INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Monitoring Protein Unfolding and DNA binding using Protein Charge Transfer Spectra

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SHORT ABSTRACT

Proteins are not expected to show electronic absorption or emission in the near UV-Visible region in the absence of aromatic amino acid residues and cofactors. However, evidence of novel protein absorbance and luminescence in the near UV-Visible region independent of the presence of aromatic amino acid residues has now been presented by multiple research groups. By employing Time-dependent density-functional theory (TDDFT) calculations, it was postulated that charge transfer involving charged atoms in the Lys/Glu sidechain and the peptide backbone gave rise to the novel absorbance observed in the 250—800 nm region for protein α_3C. This previously unknown intrinsic chromophore was termed Protein Charge Transfer Spectra (ProCharTS) by Prasad *et al*. Characteristic features of the observed blue luminescence include low to moderate quantum yields, excitation-emission spectral overlap among the multiple proteins studied, large Stokes shifts, and similar mean lifetimes for luminescence intensity decays.

Absorbance and luminescence of the proteins α_3C, α_3W, and PRM (rich in charged amino acid residues) was measured, and photophysical characteristics of the novel luminescence like Stokes shifts and quantum yields analysed. The effect of change in solvent parameters on ProCharTS absorbance and luminescence was also characterized. In the presence of α_3C CT states, the fluorescence decay of NATA (Trp analog) was no longer a single exponential but fit to a sum of two exponentials. A short component was observed in α_3W Trp fluorescence decay upon analysis by maximum entropy method, hinting toward detection of CT luminescence along with Trp fluorescence.

The chemical denaturant-induced unfolding of folded proteins α_3C, α_3W, and HSA was characterized using ProCharTS along with other conventional methods such as CD spectroscopy, α_3W and HSA Trp fluorescence, α_3C-Dansyl and HSA-Dansyl fluorescence. A decrease in α_3C, α_3W, and HSA ProCharTS absorbance was observed upon protein unfolding. Based on denaturation midpoint quantitative analysis of GdnHCl-induced protein unfolding, it was deduced that the disruption of charged amino acid contacts preceded the loss of secondary structure and Trp/Dansyl solvent exposure. Additionally, the previously reported molten globule-like state in the presence of sub-denaturing GdnHCl concentrations was detected using increase in HSA ProCharTS absorbance.

The potential application of PRM ProCharTS absorbance to monitor genomic DNA-PRM binding was also illustrated. Mixing calf thymus genomic DNA with PRM results in DNA condensation and nucleoprotein complex formation. At low HEWL/PRM concentration, the nucleoprotein complex remained soluble, but in the presence of moderate or high protein concentration, precipitation of the nucleoprotein complex was observed. The decrease in PRM ProCharTS absorbance, HEWL absorbance, HEWL Trp fluorescence, and gDNA absorbance due to nucleoprotein complex precipitation was used to monitor DNA-protein binding indirectly. This study is thus the first to use ProCharTS to monitor DNA-protein binding.

In summary, the presence of novel intrinsic absorbance and luminescence was observed in α_3C, α_3W, PRM, and HSA proteins. The effect of solvent parameters on ProCharTS and the possible influence of CT states on Trp fluorescence was investigated. Lastly, the potential applications of ProCharTS to monitor protein unfolding and DNA-protein binding were also illustrated.