INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
PhD-17 SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Spectroscopic techniques such as electronic absorption and fluorescence are fundamental for probing protein structures and dynamic processes. Traditionally, these methods have focused on intrinsic chromophores, particularly the aromatic amino acids tryptophan, tyrosine, and phenylalanine, which absorb and emit light in the UV-Visible spectrum, providing information on local environments and conformational changes. However, reliance on extrinsic fluorescent probes to broaden optical readouts can perturb native protein structure, driving interest in label free, intrinsic alternatives. Recent research has shown that proteins lacking aromatic residues can still display significant UV-Visible absorption and fluorescence, challenging conventional assumptions about protein optics. Significantly, the synthetic monomeric protein α3C, which contains no aromatic amino acids, exhibits broad absorption from 250 to 800 nm and emits deep-blue fluorescence upon UV excitation. This behaviour arises from charge transfer interactions between charged side chains, especially lysine and glutamate, where electron donors and acceptors engage in photoinduced electron transfer followed by charge recombination, producing luminescence without conventional chromophores. This phenomenon, termed Protein Charge Transfer Spectra (ProCharTS), is supported by theoretical studies showing that charged groups can act as intrinsic chromophores and generate optical transitions even in the absence of aromatic residues. ProCharTS broadens our understanding of protein photophysics and offers a novel, label-free method to monitor conformational changes such as folding, aggregation, and interactions in proteins rich in charged residues. This thesis explores the application of ProCharTS to detect key molecular events, including phosphorylation, aggregation, and unfolding. In Chapter 3, ProCharTS absorbance and luminescence were examined in both phosphorylated and unphosphorylated peptide pairs (LG7, LG7 P, KK9, KK9 P2) and in the highly charged phosphoproteins alpha casein and beta casein. These caseins, with high proportions of charged residues, are ideal for studying charge transfer effects. Significant differences in ProCharTS signals between phosphorylated and unphosphorylated forms indicate that ProCharTS sensitively reports changes in phosphorylation state. Higher extinction coefficients in proteins compared to peptides suggest that proximal charged residue networks enhance electron transfer, highlighting ProCharTS as a non-invasive tool for studying protein dynamics. Chapter 4 applies ProCharTS to protein aggregation in alpha synuclein and human lysozyme (HuL). Aggregation increases intermolecular charge contacts, amplifying ProCharTS absorbance and luminescence. These trends correlate with conventional assays but show greater sensitivity to early oligomeric species, positioning ProCharTS as a powerful label-free aggregation monitor. In Chapter 5, ProCharTS was used to detect protein unfolding in HuL and ERK2. Disruption of charged residue proximity during unfolding led to marked decreases in ProCharTS signals, consistent with changes observed using other structural probes. Overall, these studies demonstrate ProCharTS as a versatile and sensitive label-free spectroscopic approach for probing structural transitions in proteins, especially those lacking traditional chromophores.

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