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13TH EAST MIDLANDS PROTEOMICS WORKSHOP

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Wednesday 19th November 2014

Stamford Court, University of Leicester

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13th East Midlands Proteomics Workshop
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The East Midlands Proteomics Workshop (http://www.empw.co.uk) works towards creating a network of investigators active in the proteomics area. We aim to enhance awareness of facilities available in the Midlands, disseminate information relating to proteomics technologies, highlight examples of proteomics research and to provide a forum to expand research interactions.

The meeting will focus on how proteomics-based approaches are being used in biological and medical research. Keynote presentations will be given by invited speakers describing the latest advances.

The Workshop is supported by the British Society for Proteome Research (http://www.bspr.org/) and the British Mass Spectrometry Society (http://www.bmss.org.uk/).

Location
The meeting will be held at Stamford Court, Manor Road, University of Leicester, LE2 2LH

Directions and Parking
Directions to Stamford Court (post code LE2 2LH) are available at
http://www2.le.ac.uk/offices/conference/information-for-delegates/how-to-find-us-leaflet
There is **parking in the car park to the right hand side of Stamford court**. Please **note that the building is set back from the road and is not visible until you are very near it**.

Public Transport
Bus numbers **31/A** and **80/A** service the local area:
http://www.leics.gov.uk/n0383_oadby_august_14.pdf

Registration
Registration opens at 8:30 am in the **reception of Stamford Court** - which is found to the **right hand side** of the building (not the front door).

Posters
There will be a display of posters in the back of the Gilbert Hall located within Stamford Court. Presenting authors are asked to attend their posters between 13:00 and 14:00.

Trade Exhibition and Great Exhibition Prize Draw!
There will be a trade exhibition by sponsoring companies. Please visit the trade exhibition at the coffee and lunch breaks, **take along the prize draw form received at registration to have it marked by the sponsors.**
Prize Draw: There is a £50 cash prize for the first completed form picked at random at the end of the meeting. The winner **must be present** to collect the prize.

The organisers wish to thank the following companies for their generous sponsorship:

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Organising Committee
Dr Andrew Bottrill Chair EMPW2014 (University of Leicester)
Dr David Boocock (Nottingham Trent University)
Professor Helen Cooper (University of Birmingham)
Ms Clare Coveney (Nottingham Trent University)
Dr Don Jones (University of Leicester)
Dr Rob Layfield (University of Nottingham)
Dr Susan Liddell (University of Nottingham)
Dr Sharad Mistry (University of Leicester)
Dr Susan Slade (University of Warwick)

13th East Midlands Proteomics Workshop
Wednesday 19th November 2014, University of Leicester

8:30 - 9:30 **Registration**

Session 1: Chair: Andrew Bottrill

9:30 Welcome: Andrew Bottrill, chair of EMPW2014

9:35 **Keynote Presentation**
Molecular dissection of protein misfolding mechanisms
Sheena E. Radford, University of Leeds

10:15 *Purification of unanchored polyubiquitin chains with a synthetic ubiquitin linkage-selective protein and observations by ESI-MS*
Daniel Scott, University of Nottingham

10:35 Speaker for **Agilent Technologies**, GOLD SPONSORS OF EMPW2014
Only Quant What You Want
Anthony Sullivan

10:55 **Coffee / Tea / Exhibition**

Session 2: Chair: David Boocock

11:30 *Proteomics and the evolution of metabolism*
Markus Ralser, University of Cambridge & MRC National Institute for Medical Research

12:00 *A novel lipoproteomic discovery workflow for the investigation of the pleiotropic effects of statins*
Sanjay Bhandari, University of Leicester

12:20 Speaker for **Thermo Scientific,** GOLD SPONSORS OF EMPW2014
Continuing Innovation in Proteomics
Martin Hornshaw

12.40 Introduction to BSPR : **Andy Pitt**

12:45-14:15 Lunch / Exhibition
13:00 to 14:00 Poster Session

Session 3: Chair: Rob Layfield

14:15 Introduction to BMSS : **Helen Cooper**

14:20 *What's at the surface? Latest developments in LESA mass spectrometry of biological substrates*
Helen J. Cooper, University of Birmingham

14:50 *Detailed characterisation of photoactivatable metallodrug interactions with Peptides, Proteins, and DNA by high resolution tandem FT-ICR MS*
Christopher A. Wootton, University of Warwick

15.10 Coffee / Tea / Exhibition

Session 4: Chair: Sue Slade

15:40 *Proteomics of a fuzzy organelle: interphase chromatin*
Georg Kustatscher, University of Edinburgh

16:00 *Tools to aid characterizing the phosphoproteome*
Shabaz Mohammed*,* University of Oxford

16:30 Announcement of prizes

16:40 Close of meeting

13th EMPW : Oral Presentation Abstracts

Molecular dissection of protein misfolding mechanisms

Sheena E. Radford

Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

Most proteins fold efficiently to their native structures *in vivo*, assisted by molecular chaperones. It is widely known, however, that proteins do misfold and that misfolding events can result in conformational disease. Work in our laboratory aims to elucidate the mechanisms by which proteins fold, or misfold and aggregate. Our aim is to understand the fundamental principles that govern the search of the polypeptide for the native state and to inform the development of therapeutics against misfolding disease. In the lecture I will describe our recent results which have provided new insights into the very early stages of protein aggregation that reveal why a protein is amyloidogenic and how biomolecular collisions between different protein sequences can either turn an initially innocuous protein into an amyloidogenic state, or inhibit the progress of assembly. In addition, I will present new experiments using ESI-MS that we hope will assist in the search for small molecules able to inhibit defined steps in the aggregation process.

Purification of unanchored polyubiquitin chains with a synthetic ubiquitin linkage-selective protein and observations by ESI-MS

Daniel Scott[1], Thomas Garner[2], Joanna Strachan[3], Barry Shaw[1], Jed Long[4], Mark Searle[4], Neil Oldham[4], and Robert Layfield[1]

[1]School of Life Sciences, University of Nottingham, UK [2]Albert Einstein College of Medicine, Yeshiva University, USA [3]Institute of Cell Biology, University of Edinburgh, UK [4]School of Chemistry, University of Nottingham, UK

The covalent post-translational modification of proteins with polymeric ubiquitin chains, varying in length and isopeptide linkage, is critical to numerous biological pathways in eukaryotes. However a growing body of evidence also advocates a role for unanchored (or substrate-free) polyubiquitin chains in cell physiology. Insights into the biological significance of different polyubiquitin modifications have been afforded by affinity-enrichment strategies exploiting inherent non-covalent binding specificities of ubiquitin-binding domains (UBD) to purify ubiquitin-modified proteins. However natural UBDs capable of recognising specific isopeptide linkages are still largely to be identified. Here we describe the design and modelling of a completely synthetic tandem-UBD protein which affords the linkage-selective purification of unanchored polyubiquitin chains. Desired functionality of the synthetic protein is confirmed by in vitro qualitative pull-downs. Using a quantitative native ESI-MS approach to determine binding affinities in the gas phase, we show that we are able to generate an artificial avidity effect in which two UBDs work cooperatively to afford higher affinity binding. Applications in the purification of endogenous unanchored polyubiquitin from mammalian cells are described.

PRESENTATION BY Agilent Technologies GOLD SPONSOR OF EMPW2014

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Anthony Sullivan

Translational Research and all forms of clinical research involving mass spectrometric approaches require higher standards of rigour in the statistical evaluation of results, in terms of reproducibility, variance and significance. This requirement, with the associated large increase in sample cohort size needed to achieve high statistical power, gives us reason to critically challenge conventional experimental workflows in qualitative and quantitative proteomics. This presentation will discuss a fast, reproducible and statistically-powerful discovery –to-verification workflow with a reduced emphasis on identification.

Proteomics and the evolution of metabolism

Markus Ralser

University of Cambridge & MRC National Institute for Medical Research

The metabolic network is providing our cells with their chemical building blocks, and is thus centre stage for their growth and survival. To fulfil this function in the ever changing environments, the metabolic network has evolved as a highly flexible and regulated system. It is this flexibility that has recently caught attention in the medical sciences, as it opens new possibilities to target specific cells on the basis of their metabolism, and may open new perspectives in the treatment of cancer and age-associated disease. In this talk I'll discuss about how the combination of targeted metabolomics and semi-targeted proteomics helps to uncover the origins of the metabolic network, that date back to the Archean eon ~4 billion years ago. In the second part, I'll present results on our studies on the flexibility of modern metabolism. These results give insights into the

constraints that drive metabolism in our cells, and give an overview which genes, pathways and organelles are behind the regulation of metabolism.

A novel lipoproteomic discovery workflow for the investigation of the pleiotropic effects of statins

Sanjay S. Bhandari, Pankaj Gupta, Paulene Quinn, Jatinderpal K. Sandhu, Amirmansoor Hakimi, Donald J.L. Jones, and Leong L. Ng

John and Lucille van Geest Biomarker Facility
Department of Cardiovascular Sciences, University of Leicester

Introduction: Our understanding of lipoproteins has evolved over the last decade from a simplistic role centred on the transportation of lipids, to roles involving inflammation, coagulation and redox reactions, due to their unique protein cargo. The introduction of statins has revolutionised the treatment of cardiovascular disease. In this study we sought to determine the impact of statin therapy on lipoprotein remodelling in subjects with hypercholesterolemia. Using a sub-proteome of the plasma proteome we plan to investigate the modulation of proteins associated with cardiovascular disease.
Methods: 11 subjects with hypercholesterolemia were recruited into this study. Blood was withdrawn at baseline and after a minimum of 6 weeks statin therapy. Lipoproteins were isolated using a novel affinity resin and quantitatively analysed using label-free LC-MS/MS.
Results: 220 proteins were identified, of which 33 proteins demonstrated significant differential expression between the pre-statin and the statin group. ApoB100, tenascin X and complement D were down-regulated. Vinculin, integrin beta-3 and peroxiredoxin-2 were up-regulated by statins.
Discussion: Statins were associated with an up-regulation of vinculin, which may limit the cytoskeletal disarray that occurs in atherosclerosis and promote plaque stability. An up-regulation of integrin beta-3 and peroxiredoxin-2 by statins may confer protection, as knockout mice models displayed greater atherosclerosis. The benefit of statins goes beyond their lipid lowering effects. This study identifies novel pleiotropic effects of statins.

PRESENTATION ON BEHALF OF Thermo Scientific GOLD SPONSORS OF EMPW2014

Continuing Innovation in Proteomics

Martin Hornshaw

Recent technology innovations have led to the ability to perform extensive proteome characterisation with less than a day of ‘effort’. Much of that innovation has been in the area of mass spectrometry. In this presentation a brief history of the development of the orbitrap mass analyser and its hybridization with other mass analysers will be discussed. Recent progress and its impact on proteomics and proteogenomics performance will be described.

What’s at the surface?
Latest developments in LESA mass spectrometry of biological substrates

Helen J. Cooper

University of Birmingham

Liquid extraction surface analysis (LESA) is an ambient surface mass spectrometry approach which looks particularly promising for protein analysis. The benefits of LESA are speed of analysis, reduced sample preparation requirements and sample loss, potential for multiple sampling of the same location and, in the case of tissue sections, the potential to image multiple analytes.
The applications of LESA in top-down and bottom-up proteomics of a range of biological surfaces, including dried blood spots, thin tissue sections and bacterial colonies growing on agar, will be discussed. Latest developments incorporating LESA with ion mobility spectrometry will be presented.

Detailed characterisation of photoactivatable metallodrug interactions with Peptides, Proteins, and DNA by high resolution tandem FT-ICR MS

Christopher A. Wootton, Andrea F. Lopez-Clavijo, Evyenia Shaili, Mark P. Barrow, Peter J. Sadler, and Peter B. O’Connor

Department of Chemistry and Warwick Centre for Analytical Science (WCAS)
University of Warwick, CV4 7AL, UK

Metal based Chemotherapeutics are highly effective and are used worldwide to treat various strains of cancer, unfortunately (often due to selectivity issues) a wide range of dangerous side effects are experienced by patients. A new approach is to use localised activation of an inert metallo-pro-drug to ensure these effective compounds are only active where they are needed – near cancerous tissue. Meaning less of the drug is wasted

reacting in other parts of the body, causing fewer side effects in these areas, this can also translate into reduced dosages, especially since some of these drugs have been shown to be an order of magnitude more potent than Cisplatin under comparable conditions.

FT-ICR MS offers ultra-high resolving power and unparalleled mass accuracy providing the highest possible confidence during analysis of these unique and previously unexplored biomolecule modifications. We show tandem FT-ICR mass spectra of novel peptides, proteins, DNA, and their metallodrug-modified counterparts in order to deduce not only the binding sites of said drugs to biopolymers, but also to investigate the varying identity of the modifications post-photoactivation and present examples of their unique behaviour under electron based dissociations. ECD in particular was found to be critically important for retaining these modifications during MS/MS analysis. We thank the ERC, EPSRC, BBSRC, WCPRS and Bruker Daltonics for support, and all our collaborators.

Proteomics of a fuzzy organelle: interphase chromatin

Georg Kustatscher[1], Juri Rappsilber[1 &2,] Nadia Hegarat[3], Karen L. H. Wills[1], Cristina Furlan[1], Jimi-Carlo Bukowski-Wills[1] and Helfrid Hochegger[3]

[1]Wellcome Trust Centre for Cell Biology, University of Edinburgh, EH9 3JR, UK
[2]Department of Biotechnology, Technische Universität Berlin, Germany
[3]Genome Damage and Stability Centre, University of Sussex, BN1 9RQ, UK

Chromatin proteins mediate replication, regulate expression and ensure integrity of the genome. So far, a comprehensive inventory of interphase chromatin has not been determined. This is largely due to its heterogeneous and dynamic composition, which makes conclusive biochemical purification difficult, if not impossible. As a fuzzy organelle it defies classical organellar proteomics and cannot be described by a single and ultimate list of protein components. Instead we propose a new approach that provides a quantitative assessment of a protein's probability to function in chromatin (EMBO Journal, 2014). Our analysis followed a three-stage process. (1) We developed a new protocol to biochemically isolate chromatin enriched fractions. (2) We used machine learning to integrate chromatin composition over a range of biochemical and biological conditions, thereby encapsulating different degrees of involvement of proteins with chromatin. (3) We derived for each protein its probability of having a general chromatin-based function. This resulted in interphase chromatin probabilities for 7635 human proteins, including 1840 previously uncharacterized proteins. We demonstrated the power of chromatin probabilities to predict novel chromatin factors during analyses of CDK regulation (with Helfrid Hochegger, University of Sussex) and DNA replication (with Anja Groth, University of Copenhagen; Nature Cell Biology, 2014).

Tools to aid characterizing the phosphoproteome

Shabaz Mohammed

University of Oxford and Utrecht University

Currently, the preferred analytical strategy for phosphoproteome studies involves an enrichment step coupled to 2D LC. However, such a strategy is known to have a number of weaknesses including insufficient separation power and a bias against phosphosites which are generated by basophilic kinases. I will demonstrate how these biases can be alleviated by modifying enrichment and MS conditions and how separation power can be increased through an extra chromatographic step.

We have demonstrated that a refined SCX chromatographic system can isolate multiply phosphorylated, N-acetylated and singly phosphorylated peptides. Analysis of these near pure populations, once again, emphasized their inherent complexity and the deficiencies of the final LCMS step. In order to make these pools more palatable by LCMS we developed additional fractionation strategies based on HILIC or WAX. Taking a single SCX fraction and then subjecting it to WAX we were able to dramatically increase the number of phosphosites identified. The SCX system does not allow purification of phosphopeptides containing multiple basic residues. Such peptides are often products of basophilic kinases. Unfortunately, these peptides are often poorly enriched by chelation strategies. Here, we show a careful choice of material (Ti-IMAC) and solvent conditions can dramatically improve enrichment. We also found that ETD of such peptides is far superior to classical HCD or CID sequencing. I will demonstrate strategy with an application involving Rap signaling.

Our optimised enrichment protocol now allows over 90% enrichment on a lysate. I will demonstrate that enrichment followed by a 3 hour single LCMS analysis one can obtain ~5000 sites or 15,000 in a simplified 2DLC strategy.

I will finish by discussing a new sequencing method we have developed (EThcD) that often allows unambiguous site localization. I hope to show that there are many (complementary) tools available to interrogate the phosphoproteome.

13th EMPW : Poster Presentation Abstracts

1) Exoproteomics of marine *Synechococcus*: the novelty is in their secreted proteome

Joseph A Christie-Oleza[1], J Armengaud[2] and DJ Scanlan[1]

[1]School of Life Sciences, University of Warwick, Coventry, UK
[2]CEA, DSV, IBEB, Lab Biochim System Perturb, Bagnols-sur-Cèze, France

Microorganisms continuously exchange compounds with their environment and exert an influence on it by secreting functional proteins. Hence, the secreted protein fraction is how a microorganism will be perceived by other members of the community and will determine its life strategy in the environment. Bioinformatic prediction of the secreted panproteome of various *Prochlorococcus* and *Synechococcus* lineages demonstrated that the secretome had a much higher incidence of unknown proteins and with cytoplasmic functions much more conserved throughout the group, suggesting that the genomic and functional diversity of these organisms lies largely in their secretome. Biological implications would be higher diversity against predators, a step into niche partitioning, and a diverse pool of novel functions we are still far from understanding. Experimental exoproteome analysis of marine *Synechococcus* showed a large number of transport systems for inorganic nutrients, a large array of strain-specific exoproteins involved in mutualistic or hostile interactions, and exoenzymes with an ultimate mixotrophic goal. Marine *Synechococcus* can remodel its exposed proteome by increasing its repertoire of interaction proteins when grown in presence of a heterotroph or decrease its exposure to predators when grown in the dark. Finally, our data indicates *Synechococcus* is a leaky system given the accumulation of large amounts of non-secreted proteins that can well support heterotrophic life.

2) Proteomic response of Saccharomyces species to fermentation stress of formic acid in bioethanol production

Cyprian E Oshoma[1], Susan Liddell[1], Kenneth G Davis[1], Trevor Phister[2], Katherine A Smart[3], Chris D Powell[1] and Chenyu Du[1]

BBSRC Sustainable Bioenergy Centre Programme LACE
[1]School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK [2]PepsiCo Int. Beaumont Park, Leicester LE1 4ET, UK [3]SAB Miller PLC, Surrey GU21 6HS, UK

One of the current challenges associated with bioconversion of the lignocellulose hydrolysates to ethanol is the inhibitory compounds generated during pre-treatment of the lignocellulose biomass. These inhibitors are toxic to yeasts in the fermentation step. Formic acid is one of the weak acid inhibitors released into the hydrolysates at concentrations of 10 - 30 mM, and much is yet to be known about the yeasts response to this acid. In this study, *Saccharomyces cerevisiae* NCYC2592 and *Saccharomyces arboricolus* 2.3319 were used to evaluate changes at protein level in response to formic acid during bioethanol fermentations. The fermentation profile in the formic acid medium was compared with the control (without formic acid). Samples were analysed for cell counts, glucose utilisation, ethanol and glycerol productions during time interval. Cytosolic proteins of Saccharomyces spp at the early stage of fermentation (4 hours) were analysed using 2D-SDS PAGE and Progenesis Samespots software. Cytosolic proteins that were abundantly expressed in the 40 mM formic acid condition were identified. Protein spots with a fold increase of greater than 1.5 and $p < 0.05$ were considered for spot picking. 11 proteins from *S. cerevisiae* NCYC2592 and 8 proteins from *S. arboricolus* 2.3319 were identified by LC-MSMS and found to be related to formic acid stress response. Among these identified proteins, LEU2 was observed to be present in both strains suggesting it to play a key role in formic acid stress response and cell survival during fermentation.

3) Detection of Angiotensinogen Cysteine Peptides Involved in the Redox Switch Associated with the Pathogenesis of Pre-eclampsia by High Resolution Mass Spectrometry

Lina Dahabiyeh[1], David Tooth[2], Robert Layfield[2], Aiwu Zhou[3], Robin W. Carrell[4] Yahui Yan[3], Randy J Read[3], and David A Barrett[1]

[1]Centre for Analytical Bioscience, School of Pharmacy, University of Nottingham, NG7 2RD UK
[2]School of Biomedical Sciences, University of Nottingham NG7 2UH UK [3]Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, CB2 0XY, UK [4]Department of Medicine, Cambridge Institute for Medical Research, University of Cambridge, CB2 0XY, UK

Angiotensinogen (AGT) is a critical protein in the renin angiotensin system and has an important role in the pathogenesis of pre-eclampsia. The recently resolved AGT crystal structure revealed a key role for the disulphide linkage between cysteine (Cys) 18 and 138 in the redox switch of AGT that modulates angiotensin

release, and hence blood pressure, with an increased percentage of the oxidized form of AGT present in the plasma of pre-eclamptic women.
Using the human recombinant form of AGT, methods for the quantification of the two key Cys18 and Cys138 were evaluated using in-solution as well as in-gel digestion with trypsin and chymotrypsin. Cys peptides were either alkylated with iodoacetamide or N-ethylmaleimide and the AGT digest was analysed using high resolution LC-MS analysis. The chymotryptic digest of AGT from in-solution digestion resulted in the best protein sequence coverage of 57%. Good protein recovery and efficient digestion was also achieved and both modified Cys18 and Cys138 peptides were detected with good signal intensity and confirmed within 2 ppm of theoretical mass applying a fast LC-MS method. The identity of the detected alkylated Cys peptides was further confirmed with peptide sequencing by LC-MS/MS. The consistent and reproducible detection of the two key peptides with this methodology provides a good level of confidence for the detection of these peptides from human plasma, progressing towards the ultimate goal of applying targeted proteomic approach to quantify the reduced and oxidized form of AGT in human plasma.

4) Proteomics comparison of naphthalene metabolism of different *Pseudomonas stutzeri* strains

Isabel Brunet-Galmés[1], JA Christie-Oleza[2], B Nogales[1], and R Bosch[1]

[1]Microbiologia, Departament de Biologia, Universitat de les Illes Balears (UIB), Palma de Mallorca, Spain
[2]School of Life Sciences, University of Warwick, CV47AL, UK

Naphthalene is a polycyclic aromatic compound with high eco-toxicity and whose presence has been increased on the marine environment due to human activity. Naphthalene degradation by members of the species *Pseudomonas stutzeri* has been intensively studied. The genetic analysis of their naphthalene catabolic genes has revealed identical degradation strategies: naphthalene is channelled to salicylate and catechol, and this last one is further meta-cleaved to TCA cycle intermediates. However, we are not able to identify accessory proteins (transporters, efflux pumps, etc.) involved in this pathway that may explain the different behaviour of different *P. stutzeri* strains in the use of salicylate, the inducer of the catabolic pathway. To complete this study, a proteomic analysis of five naphthalene degrader strains has been performed (4 *P. stutzeri* and a strain of *P. balearica*). The comparison of their protein expression patterns when grown on naphthalene and salicylate as sole sources of carbon and energy allowed us to identify the accessory proteins involved in the degradation of naphthalene, determine the cellular trafficking of these aromatic compounds, and extend our findings to polluted natural systems.

5) How and why is the N-terminal cysteine of plant N-end rule substrates oxidised?

Daniel J Rooney[1], Susan Liddell[1], Neil J Oldham[2], Michael J Holdsworth[1].

[1]School of Biosciences, Sutton Bonington, LE12 5RD
[2]School of Chemistry, University Park Nottingham, NG7 2RD

The N-end rule pathway is a targeted proteolysis component of the ubiquitin proteasome system. Specific proteins bearing an N-terminal cysteine residue are substrates of the N-end rule pathway, requiring oxidation for degradation to occur. The ability of N-terminal cysteine proteins to 'sense' both oxygen and nitric oxide in the environment may help develop crops which can tolerate low oxygen conditions. This study aims to identify how N-terminal cysteine is oxidised using an in vivo and in vitro approach. A tagged artificial reporter protein was extracted from 7 day old transgenic seedlings of *Arabidopsis thaliana*, the protein was purified and used to perform 1D gel electrophoresis. The 'target' protein gel band was visible after comparing the coomassie stained protein profile with the western blot profile. The protein band was excised and digested then analysed by ESI-MS/MS, precursor and fragment masses were recorded using the MASCOT ions search server. Successful identification of peptides from the N-terminal cysteine reporter protein was observed although the N-terminal peptide has not yet been detected. To complement the *in vivo* approach, a synthetic peptide with an N-terminal sequence matching that of the *in vivo* reporter was also analysed by ESI-MS/MS. After exposure to hydrogen peroxide (H_2O_2), the peptide mass was observed to be increased by 48 Da. This mass correlates with a shift from Cys-thiol (-SH) to Cys-sulphonic acid (Cys-SO_3H), indicating that oxidation had occurred. Controlling oxidative conditions in vitro should help us understand the oxidative changes which occur to the N-terminal cysteine in vivo.

6) Evaluation of a modified FAIMS Interface: Improvement in Nonredundant Peptide Identification in Proteomic Analyses

Hongyan Zhao[1], Andrew J Creese[1], Michael W Belford[2] and Helen J Cooper[1]

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[2]Thermo Fisher Scientific, San Jose, CA 95134, USA

Field Asymmetric Ion Mobility Spectrometry (FAIMS) separates ions based on differences in mobility in high and low electric fields, providing an extra dimension of selectivity prior to mass spectrometric analysis. FAIMS

can significantly improve S/N, facilitating identification of low abundant peptides. Nevertheless, low transmission efficiency results in a reduction in ion signal. The standard Thermo FAIMS device comprises of cylindrical electrodes with a 2.5 mm gap. Recently, a new FAIMS interface has been introduced with reduced electrode gap (1.5 mm) and novel electrode design (1). The reduction in gap distance increases the electric field and reduces residence time of ion. We evaluated the performance of the two FAIMS interfaces using a standard 6- protein digest and whole cell lysate from breast cancer SUM52 cells. Samples were analysed by external CV-stepping LC-FAIMS-MS/MS. Similar sequence coverage was observed from the standard digests, but a 71.3% increase in PSM and 2-fold increase in SEQUEST score was found for the modified FAIMS. For the SUM52 samples, the number of peptides identified via the modified FAIMS was 28.0% greater than the number identified via standard FAIMS. The number of peptides identified that were unique to a particular CV was 57% greater for the modified FAIMS than the standard FAIMS. (1) Prasad, S.; Belford, M. W.; Dunyach, J.-J.; Purves, R. W. J. Am. Soc. Mass Spectrom. 2014. [Epub ahead of print]

7) A proteomic approach to assess alcohol-related brain damage in humans

Amaia M. Erdozain[1,2], Benito Morentin[3], Lynn Bedford[4], Emma King[4], David Tooth[4], Charlotte Brewer[1], Declan Wayne[1], Peter Wigmore[4], Luis F. Callado[2], and **Wayne G. Carter**[1]

[1] School of Medicine, University of Nottingham, Royal Derby Hospital Centre, DE22 3DT, UK [2]Department of Pharmacology, University of the Basque Country, and Centro de Investigación Biomédica en Red de Salud Mental, Spain [3]Section of Forensic Pathology, Basque Institute of Legal Medicine, Bilbao, Spain [4]School of Life Sciences, University of Nottingham, NG7 2UH, UK.

Chronic excessive alcohol intoxications evoke cumulative damage to tissues and organs. We examined prefrontal cortex (Brodmann's area (BA) 9) from 20 human alcoholics and 20 age, gender, and postmortem delay matched control subjects. H & E staining and light microscopy of prefrontal cortex tissue revealed a reduction in the levels of cytoskeleton surrounding the nuclei of cortical and subcortical neurons, and a disruption of subcortical neuron patterning in alcoholic subjects. BA 9 tissue homogenisation and one dimensional polyacrylamide gel electrophoresis (PAGE) proteomics of cytosolic proteins identified dramatic reductions in the protein levels of spectrin β II, and α- and β-tubulins in alcoholics, and these were validated and quantitated by Western blotting. We detected a significant increase in α-tubulin acetylation in alcoholics, a non-significant increase in isoaspartate protein damage, but a significant increase in protein isoaspartyl methyltransferase protein levels, the enzyme that triggers isoaspartate damage repair in vivo. There was also a significant reduction inproteasome activity in alcoholics. One dimensional PAGE of membrane-enriched fractions detected a reduction in b-spectrin protein levels, and a significant increase in transmembranous α3 (catalytic) subunit of the Na+,K+-ATPase in alcoholic subjects. However, control subjects retained stable oligomeric forms of α-subunit that were diminished in alcoholics. In alcoholics, significant loss of cytosolic α- and β-tubulins were also seen in caudate nucleus, hippocampus and cerebellum, but to different levels, indicative of brain regional susceptibility to alcohol-related damage. Collectively, these protein changes provide a molecular basis for some of the neuronal and behavioural abnormalities attributed to alcoholics.

8) Characterisation of a novel spontaneous model of epithelial to mesenchymal transition using a primary prostate cancer cell line using quantitative label-free proteomics (SWATH™)

David J Boocock[1], Naomi Dunning-Foreman[1], Jayakumar Vadakekolathu[1]
Clare Coveney[1], Matthew Nicklin[1], Graham J Hickman[1], Thomas Knapman[2]
Sibylle Heidelberger[2], A Graham Pockley[1] and Robert C Rees[1]

[1]John van Geest Cancer Research Centre, Nottingham Trent University, NG11 8NS, UK
[2]AB SCIEX, Warrington, UK

Introduction and Objectives: Current evidence suggests that cancer cells reactivate the latent embryonic programme of epithelial to mesenchymal transition (EMT) in order to acquire the invasive and migratory/metastatic properties that are associated with aggressive disease and its progressive in cancer. We have developed a spontaneous model of EMT using a primary human prostate cancer cell line (OPCT1), and used High Resolution Accurate Mass mass spectrometry and data independent acquisition (SWATH™) to quantify the secretome of the epithelial and mesenchymal clonal progeny.
Methods: The epithelial and mesenchymal model cell lines have previously been characterised on the basis of their expression of classic markers for epithelial/mesenchymal traits (including vimentin, E-cadherin, fibronectin, SLUG, SNAIL, TWIST, ZEB1, OCT, SOX2, NANOG) using qRT-PCR, Western blot, immunofluoresence and other functional assays. For this study, supernatants from epithelial and mesenchymal model cell lines that had been grown to 70% confluency in a complete KSF media with and without serum were analysed using an AB SCIEX 5600+ TripleTof instrument using SWATH™ methodology.
Results: A total of 822 proteins (n=10 biological replicates) derived from the harvested secretome of OPCT1 parental, epithelial and mesenchymal clones were quantified by SWATH™ from an in-house ion library containing 1356 proteins (1% FDR). 43 of these proteins were categorised as being significantly

downregulated and 38 as being upregulated in the mesenchymal clone on the basis of a 0.7 log fold change and $p<0.05$.
Conclusions: Classical markers of mesenchymal and epithelial clones cell types, such as VIME, COCA1, TSP1, CO5A1, K2CB, MMP2, PCDH1 among others were identified as being the top differentially expressed proteins using quantitative mass spectrometry. This technique could therefore be used as a substitute for conventional laboratory-based techniques for cell characterisation.

9) Detection and quantification of apolipoprotein F in human plasma by selected and parallel reaction monitoring using Skyline

Abhinav Kumar, Bevin Gangadharan, Raymond A. Dwek and Nicole Zitzmann

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Liver biopsy is the reference standard for determining the stage of liver fibrosis and no reliable non-invasive approach is currently available to differentiate between neighbouring fibrosis stages. We have previously identified apolipoprotein F (APO-F) as a potential biomarker for liver fibrosis by analysing plasma samples from hepatitis C patients. APO-F is a low abundant protein in normal human plasma and its concentration decreases as the severity of liver fibrosis progresses.
We have developed a fast, sensitive and robust antibody-free method to detect and quantify APO-F in human plasma to help determine the stage of liver fibrosis. We have used the freely available Skyline software for data analysis which accepts data acquired on multiple mass spectrometers. Selected reaction monitoring (SRM) and parallel reaction monitoring (PRM) were used to detect and quantify APO-F in human plasma. We explain the steps involved in targeted quantitation using Skyline. For absolute quantitation a digest of foetal calf serum was used as blank matrix, which does not contain human APO-F. Different concentrations of AQUA peptides of APO-F were spiked into this matrix to plot a calibration curve. The concentration of APO-F in normal human plasma was found to be 44 ng/mL.
We propose to use this approach as a potential diagnostic tool to aid clinicians in determining the severity of hepatic fibrosis and reducing the need for invasive liver biopsies.

10) Mice and Bats: What can they tell us about mammalian ageing?

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Mammalian brain ageing has been associated with impaired mitochondrial function. Our study investigates ageing by comparing the mitochondria of two small mammals. The metabolic theory of ageing proposes that the higher the metabolic rate of an organism, the shorter the lifespan. Bats are an anomaly as they have a high metabolic rate but are exceptionally long-lived. The little brown bat, *Myotis lucifugus*, has a life span of up to 34 years - in contrast mice have a maximum life span of 4 years. We have begun by comparing the mouse and bat mitochondrial proteome. Mitochondria were extracted from brain and skeletal muscle from mice (C57BL/6, age 4 weeks-18 months) and bats (*Pipistrellus pipistrellus*, juvenile and mature). 2D gel electrophoresis was used to compare the proteomes and gel profiles were analysed using SameSpots software (Totallab Ltd). We aim to identify differences between the mitochondrial proteome of these mammals.

11) Processing of rapeseeds affects the ileal digestibility of dietary protein

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Rapeseed meal (*Brassica napus*, RSM) is primarily utilized by the feed industry as a protein source. The nutritional quality of rapeseed protein is as high as that of animal protein, but the digestibility of RSM protein is lower, a finding rationalised by the supposed reduced digestibility of napin. Napin belongs to albumin family, and consists of a small (~4.5 kDa) and a large chain (~10 kDa). Sixteen cultivars of RSM, processed with cold (n=4) or hot (n=12) oil extraction were formulated in diets at 500 g/kg and fed to 14-days-old boiler chickens. After the ileal digesta were collected, the apparent ileal digestibility of total amino acids (TAA) and protein profiles (SDS-PAGE) were analysed. Apparent ileal digestibility was substantially higher after feeding the cold de-oiled RSMs compared to hot de-oiled RSMs (88-91% vs. 76-83%). SDS-PAGE profiles showed that hot oil extraction led to a loss of band intensity of napin in ileal digesta, whereas only faint bands of napin remained

in ileal digesta of cold de-oiled RSMs. Our study showed that the apparent ileal digestibility of TAA is influenced by the processing but not by degradation of napin.

12) Distinguishing cryptdin protein isoforms using ETD/PTR ion trap mass spectrometry based top down sequencing

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Infection of mice with the parasitic nematode *Trichinella spiralis* recapitulates aspects of human chronic inflammatory diseases of the intestine and represents a useful model system to study inflammatory responses in the small intestine. Following exposure to the parasite, immune effector molecules including cryptdins are released by Paneth cells. Cryptdins are anti-microbial peptides of the α-defensin family. There are more than 20 different forms of mouse cryptdin cDNAs, but only 6 of these proteins have been isolated and characterised. Cleavage of the pre-pro domains produces active cryptdin molecules 33-35 residues long. The deduced amino acid sequences of the mature peptide isoforms are similar with up to 91% sequence identity, some isoforms differ by only 2 or 3 residues.
Our aim in this study was to molecularly characterise the population of cryptdins in T. spiralis infected mouse intestinal epithelial cells. Protein samples were subjected to reverse phase high performance liquid chromatography. We attempted to establish which isoforms were present in different HPLC fractions using a variety of MS-based techniques including LC-MSMS of tryptic peptides, intact mass determination using ESI-MS, and top down sequencing using CID or ETD/PTR. Only ETC/PTR top down sequencing provided data that distinguished between the isoforms.

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