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**Role of Transient Tunnels in Function of Enzymes with Buried Active Sites**

The enzymes with buried active sites have pathways leading to them which are called tunnels. By focusing on the hydrolase family of enzymes and emphasizing the importance of water within these tunnels, this study provides valuable insights into their functional implications. Through the utilization of molecular dynamics simulations and adaptive sampling techniques, the conformational states of the systems are thoroughly investigated. Notably, the calculation of tunnels leading to the active site is conducted for all frames of the extensive simulation, leading to the identification of previously unrecognized tunnels. By tracking the movement of water molecules from the bulk to the defined region (active site) of the protein, the transport pathways employed by the enzyme during the simulation are determined. To facilitate the analysis of vast amounts of data related to tunnels and water molecules, a Python module called TransportTools is developed. The module streamlines the analysis of extensive tunnel and water molecule data. It aids in assessing water molecule utilization, transport efficiency, and functionality within protein structures. This tool has uncovered new water-transporting tunnels in enzymes like haloalkane dehalogenase, enhancing our understanding of tunnel dynamics. Additionally, it is discovered that water molecules can effectively navigate narrow regions within the tunnels through hydrogen bonding interactions. These findings are validated through a comparison between wild type epoxide hydrolase and its mutant E470G, where an increase in the utilization of alternate tunnels is observed in the mutant. Moreover, the choice of explicit water models such as OPC, TIP3P, and TIP4P-Ew significantly impacts the utilization of enzyme tunnels by water molecules. The observed differences between the three water models can be attributed to variations in their diffusion properties and charge distributions. With TIP3P model, water migration was noticeably accelerated, transporting about 2.5– and 2.0-times more water molecules than with OPC and TIP4P-Ew models, respectively. This phenomenon primarily arises from the observed quicker transit times and more water molecules concurrently migrating through tunnels when employing the TIP3P model. The consistency of these findings across various enzymes, such as alditol oxidase and cytochrome P450 2D6, along with tunnel geometries, highlights their broader relevance. This emphasizes water model importance for accurate simulations of enzymes with buried active sites which are critical for understanding their catalytic cycles, enzyme design, and predictions of drug residence times.