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Quantum dot-Eu^{3+} conjugate as a luminescence turn-on sensor for ultrasensitive detection of nucleoside triphosphates

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ABSTRACT

We report a conjugate of thioglycolic acid (TGA) capped CdTe quantum dot and Eu^{3+} ion (TGA-CdTe QD-Eu^{3+}) that can be used as an ultrasensitive luminescence turn-on sensor for nucleoside triphosphates (NTPs). The TGA-CdTe QD-Eu^{3+} conjugate is a weakly luminescent species as a result of the strong quenching effect of Eu^{3+} ion on the luminescence of TGA-CdTe QDs. The conjugate's luminescence can be readily restored by its reaction with adenosine triphosphate (ATP) and other NTPs, and thus gives an ultrasensitive detection of NTPs, with a detection limit of 2 nM. The sensing mechanism has also been explored, and the effective quenching of TGA-CdTe QDs emission by Eu^{3+} ions has been attributed to photoinduced electron transfer (PET). ATP, as the representative of NTPs, can remove Eu^{3+} from the surface of TGA-CdTe QDs, leading to restoration of the TGA-CdTe QDs luminescence.

1. Introduction

Colloidal semiconductor quantum dots (QDs), with unique optical properties, have been attracting much attention in bioanalytical and imaging application. QDs are crystals a few nanometers in diameter whose size and shape can be controlled by the duration, temperature, and ligand molecules used in the synthesis. Due to quantum confinement effects, they show unique property, such as, broad excitation and narrow size-tunable emission spectra, high photochemical stability, and long luminescence lifetime (>10 ns) [1–4]. During the past decade, QD-based luminescent probes have been extensively studied, and many probes displayed luminescence quenching (turn-off or switch-off). By a turn-off recognition, undesired emission quenching is a significant problem in practical application. Recently, scientists have made a growing effort in developing luminescence "turn-on" QD-based sensors for higher selectivity and sensitivity [2,3]. By a turn-on responsive mode, reversible quenching is generally designed to weaken the luminescence of QDs, and the quenching can be eliminated by analyte recognition. Most reversible quenching has been based on either electron/charge transfer or energy transfer process. When the electron/charge-transfer process between QDs and surface-bound receptors occurs, the exciton (electron–hole pair) recombination process is blocked, leading to luminescence quenching. The redox potential of the attached receptor is considerably changed upon analyte recognition. This change in redox potential can be sufficient to modulate the electron transfer process responsible for the quenching and switch on luminescence [5–9]. Another electron/charge transfer modulating mode can be related to the removing of the attached receptor from the surface of QDs by an analyte, blocking the electron transfer interaction [10,11]. Fluorescence resonance energy transfer (FRET) QD-based sensing [4], using QDs as energy donor, and gold nanoparticles [12–15] or organic fluorophores [16–19] as energy acceptor, is another favorable mechanism. The FRET processes are usually modulated by removing of energy acceptor through cleavage of the peptide [12,16] or competitive binding [13–15]. Additionally, reacting with analyte could cause a dramatic shift of the energy acceptor's absorption spectrum, shutting down the energy transfer pathway [17–19]. These luminescence turn-on responses based on QDs have been applied to sense cations (Cd^{2+}, Zn^{2+}, Pb^{2+}) [5,6,14], anions (F^-, CN^-, CO_3^{2-}) [7–9], nitric oxide [17], pesticide [19], and biomolecules [10–13,15,16,18], including avidin, glucose, histidine, tyrosinase and protease.

Nucleoside triphosphates (NTPs), play pivotal roles in various physiological events. It is well known that ATP is the source of free energy used to maintain order within most cells. In addition, NTPs have been recognized as signaling molecules. NTPs also serve as phosphoryl donors in kinase-catalyzed phosphorylation [20–23]. The detection of NTPs is significant for the exploration of cellular physiological processes. Fluorescent detection has

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multiple advantages including high sensitivity, low cost, ease of application and versatility. It has been of interest to develop more sensitive, higher-resolution, and more robust fluorescent detection of these NTPs in recent years [24–32], but only a few of them show turn-on recognition and have a large fluorescence response to analytes in aqueous solution [24,27].

Europium (Eu^{3+}) ions, with their partially filled orbits, are possible to quench the QDs emission through electron transfer when adsorbed onto the surface of thioglycolic acid (TGA)-capped CdTe QDs (TGA-CdTe QDs). The $P{=}O$ bonds in phosphate-containing compounds show strong coordination with lanthanide ions (Ln^{3+}) [33]. Some methods of detecting phosphate-containing compounds have been based on the binding of phosphate groups of these analytes with Tb^{3+} or Eu^{3+} complexes, enhancing or quenching their lanthanide luminescence [32,34–39]. In a recent paper, Eu^{3+} induced carbon dots (CDs) aggregated, and quenched the luminescence of CDs. Phosphate could disrupt the Eu^{3+}-induced aggregates of CDs, leading to restoration of CDs luminescence [40]. It is known that polyphosphates can chelate Eu^{3+} with even higher affinity [41]. The chelating of NTPs with Eu^{3+} can disturb the electron transfer interaction between QDs and Eu^{3+}, and the restoration of the QDs emission can be expected. Thus a turn-on QD-based sensor for NTPs can be developed.

2. Experimental section

2.1. Preparation of TGA-CdTe QDs

TGA-CdTe QDs were synthesized in the aqueous phase according to the reported method [42]. Briefly, 128 mg of tellurium powder and 143 mg of $NaBH_4$ were added to 1.5 mL of ultrapure water, which was then stirred for 8 h in an ice bath till the black color disappeared and white $Na_2B_4O_7$ was produced. The supernatant containing NaHTe was used as the precursor for the preparation of TGA-CdTe QDs. Then the freshly prepared supernatant NaHTe solution was added into a N_2-saturated $CdCl_2$ solution (pH = 11) in the presence of TGA as the stabilizing agent. The molar ratio of Cd/Te/TGA was fixed at 1:0.5:2.4. The solution was then refluxed under nitrogen flow for about 1 h to get green luminescent TGA-CdTe QDs. The concentration of TGA-CdTe QDs was estimated by the absorbance value at the first absorption peak and the molar extinction coefficient according to the method of Yu et al. [43]. The TGA-CdTe QDs were then dissolved in 10 mM Tris–HCl buffer at pH 7.4 for all measurements.

2.2. Apparatus

The steady-state fluorescence was recorded on a RF-5301PC spectrofluorophotometer (Shimadzu). The excitation wavelength was 360 nm, and the fluorescence intensity of TGA-CdTe QDs was acquired at 540 nm. Decays of TGA-CdTe QDs luminescence were recorded on a FluoroMax-4 spectrofluorometer (HORIBA Jobin Yvon), using the TCSPC (time correlated single photon counting) method. The excitation source was a pulsed 339 nm NanoLED, and the photons were collected at 540 nm. The deconvolution and exponential fitting were performed using the DAS6 software (HORIBA Jobin Yvon); the average lifetimes were calculated using the equation: $\overline{\tau} = \left(\sum_{i=1}^{n} \alpha_i \tau_i^2 / \sum_{i=1}^{n} \alpha_i \tau_i \right)$, where τ_i is the fluorescence lifetime and α_i is the pre-exponential factor.

2.3. Ultrafiltration of TGA-CdTe QDs

2-mL solutions containing 50 nM TGA-CdTe QDs and different concentrations of Eu^{3+} and ATP were ultrafiltrated using Sartorius Vivaspin centrifugal concentrators with polyethersulfone (PES) membrane of 5 kDa Molecular Weight Cut-off (MWCO) under centrifugation (3000 *g*, 8 min). The as-prepared TGA-CdTe QDs could not pass the 5 kDa MWCO membrane, monitored by measuring the fluorescence of the filtrate. The determination of the concentration of Eu^{3+} ions in the filtrate was carried out by ICP-MS, which was performed on ELAN DRC II (PerkinElmer SCIEX).

3. Results and discussion

3.1. Formation of TGA-CdTe QD-Eu^{3+} conjugate and its response to NTPs

The as-prepared TGA-CdTe QDs gave a strong emission band at 540 nm. Its luminescence was strongly quenched by titration of $EuCl_3$ in pH 7.4 Tris–HCl buffer (Fig. 1). Weakly luminescent TGA-CdTe QD-Eu^{3+} conjugate was prepared by simply mixing the 50 nM TGA-CdTe QDs and 165 nM $EuCl_3$ in pH 7.4 Tris–HCl buffer. This QD-Eu^{3+} conjugate showed a turn-on luminescent response to ATP (Fig. 2). The kinetics of the formation of preluminescent conjugate and the luminescence turn-on response to ATP was also monitored (Fig. S1). Both processes of the formation of the preluminescent conjugate and the response to ATP were so fast that they were completed immediately following the mixing of solutions. Other NTPs, including cytidine triphosphate (CTP), guanosine triphosphate (GTP) and uridine triphosphate (UTP), showed similar behavior to ATP in turning on the QD-Eu^{3+} conjugate emission (Fig. 3). The results agreed with the general principle that polyphosphates are strong complexing agents for lanthanide ions [33].

3.2. Sensitivity and selectivity of the probe for NTPs

Luminescent responses of the TGA-CdTe QD-Eu^{3+} conjugate to other phosphate-containing derivatives were also tested and the results were shown in Table 1. HPO_4^{2-} and AMP showed little influence on the luminescence of TGA-CdTe QD-Eu^{3+} conjugates. ADP and pyrophosphate (PPi) also turned on the TGA-CdTe QDs emission, but to a much lower extent than ATP. The interference

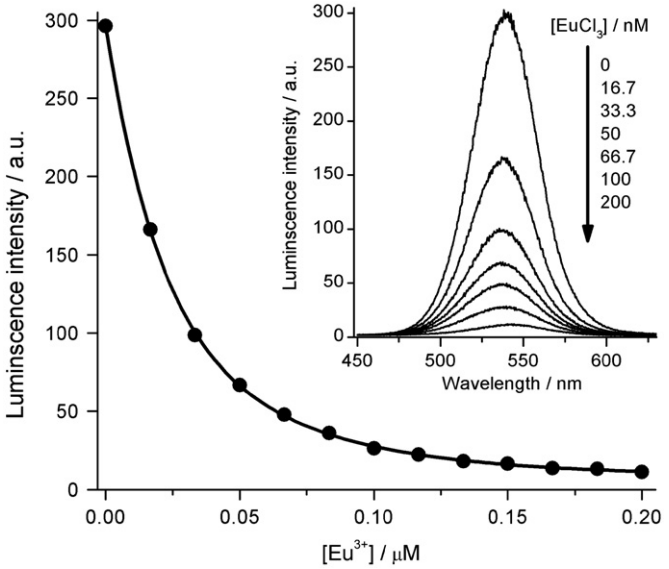


Fig. 1. Luminescence intensity (λ_{ex} = 360 nm, λ_{em} = 540 nm) of 50 nM TGA-CdTe QDs in 10 mM Tris–HCl buffer at pH 7.4 as a function of $EuCl_3$ concentration. (inset) Luminescence spectra of TGA-CdTe QDs in the presence of different concentrations of $EuCl_3$ (λ_{ex} = 360 nm).

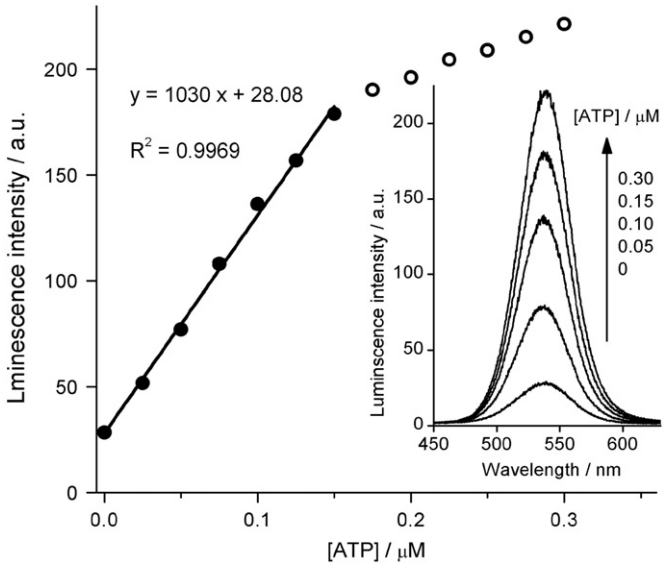


Fig. 2. Luminescence intensity of TGA-CdTe QD-Eu^{3+} conjugate as a function of ATP concentration (TGA-CdTe QDs: 50 nM, Eu^{3+}: 0.165 μM, in 10 mM pH 7.4 Tris–HCl buffer, λ_{ex}=360 nm, λ_{em}=540 nm), and the linear correlation of the data of solid circles. (inset) Luminescence spectra of TGA-CdTe QD-Eu^{3+} conjugate in the presence of different concentrations of ATP (λ_{ex}=360 nm).

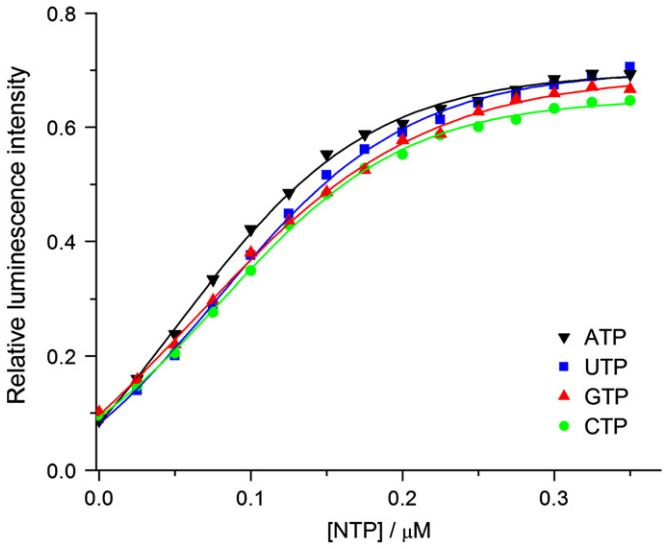


Fig. 3. Luminescence intensity of TGA-CdTe QD-Eu^{3+} conjugates as a function of the concentrations of different NTPs (λ_{ex}=360 nm, λ_{em}=540 nm). TGA-CdTe QD: 50 nM; Eu^{3+}: 165 nM.

of common anions and some compounds that potentially chelate Ln^{3+} complexes was also tested (Table 1). Only citrate had some effect on the TGA-CdTe QDs emission. The luminescent responses of TGA-CdTe QD-Eu^{3+} conjugate to these anions were consistent with their complexation strengths with Eu^{3+}. These anions show weak complexation strengths with Eu^{3+} except citrate [33].

The response of this TGA-CdTe QD-Eu^{3+} conjugate to different NTPs can be considered equal. So when this conjugate is applied in practical measurement with ATP as standard, the overall NTPs amount can be obtained. The luminescence enhancement of the preluminescent TGA-CdTe QD-Eu^{3+} conjugate was proportional to the ATP concentration in the range of 70∼150 nM (Fig. 2). Defining the detection limit as the concentration corresponding to three standard deviations of the background signal, a detection limit of 2 nM was obtained when this conjugate was used to

Table 1
Luminescence intensity enhancement of TGA-CdTe QD-Eu^{3+} conjugates against various anions (relative to ATP).

Anion	Relative luminescence enhancement (%)	Anion	Relative luminescence enhancement (%)
ATP	100	AMP	3.2
ADP	11.7	PPi	26.8
HPO_4^{2-}	1.4	CH_3COO^-	2.1
HCO_3^-	2.2	NO_3^-	1.4
SO_4^{2-}	2.2	SCN^-	3.7
F^-	1.6	Cl^-	0.8
Br^-	1.3	I^-	4.4
Citrate	17.3	Lactate	3.2
BSA	1.5	DNA	3.9
H_2O_2	1.7		

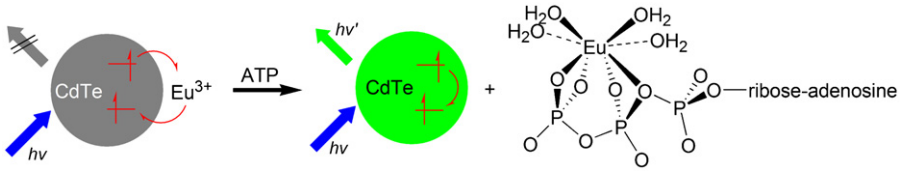
TGA-CdTe QDs: 50 nM; Eu^{3+}: 165 nM; citrate, HPO_4^{2-}, AMP, ADP and ATP: 100 nM; lactate: 1 μM; BSA and DNA: 1 mg/L; and others: 10 μM. λ_{ex}=360 nm, λ_{em}=540 nm.

quantify the NTPs in neutral aqueous solution. These results demonstrated that the TGA-CdTe QD-Eu^{3+} conjugate can be a good luminescence turn-on probe for the amount of NTPs.

3.3. Sensing mechanism

The absorption spectra of TGA-CdTe QDs did not change after adding Eu^{3+} ions (Fig. S2), which excluded the Eu^{3+} induced aggregation. To further understand the quenching mechanism, the TGA-CdTe QD-Eu^{3+} solutions were subjected to an ultrafiltration membrane of 5 kDa MWCO, through which only Eu^{3+} and Eu^{3+}-NTPs complex free in the solution but not those adhered to the surface of QDs could pass. The concentration of Eu^{3+} in the filtrate was determined by ICP-MS (Table S1). When Eu^{3+} ions relative to QDs was insufficient, Eu^{3+} was practically absent in the filtrate (No. 1 in Table S1). When the ratio of Eu^{3+} to QDs increased to 3.3, only 1.3% of Eu^{3+} was found in the filtrate (No. 2 in Table S1). This is evidence of strong binding of Eu^{3+} ions onto the surface of QDs. We explored ATP's effect (as the representative of NTPs) on the luminescence restoration of TGA-CdTe QD-Eu^{3+} conjugate. It has been reported that ATP and Eu^{3+} could form the very stable Eu^{3+}–ATP complex [41,52]. In the ultrafiltration experiments (Table S1), the Eu^{3+} in the filtrate were increased from 1.3% (in the absent of ATP) to 63.1% with addition of increasing amount of ATP to the TGA-CdTe QD-Eu^{3+} conjugate solution. This demonstrated that ATP intensively coordinated with Eu^{3+} and could remove Eu^{3+} from the surface of TGA-CdTe QDs, leading to restoration of TGA-CdTe QDs luminescence (Scheme 1).

The interaction of QDs and metal ions have been reported before [3]. In most cases, the quenching of QDs luminescence by metal ions were observed [44–48], including Cu^{2+}, Ag^+, Hg^{2+}, Pb^{2+}, and the quenching were irreversible. The quenched emission of TGA-CdTe QDs by Eu^{3+} was turned on again by titration of NTPs into the solution. This evidenced a reversible quenching mechanism, and a permanent metamorphosis of TGA-CdTe QDs can be excluded. The shortening of luminescence lifetime concomitant with the quenching of TGA-CdTe QDs emission was also observed (Fig. 4), indicating that Eu^{3+} ions quenched the excited states of TGA-CdTe QDs. Considering that the redox potential of the conduction band of the CdTe QDs is about −1.0 V [49,50], the redox potential of Eu^{3+} (−0.34 V, when its oxidation state changes from +3 to +2) [51] is high enough to induce electron transfer from the conduction band of CdTe QD to the partially occupied band of Eu^{3+} and then transfers back to the valence band of CdTe QD (Scheme 1). And thus, the partially occupied orbit of Eu^{3+} can perform as a shuttle for the photoinduced



Scheme 1. Schematic diagram of the quenching mechanism of TGA-CdTe QDs luminescence by Eu^{3+}, and ATP-induced luminescence enhancement of TGA-CdTe QD-Eu^{3+} conjugate.

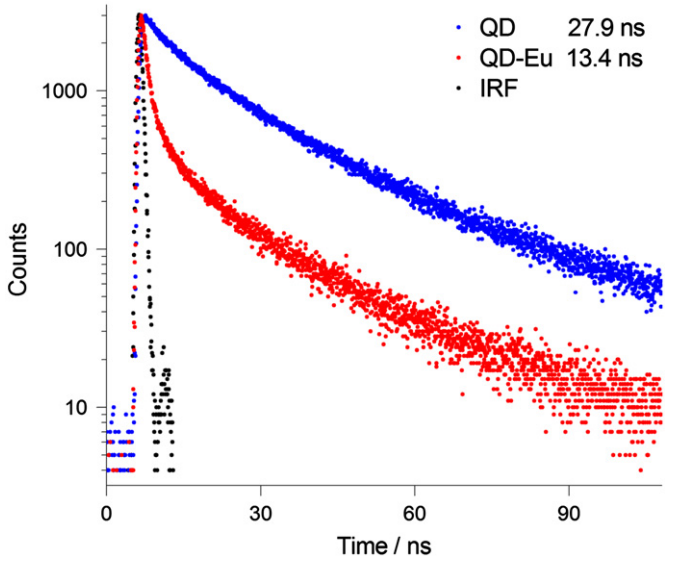


Fig. 4. Fluorescence decays of TGA-CdTe QDs before and after addition of $EuCl_3$. The photons were collected at the wavelength of 540 nm; the instrument response function (IRF) was also presented.

electron transfer (PET) from the conduction band to the valence band of the CdTe QD, and disrupts the radiative recombination process. X-ray photoelectron spectroscopy (XPS) technique has been used to elucidate the proposed mechanism, and no typical Eu 3d core-level peak of divalent Eu at 1124 eV could be observed in the XPS spectrum of TGA-CdTe QD-Eu^{3+} conjugate (Fig. S3). This result was consistent with the shuttle mechanism of Eu^{3+}, since the shuttle mechanism does not change the state of Eu^{3+} permanently. It is known that the redox potentials of Tb^{3+} (-3.7 V) and Sm^{3+} (-1.55 V) are much lower than that of Eu^{3+} when their oxidation states change from $+3$ to $+2$. Both redox potentials are lower than that of conduction band of CdTe QDs, so we expected that no PET process would happen. When Tb^{3+} ions and Sm^{3+} ions were added instead of Eu^{3+} ions in the TGA-CdTe QDs solution, respectively, both Tb^{3+} and Sm^{3+} ions showed only a little influence on the luminescence of QDs (Fig. S4).

4. Conclusions

A preluminescent conjugate of TGA-CdTe QD-Eu^{3+} was prepared by simply mixing TGA-CdTe QDs and $EuCl_3$ at a physiological pH in water. This TGA-CdTe QD-Eu^{3+} conjugate showed luminescence turn-on response to NTPs, which has proved to be selective and ultrasensitive. The quenching effect of Eu^{3+} on the luminescence of TGA-CdTe QDs is attributed to PET. The addition of ATP can remove Eu^{3+} from TGA-CdTe QDs as a result of the strong complexation of ATP and Eu^{3+}, leading to luminescence restoration. Multiple factors are contributing to the high sensitivity and selectivity, including the intense luminescence of TGA-CdTe QDs, the effective quenching of TGA-CdTe QDs luminescence by Eu^{3+}, and the strong complexation of NTPs and Eu^{3+}.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.062.

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