

9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

How does domain motion contribute to transition-state stabilization? Combinatorial thermodynamic cycle analysis of conformational coupling during tryptophan activation

Charles W Carter

University of North Carolina at Chapel Hill, USA

Enzyme mechanisms, especially those that couple NTP hydrolysis to mechanical work and information, use sophisticated dynamic networks to transduce active-site chemistry into domain motions that change binding affinities. We measured and cross-validated the energetics of such networks in *B. stearothersophilus* Tryptophanyl-tRNA synthetase (TrpRS) using both multi-mutant and modular thermodynamic cycles. Coordinated domain motions develop shear in a core packing motif conserved in >125 different protein superfamilies. Multi-dimensional combinatorial mutagenesis showed that four side chains from this “molecular switch” move coordinately with the active-site Mg²⁺ ion in the transition state for amino acid activation. A modular thermodynamic cycle consisting of full-length TrpRS, Urzyme, and Urzyme plus each of the two domains deleted in the Urzyme gives similar energetics. These complementary experiments establish that catalysis and specificity in full-length TrpRS are both coupled by 5 kcal/mole to: (i) the core packing region where domain movement generates shear, and (ii) the simultaneous motion of the two domains relative to the Urzyme. Theory shows that the minimum action path algorithm estimates thermodynamically meaningful contributions of domain movement to kinetic rates. Correlations between those parameters, the experimental rates, and structural variations induced in the combinatorial mutants confirm that these estimates are realistic. These results validate our previous conclusion that catalysis by Mg²⁺ ion is coupled to the overall domain motion. Computational free energy surfaces demonstrate that TrpRS catalytic domain motion itself is endergonic but is driven thermodynamically by PP_i release. Comparison of the impact of combinatorial mutagenesis on pre-steady state and steady-state rates confirm that dynamic active-site pre-organization endows TrpRS with the elusive conditionality of NTP utilization on domain motion.

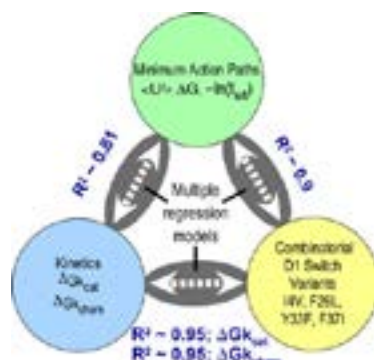


Figure1: High Correlations between structures (yellow), steady-state kinetics (blue) and computed trajectories (green) for WT and 15 combinatorial variants of typtophanyl-tRNA synthetase.

Biography

Charles W Carter is an X-ray Crystallographer who studies the origin, evolution, and structural biology of aminoacyl-tRNA synthetases. His research group introduced the use of urzyme-highly conserved structural cores that retain large fractions of the transition-state stabilization free energies of full length enzymes as experimental models of ancestral enzymes.

carter@med.unc.edu