3.4

**Time-controlled Transcardiac Perfusion Crosslinking (tcTPC): A Novel Tool for the In Vivo Study of Protein Interactions in Complex Tissues**

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Due to their favorable accessibility, membrane proteins play a prominent role for disease diagnostics and are targeted by 50% of currently known therapeutics. The importance of membrane proteins is contrasted by the sobering reality that their interactions with other proteins are the most difficult to study. Most approaches study membrane proteins outside their physiological milieu or require them to be solubilized by the addition of detergents. We have developed a novel method that covalently conserves protein interactions through time-controlled transcardiac perfusion crosslinking (tcTPC) prior to the disruption of tissue integrity. The method was validated with the gamma-secretase enzyme complex as a target. Subsequently tcTPC was employed to generate a comprehensive protein interaction map of the cellular prion protein. Our data hint at an involvement of the cellular prion protein in processes related to cell adhesion/neuritic outgrowth. Our data further establish the in vivo existence of specialized membrane regions enriched in molecules that share with the prion protein the use of a glycosylphosphatidylinositol anchor for membrane attachment. The value of tcTPC for the in vivo analysis of protein interactions will be discussed.

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3.5

**Activity-based Protein Profiling: Chemical Approaches for Functional Proteomics**

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The field of proteomics aims to characterize dynamics in protein function on a global scale. However, several classes of enzymes are regulated by posttranslational mechanisms, limiting the utility of conventional proteomics techniques for the characterization of these proteins. Our research group has initiated a program aimed at generating chemical probes that interrogate the state of enzyme active sites in whole proteomes, thereby facilitating the simultaneous activity-based profiling of many enzymes in samples of high complexity. Progress towards the generation and utilization of active site-directed chemical probes for the proteomic characterization of several enzyme classes will be described. These enzyme classes fall into two general categories: 1) enzymes for which active site-directed affinity agents have been well-defined, and 2) enzymes for which active site-directed affinity agents have been lacking. The application of activity-based protein profiling to the functional characterization of enzyme activities that vary in human cancer specimens will be highlighted, as will be the use of this strategy as a screen to discover potent and selective reversible enzyme inhibitors.

4.1

**Quantitative Proteomics: Current Status, Challenges and New Directions**

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The objective of proteomics is the systematic analysis of the proteins expressed by a cell, tissue or organism. It is expected that such analyses will define comprehensive molecular signatures of tissues, cells and body fluids in health and disease. Such signatures will impact a wide range of biological and clinical research questions, such as the systematic study of biological processes and the discovery of molecular clinical markers for detection, diagnosis and assessment of treatment outcome. The application of proteomics technology has proven particularly beneficial in cases in which differences between the proteomes (or fractions thereof) isolated from cells at different states have been analyzed. The detection of differences between proteomes implies that the measurements include accurate quantification. A number of new methods based on stable isotope tagging and tandem mass spectrometry have been developed that now support robust quantitative proteome analysis.

In this presentation, we will discuss recent advances in quantitative proteomics technology, its current status and limitations. Specific applications will illustrate the potential of the technology to define comprehensive molecular signatures of tissues, cells and body fluids in health and disease. Finally, we will discuss new experimental approaches that have the potential to significantly improve the performance and sample throughput of mass spectrometry based proteomics technology.
4.2 Mass Spectrometry-based Functional Proteomics: A Maturing Tool in Drug Target Identification and Validation

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Protein drug target identification and validation, in many ways, can be defined as a process to determine a protein’s function in the normal physiological context and its functional implications in certain diseases. Genomic technologies such as gene knockout, transcriptional profiling, RNA interference, have been routinely applied to discover and validate new drug targets. However, much useful information on the biology of a protein target, such as its direct and indirect physical interactions with other proteins, can only be obtained at the protein level. Since the function of a protein is often defined by its interactions with other proteins, mapping of protein interactions can highlight new functionalities of a known protein or even define the function of novel proteins, and thus can help us tremendously in the prioritization of drug targets, further validation of targets that are already in the pipeline, and a greater understanding of the pharmacological consequences of targeting specific proteins.

Mass spectrometry-based functional proteomics elucidates protein-protein interactions in a physiologically relevant context using modern sensitive mass spectrometers to differentially analyze complex protein mixtures enriched in functional protein complexes by affinity purification. Using this approach, the protein compositions of numerous functional protein complexes have been identified and characterized. Recently, two high-throughput analyses of protein complex composition in S. cerevisiae have also been carried out and generated an unprecedented amount of protein interaction information. However, successful MS-based functional proteomics applications are still far from routine due to many reasons such as failure to capture the protein complexes of interest, limited dynamic range and sensitivity of MS analysis, incomplete interpretation of mass spectra, and inability to analyze large data sets obtained from database searching, etc. Here we describe our efforts in establishing a streamlined MS-based functional proteomics platform and its applications in the elucidation of the estrogen receptor complex, the lymphoxygen beta receptor complex, and the GDF-8 complex as well as protein complexes involved in its downstream signaling pathways. These applications provided many unique insights into the biology of these proteins and helped us identify potential new intervention points for treatment of relevant diseases. They also demonstrated that mass spectrometry-based functional proteomics could be and should be used as a routine tool in understanding the biology of the existing targets of interest and providing great opportunities to find potential new protein targets.


4.3 A Human Proteome Resource Initiative

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A systematic approach to convert genomics data into biological knowledge based on protein profiling is described. The strategy relies on a high-throughput method for the recombinant production of non-homologous regions of the proteome selected by whole genome bioinformatics. Such protein fragments are individually used to generate and enrich mono-specific antibodies for systematic analysis of protein profiles (expression and localization) in different human organs using tissue arrays. The results suggest that this Affinity Proteomics strategy can be used to produce a proteome atlas, describing distribution and expression of proteins in normal tissues as well as in common cancers and other forms of diseased tissues.

B.1 Subproteomics at a Single Cell Level: Active Pathways in Arabidopsis thaliana Trichomes

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Plants are continually exposed to diverse environmental challenges, including mechanical injury, pathogen attacks and damage from a wide range of herbivores. Tissue injury affects defined self-defense systems where trichomes might be involved. There is also evidence that trichomes may play additional or alternative roles in both the detoxification of heavy metals and in response to various other stress conditions. Recently, it has been reported that proteins involved in sulfate metabolism are being up-regulated in trichomes. However, not much is known about the trichome proteome. To provide evidence about the function and the role of plant leaf trichomes, such as their role as a defense system, it is important to gain insight into their proteome. Currently, alternatives to 2-D PAGE for proteome analysis, such as 1-D and 2-D HPLC separation are being developed. In addition to several advantages, such as high throughput, no bias against membrane proteins, the ability to handle proteins with extremes in pI and molecular weight, nano HPLC technology allows lower total protein amounts for comprehensive qualitative proteome analysis compared to gel electrophoresis prior to MS analysis. In plants, no successful attempt towards proteomic approach on a single-cell level has yet been published. Here we demonstrate that a 1-D nano HPLC peptide separation strategy enhances the sensitivity of peptide detection, which enables protein identification of single-cell trichomes from A. thaliana leaves. Trichome cells were collected and 59 proteins involved in the sulfate metabolism or known to be implicated in the oxidative stress response were identified, such as the glutaredoxine. Here we provide new insights into the characterization of the proteome of trichome cells in A. thaliana leaves.