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Visualizing bi-enzyme complex dynamics by time-resolved crystallography
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Abstract
Bi-enzyme complexes with tunnel-mediated product/substrate transport between two active sites are intricate enzyme factories, in which two spatially separated catalytic steps are coordinated by sophisticated allosteric mechanisms. Tryptophan synthase (TS) assembles into a heterotetrameric TrpA/(TrpB)2/TrpA complex, in which the TrpA product indole is transported to the TrpB subunit through a ~25 Å long intermolecular tunnel to form the final product tryptophan[1,2]. We used TS as a pilot system to apply Time-Resolved serial crystal X-ray crystallography (TRX)[3] and Cryo-Trapping X-ray crystallography (CTX) to investigate the structural details of catalysis, allostery and product/substrate transport within a timely resolution that matches its biochemical properties. To date, we have structurally characterized eight out of 13 previously established reaction states by TRX and CTX[4,5]. By ensuring indole delivery from the TrpA active site into the intermolecular tunnel to the TrpB active site, we have identified distinct indole locations within this tunnel. In summary, our data provide an well-defined basis to unravel the biochemistry of a complex bi-enzyme system by a structure-based molecular movie presentation.

References
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