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Summing Up....

The goal of this thesis is to establish the spectroscopic signatures of QDs in nanoscale systems and to suggest notable aspects of their spectroscopy. Ever since the pioneering work of Förster, fluorescence resonance energy transfer (FRET) has become a basic experimental tool extensively used in a variety of fields. Undoubtedly, understanding any phenomena on a molecular scale has always been one of the major goals of all physical, chemical and biological quests. The strong dependence on distance, makes FRET a "nature made" nanometric ruler used in biological and chemical systems to determine distances on the molecular scale. However, at present when there is great interest in characterizing nano-materials, its achievement has gained immense priority as never before. 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 10-100Å, thereby providing us with invaluable information about structures and dynamics of macromolecules.
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The high sensitivity of 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 behavior that occurs on the nanometer 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 of 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 control, high emission quantum yields (QYs), which determine the spectroscopic states of the material and its photochemical properties. 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. Hence, a key advantage of using QDs for FRET is the ability to connect to them several donors (or acceptors), 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. Furthermore, the study of FRET properties
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of quantum dots (QDs) in solutions and arrays is presented in the introduction section.

VI.1 Overall summary

This thesis presents a comprehensive study of the spectroscopic properties of CdSe and CdSe/ZnS quantum dots with special focus on understanding how their optical properties and surface quality influence, the FRET parameters with organic dye acceptors. We have chosen CdSe and CdSe/ZnS nanocrystals, because their optical properties can selectively be tuned in visible region, making them particularly valuable as energy donors in FRET experiments.

In the first part of work (Chapter-III), the spectrally resolved energy transfer process was explored using steady-state and time-resolved fluorescence spectroscopy. From our systematic study of the fluorescence spectra and kinetics as a function of QDs, excitation energy and detection wavelength, we have determined the $\lambda$-dependent FRET rate with respect to spectral overlap of red and blue counterpart of QDs. The two different sized QDs display different emission spectra and as the emission of QDs shift to longer wavelengths the overlap with the absorption spectra of acceptor (F27) dye increases. Consequent to increase in the QD size, the QDs undergo different FRET quenching efficiency due to difference in their spectral overlap. It is interesting to note that a decrease in efficiency even with better spectral overlap is due the heterogeneity in the $\lambda$-dependent FRET rate and acceptor absorption spectra. In order to analyse the
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Effect of size and wavelength reliant variation upon FRET, we have measured the donor lifetime over the emission spectrum (for different emission wavelength) of QDs population for each FRET pair without and with acceptor. In time-resolved measurement, the FRET dynamics are certainly not homogeneous within QD population, where QD emission overlaps with blue edge of the acceptor absorption than bluer QDs. This in turn results in a gradually faster FRET dynamics as we move through the bluer QDs to redder QDs. In the second pair (QD2-F27) the exciton decay becomes gradually slower as we move from the bluer QDs to the redder QDs, since the bluer counterparts possess a better spectral overlap than the redder QDs in this pair. It is clearly seen that energy transfer is highly efficient (43-51%) extending over a range of 34-36 Å ($R_0$).

Chapter-IV explores 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 studying the role of surface states in QD based energy transfer process; a surface-related emission due to the 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 a much
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greater chance of going to the surface rather than staying inside the core of the QD. This "delocalized" feature of the electrons can be further confirmed by the fact that the PL quantum yield of colloidal QDs increased 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.

The observed non-linear dependency of the energy transfer efficiency on spectral overlap is possibly due to the intermediate states of the QDs. On the contrary CdSe/ZnS QDs show non-linear enhancement in fluorescence energy transfer efficiency as function of spectral overlap as compared to CdSe core QDs, which could again be explained by the involvement of QDs surface states as the time-resolved data reveals that, the shorter lifetime is generally attributed to the intrinsic recombination of initially populated internal core-states and the longer lifetime component in the PL decay is caused by the radiative recombination of electron and holes on the surface involving localized states. The important observation is that the amplitude of $A_3$ with longer lifetime accounts for the larger share of the longer lifetime component is a clear indication of major surface related emission caused by the radiative recombination of charge carriers involving surface states and the overall faster recombination may indicate that trap states are more delocalized. Hence, ZnS shell and its capping agent does not completely passivate the states present on the surface of core sample. However,
the nature of the recombination process is a complex issue that requires much further investigation.

Chapter-V discusses the new spectroscopic method, synchronous fluorescence scan (SFS) spectroscopy for simultaneous determination of individual mixtures of CdSe and CdSe/ZnS QDs of various sizes by applying second derivative to SFS technique. The combination of synchronous and derivative fluorometry enhances minor special features and allows more reliable identification and characterizing of chemical species. By this technique we can interpret the spectroscopic properties of nano-materials in great detail, as observed with CdSe QDs, photoluminescence surface states of CdSe QDs play a major role in fluorescence emission. In a conventional fluorescence emission spectrum there are two kinds of emission bands. The first one is the band edge emission, which is size dependent and excitation wavelength independent in certain wavelength region. The second one is the deep trap emission in the longer wavelength and is less size-dependent and we need to suppress this trap emission band. Hence, the use of synchronous scan fluorescence (SFS) technique allows the stronger peaks (band-edge emission) to be increased/enhanced selectively by the use of suitable offset wavelength interval ($\Delta \lambda$). Hence CdSe 480 QDs the surface state emission or deep trap emission at the offset of $\Delta \lambda = 10$-100 nm became less pronounced and even disappeared at $\Delta \lambda = 125$-150 nm, thus providing deeper understanding of spectroscopic measures of QDs with the help of powerful computational and new analytical techniques. Efforts are made
to develop analytical methods for continuous sensing of analytes in real time and in situ.

In conclusion, an exciting aspect of nanoparticles is that relationships between structure and electronic properties are being revealed through a combination of structural characterization, chemical physics and theory. Spectroscopy is a powerful tool that is sensitive to aspects of the electronic structure of materials. As such, spectroscopic studies of nanoscale systems often provide insights into the collective absorption and redistribution of excitation energy and solid state materials acquire molecular like spectroscopic features.

VI.2 Scope for future work

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. FRET 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 staple 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. HomoFRET, in contrast to heteroFRET, requires only a single fluorophore moiety for labelling
and is detectable by measuring the loss of anisotropy (i.e. loss of polarization) in the emission upon excitation with polarised light. Measurements of homoFRET by steady-state fluorescence anisotropy, fluorescence anisotropy imaging microscopy (FAIM) have great potential for measuring molecular self-assembly in cells.

In the near future we plan to study the homoFRET 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 that are vital to the successful transition from proof-of-concept experiments to new biological remarks.