: 7:00 am
Workshop: 7:30 am - 9:00 am

1. **New Developments of MS Reagents for Quantitative Proteomics by Dr. Rosa Viner**

   Mass spectrometry has become a prominent technique in biological research by enabling identification and quantification of proteins. Shotgun proteomics is a common strategy to identify proteins in complex mixtures, but the quality and consistency of sample preparation greatly influences the reliability and accuracy of the results and increases the time and cost of the analysis. For MS-based proteomics to reach its full potential as a routine technology in research and clinical settings, the variability associated with the sample preparation steps that precedes MS analysis must be addressed. We will present new reagents, standards and workflows which address normalization, standardization and reproducibility of sample preparation for quantitative proteomics.

2. **“Proteomics goes viral - understanding the host pathogen interactions that dictate susceptibility to merging human pathogens” by Professor Tony Purcell**

3. **Brief intro of Thermo’ s new Vanquish HPLC by Darren Jones**

SATURDAY 7TH FEBRUARY

AB SCIEX BREAKFAST WORKSHOP

Breakfast/Workshop: 7:30 am - 9:00 am

**AB SCIEX SWATH / OneOmis**

Presenter - Chris Hodgkins, AB SCIEX

Label-free quantitation using highly reproducible Data Independent Acquisition - MS/MS\textsuperscript{ALL} with SWATH\textsuperscript{TM} Acquisition - enables researchers to profile the abundance of thousands of peptides and proteins across large groups of samples. However, efficient processing of this data to provide meaningful biological results requires large amounts of computing power, creating a potential bottleneck in the road from sample to result. To overcome this, AB SCIEX and Illumina have partnered to create the world’s first multi-omics cloud-computing environment for easy, secure analysis and visualization of large and complex proteomics data sets. See SWATH\textsuperscript{TM} Acquisition 2.0 processing in action and take a tour of BaseSpace\textsuperscript{®} in this live software demonstration, presented by Chris Hodgkins, Senior Field Application Specialist, AB SCIEX ANZ.

*Continental Breakfast provided*
PRE-LORNE WORKSHOPS

WEDNESDAY 4TH FEBRUARY

MaxQuant Workshop
“Shotgun proteomics data analysis using MaxQuant and Perseus”
Presenter: Prof Juergen Cox
Location: The VRI Lecture Theatre, The Bio21 Institute, The University of Melbourne

During this one-day workshop you will learn the theory and practice of the MaxQuant software package for computational proteomics. The morning session provides an introduction to MaxQuant and Perseus and will familiarize the participants with basic and more advanced concepts. In the afternoon, hands-on examples will be worked through. Participants are encouraged to bring their own windows laptops for this practical parts.

Program:
9:00am Registration
9:20am Welcome and Introduction
9:30am Theory Session
12:00pm Lunch
1:00pm Practical Session
3:45pm Summary and Close

Proudly Sponsored by

AB SCIEX

AB SCIEX are kind sponsors of the workshop, would like to announce that AB SCIEX data can now be processed with MaxQuant software

THURSDAY 5TH FEBRUARY

Pre-Conference Tutorial Lecture MASS SPEC ‘101’

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

Where: Mantra Lorne

12:30PM - 1:50PM - Lecture 1 General Mass Spec concepts
Isotope and charge series
Buffer compatibility
Electrospray
MALDI
Charge deconvolution

1:50PM - 2:10PM - Coffee Break

2:10PM - 3:30PM - Lecture 2 Proteomic Mass Spec
Sample preparation
MS/MS sequencing
Mass estimates on recombinant proteins
Testing synthetic peptides
How to do gel spots
PROGRAM

THURSDAY, 5 FEBRUARY 2015

Registration Opens
1:00 PM
Mantra Lobby

Pre-Conference Tutorial Lecture: Mass Spec 101
12:30 PM - 3:30 PM
Heritage Dining
Chair: Nick Williamson

Welcome Address
4:00 PM - 4:10 PM
Heritage Ballroom

Richard Simpson, President of the Australasian Proteomics Society

Keynote Lecture
4:10 PM - 5:00 PM
Heritage Ballroom

Chair: Richard Simpson

Albert Heck
Exploring alternative proteases and fragmentation methods for proteomics abs#001

Ken Mitchellhill Young Investigator Award Lecture
5:00 PM - 5:45 PM
Heritage Ballroom

Chair: Richard Simpson

Benjamin Parker
Bulking up muscle proteomics: the exercise-regulated phosphoproteome and the control of muscle growth abs#002

Symposium One: 20 years of Proteomics
5:45 PM - 6:30 PM
Heritage Ballroom

Chairs: Lindsay Sparrow

5:45 PM
Marc Wilkins
Twenty Years of the Proteome abs#003

6:10 PM
Rob Moritz
Advent of Proteomics Discourse in Australia: The Lorne Proteomics Symposia abs#004

Welcome Reception
6:30 PM - 9:00 PM
Exhibition Hall
## FRIDAY, 6 FEBRUARY 2015

### Breakfast Workshop (Thermo Fisher Scientific)
7:00AM breakfast to then start at 7:30 AM - 9:00 AM
Heritage Ballroom

---

### Symposium Two: Glycoproteomics
9:00 AM - 10:30 AM
Heritage Ballroom

**Session sponsored by**
Thermo Fisher Scientific

**Chairs:** Nicki Packer & Stuart Cordwell

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Abstract/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 AM</td>
<td>Daniel Kolarich</td>
<td>The Hitchhiker’s guide to Glycoproteomics and the human Immunoglobulome</td>
<td>abs#005</td>
</tr>
<tr>
<td>9:30 AM</td>
<td>Daryl L Fernandes</td>
<td>Development of LongBow TM - an integrated system for high throughput glycomics to support biopharmaceutical realisation and clinical diagnostics</td>
<td>abs#006</td>
</tr>
<tr>
<td>10:00 AM</td>
<td>Matthew P Campbell</td>
<td>UniCarbKB: a glycobioinformatics infrastructure for data discovery</td>
<td>abs#007</td>
</tr>
<tr>
<td>10:15 AM</td>
<td>Hannes Hinneburg</td>
<td>N- and O-glycomics of FFPE tissues from histopathological slides</td>
<td>abs#008</td>
</tr>
</tbody>
</table>

---

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

### Symposium Three: Plant Proteomics
11:00 AM - 12:30PM
Heritage Ballroom

**Session sponsored by**
Shimadzu

**Chairs:** Harvey Millar & Paul Haynes

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Abstract/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:00 AM</td>
<td>Harvey Millar</td>
<td>Tracking protein turnover in plants: a new frontier in understanding the cost of proteins to plant growth</td>
<td>abs#009</td>
</tr>
<tr>
<td>11:25 AM</td>
<td>Paul Haynes</td>
<td>Proteomic Analysis of Abiotic Stress in Plants</td>
<td>abs#010</td>
</tr>
<tr>
<td>11:50 AM</td>
<td>Michelle Colgave</td>
<td>There’s a hole in my assay, dear ELISA, dear ELISA: Using MS to detect hydrolysed gluten in beer that is responsible for false negatives by ELISA</td>
<td>abs#011</td>
</tr>
<tr>
<td>12:10 PM</td>
<td>Kris Ford</td>
<td>Identification of N-linked glycosylation sites on plant glycoproteins</td>
<td>abs#012</td>
</tr>
</tbody>
</table>

---

### Lightning Talks One
12:30 PM - 1:00 PM
Heritage Ballroom

**Chair:** Ben Crossett & Michelle Colgrave

1st: Samira Ali
Characterisation of the peptide and protein content of ant venoms for use as bioinsecticide and antimicrobial leads  

2nd: Kathirvel Alagesan
A novel, ultrasensitive approach for quantitative carbohydrate composition and linkage analysis using nanoLC-ESI ion trap tandem mass spectrometry  

3rd: Joel Cain
Evaluating the relationship between n-glycosylation and protein stability in campylobacter jejuni  

4th: Harish Cheruku
TGFβ-1 increases cell proliferation, wound healing and induces changes to membrane proteome in colorectal cancer cells with varying integrin B6 expression  

5th: Keyur Dave
Comprehensive characterization of phosphorylation sites of Newcastle disease virus proteins by a multitude of fragmentation techniques  

---

20th Lorne Proteomics Symposium 2015 – Page 20
6th Iniga George
Quantitative proteomic analysis of Cabernet Sauvignon grape cells exposed to thermal stresses reveal alterations in sugar and phenylpropanoid metabolism abs#105

These 6 lightning talks will be the first 6 posters in poster session one.

Lunch/Poster Session One
1:00 PM - 2:30 PM Exhibition Hall

Free Afternoon
2:00 PM - 5:00 PM

Thermo Fisher Scientific: An afternoon with Albert
3:30 PM - 5:00PM Heritage Ballroom
“Exploring the benefits of unrestrained mass range and high resolving power on an Orbitrap (Jumbo)”
Prof Albert Heck, Utrecht University, Netherlands

*Afternoon drinks and snacks will be served

The AB SCIEX “Answer for Science, Knowledge for Life” Forum
3:30 PM - 5:00PM Heritage Dining Room

What is “Next-Generation-Proteomics”? What is the OneOmics™ project and what research will it enable in the near future? The answers to these questions and more can be found at the Answers for Science, Knowledge for Life™ Forum (replacing the AB SCIEX Lorne User-Group meeting). Hear about the latest developments in mass spectrometry-based technology for Systems Biology analysis and discuss the future of the field with Dr. Christie Hunter (Director of Proteomics Applications, AB SCIEX, USA) and your industry colleagues.

Symposium Four: Students of APS (SoAPS)
5:00 PM - 6:00 PM Heritage Ballroom
Session sponsored by SIGMA-ALDRICH

Chairs: Michelle Colgrave & Stuart Cordwell

5:00 PM Melissa Partridge
A Top-Down Proteomic Approach for Understanding Multiple Sclerosis abs#013

5:15 PM Alok Shah
Serum glycoprotein biomarkers for oesophageal adenocarcinoma abs#014

5:30 PM Daniel Stoessel
Revealing metabolic actions of novel compounds against African trypanosomes by high resolution mass spectrometry abs#015

5:45 PM Amanda Woon
Harbouring a killer: a proteomic approach to comparing host responses to Hendra virus abs#016

Student of APS (SoAPS) Dinner with Invited Speakers
6:00 PM - 7:00 PM Mantra Dining Area
Session sponsored by CSL

The AB SCIEX Pizza and Music Night
7:30 PM - 10:30PM Mantra Lawn Area

Enjoy live music, beverages & pizzas with the AB SCIEX Team
**SATURDAY, 7 FEBRUARY 2015**

**Breakfast Workshop AB SCIEX SWATH/OneOmics**

7:30 AM - 9:00 PM  
Heritage Dining Room

**Symposium Five: Metabolomics**

9:00 AM - 10:40 AM  
Heritage Ballroom

*Session sponsored by [Thermo Fisher Scientific](#)*

**Chairs:** Gavn Reid & Peter Hoffmann

9:00 AM  
**Steve Blanksby**  
Facing the fats: Can mass spectrometry unmask the true structural diversity of the lipidome? *abs#017*

9:20 AM  
**Berin Boughton**  
Identification of Plasmodium berghei markers of infection in the arthropod host by TLC-MALDI coupled to Imaging Mass Spectrometry *abs#018*

9:40 AM  
**Peter Meikle**  
Lipidomics is providing new strategies for risk assessment and therapeutic intervention in cardiovascular disease *abs#019*

10:00 AM  
**Malcolm McConville**  
Probing microbial pathogen interactions and metabolism using metabolomics *abs#020*

**Morning Tea**

10:40 AM - 11:00 AM  
Exhibition Hall

**Symposium Six: New Technology**

11:00 AM - 12:30 PM  
Heritage Ballroom

*Session sponsored by [AB SCIEX](#) + [SHIMADZU](#)*

**Chairs:** Peter Hoffmann & Andrew Webb

11:00 AM  
**Juergen Cox**  
Analysis of large proteomics datasets: pitfalls, challenges and solutions *abs#021*  

11:20 AM  
**Christie Hunter**  
Data Independent Acquisition – The Next Revolution in Proteomics *abs#022*  

11:40 AM  
**Andreas Roemp**  
Mass spectrometry imaging of tryptic peptides: towards cellular resolution in fresh-frozen and FFPE tissue *abs#023*  

12:00 PM  
**Helen Montgomery**  
The use of Axial Spatial Distribution Focussing for the generation of High Resolution and High Energy MALDI tandem mass spectra *abs#024*  

12:15 PM  
**Blaine Roberts**  
2-D Metalloprotein maps: application of quantitative metal and protein proteomics to define the metalloproteome of the human brain *abs#025*  

**Lightning Talks Two**

12:30 PM - 1:00 PM  
Heritage Ballroom

*Chair: Ben Crossett & Michelle Colgrave*

1st  
**Nathan Croft**  
Tracking the intricate dynamics of antigen and epitope kinetics during virus infection *abs#200*  

2nd  
**Kasper Engholm-Keller**  
Depolarisation-induced reprogramming of the presynaptic phosphoproteome mediates changes in neurotransmitter release *abs#201*  

3rd  
**Christoph Krisp**  
Enhancing discovery and quantitative proteomics by use of online multiphase chip LC fractionation *abs#202*  

4th  
**Erin Sykes**  
Moonlighting with Melanoma and the Unfolded Protein Response *abs#203*
5th  Cassandra Pegg
Characterisation of glycosylation of the Newcastle disease virus haemagglutinin-neuraminidase surface glycoprotein abs#204

6th  Aidan Tay
Validation of mesenchymal stem cell transcripts assembled from RNA-Seq using proteomics data abs#205

_These 6 lightning talks will be the first 6 posters in poster session two._

**Lunch/Poster Session Two**
1:00 PM - 2:30 PM  
Exhibition Hall

**Annual General Meeting**
2:15 PM  
Exhibition Hall

**Free Afternoon and Erskine Falls Tour**
2:30 - 4:15 PM  
Exhibition Hall

**APS International Awards for Early Career Researchers**
4:15 PM - 5:00 PM  
Heritage Ballroom

_Chairs: Ben Crossett & Steven Djordjevic_

4:15 PM  
Zon Wen Lai
Proteomic Determinants of Metastasis Formation in Pancreatic Adenocarcinoma abs#026

4:35 PM  
Todd M Grecco
Interactome profiling of the human histone deacetylases: Insights into the stability and regulation of chromatin remodeling complexes abs#027

**Symposium Seven: Signalling**
5:00 PM - 7:00 PM  
Heritage Ballroom

_Chairs: Mark Molloy & Hubert Hondermark_

5:00 PM  
Pedro Cutillas
Empirical inference of topology and plasticity in cancer signalling networks abs#028

5:30 PM  
Mark Graham
Proteomic profiling of activity-dependent presynaptic signalling abs#029

5:55 PM  
Mark Baker
The Prophetic Spermatozoa. How Male Infertility is Predicting the Health and Life Expectancy in Men abs#030

6:20 PM  
Michael Bank
Using novel data independent acquisition methods to obtain characterization of a biological pathway abs#031

6:45 PM  
Laura Dagley
A mass spectrometry-based approach for studying kinase activity abs#032

**APS Carnival**
7:00 PM - 12:00 AM  
Mantra Dining & Seagrass Lawn Area
Symposium Eight: Microbial Proteomics
9:00 AM - 10:30 AM  Heritage Ballroom

Chairs: Stuart Cordwell & Steven Djordjevic

9:00 AM  Steven Djordjevic
Protein cleavage generates functional diversity on the surface of bacterial pathogens  abs#033

9:30 AM  Natalie Marshall
On the hunt for mitochondrial proteolysis: Profiling changes in the mitochondrial N-terminal proteome during bacterial infection  abs#034

9:50 AM  Jeffrey J Gorman
Proteome-Wide and Proteoform-Specific Host Cell Responses to Respiratory Syncytial Virus Infection  abs#035

10:10 AM  Morton Thaysen-Andersen
Secretion of Bioactive Compartment-Specific Neutrophil Proteins Displaying a New Type of Glycosylation in Pathogen-Infected Sputum  abs#036

Morning Tea
10:30 AM - 10:40 AM  Exhibition Hall

Symposium Nine: Disease Proteomics
10:40 AM - 12:20 PM  Heritage Ballroom

Session sponsored by  ABSCIEX

Chairs: Tony Purcell & Michelle Hill

10:40 AM  David James
The Metabolic Signalling Landscape  abs#037

11:00 AM  Angus Grey
Revealing proteomic and metabolomic changes associated with lens cataract formation with Imaging Mass Spectrometry  abs#038

11:20 AM  Mark S Baker
Translation of Membrane Proteome Interactions into Novel Colorectal Cancer Targets  abs#039

11:40 AM  Jarrod Sandow
Proteomic profiling of an inducible model of acute myeloid leukaemia reveals novel insights into leukaemogenesis  abs#040

12:00 PM  Patricia Illing
Functional and biochemical analysis of HLA ligands reveals the molecular basis of HLA associated adverse drug reactions  abs#041

Closing Lecture
12:20 PM - 1:10 PM  Heritage Ballroom

Chair: Tony Purcell

Chris Overall
Tales from protein TAILS: the protease web deciphers the N terminome  abs#042

Closing Ceremony/Award Presentation
1:10 PM - 1:30 PM  Heritage Ballroom

Chair: Stuart Cordwell

Buses Depart
Buses depart at 2:00 PM SHARP  Mantra Lobby
A New Dimension in MALDI TOF-TOF Design

High Resolution
- Novel patented technology
- Established MALDI TOF-TOF expertise

High-Throughput
- Freedom to access the MALDI-7090 from any network workstation
- Multiplex Platform: integrated 10 plate loader Ultra Fast UV laser, MALDI solutions multi-user software

High-Energy CID
- True high-energy CID
- Fragmentation further enriched by retaining all metastable decay ions

Contact Information:
Freecall AUS 1800 800 950
Freecall NZ 0800 127 446
www.shimadzu.com.au
Email: info@shimadzu.com.au
POSTER LISTING

POSTER SESSION ONE

Samira Ali  
Characterisation of the peptide and protein content of ant venoms for use as bioinsecticide and antimicrobial leads  

Kathirvel Alagesan  
A novel, ultrasensitive approach for quantitative carbohydrate composition and linkage analysis using nanoLC-ESI ion trap tandem mass spectrometry  

Joel Cain  
Evaluating the relationship between n glycosylation and protein stability in campylobacter jejuni  

Harish Cheruku  
TGFβ-1 increases cell proliferation, wound healing and induces changes to membrane proteome in colorectal cancer cells with varying integrin B6 expression  

Keyur Dave  
Comprehensive characterization of phosphorylation sites of Newcastle disease virus proteins by a multitude of fragmentation techniques  

Iniga George  
Quantitative proteomic analysis of Cabernet Sauvignon grape cells exposed to thermal stresses reveal alterations in sugar and phenylpropanoid metabolism  

Meekyung Ahn  
Understanding ovine skin properties using MALDI IMS and nano-LCM  

Nicolai Bache  
LC-MS timescale intact proteoform profiling  

Iain Berry  
N-terminal Protein Sequences Highlight the Extent of Proteolytic Processing in Pathogenic Bacteria  

Amber Boyatzis  
Hydrolysis of maleimide-peptide adducts reduces sensitivity when interrogating proteomes for thiol oxidation  

Edmond Breen  
The Analysis of bead-based multiplex immuno-assays for accurate discovery of cytokine, chemokines, and growth factors expression within plasma  

Matthew Briggs  
MALDI imaging mass spectrometry of N-linked glycans on formalin-fixed paraffin-embedded murine kidney  

Stuart Brown  
Improvements in 2D gel electrophoresis for biomarker discovery in plasma proteins  

Christopher Buck  
A Label-Free Multi-Omic Study of a Glucosylceramide Inhibitor Effects on Obesity  

Christopher Buck  
Improved HDX Workflow for On-line Digestion, Separation and Data Analysis  

David Cantor  
Overexpression of αvβ6 integrin alters the colorectal cancer cell proteome in favour of elevated proliferation and a switching in cellular adhesion which increases invasion  

Natasha Care  
Characterisation of Monoclonal Antibody Drug Candidates and Biosimilars Using Mass Spectrometry  

Peter Cathro  
Isolation and identification of Enterococcus faecalis membrane proteins using membrane shaving and one-dimensional SDS-PAGE coupled with mass spectrometry  

Harish Cheruku  
Characterisation of the interaction of heterodimeric αvβ6 integrin with urokinase plasminogen activator receptor (uPAR)  

Mark Condina  
Characterization of an improved Ultra High Resolution Quadrupole Time of Flight (UHR-Q-Tof) Instrument for Proteomic applications
<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Abs#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darren Creek</td>
<td>Discovering new metabolic pathways in protozoan parasites using stable isotope labelled metabolomics</td>
<td>120</td>
</tr>
<tr>
<td>Samantha Emery</td>
<td>Discovery of virulence factors in giardia duodenalis host-cell interactions</td>
<td>121</td>
</tr>
<tr>
<td>Arun Everest-Dass</td>
<td>A platform for the structural characterization of glycans enzymatically released from glycosphingolipids extracted from tissue and cell</td>
<td>122</td>
</tr>
<tr>
<td>David Greening</td>
<td>YBX1 mediates partial EMT to promote tumourigenicity regulated by the extracellular environment</td>
<td>123</td>
</tr>
<tr>
<td>Joshua Hamey</td>
<td>Discovery of protein lysine methyltransferases that act on translation elongation factor EF1α in Saccharomyces cerevisiae</td>
<td>124</td>
</tr>
<tr>
<td>Franziska Hundt</td>
<td>Determination of Rab GTPases</td>
<td>125</td>
</tr>
<tr>
<td>Giuseppe Infusini</td>
<td>A systematic label-free quantitative approach to discover novel substrates of E3 ubiquitin ligases</td>
<td>126</td>
</tr>
<tr>
<td>Eugene Kapp</td>
<td>Comprehensive protein sequence databases and the advent of personalized sequence databases</td>
<td>127</td>
</tr>
<tr>
<td>Liisa Kautto</td>
<td>Sialic acid involvement in bacterial binding to different body fluids</td>
<td>128</td>
</tr>
<tr>
<td>Albert Lee</td>
<td>Plasma biomarkers for the detection of human growth hormone abuse in sports</td>
<td>129</td>
</tr>
<tr>
<td>Desmond Li</td>
<td>Redox modifications of cysteine in the liver of type 2 diabetes mellitus</td>
<td>130</td>
</tr>
<tr>
<td>Chi-Hung Lin</td>
<td>Toward a better understanding of glycosylation machinery by subcellular proteomics and glycomics</td>
<td>131</td>
</tr>
<tr>
<td>Ian Loke</td>
<td>Paucimannosylation in human neutrophils: Insights into the biosynthesis and immunological role of a novel type of protein -glycosylation</td>
<td>132</td>
</tr>
<tr>
<td>Andrew Lonsdale</td>
<td>Bioinformatics approaches to the proteomics of secreted proteins in Arabidopsis thaliana</td>
<td>133</td>
</tr>
<tr>
<td>Amber Lothian</td>
<td>Metalloproteomic profile of natively purified alpha synuclein from blood and brain, including the identification of the associated protein truncations by mass spectrometry.</td>
<td>134</td>
</tr>
<tr>
<td>Shiyong Ma</td>
<td>Predicting Protein Abundance based on Mass Spectrometry using Machine Learning</td>
<td>135</td>
</tr>
<tr>
<td>Nyuk Ling Ma</td>
<td>Detection of oxidative modified proteins in rice plant</td>
<td>136</td>
</tr>
<tr>
<td>Sadia Mahboob</td>
<td>A proteomic investigation for detection of early stage CRC biosignatures</td>
<td>137</td>
</tr>
<tr>
<td>Peter McCarthy</td>
<td>A workflow to identify targets of the E3 ubiquitin ligase Nedd4 involved in controlling neural crest cell fate.</td>
<td>138</td>
</tr>
<tr>
<td>Matthew McKay</td>
<td>Spectral Libraries for SWATH: Establishing a Comprehensive Database for Cancer Research</td>
<td>139</td>
</tr>
<tr>
<td>Mehdi Mirzaei</td>
<td>Understanding the molecular mechanisms of water stress tolerance induced by Piriformospora indica in barley</td>
<td>140</td>
</tr>
<tr>
<td>Parul Mittal</td>
<td>Mass Spectrometry Analysis of Endometrial Cancer</td>
<td>141</td>
</tr>
<tr>
<td>Rajesh Gupta</td>
<td>Identification of diagnostic biomarkers to improve the management of diabetic related foot ulcers</td>
<td>142</td>
</tr>
</tbody>
</table>
## POSTER SESSION TWO

<table>
<thead>
<tr>
<th>Poster Title</th>
<th>Author(s)</th>
<th>Abstract Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracking the intricate dynamics of antigen and epitope kinetics during virus infection</td>
<td>Nathan Croft</td>
<td>abs#200</td>
</tr>
<tr>
<td>Depolarisation-induced reprogramming of the presynaptic phosphoproteome mediates changes in neurotransmitter release</td>
<td>Kasper Engholm-Keller</td>
<td>abs#201</td>
</tr>
<tr>
<td>Enhancing discovery and quantitative proteomics by use of online multiphase chip LC fractionation</td>
<td>Erin Sykes</td>
<td>abs#202</td>
</tr>
<tr>
<td>Moonlighting with Melanoma and the Unfolded Protein Response</td>
<td>Cassandra Pegg</td>
<td>abs#203</td>
</tr>
<tr>
<td>Characterisation of glycosylation of the Newcastle disease virus haemagglutinin-neuraminidase surface glycoprotein</td>
<td>Christoph Kris</td>
<td>abs#204</td>
</tr>
<tr>
<td>Validation of mesenchymal stem cell transcripts assembled from RNA-Seq using proteomics data</td>
<td>Aidan Tay</td>
<td>abs#205</td>
</tr>
<tr>
<td>Virulence factors of pathogenic Escherichia coli are injected into human cells and affect the human cellular N-terminal proteome</td>
<td>Natalie Marshall</td>
<td>abs#206</td>
</tr>
<tr>
<td>Sirtuin 4 is a lipoamidase regulating the activity of the pyruvate dehydrogenase complex</td>
<td>Rommel Mathias</td>
<td>abs#207</td>
</tr>
<tr>
<td>Glycopeptide analysis of pentameric and hexameric immunoglobulin</td>
<td>Edward Moh</td>
<td>abs#208</td>
</tr>
<tr>
<td>Proteomic Analysis and Profiling of Malaysian Upland rice</td>
<td>Shahkila Mohd Arif</td>
<td>abs#209</td>
</tr>
<tr>
<td>$\text{ISD: in-source decay and MS of proteins with post-translational modifications using Matrix-Assisted Laser} \quad \text{Desorption Ionization-Quadrupole Ion-Trap Time-of-Flight Mass Spectrometer}$</td>
<td>Marcelo Moreno</td>
<td>abs#210</td>
</tr>
<tr>
<td>Moonlighting proteins of Mycoplasma hyopneumoniae and their role in pathogenesis</td>
<td>Sridevi Muralidharan</td>
<td>abs#211</td>
</tr>
<tr>
<td>Quantitative shotgun proteomics analysis of high and low antioxidant expressing Australian recombinant inbred lines of Arachis hypogaea.</td>
<td>Helen Montgomery</td>
<td>abs#212</td>
</tr>
<tr>
<td>Identification of protein-protein interactions of PfRh5 required for the invasion of human red blood cells by malaria parasites</td>
<td>Thomas Nebl</td>
<td>abs#213</td>
</tr>
<tr>
<td>Characterization of Non-Competitive Imidazoline Inhibitors of the Human Proteasome by LC-MS/MS</td>
<td>Shuai Nie</td>
<td>abs#214</td>
</tr>
<tr>
<td>The secretome of Wallemia ichthyophaga</td>
<td>Gillian Norris</td>
<td>abs#215</td>
</tr>
<tr>
<td>A Reliable and Repeatable sublimation based protocol for the analysis of Formalin Fixed Paraffin embedded (FFPE) tissue via Matrix Assisted Laser Desorption Ionisation Imaging Mass Spectrometry (MALDI-IMS)</td>
<td>Matthew O’Rourke</td>
<td>abs#216</td>
</tr>
<tr>
<td>Comparisons and optimizations on the TripleTOF 6600 and Orbitrap platforms</td>
<td>Benjamin Parker</td>
<td>abs#217</td>
</tr>
<tr>
<td>Evaluation of a lipid profiling system using reverse-phase liquid chromatography coupled to high-resolution orbitrap mass spectrometry and automated lipid identification software</td>
<td>Russell Pickford</td>
<td>abs#218</td>
</tr>
<tr>
<td>Investigation of heat stress in cattle using multiple reaction monitoring (MRM) mass spectrometry to measure cytokine levels in plasma.</td>
<td>Pedro Pires</td>
<td>abs#219</td>
</tr>
<tr>
<td>Proteomic exploration of cyclic peptide diversity among plants using peptide labelling and orthogonal separation strategies</td>
<td>Aaron Poth</td>
<td>abs#220</td>
</tr>
</tbody>
</table>
Zaidah Rahmat  
Identification of seed proteome of malaysian upland rice  

Gavin Reid  
Development of an ‘Immuno-LC-MS/MS’ Assay for the Quantitative Characterization of Oxidized and Truncated Parathyroid Hormone (PTH): Implications for the Treatment of Patients with Chronic Kidney Disease  

Ralf Schittenhelm  
Revisiting the arthritogenic peptide theory: Quantitative - not qualitative - changes in the peptide repertoire of HLA-B27 allotypes  

Crystal Semaan  
Detection of novel beta-catenin protein interactors in colon cancer  

Brian Shiell  
Zinc-α-2-glycoprotein (ZAG) is over expressed in the milk of a Pteropid bat (Pteropus alecto)  

Lauren Smith  
Irreversible Oxidative Modification of Cysteines Resulting from a Prolonged High Fat Diet Model  

David Stapleton  
Identification of apoE and amyloid beta complexes in human plasma.  

Tim Zhiduan Su  
Dissecting protein dynamics involved in reactive oxygen species-induced insulin resistance in adipocytes using SILAC-based quantitative proteomics  

Jess Tacchi  
Post-translational processing, multifunctionality and moonlighting: The not-so-simple proteome of a genome-reduced pathogen  

Vineet Vaibhav  
Identification of disease resistance genes in Sydney rock oysters using quantitative label free shotgun proteomics.  

Delphine Vincent  
Analysis of cow’s milk intact proteins using liquid chromatography-mass spectrometry  

Roza Viner  
Comprehensive Analysis of Recombinant Human Erythropoietin Glycoforms by Capillary Electrophoresis and Nanoflow Liquid Chromatography Coupled with Middle-Down Mass Spectrometry  

Valerie Wasinger  
Reverse-Polynomial Dilution Calibration Methodology extends Lower Limit of Quantification and Reduces Error in Targeted Peptide Measurements in Blood Plasma.  

Martin Whitham  
Use of in vivo quantitative proteomics for the screening of candidate myokines  

Michael Widjaja  
The accessory protein, HMW1, of Mycoplasma pneumoniae is a multifunctional protein  

Cassandra Wigmore  
Direct qualitative analysis of native human peptides in complex secretome samples with PEAKS  

Lyon Winderbaum  
Feature Extraction in MALDI Imaging using DIPPS  

Daniel Winter  
Lysine Methylation Modulates the Protein-Protein Interactions of Yeast Cytochrome c Cyc1p  

Ting Wu  
Antigen Presentation during Influenza Virus Infection  

Yunqi Wu  
Proteomic response of two different varieties of rice exposed to drought stress and recovery  

Thiri Zaw  
Evaluation of SWATH-MS for proteome profile quantitative analysis  

Chao Zhang  
Comprehensive proteomics analysis of vulvar cancer tumour progression  

Ling Zhong  
Tyrosine phosphorylation profiling and phosphoproteome mapping of three mouse tissues
SWATH™ Acquisition 2.0 with variable windows is here.

The new AB SCIEX TripleTOF® 6600 System with SWATH™ 2.0 captures virtually every detectable peptide and protein in every run, with MRM-quality quantitation and sample-to-sample reproducibility that accelerate discovery.

Maximize sample information for a more complete view of the proteome. Quantify thousands of proteins across hundreds of samples with almost no method development. Archive a digital record of every proteome you can re-interrogate at any time. Only the TripleTOF® 6600 combines increased dynamic range with the high speed, sensitivity and resolution to enable SWATH™ 2.0, unlocking the full power of data independent acquisition.

The next-generation proteomics platform has arrived.
To learn more, visit absciex.com/swath+6600

© 2014 AB SCIEX. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB SCIEX Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.
EXPLORING ALTERNATIVE PROTEASES AND FRAGMENTATION METHODS FOR PROTEOMICS
Albert Heck
1

1. Utrecht University, Utrecht, NL, Netherlands

Proteome analysis heavily relies on a broad mixture of analytical techniques, starting from sample preparation, to separation and enrichment and last but certainly not in the least mass spectrometry. Through developments in these enabling technologies MS-based proteomics has matured and start to deliver biological relevant information. Still our measurements are still far from comprehensive, and the optimal matured workflow does not (yet) exist for proteomics. In my laboratory we try to explore alternative complementary methods to explore better the richness of the proteome. In more detail I will describe methods enabling 1) enhanced peptide coverage based on a combination of HCD and ETD that leads to improved analysis of phosphopeptides, MHC peptides 1 and protein disulfide bridges 2, 2) enhanced protein and proteome coverage, by using multiple proteases and 3) improved analysis of intact proteins and protein complexes enabling to visualize all proteoforms of a given protein at once and following kinase phosphorylation in real-time using top-down approaches 3.

1. Mommen et al. PNAS 111 (2013) 4507-4512

BULKING UP MUSCLE PROTEOMICS: THE EXERCISE-REGULATED PHOSPHOPROTEOME AND THE CONTROL OF MUSCLE GROWTH
Benjamin Parker 2, 1, Nolan Hoffman 2, 1, Jonathan Davey 3, Rima Chaudhuri 2, Kelsey Fisher-Wellman 1, Max Kleinert 4, Pengyi Yang 5, Sean Humphrey 6, Daniel Fazakerley 2, Paul Gregorevic 3, Erik Richter 4, David James 2

1. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
2. The University of Sydney, Sydney, NSW, Australia
3. Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia
4. University of Copenhagen, Copenhagen, Denmark
5. National Institutes of Health, North Carolina, USA
6. Max Planck Institute for Biochemistry, Martinsried, Germany

Exercise plays an essential physiological role in the regulation of acute and long-term muscle health, insulin sensitivity and whole body metabolic homeostasis. However, only a few signalling pathways and downstream substrates are known to trigger these beneficial effects. We performed the first mass spectrometry (MS)-based phosphoproteomic analysis of human skeletal muscle biopsies obtained from healthy male subjects before and after an acute bout of high-intensity bicycle exercise. We identified 1,004 phosphosites that were significantly regulated with acute exercise. More than 900 of these phosphosites have not previously been associated with exercise and the upstream kinase is unknown. Given the known therapeutic potential of the AMPK pathway, we performed two additional MS screens to specifically pinpoint novel AMPK substrates in the human muscle including: (1) global MS-based phosphoproteomic analysis of myotubes with pharmacological AMPK activation; and (2) a novel global AMPK in vitro kinase assay combined with targeted phosphopeptide quantification with data-independent acquisition MS. Integration of these data sets revealed a number of novel AMPK substrates including A-kinase anchor protein 1 (AKAP1) that was validated in vivo. Phosphosite mutations identified a novel role for AMPK-phosphorylation of AKAP1 in mitochondrial respiration. We next investigated the use of proteomics to identify substrates involved in the regulation of long-term muscle growth. Follistatin, a potent inhibitor of TGFbeta-signalling, regulates muscle hypertrophy through an incompletely understood mechanism. Proteomic analysis of mice muscle-specific and inducible adeno-associated virus (AAV)-mediated overexpression of follistatin was performed over a time-course. Numerous proteins not previously implicated in the regulation of muscle growth were identified including the E3-ubiquitin ligase, ASB2. AAV-mediated overexpression of ASB2 induced significant muscle atrophy highlighting a novel regulator of muscle mass. These data and developed methodology will serve as an invaluable resource for future studies of muscle physiology and the development of novel therapeutics.
TWENTY YEARS OF THE PROTEOME
Marc Wilkins¹
1. Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW

On Monday the 5th of September in 1994, during the first Siena meeting (2D Electrophoresis – from protein maps to genomes), I presented the concept of the proteome and the term itself. Since that time, the field we now know as proteomics has expanded dramatically. Proteomic technology, and the new experimental paradigms that this technology supports, has revolutionised the way in which proteins can be studied in the challenging context of a cell or tissue. It has provided stunning insights into the diversity of proteins, their expression and many aspects of their function. This presentation will discuss 20 years of the proteome and of proteomics. It will discuss some major achievements of the field, some of the grand challenges that remain, and will speculate a little about what the future might hold.

ADVENT OF PROTEOMICS DISCOURSE IN AUSTRALIA: THE LORNE PROTEOMICS SYMPOSIA
Rob Moritz¹
1. Institute For Systems Biology, Seattle, WA, United States

This year marks the 20th anniversary of the Lorne Proteomics Symposium, an annual meeting of the Australasian Proteomics Society. From its humble beginnings, the APS meeting (for which it is now commonly referred to) has grown into an internationally recognized meeting for proteomics scientists to exchange ideas, start new collaborations, promote new technologies and to gather socially amongst friends and start up new friendships and collaborations on a global scale. The Lorne Proteomics Symposium of the APS is now considered a world-class meeting and has hosted well over 100 international guests over the last 20 years. The concept of the meeting was originally to provide a technology focused day prior to the Lorne Proteins Conference (a world class meeting in itself now in its 40th year. These “technology days” proved to be so successful, the meeting soon found itself expanding to include many aspects of protein identification and quantitation to also promote biological, clinical and agricultural applications of the technology. Lending from the phrase “proteomics” coined by Marc Wilkins for part of its name, this meeting now encompasses all aspects of proteome based studies and has become a fertile and important international meeting as part of the world wide proteome organization. We celebrate the 20th anniversary of the Lorne APS meeting.
THE HITCHHIKER'S GUIDE TO GLYCOPROTEOMICS AND THE HUMAN IMMUNOGLOBULOME

Daniel Kolarich

1. Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Glycosylation is well known to alter and fine-tune the functions of glycoproteins. A wide range of biological functions has been described such as conferring proteolytic resistance and mediating or inhibiting inter- and intracellular interactions [1]. As a matter of fact, protein glycosylation does not introduce a single, universally definable functionality. Thus detailed knowledge on the protein specific glycosylation and its site distribution is crucial for any systematic investigation of the functional aspects that protein glycosylation is playing on individual glycoproteins.

In that context mass spectrometry has become the method of choice for the identification and in depth primary structure characterisation of glycoconjugates. Nevertheless, it lacks the intrinsic ability to easily distinguish structural isomers of isobaric compounds, and the non-template based bio-synthesis of glycans as well as their non-linear structures pose additional challenges in glycan structure assignment by MS.

This presentation will give an overview on the peculiarities of protein glycosylation analysis and will focus in particular on advantages, challenges and limitations for in-depth glycoconjugate characterisation by MS. Synthetic glycopeptides, where both the peptide and glycan moieties can be adjusted, have become essentials tools for the systematic investigation, benchmarking and development of novel approaches for qualitative and quantitative glycopeptide analysis [2, 3]. In combination with orthogonal approaches for the analysis of the released N-glycans (porous graphitized carbon nanoLC ESI-MS/MS & CGE-LIF) and O-glycans (PGC nanoLC ESI-MS/MS) [4, 5], a detailed glycomics and glycoproteomics map of the entire human Immunoglobulome (IgA, sIgA, IgD, IgE, IgG and IgM) has been established, revealing the potential type II receptor sites present on human Immunoglobulins.

DEVELOPMENT OF LONGBOW ™ - AN INTEGRATED SYSTEM FOR HIGH THROUGHPUT GLYCOMICS TO SUPPORT BIOPHARMACEUTICAL REALISATION AND CLINICAL DIAGNOSTICS

Daryl Fernandes

1. Chief Executive, Ludger Ltd, Oxford, UK

‘I have umpteen thousand samples to analyse. How can I get reliable, detailed, quantitative maps of the complex glycosylation for each of them without spending a great deal of time and vast sums of money?’

This talk gives an overview of LongBowl - an integrated glycomics platform being developed to address this problem - which is becoming increasingly important in two areas of modern healthcare:

a. Biopharmaceutical realisation: Glycomics studies are essential throughout the development and biomanufacturing of most glycoprotein therapeutics because changes in the glycosylation can greatly influence the safety and efficacy of the drugs in patients.

b. Clinical diagnostics: Glycans play key roles in many critical biological processes and changes in glycosylation patterns of serum glycoproteins are potential early-warning biomarkers for a number of chronic diseases. These include cancers and serious inflammatory conditions such as inflammatory bowel disease (IBD). In both cases, we are interested in monitoring the (often subtle) changes in glycosylation patterns that correlate either with altered clinical performance (for the drugs) or disease progress in the patient. However, determining glycosylation patterns is still very challenging because of the complexity and heterogeneity of glycan populations. The complexity arises from the diversity in glycan monomer types and the ways they can be assembled with branched topologies. Regarding heterogeneity, both biopharmaceuticals and biological samples may be bear tens to hundreds of different types of glycans. For full glycomics studies we need to identify the complete structure of each glycan and determine its relative or absolute abundance to a high level of accuracy. Currently, there is no single analytical platform that can do this reliably, quickly and affordably. LongBowl is being designed as a first step towards achieving this and implements concepts developed in the HighGlycan programme.

This is a five year, €6m, EU-FP7 funded collaborative research project involving nine glycoscience organisations across Europe. The main topics covered in this overview will be:

1. Overall design of LongBowl, how the components fit together, flexibility for chemistry and instrumentation
2. Types of samples: biopharmaceuticals, plasma, serum glycoproteins, cells and tissues
3. Instrumentation - robotics and orthogonal analytics (UHPLC, MS, LC/MS and xCE)
4. Sample processing workflows for glycopeptides, N-glycans, O-glycans, sialic acids and neutral monosaccharides
5. Integration of modules, performance and validation
6. VTag ™ system for glycopeptide analysis by UHPLC and MS
7. Procainamide system for glycan analysis by UHPLC and MS
8. Applications: How LongBowl is being used at Ludger for biopharmaceutical glycoprofiling to support drug comparability studies and regulatory submissions and for medical glycomics for stratification of patients with chronic inflammatory conditions
9. Transfer of LongBowl technology for glycomics studies in partner labs
10. Advantages and limitations of LongBowl, future improvements.
UNICARBKB: A GLYCOBIOINFORMATICS INFRASTRUCTURE FOR DATA DISCOVERY

Matthew P Campbell1, Robyn Peterson1, Yukie Akune3, Jodie L Abrahams4, Chi-Hung Lin1, Julien Mariethoz3, Elisabeth Gasteiger5, Kiyoko F Aoki-Kinoshita2, Frederique Lisacek3, Nicolle H Packer1

1. Biomolecular Frontiers Research Centre, Macquarie University, Sydney, NSW, Australia
2. Department of Bioinformatics, Soka University, Tokyo, Japan
3. Proteome Informatics Group, Swiss Institute of Bioinformatics, Geneva, Switzerland
4. Swiss Institute of Bioinformatics, Geneva, Switzerland

In partnership with leading international research groups we are involved with the development of the UniCarb KnowledgeBase (UniCarbKB); an effort to develop and provide an informatic framework for the storage of high-quality data collections including informative meta-data and annotated experimental datasets.

UniCarbKB is a new database that aids the exploration of glycoproteins pertinent to current research strategies that strives to be a comprehensive resource. To sufficiently address research questions we aim to: 1) build on the knowledge originating from GlycoSuiteDB by carefully including selected and filtered glycoprotein data; 2) organise data to enable user-friendly interaction and querying by adopting standardisation and ontology guidelines; and 3) build a platform that will support the inclusion of new data mining tools and connect disparate resources. Here, we shall demonstrate the functionality of UniCarbKB and our strategy to increase the content and value of data provided by UniCarbKB, and our efforts to build and provide open APIs for the glycobioinformatic community.

Glycobioinformatics databases and tools are co-operatively adopting semantic technologies for managing data content, called GlycoRDF. The availability of GlycoRDF and access to RDFized databases is opening new and exciting avenues for connecting and interrogating large volumes of publicly accessible data. Examples of the capability of UniCarbKB-RDF for exploring and correlating glycomics structural and experimental data will be presented. Furthermore, the utility of semantic technologies to connect glycan-related knowledge bases with other omics resources (UniProtKB and NeXtProt) to enhance data discovery and inference of protein and glycan biological function will be highlighted.

The development of UniCarbKB and supporting technologies continues to provide a new perspective on glycobioinformatics, which extends access to high-quality annotations with interfaces to supporting analytical data sets. The initiative will be driven as a community endeavour to promote data sharing to ensure its future development and growth. Especially, our efforts to align data capture with the GlycoRDF, MIRAGE and international glycan repository initiatives.


N- AND O-GLYCOMICS OF FFPE TISSUES FROM HISTOPATHOLOGICAL SLIDES

Hannes Hinneburg1,2, Petra Korac1, Slavko Gasparov4,5, Peter H. Seeberger1,2, Vlatka Zoldoš3, Daniel Kolarich1

1. MPI for Colloids and Interfaces, Berlin, Germany
2. Free University Berlin, Berlin, GERMANY
3. Faculty of Science, Department of Biology, Division of Molecular Biology, University of Zagreb, Zagreb, CROATIA
4. Institute for Pathology and Cytology, University Hospital Merkur, Zagreb, CROATIA
5. Department of Pathology, Medical School Zagreb, University of Zagreb, Zagreb, CROATIA

Protein glycosylation is a ubiquitous post-translation modification well known to functionally influence many biological processes such as immune modulation, cell-cell interactions or signal transduction. The composition of a cell's glycome is dependant of numerous factors such as age, health or disease state, and several examples successfully demonstrated the applicability of individual glycosylation alterations as specific disease markers. This makes glycosylation a prime target for biomarker discovery, nevertheless the availability of well-defined clinical specimens in sufficient numbers often represents a serious obstacle in glyco-biomarker analyses. The possibility to use formalin-fixed paraffin-embedded (FFPE) clinical specimens presents an attractive alternative for glyco-biomarker research given that any glyco-epitopes remain unaltered and sufficient glycan amounts can be obtained from conventional FFPE tissue sections frequently used by pathologists.

Here we present an approach for the in-depth glycomic profiling of both, N-and O-glycans starting from single FFPE tissue sections as thin as 3 μm. Porous graphitized carbon (PGC) nanoLC ESI-MS/MS provides excellent isomer separation, semi-automated structure identification via spectral matching and relative quantitation of individual structures. Unstained as well as hematoxylin and eosin (HE) stained FFPE tissue samples derived from routinely taken clinical preparations were analysed and compared to their corresponding frozen counterparts to identify any potential FFPE and staining induced alterations on the N- and O-glycome. Furthermore, N- and O-glycans could successfully be retrieved from cells isolated by laser micro dissection from FFPE sections (ranging from 10000 down to 1000 cells). Overall around 90 individual glycan structures including structural isomers were identified from human hepatocellular carcinoma cells and non-tumour liver tissue. Major glyco-epitopes containing neuraminic acid and/or fucose were not affected in their structural integrity. These results emphasise the potential provided by FFPE tissue slides as a valuable source for glyco-biomarker studies from clinical specimens.

20th Lorne Proteomics Symposium 2015 – Page 35
TRACKING PROTEIN TURNOVER IN PLANTS: A NEW FRONTIER IN UNDERSTANDING THE COST OF PROTEINS TO PLANT GROWTH

Harvey Millar1, Clark Nelson1, Lei Li1, Ralitza Alexova1, Richard Jacoby1

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

Shotgun approaches or targeted SRM analyses have dominated proteome studies as tools to find changes in the proteome. These approaches, however, focus just on the proteins that are changing in abundance in order to find biological insights. They also require statistically significant changes in the total accumulated protein pool size in order to identify that ‘anything has occurred’. Analysing protein synthesis and degradation rates with progressive stable isotope labelling provides a new window on the control of protein abundance. With this approach we can determine the ‘relative age’ of the proteins that we see and define the energetic effort employed by the cell to build or maintain particular activities. We are using progressive 15N labelling of plant cells from nitrate and ammonia salts and modelling incorporation fits, to calculate the rate at which proteins which are often static in abundance in the proteome are turning over. We have developed pipelines to undertake these studies in plant cells, plant leaves and in whole plants through the use of hydroponics. Projects assessing the impact of leaf age, phosphorus limitation and groundwater salinity on protein turnover changes in plants will be discussed. Through combining such labelling with separation of protein complex and subcomplexes by native electrophoresis, we can observe the in vivo turnover rate of assembly intermediates of protein complexes. Combined these approaches provide new avenues for peptide mass spectrometry to provide answers to a wide range of questions in plant biology, and allows researchers to assess the cost of environmental factors on protein turnover and plant growth efficiency.

PROTEOMIC ANALYSIS OF ABIOTIC STRESS IN PLANTS

Paul Haynes1, Yunqi Wu1, David C.L Handler1, Samantha Emery1, Vineet Vaibhav1, Brian J Atwell1, Iniga S George1, Mehdi Mirzaei1

1. Macquarie University, North Ryde, NSW, Australia

Proteomic studies in plants including rice (Oryza sativa) and grapes (Vitis vinifera) have become increasingly prevalent in recent years now that complete genome sequences are available for both species. In our laboratory we have carried out a number of different studies involving analysis of differential protein expression in both plants and cell cultures, induced by exposure to different abiotic stress conditions. This has included detailed analysis of the response of grape cells to a wide range of temperature stresses, identification of proteins affected by changes in day length in riverbank grapes, and detailed quantitative analysis of temperature and drought stress in rice. This work was all performed using shotgun proteomics analysis with nanoLC-MS/MS, coupled with calculation and measurement of normalised spectral abundance factors using software developed in-house. This analytical pipeline enables the identification and quantification of thousands of proteins simultaneously without the need for metabolic or chemical isotopic labelling. In this presentation we will present some highlights from these studies, focusing on the commonalities of the biological responses observed in these and other studies. We will also discuss statistical validation of protein expression changes, including recent work on the development of a procedure for estimation of false discovery at the protein quantitation level.

THERE’S A HOLE IN MY ASSAY, DEAR ELISA, DEAR ELISA: USING MS TO DETECT HYDROLYSED GLUTEN IN BEER THAT IS RESPONSIBLE FOR FALSE NEGATIVES BY ELISA

Michelle L Colgrave1, Hareshwar Goswami2, Crispin A Howitt2, Greg J Tanner2

1. Agriculture Flagship, CSIRO, St Lucia, QLD, Australia
2. Agriculture Flagship, CSIRO, Black Mountain, ACT, Australia

Gluten is the collective name for a class of proteins found in wheat, rye, barley and oats. Eating gluten triggers an inappropriate auto-immune reaction in ~70 million people globally affected by coeliac disease, where the gut reacts to gluten proteins and this triggers an immune response, resulting in intestinal inflammation and damage. Gluten-free foods are now commonplace, however, it is difficult to accurately determine the gluten content of products claiming to be gluten-free using current methodologies as the antibodies are non-specific, show cross-reactivity and have different affinities for the different classes of gluten. The measurement of gluten in processed products is further confounded by modifications to the proteins that occur during processing and in some case hydrolysis of the proteins. In this study, LC-MS/MS analysis of barley-derived beers revealed that certain classes of hordein were prone to hydrolysis (B- and D-hordein). Strikingly, those beers that contained high levels of B-hordein fragments gave near zero values by ELISA. The hydrolysed fragments that persist in beer show a dose-dependent suppression of ELISA measurement of gluten despite using a hordein standard for calibration of the assay. Using 2D gels the complete suite of hordeins present in barley grain, the precursor to malt using in brewing, were visualised. Further, using a combination of high resolution discovery proteomics and targeted quantitative proteomics the “gluteome” was characterised facilitating selection of peptide markers specific to each hordein family. The development of MS-based methodology for absolute quantification of gluten is required for the accurate assessment of gluten, including hydrolysed forms, in food and beverages to support the industry, legislation and to protect consumers suffering from coeliac disease.
IDENTIFICATION OF N-LINKED GLYCOSYLATION SITES ON PLANT GLYCOPROTEINS

Kris Ford¹, Yin Ying Ho¹, Wei Zeng¹, Monika S Doblin¹, Antony Bacic²,¹
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 catalytic activity, folding, subcellular location and secretion, as well as in plant pathogen interactions¹. 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. We describe a method for the enrichment of N-linked glycopeptides from plants, followed by LC-MS/MS analysis using higher-energy collisional dissociation (HCD) and electron transfer dissociation (ETD) for peptide identification, glycan site attachment and the type of glycans attached.

A TOP-DOWN PROTEOMIC APPROACH FOR UNDERSTANDING MULTIPLE SCLEROSIS

Melissa A Partridge¹,², Simon J Myers²,³, Sumana Gopinath²,⁴, Jens R Coorsen¹,²
1. Department of Molecular Physiology, School of Medicine, University of Western Sydney, Penrith, NSW, Australia
2. Molecular Medicine Research Group, School of Medicine, University of Western Sydney, Penrith, NSW, Australia
3. Neuro-Cell Biology Laboratory, School of Science and Health, University of Western Sydney, Penrith, NSW, Australia
4. Department of Neurology, Campbelltown Hospital, Campbelltown, NSW, Australia

Multiple Sclerosis (MS) is conventionally thought to be initiated by an autoimmune T cell response. However, there is an alternate hypothesis that MS may begin as a slow, progressive (i.e. clinically undetected) degeneration of oligodendrocytes [1-3]. Currently the molecular mechanism(s) behind such a degeneration are poorly understood. To address this we have initiated a systems-wide analysis of an animal model of oligodendrocyte degeneration: the cuprizone model. We have undertaken a top-down analytical approach to investigate the breadth of proteomic alterations that cuprizone treatment has on the cortex, skeletal muscle, spleen, and peripheral blood mononuclear cells. Using 2-dimensional gel electrophoresis, deep imaging, and mass spectrometry we have identified 43 protein species as uniquely present or absent in the cuprizone treatment group. Protein species identified in the cortex are potentially linked to primary oligodendrocyte degeneration and secondary axonal damage. Furthermore, we identified alterations in the proteomes of skeletal muscle, spleen, and peripheral blood mononuclear cells following cuprizone treatment; arginase-1 was a notable hit in the spleen and has previously been linked to T cell suppression in an animal autoimmune model of MS [4-6]. A further notable finding was the absence of protein disulphide isomerase subunits in the spleen and peripheral blood mononuclear cells of the cuprizone treatment group; this may further contribute to impaired MHC class I assembly leading to reduced antigen presentation and potentially contributing to the lack of T cell response in this model [7]. Overall, the results of this study suggest that findings from the cuprizone model and likely others need to be carefully considered relative to clinical MS.

REVEALING METABOLIC ACTIONS OF NOVEL COMPOUNDS AGAINST AFRICAN TRYPSANOSOMES BY HIGH RESOLUTION MASS SPECTROMETRY

Daniel Stoessel1, Cameron J Nowell2, Katherine M Ellis1, Malcolm J McConville3, Jonathan Baell4, Darren J Creek3

1. Drug Discovery, Monash Institute of Pharmaceutical Sciences, Melbourne
2. Biochemistry and Molecular Biology, Bio 21 Molecular Science and Biotechnology, Melbourne
3. Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Melbourne
4. Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Melbourne

Trypanosoma brucei causes human African trypanosomiasis (HAT), which is transmitted by a bite of the Tsetse fly. This disease causes significant disability and is fatal for around ten thousand people every year. The armory of medication for HAT is out of date and the development of resistance, high toxicity and treatment costs are ongoing problems. High throughput screening of approximately 87,000 compounds against T. brucei identified a novel compound 3-(Oxazolo[4,5-b]pyridine-2-yl)anilide (OXPA) which shows high activity against this organism and minimal toxicity to mammalian cells. The aim of this study was to determine the biochemical actions of this compound in T. brucei.

Metabolomics with HILIC/RP-chromatography in combination with high resolution mass spectrometry was applied to in vitro cell cultures of T. brucei treated with this novel compound. The investigation of polar metabolites with untargeted HILIC metabolomics approach revealed that OXPA interacts with the sphingolipid pathway in T. brucei, most likely by inhibiting the parasitic sphingolipid synthase, which has been validated as a potential drug target in previous studies.
HARBOURING A KILLER: A PROTEOMIC APPROACH TO COMPARING HOST RESPONSES TO HENDRA VIRUS
Amanda P Woon1, James W Wynne2, Michelle L Baker2, Linfa Wang2,3, Nadine L Dudek1, Anthony W Purcell1
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 source of zoonotic pathogens, harbouring over 60 different viruses including Ebola, SARS, MERS and Hendra (HeV), many of which pose a serious threat to human and animal health. HeV is an emerging zoonotic virus currently only found in Australia. It causes respiratory and neurological diseases in horses and humans and is characterised by high morbidity and mortality. However in its natural fruit bat host (Pteropus spp.), HeV does not seem to cause any symptoms of illness, with high titres of virus shed in bat excretions. Currently, there is no vaccine or treatment against HeV for humans. We hypothesise that comparative studies of the bat and human immune response to HeV may provide valuable insight into deciphering the elements of an effective anti-viral strategy.

In this study, two hosts, bats (Pteropus alecto) and ferrets (Mustela putorius furo) (human disease model) were challenged with HeV and tissues were extracted at several time points. Tissues were homogenised and analysed by mass spectrometry (LC-MS/MS). Identified proteins were then quantified using label free approaches. Preliminary data has highlighted a difference between the bat and ferret host response to HeV infection. In bats, there was increased expression of proteins involved in innate immune responses such as the type I interferon signalling pathway. However, in ferrets, there was a strong skewing towards activation of adaptive immune responses including proteins involved in antigen processing and presentation. In both models, there was an overlap in the induction of proteins involved in the complement system. These data provide candidate pathways for further examination using targeted mass spectrometry and orthogonal techniques. Validation using these sensitive and quantitative approaches will further elucidate the mechanisms by which bats are able to control viral infection while humans succumb to disease. Ultimately, it will also address the question of whether bats tolerate virus due to superior innate immunity or whether a propensity towards immunopathology in humans contributes to the mortality of HeV.

FACING THE FATS: CAN MASS SPECTROMETRY UNMASK THE TRUE STRUCTURAL DIVERSITY OF THE LIPIDOME?
Stephen Blanksby1, M Bhujel1, H.T Pham2, T.W Mitchell3
1. Central Analytical Research Facility, Queensland University of Technology, QLD
2. Department of Chemistry, University of California, Riverside, USA
3. School of Medicine, University of Wollongong, NSW

Lipids play pivotal and diverse roles in cellular function, ranging from structuring cell membranes to energy storage and signaling. Understanding the full range of the biological functions of lipids, and the associated mechanisms, requires a detailed understanding of the diverse array of molecular structures that constitute the lipidome.[1] Recent advances in mass spectrometry – notably the increases in sensitivity and resolving power – have revealed the staggering molecular complexity of lipids in biological extracts. Elucidating the molecular structure of these lipids within such rich mixtures however, represents a formidable challenge to conventional mass spectrometry. The recent combination of collision-induced dissociation (CID) with ozone-induced dissociation (OzID) has been shown to be a powerful approach to providing a near-complete structure of complex lipids including the assignment of position(s) of unsaturation and relative acyl chain positions in glycerolipids.[2] Importantly, these novel ion activation strategies identify the presence of isomeric lipids within complex extracts and examples from investigations of lipid extracts from humans and insects indicate functional significance of specific isomeric forms.

IDENTIFICATION OF PLASMODIUM BERGHEI MARKERS OF INFECTION IN THE ARTHROPOD HOST BY TLC-MALDI COUPLED TO IMAGING MASS SPECTROMETRY

Berin A Boughton¹, Daniel Sarabia¹, Dean Goodman³, Mark Condina¹, Geoff McFadden², Anthony Bacic⁴,¹, Ute Roessner¹

1. School of Biosciences, Metabolomics Australia, The University of Melbourne, Melbourne, Vic
2. School of Biosciences, School of Botany, University of Melbourne, Parkville, Vic
3. Bruker Pty Ltd, Preston, Melbourne, Vic
4. ARC Centre of Excellence in Plant Cell Walls, School of Botany, The University of Melbourne, Parkville, Melbourne, Vic

Thin Layer Chromatography (TLC) coupled to Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) offers the ability to couple a rapid and versatile separation technique with routine MALDI MS analysis providing an effective strategy for the analysis of lipid mixtures. Due to the varying detectability of individual lipid classes when using a MALDI Imaging Mass Spectrometry (MALDI-IMS) approach, prior separation using TLC offers advantages including detection of either low abundance or suppressed ions coupled with the ability to analyse multiple samples (e.g. control and infected) at the same time. The complement of lipids extracted from excised midguts of control and mosquitoes infected with GFP labelled Plasmodium berghei ANKA, were separated by TLC using optimised separation and matrix application conditions prior to MALDI Fourier Transform MS (FTMS) analysis. Using this approach we putatively identified a phosphatidylethanolamine (PE(38:5)) as a marker of the infection status. Subsequent tissue imaging using MALDI-FTMS and classification using Receiver Operating Characteristic (ROC) curve analysis of control and infected mosquitoes tissue sections confirmed the PE(38:5) to be collocated with the site of infection within the midgut. Further, a number of polyunsaturated fatty acids corresponding to eicosapentaenoic acid and eicosatetraenoic acid, implicated in both parasite development and host immune response, were found to be highly abundant in infected tissues sections.

LIPIDOMICS IS PROVIDING NEW STRATEGIES FOR RISK ASSESSMENT AND THERAPEUTIC INTERVENTION IN CARDIOVASCULAR DISEASE

Peter Meikle¹

1. Baker IDI Heart & Diabetes Institute, Melbourne, VIC, Australia

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

We have developed a targeted lipidomics platform using liquid chromatography electrospray ionization-tandem mass spectrometry to profile 300-400 lipids from 10 mL plasma. We have applied this technology to multiple clinical and population based cohorts to define the plasma lipid profiles associated with T2D and CVD and evaluate the potential application of these profiles to diagnose, assess disease risk and inform on disease pathogenesis.

Regression analysis adjusting for covariates (age, sex, systolic blood pressure and obesity) identified multiple lipid species that were significantly associated with prevalent and incident T2D or CVD. Multivariate analysis incorporating unsupervised feature correlation minimization and reliefF feature selection was employed to create and test multivariate classification and risk assessment models incorporating different numbers of lipids and other risk factors. Models incorporating lipids performed better than models based solely on traditional risk factors.

Plasmalogens, a class of lipids with anti-oxidative and anti-inflammatory properties, were negatively associated with prevalent and incident CVD. We subsequently investigated the potential of batyl alcohol (BA, metabolic precursor to plasmalogen) to modulate plasmalogens level and attenuate the development of atherosclerosis in ApoE−/− and ApoE−/−Gpx1−/− mice fed a high-fat diet. The mice without BA developed extensive atherosclerotic plaques throughout the aorta. This was reduced by 70% (P<0.001) in the BA-treated mice.

Plasma lipid profiling can provide insight into disease pathogenesis and may contribute to a new approach to risk stratification and therapeutic intervention in T2D and CVD.
PROBING MICROBIAL PATHOGEN INTERACTIONS AND METABOLISM USING METABOLOMICS

Malcolm McConville1, Joachim Kloehn1, Eleanor Saunders1, Martin Blume1, Jame MacCrae1, Simon Cobbold1, Charlie Hwa Chua1, Michael Dagley1, Fleur Sernee1, Julie Ralton1, Fiona Sansom1

1. Department of Biochemistry and Molecular Biology, Bio21 Institute of Molecular Science and Biotechnology, University of Melbourne, Parkville, VIC

Protozoan parasites are an evolutionarily diverse group of single-celled eukaryotes that cause a number of important diseases, such as malaria, toxoplasmosis and leishmaniasis, that collectively affect hundreds of millions of people worldwide. There are no vaccines for any of these diseases and current drug therapies are extremely limited and/or being undermined by the emergence of drug-resistant parasite lines, and there is a urgent need to identify new drug targets. While metabolic enzymes are high priority drug targets, our current understanding of the cellular metabolism and physiology of intracellular parasite stages is limited. In particular, while genome-wide annotations have provided broad insights into the metabolic potential of these pathogens, the majority of protein-encoding genes in these genomes (60-70%) have no known function. Furthermore, attempts to infer stage-specific changes in metabolism from transcriptional or proteomic analyses have also been hampered by the reliance of these organisms on post-translational responses. In order to identify metabolic pathways that are active in pathogenic stages we have developed a comprehensive suite of MS-based metabolomic approaches for characterizing parasite metabolism in situ. In this talk, examples will be provided of how MS-based metabolite profiling coupled with 13C-stable isotope labelling approaches have revealed novel aspects of parasite metabolism that are essential for intracellular survival. We have also developed new approaches for measuring parasite and host responses in infected animal tissues using heavy water (2H2O) labelling and high resolution FTICR-imaging mass spectrometry. The latter approaches are being used to identify metabolically distinct populations of pathogens in tissues that may be differentially susceptible to anti-parasite drugs.

ANALYSIS OF LARGE PROTEOMICS DATASETS: PITFALLS, CHALLENGES AND SOLUTIONS

Jurgen Cox1

1. Max Planck Institute of Biochemistry, ., Germany

Since it is becoming feasible to collect shotgun proteomics data on the scale of the whole human genome it is crucial that computational workflows for the identification and quantification of peptides and proteins are equipped for dealing with mass spectral datasets of enormous sizes. The challenges are two-fold: 1) the requirements for the computational efficiency are daunting including demands for high degree of parallelization and efficient I/O. 2) Prescriptions for restricting the false discovery rate for peptides and proteins need to be utilized in order to ensure validity of the results. We show how these and other crucial problems are solved in the MaxQuant software and present examples of its application to several large-scale proteomics datasets.

DATA INDEPENDENT ACQUISITION – THE NEXT REVOLUTION IN PROTEOMICS

Christie Hunter1

1. AB SCIEX California, Redwood City, CA, United States

As the field of proteomics research has evolved over the past ten years, from identification focused experiments to today’s highly quantitative strategies, different techniques have been developed to meet the workflow needs. Most recently, SWATH™ Acquisition, a data independent acquisition workflow, has been developed that increases the comprehensiveness of data collection while maintaining high quantitative reproducibility. Now, thousands of proteins can be profiled across large sample sets with high data completeness and quantitative accuracy approaching that of the gold standard MRM approach. In DIA, larger 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. Post-acquisition, MS/MS data is interrogated using spectral libraries to generate quantitative XICs, providing MRM-like data for better specificity and quantitative accuracy. Significant progress has been made in understanding and improving the analytical attributes of this DIA technique, using key sample types. For a new technique to have impact on biomarker research however, it must be routinely implemented across multiple labs. Progress to date on the use of DIA workflows for biomarker research will be discussed.
MASS SPECTROMETRY IMAGING OF TRYPTIC PEPTIDES: TOWARDS CELLULAR RESOLUTION IN FRESH-FROZEN AND FFPE TISSUE

Andreas Roempf1, Dhaka Bhandari1, Katharina Huber1, Bernhard Spengler1

1. Justus Liebig University Giessen, Germany

The direct detection and identification of intact proteins in MALDI imaging remains a challenging task due to limited sensitivity and mass range. Here we present new approaches for on-tissue tryptic digestion of proteins. We focus on optimizing the spatial resolution and reliability of peptide identification. We also present strategies for data processing based on the common data format imzML.

Trypsin solution was deposited in several cycles on tissue with a spraying device. An atmospheric pressure matrix assisted laser desorption (AP-MALDI) ion source coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific GmbH, Bremen) was used for imaging experiments. Tryptic peptides were identified by matching imaged m/z peaks to peptides which were identified in complementary LC-MS/MS measurements of an adjacent tissue sections. All MS measurements were based on accurate mass (< 3 ppm RMS).

A coronal mouse brain section was measured at 50 µm pixel size. Peptide peaks were detected on tissue with a mass resolution of R=80000 (@m/z700). This measurement reveals detailed histological structures such as the ependyma (consisting of a single cell layer) which is clearly defined by several identified peptides. Highly reliable information about protein distribution was also obtained for clinical human tissue originating from brain and gastric cancer biopsies. This data is used to investigate intratumor heterogeneity on a molecular level. A whole body section of an infant mouse was imaged at 50 µm pixel size. Strategies for processing this 40 GB data set by conversion to the common data format imzML will be discussed.

A coronal mouse brain section was imaged at a pixel size of 25 µm. The resulting ion images of tryptic peptides showed excellent correlation with myelin and H&E staining. Peptide peaks were detected on tissue with a mass resolution of R=40000 (@m/z700). The sensitivity could be significantly improved compared to previous experiments and about 150 tryptic peptides which show a clear spatial distribution were identified.

Initial results for formalin-fixed paraffin-embedded tissue will be discussed. Different protocols for deparaffinization and antigen retrieval were evaluated. Tryptic peptides were detected with accurate mass at 35 µm pixel size in mouse brain tissue.

Some of the datasets discussed here were up 40 GB. This requires efficient ways for data processing. An example on how to use the common data format imzML in order to make use of multiple software tools is discussed.

2-D METALLOPROTEIN MAPS: APPLICATION OF QUANTITATIVE METAL AND PROTEIN PROTEOMICS TO DEFINE THE METALLOPROTEOME OF THE HUMAN BRAIN.

Blaine R Roberts1, Amber Lothian1

1. Florey Institute of Neuroscience and Mental Health, Parkville, VIC, Australia

Despite the importance of metal cofactors, techniques to measure metal status in a proteomic fashion are not readily available. One major challenge for metalloproteomics is determining the identity of the metalloprotein. Thus, we have been pursuing the development of tools that will allow the direct identification and quantitation of individual metalloproteins from biological samples. The tools we have developed allow for comparative metalloproteomics and provide a metalloprotein map that is reminiscent of 2D polyacrylamide gels. The ability to resolve and measure metalloenzymes in detail have practical implications for a variety of research areas including neurodegeneration, cancer, aging, heavy metal toxicity and therapeutic development. Here we present metalloprotein maps for Cu, Fe, Zn, Mn, Co, Ni and P from human brain tissue. The combination of multidimensional chromatography and traditional proteomics has provided one of the first in-depth looks at the human metalloproteome and has resulted in the discovery of novel metalloproteins. The future development and application of this technique promises to yield important insights into the role of metals in biological and pathological processes.
THE USE OF AXIAL SPATIAL DISTRIBUTION FOCUSING FOR THE GENERATION OF HIGH RESOLUTION AND HIGH ENERGY MALDI TANDEM MASS SPECTRA

Helen Montgomery1, Philippa Hart1, Mathew E Openshaw1, Yuzo Yamazaki2, Omar Belgacem1, Andrew Bowdler1
1. Shimadzu, Kratos, Manchester, UK
2. Shimadzu, GADC, Kyoto, Japan

The curved field reflectron has been in existence for nearly two decades since it was introduced by Cotter and Cornish. For several years the CFR was used in the only commercially available instrument capable of MALDI TOF MS/MS without scanning the reflectron. Seamless PSD provided advantages in terms of both sensitivity and speed of acquisition. However, the key advantage of the CFR over conventional linear field reflectrons, even those used in TOF/TOF instruments has been the ability to produce MS/MS at the full extraction energy. This is because there is no need with the CFR to re-accelerate the ions after the first TOF. So that, not only is very high sensitivity achieved for MS/MS but also that true high-energy CID is possible with lab collision energies of 20keV or more.

However, even when used in MALDI TOF instruments with in excess of 20,000 mass resolutions for MS, the mass resolution using the CFR for MS/MS was limited to isotopic resolution up to about 1000 Da. This has been known for a long time to be due to the increase in the laser intensity required to produce the meta-stable ions required for MS/MS (even with high-energy CID) which is much higher than the threshold for MALDI TOF MS. However, until recently, the effect had not been understood or quantified. More importantly, the reduced mass resolution has meant that the CFR instruments were not able to match the ultimate performance of other TOF/TOF instruments.

In this paper, we present the basic theory behind Axial Spatial Distribution Focussing (ASDF) as well as its applications for various structural elucidations and applications problem. Lipids fragmentations data showing C-C backbone fragmentation as well as high energy tandem Mass spectra of Peptides allowing differentiation of isomeric species will be presented. With the technique of Axial Spatial Distribution Focussing in combination with the Curved-Field Reflectron, fragment mass resolutions in excess of 10,000 can be achieved for MS/MS of meta-stable ions and CID at lab energy of 20keV. By using ASDF, the Curved-Field Reflectron is again capable of defining the state of the art for MALDI TOF-MS/MS and can really be said to have come of age.

PROTEOMIC DETERMINANTS OF METASTASIS FORMATION IN PANCREATIC ADENOCARCINOMA

Zon Lai1, Peter Bronsert2, Mirjam Damen3, Arjen Scholten1, Oliver Schilling 1
1. Institute for Molecular Medicine and Cell Research, University of Freiburg, Freiburg, BW, Germany
2. Department of Pathology, University Medical Centre, Freiburg, BW, Germany
3. Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Utrecht, The Netherlands

Pancreatic cancer is currently the fourth most common internal cancer worldwide. With more than 200,000 reported cases annually, its mortality is almost equal to the incidence rate. Pancreatic cancer is an aggressive malignancy with one of the worst outcomes of all cancers and has a very poor prognosis: with a 1-year survival of less than 25%, and once the tumors have metastasized, a five year survival of less than 5%. In our study, we aim to investigate the key proteins from the primary tumor of pancreatic ductal adenocarcinoma (PDAC) that drives metastatic spread. Quantitative profiling of proteins using dimethyl labeling followed by 2D-LCMS/MS analysis of five primary tumours from patients with observed lymph node metastases (TNM classification=N1) were compared against five primary tumours from patients with no lymph node metastases (TNM classification=N0). We have identified and quantified a total of 2451 proteins in at least 3 of 5 patients, of which 105 proteins displayed altered levels in N0 versus N1 (p < 0.1, delta fold change >0.5). For selected proteins, the findings were validated by immunohistochemistry and Western blotting. Our data revealed a number of proteins that had already been related to cancer metastasis and tumour cell invasion such as dermatopontin, biglycan, activated leukocyte cell adhesion molecule (ALCAM), but also other potential new prognostic biomarkers such as DNA binding protein A and guanine nucleotide-binding protein G(1)/G(5)/G(7)/G(T) subunit beta-2. Furthermore, our data indicate a number of proteins which were related to immune responses and tumour microenvironment. Taken together, the preliminary findings from this study shed lights on novel candidates, which may play pivotal roles in cancer cell invasiveness and metastatic spread in pancreatic cancer.
INTERACTOME PROFILING OF THE HUMAN HISTONE DEACETYLASES: INSIGHTS INTO THE STABILITY AND REGULATION OF CHROMATIN REMODELING COMPLEXES

Todd M Greco1, Preeti Joshi1, Amanda J Guise1, Alexey I Nesvizhskii2, Ileana Cristea1
1. Department of Molecular Biology, Princeton University, Princeton, NJ, USA
2. Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

Histone deacetylases (HDACs) are a family of 11 essential transcriptional regulatory enzymes. HDAC dysfunction contributes to many human disease states, including cancers and viral infections. Indeed, small molecule HDAC inhibitors are in clinical trials for treatment of cutaneous T-cell lymphomas. However, these inhibitors are relatively non-selective, and the functions and interactions of many HDACs remain poorly understood. A systematic characterization of unique and shared protein complexes among HDAC family members would provide insight into HDAC-specific functions and improved targets for therapeutic intervention. Towards these goals, we integrated fluorescence microscopy, immunooaffinity purifications, quantitative mass spectrometry, functional network analysis, and improved bioinformatics tools to build the first global protein interaction network for all eleven human HDACs in T-cells. Through optimization of label-free SAINT modeling of interaction specificity, we detected many well-known HDAC interactions as well as 200 previously unreported interactions. For instance, we identified deleted in breast cancer 1 (DBC1), a protein associated with tumor progression and known to inhibit HDAC3 and SIRT1. Our finding that DBC1 also interacts with HDAC5 and HDAC9 suggests a broader function, which we further characterized using molecular biology and biochemistry approaches. Apart from HDACs’ transcriptional roles, functional interaction profiling of novel interactions highlighted emerging roles in spliceosomal snRNP assembly and cell cycle regulation. To provide a deeper functional understanding of HDAC regulation, we designed a hybrid approach integrating label-free and isotope-labeling quantification to profile relative interaction stability across HDAC protein complexes. Interestingly, HDAC1 interactions were either highly stable within chromatin remodeling complexes or fast-exchanging associations with DNA-binding proteins and transcription factors. Collectively, our work demonstrates the effectiveness of an integrative proteomics approach in extrapolating protein functions based on interaction networks. Our global HDAC interaction dataset led to the identification of new HDAC functions and provides a rich resource for investigating HDACs in health and disease.

EMPIRICAL INFERENCE OF TOPOLOGY AND PLASTICITY IN CANCER SIGNALLING NETWORKS

Pedro Cutillas1
1. Integrative Cell Signalling and Proteomics, Barts Cancer Institute, London, UK

Signalling pathways driven by the activity of lipid and protein kinases regulate key cell biological functions. Such pathways form complex networks of biochemical reactions that decode and integrate a multitude of inputs from the cell environment in order to produce an appropriate response. The topology of signalling networks has been inferred from experimental data collated from different studies using computational methods. As a complementary approach to network biology, this presentation will discuss our efforts in using empirical data (from MS-based phosphoproteomics) to infer signalling network topology de novo and without a preconception of how such networks may be wired in specific cell types. Studies in which this approach was used to investigate network plasticity in cancer will also be presented.

PROTEOMIC PROFILING OF ACTIVITY-DEPENDENT PRESYNAPTIC SIGNALLING

Mark E Graham1, Phillip J Robinson1, Kasper Engholm-Keller1
1. Children’s Medical Research Institute, Westmead, NSW, Australia

Protein phosphorylation and dephosphorylation is a crucial fast signalling mechanism following depolarisation of presynaptic nerve terminals. The strength of connections in neural circuits is adaptable, depends on brain activity and underlies learning and memory. Depolarisation of axons and presynaptic terminals causes neurotransmitter filled synaptic vesicles to fuse with the membrane and release neurotransmitter into the synaptic cleft. The amount of neurotransmitter released can be regulated by a range of different biological processes directly and indirectly related to the cycle of synaptic vesicle fusion and biogenesis. In the past, 32P metabolic labelling has provided a view of how presynaptic protein machinery engages with activity-dependent signalling. We have used chemical dephosphorylation of isolated presynaptic nerve terminals to profile presynaptic signalling and compared this to 32P metabolic labelling. Peptide derivatisation by iTRAQ or dimethylation with isotopes of different mass allowed quantification of phosphorylation levels over time. We have shown that the type and level of stimulus determines the presynaptic response and have identified regulators of the synaptic vesicle cycle and other biological processes as targets for activity dependent phospho-signalling.
THE PROPHETIC SPERMATOZOA. HOW MALE INFERTILITY IS PREDICTING THE HEALTH AND LIFE EXPECTANCY IN MEN.

Mark Baker¹, Anita Weinberg¹, Louise Hetherington¹

¹. University of Newcastle, Newcastle, NSW, Australia

Male infertility is a very common condition, with reports suggesting that one in 15 men of reproductive age is affected¹. The diagnosis of male-factor infertility is difficult and involves discounting female infertility through hormone measurements, pelvic examination and invasive laparoscopy. A semen profile analysis can suggest male infertility, if sperm counts are <15-20 million/ml, or <50% of sperm possess forward progressive motility (and < 25% rapidly progressive sperm) or <4% good morphology sperm. However, for many couples (20-30%), infertility remains largely unexplained.

Large scale cohort reports have shown that infertile men on average are dying younger than their fertile counterparts. In addition, infertile men on the whole demonstrate three times the average rates of cancer compared to the general population². As such, it appears that spermatozoa may give a “prophetic” insight into the overall health of men with a current leading hypothesis suggesting that sperm cells may act as a “canary in the coalmine” for the future prediction of men’s health².

As such, we have used quantitative proteomics analysis to compare spermatozoa taken from healthy, fertile individuals and compared the proteome to that of an infertile male. Several proteins were found to be altered, including, the sperm specific protein, Outer Dense Fibre 1, which was virtually absent from the gametes of the infertile male. Of particular note however, the epigenetic regulation of histones was shown to be very different in the infertile sample, compared to the fertile sample. This suggests that erroneous post-translational modifications on Histones within infertile men may represent the first insight into not only male infertility, but also may explain the link between infertile gametes and the future prediction of men’s health.

2. Eisenberg, M.L., Betts, P et al., 2013. Increased risk of cancer among azoospermic men. Fert. Ster. 100. 681-685

USING NOVEL DATA INDEPENDENT ACQUISITION METHODS TO OBTAIN CHARACTERIZATION OF A BIOLOGICAL PATHWAY

Michael Blank¹, Ryan Bomgarden², Neelu Puri³, Rosa Viner¹, Bhavin Patel², Iman Mohtashemi¹, Andreas Huhmer¹

¹. Thermo Fisher Scientific, San Jose, CA, United States
2. Thermo Fisher Scientific, Rockford, IL, United States
3. University of Illinois at Chicago, Rockford, IL, United States

Publish consent withheld

3. Egerton et al. Nat. Methods, 2013, 10, 744-766
A MASS SPECTROMETRY-BASED APPROACH FOR STUDYING KINASE ACTIVITY

Laura F Dagley1, Tatiana B Kolesnik2, Phillip P Sharp1, Edmond M Linossi2, Simon Chatfield2, Gabrielle L Goldberg1, Andrew I Webb3, Christopher J Burns2, Nicos A Nicola1, Jeffrey J Babon1, Isabelle S Lucet1, Sandra E Nicholson1

1. The Walter and Eliza Hall Institute of Medical Research (WEHI), PARKVILLE, VIC, Australia
2. Department of Medical Biology, University of Melbourne, PARKVILLE, VIC, Australia

Protein kinases play a key role in numerous cell signalling pathways. They have emerged as an important class of enzymes since aberrant kinase activity is associated with human cancers and inflammatory disorders. Whilst the human kinome is comprised of over 500 members, only a fraction have been exploited as drug targets for therapeutic interventions. Thus, many members of the kinase superfamly and their role in human diseases remain poorly understood. This can be attributed to the fact they are in low cellular abundance in complex protein samples and their detection is reliant on the quality of available phosphoantibodies. In recent years, efforts have been made to develop immobilized small-molecule kinase inhibitors as kinase-capturing reagents. When coupled with highly sensitive mass spectrometry analysis, this kinase enrichment step allows for the comprehensive scanning of the kinome and the identification of dysregulated kinase signaling networks in disease states1.

In this study, we have used two kinase-capturing reagents to profile the kinome from various cellular sources including HEK293Ts, primary human neutrophils, murine natural killer (NK) cells and bone marrow-derived macrophages (BMDMs). Kinome enrichment was achieved using CTx-0294885 resin (KiNET-1 beads, SYNkinase) and a pan-JAK1/2 inhibitor CYT387 (Cytopia/Gilead) coupled to NHS-sepharose beads. In this way we identified a number of novel kinases within specific cell types, some of which may play important roles in the context of inflammatory diseases such as Gout and Rheumatoid arthritis. This kinome profiling strategy was performed alongside a phosphopeptide enrichment strategy for quantitative mass spectrometry analysis. Using this approach we identified a number of novel phosphopeptide sites within clinically-relevant kinases including the JAK protein tyrosine kinases. We identified 40 phosphorylation sites in murine JAK2 (23 novel and 17 known sites), whilst 43 phosphorylation sites were identified in murine JAK1 (32 novel and 11 known sites). Mutational studies are currently underway to screen a selection of evolutionarily-conserved candidate phosphorylation sites for functional relevance.

The authors would like to acknowledge Leanne Daly from Synkinase for supplying the KiNET-1 beads used in this study.


PROTEIN CLEAVAGE GENERATES FUNCTIONAL DIVERSITY ON THE SURFACE OF BACTERIAL PATHOGENS

Steven P Djordjevic1

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

Bacterial pathogens are known to post-translationally cleave large mass, surface molecules during maturation but the extent to which processing occurs on the cell surface has largely remained unexplored. We have been characterising post-translation cleavage events in cell surface proteins produced by several pathogenic mycoplasma species. Cell shaving and cell surface biotinylation protocols in combination with LC-MS/MS and 2D-PAGE have been used to generate comprehensive datasets that identify the complement of cell surface proteins and predict cleavage sites, such as the now well-characterised S/T-X-F↓X-D/E motif found in the P97 and P102 adenin families (1-7). Our complementary, orthogonal approaches show that adhesins, lipoproteins, non-classically secreted proteins, and proteins predicted by PSORTb to localise to the cytosol can be cleaved. To investigate the functional significance of protein processing, we have developed a series of affinity-capture protocols using heparin, fibronectin, plasminogen, actin and host epithelial cell surface antigens as bait (1-7). Cleavage fragments representing different regions of the same molecule have been shown to bind to different extracellular matrix components and host surface-associated molecules (1-7). Independent studies show that these interactions occur with physiologically relevant binding affinities suggesting that processing may be a mechanism to increase the repertoire of functionally diverse proteins on the cell surface of these genome-reduced pathogens.

3. Deutscher et al., 2012. Journal of Proteome Research. 11:1924
4. Seymour et al., 2012. Cellular Microbiology.14: 81
5. Raymond et al., 2013. Journal of Proteome Research 12:5891
PROTEOME-WIDE AND PROTEOFORM-SPECIFIC HOST CELL RESPONSES TO RESPIRATORY SYNCTIAL VIRUS INFECTION

Jeffrey J Gorman1,2, Keyur A Dave1, Emma L Norris1, Madeleine J Headlam1, Marcus L Hastie1, Keith Chappell, Alexander A Bukreyev2, Ursula J Buchholz2, Peter L Collins3, Toshna Singh3

1. Protein Discovery Centre, Queensland Institute of Medical Research, Herston, QLD, Australia
2. QIMR Berghofer, Herston, QLD, Australia
3. National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Human respiratory syncytial virus (hRSV) is the most serious cause of lower respiratory tract infection in infants, young children and immunocompromised individuals. There are no licensed vaccines or efficacious therapeutics to combat hRSV. Innate immune responses are initiated upon host cell detection of viral-specific molecular patterns, such as virus-specific RNA features. This detection system activates the production of immunomodulatory cytokines (induction), which in turn stimulate the production of antiviral protein effectors (signalling), some of which amplify the cytokine induction process. hRSV produces two small non-structural proteins (NS1 and NS2) which dampen the host cell innate immune system by targeting key players in both induction and signalling pathways. Therapeutic and vaccine developments to combat hRSV may emerge by gaining a better understanding of the interactions between hRSV NS1 and NS2 and host cell antiviral pathways.

We have developed a comprehensive suite of proteomic approaches to identify specific targets of hRSV NS1 and NS2. This includes workflows based on in-solution protein IEF of cell lysates with downstream bottom-up mass spectrometric label free quantification of the differences between hRSV-infected and uninfected cells. Another workflow involves bottom-up analyses of digests of unfractonated whole cell lysates combined with label free quantification. Our infectious experiments include hRSV encoding the complete wild-type genome and genotypes lacking NS1 and/or NS2 coding capacities. As a consequence we have identified key pathways that are promoted and/or blocked in order to achieve survival of the infected host cell in a state conducive to hRSV propagation. Although some observations were common to both proteomic workflows, the in-solution IEF workflow uniquely enabled observation of some regulation at a global level due to selective enrichment of low abundance proteins. Furthermore, the in-solution IEF workflow provided information on regulation at a proteoform level that was not evident on a global quantification level. Of particular interest were the observations that NS1 appears to directly suppress the activation of NF-κB1 and to promote the survival of pro-apoptotic proteins within the infected cell. Furthermore, it was evident that hRSV has tactics to suppress the innate antiviral responses of the infected cell independently of NS1 and/or NS2. The strengths and weaknesses of these workflows will be discussed along with how specific observations provide a better understanding of how hRSV subverts host cell antiviral pathways.

SECRETION OF BIOACTIVE COMPARTMENT-SPECIFIC NEUTROPHIL PROTEINS DISPLAYING A NEW TYPE OF GLYCOSYLATION IN PATHOGEN-INFECTED SPUTUM

Ian Loke1, Vignesh Venkatakrishnan1, Benjamin L. Parker2, Simone Diestel3, Nicki H. Packer3, Morten Thaysen-Andersen3

1. Macquarie University, North Ryde, NSW, Australia
2. Diabetes and Obesity Program, Garvan Institute of Medical Research, Sydney, NSW, Australia
3. Institute of Nutrition and Food Sciences, University of Bonn, Bonn, Germany

Unlike plants and invertebrates, vertebrates reportedly lack proteins displaying truncated N-linked glycosylation (paucimannosylation) with the monosaccharide composition mannose1,3:fucose2:1 N-acetylglucosamine2. Enabled by technology advancements in system-wide glycopeptide characterization (glycoproteomics), we document as the first that protein paucimannosylation is a significant host-derived molecular signature in neutrophil-rich sputum from pathogen-infected human lungs and is negligible in pathogen-free sputum. Six paucimannose N-glycans were carried by inflammation-associated proteins of human neutrophil origin including myeloperoxidase, azurocidin and neutrophil elastase that localized specifically to azurophilic granules. Paucimannose in vitro synthesis by human azurophil-specific β-hexosaminidase A, together with promyelocyte stage-specific expression of paucimannosylated proteins and their biosynthetic enzymes, indicated a novel spatio-temporal biosynthetic route in early neutrophil maturation. Exogeneous generation of paucimannose was excluded by absence of bacterial exoglycosidase activity or paucimannosidic glycans. P. aeruginosa induced virulence-dependent secretion of paucimannosidic proteins from isolated and sputum neutrophils. Interestingly, paucimannosidic proteins displayed virulence-specific bacteriostatic effects towards P. aeruginosa and showed affinities to mannan-binding lectin suggesting multiple immune-related functions of paucimannosylation in activated neutrophils.
THE METABOLIC SIGNALLING LANDSCAPE

David James¹, Ben Parker¹, Nolan Hoffman¹, Erik Richter¹, Rima Chaudhuri¹, Pengyi Yang¹
¹. Charles Perkins Centre, School of Molecular Biosciences, and Sydney Medical School, The University of Sydney, Sydney, NSW

Metabolic homeostasis is subject to major challenges particularly those imposed by starvation, diet and exercise. There has been considerable interest in dissecting the adaptations that occur in response to these kinds of perturbations and which of these are most likely to contribute to defects or constitute major points of regulation. As one approach toward addressing this challenge we have embarked upon global analysis of protein phosphorylation networks in either adipose tissue or skeletal muscle under different perturbation states including insulin stimulation, exercise or insulin resistance. This has unveiled the complex nature of these perturbations as well as a surprising heterogeneity in terms of the biological pathways that participate in metabolic homeostasis.

The details of some of these networks will be presented together with specific examples of some of the novel features that have emerged from this kind of research.

REVEALING PROTEOMIC AND METABOLOMIC CHANGES ASSOCIATED WITH LENS CATARACT FORMATION WITH IMAGING MASS SPECTROMETRY

Angus C Grey¹, Mitchell G Nye-Wood¹, Jeffrey M Spraggins², Richard M Caprioli², Kevin L Schey², Paul J Donaldson¹
¹. University of Auckland, Auckland, New Zealand
². Mass Spectrometry Research Centre, School of Medicine, Vanderbilt University, Nashville, TN, USA

The lens is a unique structure in the eye that focusses light onto the retina in order to form a sharp image, and has several adaptations to do this effectively. It lacks a blood supply, has an ordered cellular structure, and loses light scattering cellular organelles as lens fibre cells mature. Since it is a large avascular tissue, the lens requires a unique transport system that delivers nutrients and removes waste products from the lens centre. Breakdown of this system is thought to lead to lens protein modification and cataract formation, the leading cause of blindness worldwide. Cataract is characterised by opacities that form in distinct regions of the lens, and results from both genetic and environmental factors. Furthermore, biochemical studies into the aetiology of lens cataract have shown that metabolic changes associated with oxidative damage precede the proteomic changes that lead to cataract formation.

MALDI imaging mass spectrometry was utilised to map metabolomic and proteomic changes that lead to cataract formation in a laboratory model of age-related cataract. Bovine lenses were removed from the ocular globe and subjected to hyperbaric O₂ treatment to simulate age-related cataract. Lens cryosections were collected on MALDI targets, and matrix (9-aminoacridine or sinapinic acid) applied. MALDI imaging data sets were collected at 150-250mm spatial resolution using a MALDI-FTICR mass spectrometer (for metabolites) or a MALDI-TOF mass spectrometer (for proteins).

Hundreds of signals for small molecules were detected, including major lens antioxidants, nucleotides and purinergic signalling molecules. For proteomic studies, unmodified and modified forms of the abundant lens protein alpha crystallin were mapped. The effect of hyperbaric oxygen treatment on metabolite and protein distributions will be discussed. This study highlights the complex aetiology of lens cataract and may suggest novel targets for anti-catarract therapies.
TRANSLATION OF MEMBRANE PROTEOME INTERACTIONS INTO NOVEL COLORECTAL CANCER TARGETS

Mark S. Baker1, Charlie Anh1, David Cantor1, Harish Cheruku1, Abidali Mohamedali, Shoba Ranganathan1, Pierre Chapuis2, Charles Chan2, Owen Dent2, Ed Nice3

1. Australian School of Advanced Medicine and Dept of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW
2. Dept of Surgery/Diagnostic Pathology Unit, Concord Repatriation General Hospital, Sydney, NSW
3. Dept of Biochemistry & Molecular Biology, Monash University, Australia

Our past works have shown that integrin (avb6) and protease receptor (uPAR) expression are upregulated in colorectal cancer (CRC), allowing us to understand changes associated with the epithelial metastatic phenotype. Here, we show how immunoprecipitation allows identification of uPAR and avb6-interacting membrane proteins (i.e., metastasome) and downstream signaling events are linked. We also demonstrate how the metastasome is involved in regulation of cellular invasive characteristics, including the switch of TGFβ1 from a suppressive to an aggressive factor in late stage colorectal cancer. Analyses of the precise sites of protein interaction between uPAR and integrin avb6 using a plethora of methods, including peptide arrays, peptide competition assays, in silico modelling, inhibitory peptide (iPEP) studies and proximity ligation confirm the interaction and suggest interesting biologies that have implications for development of new therapies targeted at this interaction. These studies have developed lead iPEPs and are now focussed on small molecule compounds that antagonise this particular metastasome. Both lynchpin proteins (uPAR and avb6) are independently shown to be poor epithelial prognostic indicators, especially in Stage B rectal cancer. Despite this data, little is known regarding patient outcome when both factors are co-expressed. We propose a new model for how signal transduction “switches” require pre-formation of a precise membrane interactome composition. In addition, our recent rectal cancer study demonstrates that uPAR is expressed both in the stromal and/or epithelial compartments (presumably with different interactomes since avb6 is epithelialy-restricted) and that high expression in these different compartments results in opposite clinical survival outcomes (high epithelial rectal cancer uPAR levels poorer prognosis; high stromal expression better prognosis). We are exploring if the ratio of these uPAR measurements provides a better overall clinical prognostic indicator. Collectively, our data demonstrate combination(s) of clinical proteomics and cell biological methods result in deep, comprehensive membrane proteome understanding in cancer, providing a potential new CRC therapeutic target.

PROTEOMIC PROFILING OF AN INDUCIBLE MODEL OF ACUTE MYELOID LEUKAEMIA REVIEWS NOVEL INSIGHTS INTO LEUKAEMOGENESIS.

Jarrod Sandow1, Gabriela Brumatti1, Giuseppe Infusini1, Andrew Webb1, Paul Ekert1

1. Walter & Eliza Hall Institute, Parkville, VIC, Australia

The Mixed Lineage Leukemia (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 leukemias 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-translocation gene expression through quantitative proteome analysis using a leukaemia model. Our model of acute myeloid leukaemia (AML) is controlled by the regulated expression of a common MLL-translocation gene, MLL-AF9. Initially, surface captured proteins were analysed over a time course using a SILAC quantitative proteomics workflow and high-resolution mass spectrometry 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 entire leukaemia proteome during MLL-AF9 regulation. The results from these two techniques have not only confirmed several known targets in AML but highlighted novel pathways and targets that are upregulated when MLL-AF9 is expressed. These pathways include epigenetic regulators, cell signalling 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 biomarkers and novel drug targets.
FUNCTIONAL AND BIOCHEMICAL ANALYSIS OF HLA LIGANDS REVEALS THE MOLECULAR BASIS OF HLA ASSOCIATED ADVERSE DRUG REACTIONS.

Patricia Illing¹, Nicole Mifsud¹, Nathan Croft¹, Nadine Dudek¹, Jamie Rossjohn¹, Julian Vivian¹, Anthony Purcell¹

1. Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

Adverse drug reactions (ADRs) cost the Australian health care system over $660 million annually and complicate the use and public trust of key pharmaceuticals. In recent years numerous associations between immune mediated ADRs and specific Human Leukocyte Antigen (HLA) alleles have been identified. These molecules are cell surface glycoproteins responsible for the presentation of peptides, derived from the breakdown of both endogenous and exogenous proteins, at the cell surface for immune surveillance. In health, constitutive HLA-peptide complexes are inert, whilst HLA molecules in complex with novel or foreign peptides (e.g. those derived from invading pathogens) may stimulate a T cell response. We recently defined the mode of interaction of the anti-retroviral drug abacavir with HLA-B*57:01, the allele associated with the potentially lethal ADR, abacavir hypersensitivity syndrome. Using a combination of mass spectrometry and structural biology, we showed that abacavir bound within the peptide-binding groove of HLA-B*57:01 altering the selection of self-peptides, causing significant perturbation of the immunopeptidome (the peptides displayed by the HLA), and presenting T cells with countless novel, immunogenic, HLA-B*57:01-abacavir-self-peptide complexes¹. Here, we extend these studies to other ADRs and use mass spectrometry (targeted and data dependent acquisition methods) and T cell activation assays to monitor drug induced changes in the immunopeptidome and the immunogenicity of ADR associated HLA molecules. Recent data on the application of these techniques to investigate interactions between allopurinol (and its metabolites) and HLA-B*58:01, implicated in allopurinol hypersensitivities such as the severe cutaneous reactions Stevens-Johnson syndrome and Toxic Epidermal Necrolysis, will be discussed.


TALES FROM PROTEIN TAILS: THE PROTEASE WEB DECIPHERS THE N TERMINOME

Chris Overall¹

1. University of British Columbia, Vancouver, BC, Canada

Biological systems are complex and so model systems leading to models to predict human biological processes are required for mechanistic understanding of physiological pathways in all biological events and responses, signaling pathways and their perturbations in disease, and drug target validation. For example, interactions of proteases and inhibitors through protein cleavage and inhibition play crucial roles in the regulation of biological systems. Well studied as classically described protein cascades e.g. the complement system and coagulation, or newer recognized cascades of kallikreins in skin and caspases in apoptosis, these interactions have been proposed 10 years ago to expand pervasively in a network termed the protease web.

We compiled large-scale data of protease web interaction and expression to investigate the regulatory potential of the protease web. Using graph theory to represent the protease web of annotated cleavage and inhibition information, we investigated connectivity in the protease web. We identified a pervasive human protease web, where interactions between protease classes and cascades are common with protease inhibitors, in particular serpins, often forming network hubs. The model describes how proteases can influence the cleavage of many more proteins than their direct substrates through protease activation pathways or inactivation of protease inhibitors. Our model successfully predicted a perplexing proteolytic pathway in vivo, where activities of MMP8 and neutrophil elastase are linked by an inactivating cleavage of serpin A1 by MMP8 that we experimentally validated. We also found that protein interaction databases frequently contain protease interactions, in particular inhibitions, and improved our model of the protease web. Degradomics analysis using TAILS N-terminomics allowed us to dissect proteolysis in skin inflammation and demonstrated the interconnectivity of the protease web and new roles for proteases in vivo. By removing serpin blocks, MMPs flip the “Metallic Serpin Switch” and so dynamically regulate serine protease cascades by altering the balance between intact inhibitory serpins and inactivated cleaved serpins.

Our findings supply systematically derived and validated evidence for the existence of the protease web, where interactions are potentially broad but are fine tuned by expression and localization of interactors leading to specific interactions in- and outside cells and in specific tissues.
CHARACTERISATION OF THE PEPTIDE AND PROTEIN CONTENT OF ANT VENOMS FOR USE AS BIOINSECTICIDE AND ANTIMICROBIAL LEADS

Samira Alli1, Axel Touchard2, Matt Padula1, Alain Dejean3, Jerome Orivel1, Pierre Escouzas2, Graham Nicholson1

1. University of Technology, Sydney, UTS, Broadway, NSW, Australia
2. VenomeTech, 473 Route des Dolines, Valbonne, France
3. CNRS, UMR Ecologie des Forêts de Gayane (EcoFoG), 97379 Kourou Cedex, France

Animal venom peptides are currently being developed as novel drugs and bioinsecticides. Given that ant venoms are used for predation and defence, their venom is a potential source of these peptides. Also, venomous ants are an untapped source of these lead compounds as they remain extremely understudied. Initially, the protein/peptide components of seven different ant species were characterised. 1D and 2D gels of the venoms revealed proteins ranging from <10 kDa to >250 kDa. NanoESI-QTOF-MS/MS analysis confirmed the presence of common venom proteins and many undescribed proteins. C18 reverse-phase (RP) HPLC separation followed by LC-MALDI-TOF MS of the venoms was then undertaken, this revealed considerable heterogeneity in the HPLC and mass profiles of the five venoms. After optimisation of the MALDI matrices, it was found that venoms contained between 144–1032 peptides. Most of these peptides lie between 1–5 kDa. Disulfide-bonded peptides were also identified in all ant venoms (range 2–28) using the reducing MALDI matrix, 1,5-DAN. Insecticidal activity of whole venom was determined using house crickets. Of the four venoms tested thus far, three venoms showed significant paralytic and lethal activity. Bioassay-guided fractionation using cation exchange and RP-HPLC of three venom currently being undertaken to identify insecticidal neurotoxins.

The antimicrobial activity of venoms and toxins is also presently being investigated using MIC (minimum inhibitory concentration) assays with both gram positive (Staphylococcus aureus) and gram negative bacteria (Escherichia coli) to identify potential anti-microbial agents. In conclusion, these venoms represent a vast and untapped source of potentially novel bioactive drug and insecticide leads.

A NOVEL, ULTRASENSITIVE APPROACH FOR QUANTITATIVE CARBOHYDRATE COMPOSITION AND LINKAGE ANALYSIS USING NANOLC-ESI ION TRAP TANDEM MASS SPECTROMETRY

Kathirvel Alagesan1, Daniel Varón Silva2, Peter H Seeberger2, 1, Daniel Kolarich2

1. Institute for Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany
2. Max Planck Institute for colloids and Interfaces, Berlin, Germany

Abstract

Introduction Glycosylation is one of the most complex yet common post translational modifications, which drastically enhances the functional diversity of proteins and influences their biological activity. Identification and characterisation of glycans is an important step in correlating glycan structure to its biological function. Structural assignment of glycans (glycomics) and their compositions based on MS analyses is often based on taking well studied glycosylation pathways for granted. Nevertheless, many monosaccharide building blocks are indistinguishable by mass alone and detailed linkage information is also not easily obtained by MS/MS analyses. In particular when studying glycosylation from less well studied organisms such as prokaryotes, monosaccharide analysis is indispensable to profile the composition of complex carbohydrates present in glycoconjugates.

Methods Carefully designed sequential permethylation and reductive amination steps prior and after acid hydrolysis enable separation and differentiation of the various monosaccharides and their respective linkage positions. Separation of various derivatised monosaccharides was achieved using Reversed Phase – Liquid Chromatography (RP-LC)-ESI-MS/MS. In addition, absolute quantitation is accomplished including a set of internal standards, thus providing qualitative and quantitative information on the monosaccharide residues present in a specific sample.

Results Various derivatised monosaccharides alditols were identified based upon their retention time along with mass spectrometric detection. The resulting fragment ions observed in the MS/MS spectra arise from the cleavage of methyl groups are diagnostic for the each substitution pattern. These differences allowed establishment of ‘fragmentation pattern’ for these derivatised monosaccharide alditols. Furthermore, we have established fragmentation pattern library for various biologically important N-glycans for automated linkage analysis.

Conclusion The method reported herein has a limit of detection of ≤ 250 fmol for all the monosaccharide analysed and is sensitive to as low as 12 pmol of initial analyte, which now allows to perform both, glycomics and monosaccharide analyses from low µg amounts of initial glycoprotein. The present method reported here does provide more confidence in correlating the structure and stereochemistry of monosaccharides present in any glycoconjugates, thereby increasing the confidence in glycan structure assignments obtained from glycomics experiments.

Novel Aspect The methodology reported describes for the first time, a simple and sensitive method using Reversed Phase – Liquid Chromatography (RP-LC)-ESI-MS/MS for unambiguous identification and linkage determination of various monosaccharides (including N-acetylglucosaminic acids).
EVALUATING THE RELATIONSHIP BETWEEN N-GLYCOSYLATION AND PROTEIN STABILITY IN CAMPYLOBACTER JEJUNI

Joel JA Cain1,2, Nicholas NE Scott1,2, Nestor N Solis3,1,2, Melanie MY White1,2, Stuart SJ Cordwell1,2,4,5
1. Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia
2. School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia
3. University of British Columbia, Vancouver, BC, Canada
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. C. jejuni was the first prokaryotic organism found to possess an N-linked protein glycosylation system. Approximately 100 C. jejuni proteins to date have been shown to be targets of this post-translational modification1,2, and this system has been shown to be a crucial for pathogenicity albeit through an as yet undefined mechanism. Here, we employed iTRAQ-based labelling to determine the effect of either loss of the oligosaccharyltransferase (ΔpglB), or biosynthesis of the glycan (ΔpglDEF) on whole protein abundance in a relatively recent clinical isolate, C. jejuni strain JH11. Of the 1077 C. jejuni proteins quantified, only 57 were deemed to have a significant change in abundance in either of the Δpgl strains relative to the wild-type isolate. A large proportion of known glycoproteins were quantified, with ~17% displaying an altered abundance in the N-glycosylation negative strains.

N-terminal amine isotopic labelling of substrates (N-TAILS3) was also employed for pair wise comparisons of the N-degradome of wild-type JH11 and individual pgl knock out strains to address the hypothesis that the addition of the N-linked glycan may provide protection from proteolytic degradation for the largely unstructured regions of the protein on which they're commonly attached. We were able to identify and quantify 4122 unique N-termini from 766 C. jejuni proteins. From those derived from known N-linked glycoproteins, a number were found to be in close proximity to or contained the sites of N-linked glycosylation and in turn displayed a significant difference in their relative abundance in the Δpgl mutants. These proteomics-based approaches were complemented with various standard phenotypic tests to establish if the observed effects of loss of N-glycosylation extended to broader changes in C. jejuni’s physiology.


TGFB-1 INCREASES CELL PROLIFERATION, WOUND HEALING AND INDUCES CHANGES TO MEMBRANE PROTEOMES IN COLONECTRAL CANCER CELLS WITH VARYING INTEGRIN B6 EXPRESSION

Harish R Cheruku1, David I Cantor1, Abidali Mohamedali2, Sock-Hwee Tan3, Seong B Ahn1, Edouard C Nice3, Mark S Baker3
1. Australian School of Advanced Medicine, Macquarie University, North Ryde, NSW, Australia
2. Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW, Australia
3. Monash University, Clayton, Victoria, Australia

It is well understood that Transforming Growth Factor-β (TGFβ) signalling is a potent regulator of cell growth, differentiation, migration and tumour suppression. However, during colorectal cancer (CRC) and other cancers TGFβ mRNA and protein show marked increases are associated with tumour growth. The involvement of TGFβ as a tumour promoter is poorly understood. We hypothesise that the increased expression of TGFβ can be achieved by high expression levels of integrin αvβ6, which can activate latent-TGFβ and MMP-9 both of which are activators of latent-TGFβ. High expression of integrin αvβ6 has been observed in various cancers including CRC. Cell proliferation and wound healing assays showed significant differences when treated with active TGFβ. To gain more in-depth understanding of role of TGFβ, αvβ6 and uPAR in CRC we compared the membrane proteome of TGFβ1 (10ng/mL) stimulated SW480 cells with none or overexpressing integrin αvβ6 using iTRAQ. We identified 2666 proteins, out of which 510 proteins were from the plasma membrane. The IPA analysis with significantly altered proteins revealed cellular assembly and organization, cell-to-cell signalling and interaction as the top-ranked altered biological function. Ingenuity pathway analysis also showed RAN and EIF2 signalling were amongst top 5 canonical pathways identified. We identified various proteins including integrins (α3β1, α4β1, α6β1, α5β1, αv), ERK1/2, TGFβ and MAPK1/2 through IPA network analysis. All these molecules have been previously implicated in various cancers including CRC. The results show that TGFβ signalling is enhancing the ability of the cancer cells towards progression and metastasis. Therefore, it is very important to study the TGFβ associated protein-protein interactions during CRC which will serve to identify novel key players for metastasis, and serve as biomarkers and/or drug targets to improve CRC therapy.
COMPREHENSIVE CHARACTERIZATION OF PHOSPHORYLATION SITES OF NEWCASTLE DISEASE VIRUS PROTEINS BY A MULTITUDE OF FRAGMENTATION TECHNIQUES

Keyur A Dave, Buddhika A Jayakody, Jeffrey J Gorman
1. Protein Discovery Centre, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

Paramyxoviruses are a family of negative stranded RNA viruses whose members comprise of serious human pathogens, such as measles virus, mumps virus and respiratory syncytial virus; and in animals, such as Newcastle disease virus (NDV) and rinderpest virus. Apart from its potential economic impact on the poultry industry worldwide, NDV is becoming a promising agent for oncolytic virotherapy of mammalian cancers.

For productive infection viruses must hijack the host cellular machinery as they cannot self-reproduce. To avoid elimination they have to weaken host antiviral responses but at the same time utilize the host intracellular trafficking and biosynthetic systems to enter and exit infected cells while producing new viral particles. These variegated interactions expose viral proteins to numerous host modifications. In addition, cellular localization of these interacting host proteins indicates intracellular trafficking behaviour of the viral proteins during the infectious cycle.

In this pursuit, we have extensively characterized phosphorylation sites of the avian paramyxovirus-1, NDV, proteins by using an array of fragmentation techniques on several advanced mass spectrometry (MS) platforms. This includes analysis of unfraccionated and phosphopeptide enriched digests from lysates of purified virions by ion trap (IT) CID with multi-stage activation, IT-ETD, IT-EThCD, IT-EThCD, Orbitrap (OT)-HCD and IT-HCD. These multitudes of fragmentation techniques on different MS platforms have provided common and unique phosphopeptide identifications in NDV proteins. Importantly, diverse and distinctive peptide backbone cleavage favoured by different fragmentation techniques has enabled unequivocal assignment of phosphosites detected in NDV proteins.

QUANTITATIVE PROTEOMIC ANALYSIS OF CABERNET SAUVIGNON GRAPE CELLS EXPOSED TO THERMAL STRESSES REVEAL ALTERATIONS IN SUGAR AND PHENYLPROPANOID METABOLISM

Iniga S George, Dana Pascovici, Mehdi Mirzaei, Paul A Haynes
1. Macquarie University, Sydney, NSW, Australia
2. Australian Proteome Analysis Facility (APAF), Macquarie University, Sydney, NSW, Australia

Grapes (Vitis vinifera) are a valuable fruit crop and wine production is a major industry. Global warming and expanded range of cultivation will expose grapes to more temperature stresses in future. We investigated quantitative shotgun proteomic changes induced by temperature stresses on Cabernet Sauvignon cells to provide insights into metabolic pathways related to temperature stress in grapevines. Our study examined proteomic changes induced by the impact of four different temperature stress regimes, including both hot and cold temperatures, on cultured grape cells. Cabernet Sauvignon grape cell suspension cultures grown at 26°C were subjected to 14 hours of exposure to 34°C and 42°C for heat stress, and 18°C and 10°C for cold stress. Cells from the five temperatures were harvested in biological triplicates. Proteins were extracted using methanol-chloroform and digested by filter aided sample preparation (FASP) protocol [2]. Proteins were identified with a Velos-Pro linear ion-trap mass spectrometer by gas phase fractionation (GPF) [3], and spectra were searched against UniProtKB Vitis vinifera database and quantified by spectral counting. A total of 1755 non-redundant proteins were identified from the five temperature points. Ninety-eight proteins were identified exclusively in extreme heat stress (42°C) and ninety-five proteins were identified exclusively at extreme cold stress (10°C).

Gene Ontology (GO) annotations of differentially expressed proteins provided insights into the metabolic pathways that are involved in temperature stress responses in grape cells. Sugar metabolism displayed switching between alternative and classical pathways during extreme temperature stresses. Nine proteins involved in the phenylpropanoid pathway were highly up-regulated at extreme cold stress, and numerous other proteins involved in phenylpropanoid metabolism and anthocyanin synthesis were found to be cold-responsive proteins. This is the first label-free shotgun proteomic study on grapes exposed to hot and cold temperature stress.

UNDERSTANDING OVINE SKIN PROPERTIES USING MALDI IMS AND NANO-LCMS

Meekyung Ahn1, Santanu Deb Choudhury2, Catherine Maidment1, Geoff Holmes Holmes1, Gill Norris3

1. Leather and Shoe Research Association, Palmerston North, Manaw, New Zealand
2. AgResearch, Christchurch, New Zealand
3. IFS, Massey University, Palmerston North, New Zealand

New Zealand produces large quantities of sheep pelts, which are largely used to make garment leather. Leather from conventionally processed sheep skins is not strong enough for footwear. Therefore, converting them to footwear leather would not only increase their raw material value but also be of more benefit to the NZ sheep leather industry as it targets the more stable footwear market rather than that of fashion-dependent clothing.

Although much research has been carried out into the physical structure and arrangement of collagen fibers/fibrils of leather and the effects of this on strength, the chemical characteristics and molecular composition of leather is less well understood.

It is known that raw sheep skins are strong but that their strength is lost in the process of leather making, which removes unwanted skin protein components in order to achieve desired properties, especially softness and flexibility in the leather. Using a proteomics approach would allow us to identify structural proteins in raw sheep skin. Comparing proteins from raw sheep skin to pickled sheep skin will give us information on what proteins have been lost during the leather process.

Here, we describe a survey of the identified skin proteome in raw and pickled sheep skins using a gel approach based on chip based nano-LC-MS/MS (Agilent Q-TOF 6520) and MALDI IMS (Autoflex III, Bruker). In the former method, solubilised skin proteins were separated by conventional 1D-gel electrophoresis prior to trypsin digestion. Trypsin digested peptides were then separated by nano-LC and identified using TPP (trans-proteomics pipeline), searching against the mammalian NCBI database. Protein matches were retained less than 5% FDR with at least two different unique peptides. The second method was an on-tissue approach applying trypsin directly on to the tissue sections. This is a powerful technique for mapping biological molecules such as endogenous proteins and peptides as it detects molecular species present and visualizes their distributions in a single tissue section. The two techniques combined provide us with high confidence in any identified proteins.

LC-MS TIMESCALE INTACT PROTEOFORM PROFILING

Nicolai NB Bache1, Pierre-Olivier POS Schmit2, Christian CR Ravnsborg1, Ole OV Vorm1

1. Bruker, Odense, Denmark
2. Bruker Daltonics, Bremen

All along their life cycle, proteins undergo various transformations that can alter their functions while keeping a good part of their primary sequence intact. These multiplication of PTM patterns, alternative splicing forms or products of proteolytic processing cannot be simply resolved with a bottom-up approach, as very few peptides are specific from the given proteoform. However, the information relative to the distribution of different proteoforms is encoded in their intact masse. Being able to catch this information efficiently will drive the biologist into a new analytical dimension, far beyond the traditional gel-based approaches.

Bruker has an history with the analysis of intact proteins, whatever it is with NMR, X-ray Crystallography, Infrared or MS, accessing all structural information, from the primary to the quaternary structure. Bruker’s UHR ESI-Q-TOF systems are now capable of delivering accurate mass and isotopic pattern information for hundreds of protein in a mixture within minutes. In the exemple we present, the combination of dynamic range, broadband transmission and sensitivity makes it possible to resolve several hundreds of proteins from a E.Coli and yeast Lysates in less than 20 minutes. The Dissect™ and SNAP™ algorithms enable to extract the information of co-eluting proteins and retrieve the monoisotopic mass from any isotopically resolved protein, therefore enabling to reveal the proteoforms distribution patterns. First insights in the quantitative capabilities of the approach will be illustrated.
N-TERMINAL PROTEIN SEQUENCES HIGHLIGHT THE EXTENT OF PROTEOLYTIC PROCESSING IN PATHOGENIC BACTERIA

Iain J Berry1, Jess L Tacchi1, Veronica M Jarocki1, Benjamin B A Raymond1, Matt P Padula2,1, Steven P Djordjevic1

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

Proteolytic cleavage is a ubiquitous post-translational modification of proteins, responsible for protein signalling, activation, localisation and ultimately degradation. Due to a variety of experimental limitations this important physiological process has been largely understudied, particularly in prokaryotes and archaea. Proteases and proteolytic processing have been linked to virulence in a variety of infectious diseases. Therefore experimental investigation of proteolytic cleavage is needed to determine the extent and functional roles of protein processing in the production of mature proteins.

The primary focus of this study is to sequence the N-terminal sequences of mature protein products of prokaryotic pathogens, and to characterise the resulting mature protein fragments. N-terminal sequence data can be used to identify true protein start sites and any downstream post-translational processing. The genome-reduced agriculturally-important pathogen *Mycoplasma hyopneumoniae*, was selected as a model organism for these studies. There is a large body of literature demonstrating extensive proteolytic processing of many highly expressed adhesins which are critical for pathogenesis.

Using dimethyl labelling of protein amine groups, including native N-termini, the data obtained from mass spectrometry analysis has provided evidence confirming the start sites of protein translation and complementary data pinpointing the exact sites of proteolytic cleavage. In addition, the data indicates that an unprecedented number of cytosolic proteins are targets for post-translational processing on the cell surface of pathogenic bacteria. Many of these proteins have been described as “moonlighting” proteins in the literature and have been implicated in pathogenesis in other organisms.

HYDROLYSIS OF MALEIMIDE–PEPTIDE ADDUCTS REDUCES SENSITIVITY WHEN INTERROGATING PROTEOMES FOR THIOL OXIDATION

Amber E Boyatzis1, Scott Bringans2, Matthew J Piggott3, Richard Lipscombe2, Peter G Arthur3

1. University of Western Australia, Crawley, WA, Australia
2. Proteomics International, Perth, Western Australia, Australia
3. The University of Western Australia, Crawley, WA, Australia

Oxidative stress, caused by reactive oxygen and nitrogen species (RONS), is widely recognized as important in both the pathogenesis and subsequent pathophysiological sequelae of many diseases, including diabetes, cancer, and muscular dystrophy. A key target of RONS is the thiol group of protein cysteine residues. As thiol oxidation can affect protein function, mechanistic information about how oxidative stress affects tissue function can be ascertained by identifying oxidized proteins.

The most popular proteomics-based method for interrogating protein cysteine oxidation is via the alkylation of thiols using iodoacetamide chemistry, as in the OxICAT adaptation of the ICAT kit. However, this method is suboptimal for assessing the labile oxidative modification of cysteine residues as iodoacetamide chemistry has been shown to result in incomplete, non-specific, and slow alkylation of protein thiols.

Alkylation by maleimide chemistry is complete, highly selective, and rapid. It is therefore preferred to iodoacetamide. However, we find that in a standard proteomic workflow, significant hydrolysis of the maleimide adducts (succinimides) occurs with associated +16 m/z and +18 m/z peaks in the mass spectrum.

Indeed, when losses during a protein sample preparation workflow were tracked using 1D gel electrophoresis and MALDI-TOF mass spectrometry, it was found that up to 89% of the alkylated peptide can be hydrolysed during sample preparation. Gel electrophoresis alone resulted in an average 6.4% ± 3.9% (n = 3, mean ± SEM) hydrolysis; coomassie staining of gels 25% ± 6.8%, tryptic digests of gel bands 17% ± 21%; extraction of peptides from gel bands 13% ± 2%; and biotin/streptavidin purification 30% ± 2%. The cumulative loss of signal for the peptide adduct at the expected m/z is so large that it can it to fall under the limit of detection, leading to incomplete profiling of the redoxome. Strategies to minimize succinimide hydrolysis will be presented.
THE ANALYSIS OF BEAD-BASED MULTIPLEX IMMUNO-ASSAYS FOR ACCURATE DISCOVERY OF CYTOKINE, CHEMOKINES, AND GROWTH FACTORS EXPRESSION WITHIN PLASMA

Edmond J Breen¹, Alamgir Khan¹

1. APAF, North Ryde, NSW, Australia

Small proteins found in plasma such as cytokines, chemokines and growth factors are involved in aging, disease pathogenesis, embryonic development, non-specific response to infection, specific response to antigens, and changes in cognitive functions. In addition, such analytes have roles in stem cell differentiation, vaccine efficacy and allograft rejection. Often the expression of these analytes are obtained from bead based multiplex immunoassays such as Luminex’s xMAP® technology. Software systems such as xPONENT® from Luminex and Bio-Plex® Manager™ output tabulated sheets containing observed concentration values, fluorescent values and standards and expected concentration values as well as background corrected values for test, controls, standards and blanks samples. However, from such systems much of the life scientist’s test data can end up classified as being out-of-range leaving him or her with the problem of working with sparse and unbalanced data that in turn reduces their power of discovery and analysis. We have developed new approaches to the analysis of bead-based immunoassays that can reduce the number of out-of-range values by 50%. We show that basing the analysis on raw and mean fluorescent values is often a better choice than relying on the derived concentrations values obtained from commercial software systems. We show how to analyse and to normalize the test data to compensate for plate-to-plate variations, how to check the reliability of test samples even if all or most of the concentration data is deemed out-of-range, and we show how to get improved estimates of analyte concentrations. In this study we use the expression of 14 analytes, using 6 commercially available kits, across 177 patients, recorded at two time points and the associated analyte standards, controls and blanks. In total 60 micro titre plates are used resulting in 4965 readings. We show that the common practice of subtracting analyte blanks from test samples has no rational basis. We argue for our analysis of protein expression that the fluorescent values are a better choice than absolute concentration values. The significance of this work for the life scientist means higher statistical power and even lowers experimental costs.

MALDI IMAGING MASS SPECTROMETRY OF N-LINKED GLYCANS ON FORMALIN-FIXED PARAFFIN-EMBEDDED MURINE KIDNEY

Matthew Briggs¹, OJ Gustafsson¹, MR Condina², LJ Winderbaum¹, M Pelzing², SR McColl³, AV Everest-Dass⁴, NH Packer⁴, P Hoffmann³

1. Adelaide Proteomics Centre, School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA
2. Bruker Pty. Ltd., Preston, Vic
3. Chemokine Biology Laboratory, School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA
4. Faculty of Science, Biomolecular Frontiers Research Centre, Macquarie University, Sydney, NSW

Recent developments in spatial proteomics have paved the way for retrospective in situ mass spectrometry (MS) analyses of formalin-fixed paraffin-embedded clinical tissue samples. This type of analysis is commonly referred to as matrix-assisted laser desorption/ionization (MALDI) imaging. Recently, formalin-fixed paraffin-embedded MALDI imaging analyses were augmented to allow in situ analyses of tissue-specific N-glycosylation profiles. In the present study, we outline an improved automated sample preparation method for N-glycan MALDI imaging, which uses in situ PNGase F-mediated release and measurement of N-linked glycans from sections of formalin-fixed murine kidney. The sum of the presented data indicated that N-glycans can be cleaved from proteins within formalin-fixed tissue and characterized using three strategies: (i) extraction and composition analysis through off-target MALDI MS and liquid chromatography coupled to electrospray ionization ion trap MS; (ii) MALDI profiling, where N-glycans are released and measured from large droplet arrays in situ; and (iii) MALDI imaging, which maps the tissue specificity of N-glycans at a higher resolution. Thus, we present a complete, straightforward method that combines MALDI imaging and characterization of tissue-specific N-glycans and complements existing strategies.
IMPROVEMENTS IN 2D GEL ELECTROPHORESIS FOR BIOMARKER DISCOVERY IN PLASMA PROTEINS

Stuart Brown¹, Gillian Norris¹

1. Massey University, Palmerston North, NZ, New Zealand

Publish consent withheld


A LABEL-FREE MULTI-OMIC STUDY OF A GLUCOSYLCERAMIDE INHIBITOR EFFECTS ON OBESITY

Lee Gethings¹, John Shockcor², Robert Beynon³, Johannes Aerts⁴, Christopher Buck⁵

1. Waters Corporation, Manchester, UK
2. Waters Corporation, Milford, MA, USA
3. Institute of Integrative Biology, University of Liverpool, Liverpool, UK
4. Dept of Medical Biochemistry, University of Amsterdam, Amsterdam, Netherlands
5. Waters Australia, Burnsside, VIC, Australia

Obesity is one of the risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. It has previously been demonstrated that glucosylceramides play a crucial part in such metabolic syndromes. The manipulation of the function of glucosylceramides with small molecule drug compounds within mouse models has shown that symptoms can be negated. Knowledge relating to the proteome, metabolome and lipidome during development is still to be fully explored. The work presented here is to provide a multi-omic analysis of protein and lipid liver extracts from control and obese mouse models undergoing treatment to prevent or revert obesity.

IMPROVED HDX WORKFLOW FOR ON-LINE DIGESTION, SEPARATION AND DATA ANALYSIS

Asish Chakraborty1, Ying Qing Yu1, Michael Eggertson1, Keith Fadgen1, Weibin Chen1, Christopher Buck2

1. Waters Corporation, Milford, MA, USA
2. Waters Australia, Burnside, VIC, Australia

Hydrogen Deuterium Exchange (HDX) experiments on proteins can reveal detailed information about protein tertiary structure (folding/packing) and quaternary structure (protein-protein interactions). Several key components of a Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS) workflow include reproducible sample preparation, on-line digestion, chilled chromatography, MS and dedicated informatics tools. Each of these components have been improved in recent years and in this study we demonstrate optimizations in microscale chromatography that improve both pepsin digestion efficiency and the resolution of the reverse phase peptide separation. The practical results are improved protein sequence coverage and more reproducible HDX experiments.


OVEREXPRESSION OF AVB6 INTEGRIN ALTERS THE COLORECTAL CANCER CELL PROTEOME IN FAVOUR OF ELEVATED PROLIFERATION AND A SWITCHING IN CELLULAR ADHESION WHICH INCREASES INVASION

David Cantor1, Iveta Slapetova2, Alison Kan1, Leon McQuade2, Mark S. Baker1

1. Macquarie University, Sydney, NSW, Australia
2. University of New South Wales, Sydney
3. Australian Proteome Analysis Facility, Sydney

Many proteins enhance cancer progression towards life-threatening metastases. These include linking proteins called integrins which mediate cell adhesion to the extracellular matrix (ECM), consequently altering both function and phenotype. Specific neoexpression of the β6 integrin subunit correlates with the epithelial-to-mesenchymal transition, metastasis and with poor overall patient survival. Whilst β6 is implicated in these processes, exactly how it affects signaling and/or proteolytic pathways in metastasis remains unclear. A membrane-enriched peptide IPG-IEF shotgun proteomics study was undertaken comparing subclones of the SW480 CRC cell line transfected with a vector inducing unregulated β6 integrin overexpression against the ‘empty’ mock vector control cell line. β6 overexpression induced a significant change in 708 proteins found localised across most intracellular locations, some involving cellular processes and pathways underpinning cancer progression. β6 expression increased cell proliferation 4-fold, while decreasing cell adhesion to many integrin-ECM substrates. β6 expression also enhanced cell invasion and promoted the expression/repression of many established cancer-related pathways.
CHARACTERISATION OF MONOCLONAL ANTIBODY DRUG CANDIDATES AND BIOSIMILARS USING MASS SPECTROMETRY

Natasha Care\textsuperscript{2,1}, Matthew Fitzhenry\textsuperscript{2,1}, Mark P Molloy\textsuperscript{2,1}, Xiaomin Song\textsuperscript{2,1}
1. APAF, Sydney, NSW, Australia
2. Macquarie University, Sydney, NSW, Australia

The selective and specific recognition properties of antibodies are fundamental to many applications in biomedical research. The use of monoclonal antibodies (mAbs) as therapeutic drugs has proved to be highly successful, and as patent rights run out, an increasing number of biosimilar antibodies are being developed.

The structural characterisation of mAbs is one essential requirement for their commercialisation. Mass spectrometry (MS) is a proven analytical tool for proteomics and allows the sensitive and high resolution characterisation of proteins, including mAbs.

In our work, we have applied an array of MS techniques to characterise several commercially available therapeutic mAbs. To first achieve baseline resolution of an IgG mAb, we performed intact protein mass measurement on a high resolution mass spectrometer. The relative abundance of glycans present on the antibody heavy chain was then determined using a mAb-Glyco chip LC-MS system (Agilent Technologies). To characterise the primary structure the antibody was reduced and alkylated then digested using three different enzymes (Trypsin, Glu C, Lys C) and microwave assisted acid hydrolysis. The acquired peptide MS/MS data was matched against the predicted peptide fragments for the antibody heavy and light chains. The combined results from each enzyme digestion achieved close to 100% sequence coverage for both the heavy and light chains. Disulfide bridges were determined by digestion with trypsin or GluC/Trypsin, followed by LC ESI MS/MS. A theoretical disulfide bond peak list was generated using in-house software and MasterView (AB Sciex) was used to screen the LC ESI MS/MS data and identify peptides with disulfide bonds.

This workflow demonstrates how MS can be used to generate accurate and comprehensive structural information for biomedically relevant mAbs.

ISOLATION AND IDENTIFICATION OF ENTEROCOCCUS FAECALIS MEMBRANE PROTEINS USING MEMBRANE SHAVING AND ONE-DIMENSIONAL SDS-PAGE COUPLED WITH MASS SPECTROMETRY.

Peter Cathro, Peter McCarthy, Peter Hoffmann, Peter Zilm

Background: Enterococcus faecalis is a significant nosocomial pathogen which is able to survive in diverse environments and resist killing with antimicrobial therapies. The expression of cell membrane proteins play an important role in how bacteria respond to environmental stress. As such, the capacity to identify and study membrane protein expression is critical to our understanding of how specific proteins influence bacterial survival. Here we describe a combined approach to identify membrane proteins of E. faecalis ATCC V583 using membranes fractionated by either 1D-SDS-PAGE or membrane shaving, coupled with LC-ESI mass spectrometry.

Results: Two hundred and two membrane-associated proteins were identified which represents approximately 24 percent of the predicted membrane-associated proteome. 170 were isolated using 1D-SDS-PAGE and 68 with membrane shaving, with 36 proteins being common to both techniques. Ninety seven percent of the proteins identified by membrane shaving were membrane associated with the majority being integral membrane proteins (89%). The majority of proteins identified with known physiology are involved with transportation across the membrane.

Conclusion: The combined 1D-SDS-PAGE and membrane shaving approach has produced the greatest number of membrane proteins identified from E. faecalis to date.
CHARACTERISATION OF THE INTERACTION OF HETERODIMERIC AVB6 INTEGRIN WITH UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR)

Seong B Ahn¹, Samyuktha Anand², Abidali Mohamedali², Harish Cheruku¹, Debra Birch², Gopichandran Sowmya², David I Cantor³, Shoba Ranganathan⁴, David W Inglis⁵, Ronald Frank⁶, Miachael Agrez⁷, Edouard C Nice⁸, Mark S Baker⁹

1. Australian School of Advanced Medicine, Macquarie University, North Ryde, NSW, Australia
2. Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW, Australia
3. Department of Engineering, Macquarie University, North Ryde, NSW, Australia
4. Department of Chemical Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany
5. Division of Surgery, John Hunter Hospital, Newcastle, NSW, Australia
6. Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

Urokinase plasminogen activator receptor (uPAR) and the epithelial integrin αvβ6 play critical roles in cancer metastasis. Importantly, both these interacting molecules have been implicated in epithelial-mesenchymal transition (EMT) changes that facilitate escape of metastatic cells from tissue barriers. This study aims to characterise the uPAR•αvβ6 in ovarian and colon cancer cells.

The study uses orthogonal in cellulo and in vitro approaches to characterise the uPAR•αvβ6 interaction on OVCA429 (ovarian cancer cell line) and four different colon cancer cell lines with modified β6 subunit expression (SW480Mock, SW480β6OE, HT29Mock and HT29β6AS). Proximity ligation assays (PLA) was used to confirm the previously identified uPAR•αvβ6 interaction site. Peptide arrays were used to identify the specific sites of interaction which also validates our PLA studies. In silico structural analysis was then used to identify the most likely uPAR•αvβ6 interaction sites based on the peptide array data. PLA study confirmed the uPAR•αvβ6 interaction in our cell lines. PLA studies were further validated using peptide arrays to identify potential physical sites of uPAR•αvβ6 interaction and as well as interactions with other known uPAR partners (e.g., uPA and vitronectin) and individual integrin subunits (i.e., αv, β1, β3 and β6). Our data suggests that interaction with uPAR requires expression of the complete αβ heterodimer rather than either individual subunits (i.e., αv, β1, β3 or β6). Finally, from the in silico structural analysis, it appears the most likely unique site/s of interaction of αvβ6 with uPAR are located in uPAR domain II and domain III. Functional consequences of disrupting uPAR•αvβ6 as a novel therapeutic target to ablate metastasis are explored. (Pubmed ID: 25318615)

CHARACTERIZATION OF AN IMPROVED ULTRA HIGH RESOLUTION QUADRUPOLE TIME OF FLIGHT (UHR-Q-TOF) INSTRUMENT FOR PROTEOMIC APPLICATIONS.

Pierre-Olivier SCHMIT⁴, Mark Condina⁵, Annette Michalsky⁶, Stephanie Kaspar⁷, Markus Lubeck⁷, Christoph Gebhardt⁷, Oliver Raether⁷

1. Bruker Daltonics, Wissembourg, France
2. Bruker Pty Ltd, Preston
3. Bruker Daltonik GmbH, Bremen

In shotgun proteomics it is desirable to identify and quantify a large number of individual peptides from complex samples, such as tryptic digests of human plasma samples or whole cell lysates 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.

To test the impact of these modifications on proteomics performance, different complex tryptic digests (Lysates of Escherichia coli, Saccharomyces cerevisiae, human plasma) were mixed with stable isotope labeled peptides or digests of standard proteins at known concentrations, spanning a range of several orders of magnitude. Samples were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to with nano-UHPLC MS/MS on the modified UHR-TOF. For peptide identification and quantitative analysis the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)).

For higher sensitivity at fast acquisition speed, ion extraction from the collision cell into the orthogonal acceleration of the TOF-analyzer was improved by using a novel collision cell design was used. Increased resolution without changing the effective flight path could be achieved with a modified reflectron. In addition, a faster detector (reduced width of individual ion scans) was used. Using an optimized detector digitizer combination, a threefold higher dynamic range was observed. However in complex samples, the dynamic range is also limited by the capability of the instrument to resolve nearly isobaric compounds. The performance improvements were analyzed in a label-free quantification experiment, evaluating in particular the number of quantifiable peptides over the entire dynamic range. As a defined model system for complex proteomics samples, a mixture of 48 standard proteins spanning a concentration range of five orders of magnitude (Universal Proteomics Standard, UPS-2, Sigma) was spiked into samples of 500ng E. coli, S.Cerevisiae (higher complexity) or human plasma (wider dynamic range) digests at a concentration of 1:2. During separation in with two hour gradients, the UPS-2 peptides could be quantified based on the MS full scans at levels from 500fmol down to the low attomole range. Similar analyses with background samples of higher complexity (S. cerevisiae) and wider dynamic range (human plasma) were carried out to further evaluate the benefit of the novel hardware features.
DISCOVERING NEW METABOLIC PATHWAYS INPROTOZOAN PARASITES USING STABLE ISOTOPE LABELLED METABOLOMICS

Darren J Creek1, Rainer Brietling2, Malcolm J McConville3, Dominique Soldati-Favre4, Michael P Barrett5
1. Monash University, Parkville, VIC, Australia
2. University of Manchester, Manchester, UK
3. Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Vic, Australia
4. University of Geneva, Geneva, Switzerland
5. University of Glasgow, Glasgow, UK

Metabolomics is the global measurement of metabolites in a biological system. Established metabolomics methods allow relative quantitation of a broad range of metabolites, providing a snapshot of the metabolic state of the system under investigation. However, these methods do not reveal the connectivity of metabolites or the flux through metabolic pathways. A combination of high resolution untargeted metabolomics with stable-isotope labelling allows a system-wide analysis of metabolic flux and the subsequent discovery of active metabolic pathways within a cell.

The protozoan parasites *P. falciparum* and *T. brucei* are the causative agents of malaria and human African trypanosomiasis, respectively. These parasites salvage many nutrients from the host, or from the medium in cell culture experiments, but also possess numerous active metabolic pathways.

U-13C-glucose labelling in cell cultures, combined with high-resolution mass spectrometry-based metabolomics, allowed direct observation of the fate of glucose utilization in these protozoan pathogens. According to published models, *T. brucei* was thought to have a streamlined glucose metabolism, limited to glycolysis. However, our approach demonstrated incorporation of glucose-derived carbon into over 100 metabolites, revealing the presence of novel active metabolic pathways in these organisms.

Extensive post-glycolytic metabolism was also observed in *P. falciparum*, and stable isotope-labelled metabolomics analysis of knockout lines revealed a novel activity for the enzyme putatively annotated as branched chain keto-acid dehydrogenase (BCKDH) linking glycolysis to the TCA cycle.

Stable isotope labelling coupled with untargeted metabolomics enabled system-wide evaluation of active metabolic pathways in protozoan pathogens. Several novel pathways and enzyme activities were identified in *T. brucei* and *P. falciparum*, allowing significant extensions to previous models of glucose metabolism. This experimentally-derived, systems-based depiction of the active metabolic networks in these pathogens will assist in the elucidation of potential drug targets for the treatment of parasitic diseases.

DISCOVERY OF VIRULENCE FACTORS IN GIARDIA DUODENALIS HOST-CELL INTERACTIONS

Samantha J Emery1, Mehdi Mirzaei1, Daniel Vuong2, Joel M Chick3, Ernest Lacey3, Paul A Haynes1
1. Macquarie University, North Ryde, NSW, Australia
2. Microbial Screening Technologies Pty. Ltd., Smithfield, NSW, Australia
3. Harvard Medical School, Boston

*Giardia duodenalis* is a protozoan parasite and major contributor worldwide of diarrhoea and gastroenteritis in vertebrates, including humans. Giardiasis is characterised by proliferation of trophozoites in the small intestines, where parasites attach to the gut mucosa via a ventral disk resulting in infection pathology. Our experiment quantifies protein changes in trophozoites exposed to host soluble factors (HSF) from HT-29 intestinal epithelial cells (IEC), compared to the co-incubation with the cell-monolayer itself. This host-cell co-incubation permits attachment of trophozoites to host cells. All treatments, including the control, were incubated in serum-free DMEM for 6 hours at 37°C in 5% CO2. Two complementary quantitative approaches; Tandem Mass Tags (TMT) labelling and label-free based on spectral counting approach were performed to quantify the differentially expressed proteins of biological triplicates between conditions. We identified a total of 1650 proteins, of which a small subset of 21 up-regulated and 14 down-regulated proteins were reproducibly differentially expressed, including up-regulation of known and putative virulence factors. Cathespin B precursor, a confirmed immunomodulatory protein, was up-regulated in trophozoites exposed to HSF, but was not changed during co-incubation with host cells. Similarly, VSP antigenic switching was increased in trophozoites incubated with HSF compared to co-incubation. Several signalling proteins were up-regulated, in particular the Tenascins, during incubation with HSF.

These results indicate that induction of virulence factors from several important protein families occurs independent of trophozoite attachment to host cells. Significantly, we observed equivalent up-regulation, in both HSF-exposed and co-incubated trophozoites, of the *Giardia duodenalis* cystatin homologue and protease inhibitor, which may represent a novel pathogenicity factor in *Giardia*. 
A PLATFORM FOR THE STRUCTURAL CHARACTERIZATION OF GLYCAN ENZYMATICALLY RELEASED FROM GLYCOSPHINGOLIPIDS EXTRACTED FROM TISSUE AND CELLS

Merrina Anugrah1, Arun Everest-Dass1, Nicki Packer1

1. Macquarie University, NSW, Australia

Glycosphingolipids (GSLs) constitute a highly diverse class of glyco-conjugates which are involved in many aspects of cell membrane function and disease. The isolation, detection and structural characterization of the carbohydrate (glycan) component of GSLs are particularly challenging given their structural heterogeneity and thus rely on the development of sensitive, analytical technologies. In this study, neutral and acidic GSL standards were immobilized onto polyvinylidene difluoride (PVDF) membranes and glycans were enzymatically released using Endoglycoceramidase II (ECase II), separated by porous graphitized carbon (PGC) liquid chromatography and structurally characterized by negative ion mode electrospray ionization mass spectrometry (PGC-LC-ESI-MS/MS). This approach was then employed for GSLs isolated from 100 mg of serous endometrioid cancer tissue and from cell line (107 cells) samples. Glycans released from GSL standards comprising of ganglio-, asialo-ganglio- and the relatively resistant globo-series glycans, using as little as 1 mU of enzyme and 2 μg of GSL. The platform of analysis was then applied to GSLs isolated from tissue and cell line samples and the released isomeric and isobaric glycan structures were chromatographically resolved on PGC and characterized by comparison with the MS2 fragment ion spectra of the glycan standards and by application of known structural MS2 fragment ions. This approach identified several (neo-)lacto-, globo- and ganglio-series glycans and facilitated the discrimination of isomeric structures containing Lewis A, H Type 1 and Type 2 blood group antigens and sialyl-tetraosylceramide. We describe a relatively simple, detergent-free, enzymatic release of glycans from PVDF-immobilized GSLs, followed by the detailed structural analysis afforded by PGC-LC-ESI-MS/MS, to offer a versatile method for the analysis of tumour and cell-derived GSL-glycans. The method uses the potential of MS2 fragmentation in negative ion ESI mode to characterize, in detail, the biologically relevant glycan structures derived from GSLs.

YBX1 MEDIATES PARTIAL EMT TO PROMOTE TUMOURIGENICITY REGULATED BY THE EXTRACELLULAR ENVIRONMENT

David Greening1, Shashi K Gopal1, Rommel A Mathias1, Hong Ji1, Hong-Jian Zhu2, Richard J Simpson1

1. Department of Molecular Science, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Vic
2. Department of Surgery, The University of Melbourne, Melbourne, Vic

Epithelial-mesenchymal transition (EMT) describes a morphogenetic program which confers mesenchymal cell properties such as reduced cell-cell contact and increased cell migration and invasion, to epithelial cells. Here we investigate the role of the pleiotropic transcription/splicing factor and RNA-binding protein nuclease-sensitive element-binding protein 1 (YBX1/YB-1) in inducing EMT and increasing the oncogenic potential of epithelial MDCK cells. Stable expression of YBX1 in MDCK cells (MDCK\textsuperscript{YBX1}) induced partial EMT, including cytosolic relocalization of E-cadherin, increased cell scattering, and anchorage-independent growth. A critical feature of this study was the observation that MDCK\textsuperscript{YBX1} cells were pro-oncogenic and established tumour xenograft growth, in contrast to parental MDCK/ MDCK\textsuperscript{YBX1} cells. We further sought to identify the molecules that may confer these properties, and used an in-depth proteomic approach (UPLC Orbitrap Elite, MaxQuant workflow) to profile and quantitate (label-free quantitation) the cell lines from \textit{in vitro} cell culture (cellular and secreted molecules), as well as the \textit{in vivo} MDCK\textsuperscript{YBX1} cell tumour xenograft directly. We reveal that YBX1 regulates the extracellular environment through expression levels of secreted/ extracellular proteins. The increased tumourigenicity of MDCK\textsuperscript{YBX1} cells correlated with the identification of elevated levels of secreted factors (TGF-β, CSF-1, NGF, and VGF) and proteases (ADAM9 and ADAM17), known to enhance angiogenesis and cancer progression. Treatment of recipient 2F-2B endothelial cells with MDCK\textsuperscript{YBX1} cell conditioned medium containing both soluble proteins and extracellular vesicles increased their motility. These findings contribute to our understanding of how YBX1 as an oncogenic enhancer mediates partial EMT in epithelial cells and promotes tumour angiogenesis via the elevated release of a new suite of secreted molecules.
DISCOVERY OF PROTEIN LYSINE METHYLTRANSFERASES THAT ACT ON TRANSLATION ELONGATION FACTOR EF1α IN SACCHAROMYCES CEREVISIAE

Joshua J Hamey1, Gene Hart-Smith1, Marc Wilkins1
1. University of New South Wales, Sydney, NSW, Australia

Lysine methylation is a post-translational modification with emerging proteome-wide functions. Like its counterpart, arginine methylation, it is known to affect protein-protein interactions, and this has implications for its role in modulating the dynamics of protein-protein interaction networks. To uncover the mechanisms by which lysine methylation modulates protein-protein interactions, it is essential to discover and characterise the enzymes which catalyse methylation. Through mass spectrometry-based screening of knockouts of four putative yeast protein methyltransferases, we have discovered three novel lysine methyltransferases that methylate the translation elongation factor EF1α – YGR001C, YNL024C and YLR285W. YGR001C trimethylates lysine 79, YNL024C monomethylates lysine 390 and YLR285W is responsible for the di- and tri-methylation of two novel sites – lysines 3 and 5. All three methyltransferases belong to the seven-beta-strand family of methyltransferases, with YGR001C being previously putatively assigned as a N(6)-adenine-specific DNA methyltransferase. N6AMT2, the human homolog of YGR001C, is likely to also be a lysine methyltransferase, as lysine 79 is known to be trimethylated in human EF1α. The structural contexts of the modifications suggest that they may have distinct roles in modulating the function of EF1α. Besides its canonical role in facilitating delivery of tRNA to the ribosome, EF1α is known to have a number of so-called ‘moonlighting’ functions – including regulating the actin cytoskeleton and export of tRNA – which are facilitated by its numerous protein interaction partners. Understood in this context, it is likely that these methyltransferases serve to control which of the many protein-protein interactions EF1α partakes in, and thereby have proteome-wide functional implications.

DETERMINATION OF RAB GTPASES

Franziska C. Hundt, Marina Wotske, Dirk A. Wolters

Isoprenylation is a form of posttranslational modification (PTM), which constitutes the attachment of isoprenoid groups to cysteine of the C-terminus of proteins. Two forms of isoprenylation are distinguished: Three isoprenoid units form the farnesyl group (C15H25) with a mass of 205 Da, whereas the geranylgeranyl group (C20H33) represents four isoprenoid units with a mass of 273 Da. Prominent examples of isoprenylated proteins are the Ras and Ras-related proteins. Like other small GTPases, these proteins change between two notable conformational stages: the active membrane-associated GTP- and the inactive cytosolic GDP-form. In this context, the isoprenylation serves as a membrane anchor and allows the interaction between isoprenylated Ras and Ras-related proteins with their effector proteins.

All three isoforms of the Ras protein are farnesylated, and the members of the Rho protein superfamily are geranylgeranylated. The Ras-related superfamily of Rab proteins takes an exceptional position: Rab proteins receive two geranylgeranyl moieties from the Rab-geranylgeranyltransferase (Rab-GGTase). After double geranylgeranylation, Rab is integrated into the cell membrane and serves as a molecular switch in intracellular trafficking. Several Rab proteins are aberrantly expressed in tumors but no Rab-specific drugs have been developed yet. Because of the essential isoprenylation for correct localization and function of Rab, inhibitors of Rab-GGTase have been found. Nonetheless, isoprenylated proteins are low-abundant in complex cell samples. This is why it is extremely difficult to detect them with mass spectrometry, despite sensitive measuring devices. Therefore, enrichment strategies are indispensable.

We have established an elutropic series by using liquid chromatography-mass spectrometry (LC-MS), which discriminates farnesylated, singly and doubly geranylgeranylated peptides. Furthermore, we investigated characteristic gas phase reactions of isoprenylated peptides during ionization and fragmentation. These results pave the way for future studies on e.g. prenyltransferase inhibitors without further enrichment steps.

A SYSTEMATIC LABEL-FREE QUANTITATIVE APPROACH TO DISCOVER NOVEL SUBSTRATES OF E3 UBIQUITIN LIGASES.

Giuseppe Infusini¹, Pauline Y Huang², Alan C Ching², José A Villadangos²

1. Proteomics Laboratory, Walter and Eliza Hall Institute, Parkville, VIC, Australia
2. Biochemistry and Molecular Biology, The University of Melbourne, Parkville, VIC, Australia

Untargeted label-free quantitative proteomics has become an increasingly powerful methodology that can provide vital quantitative information, either relative or absolute, on large parts of the expressed proteome. Contrarily to label-based methodologies, label-free quantitation is applicable to any kind of sample and, in principle, has no limitation on the number of samples that can be compared. These advantages make label-free quantitative proteomics the method of choice for analysis of clinical samples, primary cells and tissues.

In this study we set out to identify novel substrates for the family of Membrane-associated RING-CH (MARCH) E3 ubiquitin ligases in vivo. Regulation of cell surface receptors is essential for the maintenance of the cell proteostasis, and ubiquitination at the plasma membrane has been observed to be a fundamental post-translational mechanism regulating a wide variety of surface proteins, including immune receptors. The MARCH ligases are a subfamily of the RING E3 ligases originally identified in viruses to be involved in immune evasion strategies. Numerous in vertebrate studies suggest that the MARCH ligases’ main role is to regulate trafficking, surface expression and turnover of immune receptors, however most of their substrates remain unknown.

In order to identify novel surface targets of the MARCH ligases we developed a plasma-membrane enrichment strategy from primary antigen presenting cells (DCs), purified from wild-type and MARCH-deficient mouse spleens. Quantitative analysis of LC-MS/MS data was performed using MaxQuant for peptides/proteins identification and for feature detection, while methods for the quantitative analysis and statistics were developed in house. Through this approach we were able identify potential novel targets for most of the MARCH ligases as well as to confirm previously characterised ones. With this work we have established a robust methodology to systematically identify potential targets of ubiquitin ligases in vivo.


COMPREHENSIVE PROTEIN SEQUENCE DATABASES AND THE ADVENT OF PERSONALIZED SEQUENCE DATABASES

Eugene Kapp¹

1. WEHI, Parkville, VIC, Australia

“Which sequence database should I use for my proteomics analysis?” is a question that is perhaps not asked often enough. Reference protein sequence databases (such as Ensembl, UniProtKB, NCBIinr or LudwigNR) all have their own advantages, disadvantages and quirks. Based on 3 different proteomic applications from protein characterisation, pathogen detection and OMICS integration (RNASeq and Proteomics), I will illustrate the advantages and disadvantages of these publically available reference sequence databases and hopefully shed some light and provide insight into the questions that should be asked, which ultimately of course depends on the experiment at hand and the biological questions being asked. More recently, the integration of datasets sourced from different technologies (such as RNASeq and Proteomics) has become a reality. Based on 2 different projects I will share some insight into the benefits and methodology involved in facilitating this integration and of course the pitfalls associated with this task.
SIALIC ACID INVOLVEMENT IN BACTERIAL BINDING TO DIFFERENT BODY FLUIDS

Liisa Kautto¹, WY Cheah¹, C-H Lin¹, J Grinyer¹, MDP Wilcox², NP Packer¹

1. Biomolecular Frontiers Research Centre, Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW
2. School of Optometry and Vision Science, University of NSW, Sydney, NSW

One of the key initial processes involved in bacterial infection is the attachment to host cell receptors, typically glycans (sugars) conjugated to proteins or lipids on epithelial cell surfaces. Accordingly, many pathogens have developed adhesins or lectins that attach to specific glycans epitopes characteristic of the host cells they target. In turn, we propose that the host provides an ingenious innate defence mechanism that uses the glycoproteins in their secreted fluids to competitively bind to the bacterial pathogens to prevent infection.

Our research has demonstrated that bacteria bind differentially to glycan moieties on secreted glycoproteins from human tears, milk, saliva and sweat, potentially providing decoys for selective adhesion and clearance of pathogens as the secretory fluids wash the epithelial cell surface. Here we demonstrate the differential involvement of sialic acid in the adhesion of pathogenic and commensal bacteria to secreted glycoproteins in these human secretions. Our techniques include a 96 well plate bacterial adhesion assay in which the glycoproteins bind to the PVDF membrane in the wells through hydrophobic interactions, leaving the glycan molecules exposed on the membrane surface. The presented glycans are then probed with fluorescently-labelled bacteria and bacterial binding quantified using a fluorescent plate reader. The importance of terminal sialic acid in the bacterial–secreted glycoprotein interaction was determined by detecting any change in the bacterial binding following neuraminidase treatment.

The work demonstrates a natural mechanism by which sialic acid contributes to the attachment of pathogenic bacteria to the glycoproteins secreted in a diverse range of body fluids and suggests there is a glycan-mediated protective process against infection in all human secretions.

PLASMA BIOMARKERS FOR THE DETECTION OF HUMAN GROWTH HORMONE ABUSE IN SPORTS

Sock Hwee Tan¹, Albert Lee¹, Vita Birzniece², Ken Ho², Mark Molloy¹, Alamgir Khan¹

1. Australian Proteome Analysis Facility, North Ryde, NSW, Australia
2. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Human growth hormone (hGH) is a naturally occurring, 191 amino acid peptide hormone secreted by the pituitary gland with anabolic and growth-promoting activity. Since the increased availability of rhGH in the late 1980s for the treatment of hGH-deficient patients, rhGH has become a drug of abuse in sport and it is prohibited by the World Anti-Doping Agency (WADA). Currently, there is no method available that can clearly discriminate between GH user and nonuser due to difficulties in direct differentiation between native and recombinant iso-forms of hGH, short half-life in circulation, and not enough biomarkers reported. We have identified new GH-dependent plasma biomarkers by two-dimensional difference gel electrophoresis (2-D DIGE) and Isobaric tags for relative and absolute quantitation (iTRAQ) analyses which have longer half-life than rhGH itself. We have validated the identities of candidate markers by using orthogonal methods such as western blotting and selected reaction mass spectrometric (SRM) analysis. By combining bio-markers reported by others and new markers that we have identified in our work may provide us an opportunity to develop a better rhGH abuse detection method in sports or monitoring changes in plasma proteome after GH-administration in clinical trials.
REDOX MODIFICATIONS OF CYSTEINE IN THE LIVER OF TYPE 2 DIABETES MELLITUS

Desmond K Li¹, Lauren E Smith², Joel A Cain¹, Jana Paulech¹, Stuart J Cordwell¹,², Melanie Y White¹,²

1. School of Molecular Bioscience, The University of Sydney, Camperdown, NSW, Australia
2. Discipline of Pathology, School of Medical Sciences, The University of Sydney, Camperdown, NSW, Australia

Reactive oxygen species (ROS) and associated redox modifications have normal physiological roles in signalling but are also implicated in a number of pathologies. Oxidative stress associated with type 2 diabetes mellitus (T2DM) has been attributed to increased generation/reduced clearance of ROS in insulin sensitive tissues including the liver¹. During oxidative stress, proteins undergo redox modifications through the thiols of cysteine, which may alter structure, functionality and signalling pathways. To determine proteins of the liver which undergo redox modifications with T2DM, we investigated a number of associated metabolic disorders using redox proteomics. Rats were subjected to a high fat diet (HFD) to induce peripheral insulin resistance, as well as streptozotocin (STZ) to create β cell dysfunction, thereby generating the T2DM pathology. Rats treated with only HFD or STZ were utilised to analyse the pre-diabetic state. To enrich low abundance redox modified peptides, thiol disulfide exchange was utilised for reversibly modified cysteines² with quantitation by iTRAQ. Strong cation exchange was used to select for irreversibly modified cysteines³, combined with label-free quantitation. Enriched and non-captured fractions were subjected to 2D-LC to increase depth of coverage before identification by tandem mass spectrometry. Using this approach, the TripleTOF 5600 (AB Sciex) identified 8038 redox-modified sites in the liver proteome, with over 2223 sites quantified in response to T2DM (HFD+STZ). Oxidative modifications to proteins which maintain redox balance suggests dysregulation, contributing to oxidative stress in T2DM. Observed changes in enzymes involved in glucose metabolism suggest a role of redox modifications in glucose utilisation/production. Modifications to enzymes in fatty acid metabolism correlate with the hyperlipidemia associated with T2DM. Many proteins showed increased levels of irreversible oxidative modifications contributing to protein damage. Changes in the redox status of these proteins could be indicative of these protein species prone to oxidative damage, contributing to pathogenesis or a level of cellular regulation in response to the metabolic disturbance.


TOWARD A BETTER UNDERSTANDING OF GLYCOSYLATION MACHINERY BY SUBCELLULAR PROTEOMICS AND GLYCOMICS

Chi-Hung Lin¹, Jenny HL Chik¹, Nicolle H Packer¹, Mark P Molloy¹,²

1. Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia
2. Australian Proteome Analysis Facility, Macquarie University, Sydney, NSW, Australia

Publish consent withheld
PAUCIMANNOSYLATION IN HUMAN NEUTROPHILS: INSIGHTS INTO THE BIOSYNTHESIS AND IMMUNOLOGICAL ROLE OF A NOVEL TYPE OF PROTEIN N-GLYCOSYLAION

Ian Loke¹, Vignesh Venkatakrishnan¹, Nicki Packer¹, Morten Thaysen-Andersen¹
¹. Macquarie University, North Ryde, NSW, Australia

The biosynthetic pathway of mammalian protein asparagine (N)-glycosylation is known to produce three classes of N-glycans of the high mannose, hybrid and complex type that are abundantly expressed on glycoproteins. A fourth class of N-glycosylation known as paucimannosylation, defined as the addition of a full or partial chitobiose core to asparagine residues, is considered to be a glycosylation feature of invertebrates. However, recent literature has reported the presence of paucimannosidic proteins in humans and in mice during inflammation-related conditions, suggesting a potential immunological link to these novel glycoproteins in mammals. Given the importance of neutrophils in innate immunity, we here perform an in vitro based investigation of the biosynthesis and potential immunological functions of protein paucimannosylation in human neutrophil-like HL60 cells. Moderate to strong co-localisation of paucimannosidic epitopes, β-hexosaminidase A (Hex A) and azurophilic granule resident myeloperoxidase using immunofluorescence indicates involvement of Hex A in the synthesis of protein paucimannosylation, suggesting an azurophilic-specific localisation of paucimannosidic proteins in neutrophils. Additionally, HL60 and P. aeruginosa co-culture and the use of mass spectrometry showed that the azurophilic compartments of the neutrophil can be mobilised upon virulent stimuli, resulting in a secretion of paucimannosidic proteins into the extracellular milieu. Finally, we show that human proteins carrying these novel glycoepitopes display significant bacteriostatic activities towards specific P. aeruginosa strains under biologically relevant concentrations, as shown by the effect of purified paucimannosidic proteins on bacteria growth. Taken together, we here provide evidence supporting functionally important, previously un-described, roles of protein paucimannosylation in human neutrophils.

BIOINFORMATICS APPROACHES TO THE PROTEOMICS OF SECRETED PROTEINS IN ARABIDOPSIS THALIANA

Andrew Lonsdale¹
¹. ARC Centre of Excellence in Plant Cell Walls, School of Botany, University of Melbourne, Parkville, VIC, Australia

METALLOPROTEOMIC PROFILE OF NATIVELY PURIFIED ALPHA SYNUCLEIN FROM BLOOD AND BRAIN, INCLUDING THE IDENTIFICATION OF THE ASSOCIATED PROTEIN TRUNCATIONS BY MASS SPECTROMETRY.

Amber Lothian¹, Alan Rembach¹, Catriona A McLean², Robert A Cherny¹, Malcolm Horne¹, Roberto Cappai³, Colin L Masters⁴, Blaine R Roberts¹

1. The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia
2. National Trauma Research Institute, The Alfred Hospital, Melbourne, Victoria, Australia
3. Department of Pathology and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria, Australia

Alpha synuclein is an abundant neurological protein that has been implicated in a number of neurodegenerative diseases. It has been proposed that the mechanism by which this protein elicits its effects in these diseases is through metal-protein interaction causing conformational changes which results in aggregation and oxidative stress. However the exact pathological role of alpha synuclein is yet to be elucidated and the interactions between it and metals have only been shown using recombinant proteins. Most recombinant protein techniques lack the machinery for incorporating post-translational modifications common to eukaryotic proteins, including chaperones for delivering copper. To investigate the metal status of native alpha synuclein we have purified it from both human erythrocytes and human brain tissue using non-denaturing techniques. Using size exclusion chromatography coupled to inductively coupled plasma – mass spectrometry we were able to directly determine the metal status of alpha-synuclein. Results indicated that alpha synuclein does not bind significant amounts of copper, iron or zinc, even when these metals were added to the protein in excess. In addition to this we used intact mass spectrometry to identify alpha synuclein, which was present in different truncated forms in the blood and the brain. Based on these results native alpha synuclein purified using these techniques is not a metalloprotein and can is present in multiple truncated versions in the blood and brain.

DETECTION OF OXIDATIVE MODIFIED PROTEINS IN RICE PLANT

Nyuk Ling Ma¹

1. University Malaysia Terengganu, Kuala Terengganu, TRG, Malaysia

Plant is sessile as it is constantly being exposed to environment stresses. These stresses can cause the build-up of reactive oxygen species (ROS) in plant cell that will cause oxidative damage on the DNA, lipid and also proteins. There are two types of oxidative modified protein, one being reversible and another one could lead to permanent damage to the plant cell. The reversible proteins also usually act as signalling proteins. Hence, detection and identification of these proteins is prime target for crop improvement. In this study, 5-Iodoacetamidofluorescein (5’IAF) that competes with hydrogen peroxide was used to identify oxidative modified cysteine proteins following the application of oxidative stress. This approach enables the identification of six groups of proteins that regulate the plant stress mechanisms. The six groups of proteins include L-ascorbate peroxidase redox protein, S-adenosylmethionine synthase and cysteine synthase that regulate amino acid metabolism, salt stress root protein RS1, photosynthesis proteins and seed storage proteins. The interaction of the key proteins observed in this study with the downstream of metabolites in combination with other phenotypes will provide a full picture of the role of these proteins in stress adaptation. The findings provide useful information as to production of stress tolerance plant in the future.
PREDICTING PROTEIN ABUNDANCE BASED ON MASS SPECTROMETRY USING MACHINE LEARNING

Shiyong Ma¹, Jason Wong¹

1. Prince of Wales Clinical School and Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW, Australia

Motivation:

Protein quantification has long been an interesting and paramount area in proteomics field. MaxQuant, currently one of the most commonly used softwares for protein quantification, employs state-of-the-art peptide intensities based methods. Using state-of-the-art protein quantification methods such as iBAQ, it has been shown that the accuracy of protein quantification is dependent on its abundance such that highly abundant proteins can be much more accurately quantified compared with low abundance proteins. Other peptide ion intensities based quantification methods have the same problem. (Wilhelm, et al. 2014)

Protein peptide mass spectrometry intensities have been verified to be correlated with its protein abundance and chemical and biophysical features (Scherbart, et al. 2009). In this study, we develop a machine learning to model the relationship between chemical and biophysical peptide features and its protein abundance in an attempt to improve MS-based protein quantification accuracy.

Results:

Machine learning models including Support Vector Machine and Artificial Neural Networks (including Deep Learning Models) with various topologies was developed and used to model a set of 690 chemical and biophysical features including AAIndex features (Kawashima, et al 2008) associated with each peptide.

First, our machine learning models are trained on AQUA data of U2OS (4490 proteins), and the pearson correlation coefficient R between our predicted protein abundance using cross validation and AQUA abundance is substantially higher than that between AQUA and iBAQ abundance (R\textsubscript{Pearson} increases from 0.806 to 0.90578). This indicates our developed protein and peptide feature set can assist improving the accuracy of calculating protein abundance via machine learning models.

Second, we compared our peptide feature-based protein abundance prediction model with MaxLFQ (Cox, et al. 2014), using, a dataset consisting of proteins spiked in with known ratios of SILAC labelled protein. Our model was trained using a set of proteins where the SILAC ratio accurately corresponded with the expected ratio. This model was then applied in predicting abundance of all the proteins (4446 proteins) in the dataset. To date, the predicted protein abundances also shows the potential of outputing protein abundance ratio distribution better than MaxLFQ and our research is continuing for further optimizing the performance of the models.

Conclusion:

Our study shows that by considering peptide features when computing protein abundance estimation from MS-based data the accuracy of the measurement can be improved. This has important implications particularly in label free protein quantification and studies to correlate gene expression and protein abundance.


A PROTEOMIC INVESTIGATION FOR DETECTION OF EARLY STAGE CRC BIOSIGNATURES

Sadia Mahboob¹, S B Ahn¹, Abidali Mohamedali², Mark S Baker¹

1. Australian School of Advanced Medicine (ASAM), Macquarie University, NSW
2. Macquarie University, NSW, Australia

The current methods widely deployed for colorectal cancer (CRC) screening are grossly inadequate on sensitivity and specificity ground. Cancer specific biomarkers are found in low concentrations (pg- ng/mL) in plasma as a result of structural changes in the microenvironment of cancer tissues followed by dilution in plasma. This study was designed to meet the longstanding unmet clinical need for cancer screening and surveillance using minimally invasive techniques. Expression of 92 potential biomarkers were measured from clinically stage CRC (Duke’s A, B, C, D) and control (i.e., unaffected) EDTA plasma samples utilizing PEA based Proseek® Multiplex Oncology I kit. A duplicate set of samples were analysed by Bio-Plex Pro™ human cytokine 27-plex immunoassay kit. Expression of CEA (a diagnostic biomarker for CRC) was found to be significantly high in malignant stage while IL 8 and prolactin had significant level of expression between control, benign and malignant stages. Additional experiments are being conducted on the same set of plasma using commercially available MARS 14 followed by in house immune based depletion system. The aim is to detect low abundance novel biomarkers using state-of-the-art LC-MS instrumentation housed in Australian Proteome Analysis Facility. In addition, uHPLC based analysis is being conducted on these samples to identify any fluctuation trend in kynurenine pathway in various cancer stage. The outcomes from this study will be followed up with a subsequent investigation where expression of 17 new proteins will be measured by PEA technology as well as statistical correlation will be established between the expression and clinical data. We believe these findings will enable to establish new, improved and volume-sparing plasma biomarkers/biomarker signature panels. The detailed results will be presented at the upcoming Lorne Proteomics conference which would specifically provide useful information to researchers about a longstanding unmet clinical need for early stage CRC detection.
A WORKFLOW TO IDENTIFY TARGETS OF THE E3 UBIQUITIN LIGASE NEDD4 INVOLVED IN CONTROLLING NEURAL CREST CELL FATE.

Peter McCarthy¹, Sophie Wisniak², Roger Daly², Oded Kleinfeld², Quenten Schwarz¹

1. Center for Cancer Biology, University of South Australia, Adelaide, SA, Australia
2. Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

Advances in diglycine remnant affinity enrichment techniques have seen ubiquitylation studies enjoy a significant amount of attention in recent proteomic research. Here we report our findings from experiments aimed at identifying targets of the HECT E3 ubiquitin ligase, Nedd4. We have previously shown Nedd4 plays a critical role in neural crest cell development. Our studies in Nedd4⁻/⁻ mice reveal striking defects in the development of neural crest cell derived tissues, including craniofacial and cardiac. In combination with in vitro data, these studies implicate Nedd4 as an important factor in the maintenance of neural crest cell identity, survival and/or stemness. To begin addressing how neural crest cells are affected by loss of Nedd4, we needed a workflow to identify targets. To this end, we have carried out siRNA knockdown of Nedd4 in a SILAC labeled neural crest cell line and used a diglycine remnant enrichment strategy to reveal diglycine peptides that are less abundant following Nedd4 knockdown compared to controls. We compare these data to total protein abundance data gathered from samples by LC-MS/MS prior to diglycine remnant enrichment, to discern changes specifically in diglycine peptide abundance from changes in total protein. And finally, we cross check our proteomic data to expression data obtained from the same experimental model to better distinguish whether changes in protein abundance arise from changes in stability or from changes in gene expression. Taken together, we present a workflow for the identification of protein targets of E3 ubiquitin ligases and reveal a number of interesting and novel putative Nedd4 targets.

SPECTRAL LIBRARIES FOR SWATH: ESTABLISHING A COMPREHENSIVE DATABASE FOR CANCER RESEARCH

Matthew McKay¹, Mark Molloy¹, Christoph Krisp¹

1. Australian Proteome Analysis Facility, Macquarie University, NSW, Australia

SWATH represents an important development for establishing a comprehensive LC/MS based quantitative strategy for cancer based proteome projects. The approach currently relies on the development of spectral libraries containing both product ion data and retention time information, and provides the basis for extensive data-independent quantitation. Although significant progress has been made recently towards mapping the human proteome, spectral libraries remain an important target resource for proteomics. Here we report on the assessment of new 6600 Triple TOF technology in providing extended proteome coverage and the establishment of a comprehensive spectral library for cancer research. We have observed a two-fold increase in protein coverage using a 6600 Triple TOF compared with a 5600 Triple TOF, and now detect over 20,000 distinct peptides from 3000 proteins in a single two-hour 1-dimension chipLC/MS analysis. Utilising this additional capacity in proteome coverage, we have developed a comprehensive spectral library for SWATH using a variety of human cancer cell lines, including melanoma, colorectal, thyroid, cervical and transformed embryonic kidney cells. We demonstrate the utility of this extended cancer database by characterising quantitative differences of each cancer cell line via SWATH.

UNDERSTANDING THE MOLECULAR MECHANISMS OF WATER STRESS TOLERANCE INDUCED BY PIRIFORMOSPORA INDICA IN BARLEY

Mehdi Mirzaei¹, Mehdi Ghabooli², Paul A Haynes¹, Mohammad-Reza Hajrezai³, Ghasem Hosseini Salekdeh²

1. Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia
2. System Biology, Agricultural Biotechnology Research Institute, Tehran, Iran
3. Physiology and Cell Biology, Institute of Plant Genetics and Crop Plant Research IPK, Gatersleben, Germany

Piriformospora indica is a mutualistic root endophytic fungus, which transfers several benefits to hosts including enhance plant growth and increase yield under both normal and stress conditions. It has been shown that P. indica root-colonization enhances water stress tolerance based on general and non-specific plant-species mechanism. To better understand the molecular mechanism of P. indica-mediated drought stress tolerance, we designed a set of comparative experiments to study the impact of P. indica on barely plants cultivar “Golden Promise” grown under different drought levels [Filed capacity (F.C.), 25% F.C and 50%]. P. indica enhanced root and shoot biomass of colonized plants under both well-watered and water-deficit conditions. Proteome analysis of P. indica-colonized barley leaves under well-treated and water-deficit conditions resulted in detection of 726 reproducibly protein spots. Mass spectrometry analysis resulted in the identification of 45 differentially accumulated proteins involved in photosynthesis, reactive oxygen scavenging, metabolisms, signal transduction, and plant defense responses. Interestingly, P. indica increased the level of proteins involved in photosynthesis, antioxidative defense system and energy transport. We propose that P. indica-mediated drought stress tolerance in barely is through photosynthesis stimulation, energy releasing and enhanced antioxidative capacity in colonized plants.
MASS SPECTROMETRY ANALYSIS OF ENDOMETRIAL CANCER

Parul Mittal¹, Manuela Klingler Hoffmann¹, Georgia Arentz¹, Gurjeet Kaur Chatar Singh², Martin Oehler¹, Chao ZHANG¹, Peter Hoffmann¹

1. The University of Adelaide, Adelaide, SA, Australia
2. Institute for Research in Molecular Medicine, University Sains Malaysia, Pulau Pinang, Malaysia

“Endometrial cancer with regional lymph node metastasis has a high mortality rate. Lymph node metastasis is therefore a crucial factor in the prognosis and choice of treatment for patients with endometrial cancer. Based on the assumption that metastasis is mainly determined by properties of the primary tumor and its interaction with the surrounding structures we aim to explore the possibility of predicting the presence of metastasis based on the molecular features of the primary tumor.

A tissue cohort comprising of endometrial primary tumors with metastasis (n=41) and without lymph node metastasis (n=51) was analysed to identify proteins associated with regional lymph node metastasis. For this, two complementary methods MALDI Imaging and Label free quantitative proteomics were used. Though MALDI-IMS allows the analysis of hundreds of peptides and lipids simultaneously, MALDI is not considered quantitative. Therefore, quantitative proteomic profiling by label free analysis will be used in conjunction with MALDI-IMS to identify proteins and peptides that are differentially expressed in primary tumours and have been metastasized. Our preliminary data of tryptic peptide imaging on FFPE tissue micro arrays indicates the metastatic potential of the primary tumor. Using the label free quantitative approach, we have identified three proteins which are differentially expressed between primary tumors with and without lymph node metastasis. In conclusion, we expect that the results from this study will provide crucial new tools to assist in the diagnosis and prognosis of Endometrial cancer and would prevent overtreatment of patients, whose primary tumor does not have metastatic potential.”

IDENTIFICATION OF DIAGNOSTIC BIOMARKERS TO IMPROVE THE MANAGEMENT OF DIABETIC RELATED FOOT ULCERS

Rajesh Gupta¹, Srihari Sharma², Dayle Sampson², Arnulf Compay², Melissa Fernandez², Gary Shooter¹

1. Central Analytical Research Facility, IFE, QUT, Brisbane, QLD, Australia
2. Institute of Health and Biomedical Innovation, QUT, Brisbane, QLD, Australia

Diabetic related ulcers are a common and severe complication of diabetes that can lead to severe health complications and prove fatal if mistreated or undiagnosed. Currently, there is lack of understanding regarding the underlying biochemical and physiological processes involved during chronic wound healing. The objective of this project was to understand and investigate the underlying factors in the wound environment that could be used to distinguish healing from non-healing diabetic foot ulcers. Bottom up proteomics was performed to analyse healing and non-healing representative pooled samples using SDS-PAGE and LC-MS/MS by AB SCIEX TripleTOF® 5600 System. The data analysis identified more than 400 proteins each from healing and non-healing samples, and 15 and 16 proteins unique to healing and non-healing respectively. A semi-quantitative approach involving spectral counting revealed more than 200 differentially regulated proteins in the samples. Proteins were grouped based on different functional properties and oxidative stress related proteins were selected for further analysis as there is growing evidence to suggest their vital role in the pathogenesis of diabetes related ulcers. The top 6 abundant proteins, catalase, peroxiredoxin 1, peroxiredoxin 2, superoxide dismutase-2 (SOD-2), S100-A9 and myeloperoxidase (MPO), were chosen on the basis of semi quantification results for further validation using enzyme-linked immunosorbent assay (ELISA).
TRACKING THE INTRICATE DYNAMICS OF ANTIGEN AND EPITOPE KINETICS DURING VIRUS INFECTION

Nathan P Croft1, Danielle A de Vertuil1, Stewart A Smith2, Ralf B Schittenhelm1, David C Tscharke2, Anthony W Purcell1

1. Monash University, Melbourne, VIC, Australia
2. Research School of Biology, The Australian National University, Canberra, ACT, Australia

The CD8+ T cell response to virus infection is dependent upon recognition of peptide epitopes complexed to MHC molecules and presented on the surface of infected cells. Whilst the mechanisms underlying this processing pathway are known, there is a paucity of quantitative information regarding the dynamics of presentation and how this may impact upon the magnitude of the T cell response. Here we have used targeted mass spectrometry to investigate the display of epitopes during vaccinia virus infection of three different cell types; namely in vitro fibroblasts and dendritic cells, and ex vivo bone marrow-derived dendritic cells. LC-MRM was used to simultaneously track the kinetics of 47 vaccinia-derived epitopes presented to the immune system, incorporating stable isotope-labeled peptides in order to provide measures of absolute abundance, whilst data-independent SWATH-MS was used to track antigen levels and profile virus proteome expression. The CD8+ response to each epitope was measured in parallel from infected mice in order to determine the immunodominance hierarchy each peptide elicits.

We reveal that the nature of vaccinia epitope display is highly diverse during infection, with peptide levels spanning many orders of magnitude. Further, whilst the relative kinetic of each epitope was remarkably similar across each cell type, abundance levels were found to vary considerably, with notably diminished levels on fibroblasts. Antigen expression and epitope kinetics were tightly coupled, as is predicted in order to engender a rapid T cell response to infection. However, correlations between epitope abundance and CD8+ response magnitudes remain difficult to predict, despite here using ex vivo dendritic cells as a model of in vivo infection. This study represents an advancement in our understanding of virus infection in the context of the immune system and will be necessary to drive further investigation into factors that can tailor immunity to pathogens for therapeutic benefit.

DEPOLARISATION-INDUCED REPROGRAMMING OF THE PRESYNAPTIC PHOSPHOPROTEOME MEDIATES CHANGES IN NEUROTTRANSMITTER RELEASE

Kasper Engholm-Keller1,2, Phil Robinson1, Mark Graham1

1. Children’s Medical Research Institute, Westmead, NSW, Australia
2. University of Southern Denmark, Odense, Denmark

Learning and memory result from synaptic plasticity - the ability of synaptic transmission to be modulated over time in response to stimuli. In contrast to the well-studied postsynaptic changes, presynaptic change in neurotransmitter release (exocytosis) from synaptic vesicles (SVs) is a mechanistically less understood form of synaptic plasticity. Exocytosis is regulated by phosphorylation, and the phospho-status of several exocytic proteins affects transmitter release. Exocytosis is followed by synaptic vesicle endocytosis which is controlled by proteins that are rapidly dephosphorylated upon nerve stimulation. We have used large-scale quantitative phosphoproteomics to study proteins from isolated nerve endings from rat brain (synaptosomes) that were at rest, depolarised for 10 sec. using KCl, or depolarised and allowed to recover for up to 15 min after depolarisation. After stimulation, the synaptosomes were lysed, subjected to protein precipitation and digested using trypsin. Phosphopeptides were enriched and fractionated using a combination of TiO₂, sequential elution from IMAC (SIMAC) and hydrophilic interaction liquid chromatography (HLIC) (collectively abbreviated TiSH). Peptides were analysed by LC-MS/MS on an LTQ-Orbitrap Velos/Elite and a 5600 TripleTOF instrument. The results show a prolonged, global reprogramming of the synaptic phosphoproteome, with an overrepresentation of exo- and endocytic proteins showing altered phosphorylation levels, and we therefore hypothesise that the initial short-term depolarisation changes the potential for subsequent neurotransmitter release on a much longer time-scale. Neurotransmitter release experiments are currently being performed and preliminary data seem to confirm the hypothesis – showing that the level of glutamate release from synaptosomes at a second stimulation 15 min after the initial one depends on the strength of the initial stimulation. In conclusion, our results indicate that depolarisation-induced phosphorylation changes in the pre-synaptic proteome constitute a previously undescribed form of pre-synaptic plasticity.
ENHANCING DISCOVERY AND QUANTITATIVE PROTEOMICS BY USE OF ONLINE MULTIPHASE CHIP LC FRACTIONATION

Christoph Krisp¹, Robert Parker², Dana Pascovici², Hao Yang³, Remco van Soest³, Mark P Molloy¹

1. Australian Proteome Analysis Facility, Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, New South Wales, Australia
2. Department of Respiratory Medicine, Imperial College of Science, Technology and Medicine, London, United Kingdom
3. Eksigent part of AB SCIEX, Redwood City, California, USA

Online strong cation exchange (SCX) coupled with reversed phase (RP) mass spectrometry (MS) also known as MudPIT (multi-dimensional protein identification technology) is widely used in discovery-based proteomics. We combined MudPIT with selected reaction monitoring (SRM) MS to avoid off-line sample handling and showed a significant boost in signal response (90% increase).¹

Encouraged by these findings, we developed chip-based SCX coupled RP columns to achieve higher column-to-column reproducibility and greater robustness of automated LC-MS/MS workflows. Technical proofs were established using cell lysates and plasma (non-immunodepleted) digests (5 µg). Five-step salt-based fractionation followed by 1 h acetonitrile gradients revealed significant peptide identification increases compared with 1 h RP-LC-MS/MS (5 µg, ~80% increase) and more accumulated unique peptides compared with 5x 1 h RP-LC-MS/MS runs (25 µg, ~10% increase). Multiphase separated samples demonstrated remarkable elution consistency (less than 6% in more than 1 fraction in cell lysates, and less than 13% in plasma). Highly reproducible separation enabled multiphase chip integration into high resolution multiple reaction monitoring (MRM-HR) applications. Fraction-specific and retention time scheduled peptide lists (503 peptides from 173 plasma proteins) were generated. All targeted peptides were quantifiable in multiphase MRM-HR while 16% (85 peptides) were undetectable in conventional MRM-HR and demonstrated 70% mean peak area increases. Interestingly, predominantly early eluting peptides demonstrated greatest increases (>10-fold).

Multiphase fractionation was also beneficial for spectral library generation in data independent quantitation (SWATH-MS). Five-step fractionation of thyroid cancer cell lysates led to spectral library generation for 3000 proteins. Analysing SWATH-MS acquisitions of same lysates resulted in reliable quantitation of 85% of all proteins in the library. Using multiphase fractionation with SWATH-MS to analyse 10 melanoma cells with different MAPK pathway mutations demonstrated (principle component analysis) explicitly segregation of MEK inhibitor sensitive cells from MEK insensitive cells with distinct marker proteins for these phenotypes (confirmed by SRM-MS).


MOONLIGHTING WITH MELANOMA AND THE UNFOLDED PROTEIN RESPONSE

Erin K Sykes¹, Swetlana Mactier¹, Kimberley L Kaufman³, Duthika M Mallawaaratchy¹, Munther Alomari¹, Richard I Christopherson¹

1. University of Sydney, Darlington, NSW, Australia
2. Brain and Mind Research Institute, The University of Sydney, Darlington, NSW, Australia

Introduction and Objectives - Metastatic melanoma is a lethal neoplasm with rapid progression and systemic dissemination. The 5-year survival rate for patients with distant metastases is just 6%-18%. This is due not only to the aggressive nature of the disease but also a lack of effective therapies. Activation of the unfolded protein response (UPR), a stress response, in melanoma positively correlates with tumour progression, metastasis and poor outcome. The complete action of the UPR is yet to be determined, and its role in cancer progression is undefined.

Method - In the present study, proteomic analyses have been used to identify proteins involved in the UPR and to elucidate its role in melanoma. Subcellular proteomes of human melanoma cell lines (Mel-RM and WMM1175) treated with thapsigargin (TH), an inducer of ER stress, were labeled with iTRAQ and analysed using 2D LC-MS/MS. Differentially abundant proteins were validated using selected reaction monitoring (SRM) mass spectrometry and western blotting. Membrane-associated progesterone receptor component 1 (PGRMC1) and 4F2-cell surface antigen were labeled with Cy5-conjugated antibody and the sub-cellular trafficking with TH treatment monitored by fluorescence microscopy. Finally pull-downs of PGRMC1 and 4F2 were performed to elucidate their role in the UPR.

Results and Discussion - Following activation of the UPR, 80 differentially abundant proteins were identified by mass spectrometry. These proteins are involved in stress response, cell migration and adhesion, and apoptosis/survival. PGRMC1 and 4F2 are up-regulated and translocated as a result of UPR activation, both proteins have been linked with cancer progression and their association with the UPR novel.

Conclusions - The UPR affects several important oncogenic proteins and pathways, such as the Akt and MAPK pathways, showing the importance of the UPR in melanoma progression. The differentially abundant proteins identified here extend our understanding of how the UPR can modulate protein function and contribute to melanoma tumourigenesis.
CHARACTERISATION OF GLYCOSYLATION OF THE NEWCASTLE DISEASE VIRUS HAEMAGGLUTININ-NEURAMINIDASE SURFACE GLYCOPROTEIN

Cassandra L Pegg1,2, Christine Hoogland1, Jeffrey J Gorman1,2
1. QIMR Berghofer, HERSTON, QLD, Australia
2. School of Chemistry and Molecular Biology, University of Queensland, St Lucia, QLD, Australia

Newcastle disease virus (NDV) is an avian virus belonging to the family Paramyxoviridae and the causative agent of Newcastle disease. The desire to study NDV is due not only to the significant economic impact it has on the poultry industry worldwide, but also its potential use as an oncolytic agent and vaccine vector for human and animal use.1-3 Of great importance to NDV is the attachment surface glycoprotein, haemagglutinin-neuraminidase (HN). HN recognises and binds sialylated structures on host cells and facilitates virus-cell membrane fusion. It has been shown that the N-linked glycans present on HN can modulate the ability of the virus to infect cells and stimulate the host immune system.4 However, characterisation of site-specific glycan occupancy and heterogeneity remains one of the few unexplored areas related to NDV HN. Accordingly, mapping the repertoire of glycans from HN may help elucidate mechanisms of infectivity and immune evasion. Not only with respect to NDV, but also within closely related viruses that cause disease in humans, such as parainfluenza viruses. Liquid chromatography-MS/MS strategies were implemented to structurally characterise the digested glycoprotein. Dissociation techniques included higher collision energy dissociation (HCD) and electron transfer dissociation (ETD) using high mass accuracy Orbitrap mass analysis and collision induced dissociation (CID) with linear ion trap mass analysis. Examination of NDV HN revealed considerable diversity amongst the HN-linked structures, with up to sixty-three glycoforms present at one site. These included high mannose, fucosylated, sialylated and mono- and disulphated N-linked glycans. In addition, a novel sialylated O-linked glycan was also discovered, which may have implications in the functional interactions of the protein.


VALIDATION OF MESENCHYMAL STEM CELL TRANSCRIPTS ASSEMBLED FROM RNA-SEQ USING PROTEOMIC DATA

Aidan Tay2,1, Chi Pang2,1, Natalie Twine2,1, Gene Hart-Smith2,1, Linda Harkness3, Moustapha Kassem3, Marc Wilkins2,1
1. School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia
2. Systems Biology Initiative, The University of New South Wales, Sydney, New South Wales, Australia
3. Department of Endocrinology and Metabolism, Odense University Hospital & University of Southern Denmark, Odense, Denmark

Alternative splicing of mRNA diversifies the function of human proteins, with tissue- and cell-specific protein isoforms being the most difficult to validate. While transcriptomic experiments enable the detection of many alternatively spliced transcripts, it is not known if these transcripts have protein-coding potential. We recently published the PG Nexus pipeline1, which facilitates high confidence validation of exons and exon-exon junctions of spliced transcripts by integrating transcriptomics and proteomics data. In this study, we applied PG Nexus towards the analysis of an undifferentiated human mesenchymal stem cell line and compared the number of protein isoforms validated using different protein sequence database, including public online databases and RNA-seq derived databases. With significant overlaps with other databases, we identified 8,011 exons and 3,824 splice junctions from 2,379 genes with the Ensembl database. The Ensembl database consistently outperformed the other data sources, but predicted open reading frames from RNA-seq derived transcripts were comparable, with only 6 less splice junctions validated. Using proteotypic and isoform-specific peptides, we validated 462 protein isoforms and a higher number is possible if we included multiple proteotypic peptides. Multiplexing proteotypic peptides in SRM assays or similar experiments will increase the confidence and coverage of protein isoform validation experiments.

VIRULENCE FACTORS OF PATHOGENIC *ESCHERICHIA COLI* ARE INJECTED INTO HUMAN CELLS AND AFFECT THE HUMAN CELLULAR N-TERMINAL PROTEOME

Natalie C Marshall1,2, Theo Klein3, Michael J Thejomayen1,2, Niklas von Krosigk2, Nikolay Stoynov4, Leonard J Foster4,5, Christopher M Overall1,2, B Brett Finlay1,2,5

1. Department of Microbiology & Immunology, University of British Columbia, Vancouver, BC, Canada
2. Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada
3. Department of Oral Biological and Medical Sciences, University of British Columbia, Vancouver, BC, Canada
4. Centre for High-Throughput Biology, University of British Columbia, Vancouver, BC, Canada
5. Department of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, BC, Canada
6. Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada

Enteropathogenic *Escherichia coli* (EPEC) infects human intestinal cells and causes severe diarrheal disease worldwide. During infection, EPEC utilizes a type-1-like secretory system (T1SS) to inject virulence factors directly into the cytoplasm of the infected human cell. Identifying the functions and mechanisms of ≥23 T3SS effector proteins is a substantial challenge. Characterizing human cellular proteome dynamics during infection could substantially contribute to our understanding of how T3SS effectors contribute to EPEC-mediated disease.

This study aims to characterize T3SS-mediated alterations in the human proteome using an N-terminal proteomics approach. Proteases regulate human cellular processes relevant to infection, including inflammation, innate immunity, and cell death. N-terminal proteomics enriches proteolytic events, facilitating the study of protease activity and dysregulation. This study may reveal how these and other global pathways are affected by T3SS effectors and may identify novel substrates of T3SS proteases.

Cultured human epithelial cells were infected for 1.5 hours with wild-type EPEC or a mutant unable to secrete T3SS effectors. The N-terminal proteomes of infected human cells were quantitatively compared using terminal amine isotopic labeling of substrates (TAILS) and tandem mass spectrometry on an Orbitrap Velos mass spectrometer. Data from three independent experiments were analyzed by Mascot and quantified by MaxQuant.

We identified 2112 unique modification-specific N-terminal peptides from 1359 unique proteins at 1% FDR. Preliminary data indicate T3SS-dependent proteolysis of proteins involved in known T3SS-mediated pathways, including innate immunity and cytoskeletal regulation, as well as novel proteolytic events in proteins known to interact with EPEC T3SS effectors. Finally, novel proteolytic events in beta-actin may provide further mechanistic details on how the T3SS mediates host cytoskeletal rearrangements during infection, a hallmark of EPEC infection.


---

SIRTUIN 4 IS A LIPOAMIDASE REGULATING THE ACTIVITY OF THE PYRUVATE DEHYDROGENASE COMPLEX

Rommel Mathias1,2, Todd M Greco1, Adam Oberstein1, Hanna G Budayeva1, Rumela Chakrabarti1, Elizabeth A Rowland1, Yibin Kang1, Thomas Shenk1, Ileana M Cristea3

1. Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA
2. Department of Biochemistry, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Vic
3. Department of Biochemistry, Princeton University, Princeton, New Jersey, USA

Sirtuins (SIRTs) are a critical family of seven mammalian nicotinamide adenine dinucleotide (NAD+)-dependent enzymes which govern genome regulation, metabolism, and aging. SIRTs display widespread subcellular distributions; SIRT1, SIRT6, and SIRT7 are nuclear, SIRT2 is predominantly cytoplasmic, and SIRTs3-5 are mitochondrial. Despite all SIRTs containing a conserved deacetylase domain, only SIRTs1-3 show robust deacetylation activity, while mitochondrial SIRT4 and SIRT5 have little to no deacetylase activity. Emerging evidence has revealed that several SIRTs can hydrolyze alternative lysine modifications more efficiently than acetyl. Specifically, SIRT5 preferentially desuccinylates and demalonylates proteins, while SIRT6 can hydrolyze long-chain fatty acyl lysine modifications. A catalytic activity for SIRT4 has not been identified, however, here, we identify that SIRT4 is a robust lipoamidase. SIRT4s catalytic efficiency for lipoyl- and biotinyl-lysine modifications is superior to its deacetylase activity. The pyruvate dehydrogenase complex (PDH), which converts pyruvate to acetyl-CoA, is a biological substrate of SIRT4. We demonstrate that SIRT4 can enzymatically hydrolyze the lipoamide cofactors from the E2 component dihydrolipoamide acyltransferase, and diminish overall PDH activity. For over 40 years, PDH has only been known to be regulated by phosphorylation of its E1 component. However, we now reveal its regulation by SIRT4, and establish SIRT4 as the first mammalian cellular lipoamidase, and a crucial guardian of metabolism.

GLYCOPEPTIDE ANALYSIS OF PENTAMERIC AND HEXAMERIC IMMUNOGLOBULIN M

Edward Moh1, Morten Thaysen-Andersen1, Nicki Packer1

1. Macquarie University, Sydney, NSW, Australia

Human immunoglobulin (Ig) M is the largest antibody that exists naturally as a pentamer (with J-chain) or a hexamer form in blood circulation. They are the first antibodies to respond when a new or self-antigen is detected. A subset of circulating IgM, termed natural IgM, is responsible for the quality control of healthy cells and has reported anti-cancer therapeutic capabilities.

To this end, a monoclonal natural IgM that targets cancer cells has been recombinantly expressed in human cell line PER.C6. Pentamer and hexamer forms of this IgM were purified for full glycosylation profiling.

In IgG, differences in glycosylation profile and site occupancy at the single N-glycosylation site at Asn297 have been reported widely to implicate host immune response. IgM has 5 N-linked glycans on each heavy chain, with a total of 51 N-glycosylation sites on the pentamer and 60 N-glycosylation sites on the hexamer. The effects of glycosylation on IgM-mediated immune response have not been explored thoroughly.

Using LC-MS/MS, we show that glycopeptide analysis of both pentameric and hexameric forms of the IgM contain differences in glycosylation site occupancy and glycosylation profile, despite having identical amino acid sequences.

In conclusion, characterization of the full glycosylation profile of IgM provides a stepping stone towards understanding effects of glycosylation on IgM-mediated immune response.

PROTEOMIC ANALYSIS AND PROFILING OF MALAYSIAN UPLAND RICE

Shahkila Mohd Arif1, Ma Nyuk Ling2, Zaidah Rahmat1

1. Universiti Teknologi Malaysia, Johor Bahru, JOHOR, Malaysia
2. Department of Biological Science, Faculty of Science and Technology, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

In this era, proteomics is one of the omics research fields that are voraciously studied in order to understand more on gene expression and genome annotation. Rice has played a very prominent role over centuries as a staple food and nutrient provider consumed by more than half of world populations. Even though there are many studies being carried out for wetland rice protein identification, none has been reported for upland rice. This preliminary study aims to help establish the proteome profile of Malaysian upland rice in the hope that it would be beneficial in understanding the proteins with diverse functions and predicting function of unknown proteins.

In this study protein from upland rice was extracted by using TCA/Acetone based method. The protein pattern was compared on 1D SDS-PAGE, the excised gel bands and total protein were digested with trypsin and analyzed by Liquid chromatography-tandem mass spectrometry (LC-MS/MS). The MS/MS spectra obtained were subjected to search against Oryza sativa database. Comparison of the protein on 1D SDS-PAGE showed similar pattern for all cultivars. However, analysis via mass spectrometry revealed that the same protein presented may not be detected in all cultivars. More than 200 proteins have been identified and most of the proteins found are responsible for the growth and development of the plant, stress tolerance, antioxidant and natural sweetener. This finding gives insightful information with regards to the various proteins expressed and synthesized in upland rice that could contribute to a better understanding of its physiological significance. It could bring us one step closer to determine the function of every gene in upland rice particularly the one that is closely related with its growth and development.
MOONLIGHTING PROTEINS OF MYELOPLASMA HYOPENUMONIAE AND THEIR ROLE IN PATHOGENESIS

Marcelo Moreno1,², Jerran Santos2,³, Jessica Tacchi2,³, Benjamin Raymond2,³, Matthew Padula2,³, Steven Djordjevic2,³

1. the three institute, Sydney, NSW, Australia
2. UTS, Sydney, NSW, Australia

Mycoplasma species are strictly parasitic bacteria that have undergone extensive genomic reduction and therefore biochemical efficiency is essential for their survival. They require their host for the essential supply of lipids, amino acids and nucleotides for growth. The pig pathogen Mycoplasma hyopneumoniae infects the respiratory system of pigs and causes significant agricultural losses. One hypothesis is to explain their success as chronic pathogens suggests that the Mollicutes have evolved multifunctional proteins as a means to increase protein function without expanding gene content.

Multifunctional proteins were first described in 1995 and have since been found in all biological kingdoms. Bacterial moonlighting proteins have been found to participate in key host-pathogen interactions and primarily function as adhesins, metabolic enzymes, ribosomal proteins, chaperones, and proteases. Moonlighting proteins can be detected in cellular fractions other than those stated by initial annotation and online databases, and by identifying the secondary functions of known proteins. The extracellular function of these proteins expressed by bacteria are predominantly roles in host interaction, primarily binding to extracellular matrix or host cell surface molecules, but also in modulating host immune function. Studies have shown that glycolytic enzymes which are conserved in both the pathogen and the host can introduce autoimmune in the host organism.

Specific surface-exposed proteins from M. hyopneumoniae identified by cell-surface proteomic analyses were selected as possible moonlighting proteins because of their annotations and bioinformatics predictions to be cytosolic. The natively purified recombinant proteins were characterised for heparin, plasminogen and fibronectin binding using microscale thermophoresis, blotting techniques and heparin affinity chromatography to elucidate their functional role as moonlighting proteins. With the use of protein modelling programs and bioinformatics software, the host molecule binding motifs specific for heparin and therefore potential binding sites on these putative moonlighting proteins can be identified.
QUANTITATIVE SHOTGUN PROTEOMICS ANALYSIS OF HIGH AND LOW ANTIOXIDANT EXPRESSING AUSTRALIAN RECOMBINANT INBRED LINES OF ARACHIS HYPOGAEA.

Sridevi Muralidharan¹, Graeme Wright², Alice Lee¹

1. ARC Training Centre for Advanced Technologies in Food Manufacture, University of New South Wales, Kensington, NSW 2052, Australia
2. Peanut Company of Australia, Kingaroy, QLD 4610, Australia

Peanuts are rich in essential nutrients, including proteins and antioxidants and are favoured by consumers seeking healthy food options. Peanut breeders are keen to develop cultivars with enhanced valuable functional food traits, in addition to disease resistance and high kernel yield potential. For example, peanut cultivars with high oleic acid content >75% have recently been developed by hybridisation using a naturally occurring mutant line developed by the University of Florida in the USA. In an effort to breed a stable peanut cultivar with enhanced antioxidant content, a population of recombinant inbred lines (RILs) was developed by the Australian Peanut breeding program through hybridisation by crossing a full season peanut genotype consistently expressing high antioxidant capacity (D147-p3-115) with another line consistently expressing low-mid level antioxidant capacity (Farnsfield).

A subset of RILs (p27-272, p27-363, p27-036) showing very high and very low total antioxidant capacities and parent lines (D147-p3-115 and Farnsfield) were selected for proteomics analysis. A label-free quantitative shotgun proteomics approach coupled with nano-MS/MS was adopted to study functional proteins altered in peanuts with different antioxidant capacities. Due to the lack of the complete peanut genome, peptides were identified using a customised peanut protein sequence database. Statistically significant differentially expressed proteins between peanut lines with high and low antioxidant capacities were identified, and mapped to biological pathways. Protein abundance changes in biological pathways reveal the molecular fluctuations mediating cellular processes underlying each of the different RILs. Potential protein biomarkers were identified for high antioxidant expressing peanut lines of Arachis hypogaea. These markers could help breeders in developing correlations between antioxidant responsive proteins and genes that account for the observed phenotype. This information could potentially enable breeders to integrate this with marker assisted selection for the breeding of high antioxidant cultivars.

Keywords: Peanuts, antioxidant capacity, Shotgun proteomics, nano LC-MS/MS, biomarker discovery.

IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS OF PFRH5 REQUIRED FOR THE INVASION OF HUMAN RED BLOOD CELLS BY MALARIA PARASITES

Thomas Nebl¹, Lin Chen¹, Danny Wilson¹, Brian J Smith², Alan F Cowman¹

1. Walter & Eliza Hall Institute, Parkville, VIC, Australia
2. Department of Chemistry, La Trobe Institute for Molecular Science, Bundoora, Victoria, Australia

Plasmodium falciparum causes the most severe form of malaria in humans causing over 700,000 deaths each year. Invasion of human erythrocytes is essential for its survival and this process involves the interaction of multiple host-parasite receptor-ligand interactions. One of the most important is that of the parasite ligand PfRh5 with the host receptor basigin. PfRh5 is the leading blood-stage vaccine candidate that elicits potent strain-transcending invasion inhibitory antibodies. We have determined the crystal structure of PfRh5 to a resolution of 2.18 Å (Chen et al., PNAS 2014). The PfRh5 adopts a novel fold with the core structure consisting of seven α-helices winding around two β-sheets in an anti-parallel fashion to form a flat and compact molecule. Chemical cross-linking mass spectrometry and molecular modelling of the PfRh5-basigin interaction suggested that the receptor binds at one end of PfRh5, providing other areas for interaction with other proteins, such as PfRh5 interacting protein, PfRipr1 (RHS-interacting protein 1). Quantitative MS analyses of Co-IPs using HA-tagged PfRipr1 or pull-downs using recombinant Rh5 as a bait demonstrated that PfRh5 and PfRipr1 associates with a novel conserved protein (PfRipr2) to form a complex on the merozoite surface. This discovery elucidates a novel protein that may tether PfRh5 to the merozoite surface and facilitate its binding with basigin. Our insights open potential avenues for novel anti-malarial strategies that may inhibit the formation of the crucial basigin-PfRh5-PfRipr1/2 invasion complex.
CHARACTERIZATION OF NON-COMPETITIVE IMIDAZOLINE INHIBITORS OF THE HUMAN PROTEASOME BY LC-MS/MS

Shuai Nie¹, Evert Njomen¹, Jetze. Tepe¹, Gavin E. Reid²,³,¹
1. Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, United States
2. School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia
3. Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

Inhibition of the ubiquitin-proteasome pathway using bortezomib has emerged as a rational strategy for the chemotherapeutic treatment of multiple myeloma (MM). However, the competitive inhibition mechanism of bortezomib results in >97% of patients becoming resistant or intolerant to treatment in the first few years, after which survival is typically less than a year. Non-competitive proteasome inhibitors, such as imidazoline scaffolds, that regulate the proteasome via a mechanistically distinct protein:ligand interaction, and that overcome resistance and are highly effective in vivo, have therefore received recent interest for overcoming the limitations of competitive inhibitors. However, little is known about the site(s) of non-competitive inhibitor binding to the proteasome, or how they exert their biological activity. Here, we have employed a strategy involving immunoprecipitation and quantitative LC-MS/MS analysis of ubiquitylated substrates, to gain insights into the non-competitive human proteasome inhibition mechanism of imidazoline scaffolds TCH-013 and TCH-165, compared to the competitive inhibitor bortezomib. Cytosolic proteins extracted from RPMI cells incubated with 1.0 µM TCH-165, 10.0 µM TCH-013, 0.1 µM Bortezomib or 1.0 % DMSO for 1 hour were treated with ubiquitin antibody conjugated to agarose beads, then eluted with 0.1 M glycine at pH 2.5 and 4 °C for 10 min. Each protein sample was then digested using trypsin, Lys-C and Asp-N. Quantitative analysis of differential protein abundances between the different treatment groups was performed via dimethyl labelling, prior to LC-MS/MS using a Thermo Q-Exactive Plus mass spectrometer. Protein identification and quantitation was achieved using Mascot database search software. Preliminary results from this study indicate that the competitive proteasome inhibitor bortezomib presents vastly different and more abundant ubiquitylated protein profiles compared to the non-competitive inhibitors, indicating a lack of accumulation of some proteins with non-competitive inhibition, and supporting the hypothesis that imidazoline scaffold inhibitors present a drug-induced substrate specific inhibition of proteasome mediated protein degradation.

THE SECRETOME OF WALLEMIA ICHTHYOPHAGA.

Gillian E Norris¹, Taryn A Miller¹, Trevor S Loo¹, Mark I Patchett¹, Meekyung Ahn²
1. Massey University, Palmerston North, New Zealand
2. Leather and Shoe Research Association, Palmerston North, New Zealand

The subphylum Wallemiomycetes contains three species distinguished by both genetic and morphological characteristics: W. ichthyophaga, W. sebi and W. muriae. These species have been isolated from environments with low water availability and are considered to be the most xerophilic and halophilic fungi isolated to date. W. ichthyophaga requires at least 10% NaCl to grow and thrives in saturated salt media. We isolated a strain of W. ichthyophaga, W. sebi which requires at least 10% NaCl to grow and thrives in saturated salt media. We isolated a strain of W. ichthyophaga, W. sebi which requires at least 10% NaCl to grow and thrives in saturated salt media. We isolated a strain of W. ichthyophaga, W. sebi which satisfies all these requirements. In this study, we hypothesised that W. ichthyophaga, W. sebi and W. muriae, which thrive in environments with low water availability and are considered to be the most xerophilic and halophilic fungi isolated to date, would be N-glycosylated. We therefore repeated the experiment without pre-fractionation (i.e. using LC-MSMS on the trypsinned desalted concentrated secretome), and these results will be reported. One of the identified glucosidases was further purified using IEC and SEC, and characterised. The enzyme was shown to be N-glycosylated, was most active at 10% NaCl, and interestingly, appeared to be able to hydrolyse both beta-(1,4) and alpha-(1,4) linkages.

References.
A RELIABLE AND REPEATABLE SUBLIMATION BASED PROTOCOL FOR THE ANALYSIS OF FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) TISSUE VIA MATRIX ASSISTED LASER DESORPTION IONISATION IMAGING MASS SPECTROMETRY (MALDI-IMS)

Matthew B O’Rourke¹, Matt P Padula²

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

Matrix Assisted Laser Desorption Ionisation Imaging Mass Spectrometry (MALDI-IMS) is a robust technique that has seen great improvement in recent time and has the potential to further the field of diagnostic imaging. However with automated sample preparation approaches becoming more and more prevalent, little work is being done to understand and standardize the critical matrix application and co-crystallisation techniques in use, or investigate more economical non-machine based approaches. Our research has led us to dissect and define the conditions responsible for the successful implementation of a sublimation based approach for sample preparation. By quantifying each condition and empirically determining their ideal conditions, we propose a repeatable and cost effective method for the universal MS imaging of Formalin fixed paraffin embedded (FFPE) tissue. In this work, we have demonstrated a universal tissue preparation and matrix application technique with low startup costs that can be operated by “walk-up” users and provide explanation and background information for further customization and optimization by experienced and inexperienced operators alike. We as also describe a novel technique for a non-delocalising method of Trypsin application. The application of such a technique has also opened up new avenues of research that allow for the combination of disparate methodologies to incorporate IMS as a standard indicative technique; We are aiming for the integration of IMS as a standard operating protocol in the same way other techniques have become ubiquitous in the lab. Only through the dissemination of complete and unabridged protocols and the empirical determinations for the conditions that govern them, will integration of disparate techniques create workflows with global perspective and therefore global results.

COMPARISONS AND OPTIMIZATIONS ON THE TRIPLETOF 6600 AND ORBITRAP PLATFORMS

Benjamin Parker¹

1. The University of Sydney, Sydney, NSW, Australia

The choice of a specific mass spectrometer for a specific proteomic application is critical. Furthermore, the combination of optimized settings with efficient data analysis pipelines or parameters can heavily influence results. The aim of this study was to maximize protein identification on instruments available in the Mass Spectrometry Core Facility at the Sydney University. A single tryptic digest of mouse liver was analysed using highly similar chromatography conditions on a variety of instruments including the TripleTOF 6600, Q-Exactive and LTQ-Orbitrap Velos Pro. Initially, optimization of settings was performed on the TripleTOF 6600 to maximize protein identification with nanoUHPLC and long gradients. A “Fast” and “Sensitive” acquisition strategy was devised similar to (Kelstrup et. al., J. Proteome Res., (2012), 11(6); 3467) and used to compare triplicate high load injections for single-shot proteome analysis. Next, data-analysis pipelines were directly compared including Proteome Discoverer, MaxQuant and Protein Pilot. These data will provide valuable insights into efficient protein identification with various platforms.

EVALUATION OF A LIPID PROFILING SYSTEM USING REVERSE-PHASE LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION ORBITRAP MASS SPECTROMETRY AND AUTOMATED LIPID IDENTIFICATION SOFTWARE

Russell Pickford¹

1. UNSW Australia, Sydney, NSW, Australia

A lipid is generally considered to be any molecule that is insoluble in water and soluble in organic solvents. Biological lipids usually refer to a broad grouping of naturally occurring molecules which includes fatty acids, waxes, eicosanoids, monoglycerides, diglycerides, triglycerides, phospholipids, sphingolipids, sterols, terpenes, prenols, fat-soluble vitamins (such as vitamins A, D, E and K) and others. The main biological functions of lipids include their central role in energy storage, as structural components of cell membranes, and as important signaling molecules Mass spectrometry is used frequently for lipidomics, but is not currently in general use at UNSW. As a facility we frequently face problems such as inexperienced instrument users, poor sample preparation practices and a lack of understanding of the experiments involved. Technical advances in instrumentation (UHPLC and Orbitrap mass analysers) and in software for qualitative and quantitative lipidomics have provided us with a potential ‘turnkey’ system with robust characteristics and production of high quality data. This type of approach cannot replace dedicated lipidomics laboratories and specialists, but it is hoped that it will provide a general lipidomics solution in a facility environment. Initial results and evaluations of the system combining LipidSearch software with a Q-Exactive Plus mass spectrometer and C18 UHPLC are presented and discussed. Results obtained from mouse liver extracts will be compared with those obtained from the same samples measured using the current gold standard - ‘shotgun’ direct infusion analysis on a QTrap instrument.
INVESTIGATION OF HEAT STRESS IN CATTLE USING MULTIPLE REACTION MONITORING (MRM) MASS SPECTROMETRY TO MEASURE CYTOKINE LEVELS IN PLASMA.

Pedro RL Pires, Harry Goswami, Gene Wijffels, Michelle Colgrave

1. Agriculture Flagship, CSIRO, Brisbane, Queensland, Australia
2. Department of Surgery, University of São Paulo, São Paulo, Brasil

Feedlotting cattle is a common strategy to increase growth rate and the quality of the meat from beef cattle. However, the combination of tropical high humidity and climatic temperatures with the carbohydrate and protein rich diet supplied to cattle in this production system can result in deleterious effects on metabolism, heat stress and even heat stroke in extreme cases. Heat stress triggers an inflammatory process which in turn triggers the production of a group of key plasma cytokines: interleukin-1 beta (IL-1β); interleukin-6 (IL-6); interferon gamma (INF-γ) and tumor necrosis factor alpha (TNF-α). These plasma cytokines are found in low concentrations (0.03 to 0.3 pmol/µL) as measured by enzyme-linked immunosorbent assays (ELISA). In this research, we aimed to develop and optimize a mass spectrometry-based assay employing multiple reaction monitoring (MRM-MS) to detect and quantify plasma cytokines at low concentrations. For MRM-MS method development commercially available recombinant cytokines were digested with trypsin and analysed by high resolution MS/MS analysis to select peptides useful for quantification and for MRM design. Samples were chromatographically separated on a Shimadzu Nexera HPLC system (Shimadzu, Australia) using a Phenomenex Kinetex C18 (2.1 x 100 mm) column and a linear gradient of 5-45% acetonitrile over 6 min with a flow rate of 400 µL/min. The eluent from the HPLC was directly coupled to a 6500 QTRAP MS/MS system (AB/Scieix, Foster City, USA) equipped with a TurboV ionization source operated in positive ion mode. Three MRM transitions for each candidate peptide biomarker were selected based on MS response and the collision energy (CE) for each transition was optimised. Using five peptides per cytokine the dynamic range of detection was assessed in aqueous solution. Cytokine extraction will be assessed and optimised using established protocols including solid-phase extraction (SPE) or acetonitrile precipitation. Method development and optimisation experiments have demonstrated that MRM-MS is a sensitive and selective alternative to ELISA capable of identifying and quantifying cytokines at concentrations below 2 nmol/µL.

PROTEOMIC EXPLORATION OF CYCLIC PEPTIDE DIVERSITY AMONG PLANTS USING PEPTIDE LABELLING AND ORTHOGONAL SEPARATION STRATEGIES

Aaron Poth, Michelle Colgrave, David Craik

1. CSIRO Animal, Food and Health Sciences, St Lucia, QLD
2. Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia

Plants have evolved various defense strategies to counter the constant threat from predators and infectious organisms. Aside from physical deterrents such as thorns, plants produce a chemical arsenal consisting of small molecules typically assembled from isoprene units, alongside a range of defense peptides. Cyclotides, found in a range of plant species are ribosomally synthesised defense peptides defined by their unique combination of a cyclic peptide backbone and cystine-knotted topology. Challenges faced in the proteomic analysis of cyclotides stem from difficulties in their chromatographic separation, the necessity for linearisation prior to MS analysis, and their typically poor fragmentation. Here we examine the benefits of orthogonal separation and chemical derivatisation prior to tandem MS in the proteomic characterisation of cyclotides.
IDENTIFICATION OF SEED PROTEOME OF MALAYSIAN UPLAND RICE

Zaidah Rahmat1, Nurul A. Zaidi1, Nyuk Ling Ma1, Pick Kuen Chan4

1. Universiti Teknologi Malaysia, Johor Bahru, JOHOR, Malaysia
2. International Islamic University Malaysia, Kuantan, Pahang, Malaysia
3. Department of Biological Science, Faculty of Science and Technology, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia
4. Agro-Biotechnology Institute Malaysia, Serdang, Selangor, Malaysia

Rice (Oryza sativa L.) is immensely consumed by global population and becoming the staple food in many countries. Providing a lot of nutrient content, rice comes in various variety, shapes, color and taste. Typical rice breeding is in flooded condition with good irrigation system referred to as wetland variety hence, requiring substantial amount of land. Our focus is on varieties that can sustain drought condition and require minimal amount of water for its growth known as upland rice. Upland rice in Malaysia is mostly grown either on hilly side or low land area without subjecting the plants to flooded condition where the source of water comes from rainfall. We set out to analyze a few of Malaysian upland rice varieties to identify the proteins to establish seed proteome database for rice from Malaysia. Protein were extracted from the seeds using suitable method adapted from Singh et al (2004) followed by separation on 1D SDS-PAGE, tryptic digestion and peptide sequencing by LC-MS/MS. Identified proteins were then searched against Oryza sativa databases. We confirmed the presence of water stress inducible protein which confers the upland variety tolerance to drought aside from many other proteins such as storage proteins, embryo-specific proteins, stress-related proteins, signaling proteins and several antioxidants. Responses to stress, biotic and abiotic stress, and protein metabolism are the largest representation of GO analysis. Our findings presents a preliminary insight into the seed proteome of Malaysian upland rice which could provide an alternative to typical rice breeding due to land scarcity.

DEVELOPMENT OF AN ‘IMMUNO-LC-MS/MS’ ASSAY FOR THE QUANTITATIVE CHARACTERIZATION OF OXIDIZED AND TRUNCATED PARATHYROID HORMONE (PTH): IMPLICATIONS FOR THE TREATMENT OF PATIENTS WITH CHRONIC KIDNEY DISEASE

Gavin E Reid1, Li Cui1, Fabrizio Bonelli3, John Wall1, Angela Podgorski1, Marie Philipneri4, Anjana Jagalur4, Amy Kreig5, Mustafaa Mahmood4, Kevin J Martin1

1. The University of Melbourne, Parkville, VIC, Australia
2. Michigan State University, East Lansing, MI, USA
3. Diasorin Inc., Stillwater, MN, USA
4. Division of Nephrology, Saint Louis University, St Louis, MO, USA

Parathyroid hormone (PTH) plays a critical role in the regulation of circulating blood calcium levels, and serves as a biomarker of secondary hyperparathyroidism associated with the diagnosis of disorders such as vitamin D deficiency or chronic kidney disease (CKD), and for monitoring the effectiveness of treatment e.g., hemodialysis. Immunochemiluminescent assay of full length (i.e., 1-84) PTH has historically been problematic due to variable standardization, cross reactivity with truncated PTH fragments (e.g., 7-84), and variable correlations with biological parameters. A recent report that PTH may be oxidized in vivo further complicates these issues, since oxidized PTH is biologically inactive.

To address these challenges, an immunocapture liquid chromatography–tandem mass spectrometry (‘immuno-LC-MS/MS’) analysis strategy was developed and optimized, together with a novel dual isotope-labeled internal standard approach (i.e., [15N]-labeled full length and truncated PTH and [13C6,15N] isotope-labelled oxidized PTH tryptic peptides), in order to (i) quantitatively determine the nature of circulating full length and truncated PTH and their individual oxidized isoforms, (ii) differentiate between in vivo and ex vivo oxidative modifications, and (iii) examine the effects of PTH oxidation on current immunochemiluminescent PTH assays. First, using a series of purified full length, truncated and individual oxidized isoforms of PTH spiked into depleted plasma, the recovery of the overall analysis strategy was determined to be 66%, with a wide linear range for detection from 5-2000 pg/mL.

Then, two series of plasma samples from patients on hemodialysis were analyzed. In Set A, oxidized full length and truncated PTH were commonly observed, with oxidation ranging from 0 to 80% of the total PTH observed, and with 34 of the 41 samples containing more than 10% oxidation. In general, there was more oxidized PTH 7-84 than oxidized PTH 1-84. In contrast, in Set B, only minimal amounts of oxidized PTH peptides were observed, with only 3 of 29 samples containing >10% oxidation. The marked difference in oxidized PTH peptides between Set A and Set B suggests that oxidation of PTH may be occurring ex-vivo. Further studies are required to fully define the possibility and origin of ex-vivo oxidation. The immuno LC-MS/MS results correlated extremely well with the assay of PTH 1-84 and less well with other “intact PTH” assays. The effects of oxidation on the results obtained using current intact PTH assays may represent an additional factor contributing to the variability of PTH assay results and their correlation with biological parameters.
REVISITING THE ARTHRITOGENIC PEPTIDE THEORY: QUANTITATIVE - NOT QUALITATIVE - CHANGES IN THE PEPTIDE REPERTOIRE OF HLA-B27 ALLOTYPES

Ralf B. Schittenhelm¹, Terry C.C. Lim Kam Sian¹, Pascal G. Wilmann³, Nadine L. Dudek¹, Anthony W. Purcell¹

1. Monash University, Clayton, VIC, Australia

The association of Human Leukocyte Antigen (HLA) B27 with spondyloarthropathy is one of the strongest documented for any autoimmune disease. A common hypothesis for this association is the arthritogenic peptide theory. It dictates that differences in the peptide binding preferences of disease-associated and non-associated HLA-B27 allotypes underpin the presentation of bacterial and self-peptides leading to cross-reactive T cell immunity and subsequent autoimmune attack of affected tissues.

Using high-resolution mass spectrometry in combination with SWATH-MS and MRM, we have analyzed and comprehensively quantified the peptide repertoires derived from the 8 most common HLA B27 allotypes, HLA-B*27:02 to HLA-B*27:09. Differences in the peptide binding pockets of these molecules manifest largely as quantitative, but not qualitative changes in the peptide cargo. Thus, absolute binding preferences do not explain disease-association and the arthritogenic peptide theory needs to be reassessed in terms of quantitative changes in self-peptide presentation, T cell selection and altered conformation of bound peptides.

DETECTION OF NOVEL BETA-CATENIN PROTEIN INTERACTORS IN COLON CANCER

Crystal Semaan¹, Manisha Sharma², Beric Henderson², Mark P Molloy¹,³

1. Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia
2. Westmead Institute for Cancer Research, The University of Sydney, Westmead Millennium Institute at Westmead Hospital, Sydney, Australia
3. Australian Proteome Analysis Facility, Macquarie University, Sydney, Australia

Beta-catenin is a protein associated with several human cancers. The progression of cancer is correlated with nuclear accumulation of beta-catenin, a process which is regulated by phosphorylation. Protein phosphorylation also alters function through changing protein-protein interactions. In the context of colon cancer we are interested in furthering our understanding of these two aspects of beta-catenin regulation.

We generated recombinant human full-length beta-catenin constructs to achieve phospho-mimic and phospho-dead Y654 in the Arm 10-12 domain. These constructs were used along with wildtype beta-catenin to enrich for protein binding-partners in SILAC labelled SW480 and HT29 colon cancer cell lines in triplicate. Eluates were recovered, separated by SDS-PAGE and analysed by mass spectrometry (Orbitrap ELITE). Proteome Discoverer 1.3 was used for data analysis and candidates selected based on presence in all replicates at least 2-fold more than the control. Detection of at least 2 peptides and replicates having a p-value <0.05 (one-sided t-test) was also applied.

For SW480, candidates include 225 for the wildtype, 49 for the phospho-mimic and 62 for the phospho-dead. For HT29, candidates include 66 for the wildtype, 146 for the phospho-mimic and 22 for the phospho-dead. Several well-known beta-catenin interactors were detected including catenin alpha-1, catenin delta-1, adenomatous polyposis coli protein (APC), four and a half LIM domains protein 2, ruvB-like 1, and paxillin.

Novel candidate interactors were grouped into processes and functions including nuclear import and export, regulation of cell cycle and mitochondrial proteins. We selected 3 candidate proteins (OPA1, Cdk1, Hsp70) as potential novel interactors and attempted to validate by immunofluorescence microscopy and an in situ proximity ligation assay (DuoLink). This presentation will report our progress towards confirming these proteins as novel beta-catenin effectors.
ZINC-A-2-GLYCOPROTEIN (ZAG) IS OVER EXPRESSED IN THE MILK OF A PTEROPID BAT (PTEROPUS ALECTO)

Brian Shiell1, Grant Peck1, James Wynne1, Gary Beddome1, Lawrence Mok1, Colgrave Michelle2, Michelle Baker3, Wojtek Michalski1
1. CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia
2. CSIRO, Agriculture Flagship, St Lucia, Queensland, Australia

Bats have been identified as reservoirs for a number of zoonotic viruses, including Ebola virus, Henipah viruses, SARS-like coronavirus and Australian bat lyssavirus. Bats have the ability to transmit these viruses (a process known as spillover) to other susceptible species leading to severe illness and in some cases death. While these bat borne viruses may cause significant disease in their spillover host, bats remain asymptomatic and rarely show any clinical signs of disease. The molecular mechanism by which bats control viral infection is currently unknown and is therefore of significant value.

Mammalian breast milk is rich in nutrients, hormones, growth factors and immunoactive molecules, which influence the growth, development and immune status of a newborn infant. It is hypothesized that the nutritional function of milk evolved subsequent to its protective function; that is, the mammary gland evolved from the innate immune system.

The comprehensive proteomic analysis of Pteropid bat milk, presented in this study, revealed that it is very concentrated and rich in proteins, with the majority of typical milk proteins identified. The most distinctive and interesting feature of P.alecto milk was the over production of zinc-a-2-glycoprotein (ZAG). This protein was detectable, albeit in much lower level, in human milk but was not detected in milk of other mammals analysed, including an Australian microbat (Chalinolobus gouldii).

ZAG is a protein of interest due to its postulated multifunctional role, although it is still considered a protein with an unknown function. One role postulated is as a novel adipokine (lipid metabolism regulatory molecule), whereas another proposed role is, due to structural organization and fold similar to MHC class I antigen-presenting molecule, in the expression of the immune response. Hence the overexpression of ZAG in bat milk may be related to the molecular mechanism by which bats control viral infection.

IRREVERSIBLE OXIDATIVE MODIFICATION OF CYSTEINES RESULTING FROM A PROLONGED HIGH FAT DIET MODEL

Lauren E Smith1, Kasper Engholm-Keller2, Jana Paulech1, Stuart J Cordwell1,3, Melanie Y White1,3
1. Discipline of Pathology, University of Sydney, Sydney, NSW, Australia
2. Children’s Medical Research Institute, Westmead, NSW
3. School of Molecular Bioscience, University of Sydney, Sydney, NSW

The extent of oxidative stress on the myocardial molecular environment due to obesity is yet to be fully understood. The cellular response to increased oxidative stress is regulated in part, by highly reactive thiol groups on cysteine residues undergoing various oxidative modifications that regulate protein function. Pathologies such as obesity lead to the formation of aberrant oxidative modifications on proteins, such as the largely irreversible sulfinic and sulfonic acids. This study aims to utilise a novel enrichment strategy to identify proteins that undergo these oxidative modifications as a result of obesity.

Rats were fed a CHOW (12% fat) or high fat (HF) (42% fat) diet for 6 months. To quantify alterations in protein abundance, samples were isobarically tagged prior to mass spectrometry (MS) analysis. In parallel, irreversibly oxidised peptides were negatively selected utilising low pH loading conditions for strong cation exchange (SCX), whereby the negative charge imparted by the over-oxidation of Cys limits interaction with the chromatography. The resulting SCX fractions were further partitioned by hydrophilic interaction chromatography prior to MS analysis.

Irreversibly oxidised Cys peptides were increased 2.5 fold in the HF diet, with 10 unique irreversibly oxidised Cys-containing peptides observed in the CHOW diet (10 unique proteins) compared with 22 observed in the HF diet (arising from 20 unique proteins). 55 discrete proteins were observed to be significantly changed in abundance as a result of the HF diet. These proteins were largely found to be associated with fatty acid metabolism, degradation and oxidation. The most prevalent of these proteins identified as oxidised due to the HF diet was β-actin. This irreversible oxidation may cause stiffening of the cytoskeleton, impacting the arrangement and functionality of the z-disk, as shown in hypertrophic models. Given that hypertrophy is a comorbidity associated with obesity, we suggest that this alteration to beta-actin has functional implications in this model.

This study is the first application of this novel method of enrichment for irreversibly oxidised Cys proteins to obesity, identifying key proteins associated with important functional groups. Understanding these modifications and their impact on myocardial function may provide novel mechanisms of treatment to improve recovery from AMI in the high-risk obese population.
IDENTIFICATION OF APOE AND AMYLOID BETA COMPLEXES IN HUMAN PLASMA.

David I Stapleton¹, Scott Laffoon², Paul A Grieco², Edward A Dratz², Anne Roberts¹, Colin I Masters¹, AIBL Research Group², Blaine R Roberts¹

1. Florey Institute of Neuroscience and Mental Health, Parkville, VIC, Australia
2. Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA
3. www.aibl.csiro.au, Parkville, Victoria, Australia

The cholesterol and lipid transporter, apolipoprotein E (ApoE), has an important although uncertain role in the pathogenesis of sporadic Alzheimer’s disease (AD). ApoE’s importance is demonstrated by the significantly increased risk of developing Alzheimer’s disease between the three main alleles of APOE, ε2, ε3, ε4. ApoEε4 is the most jeopardizing allele, conferring a dose-dependent increase to the risk for AD and reducing the mean age of onset from 84 to 68 years old in homozygous ε4 persons, compared to persons with no ε4. Whereas ε4 is second only to age in importance amongst AD risk factors, its presence is neither necessary nor sufficient to cause AD, suggesting that environmental or other genetic elements contribute to the disease. Here we show using large-format, narrow pI range (4.7-5.9), 2D-PAGE proteomics of heparin-Sepharose binding proteins from homozygous (ε2/ε2, ε3/ε3, ε4/ε4) human sera. We find no differences in total spot volumes from ApoE spot trains between heparin-Sepharose enriched sera from healthy or Alzheimer’s disease ApoE carriers. Furthermore, there were no significant differences found between ε2, ε3 or ε4 carriers of native ApoE migrating at 34 kDa. However, a reduction-resistant variant migrating at 45 and 55 kDa were found in ε3/ε3 and ε2/ε2 individuals respectively. Mass spectrometry identified these species to be complexes of ApoE, ApoAII and amyloid β. These data suggest that ApoE isoforms have unique functions that require further investigation.

DISSECTING PROTEIN DYNAMICS INVOLVED IN REACTIVE OXYGEN SPECIES-INDUCED INSULIN RESISTANCE IN ADIPOCYTES USING SILAC-BASED QUANTITATIVE PROTEOMICS

Tim Zhiduan Su¹, Kelsey Fisher-Wellman¹, Benjamin Parker¹, David James¹

1. Charles Perkins Centre, School of Molecular Bioscience, The University of Sydney, Sydney, NSW

The production and accumulation of reactive oxygen species (ROS) may contribute to the development of insulin resistance, which is the vital feature of type 2 diabetes. Thioredoxin and glutathione are the major thiol antioxidants protecting cells from ROS-induced cytotoxicity. In this study, carmustine (BCNU) was used to specifically inhibit glutathione reductase and auranofin (AF) was used to specifically inhibit thioredoxin reductase. In assay1, BCNU was used to stimulate 3T3L1 adipocytes for 2h and 24h. Glucose uptake was significantly inhibited by 2h-long stimulation, but not affected by 24h-long stimulation. In assay2, BCNU and AF were used together to stimulate 3T3L1 adipocytes for 2h and 24h. Glucose uptake was dramatically inhibited by both 2h- and 24h-long stimulation. Using SILAC-based quantitative proteomics, we quantified 4533 and 4111 proteins respectively. Based on fuzzy c-means analysis, these proteins were clustered into eight groups, and some of them were tightly associated with glucose uptake changes. In BCNU-stimulation assay, proteins with negative glucose uptake correlation were mainly located in mitochondria, enriched in oxidative phosphorylation pathway. However, proteins with positive glucose uptake correlation were mainly enriched in Proteasome. In BCNU/AF-stimulation assay, proteins with both negative and positive glucose uptake correlation were mainly located in mitochondria. The former were enriched in oxidative phosphorylation pathway, but the latter played key roles in generation of precursor metabolites and energy, enriched in glycolysis/gluconeogenesis pathway and some other metabolism-related pathways. Several dynamic key proteins and well-known phosphorylation sites perhaps involved in insulin resistance were validated by western blot.
POST-TRANSLATIONAL PROCESSING, MULTIFUNCTIONALITY AND MOONLIGHTING: THE NOT-SO-SIMPLE PROTEOME OF A GENOME-REDUCED PATHOGEN

Jess Tacchi\(^1\), Benjamin Raymond\(^1\), Veronica Jarocki\(^1\), Michael Widjaja\(^1\), Iain Berry\(^1,2\), Matt Padula\(^1,2\), 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 an economically significant pathogenic bacterium that chronically infects the respiratory tract of swine. This infection leads to pneumonia and secondary infections, costing agricultural industries significantly in the use of antibiotics and vaccines, which are currently largely ineffective. An improved understanding of the molecular mechanisms behind the infection process is essential to our ability to rationally design better vaccine and therapeutic interventions. With fewer than 700 predicted protein coding sequences, M. hyopneumoniae possesses one of the smallest genomes of any free-living organism. As such, it lends itself well to thorough proteomic interrogation.

A slew of proteomic techniques have been used to investigate the global proteome and surface-proteome at the protein and peptide level, including surface shaving and labelling techniques, ligand and immuno-blotting and affinity chromatography, as well as N-terminal dimethyl labelling to determine true N-terminal of mature proteins. This conceptually unbiased, function-oriented approach has revealed an unexpected level of complexity in the use of proteolytic processing, multifunctional proteins and moonlighting to compensate for reduced coding capacity at the genome level. While transcriptome studies suggest that the majority of genes are transcribed, our analyses identified less than 400 detectable expressed protein products. However, a significant number of these were discovered to be post-translationally modified, multifunctional or present at the cell surface, despite not possessing traditional signal peptides. Many of these proteins, predominantly cell surface adhesins, we have already described in the literature, however a surprising number of cytoplasmic “housekeeping” proteins are also found to be post-translationally cleaved, multifunctional or moonlighters. These findings can be applied to the development of vaccines and therapeutics in Mycoplasma, as well as having wider implications for the field of biology, if this level of post-translational regulation can be found in other bacterial pathogens.

IDENTIFICATION OF DISEASE RESISTANCE GENES IN SYDNEY ROCK OYSTERS USING QUANTITATIVE LABEL FREE SHOTGUN PROTEOMICS.

Vineet Vaibhav\(^2,1\), Emma Thompson\(^2,3\), David Raftos\(^2,3\), Paul Haynes\(^1\)

1. Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW, Australia
2. Sydney Institute of Marine Sciences, Sydney, NSW, Australia
3. Biological Sciences, Macquarie University, North Ryde, NSW, Australia

The Oyster industry in Australia is one of the biggest contributing industries to Aquaculture. Sydney rock oyster (SRO) industry in particular has been severely affected by Queensland Unknown (QX) disease outbreaks, which is caused by a single celled protozoan, Marteilia sydneyi. Continuous outbreaks of this disease pose great risk for the sustenance of the industry, and hence development of disease resistant lines holds the key for the future of the industry. In 1990s NSW Department of Primary Industries started a selective breeding programme to develop a line of QX resistant SRO by performing interbreeding amongst the survivors of the QX outbreak. These oysters showed good disease resistance, but very little is known about the molecular mechanisms involved. Previous studies have revealed that genes for disease resistance are heritable, so molecular level knowledge, will be a great help in the development of disease resistant lines. Using Marker assisted selection. We have performed proteomic comparison of QX resistant selected lines with Wild type oysters, using both 2D electrophoresis and label free shotgun proteomics. To get an insight into disease progression, we analysed the temporal proteomic variation of n=20 oysters of each type, collected from Hawkesbury River at two different time points during the disease progression. In an initial study using 2D gel comparison of QX resistant lines and wild type oysters, we found 38 proteins to be significantly (p<0.05) altered in expression in the disease resistant oysters. Quantitative label free shotgun proteomics will also be performed on the same protein samples to substantiate the findings further.
COMPREHENSIVE ANALYSIS OF RECOMBINANT HUMAN ERYTHROPOIETIN GLYCOFORMS BY CAPILLARY ELECTROPHORESIS AND NANOFLOW LIQUID CHROMATOGRAPHY COUPLED WITH MIDDLE-DOWN MASS SPECTROMETRY

Roza Viner1, A M Heemskerk2, D Horn1, J Saba1, M Bern3, D Bush4, M Santos5, H Dewald5, A R Ivanov4, B L Karger4

1. Thermo Fisher Scientific, San Jose, CA, United States
2. Leiden University Medical Center, Leiden, The Netherlands
3. ProteinMetrics, Palo Alto, CA, United States
4. Barnett Institute, Northeastern University, Boston, MA, United States
5. AB Sciex, Brea, CA, United States

Introduction

Erythropoietin (EPO) is an essential glycoprotein for hormone red cell production. Recombinant human Erythropoietin (hrEPO) is used for treatment of anemia resulting from chronic kidney disease. EPO has three N-glycosylation sites and one O-glycosylation site which leads to high heterogeneity, making glycoform profiling challenging. Multiple formulations of hrEPO have been studied extensively by different approaches, including capillary electrophoresis (CE) and nanoflow liquid chromatography (nLC) coupled to advanced mass spectrometry (MS) analysis. Although CE-MS has been used to characterize intact EPO glycoforms, quantitative mapping of each glycosylation site by CE-MS has not been previously accomplished. Here, we report that sheathless CE- and nLC-based separations coupled to Orbitrap middle-down MS provide complementary information and allow for comprehensive site-specific glycan mapping of rhEPO.

Methods

Reduced and alkylated rhEPO (Erythropoietin-Alpha, ProSpec, NJ) was digested with LysC (Roche, CA). Digested rhEPO was analyzed on an LTQ Orbitrap Elite using CESI-MS or LC/MS coupled with high resolution high mass accuracy (HRAM) FT scanning of ETD or HCD MS2 fragment ions. Low flow sheathless CESI (Beckman Coulter, CA) employed both neutral coated and bare fused silica capillaries with integrated porous tip ESI sprayers. ProsightPC 3.0, Protein Deconvolution 3.0, Proteome Discoverer 2.0 (Thermo Fisher Scientific) and Byonic (ProteinMetrics, CA) software packages were used for data analysis. A custom EPO glycan database was generated using proteinase K (Roche) digest and WAX RP-nLC-MS/MS analysis on Orbitrap Fusion. SimGlycan (PremierBiosoft, CA) was used for glycan identification.

Preliminary results

To generate the EPO glycan database, we used proteinase K to preserve site localization information. Short proteinase K glycopeptides were separated by a novel mixmode WAX-RP column. (Thermo Fisher), detected by HCDpdCID analysis on an Orbitrap Fusion and identified by SimGlycan. Identified glycans were used to compile the custom glycan database for glycopeptides identified by Byonic or Prosight PC. To perform complete quantitative glycan site-specific mapping of rhEPO, we employed limited Lys-C digest to yield one glycosylation site per peptide fragment of 3-10 kDa size for middle-down analysis. This enabled more complete sequencing of glycoforms compared with top-down analysis. The rhEPO glycopeptides were well separated from non-glycosylated peptides by CESI and resolved over 20 min in a 50 min long run. Glycoform resolution was mostly based on differences in the number of sialic acid residues. The two predominant O-glycosylated peptides (N-acetylhexosamine-hexose with one or two sialic acids) migrated as completely resolved peaks. Due to high efficiency separation, we identified 14 glycoforms on Ser126 by CESI-MS but only 4 by LC-MS using a Magic C18 AQ nano-LC column. Although our method demonstrated high efficiency separation and detection sensitivity of glycopeptides, we were initially unable to identify double N-glycosylated peptides containing Asn124 and Asn19. Ultimately, we determined our EPO sample protein sequence was different from the one provided by the manufacturer. Using HRAM ETD and HCD fragmentation in combination with manual de novo sequencing and wild card searches (Byonic), we were able to identify the actual sequence of the EPO standard and N-linked glycopeptides. These results suggested that CESI-HRAM middle-down is a sensitive and efficient method for characterization of proteins or biopharmaceuticals with multiple glycosylated endogenous proteoforms.
REVERSE-POLYNOMIAL DILUTION CALIBRATION METHODOLOGY EXTENDS LOWER LIMIT OF QUANTIFICATION AND REDUCES ERROR IN TARGETED PEPTIDE MEASUREMENTS IN BLOOD PLASMA.

Valerie C Wasinger¹, Yunki Yau², Xizi Duo², Rupert W Leong³

1. UNSW Australia, Sydney, NSW, Australia
2. The University of NSW, Kensington, NSW, Australia
3. Endoscopy, Concord Hospital, Concord, NSW, Australia

Matrix effect is the alteration of an analyte’s concentration-signal response due to co-existing ion components. It is one of the most common problems leading to inaccuracy and imprecision in quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). With Electrospray Ionization (ESI) as the ion source, matrix effects are believed to be a function of the relative concentrations, ionization efficiency, and solvation energies of the analytes within the ESI droplet. For biological matrices such as plasma (with ~10⁷ unique proteins when unfractionated and non-depleted), the interactions between droplet components is immensely complex and the subsequent effect on analyte signal response not well elucidated. This study comprised of three sequential quantitative analyses: We investigated whether there is a generalizable correlation between the range of unique ions in a sample matrix (complexity), the amount of matrix components (concentration), and matrix effect, by comparing an E.coli peptide-digest matrix (an approximate 2600 protein proteome) with phospholipid depleted human blood plasma, and unfractionated, non-depleted human plasma matrices (~10⁷ proteome) for six human plasma peptide Multiple Reaction Monitoring (MRM) assays. Our dataset demonstrated significant analyte-specific interactions with matrix complexity and concentration properties resulting in significant ion suppression for all peptides (p<0.01), with 0>non-uniform effects on the ion signals of the analytes and their stable-isotope analogs. These matrix effects were then assessed for translation into error and precision affects in a low concentration (~0-250ng/mL) range across no-matrix, complex matrix (E.coli and phospholipid depleted plasma), and highly complex matrix (non-depleted plasma), when a standard addition Stable Isotope Dilution (SID) calibration method was used. Back-calculated error (%) and precision (CV%) across all matrices by SID were within <20%, however error in phospholipid depleted 20>20>and non-depleted plasma matrices were significantly higher compared to no-matrix and E.coli matrices (p=0.007). Finally a novel Reverse-Polynomial Dilution (RPD) calibration method with and without phospholipid-depletion was compared to SID for relative error and precision in a ~0-250ng/mL range in plasma. RPD techniques extend the Lower Limit of Quantification and reduces error (p=0.005) in low-concentration plasma peptide assays and is broadly applicable for verification phase Tier 2 multiplexed MRM assay development within the FDA-National Cancer Institute (NCI) biomarker development pipeline.

USE OF IN VIVO QUANTITATIVE PROTEOMICS FOR THE SCREENING OF CANDIDATE MYOKINES

Martin Whitham¹, Martin Pal¹, Matthew J McKay², Ardishir Amirkhani², Peter Iliades¹, Mark P Molloy², Mark A Febbraio¹

1. BakerIDI, Melbourne, VIC, Australia
2. Australian Proteome Analysis Facility, Sydney, NSW, Australia

Since the characterisation of Interleukin-6 as a myokine, the concept that skeletal muscle is a bona fide endocrine organ has gained considerable support. Here, we adopted the use of fully labelled [¹³C]Lysine SILAC mice to screen skeletal muscle for myokine candidates. We pooled the gastrocnemius muscles from three, unlabelled C57/BL6 mice either exposed to 90 minute treadmill running or non-exercised controls and combined 1:1 with a spike-in SILAC gastrocnemius standard. After separation of 10 fractions by SDS-PAGE and trypsin/Lys-C enzymatic digestion, the resultant peptides were analysed by LC-MS/MS using an Orbitrap Elite. In all, we identified 2280 proteins with a 5% peptide false discovery rate. 593 of these proteins were quantified across exercise and control experiments, 337 of which contained at least two SILAC ratios. Enrichment of the data set for the gene ontology cellular component, extracellular region or space, distinguished a selection of candidate myokines such as Decorin, Annexin A2 and Mimocan that are currently undergoing validation.
THE ACCESSORY PROTEIN, HMW1, OF MYCOPLASMA PNEUMONIAE IS A MULTIFUNCTIONAL PROTEIN

Michael Widjaja1, Matt Padula1, Steven Djordjevic1

1. i3 institute, Sydney, NSW, Australia

Mycoplasma pneumoniae (Mpn) is a major cause of community acquired pneumonia predominantly in children and the elderly. Mpn is remarkable in that it produces an attachment organelle that dictates how Mpn adheres to the human respiratory epithelium and the direction of motility. The attachment organelle focuses several key adhesion molecules to the tip of the organelle including the P1 and P30 adhesins. Three accessory proteins known as High Molecular Weight proteins HMW1, HMW2 and HMW3 as well as the product of the mpn142 gene are thought to play a significant role in localising the adhesins to the attachment organelle. At present there is no data to suggest that these accessory proteins have a direct role in adherence and their cellular location is still unresolved. In this study we characterised the function of HMW1. HMW1 is 112 kDa protein that migrates abnormally during SDS-PAGE at approximately 250 kDa. Four recombinant fragments spanning HMW1 were constructed as polyhistidine fusion proteins, expressed in Escherichia coli and purified using nickel-affinity chromatography. Rabbit antisera were raised to each to these purified fragments and used to localise HMW1 in the attachment organelle by immunofluorescence microscopy. In preliminary experiments aimed at determining if HMW1 plays a role in binding host molecules, affinity chromatography columns separately loaded with fentin, actin, fibronectin, heparin or plasminogen were constructed. Binding interactions between these bait proteins and native Mpn proteins were identified by LC-MS/MS. Our data suggests that HMW1 contains binding domains for several of these host molecules. Specific binding affinity involving different regions of HMW1 and host molecules will be measured by thermophoresis.

FEATURE EXTRACTION IN MALDI IMAGING USING DIPPS

Lyron J Winderbaum1

1. Adelaide Proteomics Centre, The University of Adelaide, Adelaide, SA, Australia

MALDI Imaging is a promising new technology that allows for spatial heterogeneity in samples to be addressed. Signal intensity can be highly variable in MALDI Imaging experiments and adjusting for this variability can be difficult. We bypass the issue of signal intensity by considering presence/ absence data. This simplification allows proportions of occurrence, i.e. proportions of spectra with a peak at a particular m/z value, to be calculated. Using these proportions, signals that differentiate known spatial regions of interest can be identified by difference in proportion of occurrence statistics, or DIPPS. In many situations, this is ideal as it identifies a set of signals of interest that can serve as starting points for further analyses. We provide two example applications. The first example relates to an experiment designed to test if it is possible to detect N-linked glycans using MALDI Imaging by in situ application of PNGase. A control region of tissue was included, that was not treated with PNGase, and potential glycan signals are identified by difference in proportions in the tumour region. The tumour region is not annotated to begin with, and so is first separated by k-means clustering. Further analyses, including MS/MS identification, have been used to validate selected results in both example applications.

LYSINE METHYLATION MODULATES THE PROTEIN-PROTEIN INTERACTIONS OF YEAST CYTOCHROME C CYC1P

Daniel L. Winter1, Dhanushi Akeygunawardena2, Gene Hart-Smith1, Melissa A. Erce1, Marc R. Wilkins1

1. Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, UNSW, Sydney, New South Wales, Australia
2. Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia

In recent years, protein methylation has been established as a major intracellular post translational modification (PTM). It has also been proposed to modulate protein-protein interactions (PPIs) in the interactome. To investigate the effect of PTMs on PPIs, we recently developed the conditional two hybrid (2H) system. It allows the co-expression of a modifying enzyme such as a methyltransferase, acetyltransferase, or kinase alongside the bait and prey proteins. Any increase or decrease in interaction due to the modification of the proteins can be measured by an increased or decreased level of reporter gene expression. With this, we demonstrated that arginine methylation can modulate PPIs in the yeast interactome. Here, we used the 2H system to investigate the effect of lysine methylation. Specifically, we asked whether Ctm1p-mediated trimethylation of yeast cytochrome c Cyc1p, on lysine 78, modulates its interactions with Erv1p, Ccp1p, Cyc2p and Cyc3p. We show that the interactions between Cyc1p and Erv1p, and between Cyc1p and Cyc3p, are significantly increased upon trimethylation of lysine 78. This increase of interaction helps explain the reported facilitation of Cyc1p import into the mitochondrial intermembrane space upon methylation. This is one of the first demonstrations in a two-hybrid system that lysine methylation can modulate PPIs. It will be of interest to understand how widespread this process is in the eukaryotic cell.
ANTIGEN PRESENTATION DURING INFLUENZA VIRUS INFECTION

Ting Wu1, Jing Guan2, Nathan P Croft1, Nadine L Dudek1, David C Tscharke3, Nicole L La Gruta2, Anthony W Purcell1

1. Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia
2. Department of Microbiology and Immunology, the University of Melbourne, Melbourne, VIC, Australia
3. Research School of Biology, Australian National University, Canberra, Australia

CD8+ cytotoxic T cell (CTL) mediated immunity plays an important role in controlling and eradicating influenza virus in the host. CTL recognize major histocompatibility complex class I molecules (MHCI) in complex with viral peptides that are presented on the surface of antigen-presenting cells (APCs). T cell immunity directed against conserved CTL epitopes from internal influenza proteins provides an alternative vaccination strategy to current whole/split virus or subunit vaccines. However, the limitation in the development of suitable peptide-based vaccines is incomplete information on viral peptide processing and presentation and the impact of antigen presentation on the specificity of T cell responses. In this study we have quantified viral epitopes presented on the surface of H1N1-infected mouse dendritic and lung epithelial cells through a combined QTOF and triple quadrupole mass spectrometry approach. In total, 21 viral peptides were identified including two known immunodominant epitopes (NP366-374 and PA224-233), 10 known subdominant epitopes and 8 novel peptides. One of these novel epitopes was uniquely presented on the surface of infected epithelial cells. Compared to dendritic cells, lung epithelial cells were generally less efficient at peptide presentation: 2 peptides were not expressed at all, whilst 14 epitopes showed a level of expression less than half of that observed on dendritic cells. Across both cell lines, NP366-374 abundance was the highest (over a thousand copies per cell), whereas PA224-233 abundance was the lowest (~10 copies per cell), despite the co-dominant response to these NP and PA epitopes during primary infection in mice. These results indicate a disparity in the absolute levels of peptide presentation by different cell types, and their potential to affect T cell responses. The disconnect between epitope abundance and immunodominance provides an important insight into how T cells respond to infection. Moreover, the newly identified candidate viral peptides provide potential targets for the development of peptide-based vaccines.

PROTEOMIC RESPONSE OF TWO DIFFERENT VARIETIES OF RICE EXPOSED TO DROUGHT STRESS AND RECOVERY

Yunqi Wu1, Mehdi Mirzaei1, Brian Atwell2, Paul Haynes1

1. Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, New South Wales, Australia
2. Department of Biological Sciences, Macquarie University, Sydney, New South Wales, 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. Cereal crops must be grown in hotter, drier and more hostile climates as expansion of the range of food crops accelerates, and climate change makes existing cropping zones more unreliable. 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 temperate-climate 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. We have grown both varieties and subjected them to drought stress and subsequent recovery, and collected material at each timepoint. Vegetative growth is critical for high productivity crops. We have focused on cell division – the variable for growth of any organism. The very small cohorts of dividing cells in the plant meristem have been collected via micro dissection and analyzed separately. We contrast this with protein expression patterns in the mature leaf blades, using both label free quantitative shotgun proteomic analysis and Tandem Mass Tags labelling. Preliminary results indicate 275 drought response proteins in Nipponbare while in IAC 1131 205 drought response proteins are identified. Cell biology analysis reveals that cell division in IAC 1131 is hardly affected by drought stress, however, the number of dividing cells in Nipponbare decreases significantly. The wealth of detailed information produced in this study will provides insight into the key proteins involved in leaf growth and drought tolerance.
EVALUATION OF SWATH-MS FOR PROTEOME PROFILE QUANTITATIVE ANALYSIS

Thiri Zaw¹, Jemma Wu¹, Dana Pascovici¹, Natasha Care¹, Mark P Molloy¹, Xiaomin Song¹

1. Australian Proteome Analysis Facility, Macquarie University, NSW, Australia

MS/MS with SWATH acquisition is a data-independent acquisition strategy that generates high resolution MS/MS spectra for all detectable analytes in a biological sample within a pre-defined mass range. This technique enables high specificity MRM-like quantitation by extracting high resolution product ions of target analytes.

To evaluate SWATH as a proteome profile differential analysis technique, we acquired two sets of SWATH-MS data using two TripleTOF 5600 mass spectrometers for samples of human K562 whole cell protein digest spiked with 0.1%, 0.5%, 2%, 5% and 10% yeast protein digest. We extracted SWATH data using single IDA run library, comprehensive sample and instrument specific library, comprehensive sample specific library from other instrument, as well as publicly available generic libraries. We tested the effect of using different libraries to the quantitation results and determined the cut-off thresholds which would generate most true positives and most true negatives with least false positives reports for differential expressed protein discovery. The quantitation accuracy was also examined.

Our result demonstrated that it is feasible to extract SWATH data using comprehensive SWATH library built from IDA data acquired on different instruments or from publicly available MS/MS data. For our tested human protein spiked with 2% and 10% yeast samples, when a comprehensive generic library was used to extract SWATH-MS data and a cut-off threshold of protein fold change larger than 1.5 with protein area T-Test P-value smaller than 0.05, 3785 human proteins were quantified correctly as not changing in quantities between samples and 155 yeast proteins were correctly quantified as being changed in quantities with false positive discovery rate of 1.37%. We observed that false positive discovery rate would be significantly reduced with more stringent cut-off criteria that measure peptide area T-Test P-values.

COMPREHENSIVE PROTEOMICS ANALYSIS OF VULVAR CANCER TUMOUR PROGRESSION

Chao ZHANG¹, Manuela Klingler-Hoffmann¹, Georgia Arentz¹, Lyron Winderbaum¹, Parul Mittal¹, Martin Oehler², Peter Hoffmann¹

1. Adelaide Proteomics Centre, Adelaide, SA, Australia
2. Discipline of Obstetrics and Gynaecology, School of Paediatrics and Reproductive Health, Research Centre for Reproductive Health, Robinson Institute, The University of Adelaide, Adelaide, South Australia, Australia

Vulvar cancer is a rare gynaecological cancer with increasing incidence rate in the past decades, especially among the Indigenous population and human papillomavirus (HPV) prevalent areas. However, due to low incidence rate, only limited numbers of tumour samples are available, here we use complementary mass-spectrometric approaches to identify tumour specific diagnostic and prognostic biomarkers. Mass spectrometry based proteomics analysis for protein discovery has been successfully applied on gynaecological cancers. MALDI-Imaging MS and Label-free quantification MS are able to quantify numerous proteins from different morphological features, which are ideal tools for studying current vulvar cancer proteomics patterns. To investigate vulvar cancer tissue unique proteomics patterns, MALDI-Imaging and LFQ MS have been performed using FFPE tissue blocks from four vulvar cancer patients. Statistics software was used for spectra training and peak matching on different platforms. We have identified 1400 proteins from all samples and half of them are unique to specific tissue types. Based on these analyses, we can discriminate between healthy and tumour and have identified a few proteins which are up regulated during cancer development. Furthermore, RNA-sequencing will be introduced to complement current protein databases with transcriptomic data. In summary, our approach provides valuable insight into vulvar cancer development.
TYROSINE PHOSPHORYLATION PROFILING AND PHOSPHOPROTEOME MAPPING OF THREE MOUSE TISSUES

Ling Zhong1, Mark Raftery1
1. Bioanalytical Mass Spectrometry Facility, University of New South Wales, Sydney, NSW, Australia

Reversible tyrosine phosphorylation is a key biochemical process that regulates many responses and is often deregulated in cancer and immunological disorders. In this study, we report a large-scale enrichment and immunoprecipitation of phospho-tyrosine-containing peptides from three mouse primary tissues, brain, lung and liver with two commercial available anti-phosphotyrosine antibodies, pY100 and 4G10, to determine complementary enrichment patterns of these antibodies.

In order to obtain more complete phosphoproteome information of each tissue, phosphorylated serine, threonine (pST) and phospho-sylated tyrosine (pY) were enriched via titanium dioxide (TiO2). We analysed a small portion of these fractions and identified 2256 pST phosphoproteins and 5168 unique phosphopeptides across the three tissues. After pY enrichment, we identified 715, 529 and 274 phosphotyrosine containing peptides and 491, 338 and 203 phosphoproteins from brain, lung and liver tissue samples, using pY 100 and 4G10 antibodies with 1% FDR. Brain tissue had the largest number of unique phosphotyrosine peptides (43.8%) and phosphoproteins (42.7%) of the three tissues examined. Without the pY-enrichment, the distribution of phosphorylated serine, threonine and tyrosine was calculated to be 86.1%, 11.4% and 2.3% for brain tissue, 89.8%, 9% and 1.0% for lung and 84.7%, 12.1% and 2.5% for liver tissue. Combining the identifications from pST and enriched pY-containing peptides, a total of 1683, 861 and 1379 phosphopeptides were identified from brain, lung and liver tissue, respectively. The overlap of pY proteins and pST proteins is around 9% for brain and lung samples and 4% for liver tissue. Each antibody enriched different sets of pY-containing peptides and a recognition specificity analysis shows the 4G10 seems to prefer the sequence window containing negatively charged amino acids. An Ingenuity Pathway Analysis of the pY-containing proteins shows that leukocyte extravasation signaling and integrin signaling are enriched the most in the lung tissue while the Fcγ receptor-mediated phagocytosis in macrophages and monocytes signaling enriched in similar level across the three tissues.

ANALYSIS OF COW’S MILK INTACT PROTEINS USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY.

Delphine Vincent1, Aaron Elkins1, Mark Condina2, Vilnis Ezerneiks1, Nga Nguyen1, Benjamin G Cocks1, 3, Simone Rochfort1, 3
1. Department of Environment and Primary Industries, Bundoora, VIC, Australia
2. Bruker Pty Ltd, Preston, VIC, Australia
3. La Trobe University, Bundoora, VIC, Australia

Cow’s milk is a complex fluid whose proteome displays a diverse set of proteins of high abundance such as caseins (CN) and medium to low abundance whey proteins such as β-lactoglobulin (β-LG), α-lactalbumin (α-LA), lactoferrin, immunoglobulins, glycoproteins, peptide hormones and enzymes. CNs represent 80% of cow’s milk proteins and possess important nutritional and functional properties, whilst β-LG (largely unknown function) and α-LA (involved in lactose biosynthesis) represents approximately 10 and 4%, respectively. In addition, a variety of post-translational modifications of these proteins introduces further complexity to the composition of the milk proteome. As genetic variants of CNs and whey proteins influence many properties of milk that are essential to the dairy industry, the development of robust analytical methods for the separation and quantification of variants of milk proteins within single protein fractions is of interest.

Several LC and/or gel (1D- and 2D-PAGE) coupled ESI- and MALDI-based strategies have been applied for the separation and subsequent characterisation of milk protein variants [1-4]. Whilst both top down and bottom up methods could be exploited, the majority of reported analytical workflows have applied bottom-up strategies, due to the high resolution, mass accuracy and the need for large ion fragmentation capability of complex mixtures [1].

This work aims at establishing a robust LC-MS-based method to study intact major proteins from cow’s skim milk. The effects of column temperature, mobile phase composition, and gradient conditions were evaluated to improve protein separation prior to analysis using ESI-Qq-TOF MS. High resolution spectra subsequently allowed accurate mass determination and sequencing of major proteins. This top down LC-MS workflow enables a high-throughput profiling of abundant proteins of cow’s milk with minimal sample preparation.

DIRECT QUANTITATIVE ANALYSIS OF NATIVE HUMAN PEPTIDES IN COMPLEX SECRETOME SAMPLES WITH PEAKS

Cassandra Wigmore¹, Martijn Pinkse², Bharath Kumar Raghuraman², Baozhen Shan¹, Peter D Verhaert²

¹. BSI, Waterloo, Ontario, Canada
². Delft University of Technology, Delft, Netherlands

Introduction

Native (poly)peptides released into body fluids contain highly relevant information as regulatory biomolecules with both diagnostic and therapeutic potential. A major analytical challenge in secretory peptide researches is the low abundance of the analyte proportional to the large volume of extracellular matrix proteins. The current analytical solution to this problem is to enrich the peptide fraction of a complex sample by physical removal of the most abundant proteins. Here we report a direct approach to quantitative analysis of endogenous peptides in complex secretomes, without removal of highly abundant background proteins.

Methods

A high-resolution mass spectrometric workflow combining database search with de novo sequencing was proposed to facilitate analysis of the secretome beyond the dominant tryptic fragments. After a first round of database searching, with the tryptic enzyme specificity, all confident matches were filtered out. The remaining unidentified MS/MS spectra with good de novo sequencing tags were performed second round database search without enzyme specificity. This allows the identification of endogenous peptides with high sensitivity and accuracy. Peptides were quantified with intensity-based label-free quantification.

Results

Samples were conditioned FCS-containing medium of a selected population of human T-cells, which were reduced, alkylated, fractionated over C4 RP-HPLC, digested with trypsin and run by LC-MS with an LTQ Orbitrap Velos. Given a complex two-dimensional LC-MS/MS dataset (>130 GB) of conditioned medium of two differently treated primary human cell cultures sampled at three time points, PEAKS was supplemented with the described novel data analysis workflow. This allows the identification of small endogenous peptides in the presence of a very busy background of predominant tryptic peptides, which made the former invisible in standard analyses. Label-free quantitation shows that these (poly)peptide profiles contain biomarkers for specific physiological or pathological conditions.

Conclusions

Direct analysis of endogenous peptides in complex secretomes
AB SCIEX

Web: www.absciex.com

AB SCIEX is a global leader in the development of best-in-class mass spectrometer-based technologies, enabling scientists and analysts to push the limits of their fields and answer complex questions, thereby improving our world. We partner with leaders in many disciplines to create instrumentation, software, reagents, and services that are reliable, sensitive, and intuitive. We take pride in playing a supportive role in advancing science and improving our world by empowering our customers with technologies that ultimately create wellness, confidence, and trust. With a more than 20-year history of innovation, we operate as AB SCIEX, a company within the Danaher Corporation.
AGILENT TECHNOLOGIES
Web: www.agilent.com
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 is helping life science researchers learn more about cancer, cardiovascular diseases, diabetes, Alzheimer’s, Parkinson’s, autism and other ailments. Our instruments, software and sample preparation solutions help scientists conduct faster, more accurate research in multi Omics area.

APAF
Web: www.proteome.org.au/
APAF Ltd - Australian Proteome Analysis Facility, was the birthplace of the term proteomics in 1995 and was the world’s first dedicated high throughput proteomics laboratory. APAF has over 18 years of experience in providing proteomic services, and combined with our leading edge infrastructure and expertise we provide total solutions for all your proteomic research needs. APAF’s mission is to assist the scientific community address their protein analysis needs.

BRUKER BIOSCIENCES P/L
Web: www.bruker.com
Bruker continues to expand on its comprehensive range of innovative mass spectrometry systems. The new compact, impact II™, and maXis II™ ESI-QTOF Instant Expertise™ mass spectrometers are the showcase instrument platforms for life science research, drug discovery & development, and screening applications involving the analysis of unknown compounds in complex matrices. The systems provide cutting edge performance in one-shot analysis for identification and quantitation from small molecules up to antibodies.
Visit our booth for more information on these new ESI-QTOF mass spectrometers, as well as information on the new solarix XR FTMS, amaZon speed ETD Ion Trap MS, ultrafleXtreme MALDI TOF/TOF MS, and MALDI Biotyper.

BIOINFORMATICS SOLUTIONS INC
Web: www.bioinfor.com/
Bioinformatics Solutions Inc. is the creator of the industry standard PEAKS de novo software for proteomics mass spectrometry data analysis. Starting from raw mass spectrometry data, PEAKS effectively performs peptide and protein identification, PTM and mutation characterization, quantification (label and label free) as well as result validation, visualization and reporting.

CSL
Web: www.csl.com.au
CSL is a global specialty biopharmaceutical company that researches, develops, manufactures and markets biotherapies to treat and prevent serious and rare medical conditions. We produce safe and effective therapies for patients who rely on them for their quality of life and sometimes for life itself, enabling many thousands of people around the world to lead normal healthy lives. CSL employs over 13,000 staff in more than 27 countries. Our headquarters are in Australia and we have substantial manufacturing operations in the US, Germany, Switzerland and Australia. CSL also has one of the largest plasma collections networks in the world and operates the only influenza vaccine manufacturing facility in the Southern Hemisphere.

GENESEARCH
Web: www.genesearch.com.au
Genesearch is the Australian distributor of New England Biolabs molecular biology reagents; Cell Signaling Technology phospho, total, and motif antibodies; Cisbio Bioassays tools for immunoassay development; BioSilta pre-sterilized media for expression of recombinant proteins; Thomson Instrument Company consumables; and DNA2.0 custom gene synthesis and Electra vector systems.

MIMOTOPES
Web: www.mimotopes.com
Established and located in Melbourne, Australia in 1988 Mimotopes is a vibrant leading peptide, antibody and discovery chemistry research company. Mimotopes focused on its core expertise in peptide synthesis and the manufacturing of its patented SynPhase™. Our core service and product offering includes: B cell and T cell Epitope Mapping, Packs of Streptavidin Plates, Cell Penetrating TAT Peptides, Custom Peptide Libraries (PEPSETs), Custom Peptide Antibodies, PEG Peptides, Antimicrobial Peptides, Stapled Peptides, Cyclic Peptides, Multiple Disulphide Bridges, over 5,000 Catalogue Peptides and Reagents, Anything Peptide.
New::: KITS, GENE EXPRESSION DNA and PROTEIN EXPRESSION, PCR reagents, Labelling
NOVACHEM
Web: www.novachem.com.au
A supplier of research materials, standards and labelled compounds, Novachem has serviced the analytical and research industry for almost 40 years. As distribution partner for Cambridge Isotopes Limited (CIL), Novachem provides a specialised service for the proteomics and metabolomics research institutions by providing labelled amino acids, proteins and peptides, and labelling kits for both quantitative and qualitative analysis.

PEAK SCIENTIFIC INSTRUMENTS
Web: www.peakscientific.com
Peak Scientific Instruments are a manufacturer of laboratory gas generators including nitrogen, hydrogen and zero air suitable to operate most laboratory analytical applications such as LCMS (liquid chromatography mass spectrometry) and GC (gas chromatography).

PROTEOMICS INTERNATIONAL
Web: www.proteomics.com.au
Proteomics International is a specialist technology-driven life science company based in Perth, Western Australia. The Company operates in three synergistic business units: Diagnostics (biomarker discovery), Therapeutics (drug discovery of peptide medicines) and Analytical services.
PI was the first company globally to receive ISO 17025 accreditation in proteomics services.

SHIMADZU
Web: www.shimadzu.com.au
Our products and solutions serve the research community and analysts in diverse fields such as chemistry, molecular biology, medical science environmental analysis and industry. It has resulted in our leadership in spectroscopy, chromatography, mass spectrometry, environmental analysers, medical systems, aircraft equipment, semiconductors and flat panel display equipment. Our Excellence is proven in the Science, Shimadzu.

SIGMA-ALDRICH
Web: www.sigma-aldrich.com
Sigma-Aldrich is a leading life science and high technology company supplying the research community with over 270,000 products including reagents and consumables for analytical, biochemical and proteomics research. Sigma’s comprehensive offering in analytical standards and bio-separation research is world-class and includes the Supelco® HybridSPE range. For further information, visit sigma-aldrich.com

THERMO FISHER SCIENTIFIC
Web: www.thermofisher.com.au
Thermo Fisher Scientific is the world leader in serving science, enabling our customers to make the world healthier, cleaner and safer. We are the leading provider of analytical instruments, equipment, reagents and consumables, software and services for research, analysis, discovery and diagnostics.
In Australia and New Zealand, you can access the renowned Thermo Scientific portfolio as well as premium brands from our supplier partners through Thermo Fisher Scientific. Our comprehensive range includes high-end analytical and process instrumentation, laboratory equipment, a complete range of consumables, chemicals and reagents as well as the service and support to optimise your business.

WATERS AUSTRALIA
Web: www.waters.com
Waters creates business advantages for laboratory-dependent organisations by delivering practical and sustainable scientific innovation to enable significant advancement in healthcare, environmental management, food safety, and water quality. Waters products are used by pharmaceutical, life science, biochemical, industrial, academic and government organizations working in R&D, quality assurance and other laboratory applications.
ATTENDEE LIST

Jodie Abrahams
Macquarie University, NSW
Australia
jodie.abrahams@mq.edu.au

Doreen Agyapomaa
The Walter & Eliza Hall Institute of Medical Research, VIC
Australia
agypomaa@wehi.edu.au

Samira Aili
University of Technology, Sydney, NSW
Australia
aili_r@yahoo.fr

Kathirvel Alagesan
Max Planck Institute for colloid and interfaces, Germany
kathirvel.alagesan@mpikg.mpg.de

Ching-Seng Ang
University of Melbourne, VIC
Australia
ching-seng.ang@unimelb.edu.au

Peter Arthur
University of Western Australia, WA
Australia
peter.arthur@uwa.edu.au

Ali Azimi
Sydney University, NSW
Australia
aazi9158@uni.sydney.edu.au

Nicolai Bache
Bruker, Denmark
nicolai.bache@bruker.com

Mark Bailey
Zeotis Aust Research & Manufacturing Pty Ltd, VIC
Australia
mark.bailey@zoetis.com

Mark Baker
University of Newcastle, NSW
Australia
mark.baker@newcastle.edu.au

Nick Bartone
CSIRO, VIC
Australia
nick.bartone@csiro.au

Iain Berry
UTS, NSW
Australia
iain.j.berry@student.uts.edu.au

Geoff Birrell
Australian Army Malaria Institute, QLD
Australia
Geoff.Birrell@defence.gov.au

Stephen Blanksby
Queensland University of Technology, QUEENSLAND
Australia
stephen.blanksby@qut.edu.au

Megan Borg
AB SCIEX Australia, VIC
Australia
megan.doherty@absciex.com

Berin Boughton
The University of Melbourne, VIC
Australia
baboug@unimelb.edu.au

Amber Boyatzis
University of Western Australia, WA
Australia
10507456@student.uwa.edu.au

Stuart Brown
Massey University, NZ
New Zealand
Stuart_Brown@xtra.co.nz

Christopher Buck
Waters Australia, VIC
Australia
Christopher_Buck@waters.com

Matthew Campbell
Macquarie University, NSW
Australia
matthew.campbell@mq.edu.au

Marcia Chandler
Shimadzu Scientific, NSW
Australia
marcia.chandler@shimadzu.com.au

Mark Condina
Bruker, VIC
Australia
mark.condina@bruker.com

Jens Coorssen
School of Medicine, UWS, NSW
Australia
j.coorssen@uws.edu.au

Darren Creek
Monash University, VIC
darrencreek@gmail.com

Nathan Croft
Monash University, VIC
Australia
nathan.croft@monash.edu

Ben Crossett
University of Sydney, NSW
Australia
ben.crossett@sydney.edu.au

Laura Dagley
Walter and Eliza Hall Institute of Medical Research, VIC
Australia
dagley.l@wehi.edu.au

Arun Dass
Macquarie University, NSW
Australia
adass@science.mq.edu.au

Keyur Dave
QIMR Berghofer, QLD
Australia
keyur.dave@qimr.edu.au

Steven Djordjevic
University of Technology, Sydney, NSW
Australia
steven.djordjevic@uts.edu.au

Richard Edwards
University of New South Wales, NSW
Australia
richard.edwards@unsw.edu.au

Aaron Elkins
Department of Primary Industries, VIC
Australia
aaron.elkins@depi.vic.gov.au

Samantha Emery
Macquarie University, NSW
Australia
samantha.emery@mq.edu.au
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasper Engholm-Keller</td>
<td>Children's Medical Research Institute, NSW</td>
<td>kasper <a href="mailto:ENgholmKeller@gmail.com">ENgholmKeller@gmail.com</a></td>
</tr>
<tr>
<td>Madeleine Headlam</td>
<td>QIMR Berghofer Medical Research Institute, QLD</td>
<td><a href="mailto:madeleine@qimrberghofer.edu.au">madeleine@qimrberghofer.edu.au</a></td>
</tr>
<tr>
<td>Andrew Heath</td>
<td>Novachem, VIC</td>
<td><a href="mailto:andrewh@novachem.com.au">andrewh@novachem.com.au</a></td>
</tr>
<tr>
<td>Michelle Hill</td>
<td>University of Queensland, QLD</td>
<td><a href="mailto:m.hill2@uq.edu.au">m.hill2@uq.edu.au</a></td>
</tr>
<tr>
<td>Thomas Hennessy</td>
<td>Agilent Technologies, VIC</td>
<td><a href="mailto:thomas.hennessy@agilent.com">thomas.hennessy@agilent.com</a></td>
</tr>
<tr>
<td>John Hewetson</td>
<td>Shimadzu Scientific Instruments, NSW</td>
<td><a href="mailto:john.hewetson@shimadzu.com.au">john.hewetson@shimadzu.com.au</a></td>
</tr>
<tr>
<td>Hannes Hinneburg</td>
<td>MPI for Colloids and Interfaces, Germany</td>
<td><a href="mailto:Hannes.Hinneburg@mpikg.mpg.de">Hannes.Hinneburg@mpikg.mpg.de</a></td>
</tr>
<tr>
<td>Peter Hoffmann</td>
<td>University of Adelaide, SA</td>
<td><a href="mailto:Peter.Hoffmann@adelaide.edu.au">Peter.Hoffmann@adelaide.edu.au</a></td>
</tr>
<tr>
<td>Christine Hoogland</td>
<td>QIMR Berghofer Medical Research Institute, QLD</td>
<td><a href="mailto:Christine.Hoogland@qimrberghofer.edu.au">Christine.Hoogland@qimrberghofer.edu.au</a></td>
</tr>
<tr>
<td>Colin House</td>
<td>Peter MacCallum Cancer Centre, VIC</td>
<td><a href="mailto:colin.house@petermac.org">colin.house@petermac.org</a></td>
</tr>
<tr>
<td>Franziska Hundt</td>
<td>Ruhr University Bochum, Germany</td>
<td><a href="mailto:franziska.hundt@rub.de">franziska.hundt@rub.de</a></td>
</tr>
<tr>
<td>Christie Hunter</td>
<td>AB SCIEX California, CA</td>
<td><a href="mailto:Christie.Hunter@absciex.com">Christie.Hunter@absciex.com</a></td>
</tr>
<tr>
<td>Patricia Illing</td>
<td>Monash University, VIC</td>
<td><a href="mailto:patricia.illing@monash.edu">patricia.illing@monash.edu</a></td>
</tr>
<tr>
<td>Giuseppe Infusini</td>
<td>Walter and Eliza Hall Institute, VIC</td>
<td><a href="mailto:infusini@wehi.edu.au">infusini@wehi.edu.au</a></td>
</tr>
<tr>
<td>Kris Ford</td>
<td>University of Melbourne, VIC</td>
<td><a href="mailto:kferg@unimelb.edu.au">kferg@unimelb.edu.au</a></td>
</tr>
<tr>
<td>Jeff Gorman</td>
<td>QIMR Berghofer, QLD</td>
<td><a href="mailto:jeff.gorman@qimr.edu.au">jeff.gorman@qimr.edu.au</a></td>
</tr>
<tr>
<td>Michelle Hill</td>
<td>University of Queensland, QLD</td>
<td><a href="mailto:m.hill2@uq.edu.au">m.hill2@uq.edu.au</a></td>
</tr>
<tr>
<td>Hannes Hinneburg</td>
<td>MPI for Colloids and Interfaces, Germany</td>
<td><a href="mailto:Hannes.Hinneburg@mpikg.mpg.de">Hannes.Hinneburg@mpikg.mpg.de</a></td>
</tr>
<tr>
<td>Eugene Kapp</td>
<td>WEHI, VIC</td>
<td><a href="mailto:kapp@wehi.edu.au">kapp@wehi.edu.au</a></td>
</tr>
<tr>
<td>Liisa Kautto</td>
<td>Macquarie University, NSW</td>
<td><a href="mailto:liisa.kautto@mq.edu.au">liisa.kautto@mq.edu.au</a></td>
</tr>
<tr>
<td>Zon Lai</td>
<td>University of Freiburg, BW</td>
<td><a href="mailto:zon.lai@mol-med.uni-freiburg.de">zon.lai@mol-med.uni-freiburg.de</a></td>
</tr>
<tr>
<td>Chi-Hung Lin</td>
<td>Macquarie University, NSW</td>
<td><a href="mailto:mealbox@gmail.com">mealbox@gmail.com</a></td>
</tr>
<tr>
<td>Hadi Lioe</td>
<td>CSL Limited, VIC</td>
<td><a href="mailto:hadi.loie@csl.com.au">hadi.loie@csl.com.au</a></td>
</tr>
<tr>
<td>Ian Loke</td>
<td>Macquarie University, NSW</td>
<td><a href="mailto:ian.loke@students.mq.edu.au">ian.loke@students.mq.edu.au</a></td>
</tr>
<tr>
<td>Amber Lothian</td>
<td>Florey Institute of Neuroscience and Mental Health, VIC</td>
<td><a href="mailto:amber_lothian@hotmail.com">amber_lothian@hotmail.com</a></td>
</tr>
<tr>
<td>Shiyong Ma</td>
<td>UNSW, NSW</td>
<td><a href="mailto:felix.ma@student.unsw.edu.au">felix.ma@student.unsw.edu.au</a></td>
</tr>
<tr>
<td>Authors</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Abeygunawardena, D</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Abrahams, J.L</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Aerts, J</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Agrez, M</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Ahn, M</td>
<td>106,215</td>
<td></td>
</tr>
<tr>
<td>Ahn, S</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>Ahn, S.B</td>
<td>103,118</td>
<td></td>
</tr>
<tr>
<td>Aili, S</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Akune, Y</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Alagesan, K</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Alexova, R</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Alomari, M</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Amirkhani, A</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Anand, S</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Anh, M</td>
<td>106,215</td>
<td></td>
</tr>
<tr>
<td>Anh, S.B</td>
<td>103,118</td>
<td></td>
</tr>
<tr>
<td>Anh, S</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Akune, Y</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Alagesan, K</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Alexova, R</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Alomari, M</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Amirkhani, A</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Anand, S</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Anh, C</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Anugraham, M</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Aoki-Kinoshita, K.F</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Arentz, G</td>
<td>142,240</td>
<td></td>
</tr>
<tr>
<td>Arthur, P.G</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Atwell, B</td>
<td>238</td>
<td></td>
</tr>
<tr>
<td>Atwell, B.J</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Babon, J.J</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Bache, N.N</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Bacic, A</td>
<td>12,18</td>
<td></td>
</tr>
<tr>
<td>Baell, J</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Baker, M.S</td>
<td>103,118</td>
<td></td>
</tr>
<tr>
<td>Baker, M</td>
<td>115,138,22</td>
<td></td>
</tr>
<tr>
<td>Baker, M.L</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Barbier, P</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Barbour, A</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Barrett, M.P</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Beddome, G</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Belgacem, O</td>
<td>210,25</td>
<td></td>
</tr>
<tr>
<td>Bern, M</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Berry, I</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>Berry, I.J</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Beynon, R</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Bhandari, D</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Bhujel, M</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Birch, D</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Birzniece, V</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Blank, M</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Blanksby, S</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Blume, M</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Bomgarden, R</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Bonelli, F</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Boughton, B.A</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Bowdler, A</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Boyatzis, A,E</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Breen, E.J</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Brietling, R</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Briggs, M</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Bringans, S</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Bronsert, P</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Brown, S</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Brumatti, G</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Buchholz, U.J</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Buck, C</td>
<td>113,114</td>
<td></td>
</tr>
<tr>
<td>Budayeva, H.G</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Bukreyev, A,A</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Burns, C.J</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Bush, D</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Cain, J.A</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Cain, J.J</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Campbell, M.P</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Cantor, D</td>
<td>115,39</td>
<td></td>
</tr>
<tr>
<td>Cantor, D.I</td>
<td>103,118</td>
<td></td>
</tr>
<tr>
<td>Cappai, R</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Caprioli, R,M</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Care, N</td>
<td>116,239</td>
<td></td>
</tr>
<tr>
<td>Cathro, P</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Chakrabarti, R</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Chakraborty, A</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Chan, C</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Chan, P</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>Chappell, K</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Chapuis, P</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Chatar Singh , G</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Chaffield, S</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Chaudhuri, R</td>
<td>2,37</td>
<td></td>
</tr>
<tr>
<td>Cheah, W</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Chen, D</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Chen, L</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Chen, W</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Cherry, R.A</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Cheruku, H</td>
<td>118,39</td>
<td></td>
</tr>
<tr>
<td>Cheruku, H.R</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Chick, J.M</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Chik, J.H</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Ching, A.C</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Choi, E</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Christopherson, R.I</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Cobbold, S</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Cocks, B.G</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Colgrave, M</td>
<td>219,220</td>
<td></td>
</tr>
<tr>
<td>Colgrave, M.L</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Collins, P,L</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Compay, A</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Condina, M</td>
<td>111,120,18</td>
<td></td>
</tr>
<tr>
<td>Coorssen, J.R</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Cordwell, S.J</td>
<td>131,226</td>
<td></td>
</tr>
<tr>
<td>Cordwell, S.S</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Cowman, A.F</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Cox, J</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Craik, D</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Creek, D.J</td>
<td>121,15</td>
<td></td>
</tr>
<tr>
<td>Cristea, I</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Cristea, I,M</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Croft, N</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Croft, N.P</td>
<td>200,237</td>
<td></td>
</tr>
<tr>
<td>Cui, L</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Cutilias, P</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Dagley, L.F</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Dagley, M</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Daly, R</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Damen, M</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Dave, K.A</td>
<td>104,35</td>
<td></td>
</tr>
<tr>
<td>Davey, J</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>de Vertuill, D.A</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Deb Choudhury, S</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Dejean, A</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Dent, O</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Dewald, H</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Diestel, S</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Djordjevic, S</td>
<td>211,229,</td>
<td></td>
</tr>
<tr>
<td>Djordjevic, S,P</td>
<td>234</td>
<td></td>
</tr>
<tr>
<td>Doblin, M.S</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Donaldson, P.J</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Dratz, E.A</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Dudek, N</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Dukeek, N.L</td>
<td>16,237</td>
<td></td>
</tr>
<tr>
<td>Dudek, N.L</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Duo, X</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>Eggertson, M</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Ekert, P</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Elkins, A</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Ellis, K.M</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Emery, S</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Emery, S.J</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Engholm-Keller, K</td>
<td>201,226,29</td>
<td></td>
</tr>
<tr>
<td>Erce, M.A</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Escoubas, P</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Everest-Dass, A</td>
<td>111,123</td>
<td></td>
</tr>
<tr>
<td>Ezerneiks, V</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Fadgen, K</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Fazakerley, D</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Febbraio, M.A</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Fernandes, D</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Fernandez, M</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Finlay, B</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Fisher-Wellman, K</td>
<td>2,228</td>
<td></td>
</tr>
<tr>
<td>Fitzhenry, M</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Ford, K</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Foster, L.J</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Frank, R</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Gasparov, S</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Gasteige, E</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Gautier, B</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Gebhardt, C</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>George, I.S</td>
<td>10,105</td>
<td></td>
</tr>
<tr>
<td>Gehling, L</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Ghabooli, M</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Goldberg, G.L</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Goodman, D</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Gopal, S.K</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Gopinath, S</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Gorman, J.J</td>
<td>104,204,35</td>
<td></td>
</tr>
<tr>
<td>Goswami, H</td>
<td>119,219</td>
<td></td>
</tr>
<tr>
<td>Graham, M</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>Graham, M.E</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Greco, T.M</td>
<td>207,27</td>
<td></td>
</tr>
<tr>
<td>Greening, D</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Gregorevic, P</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Grey, A.C</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Grieco, P.A</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Grinyer, J</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Guan, J</td>
<td>237</td>
<td></td>
</tr>
<tr>
<td>Guise, A.J</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Gupta, R</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Gustafsson, O</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Hajrezaei, M</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Hamey, J.J</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Handler, D.C</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Hankness, L</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Hart, P</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Hart-Smith, G</td>
<td>125,205,236</td>
<td></td>
</tr>
<tr>
<td>Hastie, M.L</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Haynes, P</td>
<td>10,230,238</td>
<td></td>
</tr>
<tr>
<td>Haynes, P.A</td>
<td>105,122,14</td>
<td></td>
</tr>
<tr>
<td>Headlam, M.J</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Heck, A</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Heemskerk, A.A</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Henderson, B</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>Hetherington, L</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Hill, M</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Hinneburg, H</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ho, K</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Ho, Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Hoffman, N</td>
<td>2,37</td>
<td></td>
</tr>
<tr>
<td>Hoffmann, M</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Hoffmann, P</td>
<td>111,117,142,240</td>
<td></td>
</tr>
<tr>
<td>Holmes, G</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Hoogland, C</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Horn, D</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Horne, M</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Hosseini Salekdeh, G</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Howitt, C.A</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Huang, P.Y</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Huber, K</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Huhmer, A</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Humphrey, S</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hundt, F.C</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Hunter, C</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Hwa Chua, C</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Illiades, P</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Illing, P</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Infusini, G</td>
<td>127,40</td>
<td></td>
</tr>
<tr>
<td>Inglis, D.W</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Ivanov, A.R</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Jacoby, R</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Jagalur, A</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>James, D</td>
<td>2,228,37</td>
<td></td>
</tr>
<tr>
<td>Jarocki, V.C</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>Jayakody, B.A</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Ji, H</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Joshi, P</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Kan, A</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Kang, Y</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Kapp, E</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Karger, B.L</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Kaspar, S</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Kassem, M</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Kaufman, K.L</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Kautto, L</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Khan, A</td>
<td>110,130</td>
<td></td>
</tr>
<tr>
<td>Kleinfeld, O</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Klein, T</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Kleinert, M</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Klinger-Hoffmann, M</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>Kloehn, J</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Kolarich, D</td>
<td>101,5,8</td>
<td></td>
</tr>
<tr>
<td>Kolesnik, T.B</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Korac, P</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Kreig, A</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Krisp, C</td>
<td>140,203</td>
<td></td>
</tr>
<tr>
<td>La Gruta, N.L</td>
<td>237</td>
<td></td>
</tr>
<tr>
<td>Lacey, E</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Laffoon, S</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Lafitte, D</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Lai, Z</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Le Cao, K</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Lee, A</td>
<td>130,212</td>
<td></td>
</tr>
<tr>
<td>Leong, R.W</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>Li, D.K</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Li, L</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Lim Kam Sian, T.C</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Lin, C</td>
<td>129,132,7</td>
<td></td>
</tr>
<tr>
<td>Linossi, E,M</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Lipscombe, R</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Lisacek, F</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Loke, I</td>
<td>133,36</td>
<td></td>
</tr>
<tr>
<td>Lonsdale, A</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Loo, T.S</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Lothian, A</td>
<td>135,24</td>
<td></td>
</tr>
<tr>
<td>Lubeck, M</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Lucet, I.S</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Ma, N</td>
<td>136,221</td>
<td></td>
</tr>
<tr>
<td>Ma, S</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>MacCrae, J</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Mactier, S</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Mahboob, S</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>Mahmood, M</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Maidment, C</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Mallawaaratchy, D.M</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Mariethoz, J</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Marshall, N.C</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Martin, K.J</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Masters, C.L</td>
<td>135,227</td>
<td></td>
</tr>
<tr>
<td>Mathias, R</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Mathias, R.A</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>McCarthy, P</td>
<td>117,139</td>
<td></td>
</tr>
<tr>
<td>McColl, S</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>McConvil, M</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>McConvil, M.J</td>
<td>121,15</td>
<td></td>
</tr>
<tr>
<td>McFadden, G</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>McKay, M</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>McKay, M.J</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>McLean, C.A</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>McQuade, L</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Meikle, P</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Michalski, W</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Michalski, A</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Michelle, C</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Mifsud, N</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Millar, H</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Miller, T.A</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Mirzaei, M</td>
<td>10,105,122,141,238</td>
<td></td>
</tr>
<tr>
<td>Mitchell, T</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mittal, P</td>
<td>142,240</td>
<td></td>
</tr>
<tr>
<td>Moh, E</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Mohamedali, A</td>
<td>103,118,13</td>
<td></td>
</tr>
<tr>
<td>Mohd Arif, S</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>Mohtashemi, I</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Mok, L</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Molloy, M</td>
<td>130,140</td>
<td></td>
</tr>
<tr>
<td>Molloy, M.P</td>
<td>116,132,20,322,233,</td>
<td></td>
</tr>
<tr>
<td>Montgomery, H</td>
<td>210,25</td>
<td></td>
</tr>
<tr>
<td>Moreno, M</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td>Moritz, R</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Muralidharan, S</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Murphy, J.M</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Myers, S.J</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Nancarrow, D</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Nebl, T</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Nelson, C</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Nesvizhskii, A.I</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Nguyen, N</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Nice, E</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Nice, E.C</td>
<td>103,118</td>
<td></td>
</tr>
<tr>
<td>Nicholson, G</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Nicholson, S.E</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Nicola, N.A</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Nie, S</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Njomen, E</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Norris, E.L</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Norris, G</td>
<td>106,112</td>
<td></td>
</tr>
<tr>
<td>Norris, G.E</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Nowell, C.J</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Nye-Wood, M.G</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Nyuk Ling, M</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>O’Rourke, M.B</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>Oberstein, A</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Oehler, M</td>
<td>142,240</td>
<td></td>
</tr>
<tr>
<td>Openshaw, M.E</td>
<td>210,25</td>
<td></td>
</tr>
<tr>
<td>Orivel, J</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Overall, C</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Overall, C.M</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Packer, N</td>
<td>111,123,12,9,133,208</td>
<td></td>
</tr>
<tr>
<td>Packer, N.H</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Packer, N.H</td>
<td>132,7</td>
<td></td>
</tr>
<tr>
<td>Padula, M</td>
<td>100,211,229,234</td>
<td></td>
</tr>
<tr>
<td>Padula, M.P</td>
<td>108,216</td>
<td></td>
</tr>
<tr>
<td>Pal, M</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Pang, C</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Pang, E</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Parker, B</td>
<td>2,217,228,37</td>
<td></td>
</tr>
<tr>
<td>Parker, B.L</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Parker, R</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Partridge, M.A</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Pasovcici, D</td>
<td>105,203,239</td>
<td></td>
</tr>
<tr>
<td>Patchett, M.L</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Patel, B</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Paulech, J</td>
<td>131,226</td>
<td></td>
</tr>
<tr>
<td>Peck, G</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Pegg, C.L</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Pelzing, M</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Peterson, R</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Pham, H</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Philipneri, M</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Pickford, R</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>Piggott, M.J</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Pinkse, M</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>Pires, P.R</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>Podgorski, A</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Poth, A</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Purcell, A</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Purcell, A.W</td>
<td>16,200,237</td>
<td></td>
</tr>
<tr>
<td>Purcell, A.W</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Puri, N</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Raether, O</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Raftery, M</td>
<td>241</td>
<td></td>
</tr>
<tr>
<td>Raftos, D</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Raghuraman, B</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>Rahmat, Z</td>
<td>209,221</td>
<td></td>
</tr>
<tr>
<td>Ralton, J</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Ranganathan, S</td>
<td>118,39</td>
<td></td>
</tr>
<tr>
<td>Ravnsborg, C.C</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Raymond, B</td>
<td>211,229</td>
<td></td>
</tr>
<tr>
<td>Raymond, B.B</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Reid, G.E</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Reid, G.E</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Rembach, A</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Research Group, A</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Richter, E</td>
<td>2,37</td>
<td></td>
</tr>
<tr>
<td>Roberts, A</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Roberts, B.R</td>
<td>135,227,24</td>
<td></td>
</tr>
<tr>
<td>Robinson, P</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>Robinson, P.J</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Rochfort, S</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Roempp, A</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Roessner, U</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Rossjohn, J</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Rowland, E.A</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Saba, J</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Sampson, D</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Sandow, J</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Sansom, F</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Santos, J</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td>Santos, M</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>Sarabia, D</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Saunders, E</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Saunders, N</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Schey, K.L</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Schilling, O</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Schittenhelm, R.B</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Schittenhelm, R.B</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>SCHMIT, P</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Schmit, P.P</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Scholten, A</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Schwarz, Q</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Scott, N.N</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Seeberger, P.H</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Seeberger, P</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Selami, L</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Semaan, C</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>Sernee, F</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Shah, A</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Shan, B</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>Sharma, M</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>Sharma, S</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Sharp, P.P</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Shenk, T</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>Shiell, B</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Shockcor, J</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Shooter, G</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Silva, D</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Simpson, R.J</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Singh, T</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Slapetova, I</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Smith, B.J</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Smith, L.E</td>
<td>131,226</td>
<td></td>
</tr>
<tr>
<td>Smith, S.A</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Soldati-Favre, D</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Solis, N.N</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Song, X</td>
<td>116,239</td>
<td></td>
</tr>
<tr>
<td>Sowmya, G</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Spengler, B</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Spraggins, J.M</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Stapleton, D.I</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Stoeessel, D</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Stoynov, N</td>
<td>202,206</td>
<td></td>
</tr>
<tr>
<td>Su, T</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>Sykes, E.K</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Tacchi, J</td>
<td>211,229</td>
<td></td>
</tr>
<tr>
<td>Tacchi, J.L</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Tan, S</td>
<td>103,130</td>
<td></td>
</tr>
<tr>
<td>Tanner, G.J</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Tay, A</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Tepe, J.J</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Thaysen-Andersen, M</td>
<td>133,208,36</td>
<td></td>
</tr>
</tbody>
</table>
Thejomayen, M.J 206 | Yang, P 2,37
Thejomayen, M.P 202 | Yau, Y 232
Thompson, E 230 | Yu, Y.Q 114
Touchard, A 100 | Zaidi, N.A 221
tscharke, D.C 200,237 | Zaw, T 239
Twin, N 205 | Zeng, W 12
vaibhav, V 10,230 | ZHANG, C 142,240
van Soest, R 203 | Zhong, L 241
Venkatakrishnan, V 133,36 | Zhu, H 124
Verhaert, P.D 243 | Zilm, P 117
Villadangos, J.A 127 | Zoldoš, V 8
Villard, C 210
Vincent, D 242
Viner, R 231,31
Vivian, J 41
von Krosigk, N 202,206
Vorm, O.O 107
Vuong, D 122
Wall, J 222
Wang, L 16
Wasinger, V.C 232
Webb, A 40
Webb, A.I 32
Weinberg, A 30
White, M.M 102
White, M.Y 131,226
Whiteman, D 14
Whitham, M 233
Widjaja, M 229,234
Wigmore, C 243
Wijffels, G 219
Wilcox, M 129
Wilkins, M 125,205,3
Wilkins, M.R 236
Wilmann, P.G 223
Wilson, D 213
Winderbaum, L 111,240
Winderbaum, L.J 235
Winter, D.L 236
Wiszniaik, S 139
Wolters, D.A 126
Wong, J 137
Woon, A.P 16
Wotske, M 126
Wright, G 212
Wu, J 239
Wu, T 237
Wu, Y 10,238
Wynne, J 225
Wynne, J.W 16
Yamazaki, Y 25
Yang, H 203