Sunday, September 15, 8:15 - 9:30 am

PSI/ProteomeXchange/KBpC + C-HPP (Part 1)

Room 303-304

The HUPO Proteomics Standards Initiative Standard Formats and Their Use in Current Software

Eric Deutsch

Institute for Systems Biology, Seattle, WA, USA

The HUPO Proteomics Standards Initiative (PSI) defines community standards for data representation in proteomics to facilitate data comparison, exchange and verification. We will present the recent activities of the PSI, including the development of the TraML, mzIdentML, mzQuantML, and mzTab formats. We will describe the new minimum information specification MIAPE - Quant, as well as updates for other MIAPE modules. For most of the community, these formats and specifications are only as useful as the software that supports the formats. Therefore, we will discuss the implementation of these formats in currently available software and software in progress. We will conclude with current and future projects of the PSI and how you can become involved.

Update on ProteomeXchange

Henning Hermjakob

European Bioinformatics Institute, Cambridge, UK

The ProteomeXchange consortium (PX) provides a globally coordinated infrastructure for the deposition and public dissemination of MS-based proteomics data. For each dataset, PX aims to capture raw data, metadata, and processed results, to allow different views of the submitted data, from the original author analysis and interpretation in e.g. PRIDE to re-processed views in e.g. PeptideAtlas to higher abstraction levels in molecular biology resources like UniProtKB.

For all types of PX datasets, all the data remains private by default and each submission becomes publicly available only on author instruction or publication of the manuscript supported by the dataset. When this happens, a short summary announcement is released though a public announcement system, as a RSS feed. All the PX announcement messages are stored and searchable in ProteomeCentral. This resource generates a unique identifier and constitutes a registry for each PX data dataset. In addition, it provides the users with an efficient way to identify datasets of interest.

ProteomeXchange has been in production mode since summer 2012. As of July 2013, 310 submissions with a total volume of 20 TB from 28 countries have been received, ranging from a few hundred spectra to 4 TB in a single submission. A significant fraction of depositions stems from HPP participants. ProteomeXchange submission and dissemination sites are accessible from www.proteomexchange.org/

Integration of Proteomics Data in neXtProt

Lydie Lane

Swiss Institute of Bioinformatics, Geneva, Switzerland

neXtProt (www.nextprot.org/) is a web-based knowledge platform dedicated to human proteins. It is built upon the corpus of data from Swiss-Prot, to which are added selected high quality data originating from a variety of high-throughput approaches. In addition to mass-spectrometry-based protein and post-translational modification (PTM) site identifications, those data include coding polymorphisms, splice variants, as well as information about protein interactions, structure, localization and function.

We have established a pipeline to integrate all the human peptide identifications from PeptideAtlas. Other large-scale proteomics datasets are integrated from direct submissions and original papers. Each annotated peptide or PTM site identification is accompanied by metadata that provides details on the experimental setup using controlled vocabularies, and by a description of their confidence (Gold meaning <1% error rate, Silver meaning <5% error rate). We have integrated 32521 PTM sites (N-glycosylation, phosphorylation, S-nitrosylation, ubiquitination and sumoylation), which corresponds to 17410 new PTM annotations. We have also mapped 279,933 identified peptides to 14,131 proteins.

neXtProt data is available in XML and PEFF formats ("PSI extended FASTA format"), as well as through an API (www.nextprot.org/rest/) that allows users to retrieve the complete set of PTMs or variants for all isoforms of a protein, along with their experimental evidence and Gold/Silver data confidence assessment.

Sunday, September 15, 8:15 - 9:30 am

EyeOME

Room 301

Introduction to the Human Eye Proteome Project

Richard Semba[1]; Jan J. Enghild[2]; Vidya Venkatraman[3]; Thomas F. Dyrlund[2]; Jennifer E. Van Eyk[3]

1Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark; 3Johns Hopkins Bayview Proteomics Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

There are an estimated 285 million people with visual impairment worldwide, of whom 39 million are blind. The pathogenesis of many eye diseases remains poorly understood. The human eye is currently an emerging proteome that may provide key insight into the biological pathways of disease. We review proteomic investigations of the human eye and present a catalogue of 4842 non-redundant proteins identified in human eye tissues and biofluids to date. We highlight the need to identify new biomarkers for eye diseases using proteomics. Recent advances in proteomics now allow the identification of hundreds to thousands of proteins in tissues and fluids, characterization of various post-translational modifications, and simultaneous quantification of multiple proteins. To facilitate proteomic studies of the eye, the Human Eye Proteome Project (HEPP) was organized in September 2012. The HEPP is one of the most recent components of the Biology/Disease-driven Human Proteome Project (B/D-HPP) whose overarching goal is to support the broad application of state-of-the-art measurements of proteins and proteomes by life scientists studying the molecular mechanisms of biological processes and human disease. The large repertoire of investigative proteomic tools has great potential to transform vision science and enhance understanding of physiology and disease processes that affect sight.

Proteome of Human Tears and the Mouse Retina

Zhou Lei[1,2,4], Roger Beuerman[1-4]

[1]Singapore Eye Research Institute, [2]Duke-NUS, SRP Neuroscience and Behavioral Disorders, [3]Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, [4]Ophthalmology, University of Tampere Medical Center, Tampere, Finland

Current mass spectrometry technologies have significantly improved the coverage of the proteome, which provides more systemic insights into the pathogenesis of the disease. Here, we gave two examples of such in-depth analysis of human tear proteome and mouse retina proteome. Tears, an easily accessible body fluid covering the ocular surface, provide valuable information of different ocular diseases. Recently, we showed that more than 1500 proteins could be identified from normal human tears. A quick 1D-RPLC-MS/MS using a 90-mins gradient was able to identify around 500~700 tear proteins using 4 mg of total tear proteins equivalent to about 0.5 ml of tears. MS/MSALL with SWATH acquisition enables MRM-based quantitation (high reproducibility with CV% < 20%) of around 500 tear proteins using the same 1D LC-MS/MS setup. In another study, iTRAQ-based quantitative proteomics was utilized to investigate the changes of the mouse retina proteome in a lens-induced myopia model and in response to atropine treatment. Approximately 4000 proteins were identified with high confidence (<1% FDR) from mouse retina tissue and 3300 of them had quantitative information. Pathway analysis revealed that GABA transporters (GATs) that were upregulated in experimentally-induced myopic mouse retina and chronic administration of atropine were found to restore these levels back to normal. This study indicated that GATs could be a potential target for regulating the anti-myopic effects of atropine in mouse eyes. In conclusion, the ability for in-depth and quantitative analysis of the proteome will provide more information for understanding the disease and useful for biomarker research.

The Proteome of the Anterior Segment in Relation to Glaucoma

Deepak Edward[1,2]; Rachida Bouhenni[3]

[1]King Khalid Eye Hospital, Riyadh, Kingdom of Saudi Arabia; [2]Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [3]Summa Health System, Akron, OH, USA

Glaucoma, a heterogeneous group of diseases involving impairment of aqueous humor (AH) outflow from the anterior chamber, includes alterations in AH, ciliary body, iris, and trabecular meshwork (TM). The AH proteome of control and primary congenital glaucoma (PCG) patients and iris proteome of normal eyes were examined using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). Proteomic alterations in anterior segment tissues in primary open angle glaucoma (POAG) were reviewed. The proteomes of AH in PCG and control revealed differential expression of 7 proteins, including apolipoprotein A-IV (ApoA-IV), albumin (Alb), and antithrombin III (ANT3), which were detected at significantly higher levels in PCG AH compared to control, and transthyretin (TTR), prostaglandin-H2 D-isomerase (PTGDS), opticin (OPT), and interphotoreceptor retinoid binding protein (IRBP), which were detected at significantly lower levels in PCG. Normal iris proteome included 550 proteins that consisted of cytoskeleton and extracellular matrix (ECM) proteins (10%), development (25%), adhesion (4%), antioxidant activity (2%) and other cellular processes such as growth. Proteins previously implicated in glaucoma such as PTGDS, OPT, TTR were detected. In POAG, elevated levels of transforming growth factor beta 2, abnormal accumulation of ECM proteins such as cochlin and secreted protein acidic and rich in cysteine, oxidative stress proteins, and abnormal protein expression and changes in cytoskeletal interactions with the TM have been described in the literature. Establishment of the normal proteome of AH and iris and alterations of the proteome of AH and TM will help in further understanding of the pathophysiological processes and pathways involved in glaucoma.

Vitreous Proteome in Diabetic Retinopathy

Edward P. Feener[1,2], Lloyd P. Aiello[1,3]

1Joslin Diabetes Center and Departments of 2Medicine and 3Ophthalmology, Harvard Medical School, Boston, MA, USA

Diabetic retinopathy (DR) a leading causes of vision loss worldwide. DR includes nonproliferative diabetic retinopathy and can progress to proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME). Proteomic analyses of vitreous have begun to characterize the vitreous proteome in normal subjects and identify changes in vitreous protein abundance associated with PDR and/or DME. The gel-like composition of the vitreous is derived mainly from a hydrated network of fibular macromolecules, including glycosaminoglycans, proteoglycans, and collagen fibrils. Proteomics has identified several hundred soluble proteins that are reproducibly detected within this vitreous fluid/matrix and many more that are detected randomly or in a small subset of vitreous samples, suggesting that levels of certain proteins can markedly differ among vitreous samples. Vitreous proteins have been annotated to mediate signaling, transport, chaperone, and proteolytic functions. These findings suggest potential roles for vitreous proteins in the maintenance of vitreous structure, intraocular metabolism, complement activation, redox balance, inflammation, coagulation, and cell survival and growth. Several groups have shown the vitreous proteome is markedly altered in advanced stages of DR and is often accompanied by a 3-4 fold increase in total vitreous protein. Changes in the DR vitreous proteome appear to be mediated by a number of factors including the breakdown of blood retinal barrier function and extravasation of circulating proteins, intraocular hemorrhage, cell lysis, and alterations in the production and/or clearance of locally expressed proteins. Functional studies are underway to identify vitreous proteins that may contribute to, or protect against, the incidence and/or progression of DR.

Personalized Proteomics for Inflammatory Retinal Disease Therapy

Vinit B. Mahajan[1,2]; Jessica M. Skeie[1,2]

[1]Omics Laboratory, [2]Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA, USA

The causative molecules for inflammatory vitreoretinal disease are not known. We applied proteomic strategies to identify biomarkers and therapeutic targets in patients with genetically identical and genetically heterogeneous proliferative vitreoretinopathy (PVR). After establishing a surgical vitreous biorepository, we used proteomic profiling to identify biomarkers and therapeutic targets in patients with inherited and non-inherited proliferative vitreoretinopathy (PVR). The clinical phenotype of patients was ascertained using standardized grading methods. Vitreous cytokine expression was analyzed using antibody arrays and label-free proteomics. Pathway analysis was performed using MetaCore software. The clinical response of patients treated with anti-cytokine therapy was studied. Bioinformatic analysis revealed statistically significant, stage-specific cytokines in the inherited CAPN5-associated vitreoretinal disease. Following personalized therapeutic targeting, phenotypic ascertainment and proteomic profiles revealed drug responsive and nonresponsive pathways. For example, anti-VEGF therapy was highly effective in controlling neovascularization as was anti-IL-17 therapy for vitreous inflammation. The absence of TNFalpha and B-cell markers correlated with treatment failure using infliximab and B-cell immunosuppression. Failure to control pathologic fibrosis was associated with persistent expression of specific cytokines that escaped steroid therapy, which were also found in comparative profiles of non-inherited PVR. Personalized proteomic profiling of surgical tissue can identify the specific proteins and pathways involved in the progression of PVR. This data can be used to select rational therapies, avoid drugs where the target is nonexistent, and overcome the limitations of large clinical trials. Studies that take advantage of inherited disease may help convert biomarkers into therapeutic targets for common non-inherited disease.

The Tears as a Source for Proteomic Biomarkers of Human Eye Diseases

Roger Beuerman[1-4]; Zhou Lei[1,2,4]

1Singapore Eye Research Institute, 2Duke-NUS, SRP Neuroscience and Behavioral Disorders, 3Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, 4Ophthalmology, University of Tampere Medical Center, Tampere, Finland

Tears reflect the health of the ocular surface and can be easily collected for evaluation and screening. Proteomic studies of tears have identified key molecules associated with inflammatory diseases such as dry eye, Meibomium gland disease (MGD), and pterygium. Upregulated proteins include S100 proteins such as alpha-enolase, alpha-1-acid glycoprotein 1, S100 A8 (calgranulin A), S100 A9 (calgranulin B), S100 A4 and S100 A11 (calgizzarin) and down-regulated proteins, include prolactin-inducible protein (PIP), lipocalin-1, lactoferrin and lysozyme. Receiver operating curves (ROC) were evaluated for individual biomarker candidates and a biomarker panel, which had a diagnostic accuracy of 96% (sensitivity, 91.0%; specificity, 90.0%). The clinical classification of the severity of the dry eye condition was successfully correlated to the proteomics by using three proteins that are associated with inflammation, alpha1-acid glycoprotein 1, S100 A8, and S100 A9. In MGD, levels of S100A8 and S100A9 were correlated to disease severity and levels of S100A8 protein were significantly correlated to sensations of grittiness, whereas S100A8 and S100A9 were correlated to symptoms of redness and transient blurring. Higher levels of S100A6, S100A8, and S100A9 expressions were found in pterygium relative to normal conjunctiva. In addition, a distinct alteration of localization of S100A11 expression was observed in pterygium epithelium compared to the conjunctiva. In each disease, severity of disease was reflected in expression of proteomic biomarkers. Proteomic biomarkers must undergo thorough clinical trials for application, but in the future could replace lengthy and often inconclusive clinical observations and be applied in general clinical use.

Sunday, September 15, 8:15 - 9:30 am

Proteome Biology of Stem Cells

Room 311-312

Deep Subcellular Proteome Profiling of Human Induced Pluripotent Stem Cell by One-Shot NanoIc-MS/MS Analyses with Meter-Scale Monolithic Silica Columns

Mio Iwasaki[1], Masato Nakagawa[1], Yasushi Ishihama[2], Shinya Yamanaka[1,3]

[1]Center for iPS Cell Research and Application, Kyoto University, Japan; [2]Graduate School of Pharmaceutical Sciences, Kyoto University, Japan; [3]Gladstone Institute of Cardiovascular Disease, San Francisco, USA

The molecular mechanisms for self-renewal and pluripotency of human induced pluripotent stem cell (hiPSC) still remain unclear. Previously, some researchers have been performed to characterize hiPSCs using high-resolution

MS-based proteome analyses. However, the technology of proteome analysis is still immature to uncover the real proteome state in the cells including low expressing proteins. On the other hand, proteome profile in each subcellular location is a valuable knowledge of cellular functions. We thought fractionation of cellular organelle is effective approach to increase the proteome coverage and to get the knowledge of localization.

In this study, we improved the identification efficiency of low expressing proteins using subcellular fractionation technique combined with high sensitive one-shot nanoLC-MS/MS analyses. Cellular organelle of nucleus, cytoplasm and membrane were isolated and analyzed by nanoLC-MS/MS using meter-scale monolithic silica columns. Finally, we successfully identified more than 6,000 unique proteins from the nuclear fraction including 400 transcription factors which are known as very low expression levels. This deep subcellular proteome analysis data disclosed more detailed differences between iPSC and fibroblast cells and elucidated the key components governing the cellular functions of iPSC.

Proteomic Analysis of LGR5+ve Intestinal Adult Stem Cells and Their Immediate Undifferentiated Daughters

Javier Muñoz[1,2], Daniel E. Stange[3], Marc van de Wetering[3], Shabaz Mohammed[1,2], Hans Clevers[3], Albert J. R. Heck[1,2]

[1]Biomolecular Mass Spectrometry and Proteomics Group, Utrecht University, The Netherlands; [2]Netherlands Proteomics Center, Utrecht, The Netherlands; [3]Hubrecht Institute, KNAW, Utrecht, The Netherlands

The identification of Lgr5 as an intestinal stem cell marker has made it possible to isolate and study modest amounts of primary intestinal stem cells. Here, we show that starting with only 300,000 cells quantitative mass spectrometry, using a chemical isotope labeling approach at the peptide level, can be used to profile in-depth directly at the protein level changes between FACS-sorted Lgr5+ve stem cells and their immediate undifferentiated daughter cells, enabling quantitation of 3,224 proteins. The data revealed the increased presence in Lgr5+ve stem cells of several previously reported stem cell markers including Ascl2 and Olfm4. The overall comparison of mRNA and protein levels indicated a high level of correlation, authenticating the novel proteomics approach and implying that the initial control of intestinal stem cell biology occurs largely at the mRNA level. Our study presents a valuable resource for the study of intestinal stem cell biology.

Stem Cells and Neural Development

Akhilesh Pandey

Johns Hopkins University, Baltimore, USA

The ability to derive neural progenitors, differentiated neurons and glial cells from human embryonic stem cells (hESCs) with high efficiency holds promise for a number of clinical applications. However, investigating the temporal events is crucial for defining the underlying mechanisms that drive this process of differentiation along different lineages. I will discuss our efforts using quantitative proteomics to monitor the temporal dynamics of protein abundance as human embryonic stem cells differentiate into motor neurons and astrocytes. We identified a number of proteins whose expression was largely confined to specific cell types, embryonic stem cells, embryoid bodies and differentiating motor neurons. I will also describe our proteomic analysis of ESCs differentiating into oligodendrocytes, which are glial cells of the central nervous system that produce myelin. This is exciting because cultured oligodendrocytes provide immense therapeutic opportunities for treating a variety of neurological conditions.

Sunday, September 15, 8:15 - 9:30 am
Human Brain Proteome Project (HBPP)
Room 313-314

The Role of Neuroproteomics to Elucidate Neurodegenerative Disease Mechanisms

Lea T. Grinberg

University of Sao Paulo (Brazil) and University of California, San Francisco (USA)

Neurodegenerative diseases demonstrate lesions of opposite nature. Abnomal protein deposits are known as positive lesions, and neuronal loss comprise the negative lesions. Proteomics methods were instrumental to identify the pivotal proteins that compose inclusions in frontotemporal lobar degeneration: TDP-43 and FUS. In this lecture, we will discuss which fundamental questions on neurodegenerative diseases are still open and how proteomics tools may help to find the answers

Biomarker Discovery for Alzheimer and Parkinson Disease

Helmut E. Meyer[1], Caroline May[1], Andreas Schrötter[1], Michael Turewitz[1], Martin Eisenacher[1], Dirk Woitalla[2], Helmut Heinsen[3], Jens Wiltfang[4], Renata Leite[5] and Lea T. Grinberg[5]

[1]Medizinisches Proteom-Center, Ruhr-University Bochum, Germany, [2]Neurologische Universitätsklinik der Ruhr-Universität Bochum, St. Josef Hospital, Germany, [3]University Clinic Würzburg, Morphological Brain Research, Würzburg, Germany, [4]Klinik für Psychiatrie und Psychotherapie, Univ. Duisburg-Essen, [5]Brain Bank, Brazilian Aging Brain Study Group, Univ. of São Paulo, Brazil

Besides known molecular markers for Alzheimer's disease (AD) and Parkinson's disease (PD) like Aß-peptides, tau, phospho-tau and α -synuclein, which are used to diagnose dementia patients already affected or in an early stage of the respective disease, new validated biomarkers for AD or PD are urgently needed.

In an approach to identify new biomarker candidates we started a collaboration with the brain bank in São Paulo, Brazil, and the University Clinic, Würzburg, Germany, to focus our proteomic strategies on those brain regions of interest which are usually affected by AD or PD. Data from literature suggest that autoantibodies from sera of affected individuals showed immuno-reactivity in neurons in disease specific brain regions. Especially membrane proteins are of great interest, since they may represent first targets of autoimmune reactivity.

Therefore, in case of AD the raphe nucleus, the entorhinal region, the hippocampus and cortex layer 3b, in case of PD the enteric neuronal system, the dorsal motor nucleus of the vagal nerve, the locus coeruleus and the substantia nigra pars compacta and in case of ALS primary and secondary motor neurons are of primary interest. Different types of neurons will be isolated from post mortem human control brain by laser microdissection. These samples will then be analyzed by proteomic technologies, i.e. 1D-SDS/LC-MSMS and Western blot analysis. We are focusing on the identification of auto-antigen targets in AD and PD verifying the presence of pathologically effective autoantibodies in the sera of affected patients.

This work was supported by the European Regional Development Fond (ERDF) of the European Union and the Ministerium für Innovation, Wissenschaft und Forschung des Landes Nordrhein-Westfalen (ParkChip, FZ 280381102 and PURE, Az. 131/1.08-031).

Monday, September 16, 8:15 - 9:30 am
Liver (HLPP and B/D-HPP-Liver)
Room 303-304

Urine as a Source of Liver Disease Biomarkers and Proteomics Studies with Human Hepatoblastoma Samples

Felix Elortza

CIC bioGUNE, CIBERehd, ProteoRed-ISCIII, Technology Park of Bizkaia, Derio, Spain

Liver proteomic research keeps being one of the most active topics at CIC bioGUNE´s proteomics platform. We keep involved in the liver disease biomarker discovery in urine by analyzing urine peptides and exosome proteomes. Urine samples are ideal for diagnostic purposes because of the ease of sample collection using standardized protocols without the need for invasive methods, manipulation and storage. We explored this biofluid as a source to identify makers for liver pathologies by using two different approaches: exosome proteomics and natural occurring peptidome profiling.

Besides, we are involved in a collaboration with Dra. Carolina Armengol at IGTP-CIBERehd, and we have performed differential proteomics experiments

with hepatoblastoma human samples. Hepatoblastoma is a malignant embryonal tumor of the liver usually diagnosed in children younger than 3 years of age. We have combined DIGE based analysis together with the nLC MS/MS label free quantitation approach searching for new prognostic biomarkers. The results obtained from both techniques are complementary and show interesting aspects when comparing with previously published related studies in liver cancer context.

Identification of Proteins Driving the Progression of Liver Injury and Potential Biomarkers

Fernando J. Corrales

Division of Hepatology and Gene Therapy, CIMA, University of Navarra, Pamplona, Spain

The liver is one of the main topics in the B/D-SpHPP as there is an urgent need for novel clinical strategies to improve the management of liver diseased patients. In our lab, studies have been performed to identify mechanisms of liver disease progression. Proteomic analyses lead us to the identification of mitochondrial Prohibitin 1 as a central protein to preserve liver homeostasis and its down-regulation correlates with the development of non-alcoholic steatohepatitis. Defective prohibitin 1 induces apoptosis in human hepatoma cells and its partial deletion in mice induces liver damage and an exacerbated inflammatory response. The use of proteomic approaches led us to define a collection of proteins that might be relevant in the progression of human hepatocellular carcinoma (HCC). VASP has emerged as a biomarker candidate for the early detection of HCC as its plasma levels increase significantly and this change can be detected in cirrhosis, which is considered as a pretumoral condition. Preliminary results will be also presented relative to the follow-up of a group of HCC patients subjected to radioembolization.

Towards the System Medicine of Non-Alcoholic Fatty Liver Disease

Tommy Nilsson

The Research Institute of the McGill University Health Centre, the McGill University Health Centre & McGill University, Montreal, Quebec, Canada

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of disease states ranging from benign non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) associated with increased mortality rates. As a chronic polygenic and complex disease, it places a large portion of the general population at risk of developing terminal cirrhosis, hepatocellular carcinoma and/or hepatic decompensation. Significantly, between 3-19% of the general population have some form of NASH (inflammation) depending on their body mass index and when coupled with increased fibrosis, over time, are at high risk of advancing towards terminal liver disease. Through funds obtained from FRQ-S (lead PI-T. Nilsson), The Research Institute of the McGill University Health Centre (RI-MUHC), the McGill University Health Centre & McGill University (McGill), we have undertaken a translational study to delineate underlying pathophysiologies of involved disease states associated with NAFLD. Central to the project has been the establishment of a liver biobank (lead PI-P. Metrakos) designed such that it enables immediate processing of procured samples (from patients and donors) through sub-cellular fractionation to enrich for organelles (lead PI-J. Bergeron) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and advanced bioinformatics (lead PI-T. Nilsson) to assign hepatic proteins to their cognate organelles and to map how these change in relation to NAFL and NASH. In addition, to compare at the genomic and transcriptional level, changes with respect to disease (lead PI-R. Sladek). The aim of the project is to develop non-invasive patient-centric approaches to diagnose and monitor disease progression and to highlight avenues for early intervention. To date, following 18 months of analysis, we have identified 6,053 proteins (FDR below 0.1% as deduced through Protein Prophet/Scaffold) each corresponding to a unique gene based on a minimum of two independent and unique peptides (threshold 95%). In this, we assigned a total of 106,530 unique peptides based on 7,278,890 assigned spectra. An additional 3,556 proteins were detected with one unique peptide (threshold 95%) based on 6,579 peptides and 227,403 spectra. A large portion of identified proteins has been assigned to their cognate organelles through hierarchal clustering (an approach we term "The Protein Microscope") awaiting confirmation through antibody-based methods. High fidelity peptide and protein assignment was made possible through the use of the human centric database NeXtProt combined with high mass accuracy/high resolution LC-MS/MS. A complete progress report with selected highlights will be presented. Additional PIs involved in the projects are: Rima Rozen (McGill); Vassilios Papadopoulos (RI-MUHC); Silvia Vidal (McGill); Brian Gilfix (RI-MUHC); Barry Posner (McGill); Bartha-Maria Knoppers (McGill); Yann Yoly (McGill).

Monday, September 16, 8:15 - 9:30 am

Cancer

Room 302

CPTAC - a Proteogenomics Network for Cancer

Christopher Kinsinger

Clinical Proteomic Tumor Analysis Consortium (CPTAC), National Cancer Institute, USA

Molecular characterization of human cancers has generated large volumes of genomic data through comprehensive and coordinated efforts such as The Cancer Genome Atlas (TCGA) project. However, the mechanisms by which cancer genes singly or cooperatively transform cells remain poorly understood. Proteomic characterization of cancer tissues with genomic data represents a key step in not only verifying the genomic alterations at the protein level, but also allowing for the analysis of unique features that are inherent to proteins including post-translational modifications. The proteomic characterization of cancer tissues is therefore essential for the development of a successful strategy to reduce cancer mortality. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) was launched by the National Cancer Institute (NCI) as a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust and quantitative proteomic technologies and workflows. CPTAC aims to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provides this data with accompanying assays and protocols to the public. In this initiative session for the Cancer Proteome-Human Proteome Project (CP-HPP), we will describe the specimens, proteomic technologies, workflows, and public data releases of the CPTAC program.

Analysis of Tissue Biopsies by PCT-SWATH

Guo Tiannan

ETH Zurich, Institute of Molecular Systems Biology, Switzerland

Fast, quantitatively accurate, deep and reproducible proteomic analysis of complex biological samples remains a major hurdle in systems biology and systems medicine. Here we propose a method to convert the proteome contained in small (biopsy) cell or tissue sample into a digital data set that contains signature fragment ion patterns for all peptides derived from the sample. These datasets can be then perpetually mined in silico. In essence, the method converts the physical proteome in a (clinical) sample into a permanent digital representation. The method is built on pressure-cycling technology and SWATH-MS. The entire workflow can be completed within 12 hours. Subsequent targeted data analysis offers unprecedented performance in identifying and quantifying low-abundance signaling protein networks in human tissues in an extendable manner. We have applied this method to a kidney cancer cohort, and uncovered dis-regulated signaling protein networks.

Proteomics-Based Studies on Colorectal Cancer

Edouard Nice

Clinical Biomarker Discovery and Validation, Monash Antibody Technologies Facility, Monash University, Australia

Colorectal cancer (CRC) is currently the third most common cause of cancer death worldwide. If detected early while the disease is localised, 5 year survival following simple surgical resection is greater than 90%. By contract, if the cancer has metastasised, 5 year survival is less than 10%. Improved early detection and understanding of the metastatic process is clearly urgently required.

In this presentation I will describe some of our recent proteomics-based studies to both identify novel faecal biomarkers for detection and surveillance of the disease and also describe how we have we have integrated shotgun proteomics approach with a genome-wide transcriptomic approach (RNA-Seq) on a set of human colon cancer cell lines (LIM1215, LIM1899 and LIM2405) to both identify cancer associated proteins with differential expression patterns as well as protein networks and pathways which appear to be deregulated in these cell lines.

Integrative Analysis - Bridging the Gaps in Genetic Information Flow between Genomic and Proteomic Data

Zhen Zhang

Biomarker Discovery and Translation, Johns Hopkins University, USA

Molecular characterization of human cancers has generated large volumes of genomic and proteomic data through comprehensive and coordinated efforts such as TCGA and CPTAC. However, analyses of such genomic and proteomic data, even when comparing the same phenotypic differences, often yield results that do not overlap much in terms of the corresponding genes. We will present our effort in developing an integrative analysis approach and associated bioinformatics tools to take advantage of existing knowledge and databases such as pathways and interactomes, to search for the missing layer of molecular entities that could provide the most plausible linkage between the observed phenotype-dependent genomic alterations and proteomic expression changes. Results from such tools will not only provide plausible interpretations of the analysis results but also generate meaningful hypotheses for further verification.

Breast Cell Index and Atlas Projects

Peter James

Department of Immunotechnology, Lund University, Sweden

We have analysed over 450 primary human breast tumours and seven cells lines that represent the most common types of breast cells. These include model human cell lines representing the main molecular portraits of breast cancer as defined by mRNA expression (Luminal A and B, Basal, ErbB2, and normal-like) as well as a breast adipocyte and fibroblast cell line. In addition to cataloguing protein expression levels and modifications, we have been examining the intracellular distribution of proteins amongst the organelles. We have built up a large spectral library that enables us to create and validate rapid SRM assays for various aspects of breast cancer development and therapy choice and response monitoring. For example we have developed SRM assays for the majority of the proteins involved in DNA repair to allow monitoring of patient response to chemo- and radiotherapy. SRM analysis also allows a rapid determination of the levels of the hormone receptors (Estrogen, Progesterone and HER receptors) as well as known proteins involved in therapy resistance. We have been analysing the changes in protein expression during cancer advance using paired sets of primary tumour and local recurrence and comparing this to primary tumour and distal metastasis. This will enable us to more accurately define the degree of aggressiveness of a tumour and to develop a method to test for the presence of cancer cells in the margins of the primary tumour and in the sentinel node.

Human Proteome Knowledge Discovery Gateway：Progress and Perspective

Dong Li and Fuchu He

State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, National Center for Protein Sciences, National Engineering Research Center for Protein Drugs, China

High-throughput proteomic research approaches, such as mass spectrometry and antibody-based experiments, have generated a great amount of heterogeneous datasets. Mining knowledge from these datasets presents significant challenges, including the quality control, storing, processing, integrating and visualization of various types of proteomic data.

To address these challenges, we are constructing a human proteome knowledge discovery gateway. First, an interactive, configurable and extensible, configurable and interactive workflow-based platform will be constructed to analyze the proteome datasets based on the open-source galaxy framework on a cloud infrastructure. And now, this platform has integrated the analyses tools for identifying missing proteins, mapping peptides onto chromosomes, integrating peptide with ENCODE datasets, protein annotation, Gene Ontology enrichment analyses, protein biological network/pathway analyses, fundamental statistical analyses and machine learning. More importantly, this platform also integrates a configurable workflow system to combine these software tools, supporting workflow construction, editing, running, sharing and viewing. Second, bioinformatics browsers will be integrated into this gateway including the chromosome-based proteome browser (CAPER) and pathway-based proteome browser (PAPER). And now CAPER has been available online as a web-based, user-friendly web service, which can display proteomic data sets and related annotations comprehensively, CAPER employs two distinct visualization strategies: track-view for the sequence/site information and the correspondence between proteome, transcriptome, genome, and chromosome and heatmap-view for the qualitative and quantitative functional annotations. The PAPER will assign the proteome datasets to the human biological pathways, and this service will be online as soon as possible. Third, application software will be developed to annotate the human genome, such as finding novel protein-coding genes and presenting the correlation between the chromosome to genes, epigenome, proteomes, and phenotype, and also to find the key regulatory molecules/modules, the potential drug target and biomarkers by mapping proteome profile to human pathways.

Taken together, the human proteome knowledge discovery gateway will greatly facilitate the complete annotation and functional interpretation of the human genome by proteomic approaches, thereby making a significant contribution to the Human Proteome Project and even the human physiology/pathology research.

Monday, September 16, 8:15 - 9:30 am

HKUPP+HPPP

Room 311-312

Proteomic Analysis of Nephron Segments of Formalin-Fixed Paraffin-Embedded Human Kidney Tissues

Tadashi Yamamoto, Keiko Yamamoto, Yutaka Yoshida, Bo Xu, Ying Zhang, Sameh Magdeldin

HKUPP

Nephrons are the functional units of the kidneys and consist of several parts (glomerulus, proximal tubule, descending and ascending loop of Henle and distal tubule) connected to collecting ducts. Knowing of proteome profile of each nephron segment will contribute to understand characteristics and functions of the segment. Nephron segments (glomeruli, proximal tubules, descending and ascending loops of Henle and distal tubules) and collecting ducts were separated from formalin-fixed paraffin-embedded human kidneys by laser microdissection after immune-labeling of each part with antibodies against unique proteins in each part. Tissue sections of total area of ~1 mm2 each were digested with trypsin after autoclave treatment (On-site direct digestion, OSDD method). Peptides were collected from the segments (three samples each) and purified by C-18 Stage-Tip and analyzed by LC-MS/MS (Thermo Orbitrap LTQ) and proteins were identified by Mascot with FDR less than 1%.

More than 1000 proteins were identified by a single MS analysis of each sample and proteins uniquely expressed in each nephron segment were selected in glomerulus; 153, proximal tubule; 306, distal tubule; 58, and collecting duct; 40. Gene Ontology annotation analysis showed that the most enriched cellular components were cytoskeleton proteins in the glomerulus, cytoplasm proteins in the proximal tubule, mitochondrion proteins in the distal tubule, and cytoplasmic part proteins in the collecting duct.

The proteome analysis of nephron segments demonstrated their significant features and provided useful knowledge of their functions.

A Collective Analysis of Three Human Subproteomes using PeptideAtlas

Eric Deutsch, Terry Farrah, Tadashi Yamamoto, Julian Watts, Micheleen Harris, Zhi Sun, Gil Omenn

HPPP

The kidney, urine, and plasma subproteomes are intimately related: waste products are filtered from the plasma by the kidney and excreted via the urine, while some kidney proteins pass directly into the urine. Human kidney, urine, and plasma samples were measured using shotgun proteomics and all peptides were mapped to neXtProt. A software tool was developed within PeptideAtlas to show which neXtProt entries were unique to, or highly enriched in, each of the subproteomes relative to the others. The tool also performed detailed comparisons to reveal which urine proteins were likely derived from kidney, which were likely derived from plasma, and which were likely not derived from either. Gene Ontology analysis confirmed what is already known about the relationships among these subproteomes and revealed some new findings.

Different Levels of Variability in the Human Plasma Proteome

Yansheng Liu, Ben Collins, Ludovic CJ Gillet, Alfonso Buil, Emmanouil T. Dermitzakis, Lin-Yang Cheng, Olga Vitek, Ruedi Aebersold

Institute of Molecular Systems Biology, ETH Zurich

Human plasma is an appealing sample for translational medicine and biomarker discovery studies. However, fundamental questions of the variability of the plasma proteins in a population and their longitudinal pattern over years remain poorly characterized. 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 this technology we analyzed above 200 healthy plasma samples from monozygotic (mz) and dizygotic (dz) twins that were collected with 2-7 year intervals. 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. Using the set of linear mixed effects models provided by MSstats software, we aim to systematically investigate and decompose the technical (transition-, peptide- and protein-level) and biological variance in human plasma proteome profiles. The data shows an unprecedented degree of reproducibly, with the median peptide CV of 11.2% for technical replicates. We estimate that the stable variation, comprising familial and individual-environmental factors, accounts in general for considerable fractions of biological variation in plasma protein concentrations.

Quantification of Peptides in Clinical Samples Based on High-Resolution Mass Measurements

Bruno Domon

Luxembourg Clinical Proteomics Center, Luxembourg

New hybrid mass spectrometers with high resolution and accurate mass (HR/AM) capabilities have opened new avenues in quantitative proteomics. Targeted analyses, routinely performed on triple quadrupole mass spectrometers using the selected reaction monitoring (SRM) mode, were replicated on a high-resolution quadrupole-orbitrap instrument (Q-Exactive) to improve the selectivity of the measurements. In this context, targeted measurements benefit from a narrow mass filtering window of the precursor ions together with the orbitrap HR/AM measurement of the fragment ions, while the multiplexing capability of the instrument was leveraged to measure precisely, using internal standards, a large number of peptides in a single LC-MS run. The quantification of peptides was performed in this parallel reaction monitoring mode (PRM), by extracting post-acquisition ion traces of specific fragment ions. The performance of the PRM technique was benchmarked against that of the reference SRM approach, and more specifically, the trapping capability proved beneficial for the enrichment of precursor ions of peptides in very tiny amounts (sub-amol level) and thus dramatically increase the signal-to-noise ratio. The PRM technique was applied to the analysis of clinical samples with a complex background (plasma and urine) to demonstrate the benefits of the gain in selectivity and the identification of the fragments through accurate mass, to increase confidence in the measurements. More specifically, lung cancer candidate markers were analyzed by this technique to differentiate the disease stages and subtypes. The results obtained by this technique allowed a clear discrimination of the different disease stages.

Monday, September 16, 8:15 - 9:30 am

Human Diabetes Proteome Project (HDPP)

Room 313-314

The Human Diabetes Proteome Project (HDPP)

D. Schvartz[1], F. Topf[1,2], P. Gaudet[3], F. Priego-Capote[4,5], A. Zufferey[1], N. Turck[1], PA. Binz[2,6], P. Fontana[1,7], A. Wiederkehr[8], F. Finamore[1], I. Xenarios[2,6], D. Goodlett[9,10], M. Kussmann[8,11,12], P. Bergsten[13], J-C. Sanchez[1]

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Diabetes mellitus is a complex multifactorial disease characterized by hyperglycemia and deranged lipids, which have been linked to diabetes-related complications. The Human Diabetes Proteome Project (HDPP) consortium was created at HUPO 2012 to unravel molecular mechanisms leading to diabetes and to understand the dysfunctions induced by glucose and free fatty acids. During the first year, the partners of HDPP identified the short-to-mid term objectives of the project. Various omics datasets will be collected, mainly by analyzing insulin producing cell lines, islets, and human blood from different conditions. Data integration as well as network biology approaches will be applied to enhance our knowledge of pathways centrally involved and deregulated in diabetes. Existing projects from the partners are already delivering omics data on human islets, rodent beta-cells, mitochondria, glycation in human blood as well as key results on modifications associated to beta-cell dysfunction. Based on the three pillars of the B/D-HPP projects, HDPP has already made publicly available (www.hdpp.info) three key protein resources [1]: (1) the 1' 000 diabetes-associated protein (the 1000-HDPP) database with links to their neXtProt, Peptide Atlas and Human Protein Atlas references; (2) a list of 5' 300 human islet proteins; and (3) a list of 2' 500 rodent beta-cell proteins. All results obtained so far through the HDPP initiative will be presented in the HDPP workshop held at the 12th HUPO world congress.

[1] Topf F., Schvartz D. et al. The Human Diabetes Proteome Project (HDPP): From Network Biology to Targets for Therapies and Prevention. Translational Proteomics 2013, in press.

Tuesday, September 17, 8:15 - 9:30 am

HGPI Glycoproteomics

Room 301

Summary of the First to Third Pilot Studies of Human Disease Glycomics/Proteome Initiative (HGPI)

Hiromi Ito

Department of Biochemistry, Fukushima Medical University

The Human Disease Glycomics/Proteome Initiative (HGPI) aims to standardize the data presentation in glycomics associated with diseases and promote data exchange, comparison, and verification among researchers. We have done three analytical pilot studies to date. The first and second studies were analyzed N- and O-linked glycans using standard glycoproteins (i.e. 1st: IgG and transferrin, 2nd: IgA), respectively. The purpose of these pilot studies was verification of comparing different methods (e.g LC-based and MS-based technologies) for quantitation of N- and O-linked glycans [1,2]. Then, the third study was attempted the following two analyses under the theme of 'Glyco-Biomarker Discovery'. One is the structural analyses of glycans derived from three different cancer cell lines (i.e. L428: Hodgkin's Lymphoma cell, U937: Lymphoma cell, and SK-N-SH: Neuroblastoma cell). Second is the identification of carbohydrate antigen-carrier proteins which is based on interest of each participant. The comprehensive analysis of glycans derived from cancer cells is helpful for understanding the activity of the glycosylation machinery of the cell. Here, we summarized the results of the third pilot study in addition to the first and second study.

[1] Comparison of the methods for profiling glycoprotein glycans – HUPO Human Disease Glycomics/Proteome Initiative multi-institutional study. Glycobiology 2007, 17, 411 - 422.
[2] Comparison of Methods for Profiling O-glycosylation: HUPO Human Disease Glycomics/Proteome Initiative Multi-Institutional Study of IgA1. Mol. Cell. Proteomics 2010, 9, 719 - 727.

Proposal of a New International Collaboration under the HPP: Biology/Disease-driven Glycoproteome Project (B/D-GPP) and Current Resources

Hisashi Narimatsu

Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST)

In Boston Congress 2012, I have proposed a new international collaboration for systematic uncovering of protein glycosylation as a part of B/D-HPP under the HPP of HUPO to expand the past studies in HGPI. This activity is named as Biology/Disease-driven Glycoproteome Project (B/D-GPP) aiming at construction of the open-access Glycoproteome Atlas that contains following information. First, we would like to collect data on mouse as a model organism, because common sources are available worldwide and a wide range of gene-deleted mice would be useful to reveal the structures and functions of glycans. Next or in parallel, human cell lines would be appropriate for the project. We should establish the Glycoproteome Atlas as a database, and collect the data for each of the tissues, body fluids, and cells. The Glycoproteome Atlas should encompass: 1) glycan profiles by lectin microarray, 2) mass spectra of glycans (MS and MSn), 3) expression profile of glycogenes (glycan biosynthesis-related genes), 4) tissue images stained with a series of lectins, 5) LC and CE (capillary electrophoresis) profiles of glycans, and 6) N- and O-glycoproteome profiles that provide protein ID and actual glycosylated sites. In this talk, the basic plan and current data resources such as N-glycoproteome profiles by the IGOT-LC/MS method and glycan profiles by lectin array analyses for mouse tissues are presented to call for the participation to this project.

Dissection of the Human GalNAc O-glycoproteome? Mapping Specific Functions of Individual Polypeptide GalNAc-transferase Isoforms by Zinc-finger Gene Engineering of Human Cells

Katrine ter-Borch Gram Schjoldager

Department of Cellular and Molecular Medicine, University of Copenhagen

Posttranslational modifications (PTMs) greatly expand the function and regulation of proteins, and glycosylation is the most abundant and diverse PTM. Deficiencies in protein glycosylation lead to a number of severe diseases and multisystemic disorders. Recently we have begun to uncover more subtle disease phenotypes associated with deficiencies in glycosyltransferase genes that are members of large homologous gene families with partial redundancies. Thus deficiencies in protein O-GalNAc glycosylation, where the first initiation step is regulated by 20 distinct GalNAc-transferases, produce cell and protein specific effects and subtle distinct phenotypes such as hyperphosphatemia with hyperostosis and dysregulated high density lipoprotein cholesterol (HDL-C) and lipid metabolism. We uncovered a possible mechanism by which one of the GalNAc-transferases, GalNAc-T2, co-regulates proprotein convertase (PC) processing and activation of the lipase inhibitor ANGPTL3, which can affect HDL-C and lipid plasma levels, both of which are heritable risk factors for coronary artery disease (CAD). More recently we have used the zinc-finger nuclease (ZFN) gene targeting of the human C1GalT1 chaperone COSMC to generate stable HepG2 SimpleCells with homogenous truncated GalNAc O-glycans in order to characterize the simplified O-glycoproteome. We have now applied this strategy to show non-redundant O-glycosylation performed by a single polypeptide GalNAc-T using differential analysis of O-glycoproteomes produced in an isogenic cell model with and without knock-out or knock-in of GalNAc-transferases. We have found several isoform specific substrates for GalNAc-T1, -T2 and -T3 that serve as potential biomarkers for disease caused by dysfunctional O-GalNAc-glycosylation and demonstrated that the human O-glycoproteome is differential and dynamic.

Tuesday, September 17, 8:15 - 9:30 am

iMOP - Initiative on Model Organism Proteomes

Room 302

iMOP – Initiative on Model Organism Proteomes

Sabine P. Schrimpf[1], Andreas Tholey[2], Emøke Bendixen[3], Michael O. Hengartner[1]

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Fundamental pathways and biological processes are conserved across species, and studies of model organisms play an important role for understanding human biology and health. The continuous improvement and use of mass spectrometry have led to a dramatic increase in proteome coverage, and to the inclusion of an increasingly broad range of model organisms over the recent years. However, new model organism species are typically supported by only small communities. To form a broader model organism proteomics community, the initiative on model organism proteomes (iMOP; www.imop.uzh.ch) was integrated into HUPO. The research interest of the iMOP community is already quite diverse; however, we still encourage more research groups to join iMOP. Current iMOP members either focus on the interaction between humans and other organisms at the proteome level or and they are interested in the relevance of model organism proteomes to the human proteome. They run inter- and intra-species proteome and transcriptome comparisons to address evolutionary aspects and pathway development. iMOP members also focus on species that are important for food production and on host-pathogen-interactions. The iMOP initiative distributes proteomics knowledge to a wide range of model organism communities that are otherwise not closely linked. iMOP will contribute to the human proteome project (HPP) by providing comparative studies across species.

The Methylproteome Network of *Saccharomyces cerevisiae*

Marc R. Wilkins

University of New South Wales, Australia

Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. This presentation outlines our efforts to construct the first 'methylproteome network' for a eukaryotic cell and provides evidence that arginine methylation modulates protein-protein interactions in this network. We analysed the yeast methylproteome to identify methylated proteins and precise modification sites. Immonium ion-based scanning and targeted data acquisition - electron transfer dissociation MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted onto microscope slides). This showed that protein methylation is widespread in the eukaryotic cell. To build the intracellular methylation network, all known and putative

methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzyme was responsible for which methylation event. This led to the discovery of a new lysine methyltransferase, we named Efm2. Enzyme-substrate links were further investigated by the analysis of recombinant substrate proteins methylated by recombinant enzymes, by in vivo methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with the yeast protein-protein interaction network to generate the first 'methylproteome network'. Interestingly, this suggested that many protein-protein interactions could be controlled by protein methylation. To test this, we constructed a new 'conditional two-hybrid' (C2H) system. Interactions of proteins were tested in the presence of a methyltransferase or in the presence of the same enzyme with active site knocked out. Of the protein-protein pairs involving arginine methylated proteins, half of those tested to date have shown increases in interaction in association with methylation.

Secretome Protein Profiling of Growth Interaction between *Listeria monocytogenes* and *Lactobacillus lactis* subsp. *lactis*

Paola Roncada

Lazzaro Spallanzani, Italian Experimental Institute, Milan, Italy

It is well known that starter cultures produce a wide range of anti-microbial metabolites which include organic acids, diacetyl, acetoin, hydrogen peroxide, antibiotics and bacteriocins. This anti-microbial activity can contribute in a number of ways towards improving the quality of fermented foods, for example, through the control of pathogens, extending shelf life and improving sensory qualities. The aim of this work is to explore the inhibition power of lactic acid bacteria in vitro against a pathogen, Listeria monocytogenes, that can occur on the rear of some type of cheese.

It has already been documented how bacterial competition can be used to overcome the growth of foodborne pathogens. As inter-cell communication can occur among bacteria for quorum sensing mechanisms, the secretion of extracellular proteins for other bacterial-growth inhibition has been as well documented.

In this project it has been decided to study the putative mechanisms of *Lactobacillus lactis* subsp. lactis in the inhibition of *Lysteria monocytogenes* growth. In particular it is already been documented that some species of lactic acid bacteria (LAB) are able to produce bacteriocins responsible for listeria monocytogenes growth inhibition. Briefly, cell culture filtrate of LAB, *Listeria monocytogenes*, and of LAB growing together with listeria have been analyzed from the peptidomic and proteomic point of view. Image analysis interestinguily revealed the presence of some specific proteins that are secreted only from one or both bacteria just when they are growing in the same medium. This demonstrates that inter-specific cell-cell communication is occurring for bacterial competition mechanisms. The bands and the spots of interest have been excised from the gels to be analyzed and identified through mass spectrometry.

Acetyl-phosphate Links Metabolism to Global Acetylation Dynamics in *E. coli*

Brian Tate Weinert

University of Copenhagen, Denmark

Recent proteomic studies identified thousands of lysine acetylation sites in diverse organisms from bacteria to humans. However, little is known about their origin and regulation. We used a quantitative mass spectrometry-based quantitative proteomics approach to study acetylation dynamics at thousands of sites in the model bacterium Escherichia coli (E. coli). We identified more than 8,000 unique acetylation sites and found that acetylation occurred at a low level and accumulated in growth-arrested cells in a manner that depended on the formation of acetyl-phosphate (AcP) through glycolysis. Mutant cells unable to produce AcP had significantly reduced acetylation levels while mutant cells unable to convert AcP to acetate had significantly elevated acetylation levels. We showed that AcP can chemically acetylate lysine residues in vitro and that AcP levels are correlated with acetylation levels in vivo, suggesting that AcP acetylates proteins nonenzymatically in cells. Most acetylation occurred independently of the YfiQ acetyltransferase and the CobB deacetylase suppressed acetylation at ~10% of sites. CobB-regulated acetylation sites were more sensitive to increased acetylation in growth-arrested cells and to AcP in vitro, suggesting that CobB suppresses acetylation at these sites to maintain very low levels of acetylation. These results uncover a critical role for AcP in bacterial acetylation and indicate that most acetylation in E. coli occurs at a low-level and is dynamically affected by metabolism and cell proliferation in a global, uniform manner.