Preface

An Overview of Chemical Proteomics: Methods and Applications

The multidisciplinary science of chemical proteomics studies how small molecules of synthetic or natural origin bind to proteins and modulate their function. Scientists in the field have different backgrounds including molecular and cell biology, biochemistry, pharmacology, organic chemistry, and physics. *Chemical Proteomics: Methods and Applications* is directed at molecular biologists and biochemists with either an interest in small molecules themselves, e.g., in drug discovery projects, or in using small-molecule probes as research tools to study protein function. The book may also be useful for organic chemists with an interest in biology and for specialists in protein mass spectrometry.

In the introductory chapters, we discuss analytical strategies for chemical proteomics projects, with a focus on the current state-of-the-art in protein mass spectrometry, and describe several examples how chemical proteomics can impact the field of drug discovery. The consecutive chapters provide detailed experimental protocols. Most chemical proteomics projects consist of three parts. In the first part, the chemical probe is selected or designed, and then synthesized. In the second part, the probe compound is exposed to the protein sample or cell extract. In the final part, proteins binding to the probe compound are identified and often quantified. In simple applications, this is often achieved by antibody-based detection, but if the aim is the discovery of targets in an unbiased fashion, mass spectrometry is the method of choice. The following chapters cover all of these aspects.

The first set of chapters describes how probes are generated from commercially available reagents without elaborate chemical synthesis procedures, and how the proteins binding to the probes can be analyzed by immunodetection or by mass spectrometry. Rix et al. and Saxena provide protocols for direct noncovalent affinity capture using protein kinase inhibitors as an example, which serves to profile the targets of these compounds and provides probes for kinase expression and activity. Ge and Sem have developed a target class-specific probe for the labeling of dehydrogenase enzymes. Kawamura and Mihai, and Codreanu et al. describe the use of biotin-conjugated probes which form covalent adducts with defined subproteomes, here consisting of adenine-binding proteins and targets of lipid electrophiles. Lenz et al. combine features of noncovalent and covalent capturing strategies in their bifunctional ligands designed to target methyltransferases, an emerging class of drug targets.

The second set of chapters is concerned with techniques for the discovery of small-molecule targets and the probing of target function. Ong et al. describe the use of stable isotope labeling of amino acids in cell culture (SILAC) in identifying proteins that bind small-molecule probes in cell extracts. Hopf et al. perform affinity enrichment of target proteins on a probe matrix in the presence of competing free test compound in solution, thus enabling determination of binding potencies of the free test compound to affinity-captured proteins from cell extracts. The method employs quantitative mass spectrometry with isobaric mass tags to determine the potencies for a large number of targets in a single analysis. Ge and Sem have developed a protocol for the detection and purification of dehydrogenase

enzymes, which may represent targets, but also unwanted off-targets, for certain types of drugs. Kovanich et al. describe a combination of cAMP-based affinity chromatography with quantitative mass spectrometry to investigate protein kinase A complexes in extracts of cells and tissues. De Jong et al. use activity-based chemical probes to profile the activity of the proteasome, which has recently emerged as an important cancer target, in cells and tissues. The next three chapters provide innovative protocols for the study of potential drug targets by chemical cross-linking and mass spectrometry. Mueller et al. provide a method to study protein–drug interactions, and Gasilova et al. employ cross-linking and MALDI-mass spectrometry to study ligand modulation of protein–protein interactions. Jeon et al. provide a protocol for in vivo cross-linking via time-controlled transcardiac perfusion, which in principle enables the direct analysis of protein targets in animal models.

The final set of chapters is concerned with small-molecule ligand and drug discovery. Casalena et al. describe the discovery of probe compounds by utilizing compound libraries immobilized on microarrays. Wolf et al. delineate general guidelines for working with small molecules, including aspects like storage, the preparation of solutions, and the determination of solubility. The chapter by de Matos et al. provides guidelines for the use of the ChEBI database, which should be very helpful for researchers tasked with the selection of a particular probe or with building a small molecule collection to purpose. They describe the Chemical Entities of Biological Interest (ChEBI) database which helps to find probe molecules with the desired structural or biological features. Finally, many researchers will consider whether their research tool compound might have the potential to be developed into a drug. Zhang delivers a lucid analysis of the features that distinguish drugs from probe molecules, and lays out a set of rules for "drug likeness."

Affinity- and activity-based chemical probes, combined with quantitative immunodetection and mass spectrometry techniques, are increasingly gaining appreciation as powerful strategies for the molecular analysis of complex biological systems in homeostasis and disease. We hope that the methodologies described in this volume will contribute to a wider application of chemical proteomics methods in biochemical and cell biological laboratories.

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