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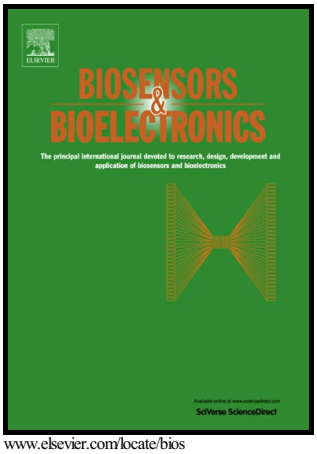

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A ratiometric electrochemiluminescent biosensor for Con A detecting based on competition of dissolved oxygen

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Abstract

A novel ratiometric electrochemiluminescent (ECL) biosensor was designed for the detection of concanavalin A (Con A) based on two ECL emitters competing for the dissolved oxygen (O_2). In this strategy, CdTe quantum dots (QDs) were used as the cathodic emitter and N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was used as the anodic emitter. With the presence of dissolved O_2 utilized as co-reactant, CdTe QDs showed an ECL emission at -1.7 V, and ABEI showed an emission at +0.6 V. Phenoxy-derivatized dextran (Dexp), as a recognition element, was immobilized by graphene oxide functionalized CdTe QDs (G-CdTe QDs) to recognize Con A, and further combined with Dexp, gold and platinum nanoparticles decorated ABEI

(Dexp-Au-Pt-ABEI) to form a sandwich structure. With the increasing concentration of analyte Con A, the ECL signal of cathodic CdTe QDs decreased and the anodic response of ABEI increased, thus achieving a ratiometry detection of Con A with a wide linear range from 1.0×10^{-4} ng/mL to 10 ng/mL. The detection limit was low to 3.0×10^{-5} ng/mL. This proposed ratiometric ECL biosensor not only conquered the false errors caused by external interferences, but excluded the disability caused by exogenous co-reactant via the application of common dissolved O_2. It also exhibited an excellent stability, selectivity and reproducibility.

Keywords: Con A; Electrochemiluminescent; Biosensor; Ratiometric; ABEI; CdTe QDs

1. Introduction

Electrochemiluminescence (ECL), which also called electrogenerated chemiluminescence, is a kind of luminescence merged with electrochemistry (Zhang et al., 2018). It combines the advantages of both chemiluminescence and spectroscopy, and presents outstanding advantages over other methods due to its versatility, simplified device, good controllability for time and space (Li et al., 2017; Dai et al., 2015; Zhang et al., 2017). ECL is usually observed using along with co-reactant that can remarkably enhance the ECL intensity of the ECL materials (Richter., 2004; Miao., 2008; Serena et al., 2017). As an increasing popular analytical technique, ECL

has been proved to be effective in analytical and bioanalytical applications such as metal ions detection (Emanuela et al., 2009), molecules recognition (Paola et al., 2009), DNA detection (Lynn et al., 2004) and immunoassays (Frédérique et al., 2009).

However, the strategies of the ECL signal are mostly single signal, and this kind of assay may provide false positive or negative errors owing to the environmental changes (Zhang et al., 2013). And it is also difficult to exclude the error that caused by these interferences. Therefore, to seek a novel and efficient ECL strategy that can exclude the errors is of great significance. The fluorescence ratio method is based on dual emission or dual excitation dyes, which have two or more emission peaks, and the ratio of fluorescence intensities at different wavelengths is related to the concentration of the target analyte (Zhang et al., 2013). This fluorescence ratio method provides a more accurate and reliable quantitative measurement method for modern biological analysis than a single measurement owing to its increasing signal-to-noise ratio, avoiding some changes caused by the external environment, such as the concentration of co-reagents, light scattering from the sample matrix, pH, and instrumentation (Wu et al., 2016; Fan et al., 2013; Lee et al., 2015). Inspired by the ratiometric fluorescent strategy, many ratiometric ECL systems were proposed for bioanalysis. The first ECL ratiometric biosensor was established by Xu's group for DNA analysis in 2013, and in this sensing approach two ECL emitters CdS NCs and luminol worked as the cathodic and anodic emitters, respectively, and H_2O_2 worked as the common coreactant. Pt NPs was employed in this system to quench the ECL signal of CdS NCs and enhance the ECL intensity of luminol (Zhang et al., 2013).

After that, several ECL ratiometric studies depending on dual-wavelength ratiometry or dual-potential ratiometry have been reported. Generally, the ratiometric ECL studies were based on the ECL resonance energy transfer (ECL-RET) between two ECL emitters or between the emitter and mental nanoparticles. Furthermore, ECL-RET usually depended on the spectra overlap of the ECL energy donor and acceptor, and it's always restricted with the distance between the energy donor and acceptor. Thus, it is not easy to fulfil the requirements that ECL-RET strategy request. Fortunately, the ratiometric ECL strategies based on the competitive consumption of the co-reactant could conquer the restrictions of the ECL-RET. To date the ratiometric ECL strategies based on the competitive consumption of the co-reactant were not as many as the reports based on the ECL-RET. For example, a ratiometric ECL strategy based on the competitive consumption of co-reactant H_2O_2 between the CdS QDs and luminol was proposed for immunosensing (Huang et al., 2016). In this sensing, with the increase amount of target model carcino-embryonic antigen (CEA), the cathodic ECL signal of CdS QDs at -1.5 V was decreased and the anodic ECL signal of luminol at +0.3 V was increased for competition of H_2O_2. However, for the application of the exogenous co-reactant, the catalytic efficiency is relatively low, furthermore, the consumption of reagents will increase. At the same time, there are certain safety risks and measurement deviation for some small molecular co-reactant with volatility and toxicity. Thus, it is of great significance and prospect to develop a ratiometrci sensing excluding ECL-RET and the addition of exogenous co-reactant.

Quantum dots (QDs) are small semiconductor nanoparticles with nanometers

from 1 to 10 nm (Alivisatos., 1996). QDs will emit the light of special frequency with the application of electricity and light, and the frequency of the light will change with the size of QDs (Wang et al., 2018; Dworak et al., 2018). Because of the tunable properties and excellent optical properties of QDs, they exhibit potential applications in photovoltaic devices (Wang et al., 2008), light emitting diodes (LED) (Dai et al., 2014) and biological analysis (Michalet et al., 2005). Since the silicon semiconductor was investigated by Bard for its ECL properties (Ding et al., 2002), several QDs were applied into ECL fields. Moreover, QDs are also of wide interest in recent investigation of ratiometric ECL field. For example, a dual-potential ratio ECL approach was designed for protein kinase activity detection by graphene quantum dots (GQDs) and luminol utilized as two emitters with the common co-reactant H_2O_2, and the introduction of AuNPs in this approach was observed as the enhancement factor of luminol and also served as a decrease factor of GQDs (Zhao et al., 2015). Thus, two ECL signals at the different potentials were changed oppositely to form a ratio ECL biosensor. At the same period, a potential-resolved biosensor was constructed for the metallothionein detection using CdTe QDs as the cathodic emitter and $Ru(bpy)_3^{2+}$ as anodic emitterwith $K_2S_2O_8$ as the coreatctant (Dai et al., 2015). These explored methods show their intriguing ECL properties in biosensing and applications, and exhibited their promising potential in further studies. However, these reports all involve the ECL-RET and the addition of exogenous coreactants.

N-(aminobutyl)-N-(ethylisoluminol) (ABEI), a derivative of luminol, has relatively high ECL efficiency when compared to luminol (Shen et al., 2011). With

the merits of nontoxicity, more active amino groups, chemical stability, ABEI has been widely applied in ECL field for bioanalysis (Jiang et al., 2016). H_2O_2 as a typical ECL reaction coreactant work with ABEI to enhance the ECL efficiency, then ABEI-H_2O_2 has been employed as a novel ECL system in bioassays (Liu et al., 2016). For example, an ECL immunosensor was explored using functionalized ABEI for N-terminal pro-brain natriuretic peptide (NT-proBNP) detection, which showed good ECL performance and great application in bioanalysis (Zhang et al., 2015). Moreover, recently a report described that ABEI can be utilized for mucin1 detection with H_2O_2-free strategy, and the ECL intensity can be enhanced by the dissolved oxygen (O_2) (Jiang et al., 2017). Such a H_2O_2-free strategy expanded the employment of ABEI in ECL clinical determination. In addition, ABEI could not only be a popular ECL reagent but also be a good reductant (Tian et al., 2011).

In this work, a ratiometric ECL approach was developed based on two ECL emitters competing for the common co-reactant (dissolved O_2). CdTe QDs were used as the cathodic emitter and ABEI was used as the anodic emitter. As well, ABEI served as the reducing agent to in situ reduce $HAuCl_4$ and H_2PCl_6 for preparing the ABEI-Au-Pt nanoflowers. Such a nanoflower structure not only could achieve the high loading of the anodic emitter ABEI but also could serve as a matrix for immobilizing phenoxy-derivatized dextran (Dexp) via π-π stacking between Dexp and ABEI. Con A was chosen as the model biomolecule since it can mediate pathophysiological and physiological responses (Zhang et al., 2017). Graphene decorated CdTe QDs (G-CdTe QDs) were immobilized onto the electrode as a matrix

for combining recognition element Dexp via the π-π stacking between graphene and Dexp, and then recognizing the analyte Con A via the specific carbohydrate-protein interaction. Finally, Dexp-ABEI-Au-Pt nanoflower was incubated onto the modified electrode to form a sandwich structure. With the increase concentration of Con A, the amount of ABEI immobilized on the electrode was increased, an opposite change could be seen as the ECL intensity of CdTe QDs decreased and ECL intensity of ABEI increased due to the competition for dissolved O_2 between ABEI and CdTe QDs. Such a ratiometric strategy not only overcame the limitations of both ECL-RET and the use of exogenous coreactants, but also was a pioneering for ABEI employed as the cathodic ECL emitter for designing a dual-potential ECL biosensor.

2. Experiment

2.1 Reagents and materials

Sigma Chemical Co. (USA) provided 1,2-epoxy-3-phenoxypropane (Epoxy), hydrogen tetrachloroaurate ($HAuCl_4 \cdot 4H_2O$), chloroplatinic acid (H_2PtCl_6), 3-mercaptopropionic acid (MPA) and Con A. Alfa Aesar Chemical Co., Ltd. (Tianjin, China) supplied Na_2TeO_3 and $CdCl_2 \cdot 2.5H_2O$. Graphene oxide (GO), bovine serum (BSA) and dextran were obtained from Aladdin Ltd. (Shanghai, China). N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was purchased from TCL Development Co., Ltd. (Shanghai, China). Phosphate-buffered saline (PBS, 0.10 M) solutions used during the detection.

2.2 Apparatus

Xi'an Remax Electronic provided a MPI-A electro-chemiluminescence analyzer

for ECL measurements. The voltage of 800 V for photomulti-plier tube, the scanning range of -1.6 V~+0.6 V and the rate of 0.5 V/s were used during ECL detection. Shanghai CH Instruments provided a CHI600D electrochemical work station for the cyclic voltammetry (CV) measurements. A three-electrode system was used with a modified electrode, Ag/AgCl and Pt wire as the working electrode, reference electrode and counter electrode, respectively. Shimadzu Corp. (Tokyo, Japan) supplied a UV-2450 UV-VIS spectrophotometer for recording UV-vis absorption spectrumfrom. The scanning electron micrographs (SEM) were supplied by the scanning electron microscope (Hitachi, Japan), and the image of the transmission electron microscope (TEM) was performed by Hitachi H-800 microscope (Japan). X-ray photoelectron spectroscopy (XPS) was studied by Thermo ESCALAB 250 spectrometer (SID-Molecular).

2.3 Preparation of G-CdTe QDs

The synthesis of G-CdTe QDs is based on the following method (Fu et al., 2017). 36.89 mg $CdCl_2 \cdot 2.5H_2O$ was dissolved using 50 mL deionzed water. Then, under stirring, GO dispersion (220 μL, 1 mg/mL) was added into the above solution and mixed for 1 h. After that, 1 mL 0.01 M Na_2TeO_3, 50 mg $C_6H_5Na_3O_7 \cdot 2H_2O$, 33 μL MPA and 100 mg $NaBH_4$ were injected into the mixture solution still under stirring. Next, this mixture solution was refluxed at 130 ℃ for about 10 h, followed by purifying, the product was washed by ethanol and water with the volume ratio of 1:1 and centrifuged. At last, the final sediment was dispersed in deionized water and stored at 4 ℃before use.

2.4 Preparation of Dexp-ABEI-Au-Pt composites

The phenoxy-derivatized dextran (Dexp) was synthesized using the previously reported protocol (Barone and Strano, 2006). In brief, 4 g dextran was dissolved in 32 mL 1 M NaOH solution and heated to 40 ℃, then 3.34 mL Epoxy was added. After that the reacting mixture was flocculated with ethanol and then washed by doubly distilled water, thus Dexp was obtained.

As-prepared ABEI was diluted into a 10 mM solution. 1 mL 1% $HAuCl_4$ solution was added into the diluted ABEI rapidly with vigorously stirring. The mixed solution was maintained stirring for 2 h at room temperature. Then another 1 mL 1% H_2PtCl_6 stock solution was added into the mixture and also kept on for 2 h with stirring. After that the final mixture was centrifuged at 10000 rpm for 15 min, and washed with deioned water for three times. The obtained product (ABEI-Au-Pt) was dispersed in double distilled water and then mixed with as-synthesized Dexp solution under stirring for 2 h at room temperature. Subsequently, BSA (1%) was added into above mixture solution to block possible remaining active sites of Au-Pt nanoparticles, thus eliminating the non-specific adsorption effect of Dexp-Au-Pt-ABEI. Finally, the nanocomposite was centrifuged and washed with deioned water for three times. The obtained product (Dexp-Au-Pt-ABEI) was dispersed in double distilled water and stored in 4℃ before use.

2.5 Fabrication of the ratiometric ECL biosensor

The fabrication process of the ratiometric biosensor was depicted in Scheme 1. The bare glassy carbon electrode (Φ=4.0 mm) was polished with alumina powders

(0.3 μm and 0.5 μm) on silk, and followed by rinsing with ultrapure water and ethanol in turn before modification. The surface-cleaned GCE was then modified with 10 μL of G-CdTe QDs dispersion and dried at room temperature. Afterwards, 10 μL of Dexp was cast onto the electrode via the π-π stacking reaction between G-CdTe QDs and Dexp. Following, 10 μL of BSA (1%) was cast onto the GCE for blocking nonspecific binding sites. Subsequently, 10 μL of Con A with different concentration was incubated onto the electrode through the biospecific interaction with Dexp for 1 h. Finally, the modified electrode was incubated with 10 μL of prepared Dexp-ABEI-Au-Pt at 4 ℃ for 2 h to form the dual-potential ECL biosensor. The GCE was washed with double distilled water after each step of modification and moreover the prepared electrode was stored in refrigerator before investigation.

3. Results and discussion

3.1 Characterization of G-CdTe QDs and ABEI-Au-Pt

As shown in Fig. 1, G-CdTe QDs and ABEI-Au-Pt were characterized by TEM and SEM. In the TEM images of G-CdTe QDs (Fig. 1A), it can be clearly observed that quantities of CdTe QDs was doped on the surface of graphene. And the enlarged morphology of G-CdTe QDs was presented in Fig. 1B, and several CdTe QDs could be seen with the size around 2.2 nm. SEM was conducted to record the morphology of ABEI-Au-Pt. As depicted in Fig. 1C, the ABEI-Au-Pt nanoflowers were well-synthesized, and each nanoflower was composed layer by layer via the in situ reduction of $HAuCl_4$ and H_2PtCl_6 due to the reductant of ABEI.

X-ray photoelectron spectroscope (XPS) was also employed to confirm the

synthesis of ABEI-Au-Pt, and the results were depicted in Fig. 1D. The characteristic peaks at 398.3 eV and 293.9 eV were belong to ABEI, and the characteristic peaks of Au 4f were observed at 84.25 eV and 87.75 eV. The peak at 72.1 eV was classified to Pt 4f. All these characteristic peaks suggested that the ABEI-Au-Pt nanocomposite was successfully obtained. Fig. S1 presented the ECL spectrum of ABEI (curve a) and the UV-vis absorption spectrum of G-CdTe QDs (curve b) . It could be evidently observed that there was no spectral overlap between these two spectra, which meat that there was no ECL energy transfer between the two ECL emitters.

3.2 ECL and CV measurements of the modified electrode

The ECL behavior of stepwise modification process was confirmed in 3 mL 0.10 M PBS (pH 7.4). Fig. 2A displayed the ECL intensity-potential curves. As shown, no anodic ECL response was observed but a strong cathodic ECL response at -1.7 V was observed for G-CdTe QDs modified electrode (curve a). After the incubation of Dexp (curve b) and BSA (curve c), the modified electrode exhibited a gradual decreased ECL emission. And when the analyte Con A (curve d) was cast onto the above mentioned electrode, there was also an obvious decrease in the cathodic ECL response. The decrease in ECL intensity was due to the fact that these additives were nonelectroactive materials which hindered the electron transfer. Surprisingly, a new anodic ECL intensity appeared at about +0.6 V and a decline of cathodic ECL emission was observed owing to the presence of ABEI (curve e) when the electrode was modified with nanocomposite Dexp-ABEI-Au-Pt. Above results indicated that a dual-potential ECL ratometric biosensor was well-constructed.

To get the information on interface changes of the electrode during the modification process, the cyclic voltammetry (CV) response for each modification was performed with $[Fe(CN)_6^{4-}]/[Fe(CN)_6^{3-}]$ as redox probe. The corresponding results were described in Fig. 2B. A well-defined redox peak (curve a) was observed at the bare electrode. A manifest decrease of redox peak current was observed after G-CdTe QDs (curve b) was modified onto the electrode due to the properties of attenuating the electron transfer. And when Dexp (curve c) and BSA (curve d) were sequentially incubated onto the modified electrode, there were stepwise decline of the redox peak currents. When Con A (curve e) was cast onto the electrode, the redox peak currents also showed a decrease evidently. These continuous decline trend was because of the impedance of Dexp, BSA and Con A. However, a significant increased peak current appeared with the incubation of Dexp-ABEI-Au-Pt (curve f) due to the promotion effect of it.

3.3 Optimal condition of the ECL biosensor

The pH often affected the performance of ECL approach. To comply the excellent ECL behavior of G-CdTe QDs and Dexp-ABEI-Au-Pt, the effect of pH of PBS solution on the ECL response of two emitters was investigated in 0.10 M PBS solution in the pH range from 6.5 to 8.5. As seen in Fig. 3, with the increase of pH, the ECL responses of both G-CdTe QDs and ABEI were increased. But considering the biological activities of Con A, pH 7.4 was chosen as the optimal experimental pH.

3.4 ECL performance of the biosensor

The ECL performance of the biosensor was investigated under the optimal

experiment condition. Fig. 4A described a change trend of the dual-potential ECL intensity with the concentration of target analyte Con A. It can be observed that the cathodic ECL emission of G-CdTe QDs decreased at around -1.7 V and the anodic ECL emission of ABEI increased at +0.6 V with the increase in the concentration of Con A. The ratio value of G-CdTe QDs ECL intensity to ABEI ECL intensity had a good linear relationship to the logarithm of Con A concentration when the concentration of Con A ranged from 1.0×10^{-4} ng/mL to 10 ng/mL. The linear regression equation was $I_{\text{G-CdTe QDs}}/I_{\text{ABEI}} = -0.5189+0.6459\log c$ and the correlation coefficient was 0.995. Here, I represents the ECL response of the emitter, and c represents the detecting concentration of Con A). According to the calculation formula of the limit of detection *(LOD*): $LOD = 3S_B/m$ (Fu et al., 2017), *LOD* was calculated as 3.0×10^{-5} ng/mL for our biosensor. Here, m means the slope of the linear regression equation and S_B means the standard deviation of the blank experiment.

The opposite changes of ECL signals at two potentials were ascribed to the fact that both G-CdTe QDs and ABEI depended on dissolved O_2 as the common co-reactant. When the decorated ABEI nanocomposite was introduced into the ECL system, it could competitively consume the co-reactant dissolved O_2 with G-CdTe QDs, thus the cathodic ECL response decreased while the anodic response increased with the increase in the immobilization amount of ABEI. The luminescence mechanisms of two ECL emitters are possibly as follows (Fu et al., 2017; Jiang et al., 2017):

$$\text{G-CdTe QDs} + e^- \rightarrow \text{G-CdTe QDs}^{\bullet-} \quad (1)$$

O_2 + 2G-CdTe QDs$^{\bullet-}$ + 2H^+ → H_2O_2 + 2G-CdTe QDs* (2)

G-CdTe QDs* → G-CdTe QDs + hν (3)

ABEI - e^- → ABEI$^{\bullet+}$ (4)

O_2 + e^- → $O_2^{\bullet-}$ (5)

ABEI$^{\bullet+}$ + $O_2^{\bullet-}$ → ABEI* (6)

ABEI* → ABEI + hν (7)

In order to verify that the dissolved O_2 served as the common co-reactant of both G-CdTe QDs and ABEI, a comparative experiment was performed by bubbling nitrogen to remove oxygen. It could be clearly seen that two ECL emitters exhibited a significantly lower ECL responses when fulled with nitrogen (Fig. S2). Thus, dissolved oxygen could be recognized as the common co-reactant of the two different emitters. Additionally, comparison experiment was also investigated to certify that Au-Pt nanoparticles in this system were not the factor to quench the luminescence of ECL emitter (Fig. S3).

Compared with previous work studying on Con A analysis, this constructed dual-potential ratiometric ECL approach exhibited a lower detection limit (Table S1), demonstrating a promising potential of this strategy for further bioanalysis.

3.5 Feasibility of ratiometric ECL biosensor

Outstanding stability is important for ECL sensing. The stability of this ratiometric sensing approach was studied in 0.10 M air-saturated PBS (pH 7.4) using the biosensor incubated with 1.0×10^{-3} ng/mL Con A. As shown in Fig. 5A, the relative standard deviation (RSD) for 8 consecutive cycle potential scans was 2.7%, indicating

a good stability of this biosensor.

To estimate the selectivity of the established biosensor, alpha-fetoprotein (AFP, 50 ng/mL), phytohemagglutinin (PHA, 50 ng/mL), BSA (1%) and carcinoma antigen (CA, 0.1 U/mL) were selected as possible interfering substances. In this test, the ECL responses of the biosenosr towards Con A (0.1 ng/mL) were detected with and without these interfering substances, respectively. As shown in Fig. 5B, no evident difference among these data in the histogram was observed, indicating that the influence of above interfering substance can be ignored. Additionally, we used other protein like AFP instead of Con A to investigate whether it could interact with Dexp-ABEI-Au-Pt to form a sandwich-type configuration. Corresponding result was shown in Fig. S4 in the supplementary materials. As expectedly, no anodic ECL response was observed in the absence of Con A. Above experiments demonstrated the prepared biosensor exhibited a superior selectivity to Con A detection, which was ascribed to the great biospecific recognition between Dexp and Con A.

Under the optimal experimental conditions, the reproducibility of the potential-resolved ECL biosensor was also tested by 5 proposed biosensors incubated with the same concentration of Con A (0.1 ng/mL). Among the 5 biosensors, the RSD were 3.4% for the cathodic ECL emissions and 4.7% for the anodic ECL emissions, showing an acceptable reproducibility.

3.6 Analysis of Con A in human serum

The potential applicability of the dual-potential ratiometric ECL biosensor was studied in human serum samples, and the standard addition method was used to test

the recoveries. Human serum samples were diluted by 0.10 M PBS (pH 7.4) before the detection. The corresponding results were shown in Table S2, and the recoveries were in the range from 95.3% to 105.7%, indicating its potential in real biological analysis.

Conclusion

In this work, a dual-potential ratiometric ECL sensing approach was designed for Con A detection based on the competition for dissolved oxygen (O_2) between G-CdTe QDs as the cathodic ECL emission and ABEI as anodic ECL emission. Particularly, ABEI not only served as the anodic emitter, but also employed as the reductant for in situ reducing of $HAuCl_4$ and H_2PtCl_6 to form the nanocomposite of ABEI-Au-Pt. In the strategy, both G-CdTe QDs and ABEI rely on the dissolved O_2 as common co-reactant, with the increase amount of Con A, the ECL intensity of cathode decreased while the intensity of anode increased because of the competitive consumption for dissolved O_2 between G-CdTe QDs and ABEI, and the ratio for the two kind of intensity ($I_{\text{G-CdTe QDs}}$ / I_{ABEI}) was logarithmically related to the concentration of Con A, thus demonstrating a successful conformation of this sensing approach. Additionally, this strategy is a H_2O_2-free strategy, only consumes the dissolved O_2 and shows no need for exogenous co-reactant. It was a pioneering for designing a ratiometric strategy only based on two ECL emitters competing for the dissolved O_2.

Acknowledgments

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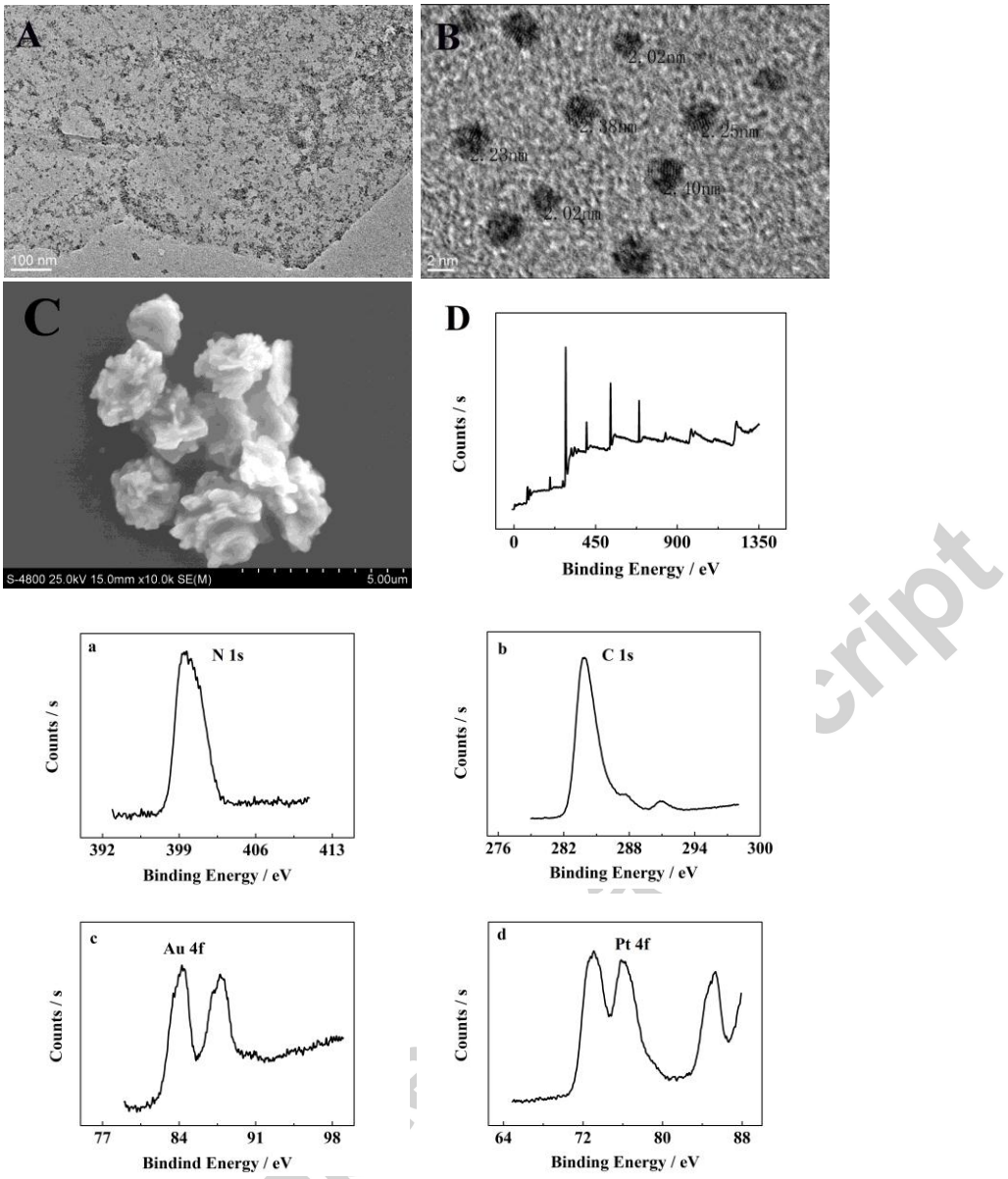
Fig. 1 (A) The TEM image of G-CdTe QDs and (B) enlarge TEM of CdTe QDs; (C) SEM image of ABEI-Au-Pt; (D) XPS spectra of ABEI-Au-Pt composite: (a) N 1s region, (b) C 1s region, (c) Au 4f region, (d) Pt 4f region

Fig. 2 (A) ECL intensity-potential profiles of (a) G-CdTe QDs/GCE, (b) Dexp/G-CdTe QDs/GCE, (c) BSA/Dexp/G-CdTe QDs/GCE, (d) Con A/BSA/Dexp/G-CdTe QDs/GCE, and (e) Dexp-ABEI-Au-Pt/Con A/BSA/Dexp/G-CdTe QDs/GCE in 0.10 M PBS (pH 7.4); (B) CV curves of (a) bare GCE, (b) G-CdTe QDs/GCE, (c) Dexp/G-CdTe QDs/GCE, (d) BSA/Dexp/G-CdTe QDs/GCE, (e) Con A/BSA/Dexp/G-CdTe QDs/GCE and (f) Dexp-ABEI-Au-Pt/Con A/BSA/Dexp/G-CdTe QDs/GCE in 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1: 1). Scan rate: 50 mV/s Effect of the pH of PBS on the ECL intensity of ABEI and G-CdTe QDs

Fig. 4 (A) ECL response of the ratiometric biosensor to (a) 1.0×10^{-4} ng/mL, (b) 1.0×10^{-3} ng/mL, (c)1.0×10^{-2} ng/mL, (d) 0.1 ng/mL, (e) 1.0 ng/mL, and (f) 10 ng/mL Con A in 0.10 $mol\cdot L^{-1}$ PBS (pH 7.4); (B) Calibration curve at different concentration of Con A

Fig. 5 (A) The ECL stability of the ratiometric biosensor for the detection of 1.0×10^{-3} ng/mL Con A; (B) ECL response of the biosensor incubated with (a) Con A (0.1 ng/mL), (b) Con A (0.1 ng/mL) + BSA (1%), (c) Con A (0.1 ng/mL)+PHA (50 ng/mL), (d) Con A (0.1 ng/mL)+CA (0.1 U/mL), and (e) Con A (0.1 ng/mL)+AFP (50 ng/mL)

Scheme 1 Schematic illustration of (A) preparation of G-CdTe QDs and Dexp-ABEI-Au-Pt; (B) The stepwise fabrication process of ratiometric ECL sensor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2.38nm
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2.23nm
2.40nm
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2 nm
C
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5.00um
D
Counts / s
0
450
900
1350
Binding Energy / eV
a
N 1s
Counts / s
392
399
406
413
Binding Energy / eV
b
C 1s
Counts / s
276
282
288
294
300
Binding Energy / eV
c
Au 4f
Counts / s
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d
Pt 4f
Counts / s
64
72
80
88
Binding Energy / eV

Fig. 1

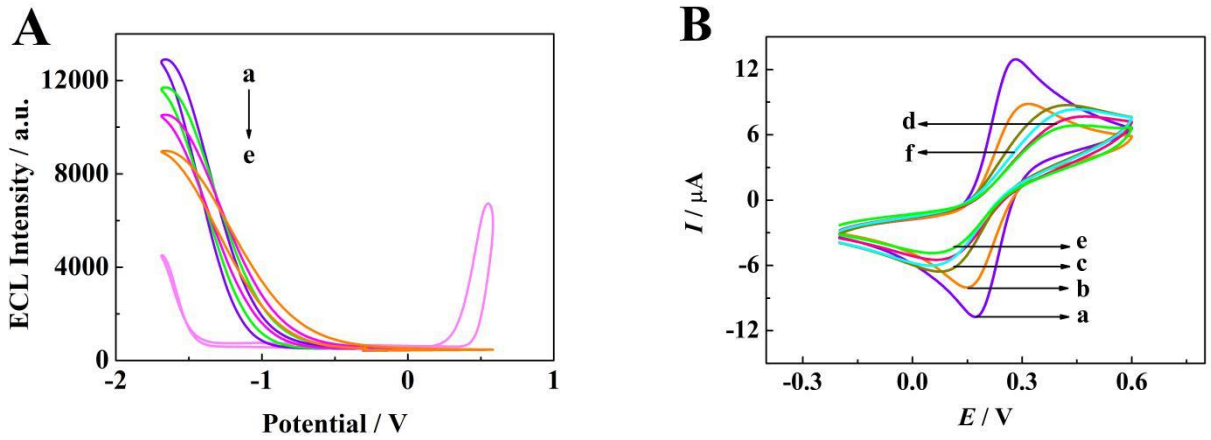


Fig. 2

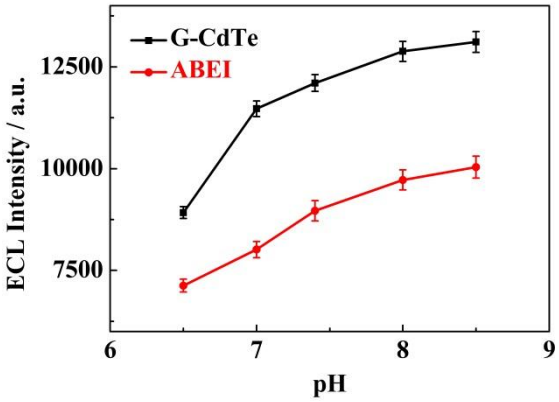


Fig. 3

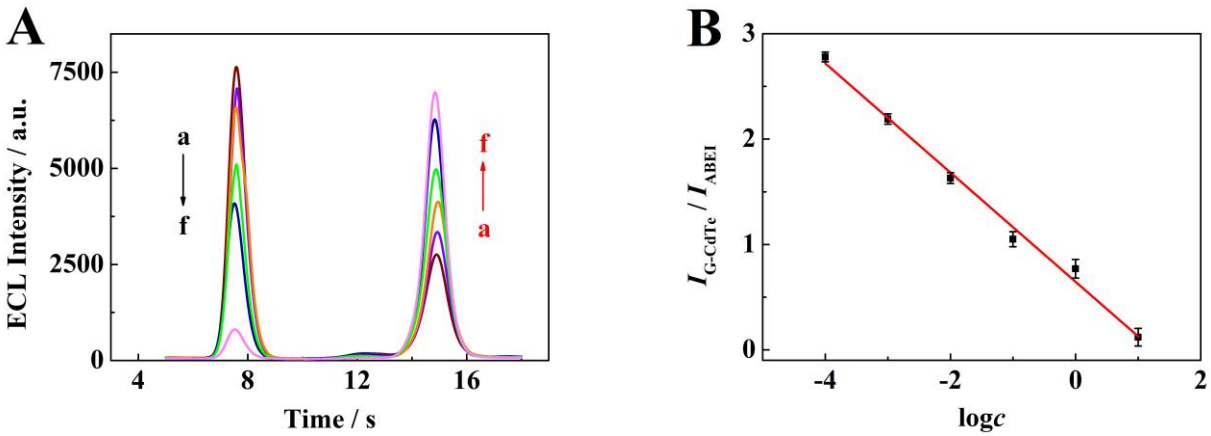


Fig. 4

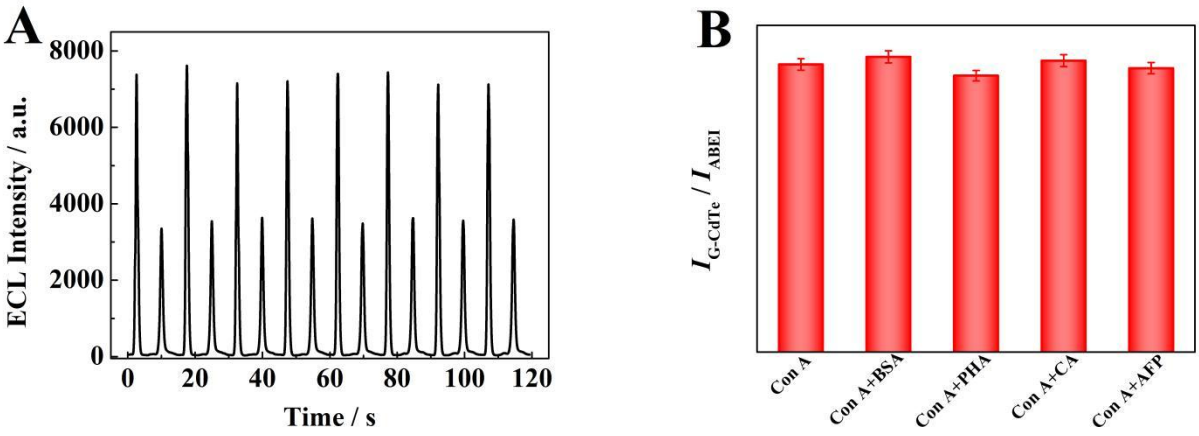
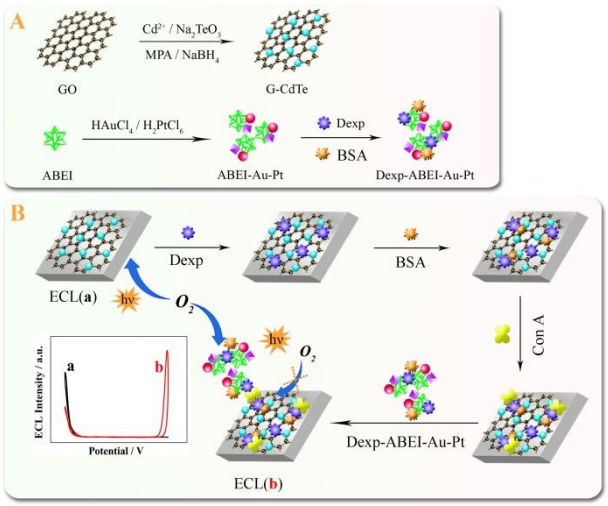


Fig. 5



Scheme 1

Highlights

- The ratiometric biosensing for Con A detection was the first time.
- In this proposed strategy, two emitters CdTe QDs and ABEI competed for dissolved oxygen (O_2) as co-reactant to form the ratiometric ECL biosensor.
- This proposed ratiometric ECL biosensor not only conquered the false errors caused by external interferences, but excluded the disability caused by exogenous co-reactant via the application of common dissolved O_2.