1.1 Proteomics as a Genomic Science

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Proteomics attempts to analyze the protein complement of the genome comprehensively, and it can therefore be considered a discipline of the genomic sciences. Proteomics is challenging, because proteomes change quantitatively and qualitatively to reflect changes in the physiological state of cells, organs and organisms. As a consequence of post-transcriptional events (splicing) and post-translational changes (modifications, proteolytic processing, complex formation and others), the number of proteins that comprise a proteome is vastly larger than the number of genes that constitute a genome. It is therefore not surprising that the proteome of any species remains to be completely described.

Most current MS based proteomic strategies in every experiment chart the proteome anew, i.e. they do not take advantage of information collected in prior experiments from the same or related proteomes and are therefore very inefficient. There exist only partial descriptions of the proteome. In this presentation we will describe the development of a suite of computational tools that support the integration of prior data into the analysis of present experiments and the integration of statistically validated data into relational databases. We will also discuss the consequences of these tools for current proteomics technologies and for the catalysis of new experimental approaches for high throughput quantitative proteomics. Finally we will discuss new insights gained from large, integrated proteomic datasets for the annotation of genomes and proteomes.

References
1. PeptidAtlas, Desiere et al., Genome Biology 2005

1.2 Towards Standardised Capturing of Proteomics Data and Proteomics Repositories

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The completion of many genomes has shifted the attention from deciphering the sequences to the identification and characterization of the encoded components on our way towards a better understanding of cellular processes. The identification and functional annotation of the proteomes starts with the identification of genes and transcripts as a prerequisite of proteome annotation. Ab initio gene predictions are very powerful in predicting most of the exons in a genome, but reliable gene structure predictions of both known and novel genes are dependent on existing transcription and protein information. An enormous amount of data already exists on the function of many proteins but this is scattered over many resources. Public domain databases are required to manage and collate this information and present it to the user community in both a human and machine readable manner. Standardised capturing of Proteomics data is an essential step towards Proteomics repositories useful for proteome annotation. This talk will give an overview about the development of Standards for reporting Proteomics data and Proteomics repositories, as well as the relationship of these new resources with the already existing biomedical database landscape.

1.3 Nanobiotechnology in Proteomics

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We developed a novel method for proteome and glycoproteome analysis based on the nano-biodevice. Nano-device developed for an integration of a chip for pretreatment of cell and a microchannel array electrophoresis chip enables to pre-treat cell as well as to separate many biological samples (12–96 samples) involving complex mixture of proteins (>20 proteins, 10 to 200 kDa) at a single run only within 15 seconds(s) [1, 2]. Although microchannel electrophoresis relies almost exclusively on electrokinetic force and some loss of resolution is unavoidable, our developed method employing hydrodynamic force has enabled the drastic improvement of separation time without compromising the resolution. Utilizing this technique, 12 samples of complex protein mixture extracted from human T cell line, lymphoblastic Jurkat cells, were separated within 15 s at a single run by using 12-lane integrated microchip, with high reproducibility. The nano-biodevice will be applicable to fast-analysis of sugar chain on the glycoproteins [2]. We developed high-sensitive LED induced fluorescence detection system for nano-biodevice and applied to analysis of fluorescent labeled sugar chain. Complex mixture of sugar chains digested from glycoproteins (AGP and IgG) is separated and detected within 60 s with high-resolution. Coupling of quantum dot and siRNA is applied to the functional analysis for bcr-abl tyrosine kinase in chronic myelogenous leukemia (CML) [4–6]. We found quantum dot anti-CD conjugates is the powerful tool for the diagnosis of cancer in the early state and will be the potential photosensitizer in photodynamic therapy of cancer [4]. Nanobiotechnology will offer the enormous advantages for high throughput proteomic analysis and proteomic medicine for cancer research.

References
1.4 From Sequences to Knowledge, the Role of the Swiss-Prot Component of UniProt

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Swiss-Prot [1] is a protein sequence and knowledge database that is valued for its high quality annotation, the usage of standardised nomenclature, direct links to specialised databases and minimal redundancy. Its development started almost 20 years ago in 1986 and, since 2003, it is the cornerstone of the Universal Protein Resource (UniProt) [2]. Representing the complexity of the protein world is a challenge that drives the evolution of Swiss-Prot. Thanks to technologies from the field of proteomics it is now possible to extend our knowledge of proteins in directions not addressed by the massive flood of genomic and transcriptomic data. In this respect, the correct representation of post-translational modifications is crucial to the understanding of the variability of proteins and of the modulation of their functions. We will describe how we attempt to capture a wealth of information relevant to proteins in Swiss-Prot in the context of a specific annotation program, the Human Proteomics Initiative (HPI). The goal of HPI is to annotate with a very high level of quality and in-depth information, knowledge on human proteins and their mammalian orthologs.

References

1.5 Deciphering Membrane Proteomes in the Model Plant Arabidopsis thaliana


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Plant proteomics has become a complementary technology for functional genomics. In particular, sub-proteomes dedicated to plant cell organelles (mitochondria, chloroplasts, vacuoles . . . ) led to relevant identification of proteins associated with a cell compartment and enhanced our understanding of plant organelle functions. The characteristics of Arabidopsis thaliana, including availability of whole genome sequence and genomic resources, have made this small weed a model organism for studies of the cellular and molecular biology of flowering plants. We have set up a general strategy for membrane proteomics and we analyzed the proteomes of different Arabidopsis membrane systems, namely the chloroplast envelope membranes (Ferro et al., Mol. Cell Proteomics 2003, 2, 325–345) the plasma membrane (Marmagne et al., Mol. Cell Proteomics 2004, 3, 675–691) and membranes from mitochondria (Brugière et al., Phytochemistry 2004, 65, 1693–1707). The interest to combine several complementary extraction procedures to take into account specific features of membrane proteins will be discussed in the light of our recent proteomics data (Ephritikhine et al., Plant Physiol. Biochem. 2004, 42, 943–962). These examples also illustrate how proteomics can feed bioinformatics for a better definition of prediction tools and give valuable information for biological investigations. In particular, membrane proteomics brings new insights over plant membrane systems, on the membrane compartment where proteins are working and on their putative cellular functions.
1.6 Proteomics of the Early Secretory Pathway

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The CAP principle of Brenner is a challenge to high throughput proteomics. Meeting the criteria of this principle which stands for a Comprehensive Accurate and Permanent representation of proteomics data has been attempted in the Cell Map Project. As an illustrative example, we have analyzed organelles representing the early secretory pathway of rat liver parenchyma. Using a quantitative approach to account for the assignment of tandem mass spectra has resulted in the characterization of over 4000 proteins representing 5 different compartments, the discovery of over 200 "novel" proteins linked to the early secretory pathway as well as testing the limits of a key hypothesis (the maturation hypothesis) to explain biosynthetic cargo transport through the early secretory pathway.

1.7 Proteomic Approach to Define Multivariate Protein Markers in Lung Diseases

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Diffuse interstitial lung diseases (DLD) are a heterogeneous group of diseases with a different pathogenesis but a common evolution towards pulmonary fibrosis of various degree of severity. Due to its multifactorial character DLD can be studied much better with a global analysis and specifically at the protein level with a proteomic approach. Proteomics is now widely accepted as the complementary technology for genetic profiling. It is really a powerful approach for the study of diseases and to identify and characterize multivariate protein markers that can characterize specific pathological states or drug effects much better than the analysis of individual or small numbers of proteins.

BronchoAlveolar Lavage Fluid (BALF) is presently the most common way of sampling the components of the epithelial lining fluid and the most faithful reflect of the protein composition of the pulmonary airways. For this reason we performed a 2D electrophoretic/MS proteomic study on BALF of three different DLD, such as sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis in order to provide a more comprehensive picture of alveolar events and to define specific multivariate protein markers to be used for a most sensitive and specific diagnosis and prognosis. The results obtained enabled us to identify the main profiles of the three different diseases and the multivariate protein markers between them. Interestingly the three diseases showed statistically different levels of antioxidant proteins and distinct patterns of oxidized proteins that were extensively studied.

1.8 Function Prediction at the Protein, Proteome, and Community Level

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Biological function comes in many flavors and works at different levels, ranging from residues in proteins via cell types to species communities. I will introduce in function prediction for individual proteins, proteomes from many organisms and even for large collections of proteins from complex environments such as water and soil. So far, function prediction has been mostly qualitative but proteins function in space and time. Thus, I will also hint at approaches that aim at temporal and spatial aspects of function.
1.9 Integration of Experimental Data on Gene Expression and Protein-Protein Interaction with Predicted Functional Associations

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Improved understanding of many complex, cellular mechanisms, such as the eukaryotic cell cycle, necessitates the identification of genes and proteins that belong to broad “systemic” categories, where the diversity in terms of molecular functionality is high. This also holds true for most cellular compartments, for example the nucleus or the mitochondrion. Similarly, proteins that are transported, for example secreted, share properties no matter whether they are translocated by the classical secretory pathway, or by alternative, non-classical pathways.

For this kind of functional classification, we have constructed a new approach where putative posttranslational modifications, sorting signals, predicted structural features, and calculated features such as chain length, amino acid composition, isoelectric point, hydrophobicity are integrated and used to infer the functional class, which may be broad, like “cell cycle regulated,” “secreted,” “nuclear,” or “stress response,” but also narrow as for the conventional Gene Ontology categories, like ligand-gated ion channel etc.

The approach predicts functional role categories in the “feature” space of the proteome, rather than using the “sequence” space of the genome. One remarkable result from the work is that many proteins seem to display conservation in feature space rather than in sequence space, and the method will therefore be able to transfer functional information from one species to another in new ways.

This type of prediction can be integrated with experimental data, such as gene expression data and protein-protein interaction data, and interaction networks can be extracted and characterized. The talk will focus on a protein feature analysis of cell cycle regulated proteins in yeast and man, in addition to a compartment-specific analysis of the human nucleolus.

References


1.10 Proteome Analysis of Butyrate-treated HCT-116 Colorectal Cancer Cells

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Colorectal cancer is among the most common cancers in many developed countries. Studies have shown that environmental factors, especially dietary habits, contribute to colorectal cancer. Butyrate, a fermentation product of fibre, is involved in the association of fibre-rich diet and low colorectal cancer risk. To better understand the molecular mechanism of butyrate’s chemo-preventive role in this disease, we undertook a functional proteomics approach to identify candidate proteins regulated by butyrate in HCT-116 cells. Expression proteomics of butyrate-treated HCT-116 whole cell extracts was initially conducted using pH 3–10 IPG in 2-D DIGE. 20 protein spots were detected to be differentially expressed by more than 2-fold ratio, of which only 6 were identified by MALDI-TOF MS. To extend the proteome coverage, heparin affinity chromatography was used as a pre-fractionation step. This enrichment procedure coupled with narrow range IPGs (pH 4–7 and pH 6–11) in 2-D DIGE led to the detection of 22 and 24 differentially expressed spots respectively. The identities of 25 protein spots were obtained, of which 5 (all with different ps) were shown to be heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1). Three (with M, ~38kDa) were down-regulated and two (with M, ~26kDa) were up-regulated. Using western blot analysis, we verified that native hnRNP A1 underwent post-translational modification upon butyrate treatment leading to changes in its pI values. In addition, analysis of the subcellular compartment of these cells indicated the presence of a cleaved hnRNP A1 in the cytosolic fraction induced by butyrate, thus suggesting that its nuclear translocation was inhibited upon cleavage. Modulations of hnRNP A1 may play a significant role in the mediation of growth arrest and apoptosis by butyrate.

1.11 Proteomics of the Transplanted Heart

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Heart transplantation has become an effective procedure for treating end-stage heart failure due to improvements in immunosuppressive therapy, with more than 3,000 operations being carried out annually. However, rejection of the grafted organ remains a major challenge. Heart mediated acute rejection is the major risk for survival during the first year, it is generally manageable using current immunosuppressive therapies. However, long-term survival is severely compromised by chronic rejection, with 42% of grafts being affected after 5 years. Current methods for monitoring acute (endomyocardial biopsy) and chronic rejection (angiography or intravascular ultrasound) are highly invasive, very expensive, and are unable to predict disease onset. We have used proteomics to develop minimally-invasive methods to monitor rejection based on the analysis of serum samples. Most serum proteomic studies have been severely compromised by the high dynamic range of the proteins present. To overcome this limitation, we adopted strategies that avoided direct “global” analysis of serum. We have identified several potential biomarkers of both acute and chronic rejection. Some of these have been implemented in ELISA format and have been used to assess their utility as clinical assays.

In spite of the high rate of incidence of chronic rejection (manifested as transplant-associated coronary artery disease; Tx-CAD), there is a small cohort of patients who remain disease-free more than 10 years following transplantation. We have used proteomics to investigate whether these patients express proteins within their hearts that provide protection against the development of Tx-CAD. We demonstrated that vascular expression of a specific diphosphorylated form of the 27kDa heat shock protein (Hsp27) is associated with freedom from vascular disease after cardiac transplantation. Understanding the mechanism of this protective effect may provide new opportunities for therapeutic intervention.
1.12
Clues to Composition and Conformation of Peptides and Proteins from Their Adduction Behavior

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The extent of adduction to peptides and proteins of molecules such as water, alcohols, and amino acids, as well as various anions and cations, can provide information and insight on the structure, composition and conformation of both the parent species and the resulting complexes. Thus, in some early experiments of this type we discovered that what we had thought were singly charged ions of a small peptide were in fact doubly charged dimers of that peptide. In a subsequent study we found that the gas phase hydration behaviour of a mixed dimer or two peptides could provide insight on the conformation of that dimer. This report summarizes some more recent results of such adduction studies.

1.13
Miniaturization Technology and Mass Spectrometry Coupling

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Instrument miniaturization and microfabrication is an important trend in the development of analytical technology. Besides size reduction the microfabrication allows creation of integrated fluidic systems with zero dead volume channel junctions, difficult to achieve by traditional tubes and connectors. The smaller system volumes also lead to lower reagent and sample consumption and faster analysis times. Once optimized, the microfabrication provides means for inexpensive and reproducible large scale production of the microdevices. Typical applications involving chemical sensors, DNA and protein arrays or microchannel separations have recently been complemented by coupling with mass spectrometry. It can be anticipated that the importance of the microfluidic systems suitable for direct coupling with mass spectrometry will grow especially in genomics, proteomics and drug development applications. In this respect the microfluidic devices can be viewed as a part of the sample preparation system. Microfluidics designed for both MALDI and/or electrospray is currently under development. An important part of the design for coupling with ESI/MS is the electrospray interface, which facilitates stable and efficient sample ionization and transport of the analytes into the mass spectrometer. While the common preparation of the electrospray emitters from glass or fused silica capillaries is quite straightforward, the microfabrication of fine electrospray tips seems to be more demanding. This is mainly true for the case where the electrospray interface is an integral part of a more complex microfluidic system for sample preparation and/or separation. This presentation will review the theoretical and practical aspects of the different approaches to the microfluidics/ESI/MS coupling.

1.14
A Mammalian Organelle Map by Protein Correlation Profiling

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Localization of proteins to membrane-enclosed organelles is a central feature of cellular organization. Using protein correlation profiling to track the abundance of thousands of peptides through centrifugation gradients, we mapped 1502 proteins to subcellular locations in mouse liver. Ten major clusters emerged, which corresponded to well-characterized cellular compartments based on a combination of enzymatic assays, marker protein profiles and confocal microscopy. Protein correlation profiles validated genuine organelar components and enabled us to assess the specificity of previously published organelar proteomic inventories. Remarkably, 41% of all organelar proteins were found in more than one location. Integration of the organelar proteomic data with atlases of RNA abundance and genome sequence enabled us to identify networks of co-expressed proteins, cis-regulatory motifs, and putative transcriptional regulators involved in organelle biogenesis. Our analysis ties classical biochemistry, cell biology and genomics into a common framework for the analysis of organelles.

1.15
2D Electrophoresis for Proteome Analysis

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Despite being less than perfect, and in face of emerging complementary technologies (e.g., MudPIT, stable isotope labelling, protein arrays) that have emerged, two-dimensional gel electrophoresis with immobilized pH gradients (IPG-Dalt) is actually the workhorse for most ongoing proteome projects.

With the introduction of O’Farrels 2D electrophoresis, based on carrier-ampholyte generated pH gradients in the first dimension, for the separation of total cell lysates in presence of detergents and chaotropes in 1975, a decade of most ambitious 2D projects (e.g., Andersons “Human Protein Index”) followed. However, only with the advent of immobilized pH gradients for 2D electrophoresis (Görg et al., Electrophoresis 1988 & 2000), highly sensitive mass spectrometry for spot identification in routine, and proteomics databases/tools on the World Wide Web, parallel quantitative expression profiling of large sets of complex protein mixtures for proteome analysis became daily practise. No matter what new technique will come up, 2D electrophoresis technology has never been as good as today with respect to reproducibility, resolution (ΔpI = 0.001) by using narrow overlapping IPGs over 24 cm separation distance, coverage of pH range from 2.5 to 12 and quantitation of differentially expressed proteins by using DIGE technology. In addition, by combining sample prefractionation techniques with narrow IPGs, even low abundance proteins - not amenable in routine by any technique up to now - can be detected. As long as a breakthrough of new technologies to measure up the proteomic approach with the speed/automation of genome analysis is still await, the workhorse of proteomics - the 2D/MS technology - is still indispensable for parallel quantitative protein expression profiling.
1.16
The EU Interaction Proteome Project
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Interaction Proteome is a large Integrated Project funded by the European Commission within Framework 6. The consortium comprises 13 partners in 6 European countries and began its work in 2004. The aim of Interaction Proteome is to establish Europe as an international leader in functional proteomics, the analysis of protein-protein interactions. Major objectives include the development of a broadly applicable platform of routine methods for the analysis of protein-interaction networks. A multi-disciplinary approach is laid out to address different aspects of the generation of protein-interaction data; their validation by cell biological, biochemical and biophysical methods; their collection in a new type of public data base; and their exploitation and use for in silico simulations of protein-interaction networks. The project will be introduced using recent insights into the function of molecular chaperones in protein folding gained from the proteomic analysis of their protein substrates.

1.17
Plan and Progress of Human Liver Proteome Project
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HLP has set up seven initiatives so far. HLPP was its second initiative, but the first for a specific organ of human beings. Its main scientific aim of HLPP is to generate an integrative approach leading to several comprehensive atlas of the human liver including two profiles (proteome expression profile and protein modification profile) and two maps (cellular localization map and protein linkage map).

The progress of HLPP includes the evaluation of technologies, bioinformatics set-up, sampling and preparation, preview with human fetal liver and the primary analysis of French liver tissues. By using the standard proteins, we evaluate the false positive rate, false negative rate, concentration scale and the sensitivity of the selected technologies routes. My-WorkSpace project management information system has been developed to collect detailed experimental information, proteins/peptides identification quality control and local data management. We also use the liver tissue of the C57 mouse to find the optimum method of subcellular preparation. As the previews of HLPP, systematic identification and characterization of human fetal liver (HFL) proteome was performed, including protein expression profile, phosphorylated protein profile (Phosphoproteome), protein interaction linkage (Interactome), comparison between HFL proteome and its transcriptome and novel protein mining. The second step of HLPP is the systematic analysis of the protein expression profile of normal liver sample from France. The undergoing step of HLPP is systematic identification and characterization of normal liver sample from China, including the protein expression profile, transcriptome, protein interaction and protein post translation modification analysis.

1.18
Quantitative Proteomics by Metabolic Stable Isotope Labeling and Mass Spectrometry
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One of the major aims of proteomics is to provide quantitative data on differential protein expression levels. Recently, mass spectrometry-based methods have been introduced that can provide quantitative data on differential protein expression, mostly using stable isotope labeling (1). We opted for metabolic labeling as this provides efficient means to quantify differential protein expression, and has the advantage that all proteins are labeled universally (2).

In a first application we investigated the effect of carbon and nitrogen limitation on Saccharomyces cerevisiae. Yeast was metabolically labeled in well-controlled chemostat cultures. 14N and 15N labeled proteins were separated using 1D gel electrophoresis followed by RP-LC-ESI-MS on a LC-Q. Relative quantification was performed by using Relex software (3). We quantified 759 proteins, using on average 8 peptide peak pairs per protein. This analysis revealed that 419 proteins showed a significant increase/decrease in expression level. The functional annotation of these proteins revealed that the yeast cells change expression levels of enzymes involved in metabolism of the growth-limiting compound. The protein expression ratios were compared with corresponding transcript levels. Moreover, we compared the accuracy of quantification by stable isotope labeling with that achieved by DIGE analysis of the same yeast samples (4).

Additionally, we labeled Drosophila M. using metabolically labeled yeast as food-source (5), and analyzed at the proteome level the early embryonic development of the fruitfly. Here, we used nanoflow RP-LC-ESI-MS on a LTQ-FT-ICR MS. There was an added benefit of the FT-ICR approach in improvement of confidence scores for identification, sensitivity in detection, and increase of number of peptide pairs that were used for quantification. Relative protein expression quantification was performed using MSQuant software that we modified to enable the quantification of 15N labeled proteins. References

Towards a Comprehensive Understanding of Bacterial Physiology by Proteomics

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The genome sequence represents the “blue-print of life” only, functional genomics approaches are necessary to bring this genome sequence to life. Because of their low complexity Bacteria (Bacillus subtilis, Staphylococcus aureus) are useful model systems to show how the “virtual life of the genes can be transferred to the real life of the proteins,” the players of life.

In the first part the “vegetative proteome” of growing cells will be presented that is a useful tool for analysing the regulation of entire metabolic pathways. In the second part the gene expression pattern of non-growing cells will be analysed which is organised in a complex network of overlapping regulation groups (stimulons, regulons, modulons). Proteomics in combination with transcriptomics is the “state of the art” to assign the single genes to the various regulons active in stressed or starved non-growing cells and thereby to define the structure and function of already known and even unknown regulons. Growth and developmental processes such as the transition of growing cells into non-growing cells caused by stress or starvation can be followed at a proteomic scale in kinetic studies. Furthermore, proteomic signatures for stress and starvation stimuli can be used to predict the physiological state of cells grown in natural ecosystems or in a bioreactor. The proteomic signatures offer various industrial applications from fermentation monitoring up to drug research.

In the second part of the talk it will be demonstrated how the proteomic approach can be used for analyzing global control of protein stability, protein secretion, posttranslational protein modifications or protein damage.

Finally, first results on gel free proteomics will be compared to gel-based proteomics data. The combination of both techniques is the only way towards the description of the entire proteome of bacteria.

3D Structure of Human Proteins; from Narrow Focus to Large-scale Data Gathering and Back

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Structural genomics (structural proteomics) is concerned with the 3D structure determination of all proteins encoded by the genomes of key organisms. To achieve this ambitious goal, structural genomics initiatives are challenged to develop high-throughput innovations for all preparative and analytic steps of NMR or crystal structure analysis of proteins. The Protein Structure Factory, a Berlin-area structural genomics consortium, is focussed on the structure analysis of human proteins (Heinemann et al., Acc. Chem. Res. 2003, 36, 157–163). In order to meet its research goals, the Protein Structure Factory has implemented protocols and facilities for all aspects of protein structure analysis, including a robotic station for protein crystallization and beamlines for protein crystallography at the Berlin synchrotron BESSY. Recently, the efforts of the Protein Structure Factory have been refocussed on a smaller number of well defined biological targets such as the human TRAPP tethering complex (Turnbull et al., EMBO J. 2005, 24, 875–884). The high-throughput innovations made in the earlier phase prove extremely useful for this more narrowly focussed approach as well.

MALDI Mass Spectrometry in Proteomics; Past, Present, and Future

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Over the last ten to fifteen years mass spectrometry has evolved as the central analytical technique in protein analysis. It spans the whole range from primary sequence through higher order structures to posttranslational modifications. This development is a result of the introduction of the soft ionization techniques of ESI and MALDI. For both proteomics is by far the major field of application.

The majority of basic features and procedures of MALDI-MS have been established early on. Most of the commonly used matrices were found early on, as have the key features mass range, sensitivity and ion suppression in mixture analysis. Sample preparation and heterogeneity are still the main difficulties in a routine application of MALDI-MS. More recently a number of different procedures for a “matrix-less” laser desorption with porous silicon (DIOS), carbon nanoparticles and –tubes and other structured surfaces have been developed. So far none of them seems to quite match the MALDI performance even in the mass range of peptide mapping.

Much attention has been paid to combining different prefractionation techniques with MALDI and great advances have been achieved with on- and offline coupling of electrophoretic or chromatographic systems with MALDI analyzers. Instrumentation for MALDI-MS has seen a strong development over the years and is still going on. While axial extraction time-of-flight analyzers are still the most commonly used analyzers, other types such as orthogonal extraction time-of-flight instruments, ion traps and Fourier Transform spectrometers are quickly catching up and may be the main players in the future. Atmospheric pressure MALDI sources are also an interesting addition to the available tool kit. Considerable progress has also been made towards high throughput and automation.

Proteomics and Clinical Chemistry

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Proteomics delivers if general clinical chemistry principles and rules are fulfilled. Clinical proteomics could be considered as a subspecialty of clinical chemistry or biochemistry. From pre-analytical aspects to results interpretation, each step should be well defined and planned. Accuracy and precision of each measurement should be known. Uncertainty should be quantified. LIMS (laboratory information management systems) should provide the necessary tools to store, to display and to compute the large volume of data generated in the proteomics workflow and should link the results to anonymized patient record. Clinical example will be shown to highlight clinical proteomic applications and development, to demonstrate how clinical chemistry principles apply and how new bioinformatic applications and software will contribute.
Proteomics of Breast Cancer for Signal Pathway Profiling and Target Discovery

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Proteomics is starting to lead to the identification of new markers and potential targets in oncology, but the intrinsic cellular and molecular complexity of cancer remains the major difficulty to descriptive and functional explorations. Differential proteomics of cancer biopsies versus normal breast tissues has several limitations, due to both the cellular diversity of breast tumors and the limited size of the biopsies and we found appropriate to start from tumor cell lines and primary cultures to primarily select potential markers that can subsequently be validated by studying breast tumor biopsies. A global analysis of normal versus cancer cells, using various technological approaches like 2DE or SDS-PAGE-LC/MS-MS, indicated a limited number of proteome changes, confirming the fact that cancer cells are molecularly similar to their normal counterparts. Several changes in individual proteins that we observed in vitro were confirmed in tumor biopsies. This is for example the case of the molecular chaperone 14-3-3 and the serine protease inhibitor Maspin that both appeared to be down-regulated in breast cancer. A major advantage of working primarily with cell cultures is that it allows physiological experimentation such as growth factor or hormone stimulation that can uncover new markers and targets. The Nerve growth factor (NGF) was previously found as a stimulator of breast cancer cell survival and proliferation and using LC-MS/MS we were able to detect its autocrine production in breast cancer cells. Interestingly, NGF is produced in high amount in cancer cells whereas normal breast epithelial cells do not express this growth factor. This differential expression was found both in cell cultures and in tumor biopsies and therefore, NGF appears to be a marker of breast cancer with potential clinical interest. Proteomics based deciphering of NGF signaling led to the identification of specific targets downstream to NGF receptors (p140TrkA and p75NTR), such as the tumor necrosis factor-associated domain protein TRADD that is essential to p75NTR signaling or the IKK signaling necessary to the activation of the Akt/NF-kB pathway. Pharmacological inhibition and RNA interference-based modulation of NGF signaling resulted in a strong inhibition of breast cancer cell growth and a correlative reduction in tumor volume and metastasis was observed in SCID mice, indicating that targeting this pathway is a rational approach for future therapeutic strategies. Together, these data show that NGF and its signaling are of potential clinical interest in breast cancer and that proteomics, when used in combination with other approaches, can be the driving force of a descriptive and functional exploration of cancer. Whereas proteomics will rapidly translate into practical applications is still a matter of debate, but there is no doubt that significant outcomes have already emerged toward the development of new potential strategies for detection and treatment of breast tumors.

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Quantification of Post-Translational Modifications in Proteomics by Mass Spectrometry

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The regulatory role of a variety of post-translational modifications of proteins and the significance of the functional interplay between modifications is now only beginning to be realized and worldwide much research is being directed towards this goal. However, progress in this field of research relies on availability of sensitive and specific analytical techniques to monitor and establish the nature of modifications on proteins and the dynamic changes they undergo as a function of time and environment, in health and disease. A completely new set of analytical strategies and technologies have to be developed and applied in biological and biomedical studies to reveal multisite protein modification and explain their functional role. We are using the concept of “modification-specific” proteomics to investigate protein phosphorylation, glycosylation, acylation and other types of post-translational modifications. We have recently reported on a quantitative phosphoproteomic study of the yeast pheromone signaling pathway, where more than 700 phosphopeptides were identified, among which 131 could be quantified. This study demonstrated that it is feasible to monitor cell signaling processes as a response to environmental factors, such as hormones or growth conditions.

We have also acquired experience in determination of acetylation and methylation sites in core histones by mass spectrometry. Tandem mass spectrometry studies using LC-ESI-MS/MS and MALDI MS/MS have shown that acetylation and trimethylation of lysine residues (same nominal mass) can be distinguished by observation of diagnostic reporter ion signals (immonium ions and neutral loss ions) and by accurate determination of peptide masses. Similarly, mono-, di- and trimethylation of lysines and dimethylation of arginine has been detected and characterized.

The concept of “modification-specific proteomics” will be presented as well several applications to biological systems.

References
1.25
Proteomics of Plant Responses to Biotic Stresses; Progress, Problems, and Challenges

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Application of proteomics to plant biology research has, so far, been very limited when compared with other systems, mainly humans and yeast. No more than 260 plant proteomic papers have been published since 1995, with an high percentage (62 out of the 260) being reviews, indicative of its enormous potential and challenges better than achievements. Published papers mainly deal with the model plant systems Arabidopsis thaliana or Medicago truncatula, and with the major crop plants rice, maize, wheat and tobacco. The studies carried out focus on different aspects of the plant biology, with some of them, in number of 23, covering, in more or less extent, plant responses to biotic stresses (virus, bacteria, fungi and parasitic plants). Proteomics will complement genomics and classical biochemical studies, providing additional data that will help in the understanding of the molecular bases of the host plant-pathogen/parasite interaction and that of the resistance and will allow the identification of key resistance proteins (pathogenesis-related, PRs) that can be used as markers in breeding programmes or to obtain designed crops by genetic engineering. Defense proteins are constitutively present in specific plant organs, parts or subcellular fractions such as the seeds, xylem sap, cell wall or apoplast. However, most of them are induced in response to attack, and its study is based on comparative proteomic approaches using susceptible and resistant genotypes, mutants and transgenic plants, mainly by using the 2-DE technological platform. Other proteomic areas applied to the study of plant responses to biotic stresses is that of the post-translational modifications, mainly phosphorylation and redox modification, very important when deciphering the complex network signalling conducting to the local or systemic defense gene activation. Resistance or susceptibility is the final result of a continuous interplay between host plant cells and aggressor organism that can be analyzed and understood at the molecular level by using a interactomic approach. All the mentioned aspects (pathosystemes, objectives, technological platforms) will be covered in detail during the presentation, differentiating between what has been done or published and emphasizing on conceptual or technical errors, problems, and future challenges.

1.26
Regulation of the Raf Signalling Pathway by Protein Interactions

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The Ras-Raf-MEK-ERK/MAPK pathway regulates fundamental biological processes including cell proliferation, malignant transformation, differentiation, survival, motility and invasion. While the basic biochemistry of this pathway is well worked out, it is still enigmatic how this pathway can deliver these diverse biological results and how it is coordinated with other pathways. To answer these questions we use a combined approach of classical biochemistry and molecular biology together with mathematical modelling, microscopical imaging and proteomics. Our proteomics work focuses on the identification and characterisation of the components of multiprotein signalling complexes within the Ras-Raf-MEK-ERK/MAPK pathway. A main approach towards the functional analysis is the comparison of signalling complexes isolated under different cellular conditions (e.g., mitogen or proapoptotic stimulation, stress etc). Such comparisons can pinpoint dynamic protein associations that are functionally involved in the specific cellular response. This approach has led to the description of a new Raf-1 effector pathway that controls apoptosis via MST2, and new regulators of Raf-1 kinase activity. These results will be discussed.

1.27
Functional and Chemical Proteomics for Drug Discovery

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Current proteomics research of Cellzome is focused on a number of functional and chemical proteomics projects. The goal of the functional proteomics projects is to identify novel protein targets in therapeutically relevant human disease pathways. As an example, we have used tandem affinity purification and mass spectrometry (TAP-MS) to map the protein interaction network underlying the Tumor Necrosis Factor alpha (TNFa) signalling pathway. The cytokine TNFs triggers a key pro-inflammatory signaling pathway, which forms the basis for numerous physiological and pathological processes (Bouwmeester et al. Nature Cell Biology 6, 97–105 2004). Several protein interactors were found to modulate the pro-inflammatory response and therefore represent novel opportunities for therapeutic intervention in diseases, like rheumatoid arthritis and psoriasis, where inflammatory processes play a major role.

Chemical proteomics projects center around the elucidation of the mechanism of action of bioactive small molecules and drugs as well as their molecular selectivity against particular groups of protein targets. For example, we have screened an extended set of novel and known kinase inhibitors against the proteome and have in this way determined their respective target profiles, leading to novel target-lead combinations with defined selectivity profiles that represent drug discovery opportunities. Alongside chemical lead optimization, we are using chemical proteomics to profile leads against the proteome to confirm their mode-of-action and to exclude potential off-target liabilities.
1.28

Cell Fate Decisions Revealed by Quantitative Phosphoproteomics

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How does the cell integrate multiple signals as well as its own state to “decide” to proliferate, differentiate or go into apoptosis? Many scientists are trying to answer these questions and they use diverse strategies such as in-depth biological as well as large-scale “omic” approaches and mathematical model building. Here we report on our work combining quantitative phosphoproteomic and chemical approaches, which we believe offer a uniquely direct way to address cell fate decisions.

First the Stable Isotope Labeling in Cell culture (SILAC) technology will be introduced as a way to accurately quantify proteins between two or three cellular states. Using this system post-translationally modified peptides, such as phosphopeptides can also be analyzed in a quantitative fashion. This will be illustrated using growth factor signaling as an example. By comparing phosphoproteomes at different time points after EGF stimulation, kinetic curves of activation covering whole signaling cascades can be obtained. Such data will be indispensable for modeling such cascades.

We used mesenchymal stem cells as models for cellular differentiation. These adult stem cells have already been applied clinically for bone regeneration and we investigated the role of growth factors in differentiation of these cells into bone forming cells. EGF but not PDGF, a closely related growth factor, stimulated the differentiation (Kratchmarova, et al., Science, June 3rd 2005 issue). Using quantitative phosphoproteomics to compare the entire tyrosine phosphoproteome of EGF and PDGF stimulated cells, we found a critical control point activated by one but not the other growth factor. Chemical inhibition of the corresponding pathway conferred full differentiation ability on PDGF. These studies show that cell fate decisions can be taken far upstream in a signaling cascade and that quantitative proteomics is a promising tool to elucidate these decisions.

1.29

Progress in Proteome Profiling of Hepatocellular Carcinoma Tissues from Patients Infected with Hepatitis C Virus and Its Possible Clinical Application

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Chronic infection with hepatitis C virus (HCV) is known to be a risk factor for liver cirrhosis, steatosis and hepatocellular carcinoma (HCC). To understand molecular mechanisms of hepatocarcinogenesis and to find biomarkers for HCV-HCC we performed proteomic studies of cancerous and non-cancerous tissues from HCC patients with HCV infection by means of two-dimensional gel electrophoresis and tandem mass spectrometry. Transcriptomic studies of the tissues were also carried out for evaluation of data from the proteomic studies. In cancerous tissues, heat shock protein (HSP)-70 family such as GRP78, HSC70, GRP75 and HSP70.1, HSP60, triosephosphate isomerase, phosphoglycerate mutase-1, alpha-enolase, ATP synthetase beta-chain and glutamine synthetase isoforms were increased whereas albumin, ferritin light chain, smoothelin, tropomyosin beta-chain, arginase-1, aldolase-B and ketohexokinase were decreased. It has been reported that HCV core protein in the transgenic mice give mitochondrial and ER stress to enhance lipid peroxidation and to generate HCC. These results suggest that 1) chronic infection of HCV in hepatocytes gives continuous oxidative stress generating reactive oxygen species (ROS), 2) ROS gives DNA damages causing gene mutations for cell dedifferentiation, and 3) the increase of glycolytic enzymes supply ATP for cell proliferation. Further examinations indicated that alpha-enolase was significantly higher in poorly differentiated HCC which was also documented by immunoblot analysis and immunohistochemistry. The expression of alpha-enolase also correlated positively with tumor size and venous invasion. These results suggest that alpha-enolase is one of the candidates for biomarkers of tumor progression of HCV-HCC.
1.30

Activity-based and Affinity-based Approaches Using Stable Isotope Labeling in Drug Discovery

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The most of primary targets of drugs are proteins, therefore protein-compound interaction studies are one of key steps in drug discovery. The identification of drug targets elucidates the mode-of-action of the main effect/side effects of the drug. For identification of specific targets of a drug candidate, target proteins are enriched or isolated by affinity techniques using compound-conjugated matrix, but the affinity and specificity of synthetic small molecules to their protein targets are rather low in many cases. Thus, nonspecific interactions between a synthetic compound and its binding proteins often lead to difficulty in specifying the primary binding partners. Stable isotope labeling strategies have proven particularly advantageous for the discrimination of proteins specifically associated with the target population from non-specifically co-purified contaminants. Now we have expanded this approach to evaluate specificity to multiple compounds versus multiple target proteins.

Studies in protein phosphorylation may hold particular promise for dissecting signaling pathways and molecular classification of diseases. Especially, profiling of a novel kinase-inhibitor is quite important, because the target kinase may have unknown substrates and the inhibitor may influence unpredicted pathways. Therefore there is a great need for methods capable of accurately elucidating sites of phosphorylation. Here, we have developed a phosphoproteomic approach, which does not require any chemical reaction, but can increase the specificity of immobilized metal ion affinity chromatography with simple procedures, that is suitable for both small-scale phosphoprotein analysis and large-scale phosphoproteomics and should be useful in acquiring phosphorylation maps to elucidate signal transduction pathways in a variety of diseases.

1.31

A Successful Thesis?

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I will talk about the start of proteomics. A quarter of century before the word was coined, the only gene expression profiles were of early versus late bacteriophage genes and there were no “omics” –not even genomics. I was trying to start my thesis, not a field. However, I had ambitions of doing molecular biology in eukaryotic systems and felt that a transition from one to two-dimensional gel electrophoresis would provide the resolution needed for meaningful gene expression studies. The key insights were mundane –recognition of a need for solubilizing denaturants and for sensitive detection, and a respect for the chemical fragility of proteins –however, when approached with great technical care, the results were spectacular. I will discuss the introduction of the technique, the rejection of the paper and initial reactions. I will review the first real proteomic experiment, done in 1974 but only published years later in a book chapter*. I determined how many E. coli genes showed altered expression in response to cyclic AMP. In the process, I learned the importance of controlling the genetic background, the need for experimental care, and the power of combining genetics with a global analysis. Many lessons-learned have been lost and unanswered questions brushed aside in the deluge of progress: I will review some that have ripened to maturity. But the big question is –is proteomics a science or a technology? If proteomics is a science, what are its important questions? My thesis committee wants to know.


1.32

Analysis of the Budding Yeast Proteome

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To take advantage of the information provided by genome sequences, in collaboration with Jonathan Weissman’s lab (HHMI, UCSF), we have developed reagents and methods to study the complete complement of proteins from budding yeast. Ultimately, we seek to be able to monitor dynamic changes in the protein abundance, localization, and post-translational modification of the proteome. To enable these studies, we have constructed two collections of ~5000 yeast strains, each expressing a single protein fused to an epitope tag for measurement of protein abundance (the TAP tag), or fused to the green fluorescent protein (GFP) to facilitate studies of protein localization. With the Weissman lab, we completed two studies characterizing protein abundance and subcellular localization in the yeast proteome (Ghaemmaghami et al., Nature 2003; Huh et al., Nature 2003). We are now focused on the following studies: (1) characterizing protein lifetimes; (2) screening the proteome for protein kinase substrates; (3) investigating dynamic changes in protein subcellular localization.

1.33

In Search for the Biomarker Proteins from the Liver and Lung Cancer Specimens

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Our presentation will be divided into two parts: The first will focus on our work-in-progress report on the comprehensive proteome analysis of hepatocellular carcinoma (HCC), including certain functional aspects. Paired liver tumor tissues and plasma samples corresponding to the tissues of several HCC patients were analyzed using various proteomic tools including two-dimensional electrophoresis, 2D-Nano LC-MS/MS, and MALDI-TOF/TOF. For efficient mass spectral analysis, glycoproteins present in plasma were pretreated with PNFase F in order to remove N-linked glycans. Summary results of proteomic analyses will be discussed, with emphasis on the metabolic map of proteins involved in energy production and detoxification pathways. Interestingly, we observed certain discrepancies in the relative expression between some differentially expressed proteins and their tyrosine phosphorylated proteins. In addition, recent development of sample preparation and profiling methods for highly alkaline proteins of HCC or other cells, using a non-2D separation system such as PF2D, will be introduced. The second part of our presentation deals with proteome analysis of lung cancer specimens in which certain signaling protein like 14-3-3 and its isoforms were investigated with respect to their roles for carcinogenesis. To understand a possible function of 14-3-3 isoform, a specific siRNA was designed and transfected into lung cancer cell lines (A549) and examined for their differential protein profiles. We will present some preliminary observation from this experiment in regard to metabolic consequences of carcinogenesis in vitro in lung cancer cells.
**1.34 Myocardial Ischemic Injury and Proteasome Function**

P. Ping

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Cardiac cells conduct their beat to beat function through integration of their cellular networks and pathways. Disruption of these pathways leads to the manifestation of cardiovascular diseases. One of the essential pathways in the cardiac cells that are responsible for regulating protein degradation is formed on the basis of an array of multiprotein complexes, namely, the 20S and the 26S proteasomes. In a series of proteomic based investigations, our research team has characterized these multiprotein complexes with respect to their molecular composition, function, and regulation. In addition, we have begun our query to decode how this cellular pathway contributes to the manifestation of cardiovascular diseases. For instance, in control human sera, 2DE maps stained with micellar Coomassie reveal only 195 spots, but 523 are detected in the same amount of "equalized sera." Similar improvements on spot detection can be appreciated in egg white, in which only six proteins make up some 99% of the total proteome. All these data are confirmed using MS based proteomics: such as SELDI-TOF-MS especially with respect to small sized peptides complementing thus 2DE data. Results will also be presented on the major improvement in species detected in ligand library-treated human urines, cerebrospinal fluid and other tissues. The increased number of species revealed by the described technology allows an ease detection of proteins that are currently difficult to evidence due to their very low abundance. It is felt that this novel method could offer a strong step forward in "mining below the tip of the iceberg" for detecting the "unseen proteome."

**References**


**1.35 Carboxylation of Protein During Oxidative Stress**

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This paper reports studies on carbonylation of proteins in yeast and mammals during environmental stress. Oxidized proteins were isolated by (1) biotinylation of oxidized proteins with biotin hydrazide, (2) affinity selection using monomeric avidin affinity chromatography, and (3) further fractionation by reversed-phase chromatography (RPC). Fractions from RPC were then trypsin digested and the tryptic peptides identified by MS/MS. More than 400 proteins were identified, among them 80% of the ribosomal proteins. Oxidation sites in one fourth of these proteins were identified and found to occur at a particular site on the structural periphery. Multiple amino acids in the same tryptic peptide were often oxidized. Over 10% of the proteins identified appeared in more than one RPC fraction. Based on the position of the peptides identified in the primary structure of a protein parent it was concluded that this occurred by primary structure fragmentation, perhaps of enzymatic or oxidative origin. Finally, peptides from two or more proteins occurred together in more than one RPC fraction with 2% of the oxidized proteins. This data was interpreted to mean that protein cross-linking had occurred. At least a portion of this cross-linking appears to occur through RNA.

**1.36 Equalizer Beads; the Quest for a “Democratic Proteome”**

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In a recent communication [1] novel powerful approaches to decipher proteome compositions have been reviewed. Among them a method capable to “compress” the difference in protein concentration using ligand libraries has been described. When these large ligand libraries are impregnated with complex proteomes (e.g., human sera) of widely differing protein composition, a modification of protein concentration is easily obtained thus greatly enhancing the concentration of the most dilute components [2] while reducing the presence of high abundance species. For instance, in control human sera, 2DE maps stained with micellar Coomassie reveal only 195 spots, but 523 are detected in the same amount of "equalized sera." Similar improvements on spot detection can be appreciated in egg white, in which only six proteins make up some 99% of the total proteome. All these data are confirmed using MS based proteomics: such as SELDI-TOF-MS especially with respect to small sized peptides complementing thus 2DE data. Results will also be presented on the major improvement in species detected in ligand library-treated human urines, cerebrospinal fluid and other tissues. The increased number of species revealed by the described technology allows an ease detection of proteins that are currently difficult to evidence due to their very low abundance. It is felt that this novel method could offer a strong step forward in "mining below the tip of the iceberg" for detecting the "unseen proteome."

**References**


**1.37 Modification-specific Proteomics, a Concept for Subproteomics**

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The complexity of the proteome in terms of dynamic range and number of variants of given gene products due to alternative splicing and co- and post translational modification makes it impossible to cover the complete proteome of a cell or tissue type in a single experiment. To reduce the complexity, many proteomics studies are now performed on isolated organelles. However, the post translational modifications on a given protein may be extremely heterogeneous in terms of site as well as in terms of the modifying group. Since the localization of a protein often depend on its modification state, methods are needed to determine the relationship between the modification state of a protein and its localization.

We have developed a concept, which we term modification specific proteomics that allows specific assignment of a given type of modification in proteomics studies. By combining this with organelle specific proteomics, perspectives to determine the modification state of a protein is needed for its presence in a given localization in the cell. The methods used for modification specific proteomics for studies of and applications to organelle specific proteomics, e.g., membranes, will be described.
1.38
Plasma Proteomics; Challenges and Windows of Opportunities
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Currently, plasma proteomics is in its optimistic phase. However, serious drawbacks such as sensitivity, accuracy, assay robustness or competition with existing multiplex immunoassay platforms delay realization in the routine laboratory. Plasma proteomics has still to identify its diagnostic niches.
Serum is a widely used blood material for laboratory analyses but interindividual variability of coagulation proteases and release of cell constituents often affect quantitative measurements. Moreover, anticoagulant treatment may interfere with the coagulation process in vitro. Sample preparation and storage is a further issue. In particular, the time frame for specimen transport and serum preparation has to be considered. A higher stability of centrifuged or filtered material can be anticipated for plasma. Serum-related problems can definitely be avoided by use of citrate or EDTA plasma. To cope with the transition of basic proteomic to laboratory routine, technical problems have also to be solved. Current proteomic instruments resemble rather advanced prototypes which need careful reengineering to realize robust automated high-throughput instruments for laboratory medicine. In addition, standardization and the availability of calibrator preparations are unresolved. Finally, proteomics has just begun to search for diagnostic niches, such as the analysis of regulatory networks of agonistic and antagonistic peptide hormones and other metabolic peptide regulators.
In the near future, proteome science and bioinformatics will hopefully meet disease-specific questions to open laboratory medicine for proteomics.

1.39
SmartCell; a Cell Network Simulation Program
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SmartCell has been developed to become a general framework for modeling and simulation of reaction-networks in a whole-cell context. The originality of SmartCell lies in the general approach to modeling biological processes, both in how reactions are described and how the geometrical aspects of the system are handled. In this respect, SmartCell supports localization and diffusion by using a mesoscopic stochastic reaction model. In this work we describe the algorithm, and use it to determine the impact of diffusion and localization on the behavior and noise of simple well-defined networks, previously analyzed with differential equations. We found that these factors could have a major impact on the behavior of networks.

1.40
Proteomic Analysis of Colorectal Cancer; Strategies for Novel Biomarker Discovery
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Colorectal cancer (CRC) is a leading cause of cancer death in the Western World. Early detection is the single most important factor influencing outcome of CRC patients. If identified while the disease is still localized, CRC is treatable. To improve outcomes for CRC patients, there is a pressing need to identify biomarkers for the early detection (diagnostic markers), prognosis (prognostic indicators), tumor responses (predictive markers) and disease recurrence (monitoring markers). Despite recent advances in the use of genomic analysis for risk assessment, in the area of biomarker identification, genomic methods have yet to produce reliable candidate markers for CRC. For this reason, attention is now being directed towards protein chemistry or proteomics as an analytical tool for biomarker identification. Here, we discuss various proteomics technologies, especially those relating to definition of plasma membrane proteins, the secretome, peptidomics (or <10KDa polypeptides) with reference to how they may contribute to CRC biomarker discovery.

1.41
Strep-Tag and Anticalins; Molecular Tools for Structural Proteome Research
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The Strep-tag is a nine amino acid peptide (AWRHPQFGG) that specifically binds to streptavidin in a reversible manner such that it can be employed for the efficient purification of corresponding fusion proteins on an affinity column. Elution of the bound recombinant protein is effected under mild buffer conditions in a biochemically active state by competition with biotin or suitable derivatives. The Strep-tag can be easily fused to a recombinant polypeptide during subcloning of its cDNA or gene and it usually does not interfere with protein function, folding, secretion or even crystallisation. The development of the Strep-tag II (NWSHPQFEK) permits fusion to either end of a protein or internal insertion, as a linker between two protein domains. High affinity antibodies and detection reagents are available (http://www.iba-go.com/prottools) for Western blots, ELISAs, and histochemical staining. Hence, the Strep-tag system provides a reliable tool for the parallel isolation and functional analysis of multiple gene products.
Anticalins are a novel class of artificial ligand-binding proteins, which are prepared from members of the lipocalin family via targeted random mutagenesis and phage display selection against prescribed haptons or antigens. Whereas the characteristic β-barrel structure of lipocalins is highly conserved, the loop region at the open end of the barrel is hyper-variable and can be reshaped for molecular recognition. The first anticalins were selected against small ligands, but libraries have recently been constructed to generate anticalins with high affinities and specificities towards protein targets. Anticalins are much smaller than antibodies or their antigen-binding fragments and consist of a single polypeptide chain. Thus, anticalins offer benefits for substituting conventional antibodies in several areas, such as for the detection, immobilization or functional characterization of recombinant proteins in proteome research.
1.42

A Novel Cell Signalling Pathway in Arabidopsis Revealed by Proteomics

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Previous experimentation in our laboratory focussed on identification of protein components of the plant extracellular matrix (Chivasa et al. (2002) Electrophoresis 23, 1754–1765; Ndimba et al. (2003) Proteomics 3, 1047–1059). These initial studies revealed a number of proteins not expected to reside in the extracellular matrix due to their classical function previously thought to be restricted to the intracellular compartments. This raised the possibility of the existence of hitherto undiscovered signalling pathways in plants. In this presentation, recent results from our laboratory will point to a central role of the extracellular matrix in the maintenance of plant cell viability via the coordinated control of highly conserved extracellular metabolites. We also show a novel role of the extracellular matrix in the mediation of fungal toxin-induced death in Arabidopsis thaliana. This constitutes an example of novel function discoveries enabled by the formulation of hypotheses based on organellar proteomic studies.

1.43

Protocol for NMR-based Structural Proteomics

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NMR-based structural proteomics (genomics) requires high-throughput (HTP) structure determination (Nature Struc. Biol. 2000, 7, 982–984). Data for determining high-quality structures of proteins up to ~20 kDa can nowadays be collected within a few days on spectrometers equipped with cryogenic probes, provided that protocols are implemented to avoid sampling limited data acquisition (PNAS 2002, 99, 8009–8014). G-matrix Fourier transform (GFT) NMR spectroscopy (JACS 2003, 125, 1385–1393) effectively solves the associated “NMR sampling problem” (Methods Enzymol. 2005, 394, 78–108) by joint sampling of several indirect dimensions of multidimensional NMR experiments. This leads to the detection of “chemical shift multiplets” in which each multiplet component encodes a defined linear combination of shifts. In order to avoid spectral congestion, G-matrix transformation enables editing of the multiplet components in separate sub-spectra. Since chemical shifts are encoded in a redundant manner, the multidimensional NMR spectral information is obtained with high precision. Employment of GFT NMR can accelerate data collection by factors of ~10–100, and routinely provides precise four, five and even six-dimensional spectral information. Using the GFT NMR approach for complete protein resonance assignment (PNAS 2004, 101, 9642–9647), including Nuclear Overhauser Enhancement Spectroscopy (JACS 2005, 127, in press), allowed us to devise a standardized semi-automated protocol for NMR HTP structure determination (PNAS 2005, 102, in press). The protocol was employed to solve numerous structures of the Northeast Structural Genomics Consortium (http://www.nesg.org) pipeline with molecular masses between 9 and 22 kDa (average ~ 15 kDa). Recently, novel GFT NMR experiments were introduced (JACS 2005, 127, 4554–4555) to (i) pave the way for structural genomics of membrane proteins and (ii) efficiently assign resonances of proteins with extreme shift degeneracy such as (partially) unfolded proteins. The impact of GFT NMR on structural biology and proteomics will be discussed.
Role of Branching N-Glycans and the Functional Glycomics

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Over 50% of proteins undergo glycosylation and functional glycomics is one of the most important strategies for proteomics in order to explore the protein functions.

Our group has been interested in the role of glycosyltransferases involved in the Biosynthesis of N-glycan branching such as GnT-III, V, and Fut 8 and found the several target molecules for the glycosyltransferase genes which play pivotal roles in cancer metastasis, cancer suppression and antibody dependent cellular cytotoxicity activity which is a key role in the antibody therapy against cancer. We developed null mice of Fut 8 gene and the null mice lack core fucose of the glycoproteins as judged by Mass spectrometry and develop the emphysematous changes of the lung. This phenotypic change was found to be due to the constitutive activation of matrix metalloproteinase (MMP) gene(s) due to aberrant glycosylation of a growth factor receptor which may otherwise suppress the MMP gene expression.

The null mice were rescued by the treatment of the growth factor indicating that the development of lung emphysema in the null mice was actually dysregulation of the growth factor signaling as judged by the functional glycomics approach. These data indicate that functional glycomics may open a new insight into the role of glycoproteins in vivo.

A Human Protein Atlas for Normal and Disease Tissue

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Here, we present a new protein atlas database (www.proteinatlas.org) showing the expression and localization of human protein in normal and cancer tissues. The atlas is based on the use of antibodies (1) to generate high-resolution immunohistochemistry images representing 48 normal tissues and 20 different cancer types (2). Each antibody is used to generate more than 500 individual images and each image has been annotated by a pathologist (3). The database has been created by the Swedish Human Proteome Resource (HPR) program funded by the non-profit Knut and Alice Wallenberg Foundation and the program has been set-up to allow the exploration of the human proteome with Antibody-based Proteomics (4). The basic concept is to generate, in a systematic and high-throughput manner (2), specific antibodies to all human proteins, and subsequently used these for functional analysis of the corresponding proteins in a wide range of assay platforms, including (i) a protein atlas for tissue profiles (3), (ii) specific probes to evaluate the functional role of individual proteins, and (iii) affinity reagents for purification of the specific proteins and their associated complexes for structural and biochemical analyses.

References
1.46 Functional Proteomics on Cell Lines and Tissues

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Proteomics technologies are beginning to make a major contribution to pharmaceutical research. High-throughput protein identification is one important aspect, but at the same time only the basis for expanding our knowledge of protein function, which requires in-depth characterization, confirmation and validation. Whereas mapping and identification of proteins can yield valuable insights, in many cases they tell only part of the story. In mammalian systems a large part of signal transduction and regulation of the appropriate response is not based on “true” differential protein expression, but on modification of the existing pool of proteins. Therefore a proteomics analysis of such systems must encompass the identification of differentially expressed proteins and their characterization with respect to functionally important modifications. In addition, evolving from a focused proteomics technology platform to an integrated approach encompassing various functional genomics technologies are pivotal for translating proteomics results into novel biological insight.

1.47 Combined Fractional Diagonal Chromatography (Cofradic); a Multipurpose Tool for Peptide-Centric Proteomics

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We recently introduced COmbined FRActional DIagonal Chromatography (COFRADIC) as a multi-purpose separation tool in peptide-centric proteomics. COFRADIC is based on the principle of diagonal chromatography in which a defined subset of peptides is modified in between two consecutive identical chromatographic separations. Altered peptides will now elute differently from their original position and can be isolated and identified by MS-based techniques.

This method allows specific sorting of peptides carrying a modifiable functional group such as Met-, Cys- or alpha-NH2-groups. The original peptide mixture is now less complex, while still keeping the representative aspect of the proteins present in the starting lysate. Integrated with ion-exchange chromatography, the fractionation power of COFRADIC is dramatically increased leading to up to 70% sample-to-sample coverage with several thousands of proteins identified.

COFRADIC is also able to select peptides carrying post-translational modifications such as phosphorylation, glycosylation, nitration and S-nitrosylation. A similar work flow is also used to search for target proteins that covalently interact with drugs in total cell lysates.

1.48 Toward Error-free Proteomics

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Bottom-up protein identification using tandem mass spectrometry (MS/MS) commonly encounters two difficulties: assessing the reliability of identified data and the efficiency problem. The latter issue reflects the fact that, in usual approaches, a large portion (80–90%) of peptide MS/MS data fails to produce useful database identification. These problems are inter-related, and low efficiency usually means poor validity. We address these issues through hardware and software developments. On a hybrid LC/MS Fourier transform mass spectrometer LTQ FT (Thermo), we utilize together with the traditional Collision-Activated Dissociation (CAD) an orthogonal fragmentation technique Electron Capture Dissociation (ECD). Comparing CAD and ECD data for the same peptide, consensus sequence information can be extracted, which greatly (10–100 times) increases the data validity. Alternatively, keeping the same margin for false positives, one increases 2–3 times the number of identified proteins. We also introduced and evaluated a new scoring principle (S-score) that is based on the maximum length of a peptide sequence tag found in a given MS/MS spectrum. S-score allows filtering out poor-quality MS/MS spectra before database submission and either confirms or rejects poorly matched identifications. As a result, up to 30% of all MS/MS data produce now useful database identification. At a high S-score threshold, the database search becomes binary: any match is 95% reliable, while no match means 95% probability of a mutation, modification or novel protein. The sequence tag derived for S-score can further be used for de novo sequencing. The prospects of reliable high-throughput de novo peptide sequencing as well as mapping posttranslational modifications using these and similar approaches will be presented, and the benefits of orthogonal fragmentation techniques for proteomics will be discussed.