Application of Total Serum N-Glycome Profiling to Diagnosis of Liver Fibrosis and Cirrhosis in Patients with Chronic Hepatitis B Infection

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Liver fibrosis, which progressively develops into cirrhosis, is an impending threat to the millions of patients infected with hepatitis. Non-invasive biomarkers are urgently needed. Liver produces majority of serum glycoproteins. Any abnormalities in N-glycome of total serum glycoproteins may indicate the presence of liver diseases, and may be used as biomarkers. In this study, we have established a quantitative high-throughput assay for profiling total serum N-glycome by MALDI-TOF MS, and applied it to the identification of N-glycans for diagnosis of liver fibrosis and/or liver cirrhosis in patients with chronic hepatitis B (CHB) infection. The intra- and inter-assay coefficients of variation were less than 8% and 17% respectively. Total serum N-glycome profiles from 46 CHB patients with different degrees of liver fibrosis were obtained and subjected to bioinformatics analyses. Sixty three N-glycans were identified; twenty one were correlated with fibrosis stages. A glycan of m/z 1829 (P = 0.0005) had a sensitivity of 82% and a specificity of 84% for detecting liver fibrosis, while a glycan of m/z 1444 (P < 0.0005) had a sensitivity of 76.5% and a specificity of 69% for detecting liver cirrhosis. An artificial neural network model comprising 21 glycans was developed. It could effectively detect both liver fibrosis and cirrhosis with sensitivities > 92% and specificities > 72%. The structures of 9 glycans were predicted by searching the N-glycan mass database. Fucosylated glycan species with a bisecting N-acetyl-glucosamine were increased in liver fibrosis patients. This is the first study to demonstrate the quantitative nature of MALDI-TOF MS in N-glycome profiling, and the first study to reveal the importance of serum N-glycome profile in liver fibrosis diagnosis. (The authors are supported by the Research Fund for the Control of Infectious Diseases from the Health, Welfare and Food Bureau of the Hong Kong SAR Government).

Structural Proteomics of the Human Protein Tyrosine Phosphatase Family

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Protein tyrosine phosphorylation plays a major role in many cellular functions including cell survival, proliferation, differentiation and mobility. Protein tyrosine phosphatases (PTPs) are a key group of signal transduction enzymes which, together with protein tyrosine kinases, control the levels of cellular protein tyrosine phosphorylation. PTPs have a conserved catalytic domain with HCXXGXXR motif and show some subtle structural differences in the residues surrounding active site. Detailed informations of PTP structures will enable us to verify the interaction between substrates and the active site and provide a solid foundation for rational PTP inhibitor design. There are at least 107 genes coding for PTPs in the human genome. Recently, about half of all PTP genes have been implicated in human disease or are recognized as potential drug targets. 81 out of 107 target genes were well expressed in E. coli and 70 soluble PTPs were obtained; of these 40 were purified to homogeneity and then were subjected to the crystallization screening. 14 PTPs out of the screening proteins were successfully crystallized. Based on the structure of PTPs, we are analyzing several PTPs to find out their exact functions in vivo and vitro by biochemical and cellular biological assays.

Quantitative Proteomic Analysis of Low Molecule Weight Plasma Proteins by Using Ultra Filtration and Large Gel Second Dimensional Electrophoresis

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The present proteomic study summarizes technologies for fractionation using a cut off membrane (30 KDa) to obtain low molecule weight plasma proteins and statistical quantitative analysis using 1-DE and 2-DE image assay to choose the target plasma protein molecules. Plasma proteomic analysis was performed on one reference specimen from a total of 10 healthy donors, which sample was made from ten-pooled plasma. Also, large gel 2-DE system was applied to separate plasma proteins and the low molecule weight plasma proteins (about 1,500) were identified by MALDI-TOF or MALDI-TOF/TOF.
Identification of Ubiquitin-interacting Proteins from Mouse Brain Using Multidimensional Protein Identification Technique Following Ubiquitin Affinity Chromatography

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Ubiquitination is a novel post-translational modification, which regulates the stability, activity, localization, and interaction of target substrate. The discovery of a new ubiquitinated protein as well as its new function seems to be everlasting, which graced ubiquitin (Ub) with Darwin’s phosphate. It is obvious that Ub interacts with a variety of proteins for its multifunctional role. In order to analyze the ubiquitin-interacting proteins (Ub-IP) systematically, we first tried to isolate Ub-IPs using affinity chromatography. Since most of the proteins are known to interact with Ub through the hydrophobic patch on the surface of Ub formed by Leu8, Ile44, and Val70, we prepared both Ub- and mutant Ub (mUb; Leu8Ala, Ile44Ala, Val70Ala)-affinity chromatography and isolated the interacting proteins separately. Isolated proteins were then identified by tandem liquid chromatograph coupled to tandem mass spectrometry (multidimensional protein identification technology). Proteins identified only from Ub-affinity chromatography with the exclusion of proteins identified from mUb-affinity chromatography were selected as Ub-IPs and further analyzed in this study. Total 110 Ub-IPs were selected including enzymes involved in ubiquitination and deubiquitination. Many Ub-IPs contain ubiquitin binding domains (UBDs) such as UBA, UIM, CUE, PAZ, GAT, and VHS. Proteins related to endocytosis and ERAD were detected as the relatively abundant Ub-IPs in mouse brain by the semi-quantification based on the number of detected mass spectrums. Now we perform the comparative analysis of Ub-IPs between wild type and 2X AD Tg mouse brains. (This work was supported by 21C Frontier Functional Proteomics Project and by the grant for the study of Ubiquitome Functions from the Korea Ministry of Science & Technology).
An important technique for protein characterization is isoelectric focusing, which separates proteins by their intrinsic isoelectric points (pI values). Isoelectric focusing is widely used for pI determination of unknown proteins, as well as to determine charge heterogeneity in a single protein sample. Due to the high resolution capabilities of the capillary electrophoresis format, recent years have seen an increased interest in capillary isoelectric focusing (CIEF). In this report, CIEF is used to characterize proteins of interest isolated from a bovine skeletal muscle proteome of soluble proteins. However, the inherent complexity and dynamic range of this proteome necessitates some “upstream” separation, to reduce complexity before the CIEF separation. A two-dimensional liquid fractionation of this proteome is performed, with the first dimension using pH and the second dimension using hydrophobicity to separate the proteins. This fractionation creates a pH/hydrophobicity profile for the proteome. Each fraction in this profile is now suitable to CIEF. CIEF is performed by mixing the sample with a CIEF gel and 3–10 ampholyte solution, filling a capillary with this mixture, dipping the capillary in appropriate electrolytes, and applying a voltage on both ends. This separation identifies the pls of individual proteins in the mixture. Once the pl value is known, CIEF can be repeated with narrow range ampholytes added to the mixture to “zoom in” to a specific pl range. For example, if the pl of the protein is 8.5, an 8–10 narrow range ampholyte is used to better resolve the protein peaks. Better resolution leads to precise information about charge heterogeneity in the protein. These results demonstrate the importance of CIEF to characterize proteins that are isolated from a more complex proteome by a two-dimensional fractionation technique.

Protein Isoforms in Cancer, Identified and Quantified by the ICPL (Isotope-coded Protein Labelling) Technology

Truncated protein isoforms have been reported as potential diagnostic markers for a variety of cancers, e.g. breast and bladder cancer. However, they can only be detected and quantified if the protein sizes are retained. T. M. Halder, E. Keidel, D. Dosch, M. Kersten, J. Kellermann, and F. Lottspeich

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Here we investigated the application of ProteoChip in expression proteomics. Antibody microarrays constructed by immobilization of 60 distinct monoclonal antibodies on ProteoChip base plates were used to analyze differentially expressed pattern of cell signaling proteins in lysates of HUVEC in the presence and absence of angiogenin. The antibody microarray approach showed angiogenin-induced upregulation and downregulation of several cellular regulators related with proliferation. Consistent with these findings, the regulatory mechanism of the cell proliferation by angiogenin was evaluated. Differentially expressed proteins determined by the antibody microarray were validated by western blot analysis. Taken together, these data suggests that antibody microarrays using ProteoChip technology can be a powerful tool for high-throughput analysis of proteome in human cells and tissues.

Protein Isoforms in Cancer, Identified and Quantified by the ICPL (Isotope-coded Protein Labelling) Technology

T. M. Halder, E. Keidel, D. Dosch, M. Kersten, J. Kellermann, and F. Lottspeich

TopLab, Martinsried, Germany; Max-Planck-Institute of Biochemistry, Martinsried, Germany

ProteoChip has been developed as a novel protein microarray technology. Its application has been determined in new field screening and molecular diagnostics so far and should be gradually enlarged in the field of biology. Here we investigated the application of ProteoChip in expression proteomics. Antibody microarrays constructed by immobilization of 60 distinct monoclonal antibodies on ProteoChip base plates were used to analyze differentially expressed pattern of cell signaling proteins in lysates of HUVEC in the presence and absence of angiogenin. The antibody microarray approach showed angiogenin-induced upregulation and downregulation of several cellular regulators related with proliferation. Consistent with these findings, the regulatory mechanism of the cell proliferation by angiogenin was evaluated. Differentially expressed proteins determined by the antibody microarray were validated by western blot analysis. Taken together, these data suggests that antibody microarrays using ProteoChip technology can be a powerful tool for high-throughput analysis of proteome in human cells and tissues.
Large Scale Comparative mRNA and Protein Expression Profiling

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How well do the mRNA levels correlate with the corresponding protein levels in cells and tissues? This question has been addressed more and more often with the massive amount of data on relative mRNA levels generated by DNA microarrays during the last years. Several comparisons between mRNA and protein levels have also been performed, with varying results and mainly for small sets of transcripts and proteins.

We present a large scale comparative expression profiling, which was achievable through the Human Protein Atlas (HPA) project (www.proteinatlas.org). HPA utilizes a high-throughput generation of monospecific antibodies (msAb) for the profiling of protein expression and localization in human normal and cancer tissues. A cell atlas will also be introduced, where the protein expression and sub-cellular localization in cell lines will be displayed. The cell atlas is based on a recently developed cell tissue microarray (cell-TMA), where cell lines are solidified, sliced and printed on arrays and used for detecting proteins using the manufactured antibodies. The cell-TMA was stained with msAbs and a new automated image analysis software enables a relative quantification of the protein expression among different cell lines.

The cDNA fragments used to produce the recombinant protein antigens for immunizations and subsequent generation of msAbs were amplified and spotted on microarrays. Transcript profiles from 27 cell lines were retrieved from these microarrays and analysed. The results from a correlation analysis for hundreds of transcripts and proteins, based on the variation of expression among the cell lines, will be presented.

Hyperglycemia-induced Proteome Alterations in Rat Cardiomyocytes

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Hyperglycemia is the most common problem in diabetes with consequent various oxidative stress related events. This oxidative stress induced reactive oxygen species is major contributor in cell apoptosis and/or necrosis. Cardiac tissue, as a vulnerable candidate, is greatly impacted by this scenario. Comparative proteomics affords reasonable approach in disclosing many mysteries related to progression of these events on molecular basis. The influence of high glucose (30 mM) Tyrode’s perfusion on rat heart compared with control (5.5 mM glucose Tyrode’s perfused rat heart) was examined. Possible hyperosmotic effect was tested by perfusion of osmotically equivalent mannitol solution. 2-DE proteomics followed by MALDI-TOF MS analysis were performed in all groups. Protein confirmation was assisted by immunoblotting and RT-PCR. High glucose perfusion results in 11 up-regulated and 13 down-regulated protein spots, respectively. General tendency of decreased glycolysis, accelerated TCA, a-keto acid and fatty acid breakdown was observed. Interestingly, whilst members of chaperone like heat shock proteins and structural proteins were down regulated on glucose perfusion they showed marked elevation in mannitol perfused group. The extent by which glucose over load influences the progression of cardiac infarction was evaluated on tissue level with triphenyl tetrazolium chloride (TTC). Discrimination between apoptosis and necrosis on was performed by FACS-assisted annexin V and propidium iodide combination on isolated cardiomyocytes. Both TTC staining and FACS results confirmed the increased rate of apoptosis on glucose over load with Bax-independent mechanism, as indicated from RT-PCR data. Confocal microscopic tracing of isolated cardiomyocyte revealed mitochondrial membrane potential stability with increased cytoplasmic calcium after glucose loading. Additionally, neither NO nor mitochondrial superoxide level was influenced by high glucose. To conclude glucose overload in cardiomyocytes blunted many anti-apoptotic proteins e.g. chaperones expression. In doing so it eventually mediates, at multiple metabolic sites, rearrangement changes that aggravate the susceptibility of cardiac myocyte for apoptosis.
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The Use of Comparative Proteomics, Functional and Morphological Adaptive Changes in Elucidation of Mitochondrial Alterations in Gastric Carcinoma Cell Line

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Mortality rates of gastric cancer are particularly increasing in many parts in the world including Asia, South America, and parts of Europe. Since mitochondrial contribution in regulation of apoptosis is promising approach as anti-cancer target, elucidation of mitochondrial changes is first step for this recruitment in gastric cancer. The study was designed to clarify the impact of gastric cancer on mitochondrial structural and functional as well as proteome adaptations in human gastric cancer cell line (AGS) using normal rat gastric cell line (RGM-1) as control. Consistent with significant decrease in oxygen consumption rate, as reported by Clark’s oxygen electrode, both mitochondrial count and size showed marked reduction, as revealed by electron microscopic study, in cancer cell line. The confocal microscopic tracing proved an elevated mitochondrial membrane potential and increased matrix Ca\(^{2+}\) concentration. Moreover, the 2DE-MALDI-TOF proteomics data indicates many structural and functional proteins expression difference in mitochondria of human gastric cancer cells when compared with that of control. Interestingly, ubiquinol-cytochrome c reductase (Complex III), that functions as the O\(_2\) sensor during hypoxia by regulating ROS generation showed up regulation in AGS rather than RGM-1 control cells. The over expressed complex III protein may partly contribute, as internal stimulant, in adaptive changes concerning hypoxia induced factor (HIF) with subsequent cell survival in human gastric cancer cell line (AGS). Mitochondrial short-chain enoyl-Coenzyme A hydratase up regulation overestimates the role of \(\beta\)-oxidation as alternative supply of reducing equivalent in gastric cancer cells.

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A Protein Interaction Network of Human Liver

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With the initiative of Human Liver Proteome Project (HLPP), one major goal is to clone all of the ORFs expressed in human liver and study the binary protein-protein interactions between them. More than 5000 unique ORFs were cloned till now. More than 1500 ORFs were used as baits to screen human liver cDNA library by yeast two hybrid technology. Total 1245 unique protein-protein interactions were obtained, which corresponds to 531 baits. For our surprising, our data has little overlap (less than 5%) with two published large-scale human interactome dataset, which suggest it is complementary between different research and diverse methods. A few liver diseases-related interacted protein pairs were used for further molecular and cellular mechanism research. The initial research of protein linkage map in human liver may be valuable source for human protein-protein interactome and liver diseases study.

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Analysis of Proteome in Human Follicular Fluid Derived from Polycystic Ovary Syndrome (PCOS) Patients

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Polycystic ovary syndrome (PCOS) represents the most common cause of anovulatory infertility and affects 5–10% of women of reproductive age. The etiology is still unknown exactly. To investigate the differentially expressed proteins from PCOS versus normal patients, the protein expression in follicular fluid was analyzed using two-dimensional electrophoresis (2-DE). Follicular fluid contains a variety of biologically important proteins of the requirements for oocyte fertilization and follicle maturation in the mammalian reproductive process. Therefore, it may be used as a provisional source for identifying proteins related with PCOS. Over-expressed six proteins (kininogen 1, cytokeratin 9, antithrombin, fibrinogen, apolipoprotein A-IV precursor, and a-1-B-glycoprotein) in follicular fluids from PCOS patients were identified with matrix assisted laser desorption/ionization-time or flight-mass spectrometry (MALDI-TOF-MS) and nano-LC MS/MS. Western blot analysis confirmed that the protein expression level of apolipoprotein A-IV precursor and a-1-B-glycoprotein was increased in follicular fluid from PCOS patients than those from normal controls. This data may provide valuable information for understanding the underlying mechanisms and use as biomarkers for PCOS.

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A Simple and Robust Two-dimensional Micro-column Liquid Chromatography Platform for Proteome Analysis

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For proteome research, a comprehensive analysis platform is independent. Although 2D-PAGE is one of the most commonly used method in many proteomics laboratories, it still suffers from some unavoidable drawbacks, pushing researchers to develop alternative approaches.

In our recent study, a simple and robust two-dimensional micro column high performance liquid chromatography (2D-microHPLC) platform was established by utilizing one autosampler and one HPLC system. For the first dimensional separation, ion exchange chromatography was employed with an SCX trap column. The stepwise gradient elution of different salt concentration was performed via an autosampler, and the eluents were collected by a C8-trap column through a two-position ten-port valve. After desalting, each fraction was separated by RPLC with a C18 column under linear gradient, and further identified by MS. To evaluate this platform, the digests of five proteins were analyzed, and quite good reproducibility was obtained. Furthermore, such a platform was applied into the analysis of excreted proteins from lung cancer tissue.
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ITRAQ-Quantification as an Analytical Tool to Describe Proteome Changes in Rat Liver Peroxisomes after Bezafibrate Treatment

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Subcellular organelles represent attractive targets for proteome analysis because they represent discrete functional units, which make them suitable targets for the enrichment and identification of low abundant proteins in a functional protein network. However, organelar proteomes rapidly change in accordance to extra- and intracellular stimuli, making it necessary to compare different organelle samples, e.g. before and after drug treatment, to understand their physiological role in their cellular environment. In this context we analyzed isolated rat liver peroxisomes, fulfilling important reactions in lipid metabolism, after treatment with the hypolipidemic drug bezafibrate, inducing both β-oxidation and peroxisome proliferation. To quantify these changes on the proteome level, subfractions of matrix, peripheral and integral membrane proteins were independently digested with trypsin, labelled with iTRAQ reagent and analyzed by Maldi-TOF/TOF mass spectrometry.

As results, we detected at first distinct changes in pathways of fatty acid breakdown. However, the enzyme content was modulated in a highly specific fashion, inducing classical enzymes for β-oxidation of straight chain fatty acids, but down regulating enzymes for the oxidation of branched chain fatty acids. Comparably, the detected proteins involved in peroxisome biogenesis showed differential regulation, as well. Peroxins with potential involvement in the process of peroxisomal membrane biogenesis tended to be up regulated in response to the treatment, whereas peroxins for the import of matrix proteins stayed more or less equal.

Moreover, the MS-analysis revealed several new potential peroxisomal proteins, from which seven could be verified by targeting experiments using myc-tagged protein constructs. Interestingly, in two cases overexpression experiments changed the morphologic appearance of peroxisomes, leading to a potential role in the process of peroxisome biogenesis or maintenance. Further, proteins previously described as localized to other compartments were detected in significant amounts. Their peroxisomal localization was verified by targeting experiments using tagged constructs as well.

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A Comprehensive Proteomic Analysis of Dauer and Cholesterol Signaling Pathway in Caenorhabditis elegans

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With the availability of its complete genome sequence and unique biological features relevant to human disease, C. elegans has become an invaluable model organism for the studies of proteomics, leading to the elucidation of nematode gene function. We have focused on two key metabolic regulators of C. elegans: dauer-inducing pheromone (daumone: Jeong et al., 2005, Nature, 433, 541–545) and cholesterol (Choi et al, 2003, MCB, 2, 1086–95). First, using various proteomic tools including 2D-LC-MS/MS and DIGE, we analyzed proteomic profiles upon treatment of daumone, which signals C. elegans to enter the dauer stage, an enduring and non-ageing stage of the nematode life cycle. We found that there are multi-level changes in protein expression in the area of energy metabolism (decreased) and oxidative defense system (increased), suggesting that differential changes in various metabolic pathways appear to accommodate this non-aged state. This proteomic data was also paralleled with DNA microarray studies, which provided a window for direct comparison of proteomic and genomic profiles in dauer state. Second, we have launched a global C. elegans proteome project (CEPP) in order to analyze a comprehensive map of proteins involved in cholesterol signaling pathway in embryonic stage of C. elegans. In this talk, we will discuss a few lessons as well as some progress obtained from the pilot phase of CEPP with respect to data management and functional annotation in C. elegans (Supported by grants from BioGreen21 Project [to YKP] and KOSEF [YHS]).

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High Sensitive Detection Methods for Phosphorylated Peptides Analysis

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Protein phosphorylation, which controls basic cellular processes, is one of the most important ways of regulating protein function in cells. However, the determination of phosphopeptides and the identification of phosphorylation sites are still difficult tasks, because of the low abundance of phosphorylated peptides and the poor ionization capacity under common MS conditions. Usually, the selective isolation or enrichment before the analysis is indispensable for phosphopeptide analysis.

To improve the detection sensitivity of phosphorylated peptides, in our recent work, the selective concentration of phosphorylated peptides was performed by using a short microcolumn packed with titanium dioxide (TiO₂), which was an alternative to IMAC prior to LC-MS analysis. To obtain good results, experimental conditions for the analysis of standard peptides and their corresponding phosphorylated ones were optimized, including the pH values of loading buffer and washing buffer. Under the optimal conditions, with GTPyG as the testing sample, the recovery on the TiO₂ column was found to be 65.9%, and the enrichment capacity was proven to be more than 70 nmol. Furthermore, the digest of β-casein was analyzed, and the mono-phosphorylated sequences were successfully identified using LC-MS/MS followed by database searching. However, for multi-phosphorylated sequence, the detection sensitivity was rather poor. Therefore, in our study, the alkaline phosphatase treatment was applied, resulting in obvious improvement on the signal to noise ratio and MS/MS spectra identification capacity.
Quantitative Proteomic Analysis Based on Multidimensional Chromatographic Separation of Intact Proteins and Mass Spectrometric Identifications

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Quantitative proteomic analysis is important for proteome studies. Although two-dimensional gel electrophoresis (2DE) provides quantitative information for proteins by silver stain, it is limited by its dynamic range. Also, stable isotopic labeling like ICAT, etc., can provide quantitative identification. However, it only gives a relative quantitative information for comparison purpose. It is necessarily to develop an approach to knowing absolute expression level of proteins for concentration quantification, disease diagnosis, and variety of other uses. In this study, multidimensional chromatographic separations are carried out based on systematic selectivity optimizations. Size exclusion chromatography, SEC, is employed as first dimensional fractionation. Ion exchange chromatography and reverse phase liquid chromatography, IEC-RPLC, are use as the second and the third dimensions. UV absorbance and laser induced fluorescence are used as quantitative detections. Thousands of proteins with a concentration difference in 5–7 orders span are quantified. In addition, new technologies for fast on-line protein digestion and selective concentration of peptides are developed and applied to proteins identification after quantitative analysis. Based on intact protein separations, a top-down strategy using peptide mapping of fingerprint, PMF, and MS/MS peptide mapping are further proved to be an effective method for protein identification, especially for those proteins with post-translational modifications. This approach has been applied to the proteomic analysis of the liver tissue samples.

The Ontology Lookup Service, a Centralized Point of Access for Interactive and Programmatic Controlled Vocabulary Queries

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High-throughput proteomic analysis methods are generating data volumes that are difficult to systematize due to their size and complexity. For information and reproducibility, the inclusion of sample and method metadata is required but such information is heterogeneous in nature and can be referred to in multiple ways. The solution is provided through the use of ontologies because they provide a standard, controlled vocabulary. Unfortunately, few tools are available to facilitate their use. When tools exist, they tend to be limited to a specific ontology and make it difficult to extract the information in a consistent fashion.

The Ontology Lookup Service (OLS) was developed as a requirement of the PRoteomics IDEntification (PRIDE) database to provide it with a powerful, centralized interface to query the ontologies used to annotate data submissions.

The OLS provides an integrated entry point for all ontologies published by the Open Biomedical Ontology (OBO) project and the Proteomics Standards Initiative (PSI). Currently available ontologies cover model organism anatomy and development, phenotypes and diseases, experimental procedures and methods, chemical compounds, gene function, molecular interactions, post-translational modifications and many others.

The OLS can be used interactively (http://www.ebi.ac.uk/ontologylookup) to obtain information on a single term using a rich web browsing interface that provides auto-completion of queries from over 135,000 indexed ontology terms. In addition, an ontology viewer is available to browse a complete ontology or subsets of it.

A public programmatic interface is also available to query the OLS web service using SOAP. Clients that connect to the web service can be used to harness the power of the OLS and quickly integrate its functionality in any application developed with a modern programming language (such as Java, Perl, C++ or VB). OLS source code and clients are available online under the open source Apache License.
Nano-HPLC-MS/MS with Double Splitting Injection System for the Analysis of Proteins

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Nano-scale HPLC coupled with tandem MS is currently one of the most versatile and popular techniques in proteome research. To obtain constant and stable flow-rate, single splitting system was utilized in most cases, resulting in either sample waste or serious gradient delay. In our study, a nano-HPLC-MS/MS system with double splitting injection system was established for protein analysis. The novel injection system could not only assure large volume injection, but also avoid the gradient delay of the mobile phase, which might result in the excess dead volume between the splitter and the separation column. Comparison between the performance of the developed system and other commonly use ones was carried out. The experimental result demonstrated that by the former way the detection limit of proteins could be lowered to sub-pg/μL with several μL injection volume. In addition, over 10 min’s gradient delay of the mobile phase resulted from the washing volume before separation could be avoided. To evaluate such a platform, excreted proteins from human lung cancer tissue with the total protein concentration of 700 ng/μL was digested, and 2 μL of the protein digests was analyzed by a length of 18 cm reversed phase column packed with 3 μm C18 silica beads, followed by MS/MS detection and database searching. In our experiments, over 70 proteins were identified, and the distribution of molecular weight of the identified proteins was analyzed and compared with that obtained from SDS-PAGE.

Fast Protein Digestion by Monolithic Enzymatic Microreactor and Analysis by Double Splitting Nano-HPLC-MS/MS System

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With the acceleration of complete genome discovery, the challenge of proteomics is to identify and characterize proteins encoded by the genomes as efficiently and rapidly as possible. One of the most important processes in protein identification accomplished with MS is the proteolytic digestion of proteins. The traditional digestion was performed in solution for at least several hours, because, to avoid the autodigestion of trypsin, the digestion is generally performed with low concentration enzyme. To solve these problems, in our recent work, a monolithic enzymatic microreactor was prepared in a fused-silica capillary by in situ polymerization of acrylamide (AA), N-acryloxysuccinimide (NAS) and ethylene dimethacrylate (EDMA) in the presence of a binary porogenic mixture of dodecanol and cyclohexanol, which could offer very low back pressure, enabling the fast digestion of proteins. Bovine serum albumin was digested, and 2 μL of the protein digests was analyzed by a length of 18 cm reversed phase column packed with 3 μm C18 silica beads, followed by MS/MS detection and database searching. In our experiments, over 70 proteins were identified, and the distribution of molecular weight of the identified proteins was analyzed and compared with that obtained from SDS-PAGE.

Functional Analysis of a Histone Deacetylase-like Protein of Thermus caldophilus GK24 using a Proteomics Approach

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Histone deacetylase (HDAC) is one of the key enzymes for down-regulating gene expression via chromatin modification in eukaryotes. However, the function of the prokaryotic counterpart is not fully understood yet. Previously, we cloned HDAC-like protein in Thermus caldophilus GK24 (THDAC) from a genomic library of the microorganism based on homology analysis with human HDAC1. The purified THDAC showed a strong HDAC activity and was sensitive to mammalian HDAC inhibitors, such as SAHA. To investigate the effect of HDAC inhibitors on the phenotype of T. caldophilus, SAHA was directly administered to the culture media of the microorganism. As a result, an elongated morphology of T. caldophilus was observed after treatment with SAHA implying that a systemic change occurred after the inhibition of HDAC in the microorganism. To identify target proteins of THDAC which may cause the major phenotype changes in T. caldophilus, we conducted 2D-gel electrophoresis using protein extracts from SAHA-treated T. caldophilus. Some of chaperon proteins and energy metabolic enzymes were significantly up- or down-regulated by the inhibition of THDAC activity. These results suggest that THDAC in T. caldophilus may play a role in the protein folding and the energy metabolism of the microorganism.
Dysfunctional Kinase-driven Signaling Pathways in Cystic Fibrosis Lung Epithelial Cells


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Background: Cystic fibrosis is a proinflammatory disease characterized by high levels of cytokines in the airway. The mechanism by which CFTR mutations have proinflammatory consequences is unknown. It is possible that dysfunctional signaling pathways play an important mechanistic role in CF pathophysiology.

Hypothesis: We have hypothesized that the proinflammatory phenotype of CF cells may be driven by dysfunctional phosphorylation.

Methods: CF epithelial IB3–1 and CFTR-repaired cells were pulsed with [32P]-orthophosphate. Phosphoproteins were purified using IMAC and immunoaffinity chromatography and separated by 2D gel electrophoresis. Proteins were visualized using silver staining and phosphorimaging and differentially phosphorylated proteins were determined using the Progenesis software package and MALDI-TOF mass spectrometry.

Results: Many proteins associated with the CFTR trafficking defect were aberrantly phosphorylated in CF cells including HSP96 (P = 0.0005), proteasomal activator subunit 2 (P = 0.001) and proteasomal subunit β (P = 0.0006). Many proteins associated with the cytoskeleton were also differentially phosphorylated in IB3–1 cells. These included cytokeratin 8 (KRT8; P = 0.06), myosin regulatory light chain (MRLC1; P = 0.0002), actin capping protein CapZ (CapZ-β; P = 0.02), translationally controlled tumor protein (TCTP; P = 0.00001), phosphogluconolactonase (6PGL; P = 0.002) and nuclear transport factor 2 (NTF2; P = 0.0033). Many of these proteins may interact with CFTR through known binding sites or partners. NTF2 has been shown to provide a link between the actin cytoskeleton and nuclear transport.

Conclusions: Hyper-phosphorylation of HSP96 may be crucially important for understanding the CF disease phenotype. HSP96 is an endoplasmic reticulum-resident chaperone which interacts with nascent or aberrantly folded proteins. HSP96 also interacts with the TLR signaling pathways, thereby playing a role in innate immunity. Dysfunctional cytoskeleton-mediated signaling between the plasma membrane and the nucleus may also be linked by NTF2, leading to the characteristic proinflammatory state of the CF lung.

High Capacity Enrichment Columns and Novel Packing Material with Increased Peak Capacity on HPLC-Chip/MS to Improve Identification in Proteomic Samples

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HPLC-Chip/MS is a novel technology that integrates sample enrichment, analyte separation and the nanospray emitter on a single μ-fluidic polymeric chip device. Short connecting channels and the absence of connections and fittings provide significantly reduced pre- and post-column peak dispersion. This, results in increased resolution and sensitivity and leads to a higher number of identified proteins and peptides in complex samples.

Severe limitations in liquid-based proteome analyses are resolving power and dynamic range. Two approaches are presented how to decrease those obstacles.

Increasing loading capacity of the chip enrichment column in combination with a long analytical separation column resulted in an increased number of identified proteins and the detection of additional low abundant proteins in highly complex samples. In addition the enrichment column was more tolerant to sample mass overloading exhibiting a much lesser degree of chromatographic performance loss compared to the usage of small enrichment columns.

In a second approach we evaluated different column packing materials including a newly developed material in order to increase total peak capacity. Under identical gradient conditions the new material was superior to the other materials showing a wider elution window and exhibiting smaller peak width.

Both, high capacity enrichment columns and packing material with new selectivity were investigated using complex standards and real samples such as E. coli extracts and serum.
Total 4829 proteins were identified from the two cohorts. Label-free analysis based on peptide spectral counts offers a fast and sensitive approach to quantitative level.

Furthermore, we combined the Yin-Yang-MDLC methods with the SILAC reactive isobaric tagging reagent (iTRAQ) was used. MS/MS experiments, where two cell lines were compared, an amino-reactive isobaric tagging reagent (ITRAQ) was used.

It triggers signal transduction, followed by gene regulation and protein expression. Therefore, the protein expression and protein phosphorylation appear to be the most important targets analyzed by current proteomics. Our work showed the effective enrichment and identification of phophopeptides using pH-based strong cation exchange (SAX) combined with reverse phase LC-MS/MS. Furthermore, we designed an strategy to obtain the total information of peptides, using a so-called Yin-yang MDLC-MS/MS. The protein digests were first loaded on strong cation exchange columns, in which most of the relatively basic peptides were captured. The flow-through part was collected and further loaded on SAX column which can bind more acidic peptides and phosphopeptides. Both columns were eluted by offline pH steps or online linear pH gradient, followed by reversed phase LC-MS/MS. The most complete protein identification including the phosphorylation was achieved by the Yin-yang MDLC-MS/MS. Furthermore, we combined the Yin-Yang-MDLC methods with the SILAC quantitation assay, to quantify the protein and protein phosphorylation, in 3T3L1 cell with and without insulin stimulation. More than 1000 phosphorylated sites were identified by this method including many novel sites. The protein and protein phosphorylation dynamics were elucidated on quantitative level.
Characterization of the Sialiome Using Titanium Dioxide and Mass Spectrometry; Identification of Sialic Acid Containing Glycopeptides

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Abnormal glycosylation can lead to protein malfunction, resulting in severe diseases e.g., cancer. Sialic acids are found in the reducing end of glycans attached to proteins on the surface of cells or in body fluids. They are participating in a large number of biological and pathological phenomena including cell-cell communication, epitope recognition, migration and cancer metastasis. The amount of Sialic acids is known to differ in sera from patients with various diseases, and therefore sialic acids containing peptides could be potential biomarkers. Here we describe a new technique which uses the high affinity of the sialic acids towards titanium dioxide in a non-HILIC manner to selectively purify the sialic acids containing peptides after proteolytic processing of glycoproteins e.g., from plasma.

The result shows that the sialic acid containing glycopeptides are specifically retained under special buffer conditions whereas neutral glycopeptides are not retained. This retention was found not to be due to the HILIC properties of the TiO₂ material but is most likely a result of a multidentate binding between the sialic acid in itself or in combination with closely spaced OH groups and the TiO₂ surface.

The optimized method was applied to more complex samples e.g., tryptic digestion of proteins from depleted plasma. The purified sialic acid containing glycopeptides were treated with PNGase F in the presence of O¹⁸ buffer and the resulting peptides were analyzed by LC-MSMS. A total of 200 glycosylation sites in 105 proteins have been found in depleted plasma from control patients. In combination with O¹⁸ labeling during trypsin digestion we have used the presented strategy to identify and quantify a large number of sialic acid containing glycopeptides in plasma from patients with Insulin dependent Diabetes mellitus and Bladder cancer.

Isolation of Plasma Microparticles from Patients with Systemic Lupus Erythematosus for Protein Marker Discovery

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Searching for blood-borne biomarkers in systemic lupus erythematosus (SLE) we have investigated the microparticle fraction of blood in patients with SLE and in healthy controls. SLE is an autoimmune disease characterised by the presence of autoantibodies and impaired clearance of apoptotic cells. Microparticles are membranous fragments smaller than 1 micrometer, which primarily originate from cells undergoing apoptosis or activation in diseased tissues. Thus, microparticles are likely to contain proteins that carry information about tissue origin and disease pathology; and the microparticle fraction of blood in patients with SLE is therefore suspected to differentiate from the microparticle fraction in healthy controls.

Microparticles were isolated by ultracentrifugation (250 000 g, 1 hr) of clarified and 10X diluted plasma (centrifuged 3 500 g, 10 min, to remove any remaining platelets and cell debris). The pellet including microparticles was washed twice in PBS by resuspension and repeated ultracentrifugation to wash out soluble plasma proteins. The proteome of the isolated microparticles was visualised by SDS-PAGE and 2-D gel electrophoresis and showed changed patterns relative to the 2-D gel electrophoresis pattern obtained from clarified (microparticle-depleted) plasma. Differences between the 2-D gel electrophoresis pattern from SLE microparticles and microparticles from healthy controls are currently under investigation and will be examined by image analysis and mass spectrometry.

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Normal Phase LC-ESI MS Analyses for N-Glycans at the Femtomole Level
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Comprehensive analyses of glycans are important for understanding protein functions. We have reported methods for the separation of complex mixtures of neutral and sialylated pyridylamino (PA-) oligosaccharides based upon differences in the net carbohydrate content (size) and the number of sialic acid moieties (charge), using an HPLC column with amine-bearing resin (NP-HPLC) (Kondo et al. Anal. Biochem. 219:21, 1994, Nakano et al. Glycobiology 14: 431, 2004). Moreover, we have also established a hyphenated technique of NP-HPLC and ESI MS and determined the structure of N-glycans in haptoglobin purified from sera of patients with pancreatic cancer and normal volunteers. We found that the α1-3/α1-4/α1-6 fucosylation on N-glycans of haptoglobin was increased in patients with pancreatic cancer. These findings suggest that fucosylated haptoglobin could serve as a novel marker for this cancer (Okuyama et al. Int. J. Cancer 118:2803, 2006).

In this presentation, we tried to develop a method for higher sensitive analysis using NP-HPLC-ESI MS technique in order to achieve high throughput analysis of N-glycans with a limited amount of samples. Since protein(s) may have multiple N-glycans with diverse structures (micro-heterogeneity), this is a major technical breakthrough. In this study, we established a method using micro-flow HPLC (LC: Agilent 1100 Series LC, column: Asahipak 0.3 × 100 mm, Shodex) with MS (HCT, Bruker-Daltonics). This method is rapid and simple because it requires only one injection of a sample and is useful for analyses at the femtomole level of complex mixtures of glycans. (Supported by the 21st Century COE program).

Rapid Protein Identification Using Monolithic Column HPLC Combined with High Resolution/High Mass Accuracy Linear Ion Trap Orbitrap Mass Spectrometry
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Identification of complex digest mixtures is best done by high mass accuracy/high resolution mass spectrometry combined with extensive HPLC separation. The last step of a proteomics experiment is most often C18 reversed phase chromatography coupled to a mass spectrometer. An MS system with high mass accuracy/high resolution capabilities will enable high protein identification rates while minimizing false positive results. Since time is a factor in comprehensive studies, it is optimal to perform fast HPLC-MS/MS experiments.

Polymeric monolithic stationary phases offer an alternative to the classical micro particulate sorbents, bringing important advantages to sample analysis. In contrast to traditional stationary phases that consist of packed particles, the monolithic separation medium is a continuous, rigid, polymeric rod with a porous structure. The lack of intraparticular void volume improves mass transfer and separation efficiency, which allows for very fast separations of biopolymers. The peak width within those LC runs are in the range of 3–6 s at half height, necessitating fast scanning mass-spectrometers.

In this work we show that we can reduce total sample analysis cycle time from 60 minutes to 20 minutes without making compromises with respect to the number of identified peptides/proteins. An LTQ Orbitrap MS system was used in ”parallel” operation mode, providing one survey scan with high mass accuracy at R = 60000 and, simultaneously, 6 data dependent LTQ-MS/MS fragment spectra. We show examples from artificial protein digest mixtures and from highly complex native peptides mixtures from body fluids.
Photo-initiated Entrapping of Functionalized Microspheres for Nanospray Applications

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Microfluidic/capillary analytical devices coupled with mass spectrometry are possible solutions to the current analysis bottleneck associated with proteomic analysis. We have examined the coupling of capillary and microfluidic devices to mass spectrometry using a microsphere based nanospray emitter. Following packing the microspheres against a porous frit within the device or capillary, the fluidic channels are filled with a polymerization mixture composed of monomer cross linker, initiator and porogen. The device or capillary is then photo masked and illuminated to initiate polymerization which leads to micro-spheres that are linked at specific bead to bead to capillary contact points. Once the retaining frit is removed the exposed spheres provide a pore network, formed by the interstitial spaces between spheres, at the exit aperture of the capillary or microfluidic device. The spheres/pores function to divide the flow and perform in a similar manner to conventional tapered nanospray emitter at flow rates > 300 nL/min., however contribute to the formation of a “Taylor mist” at low flow rates (10–200 nL/min). In addition we will demonstrate that the microsphere based emitter provides an increased robustness to clogging compared to conventional tapered capillary emitters. The entrapped microsphere material can also be used for sample preparation. The photo initiated webbing is “ideal” from a chromatographic standpoint since the beads are covalently attached preventing movement, and the majority of the surface is left to interact with the surrounding fluid. We will show the effects of monomer, microsphere surface chemistry, and porogenic solvent composition on the entrapped microsphere material. We have currently developed webbing conditions to entrap ODS chromatographic particles and have utilized them to facilitate on-line desalting and solid phase extraction of protein and peptide samples prior to MS analysis.

Hydrophobicity Measurement via Optimal Retention Time Alignment

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Many long-term proteomics efforts have amassed large databases containing retention time information for identified peptides. Initial studies have shown that the variation in retention times maintains a linear correlation between experimental runs, preserving information about the relative hydrophobicities of the observed peptides.

In the present study, we describe a method for the alignment of retention times, and demonstrate its use in a data set consisting of ~100,000 peptide identifications of ~6,000 unique peptides, each identified multiple times in ~3,000 experimental LC-MS/MS runs. This method is optimal in the sense that every peptide identification is used to determine how each LC run aligns onto the reference scale. The method is statistical in nature and thus automatically accounts for random errors in the retention time measurements. It estimates the relative hydrophobicities of the each peptide in the data set and provides ranges of uncertainty for the estimated hydrophobicities. To assess the quality of the hydrophobicities, correlation coefficients for hydrophobicity versus measured retention times are calculated for every LC run. Over 90% of experimental runs have correlation coefficients above 0.90, indicating that the hypohophobicities are a high quality summary of the information in the data set. Exact mass determination by FTICR or Orbitrap MS may then be used to confirm the tandem MS assignments via retention times and also confirm predicted elution times for specific sequences.

Protein Profile of Bovine Longissimus Dorsi Muscle

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The purpose of this study was to construct protein profile of bovine longissimus dorsi and to evaluate biomarker proteins based on estimated carcass value to improve meat quality. In order to achieve this purpose, we applied a proteomic approach to investigate protein composition and to establish a 2-DE reference map for Hanwoo longissimus dorsi muscles proteins. We initiated the construction of a protein reference map of Hanwoo longissimus dorsi muscles. A total of 150 protein spots were separated by 2-DE, and 102 protein spots representing 75 proteins were characterized with mass spectrometry. The largest portion of these proteins was involved in metabolism (44%). The rest proteins were consisted of cell structure (12.8%), contractile apparatus (20.5%) and others (27.5%).

Based on bovine protein map, proteins associated with high carcass value were screened in comparison with that of low carcass value. Six proteins were nominated as candidate biomarkers, which were expressed significantly different depending on carcass value. Taken together, these results would be contributed to improve meat quality and will be served as biomarkers to improve cattle breeding.
The Testicular Stem Cell Proteome Pilot Project; Focus on the Automated MS Data Processing Pipeline

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Spermatogenesis is a complex process involving cell proliferation, differentiation and apoptosis. The control systems underlying this process still remain to be elucidated. Our project aims at the defining and deciphering of normal testicular proteome with the objectives to conduct an exhaustive and systematic analysis of testicular interactions at the molecular level. A priority was given to spermatogonia since, as a key cell in spermatogenesis, the latter probably contains an entire arsenal of proteins common to other pluripotent stem cells in the body, but is also likely to contain specific proteins, responsible for conferring on this cell its functional identity as the stem cell involved in spermatogenesis.

To technically comply with our objectives, we focused on the necessary automatization for collection, storage and automatic processing of mass spectrometry data, and peptide/protein validation. Several optimisation steps for automatic MS and MS/MS data acquisition on an Ultraflex MALdi-TOF/TOF instrument were evaluated and we have chosen to use the proven ProteinScape software for data collection processing and storage. To enhance the results, Mascot and Phenyx search engines, installed onto a Linux cluster, were used for generating the protein list and different approaches were elaborated to find the best search strategies, with little or no human intervention.

Our work has lead to the identification of numerous spermatogonia biomarkers and we are currently gaining new insights on the role of these proteins in testicular physiology and pathophysiology. The present project should contribute to a better knowledge on testicular stem cell behaviour and allow to explain certain cases of infertility as well as the aetiology of some testicular cancers. The use of ProteinScape appears quite valuable for technically sustaining this long term project.

Nuclear Proteome Dynamics in Susceptibility to Cancer Development

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A DNA double strand break (DSB) is the type of DNA damage that most severely affects the genomic integrity of the cell. If not repaired efficiently, this results in genomic instability and eventually the development of cancer. Risk factors for head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol intake. However, the large majority of smokers and alcohol drinkers does not develop HNSCC, since the capacity to deal with the induced DNA damage plays an important role besides the exposure to these exogenous agents. A good biomarker of susceptibility to multiple cancer types, including HNSCC, is the hypersensitivity to chromatid breaks after exposure to bleomycin or gamma-irradiation. However, the exact pathways and involved proteins that could explain this hypersensitive phenotype are not known.

Therefore, human lymphoblastoid cell lines were challenged with bleomycin, which induces DNA DSBs. The proteomic response was analyzed in time using 2D DiGE, allowing simultaneous analysis of 56 samples. According to the general response to bleomycin, we established that about 60 protein levels showed a statistically significant change, most of which occurred rapidly after bleomycin challenge (1). On the other hand, the analysis of the hypersensitive phenotype versus control revealed about 20 proteins of interest. Many of the identified proteins could be assigned to known DNA DSB response processes, such as sensing DSBs, DNA repair through effectors, or cell cycle arrest at the G2/M phase checkpoint. Strikingly, and not reported previously, the nuclear levels of the INHAT complex proteins were affected, suggesting that this complex plays a role in changing the chromatin structure allowing the DNA repair enzymes to gain access to the DNA lesions.

Reference
Glycoprofiling of the Human Salivary Proteome

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Glycosylation is important for a number of biological processes and is the most common and most complicated post-translational modification found on proteins. Therefore it is not surprising that changes in the glycoform population have been linked to many diseases. By understanding the glycosylation profile in both healthy and diseased individuals, we can detect markers that can be used in diagnosing and monitoring the progression of disease. Saliva provides an easy, painless reservoir for detecting potential biomarkers of disease. Our group is generating a complete catalogue of the salivary proteome and its associated glycosylation. This work combines two-dimensional gel electrophoresis, lectin blotting, and mass spectrometry to map the salivary glycome. Lectins are a class of proteins that recognize a wide variety of specific oligosaccharide motifs. A panel of 15 lectins from seven sugar-specific categories (fucose, galactose, glucose, mannose, N-acetyl-galactosamine, N-acetyl-glucosamine, and sialic acid) was used to determine the type and extent of glycosylation from two healthy male individuals. Lectin blots were compared to 2D gels stained either with Sypro Ruby (protein stain) or Pro-Q Emerald 488 (glycoprotein stain). The proteins from 166 spots on the Sypro Ruby stained gel were identified by MS and then catalogued with their reactivity to the lectin panel. Each lectin shows a distinct pattern even if they belong to the same sugar-specific category. This is not surprising since lectins not only recognize a particular oligosaccharide but also its specific linkage. In addition, the glycosylation profiles generated from the lectin blots show that most of the salivary proteins are glycosylated and that the pattern is more widespread than is demonstrated by the glycoprotein stained gel. To look for potential targets for diagnostic tools and therapeutic treatments, future work will compare the salivary glycosylation profile of healthy individuals versus individuals with a large number of decayed teeth.

Large-Scale Proteomics Experiments in Context

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A major challenge in the field of proteomics is extracting knowledge from the enormous amount of data generated. While the infrastructure to store and exchange experimental data is slowly emerging proteomics is still lacking bioinformatics tools and resources to enable the integrated analysis of proteomics experiments. Typically such experiments result in long lists of candidate proteins which need to be put into a biological context to facilitate interpretation of the results. However, most publicly available resources do not offer set based analyses of different aspects of a protein list required for a straightforward interpretation of large-scale experiments. Instead information has to be gathered from different disperse data sources, integrated and analysed using a variety of different tools. This is a time-consuming process complicated further by constantly changing sequence and annotation resources, in particular when time-separated experiments are to be compared. As part of the HUPO Brain Proteome Project (BPP) pilot study we have set up a framework to integrate and analyse information from different, mainly protein centric data sources. A list of anonymous protein sequences is put into a broader biological context by providing an overview on different aspects of the protein set such as genomic context, protein sequence features, protein families and domains, functional categories, biological pathways, tissue expression, protein-protein interactions and disease association. Application of the annotation pipeline is demonstrated by the analysis of proteins identified during the HUPO BPP pilot study.
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Improving Proteome Analysis Using Cleavable Surfactants

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This study describes a new reagent used to prepare hydrophobic samples for analysis by mass spectrometry. PPS Silent™ Surfactant is a cleavable detergent that was developed to improve the sensitivity of MALDI analysis of hydrophobic proteins. Recently, application of this reagent has been extended to include analysis of complex protein mixtures using multidimensional LC/MS/MS. The advantages of using such a reagent will be highlighted, and the reagent will be compared to similar reagents, including RapiGest™ SF (Waters Corp., Milford, MA) and Invitrosol™ (Invitrogen, Carlsbad, CA).

Complex mixtures of proteins derived from a variety of sample types including cells grown in culture and tissue extracts were analyzed using PPS Silent™ Surfactant. Cell extractions are performed using 0.2% PPS in buffer (pH 7.8). Proteins were reduced and alkylated prior to digestion using trypsin. Prior to mass spectrometry analysis, HCl is used to cleave the detergent. Tryptic peptides were analyzed using nano- LC/MS/MS with an ion trap mass spectrometer. Peptides and proteins identified were compared to evaluate reagent performance. All experiments were repeated in replicate.

The analysis of E. coli cell extracts using PPS Silent™ Surfactant showed the following performance characteristics. Using PPS, 571 proteins were identified from the analysis. When the results obtained from RapiGest to those obtained using PPS, it was found that an increase in 30% was observed. (571 proteins identified compared to 428 with RapiGest) However, it is noted that 355 proteins were observed using both reagents while 216 proteins were found only in the PPS sample and 73 proteins were found only in the RapiGest sample. This result demonstrates that, although overall performance differs between the two reagents, they can have complementary uses in proteomics applications.

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ProteomicsPortal.org; Building a Gateway to Proteomics Resources

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Organizing resources in the web is a fairly difficult task. First, the URI that represents the resource is often unstable; it can change over time for either technical or social reasons. Second, most resources, such as software, databases, protocols etc., are, in essence, not the information resources (IR) as defined in [1]. Hence, using an HTML document to represent a non-IR resource will easily lead to the identity crisis [2] that will hamper the automated resource discovery in semantic web. Here, we described the design and implementation of a portal site http://proteomicsportal.org that is aimed to solve the above issues. ProteomicsPortal.org provides each registered resource a persistent URI. Dereferencing the URI will lead to either an HTML document or an RDF document depending on how HTTP content is negotiated. Such a dual response model is aimed to cater the need of both human and machine so that the portal site is not only useful in the current web but also capable of embracing the full potential of the semantic web. Furthermore, ProteomicsPortal.org also supports open discussion on the registered resources so that the site can serve not only as a central registry but also as a public forum for proteomics resources.

References

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Alternative Chaperone Machinery May Compensate Calreticulin/Calnexin Deficiency in Caenorhabditis elegans

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Proper folding and maintenance of the native structure is central to the protein function, is assisted by a family of proteins called chaperones. Calreticulin and calnexin are endoplasmic reticulum resident chaperones well-conserved from worm to human. Calreticulin/calnexin knock-out mice exhibit severe phenotype; whereas in Caenorhabditis elegans, calreticulin [crt-1(jh101)] and calnexin [cnx-1(nr2009)] null mutant worms exhibit only mild phenotype, suggesting the possible existence of alternative chaperone machinery which can compensate the deficiency of calreticulin and/or calnexin. In order to rapidly identify the compensatory chaperone components involved in this process, we analyzed the proteome of crt-1(jh101) mutants and crt-1(jh101);cnx-1(nr2009) double mutants. When worms were grown at 20°C, we found that five proteins were up-regulated and two proteins were down-regulated in crt-1(jh101) mutant; nine proteins were up-regulated and five proteins were down-regulated in crt-1(jh101);cnx-1(nr2009) double mutant. In addition, elevation of the cultivation temperature to 25°C, which is still permissive to growth but causes specific defects in mutants led to identify several additional proteins. Interestingly, consistent increment of heat shock protein-70 family members (hsp70) together with protein disulfide isomerase (PDI) at all the examined conditions suggest the possible compensatory function imparted by hsp70 and PDI family members in the absence of calreticulin and/or calnexin.