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Highly efficient quenching of electrochemiluminescence from CdS nanocrystal film based on biocatalytic deposition

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ABSTRACT

Aiming to find an alternative electrochemiluminescence (ECL) quenching route of high efficiency, biocatalytic precipitation (BCP) was firstly coupled with ECL for investigating its insulating effect on ECL. Experimental data revealed that an insulating layer could be formed via BCP onto the electrode surface, inhibiting the reaction between the coreactant and luminophore and thereby impairing the ECL emission dramatically. Since the extent of insulating effect associated strongly with analyte concentration, a new ECL biosensor was successfully realized. In a model horseradish peroxidase (HRP)-based system, the fabricated biosensor possessed high sensitivity and wide linear range from 1.0×10^{-10} M to 1.0×10^{-6} M for H_2O_2 determination with a detection limit of 4×10^{-11} M ($S/N = 3$). This method has great potential in extending the application of ECL biosensor for various bioanalytes.

1. Introduction

ECL involves the generation of species at electrode surfaces that then undergo electron-transfer reactions to form excited states that emit light [1]. With high sensitivity, no background from unnecessary photoexcitation, excellent temporal and spatial controllability [2], ECL has attracted substantial research interests in enzyme catalytic [3,4], immune [5,6] and DNA biosensors [7–10]. In the past decade, with inherent advantages including high quantum yields of fluorescence, size or surface trap-control luminescence as well as good stability, semiconductor nanocrystals (S-NCs) have been widely used as ECL emitters for numerous bioanalysis [11–13]. The ECL of S-NCs is usually generated by one-directional potential scanning on the electrode in the presence of both the luminophore and coreactant. Upon oxidation or reduction, the coreactant would produce as intermediate to react with the ECL luminophore to create excited states [14]. To date, most of the quenching type ECL biosensors were accomplished by the inhibition of electron transfer or by energy transfer [15,16]. These methods lead to a target concentration-dependent decrease of ECL emission. The traditional quenching type of ECL-biosensoring by the inhibition of electron transfer mainly come from the enhancement of steric hindrance effect from the formation of bioconjugates, such as immunocomplex [17–19] or the consumption of ECL coreactant in an biorecognition events, such as enzymatic reaction [20–22]. Nevertheless, these traditional quenching routes are usually unable to achieve the great signal diminution. Hence, to find alternative ECL route with higher quenching efficiency would be desirable.

Enzyme-stimulated BCP [23–25], involving the formation of insoluble products on the electrode surface, has been demonstrated as an effective strategy to influence the electron transfer in the photo-current conversion process [26]. Since ECL represents the process from electrochemistry to optical signal [2,27], one could expect that the insoluble coverage layer would probably open a different perspective for ECL bioassay and biosensor design. However, this kind of insulating effect has never been exploited in ECL analysis to the best of our knowledge.

With the specific interest, this work firstly integrated the BCP with ECL biosensor for the exploitation of insulating effect with the aim to searching for advanced ECL bioanalysis route. As shown in Scheme 1, HRP-induced BCP on the CdS nanocrystals (NCs) modified glassy carbon electrode (GCE) were used for the ECL detection of H_2O_2, based on the HRP accelerated 4-chloro-1-naphthol (4-CN) oxidation by H_2O_2 to produce the insulating barrier benzo-4-chlorohexadienone on the GCE surface [28,29]. The experimental results demonstrated that the thin precipitate coverage could effectively inhibit the reaction between the coreactant $K_2S_2O_8$ and CdS NCs for the consequent highly efficient ECL quenching. Because various enzymes could also stimulate the formation of BCP, this simple model work probes the new possibility of using BCP reaction to produce insulating effect for the development of advanced sensitive ECL bioassay.

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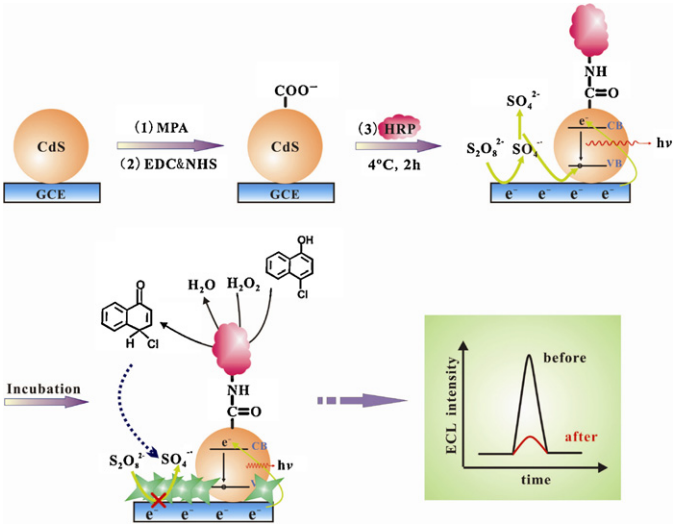

Scheme 1. Diagram of process to fabricate BCP integrated ECL biosensor.

2. Experimental

2.1. Reagents and apparatus

HRP (MW 44,000, ~250 units/mg protein), 4-CN, 1-methylimidazol, 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were of commercial source (Sigma–Aldrich, St. Louis, MO). 0.1 M phosphate buffer KH_2PO_4–K_2HPO_4 containing 0.1 M NaCl (pH 7.4, PBS) was used for immobilization and incubation of HRP. All other reagents were of analytical grade and used without further purification. Millipore ultrapure water (resistivity ≥ 18.2 MΩ cm) was used throughout the experiment.

The ECL emission measurements were conducted on a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science and Technology Co. Ltd., Xi'An, China). Electrochemical impedance spectrosocopy (EIS) was carried out with an Autolab potentiostat/galvanostat (Eco Chemie B.V., Netherlands). Atomic force microscopy (AFM) images were performed in ambient conditions using a molecular imaging Pico SPM in tap mode with a 10 μm scanner.

2.2. Preparation of CdS NCs

CdS NCs with the diameter of 5 nm were synthesized as in our previous work [9]. Briefly, $Cd(NO_3)_2{\cdot}4H_2O$ (0.1683 g) was dissolved in 30 mL ultra-pure water, and heated to 70 °C under stirring, then injected into a freshly prepared solution of Na_2S (0.5960 g) in 30 mL ultra-pure water. Instantly, orange–yellow solution was obtained. The solution was held at 70 °C for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol two times and ultrapure water two times. Then, the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. The final solution was rather stable for 2 months when stored in a refrigerator at 4 °C.

2.3. Preparation of CdS NCs film

The GCE (3 mm) were used as working electrodes in typical three-electrode system. GCE was pretreated by polishing its surface with successively finer grades sand papers and then polished to a mirror smoothness with aqueous slurries of alumina powders (average particle diameters: 0.3 μm and 0.05 μm Al_2O_3) on a polishing silk. Finally, the GCE was thoroughly rinsed with water and then sonicated in ethanol and ultrapure water in turn. The modification of CdS NCs film was achieved by dropping 10 μL of CdS NCs solution onto the pretreated surface of GCE and evaporated in air at room temperature. Owning to the abundant active sites on the fresh surface of pretreated GCE, the CdS NCs could adsorb on it firmly. The CdS NCs modified GCE was stored in PBS buffer for characterization and further modification.

2.4. Fabrication of the ECL biosensor

Due to the presence of both mercapto group and carboxylic acid group in MPA, it is used for the connection of HRP to the CdS NCs modified GCE surface. In detail, the CdS NCs modified GCE was dipped in 3 mM MPA for 5 h at 4 °C since the MPA mercapto group had strong coordinative ability with Cd^{2+} on the NCs surface. Subsequently, the electrode was immersed in 1.0 mL of 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 2 h at 4 °C to active the terminal carboxylic acid group of MPA. Due to the covalent reaction between the activated carboxylic acid group and amino-containing HRP, the HRP could successfully immobilized onto the CdS NCs film via immersing the modified electrode in 100 μL 0.1 M PBS buffer at pH 7.4 containing 5.0 mg/L HRP at 4 °C for 2 h. The electrode surface was rinsed with 0.1 M PBS buffer after each step to remove nonspecifically adsorbed species.

2.5. Analytical procedure

4-CN was dissolved initially in ethanol and then diluted with 0.1 M PBS buffer, yielding the developing solution that included 1.0 mM 4-CN and 2% (v/v) ethanol. The HRP/CdS modified GCE was then incubated in the developing solution consisting of H_2O_2 with various concentrations for 10 min. Before and after the incubation, the ECL responses of the electrode were recorded in 0.1 M PBS (pH 8.0) containing 0.05 M $K_2S_2O_8$ as a coreactant. The linear scan potential was applied with a scan rate of 100 mV/s and the voltage of the photomultiplier tube (PMT) was set at −600 V. ECL signals related to the H_2O_2 concentrations could be measured. EIS and AFM analyses were also applied for the characterization of biosensor fabrication.

3. Results and discussion

3.1. Insulating effect on ECL

The new biosensing protocol was depicted schematically in Scheme 1. The ECL emitters of CdS NCs modified on the GCE could generate an ECL peak at ca. −1.20 V with coreactant $K_2S_2O_8$, the presence of which is crucial to the strong and stable ECL emission (Fig. 1, curve a). Upon the potential scan with an initial negative direction, the CdS NCs were reduced to nanocrystal species ($CdS^{-\bullet}$) by charge injection, while the coreactant $S_2O_8^{2-}$ was electrochemically reduced to the strong oxidant $SO_4^{-\bullet}$. Thereafter, $SO_4^{-\bullet}$ reacted with the negatively charged $CdS^{-\bullet}$ by injecting a hole into the highest occupied moleculoar orbital to produce an excited state of CdS NCs (CdS^*), and emitted light in the aqueous solution. The corresponding ECL processes are as follows [30,31]:

$$CdSNCs + ne \rightarrow nCdS^{-\bullet} \quad (1)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet} \quad (2)$$

$$CdS^{-\bullet} + SO_4^{-\bullet} \rightarrow CdS^* + SO_4^{2-} \quad (3)$$

$$CdS^* \rightarrow CdS + h\nu \quad (4)$$

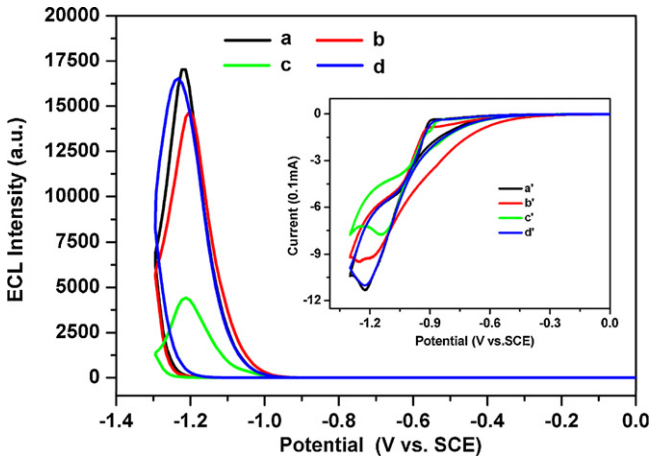

Fig. 1. Cyclic ECL on potential curves in various cases. (a) CdS NCs film on GCE; (b) HRP/CdS–GCE; (c) precipitation/HRP/CdS–GCE; (d) BCP without HRP. Inset: the corresponding CV. ECL detection buffer: 0.1 M PBS (pH 8.0) containing 0.05 M $S_2O_8^{2-}$. Scan rate, 100 mV s^{-1}.

After HRP immobilization, the ECL emission exhibited a small decrease as shown in curve b of Fig. 1, which should be attributed to the retarded electron-transfer in ECL reaction caused by the enhanced steric hindrance of the protein. Interestingly, the ECL intensity decreased dramatically after the further incubation of the HRP/CdS electrode in BCP solution (curve c). HRP would accelerate the oxidation of 4-CN by H_2O_2, yielding the insoluble and insulating product of benzo-4-chlorohexadienone on the transducer surface. The formation of an insulating coverage layer would greatly inhibit the transfer of $K_2S_2O_8$ to the electrode surface and hence impair the ECL emission. This impediment effect was further confirmed by the cyclic voltammograms (CV). As shown in the inset of Fig. 1, the peak current decreased with the progressive modification processes, especially after BCP reaction. To better clarify this effect, a control experiment without the involvement of HRP was carried out. It was found that the pure CdS NCs modified electrode could not activate the BCP reaction and the ECL intensity experienced no attenuation accordingly (curve d).

3.2. Characterization of biosensor fabrication

AFM technique was then employed to reveal the morphology of fabricating process using bare silicon wafer. The silicon wafer was smooth and flat (Fig. 2A inset). Fig. 2B showed the AFM image of CdS NCs film on the wafer, with the undulate height increased to ca.15 nm. When HRP was assembled on the CdS NCs film, the thickness of all the layers increased to ca.60 nm as well as the enhanced roughness (Fig. 2C). Finally, after the incubation in BCP solution, the thickness of the entire surface was further increased to ca.70 nm, but the surface was flattened (Fig. 2D). The significant differences in the morphology before and after the BCP reaction demonstrated the generation of an insoluble cover layer on the electrode surface.

As an effective method to monitor the electrode interfacial feature, EIS was then utilized to follow the fabrication process of the biosensor. Fig. 3 showed the Nyquist polt of impedance for the stepwise modification process. For the bare GCE, it exhibited an almost straight line (curve a), which was characteristic of a diffusional process. After the anchor of CdS NCs onto the electrode surface, the EIS showed a low interfacial electron-transfer resistance R_{et} (curve b) due to the conductivity of the CdS NCs. The subsequent immobilization of HRP on the CdS NCs film resulted in the further increased resistance (curve c). After BCP, the EIS showed a significant increase in diameter (curve d), indicating that the insulating layer greatly prevented the diffusion of redox probe to the electrode surface.

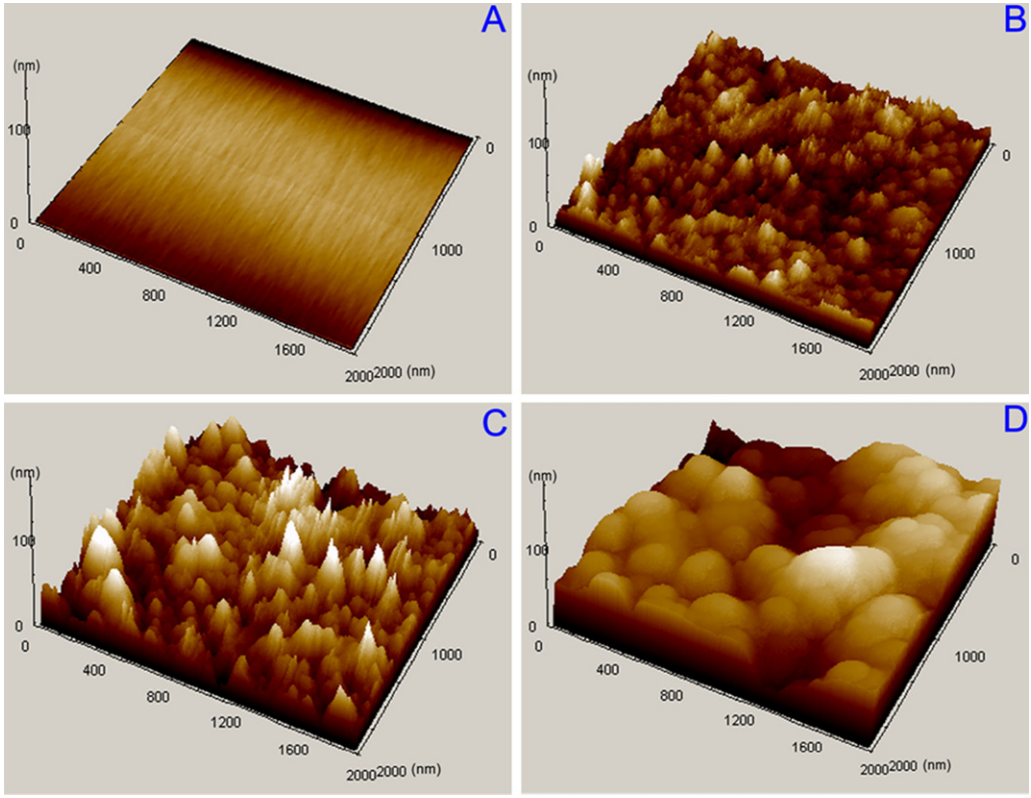

Fig. 2. AFM images of (A) bare silicon wafer; (B) CdS NCs film; (C) HRP/CdS; (D) precipitation/HRP/CdS on smooth silicon wafers.

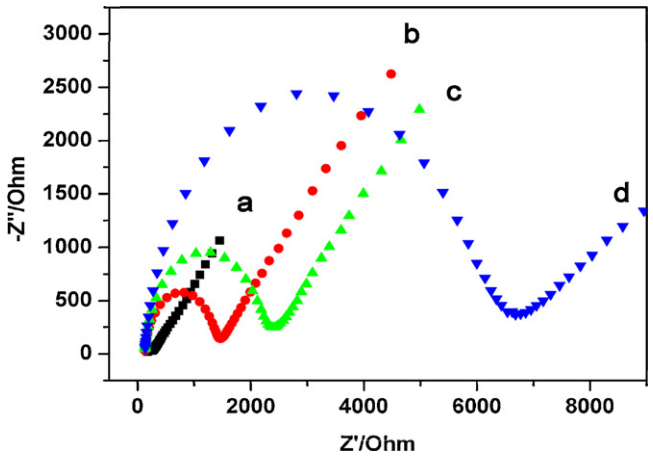

Fig. 3. EIS of modified ECL biosensor: (a) bare GCE; (b) CdS–GCE; (c) HRP/CdS–GCE; (d) precipitation/HRP/CdS–GCE. EIS were measured in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ with the frequency range from 0.005 Hz to 10000 Hz and signal amplitude of 5 mM.

These results were in good agreement with the results of ECL and CV measurements.

3.3. Optimization of experiment conditions

In biosensor preparation, the enzyme immobilization on the CdS NCs-electrode surface was a crucial step because the density of HRP associated intimately with the sensor performance. The immobilization time ranging from 0.5–5 h was investigated. The densities of HRP increased with the immobilization time and reached a saturation value after 2 h. Thus, 2 h was applied for the HRP immobilization.

Another fact that can impact the sensor sensitivity is the BCP incubation time, which was closely related to the extent of precipitate coverage. The decrement of ECL intensity increased with the increment of incubation time from 2 to 10 min, and then leveled off. This may be because the precipitate covered the biocatalytic sites and consequently blocked the further accumulation of the insoluble product [29]. Therefore, 10 min was accepted as the incubation time.

3.4. Analytical performance of the biosensor in H_2O_2 detection

After assembled HRP on CdS NCs, the constructed enzyme biosensor exhibited desirable reliability and stability. Fig. 4A inset showed the ECL emission from this biosensor upon continuous potential scanning for 10 cycles without intensity change. After the storage in pH 7.4 PBS in the refrigerator at 4 °C for 1 month, the biosensor showed a quite satisfying stability, which retained about 94.5% of its initial response. Due to the high and stable ECL signals, this enzyme biosensor was very suitable for ECL detection.

Because the insulating effect was directly related with analyte concentration and thus a new ECL biosensor could be realized by monitoring the ECL signal that reflected the extent of insulating effect. Fig. 4A presented the ECL signal change of the HRP/CdS modified electrode after incubation with different concentrations of H_2O_2 (0–10^{-5} M). The decrement of the ECL intensity originated from the enhanced insulating effect with the increase of H_2O_2 concentration. The decrement was logarithmically related with the H_2O_2 concentration in the wide range from 1.0×10^{-10} M to 1.0×10^{-6} M ($R = 0.9934$) with a detection limit of 4×10^{-11} M at S/N ratio of 3 (Fig. 4B).

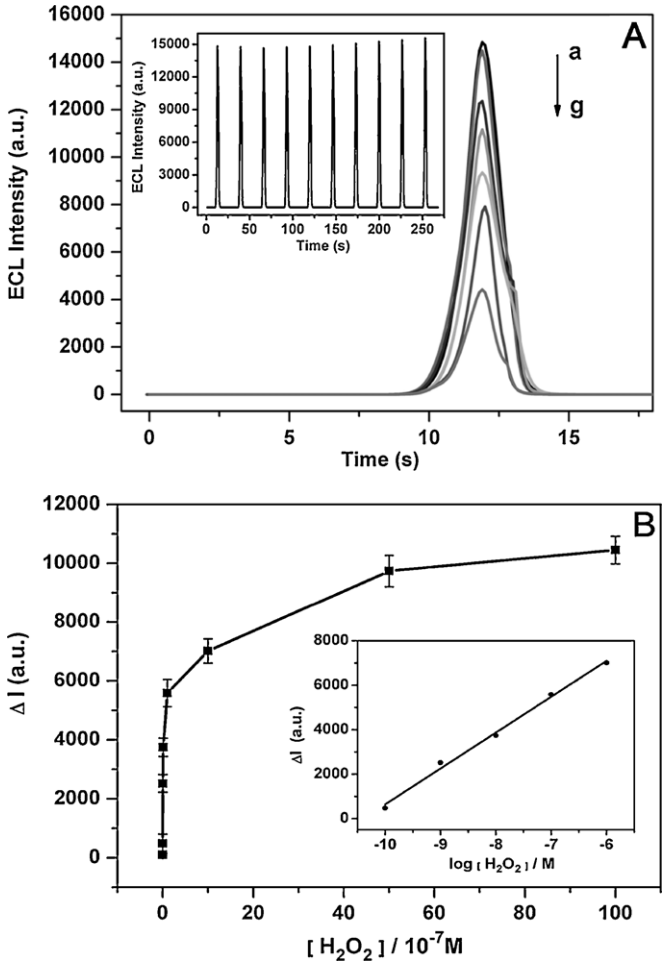

Fig. 4. A: The ECL signals of the biosensor incubated with BCP solution containing different concentrations of H_2O_2 (a–g: 0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M, respectively). Inset: Stabilization of ECL emission from HRP/CdS–GCE under continuous cyclic potential scan for 10 cycles. B: Relationship between ΔI and H_2O_2 concentration, three measurements for each point. Inset: logarithmic calibration curve for H_2O_2.

Moreover, due to the separation of enzyme incubating and ECL detecting solution, the interference could be avoided easily and therefore improving the sensitivity of biosensor [21]. To investigate the specificity of the ECL biosensor, we measured the ECL response of the mixture containing uric acid (UA), ascorbic acid (AA) and H_2O_2 at the same concentrations of 1.0×10^{-7} M. Compared with the ECL response obtained from the pure H_2O_2, no significant difference was found, indicating that this BCP-based ECL biosensor has high selectivity. The reproducibility of the ECL biosensor was evaluated from the response to 1.0×10^{-6} M H_2O_2 at five independent electrodes. A series of measurements resulted in a relative standard deviation (R.S.D.) of 9.3%, indicating good electrode-to-electrode reproducibility of the fabrication protocol.

3.5. Real sample analysis

To evaluate the analytical reliability and application potential of the proposed method, the biosensor was used to detect rainwater, disinfectant and contact lens solution respectively, which contain different concentration of H_2O_2. The results were summarized in Table 1. The recoveries range from 97.00% to 104.00%, which were satisfactory for practical applications of this simple ECL biosensor.

Table 1
Results of analysis of H_2O_2 in real samples using proposed ECL biosensor.

Sample	Added	Found	Recovery (%)	R.S.D.[a] (%)
Rainwater	–	3.82 μM	–	1.92
	1.00 μM	4.79 μM	97.00	2.28
Disinfectant[b]	–	20.50 nM	–	1.69
	1.00 nM	21.54 nM	104.00	3.45
Contact lens solution[b]	–	4.93 nM	–	2.02
	1.00 nM	5.91 nM	98.00	3.17

[a] R.S.D.: relative standard deviation ($n = 3$).
[b] Samples diluted 10^8 times.

4. Conclusions

In summary, we demonstrated the possibility of coupling BCP with ECL for the development of sensitive ECL biosensors with highly quenching efficiency. Since various enzymes which could induce the BCP of insoluble products may also be introduced into ECL bioassay, the present method might be one general approach for the development of the highly sensitive ECL biosensor.

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