Preface: Evolutionary Nature of the Proteomic Revolution

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For the past decade we have witnessed success and broad adoption of proteomic techniques, fueled by rapid innovations in mass spectrometry and bioinformatics. Proteomics is currently applied to the discovery of new protein biomarkers of disease,[1,2] toxicity,[3] and drug efficacy.[4] Recently, numerous attempts were undertaken to use proteomic analysis workflow in early diagnostics.[5] Proteomics, in conjunction with gene expression and metabolic profiling, forms the foundation of a systems biology approach to understanding and modeling fundamental mechanisms of life, essentially driving biology closer to the realm of exact sciences.[6]

Regardless of its wide adoption, proteomics is a constantly evolving field. It is interesting to note, that while instrument development has received enormous attention in recent years, the separation techniques used for analysis of proteins have remained fairly conservative. However, the debates on which separation technique is best suitable for proteomic analysis continue to stir the scientific community.

There are generally three distinct directions in proteomics. The first approach, the historical two-dimensional gel electrophoresis (2DE) approach, well regarded for its resolving power, massively parallel separation, quantitative nature, and instant visualization of thousands of protein species, including distinct posttranslationally modified protein isoforms, has been criticized for the labor-intensive process and the lack of reproducibility.

The second approach, based on variations of multidimensional chromatography, apparently is easier to automate, although analysis typically takes longer due to the serial nature of the separation process.

The third emerging approach calls for using various microarray technologies for protein profiling, primarily driven by the diagnostic factions of proteomics.[5,7,8] Because this approach does not typically utilize any separation techniques at the time of analysis, it will not be covered in this book.

There are several permutations of the first and second approach being reported from time to time. These methods generally combine the chromatography and electrophoresis steps, either an ion-exchange chromatography fractionation followed by a one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or isoelectric focusing followed by a reverse phase chromatography.[9–12]

Separation steps are typically coupled with either rapid matrix-assisted laser-desorbtion ionization-time of flight (MALDI-TOF) peptide mass fingerprinting (PMF) or more versatile electrospray ionization-tandem mass spectrometry (ESI-MS/MS) protein identification.[8]

It is now becoming apparent that all of the approaches described above are optimized for particular applications or user preferences and should be considered complimentary to each other. As high-end tandem instruments capable of accurate mass determination and data-dependent analysis of posttranslational modifications become affordable, diversity of liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques allow automated data acquisition and confident protein identification from complex samples. On the other hand, considering the wide adoption of proteomic techniques by biochemists, electrophoresis is still arguably the most frequently used method of protein separation to date.

"2D OR NOT 2D?"

That was the question posed in Shakespearian satire by Fey and Larsen in a *Current Opinions in Chemical Biology* review article published in 2001.[13] This marked a period that seemingly began a movement away from 2DE, or at least an attempt by some researchers to avoid using it.

However, as one of the major contributors to the field of protein separation, Pier Giorgio Righetti, has recently illustrated in his review of the history of electrophoresis,[14] this technology is a prolific area of research and remains an integral part of proteomic analyses today and most likely for some time to come.

In the past, the technique was viewed as procedurally complex, highly irreproducible, and incapable of eliciting all of the protein constituents of a proteome, particularly low abundance and membrane proteins. For years, there were concerns expressed by many groups that subpopulations of proteins, such as hydrophobic membrane proteins, possibly clinically significant biomarkers, or targets of therapeutic treatment, might not be accurately represented in the two-dimensional (2D) array. This criticism has been largely dismissed by recent critical advances in 2DE, a technique 30 years old, but still in its formative years. For example, the implementation of neutral pH buffer systems has made possible the commercial mass production of stable polyacrylamide gels making the second dimension of electrophoresis both reproducible and procedurally benign. Likewise, the refinement of immobilized pH gradients has eliminated the problems inherent to carrier ampholyte-driven isoelectric focusing (IEF) and because the pH gradient is insusceptible to drift, proteins are resolved reproducibly along the x-axes of the 2D array.

Some efforts have been made to increase the capacity of 2DE for resolving the entire protein content of cells. The synthesis of linear sulfobetaine surfactants has facilitated the isolation of even the most recalcitrant membranes proteins, and it is this segment of the cellular proteome that is often thought to represent the majority of potential drug targets. Zwitterionic detergents have replaced the nonionic ones used earlier for protein solubilization. It is the increased stringency of these new surface reactive agents that not only enables the isolation of membrane associated proteins, but ensures their solubility is maintained during the first dimension of electrophoresis.

It is the purpose of this volume, nearly two years in the making, to provide a comprehensive overview of the separation methods currently employed in proteomic analyses.

Regardless of the chosen analytical approach, a common concern of missing the low abundance components of the proteome still remains prevalent in proteomics. Hence, the need for complexity reduction prior to proteomic analysis remains the most popular paradigm at the moment.

The first section of this book deals with sample preparation. It includes several descriptions of immunological depletion of high-abundance proteins, specifically from plasma and sera, but applicable to any species for which there are available antibodies. As communicated in subsequent chapters, the complexity of proteomes can be diminished by fractionation into smaller, less complicated "subproteomes" that can be analyzed individually. Ion-exchange chromatography, free flow electrophoresis (FFE), and solution-phase IEF are all such "divide and conquer" strategies for the isolation of disparate fractions of defined isoelectric point (pl) intervals from complex samples.

Several applications to specific areas of research are described in the following chapters. These chapters provide valuable insights, often derived empirically, for several representative samples. For example, the extraction of proteins from myelinated tissues such as the optic nerve as described by Bhattacharya et al. (Chapter 9), indeed a challenge in terms of sample preparation, will prove invaluable to the researcher trying to elucidate proteins from recalcitrant samples that are resistant to usual solubilization schema.

Reduction and alkylation of protein disulfides is reviewed in Chapter 12 by Bai et al. who prescribe new reagents that alkylates nearly 100% of the cysteines. Further, this chapter explains the rationale of reduction and alkylation before IEF, further improving the reproducibility of 2DE separation.

In recent years, rapid development of novel, more affordable mass spectrometry instrumentation capable of producing data of high-mass accuracy at great resolution, has strengthened the idea to alleviate the need for high-quality separations prior to analysis, shifting the burden of separation onto a mass spectrometer.

Several comprehensive reviews have been written recently on using automated liquid-phase separation methods.[15–21] We allocate three chapters of this book to cover some of the new trends in chromatographic separations coupled with mass spectrometry, such as a utility of accurate mass determination to enable identification of proteins in complex mixtures, creative methods combining classical top-down and bottom-up approaches to assure reliable automated protein identification, and the novel, promising area of high-resolution monolithic capillary columns.

Many approaches have been developed to challenge the most famous claim of 2D gels, an ability to quantitatively assess protein expression, by development of mass spectrometric methods for relative[22–24] or absolute[25] quantification of differentially expressed proteins. However, most successful efforts on automated LC-MS/MS methods are still being pursued by a fairly small community of technology champions and frequently are applicable to the samples of relatively low complexity. It is also important to note that one of the biggest adoption barriers for quantitative LC-MS/MS and multiple permutations of classical Multidimensional Protein Identification Technology (MudPIT) techniques has been related to the bottlenecks in data analyses such as limitations of commercially available

software tools to handle reliable automated data-dependent data acquisition, protein identification, and validation of the results thus obtained applicable for the analysis of complex biological samples.[26]

Recent advancements in functional proteomics call for shifting the experimental efforts to the detection of protein interactions. Conventional approaches to protein isolation and analysis portrays proteins in their "naked," unnatural form and completely ignores protein-DNA, protein-lipid, and protein-carbohydrate interactions of biological relevance. It is this growing interest in protein interactions that will be the impetus for the development of new technologies capable of analyzing proteins in their native state. Accordingly, two chapters in this book discuss the application of nuclear magnetic resonance (NMR) for the analysis of such interactions.

We truly believe that proteomics is yet to bring its best results. The "Proteomic Revolution" all of us have been waiting for is finally over. Proteomics is now widely accepted as one of the essential techniques for biomarker discovery. Now it is the time for the evolutionary process: selection of the best, most successful technologies that can deliver high-quality knowledge in our quest for understanding life.

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