Resonance energy transfer in conjugates of semiconductor nanocrystals and organic dye molecules

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Abstract. I analyze the efficiency of Förster resonance energy transfer (FRET) in luminescent donor-acceptor complexes based on conjugates of CdSe/ZnS quantum dots and nanorods and the luminescent dyes. Semiconductor nanocrystals serve either as FRET donors or acceptors. Experimentally observed reduced FRET efficiency in complexes of nanorods and dye molecules as compared to quantum dots are found to be attributable to a distance-limited energy transfer rate in case of point-like dye dipoles and extended nanorod dipole.

Keywords: quantum dots; excitons; fluorescence.

1 Introduction

Förster resonance energy transfer (FRET) in a system of semiconductor quantum dots (QDs) and organic dye molecules is important for practical applications in biology, medicine, sensing, photovoltaic devices, etc.\textsuperscript{1-7} Conjugates of QDs and organic molecules serve as pH and redox sensors\textsuperscript{1-5} and multiplexed markers.\textsuperscript{6-9} Most experiments are done with CdSe or CdTe based core-shell QDs serving as FRET donors and various luminescent dye molecules serving as acceptors.\textsuperscript{9-12} Quantum dots are almost ideal candidates for FRET donors or acceptors, due to their extended optical absorption, tunable and narrow emission band, and relatively high luminescence quantum yield. Semiconductor nanorods (NRs) attract considerably less attention, although they possess unique optical properties.\textsuperscript{13} NRs absorb and emit strongly polarized light, due to the optical dipole oriented and extended along the NR long axis.\textsuperscript{14} FRET in the system of oriented dipoles is an important tool for studying the relative orientation and dynamics of chromophores. Additionally, an extended optical dipole in NRs causes a much larger molar absorption coefficient, which is important for achieving efficient FRET.

Usually, FRET is studied between different organic molecules, inorganic ions, or small nanoparticles of more or less similar size. In this case, the classic Förster theory describing interaction between point-like optical dipoles is valid. In case of semiconductor NRs, their length is about two orders of magnitude larger than the size of an organic dye molecule. Taking into account that the optical dipole in a semiconductor NR is extended over the whole NR length, the FRET problem in case of an NR-dye conjugate can no longer follow the Förster rules. Since I am interested mainly in FRET efficiency in dye-nanoparticle conjugates for practical applications, the nanoparticle geometry and dimension can play a very important role. In this paper, I make a comparative study of the FRET efficiency in conjugates of fluorescent dye molecules and CdSe/ZnS core-shell QDs and NRs where both QDs and NRs serve as either the FRET donors or acceptors.

2 Experiment and Materials

Hydrophobic CdSe-ZnS core-shell QDs (4 nm in diameter) and NRs (4 nm in diameter and 23 nm in length) were synthesized according to previously published procedures based on...
high-temperature reaction between organometallic precursors in coordinating solvents. The nanocrystals were solubilized in water with D,L-cysteine (Cys), or a mixture of aminoethanethiol and dimethylaminoethanethiol. Briefly, dry nanocrystals (ca. 5 mg) were dissolved in chloroform (2 ml), and methanol (3 ml) was then added. The solid phase was centrifuged, washed with methanol, and dissolved in chloroform (3 ml). Next, an equimolar mixture of aminoethanethiol hydrochloride (Aldrich, 0.3 ml) and dimethylaminoethanethiol hydrochloride (Aldrich, 0.3 ml) in methanol (0.1 mmol/mL each) was added to the nanocrystals in chloroform, and the mixture was stirred for 20 min to complete the binding of thiols to the surface of the nanocrystals.

In another option, 0.5 mL of D,L-cysteine hydrochloride (Cys) in methanol (5 mg/mL) was added to nanocrystals in chloroform, and the mixture was stirred for 10 to 15 min to complete the binding of Cys molecules to the surface of nanocrystals.

The solid phase containing the nanocrystals with the monolayer of aminothiols on their surfaces was centrifuged, washed two times with methanol, and dissolved in anhydrous dimethylsulfoxide (DMSO, 3 ml). Alexafluor®647 carboxyl acid succinimidyl ether (Invitrogen) was dissolved in anhydrous DMSO at the concentration of 0.1 mM, and 100 μL of this solution was added to the nanocrystals in DMSO solubilized with the aminoethanethiol/dimethylaminoethanethiol mixture. The mixture was stirred in the dark at room temperature for 2 h in order to complete the reaction between the succinimidyl ether groups of Alexafluor®647 and amino groups of aminoethanethiol on the surface of the nanoparticles. The conjugates of nanocrystals with dye molecules where dialyzed against bidistilled water. During the dialysis, the conjugates formed the aggregates, which were removed from the solution by centrifugation (5000 rpm, 3 min). The solid phase was washed several times by bidistilled water, in order to remove unbound dye molecules, and redispersed in a fresh portion of DMSO (3 ml).

For Cys-stabilized nanocrystals, fluorescein isothiocyanate (FITC, Sigma) was conjugated with NRs and QDs according to the published protocol. Briefly, 1 mg of FITC was dissolved in 5 ml of anhydrous DMSO and stored at 4°C. Then two 20-μL aliquots of FITC solution were added to 2-mL aliquots of aqueous solutions of QDs and NRs at room temperature and stirred for 1 h in the dark. Then both solutions were purified by dialysis against 1 : 100 diluted phosphate buffer in order to remove unbound FITC. QD-FITC and NR-FITC conjugates were embedded into the polymeric matrices and studied in a solid form.

To prepare polymeric films containing QD-FITC and NR-FITC conjugates, a polymer polyvinylpyrrolidone (PVPR, Aldrich, MW = 1300000) was added to each solution to the concentration of 5 mg/mL. Additionally, three reference aqueous solutions with the same concentration of PVPR and pH were prepared with Cys-solubilized QDs, Cys-solubilized NRs, and pure FITC. Finally, all five solutions were drop-cast on to round quartz substrates 2.5 cm in diameter and dried at room temperature in the dark to obtain polymeric films. The average thickness of all polymeric films was about 10 μm, and the optical density of all samples at the emission range of nanocrystals (ca. 600 to 650 nm) was kept below 0.3 in order to avoid the emission’s reabsorption.

The photoluminescence (PL) spectra of all samples were recorded at room temperature with the Jobin-Yvon Fluoromax-2 spectrofluorimeter, and the optical absorption spectra were recorded with the Ocean Optics HR 2000 spectrophotometer.

### 3 Results and Discussion

Semiconductor QDs and NRs possess extended optical absorption above their band edge. This causes efficient excitation of QD and NR PL at different wavelengths and makes the studied CdSe QDs and NRs ideal candidates for FRET donors. Since dye molecules serving as FRET acceptors have a relatively narrow absorption band, one may easily achieve selective QD or NR optical excitation behind the optical absorption of dye molecule. Figure 1 shows the room temperature optical absorption and PL spectra of CdSe/ZnS core-shell QDs and NRs and their conjugates with AlexaFluor®647 dye.

The FRET efficiency in QD-dye and NR-dye conjugates may be determined according to the formula:
where $F_{DA}$ and $F_D$ are the fluorescence intensities of FRET donors with and without the acceptor, respectively, normalized to the nanoparticles concentration. Based on the data of Fig. 1, the FRET efficiencies ($E$) for QD-dye and NR-dye conjugates were determined to be $E = 0.96$ and $E = 0.87$, respectively. However, the different concentration of dye molecules per single QD and NR must be taken into account. Since the surface area of QDs is much smaller than that of NRs, the number of attached dye molecules is different. In order to estimate the number of dye molecules per single nanocrystal, the concentrations of QDs, NRs, and dye were determined from the magnitude of absorption peak (first excitonic transition for semiconductor nanocrystals) and the

$$E = 1 - \frac{F_{DA}}{F_D},$$

Fig. 1 Room temperature absorption (a) and PL (b) and (c) spectra of CdSe/ZnS QDs (red curves), NRs (green curves), and their conjugates with AlexaFluor®647 dye in DMSO. The blue and cyan curves in (a) correspond to the absorption and PL spectra of free AlexaFluor®647 dye in DMSO. The blue curves in (b) and (c) correspond to the PL spectra of AlexaFluor®647 dye conjugated with QDs and NRs, respectively. The PL excitation wavelength $\lambda_{ex} = 450$ nm.
reference data on the molar absorption coefficient for AlexaFluor®647, CdSe/ZnS QDs, and NRs\textsuperscript{18} (the molar absorption coefficient for CdSe NRs demonstrates simple volume-dependent behavior for fixed diameter\textsuperscript{19}).

The average number of dye molecules per nanocrystal for each conjugate is 2.7 for QDs and 12 for NRs, respectively. Therefore, the FRET efficiency is much lower in NR-dye conjugates than it is in QD-dye conjugates, taking into account the larger amount (roughly five times) of acceptor molecules per donor in the NR-dye complex.

In the case of multiple acceptors, one should take into account possible nonlinear effects, like FRET saturation. To study the saturation effect in QD-dye and NR-dye conjugates, the series of conjugates were prepared with the average number of AlexaFluor®647 molecules varying from 1.5 to 27 per NR and from 1.5 to 3.4 per QD. The protocol for the preparation of conjugates with different dye/nanoparticle ratios was the same as that described above, and only the amount of dye used for conjugation was varied. Using Eq. (1), the FRET efficiency was determined within the prepared conjugates, and these results are presented in Fig. 2 in the form of FRET efficiency \( E \) plotted versus the average number of dye molecules per NR or QD.

Figure 2 clearly shows the presence of the saturation effect in FRET efficiency with an increased number of dye molecules per CdSe NR. It must be pointed out that the saturation effect is inherent to QDs, NRs, and other types of FRET pairs. However, the FRET efficiency in QD-dye conjugate appears to be already above about three dye molecules per QD. The origin of slower FRET saturation for NR-dye conjugates in Fig. 2 is not clear yet. It’s possible that the dye–dye interaction growing with the increased amount of dye molecules bound to the surface of each NR causes the reorientation of optical dipoles of dye molecules toward less favorable FRET conditions.

Another important conclusion is that the \( E \) values for QD-dye and NR-dye conjugates prepared at 1:1 dye/nanoparticle ratios and derived from the corresponding fitting curves point to the FRET efficiency from a single NR to a single dye (\( E = 0.18 \)) being less than half that of a QD-dye pair (\( E = 0.42 \)). The reason for the different FRET efficiencies in QD-dye and NR-dye complexes lies in the specific dipole–dipole interaction between the extended optical dipole of a single CdSe NR and the point-like dipole of a single dye molecule (in the case of a QD-dye complex, the classic FRET between two point-like dipoles is achieved). To study this effect in a more complex way, the reverse situation was realized experimentally, where CdSe QDs and NRs served as FRET acceptors, while FITC molecules served as FRET donors. FITC molecules were chemically bound to the surface of Cys-solubilized nanocrystals, and the resultant conjugates were embedded in solid polymeric film. Figure 3 shows the experimental PL and photoluminescence excitation (PLE) spectra of QD-FITC and NR-FITC conjugates in PVPR.
Both types of CdSe nanocrystals exhibit PL peaking at around $\lambda = 620$ nm, while the FITC PL band is located at around $\lambda = 540$ nm. The PLE spectra, shown by black curves in Fig. 3(b) and 3(c), show that the excitonic emission both from QDs and NRs in their conjugates with FITC can be efficiently excited in the region of the absorption band of FITC molecules. This fact demonstrates the presence of very efficient FRET from FITC molecules to nanocrystals. The

![Graph showing PL and PLE spectra](image)

Fig. 3 The PL (blue curves, $\lambda_{\text{exc}} = 400$ nm) and PLE (red and black curves) spectra of free FITC (a), QD-FITC (b), and NR-FITC (c) in PVPR films. For the red curves, $\lambda_{\text{detect}} = 535$ nm, and for the black curves, $\lambda_{\text{detect}} = 620$ nm.
FRET efficiency can be determined with the same Eq. (1) using the values of the PL signal from free FITC molecules and FITC bound to the acceptors. Again, the estimated FRET efficiency $E$ derived from the data of Fig. 3 is higher for the QD-FITC complex ($E = 0.8$) than it is for NR-FITC ($E = 0.6$). These values were calculated taking into account the relative concentration of FITC molecules in all studied samples.

The number of FITC molecules per QD is estimated at around 6, while it is around 75 for NRs. Such large discrepancies in the number of dye molecules per nanocrystal in the case of FITC and AlexaFluor®647 dye molecules is due to the much smaller size of FITC (molecular weight of 389, compared with 1300 for AlexaFluor®647) and the more efficient conjugation of FITC molecules with amino groups of Cys molecules on the surface of CdSe/ZnS nanocrystals. Taking into account the much larger concentration of FITC molecules per NR, one may conclude that the FRET efficiency from dye to NR in our NR-dye conjugates is much smaller than that from dye to QD. The physical form of the studied conjugates (a solid sample in the case of FITC and a colloidal solution in the case of AlexaFluor®647) does not affect significantly the general conclusion that semiconductor NRs are less efficient FRET donors or acceptors than QDs. The reason of such weak efficiency lies in the nature of dipole–dipole interaction between dye molecules and nanocrystals. The length of the effective region in the NR to which the energy from/to dye molecule may be transferred is of the order of $R$—the distance between dye and the NR center (Fig. 4). It is about 3 to 4 nm and close to the average diameter of our NRs.

The conventional point-dipole FRET theory is not valid when the FRET-based configuration involving extended oriented dipoles is concerned. A number of sophisticated FRET theories and models have been developed for the systems of interacting extended and point-like optical dipoles. The fact that the energy transfer rate is distance-limited in the case of the energy transfer between extended and point-like dipoles reduces the NR region from which the efficient FRET may occur to only a few nanometers. For example, the one-dimensional origin of the optical dipole in an NR-based FRET system may cause the slower spatial decay of energy transfer rate $\gamma \sim 1/R^3$, compared with $\gamma \sim 1/R^6$ for conventional dipole–dipole interaction. However, a much larger number of dye molecules per NR compared with QDs equalizes the situation and brings the total FRET efficiency in an NR-dye complex close to 100%.

4 Conclusion

I compared the efficiency of resonance energy transfer in the conjugates of CdSe/ZnS quantum dots and nanorods, and different dye molecules served either as FRET donors or acceptors. A sufficiently smaller FRET efficiency for nanorod-dye complexes was observed and explained by the distance-limited energy transfer rate in the case of extended and point-like dipoles. However,
the fact that the molar absorption coefficient of CdSe nanorods is almost an order of magnitude larger than that of quantum dots, together with the bigger surface area that allows more dye molecules to be attached to their surface, make nanorods-based FRET complexes very efficient and perspective as fluorescent sensors.

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References


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