INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title:
Investigating the unique features of Self-assembled Hen Egg White Lysozyme Nano-aggregates using Biophysical approaches

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SHORT ABSTRACT

We report a novel strategy for synthesis of Hen-egg-white Lysozyme nanoparticles by alkaline pH induced aggregation at room temperature (298 K). Aggregation at alkaline pH is characterized by an absence of lag phase or nucleation phase. Thus, aggregation proceeds without any requirement of critical concentration of the nucleus. These nanostructures offer the advantage of synthesis in a size controlled manner. Intermolecular association by disulfide linkage (s-s) renders the protein conformation to be further stable against any electrostatic repulsion faced by the oligomeric units when transferred from pH 12.2 to pH 7.0.

Detailed characterization of a complex self-assembling system presents an enormous challenge due to the heterogeneous nature of oligomeric species involved in the aggregation of HEWL. Biophysical methods like steady state fluorescence **anisotropy**, **AFM**, **TEM**, **SAXS** and **DLS** were employed to characterize the nanoparticles. In order to address the issue of size heterogeneity, chromatographic methods such as ion exchange and size exclusion have been used. **Accessible hydrophobic regions in** HEWL nanoparticles were assessed by ANS and Tryptophan fluorescence. Sensitivity of Pyrene monomer fluorescence has been used to estimate the probe microenvironment as a result of conformational changes during aggregation of HEWL. DLS was used as characterization technique to analyze the size distribution of polydisperse HEWL oligomers. Small angle X-ray scattering has been used to extract structural parameters and track conformational transition of HEWL aggregates.

Significant drop in steady state anisotropy of **Fluorescein conjugated** fractions shows that aggregates labeled with multiple fluorophores exhibit HOMO-FRET. Chemical incorporation of $-CF_3$ group into Hen egg white lysozyme protein was used as the strategy to develop **fluorinated conjugates**. Here, we report the development of fluorinated HEWL polydisperse nanoparticles and fluorinated HEWL monomer. It was achieved by covalent linking of SETFA (S-ethyl trifluorothioacetate) to solvent accessible lysine residues of the protein.