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Single-Molecule Analysis of Protein Large-Amplitude Conformational Transitions

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Proteins have evolved to harness thermal fluctuations, rather than frustrated by them, to carry out chemical transformations and mechanical work. What are, then, the operation and design principles of protein machines? To frame the problem in a tractable way, several basic questions have been formulated to guide the experimental design: (a) How many conformational states can a protein sample on the functionally important timescale? (b) What are the inter-conversion rates between states? (c) How do ligand binding or interactions with other proteins modulate the motions? (d) What are the structural basis of flexibility and its underlying molecular mechanics? Guided by this framework, we have studied protein tyrosine phosphatase B, PtpB, from M. tuberculosis (a virulence factor of tuberculosis and a potential drug target) and adenylate kinase, AK, from E. coli (a ubiquitous energy-balancing enzyme in cells). These domain movements have been followed in real time on their respective catalytic timescales using high-resolution single-molecule Förster resonance energy transfer (FRET) spectroscopy. It is shown quantitatively that both PtpB and AK are capable of dynamically sampling two distinct states that correlate well with those observed by x-ray crystallography. Integrating these microscopic dynamics into macroscopic kinetics allows us to place the experimentally measured free-energy landscape in the context of enzymatic turnovers.