The Power of Physical Chemistry Unleashed on Proteins

ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · FEBRUARY 2011

Impact Factor: 7.46 · DOI: 10.1021/jz200086z

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THE JOURNAL OF PHYSICAL CHEMISTRY Letters

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The Power of Physical Chemistry Unleashed on Proteins

Understanding how living systems work demands deep insights from physics, and these insights come from powerful physical methods. This issue of *J. Phys. Chem. Lett.* offers three Perspectives that describe novel spectroscopic methods and how they are applied to major questions about protein structure, function, and folding. The authors illustrate how they can explore protein energy landscapes, intricacies of protein−metal interactions, and protein folding in the cell with these spectroscopic approaches and thereby give a tantalizing taste of how the power of physical chemistry can address knowledge gaps in biology.[1−3]

Protein structures are not static, despite the impression given by atomic-resolution images from X-ray crystallography. Indeed, it is increasingly clear that protein functions rely on their dynamic nature.[4] For example, the dynamic properties of proteins enable their catalytic efficiencies to be tuned by regulatory molecules, their binding specificities to be switched to create specialized signal transduction networks, and their mechanical movements to be coupled to energy sources in order to perform tasks like muscle contraction and ion movements across membranes. Understanding the intrinsic dynamics of proteins requires methods that yield information on a wide range of time scales. A growing arsenal of nuclear magnetic resonance,[5] electron spin resonance,[6] single-molecule fluorescence,[7] and computational[8] methods help to shed light on dynamics from the millisecond to picosecond time scales. However, each has its limitations. It is abundantly clear that application of new methods and integration of data from multiple methods will help conquer the challenges of complex biological systems. The Perspectives in the issue describe some less widely used approaches that have experienced great advances in recent years. Their use greatly expands the capabilities of the biophysical chemist.

In one Perspective in this issue, Asher and co-workers[1] present an informative review on developments and applications of UV resonance Raman (UVRR) spectroscopy of proteins. This method has the potential to explore in detail the conformational landscape of a polypeptide backbone by direct excitation of the peptide bond or, alternatively, to interrogate in depth the behavior of protein cofactors such as hemes. Adding to the power of UVRR is the use of temperature jumps to trigger conformational re-equilibration and extract kinetic descriptions of folding and unfolding events. This Perspective abundantly demonstrates ways in which UVRR can provide information that is elusive by other methods.

The Perspective by Aziz[2] gives an overview of how the structure and function of metal centers in proteins can be explored using X-ray spectroscopies enhanced by synchrotron light sources and innovative microjets. The power of these approaches is multifold. Not only can proteins be studied in solution, but also, the electronic states of protein-bound metals can be explored in exquisite detail, elucidating the nature of their energy levels and ligand binding. Aziz describes new methods such as core−hole X-ray absorption spectroscopy (XAS), which yields structure and dynamics in femtoseconds, and L-edge XAS of transition metals, which has revealed details about the active centers of respiratory and photosynthetic metalloproteins. He reports on observation of sub-background features in fluorescence yield mode of XAS (XAS-FY) and how this is applicable to electron-transfer reactions and solute interactions.

A major frontier in biology is the exploration of detailed molecular events in the native complex environment of the cell. In another Perspective in this issue,[3] Ebbinghaus and Gruebele describe recent efforts to follow protein folding in vivo and review how the complex nature of the cellular environment makes such experiments daunting. Nonetheless, their importance is undeniable as it is highly likely that the folding landscape will be altered by cellular factors such as macromolecular crowding and molecular chaperone interactions.[9] This Perspective provides a description of the authors' own very fruitful development of methods to spatially resolve the response of a protein inside of a mammalian cell to small temperature jumps. The signal observed arises from resonant energy transfer between genetically encoded fluorescent probes on the protein of interest, and images are mapped onto the cell using a fluorescence microscope. In this way, protein denaturation curves have been obtained for a protein within a cell. These authors found that phosphoglycerate kinase was 8 kJ/mol more stable inside a cell than in solution. The impact of the cellular environment may not be the same for all proteins as our laboratory had previously observed a decrease in stability for a small single-domain protein in *E. coli* cells.[10,11] Extension of these studies to other proteins and cell types is needed to develop a clear picture of the folding landscape in vivo.

Taken together, these three Perspectives[1−3] provide the interested reader with a smorgasbord of innovative and informative physical methods and illustrates how they can rewardingly be applied to current problems in protein biochemistry.

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Received Date: January 19, 2011
Accepted Date: January 19, 2011
Published on Web Date: February 17, 2011

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DOI: 10.1021/jz200086z | *J. Phys. Chem. Lett.* **2011,** *2*, 327–328