Preface

Proteins are the cellular workforce of the critical pathways that are needed to maintain homeostasis and regulate a wide array of biological processes. These include modulating cell to cell communication, a host of events in the cytoplasm to drive metabolism and biological molecule creation, and nuclear processes to attenuate gene expression patterns and repair the genome during DNA damage. Essential to these actions are the successful completion of numerous biochemical reactions mediated by the catalytic activity of enzymes. Developments in technology have played significant roles in advancing our understanding of activity. Creation of recombinant DNA technology, molecular biology techniques, and specialized spectroscopy methods have allowed for tracking of enzymatic activities both *in vitro* and *in vivo*. Here in this edition of Methods in Enzymology, we have assembled a list of chapters that highlight various techniques and methodologies that can be used to characterize a multitude of aspects concentrated on protein post-translational modification (PTM) and this associated enzymatic activity.

Snyder and co-workers describe the latest advances using high resolution mass spectrometry (MS) to quantify acetyl-CoA and related species. This metabolite is highly utilized in the cell for many reactions, such as for modification of proteins such as histones. In a related vein, Hsu and co-workers also describe the metabolomics analysis of lipid modifying enzyme products by MS. Gottlieb et al. describe methodologies for expression and purification of recombinant N-terminal acetyltransferases, enzymes that utilize acetyl-CoA to modify the N-termini of many proteins. Approaches to determine the acetylation stoichiometry of proteins on a proteome-scale or to quantify the levels of protein acetylation during viral infection are nicely written by the Chen and Cristea labs, respectively. Additionally, the Christianson lab describes the preparation, purification, and crystallization of HDAC6 and HDAC8 inhibitor complexes, while Adams et al. describe the purification and enzymatic assessment of the class I HDACs, HDAC1 and HDAC2, all enzymes that remove protein acetylation marks.

Protein modification is central to transduction of activated signaling pathways. Kreuzer et al. describe the use of tandem mass tagging strategies to quantify over 20,000 phosphorylation sites from cultured cells or tissue. Parker and co-workers focus on the use of peptide substrates to monitor

tyrosine kinase activity from human cells. Li et al. detail methods using MS to identify and quantify poly-ADP-ribose modification on proteins. Fenselau and co-workers tackle the characterization of branched chain ubiquitin polymers, a modification important for DNA damage and protein degradation. Kashina and co-workers develop biochemical assay to follow protein arginylation activity *in vitro*. Dyer et al. discuss proteomic strategies aimed at detection of two elusive types of modification, S-nitrosylation and S-glutathionylation. Lund et al. focuses on obtaining quantitative MS of protein lysine methylated substrates. The Overall research group updates their Terminal Amine Isotopic Labeling of Substrates (TAILS) approach, a strong method for the enrichment of N-terminal peptides.

We have several chapters that deal with methodologies for protein glycosylation. Wu and co-workers concentrate their efforts on analysis of N-glycoproteins from the cell surface, while Liu et al. describe experimental and computational methods for glycosylated neuropeptides. Huang and co-workers use small molecules to silence the functions mediated by heparan sulfate glycosaminoglycans. Other chapters reveal protocols to understand peptide isomers and epimers (Julian and co-workers), modified troponin isoforms (Ge and co-workers) or review the latest in characterization of RNA post-transcriptional modifications (Liu and co-workers). Lastly, we also have two chapters based on creation of modified proteins. David and co-workers utilize the intein trans-splicing system for *in vivo* generation of modified proteins such as histones, while Rinehart and co-workers develop genetic code expansion techniques for enabling site-specific incorporation of phosphorylated amino acids onto recombinant proteins. Overall, we feel that these chapters will allow for others across many fields to characterize many different modified proteins and their enzymatic activity with detailed protocols that will lay the blueprints for their analyses.

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