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Fluorescence resonance energy transfer sensor between quantum dot donors and neutral red acceptors and its detection of BSA in micelles

Shenguang Ge [a,*], Juanjuan Lu [a], Mei Yan [a], Feng Yu [b], Jinghua Yu [a,*], Xiujin Sun [a]

[a] Shandong Provincial Key Laboratory of Fluorine Chemistry and Chemical Materials, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China
[b] School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798 Singapore, Singapore

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ABSTRACT

Bovine serum albumin in micelles was effectively detected by a fluorescence resonance energy transfer -based molecular sensor using CdTe quantum dots as energy donors and neutral red as energy acceptors. The fluorescence resonance energy transfer efficiency was enhanced by the construction of cetyltrimethylammonium bromide micelles, which significantly reduced the distance between the donor and the acceptor. The results indicated that the addition of the albumin easily leads to the fluorescence quenching of CdTe-neutral red fluorescence resonance energy transfer due to the formation of CdTe-albumin complexes which have weak affinity to CdTe and repels the dyes from CdTe surface. The fluorescence intensity of the CdTe-neutral red sensor quenched with the increasing concentration of albumin. The linear range of albumin was from 0.4×10^{-3} to 11.00×10^{-3} g L^{-1}. The detection limit was 0.13 g L^{-1}.

1. Introduction

Fluorescence resonance energy transfer (FRET) is a process in which an excited-stated donor chromophore can transfer energy to a proximal acceptor chromophore (typically <10 nm) through a long-range nonradiative dipole-dipole coupling. It has been extensively and intensively studied as a powerful analytical technique to interrogate changes in molecular conformation, association and the assembly or disassembly of biomolecular machinery [1–5]. Semiconductor quantum dots (QDs) are inorganic nanoparticle fluorophores with unique optical and spectroscopic properties that make them be excellent fluorescent labels for bioimaging, sensing, and diagnostics. QDs possess a variety of advantages over conventional organic dyes, such as high quantum yields, that render them excellent photo and chemical stability, and size-dependent, broad absorption with large molar extinction coefficients, narrow symmetric emission spectra, tunable absorption and emission [6–10]. Due to the aforementioned advantages QDs have been widely used as donors and acceptors in energy transfer studies. In recent years, numerous advances have been achieved in QD-based FRET investigations, regarding both fundamental theoretical analyses and biological applications, including the design of biosensor and the analysis of biomolecular conformation and interaction [11,12]. However, a limitation of FRET studies usually stems from the fact that it only permits the analysis of interactions between two molecular entities.

Inorganic semiconductor quantum dots and π-conjugated organic molecules (Dyes) are being increasingly considered for optoelectronic and sensing applications [13–15]. Due to that the effectively conjugation of QDs with neutral red (NR) has some advantages, FRET permits one to tune the degree of spectral overlap between donor and acceptor. Furthermore, there was a unique configuration, where a single donor can interact with several acceptors simultaneously. The interest has been growing quickly amongst researchers in the field of FRET sensors, which are used to quantify molecular dynamics in protein-protein interactions, protein-DNA interactions, and protein conformational changes.

In this article, the biosensing platform is constructed according to the noncovalent assembly. The aforementioned FRET-based molecular sensor was employed to detect bovine serum albumin (BSA), which is widely used in medical and biological fields. It was found that the FRET phenomena had good sensitivity and stability in cationic cetyltrimethylammonium bromide (CTMAB) micelle. Moreover, the FRET efficiency was investigated in the micelles, which directly affected the distance between the CdTe QDs donors and the dye NR acceptors. The CTMAB micelles was proved to

* Corresponding authors. Tel.: +86 531 82767161.
E-mail addresses: ujn.yujh@gmail.com, chm_gesg@ujn.edu.cn (S. Ge).

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contribute to diminish the distance between the CdTe QDs donors and the dye NR acceptors, improve the FRET efficiency and enhance sensitivity of detecting BSA.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed on a LS-55 spectrofluorimeter with 1.0 cm quartz cell (Perkin–Elmer, USA). Absorption spectra were recorded on a UV3101 spectrometer (Shimadzu, Japan). The pH measurements were made by using a PT-10 digital pH-meter (Sartorius, Germany) with a combined glass-calomel electrode. The transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images of QDs were performed with a JEM-2100 (JEOL, Japan) transmission electron microscope.

2.2. Reagents

All chemicals used were of analytical grade or of the highest purity available. All solutions were prepared using Milli-Q water (Millipore water purification system (≥18 MΩ, Milli-Q, Millipore, Billerica, MA)) as a solvent. Tellurium (reagent powder, 99.999%), thioglycolic acid (TGA), bovine serum albumin (BSA) were purchased from Kemiou (Tianjin, China); $CdCl_2 \cdot 2.5H_2O$ and sodium borohydride were purchased from Shanghai Chemical Reagent Company (Shanghai, China); HCl, NaOH, potassium hydrogen phthalate and citric acid was obtained from Aladdin (Shanghai, China); stock solutions of BSA were prepared by directly dissolving commercial BSA in Milli-Q water at 0–4 °C.

2.3. Preparation of water-soluble CdTe QDs

CdTe QDs were prepared by using the reaction between Cd^{2+} and NaHTe in the presence of thioglycolic acid (TGA) as the stabilizing agent following the method described previously [16,17]. Sodium borohydride was reacted with tellurium powder (2:1 molar ratio) in water to produce NaHTe. The molar ratio of Cd^{2+}:Te^{2-}:TGA was 1:0.5:2.5. The pH value of the solution was adjusted to 7.6 with 0.1 M NaOH. The oxygen in the system was removed by flowing nitrogen when the system was maintained under distillation reflux at 100 °C for 2 h.

2.4. FRET between QDs and NR

NR has amino groups available for the conjugation with carboxylic acid group capping QD nanoparticles via an electrostatic interaction [18–20]. Under pH 8.0 conditions dyes expose amino moieties for binding to the QD surface. Conjugates, CdTe-NR, were prepared by adding appropriate amounts of NR to 100 μL of 50 μmol L^{-1} CdTe solutions in CTMAB micelle (pH 8.0) and allowed to self-assemble for 1 h at room temperature. Molar ratios of NR to QDs were discretely varied among samples from 0 to 50. The individual samples were then diluted with Milli-Q water to a total volume of 5 mL. The conjugates were loaded onto an Amicon Centricon spin dialysis tube having a cutoff molecular weight, MW, of 300 kDa and centrifuged at 1000 × *g* 2 times for 20 min each, with a Milli-Q water wash between the spins. The ratio of conjugated NR to QD was controlled by varying the amount of added NR and was determined by ultraviolet/visible (UV/vis) absorption spectrophotometry [2,21,22].

2.5. Preparation of BSA-coated QD

QD-BSA conjugate was prepared according to the literature [23] with a slight modification. Firstly, 100 μL of 50 μmol L^{-1} QDs were added to the preformed solution, which employed phosphate buffer saline (PBS) buffer solution (pH 8.0) in micelle with adding appropriate amounts of BSA, allowed to self-assemble for ~15 min at room temperature, and then stored it in air. Molar ratios of BSA to QDs were discretely varied among samples from 0 to 10. The individual samples were then diluted with PBS buffer to a total volume of 3.0 mL. The final solutions were placed in a 10 mm optical path quartz fluorescence cuvette, and measured by Fluorescence spectrometer with the excited wavelength of 345 nm.

3. Results and discussion

3.1. TEM image of CdTe QDs

TEM image of the as-prepared sample is shown in Fig. 1, which indicates that the as-prepared sample is composed of uniform dispersed QDs with ~3.1 nm in diameter. HRTEM image of this CdTe show well-resolved crystal lattice fringes, demonstrating the highly crystalline nature of the synthesized QDs.

3.1.1. Absorption and fluorescence spectra of CdTe QDs (donor) and NR (acceptor)

NR was selected as the acceptor dye since its absorption spectrum has a high degree of overlap with the emission spectrum of the QDs. The appropriate size of CdTe QDs was chosen so as to maximize the overlap of the emission spectra of the donor and absorption spectra of the acceptor while still maintaining good resolution of their emission spectra. The maximal absorption and emission peaks of the QDs are at 491 and 544 nm, respectively, while those of NR are at 532 and 620 nm, respectively. So there is an appreciable overlap between the emission spectrum of CdTe QDs (donor) and the absorption spectrum of NR (acceptor). In order to minimize the contribution of the acceptor fluorescence coming

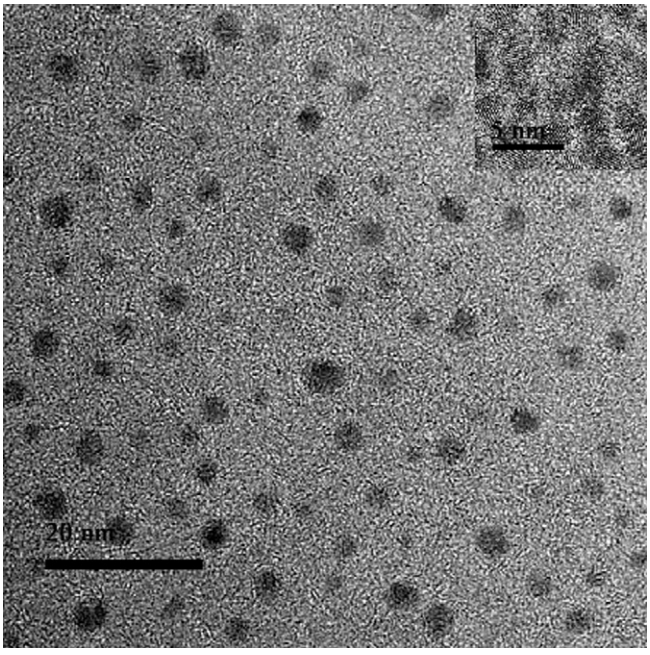

Fig. 1. TEM image of CdTe QDs (Inset: HRTEM).

from direct excitation of the acceptor, the FRET measurements were performed by exciting the donor at 345 nm.

3.2. Interaction of CdTe QDs (donor) and BSA

BSA can be coupled with TGA-capped CdTe quantum dots via electrostatic interactions between its amino group and carboxylic acid group of TGA. In PBS buffer (pH = 8.0), TGA-capped CdTe formed a stable conjugate with BSA. Excitation wavelength and emission wavelength of this system were 345 nm and 555 nm respectively. It was observed that the fluorescence intensity increased with increasing the amount of BSA, as shown in Fig. 2. BSA was linked to the surface of CdTe QDs, which reduced the defects of CdTe QDs, and increased the fluorescence intensity of CdTe QDs.

3.3. Construction of the FRET system in micelles

Simple mixing of CdTe QDs and NR in PBS buffer solution led to no obvious increase in the fluorescence intensity of the acceptor. The result indicated that the interaction (mainly the electrostatic force) between the electronegative CdTe QDs and cation dye NR was too weak to draw them close enough.

A Surfactant sphere was used to act as a nano-vessel for TGA-capped CdTe QDs and NR. On account of different chemical environments, polarity and lipophilicity of the inside and outside of micelles are remarkably different [24–26]. It is expected that chemical behavior such as the rate of reaction and the solubility of the compound in the micellar system would be different from those in the bulk solution. Moreover, the electronic energy state which is stabilized by the surfactant tends to change in the excitation or relaxation process of the QDs and would result in an increase of the fluorescence intensity. Considering that the surfactant could lower the surface tension of the solution [27–29], several surfactants in common use were investigated.

Herein, three types of surfactants, neutral (Tween 80), anionic sodium dodecyl sulfate (SDS) and cationic (CTMAB) surfactants were homogeneously dispersed in 0.1 mol L^{-1} PBS buffer pH 8.0, were studied. The concentration of each surfactant used was twice its critical micelle concentration (cmc): 0.2×10^{-3} mol L^{-1} Tween 80, 1.8×10^{-3} mol L^{-1} SDS and 2×10^{-3} mol L^{-1} CTMAB. NR-CdTe was mixed with three surfactants respectively to check their PL intensity. The fluorescence intensity of NR acceptor increased by 3.8% and 1.9 in the presence of CTMAB and Tween 80 micelles, respectively, while the fluorescence intensity decreased by 0.5 in the presence of SDS. The enhancement of the fluorescence intensity of NR acceptor in the presence of cationic and neutral surfactants may be due to a partial incorporation of CdTe-TGA QDs and NR into the surfactant micelle. The incorporation leads to a separation of CdTe-TGA QDs and NR from the bulk solution, which produce a conjugation of NR molecules on the surface of the QDs. Moreover, the concentration of the QDs and NR inside the micelle is relatively higher than those in the regular solution. In contrast, in the presence of SDS, the decrease in fluorescence intensity can be ascribed to the electrostatic repulsion between the negative charge on the surface of CdTe-TGA QDs and the anionic head of SDS, which limits the incorporation of CdTe-TGA QDs and NR into the surfactant micelle. A strong FRET phenomenon occurred when CTMAB was used. Therefore, CTMAB was employed to construct the FRET system in this work.

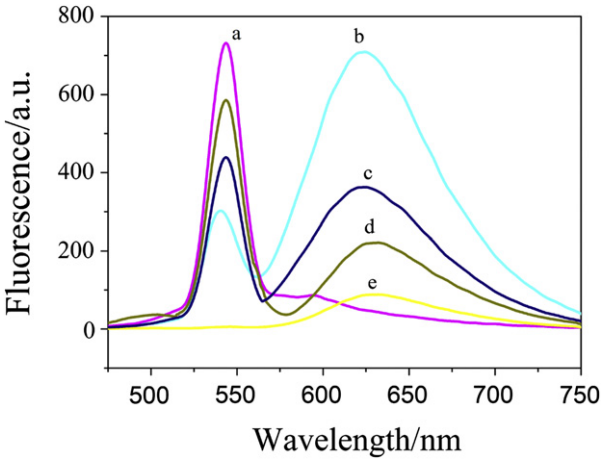

Fig. 3. Fluorescence spectra of CdTe-NR in micelles; a. CdTe, b. CdTe-NR-CTMAB, c. CdTe-NR-Tween 80, d. CdTe-NR, e. CdTe-NR-SDS.

As shown in Fig. 3, the fluorescence spectra change when CdTe QDs interact with NR in CTMAB solution. A significant enhancement of NR fluorescence intensity and the corresponding quenching of the CdTe QDs are observed. The blue shift of the CdTe emission peak from 542 to 535 nm also confirms the FRET process, in which the microenvironment around the donor is altered.

Fig. 4 shows a series of emission spectra obtained for different Dye/QD ratios. The given ratios are the nominal values of the starting mixtures, and for each ratio the precipitation-dissolution

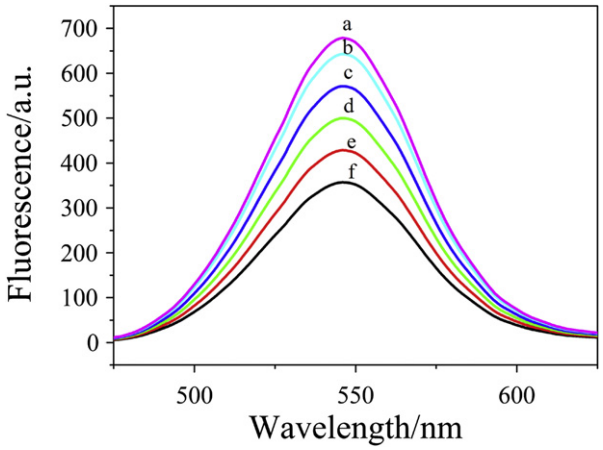

Fig. 2. Fluorescence spectrum of CdTe QDs and BSA.

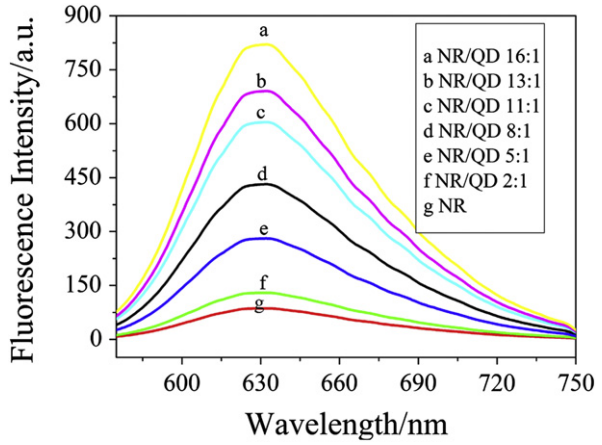

Fig. 4. Emission spectra of NR with different Dye/QDs ratios; Ratio of NR/QDs (a) 16:1, (b) 13:1, (c) 11:1, (d) 8:1, (e) 5:1, (f) 2:1, (g) NR.

step has been carried out. As the dye amounts increased, the QD emission is successively quenched and sensitized dye emission increased, indicating efficient energy transfer happened in the complexes, which will be analyzed in full detail below. All spectra have been corrected for the small contribution from direct excitation of the dye. For a given Dye/QD ratio in micelle, emission spectra remained almost constant for up to 6 months corroborating the high stability of the complexes.

3.4. Energy transfer between CdTe QDs and NR in CTMAB micelles

To quantify the efficiency of energy transfer, the energy transfer efficiency (E) was calculated according to Eq. (1) [3]. It is defined as the number of quanta transferred to the acceptor divided by the number of quanta absorbed by the donor. E can be measured experimentally and is commonly expressed as:

$$E = 1 - \frac{I}{I_0} \tag{1}$$

where I is the fluorescence intensity of the donor in the presence of the acceptor and I_0 is the fluorescence intensity of the donor in the absence of the acceptor.

The relationship between E and r (the distance between the donor–acceptor pair) is given as Eq. (2) [3]. So, the distance r can be calculated from the value of E according to the following equation:

$$E = \frac{R_0^6}{R_0^6 + r_0^6} \tag{2}$$

where R_0 is the critical distance when the energy transfer efficiency is 50%, which is expressed as [3]:

$$R_0^6 = 8.8 \times 10^{-25} K^2 n^{-4} \Phi_D J(\lambda) \tag{3}$$

where the spatial orientation factor K^2 and the refraction index n are accepted as 2/3 and 1.336, respectively [30,31]. The quantum yield Φ_D of CdTe QDs was measured to be 56.0% using rhodamine 6G as the reference standard [32–34]. And $J(\lambda)$ is the spectral overlap integral which can be calculated from the following equation:

$$J(\lambda) = \frac{\int I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int I_D(\lambda) d(\lambda)} \tag{4}$$

where I_D is the fluorescence intensity of the donor and ε_A is the molar absorptivity of the acceptor. The spectral overlap of the fluorescence emission spectrum of CdTe QDs and the absorption spectrum of NR is shown in Fig. 5. The $J(\lambda)$ is calculated to be 5.87×10^{-14} cm^3 L mol^{-1} for this FRET system by integrating the spectra for 410–650 nm in Fig. 5. So, the R_0 is calculated to be 4.7 nm from Eq. (3). In this study, the E could reach 72.5% (calculated using Eq. (1)). By fitting the data with Eq. (2), the r is found to be 4.0 nm, which is much smaller than 7 nm, a criterion value for energy transfer phenomenon to occur, indicating that the energy transfer from CdTe QDs to NR occurred with high probability.

4. Interaction of BSA and CdTe-NR in micelle

In addition, the reactions between BSA and CdTe-NR in the three surfactant micelles were also investigated in order to improve the fluorescence quenching sensitivity. The concentration of BSA was fixed at 5.00×10^{-3} g L^{-1}. The fluorescence quenching ability of BSA was represented in terms of relative fluorescence quenching (I_0/I),

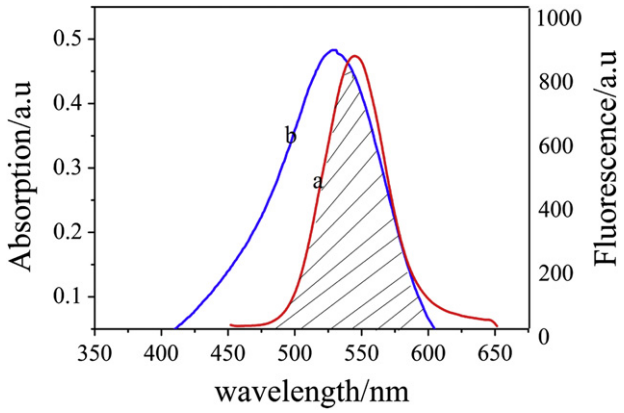

Fig. 5. Spectral overlap between the fluorescence spectra of CdTe QDs(a) and the absorption spectra of NR(b).

where I_0 and I are the fluorescence intensities of CdTe-NR in the absence and in the presence of BSA, respectively. While a slight quenching in fluorescence intensity was observed when BSA was introduced to the SDS system, the intensities decreased in the Tween 80 and CTMAB systems. A maximum quenching efficiency was observed in the presence of CTMAB, suggesting that the cationic surfactant was a suitable surfactant that could be used to increase the fluorescence quenching ability of BSA. The quenching efficiency of the CdTe-NR was substantially lower upon the opening of NR by the competition of BSA (See Fig. 6), presumably, because of the increased distance separating the NR from the QDs, the CdTe and NR FRET could not occur.

The fluorescence intensities of the acceptor were quenched when BSA was added. The quenching extent of NR was much greater. In addition, the characteristic emission peak of NR was shifted to a longer wavelength by 76 nm than that of CdTe. So, the monitoring of the spectral change of NR was more suitable for the determination, especially in biologic samples, because long emission wavelength could be distinguished easily from the sample background. We developed a fluorescent biosensing platform for BSA detection using the CdTe-NR FRET sensor. The fluorescence intensity of the CdTe-NR sensor quenched with the increasing concentration of BSA. The linear range is from $0.4 \times 10^{-3} \sim 11.00 \times 10^{-3}$ g L^{-1} with linear equation $\Delta I_F = 5.85 + 34.05\rho$ ($\times 10^{-3}$ g L^{-1}), where ΔI_F is the relative fluorescence intensity and ρ is the concentration of BSA (regression coefficient $R = 0.9961$). Detection limit is 0.13×10^{-3} g L^{-1}. Further, control studies showed no significant changes of fluorescence intensity when adding the same volume of BSA to the NR without CdTe, coinciding with the above observation that the fluorescence quenching was induced by BSA interacting with CdTe-NR FRET sensor. The quenched relative fluorescence has good reproducibility with standard deviation usually less than 5%. Possible reasons may explain this excellent

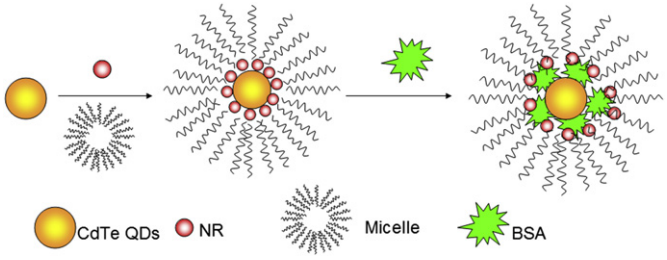

Fig. 6. Schematic illustration of the interaction of CdTe-NR and BSA in micelles.

performance of CdTe-NR FRET sensor. The quenching of fluorescence is highly dependent on the energy transfer between dyes and CdTe, which is critical for CdTe FRET sensor. The high fluorescence quenching efficiency leads to the low background of signals and results in the high sensitivity.

5. Conclusion

In summary, a facile approach has been demonstrated for the detection of BSA, which is based on a NR and CdTe QDs FRET in micelles. There is an appreciable overlap between the emission spectrum of the QDs (donor) and the absorption spectrum of the NR (acceptor). A strong FRET phenomenon occurred when the cationic surfactant CTMAB was used, and the energy transfer efficiency was about 72.5%. Moreover, the FRET phenomenon has excellent sensitivity and stability in micelles. The developed method provides a new perspective to the FRET and is expected to be used for the biological and environmental analysis.

Acknowledgments

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