

BSPR Annual Scientific Meeting

Integrative Proteomics:

Protein Function,
Cellular Systems
and Big Data



1st - 3rd July 2019 • Southampton

Programme



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BSPR Annual Meeting 2019

'Integrative Proteomics: Protein Function, Cellular Systems and Big Data'

Southampton, UK 1st – 3rd July 2019

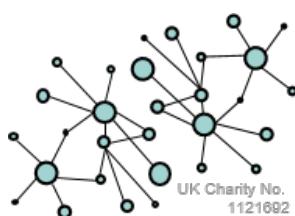
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ABOUT THE BSPR

The British Society for Proteome Research (BSPR) is the UK and Ireland society for all people using or interested in proteomics, and aims to advance the science of proteomics, to further public education therein and to promote study and research work in proteomics and related subjects for public benefit. The BSPR represents both the Human Proteome Organization (HUPO) and the European Proteomics Association (EuPA) in the UK and Ireland and is affiliated to the Society of Biology.

The BSPR is a registered incorporated charity (Registered Company Number 6319769, UK Charity Number 1121692).



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Research



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WELCOME ADDRESS

Dear Colleagues,

As President of the British Society for Proteome Research and on behalf of this year's Conference organisers, it is my great pleasure to welcome you to this the 16th Annual Meeting of the Society which is being held here in the Solent Conference Centre, Southampton.

Southampton is noted for its association with the RMS Titanic, the Spitfire and more generally in the World War II narrative as one of the departure points for D-Day, and more recently as the home port of a number of the largest cruise ships in the world.

Innovative experimental approaches that have advanced the field of functional proteomics continue to expand in scale and complexity. Proteomic technology is increasingly used to measure further protein properties affecting cellular function and disease mechanisms, including post-translational modifications, protein-protein interactions and subcellular and tissue distributions. Interrogation of biological systems at multiple omics levels are also proving particularly powerful, driven by the development of innovative tools to analyse, visualise and explore large, complex proteomics data. We hope that in the next few days, the programme theme, "**Integrative Proteomics: Protein Function, Cellular Systems and Big Data**" will bring together these aspects exploring current and emerging technologies; and challenges in proteome research.

The organising team for this year's meeting has brought together a diverse, international line-up of speakers, from the established leaders in the field to some of our newest and brightest young scientists. Combined with an Institute for Systems Biology training workshop tutored by Luis Mendoza, a trade exhibition and poster presentations, we anticipate this will provide us all with a stimulating and dynamic environment to discuss the latest developments in proteomics.

Finally, I'd like to take this opportunity to thank those individuals who made the meeting possible. Paul for organisation of the conference and Rob for coordinating the scientific programme, – they have done a lot of work very efficiently. Thanks also to members of the BSPR committee for their support. Of course, the meeting would not be possible without our generous contribution of sponsors and so we thank them and I encourage you to engage with them – not least to try to get the answers needed to win the Trade Exhibition Quiz!

I hope that you have an entertaining and rewarding meeting and that you enjoy your time here in Southampton.

Kindest regards,

Prof Stephen Pennington, President, BSPR

ACKNOWLEDGEMENTS

Organising Committee Paul Skipp (Chair), James Waddington, Josie Beeley, Stephanie Baker

Scientific Programme Rob Ewing (Chair), Sara Zanivan, Juan Antonio Vizcaino, Karin Barnouin, Steve Pennington, Rob Beynon, Kathryn Lilley

Promoters Roz Jenkins, Karin Barnouin, Chuqiao Gong.



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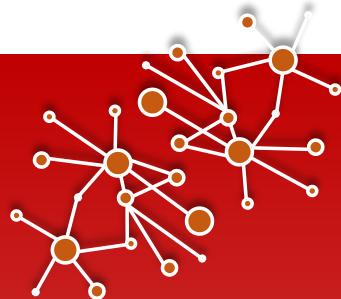


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EXHIBITORS AND SPONSORS



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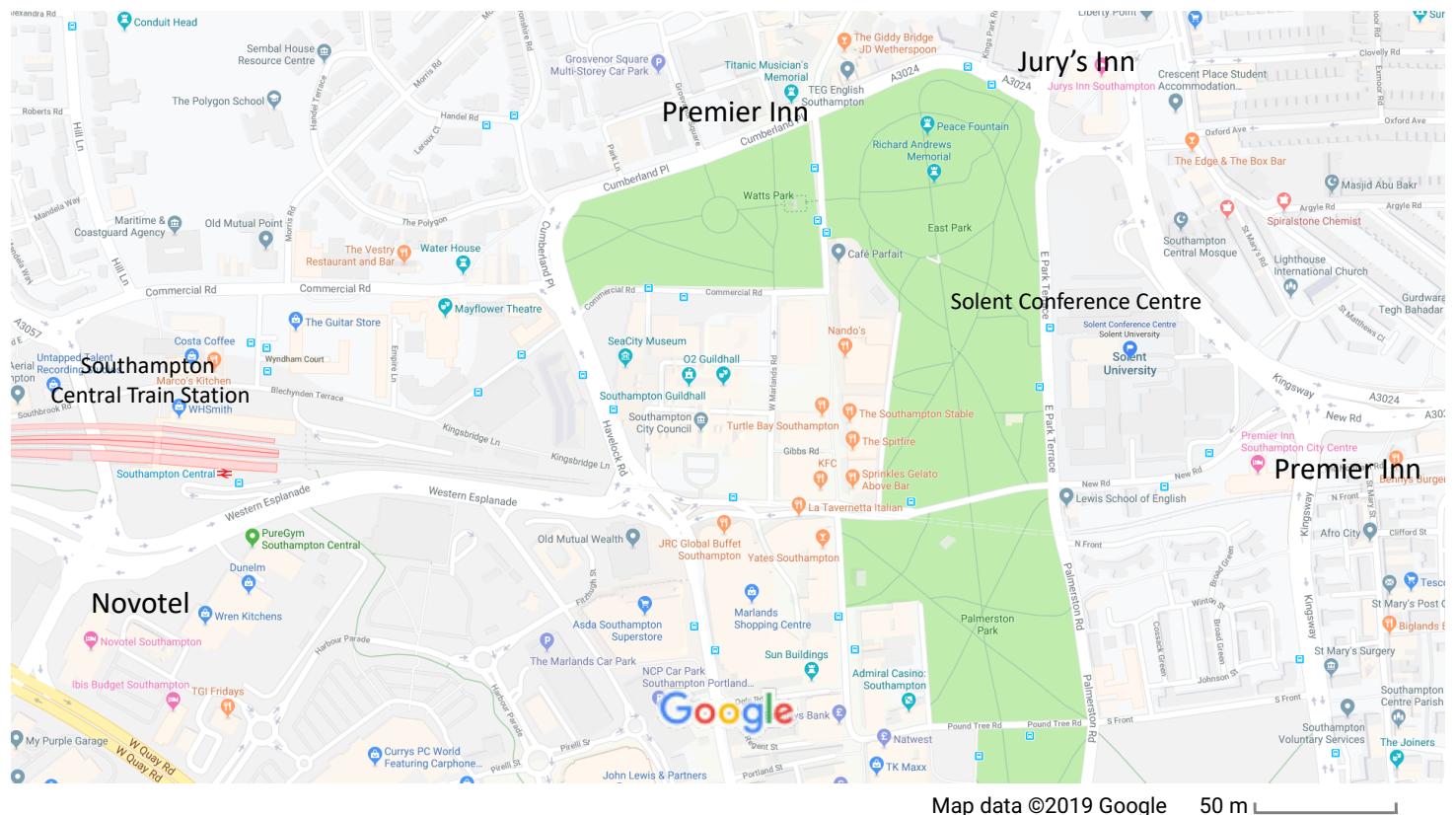
Waters



British Society for Proteome Research Annual Scientific Meeting 2019

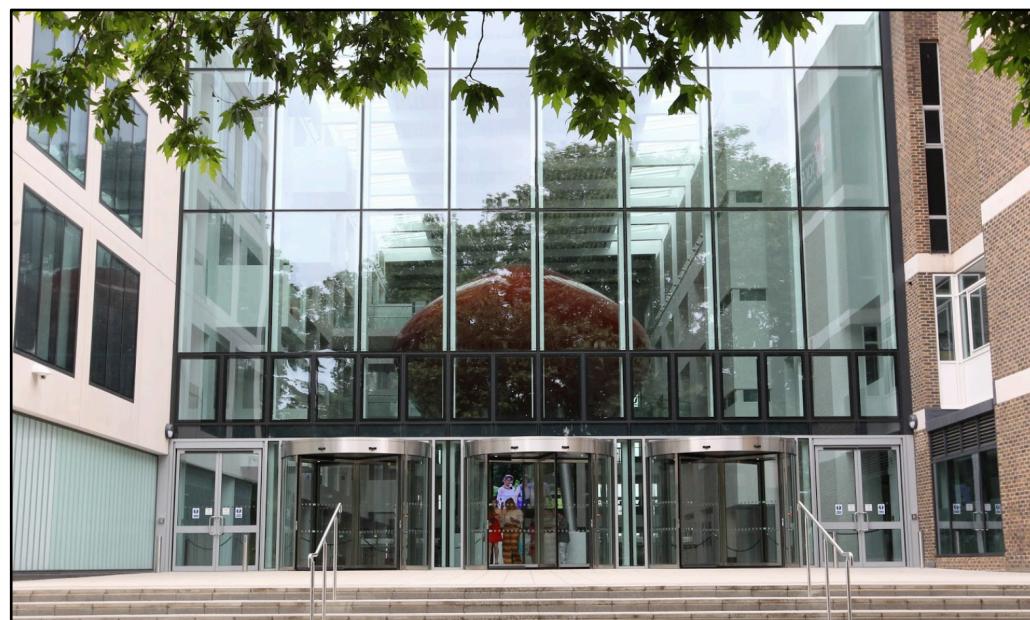
MAPS

Southampton City Centre



Map data ©2019 Google 50 m

Solent Conference Centre

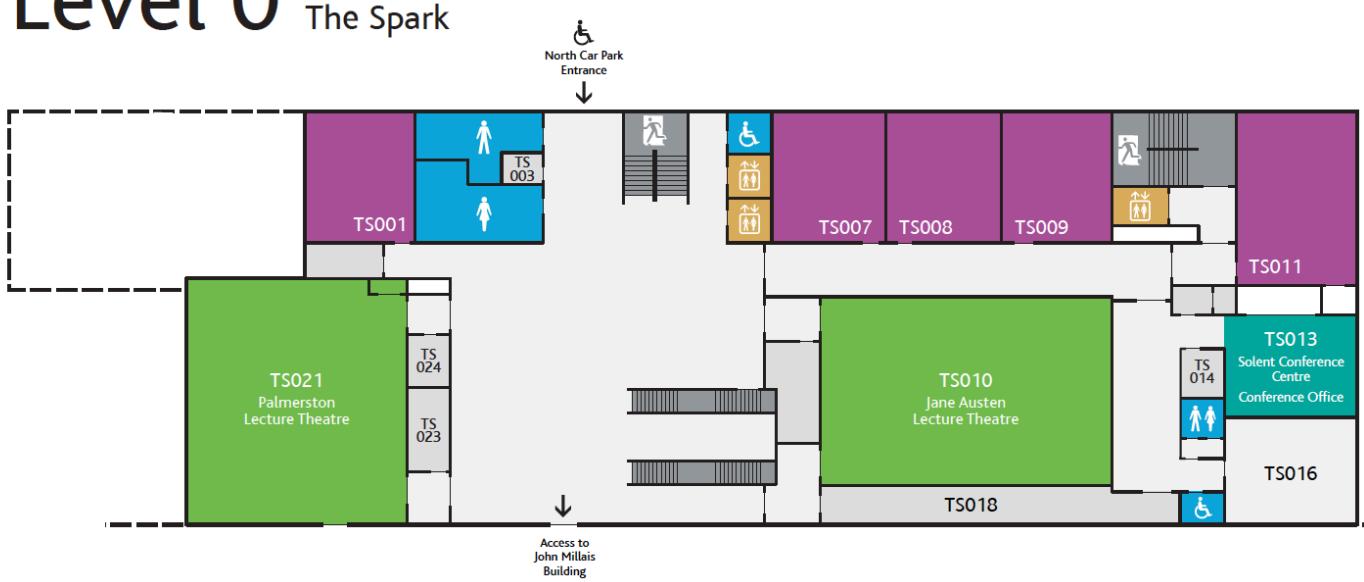


Solent Conference Centre



Solent Conference Centre, Jane Austen auditorium

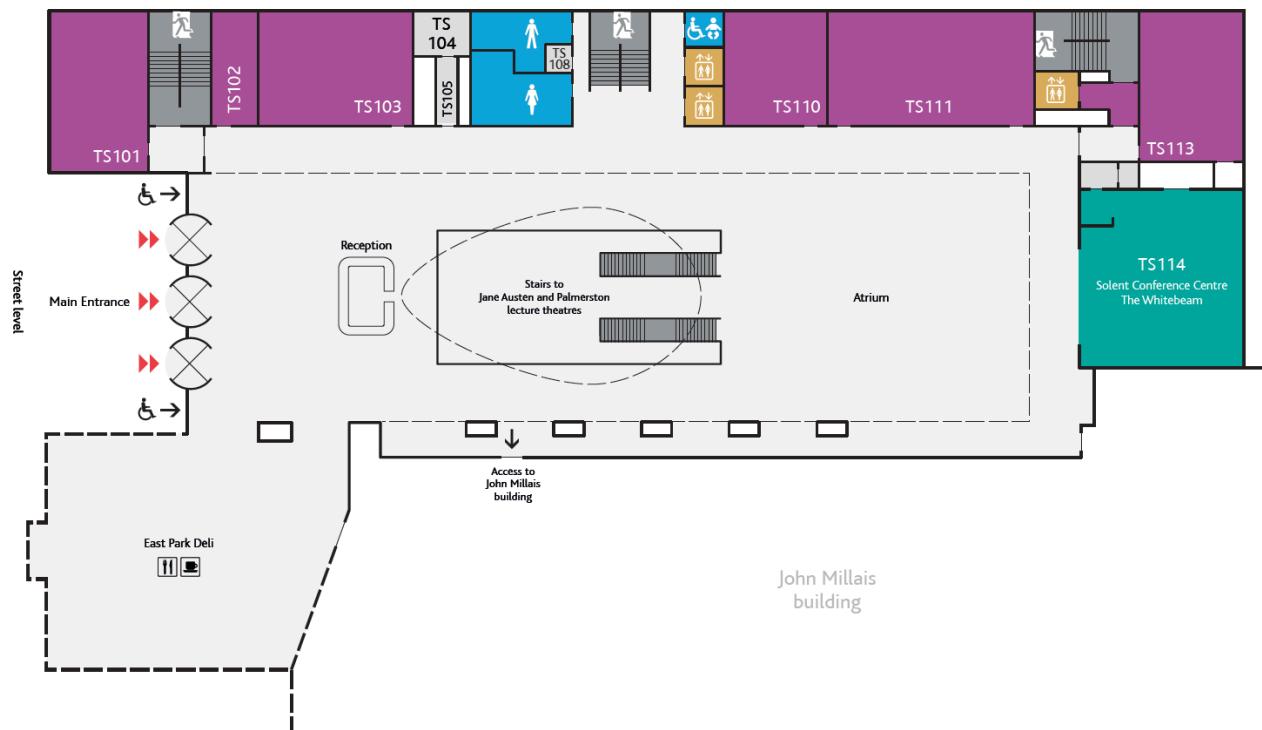
Level 0 The Spark



Poster/exhibition/lunch/coffee/tea breaks – level 1 (main entrance level)

Level 1

The Spark



CONFERENCE INFORMATION

Getting to the Solent Conference Centre, Solent University.

By Train (Southampton Central)

If you exit via Platform 1, turn right, walk up the hilly footpath towards the Civic Centre building (large white stone building with clock tower). Then follow Civic Centre Road and New Road straight on until you see the University ahead of you.

If you exit on Platform 4, turn left, walk up the hill towards the Civic Centre building (large white stone building with clock tower). Then follow Civic Centre Road and New Road straight on until you see the University ahead of you.

By Plane

From Southampton International Airport

There are regular buses, trains and taxis from the airport to the centre of Southampton.

Buses and taxis are right outside the entrance, whilst the train station is located a couple of minutes' walk away and is called Southampton Airport Parkway.

The airport is approximately four miles from Solent Conference Centre (Solent University) and train services to the city centre run frequently with journey times of ten minutes.

Taxis are available from outside the terminal but need to be booked at the taxi desk inside the airport.

By Car

Southampton is just 75 miles (120km) from London, with excellent road links. Southampton sits at the southern end of the M3 motorway.

Those who are travelling with a sat nav should use the postcode SO14 0YN.

Parking

While the University has limited on-campus parking, there are several car parks in the immediate vicinity. If you are a blue badge holder, please email us to reserve a parking space on the main campus.

There are over 200 free to use cycle spaces on the main campus.

Social Events

Wine Reception at Solent Conference Centre

Wine Reception at the Solent Conference Centre (Monday 1st July 2019). Following the poster lightning talks there will be an opportunity for poster presenters, delegates and vendors to engage with colleagues over wine and canapes.

Conference Dinner in the Bow room at The Harbour Hotel, 19.30 on Tuesday 2nd July. Attendance by ticket only.

The Harbour Hotel features stunning contemporary interiors, the city's most exciting rooftop destination in Southampton's thriving marine culture and offers a gateway to the beautiful Solent. The evening will begin at 19.00 with pre-dinner drinks, followed by a 3-course meal and wine. This is a fantastic opportunity to network in an informal environment and to participate in the not-to-be-missed, world famous Professor Lilley trivial pursuit quiz.

A coach has been arranged from the Solent Conference Centre which will leave promptly at **19.00**. For those of you who wish to make their own way to The Harbour Hotel at Ocean Village, the post code is SO14 3QT.



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PROGRAMME AT A GLANCE

Day 1 – 1st July 2019

8.00 – 10.00	TRADE EXHIBITION SET-UP
9.00 – 10.00	REGISTRATION AND POSTER SETUP
10.00 – 10.15	WELCOME ADDRESS: Steve Pennington, UCD Conway Institute, Ireland

10.15 – 11.00	<i>Chair: Steve Pennington, UCD Conway Institute, Ireland</i> BSPR LECTURE Angus Lamond, University of Dundee, UK iPS Proteomes in Health and Disease
11.00 – 11.45	PLENARY Michael Snyder, Stanford University, USA Big Data and Health

12.00 – 13.45 LUNCH/TRADE EXHIBITION

13.45 – 15.15	<i>Chair: Karin Barnouin, BioApicem Ltd/UCB</i> SESSION 1 Interaction Proteomics
13.45 – 14.15	KEYNOTE Jyoti Choudhary, The Institute of Cancer Research, UK Deriving cancer cell networks from quantitative proteomics data
14.15 – 14.35	SELECTED TALK 1 Andrew Pitt, Aston University, UK Changes in the PTEN interactome as a result of redox regulation.
14.35 – 14.55	SELECTED TALK 2 Walter Kolch, University College Dublin, Ireland Mutant RAS causes extensive re-wiring of protein-protein interactions and information flow in the EGF-receptor signaling network
14.55 – 15.15	SELECTED TALK 3 Mark Collins, University of Sheffield, UK S-acylated Golga7b stabilises DHHC5 at the plasma membrane to regulate cell adhesion

15.15 – 15.45 TEA-COFFEE BREAK/TRADE EXHIBITION

15.45 – 17.15	<i>Chair: Rob Beynon, University of Liverpool, UK</i> SESSION 2 British Mass-Spectrometry Society: Emerging Technologies
15.45 – 16.15	KEYNOTE Rainer Cramer, University of Reading, UK Omic profiling of bacteria using liquid AP-MALDI MS
16.15 – 16.35	SELECTED TALK 4 Robert Van Ling, PharmaFluidics, Belgium Driving chromatography towards single cell proteomics using micro-Pillar Array Column technology
16.35 – 16.55	SELECTED TALK 5 Jonathan Phillips, University of Exeter, UK Protein choreography: dynamic control of protein function

16.55 BREAK/TRADE EXHIBITION

17.15 – 18.00	FLASH TALKS
18.00 – 20.00	BEER/WINE RECEPTION/APPETIZERS/POSTER SESSION

DAY 2: 2nd July 2019

9.00 – 10.30	Chair: Paul Skipp, University of Southampton, UK SESSION 3 Clinical & Cancer Proteomics
9.00 – 9.30	KEYNOTE Sara Zanivan, CRUK Beatson Institute, UK CAFs are key mediators of tumour-stroma interactions: role of extracellular vesicles
9.30 – 9.50	SELECTED TALK 6 Harvey Johnston, UCL, UK (Early Investigator Talk) Integrated Tumour and Plasma Proteomics of Contrasting B-cell Cancers Identifies Common and Specific Tumour Signatures and Divergent Mechanisms of Biomarker Emergence
9.50 – 10.10	SELECTED TALK 7 Sidra Saeed, University of Bradford, UK (Early Investigator Talk) Proteomic profiling of matched normal and tumour tongue biopsies from smokers and non-smokers
10.10 – 10.30	SELECTED TALK 8 John Timms, UCL, UK Longitudinal and Network Serum Biomarker Models for the Early Detection of Ovarian Cancer
10.30 – 11.15	TEA-COFFEE BREAK/POSTERS/TRADE EXHIBITION
11.15 – 12.45	Chair: Rob Ewing, University of Southampton, UK SESSION 4 Computational Proteomics & Big Data
11.15 – 11.45	KEYNOTE Luis Mendoza, Institute for Systems Biology, USA Building a Robust Discovery Pipeline in the Age of Big Data
11.45 – 12.05	SELECTED TALK 9 James Schofield, University of Southampton, UK TopMD: Pathway Biomarkers
12.05 – 12.25	SELECTED TALK 10 Andrew Jarnuczak, European Bioinformatics Institute, UK Evaluation of cancer cell lines as tumour models through meta-analysis of public proteomics data
12.25 – 12.45	SELECTED TALK 11 Maruan Hijazi, QMUL, UK Chemical phosphoproteomics systematically identifies circuitries of cancer kinase networks
12.45 – 14.15	LUNCH/POSTERS/TRADE EXHIBITION
14.15 – 15.45	Chairs: Juan Vizcaino & Sandra Orchard, EBI, UK SESSION 5 European Bioinformatics Institute Workshop
14.15 – 14.30	Emily Bowler-Barnett, European Bioinformatics Institute, UK (Early Investigator Talk) UniProt
14:30 – 14.45	Juan A. Vizcaino, European Bioinformatics Institute, UK The PRIDE database: storing, disseminating and integrating proteomics data in the public domain.
14:45 – 15.00	Sandra Orchard, European Bioinformatics Institute, UK IntAct and the Complex Portal
15:00 – 15.15	Henning Hermjakob, European Bioinformatics Institute, UK Reactome
15:15 – 15.30	Denise Carvalho-Silva, European Bioinformatics Institute, UK Open Targets: Identifying Drug Targets in a Pre-competitive Framework
15:30 – 15.45	Questions, overall discussion
15.45 – 16.15	TEA/COFFEE/POSTERS/TRADE EXHIBITION
16.15 – 17.00	Chair: BSPR Secretary Peter Kilby, Syngenta, UK BSPR AGM
17.00 – 17.45	PLENARY Tim Elliott, University of Southampton, UK Peptide ligand selection in the MHC I antigen processing pathway
19.00 – 22.30	CONFERENCE DINNER/KATHRYN LILLEY QUIZ

DAY 3: 3rd July 2018

9.00 – 10.10	<i>Chair: Roz Jenkins, University of Liverpool, UK</i> SESSION 6 Post-translational Modifications and Functional Proteomics
9.00 – 9.30	KEYNOTE Pedro Beltrao, European Bioinformatics Institute, UK Functional relevance of eukaryotic protein phosphorylation
9.30 – 9.50	SELECTED TALK 12 Sabrina Samuel, University of Hull, UK (Early Investigator Talk) Investigating the effect of arginine methylation inhibitors in brain tumours, using microfluidic approaches
9.50 – 10.10	SELECTED TALK 13 Anthony Zerlin, Agilent Technologies, Waldbronn, Germany. Human Breast Cancer Cell Line Phosphoproteome Revealed by an Automated and Highly Selective Enrichment Workflow
10.10 – 10.40 TEA/COFFEE/POSTERS/TRADE EXHIBITION	
10.40 – 11.50	<i>Chair: Andy Pitt, Aston University, UK</i> SESSION 7 Post-translational Modifications and Chemical Modification
10.40 – 11.10	KEYNOTE Tiziana Bonaldi, Istituto Europeo di Oncologia, Italy Protein methylation on and beyond histones, in health and cancer: a proteomic perspective
11.10 – 11.30	SELECTED TALK 14 Kareena Adair, University of Liverpool, UK (Early Investigator Talk) A proteomics-based approach to assess the immunogenicity of drug protein adducts in idiosyncratic liver injury
11.30 – 11.50	SELECTED TALK 15 Alexander Eckersley, University of Manchester, UK (Early Investigator Talk) Proteomic approaches for identifying fingerprints of in vitro and in vivo damage in extracellular matrix assemblies
11.50 – 12.45 LUNCH/POSTERS/TRADE EXHIBITION	
12.45 – 13.00 POSTER AND YOUNG INVESTIGATOR AWARDS	
13.00 – 14.10	<i>Chair: Chris Sutton, University of Bradford, UK</i> SESSION 8 Cellular Dynamics
13.00 – 13.30	KEYNOTE Benedikt Kessler, University of Oxford, UK Comprehensive landscape of active deubiquitinating enzymes profiled by advanced chemoproteomics
13.30 – 13.50	SELECTED TALK 16 David-Paul Minde, University of Cambridge, UK BioProX reveals comprehensive cellular conformational dynamics
13.50 – 14.10	SELECTED TALK 17 Matthias Trost, Newcastle University, UK Proteomics analysis of ubiquitylation on the phagosome: a tale of inflammatory responses and vesicle trafficking
14.10 – 14.40	PLENARY Rob Beynon, University of Liverpool, UK Making proteomics add up – the hard challenge of adding hard numbers to hard proteomes
14.40 – 14.50	BSPR2020 & HUPO 2019 advertisement

INVITED TALKS AND ORAL ABSTRACTS

Day 1 – 1st July 2019

10.15 – 11.00 BSPR LECTURE

iPS Proteomes in Health and Disease

Angus Lamond

Centre for Gene Regulation and Expression, MSI/WTB/JBC Complex, University of Dundee, Dundee, DD1 5EH, UK.



Deep mining of proteomes, using mass spectrometry (MS) based proteomics technology, can provide invaluable insights, at a systems level, into both physiological responses in healthy cells and mechanisms causing disease phenotypes. This allows unbiased, global, quantitative measurements linking cellular phenotypes with changes in protein dynamics in both healthy and diseased cells. A major challenge that emerges from this ability to generate very large sets of proteomics and

parallel ‘poly-omics’ data is how to manage, analyse and integrate the huge resulting volumes of complex information. I will describe our progress in a large-scale, poly-omics project where we have used quantitative proteomics to analyse many different human induced pluripotent stem cell lines (iPSCs), derived from both healthy donors and patient cohorts with inherited genetic disorders. This project highlights some of the technical and analytical challenges inherent in performing proteomic analyses at this scale. I will also describe user-friendly, computational tools we have built for the effective management and sharing of these large, multidimensional data sets (see; www.peptracker.com/epd). Our results show that disease causing mutations result in alterations in the proteomes of iPSC lines that reflect phenotypic defects observed in differentiated adult tissue in patients.

11.00 – 11.45 PLENARY LECTURE 1

Big Data and Health

Michael Snyder

Stanford University School of Medicine, Stanford, USA



Recent technological advances as well as longitudinal monitoring not only have the potential to improve the treatment of disease (Precision Medicine) but also empower people to stay healthy (Precision Health). We profiled 107 participants using multiomics technologies (genomics, immunomics, transcriptomics, proteomics, metabolomics, microbiomics and wearables etc) for up to eight years and made 49 major health discoveries. Altogether, we conclude that deep longitudinal profiling using advanced technologies can lead to actionable health discoveries and provide important information relevant for precision health.

SESSION 1 - INTERACTION PROTEOMICS

13.45 – 14.15 KEYNOTE LECTURE

Deriving cancer cell networks from quantitative proteomics data

Jyoti Choudhary

The Institute of Cancer Research, UK



The Functional Proteomics Team is interested in solving questions about the biology underlying cancerous disease by studying molecular processes and pathways. To do this, the team use a range of techniques including mass spectrometry, biochemistry, molecular biology and informatics, to study protein function, proteome composition and organisation. The team develops advanced mass spectrometry and novel proteomics techniques to further an understanding of cancer biology. Taking a multi-omics approach, the group examines both the genome and the proteome (entire set of expressed proteins) to investigate the heterogeneity of tumours. Through its research and collaborations the group explores protein-protein interactions, cell signalling and protein expression to investigate cancer in a range of human tissues, cell lines, and model organisms.

14.15 – 14.35 SELECTED TALK 1*Changes in the PTEN interactome as a result of redox regulation.*

Ivan Verrastro¹, Karina Tveen-Jensen¹, Panashe Kativu¹, Corinne M Spickett¹ and Andrew R Pitt¹

¹ Life and Health Sciences, Aston University, Birmingham, B4 7ET

Introduction:

PTEN (Phosphatase and tensin homolog) is a redox-sensitive, dual-specificity protein phosphatase involved in regulating a number of cellular processes including metabolism, apoptosis, cell proliferation and survival by down-regulating the PI3K/Akt pathway. Although it is known that PTEN can be reversibly inactivated by oxidation of the catalytic cysteine residue to form an intramolecular disulfide, the effect of this oxidation on the PTEN interactome had previously not been studied in detail.

Experimental approach:

We investigated changes in the specific redox interactome of PTEN by a new in vitro approach. PTEN-GST fusion purified from Escherichia coli was prepared in reduced and hydrogen peroxide-oxidized and dithiothreitol-reduced forms, oxidative modifications fully characterized by mass spectrometric and activity-based methods, and the protein immobilized on a glutathione-sepharose-based support. These resins were then used to capture interacting proteins from a HCT116 cell lysate. Captured proteins were identified by LC-MSMS and selective binding to oxidized or reduced forms of PTEN quantified using label-free methods.

Results:

We identified 86 interactors for PTEN, the abundance of fourteen of which varied significantly with the redox status of PTEN. These including thioredoxin, peroxiredoxin-1, DDB1 and Annexin A2; all except one showed higher binding to oxidized PTEN. The interaction of a small number of partners were validated both in vitro and in cellulo using western blotting.

Conclusions:

The development of in vitro capture methods for modified proteins where the modification status of the capture protein can be fully characterized has great potential for identifying modification-dependent protein interactions. Our results suggest that the redox status of PTEN causes a functional variation in its interactome that may be important in the role of PTEN in signaling and disease.

14.35 – 14.55 SELECTED TALK 2*Mutant RAS causes extensive re-wiring of protein-protein interactions and information flow in the EGF-receptor signaling network*

Susan Kennedy¹, Mohammed-Ali Jarboui^{2,3}, Sriganesh Srihari⁴, Cinzia Raso¹, Kenneth Bryan⁴, Layal Dernayka², Theodosia Charitou⁴, Manuel Bernal-Llinares⁴, Carlos Herrera-Montavez¹, Aleksandar Krstic¹, David Matallanas¹, Max Kotlyar⁵, Igor Jurisica^{5,6,7}, Jasna Curak^{8,9,10}, Victoria Wong^{8,9,10}, Igor Stagljar^{8,9,10}, Thierry LeBihan¹¹, Lisa Imrie¹¹, Priyanka Pillai⁴, Miriam A. Lynn⁴, Erik Fasterius¹², Cristina Al-Khalili Szigyarto¹², Christina Kiel^{1,13,14}, Luis Serrano¹³, Nora Rauch¹, Ruth Pilkington¹, Patrizia Cammareri¹⁵, Owen Sansom^{15,16}, Steven Shave¹⁷, Manfred Auer¹⁷, Nicola Horn², Franziska Klose², Marius Ueffing², Karsten Boldt², David J. Lynn^{4,18}, **Walter Kolch**^{1,14,19}.

¹ Systems Biology Ireland, University College Dublin, Dublin 4, Ireland; ² Institute for Ophthalmic Research, University of Tübingen, Germany; ³ Werner Siemens Imaging Center, University of Tübingen, Germany; ⁴ EMBL Australia Group, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia; ⁵ Krembil Research Institute, University Health Network, Toronto, Canada; ⁶ Departments of Medical Biophysics and Computer Science, University of Toronto, Canada; ⁷ Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic; ⁸ Donnelly Centre, University of Toronto, Canada; ⁹ Department of Biochemistry, University of Toronto, Canada; ¹⁰ Department of Molecular Genetics, University of Toronto, Canada; ¹¹ Synthetic and Systems Biology, University of Edinburgh, UK; ¹² School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden; ¹³ Centre for Genomic Regulation, Barcelona Institute of Science and Technology, Spain; ¹⁴ School of Medicine, University College Dublin, Dublin 4, Ireland; ¹⁵ Cancer Research UK Beatson Institute, Glasgow, UK; ¹⁶ Institute of Cancer Studies, Glasgow University, UK; ¹⁷ School of Biological Sciences and School of Biomedical Sciences, University of Edinburgh, UK; ¹⁸ School of Medicine, Flinders University, Bedford Park, SA 5042, Australia; ¹⁹ Conway Institute, University College Dublin, Ireland.

Protein-protein interaction networks (PPINs) organize fundamental biological processes, but how oncogenic mutations impact these interactions at a network-level scale is poorly understood, as are the consequences of network rewiring. Here, we have analyzed how a common oncogenic KRAS mutation (KRASG13D) affects PPIN structure and function. Using large-scale quantitative mass spectrometry (qMS) we have mapped >6,000 PPIs in the Epidermal Growth Factor Receptor (EGFR) network, arguably one of the most important signaling networks in cancer. We show that the EGFR network is extensively rewired in colorectal cancer (CRC) cells expressing transforming levels of KRASG13D (mtKRAS), demonstrating that these cells adaptively reconfigure network-wide PPIs far downstream of the mutant protein itself. The factors driving PPIN rewiring are multifactorial including changes in protein expression and phosphorylation. Mathematical modelling also suggests that the binding dynamics of low and high affinity KRAS interactors contribute to rewiring. PPIN rewiring substantially alters the composition of protein complexes, signal flow, transcriptional regulation, and cellular phenotype. These changes were validated by targeted and global experimental analysis. Importantly, genetic alterations in the most extensively rewired PPIN nodes occur frequently in CRC and are prognostic of poor patient outcomes. These PPIN maps are a high quality and functionally validated resource for studying mutant KRAS signaling at a network scale, providing novel insights into disease mechanisms and opportunities to design diagnostic and therapeutic interventions.

14.55 – 15.15 SELECTED TALK 3

S-acylated Golga7b stabilises DHHC5 at the plasma membrane to regulate cell adhesion

Keith T. Woodley¹ and **Mark O. Collins**^{1, 2}

¹ Department of Biomedical Science & Centre for Membrane Interactions and Dynamics (CMIAD), Firth Court, Western Bank, University of Sheffield, S10 2TN, United Kingdom; ² Faculty of Science Mass Spectrometry Centre, University of Sheffield, Brook Hill Road, Sheffield, S3 7HF, United Kingdom.

S-acylation (palmitoylation) is the only fully reversible lipid modification of proteins however little is known about how protein S-acyltransferases (PATs) that mediate it are regulated. DHHC5 is a PAT that is mainly localised at the plasma membrane with roles in synaptic plasticity, massive endocytosis and cancer cell growth/invasion. Here we demonstrate that DHHC5 binds to and palmitoylates a novel accessory protein Golga7b. Palmitoylation of Golga7b prevents clathrin-mediated endocytosis of DHHC5 to stabilise it at the plasma membrane. Proteomic analysis of the composition of DHHC5/Golga7b-associated protein complexes reveals a striking enrichment in adhesion proteins, particularly components of desmosomes. We show that Desmoglein-2 and Plakophilin-3 are substrates of DHHC5 and that DHHC5/Golga7b are required for localisation of Desmoglein-2 to the plasma membrane and for desmosomal patterning. Loss of DHHC5/Golga7b causes functional impairments in cell adhesion suggesting these proteins have a wider role in cell adhesion beyond desmosome assembly. This work uncovers a novel mechanism of DHHC5 regulation by Golga7b and demonstrates a role for the DHHC5/Golga7b complex in the regulation of cell adhesion.

SESSION 2 - British Mass-Spectrometry Society: Emerging Technologies**15.45 – 16.15 KEYNOTE LECTURE***Omic profiling of bacteria using liquid AP-MALDI MS*

Sophie Lellman¹ and Rainer Cramer¹

¹ Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK



Proteomic profiling of bacteria using mass spectrometry (MS) has become an invaluable tool in the hospital laboratory. Commonly used commercial instruments typically analyse the peptidomic/proteomic fingerprint of the bacterium in the m/z range of 2-20 kDa, allowing the detection of peptides and ribosomal proteins. In this study, we present the first 'omics' data collected using liquid atmospheric pressure (AP) matrix-assisted laser desorption/ionisation (MALDI) MS for bacterial profiling. Several bacterial strains were analysed, including health-relevant species such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Lactobacillus brevis*. Only 1-10 colonies of each strain were collected and processed using a simple and fast chemical protein extraction technique. Samples were then spotted with a liquid support matrix and analysed using a Synapt G2-Si with an in-house modified AP-MALDI source.

Initial data shows each species produces a unique 'omic' profile in the m/z range of 500-1100, allowing species discrimination. The analysis of this mass range is typically excluded from commercial MALDI-TOF biotyping instruments due to their inherent difficulties in recording adequate analyte ion signals in this range but can be exploited in Q-TOF type instruments with AP-MALDI ion sources and ion mobility separation. Using AMX model builder, the bacterial species tested form distinct clusters using principle component analysis (PCA). This study demonstrates the potential of using this low m/z range profile to provide initial, rapid species identification. Additional benefits of this instrumental setup include analysing liquid samples at AP, which further reduces time for sample introduction and analysis.

16.15 – 16.35 SELECTED TALK 4

Driving chromatography towards single cell proteomics using micro-Pillar Array Column technology

Robert Van Ling¹, Jeff Op de Beeck¹, Geert Van Raemdonck¹, Kurt Van Mol¹, Bo Claerebout¹, Natalie Van Landuyt¹, Wim De Malsche², Gert Desmet², Paul Jacobs¹

¹PharmaFluidics, Belgium; ²VUB - Free University Brussels, Belgium

Micromachined chip columns, known as micro-Pillar Array Columns or μ PAC™, are a promising substitute for packed bed nano LC columns in bottom-up proteomics. The high permeability and low ‘on-column’ dispersion obtained by the perfect order of the separation bed makes μ PAC™ based chromatography unique in its kind. Eliminating peak dispersion, originating from heterogeneous flow paths, components maintain a much higher concentration resulting in unprecedented separation performance. The freestanding micro-pillar structure also results in much lower backpressures, allowing increased flow flexibility with exceptional peak capacities and robustness.

In this presentation, we will demonstrate the possibilities of the μ PAC™ in routine proteomic research settings. Utilizing a 50 cm long μ PAC™, with an internal volume of 3 μ L, high throughput analyses with shorter gradient times (30, 60 and 90 minute gradients) are possible over a wide range of flow rates, between 100 and 2000 nL/min. Examples using 500 ng of HeLa cell digest, indicate an increase in protein identifications up to 50% and a gain of 70% in peptide identifications can be achieved when comparing the 50 cm μ PAC™ column to the current state-of-the-art in packed bed columns under the same separation conditions.

16.35 – 16.55 SELECTED TALK 5

Protein choreography: dynamic control of protein function

Jonathan Phillips¹

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Protein molecules are constantly moving – and it is this movement and plasticity that underpins many processes in life, disease and in medicine. But how are these movements directed to control function: how do mutation, phosphorylation and ligand binding regulate enzyme catalysis? Here we quantify the allosteric control of glycogen phosphorylase at near-amino acid resolution. This provides the first direct evidence of the entropic lever mechanism that gates access to the active site in response to phosphorylation and ligand binding events up to 6 nm away. To enable this, we have developed a new fully-automated and highly flexible implementation of millisecond-labelling hydrogen/deuterium-exchange mass spectrometry (HDX-MS). This makes it possible to observe protein dynamic changes in a millisecond time-resolved manner.

DAY 2: 2nd July 2019

SESSION 3 Clinical & Cancer Proteomics

9.00 – 9.30 KEYNOTE LECTURE

CAFs are key mediators of tumour-stroma interactions: role of extracellular vesicles

Sara Zanivan, Cancer Research UK Beatson Institute, Glasgow G611BD, UK



Background: It is becoming clear that intercellular communication among different tumour cell types plays key roles in cancer progression and metastasis. In solid tumours, cancer associated fibroblasts (CAFs) are a major stromal cell type and important contributors of these interactions thanks to their unique ability to secrete plethora of different factors. CAF-secreted factors can alter the tumour vasculature, which is fundamental to fuel cancer cell growth, provide a route to the cancer cells to form metastases and to recruit circulating myeloid cells into the tumour. CAF-derived extracellular vesicles (EVs) have also emerged as positive mediators of cancer progression. EVs carry different types of molecules from one cell to another and strongly modify the phenotype of the recipient cells. Understanding how CAF-derived EVs support cancer may provide important hints to block this mechanism to halt tumour progression and metastasis.

Approach and Methods: Protein and peptide labelling techniques have revolutionized MS-proteomics providing excellent tools for accurate protein and peptide quantification. These techniques are also largely exploited to investigate protein localisation and intercellular communication. We have used trans-SILAC and developed a novel labelling technique for EVs to elucidate the intercellular communication between CAFs and other tumour cell types.

Results and Conclusions: We have found that CAF-derived EVs mediate the transfer of fibroblasts' proteins to endothelial cells, which are the major cellular component of the blood vessels, and that this alters the endothelial functions. I will discuss the relevance of this mechanism in tumours.

9.30 – 9.50 SELECTED TALK 6

Early investigator talk

Integrated Tumour and Plasma Proteomics of Contrasting B-cell Cancers Identifies Common and Specific Tumour Signatures and Divergent Mechanisms of Biomarker Emergence

Harvey E. Johnston ^{1,2,3}, Matthew J. Carter ², Kerry L. Cox ², Melanie Dunscombe ², Antigoni Manousopoulou ^{3,4}, Paul A. Townsend ⁵, Spiro D. Garbis ^{3,4}, Mark S. Cragg ²

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Burkitt's lymphoma and chronic lymphocytic leukaemia (CLL) are B-cell cancers with very contrasting presentations; Burkitt's lymphoma is a highly aggressive lymphoid tumour, frequently affecting children, whereas CLL typically presents as an indolent, slow-progressing leukaemia affecting the elderly. Overexpression of the myc and TCL1 oncogenes in the B-cells of 'E μ -myc' and 'E μ -TCL1' mice induce spontaneous malignancies, which are widely used to model Burkitt's lymphoma and CLL, respectively.

To examine the biology underpinning contrasting cancers and interrogate biomarker emergence, E μ -myc and E μ -TCL1 B-cell tumours, controls and corresponding plasma samples were evaluated using isobaric tags and 2DLC-MS/MS proteomics. Plasma samples were analysed using a 3DLC sub-proteome enrichment strategy isolating the low molecular weight plasma proteome. Over 8000 cellular and 2000 plasma proteins were quantitatively profiled across all samples ($q < 0.01$). Almost 700 proteins exhibited overexpression in both of the contrasting tumour tissues suggesting a shared patterns of dysregulation across B-cell cancers. Mechanisms typical of proliferating cells such as chromosome segregation and ribosome biogenesis were most prevalent.

E μ -myc tumours overexpressed methylating enzymes and underexpressed cytoskeletal components. E μ -TCL1 tumours specifically overexpressed ER stress response proteins and signalling components including the interleukin-5 (IL5) receptor. IL5 was subsequently shown to promote E μ -TCL1 tumour proliferation.

Tumour plasma contained a substantial tumour lysis signature, most prominent in E μ -myc plasma, whereas E μ -TCL1 plasma contained signatures of immune-response, inflammation and microenvironment interactions, with putative biomarkers in early-stage cancer. In summary, integrated proteomics allowed the dissection of a systemic response and a tumour lysis signature present in aggressive and indolent cancers, respectively.

9.50 – 10.10 SELECTED TALK 7**Early investigator talk**

Proteomic profiling of matched normal and tumour tongue biopsies from smokers and non-smokers

Sidra Saeed¹, S. Shaheed¹, W. Burrill², L. H. Patterson¹, K. Pors¹, C. Sutton¹

¹Institute of Cancer Therapeutics and ²Ethical Tissue, University of Bradford, UK

Introduction

Head and neck squamous cell carcinoma (HNSCC) has a yearly incidence of 600,000 cases worldwide, with ≥40% mortality rate. Due to late detection, survivors often have significant debilitating physical impairment to their day-to-day life. Hence, there is an unmet clinical need to identify biomarkers for early detection and intervention that can lead to less detrimental impact on lifestyle. An iTRAQ proteomic approach was used to profile protein changes in matched normal and tumour tissues for clinical applications.

Materials and Methods

Matched normal and tumour samples from non-smoking and smoking patients with tongue carcinomas were subject to cryo-pulverisation and protein determination. Extracts were pooled, trypsin digested and iTRAQ 4-plex labelled. Data generated by 2D-LC/MS on an Orbitrap Fusion, searched by Mascot Server and relative protein changes were quantified by LAMMA. Significantly changed (\pm SD) proteins associated with cause were evaluated using bioinformatics tools (STRING, DAVID, PANTHER).

Results

A total of 3426 proteins were identified and quantified. Comparison of non-smoker tumour with smoker tumour identified 64 proteins upregulated and 62 downregulated, smoker tumour vs smoker normal identified 349 proteins upregulated and 395 downregulated, non-smoker tumour vs non-smoker normal identified 469 proteins upregulated and 431 downregulated. Collagen PTM enzymes and apoptotic proteins were differentially expressed between non-smokers and smokers.

Discussion

The biological significance of the results indicated the importance of using biopsies with good clinicopathological data for experimental design. The approach provides an important step towards comprehensive stratification of HNSCCs based on cause and region-specific pathology.

10.10 – 10.30 SELECTED TALK 8

Longitudinal and Network Serum Biomarker Models for the Early Detection of Ovarian Cancer

Harry J Whitwell¹, Jenny Worthington¹, Oleg Blyuss¹, Aleks Gentry-Maharaj¹, Andy Ryan¹, Richard Gunu¹, Jatinderpal Kalsi¹, Usha Menon¹, Ian Jacobs¹, Alexey Zaikin^{1,2}, and John F. Timms¹.

¹Institute for Women's Health, University College London, Gower St, London, W1CE 6BT, UK; ²Department of Mathematics, Gower St, London, W1CE 6BT, UK.

Background

Ovarian cancer survival rates are dismal with the majority of cases diagnosed at late stage and early detection markers are urgently needed. The UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) demonstrated earlier detection by annual screening using serial CA125 measurements, reporting a 15% mortality benefit, although this was not significant. Our aim was to improve upon current serological methods for ovarian cancer detection.

Methods

We performed biomarker discovery on a case control set of pre-diagnosis serum samples nested within UKCTOCS using quantitative 3D-LC-MS/MS. Potential biomarkers, including candidates from previous studies, were quantified by ELISA in 490 serial serum samples from 39 cases and 31 controls. Changes over time were described mathematically and logistic regression used to generate longitudinal models for cancer prediction. A network topological approach was also employed on a larger dataset for a subset of Type II cases.

Results

The best performing models incorporating CA125, HE4, CHI3L1, PEBP4 and/or AGR2, provided 85.7% sensitivity at 95.4% specificity for detecting all cases at 1 year before diagnosis, significantly improving on using CA125 alone. For Type II cases, models achieved 95.5% sensitivity at 95.4% specificity. Predictive values of models were elevated earlier than CA125, showing potential for improving lead time. The network approach showed promise for early detection and understanding the biology of cancer progression.

Conclusions

We have developed novel predictive models for ovarian cancer based on serial serum biomarker measurements that significantly improve upon using CA125 alone. These models warrant independent validation in a larger case control study.

SESSION 4 - COMPUTATIONAL PROTEOMICS & BIG DATA

11.15 – 11.45 KEYNOTE LECTURE

Building a Robust Discovery Pipeline in the Age of Big Data

Luis Mendoza

Institute for Systems Biology, Seattle, USA



The digitization of scientific data has opened the doors to unprecedented avenues for testing hypotheses across many disciplines. As technology and methods advance, and the ability to gather millions of data points becomes easier, the need for robust analytical pipelines becomes paramount. We look back at an early and highly successful project in scientific Big Data and see what lessons can be applied to the development of modern analysis pipelines in proteomics.

11.45 – 12.05 SELECTED TALK 9

TopMD: Pathway Biomarkers

James P R Schofield¹, Fabio Strazzeri¹, Paul J Skipp¹

¹Centre for Proteomic Research, University of Southampton, SO17 1BJ, UK

Biomarkers are typically identified as high fold change, high significance molecules which classify disease in separate populations. They promise great advances to drug development and healthcare, but, to date, very few biomarkers have been validated and translated to clinic, presumably because of high variability in individuals, populations, diseases and measurement accuracy. The consideration of molecules in isolation ignores the value of 'omics data, where perturbations to the proteome cause ripple effects throughout the global biological network. A patient's proteome mapped onto a global biological network is a 3D landscape which accurately represents the molecular pathology of a patient. Diseases result from changes to the activities of pathways, not from changes in expression or activity of discrete molecules. TopMD measures the 3D landscape of the proteome on a global biological network using topology and plots the profile of differentially modulated pathways. This TopMD Map is a pathway biomarker with increased accuracy as a diagnostic compared to traditional, discrete biomarkers.

12.05 – 12.25 SELECTED TALK 10

Evaluation of cancer cell lines as tumour models through meta-analysis of public proteomics data

Andrew F Jarnuczak¹, Hanna Najgebauer¹, Mitra Barzine¹, Deepti J Kundu¹, Fatemeh Ghavidel², Yasset Perez-Riverol¹, Irene Papatheodorou¹, Alvis Brazma¹, Juan Antonio Vizcaíno¹.

¹EMBL-European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom; ²Department of Informatics, University of Bergen, Bergen, Norway

Introduction

On a molecular level, cell lines have been shown to broadly resemble primary tissues using DNA and RNA-based omics technologies. While these studies often included hundreds of samples, proteomics efforts are usually much smaller in scale. However, given the amount of MS-based data in the public domain we can now employ in-silico analyses to integrate and reuse this valuable resource.

Methods

We have collected and manually curated 7,171 MS-based raw files from 11 large-scale cancer proteomics studies. The raw data was reprocessed using MaxQuant software. A multi-step procedure was devised to normalise and integrate the resulting quantification data. Protein abundance was also integrated with RNA expression and drug sensitivity data.

Results

From the combined re-analysis of 173 Million spectra, we obtained protein expression values from 191 cell lines and 246 clinical tumour samples, across 13 different cancer lineages. The reacquisition of this data would take over 538 days of mass spectrometer time.

We found that baseline protein expression in cell lines was generally representative of clinical tumour samples. However, when considering differential expression between cancer subtypes, as exemplified in the breast lineage, many were not well recapitulated in the cell line models.

Integration of RNA-seq data suggested that the level of transcriptional control in cell lines changed significantly depending on their lineage. Additionally, in agreement with previous studies, we found that variation in mRNA levels was generally a poor predictor of changes in protein abundance.

Conclusions

This work constitutes the first such meta-analysis of protein expression in cancer and provides a valuable quantitative resource.

12.25 – 12.45 SELECTED TALK 11

Chemical phosphoproteomics systematically identifies circuitries of cancer kinase networks

Maruan Hijazi¹, Ryan Smith¹, Vinothini Rajeeve¹, Conrad Bessant²⁻³, and Pedro R. Cutillas¹⁻³.

¹Signalling & Proteomics Group, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, EC1M 6BQ, UK; ²School of Biological and Chemical Sciences, Queen Mary University of London, Mile End, E1 4NS, UK; ³The Alan Turing Institute, British Library, 96 Euston Road, London, NW1 2DB, UK.

Kinase signalling is aberrantly regulated in cancer, but the kinase network elements that determine cancer phenotypes are not fully characterised. Here, by comparing the *in vitro* specificity profiles of 36 kinase inhibitors with their effects on cellular phosphoproteomes, we identified 6,206 kinase-phosphosite relationships for 106 kinases and 1,508 network edges. Comparative analysis of these markers of network circuitry revealed signalling axes in target and compensatory pathways that accurately modelled sensitivity to targeted drugs in primary tumours. Kinase activity was not associated with kinase gene expression but, for kinases with several phosphorylation sites, with global phosphorylation extent. Differences in kinase activation existed in PIK3CA, FLT3 and NRAS mutated tumours and in triple negative breast cancer, revealing potential therapeutic targets for clinically defined tumours. Our study provides insights into kinase network regulation and represents a unique resource to investigate the relationships between kinase network topology and cell phenotypes.

SESSION 5 EUROPEAN BIOINFORMATICS INSTITUTE WORKSHOP**14.15 – 14.30 - EBI WORKSHOP TALK Early investigator talk***UniProt; programmatic access via the Proteins API***Emily H. Bowler-Barnett¹, Sandra. Orchard¹, UniProt Consortium^{1,2,3}.**¹European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SD, UK;²Swiss Institute of Bioinformatics, Centre Medical Universitaire, 1 rue Michel Servet, 1211 Geneva 4, Switzerland;³Protein Information Resource, Georgetown University Medical Center, 3300 Whitehaven St. NW, Suite 1200, Washington, DC 20007, USA and University of Delaware, 15 Innovation Way, Suite 205, Newark, DE 19711, USA.

Emily is a scientific database curator in the Protein Function Content Team at EMBL-EBI. Her role focuses on the expert curation of scientific literature to maintain the accuracy and quality of the UniProt Knowledgebase, she specializes in the curation of mammalian proteins.

Prior to joining the UniProt team Emily obtained her PhD in Proteomics and Systems Biology from the University of Southampton, where her research focused on the remodelling of the Wnt signalling pathway in colorectal cancer. Her previous experience includes annotation of single nucleotide variants as part of the GWAS Catalog (EMBL-EBI), an MSc in Molecular Medicine from the University of Sheffield, and a BSc in Biomedical Sciences from Brunel University, London.

Emily's talk will focus on the programmatic access of the UniProt database via the UniProt Proteins API. This allows researchers to access and download UniProt entry data alongside large-scale genomic, proteomics, and variation data to facilitate further investigation of their target proteins.

14:30 – 14.45 – EBI WORKSHOP TALK*The PRIDE database: storing, disseminating and integrating proteomics data in the public domain***Juan A. Vizcaino¹**¹EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD, UK

We are responsible for the maintenance and further development of the PRIDE database of mass spectrometry-based proteomics data, and related tools and resources. We are leading the global ProteomeXchange Consortium, standardising public proteomics data submission and dissemination worldwide. Additionally, we heavily contribute to the activities of the Proteomics Standards Initiative. We also develop open data analysis pipelines for different proteomics approaches, with the aim of improving scientific reproducibility. Finally, we integrate proteomics data from PRIDE into other EMBL-EBI resources such as Expression Atlas, Ensembl and UniProt. Our goal is to help researchers to make the most of public proteomics data!

14:45 – 15.00 – EBI WORKSHOP TALK

IntAct and the Complex Portal

Sandra Orchard¹, the IMEx Consortium, the UniProt Consortium

¹European Bioinformatics Institute, European Molecular Biology Laboratory (EMBL-EBI), Hinxton, Cambridgeshire, UK



The UniProt knowledgebase (www.uniprot.org) is the key reference resource used by proteomics scientists. Not only does the database provide the user with the high-quality, non-redundant protein sequences which underpin the mapping of peptides to proteins by database search engines and complete proteome datasets for a broad taxonomic range of species, but it is also possible to access information of the molecular interactions made by these proteins. A filtered set of high-quality binary protein-protein interactions are imported from the IMEx Consortium, a collaboration between interaction databases established to provide researchers with a single corpus of experimentally verified protein interaction evidences

annotated to a highly detailed curation standard. An adjacency graph is available in relevant UniProtKB entries to visualise these data. The binding of small molecule cofactors has been improved by links to the ChEBI ontology, making hierarchical searching of these molecules now possible. Finally, UniProtKB has recently adopted the Rhea knowledgebase of biochemical reactions as the reference vocabulary for enzyme annotation, enabling a systematic description of enzyme interactions with both substrates and metabolite products allowing users to search, browse, and mine enzyme data in new ways, combining approaches from the fields of cheminformatics and proteomics.

15:00 – 15.15 – EBI WORKSHOP TALK

Reactome - Mapping Expression Data into Pathway Space

Henning Hermjakob¹

¹European Bioinformatics Institute, European Molecular Biology Laboratory (EMBL-EBI), Hinxton, Cambridgeshire, UK



Reactome is a free, open-source, curated and peer-reviewed pathway database. It now covers almost 11,000 human genes, extended by ca. 5,000 medium and high confidence interactors from the IntAct database, fully integrated and searchable. We will present recent additions to the Reactome platform, enhancing its capability for efficient analysis of protein expression datasets in a genome-wide pathway space. In addition to qualitative pathway overrepresentation, Reactome now offers quantitative gene set enrichment analysis (GSEA) through an R interface, linking R's advanced analysis capabilities to Reactome's rich visualisation interface. We also present a new interactive visualisation of pathway analysis results, based on Voronoi maps, as well as comprehensive filtering of analysis results.

15:15 – 15.30 – EBI WORKSHOP TALK

Open Targets: Identifying Drug Targets in a Pre-competitive Framework

Denise Carvalho-Silva¹

¹European Bioinformatics Institute, European Molecular Biology Laboratory (EMBL-EBI), Hinxton, Cambridgeshire, UK



The path through drug discovery is lengthy, costly and characterised by low success rates, with many potential drugs failing due to lack of safety or efficacy. Generating and interpreting the data required to identify a good drug target demands a diverse set of skills, evidence types and technologies, which rarely exist today in any single organisation. Open Targets brings expertise from EMBL-EBI and the Wellcome Sanger Institute, and five pharmaceutical companies (Biogen, Celgene, GSK, Sanofi, and Takeda) to systematically identify and prioritise targets from which safe and effective medicines can be developed. In this talk, I will highlight the work we are carrying out in two major areas of research in Open Targets, data generation and data integration, through a portfolio of experimental and bioinformatics projects. We are committed to openly sharing our data with the scientific community through publications and Open Targets web resources, such as the Open Targets Platform and Open Targets Genetics.

17.00 – 17.45 PLENARY LECTURE 2

Peptide ligand selection in the MHC I antigen processing pathway

Timothy Elliott¹

¹Centre for Cancer Immunology, Faculty of Medicine, University of Southampton, Southampton SO16 6YD



Tim Elliott left the University of Oxford with a first in Biochemistry in 1983 and completed his PhD in cancer immunotherapy at the University of Southampton in 1986. He did his postdoctoral training at MIT with Herman Eisen at the Center for Cancer Research. In 1990 he returned to the University of Oxford to join the Institute for Molecular Medicine as a Wellcome Trust Research Fellow, joining a key group of immunologists studying antigen presentation at the molecular level: where he continues to be a world leader with over 150 research articles on the subject. In 1993 he was appointed to a lectureship and later a Professorship at Balliol College, University of Oxford, as a Wellcome Trust Senior Fellow in Basic Biomedical Science. In 2000, he moved to the University of Southampton as Professor of Experimental Oncology and five years later became Associate Dean for the Faculty of Medicine. In 2015 he stepped down from this role to take up interim Pro Vice Chancellor (Research) for the University of Southampton. He is Director of the new Southampton Centre for Cancer Immunology which opened in 2018, and Deputy Director of the interdisciplinary Southampton Institute for Life Sciences. He is a Fellow of the Royal Society for Biology and Fellow of the Academy of Medical Sciences. He has incorporated discoveries in the areas of antigen processing, T cell regulation and immunodominance into the development of new cancer immunotherapies and is the recipient of a Royal Society/Wolfson Research Merit Award.

SESSION 6 POST-TRANSLATIONAL MODIFICATIONS AND FUNCTIONAL PROTEOMICS**9.00 – 9.30 KEYNOTE LECTURE**

Functional relevance of eukaryotic protein phosphorylation

Pedro Beltrao¹

¹EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD, UK



Cells need to constantly adapt to changes in conditions and use post-translational regulation as a fast way to transfer information from sensors to effectors of cellular responses. Advances in mass-spectrometry now allow us to identify post-translational modification (PTMs) sites in large scale and to quantify their changes across different conditions. However, little is known about how the thousands of recently discovered PTMs modulate protein function, coordinate cellular responses and change in disease. We have been working to study the evolution, dynamics and genetic determinants of protein phosphorylation. We have observed that only a small fraction of phosphosites are ancient in origin and we have suggested that a fraction of these may be non-functional. To study this, we have generated a library of 500 yeast phosphosite deficient mutants that we

screened, together with the gene deletion library, for fitness defects in 100 diverse stress conditions. Around 50% of mutants show phenotypes in our screens with a fraction of these mimicking the effect of a full knock-out. In parallel, we have developed a machine learning approach to score human phosphosites according to their relevance for organismal fitness. This functional score can identify phosphosites that are more likely to impact on interactions, show phenotypes when mutated or be associated with human diseases. Together, such genetic and computational approaches can identify the phosphosites most likely to contribute to fitness.

9.30 – 9.50 SELECTED TALK 12

Early investigator talk

Investigating the effect of arginine methylation inhibitors in brain tumours, using microfluidic approaches

Sabrina Samuel¹, Srihari Deepak², John Greenman¹, Pedro Beltran-Alvarez¹.

¹Faculty of Health Sciences, University of Hull, HU6 7RX, UK, ²Hull and East Riding of Yorkshire NHS Trust, HU3 2JZ

Background: Glioblastoma (GBM) is the most aggressive form of brain tumours with the median patient survival being only 1.5 years. It is therefore imperative to identify new therapeutic targets and evidence has suggested a role for protein arginine methyltransferases (PRMTs), a group of enzymes responsible for the transfer of methyl groups to arginine residues.

Aims: Here, we aim to determine the clinical application of PRMT inhibition, by measuring the anti-proliferative effects of PRMT inhibiting drugs on patient tissue using a microfluidic set up. Due to the interactive nature of post-translational modifications (PTMs), we also investigated possible cross-talk events between PRMTs in GBM tissue.

Methods: Patient samples were collected following surgical removal and placed in a microfluidic chip where they were perfused with the type 1 PRMT inhibitor, MS023, for 192 hrs. LDH assays were used to follow cell viability and western blotting to confirm inhibition of protein arginine methylation. We have used methyl-SILAC labelling to identify proteins that undergo methylation and demethylation in GBM samples on chip.

Results: We demonstrate that GBM tissues can be maintained in a viable state for at least 8 days in microfluidic devices. This provides us with an excellent window of opportunity to treat GBM biopsies with novel drugs, such as arginine methylation inhibitors. We show off-target effects of arginine methylation inhibitors and cross-talk with methylation, using western blot and methyl-SILAC.

Our results are very timely and will help identify molecular targets and mechanisms that could help define novel therapeutic approaches to GBM treatment.

9.50 – 10.10 SELECTED TALK 13

The Agilent AssayMAP Bravo platform provides a fully automated, highly selective, and reproducible enrichment workflow for phosphopeptides using high-capacity Fe(III)-NTA cartridges.

Anthony Zerlin¹, Wu Shuai¹, Wu Linfeng¹

Agilent Technologies, Waldbronn, Germany; Agilent Technologies, Inc., Santa Clara, CA, USA

The Agilent AssayMAP Bravo provides a highly selective, and reproducible enrichment workflow using high-capacity Fe(III)-NTA cartridges. To evaluate how the ratio of total peptide sample amount to affinity resin affects the performance and reproducibility of phosphopeptide enrichment, and monitor the yield and reproducibility of the enrichment using phosphopeptide standards, Agilent joined the part II initiative proposed by the EuPA. The samples consisted of a tryptic digest of the human MCF7 breast cancer cell line (C-18 purified), prespiked with a mixture of 20 human phosphopeptide standards containing light isotopes. The cell line digest samples were loaded onto the cartridges with four different sample/resin ratios. The enriched samples were analyzed Agilent 6550 iFunnel Q-TOF and Agilent 6495B triple quadrupole (TQ).

The AssayMAP enabled high-throughput and reproducible phosphopeptide enrichment. The enrichment result showed excellent selectivity (>90 %) across all samples with different sample/resin ratios.

Phosphopeptide selectivity increased from 1.5 % before enrichment to over 90 % after enrichment for all samples. Across the four sample/resin ratios, we found that the distinct number of phosphopeptides increased with more sample loading, but not proportional to the ratio.

The yield for each phosphopeptide within the same sample varied, ranging from less than 10 % to nearly 100 %. However, the same phosphopeptide standards showed consistent yield across different sample/resin ratios, with approximately 63 % overall recovery for the 20 phosphopeptide standards. The yield for each phosphopeptide standard showed excellent reproducibility, with less than 5 % RSD of the enrichment and robustness of the peptide quantitation.

SESSION 7 POST-TRANSLATIONAL MODIFICATIONS AND CHEMICAL MODIFICATION**10.40 – 11.10 KEYNOTE LECTURE***Protein methylation on and beyond histones, in health and cancer: a proteomic perspective*

Roberta Noberini¹, Camilla Restellini¹, Evelyn O Savoia¹, Roberto Giambruno¹, Valeria Spadotto¹, Marianna Maniaci¹, Enrico Massignani¹ and **Tiziana Bonaldi¹**

¹Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy



Background: Chromatin is a dynamic, well-organized protein-DNA-RNA structure that controls various DNA-dependent processes. A large number of site-specific post-translational modifications of histones (hPTMs) contribute to the maintenance and modulation of chromatin plasticity, gene activation, DNA replication and repair. Aberrations in histone PTMs, as well as in the histone modifying enzymes (HMEs) have been reported in many tumors, and many epigenetic inhibitors are currently developed for cancer treatment. Therefore, profiling epigenetic features in cancer is crucial for the discovery of both biomarkers for patient stratification and novel epigenetic targets. HMEs, however do not only target histones and a more systematic analysis of non-histonic K/R- methylation is essential to better dissect epigenetic drugs' MoA.

Approach and Methods: Recent achievements made MS-based proteomics an excellent tool to help understanding how histone and non-histonic PTMs affect the structural-functional state of chromatin and modulate gene expression. We set up novel biochemical protocols of histone and methyl-protein enrichment followed by ad hoc MS-methods and bioinformatic tools to characterize the epigenetic landscapes of various cancer model systems, including the investigation of non-histone protein methylation.

Results and Conclusions: The hPTM signatures emerging from the analysis of BC samples revealed insights into the epigenetic mechanisms that underlie this tumor, and particularly TN, suggesting novel epigenetic pathways targetable for therapy. The methyl-proteome profiling during the replicative stress response and upon pharmacological inhibition of distinct PRMTs revealed new roles of PRMT1 and PRMT5 in cellular processes such as SASP and miRNA biogenesis.

11.10 – 11.30 SELECTED TALK 14 Early investigator talk

A proteomics-based approach to assess the immunogenicity of drug protein adducts in idiosyncratic liver injury

Kareena Adair¹, Arun Tailor¹, James Waddington¹, Monday Ogese¹, Rosalind Jenkins¹, B Kevin Park¹, Xiaoli Meng¹, Dean J Naisbitt¹.

¹MRC Centre for Drug Safety Science, Dept. Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK.

Introduction. Idiosyncratic drug-induced liver injury (iDILI) is a serious adverse reaction. Flucloxacillin and amoxicillin are both common culprits of iDILI and the pathogenesis is putatively mediated by the adaptive immune system. Several GWAS studies have associated specific HLA alleles with DILI including HLA-B*57:01 with flucloxacillin and HLA-A*02:01 with amoxicillin. Further evidence for the immune pathogenesis includes drug-specific T-cells in iDILI patients. However, the exact antigen, which is responsible for activating T-cells, has not yet been defined. Several proteomics based approaches in our lab have been carried out to identify and characterise these drug associated antigens including 1) MHC-peptide elution to assess the naturally expressed immunopeptidome of flucloxacillin treated C1R-B*57:01 antigen presenting cells and 2) a bottom-up proteomics approach to interrogate exosomal fractions derived from amoxicillin treated primary human hepatocytes. The aim of this work was to synthesise and fully characterise these antigens to assess their role in activating T-cells in HLA-restricted volunteers and patients.

Methods. Drug-modified synthetic peptides were designed in-house, purified using HPLC and validated using LC/MS/MS. A naïve T-cell priming assay was utilised to assess the immunogenicity of one candidate peptide in healthy volunteers expressing the risk allele HLA-A*02:01.

Results. Naturally processed flucloxacillin-modified peptides were detected on the surface of antigen presenting cells expressing HLA-B*57:01. Multiple amoxicillin-modified proteins including human transcription factor SOX3 were detected in hepatocyte derived exosomes. An amoxicillin-modified peptide activated T-cells from HLA-A*02:01 donors.

Conclusions. Drug-modified peptides derived from MHC epitopes and exosomal fractions have immunogenic potential in iDILI

11.30 – 11.50 SELECTED TALK 15

Early investigator talk

Proteomic approaches for identifying fingerprints of in vitro and in vivo damage in extracellular matrix assemblies

Alexander Eckersley^{1,3}, Matiss Ozols^{1,3}, Ronan O'Cualain³, April R Foster^{2,3}, Suzanne M Pilkington^{2,3}, Christopher EM Griffiths^{2,3,4}, David Knight³, Rachel EB Watson^{2,3,4}, Michael J Sherratt^{1,3}.

Divisions of ¹Cell Matrix Biology & Regenerative Medicine and ²Musculoskeletal and Dermatological Sciences, ³School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester and ⁴NIHR Manchester Biomedical Research Centre, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK

Existing immunohistological and ultrastructural approaches are poorly-suited to characterising age-induced molecular damage within long-lived extracellular matrix assemblies. In skin, photoageing is characterised by immunohistological remodelling of fibrillin, but not collagen VI, microfibrillar networks. We have demonstrated previously that ultraviolet (UV) radiation affects fibrillin, but not collagen VI, microfibril ultrastructure. Here, we aimed to determine if proteomic methods (peptide fingerprinting and domain mapping) can detect molecular-scale UV-induced damage (in vitro) and photoageing-specific damage (in vivo) within fibrillin-1 (FBN1) and collagen 6 alpha-3 (COL6A3) proteins.

Fibrillin and collagen VI microfibrils were co-purified from human dermal fibroblasts (N=3; donors aged 75-79 yrs) and from photoprotected and photoexposed skin biopsies (buttock and forearm) (N=6; 62-79). Samples were either untreated or UV irradiated with solar simulated radiation (SSR; 30 J/cm²) or UVB (0.1 J/cm²). Ultrastructural damage was assessed by atomic force microscopy and molecular damage by proteolytic peptide generation followed by LC-MS/MS.

Fibrillin, but not collagen VI, microfibril ultrastructure was significantly altered by in vitro exposure to SSR and UVB irradiation; proteomic analysis showed that irradiation consistently enhanced the yield of FBN1 but not COL6A3, peptides. However, in vitro UVB and SSR irradiation and in vivo photoageing was associated with characteristic peptide “fingerprints” within not only FBN1 but also COL6A3 proteins, some of which were conserved between in vitro and in vivo damage.

This study demonstrates the potential of proteomic approaches for identifying patterns of peptides characteristic of both acute and chronic damage within matrix proteins. This study was supported by Walgreens Boots Alliance.

SESSION 8 CELLULAR DYNAMICS

13.00 – 13.30 KEYNOTE LECTURE

Comprehensive landscape of active deubiquitinating enzymes profiled by advanced chemoproteomics

Adan Pinto-Fernandez¹, Simon Davis¹, Abigail Schofield¹, Hannah C Scott¹, Eidarus Salah², Sebastian Mathea², Philip Charles¹, Andreas Damianou¹, Roman Fischer¹, **Benedikt M Kessler¹**.

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Enzymes that bind and process ubiquitin, a small 76 amino acid protein, have been recognized as pharmacological targets in oncology, immunological disorders and neurodegeneration. Mass spectrometry technology has now reached the capacity to cover the proteome with enough depth to interrogate entire biochemical pathways such as DUBs and E3 ligase substrates. We have recently characterized the breast cancer cell (MCF-7) deep proteome by detecting and quantifying ~10,000 proteins, and within this data set, we can detect endogenous expression of 66 deubiquitylating enzymes (DUBs), whereas matching transcriptomics detected 78 DUB mRNAs across all six DUB enzyme families. Since enzyme activity provides another useful layer of information in addition of the expression levels, we have combined advanced mass spectrometry technology, pre-fractionation and more potent/selective ubiquitin active-site probes with propargyl based electrophiles to profile 74 DUBs in MCF-7 crude extract material. Competition experiments with cysteine alkylating agents, pan-DUB inhibitors of ubiquitin combined with probe labelling revealed the proportion of active cellular DUBs directly engaged with probes by label-free quantitative (LFQ) mass spectrometry, demonstrating that USP13, USP39 and USP40 are non-reactive to probe, reflecting low or complete biological inactivity under these cellular conditions. Our extended chemoproteomics workflow increases depth of covering the active DUBome, including isoform-specific resolution, and provides the framework for more comprehensive cell-based DUB activity and small molecule selectivity profiling.

13.30 – 13.50 SELECTED TALK 16

BioProX reveals comprehensive cellular conformational dynamics

David-Paul Minde¹, Manasa Ramakrishna², Ben F. Luisi¹, Kathryn S. Lilley¹

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Mass spectrometry approaches that aim to deliver protein conformation involving labelling by hydrogen /deuterium exchange or hydrogen radicals do not comprehensively capture conformational protein dynamics inside intact cells. This is due to prohibitive sample complexity or acute toxicity of the reactions. Here, we present Biotin Proximity eXchange (BioProX), a method based on *in vivo* biotinylation upon application of a nontoxic biotin label that selectively targets lysine residues, thereby simplifying sample complexity and enabling relative quantification when applied to dynamic studies. As a proof of concept we reproducibility detected hundreds of abundance changes among thousands of biotin-peptides detected in intact bacterial cells under mild and toxic heat-shock conditions suggesting gross protein conformational changes. We also applied BioProX *in vitro* to yield comprehensive insights into conformational dynamics of purified ribosomes based on their interaction with magnesium cations. Consistent with previous HDX-MS data, ribosomes are stabilized at high magnesium while our data add additional local resolution and quantitatively reproducible insights into the dynamics of this essential molecular machine. Intrinsic compatibility of BioProX with complex samples as well as high sensitivity for purified complexes and compatibility with widely accessible mass spectrometry workflows make it an attractive complementary method to study protein conformations *in vivo* and *in vitro*.

13.50 – 14.10 SELECTED TALK 17

Proteomics analysis of ubiquitylation on the phagosome: a tale of inflammatory responses and vesicle trafficking

Tiaan Heunis¹, O. Bilkei-Gorzo², J. Peltier¹, A. Härtlova¹, **Matthias Trost**¹

¹Newcastle University, Institute for Cell and Molecular Biology, Newcastle upon Tyne, NE24HH; ²MRC Protein Phosphorylation Unit, University of Dundee, Dundee, DD15EH.

Phagocytosis is an evolutionarily conserved key process required for innate immunity and homeostasis. During phagocytosis, particles are internalised into a de novo, membranous organelle, the phagosome, which fuses with early and late endosomes and, finally, lysosomes in a process called phagosome maturation. Our data showed that phagosomes are enriched in polyubiquitylation, which is further enhanced by IFN- γ . We therefore explored the role of ubiquitylation on phagosome functions using a range of ubiquitin proteomics tools. Applying targeted mass spectrometry approaches, we quantified ubiquitin chain linkage peptides from phagosome samples. All chain linkages apart from linear chains are present on phagosomes and IFN- γ activation enhanced K11, K48 and K63 chains significantly. In order to identify the molecular function of this polyubiquitylation, we characterized the ubiquitinome of phagosomes of IFN- γ activated macrophages and can demonstrate that ubiquitylation is preferentially attached to proteins involved in vesicle trafficking, thereby delaying fusion with late endosomes and lysosomes. We further show that phagosomal recruitment of the E3 ligase RNF115 is enhanced upon IFN- γ stimulation, which is responsible for most of the increase of K63 phagosomal polyubiquitylation. Loss of RNF115 also affected proinflammatory cytokine production and replication of bacterial pathogens. Detailed understanding of phagosomal ubiquitylation could not only increase our understanding of vesicle trafficking but could also serve as possible targets for antibacterial host-directed therapies.

14.10 – 14.40 PLENARY LECTURE 3

Making proteomics add up – the hard challenge of adding hard numbers to hard proteomes

Rob Beynon¹

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A primary effort in proteomics lies in the development of methodologies, or application of those methodologies to quantity changes in a proteome. But, to place proteomics at a systems level, we also need to be able to define the proteome in a steady state, where protein abundances do not change. Under these circumstances we first need to know the absolute numbers (in copies per cell) of the proteins of interest; challenging because mass spectrometry-based proteomics is not inherently quantitative. Secondly, to properly understand the potential for change, we also need to understand the dynamics of the proteome. Knowing the size of a protein pool, we need to be aware of the rate at which newly synthesised protein molecules enter the pool, as well as the rate at which they exit to be degraded. In the steady state, these two processes balance each other, but if a protein changes in abundance, we cannot know how this was brought about without understanding of the dual processes of protein synthesis and protein degradation.

In this lecture, I want to address some of the issues associated with the challenge of the parametric definition of the proteome – how we can address proteome wide absolute quantification, and how we can gain a numerical understanding of the flux through the proteome. I'll discuss how reliable protein turnover measurements are at the level of the proteome, and explain some new developments that we are exploring for absolute quantification of the proteome, capitalising on the tools and strategies of synthetic biology.

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POSTERS

P1 Functions of the BBSome protein complex in Leishmania mexicanaSarah L Berry,² **Sarah R Hart**,¹ Helen Price,²¹Institute for Science and Technology in Medicine, Keele University, Keele, ST5 5BG, UK; ²School of Life Sciences, Keele University, Keele, ST5 5BG, UK.

The neglected tropical disease leishmaniasis is caused by infection with the protozoan parasite *Leishmania* spp. With over 1 million new cases per year, this disease is a significant cause of mortality and morbidity in endemic areas. *Leishmania* parasites' lifecycle is complex, with multiple insect and mammalian intracellular (macrophage) phases.

The BBSome is a protein complex associated with molecular trafficking to/from primary cilia and flagella in other eukaryotes. Previous work (Price et al., 2013) showed that deletion of one of the subunits, BBS1, from *L. major* severely reduces parasite virulence in mice. We hypothesise that the *Leishmania* BBSome is involved in the transportation of macromolecules to the parasite cell surface. We are in the process of testing this hypothesis by analysis of transgenic parasite cell lines with disrupted BBSome function. We have targeted BBS9, a core protein subunit of the BBSome. Our data shows that knocking out the BBS9 gene in *L. mexicana* causes a significant decrease in cell size, flagellum length and motility in insect infective-stage promastigotes. The ability of stationary phase promastigotes to infect THP-1 monocytes (as a proxy for macrophage infection) is also significantly reduced in BBS9-/ mutant lines compared to the parental line. The next steps in this work are to analyse the effect these changes have on the distribution of macromolecules on the cell surface. Here, we are using biotinylation and streptavidin pull down of cell surface proteins, with analysis using TMT and mass spectrometry to observe differences in protein levels.

P2 Identifying the molecular networks underpinning the aggressive nature of Childhood Grade IV Brain Tumours**Matt L Sherwood**¹, Keith Okamoto², Paul Skipp¹ and Rob M. Ewing¹¹School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, SO17 1BJ; ² Department of Genetics and Evolutionary Biology, University of São Paulo, Brazil

Brain tumours are the largest cause of cancer-related deaths in those under 40 due to the complexity of surgically accessing and therapeutically targeting the brain. Grade IV brain tumours, such as medulloblastoma and atypical teratoid rhabdoid tumour (ATRT), are highly aggressive and invasive. Medulloblastoma and ATRT have a significantly higher frequency in children and treatment side-effects often lead to severe life-long disability. In 2003, malignant cells with stem-like properties which can generate heterogeneous tumours with elevated metastasis and therapeutic resistance were first isolated from brain tumours. I am applying mass spectrometry proteomics, bioinformatics and computational biology to generate a comprehensive analysis of the protein networks and cellular profiles of novel human medulloblastoma and ATRT stem-like cells. Initial results suggest these cells have significant upregulation of metabolomic proteins primarily implemented in aerobic glycolysis, implying enhanced energetic demands when compared to less aggressive brain tumour cells. These cells repress the expression of the key epigenetic regulators DNMT1 and DNMT3?, suggesting dysregulation of gene expression through a loss of DNA CpG methylation; a core component of neoplastic transformation. Culturing under 1% oxygen recently highlighted an interesting response of the cells to the pathophysiological oxygen concentration of a brain tumour. The data acquired warrants investigation, via proteomic and complementary techniques, to further unravel the molecular mechanisms underpinning the aggressive nature of these brain tumour stem-like cells. This in-depth proteomic study is vital for the appropriate use of these cells in rare cancer stem cell-based models to study tumorigenesis and to inform therapeutic development.

P3 Metabolic adaptation of *E. coli* to cyanobacterial sesquiterpene synthase

Faith O Robert,¹ Esther Karunakaran,²

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Introduction

E. coli has been employed as an organism of choice for production of many recombinant proteins as well as metabolites for reasons ranging from cheap and rapid growth, to abundance of genomic, transcriptomic, proteomic and metabolic data.

There are many variables that determine efficiency of a heterologously expressed pathway such as consumption of intermediates by native pathways, mRNA abundance and half-life, protein abundance. Proteomics methodologies can help to identify pathway bottlenecks which can then be metabolically engineered to increase product titres.

Targeted proteomics methodologies have recently been applied to metabolically engineer a more than three-fold increase of amorpha-4,11-diene titre to greater than 50mg/ml in *E. coli*. Several other quantitative proteomics methodologies are presently widely employed, including iTRAQ.

Method

E. coli cells containing the sesquiterpene synthase and control were grown overnight and harvested. Proteins extraction was done, quantified and then subjected to tryptic digestion. Digested and vacuum dried peptides were labelled with a 4-plex iTRAQ according to the manufacturer's instruction and then fractionated. Followed by mass spectrometric analysis.

Results

Results show there is growth retardation as a result of reduction of the biosynthetic machinery of the cell for cell wall components, energy depletion, amino acids as well as nucleotides. There is a mobilization of metabolic resources and energy for the production of recombinant protein.

Conclusion

Growth retardation and mobilization of cell resources towards recombinant protein synthesis at the expense of the cell would be ameliorated if an external source of isoprene monomers can be supplied to the cell.

P4 Multi-batch TMT reveals false positives, batch effects and missing values

Alejandro Brenes¹, Jens Hukelmann¹, Dalila Bensaddek¹, Angus Lamond¹

¹Laboratory for Quantitative Proteomics, School of Life Sciences, University of Dundee

Introduction: Multiplexing strategies for large-scale proteomic analyses have become increasingly prevalent, TMT in particular. Here we used a large iPSC proteomic experiment with twenty-four 10-plex TMT batches to evaluate the effect of integrating multiple batches within a single analysis.

Methods: 24 batches of 10-plex TMT ran on the same orbitrap fusion using SPS-MS3, quantified in a single MaxQuant run with 1% FDR.

Results: We identified an inflation rate of protein missing values as multiple batches are integrated and show this is aggravated at the peptide level. We also show that the precision of quantitation within a single TMT batch is not reproduced when data are integrated from multiple batches, unless normalisation strategies to address the batch effects are used. Furthermore, we demonstrate the incidence of false positives by using Y chromosome specific peptides as a control. These Y chromosome-specific peptides were consistently detected in the female channels of all TMT batches. We then used the same Y chromosome specific peptides to quantify the level of ion co-isolation as well as the effect of primary and secondary reporter ion interference. These results were used to propose solutions to mitigate the limitations of multi-batch TMT analyses.

Conclusions: While single TMT batches can provide precise results within the multiplexed experiment, we found that this is often not reproducible across multiple batches. This study has also highlighted the issue of false positives, reporter ion interference and co-isolation interference. TMT is a very powerful methodology, however studies must be planned understanding its limitations.

P5 C-omics Explorer: A versatile web-based resource for integrated analysis, exploration and visualisation of cancer -omics datasets

Grigoris Koulouras¹ and Sara Zanivan¹

¹Cancer Research UK Beatson Institute, Glasgow G61 1BD, UK.

The increasing number of proteomics studies has undoubtedly provided unprecedented opportunities for research and shed new light on functional mechanisms, signalling transductions and biomarker identification over the past years. This fact has motivated us to develop C-omics Explorer, a web-based platform and open-access data repository of proteomics datasets derived from studies that have been performed at the CRUK - Beatson Institute. The C-omics Explorer provides an all-in-one suite for the analysis, visualisation and exploration of multiple cancer studies, under various experimental conditions, with a particular emphasis on readily understandable outcomes via interactive features. The effortless navigation through the intuitive user interface of C-omics Explorer allows researchers to retrieve comprehensive and comparable information of protein regulation and abundance across different samples, thus paving the path to a better understanding of meaningful cellular processes. Both quantitative and qualitative information of proteomic profiles across experiments is provided, coupled with highly interactive sequential and structural viewers, a handy incorporated pathway analysis tool (Reactome), as well as a fully automated curation procedure. C-omics Explorer provides a publicly available and continuously enriched resource of cancer proteomics datasets of human cells and other organisms. The modular design and scalable architecture of C-omics Explorer offers an environment suitable for future integration of proteomics datasets with other related -omics data aiming to serve as a flexible analysis tool for the effective investigation of the functional properties that affect cellular mechanisms and disturb subcellular functions.

P6 mzMLb: a PSI standards compatible binary mass spectrometry data format for efficient read/write speed and storage space requirements

Ranjeet S. Bhamber¹, Andris Jankevics,^{1, 2} Eric W Deutsch,³ Andrew R Jones,⁴ Andrew W Dowsey,¹

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³Institute for Systems Biology, Seattle, Washington 98109, United States; ⁴Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom.

Introduction: With the advent of next-generation data analysis pipelines, the need for an open format possessing both file size efficiency and read/write speeds has become paramount. The Proteomics Standards Initiative established an XML representation for data interchange, mzML, receiving substantial uptake; nevertheless, storage and IO efficiency was not the main focus. We propose a simple and future-proof HDF5 file format ‘mzMLb’, based on mzML, which is both storage and IO optimised.

Methods: mzMLb utilises standard HDF5 functionality compatible across all operating systems and languages. All Base64-encoded data from mzML are stored natively as HDF5 datasets. The mzML metadata is stored as a HDF5 character array dataset, while the spectrum and chromatogram indices are replaced by native HDF5 versions. We implemented a simple lossy compression method based on the binary representation of data and a linear prediction that results in file size reductions equivalent to Numpress compression.

Results: We compared our new mzMLb file format to vendor raw file, mzML, mz5, and Numpress within both mzML and mzMLb. Benchmarks were performed including write time and random read of single and sequential compressed data. Our approach demonstrates both faster write/read times and smaller compressed output file data across both random and sequential access of spectral data when compared to all previous approaches.

Conclusions: We demonstrate that by using a binary file format to store MS data it is possible to improve data reading/writing speed and keep all related metadata. We have fully implemented mzMLb in ProteoWizard package across supported platforms.

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P7 Tools to search, explore and interrogate your proteomics data with UniProt.

Emily H. Bowler-Barnett¹, Emanuele Alpi¹, Michele Magrane¹, Sandra Orchard¹, UniProt Consortium^{1,2,3}.

¹European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SD, UK; ²Swiss Institute of Bioinformatics, Centre Medical Universitaire, 1 rue Michel Servet, 1211 Geneva 4, Switzerland; ³Protein Information Resource, Georgetown University Medical Center, 3300 Whitehaven St. NW, Suite 1200, Washington, DC 20007, USA and University of Delaware, 15 Innovation Way, Suite 205, Newark, DE 19711, USA.

The analysis of proteomics data is inherently reliant on high-quality protein sequence databases. UniProt is a comprehensive, expert-led, publicly available database of protein sequence, function and variation information. It currently holds over 13,000 reference proteomes, that are constantly updated and reviewed based on collaborations with a variety of sources such as Ensembl, RefSeq, ENA and proteomics repositories such as ProteomicsDB, Peptide Atlas and MaxQB.

To facilitate searching of proteomics data, reference proteomes can be downloaded in FASTA format or queried programmatically using the UniProt Proteins API which allows researchers to access UniProt data alongside large-scale genomic, proteomics and variation data. Data is available for download and querying in a range of formats; including XML, FASTA and PEFF.

To facilitate further investigation of target proteins, mapped proteomics data pertaining to unique peptides is available graphically alongside variant, domain, and post-translational modification sites for each canonical protein sequence. This allows researchers to reference their data within the context of a proteins' peptide sequence and understand the current functional biological information available for their proteins of interest.

All data are freely accessible from www.uniprot.org

P8 Dracula: An online mass spectrometry search engine

Andrew Collins,¹ Andrew R Jones,¹

¹University of Liverpool, UK

In this work we introduce a new web-based, proteomics search engine called Dracula that can utilise volunteer computation to provide a custom-built high-performance computing platform in a distributed and on-demand manner. To do this Dracula consists of a central server node that is responsible for the initial acceptance of search queries, indexing and result assembly. The analysis of spectra is provided by volunteer client nodes which request, analyse and return data back to the central server. We show that through using Dracula it is possible to quickly generate high-quality identifications with no computation costs to the submitter.

Firstly, Dracula's computation model is like various existing GRID computation models, where by many highly distributed volunteers provide computing power to solve a given problem. In our case, our client is written in JavaScript that can be embedded within any website to utilise the computing of any passing web traffic. The result of this is there are no installation or high-performance computing requirements on the user.

Further, Dracula uses a statistically-based scoring algorithm to provide high separation between true and false allowing for large numbers of high-quality identifications to be detected at 1% FDR. We show that performance of Dracula is not only capable of identifying large numbers of identifications, but that via the ability to scale-up, it is particularly appropriate for searches with many spectra or large databases, such as in proteogenomics.

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P9 Predicting crosstalk between phosphosites from large scale quantitative phosphoproteomics data

Augustine C Amakiri¹, Anton Kalyuzhuy, ¹, Andrew Jones, ¹.

¹ Institute of Integrative Biology, University of Liverpool, UK.

Proteins undergo different kinds of post translational modifications (PTMs) which enable them to perform more specific functions in the body. There are over 480 types of PTM recorded in www.uniprot.com including phosphorylation, acetylation, methylation and many others. Phosphorylation and dephosphorylation of proteins is the hallmark of cell signalling. Several studies have shown that while an interplay between some of these PTMs lead to important biological processes, others have been implicated in disease pathways including cancer. In this research, we aim to predict crosstalk between phosphosites from large scale cancer phosphoproteomics datasets – indicative that different sites within the same protein are under coordinated control. Our method normalises quantitative signals across multiple conditions (on a per protein basis), enabling us to detect significant correlations in quantitative profiles between sites. Where significant correlations are observed, we can then compare the predicted responsible kinases to infer that the same kinase is phosphorylating multiple sites on the same protein, or potentially that different kinases are working together. Initial results suggest that particular pairs of kinases e.g. CDK1 and CDK2a2 appear to be working together based on enrichments for pairs of phosphosites putatively phosphorylated by these kinases having correlated quantitative patterns.

P10 IcmsWorld: New Software for 3d visualisation of LC-MS data

Antony McCabe¹, Dean Hammond¹, Andrew Jones¹.

¹Computational Biology Facility, University of Liverpool

We have developed novel new software, IcmsWorld, for PC and Mac, which provides efficient 3d-visualisation of mass-spectrometry data.

One unique feature of the software is that it allows the data for multiple files to be viewed simultaneously. Here, we use this feature to examine data from a dynamic SILAC experiment, conducted to investigate the secretion kinetics of proteins in cultured cells. In this experiment, peptides acquire labels over a time course and by using IcmsWorld we are able to view the data for 6 timepoints simultaneously (0, 30 mins, 1 hour, 2 hours, 6 hours, 24 hours). Hence, for any peptide of interest, we can quickly establish the profile of the label acquisition over the timespan.

Furthermore, we can use IcmsWorld to automatically generate still comparison images of the LC-MS data from all of the peptides that were identified by MaxQuant - this allows us to quickly scan for particular acquisition profiles by looking at the raw LC-MS data, without needing to rely on software-generated chromatograms. We are also able to easily examine the raw data directly, across the different timepoints, and can see features that appear to show relevant label acquisition profiles, but that were not previously identified as peptides of interest by MaxQuant.

We believe that our new visualisation software will prove to be a useful tool in examining LC-MS datasets, particularly for quality control purposes and the manual verification of software-generated annotations.

P11 Liquid AP-MALDI MS profiling for rapid assessment of milk quality and animal welfare

Cristian Piras¹, Oliver J. Hale², Chris Reynolds³, A K (Barney) Jones³, Nick Taylor⁴, Mike Morris⁵, Rainer Cramer¹

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling is rapid, cost effective and applicable to the large-scale identification of peptides/proteins and metabolites. However, some work still needs to be done for the successful application of this technique to the analysis of liquid biological specimens.

In this work, we propose a high-throughput method for liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry (AP-MALDI MS) profiling that has been applied to the analysis of milk adulteration and animal welfare. AP-MALDI MS works on a homogenous droplet and is capable to produce ions with high charge states that allow, in the same analysis, the profiling of lipids and entire proteins/proteoforms in an m/z range below 2000.

With this technique, milk from cow, goat, sheep and camel were classified with 100% accuracy in 10-second scans. It was possible to detect goat milk adulterated by 5% cow milk with 92.45% sensitivity and 94.53% specificity. Goat milk adulterated by 10% cow milk was detected with 99.15% sensitivity and 99.10% specificity. All these MS analyses were performed within 30 seconds.

Specific ion signatures of bovine mastitis have been detected up to 6 days before the clinical event in 1-minute analyses. All signals were related to the differential presence of intact proteins/proteoforms. These three results represent just a few examples of the potential applications of this versatile technique that couples lipids and top-down protein analysis of complex biological matrices such as milk.

P12 Driving chromatography towards single cell proteomics using micro-Pillar Array Column technology

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Recent current technological innovations focus on profiling complex systems at the single cell-level. The rapidly advancing field of single-cell proteomics aims at comprehensively quantifying protein expression levels from individual mammalian cells, complementing single cell transcriptomics and genomics analyses. A single mammalian cell contains about 0.3 pg of protein, which is 1000 times less than what is commonly used in proteomics analysis. Robust analysis of such very low input samples thus poses one of the key challenges to the current developments of new mass-spectrometry based proteomic workflows, including sample preparation techniques and instrumentation.

Here we carefully evaluated the potential of newly emerging chromatographic technologies to significantly improve sensitivity for low input proteomics samples. We compared the performance of the new micro-Pillar Array Column or μ PAC technology, 50cm reversed phase C18, to the widely used proteomics-standard, 2 μ m, 75 μ m x 50 cm column in the analysis of 10 ng HeLa Pierce tryptic digest. The trapping configuration in both cases allows effective removal of sample matrix components such as salts and contaminants which can interfere with downstream MS analysis, hereby increasing analytical column lifetime and at the same time improving spectral quality that is generated for a sample. A recently released trapping column employing the micro-pillar system was used in combination with the μ PAC column. Chromatographic support was installed in a generic proteomics nanoLC-ESI-MS/MS setup, comprising the respective pre- and analytical columns and Q Exactive HF-X mass-spectrometer.

P13 Full Spectrum Complexome Analyses: Towards the comprehensive characterisation of cellular protein complexes by Size Exclusion Chromatography

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We have developed an efficient approach for the systematic characterization of cellular protein complexes using uHPLC-based Size Exclusion Chromatography (SEC). Recently, we have shown that even very large cellular complexes, including ribosomes and polysomes, can be separated efficiently by uHPLC using SEC columns with a pore size of 2,000Å (Yoshikawa et al., eLife 2018; 7:e36530). Conversely, however, these very large pore size SEC columns provide poor resolution of smaller protein complexes. We show here that Full Spectrum Sampling of all size classes of cellular protein complexes is enabled in a single shot uHPLC analysis by combining two SEC columns with different pore sizes, run in series. Thus, the void volume containing larger protein complexes that are not resolved by the smaller pore size SEC column are separated by the larger pore size SEC column, providing a Full Spectrum Complexome profile for the cell or tissue extract being analysed. This approach is highly reproducible and provides a detailed protein complex fingerprint that can be used to compare any combination of cell/tissue types and/or growth conditions, drug treatments etc. For example, we detect clear differences in the Complexome Fingerprints for human cell lines comparing normal conditions and exposure to glucose starvation. The resulting SEC fractions can be analysed further by targeted LC-MS/MS to identify protein and PTM-level changes in the respective extracts, with increased sensitivity.

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P14 METAPROTEOMICS PILOT STUDY - BONE DEGRADING MARINE BIOFILM

Carmen Ioana Muresan¹, Alina Nistor¹, Ioana Ecaterina Pralea², Antonio García-Moyano³, Erik Borchert⁴, Ute Hentschel⁴, Manuel Ferrer⁵, Gro Elin Kjæreng Bjerga³, Cristina Adela Iuga^{2,6}, Ramona Suharoschi¹.

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³NORCE, Norwegian Research Centre, Bergen, Norway (Coordinator of ProBone); ⁴GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany; ⁵CSIC, Institute of Catalysis, Madrid, Spain; ⁶Department of Pharmaceutical Analysis, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy of Cluj-Napoca, Romania.

According to FAO projection in 2027 global meat production will increase by 13% (2014-2016). In this perspective, in the next decades, the meat industry residual deboning material will follow this growth trend. Thus, the ProBone Project (<http://www.marinebiotech.eu/ProBone>) is aiming at the development of tools for bone degradation which is safe for people and the environment. In nature, bones are degraded by the concerted action of free-living bacteria and symbiotic microorganisms associated with bone-thriving invertebrates. In ProBone, bone degradation in the marine environment is explored. In particular the enzymatic mechanisms. An enzymatic hydrolysis is an attractive industrial process that can be used to obtain valuable products from residual bone materials.

Metaproteomic tools will be employed in order to discover relevant enzymes from the bone-degrading marine biofilm. Cow, swine and turkey bones were artificially immersed in the depths (~70 m) of the Byfjorden outside Bergen, Norway for several months and the biofilm that developed on the bone surface was sampled. Total proteins extracted from the samples will be further separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and analysed using a label-free shotgun mass spectrometry based proteomics approach (LC-ESI-QTOF-IMS). Raw data will be processed and the recorded spectra will be searched against a Uniprot/ Swissprot protein database. Proteins identifications and taxonomic assignment will be linked with phylogenetic and metagenomic information. By this approach, we expect to identify bone hydrolytic enzymes which will have a great impact on biotechnology in terms of suitable commercial enzymes able to access the recalcitrant bone components.

P15 A modified Orbitrap™ Tribrid mass spectrometer with real-time search and advanced spectral processing enhances multiplexed proteome coverage and quantification accuracy.

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We present new hardware and software features that enhances proteome coverage and quantification accuracy for Tandem Mass Tags™ (TMT™). To assess the accuracy, precision, and sensitivity of the modified Orbitrap Tribrid mass spectrometer for TMT based quantitation, we utilized the TMT11plex yeast digest standard. Data was collected using Synchronous Precursor Selection (SPS) and with and without a FAIMS Pro™ interface. Raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.3 software using the SEQUEST® HT search engine. To improve upon existing SPS methods, we implemented a Real Time Search (RTS) filter between the MS2 and MS3 scans. This feature benefits TMT SPS-MS3 methods in two distinct ways. First, MS3 scans are only triggered if a peptide-spectrum match (PSM) is identified from the preceding MS2. This increased the number of peptides identified with SPS-RTS-MS3 by 30%. Secondly, RTS identifies precursors for MS3 on-line that are generated from the identified peptide. Thus, TMT SPS-RTS-MS3 quantitation can be improved to be 95% isolation interference free. Next, we evaluated a new feature called TurboTMT, powered by the ?SDM algorithm. ?SDM is an advanced spectra processing algorithm that increases resolution within a range of the spectrum without requiring a longer transient. Applying ?SDM specifically to the TMT reporter ions increased the resolution sufficient to baseline resolve TMT isotopologues even when using transients that produce a 30,000 or 15,000 resolving power MS2 scan. ?SDM increased both the spectral acquisition rate for TMT11plex experiments and the number of identifications for SPS-MS3.

P16 Towards turnkey targeted proteomics solution using internal standard triggered acquisition on modified Orbitrap mass spectrometers

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An extension of PRM (Parallel Reaction Monitoring) involves the addition of internal standards to samples to dynamically control the acquisition process and maximize its efficiency. The original prototype implementation of this internal standard triggered-PRM (IS-PRM) on quadrupole-Orbitrap instrument enabled the systematic monitoring of large sets of proteins with higher analytical performance. Here, the acquisition scheme of the approach has been revisited, while keeping the same rationale, to enable a more generic implementation on latest generation Orbitrap-based instruments. The analyses were performed by nanoLC coupled to either a modified Q Exactive™ or Orbitrap Fusion™ Tribrid™ mass spectrometers. The IS-PRM method toggled between i) a “watch mode”, in which internal standards (IS) were continuously measured through PRM scans over their (dynamically corrected) elution time monitoring windows at fast scanning rates, and ii) a “quantitative mode” (triggered by the real-time detection of the IS) which measured the corresponding pairs of IS and endogenous peptides over their pre-defined elution profiles with optimized PRM acquisition parameters. In comparison with conventional PRM, the technique significantly enhanced the acquisition efficiency (typically 5-fold higher), which was leveraged to expand the scale and data quality of targeted experiments. The method was applied to various types of samples supplemented with different sets of SIL peptides in various formats (30-800 IS). The ability to embed in instrument software pre-set (optimized) methods associated with predefined kits of IS represents a pivotal step towards a turnkey targeted proteomics solution.

P17 *Polyomics analyses reveal a role for nucleobase transporter in gentamicin-attenuated Leishmania mexicana*

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Leishmania have the ability to subvert the host immune system and adopt sophisticated strategies to develop and survive within macrophages in mammalian host. The molecular communication between host and parasite decides the outcome of infection, but is incompletely understood. We have compared genotype and phenotype of an attenuated Leishmania mexicana line with a virulent, isogenic wild type precursor. We aim to identify key virulence factors and to explore the potential of the attenuated line as a vaccine candidate.

This study was involved comparative polyomics approaches to identify the molecules that contribute to Leishmania virulence. Log phase promastigotes of wild-type and gentamicin-attenuated (H-line) were grown in parallel in media containing 10% FBS, and they were harvested for polyomics analyses. For comparative proteomic analysis, protein extracts were labelled using 6-plex TMT and analyzed with LC-MS/MS. For metabolomics, metabolites were extracted with Chloroform/ Methanol/ Water (1:3:1) and analyzed with LC-MS. For transcriptomics, RNA was isolated and converted into a library of cDNA molecules for cluster generation and DNA sequencing. We found 18 proteins, 26 identified metabolites, and 481 transcripts were differentially expressed in H-line. A threshold of $FC \geq 1.5$ and $FDR \leq 0.05$ was set up for polyomics data analyses. Correlation of polyomics datasets reveals that nucleotide metabolism is significantly altered in H-line. Furthermore, nucleobase transporter was significantly down regulated in proteomics analysis. Modulation of gene expression, observed through polyomics analyses, may relate to gentamicin selection. $\Delta NT3$ cells become more sensitive to allopurinol (purine analogue) at lower concentrations ($EC_{50} = 12.4$) comparing to the wild-type cells ($EC_{50} = 141.1$), suggesting that this may contributes to Leishmania avirulence.

P18 Identification of tumour-specific neo-epitopes in oesophageal adenocarcinoma

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Introduction: Adenocarcinoma is the predominant subtype of oesophageal cancer in the west and incidence is rising. Oesophageal adenocarcinoma (OAC) usually presents at a late stage, with 5 year survival rates <15%, and is often resistant to chemotherapy. When compared to other cancer types, OAC is characterised by high mutation rates, but few recurrently mutated genes. Personalised immunotherapy approaches targeting individual tumour mutations are a promising area of investigation for this tumour type. Our aim was to identify neo-epitopes presented by MHC-I and –II in tumour samples suitable for personalised CD8 peptide vaccine development.

Methods: MHC-I and –II immune-peptides were purified from five matched OAC tumour and healthy tissue samples and analysed by tandem MS. Tumour-specific mutanomes for each patient were derived from whole exome sequencing data of healthy and tumour tissues. Tumour immunopeptidomes were searched against each individualised mutanome using Peaks software to find neoantigens consisting of single amino acid substitutions.

Results: Two neoantigens were found, one MHC-I and one MHC-II, both in the same tumour sample. This was the largest tumour analysed and also contained the highest number of tumour-specific mutations. A further potential neo-antigen was found in the proteome of this tumour, but has not yet been confirmed as being presented by MHC.

Conclusions: Our data demonstrate the feasibility of the immunopeptidomics approach to find tumour-specific neoantigens in OAC. Functional assays of T cell reactivity to these neo-epitopes are underway to determine their suitability for peptide vaccine development.

P19 High throughput Capillary-flow LC-MS for large scale Clinical Proteomics Research Studies from Crude Blood Extracts

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The availability and ease of sampling of blood and its constituents make it ideal for large cohort clinical research studies. Proteins, as major players in catalytic and structural as well as many signaling functions, are essential research targets. Whilst nano-LCMS is capable of both specific and significant proteome depth, its widespread adoption has been limited by the low throughput and insufficient robustness of the methods employed.

Here we demonstrate a new set of capillary-flow LC-MS (capLC-MS) methods capable of large sample cohort analysis using a Thermo Scientific™ UltiMate™ 3000 RSLCnano system coupled to a Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ mass spectrometer.

The five low-flow LC-MS methods are capable of throughputs of 180, 100, 60, 30 and 24 samples per day affording MS utilization from 75 to over 90% respectively. These were validated using HeLa protein and crude plasma digests and showed excellent long-term reproducibility and good protein coverage with more than 150 protein groups identified using the 8 min LC-MS method increasing to over 250 protein groups for the 60 min plasma runs.

The low-flow LC-MS methods provide sharp, symmetric peaks that, in combination with the high sensitivity and fast acquisition speed of the Q Exactive HF-X mass spectrometer deliver robust, fast and deep profiling for crude plasma protein digests and cell lysates. Furthermore, the consistent results generated over hundreds of replicate injections, prove that the methods are suited to the analysis of large sample cohorts.

For Research Use Only. Not for use in diagnostic procedures.

P20 Protein extraction for quantitative proteomics from formalin fixed paraffin embedded (FFPE) tissue on the μm^3 scale.

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Preservation of tissues by FFPE processing has been, and remains, common practice for clinical pathology diagnostic samples, stabilising the structure/architecture of the tissue for prospective analysis.

Consequently, there are large archives within Histopathology Departments comprising samples, derived from a myriad of diseases often with adjacent healthy tissues that can be used to understand disease aetiology and for in-depth characterisation of specific tissue types. However, individual isolates are small (consist of less than a gram in some instances), and regions or structures of interest within these samples will typically be much smaller. Commercial kits for extraction of proteins for proteomics analysis from FFPE tissues require 1-2 mm³ of tissue, which may not be feasible when the sample is limited, or if analysis requires isolation of sub-structures.

Here we demonstrate efficient extraction of proteins from FFPE human skin tissue sections of 0.01 mm³ following laser capture micro-dissection, permitting identification of over 600 proteins by LC-MS/MS. Moreover, we demonstrate that our protocol is suitable for isobaric labelling-based protein quantification enabling protein component of different tissue sub-structures to be defined. We suggest this is suitable for efficient proteomics analysis of small sections of FFPE tissue, ensuring efficient use of finite clinical samples deposited in archives and biobanks.

P21 Non-invasive profiling of the breast micro-environment to monitor breast health

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Introduction. Nipple aspirate fluid (NAF) is a breast-specific proximal fluid, secreted by the epithelial cells lining the breast ductal system from which 85% of breast cancer cases arise. NAF is, therefore, an attractive resource for breast cancer biomarker discovery as it can be attained simply and non-invasively through breast massage and screened for biomarkers indicative of disease development. Clinical biomarkers must be identified using a reproducible, high-throughput method, allowing disease indicators to be identified rapidly.

Methods. To accelerate our understanding of the protein composition and consistency of NAF, 20 matched pairs from both healthy volunteers and invasive carcinoma (IC) patients were reduced, alkylated, and trypsin digested prior 1D-LC/MS analysis on Orbitrap Fusion. One IC NAF pair was selected as an inter-experiment control, analysed in triplicate within each experiment for qualitative proteomic profiling. MS/MS fragment mass lists were searched against the Swiss-Prot database (*Homo sapiens*) through Proteome Discoverer 2.2, using Mascot software for protein identification.

Results. Overall, 1320 different proteins were identified between the 20 NAF samples. Analysis of the inter-experiment control IC patient samples recognised 270 proteins that were consistently identified regardless of breast origin or experiment, 17 were unique to the diseased breast. Bioinformatics analysis using STRING highlighted the consistently recognised proteins as playing key roles in cell adhesion ($p=0.0273$), cell migration ($p=0.0272$), tissue morphogenesis ($p=0.0273$) and angiogenesis ($p=0.0273$).

Conclusion. The results highlight the great potential NAF carries as a liquid-biopsy for monitoring breast health. Analysis in 1D-LC/MS provides relatively rapid insight into the breast microenvironment.

P22 Proteomic profiling of precision cut liver slices: A model for chronic liver disease

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Introduction

Fibrosis is a pathological state characterised by the excessive deposition of extracellular matrix components, scar tissue formation and impaired tissue function. In the context of hepatology, fibrosis occurs as a consequence of persistent liver injury, and is a major risk factor associated with progression to cirrhosis. Therefore, improved understanding of liver fibrosis is imperative.

Methods

Precision cut liver slices (PCLS) represent an improved model system for the study of liver disease. A novel bioreactor has previously been developed by our group, which has the capacity to maintain viable PCLS for up to six days. Here, proteomics was performed on human PCLS over a 96 hour time-course to investigate whether the prolonged culture process induces proteome-wide changes, which may confound results in differential analyses. Secretome samples from PCLS cultured in serum-free media were also analysed by mass spectrometry.

Results

LC-MS/MS analysis of the PCLS proteome on a TIMS-TOF Pro mass spectrometer identified 3,121 protein groups, where only transferrin receptor protein 1 was found to be differentially expressed. LC-MS/MS analysis of the PCLS secretome identified just under 900 protein groups, indicating that despite high levels of albumin in the liver secretome, relatively in-depth coverage is achievable.

Conclusions

This study verifies the suitability of the PCLS bioreactor as a model system for studying liver disease. Next steps include stimulation of PCLS with TGF- β , a known inducer of fibrosis in PCLS, to investigate how the hepatic epithelium modulates its phenotype on both the proteome and secretome level in response to chronic damage.

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P23 *Proteomics analysis of air-surface liquid from a 3D-reconstituted airway epithelium shows reduced effect of 4-weeks exposure to tobacco heating product in comparison with cigarette smoke*

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Cigarette smoking is a risk factor for many diseases. Recently there has been an increase in the prevalence of next generation nicotine and tobacco products (NGPs) including tobacco-heating products (THPs). THPs heat tobacco at <350°C instead of burning (900°C), having the potential to significantly reduce the majority of cigarette smoke toxicants.

In this study, we compared the proteomic profile of a 3D-reconstituted human airway epithelium (MucilAirTM) after a 4-weeks exposure regimen to THP aerosol or smoke from a scientific reference cigarette (3R4F). Using both a targeted LC-MS Multiple Reaction Monitoring (MRM) and “shotgun” exploratory LC-MS proteomic approach, we quantified proteins in the air-surface liquid (ASL) collected from MucilairTM tissues relative to air control. For a selected panel of 19 proteins analyzed in the ASL of MucilairTM exposed to 3R4F smoke in targeted LC-MS mode, we detected significant changes in the abundance of 3 proteins associated with COPD pathophysiology (LPLC1, NGAL and PIGR). In comparison, no response was observed when the same protein panel was quantified in the ASL from THP aerosol-exposed MucilairTM tissues against air control. Exploratory “shotgun” proteomics analysis of ASL from MucilairTM exposed to 3R4F revealed an increased overall disturbance and several instances of significant differences in protein abundance compared to the untreated air control, but minimal responses were observed for THP aerosol.

In conclusion, both targeted and exploratory proteomic analysis confirmed that THP aerosol is associated with a reduced proteomic response in MucilairTM ASL compared to a conventional cigarette, after a 4-weeks exposure regimen *in vitro*. Further pre-clinical and clinical assessments are required to further understand the risk reduction potential of these novel products at individual and population levels.

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P24 *In vitro toxicogenomic and proteomic assessment of e-cigarette aerosol compared to cigarette smoke*

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E-cigarette use has increased globally and could potentially offer a lower risk alternative to cigarette smoking. Differential gene and protein expression studies may offer perspectives for assessing the reduced risk potential of e-cigarettes. In this study, we compared the transcriptional and proteomic profile of primary 3D human airway tissues (MucilAirTM) exposed to e-cigarette aerosols or smoke from a scientific reference cigarette (1R6F), using an acute exposure regimen. Transcriptomic analysis was conducted using next-generation sequencing, and the functional profile of differentially expressed RNAs was assessed using gene set enrichment analysis. Using both a targeted and “shotgun” exploratory LC-MS proteomic approach, we quantified proteins in the air-surface liquid (ASL) collected from exposed MucilAirTM tissues relative to air control.

Using pFalse Discovery Rate (FDR) <0.01 and fold change (FC)>2, 5603 and 2180 genes differentially expressed RNAs were identified at 24hrs and 48hrs post- 1R6F exposure, respectively. No differentially expressed genes were detected when comparing e-cigarette exposed RNA-seq data to the air control. Gene set enrichment analysis revealed a clear response from lung cancer, inflammation and fibrosis-associated genes upon 1R6F exposure, and a low-confidence response from metabolic/biosynthetic, extracellular membrane, apoptosis and hypoxia genes upon e-cigarette exposure, suggesting a reduced impact of e-cigarette acute exposure on gene expression.

Using a [FC]>1.5 and p-value<0.05 threshold, for a selected panel of 21 ASL proteins, significant changes were observed 24hrs post- 1R6F exposure for 1 protein involved in angiogenesis (ANGL3), and at 48hrs post- 1R6F exposure for 2 proteins involved in adaptive immune response at mucosal surfaces (PIGR and MUC1), while no response was observed for e-cigarette aerosol. Using a FC >1.5 and p-value<0.05 threshold, exploratory “shotgun” proteomic analysis of ASL proteins revealed a robust change in protein expression at both 24hrs and 48hrs post-1R6F exposure, while minimal changes were observed for e-cigarette aerosol, relative to air controls. In conclusion, e-cigarette aerosol induced minimal protein and gene expression compared to a conventional cigarette after acute exposure *in vitro*.

P25 Integrative -Omics Reveals Novel Targets Underlying the Pathomechanisms of Uterine Fibroids and Associated Heavy Menstrual Bleeding

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Introduction: Uterine fibroids (UF's) are benign tumours affecting up to 80% of women of reproductive age. Approximately 30% of fibroid patients suffer severe symptoms including painful heavy menstrual bleeding (HMB). Although mutations in MED12 or HMGA2 account for the majority of UF occurrence, the processes by which these lead to UF's and HMB remain poorly understood. Using an array of -omics technologies, we undertake the first comprehensive systems biology study of the pathomechanisms and vascularisation underlying UF formation and maintenance.

Methods: Fibroid, endometrium, myometrium, pseudocapsule, and healthy control samples were obtained from patients undergoing surgery at the John Radcliffe Hospital, Oxford, UK. Gene and protein expression was investigated by RNA-Seq and quantitative proteomics. Differential expression of candidate angiogenic factors was validated on FFPE tissues using immunohistochemistry, and tested in vitro on human uterine microvascular endothelial cells. Their angiogenic potential was evaluated in proliferation and tube formation assays. Vascularised fibroid tissue architecture was imaged by light sheet microscopy.

Results: Uterine tissue transcriptomics and proteomics reveal altered biological pathways between different uterine tissue types, serving as novel druggable targets for the treatment of UF's and HMB. Immunohistochemistry staining of UF vasculature confirmed the presence of candidate angiogenic factors, which were validated using tube length, endothelial mesh, and cell proliferation assays. Cleared whole mice uterus showed a distinct blood vessel pattern upon visualisation with 3D reconstruction imaging.

Conclusion: Using our multi -omics approach, we have identified several exciting leads in the understanding of the pathomechanisms of UF's and associated HMB.

P26 Identifying the interaction partners and substrates of oncogenic deubiquitinating enzyme USP7 in colorectal cancer

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The ubiquitin-specific protease USP7 is a deubiquitinase that interacts and targets various substrate proteins. USP7 stabilises many substrates by the deubiquitination process that preserves them from proteasomal degradation and is an important regulator of cell homeostasis and survival. There is also significant interest in therapeutically targeting USP7 and defining its substrates and interaction partners is an important goal. Our goal is to define the USP7 associated protein network and understand USP7's role in the regulation of oncogenic proteins in colorectal cancer.

As a cell-model for proteomic analysis, we are using LS174T cell expressing inducible siRNA to knock-down expression of USP7. Depletion of USP7 significantly decreases the viable cells and cell growth in this model and also decreases the abundance of known interaction partners such as DNA Methyltransferase I (Dnmt1). We are using quantitative LC-MS/MS in conjunction with this inducible knock-down and in this presentation will present preliminary results from this study. Our findings may offer a new approach for the determination of the molecular effects of USP7 on DNMT1 and cell survival. This may open up new horizons for targeting USP7 as new molecular targets for the treatment of colorectal cancer.

P27 Multi-faceted chemical and genetic knock-down approaches to mapping functional protein networks of DNA methyltransferase I (DNMT1)

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Patterns of DNA methylation, a key epigenetic mark across the eukaryotic genome, are established and maintained by the DNA methyltransferase proteins (DNMTs). DNA Methyltransferase I (DNMT1) is the principal DNA methyltransferase in eukaryotic cells, preferentially methylating hemi-methylated CpG sites to replicate patterns of methylation after DNA replication. A key question, given the important role of DNMTs, is how their function and activity is regulated. DNMT1 abundance and stability is primarily regulated through post-translational level gain or loss of protein-protein interactions and addition or removal of post-translational modifications. DNMT1 protein-protein interactions include proteins that function in the DNA replication machinery and chromatin modifying proteins as well as proteins that directly regulate the stability and abundance of DNMT1 through modification of PTMs. We previously showed that DNMT1 is regulated by the activity of the Wnt signalling pathway via a protein-protein interaction between DNMT1 and β-catenin. In this work, we are using multiple approaches to characterize the DNMT1 protein functional network in colorectal cancer cells. First, chemical knock-down of DNMT1 using the nucleoside analog 5-azacytidine was used to induce selective proteasomal degradation of DNMT1. Second, colorectal cancer cells expressing DNMT1 hypermorphic alleles were compared to cells expressing wild-type DNMT1. We performed quantitative label-free LC-MS/MS in both studies using Nano-LC separation and a Waters Synapt G2-S mass-spectrometer. These studies identified multiple perturbations of pathways and processes induced by DNMT1 knock-down. We identified multiple chromatin-structure, metabolic and epigenetic-related proteins that are sensitive to 5-azacytidine including the epigenetic regulator UHRF1. Analysis of the proteome of DNMT1 hypermorphic cells indicates an important, potentially non-methylation dependent role for DNMT1 in the regulation of epithelial-mesenchymal transition. In summary, deep, functional analysis of cells with perturbed DNMT1 protein expression identifies novel functional DNMT1-related protein networks in cancer cells.

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P28 *Dissecting WISP1-associated networks in Idiopathic Pulmonary Fibrosis using RNA-Seq and Affinity-Purification Mass-Spectrometry (AP-MS)*

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and fatal lung disease of unknown cause affecting older adults. IPF physiology is characterised by build up of stiffened, collagenous extracellular matrix (ECM) in the lung interstitium, leading to impaired gas exchange. This ECM is produced by aggregates of fibroblasts known as fibroblast foci. IPF pathogenesis is driven by chronic activation of tissue repair pathways, such as Wnt and TGF- β signalling. Wnt inducible signalling protein 1 (WISP-1) is a protein that has been implicated in IPF pathogenesis, although its role remains unclear. Genetic risk factors associated with IPF include mutations in the telomere reverse transcriptase (TERT) enzyme, and telomere dysfunction has been identified as contributing to IPF progression.

We have used RNAScope in-situ– hybridisation techniques combined with RNA-seq data from laser-capture microdissected lung tissue to identify WISP-1 localisation in IPF tissue, and an affinity purification mass spectrometry (AP-MS) approach on GFP-tagged, overexpressed WISP-1 to identify key protein interaction partners which may contribute to IPF pathogenesis.

In situ hybridisation and laser capture RNA-seq data identifies WISP-1 as being highly expressed in the fibroblast foci in IPF tissue. AP-MS of WISP-1 in HEK-293t cells reveals interaction partners associated with the DNA damage response and telomerase regulation, including chaperonin protein complexes. AP-MS also confirms that WISP-1 is secreted as it is enriched for secretory cellular compartment gene ontology terms.

WISP-1 interaction with telomerase associated proteins suggests a mechanism by which it can contribute to IPF, and demonstrates potentially novel intracellular roles for WISP-1 protein.

P29 *Investigating the ways in which the novel PRMT1 inhibitor furamidine inhibits arginine methylation in human platelets.*

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Background: Arginine Methylation (ArgMe) is one of over 200 known post-translational modifications which are known to occur in mammalian cells. Facilitated by Protein Arginine Methyltransferases (PRMTs), a CH₃ group is added to the Arginine amino acid. Furamidine is a novel PRMT1 inhibitor which has been found in this study to reduce the ArgMe of platelet proteins, and functionally inhibit platelet aggregation.

Results: Bioinformatic analysis of proteomic data showed that out of 4058 platelet proteins, 260 are modified by arginine methylation. Included in these proteins was the platelet protein VASP which was found to be methylated at R68 and R298. These proteins found to be arginine methylated in platelets were enriched in the gene ontology term “platelet aggregation”. Platelet aggregation was found to be inhibited upon ex vivo incubation with Furamidine for a period of 4 hours with an IC₅₀ of 13.4μM. Platelet proteins including VASP were determined to be ArgMe by western blot, which was then reduced upon Furamidine incubation.

Conclusion: Through this study, the dysfunction of platelets has been linked to ArgMe through inhibition of PRMT1 by furamidine, which impairs aggregation of platelets ex vivo. As the dysfunction of platelets in vivo has been linked to the onset and progression of cardiovascular disease, further research into the arginine methylome of platelets and the possibility of using furamidine as a possible, novel antiplatelet treatment may provide a more effective mechanism for treating the 7 million people who are fighting heart disease in the UK every day.

P31 *Effect of short-chain aldehydes on the structure and enzymatic activity of the glycolytic enzyme pyruvate kinase*

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Introduction: Pyruvate kinase (PK) catalyses the last step in glycolysis, and contributes to the regulation of aerobic glycolysis in cancer cells, generating a more pro-oxidant state. This results in the formation of electrophilic fragmentation products from oxidised lipids, including 4-hydroxy-2-hexenal (HHE), malondialdehyde and acrolein. Little is known about the interaction of these molecules with pyruvate kinase, so we have investigated their reactions with and effects on the activity of the enzyme.

Methods: Purified rabbit muscle PK or MCF7 cells were treated with various concentrations of lipid oxidation product for a range of times. PK activity was measured using a lactate dehydrogenase/NADH coupled assay. For in vitro experiments, modifications were identified using standard LC-MSMS based proteomics approaches. For cells, pyruvate kinase was enriched from lysates using PAGE followed by in-gel digestion and LC-MSMS analysis. Data was searched using Mascot and also manually searched using reporter ions. All MSMS data was manually validated.

Results: Data showed unique modification profiles for each aldehyde, with only Lys 393 and 475 modified by all 3 compounds. Acrolein was the most potent inhibitor, and even at pathophysiological concentrations (2-10 µM) induced significant effects. Treatment of MCF-7 cells caused similar losses of pyruvate kinase activity, with time and dose-dependent effects. Cys326, Cys358, and Cys474 were found to be modified; these are located at or near the allosteric or active sites.

Conclusions: These results suggest that reactive short-chain aldehydes could be involved in the metabolic changes observed in cancer cells through alteration of pyruvate kinase activity.

P32 Detection of flucloxacillin modified peptides naturally presented by HLA-B*57:01, a risk allele for flucloxacillin-induced liver injury

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Introduction: Flucloxacillin is a β -lactam antibiotic associated with a high incidence of idiosyncratic drug-induced liver reactions. Although expression of HLA-B*57:01 increases susceptibility, little is known of the pathological mechanisms involved in the induction of the clinical phenotype. Irreversible protein modification is suspected to drive the reaction through the modification of peptides that are presented by the risk allele. In this study we investigated the precise nature of the MHC peptides presented by HLA-B*57:01.

Methods: Flucloxacillin protein binding was assessed in HepaRG liver like cells, and C1R-B*57:01 cells; B-lymphoblast cells transfected with HLA-B*57:01. To identify drug modified MHC peptides, C1R-B*57:01 cells were incubated with flucloxacillin for 48h and HLA peptide complexes were subsequently eluted and processed for mass spectrometric analysis.

Results: Direct modification of multiple proteins was observed, which could lead to neo-antigens being presented. Flucloxacillin was shown to disrupt transporter activity in HepaRG cells with localization appearing in bile canaliculi regions. Of the peptides eluted from flucloxacillin treated C1R-B*57:01 cells, 6 peptides were fully annotated to show flucloxacillin-lysine covalent binding, with other partially annotated peptides indicating modifications.

Conclusions: We have demonstrated that localization of flucloxacillin occurs in the site of clinical disease during flucloxacillin-induced liver injury. We also demonstrated that drug-modified peptides are presented by HLA-B*57:01. Further investigation into the immunogenicity of haptenated proteins and MHC peptides in the onset of iDILI is ongoing.

P33 Regulation of synaptic protein function by the lysine deacetylase Sirtuin-2

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Recent studies indicate an emerging role of lysine acetylation in the regulation of synaptic protein stability. Acetylation is a reversible process controlled by families of acetyltransferase and deacetylase (KDAC) enzymes. Owing to recent developments in mass spectrometry-based proteomics, large numbers of acetylated proteins and individual sites of acetylation in different cell types and tissues have been identified and revealed that acetylation has particular enrichment in the brain tissues that explain the role of acetylation in neurophysiological functions. 60% of synaptic proteins are modified by acetylation on >13,000 sites. SIRT2 is a NAD-dependent KDAC enzyme highly expressed in brain tissue and known for its association with many neurodegenerative diseases. Analysis of synapse proteome of mice and zebrafish datasets showed that SIRT2 is highly expressed in synapses. However, its function in the brain is not well understood. SIRT2 deacetylation of the AMPA receptor and Arc protein regulates their stability and their function in synaptic plasticity by maintaining the balance between acetylation and ubiquitination at individual lysine residues. Brain tissue from SIRT2 knockout mice along with specific lysine acetylation antibodies and SIRT2 inhibitors are used to allow in-depth characterisation of SIRT2 substrates and function at synapses using nano-flow (LC-MS/MS). Our lab was able to identify novel substrates of SIRT2 that will be further investigated to advance the understanding of the involvement of SIRT2 in neurodegenerative diseases and link those substrates with processes downstream of SIRT2.

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