OL-01 Innovative Technology for Sequence Analysis of Intact Proteins on a Chromatographic Time-Scale

Donald F. Hunt

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This lecture will focus on data generated with a new ion source that facilitates simultaneous generation of positively charged sample ions by electrospray ionization and negative charged reagent ions for both electron transfer dissociation (ETD) and ion-ion proton transfer (IIPT) reactions on Orbitrap mass spectrometers. Chemical derivatization of intact proteins to enhance charge and direct fragmentation will also be discussed. Implementation of multiple C-trap fills, peak parking, and ion ejection strategies to enhance sequence coverage of intact proteins will also be described. Use of IIPT/ETD facilitates near complete sequence coverage on many intact proteins and is ideally suited for locating multiple posttranslational modifications on the same protein molecule.

OL-02 New Gene Expression Regulation World Based on Transcriptome Network

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The development of next-generation sequencers has brought not only high-throughput sequencing but also new possibilities for various kinds of analysis methods of genetic information. Now, a next-generation sequencer is not just a machine to detect nucleotide bases. It has evolved into an analyzer for rapid conversion of analogue information extracted from biological molecules into genome-wide digital data. Our center organized FANTOM (Functional Annotation of Mammalian Genome), an international consortium, which is leading transcriptome research in the world. Using the next-generation sequencers, we developed a unique technique, CAGE (Cap Analysis of Gene Expression), which enabled us to conduct comprehensive large-scale promoter analysis (Omics research); for example, the analysis of transcription starting sites. In the FANTOM4 project we analyzed transcriptional regulatory networks during a process of cell differentiation based on actual measurement value using the 'Basin Network' concept. This was achieved using the CAGE technology; an analysis of 3D relationships among the regions of DNA using Chip-seq and various bioinformatics techniques. This network analysis method enabled us to make a complete catalog of networks that regulate distinct cellular states. The aim of our current project, FANTOM5, is to gain a full understanding of transcriptional regulation in a human system by generating transcriptional regulatory networks that define every human cell type. We have succeeded in creating cells with a specific function without going through the iPS cell. This is pioneering the way for various medical applications. We have found approximately 100,000 new promoters which are involved in 'Basin Network' in each cellular state. We also identified 2,113 bidirectional promoters from 42,887 human transcriptional units (TUs). Bidirectional promoters were characterized as 'Enhancer' and shown to be highly tissue specific. Another major discovery is that ncRNAs constitute the majority of the RNA population as shown by our analyses. Through FANTOM activities we found that huge amounts of ncRNAs play important roles in the gene expression regulatory network at all stages including DNA, transcription, RNA, translation and protein. These discoveries replace the current central dogma of molecular biology by a new one.

PL01-01 New Technologies for MS-Based Proteomics and their Application in Biology and Biomedicine

Matthias Mann

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Mass spectrometry-based proteomics, particularly in a quantitative and high resolution format, has become a very powerful technology to study gene expression at it 'end point' - the level of proteins. The technology of high resolution mass spectrometry (MS)-based proteomics has progressed tremendously over the last ten years[1]. Our group has worked intensively on improving and streamlining the shot-gun proteomic workflow, especially in the areas of sample preparation, chromatography, mass spectrometry and computational analysis. Together, proteomic advances now allow near complete quantification of the yeast proteome in just a few hours[2] - a task that previously took weeks of measurement time[3].

This new capability relies on 'single-run' or 'single-shot' proteomics, in which we couple a relatively long HPLC column to a high resolution, bench-top mass spectrometer with very high sequencing speed[4]. Single-run proteomics occupies a middle ground between deep shot-gun proteomics and targeted proteomics. This third way of doing bottom-up proteomics has several attractive features. It uses minimal amounts of sample and allows measurement of many states of the proteome while remaining unbiased in the sense that no subgroup of the proteome needs to be selected for analysis. As such, single-run proteomics is applicable to focused biological studies as well as for systems biology. In our laboratory, this approach is already used routinely for yeast. In this talk I will present the current technological state of the approach and its application to yeast systems biology. Furthermore, I provide an update on how far its realization has progressed to much more complex mammalian system, exemplified here by human cancer cell lines.

MS-based proteomics can also analyze post-translational modifications on a very large scale - for example, more than 50,000 phosphorylation sites can readily be detected in a cell line. Our laboratory extensively uses quantitative proteomics data not only for protein expression measurement but also for the detection of specific protein interactions. In this format, protein quantification (by SILAC labeling or in a label-free format) is applied to distinguish background binders from true binders. By quantification of binders to bait molecules vs. a control bait, the need for stringent washes is reduced and transient binders can still be detected. We will describe application of our generic workflow to interactions with specific DNA elements in the genome (such as GWAS derived SNPs or QTLs), RNA structures, post-translational modifications as well as to stimulus dependent interactions in signaling pathways.

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PL01-02 Defining the Human Tissue-Specific Proteomes Based on Transcriptomics and the Human Protein Atlas

Mathias Uhlen

Science for Life Laboratory, Royal Institute of Technology (KTH), Sweden

Central questions in human biology relate to how cells, tissues and organs differ in the expression of genes and proteins and what consequences the global expression pattern has for the phenotype of various cells with different functions in the body. Here, we have made an attempt to define the human tissue-specific proteome based on parallel transcriptomics analysis (RNA-seq) of dissected samples from most major organs and tissues in the human body. This allowed us to identify, on a genome-wide level, tissue-specific expression of transcripts for all putative protein-coding genes (n=20,050) in these organs and tissues. This data has been integrated with Human Protein Atlas (www.proteinatlas.org) data with the aim is to generate a first draft on a whole-proteome level of protein localizations in human cells, tissues and organs, including various disease-related tissues. The current version 11.0 of the Human Protein Atlas contains data from more than 18,000 validated antibodies targeting 15,000 genes corresponding to 75% of the protein-encoded genes in humans. The portal contains more than 13 million high-resolution images generated by immunohistochemistry and confocal microscopy. Integration of the antibody-based profiling and the transcriptome analysis has allowed us to build a human body map across all major human organs and tissues to allow the systematic exploration of the protein-coding genome, including an attempt to define the tissue-specific and "house-keeping" proteomes of humans.

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PL02-01 Proteomics and Neurobiology

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A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale quantitative analysis of protein data from experiments. We've been applying mass spectrometry based methods to the study of brain function and disease. Application of these methods to the study of Alzheimer's mouse models, brain development, schizophrenia, cortical barrel cortex by sensory deprivation during synaptogenesis, and the identification of trans-synaptic ligand receptor interactions will be described.

PL03-02 neXtProt: The Human Protein Knowledge Platform in the Context of HPP

Amos Bairoch

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neXtProt (www.nextprot.org) is a web-based protein knowledge platform developed within the SIB -Swiss Institute of Bioinformatics to support research on human proteins. As such, its role is analogous to that of Model Organism Databases (MODs) for model species. The core data set in neXtProt is the whole corpus of manually curated annotations extracted from UniProtKB/Swiss-Prot5 for human proteins. This set is continuously being complemented with a wide range of quality-filtered data from high throughput studies. Special attention is given to the quality of the data integrated in order to avoid flooding the system with noisy data.

neXtProt actively supports the efforts of HUPO Human Proteomics Project (HPP). In this context we are integrating into neXtProt data originating from proteomics experiments, including peptides and PTMs identification. We are also distributing regularily updated tables that allow to track the progress of HPP chromosome per chromosome. To allow our users to make the best use of the information that is available in the platform we have developed exports options (PEFF, XML) as well as programmatic access (API). We are currently developing a new search interface that will allow precise querying and retrieval of any type of information stored in the platform.

There is a very long road in front of us and the challenge of integration the mass of heterogeneous knowledge on human proteins is a distant perspective and we hope you will enjoy working with neXtProt and will help us making it evolve by telling us of your specific needs.

PL03-01 SWATH-MS: Principles and Applications to Quantitative Biology

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A range of proteomics technologies has been highly successful in supporting projects in molecular and cell biology and in biochemistry. For most of these projects proteomics has focused on the identification and quantification of proteins and their modifications in a small number of samples. For some fields of research, exemplified by clinical (e.g. biomarker) or systems biology studies, it is essential to accurately quantify specific sets of proteins across large numbers of samples at a high degree of reproducibility and high sample throughput.

To address these needs we recently introduced SWATH-MS. Like other Data Independent Acquisition (DIA) methods, SWATH-MS essentially converts all physical specimens in a sample. In the case of proteomics, the proteolytic peptides of a protein extract convert into a digital file that can be perpetually interrogated for the presence and quantity of any protein that is in the detection range of the system. In the process, high mass accuracy fragment ion maps are acquired for all sample analytes within a user-defined retention time and mass range window, by repeatedly cycling through consecutive precursor isolation swaths. The thus generated complete and permanent fragment ion records are then, in a second step, queried for the presence and quantity of specific peptides, using spectral libraries as prior information. Essentially, SWATH-MS combines DIA and high throughput targeted data analysis.

In the presentation we will discuss with specific applications how the favorable performance characteristics of the SWATH-MS technique translate into new biological knowledge. Examples include the accurate quantification of proteins across numerous samples to identify pQTL's and to measure the dynamics of signaling systems and of protein interaction networks, as well as the use of a chromatographic retention time dimension to improve the assignment of protein phosphorylation sites in phosphoproteomic studies.

PL04-01 Unleashing the Power of Proteomics to Develop Blood Based Cancer Markers

Samir Hanash

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There remains an urgent need for simple non-invasive tests to detect cancer, to personalize treatment and to monitor for disease regression or progression. Over the past decade, the depth of analysis currently possible using proteomics has increased substantially, allowing the identification of well over 2,000 proteins in plasma, reaching protein concentrations well below the ng/ml. We have applied an integrated strategy for the discovery of blood based cancer biomarkers using rigorous experimental design applied to biospecimens that minimize bias and that are highly relevant to the intended clinical applications. The major targeted cancers are lung, breast, colon and pancreas. This effort has resulted in the identification of novel protein products encoded in the genome, novel protein forms associated with disease development. Using training and testing approaches, panels of markers have been assembled that have utility for cancer early detection and assessment of treatment response. Success in discovery and initial validation is leading the way to prospective clinical trials to determine the utility of blood based cancer markers in complementing imaging modalities for cancer detection and for disease monitoring.

PL04-02 A Decade of Adventures of CNHUPO and HLPP

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Following its inception in 2002, CNHUPO organized the 1st international forum in liver proteomics and then sent delegates to attend HUPO 1st congress. In the congress, the HLPP was initiated as the first international cooperative project in organ proteomics. This marked the beginning of CNHUPO and HLPP. For the past decade, CNHUPO has been at the forefront of pushing forward proteomics in China and the world. We introduced the young proteomics field to Chinese people by organizing national/international meeting, training young investigators, establishing comprehensive collaborations in the nation and beyond. We are the strong advocates for funding support from the Chinese government. With the strong funding support and great collaborative efforts from scientists around the world, we have made proteomics widely applied in almost all fields of biological research in China. On the front of HLPP, we proposed the concept of looking at the liver proteome at physiological and pathological conditions by comprehensively measuring protein abundance, PTM, and PPI. The concept has become the cornerstone of the new HPP. Despite the limitation of technology development, we have developed a series of novel proteome strategies to draw the 1st version of a map of the human liver reference proteome at a depth of ~10k proteins. Reflect on the success of CNHUPO and HLPP, the following marks might deserve to be highlighted: 1) Proteomics is ready to take off in the age of grand discoveries. 2) Proteomics as a big science needs the support of big projects and big core facilities. 3) Supports from scientists and public are as important as the one from government. 4) Powerful organizations/consortiums play essential role in big "Omics" projects. 5) Close-collaboration between national and international organization/consortium can significantly accelerate progression of the proteomics field. 6) Technology is a driving force in proteomics. 7) Young investigators are the future of proteomics.

Keywords: CNHUPO, HLPP, Proteome

PL05-02 Evolution of Alternative Splicing and Transcriptional Regulation by Transposable Elements

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Transposable elements are present in numerous copies in mammalian genome and comprise up to 45% of the human genome. They can influence gene transcription and biological function through various mechanisms. Long terminal repeats (LTRs) of human endogenous retroviruses (HERVs) have been shown to influence the expression of neighboring genes. Solitary LTRs contain various transcriptional regulatory elements including promoters, enhancers, and polyadenylation signals. Functional LTR transcription start sites are located between the R and U5 region. Hypomethylation of the LTR element allows the neighboring functional gene to have tissue specific expression. Accumulated changes of the LTR elements in gene regulation are likely to be functional factors for the process of diversification, speciation and evolution consequences. A small minority of such sequences has acquired a role in regulating gene expression, and some of these may be related to differences between individuals, and to expression of disease. They seemed to be a source of alternative splicing, structural change of genomes, and could be related to genetic variation and epigenetic regulation linked to diseases.

PS01-01 Proteomic Approaches for Quantitative Protein Complex Analysis

Marius Ueffing[1,2], Andreas Vogt[1], Yves Texier[1], Bettina Fuerholzner[1], Grischa Toedt[3], Toby Gibson[3], Norbert Kinkl[1], Karsten Boldt[1]

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Analyzing the molecular architecture of native multi-protein complexes via biochemical methods has so far been difficult and error prone. Protein complex isolation by affinity purification in combination with mass-spectrometry (MS) is a commonly used technique and interaction data derived thereof are the basis for predictions of biological pathways or disease mechanisms concerning those proteins. Selectivity and specificity of pull-downs based on tag-fusion-proteins as well as immunoprecipitations (IP) suffer are compromised by non-specific binders to the capture agent or carrier beads. Due to this lack of specificity, a large proportion of reported protein interactions in the literature as well as in databases gathering interaction data are likely to be compromised by false positives.

Isotope labeling in combination with affinity-based isolation has increased accuracy and reproducibility; yet, larger organisms - including humans - are hardly accessible to metabolic labeling. With the aim to analyse protein interactions in at endogenous levels in primary tissue and combine the selectivity of affinity-based isolation with the accuracy and reproducibility of MS, we combined isotope coded protein labeling (ICPL) with IP and quantitative MS. ICPL-IP allows sensitive and accurate analysis of protein interactions from primary tissue including human specimens. Using a pull down strategy (SF-TAP) we have further refined a pre-existing method to destabilize protein complexes by treatment with very low concentrations of SDS. By combining SDS concentration gradient-induced decomposition of protein complexes coupled to quantitative MS and in silico elution profile distance analysis we can now determine protein complex composition as well as complex topology. By applying these new methods to a cellular transport module, the IFT/Lebercilin complex we demonstrate its ability to determine modular composition as well as sensitive detection of known and novel complex components.

PS01-02 Workflows and Solutions for Targeted Quantification to Increase Throughput and Sensitivity

Amol Prakash

Thermo Fisher Scientific, USA

A typical mass spectrometry based biological experiment requires large number of experiments, which translate to high throughput. We have developed some novel ideas on the hardware, sample prep and data analysis side which will unify all these thoughts into a multiplexing concept which will increase knowledge throughput without requiring many days of instrument time or analysis time. I will present some these via real world examples to show how we were able to translate these into practical improvements in our workflow to achieve both throughput and sensitivity.

PS01-03 Proteomic Analysis of Colon Cancer Phenotypes

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Microarray and next generation sequencing-based high-throughput genomic technologies have allowed comprehensive characterization of cancer phenotypes. These studies have resulted in improved understanding the biological behavior of cancer cells and important advances in personalized treatment of this disease. However, genes and transcripts execute most of their functions through the proteins they encode and insight into the global proteome remains relatively unexplored because of technical limitations in quantitatively assessing large numbers of proteins in complex mixtures. Because proteins are the ultimate effector molecules in cells and undergo changes that cannot be captured by genomics and transcriptomics, global proteomic analysis can serve as an orthogonal analysis strategy for biological discovery that complements genomic and transcriptomic analyses.

Recent technological advances allow thousands of proteins to be routinely identified from small quantities of tissues or cells through shotgun proteomics. Studies by the National Cancer Institute-supported Clinical Proteome Technology and Assessment of Cancer (CPTAC) network (proteomics.cancer.gov) have laid the foundation of a standardized and reproducible shotgun proteomic methodology. For this approach, proteins are digested in to peptides that separated using multi-dimensional liquid-chromatography and identified using mass-spectrometry. We employed this approach for the analysis of colorectal cancer cell lines and primary tumor tissues and derived proteomic signatures associated with different sub-types of the colorectal cancer phenotype using enrichment analysis (www.webgestalt.org). Expression profiles of proteins of interest were further quantified using targeted mass-spectrometry analyses using multiple-reaction monitoring (MRM). These analyses confirmed our initial observations by shotgun proteomics in the large majority of cases. Novel biological insights were confirmed using *in vitro* biological assays for specific biological properties of colorectal cancer cell lines.

Of particular importance is the combination of genomic and proteomic data to study the expression of predicted genomic features in the proteome. Examples are expression profiles of proteins located in amplified regions and the capability to identify variant protein sequences and the global proteomic effects of mutations. Our studies identified expression of important cancer-associated mutations such as K-ras codon 12 and B-raf codon 600 mutations at the protein level, the presence of somatically acquired peptide variants, in addition to known variants that could be mapped to single nucleotide polymorphisms (SNPs) in the genome. Proteomic profiles in cells with increased levels of variant peptides suggest a coordinated program of cellular adaptation resulting in degradation of abnormal proteins.

PS01-04 Quantitative CID Cleavable Crosslink Strategy on a Benchtop Instrument Using All-Ion-Fragmentation and SILAC

Richard Alexander Scheltema, Herbert Schiller, Matthias Mann

Max Planck Institute for Biochemistry, Germany

Mass spectrometry based crosslinking experiments have been one of the major successes in proteomics in recent years and have as a result become part of the standard proteomics toolkit. However, most current approaches rely on full database searches to determine the sequence identities of the crosslinked pair and are therefore limited in sample complexity, while lacking deterministic and quantitative properties. Alternative approaches use CID labile linkers, but these require expensive state-of-the-art mass spectrometry platforms and lack in sensitivity. Here we present a novel crosslinking approach designed to overcome these limitations with SILAC labeling in conjunction with CID cleavable crosslinkers on the Q Exactive benchtop mass spectrometry platform. As this platform lacks true ms3 capabilities, we utilize pseudo-ms3 as All-Ion-Fragmentation (AIF) to separate the crosslinked peptides prior to further analysis. The combination of full scans and AIF scans allows for deterministic assignment of the two crosslinked peptides both during acquisition as well as during the analysis of the recorded data. Analysis of the recorded data is performed with MaxQuant, which has been extended to support this type of data. The method is validated on the recently published TRIC/CCT chaperone complex for which we approach the sensitivity of non-cleavable crosslinking approaches, while opening up the possibility to analyze higher complexity samples for which MaxQuant Real-time is used to improve the acquisition.

Keywords: Crosslinking, LC/MS, Real-time control

PS02-01 Design, Operation and Application of an Automated High Throughput Monoclonal Antibody Facility to Support Global Proteomics Initiatives

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Monoclonal antibodies (Mabs) are a key reagent to support the Chromosome-Centric Human Proteome Project which aims to define the full set of proteins encoded by each chromosome, including PTMs, ASTs and protease-processed protein variants. Fundamental to this initiative is the generation and validation of large numbers of high affinity renewable Mabs, and making them available to the research community at an affordable price. This will be particularly relevant for proteins which have not yet been formally identified by mass spectrometry or using currently available antibodies. Monash University has established a state of the art high throughput robotic platform (Monash Antibody Technologies Facility; MATF) to produce custom made, high quality, high-affinity mouse or rat Mabs against protein or peptide targets with the capacity to provide thousands of novel antibodies per year to a global clientele. Using Antigen-MicroArray (AMA) technology for primary screening, multiple antigens can be screened simultaneously for specific binding and, by differential staining, IgG secreting clones can be specifically selected. Chr 7 has been formally selected by a consortium of Australian and New Zealand researchers since it contains a number of disease-associated genes or loci, including the EGFR which has been a long term focus of a number of research groups in Australia. Preliminary bioinformatics screening has indicated that of the 946 protein encoding genes listed in neXtProt, 170 have not yet been experimentally confirmed. It is proposed that, using recombinant proteins or defined peptides (e.g. peESTs), customised antibodies would be raised to these proteins for use in affinity proteomics, western blotting, IHC and ELISA approaches. In this presentation we will describe the design and operation of the antibody facility, discuss operating and financial models, discuss antibody validation and a strategy for the use of such antibodies to identify "missing proteins".

Keywords: Monoclonal antibodies, Robotics, Missing Proteins

PS02-02 Exploration of the Protein Coding Genome

Caroline Kampf[1], Fredrik Ponten[1], Linn Fagerberg[2], Bjorn Hallstrom[2], Emma Lundberg[2], Kalle Jonasson[2], Mathias Uhlen[2]

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Since 2003 the Human Protein Atlas project has worked systematically towards completing a proteomic map of gene expression in a range of normal tissues, cancers and cell lines an antibody-based approach. The output of the project is a publically available Protein Atlas (www.proteinatlas.org), where all IHC data, images along with annotations and validation data are published. Recently, a large effort was made to also generate data on a transcript level using RNA-sequencing, and complete transcriptomic data is now available for 27 normal tissues and 44 cell lines included in the Human Protein Atlas. The quantitative transcriptomics approach across all major human tissues allows us to systematically explore the protein-coding genome, generating a first draft of a characterization of "house-keeping" as well as tissue-specific proteins. In addition, the large-scale profiling on both transcript and protein level allows for an additional level of antibody validation. The Human Protein Atlas project now aims to set up a new gene expression portal, built to serve as a repository of information on transcript expression levels combined with spatial cellular and subcellular information on the protein expression.

Keyword: Immunohistochemistry, Human Protein Atlas, proteomics, transcriptomics

PS02-03 Chemical Proteomics for Target Identification and for the Development of Therapeutic Antibodies: From the Bench to the Clinic

Dario Neri

Swiss Federal Institute of Technology (ETH Zürich), Switzerland

Antibodies can be used to deliver bioactive molecules (drugs, cytokines, photosensitizers, radionuclides, fluorophores etc.) to sites of disease (e.g, cancer or chronic inflammatory conditions), thus enabling molecular imaging applications or therapeutic interventions which spare normal tissues. The antibody-based targeting of markers of pathology which are readily accessible from the blood-stream (e.g., markers of angiogenesis or components of the modified extracellular matrix) is particularly attractive for biomedical applications.

In this lecture, I will present results of chemical proteomics methodologies developed in my laboratory, which have allowed the development of antibody-based therapeutic agents, which are currently being investigated in clinical trials. A main technology used in the lab for target identification purposes consists in the terminal perfusion of animal models of pathology (e.g., tumor-bearing mice) with a reactive derivative of biotin, which covalently modifies accessible proteins. Alternatively, the biotinylation reaction can be performed by *ex vivo* perfusion of surgically resected human organs with cancer. Biotinylated proteins can be purified on streptavidin resin in the presence of stronge detergents, digested and the resulting tryptic peptides separated by HPLC and analyzed by mass spectrometry in the presence of internal standards for quantification purposes.

I will show how novel targets have been discovered using chemical proteomics methodologies (in collaboration with Philogen, a Swiss-Italian biotech group) and how the corresponding monoclonal antibodies can be used for imaging and therapeutic applications.

PS02-04 Development of an Automated Immuno-MALDI Assay for the Clinical Measurement of Plasma Renin Activity

Robert Popp[1], David Malmstrom[1], Alexander G. Camenzind[1], Daniel T. Holmes[2], J. Grace van der Gugten[2], Christoph H. Borchers[1,3]

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The renin angiotensin aldosterone system (RAAS) is crucial for the regulation of blood pressure. Dysfunctions of the RAAS can lead to severe diseases related to hypertension. A well-established biomarker for the diagnosis of primary aldosteronism, a form of secondary hypertension, is plasma renin activity (PRA). It is commonly determined by radioimmunoassay (RIA), which has the disadvantages of using radioisotopes and the possibility of cross-reactivity.

To overcome these issues we have developed a mass spectrometric PRA assay based on immuno-MALDI (iMALDI) with the final goal of implementing it in the clinic. Plasma samples are split into two aliquots. The first aliquot is incubated at 37 ˚C for Angiotensin I (Ang I) generation, the second one is placed on ice as a blank. The aliquots are then incubated for 1 hour with stable isotope-labeled Ang I analogues and anti-Ang I antibodies bound to magnetic beads. The beads are washed and spotted directly onto a MALDI target

with the peptides being eluted from the beads by addition of HCCA matrix. Ang I quantitation of both aliquots allows for PRA determination.

64 clinical samples were prepared manually and analyzed using our iMALDI method. Results were compared with RIA (R^2 = 0.9412) and LC-MS/MS (R^2 = 0.9471) results determined at St. Paul's Hospital in Vancouver, Canada, exhibiting strong correlation to clinical measurement.

In order to achieve the requirements for clinical assays (robustness, high throughput and accuracy) we have optimized and automated the sample preparation on an Agilent Bravo liquid handling platform for analysis on a Bruker Microflex MALDI instrument.

Keywords: iMALDI, Plasma Renin Activity, Automation

PS03-01 Single Cell Membrane Protein-Protein Interactions on Human Cancer Cells May Change Antigen Availability to Immunohistochemical Detection

Mark S. Baker, David Cantor, Harish Cheruku, Rohit Saldanha, Leon McQuade, Abidali Mohamedali, Charlie Ahn, Iveta Slapetova, Samuykta Anand, Shoba Ranganathan, Ed Nice

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Proteomics delivers more comprehensive understand of biology. Recent proteomics studies on colorectal cancer (CRC) cell lines where integrins and protease receptor expression have been deliberately altered are presented in the context of attempts to understand changes associated with the invasive/metastatic phenotype.

Immunoprecipitation proteomics on epithelial cells in our lab has now identified uPAR- and $\alpha v\beta 6$ integrin interacting proteins (i.e., P-P metastasome) and showed how many of these proteins may be involved in the regulation of TGFβ1 activation and downstream effects. Subsequent detailed analyses of the sites of protein interaction between uPAR-$\alpha v\beta 6$ using overlapping peptide array binding blots, ELISA-type peptide competition assays, structural modelling and proximity ligation assays suggest interesting biologies may also be associated with these interactions.

In addition, we demonstrate a potential solution to a very old argument regarding whether the epithelial and/or the stromal cells found in human CRC tissues are uPAR+. In detail, we can report data that suggests a reason why both sides of the "debate" may be accurate - namely that different anti-uPAR MAbs do indeed differentiate the apparent presence of uPAR on both of these cells types in CRC tissues. We show that uPAR is "visible" to different MAbs on both epithelial and stromal cells but not to the other MAb. We propose that uPAR-P interactions may "shield" epitopes recognised by certain epitope-specific anti-uPAR MAbs whilst allowing detection by others directed against distant epitopes. This data raises the conundrum that P-P interactions may shield detection of cell-surface antigens involved in P-P interactions a single cell type specific manner.

PS03-02 Single Cell Analysis of Proteins from Clinical Samples Relevant to Human Health

David R. Klug

Department of Chemistry, Imperial College London, UK

There are a range of motivations driving the development of single cell analysis in general, and single cell protein analysis in particular and I will discuss some of these. My group has up to now focussed on the development of microfluidic devices incorporating antibody capture and single molecule detection. I will outline how these work, their strengths and limitations and discuss some possible applications. This will be followed by some preliminary data on both cell lines and clinical material from prototype devices to highlight how one can put such devices into operation in the context of biological and biomedical research.

PS03-03 Analysis of Single Cell Signaling Through Time and Space by Mass Cytometry

Bernd Bodenmiller

IMLS, University of Zurich, Switzerland

Tissues and tumors are complex assemblies of multiple cell types that interact and communicate with each other to achieve disease states.
In cancer, many aspects of tumor development to metastasis depend on these cell interactions in unique microenvironments. Especially signaling networks, forming the core of cellular decision making, are shaped by cell interactions. To study and understand the decision making in the microenvironments, single cell analysis technologies are needed that allow to measure cell type, signaling network state and other cellular processes with spatial resolution.
Mass cytometry is a recent single cell mass spectrometry approach that enables to measure up to 100 molecules simultaneously using isotopically pure rare earth metals as reporters. Previously, only cells in suspension could be analyzed using mass cytometry, and thus essential information on cell location and cell-to-cell interactions was lost. We have now coupled immunocytochemical and immunohistochemical methods with high-resolution laser ablation to mass cytometry. The approach now enables the simultaneous imaging of up to 100 proteins and phosphorylation sites at a sub-cellular resolution. We mass cytometry to study the signaling networks activated during the epithelial-mesenchymal transition (EMT), a process driving the formation of metastasis, in model systems and within their native microenvironment in breast cancer tumors.
Imaging mass cytometry revealed an unexpected complexity of cell-to-cell interactions and cellular (EMT) states in the analyzed tumors. The approach also allowed to accurately classify patients based on the visualized single cell marker expression and cell interactions. Imaging mass cytometry will enable the analysis into how cellular assemblies generate phenotypes in health and disease and will support the transition of medicine towards individualized molecularly targeted therapies.

PS03-04 Monitoring Protein Synthesis in Living Cells with Fluorescent Labeled tRNA FRET Pairs

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We introduce Protein Synthesis Monitoring (PSM) - a technique to monitor protein synthesis in living cells. In PSM, we transfect cells with tRNAs labeled as FRET donors and acceptors. A FRET signal is generated only when a donor- and an acceptor-labeled tRNA come in close contact (< 7nM), as they do on the ribosome during elongation. The intensity of the FRET signal correlates with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis. PSM can monitor general protein synthesis using bulk tRNAs, or the synthesis of a specific protein, using specific pairs of tRNA. PSM has sub-micron spatial and sub-second temporal resolutions. Cells continue to live and grow normally, and the synthesized proteins are unchanged since the labeling is on the tRNA itself and not on the amino acid. The cells uptake the tRNAs using liposomes or other common methods. The specificity of PSM arises from the large number of distinct tRNA pairs - 1176 in humans (corresponding to 48 isoacceptors). For about 83% of all proteins, an adjacent pair of tRNAs can be found that is enriched in the synthesis sequence of that protein compared to its frequency in the average, or background protein. The enrichment, or E-factor, can indicate the expected signal to background value for a given protein. With this approach, cells can be monitored for the exact timing of synthesis of a protein of interest, provided it is synthesized at sufficient rates (e.g. a sufficient number of ribosomes is engaged in synthesis of this protein). We have demonstrated specific PSM for monitoring synthesis of a viral protein (NS3) during viral infection using Isoleucine tRNA, and for monitoring synthesis of collagen during fibrosis in mouse fibroblasts using tRNA-Gly and tRNA-Pro. We will discuss these results as well as additional applications of PSM in stem cells, drug discovery, cell sorting and basic research.
Keywords: Protein Synthesis, ribosome, tRNA

PS04-01 (Keynote) Importance of Systems Glycobiology for Understanding Disease Onset, Biomarker and Therapeutics

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Glycans are implicated in the onset of infectious diseases, as well as life-style related diseases such as diabetes and chronic obstructive lung disease, autoimmune diseases, neuromuscular disease, and cancer. Glycosylated proteins are also used for cancer biomarkers. The use of modified recombinant glycoproteins can be useful for various therapeutics including antibody therapy against cancers. Our group has been focused on the role of glycosyltransferases in the biosynthesis of N-glycan branching and the identification of their target proteins in relation to diseases. We recently proposed the concept of the "Glycan cycle" as a functional unit of glycans for understanding glycan functions, which will permit the integration of glycan functions in relation to diseases. This conceptual "functional unit of the glycan cycle," such as just described for GlcNAc or fucose, is intended to help in developing our understanding of the integrative and dynamic analysis of glycan functions, an important approach to systems glycobiology. I will also discuss some of our recent studies dealing with the metabolism of nucleotide sugars using chemical biology techniques. In previous studies, we developed a method for the simultaneous analyses of nucleotide sugars, Moreover, in order to investigate the fate of UDP-GlcNAc, we developed a tracing method for following the synthesis of UDP-GlcNAc and its utilization, and GlcNAc utilization using $^{13}C_6$-glucose and $^{13}C_2$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry. Thus systems glycobiology is one of the promising approaches for understanding glycan functions.
Keywords: Systems Glycobiology, Glycans

PS04-02 Challenges of Disease Biomarker Discovery: A Perspective from Developing Countries

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Biomarkers are biomolecules that can aid in early disease diagnosis, discrimination between diseases with overlapping clinical manifestations as well as useful in monitoring disease progression. We are investigating proteomic alterations in various types of biological samples in different tropical infectious diseases and human brain cancers to gain mechanistic insight about the disease pathogenesis and identification of diagnostic and prognostic protein markers. Our comparative analysis of malaria, dengue and leptospirosis revealed alterations in expression levels of quite a few serum proteins in multiple diseases, which could be an upshot of inflammation mediated acute phase response signaling, while the uniquely differentially expressed candidates were also identified in each pathogenic infection indicating unique responses. With a panel of identified proteins we are able to distinguish different infectious diseases or different grades of brain tumors.
There are several challenges associated with proteomics-based clinical research in developing countries. Considering over 80% of global burden of disease resides in developing countries, biomarker discovery research should be accelerated to improve diagnostic and therapeutics in countries with low-resource settings. The organized collection and storage of biospecimens thorough biobanking initiatives are required for "omics-based" translational studies. Apart from governmental funding resources, there is an urgent need of non-governmental funding resources in developing world to accelerate clinical research. Moreover, collective improvement of biomarker discovery and validation process requires sharing of scientific data among different research groups across the world. To this end, Data-Enabled Life Sciences Alliance is and important initiative to establish a common data bank for biological sciences to share scientific data among different research groups across the world.
Keyword: Biomarker, Cancer, Infectious diseases, Developing countries

PS04-03 Proteomics and Nephrolithiasis

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In addition to defining biomarkers for diagnostics and prognostics, biomarker discovery in nephrolithiasis (kidney stone disease) is also directed to characterizations of target molecules that play critical roles in kidney stone formation. Our group has performed proteomic investigations of kidney stone disease to screen for such target molecules that can modulate calcium oxalate (CaOx) crystals, which are the major crystalline compositions in kidney stones. Various functional analyses have been developed and optimized to address modulatory function (either inhibition or promotion) of these target molecules in CaOx crystal growth, aggregation, adhesion and invasion. In this session, some successful characterizations of such target molecules or kidney stone modulators will be highlighted. For example, the most recent findings were obtained from characterizations of changes in secretome of renal tubular epithelial cells after exposure to CaOx crystals. One of the altered secreted proteins identified from this study was enolase-1. To address functional significance of enolase-1 in the disease mechanisms, we have developed a novel assay to investigate CaOx crystal invasion through the renal interstitium [Chiangjong W, Thongboonkerd V. Talanta. 101:240-5, 2012]. The data revealed that enolase-1 could bind to CaOx crystals, resulting to crystal invasion through extracellular matrix (ECM) via plasminogen/plasmin system in a dose-dependent manner. These data may lead to further development of therapeutic targets for prevention of kidney stone formation in high-risk group and/or its recurrence.

PS04-04 High Throughput Cell-Based Studies and Protein Microarrays for Biomarker and Target Discovery

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One of the most compelling steps in the post-genomic era is learning the functional roles for all proteins. The DNASU repository accelerates discovery by providing nearly 15,000 full-length clones for human genes enabling high-throughput protein function studies. To exploit this resource, we developed a novel protein microarray method, called nucleic acid programmable protein array (NAPPA). In lieu of printing purified proteins, NAPPA translates proteins in situ from printed cDNAs by extracts containing human ribosomes and chaperones. This obviates the need to purify proteins, produces human proteins in their natural milieu, and ensures protein stability on the array as the proteins are made just-in-time for assay. NAPPA arrays have been used in the discovery of disease biomarkers, protein-protein interactions and enzyme substrates. Recent experiments have focused on the search for autoantibody responses in breast cancer patients. The prevalence of autoantibodies to specific proteins is typically in the 20% range; thus a panel of autoantibodies will be needed to achieve high sensitivity. Using 155 cases/130 controls in a three phase study, including a blinded validation, we have discovered a panel of 28 autoantigens in breast cancer with sensitivities ranging from 5-40% and specificities ranging from 80-100%. We are further advancing this methodology using photolithographically etched discrete silicon nanowells coupled with next generation piezoelectric printing to achieve very high densities. High density protein expression and display, as well as functional protein-protein interactions, was shown in 8000 nanowell arrays, the highest reported density of individual proteins in nanovessels on a single slide.

Keyword: autoantibodies, biomarker, cancer

PS05-01 (Keynote) Towards Novel Separation and Fragmentation Techniques for Top-Down Proteomics

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Top-down proteomics is based on separating proteins from complex mixtures, ionizing them and fragmenting their molecular ions inside a mass spectrometer. The bottlenecks of the approach appear to be the insufficiently potent methods of protein separation and fragmentation. Our laboratory works towards developing novel approaches to solving these bottlenecks.

Ideally, a separation method should be orthogonal to reversed-phase liquid chromatography (rpLC), an efficient separation technique often coupled directly to mass spectrometry. Electrophoretic focusing is such a method, but its use is restricted by the absence of a commercial device directly compatible with rpLC and suitable for proteomics. We have recently designed such a device based on a multiple-junction capillary fractionator [1]. The device separates a mixture of polypeptides by their isoelectric point into a desirable number of rpLC-orthogonal fractions (usually 4-20) that can be directly injected onto a chromatographic column. We have now thoroughly tested this device in 2D proteomics and achieved a significant increase in proteome coverage.

The ideal fragmentation method should efficiently cleave polypeptide bonds in large molecules that may be in low charge states, as in native mass spectrometry. Electron-capture based methods, such as ECD and ETD, are attractive candidates, but their efficiency for lower charge states needs to be improved. Increasing the precursor charge state in the gas phase before fragmentation (i.e. charge increase followed by charge reduction) would solve the problem. We are pursuing this goal using a novel combination of a plasma source and a high-resolution mass spectrometer (benchtop Orbitrap). Preliminary experiments are encouraging [2]. Recent results on such a combination will be presented.

1. Chingin, K.; Astorga-Wells, J.; Pirmoradian Najafabadi, M.; Lavold, T.; Zubarev, R. A. *Separation of polypeptides by isoelectric point focusing in electrospray-friendly solution using multiple-junction capillary fractionator, Anal. Chem*. 2012, 84, 6856-6862.

2. Chingin, K.; Makarov, A.; Denisov, E.; Zubarev, R. A. *Fragmentation of Positively Charged Biological Ions Activated with A Beam of High-Energy Cations, in revision*.

PS05-02 (Keynote) Top-down Analysis of Modified Proteins and Glycoproteins Using Various Dissociation Methods

Mark E. McComb, Roger Theberge, Sandrine Bourgoin-Voillard, Yan Jiang, Stephen A. Whelan, Xiaobin Xu, Xiang Yu, Christian F. Heckendorf, Jean L. Spencer, Cheng Lin, Catherine E. Costello

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For determinations that must take into account the presence of specific proteoforms, top-down sequencing provides the most information on amino acid sequence (including details on variants) and the type and location of post-translational modifications. It is thus ideal for proteomics and for clinical and functional studies that can reveal information about the inter-relationships of various potential modifications. We are optimizing the operating conditions for our 12-T SolariX hybrid Qh-FTICR MS (Bruker) utilizing several ExD modes, with or without IR activation, our Q-Exactive orbitrap MS with HCD (Thermo Fisher) and our UltrafleXtreme MALDI-TOF/TOF MS (Bruker) to perform top-down MS/MS studies of proteins and glycoproteins and their fragments produced by ISD. The techniques are also useful for characterization of chemically cross-linked proteins and peptides. To facilitate data interpretation, we have modified our BUPID software to accommodate the needs of top-down spectral interpretation (BUPID-Topdown). The experimental strategies will be illustrated with data from ongoing studies.

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Keyword: top down sequencing

PS05-03 Top-Down and Flexible Analysis of Protein Using MALDI In-Source Decay

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Mass spectrometry (MS) provided fascinating ionization tools such as ESI and MALDI methods. Recent topics relating to radical ion chemistry in MS are MALDI-ISD and ESI-ECD/ETD, resulting in $c^{\cdot}$ - and $z^{\cdot}$ -ions originated from the specific cleavage at N-Ca bond on the peptide backbone. MALDI-ISD is used for identifying intact proteins as a tool of top-down proteomics. MALDI-ISD uses the hydrogen radicals to form the protein radicals. Hydrogen radicals are produced from matrix activated with UV laser photons. The resulting hydrogen radicals bind to carbonyl oxygen on the backbone and result in protein radicals. Recent advance of MALDI-ISD gives information about susceptible amino acid residues to the N-C^{α} bond cleavage. The susceptible residues Xxx-Asp/Asn and Gly-Xxx which give relatively intense $c^{\cdot}$ -ion peaks can be rationalized from a criterion that those residues are preferred in flexible backbone structures free from intramolecular hydrogen-bonded structures such as α-helix and β-sheet. This presence of more susceptible amino acid residues than the rest in protein is of interest from the standpoints of interaction(s) between peptide backbone and matrix molecules and of protein flexibility. Protein flexibility is relating to the interactions with drugs, nucleic acids, peptides and proteins. Such a so-called "intrinsically disordered protein [6]" is relating to wide variety of biological functions. Here I describe a relationship between ISD flexible residues and protein backbone flexibility which can be estimated by using hydrogen/deuterium exchange (HDX) by NMR spectroscopy and the B-factor by X-ray crystallography.

Keyword: MALDI, In-source decay, Flexible amino acid, Asp, Asn, Gly

PS05-04 Top-down Proteomic Analysis of Enzymes and Complexes in *Trichoderma* Fungal Secretomes

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Biomass degrading enzymes produced by fungi must be understood in relation to their structural and functional properties in order to become fully usable for biotechnological applications, including biofuel production. Recently, we showed that *Trichoderma harzianum* and *Trichoderma reesei* secretomes contain cellulases and hemicellulases assembled as multienzymatic complexes as revealed by blue native-PAGE and LC-MS/MS bottom-up techniques. Herein, we report on a novel use of top-down proteomics for the identification of intact proteins and native complexes in the *Trichoderma reesei* secretome. The sample was collected after nine days of growth on sugarcane waste, and separated using SDS-PAGE and BN-PAGE followed by electroelution. Fractions were processed using methanol/chloroform/water precipitation prior to nano-LC analysis. Top-down mass spectra were collected on an Orbitrap Elite mass spectrometer with fragmentation performed by ETD and HCD. Proteins were identified using ProSightPC 3.0. Data from SDS-PAGE coupled to denatured electroelution yielded top-down MS identification of 531 proteoforms within 95 different protein accession numbers so far. Current work is aimed at applying blue native PAGE coupled to native electroelution, followed by top down LC-MS/MS to identify the protein subunits of high molecular weight complexes present in the *T. reesei* secretome.

Keywords: Top-down proteomics, Trichoderma secretome, Complexes

PS06-01 Molecular Tissue Imaging Under Ambient Conditions Using a Novel Imaging Source Coupled to High Resolving Power Mass Spectrometry

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Mass Spectrometry Imaging (MSI) is used to simultaneously elucidate the spatial distributions of endogenous compounds (*e.g.*, metabolites, lipids, glycans, peptides, and proteins) as well as xenobiotics including drugs and their metabolites with unparalleled molecular specificity. While these analyses are typically performed under vacuum using MALDI-MSI, novel ambient ionization sources have been developed allowing for molecular imaging under ambient conditions. Ambient imaging permits the analysis of samples that are not amenable to vacuum with little to no sample preparation. Matrix-assisted laser desorption electrospray ionization (MALDESI) is the first hybrid ambient ionization source that combines features and benefits of MALDI and ESI. This technique involves using a pulsed laser to excite an endogenous or exogenous matrix that facilitates desorption of neutral analyte molecules from the sample. These neutral analytes partition into the charged solvent droplets of an electrospray plume and become ionized via an ESI-like mechanism. Different modes of the MALDESI technique have been demonstrated using various laser wavelengths including ultraviolet (UV-MALDESI) and infrared (IR-MALDESI). For each mode, a corresponding matrix is chosen that strongly absorbs in the wavelength region of the laser emission. In IR-MALDESI, with laser emission at 2.94 μm, liquid water or ice can be used as the laser absorbing matrix which nearly eliminates spectral interference from matrix related ions. The use of ice as a matrix for IR-MALDESI-MSI was systematically investigated using shadowgraphy imaging techniques correlated with corresponding mass spectra. IR-MALDESI-MSI has been applied to drug and metabolism studies as well as hypothesis driven and discovery based investigations to discern the dissimilarities between tissue sections from various biological states. In addition, a freely available and vendor neutral software has been developed to process MSI datasets.

PS06-02 Imaging Mass Spectrometry of Clinical Samples

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Development of molecular imaging technology has potently progressed biology and medicine. Main stream of such methodologies has been staining methods such as immunohistochemistry and genetic manipulation to incorporate fluorescent protein tags into the target proteins. Antibodies do not have one-to-one correspondence to the genetic codes. To this problem, imaging mass spectrometry is one of the solutions. We developed microscopy combined with matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS), a two-dimensional MS technique, in collaboration with Shimadzu. This is a molecular imaging technique to investigate directly the spatial distribution of biomolecules on tissue sections without any time-consuming extraction, purification, and separation procedures [1]. We have enabled visualization and direct on-tissue identification [2, 3]. Innovation in ionization methods and equipments supported these achievements [4]. Having started with microanatomical, physiological analysis of molecular distribution, we are currently extending the research target to the clinical fields including [5]. I would like to present the methodology, application, and recent advances in our MALDI-IMS study.

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PS06-03 A Study of Drug Distribution in Malignant Melanoma Tissue by MALDI Mass Spectrometry Imaging for Evaluation of Drug Efficacy

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Malignant melanoma (MM) is a disease with ever-increasing detection rates in the industrialized countries. However, the elevated incidence frequency has not been paralleled by the development of novel therapeutic agents with a significant impact on survival. Currently, limited therapeutic options exist for patients with metastatic melanomas, and all standard combinations used in metastasis therapy have low efficacy. Therefore, to develop the novel therapeutic agents is required for improving outcome of MM patients. In the process of drug development for therapy purposes, one of the key objectives is to optimize the efficacy and safety. Mass spectrometry imaging (MSI) is a powerful tool for pharmacokinetics/pharmacodynamics. MSI will provide a way forward in characterizing drugs and their spatial localization in tissue sections. The aim of this study was to examine the drug distribution within melanoma tissue sections using a pharmacokinetic model that allows quantitative analysis by MALDI MSI. MM tissues were prepared in sections of 10 μm in thickness prior to be exposed to drug by our in-house designed microdispenser platform. Samples coated with a matrix (α-cyano-4-hydroxycinnamic acid) were analyzed by a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany). The drug fragment ions were visualized by the ImageQuest software (Thermo Scientific) and quantified by QUANTINETIX (Imabiotech, Loos, France). We examined the tissue localization of several drugs, which have previously reported in MM studies, and detected the parent and fragment ion mass signals with high sensitivity. These compounds were evaluated for further investigations to measure the drug efficacy in clinical settings.

Keywords: imaging, mass spectrometry, malignant melanoma

PS06-04 Peptide MALDI Imaging - How to Get Most Out of Your Sample?

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Introduction: Matrix-assisted laser desorption ionization (MALDI)-Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection especially peptide IMS. The overall goal of this study was to test and compare various protocols of tissue digest in order to improve the quality of IMS data.

Material and Methods: In the presented work the MALDI-ImagePrep™ device from Bruker Daltonics was used for automated spraying of trypsin and matrix. Ten μm thin rat brain tissue sections served as samples. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics). The experimental set up consisted of different experiments varying the trypsin incubation time, the matrix, the protease and the raster width of the laser. Every obtained image was analyzed using the flexImaging™ and the SCiLSLab software (Steinbeis Innovation Center SCiLS, Bremen).

Results: A total of 73 slides were processed and measured. It has been noted that the type of matrix has a significant impact on the number and quality of resolved structures. Similar observations were made for the experiments with trypsin overnight digestion. However it also became clear that the vast amount of external factors can have a large impact on the overall performance and outcome of an on-tissue digestion protocol. The SCiLS Lab software facilitated the comparison of sample sets due to the analysis of more than one sample simultaneously.

Conclusion: A crucial aspect is without doubt the quality of the sample. Analyzing such a comprehensive dataset is time consuming and difficult due to the variability of the parameters and more importantly the lack of tools for objective post-processing. Help may be provided by the SCiLS Lab software allowing the analysis of many samples in parallel.

Keywords: MALDI imaging, method improvement, rat brain

PS07-01 Global Mapping of Mitochondrial Interaction Networks Connects Mitochondrial Function to Neurodegenerative Disease

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Proteomic approaches such as affinity purification in combination with mass spectrometry (AP-MS) and yeast two-hybrid have been proven successful in the isolation of native soluble or membrane protein-protein interactions and complexes in model organisms, including the yeast *Saccharomyces cerevisiae* and the eubacterium *Escherichia coli*. In this talk, I will discuss how the AP-MS purification strategy, in particular, can be a powerful tool for generating interactome maps for disease-causing mitochondrial proteins (MPs). MPs are involved in many cellular processes, as a result of their propensity to interact with each other and with other extra-compartmental proteins, and hence defects in their function have emerged as causative factors for diverse human disorders, particularly neurodegenerative (ND) diseases like Parkinson's, Alzheimer's and Huntington's. To date, biochemical and genetic investigations have uncovered only a small number of MPs involved in ND diseases. Given the diversity of processes affected by mitochondrial function, and because it is difficult to pinpoint the role of mitochondrial dysfunction in human diseases, many more remain unknown. We are addressing this deficit by focusing on over 600 putative disease-causing MPs, of which we have affinity purified roughly 60 lentiviral tagged proteins in the mammalian model human embryonic kidney (HEK293) cell line. Interactors of the purified proteins were then identified with a high performance Orbitrap Elite mass spectrometer. Our assay captured both previously known interacting proteins, as well as several new associations that have not been reported previously. The data we have generated so far provides new insight into the complex etiologies of ND disease, and opens avenues for identifying new therapeutic drug targets that could ameliorate many diseases all together.

PS07-02 Decoding Ligand Receptor Interactions

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Ligand-induced changes in cell surface receptors result in physiological responses, which constitute the biological activity of various ligands such as proteins, peptides, pharmaceutical drugs, toxins or whole pathogens. However, traditional approaches for the ligand-based identification of corresponding receptors are usually limited to non-transient, high affinity interactions and highly artificial experimental set-ups. Therefore, many signaling molecules remain orphan ligands without a known primary molecular target – invaluable information in understanding the respective mechanisms of signal transduction, drug action or disease. Previously, we have developed the cell surface capturing (CSC) technology for the unbiased identification and quantification of cell surface N-glycoproteomes by mass spectrometry (MS). This demonstrated the powerful applicability of chemical reagents in the tagging of cell surface glycoproteins at carbohydrate groups and the subsequent purification of the corresponding peptides for MS analysis. Based on these results we now synthesized trifunctional cross-linkers for the ligand-based tagging of glycoprotein receptors on living cells and the purification of receptor-derived peptides for MS analysis. Through quantitative comparison to a sample generated with an unspecific control probe, this ligand-based receptor capturing (LRC) approach allows for the highly specific and sensitive detection of ligand interactions with their corresponding receptors under near-physiological conditions. Experiments with ligands ranging from peptide hormones to clinical antibodies demonstrate the potential of this approach to specifically identify one or more target receptors for a given ligand with great statistical power. Advanced discovery-driven applications reveal potential receptors and receptor panels for ligands ranging from protein domains to intact viruses. Together, I will present a short summary of our recent biomedical research to understand the surfaceome as a cellular signaling gateway and a chemoproteomic technology for the unbiased detection of ligand-receptor interactions on living cells.

PS07-03 A Plasma Membrane Proteomic Analysis of Mouse and Human Cardiovascular Proteins

Parveen Sharma, Nicolas Bousette, Savo Lazic, Cynthia Abbasi, Nicole Dubois, Alex Ignatchenko, Vladimir Ignatchenko, Jie Liu, Allen Teng, Melissa Noronha, Robert Hamilton, Peter P. Liu, Peter H Backx, Gordon Keller, Igor Stagljar, Ian C. Scott, Thomas Kislinger, Anthony Gramolini

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We employed cationic silica-bead coating coupled with shotgun proteomics to enrich and identify cell-surface associated proteins from primary mouse neonatal and human fetal ventricular cardiomyocytes, endothelium, and smooth muscle cells. Human coronary artery smooth muscle and endothelial cells, and human cardiac muscle derived cardiomyocytes were cultured. Human fetal ventricular myocytes were acutely dissociated. Membrane proteins were cross-linked to cationic silica beads to isolate cytosolic proteins and a membrane fraction attached to the beads. Samples were analyzed by LC-MS MuDPIT strategies on a Thermo LTQ or LTQ Orbitrap. Shotgun proteomics identified >3,000 mouse and >2,500 human proteins. Organelle enrichments were confirmed by immunoblotting. Mapping of orthologous proteins between mouse and human resulted in 1717 proteins. In the cardiomyocytes, QSpec statistical analysis calculated differential spectral counts between proteins found in the membrane enriched and membrane depleted fraction and provided a dataset of 555 cardiomyocytes proteins. Bioinformatic integration with transmembrane helix predictions, Phenotype Ontology (PO), and available microarray data identified a rank ordered set of cardiac-enriched surface proteins; select examples of which the subcellular location were further confirmed using confocal microscopy, immunogold electron microscopy, and sucrose density gradients. For several of the highly ranked membrane proteins, lentiviral-based shRNA knock-down demonstrated significantly altered Ca^{2+} transient amplitude, release rates, and uptake rates. Initial knockouts in Danio rerio (zebrafish) support the cultured myocyte studies. In conclusion, we have provided the first comprehensive analysis of membrane cell surface-associated proteins in all three major cardiac cell types, and provided a pipeline to validate surface proteins in cardiomyocytes that might be involved in Ca^{2+} dynamics.

Keywords: cardiomyocyte, membrane proteomics, cell surface

PS07-04 Development of Novel Membrane Protein Marker for Lung Cancer Diagnosis and Therapy

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Lung cancer is the one of common cancers and the fetal disease causing high death rate. Lung cancer is histologically divided into four types: adenocarcinoma, large-cell carcinoma, small-cell carcinoma, and squamous-cell carcinoma. High death rate of lung cancer is because of late diagnosis and absence of effective treatment. Thus, it is important to develop an efficient method for diagnosis and treatment. Targeted therapy is a rising method for cancer therapy and has a potential clinical benefit. Especially, cancer specific membrane proteins are useful as biomarker. In this study, we found candidate proteins for biomarker of lung cancer and confirmed that they can target four types of human lung cancers.

We analyzed proteins expressed in normal human lung tissue and in four types of human lung cancer cell lines. We got the list of 1,340 proteins by MS/MS. Among them we chose four promising proteins which can be used as biomarker of lung cancer. For further validation, we prepared antibodies against candidate proteins and carried out various experiments to verify the specific expression of candidates in the membrane of lung cancer cell. Also, we performed antibody treatment in lung cancer cells and in the xenograft model to analyze whether antibody can regulate proliferation of lung cancer cells.

According to results of western blot and immunocytochemistry and immunohistochemistry, we concluded that candidate proteins are expressed in human lung cancer cell specifically. In addition, the result of flow cytometry showed candidate proteins are located in membrane of human lung cancer cell lines. Furthermore, we found that antibodies against candidate proteins can kill lung cancer cells and block the growth of lung cancer tumor in xenograft model. Besides, in vivo and ex vivo imaging represented antibodies can target lung cancer tumor. These results suggest four candidates can play a significant role in targeting lung cancer for diagnosis and treatment.

Keywords: lung cancer, membrane protein, biomarker

PS07-05 Proteomic Profiling of Ganglioside-Associated Microdomain in Malignant Melanomas

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Melanoma is difficult to cure because of its malignant properties. Ganglioside GD3 levels are highly elevated in melanomas. It has been shown that GD3 enhances cell proliferation and invasion using GD3 synthase-transfectant cells of a GD3-negative (GD3-) mutant line SK-MEL-28 N1. p130Cas, paxillin and FAK were identified as highly tyrosine-phosphorylated molecules involved in the increased cell proliferation and invasion with GD3 expression. However, remaining issue to be clarified is how GD3 interacts with known/unknown molecules in the vicinity of cell membrane. To clarify these mechanisms, we isolated the glycosphingolipid-enriched microdomain (GEM)/rafts with sucrose density-gradient ultra-centrifugation of Triton X-100 extracts from GD3+ and GD3- cells. We also labeled cell surface molecules present in the vicinity of a target molecule in living cells with EMARS reaction (Honke et al.). Isolated molecules as components in GEM/rafts and EMARS products were comprehensively analyzed with LC/MS (LTQ-Orbitrap). In the GEM/rafts, 73 membrane proteins were identified in GD3+ and GD3- cells. Among them, 50 membrane proteins were common. In the EMARS with GD3, 9 molecules such as neogenin, integrin $\alpha 3$, $\beta 1$ and MCAM were identified as GD3-interacting molecules. They were also identified in the GEM/raft fraction. To check the results of LC/MS, we performed immuno-blotting and confocal microscopy analysis. Neogenin and MCAM were found in GEM/raft fraction of GD3+ cells and labeled with EMARS reaction in immuno-blotting. They were co-localized with GD3 in confocal microscopy analysis. Differences in the molecular profiles identified in GEM/rafts and as EMARS products suggest the heterogeneity in GEM/rafts.

Keywords: Lipid raft, Glycosphingolipid, EMARS

PS08-01 Integrated Site Specific Mapping of N-linked Glycopeptides for Cancer Biomarker Discovery in Human Plasma by Mass Spectrometry

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The glycoprotein analysis (GPA) system was developed to identify the N-linked glycopeptide including glycan compositions and amino acid sequences and quantify each glycopeptides. Three scoring algorithms was used for automated high-throughput glycopeptide identification; M-score for glycopeptide selection, S-score for glycopeptide match, and Y-score for glycopeptide identification. The GPA platform were basically designed to utilize MS and MS/MS data from most type of MS spectrometer such as QTOF-MS, Orbitrap-MS, and FT-MS.

Typical standard glycoproteins were digested with trypsin and analyzed by LC/MS to obtain high resolution MS and MS/MS spectra with HCD or CID fragmentation. In order to select N-glycopeptides in the GPA system, the glycan oxonium ions and accurate MS spectra were used. Label-free quantification of the identified N-glycopeptides between normal and cancer was performed.

This GPA platform has been successfully evaluated for the site-specific mapping of different N-glycoproteins for cancer biomarker discovery to identify and quantify different glycoproteins in human plasma including medium and low abundant proteins, such as TIMP-1 with HILIC enrichment and LC/MS/MS analysis. The increased levels of fucose, sialic acid, N-acetyl glucosamine, and highly branched N-linked glycans were found to be associated with cancer more than normal case.

PS08-02 Nerve Growth Factor and Its Precursor as Cancer Biomarkers and Targets: Using Proteomics from Discovery to Validation

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Nerve growth factor (NGF) and its precursor (proNGF) are well know for their role in the development of the nervous system where they participate in neuron survival and differentiation. We have discovered that NGF can stimulate breast cancer cell growth and using proteomics as well as other molecular biology approaches we have shown that it is overexpressed in breast tumours. Moreover, ProNGF is also secreted by breast cancer cells and has the effect of enhancing tumour cell invasion. In animal models, targeting NGF and proNGF or their receptors TrkA, $p75^{NTR}$ or sortilin resulted in an inhibition of tumour growth and metastasis and therefore NGF and proNGF are potential clinical biomarkers and targets in breast cancer. Bench to bedside translation of biomarkers involves validation studies with large cohorts of cancer samples as well as the development of tools for clinical applications. An integrative approach involving immunohistochemistry, ELISA, and multi reaction monitoring (MRM) mass spectrometry has been developed to analyse tumours and blood samples and to explore correlation between the levels of proNGF/NGF and their receptors with clinicopathological parameters including tumour subtype, estrogen and progesterone receptors, HER2, node invasion and patient survival. These studies have highlighted a number of features, such as a higher level of proNGF in the blood of breast cancer patients as compared to normal controls, suggesting proNGF as a possible biomarker for diagnosis, and a relationship with node invasion indicating that it may also be a prognosis biomarker for the risk of developing metastasis. Interestingly, overexpression of NGF/ProNGF was also found in lung, thyroid, and prostate tumours, expanding their clinical value to other cancers.

Keywords: Cancer, Breast, Lung, Thyroid, Prostate, Biomarkers and Therapeutic Targets

PS08-03 Convergence of Genomics with Proteomics to Better Understand Cancer Biology - The NCI Clinical Proteomic Tumor Analysis Consortium

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The National Cancer Institute (NCI), part of the National Institutes of Health (NIH), has established a consortium of research centers that are focusing on identifying proteins that derive from cancer genomes. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) adds to NCI's ongoing initiatives in molecular biology technology-based research programs, such as The Cancer Genome Atlas (TCGA), that comprehensively characterize tumors and make its findings available to the public. Initiatives such as TCGA have characterized and sequenced the genomic alterations from several types of cancer, providing a catalog of alterations in a cancer genome and setting the stage for the development of more molecular interventions that attack cancer cells based on their specific genetic makeup. CPTAC is leveraging its state-of-the-art, proteomic technologies to comprehensively connect genomic alterations to cancer biology with proteomics. A unique feature of CPTAC is the utilization of genomically characterized biospecimens (such as those from TCGA) in conjunction with an independent prospective biospecimen cohort to confirm unique biological findings. Data, assays, and protocols produced from this program will be made available to the public. This seminar will discuss the questions being addressed by the program and what it hopes to accomplish in furthering our understanding of cancer biology.

Keyword: genomics, proteomics, proteogenomics, cancer

PS08-04 Label-free Quantitative Personalized Tissue Membrane Proteomics and Targeted Membrane Glycoprotein Profiling for Gastric Cancer Biomarker

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The glycoproteins and/or membrane proteins hold promises for discovery of cancer biomarker because most FDA-approved cancer biomarkers are secreted glycoproteins. Unfortunately, some of the current cancer biomarkers, such as CEA, CA 19-9, and CA 72-4 have low sensitivity and specificity for gastric cancer (GC) detection. Recent studies suggest that analysis of tumor tissues can directly lead to the investigation of the origin of cancer and generate potential markers with higher specificity and sensitivity. In this study, we applied an informatics-assisted label-free quantification method for personalized membrane proteomics analysis of paired of cancerous and adjacent normal tissue from patients with gastric cancer. In this study, individual membrane proteins were first purified from paired tumor and adjacent normal tissues of 24 GC patients with different stages. The purified membrane proteins were mixed with internal standard protein, subjected to gel-assisted digestion. Finally, extracted peptides were analyzed in triplicate by LC-MS/MS. The analysis quantified 1752 proteins across different patients; 1058 were classified as membrane proteins or membrane-associated proteins including 525 plasma membrane proteins annotated by Gene Ontology, Ingenuity Pathway Analysis Knowledge Base, and TMHMM prediction. There are 497 significantly up-regulated expressed proteins in the four different stages of GC patients including the well-documented GC biomarkers: EGFR, ENO1 and PPIA. These previous biomarker candidates show up-regulation in <40% patients, suggesting their low sensitivity in for clinical utility. Therefore, we select 8 biomarker candidates which satisfy criteria including structural characterization as glycosylated membrane proteins and ability to secret or shed in serum; five of these proteins had been reported to associate with GC. Those candidates will be validated extensively in well-defined retrospective and prospective clinical samples.

Keywords: membrane protemoics, gastric cancer, biomarker

PS09-01 Towards a Comprehensive Characterization of a Human Cancer Cell Phosphoproteome

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We recently demonstrated that by using a refined strong cation exchange (SCX) chromatographic system one can isolate multiply phosphorylated, N-acetylated and singly phosphorylated peptides. Analysis of these near pure populations, once again, emphasized their inherent complexityand the deficiencies of the final LCMS step. In order to make these pools more palatable by LCMS we developed additional fractionation strategies based on hydrophilic interaction liquid chromatography (HILIC) or weak anion exchange (WAX). Taking a single SCX fraction and then subjecting it to WAX we were able to dramatically increase the number of phosphosites identified.

The SCX system does not allow purification of phosphopeptides containing multiple basic residues (basic phosphopeptides). Such peptides are often products of phosphosites that are adjacent to basic residues and are often generated by basophilic kinases. Unfortunately these peptides are often poorly enriched by chelation strategies since there is frequently a salt bridge present between the phosphate and amino/guanido groups hindering coordination and enrichment. Here, we show a careful choice of chelation material (Ti-IMAC) and solvent conditions can dramatically improve enrichment. In our hands we have found that ETD of such peptides is far superior to classical HCD or CID sequencing.

Our removal of biases in phosphopeptide enrichment allowed a protocol to be formed that allows over 90% enrichment on a lysate. I will demonstrate that enrichment followed by a 3 hour single LCMS analysis one can obtain approx. 5000 sites or 15,000 phosphosites in a simplified 2DLC strategy.

I will finish by discussing a new sequencing method we have developed (we refer to as EThcD) that provides excellent tandem mass spectra of phosphopeptides and often allows unambiguous site localization. In this presentation I hope to show that there are many (complementary) tools available to interrogate the phosphoproteome.

PS09-02 Sequential Phosphoproteomic Enrichment through Complementary Metal-Directed Immobilized Metal Ion Affinity Chromatography

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There have been recent advances in instrumentation and analytical strategies aimed at the identification and quantitation of protein phosphorylation. Despite these advances, methodologies to enrich heterogeneous types of phosphopeptides are critical for comprehensive mapping of the under-explored phosphoproteome. Taking advantage of the distinct binding affinities of Ga^{3+} and Fe^{3+} for phosphopeptides, we designed a tip-based, metal-directed immobilized metal ion affinity chromatography (MD-IMAC) procedure for the sequential enrichment of phosphopeptides. In Raji B cells, up to 92% of the 6,283 phosphopeptides were uniquely enriched in either the 1st Ga^{3+}-IMAC (41%) or 2nd Fe^{3+}-IMAC (51%). The complementary properties of Ga^{3+} and Fe^{3+} were further demonstrated through the exclusive enrichment of almost all of the 1,214 multiply phosphorylated peptides (99.4%) in the Ga^{3+}-IMAC, whereas only 10% of the 5,069 monophosphorylated phosphopeptides were commonly enriched in both fractions. The application of our sequential Ga^{3+}-Fe^{3+}-IMAC approach to human lung cancer tissue allowed the identification of 2,560 unique phosphopeptides. In addition to the above mentioned monophosphorylated peptides and multiply phosphorylated peptides, this fractionation ability was also demonstrated on the basic and acidic phosphopeptides This MD-IMAC strategy provided complementary mapping of different kinase substrates and their phosphorylation sites in multiple cellular pathways related to cancer invasion and the metastasis of lung cancer. Given the fractionation ability, reproducibility, sensitivity and ease of tip preparation demonstrated for this Ga^{3+}-Fe^{3+}-IMAC technique, we propose that this strategy allows more comprehensive characterization of the phosphoproteome both *in vitro* and *in vivo*.

PS09-03 Enhanced Phosphopeptide Identification in Escherichia Coli by Stepwise Hydroxy Acid-Modified Metal Oxide Chromatography with Elevated Sample Loading Capacity

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Protein phosphorylation in bacteria is as important as in other living organisms to regulate physiological processes. However, the much lower phosphoprotein stoichiometry severely hampered the progress in phosphoproteomic field in microorganisms. Here, the stepwise enrichment with large-scale hydroxy acid-modified metal oxide chromatography (HAMMOC) was integrated into phosphoproteomic analysis in gram-negative model organism, *Escherichia coli*. The large-scale HAMMOC (200-μL C8 StageTip) showed the comparable enrichment efficiency with the conventional one (10-μL C8 StageTip). From 100 μg protein lysates, 31 and 38 phosphopeptides accompanied with 137 and 122 non-phosphopeptides were identified in conventional and large-scale HAMMOC, respectively. Also, these two methods had similar enrichment specificity with an average of 48.8 % and 44.6% in conventional and large-scale HAMMOCs. As increasing the lysates from 500 to 1000 μg with 100 μg interval, 998 unique phosphopeptides accompanied with 4,186 non-phosphopeptides were identified in total by large-scale HAMMOC. 268 serine, 104 threonine, and 38 tyrosine phosphorylation sites were found from 318 phosphoproteins yielding a Ser/Thr/Tyr phosphorylation ratio of 65.4/25.4/9.2%. Since the large number of non-phosphopeptides would suppress the phosphopeptide ionization and thus hindering the identification of phosphopeptides, further development on the stepwise strategy was adopted to reduce the non-phosphopeptides. Successfully, the enrichment specificity was significantly improved to higher than 95%. This approach exhibited its advantages in higher enrichment efficiency in aspect of less sample amount and LC-MS/Ms analysis time and simpler manipulation with higher number of identified phosphopeptides and phosphoproteins. Therefore, this method displayed its feasibility in bacterial phosphoproteomic analysis.

Keywords: bacterial phosphoproteomics, phosphopeptide enrichment

PS09-04 Unravelling Cell Signaling Events with Sub-Minute Temporal Resolution

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Large-scale phosphoproteomics studies enable the profiling of more than 15000 phosphosites from mg size of cell extracts. Investigation of dynamic changes in protein phosphorylation is a promising approach to dissect signaling events and correlate interactions between kinases, phosphatases and their substrates. Many protein phosphorylation/dephosphorylation events take place rapidly in response to environmental perturbation and thus require special sample handling techniques. Here, we present a novel sample collection protocol tailored for phosphoproteomic analysis of fast signaling events. We measured changes in phosphorylation within the first minute following osmotic shock in *Saccharomyces cerevisiae* with a temporal resolution of 5 sec. Our approach provided an unprecedented temporal resolution enabling the collection of high quality phosphorylation profiles for ~5,500 phosphosites on ~1,600 proteins. Evolutionary analysis showed that our dynamic phosphosites are more conserved than static ones, and are more functionally significant. We also found high enrichment of these sites on regulatory proteins such as kinases and phosphatases. Particularly we detected dynamic changes in phosphorylation on more than 25 % of the 128 kinases from *S. Cerevisiae* proteome. Temporal resolution achieved in our experiments enabled the dissection of dynamic events within a MAPK pathway responsible for high osmolarity including dual phosphorylation of MAPK Hog1. We also for a first time identified dynamic changes in phosphorylation on the majority of key players such as Sla1 (S785, S996), Abp1 (S357), Akl1 (S12, S985), and Syp1 (S347, S405) involved in clathrin mediated endocytosis. Subsequent site mutagenesis experiments confirmed our findings and allowed us to get deeper understanding of interplay between osmotic shock and endocytosis events.

Keywords: phosphoproteomics, dynamics, osmotic shock

PS10-01 Dissecting the Sub Cellular Proteome

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Many proteins exist in highly controlled micro-environments, which range in scale from organelles, sub-organelle compartments, and clusters of membrane bound proteins to multi-protein complexes. The components of such micro-environments vary in a role-dependent manner increasing functional diversity. Our knowledge of the constituent proteins in all of these niches is important to the analysis of protein function and our understanding of cellular systems. Moreover, the ability to look at changes in niche components is vital to the elucidation of the mechanisms associated with disease.

The characterization of a protein's location has traditionally involved relatively low throughput microscopy or co-isolation of proteins in complex coupled with antibody based detection methods. Such approaches tend to be targeted and require high quality reagents to ensure robust data. More recently, the emergence of high throughput quantitative mass spectrometry has allowed analysis of both location and interaction partners on a proteome-wide scale.

The comprehensive determination of sub-cellular niches is beset with numerous technical challenges including the necessity to express proteins at non-physiologically relevant abundance levels, or to express tagged proteins which may behave differently than their native counterparts. Moreover, it is often impossible to purify sub-cellular niches and complexes without a significant level of contamination from unrelated proteins leading to false discoveries. Furthermore, many interactions of interest are weak or transient, and highly dynamic and thus difficult to capture.

In this presentation, I will describe emerging quantitative proteomics tools that specifically target membrane proteins giving information about their sub-cellular locations and binding partners. These methods allow simultaneous and accurate protein localization of membrane proteins across multiple locations and do not rely on the complete purification of any niche of interest. I will describe tools that we have developed which employ novel machine learning methods for data analysis. I will also introduce a novel method for defining the local environments of proteins within membranes.

PS10-02 How to Submit MIAPE Compliant Data to ProteomeXchange Repository in the Context of the Human Proteome Project

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The Chromosome-Centric HPP is one component of the HPP and focuses on constructing the proteomic catalog in a chromosome-by-chromosome fashion (www.c-hpp.org). This implies to generate and create publicly accessible data and informational resources supporting better proteomics experiments for a wider range of the life science community. It is expected the amount of submitted data will continually grow as HPP begins to produce data from many teams. Metadata about sample processing and experimental procedures becomes crucial for HPP project where information about how each proteoform has been experimentally detected from a certain sample/tissue should be part of the aimed human proteome map. The ProteomeXchange consortium was launched with the aim of providing scientific community a unique entry point for sharing proteomics data, developing the appropriate tools based on HUPO-PSI standards. Metadata about sample and experimental equipment and protocols that is currently required seems to be crucial for the HPP submissions.

Here we present the ProteoRed MIAPE Extractor, which prepares data for a ProteomeXchange submission and additionally provides the way for assuring MIAPE compliance of the submitted data.

The tool is able to firstly extract MIAPE data from PSI standard data files. Secondly, it provides a way for completing the required metadata to reach the MIAPE compliance. Thirdly, it compiles and integrates data coming from several experiments, allowing its filtering, keeping just the reliable data. Finally the MIAPE Extractor prepares all data for a ProteomeXchange submission by compiling all required files in an output folder and creating a PRIDE XML file from each processed experiment data, also including human readable MIAPE compliant reports. Then, an automatic submission using the ProteomeXchange submission tool can be done. This tool is being used by the Spanish Human Proteome Project (SpHPP) consortium and its open for its use from any other consortium.

PS10-03 SOMAmer Capture Coupled to Mass Spectrometry for Plasma Protein Quantification

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Proteins can be quantified with high specificity and sensitivity by mass spectrometric measurement of immunoaffinity-enriched proteins or proteotypic peptides. Such assays are either termed mass spectrometric immunoassays (MSIA) or stable isotope standards and capture by anti-peptide antibodies (SISCAPA) depending on whether the quantification is performed at protein or peptide level.

However, capture molecules are still the limiting resource despite extensive activities in the field of affinity reagent generation. Slow off-rate modified aptamers (SOMAmers) are synthetic protein binders, which are isolated from artificial binder libraries. Availability of such binders is virtually unlimited and their production is more cost efficient compared to e.g. antibody binders. We demonstrate a workflow where plasma proteins and their isotopically labeled counterpart are precipitated using SOMAmers. The enriched proteins are digested on-bead and quantified by selected ion monitoring mass spectrometry with peptide redundancy for each protein. Advantages and disadvantages compared to peptide-centric approaches such as SISCAPA will be discussed by means of comparative data generated with SOMAmers.

Keywords: Mass spectrometric Immunoassay, Slow off-rate modified aptamers, Plasma proteins

PS10-04 Protein Quantitative Trait Locus (pQTL) Analysis in Mouse by Targeted Proteomics

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The hallmark of targeted proteomics is the quantification of a set of predefined peptides in a complex sample by the acquisition of specific precursor ion to fragment ion transitions over time. As the main implementation of this concept, selected reaction monitoring (SRM) has become a technology that ideally complements some limitations of shotgun strategies by its unique potential of SRM for reliable and reproducible quantification of proteins of low abundance in complex mixtures. To correlate protein abundance with genetic variations, a protein quantitative trait locus (pQTL) analysis relies on consistent and precise quantification of a set of peptides throughout a large number of samples. Here we applied SRM to a pQTL analysis across 58 liver samples of 29 diverse mice strains (BXD type derived from C57BL/6J and DBA/2J strains) to study the genetic control of 155 metabolic proteins. In total, we found 130 QTLs under chow diet or high fat diet, among which DHTKD1 and 1433B map significantly to the same QTL under both conditions. DHTKD1 is one component of the 2-oxoglutarate dehydrogenase complex, and its mutations cause 2-aminoadipic and 2-oxoadipic aciduria in human. We found a strong correlations between DHTKD1 protein levels in the liver, α-aminoadipic acid levels in plasma, and α-ketoadipic acid in the urine. These modulated metabolite levels, which are also observed in humans with malfunctioning DHTKD1, appear to be dependent on sequence variants in the Dhtkd1 gene between the two parent strains. Furthermore, most proteins were not cis-regulated (i.e. by their own gene) and were often differentially regulated between the two dietary conditions. Our results suggest targeted proteomics-based QTL analysis as a powerful strategy to study correlation among genetic variance, protein abundance and phenotypes in complex systems and to reveal regulatory networks of metabolic diseases.

Keywords: protein quantitative trait locus (pQTL), targeted proteomics, murine genetic reference populations

PS11-01 (Keynote) The Ubiquitin-Proteasome System

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The ubiquitin-proteasome system (UPS) controls diversed cellular processes - such as progression through the cell cycle, signal transduction, cell death, immune responses, metabolism, protein quality control, and developmental programs - by degrading short-lived regulatory or structurally aberrant proteins. I have been studying the UPS for the past 30 years, focusing on the clarification of the ubiquitin chain puzzling and the structure and functions of the proteasome. The former ubiquitin functions as a key molecule in an elegant post-translational protein modifying system. The latter proteasome is a 2.5-MDa sophisticated multisubunit complex that contains a catalytic core particle (CP) and two terminal regulatory particles (RPs), which associate with the termini of the central CP at opposite orientations. The CP consists of four heptameric rings (two outer α-rings and two inner β-rings), which are made up of seven structurally related α and β subunits. The CP contains catalytic threonine residues (β1, β2, and β5 with caspase-like, trypsin-like, and chymotrypsin-like activities, respectively) on the surface of a chamber formed by two abutting β-rings. The RP recognizes polyubiquitylated substrate proteins and then unfolds and translocates these proteins into the interior of the CP for degradation. One longstanding question is how the complex structure of the proteasome is organized with a high fidelity. Recently, we proposed a novel assembly mechanism that is assisted by multiple proteasome-dedicated chaperones. Moreover, we found the diversity of proteasomes named the immunoproteasome and the thymoproteasome whose catalytic subunits are replaced by homologous counterparts. These two isoforms perform specialized functions that help discriminate self from non-self in cell-mediated immunity. Collectively, emerging evidence suggests that the UPS plays an important role in various intractable diseases that have been increasing in today's aging society.

PS11-02 (Keynote) Molecular Dissection of Autophagy - Intracellular Recycling System -

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Every cellular process is mediated by a balance between synthesis and degradation of proteins. Recently it has become clear that degradation is a highly regulated process, playing critical roles in cell physiology. Proteasome degrades every target protein after strict recognition by ubiquitination reactions, while the lysosome/vacuole system, autophagy, facilitates bulk and non-selective degradation. Under starvation recycling of own proteins becomes crucial for survival. Research also indicates that selective elimination of harmful proteins, organelles, and intracellular bacteria via autophagy is important for maintenance of cellular homeostasis.

More than 20 years ago we first observed autophagy induced by nutrient starvation in the yeast, *S. cerevisiae*, under a light microscope. Taking this advantage of the yeast, we succeeded in isolation of many autophagy-defective mutants. We know now that 18 *ATG* genes are essential for starvation-induced autophagy. These Atg proteins function concertedly in the sequestration of cytoplasmic constituents during the formation of a specialized membrane, known as the autophagosome. The Atg proteins consist of six functional units, namely the Atg1 protein kinase and its regulators, the PI3 kinase complex, the Atg2-Atg18 complex, the membrane protein Atg9, and two unique ubiquitin-like conjugation systems. Since these *ATG* genes are well conserved from yeast to mammals and plants, a vast range of studies in autophagy have recently been undertaken in various organisms.

We are attempting to elucidate the function of these Atg proteins, helping us to understand the unique membrane dynamics during autophagosome formation using the yeast system. To unveil the remaining mysteries of autophagy comprehensive analyses are required, including by microscopy, biochemistry, molecular biology, cell biology, and structural biology. Our present knowledge on the molecular mechanism and physiological roles of autophagy will be presented.

PS11-03 How Unspecific is Protein Degradation by Stress-Induced Autophagy?

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Stress-induced autophagy is regarded as a model for unspecific, autophagosomal bulk degradation of proteins by lysosomes. However, the autophagic flux is generally measured by western blot analysis of single proteins, microscopic techniques addressing autophagosome formation/degradation, or "long-lived protein degradation" assays using radioisotope tracers. These techniques monitor single proteins, whole organelles, or bulk protein degradation, but are unable to characterize the degradation of multiple individual proteins. Using mass spectrometry-based proteomics, we have studied protein abundance differences, turnover, and degradation in cells undergoing autophagy. In addition, we monitored the proteomic composition of autophagosomes by organellar proteomics approaches. Depending on the stress-inducing stimulus as well as the timeframe of stimulation, proteins from different subcellular compartments exhibit distinct dynamics. Also the composition of the autophagosome reflects the cellular needs under the specific stress conditions. Thus, global proteomics analyses highlight stimulus-specific aspects of stressed-induced autophagy. Currently, we aim at deciphering underlying autophagosomal targeting mechanisms responsible for specific protein degradation by autophagy.

Keywords: autophagy, degradation, SILAC

PS11-04 The True Story of How MHC Peptides are Produced

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Cellular protein degradation can be followed by analysis of their degradation products, the HLA peptides. This immunopeptidomes provide an unsurpassed outlook on the degradome of the cells and enables following the dynamics of degradation of the cells' proteins. We performed large-scale immunopeptidome analyses, using dynamic-SILAC labeling by immunoaffinity purification of the HLA molecules and Orbitrap MS. Using specific inhibitors we were able to define the main proteolytic pathways that lead to the formation of these immunopeptidomes. The more interesting effects were observed by inhibiting the proteasomes with inhibitors, such as epoxomicin and bortezomib (Velcade), which affected in a complex manner the rate of synthesis of the cellular proteins and of their degradation, and formation of MHC peptides. While (as expected) the proteasome inhibitors reduced the rates of degradation of many cellular proteins, they increased the degradation (and synthesis) rates of others. Correlating between the rates of production of the source proteins and their derived HLA peptides suggests that the contribution of the proteasomal proteolysis to the production of the HLA immunopeptidome should be reevaluated and that it may contribute more significantly to the production of HLA peptides derived from newly synthesized proteins, possibly including defective ribosome products (DRiPs) and short lived proteins (SLiPs), while a non-proteasomal pathway (such as the autophagosomal pathway) may contribute more to the production of HLA peptides derived from stable, long-lived proteins.

Keywords: HLA peptidome, dynamic-SILAC, proteasome

PS12-01 Quantitative Assessment of the Interactome, Phosphoproteome and Regulome in Bacterial Infection

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Interactome maps are typically generated using affinity purification-mass spectrometry, yeast two-hybrid screening or protein-fragment complementation assay. We have developed a novel method to monitor the interactome that combines SILAC and size exclusion chromatography to derive protein complex composition based on chromatographic co-elution; the use of triplex SILAC also allows measurement of the interactome's response to stimuli. This approach is as sensitive and as specific as more conventional techniques but avoids tagging artifacts and requires two orders of magnitude less work and instrument time. We apply it here to measure the response various interactomes to diseases states, including infection by the facultative intracellular bacterium *Salmonella enterica*, serovar Typhimurium. Presumably through its type 3-secreted effectors, *Salmonella* infection leads to wholesale rearrangement of the interactome. Many of the changes occur in proteins and complexes that are known targets of *Salmonella* effectors but several novel complexes also appear to be targeted. We will also present how the method can be adapted to monitor membrane protein complexes, such as those from the mitochondria and how they change during apoptosis.

Keyword: Interactome

PS12-02 Surrogate Markers for Abeta as Biomarkers for Alzheimer and T-ALL

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The 42 amino acid amyloid β-peptide (Aβ42) is the major constituent of senile plaques, which is the pathological hallmark of Alzheimer disease (AD) and is generated by proteolytic processing of the β -amyloid precursor protein (βAPP). Remarkably, in the CSF of AD patients Aβ42 levels are low, whereas this peptide accumulates within the brain. It has been believed that this discrepancy may be due to the fact that Aβ 42 is largely deposited in insoluble plaques within the brain and that clearance into the CSF is therefore reduced. Although the reverse relationship of Aβ42 levels can be used as a biomarker to some extent after disease onset, better surrogate markers specifically for presymptomatic diagnosis are desperately required.

The canonical Notch signaling is transmitted intracellularly via sequential endoproteolysis of Notch receptors in a ligand-dependent manner. The receptors undergo extracellular shedding by metalloproteases, followed by intramembrane proteolysis by presenilin (PS)/g-secretase. As a result, the intracellular signaling molecule Notch intracellular domain (NICD) is liberated. Because the degradation of the Notch-1 transmembrane domain is thought to require an additional cleavage near the middle of the transmembrane domain, extracellular small peptides (Notch-1 Aβ-like peptide [Nβ]) may be produced. We discovered APL1β28 as a novel and highly sensitive biomarker. This peptide is generated by the same proteolytic mechanism as Aβ42, except that it is derived from a divergent substrate, namely the βAPP-like protein, APLP1. Non-amyloidogenic APL1β28 can be detected in the CSF and its levels correlate with Aβ42 production. Remarkably, the ratio of APL1β28 to total APL1β are significantly increased in familial and sporadic AD patients. We propose using the levels of APL1β28 as a surrogate marker for A β 42 production in the central nervous system. This has clinical importance for the diagnosis and early detection of sporadic AD.

Similarly, we found that the Nβ species are aberrantly secreted in T-lymphoblastic leukemia (T-ALL) cells. The overexpression of pathogenic Notch-1 mutants or the artificial upregulation of S2 cleavage increased the levels of Nβ secretion. Therefore, we considered that high levels of Nβ may be secreted from human T-ALL cells, with abnormally enhanced Notch signaling due to the Notch-1 mutations. These results indicate that N β is a candidate surrogate marker for Notch signal transduction, and potential biomarker for T-ALL.

PS12-03 In Deep Characterization of the Host Pathogen Interaction during Internalization of *Staphylococcus aureus* by A549 Cells

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S. aureus, a Gram-positive coccus, can cause a broad range of diseases in humans ranging from milder skin infections to severe diseases such as the toxic shock syndrome or systemic infections like sepsis or endocarditis. Though widely considered an extracellular pathogen, it became recently obvious that *S. aureus* is able to invade and persist in non-professional phagocytic cells. We therefore developed a protocol which allows the quantification of proteins from bacteria and host cells with cell sorting and LC-MS/MS based proteomics. The host model chosen in the study presented here employs the A549 cell line isolated from a human lung cancer. First, we observed an increase of the intracellular number of bacteria per infected cell until 6.5 h post infection. Second, 842 *S. aureus* proteins were identified and quantified using our proteomics workflow. As part of this data set, bacterial proteins related to the uptake of iron or to the peptidoglycan biosynthesis showed a higher level during infection, while virulence factors and ribosomal proteins were reduced in abundance. Third, proteomic analysis of the host side resulted roughly in the identification of 2,000 and quantitation of 1,000 proteins. Ten proteins belonging to the pigment granule and 27 proteins of the membrane-enclosed lumen were up-regulated. Contrarily, proteins of the non-membrane-bound organelle and of protein biosynthesis were down-regulated when compared to non-infected control cells. Finally, we quantified twelve different cytokines in the cell culture supernatants and observed increased levels of IL-8, IL-6 and IFN-γ in a time frame of 48 h post infection.

Keywords: Host-Pathogen Interaction, isotopic labeling, Voronoi Treemap Visualization

PS12-04 Proteomic Study of Biomarkers for Amyotrophic Lateral Sclerosis in Human Muscle Biopsies

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Amyotrophic lateral sclerosis (ALS) is a serious neurodegenerative disorder characterized by progressive loss of motor neurons causing muscle atrophy. Many research groups looked for ALS protein biomarkers applying proteomic and genomic approaches, using different tissues and body fluids, animal models and real patients. In present study we used muscle biopsies from patients diagnosed with ALS in order to find the altered proteins specific for this disease. We compared three groups of patients: ALS diagnosed, control objects, and patients with other diseases causing denervation (one of them is post-polio syndrome). Our gel-free proteomic approach involved detergent-based protein extraction from the muscle tissue, in-solution digestion of proteins, and isotope dimethyl labelling of tryptic peptides, followed by protein identification and relative quantification by nano-liquid chromatography - high resolution mass spectrometry (nanoLC-MS/MS, Fourier-transform ion cyclotron resonance mass spectrometer, FT-ICR, has been used). We found an array of protein candidates which were: (i) downregulated exclusively in ALS patients' biopsies, e.g. FHL1, Four and a half LIM domains protein 1, (ii) upregulated exclusively in ALS patients' biopsies, e.g. PADI2, Protein-arginine deiminase type-2, (iii) altered in biopsies of patients with other diseases, but not changed in ALS patients' biopsies. These proteins, individually or in combination, could be used as disease biomarkers revealing molecular mechanisms of ALS development. Importantly, after additional verification, these potential biomarkers can be used for diagnosis of ALS from muscle biopsy without a risk to confuse ALS with other denervation (presently ALS cannot be diagnosed though muscle biopsy).

Keywords: ALS, muscle, proteomics

PS12-05 Mass Spectrometry Methods for Surrogate Biomarker Discovery in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most common and severe form of childhood muscular dystrophies affecting about 1 in 3,500 boys. The need to define surrogate biomarkers to monitor DMD progression and response to treatments is becoming crucial as promising treatment strategies for DMD are entering phase II and III clinical trials. The most commonly used surrogate endpoint for DMD to date is the 6 minute walk test. However, this test seems to be not sensitive enough for clinical trials with a short a period of time and also impractical for DMD patients who lost ambulation. In this study we have initiated proteome and metabolome profiling on serum samples from both DMD patients and dystrophin deficient mouse model (mdx). Using nano-hydrogel particle proteome profiling on serum samples from DMD donors (n = 10) and age matched healthy controls (n = 10) we identified 11 candidate biomarkers that were highly elevated (p value < 0.05) in serum of DMD patients relative to controls. Whole serum proteome profiling of mdx (n = 3) and wild type mouse (n= 3) using SILAC mouse spike-in strategy led to the identification of an additional 19 candidate biomarkers that were associated with dystrophin deficiency. Of these 8 were validated in human serum samples. Furthermore metabolomics analysis of DMD serum (n = 5) versus healthy control serum (n = 5) revealed dramatic alterations in the levels of specific amino acids and lipids. Biomarkers associated with disease progression were evaluated and validated in the mouse model.

Keywords: Duchenne muscular dystrophy, serum, surrogate biomarkers

PS13-01 Quantitative Mass Spectrometry (SRM/MRM) to Amyloid Peptides, Tau Protein, and Apolipoprotein E in Human Cerebrospinal Fluid for Alzheimer Disease Diagnosis

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BACKGROUND: Recent improvements in mass spectrometry (MS) allow this technology to quantify with clinical grade analytical sensitivity and specificity, peptides and proteins in biological fluids. We believe that in some cases MS will represent a valuable alternative to immunodetection methods. We followed this path for biomarkers in Alzheimer disease (AD) which represents major cause of dementia. AD is associated with specific apolipoprotein E (ApoE) isoforms, and with alteration of cerebrospinal fluid (CSF) biomarkers. As a matter of fact, the decrease of amyloid peptides (Aß) and the increase of Tau proteins in CSF are currently use for AD diagnosis. Many isoforms of these molecules exist and MS represent an interesting tool to quantify their diversity, and therefore, to improve AD diagnosis and follow-up.

METHODS: For this purpose, quantitative targeted mass spectrometry (SRM/MRM) was developed using a triple quadripole. SPE, trypsic digestion and sample clean-up were realised using an automated liquid handling robot. Quantotypic peptides (Aß1-40, Aß1-42, tau, ApoE..) were synthetized in light and heavy ($^{13}C/^{15}N$) versions and used in calibration curve to evaluate LOD and LOQ. Experiments were run on series of human biological samples.

RESULTS: Optimal MRM methods for the different analytes were developed. Detection of specific Apo E peptides resulted in a rapid method for e2/e3/e4 phenotyping. Different isoforms of Aß and Tau proteins were detected with sensibility compatible with pathophysiological variations. Correlation with immunodetection methods and validation of the clinical relevance of the results are on-going.

CONCLUSIONS: The MS detection of several isoforms of Aß, Tau protein, and Apo E in CSF represents an important achievement that opens new avenue for quantitative Clinical Chemistry Proteomics (qCCP). The perspective is to exploit these results to improve phenotyping, diagnosis and follow-up of dementia.

Keywords: Quantitative Clinical Mass Spectrometry, Neurodegenerative diseases, Alzheimer

PS13-02 High Resolution MRM Quantification of 300 Tear Proteins Using MS/MS^{ALL} with SWATH™ Acquisition and Its Application to Biomarker Discovery

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Many studies have demonstrated that the tear fluid is an accessible and useful source in studying ocular surface disorders and biomarker discovery. The aim of this study was to establish a robust, reproducible and rapid quantitative method for tear protein biomarker study from a large number of clinical samples. Human tear samples were collected from 1000 patients with no eye complaints (411 male, 589 female, average age 55.5 years, SD 14.5 years) using the Schirmer tear test strips and pooled into a single global control sample. A 2-hour nanoLC-MS/MS run was used to separate the tryptic peptides and MS data was recorded using MS/MS^{ALL} with SWATH acquisition on a TripleTOF® 5600 system. Data from three information dependent acquisition (IDA) experiments were combined and used as ion library for subsequent processing of SWATH acquisition data. This method was applied to compare the levels of tear proteins in both post-trabeculectomy glaucomatous eyes (n=11) and normal control eyes (n=8). For quantitation, 1487 peptides representing 474 proteins from IDA were used and the coefficient of variation (CV) of 808 peptides representing 298 proteins was below 20% for five replicates. Quantitative analysis revealed that 27 tear proteins were upregulated (ratio > 2.0) and 20 tear proteins were downregulated (ratio < 2.0) in post-trabeculectomy group as compared to control group. Tear proteomics results suggest that prolonged ocular surface inflammation after trabeculectomy may lead to increased wound healing and reduced surgical success. This study demonstrated that high resolution MS/MS based quantification of hundreds of tear proteins using MS/MS^{ALL} with SWATH acquisition is a powerful workflow for biomarker discovery.

Keywords: SWATH, Tear Proteomics, High Resolution MRM

PS13-03 Development of an LC-MRM Based Assay of Prostate Specific Antigen (PSA) in Blood Samples

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Introduction Quantitative determination of Prostate Specific Antigen (PSA) in the human serum has been used for the early diagnostics and prognostics of prostate cancer. Most of the existing methods utilize enzyme-linked immunosorbent assay (ELISA) to measure the PSA in the serum. The ELISA method, while providing good sensitivity and high throughput for the PSA diagnostics, suffers from low specificity. There is a great deal of interest to find a more reliable diagnostic and prognostic method. In this paper we reported a method to quantify PSA in serum using a simple sample preparation followed by the LC-MS/MS analysis operated in multiple reaction monitoring (MRM) mode. The MRM method provides high sensitivity, high accuracy and good specificity. **Methods** PSA standard was trypsinized and analyzed for peptide identification on an ion trap mass spectrometer initially. The obtained MS/MS data was searched using Mascot software. Two signature peptides from PSA standard were selected for MRM analysis. The MRM method and ion source parameters were further optimized for sensitivity and interference, and linear responses. For serum samples, high abundant proteins such as albumin were removed by solid phase extraction (SPE) to reduce the matrix interference. After trypsin digestion, the PSA peptides were analyzed with the MRM method with external calibration. **Preliminary results** An initial MS/MS study on an ion trap mass spectrometer was carried out on the tryptic digested PSA standard. Based on the high relative abundance and spectra quality and other reported exclusion criteria, two peptides were chosen with their MRM transitions for further optimization. For PSA standard, the LOQ (S/N>10) of the developed MRM assay are at pg/mL level. For PSA in serum after the removal of high abundance protein, the LOQ is at high pg/mL level, sufficient for clinical diagnosis purpose.

Keywords: Clinical diagnostics, MRM triple quadrupole, Biomarker

PS13-04 Improved PhosphoTau SRM Assay Sensitivity Enables Multi-site Tau Phosphorylation Quantitation in a Preclinical Model of AD Treated with Novel Small Molecule Inhibitors of Casein Kinase 1 Delta

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Neurofibrillary tangles, comprised of paired helical filaments (PHFs) of hyper-phosphorylated tau, are a pathological characteristic of Alzheimer's disease (AD). We previously characterised PHF tau isolated from post-mortem AD brain tissue by mass spectrometry. Subsequently, we developed the Phospho-Tau SRM assays to measure tau phosphorylation levels in preclinical AD models. The Phospho-Tau SRM 6plex assay enables the quantitation of total tau plus five phosphorylation sites; distinct human and mouse pThr181 measurements, pSer199, pThr231, pSer262 and pSer396 (human 2N4R numbering). The Phospho-Tau SRM 7plex assay quantifies six phosphorylation sites, pSer46, pThr50, pSer113, pSer396, pSer404, pSer433, as well as distinct measurements covering the R406W mutation, present in the TMHT tau transgenic mouse model. Prior to SRM analysis, phosphopeptides were resolved by microflow (100 μL/min) reversed phase chromatography (XBridge C18 3.5 μM, 1.0 x 100mm, Waters). The linear working range of the microflow-PhosphoTau SRM 6plex assay was 5-1000fmol on column (o/c), with CVs ranging from 5-20%. Per analysis up to 10 μg total protein per sample was consumed. Herein we describe the miniaturisation of the PhosphoTau SRM assays to nanoflow (200nl/min; Easy C18 3 μM ID 75 μM x 100mm, ThermoFisher). Preliminary data demonstrates a 50 fold improvement in linear working range, down to 100 atmol o/c, and detection of endogenous Tau phosphorylation levels in preclinical AD models from as little as 1 μg of material. The utility of the nanflow-PhosphoTau SRM assays to quantify multiple site specific phosphorylation events will be demonstrated in a tau transgenic mouse model treated with novel small molecule inhibitors of Casein Kinase 1 delta.

Keywords: Selective Reaction Monitoring, Targeted Quantitative Phosphoproteomics, Alzheimer's, disease

PS14-01 The Structural Proteomics of Glycoproteins

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It is estimated that approximately half of all proteins encoded by the human genome have attached sugars. Many of these molecules are important cell surface receptors and the target of disease modifying antibodies. Obtaining structural information from glycoproteins presents presents two major technical challenges for structural biology (1) protein production requires eukaryotic expression which is time-consuming and relatively expensive and (2) products are heterogeneously glycosylated which generally militates against crystallization. We have developed methods to address these issues and have assembled a pipeline for producing and crystallizing glycoproteins. The pipeline includes parallelized ligation independent cloning, semi-automated small scale transient expression screening in HEK 293 cells; scale-up of protein production in HEK cells; automated protein purification; quality assessment by mass spectroscopy; and nanodrop crystallization. The application of these processes to solving the structure of a number of glycosylated proteins will be presented.

Review

Aricescu AR, Owens RJ. Expression of recombinant glycoproteins in mammalian cells: towards an integrative approach to structural biology Curr Opin Struct Biol. 2013 Apr 25. pii: S0959-440X (13) 00055-9. doi:10.1016/j.sbi.2013.04.003. [Epub ahead of print]

PS14-02 Structural Insights into the EGFR Pathway Substrate Dephosphorylation by Protein Tyrosine Phosphatase N3

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Reversible tyrosine phosphorylation is an essential post-translational modification in the regulation of numerous cellular activities. This fundamental mechanism is controlled by protein tyrosine kinases, protein tyrosine phosphatases (PTPs) and thousands of their substrates in cells. PTPs represent a large family of evolutionarily conserved enzymes, which are responsible for the phosphate removal via a phosphoryl-cysteine intermediate. In this project we have focused on PTPN3, a member of the membrane-associated tyrosine phosphatase sub-family contains an N-terminal FERM domain, a PDZ domain and a C-terminal PTP domain. Our recent findings have indicated that PTPN3 is a specific phosphatase for an epidermal growth factor receptor (EGFR) pathway substrate (EPS). Dephosphorylation of EPS by PTPN3 is important for the regulation of EGFR internalization in non-small cell lung cancer. To determine the molecular basis of PTPN3 substrate recognition, we have solved the crystal structure of PTPN3 catalytic domain in complex with EPS phosphopeptide at 1.6 Å resolution. Binding of EPS phosphopeptide to the PTPN3 active site reveals a novel conformation, which is different to other PTP-phosphopeptide structures. Our phosphatase activity confirmed a high level of substrate specificity between PTPN3 and EPS. Employing the biochemical approach and structural analyses, we have identified several key PTPN3 residues involved in the recognition of EPS. Its effect *in vivo* is currently being investigated. In a parallel study, we have also determined the crystal structure of PTPN3 in complex with the MAPK12 phosphopeptide at 2.5 Å resolution. The interaction between PTPN3 and the MAPK12 phosphopeptide reveals a similar binding pattern to other PTP-phosphopeptide structures, which further emphasize the novelty of EPS recognition by PTPN3.

Keywords: Dephosphorylation, Phosphatase, EGFR

PS14-03 Cooperative Formation of a Multiple Transcription Factor Assembly on an Enhancer DNA and Its Regulation by Phosphorylation of a Transcription Factor via a Cell Signaling

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Eukaryotic transcription factors (TFs) activate target genes by forming specific TFs-DNA complexes on target gene enhancers. These events are characterized by a cooperative TFs binding on the target DNA and also considered to be regulated by chemical modifications of TFs within the complex.

To understand the mechanism for regulation of higher order TFs-DNA complexes, we focused on a complex formed on the *TCRα* gene enhancer, which is comprised of multiple hematopoietic TFs including Ets1, Runx1/CBFβ and LEF1. Among these TFs, Ets1 is known to be phosphorylated via a Ca^{2+}-dependent cell signal, and the phosphorylated Ets1 appreciably inhibits its own DNA binding activity. We recently performed crystallographic and biochemical analyses of the Ets1-Runx1-CBFβ-DNA complex formed on the *TCRα* gene enhancer and found that Runx1 allosterically modulates the conformation of Ets1 through the DNA molecule, thereby enhancing the DNA binding activity of Ets1 and counteracting the autoinhibitory effect of phosphorylation of Ets1 for the DNA binding.

Now, to understand the mechanism in detail, we have engaged in analyses of the inhibitory mode by which phosphorylation of Ets1 affects its DNA binding. In parallel, to reveal a complete picture of the TFs-DNA complexation on the *TCRα* gene enhancer, we proceed with crystallographic analyses of the LEF1-Runx1/CBFβ-DNA and the Ets1-$(Runx1/CBF\beta)_2$-DNA complexes.

We will discuss a possible mode of cooperative DNA binding of these TFs and their interplay with phosphorylation.

PS15-01 Chemical Proteomics for Drug Discovery: Drug Targets, Drug Selectivity, Drug Mechanism of Action and Drug Resistance

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Preclinical stages in the drug discovery process require a multitude of biochemical and genetic assays in order to characterize the effects of drug candidates on cellular systems and model organisms. Early attempts to apply unbiased proteomic techniques to the identification of protein targets and off-targets as well as to elucidate the mode of action of candidate drug molecules suffered from a striking discrepancy between scientific expectations and what the technology was able to deliver at the time. Dramatic technological improvements in mass spectrometry-based proteomic and chemoproteomic strategies have radically changed this situation in the past few years. On the example of kinase inhibitors, this presentation highlights chemical proteomic approaches suitable for differnt aspects of drug discovery. These are illustrated by examples from my laboratory including the identification of drug targets, the determination of the selectivity of drugs, the elucidation of the mechanism of action of a drug and the detection of resistance mechanisms.

Reference:

Schirle, Bantscheff and Kuster (2012) Mass Spectrometry-Based Proteomics in Preclinical Drug Discovery. *Chemistry & Biology*, **19** 72-84.

PS15-02 Systems-Level Analysis of Inflammatory Proteolytic Events In Vivo by N-Terminomics Analyses

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During inflammation, local tissue responses are augmented by complement and acute phase proteins that exude into the tissue because of increased blood vessel permeability mediated by bradykinin, which is proteolytically released from kininogen. We quantified changes in the proteome and the nature of protein N-termini (the N-terminome) and the altered abundance of murine proteases and inhibitors during skin inflammation. Through analysis of the N-terminome by iTRAQ-terminal amine isotopic labeling of substrates (TAILS), we identified cotranslational and posttranslational αN-acetylation motifs, quantitative increases in protein abundance, and qualitative changes in the proteolytic signature during inflammation. Of the proteins identified in normal skin, 50% were cleaved, which increased to 60% during inflammation caused by phorbol esters, including chemokines and complement in which we identified previously uncharacterized cleavage sites. In mice deficient in matrix metalloproteinase 2 (MMP2), exudation of serum proteins was diminished compared to that in wild-type mice, and their proteolytic networks differed. Quantitative analysis of the neo-N terminal peptides revealed a novel MMP2 cleavage site in complement 1 (C1) inhibitor that was detected *in vivo*. Cleavage and inactivation of the C1 inhibitor by MMP2 increased complement activation and bradykinin generation by plasma kallikrein in wild-type mice, leading to increased vessel permeability during inflammation. In the absence of MMP2, the intact C1 inhibitor levels rose and exerted negative regulatory effects on generation of bradykinin by reducing plasma kallikrein activity and kininogen cleavage. The knock out mice also had reduced complement activation through pathways controlled by C1 inhibitor. Our degradomics analysis dissecting proteolysis in skin inflammation demonstrated perturbance of the proteolytic signaling network and its functional consequences arising from lack of a single protease. The roles of proteases in vivo can therefore be unravelled and either the protease or the pathways they regulate can then be targeted for drug development to restore normal homeostasis.

PS15-03 Cell-Surface Proteins as Drug Targets

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Cell-surface receptors represent attractive targets for drug intervention and numerous cancer therapies are directed towards these molecules using either monoclonal antibodies or low molecular weight inhibitors thereby modifying the functionality of these receptors. Analytical proteomics provide the tools to identify quantify and structurally characterize these cell-surface proteins, of which the majority is glycosylated. A detailed characterization is essential as post-translational modifications, mutations, deletions, and the formation of protein complexes are frequently associated with the biological activity of the receptors.

Targeted high-resolution mass spectrometry methods have been developed to precisely analyze glycosylated cell surface proteins. These have been applied to monitor changes in glycosylation patterns of the primary cell surface receptor targets in health and disease, as well as their downstream "partners" involved in signaling pathways.

The methodology based on the specific isolation of glycoproteins from isolated cells or tissues and the LC-MS characterization will be demonstrated on the EGF-receptor, an important drug target in various forms of cancer. Furthermore, down-stream signaling pathway profiling of wild-type EGF receptor and clinically relevant mutated EGF receptor forms using reversed protein arrays have been employed to trace sensitivity and acquired resistance of tumors towards EGF receptor selective tyrosine kinase inhibitors.

PS16-01 Integrative Multi-Platform Analysis of Cancer Kinome Networks

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Biological systems are composed of highly dynamic and interconnected molecular networks that drive biological decision processes. The goal of network biology is to describe, quantify and predict the information flow and functional behaviour of living systems in a formal language and with an accuracy that parallels our characterisation of other physical systems such as Jumbo-jets. Decades of targeted molecular and biological studies have led to numerous pathway models of developmental and disease related processes. However, so far no global models have been derived from pathways, capable of predicting cellular trajectories in time, space or disease. The development of high-throughput methodologies has further enhanced our ability to obtain quantitative genomic, proteomic and phenotypic readouts for many genes/proteins simultaneously. Here, I will discuss how it is now possible to derive network models through computational integration of systematic, large-scale, high-dimensional quantitative data sets. I will review our latest advances in methods for exploring phosphorylation networks. In particular I will discuss how the combination of quantitative mass-spectrometry, systems-genetics and computational algorithms (NetworKIN [1] and NetPhorest [4]) made it possible for us to derive systems-level models of JNK and EphR signalling networks [2,3]. I shall discuss work we have done in comparative phospho-proteomics and network evolution [5-7]. Finally, I will discuss our most recent work in analysing genomic sequencing data from NGS studies and how we have developed new powerful algorithms to predict the impact of disease mutations on cellular signaling networks [8,9].

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PS16-02 The Genetic Landscape of a Cell

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We are constructing a genome-scale genetic interaction map, examining all ~18 million gene-gene pairs for synthetic genetic interactions, which has generated quantitative genetic interaction profiles for ~75% of all genes in the budding yeast, *Saccharomyces cerevisiae*. A network based on these profiles reveals a functional map of the cell in which genes of similar biological processes cluster together in coherent subsets and highly correlated profiles delineate specific pathways to define gene function. Most recently, we've been focussing on the essential gene network, mapping genetic interactions for conditional temperature sensitive alleles of essential genes. The resultant global network identifies functional cross-connections between all bioprocesses, mapping a cellular wiring diagram of pleiotropy. Genetic interaction degree correlated with a number of different gene attributes, which may be informative about genetic network hubs in other organisms. Large-scale genetic interaction mapping in human cancer cells carrying defined mutations revealed networks resembling the yeast network and identifies potential drug targets for synthetic lethal cancer therapies.

PS16-03 Ca^{2+} Induced Phosphorylation Signalling in Nerve Terminals

Phillip J. Robinson

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Synaptic plasticity is how neurons adapt over time, as occurs when the brain is learning and storing memories. The main adaptation is the amount of synaptic transmission between presynaptic nerve terminals and postsynaptic dendrites. Presynaptic nerve terminals are small organelles of about 1 micron in diameter containing all the machinery and subcellular organelles required for synaptic transmission. They respond to differing depolarising stimulus by increasing exocytosis of synaptic vesicles (SVs), and produce new SVs by two recycling paths: clathrin-mediated endocytosis or bulk endocytosis following by budding of new SVs. Initially phosphorylation of dynamin was found to mediate bulk endocytosis. We have now shown that depolarising stimuli of different intensities stimulates unique patterns of global phosphorylation changes in the nerve terminal. The proteins are involved in multiple SV functions including different endocytic modes and SV biogenesis. We have uncovered evidence for the global integration of these signal transduction pathways by protein phosphatase regulatory proteins. We aim to increase knowledge of presynaptic plasticity at the molecular level. Better understanding the molecular mechanisms of synaptic transmission may lead to new therapeutic approaches for treatment of disorders of synaptic transmission.

PS17-01 Conformational Changes of Multi-Protein Complexes Determined by Mass Spectrometry

Juri Rappsilber

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Life is a marvellous and complex network of dynamic processes. Structural biology currently provides wonderful insights on highly purified proteins (often trimmed to remove flexible domains) yielding snapshots that reveal much about how protein domains interact and how ligands are bound. However, I cannot help but imagine how much more we could learn if we were to study intact proteins in their native environments as parts of dynamic processes possibly within huge macromolecular assemblies. I think with quantitative cross-linking/mass spectrometry (CLMS) we start holding a tool in our hands that will make important contributions to this. We have shown recently that cross-linking analysis is compatible with quantitation by stable isotope labelling (Fischer et al. 2013). Quantitative CLMS shows its full power as part of an integrated structural biology approach that includes tools that outline the shape of protein complexes (cryoEM and SAXS) and tools that reveal high-resolution structures of subunits (X-ray crystallography, NMR). I will present here how quantitative CLMS as part of integrated structural biology can provide structural and biological insights into key proteins.

Fischer L, Chen ZA, Rappsilber J. Quantitative cross-linking/mass spectrometry using isotope-labelled cross-linkers. J Proteomics. 2013 Aug 2;88:120-8.

PS17-02 Protein-Phosphoprotein Complexes in DNA Damage and Cancer Signaling

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The forkhead-associated (FHA) domain is the only known phosphoprotein-binding domain that specifically recognizes phosphothreonine (pThr) residues, distinguishing them from phosphoserine (pSer) residues. In contrast to its very strict specificity toward pThr, the FHA domain recognizes very diverse patterns in the residues surrounding the pThr residue. Also, the FHA domain exists in various proteins with diverse functions and is particularly prevalent among proteins involved in the DNA damage response and cancer signaling. This lecture will highlight the diversity of biological functions of two FHA domain-containing proteins in DNA damage response and cancer signaling. Phosphorylation sites and binding proteins are identified by mass spectrometry, and then the structures of protein-phosphoprotein or protein-phsphopeptide complexes are solved by NMR or X-ray crystallography.

Keyword: FHA

PS17-03 From the Top Down; Mass Spectrometry and Its Role in Studying Intact Proteins and Protein Complex Structure, Dynamics, and Assembly

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Around for more than a century the analytical technique of mass spectrometry is blooming more than ever, and applied in nearly all aspects of the natural and life sciences. In the last two decades mass spectrometry has become routine for the high-throughput analysis of peptides. However, also intact proteins and even complete protein complexes can nowadays be analyzed, enabling MS to enter the field of structural biology. Here, I will describe the emerging role of mass spectrometry with its different technical facets in structural biology, focusing especially on the analysis of viruses, dynamic protein assemblies and therapeutic antibodies. I will describe how mass spectrometry has evolved into a tool that can provide unique structural and functional information about viral protein and protein complex structure, conformation, assembly and topology, extending to the direct analysis of intact virus capsids of several million Da in mass. Mass spectrometry is now used to address important questions in virology ranging from virus structural topology to how viruses assemble. The unique features of native MS will further be highlighted in a study of the macromolecular complex controlling circadian timing. I will also describe recent developments in mass spectrometry technology that have allowed us to analyze intact native proteins and protein complexes using Q-ToF and Orbitrap mass analyzers with very high sensitivity and mass resolving power, and how that has enabled us to profile therapeutic antibody quality and structure in their native state without requiring any sample preparation. Prospectively, I will describe what I think will be the huge contribution of this breakthrough for the future of proteomics.

Keywords: protein assemblies, top-down proteomics, native mass spectrometry

PS17-04 In Vivo Crosslinking Combined with Label-Free Quantitative Proteomics to Determine the Subcellular Distribution and Stoichiometry of Proteasome Complexes

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The proteasome is a large protein complex involved in the degradation of intracellular proteins. It thus plays a crucial role in the regulation of many cellular processes and in antigenic peptides presentation. A 20S core particle, that contains the catalytic activity, can be associated to one or two regulatory particles (RPs) of identical or different protein composition. Several RPs exist but their precise subcellular distribution remains to be determined. Here we present an efficient integrated workflow combining *in vivo* crosslinking with cell fractionation and an affinity purification-mass spectrometry strategy using the 20S core particule as a bait and label-free quantitative proteomics to determine the endogenous subcellular distribution of human proteasome complexes[1].

Labile proteins interactions of RPs with the 20S complex have been successfully stabilized by *in vivo* crosslinking using formaldehyde before cellular factionation while maintaining proteasome activity. Label-free quantitative data were then acquired using a high sequencing speed, high resolution Orbitrap mass spectrometer and analyzed using the home-developped MFPaQ software. In two leukemic cell lines we showed that there was a high proportion of 20S complex not associated with RPs and that the 19S RP was the main associated activator in all cellular compartments (cytosol, microsomes, nucleus). This result was confirmed in total cell extracts of 7 various additional cell lines. However, the determination of the precise distribution of free and RPs associated 20S complexes revealed differences that could be correlated with variations in proteasome activity, including in fractions where proteasome complexes are present in low amounts (nucleus, microsomes). Moreover, this optimized workflow allowed to follow the dynamics and to highlight the specificity of RPs association with the 20S complex.

1. Fabre et al, Mol Cell Proteomics 2013, 12, 687

Keywords: Protein complex, Human Proteasome, Crosslink

PS18-01 Utilizing Carbohydrate Fragmentation Database (UniCarb-DB) for Glycoresearch Discovery

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Glycomic analysis of *O*-linked oligosaccharides using mass spectrometry are generating large amount of data that is difficult to overview and extract the important information that could be used to answer biological questions. The exercise can simplistically be described as being able to identify the core types (usually core 1-4), branching (I and i) and/or elongation (type 1 and type 2) pattern, and terminal epitopes (eg sialylation, blood groups). Branching/elongation analysis also includes the exercise in identifying non-terminating such as sulfation, and internal sialic acid.

In order to address these question we have accumulated structural information together with mass spectrometric data (MS^2 spectra) and metadata in the resource UniCarb-DB (www.unicarb-DB.com). This resource is now the platform for future development of softwares for interpretation of glycomic LC-MS and LC-MS^2 data. The utilization of peak matching tools for MS^2 spectra allows identification of oligosaccharide candidate structures based on matches found with structurally assigned spectra in the database The peak matching tool can also be used to identify the type of terminal structures present. This is exemplified by the approach to identify 3 or 6 linked sulfate based on *m/z* regional matching of MS^2 spectra.

Including appropriate metadata to UniCarb-DB would allow advanced bioinformatic mining of the database. For instance, including also intensity information and type of instruments in the database, datasets using similar analytical methods can be compared for such things as tissue typing and biomarker discoveries, where it is obvious from comparison pulmonary infection, that its is not only a switch between sulfate and sialic acid but also an alteration of the core type.

The challenge for the future is to utilize current glycomic resources, such as UniCarb-DB to develop the workflows for glycomic analysis to reach the sophistication to be utilized for biodiscovery.

PS18-02 Challenges & Solutions for Glycoproteomics

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Glycoconjugates are well known to be key players of intercellular communication [1]. Comprehensive knowledge on these important molecules and their particular glycan structures present on the cell's surface is a fundamental requisite for deciphering glycan mediated intercellular communication signals. Glycoproteomics provides this information by identifying, characterising and cataloguing glycoproteins present on cell surfaces or biological fluids [2, 3, 4]. In this context detailed glycoproteomic maps of major immunoglobulins such as sIgA [5] and IgM [Kolarich *et al*, manuscript in preparation] have just been acquired recently.

Despite tremendous recent advances in qualitative glycoproteomics applications particular quantitative questions regarding the MS signal intensity relationships between various forms of un-/de-/glycosylated peptides remained unanswered. To determine the quantitative information label free techniques provide on glyco-microheterogeneity an in-house produced synthetic library of peptides and N-glycopeptides was investigated using various ionisation sources/MS detector combinations [4]. These N-linked glycopeptides were found to exhibit up to 10x lower signal intensities compared to their unglycosylated counterparts when equimolar mixtures were analysed. This finding emphasises the importance of glycopeptide specific sample preparation steps prior analysis.

Combining glycoproteomics findings with well-established solid phase (glyco) peptide synthesis provides unique opportunities for numerous glycobiology aspects such as method development, quantitative glycoproteomics and functional glycobiology.

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PS18-03 Connecting Proteomics with Glycomics

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As the proteomics field starts to encompass glycomics and glycoproteomics, specific new methodologies and informatics databases and tools need to be developed to interpret the data and ultimately relate it to the function of these glycans and their glycoconjugates.

For glycoproteomics analysis of a complex biological mixture of proteins in sputum we present a novel glycomics and glycoproteomics strategy for the site-specific analysis of glycoproteins. N-glycosylation heterogeneity was determined by combining global N-glycome characterization followed by the analysis of deglycosylated and glycosylated peptides with orthogonal fractionation, complementary tandem mass spectrometry (MS/MS) and advanced data-processing tools.

For glycoprotein data analysis, in 2011 we introduced UniCarbKB as an international initiative that aims to collect, distribute and extend resources and practices from glycobiologists to the whole biological research community (1). The mission is to provide a comprehensive, high quality catalogue of published and experimental information on the carbohydrates attached to proteins, and to integrate this data with the other '-omics' knowledgebases. I will present on behalf of many participants, a summary of the start we have made on establishing the infrastructure and content of the publically available UniCarbKB. The establishment of a glycomics bioinformatics hub on the ExPASy server and the linking of UnicarbKB to the proteomics knowledgebase UniProt will be described. Other data initiatives, such as the establishment of UniCarb-DB an annotated data repository of MS/MS spectra, currently in its infancy, will also be reported on. In the future it is hoped that the UniCarbKB knowledgebase, centered on a reference database of curated glycan structures, will become the key resource of quality information for glycoproteomic research.

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PS18-04 N-Glycoproteome Analysis from Identification of Glycosylation Sites to Intact Glycopeptides

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The comprehensive analysis of N-glycoproteome requires the high-throughput determination of glycans structures and their precise attachment sites on protein sequences. By using the combined technology and method, we have identified about 5,000 glycosylation sites to generate the largest dataset of protein glycosylation for human liver. We also developed the solid-phase based technology by integrating all of the digestion, enrichment, deglycosylation together with LC-MS analysis for glycoproteome analysis, and by which both the identification sensitivity and through-put was improved greatly. Almost no glycopoteomic method has been developed for identification of both glycan structures and peptide backbone, finally we present a novel high throughput strategy that combines MS spectra of deglycosylated peptides and MS spectra of intact glycopeptides to identify glycopeptide sequences, glycan structures, and specific attachment sites. Application of this strategy to a complex proteome led to the identification of the largest dataset of glycosylation from mammalian samples.

Keywords: Glycoproteomics , N-glycosylation site, Glycan structure

PS19-01 (Keynote) Exploring Communication in the Tumor Microenvironment

Catherine Fenselau[1], Meghan Burke[1], Waeowalee Choksawangkarn[1], Rebecca Rose[1], Avantika Dhabaria[1], Yan Wang[1], Nathan Edwards[2], Suzanne Ostrand-Rosenberg[3]

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Myeloid-derived suppressor cells (MDSC) inhibit both innate and adaptive immunity to cancer cells. Inflammation enhances accumulation and activity of MDSC in the tumor microenvironment. We postulate that exosomes, nanoscale membrane-bound extracellular vesicles that are shed by MDSC, participate in chemotaxis and immunosuppression in the tumor microenvironment. In search of mechanistic insights to test this hypothesis, qualitative and quantitative proteomic strategies have been used to identify the protein cargo of these exosomes and to analyze differential effects of inflammation on these proteins. The activities of proteins found to be of high interest have been confirmed biologically and tested for activity.

Exosomes were shed by MDSC induced in BALB/c mice by the 4T1 mammary carcinoma and developed in either a conventional tumor microenvironment or a microenvoronment with increased inflammation due to high levels of the pro-inflammatory cytokine IL-1β. More than 1000 proteins have been identified from MDSC exosomes thus far. The majority of these proteins are assigned as originating from the cytosol, nucleus, cytoskeleton and plasma membrane in parental MDSC. Functional ontology assigned the majority as protein binding, nucleotide binding, ion binding and hydrolytic. In replicate iTRAQ experiments, the concentrations of S100-A9, S100-A8 and neutrophilic granule protein were found to change in concert in the exosomes, though not in the parental MDSC. Functional studies showed that S100 A8/A9 proteins in exosomes mediate MDSC chemotaxis and alter the function of macrophages. Abundances of histones and several enzymes involved in the production of NADPH+ were also found to be altered by inflammation.

Keyword: exosomes, cancer

PS19-02 Oncogenic H-Ras Reprograms Madin-Darby Canine Kidney (MDCK) Cell-Derived Exosomes During Epithelial-Mesenchymal Transition

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Epithelial-mesenchymal transition (EMT) is a highly conserved morphogenic process defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. EMT is associated with increased aggressiveness, invasiveness, and metastatic potential in carcinoma cells. To assess the contribution of extracellular vesicles during EMT, we conducted a proteomic analysis of exosomes released from Madin-Darby canine kidney (MDCK) cells, and MDCK cells transformed with oncogenic H-Ras (21D1 cells). Exosomes are 40-100 nm membranous vesicles originating from the inward budding of late endosomes and multivesicular bodies (MVBs) and are released from cells upon fusion of MVBs with the plasma membrane. Exosomes from MDCK cells (MDCK-Exos) and 21D1 cells (21D1-Exos) were purified from cell culture media using density gradient centrifugation (OptiPrep™), and protein content identified by GeLC-MS/MS proteomic profiling. Both MDCK- and 21D1 Exos populations were morphologically similar by cryo-electron microscopy and contained stereotypical exosomes marker proteins such as TSG101, Alix and CD63. In this study we show that the expression levels of typical EMT hallmark proteins seen in whole cells correlate with those observed in MDCK- and 21D1-Exos - i.e., reduction of characteristic inhibitor of angiogenesis, thrombospondin-1 and epithelial markers E-cadherin, and EpCAM, with a concomitant up-regulation of mesenchymal makers such as vimentin. Further, we reveal that 21D1-Exos are enriched with several proteases (e.g., MMP-1, -14, -19, ADAM- 10, DAMTS1), and integrins (e.g., ITGB1, ITGA3, ITGA6) that have been recently implicated in regulating the tumour microenvironment to promote metastatic progression. A salient finding of this study was the unique presence of key transcriptional regulators (e.g., the master transcriptional regulator YXB1) and core splicing complex components (e.g., SF3B1, SF3B3 and SFRS1) in mesenchymal 21D1-Exos. Taken together, our findings reveal that exosomes from Ras-transformed MDCK cells are reprogrammed with factors which may be capable of inducing EMT in recipient cells.

PS19-03 EVpedia: An Integrated Proteome Database for Systemic Analyses of Prokaryotic and Eukaryotic Exosomes

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The secretion of extracellular vesicles, also known as exosomes and microvesicles, is a common cellular activity observable not only in simple unicellular organisms (e.g. archaea, Gram-positive, and Gram-negative bacteria) but also in complex multicellular ones. Extracellular vesicles are spherical bilayered proteolipids with a mean diameter of 20-1,000 nm, which are known to contain various bioactive molecules including proteins, lipids, and nucleic acids [1]. Here, we present EVpedia ((http://evpedia.info), which is an integrated database of high-throughput datasets (proteins, mRNAs, miRNAs, and lipids) from prokaryotic and eukaryotic extracellular vesicles [2]. EVpedia provides an array of tools, such as the search and browse of vesicular components, Gene Ontology enrichment analysis, network analysis of vesicular proteins and mRNAs, and a comparison of vesicular datasets by ortholog identification. Moreover, publications on extracellular vesicle studies are listed in the database. This free web-based database of EVpedia might serve as a fundamental repository to stimulate the advancement of extracellular vesicle studies and to elucidate the novel functions of these complex extracellular organelles.

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PS19-04 Extracellular Vesicle (EV) Array: Microarray Capturing of Exosomes and Other Extracellular Vesicles for Multiplexed Phenotyping

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Based on the technology of protein microarray, we hereby present a highly sensitive Extracellular Vesicle (EV) Array capable of detecting and phenotyping exosomes or other microvesicles from unpurified starting material in a high-throughput manner. The EV Array utilizes the possibilities to detect and profile microvesicles for 21individual surface exposed antigens simultaneously using only small amounts of starting material.

Exosomes are extracellular vesicles (40-100 nm) secreted by various cell types. The quantity and molecular composition of exosomes shed from different cell types differs considerably. Until now, the "gold standard" for quantification, characterization and phenotyping of exosomes is either by WB or FACS. These types of analyses requires considerable amounts of exosomal material (20-30 μg of protein derived from appr. 10^8 cells) and are only capabale of producing phenotypical data on one antigen per experiment. The EV Array exploits the potentials to detect and profile exosomes for 21 antigens simultaneously using unpurified exosomes from cell culture medium from 10^4 cells.

The EV Array is based on the antibody capture of microvesicles and subsequent detection of the captured subtypes of microvesicles by labeled anti-tetraspanin antibodies (CD9, CD63 and CD81 for exosomes). Antibodies used to capture these targeted exosome biomarkers are specific to membrane proteins for: exosomes in general (CD9, CD63, and CD81), and exosomes from cancer cells (EpCam, CD276) and 11 other membrane markers. Compared to NanoparticleTracking Analysis (NTA), EV Array revealed a higher specificity and sensitivity for exosomes, regardless of the pre-analytical circumstances as two-steps centrifugation or freezing/storage of the samples. Among others, the method was used to generate an extensive phenotyping of plasma-derived exosomes from 80 healthy donors.

Keywords: Protein Microarray, Exosome, Extracellular Vesicle (EV) Array

PS20-01 Phosphoproteomics and Cancer

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Lung cancer is the leading cause of cancer-related death in the USA and worldwide. Due to the late stage of diagnosis, poor efficacy of chemotherapies, and development of drug resistance, the 5-year survival rate is 16%. Genomic sequencing has revealed mutations in human lung cancers, e.g. EGFR, KRAS, ALK, and RET, that control oncogenic processes including evasion of apoptosis, cellular proliferation and cellular invasion. Unfortunately, targeted therapies for these kinases ultimately fail due to acquired resistance. To identify additional targets for treatment inherently transparent to genomic sequencing, new technologies must be developed, implemented and validated at the level of proteins and post-translational modifications.

Quantitative proteomics and phosphoproteomics are poised to improve our understanding of oncogenic signaling. We implemented a quantitative phosphoproteomic strategy to identify mitotic substrates of two families of mitotic kinases, Polo-like kinases and Aurora kinases, which are amplified or overexpressed in lung cancer. We increased the number of known phosphorylation loci attributed to Polo-like kinase 1, Aurora A, and Aurora B by 10-fold.

We then implemented a robust and accurate quantitative phosphoproteomics strategy to interrogate the global phosphorylation profile of primary human lung tumors. We quantified ~9000 phosphorylation sites across signaling pathways including KRAS, PI3K, and ERBB2, and identified the enrichment of PLK1-specific substrates in one of these human tumors. To ensure that the proteome and phosphoproteome of patient tumors was stable during processing by pathologists, we quantified protein and phosphopeptide abundances after tumor resection. Having established a robust analytical workflow for kinase network profiling by quantitative phosphoproteomics, it should now be possible to identify patient-specific phosphoproteomic profiles to aid informed decision making for personal treatment strategies.

PS20-02 How Targeted Therapies Exploit Signaling (Src) and Metabolic (Glucose) Over-Dependencies in Cancer Homeostasis

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Unbiased inquiries into signaling and metabolism using mass spectrometry-based phospho-proteomics have repeatedly pointed us to networks involving negative and positive feedback, cross-talk, synergy, and unexpected results. Examples include negative feedback of Src signaling in kinase inhibitor-resistant Bcr-Abl-driven leukemias (Rubbi et al.); feed-forward, synergistic amplification of signaling upon metabolic stress leading to catastrophic death (Graham et al.); synergistic co-treatments that can prevent kinase inhibitor resistance; and the identification of druggable tyrosine signaling in prostate cancer, a tumor type in which tyrosine signaling mutations are rare (Drake et al.). In sum, these examples provide illustrations of how the signaling and metabolic states of cancer cells, while homeostatic, can be relatively unstable to disruptive perturbations.

PS20-03 Development of Universal Protocols for Clinical Tissue Phosphoproteomics Optimized for Formalin-Fixed Paraffin-Embedded Specimens

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Archives of clinical tissues have been stocked typically in formalin-fixed and paraffin-embedded (FFPE) state in almost all clinical institutes for several decades, and therefore the accumulated specimens can be useful for retrospective research. This fact motivates us to achieve proteome and phosphoproteome analysis of FFPE clinical samples. However, FFPE specimens have inadequate properties to be analyzed, e.g. low recovery of proteins and artificial modifications by formaldehyde-induced cross-linking, leading to the low efficiency in protein identification as well as the significant decrease in quantitative accuracy. Recently, we introduced a novel protocol for protein extraction and digestion to maximize the proteome coverage, which we call phase transfer surfactant (PTS)-aided method. PTS method made it possible to extract 100 μg of protein from a few FFPE slides and identify more than 1500 phosphopeptides in combination with hydroxy acid-modified metal oxide chromatography (HAMMOC) using titania. In this study, we further improved our PTS method by utilizing novel deparaffinization protocols, to achieve more effective protein extraction and reduce the processing time. The optimized protocol was applied to FFPE slides of various human cancers and healthy control samples, and the obtained phosphoproteomes were compared. Furthermore, phosphoproteomes of freshly frozen tissues from cancer patients and healthy controls were also analyzed, and the difference between FFPE specimens was evaluated. In our system, almost all clinical samples including FFPE and freshly frozen specimens can be retrospectively analyzed without any analytical and biological discrimination.

Keywords: phosphoproteomics, FFPE, clinical tissue

PS20-04 Identification of Dysregulated Kinase-Mediated Pathways in Hepatocellular Carcinoma by a Quantitative Phosphoproteome Approach

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Phosphorylation is one of most common post-translational modifications in mammalian cells. It regulates protein activity, cellular localization, conformation, and binding affinity and further mediates diverse key physiological functions. Numerous literates have established the close association between aberrant phosphorylation and many diseases, including cancer.

Hepatocellular carcinoma (HCC) is a common and poor prognostic cancer worldwide. Surgical resection is the most effective modality, but there are many limitations, and unfortunately, the recurrence rate is about 50% even after resection. In previous studies, various kinases and phosphorylation-mediated signaling pathways such as HGF/c-Met signaling pathway, ERK/MAPK pathway and Wnt/β-catenin signaling pathway have been found to be significantly altered in HCC patients. Conceivably, phosphorylation plays an important role in these molecular mechanisms that can affect tumorigenesis. Therefore, exploring the tissue phosphoproteome profiles will facilitate the identification of critical factors involved in HCC.

In this study, we established a technology platform for quantitative phosphoproteome analysis via combining stable isotope dimethylation labeling and online SCX-TiO_2/RP-LTQ-Orbitrap, and then compared tissue proteome and phosphoproteome between tumor tissues and paired adjacent non-tumor counterparts in three HCC patients. The results yielded 3100-4700 quantifiable phosphopeptides corresponding to over 2600 proteins with high confidence. In order to check the accuracy of our platform, we proceeded to confirm the quantitative results by Western blotting using phospho-specific antibodies.

Furthermore, key upstream protein kinase(s) responsible for the phosphorylation of those phosphosites dysregulated in tumor tissues were predicted, enabling us to unravel aberrant active kinases as biomarkers, as well as discover potential therapeutic targets for HCC patients.

Keywords: phosphoproteome, hepatocellular carcinoma, dimethylation labeling

PS21-01 Integration and Data-Mining of Human Transcriptome and Proteome Databases in H-InvDB

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Based on comprehensive analyses of human transcriptome, we developed the H-Invitational Database (H-InvDB; http://hinv.jp/), an integrated database of all human genes and transcripts. The latest release of H-InvDB defines 36,789 protein-coding genes, 692 transcribed pseudogenes, and 8,366 potential non-protein-coding genes. The protein-coding genes include 13,320 genes for hypothetical proteins that require further experimental validation at the protein level. H-InvDB also defines a total of 61,403 alternative splicing (AS) isoforms of human protein coding genes. Detailed annotation of human AS isoforms has been compiled in a specialized database, H-DBAS (http://hinv.jp/h-dbas/). These information resources are useful for future experimental identification of novel human proteins, especially in C-HPP. We thus developed the H-Inv Extended Protein Database (H-EPD; http://hinv.jp/hinv/h-epd/) that provides comprehensive, non-redundant human protein sequences, including both curated and predicted human proteins in H-InvDB and other protein databases. Another newly released database that we constructed is a protein complex database (PCDq; http://h-invitational.jp/hinv/pcdq/) that provides information for both curated and predicted human protein complexes based on protein-protein interaction data and literatures. The predicted protein complexes can provide functional clues about hypothetical human proteins. We also provide useful tools for data-mining from these databases, including H-InvDB Enrichment Analysis Tool (HEAT) and ID Converter System (http://biodb.jp/).

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PS21-02 What is a Proteomic Publication?

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The amount of information submitted to accompany a proteomic publication has changed dramatically in the last ten years, largely due to the implementation of journal publication guidelines. Authors are now expected to provide access to annotated spectra of at least the results that have the highest potential for mis-interpretation, and it is increasingly expected that the raw data files themselves are also submitted to a public location. In this presentation I will discuss options for how data associated with a publication is made available, tools for viewing the different results formats, including a spectral viewer that supports many results file formats, and highlight examples of how this data is mined. As part of this I will touch on the graying boundary between what should be in a journal article and what is supplementary information and whether data that is not peer-reviewed should be submitted to proteomic repositories.

Keywords: Journal Guidelines, Spectral Viewers, Repositories

PS21-03 Estimation of Protein Species Number for Mammalian, Bacteria, Insecta and Yeast

Elena Ponomarenko, Stanislav Naryzhny, Ekaterina Poverennaya, Mikhail Pyatnitskii, Andrey Lisitsa, Alexander Archakov

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The sequencing of the human genome was completed over 10 years ago during the Human Genome Project. As a logical continuation of this project, Human Proteome Project was launched in 2010. At the same time, the target size of the human proteome is still obscure: from 10 000 (Adkins et al., 2002) to 1 billion (Kelleher, 2012) of different protein species assumed. The diversity of protein species arises from the appearance of single amino-acid polymorphisms (nsSNP, SAP), alternative splicing variants (AS) and post-translational modifications (PTM). During last 3 years the numbers of entries in UniprotKB concerning SAP, AS and PTM stable for *Drosophila melanogaster* and *Saccharomyces cerevisiae* S288c, while for human there is a slight increasing SAP-related entries, and number of PTM-related entries are increased for *Rattus norvegicus* and *Escherichia coli.* Multiplying the average number of variations per gene, we could estimate the number of protein species coded by one gene; applying this calculation to all genes, we could expect the number of protein species. For human, rat and E-coli the minimal number of protein species estimated as 1,9 mln., 390 000 and 45 000 respectively. We obtain approximately 307 000 protein species for *Drosophila melanogaster* and 290 000 for *Saccharomyces cerevisiae* S288c (UniprotKB, v.03_2013). As the number of possible protein variants will definitely increase due to growth of the database, here we use the term «minimal number of protein species» for mammalian and *E. coli.* The number of protein species is necessary for understanding the target size of the proteome of each organism. Moreover, such kind of data is necessary for determination the each protein species abundance. Dependence of the number of detected proteins and the analytical sensitivity could be based on a comparison of the theoretical calculations with experimental results of number of protein species detected using 2DE with various dyes types.

Keywords: Data analysis, Number of protein species, Human Proteome Project

PS21-04 Isobar: Making Sense out of Protein and Modified Peptide iTRAQ/TMT Quantitative Data

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While current mass spectrometry is able to measure thousands of non-modified and posttranslationally modified peptides, the identification of significantly regulated molecules remains challenging. We have introduced a new software package - isobar - to address this problem both at the protein and the modified peptide levels for iTRAQ and TMT data.

The isobar platform relies on carefully tested statistical models (Breitwieser et al., J Proteome Res, 2011) that separate technical variability, originating from the instrumentation, and biological variability. Significantly regulated proteins can be naturally selected by requiring a clear measure and biological significance. Isobar can also take advantage of replicates present in a single iTRAQ or TMT experiment.

We have extended isobar to analyze the regulation of PTMs (Breitwieser & Colinge, J Proteomics, 2013) by assessing the statistical methods in this special condition and by introducing necessary new features. In particular, isobar provides a generic mechanism of validating the localization of PTMs by means of the Mascot Delta Score approach or specialized external tools such as PhosphoRS. The navigation of peptide regulation results is facilitated by a sophisticated hyperlinked spreadsheet user report which integrates references to known PTMs from neXtProt and PhophoSitePlus.

Isobar can be run without programming skills and it is released as a Bioconductor R package thus allowing more advanced users and bioinformaticians to fully exploit its rich repertoire of functions. It can parse the most common file formats (Mascot, Phenyx, MSGF+, MGF, Rockerbox, MzIdentML). Isobar also supports label free quantitation by computing emPAI and dNSAF protein abundance indexes.

Web site: http://www.ms-isobar.org

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Keywords: quantitative proteomics, bioinformatics, PTM

PS21-05 FindPairs - The Protein Quantification Module of the PeakQuant Software Suite

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Accurate protein quantification is a major task in Proteomics. A wide range of stable isotope labeling techniques allow simultaneous quantification of thousands of proteins by using mass spectrometry. Here, the FindPairs module of the PeakQuant software suite is presented. It automatically determines peptide and protein abundance ratios based on the automated detection of isotopic peak patterns in stable isotope-coded mass spectrometric data. Hence it also works with SILAC and iTRAQ, the practicability of FindPairs is shown on the quantitative analysis of proteome data acquired in $^{14}N/^{15}N$ metabolic labeling experiments. This works on the one hand "database-driven", when sequences are known. On the other hand, a special feature of FindPairs is the application of an average mass shift factor to identify isotope patterns of $^{14}N/^{15}N$ peptide pairs, even if no sequence information is known. This is interesting especially for Quantitative Proteomics in unknown species, i.e. "Meta-Proteomics". Furthermore we give an overview of the features of FindPairs and compare these with already existing quantification packages. The software is publicly available at http://www.medizinisches-proteom-center.de/software and free for academic use.

Keyword: Bioinformatics, Quantitative Proteomics, metabolic labeling, 14N/15N labeling

PS22-01 Immunoaffinity Separations and Intelligent PRISM Fractionation for Achieving Ultrasensitive Targeted Protein Quantification

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Selected reaction monitoring (SRM) has been regarded as a promising high throughput targeted protein quantification technology; however, one of major limitations for current SRM technology is the lack of sufficient sensitivity for detecting low-abundance proteins present at sub-ng/mL in human plasma/serum. To address these challenges, we have developed and integrated different levels of immunoaffinity depletion techniques to enhance the overall sensitivity. More recently, we developed an antibody-free strategy that involves high-pressure, high-resolution separations coupled with intelligent selection and multiplexing (PRISM) for highly sensitive SRM-based targeted protein quantification. The strategy capitalizes on high resolution high pH reversed-phase LC separations for analyte enrichment, intelligent selection of target fractions via on-line SRM monitoring of internal standards, and fraction multiplexing prior to LC-SRM quantification. We demonstrated accurate and reproducible quantification of proteins at concentrations in the 50-100 pg/mL range in plasma/serum by coupling with IgY14 immunoaffinity depletion. PRISM-SRM provided up to ~1000-fold improvement in the LOQ when compared to conventional SRM measurements and a moderate throughput of quantification (~50 samples/week) with the use of fraction multiplexing. Applications to clinical serum samples illustrated the detection of several endogenous ng/mL-level proteins, including prostate-specific antigen (PSA), and an excellent correlation between the results obtained from the PRISM-SRM assay and those from clinical immunoassay for PSA. This approach was also applied for the verification of a number of prostate cancer candidate biomarkers in human urine, including anterior-gradient 2 (AGR2), where the data displayed a preliminary discrimination between prostate cancer and non-cancer subjects. Our results demonstrate that PRISM-SRM is an effective method for quantification of low-abundance endogenous proteins in highly complex biofluid samples. We anticipate broad applications for targeted quantification of low-abundance proteins in systems biology and candidate biomarker verification studies.

PS22-02 A High-Throughput and Reproducible Workflow for Targeted Mass Spectrometry Applied to Large Scale Analysis of Clinical Samples

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Multiple Reaction Monitoring (MRM or SRM) is a targeted mass spectrometry (MS)-based method for quantifying peptides that are unique for specific proteins in biological/clinical samples. The challenge with MRM is speed and accuracy of processing proteins into peptide and subsequent LC/MS/MS analyze of 100-1000s of samples. Numerous steps in sample preparation (protein denaturation, reduction, alkylation, digestion, peptide desalting) introduce analytical errors beyond acceptable limits for bioanalytical methods. Here, we describe the implementation of automated robotic sample processing and a dual column LC/MS/MS system to improve the reproducibility and throughput of MRM. A single or dual reverse-phase chromatography systems (MPX™-2 System, Shimadzu) was linked to a triple quadruple MS instrument (QTRAP® 5500 or 6500, AB Sciex). Pooled human sera was processed for MRM in a 96-well format using a Biomek NX^P automated liquid handling system (Beckman Coulter). The accuracy of a traditional manual MRM workflow was measured by spiking a constant amount of β-gal into 171 plasma samples. Each sample was reduced, alkylated, digested with trypsin, and spiked with heavy-isotope peptide internal standards, and desalted on a 96-well HLB™ solid phase extraction plate (Waters): 60 samples were processed per day = 70 hours for 171 samples. Samples were run in triplicate on a QTRAP® 6500 system with %CV for β-gal of 31%. While, robotic processing using a Biomek NX^P liquid handler reduced the total sample preparation time (excluding desalting) to < 18 hours including overnight trypsin digestion (which can be reduced to 2 hours) with a %CV of 5%. Moving from manual or 96 well plate desalting (%CV >20%) to LC online desalting using an online trap column or direct divert method where the salt fraction is diverted to waste prior to the QTRAP system had %CV of 9 and 4%, respectively. Furthermore, throughput for LC/MS/MS MRM assays doubled using a two parallel C-18 reversed phase columns LC system. Here peptides from one column elute into the MS while the other column is washed, regenerated and loaded. The %CV between LC columns was <5%. In conclusion, an automated high-throughput workflow with sample processing robotics and multiplexed LC/MS/MS for accurate and reproducible large-scale analysis of biological/clinical samples.

PS22-03 Mark Twain: How to Fathom the Depth of Your Pet Proteome

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The present lecture will highlight recent progresses in the technique of combinatorial peptide ligand libraries (CPPL), a methodology that has much to offer for the detection of low- to very-low abundance proteins in any proteome. In particular, advances in exploration of the urinary, plasma, CSF and tissue proteomes are discussed and evaluated. It will be shown that when treating biological fluids, such as plasma, with CPLLs, the detection sensitivity, which in the control only reaches 10ng/mL, can be enhanced to as high as 10pg/mL, with an increment of sensitivity of three orders of magnitude. Exploring such extreme low concentration intervals will allow access to those most sought after biomarkers that so far have been much elusive. Even in tissue proteome extracts, up to the present not analyzed via CPLLs, massively overloading the CPLL beads will allow exploration of very low-abundance proteins in presence of a set of highly abundant tissue proteins. The possibility of using CPLLs as a two-dimensional pre-fractionation of any proteome is also evaluated: on the charge axis, CPLL capture can be implemented at no less than three different pH values (4.0, 7.2 and 9.3), thus permitting a capture of proteinaceous analytes bearing a net positive or net negative charge, respectively. When capture is performed in absence of salts or at high levels of salts, one can favour capture of hydrophilic vs. hydrophobic proteins, respectively. This would thus be a genuine 2D protocol, working on orthogonal separation principles (charge vs. hydrophobicity). As the horizon of CPLLs is expanding, we expect major breakthroughs in, e.g., biomarker discovery, a field that as suffered a decade of failures.

Boschetti E, Righetti PG. Low-Abundance Proteome Discovery. State of the Art and Protocols, Elsevier, Amsterdam, 2013, pp. 1-341.

PS22-04 Automated Native Sample Preparation for Proteome Analysis

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Multidimensional chromatography has been adapted for fractionation of body fluids for proteomic analysis and biomarker search. Our method combines serial native size exclusion (1D), followed by parallelized anion exchange (2D) and lectin affinity (3D) chromatography. Parallelization and automation is realized with separation, spectrophotometric readout, temporary storage, hit picking, medium exchange, digest, desalting, and finally storage within an autosampler of LC-MS. The central unit is an experimental setting enabeling multichannel pipetting and robotic handling of microplates, reservoirs, and column arrays. Some tools have been developed to improve medium exchange, desalting, re-concentration, and readout. The protein input is scalable from 2 to more than 100 mg and from 2 to 1000 kDa molecular weights. Globally, protein recoveries from the corresponding load are 93.7 ± 2.7, 97.1 ± 11.2, and 88.8 ± 3.6% and best precisions are 3.3, 3.8, and 5.1% CV with 1D-, 2D-, and 3D-fractionation, respectively. For process control and all-round analytics a software package was developed. Besides automation, this method has several advantages: high proteome coverage, flexible dynamic range with respect to molecular weight and sample amount, and optional enzymatic and immunological analytics additional to mass spectrometry. All intrinsic components and information are preserved after fractionation including complex formation, fragmentation, and biological activities beneficial for biomarker search as well as for efficient evaluation. Preliminary applications show versatility with profiling plasma proteomes of humans, cattle, goat and mouse, and human cerebrospinal fluid. Application to biomarker search yields several candidate markers that could already be validated in severe inflammation and Alport syndrome.

Keywords: automated native multidimensional fractionation, parallel sample preparation

PS23-01 (Keynote) New Technology in Integrated Omics: Challenges: Concepts, Methods and Tools

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The Human Proteome Project is on its tracks and many if not most human proteins will be found in biological samples in the coming years. However, although the Human proteome project aims at the detection and the description of all human proteins encoded in the genome, it will remain a tremendous challenge to convert this new information into an increased knowledge on human biology and human physiopathology. I would like to challenge the fact that recent findings in molecular biology will be sufficient to explain biology as a whole. On the contrary, I' ll argue that systemic definition and global characterization of living systems will be usefully combined with molecular studies for a better understanding of biological functions in isolation or taken as a whole for a complete organism. For example, we need more integrative sciences between mathematics modelling, bio-mechanics, molecular biology, medicine and cognitive sciences to better address the question of heredity versus acquired traits (and possibly disorders) in cognitive brain functions. New representation of living systems will emerge in coming years, integrating new knowledge and new concepts coming from various fields. This fruitful combination will be the driving force for developing new technologies for a better monitoring of human health and, whenever necessary, better tools fur disease treatment.

Keyword: Integrated biology - interdisciplinarity

PS23-02 Insights from a Multi-Omic Analysis of a Single Human Cell Type

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Advances in the field of genomics, epigenetics, transcriptomics and proteomics have provided researchers the technical capability to carry out unbiased genomewide studies. These strategies are often used in isolation while investigating biological problems. It is now apparent that a unified approach that allows us to study genome, epigenome, transcriptome and proteome would provide better insights than any single Omics study. However, the challenging aspect of such a unified approach is the lack of appropriate computational tools to deal with such data. In an effort to determine the feasibility of embarking on a multi-Omics approach, we carried out whole genome sequencing, genomewide methylation profiling, mRNA sequencing, miRNA sequencing, proteomic and phosphoproteomic profiling of naïve CD4+ T cells from a single individual. We have generated one of the most extensive proteomic catalogs of any primary cell type in humans by obtaining data regarding protein expression, acetylation and phosphorylation of naïve CD4+ T cells. This study establishes expression of >8,000 proteins in naïve CD4+ T cells along with their various alternative forms including splice variants and post-translationally modified versions. We have obtained novel, and surprising, insights into transcription and translation of genes/proteins by correlating the methylation patterns with mRNA, miRNAs and proteins. For example, although recent studies have reported abundant, widespread RNA editing in cultured human cell lines, our analysis reveals RNA editing as a relatively infrequent event and highlights some of the caveats of next generation sequence data analysis. Overall, we believe that such multi-Omics profiling studies can inform us about basic molecular mechanisms in addition to paving the way for personalized medicine.

PS23-03 Chr 18 Quantitative Proteome and Transcriptome

Victor Zgoda, Arthur Kopylov, Olga Tikhonova, Alexander Moisa, Nadezhda Pyndyk, Tatyana Farafonova, Svetlana Novikova, Andrey Lisitsa, Alexander Archakov

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The current stage of C-HPP is focused on constructing the chromosome-centric proteomic catalogues. Representative proteins should be mapped in the tissues and quantitatively annotated. SRM is recommended for C-HPP as the method for targeted protein detection and quantification in complex samples due to its high sensitivity at the level of few protein copies per cell. Using SRM, the fragment ion maps for 277 proteins were assayed by SRM in depleted plasma, liver and HepG2 cells. Summarized SRM results for all types of biomaterial comprised in total 250 detected proteins which covered 90% of protein-coding genes of Chr 18. Analysis of tissue distribution demonstrated that a significant portion of detected proteins (51%) was observed both in human plasma and liver tissue. There were also proteins featured for plasma only (33%) or exclusively observed in the liver (16%). For 27 proteins, we observed the absence of the SRM signal or poorly reproduced signal.

Concentration of 50 proteins encoded by Chr 18 was measured by SRM using external calibration with purified nonlabeled peptides. The results on the quantitative label-free analysis of proteins in biological samples were validated using the stable isotopelabeled peptides. Generation of the correlation curve for 30 light- and isotope-labeled peptides measured in plasma, liver and HepG2 cells illustrates applicability of such an approach. The high correlation coeffcient (R^2= 0.96) between protein concentrations estimated by external calibration with light peptides and by isotope-labeled standards was observed. Therefore, we expect the external calibration with light peptides provides the suitable estimation for protein abundance.

PS23-04 Firmiana: An Integrated Platform for Mass Spectrometry-Based Proteomics Studies Based on Galaxy Framework

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Data analysis is essential and critical for proteomics studies. However, increasingly large amouns of high through-put data produced from mass spectrometry experiments pose significant challenges for computational analysis. Although lots of bioinformatics tools have been available, they are difficult to use, especially for experimental biologists with no programming experiences. Meanwhile some computational analyses are complex and integrative. Thus, user-friendly data analysis platforms integrating a broad range of tools are quite necessary.

Here we present a web-based platform called Firmiana for mass spectrometry-based proteomics study, built upon open source Galaxy framework which was originally designed for genomics research but allows easy integration of tools used in proteomics study as well as rapid configuration of proteomics data analysis workflows. This platform has integrated a collection of current and newly developed representative tools involved in most steps of mass spectrometry proteomics data analysis, from preliminary experiment management, file format conversion, spectrum identification with database searching, statistical validation of peptide and protein identification results, peptide and protein quantification, to downstream analysis such as significance test and Gene Ontology analysis. In addition, more new functions and tools can be included in this platform.

The user-friendliness of Firmiana platform will greatly facilitate mass spectrometry-based proteomic studies. The intuitive graphical user interface of tools liberates researchers from complex software operations, and the flexible construction of workflows simplifies complex data analyses. Besides, benefit from the scalability of Galaxy framework, tools can be configured to run on clusters or cloud to utilize more powerful computational resources.

Keywords: Proteomics Data Management & Analysis System, Galaxy, Mass Spectrometry Experiments

PS24-01 A Robust Method for the Relative Quantitative Analysis of Dual Labeled N-Glycans

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With the development of glycoproteomics, relative quantification of glycosylation has received considerable research attention, which is important for discovering glycosylation changes and finding potential biomarker. High throughput glycan quantitation was developed to investigate quantitative glycan changes in structure type, composition, linkage and conformation of glycans from different biological samples. In this research, we developed a novel relative quantitation method for glycans. The novel sodium borohydride assisted enzymatic ^{18}O labeling added the reducing end of all released glycans with 3 Da. After this label, the mass gap increased to 3 Da, and the partial overlap of isotope envelopes was largely reduced. Moreover, the glycan became more stable, and the labeled ^{18}O would never exchange with ^{16}O again in normal water. We further tested the stability and overlap of this label, which showed good results. We optimized the labeling, and made the method more suitable for quantitative glycomics by mass spectrometry. Moreover, we utilized this method to quantitatively determine the glycomic changes in sera from healthy individuals and patients diagnosed with hepatocellular carcinoma (HCC), and got the good results

Keywords: Dual Labeled N-glycans, Relative Quantitative, Mass spectrometry

PS24-02 An Integrated Approach of Proteomics, Glycoproteomics and Glycomics for the Structural and Functional Study of Glycoproteins

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Glycosylation is one of the most common forms of protein modifications. Each glycoprotein can be glycosylated at different glycosites and each glycosite may be modified by different glycans. This structural heterogeneity provides additional functions for each glycoprotein in physiological and pathological processes. However, the structural heterogeneity also complicates the studies of structure-function relationships of glycoproteins. To rapidly identify and quantify the glycosylation on each glycosite from complex biological mixtures and to understand the functions of the glycosylations, an integrated approach for global proteomics, glycoproteomics, and glycomics was used. We performed quantitative analyses of proteins, glycosylation site occupancy and glycan structures at specific site by the isolation of glycopeptides and glycans using chemical-enzymatic approaches and analysis of glycopeptides and glycans by liquid-chromatography followed by quantitative analysis by mass spectrometry. We showed by specific examples that upon the identification of specific changes in glycosylation, glycosites containing aberrant glycans were readily identifiable and quantifiable using the combination of quantitative global proteomics, glycoproteomics and glycomics. The application of integrated approaches may facilitate our understanding of how perturbed glycosylation impacts upon disease progression and could be used to discover glycosylated proteins that could serve as biomarkers or therapeutic targets to improve clinical outcomes of patients.

PS24-03 Use of Zinc Finger Nuclease Gene Targeting for Functional Glycomics

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Glycosylation is the most abundant and diverse posttranslational modification of proteins. While several types of glycosylation can be predicted by the protein sequence context, and substantial knowledge of these glycoproteomes is available, our knowledge of the GalNAc-type O-glycosylation is highly limited. This type of glycosylation is unique in being regulated by 20 polypeptide GalNAc-transferases attaching the initiating GalNAc monosaccharides to Ser and Thr (and likely some Tyr) residues. We have developed a genetic engineering approach using human cell lines to simplify O-glycosylation (SimpleCells) that enables proteome-wide discovery of O-glycan sites using "bottom-up" mass spectrometric analysis. We implemented this on 12 human cell lines from different organs, and present a first map of the human O-glycoproteome with almost 3,000 glycosites in over 600 O-glycoproteins as well as an improved NetOGlyc4.0 model for prediction of O-glycosylation. The finding of unique subsets of O-glycoproteins in each cell line provides evidence that the O-glycoproteome is differentially regulated and dynamic. The greatly expanded view of the O-glycoproteome should facilitate the exploration of how site-specific O-glycosylation regulates protein function.

PS24-04 Breast Cancer Tumour Transformation from Primary Tumour to Secondary Site

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Breast cancer is a very heterogeneous disease and some patients are cured simply by surgical removal of the primary tumour while other patients suffer from recurrence and spreading of the disease. A number of treatment predictive factors have been identified such as tumour size, estrogen (ER) and progesterone (PgR) receptor status and human epidermal growth factor receptor 2 (HER2) status. Lymph node involvement is also assessed during surgery to determine if the tumour has started spreading and thus determine if lymph node stripping is required. The predictive factors assessing the nature of the tumour are all based on the status of the primary tumour. However, it could be anticipated that the cancer cells undergo a molecular transformation allowing the spreading to a secondary site. If the lymph nodes are positive for cancer cells or if distant metastases are identified, this disease would likely be more successfully treated by assessing predictive markers characterizing the cells having undergone spreading. We are analysing a unique tumour material comparing a set of 18 primary breast cancer tumours with matched axillaries positive for breast cancer cells and a set of 20 primary tumours with matched distant metastases spread to different sites in the body to further understand the molecular changes during the spreading and identify novel predictive markers. We are analysing these tumours for glycoproteins. Protein glycosylation is predominant in both membrane proteins and secreted proteins. Importantly, changes in glycosylation of these proteins have been shown to correlate with cancer states. Glycopeptide capture was used in this study to selectively isolate and quantifies N-linked glycopeptides from mixtures of glycoproteins. The captured glycopeptides were subjected to mass spectrometry analysis. Glycopeptide capture gave the most satisfactory results with 1145 proteins identified in total, all samples combined.
Keywords: Glycosylation, Breast cancer, Biomarker discovery

PS25-01 Functional Validation of Biomarker Discovery from Mouse Models to Translational Research

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Animal model is very useful to discovery the disease-candidate biomarkers instead of human patients. However still there has been a couple of issue to define what can be the best model for human disease for discovering biomarkers. We found differentially expressed proteins and transcripts during adipocyte differentiation using proteomics and DNA micrarray.
To validate these proteins, we developed several lines of knock-out mice. Among them, we found obesity-related phenotypes from AHNAK knockout mouse. To investigate the functional role of AHNAK in lipogenesis, HFD (High fat diet) and LFD (Low fat diet) were fed for 12 weeks to AHNAK knock-out mice and its age matched wild type mice. Even though AHNAK knock-out mice revealed a reduced body weight at birth compared with wild type mice, ratios of major organ weight to body mass was almost same of wild type mice. Body weight of HFD-fed AHNAK mice showed significantly reduced with the rate of weight gain compared to HFD-fed wild type mice despite an identical food intake when normalized to body mass. HFD-fed AHNAK mice display a reduced epididymal fat mass. From discovering differentially expressed proteins in disease models to validate its function involved in the onset of disease, phenotypical characterization of knockout mice is one of essential issues leading to disease.

PS25-02 Biomarkers of Diabetes in Plasma of NOD Mice

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Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and it has become a global epidemic in the past years. The heterogeneity in the determining factors for the development of diabetes and its complications represent a big challenge in the advance of new strategies for prevention and treatment. In the present study we propose the investigation of potential biomarkers of diabetes, as well as biomarkers of early stages of diabetic nephropathy in plasma of non-obese diabetic mice using proteomic analysis. Plasma samples from adult diabetic (n=6) and non diabetic NOD (n=4), as well as Swiss mice (n=5), were collected and processed. Individual protein samples were analyzed by NanoUPLC tandem nanoESI-LC MS^E. A total of 141 proteins were identified in all three groups but 10 proteins were exclusively expressed in the diabetic group. Among those ten, candidates had already been associated to type 1 diabetes prevention, endothelial function impairment, retinopathy, diabetes related oxidative stress and one had been described as early biomarker for the disease. Seven proteins were only present in the Swiss strain and 8 were exclusive of the NOD mouse lineage, independent of the diabetic state. NOD mice are not born diabetic and the incidence on the colony is not 100% so our next step will be to follow NOD from early age to adulthood collecting samples at different time points to better understand the change in the proteomic profile that occurs in the diabetic state.
Keywords: Diabetes, NOD mice, biomarkers

PS25-03 Proteomic Analysis of Left Ventricular Tissues in Dilated Cardiomyopathy Mouse Models

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Dilated cardiomyopathy (DCM) is an intractable disease, and neither its radical treatment other than cardiac transplantation nor its differential diagnostic procedure has been established. To develop these methods for DCM, it is important to understand its pathogenic mechanism. Because phenotypes of causative gene mutations in the DCM are rather common regardless of the genes, it is essential to analyze proteomic changes associated with its progression. In this study, we performed proteomic analysis of left ventricles (LVs) of 4C30 mouse, a DCM model overexpressing Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 2. 4C30 mouse shows a severe symptom of DCM around 24 weeks. Frozen LV tissues were pulverized, denatured, and digested with trypsin in the presence of sodium deoxycholic acid. After desalting, the digests were analyzed by nanoLC-MS/MS (Triple TOF5600) and differential protein expression was quantitatively evaluated with a 2DICAL software. Among 894 identified proteins, 186 and 145 proteins showed significant increases or decreases in 4C30 mice, respectively, compared with age-matched wild-type mice. Proteins associated with fibrosis, remodeling of extracellular matrix and cytoskeleton, and endoplasmic reticulum stress were increased, while energy metabolic enzymes involved in the glycolysis, TCA cycle, and β-oxidization were remarkably decreased. Since β-oxidization system in particular is a main energy source for the heart, this energy depletion might underlie conditions leading to DCM. In addition, regulatory proteins of intracellular calcium levels were also decreased, suggesting malfunction of the cardiac contraction and relaxation. These proteins in the energy metabolism and calcium regulation may be useful for diagnosis and treatments of DCM. We are currently analyzing another mouse model to find common features for understanding pathogenesis of the DCM.

Keywords: dilated cardiomyopathy, 4C30 mouse

PS25-04 Plasma Proteomic Pattern Analysis for Murine Experimental Autoimmune Encephalomyelitis (EAE) Model

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Background: Multiple Sclerosis (MS) is the most common demyelinating disease of the CNS where current treatments have limited effectiveness. Experimental autoimmune encephalomyelitis (EAE), has developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Here we adopt a plasma proteomic pattern analysis using murine EAE model. **Methods**: For active induction of EAE, female SJL/J mice were immunized with myelin basic protein (MBP) derived peptides at 10 wk of age. Mice were observed for clinical signs of EAE and scored on a scale according to the severity of the clinical signs. Murine plasma peptides/proteins were purified with C8 magnetic beads using a robotics (ClinProtrobot system) and obtained spectra by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Autoflex II). The spectra were analyzed and multivariate statistics and receiver operation characteristics were calculated using ClinProTools 2.2™. **Results and Discussions**: EAE have been developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Plasma proteomic pattern was clearly altered with progression of EAE symptoms by machine learning method. By building a support vector machine classifier, an effect on plasma proteomic pattern of EAE was clearly observed with good cross validation accuracy from 13 post immunization day (pid). Of note, some peaks enabled an annotation of clinical stages such as remission and relapsing of the EAE. **Conclusion:** Plasma proteomic pattern analysis is a promising and a reliable biomarker strategy in applying murine EAE model.

Keywords: multiple sclerosis, Experimental autoimmune encephalomyelitis, proteomic pattern analysis

PS25-05 Integrated Proteomics for the Study of Metastatic Human Tongue Cancer Development in a Heterogeneous Microenvironment

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Tongue cancer has been shown to be the most metastatic of oral cancers in patient cases. To understand the behavior of metastatic cells and the mechanism of metastatic cancer cell development in a heterogeneous tumor microenvironment, we studied human tongue cancer cell lines with highly metastatic (HM) and non-metastatic (NM) properties, established from the same patient. We created an orthotopic xenograft mouse model via co-injection of dsRed NM and GFP HM clones, and observed that HM cells grew aggressively in the tumor center and formed metastases, while NM cells translocated to the tumor margin without metastasis. The cell lines were then compared using differential proteomic (iTRAQ) and transcriptomic (DNA chip) analyses, followed by network analysis (KeyMolnet) of the combined mRNA and protein data. With the statistically highest score, we detected the HIF (hypoxia inducible factor) signal pathway as being specifically upregulated in HM cells. Detailed analysis of identified signal network showed that 30 (75%) of 41 proteins displaying higher expression in HM were directly or indirectly related to HIF signal transduction. Immunoblotting and immunocytochemistry further revealed up-regulation of the expression and activation of HIF-1α in HM. For biological validation, we prepared HIF-1α knockdown HM (HM-HIF^{KD}) cells and observed the cellular proliferation and motility in 2-/3-dimensional co-culture systems of NM and HM or HM-HIF^{KD} clones. The results showed that growth of NM cells was suppressed with the co-culture of HM cells, while the NM cell growth normalized with HM-HIF^{KD} cells. In time-lapse video analysis, HM cells showed aggressive growth with crowding-induced exclusion of NH cells. HM-HIF^{KD} cells, however, had no effect on NM cell growth. These results demonstrate that HIF-1α related signals are important in the development of highly metastatic tumors in a heterogeneous microenvironment.

Keywords: cancer, metastasis, heterogeneity

PS26-01 Label-Free Proteomics for Biomarker Discovery and Validation in Proximal Fluids: Applications in Colon Cancer and Alzheimer's Disease

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Label-free mass spectrometry-based proteomics applied to biomarker-rich sub-cellular compartments in (pre)-clinical samples and human proximal biofluids in vivo is a powerful, versatile approach for discovery of tissue-derived biomarkers with close association to the disease and potential for non-invasive applications.

Our label-free workflow is based on GeLC-MS/MS and spectral counting-based quantitation combined with dedicated statistics (Pham et al., Bioinformatics 2010, 2012). With correct study design and sample handling, this workflow is reproducible (Piersma et al., JPR 2010 and associated news highlight) and allows for quantitative protein profiling in simple and more complex workflows (reviewed in Pham et al., Expert Rev Mol Diagn. 2012). We introduced the 'whole gel' procedure to speed up the in-gel digestion procedure when handling multiple samples and many gel bands (Piersma et al., Proteome Sci 2013). More importantly, we have successfully applied this workflow in multiple biomarker discovery projects, most notably in colon, lung and breast cancer and in neurodegenerative diseases.

In my talk, I will present the steps that we consider important for reliable biomarker discovery and show results from our on-going efforts in colon cancer and Alzheimer's Disease (AD). In both examples patient proximal fluids were the starting point for discovery of early detection markers: stool for colon cancer and cerebrospinal fluid for AD. Proteome profiling of stool revealed a set of proteins significantly enriched in colon cancer compared to control stool samples, of which candidate biomarkers could be verified by MRM. CSF proteomics identified promising candidates for identifying patients with mild cognitive impairment at risk of developing AD, that were validated in independent samples using proteomics as well as western blotting.

PS26-02 Clinical Proteomics MRM

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Protein quantitation is essential for diagnostic and prognostic monitoring of disease biomarkers, and for verifying and validating the candidates in human biofluids. A targeted multiplexed approach involving MRM/MS in conjunction with isotopically labeled peptide standards has demonstrated great utility in delivering precise and accurate protein concentrations. Although the methods are currently employed in pre-clinical screening studies, they were developed with an eye toward clinical translation to aid diagnostic accuracy through multiplexed marker analysis. To be presented is an overview of our methodological advancements and disease-specific applications for the pre-clinical evaluation of putative disease protein biomarkers in human biologics, including blood plasma. These enable large (>150 proteins) or condensed (*e.g.*, 40 proteins in a CVD-focused study) protein panels to be interrogated against patient samples in a rapid and inexpensive manner, while maintaining high robustness (average CVs: 7% for signal and 0.05% for retention time) and sensitivity (concentration ranges: 6 and 8 orders-of-magnitude for 1D and 2D LC-MRM/MS) for precise and relative quantitation of high-to-low abundance protein biomarkers.

PS26-03 Proteomics Strategies for Analysis of Liver Cancer

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Cholangiocellular carcinoma (CCC) is a major subtype of liver cancer that arises in the bile ducts. It is known to have poor patient outcomes, with a 5-year survival rate of about 5 %. The incidence of, and mortality from, CCC are increasing worldwide. At present, extensive surgical resection or transplantation remain the only potentially curative treatments, although most patients are considered inoperable at the time of diagnosis. Sensitive and specific biomarkers for the early detection of CCC are therefore needed.

In order to identify such biomarkers, malignant and healthy tissue from 8 CCC patients was analysed using two techniques from quantitative proteomics. 2D-DIGE (two-dimensional differential in-gel electrophoresis) was performed with minimal labelling of disrupted tissue. Differential spots were detected using DeCyder Software (GE Healthcare) and proteins of interest were identified by MALDI-TOF mass spectrometry on an Ultraflex II (Bruker). Parallel to this, a mass spectrometry-based label-free approach was adopted. For this, an RP-HPLC-MS/MS method was used to analyse the samples, with an Ultimate 3000 RSLCnano system (Dionex) online coupled to an LTQ Orbitrap Elite (Thermo Scientific). Progenesis software (Nonlinear) was used to detect differentially expressed proteins. Additionally, the proteome of bile from five CCC patients was analysed to check for the presence of proteins found to be regulated in the tissue. The bile was treated with Cleanascite Lipid Removal Reagent (Biotech) and a 2-D Clean-Up Kit (GE Healthcare) to eliminate salts and charged detergents. The proteome was subsequently analysed using RP-HPLC-MS/MS, as described above.

As expected, both strategies show complementary results, which indicates that a combination of both techniques may be very promising in the identification of biomarker candidates for CCC. Some of the proteins found using both these techniques were also detected in the bile of CCC patients. These overlapping results, from the tissue and the bile, make them especially promising candidates for minimally invasive biomarkers. In future projects these potential biomarkers will be validated using western-blots, immunohistochemical techniques and ELISA.

PS26-04 From a Brain Damage Proteomics Model to a Blood Time Indicator of Stroke Onset: The Glutathione S-Transferase-Pi Proof of Concept

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Vascular cerebral accident, or stroke, is a leading cause of death and disability in industrialized countries. An early diagnostic blood marker of stroke would allow immediate therapeutic interventions and hence reduce the extent of brain damage and risk of death. However, the discovery of novel diagnostic markers directly in the blood is limited and presents a real challenge for the development of new diagnostic tools. This can be overcome by the use of appropriate models and experimental designs. Here, we will report the use of post-mortem cerebrospinal fluid and cerebral extracelullar microdialysis fluid as models of stroke brain damage events for the discovery of potential early markers of stroke. The validation of 5 proteins found differentially expressed in model samples by proteomics strategies were compared to 25 known brain markers by immunoassays on a prospective cohort of stroke patients. Among the 30 molecules tested, GST-pi concentration was the most significantly elevated marker in the blood of stroke patients ($p<0.001$). More importantly, GST-pi displayed the best area under the curve (AUC, 0.79) and the best diagnostic odds ratios (10.0) for discriminating early (<3 h of stroke onset) vs. late stroke patients (>3 h after onset). The ability of GST-p to predict the time window after stroke onset and consequently the potential eligibility for thrombolytic therapies may open new avenues for the management of stroke patients but also more specifically for the wake-up stroke patients, representing up to 30% of stroke events and traditionally excluded from treatment because of unknown time of symptom onset.

PS26-05 Diagnosis of Male Reproductive System Disorders with Protein Biomarkers Quantified in Seminal Plasma

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Non-invasive methods for differential diagnosis of male reproductive system disorders present urgent unmet needs in the urology clinics. In this presentation, we will introduce our biomarker discovery platform, discuss in detail development of male infertility biomarkers and suggest approaches for identification of biomarkers of prostate cancer and prostatitis. In our search for biomarkers, we focus on the proteome of seminal plasma (SP), a proximal fluid suitable for identification of novel biomarkers and for development of non-invasive diagnostic tests. Using mass spectrometry, we identified more than 3,100 proteins in SP of healthy men and men with infertility, prostate cancer and prostatitis.

To develop markers for differential diagnosis of male infertility, we selected 79 biomarker candidates based on differential proteomics of SP from fertile men and men with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Using quantitative SRM assays, we verified 30 proteins and then validated 18 proteins in 148 SP samples. We identified two proteins, epididymis-specific ECM1 and testis-specific TEX101, which differentiated between OA, NOA and normal spermatogenesis with near absolute specificities and sensitivities. The performance of ECM1 was confirmed by ELISA in 188 samples, and a 2.3µg/mL cut-off distinguished OA from normal spermatogenesis with 98% specificity, and OA from NOA with 74% specificity, at 100% sensitivity. Immunohistochemistry and immunoaffinity-SRM assays revealed differential expression of TEX101 in distinct histopathological subtypes of NOA. As a result, we proposed a simple 2-biomarker algorithm for differential diagnosis of OA and NOA, and, in addition, for the differentiation of NOA subtypes. Clinical assays for ECM1 and TEX101 will replace diagnostic testicular biopsies and improve the prediction of testicular sperm retrieval, thus increasing the reliability of assisted reproduction techniques.

PS27-02 Development of iPS Cell Technology for Clinical Application

Masato Nakagawa

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Pluripotent stem cells have been thought to be useful sources for regenerative medicine. Although embryonic stem (ES) cells have abilities to differentiate into several kinds of somatic cells and grow infinitely in vitro, there are several problems with using ES cells for clinical application such as ethical issues and rejection after transplantation. To overcome these problems, pluripotent stem cells were generated from somatic cells by introduction of defined factors. These have been termed induced pluripotent stem cells (iPSCs). iPSCs have raised hopes for a new era of regenerative medicine because they can avoid the ethical problems and innate immune rejection associated with ES cells.

iPSCs have been generated from mouse and human fibroblasts by retroviral transduction of four transcription factors, Sox2, Oct3/4, Klf4, and c-Myc. Both mouse and human iPSCs are indistinguishable from ES cells. We found that the chimeras and progenies derived from mouse iPSC showed an increased incidence of tumor formations, primarily due to the reactivation of the c-Myc retrovirus. To overcome this issue, we have developed the methods for generation of safer iPSCs by episomal vectors containing L-Myc instead of c-Myc.

Human iPS cells (hiPSCs) are typically generated and maintained on feeder cells. Mouse feeder cells (SNL or MEF) are conventionally used for hiPSC culture. These cells are prepared with FBS-containing medium. For clinical use, feeder-free (Ff) and xeno-free (Xf) culture conditions seem to be better than the conventional ones. We succeeded to develop the Ff-culture conditions by using recombinant proteins and Xf-medium for hiPSC establishment and long-term culture.

We believe that this system is useful not only for producing clinical-grade hiPSCs in Cell Processing Centers for future application, but also for research, such as basic stem cell research.

PS27-03 Directed Differentiation of Stem Cells by Changing Protein Dynamics

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A holy grail of proteomics is to identify the cellular locations of all proteins. Several studies have sought to identify protein locations systematically, but an even larger task is to map and validate which of these proteins dynamically translocate as well as interact in development and disease (the translocalizome, the interactome).

We developed the novel algorithm for predicting condition-specific subcellular locations of the gene coding proteins at genome-wide level using only limited and condition-unspecified known locations. With systems biological mRNAs analysis of human stem cells using this method, the key target genes and their coding proteins which involved in maintaining pluripotency and differentiation process were predicted. Molecular biological experiments for target genes and proteins as well as novel protein function validation methods such as FCCS (Fluorescent Cross Correlation Spectroscopy), PLA (Proximity Ligation Assay), molecular imaging, gene transcription will be demonstrated for visualizing the validation process of key mechanisms. Finally, artificial translocation and interaction of novel protein complexes induced directed differentiation of human stem cells will be introduced.

PS27-04 Deep Proteome Profiling Identifies Distinct Pluripotent Cellular Populations During Somatic Cell Reprogramming into Induced Pluripotent Stem Cells

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Somatic cell reprogramming into induced pluripotent stem cells (iPSCs) is a process in which a complex network of molecular events generates a wide spectrum of either transient and stable cell states. Understanding the molecular mechanisms underlying the reprogramming phases is essential to increase its efficiency and will have important implications for improving our knowledge of cellular plasticity. Here we applied an in-depth high-resolution quantitative MS-based analysis to probe dynamic proteome changes during reprogramming. Protein levels were quantified at 13 different states over the reprogramming time-window by using isobaric labeling (i.e. TMT), SCX pre-fractionation and high-resolution LC-MS/MS (i.e. Orbitrap Velos). Using this approach, we successfully identified 504,060 PSMs at a false discovery rate lower than 1% which corresponded to 7,265 unique protein groups. Most importantly, a total of 6,915 proteins were quantified and for 65% of them we obtained temporal profiles uncovering the whole set of samples included in the analysis. Our data reveal a proteome resetting step taking place already 48 hours after transgene expression, which involved specific biological processes linked to the c-Myc transcriptional network. A second wave of major proteome reorganization occurred in a later stage of reprogramming, where we could distinguish and characterize two distinct pluripotent cellular populations. One of these represented a stable transgene-dependent cell type, which showed an altered pattern of adhesion proteins and a partial activation of pluripotency markers. The other population represented embryonic stem cell (ESCs)-like cellular precursors and showed a higher level of proteins involved in cell adhesion, epigenetic mechanisms and pluripotency.

Keywords: Cellular Reprogramming, Induced Pluripotent Stem Cells, Quantitative Proteomics

PS28-01 Whole Proteome Resources: Access and Sharing of Data through the Peptide and SRMAtlas Proteomics Suites

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A major goal of the human proteome project is to advance the characterization of all coding gene products to complete the human proteome and to enable the application and dissemination of proteomic technologies that will support the comprehensive identification and quantitation of all the proteins, their isoforms, and post-translational modifications contained within the human proteome. With recent advances in comprehensive resources in technology, informatics and reagents, the ability to measure proteins in all its forms is now realized. These developments of new technology for multiplexed quantitative protein measurements, with a throughput consistent with the needs of iterative measurements of perturbed systems, has enabled the building and sharing of tools that are generally applicable to all proteomic-based studies. The advancements in proteomic informatics provides highly curated databases that enable standardization across workflows and form a solid basis for the widespread deployment of multiplexed assays and reporting of their results in a community based manner. This sharing and re-use of data and results provides confidence in the field of proteomics. I will discuss tools that are capable of generating complete, reproducible and quantitatively accurate datasets of entire proteomes.

PS28-02 Building Your Knowledge Base of Empirically Measured Peptides with Skyline and Panorama

Brendan MacLean

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Targeted proteomics is becoming an increasingly popular method of measuring peptide quantities in experiments. It was recently chosen as method of the year by Nature Methods. Targeted proteomics relies heavily on the principle that by accumulating prior knowledge of peptides of interest, a mass spectrometrist can make highly precise quantitative measurements of the peptides, with high confidence. That prior knowledge frequently takes the form of empirical measurements of physiochemical properties of the peptide molecules which are hard to derive from the amino acid sequence alone, such as: peptide retention time on column, relative peptide expression for a protein, linear range in a given sample matrix, relative product ion expression under fragmentation, optimal collision energy for each fragment and collisional cross section. Existing tools have made storing and reusing this critical information cumbersome and error-prone. To improve this situation, developers of the targeted MS desktop application, Skyline, and collaborators at LabKey Software have implemented the Panorama repository software for laboratories already accumulating targeted proteomics experimental results in the form of Skyline documents. The Panorama software helps labs organize results, collaborate securely, and build collections empirically derived targeted assay properties to inform future experiments. Skyline now supports both publishing a document directly to a server running Panorama, and also downloading chromatogram libraries of targeted assay properties from a Panorama server. Developed as a module in the LabKey Server biomedical data management platform, Panorama is freely available and open source. Free hosted Panorama projects are also available on http://panoramaweb.org.

PS28-03 Computational Proteomics Enables Accurate Label Free Quantification of Proteins and Posttranslational Modifications

Juergen Cox

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Protein quantification without isotopic labels has been a long-standing interest in the proteomics field and is becoming a feasible alternative to label-based quantitative proteomics workflows. However, accurate and robust proteome-wide label-free quantification of pre-fractionated samples remains a challenge. Here we report on a new intensity determination and normalization procedure called MaxLFQ that is fully compatible with any peptide or protein separation prior to LC-MS analysis. Protein abundance profiles are assembled using the maximum possible information from MS-signals given that the presence of quantifiable peptides varies from sample to sample. On a benchmark dataset with two proteomes mixed at known ratios, we accurately detect the mixing ratio over the entire protein expression range, with higher precision for abundant proteins. On a second benchmark dataset, we accurately quantify fold changes over several orders of magnitudes, a task that is challenging with label-based methods.

An even more challenging task the quantification of posttranslational modification sites without labeling. We introduce a computational strategy for extracting site occupancies from label-free datasets with multiple samples. For sites that are changing between biological conditions high quality occupancy estimates are obtained. A quality parameter is introduced which indicates the reliability of the site occupancy quantification

MaxLFQ is a generic label-free quantification technology that is readily applicable to tackle many biological questions and it is compatible with standard statistical analysis workflows, and it has been validated in many and diverse biological projects. Our algorithms can handle very large experiments of 500+ samples in manageable computing time. It is implemented in the freely-available MaxQuant computational proteomics platform and works completely seamlessly at the click of a button (www.maxquant.org).

PS29-01 Site-Specific Glycan-Peptide Analysis for Determination of *N*-glycoproteome Heterogeneity in Bacteria and Mammalian Systems

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The major limitation in glycopeptide analysis is the need to separate glycans from modification sites, thus losing the context of protein glycosylation. We developed a glycan-peptide analysis schema using zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) and fragmentation by collision induced dissociation (CID) and higher energy collision dissociation (HCD) MS/MS to identify >100 intact *N*-glycopeptides from the pathogen *Campylobacter jejuni*. Analysis was simplified by the conserved nature of the *C. jejuni* heptasaccharide, although the analytical depth achieved also identified targets of a phosphoethanolamine-modified *N*-glycan. We next combined glycomics and glycoproteomics for site-specific analysis of *N*-glycosylation heterogeneity in a complex mammalian protein mixture. Initially, *N*-glycomics was performed using porous graphitized carbon LC-MS/MS and the data used to create an *N*-glycan database. Tryptic glycopeptides were then enriched using ZIC-HILIC and fractionated by reversed-phase LC. Each fraction was separated into two aliquots. The first set of aliquots was treated with PNGase F, and former *N*-glycopeptides analyzed by nano-RPLC-MS/MS. This enabled the creation of a glycopeptide-centric concatenated database for each fraction. The second set of aliquots, containing intact glycopeptides, was analyzed by nano-RPLC-MS/MS, employing CID and HCD. Assignment of glycan compositions to peptide sequences was achieved by searching the intact *N*-glycopeptide HCD MS/MS spectra against the glycopeptide-centric databases employing the *N*-glycan database. Semi-automatic annotation of HCD spectra enabled false discovery rate calculations based on the frequency of peptide backbone fragment ions that matched with high mass accuracy. Finally, CID spectra were used to assign glycan structures identified in the glycomic analysis to peptide sequences. This approach identified 863 unique intact *N*-glycopeptides from 161 rat brain proteins.

PS29-02 Tackling Sulfoglycomics and Realizing the Prospects of Sulfoglycoproteomics

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The proper functioning of a diverse range of terminal glyco-epitopes as cell surface recognition codes is not only dependent on the underlying glycan chain and protein carriers, which dictate their accessibility and valency, but also modulated by specific non-sugar substituents. Sulfation at strategic position is one such critical determinant. Time and again, glycan array screening revealed that many endogenous glycan-binding proteins including members of the selectins and Siglecs, bind the sulfated counterparts of their cognate ligands at significantly higher affinity. The prime examples being sialyl 6-sulfo-LeX displayed on high endothelial venules serving as homing ligand for leukocytes expressing L-selectins, and α2-6-sialyl 6-sulfo-LacNAc for CD22/Siglec-2 on B cells. In the presence of multiple sialylation on the termini of most complex N- and O-glycans, the extra negative charge imparted by sub-stoichiometry sulfation often renders these sulfated glycans cryptic in single dimensional glycomic mapping. In analogy to phosphoproteomics, which requires extra experimental steps to enable global mapping of otherwise under-represented phosphopeptides, we have taken the lead to establish the enabling techniques for MS-based sulfoglycomics and shown that sulfation is more widespread than currently appreciated. Many previously unknown sulfo-glycotopes were identified *de novo* and yet, paradoxically, several others predicted based on antibody staining or glycan array data remain undetectable. Unlike the latter approaches, only MS-based sulfoglycomic identification will inform the underlying glyco-structural context. A demanding next step is to further localize the expression of these functionally important sulfated glycans on key cell surface and adhesion molecules by glycoproteomics. However, several technical hurdles remain in realizing this aim and our current advances in sample preparation, MS data acquisition and analysis will be presented.

PS29-03 A New Method for Glycopeptide Assignment by Duplex-LC/MS Analyses

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Assignment of glycopeptide on both peptide sequence and glycan moiety remains technically challenging. Although some approaches including MS/MS analysis employing electron-transfer/capture dissociation (ETD/ECD) have been reported to enable the assignment, these methods are basically applicable to isolated or mixture of a small number of glycoprotein(s) and are still hard to apply for a complex mixture of glycopeptides. Thus far, we have developed a method to identify a large number of peptide sequences carrying *N*-glycan(s) by coupling the affinity capture of glycopeptide subset, enzymatic deglycosylation-mediated stable isotope-labeling on their glycosylated Asn residues, and LC/MS identification of the labeled peptides (IGOT method). Then, we designed a new method to find a series of glycopeptide signals as clusters of glycoforms from LC/MS data, and to predict the peptide sequence of each glycoform cluster by matching the observed accurate mass of glycopeptides and mass of the peptide portions identified by IGOT method. This method allows easy assignment of glycopeptide glycoforms derived from a single model glycoprotein.

Keywords: Glycoproteome, Glycoform, Glycan heterogeneity

PS30-01 Target Deconvolution of Bioactive Small Molecules via Multi-Omics Based Approach

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Many efforts have focused on target deconvolution of bioactive small molecules from phenotypic screens to explore mechanisms of action (MOA) of small molecules and target proteins towards cellular and medical applications. We have developed Multi-Omics based Target Identification and Validation (MOTIV) for harnessing target deconvolution of bioactive small molecules. MOTIV includes a direct affinity-based target protein identification using phage display biopanning or Drug Affinity Responsive Target Stability (DARTS), an indirect genomics-based profiling using yeast ORFeome, and combinations of computational network analysis and validations of these target candidates leading to identification of the biologically relevant target proteins and cellular mechanism of small molecules of interest. We have applied this approach to explore MOA of bioactive small molecules from phenotypic screens of angiogenesis and autophagy. Accordingly, new target proteins and pathways have been identified and validated to provide new insights into the biology of angiogenesis and autophagy leading to biotechnological and medical applications. In this presentation, recent results of our group using MOTIV for target deconvolution of bioactive small molecules targeting angiogenesis and autophagy will be introduced.

PS30-02 Reverse Chemical Proteomics as a Tool in Drug Discovery

Peter Karuso

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Functional proteomics has an important role to play in drug design and development. Not only for mapping proteins in health and disease but also in uncovering the mode of action of drugs (chemical proteomics).[1] Our research focuses on *reverse chemical proteomics (RCP)*, which promises to be a fast, efficient and enabling technology for understanding drug action, identifying the most avid binding partners for small molecules and the discovery of new protein drug targets for medicinal chemistry.

In *RCP* the proteome is tagged with its encoding gene and the bait tagged with and affinity reagent such as biotin. Using T7 bacteriophage as the genetic tag of the proteome has many advantages but the most important is that the proteome can be amplified as many times as needed. The most avid binding partners are purified through multiple rounds of affinity purification (biopanning).

Here, we will outline the concepts of *RCP* and our results for the discovery of protein binding partners for the natural products FK506, artemisinin, kahalalide F and daptomycin. FK506 has well characterized interactions and acts a positive control while artemisinin is an antimalarial with recently described anticancer activity. Daptomycin is a recently introduced antibiotic with notable side effects that suggest off-target associations with human proteins. The natural products were biotinylated at sites known not to affect the compound' s biological activity and the resulting probes were screened against cDNA libraries. This work aims to support our understanding of the underlying biochemical pathways of these compounds and to pave the way for better structure-based drug design and development through chemical protemics.

[1] P. Karuso, in *Comprehensive Natural Products II: Chemistry and Biology, Vol. 9* (Eds.: L. N. Mander, H. W. Lui), Elsevier, Oxford, **2010**, pp. 513-567.

PS30-03 Target Identification of Novel Anti-inflammatory Compound Using Chemical Proteomics Approach with Bait Compound

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Interleukine-12 (IL-12) and IL-23 are pro-inflammatory cytokines which induce serious pathological conditions in diverse of inflammatory diseases such as inflammatory bowel disease (IBD), psoriasis and rheumatoid arthritis. The therapeutic potential of IL-12/23 inhibition has been validated in clinical by Ustekinumab which is an approved monoclonal antibody neutralizing IL-12/23. Through the cell-based phenotypic screening and extensive medicinal chemistry campaign, we generated APY0201 as a potent and unique inhibitor for IL-12/23 production from activated macrophages, possessing significant selectivity over other cytokines including TNF-α. As a result of chemical proteomics approach using a bait compound equipped with FLAG peptide, PIKfyve kinase was identified as a biological target of our promising IL-12/23 production inhibitor. APY0201 is a potent, highly selective and ATP-competitive PIKfyve kinase inhibitor, which ameliorated inflammation in experimental model of colitis. We will disclose the design of bait compound equipped with FLAG peptide, strategy of target identification, SAR, and unique character of this novel drug target, to treat autoimmune and inflammatory diseases.

Keywords: Target identification, PIKfyve, IL-12/23

PS31-01 An Overview of Label-Free Quantitative Shotgun Proteomics in Rice and Grapes

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In recent years we have performed a number of label-free quantitative shotgun proteomics studies in rice and grape plants and cells that have revealed new information on how plants respond at the molecular level to abiotic stress in different environments. In a detailed study of temperature stress in rice cells, we observed tightly controlled switching of sugar metabolism pathways occurring in response to increased temperature, along with modifications in cell walls occurring at extremes of both hot and cold, but by different mechanisms. Perhaps the most striking feature of this study was the apparent metabolic 'panic response' of cells that was triggered between 36°C and 44°C. In a drought time-course study in six week old rice seedlings we observed a tightly coordinated response whereby aquaporins and VTPases were reduced in expression at the onset of drought and then greatly increased in expression as the drought cycle progressed. Additionally, in a complex study of split-rooted plants under drought stress we found evidence of directional long distance signaling between roots in different environments but attached to the same shoot. The availability of a complete sequenced and annotated genome for *Vitis vinifera* has allowed us to move into working in grapevines, which are a vitally important crop plant. We have undertaken a detailed proteomic analysis of water deficit stress in 2 year old Cabernet Sauvignon grapevines. One intriguing finding from this work is the detection of protein expression changes in response to water stress, before any phenotypic changes are seen at the whole plant level.
We will also present initial results from detailed studies of temperature stress in Chardonnay grape cells and photoperiodsensitivity in bud tissues from *Vitis riparia.*
Keywords: label-free quantitation, agricultural proteomics, abiotic stress

PS31-02 Green Systems Biology - From Single Genomes, Proteomes and Metabolomes to Ecosystems Research and Biotechnological Applications

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Systems biology is the approach to combine molecular data, genetic evolution, environment and species-interaction with the computer-assisted understanding, modeling and prediction of active biochemical networks. The idea relies strongly on the existence of complete genome sequences and the development of new technologies for the analysis of molecular data. Here, projection of metabolomics and proteomics data into genome-wide metabolic networks combined with metabolic modeling emerge as important technologies for improving gene annotation processes [1, 2]. Using quantitative proteomics and metabolomics we begin to investigate the genome-scale molecular phenotype and the interrelation of the metabolome, the proteome and its environment [1]. Genome-, proteome- and metabolome- as well as anatomical/morphological/physiological- data integration strategies and modeling approaches will be discussed for systems such as Arabidopsis, Tomato and *Chlamydomonas reinhardtii*, the "green yeast" as a model system for third-generation biofuels. For these approaches an extended proteomics and metabolomics platform is presented [3-6]. However, before data reveal their interrelation, extended statistical and mathematical concepts are required for the integrative analysis of multifactorial phenomena [7]. The detection of significant correlations between the different components based on principal components analysis or related techniques is the basis for biological interpretation [8-11]. We have extended this idea and developed an approach which connects systematically the predicted genotype with the statistical features of metabolomics and proteomics data [12]. By using this approach, recently, we were able to calculate the differential biochemical Jacobian from perturbed metabolomics data for the first time [5]. Results and implications of this approach will be discussed.
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PS31-03 Golgi Proteomics in Plants and Application for Biofuel Plant Development

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The production of cellulosic biofuels utilizes the energy reserves contained in plant cell walls. Plant cell walls are costly to deconstruct, contain inhibitors, have non-optimal sugar profile and are a low density material. Nonetheless, incremental modifications to this biomass can result in significant economic improvements. The plant Golgi apparatus synthesizes a significant proportion of matrix polysaccharides destined for incorporation into the plant cell wall. Recently, we developed the first plant Golgi purification strategy based on a combination of density centrifugation and charge based separation using free-flow electrophoresis. The application of this technique to the model plant Arabidopsis enabled a proteomic survey of this organelle which consistently identified around 400 proteins. This collection of Golgi resident proteins provided a list of targets for the development of engineering strategies for the manipulation of the plant cell walls. Recently, in an attempt to identify more subtle approaches for cell wall engineering, we examined the possibility that sub-compartment charge differences of *cis*- *medial*- and *trans*-Golgi components may separate during FFE. We have employed immunoblots, single fraction resolution shotgun mass spectrometry and MRM to support the hypothesis of *cis*- *medial*- and *trans* separation of plant Golgi by free-flow electrophoresis. These approaches have enabled the grouping of functional partners at the sub-Golgi level. These findings should eventually enable more subtle engineering of matrix polysaccharide biosynthesis for optimal cell wall profiles.

PS32-01 Quantitative Proteomics of Lysine Acetylation, Succinylation, and Ubiquitylation

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A great majority of proteins in eukaryotic cells are modified by different posttranslational modification (PTMs). PTMs are key constituents of cell signaling networks, comprising some of the most sophisticated control switches in the cell. Given the vast complexity and their all essential role in controlling biology of living cells, analysis of proteins and PTMs is one of the most exiting areas of research in biology.
Recent developments in high-resolution mass spectrometry (MS), computational proteomics, and optimized PTM-enrichment strategies have greatly facilitated unbiased (non-hypothesis driven) analysis of posttranslational modifications. We have applied these approaches to investigate dynamics of the three major lysine PTMs – acetylation, succinylation and ubiquitylation. Large-scale analysis of these PTMs provided systems view of signaling networks, and revealed their extensive involvement in diverse biological processes. I will discuss these recent developments and highlight applications of high resolution MS for analysis of lysine acetylation and ubiquitylation dynamics in response to cellular perturbations.

PS32-02 Comprehensive Identification of Human N-Myristoylated Proteins Using cDNA Resource and Cell-Free Protein Synthesis System

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Protein N-myristoylation is a well-recognized form of lipid modification that occurs in eukaryotic and viral proteins. Many N-myristoylated proteins play key roles in regulating cellular structure and function. However, comprehensive identification of human N-myristoylated proteins has not been accomplished. The analysis of protein N-myristoylation has been performed mainly by the cell-based strategy such as the MS analyses of proteins extracted from cells or tissues, or the metabolic labeling of the cells transfected with a particular cDNA. One possible alternative method to analyze protein N-myristoylation is the use of cell-free protein synthesis system. Recent development of novel eukaryotic cell-free protein synthesis system and improvement of mass spectrometric analysis system, coupled with the enlargement of the available well-characterized cDNA resources, enables genome-wide analyses of protein N-myristoylation. In this study, in order to establish the strategy for comprehensive identification of human N-myristoylated proteins, the susceptibility of human cDNA clones in human cDNA resources to protein N-myristoylation was evaluated by metabolic labeling in an insect cell-free protein synthesis system and in transfected mammalian cells.
As a result, the products of 35 out of ~6300 human cDNA clones (Kazusa ORFeome project (KOP) human cDNA clones) were found to be novel human N-myristoylated proteins. These novel N-myristoylated proteins contain not only physiologically important cytoplasmic proteins such as protein kinases, phosphatases, E3-ubiquitin ligases, cytoskeletal regulating proteins, apoptosis related proteins, disease related proteins, but also many integral transmembrane proteins that play critical roles in various cellular functions. Functional analyses of non-myristoylated mutants of these proteins revealed that protein N-myristoylation plays critical roles in the expression of their physiological functions. These results indicate that the strategy proposed in this study is useful for the comprehensive identification of human N-myristoylated proteins from human cDNA resources.

PS32-03 Identification and Characterization of the Posttranslational Modifications of Yeast 26S Proteasome

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The 26S proteasome is a multisubunit protease involved in the proteolysis of various proteins tagged with polyubiquitin chains and controls the expression of their functions at appropriate times and locations. Posttranslational modifications (PTMs) are expected to be involved in the dynamic regulation of the proteasome activity.
We have identified more than 110 PTM sites in the 34 subunits of the yeast 26S proteasome. For the N-terminal peptides, 21 N-acetylation sites and one N-methylation site were identified. In addition, we also found one N-myristoylation site, a lipid anchor modification on N-terminal glycine, in Rpt2 subunit. It is widely accepted that the N-myristoylation is implicated in the intracellular localization or intermolecular interaction of modified proteins. For the functional characterization of the N-myristoylation on Rpt2, we constructed the yeast strains carrying site-specific substitution/deletion mutation. Both mutants showed abnormal sensitivity to the protein misfolding and increased accumulation of polyubiquitinated proteins. Fluorescence microscopy of the GFP-tagged proteasomes showed the nuclear localization of proteasome in normal cells, while the mutation resulted in the diffusion of the nuclear proteasome into the cytoplasm, where it formed aggregates. In contrast, assembly and peptidase activity of the 26S proteasome were totally unchanged, as revealed by the MS analysis of the TAP-purified proteasome and in-gel peptidase assay. This is the first report indicating that PTM on a single subunit influences the subcellular localization of a huge protein complex unless disturbing its assembly or activity.
Keywords: Posttranslational modification, Myristoylation, Proteasome

PS32-04 Mapping the Human Methyltransferasome Reveals the Existence of a Posttranslational Modification Code that Targets Molecular Chaperones to Regulate Functional Organization of the Human Proteome

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In efforts aimed at identifying and characterizing protein substrates and regulators of all known and putative human methyltransferases (the methyltransferasome) using multiple cell compartment affinity purification coupled with mass spectrometry (MCC-AP-MS), our laboratory has discovered a novel family of lysine methyltransferases that preferentially target and regulate molecular chaperones. Our results indicate that methylation of molecular chaperones VCP by METTL21D and Hsp70 by METTL21A regulates the activity of both chaperones. In the case of VCP, trimethylation of lysine 315 by METTL21D is stimulated by the addition of the UBX cofactor ASPSCR1, which we show directly interacts with the methyltransferase. This stimulatory effect was lost when we used VCP mutants (R155H, R159G and R191Q) known to cause Inclusion Body Myopathy with Paget's disease of bone and Fronto-temporal Dementia (IBMPFD) and/or some familial forms of Amyotrophic Lateral Sclerosis (ALS). Lysine 315 falls in proximity to the Walker B motif of VCP's first ATPase/D1 domain. Our results indicate that methylation of this site negatively impacts the ATPase activity of this molecular chaperone. In the case of Hsp70, methylation of lysine 561 by METTL21A was shown to decrease formation of stress granules in response to arsenite treatment, a model that is widely used to study improper protein folding in degenerative disorders. In addition to having implications for the development of therapeutics for degenerative neuromuscular disorders such as ALS and IBMPFD, the discovery of a family of chaperone-targeting methyltransferases led us to propose the existence of a chaperone posttranslational modification code, that we termed the chaperone code, which is at play to orchestrate the proper folding and assembly of protein complexes that make up the human proteome. We will report on our most recent results relating to decryption of the chaperone code and its role in diseases.
Keywords: Protein-protein interactions, Methylation, Molecular chaperones

PS32-05 A Proteomic Investigation of Proteasome Malfunctioning

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Inhibition of the proteasome is pro-apoptotic in most cell types. In cancer therapeutics, the induction of apoptosis using chemical agents is widely used, for instance for the treatment of myeloma patients. Here, we aim to dissect the functional modules of the proteasome by profiling the dynamic proteome and ubiquitinome as a result of proteasome dysfunctioning. We study the effect of proteasome malfunctioning on the global cellular *Drosophila* S2 cellular proteome using a SILAC-based nanoLC-MS/MS (Q Exactive) approach. Inhibition of the proteasome is accomplished by either using chemical agents (MG132 and lactacystin) or by using selective RNAi knockdown constructs against different proteasomal subunits. For the identification of the dynamic pool of ubiquitinated proteins we use a recently developed protocol based on immunoprecipitation of peptides derived from ubiquitinated proteins. Roughly 5,000 proteins are identified and quantified routinely in these SILAC screens. After relatively short incubation times with drugs, approx. 100 proteins are specifically found to be upregulated and/or accumulated. After longer incubation times and upon RNAi of knockdown of proteasomal subunits, the abundances of several hundreds of proteins are altered. Proteins that show severe and relatively fast upregulation and/or accumulation are associated with functional categories such as stress response, cell cycle regulation, apoptosis and the ubiquitin-proteasome system. In addition, the pool of ubiquitinated proteins is upregulated after proteasome inactivation. Strikingly, there is little overlap between the sets of proteins with increased abundances and proteins showing increased ubiquitination ratios. Currently, we are investigating the target specificity of various proteasome-bound deubiquitinating enzymes by analysis of the dynamic ubiquitinome. Results are expected to give more insight into the mechanism and specificity of the proteasome functioning.
Keywords: Proteasome, Ubiquitinome, SILAC

PS33-01 Tracking Protein Turnover in Plant Cells and Intact Plants: A New Frontier is Understanding the Energy Efficiency of Plant Growth

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Shotgun approaches or targeted SRM analyses now dominate proteome studies as tools to find changes in the proteome. Key limitations, however, are that these approaches focus just on the proteins that are changing in abundance to find biological insights and they require statistically significant changes in the total accumulated protein pool size in order to identify that anything has occurred. Analysing protein synthesis and degradation rates with progressive stable isotope labelling provides a new window on the control of protein abundance as we seek to determine the 'relative age' of the proteins that we see. Through progressive ^{15}N labelling of plant cells from nitrate and ammonia salts and modelling incorporation fits, we can calculate the rate at which proteins which are static in abundance in the proteome are turning over, and thus provide an extra dimension to proteome analysis by an assessment of what is controlling protein abundance in cells. Through combining such labelling with separation of protein complex and subcomplexes by native electrophoresis, we can observe the *in vivo* turnover rate of assembly intermediates of protein complexes. We have developed pipelines to undertake these studies in plant cells, plant leaves and in whole plants through the use of hydroponics. Projects assessing the impact of phosphate limitation, groundwater salinity and nitrogen fertilization on protein turnover changes in plants will be discussed. Combined there approaches provide new avenues for peptide mass spectrometry to provide answers to a wide range of questions in plant biology, and allows researchers to assess the cost of environmental factors on protein turnover and plant growth efficiency.

Keywords: plant proteomics, protein turnover, protein complexes

PS33-02 Functional Proteomics of Plant Signaling and Metabolic Networks

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Environmental stresses affect plant growth, development, yield and bioenergy production. Cellular molecular networks connect environment signals to phenotypes. Our knowledge about how different molecules and pathways connect to each other is very limited. Here we implement functional proteomics approaches to tackle plant molecular networks. We make use of stomatal movement and glucosinolate metabolism as model systems. Stomatal movement is essential for plant growth, yield, and interaction with the environment. Quantitative proteomics of stomatal guard cells under different hormone treatments has revealed novel redox responsive proteins, which not only highlight crosstalk between hormone signaling at the posttranslational level, but also provide nodes and edges for biotechnological applications. Glucosinolates constitute a large group of plant specialized metabolites in Brassica crops and vegetables. In addition to anticarcinogenic activities, glucosinolates play important roles in plant defense against herbivores and pathogens. Here we have implemented a systems biology approach by perturbing the expression of key genes, followed by examining changes in metabolite and proteins levels in other pathways. In this presentation, our recent progress on developing and implementing proteomics and metabolomics tools to elucidate plant molecular networks will be reported.

Keywords: Quantitative proteomics, Molecular networks, Brassica plants

PS33-03 Comparative Analysis of the Proteomes of *Ricinus communis* and *Jatropha curcas* Seeds

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The seeds of *Ricinus communis* and *Jatropha curcas* are regarded as a potential source of raw material for the production of biodiesel, but exploitation of this potential is hampered by a lack of understanding regarding key aspects of seed development and biochemistry. We have performed a detailed histological analysis of seed development in these two species and used it as a guide to undertake an in-depth proteome analysis of the inner integument, nucellus and endosperm of developing seeds, as well as of plastids isolated from these tissues. In this presentation, the many commonalities unraveled by our proteome analysis in the biochemical machinery of seeds from these species will be discussed, particularly in relation to carbon and nitrogen flow. We will also discuss results pertaining to the pattern of deposition and synthesis of ricin and phorbol esters in maternal (inner integument and nucellus) and embryo (endosperm) tissues.

PS33-04 Proteomic Analysis Showing the Features of Metabolism Regulation During Rice Seed Germination

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Rice seeds are not only the staple food resource of the world population, but also an essential material for agricultural production. Successful germination of rice seeds is a prerequisite for its cultivation. In order to uncover the mechanisms underlying rice seed germination, systematic proteomic analyses were conducted on rice seeds during this process. Metabolic pathways were constructed based on the proteome profiling data. Along with the comparative proteomic analyses, we found that the enhancement of anaerobic respiration including glycolysis and fermentation might be the main source of ATPs at the early stage of germination, and the aerobic respiration was only enhanced at the late stage. Starch granules were quickly degraded in the endosperm into glucose 6-phosphate, which was transferred into embryos either for further degradation or for biosynthesis of starch. The newly biosynthesized starches were mainly accumulated in the tissues around the bundle sheath. The genetic information for germination were programmed during seed maturation, so the initiation of transcription is unnecessary, but it can help to ensure the rapidity and efficiency of the germination. Unlike transcription, translation is absolutely needed. Furthermore, we also found that there were several GA and ABA responsive elements in the promoters of most of the genes encoding the up and down-regulated proteins respectively, which suggested that the germination of rice seed might also be regulated by GA and ABA. These studies bring us some new insights into the rice seed germination.

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Keyword: rice, proteomics, germination

PS33-05 Involvement of the Plasma Membrane in Plant Cold Adaptation: A Protomics Perspective

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The plasma membrane (PM) plays a number of important roles in determining plant survival under adverse environmental conditions. We have revealed that the PM is the primary site of freezing injury due to its central role in a freeze-thaw cycle and cold acclimation (CA) results in changes in its compositions dynamically to increase the cryostability of the PM. Responses of PM proteome to CA are well corresponded to the increase of freezing tolerance and, in fact, we demonstrated that a few PM proteins directly affect freezing tolerance during CA. We have been studying cold-induced PM proteome changes in plants using oat, rye and *Brachypodium*, all in Family Gramineae, to explore possibilities to increase agricultural productivity of the crops in cold-temperature regions. Using purified PM fractions and nano-LC-MS/MS analysis, we comprehensively catalogued cold-responsive PM proteins and subsequently categorized them according to proposed functions. *Brachypodium* PM proteome was quite similar to that of oat and rye but a few differences were apparent. Furthermore, PM proteome changes during CA is somewhat species-specific, which may be associated with differences in freezing tolerance development of the three plant species during CA. We will present profiles of PM proteome of three Gramineae plant species before and after CA and discuss how we can use PM proteome profiles to understand plant adaptation to cold stress conditions. (Supported in part by grants-in-aid from MEXT and JSPS, Japan, #22120003, #24370018 and #24-7373.)

Keywords: Plants, Plasma membrane proteome, Stress adaptation

PS33-06 Characterization of Muscadine Berry Proteome Using Label and Label Free Mass Spectrometry Approaches

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Muscadine grapes (*Vitis rotundifolia*) are well known for variety of nutraceutical and enological characteristics. Our earlier results indicated potential anticancer activity of berry extracts on human cancer cell lines. These extracts contained variety of phenolics associated with anticancer activity. Until recently, most studies were focused on viniferas grapes, while little information is available on muscadine grape berries. Advances in mass spectrometry enhanced the ability to reveal more identified proteins in tissues. The objective of this research was to investigate the proteome profile of muscadine berry using gel based and gel free separation methods. Total proteins of pericarp were extracted from different stages of berry development and ripening. Two-dimensional electrophoresis resolved approximately 350 proteins. Gel free iTRAQ label followed by mass spectrometry identified 600 proteins, while label free mass spectrometry revealed over 1600 proteins. Over 150 proteins showed differential expression during the berry development. We contemplate that, gel free and label free method of protein identification revealed more proteins in the pericarp tissue. Functional annotation revealed the proteins involved in pathways related to defense and secondary metabolite synthesis. Protein-interaction studies of these differentially expressed proteins revealed several orthologous proteins showing interactions in *Arabidopsis* interactome database. Further investigation on interaction network will determine the role of differentially expressed proteins associated with the biosynthesis of nutraceutical compounds.

Keywords: Grape berry, iTRAQ label, Mass spectrometry

PS33-07 Quantitative MSE Proteomics as a Tool for the Determination of Clinically Relevant Proteins in Wheat Grain

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The amounts of clinically relevant disease-related proteins in wheat grain are largely unknown. Developing the methods for quantitative measurement of clinically relevant proteins could support advancements in understanding exposure thresholds and clinical study design. The aim of our study was to use a data-independent mass spectrometry (MS^E) approach for quantifying gliadin and glutenin proteins in wheat grain. The biological replicated analysis yielded concentrations for 34 gliadin and 22 glutenin proteins and detected several peptides carrying four previously discovered epitopes that belong to gamma gliadin B precursor. The technical coefficients of variation ranged from 0.12 to 1.39 and indicates that MS^E proteomics is a reproducible quantitative method for the determination of gliadin and glutenin content in the highly complex matrix of protein extracts from wheat grain.

Keywords: wheat, grain, allergy

SS-02 (Keynote) The Strategy, Organization, and Progress of the Human Proteome Project

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The global Human Proteome Project aims to identify and characterize at least one protein product and many PTM, SNP, and splice variant isoforms from all 20,300 human protein-coding genes [www.thehpp.org]. The deliverables are an extensive parts list and an array of technology platforms, reagents, spectral libraries, and linked knowledge bases that advance the field and facilitate the use of proteomics by a much wider community of life scientists. Such enablement will help address the Grand Challenge of using proteomics to bridge major gaps between evidence of genomic variation and diverse phenotypes.

SS-03 (Keynote) Genome-wide Proteomics: The Role the C-HPP Initiative to Study Cancer Gene Amplicons and Proteomic Signatures

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The development of a genome-wide proteomic strategy has been elucidated by the Human Proteome Project (HPP) and promotes the integrating transcriptomic and proteomic data in the context of biology and disease studies. Also the proteomic researcher will better understand the genomic context of their observations. This lecture will illustrate this approach with a study of breast and gastric cancer driven by the oncogene ERBB2, a member of epidermal growth factor receptor (EGFR) superfamily of tyrosine kinase receptors. In this study the gastric cancer were of the intestinal type and consisted of two cancer sample sets, ERBB2 positive and negative. For both sets the control was normal tissue adjacent to the tumor. In the analysis proteins were extracted from the tissue samples separated by SDS-PAGE, then analyzed by nanoLC coupled to an LTQ-Orbitrap mass spectrometer. We also examined transcriptomic data from ERBB2 expressing cancer cell lines, SNU16 and KATOIII (gastric) and SKBR3, SUM 149 and 190 (breast cancer). While the proteomic study was of lesser depth (approximately 3,000 proteins identified) than the RNA-Seq analysis that identified 11,000 transcripts (100 million reads) proteomics can aid the identification of significant pathways and expression events identified by the transcriptome analysis. The study identified the ERBB2 amplicon, which contains a group of genes co-amplified with the oncogene as well as other pathways of potential significance such as the MEK-ERK and the proteasome degradation pathway.

SS-04 Protein Post-Translational Modifications and their Regulation of Cardiovascular Disease

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To improve our understanding of cardiovascular biology, we investigate the role of proteins under defined disease conditions via the use of model systems. Heart disease is the leading cause of mortality and morbidity worldwide, with coronary heart disease (CHD) accounting for 52% of attributable deaths. Acute myocardial infarction (AMI) is directly linked to CHD and results from myocardial ischemia, where the heart receives an inadequate supply of oxygen. The extent of damage to the heart is proportionate to the duration of ischemia, whereby transient episodes are protective, brief periods cause reversible contractile dysfunction and extended insults result in cellular necrosis and ultimately organ failure. Given the broad range of functional responses resolvable on a relatively sort time scale (1-60 minutes), we hypothesize that subtle protein post-translational modifications play an essential role in these outcomes. Proteomic technologies have facilitated our understanding of the role of protein degradation, phosphorylation, acetylation, glycosylation and oxidation in response to myocardial ischemia. To model disease, we utilise ex-vivo induction of ischemia in rat (*Rattus norvegicus*) and rabbit (*Oryctolagus cuniculus*) myocardium as model organisms. This permits investigation of the cellular events, under otherwise ideal conditions prior to validation in clinical cohorts, which are ultimately more challenging given the heterogeneous nature of AMI patients whom present with diverse co-morbidities including diabetes. An essential component of proteomic PTM investigations are the observation of non-modified counterparts to ensure that changes in the modified species are independent of changes at the protein level and to improve our coverage of the cardiac proteome. We have identified over 4,000 unique myocardial proteins of which nearly 50% are modified at one point during the progression from protection to necrosis using our ex-vivo model. In our experience, to improve our understanding of myocardial I/R injury, model systems provide the ideal biological situations from which to generate hypotheses for testing in more complex clinical situations.

JHP-01 Molecularly-informed Discovery of Proteomic Biomarkers for Cancer Detection

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Development of proteomic biomarkers, in part, has been driven by intuition and serendipity. This type of approach has led to discovery and development of biomarkers, which were later found to be compromised by the lack of reproducibility, generalizability and usefulness in clinical application. This is despite the fact that over the years the molecular knowledge of cancer initiation, causation and progression has made remarkable progress. The Whole Genome Sequencing (WGS) is rapidly generating data on gene expression profiling, copy number variation profiling, SNP genotyping, genome wide DNA methylation profiling, microRNA profiling, and exon sequencing of at least 6,000 genes, including microRNA. These efforts have led to identification of several important cancer networks and pathways that are becoming useful tools for discovering genomic biomarkers. However, proteomics offers more phenotypically-related biomarkers than genomically-related biomarkers and are more amenable to clinical applications. The Speaker will discuss the importance of genetically driven knowledge in developing strategies for protein-based biomarkers in cancer detection. For example, the NCI's Early Detection Research Network (EDRN; www.cancer.gov/edrn) investigators are proactively analyzing The Cancer Genome Atlas (TCGA) data along with their own expression data to guide the discovery of ovarian cancer-related secretome genes that can be further prioritized and subjected to scrutiny for clinical validation. The secretome will be used to interrogate large numbers of microarrays performed on a variety of cancer and control tissues including TCGA. For example, all 480 serous ovarian cancers (within the TCGA), which have mRNA expression data, will be utilized to identify differentially expressed transcripts between cancer and normal tissue. Lists of genes with significant differential expression will then be filtered using a variety of expression profiling databases of microdissected ovarian cancers and normal control tissues. Any gene found in multiple datasets will be considered high priority epithelial biomarkers. The final product will be a highly filtered list of differentially expressed genes (not found to be highly expressed in other tissues) that potentially encode proteins found in the serum. Such approaches are being employed in discovering biomarkers for ovarian, pancreatic and other types of cancers.

JHP-02 A Proteomic Approach to Decipher the Molecular Link between HIV-1 Gag and Host Proteins

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Background & Purpose: The process of assembly and release of Human Immunodeficiency Virus type1 (HIV-1) is driven by the active trafficking and assembly of Gag precursor protein (p55) in infected host cells. Although the involvement of host proteins in the regulation of Gag during the virus particle production has been proposed, the detailed molecular mechanisms are still not yet well characterized. The discovery of cellular factors that participate in this process can provide further insights into the nature of HIV-1 replication pathways and assist with identifying new targets for anti-viral therapies.
Methods & Results: In our current study, we utilized the amplified luminescent proximity homogenous assay (AlphaScreen) as an *in vitro* protein-protein interaction analysis in an attempt to identify host factor(s) that directs Gag to promote virus replication. Using full-length HIV-1 Gag and host proteins synthesized by the wheat cell-free protein production system, we found that the atypical protein kinase C (aPKC) can bind HIV-1 Gag. LC-MS/MS and immunoblotting analysis with a phospho-specific antibody confirmed both *in vitro* and *in vivo* that aPKC phosphorylates HIV-1 Gag at Ser-487. Computer-assisted structural modeling and a subsequent cell-based assay revealed that this phosphorylation event is necessary for the interaction between Gag and HIV-1 accessory protein Vpr (viral protein R), and results in the incorporation of Vpr into virus particles. Moreover, the inhibition of aPKC activity reduced the Vpr levels in virus particles and impaired HIV-1 infectivity in human macrophages.
Conclusions: Our current study sheds new light on the molecular link between Gag phosphorylation by aPKC and viral infectivity through the incorporation of Vpr into virions. The targeting of aPKC activity could be a potential option as a novel therapeutic intervention against HIV-1 infection.
Keywords: HIV-1, host factor, phosphorylation

JHP-03 Phosphoproteomics of Human Liver Cancer Analyzed by 2-Dimensional Image-Converted Analysis of Liquid Chromatography and Mass Spectrometry (2DICAL)

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Two-dimensional image-converted analysis of liquid chromatography and mass spectrometry (2DICAL), which we developed originally, is a proteomic analysis system that compares individual peptide peaks of multiple samples and selects significant peptide peaks with statistical analyses in a label-free method. We applied 2DICAL in analyses of the phosphoproteomics of human liver cancer to search for specific phosphorylation changes in human liver cancer.
106 samples were collected from patients with liver cancer in an ethically approved process. Deep-frozen raw tissue (1mg) was fixed in 100% methanol and trypsinized in 1% sodium deoxycholate solution. Phosphopeptides were extracted by the HAMMOC method and desalted. Liquid chromotography-mass spectrometry and tandem mass spectrometry (MS-MS) spectra were acquired by Triple-TOF 5600. LC-MS-MS data (n = 212) were analyzed by 2DICAL, and the peptide peaks with fragment expectation below 0.05 by Mascot analysis were statistically analyzed in liver cancer and noncancerous liver tissue. The significant phosphopeptide peaks were selected and applied to the informatics investigation.
In the detected 44,990 peptide, 2,390 were phosphopeptides with fragment expectations below 0.05 by Mascot analysis. In the differential analysis between the liver cancer and noncancerous liver tissues, 173 phosphopeptides were increased more than two-fold and 145 were decreased by less than half in liver cancer tissue compared to noncancerous liver tissue. The altered phosphopeptides were referred to the derived proteins and classified by the Gene Ontology terms. There were several phosphopeptides that were not reported to change in human liver cancer. They were considered to be not only candidate biomarkers but also potential therapeutic targets for liver cancer. A phosphoproteomics analysis of 106 human liver tissues by 2DICAL revealed specific and systemic phosphorylation changes in liver cancer.
Keywords: 2DICAL, Liver cancer, Phosphoproteomics

YI01-01 Role of CYLD Deubiquitinase in EGF Signaling Pathway

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The tumor suppressor for human cylindromatosis, CYLD, is an ubiquitiously expressed deubiquitinase that specifically hydrolyzes K63-linked polyubiquitin chains. CYLD was initially described as an inhibitor of the TNF-activated NFkB pathway through deubiquitination and subsequent inactivation of key adaptor molecules such as NEMO, TRAF2, TRAF6 or Bcl-3. Accordingly, CYLD down-regulation has been reported in several types of tumors including lung, liver or colon cancer, whereas cyld-/- mice show increased susceptibility to develop colon or skin tumors. Nevertheless, recent evidences suggest its spectrum of biological activities is not limited to the NFkB pathways. Our earlier studies revealed that CYLD could be involved in the signaling cascade initiated by the epidermal growth factor (EGF), as it was found to be strongly tyrosine phosphorylated upon EGF stimulation. The engagement of CYLD in EGF signaling has also been documented in several very recent studies. However, the functional meaning and impact of the novel role attributed to CYLD still needs to be elucidated. In order to decipher the implication of CYLD in EGF signaling, we applied SILAC-based quantitative proteomics to compare the phosphoproteome as well as the CYLD-interacting protein network in wild type- and CYLD- silenced cells. These unbiased analyses may provide better understanding of the role of the CYLD deubiquitinase in the signal transduction initiated by EGF.
Keywords: EGF signaling, CYLD deubiquitinase, Quantitative proteomics

YI01-02 ATP Accessibility Screening (AAS), A High-Throughput and High-Resolution Kinase Analysis Platform for Signaling Research

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Phosphoproteome analysis is now widely used for various signaling research. It is possible to quantitate phosphorylation level of thousands or more phosphosites. However, deep phosphoproteome analysis is low-throughput. Due to this disadvantage, it is very difficult to use phosphoproteome analysis for experiments which need high-throughput fashion, such as chemical library screening or genome-wide knockdown experiment. Phosphoproteome analysis is useful to monitor kinase activity, when target kinase has a phosphorylation site which represents its kinase activity. However, a kinase which activity is not regulated by phosphorylation, such as constitutive active kinase, cannot be monitored by phosphoproteome analysis. In that case, we might predict kinase activity from phosphosites (substrate) data using bioinformatics analysis, but such kind of analysis is low-resolution (for example, it is difficult to distinguish AKT1 and AKT2 activation). In order to overcome these challenges, we developed a high-throughput and high-resolution proteomic analysis platform, ATP Accessibility Screening (AAS). AAS is a kind of activity-based proteomic analysis targeted on ATP accessibility of kinases. We employed amine-reactive ATP-biotin tag to label lysines conserved in ATP binding pocket. After labeling, protein was digested, enriched by streptavidin beads and applied to LC-MS/MS. About 15% of identified proteins were kinases. Thus we constructed an inclusion list contains *m/z* and retention time information on peptides from 249 kinases. Using this inclusion list, we can quantified ~130 kinases in 1 hour run from 4 mg SILAC labeled Hela-S3 lysate. We will also report the effects of ionized radiation at different doses and at different time after irradiation on ATP accessibility.
Keywords: ATP accesibility, kinase, screening

YI01-03 Hidden Proteome: Multiplex Quantitation of Low- and Ultralow-Copy Number Proteins in HepG2 Cells and Human Plasma

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Using ordinary SRM approach we have registered almost complete chromosome 18th proteome in human plasma and HepG2 cells. Still, there is some portion of proteins that remaine undetectable due to their low concentration. To discover and quantitate such "hidden" proteins we designed an experiment comprising reducing sample complexity, increasing the sensitivity and simultaneous qualification and quantitation using stable isotope dilution in QED-SRM mode. The problem of complexity of biological sample was resolved by fractionation. The resulting fractions were enriched by irreversible covalent binding, which allowed proteins immobilization and enhancement of digestion with trypsin. Quantitative analysis was performed in QED-SRM mode using multiplex calibration approach with stable isotope dilution. The defined attitude permitted simultaneous verification of the targeted peptides by full MS and following progressive MS/MS scanning of the isolated precursor ions overlaying SRM transitions. Quantitative analysis in reduction energy ramping manner was performed in the case where SRM transitions match the defined criteria after dd-MS/MS scanning. The correctness of the isolated and quantified peptides was verified by,firstly,MASCOT processing of the accumulated MS/MS data and matching them to the registered peptides; co-elution of native peptides with heavy internal standard peptides; and assay of the transitions stability and conformity using MLD (Mean Logariphmed Derivative) function. Thus, we registered up to 90% of proteins after fractionation and enrichment with sensitivity of 10^{-17} M. Only 70% of the verified proteins were quantified while the remaining proteins were registered with the signal outside of the LLOQ. Bioinformatic processing of the registered and quantified proteins interaction and molecular functions has been applied in order to eunsure the possibility of their adequate presence and quntitation in HepG2 cells and human plasma.
Keywords: quantitation, QED-SRM, low-copy number

YI01-04 Targeted Proteomics to Validate and Quantify One-Hit Wonders Proteins in Human Liver

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In shot-gun proteomics, protein identification and quantification based on peptide fragment sequences commonly exclude single hit protein identifications. Although rigid guidelines ensure high quality of the reported identifications and avoid the inflation of identification lists with erroneous entries, exclusion of single hit wonders may result in the loss of potentially valuable meta-data. Since the concept of proteotypic peptides are widely used in quantitative proteomics, retrial of these one-hit wonders will replenish our knowledge in gene-centric proteomics. We employed SRM to verify the one-hit wonders proteins in Chinese Human Liver Proteome Project dataset. Crude peptides were synthesized and used to develope SRM assays for target peptides. Proteins extracted from normal human liver were separated in SDS-PAGE and digested in split gel slice. Then the digests were subjected to LC- scheldued SRM analysis. Totally, 184 SRM assays were developed and expression of 57 target proteins were confirmed in normal human liver tissues. Among the proved 57 one-hit wonders, 48 proteins are of minimally redundant set in the PeptideAtlas data base, 8 proteins even have none MS-based information before.

Keyword: SRM, one-hit wonders,human liver

YI01-05 Basic Fetoprotein is Homologous with G6PI/AMF/NLK/MF/PGI/PHI/SA-36 by the Identification of 99% AA Sequence for BFP Using MALDI-MS

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Basic fetoprotein (BFP), which is found in serum, gastro-intestinal tract and brain tissue of human fetuses, is widely used as a serum and urinary tumor marker, but its structure has not yet been investigated. Hence, we investigated the amino acid (AA) sequence of BFP, derived from the human hepatocellular carcinoma tissues grafted into nude mouse, using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS)(AXIMA Performance and AXIMA Resonance; Shimadzu/Kratos). By employing a wide variety of protease digestion (Trypsin, Lys-C, Lys-N, Asp-N, Arg-C, Trypsin & V8, Trypsin & Asp-N, Lys-C & Asp-N), selection of matrix system (3AQ/CHCA as matrix, MDPNA as additive), and MALDI-MS, we have successfully sequenced up to 99% of BFP without pre-fractionation. As a result, BFP tumor marker was found to be homologous to glucose-6-phosphate isomerase (G6PI), which revealed that BFP is also homologous to autocrine motility factor (AMF) / neuroleukin (NLK) /maturation factor (MF) /phosphoglucose isomerase (PGI) / phosphohexose isomerase (PHI) /sperm antigen (SA-36).
In conclusion, we showed that the BFP tumor marker is homologous to human G6PI protein by the identification of 99% AA sequence of BFP using MALDI-MS. Results demonstrated that the analytical system using MALDI-MS can serve as a very powerful tool for protein sequencing. Here in this session we describe details of this work.

Keywords: sequence coverage, biomarker, PTMs analysis

YI01-06 Systematic Characterization of Human Platelets in Arterial Vascular Disorders by Quantitative Proteomics

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Anti-platelet treatment is of fundamental importance in combatting functions/dysfunction of platelets in the pathogenesis of cardiovascular and inflammatory diseases. Dysfunction of anucleate platelets is likely to be completely attributable to alterations in protein expression patterns and post-translational modifications. Combining elaborate protocols for platelet isolation from fresh blood donations in conjunction with quantitative mass spectrometry, we created the first comprehensive and quantitative proteome of highly pure human platelets, comprising almost 4,000 unique proteins with copy number estimates for ~3,700 of those and relatively quantified ~1,900 proteins between four different healthy donors - with negligible contamination by leukocytes, erythrocytes and plasma, respectively. For the first time, our data allow for a systematic and weighted appraisal of protein networks and pathways in human platelets, and indicate the feasibility of differential and comprehensive proteome analysis from small blood donations. Since 85% of the platelet proteome show no variation between healthy donors, this study represents the starting point for disease-driven platelet proteomics. These findings allow for correlation to genome-wide association studies which identified in a retrospective manner a set of chromosomal regions affecting the risk of cardiovascular diseases. While respective gene products could be identified in platelets, a comprehensive and quantitative comparison of protein patterns between patients and relevant controls such as relatives and spouses to validate risk factors is still missing. In order to improve cardiovascular risk management, genomic and proteomic analyses of respective corresponding gene loci and proteins using next generation sequencing and targeted MS strategies are applied with the final goal to characterize valuable biomarkers for biomedical screenings.

Keywords: platelets, quantitative proteomics, posttranslational modification

YI01-07 Secretome Analysis of Three-Dimensional in Vitro Model Cholangiocarcinoma

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Cholangiocarcinomas (CCA) is a malignant neoplasm of biliary tract epithelium with an incidence and mortality progressively increase over the past decades. Although the occurrences of this cancer are variable amongst countries, Thailand has the highest incidence rate in the world. As this aggressive and poorly understood malignancy remains largely incurable, developing more effective biomarkers and therapeutic modalities could significantly extend survival rate for these patients. Nowadays, study of cancer cell lines secretome as a means to identify diagnostic and prognostic markers has been widely performed. However, conventional method is to collect conditioned media obtained from monolayer culture that may not be wholly representative of dynamic features in which tumors exist in vivo. At present, three-dimensional (3D) culture has been extensively used since it provides more realistic microenvironment in natural physiology than routine method. Therefore, scaffold-based 3D culture of human intrahepatic cholangiocarcinoma isolated from Thai patient (HuCCA-1) was established and differentially secreted proteins between 3D and monolayer cultures were identified. In total, 25 distinct proteins which belong to categories of metabolic enzymes, signal transduction, stress response, cytoskeleton, and protein synthesis and degradation upregulated in 3D culture. These altered proteins might indicate similarity between this model and in vivo secretion from solid tumor and would provide more valuable data about bile duct cancer pathogenesis that could improve future diagnosis and therapeutic strategies.

Keywords: three-dimensional culture, cholangiocarcinoma, secretome

YI02-01 Applying SWATH-MS to Dissect the Variability and Heritability of the Human Plasma Proteome

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The plasma proteome has attracted a lot of attention in translational medicine and biomarker discovery studies. However, fundamental questions such as the variability of the plasma proteins in a population, their inheritability and their longitudinal pattern over years remain unexplored. This is due to the lack of suitable analytical methods that can consistently identify and quantify a large number of proteins among individuals in large sample cohort. We demonstrate that our newly developed Data independent Acquisition (DIA) method, SWATH-MS [1], provides the unique and unprecedented chance to address these difficulties [2] for population proteomic studies.

Using 232 plasma samples from monozygotic (mz) and dizygotic (dz) twins that were collected with 2-7 year intervals, we aimed to systematically investigate and decompose the technical (peptide- and protein-level) and biological variance (such as genetic and individual-/common-environmental and longitudinally unstable factors) in human plasma proteome profiles. The results indicate that SWATH-MS identified and quantified at least 2500 unique stripped peptides (regardless of charge-state and modification) at an FDR of 1%, corresponding to more than 400 proteins at a high degree of reproducibility in the plasma samples. The data showed an unprecedented degree of reproducibly, with the median CV of 11.2% for technical replicates. Overall, monozygoctic twins showed significantly higher proteomic concordance in plasma compared to di-zygotic twins (P=2.97E-37). An accurate estimation protein heritability allowed by twin strategy is currently underway.

References:
[1] Gillet, L. C., et al, Mol Cell Proteomics. 2012, 11(6): O111.016717.
[2] Liu, Y. S., et al., Proteomics. 2013 Apr;13(8):1247-56.

Keywords: Plasma Proteome, SWATH-MS, Population proteomics

YI02-02 Plasma Proteome Analysis Using LC-MS/MS with Travelling Wave Ion Mobility and an Alternative Computational Solution to Protein Quantitation

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Biomarker discovery involves the analysis of highly complex biological samples. Even using chromatography coupled with mass spectrometry, many species still co-elute, causing masking of ion signals and challenging protein identification and quantitation. Many techniques for incorporating additional separation, such as pre-fractionation and electrophoresis, decrease sample throughput and involve additional sample preparation which can contribute to preanalytical variation. Travelling wave ion mobility coupled with label free data independent acquisition (DIA) requires no additional sample preparation, yet confers significant advantages in proteomic analysis. The inclusion of an ion mobility step into the workflow also allows the number of identified proteins to be significantly increased whilst precursor and product mass accuracies are maintained between the modalities The ion packeting behaviour of travelling wave ion mobility (TWIMS) affords improved and less interfered detection of lower abundant species, however, one obstacle encountered with the analysis of high dynamic range proteomic samples is signal saturation of high abundant ions, causing issues in quantitating the most abundant proteins. This abstract presents an alternative bioinformatic approach which overcomes this by calculating protein quantities from product ion data. Protein quantities calculated with this method are more in line with the widely accepted label free data independent acquisition quantities calculated from precursor ion data. It is thus shown that ion mobility enhances proteome coverage, and with an adapted data processing method, it can be reliably used for quantitation as part of a biomarker discovery pathway.

Keywords: Plasma, Ion mobility, Absolute quantitation

YI02-03 Quantifying the Dynamics of a 14-3-3 Protein Interaction Network by Affinity Purification and SWATH Mass Spectrometry

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Protein complexes and protein interaction networks are essential mediators of most biological functions. Complexes supporting transient functions such as signal transduction processes are frequently subject to dynamic remodeling. Currently, the majority of studies into the composition of protein complexes are carried out by affinity purification and mass spectrometry and present a static view of the system. To move toward a better understanding of inherently dynamic biological processes, methods which can reliably quantify temporal changes of protein interaction networks are essential. In this study we determined the capability of affinity purification combined with SWATH mass spectrometry (AP-SWATH) to quantify the reorganization of protein-protein interactions in time resolved perturbation experiments. We chose to study the dynamics of the 14-3-3β scaffold protein interactome after stimulation of the insulin/PI3K/AKT pathway. The analysis, performed using the open source software OpenSWATH, provided a complete quantitative data matrix highlighting patterns of dynamic regulation in 567 14-3-3β interacting proteins with respect to IGF1 stimulation. Quantitative changes in 14-3-3β interacting proteins clustered in to 5 distinct time profiles, 2 of which were strongly related to the activity of basophilic kinases such as AKT. To our knowledge this study represents the largest reported interactome for a single bait indicating that at least 2.8 % of the proteome is engaged by 14-3-3β containing scaffold dimers, and that a substantial portion of these are regulated after IGF1 stimulation. We therefore establish AP-SWATH as a tool to sensitively quantify dynamic changes in protein complexes and interaction networks in perturbed systems.

Keywords: quantitative interaction proteomics, data independent analysis, systems biology

YI02-04 Investigation of Time Dependent Competitive Protein Adsorption to Surfaces Using Mass Spectrometry

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Introduction

Surfaces in a complex protein solution will adsorb proteins. This event is fast and dynamic and triggers a biological response against the inserted/implanted biomaterial that eventually will lead to biofouling and encapsulation. This affects the properties of the inserted devices, such as hampered membrane functions of microdialysis (MD) probes or distortion in response of biosensors.

Methods

Untreated and coated filtration membranes were used as adsorption templates for human ventricular cerebrospinal fluid (vCSF). After adsorption in an incubation chamber, the membranes were washed, dried and the proteins were reduced, alkylated and digested. The sample preparation procedure was conducted according to an on-surface enzymatic digestion (oSED) protocol previously described by our group. The oSED digests were analyzed by nanoLC ESI-MS/MS using a 7T hybrid LTQ FT and Velos pro orbitrap mass spectrometer.

Preliminary Data

In this study, we present a time resolved map of protein adsorption. Non-coated and tri-block polymer coated, polycarbonate membranes was used as templates. As expected, a time and surface property dependent protein adsorption relationship was observed. It is not surprising that the degree of protein binding onto modified and non-modified surfaces was dependent on the properties of the protein as well as the properties of the surface. The process of biofouling for in vivo inserted materials can be postponed and thereby increasing the lifetime and use of e.g. microdialysis probes for patient monitoring. The preliminary data are very promising making it possible to identify a spectra of adsorbed proteins on different surfaces in a time dependent way.

Keywords: Competitive, Surface, Adsorption

YI02-05 Establishment and Application of a High-Quality Comparative Analysis Strategy of Low-Abundance Biomarker Peptide in Serum Based on Optimized Novel Peptide Extraction Method

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Low-abundance native peptides are an attractive target for the discovery of disease biomarkers. However, validating candidate peptides is difficult due to challenges associated with precise peptide identification and development of high-throughput assays using specific antibodies. Therefore, a highly reproducible and sensitive strategy based on effective peptide enrichment methods is needed to identify clinically useful biomarkers. We optimized our novel differential solubilization (DS) method [1] to selectively enrich peptides less than 6,000 Da, using tricine-SDS-PAGE to evaluate the optimization. The modified DS method was combined with LC-MS using conventional HPLC. The reproducibility and sensitivity of the proposed strategy were sufficient to enable discovery of low-abundance (ng/mL range) candidate biomarker peptides. A total of 40 serum samples collected pre- and post-surgery from renal cell carcinoma (RCC) patients were analyzed, resulting in discovery of 2 peptides that are upregulated and one peptide that is downregulated in pre-surgery RCC patients. These peptides were validated using 40 serum samples collected pre- and post-surgery from bladder tumor (BT) patients. Two candidate peptides that were upregulated in pre-surgery RCC patients were not upregulated in the sera of the pre-surgery BT patients. Finally, we propose 2 candidate marker peptides that could be used to detect RCC.

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Keywords: biomarker, peptide, serum

YI02-06 Quantitative Proteomic Approach to Identify Proteins Signalling Pathways in Human Cerebral Microvascular Endothelial Cells Modified with 17β-Estradiol

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Oestrogen has been shown to facilitate multi actions in regulating downstream cellular signals such as transcription, proliferation, and differentiation processes. However, the complete molecular mechanism involved in oestrogen modulation in human cerebral microvascular endothelial cells (HCMEC) is still unclear. Identification and quantification of proteomic changes will allow targeted research into protein networks associated with cerebrovascular disease related to oestrogen deficiency. In this study, interaction between 17β-estradiol and oestrogen receptors was investigated by incubating HCMEC for 24 hours with 17β-estradiol followed by Proximity Ligation Assay (PLA). Interactions were recognized as red dot formations on the cells. In order to systematically profile the changes in protein expression, the cells were fractionated into three different components; 1) membrane proteins 2) cytoplasm proteins, and 3) nuclear proteins respectively. The protein extracts were tagged using iTRAQ labeling and analyzed by LC ESI MS/MS. A total of 2350 unique proteins were identified and the expressions of 317 proteins were significantly altered ($p<0.05$) following treatment with 17β-estradiol. Ingenuity pathway analysis was performed for the regulated proteins and the signalling events directed to *Eukaryotic Initiation Factor 2 (eIF2)* signalling was identified as the major pathway. In addition, 2 other pathways were identified in membrane and cytoplasm components, i) *hypoxia signalling* and ii) *glycolysis signalling*. In nuclear fraction, i) *granzyme signalling* and ii) *Eukaryotic Initiation Factor 4 (eIF4) & p70S6K signalling* are highly stimulated. Thus, a combination of high throughput technique together with bioinformatics analysis is valuable in providing insights of molecular signalling events of 17β-oestradiol modulations in HCMEC.

Keywords: oestrogen, protein signalling pathways, protein interactions

YI02-07 Defining the Structure of Mitotic Chromosomes Using Multi-Classifier Combinatorial Proteomics Together with DT40 Genetics

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Packaging of DNA into condensed chromosomes during mitosis is essential for the faithful segregation of the genome into daughter nuclei. Although studied for over 100 years, mitotic chromosome structure and composition is yet to be fully elucidated. A novel approach called multi-classifier combinatorial proteomics (MCCP) has revealed the protein composition of mitotic chromosomes purified from chicken DT40 cells. One of the main advantages of MCCP is that it can be combined with SILAC to quantitatively compare chromosomal proteomes from different genetic knock-out cell lines. Here, we apply this method to compare chromosomal proteomes in the presence or absence of individual SMC complexes; key components that have been shown to play a crucial role in mitotic chromosome structure. While SMC1/3 and SMC2/4 form part of Cohesin and Condensin, respectively, the SMC5/6 complex is thought to be involved in DNA repair. Mitotic chromosomes were isolated from conditional genetic knockouts of Scc1, SMC2 or SMC5 cultured under SILAC conditions. Using quantitative Mass Spectrometry we were then able to rank >3,000 proteins based on whether their chromosomal association was dependent on individual SMC complex components. MCCP with using machine learning, Random Forest, will enable the first attempt at mapping the entire proteome regulation networks associated with SMC complexes, which in turn will explain how these complexes are coordinating chromosome structure.

Keywords: mitosis, chromosome, SMC

YI02-08 Targeted Analysis of *Salmonella* Effector Proteins Using Multiple Reaction Monitoring

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Salmonella enterica is a versatile pathogen capable of infecting diverse hosts and causing different diseases. In humans, *S. enterica* strains can cause typhoid or gastroenteritis, resulting in much morbidity and mortality throughout the world. Upon encountering a human cell, *Salmonella* delivers a choreographed series of virulence factors (also known as 'effector' proteins) into the cells. These effector proteins initially enable the bacteria to enter the host cell and then to target various natural processes within that host cell. To develop a clearer picture of the global effects of *Salmonella* on host cell processes, multiple reaction monitoring (MRM) assays for all known and potential new *Salmonella* effectors have been constructed by synthesizing at least two unique peptides per protein. Transitions were optimized by characterizing the synthesized peptides on a triple quadrupole mass spectrometer mainly focusing on optimizing collision energies. The three most intense fragment ions for each peptide were selected and used as transitions to detect the peptides in biological samples. A baseline level of effector proteins was established by analyzing *Salmonella* bacteria grown under effector secreting conditions. Finally, effector protein dynamics were investigated by analyzing *Salmonella* infected HeLa cells. This data shows how *Salmonella* takes control of its host cell by secreting specialized proteins at specific time points.

Keywords: Multiple Reaction Monitoring, Salmonella, Quantitative proteomics

YI03-01 Differentially Glycosylated Circulating Protein Biomarker Discovery for Barretts Esophagus and Esophageal Adenocarcinoma

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Esophageal adenocarcinoma (EAC) arises from precursor metaplastic condition Barrett's Esophagus (BE). BE patients are frequently screened using endoscopy-biopsy for early neoplastic changes. However, being an asymptomatic condition, it is very difficult to identify BE patients and to recruit them for screening. Also endoscopy is not suitable for large scale population screening due to high cost and patient non-compliance. Taken together, majority of EAC cases are diagnosed very late during pathogenesis and showed high mortality.

To facilitate early diagnosis, we focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to isolate different glycan structures on serum glycoproteins. Serum samples from control (n=9), BE (n=10) and EAC (n=10) patient groups were analyzed by lectin magnetic bead array-coupled mass spectrometry [1]. Customized database ""GlycoSelect"" was developed which incorporates outlier detection and sparse Partial Least Squares regression Discriminant Analysis[2]. We identified a ranked list of candidate glycobiomarkers that distinguish a) EAC from BE and b) BE from control group. In general, glycoproteins bound several lectins, reflecting heterogeneity and multiplicity of glycosylation. Specific glycan structure changes were observed as loss and gain of binding to a single lectin while maintaining binding to other lectins. Future work will validate the candidate protein-lectin pairs using a customized lectin-affinity array-coupled with quantitative mass spectrometry using independent cohort of 100+ patients. The specificity and sensitivity of panels of glycobiomarkers will be determined to develop a serum screening test for BE/EAC.

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[2] Lê Cao et al., BMC Bioinformatics 12, 253-268 (2011)

Keywords: Esophageal Adenocarcinoma (EAC), Barrett's Esophagus (BE), Biomarker

YI03-02 A Novel Titanium Dioxide Plate (TiO_2 Plate) for Phosphopeptide Enrichment and On-Target MALDI-TOF Analysis

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Protein phosphorylation is a major protein post-translational modification that regulates many cellular processes and activities. Because mass spectrometer (MS) signals of low abundance of phosphorylated peptides are commonly suppressed by the presence of abundant non-phosphorylated peptides, one of the major challenges in the detection of phosphopepdies is the enrichment of low-abundant phosphopeptides from complex peptide mixtures. Titanium dioxide (TiO_2) has been proven to be a highly efficient approach and is widely applied for phopshopeptide enrichment. In this study, a novel TiO_2 plate was proposed by coating TiO_2 particles onto MALDI plates, glass or plastic substrates with a simple and rapid approach. The TiO_2 plate can be used for on-target MALDI-TOF analysis or as a purification plate, on which phosphopeptides were eluted out and subjected to MALDI-TOF or nanoLC-MS/MS analysis. The detection limit of the TiO_2 plate is 10 folds lower than TiO_2-packed tips approach. The capacity of the 2.5 mm TiO_2 spot was estimated to be about 10 μg of β-casein. With TiO_2 plate enrichment of SCC4 cell lysate digests and nanoLC-MS/MS analysis, 82% of the detected proteins were phosphorylated, illustrating the TiO_2 plate is practical and effective to enrichment phosphopeptides from complex samples.

Keywords: phosphorylation, on-plate, MALDI-TOF

YI03-03 Site-Specific N-linked Glycosylation Analysis by Nano-LC Tandem Mass Spectrometry Coupled with a Spectral Library Searching Approach

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Protein glycosylation is one of the most common forms of post-translational modification in eukaryotic proteins, playing critical roles in protein structures and functions. The most common approach of protein glycosylation analysis by mass spectrometry involves chemical or enzymatic release of the glycans from glycoproteins, followed by LC-MS analyses of the glycans and the deglycosylated peptides separately. However, the glycan heterogeneity on each glycosylation site is lost in this approach. In our work, protein N-linked glycosylation is characterized at the glycopeptide level to reveal the microheterogeneity on each glycosylation site. Glycopeptides were enriched by hydrophilic affinity interaction using cellulose microcrystalline, analyzed by nano-LC/MS, and fragmented by collision-induced dissociation in data-dependent acquisition mode. For automated identification, the resulting spectra are searched against a library of reference spectra of *N*-linked glycopeptide predicted by MassAnalyzer [Zhang et al, *Anal. Chem.* **2010,** *82*, 10194-10202], using SpectraST [Lam et al, *Nat. Methods* **2008**, *5*, 873-875]. Both the m/z and the intensity information of the possible fragmentation ions are taken into consideration in our search algorithm to achieve a more reliable glycopeptide identification. We validated our method using purified glycoprotein standards, and evaluated its potential to be used in automated profiling of complex samples.

Keywords: site-specific N-glycosylation analysis, automatic glycopeptide identification and profiling, spectral library searching

YI03-04 Proteome-Wide Identification of Poly (ADP-Ribosyl)ation Targets in Different Genotoxic Stress Responses

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Poly(ADP-ribos)ylation (PARylation) is a reversible post-translational protein modification found in higher eukaryotes synthesized by the catalytic activity of poly(ADP-ribosyl)transferases (ARTDs/PARPs). PARylation is involved in various cellular processes such as DNA damage response, transcription, energy metabolism and cell death. Supporting its role in DNA repair, specific inhibition of ADP-ribosyltransferase activity has recently been shown to constitute an effective target in treatment of several types of cancer. However, despite this recent scientific progress only little is known about the actual acceptor proteins of PARylation and how the modification regulates the functional role of these target proteins in mammalian cells. We performed a sensitive proteomics approach based upon quantitative mass spectrometry (SILAC) for macrodomain-based enrichment and identification of PARylated proteins that become covalently modified under different conditions of genotoxic stress. Our screen identified novel candidates not previously reported to be targets of PARylation, while confirming the majority of known PARylated proteins. Biochemical *in vitro* and *in vivo* validation of novel acceptor proteins confirmed that our methodology targets covalently PARylated proteins. Nuclear proteins encompassing nucleic binding properties were most prominently found to be PARylated upon genotoxic stress, in agreement with the nuclear functions ascribed to ARTD1/PARP1 and ARTD2/PARP2. Distinct differences in proteins becoming PARylated upon various genotoxic insults were observed. Most significantly, proteins involved in RNA metabolism are PARylated upon oxidative and alkylation induced stress, demonstrating that post-transcriptional processes are readily controlled through specific genotoxic stress-induced PARylation.

Keywords: poly (ADP-ribos) ylation, PARylation, genotoxic stress

YI03-05 Synthesis of Galactose-Deficient IgA1 O-glycans by GalNAc-transferases: Implications for the Pathogenesis of IgA Nephropathy

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IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans (Gd-IgA1) plays a key role in the pathogenesis of IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential *O*-glycosylation sites occupied; some Gal-deficient glycans consist of terminal *N*-acetylgalactosamine (GalNAc). IgA1-producing cells derived from IgAN patients secrete more IgA1 with Gal-deficient *O*-glycans and higher content of GalNAc compared to IgA1 from cells of healthy controls (HC). IgA1 *O*-glycosylation is thought to be initiated by GalNAc-T2, but the expression of GalNAc-T2 does not differ between the cells from IgAN patients and HC. In contrast, expression of GalNAc-T14, a GalNAc-T with high structural similarity to GalNAc-T2, is elevated in the cells from patients. To determine potential contribution of these enzymes to Gd-IgA1 formation, we analyzed kinetics and site-specificities of GalNAc-T2 and -T14 for IgA1 HR using high-resolution mass spectrometry. A synthetic IgA1 HR peptide (sHR) and a panel of synthetic IgA1 HR glycopeptides (sGP) with a single GalNAc residue at different sites were used as acceptors. GalNAc-T2 had higher activity, *i.e.*, faster rate of glycosylation of sHR, than did GalNAc-T14. The sites of glycosylation in sHR catalyzed by GalNAc-T2 and -T14 were the same for the variants with up to 5 sites and appeared in a predominantly ordered fashion. Localization of GalNAc on sGP did not affect the kinetics of GalNAc-T2, but GalNAc-T14 more effectively glycosylated the sGP variant with a GalNAc at S9. In summary, GalNAc-T2 and -T14 have similar site-specificity for IgA1 HR, but differ in kinetics and in how their activity is affected by preexisting glycosylation. We speculate that the elevated expression of GalNAc-T14 could contribute to the production of Gd-IgA1 in IgAN.

Keywords: IgA nephropathy, IgA1 glycosylation, O-glycopeptides

YI03-06 A SILAC-based Approach Defines an Angiotensin II- Regulated Proteome in Primary Human Kidney Cells

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Angiotensin II (AngII), the major effector of the renin-angiotensin system, mediates kidney disease progression by signalling through AT-1 receptor (AT-1R), but there are no specific measures of renal AngII activity. Accordingly, we sought to define an AngII-regulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of AngII activity in the kidney. We utilized stable isotope labelling with amino acids (SILAC) in PTECs to compare proteomes of AngII-treated and control cells. Of 4618 quantified proteins, 83 were differentially regulated in 4 replicates. SILAC ratios for 18 candidates were confirmed by Selected Reaction Monitoring (SRM) assays. Both SILAC and SRM revealed heme oxygenase-1 (HO-1) as the most significantly upregulated protein in response to AngII stimulation. AngII-dependent regulation of HO-1 gene and protein was further confirmed by qRT-PCR and ELISA in PTECs. In order to extend these *in vitro* observations, we overlaid a network of significantly enriched gene ontology (GO) terms from our AngII-regulated proteins with a dataset of differentially expressed kidney genes from AngII-treated wild type mice and AT-1R knock-out mice. Five GO terms were enriched in both datasets and included HO-1. Furthermore, HO-1 kidney expression and urinary excretion were reduced in AngII-treated mice with PTEC-specific AT-1R deletion compared to AngII-treated wild-type mice, thus confirming AT-1R-mediated regulation of HO-1. In summary, our *in vitro* approach identified novel molecular markers of AngII activity and the animal studies demonstrated that these markers are relevant *in vivo*. These interesting proteins hold promise as specific markers of renal AngII activity in patients and in experimental models.

Keywords: renin angiotensin system, proximal tubular kidney cells, SILAC

YI03-07 An Improved Protocol for the Enrichment of Plasma Membrane Proteins Allows the Identification of Accessible Antibody Targets on Trastuzumab-Resistant Breast Cancer Cells

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Characterization of the cell surface proteome is of fundamental importance for the development of antibody-based therapies, as well as for the understanding of how cells interact with their environment. Biotinylation of cell surface proteins is one of the most frequently used procedures for their mass spectrometry-based characterization. Here, we present a study investigating the enrichment of membrane proteins of SK-BR-3 breast cancer cells by surface biotinylation performed alone or in combination with either ultracentrifugation or detergent-based fractionation. SK-BR-3 cells are a model system for HER2-positive breast cancer, clinically characterized by high metastatic potential and dismal prognosis. The anti-HER2 antibody Trastuzumab is frequently used for the treatment of these patients, but its clinical efficacy is limited by the emergence of resistance. To understand the changes in cell surface proteome, we have developed an SK-BR-3 cell line resistant to Trastuzumab-mediated growth inhibition and we have studied proteome changes by cell surface biotinylation and detergent-based fractionation. Thirteen membrane-associated proteins were found to be regulated between resistant and parental cell lines. Human monoclonal antibodies against most promising antigens, isolated from phage display libraries and conjugated to a highly potent cytotoxic agent, mediated cancer cell killing *in vitro*. In summary, the procedure described in this article allowed an efficient characterization of the cell surface proteome of closely related SK-BR-3 cell lines and led to the identification of three tumor-associated antigens which could be considered for the development of anti-breast cancer antibody products.

Keywords: cell-surface biotinylation, Trastuzumab resistance, antibody drug conjugates

YI03-08 Mass Isotopomer Analysis of Metabolically Labeled Nucleotide Sugars and N- and O-glycans for Tracing Nucleotide Sugar Metabolisms

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Nucleotide sugars are the donor substrates of various glycosyltransferases, and an important building block in N- and O-glycan biosynthesis. Their intercellular concentrations are regulated by cellular metabolic states including diseases such as cancer and diabetes. In order to investigate the fate of UDP-GlcNAc, we developed a tracing method for UDP-GlcNAc synthesis and utilization, and GlcNAc utilization using $^{13}C_6$-glucose and $^{13}C_2$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry.

Metabolic labeling of cultured cells with $^{13}C_6$-glucose and the analysis of isotopomers of UDP-HexNAc (UDP-GlcNAc plus UDP-GalNAc) and CMP-NeuAc revealed the relative contributions of metabolic pathways leading to UDP-GlcNAc synthesis and utilization. In pancreatic insulinoma cells, the labeling efficiency of a $^{13}C_6$-glucose motif in CMP-NeuAc was lower compared with that in hepatoma cells.

Using $^{13}C_2$-glucosamine, the diversity of the labeling efficiency was observed in each sugar residue of N- and O-glycans on the basis of isotopomer analysis. In the insulinoma cells, the low labeling efficiencies were found for sialic acids as well as tri- and tetra-sialo N-glycans, whereas asialo N-glycans were found to be abundant. Essentially no significant difference in secreted hyaluronic acids was found among hepatoma and insulinoma cell lines. This indicates that metabolic flows are responsible for the low sialylation in the insulinoma cells. Our strategy would be useful for systematically tracing each stage of cellular GlcNAc metabolism.

Keywords: mass spectrometry, glycosylation, glucose metabolism

CL-01 Translation of Clinical Proteomics: Opportunities and Challenges

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Most human diseases, such as cancer, are often diagnosed either in their late stages when the chance of cure is relatively low or in the form of diseases that might not have to be treated. What we need is to be able to detect lethal diseases in their early stages. Proteomic biomarkers offer the best opportunity for making significant impacts in the flights against lethal diseases. During the last decade of proteomic research, significant progress has been made in the advancement of new technologies and the discovery of potential biomarkers. However, limited successes have been shown in the translation of proteomic discovery into clinical practice. I believe that the time has come for us to focus on the translation of clinical proteomics.

In my presentation, I will discuss the opportunities and challenges for biomarker discovery, validation and translation. Case studies will be presented. To be successful, we need to develop a roadmap and identify several key steps that are critical in this process. I will discuss the 4Bs, the 4Gs and 4Ps for proteomics translation. (1) To define clearly a specific "intended use" for unmet clinical needs, (2) to generate sufficient evidence in preliminary studies to support the investment for a large-scale validation study, (3) to select and develop assays with analytical performance suitable for clinical laboratory and (4) to conduct clinical trial to demonstrate clinical utilities in order to obtain regulatory approval and gain acceptance by the clinical community. The successful translation of clinical proteomics into clinical practice will require close collaboration between researcher, industry, regulator and clinician/ clinical laboratory.

CL-02 Clinical Proteomics for Microbiology

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Until recently, bacterial identification in clinical laboratories has relied on conventional and time-consuming phenotyping. In the last few years, MALDI TOF MS has been widely applied as an identification procedure because of its diagnostic and economical benefits.

Judging from the final program of the MSACL (The Association for Mass Spectrometry: Applications to the Clinical Lab) 2013 (San Diego), two commercial systems including commercial databases are available; the Bruker Biotyper (Bruker-Daltonics) and the VITEK MS (bioMerieux). Generated unique spectra of intact cells are compared with previously collected fingerprint libraries that are commercially available. Reports from around the world have indicated genus-level identifications of 97%-99% and species level identifications of 85%-97% when testing routinely isolated bacteria and yeast using Bruker Biotyper MALDI-TOF MS.

MALDI-TOF MS identification of bacterial at species level remains unsatisfactory. One of the reasons is an incomplete database that still needs refinement and expansion. Augmentation of the commercial database by incorporating mass spectra obtained in-house from clinical isolates may increase the identification rate. Recent studies have shown that this technology can be applied to accurately identify filamentous fungi and *Mycobacterium* species providing that effective sample preparation methods are established for these microorganisms.

A rapid identification of microorganisms growing in blood culture will have a great impact on the management of bloodstream infections. In terms of detection of antibiotic resistance, MALDI-TOF MS may be a promising tool, but this technology is not mature enough to provide a whole picture of complex process of antibiotic resistance.

Keyword: Clinical Microbiology

CL-03 Proteomic Investigations of Heart and Lung Diseases

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Our research aims to understand health and disease at the molecular level. Meeting these goals requires new energy and insight, close interactions among chemists, biologists and clinicians, and the continuous evolution of technologies and tools for data handling and interpretation. Dynamic post-translational modifications of proteins, *e.g.*, glycosylation, phosphorylation, acylation, oxidative modifications, and their specific position(s), site occupancy, co-occurrence and kinetics, affect the properties of proteins and whole cells, their interactions, transport, activity, and lifetimes. Mass Spectrometry-based approaches that drive novel, emerging capabilities are essential for investigation of the healthy state and aberrations. This lecture will focus on strategies developed to facilitate investigations of metabolic causes underlying cardiovascular disease and changes that occur during development of pulmonary arterial hypertension. These examples, chosen from projects now underway in our laboratories, will illustrate promising approaches, interesting results and remaining challenges.

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CL-04 Challenges and Opportunities in Biomarker Discovery by Comparative Proteomic Analysis of Blood Circulating Proteins - Biological and Statistical Concerns

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Over the past 10 years, large amount of global efforts have been made to discover serum/plasma protein biomarkers by comparative proteomic analysis of blood circulating proteins. The most commonly used approach was the single-center case-control design. In case-control proteomic studies, quantitative profiles of serum/plasma proteins were first obtained in an untargeted manner, and then compared to identify the differences as individual potential biomarkers or a combination of differential features as diagnostic/prognostic disease-associated fingerprints. In spite of advantages of case-control design such as time-efficiency and cost-effectiveness, there are many pitfalls. Surface-enhanced laser desorption/ionization (SELDI) TOF mass spectrometry (MS) (or called ProteinChip SELDI technology) is the first high-throughput technology that allows comparison of plasma/serum proteome contents in a large number of subject samples within a short period of time. Using this technology, numerous case-control studies found serum/plasma proteomic fingerprints with over 90% accuracy in the diagnosis or prognosis of various diseases. However, criticisms and hesitations on this approach have been appearing all over the world. After accumulating more research experiences, researchers now have better understandings of characteristics and limitations of applying comparative proteomic analysis of blood circulating proteins to biomarker discovery. By using our MS-based biomarker discovery studies as examples, opportunities as well as biological and statistical concerns on applications of case-control comparative proteomic analysis to biomarker discovery will be discussed in this lecture. With rapid advancement of MS technologies and proper clinical study designs, discoveries of clinically useful biomarkers should be forthcoming.

CL-05 How Useful is Proteomics in the Clinic? A Case Study of Breast Cancer

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Breast cancer is a complex and heterogeneous disease that is usually characterized by histological parameters such as tumour size, cellular (re-)arrangements, necrosis, nuclear grade and the mitotic index leading to a set of around twenty subtypes. Together with clinical markers such as hormone receptor status, this classification has considerable prognostic value but there is a large variation in patient response to therapy. Gene expression profiling has provided molecular profiles characteristic for distinct subtypes of breast cancer that reflect the divergent cellular origins and degree of progression. Here we present a large-scale proteomic profiling study of 483 sporadic and hereditary breast cancer tumours with matching mRNA expression analysis. The subgroups formed upon unsupervised clustering agree very well with groups found on transcriptional level however the classifiers (genes or their respective protein products) differ almost entirely between the two data sets. We have also carried out an in-depth quantitative proteomic survey of five breast cancer cell lines as well as two breast derived cell lines representing fibroblasts and adipose cells. We show a poor correlation between transcriptomics and proteomics data as well as a low degree of similarity between the proteomes on samples obtained *in vivo* and *in vitro.*
The protein data can be transferred into a rapid highly multiplexed assay that is easily implemented in standard clinical chemistry practice, allowing a rapid and cheap characterisation of tumour tissue suitable for directing choice of treatment. We have studied the response to both chemo- and radiotherapy treatments. The response to DNA damage by alkylation and DNA topoisomerase inhibition was studied in two breast cancer cells lines as was the effect of ionising radiation. We present data from both a shotgun and a targeted, pathway-centric approach to highlight the different DNA repair pathway modulation in the cell lines and the correlation with viability and DNA damage assays. This type of focussed profiling may be of utility in rapidly defining non-responders undergoing systemic neoadjuvant therapy. These assays, together with the molecular classifiers and hormone receptor readouts are now being established into the clinic in a first evaluation phase.

CL-06 Mass Spectrometric Molecular Phenotyping of Tissues and Bodyfluids

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Systems biology is focused on the study of dynamic networks of interacting molecules and places such networks between genotype and phenotype. It is assumed that a specific genome encodes the molecules that constitute such a network and that the network is modulated by perturbing effects such as environmental factors. The properties emerging from the network as a whole determine observable phenotypes. Many of the molecular networks of the cell consist of or involve proteins. Therefore, the precise determination of the acute state of protein networks is highly informative as an acute phenotypic readout.

Mass spectrometry based proteomics is a central life science technology that has realized great progress towards the identification, quantification and characterization of the proteins that constitute a proteome. In this presentation we will discuss how mass spectrometry based proteomics has been applied to network biology to identify the nodes and edges of biological networks, to detect and quantify disease related network changes and to correlate dynamic network rewiring with a disease phenotype. We will also discuss future directions for mass spectrometry based proteomics within the network biology paradigm and their significance for the study of networks perturbed in human disease.

ED-01 Integrating Proteomics, Transcriptomics and miRNAs for Biomarker Discovery

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In recent years, Omics approaches have been in the frontier of biomarker discovery, although the clinical outcome of these efforts is yet to be fully realized. One of the major challenges in cancer biomarker discovery has been to successfully translate potential candidates from the discovery phase to clinical validation, on account of large heterogeneity that exists among individual tumor cases. Therefore generating robust discovery panels that carry analytical rigour as well as relate in the biological and regulatory context would be important for translation to the clinic. Integration of multiomics data and deciphering their relationships and the key pathways will not only enhance our understanding of the tumor but is also crucial for improved outcomes. However, despite advances in analytical platforms, there are still limitations to achieve linear correlation between transcripts and proteins, in sufficient numbers. Although in a limited way, we have attempted integration of altered miRNAs and their mRNA and protein targets for Glioblastoma multiforme (GBM), using transcriptomics study carried out by the Cancer Genome Atlas (TCGA) group and differential proteomics data generated from our lab. Transcriptomics analysis by TCGA group has revealed a large number of altered miRNAs associated with these tumors. When we examined the presence of predicted targets of these miRNAs in the mRNA (TCGA) and protein (our lab) datasets, we observed interesting correlations consistent with vertical regulatory linkage. Extension of this to multiple miRNAs would generate large portfolio of the target molecules with a second horizontal linkage in terms of their biological function and pathways. Such 2 Dimensional molecular maps – with a. regulatory linkage in one dimension and b. biological/functional linkage in the second dimension, would form strong panels to be integrated into clinical experimental designs. They also offer the plausibility of developing clinical assay methods at three different levels of gene expression - regulatory miRNA or target mRNA or protein, two of these are also accessible as circulatory molecules in body fluids.

ED-02 Construction and Analysis of Protein-Protein Interaction Networks: A Tutorial

Marc Wilkins

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Most proteins in the cell interact with other proteins to deliver their function. To define these interactions, large-scale studies of protein-protein interactions have been recently undertaken using two-hybrid techniques or the affinity purification of complexes followed by mass spectrometry-based protein identification. These approaches have been applied to a number of species; the best studied species is *Saccharomyces cerevisiae* (baker's yeast), however bacterial and mammalian species have also been analysed. Interaction networks can be built using software tools and interaction data. These networks can be co-analysed with other data types, for example protein expression or functional data. This can provide numerous novel insights into the function of the cell. This tutorial will introduce protein-protein interactions, relevant databases, and discuss case studies to illustrate the construction and analysis of protein-protein interaction networks.

ED-03 Adventures in Personal Genomics and Whole Omics Profiling

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Personalized medicine is expected to benefit from the combination of genomic information with the global monitoring of molecular components and physiological states. To ascertain whether this can be achieved, we determined the whole genome sequence of an individual at high accuracy and performed an integrated Personal Omics Profiling (iPOP) analysis, combining genomic, transcriptomic, proteomic, metabolomic, DNA methylomic, and autoantibodyomic information, over a 38-month period that included healthy and six virally infected states. Our iPOP analysis of blood components revealed extensive, dynamic and broad changes in diverse molecular components and biological pathways across healthy and disease conditions. Importantly, genomic information was also used to estimate medical risks, including Type 2 Diabetes, whose onset was observed during the course of our study. Our study demonstrates that longitudinal personal omics profiling can relate genomic information to global functional omics activity for physiological and medical interpretation of healthy and disease states.

ED-04 Significance of Secretome Analyses

Richard J. Simpson

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The identification of secreted proteins (secretome) in the tumour microenvironment and a detailed knowledge of their interplay with tumour cells and stroma are of critical importance for improving our understanding of fundamental tumour biology. Such an understanding of secreted '*cancer signatures*' will greatly enhance prospects of improved early diagnostic biomarkers and therapeutics. Although the term 'secretome' was introduced to define proteins released from cells grown in culture, the composition of this sub-proteome should be extended to include not only classically-secreted proteins (i.e., endoplasmic reticulum and Golgi-dependent) and non-classically secreted proteins, but also proteins released through secretion of membranous vesicles (Extracellular Vesicles, EVs) such as shed microvesicles (SMVs) and exosomes. In this lecture I will focus on methods for preparing both secreted proteins from cell lines and tumour interstitial fluid, and released EVs, discuss associated technical challenges, and also update recent efforts at delineating cancer cell associated secretomes. Finally, I will appraise recent outcomes from cancer secretome studies, particularly the exciting advances within the exosome proteomics field which highlight their pivotal role in preparing metastatic niche formation.

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ED-05 Proteomic Strategy for Development of Clinical Approach

Siqi Liu

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Protein biomarkers represent an enormous advance to our understanding of the clinical diagnosis and treatment to disease. With a great achievement in molecular pathology, we realize that most diseases are not directly resulted from or indicated as a single element, but are closely related with multiple factors, genes, proteins or metabolites. Considerable effort therefore has been expended to characterize the disease genomes, proteomes and metablomes, especially for detection of the biomarkers at early stage. Similar to the traditional approach of clinical biochemistry, the new generation of clinical methods based upon proteomics are still focused on the measurement to the samples derived from body fluids, particularly serum. The number of disease biomarkers measured by the updated techniques, however, is significantly enlarged. As regards the protein candidates in serum, the potential biomarkers are generally divided into two sets, serum proteins and autoantibodies, while in technical consideration, the updated methods are largely antibody- and mass spectrometry-based. With careful selection of immuno-signals, array-ELISA and protein chip have become the feasible approaches in clinical practices. On the basis of large screening of proteomic analysis and the resolution improvement in mass spectrometry, the approach of target proteomics upon multiple reaction monitoring (MRM) has emerged as a powerful means in clinical application.

ED-06 A New Genome-Wide Proteome Project for the Future Biomedical Sciences

Young-Ki Paik

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Proteomics is well positioned to address the gap between the genome and phenome, and complement data from the reference human genome sequence and gene expression studies. HUPO has recently initiated a new genome-wide proteome project termed 'Chromosome-Centric Human Proteome Project (C-HPP) (1). The initial goal of the C-HPP is to identify at least one representative protein encoded by each of the approximately 20,300 human genes and match it with its tissue localization and major isoforms including post-translational modification (PTM) based on quantitative mass spectrometry complemented with antibody reagents (1). Throughout this 10-year project (2012-2022), C-HPP will generate information useful for the search for new diagnostic biomarkers and drug targets and also study disease gene families clustered in each chromosome (2). Human genome studies (e.g, ENCODE), as well as transcriptome sequencing provides a basis for identification of protein isoforms generated by alternative splicing transcripts (AST) and by nsSNP, creating transformative advances for use in the proteomics community (3). Likely results of the C-HPP are: i) integrated transcriptomics/proteomic measurements, ii) a paradigm shift from individual laboratories to international research alliances, iii) the development of informatics systems and associated interfaces, and iv) powerful new MS for applications (e.g., intact protein variant analysis for biomarker discovery) (1, 3). Currently more than 25 countries participate in this C-HPP initiative by taking each chromosome and set the guidelines for data collection, collaboration and operation of the consortium (2). Special issue on the C-HPP has been published in 2013, setting a major milestone of this global project (4). We believe this new initiative will provide not only a new paradigm of education in integrated omics field but also fresh view on the genome-wide protein resources for biomedical societies in the future. I will present the ongoing exploration of the biological resources of the C-HPP for study of preeclampsia disease.

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HA-A-01 Exposing the Proteome in Full Glory Through Advances in Enabling Technologies

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Over the past decades we have learned that the proteome is extremely multifaceted due to splicing and protein modifications, further amplified by the interconnectivity of proteins into complexes and signaling networks that are highly divergent in time and space. Proteome analysis heavily relies on a broad mixture of analytical techniques, starting from sample preparation, to separation and enrichment and last but certainly not in the least mass spectrometry. Through developments in these enabling technologies MS-based proteomics has matured and start to deliver biological relevant information. Here I will portray this emerging next-generation of proteomics describing recent applications; highlighting especially some of the enabling technologies our laboratory has contributed in e.g. phosphoproteomics, quantitative proteomics and analysis of intact proteins and protein complexes.

JHA-A-01 Development and Application of Analytical Technologies for Proteomics and Phosphoproteomics

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Rapid progress has been made in identifying cellular phosphoproteins by shotgun phosphoproteomics using nanoLC-MS combined with phosphopeptide enrichment techniques. Because samples for shotgun proteomics exhibit extremely high complexity with a wide dynamic range of concentration, fractionation approaches before/after phosphopeptide enrichment prior to nanoLC-MS are often necessary to widen the phosphoproteome coverage. Recently, by using one-dimensional nanoLC-MS with meter-long, monolithic silica-C18 capillary column, we successfully identified the proteome expressed in *E. coli* cells [Iwasaki et al, Anal Chem 2010] and human induced pluripotent stem cells [Yamana et al, JPR 2013]. We also developed a highly efficient phosphopeptide enrichment protocol based on aliphatic hydroxyl acid-modified metal oxide chromatography (HAMMOC) using lactic acid-modified titania [Sugiyama et al, MCP 2007]. We combined the 'one-shot' approach with HAMMOC, and applied to STY phosphoproteomics without any fractionation, identifying more than 12,000 phosphosites (3,700 phosphoproteins) from 0.25 mg HeLa lysate. We also employed sequential enrichment of tyrosine phosphopeptides using lactic acid-modified titania chromatography followed by immunoprecipitation using pY antibody cocktails. Furthermore, we minimized the required samples down to 10,000 cells (approx. 1 microgram of proteins) by miniaturizing LC, skipping autosampler and optimizing protocol based on phase-transfer surfactants [Masuda et al, JPR 2008], resulting in identification of more than 1,000 phosphopeptides [Masuda et al, Anal Chem 2011]. Based on these approaches, phosphorylation dynamics induced by kinase inhibitors were quantitatively analyzed to elucidate the mechanism of actions of these drugs [Imami et al, MCP 2012].

JHA-A-02 Snapshot Analysis of Protein Complexes Using Proteomic Technology

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Proteomic technologies allow a comprehensive study of multi-protein complexes that carry out many cellular functions in a higher-order network in the cell. The use of tagged proteins as affinity bait, coupled with mass spectrometric identification, enables us to isolate almost any functional protein complex or its synthetic intermediates that might represent snapshots of nascent functional protein complexes at particular stages of its biogenesis and to identify their constituents-some of which show dynamic changes for association with the intermediates at various stages of the biogenesis. The idea behind this snapshot analysis is that some of the associated proteins in one initially isolated complex can also be present in other precursor complexes, and thus would allow the purification of intermediates from different stages of biogenesis. Initially, we had applied this approach to the analysis of protein constituents of pre-ribosomes, and is now expanded this to the analysis of not only protein but also RNA constituents of small nuclear ribonucleoprotein (snRNP) intermediates formed during spliceosome biogenesis in cooperation with Dr. Isobe of Tokyo Metropolitan University. In this presentation, I would like to talk about mainly current status of the snapshot analysis of snRNP complexes.

Keywords: protein-protein interaction, biogenesis of protein machinery, ribonucleoproteomics

JHA-A-03 A Success in a Diagnosis Kit for Liver Fibrosis Using Multiple Novel Technologies of Glycoproteomics

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One methodology for biomarker discovery of chronic diseases exploits the fact that glycoproteins produced by impaired cells have altered glycan structures although the proteins themselves are common, ubiquitous, abundant and familiar. Here, we describe our strategy to approach the detection of these low-level glycoproteins as serum biomarkers: 1) A quantitative real-time PCR array for glycogenes to predict the glycan structures of secreted glycoproteins; 2) Analysis by lectin microarray to select lectins which distinguish disease-related glycan structures on secreted glycoproteins; 3) An isotope-coded glycosylation-site-specific tagging (IGOT) high-throughput method to identify carrier proteins having the specific lectin epitope. 4) Selection of candidate molecules by skillful bioinformatics technology. 5) Re-selection of the best lectin distinguishing the glycan-alteration in the patient serum. 6) Final verification using more than 100 patient sera.

Using this strategy, we have identified many glycoproteins containing glycan structures altered in impaired cells. These candidate glycoproteins were immunoprecipitated from serum using commercially available antibodies, and their glycan alteration was examined by a lectin microarray. Finally they were analyzed by multistage tandem mass spectrometry (MS^n).

According to this protocol, we succeeded in establishment of a diagnosis kit for liver fibrosis. A novel marker hyperglycosylated *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA^+-M2BP) was developed for liver fibrosis using the glycan "sugar chain"-based immunoassay. This kit was named FastLec-Hepa, and automatically detects unique fibrosis-related glyco-alteration in serum M2BP within 20 min. FastLec-Hepa is the only assay currently available for clinically beneficial therapy evaluation through quantitation of disease severity.

JHA-A-04 The Analysis of Protein Post-Translational Modifications

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Post-translational modifications (PTMs) frequently play a key role in regulating protein function. The proteasome is composed of at least 33 different subunits and is a multi-catalytic protease complex that degrades ubiquitinated proteins in eukaryotic cells. The comprehensive PTM analysis of proteasome subunits using proteomic techniques indicated that 21, 1, 1 and 28 subunits were N-acetylated, N-myristoylated, N-methylated and phosphorylated, respectively. Furthermore, the study using the yeast mutants showed that N-acetylation might be involved in the chymotrypsin-like activity and accumulation level of the 20S proteasome and that N-methylation of Rpt1 might be essential for cell growth or stress tolerance in yeast. Like this, it is evident that the PTMs significantly affect the function of the proteasome. The phosphorylation of heterogeneous nuclear ribonucleoprotein K (hnRNP K) is also thought to play an important role in cell regulation and signal transduction. However, the relationship between hnRNP K phosphorylation and cellular events has only been indirectly examined, and the phosphorylated forms of endogenous hnRNP K have not been biochemically characterized in detail. The PTM analysis using 2-D Phos-tag affinity electrophoresis was successful to characterize multiple forms of hnRNP K produced by alternative splicing of the single hnRNP K gene and phosphorylation of Ser116 and/or Ser284. Furthermore, this analysis demonstrated that each form of hnRNP K was differentially modulated in response to external stimulation with bacterial lipopolysaccharide or serum. Like this, the PTM analysis is also crucial for a better understanding of the functional properties of proteins.

JHA-A-05 Development of Glycoproteomic Technologies and Identification of Glycan-Targeting Tumor Markers

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Protein glycosylation is one of the most complicated post transcriptional modifications, while it plays diverse physiological functions by controlling protein folding, charge state, ligand-receptor interactions, or immunogenicity. To date, we have focused on developing novel glycoproteomic technologies which allowed rapid and comprehensive profiling of clinically-important glycan structure disorders on both targeted glycoproteins and even whole serum/plasma glycoproteins.

Isotopic glycosidase elution and labeling on lectin-column chromatography (IGEL) technology (Mol Cell Proteomics, 2010, 9(9):1819) enabled us not only to identify N-glycosylation sites comprehensively but also to compare glycan structures on each glycosylation site quantitatively in a single LC/MS/MS analysis. Using this technology, we revealed that glycans on A2GL_Asn151, A2GL_Asn290, CD14_Asn132, CO8A_Asn417, C163A_Asn64, TIMP1_Asn30, and TSP1_Asn1049 demonstrated lung cancer-specific alterations.

Recently we developed Energy resolved oxonium ion monitoring (Erexim) technology to evaluate glycan microheterogeneities on therapeutic protein drugs (Anal Chem, 2012, 84(22):9655). The Erexim method can quantify ~50 glycan structure variations on a therapeutic antibody molecule according to energy-resolved MRM for oligosaccharide-derived oxonium ions in only 10 minutes. We discovered significant lot-to-lot variations of glycosylation profiles on Herceptin and Avastin. The existence of anaphylaxis-inducible glycan structures on Erbitux were also quantitatively determined by Erexim. This technology has already been put to practical use in R&D and CMC areas by a contract research organization (CRO).

Thus I'd like to continue facilitating the life innovation by creation of further sophisticated MS-based glycoproteomic technologies in the future.

JHA-A-06 Rapid Discrimination between Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Using MALDI-TOF Mass Spectrometry

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for nosocomial infection. The presence of MRSA in a hospital is very detrimental to patients and to hospital management. Thus, rapid identification of MRSA is needed. This study performed a prospective study of rapid discrimination of MSSA from MRSA using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) system. We evaluated 305 clinical isolates of *S. aureus* using the MALDI-TOF MS system and support vector machine. The predictive model was trained using 100 *S. aureus* isolates (50 MSSA and 50 MRSA). The identification rates were 90.2% for MSSA and 78.6% for MRSA following the 10-fold cross-validation SVM. In the blind test sets, 205 *S. aureus* isolates (95 MSSA and 110 MRSA) were correctly classified. The identification rates were 95.8% for MSSA and 81.8% for MRSA. In conclusion, the method proposed in this study using a predictive model enables detection from one colony in 5 minutes, and thus is useful at clinical sites at which rapid discrimination of MRSA from MSSA is required.

JHA-A-07 Industrialization of Clinical Proteomics Using Mass Spectrometry-Based Technologies

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Medical ProteoScope (MPS) is the company of proteomics excellence towards the better quality of life. In clinical practice, there is a need for biomarkers to help improve treatment outcomes. Based on integration of the state-of-the-art proteomics technology, we have focused on development of protein biomarkers. Label-free comparative LC-MS/MS is our core analytical platform, associated with the self-made algorithms, i-OPAL and i-RUBY, for data alignment of non-linearly fluctuated LC elution time. Under quality control (QC) operations of these technologies, collaborative studies with academic institutions and drug companies led to identification of proteins related closely to drug response, current state of the disease, progression of the disease and metastatic risk in the case of cancer patients. Posttranslational modifications (PTMs) of proteins are the promising targets for understanding disease mechanisms. Since 2008, MPS participates in the coordination funds presided by Yokohama City University. We developed differential analysis systems of PTMs including reversible phosphorylation. Formalin-fixed paraffin-embedded (FFPE) tissues archived in hospitals have a potential for discovery of the therapeutic targets as well as proteomic biomarkers. We have optimized protocols to dissect minute lesions from the FFPE tissues. Proteomic data of the collected specimens enabled to evaluate more accurately the disease state of the tissues. Thus, as a proteomics leading venture, MPS is continuously contributing to the fields of medicine.

JHA-A-08 A Novel Approach for Discovering Proteins at Extremely Low-Abundance in Serum

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The proteomic analysis of serum (plasma) has been a major approach to determining biomarkers essential for early disease diagnoses and drug discoveries. The determination of these biomarkers, however, is analytically challenging since the dynamic concentration range of serum proteins/peptides is extremely wide (more than 10 orders of magnitude). Thus, the reduction in sample complexity prior to proteomic analyses is essential, particularly in analyzing lowly abundant protein biomarkers.

Here, we demonstrate a novel approach to the proteomic analyses of human serum that uses an originally developed serum protein separation device and a subsequently linked high-performance mass spectrometer system. The hollow-fiber membrane based serum pre-treatment device we developed can efficiently deplete high-molecular-weight proteins and concentrate low-molecular-weight proteins/peptides automatically within an hour. The proteomic analysis of healthy human serum pre-treated using the device, followed by the mass spectrometer, successfully identified about 2,000 proteins. According to the concept of the pre-treatment device, 64% of the identified proteins were smaller proteins than the human serum albumin. We believe this unique serum pre-treatment device and the proteomic analysis protocol allow high-throughput and efficient discovery of serum disease biomarkers.