PREFACE

High-energy phosphate ester bonds are often thought of as the energetic currency of the cell. Phosphoryl transfer is involved in processes ranging from the biosynthesis and catabolism of carbohydrates and polysaccharides, nucleic acids, and cofactors, to the regulation of cellular pathways. In addition, phosphoryl groups on small-molecule metabolites confer important properties—providing excellent "handles" for enzyme binding, trapping metabolic intermediates within the cell, and controlling the reversibility of metabolic pathways. The phosphorylation states of proteins also afford key control points for signal transduction and the regulation of myriad critical physiological processes. For this reason, kinases are extremely important therapeutic targets and there is significant interest in developing diverse approaches to regulate their function through small-molecule inhibitors. We present here the less studied but equally important complement of phosphate ester chemistry, namely the coordinated action of phosphatases. Phosphatases represent control points in pathways and new opportunities for use in chemoenzymatic synthesis. In many cases, such critical players may be functionally unannotated. However, there are many challenges in their study—spurring on the efforts described in this volume. Phosphoryl transfer is, itself, complex chemistry to describe with highly charged phosphate ester substrates and the frequent participation of metal ions in enzyme-catalyzed reactions. Structure/function analysis and specificity are especially thorny problems to study in phosphatases because of the large-scale conformational changes that often accompany binding events. Indeed, these conformational changes can be key in achieving specificity and may also become the rate-limiting step in catalysis. In terms of assaying phosphoryl transfer, the number of direct assays is limited and special considerations must be taken into account to achieve physiologically relevant measurements of activity.

I am excited to present this volume of *Methods in Enzymology* on phosphatases which draws upon approaches from the varied disciplines of computational and organic chemistry, biophysics, biochemistry, bioinformatics, and cell biology to address the structure, mechanism, and regulation of phosphatases. The themes covered in the volume comprise many creative strategies including computational approaches for understanding phosphoryl transfer, means for assessing mechanism, conformational changes and enzyme dynamics, the assignment of enzyme function and specificity, and

the regulation of phosphatases. Although each of the methods presented here target-specific enzymes or enzyme families, there is clear application to other phosphatase targets and indeed, in many cases, more generally to other reaction types.

In the first chapter, Purg and Kamerlin tackle the mechanism of diisopropyl fluorophosphatase using the empirical valence bond approach. This computationally efficient method allows them to perform extensive configurational sampling along the entire reaction trajectory of the enzyme. In a complementary discussion, Cui and colleagues apply QM/MM free-energy simulations to elucidate the mechanisms of alkaline phosphatase and myosin. The authors point out that the computational efficiency of this approach is especially helpful in the case of these enzymes, where trends in the transition state or energetics are critical to the interpretation. The two chapters by Goldman and colleagues move the discussion of mechanism into the experimental realm, in an enzyme system in which the hydrolysis of inorganic pyrophosphate is coupled to the pumping of ions across the membrane. The coupled-vectorial processes enacted by these membrane-bound enzymes, previously captured in snapshots by crystallography, are now made dynamic by employing Förster resonance-energy transfer measurements to derive information on distance and the frequency of conformational transitions. The experimental methods presented are enhanced by molecular dynamics and computational modeling, improving both experimental design and data analysis. In the following chapter, bioinformatic approaches on the evolution and differential mechanisms of the pyrophosphatases are enhanced by a hydrolytic activity assay to assess potassium ion dependence. The focus on mechanism is continued by Allen and colleagues, who present methods for obtaining X-ray crystal structures where substrates, products, and intermediate/transition-state analogs are liganded to members of a large superfamily of phosphatases, allowing us to traverse the entire reaction coordinate.

The next four chapters are directed toward the challenges of determining substrate specificity and of defining the enzyme conformational states resulting from ligand binding. The first of these addresses the need for assignment of enzyme function, accelerated by the ever-growing release of new genomic data. In their chapter, Ghodge and Raushel outline a complete template for the assignment of function, using as examples members of the polymerase and histidinol phosphatase family of proteins. The approach leverages the shared structural and mechanistic features of the family to predict substrates and plan targeted mechanistic studies of closely related homologs. Quantitation of the binding affinities of substrates and inhibitors and

associated changes in molecular state are tackled in the chapter by Xu and Van Doren. Their described TREND NMR software package fits binding isotherms from titrations detected with NMR spectra. Protein tyrosine phosphatase 1B variants and inhibitor complexes are used to exemplify the application of principal component analysis in order to group and compare NMR-detected enzyme states. Stiers and Beamer define methods to probe the flexibility of the various conformational states associated with phosphorylation of the nucleophile in enzymes in the α-D-phosphohexomutase superfamily and describe means to control and quantify the extent of phosphorylation. The last chapter in this section, by Zhang and colleagues, addresses the question of how to study specificity when the phosphatase is highly selective for *cis*- or *trans*-amide conformations within serine/threonine–proline motifs. Their solution is to use proline-mimicking isosteres that are locked to prevent interconversion.

Phosphatases are integral to cell signaling processes and are thus themselves subject to regulation. The last chapters in this volume describe examples of regulators and regulation and methods to assess them. The contributions from Bourret and Silversmith and from Gao and Stock detail quantitative analyses of bacterial response regulator phosphatases in vitro and in vivo, respectively. The in vivo data combined with mathematical modeling allow for the quantitative assessment of intracellular phosphatase activity of sensor histidine kinases. Complementary in vitro approaches comprise assay methods for chemotaxis two-component signal transduction phosphatases and modifications for those phosphatases not involved in chemotaxis two-component systems, where the timescales differ. The following chapter by Tipton and Hannink delves into the problem of purifying and assaying dimeric and multimeric oligomerization states of a phosphoglycerate mutase family member that regulates mitochondrial dynamics. Purification and physiologically relevant assay methods are also presented in the chapter by Granade and Harris, where the phosphatidic acid phosphatase activity of lipin is measured using small unilamellar vesicles. Mrksich and colleagues describe an approach using mass spectrometry with peptide arrays to study the substrate specificities of two protein tyrosine phosphatases. As the authors highlight, the mass spectrometry method is particularly significant for phosphatases as there are few other existing assays capable of directly measuring dephosphorylation. Finally, Dempsey and Cole showcase the semisynthesis of a C-terminally phosphorylated phosphatase and tensin homolog, PTEN, and develop methods to assess the effect of phosphorylation on protein stability, structure, and enzymatic activity.

Here, the latest methods of interrogating phosphatase chemistry can be found, hopefully benefiting researchers of this multifaceted and important area of enzymology. In conclusion, I wish to thank the authors for their valuable contributions to this volume, detailing approaches spanning from the computer to the cell. I also thank the editorial and production teams at Elsevier for their assistance in bringing this volume to light.

KAREN N. ALLEN
Department of Chemistry,
Boston University,
Boston, MA, United States