INTERACT 2021

6th – 8th July 2021

CONFERENCE PROGRAMME

https://www.bspr-interact.com/

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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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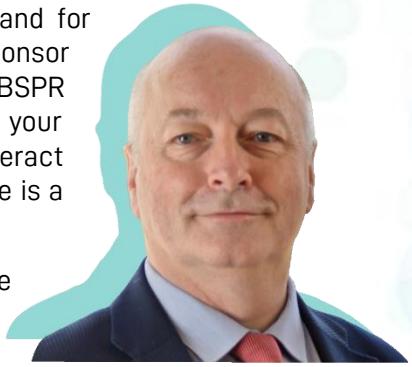
Welcome Address

Dear Colleagues,

As President of the British Society for Proteome Research and on behalf of this year's virtual organising committee, it is my great pleasure to welcome you to BSPR Interact 2021. Due to ongoing uncertainties and restrictions imposed by COVID 19, the 2021 event is being held virtually on the mornings of the 6th, 7th, and 8th July. While we wish we could all meet in person, and look forward to doing so soon, the virtual platform has given us the opportunity to develop a different meeting format and invite more speakers from further afield. Over the next few days, the conference will feature prominent and upcoming UK and international scientists presenting the latest research and methodology in the field of proteomics. A diverse range of themes have been incorporated into this year's conference including Clinical Proteomics, Structural Proteomics, Spatial Proteomics, Post-translational Modifications, New Technologies, Computational Proteomics, Single Cell Proteomics, Biomarkers and Artificial Intelligence. The BSPR is committed to providing early career researchers (ECRs) opportunities to present their research at our annual scientific meetings and it is notable that this year's meeting includes a dedicated ECR session on the 7th. The four talks in the ECR session were selected from submitted abstracts and the high quality of the abstracts received made it very difficult to select just four. Please give our selected ECRs your support by attending the session.

The planning and execution of a BSPR meeting takes considerable effort from a team. Organising a virtual meeting with its different challenges and opportunities is as demanding as organising a face-to-face meeting. I would like to take this opportunity to thank the Virtual Organising Committee (VOC) - listed below - for the time and effort they have spent over the last 6 or 7 months, including during weekly conference calls, to organise this year's meeting. I'm sure you will agree that the VOC, with support from Conference Organisers, have produced an excellent meeting.



It would not be possible to host a meeting like this without the generous support of our sponsors. We thank each of them for their continued support of the BSPR and for sponsoring BSPR Interact 2021. In these very different times we are aware that sponsor participation and engagement in virtual meetings can be challenging. So, at BSPR Interact 2021 the VOC have introduced 'gamification' to promote and track your engagement with the platform and our sponsors. I encourage you to actively Interact with the sponsors and collect points as you do so. Points mean prizes and there is a generous cash prize for the person who collects the most points!

Of course, we are all looking forward to meeting in person soon but until then we hope you will find BSPR Interact 2021 both stimulating and rewarding. With the now 'traditional' 'Kathyrn Lilley Quiz' we think you'll find it amusing too!

Have a great meeting,

Steve

Prof Stephen R Pennington,
President, BSPR

Acknowledgements

Virtual Organising Committee: Maike Langini (Chair), Kathryn Lilley (Scientific Programme), Sara Zanivan (Scientific Programme), James Waddington (Website and Promotion), Harry Whitwell (Co-Treasurer & Sponsor Liaison), Phil Green (Co-Treasurer & Sponsor Liaison), Kareena Adair (Media & Communications) and Roz Jenkins (Promotion).

Sponsors

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Day 1: 6th July 2021

09:00 - 09:30 | Break, poster viewing and networking

Opening Session | Sponsored by Covaris

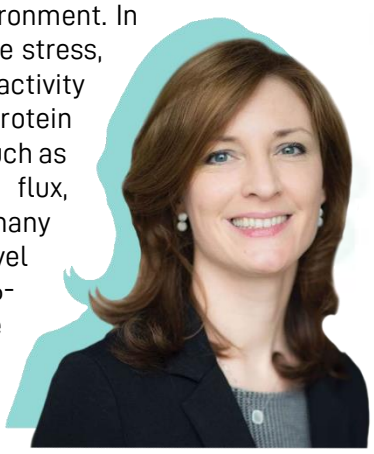
Session chair: Sara Zanivan | Session co-chair: Maike Langini

09:30 - 09:45 | Welcome address from the BSPR President, Steve Pennington

09:45 - 10:10 | Plenary talk presented by Paola Picotti, ETH Zürich Institute of Molecular Systems Biology

Proteomes in 3D

Biological processes are regulated by molecular events, such as intermolecular interactions, chemical modification and conformational changes, which do not affect protein levels and therefore escape detection in classical proteomic screens. Reasoning that these events affect protein structure, we tested whether a global readout of protein structure could detect various types of functional alterations simultaneously and in situ. We tested this idea using limited proteolysis coupled to mass spectrometry (LiP-MS), which monitors structural changes in thousands of proteins within a complex, native-like environment. In bacteria adapting to different nutrient sources and in yeast responding to acute stress, the structural readout, visualized as structural barcodes, captured enzyme activity changes, allosteric regulation, phosphorylation, protein aggregation and protein complex formation, with the resolution of individual regulated functional sites such as binding and active sites. Comparison with prior knowledge, including flux, phosphoproteomics and metabolomics data, showed that LiP-MS detects many known functional alterations within well-studied pathways. It suggested novel metabolite-protein interactions and enabled identification of a fructose-1,6-bisphosphate-based regulatory mechanism of glucose uptake in E. coli. The structural readout dramatically increases the coverage of classical protein expression profiling, generates mechanistic hypotheses, better links holistic and reductionist approaches, and paves the way for a new in situ structural systems biology.



10:10 - 10:30 | Plenary 1 question and answer session

10:30 - 10:50 | Covaris sponsored talk by Jeroen Krijgsveld, German Cancer Research Center & University Heidelberg

Automated sample preparation for reproducible proteomics in biology and the clinic

Proteome analysis is typically performed by direct integration of chromatography and mass spectrometry, allowing unattended and standardized analysis of multiple samples. In contrast, sample preparation prior to LC-MS is still largely a manual process, constituting a major source of variability in sample handling and, ultimately, in proteomic data. Here, we present how we established a generic, automated workflow for proteomic sample preparation by combining AFA-based ultrasonication and single-pot solid-phase-enhanced sample preparation (SP3), enabling routine and standardized proteome analysis with minimal hands-on time. Sonication is used for protein extraction from virtually any sample type, including cells, fresh tissue and FFPE tissues in a 96-well format, followed by protein clean-up and digestion by SP3 performed on a Bravo liquid handling platform. Sensitivity and reproducibility of the approach will be shown from low-input experiments starting from 100-1000 cells, from fresh tissues of various organs, and from FFPE tissue sections of cancer specimens. Furthermore, we used this pipeline to analyze a cohort of 96 ependymoma brain tumor samples, and integrated the obtained proteomic data with other omics layers for disease sub-classification. In conclusion, AFA-based ultrasonication combined with autoSP3 is a robust platform for standardized and parallel processing of a variety of tissue types for low-input proteomics, serving a wide range of clinical and non-clinical proteomic applications.

10:50 - 11:00 | Covaris sponsored talk question and answer session

11:00 - 11:30 | Break, poster viewing and networking

Session 2 - Cell Signalling and Emerging Technologies | Sponsored by Promega

Session chair: Andrew Pitt | Session co-chair: Lukas Krasny

11:30 - 11:31 | Session 2 introduction

11:31 - 11:35 | Promega talk presented by Hillary Pollard

11:35 - 11:50 | Helen Cooper, University of Birmingham

Native ambient mass spectrometry: Mass spectrometry imaging of intact proteins and protein complexes

Mass spectrometry imaging (MSI) provides information on the spatial distribution of molecules within a biological substrate, such as a thin tissue section, without the requirement for labelling. Ambient mass spectrometry, in which biological substrates are sampled at ambient temperature and pressure, and which requires little or no sample preparation, is ideally suited to in situ analysis of biomolecules. A suite of ambient mass spectrometry techniques exist including liquid extraction surface analysis (LESA), desorption electrospray ionisation (DESI) and nano electrospray desorption electrospray ionisation (nano-DESI), all of which have found applications in MSI.

A separate branch of mass spectrometry, native mass spectrometry, provides information relating to protein structure, including stoichiometry of protein assemblies and protein-ligand complexes, through retention of solution-phase interactions in the gas-phase. When native mass spectrometry is combined with ion mobility spectrometry, it is possible to determine rotationally-averaged collision cross sections.

Our goal is to combine native mass spectrometry with ambient mass spectrometry imaging to enable simultaneous acquisition of spatial and structural information on intact proteins directly from their physiological environment. Latest developments with LESA and nano-DESI, and their integration with ion mobility spectrometry, for the identification, structural characterisation, and imaging of monomeric proteins, protein assemblies, and protein-ligand complexes directly from a range of tissue types and pathologies will be presented.

11:50 - 12:05 | Maria Robles, Ludwig Maximilian University of Munich

Daily cycles of protein and phosphorylation abundance regulating rhythmic physiology

Circadian clocks are cell endogenous and self-sustainable oscillators found in virtually every cell in the body that play a fundamental role in cellular and tissue physiology. This internal time-keeping system anticipates daily environmental changes to thus prepare organismal metabolism for those recurrent changes. We use MS-based label free quantitative proteomics to study organismal daily dynamics of protein and phosphorylation. In my talk I will present some of our proteomics work aiming to elucidate circadian clock mechanisms from molecular to system levels in health and diseases.

12:05 - 12:20 | Joshua Coon, University of Wisconsin-Madison

New mass spectrometry technology for proteome analysis

In this presentation I will describe a variety of new mass spectrometry technologies for the analysis of proteins and proteomes. Through development of a multi-protease digestion strategy and the use of collisional and electron transfer dissociation we have achieved the deepest proteome analysis reported to date. This approach has led to the detection of over 17,000 gene products and the first ever global assessment of how mutations and splicing events are incorporated into the proteome. In another method we describe the use of ion mobility coupled with tandem mass spectrometry to directly analyze complex proteome mixtures at very high speeds. Finally, we describe a novel approach to prepare samples for transmission electron microscopy using native mass spectrometry to purify and collect protein complexes.

12:20 - 13:00 | Discussion

13:00 - 14:00 | Poster session

14:00 | Day 1 END

Day 2: 7th July 2021

09:00 - 09:30 | Break, poster viewing and networking

Session 3 - Early Career Researcher (ECR) Session | Sponsored by Thermo Fisher Scientific

Session chair: Rainer Cramer | Session co-chair: Kareena Adair

09:30 - 09:31 | Session 3 introduction

09:31 - 09:35 | Thermo Fisher Scientific talk presented by Aaron Robitaille

09:35 - 09:47 | Diana Canetti, University College London

Clinical ApoA-IV amyloid is associated with fibrillogenic signal sequence

Amyloidosis is an uncommon disease that occurs when normal circulating proteins misfold and accumulate as insoluble fibrillar aggregates. There are over 20 different circulating proteins known to cause amyloidosis. The UK National Amyloidosis Centre (NAC) operates a UKAS-validated proteomics facility to type these amyloid proteins. Apolipoprotein A-IV amyloidosis (AApoA-IV) is a rare form of the disease, mainly characterised by renal and cardiac dysfunction. In addition to its intrinsic amyloidogenicity, ApoA-IV is also deposited along with amyloid of all protein types and is one of the amyloid signature proteins. This can cause difficulties in distinguishing between ApoA-IV as the amyloid protein and an amyloid-associated signature protein. The Mayo clinic have set proteomics criteria for ApoA-IV amyloid which include a high probability identification of ApoA-IV in the absence of other candidate amyloid proteins. We have now established that clinical ApoA-IV amyloid commonly contains signal sequence: following tryptic digestion, signal sequence peptides (p.18-43, p.19-43 and p.20-43) were identified in 17/24 clinical biopsies from ApoA-IV amyloidosis patients either attending the NAC clinics or sent to us for histology review. The normal N-terminal peptide, p.21-43, was present in all cases. ApoA-IV signal was also identified in the original cardiac biopsy from a Swedish patient in which ApoA-IV amyloid was first described, and in plasma from 1/3 cardiac AApoA-IV patients by targeted mass spectrometric analysis; it was not detected in controls suggesting the circulating level of signal-ApoA-IV is low. The identity of these signal-containing N-terminal peptides were confirmed by comparison with authentic standards. ApoA-IV signal was present in only 1/266 clinical biopsies where other amyloidogenic proteins were identified as the amyloid type: signature ApoA-IV does not appear to be associated with the presence of signal. The three signal-containing tryptic peptides together with the normal N-terminal peptide (p.21-43) that were all detected in ApoA-IV amyloid were examined for the capacity to form amyloid fibrils in vitro. The p.20-43 peptide and to a lesser extent, the N-terminal peptide were fibrillogenic at physiological pH generating amyloid-like Congo Red positive fibrils. If this effect translates to the mature circulating protein in vivo, then the presence of signal may result in preferential deposition as amyloid, perhaps acting as seed for the main circulating native form of the protein. In conclusion, ApoA-IV amyloid is associated with signal-containing protein, and this is now used in our facility as a further diagnostic test for ApoA-IV amyloid. The p.20-43 peptide shows enhanced fibril formation in vitro compared with the N-terminal peptide and other signal peptides. The presence of additional signal sequence on circulating ApoA-IV may lead to enhanced amyloid deposition in vivo and could potentially influence other ApoA-IV pathologies

09:47 - 09:59 | Wael Kamel, University of Oxford

Global analysis of protein-RNA interactions in SARS-CoV-2 infected cells

As with all RNA viruses, the viral life cycle of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) relies on host RNA binding protein (RBPs). However, the complement of RBPs involved in SARS-CoV-2 infection of human lung cells remained largely unknown. To answer this question in a global scale, we employed a multi-omics approach to uncover the complement of RBPs that are involved in SARS-CoV-2 infection of Calu-3 cells. Applying the previously-established comparative RNA interactome capture (cRIC) (Garcia-Moreno et al., 2019), we discovered that the cellular RNA-binding proteome (RBPome) is profoundly remodelled upon SARS-CoV-2 infection, affecting translation initiation, splicing and antiviral pathways amongst others. Interestingly, we observed that the alterations in the RBPome are not due to changes in RBP abundance, but correlates with a pervasive alteration of the cellular transcriptome and an increase in post-translation modifications mapping to cellular RBPs. To determine the complement of cellular and viral RBPs that interact with SARS-CoV-2 RNA, we applied a novel method named viral RNA interactome capture (vRIC). This method combines 4-thiouridine labelling of viral RNA in presence of an inhibitor of cellular RNA polymerases, UV crosslinking at 365 nm, oligo (dT) capture and quantitative proteomics. vRIC identified 139 cellular and 6 viral proteins that directly interact with SARS-CoV-2 RNA. Pharmacological inhibition of these RBPs impairs SARS-CoV-2 infection, thus revealing novel potential targets for antiviral therapies against COVID-19. Finally, we identified several members of the tRNA ligase complex (tRNA-LC) that interact directly with SARS-CoV-2 RNA. The tRNA-LC colocalizes with viral replication centres upon SARS-CoV-2 infection of Calu-3 cells. Knock down of the tRNA-LC core component DDX1 hampers the synthesis of positive and negative SARS-CoV-2 strands. Importantly, this effect was also observed in other positive stranded RNA viruses, suggesting a master regulatory role in virus replication. Collectively, our results provide a comprehensive view of protein-RNA interactions in SARS-CoV-2 infected cells, revealing important cellular factors for the virus life cycle with great therapeutic potential.

09:59 - 10:11 | Martin Rusilowicz, University of Manchester

AlacatDesigner, software for the selection of appropriate peptides for protein quantitation

Protein quantitation by MS is conducted via analysis of its constituent peptides, with one or more peptide quantitations acting as a proxy for the protein quantitation. However due to the lack of a 1:1 mapping between quantitation and MS signal intensity, absolute quantitation of the peptides is not possible without the injection of a standard whose concentration is known in advance. // In Qconcat approaches, synthetic "proteins" are introduced. These Qconcats possess arrays of Qbricks. In turn, each Qbrick targets a protein of interest and possesses two peptides encoded in a nucleotide construct that can be combined with others in a synthetic biology approach. In this manner, designer-Qconcats can be "dialled up", allowing multiple proteins to be quantified at the same time and requiring only a single injection. The Qbricks can be produced in advance and assembled to order, with this "a la carte" approach giving rise to the ALACAT philosophy. // However, all Qconcat-like studies can be marred by poor selection of the Qbrick peptides. A number of factors result in divergence of the peptide and protein quantifications, ranging from post-transcriptional-modifications in vivo, different degrees of mis-cleavage during workbench digestion, and varying ionisation potentials and "flyabilities" within the mass spectrometer itself. // Our project, "AlacatDesigner", aims to locate suitable peptides for quantification of the user's target protein, and subsequently determine an appropriate Qbrick (or Qconcat) design. // To this end, the candidate peptides are subject to a barrage of tests ranging from simulations of digestion (e.g. McPred), flyability (e.g. Consequence) and whole-workflow suitability (e.g. DeepMsPeptide), as well as being queried against existing databases (e.g. PrideClusters) and literature (e.g. Google Scholar) for evidence of past performance. An internal database and deep-learning predictor are additionally queried, in order that performance from previous analyses can be fed back into the system, once the Qbricks have been tested empirically and concrete information is available. // An empircal dataset comprising proteins from yeast grown at 6 growth rates is used here to showcase AlacatDesigner. Validation shows that Qconcat peptide selections are enriched for peptides that are good quantotypic and proteotypic candidates. Additionally, the guided interface of AlacatDesigner allows human intervention at all points throughout its processing pipeline, allowing a human mediator to reflect upon test scores and focus the peptide/qbrick selections according to individual project requirements. // The software is freely available to use online via the web interface, or it can be downloaded as a Desktop application for use offline. AlacatDesigner can also be imported as a Python package, to run from the command line interface, or to integrate into custom scripts and programs.

10:11 - 10:23 | Xiaobo Tian, University of Groningen

Isotopic Ac-IP tag enables multiplexed proteome quantification in data-independent acquisition mode

Data-independent acquisition (DIA) is an increasingly used approach for quantitative proteomics. However, most current isotope labeling strategies are not suitable for DIA, as they lead to more complex MS2 spectra or severe ratio distortion. As a result, DIA suffers from a lower throughput than data-dependent acquisition (DDA) due to a lower level of multiplexing. Herein, we synthesized an isotopically labeled acetyl-isoleucine-proline (Ac-IP) tag for multiplexed quantification in DIA. Differentially labeled peptides have distinct precursor ions carrying the quantitative information but identical MS2 spectra, since the isotopically labeled Ac-Ile part leaves as a neutral loss upon collision-induced dissociation, while fragmentation of the peptide backbone generates regular fragment ions for identification. The Ac-IP labeled samples can be analyzed using general DIA LC-MS settings and the data obtained can be processed with established approaches. Relative quantification requires deconvolution of the isotope envelope of the respective precursor ions. Suitability of the Ac-IP tag is demonstrated with a triplex-labeled yeast proteome spiked with bovine serum albumin (BSA) that was mixed at 10 : 5 : 1 ratios resulting in measured ratios of 9.7 : 5.3 : 1.1.

10:23 - 11:00 | Discussion

11:00 - 11:30 | Break, poster viewing and networking

Session 4 - Computational Proteomics | Sponsored by Sciex

Session chair: Robert Ewing | Session co-chair: Harry Whitwell

11:30 - 11:31 | Session 4 introduction

11:31 - 11:35 | Sciex talk presented by Nick Morrice

11:35 - 11:50 | Mischa Savitski, EMBL Heidelberg

Understanding post-translational regulation using biophysical proteomics

Mapping gene function and interactions has been revolutionized by the advent of high-throughput reverse genetic approaches. However, due to the large number of perturbations needed to empower the functional association of genes, these approaches are limited to growth or morphological readouts. We have recently developed thermal proteome profiling (TPP) to assess protein state and interactions in vivo. TPP is based on the principle that heat-induced protein aggregation depends on its binding to ligands (metabolites, nucleic acids or other proteins) or post-translational modifications.

Here, we combine thermal proteome profiling (TPP) with a reverse genetics approach to measure abundance and thermal stability of over 1,700 proteins upon 121 genetic perturbations in Escherichia coli. This revealed that essential proteins are rarely regulated in their abundance, but commonly change in their thermal stability—with this being related to changes in their activity. We found that functionally associated proteins have coordinated abundance and thermal stability changes across mutants, which are a result of their co-regulation and physical interactions (with metabolites, co-factors or other proteins). This allows us to suggest the function of uncharacterized proteins in a guilt-by-association manner. Further, we observed that deletion mutants with a larger proportion of their proteome affected were more sensitive to chemical and environmental perturbations. We were also able to pinpoint molecular changes that explain previously determined growth phenotypes and that go beyond the deleted gene.

In conclusion, TPP provides a novel way of systematically phenotyping the cell. This platform can be used to improve our understanding of basic bacterial biology by gaining insights into gene regulation, protein complex architecture, and metabolic activity.

11:50 - 12:05 | Oliver Crook, University of Oxford

Uncertainty and choices in mass spectrometry data science

Uncertainties arise in mass spectrometry because of the technical variability inherent in the instruments we use, the biologically variability in the samples, as well as the choices made by filtering and preprocessing. These choices are often employed to control an error rate or prioritise "hits". This process can lead us to being overconfident in our results but, perhaps worse, overlook interesting biological results. Time permitting, I will introduce several examples from diverse areas of mass spectrometry including spatial proteomics, thermal proteome profiling and hydrogen-deuterium exchange mass spectrometry and show what tools we have to deal with errors and uncertainty in mass spectrometry data science

12:05 - 12:20 | Mike MacCoss, University of Washington

Can we put Humpty Dumpty back together again? What does protein quantification mean in bottom-up proteomics?

Bottom-up proteomics provides peptide measurements and has been invaluable for moving proteomics into large-scale analyses. In bottom-up proteomics, protein parsimony and protein inference derived from these measured peptides are important for determining which protein coding genes are present. However, given the complexity of RNA splicing processes, and how proteins can be modified post-translationally, it is overly simplistic to assume that all peptides that map to a singular protein coding gene will demonstrate the same quantitative response. Accordingly, by assuming all peptides from a protein coding sequence are representative of the same protein we may be missing out on detecting important biological differences. To better account for the complexity of the proteome we need to think of new or better ways of handling peptide data.

12:20 - 13:00 | Discussion

13:00 - 14:00 | Poster session

14:15 - 15:30 | BSPR Annual General Meeting

15:30 | Day 2 END

Day 3: 8th July 2021

09:00 - 09:30 | Break, poster viewing and networking

Session 5 - Single Cell and Clinical Proteomics | Sponsored by Bruker

Session chair: Juan Antonio Vizcaino | Session co-chair: Gina Eagle

09:30 - 09:31 | Session 5 introduction

09:31 - 09:35 | Bruker talk presented by Bram Snijders

09:35 - 09:50 | Erwin Schoof, Technical University of Denmark

Characterizing heterogeneity within hematopoietic cell hierarchies using quantitative Single-Cell Proteomics approaches

In recent years, life science research has experienced a significant shift, moving away from conducting bulk cell interrogation towards single-cell analysis. It is only through single-cell analysis that a complete understanding of cellular heterogeneity, and the interplay between various cell types that are fundamental to specific biological phenotypes, can be achieved. The hematopoietic system is a prime example of such a complex hierarchy, where Hematopoietic stem cells (HSC) are the origin of all cell lineages contained therein. Acute myeloid leukemia (AML), a perturbed state of hematopoiesis, is also hierarchically organized, with leukemia stem cells (LSC) at the apex. Successful eradication of AML will likely depend on specific targeting of these tumour-initiating cells, in turn requiring their molecular characterization.

Here, we have taken both healthy and malignant hematopoietic cells, and subjected them to fluorescence-activated cell sorting combined with novel single-cell proteomics (scMS) strategies to identify the protein landscapes of individual cells. We demonstrate both through pre-enrichment of cell populations and through a non-enriched unbiased approach that our workflow enables the exploration of cellular heterogeneity within developmental hierarchies. By using the latest state-of-the-art LC-MS instrumentation with intelligent data acquisition, this has resulted in unprecedented maps of protein expression in individual cells. Furthermore, we developed a computational workflow (SCeptre) that effectively normalizes the data, clusters the cells, integrates available FACS data (i.e immunophenotype) and permits the extraction of cell-specific proteins. We found a strong enrichment of cell-type specific proteins in various compartments, and the resulting protein signatures clearly distinguish the differentiation stages that exist within their respective hierarchies. The results presented here support the power of implementing global single-cell proteomics studies in proteomics labs across the world.

09:50 - 10:05 | Tami Geiger, Weizmann Institute of Science

Proteomic analysis of cancer internal heterogeneity

Cancer heterogeneity is one of the major challenges that hampers the ability to cure the disease. Tumors differ in their genetic profiles and the cellular interactions in the microenvironment, and each tumor may have multiple different clones with distinct molecular characteristics. Therefore understanding cancer heterogeneity has major translational implications. In breast cancer, different regions within single tumors may vary in the levels of key molecular markers, such as the estrogen receptor, progesterone receptor and HER2, and this heterogeneity affects metastasis and treatment response. Global analyses of internal heterogeneity have mainly concentrated on the genomic layer, revealing evolutionary trajectories, and their association with immune selection. However, focusing only on the genomic level ignores the functional proteomic layer of tumor subpopulations, and their interactions with the tumor microenvironment. Using mass spectrometry-based proteomics, we aim to understand the functional proteomic layer of cancer heterogeneity in breast cancer. We combined analysis of clinical samples with histopathological analysis and functional validations, to unravel novel regulators of cancer progression. To tackle the challenge of the very small sample amounts from tumor regions, we implemented an automated high-throughput pipeline, which combines the Single Pot Solid Phase Sample Preparation (SP3) technique with multiplexed TMT labeling. These methods enable highly sensitive sample preparation from small formalin-fixed tissue samples and from single cells in culture. Analysis of hundreds of breast cancer tumor regions unraveled the association between clinical parameters and the protein networks, and showed their heterogeneity within single tumors. Our research showed the importance of each clinical feature and the significance of the immune system in affecting tumor heterogeneity. Finally, the proteomic layer added the functional attributes that cannot be seen by any clinical measurement, thereby showing the importance of the protein complement in clinical cancer research.

10:05 - 10:20 | Christoph Messner, Francis Crick Institute

Ultra-high-throughput proteomics and its clinical applications

Biomarker discovery studies or precision medicine require the measurement of large sample series. However, conventional MS-based proteomics technologies are limited in throughput and robustness. To overcome these limitations we have developed a platform that consists of 1) a semi-automated and highly standardised sample preparation workflow that can process four 96-well plates in parallel; 2) a high flow-rate chromatographic setup (800 µl/min) for reduced overheads and increased robustness [1]; 3) the DIA-NN software package that uses deep neural networks and was specifically developed for the spectral complexity of short gradient runs [2], and 4) Scanning SWATH, a scanning quadrupole data-independent acquisition (DIA) method [3]. The latter accelerates the mass spectrometric duty cycles and exploits a continuous movement of the precursor isolation window to assign precursor masses to the MS2 fragment traces. This increases the precursor identifications of up to 70% compared to conventional DIA methods on 0.5-5 minute chromatographic gradients. We demonstrate the application of ultra-fast proteomics in COVID-19 patient classification and biomarker discovery. With gradients as fast as 1 minute, we identified a panel of plasma proteins that change depending on the severity of the disease. In conclusion, our results demonstrate a significant acceleration of proteomic experiments, facilitating large-scale clinical and epidemiological studies.

Messner, C. B. et al. Ultra-high-throughput clinical proteomics reveals classifiers of COVID-19 infection. Cell Systems (2020) doi:10.1016/j.cels.2020.05.012.

Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S. & Ralser, M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. Nat. Methods 17, 41–44 (2020).

Messner, C. B. et al. Ultra-fast proteomics with Scanning SWATH. Nat. Biotechnol. (2021) doi:10.1038/s41587-021-00860-4

10:20 - 11:00 | Discussion

11:00 - 11:30 | Break, poster viewing and networking

Closing Session | Sponsored by PreOmics

Session chair: Kathryn Lilley | Session co-chair: James Waddington

11:30 - 11:31 | Closing session introduction

11:31 - 11:35 | PreOmics talk presented by Sarah Lupton

What's your key concern, sample prep or the MS analysis? Time to look for a streamlined process

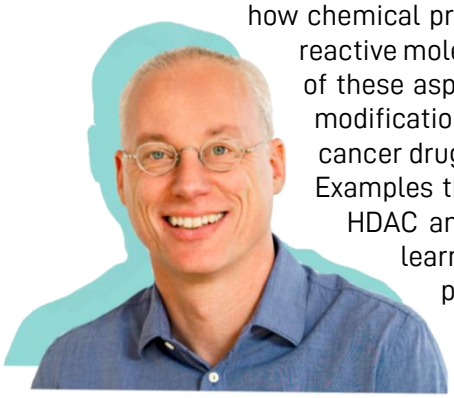
11:35 - 11:40 | ECR prizes

11:40 - 11:45 | Poster winner

11:45 - 12:20 | Plenary talk presented by Bernhard Küster, Technical University of Munich

Watching drug action in cancer cells through the proteomic burning glass

Most drugs act directly on proteins, are proteins themselves or engage cellular pathways controlled by proteins. It, therefore, comes as no surprise that proteomics has become an integral part of modern drug discovery research, particularly when screening compounds in phenotypic assays. Here, success stories from the past include target deconvolution using immobilized drugs as affinity tools, thermal proteome profiling or exploiting other biophysical properties on a protein-drug interactions.



More recently, the field of chemical biology has become to rely a great deal on proteomics to find out if and how chemical probes engage their targets in cells. Prominent examples are Cys- or Lys-reactive molecules or protein degraders such as PROTACs. This talk will touch on some of these aspects but will mostly deal with the impact of drugs on post-translational modifications such as phosphorylation, acetylation or ubiquitinylation because many cancer drugs directly or indirectly impinge on signaling pathways regulated by PTMs. Examples that will be covered are classic chemotherapeutics, kinase, phosphatase, HDAC and protease inhibitors as well as antibodies. I will discuss what can be learned from monitoring PTMs in response to drugs and show that the proteomic burning glass uncovers an entire new micro cosmos of proteins and PTMs with implications for drug discovery and patient stratification and monitoring.

12:20 - 12:45 | Question and answer session

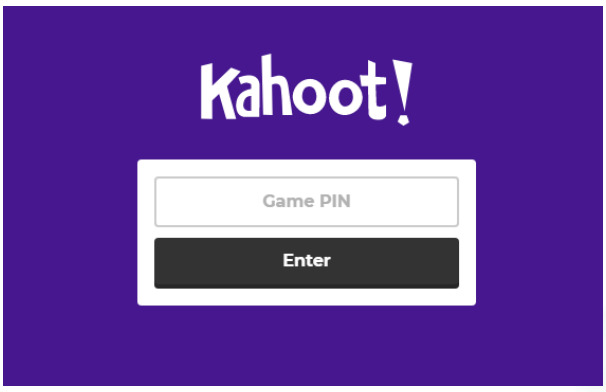
12:45 - 13:00 | Closing address from the BSPR President, Steve Pennington

13:15 – 14:30 | The Kathryn Lilley quiz!

Come and join the annual BSPR Kathryn Lilley quiz and use your proteomics knowledge to win a fabulous prize.

Simply log on to the conference and also either open up another window or use your phone to log on to:

www.kahoot.it



Kathryn will give you the pin code, enter this code, give yourself a name and then we are all set to play in real time. Send your quiz name and email address in the chat function to the host to claim your prize!

NB: the worst score gets the famous BSPR wooden spoon of shame!

Accepted Abstracts

The below abstracts have been accepted for BSPR Interact 2021 and will presented in the form of a poster. Dedicated poster sessions are scheduled for 1 pm on days 1 and 2 (6th and 7th July) of the conference.

P-BIOM-01

Biomarkers

Love is in the hair: arginine methylation of human hair proteins as novel cardiovascular biomarkers

Pedro Beltran-Alvarez, Biomedical Sciences, University of Hull

Cardiovascular disease is the major cause of death worldwide. Extensive cardiovascular biomarkers are available using blood tests but very few, if any, investigations have described non-invasive tests for cardiovascular biomarkers based on readily available hair samples. Here we show, firstly, that human hair proteins are post-translationally modified by arginine methylation (ArgMe). Using western blot and proteomic data mining, we identify tens of ArgMe events in hair proteins, and we propose that keratin-83 is one of the major proteins modified by ArgMe in the human hair. Second, using a preliminary cohort (n = 18) of heterogenous healthy donors, we show that the levels of protein ArgMe in hair correlate with serum concentrations of a well-established cardiovascular biomarker, asymmetric dimethylarginine (ADMA). Compared to blood collection, hair sampling is cheaper, simpler, requires minimal training and carries less health and safety and ethical risks. For these reasons, developing the potential of hair protein ArgMe as clinically useful cardiovascular biomarkers through further research could be useful in future prevention and diagnosis of cardiovascular disease.

P-BIOM-02

Biomarkers

A novel method for identification of insulin and processing intermediates in type 2 diabetes plasma

Rachel Foreman, Sam Galvin, Richard Kay, Claire Meek, Frank Reimann, Fiona Gribble. Wellcome MRC Institute of Metabolic Science, University of Cambridge, UK

Diabetes mellitus is a disease of insufficient endogenous insulin secretion to meet the demands to control plasma glucose. Treatment often involves administration of exogenous insulin analogues, but if not carefully dosed, can lead to hyperinsulinaemic hypoglycaemia. Immunoassays can be used to measure insulin and C peptide concentrations in patients with diabetes mellitus or suspected hyperinsulinaemic hypoglycaemia. However, many of the antibody combinations used in standard immunoassays cross react with exogenous insulin analogues as well as partly processed endogenous insulins, such as des 31,32 proinsulin, as these have overlapping epitopes with fully processed insulin. Targeted LC-MS/MS by contrast can distinguish between all these species and the aim of this study was to detect endogenous insulin, partially processed proinsulin and exogenous insulin analogues in human plasma, including samples collected during an oral glucose (75 g) tolerance test from patients with type 2 diabetes and healthy controls. Peptidomic analysis was also performed on pancreatic islets from donors with type 2 diabetes, to detect any insulin processing products. Method: Peptides were extracted from the plasma samples using a combination of organic precipitation and solid phase extraction, which are standard peptidomic techniques. They were analysed on a HPLC coupled ionkey assisted TQXS (Waters) mass spectrometer and measured using specific MRM transitions. Quantitation of insulin and C-peptide was achieved by the preparation of calibration standards, which were extracted alongside the samples. Islets were isolated from pancreatic tissue, lysed with guanidine hydrochloride and peptides were extracted using organic precipitation. Results: Our method identified endogenous insulin, C-peptide, proinsulin, des 31,32 proinsulin in the plasma samples. It could also distinguish spiked-in insulin analogue drugs, including Humalog (lispro), aspart and glargine, although none were detected in these samples, as no patients were on insulin treatment. The participants with type 2 diabetes showed a significant increase in the ratio of unprocessed and des 31,32 proinsulin to insulin in fasting plasma, when compared to controls. This increased ratio was preserved during the OGTT, when insulin secretion was stimulated by oral glucose. Surprisingly, no significant increase in the ratio of partly processed to fully processed insulins were detectable in islets from donors with type 2 diabetes. Discussion: LC-MS/MS can identify the exact peptide species in clinical samples and is particularly valuable when immunoassays give unexpected or unclear results. Early type 2 diabetes is associated with an elevation of insulin in people with obesity, but our observation of increased circulating levels of partly processed insulins may reflect increased secretory demands on the pancreatic beta cell, causing enhanced secretion of immature vesicles.

P-BIOM-03

Biomarkers

EEF2K activity determines synergy to PI3K and MEK inhibitors in combination in cancer cells

Maruan Hijazi (1), Nosheen Akhtar (1), Pedro Casado (1), Saul Alvarez-Teijeiro (1), Vinothini Rajeeve (1), Pedro R. Cutillas (1-2). (1) Cell Signalling & Proteomics Laboratory, Centre for Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, EC1M 6BQ, UK. (2) The Alan Turing Institute, British Library, 96 Euston Road, London, NW1 2DB, UK.

PI3K-mTOR and MAPK are the most frequently dysregulated pathways in cancer. Consequently, inhibitors against members of this network are actively pursued by pharmaceutical companies to treat different cancer types. However, these pathways converge to regulate downstream functions and often compensate each other, leading to drug resistance and transient responses to therapy. In order to overcome resistance, therapies based on co-treatments with PI3K/AKT and MAPK inhibitors are now being investigated in clinical trials but there is no understanding of the mechanisms of sensitivity to co-treatment. An example of pathway convergence is the control of protein synthesis through the phosphorylation of eEF2K. We found this kinase to be a key protein mediating synergism in response to PI3K and MEK inhibitors (PI3Ki and MEKi) in combination for some cancer types. We utilized different proteomic and cell biological approaches to evaluate whether eEF2K activity modulates the sensitivity of cells to PI3K and MEK co-inhibition. We finally determine that eEF2K-mediated phosphorylation is a potential biomarker to predict synergy of co-treatment in cellular models of acute myeloid leukaemia.

P-BIOM-04

Biomarkers

TMT proteomics analysis of plasma in pigs fed low dietary ω6:ω3 ratio during gestation and lactation

T.X. Nguyen1,2, A. Gelemanović3, S. McGill2, S. Weidt2, M. Piñeiro4, A. Agazzi1, G. Savoini1, P.D. Eckersall2, R. Burchmore2 1Università degli Studi di Milano, Via dell'Università, 6, 26900 Lodi, Italy, 2University of Glasgow, Bearsden Rd , G61 1QH, United Kingdom, 3Mediterranean Institute for Life Sciences (MedILS), Meštrovićevo šetalište 45, 21000 Split, Croatia, 4Acuvet Biotech, C/Bari, 25 dpdo, 50197 Zaragoza, Spain

A balanced dietary ω6:ω3 fatty acid ratio has beneficial effects during parturition and lactation by improving fetal growth and neonatal vigor. However, how plasma protein profiles are changed by supplementing a low dietary ω6:ω3 ratio during gestation (G) and lactation (L) is not well understood in pigs or other species and was investigated with a quantitative proteomic approach. Sixteen sows were assigned to receive 1 of 2 diets: a ω6:ω3 ratio of 13:1 during G and ratio of 10:1 during L (control ratio or CR, n=8) or with an increase in ω3 fatty acids and a ω6:ω3 ratio of 4:1 (low ratio or LR, n=8) from day 28 of G onwards1. Blood was collected at day 108 of G (G108) and the end of L (L-End) and plasma (EDTA) was aspirated. Plasma samples (n=5 per group) were subjected to in-solution tryptic digestion followed by isobaric labeling with tandem mass tag 11plex reagents. Labeled peptides were mixed and analyzed by LC-MS/MS. Proteins were identified by searching against the UniProtKB Sus scrofa FASTA files (104,940 sequences) using SEQUEST search engine in Proteome Discoverer 2.4. Differentially represented proteins were determined by the Mann-Whitney U test with $P<0.05$. Pathway enrichment analysis was performed using the Reactome database. We removed homologs of the 531 proteins identified in the plasma. In LR group at G108, hemopexin and clusterin were overabundant and related to antimicrobial peptides. Ceruloplasmin and haptoglobin were decreased and implicated in hemochromatosis, aceruloplasminemia and solute-carrier transporter disorders. At L-End, complement component C3 was increased and histidine-rich glycoprotein and alpha-1-antichymotrypsin were decreased in LR group. The most enriched terms in the LR group were initial triggering of complement and immunoregulatory interactions between a lymphoid and a non-lymphoid cell, while the most down-regulated pathways were platelet regulation and response. Comparing G108 to L-End, 16 proteins increased and 10 proteins decreased. Amongst these, fetuin-B, histidine-rich glycoprotein, apolipoprotein A-I, A-II and E were increased while haptoglobin, inter-alpha-trypsin inhibitor heavy chain H4 and alpha-1-antichymotrypsin were decreased in L-End. Overabundant proteins were related to the regulation of insulin-like growth factor, hemostasis, assembly and remodeling of chylomicron and plasma lipoprotein, metabolism of fat-soluble vitamins, and regulation of lipid metabolism by PPAR-α. Overall, lowering dietary ω6:ω3 ratio during gestation and lactation significantly enriched several pathways specifically related to immune system development and response. This finding provides evidence to explain the mechanism for enhanced survival rate and weight gain of piglets born to the LR sows, as shown in our previous study1. These data demonstrate the benefit of increased ω3 fatty acid in pig nutrition, which also supports human nutrition.
1Nguyen et al. 2020 Animals 10 (11):2049

P-BIOM-05

Biomarkers

Copine 3 as a potential downstream signalling candidate in ErbB2 over-expressing breast cancer.

Tendayi Samuriwo, University College London, Alexey Zaikin, University College London and Harry Whitwell, Imperial College London & John Timms, University College London

ERBB2/HER2 is a receptor tyrosine kinase considered critical in the development of breast cancers. The aberrant overexpression of ERBB2 occurs in a significant proportion of human breast cancers, where it correlates to therapeutic resistance and poor prognosis for the patient. Previously, it has been shown that certain poorly characterised gene products potentially operate downstream of ERBB2 to bring about ERBB2-dependent cellular transformation. CPNE3 is among the most interesting of these gene products, however its role and significance in ERBB2 mediated signalling remains poorly understood. Expression based profiling has shown that the expression of CPNE3 is higher in ERBB2 overexpressing breast cancer cells. Therefore, in this work we investigated the impact of CPNE3 on the phenotype of ERBB2 overexpressing human mammary luminal epithelial cells (HMLECs). We found that siRNA mediated knockdown of CPNE3 did not affect cell proliferation of HMLEC cells, albeit global protein expression profiling of the cells led to the identification of potential downstream interaction partners of CPNE3. These potential interaction partners offer insight into a potential role for CPNE3 in the early stage events of ERBB2 mediated cellular transformation in HMLECs. We sought to further understand these early stage events by evaluating a potential modulation of cell adhesion and spread on an adherent surface following siRNA mediated knockdown of CPNE3. Our results suggest that CPNE3 plays a role in these early stage events by regulating actin cytoskeletal reorganisation and possibly facilitating ERBB2 mediated epithelial to mesenchymal transition (EMT). In addition, the results establish a baseline for cell behaviour in response to CPNE3 expression and a good foundation for phosphoproteomic studies.

P-BIOM-06

Biomarkers

In-depth proteomic characterisation of different aetiologies of cardiomyopathy

Claire Tonry, The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast; Patrick Collier, Department of Cardiovascular Medicine, Cleveland Clinic, Ohio, USA; Christine Moravec, Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Ohio, USA; Mark Ledwidge, Heart Failure Unit, St Vincent's University Hospital Healthcare Group, Elm Park, Dublin, Ireland; Ken McDonald, Heart Failure Unit, St Vincent's University Hospital Healthcare Group, Elm Park, Dublin, Ireland; Ben C Collins, School of Biological Sciences, Queen's University Belfast; Chris J Watson, The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast

INTRODUCTION: The purpose of this study was to gain greater understanding of the pathogenesis of hypertrophic obstructive cardiomyopathy (HOCM), dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ISCM). These conditions lead to heart failure (HF) and the prognosis for HF differs based on the underlying aetiology. Cardiac tissue represents a challenging sample from the proteomics perspective due to the dominant signal from of a small number of high abundance proteins. Thus, a diaPASEF workflow was applied in order to achieve deep quantitative coverage of cardiac tissue from HOCM (n=12), DCM (n=9), ISCM (n=9) and age/sex matched controls (NF, n=9). RNA-seq analysis was aslso performed on these samples. METHODS: Unbiased, deep proteomic analysis of individual samples was performed using the diaPASEF workflow on a timsTOF Pro mass spectrometer. Analysis of high pH-reversed phase fractionated sample pools was performed in ddaPASEF mode to generate spectral library data. Raw data files were processed through Spectronaut 14 software for spectral library building, protein identification and quantification. Differentially expressed proteins were identified based on an observed fold change of ≥ 1.5 or ≤-1.5 and q-value ≤ 0.005. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) software. RESULTS: Label-free MS analysis led to over 4,000 protein identifications, with 3,484 proteins commonly identified across all patient samples. Over 1,000 significantly differentially expressed protein candidates were identified for comparisons between NF and DCM, HOCM or ISCM. DCM-specific protein changes were strongly associated with glutamine biosynthesis, HOCM-specific protein changes were strongly associated with LXR/RXR Activation, while ISCM-specific protein changes were most associated with tryptophan degradation pathways. DCM vs NF, ISCM vs NF and HOCM v NF had shared differentially expressed proteins that were also significantly altered at gene level (n=18). Canonical pathway analysis revealed that Choline Degradation and Lysine Degradation pathways were most strongly associated with these candidates. Expression changes for some of the top over- and under-expressed HF candidates were validated in an independent replicate dataset (PXD008934). In this dataset, combined measurement of identified HF candidates differentiated between NF and HF samples with high accuracy (0.965 [CI 0.912 – 1.000]). CONCLUSIONS: This represents one of the largest and deepest proteomic datasets for myocardial tissue reported to date. The dataset, which compliments existing transcriptomic data for these samples, has highlighted a number of significant proteins associated with different underlying aetiologies of HF. Prognosis for HF differs depending on the aetiology from which it arises. Hence, the dataset here will help in further understanding the pathogenesis of the disease, leading towards more personalised treatment.

P-BIOM-07

Biomarkers

DIA Performance in Discovery and Quantitative Analysis Using Orbitrap Exploris Mass Spectrometers

1Yang Liu, 1 Khatereh Motamedchaboki, 2Joshua Nicklay, 3 Sega Ndiaye, 1 Aman Makaju, 4 Amarjeet Flora, 4 Ryan Bomgarden, 5Jenny Ho, 1Daniel Lopez-Ferrer 1Thermo Fisher Scientific, San Jose, CA, USA; 2Thermo Fisher Scientific, NJ, USA; 3Thermo Fisher Scientific, Courtaboeuf, France; 4Thermo Fisher Scientific, Rockford, IL, USA, 5Thermo Fisher Scientific, Hemel Hempstead, UK

Introduction Data-independent acquisition (DIA) mass spectrometry (MS) provides deep proteome analysis without the bias from peak intensity. In addition to a more comprehensive coverage of identification, DIA also shows accurate label-free quantification. In this work, we evaluated the DIA performance on both Thermo ScientificTM Orbitrap ExplorisTM 480 and 240 mass spectrometers for discovery and quantitative proteomics analysis. Methods Mixed Hela:E.coli digested peptides (1:2, 1:4, 1:8 ratio) were loaded on 25cm Aurora column (25cm x 75um ID, 1.6um C18) with an Thermo ScientificTM Easy-nLCTM 1200 system, separated by a 90min LC gradient before being injected to Thermo ScientificTM Orbitrap ExplorisTM 240 or 480 MS. Mixed Hela:Yeast peptides was analyzed using the same setup at different gradient length (15min, 30min, 90min). Chromatogram spectral library was built by matching the gas-phase fractionations (GPF) to the predicted spectral library1. Acquired DIA data were analyzed by SpectronautTM 14.0. Results Spectral libraries of 1000ng Hela:E.coli mixture were built on two Orbitrap ExplorisTM platforms and the dynamic ranges were demonstrated to be up to 7 orders of magnitude. By matching with each library, 9056 and 9472 proteins were identified on Orbitrap ExplorisTM 240 and 480, respectively. The Hela:E.coli spike-in proteome acquired on both systems were quantified by directDIATM. ExplorisTM 480 exhibited better quantification accuracy than ExplorisTM 240. Furthermore, we also proved that matching with the library built at a longer gradient and a higher sample load would improve the identification coverage. Therefore, once built at ultimate setup, the spectral library can be used for universal DIA discovery of the same sample. Conclusions Both Thermo ScientificTM Orbitrap ExplorisTM 240 and 480 mass spectrometers had excellent performance in DIA analysis in discovery and quantitation. References 1. Searle, B.C., Swearingen, K.E., Barnes, C.A. et al. Nat Commun 11, 1548 (2020).

P-CLIN-01

Clinical Proteomics

Phosphoproteomics defines two biologically distinct groups of KMT2A rearranged leukaemia

Pedro Casado 1, Ana Rio-Machin 2, Juho J. Miettinen 3, Findlay Bewicke-Copley 2, Kevin Rouault-Pierre 4, Alun Parsons 3, Szilvia Krizsan 5, Vinothini Rajeeve 1, Sarah Mueller 6, Csaba Bödör 5, Jean-Baptiste Cazier 7, David Tausig 8, Dominique Bonnet 9, John Gribben 4, Caroline Heckman 3, Jude Fitzgibbon 2, Pedro R. Cutillas 1,10. 1 Cell Signalling and Proteomics Group, Centre for Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, London, UK. 2 Personalized Cancer Medicine, Centre for Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, London, UK. 3 Institute for Molecular Medicine Finland – FIMM, HiLIFE – Helsinki Institute of Life Science, iCAN Digital Precision Cancer Medicine Flagship, University of Helsinki, Helsinki, Finland. 4 Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK. 5 HCEMM-SU Molecular Oncohematology Research Group, 1st Department of Pathology and Experimental Cancer Research, Semmelweis University Budapest, Budapest, Hungary. 6 Barts Cancer Institute Tissue Bank, Barts Cancer Institute, Queen Mary University of London, London, UK. 7 Centre for Computational Biology, University of Birmingham, Edgbaston, Birmingham, UK. 8 Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, UK. 9 Haematopoietic Stem Cell Lab, The Francis Crick Institute, London, UK. 10 The Alan Turing Institute, The British Library, 2QR, 96 Euston Rd, London, UK.

Precision medicine aims to increase clinical outcomes by tailoring treatments to the molecular profile of the patient's disease. The key task of patient stratification based on predicted responses to treatment is currently performed using genomic profiling of healthy and pathologic tissues. However, techniques based on proteomics, phosphoproteomics and profiling of other modifications are starting to be included in studies that aim to improve the stratification of patients into more phenotypically homogeneous groups. Acute myeloid leukaemia (AML) is a blood cancer with no cure for most patients. Prognosis is particularly poor for patients with adverse karyotypes and no specific therapies are available for them. Here, we performed a molecular profiling of 74 poor risk AML patients that included sequencing 30 genes and quantifying 33,567 transcripts, 6,637 proteins, 26,710 phosphopeptides, 4,760 acetylated or methylated peptides and 84 kinase activities. We identified a 18 phosphopeptide signature that stratified KMT2A rearranged cases into two groups (MLLGA (n=11) and MLLGB (n=5)). MLLGA, when compared with MLLGB or other poor risk samples (No_MLL), showed higher expression of HOXA genes and elevated phosphorylation of DOT1L complex components and of proteins involved in splicing, replication and DNA damage. Using an ex vivo drug screening that included 628 compounds, we found that MLLGA was more sensitive than MLLGB and No_MLL to 15 agents including genotoxic drugs, mitotic targeting drugs and inhibitors of IMPDH, a key enzyme for GTP synthesis . Ex-vivo proliferation rate correlated with responses to 13 of the 15 drugs. However, other factors could contribute to the differential response between MLLGA and MLLGB. In KMT2A rearranged cells, sensitivity to an IMPDH inhibitor (AVN-944) correlated with higher expression of IMPDH2 and nucleolar proteins, with MLLGA presenting higher levels of IMPDH2 and key nucleolar proteins than MLLGB. To investigate causal effects, we studied the phosphoproteomes of cells treated with AVN-944. In line with the correlation data, AVN-944 increased the phosphorylation of multiple nucleolar proteins involved in rRNA processing like NCL, NOL8 or RRP8 in MLLGA but not in MLLGB. These data are consistent with IMPDH having a role in rRNA synthesis, and indicate that the AVN-944 ability to interfere with nucleolar biology is, at least partially, responsible for its higher anti-cancer activity in MLLGA cases. In summary, we identified a phosphoproteomics signature that stratified KMT2A rearranged patients into two subgroups with different sensitivity to IMPDH inhibitors, genotoxic compounds and cell cycle inhibitors. We found that the nucleolar function contributed to the differential response to IMPDH inhibitors in these patient samples. Our results provide a rationale for the use of IMPDH inhibitors to treat a subgroup of AML patients characterised by high phosphorylation of the MLLGA signature identified in this study.

P-CLIN-02

Clinical Proteomics

Development of a Workflow for Deep Proteome Profiling in Human Plasma by Micro-LC-MS/MS

Roxana Eggleston-Rangel (Phenomenex), Jason Anspach (Phenomenex), Jennifer E. Van Eyk (Cedars Sinai), Simion Kreimer (Cedars Sinai), Angela Mc Ardle (Cedars Sinai), Aleksandra Binek (Cedars Sinai), Alejandro Rivas (Cedars Sinai), Danica Manalo (Cedars Sinai), Connor Phebus (Cedars Sinai), Blandine Chazarin (Cedars Sinai), Annie Moradian (Cedars Sinai), Cory Bystrom (Cedars Sinai)

Human plasma holds a large repertoire of human proteins; besides plasma proteins, it also contains leaked tissue proteins and various immunoglobins. Plasma proteome contains the key to understanding many aspects of human biology and thus methodologies for human plasma identification are of vital importance. Here, we present a workflow for deep human plasma, depleted plasma and whole blood proteome analysis using micro flow Liquid chromatography coupled to mass spectrometry (micro-LC-MS/MS). In this workflow, five sample replicates were prepared to assess reproducibility on 3 separate days. Our results show the quantification of proteins with median intra-day coefficients of variations (CVs) ranging from 10-13 %, 4-8 % and 10-13 %; while inter-day CVs were 24%, 15 % and 18% in native plasma, depleted plasma, and blood respectively. The total number of proteins quantified were 504, 473, 578 in plasma, depleted plasma and blood respectively. Total number of peptides quantified were 2378, 2029, 1622 in plasma, depleted plasma and blood respectively. Overall, this workflow is recommended for anyone interested on profiling the human blood proteome.

P-CLIN-03

Clinical Proteomics

Proteomics of pregnancy: a story of mice and (Wo)men

Amy L. George 1, Claire Meek 1, Amanda N. Sferruzzi-Perri 2, Fiona M. Gribble 1, Frank Reimann 1, Richard G. Kay 1. 1. Wellcome MRC Institute of Metabolic Science, University of Cambridge, UK 2. Department of Physiology, Development and Neuroscience, University of Cambridge, UK

Plasma proteomics is inherently challenging due to extremely high-abundance proteins dominating the less abundant proteins of interest. Many human studies have utilised immunological-based methods to systematically remove up to 20 high abundant plasma proteins. Whilst effective, the approach is expensive and suffers from low throughput. The equivalent technology for mouse plasma is far more limited, severely impacting the dynamic range of analyses that can be performed. We present a simple and rapid method for low molecular weight (MW) protein and peptide enrichment. By disrupting protein-protein/peptide interactions, low MW components that may be bound to high abundance and high MW species are released. Many large and high abundance plasma proteins are then depleted with organic-solvent precipitation and solid-phase extraction. Our approach has been applied to both mouse and human plasma to characterise the circulating proteome during pregnancy. Analysis of mouse plasma (10-25 µL) at 16 days of gestation (term ~20 days) identified various placental-specific and pregnancy-associated proteins. These include insulin-like growth factor binding protein-4 and numerous placental lactogens/prolactins (PL/PRLs), which are important for modulating fetal growth and maternal metabolism. Prolactin-8A9 and growth hormone receptor were identified at significantly elevated levels in pregnancy plasma compared with non-pregnant plasma. Based on a quantitative profile of detected PL/PRLs, pregnancy samples could be distinguished with 100% accuracy. Human plasma (25 µL) was analysed at 28 weeks of gestation and identified proteins exclusively expressed by the placenta, such as human choriogonadotropin (an essential hormone for the maintenance of pregnancy) and pregnancy-specific glycoproteins (maternal immune-modulation). Other placental proteins detected were kisspeptin (stimulator of gonadotropin secretion and trophoblast invasion) and placental lactogen (regulates fetal nutrient supply), which are secreted by the syncytiotrophoblast. PRL, involved in mammary gland development and alpha-lactalbumin, a milk protein, were also detectable in the circulation. The ability to measure proteins with major roles in maternal physiology and fetal/offspring development in the maternal circulating proteome could enable researchers to better understand the aetiology of common pregnancy complications and devise diagnostic and therapeutic strategies. Our solvent-based extraction approach offers an effective alternative to immunodepletion at a fraction of the cost, is compatible in a 96-well format thereby increasing throughput, and is effective in both human and mouse plasma.

P-CLIN-04

Clinical Proteomics

Peptidomics of liver disease: A potential role for apolipoprotein oxidation and degradation?

Richard G Kay1, Amy L George1, Rachel Foreman1, Gabriele Moccario2, Mattia Frontini3, Toni Vidal-Puig1, Frank Reimann1, Fiona M Gribble1, Vian Azzu1,4, Michele Vacca1, Michael Allison4 1. Wellcome Trust MRC Institute of Metabolic Science, University of Cambridge. 2. Department of Biochemistry, University of Cambridge. 3. Department of Haemotology, University of Cambridge 4. Liver unit, Cambridge University Hospitals NHS Foundation Trust

Introduction: Non-alcoholic fatty Liver disease (NAFLD) is becoming more commonplace in populations with excessive consumption of western style diets. Excess calorie intake combined with an inactive lifestyle leads to increased prevalence of metabolic syndrome, and ultimately leads to an NAFLD pathology. The diagnosis of NAFLD is currently performed using plasma liver enzyme tests, whilst diagnosis of the more serious non-alcoholic steatohepatitis (NASH) requires far more invasive and challenging tests. The identification of potential circulatory biomarkers for liver disease progression (NAFLD to NASH) is therefore extremely important, which a proteomics / peptidomics type analyses could perhaps provide. Methods: Low molecular weight serum proteomics analysis was performed using organic solvent precipitation treatment with 6M GuHCl, solid phase extraction and tryptic digestion. Peptidomics was performed using a well validated organic solvent precipitation approach, also including solid phase extraction. Both proteomic and peptidomic samples were analysed on a ThermoFisher Orbitrap Q-Exactive plus, and data searched against the human Uniprot database using PEAKS 8.5. Precursor and product ion data of potential biomarkers were identified and optimised on a Waters TQ-XS triple quadrupole system. A limited validation cohort was analysed comprising of 20 controls and 95 disease samples using a 10 minute, high throughput LC-MS/MS methodology. Results: Database searching of the proteomics dataset failed to identify proteins that discriminated between NAFLD and NASH stratifications, however did discriminate between control and overall disease state. It was noted that the proteomics search results identified a number of oxidised apolipoprotein derived peptides, therefore the proteomics dataset was reanalysed using a peptide centric approach, which showed these oxidised peptides were identified as significant. The peptidomics analysis confirmed the presence of high levels of circulating oxidised peptide fragments from apolipoproteins C3 and E. The analysis of a large cohort showed these peptides generated good discriminatory power, with ROC AUC values of 0.989 and 0.987 for APOC3 and APOE fragments respectively. Whilst these protein fragments showed good discriminatory power for control and disease state, they were not able to stratify the liver disease patients, suggesting their presence could be due to factors leading to liver disease development rather than progression.

P-CLIN-05

Clinical Proteomics

Clinical Amyloid Typing by Proteomics: Performance Evaluation and Data Sharing between Two Centres.

1 Paola Nocerino, 1 Dr Diana Canetti, 1,2 Prof. Vittorio Bellotti, 1 Dr Graham W. Taylor, 3 Prof. Pierluigi Mauri. 1)Wolfson Drug Discovery Unit and National Amyloidosis Centre, University College London, London, UK; 2) Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, Pavia, Italy; 3) Proteomics and Metabolomics Laboratory, CNR-ITB, Segrate, Milan, Italy

The clinical Mass spectrometry (MS)-based proteomics is emerging as one of the best approaches for both basic research and clinical diagnosis of amyloid diseases. The use of Laser Capture Microdissection (LCM) allows the precise selection of amyloid material for MS analysis, and LCM-MS is now considered crucial for amyloid typing. However, amyloid proteomics is currently carried out in a relatively few centers worldwide and there is a need to agree on common standards and share best practice. The European Proteomics Amyloid Network (EPAN) was established in December 2017 to address these issues. In this context, we report the first inter-laboratory study carried out at National Amyloidosis Centre (NAC) based in London and Istituto di Tecnologie Biomediche-Consiglio Nazionale delle Ricerche (ITB-CNR) in Milan. Both centres are members of the EPAN and routinely apply the proteomic approach using different software and alghoritms for protein identification. Mass spectrometry raw data were exchanged between the two centers and analysed through the use of different search engines including Mascot, Scaffold, Proteome Discoverer, Sequest, different analysts and applying the algorithms currently in use at each centre. The comparison allowed a very good concordance (92 %, 37/40 total samples) between the proteomics results obtained in London and Milan. These findings confirm the robustness and sensitivity of the MS-based approach and the good accuracy of the different bioinformatics setups used in the respective centres. The collaboration between amyloid proteomic platforms is a valuable opportunity to better understand the basis of amyloidosis improving clinical diagnosis and management of the disease. NAC and ITB-CNR data sharing approach, as part of EPAN's initiative, offers a straightforward and inexpensive model for future accreditations studies.

P-CLIN-06

Clinical Proteomics

Hyperplexed Proteomics to Identify Drivers of Metastatic Pancreatic Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with limited treatment options and a dismally low five-year survival rate. Due to the high potency of early-disseminating metastatic tumors, which are often already detected at diagnosis, the majority of PDAC patients succumb to metastasis-related complications even after full resection of the primary tumor site. Thus, the ability to effectively treat PDAC largely depends on our ability to block or even reverse the process of metastasis. Despite this, a deep understanding of the molecular basis of PDAC metastasis has not yet been achieved. Recent work has identified the dysregulation of protein synthesis and degradation as critical drivers of PDAC tumorigenesis. We seek to determine if and to what extent these types of post-transcriptional gene expression regulation programs are also relevant for PDAC metastasis. To achieve this goal, we are developing a proteome-wide protein life cycle profile of metastatic-tumor-derived organoids from the KPC mouse model of PDAC using hyperplexed proteomics. With this information, we seek to identify novel proteins or pathways that may represent exploitable vulnerabilities for downstream drug discovery efforts. Specifically, we are performing a first-of-its-kind comparative analysis of matched primary- and metastatic tumor organoids derived from the LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre (KPC) mouse using dynamic SILAC-TMT (dSILAC-TMT). dSILAC-TMT allows for robust normalization and comparative analyses between samples while reducing noise and increasing data completeness, allowing us to identify potential PDAC drivers with high confidence. We are additionally performing deep sample fractionation to increase proteome coverage, paired with careful study design, including "booster" samples - which are fully heavy-SILAC-labeled - to facilitate early time point measurements. These improvements ensure that we can profile proteins with half-lives spanning the full physiological time range of minutes to days. We are pairing dSILAC-TMT measurements with both dSILAC-DIA and global expression profiles acquired with TMT (DDA) and DIA-based methods. These layered measurements will allow us to understand the biological relationship between protein abundance and protein turnover, as well as provide more insight into the relative strengths of DIA vs. DDA-based measurements in the context of proteome turnover. Through this dataset, we aim to not only create a resource for understanding the progression of PDAC, but to also further clarify the relationship between parameters such as protein abundance, protein turnover, protein usage, and essentiality in the disease context. We hypothesize that highly differentially turned over – "unstable" – proteins are most likely to represent potential therapeutic vulnerabilities in PDAC, and that these findings will be generalizable to other disease models, particularly those involving proteostatic disruptions.

P-CLIN-07

Clinical Proteomics

CAFs modify the phenotype of endothelial cells through the EV-mediated transfer of functional proteins

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Cancer associated fibroblasts (CAFs) are highly secretory cells that play an important role in cancer progression and metastasis. CAFs secrete cytokines, growth factors, metabolites, extracellular matrix (ECM) components and remodelling enzymes that increase cancer cell malignancy, modulate the immune response and promote angiogenesis. In addition to these mechanisms of intercellular communication, CAFs are able to support cancer cell proliferation and invasion through the transfer of their own proteins. The transfer of proteins from CAFs to cancer cells can occur by the secretion of extracellular vesicles (EVs) or the formation of cytoplasmic bridges. In the tumour stroma, CAFs can exist in close proximity of the vasculature, but how CAF-derived proteins affect the functions of the endothelial cells (ECs) and whether this contributes to cancer pathology has not been described yet. Here, we have investigated the crosstalk between CAFs and ECs mediated by the transfer of CAF proteins using co-culture methods and mass spectrometry (MS)-based proteomic approaches. We found that CAFs transfer proteins to ECs and we have identified them using trans-SILAC proteomics. The analysis of the transferred proteins revealed that they belong to specific intracellular compartments or are associated with the plasma membrane. Remarkably, Gene Ontology category enrichment analysis showed that the transferred plasma membrane receptors and membrane-bound ligands are mainly involved in cell migration and cell-cell adhesion. This finding suggests that CAF-derived proteins may have important implications on the functions of the tumour vasculature. In fact, the migration of ECs is fundamental for the formation of new blood vessels, which fuel tumour growth. Furthermore, the binding between ECs and cancer cells is the first step of the metastatic cascade, and the recruitment of intratumoral immune cells depends on receptors expressed on the EC surface. Notably, we found that CAF-derived Thy-1 membrane glycoprotein mostly contributed to change EC proteome and promoted the adhesion of ECs to THP-1 monocytes. Thus, CAF-derived Thy-1 endows ECs with additional cell-cell adhesive properties towards immune cells. CAF-derived EVs mediated the transfer of proteins to ECs. We observed that CAFs release different types of EVs that could either float in the extracellular fluid or bind to the ECM, and that the EVs responsible for protein transfer are primarily those located in the ECM. Finally, we found that enhanced protein transfer ability is a hallmark of CAFs. In fact, CAFs have a protein transfer ability higher than normal fibroblasts. Moreover, we observed that the myofibroblastic CAFs are the major donor of proteins to ECs. Our work unravels a novel way through which CAFs may influence cancer by modulating the tumour vasculature, and further studies are needed to understand the contribution of this mechanism to tumour growth and metastases.

P-CLIN-08

Clinical Proteomics

Employing the Zika Virus to kill paediatric nervous system tumour cells

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Malignant paediatric nervous system tumours, such as Medulloblastoma, Neuroblastoma and ATRT commonly harbour tumour cells with stem-like features which are highly tumorigenic and resistant to conventional cancer therapies. These tumours can exhibit high lethality and may result in severe sequelae, including cognitive and motor deficits that significantly affect patients' quality of life. Oncolytic virotherapy is a novel therapy class that exploits viruses that preferentially infect and destroy tumour cells. These viruses present a unique advantage in targeting highly heterogeneous cancers, such as nervous system tumours, as they possess a secondary mechanism of action through which they induce a tumour-specific immune response. Clinical studies employing oncolytic virotherapy have in general reported low toxicity and minimal adverse effects, deeming oncolytic virotherapy as a potentially attractive and safer intervention against paediatric tumours. The Zika virus (ZIKV) is capable of infecting and destroying neural stem-like cancer cells from human embryonal Central Nervous System (CNS) tumours in vitro and in vivo. Infection of CNS tumour cells with ZIKV effectively inhibits tumour metastasis in mice and, in some cases, induces complete tumour remission. Neuroblastoma arises from immature nerve cells and multiple Neuroblastoma cell lines are susceptible to ZIKV infection and oncolysis. These initial findings have demonstrated the potential for a ZIKV-based virotherapy against paediatric nervous system tumours and warrants examination into the molecular mechanisms through which ZIKV executes its oncolytic ability. My research goal is to elucidate the mechanisms which are of paramount importance for ZIKV-induced oncolysis of brain tumour and Neuroblastoma cells. Utilising global expression omics profiling of ZIKV infection and mapping of viral protein-host protein interactions will identify these mechanisms both at the cellular pathway and molecular levels. These collectively will inform our understanding of how we can employ a future ZIKV-based virotherapy against paediatric nervous system tumours.

P-CLIN-09

Clinical Proteomics

Proteomic profiling of Human Bronchoalveolar lavage fluid following intra-lung challenge with live BCG and PPD in individuals with a gradient of TB risk

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The alternative route of TB vaccine delivery in man is urgently needed if human tuberculosis's effective management and treatment are realized. The BCG vaccine is currently delivered intradermally, and the protective property of BCG wanes after ~20 years. Using a controlled human infection model stands a chance of broadening our understanding of TB disease and its pathogenesis. In this study, the human bronchoalveolar lavage fluid's proteomic profiling was done following the human lung challenge with the live BCG and Purified Protein Derivative (PPD) to understand the immune response at the infection- the human lungs. The raw proteomic data files were searched against the human Uniprot sequence database using the Andromeda search engine, integrated within MaxQuant. We identified 2248 protein groups. In Recurrent TB (RTB), the innate immune response to PPD was more robust, while the response to live BCG was very weak. The Sterilizing immunizers (SIM) cohort's live BCG and PPD challenge did not have a noticeable difference because the baseline samples displayed a robust innate immune response. The Latent TB infection (LTBI) mounted a potent innate immune response to live BCG, and the PPD was less immunogenic in the LTBI cohort. The Previous TB patients displayed a strong Innate immune response to both PPD and live BCG challenge. The strong innate immune response indicates that the innate immune cells also express the memory response to the invading pathogens in the human lungs. In conclusion, our data's four challenge groups showed a unique reaction to live BCG and PPD challenges, which was characteristic of the patient phenotype.

P-COM-01

Computational Proteomics

Uncertainty aware quantification and differential analysis with the seaMass R package

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seaMass is an R package (https://github.com/biospi/seaMass) for protein-level quantification, normalisation and differential expression analysis of proteomics mass spectrometry data after peptide identification and feature-level quantification. Using the concept of a blocked experimental design, seaMass can analyse all common discovery proteomics paradigms including label-free (e.g. Waters Progenesis input), SILAC (e.g. MaxQuant input), isotope labelling (e.g. SCIEX ProteinPilot iTraq and Thermo ProteomeDiscoverer TMT input) and data-independent acquisition (e.g. OpenSWATH input), and is able to scale to studies with hundreds of assays or more. seaMass consists of three Bayesian mixed-effects models: seaMass-sigma, which performs raw protein-level quantification; seaMass-theta, which performs protein-level normalisation across assays (label-free/DIA runs or iTraq/TMT/SILAC channels); and seaMass-delta, which performs differential expression analysis and false discovery rate estimation. A key novelty is that uncertainty is propagated downstream, as all quantifications and differential expression output by seaMass-sigma, theta and delta are accompanied by standard errors. Moreover, within each procedure multiple forms of uncertainty are handled. For example, seaMass-sigma assesses the quantification reliability of each feature and peptide across assays so that only those in consensus influence the resulting protein quantification strongly. An empirical Bayes procedure is incorporated that calibrates the uncertainty of 'one-hit' wonders using the distribution of feature and peptide variances derived from proteins with many peptides. Similarly, unexplained variation in each individual assay is captured, providing both a metric for quality control and automatic down-weighting of suspect assays. seaMass results are output as csv files for further analysis and are also presented in an interactive html report with detailed per-protein plots as well as volcano plots and novel uncertainty-aware principal components analysis. Available spike-in studies for validating informatics pipelines do not model protein digestion variation between samples, and the fold-changes often do not reflect those we aim to detect in clinical samples. To overcome these issues we developed a 4v4v4 experiment with E.coli spiked into rat lysate at ratios of 1:1, 1:1.2 & 1:1.6, with each sample then separately digested and labelled, which was subsequently run on both Thermo Orbitrap label-free and Sciex ToF Itraq platforms and then analysed with MaxQuant and ProteinPilot respectively. Performance on these and existing SWATH validation datasets (Navarro et al. 2016) demonstrate substantively improved recall compared to MSstats and MSqRob. For example, seaMass correctly flags 30% more differentially expressed proteins at the same false discovery proportion compared to MSstats for the Navarro dataset of pure technical replicates, and 50%+ for our challenging spike-ins.

P-COM-02

Computational Proteomics

lcmsWorld: High-Performance 3D Visualization Software for Mass Spectrometry

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lcmsWorld is new, high-performance 3D visualisation software for mass-spectrometry data. It takes advantage of standard PC graphics hardware to quickly and smoothly view and navigate LC-MS data as a three-dimensional terrain. A pre-processing step allows the software to subsequently access any area of the data instantly, at multiple levels of detail. The data can then be freely navigated, while the software automatically selects, loads, and displays the most appropriate level of detail, changing as the user uses their keyboard, mouse or touch-screen to move around, rotate, and zoom the data view. lcmsWorld can directly load industry-standard .mzml file, or common Thermo .raw files. Annotations, such as peptide identifications, can then be added by loading simple .csv files, mzIdentML or mzTab files (provided that retention time data is included). These annotations can then be filtered, searched, and viewed, appearing on top of the corresponding LC-MS data. Multiple LC-MS files can be viewed side-by-side, with synchronised camera controls, so that the same areas can be visually compared - for example, to check the quality of sample replication across files or to zoom-in and look at individual differences between samples. Even large (e.g., 6Gb+) data files can be easily loaded and viewed on standard laptops and workstations. Due to the storing of the pre-processed data file, when the software is subsequently re-launched, the previous data file and view can usually be displayed in around a second. lcmsWorld does not require Administrator rights and does not need to be installed. lcmsWorld is free, open-source software and available for Windows, Mac, and Linux. Windows releases, source code and example data files are available on GitHub at https://github.com/PGB-LIV/lcmsWorld

P-COM-03

Computational Proteomics

Developing the concept of quantotypicness for protein quantitation in proteomics

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Evaluating the quantitative, and even just the qualitative, aspects of biological networks can improve our understanding of the stoichiometry and complex interplay of system components, as well as aiding in the identification of disease biomarkers. Hence to measure these molecules internal peptide standards are often used in MS-based proteomics. Selecting the optimal peptides to yield the most accurate quantitation remains a challenge. Here we have analysed a large-scale proteomics dataset, of 362 OPENSWATH MS runs, on a pre-digested K562 cellular lysate, to identify peptide features that can be used as selection criteria. This dataset has minimal biological variation and represents a robust measure of the scope of technical variation in the protein sample, which makes this data well suited to isolate peptide properties that cause variance in quantitation across runs. To ensure good data quality we explored the use of quality control metric-based filtering using SwaMe on the SWATH Mzml files. In addition, we compared two protein inference pipelines, namely using an established methodology, MsStats, and a Bayesian modelling and imputation approach, seaMass, on the quantitation estimations at both a protein and peptide level. While many of the peptides in an MS library are already inherently proteotypic, being both detectable and unique to their protein antecedent, they may not be 'quantotypic'. We define this concept to mean that the peptide's quantitative signal robustly tracks that of its parent protein. We've used our MsStats and seaMass based pipelines to identify and characterise these quantotypic peptides, whose levels correlate most tightly with their parent protein therefore making them good surrogates. This concept differs markedly from proteotypicness. We have generated datasets of good and poor quantotypic peptides in order to train a Machine Learning Algorithm, which shows good predictive performance. We believe that the use of quantotypic peptides and this concept can facilitate more accurate quantitation in MS experiments that utilize synthesising synthetic peptides such as Alacats, and, potentially to, replace top-N style metrics with best/most quantotpyic-N metrics.

P-PTM-01

Post-translational Modifications

Characterization of novel synaptic substrates of the deacetylase enzyme Sirtuin-2

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Recent studies have identified a new role for non-histone lysine acetylation in the regulation of synaptic protein stability. The majority of the synaptic proteins are acetylated, which is a reversible post-translation modification of lysine residues that allows for reciprocal protein regulation. Acetylation is particularly enriched in brain tissue with a significant association with neurophysiological functions. The function of a limited number of synaptic proteins has been investigated. The AMPA receptor and Arc (Arg3.1) protein are deacetylated by Sirtuin 2 (SIRT2) on sites that can also be ubiquitinated; this balance between acetylation and ubiquitination regulates their function in synaptic plasticity, learning and memory. SIRT2 is expressed highly in brain tissue and it has been associated with many neurodegenerative diseases. However, the substrates and regulatory role of SIRT2 is poorly understood at the synapse. Here we have sought to identify putative SIRT2 substrates using quantitative analysis of the acetylome immune-enriched from SIRT2 knockout and wild type brain tissue. Using this strategy, we have identified 2,267 unique acetylation sites on 1,553 acetylated proteins. Among them, 246 sites have significantly higher acetylation levels in SIRT2 knockout brain tissues and represent putative SIRT2 substrates. 40% of these sites are also ubiquitinated indicating a substantial degree of crosstalk between these PTMs and highlights sites that may be important regulatory switches at the synapse. Our data identified novel SIRT2 substrates that will advance the understanding of the role of SIRT2 in neurodegenerative diseases and help to link those substrates with specific downstream processes.

P-PTM-02

Post-translational Modifications

Proteomic Profiling analysis of USP7 network revealed its role in protein ubiquitination pathway

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It has been identified that the deubiquitinating enzyme ubiquitin-specific peptidase 7 (USP7) is an oncogenic protein because of its role in upregulating many cellular pathways and interacting with multiple tumor suppressors. Our study aims to recognize the USP7 protein-protein interaction network and protein complexes. We used LS88 cell line, a stable colorectal cancer cell carrying a Tet repressor (TR)-construct and a pTER-USP7 shRNA construct to inducibly knock-down USP7. Treated (1ug/mL doxycycline) and untreated cells were analysed 72 hours after treatment using Thermo Orbitrap Fusion mass-spectrometry of whole cell proteomes. 6697 high confidence proteins (1% FDR) were identified, and label-free quantification performedAnalysis of altered pathways and protein annotations was then performed using Ingenuity Pathways Analysis (ref) and DAVID (ref) and this showed that the protein ubiquitination pathway is a significant cellular pathway in our cell model. We alsoe found that TRIM27, which has been indicated as an E3 ubiquitin ligase, physically interacts with USP7. We also show that USP7 is essential for cell viability and cell cycle in colorectal cancer cells.

P-PTM-03

Post-translational Modifications

Molecular atlas of Acanthamoeba castellanii remodeling during cyst formation

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Acanthamoeba spp. are free-living amoebae found in soil, water, and air. They cause fatal infections in the human central nervous system (encephalitis) and are responsible for Acanthamoeba cornea keratitis, an eye infection that can result in blindness. They are also known to host pathogenic bacteria such as Legionella spp. and Mycobacterium avium, to name a few. Acanthamoeba spp. present a two-phase life cycle: (i) an active phase that feeds on bacteria and available wastes, (ii) a dormant cyst phase triggered by stress or starvation. The latter is characterized by a round shape and double-walled protection which makes the amoeba very resistant. It is thus important to understand mechanisms involved in cyst formation in the context of medical treatment. Here, we mapped the molecular changes occurring in Acanthamoeba castellanii during cyst formation using high throughput transcriptomics, proteomics and phosphoproteomics. We identified 166,782 transcripts and 8,577 proteins that were monitored up to 8h after triggering cyst formation in vitro. RNAseq identified more than 100,000 previously undescribed transcripts that were used for protein identification. This strategy allowed the identification of 2,701 proteins absent from A. castellanii reference proteome. Overall, 3,270 proteins were quantified at transcript- and protein-level, constituting the first time-resolved molecular atlas of A. castellanii remodeling during cyst formation. We observed a delay between transcript- and protein-level regulation. 443 proteins presented significant variation at protein-level while nearly 500 were regulated by phosphorylation and/or dephosphorylation. Of these, only 11% were regulated at both protein- and phosphorylation-level. These results confirm the involvement of phospho-regulation in Acanthamoeba spp. cyst formation while providing relative quantification of 6,376 individual phosphorylation sites. This work is the first multi-OMICs data set exploring amoeba encystment from transcripts to post-translational modifications. We annotated the genes using eggNOG-mapper to provide functional information and performed statistical analysis that identifies proteins and phospho-regulations potentially involved in cyst formation.

P-PTM-04

Post-translational Modifications

Signalling rewiring and sensitisation to MEK inhibition following LSD1 targeting in AML

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Kinase-targeted therapies for the treatment of cancer have become increasingly adopted over the years, as more advanced genetic screening and patient stratification have allowed for better personalised care. However, the development of drug resistance due to adaptive intracellular signalling rewiring following treatment is a common occurrence. Epigenetic changes have been suggested as driving forces behind such rewiring effects, although the mechanisms remain poorly understood. Here, the role of epigenetic interventions in remodelling intracellular signalling was explored, with acute myeloid leukaemia (AML) chosen as a model for the relevance of epigenetic and signalling dysregulations to the disease. Using mass spectrometry-based phosphoproteomic analysis, it was found that treatment of AML cells with a variety of epigenetic inhibitors led to significant changes in the activity of kinases downstream of receptor tyrosine kinases (RTKs). Inhibition of the histone demethylase LSD1 was found to be particularly effective, as several signalling pathways, as well as key cellular functions, were impacted. Furthermore, by performing subcellular fractionation proteomic analysis, an overall switch in signalling could be observed upon LSD1 inhibition, with levels of signalling proteins belonging to the RAF/MEK/ERK signalling pathway showing an overall increase, whilst signalling proteins belonging to the PI3K/AKT/mTOR pathway showed an overall decrease. The changes observed at the proteomic and phosphoproteomic level upon LSD1 inhibition were found to translate to an overall sensitisation of AML cells to subsequent treatment with MEK inhibition. A sequential treatment of 5 days with LSD1i, followed by 3 days with MEKi was found to result in a synergistic decrease in cell proliferation and viability. This effect could be observed in a variety of AML cell lines, as well as in patient-derived primary AML cells, particularly in those carrying MLL translocations and KRAS mutations. Basal proteomic and phosphoproteomic analysis of AML cell lines confirmed a correlation of levels of expression of signalling proteins (and the associated phosphopeptides) with sensitivity to the LSD1i-MEKi sequential treatment. In particular, overall levels of MAPK1 and p70 S6K, as well as phosphorylation of MEK2 were found to correlate with sensitivity to LSD1i-MEK sequential treatment; whilst phosphorylation of mTOR and MAP3K1 were found to anti-correlate with sensitivity. This study contributes to our understanding of the role of epigenetic mechanisms in driving signalling remodelling in cancer. Importantly, changes at the proteomic and phosphoproteomic level were identified upon LSD1 inhibition in AML cells, which allowed to identify sequential treatment as a potential therapeutic strategy for combining epigenetic- and kinase-targeted interventions.

P-PTM-05

Post-translational Modifications

HLA-B*13:01-restricted activation of CD8+ T-cells from hypersensitive patients with designer nitroso dapsone-modified peptides

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Background: Dapsone (DDS) is known to cause hypersensitivity syndrome (DHS) in 0.5 to 3.6% of exposed individuals. Susceptibility to DHS is strongly associated with the expression of the HLA class I allele B*13:01. Our previous studies identified dapsone- and nitroso dapsone (DNO) (cysteine binding reactive metabolite)-responsive CD4+ and CD8+ T-cells in the peripheral blood of hypersensitive patients and healthy donors expressing the risk allele. Nitroso dapsone-responsive CD8+ T-cells were activated via a hapten mechanism, and the T-cell response was highly polymorphic; the drug metabolite interacted with several class I alleles including HLA-B*13:01 to stimulate proliferation and cytokine release. This study aimed to (1) design HLA-B*13:01 binding peptide that contains a reactive cysteine residue, (2) generate dapsone nitroso modified peptides that are free of nitroso metabolite (3) explore immunogenicity of the peptides using autologous Antigen-presenting cells (APC) and APC transfected with single HLA-B alleles. Methods: Cysteine-containing 9mer peptides with high binding affinity to HLA-B*13:01 were designed using the IEDB Analysis Resource Several positional derivatives [AQDCEAAAL (Pep1), AQDACEAAL (Pep2), AQDAECAAL (Pep3), AQDAEACAL (Pep4)] were generated, modified with nitroso dapsone, and purified by HPLC. Purity was assessed by HPLC and LC-MS. by serial dilution and mitogen stimulation, specific CD8+ T-cell clones were generated from two DNO-modified peptides (DNO-modified Pep1 and DNO-modified Pep4), and two dapsone hypersensitive patients (Patient 8 and 14). T-cells were phenotyped, characterized for proliferation and cytokine release, and tested for cross-reactivity. A panel of autologous APC and C1R cells expressing HLA-B*13:01 or other HLA-B alleles were used to determine HLA restriction. Result: Nitroso dapsone modified peptides were characterized by LC-MS/MS analysis. Adduct identification was achieved by comparing the fragmentation patterns of the unmodified peptide with the corresponding modified peptide. HPLC analysis confirmed that the modified peptide was free of dapsone and nitroso metabolite. > 50 CD8+ reactive clones were generated from DNO-modified Pep1 and DNO-modified Pep4. Clones proliferated and secreted some cytokines (mainly interferon-gamma (IFN-γ), interleukin (IL)-13, interleukin (IL)-5, and Granzyme B (GrB), with graded concentrations of DNO-modified peptides. Clones displayed no cross-reactivity with unmodified peptide, and 2 groups of cross-reactivity with dapsone (DDS) and its metabolite (DNO) as (cross-reactivity with DNO, but not DDS) and (cross-reactivity with both DNO and DDS). Glutathione (GSH) did not inhibit the reaction with DNO-modified peptides, while it is inhibited with DNO alone. T-cell activation was MHC class I restricted and restricted to HLA-B*13:01. Conclusion: These data characterize hypersensitive patient CD8+ clones that are activated with a nitroso dapsone-modified 9mer peptide in an HLA-B*13:01-restrictive manner and provide a platform for further structural analysis of the drug-HLA binding interaction.

P-SCP-01

Single Cell Proteomics

Citrus Pectin modulates chicken PBMC proteome

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Citrus pectin (CP) is a dietary fiber commonly used in animal and human nutrition as it was shown to have antioxidative, anticancer and anti-inflammatory properties. CP supplementation in broilers has improved energy utilization and nutrient digestibility in vivo and has suppressed two main inflammatory functions of chicken monocytes in vitro: chemotaxis and phagocytosis. However, limited information on the molecular mechanisms underlying the immunomodulatory effects on the chicken is available so far. To cover this gap, this study aimed to assess the effects of CP on chicken peripheral blood mononuclear cells (PBMC) proteome. Cells were purified from 7 different whole blood pools of healthy chickens and incubated with 0.5 mg/mL of CP or medium as control (untreated) for 20 h at 41°C in a humified atmosphere with 5% CO2. Proteins were extracted and concentrated with the FASP column before being analyzed by nano-LC-MS/MS. A total of 1503 of quantifiable proteins was identified by at least 2 peptides, and a supervised multivariate statistical analysis (sparse PLSDA) for paired data was applied to identify proteins that contribute to a clear separation of the CP and untreated groups. A total of 376 (25 % of the total number of proteins) proteins from both components were selected, and a Gene Ontology (GO) analysis was performed using ProteINSIDE (https://www.proteinside.org/). The main enriched GO terms in the biological process category ($P < 0.05$) were related to peptide metabolic process, actin cytoskeleton organization, actin filament organization, vesicle-mediated transport and cell migration. To further elucidate the specific role of the discriminant proteins identified by the s-PLSDA for each group, 51 proteins with the highest abundance in CP and 137 in the untreated group were selected and again mined with ProteINSIDE. Proteins with higher abundance in CP were mainly involved in actin cytoskeleton organization and cell migration, while in untreated group in metabolic processes and translation. Among the proteins with the highest abundance in CP (FC >1.2), MARCKSL1, a protein known to reduce actin plasticity and restrict cell movement, was detected, consistent with the suppression of chicken monocytes chemotaxis we observed in a previous study. On the other hand, MARCKS, LGALS3 and LGALS8 showed the lowest abundance in CP (FC ≤ 0.8), these proteins mainly being related to cell migration, phagocytosis and autophagy. Moreover, CP has demonstrated to inhibit LGALS3 in mice, a carbohydrate-binding protein expressed in monocytes and macrophages, which could explain the suppression of some of their immune responses and consequently of its anti-inflammatory effects. In conclusion, these results suggest that CP does modulate chicken PBMC proteome and support the effects observed in vitro on chicken monocytes phagocytosis and chemotaxis.

P-SCP-02

Single Cell Proteomics

Improving Nano-LC-MS/MS Data Quality Using a Trap-and-Elute Methodology

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Mass spectrometry coupled to liquid chromatography has proven to be a powerful tool in discovery proteomics. Nonetheless, proteomics research is often challenged by the complex nature of its samples. The identification and quantification of protein and their subsequent Post Translational Modifications (PTMs) can prove particularly difficult when dealing with small amounts of samples, small volumes, and complex sample matrices (biofluids and single-cell analysis). Nano-LC-MS/MS is a tool that can help overcome these challenges. Reducing column inner-diameter results in a reduction in chromatographic dilution and increased sensitivity allowing for more efficient MS/MS sampling and thus a higher number of molecule identifications. While column miniaturization effectively deals with many of the foretold challenges, nano-LC-MS/MS capabilities can be further extended by using the trap-and-elute method. When using a trap column, the sample is loaded onto the trap and can be washed during the loading step allowing the undesirable particles and substances to go to waste. Once the sample is concentrated into the trap and free of contaminants, the flow path is changed to allow the sample to be directed towards the column for analysis. A trap column not only concentrates the sample and helps to extend the column lifetime, but it can also improve the chromatographic separation. In this work, we investigate the effect of the combination of column and trap selectivity demonstrating the importance of different trap and column selectivities in the quality of data. We will show that by optimizing the column and trap selectivity can directly affect the number of protein and peptide identifications in bottom-up proteomics especially when investigating PTMs.

P-SCP-03

Single Cell Proteomics

Label-Free Proteomics Performance with New Orbitrap Exploris 480 mass spectrometer with Single-Cell Sensitivity

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Introduction LC-MS-based proteomics, a powerful technique for identification and quantification of peptides and proteins in complex samples. needs to provide robustness to analyze 1000s of samples without compromising on proteome coverage and quantitation performance. Here we demonstrate data reproducibility across different laboratories on the new Orbitrap Exploris 480 MS coupled to an High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) Interface. The sensitivity of the Orbitrap Exploris 480 MS was evaluated in a data dependent label free method from just a single Hela cell to 5000 HeLa cells with great quantitation accuracy across a wide range using a HeLa:Yeast spike-in experiment. Methods Single HeLa cells isolated via fluorescence-activated cell sorting, processed on Nanodroplet Processing in One Pot for Trace Samples (nanoPOTS) platform and Pierce HeLa digest in a range of 0.2-1000ng were analyzed to evaluate instrument sensitivity with different throughputs (30,60, 90 and 120 min gradients) on either the UltiMate™ 3000 RSLCnano (single cells) or EASY-nLC 1200 coupled to an Orbitrap Exploris 480 MS with FAIMS Pro interface. Label free, quantitation performance was evaluated to demonstrate instrument sensitivity and methods reproducibility. Preliminary data The performance of this new benchtop mass spectrometer was evaluated in a data-dependent acquisition (DDA) for sample injection amounts of just a single Hela cell to 5000 HeLa cells (~1ug). This instrument sensitivity enables identification of ~7000 protein groups with 5.5 order of magnitude dynamic range from only a 200 ng of bulk HeLa digest and great replicate reproducibility and ~800 protein groups identification from a single Hela cell in 2hr gradient. The method performance and reproducibility were also evaluated across different instruments located in different laboratories around the world with great reproducibility in peptide and protein identification.

P-SPAT-01

Spatial Proteomics

Spatial resolution of proteomic changes in lung tissue from a mouse model of acute epithelial injury

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Repeated epithelial injury and aberrant repair are considered as major drivers of idiopathic pulmonary fibrosis (IPF) leading to chronic inflammation, fibroblast activation and finally to scarring and stiffening of the lung. Since the decline of lung function is the first symptom reported by IPF patients and typically occurs in the late disease stage, animal models are required to study early disease-driving mechanisms. Several models are already available, for example the diphtheria toxin receptor/diphtheria toxin (DTR/DT) cell depletion model. However, generation of cell-specific DTR transgenic mice is time-consuming and costly.

Here, a novel and flexible DTR/DT model of acute epithelial lung injury driven by adeno-associated virus (AAV) variant 6.2 mediated human DTR (hDTR) expression was characterized. The hDTR is sensitive to DT, which induces apoptosis by blocking protein synthesis. This contrasts with the murine DTR, which is at least 10^5 times more resistant to DT due to a polymorphism. The AAV6.2 vector transduces specifically into bronchial epithelial and alveolar epithelial type II (ATII) cells leading to their depletion after DT administration.

Using laser-capture microdissection different epithelial cell regions (bronchial epithelium and normal and infiltrated alveolar epithelium; ~3000 cells each) were isolated from formalin-fixed and paraffin-embedded lung tissue of AAV-stuffer (control) and AAV-hDTR mice, which were administered intratracheally with 100 ng DT for 24 h, and analysed using tandem mass tagging and high-resolution mass spectrometry.

Results showed upregulation of proteins involved in immune and inflammatory response in all epithelial cell regions. Interestingly, this response was stronger in the bronchial epithelium. However, most deregulations were specific to the lung region. Downregulation of marker proteins, such as SP-C, SP-A and Muc1 for ATII cells, and Scgb1a1 for club cells, confirmed targeted depletion of these cells in the corresponding region. Extracellular matrix proteins were increased in infiltrated alveolar tissue, which promotes cell migration to the site of injury, while proteins involved in pulmonary surfactant synthesis, alveolar fluid clearance and alveolar-capillary barrier were downregulated in alveoli. Overall, the data provides spatially resolved insights into repair mechanisms in upper and lower airways after acute epithelial injury.

P-STRUC-01

Structural Proteomics

Proteomic characterization of food derived extracellular vesicles

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Topic: Extracellular vesicles. Introduction: Extracellular vesicles (EVs) are a heterogeneous group of cell-derived spherical structures that have gained tremendous interest in the last decade as promising and editable nanocarriers. However, the fact that most EVs are derived from immortalized cellular lines implies low obtention efficiency and safety concerns. In order to overcome these challenges, we obtained EVs from different food sources (FS) and analyzed their proteomes through unbiased discovery-driven proteomics. Methods: EVs from four different FS were isolated by implementation of the Molecular Weight Cut Off (MWCO) methodology. Protein amount was quantified by BCA (bicinchoninic acid) assay. FS-EVs constituent proteins were tryptically digested through an in-solution digestion strategy and analyzed by shotgun proteomics based liquid chromatography tandem-mass spectrometry (LC-MS/MS). Results: The results obtained indicated that EVs sources in foods highly influenced the proteome compositions of the isolated vesicles. Mammalian EVs display much higher abundance of membrane proteins compared to FS-EVs from plant or microbial origin derived foods. However, the existence of ubiquitous proteins present in all sources characterized, including enolases or GPI-anchoring proteins among others, was also found. Additionally, we observed that bacterial-derived food EVs contain certain proteins with invasive ability, that may facilitate internalization of these vesicles into recipient cells. Conclusion: Molecular compositions of food EVs are highly influenced by the source of origin, presenting significant differences from bacterial to mammalian origin. These differences on the compositions also influence the functionality of these daily consumed food features. In-depth molecular characterization of these compositions is required to further understand the roles of the different molecular patterns included in highly consumed food matrices.

P-STRUC-02

Structural Proteomics

Characterising the N-domain of cytosolic carboxypeptidases via cross-linking mass spectrometry

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The cytosolic carboxypeptidases (CCPs) are metallocarboxypeptidases that catalyse the deglutamylation of the polyglutamate side chains generated as a post-translational modification in some proteins such as tubulins. They are involved in a wide spectra of cell processes, as spermatogenesis, antiviral activity, embryonic development, and neurodegeneration. All members of this subfamily have two highly conserved domains between species: the catalytic carboxypeptidase C-terminal domain and the N-domain. This last one, exclusive of this subfamily, shows 3 conserved motifs and a folding that has not been previously described in any protein. Nevertheless, it has been impossible to assign a function to N-domain or to any of its motifs. The goal of the present study is to determine the specific function or functions of N-domain. For this purpose, we recreated on human CCP6 a naturally found mutation on motif I of the N-domain of the CCPP-1 homologue of Caenorhabditis elegans. This mutation let to a partial loss of catalytic function of the protein, depriving cells of cilia-based signal transduction which ended in neuronal deficiencies in C. elegans. Biochemical and proteomic analyses were conducted using this mutant and compared with wild type and catalytically dead versions of human CCP6. Results showed that this domain was involved in the catalytic activity of human CCPs, impairing its deglutamylating activity on cellular substrates like tubulin, but not over synthetic small substrates. Protein stability assays determined that this was not due to a misfolding of the protein. However, subsequent analysis of the mutant´s binding capacity to its substrate showed that it did not bind to tubulin. Thus, model-based in silico studies, carried out to determine how the mutation affects the general conformation, indicated that this mutation made motif I less exposed and had less conformational flexibility to recognize tubulin and the rest of its specific substrates. This suggest that loss of catalytic function in the mutant protein could be due to a lack of binding to the substrate and not by a real lack of catalytic activity. As a final step in our characterisation, cross-linking mass spectrometry was used to further analyse the general structure of our protein of interest, as well as the implication of the studied domain in the interaction between CCPs and tubulin. Overall, the results suggest that the N-domain is involved in the specific enzyme-substrate recognition of CCPs, being an essential domain for their correct catalytic activity.

P-TECH-01

New Technologies

Characterizing lysosomal proteases to design an in silico post-digestion peptide prediction tool

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Phagocytosis and protein digestion play a key role in multiple immune processes, such as antigen presentation and clearance of apoptotic cells. Recent studies have suggested that upon recruitment and phagocytosis of apoptotic cells in processes like cancer, tissue-associated macrophages (TiMas) may have the potential to recirculate back to peripheral blood (PB), carrying tissue-derived post-digestion peptides in their phagolysosomes. Screening for the presence of such tissue-derived peptides in recirculating TiMas could be used as a screening/monitoring tool in cancer. As post-digestion peptides are dependent on the proteases present in the (phago)lysosomes, information on TiMas' lysosomal protease content is key for the development of new flow cytometry-based cancer screening methods. However, no comprehensive list of these proteases has been defined for monocytes and macrophages, and the potential impact of activation, maturation, or microenvironment on lysosomal proteases is largely unknown. In addition, no bioinformatics tool is currently available for prediction of the post-digestion protein fragments, based on lysosomal proteases in different phagocytic cells. We aim at characterizing the lysosomal proteases, present in major monocytic populations and TiMas (using glioma as a model) for further development of an in silico post-digestion prediction tool. MEROPS and Degradome peptidase databases and the UniProt repository were used for identification of potential lysosomal proteases based on enzymatic activity and cell location. Protease expression was evaluated on purified PB classical (cMo), intermediate (iMo) and non-classical (ncMo) monocytes, and TiMas from glioma samples after lysis, digestion (SP3 approach), and TMT-labelling for mass spectrometry analysis. In parallel, a post-digestion prediction bioinformatics tool, including a user-friendly web-based graphical user interface (GUI), was developed taking into account cutting patterns and relative abundance of the proteases. Twenty proteins were defined as lysosomal protease candidates, of which 60% (12/20) were indeed detected in the studied populations. Quantitative analysis identified similar expression values for the three monocytic subsets, except for cathepsin B, which was downregulated in ncMo. Conversely, glioma TiMas overexpressed (up to 6-fold) almost all proteases (11/12) vs. PB monocytic populations, except cathepsin S, that showed a lower expression in TiMas. This information was employed for the design of the post-digestion prediction tool (predLyP), which includes consensus cutting sites per protease and generates in silico the potential peptides of target proteins after lysosomal digestion. In conclusion, a lysosomal protease-based in silico tool was built for the prediction of post-digestion peptides generated in glioma macrophages. This output could be broadly applicable in different fields, e.g the design of an antibody-based tool for cancer screening.

P-TECH-02

New Technologies

Unlocking Plasma Proteomics at Scale: A multi nanoparticle approach to improve the depth of coverage

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Introduction: Blood plasma is the ideal biospecimen to assess the health and diseased states of humans since it passes by almost all tissues and is accessible from a large number of individuals at different time points. However, the challenging wide dynamic range of the plasma proteome comprising thousands of proteins and their proteoforms (e.g., PTMs, isoforms) limits unbiased proteomics at depth in large-scale with current technologies. To overcome this limitation, we have developed a fast and scalable technology that employs intricate protein-coronas formed on the surface of engineered nanoparticles (NPs) to interrogate the depth of plasma proteomes. A combination of the selected 5 NP allows rapid quantification of over 2,000 proteins across 7 orders of magnitude from a set of plasma with high precision. The key to expand the capability of the NPs in proteomics is to characterize physicochemical properties driving protein corona formation while exploring biological pathways interrogated with each NP. Methods: We have engineered and tested a set of 45 functionalized NPs with specific physicochemical properties and profiled a set of plasma proteomes determining differentially enriched proteins with LC-MS/MS analysis (Orbitrap Tribrid Fusion Lumos). Based on the quantitative differences, we have modeled protein intensities and abundances of protein families as a function of NP's physicochemical makeup. Results: Protein intensities are differentially affected by physicochemical characteristics of the NPs including charge, hydrophobicity, and specific chemical groups. This allows NPs to sample the proteome at the proteoform level across a wide dynamic range by affinity and concentration. Individual NPs can be optimized to interrogate sets of proteins across biological pathways and NP panels can be compiled to facilitate unbiased and broad proteome coverage. Conclusions: NP panels capture a large and diverse set of proteins and biological pathways based on their physicochemical makeup. Our data allows to expand the NP panel to capture previously un-identifiable proteins in plasma and optimized NP panels for any tissue, PTM or molecular classes for next generation large-scale omics studies and biomarker discovery.

P-TECH-03

New Technologies

Qualitative flexibility combined with quantitative power using a novel QTOF instrument with improved sensitivity

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The key to achieving robust analytical results lies in the combination of sensitivity, selectivity, and specificity. Sensitivity ensures there is plenty of signal to identify and quantify analytes of interest. Selectivity differentiates analyte signal from noise and interferences. Specificity ensures compound identifications are accurate and confident. The technological advancements in this novel QTOF MS system combine qualitative flexibility and quantitative power for the most demanding sample types and workflows. Here we discuss a novel research grade QTOF MS instrument with significant MS/MS sensitivity gains that is also equipped with an EAD device. The system is also equipped with a detection system that allows for very wide inter- and intra-scan dynamic range and very fast acquisition rates.

P-TECH-04

New Technologies

Optimizing protein identification results on a novel QTOF instrument with improved sensitivity, using cloud processing

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Proteomics samples are some of the most complex samples analyzed by mass spectrometry, therefore the ability to collect high quality MS/MS at very high acquisition rates is key to achieving high numbers of peptide and protein identifications. One of the main acquisition workflows for discovery proteomics is data dependent acquisition (DDA), this is used for both initial characterization proteins and proteoforms present in a biological sample and to generate spectral ion libraries used in processing of data independent (DIA) quantitative data. Increasingly researchers are using microflow chromatography to accelerate studies, generating more data per day, thus requiring improvements in data processing speed and scale. Here we describe a novel research grade QTOF MS instrument with significant MS/MS sensitivity gains, so this study investigates the impact of this large sensitivity gain on protein and peptide identification rates. A design of experiments approach (DOE) was used to explore and optimize key acquisition parameters. Four different microflow gradient lengths were tested across a range of sample loadings and a series of acquisition parameters were optimized. All data processing was performed in the ProteinPilot App in OneOmics suite in the cloud. Over 40% improvements in the number of protein IDs were observed relative to previous state-of-the-art technology for identical sample loadings.