

# Clinical Proteomics- Promises and Challenges

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## Abstract

*Proteomics is an emerging field in medical science focused on the library of proteins specific to a given biosystem, the proteome, and understanding relationships therein. The application of proteomic methods in clinical research will assist discovery of biomarker that may lead to new diagnostic tests and improvements in the therapeutics. Mass spectrometry (MS) has become an essential analytical tool in proteomic field for its ability to determine molecular weight of peptides with high precision; to generate protein expression profile with rich information content; and the ability to operate at high degree of automation. The maturation of MS technologies has made significant contributions in the clinical research. In this review, several MS-based protein analytical methods are discussed in the content of their clinical applications.*

## Introduction

Prevention, early detection and early intervention are the primary aims of clinical research. The available therapeutic measures for most malignant conditions yield cures only when applied at early stages, when disease bulk is both minimal and localized. Multiple studies have shown that early intervention improves outcome. There are few malignancies for which we have highly sensitive and specific screening in procedures at this time. The field of proteomics studies proteins in an effort to catalog them and to understand their roles in biology and pathology so they may be applied to early diagnosis and to optimizing treatments.

## The Challenges

The maturation of high-resolution analytical instruments like the mass spectrometer has generated tremendous interest in using this tool in the clinical setting to detect and monitor protein biomarkers in serum, proximal fluids and tissues in humans (1). However, identifying and characterizing proteins from clinical samples have been quite difficult for biomarker discovery. Serum has a dynamic protein expression range of 10 orders of magnitude and is dominated by albumin and immunoglobulins that represent greater than 80% of the total protein content. Thus low abundance biomarkers are contained in the thousands of proteins that represent the remainder of the total serum protein mass. This poses a significant obstacle for detecting biomarkers by current mass spectrometric methods. Sample preparation methods that can reduce protein complexity while maintain analysis throughput and quantification accuracy are necessary to meet the challenges.

## Expression profiling using two-dimensional gel electrophoresis (2-DE)

Historically, 2-DE has been the tool of choice to resolve complex protein mixtures and to detect differences in protein expression patterns between normal and diseased specimens. It separates proteins based on

their mass and charge differences and the proteins are detected by staining or labeling methods. It was populated by the development of MS methods (peptide fingerprinting) that allows routine protein identification of separated proteins. In general, using 2-DE methods to study biomarker expression in clinical samples has been difficult due to the inherent limitations of 2-DE methodology. First, the hydrophobic, insoluble nature of membrane and membrane associated proteins make them incompatible with the buffers of the 2-DE system. Therefore, this class of proteins tends to be significantly underrepresented in 2-DE studies. Invariably, due to limited dynamic range of the gel method, the most-abundant soluble proteins are typically visualized and detected by 2-DE methods. This impacts biomarker discovery and characterization in disease in several ways. First, it severely limits the ability to detect and explore differences in cell-surface receptor membrane protein expression that may occur between normal and disease tissue. Second, 2-DE gel protein patterns are notoriously difficult to reproduce between laboratories. To address these shortcomings, a newer method referred to as DIGE (2), which incorporates fluorescent cyanine dyes into the protein samples differentially labeled with different fluorescent stains are processed in a single 2-DE gel so that the relative signal intensity of the different fluorescent labels can be detected by spectral analysis and, based on the ratio of signal intensities, the relative abundance of each protein in the two samples can be quantified. 2-DIGE alleviates the pattern reproducibility problem but not the other problems associated with 2-DE. Specifically, the method still requires large amounts of starting material (40 – 100 mg of total protein to generate upward of 500 spots/gel) to visualize adequate number of silver-stained protein in the gel. Thus, 2-DE methods may not represent the optimal proteomic platform to detect and study biomarkers in tissues and serum. In contrast, 2-DE methodology has and will continue to be a powerful platform for identifying potential biomarkers in animal model systems. These experimental systems can generate abundant amounts of protein, which is typically not the case when clinical samples are utilized.

### **Expression profiling using SELDI-MS and MALDI-MS**

A new MS-based proteomic approach termed SELDI coupled to the mass spectrometer has sparked tremendous excitement as a diagnostic tool in cancer research (3). The SELDI approach involved extracting protein/peptides from tissue and/or serum and applying them to an affinity capture surface located on a chip, which selectively binds a specific subset of proteins. Nonbound proteins are washed away, the capture proteins are ionized by MALDI (matrix assisted-laser desorption ionization) MS, and their unique masses are recorded in a low-resolution time-of-flight (TOF) mass

spectrometer. The SELDI-TOF method generates signature of thousands of potential protein peaks that investigators use to compare pattern differences between normal and disease samples. Computer algorithms are subsequently used to analyze and select discriminatory peaks that separate normal and diseased populations. The SELDI-TOF approach has generated promising results in an ovarian cancer study (4). It was reported that diagnostic peak patterns identified 100% of all ovarian cancer samples and correctly assigned 95% of healthy and benign subjects correctly. As anticipated, the discriminatory power of the SELDI approach has sparked tremendous excitement in its use as a diagnostic tool in detecting various types of diseases. However, there are many important issues remain to be addressed before the application of the SELDI technology as a clinical diagnostic tool. First, diagnostic peak patterns need to be confirmed independently by other research groups using the same sample preparation and analytical procedures. Second, a standardized procedure for comparing algorithms needs to be established.

A fundamental weakness of the SELDI approaches is that the chemical identity of the discriminating peaks used to separate normal and disease population are largely unknown. It is due to, in part, the low binding capacity of the affinity surface. An alternative approach, utilizing functionalized magnetic particles for sample preparation, was introduced to increase the captured protein for subsequent MALDI-MS analysis (5). Magnetic bead handling is an automation-friendly and scalable method for sample preparation. Combined with a high resolution MS, even a MS/MS MALDI analyzer, one can expect increased success rate in determining the chemical identity of potential biomarkers.

### **Expression profiling using multidimensional LC methods**

Gel-free proteomic approaches that incorporate multiple steps of liquid chromatography (LC) to reduce that protein and peptide complexity prior to protein identification by LC-MS/MS methods have gained broader acceptance over the past several years (6). Multidimensional LC approaches have several distinct advantages over 2-DE method. First, the physiochemical properties of specific classes of proteins, for example, membrane proteins, are not selected against. Second, it provides greater flexibility in sample handling and processing, while sample losses incurred by resolving protein into a gel prior to LC-MS/MS are avoided. In this strategy, the complexity of sample is progressively reduced until a stage at which the mass spectrometer can identify most of the peptides. Reducing sample complexity through enrichment steps can aid the identification of low-abundant proteins. Traditionally, standard LC-MS methods have not been very accurate for quantification. By incorporating stable isotope labels into proteins, a technology termed ICAT (7), one can start to

quantify protein expressions from different samples. However, the prohibitive high cost of ICAT technology and low throughput sample processing of LC methods make it difficult to apply on large scale clinical studies.

### Discussion

The maturation of MS technologies has given clinical scientist a powerful analytical tool to study human disease. The increased sensitivity of mass spectrometers over the past several years inevitably expedited the identification and validation of critical biomarkers. Advances in sample preparation methodology gave MS versatility in solving various types of clinical problems. Clearly, these methods compliment each other in throughput, information content and detection sensitivity. Researchers need to carefully select method or combination of methods to answer their unique questions. Despite the tremendous progress made in the recent years, at current stage, dealing with samples with high degree of protein complexity and wide dynamic range of protein concentration remains a challenging problem for MS-base technologies. A

coordinated effort to improve sample processing methods, instrumentation, bioinformatics tools is necessary to fulfill the promises of this emerging technology.

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