Dheeraj Kumar Sarkar, M.Sc.

Simulations of ligand binding processes in proteins

Biological processes are intrinsically regulated by small molecules and their interactions, particularly in the form of protein-ligand complexes. The transport of ligands in proteins plays a crucial role in many biological processes, including signal transduction, enzyme catalysis and the transport of nutrients and metabolites. Therefore, understanding ligand binding processes is of great importance for the structure-based design of drugs and the engineering of improved enzyme catalysts. Notably, a significant fraction of enzymes have their active site buried in deep cavities, and the challenges lie in successfully capturing the ligand binding and unbinding processes, as access to those cavities by small molecules is generally restricted by protein tunnels and molecular gates. Changes in protein tunnels can often lead to altered activity, selectivity, promiscuity, and stability. To address these shortcomings, in my Ph.D. research, I contributed to investigating thermal and kinetic properties by assessing the utilization of small molecules via transport tunnels in proteins with buried active sites. Enhanced sampling molecular dynamics (MD) methods were applied to effectively assess the thermodynamics and kinetics of ligand binding processes while understanding the underlying molecular mechanisms. The thesis consists of three manuscripts. The first part of the thesis focuses on developing a method by designing knowledge-based seeding schemes to study ligand interaction processes in haloalkane dehalogenase effectively. The method employs adaptive sampling simulations for high-throughput sampling of binding phenomena, guided by Markov State Models (MSMs) to generate meaningful kinetic models describing protein-ligand bound and unbound long-lived states and their interconversion processes. In the second part of the dissertation, the massive use of molecular tunnels to facilitate ligand transport was evaluated and quantified to gain more detailed insights into ligand transport processes, showcasing the applicability of the in-house developed software tool. Finally, the third part of the thesis deals with the accessibility of the Cytochrome c active site for crowded hydrotropes and the role of their binding on the thermal stability of this enzyme. The process was studied at two temperatures involving three different compositions of the hydrotropes using adaptive sampling simulations guided by Markov models. Several insights were revealed into the role of hydrotropic solvents in providing stability to functional parts of Cytochrome c and regulating the dynamics of the open and closed states. Overall, this thesis represents the application, evaluation, and development of a high-throughput molecular dynamics protocol using protein-ligand complexes to study ligand binding processes and its role in improving the understanding of the coupling between the dynamics and function of enzymes.

Keywords

Adaptive, High-throughput simulations, ligand binding processes, Markov state models, enzymes, tunnels, gates