INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Probing the Structure and Dynamics of Intrinsically Disordered c-Myc PEST Fragment Using Spectroscopic Techniques**

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SHORT ABSTRACT

Intrinsically Disordered Proteins (IDPs) do not have any well-defined stable secondary or tertiary structure under the physiological conditions. Regardless of lacking stable structure, IDPs have several structural and functional advantages over the ordered proteins and participates in many key biological functions such as signaling and cell cycle control. An extensive analysis of the structure, function and dynamics of IDPs is necessary for deciphering the elaborate physiological control of their functions and how such controls might fail in human diseases. In this context, we have chosen an IDP, human c-Myc PEST region whose structural determination and characterization of disordered properties has not been done before. The c-Myc oncoprotein is a highly unstable transcription factor which is involved in many essential cell processes. Its centrally located, PEST region is responsible for quick degradation of c-Myc protein. The exact mechanism of PEST recognition and targeting of PEST containing proteins for degradation via proteasome is poorly understood and structural analysis of PEST region can provide better insights to understand their functional mechanism.

This thesis work presents thorough study of disorder properties of the human c-Myc PEST fragment, analysis of its structure and dynamics and how PEST fragment behaves in different environments using multiple spectroscopic and biophysical techniques. We have predicted

highly disordered structure of c-Myc PEST fragment by different disorder prediction tools and experimentally confirmed various disorder properties of PEST fragment. The structural analysis of PEST fragment revealed its random coil structure. This random coil structure of PEST fragment may be responsible for rapid destruction of c-Myc protein as it will easily be accessible and recognized by proteolytic enzymes. Further, by utilizing steady state and time-resolved fluorescence and other biophysical techniques we found that lowering the pH induces folding in PEST fragment and folding was more pronounced at the C-terminus compared to the N-terminus. This pH triggered folding in PEST fragment suggests a model for stabilization of c-Myc protein in some tumors as it will not be cleaved easily by proteolytic enzymes after folding. We also discovered involvement of sole cysteine residue in dimerization of PEST fragments through disulphide bond formation and this dimer of PEST was also found highly disordered in structure. This dimer formation provides some clue about involvement of PEST fragment's single cysteine in intramolecular disulphide bond formation in c-Myc protein.

In the final part of this thesis work, we exploit the richness of charged amino acid population in IDPs to sense their structural transitions using recently discovered Protein Charge Transfer Spectra (ProCharTS). Conformational changes induced in the PEST fragments by altering pH and temperature of aqueous medium was monitored by ProCharTS and confirmed by CD spectra. Significant changes in ProCharTS spectrum was observed with changing pH in the range 3—11, which correlated with changes in secondary structure of PEST fragments. ProCharTS intensity was sensitive to temperature induced changes in the secondary structure of PEST fragments between 25—85°C. Presence of 250 mM NaCl or KCl in the medium also altered the ProCharTS spectrum.

Taken together, this thesis work reveals structural and disorder properties of human c-Myc PEST fragment and highlights the utility of ProCharTS as a new label-free intrinsic probe to monitor structural transitions in IDPs.