Title:	Ultrafast Excited-State Dynamics of Chromophores of Fluorescent Proteins in Solutions and Within Protein Scaffolds
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Abstract:	Fluorescent proteins (FPs) possess intrinsic fluorophores responsible for their bright fluorescence. FPs are widely utilized as genetically encodable markers, capable of being expressed in diverse heterologous systems. Initially extracted from the jellyfish Aequorea victoria and other marine organisms, and subsequently developed using mutagenesis, FPs have become indispensable in live-cell imaging. The captivating aspect of bright fluorescence from FPs lies in the chromophore formation through autocatalytic post-translational modifications of three consecutive amino acids. These chromophores, whether in intact proteins or in solution, exhibit various photo-induced processes, involving changes in the ground and the excited electronic states, encompassing alterations in its structure of the chromophores as well as of their neighboring environment. Thus, the subtle interaction between the protein and chromophore plays a pivotal role, leading to the remarkable modulation in chromophore fluorescence upon changing their local environment. This thesis is aimed at exploring the influence of local environmental factors, such as solvent polarity and pH, on the photoinduced electron or proton transfer phenomena involving the FP chromophores both in solution and within intact proteins. To delve into these photoinduced excited-state dynamics (occurring in ultrafast time scales) and their relaxation pathways, femtosecond transient absorption spectroscopy is employed, supported by various other experimental and theoretical studies. Following an introduction to the motivation behind this work and an overview of the methodologies used, ultrafast excited-state dynamics of chromophores in the absence of confinement within protein scaffold will be discussed where role of solvent polarity in intramolecular charge transfer dynamics within various synthetic derivatives of chromophore analogues, and planarity-induced intramolecular electron transfer dynamics leading to large Stokes shift in certain compounds (which are not chromophore analogues), were investigated. Further, the photo-physics involving ultrafast excited-state isomerization and proton transfer along with conformational alterations in certain red fluorescent proteins (mKeima and mBeRFP) will be presented, with a particular emphasis on the modulation induced by change in pH of the local environment. Most importantly, it will be discussed how presence of numerous conical intersections drive the complicated photocycle in these proteins, leading tomultiple distant emissions and large Stokes shift. The presentation will conclude with a discussion on the future prospects of this research, with an emphasis on the possible applications in bio-imaging and the development of strategies to track the structural alterations involving charge (electron and proton) transfer using broadband time-resolved impulsive stimulated Raman spectroscopy.
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