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Current Opinion in Structural Biology

Molecular modeling of conformational dynamics and its role in enzyme evolution

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With increasing computational power, biomolecular simulations have become an invaluable tool for understanding enzyme mechanisms and the origins of enzyme catalysis. More recently, computational studies have started to focus on understanding how enzyme activity itself evolves, both in terms of enhancing the native or new activities on existing enzyme scaffolds, or completely *de novo* on previously non-catalytic scaffolds. In this context, both experiment and molecular modeling provided strong evidence for an important role of conformational dynamics in the evolution of enzyme functions. This contribution will present a brief overview of the current state of the art for computationally exploring enzyme conformational dynamics in enzyme evolution, and, using several showcase studies, illustrate the ways molecular modeling can be used to shed light on how enzyme function evolves, at the most fundamental molecular level.

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Current Opinion in Structural Biology 2018, **52**:50–57

This review comes from a themed issue on **Biophysical and computational methods**

Edited by **Mark Yeager** and **Gregory A Voth**

https://doi.org/10.1016/j.sbi.2018.08.004

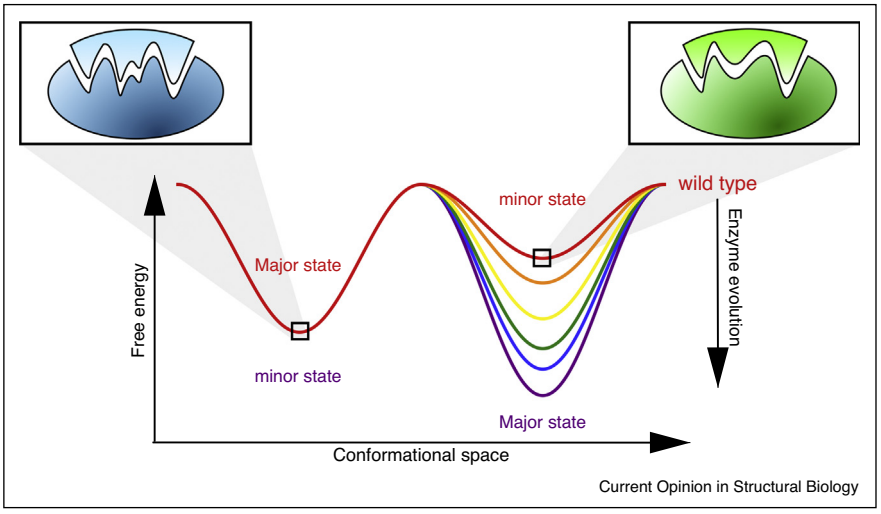
Introduction

There has been substantial debate in recent years about the extent to which conformational dynamics plays a role in facilitating enzyme catalysis [1–11]. What is even less well understood, however, is the extent to which the fine tuning of enzymes' conformational ensembles acts as a driver for molecular innovation and the evolution of new catalytic functions. The 'New View' of proteins, which was put forth by Tawfik and coworkers in the early 2000s [12••,13] has found increasing experimental and computational support [14–16]. In brief, this model argues that a single protein sequence can adopt both multiple structures and functions, which can be separated by either local conformational fluctuations (such as side chain of loop dynamics), or the global conformations of the protein. This flexibility allows enzymes to vastly expand their functional diversity, as the incorporation of mutations can alter the conformational ensemble of a protein towards conformations that are only rarely sampled in the wild-type enzyme but allow it to bind new ligands or perform new chemistry (Figure 1). Being able to understand the role of conformational dynamics in enzyme evolution is important not only to expanding our fundamental biochemical understanding of how enzymes work, but also provides a new feature that can be manipulated in the design of novel enzymes with tailored properties for use as extracellular catalysts. We recently covered the increasing evidence of the important role of conformational diversity in enzyme evolution in a separate review [11], and refer interested readers to our previous work. In the present contribution, we will discuss the important role simulations are starting to play in unraveling the secrets of molecular evolution.

Examples of relevant methodologies

Molecular simulations have a long history of being used to address complex biological problems [17–21], and understanding the role of conformational flexibility in molecular evolution is no exception. That is, while traditionally, computational approaches to studying evolution have focused on the analysis of either sequence data or approaches based on the tools of structural bioinformatics [22–24], increasing computational power now allows biomolecular simulation techniques to be used to study the evolution of protein function at the molecular level. This, of course, includes coupling hybrid QM/MM simulations [25–27] of enzyme function with overall conformational sampling using classical molecular dynamics (MD) [28–30] and enhanced-sampling approaches [31,32] such as different flavors of replica exchange simulations (e.g. temperature [33] or Hamiltonian [34]), umbrella sampling (US) [35], or metadynamics [36]. In addition, long timescale MD simulations can be used to construct Markov state models (MSM) [37,38] in order to examine different potential conformational states sampled by the system. Finally, biomolecular simulations benefit, nevertheless, from structural bioinformatics and machine learning based approaches, and can in turn also be coupled to, for example, normal mode analysis [39,40], principle component analysis (PCA) [41–43], dynamic cross-correlation matrix (DCCM) analysis [44], mutual information analysis [45,46], or approaches to study flexibility such as the dynamic flexibility index (DFI) [47•] or shortest-path

Figure 1



Conformational diversity and sampling during the evolution of enzyme function. While a hypothetical wild-type enzyme samples two states with significantly different free energies (i.e. major and minor), mutations accumulated over the evolutionary trajectory, even if located far from the active site, can influence the conformational ensemble of the enzyme's active site. In that way, evolution arranges that, what was originally the structure of a minor state in the wild-type enzyme, becomes the energetically more favorable major state in the evolved variant. Such changes in conformational dynamics and sampling can have an impact on, for example, increasing catalytic activity, shifting reaction selectivity, or enabling enzymes to bind diverse substrates and catalyze novel chemistries.

map analysis [48•], to name just a few (increasingly) commonly used examples. Our goal in this contribution is to highlight some showcase studies, using methods such as those listed above, to obtain valuable insights into the role conformational dynamics plays in determining both how new functions emerge on pre-existing scaffolds, as well as how enzyme function can emerge completely *de novo* on non-catalytic scaffolds.

Showcase systems

Understanding the role of conformational dynamics in enzyme *evolution* is a relatively young field. Despite this, there have already been several seminal pieces of work from either computational or an experimental perspective, and, as has been discussed by both ourselves and others [48•,49••,50•,51••,52,53], there is increasing evidence that conformational dynamics at different timescales, whether at the local level of side chains in the active site or global motions across the whole enzyme, can be critical for allowing for the evolution of new enzyme functions. We emphasize here that, clearly, the degree of overall flexibility can vary across the evolutionary trajectory. Therefore, the fine-tuning of dynamics in the early stages of the evolution of new functions is not inconsistent with subsequently more rigid fully-evolved enzymes. This is a separate discussion from that of whether conformational dynamics is important to catalysis itself [11]. However, shifting and fine-tuning of conformational dynamics during the evolution of new functions can allow for an enzyme to sample new potentially catalytically productive conformations, while also binding new substrates, which would in turn lead to the ability to catalyze new chemical reactions.

In our own work, we have used MD simulations to probe conformational diversity along enzyme evolutionary trajectories in different contexts. For example, we recently combined ancestral sequence reconstruction [54], biochemical and biophysical characterization, structural and computational biology in order to engineer a *de novo* active site capable of catalyzing Kemp elimination [55,56] (Figure 2a) in resurrected Precambrian β-lactamases [57]. We worked with selected systems that sampled a vast region of the sequence space of both ancestral and modern β-lactamases, going back up to ~3 billion years of evolutionary time (Figure 2b), in order to explore the structural and physico-chemical features that would allow for the emergence of new active sites. Our work demonstrated that it is possible to introduce a *de novo* active site with a novel biochemical function through a single hydrophobic-to-ionizable amino acid substitution, and that conformational flexibility can assist in the subsequent evolution of this new active site both by improving substrate substrate and transition state binding and transition state binding, and by increasing the sampling of potentially catalytically competent conformations of the active site [57]. We also showed that the subsequent loss of Kemp eliminase activity upon moving from Precambrian to modern β-lactamases is due to a loss of conformational diversity as the β-lactamases specialized for their modern functions (Figure 2c). This is in excellent agreement with previous computational work on the