# 10TH EAST MIDLANDS PROTEOMICS WORKSHOP

## Wednesday 2nd November 2011

## Sir Denis Rooke building, Holywell Park, Loughborough University

# 10th East Midlands Proteomics Workshop

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The East Midlands Proteomics Workshop (http://www.empw.org.uk) was established to create a network of investigators active in the proteomics area with a view to enhancing awareness of facilities available in the East Midlands, disseminating specialist knowledge and information relating to proteomics technologies, highlighting examples of proteomics research and providing a forum to discuss 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 external 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 in the Sir Denis Rooke building on the Loughborough University, Holywell Park Campus. Further information about Holywell Park can be found at:
http://www.welcometoimago.com/delegates/holywell-park

**Directions and Parking**
Directions to Holywell Park (post code LE11 3GR) are available at the location http://www.welcometoimago.com/contact/location/maps. There is ample parking in the car park adjacent to the Sir Denis Rooke building. A shuttle bus operates from Loughborough railway station to Holywell Park, departing the station every 10 minutes.

**Registration**
Registration opens at 8:30 in the foyer of the Sir Denis Rooke building.

**Posters**
There will be a display of posters in the exhibition area of the Sir Denis Rooke Building. 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. **Prize Draw:** There is a £50 cash prize for the first completed form picked at random. The winner must be present at the end of the meeting to collect the prize.

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

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Ms Clare Coveney (Nottingham Trent University)
Dr Don Jones (University of Leicester)
Dr Rob Layfield (The University of Nottingham)
Dr Sharad Mistry (University of Leicester)
Dr Susan Liddell (The University of Nottingham)

**Programme**

**10th East Midlands Proteomics Workshop**
**Wednesday 2nd November 2011, Loughborough University**

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

**Session 1**: Chair: Colin Creaser

9:30 Welcome – Professor Paul Chung, Dean of the School of Science, Loughborough University

9:40 **Modern phosphoproteomics; can we see everything?**
Nick Morrice, The Beatson Institute, Glasgow

10:20 **Inflammatory and proteomic changes in the circulation and adipose tissue in response to two weeks of high intensity intermittent exercise in overweight and obese males**
Wayne Carter, University of Nottingham & Loughborough University

10:40 **Proteomics of Combretastatin Treated Fibrosarcomas Using Ion-Mobility MALDI Mass Spectrometry Imaging**
Laura Cole, Sheffield Hallam University

11:00 **Coffee / tea / Exhibition**

**Session 2**: Chair: David Boocock

11:30 **Quantification; how hard could it be?**
David Knight, The University of Manchester

12:10 **End to End Proteomics – from Sample to Orbitrap**
Martin Hornshaw, Thermo Scientific

12:30 **Lunch / Exhibition**

**Poster Session from 13:00 to 14:00**

**Session 3**: Chair: Rob Layfield

14:00 **Special presentation for the 10th anniversary of EMPW**
**High resolution, high accuracy mass spectrometry for comprehensive proteome identification**
Matthias Mann, Max-Planck Institute of Biochemistry, Martinsried, Germany

14:45 **The importance of total system peak capacity for complex proteome analysis**
Robert Tonge, Waters Corporation

15:05 **Coffee / tea / Exhibition**

**Session 4:** Chair: Susan Liddell

15:40 **Non-covalent Ubiquitin-Ubiquitin Binding Domain interactions studied by Electrospray Ionisation-Travelling Wave Ion Mobility Spectrometry-Mass Spectrometry**
Kleitos Sokratous, The University of Nottingham

16:00 **Interrogating protein conformation and biomolecular complexes with IMS-MS**
Alison Ashcroft, University of Leeds

**16:40 Announcement of prizes**

**16:50 End of meeting**

# 10th East Midlands Proteomics Workshop: Oral Presentation Abstracts

## Modern phosphoproteomics; can we see everything?

***Nick Morrice***

*The Beatson Institute for Cancer Research, The University of Glasgow*

Phosphoproteomics has become more routine over the past few years and is often used to define signalling pathways that are affected by treatment of activators or inhibitors of protein kinase or protein phosphatases. Large scale phosphoproteome studies have identified up to 30000 sites and these can be quantified using either in vitro or in vivo labelling techniques such as ITRAQ, Dimethylation or SILAC. The workflows used to enrich phosphopeptides from cell lysates are varied, but the overall number of phosphorylation sites identified from a number of laboratories are similar. In this presentation I will compare the sites found for individual proteins from large scale phosphoproteome studies compared with individual phosphoprotein characterisation experiments. The benefits and limitations of phosphoproteomic workflows will also be discussed.

## Inflammatory and proteomic changes in the circulation and adipose tissue in response to two weeks of high intensity intermittent exercise in overweight and obese males

*Melanie Leggate[1], **Wayne G Carter**[2], Matthew JC Evans[1], Rebecca A Vennard[1], Sarah Sribala-Sundaram[1] and Myra A Nimmo[1]*

*[1]School of Sport, Exercise and Health Sciences, Loughborough University*
*[2]School of Graduate Entry Medicine & Health, University of Nottingham Medical School, Royal Derby Hospital*

Obesity is associated with chronic low-grade inflammation and underpins many long-term debilitating health conditions, including Type 2 diabetes mellitus and cardiovascular disease. Exercise training as a lifestyle intervention has the potential to counteract obesity, and we have examined the effects of two weeks of high intensity intermittent training (HIIT) on the inflammatory status in plasma and adipose tissue of an overweight and obese male cohort. Twelve participants (mean (SD); age 23.7 (5.2) y, body mass 91.0 (8.0) kg, BMI 29.1 (3.1) kg·m-2) undertook 6 HIIT sessions over 2 weeks. Resting blood and subcutaneous adipose tissue samples were collected and insulin sensitivity determined, pre- and post-training. Inflammatory proteins were quantified in plasma and adipose tissue. There was a significant decrease in soluble interleukin 6 receptor (sIL-6R; $p = 0.050$), monocyte chemotactic protein-1 (MCP-1, $p = 0.047$) and adiponectin ($p = 0.041$) in plasma post-training. Plasma IL-6, intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor-α (TNF-α), IL-10 and insulin sensitivity did not change. In adipose tissue, IL-6 significantly decreased ($p = 0.036$) and IL-6R increased ($p = 0.037$), whilst adiponectin tended to decrease ($p = 0.056$), with no change in ICAM-1 post-training. TNF-α, MCP-1 and IL-10 were not detectable in adipose tissue. Adipose tissue biopsies taken from participants before and after exercise training were homogenised and proteins profiled using one-dimensional gel electrophoresis. The most prominent changes in the adipose tissue proteome as a consequence of HIIT were determined. This proteomic approach identified significant reductions in annexin A2 ($p = 0.046$) and fatty acid synthase ($p = 0.016$) as a response to HIIT. The current investigation suggests two weeks of HIIT is sufficient to induce beneficial alterations in the resting inflammatory profile and adipose tissue proteome of an overweight and obese male cohort.

## Proteomics of Combretastatin Treated Fibrosarcomas Using Ion-Mobility MALDI Mass Spectrometry Imaging

***Cole LM[1]**, Bluff J[2], Claude E[4], 1Carolan VA, Paley M[3], Tozer GM[2] and Clench MR[1]*

*[1]Biomedical Research Centre. Sheffield Hallam University*
*[2]Tumour Microcirculation Group, Department of Oncology. University of Sheffield*
*[3]Academic Radiology, Department of Human Metabolism, University of Sheffield*
*[4]Waters Corporation UK. Atlas Park, Simonsway, Manchester*

The study of proteins induced following tumour vascular-targeted therapy could aid determination of both treatment response and resistance mechanisms. Proteomic profiling and imaging techniques were applied for such investigations using MALDI Mass spectrometry (MALDI-MS). Ion mobility separation coupled with MALDI provides further separation of isobaric ions to assist analysis of numerous species resulting from in- situ tissue tryptic digests. MALDI-MSI could therefore allow observation of proteins with relevance in oncology, stress resistance and actin dynamics.
MALDI-MS was performed on mouse fibrosarcoma models following treatment with the tubulin-binding tumour vascular disrupting agent, combretastatin A-4-phosphate (CA-4-P).
In situ tryptic digests were performed on frozen sections from subcutaneously transplanted fibrosarcoma120/ 188 tumours, expressing only the 120/ 188 isoform of VEGF. Mice were treated with CA-4-P or saline. α-cyano-4-hydrocinnamic acid (CHCA) matrix applied was deposited using the Portrait 630TM reagent multi-spotter or Sun Collect spraying system. MALDI-MS profiling and Imaging were performed using a Waters G2 HDMS instrument.
Co-localised images provide initial insight into the heterogeneous spatial distribution of peptides present. Numerous tryptic peptides are observable and MS/MS investigations are underway to assign identities to them, PCA and PLSDA are used for statistical analysis.

## Quantification; how hard could it be?

***David Knight***

*The Manchester Interdisciplinary Biocentre, The University of Manchester*

The ultimate aim of proteomics is to quantify as many components as possible of the biological system investigated. Mass spectrometry equipment has been developed to deeply catalogue a system and a multitude of methodologies have followed to use the data provided for quantification. One of the interests of our facility is to investigate these quantification methodologies from an analytical point of view. This presentation will describe our investigations into the fundamentals of peptide quantification using the popular techniques of triple quadrupole based MRM analysis and high resolution LC-MS analysis on the new Orbitrap Elite mass spectrometer.

## End to end Proteomics – from Sample to Orbitrap

***Martin Hornshaw***

*Thermo Scientific, Stafford House, Boundary Way, Hemel Hempstead*

Proteomics is not the fairly simple discipline it once was in its early days. Today proteomics can be further substantially subdivided (discovery, quantitative, clinical, phospho-, glyco-proteomics, etc) which requires different sample preparation techniques, as well as a range of separations and/or enrichments and finally different mass spec data acquisition and data analysis approaches. In this brief talk I will describe a small number of end-to-end approaches to some of these more focused areas of proteomic endeavour.

## High resolution, high accuracy mass spectrometry for comprehensive proteome identification

***Matthias Mann***

*Max-Planck Institute of Biochemistry, Martinsried, Germany*

Mass spectrometric technology has improved dramatically over the past years and so have all the steps connected to the shotgun proteomics workflow. Here, we will give an overview of the latest iteration of this technology and its application to a number of biological and systems biological questions. Using the SILAC technology, it is now possible to perform absolute quantification of proteins in cell lines, in addition to relative quantification. Likewise, SILAC can now be used as a 'spike-in standard' to enable quantification of human tumor samples and other tissues. The talk will mainly concentrate on recent developments in deep expression proteomics. The yeast proteome has already been quantified comprehensively, as judged against genome-wide tagging experiments. Here we describe a streamlined technology, that achieves almost the same depth of analysis but with single shot measurements on a quadrupole linear Orbitrap mass spectrometer (Q Exactive). We also report on deep proteome studies on human cancer cell lines, where more than 10,000 proteins can now routinely be identified. Finally, clinical application of these technologies is exemplified by distinguishing closely related forms of B-cell lymphoma.

## The importance of total system peak capacity for complex proteome analysis

***Robert Tonge***

*Waters Corporation, Mass Spectrometry Technology Center, Atlas Park, Manchester*

Shotgun proteome profiling is an analytically challenging discipline in which tens of thousands of peptides are analysed in a given analytical run. Usually, these peptides are not evenly distributed through the mass and chromatographic dimensions generally available, and result in areas of extreme analyte density. Analytical systems with high peak capacity are required to minimise chimeric interferences and to extract accurate and reproducible data from these samples. Total system peak capacity ($Pc_{SYSTEM}$) is determined by the respective peak capacities of LC ($Pc_{LC}$) and MS ($Pc_{MS}$) dimensions, and each of these can be significantly extended by the use of ultra high pressure multidimensional UPLC and ion mobility-MS methodologies. Orthogonality of dimensions is also an important factor to consider in the design of optimal analytical systems. Given the extreme complexity typical in proteome samples, sometimes even analytical systems with high $Pc_{SYSTEM}$ are unable to resolve all the peptides in the sample. In these cases, data-independent acquisition strategies such as $HDMS^E$ are required to deconvolute the resultant composite spectra, and can also provide quantitative data via label-free methodologies.

## Non-covalent Ubiquitin-Ubiquitin Binding Domain interactions studied by Electrospray Ionisation-Travelling Wave Ion Mobility Spectrometry-Mass Spectrometry

***Kleitos Sokratous*[1]**, *Lucy V. Roach*[1], *Robert Layfield*[2] *and Neil J. Oldham*[1]

[1]*School of Chemistry, University of Nottingham*
[2]*School of Biomedical Sciences, University of Nottingham*

Ubiquitin binding domains (UBDs) are modular protein domains that associate with ubiquitin through non-covalent interactions. This phenomenon regulates the fate of ubiquitinated proteins. Several UBDs have been identified and categorized, for example, α-helical domains (single helix and triple helix bundles), zinc finger domains and others. However, the specificity of these domains for preferred stoichiometry, polyubiquitin length and topology is believed to vary between UBDs and has not been extensively studied. Using electrospray ionization (ESI) we have screened a range of UBDs against mono-ubiquitin and di-ubiquitin (K63 and K48 linked) to develop a sensitive and selective MS-based method for studying these important interactions. Here we present the potential of ESI-MS as a tool for the determination of apparent binding affinities (Kd) for these biologically important complexes. Kd values and specificity for ubiquitin binding to four UBDs were shown to be in good agreement with those determined by other biophysical techniques.

## Interrogating protein conformation and biomolecular complexes with IMS-MS

***Alison Ashcroft***

*The Astbury Centre for Structural Molecular Biology, University of Leeds*

Key to gaining an understanding of biological processes is to be able to characterise proteins and biomolecular complexes and to determine how these species carry out their unique functions: an edict which is the basis of structural proteomics. ESI-MS is a valuable technique for biomolecular analyses, having the ability to maintain non-covalent interactions intact during the gas-phase analysis and being able to monitor reactions in real-time. More recently, ESI-MS coupled to the separation technique ion mobility spectrometry has emerged as a powerful tool for the analysis of co-populated protein conformers and non-covalent, macromolecular complexes, providing mass, stoichiometry and shape (via collision cross-sectional area) information on each species in a single experiment. Data will be presented from a range of biomolecular complexes including bacterial pili, phage baseplates and amyloid fibrils.

# 10th East Midlands Proteomics Workshop: Poster Presentation Abstracts

## 1. Impact of LC-MSE on biomarker discovery in heart failure

***J. Auluck*[1]*, P. Quinn*[2]*, R. Willingale*[3]*, P. Farmer*[1]*, L. Ng*[2] *and D. Jones*[1]

[1]*Dept of Cancer Studies and Molecular Medicine,* [2]*Dept of Cardiovascular Sciences*
[3]*Dept of Physics and Astronomy, University of Leicester*

In the UK 900,000 people suffer from heart failure, of which 30 – 40% die within 1 year of diagnosis. Current biomarkers of heart failure are available however, the accuracy and efficacy of these markers has been questioned. The detection of biomarkers in plasma is difficult, as approximately 22 proteins dominate 99% of the plasma proteome, masking the presence of lower abundance proteins. Coupled with the precision and accuracy of mass spectrometry and use of bioinformatics, protein profiles can be generated. This allows discrimination between heart failure and disease free protein profiles, potentially leading to the discovery of biomarkers for physiological conditions.

Plasma protein profiles from 12 healthy patients and 12 heart failure patients were obtained using a Waters Synapt G2 QToF mass spectrometer using immunodepletion. The Agilent human MARS14 HPLC column was used to immunodeplete plasma. The MARS14 column removes the 14 most abundant proteins in plasma via affinity interactions, fractionating plasma and leaving an elution of low abundant proteins for further analysis. Each sample was analysed using a LC-MSE experiment and run in triplicate. Statistical comparisons of the protein profiles were made to identify potential candidates for biomarkers.

The profiles were statistically analysed using ExpressionE analysis and potential protein markers were identified. Using a label-free 1D LC-MSE experiment we have found that differences in protein expression of heart failure and disease free patient profiles exist. Candidate biomarkers which can be further verified using alternative mass spectrometric strategies are discussed.

Analysis of the potential protein markers identified by ExpressionE analysis will be verified using SRM studies and verifyE as a precursor to a larger patient cohort soon to be undertaken.

## 2. Directing biological response through material properties

***Kuforiji F.O***[1]*, El-Haj A*[1]*, Clemments M.*[2]*, Jenkins G*[3]*, Hart S*[1] *and Roach P*[1]

[1]*Institute for Science and Technology in Medicine, Keele University*
[2]*School of Life Science, University of Westminster, London*
[3]*Centre for Bio-Inspired Technology, Imperial College London*

Biomaterials are used in tissue engineering to repair, replace or augment healthy tissue. Although mechanical properties derived from the material bulk are well established attention has turned towards the surface of biomaterials in order to more easily integrate these materials into the body. Cells naturally secrete proteins in order to moderate their environment, providing a route for many cellular mechanisms including attachment and proliferation. To date, little information on cellular mechanisms in relation to their interaction with biomaterial surfaces has been reported. By understanding such responses will allow for the development of advanced biomaterial coatings, controlling cellular responses with medical devices.

Mass spectrometry has been used to evaluate differences in cellular secretions in relation to a range of surface chemistries. 3T3 fibroblasts were cultured over surfaces presenting OH, COOH, NH2 and CH3 terminal chemistry, prepared using silane self assembled monolayers on glass. Cell culture media was taken at varying time points after cell seeding, being worked up via acetonitrile precipitation and ZipTip desalting procedures and analysed using electrospray mass spectrometry.

Mass spectral differences are found highlighting variation in cell secretions in relation to their interaction with the underlying surface chemistry. Cellular morphology, adhesion and proliferation rates also show varying responses of cells to surface chemistry.

3T3 fibroblasts have been shown to adhere, proliferate and have distinct morphology depending upon the surface chemistry on which they reside. Differences in secreted proteins were also observed indicating that surface chemistry controls internal cellular processes.

We acknowledge funding from EPSRC DTC programme and the National Endowment for Science, Technology and the Arts (NESTA).

## 3. Hypothesis generation of protein interactions from large scale mass spectrometry datasets using machine learning

***A. L. Swan*[1], *K. L. Hillier*[1], *J. R. Smith*[2], *D. Allaway*[3], *S. Liddell*[1], *A. Mobasheri*[1] *and J. Bacardit*[1]**

[1]*University of Nottingham, Sutton Bonington* [2]*Bruker UK Limited, Coventry*
[3]*WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds*

The identification of potential biomarkers and their interactions is an important problem across many biological/biomedical domains. The development of novel strategies for analysing –omics data can help identify new biomarkers and reiteratively improve the experimental approach used. Proteomics data generated by high throughput mass spectrometry are suitable for developing machine learning methods, as they provide a semi-quantitative analysis of a diverse range of proteins. A method was developed to classify mass spectrometry data from secretome samples of an in vitro explant model of synovial joint inflammation, using canine articular cartilage explants, based on their protein content. Mascot was used to determine proteins present in samples and the protein assignments were verified using ProteinProphet. The rule-based machine learning system BioHel was used first to classify data from four treatment classes. Subsequently, the rule sets generated by BioHel were analysed to identify the proteins most frequently used for predicting the treatment applied to the samples. Finally, pairs of proteins that were frequently found together in rules were extracted and counted and from this a protein interaction network was generated. This method is useful in determining proteins that can differentiate between classes and those that are likely to be functionally related.

## 4. A time course to optimise proteomic studies on the articular cartilage explant model secretome: investigating responses to IL-1β, TNF-α and carprofen

***Adam Williams*[1], *Julia Smith*[2], *David Allaway*[3], *Pat Harris*[3], *Susan Liddell*[1] *and Ali Mobasheri*[1]**

*1 University of Nottingham, 2 Bruker UK Limited, 3 WALTHAM Centre for Pet Nutrition*

Osteoarthritis (OA) is a highly prevalent musculoskeletal disease with significant implications for the welfare and mobility of humans and companion animals. The main characteristics of OA are cartilage degradation, joint inflammation and pain. Differential protein release from chondrocytes within cartilage may contribute towards progression of these symptoms. Previous work in our laboratory developed an explant model of equine articular cartilage and revealed that many proteins present in the secretome are abundant extracellular matrix proteins. During proteomic analysis, high abundance proteins interfere with downstream identification of lower abundance proteins. To uncover changes in lower abundance proteins due to inflammation and/or treatment, we analysed the time course of protein release during 28 days of culture. Articular cartilage explant cultures were established from metacarpophalangeal joints of 3 animals (euthanized for purposes other than research) and treated with combinations of pro-inflammatory cytokines IL-1β, TNF-α and the non-steroidal anti-inflammatory drug, carprofen. Shotgun and GeLC-MSMS analyses confirmed the presence of known abundant cartilage associated proteins and revealed that their levels are reduced by 18 days of incubation. Moreover, cytokine stimulated MMP-3 was down-regulated by carprofen after 12 days incubation. Eighteen days is a suitable time point for further in depth shotgun LC-MSMS analysis.

## 5. Determining the specificity of ketosynthase domains using mass spectrometry: A method to confirm predicted biosynthetic pathways

***Matthew Jenner*[1], *Sarah Frank*[2], *Prof. Jorn Piel*[2] *and Dr. Neil Oldham*[1]**

[1]*University of Nottingham*
[2]*Kekulé-Institute for Organic Chemistry and Biochemistry, University of Bonn, Germany*

Modular type I polyketide synthases (PKSs) are huge bacterial megasynthases, and are responsible for the biosynthesis of a myriad of natural products. Unlike cis-PKSs, which possess integral acyltransferase (AT) domains, the AT activity in trans-AT PKSs is instead supplied by free-standing proteins. These 'AT-less' PKSs also exhibit aberrant enzymatic characteristics, making bioinformatic prediction of trans-AT PKS functionality considerably harder.

Utilising electrospray ionisation- (ESI-) and matrix-assisted laser desorption ionisation- (MALDI-) mass spectrometry, the specificity of key KS domains from two trans-AT PKS clusters has been determined. A range of N-acetyl cysteamine (SNAC) thioesters, mimicking the biosynthetic intermediates, were tested for their ability to acylate isolated KS domains from the bacillaene (bae) and psymberin (psy) clusters.

ESI-MS was used to detect the covalent modification of the KS domains, allowing rapid testing of a range of SNAC substrates. SNAC structures that were observed to acylate KS domains were then subjected to proteolysis and MALDI-MS.

KS5 from bae was observed to be highly specific for unbranched acyl-SNACs, and showed preference towards an α-β double bond. In contrast, KS1 from psy showed levels of tolerance towards β-branched substrates.

## 6. Isolation and Analysis of Unanchored Polyubiquitin

***Varun Gopala Krishna*[1]*, Joanna Strachan[1], David Tooth[1], Lucy Roach[2], Neil Oldham[2] and Robert Layfield[1]***

*[1]School of Biomedical Science, University of Nottingham*
*[2]School of Chemistry, University of Nottingham*

The accepted dogma regarding ubiquitin as a post-translational modifier has been updated recently by the discovery of a possible role for unanchored (not covalently attached to target proteins) polyubiquitin chains (Xia, et al. 2009). These polyubiquitin chains were found to activate protein kinases and induce selected signalling pathways, albeit in in vitro experiments. However, previous studies relied on indirect methods to prove the polyubiquitin chains were unanchored. We have recently refined methods that enable for the first time the selective purification of these chains from cells and tissues, which are then subjected to analysis (qualitative and quantitative) including with MS approaches.
Using a ubiquitin-binding domain known to selectively bind to the C-terminal diglycine motif presented by unanchored polyubiquitin chains (and free monoubiquitin), we were able to affinity purify this fraction of the ubiquitome. The purified samples were then subjected to Western blotting and MS analyses employing AQUA (Absolute Quantification) standards for different ubiquitin isopeptide linkages.
Purified samples from human cells revealed an abundance of higher order unanchored polyubiquitin chains (ranging from 2 to an excess of 10 ubiquitin moieties) and also allowed for qualitative determination of their isopeptide linkage types using LC/MS. AQUA analysis by LC/MS using a Triple Quadrupole Mass analyser also allows determination of the relative proportion of different isopeptide linkages within the chains. Fluctuations in the composition of the cellular pool of unanchored polyubiquitin upon activation of signalling pathways would provide new insights into regulation of the ubiquitin system.
Xia ZP, L. Sun *et al* (2009) Direct activation of protein kinases by unanchored polyubiquitin chains. Nature 461: 114

## 7. Characterisation of high and low avidity T-lymphocytes isolated from a vaccination mouse model (C57Bl/6J) using transcriptomic and proteomic methods

***Jayakumar Vadakekolathu****, David Boocock*, Stephanie McArdle*, Clare Coveney*, Victoria Pudney[†], Lindy Durrant[†] and Robert C Rees****

**John van Geest Cancer Research Centre, Nottingham Trent University*
*[†]Department of Clinical Oncology, City Hospital, Hucknall Road, Nottingham*

In cytotoxic T-cells (CTL) based cancer immunotherapy, high frequency CTL response doesn't always correlated with positive clinical outcome. However, The quality of this CTL responses can be assessed by ELISPOT/chromium release assays and often the high avidity T cell responses are generally considered to be a positive indicator of therapy. So the detection of these cells in PBMCs or in tumour biopsies of patients would greatly help clinicians to decide subsequent treatment detection. High and low avidity antigen specific CTLs isolated from the TRP-2 C57Bl/6J model was used for the discovery of biomarkers of high avidity by gene array and proteomic mass spectrometry platforms and decipher the pathways involved in the differentiation and expansion of the subsets of high avidity cytotoxic T-lymphocytes (CTLs). Gene expression studies shortlisted 10 candidate genes and several noncoding RNAs as possible targets for further investigations. Proteomic characterisation using LC-MALDI-TOF analysis revealed nine candidate proteins which showed significant differences in peak intensities between the groups. Many of these have significant functional roles in T-cell functionality/development. All candidate markers will be taken further for validation using a different model prior to testing on human clinical samples.

## 8. Non-covalent Ubiquitin-Ubiquitin Binding Domain interactions studied by Electrospray Ionisation-Travelling Wave Ion Mobility Spectrometry-Mass Spectrometry

***Kleitos Sokratous*[1]*, Lucy V. Roach[1], Robert Layfield[2] and Neil J. Oldham[1]***

*[1]School of Chemistry, University of Nottingham*
*[2]School of Biomedical Sciences, University of Nottingham*

**See talk abstract**

## 9. Quantitative Proteomic Profiling of Healthy and Alzheimer's Disease Plasma to Confirm Previously Described CSF Biomarkers

*Baharak Vafadar-Isfahan[1], Christophe Lemetre[1], **Clare Coveney[1]**, David J Boocock[1], Graham Ball[1], Kevin Morgan[2] and Robert C Rees[1]*

*[1]The John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University [2]Institute of Genetics, School of Molecular Medical Sciences, University of Nottingham*

Identification of reliable biomarkers for Alzheimer's disease (AD) would be an invaluable aid to clinical diagnosis and allow detection much earlier, when drug intervention is likely to be most effective. Previously we have identified a putative panel of AD biomarkers (7 proteins) in cerebrospinal fluid (CSF) using MALDI-MS profiling (Vafadar-Isfahani *et al*., 2011). Here we investigate the potency of MS-based quantitative strategy for validation of this candidate panel of biomarkers in plasma with the prospect of detecting additional potential biomarkers of AD.

High abundant plasma proteins were depleted by immunodepletion followed by tryptic digestion and iTRAQ labelling. Furthermore, fractionation using preparative IEF, followed by reversed-phase nanoHPLC was implemented which was followed by MALDI MS/MS analysis.

Preliminary results using iTRAQ labelling identified 2095 proteins in the plasma with associated AD/control iTRAQ ratios for each protein. 58% of the proteins had no significant change between AD and control patients however, 9% and 11% of the total identified proteins showed increased and decreased levels respectively, in the plasma of AD patients in comparison to controls. In addition, the iTRAQ results were comparable to the previously described panel, indicating that this panel maybe used for plasma examination. Some of the proteins showing altered levels have previously been associated with AD, however, further experiments are required to validate these findings.

## 10. Rapid Biomarker Profiling Of *Escherichia coli* Utilising MALDI-TOF Mass Spectrometry and a Multivariate Pattern Recognition Approach

***Remi Momo***[1], *Elaine Martin OBE[1], Gary Montague[1], Ronan O'Kennedy[1], Jane Povey[2], Chris O'Malley[1] and Mark Smales[2]*

*[1]School of Chemical Engineering and Advanced Materials, Newcastle University*
*[2]School of Biosciences, University of Kent, Canterbury, Kent*

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has been exploited extensively in the field of microbiology for the investigation of bacterial species, the detection of biomarkers and early disease diagnosis. One of the key issues involved in MALDI-TOF MS studies is the need to reduce the high-dimensionality of the data generated. In the context of biomarker discovery, analysis of such complex spectra data may cause variances pertaining to the experimental hypothesis to be confounded with variations from experimental sources.

In the study reported, chemometric data analysis techniques were applied to MALDI-TOF MS data obtained from *E. coli* cell samples. The technique of primary interest was a multivariate pattern recognition technique, Partial Least Squares-Discriminant Analysis (PLS-DA). PLS-DA modelling results suggest that samples of cultures at exponential phase was separated from those at stationary phase along the second latent variable. The third latent variable separated samples of cultures at stationary phase from those at decline phase.

The mass-to-charge ratio (*m/z*) biomarker peaks responsible for their separation of these samples were also identified. A Swiss-Prot/TrEMBL database search with these biomarker peaks identified and attributed most *m/z* ion signals from exponential phase cultures to series of ribosomal proteins (L22, L23, L25, L29, L31, L32, L33, S20 and S22); those for stationary phase cultures attributed to nucleoid-associated proteins (MccB17, IhfA and Dps); and toxin-antitoxin (TA) module products (ChpS antitoxins, YefM, RelE and RelB) for death phase cultures which are stress-response molecules. The recognition of these protein *m/z* signals suggest that these proteins can be used as growth-phase-associated biomarkers to distinguish between *E. coli* cultures at different growth phases.

## 11. Plasma protein profiling for bladder cancer biomarker discovery using UPLC-HDMSE/label-free quantitation

***Amirmansoor Hakimi***[1], *Charlotte E. Daly*[1], *T.R. Leyshon Griffiths*[2], *Leong Ng*[3], *George D.D. Jones*[4] *and Donald J.L. Jones*[1]

[1]*Department of CSMM, RKCSB, LRI, University of Leicester*
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In the UK, approximately 10,000 new cases of bladder cancer (BC) have been diagnosed in 2007 and ~5000 patients have been died of the disease in 2008 (~50%) (CRUK, 2010). 70% of bladder cancer patients at diagnosis have early stage non-muscle invasive disease with high tendency of recurrence post treatment. For the clinical management of BC it is imperative to discover reliable biomarkers that can be used for both diagnose and predict the likely outcome for patients with non-muscle invasive disease. In this study we are looking for biomarkers of BC by profiling proteins of plasma sample from BC patients in two groups and comparing it with healthy controls. A Waters Synapt-G2-HDMS coupled to a nano-Acquity-UPLC was used to analyse the samples. PLGS 2.5 was used to process the data. Up to 95% of the top 14 of high abundance proteins depleted from plasma using the SEPPRO Igy-14 column from Sigma-Aldrich. Acetone precipitation was performed to exchange the buffer following by digestion of low abundant proteins. Samples then were concentrated and spiked with internal standard (ADH) before analysing with mass spectrometer. Raw data files processed with PLGS for identification and quantification of detected proteins. Discrimination of up/down-regulated proteins in every group is demonstrated using ExpressionE which ultimately help find biomarkers for prediction and progression bladder cancer. Future work will require the selective choosing of detected biomarkers using VerifyE which enables a targeted approach to biomarker validation.

## 12. Proteomic profiling of a human prostate cancer cell line and its subpopulations using LC-MALDI-TOF-TOF mass spectrometry

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Prostate cancer (PCa) is the second most common type of cancer found in men but is lacking in good prognostic and diagnostic biomarkers. This research focused on biomarker discovery in OPCT1 (PCa cell line) and three sub-populations, identified by immunofluorescence, that show differential CD44 and T21 genotypic expression. Previous studies on role of CD44 expression in prostate cancer and T21 as a novel prostate cancer antigen had suggested their role in tumour progression and metastasis (CD44). Here, we aimed at identifying differentiating proteins expressed between these cell populations by generating protein profiles using LC-MALDI-MS technique. Protein from cells were tryptically digested and peptides fractionated using reversed phase LC-MALDIMS with targeted MSMS following bioinformatic analysis with ProfileAnalysis 2.0 software resulting in identification of 14 differentially expressed proteins. From the proteins identified, further investigation on the proteins vimentin and zyxin were suggested, as they were previously studied to be directly associated with prostate proliferation, metastases and CD44 expression respectively. Their verification as candidate biomarkers may be useful for isolation of such characterised cells in heterogeneous populations. Further, this study also leads to investigation on the role of these identified proteins in the biochemical pathways involved with CD44 and T21 expression.

## 13. Accurate quantification of proteins in complex matrices for the production of matrix certified reference materials

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Mass spectrometry (MS) has evolved as an important technique to support and validate immunoassay technology in clinical, food and environmental applications by facilitating the production of matrix certified reference materials (CRM). Recently robust methods have been developed to quantify proteins in standard solutions and in complex matrices, whereby the results are directly traceable to SI, via isotope dilution mass spectrometry.

An example of the high accuracy quantification of C-reactive protein, an inflammatory marker, in standard solutions will be presented to be further used as standard for clinical measurements. An example of traceable quantification of hGH in serum by using isotopically labelled proteins and peptides will be discussed and the advantages and the problems associated to the use of isotopically labelled proteins will be highlighted.

The importance of biological reference materials in allergen analysis will be also presented and an example of feasibility of producing a lysozyme in wine reference material will be described.

## 14. Ion Mobility-Assisted Data-Independent Acquisition for Studying Bacterial Protein Extracts

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An ion mobility-mass spectrometry approach using reversed phase-liquid chromatography, data-independent acquisition and label-free quantification was evaluated for the analysis of complex bacterial proteomic samples. A non-biased comparison was made between mobility-assisted (HDMSE) and conventional data-independent (MSE) approaches on a quadrupole time-of-flight instrument with a traveling-wave ion mobility cell (Synapt G2), in the analysis of a relatively simple mixture of proteins and soluble protein extracts obtained from *Methylocella silvestris* whilst grown on different substrates.

Improved quality of product ion spectra is observed in the mobility approach; firstly, analysis of product ion spectra obtained using normal MSE mode and HDMSE mode show that there is a large (up to five-fold) decrease in the level of noise and secondly, additional consecutive ions are assigned in mobility-enabled product ion spectra. The number of peptide/protein identifications increased and the replication rate was improved in the mobility-assisted method, as was variance in precursor intensity and protein quantification. Good agreement with the conventional MSE method was obtained for quantitative measurements. As expected, a small reduction in effective experimental dynamic range was observed with the HDMSE experiment due to detector saturation under the stringent requirements of the mobility-enabled data acquisition.

## 15. In-source fragmentation of FAIMS-selected peptide ions in combination with liquid chromatography and time-of-flight mass spectrometry

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Miniaturised ultra high field asymmetric waveform ion mobility spectrometry (ultra-FAIMS) has been combined with mass spectrometry (MS) and liquid chromatography-mass spectrometry (LC-MS) for the selective transmission of peptide ions prior to in-source collision-induced dissociation (in-source CID). Peptide ions were selectively transmitted from a mixture on the basis of differential mobility, prior to fragmentation. This filtered out unrelated pre-cursor ions, simplifying the resulting mass spectrum. Peptide standards, unresolved by LC, were separated by FAIMS prior to in-source CID, enhancing fragment ion detection.

The LC-FAIMS-CID-MS method was applied to co-eluting tryptic peptides from human plasma proteins. The spectrum complexity was reduced by selectively transmitting peptides at a fixed compensation voltage (CV), at the expense of other co-eluting species. In addition, the removal of species that did not readily fragment enhanced fragments of peptides of interest, facilitating peptide identification.

## 16. Isolation of Intracellular proteins of *Brevibacterium linens* DSM 20518 and its Proteomic Characterization

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Due to the importance of *Brevibacterium linens* in the industrial production of enzymes, amino acids and vitamins and particularly in the production of a variety of cheeses, the vast majority of research carried out has focused on expression of extracellular proteins and amino acid biosynthesis. In recent years, additional topics have received attention, driven (i) by the establishment of the *Brevibacterium linens* BL2 genome sequence, (ii) by the realization that construction of the ‘‘optimal’’production strain requires knowledge on the physiology and biochemistry of the organism as a whole rather than only of selected parts, and (iii) by the possibility of using *Brevibacterium linens* as a nonpathogenic model organism to study features in common with pathogenic corynebacteria e.g. *B. immariophilum, B.seonmiso, B. tapie, B.thiogenitalis.*

Here, we identify intracellular proteins associated with nonpathogenic *Brevibacterium linens* strain DSM 20518. One-dimensional SDS PAGE gel electrophoresis of bacterial membrane extract followed by nanoLC-ESI mass spectrometry revealed different classes of proteins including dehydrogenases, oxidoreductases, synthases, aminotransferases, carboxylases and hydrolases during late exponential growth (72 h shake flask culture) in minimal medium.