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Green Fluorescent Protein (GFP) in polar aprotic organic solvents

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Wild-type Green Fluorescent Protein (Wt-GFP) exhibits two absorption peaks corresponding to neutral (A) and anionic (B) forms of the chromophore. The ratio of the peaks is found sensitive to organic solvent addition in a similar manner as observed for pH titration. There exists an active proton wire between the chromophore hydroxyl and Glu222 residue via water and Ser205 molecules. The same wire is inactive in S65T mutants. An organic solvent based spectral shift is limited to Ser65-Glu222 proteins only. It is speculated that organic solvents are perturbing the protonation-deprotonation equilibrium. To find the mechanism by which organic solvents are bringing this shift His148 and Thr203 positions are targeted for directed mutagenesis. Various mutants have been studied in the presence of organic solvents using absorbance/fluorescence spectroscopy. To see the effect on excited state dynamics femtosecond/picosecond time-resolved fluorescence study has been done. On the basis of which it is proposed that organic solvent-based spectral transition is a ground state process which needs an active Ser65-Glu222 proton wire along with the anionic form stabilizing residues at 203 and 148 positions.

Biography

Jasvir has done her M.Sc. in Molecular biology and Biochemistry from Guru Nanak dev University Amritsar, Punjab, India. Currenty she is a Ph.D. research scholar in the school of Biotechnology, Jawaharlal Nehru University, New Delhi, India. Her Ph.D. work is to study spectral perturbations in GFP in non-aqueous environment.

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