Two novel dual-vector bacterial expression systems, designed for high-throughput generation of antibodies specific for cDNA-encoded proteins, are presented. The vectors allow for two approaches to a concept that involves parallel expression of cDNA-encoded proteins, in two vector systems as fusion with different tags, both enabling single-step affinity purification. Both approaches utilize fusion tags that include a portion with documented immunopotentiating effect to stimulate antibody production, and generated fusion proteins are used to elicit antibodies. The second fusion protein, expressed using one of the two novel vectors, is used in an immobilized form as an affinity ligand to enrich, from the generated sera, antibodies with selective reactivity to the cDNA-encoded part. One of the two vectors is taking advantage of a novel Staphylococcus aureus protein A (SPA)-binding affinity tag, ZSPA-1, enabling straightforward affinity purification and blotting procedures of expressed gene products on IMAC, (ii) generated antibodies showed high specificity for three of the clones from a mouse testis cDNA library were expressed and purified and it was found; (i) that Protein A-based purification was more stringent than IMAC, (ii) generated antibodies showed high specificity for three of the clones in blotting procedures on different cell types and tissue homogenates. We thus conclude that the presented dual-vector method offers a highly stringent strategy for generation of mono-specific polyclonal antibodies.
28.3 Computer-aided Selection of Targets for Antimicrobial Drug Design Based on Comparative Analysis of Genomes
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Continuous growth of genomic databases and the need for new antimicrobials gave rise to the application of cross-genome analysis for the selection of potential targets for antimicrobial drug design.

In accordance with the current concept of such target selection the test of compliance of each gene (protein) of target microorganism to a number of biomedical and technological requirements (importance of target protein for the microorganism survival, necessary spectrum and safety of the drug to be created, possibility of target structure-based design of lead compounds, etc.) should be carried out. This task can be achieved by the comparative analysis of genomes.

We developed an approach for target selection based on comparison of genome sequences by local pair-wise alignment. In this approach the results of alignments are used to estimate the degree of correspondence of genes with requirements listed above. It was implemented in original software GenMesh and used for comparative analysis of Mycobacterium tuberculosis genomes (H37Rv and CDC1551), Mycobacterium leprae and known human protein sets and all proteins with known 3D structure. The correct valuations for targets of currently used drugs against tuberculosis were obtained and 13 new potential targets were found.

28.4 Proteomic Analysis of the Pancreas in TGZ-treated ZDF Rats
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Type 2 diabetes is one of serious metabolic disease accompanied with various type of vascular complications. There have been so many attempts for the development of new therapeutics of type 2 diabetes. However still there is a need for discovering new drug targets for type 2 diabetes. In order to identify the differentially expressed proteins in the pancreas of Zucker Diabetic Fatty (ZDF) rats, type 2 diabetic animal model, after troglitazone (TGZ) treatment, we used the proteomics technique based on two-dimensional electrophoresis and mass spectrometry (MALDI-TOF). After treatment of TGZ for 1 week, the plasma glucose and insulin level were determined. The glucose level was dramatically decreased to normal range after TGZ treatment. However insulin level was not significantly changed after TGZ treatment. We identified differentially expressed proteins in the pancreas of ZDF rats after TGZ treatment. We found 58 differentially expressed known proteins and 8 unknown or putative proteins. Now we are validating the function of identified proteins. We may provide basic information for the understanding of pathogenesis of type 2 diabetes.

28.5 Chronic Nicotine Administration in Rat Induces Proteomic Changes on Polymorphonuclear and Mononuclear Leukocytes
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The aim of this study was to establish if chronic nicotine treatment could induce proteomic changes in peripheral blood leukocytes, providing information about pathways involved in dependence. Five rats were treated for 14 days with subcutaneously-injected nicotine while five control animals received saline. Blood from each experimental group was pooled and polymorphonuclear and mononuclear leukocytes were separately purified. Maps prepared in triplicate were analysed with PDQuest software to detect differences in protein expression pattern. In both types of leukocytes several proteins resulted differentially expressed in the nicotine-treated as compared with the saline-treated group. Protein spots of interest were analysed for identification by peptide fingerprinting, using MALDI-TOF mass spectrometry. This proteomic study shows that chronic nicotine treatment influences the protein expression profile in peripheral tissues such as blood leukocytes.

28.6 Proteomic Analysis of Human Hepatic Drug-metabolizing Enzymes
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The superfamily of cytochrome(s) P450 plays a key role in oxidative hepatic and extra-hepatic drug metabolism. CYPs are membrane proteins that are expressed in varying amounts, and many forms differ very little in their amino acid sequence and catalytic properties. Since individual CYPs exhibit a broad, often overlapping substrate specificity, knowledge of the CYP composition is critical in predicting drug/drug interactions and formation of reactive intermediates. Current research approaches to the identification of individual P450 forms include: use of specific P450 substrates or inhibitors, antibody-based identification and mRNA-based analysis. All of these approaches suffer from one common disadvantage—they all are indirect methods. At the same time current developments in mass spectrometry provide a direct and reliable approach to protein identification with sensitivity in the femtomole or low picomole range. In previous work we have shown that 2DE cannot separate endoplasmic reticulum membrane proteins, particularly CYPs. On the other hand, MALDI TOF peptide mapping in conjunction with 1DE makes an excellent analytical approach for a detailed proteomic analysis of the CYP isozymes composition in various rat and rabbit liver microsomes. In this study we have used high-accuracy MALDI TOF-based peptide mapping to perform direct identification of distinct CYP isozymes in various human liver microsomes. We have used pooled as well as individual (male and female) samples of human microsomes purchased from three different companies. A detailed analysis of the data obtained will be presented.
28.7 Application of Toxicoproteomics to Drug Development: Effects of a Kinase Inhibitor on Protein Expression in Rat Liver

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Protein kinases form a large family of enzymes that serve as key regulatory components in signal transduction pathways. The development of selective protein kinase inhibitors that can block or modulate diseases caused by abnormalities in these signaling pathways is widely considered as a promising approach for drug development. Compound X is a kinase inhibitor that has been developed for treatment of asthma. In a 14-day oral toxicity study in rats, compound X was administered at 10 or 200 mg/kg/day and at the highest dose, induced hepatotoxicity. A toxicoproteomics study was initiated to elucidate the molecular mechanisms underlying the observed drug-induced effects. After protein extraction, 2D gel electrophoresis was performed using the DIGE technology. Identification of the up- and down-regulated spots was performed by mass spectrometry. Results showed that compound X at the dose of 200 mg/kg induced an up- and down-regulation of 69 and 20 proteins, respectively. Data bioanalysis showed that numerous biochemical pathways were involved such as lipid and fatty acid metabolism, amino-acid and protein metabolism and citric acid cycle. Proteins involved in oxidative stress and xenobiotic metabolism were found to be strongly affected by the treatment, suggesting that toxicity might be linked to induction of oxidative stress by a reactive metabolite in liver. In addition, opposite effects on the isoforms of carboxylesterase 4, fumarylacetoacetase and 3-oxo-5-α-steroid 4-dehydrogenase were observed at the highest dose, suggesting a compound-specific signature of the toxic effects of compound X in the liver.

28.8 New Lead Compounds Via Smart Proteomics Approach

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The post genomic era left pharmaceutical R&D with a huge number of possible disease related targets, their respective validation in accepted pharmaceutical in vitro and in vivo models, and the task to design and to develop lead candidates and new chemical entities (NCEs). Until today, proteomics technologies proved to be decisive for target discovery and validation only.

In the present study, Protagen shows that the strategic application of its proprietary integrated proteomics technology (IPT) platform allows the straightforward design of small molecule lead compounds with significant biological activity. The Protagen strategy is based on the identification of regulatory networks of cellular proteins affected by already marketed drugs in diseased and normal state. The extraction of knowledge from these proteomics studies and its correlation with the basics in cell biology, biochemistry and pharmacology can be applied on the design of small molecule drugs. In this study, an anti-proliferative and anti-inflammatory drug was used to identify a set of proteins all belonging to the major cellulary energy generating machinery. The comparison of drug structures and natural substrates as well as effectors of these proteins gave guidance for the synthesis of P@G1011. The new lead candidate has been tested in vitro and in animal models and showed efficacy in different cell lines and in rat cancer model.

These data strongly demonstrate the potential of smart designed proteomic studies in delivering superior quality of targets and lead compounds and in speeding up the pharmaceutical R&D process.
Proteomics Identification of Beneficial Actions of Grape Seed Extract on AD-linked Protein Targets

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Behavior studies have demonstrated neuroprotective effects of botanical preparations high in anti-oxidants including soy isoﬂavones and blueberries, however the molecular basis of these effects has not been understood. Recent reports have identiﬁed multiple protein alterations in Alzheimer’s disease brains, but which are involved in the pathogenesis of the disease is unknown. Using a combination of 2D electrophoresis, MALDI-TOF and LC-tandem mass spectrometry, as well as western blots of 2D gels, we have identiﬁed several proteins in rat brain that are altered following ingestion of diets supplemented with grape seed extract. The observed changes included both differential protein expression and alterations in modiﬁcations. Statistical analysis carried out on spot intensities without reference to the images conﬁrmed each of the changes. Several of the protein changes detected were previously identiﬁed as differentially expressed or modiﬁed in AD brain. The changes we noted among these latter proteins were in the opposite direction of the changes noted in AD brain, relative to non-disease human brain. This is the ﬁrst conclusive identiﬁcation of speciﬁc protein targets for a botanical, as well as the ﬁrst identiﬁcation of such targets that are speciﬁcally linked to a disease. The fact that the direction of the changes is counter to that observed in disease suggests that ingestion of GSE has speciﬁc neuroprotective activity.

Proteomics Applied to Antibacterial Drug Discovery

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In 1975 O’Farrell published a method to separate a complex mixture of cellular proteins such that individual polypeptides could be detected and quantiﬁed. Physiologists immediately saw the potential of this global protein proﬁling method as a means to study physiology at a molecular level. The potential was realized within the ﬁrst ten years with examples of the classiﬁcation of proteins into functional groups and into regulatory networks. Recently, it has been demonstrated that changes in protein expression can be correlated with physiological and genetic variation. Despite the success, the work was largely ignored because of the difﬁculty in determining which gene encoded the polypeptide being cataloged. During the last ten years, methods for global proﬁling of RNA (immediately linked to their cognate gene) have been developed and are widely used. In addition, mass spectrometry methods have been adapted to handle proteins from gels and has advanced to the point that the 200 most abundant protein spots on the gels can be identiﬁed.

Does knowing the genes that encode the molecules monitored with these global expression methods aid in the interpretation of the data? Global proﬁling reveals cell behavior at a molecular physiological level. How much of the cell’s behavior has previously been characterized at the molecular level? Most investigators use only a small portion of the global proﬁling data. The typical “human response” to a report of a global proﬁling data set is immediate “data reduction”. What and how can we expect global RNA and protein proﬁling contribute to biology?