

Electrogenerated Chemiluminescence Resonance Energy Transfer between Luminol and CdSe@ZnS Quantum Dots and Its Sensing Application in the Determination of Thrombin

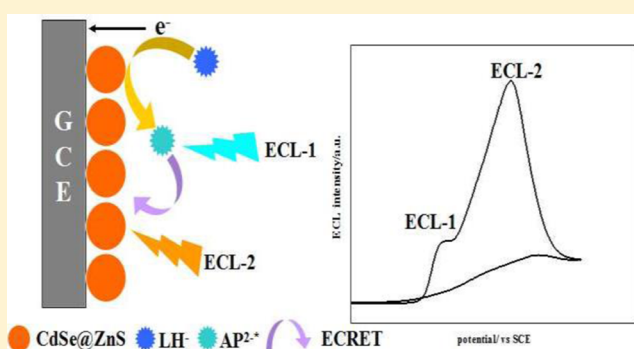
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S Supporting Information

ABSTRACT: In this work, electrogenerated chemiluminescence resonance energy transfer (ECL-RET) between luminol as a donor and CdSe@ZnS quantum dots (QDs) as an acceptor was reported in neutral conditions. It was observed that a glassy carbon electrode modified with CdSe@ZnS quantum dots (CdSe@ZnS/GCE) can catalyze the luminol oxidation to promote the anodic luminol ECL without coreactants. The intensity of anodic luminol ECL (0.60 V) at the CdSe@ZnS/GCE was enhanced more than 1 order of magnitude compared with that at the bare GCE. Another stronger anodic ECL peak observed at more positive potential (1.10 V) could be assigned to the ECL-RET between the excited state of luminol and the QDs. A label-free ECL aptasensor for the detection of thrombin was fabricated based on the synergic effect of the electrocatalysis and the ECL-RET. The approach showed high sensitivity, good selectivity, and wide linearity for the detection of thrombin in the range of 10 fM–100 pM with a detection limit of 1.4 fM ($S/N = 3$). The results suggested that the as-proposed luminol–QDs ECL biosensor will be promising in the detection of protein.



Since Bard et al. explored electrogenerated chemiluminescence (ECL) properties of silicon semiconductor nanocrystals (also known as quantum dots, QDs) in 2002, the interest for the preparation and application of various QDs with ECL activity has been growing.¹ In the presence of coreactants, such as $K_2S_2O_8$ and H_2O_2 , most of the QDs show strong cathodic ECL and can be used to develop ECL biosensors based on ECL quenching or enhancement via charge transfer or energy transfer.^{2–7} For bioanalytical applications, it is highly desirable to have QDs ECL produced in neutral media upon anodic potential scanning so that the system can be used in bioassays, and the effect of oxygen reduction from air and solution media can be eliminated.⁸ However, the anodic ECL intensity of QDs is much lower than that of conventional luminescent reagents such as luminol or $Ru(bpy)_3^{2+}$, and QDs in ECL processes with a high excited potential are unstable. Although great effort has been made for the improvement of QDs anodic ECL, it is still not strong enough compared to the cathodic ECL.^{9–11} Therefore, developing new QDs involving ECL systems with strong and stable anodic ECL intensity would be desirable.

Because luminol can produce strong anodic ECL at a wavelength of 425 nm in alkaline conditions, it can be considered as an efficient reagent to be widely used as ECL labels in bioassays.^{12,13} However, luminol ECL is extremely

weak in neutral media and often needs hydrogen peroxide acting as coreactant to enhance the ECL signals.¹⁴ To overcome these problems, the catalytic effect of some nanoparticles, such as gold and platinum nanoparticles, has been introduced to sensitize luminol ECL reaction for the determination of biomolecules.^{15–17} Although luminol ECL could be enhanced at these nanoparticles modified electrodes in neutral solutions, poor sensitivity and selectivity are still problems. Previous work has revealed that quantum dots could exhibit good electrocatalytic behavior; however, quantum dots as catalysts in ECL investigations were rarely reported.¹⁸

Luminescence resonance energy transfer (LRET) is a powerful technique for probing changes in the distance between donors and acceptors. According to the different types of the donor luminescence, three kinds of LRET, such as fluorescence resonance energy transfer (FRET), chemiluminescence resonance energy transfer (CRET), and bioluminescence resonance energy transfer (BRET), have been reported and have helped make remarkable progress in bioapplications.^{19–23} As another kind of donor luminescence, the ECL

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technique has attracted increasing interest in sensing applications due to its high sensitivity. Nevertheless, little attention has been paid to the ECL resonance energy transfer (ECL-RET) as a result of the difficulty in finding a suitable donor/acceptor pair and the electrochemical instability of the acceptor itself.²⁴ Recently, several ECL-RET systems regarding quantum dots, luminol, and Ru(bpy)₃²⁺ were reported.^{25–28} These work suggested that ECL-RET could occur between a traditional luminescent system and quantum dots. However, the reported ECL-RET systems involving luminol were usually operated in alkaline condition and unstable H₂O₂ was often required as coreactant, which would inevitably limit their applications.^{25,26} In the present work, luminol ECL was studied at a glassy carbon electrode modified with CdSe@ZnS QDs (CdSe@ZnS/GCE) in a neutral solution and strong light emission was obtained in the absence of H₂O₂. The enhancing effect showed that the QDs could promote the oxidation of luminol, and the ECL-RET between luminol and QDs can be observed. The technique can be used to fabricate a biosensor for the determination of thrombin. The thrombin binding aptamer (TBA) was covalently attached to the CdSe@ZnS/GCE through the amidation reaction between the amino group of TBA and the carboxyl group on the QDs surface. The probe DNA was immobilized on the modified electrode through the hybridization with TBA. Another layer of QDs was connected to the probe to further increase the ECL signal. The detection of thrombin can be realized because thrombin has higher affinity for TBA over the probe DNA. Therefore, a simple and convenient sensing platform is proposed based on QDs, nucleic acid aptamer, and the intercalated probe for the sensitive and selective detection of thrombin.

■ EXPERIMENTAL SECTION

Chemicals and Apparatus. Luminol, poly-(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW = 200 000–350 000), thioglycolic acid (TGA), thrombin, bovine serum albumin (BSA), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. A luminol stock solution (0.01 mol L⁻¹) was prepared by dissolving luminol in 0.1 mol L⁻¹ NaOH and stored in the refrigerator. The stock solution was used to prepare working standard solution by suitable dilution. Multiwalled carbon nanotubes (CNTs, CVD method, purity >95%, diameter 30–60 nm, length 0.5–15 μm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). All other chemicals were analytical grade, and double distilled water was used throughout. 0.1 mol L⁻¹ pH 7.4 phosphate buffer solution (PBS) was prepared by mixing the stock solution of Na₂HPO₄ and NaH₂PO₄, and then adjusting the pH with 0.1 mol L⁻¹ NaOH and H₃PO₄. The 15-mer DNA thrombin-binding aptamer (TBA) and its complementary strand (probe) were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). The sequences used are as follows:

TBA: 5'-NH₂-(CH₂)₆-GGTTGGTGTGGTTGG-3'

Probe: 5'-NH₂-(CH₂)₆-TTTTTCCAACCACCAACC-3' (The italicized part is the complementary strand of the thrombin-binding aptamer.)

The electrochemical measurements were recorded with CHI 660D electrochemical workstation (CH Instruments Co., China). The ECL emission measurements were conducted on a model MPI-M electrochemiluminescence analyzer (Xi'an

Remax Electronic Science & Technology Co. Ltd., China) at room temperature, and the voltage of the photomultiplier tube (PMT) was set at -800 V during the detection. All experiments were carried out with a conventional three-electrode system, including a modified GCE as the working electrode, a platinum wire as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode, respectively. A commercial 5 mL cylindroid glass cell was used as the ECL cell and was placed directly in the front of the PMT. High resolution transmission electron microscopy (HRTEM) was obtained by a JEOL-2100 transmission electron microscope (JEOL, Japan). The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 spectrophotometer (Shimadzu, Japan). The fluorescence measurements were carried out with RF-5301PC FL spectrophotometer (Shimadzu, Japan). The ECL spectra were obtained by collecting the ECL signals at 0.60 and 1.10 V during cyclic potential sweep with 9 pieces of filter at 400, 425, 450, 475, 500, 525, 550, 575, 600, and 625 nm, respectively.

Synthesis of CdSe@ZnS QDs. First, thioglycolic acid (TGA)-capped CdSe QD was synthesized following literature procedures.²⁹ The mole ratio of Cd:Se:TGA was 1:2.5:0.5. Then, 40 mL of aqueous solution containing mercaptopropionic acid (MPA) and ZnCl₂ was added into the core QDs solution to obtain MPA-CdSe@ZnS QDs following the method described in the literature.³⁰ The core-shell QDs were precipitated out with ethanol, centrifuged, dried under vacuum, and kept in a refrigerator at 4 °C for further use.

Fabrication of ECL Aptasensor. A glassy carbon electrode was mechanically polished with alumina pastes of 0.3 μm, and then cleaned thoroughly in an ultrasonic cleaner with alcohol and water sequentially. After it was dried with blowing N₂, 10 μL of CdSe@ZnS QDs was spread on the working electrode and dried at the room temperature to fabricate CdSe@ZnS QDs modified GCE (CdSe@ZnS/GCE).

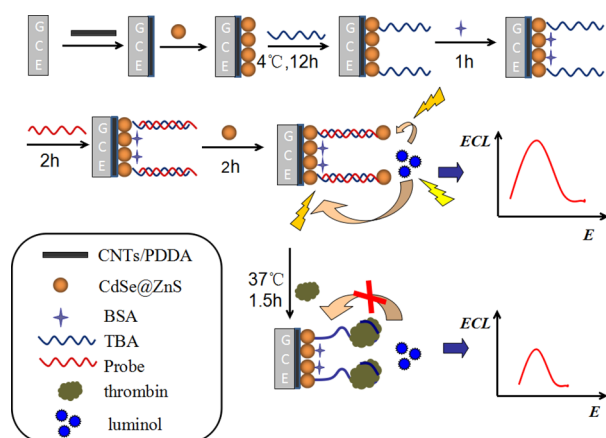
The ECL sensor was fabricated as follows: 10 μL of PDDA functionalized CNTs was spread on a cleaned bare GCE to firmly immobilize QDs.³¹ After the film was dried in the air, 10 μL of CdSe@ZnS QDs was spread on the GCE, and dried at the room temperature. The modified GCE was immersed into the 0.1 mol L⁻¹ PBS (pH 7.4) containing 10 mM EDC and 5 mM NHS (EDC/NHS) for 30 min to active the carboxylic groups on the surface of QDs. After the surface was washed with PBS, 10 μL of TBA (5 μM) was dropped onto the modified electrode surface, and the material was incubated at 4 °C overnight to connect with QDs through the reaction between -NH₂ and -COOH groups. After the electrode was washed with PBS, the modified electrode was immersed into 1% BSA solution for 1 h to block the nonspecific binding sites on the surface. Then, 10 μL of probe was dropped onto the electrode to hybridize with TBA at 4 °C for 2 h followed by washing with PBS. Finally, 10 μL of QDs was activated with EDC/NHS for 30 min and dropped to the electrode to incubate at 4 °C for 2 h.

For the detection of thrombin, the sensor was incubated with variable concentrations of thrombin for 1.5 h at 37 °C water bath. Before measurement, it was washed with PBS and ultrapure water for several times. The whole preparation process is outlined in Scheme 1.

■ RESULTS AND DISCUSSION

Electrochemistry of Luminol at a CdSe@ZnS/GCE. A high resolution transmission electron microscopy image displayed the crystalline feature of CdSe@ZnS with average

Scheme 1. Schematic Representation of the Modification of the GCE and the Detection of Thrombin



size of about 4.5 nm (Figure S1, Supporting Information). The modified process of QDs on a glassy carbon electrode was monitored by electrochemical impedance spectroscopy (EIS) (Figure S2, Supporting Information). The diameter of EIS increased with the increase of QDs modified on the electrode due to the electrostatic repulse force between the negative charged QDs and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ probe, which indicated that QDs were successfully modified onto the bare GCE.

Cyclic voltammetry (CV) measurements of a bare GCE and a $\text{CdSe@ZnS}/\text{GCE}$ were obtained in neutral PBS with the absence and presence of luminol, respectively, as shown in Figure 1.

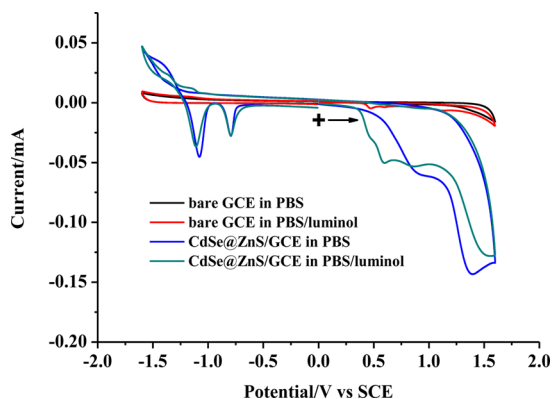


Figure 1. Cyclic voltammograms of a bare GCE, and a $\text{CdSe@ZnS}/\text{GCE}$ in PBS under N_2 atmosphere in presence and absence of luminol. Luminol, $1.0 \times 10^{-4} \text{ mol L}^{-1}$; PBS, 0.1 mol L^{-1} ; pH, 7.4; scan rate, 100 mV s^{-1} .

No CV peaks can be observed at the bare GCE in PBS. After luminol was added into PBS, one oxidation peak was observed at the bare GCE with the onset potential of 0.40 V, which could be assigned to the oxidation of luminol.¹⁵ The current increased apparently after the CdSe@ZnS QDs were modified on the bare GCE, which was also reported.³² Two sharp reduction peaks were observed at -0.80 and -1.10 V, respectively. The former could be assigned to the reduction of CdSe core while the later could be assigned to the reduction of ZnS shell, because only the former peak was observed at a CdSe QDs modified GCE. In the positive potential range, one broad CV peak located around 0.90 V with and without luminol could be

assigned to the oxidation of QDs.³³ In the presence of luminol, one strong oxidation peak was observed with the onset potential of 0.38 V at the $\text{CdSe@ZnS}/\text{GCE}$, which could be assigned to the oxidation of luminol. Compared with the counterpart of the bare GCE, the oxidation potential negatively shifted while the peak current increased nearly 1 order of magnitude, indicating the catalytic effect of CdSe@ZnS QDs on the oxidation of luminol.

ECL-RET between Luminol and QDs. It is well-known that anodic luminol ECL is extremely weak in neutral conditions without coreactants, and metal nanoparticles could enhance luminol ECL due to their catalytic effect.¹⁵ Because CdSe@ZnS QDs exhibited excellent catalytic effects on luminol oxidation, it is reasonable to deduce that a strong luminol ECL signal might be obtained at the $\text{CdSe@ZnS}/\text{GCE}$ in the absence of coreactants. Therefore, the ECL behavior of luminol was comparatively studied at the bare GCE and the $\text{CdSe@ZnS}/\text{GCE}$, as shown in Figure 2. It could be observed that one

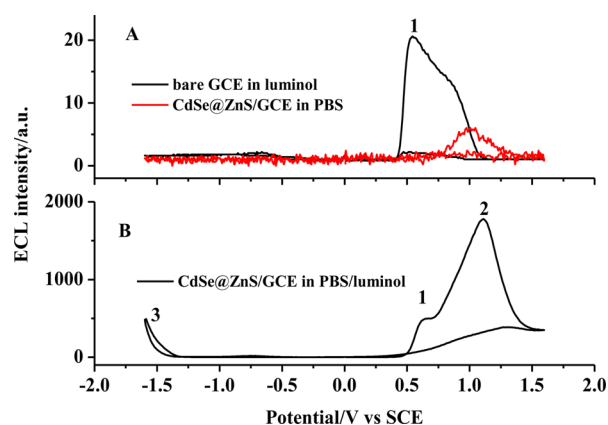


Figure 2. Electrogenerated chemiluminescence of luminol at a bare GCE and a $\text{CdSe@ZnS}/\text{GCE}$ in neutral condition. Luminol, $1.0 \times 10^{-4} \text{ mol L}^{-1}$; PBS, 0.1 mol L^{-1} ; pH, 7.4; scan rate, 100 mV s^{-1} . If not mentioned additionally, all high voltages applied to the PMT were maintained at -800 V .

weak anodic ECL (ECL-1) with the peak potential of 0.56 V was obtained at the bare GCE in PBS containing luminol, as shown in Figure 2A, which could be assigned to the anodic ECL of luminol in the presence of dissolved oxygen.¹⁵ Extremely weak anodic ECL located at 1.0 V could be observed at the $\text{CdSe@ZnS}/\text{GCE}$ in PBS without luminol, which should be assigned to the reaction between oxidized QDs and $\text{O}_2^{-\bullet}$.¹¹ In the presence of luminol, one strong anodic ECL peak (ECL-2) at the $\text{CdSe@ZnS}/\text{GCE}$ was observed at 1.10 V with a shoulder ECL peak (ECL-1) at 0.60 V, as shown in Figure 2B. The onset potential of ECL-1 was the same as that obtained at the bare GCE, revealing that ECL-1 was result from the oxidation of luminol. The intensity of ECL-1 increased more than 1 order of magnitude compared with the bare GCE, indicating the catalytic effect of QDs could significantly enhance luminol ECL signal. Extremely strong ECL-2 was probable result from the energy transfer between luminol and QDs, because ECL-2 cannot found at the bare GCE in luminol solution and is extremely weak at the $\text{CdSe@ZnS}/\text{GCE}$ in PBS. ECL-3 should be related to the reaction between luminol and electrogenerated hydrogen atoms rather than from QDs because there has no coreactant in the system, and the potential of ECL-3 located in the potential range of hydrogen

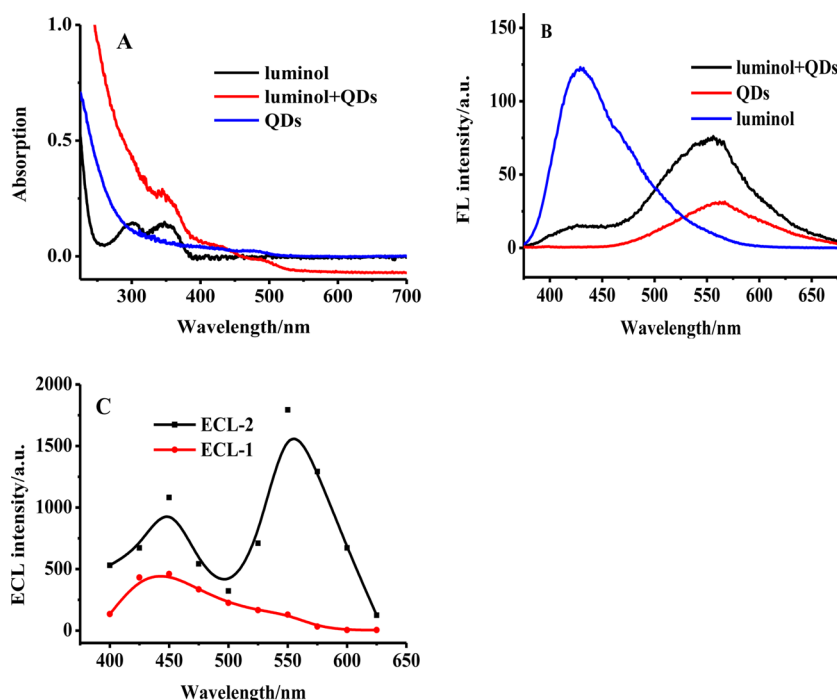


Figure 3. (A) UV-vis absorption spectra of luminol, QDs, and luminol+QDs. (B) Fluorescence spectra of luminol, QDs and luminol+QDs. (C) ECL spectra of ECL-1 and ECL-2.

evolution.¹⁵ The high intensity of anodic ECL in neutral condition in the absence of coreactant makes it suitable for the fabrication of biosensor.

Previous work reported that ECL-RET can occur between luminol- H_2O_2 ECL system and CdSe@ZnS QDs in alkaline solution.²⁵ However, two factors limit their further application in bioassay. One is that high pH value is often necessary for the generation of strong ECL. The other is that H_2O_2 is unstable and can influence the reproducibility of ECL signal. Up to date, we have not found the ECL-RET research between luminol ECL without coreactant and QDs in neutral condition. In the present study, strong anodic luminol ECL (ECL-1) at the CdSe@ZnS/GCE in neutral condition without coreactant can provide a good donor for ECL-RET. Meanwhile, extremely weak anodic ECL of QDs suggested that QDs can act as an acceptor in ECL-RET process. The extremely strong ECL emission (ECL-2) at the CdSe@ZnS/GCE in the presence of luminol indicated the possibility of ECL-RET between luminol and QDs.

During the investigation of luminol ECL, we found some metal ions, such as Ag^+ , Zn^{2+} , Co^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , Fe^{2+} , and some reductive species including cysteine, ascorbic acid, and citric acid, could seriously influence ECL signal at the bare GCE. The influence of these species on luminol ECL at the CdSe/ZnS/GCE was investigated, and the results suggested that these substances exhibited no apparent influence on ECL signal. At the bare GCE, the above substances could participate in ECL reactions of luminol and influence its ECL signal. When luminol ECL was studied at the CdSe@ZnS/GCE, luminol could transfer its energy to CdSe@ZnS QDs to generate new ECL emission, which can sufficiently avoid the influence from the above substances.

To further make sure that energy transfer could occur between luminol and QDs, UV-vis, fluorescence (FL), and ECL spectra were recorded, as shown in Figure 3. In Figure 3A, two absorption peaks of luminol were observed in the range of

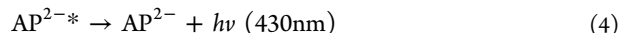
300 to 400 nm while the maximum absorption peak of QDs was at ~ 470 nm. After luminol was mixed with QDs, the absorption band of the mixture differed apparently from that of luminol or QDs, indicating the interaction between QDs and luminol. Figure 3B depicts the fluorescence of luminol, QDs, and their mixture. The maximum emission of luminol located at 425 nm while the maximum emission of QDs located at 560 nm. The absorption peak of QDs overlaps with the emission peak of luminol, indicating that energy transfer can take place.³⁴ When luminol was mixed with QDs, the FL intensity at 425 nm decreased greatly while the FL intensity at 560 nm increased, showing the energy transfer between luminol and QDs.

ECL spectra of two anodic ECL peaks (ECL-1 and ECL-2) were recorded, as shown in Figure 3C. The maximum emission of ECL-1 located at 450 nm, corresponding to the emission of 3-aminophthalate, confirmed that the ECL-1 signal was initiated by luminol reactions. In the case of ECL-2 signal, two emission peaks at 450 and 550 nm were observed respectively, indicating the existence of two luminophors from luminol and QDs, respectively. Therefore, the excited state of luminol could transfer its energy to QDs and generate strong light emission due to without other coreactants.

Mechanism for the Enhanced Anodic ECL. The mechanism of luminol ECL was presented in Richter's review.³⁵ In general, luminol (LH^-) is electrochemically oxidized to produce the diazo compound AP^{2-} . The compound is converted to its excited state, AP^{2-*} , in the presence of O_2 . AP^{2-*} goes back to the ground state, AP^{2-} , and emits light with a maximum emission of ~ 430 nm.

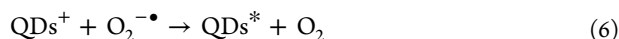
It was described previously that dissolved oxygen played an importance role during the ECL emission procedure.¹⁵ To confirm this fact, the solution was bubbled with highly pure N_2 for 30 min before measurement to remove the dissolved O_2 . The results showed that the intensities of ECL-1 and ECL-2 were extremely weak in N_2 saturated solution, indicating that the ECL emission is dependent on the dissolved O_2 . Combine

the results of the present experiments and the reference,¹⁵ the mechanism for ECL-1 can be described as follows

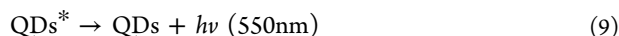


QDs exhibited a remarkable electrocatalytic effect to eq 1 and resulted in strong ECL emission in neutral conditions.

It was reported that the dissolved oxygen participated in the ECL reactions of QDs in the species of $\text{O}_2^{\bullet-}$, which could inject electrons into the hole injected QDs and generate anodic ECL.¹¹ Therefore, extremely weak ECL observed at the CdSe@ZnS/GCE in PBS should be generated as follows



Because UV-vis absorption of QDs overlaps with the emission range of luminol, the excited QDs could be produced by the energy transfer from the excited state of luminol.²⁰ The mechanism of ECL-2 should be as follows



QDs can act not only as a catalyst to enhance luminol ECL but also as an ECL energy acceptor to generate anodic ECL, which is even stronger than that of luminol and gives a new route for the bioapplication of anodic ECL in neutral conditions.

Fabrication of Biosensor. Nucleic acid aptamers are single-stranded DNA or RNA oligonucleotides with the high affinity and specificity toward their targets. Various aptamer-based biosensors have been developed using the specific identification of aptamer toward their target.³⁶ Scheme 1 depicts the protocol of the proposed ECL aptasensor based on the assembly strategy of oligonucleotide and QDs. First, multiwalled carbon nanotubes (CNTs) were cast on a bare GCE to facilitate the modification of QDs. Then, CdSe@ZnS QDs were immobilized on the CNTs modified electrode to bring enhanced ECL signal. TBA was immobilized on QDs film through the interaction between $-\text{COOH}$ groups of QDs and $-\text{NH}_2$ groups of TBA. The probe was assembled on the TBA modified electrode via the hybridization following by the immobilization of another layer of QDs, which could further increase the ECL signal.

As an effective tool for characterizing the interface properties, electrochemical impedance spectroscopy (EIS) was used to monitor the biosensor fabrication process. Figure 4 displays the Nyquist diagrams of each electrode involved during every construction step. The impedance spectra consisted of a semicircle at high AC modulation frequency and a line at low AC modulation frequency, demonstrating that the electrode process was controlled by electron transfer at high frequency and by diffusion at low frequency. The charge transfer resistance (R_{ct}), which equals the diameter of semicircle, reflects the restricted diffusion of the redox probe through the multilayer system. When the CNTs were modified on the bare

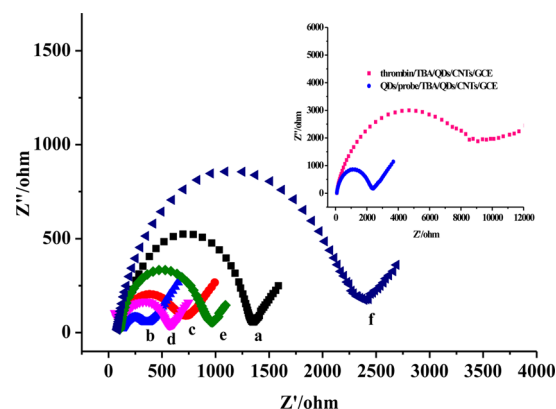


Figure 4. Nyquist diagram of electrochemical impedance spectra recorded from 0.01 to 10^5 Hz for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (10 mM, 1:1) in 10 mM pH 7.4 PBS containing 0.10 mol L^{-1} KCl at (a) bare GCE, (b) CNTs/GCE, (c) QDs/CNTs/GCE, (d) TBA/QDs/CNTs/GCE, (e) probe/TBA/QDs/CNTs/GCE, (f) QDs/probe/TBA/QDs/CNTs/GCE. The inset is the Nyquist diagrams of before and after it was incubated in thrombin.

GCE, R_{ct} decreased greatly due to the good conductivity of CNTs. R_{ct} increased when CdSe@ZnS QDs were modified onto the CNTs film, indicating that the QDs film hindered the access of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to the electrode surface. The reason is that the negatively charged QDs can repulse $[\text{Fe}(\text{CN})_6]^{3-/4-}$. When TBA was connected on the electrode, R_{ct} decreased again because the positively charged amino group of DNA is beneficial to the access of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to the electrode surface. When the probe was connected, R_{ct} increased gradually due to the insulating property of DNA. When QDs were modified on the probe DNA, R_{ct} increased greatly for the existence of electrostatic repulse force between QDs and $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Thrombin is a kind of protein, which could perturb the interfacial charge transfer.³⁷ Therefore, after the sensor was incubated in thrombin, the largest R_{ct} was obtained due to the insulating effect of protein, as shown in the inset of Figure 4. The results suggested a successful stepwise fabrication of the proposed biosensor.

The ECL responses of the modified electrodes during different stages were examined as shown in Figure 5. The ECL signal obtained at the CNTs/GCE was slightly stronger than that of the bare GCE, which is due to the good conductivity of

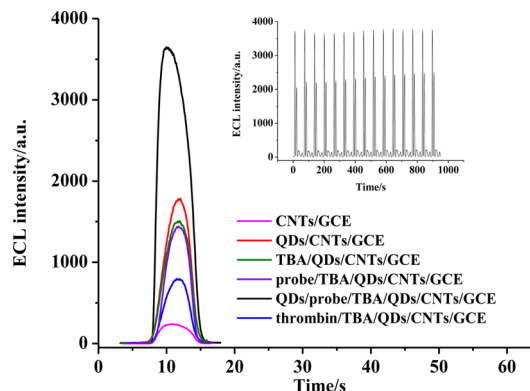


Figure 5. ECL profiles of different modified electrodes at 1.10 V. The inset is ECL emission from the biosensor under continuous cyclic voltammetry for 15 cycles. Luminol, 1.0×10^{-4} mol L^{-1} ; PBS, 0.1 mol L^{-1} ; pH, 7.4; scan rate, 100 mV s^{-1} .

CNTs. When the first layer of QDs was modified on the CNTs film, the enhanced ECL was obtained as described previously. When the TBA and the probe DNA was modified successively on the QDs/CNTs/GCE, the intensity of ECL decreased slightly due to the poor conductivity of DNA. When the second layer of QDs was modified on the probe DNA, the ECL signal was further increased due to the double enhancing effect of QDs as well as the ECL-RET between luminol and QDs. The ECL signal of the biosensor at 1.10 V remained at an almost constant value during 15 consecutive cyclic potential scanning as shown in the inset of Figure 5. The stable ECL signals can facilitate the design of ECL sensor. When thrombin was hybridized with its aptamer to release the probe DNA, the ECL-RET between luminol and the QDs was hindered, resulting in the reduced ECL intensity instantly. The inhibiting effect of thrombin on ECL signal could be used in the determination of thrombin indirectly.

Analytical Performance of the Biosensor. After the as-prepared working electrode was incubated with thrombin solution at different concentrations, the ECL intensities from the biosensor were tested. The quantitative behavior of the aptasensor for thrombin was determined by measuring the dependence of the ΔECL upon the concentration of thrombin. In Figure 6, the ECL intensity decreased gradually with an

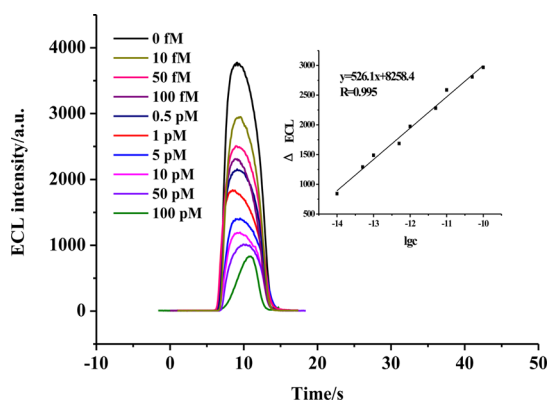


Figure 6. ECL signals of the biosensor incubated with different concentrations of thrombin (from 10 fM to 100 pM). The inset is the corresponding logarithmic calibration curve; ΔECL stands for the inhibited ECL signal of the modified electrode after the immobilization of different concentrations of thrombin. Luminol, 1.0×10^{-4} mol L^{-1} ; PBS, 0.1 mol L^{-1} ; pH, 7.4; scan rate, 100 mV s^{-1} .

increase of the concentration of thrombin. The calibration plot showed a good linear relationship between ΔECL and the logarithm of thrombin concentration in the range from 10 fM to 100 pM with the correlation coefficient of 0.995, as shown in the inset of Figure 6. Due to the synergy effects as stated above, the detection limit of the biosensor was found at levels down to 1.4 fM ($S/N = 3$). The comparison of the various ECL biosensors is listed in Table S1 (Supporting Information). It can be found that the present biosensor is superior to other reported ECL biosensors.

Specificity of the Biosensor. The specificity of the proposed strategy for thrombin was evaluated to illustrate its practicability, as shown in Figure 7. In the experiment, BSA, α -amylase, lysozyme, and the mixture of thrombin with them were employed to undergo the same process at the same concentration of thrombin. It could be observed that only the thrombin sample and the mixture of thrombin gave significant

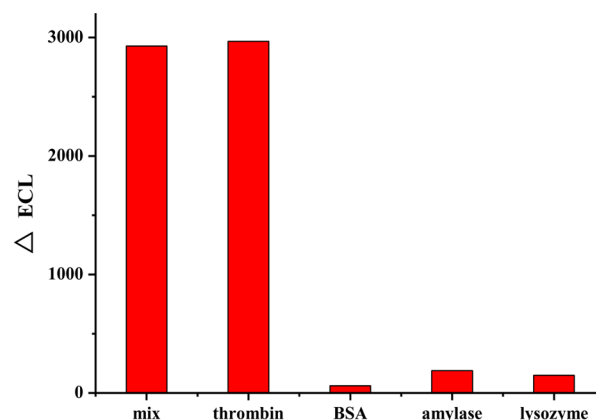


Figure 7. Selectivity of the proposed biosensor to thrombin by comparing it to the interfering proteins. BSA, α -amylase, lysozyme, and thrombin at 100 pM, respectively. Luminol, 1.0×10^{-4} mol L^{-1} ; PBS, 0.1 mol L^{-1} ; pH, 7.4; scan rate, 100 mV s^{-1} .

decreased ECL intensity. BSA, α -amylase, and lysozyme had almost negligible interference for thrombin detection. These results indicate good selectivity and high specificity of the proposed biosensor to thrombin.

Applications of the Biosensor. The potential applicability of the ECL biosensor in the detection of thrombin in real sample was investigated by determining thrombin in human blood serum samples (the samples were obtained from Nanjing Gulou Hospital). Because no thrombin was found in the serum samples, the serum 1:5 diluted with TE buffer (pH 7.4) was spiked with thrombin at different concentrations. The results in Table S2 in the Supporting Information show the acceptable relative standard deviation (RSD) and good recoveries, implying that the present biosensor has promising analytical applications in real biological samples.

CONCLUSIONS

In summary, luminol ECL could be enhanced greatly on the CdSe@ZnS QDs modified electrode due to the catalytic effect of QDs on the oxidation of luminol. The ECL resonance energy transfer between the excited state of luminol and QDs generated another more intensive and stable anodic ECL signal in neutral condition. The proposed luminol-QDs ECL-RET system can operate in neutral conditions, avoid the use of coreactants, and simplify the ECL process. A label-free ECL aptasensor for the detection of thrombin was developed based on the ECL-RET and exhibited a wide dynamic range from 10 fM to 100 pM with a low detection limit of 1.4 fM. The sensitivity of the proposed aptasensor is superior to most previously reported label-free detection methods for thrombin. The strategy could be used to develop aptasensors for various targets through just simple substitutions of aptamers.

ASSOCIATED CONTENT

Supporting Information

Characterization of CdSe@ZnS quantum dots and QDs modified electrode. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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