12TH EAST MIDLANDS PROTEOMICS WORKSHOP

Wednesday 6th November 2013

Stamford Court, University of Leicester

12th East Midlands Proteomics Workshop

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The East Midlands Proteomics Workshop (http://www.empw.co.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 at Stamford Court, Manor Road, University of Leicester.
Further information can be found at http://www.le.ac.uk/conference/ven_gm.html

Directions and Parking
Directions to Stamford Court (post code LE2 2LH) are available at
http://www.le.ac.uk/conference/loc_directions.html
There is parking in the car park to the right hand side of Stamford court – note that the building is set back from the road and is not visible until you are very near.

Registration
Registration opens at 8:30 am in the **reception of Stamford Court** - which is found to the right hand side of the building.

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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Dr Don Jones, Chair EMPW2013 (University of Leicester)
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Dr Andrew Bottrill (University of Leicester)
Professor Helen Cooper (University of Birmingham)
Ms Clare Coveney (Nottingham Trent University)
Dr Rob Layfield (University of Nottingham)
Dr Susan Liddell (University of Nottingham)
Dr Sharad Mistry (University of Leicester)
Dr Susan Slade (University of Warwick)

12th East Midlands Proteomics Workshop
Wednesday 6th November 2013, University of Leicester

8:30 - 9:15 **Registration**

Session 1: Chair: Don Jones

9:15 Welcome Don Jones, chair of EMPW2013

9.20 Prof Mike Barer, College Director of Research, University of Leicester

9:30 **Keynote Presentation**
Proteomic approaches for the analysis of multi-protein complexes and cell cycle variation of gene expression
Angus Lamond, University of Dundee

10:10 *Spatial profiling and identification of proteins from human liver using liquid micro-junction extraction coupled to a high-resolution mass spectrometer*
Joscelyn Sarsby, University of Birmingham

10:30 **Speaker for Bruker UK Ltd, GOLD SPONSORS OF EMPW2013**
Improving glycan analysis using mass spectrometry for biotherapeutics, diagnostics and biomarker discovery
Daniel Spencer, Ludger Ltd, Abingdon

10:50 **Coffee / tea / Exhibition**

Session 2: Chair: Andrew Bottrill

11:20 *The use of proteomic approaches to unravel the complexity of psychiatric disorders*
Paul C Guest, University of Cambridge

11:50 *Using high resolution MS to discover tumour stroma markers*
Sara Zanivan, The Beatson Institute for Cancer Research, Glasgow

12:10 **Speaker for Waters Corporation, GOLD SPONSORS OF EMPW2013**
An integrated strategy for panomics analysis employing three dimensions of resolution
Robert Tonge

12.30-14:10 Lunch / Exhibition

13:00 to 14:00 Poster Session

Session 3: Chair: Rob Layfield

14:10 *Characterisation of high and low avidity peptide specific $CD8^+$ T cells using transcriptomic and proteomic tools*
Jayakumar Vadakekolathu, Nottingham Trent University

14:30 *Secretomes as a Source of Bladder Cancer Biomarkers*
Doug Ward, University of Birmingham

14:50 *Enhancing the analysis of ubiquitin and SUMO modifications; a combination of chemical derivitisation and data independent acquisition*
Duncan Smith, Paterson Institute for Cancer Research, Manchester

15.20 Coffee / tea / Exhibition

Session 4: Chair: Helen Cooper

15:50 *Characterising receptor complexes and endosomal proteomes during plant defence responses*
Alex Jones, University of Warwick

16:10 *Just add FAIMS: combining field asymmetric waveform ion mobility spectrometry with mass spectrometry for peptide and protein analysis*
Colin Creaser, Loughborough University

16:40 Announcement of prizes Sue Slade

16:50 Close of meeting

12th East Midlands Proteomics Workshop: Oral Presentation Abstracts

Proteomic approaches for the analysis of multi-protein complexes and cell cycle variation of gene expression

Angus Lamond, *Mark Larance, Yasmeen Ahmad, Tony Ly, Dalila Bensaddek, Aki Endo & Armel Nicolas*

Centre for Gene Regulation and Expression, MSI/WTB/JBC Complex, University of Dundee, Dundee Email: a.i.lamond@dundee.ac.uk

We study gene and cell regulatory mechanisms using a dual strategy that combines mass spectrometry (MS) based proteomics with live cell fluorescence imaging (see www.LamondLab.com). Using quantitative proteomics we have created a flexible suite of assay formats to characterize, system-wide, 'Protein Properties', including measurements of protein abundance, subcellular protein localization, turnover rates, post-translational modifications, cell cycle variation and specific protein interaction partners in human cells and model organisms. Applying these assays allows us to determine how such protein properties vary between cell types and how they change within cells as they progress through the cell cycle and respond to different growth conditions, drug treatments, external stimuli and stress.

The systematic analysis of protein properties using quantitative proteomics inevitably generates very large data sets. An integral part of our work therefore has been the parallel development of a dedicated software environment, called PepTracker (see: http://www.peptracker.com/), to manage the collection, mining and visualization of these large scale, 'next generation' proteomics datasets and their integration with transcriptomics and genomics data. We have used computational approaches developed in the business intelligence field to model the proteomics data and to facilitate rapid and complex data mining. The integration of quantitative proteomics with state of the art 'in-database' analytics facilitates the development of the "Super Experiment" strategy. Thus, every individual experiment also contributes to building a large scale 'Super Experiment' for studying cellular mechanisms and responses at the system-wide level, drawing on information from many different cell types and experimental conditions. To facilitate large scale proteomics studies we have developed DataShop, a freely available App for the visualization and analysis of very large data sets.

Two major themes of our recent work have been the analysis of multi-protein complexes and the identification and characterization of proteins regulated by targeted degradation mechanisms. This includes extending our proteomic studies to include detection of specific protein isoforms and post-translational modifications. I will present recent data from studies using centrifugal elutriation combined with MS to characterize variation in gene expression across the human cell cycle and new methodology for studying multi-protein complexes using uHPLC and size exclusion chromatography combined with MS. All of our high throughput proteomic data sets have been incorporated into a searchable, online database called the Encyclopedia of Proteome Dynamics http://www.peptracker.com/encyclopediaInformation

Key words: Mass Spectrometry, Proteomics, PepTracker, SILAC, Organelles, Degradation, Proteasome

Spatial profiling and identification of proteins from human liver using liquid micro-junction extraction coupled to a high-resolution mass spectrometer

Joscelyn Sarsby, Nicolas J. Martin, Alan M. Race, Patricia Lalor, Josephine Bunch and Helen J. Cooper

Mass Spectrometry Imaging Research, University of Birmingham

Atmospheric pressure tissue surface sampling using automated liquid extraction surface analysis (LESA), coupled to high-resolution mass spectrometry, is presented as a versatile tool for surveying proteins in human liver tissue.

Optimised protein extraction has been conducted using solvent extraction and repeated sampling at the same location, where initial lipid extraction acts as localised tissue washing prior to protein extraction. Top-down methods using collision-induced dissociation (CID) and electron transfer dissociation (ETD) mass spectrometry were used to identify a 10 kDa heat shock protein (39% coverage), the α-chain of haemoglobin (29% Coverage) and the liver fatty acid binding protein (FABP) (44% Coverage), including the identification of post-translational modifications and a single amino-acid substitution in the L-FABP. Many more proteins (355) were identified using an automated bottom-up method in which intact proteins were extracted from 1 mm diameter regions of tissue using LESA, digested with trypsin and the resulting peptides analysed by LC/MS/MS with collision induced dissociation. Direct tissue sampling using optimised solvent solutions was performed at 60 locations across a human liver section and 2D profiles of proteins were constructed using in-house software. These experiments demonstrate the potential of this strategy for direct tissue sampling of endogenous molecules, using liquid micro-junction surface sampling techniques.

PRESENTATION ON BEHALF OF BRUKER UK Ltd GOLD SPONSOR OF EMPW2013

Improving glycan analysis using mass spectrometry for biotherapeutics, diagnostics and biomarker discovery

Daniel I R Spencer[1], Archana Shubhakar[1], Conception Badia Tortosa[1], Radoslaw Kozak[1], Manfred Wuhrer[2] and Daryl L Fernandes[1]

[1]Ludger Ltd, Culham Science Centre, Abingdon, Oxfordshire [2]Biomolecular Mass Spectrometry Unit, Department of Parasitology, Leiden University Medical School Center, Leiden, The Netherlands.

Ludger Ltd. is a company based in Oxfordshire that specializes in glycotechnology. Glycosylation is a protein modification that is an important consideration for drug developers but can be complex to analyse. We work with pharmaceutical companies to help them to accurately analyse glycan structures for therapeutics such as erythropoietin, IgG and follicle stimulating hormone where the data obtained is used at every stage of the therapeutic design to product life cycle including clone selection, quality control, comparability and regulatory authority submissions. We are also part of several European Union Framework Programme 7 grants including HighGlycan, IBD-BIOM and GlycoPar. We demonstrate here some mass spectrometry based techniques that we are automating for analysis of IgGs, human plasma, and for glycan biomarker discovery, and present a range of glycan/glycopeptide standards that we have developed to improve glycan quantitation.

The use of proteomic approaches to unravel the complexity of psychiatric disorders

Paul C Guest

Department of Chemical Engineering and Biotechnology, University of Cambridge, Tennis Court Road, Cambridge

Psychiatric disorders such as schizophrenia are heterogeneous conditions, characterized by an overlapping array of clinical manifestations. Although the most well known manifestations include serious effects on mood and behaviour, patients can also suffer from severe co-morbidities, including immune system or metabolic abnormalities. Thorough characterization of blood serum samples from schizophrenia patients using multiplex immunoassay and mass spectrometry profiling approaches has helped to de-convolute the complexity and heterogeneity of some of these conditions at the molecular level. This could lead to the development of biomarker panels for use in patient stratification through characterization of biochemically different disease subtypes. In addition, proteomic methods have recently been used for molecular characterization of the mechanism of action of psychiatric medications in both preclinical models and patients. This has resulted in identification of molecular panels that show some promise for prediction of response or for monitoring treatment outcomes. This presentation will describe how proteomic profiling methods can impact the future of diagnosis and therapeutics, and facilitate personalized medicine approaches for more effective treatment management of patients with these debilitating disorders.

Using high resolution MS to discover tumour stroma markers

Juan Ramon Hernández-Fernaud, Lisa J Neilson, Jim Norman and **Sara Zanivan**

The Beatson Institute for Cancer Research, Glasgow

Tumours are complex tissues composed by tumour cells and other distinct cell types such as fibroblasts. These cells interact with one another, directly by cell-cell interaction and indirectly by secreting soluble factors, and actively participate to the tumorigenesis process. Cancer-associated fibroblasts, CAFs, have distinct traits compared to normal fibroblasts. CAFs are frequently present in the stroma of human breast carcinoma, and have been shown to promote tumorigenesis and dictate tumour outcome.

We have combined optimised fractionation protocols, SILAC, and high resolution mass spectrometry to compare the proteome and secretome of immortalised human mammary CAFs and normal fibroblasts. These include many proteins known to regulate fibroblast activation and tumour growth, but also some previously unrelated to this processes. Amongst those we have discovered CLIC3 as a marker for CAFs, and we show that CLIC3 is expressed in primary fibroblasts and in the tumour stroma of patient samples. Furthermore we provide evidence in vitro that CLIC3 has a functional role in CAFs and therefore shed new light on a potential role of stromal CLIC3 in regulating tumour progression.

PRESENTATION BY WATERS Corp GOLD SPONSOR OF EMPW2013
An integrated strategy for panomics analysis employing three dimensions of resolution

Robert Tonge

Waters Corporation, MS Technology Center, Atlas Park, Manchester

Complementary metabolomic/lipidomic and bottom-up proteomic studies can provide a multi-dimensional view of biological processes at a molecular level. However, such analyses pose a massive analytical challenge in which thousands of structurally similar analytes are distributed over a wide dynamic range. Our unique analytical strategy combines three orthogonal techniques to separate, characterise and quantify the components of very complex biological samples. We integrate Ultra Performance Liquid Chromatography (UPLC) or Ultra Performance Convergence Chromatography (UPC^2), Travelling Wave Ion Mobility Separation (TW-IMS) and high resolution Time-Of-Flight (TOF) mass spectrometry to enable High Definition Panomics Analysis. The combination of 2D UPLC, TW-IMS & MS allows the total Peak Capacity of the analytical system to be increased by an order of magnitude compared to the conventional 2D LC & MS methodologies. Endogenous metabolite/lipid and protein profiling are performed with highly specific analytical systems where analytes are fully separated before identification and quantification, minimising the potential misleading effects of contaminant data. While the core of either system may be the same High Definition Mass Spectrometer (HDMS) the chromatography and software tools are optimised for each workflow. The Waters Omics Research Platform Solution with TransOmics Informatics is co-developed with Nonlinear Dynamics (Newcastle, UK). It delivers a common High Definition Workflow for metabolite/lipid and bottom-up protein profiling with intuitive data visualization to facilitate large panomics studies. The next generation of this unique Research Platform Solution will be previewed in this presentation.

Characterisation of high and low avidity peptide specific $CD8^+$ T cells using transcriptomic and proteomic tools

Jayakumar Vadakekolathu*, Stephanie McArdle*, Victoria Pudney†, Lindy Durrant†, Graham Pockley*, Robert Rees*, Clare Coveney*, David Boocock*

**John van Geest cancer Research Centre, Nottingham Trent University, Nottingham*
†Scancell Ltd., Department of Clinical Oncology, The University of Nottingham, Nottingham

One of the hallmarks of successful immunotherapy is the generation of high avidity T cells, which is the measure of sensitivity of T cells to recognise its cognate peptide in a dose dependent manner (high avidity T cells recognise very low concentrations of peptide, and vice versa for low avidity T cells). However current assays are laborious, samples demanding and not ideal for assessing all the cancer vaccination settings, therefore there is a need to identify markers which can quickly and reliably identify high avidity T cell responses. This study used a C57Bl/6J mouse model with one self ($TRP2_{180\text{-}188}$) and a foreign ($OVA_{257\text{-}264}$) antigenic epitope with two vaccine frame works - DNA vector (ImmunoBody®) and a peptide vaccine, for the generation of high and low avidity T cell responses respectively. Peptide specific $CD8^+$ T cells were isolated from each group and global transcriptional profiling identified several differentially expressed genes between high and low avidity groups. Six of the differentially expressed genes were further confirmed using qRT PCR, of which three genes showed a positive correlation between microarray data. Two of these were further confirmed at protein level using flow cytometry. Proteomic characterisation of pentamer sorted cells using LC-MS profiling identified 111 and 129 proteins uniquely associated with Peptide or ImmunoBody® pentamer positive T cells. Many of these proteins were associated with important T cell functional properties, further confirmations are ongoing.

Secretomes as a source of bladder cancer biomarkers

Richard T. Bryan, Neil J. Shimwell, Ashley Martin, Wenbin Wei and **Douglas G.Ward**

School of Cancer Sciences, University of Birmingham

Approximately 10,000 cases of bladder cancer are diagnosed each year in the UK and 5000 patients die from the disease. Bladder cancer is detected by cystoscopy, an unpleasant and expensive procedure. Better urinary biomarkers could reduce reliance on cystoscopy.

Despite many proteomic investigations, clinically useful biomarkers have proven elusive. Assuming that such markers exist, they are difficult to detect directly in urine due to their low-abundance in a complex background and the confounding effects of haematuria.

We have used shotgun proteomics to identify proteins released by bladder cancer cell lines. Proteins were selected for further investigation based on gene expression data; the rationale being that proteins released from bladder cancer cells and over-expressed in bladder tumours may act as tumour-specific urinary biomarkers. The candidate biomarkers were assayed by ELISA in urine collected from patients across the West Midlands.

Three candidate biomarkers were elevated in the urine of patients with muscle-invasive bladder cancer although not in non-muscle invasive disease. Urinary levels of these proteins are also prognostic for bladder-cancer specific survival (independent of stage and grade). We conclude that, at least in the case of bladder cancer where a proximal fluid is readily available, combined secretomics/transcriptomics can reveal new cancer biomarkers.

Enhancing the analysis of ubiquitin and SUMO modifications; a combination of chemical derivitisation and data independent acquisition

Duncan Smith

Paterson Institute for Cancer Research, Manchester

The proteomic analysis of ubiquitin-like modifications is challenging not least due to the fact that isopeptide products of modified protein digests don't necessarily behave like their linear peptide cousins. The current approach to deal with ubiquitinylation is to rely on the presence of a remnant diglycine tag on the modified lysine residue. However, a lack of any diagnostic fragment ion and the existence of residues isomeric to GG make false positive assignment problematic. The analysis of SUMO modification is much more challenging in most species courtesy of the typically extended isotag sequences associated with their tryptic digestion. We have developed a methodology to generate much shorter SUMO isotags without the need for genetic manipulation. In order to direct the fragmentation of both Ub and SUMO specific isopeptides towards analytically powerful diagnostic ions, we have utilised various chemical derivitisation strategies in combination with CID. Moreover, we have implemented a data independent acquisition (SWATH) approach to facilitate the simultaneous qualitative and quantitative analysis of Ub and SUMO modifications in a single workflow. Our approach allows quantitative analysis in both MS and MSMS further strengthening quantitative conclusions.

Characterising receptor complexes and endosomal proteomes during plant defence responses

Alex Jones

School of Life Sciences, University of Warwick, Coventry

A key part of the innate immune system, in both plans and animals, is the activation of plasma membrane localized receptors after binding to their cognate ligand. Ligand binding typically initiates disengagement of inhibitors from the receptor complex, recruitment of additional components and post-translational modification of proteins within the complex and of down-stream targets to mediate signal transduction. Activated receptor complexes are endocytosed and components sorted for recycling back to the plasma membrane or destruction in the vacuole. As many of these events occur before transcriptional changes proteomic approaches provide an essential tool. I will illustrate the impact of relatively simple proteomic experiments on our understanding of receptor complex activation and downstream signaling in the model plant *Arabidopsis thaliana*, using LC-MS/MS (LTQ-Orbitrap, ThermoScientific) and SRM (Xevo TQ-S. Waters Corp.). I will also discuss strategies - and limitations - in quantifying change in phosphorylation sites in transient and low abundance samples.

Just add FAIMS: combining field asymmetric waveform ion mobility spectrometry with mass spectrometry for peptide and protein analysis

Colin Creaser

Centre for Analytical Science, Department of Chemistry, Loughborough University

Field asymmetric waveform ion mobility spectrometry (FAIMS) is an electrophoretic technique that separated ions in the gas phase on the basis of differences in their mobility in the presence of alternating high and low electric fields. FAIMS is highly orthogonal to the separation of ions based on mass-to-charge ratio obtained by mass spectrometry, so combined FAIMS-MS is capable of multidimensional separations. The potential of including a FAIMS separation in the mass spectrometry analysis of peptides and proteins will be discussed.

12th East Midlands Proteomics Workshop: Poster Presentation Abstracts

1) Novel biomarkers for predicting poor treatment response in heart failure in order to guide therapy

Cao Huy Thong[1], Paulene A. Quinn[1], Jatinderpal K Sandhu[1], Adriaan A. Voors[2], Chim Lang[3], Donald J. L. Jones[4], Leong Loke Ng[1]

[1]Department of Cardiovascular Sciences, Leicester Royal Infirmary and NIHR Leicester Cardiovascular Biomedical Research Unit, University of Leicester [2]Department of Cardiology, University Medical Center Groningen, Netherlands, [3]Division of Cardiovascular and Diabetes Medicine, University of Dundee

[4]Department of Cancer Studies and Molecular Medicine, Leicester Royal Infirmary, University of Leicester

Heart failure is a major health problem in western countries with a high overall prevalence, high cost and a very poor outcome. The aim of this study is to discover proteins in plasma that are able to predict the difference in clinical response to standard therapy in patients with heart failure.

In this study, we compared plasma proteins in 50 patients who responded to standard treatment (responders) with 50 patients who died or were re-hospitalised (non-responders). Plasma samples were first pooled, depleted of 14 high abundance proteins and then digested to peptides. Peptides were analysed on 2-dimensional liquid chromatography coupled to tandem mass spectrometry.

434 proteins were identified in the plasma of heart failure patients. 220 proteins in both groups were found to be over or under-expressed significantly, with 144 up-regulated and 76 down-regulated. Several of these proteins have the potential to become novel biomarkers for predicting treatment response in heart failure.

The discovery of novel biomarkers in this study will lead to the development of a more personalised approach in predicting treatment response in order to guide therapy. In this way, unnecessary therapy to non-responders may be avoided and novel therapeutic targets could be identified to improve outcomes.

2) New clinical proteomics approaches for discovering biomarkers: searching for liver fibrosis markers in hepatitis patients

Bevin Gangadharan

Department of Biochemistry, University of Oxford

Introduction: Two-dimensional gel electrophoresis (2-DE) is often used to separate plasma or serum proteins in an attempt to identify novel biomarkers. A major difficulty with this approach is due to high abundant plasma/serum proteins which limits the detection of low abundance features. To overcome this problem a novel proteomics approach was developed and used to identify new fibrosis biomarkers in patients with different stages of liver fibrosis.

Methods: Plasma samples from healthy individuals and patients with hepatitis C virus (HCV) induced cirrhosis were analysed using 2-DE over a narrow pH 3-5.6 range, a range outside the pH of highly abundant albumin, transferrin and immunoglobulins. Novel markers identified by this approach were validated across all fibrosis stages by Western blotting.

Results: 44 candidate biomarkers were revealed of which 20 were novel. Western blot analysis with newly identified biomarkers, showed a consistent change with increasing fibrosis stage and were promising when compared to the markers used in established fibrosis tests.

Conclusion: This is the first time the pH 3−5.6 range has been used to separate plasma by 2-DE. This pH range is useful for discovering novel biomarkers in all diseases. The novel fibrosis markers identified by this new clinical proteomics approach may help to assess hepatic fibrosis and eliminate the need for invasive liver biopsies.

3) A new sensitive liquid chromatography-tandem mass spectrometry technique to measure 3-nitrotyrosine

Fozia Shaheen, Derek Wilkinson, Naila Rabbani and Paul J Thornalley

Warwick Medical School, Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry

3-Nitrotyrosine (3-NT) is a marker of protein nitration in physiological systems. It is present as 3-NT residues in proteins and 3-NT free adduct in physiological fluids. Levels of plasma 3-NT are used as a marker of nitrosative stress in diabetes and renal failure. Protein nitration is a minor quantitative modification, so extremely sensitive and specific analytical techniques are required for 3-NT quantification. Methods employed to measure 3-NT include immunoassay and HPLC with absorbance, fluorescence or electrochemical detection but have inadequate analytical performance. The gold standard reference method for trace 3-NT quantitation is stable isotopic dilution analysis-liquid chromatography tandem mass spectrometry (LC-MS/MS). We compare two LC-MS/MS systems, Acquity-Quattro PremierTM and recently introduced Acquity-Xevo-TQ-STM system with improved vapour phase sampling, for this application.

The limit of detection for 3-NT was lower for the Xevo TQ-S than the Quattro Premier LC-MS/MS system: LOD (fmol): Xevo TQ-S 0.423 ± 0.014, Quattro Premier 4.67 ± 0.14 ($P<0.001$). 3-NT residue content in plasma protein of healthy people was 8.30 ± 1.36 μmol/mol tyr. 3NT free adduct concentration in plasma of healthy people was 0.191 ± 0.170 nM.

Xevo TQ-S has improved performance and is suitable for plasma quantification of 3-NT.

4) Investigating the ageing mitochondrial proteome

Amelia Pollard[1], Freya Shephard[1], Susan Liddell[2] and Lisa Chakrabarti[1]

[1]School of Veterinary Medicine and Science and [2]School of Biosciences, University of Nottingham

Ageing and neurodegeneration are entwined through their sharing of mitochondrial dysfunction as a common feature. Our study aims to explore mitochondrial changes through the ageing process. We have begun by investigating the proteomic alterations that occur during ageing in murine mitochondria. We extracted mitochondria from young (4-14 weeks) and old (19 months) mouse brain and skeletal muscle tissue. 2D gel electrophoresis was used to compare the mitochondrial proteome and the gels were analysed using SameSpots software (Totallab). Protein spots with a fold change greater than 1.2 and a p value less than 0.15 were considered for spot picking. Six protein spots were picked from the gel and digested with trypsin before ESI-MSMS was performed. The data produced was searched against the Swissprot database using MASCOT to identify the proteins. We will present data indicating there are alterations in the mitochondrial proteome in older mouse brain and muscle.

5) Mapping the binding sites of new photoactivatable anticancer complexesto peptides and proteins via FT-ICR MS

Christopher A Wootton, Ilaria Finazzi, Evyenia Shaili, Andrea Lopez-Clavijo,
Mark Barrow, Peter J Sadler and Peter B O'Connor

FT-ICR Mass Spectrometry Group, Dept. of Chemistry, University of Warwick, Coventry

Metal based drugs have been widely employed to help combat many types of cancer. Unfortunately it has been shown that very little of the drug reaches its DNA target and up to 98% of some of the most common anticancer compounds can become protein bound within 1 day of injection. These metallodrug-protein interactions can cause changes in protein structure, limit protein function and contribute to the large array of side effects experienced by patients undergoing cancer treatment. New anti-cancer compounds offer increased cytotoxicity and different mechanisms of action to traditional cancer treatments in an effort to improve therapy by circumventing cisplatin resistance in certain strains of cancer.
Herein we present initial results from a study showing the interactions of recently developed anti-cancer compounds with peptides and proteins with different mechanisms of action, including photoactivatable metal centres, by top-down high resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). More gentle fragmentation techniques such as Electron Capture Dissociation (ECD) of the drug-peptide/protein adducts prove crucial in preserving the species during MS/MS when compared to Collisionally Activated Dissociation (CAD).

6) Proteins associated with wound healing and plasticity are up-regulated in the striatum of 6-OHDA lesioned rats at progressive stages of nigro- striatal dennervation

Heidi R Fuller[1,2] and **Monte A Gates[2]**

[1]Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry
[2]Keele University, Institute for Science and Technology in Medicine, Department of Life Sciences, Keele

Idiopathic Parkinson's disease is marked by degeneration of dopamine neurons projecting from the substantia nigra to the striatum. Although proteins expressed by the striatum can positively affect the viability and growth of dopaminergic neurons, very little is known about the proteome of the striatum as nigro-striatal degeneration progresses. Using iTRAQ labeling and mass spectrometry analysis, a quantitative comparison of the striatal proteome of rats before, during, and after 6-OHDA induced dopamine denervation was performed. Initial analysis revealed the differential expression of 99 proteins at 3 days, 62 proteins at 7 days and 77 proteins at 14 days post-lesioning, compared to the unlesioned striatum. Additional analysis, using western blotting and/or immunohistochemistry, revealed that the reduced expression of proteins characteristic of nigro-striatal degeneration (including TH and DARPP-32), was accompanied by an increased expression of proteins associated with wound healing (such as GFAP+ astrocytes), and neuronal plasticity (e.g., guanine deaminase). Such findings provide evidence for the possibility that the normal mammalian striatum can actively respond to nigro-striatal degeneration via the increased expression of proteins that may facilitate repair and growth within the basal ganglia nuclei. In the future it will be important to compare this response by the otherwise healthy striatum to dopamine denervation, with that seen within the striatum of PD sufferers to determine if the nuclei is responding in a similarly positive manner or failing to support nigro-striatal neurons in the disease.

7) Extended exposure to caffeine and sucrose results in persistent changes to locomotor behavior, neurobiology and response to acute methamphetamine challenge

JL Franklin[1], M Mirzaei[2], TA Wearne[1], MK Sauer[1], J Homewood[1], AK Goodchild[2], PA Haynes[3], JL Cornish[1]

Departments of Psychology[1], Australian School of Advanced Medicine [2] and
Chemistry and Biomolecular Sciences[3], Macquarie University, North Ryde, NSW, Australia

The orbitofrontal cortex (OFC) plays a critical role in regulating several cognitive processes associated with addictive behaviour. Caffeine is a psychostimulant commonly consumed by adults, often in combination with sugar. Exposure to psychostimulants has implications for neural function, potentially increasing the risk of developing an addiction disorder. This study examined whether caffeine and sucrose exposure alters OFC neurobiology and behavioural function. Method: Male Sprague Dawley rats were treated for 26 days with water, caffeine (0.6g/L), 10% sucrose or combination. Locomotor behaviour was measured on the first and last day of treatment. Then again after 7 days treatment free, when the animals were challenged with saline (1ml/kg, i.p.) or methamphetamine (1mg/kg, i.p). Label free quantitative shotgun proteomic analysis of the OFC was then conducted. Results: Locomotor response differed depended on pre-treatment and challenge exposure. Proteomic analyses of the OFC identified over 650 differentially expressed proteins across treatments, many relating to mitochondrial function, CDK5, dopamine, GABA and glutamate signalling. Conclusion: These data suggest there are behavioural interactions between methamphetamine-mediated locomotor activity and chronic caffeine and sucrose consumption. The protein expression patterns suggest that the abuse of these substances produce distinct neurobiological changes to protein systems important in behaviour and mental health.

8) Development and application of FAIMS for investigation of the FGF signaling pathway

Hongyan Zhao, Debbie Cunningham, Andrew J. Creese, John K. Heath and Helen J. Cooper

School of Biosciences, University of Birmingham, Edgbaston, Birmingham

High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) is a technique that focuses and separates ions at atmospheric pressure. Liquid chromatography(LC)-FAIMS-MS/MS has been applied in proteomics studies, and offers advantages including reduced chemical noise and increased signal-to-noise ratio. Several groups have demonstrated FAIMS can greatly improve the proteome coverage and has the potential to separate peptide isomers.
Fibroblast Growth Factor (FGF) and FGF signalling pathway play key roles in angiogenesis, wound healing and tumour growth. In previous work, a MS-based differential phosphoproteomics approach was used to study the FGF signalling and one of its downstream pathways-the Src mediated network. In order to gain more extensive knowledge of phosphorylation events in FGF signalling, especially those regulated by Src family kinase activity, we applied LC-FAIMS-MS/MS for the investigation of FGF signalling. Preliminary data suggests, compared to traditional LC-MS/MS, LC-FAIMS-MS/MS extends the phosphoproteome coverage by over 60%. A larger proportion of multiply-phosphorylated peptides were identified when FAIMS was applied. The use of FAIMS provides improved proteome coverage and allows efficient identification of multiply-phosphorylated peptides, which is promising for an in-depth investigation of FGF signalling.

9) Cadmium chloride inhibits cell migration and disrupts actin dynamics in cultured Sertoli cells

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Cadmium is a major environmental pollutant due to expansion in anthropogenic and industrial activities and may have damaging effects on human male fertility. Sertoli cells are directly involved in the traverse movement of developing germ cells in the seminiferous epithelium, because the later lack an architecture feature of migrating cells. In the present study, we investigated whether cadmium chloride (CdCl2) treatment affected Sertoli cells migration using live cell imaging in scratch assay method. Sertoli cells were incubated in the absence and presence (1µM and 12µM) of CdCl2 for 24 h. The results showed that exposure to CdCl2 caused disruption in Sertoli cell leading edge, which was associated with cell migration. CdCl2 had an inhibitory effect on Sertoli cell migration compared to control. The Population of invading cells per unit area decreased with time in the presence (1µM and 12µM) of CdCl2, compared to control. However, after 12 h of incubation with scratch application, there appeared to be more migrating cells with higher concentration than lower concentration of CdCl2. Indirect immunofluorescence analysis of fixed cell monolayers revealed anti-actin staining in both the cytoplasm and the nucleus, which showed reduced intensity with concentration. The level of actin detected on western blots was significantly reduced in a dose dependent manner in cytoplasmic and nuclear extracts. Coincidentally, cofilin levels on western blots showed a significant transient increase at the early time point but decreased at later time points. By contrast, the phosphorylation status of cofilin was significantly reduced at the early time point and increased at later time points.

10) Plasma proteome analysis using LC-MS/MS with travelling wave ion mobility and an alternative computational solution to protein quantitation

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Biomarker discovery involves the analysis of highly complex biological samples. Even using chromatography coupled with mass spectrometry, many species still co-elute, causing masking of ion signals and challenging protein identification and quantitation. Many techniques for incorporating additional separation, such as pre-fractionation and electrophoresis, decrease sample throughput and involve additional sample preparation which can contribute to preanalytical variation. Travelling wave ion mobility coupled with label free data independent acquisition (DIA) requires no additional sample preparation, yet confers significant advantages in proteomic analysis. The inclusion of an ion mobility step into the workflow also allows the number of identified proteins to be significantly increased whilst precursor and product mass accuracies are maintained between the modalities

The ion packeting behaviour of travelling wave ion mobility affords improved and less interfered detection of lower abundant species, however, one obstacle encountered with the analysis of high dynamic range proteomic samples is signal saturation of high abundant ions, causing issues in quantitating the most abundant proteins. This abstract presents an alternative bioinformatic approach which overcomes this by calculating protein quantities from product ion data. Protein quantities calculated with this method are more in line with the widely accepted label free data independent acquisition quantities calculated from precursor ion data. It is thus shown that ion mobility enhances proteome coverage, and with an adapted data processing method, it can be reliably used for quantitation as part of a biomarker discovery pathway.

11) Evaluation of mass spectrometry based approaches for the diagnosis of hemoglobinopathies

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Hemoglobinopathies are genetic defects that result in an abnormal structure of one of the globin chains. Three MS-based approaches were evaluated in a clinical trial incorporating 2017 patient samples. The methods used were: Intact mass measurement of the globin chains followed by MS and, if required, MS/MS measurements on tryptic peptides; UPLC-MRM experiments using diagnostic tryptic peptides; Intact mass measurement of globin chains followed by top down ETD fragmentation of selected globin- chains on a selected number of samples.

A mirrored clinical trial on patient samples was undertaken, in conjunction with a hospital, using the established method based on cation exchange LC. The existing approach identifies variants that have been established as being of clinical significance in the UK, including HbSS, HbSC, HbS, HbD-Punjab, HbE and HbO-Arab. HbA2 is also measured with elevated levels, used as an indication of β-thalassemia. All methods agreed well with the hospital results for the vast majority of samples. The hospital method gave a false positive result on three samples, two of which were identified as HbD but were unambiguously identified by MS as HbG-Philadelphia and one identified as elevated HbF which was a β-chain variant. Seven samples were found by MS to contain Hb variants not detected using the hospital method. Correlations of HbA2 levels between the mass spectrometry experiments and the hospital results were good however the absolute levels differed.

12) Bottom-up proteomics of dried blood spots by liquid extraction surface analysis and punch and elute based tryptic digestions

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Dried blood spots (DBS) have been used in the clinic as a blood sampling technique since the 1960's and in the mid 1990's mass spectrometry was used as to sample small molecules and metabolites. However in this time, very little has been done to attempt to analyse DBS from a proteomics perspective. To address this issue we present LC MS/MS based identification of large numbers of endogenous proteins from tryptic digestions of DBS. Two different methods are investigated, the first via a liquid extraction surface analysis (LESA) based automated trypsin digest based on the Triversa Nanomate nanoelectrospray platform and the second a more traditional punch and elute strategy. The latter will also be used with an immunodepletion stage as a sample enrichment process. This approach is used as an attempt to try and increase the number of lower abundance plasma proteins that can be detected from a dried blood spot.

13) Validation of bottom-up matrix-assisted laser desorption/ionisation ion mobility separation mass spectrometry imaging using recombinant "IMS TAG" proteins

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Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) provides a methodology to map the spatial distribution of peptides generated by in situ tryptic digestion of biological tissue.
It is challenging to correlate these peptides to the proteins from which they arise because of the many potentially overlapping and hence interfering peptide signals generated. A recombinant protein has been synthesised that when cleaved with trypsin yields a range of peptide standards for use as identification and quantification markers for multiple proteins in one MALDI-IMS-MSI experiment. Mass spectrometry images of the distribution of proteins in fresh frozen and formalin-fixed paraffin-embedded tissue samples following in situ tryptic digestion were generated by isolating signals on the basis of their m/z value and ion mobility drift time, which were correlated to matching peptides in the recombinant standard. Current experimental focus is aimed to assess the apparent influence of a 'cancer stem like' population of cells possibly responsible for a switch back to viable tissue in tumours.
Further development is also underway of the recombinant standard to enable quantitation of proteomic response via MALDI-MSI, a concept which up to now has only been observable as a measure of intensity using heat mapping imaging software.

14) Increased sub-proteome coverage of *Myxococcus xanthus* using a combination of 1D and 2D-chromatography incorporating mobility-enabled Data Independent Acquisition

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Myxococcus xanthus, a bacterial predator, is able to consume a variety of micro-organisms including *E. coli* in vitro. *M. xanthus* secretes outer membrane vesicles (OMVs) enriched with a subset of outer membrane and periplasmic proteins presumably due to a specific (but unknown) targeting mechanism, but the molecular basis of killing remains uncharacterised. We employed quantitative analysis on three sub-proteomes (cytosolic, OMVs and exoproteome) using a combination of 1D and 2D-(RP/RP) chromatography incorporating mobility-enabled data independent acquisition (HDMSE) to significantly improve coverage and confidence.
Comprehensive sub-proteome coverage was achieved using 2D (RP/RP) HDMSE. For OMVs the number of identified proteins increased 3-fold from 1D-MSE to 2D-HDMSE with average sequence coverage of 20.2%.
Similarly for exoproteome, the protein identification rate increased four-fold with 2D-HDMSE. Sequence coverage and peptides/protein increased when mobility-enabled acquisitions were performed. Protein identifications from a cytosolic sub-proteome increased 2-fold with 12 peptides/protein and 28.5% coverage from 2D-HDMSE. All proteins identified by 1D-MSE were also observed in 2D-HDMSE experiments.
Over three hundred Myxococcus proteins were in this study, but only 13 were observed in all sub-proteomes. We demonstrated that the exoproteome was not a result of cell lysis with OMVs having a distinct unique proteome.

15) Investigating the structure of hemoglobin by means of travelling wave ion mobility mass spectrometry

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Hemoglobin (Hb) is a tetrameric noncovalent complex two α- and β- chains each associated with a single heme group. Disorders associated with haemoglobin, termed hemoglobinopothies, are amongst the more prevalent inherited disorders. There are more than 1000 such disorders, the most prevalent being sickle-cell disease. It is estimated that 7% of the world's population are carriers of the sickle trait.
Previous work from this group used travelling wave ion mobility mass spectrometry (TWIM-MS) to estimate the rotationally averaged collision cross sections (CCS) of the monomeric, dimeric and tetrameric species and compared these values with the CCS values calculated from x-ray crystal diffraction data. In recent years new information on native calibration standards has been published which allows for more accurate estimation of the rotationally CCS's of native proteins using TWIM-MS. Sickle (HbS) and normal (HbA) samples were analysed by TWIM-MS using these new calibration standards and compared with previously published values.
The data indicates that CCS's acquired using the previous calibration standard underestimated the CCS values compared to the new native protein standards.
Most published CCS values have been obtained experimentally using an elevated source temperature and pressures. The effect of source temperature on observed CCS has been investigated by studying hemoglobin.

16) Glycation of HDL and LDL by methylglyoxal causes structural remodelling linked to increased atherogenicity in diabetes

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Cardiovascular disease (CVD) is a major cause of mortality wo rldwide. Increased risk of CVD and atherosclerosis is associated with high levels of LDL and low level of HDL. The processes associated with transformation of LDL to atherogenic forms and decline in quality of HDL are not fully understood. In recent studies we have investigated effect of glycation of LDL and HDL by MG to physiological, minimal extent on particle size, structural remodelling and catabolism.
LDL minimally modified by MG (MGmin-LDL) in vitro had decreased particle size – small and dense. It had increased binding to proteoglycans and increased aggregation in vitro. Mass spectrometry peptide mapping identified arginine-18 as the hotspot site of apolipoprotein B100 modification in MGmin-LDL. A computed structural model predicted that methylglyoxal modification of apolipoprotein B100 induces distortion, increasing exposure of the N-terminal proteoglycan binding domain on the surface of LDL. HDL modified minimally by MG had decreased particle size and stability. Mass spectrometry peptide mapping identified critical arginine residues that were hotspot sites of apolipoprotein A-1 modification in MGminHDL. Glycation of LDL and HDL by MG represent new non-oxidative routes to dysfunctional lipoprotein that renders LDL more atherogenic and impairs the quality of HDL.

17) Temporal changes in the secretome of articular cartilage exposed to interleukin-1 beta and carprofen

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Proteins released during cartilage explant inflammation and degradation can aid our understanding of degenerative diseases like osteoarthritis. High-throughput MS analysis compared different culture times to evaluate changes in inflammatory processes, and to assess if this approach could be used to screen effects of anti-inflammatory compounds.
Equine explant cultures derived from animals euthanized for purposes other than research were treated with pro-inflammatory cytokine IL-1β (10 ng/ml) or a COX-2 specific NSAID carprofen (100 µg/ml) + IL-1β. Tryptic digestions of cartilage proteins released into supernatants during 0-6 days or 12-18 days of incubation were analysed by nanoLC-MS/MS using an amaZon speed ETD instrument.
Extracellular matrix (ECM), non-ECM secreted and intracellular proteins were present in culture fluid at both time points. Semi-quantitative analysis using mascot scores indicated that carprofen reduced the level of some ECM proteins at 12-18 days. Certain non-ECM secreted proteins were identified at only one time point (YKL-40 & PCOC-2 early, CCL20 later). Evidence from intracellular protein presence indicated cell death was highest with IL-1β stimulation. Presence of intracellular proteins was lower in carprofen cultures, especially after 18 days, suggesting it decreased inflammation and associated cell death. Sampling at different time points impacted on proteins meeting the identification cut-off, but did not provide sufficient information to merit the additional resources involved.

18) Stable isotopic dilution analysis of AGEs and amino acids metabolome by liquid chromatography-tandem mass spectrometry

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Glycation of proteins by methylglyoxal and related physiological dicarbonyls forms advanced glycation end products (AGEs). These are often the most quantitatively important AGEs in physiological systems. Quantifying AGE residues in proteins and AGE free adducts in body fluids (plasma, urine, synovial fluid, cerebrospinal fluid) has revealed the often functionally important dicarbonyl-derived AGEs in physiological systems. A key analytical advance has been use of stable isotopic dilution analysis-liquid chromatography tandem mass spectrometry (LC-MS/MS) for quantitation of AGE residues in proteins (after exhaustive enzymatic hydrolysis) and AGE free adducts in fluid and tissues ultrafiltrates. We led derivatisation-free quantitation of AGEs in 2000. Since then the performance of LC-MS/MS systems has improved such that limits of detection (LODs) have decreased down to 100-fold lower compared to early studies. Herein we describe the current state-of-the-art AGE analysis. We routinely perform quantification of 14 protein glycation adducts, 5 oxidation adducts and the full amino acid metabolome in each run. The method uses graphitic HypercarbTM chromatography with an Acquity-Xevo-TQ-STM LC-MS/MS system. Typical limits of detection are (3 x SD of zero analyte control from regression of calibration curves) are: MG-H1 67 fmol, G-H1 31 fmol, 3DG-H1 60 fmol, MOLD 9 fmol, glucosepane 35 fmol, ornithine 79 fmol, CML 110 fmol, CEL 25 fmol, CMA 150 fmol and FL 174 fmol. The Xevo-TQ-STM system has improved performance, requires less material and is suitable for quantification of AGEs.

19) Detection of novel biomarkers in the cerebrospinal fluid of multiple sclerosis patients

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A biological biomarker may provide a valuable means to detect, measure, or quantify normal biological activity or that associated with a disease, or assess the effects of a disease treatment. Differential proteome profiling is often used to provide a basis for mining suitable biomarkers of disease. However, patho-physiological changes may be manifested as either a difference in protein post-translational modification (PTM) levels and/or alteration in protein associations. These processes may arise in the absence of protein level changes, and may therefore be missed by conventional biomarker proteomics. We have assessed protein PTM in the cerebrospinal fluid of multiple sclerosis (MS) patients and control subjects. We report the detection of novel protein biomarkers in MS patients via radiolabelling of PTMs. The sensitivity afforded by protein PTM radiolabelling and autoradiography provided a basis to detect novel protein biomarkers, and characterise the proteins by one dimensional and two dimensional separation techniques. This sensitivity and detection of protein PTM provided a viable means to track these novel biomarkers, and enable purification to a level sufficient for mass spectrometry identification.

20) **Application of FAIMS for identification of novel glycans and glycosylation sites in flagellin A of *Campylobacter jejuni***

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High Field Asymmetric Wave Form Ion Mobility (FAIMS) is emerging as a powerful technique for bimolecular analysis. FAIMS relies on differences in ion mobility in high and low electric fields to achieve gas phase separation of ion at atmospheric pressure. FAIMS coupled with tandem mass spectrometry (MS/MS) offers benefits for structural elucidation of posttranslational modifications (PTMs) of proteins and peptides. These advantages include increased signal to noise, reduced interference from ions of similar mass per charge (m/z), and separation of isomers and positional variants. Glycosylation is one of the most important PTMs in both eukaryotes and prokaryotes. Glycosylation in bacteria is of key interest in biology because of its relevance to pathogenicity in the host immune system.

Here we combine FAIMS with liquid chromatography tandem mass spectrometry (collision induced dissociation (CID) and electron transfer dissociation (ETD)) for the analysis of O-linked glycosylation in *C. jejuni* flagellin. Our aims were specifically to target the region of the protein [388-463] (inaccessible by trypsin) by treating flagellin with the protease proteinase K, and analysing the resulting digest by the use of liquid chromatography (LC) FAIMS MS/MS. The results show that the region of the protein [388-463] of *C.jejuni* is heavily glycosylated with multiple glycans. We also show isomeric separation of glycopeptides from proteinase K digest of *C.jejuni* flagellin with 20% improved sequence coverage using LC FAIMS MS/MS.

21) **Atomic Force Microscopy provides a novel insight in probing the effect of humidity on a therapeutic IgG antibody (alone and with sucrose)**

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Atomic force microscopy (AFM) is a broad spectrum technique able to collect information at nanoscale level, about cellular, subcellular and molecular structures and the underlying processes. Specifically, in the field of nanomedicine, here, we introduce the novelty of AFM in the molecular imaging of therapeutic protein (IgG antibody) coupled with mapping the dynamics in the dimensions and morphology of antibody when subjected to a chronological increase in moisture during real time imaging. Moreover, AFM demonstrated a novel insight into the potential immunity provided by non-reducing sugar (sucrose), against moisture lead denaturation which otherwise eventually leads to aggregation. There occurred a gradual increase in the molecular dimensions of therapeutic IgG features associated with the growth of crystalline material when therapeutic IgG lyophilized (alone and with sucrose) was exposed to a step wise increase in relative humidity (rH) from 20-80 %, each step up with (rH 20%). IgG containing sucrose displayed lesser growth in crystalline areas and the aggregates were relatively lesser in volume when compared to IgG on its own. However, under extreme humid conditions (rH 80%), IgG lyophilized with sucrose were found extremely hydrated and swollen compared to IgG on its own.

This insight can be a useful contribution towards better understanding for the development of novel screening methodology at nanoscale for assessing the impact of various interacting factors on the complexity of stabilizing proteins, both in therapeutic as well as in proteins whose aggregation is responsible for several pathological conditions in humans such as Alzheimer's and Parkinson's etc.