FUNCTIONAL TRANSITIONS IN ENYZME EVOLUTION: BALANCING STABILITY, FOLDING AND CATALYTIC SPECIFICITY

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Evolutionary pathways by which proteins have evolved in Nature over billions of years have resulted in an impressive diversity of structures that carry out many functions with unrivalled efficiency. Directed protein evolution in the test tube can emulate natural evolution, but is often limited by low hit rates and small improvements during evolutionary cycles. Furthermore, the combination of mutations that is needed for large improvements cannot always be reached by one-by-one mutational steps due to the occurrence of general loss-of-function mutations or epistatic ratchets. The question then arises how evolutionary dead ends can be avoided. Important parameters that shape these fitness landscapes are e.g. expression level, stability and catalytic activity/specificity. We are currently probing these parameters for ancestral sequences inferred from phylogenetic relationships between members of the catalytically diverse metallo-β-lactamase[1] and alkaline phosphatase[2-4] superfamilies. Mapping of substrate specificity profiles on the genetic relationships allowed the identification of the ancestral nodes between which transitions in primary function most likely occur. The latter is one of the key processes in evolution of new functions. The substrate specificity profiles of the current enzymes suggest that the change in primary function is the result of a shift in substrate preference rather than *de novo* evolutionary invention of a novel activity. Furthermore several characterized ancestral sulfatases suggest a shift toward increased specificity over evolutionary time, as well as a trend that ancestral enzymes are more stable than present day enzymes.

References

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