INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	SHRUTIDHARA BISWAS
Roll Number	:	11610609
Programme of Study	:	Ph.D.
Thesis Title	:	**INVESTIGATING THE STRUCTURE, FUNCTION AND DYNAMICS OF AN INTRINSICALLY DISORDERED ENZYME**
Name of Thesis Supervisor(s)	:	PROF. RAJARAM SWAMINATHAN
Thesis Submitted to the Department/ Center	:	BIOSCIENCES AND BIOENGINEERING
Date of completion of Thesis Viva-Voce Exam	:	02-03-2020
Key words for description of Thesis Work	:	Intrinsically disordered proteins, disordered enzymes, Fluorescence Spectroscopy, Monomeric Chorismate Mutase, Site-specific Mutations

SHORT ABSTRACT

The engineered intrinsically disordered enzyme monomeric chorismate mutase (mCM) catalyses the rearrangement of chorismate to prephenate and hence, emerged as a valuable model system to study enzymatic catalysis. mCM displayed all the hallmarks of a molten globule, a functionally inactive protein folding intermediate, but surprisingly shows near-native catalytic activity. The nature of structural plasticity mechanisms in mCM that enable catalysis were investigated in this thesis work. We probed the structure and dynamics of mCM using the intrinsic fluorophore tryptophan and the extrinsic fluorophore dansyl (labeled to cysteine residue), which also served as a FRET pair in our experiments. Through site-directed mutagenesis, single tryptophan and single cysteine mutant pair of mCM protein were generated at specific locations (based on NMR structure with substrate analogue) such that FRET between a Trp (donor)-Dansyl (acceptor) pair can be measured effectively and without perturbation of the substrate binding site of the enzyme. Three mutants namely Mutant1 (W24K), Mutant2 (W24K/C69A) and Mutant3 (W24K/C69A/A6C) were extensively studied and their structural and functional properties compared with the parent wild-type mCM enzyme (Wt mCM). Time-Resolved Fluorescence measurements could sensitively detect local changes in structure of mCM after TSA binding. Time-resolved fluorescence anisotropy decay measurements revealed global structural alternations in mCM after ligand (TSA) binding in the Wt mCM as well as the mutants. Mutant1 retained 85% of the enzyme activity, whereas drastic drop in enzyme efficiency to ~2.5% in Mutant2 and ~11% in Mutant3 was observed. These experimental results also emphasize the role of the single cysteine residue (Cys 69) in maintaining the structural and functional integrity of mCM, even though Cys 69 doesn't form a part of the enzyme active site The single tryptophan mutant (W24K) of mCM displayed a substantial shift between the Trp-Cys FRET pair of approximately 6 Å after addition of the ligand. The overarching theme that has emerged from the series of carefully designed experiments in my thesis is that the integrity of the secondary structure and ligand-induced global compaction (disorder-to-order transition) play a crucial role in catalysis and that even minor perturbation in regions distant from the active site can significantly impact the enzyme efficiency.