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## Preface

*Posttranslational Modifications of Proteins: Tools for Functional Proteomics* is a compilation of detailed protocols needed to detect and analyze the most important co- and posttranslational modifications of proteins. Though, for reasons of simplicity not explicitly mentioned in the title, both kinds of modifications are covered, 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 the characterization of pharmaceutical proteins.

The majority of all proteins undergo co- and/or posttranslational modifications. Knowledge of these modifications is extremely important, since they may alter physical and chemical properties, folding, conformation distribution, stability, activity, and, consequently, 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 interactions, 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 on its amino acid sequence, which is determined by the corresponding DNA sequence, but also on the presence and structure of protein modifications.

The individual chapters provide detailed step-by-step instructions for the analysis of the most important protein modifications, especially the assignment of disulfide bond sites in proteins (Chapter 1). Analysis of protein glycosylation is treated in great detail. Starting with the analysis of carbohydrate composition (Chapter 2), the methods for cleavage, labeling, separation, and sequence analysis of N-linked glycans are described (Chapters 3–5). Analysis of protein O-glycosylation in general and specific detection of O-linked N-acetylglucosamine residues follow (Chapters 6 and 7, respectively). Finally, Chapter 8 provides a method to analyze the oligosaccharides present at specific single glycosylation sites in a protein.

A method for analysis of glycosylphosphatidylinositols describes analysis of the carbohydrate and lipid portions as well (Chapter 10). Two more protocols on the analysis of lipid modifications, in particular S-acylation (Chapter 11) and ubiquitination (Chapter 12) follow.

Further protein modifications of interest and different nature complete the book: analysis of protein methylation, acetylation, phosphorylation, and sulfation

(in Chapters 13–15, respectively), and analysis of  $\alpha$ -amidation,  $\gamma$ -glutamate, iso-aspartate, and lysine hydroxylation in Chapters 16–19. Topics of more general interest are treated in the final two chapters. Chapter 20 describes the use of a heterologous expression system for the analysis of posttranslational modifications and Chapter 21 shows how to detect the influence of glycosylation on protein spot patterns in 2D gel electrophoresis.

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 led 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 proteomics research, the description of expression levels of proteins related to a defined cell or tissue status will be incomplete without knowledge of the posttranslational modifications of those proteins.

In addition to possible changes in the activity or function of a protein, changes in its molecular weight or charge caused by protein modifications will influence the separation of proteins during 2D gel electrophoresis. Protein spot patterns generated by 2D electrophoresis will change because of altered protein expression or changes in the protein modifications. As an example, Chapter 21 describes detection and influence of sialylation and *N*-glycosylation on the protein spot pattern obtained by 2D gel electrophoresis.

(2) An additional important practical application of posttranslational modification analysis is to ensure the product quality of therapeutic pharmaceutical proteins. Recombinant proteins intended for therapeutic use in humans must be accorded particularly thorough investigation. Product quality depends on accurate posttranslational modification in the respective expression system during production, e.g., insect or several mammal cell lines. Note that different expression systems may vary in their ability to carry out posttranslational modifications and that changes in cell culture conditions also influence these modifications. Thus, posttranslational modifications of recombinant proteins have to be monitored during production and documented for registration. Directly related to this topic, Chapter 9 shows how to monitor glycosylation in order to ensure product consistency.

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 posttranslational modifications of proteins. *Posttranslational Modifications of Proteins: Tools for Functional Proteomics* intends to serve as a 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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