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Cooperativity and flexibility in enzyme evolution

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Enzymes are flexible catalysts, and there has been substantial discussion about the extent to which this flexibility contributes to their catalytic efficiency. What has been significantly less discussed is the extent to which this flexibility contributes to their *evolvability*. Despite this, recent years have seen an increasing number of both experimental and computational studies that demonstrate that cooperativity and flexibility play significant roles in enzyme innovation. This review covers key developments in the field that emphasize the importance of enzyme dynamics not just to the evolution of new enzyme function(s), but also as a property that can be harnessed in the design of new artificial enzymes.

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Introduction

The classical picture of enzymes has been that they are highly specific catalysts, with one structure correlating to one function [1]. This view was challenged, however, with the realization that many, if not even most, enzymes are catalytically promiscuous, and can catalyze one or more reactions in addition to their native activities [2••,3•,4]. As early as 1976, Jensen (and later O'Brien and Herschlag [3•]) surmised that this promiscuity provides a stepping stone for the evolution of enzyme function, allowing for greater flexibility to acquire novel activities. Indeed, the exponential increase in the number of publications on biocatalysis that occurred between the 1970s and the late 1980s was to a large extent linked to the realization that many enzymes were not as substrate-specific as previously thought, and thus to the emergence of the exploitation of protein promiscuity in biotechnological applications [5,6]. Finally, Tawfik and coworkers [7••,8••] presented an "avante garde" new view of proteins, in which they argued that one sequence can adopt both multiple structures and multiple functions, and that this flexibility forms the cornerstone of the evolution of new enzyme functions. That is, by harnessing conformational diversity and catalytic promiscuity, enzymes can vastly expand the functional diversity of a limited repertoire of sequences, and in this way allow for new functions to evolve in old scaffolds.

Recent years have seen an explosion of interest in this area, focusing on both the role of conformational dynamics in the evolution of enzyme function [7••,8••,9,10••,11,12••,13] as well as on how an enzyme's dynamical properties are altered along evolutionary trajectories [14–17]. Based on work by both ourselves [14,17–22] and others [7••,8••,10••,12••,15,23,24•,25], we propose a model for enzyme evolution that involves a tightrope balance between flexibility, rigidity, cooperativity, and modulation of active site polarity, that controls not only an enzyme's specificity, but also the evolution of new active sites with novel functionalities.

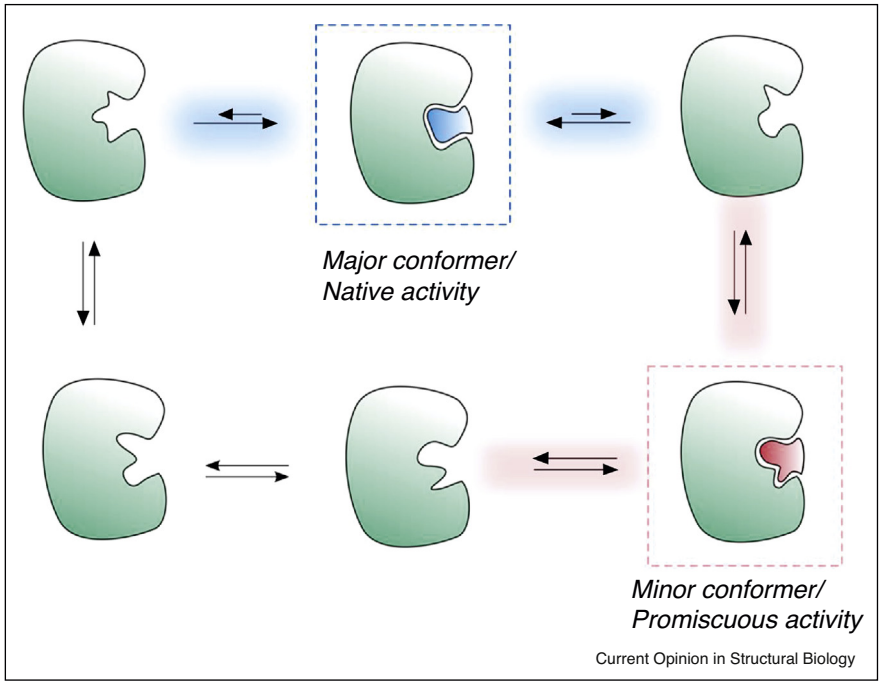
Conformational dynamics and the evolution of new enzyme functions

Enzymes are dynamical entities, that can change their conformation in many different ways, from local fluctuations of side chains, through to large scale loop and even domain motions [26]. These changes can be intimately linked to an enzyme's function: for example, many enzymes undergo conformational changes to attain catalytically active conformations [27•,28], allosteric regulation is critical to the function of many enzymes [29], and several proteins undergo order-disorder transitions to facilitate chemistry (see *e.g.* refs. [30–36]). These conformational transitions also facilitate catalytic promiscuity, allowing enzymes to adapt to bind substrates at the same (or sometimes even multiple) active site(s) [7••,8••,37], and fine-tuning these conformational ensembles can lead to the evolution of new functions (Figure 1) [8••]. To illustrate this point, we present a number of case studies where conformational dynamics clearly plays a critical role in different enzymes' functional evolution.

Dihydrofolate reductase

Dihydrofolate reductase is a monomeric catalyst of the NADPH-assisted conversion of dihydrofolate (DHF) to

Figure 1



Schematic overview of the relationship between conformational dynamics and protein evolvability. In this model, proteins can interchange between multiple conformations, with the dominant conformation being considered to be the native state, which interacts with the native ligand (blue). Conformational fluctuations such as, for example, side chain or loop dynamics, can then lead to multiple alternative conformations which can either interact with the native ligand, or with promiscuous ligands (red). These alternative conformations may be only rarely sampled in the wild-type enzyme; however, mutations can gradually shift the balance of populations such that any of these alternate conformations becomes the dominant conformation in evolved enzymes, leading to a shift in activity. This figure is adapted from Ref. [8••]. Reproduced with permission from Ref. [8••].

tetrahydrofolate (THF) *via* hydride transfer [38]. This enzyme has a catalytically important and mobile active site loop (the Met20 loop, Figure 2) [39]. The unusual temperature-dependence of the kinetic isotope effects for the hydride transfer reaction catalyzed by this enzyme [40,41] have made DHFR a historically important model system for the study of tunneling and dynamical effects in enzyme catalysis [10••,16,24•,42–51].

Interestingly, even though the human (*h*DHFR) and *E. coli* (*ec*DHFR) enzymes are highly structurally similar, they have significant differences in their sequences, and also their reaction kinetics and rate-limiting steps under physiological conditions [52–54]. To address these apparent discrepancies, Wright and coworkers used a combined structural biology, cell biology, bioinformatics and mutagenesis analysis to probe dynamical differences during the evolution of enzymes in the DHFR family [24•]. Based on this analysis, the authors were able to demonstrate subtle but significant differences in loop dynamics in the two enzymes, that were used to rationalize why *h*DHFR is unable to function efficiently in the environment of an *E. coli* cell. In particular, significant differences in the flexibility of the active site loop in the two enzymes, as exemplified by *h*DHFR lacking the critical closed-to-occluded conformational transition observed in *ec*DHFR, was argued to have a major impact on ligand flux, as well as the overall catalytic cycle, allowing evolution to fine tune the two different enzymes for two different types of cellular environment [24•]. Kohen and Klinman have similarly used DHFR as a model system to probe the evolutionary aspects of enzyme dynamics [10••], through examining evolutionary-dependent (coevolving) residues as well as the preservation of functional dynamics across broad spans of evolutionary time. Based on their analysis, they have argued that DHFR dynamics evolved with time in order to optimize the catalyzed reaction, and that there is a possible evolutionary conservation of functional dynamics at different timescales in the enzyme, which plays a regulatory role in both general biological function of this enzyme as well as in the enzyme-catalyzed reaction. Finally, based on combined isotope labeling and QM/MM studies, Alleman and coworkers have argued for a minimization of dynamical effects during the evolution of DHFR, in order to optimize a nearly-static, reaction-ready and electrostatically optimal ground state during the course of evolution [16].