Allosteric Regulation of Transient Ammonia Tunnel in Bi-functional Enzyme FGAM Synthetase

Ruchi Anand

Department of Chemistry, Indian Institute of Technology Bombay, Mumbai-400076, India

Email: ruchi@chem.iitb.ac.in

Molecular tunnels regulate delivery of substrates/intermediates in enzymes which either harbor deep-seated reaction centers or for transport of reactive/toxic intermediates that need to be specifically delivered.[1,4] These tunnels can either be pre-formed, permanently visible within the protein structure or can be transient where a fine-tuned signal transduction relay makes the tunnel accessible during catalysis.[4] We investigated the allosteric signal transmission that forms a transient tunnel in a purine metabolic enzyme called FGAM synthetase. It is a multidomain bifunctional enzyme that harbours two active sites, which are about 25Å away. The first active site (G-site) produces ammonia via glutamine hydrolysis, which is then channelled to the second active site (F-site), where purine intermediate FGAR is converted to FGAM.[2] This process is allosterically regulated, and several structural elements in this enzyme, such as the N-terminal domain and the catalytic loop (C-loop), facilitate cross-talk.[3] We probed dynamics of a 20-amino acid long C-loop, which serves as an allosteric switch, by site-specific incorporation of unnatural amino acids and determined its role in initiating allosteric cues, thereby regulating the distant G-site activity[4]. Using a combination of cryogenic electron microscopy and time-resolved fluorescence resonance energy transfer (FRET), we delineated the entire allosteric mechanism that operates in FGAM synthetase and could trap a transient ammonia tunnel in the cryo-EM structure. Here, we discuss how the extensive rearrangement of structural elements in the protein modulates the inter-domain interactions, enables signal transduction and finally opens the transient ammonia tunnel that connects the two active sites.

References:

1. N. Sharma, S. Singh, A.S. Tanwar, J. Mondal and R. Anand, *ACS Catal.* **2022,** *12(3),1930-1944.*
2. N. Sharma, N. Ahalawat, P. Sandhu, E. Strauss, J. Mondal and R. Anand, *Sci. Adv.* **2020**, *6*, eaay7919.
3. S. Singh and R. Anand, Curr. Opin. Chem. Biol. 2023, 73,102261
4. S. Singh, S., T. Kistwal, A. Datta, A., & R. Anand, *JPCL Letters, **2025**,* 16, 1582-1589