

The Proteomics Protocols Handbook

EDITED BY

John M. Walker



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University of Hertfordshire, Hatfield, UK

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**The
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Handbook**

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Preface

Recent developments in the field of proteomics have revolutionized the way that proteins, and their contribution to cellular functions, are studied. The subsequent increased understanding of the mechanisms of cellular function and malfunction will have particular impact in the area of medical research, where disease processes will be better understood, many new (protein) therapeutic targets identified, and novel therapeutic agents developed. At the basic research level, phenotype will be explained in terms of cellular mechanisms.

The completion of the sequences of an ever-widening range of genomes—not least of all, the human genome—has provided the molecular biologist with a wealth of data that needs to be analyzed and interpreted. For a variety of reasons (including alternative mRNA splicing, varying translational stop/start sites, frameshifting, and the inability to deduce posttranslational modifications), complete sequences of genomes are insufficient to elucidate the protein components of cells. The focus of attention has therefore turned to directly examining these protein components as the means of understanding cell function, as well as the cellular changes involved in disease states. However, the wealth of gene sequencing data now available has produced a glut of information that challenges the protein chemist to develop new tools to utilize this flood of genomic data.

From the beginning, the cornerstone of proteomics has been the use of two-dimensional gel electrophoresis to compare proteomes of different tissues (for example, normal and diseased tissue) with the subsequent identification of protein differences by the use of mass spectrometry and database searching. These still remain valuable techniques and receive appropriate coverage in this book. However, the term proteomics now encompasses a range of newly developed methodologies for determining the structure and function of a protein. I have therefore included in *The Proteomics Protocols Handbook* a number of novel mass spectrometry and LC-MS techniques, protein array technology, new bioinformatics tools, and the range of techniques central to structural and functional proteomics that are needed to deduce the function of newly discovered protein sequences. The use of these techniques, and no doubt further ones that will be developed in the coming years, will lead to achieving the ultimate goal of proteomics, namely to catalog the identity and function of all proteins in living organisms.

The Proteomics Protocols Handbook should prove a valuable resource for molecular biologists, protein chemists, clinical/medical researchers, structural chemists/biochemists, and microbiologists, as well as those involved in bioinformatics and structural/functional genomics.

John M. Walker

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Extraction and Solubilization of Proteins for Proteomic Studies

Richard M. Leimgruber

1. Introduction

For any proteomic study involving various control and experimental specimens, several factors need to be in place. A critical one is the extraction and solubilization of all components, regardless of whether a chromatographic (1,2) or two-dimensional (2-D) gel electrophoretic fractionation (3–6) is performed prior to analysis of proteins of interest by mass spectrometry of protein digests. All proteins must not only be extracted, but they must also be completely soluble, free from interacting partners (such as protein–RNA/DNA and protein–protein interactions, metabolites, and so on), and, in the case of 2-D gel electrophoresis, they must remain soluble as they approach their isoelectric points. The solubilization process should extract all classes of proteins reproducibly, such that statistically significant quantitative data can be obtained and correlated with experimental perturbations and the resulting biological responses.

To accomplish this task, various approaches have been presented in the literature (7–11), and many solubilization cocktails are now available commercially. However, it should be noted that currently, despite several attempts by multiple groups, there is no single solubilization cocktail that works perfectly for all conditions and samples, due to sample source-related interfering compounds and a high degree of heterogeneity among samples. This heterogeneity can lead to differing protein solubilities. Also, the presence of highly abundant proteins complicates the extraction, solubilization, and analysis of the less abundant species. Extracts from certain plant tissues also present their own set of unique issues (12). In addition to solubilization of all proteins, the solubilization agents used must also be compatible with the subsequent fractionation/analytical method employed. To date, the most efficient solubilization cocktails consist of a mixture of chaotropic agents, a mixture of detergents containing 13–15 carbon long hydrophobic chains, and a reductant (13–17).

It is important to note that the effectiveness of solubilization is not the only factor that affects the quality of the 2-D protein patterns. Gel strip rehydration, sample application method, sample load, electrophoresis conditions, and so on all have an impact on the quality of the 2-D protein fingerprint or pattern.

Recently, there has been a renewed interest in quantitative protein profiling, a process that is critical for an understanding of biological function (18,19). Because the proteome is a very complex, dynamic process that represents events at the functional