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PROJECT TITLE: Novel methods for efficient simulation of ligand transport in enzymes and their application

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Research project objectives/Research hypothesis

The primary goal of this project is to unveil molecular bases of largely unexplored factors notably affecting biological function of enzymes with buried active sites, i.e., substrate inhibition, cooperativity and interference between molecules of substrates and products during their simultaneous transport via molecular tunnels. First, we will generate comprehensive kinetics models of ligand transport in model enzymes derived from iteratively guided molecular dynamics simulations. These models will enable us to perform detailed analysis of the structure-dynamics-function relationships governing the transport of multiple ligands via the tunnels, revealing roles of direct interactions among ligands as well as allosteric effects on the tunnels mediated by protein-ligand interactions. This analysis will identify structural features responsible for the substrate inhibition, cooperativity and ligands' interference. Finally, the predictions from these models will be validated by designing mutations in the identified sites and characterizing them through combined experimental and computational approaches.

Research project methodology

Initially, we will carry out series of independent unbiased and accelerated molecular dynamics simulations of haloalkane dehalogenase LinB and its tunnel mutants to study structure and dynamics of their tunnels, and allosteric pathways possibly connected to the tunnels. Next, we will study transport processes for substrate and products separately. We will perform enhanced simulations with various starting configuration, followed by unbiased adaptive simulations to construct Markov state models of transport processes to identify kinetically meaningful metastable states and their transition probabilities, describing relevant steps involved in the transport processes. Then we will include multiple molecules of ligands into the simulations to learn how the transport processes are influenced by interactions among of several ligand molecules providing insights into the structural bases of the substrate inhibition and cooperativity s. Subsequently, the same simulation procedure with a mixture of substrates and product will be performed to study how the mechanisms and kinetics of transport processes in the studied enzyme variants change when multiple competing chemical species coexist.

Finally, we will replicate the analysis also for related DhaA enzyme and its two tunnel mutants and compare similarities in the transport processes between DhaA and LinB variants to dissect components of the transport mechanisms that are conserved within the protein family. Throughout the project, we will systematically validate our predictions against the existing experimental data, and confirm any novel findings by designing new mutants and performing their combined computational and experimental characterization.

Expected impact of the research project on the development of science

The project will provide a valuable addition to our current fundamental knowledge on the structure-dynamicsfunction relationships in the dehalogenases that represent a suitable

model system for enzymes with buried active sites as well as the large superfamily of hydrolases. We also expect to obtain novel insights into the origins of the substrate inhibition and cooperativity – phenomena necessary for appropriate *in vivo* functions of many enzymes. The obtained knowledge could later be exploited to target these properties in research aiming at engineering improved enzymes or developing novel inhibitors.
Finally, the findings on the mutual interference among different ligands and effects on their transport could facilitate more accurate studies of enzyme-drug association/dissociation processes enabling optimization of drugs residence times.