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Protein dynamics: dancing on an ever-changing free energy stage

Editorial overview

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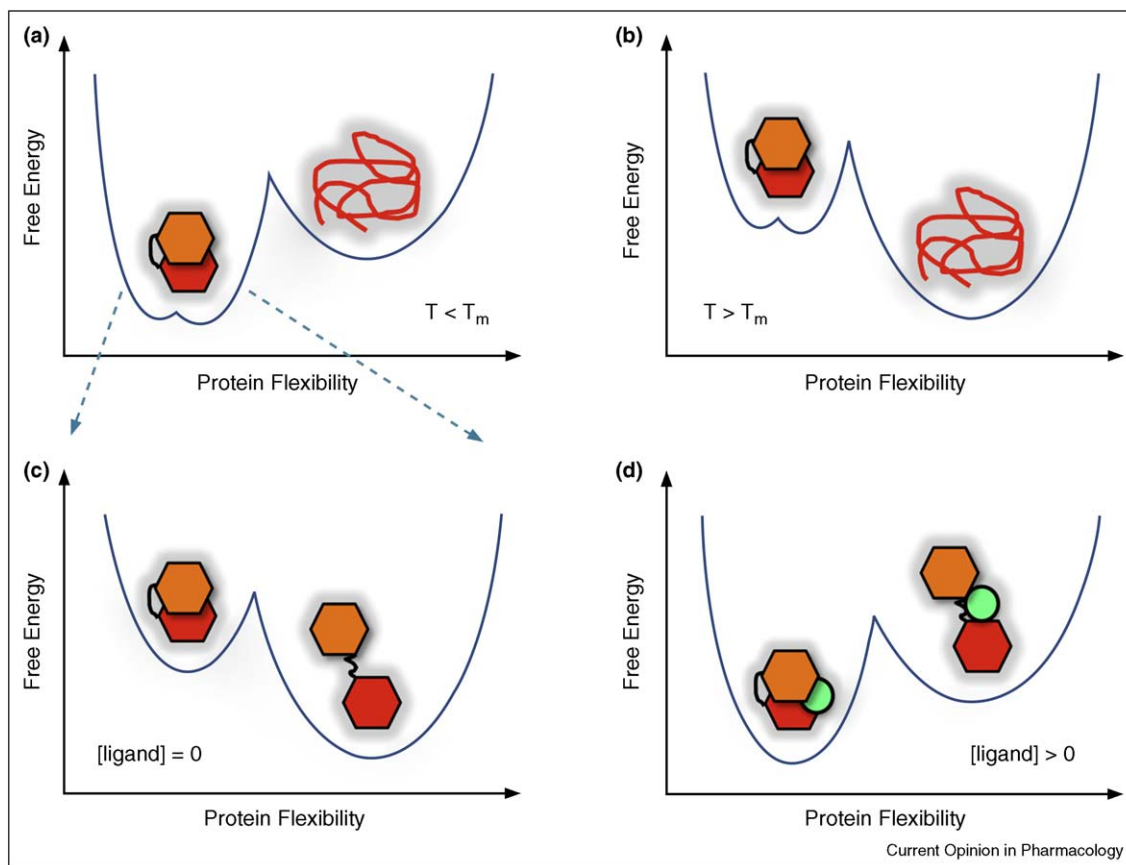
Dr. Dennis R. Livesay is an Associate Professor of Bioinformatics and Genomics at the University of North Carolina at Charlotte. He received his PhD in Physical Chemistry from the University of Illinois at Urbana-Champaign in 2000, and joined the chemistry faculty at California State Polytechnic University immediately thereafter. He moved to his current position in Charlotte in 2006. Dr. Livesay's research is focused on elucidating the physical principles that underlie protein family evolution. Past efforts have focused on the importance of protein electrostatics, and current emphasis is on the give-and-take between protein stability and flexibility.

The importance of protein dynamics has long been recognized [1–5]; however, methods to accurately describe dynamical changes are relatively recent, leading to a sea change in structural biology. Discussions of dynamics, ensembles and population shifts are now ubiquitous within protein science [6], which advocate more nuanced descriptions of pharmacological protein receptors [7]. In the same way that static photos of a dance recital certainly fail to reflect the completeness and grandeur of the performance, discrete structural snapshots lack sufficient information to completely describe protein dynamics. A proper description of protein structure and function requires more complete characterizations of this shapeshifting dance. In this set of reviews, recent advances in studying protein dynamics are presented, with the goal of encouraging pharmacologists to incorporate ensemble-based views of dynamics into their research and drug development assays. That is, significant advances that provide the technical and theoretical tools to overcome the challenges associated with the immense range of dynamical timescales are surveyed.

The importance of the free energy landscape (FEL) is a common theme throughout all of the reviews [8]. The FEL is a hyperdimensional surface that defines all possible protein conformations and their corresponding free energies, which are inversely related to their probabilities via the Boltzmann relationship. Free energy basins that correspond to the most populated states group similar, yet slightly distinct conformations. Importantly, equilibrium fluctuations occurring within the native-like basin are related to protein function and ligand-binding [9], thus obsoleting discrete models of protein conformation change. An equally important feature is that the FEL changes shape dependent upon thermodynamic and environmental conditions. For example, as depicted across the top row of Figure 1, the natively folded protein is the most stable at low temperatures; however, at temperatures above the melting point, T_m , a population shift occurs such that the denatured protein becomes the thermodynamically most favorable state. Note that FELs are often expressed in reduced dimensions on the basis of on various global measures of protein foldedness for convenience. Two common reduced dimensionality order parameters include the number of native contacts present and the global flexibility of the protein.

An alternate way of thinking about the changing free energy landscape is that it is not changing at all. Rather, temperature is yet another dimension within the hyperdimensional surface, meaning that panels (a) and (b) represent two slices through the surface. While conceptually difficult, this abstract view stresses that thermodynamic and environmental conditions are intimately and innately related to conformational probabilities. Other common 'dimensions' that are regularly manipulated include: pressure, pH, ionic strength and co-solute concentration [10]. From a pharmacological

Figure 1



The protein free energy landscape (FEL) is a hyperdimensional surface that defines all possible conformations and corresponding free energies. Here, the hyperdimensional surface is expressed as function of a global flexibility order parameter to aid visualization. **(a)** As indicated by the structural fuzziness, multiple conformations are grouped within free energy basins. In this example, the protein ensemble includes a major native-like state and a minor unfolded state. The FEL and consequent probabilities change based on temperature, as shown across in panel **(b)**. Myriad other thermodynamic and/or solvent properties can likewise be manipulated. Panel **(c)** zooms in on the native basin, revealing two distinct conformational states. Assuming the free energy difference and straddling barrier are small, the protein will continually interconvert between the 'closed' and 'open' sub-states. In panel **(d)**, the ligand (green circle) concentration is increased from zero to some appreciable amount. There is a population shift upon introduction of ligand that stabilizes the closed relative to the open. As discussed within the text, an alternate and more abstract view includes the set of physically manipulatable quantities within the FEL hyperdimensionality; however, the two viewpoints are practically equivalent.

point of view, ligand concentration is a critical dimension to consider. Note that the bumpy native basin in panel (a) suggests that two distinct conformational sub-ensembles, separated by a relatively small free energy barrier, is a better description of our protein. The lower left panel indicates that the protein fluctuates back and forth between the 'closed' and 'open' states, which is consistent with discrete views. However, functionally important fluctuations also occur within each state. That is, fast fluctuations within the basin and slow barrier-hopping fluctuations occur concurrently. Upon introduction of ligand in panel (d), these concurrent dynamical processes continue, but a population shift has occurred that stabilizes the closed state relative to the open. These nuanced views of structure and dynamics have provided immense insight into protein function, and make up our modern view of protein dynamics. These general concepts and

their importance to pharmacology are excellently surveyed in a pair of reviews from Schug and Onuchic and Kar *et al.*

Most of the reviews included here focus on the methods to describe protein dynamics. Some of the methods characterize the fast equilibrium fluctuations occurring on the ps–μs timescale within a given basin, whereas others characterize slower processes (ms or beyond) that require crossing from one basin to another. For example, the NMR methods surveyed by Sapienza and Lee are excellent for probing the former, whereas the spectroscopic methods discussed by Yengo and Berger are better suited for the latter. Reviewed by Salsbury, molecular dynamics (MD) simulations provide exquisite detail regarding the conformational fluctuations occurring within a basin. However, as discussed by Zwier and

Chong, the inherently serial MD algorithm does a poor job of crossing free energy barriers, necessitating algorithmic changes that promote barrier crossing for the simulation of slower processes. Highlighting the interconnectedness of the presented topics, Sapienza and Lee conclude their review by stressing the synergy within the fast fluctuations described by NMR and MD.

A pair of reviews by Rader and Jacobs presents alternate computational methods for studying protein dynamics. Rader focuses on coarse-grained methods that sacrifice model accuracy for computational efficiency, thus facilitating characterization of longer timescale processes. Juxtaposed to simulation methods that produce the conformational space, Jacobs presents a set of computational methods that start *a priori* from descriptions of the ensemble. Because the size of the ensemble is astronomically large, the computational challenge is to identify and focus on the most probable conformations. Moreover, since conformational probabilities are related to free energy, this requires accurate free energy calculations, which is a limiting factor of such methods.

The set of reviews conclude with three surveys of topics specifically related to pharmacology. The first by Durrant and McCammon presents recent advances in designing drugs for a moving target. In order for computer aided drug design to advance further, improved considerations of drug and receptor flexibility are absolutely vital. Vaidehi and Kennakin discuss conformational ensembles within the context of seven transmembrane receptors, which are the most common pharmacological target [11]. Specifically, they demonstrate how conformational plasticity is related to agonist affinity, elegantly highlighting the pharmacological importance of dynamics. Finally, Dunker and Uversky review the tendency for transcription factors to be intrinsically disordered, which challenges our traditional view of protein structure/function relationships. Notably, these intrinsically disordered transcription factors present novel and potentially revolutionary therapeutic strategies that are just now being exploited.

In summary, studying protein dynamics is technically and theoretically difficult because conformational changes are

occurring concurrently over a vast continuum of time-scales, which are defined by the roughness of the underlying FEL. Important aspects of protein stability, function, regulation and, by extension, druggability are related to different dynamical timescales. As such, a complete view of protein structure requires a nuanced view of dynamics that reflects the intricacies of the protein dance. Fortunately, recent technical advances are providing several experimental and computational methods for interrogating these events, which is leading to important advances in our understanding and ability to cure disease.

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