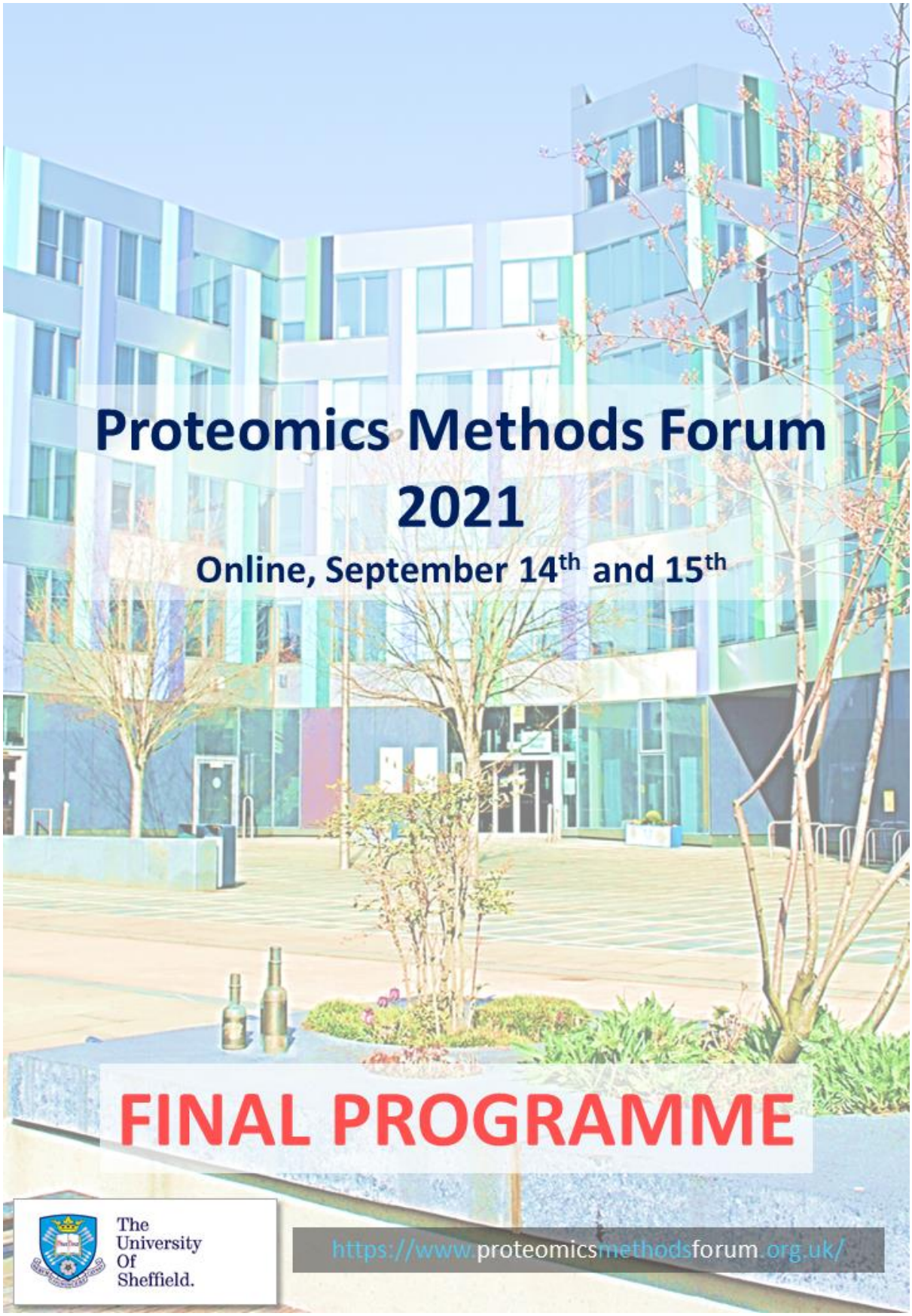

Proteomics Methods Forum 2021

Online, September 14th and 15th

FINAL PROGRAMME

The University Of Sheffield.

https://www.proteomicsmethodsforum.org.uk/

Tuesday, 14th September 2021

11:00	**-**	**11:10**	**Welcome to cPMF session**
			Adelina E Acosta Martin (University of Sheffield)
11:10	**-**	**11:40**	**Core facilities staff**
			Career progression in core facilities
			David Knight (University of Manchester)
			Open discussion
11:40	**-**	**12:10**	**Core facilities modus operandi**
			Valuing the contribution of core facilities to education and their role in the postgraduate curriculum
			Adelina E Acosta Martin (University of Sheffield)
			Open discussion
12:10	**-**	**12:30**	**Impact of Brexit and COVID on core facilities**
			Open discussion
			David Knight (University of Manchester) and Adelina E Acosta Martin (University of Sheffield)
12:30	**-**	**13:00**	**Long break**
13:00	**-**	**13:10**	**Welcome to main PMF session**
			Caroline Evans (University of Sheffield)
13:10	**-**	**13:15**	**Engagement activity I**
			Research I: Experimental design
13:15	**-**	**13:50**	**Chair:** *Duncan Smith (Cancer Research UK Manchester Institute)*
			Using MITO-Tag-based proteomics to assess impact of LRRK2 and PINK1 mutations on mitochondrial function
			Rosamund Clifford (University of Dundee)
			Development and Optimisation of an MRM-LC-MS/MS Assay for Investigating the Relative Expression of Nuclear Lamins
			Amy Grayson (Sheffield Hallam University)
			Panel discussion with additional experts
			Roman Fisher (University of Oxford) and Robert van Ling (Pharmafluidics)
			Technology I: Sample preparation
13:50	**-**	**14:15**	**Chair:** *Caroline Evans (University of Sheffield)*
			New solutions to old problems: Enhancing availability of methods with our publicly accessible resource library, & EMERGE: a platform for emerging researchers to present their innovative solutions to omics challenges
			Stoyan Stoychev (Resyn Bio)
			A Short Tale of Proteases, Surfactants and Standards
			Hillary Pollard (Promega)
			Questions
14:15	**-**	**14:35**	**Short break**
14:35	**-**	**14:40**	**Engagement activity II**
			Research II: Clinical proteomics
14:40	**-**	**15:15**	**Chair:** *Adelina E Acosta Martin (University of Sheffield)*
			SARS-COV-2 Detection by Mass Spectrometry - Considerations of Method Development and Clinical Implementation
			Dan Lane (University of Leicester)

			Targeted quantification of Factor H-Related proteins to link genotype to phenotype in Age-Related Macular Degeneration *Richard Unwin (University of Manchester)* Panel discussion with additional experts *Nicolas Autret (Covaris) and Liam Bell (CPGR, South Africa)*
15:15	**-**	**15:40**	**Technology II: Liquid chromatography** **Chair:** *Caroline Evans (University of Sheffield)*
			Maximizing Recovery of Phosphopeptides in LC-MS Studies with ACQUITY Premier *Chris Hughes (Waters)* Pushing sensitivity for LCMS Proteomics, a novel nano-capillary LC setup *Peter Mowlds (Thermo Fisher Scientific)* Questions
15:40	**-**	**16:20**	**Long break**
			Opportunity to meet with vendors in themed breakout rooms or discuss with presenters
16:20	**-**	**16:30**	**Engagement activity I - review**
16:30	**-**	**17:00**	**Plenary talk** **Chair:** *David Knight (University of Manchester)*
			Proteomics goes forensics *Simona Francese (Sheffield Hallam University)* Questions
17:00	**-**	**17:15**	**Short break**
17:15	**-**	**18:00**	**Evening social activity**

Wednesday, 15th September 2021

13:00	**-**	**13:05**	**Engagement activity III**
13:05	**-**	**13:40**	**Research III: Data analysis** **Chair:** *Richard Unwin (University of Manchester)*
			PROTREC: A probability-based approach for recovering missing proteins based on biological networks *Weijia Kong (Nanyang Technological University)* TMT Issues on Orbitrap Fusion *Michele Tinti (University of Dundee)* Panel discussion with additional experts *Mark Skehel (Francis Crick Institute) and Adam Dowle (Universtiy of York)*
13:40	**-**	**14:05**	**Technology III: Data analysis** **Chair:** *Adelina E Acosta Martin (University of Sheffield)*
			Disulfide bond analysis with Mascot *Ville Koskinen (Matrix Science)* Workflows for the analysis of CID and EAD zenoTOF 7600 proteomics datasets *Nick Morrice (Sciex)* Questions

14:05	**-**	**14:25**	**Short break**
14:25	**-**	**14:30**	**Engagement activity IV**
			Research IV: PTM and structural analysis
14:30	**-**	**15:05**	**Chair:** *Mark Dickman (University of Sheffield)*
			Phosphorylation site quantification by Parallel Reaction Monitoring focusing on phosphosite specific fragment ions *Duncan Smith (CRUK, Manchester)*
			State-of-the-art LC-MS methodologies for protein intact mass analysis in the biopharmaceutical industry *Giulia Lambiase (University of Sheffield)*
			Panel discussion with additional experts *Paula Meleady (Dublin City University) and Zuzana Demianova (Preomics)*
			Technology IV: Mass spectrometry and automation
15:05	**-**	**15:30**	**Chair:** *Adelina E Acosta Martin (University of Sheffield)*
			Ultra-high sensitivity on the Bruker timsTOF platform *Angela Paul (Bruker)*
			Determining calibration curves for targeted, high throughput proteomics with an Evosep One *Wendi Hale (Agilent/Evosep)*
			Questions
15:30	**-**	**16:10**	**Long break**
			Opportunity to meet with vendors in breakout rooms or discuss with presenters
16:10	**-**	**16:20**	**Engagement activity III - review**
16:20	**-**	**16:50**	**Discussion meeting round up and next steps**
			David Knight (University of Manchester)
16:50	**-**	**17:00**	**Close meeting and farewell**
			Adelina E Acosta Martin and Caroline Evans (University of Sheffield)

More information about chairs and moderators can be found at the end of the programme.

cPMF Session:

Career progression in core facilities.

David Knight (University of Manchester)

Career progression for technical experts has been identified as an issue nationally as a part of the concept of 'Team Science'. This presentation will discuss the national initiatives, look at potential institutional examples and describe personal experience in successfully using the HERA process for re-grading.

Keywords: Core facilities, careers, staff

Valuing the contribution of core facilities to education and their role in the postgraduate curriculum.

Adelina E Acosta Martin (University of Sheffield)

This study reveals that core facilities and their staff are vital elements of postgraduate education, showing how students and other users are aware of and value the educational role of core facility staff within a research environment. Postgraduate students are the major group of core facility users, but their grants do not cover the core facilities' FEC rates, so they are often charged with much lower rates, which causes strong adverse implications for the adequate funding of core facilities. The role and value of core facilities and their staff in universities needs an urgent review in order to challenge the reductionist vision of core facilities as instrument driven research factories. This will enable a sustainable funding model that matches their reality as synergistic environments of research and education.

Keywords: Core facilities, postgraduate education, funding gaps

Main PMF Session - Research I: Experimental design

Chair: *Duncan Smith (Cancer Research UK Manchester Institute)*

Using MITO-Tag-based proteomics to assess impact of LRRK2 and PINK1 mutations on mitochondrial function.

Rosamund Clifford (University of Dundee)

I will discuss the use of the MITO-tag rapid mitochondrial isolation technique to selectively retrieve mitochondrial proteins from mouse brain, heart and lung. We prepared these proteins for MS by loading onto an S-Trap and performing on-trap Trypsin/LysC digestion, before injecting into an HPLC system coupled to a Thermofisher Exploris 480 instrument in data-independent acquisition mode. I will discuss how the data was searched using a directDIA strategy in Spectronaut version 15 and the downstream analysis which I performed in Perseus.

Keywords: dDIA, MITO-Tag, quantification

Areas of application: Sample preparation, throughput & automation, Quantification, Informatics & data analysis, Experimental desing & proteomics workflows

Development and Optimisation of an MRM-LC-MS/MS Assay for Investigating the Relative Expression of Nuclear Lamins.

Amy Grayson (Sheffield Hallam University)

Nuclear lamins are filament proteins underlying the inner nuclear membrane and are important in several biological processes including cellular maturation and differentiation, and tissue mechanosensing. Furthermore, aberrant expression of these proteins has been associated with the development of laminopathies and malignant transformation. This presentation describes the process of developing and optimising a multiple reaction monitoring-based liquid chromatography-coupled tandem mass spectrometry (MRM-LC-MS/MS) assay using an Agilent 1290 Infinity II LC system coupled to an Ultivo LCTQ mass spectrometer, and data analysed using Skyline. Transition-dependent factors and electrospray source parameters were optimised to increase assay sensitivity. Finally, this method was applied to cell lysates of human cell lines and primary mouse lymphocytes to investigate the relative protein expression of various nuclear lamin isoforms in these biological samples.

Keywords: LC-MS/MS, multiple reaction monitoring, lamins

Areas of application: Quantification, Experimental design & proteomics workflows

Panel discussion with additional experts:

Roman Fisher (University of Oxford)

Roman Fischer (RF) is an Associate Professor and Senior Group Leader in Clinical Proteomics at the University of Oxford. He leads the Discovery Proteomics Facility at the Target Discovery Institute. RF studied Biotechnology at the Technical University Braunschweig and obtained his PhD at the Helmholtz Centre for Infection Research for the analysis of host-pathogen interactions of Listeria monocytogenes using proteomic methods (2007). After postdoctoral studies on Il-1 signalling in the laboratory of Professor Sir Philip Cohen in Dundee (Scotland), RF started to develop clinical proteomics at the University of Oxford in 2009. Since 2013 RF leads the Discovery Proteomics Facility and applies proteomic methods to a multitude of scientific questions (>150 peer-reviewed papers). Currently, RF focusses his research on the development of high-throughput and spatial proteomics methods and their application to large clinical cohorts and specimen.

Areas of expertise: Clinical Proteomics, Spatial Proteomics, High-Throughput Proteomics, Method Development

Robert van Ling (Pharmafluidics)

Robert has always worked on the chromatography aspect of proteomics, from an instrument/column manufacturer's perspective. The first products he worked with were the PepMap nanoLC and PepSwift monolithic columns, and the UltiMate/Famos/Switchos combinations from LC Packings in the 1990s. This continued via Dionex (another name from the past) to the UltiMate 3000 RSLCnano systems with EASY-Spray columns from Thermo Scientific that are often used nowadays in proteomics. With the µPAC columns (from PharmaFluidics) he is keen to continue working especially

in single cell proteomics, and biopharmaceutical peptide mapping using proteomics/low flow chromatography workflows. Finally, he has his own interest in top-down proteomics and intact protein analysis.

Areas of expertise: nanoLC chromatography

Main PMF Session - Technology I: Sample preparation

Chair: *Caroline Evans (University of Sheffield)*

New solutions to old problems: Enhancing availability of methods with our publicly accessible resource library & EMERGE: a platform for emerging researchers to present their innovative solutions to omics challenges.

Stoyan Stoychev (Resyn Bio)

At ReSyn Bio our emphasis is on improving the efficiency of bioseparation and mass spectrometry-based workflows. In order to ensure researchers are always up to date on the latest methods and workflows we are currently driving two initiatives, making our methods more accessible through a new Methods Library, and new EMERGE webinar series. The EMERGE webinar series provides a platform for emerging researchers to highlight innovative solutions for current omics problems that they are developing, while the Method Library is an annotated online repository of resources such as publications, posters and application notes, that allows simplified identification and delimitation based on a user's specific sample preparation needs. In the presentation we will demo the library to illustrate how to efficiently extract information and match to the application area by delimiting based on several criteria including, sample source, extraction and digest preparation conditions, and where relevant the type of quantification strategy and whether these have been automated for high-throughput applications.

Keywords: Methods Library

Areas of application: Sample preparation workflows

A Short Tale of Proteases, Surfactants and Standards.

Hillary Pollard (Promega)

An overview of four Promega sample prep products, including three new products and one best seller highlighted with customer data.

Keywords: New Recombinant Trypsin

Areas of application: Sample preparation

Main PMF Session - Research II: Clinical proteomics

Chair: *Adelina E Acosta Martin (University of Sheffield)*

SARS-COV-2 Detection by Mass Spectrometry - Considerations of Method Development and Clinical Implementation.

Dan Lane (University of Leicester)

The standard for COVID-19 screening, RT-PCR, relies on amplification and detection of viral RNA in nasopharyngeal swabs. Mass spectrometry (MS) approaches, developed in collaboration with the MS Coalition through 'Operation Moonshot', provides an alternative detection platform that instead targets SARS-COV-2 tryptically digested proteins. In the relatively untouched field of infectious disease screening by MS, we discuss considerations of method development (identification, tune settings, tryptic conditions, and capture-based sample preparation), and clinical application in different human biomatrices.

Keywords: SARS-COV-2, immunoaffintiy, optimisation

Areas of application: Sample preparation, throughput & automation, Quantification, Clinical proteomics

Targeted quantification of Factor H-Related proteins to link genotype to phenotype in Age-Related Macular Degeneration.

Richard Unwin (University of Manchester)

Age-related Macular Degeneration (AMD) is a leading cause of blindness. A risk locus on Chr1 contains 6 related genes, producing 7 products; Complement Factor H (FH), a splice variant Factor H-Like 1 (FHL-1), and five Factor H-Related proteins (FHR-1 to -5). We developed a MS assay to quantify all seven products to study the link between genotype, protein expression and AMD. 604 plasma samples were analysed by SRM-MS (352 AMD cases vs 252 controls), with batch QCs to assess performance giving a %CV for all proteins <15% across 37 sample batches. Both FHR-1 and FHR-2 were increased in AMD (p=2.4x10-10 and p=6.0x10-10), with modest associations of FHR-3 to -5, and FHL-1. Genome Wide Association Studies showed that SNPs within the CFH gene drove increases in circulating FHR proteins. This study confirms the mode of action by which AMD risk SNPs impact AMD development, and highlights FHRs as new targets for AMD treatment.

Keywords: Selected Reaction Monitoring; Plasma; GWAS

Areas of application: Quantification, Clinical proteomics

Panel discussion with additional experts:

Liam Bell (Centre for Proteomic and Genomic Research, South Africa)

Liam is the current proteomics manager at the Centre for Proteomic and Genomic Research (CPGR), an ISO accredited core facility based in Cape Town, South Africa. He started his career in proteomics during his PhD which was completed in Prof. Jonathan Blackburn's lab at the Institute for Infectious Diseases and Molecular Medicine (IDM) at UCT. This work focused on the differing phenotypic outcomes of peripheral blood mononuclear cells (PBMCs) in patients dually infected with HIV and TB but who suffered vastly different clinical outcomes post initiation of HIV treatment. These patients were clinically categorised as suffering from TB associated immune reconstitution syndrome (TB-IRIS). He continued his proteomics work at the Kwa-Zulu Natal Research Institute for TB and HIV (KRITH) as a post-doctoral researcher from 2013 – 2015. During this time, he focused on phospho proteomic and targeted proteomic workflows concentrating on the signalling cascades in the lung associated with TB infection. In 2015 he moved to the CPGR as an application specialist before becoming the Proteomics Manager a few months later. During his time there in this role, he has had

direct input in over 120+ proteomics projects ranging from discovery-based projects to market ready development and implementation products and has worked with both academic and industry clients alike. He has been directly involved in developing and implementing pipelines to automate and scale proteomics services solutions in the pre-analytical, analytical and post-analytical stages at the CPGR.

Areas of expertise: Biological Mass Spectrometry (MS); Quantitative proteomics; Assay development; Scaling systems and processes; Clinical and agricultural proteomics; High throughput proteomics; Infectious diseases; Non-infectious diseases.

Nicolas Autret (Covaris)

Nicolas' background is in pre-analytical and analytical methods in proteomics, and his personal interest is to advance use of proteins as biomarkers in precision medicine. He develops collaborations with public and private labs to find solutions that improve existing workflows and processes in protein sample prep. The ultimate goal (dream? ☺) is to simplify and speed protein analysis (through automation and throughput), increase methods' sensitivity, to build high confidence in results, which should finally give proteins the place they deserve in patient care.

Areas of expertise: Sample preparation, Automation, High Throughput, Biomarker discovery, Precision medicine

Main PMF Session – Technology II: Liquid chromatography

Chair: *Caroline Evans (University of Sheffield)*

Maximizing Recovery of Phosphopeptides in LC-MS Studies with ACQUITY Premier.

Chris Hughes (Waters)

Protein phosphorylation is the most common post translational modification, affecting every basic cellular process. However, detection of phosphopeptides by LCMS still remains very challenging. One problem is that phosphopeptides do not protonate efficiently due to the presence of one or more acidic phosphate groups. Incomplete recovery from the wetted components of an LC system is another cause of compromised detection. Current strategies to improve recovery include the addition of EDTA or citrate to samples in an attempt to passivate the fluidic path. However, these strategies can cause issues with chromatographic performance.

Keywords: Novel Surface Technology, Phosphopeptides

Areas of application: Phosphopeptide analysis

Pushing sensitivity for LCMS Proteomics, a novel nano-capillary LC setup.

A Peter Mowlds (Thermo Fisher Scientific)

For researchers pursuing the next scientific breakthrough, the next generation low-flow Thermo Scientific™ UHPLC system delivers maximum performance 24/7 for nano-, capillary-, and micro-flow LCMS applications. Thermo Scientific™ ProFlow™ XR 1500 bar pump technology ensures excellent retention time precision from nano- up to micro-flow, the unique low-flow split-loop autosampler enhances sample throughput and injection performance, and integrated system intelligence streamlines method editing and simplifies operation. From proteomics and biopharma to high-

throughput translational research, the hyphenation of the new system with state-of-the-art Thermo Scientific™ Orbitrap™ and triple quadrupole mass-spectrometers is pushing the limits of sensitivity and productivity.

Keywords: nanoLC, capLC, Vanquish

Areas of application: proteomics, peptide separation

Main PMF Session – Plenary talk

Chair: *David Knight (University of Manchester)*

Proteomics goes forensics.

Simona Francese (Sheffield Hallam University)

Simona Francese[1], Cameron Heaton[1], Katie Kennedy[1], Cristina Russo[1], Laura Cole[1], Richard McColm[2], Jason Eyre[3], Lynda Wild[4], Glenn Langenburg[5]

[1]Centre for Mass Spectrometry Imaging, Biomolecular Science Research Centre, Sheffield, UK. [2]Defence Science and Technology Laboratory, Porton Down, UK. [3]BMS Haemolysis Lab, Haematology Department, Sheffield Teaching Hospital NHS Foundation Trust, Sheffield, UK. [4]Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK. [5]Elite Forensic Services LLC, Minneapolis, Minnesota, US

For at least the first three decades since its advent, proteomics has largely belonged to a clinical, diagnostic or fundamental biology context. However, the range and the significance of information that proteomes can disclose have led this discipline to be also applied to forensics, ranging from human identification from hair samples, identification of bodily fluids and microbial forensics to doping investigations[1]. Fingermarks are a relatively new specimen for proteomic studies with any form of proteomic investigation only appearing in 2012 with the analysis of intact peptides and small proteins in situ[2] for determination of sex. It was not until 2015 that further developments allowed bottom up proteomics to be also applied directly in situ. Fingermarks have proven to be a huge source of information to profile suspects and peptides and proteins are quickly becoming very interesting story tellers. Whilst in situ proteomics of fingermarks has many advantages, encompassing simplified sample preparation protocols, speed and the opportunity to perform molecular imaging analyses, this area remains under-investigated. This is probably due to the unique challenges working with fingermark specimens. In this presentation the successes and the challenges of proteomics methods applied to fingermarks[3] and blood fingermarks[4] will be illustrated and discussed including recent developments.

References:

1. Merkley E.D., Wunschel D.S., Wahl K.L., Jarman K.H. Forensic Sci. Int., 297, 350–363, 2019
2. Ferguson L., Wulfert F., Wolstenholme R., Fonville J.M., Clench M.R., Carolan V.A., Francese S. Analyst, 137(20):4686-92, 2012
3. Patel E., Clench M.R., West A., Marshall .S., Marshall N, Francese S., J. Am. Soc. Mass Spectrom. 26(6): 862–872, 2015
4. Deininger L., Patel E., Clench M.R., Sears V., Sammon C., Francese S. Proteomics, 16(11-12):1707-17, 2016

Keywords: Forensics, Profiling, MALDI

Areas of application: Forensic Mass Spectrometry, Forensic Proteomics

Main PMF Session - Research III: Data analysis

Chair: *Richard Unwin (University of Manchester)*

PROTREC: A probability-based approach for recovering missing proteins based on biological networks.

Weijia Kong (Nanyang Technological University)

Despite technological advances in proteomics, incomplete coverage and inconsistent protein-reporting issues persist, resulting in proteins which are difficult or non-reproducibly observed. We collectively term these 'missing proteins" (MPs). To address this issue, we develop 'PROTREC', a missing protein recovery algorithm that uses biological networks (in particular, protein complexes) integrated with a probability-based scoring scheme. Via PROTREC, we can confidently recover proteins previously not found in the original proteomic screen. Across a variety of proteomic acquisition paradigms, our results show PROTREC dominates over several existing network-based methods. Since PROTREC relies on non-tissue specific protein complexes, we further show that performance can be further improved by optimizing the protein complex set. PROTREC is a quantum leap for missing protein prediction algorithms with wide application potential.

Keywords: protein complexes, proteomics, missing proteins

Areas of application: Informatics & data analysis

TMT Issues on Orbitrap Fusion.

Michele Tinti (University of Dundee)

The quality controls of our TMT samples analysed on the Orbitrap Fusion (MS3) show low identification rates at the beginning of the gradient. Particularly affected are the +3 charge Ions. In this talk, we would like to discuss this issue's possible causes and solutions with the proteomic community.

Keywords: TMT Orbitrap Conversion Rate

Areas of application: Quantification, Informatics & data analysis, Liquid Chromatography performance

Panel discussion with additional experts:

Mark Skehel (Francis Crick Institute)

Mark's research aims focus on the development and application of quantitative proteomic techniques, both differential labelling (TMT, SILAC) and label-free (DIA) approaches, which are used as force multipliers across a number of research themes at the Crick. He has a particular interest in the application of hydrogen deuterium exchange and chemical cross-linking combined with mass spectrometry for the structural analysis of proteins and protein complexes. His goal is to increase the subunit complexity that can be analysed by these techniques, so allowing his group to understand complex biological phenomena in terms of the molecular interactions that drive and regulate them. This is exemplified in recent HDX/MS studies of the mitochondrial inner membrane enzyme NADH:ubiquinone oxidoreductase, a 1 mDa 45 subunit protein complex.

Areas of expertise: Liquid chromatography-MS, HDX/MS, cross-linking, proteomic data analysis, post translational modification, clinical proteomics, molecular biology, analytical chemistry.

Adam Dowle (University of York)

Adam has worked in proteomics service provision at the University of York for 16 years. During this time, he has gained experience in many different techniques and applications, from protein QC to metaproteomics and many weird and wonderful things between; including a multitude of PTM classes, chemical modifications and quantification strategies, using a range of mass spectrometry instrumentation. Coupled to these workflows he has used many data analysis softwares and platforms from single spectra *de novo* sequencing to complex PTM quantification approaches.

Areas of expertise: Proteomics, LC-MS, Quantification, PTMs, MALDI, Service, Data analysis, Experimental design

Main PMF Session - Technology III: Data analysis

Chair: *Adelina E Acosta Martin (University of Sheffield)*

Disulfide bond analysis with Mascot.

Ville Koskinen (Matrix Science)

Mascot Server supports identifying intact crosslinks, including disulfide bonds. I'll illustrate the analysis steps with the NIST monoclonal antibody standard (NISTmAb). The protein has two heavy chains and two light chains and contains native crosslinks within each chain (intralinks) and between chains (interlinks). Mascot identifies all native disulfide bond sites.

Keywords: disulfide, crosslinking, Mascot

Area of application: informatics and data analysis

Workflows for the analysis of CID and EAD zenoTOF 7600 proteomics datasets.

Nick Morrice (Sciex)

The ZenoTOF 7600 is an exceptionally fast LC-MS system (133Hz), while still maintaining high resolution (>35k) in MS and MSMS modes and can be used for conventional CID as well as EAD fragmentation. This presentation will show how to process proteomics DDA, Swath and MRMhr data for both fragmentation modes generating high quality qualitative and quantitative results. Data processing of DDA and Swath data can also be performed in the cloud using OneOmics suite and a brief summary of this workflow will be shown.

Keywords: EAD, Swath and ZenoTOF

Areas of application: Proteomics workflows

Main PMF Session - Research IV: PTM and structural analysis

Chair: *Mark Dickman (University of Sheffield)*

Phosphorylation site quantification by Parallel Reaction Monitoring focusing on phosphosite specific fragment ions.

Duncan Smith (Cancer Research UK Manchester Institute)

Mapping sites of phosphorylation in a quantitative manner is a challenging endeavour. Unfortunately, this challenge can be disappear in the context of the huge phosphoproteomic datasets claiming to have achieved site specific quantitation. In the vast majority of cases, these quantitative datasets are not site specific as they rely on precursor ion or TMT/iTRAQ reporter ions, neither of which can differentiate ambiguity introduced in peptides that carry other residues with the potential for phosphorylation. Here we demonstrate an approach where phosphorylation sites from immunoprecipitated proteins can be quantified in a site specific manner using a Parallel Reaction Monitoring (PRM) approach followed by extraction of site specific fragment ions in an LCMS timeframe.

Keywords: phosphorylation, PRM and Quantification

Areas of application: Quantification

State-of-the-art LC-MS methodologies for protein intact mass analysis in the biopharmaceutical industry.

Giulia Lambiase (University of Sheffield)

The introduction of high-resolution mass spectrometers (HRMS) with electrospray ionisation (ESI) has favoured the rise of native intact mass analysis in the biopharmaceutical industry. Native ESI-HRMS represents key advances in biopharmaceutical characterisation of higher-order structures, glycosylation, size and charge variants providing simpler and faster information acquisition with no sample preparation. Moreover, such analytical approaches maximise the information obtained with one single analysis by monitoring multiple quality attributes at the molecular level with the selectivity and sensitivity of MS detection. Native intact mass analysis tools are particularly advantageous during the early-stage developability assessment, which aims at identifying potential product CQAs whilst maintaining the analysis throughput. This talk provides an overview of the state-of-the-art LC-MS methodologies for protein intact mass analysis relevant in the biopharmaceutical industry.

Keywords: Biopharmaceuticals, protein intact LC-MS, nLC-MS

Areas of application: Sample preparation, throughput & automation, Intact protein analysis, Protein modification & structural analysis, Liquid Chromatography performance

Panel discussion with additional experts:

Paula Meleady (Dublin City University)

Dr. Paula Meleady is a Principal Investigator at the National Institute for Cellular Biotechnology (NICB), School of Biotechnology, Dublin City University, Dublin, Ireland. She leads the Proteomics and

Mass Spectrometry Core Facility at the NICB, which is equipped with state-of-the-art instrumentation for protein mass spectrometry applications. Her research interests are focused on the application of advanced proteomic and mass spectrometry methods, including the characterisation of cellular post translational modifications, to understand biological systems. Specifically, her research focuses on the analysis of recombinant mammalian cell lines in order to gain insights to improving the efficiency of production of biopharmaceuticals. She also has research interests in clinical proteomics, specifically uveal melanoma and pancreatic cancer.

Areas of expertise: Biomarkers, pancreatic cancer, phosphorylation, proteomics, recombinant mammalian cell lines, biopharmaceutical production.

Zuzana Demianova (Preomics)

Zuzana's research expertise is around proteins and proteomics, from sample preparation to data analysis and reports. Since her university, she has enjoyed the challenging world of proteins, their separation and characterization. Also, she has learned a lot about small molecules from cell culture and feeding to produce a quality product. She has been a long carrier academic scientist and switch to the business world after her 2nd PostDoc. After that, she helped with AI and medical projects, worked from MS-vendor, where she started as an application specialist and global proteomics lead. Later, she was promoted to biopharma applications scientist. Since June 2021, she has been working as market development manager for BioPharma for a small startup that focuses on protein sample preparation.

Areas of expertise: Biopharma, qualitative and quantitative proteomics, sample preparation, mass spectrometry, peptide mapping, intact proteins.

Main PMF Session - Technology IV: Mass spectrometry and automation

Chair: *Adelina E Acosta Martin (University of Sheffield)*

Ultra-high sensitivity on the Bruker timsTOF platform.

Angela Paul (Bruker)

The unique and proprietary PASEF mode of operation on the timsTOF achieves near 100% duty cycle. Time and space focusing of ions in the Trapped Ion Mobility Spectrometry (TIMS) tunnel, result in a significant boost in sensitivity and sequencing speed >120 Hz. The timsTOF SCP further enhances the sensitivity with a robustly modified ion optic design enabling measurement of sub-nanogram peptide loads. The timsTOF SCP is the first dedicated instrument for unbiased quantitative single cell 4D-proteomics research, immunopeptidomics and PTM analysis with higher throughput and unprecedented robustness. More than 1500 proteins are quantified from 250picograms, covering an abundance range of 4 orders of magnitude. Bruker's collaborators are making major progress in unbiased single cell proteomics, phosphoproteomics and plasma proteomics, leveraging the speed, sensitivity and dynamic range of large-scale CCS-enabled 4D-Proteomics and 4D-Epiproteomics.

Keywords: 4D-Proteomics, Immunopeptidomics, Epiproteomics

Areas of application: single cell proteomics

Determining calibration curves for targeted, high throughput proteomics with an Evosep One.

Wendi Hale (Agilent/Evosep)

Using a commercially available complex proteomics sample (PeptiQuant Plus 125 from MRM Proteomics), calibration curves were determined to characterize the setup for linearity, precision, accuracy, specificity with a targeted, high throughput proteomics application.

Keywords: sensitivity, precision, linearity

Areas of application: targeted proteomics

Moderators and Chairs

Working in the background of Blackboard Collaborate, *Heather Walker (University of Sheffield)* and *Julian Selley (University of Manchester)* are the moderators of the event.

Duncan Smith (Cancer Research UK Manchester Institute)

Duncan performed his PhD studying the effects of oncogenic tyrosine kinases on proteomes and phospho proteomes in the lab of Simon Gaskell from 1998- 2001. He studied the preclinical efficacy of a promising new rationally designed Abl kinase inhibitor, STI571 on Bcr-Abl driven proteomic changes. STI571 went on to become the frontline therapy for CML now used under the names Gleevec and Imatinib. He then went on to setup MS and proteomic capabilities in a small biotech company called Renovo where he used the platform he built to interrogate the effects of TGFb3 in a wound healing model. In 2004, Duncan returned to academia as a postdoc at the Paterson Institute with the joint remit of co-building a local proteomics capability and utilising it in the study of stem cell differentiation in collaboration with George Lacaud, Valerie Kouskoff & Tony Whetton. In 2006, Duncan was appointed head of the Mass Spec Core facility of CRUK Manchester institute (formerly the Paterson Institute) and offers support for a whole range of LCMS based proteomics in addition to bespoke method development to enhance the impact of Cancer research at CRUKMI. In 2008, Duncan co-founded the training and consultancy company MS-Insight where he is a director.

Areas of expertise: LCMS, Proteomics, Phosphorylation, PTM, Quantitation, Phosphorylation, MS, Chromatography

Caroline Evans (University of Sheffield)

Caroline Evans is Facility Manager for the Bioanalytical Facility in the Faculty of Engineering, University of Sheffield. Prior to moving to Sheffield in 2008, Caroline co-directed the Leukaemia Research Fund Proteomics Facility at the University of Manchester. Caroline develops and applies quantitative proteomics and PTM analysis (DDA, DIA based) to a range of bioengineering and biomedical projects. Research interests include the use of E. coli, CHO and microalgal 'cells as factories' for bioproduction as well as clinical focussed research such as colon biopsies and exosomes. Caroline has a PhD in biochemistry and enjoys the diversity of projects afforded by working in the department of Chemical and Biological Engineering.

Areas of expertise: Quantitative proteomics, bioengineering, mass spectrometry, bioseparations, post-translational modifications

Adelina E Acosta Martin (University of Sheffield)

Adelina has an international research career that has allowed her to use proteomics and mass spectrometry to study different human diseases, including cardiovascular diseases, cancer, haemoglobin disorders and metabolic disorders. From when she started her MSc, and later on during her PhD and postdoc experience, she has applied and developed MS-based methods (DDA, DIA, PTM analysis, intact protein analysis, SRM, isobaric labelling, label free) and used different MS instruments (MALDI-TOF/TOF, ESI-LTQ-Orbitrap, ESI-Ion trap, QqQ, Qq-Orbitrap). In 2016, she became mass spectrometry facility manager for proteomics applications at the biOMICS Facility (University of Sheffield), where she closely works with users to achieve the best outcomes for their research projects. These projects are very diverse and go from archaeology to engineering, agriculture, ecology, microbiology, and biomedical and clinical research. She is also passionate about how postgraduate education develops within a research environment and the role of core facilities in education.

Areas of expertise: Proteomics, mass spectrometry, liquid chromatography, quantification, method innovation, postgraduate education, core facilities

David Knight (University of Manchester)

David is a chromatographer by trade, obtaining his degree in Applied Chemistry and PhD in Analytical Biochemistry part time whilst working in a bioanalytical CRO. He has run the Biological Mass Spectrometry Facility in the University of Manchester for 20 years, which has expanded to include equipment and staff for protein characterisation, proteomics, ligand binding assays, small molecule quant, metabolomics, informatics and soon MS Imaging. His current interests are in how these technologies can be applied across diverse applications, from environmental to clinical, in a manner that encourages adoption and risk as well as looking at how core facilities can bridge academic, commercial and clinical labs. He is also a technical lead for Research Data Management (RDM) in his Faculty, designing strategies for RDM that co-ordinate resources from across the university. Finally, he is active in trying to improve the recognition and cohesion of technical staff via groups such as Manchester's Technical Commitment and Faculty Committee.

Areas of expertise: proteomics, metabolomics, analytical biochemistry, mass spectrometry, research data management, technical management and leadership

Richard Unwin (University of Manchester)

Richard graduated from the University of Nottingham with a BSc in Biology and MSc in Oncology before obtaining his PhD from the University of Leeds in what was then the new field of proteomics. He joined The University of Manchester in 2001, developing new methods for analysing cancer proteomes, including working with industry to establish new mass spectrometry tools for protein measurement. In 2010 he moved to manage a mass spectrometry research laboratory within the NHS, where he worked on the study of proteins and metabolites chronic diseases such as diabetes and Alzheimer's disease, and developed new assays to measure specific molecules of interest which have now been moved into the clinical laboratories. Richard moved back to the University four years ago, becoming Deputy Director of SBDC in 2019. He collaborates widely with academic and

commercial partners and has also established a spin-out company, Complement Therapeutics, to develop a series of novel therapeutics and an accompanying precision medicine platform for treatment of conditions characterised by dysregulation of the complement system, most notably Age-Related Macular Degeneration.

Areas of expertise: Mass spectrometry, Plasma, Clinical, Targeted, Selected Reaction Monitoring, Quantitation, Assay development, Isobaric tagging, SWATH-MS

Mark Dickman (University of Sheffield)

Mark obtained a First Class Honours Degree in Biochemistry/Chemistry and a PhD at the Kreb's Institute, University of Sheffield. Post PhD Mark joined a biotechnology company, Transgenomic LTD , working as a research scientist developing analytical techniques including DNA/RNA Chromatography. Mark joined the Dept of Chemical and Biological Engineering in 2003. His research focuses on the development and application of analytical techniques to study biological systems. In particular, biological mass spectrometry in conjunction with bioseparations have been utilised to study a wide variety of biological systems. Using these analytical approaches to identify and characterise protein complexes, protein-RNA/DNA complexes, protein post translational modifications and RNA post transcriptional modifications.

Areas of expertise: Biological mass spectrometry, Bioseparations, Post-translational and post-transcriptional modifications, Proteomics, CRISPR systems, oligonucleotide analysis.