Preface

With the successes of the myriad genome sequencing projects—more than 150 published completed projects, including the human genome—the attention of the international bioscientific community is now focusing more on the structure and function of the encoded proteins in the various genomes, i.e., trying to establish what genes really do! In response to the growing interest in this field, *Proteins and Proteomics: A Laboratory Manual* (Cold Spring Harbor Laboratory Press 2003) was published, providing researchers with a comprehensive introduction to proteomic methods, including those for isolating proteins, especially from one- and two-dimensional gels (2D gels), as well as classical "pull-down" procedures, using affinity tags, for isolating proteins and their interacting partners. Procedures for identifying proteins isolated by these means (and their posttranslational modifications, especially phosphorylation sites) using classical amino- and carboxy-terminal sequence analysis, as well as mass-spectrometry-based methods, were well covered.

Now that the field of proteomics is beginning to mature, it is clear that separation tools, other than the traditional one- and two-dimensional gels, are required for proteome research, especially if we aim to see beyond the tip of the iceberg where the vast majority of precious and rare proteins are still hidden, awaiting discovery. This is especially the case for low-abundance biomarkers of disease and certain classes of proteins that are essentially refractory to 2D gels, such as membrane proteins. For instance, for the majority of protein expression profiling experiments using 2D gels, only the most abundant proteins in a cell or tissue (i.e., the general "housekeeping" and structural proteins) are observed. This is not unexpected for the simple reason that the dynamic range of protein abundances in a cell is on the order of 10^5 to 10^6. As an example, actin—one of the most abundant proteins in a cell—has a concentration of 10^8 molecules per cell, whereas some cellular receptors or transcription factors are present at only 10^2 to 10^3 molecules per cell. This problem can be exacerbated when studying biological specimens such as blood, where albumin is present at ~40 mg/ml and cytokines at low pg/ml, a dynamic range of protein abundances of ~10^9. Because the dynamic range for detection of proteins in 2D gels is ~10^4, clearly, some prefractionation techniques, preferably in combination with abundant protein depletion methods, must be invoked to move low-abundance proteins of interest onto the "radar screen."

The aim of this book, *Purifying Proteins for Proteomics: A Laboratory Manual*, which is a companion to *Proteins and Proteomics: A Laboratory Manual*, is to provide the researcher with important purification strategies (Section I) and prefractionation approaches, both chromatographic (Section II) and electrophoretic (Section III)), to circumvent the dynamic range impasse. Along with these prefractionation approaches, a variety of classical protein separation methods are covered. These are methods designed to facilitate the high-through-

put production of purified proteins for the purpose of high-resolution three-dimensional structure analysis, i.e., "structural genomics" (also referred to as structural proteomics) and protein microarray (protein chip) analysis. In addition, Section IV describes methods for determining the functional integrity of purified proteins (e.g., measurement of conformational stability, accurate molecular weight, aggregation state, and binding characteristics using surface plasmon resonance). Included in Section IV is a chapter devoted to the analysis of carbohydrate from glycoproteins; protocols are provided that detail the removal of glycans from glycoproteins and the preparation of monosaccharides for identification by gas chromatography coupled to mass spectrometry.

Purifying Proteins for Proteomics: A Laboratory Manual is aimed at those investigators who wish to isolate proteins and peptides for subsequent proteomic analysis. It is written for an audience ranging from early graduate students to experienced investigators studying problems in a broad range of disciplines, from clinical to basic sciences, and applied to a vast array of proteomic problems. I felt that a single author would be unable to do justice to the entire panoply of currently available methods. Accordingly, the book contains contributions from some of the experts in each of the specific fields that are covered.

In addition to the authors of the various contributions included in this book, I am greatly indebted to a number of people who have made significant contributions to bring this project to fruition. First, I thank the editorial and production staff at Cold Spring Harbor Laboratory Press for their dedication and tireless efforts in checking references, facts, and faulty constructions, and for keeping the book (and myself) on schedule. A special debt of gratitude goes to Inez Sialiano for coordinating the project, Dorothy Brown for editorial assistance, Susan Schaefer for page layout, Denise Weiss for her elegant design of the book, and most of all, Michael Zierler for his unstinting support as Project Manager in steering the book to completion. I also acknowledge the generous support of Jan Argentine, my Managing Editor, and John Inglis, the Director of Cold Spring Harbor Laboratory Press, for overseeing the project. I am indebted to a number of my colleagues in the "Parkville Strip" (especially, Tom Garret, Ray Norton, Geoff Howlett, Heung-Chin Cheng, Lindsay Sparrow, and Herbert Treutlein) for their critical reading of various chapters of the book. In addition, I wish to express my gratitude to Professor Joe Sambrook, senior author of the extraordinary manual *Molecular Cloning*, whose practical editorial guidance, interest, and encouragement throughout the writing of both of the Proteomics Laboratory Manuals have been invaluable. I must also thank Mary Whitham for her secretarial help in the early stages of this book and, more recently, my personal assistant, Simone Pakin, for her secretarial excellence and superb informatics skills. Finally, I would like to thank my partner, Donna Dorow, for her never-failing support during this endeavor.

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