

# **Fitness through Simplicity: Dominance of the HAD Phosphotransferase Family**

*Karen N. Allen<sup>1</sup> & Deborah Dunaway Mariano<sup>2</sup>*

<sup>1</sup>*Department of Physiology and Biophysics, Boston University School of Medicine, Boston MA 02493 - USA*

<sup>2</sup>*Department of Chemistry and Chemical  
Biology, University of New Mexico, Albuquerque, NM 87131-0001 – USA*

The evolution of new catalytic activities and specificities within an enzyme superfamily requires the exploration of sequence space for adaptation to a new substrate with retention of those elements required to stabilize key intermediates/transition states. Phylogenetic analysis, mechanistic information, and structure determination is used to reveal novel ways in which the catalytic scaffold of a mechanistically diverse superfamily, the haloalkanoic acid dehalogenase enzyme superfamily (HADSf), is tailored to new biochemical functions. Steps (and mis-steps) in the understanding of substrate specificities in capless members of the superfamily are highlighted. We propose that core residues in the large enzyme family, form a “mold” in which the trigonal bipyramidal transition-states (TBPST) formed during phosphoryl transfer are stabilized by electrostatic forces. As a test for the operation of the trigonal bipyramidal phosphorane mold, X-ray crystal structure determination has been performed on a phosphatase complex that contains a tungstate ligand that is stabilized in an otherwise high-energy coordination state. The complex is compared to that of vanadate, as an example of a ligand that can more easily expand coordination geometry. A composite TBPST derived from the analysis of 12 liganded HADSf structures, reveals absolutely conserved elements which serve to stabilize the axial and equatorial atoms of the phosphoryl group-surprisingly many originate from the enzyme main chain. All work was supported by NIH GM061099.