University of Massachusetts UMASS Medical School

GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY

Ph.D. THESIS DEFENSE

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"The Complex Role of Sequence and Structure on the Stability and Function of TIM Barrel Proteins"

Sequence divergence of orthologous proteins enables adaptation to a plethora of environmental stresses and promotes evolution of novel functions. As one of the most common motifs in biology capable of diverse enzymatic functions, the TIM barrel represents an ideal model system for mapping the phenotypic manifestations of protein sequence. Limits on evolution imposed by constraints on sequence and structure were investigated using a model TIM barrel protein, indole-3-glycerol phosphate synthase (IGPS). Exploration of fitness landscapes of phylogenetically distant orthologs provides a strategy for elucidating the complex interrelationship in the context of a protein fold.

Fitness effects of point mutations in three phylogenetically divergent IGPS proteins during adaptation to temperature stress were probed by auxotrophic complementation of yeast with prokaryotic, thermophilic IGPS. Significant correlations between the fitness landscapes of distant orthologues implicate both sequence and structure as primary forces in defining the TIM barrel fitness landscape and suggest that fitness landscapes can be translocated in sequence space.

Analysis of a surprising class of beneficial mutations in all three IGPS orthologs pointed to a long-range allosteric pathway towards the active site of the protein. Biophysical and biochemical analyses provided insights into the molecular mechanism of these beneficial fitness effects. Epistatic interactions suggest that the helical shell may be involved in the observed allostery. Taken together, knowledge of the fundamental properties of the TIM protein architecture will provide new strategies for *de novo* protein design of a highly targeted protein fold.

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