Article

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Bioactivity-Protected Electrochemiluminescence Biosensor Using Au Nanoclusters as the Low-Potential Luminophor and Cu_2S Snowflake as Co-Reaction Accelerator for Procalcitonin Analysis

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ABSTRACT: The expansion of electrochemiluminescence (ECL) technology to immunoassay at core of care emphasizes all immune molecules will not be inactivated in analysis process. That poses a major challenge to ECL-based biosensors due to the deoxynucleotide sequences of antigen or antibody could be oxidized root in excessive cyclic potential. Herein, an ultrasensitive ECL biosensor was developed based on a novel bioactivity-protected sensing strategy utilizing Au nanoclusters (Au NCs) as low-potential luminophor for detection of procalcitonin (PCT). Bovine serum albumin (BSA)-templated Au NCs exhibited low-potential anodic ECL signal in triethylamine (TEA) solution at 0.87 V, where is suitable for the survival of immune molecules. Taking advantage of good conductivity and high surface area, Cu_2S snowflake not only function as satisfying substrate for connecting immune molecules, but act as co-reaction accelerator to produce more cationic radicals $TEA^{\bullet+}$, which could improve ECL intensity to meet the requirements of trace analysis. Otherwise, HWRGWVC (HC-7) heptapeptide as specific antibody immobilizer for site-oriented fixation was introduced to further maintain the bioactivity of antibody. In view of above, the obtained biosensor exhibited ultrahigh immune recognition to targets so that the detection limit was as low as an unprecedented value of 2.36 fg/mL, which will be of great significance to the application and development of biosensor in the future.

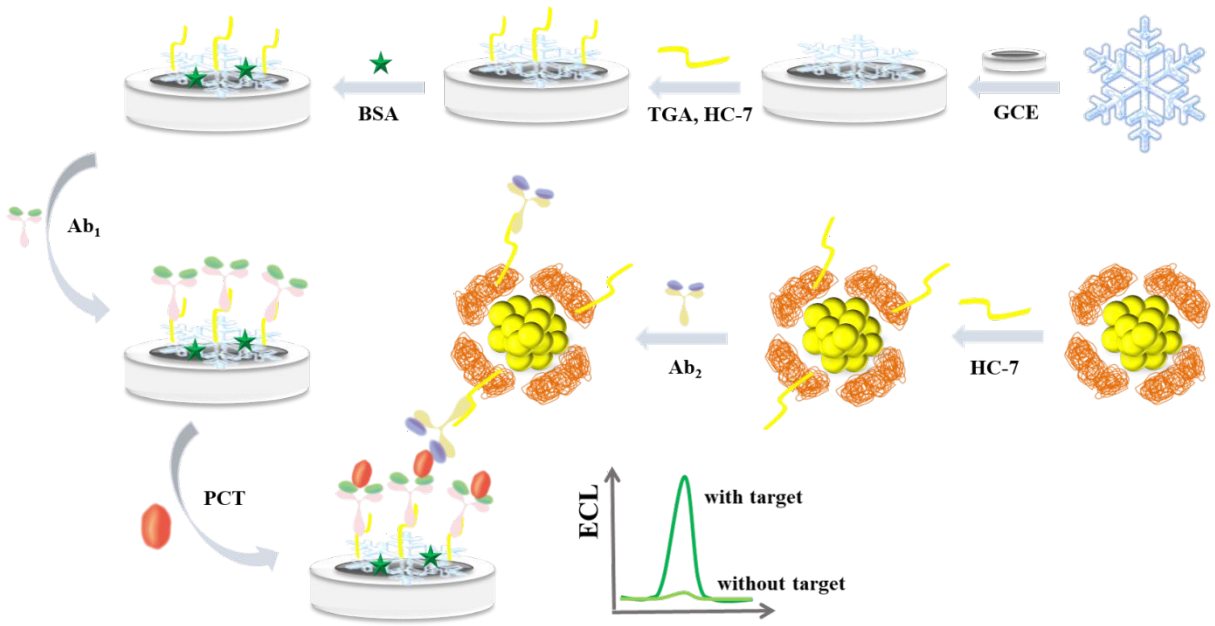
KEYWORDS: *electrochemiluminescence biosensor, bioactivity-protection, low-potential-excited, Au NCs, co-reaction acceleration, site-oriented antibody fixation*

Ultrasensitive detection of disease-related biomarkers provides a great value for the early diagnosis and treatment of underlying diseases.[1] To achieve the promise, immunoassay tools such as biosensors, as in aptasensor, enzyme-linked immunosensor, have been widely focused.[2-7] Also, electrochemiluminescence (ECL) sensors are widely developed and applied in immunoassay due to their unique advantages of simple structure, high sensitivity and low detection limit.[8,9] However, the core of immunosensor fabrication in the future should be focused on how to maintain the bioactivity of immune molecules in analysis process instead of exploring diversiform novel ECL materials.[10] Although satisfactory results have been yield by introducing optical materials with good biocompatibility instead of toxic signal probe, oligonucleotide sequences could still undergo irreversible oxidative damage when the excited potential is excessive. This means that the scanning potential needs to be strictly controlled when extending ECL technology to immunoassay.[11] Nevertheless, reports on such proposition are infrequent, to our understanding, only one report had pointed to this fact. Hence, relevant verification is indispensable, and if so, the application of some ECL luminophor in immunoassay will be limited.

Au nanoclusters (Au NCs), a common fluorescent probe, is one of potential optical materials due to its excellent optical stability, large stokes shift and high quantum yield. Au NCs could be synthesized using various ligand such as polymer, nucleic acid, protein and so on.[12-14] Particularly, the anodic or cathodic ECL signal of bovine serum albumin (BSA)-templated Au NCs was observed in various co-reactive systems, which broaden their application significantly.[13] Li et al. observed cathodic ECL signal at -1.5 V utilizing effective electron transfer between the conduction-band of excited indium tin oxide (ITO) and Au NCs in the presence of $K_2S_2O_8$, which ameliorated the thermionic induction theory at extreme potential proposed by Diez in 2009.[15,16] Instead, Yuan's groups obtained the anodic ECL signal of the Au NCs at 1.198 V using tris(3-aminoethyl)amine as coreactant.[17] Currently, it has been reported that a distinct ECL signal can be excited under lower-potential when the Au NCs coexist with triethylamine (TEA).[18] With other advantages of good biocompatibility and abundant functional groups, Au NCs has become most promising ECL luminophor for immunoassay. However, their electrochemical progresses are limited root in poor conductivity of BSA, thus some auxiliary methods should be included to ameliorate ECL efficiency.

Controlling the ECL intensity within an appropriate range is a prerequisite for meeting the requirements of trace analysis. Almost all of strategies such as noble metal ion adsorption, Forster resonance energy transfer, nanosurface energy transfer and so on, are used in ECL biosensors to enhance or quench the ECL signal all for this purpose.[19-21] Amplification of ECL intensity by co-reaction accelerated strategy with good controllability and stability is widely favored as opposite to other methods.[22] Related reports here:[23,24] Wang et al. employed a Co-MOFs to decompose H_2O_2 and accelerate the ECL process of N-(aminobutyl)-N-(ethylisoluminol) successfully. Zeng's

Scheme 1. Schematic Representation of the Fabrication of Immunosensor.



group designed an ECL aptasensor utilizing hemin as co-reaction promoter to catalyze $S_2O_8^{2-}$ and generated more radical anions ($SO_4^{\bullet-}$) for accelerating the oxidation process of PTCA. However, the investigation on Cu-based compound as the coreaction accelerator of TEA is still in its infancy. That is not difficult to achieve owing to excellent redox reversibility of Cu^+/Cu^{2+}, which could be recycled via the electrochemical redox reaction.[25]

In this work, the effect of potential on protein bioactivity was confirmed by detecting the circular dichroism (CD) spectra of procalcitonin (PCT) samples scanned under different potentials or times. Upon above conclusions, the Au NCs was synthesized utilizing BSA as reductant and employed to mark secondary antibody (Ab_2). Before that, the ECL property of Au NCs was explored in TEA solution, resulting the strong ECL signal was obtained at 0.87 V. Besides, Cu_2S snowflake was prepared by controlling reaction conditions and used as a novel co-reaction accelerator in Au NCs/TEA system. After incubation with the antibody capturer of HWRGWVC (HC-7) via amine reaction, the obtained Cu_2S/HC-7 and Au NCs/HC-7 can specifically identify the Fc portion of initial antibody (Ab_1) and Ab_2, respectively. After all strategies were performed, the detection limit of this bioactivity-protected biosensor towards PCT was down to 2.36 fg/mL, which decreased obviously compared with other ECL biosensors, providing a forward strategy expanding ECL technology to immunoassay.

■ EXPERIMENTAL SECTION

Materials, reagents and apparatus are included in Supporting Information.

Synthesis of Au NCs. The Au NCs was synthesized utilizing BSA as reductant and the specific processes referred to the previous reports with minor modifications:[13] 5 mL of $HAuCl_4$ (10 mM) was added to BSA aqueous solution (5 mL, 50 mg/mL) under vigorous stirring. After balance, 0.5 mL of NaOH (50 mg/mL) was introduced, and the mixture was transferred to water bath maintain stirring at 37 °C for 12 h. Next, the product with deep yellow was collected and dialyzed against deionized water for 24 h to obtain the pure Au NCs.

Preparation of Au NCs/HC-7/Ab_2 Bioconjugates. First, the Au NCs/HC-7 bioconjugates relied on amine reaction, where carboxyl was supplied from cysteine on HC-7 and the amino came from BSA. The specific processes are as follows: 100 μL of HC-7 (10 μg/mL) activated by EDC/NHS (2 M of EDC, 0.4 M of NHS) was mixed with 1 mL of pure Au NCs. After incubation at 4 °C for 8 h, the obtained mixture was centrifuged under 12000 rpm. Separating the upper solution, 100 μL of Ab_2 was added and shaken gently for 30 min to obtain the end-product of Au NCs/HC-7/Ab_2.

Synthesis of Cu_2S Snowflakes. The hydrothermal method was used to synthesize Cu_2S snowflakes:[26] 1 mmoL $CuCl_2$ was dissolved in 30 mL ethanediamine (EDA) utilizing ultrasound until the solids were dissolved completely. Then, 3 mmol of $(NH_2)_2CS$ was added to above clear liquid under stirring to ensure the homodisperse of each reagent. Next, the prepared mixture was transferred to a 50 mL Teflon-lined stainless steel autoclave and heated to 80 °C for 8 h. After centrifugation and washing, the obtained black powder was stored in 4 °C refrigerator for further use.

Fabrication of ECL Biosensor. The glassy carbon electrode (GCE, Φ= 4 mm) was used as the working electrode, which was burnished into a mirror-like surface by using Al_2O_3 slurries. Then the prepared GCE was blown dry by nitrogen after being washed with deionized water. Subsequently, the specific fabrication process was shown in Scheme 1. First, 6 μL of Cu_2S (2 mg/mL) was modified on GCE surface as substrate. After airing, the GCE/Cu_2S was immersed into a mixed solution containing 3 mM mercaptoacetic acid (TGA) and 0.1 M NaCl to introduce the carboxyl groups for 5 h at 4 °C (the feasibility of this method has been verified and related

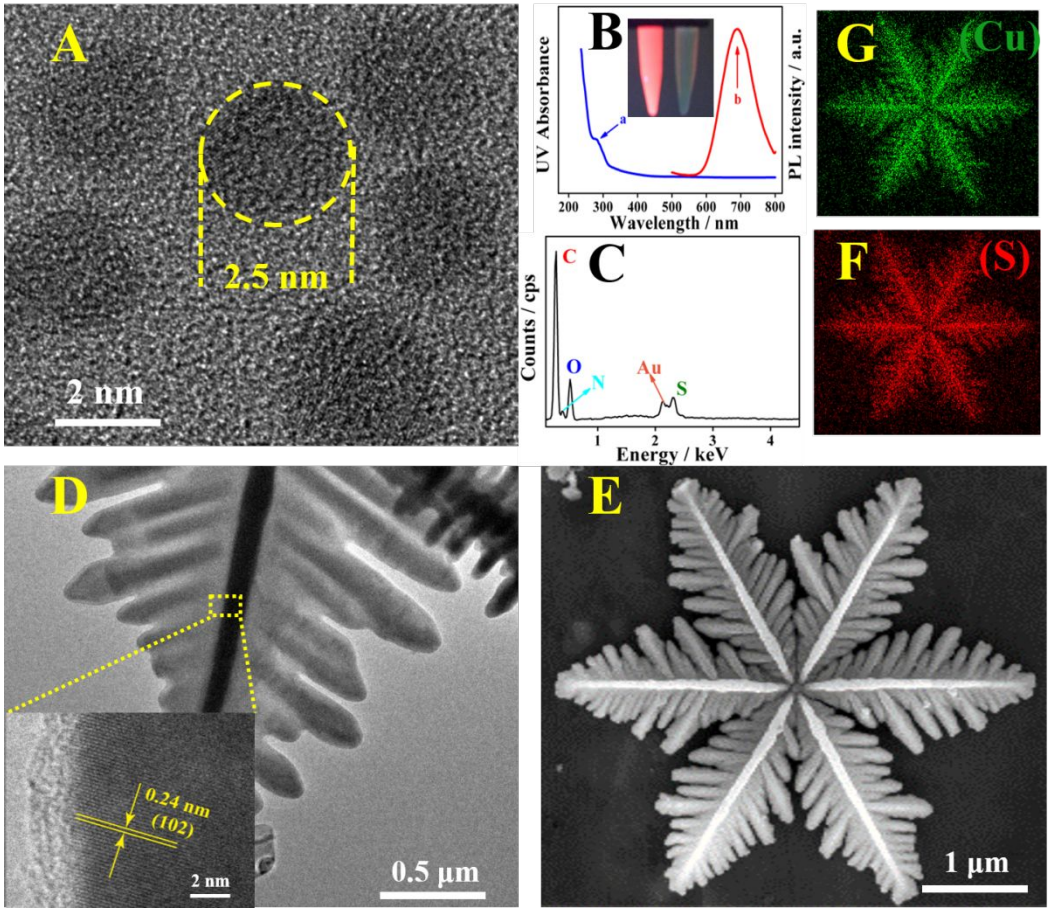


Figure 1. (A) TEM image, (B) UV-vis absorbance (line a), PL spectrum (line b) and (C) EDS spectrum of Au NCs. (D) TEM and HRTEM images of Cu_2S. (E) SEM and element mapping images of S (F) and Cu (G) in Cu_2S snowflake.

discussions were shown in Supporting Information).[27] After activation with EDC/NHS, the GCE was immersed in 10 μg/ml HC-7 solution at 4 °C for binding HC-7 via amine reaction. Then, 6 μL of Ab_1 was dripped onto the electrode and formed film. After blocking the remaining nonspecific binding sites with BSA (10 μL, 0.1wt%), PCT with different concentrations were incubated on electrode and maintained at 4 °C for 2 h. Finally, the Au NCs/HC-7/Ab_2 as signal label was combined with PCT to complete the GCE modification.

ECL Measurement Procedure. The biosensors were measured utilizing a RST electrochemical workstation as excitation source and a MPI-I weak-light detection system as ECL signal collector. The three-electrode system was composed of a working electrode (GCE), a counter electrode (platinum wire) and a reference electrode (Ag/AgCl electrode). The cyclic voltammetry (CV) voltage was set from 0 to 0.9 V with an appropriate scan rate of 150 mV/s. The ECL signals were released in 10 mL PBS (1/10 M, pH=7.8) containing 0.04 M of TEA and enlarged by a photomultiplier, which the potential of was set at 700 V and amplification level was adjusted to IV.

CD Spectra Detection of PCT Bioactivity. In order to explore the effect of potential on protein bioactivity, the PCT samples were filled in a quartz cuvette with 1 mm optical path and detected by a V100 CD system in far UV region, which was from 190 nm to 260 nm. Before that, all PCT samples (10 mL, 0.5 mg/mL) were diluted with PBS (pH=7.4) and packed into an electrolytic cell. Then, the PCT samples were bubbled with high-purity nitrogen for 30 min to empty oxygen and ensure the experimental results were not affected by superoxide radical and other reactive oxygen species. Subsequently, the PCT samples saturated with high-purity nitrogen were scanned using CV model in a three-electrode system by adjusting the potential (0 ~ 1 V, 0 ~ 1.2 V, 0 ~ 1.5 V, 0 ~ 2 V) or scanning time (0.5 min, 1 min, 2 min, 5 min) at 4 °C. Furthermore, the other experimental conditions were consistent. The data obtained are expressed as mean residue ellipticity (MRE).

Preparation of Serum Sample. The serum sample were provided from the hospital of university of Jinan. In order to evaluate the clinical feasibility of the biosensor, no further purification of the sample was required. Instead, seven different concentrations of standard PCT at 0.05, 0.1, 0.2, 0.5, 1, 5, 10 ng/mL were separately spiked into 10% diluted serum sample. After well mixing, the prepared samples were stored in a 4 °C refrigerator for further analysis.

■ RESULTS AND DISCUSSION

Characterization of Au NCs and Cu_2S. Transmission electron microscope (TEM) was employed to observe the

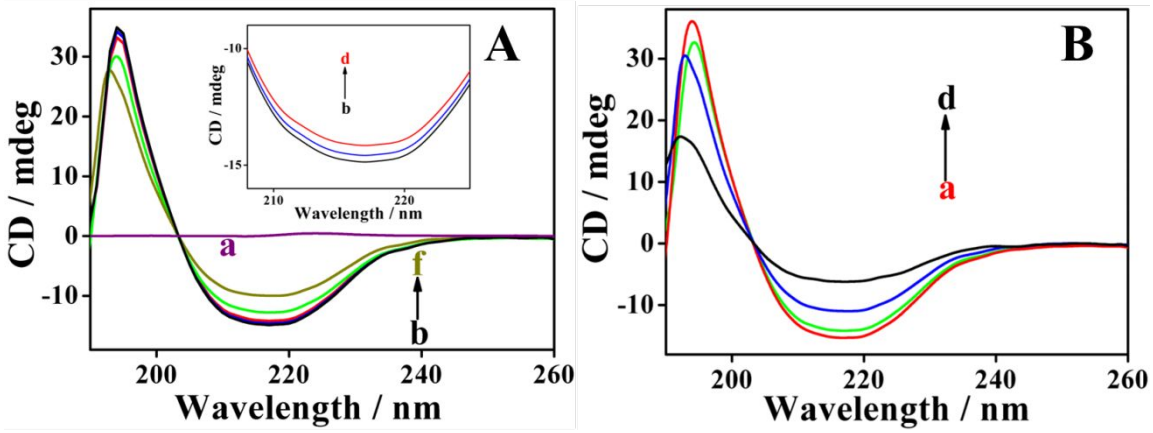


Figure 2. CD spectra of PCT with 0.5 mg/mL stimulated by different (A) potential and (B) scanning time.

morphology of Au NCs. With excellent dispersion, spherical Au NCs exhibited uniform diameters around 2.5 nm (Figure 1A). Then, the UV-vis and fluorescence spectra (Figure 1B) were used to further prove the character of Au NCs. A distinct UV-vis absorption peak was found at 275 nm (spot a), which could be interpreted as belonging to BSA. It is also proved that have no localized surface plasmon resonance in the absorption range of Au NCs due to nothing peaks appeared at 520 nm.[28-30] And then, the fluorescence spectrum showed an emission peak with the wavelength of 700 nm (spot b), which was consistent with the characteristic peak of Au NCs.[31] Moreover, the prepared Au NCs could emit red fluorescence by irradiating with a portable UV lamp, compared with pure BSA without any change (Figure 1B inset). Besides, energy dispersive spectrometer (EDS) showed C, N, O, S, Au signals (Figure 1C), which was consistent with the elementary composition of BSA-templated Au NCs. The above characterization results indicated the successful preparation of Au NCs.

X-ray diffraction (XRD) was used to characterized the crystallization of Cu_2S. Multiple characteristic peaks with different intensities were found at 2θ=26.5°, 29.2°, 37.4°, 45.8°, 48.5° and 54.6° (Figure S2), which corresponded to the (002), (101), (102), (110), (103) and (004) planes of Cu_2S, respectively. The experimental results confirmed that Cu_2S snowflakes were successfully prepared.[32]

The morphology of Cu_2S were further characterized utilizing TEM and scanning electron microscope (SEM) As shown in Figure 1D, the TEM image indicated the formation of leaf-shaped Cu_2S architecture, which equipped homogenous veins and needle-like branches with the length about 3 ~ 4 μm. Most importantly, some bulges and gaps were clearly visible on the Cu_2S surface, which could increase its specific surface area and provide more binding sites for HC-7 fixation. Besides, an area was selected for high resolution transmission electron microscope (HRTEM) scanning (Figure 1D inset), the single crystallites architectures with good crystallinity was revealed, and the lattice spacing was measured to be 0.24 nm, which was corresponded to the (102) plane of Cu_2S.[32] Meanwhile, SEM image of Cu_2S also confirmed the regularity of snowflake architectures, as shown in Figure 1E, which perfected results of TEM images, corresponding element mapping analysis (Figure 1F and G) also indicated the uniform distribution of Cu and S elements, further confirmed the successful synthesis of Cu_2S.

CD Analysis of PCT Bioactivity. The sensitivity of the biosensor depends on the bioactivity of the immune molecules, which can be reflected by observing the change of conformation utilizing CD spectra. Figure 2A showed the CD spectra of PCT with 0.5 mg/mL stimulated by different potentials. Prior to the start, the CD spectrum of pure PBS was detected (curve a). This flat line proved that PBS had no any CD absorption and it was reasonable to be used as diluent. Other curves showed the analogous β-sheet conformation due to their characteristic peaks appeared at 216 nm and 195 nm.[33-35] Concretely, the original sample without any dispose exhibited maximum MRE (curve b), which could be regarded as reference for analyzing other samples. After being scanned under 0 ~ 1 V and 0 ~ 1.2 V, the MRE of PCT decreased slightly (curve c and d), which proved that low-potential had little effect on the PCT conformation. And then, the CD intensity of PCT exhibited a trend of rapid decline (curve e) when the potential range was broadened to 0 ~ 1.5 V. Fabulously, the MRE of PCT decreased by nearly half (curve f) compared to the original sample when the excitation potential was 0 ~ 2 V. Besides, the effect of stimulated time on antibody bioactivity was explored and the results were shown in Figure 2B. All samples were stimulated under 0 ~ 1.0 V. Curve (a) represented the CD spectrum of PCT with 0.5 mg/mL stimulated for 30 s, and it can be clearly seen that has the maximum MRE compared with other samples, which proved that the low-potential in a short time does not have a significant impact on PCT bioactivity. Furthermore, curve (b), (c) and (d) respectively represented the CD spectra of PCT with stimulation for 1, 2 and 5 min, resulting the decreased significantly of MRE, which proved that long-term cyclic voltammetry scan also can inactivate the PCT. The above experimental results showed that the degree of the potential and the scanning time can lead to irreversible damage of PCT, especially the potential over 1.2 V or prolonged galvanism. In view of these, more attention should be paid to protect the bioactivity of protein rather than widely explore ECL materials in immunoassay.

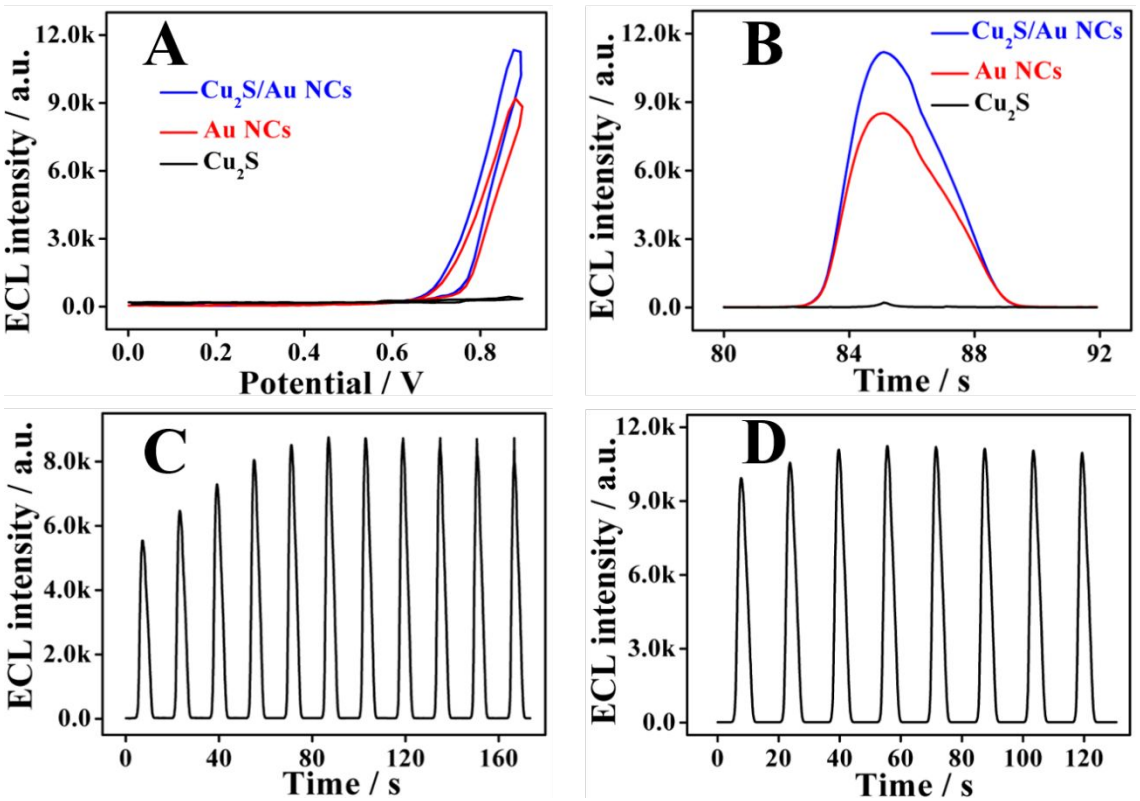


Figure 3. (A) ECL-potential and (B) ECL-time curves of Cu_2S (black curve), pure Au NCs (red curve) and Cu_2S/Au NCs (blue curve) in 10 mL of PBS (pH=7.8, 0.1 M) containing 0.04 M of TEA. The ECL stability of Au NCs (C) and Cu_2S/Au NCs (D) under the same conditions with (B).

Feasibility Analysis of Au NCs and Cu_2S for ECL Immunoassay. The ECL signal of Au NCs was explored in TEA solution, a stable anode ECL signal was observed at 0.87 V, and their ECL-potential and ECL-time profiles were shown in Figure 3A and B, respectively. Although the high-efficiency ECL of Au NCs appeared under low-potential around 0.87 V (red curve), their stabilization time took around 80 s (Figure 3C), which can still cause inactivation of protein. After loading with the substrate of Cu_2S, the obtained Cu_2S/Au NCs had a significant ECL increase (blue curve), and the stable period of signal was shortened to 30 s (Figure 3D), which proved the Cu_2S as co-reaction accelerator for remedying the defect of Au NCs was feasible.

ECL Enhancement Mechanism Discussion. In order to determine the catalytic effect of Cu_2S in Au NCs/TEA system, ECL-potential profiles (Figure 3A) were employed to analyze again. Under same experimental conditions, the ECL intensity of Cu_2S, pure Au NCs and Cu_2S/Au NCs were measured, respectively. It can be clearly seen that the Cu_2S (black curve) has no ECL signal and the ECL intensity of the Au NCs loaded with Cu_2S (blue curve) was obviously higher than that of the pure Au NCs (red curve). The possible ECL enhancement mechanism was explored as follows:[18,36,37] when the concentration of the co-reactant was higher than the luminophor, it has been indicated the photon release was based on the electrochemical oxidation of TEA. Specifically, with deepened anodic scanning, TEA was electrochemically oxidized to form cationic radicals ($TEA^{\bullet+}$) (eq 3), which can oxidize Au NCs to obtain radical cation ($Au\ NCs^{+}$) (eq 4). Besides, the redundant $TEA^{\bullet+}$ can be protonated to form reductive radicals ($TEA^{\bullet}$) (eq 5), which can reduce the Au NCs for forming $Au\ NCs^{-}$ (eq 6). As a result, the $Au\ NCs^{-}$ reacted with $Au\ NCs^{+}$ to generate high-energy-state $Au\ NCs^{*}$ (eq 7). At the end of the reaction process, $Au\ NCs^{*}$ decayed to the ground state to finish ECL emission (eq 8). It is worthy to notice that the core of the whole process depended on how much TEA were oxidized. In a word, the production of $TEA^{\bullet+}$ determined the ECL efficiency, while Cu_2S can catalyze TEA and generate more intermediate $TEA^{\bullet+}$ (eq 1 and 2) to replace eq 3, accelerating the subsequent electrode reaction.

$$Cu^{+} - e \rightarrow Cu^{2+} \quad (1)$$

$$Cu^{2+} + TEA \rightarrow Cu^{+} + TEA^{\bullet+}\ (more) \quad (2)$$

$$TEA - e \rightarrow TEA^{\bullet+} \quad (3)$$

$$Au\ NCs + TEA^{\bullet+} \rightarrow TEA + Au\ NCs^{+} \quad (4)$$

$$OH^{-} + TEA^{\bullet+} \rightarrow TEA^{\bullet} + H_2O \quad (5)$$

$$TEA^{\bullet} + Au\ NCs \rightarrow Et_2N{+}CHCH_3 + Au\ NCs^{-} \quad (6)$$

$$Au\ NCs^{+} + Au\ NCs^{-} \rightarrow Au\ NCs^{*} + Au\ NCs \quad (7)$$

$$Au\ NCs^{*} \rightarrow Au\ NCs + h\nu \quad (8)$$

Optimal ECL Conditions of Au NCs/TEA System. As a novel ECL system, the optimal ECL conditions should be explored, which could effectively enhance the ECL efficiency and maintain the immune molecules with an active-state.

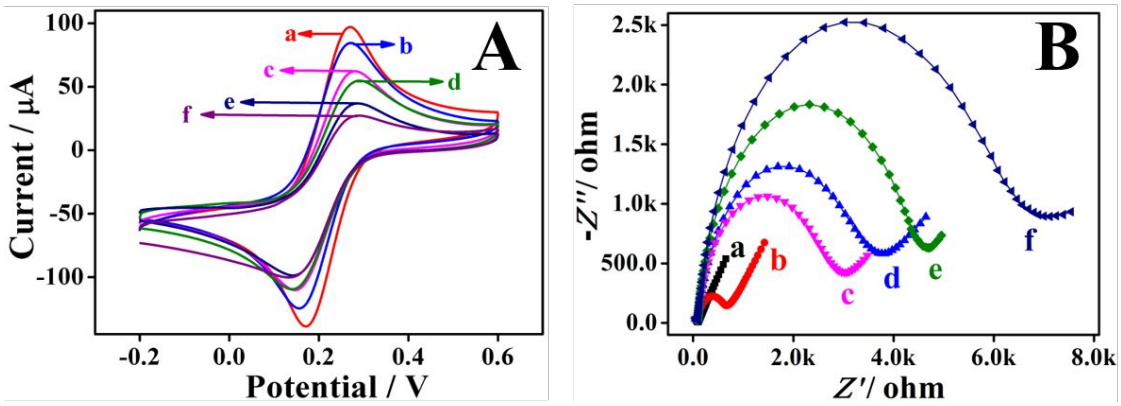


Figure 4. (A) CV and (B) EIS profiles of stepwise modified electrodes in 10 mL electrolyte containing KCl (0.1 M) and $[Fe(CN)_6]^{3-/4-}$ (5 mM): (a) Cu_2S, (b) Cu_2S/HC-7, (c) Cu_2S/HC-7/Ab_1, (d) Cu_2S/HC-7/Ab_1/BSA, (e) Cu_2S/HC-7/Ab_1/BSA/PCT, (f) Cu_2S/HC-7/Ab_1/BSA/PCT/Au NCs/HC-7/Ab_2.

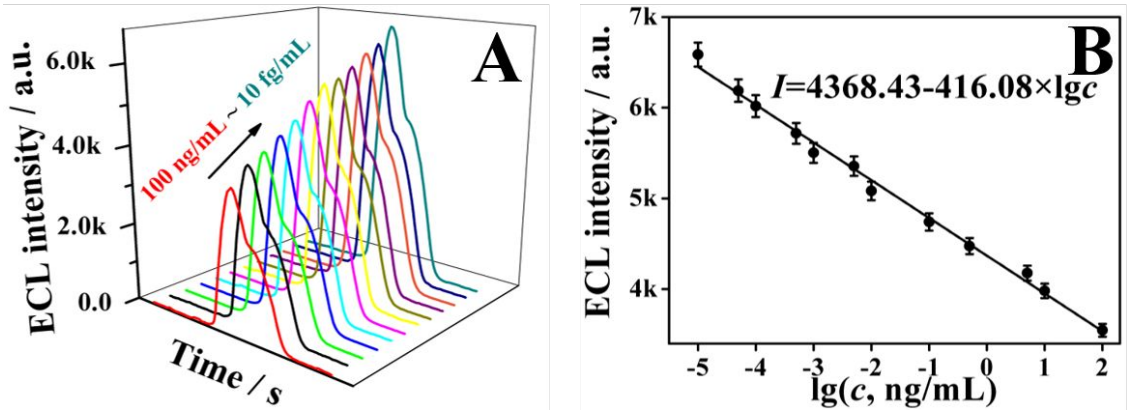


Figure 5. (A) ECL-time curve and calibration curve (B) of biosensor detected standard PCT with different concentrations: 100 ng /mL, 10 ng/mL, 5 ng/mL, 500 pg/mL, 100 pg/mL, 10 pg/mL, 5 pg/mL, 1 pg/mL, 500 fg/mL, 100 fg/mL, 50 fg/mL, 10 fg/mL.

First and foremost, the optimal pH value of Au NCs/TEA system was explored in a series of PBS with different pH range from 5.0 to 9.0, and the results were presented in Figure S3A. It has been clearly seen that a drastic increase of ECL signal when pH value improved from 5.0 to 7.8, which could be explained that abundant OH^- accelerated the process of eq 5 and eq 6 to produce more Au NCs^-. The ECL intensity still increased slightly when pH was over 7.8, but 7.8 was chosen as the optimal pH for PCT detection owing to excessive pH could cause protein metamorphic. Also, the concentration of TEA was also closely related to the ECL intensity, which was involved in the ECL process. Trace amounts of TEA would result in incomplete process of eq 5 and insufficient high-energy-state Au NCs^*. But superfluous TEA can actuate the reverse reaction of eq 4, and hindered the ECL process. The theoretical speculation was corresponded to the experimental results (Figure S3B), a strongest ECL signal was found when the TEA concentration was 40 mmol/L, which was selected as the optimal value of this condition.

Electrochemistry Characterization of Biosensor Fabrication. To demonstrate the sensor fabrication process, the change of the CV spectra of the proposed sensor was shown in Figure 4A. The larger peak currents can be clearly seen when Cu_2S (a) and Cu_2S/HC-7 (b) are fixed on the electrode, which is based on the good conductivity of Cu_2S and simple immune structure of HC-7. Subsequently, with the modification of non-conductive proteins of Ab_1 (c), BSA (d), PCT (e) and Au NCs/HC-7/Ab_2 (f) on the electrode surface, CV peak currents decreased significantly, and peak potential shifted to the right slightly, which could be explained that the protein prevented electron transfer. The above experimental results proved that the sensor was fabricated successfully.

Electrochemical impedance spectroscopy (EIS) is often used to characterize the construction of sensors as a dynamical method for analyzing electrode processes. Figure 4B showed the EIS profiles of the electrode with different substances modified. Two spectra of Cu_2S (curve a) and Cu_2S/HC-7 (curve b) were found to exhibit low impedance due to their high conductivity. After the ordinal modifications of nonconductive Ab_1, BSA and PCT on the electrode surface (curve c, d, e), resistance increased significantly. When the signal label of Au NCs/HC-7/Ab_2 was coupled on GCE surface, the resistance further increased (curve f). The above experimental results were consistent with CV profiles, which further proved that the biosensor was fabricated successfully.

ECL Response Towards PCT. The sensitivity of biosensor was investigated by measuring the ECL intensity with the concentration of PCT in the range from 100 ng/mL to 10

fg/mL. As shown in Figure 5A, the ECL intensity of biosensor increased orderly upon the decrease of PCT concentration, which showed an almost linear correlation (R^2=0.993), as depicted in Figured 5B, and thus the limit of detection (LOD) was calculated as 2.36 fg/mL (signal-to-noise (S/N) ratio = 3). The experimental results showed that the biosensor had excellent sensitivity to detect femtogram level of PCT, which was satisfactory compared with previous reports (Table S1).[38-42] However, the practical value of the sensor was uncertain due to the human serum is more complex than pure PCT, thus some performance testing and real sample analysis should be included.

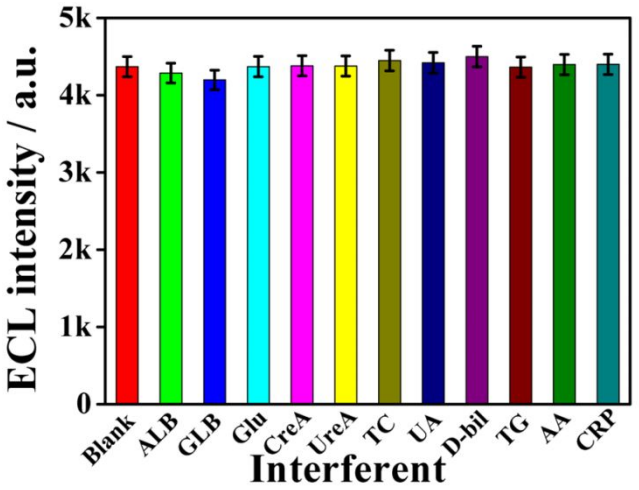


Figure 6. Anodic ECL intensity observed at 0.87 V for the 1 ng/mL of PCT with different interference: noting (Blank), albumin (ALB), globulin (GLB), glucose (Glu), creatinine (CreA), urea (UreA), total cholesterol (TC), uric acid (UA), direct Bilirubin (D-bil), triglyceride (TG), ascorbic acid (AA) and c-reactive protein (CRP). Error bars represent triplicate data points.

Analytical Performance. Given the complex composition of substances in human serum, which posed a major challenge toward the capability of sensor, especially the specificity. Common species that might interfere the selectivity of the biosensor should be detected separately. Albumin, globulin, glucose and so on, commonly encountered species within blood samples, were used as interference to evaluate the clinical viability and cross-reactivity of this system with the responses shown in Figure 6. It can be clearly seen that the addition of interference had no impact on the detection of PCT, which confirmed that the biosensor had a good specificity. Besides, with good stability and repeatability (Figure S4A and B), the biosensor could be used for clinical testing theoretically after detecting real serum samples.

Trace Detection of PCT in Human Serum and Evaluation of Method Accuracy. In this section, the standard addition method was used to evaluate the accuracy of the biosensor by detecting ECL intensity of the serum sample, which was added several standard concentrations of PCT (0.05, 0.1, 0.2, 0.5, 1, 5, 10 ng/mL).[43] Before that, the serum of human was obtained from the school hospital and its PCT was detected to be 0.031 ng/mL by this biosensor. All data are summarized in Table 1, the recoveries were acceptable with the value range from 90.0% to 99.8%, which proved that the biosensor was feasible for clinical detection.

Table 1. The recoveries of PCT in human serum using the proposed ECL biosensor.

Simple number	Origin number (ng/mL)	Spiked samples (ng/mL)	Amount found (ng/mL)	RSD (%,n=3)	Recovery (%)
1	0.031	0.05	0.076	0.58	90.0
2		0.10	0.130	0.39	99.0
3		0.20	0.228	0.25	98.5
4		0.50	0.530	0.66	99.8
5		1.00	1.027	2.66	99.6
6		5.00	5.017	1.29	99.7
7		10.00	10.009	1.22	99.8

■ CONCLUSIONS

To summarize, a sensitive and high-efficient ECL biosensor was developed by lowering the excited potential and time based on the principle of maintaining the bioactivity of protein. A misgiving, the effect of potential on protein bioactivity, had been raised and confirmed by CD spectra. Based on the fact, Au NCs was selected as the ECL emitter for biosensor fabrication, which has excellent ECL property and biocompatibility under low anodic potential with 0.87 V. Also, the Cu_2S snowflake with high specific surface area not only was used as co-reaction promoter for decomposition of TEA, but provided abundant binding sites for immune molecules fixation. The above strategies allowed the LOD of biosensor to be down to 2.36 fg/mL, which meant that the PCT ultrasensitive analysis can be achieved without any purification of the serum sample. It is expected that the biosensor can be applied in clinic.

■ ASSOCIATED CONTENT

Supporting Information

Materials and reagents; apparatus; FTIR spectra of Cu_2S and carboxylic Cu_2S; the feasibility analysis of introducing carboxyl groups on Cu_2S surface using TGA; XRD spectrum of Cu_2S; optimization of the Au NCs/TEA ECL system; stability and repeatability of the ECL biosensor for PCT detection; comparison of PCT analysis using other sensors with the proposed ECL biosensor.

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Notes

The authors declare no competing financial interest.

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