Chapter VI

Summary and Inferences

The high sensitivity of Fluorescence Resonance Energy Transfer (FRET) in the nanometric regime makes it a natural tool for investigation of systems involving nano-materials. The wealth of novel physical, chemical and biological behaviour that occurs on the nanometre scale has resulted in increasing interest in the application of nano-materials as an improved alternative to conventional organic luminescent fluorophore. Among these nanostructures, luminescent semiconductor nanocrystals or quantum dots “QDs”, have many fascinating optoelectronic properties with substantial promise for the construction of a new generation optical probes.

The use of semiconductor QDs as donors and dye acceptors for FRET applications is progressively increasing and stimulated by the rapid development in synthesis of colloidal semiconductor nanocrystals; and also the improved ability to design and control their properties through chemical procedures. QDs are suitable in many applications because of the ability to modify their absorption and emission through size and composition control as well as, high emission quantum yields (QYs). In particular, QDs are excellent long-lived energy donors due to their narrow emission peaks, wide and continuous absorption spectra above the band edge, and high stability under illumination.
VI. Summary and Inferences

Hence, a key advantage of using QDs for FRET based sensors is the ability to connect several donors (or acceptors) to them, which leads to increased FRET efficiency. FRET using semiconductor QDs has been utilized for variety of applications including sensing, bio-labeling, and energy funnelling for light-harvesting devices.

The focus of the present thesis is to establish the spectroscopic signatures of QD based FRET sensors on the nanometer scale and to underscore notable aspects of their spectroscopy. A sensor can be defined simply as a tool/mechanism that responds to a physical property in order to measure or detect it. A chemical sensor is a device or instrument that determines the detectable presence or concentration of a given analyte. Likewise a biosensor can detect disease related molecules using biological recognition elements. The analyte can also be labeled with a biomarker or tag, such as an enzyme, a radioisotope or a dye. If the dye is fluorescent, the device is then known as a fluorescence-based biosensor. FRET is a unique phenomenon that combines the sensitivity and selectivity of fluorescence with the strong dependence of FRET efficiency on the distance between donor and acceptor molecules as well as their orientation and it makes a “nature made” nanometric ruler used in biological and chemical systems to determine distances on the molecular scale.

FRET is commonly applied in biology and chemistry and has been used to measure distance and detect molecular interactions in a number of systems. In this sense, it has been used to provide information about protein conformation because it measures distances between domains in a single protein. Recent dramatic improvements in the development of fluorophores, such as fluorescent proteins and nanoparticles, along with
the availability of advanced optical detection capabilities have enhanced the strength of this technique and resulted in its increasing popularity.

The quantum dot based FRET sensors are based on FRET interaction between quantum dots, which serve as donors, and molecular fluorophores, which are attached to quantum dot surface, serving as fluorescent acceptors. These sensors have proven invaluable in providing the sensitivity and flexibility needed for analyzing the molecular interactions, determining the enzyme activity of proteins and also protein conformational changes. To understand a phenomenon on a molecular scale requires information about the spatial relationships between the molecules and this is where FRET’s performance is the best, i.e., to quantitatively measure distances between molecules in the range of 10-100 Å, thereby providing us with invaluable information about structure and dynamics of macromolecules.

VI. 1 Overall summary

The thesis presents the comprehensive study of spectroscopic properties of quantum dots with special focus on understanding how their size, composition and surface quality influence the QD-dye FRET parameter. We have chosen CdSe, CdSe/ZnS and water soluble alloyed CdSeS/ZnS quantum dots as donors, in order to initiate research work on development of FRET based chemical and biosensors; owing to their optical properties that can very selectively be tuned in visible region. The thesis also explores the biological work to study the protein-QD interaction. The protein selected for interaction studies is bovine serum albumin (BSA).
VI. 1.1 Important inferences:

1. The first part of the work as described in Chapter-III, examines the effect of core and core-shell QDs on the FRET efficiency. Experimental photoluminescence spectra of core CdSe QDs often exhibit broad tail at near-infrared energies, well below the fundamental band gap, the so-called deep trap emission. The intensities of these broad bands are sensitive to the surface termination, thus suggesting that they arise from deep trap states that reside on the crystal surface. These underscore the importance of the role of surface states in QD based energy transfer process; a surface-related emission due to recombination of electrons and holes on the surface strongly implies the involvement of surface states in the recombination process in colloidal QDs, which is a topic of current interest. Because the electron has a much smaller effective mass than the hole in a QD, once generated, it has much greater chance of going to the surface rather than staying inside the core of the QD. This “delocalization” feature of the electrons can be further confirmed by the fact that the PL quantum yield of colloidal QDs increases upon passivation of the surface utilizing core/shell structure. A comparative analysis of the FRET dynamics and dependence of the energy transfer efficiency on the spectral overlap integral and QY provides a way to investigate the role of intermediate QD surface states in energy transfer.

2. The present work opens up avenues towards the utility of effect of core and core-shell QDs on FRET efficiency. The important observation of the study being non-linear dependence of the energy transfer efficiency on both the spectral overlap as well as quantum yield of donor which is possibly due to the intermediate states of
the QDs. CdSe/ZnS QDs show enhancement in energy transfer efficiency as function of spectral overlap and quantum yield of donor QDs in contrast to CdSe core QDs. This enhancement being non-linear could again be explained as due to the involvement of surface states of QDs. The effect of surface trap state on band-edge recombination of core and core-shell QDs determined by time-resolved fluorescence spectroscopy revealed the dependence on electron-hole recombination of nanocrystals. The short lived ($\tau_1$) decay component, representing the lifetime of fluorescence decay at the band-edge, is comprised of both a radiative decay from electron-hole recombination and nonradiative decay via trap states. An increase in QD size is accompanied by increase in $\tau_1$, a behaviour that is explained as due to the decrease in accessible trap state through a reduction in surface to volume ratio because of the poor overlap of the carrier wave functions.

The central findings in the present work are: (i) surface state of quantum dots play an additional role in FRET efficiency and (ii) FRET efficiency depends on both spectral overlap as well as the quantum yield of donor. Therefore, the present study demonstrates that such QD-dye pairs can perform as very sensitive chemical sensors. The possibility of their use in biological application is being explored.

3. As described in Chapter IV, the photoluminescence spectroscopy of alloyed quantum dots (QD) is also used in FRET investigation, one of the unique properties of binary QD is size tenability: as the size of the nanocrystal increases PL emission is shifted to longer wavelength. However, these tiny size tunable
QDs are still facing difficulties in successful application in biological molecular dynamics study using FRET concept and also in multiflexing experiments. Because QDs sizes are sufficiently larger in size than biological molecules, new kinds of nanocrystals have been invented. Optical properties of these nanoparticles (NPs) are found to be different from those of binary or core/shell nanocrystals and the observed Stokes shifts are in the rage of 80-100 nm, which is considerably larger than that for most semiconductor nanocrystals. The colour-tuning emission properties are controlled by changing their constituent stoichiometry without changing the particle size. Here the emission properties of alloyed QD are tuned by varying the composition of sulfur and selenium present in the QD. By changing the ratio of selenium to sulfur composition the emission wavelength of QD is shifted to redder region.

Steady-state and time-resolved fluorescence spectroscopy of CdS$_x$Se$_{1-x}$/ZnS samples resulted in linear dependence of the energy transfer efficiency on the spectral overlap as well as on quantum yield of QD. We have demonstrated that there is a possibility of tuning the transfer efficiency up to 52 % as the acceptor-donor concentration ratio is varied. The data and analysis presented here strongly support the quenching of the alloyed QDs by Rhodamine dye via a dynamic quenching mechanism. These experiments indicate that energy transfer occurs via nonradiative pathway from the CdSeS/ZnS QDs to the Rhodamine dye. The observed values of Forster distance $R_0$ are in the range of 4.42 – 6.23 nm and are comparable to the size of biomolecules and the distance between the sites on multisubunit proteins. Therefore, our studies indicate that the present Donor-
Acceptor pairs are suitable for studies of biological macromolecules. The entire work was carried out in aqueous solution with a view to explore its applicability in biological systems.

4. For the first time we have reported that composition of quantum dots also plays a role in FRET process. FRET efficiency can be controlled by tuning the QD photoemission by varying the composition present in the QD. Our results have showed a clear dependence of the efficiency on the spectral overlap between the QD donor and dye acceptor. In this present work we observe a linear enhancement and also we have been able to achieve 50% FRET efficiency for the present QD-dye pair in aqueous system. Thus, the present work opens up new possibilities for designing light harvesting nanostructures for future applications.

5. Nanotechnology is an extremely powerful emerging technology, which is expected to have a substantial impact on the fields of pharmaceutical and medical diagnostics; there is an intensive interest in understanding the interaction between nanomaterials and biomolecules like proteins. The Chapter V discusses the spectroscopic investigation of interaction between protein and-QD by using various spectroscopic techniques and concept of FRET discussed above has been applied. Bovine serum albumin (BSA) was selected since it is well studied, most abundant in plasma and one of the most used model protein. It has a high percentage of the total plasma proteins with its major physiological role being to carry various ligands to their respective target organs. There are a number of techniques to study the interaction between quantum dots /nanocrystals and BSA, but the most convenient method is to study the fluorescence quenching of BSA.
Fluorescence quenching measurement of albumin is an important method to investigate the interactions of drugs with serum albumins. It can reveal the accessibility of quenchers to albumin’s fluorophore groups, help to understand the binding mechanisms of albumins with drugs and provide clues to the essentials of the binding phenomenon.

The present investigation emphasizes on understanding the biophysical mechanism of interaction between CdSeS/ZnS QDs and BSA. We find that BSA intrinsic fluorescence is quenched by both static and dynamic quenching mechanisms. The presence of a BSA-QD complex was confirmed by resonance light scattering (RLS) and absorption spectra of BSA. The extent of quenching of BSA fluorescence increases with an increase in temperature which further highlights the dynamic quenching mechanism. The binding constant and binding sites for BSA-QD have been calculated. The thermodynamic parameters ($\Delta H^0$, $\Delta S^0$ and $\Delta G^0$) were evaluated from Vant’t Hoff plots. The positive values of enthalpy and entropy change indicated that the interaction of BSA and QD was driven mainly by hydrophobic forces. The process of binding is spontaneous as Gibb’s energy change was found to be negative. Synchronous fluorescence spectra indicate a small change in the microenvironment of tryptophan residues. The binding sites values obtained close to unity suggest that number of QDs per BSA is unity. Thus, this work can be of great significance in advancement of protein NPs/QDs technology and understanding their potential use in drug delivery applications.
VI. 2 Future Direction

Förster resonance energy transfer (FRET) is being extensively used in many laboratories around the world to investigate and understand the phenomena that occur on the molecular scale. It is compatible with a range of microscopy techniques, improving on the resolution limit dictated by diffraction (typically 250 nm for visible light) by close to two orders of magnitude, opening our eyes to the molecular physiology of living systems and conformational dynamics.

FRET is now a stable method in biology for probing molecular interactions, conformations and subcellular organisation. However, in the particular context of imaging the self-assembly of biological molecules in live cells, applications are only just emerging. A useful variant of FRET for this purpose is homo-FRET, referring to the energy exchange between like fluorophores.

In the near future we plan to study the electronic energy transport between chemically identical fluorophores (i.e., donors) in studies of various protein systems. Measurements of intra- and interprotein energy migration by steady-state fluorescence anisotropy and fluorescence anisotropy imaging microscopy (FAIM) which have great potential for measuring molecular self-assembly in cells. Homo energy migration anisotropy detection and imaging for molecular self-assembly, and focus on aspects of its practical implementation coupled with the development of new fluorescent probes like quantum dots are vital to successful transition from proof-of-concept experiments to new biological aspects.