INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Spectroscopic, structural and functional characterization of Intrinsically Disordered Protein DHN1 from *Zea mays***

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SHORT ABSTRACT

Intrinsically Disordered proteins (IDPs) emerged as an essential class of proteins in the past two decades due to their functional relevance without having a proper 3-dimensional structure. Here, we investigated dehydrin (DHN1) protein from *Zea mays*, an IDP that belongs to the Late Embryogenesis Abundant (LEA) protein family. This thesis work focused on studying DHN1 structural dynamics and functional role using the novel spectroscopic tool Protein Charge Transfer Spectroscopy (ProCharTS) and other spectroscopic techniques like UV-visible and fluorescence spectroscopy. To investigate the structural change and functional role of DHN1, two mutants: DHN1 CW1 (Trp^{122} –Cys^{62}) and DHN1 W3 (Trp^{3}), were generated using site-directed mutagenesis. The structural analysis of the DHN1 protein divulges its complete random coil conformation in the native state. ProCharTS absorption was found to be sensitive to the conformational changes in DHN1 induced by the changes in pH and temperature of aqueous medium. Next, we investigated the luminescence characteristic of this novel ProCharTS in the DHN1 protein. This study suggests that ProCharTS in DHN1 protein is luminescent in nature. We found that the observed luminescence is excitation wavelength dependent and possesses a significant presence in the UV-Visible region. ProCharTS luminescence has a low quantum yield and lower luminescence lifetime. The origin behind this luminescence could be charge

recombination. Further, ProCharTS luminescence found to modulate the fluorescence of other chromophores like Trp in the same spectral region. Spectroscopic studies reveal that DHN1 and its mutant proteins are highly dynamic in nature and displays the disorder-to-order structural transitions in presence of TFE, SDS and at high temperatures. Further, the overall structural change in DHN1 protein in presence of SDS was measured using the Förster Resonance Energy Transfer (FRET) from excited Trp^{122} (donor) to dansyl-labelled Cys^{62} (acceptor). Intramolecular FRET distance between Trp—Dansyl indicated that the distance between Trp^{122} and Cys^{62} in DHN1 CW1 was reduced from 34 Å to 25 Å in the presence of SDS. Finally, the functional studies of DHN1 and its mutant protein indicate that DHN1 has significant cryoprotective and heat-protective functions via potential weak electrostatic binding to the target enzyme. Taken together, ProCharTS can serve as a label-free tool to study and detect structural transitions in the IDPs rich in charged amino acid residues and devoid of aromatic chromophores. The structural changes and the cryoprotection and heat-protection function of DHN1 protein could be attributed to its significant role in the stress tolerance mechanism in plants.