Characterization of Protein Kinase A Free Energy Landscape by NMR-Restrained Metadynamics

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The free-energy landscape of a protein underlies the conformational transitions that are vital to its biological function. Recent advances in experimental and computational methods are making it possible to characterize these free energy landscapes. In particular, the use of enhanced sampling techniques in molecular dynamics simulations, including the replica average metadynamics (RAM) method, have partly alleviated the sampling bottleneck and bridged the gap between simulations and experiments. Here we applied RAM to study the free energy landscape of the catalytic subunit of protein kinase A in the apo, binary (with ATP), and ternary (with ATP and pseudosubstrate, PKI_{5-24}) forms. We used backbone NMR chemical shift restraints in all three states to bias the conformational search toward conformations more reflective of experimental observables. Through this rigorous approach, we were able to characterize the rugged free energy landscape of protein kinase A and identify its modulation by ligand binding: whereas the apo state exhibits heterogeneous conformations, nucleotide binding partly reduces the conformational plasticity, and subsequent inhibitor binding further quenches the fluctuations. We conclude that **NMR-Restrained Metadynamics** is a promising approach to describe the free energy landscapes of complex proteins at atomic resolution through the integration of experiments and simulations.