Publishable summary report
Proposal No. 237065
Title: Application of directed evolution to the study of structural enzyme dynamics

The aim of the project was to utilize the technique of directed evolution to generate a range of mutant enzymes with different catalytic properties and to use a variety of biophysical approaches to characterize the structural dynamics of these molecules in order to determine the role of structural dynamics in catalysis.

Conformational sampling, catalysis, and evolution of the bacterial phosphotriesterase

To efficiently catalyze a chemical reaction, enzymes are required to maintain fast rates for formation of the Michaelis complex, the chemical reaction and product release. These distinct demands could be satisfied via fluctuation between different conformational substates (CSs) with unique configurations and catalytic properties. However, there is debate as to how these rapid conformational changes, or dynamics, exactly affect catalysis. As a model system, we have studied bacterial phosphotriesterase (PTE), which catalyzes the hydrolysis of the pesticide paraoxon at rates limited by a physical barrier—either substrate diffusion or conformational change. The mechanism of paraoxon hydrolysis is understood in detail and is based on a single, dominant, enzyme conformation. However, the other aspects of substrate turnover (substrate binding and product release), although possibly rate-limiting, have received relatively little attention. This work identifies ''open'' and ''closed'' CSs in PTE and dominant structural transition in the enzyme that links them. The closed state is optimally preorganized for paraoxon hydrolysis, but seems to block access to/from the active site. In contrast, the open CS enables access to the active site but is poorly organized for hydrolysis. Analysis of the structural and kinetic effects of mutations distant from the active site suggests that remote mutations affect the turnover rate by altering the conformational landscape. *This work has been published in the Proceedings of the National Academy of Sciences, USA, 2009, Volume 106, Pages 21631-21636, C. J. Jackson et al.*

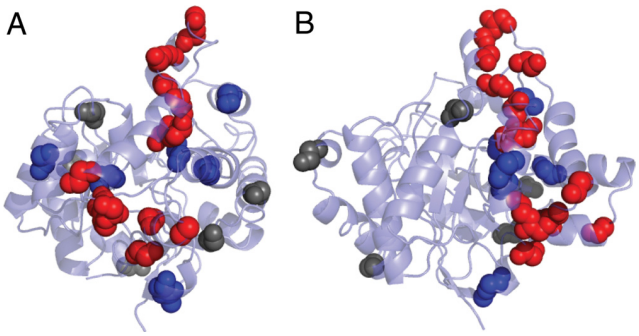

Figure 1: The locations of mutations (red) that result in changes to the activity and structural dynamics of the bacterial PTE. These mutations are over-represented in the two most dynamic regions of the protein: an active site loop and a bundle of helices at the rear of the protein.

Minor conformations accessed by structural dynamics can serve as starting points for the evolution of new activities

Any enzymes exhibit additional, promiscuous, catalytic activities in addition to their primary catalytic function. In this work we have studied a full evolutionary transition of a phosphotriesterase into an arylesterase (with N. Tokuriki and D. Tawfik). By obtaining crystal structures of a series of mutants across nineteen generations of directed evolution we have been able to map subtle changes in the conformational landscape and dynamics of the protein. The results reveal that a minor conformation is responsible for the promiscuous arylesterase activity in the early generations, and that this conformation is occasionally accessed through the structural dynamics of the protein. Over the full course of the evolution, a series of mutations progressively stabilize this minor conformation, as it becomes the dominant conformation, consistent with the overall change in function from a phosphotriesterase to an arylesterase. This work highlights the importance of structural dynamics and a complex energy landscape of conformations to evolution and provides new insights for protein engineering and design. *This work is in preparation for publication with N. Tokuriki, M. Weik and D. Tawfik.*

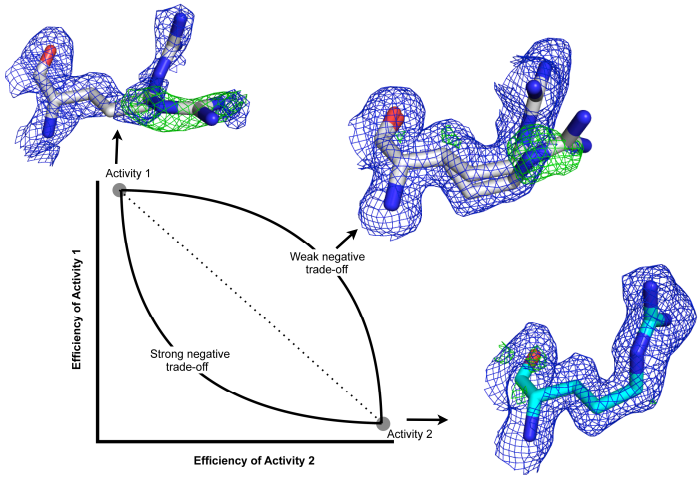

Figure 2: The conformational equilibrium between bent and extended conformers of Arg254 at the active site of phosphotriesterase during the evolutionary transition. It is clear that the bent conformation gradually eclipses the extended conformation as the new arylesterase activity begins to dominate.

Summary

We have made significant progress in establishing the role that rapid conformational change, or 'structural dynamics' play in both the function of proteins and in how they evolve. We have established that in the bacterial phosphotriesterase rapid opening and closing of an active site loop appears to allow the enzyme to satisfy the requirements of maintaining both a high affinity, pre-organized state for catalysis and low affinity state for maximum diffusion rates. Similarly, we have been able to demonstrate that conformational fluctuations can allow the protein to access minor conformations that can serve as starting points for evolution of new activities.