Molecular self-assembly is a key concept in supramolecular chemistry and which has been proven to be a reliable approach for the synthesis of nanomaterials for a variety of applications. The term self-assembly implies spontaneity, in which an ordered pattern forms itself from a disordered state, which is driven by non-covalent interactions (van der Waals, electrostatic, and hydrophobic interactions, hydrogen and coordination bonds). The focus of self-assembly as a strategy for the synthesis has been confined largely to molecules, because of the importance of manipulating the structure of matter at the molecular scale. It is believed that application of these simple and versatile molecular self-assembly systems will provide us with new opportunities to study some complex and unknown biological phenomena. The molecular engineering through design and self-assembly of biological building blocks is an enabling technology that will likely to play increasingly important role in the future technological applications. In this context, our efforts are to utilize the self-assembly of the proteins for the preparation of nano-bio conjugates and use of novel self-assembled functional organic chromophores for applications in biology and medicine. The present thesis has been divided into four chapters, of which the first chapter gives an overview of the recent developments in the molecular self-assembly with a particular emphasis on nanomaterials and their applications. The overall objectives of the thesis are also briefly described in this chapter.

The second chapter of the thesis describes the development of nano-biohybrid systems, using γ-globulin, a blood plasma protein as a capping, reducing as well as a
templating agent. The γ-globulin having amino and thio groups not only found to reduce the Au(III) ions, but also stabilize the nanoparticles. We have investigated the influence of temperature and pH, in addition to the concentration of the capping agent used for the formation of the nano-bio conjugates. For example, the formation of the narrower size distribution of the nanoparticles was observed with the increase in the concentration of the protein, which supports the fact that γ-globulin acts both as a controller of nucleation as well as stabiliser.

As analyzed through various photophysical, biophysical and microscopic techniques such as TEM, AFM, C-AFM, SEM, DLS, OPM, CD and FTIR, we observed that the initial photoactivation of γ-globulin at pH 12 for 3 h resulted in small protein fibres of ca. 20 ± 5 nm in size. Subsequently, these fibres found to assist in the alignment of the colloidal gold nanoparticles of average diameter of ca. 2-2.5 nm on the surface of the protein. At this particular irradiation time, the nano-bioconjugate thus formed exhibited negligible surface plasmon resonance absorption but showed an intense photoluminescence at 680 nm. Further irradiation for 24 h, led to the formation of self-assembled long fibres of the protein of ca. 2 ± 0.5 μm in diameter and ca. 18 μm in length. Interestingly, such a morphological transformation resulted in the sequential knock out of the anchored gold nanoparticles resulting in the clustering of the nanoparticles of size ca. 5-6 nm and observation of surface plasmon resonance band at around 520 nm with the concomitant quenching of luminescence intensity at 680 nm. The observation of light triggered self-assembly of the protein and its effect on controlling the fate of the anchored nanoparticles can be compared with the naturally
occurring process such as photomorphogenesis. Furthermore, our approach offers a way to understand the role played by the self-assembly of the protein in ordering and knock out of the metal nanoparticles and also in the design of nano-biohybrid materials for medicinal and optoelectronic applications.

Investigation of the potential applications of NIR absorbing and water soluble squaraine dyes 1-3 for protein labeling and anti-amyloid agents forms the subject matter of the third chapter of the thesis. These dyes showed good solubility in the aqueous medium and exhibited favorable photophysical properties. The study of their interactions with various proteins revealed that 1-3 showed unique interactions towards serum albumins as well as lysozyme. Detailed investigation of the lysozyme interactions indicated that this protein induces hypochromicity of ca. 69%, 71% and 49% in the absorption spectra as well as significant quenching in the fluorescence intensity of the dyes 1-3, respectively. Half-reciprocal analysis of the absorption data and isothermal titration calorimetric (ITC) analysis of the titration experiments gave a 1:1 stoichiometry for the complexes formed between the lysozyme and squaraine dyes with association constants ($K_{ass}$) in the range $10^4$-$10^5$ M$^{-1}$. We have determined the changes in the free energy ($\Delta G$) for the complex formation and the values are found to be -30.78, -32.31 and -28.58 kJmol$^{-1}$, respectively for the dyes 1, 2 and 3. Furthermore, we have observed a strong induced CD (ICD) signal corresponding to the squaraine chromophore in the case of the halogenated squaraine dyes 2 and 3 at 636 and 637 nm confirming the complex formation in these cases. In contrast, the parent dye 1 showed negligible ICD in the presence of lysozyme indicating thereby its different mode of interaction. To understand the nature of interaction of the squaraine dyes 1-3 with
lysozyme, we have investigated the interaction of dyes 1-3 with different amino acids. These results indicated that the dyes 1-3 showed significant interactions with cysteine and glutamic acid which are present in the side chains of lysozyme. In addition the temperature dependent studies have revealed that the interaction of the dye and the lysozyme are irreversible.

Furthermore, we have investigated the interactions of these NIR dyes 1-3 with β-amyloid fibres derived from lysozyme to evaluate their potential as inhibitors of this biologically important protein aggregation. These β-amyloid fibrils were insoluble protein aggregates that have been associated with a range of neurodegenerative diseases, including Huntington, Alzheimer’s, Parkinson’s, and Creutzfeldt-Jakob diseases.

We have synthesized amyloid fibres from lysozyme through its incubation in acidic solution below pH 4 and by allowing to form amyloid fibres at elevated temperature. We have monitored the changes in the absorption and emission spectra of the squaraine dyes 1-3 with the increasing addition of β-amyloid. The titration of the dye 1 with amyloid fibre resulted in ca. 74% hypochromicity, while with 2 and 3, it showed ca. 76% and 68% hypochromicity, respectively, with a red shift of ca. ~23-28 nm. To quantify the binding affinities of the squaraine dyes 1-3 with β-amyloids, we have carried out the isothermal titration calorimetric (ITC) measurements. With increasing concentration of amyloid at 25 °C, we observed the enthalpy change associated with each injection and it was found to be exothermic in nature. The association constants were determined and are found to be $1.2 \times 10^5$, $3.6 \times 10^5$ and $3.2 \times 10^5$ M$^{-1}$ for the dyes, 1-3, respectively.
To gain more insights into the amyloid inhibiting nature of the squaraine dyes under investigations, we have carried out thioflavin assay, CD, isothermal titration calorimetry and microscopic analysis. The addition of the dyes 1-3 (5µM) led to the complete quenching in the apparent thioflavin fluorescence, thereby indicating the destabilization of β-amyloid fibres in the presence of the squaraine dyes. In addition, the circular dichroism studies have provided the direct evidence for the perturbation of the secondary structure of the amyloid structure and the conversion of its β-sheet rich structure to α-helical rich profile. Further, the inhibition of the amyloid fibres by the squaraine dyes 1-3, has been evidenced though the DLS, TEM AFM and SAED, wherein we observed the complete destabilization of the amyloid fibre and transformation of the fibre into spherical particles of ca. 350 nm in the presence of 1-3. These results demonstrate the fact that the squaraine dyes 1-3 can act as protein labeling agents as well as the inhibitors of the protein amyloidogenesis.

The last chapter of the thesis describes the synthesis and investigation of self-assembly as well as bio-imaging aspects of a few novel tetraphenylethene conjugates 4-6. These conjugates consist of tetraphenylethene (TPE) as the electron donor (D) and benzothiazole core as the electron acceptor (A) unit. These systems showed two absorption bands in the region 320-335 nm and 425-450 nm, wherein the first band can be attributed to the characteristic absorption of the TPE chromophore, while the latter to the intramolecular charge transfer (ICT) band. Expectedly, these conjugates showed significant solvatochromism and exhibited a hypsochromic shift (negative
solvatochromism) as the solvent polarity increased, and these observations were justified though theoretical studies employing the B3LYP/6-31g* method.

We have investigated the self-assembly properties of these D-A conjugates through variation in the percentage of water in acetonitrile solution due to the formation of nanoaggregates. We have observed a prominent enhancement in the fluorescence intensity, when the water fraction \((f_w)\) was increased to ca. 70%, 50% and 95% in acetonitrile solutions of 4, 5 and 6, respectively. The time-resolved fluorescence studies of the conjugates by varying the percentage of water \(f_w\) in acetonitrile solution showed a double exponential decay. The major component (91%) with enhanced lifetime of 5.66 ns, was attributed to the aggregated species. The short lived component (9%) was with a lifetime of 1.4 ns, which corresponds to the monomeric state. With a view to understand the nature of the self-assemblies formed, we have carried out the temperature and time-dependent studies as well as varied ionic strength of the medium. From these experiments, it was clear that the assemblies formed were J-type and were thermodynamically stable. Further the contour map of the observed fluorescence intensity as a function of the fluorescence excitation and emission wavelength confirmed the formation of J-type aggregates in these cases.

To have a better understanding of the type of self-assemblies formed from the TPE conjugates 4-6, we have carried out the morphological analysis through various microscopic techniques such as DLS, SEM and TEM. In the case of the conjugate 4, when the water fraction was raised to ca. 70%, we observed rod shape architectures having ~ 780 nm in diameter and ~ 12 µM in length as evidenced through TEM and SEM analysis.
We have made similar observations with the dodecyl conjugate 5 at *ca.* 50% water fraction in acetonitrile solution. In contrast, the non-alkylated conjugate 6 showed nanoflower architectures at *ca.* 95% water/acetonitrile mixture. On further increasing the water percentage to *ca.* 99%, the self-assembled nanostructures of the conjugates 4-6 were found to be destabilized to give rise to the nanoparticles (NPs) of average size ~10 nm. To gain more understanding of the formation of the nanoarchitectures with the change in the percentage of water fraction, we have carried out electron diffraction (ED) studies. These studies have revealed that at *ca.* 70% and 50% water/acetonitrile mixtures, the aggregates formed from 4 and 5 were found to be highly crystalline and such structures were transformed to amorphous nature as the water fraction was increased to 99%.

To evaluate the potential of the conjugate as bio-imaging agents, we have carried out their *in vitro* cytotoxicity and cellular uptake studies though MTT assay, flow cytometric and confocal laser scanning microscopic techniques. PC-3 human prostate cancer cells were chosen as model cells to demonstrate the ability of these TPE derivatives for *in vitro* cell tracing applications. Evidently, from the concentration dependent MTT studies we have observed that the nanoparticles derived from TPE conjugates 4-6 were found to be nontoxic upto 50 µM concentrations for 4 and 6 while 5 showed the cytocompatible nature upto 25 µM. Through the time-dependent flow cytometric analysis with these TPE-NPs we have observed that almost *ca.* 98% labeling of this cell lines within 5 min of incubation at 5 µM concentration. In addition the 3D analysis of the CLSM image confirmed the cell internalization of NPs. The nanoparticles
of these systems were found to have unique cellular uptake and were found to be localized within the cellular cytoplasm. Thus nanoparticle of these conjugates which exhibited efficient emission, large stoke shift, good stability, biocompatibility and excellent cellular imaging properties can have potential applications for tracking cells as well as in cell-based therapies.

In summary we have synthesized novel functional organic chromophores and have studied systematic investigation of self-assembly of these synthetic and biological building blocks under a variety of conditions. These nano-biohybrid systems were synthesized using γ-globulin, a blood plasma protein, wherein the protein acts as a capping, reducing as well as a templating agent. The investigation of interaction of water soluble NIR squaraine dyes with lysozyme indicates that these dyes can act as the protein labeling agents and the efficiency of inhibition of β-amyloid indicate, thereby their potential as anti-amyloid agents. Finally, the novel D-A systems based on tetraphenylethene and benzothiazole exhibited interesting photophysical and self-assembling properties. Thus nanoparticles of these conjugates exhibited superior stability, fluorescence efficiency and good biocompatibility in the aqueous media thereby their potential as cell tracing and imaging applications.

*Note: The numbers of various compounds given here correspond to those given under the respective Chapters.*