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Efficiency of Energy Transfer from Organic Dye Molecules to CdSe–ZnS Nanocrystals: Nanorods versus Nanodots

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Abstract: We report on comparative experimental study of FRET efficiency in two different systems: organic dye molecules (donors) and CdSe–ZnS core–shell nanodots or nanorods (acceptors). Fluorescein isothiocyanate was bound chemically to the surface of nanocrystals using cysteine as a linker and the conjugates were embedded into the polymeric films. Contrary to intuitive presumptions based on the order of magnitude larger molar absorption coefficient for nanorods, the experiment demonstrated almost equal efficiency in the energy transfer from FITC to nanorods and nanodots. This effect is attributed to a distance-limited region of nanorod to which an efficient FRET from dye molecule can be achieved. These results may pave the way to hybrid materials with FRET efficiency controlled by the geometry of nanocrystals.

Introduction

Energy transfer in the system of semiconductor quantum dots and organic dye molecules attracts a considerable attention due to important practical applications in biology, medicine, sensing, photovoltaic devices, and other areas.[1–7] Numerous papers have been published where the basic FRET properties in quantum dot–dye systems are studied in detail.[8–13] Most of the experiments were done with CdSe–ZnS or CdSe–CdS core–shell nanodots (NDs) serving as FRET donors and various luminescent dye molecules as acceptors.[1–15] CdSe core–shell nanocrystals are routinely prepared now as aqueous colloids with the luminescence quantum yield high enough to realize even single-dot detection. They may carry on their surface different chemical groups suitable for conjugation with various biological and chemical molecules, including dyes, proteins, DNA, etc. The narrow emission spectral line width together with the ability to precisely adjust the emission wavelength in the visible region simply by changing the size of nanocrystals makes them almost ideal donor partners for FRET donor/acceptor pairs. The nanocrystals can be excited far away from the dye molecule absorption region thus sufficiently decreasing the background autofluorescence emission of the acceptor.

So far, most attention has been paid to spherical semiconductor NDs leaving elongated nanocrystals or nanorods (NRs) behind.[1–15] Generally, basic optical properties of NRs are also good for FRET applications, while differing from those of NDs in two principal points:

- NRs may be excited more efficiently than NDs due to ca. 10 times larger absorption cross-section;
- NRs absorb and emit light with a high degree of linear polarization even at room temperature.

Polarized absorption and emission of CdSe NRs arises from the orientation of 1D optical dipole moment along the longer NR axis due to specific symmetry of excitonic states in elongated nanocrystals.[16,17] The dipole–dipole interaction in

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FRET pairs is extremely sensitive to the relative spatial orientation of donor and acceptor dipoles.[18] That is why the utilization of NRs for FRET-format applications may provide additional information about orientation of chromophores serving either as the donors or acceptors. Hence, the efficiency of FRET may be additionally controlled by the geometry of interacting donors and acceptors.

In this paper we present the results of comparative experiments with two types of FRET pairs: CdSe−ZnS core−shell NDs and NRs conjugated with organic dye fluorescein isothiocyanate FITC. Contrary to most previous works we utilized here a FRET configuration when the dye molecules serve as the donors and nanocrystals as acceptors. We selected this configuration as a starting point in the experimental investigation of a model FRET scheme where the donor (dye molecule) represents the pointlike optical dipole and the NR acceptor has the 1D-oriented optical dipole moment. Conjugates of FITC with NDs and NRs have been embedded into the polymer films and analyzed in terms of FRET efficiency.

Experimental Section

Hydrophobic CdSe−ZnS core−shell NDs (4.2 nm in diameter) and NRs (4.5 × 27 nm; aspect ratio ≈ 6) were synthesized according to a previously published procedure based on high temperature reaction between organometallic precursors in coordinating solvents.[19] The nanocrystals were solubilized in water with cysteine treatment as published earlier.[20] Briefly, ca. 5 mg of dry nanocrystals were dissolved in 2 mL of chloroform and 3 mL of methanol was then added. The solid phase was centrifuged, washed with methanol and dissolved in a fresh portion of chloroform (3 mL). Next, 0.5 mL of D,L-cysteine hydrochloride (Cys) in methanol (5 mg/mL) was added to the chloroform solution and the mixture stirred for 10−15 min to complete the binding of Cys molecules to the surface of nanocrystals. The solid phase containing the nanocrystals with monolayer of Cys molecules on their surface was centrifuged, washed two times with methanol and dissolved in 3 mL of bidistilled water. Finally, the pH of the solution was adjusted to 8 with phosphate buffer.

Fluorescein isothiocyanate (FITC, Sigma) was conjugated with Cys-solubilized NRs and NDs according to published protocol.[21] 1 mg of FITC was dissolved first in 5 mL of anhydrous dimethylsulfoxide DMSO and stored at +4 °C. Then, two 20 μL aliquots of FITC solution were added to 2 mL aliquots of aqueous solutions of NDs 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 to remove unbound FITC. It is important to use highly diluted buffer solution in order to avoid the crystallization of salts in polymeric films.

To prepare polymeric films containing NDs−FITC and NRs−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:

- Cys-solubilized NDs,
- Cys-soluibilized NRs, and
- pure FITC.

Finally, all five solutions were drop-cast onto 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. 600−650 nm) was kept below 0.3 in order to avoid emission reabsorption.

The optical absorption spectra of the samples at room temperature were obtained with UV−vis spectrophotometer SOLAR Systems; photoluminescence (PL) and photoluminescence excitation (PLE) spectra were recorded with Jobin-Yvon Fluoromax-2 spectrofluorimeter. All PLE spectra were corrected on the emission spectrum of a Xe lamp and the spectral sensitivity of the detector. In order to avoid possible FITC photodegradation, all manipulations proceeded in a semidark room.

Results and Discussion

Figure 1a,b demonstrates the absorption spectra of FITC alone, NDs−FITC conjugate and NDs alone in PVPR film. Note that the PVPR film itself does not show remarkable absorption between $\lambda = 400$ and 800 nm. Similarly, Figure 1c shows the absorption spectra of NRs alone and NRs−FITC conjugate.

The absorption band of FITC is centered at $\lambda = 510$ nm while the first excitonic transitions for NRs and NDs are located at $\lambda = 605$ nm (Figure 1c) and 595 nm (Figure 1b), respectively. We calculated the relative concentration of NRs, NDs and FITC in each sample using the reference data on the molar absorption coefficients of FITC and that for the first excitonic band of NDs. To the best of our knowledge there is no experimental data on the molar absorption coefficients for CdSe NRs of different diameter and length. That is why we roughly estimated this value from the NRs molar weight suggesting that the molar absorption coefficient was proportional to the nanocrystals' molar weight. The molar weight of NDs and NRs was calculated from the average geometrical size of corresponding nanocrystals determined by TEM assuming ND to be ideal sphere and NR ideal cylinder. The values of the molar absorption coefficient at the first excitonic band and the molar weight of CdSe-core NDs were taken as the reference. These parameters are summarized in Table 1.

It needs to be pointed out that the determination of the molar absorption coefficients for NRs is more difficult than that for NDs. The spectral position of the first excitonic band as well as the absorption coefficient for NRs depends not only on their diameter but on the length too.[22] The error in the determination of NR concentration in the polymeric film by their optical absorption may be noticeable (we estimate it to be about 30%).

Figure 1 shows that the absorption band of FITC molecules does not overlap remarkably with the first excitonic bands of NDs and NRs. Therefore, the relative concentration of nanocrystals in the polymeric film may be determined by dividing the optical density at the excitonic band maximum by their molar absorption coefficient taking into account the equal thickness of all polymeric films. Similar determination of the relative concentration of FITC in NDs−FITC and NRs−FITC samples can be done if one takes into account the intrinsic absorption of nanocrystals in the spectral region of the FITC band. First, we calculated the optical density of FITC band alone in both NDs−FITC and NRs−FITC samples by the formula

$$D_{\mathrm{FITC}} = D_{\mathrm{blue}}^{510\mathrm{nm}} - (D_{\mathrm{blue}}^{1\mathrm{exc}}/D_{\mathrm{red}}^{1\mathrm{exc}} * D_{\mathrm{red}}^{510\mathrm{nm}})$$

where "blue" and "red" subscripts relate to the corresponding curves in Figure 1, "1exc" relates to the wavelength of corresponding first excitonic absorption peak in Figure 1. Then, the relative concentration of FITC was obtained by dividing

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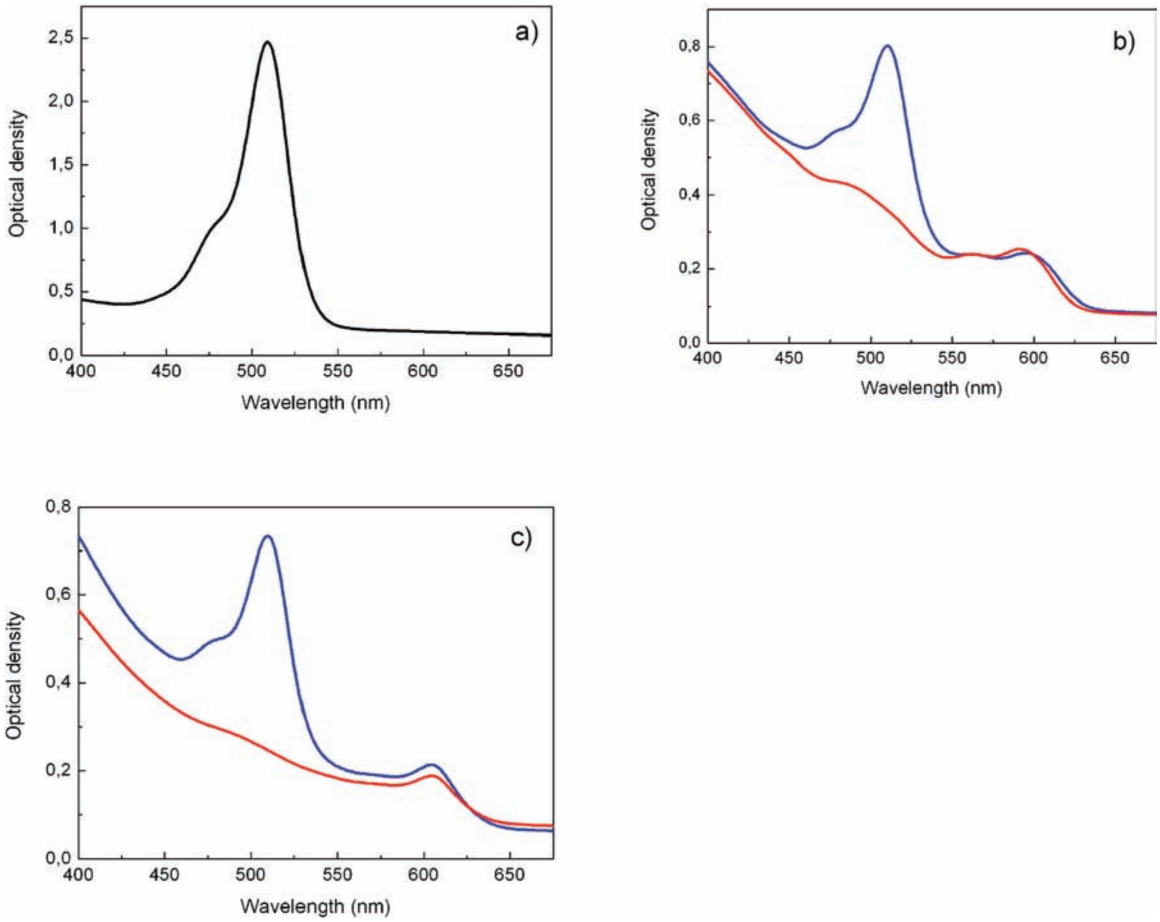

Figure 1. Absorption spectra of PVPR films containing FITC alone (a), CdSe−ZnS NDs (b, red curve), NDs−FITC conjugates (b, blue curve), CdSe−ZnS NRs (c, red curve) and NRs−FITC conjugates (c, blue curve).

Table 1. Physical and Optical Parameters of FRET Donor (FITC) and Acceptors (CdSe NDs and NRs)

type	av diam (nm)	av length (nm)	mol wt[a]	molar absorption coeff (cm^{-1} M^{-1})	surface area[a] (nm^2)
NDs	4.2		170000	280000[b]	55
NRs	4.5	27	1600000	2700000[c]	410
FITC			389	80000[d]	

[a] Calculated from the geometrical size of nanocrystals taking ND as ideal sphere and NR ideal cylinder. [b] Calculated according to Yu, W. W.; Qu, L.; Guo, W.; Peng, X. *Chem. Mater.* **2003**, *15*, 2854−2860; *Chem Mater.* **2004**, *16*, 560. [c] Estimated value (see text for details). [d] Reference 21, p 304.

Table 2. Relative Concentrations of FITC and CdSe−ZnS Nanocrystals in the Polymeric Films

rel concn	FITC	NDs	NDs−FITC	NRs	NRs−FITC
nanocrystals		6.5×10^{-7}	6.2×10^{-7}	4.4×10^{-8}	5.6×10^{-8}
FITC	2.9×10^{-5}		4.1×10^{-6}		4.2×10^{-6}
FITC per one nanocrystal			6		75

D_{FITC} value by the molar extinction coefficient for FITC. All the data are summarized in Table 2.

Data in Table 2 show that the relative concentration of FITC in NDs−FITC and NRs−FITC samples is almost identical. At the same time the concentration of NRs in NRs−FITC sample is ca. 10 times lower than of NDs in NDs−FITC. The calculated amount of FITC molecules per one NR is about 12 times higher than that of ND. On the other hand the total surface area of a single NR was calculated to be only 7.5 times higher (assuming the ideal cylindrical shape of NR). The less surface concentration of FITC molecules per one ND may be explained by higher surface roughness of real NRs which can be clearly seen in high resolution TEM images.[19,22]

Figure 2 shows both PL and PLE spectra of FITC, NDs−FITC and NRs−FITC samples. Both types of CdSe nanocrystals show emission in the region of $\lambda = 620$ nm, while FITC PL band is located at $\lambda = 535$ nm for NDs−FITC and NRs−FITC samples and at $\lambda = 541$ nm for pure FITC. Blue spectral shift in the PL maximum of FITC attached to the surface of nanocrystals may be attributed to conformational changes in FITC attached to the solid surface. However, such a small spectral shift does not affect remarkably the analysis of FRET parameters in studied systems.

All PL and PLE spectra presented in Figure 2 were obtained with the same experimental parameters (excitation intensity and wavelength, integration time, slit width) which allowed direct comparison of the FRET efficiency in NDs−FITC and NRs−FITC samples. For that we analyzed two parameters:

(i) FRET efficiency $E = 1 - (F_{DA})/(F_D)$, where F_{DA} and F_D are the donor fluorescence intensities with and without acceptor, respectively;[18]

(ii) magnitude of PLE signal at $\lambda_{exc} = 511$ nm for blue spectra in Figure 2b,c normalized to the PL quantum yield for corresponding nanocrystals.

The first parameter may be easily determined from the magnitude of corresponding peaks in Figure 2. Since the spectral line shape of FITC emission is assumed to be independent of

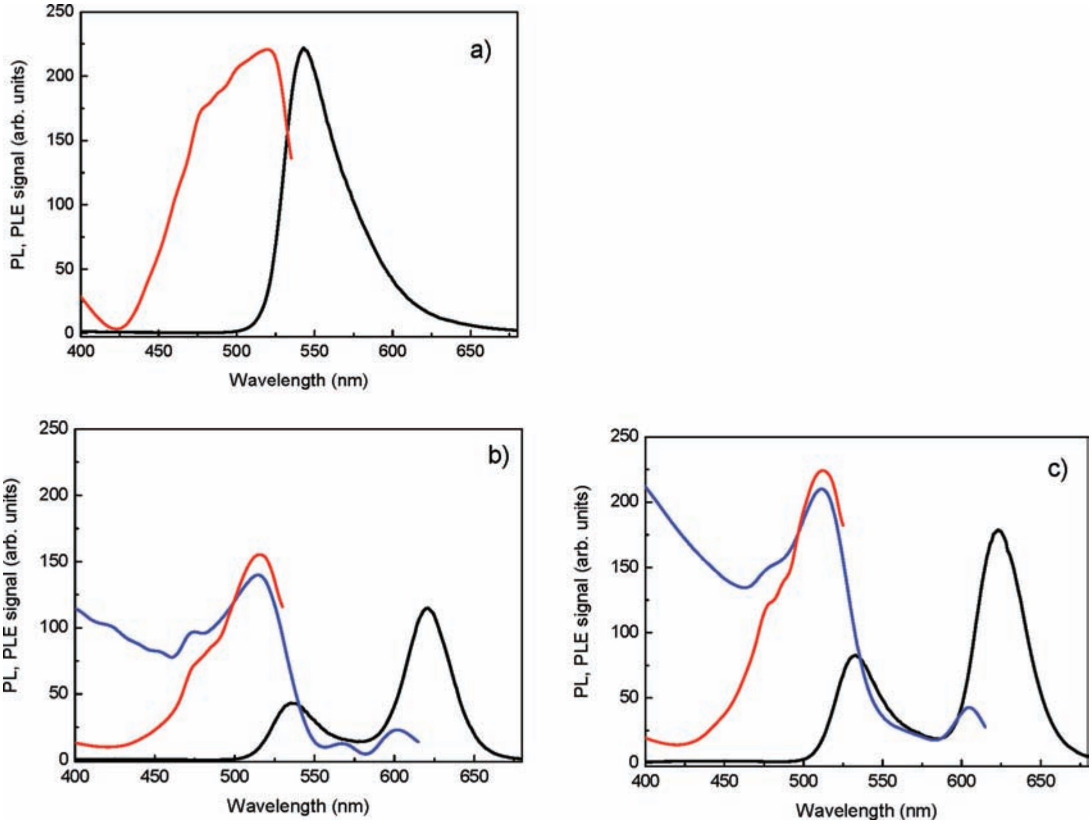

Figure 2. PL (black curves) and PLE (red and blue curves) spectra of FITC (a), NDs−FITC (b) and NRs−FITC (c) conjugates in PVPR films. Red curves are the PLE spectra for $\lambda_{\text{detect}} = 535$ nm and the blue curves the PLE spectra for $\lambda_{\text{detect}} = 620$ nm. For black curves $\lambda_{\text{exc}} = 400$ nm. Fine PLE structure around $\lambda = 470$ nm in blue and red spectra is an artifact resulting from the strong scattering of Xe-lamp lines by polymeric films.

Table 3. Parameters of Emission for NDs−FITC and NRs−FITC Samples Derived from Figure 2

type of conjugates	FRET efficiency, *E*	magnitude of PLE signal at $\lambda_{\text{exc}} = 511$ nm, $\lambda_{\text{detect}} = 620$ nm[a] (arb units)	rel quantum yield of excitonic emission	normalized PLE signal at $\lambda_{\text{exc}} = 511$ nm, $\lambda_{\text{detect}} = 620$ nm[b]
NDs−FITC	0.8	22	0.17	130
NRs−FITC	0.6	28	0.27	103

[a] Only FITC component. [b] Normalized to the relative PL quantum yield.

the sample type, the emission peak value instead of spectrally integrated emission may be used.

The calculation of the second parameter is more difficult. It needs to take into account the complex character of PLE signal at $\lambda_{\text{exc}} = 511$ nm which includes both the intrinsic PLE contribution from the nanocrystals and PLE signal from FITC. We subtracted the intrinsic PLE signal from the nanocrystals using PLE spectra of NDs and NRs alone normalized to the corresponding PLE signals at the first exctionic bands. The absolute value of the PLE signal for any emitting object is determined by the emission quantum yield. Therefore, we normalized the magnitude of the PLE signal to the value of the relative PL quantum yield for the corresponding nanocrystals. All the calculated data are summarized in Table 3.

Data of Table 3 show that both parameters, *E* and normalized PLE signal, point to the nearly equal FRET efficiency in NDs−FITC and NRs−FITC pairs within the assumed experimental error. However, conventional point−dipole FRET theory tells us that FRET efficiency between single donor and acceptor depends on the distance *R* between the donor and the acceptor dipoles, the PL emission quantum yield Q_D of the free donor, the overlap integral *J* and the orientation factor κ_p.[18] We may assume the identical value of *R* for both samples because the same linker molecule is used to attach FITC to the surfaces of ND or NR. The overlap integral expresses the degree of spectral overlap between the donor fluorescence and the acceptor absorbance according to a formula,

$$J = \int f_D(\lambda)\, \varepsilon_A(\lambda)\lambda^4\, d\lambda \qquad (1)$$

where $\varepsilon_A(\lambda)$ is the molar extinction coefficient of acceptor and $f_D(\lambda)$ the normalized fluorescence spectrum of the donor.[18] It is reasonable to assume that $f_D(\lambda)$ is the same for NDs−FITC and NRs−FITC because the absorption spectra for both samples in the region of FITC emission are almost featureless and nearly identical. The estimated value of ε_A at the first excitonic transition for single NR is about 10 times larger than that for single ND (Table 1). In the spectral region of FITC emission the absorption spectra of NDs and NRs are almost identical and rising up smoothly from 550 to 450 nm. Therefore, for semiquantitative estimations we may take the same 10-fold larger value of $\varepsilon_A(\lambda)$ for single NR relatively to single ND. Such a large value of $\varepsilon_A(\lambda)$ for single NR should result in the order of magnitude more efficient FRET in NRs−FITC which is in contradiction with our experimental data. From the first glance this effect can be readily explained by possible orthogonal orientation of donor and acceptor dipoles in NR−FITC pair which may reduce the FRET efficiency down to zero.[18] Unlike fixed orientation for 1D-oriented optical dipole moment in each NR the orientation of the optical dipole of spherical symmetry in single CdSe ND is almost random which follows from the depolarized excitation and emission of single ND at room temperature.[23,24] Unfortunately, the exact orientation of the optical dipole in FITC molecules relative to the center of nanocrystals is unknown which leaves the field for speculations. However, we suppose that the combination of fixed in some plane orientation of pointlike FITC dipole and the optical dipole of spherical symmetry in ND is more favorable for efficient FRET than the combination of two fixed and probably orthogonally oriented dipoles in NR−FITC pair.

From the other side in order to explain correctly the energy transfer in the nanowire systems conventional point−dipole FRET theory must be modified. Detailed theoretical analysis of FRET in nanowire−nanowire, ND−nanowire and dye−nanowire systems was presented in refs 25−27. For example, one-dimensional character of the optical dipole in the nanowire system causes slower spatial decay of dipole−nanowire energy transfer rate $\gamma \propto 1/R^5$, as compared to $\gamma \propto 1/R^6$ for conventional dipole−dipole FRET.[27] Likewise, the energy transfer rate from point dipole to nanowire is expected to depend on the nanowire absorption cross section per unit length ε'_A, rather than total absorption cross section which can be illustrated by scheme in Figure 3.

The length of the effective region in NR to which the energy from FITC may be transferred is of the order of R − the distance between FITC and NR centers which is about 3−4 nm and close to the average diameter of our NDs. Formally, this can be taken into account by reducing the value of the molar absorption coefficient of NRs in eq 1 approximately by a factor of R/L. In such a case the value of $\varepsilon_A(\lambda)$ in eq 1 for both ND−FITC and NR−FITC becomes comparable causing almost equal FRET efficiency E observed in our experiments.

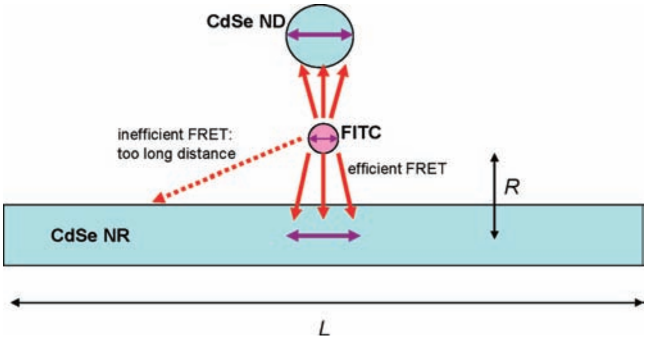

Figure 3. Scheme for FRET in ND−FITC and NR−FITC systems. Unlike ND, the efficient FRET in NR−FITC pair may be achieved only within the distance-limited region of NR marked by the purple arrow.

Conclusion

We compared the relative efficiency of the resonance energy transfer in the system of organic dye FITC molecules (donors) chemically bound to the surface of CdSe−ZnS nanodots or nanorods (acceptors). Contrary to intuitive presumptions based on the order of magnitude larger molar absorption coefficient for nanorods, the semiquantitative analysis shows almost equal efficiency in the energy transfer from FITC to nanorods and nanodots. The reason for that may be in a limited region of nanorod to which the efficient FRET from pointlike dipole can be achieved. Further experiments with CdSe nanorods different in length must be done in order to elucidate the details of FRET in the dye−nanorod pairs. Our results may pave the way to hybrid materials, sensors and chips with FRET efficiency controlled by the geometry of the nanocrystals.

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