Summary

Multiple metabolic pathways precisely regulate the metabolome and are coordinated by the upregulation and downregulation of metabolic fluxes. Metabolites function by employing specific mechanisms like allostery and direct modulation of enzymes. CSC is associated with the regulation of sulphur transport, reduction and biosynthesis of cysteine in plants and bacteria. This study describes different aspects cysteine synthase complex regulation. We studied oligomeric nature of CSC to the catalytic properties of its individual components (OASS and SAT). For the first time, we reported here that, CSC exists in dual assembly states and stability of each state is controlled by OASS and two substrates, OAS and sulfide (S^{2-}). Molecular mass based analyses clearly show that CSC from *Salmonella typhimurium* exists in both low-molecular-weight (CSC_1) as well as in high-molecular-weight state (CSC_2) when the OASS is in present excess to SAT. The concentration of OAS between 0.5-2.0 mM completely dissociates the CSC_2 and stabilizes the CSC_1 complex, whereas OAS concentrations above 5 mM can easily dissociate the entire complex into individual components. The experiments performed at the low concentrations of OAS (100 µM and 50 µM) specifically dissociates the low molecular weight complex (CSC_1). Another important finding of this study shows that effect of Na_2S (S^{2-} source) on the oligomeric property of CSC which was not reported in any study before. Here we demonstrated that Na_2S specifically stabilizes low molecular weight (CSC_1) and is able to dissociate CSC_2 to a good extent and results in formation of homogenous CSC_1 complex. Based on the current information and results presented here on CSC, we used two major parameters to build a model of a dynamic cycle of CSC. The first parameter is the concentration of $[OASS]_{dimer}$ and the second parameter is the net concentration of OAS and sulfide.

In our next study, we propose a novel mechanism of substrate facilitated dissociation of inhibitor. The competitive inhibition of O-acetylserine sulfhydrylase (OASS) by C-terminal of serine acetyltransferase (SAT) presents a paradox because the C-terminal of SAT binds to the active site of OASS with 4-6 log fold (10^4 -10^6) more affinity than that of the substrate. Therefore, it is not clear as to how substrate gains access to OASS active site under physiological conditions. Our SPR and stop flow studies demonstrate that OAS can dissociate the complex at as low as 100 µM (also reported previously Kredich et al.). The series of crystal structures along with kinetic studies in pressences of substrate (OAS), demonstrate the

competitive-allosteric mechanism in which first OAS bind to an N-terminal domain of OASS and induce allosteric conformational changes and facilitate the dissociation of inhibitor. It is interesting to note that all the previous studies suggested that only after the substrate reaction, the active closes, however, in this study we clearly shown that, the binding of OAS to the N-terminal domain, is itself a trigger for the closure of the active site in *Hi*OASS (PDB: 4ORE).

The interesting findings from results obtained from the above study where we resolved a ternary complex (PDB: 4ORE) and Binary complex (PDB: 5DBH) showed that evolutionarily conserved methionine-trio (M120, M96, and M92) of the movable domain could control the OAS recruitment. We constructed thirteen mutants at three positions in *St*OASS, *Mt*OASS and *Hi*OASS. Our mutagenesis studies, combined with structural, thermodynamic and kinetics studies performed in the presence of OAS (substrate) and inhibitor (SAT C-terminal C10 peptides) reveal that both M120 and M92 are involved in substrate sensing and protein-protein interactions. From this study we concluded that M120 is the primary substrate recruiter and is involved in substrate-specific active site closing. and we also show the role of methionine-trio in the substrate-sensitive remodelling of OASS active site. The results from crystallographic studies show that the conformation of TSGNT loop may be playing an important role in the activity of the OASS.