Enzyme Evolution and Design with Hamiltonian Replica Exchange Molecular Dynamics

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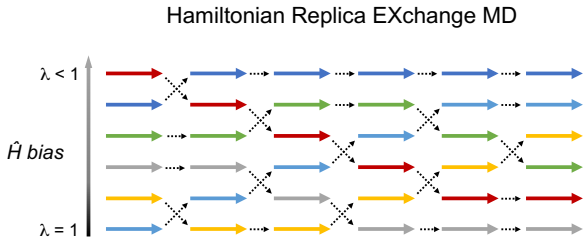
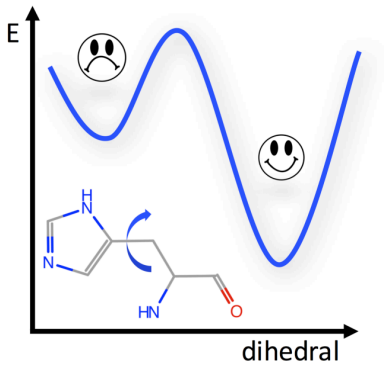
Molecular dynamics (MD) simulations are a powerful tool for computational enzymology.[1] However, classical MD is typically unable to cross high energy barriers and explore the complete conformational space at the commonly used nanosecond time scales.[2] Therefore, various enhanced sampling methods have been developed. Hamiltonian replica exchange (HREX) MD is a potent sampling method where *n* replicas of a system are simulated, with varying Hamiltonians, and are allowed to exchange the coordinates from time to time.[3] We studied how protein flexibility affects the catalytic activity over the directed laboratory evolution of glucose oxidase. The active site histidine can adopt two conformations: a catalytic and a non-catalytic one. While classical MD simulations were able to sample both, only one transition between the two conformations would typically happen during 100 ns simulation. This made it impossible to determine the relative populations of the two. On the other hand, even short HREX-MD runs were able to reproduce the experimental kinetic data for the glucose oxidase mutants. In another application, we used HREX-MD simulations to study the binding of a macrocycle to cytochrome P450. The substrate docking was not conclusive as many different binding poses were evaluated with similar scores. Due to the size and chemistry of the substrate, classical MD would not be an efficient method to explore ligand binding in this case. Therefore, we biased the substrate's Hamiltonian in the HREX simulations and, based on the identified low-energy binding modes, we performed enzyme design to manipulate the chemo-, regio-, and stereoselectivity of this enzyme.





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[2] J.L. Klepeis, K. Lindorff-Lasen, R. O Dror, D.E. Shaw, *Curr. Opin. Struct. Biol.*, **2009**, *19*, 120-127.
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