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Laboratory evolution of protein conformational dynamics

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This review focuses on recent work that has begun to establish specific functional roles for protein conformational dynamics, specifically how the conformational landscapes that proteins can sample can evolve under laboratory based evolutionary selection. We discuss recent technical advances in computational and biophysical chemistry, which have provided us with new ways to dissect evolutionary processes. Finally, we offer some perspectives on the emerging view of conformational dynamics and evolution, and the challenges that we face in rationally engineering conformational dynamics.

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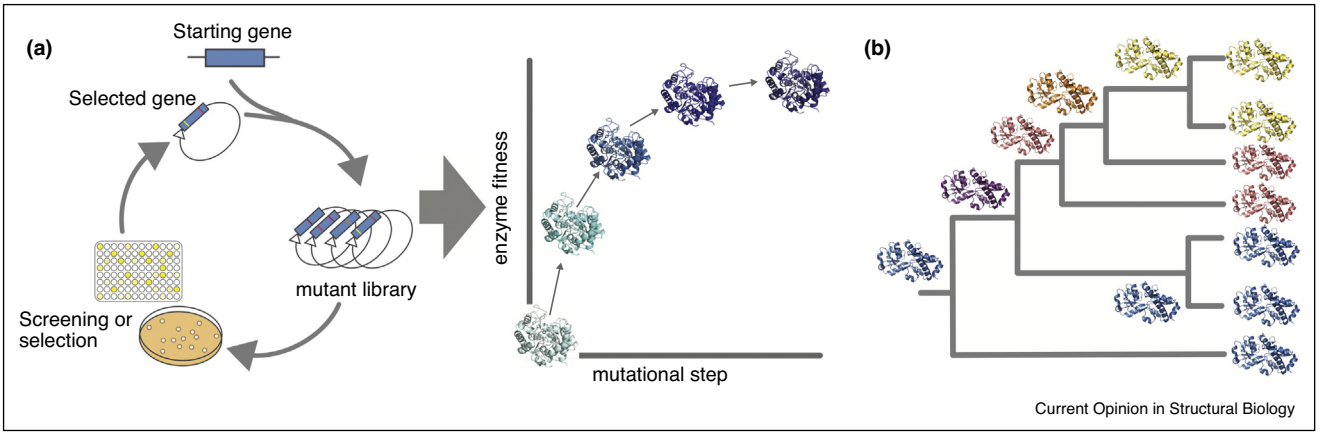
Introduction

The conformational dynamism of proteins is well established. Polypeptide chains are inherently flexible and undergo conformational change in solution at a variety of time scales. On the shortest of these timescales (fs–ps), bonds vibrate and side chains rotate. On longer time scales (ns–ms), macroscale motions can take place; loops 'open' and 'close', and domains can twist relative to each other or move on hinge-like regions. One important question in modern protein science asks how these conformations affect the function of the enzyme. The continued improvement in available biophysical techniques, including X-ray crystallography and NMR, in combination with advances in computational protein simulations, has allowed deeper analysis of protein motions. For example, the role of protein dynamics in substrate binding and product release is well studied [1–4], and cascades of conformational change are now known to underpin numerous biological functions [5]. There remains some controversy around the role of conformational dynamics in the catalytic step of enzymes; some works have proposed a role for conformational dynamics in the chemical step [6], while others suggest that experimental models have not yet conclusively demonstrated this link [7].

Given that protein structural dynamics clearly play important roles in several aspects of protein function, it is reasonable to assume that they must have evolved, or become optimized through selective pressure. Thus, one of the biggest questions relating to protein structural dynamics regards the role of molecular evolution, and how/if pathways for conformational change can be altered. During the evolution of new enzyme function, an enzyme active site must reorganize and adapt to a new substrate and/or new chemical reaction. It is generally, and reasonably, assumed that the adaptation of an enzyme to catalyze a new chemical reaction predominantly involves modification of the active site via mutation to better stabilize the transition state. However, the composition of active sites among homologous enzymes is often very similar, despite markedly different catalytic specificities [8], and laboratory (directed) evolution routinely demonstrates that remote mutations somehow have drastic effects on enzyme turnover rates or substrate preference [9–11]. These observations — in addition to our established understanding of allosteric communication between remote sites [12] — imply an important role for second/third/outer shell residues in modulating enzyme function, perhaps via control of protein structural dynamics/conformational sampling. The difficulty in studying the evolution of any trait, but especially structural dynamics, when relying on comparison between different extant proteins, is that we are comparing already highly evolved (and complex) states. To understand how something can change or evolve, it is much more informative to study the evolutionary process directly.

[5] Equal author contribution.

Figure 1



(a) The workflow of laboratory directed evolution involves the generation of a library of mutants of the gene of interest, and a screening or selection process to iteratively enhance a desired phenotype. **(b)** Ancestral protein reconstruction requires that the phylogenetic relationships between extant proteins are established, allowing ancestral sequences to be inferred and constructed.

Laboratory directed evolution and ancestral protein reconstruction

Laboratory directed evolution [Figure 1a] has been extensively used in an engineering context to produce many different proteins with a variety of improved functions, such as increased or novel catalytic activity [13], increased thermostability [14], and enhanced spectral properties [15]. However, directed evolution also provides great advantages over the study of natural homologs when it comes to the study of evolutionary processes. First, we focus on the evolution of (often) a single gene/protein of interest, rather than proteins that evolved in concert with the whole organism, which can involve complicated inter-gene epistatic relationships. Second, the high throughput screening of randomly generated mutants of a particular gene can result in the rapid enhancement of a desired phenotype over far fewer generations than is typical in natural systems, because directed evolution experiments allow for tight control of selection pressure, while natural evolutionary processes typically must balance several requirements to maximise the reproductive success of the organism. This results in significantly less neutral sequence variation, which can confound functional analysis. Finally, perhaps the most important advantage of laboratory evolution is that it allows for the study of as many intermediates along an evolutionary trajectory as desired, which can provide novel insights that cannot be gleaned through comparison of extant enzymes where only one current state can be assessed.

Ancestral protein reconstruction [Figure 1b] also seeks to remedy the shortcomings of studying extant proteins in isolation. Through the alignment of related sequences and the calculation of phylogenetic relationships between those sequences, points of diversification, or nodes, representing the predicted ancestors of extant proteins can be identified and probable sequences for these ancestral states inferred. This allows for the expression and characterization of these ancestral proteins that represent evolutionary intermediates, which can facilitate the study of evolutionary divergence [16].

Directed evolution and ancestral protein reconstruction have been instrumental in revealing fundamentally important molecular processes that underlie many protein functions. For example, our understanding of catalytic promiscuity has been substantially broadened through studying the evolution of substrate preference [17•,18]; we have gained insights into the complex relationships between thermostability and activity observed during the acquisition and optimization of new function [19,20]; and the constraints of epistasis on evolutionary trajectories can be more readily analysed thanks to the accessibility of evolutionary intermediates [21]. Most recently, attention has shifted to the study of how structural dynamics of proteins can change throughout an evolutionary trajectory, which has been facilitated by developments in computational structural biology and biophysical techniques.

Biophysical and computational analysis of protein structural dynamics

Studying the evolution of protein structural dynamics would not be possible without the use of computational and biophysical methodologies that allow structural dynamics to be dissected in different protein variants. To provide some context to the subsequent discussion of recent discoveries related to structural dynamics through evolutionary studies, we must first provide a brief