Preface

Post-translational Modifications of Proteins: Tools for Functional Proteomics, Second Edition, is a compilation of detailed protocols needed to detect and analyze the most important co- and post-translational modifications of proteins. For reasons of simplicity, although not explicitly mentioned in the title, both kinds of modifications are covered, regardless of whether they occur during or after biosynthesis of the protein. My intention was to cover the most significant protein modifications, focusing on the fields of protein function, proteome research, and characterization of pharmaceutical proteins.

The majority of all proteins undergo co- and/or post-translational modifications. The protein's polypeptide chain may be altered by proteolytic cleavage, formation of disulfide bonds, or covalent attachment of phosphate, sulfate, alkyl groups, lipids, carbohydrates, polypeptides, and others. Knowledge of these modifications is extremely important, because they may alter physical and chemical properties, folding, conformation distribution, stability, activity, and, consequently, the function of the proteins. Moreover, the modification itself can act as an added functional group. Examples of the biological effects of protein modifications include: phosphorylation for signal transduction, ubiquitination for proteolysis, attachment of fatty acids for membrane anchoring or association, glycosylation for protein half-life, targeting, cell–cell and cell–matrix interaction, and carboxylation in protein–ligand binding, to name just a few. Full understanding of a specific protein structure–function relationship requires detailed information not only about its amino acid sequence, which is determined by the corresponding DNA sequence, but also on the presence and structure of protein modifications. Consequently, analysis of post-translational modifications of proteins is essential for proteomic research, the development of new drugs, and for the production, registration, and monitoring of therapeutic pharmaceutical proteins.

In general, post-translational modifications of proteins can be classified according to their chemistry or the targeted amino acid. They can be subdivided into reversible or irreversible reactions, enzymatic or nonenzymatic reactions, according to their subcellular location or functional aspects of the modification. Though the organization of the chapters considers both the frequency and the chemical nature of the particular post-translational modification, it still remains arbitrary. The individual chapters of this book provide detailed step-by-step instructions for

the analysis of the most important protein modifications, e.g., the assignment of disulfide bonds in proteins (Chapter 1). The detection and analysis of protein phosphorylation by selective fluorescent staining in 2D-gels and by advanced mass spectrometry, respectively, is covered by Chapters 2 and 3. Chapters 4 to 7 describe analysis of protein sulfation, α-amidation, γ-glutamate, ß-hydroxyaspartate, and lysine hydroxylation. Protein ubiquitination, sumoylation, and ISGylation are covered by Chapters 8 to 10, analysis of protein methylation and acetylation by Chapter 11. Methods for analysis of lipid modifications to both the carbohydrate and lipid portion as well are given in Chapters 12 and 13 on S-acylation and glycosylphosphatidylinositols respectively. Chapters 14 to 21 describe analysis of protein glycosylation in great detail. Starting with the detection of protein glycosylation (Chapter 14), analysis of carbohydrate composition (Chapter 15) cleavage, labeling, separation, and sequence analysis of *N*-linked glycans are described (Chapters 16 to 18). Analysis of protein *O*-glycosylation in general and specific detection of *O*-linked N-acetyglucosamine residues follow (Chapters 20 and 19, respectively). Analysis of *O*-glycosidically linked *N*-acetylglucosamine (O-GlcNAc) deserves special mention. *O*-GlcNAc is a transient modification, which is involved in several cellular functions as transcription, translation, nuclear transport, and cell signalling. Because of its exceptional position within the glycosylation of proteins it is treated in a separate chapter. Chapter 21 provides a method to analyze the oligosaccharides that are present at specific single glycosylation sites in a protein. Chapters 22 to 24 give practical approaches, i.e., how to analyze and monitor glycosylation of recombinant proteins from different cell lines. Finally, a topic of general interest is treated in the last chapter. Chapter 25 describes the use web-based protein databases for analysis of post-translational modifications of proteins. Web-based databases give information on protein modifications and allow the prediction of post-translational modifications on yet uncharacterized proteins, based on the fact that post-translational modifications occur at specific amino acids, amino acid sequences, or specific 3D-structures of the protein, respectively.

Let me give special mention to two areas of research of high current interest: the fields of (1) proteomics and (2) the characterization of biological pharmaceuticals. (1) With respect to proteomics, research in the field of genomics has lead to knowledge of the complete human DNA sequence. Measurement of the mRNA pool at a specific status of the cell, the "transcriptome," was found to not necessarily reflect the cells' actual protein expression pattern. In proteomic research, the description of expression levels of proteins related to a defined cell or tissue status will be incomplete without knowledge of post-translational modifications of those proteins. The increasing interest in post-translational modifications of proteins in this field is reflected by use of the term "phosphoproteomics." Phosphoproteomics describes the analysis of the sites and amount of protein phosphorylation under different biological conditions. (2) An additional important practical application of post-translational modification analysis is to ensure product quality of therapeutic pharmaceutical proteins. The exact structure of a protein pharmaceutical cannot be defined without knowledge of all post-translational modifications. Recombinant proteins intended for therapeutic use in humans must be accorded particularly

thorough investigation. Product quality depends on accurate post-translational modification in the respective expression system during production, e.g., insect, several mammal, or human cell lines. Note that different expression systems may vary in their ability to carry out post-translational modifications and that the applied cell-culture conditions also influence these modifications. Thus, post-translational modifications of recombinant proteins have to be monitored during production and documented for registration. In their guidance Q6A for the pharmaceutical industries, the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) states, that "For desired product and product-related substances, details should be provided on primary, secondary and higher-order structure; post-translational forms (e.g., glycoforms); biological activity, purity, and immunochemical properties, when relevant." Consequently, almost each and every post-translational modification of a protein is of concern for the regulatory agencies. Moreover, glycoengineering, the directed modification of protein glycosylation, or the artificial attachment of polymers to therapeutic proteins demand analytical tools for their characterization as well.

Growing knowledge of the biological roles of protein modifications, on the one hand, and the development and availability of sophisticated, sensitive analytical methods on the other hand, are already leading to increased interest in co- and post-translational modifications of proteins. *Post-translational Modifications of Proteins: Tools for Functional Proteomics* intends to serve as practical guide for researchers working in the field of protein structure–function relationships in general, in the rapidly growing field of proteomics, as well as scientists in the pharmaceutical industries.

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