SUMMARY

Proteins and enzymes are essential for the efficient governing of cellular processes. They often work by forming multiprotein assemblies where their actions are regulated through protein-protein interactions. These are formed either transiently or permanently and play diverse roles in cell signaling, gene transcription, and metabolism. One of the main purposes of forming a multi-enzyme assembly is the favourable metabolic channeling of substrates and products between enzymes in the assembly which catalyze consecutive reactions in the pathway. Metabolic channeling protects reactive or labile reaction intermediates from escaping into the cellular milieu and hence speed-up the metabolic flux. The other important property of multi-enzyme assembly is to modulate activities of component enzymes. Regulation of enzyme activities in response to metabolic flux is necessary to maintain homeostasis of the cell. Therefore, understanding structural, energetic, and kinetic landscape of complex formation and regulatory mechanism of their activities are of paramount importance in order to understand the biochemistry of pathways. Due to their complex nature and difficulties in experimental approaches to study them, very few molecular and structural details are available in literature on these assemblies. The cysteine synthase complex (CSC) is a transient multi-enzyme assembly, formed by the physical association between serine acetyltransferase (SAT) and O-acetylserine sulfhydrylase (OASS). CSC stability is also sensitive to OAS concentration, suggesting a complex landscape for this complex. Our studies employing structural, biochemical, and analytical approaches have provided more insights into mechanism of CSC complex formation in *Salmonella*, specificity, assembly state, and regulatory mechanism. We resolved several structures of C-terminal peptide of SAT bound to OASS and investigated energetic of protein-peptide and protein-protein interactions. By employing an integrative approach, our studies unraveled the allosteric network that determines the species specificity and recognition mode of SAT C-terminal by OASS. We demonstrated that molecular weight of saturated CSC is ~ 440 kDa and it is composed of four OASS dimers bound to one SAT hexamer. Our results also revealed that CSC complex formation is multi-stage process, due to

negative cooperativity between SAT monomers while binding to OASS. Our kinetic analyses showed that enzyme activities of both SAT and OASS were modulated upon protein-protein interaction and slight increase in the acetyl CoA catalysis activity of SAT is noted in the presence of OASS.

Multiple mutations in *Hi*OASS were created and biophysical properties and structural details of low-affinity and high-affinity subtypes were elucidated. The crystallization of these *Hi*OASS mutant structures led to the discovery of multiple intermediates which depicts the mechanism of evolution of OASS having low-affinity stereotypes and high-affinity stereotypes. Both structural and energetic analyses also provided a plausible explanation for the inability of the *Hi*OASS protein to maintain a high affinity CSC with SAT. Structural studies also revealed signatures of OASS mutants which have either no affinity or diffusion limited affinity for SAT C-terminal. Through systematic mutagenesis and structural studies, we demonstrated how epistatic coupling and allosteric networks work together to recycle structural features that represent native-like and non-native like phenotypes.