

PREFACE TO THE THIRD EDITION

Most will agree that the major achievement in bioscience since 1998, when the second edition of this book was published, is sequencing of the human genome. Rather than diminishing interest in proteins, this has led to a revival in protein exploration and an intensive search for better understanding of molecular processes in health and disease. During this time, industrial exploitation of proteins in health-care has hardly declined. The application of monoclonal antibodies targeted against rheumatoid arthritis and cancer has been booming, many second- and third-generation biopharmaceuticals have been approved, and modern technologies for vaccine production based on protein engineering and cell culture are being developed on a wide front.

There are approximately 21,000 protein-encoding genes, and the human proteome is much larger than this. Although mapping the genome revealed what was in the box, the jigsaw puzzle is far from complete. Several major research projects exemplify the revitalized interest in proteins. One is the Protein Atlas initiative (www.proteomatlas.org), aimed at providing a comprehensive database of high resolution microscopic images identifying proteins in normal and cancer tissues. Others involve an ever-widening range of refined tools exploiting protein profiling micro arrays, surface plasmon resonance, mass spectrometry, ELISA, quantitative 2D electrophoresis, and so on. Many technologies are aimed at parallel processing of thousands of targets, and this is profoundly changing the way structural biology projects are managed. Streamlined, miniaturized, automated high throughput (HTP) protocols are becoming the standard, but there is still a fundamental need for protein expression and purification, not least for X-ray structural studies. Many "proteomic" projects exploit high throughput purification of tagged proteins or antibodies.

On the industrial side, particular in healthcare, protein production is rapidly maturing. Platform technologies are being applied both upstream and downstream, allowing faster and

leaner implementation as well as better control. Expression of monoclonal antibodies in mammalian cells is at the multi-gram per liter level, with cell densities of more than twenty million per milliliter, specific productivity over 20 picograms per cell per day, in bioreactors with capacities up to 20,000 liters. This several-hundred-fold increase in productivity has changed the pressures on downstream purification, resulting in the development of very high capacity chromatography media for product capture and highly selective media (frequently "multimodal") for polishing. Downstream purification of biopharmaceuticals uses platform modules for assuring virus safety and for removal of host cell proteins, aggregates and critical contaminants. Regulatory agencies are encouraging greater understanding and control of production processes, a quality by design (QbD) doctrine, and the use of modern risk management techniques and experimental design—all of which is impacting the development of purification methods.

Compared to the second edition of this book, four chapters have been deleted (Chromatofocusing, Affinity Partitioning, Immunoelectrophoresis, and Large-Scale Electrophoresis). Three chapters have been totally rewritten by new authors: Chapter 5 (High Resolution Reversed-Phase Liquid Chromatography of Proteins), Chapter 15 (Electrophoresis in Gels), Chapter 16 (Conventional Isoelectric Focusing in Gel Slabs and Capillaries and Immobilized pH Gradients). Six new chapters have been added: Chapter 10 (Affinity Ligands from Chemical Combinatorial Libraries), Chapter 11 (Affinity Ligands from Biological Combinatorial Libraries), Chapter 12 (Membrane Separations), Chapter 13 (Refolding of Inclusion Body Proteins from *E. coli*), Chapter 14 (Purification of PEGylated Proteins), and Chapter 20 (High Throughput Screening Techniques in Protein Purification). These new chapters have been written by leading experts in their respective fields. All other chapters have been thoroughly revised and updated regarding recent

applications. A new section on the history of protein chromatography has been added to Chapter 2 (Introduction to Chromatography).

It is my hope that the third edition will receive the same overwhelmingly positive response as the first and second editions, and I would like to express my appreciation to

all contributing authors and to Ms Anita Lekhwani and her staff at John Wiley & Sons, Inc., Hoboken, New Jersey, for their patience and never-failing support of this project.

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