RESEARCH ARTICLE

QUANTUM DOT BASED FRET TO FLUORESCEIN 27: A SPECTRALLY RESOLVED ENERGY TRANSFER STUDY

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ABSTRACT

Fluorescence resonance energy transfer between CdSe (480 nm) quantum dots (donor) and fluorescein 27 (acceptor) is studied using steady state and time-resolved fluorescence measurements at room temperature in toluene based solutions. Decrease in the fluorescence intensity of QD donor and increase in the dye emission intensity with increasing concentration of acceptor dye and a significant reduction of the fluorescence lifetime of the QDs in the presence of acceptor are noted. The results indicate the occurrence of efficient FRET in this system and also a clear dependence of FRET efficiency on the spectral overlap between the QD emission spectrum and the dye absorption spectrum.

INTRODUCTION

Communication, coupling between quantum dot and dye are central themes in numerous scientific efforts of present physical and technological interest in physics, chemistry and biology because of the extraordinary photophysical properties quantum dots [Crooker *et al.,* 2002, Rogach *et al.,* 2009, Somers *et al.,* 2007, Clapp *et al.,* 2006, Pons *et al.,* 2006]. In the range of 10-100 Å Förster limit i.e., "spectroscopic ruler" [Stryer *et al.,* 1978, Lackowicz *et al.,* 2006, Huachang Lu *et al.,* 2008] colloidal semiconductor quantum dots provide an alternative replacement to molecular dyes as FRET donor, an alternative approach that exploits the readily achievable coupling via long-range dipolar interaction which allows QD donor-dye acceptor communication via Förster formalism [Dezhurov *et al.,* 2011, Medintz *et al.,* 2009, Livingston *et al.,* 1957, Pons *et al.,* 2006, Rogach *et al.,* 2008]. Here the donor exciton couples with nearby acceptor through coulombic interaction effectively in transferring the excitation energy to the lower-energy fluorophore.

Resonance energy transfer provides molecular information that is different from that revealed by solvent relaxation, excited-state reactions and fluorescence quenching or fluorescence anisotropy. These other fluorescence phenomena depend on interactions that are less important for energy transfer, except for their effect on the spectral properties of the donor and acceptor [Lakowicz *et al.,* 2006]. Further Förster energy transfer rate depends critically on the spectral overlap integral between normalized donor emission and acceptor absorption shapes and on the donor-acceptor coulomb coupling $J(\lambda)$ [Scholes *et al.,* 2003]. However, the QD optical properties are dictated by the quantum mechanical color-size effect, due to strong confinement of the electron-hole pairs [Alivisatos *et al.,* 1996]. Single QDs possess very narrow emission spectra with size dependent central wavelengths spanning the whole visible spectrum from blue to red. Because of small inherent inhomogeneities, even a narrow size distribution population is always composed of individual nanocrystals of different sizes and thus distinct optical properties. In comparison,

molecules in an organic dye sample are identical and heterogeneity is the result of uncontrolled environment perturbations [Pons *et al.,* 2006, Wang *et al.,* 2001]. When using QDs as FRET donors [McGrath *et al.,* 2008], we take advantage of this size heterogeneity within a population to extract detailed information on effects of the spectral overlap on the Förster energy transfer between QD donors and dye acceptors. Because of the distinct narrow emission spectra of single nanocrystals, the spectral overlap for a QD population actually varies from one individual QD-dye pair to another, which implies that a QD emitting in a region of higher acceptor absorbance undergoes a significantly higher FRET quenching than a nanocrystal emitting in a region of lower absorbance [Pons *et al.,* 2006, Hoefling *et al.,* 2011]. We present here a spectroscopic study of the energy transfer from excitons confined in CdSe QDs to Fluorescein 27 dye. T. Förster [Förster *et al.,* 1948] has described inverse sixth power distance dependence of resonance energy transfer wherein at Förster distance (R_0), half the donor molecules decay by energy transfer and half decay by the usual radiative and non-radiative rates [Van der Meer *et al.,* 1982, Förster *et al.,* 1959, Andrews *et al.,* 1999, Agranvich *et al.,* 1982, Latt *et al.,* 1965]

$$R_0 = 0.211(k^2 n^{-4} Q_D J(\lambda))^{1/6} \ (\text{in Å}) \qquad \ldots\ldots\ldots 1$$

and the rate of transfer for donor and acceptor separated by distance r is given by

$$k_{FRET}(\lambda) \propto k^2 J(\lambda) r^{-6} k_r \qquad \ldots\ldots\ldots 2$$

where k^2 is orientation factor and k_r is the donor radiative rate. $J(\lambda)$ is the spectral overlap integral where corrected donor emission spectrum with its area normalized to unity and the acceptor absorption profile [Clapp *et al.,* 2004] which is reduces to

$$J(\lambda) \approx \varepsilon_A(\lambda)\lambda^4 \qquad \ldots\ldots\ldots 3$$

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Due to the distinct narrow emission spectra of single nanocrystals, the spectral overlap for a QD population actually varies from one individual QD-dye pair to another [Clapp *et al*., 2004, Dennis *et al*., 2008]. This implies that QD emitting in a region of higher acceptor absorbance undergoes a significantly higher FRET quenching than a nanocrystal emitting in a lower absorbance region. Thus QD emission spectrum will change in both intensity and shape with increasing FRET rate and the signal from a population of QDs conjugated to acceptor is given by

$$S(\lambda) = S_0(\lambda)\left(1 - \frac{k_{FRET}(\lambda)}{k_{FRET}(\lambda) + k_r + k_{nr}}\right) \quad \ldots\ldots\ldots\ 4$$

where $S_0(\lambda)$ is the signal from the QDs alone and one can derive FRET rate for QDs emitting at specific wavelength λ from variation in the ensemble emission spectrum $S(\lambda)$:

$$k_{FRET}(\lambda) \propto \left(\frac{S_0(\lambda)}{S(\lambda)} - 1\right) \quad \ldots\ldots\ldots\ 5$$

Equations 2, 3 and 5 indicate that the FRET rate is also proportional to $\varepsilon_A(\lambda)\lambda^4$ which can be measured independently.

MATERIALS AND METHODS

Fluorescence spectra of the donor (QDs), acceptor (dye) and the mixed solutions were recorded using spectrofluorometer (Hitachi, Model-F7000) and the uv/vis absorption spectra were obtained using spectrophotometer (Hitachi, Model-U2800). The fluorescence lifetimes were measured employing picosecond time domain spectrometer based on Time Correlated Single Photon Counting (TCSPC) technique (IBH Jobin Yvon6.1), described elsewhere [Kumaran *et al*., 2006]. The samples of CdSe 480 nm (2.1 nm size) quantum dots were excited at 375 nm using Nano LED in an IBH Fluorocube apparatus. The fluorescence emission at the magic angle (54.7) was dispersed in a monochromator (f/3) aperture and counted by a Hamamatsu Micro Channel Plate Photo-multiplier tube (R3809 MCP-PMT). The instrument response function for this system is ~52 ps. An iterative fitting program provided by IBH (DAS-6) analyzed the fluorescence decay curves. Toluene solutions of CdSe 480 nm core QDs (Lumidot, 2.1 nm size) were purchased from Sigma-Aldrich Chemicals Co. USA and Fluorescein 27 dye from Lambdaphysik, Inc. All the solvents used were of HPLC grade and were used without further purification.

RESULTS AND DISCUSSION

Steady-State Measurment

With a view to develop FRET chemical and biosensors based on semiconductor QDs, CdSe 480 nm Core QDs are chosen as donor in the present study with Fluorescein 27 dye as acceptor. Each molar concentration was studied in free solution under steady state condition. (Fig. 1) shows the normalized emission spectra of CdSe 480 nm core quantum dots in toluene excited with 375 nm light along with the absorption spectrum of F27 dye. F27 dye does not exhibit any appreciable absorption at 375 nm and therefore allows exclusive excitation of the donor. The photon induced energy transfer from donor to acceptor is monitored. A necessary factor to achieve FRET phenomena is good spectral overlap between the absorption of acceptor and emission spectrum of donor. It is observed that the addition of F27 dye to the CdSe solution does not lead to any change in the absorption spectrum of QDs. Fig. 2 shows fluorescence spectra of CdSe QDs in the presence of varying concentration of F27 dye. Upon addition of F27 dye a gradual and significant quenching of the

donor (CdSe QDs) fluorescence was observed with a simultaneous enhancement in the acceptor emission indicating some form of energy transfer to acceptor (F27). Consequently, the addition of the acceptor in increased proportion results in the presence of more acceptor molecules. Hence, this quenching of QD emission and simultaneous enhancement in F27 emission validates and confirms the nonradiative resonance energy transfer from CdSe QD to F27 dye. The generously proportioned quenching of donor and the use of high acceptor to QD ratios will compensate the large separation distance which improves the FRET efficiency. Further Pons et.al.,[2008] have observed that the FRET efficiency is not constant throughout the PL spectrum of a QD population, but is wavelength dependent. Furthermore, heterogeneity in the λ-dependent FRET rate measured for a sample depended upon the QD emission overlap and on whether the QD emission overlaps with red or blue regions of the acceptor spectrum.
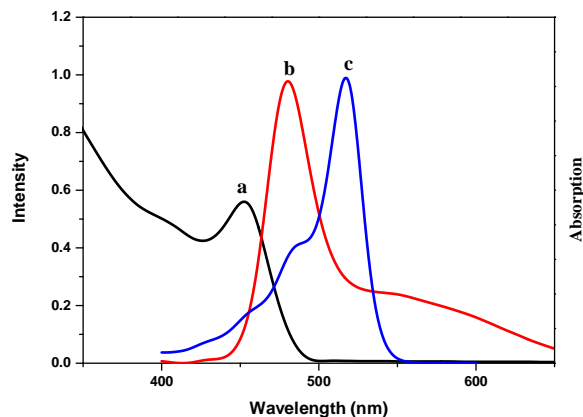

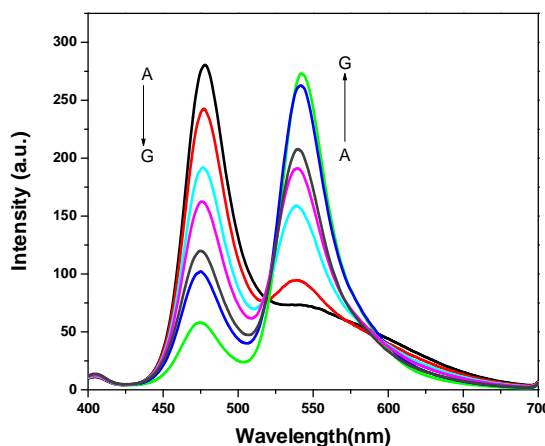**Fig. 1. Absorption (a) and emission spectra (b) of CdSe 480 nm QD along with absorption of F27 (c)**

Fig.2. Fluorescence spectra of CdSe 480 (1.0 x 10^{-3} M) in the presence of varying concentrations of F27 dye (acceptor); A) 0, B) 0.8 x 10^{-6} M, C) 1.0 x 10^{-6} M, D) 3.0 x 10^{-6} M, E) 6.0 x 10^{-6} M, F) 8.0 x 10^{-6} M, G) 10 x 10^{-6} M
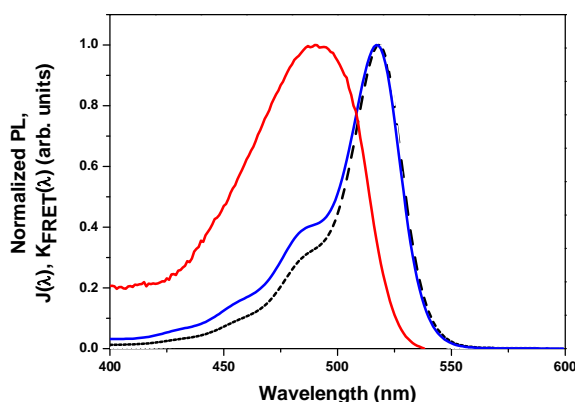

Fig. 3. Normalized plot of the overlap $J(\lambda) \approx \varepsilon_A(\lambda)\lambda^4$ for CdSe 480 nm QD (Plain Blue) together with FRET rate curve (Plain, Red) and F27 absorption (dashed black)

Table 1. FRET parameters obtained from steady-state and time-resolved measurements

System	$J(\lambda)$ $(M^{-1}cm^{-1}nm^4 \times 10^{-14})$	R_0 (Å)	r (Å)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	A_1	A_2	A_3	Average $\langle\tau\rangle$ $(A_1\tau_1 + A_2\tau_2 + A_3\tau_3)$ (ns)
CdSe 480				1.07	8.20	60.12	0.03	0.09	0.88	53.67
CdSe 480-F27	5.064	36.61	19.39	0.63	4.12	36.93	0.07	0.16	0.77	29.13

* A_1, A_2, A_3 are normalized amplitude components
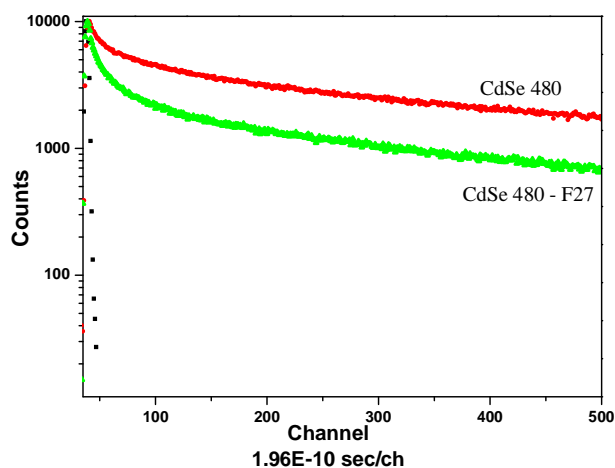

Fig. 4. Log scale plot of time-resolved photoluminescence trace for CdSe 480 nm QD with and without F27 dye (donor emission wavelength fixed at 480 nm with excitation at 375 nm)
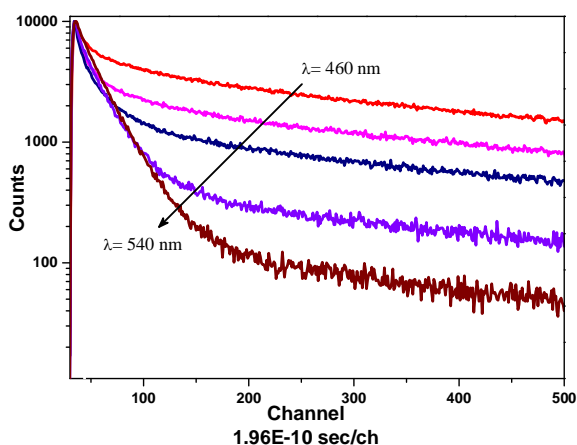

Fig. 5. Plot of the time-resolved photoluminescence trace of CdSe 480 nm QD with F27 for different emission wavelengths from 460 to 540 nm

It is possible to describe this evidently by noticing spectral overlap of donor QDs and acceptor, i.e., CdSe 480-F27 pair exhibits some sort of redder counterpart of QD emission overlapping with bluer counterpart of F27 acceptor, leading to redder QDs population being quenched to a greater extent and FRET rate curve (Fig. 3) clearly traces the acceptor absorption spectrum. We estimate the Förster distance (R_0) and intermolecular distance for this pair using Förster method as 36.61 Å and 19.39 Å, respectively. Alphandrey *et al.,* [2004] reported the Förster distance of ~30-35 Å between CdTe and Rhodamine B dye. Kagan *et al.,* [1996] reported the same order of magnitude for highly efficient FRET between QDs of different sizes. Medintz and co-workers [2006] have reported the Förster distance in the range 42-55 Å for QDs with peptide/dye conjugates. Hence, it reveals that the effect of spectral overlap between QDs emission and dye absorption is appreciable.

Time -Resolved Measurements

The nonradiative resonance energy transfer is known to substantially alter the exciton lifetime properties of the donor (τ_D). Thus, the energy transfer process was further examined using fluorescence

decay analysis by TCSPC Technique. Fig. 4 shows the fluorescence decay curves of QDs with and without F27 dye. The lifetimes in the presence and absence of acceptor dye are likely to throw light on the FRET efficiency in the chosen donor-acceptor pairs. The samples were excited with 375 nm light from LEDs and fluorescence decay traces required a sum of three exponential functions to reach low χ^2 values as well as random distributions of the weighted residuals, which are indicators of the goodness of the fits. Decay curves of the CdSe 480 nm core QD are multiexponential with three decay components as shown in Table 1. The photoluminescence decay of CdSe QD is tri-exponential with the average decay time being 53.67 ns. However, in presence of acceptor its average decay time in the presence of the acceptor is 29.13 ns. Thus the lifetime of CdSe QDs are significantly shortened upon addition of the F27 dye and it is one of the characteristic features of efficient energy transfer in the donor-acceptor systems. The reduction in lifetime also heralds the quenching of the QD emission by the acceptor dye molecules with respect to spectral overlap (Fig. 1) where the QD emission overlaps with the blue edge of the acceptor absorption spectrum i.e., the redder QDs have better overlap with acceptor absorption spectrum than the bluer QDs. This results in the decay of the intensity ratio being faster for increasing wavelength (Fig. 5) and faster FRET dynamics as we move from bluer QDs to the redder QDs.

Conclusion

The higher potential of the QDs-dye nano assembly combination than the typical organic donor and acceptor dye pairs enhance the scope of FRET analysis particularly in biological applications. In this study we have explored the energy transfer between CdSe QD (480 nm) as donor and F27 dye as a acceptor. The results clearly illustrate the dependence of FRET efficiency on spectral overlap as depicted in (Fig. 1) and demonstrated that the wavelength dependent energy transfer rate matches the dye acceptor absorption spectrum, as predicted by Förster formalism. These spectral dependencies of the rate of energy transfer are expected to extend to other mechanisms of energy transfer from QDs, such as electron transfer and metallic nanoparticles quenching [Yun *et al.,* 2005].

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