PREFACE

The computational study of enzyme structure and function has reached exciting and unprecedented levels. This state of affairs is due to a combination of powerful new computational methods, a critical evolution of ideas and insights, the ever-increasing power of computers, and important new experimental results for validation. In these two volumes of *Methods in Enzymology*, many of the leading computational researchers present their latest work, representing a range of state-of-the-art topics in computational enzymology. Generally speaking, the two volumes are divided into two general areas, the first being mostly devoted to the calculation of the free energy barriers and reaction pathways for enzymes—often using powerful quantum mechanics/molecular mechanics (QM/MM) methods—while the second volume contains a broader range of topics, including the role of enzyme dynamics and allostery, electrostatics, ligand binding, and several specific case studies.

The topic of computational enzymology—and the field of computational biophysics and biochemistry in general—has grown enormously since its inception in the early 1970s, with some of these original work being recognized by the 2013 Nobel Prize in Chemistry. It is tempting to conclude that the field is now "mature" and all that remains is for researchers in it to carry out increasingly detailed and accurate set of applications of the powerful computational methods presented herein. However, nothing could be farther from the truth. Real enzymes exist and function in highly complex, multiscale biological environments. Often several enzymes function cooperatively, and they can be influenced by, or respond to, their local environment, whether be it a lipid membrane or the crowded cellular interior. In the venerable theories of activated dynamics for condensed-phase chemical kinetics, such as transition state theory, it is tacitly assumed that the chemical reaction dictates the slowest dynamical time scale of the system, thus corresponding to the highest free energy barrier. This basic assumption also allows one to apply these simpler theories to calculate quantities such as the free energy profile, ie, free energy barrier, for a reaction along a chemical pathway in an enzyme (the so-called potential of mean force). Yet, in complex biological systems there are a wide range of timescales associated with *numerous* processes, some of which may be intrinsically coupled to the reactive process of the enzyme. In that light a key question then arises for the future.

Can we better understand enzyme kinetics in the larger biological context of the living cell through computation? This challenge awaits us.

There is also the important fact that real biological systems are *not in a state of equilibrium*. Indeed, they can be rather far from it. Much of the standard condensed-phase kinetic theory developed in the last century—and applied in present-day computational enzymology—relies on the key notion of the famous fluctuation-dissipation theorem, ie, the behavior of systems perturbed out of equilibrium can be understood from studies of ones that are actually *in equilibrium*. This so-called linear response assumption leads us to powerful mathematical formulas for observables such as kinetic rate constants, as well as the algorithms one uses to compute them, which are based on *equilibrium* molecular dynamics simulation. However, much work remains to be done to develop theories and computational algorithms for enzymes functioning in a nonequilibrium biological context, albeit some important work in that direction, motivated by experiments, has already been initiated.

The great degree of progress to date on the topic of computational enzymology is reflected in these two volumes of *Methods in Enzymology*. Moreover, this field of research continues to evolve at an increasingly rapid pace. The scope of the enzyme systems presently under study, and the elaboration of their complex behaviors, is remarkable. As an example, I can point to some of the research completed by talented young theorists as they passed through my own research group [see McCullagh, M., Saunders, M. G., & Voth, G. A. (2014). Unraveling the mystery of ATP hydrolysis in actin filaments. *Journal of the American Chemical Society*, *136*, 13053–13058.]. In this work, QM/MM, molecular dynamics, advanced free energy sampling, and insights from coarse-grained modeling were all combined to explain the origins of the $>10^4$ acceleration of ATP hydrolysis in actin filaments (F-actin) over the free monomeric form (G-actin). This ATP hydrolysis by F-actin, which has been a mystery for years, is critical to the functioning of the actin-based eukaryotic cellular cytoskeleton and now computation has solved it.

Nevertheless, in light of the great remaining challenges described earlier, it is abundantly clear that much remains to be done to further advance computational enzymology, in some cases even at a qualitative level of basic understanding. It will certainly be both important and fascinating to survey future volume(s) of *Methods in Enzymology* devoted to this topic—perhaps 10 or even 20 years from now—and to celebrate what I am sure will the outcomes from an exciting and continual evolution of this important field of research.

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