

## W0561

**High Transition State Affinity by TrpRS Coincides with an Unstable Protein Conformation.** C.W. Carter, Jr., Violetta Weinreb, Maryna Kapustina, Dept of Biochem. Biophys., CB 7260, UNC Chapel Hill, Chapel Hill, NC.

Pre-transition state (PreTS) tryptophanyl-tRNA synthetase crystal structures are almost indistinguishable (RMSD  $\sim 0.65$  Å) with that of a complex with adenosine-5' tetraphosphate (AQP). AQP has high affinity characteristic of a transition state analog inhibitor, and its  $\gamma$ - and  $\delta$ - phosphates mimic the PPi leaving group, binding more intimately to active-site lysine and serine residues than in the PreTS state ATP complex. Free, unliganded molecular dynamics simulations of both relax to other structures within 1 ns. The unliganded PreTS state reverts *backward* along the reaction path to the Open, while the AQP structure progresses *forward* to the Products conformation. The two, quite similar structures therefore lie on opposite sides of a steeply sloping conformational free-energy maximum, characteristic of a transition state. In free simulations with  $Mg^{++}$ ATP (without Trp)  $Mg^{++}$ -O distances change from  $\sim 2.5$  Å to within  $\sim 1.9$  Å, releasing two active-site lysine residues from their interactions with the phosphate oxygen atoms, coordinately with interdomain relaxation to the *Product* state. Two kinds of restraints preserve the high energy, PreTS conformation, which is also stable in free, fully-liganded trajectories. Either the centers of mass of the two domains or the  $Mg^{++}$ -O distances can be restrained to their unstable, crystallographically observed values. Thus, the high energy  $Mg^{++}$  coordination and conformational state are jointly imposed by catalytically productive interactions of active-site lysines with the PPi leaving group (1). Supported by NIGMS.

### References

1. Kapustina M, Carter CW, Jr. Computational Studies of Tryptophanyl-tRNA Synthetase Ligand Binding and Conformational Stability. *Journal of Molecular Biology* 2006;In Press.