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Two-photon-excited fluorescence resonance energy transfer in an aqueous system of CdTe quantum dots and Rhodamine B

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Two-photon excited fluorescence resonance energy transfer (FRET) between CdTe quantum dots with different emission peaks and Rhodamine B in aqueous solution are investigated both experimentally and theoretically. The photoluminescence and lifetime are measured using a time-resolved fluorescence test system. The two-photon excited FRET efficiency is found to increase as the degree of spectral overlap of the emission spectrum of CdTe and the absorption spectrum of Rhodamine B increases, which is due to the increase of Forster radius of the sample. Moreover, FRET efficiency increases when the ratio of acceptor/donor concentration increases. The two-photon excited FRET efficiency was found to reach 40%. © 2014 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4904356]

I. INTRODUCTION

Semiconductor quantum dots (QDs), with their unique optical and electrical properties, have attracted considerable research attention and present wide application prospects in the photovoltaic device and biomarker fields.1,2 When compared with CdS and CdSe QDs, CdTe QDs have a larger exciton Bohr radius and thus have a stronger quantum size effect under the same molecular dimension conditions.3 The inherent toxicity of CdTe QDs can be minimized by the growth of a biocompatible shell.4 Also, CdTe QDs have large two-photon absorption cross section that can be adjusted by varying the sizes of the QDs, and the attachment of a relevant targeting moiety can ease the selective incorporation of CdTe QDs in cells and tissues. This means that CdTe QDs have wide application prospects in the bio-probing, bio-imaging, bio-labelling, and drug delivery fields.5–7 Fluorescence resonance energy transfer (FRET) is a non-radiation-based energy transfer process that occurs between a donor (D) molecule in the excited state and an acceptor (A) molecule in the ground state by dipole–dipole interactions. The efficiency of FRET is dependent on the extent of the spectral overlap between the donor photoluminescence (PL) peak and the absorption spectrum of the acceptor, the quantum yield of the donor, and the distance between the donor and acceptor molecules.8 As present, FRET is commonly used in applications that include the determination of metal ion,9,10 the analysis of proteins,11–13 and biological molecular fluorescence probes.14,15

In this paper, a two-photon FRET aqueous system, using CdTe QDs as the donors and Rhodamine B (RhB) as the acceptor, is investigated and analyzed. Steady-state photoluminescence (SSPL), time-resolved two-photon PL, and fluorescence lifetime measurements are performed to enable analysis of the kinetics of the two-photon excited luminescence. The FRET efficiency and the Förster radius are calculated to demonstrate the relationship between the extent of the spectral overlap and the FRET efficiency under two-photon excitation.

II. EXPERIMENTAL

In the experiments, the photostable thiol-capped CdTe QDs that were chosen as donors were synthesized in three sizes in an aqueous solution by reaction of cadmium perchlorate with H2Te gas.16 RhB, the acceptor, was purchased from Aladdin Reagent Co., Ltd. and was prepared as an aqueous solution. The concentrations (unit: mol/l) of the two solutions are of the 10–5–10–6 order of magnitude to ensure that there is little reabsorption during the PL measurements. The SSPL was studied using a JASCO FP-6500 Fluorescence Spectrometer with 1 mm quartz cuvettes at room temperature. The fluorescence spectrometer uses a xenon lamp as an excitation source and thus the fluorescence spectroscopy result can be considered to be measured under one-photon excitation. To avoid any interference caused by the absorption of the RhB acceptor, the excitation wavelength was kept at 400 nm, which is away from the RhB absorption peak. The slot set at 3 mm-3 mm was considered to be suitable. The absorption measurements of solutions were undertaken using a PerkinElmer Lambda 35 UV-Vis spectrophotometer.

To analyze the kinetics of the two-photon excited luminescence, time resolved two-photon PL and fluorescence lifetime measurements were performed using the time-resolved fluorescence test system. The samples were excited at a wavelength of 800 nm with femtosecond laser pulses, produced by Ti: sapphire femtosecond laser with pulse widths of 130 fs, a repetition frequency of 76 MHz, and fixed power of 500 mW. The fluorescence spectra were measured by the fluorescence

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spectrometer and the fluorescence decay curves were recorded using a single photon counter. The samples were placed in a 10-mm cuvette, and to avoid the interference from the excitation source, the cuvette was installed at a 45° angle to allow the spectrometer to collect the reflection fluorescence of the sample. Filters were also used for the same purpose.

III. RESULTS AND DISCUSSION

A. Absorption spectra and steady-state photoluminescence

The normalized RhB absorption spectrum and the normalized SSPL of the QDs are given in Fig. 1, which indicates that the fluorescence peak wavelengths for the three sizes of QDs are located at 530 nm, 607 nm, and 650 nm. The absorption peak wavelength of RhB is 553 nm. Spectral overlap between the donor emission spectrum and the acceptor absorption spectrum is essential for FRET, and the energy transfer efficiency is closely related to the extent of this spectral overlap. Figure 1 shows that the largest spectral overlap is found between the fluorescence spectrum of QDs1 and the RhB absorption spectrum, followed by that with the spectrum of QDs2, while QDs3 has the smallest spectral overlap with the RhB absorption spectrum.

The SSPL spectra of the samples with different acceptor to donor (A/D) concentration ratios are shown in Fig. 2. This figure shows that the fluorescence intensity of the QDs decreases when the A/D concentration ratio increases, indicating significant fluorescence quenching of the donor and enhancement of the acceptor.

B. Time-resolved photoluminescence (TRPL)

Figures 3 and 4 show the two-photon fluorescence spectra of the QDs-RhB solutions. Because of the experimental error caused by sample replacement, the spectra have been normalized and plotted for the sake of the discussion. Because QDs1 has the largest spectral overlap of the three sizes of QDs, QDs1 is chosen to measure both the TRPL and the fluorescence lifetime for various A/D concentration ratios, and the results are plotted in Fig. 3. Fig. 3 indicates that the PL peak intensity of the QDs decreases until it almost disappears, whereas the A/D concentration increases. Therefore, the FRET between the QDs and the RhB in solution can be considered to be saturated at high A/D concentration ratio. The two-photon fluorescence spectra of the mixture solutions of different QDs and RhB are shown in Fig. 4. Because the fluorescence peaks of RhB and QDs2 are too close together, peak-fitting analysis is performed in Fig. 4(b) for ease of comparison. Figure 4 shows the sequence of degree of fluorescence quenching ranked in order of QDs1, QDs2, and QDs3. It can be concluded that the fluorescence is quenched with increasing spectral overlap.

C. Two-photon fluorescence lifetime

The fluorescence decay curves of the QDs1 and RhB mixed solutions with various A/D concentration ratios are measured using a single photon counter which selects the
fluorescence peak wavelengths of RhB (575 nm) and QDs (530 nm) as the sampling points, with the integration time set at 900 s. The measurements were performed in a darkroom at room temperature to reduce the measurement error caused by natural light. The decay curves were fitted using the following exponential decay curve:

$$y = A \exp(-x/t) + y_0,$$  

where \( t \) is the two-photon fluorescence lifetime. Figure 5 shows each decay curve with its corresponding exponential fitting curve, which shows that the fitting result matches the experiment data well. Figure 5(a) shows that higher the A/D concentration ratio becomes, the higher the resulting photon count can be obtained. Therefore, it can be concluded that a higher A/D concentration ratio produces a stronger RhB fluorescence intensity. Table I shows the lifetimes obtained from the fitting curve, as shown in Fig. 5(a). The lifetime of RhB is shown to be prolonged with increasing A/D concentration ratio, while the lifetimes of the QDs are reduced; also, the presence of FRET is initially confirmed here.

To measure the fluorescence decay curves of the QDs-RhB solutions with the different QD emission wavelengths, the peak emission wavelength of 575 nm for RhB, and the peak wavelengths of 530 nm, 607 nm, and 650 nm for the QDs are chosen as sample points. Using the same experimental conditions, Fig. 6 shows the measurement results. Figure 6(a) indicates that the photon counts for RhB in the samples increase with increasing spectral overlap. This result suggests that the RhB fluorescence intensity varies directly with the degree of spectral overlap. Abnormally high photon counts for the sample QDs2&RhB3 are shown in Fig. 6(b). This phenomenon may be caused by the closeness of the sample point to the fluorescence peak of RhB which indicates that there may be some interference. The fluorescence lifetimes are calculated by fitting the exponential decay curve of Eq. (1) and the results are shown in Table II. The prolonged RhB lifetime and the reduced QDs lifetimes can also be found in Table II.

Based on analysis of the results of the two-photon TRPL measurements, a reference method is introduced to estimate the two-photon absorption cross-section, and the corresponding formula is presented by the following equation:

$$\delta_2 = \delta_1 \frac{F_2 \phi_1 c_1}{F_1 \phi_2 c_2},$$  

where the subscript 1 represents the reference sample and subscript 2 represents the measured sample. \( \delta \) is the two-photon absorption cross-section and has unit of GM (where 1 GM = \( 10^{-50} \mathrm{cm}^4 \cdot \mathrm{s/\text{photon}} \)). \( F \) is the two-photon photoluminescence intensity. \( \phi \) is the quantum yield and \( c \) is the sample concentration. RhB is chosen as the reference sample because its two-photon absorption cross-section is approximately 210 GM.\(^{18}\) From Eq. (2), the two-photon absorption cross-sections of QDs1, QDs2, and QDs3 are estimated to be 4.68 \( \times 10^5 \) GM, 8.20 \( \times 10^5 \) GM, and 1.45 \( \times 10^6 \) GM, respectively. These results show that the CdTe QDs that have high two-photon absorption cross-sections are suitable as donor for two-photon FRET.

The FRET characteristics of the QD-RhB system can be calculated using the following equation:

$$R_0^6 = \frac{9000 \ln(10) \kappa^2 Q_{D}(\lambda)}{128 \pi ^2 n ^4 N_A^2},$$  

where the Förster radius, \( R_0 \), means that half of the donor molecules decay by energy transfer while the other half decay by
conventional radiative and non-radiative mechanisms at this distance. In other words, the energy transfer efficiency is 50% when the distance between donor and acceptor is $R_0$. $\kappa$ is a factor that describes the relative spatial orientation of the transition dipoles of the donor and the acceptor. When considering the random orientation in solutions, $\kappa^2$ is usually assumed to be equal to $2/3$. $Q_D$ is the quantum yield of the donor in the absence of the acceptor and can be given by a reference method. $N_A$ is the Avogadro’s number. $n$ is the refractive index of the medium and $J(\lambda)$ is spectral overlap integral, which describes the degree of spectral overlap between the PL of the donor and the absorption of the acceptor, and can be calculated using the following equation:

$$J(\lambda) = \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda,$$

where $F_D(\lambda)$, as a dimensional number, is the normalized fluorescence spectrum. Note that the source of the data for $F_D(\lambda)$ is the two-photon results obtained in this experiment. $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor and has units of M$^{-1}$ cm$^{-1}$. $\varepsilon_A(\lambda)$ can be determined by the Lambert-Beer law. $\lambda$ is the wavelength which is expressed in units of nm. The values of $J(\lambda)$ and $Q_D$ are presented in Table III. Note that RhB is selected as the standard sample. The $Q_D$ of RhB in an aqueous solution is 0.31.19

![FIG. 5. Fluorescence decay curves of QDs1-RhB solutions with various A/D concentration ratios and their corresponding fitting curves at (a) a wavelength of 575 nm (the RhB fluorescence peak wavelength) and (b) a wavelength of 530 nm (the fluorescence peak wavelength of QDs1). In (b), all curves except for the curve for “QDs1” move along the vertical axis. This explicitly demonstrates that the units used for the vertical axis are arbitrary.](image)

**TABLE I.** The fitted fluorescence lifetime of QDs1 and RhB mixed solutions with various A/D ratios.

<table>
<thead>
<tr>
<th>Sample</th>
<th>QDs lifetime (ns)</th>
<th>RhB lifetime (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RhB</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>QDs1</td>
<td>2.99</td>
<td></td>
</tr>
<tr>
<td>QDs1&amp;RhB1</td>
<td>2.21</td>
<td>1.95</td>
</tr>
<tr>
<td>QDs1&amp;RhB2</td>
<td>1.86</td>
<td>1.99</td>
</tr>
<tr>
<td>QDs1&amp;RhB3</td>
<td>1.84</td>
<td>2.06</td>
</tr>
<tr>
<td>QDs1&amp;RhB4</td>
<td>1.81</td>
<td>2.13</td>
</tr>
<tr>
<td>QDs1&amp;RhB5</td>
<td>1.81</td>
<td>2.18</td>
</tr>
<tr>
<td>QDs1&amp;RhB6</td>
<td>1.79</td>
<td>2.24</td>
</tr>
</tbody>
</table>

![FIG. 6. Fluorescence decay curves and their corresponding fitting curves at (a) a wavelength of 575 nm (the fluorescence peak wavelength of RhB) and (b) 530 nm, 607 nm, and 650 nm (the fluorescence peak wavelengths of the three sizes of QDs).](image)
Equation (3) can be simplified into Eq. (5) in the form

\[ R_0^6 = 8.79 \times 10^{-5} \kappa^2 n^{-4} Q_D J(\lambda), \]  

where \( R_0 \) is expressed in angstroms (Å). It is noticeable that Eq. (5) is only used when \( \lambda \) is expressed in units of nm and \( \kappa_0 J(\lambda) \) is expressed in M^{-1}·cm^{-1}. Equation (5) indicates that \( R_0^6 \) is proportional to both \( J(\lambda) \) and \( Q_D \), which means that a higher degree of spectral overlap and a higher donor quantum yield make the Förster radius larger, i.e., a wider energy transfer range can lead to higher FRET efficiency. Using Eq. (5), the calculated results for the Förster radius \( R_0 \) are 7.40 nm, 6.03 nm, and 5.56 nm for QDs1, QDs2, and QDs3, respectively. Based on this observation, QDs1 has the widest Förster interaction range and is the best match to the acceptor among the three QD sizes.

The FRET efficiency \( E \) can be calculated using the following equation:

\[ E = 1 - \frac{\tau_{DA}}{\tau_D}, \]  

where \( \tau_{DA} \) and \( \tau_D \) are the fluorescence lifetime of the donor with and without the presence of the acceptor, respectively. Based on the values of \( R_0 \) from Eq. (5) and \( E \) from Eq. (6), the donor-to-acceptor distance \( r \) can be calculated using the following equation:

\[ E = \frac{R_0^6}{R_0^6 + r^6}. \]  

The FRET efficiency is calculated for the QDs1 and RhB mixed solutions with various A/D concentration ratios using Eq. (6), and the results are shown in Fig. 7. It is shown that as the A/D ratio increases, the lifetime of the donor (QDs) drops accordingly, while the FRET efficiency increases logarithmically and can be as high as 40%. The acceptor lifetime increases linearly. It can be concluded that the FRET gradually saturates with increasing A/D ratio.

Table IV shows the results for FRET efficiency and the donor-to-acceptor distance for the mix solutions of QDs with different emission wavelengths and RhB and shows that there is a proportional relationship between the FRET efficiency and the degree of spectral overlap. The experimental FRET efficiency results agree approximately with the theoretically predicted values. While the distance \( r \) of the QDs1&RhB3 sample is the longest among the three samples, it is numerically closest to the corresponding \( R_0 \) and thus the QDs1&RhB3 sample can reach the highest FRET efficiency.
IV. CONCLUSION

We achieved FRET in QDs-RhB aqueous solutions using two-photon excitation and calculated the Förster radii of the samples to be 7.40 nm, 6.03 nm, and 5.56 nm for QDs1, QDs2, and QDs3, respectively. The experimental results and the theoretical analysis indicated that the FRET efficiency increases with increasing spectral overlap, which is caused by an increase in the Förster radius $R_0$. In addition, we showed that the FRET efficiency increases with increasing acceptor/donor concentration ratio. The transfer efficiency can be as high as 40%. This study shows that there is a bright future for these materials in bio-probing, bio-imaging, bio-labelling, and other biological and optoelectronic applications.

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