**INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI**
**SHORT ABSTRACT OF THESIS**

| | | |
|---|---|---|
| Name of the Student | : | SAUMYA PRASAD |
| Roll Number | : | 10610623 |
| Programme of Study | : | Ph.D. |

Thesis Title: The Role of Charged Amino Acids in the Origin of UV-Visible Electronic absorption in proteins

| | | |
|---|---|---|
| Name of Thesis Supervisor(s) | : | Prof. R. Swaminathan |
| Thesis Submitted to the Department/ Center | : | Biosciences and Bioengineering |
| Date of completion of Thesis Viva-Voce Exam | : | 07-06-2017 |
| Key words for description of Thesis Work | : | Charged amino acid chromophores, Non-aromatic amino acid chromophores, Charge transfer absorption, Photoinduced electron transfer, Lysine Glutamate interactions, Intrinsically disordered proteins Protein spectroscopy, Spectral marker for protein structure, Protein backbone, Protein side chain |

## SHORT ABSTRACT

Proteins are the most abundant intracellular macromolecules which perform a diverse range of functions within the living cell. They are known to absorb in the UV region of the electromagnetic spectrum owing to the presence of aromatic amino acids (Trp, Tyr and Phe) in them. This absorption has been well characterized using UV-Visible spectroscopy. However, L-Lys.HCl (Lysine monohydrochloride), a non-aromatic amino acid was reported to display a unique absorption at 270 nm and luminescence feature at high concentrations (~0.5 M) in aqueous medium. These features could not be accounted for by any chromophore present in the Lys molecule and a possible role of the ε-$NH_2$ moiety in Lys was anticipated. Similar observations arising from interactions between two or more lysine residues present in close spatial vicinity in lysine rich proteins like Human Serum Albumin have also been reported. However the origin of these novel spectra in the absence of any aromatic moiety has remained unanswered till date.

The work reported in this thesis is an attempt to understand the nature of the chromophore and underlying mechanism involved behind these unusual spectral signatures. Initial part of the thesis deals with studies of aliphatic compounds and short peptides devoid of any aromatic amino acids. In this study we show that the $NH_2$ moiety is actually crucial in order to see any significant absorption in the near UV region (270 nm) as compounds lacking the $NH_2$ moiety remained silent with no significant absorption in this region. To explore the effect of molecular interactions among the ε-$NH_3^+$ groups of two lysine residues behind the unusual spectral signatures which were observed in lysine rich proteins like HSA, investigations with short peptides (4-7 amino acids) containing pair of lysine residues placed at different positions in the sequence were carried out. Experiments on single lysine amino acids and peptides without any lysine residue served as controls. Compared to Lys.HCl all the peptides show ~100 fold increase in the absorptivity thereby hinting towards the possible role of peptide backbone and interaction among the Lys residues towards the unusual spectral features. Besides Lys all other non-aromatic amino acids were also studied which revealed that the charged amino acids namely Lys, Glu, Arg, His and Asp show unique absorption signatures above 250 nm, which extend up to 400 nm unlike their uncharged counterparts. Also Lys.HCl shows absorption intensities ~6 times lower than pure Lys which supports the role of participation of charged head group behind the observed spectral signatures. These features were insensitive to pH and also to the $D_2O$ exchange, which reveal that proton transfer does not play a role in the absorption spectra. All these studies gave us initial clues to understand the phenomena involved. However, in order to characterize the transitions involved we required a protein which was devoid of aromatic amino acids and rich in charged amino acids. In the hunt for such a protein we devised a new methodology wherein unique prime numbers were allotted to each amino acid based on their hydrophobicities. This was then used to generate a unique numerical score for the protein (ProtID and PS-Score) which reflect the amino acid composition of a given sequence. This methodology was applied to search and hunt down a synthetic protein (Alpha 3C) from the PDB database. The protein is made up of 54 % of charged amino acids and was devoid of any aromatic amino acids. The last part of the thesis deals with employing $\alpha_3C$ protein to gain further insights into the mechanism of unusual absorption spectra. Despite lacking the conventional aromatic chromophores, $\alpha_3C$ exhibits moderate absorption features around 270 nm with a broad tail extending well into the visible spectrum. The role of the three dimensional fold of the protein

behind the observed UV-Vis spectral features in the protein was examined. Elaborate theoretical studies revealed that interactions between spatially proximal Lys-Lys, Glu-Lys and Glu-Glu head-groups modulate the spectral transitions above 300 nm in the protein. The excited state TDDFT calculations on monomer and dimer forms of amino acids of the $\alpha_3$C protein revealed that the unique spectral signatures of Lys and Glu amino acids arise from charge transfer transitions involving the amino ($NH_3^+$)/carboxylate ($COO^-$) head groups of Lys/Glu residues and the peptide backbone. The strength of absorption in the simulated spectra of Glu was more intense than that of Lys. This was attributed to the presence of a much stronger and potent electron donating head group in Glu and also to the shorter bridge between the electron donor state and the electron acceptor state, which makes the photo induced charge transfer. In summary, work from this thesis could propose an explanation for the unusual absorption spectra in Lys rich proteins observed in the past.