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In Situ Generation of Self-Enhanced Luminophore by β-Lactamase Catalysis for Highly Sensitive Electrochemiluminescent Aptasensor

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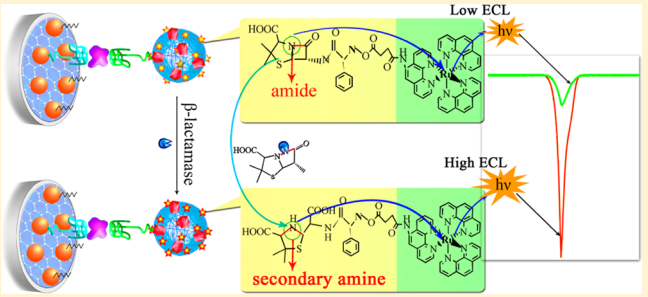
Supporting Information

ABSTRACT: This work described a new electrogenerated chemiluminescence (ECL) aptasensor for ultrasensitive detection of thrombin (TB) based on the in situ generating self-enhanced luminophore by β-lactamase catalysis for signal amplification. Briefly, a ruthenium complex (Ru−Amp), including two regions of $[Ru(phen)_2(cpaphen)]^{2+}$ and ampicillin (Amp), was synthesized as a self-enhanced ECL luminophore, which can produce an ECL signal through intramolecular interactions. Then, carbon nanotubes (CNTs) were used for immobilization of Ru−Amp via π−π stacking interactions to form the Ru−Amp@CNTs nanocomposite. Using poly(ethylenimine) (PEI) as a linkage reagent, Au nanocages (AuNCs), owing to their electronic property and large surface areas, were decorated to the CNTs to form the Ru−Amp@CNTs−PEI−AuNCs nanocomposites, which were further used to immobilize thrombin binding aptamer II (TBA II) to form a signal probe (Ru−Amp@CNTs−PEI−AuNCs−TBA II). Through "sandwich" tactics, TBA II bioconjugates, TB and TBA I were immobilized onto the gold nanoparticles modified electrode. Then, with the enzyme catalysis of β-lactamase, a novel self-enhanced ECL luminophore (Ru−AmpA) was in situ produced, which could exhibit a significant enhancement of ECL signal, due to the structure transformation of an amide bond into a secondary amine. A sandwich ECL assay for TB detection was developed with excellent sensitivity of a concentration variation from 1.0 fM to 1.0 pM and a detection limit of 0.33 fM. Therefore, the self-enhanced ECL luminophore, combining the further enhancement by in situ enzymatic reaction, is expected to have potential applications in biotechnology and clinical diagnosis.



Electrogenerated chemiluminescence (ECL), a very powerful analytical technique, is the process of producing an excited state in a photoactive molecule at the electrode surface, resulting in luminescence upon its return to the ground state.[1−3] Ruthenium(II)-based complexes, as one of the most popular ECL luminophores, have received considerable attention in clinical diagnosis and scientific research because of their excellent stability and high efficiency in aqueous phase.[4,5] In a typical Ru(II) ECL process, the electron transfer reaction occurs between electrochemically formed Ru(II) species and coreactants, such as tripropylamine (TPA),[6,7] peroxydisulfate[8] and 2-(dibutylamino)ethanol (DBAE).[9] However, the coreactants, owing to their water-solubility, have been rarely used for the application of reagentless ECL sensors. Considering all of this, the new superior ECL reagents, designed by covalently linking the two parts of ECL-active luminophore and coreactant, are expected to enhance both ECL efficiency and electron transfer performances through intramolecular interaction. Using this approach, Sun and coworkers have patented an ECL reagent of ruthenium linked to tripropylamine through an amide bond.[10,11] Kehr's group has synthesized a series of ruthenium(II) tris-bipyridyl complexes covalently linked with different amine reductants for an ECL system and studied intra- and intermolecular interactions of novel ruthenium complexes.[12,13] In our previous work, we have designed a novel ECL immunosensor based on a Ru(II) complex (Ru−PEI) for the detection of APE-1.[14] They all indicated that the ruthenium complex containing two regions of ECL-active luminophore and coreactant, named self-enhanced ECL luminophore, can generate an efficient ECL signal through intramolecular interactions.

Ampicillin (Amp), one of β-lactamase antibiotics containing the structure of amide and primary amine, could slightly enhance the ECL efficiency of ruthenium.[15] On the basis of this property, a self-enhanced ECL luminophore (Ru−Amp) was designed including two regions of $[Ru(phen)_2(cpaphen)]^{2+}$ and Amp. More importantly, when they are hydrolyzed by β-lactamase, the structure transformation of an amide bond into a secondary amine in situ occurred to produce a novel self-enhanced ECL luminophore (Ru−AmpA), which could

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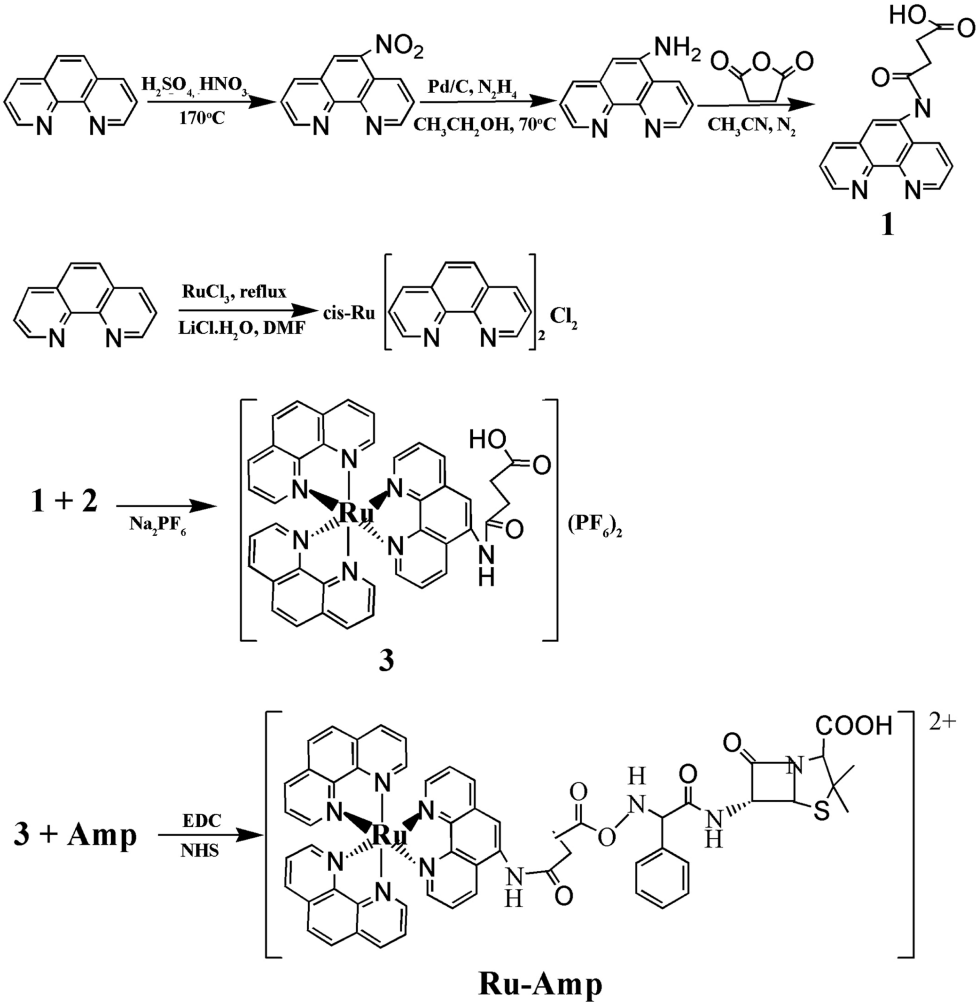
Scheme 1. Reaction Scheme for the Synthesis of Ru−Amp



significantly enhance the ECL efficiency due to the amine-to-ruthenium electron transfer through intramolecular reactions by the covalent approximation of the two reacting species. Considering all of this, Ru−Amp was synthesized and first applied to construct a highly sensitive ECL aptasensor coupling with the in situ enzymatic reaction by β-lactamase as a signal amplification strategy.

In this work, we developed an ECL aptasensor based on the self-enhanced ECL luminophore of Ru−Amp and in situ enzymatic reaction by β-lactamase for signal amplification to detect the concentration of thrombin (TB). Here, Ru−Amp wrapped on carbon nanotubes (CNTs) via $\pi-\pi$ stacking interactions to form the Ru−Amp@CNTs nanocomposites.[16] Then, Au nanocages (AuNCs) with good biocompatibility, electronic property and large surface areas,[17] were conjuncted on Ru−Amp@CNTs by the linkage reagent poly(ethylenimine) (PEI). The resultant AuNCs loaded Ru−Amp@CNTs−PEI were further employed as carriers to immobilize thrombin binding aptamer II (TBA II) to obtain TBA II bioconjugates (Ru−Amp@CNTs−PEI−AuNCs−TBA II). For constructing the aptasensor, graphene−Nafion (Gr−Nf) film was fabricated by dropping a Gr−Nf homogeneous suspension to enlarge the surface of the electrodes. Then gold nanoparticles (AuNPs) were electrodeposited on the modified electrode, which could provide a rough and stable surface that was more favorable for immobilization of thrombin binding aptamer I (TBA I). Then the TBA II bioconjugates were linked to the electrode surface via "sandwich" tactics. When β-lactamase existed, the desirable Ru−AmpA was in situ generated on the surface of electrode in high yields and exhibited high ECL efficiency, which could improve the sensitivity of the sensors. The in situ enzymatic reaction is an especially promising method because it avoids complicated labeling procedures and has the advantages of high efficiency, simplicity and low toxicity. Thus, coupling with the attractive self-enhanced ruthenium complex and the signal amplification of in situ enzymatic reaction by β-lactamase, the tactics hold promise as a great choice for sensitive and selective detection of targets studied.

EXPERIMENTAL METHODS

Reagents. Thrombin (TB, enzymatic activity of 40−300 NIH units/mg, lyophilized power), β-lactamase (1000 units/mg), ampicillin (Amp), 1-hexanethiol (HT, 96%), chloroauric acid ($HAuCl_4 \cdot 3H_2O$), Nafion (Nf), *N*-hydroxy succinimide (NHS) and *N*-(3-dimethyl aminopropyl)-*N*-ethylcarbodiimide-hydrochloride (EDC) were received from Sigma-Aldrich Co. (St. Louis, MO, USA). Poly(ethylenimine) (PEI, 50%) was obtained from Fluka (Switzerland). Graphene (Gr) and the carbon nanotubes (CNTs, 95% purity) were purchased from Nanjing Xianfeng nano Co. (Nanjing, China). Prior to use, the CNTs were pretreated with concentrated nitric acid in order to

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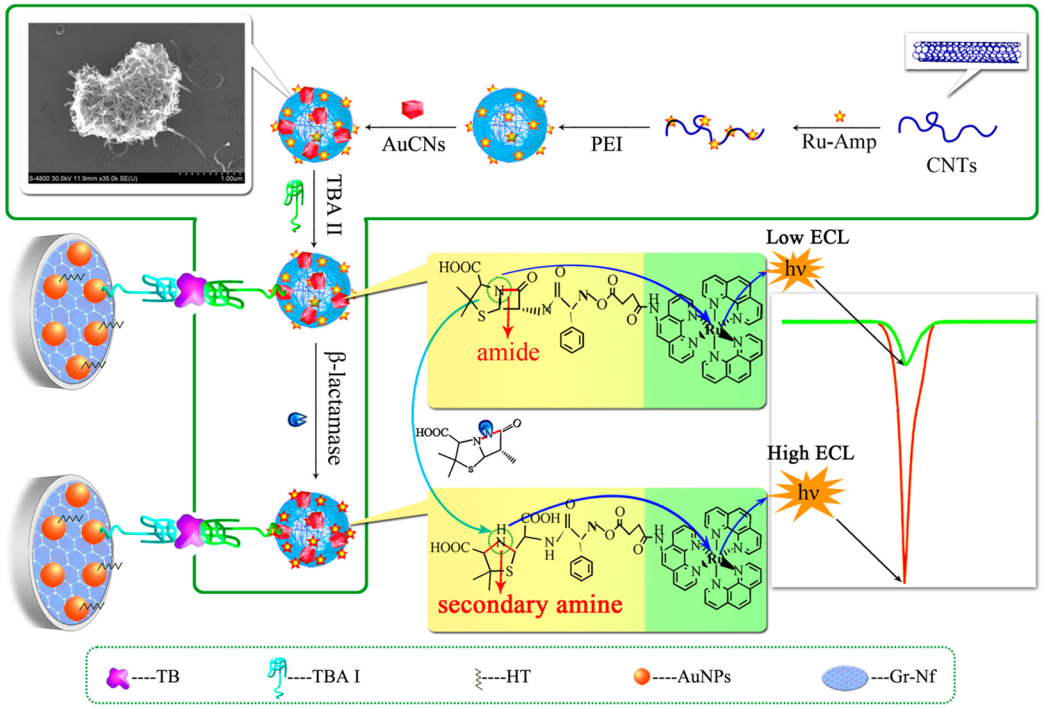
Scheme 2. Schematic Diagrams of the Aptasensor



introduce carboxylic acid groups according to the previous literature.[18] γ-Glutamyltranspeptidase (γ-GTP) antigen and alpha-L-fucosidase (AFU) antigen were bought from Biocell Company (Zhengzhou, China). Polyvinylpyrrolidone (PVP), $RuCl_3 \cdot 3H_2O$ and 1,10-phenanthroline (phen, 99%) were purchased from Chengdu Chemical Reagent Company (Chengdu, China). Thrombin binding aptamers (TBA) were received from TaKaRa (Dalian, China), and the sequences of the oligonucleotide were as follows:

TBA I: 5′-SH-$(CH_2)_6$-GGTTGGTGTGGTTGG-3′.

TBA II: 5′-NH_2-$(CH_2)_6$-AGTCCGTGGTAGGGCAG-GTTGGGGTGACT-3′.

Tris−HCl buffer, contaning 140 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$, was used to prepare the aptamer solution. Phosphate buffer solution (PBS) (pH 7.4) was prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.1 M KCl. Double-distilled water was used throughout every experiment. All other reagents were of analytical grade and used as received without further purification.

Apparatus. Cyclic voltammetry (CV) was monitored by a CHI Instruments Model 660D electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., China). The ECL emission was carried out with an MPI-A electrochemiluminescence analyzer (Xi′an Remax Electronic Science & Technology Co. Ltd., Xi′an, China) in PBS (0.1 M, pH 7.4) with the voltage of the photomultiplier tube (PMT) at 800 V in the air. A conventional three-electrode system was used with a glassy carbon electrode (GCE, φ = 4 mm) as the working electrode, a platinum wire as the counter electrode and Ag/AgCl (saturated KCl) as the reference electrode in the experiment. The morphologies of various nanomaterials were conducted by a scanning electron microscopy (SEM) system (SEM, S-4800, Hitachi) with an acceleration voltage of 10 kV. X-ray photoelectron spectroscopy (XPS) measurement was performed by a VG Scientific ESCALAB 250 spectrometer (Thermoelectricity Instruments, USA) with Al Kα X-ray (1486.6 eV) as the light source.

Preparation of Ru−Amp Complex. The synthetic procedure of the Ru−Amp complex is given in Scheme 1. 5-(3-Carboxypropionyl)amino-1,10-phenanthroline (cpaphen) (**1**) was prepared by reaction of the 1,10-phenanthrolin-5-amine with succinic anhydride. *cis*-$Ru(phen)_2Cl_2$ (**2**) was synthesized by an analogous route to that in the literature.[19] $[Ru(phen)_2(cpaphen)](PF_6)_2$ (**3**) was synthesized by a reaction of **1** with **2**. The Ru−Amp complex was synthesized by reaction of the appropriate Amp with **3** using EDC and NHS as the linkage reagents. Detailed information is shown in the Supporting Information.

Preparation of Gold Nanocages (AuNCs). AuNCs were prepared by the galvanic replacement reaction between Ag nanocubes and $HAuCl_4$ solution according to the literature[20] with a little modification. The first step: synthesis of Ag nanocubes (AgNCs). Ethylene glycol (EG) (30 mL) was placed into a three-neck flask and the temperature approached but did not exceed 160 °C. With magnetic stirring, 10 mL of PVP/EG (0.2 g) solution was added at 160 °C. Then, 400 μL of 3 mM Na_2S/EG solution was added dropwise. Lastly, 3 mL of Ag/EG (282 mM) was added dropwise. The color of the solution changed from white to brown. Then the dispersion was collected by centrifugation and washed several times by acetone. The second step: synthesis of AuNCs. The obtained AgNCs were suspended in 30 mL of double-distilled water and refluxed for 10 min. Then, 100 μL of $HAuCl_4$ (10 $mg \cdot mL^{-1}$) was added slowly and the reflux continued for 30 min. The color of the solution changed from brown to pink. Then the pink dispersion was collected by centrifugation and washed several times by double-distilled water. The resultant AuNCs were dispersed in double-distilled water and used in subsequent experiments.

Preparation of Ru−Amp@CNTs−PEI−AuNCs−TBA II (TBA II Bioconjugates). First, the pretreated CNTs (0.5 mg/

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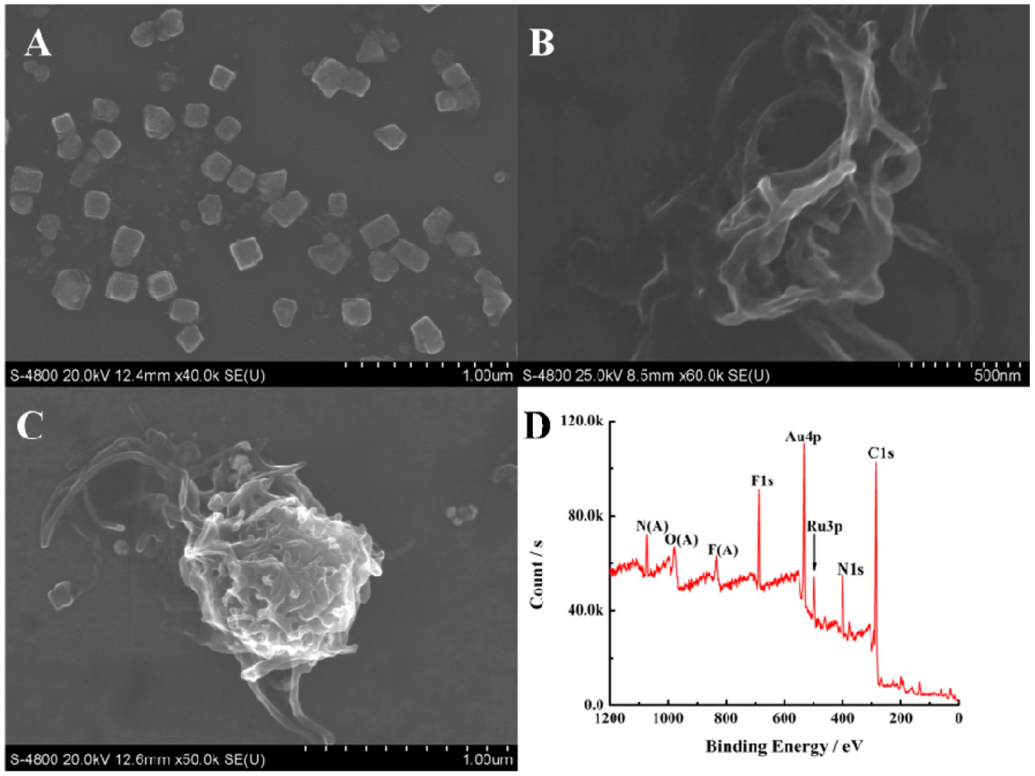

Figure 1. SEM of AuNCs (A), Ru−Amp@CNTs−PEI (B) and Ru−Amp@CNTs−PEI−AuCNs (C). XPS analysis of Ru−Amp@CNTs−PEI−AuCNs nanocomposite (D).

mL) were dispersed and sonicated in double-distilled water to obtain a homogeneous dispersion. The nanocomposite Ru−Amp@CNTs was achieved by simply stirring a solution of Ru−Amp (400 μL, 0.5 mg mL^{-1}) together with homogeneous CNTs (2 mL) at 4 °C for about 16 h through noncovalent $\pi-\pi$ stacking interactions.[21] Following this, the precipitates were collected by centrifugation and washed several times with double-distilled water. After that, Ru−Amp@CNTs were dispersed in 1 mL 1% PEI with the aid of agitation for 6 h to obtain more active $-NH_2$ groups via electrostatic interactions.[22,23] AuNCs were attached on the Ru−Amp@CNTs−PEI via Au−N junctions, which were further employed as carriers for the immobilization of TBA II (2.5 μM) to form Ru−Amp@CNTs−PEI−AuNCs−TBA II bioconjugates.

Fabrication of the Sensing Interface. Before modification, the bare electrodes were pretreated according to the previous literature.[24] Then, 1 mg of Gr was added into 2 mL of 0.5% Nf ethanol solution and sonicated to obtain a homogeneous suspension (Gr−Nf). Next 3 μL of Gr−Nf was cast on the pretreated electrode and dried in the air to obtain a Gr−Nf film. Afterward, electrodeposition was performed in the mixture solution of 1% $HAuCl_4$ to construct the AuNPs layer on the electrode surface to get AuNPs/Gr−Nf modified electrode with constant potential −0.2 V for 30 s. Subsequently, the AuNPs/Gr−Nf modified electrode was incubated into 10 μL of 2.5 μM TBA I for about 16 h at 4 °C in a moist environment. Finally, 10 μL of HT (1.0 mM) was drop-coated onto the modified electrode surface and incubated for 40 min to block the nonspecific binding sites to obtain HT/TBA I/AuNPs/Gr−Nf modified electrode.

Measurement Procedure. The proposed aptasensor was incubated in 10 μL of TB with different concentrations for 50 min at room temperature. Based on the specific binding between TB and TBA, 10 μL of prepared Ru−Amp@CNTs−PEI−AuNCs−TBA II homogeneous suspension was drop-coated onto the modified electrode surface and incubated for 50 min. Then, 6 μL of β-lactamase (100 ng/mL), prepared by Tris−HCl buffer, was drop-coated onto the modified electrode surface and incubated for 1 h. The resultant electrodes were rinsed with PBS each step. A schematic representation of the fabrication of the sandwich ECL aptasensor is shown in Scheme 2. Finally, the ECL intensity of the electrode was performed in PBS (0.1 M, pH 7.4) at room temperature in the process of detection.

RESULTS AND DISCUSSION

Characteristics of Different Nonmaterials. The as-synthesized nonmaterials were characterized by SEM (Figure 1). Figure 1A shows the SEM image of AuNCs, from which one can easily observe the hollow and square structures, which is similar to the previous description in the literature.[20] And the SEM profiles of CNTs and Ru−Amp@CNTs are shown in Figure S1 (Supporting Information), which indicated that the Ru−Amp@CNTs nanocomposites were successfully prepared. When PEI was wrapped on Ru−Amp@CNTs, the structure of Ru−Amp@CNTs−PEI was curved and bonded together due to the glutinosity of PEI (Figure 1B). Then AuNCs were decorated on the CNTs via Au−N junctions. As we can see from Figure 1C, the bright slug structure of Ru−Amp@CNTs−PEI−AuNCs was reserved. It suggested that the surface of AuNCs was coated by the Ru−Amp@CNTs−PEI nanomaterial matrix. Furthermore, the Ru−Amp@CNTs−PEI−AuNCs nanomaterial was also characterized by means of XPS (Figure 1D). The characteristic peaks for Ru 3p, C 1s, Au 4p, N 1s, F 1p, N (A) and O 1s core level regions could be obviously observed on the nanocomposite. The Ru 3p, C 1s, O 1s and F

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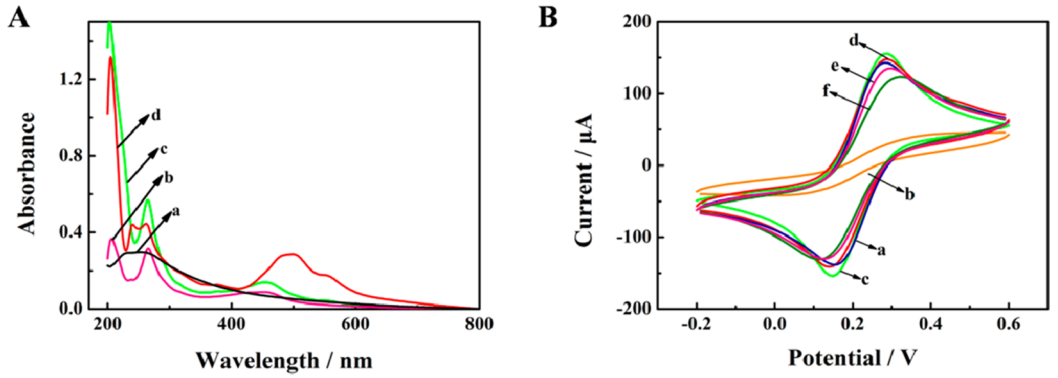

Figure 2. (A) UV–vis absorption spectra of (a) CNTs, (b) Ru–Amp@CNTs, (c) Ru–Amp@CNTs–PEI composite and (d) Ru–Amp@CNTs–PEI–AuCNs in double-distilled water. (B) Cyclic voltammograms of the electrode at different stages in 0.1 M PBS (pH 7.4) + 0.1 M KCl + 2.5 mM $[Fe(CN)_6]^{3-/4-}$ solution for (a) bare electrode, (b) Gr–Nf/GCE, (c) AuNPs/Gr–Nf/GCE, (d) TBA I/AuNPs/Gr–Nf/GCE, (e) HT/TBA I/AuNPs/Gr–Nf/GCE and (f) TB/HT/TBA I/AuNPs/Gr–Nf/GCE.

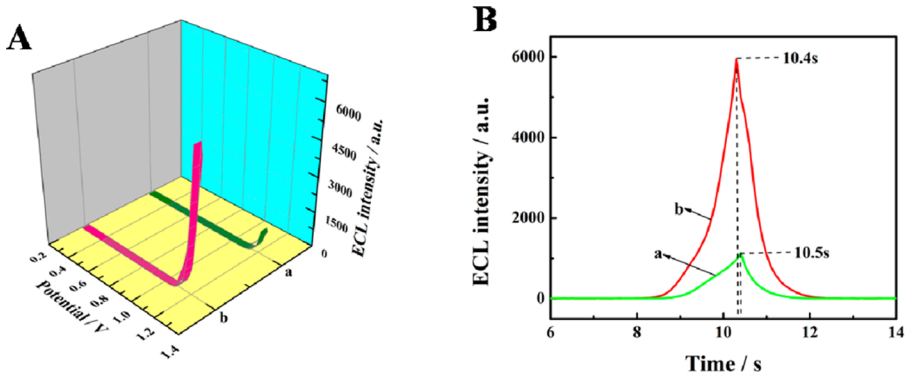

Figure 3. (A) ECL curves of the aptasensor incubated without (a) and with (b) β-lactamase. (B) ECL dynamic curve of the aptasensor incubated without (a) and with (b) β-lactamase. Applied potential: 0.2–1.25 V.

1p core level were mainly derived from the Ru–Amp@CNTs and the Au 4p doublet indicated the presence of AuCNs. The results all indicated that the nanocomposite was successfully synthesized.

UV–Vis Characterization of TBA II Tags. The absorption spectrum of the as-formed composite of Ru–Amp@CNTs–PEI–AuCNs has been measured, which was compared with the spectra of pure CNTs, Ru–Amp@CNTs and Ru–Amp@CNTs–PEI, respectively. As shown in Figure 2A, there was no obvious absorption peaks for pure CNTs (Figure 2A, curve a). However, the characteristic absorption peaks of the Ru–Amp@CNTs composite (Figure 2A, curve b) appeared at 206 and 265 nm because the complex of Ru–Amp has several benzenes and heterocyclic, which could prove the $\pi-\pi$ interactions between CNTs and Ru–Amp. After PEI was covered on the CNTs, the characteristic absorption peak at 210 nm became higher and the absorption band was red-shifted (Figure 2A, curve c), owing to the amide bond between the CNTs and PEI. The result indicated that the Ru–Amp@CNTs–PEI composite was successfully synthesized. When AuCNs were conjuncted on the Ru–Amp@CNTs–PEI composite, there a new absorption peak appeared at 517 nm (Figure 2A, curve d), which was attributed to the presence of AuCNs. These results revealed that the Ru–Amp@CNTs–PEI–AuCNs composite was successfully prepared.

Electrochemical Behaviors of the Aptasensor. Cyclic voltammetry (CV) was employed to indicate the electrochemical behaviors of the modified electrode at different stages using the redox probe of ferycyanide ($[Fe(CN)_6]^{3-/4-}$). As shown in Figure 2B, a couple of quasi-reversible, well-defined redox peaks of $[Fe(CN)_6]^{3-/4-}$ were observed on the bare electrode (Figure 2B, curve a). When the Gr–Nf was first casted onto the electrode, the redox peak current (Figure 2B, curve b) obviously decreased, as the bulky Nf perturbed the interfacial electrons transfer. After the electrodeposition of the AuNPs onto the electrode, the redox peak current (Figure 2B, curve c) obviously increased because the good conductivity of the AuNPs monolayer. When the TBA I was drop-coated onto the modified electrode, a decrease in the peak current was noted (Figure 2B, curve d). The reason may be that the TBA 1 can hinder the electron transfer capability of the electrode. After the electrode was blocked with HT, the redox peak current was decreased continuatively (Figure 2B, curves e). Finally, the modified electrode was incubated with TB and the peak current of curve f decreased further, because the captured TB could hinder the electron transfer.

Effect of β-Lactamase on the Response of the Aptasensor. To evaluate the ECL efficiency of in situ enzymatic reaction by β-lactamase, we conducted the contrast experiment to compare the ECL responses of the aptasensor incubated without and with β-lactamase. As illustrated in Figure 3A, when the potential reached higher than 1.13 V, an obvious ECL emission was observed with the peak intensity of 1129 a.u. (Figure 3A, curve a), indicating that the ECL was from Ru–Amp light emission. After the incubation with β-lactamase, an obvious ECL emission of Ru–AmpA was observed from the potential of 1.07 V with the peak intensity of 6102 au (Figure 3A, curve b). The results indicated that the emission process of

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Ru−AmpA was easier and more efficient than that of Ru−Amp. Moreover, when β-lactamase was present, a faster ECL reaction rate (Figure 3B, curve b) was achieved compared with that without β-lactamase (Figure 3B, curve a). Thus, the comparison results adequately indicate that the in situ enzymatic reaction by β-lactamase could enhance the ECL efficiency of the proposed aptasensor for the ultrasensitive detection of TB.

The mechanism of the ECL reaction of the self-enhanced ECL luminophore of Ru−Amp or Ru−AmpA is thought to involve a series of steps including electrode oxidation through intramolecular electron transfer between the L (L on behalf of Amp without β-lactamase or AmpA with β-lactamase) and Ru(II). We propose the probable sensing ECL mechanism of the self-enhanced luminophore as the following:

$$Ru(II)-L - 2e \rightarrow Ru(III)-L^{\bullet +}$$

$$Ru(III)-L^{\bullet +} \rightarrow Ru(III)L^{\bullet} + H^{+}$$

$$Ru(III)-L^{\bullet} \rightarrow Ru(II)^{*}-L$$

$$Ru(II)^{*}-L \rightarrow Ru(II)-L + h\nu$$

Optimization of the β-Lactamase Concentration. The concentration of β-lactamase has an important role on the analytical performance of the proposed ECL aptasensor. As shown in Figure 4, the ECL intensity was enhanced with

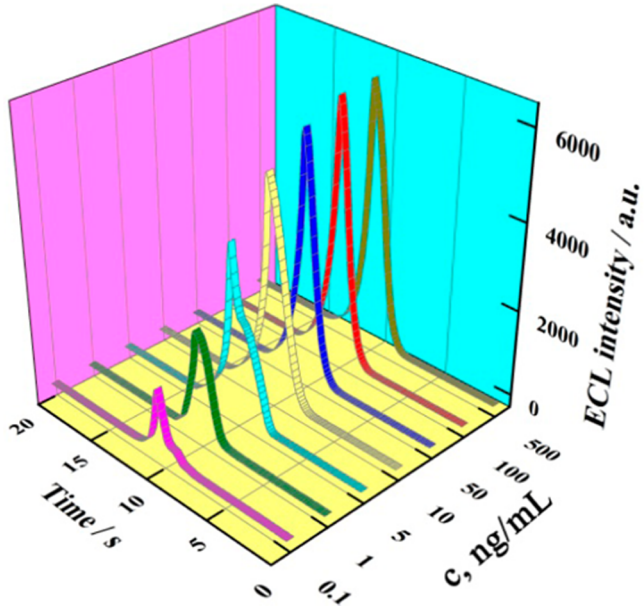

Figure 4. ECL effects of aptasensor with the different concentrations of β-lactamase. Applied potential: 0.2−1.25 V.

increases to the concentration of β-lactamase. When the concentration of β-lactamase reached 100 ng/mL, the ECL intensity increased slightly and then reached a constant value. Thus, 100 ng/mL was chosen as the optimal concentration of β-lactamase in this experiment.

Detection of TB with the Aptasensor. The relationship between ECL signals and the concentrations of TB is shown in Figure 5. As expected, the ECL intensity increased when the concentration of TB was increased. (Figure 5, curves a−h). Thus, TB could be quantitatively measured over a concentration range from 1 fM to 1 pM, with an achieved detection limit of 0.33 fM and the signal-to-noise ratio was 3. The

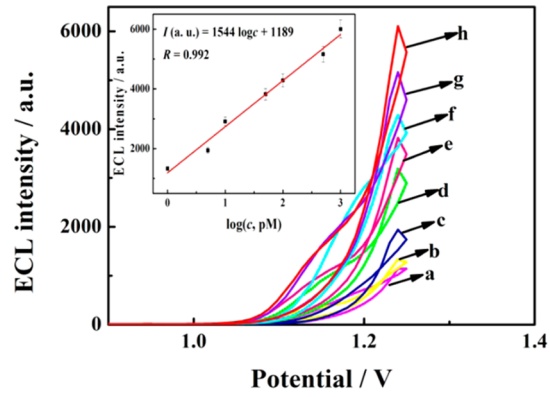

Figure 5. ECL-potential curves of the aptasensor with different concentrations of TB in 2 mL of 0.1 M PBS (pH 7.4). TB concentration: (a) 0 fM, (b) 1 fM, (c) 5 fM, (d) 10 fM, (e) 50fM, (f) 100fM, (g) 500fM and (h) 1 pM. The insert is a calibration curve for TB determination.

regression equation was I (a.u.) = 1544 log c + 1189 with a correlation coefficient of 0.992. This suggests that this strategy is highly sensitive and has great potential for early detection of TB. Additionally, we also made a comparative study between proposed aptasensor and previous reported aptasensor (Table 1). We can see that the present work for TB detection shows a low detection limit over other approaches.

Stability and Repeatability of the Aptasensor. Stability is one of the important factors to measure the performance of the aprasensor. It was evaluated by employing one aptasensor for consecutive cyclic potential scans. As we can see from Figure 6A, under continuous cyclic potential scans for 13 cycles, the relative standard deviation (RSD) of ECL was 0.93%. The result indicated that the proposed aptasensor had excellent stability. The repeatability of the aptasensor was evaluated by using five proposed aptasensors incubated with 1 pM TB. All electrodes exhibited close ECL responses and relative standard deviations of 1.24%, which was acceptable for detection of TB.

Selectivity of the Aptasensor. To investigate the selectivity and specificity of the present aptasensor, the aptasensor was incubated with different interfering substances such as γ-GTP and AFU. As shown in Figure 6B, the control experiments were performed by using γ-GTP (100 pg/mL) and AFU (100 pg/mL) to replace TB (1 pM), respectively. We can see that the ECL signal of AFU and γ-GTP did not exhibit any obvious increase compared with the blank. The aptasensor was also incubated with 1 pM TB containing 100 pg/mL γ-GTP and 100 pg/mL AFU. When the ECL response of the mixture was compared with that obtained from the 1 pM TB only, no remarkable difference was found. They all indicated that the ECL intensity in the presence of TB was much stronger than those of the others, which meant that γ-GTP and AFU had no obvious influence on the response to TB, and indicated that the aptasensor displayed good selectivity and specificity for the determination of TB.

CONCLUSION

In the present study, a sensitive ECL aptasensor based on the self-enhanced ECL luminophore and the in situ enzymatic reaction for the signal amplification was developed for TB detection. The self-enhanced ECL luminophore could overcome the shortage of high concentration of coreactant needed in the ECL system and enhance both the ECL efficiency and

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Table 1. Comparison of the TB Detection with Some Different ECL Emitters

method	label	linear range	LOD	ref
fluoresce	MCMP/FAM-ssDNA	0.25 nM−100 nM	0.25 nM	25
fluoresce	FAM-labeled TA	1 nM−100 nM	1 nM	26
SERS	AuNR−AuNP	1.74 nM−58 nM	1.74 nM	27
chemiluminescence	luminol−H_2O_2−HRP	4 fM−400 pM	2 fM	28
ECL	Fe3O4@CdSe/TPA	1 pM−5 nM	0.12 pM	29
ECL	Ru@CNTs−AuCNs	1 fM−1 pM	0.33 fM	present work

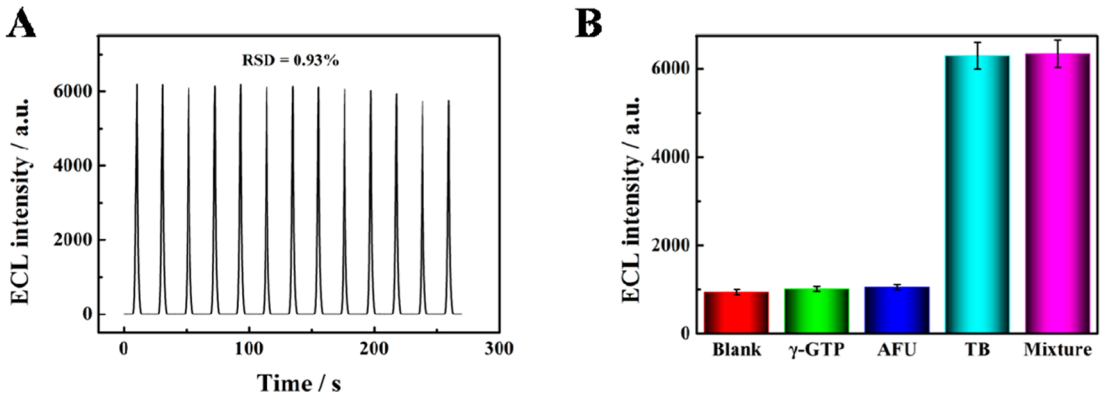

Figure 6. (A) Stability of the proposed ECL aptasensor incubated with 1 pM TB under consecutive cyclic potential scans for 13 cycles. (B) Selectivity of the proposed ECL aptasensors with different targets: blank, γ-GTP (100 pg/mL), AFU (100 pg/mL) and TB (1 pM), a mixture (1 pM TB + 100 pg/mL γ-GTP + 100 pg/mL AFU). (Error bars: SD, n = 3).

electron transfer performances through intermolecular interactions. Moreover, the ECL signal of the Ru−Amp here could be further enhanced by in situ enzymatic reaction with the aid of β-lactamase, which could effectively improve the sensitivity of the ECL aptasensor. On the basis of their excellent performance, this aptasensor achieved a detection limit of 0.33 fM and exhibits excellent selectivity. The self-enhanced ECL luminophore, coupled with the further enhancement by in situ enzymatic reaction, is expected to have potential applications in biotechnology and clinical diagnosis.

■ ASSOCIATED CONTENT

Supporting Information

Details of the preparation of Ru−Amp complex and the SEM profiles of CNTs and Ru−Amp@CNTs. 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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