## 33.32

# Functional Proteomic Platform for Mapping Cardiac Subproteomes in the Mouse: A Strategy for the Human Cardiac Proteome Project

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Significant challenges surround large-scale analysis of the human myocardial proteome, including: limited availability of tissues, absence of tightly controlled experimental groups, and lack of a concerted strategy to deconstruct protein networks underling cellular function. Thus, we propose an approach to map the human myocardial proteome based on the use of a murine model system. Furthermore, we hypothesize that the mammalian cardiac proteome is organized on the basis of multiple integrated subproteomes. We have developed a functional proteomic platform that interfaces proteomic techniques (e.g., electrophoresis/mass spectrometry) with biochemical and physiological assays to study how proteins and protein interactions engender phenotype. This platform characterizes a subproteome on four levels: 1) protein-profiling (networking proteins in a signaling system); 2) spatial-profiling (role of subcellular localization); 3) temporal-profiling (protein alterations over time following a stimulus); and 4) functional-profiling (changes in protein function associated with altered phenotype). As an example, we have validated this platform by characterizing the PKC$\epsilon$ signaling subproteome in the heart (however, the platform is applicable across cell type). PKC$\epsilon$ (a known cardiac protective protein) was found to form multiprotein complexes with $\sim$93 proteins. Assembly of these complexes appears to alter protein localization and affect protein function. Importantly, the physiological phenotype of cardiac protection appears to be directly linked to modulation of the PKC$\epsilon$ subproteome. Integration of studies examining other subproteomes will provide a more complete blueprint of cardiac cell function. Importantly, knowledge gained from these mouse studies may be exploited to render analyses of the human cardiac proteome more focused and attainable.

## 35.1

# Proteomics in Biomarker Discovery

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Proteomics, the quantitative study of the protein content of a cell in a specific state, is a promising new field of scientific endeavor fueled by high resolution separation methods, mass spectrometry and informatics. Progress is being made in the application of proteomics to biologically relevant problems, but significant technological challenges remain that are akin to the hurdles faced by the Human Genome initiative a decade ago. In this talk I will discuss some of these challenges, and describe how proteomics is contributing to the drug discovery and development process at Millennium Pharmaceuticals. I will concentrate on the roles that proteomics is playing in the discovery and validation of biomarkers of disease and pharmacodynamic markers of drug efficacy and mechanism of action. The impact of some recent advances in separation modalities, mass spectrometry and informatics will be highlighted.

## 35.2

# Selecting Targets for Therapeutic Validation Through Differential Protein Expression Using Chromatography-Mass Spectrometry

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The identification of potential targets for therapeutic intervention can be accomplished through analysis of differences in the expression levels of nucleic acid and protein gene products. These potential protein drug targets could either be directly causative of disease, or reveal biochemical pathways that could be modulated by therapeutic molecules. However, it is necessary to validate potential targets by establishing their firm association with disease, and their minimal distribution in non-diseased tissues of any type. This requirement suggests that emphasis on true and reproducible quantitation of protein expression levels in a variety of samples will be an effective and highly efficient method of generating drug targets with a high degree of utility. To achieve this aim, we have established an industrial-scale proteomics-based discovery platform consisting of cell biology, protein chemistry, and mass spectrometry technical groups using LC-MS for quantitation and MS/MS for identification. To evaluate this approach on a large-scale, we have applied it to a study of continuous cell lines derived from human pancreatic adenocarcinomas. We have been able to establish processes for target discovery for small molecule drug targets, therapeutic antibody target identification for cell surface proteins and identification of serum markers based upon standardized fractionation procedures. The results of these analyses will be presented together with the some of the issues that need to be addressed in such an undertaking.