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Conformational footprints

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Abstract

The mechanisms by which proteins evolve new functions can be slow and mysterious. Comprehensive structural analysis of enzyme variants reveal how gradual enrichments of pre-existing populations with the right productive dynamics for new functions can accomplish this aim.

Protein enzymes are powerful molecular machines that catalyze specific chemical reactions. Unlike deterministic steel-made machines, protein machines populate conformational ensembles that collectively determine functional states^{1,2}. The distributions of these states on the energy landscape are determined by thermodynamics; however, evolution tweaks them for biological functions¹. Current theories of protein structure and function postulate that several functional states may coexist even for a highly specific enzyme, and that specificity is determined by the population of a distinct conformation. The conformational ensembles and their time-dependent dynamics are essential for stabilizing transition states in chemical reactions and for moving raw materials in and products out; they are also capable of catalyzing not only one but two or more distinct chemical reactions, which are executed by different dominant functional states. A change of protein function can be realized through a shift of the population from one pre-existing state to another (Fig. 1a)¹. A protein may have minor states whose functions are effectively hidden due to their low population (Fig. 1b); the questions are whether a minor state can be gradually tuned to become a major state and whether the productive step-by-step trajectory of intermediates can be observed. While there are numerous reports on changing protein function by engineering dynamic states³, the work by Campbell *et al.*⁴ clearly demonstrates that changes to the free energy landscapes of proteins are essential for molecular evolution and that a change in function can be achieved through enrichment of pre-existing conformational sub-states—that is, population shift. As a model system, the authors show how a phosphotriesterase (PTE) can evolve into an arylesterase (AE) through multiple rounds of mutation and selection. This process illustrates how remote (non-active-site) mutations can control the evolution of catalytic activity in a stepwise, gradual way, by shifting the equilibrium between conformations.

Starting from the wild-type PTE (round zero; R0), the authors converted the enzyme into an efficient and specialized AE in 22 laboratory evolution generations; then, starting with the AE (R22), they reversed the process over 12 generations to obtain an enzyme similar to

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PTE. The crystal structures of R0 and R22 are almost identical, but there is a 10^9 -fold switch in the ratio of their catalytic specificities. The new activity is accomplished in the absence of any active site substitutions during the mid- to late-lag period of the evolution trajectory (R6-R22). The key difference in the conformations involves the His254Arg substitution, introduced in the first generation; this was followed by gradual tuning of the conformational dynamics of loops 7 and 5, which are essential for PTE and AE specificity, respectively. R6 consists of true dynamic intermediates of R0 and R22; with R0-like and R22-like enzyme ground sub-states, they are able to rapidly interconvert.

Two additional important observations emerge from the trajectory between R0 and R22. First, the biophysical basis of the apparent catalytic ‘promiscuity’ reflects coexisting states with different specificity, supporting the paradigm that distinct functions are encoded by specific states. The relative population of a state and its associated catalytic function can be adjusted by mutations. Nonproductive conformations can be sampled during evolution, natural or engineered; however, the authors show that these nonproductive conformations are ‘frozen out’ on the trajectory. Second, instead of direct change in the active site, remote mutations can control the conformational sampling. Although the efficiency of R22 is due to the ability of the active site to catalyze the reaction, it is the gradual evolutionary optimization of the configuration of the active site via remote mutations that leads to this effect. The allosteric control of the conformational dynamics and the corresponding enzymatic activities are accomplished by adjusting the internal protein interaction network (hydrogen bonds, salt bridges and hydrophobic interactions), leading to altered communication paths between loops that result in different enzymatic specificity.

These observations establish that modifications of the conformational landscape are essential for functional change. Dynamic enrichments of pre-existing but low-occupancy catalytic states—not direct mutational active site remodeling—are the key players. The same principles elucidated here for enzymatic catalysis also apply to non-enzyme proteins and other biomacromolecules and their assemblies, such as nucleosomes⁵. While enriched conformational ensembles can evolve new functions, mutational perturbations of the molecular energy landscape often lead to dysfunction. Diseases often trace to mutations of single amino acids near to and away from the functional site. One such example is the Gln61Leu cancer mutation in the Ras protein, which allosterically disables GTP hydrolysis in the presence of the Raf protein⁶.

The concept of the free energy landscape and its extension to the population shift of pre-existing states¹ lays out one of the fundamental mechanisms of biomolecular function⁷, which forms the basis for at least hundreds of research papers^{8–10}. The Campbell *et al.* paper⁴ is striking for demonstrating, through an impressive collection of crystal structures and their comprehensive analysis, the role of protein dynamics in the evolution of new enzyme functions.

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Figure 1 |.

Population shifts of pre-existing protein functional states determine changes in activity. **(a)** An enzyme with catalytic function E1 can be engineered through multiple cycles to execute function E2 through alteration of which state has a dominant population. A mutation can gradually or drastically change the relative populations of the different states and thereby modify protein function. **(b)** Both states pre-exist in the ensemble, but a protein state with lower energy (deep blue) on the energy landscape has a higher population. Transitions among different states need to cross energy barriers or go through intermediate states, which often share similar conformational dynamics with other functional states (E1/E2).