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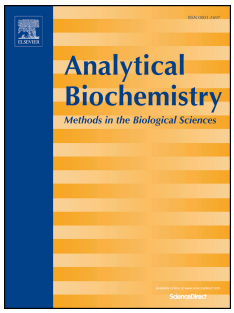

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“off-On”Switching Electrochemiluminescence biosensor for mercuy(II) detection based on molecular recognition technology

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Abstract:

A novel "off-On" electrogenerated chemiluminescence (ECL) biosensor has been developed for the detection of mercury(II) based on molecular recognition technology. The ECL mercury(II) biosensor comprises two main parts: an ECL substrate and an ECL intensity switch. The ECL substrate was made by modifying the complex of Ruthenium(II) tris-(bipyridine)($Ru(bpy)_3^{2+}$)/Cyclodextrins-Au nanoparticles(CD-AuNps)/Nafion on the surface of glass carbon electrode (GCE), and the ECL intensity switch is the single hairpin DNA probe designed according to the "molecular recognition" strategy which was functionalized with ferrocene tag at one end and attached to Cyclodextrins (CD) on modified GCE through supramolecular noncovalent interaction. We demonstrated that, in the absence of Hg(II) ion, the probe keeps single hairpin structure and resulted in a quenching of ECL of $Ru(bpy)_3^{2+}$. Whereas, in the presence of Hg(II) ion, the probe prefers to form the T-Hg(II)-T complex and lead to an obvious recovery of ECL of $Ru(bpy)_3^{2+}$, which provided a sensing platform for the detection of Hg(II) ion. Using this sensing platform, a simple, rapid and selective "off-On" ECL biosensor for the detection of mercury(II) with a detection limit of 0.1 nM has been developed.

Keywords:Electrochemiluminescence, biosensor, mercury(II) , molecular recognition technology,Switch.

1. Introduction

Mercury has been widely recognized as one of the most hazardous pollutants and highly dangerous elements due to its accumulative and toxic properties in the environment, and long-term exposure to high levels of Hg(II) ion based toxins leads to serious and permanent damage of the central nervous system and other organs [1-2]. Thus, the development of methods for the highly sensitive and selective determination of Hg(II) ion is therefore of significant importance for the environment and human health and is the subject of current analytical chemical research.

Various methods, including gas chromatography-atomic fluorescence spectrometry [3], atomic absorption spectrometry [4-6], fluorescence oligonucleotide beacons [7], and isotope dilution cold vapor inductively coupled plasma mass spectrometry [8-9] have been reported for monitoring Hg(II). However, these methods have several disadvantages such as costly instrumentation, complicated sample preparation, and the need for well-trained operators. Therefore, there is a growing need for developing a low-cost and portable sensing system for analysis of trace mercury.

Since Ono et al. reported that the Hg(II) ion possessed a unique property to bind specifically to two DNA thymine bases (T) and could form stable thymine Hg(II) thymine (T-Hg(II)-T) base pairs [10], various methods for the detection of Hg(II) ion, based on this property of T-Hg(II)-T coordination chemistry, have been developed in recent years [12-14]. In detail, T-T mismatches in DNA duplexes selectively and strongly capture Hg(II) ion (a binding constant higher than that of AT), and the metalmediated T-Hg(II)-T forms stable DNA duplexes.

In contrast, other metal ions, such as Cu^{2+}, Ni^{2+}, Pb^{2+},Co^{2+},Mn^{2+},Zn^{2+},Pb^{2+},Cd^{2+},Mg^{2+},Ca^{2+},Fe^{2+} and Sn^{2+}, do not show any notable effect on the stability of T-Hg(II)-T DNA duplex [14]. This strategy could provide a high selectivity toward environmental Hg(II) detection over other related heavy metal ions. More recently, a series of Hg(II) DNA biosensors based on T-Hg(II)-T are developed. Kong et al. [15] fabricated an electrochemical label-free biosensor for Hg(II) detection with AuNPs-functionalized rDNA as a signal amplifier, which showed a detection limit of 0.5 nM. Park et al. [16] implemented an electrochemical detection of Hg(II) using graphene oxide as the active indicator with a detection limit of 1 nM. In addition, Niu et al. [17] designed an electrochemical biosensor for Hg(II) determination based on target-induced hybridization and the detection limit of 0.6 nM was observed. A most recent report by Zhuang et al. [18] presented an Hg(II) electrochemical biosensor, which used DNA hairpins as recognition elements and exhibited a detection limit of 2.5 nM. It is indicated that all these electrochemical DNA (E-DNA) biosensors could meet the EPA criterion of Hg(II) detection in drinking water. However, the Hg(II) tends to bio-accumulate in the mammals' body which could gradually accumulate to higher concentrations and eventually cause serious health damage. Therefore, there is an urgent demand to design and develop novel electrochemical biosensors to achieve higher sensitivity for Hg(II) detection.

Electrochemiluminescence (ECL), the generation of an optical signal triggered by an electrochemical reaction has attracte much attention during the past several decades due to its sensitive, simplified optical setup, very low background signal, and good temporal and spatial control, and it has become an important and valuable detection method in biosensors [19]. Ruthenium(II) tris-(bipyridine) ($Ru(bpy)_3^{2+}$) with the advantages of chemical stability, reversible electrochemical behavior, high luminescence efficiency, and high sensitivity under moderate conditions[19] has been widely utilized in a number of bioanalytical arenas, such as immunoassays, DNA analyses, chemical sensing, imaging, and PCR product detection [21-27].

Here, we utilized molecular recognition technique and specific T-Hg(II)-T interaction to construct "off-on"ECL biosensor for mercury(II) detection. The molecular recognition technology, defined as the supramolecular noncovalent interaction between the"host"and"guest"molecules, has played an important role in the chemical sensing field, such as the supramolecular sensor chip for the metal ion detection and the fluorescent DNA detection [28-29]. Cyclodextrins (CDs) are one kind of special oligosaccharides which consist of six, seven, or eight glucose units (named α, β or γ-CD, respectively), and characterized by a toroidal form with a hydrophobic inner cavity and a hydrophilic outer side.

This biosensor employs a ferrocene-labeled hairpin-DNA as probe DNA, in which the ferrocene (Fc) molecular not only can interaction with cyclodextrins [30] but also quench the ECL of the $Ru(bpy)_3^{2+}$ molecule [31-32]. Gold nanoparticles were used as the amplification platform because of their unique properties such as fascinating electrocatalytic activity, large surface area,

excellent conductivity, and stability [33]. As figure 1 showed, the sensing platform was developed by modifying the composite of and followed by immobilizing probe DNA through host-guest recognition between Fc with CD modified on Au nanoparticles. In the absence of Hg(II), retained the single stem-loop structure and Fc quenched the ECL of the $Ru(bpy)_3^{2+}$ molecule. After incubated with Hg(II), the linear-shaped part of probe DNA folds into the T-Hg(II)-T mediated hairpin structure and this conformational change leading the quencher to leave the electrode and resulting in the remarkable increase of the ECL intensity obtained from the resulted electrode in 0.1 M phosphate buffer saline (PBS, pH 7.4) containing tri-n-propylamine (TPA). Thus, the change of ECL intensity indirectly reflected the concentration of Hg(II) ion. This new approach would be a promising Hg(II) ion detection procedure performed directly in the solution.

Figure 1

2. Experimental section

2.1 Materials

Ferrocene-Labeled DNA oligonucleotides(Fc-Probe DNA) were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China) and were purified via C18 reversed-phase HPLC and polyacrylamide gel electrophoresis (PAGE). The sequences of these oligonucleotides employed are given below.

38-mer Fc modified Hairpin Probe DNA: Fc-5′-A TT GT T GTC CCC TCT TCT TTT GGCTG GTGAGAT CAGCC-3′

The β-cyclodextrins were purchased from Wacker Chemical Corporation. The sodium hydroxide, sodium acetate buffer (0.1 M, pH 5.3) and other reagents were commercially available and of analytical reagent grade. 0.1 M phosphate buffer saline (PBS, pH 7.4) containing tri-n-propylamine (TPA) was used as a detecting buffer solution. All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

2.2 Apparatus

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) at room temperature. The electrochemical measurements were recorded with autolab electrochemical workstation (Metrohm Instruments Co., Swiss). All experiments were carried out with a conventional three-electrode system. The working electrode was a 2 mm diameter GCE; Pt wire and an Ag/AgCl electrode with saturated KCl solution served as the counter and reference electrodes, respectively. The trans-mission electron microscopy (TEM) (JEOL,2000FX Japan) and Nuclear Magnetic Resonance Spectrometer-Bruker 400MHZ(Swiss).

2.3 Preparation of the β-cyclodextrin-Functionalized Au Nanoparticles

Per-6-thio-β-cyclodextrin (SH-β-CD) is synthesized according to the literature [34], but step slightly improved (see supporting information).The β-cyclodextrin-Au Nanoparticles (CD-AuNps) were synthesized according to previous literature report with a modification [35]. Generally, 20 mL $HAuCl_4$ (0.6 mM) aqueousdimethyl sulfoxide (H_2O:DMSO=1:4) solution was quickly mixed with another 16 mL of DMSO containing 8.0 mg SH-CD and 60.4 mg $NaBH_4$ with stirring. The reaction mixture was allowed to continue stirring for 24 h at room temperature. Then, 32 mL CH_3CN was added to the reaction mixture and the precipitate was collected by centrifugation. Then the precipitate was washed with 50 mL of CH_3CN:DMSO (1:1, v/v) and 50 mL of ethanol by centrifugation, respectively. The sample was dried under vacuum at 60 ◦C overnight, and stored at room temperature. The SH-CD modified gold nanoparticles were studied with The trans-mission electron microscopy (TEM) (JEOL,2000FX Japan).The diameters of β-cyclodextrin-Functionalized Au Nanoparticles were about 16 nm. (see Supporting Information (SI) for details).

2.4 Fabrication of the ECL-Hg(II) ion Biosensor

A glassy carbon electrode (GCE, 2.0 mm diameter) was polished successively with 0.3, 0.05 μm alumina slurry, followed by rinsing thoroughly with water. The CD-AuNPs-Nafion mixture was prepared by mixing 0.5% Nafion and the CD-AuNPs suspension prepared above (v/v = 1:2) and sonicating for 30 min. Then, 10 μL of this mixture was spincoated onto the surface of the cleaned GCE with a microsyringe and allowed to dry at room temperature to obtain CD-AuNPs/Nafion modified GCE (CD-AuNPs/Nafion/GCE). After that, CD-AuNPs/Nafion/GCE was dipped into 1 mL of 1 mM $Ru(bpy)_3^{2+}$ for 30 min and then thoroughly rinsed with 10 mM phosphate buffer (PBS, pH 7.4) to form an ECL layer of $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE. The ECL Hg(II) biosensor was fabricated by dipping $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE in 0.3 mL of 0.1 mM Fc-Probe DNA solution for 60 min and then washed with 10 mM PBS (pH 7.4) thoroughly. The resulting Fc-Probe/$Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE was then thoroughly washed with 10 mM PBS(pH 7.4) and used as the ECL Hg(II) ion biosensor.

2.5 ECL sensing performance

The fabricated ECL-Hg(II) biosensor was immersed in 100 μL of 10 mM PBS (pH 7.4) containing a fixed concentration of Hg(II) for 40 min, followed by a thorough washing with 10 mM PBS (pH 7.4). A commercial cylindroid glass cell was used as an ECL cell, which contained a conventional three-electrode system consisting of a GCE, or a modified electrode or an ECL-Hg(II) ion biosensor as the working electrode, a platinum plate as the counter electrode, and an Ag/AgCl (saturated KCl) as the reference electrode, respectively. All potentials in this work were referred to the reference electrode. The ECL measurement was performed using a triangular potential scan with the rate of 100 mV/s in 2.0 mL of 0.1 M PBS (pH 7.4) containing 50 mM TPA. The concentration of Hg(II) ion was quantified by an increased ECL intensity ($\Delta I = I_s - I_0$), where I_0 and I_S were the ECL intensity of the ECL-PB biosensor before and after incubation with Hg(II), respectively. All experiments were carried out at room temperature.

3. Results and Discussion

3.1 Characterization of Controllable Solid-State $Ru(bpy)_3^{2+}$-ECL Film

The corresponding ECL intensity-potential curves of the various electrodes in 0.1 M PBS containing 50 mM TPA are presented in Figure 2(A). No ECL signal is obtained on a bare electrode (Figure 2(A) curve a). A strong ECL signal appears when the ECL substrate of $Ru(bpy)_3^{2+}$/CD-AuNPs was immobilized on the electrode (Figure 2(A) curve b), while a sharp decrease of the ECL signal is observed when the quenching monolayer of ferrocene was formed on the $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion electrode (Figure 2(A) curve c). The results verify that the ECL of the $Ru(bpy)_3^{2+}$/CD-AuNPs can be efficiently quenched by the Fc molecule.

The mechanism for quenching ECL has been explained as bimolecular energy or electron transfer between $Ru(bpy)_3^{2+}$ and ferrocerium (Fc^+), the oxidized species of Fc, along with suppression of radical reactions [36].

Obviously, the quenching efficiency of the ECL of $Ru(bpy)_3^{2+}$

is dependent on the coverage of the Fc-Probe DNA on the electrode surface. According to the method suggested by Steel et al. [37-38] the coverage of the DNA on electrode surface can be calculated by measuring the number of cationic redox molecules, such as tris(2,2,-bipyridyl)cobalt(III) perchlorate, electrostatically associated with the anionic DNA backbone. The method is used to estimate the average of surface coverage of the Fc-Probe DNA on the $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE . Figure 2(B) shows the average of surface coverage of the Fc-Probe DNA with self-assembly time for the $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE. The results indicate that the average of surface coverage of the Fc-Probe DNA increases steeply during the first 50 min and then gently on the $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE (Figure 2(B) curve a). As shown in Figure 2(B) curve b, the ECL signals of the Fc-Probe DNA/$Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE electrode are synchronously decreased with the increment of surface coverage of the Fc-Probe DNA. It means that the quenching efficiency of ECL of $Ru(bpy)_3^{2+}$ is dependent on the surface coverage of the Fc-Probe DNA on the electrode surface. Greater than 85% quenching efficiency of ECL of $Ru(bpy)_3^{2+}$ has been obtained when the $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE was assembled in the 1.0 mM Fc-MB for about 1 h.

Figure 2

3.2 Optimization of detection condition

A series of experimental was performed to establish optimal conditions for the ECL detection of Hg(II) ion, including hydrogen ion concentration (pH) and incubation time.

As shown in Figure 3A, the incubation time of ECL sensor with Hg(II) plays an important role in the detection. In the first 40 min, the ECL intensity increased rapidly. Afterward, the ECL signal increased slowly and arrived a platform. Thus, we chose 40 min as the incubation time. (Figure 3A). For PH, the CL intensity increased over the range of 6.5-7.4 and then decreased (Figure 3B). Hence, PH 7.4 was selected for subsequent experiments.

Figure 3

3.3 Analytical Performance of the EC L-Hg(II) ion Biosensor.

The quantitative behavior of this assay under the optimized experimental conditions was assessed by monitoring the dependence of the dependence of the ECL intensity on the concentration of Hg(II). As shown in Figure 4(A), the ECL intensity was proportional to the concentration of Hg(II) ion. The linear curve fitted a regression equation of y =815.4x+9525.2 within a range from 0.02 to 800 ng/mL with a correlation coefficient of R^2 = 0.9958(Figure 4(B)), where I is the ECL intensity and C is the concentration of Hg(II) ion. The limit of detection was 0.1 nM (0.02 ppb) at a signal-to-noise ratio of 3 , which is comparable with most previous assay techniques (Table 1). However, this technique has fewer steps and a shorter assay time than the other techniques.

Figure 4, table 1

The effect of 11 foreign ions including Sn^{2+}, Cd^{2+}, Ba^{2+}, Pb^{2+} ,Ni^{2+} ,Cu^{2+}, Zn^{2+} ,Mg^{2+}, Fe^{3+}, Li^{+} and Sn^{2+} on this new sensor was investigated, respectively. Remarkably, the ECL intensity was not observed with the addition of a number of metal ions at a concentration 2 orders of magnitude higher than the Hg(II) concentrations (Figure 5). These results demonstrate this method has excellent selectivity for Hg(II) over alkali, alkaline earth, and heavy transition-metal ions, owing to the high affinity and specificity of T-Hg(II)-T .Among the various metal ions studied, Hg(II) shows the highest signal gain change compared with the other metal ions.

Figure 5

To demonstrate the applicability of this new ECL sensor for the analysis of Hg(II) ions in traditional chinese medicinal plant samples, a lily sample was collected on the WanZai (JiangXi,China). The sample was prepared and then tested by the proposed technique（see supporting information). No ECL change was observed in this sample,which indicates that Hg(II) ions were not detected. The lily sample was then spiked with Hg(II) ion at different levels, and the recoveries of 800, 100, and 50 ng/mL Hg(II) ion were 104.1±10.22%, 101.2 ± 8.12%, and 99.1±2.28%, respectively. Therefore, the proposed method is particularly attractive for monitoring very low levels of Hg(II) ion in traditional chinese medicinal plant samples.

3.3 Sensor Regeneration

The prepared ECL-Hg(II) biosensor with Fc-Probe DNA were easily regenerated, because the Fc-Probe DNA were anchored to the electrode though molecular-recognition and the simple operation immersing electrode in which containing Fc-Probe DNA solution (0.1 mM) for 60 min could make regenerate the sensors easily. As shown in Figure 6, after being challenged with Hg(II) ion and regenerated for at least 6 cycles, the sensor signal was almost recovered to the original value, suggesting the prepared ECL-Hg(II) biosensor were reuseable.

Figure 6

4. Conclusion

In summary, we completed a “off-on” electrogenerated chemiluminescence (ECL) biosensor for the detection of mercury(II) based on molecular recognition technology. This sensor achieves impressive sensitivity and selectivity, with a detection limit of 0.1 nM and negligible response to other metal ions. It also offers simple operational convenience and cost-effectiveness. In addition, the electrochemical sensor could be easily and rapidly regenerated and could be re-used without any discernible loss in activity.

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Figure

Figure 1,Schematic diagram of the fabrication process of ECL-biosensor for the determination of Hg(II).

Figure 2. (A) ECL intensity-potential profiles obtained at different electrodes in 0.1 M PBS (pH 7.4) containing 50 mM TPA. (a) GCE, (b) $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE,(c)Fc-Probe/$Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE. (B) The average of surface coverage of the Fc-Probe DNA on (a) $Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE with self-assembled time, (b) ECL signals of the Fc-Probe/$Ru(bpy)_3^{2+}$/CD-AuNPs/Nafion/GCE with self-assembled time.

Figure 3. (A) ECL responses to 200 ng/ml Hg(II) ion with different incubation time. (B) ECL responses to 200 ng Hg(II) ion with different PH.

Figure 4. (A) ECL responses to different concentrations of Hg(II) ion. (B) Calibration curve of Hg(II) ion.

Figure 5. Selectivity of the analysis of Hg(II) ion by the method depicted in Scheme 1 with the following metal ions: 1, blank 2, Sn^{2+}; 3, Cd^{2+}; 4, Ba^{2+}; 5, Pb^{2+}; 6, Ni^{2+}; 7, Cu^{2+};

8, Zn^{2+}; 9, Mg^{2+} ; 10, Fe^{3+}; 11, Li^{+}; 12, Sn^{2+}; 13, Hg(II). The concentration of Hg(II) was 100 ng/ml. The concentrations of the other metal ions were 10 μg/ml. The illustrated error bars represent the standard deviations of measurements taken from at least three independent experiments.

Figure 6. Graphics for the regeneration of the ECL-biosensor with the stepwise challenge with the 200 ng/ml Hg(II) then incubated with the Fc-Probe DNA (0.1 mM). N represents the times for the sensor regeneration.

Table 1. Comparison of Sensitivity for Different Hg(II) Ion Assay Methods

Figure 1

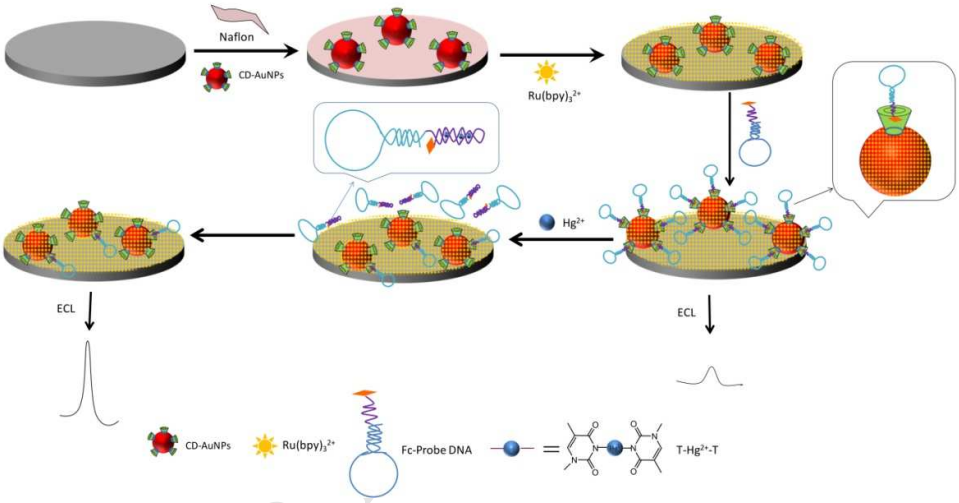

Figure 2

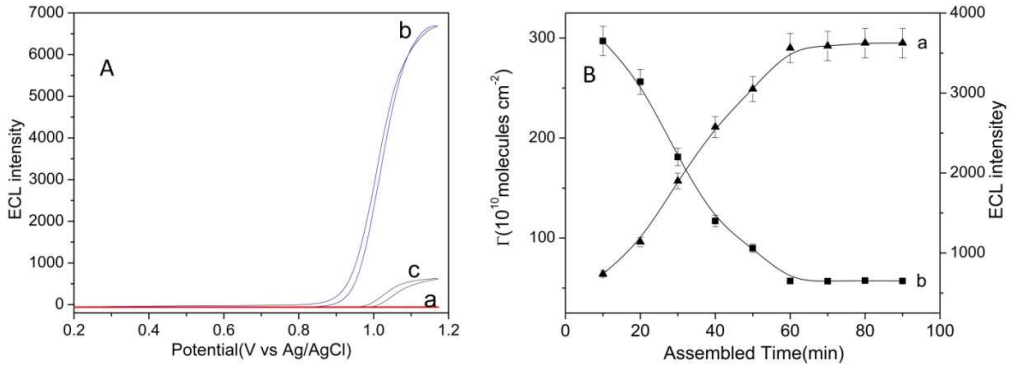

Figure 3

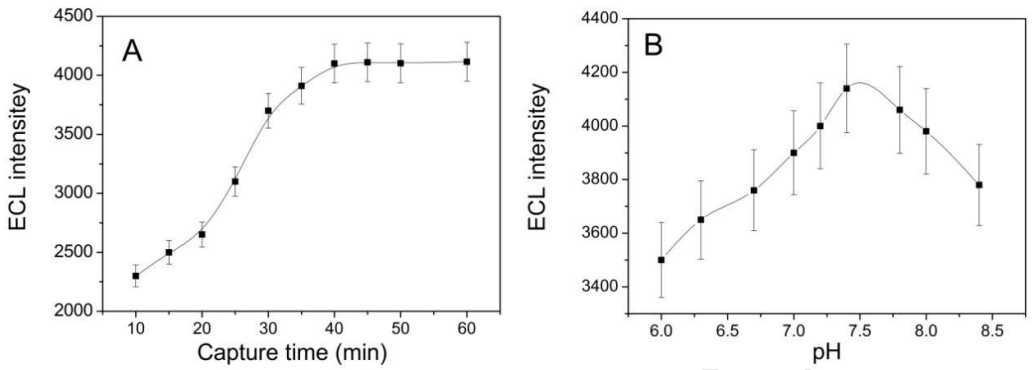

Figure 4

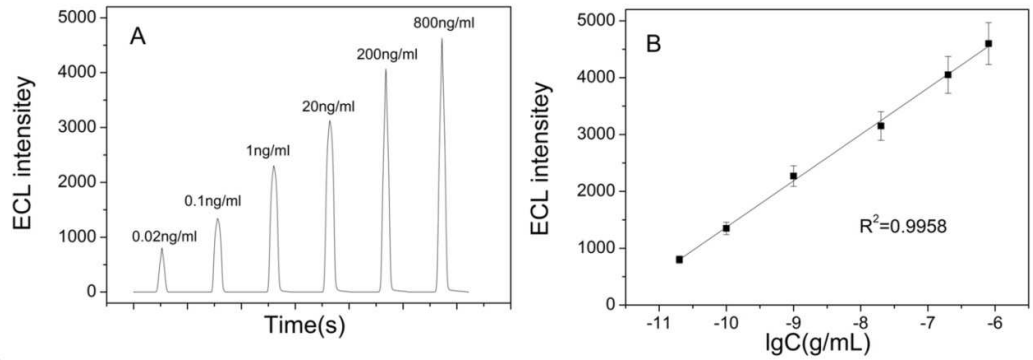

Figure 5

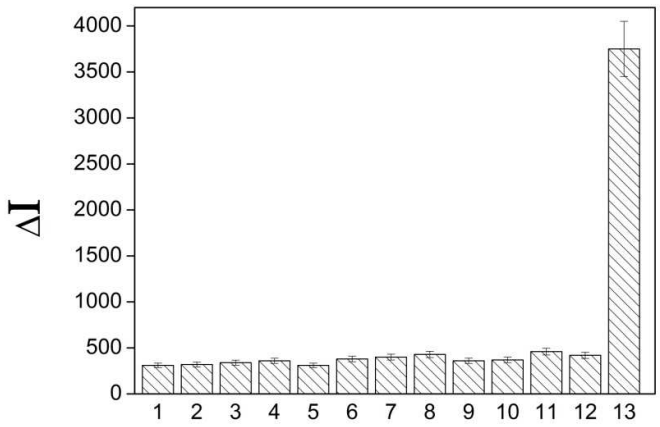

Figure 6

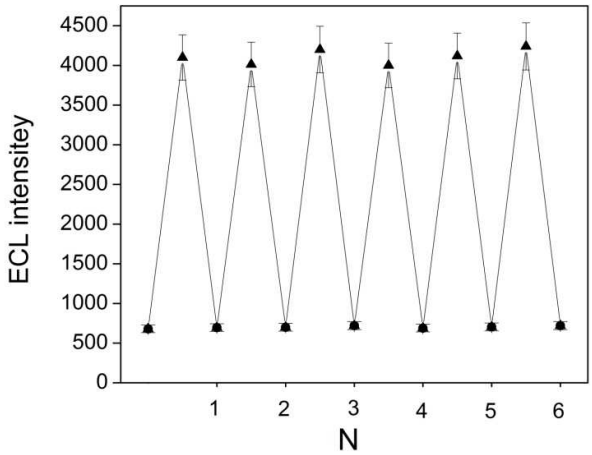

Table 1

analytical method	label	detection limit	Refs
colorimetry	Au NPs	1 nM	[39]
colorimetry	ruthenium complexes	20 ppb	[40]
colorimetry	metal NPs	5 nM	[41]
resonance scattering	Au NPs	1.3 nM	[42]
colorimetry	hemin	50 nM	[12]
fluorescence detection	quantum dot/Au NPs	0.4 ppb	[11]
fluorescence detection	phthalocyanine-T conjugate	32 nM	[43]
fluorescence detection	Au clusters	10 nM	[44]
fluorescence detection	Molecular Beacon	19 nM	[7]
electrochemical	Au NPs	0.5 nM	[13]
electrochemical	Au NPs	0.5 nM	[15]
electrochemical	glucose oxidase	100 pM	[46]
electrochemical	nanoelectrode	0.02 ppb	[47]
hyper-Rayleigh Scattering	Au NPs	5 ppb	[48]
fluorescence detection	seminaphthofluorescein	50 nM	[49]
fluorescent/colorimetric	rhodamine derivative	1 ppb	[50]
Electrochemiluminescence	$Fc\text{-}Ru(bpy)_3^{2+}$ system	0.1 nM	