A "turn-on" electrochemiluminescent biosensor for detecting Hg^{2+} at femtomole level based on the intercalation of $Ru(phen)_3^{2+}$ into ds-DNA†

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A well-designed oligonucleotide functionalized for Hg^{2+} identification and $Ru(phen)_3^{2+}$ intercalation is used to develop a "turn-on" electrochemiluminescent (ECL) biosensor for the determination of Hg^{2+} in a drop (10 μL) of sample.

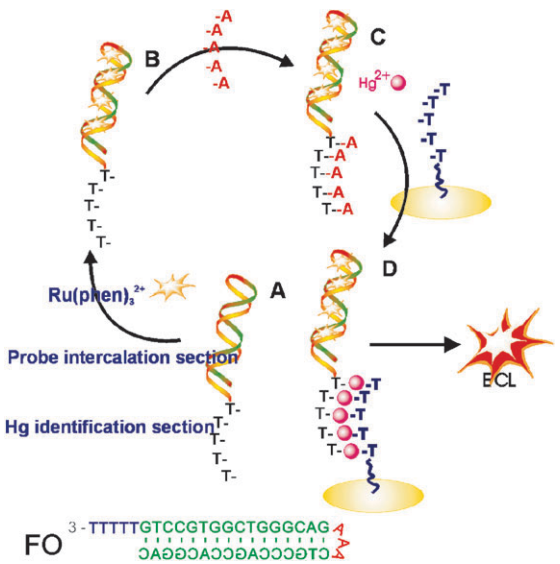
Mercury is one of the most toxic elements that affects human health and is listed in the third place on the "Priority List of Hazardous Substances" as one of 30 "precarious dangerous pollutants".[1] It is commonly incorporated into the human body through drinking water.[1,2] U. S. Environmental Protection Agency regulations state the level of Hg^{2+} in water should be <10 nM[3] and the European Community recommend it less than 5 nM.[4] Although the upper limit for Hg^{2+} concentration in drinking water is 5 nM, the estimated Hg^{2+} concentration in water ranges from 0.02 to 15 nM.[4]

However, the most widely used techniques, inductively coupled plasma optical emission spectrometry (ICP-OES) and cold vapor atomic absorption spectrometry (CV-AAS), have detection limits incompatible with the low concentration of Hg^{2+} in drinking water.[4] ICP-mass spectrometry is sensitive,[5] but it requires expensive instrumentation and suffers from "memory effects".[6] Thus, electrochemistry,[7] neutron activation analysis,[8] luminescence,[2,9] and circular dichroism[10] have been used to develop mercury sensors. Some new materials including quantum dots, DNAzymes, proteins, metal nanoparticles, oligonucleotides,[9] and carbon nanotubes were also applied to probe Hg^{2+}.[10] Although each strategy has its distinct advantages, each also presents unique limitations in terms of sensitivity, selectivity or simplicity.

Here, we report a facile strategy for the construction of a "turn-on" electrochemiluminescence (ECL) biosensor for detecting Hg^{2+} at femtomole level. This sensor was based on the formation of thymidine–Hg^{2+}–thymidine (T–Hg^{2+}–T)[9] and the intercalation of $Ru(phen)_3^{2+}$ (phen = phenanthroline), which acts as ECL probe, into double stranded DNA.[11] The high ECL sensitivity[12] and T–Hg^{2+}–T stability[9] allow the ECL sensor to detect Hg^{2+} with high selectivity (20 000-fold higher for Hg^{2+} than competing metal ions), as well as a very low detection limit (20 pM, corresponding to 0.2 femtomole or 1.2×10^8 Hg^{2+} ions). Besides, only 10 μL sample solution is needed for each assay.

A well-designed functional oligonucleotide (hereafter referred to as FO) containing two functional sections is adopted (Scheme 1) for the mercury sensor. Five thymidine bases (T_5), as Hg^{2+} identification section, was used to form the T–Hg^{2+}–T construction with the immobilized T_5 *via* Hg^{2+} ions. The probe intercalation section was a double stranded (ds)-DNA of sixteen base pairs (bps). Every four bps can embed one $Ru(phen)_3^{2+}$ molecule,[11] so four $Ru(phen)_3^{2+}$ molecules can be intercalated into the probe intercalation section (denoted as FO/$Ru(phen)_3^{2+}$ complex). A piece of oligonucleotide, A_5, was used to pre-form ds-DNA with the Hg^{2+} identification section (Scheme 1C) to avoid the interaction of Hg^{2+} ions and two FOs *via* their Hg identification sections.

To determine Hg^{2+} ions, 10 μL of sample and 5 μL of FO/$Ru(phen)_3^{2+}$ complex were dropped onto the T_5-modified Au electrode. During the incubation period (30 min at 36 °C), T–Hg^{2+}–T construction occurred and the probe, $Ru(phen)_3^{2+}$, was introduced onto the electrode surface (Scheme 1D). ECL emission related to the content of Hg^{2+} ions was observed when the modified electrode was swept from 0 to 1.25 V with 20 mM tripropylamine (TPA) as co-reactant. No chemical syntheses are required for this approach, and consequently it is simple, fast and economical compared with other methods.[9,12a] The "turn-on" mode leads to the sensor not suffering from the problem of false positive and limited signal intensity that are present in the "turn-off" systems.[9c,12a] The stability of the



Scheme 1 Schematic illustration of the ECL biosensor for detecting Hg^{2+}. (A) The functional oligonucleotide (FO) containing Hg^{2+} identification and probe intercalation sections; (B) the intercalation of $Ru(phen)_3^{2+}$ into FO; (C) the preformed A–T duplex; and (D) formation of T–Hg^{2+}–T, which results in the introduction of the $Ru(phen)_3^{2+}$ probe onto the electrode surface for ECL determination of Hg^{2+}.

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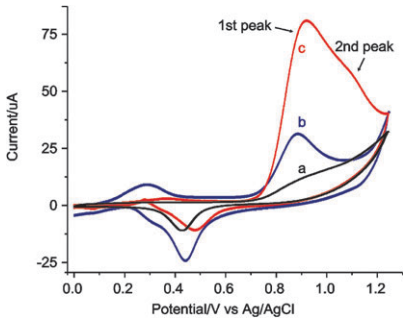

Fig. 1 Cyclic voltammograms at (a) the bare Au electrode; (b) the T_5-modified Au electrode; and (c) after incubation with the functional oligonucleotide and 500 pM Hg^{2+} ions. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA; Scan rate: 50 mV s^{-1}.

FO/$Ru(phen)_3^{2+}$ complex was investigated (Fig. S1–S3, ESI†) and confirmed its practicability.

Cyclic voltammetry (CV) in 0.1 M phosphate buffer saline (PBS, pH 7.5) containing 20 mM TPA was recorded for the modified electrodes for sensing Hg^{2+}. The attachment of T_5 onto the electrode surface increased the anodic current of TPA oxidation at 0.9 V (Fig. 1b). A large increase in the anodic current was observed after the FO/$Ru(phen)_3^{2+}$ complex was attached onto the electrode surface *via* Hg^{2+}. Meanwhile a shoulder peak appears at *ca.* 1.10 V (Fig. 1c), which relates to the oxidation of $Ru(phen)_3^{2+}$. These results confirm that the $Ru(phen)_3^{2+}$ probe is successfully introduced onto the electrode surface by the formation T–Hg^{2+}–T. Earlier study[11a] had indicated that ds-DNA provides a micro-environment to aid oxidation of TPA. It contributes to the improved oxidation current of TPA when the FO is attached onto the electrode surface *via* T–Hg^{2+}–T formation.

CVs at different scan rates was used to investigate the oxidation of TPA and the intercalated $Ru(phen)_3^{2+}$. The anodic peak currents , I_{pc}, of TPA oxidation (1st peak in Fig. 2A) were directly proportional to the square root of the scan rates (Fig. 2B), which indicates TPA oxidation is controlled by diffusion through the DNA film. The oxidation peak of $Ru(phen)_3^{2+}$ (2nd peak in Fig. 2A) appeared after the FO/$Ru(phen)_3^{2+}$ complex was introduced onto the electrode surface. The linear relationship between peak currents and the potential scan rates (Fig. 3C) shows that the $Ru(phen)_3^{2+}$ electrochemistry is a surface-controlled process and that $Ru(phen)_3^{2+}$ is stably intercalated into the ds-DNA section. This is consistent with the result for ECL modified electrodes.[13] For comparison, Fig. S4 (ESI†) presents the CVs of T_5-modified Au electrode at different scan rates. Only an anodic peak appears and no 2nd anodic peak like those in Fig. 2A is observed. The peak current is also lower than that in Fig. 2A at the same scan rate because of less TPA oxidation at the single stranded (ss)-DNA modified electrode.[11a] The intrinsic conductivity of ds-DNA also promoted the oxidation of TPA and $Ru(phen)_3^{2+}$.[12a,14] The improved oxidation of the ECL species and the increase in number of $Ru(phen)_3^{2+}$ probe molecules contribute to the enhanced ECL emission.

The emission signals were recorded with varying concentration of Hg^{2+} ions (Fig. 3). With no Hg^{2+} only a very low ECL response was observed, which was possibly due to the

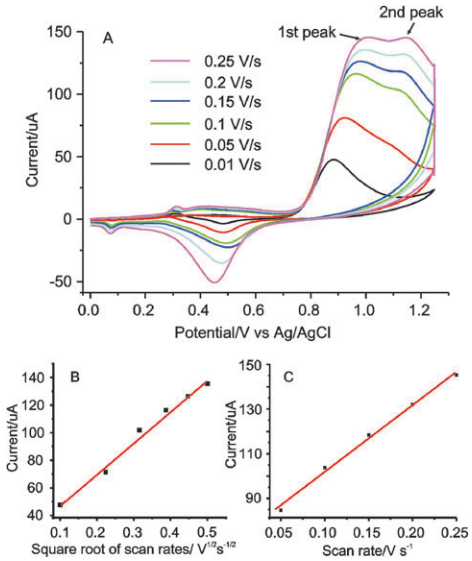

Fig. 2 (A) Cyclic voltammograms of the Au electrode modified with the FO /$Ru(phen)_3^{2+}$ complex *via* Hg^{2+} ions at different scan rates. (B) The relationship between the first anodic peak currents and the square root of scan rate. (C) The relationship between the second anodic peak currents and scan rates. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA.

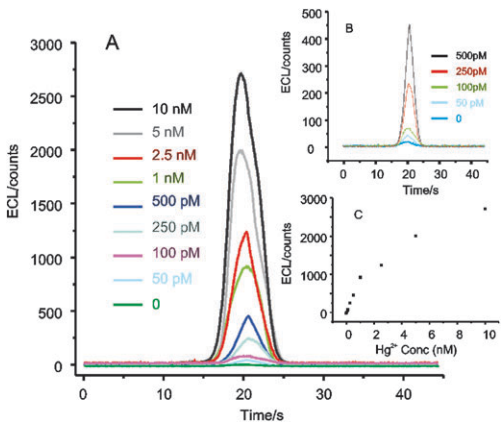

Fig. 3 Sensitivity of the Hg^{2+} biosensor. (A) ECL increase in the presence of varying concentrations of Hg^{2+} ion. (B) ECL responses at low Hg^{2+} ion concentrations. (C) ECL profile for varying concentrations of Hg^{2+}.

non-specific adsorption of the FO/$Ru(phen)_3^{2+}$ complex. A concentration-dependent response was observed from 50 pM to 10 nM and was comparable with Hg^{2+} levels in water.[4] The sigmoid shape in Fig. 3C suggested that the binding of Hg^{2+} ion on the electrode surface was a cooperative process.[9a] A detection limit of 20 pM or 4 ng Hg^{2+} L^{-1} is achieved. A precision (RSD, $n = 6$) of 2.1% was obtained (Fig. S5, ESI†), which indicates the ECL biosensor has good reproducibility. A comparison of the detection limits of various Hg^{2+} ions sensors based on T–Hg^{2+}–T is listed in Table S1 (ESI†). Among all the biosensors, this ECL strategy presents the lowest detection limits with a simple construction.

To test the selectivity, the ECL responses in the presence of competing metal ions were recorded (Fig. 4). Although there is no evidence that these ions are inserted into the T–T base pair,

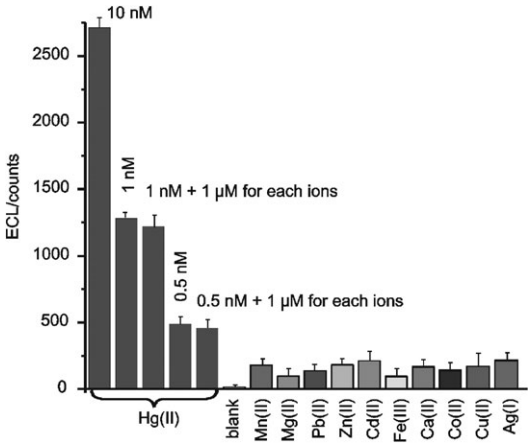

Fig. 4 Selectivity of the Hg^{2+} ion biosensor. All competing ion solutions were 10 μM. As a comparison, the responses of 0.5, 1.0, 10 nM Hg^{2+} ions, and mixtures of different concentrations of Hg^{2+} with 1 μM for each ions are presented.

they produce high responses over the blank at 10 μM level, and this is particularly evident for Cd^{2+}, Cu^{2+}, Mn^{2+}, Ag^{+} and Zn^{2+} ions. However, none of 10 μM metal ions gave the ECL emission higher than one third of that produced by 0.5 nM Hg^{2+} ions. Consequently, the selectivity was at least 20 000-fold higher for Hg^{2+} ion than the competing metal ions. Very similar ECL emission was observed for 1 or 0.5 nM with and without the mixture of competing ions (at their 1 μM level), which indicates this sensor can be applied in a complex solution. Because of the high selectivity of the biosensor for Hg^{2+} ions, a chelating reagent is not needed to overcome the issue of interference.[15] Before determination, the FO/$Ru(phen)_3^{2+}$-modified electrode was thoroughly cleaned to remove the matrix from the electrode surface. Therefore, this sensor does not suffer environmental interference, which is often encountered during fluorescence sensing of Hg^{2+}.[9]

The accuracy of the biosensor was demonstrated by comparison of the results obtained with it and those from CV-AAS. The results were in good agreement with those obtained by CV-AAS (Table S2, ESI†), showing the practicability of the proposed method for real samples. The recoveries ranged from 95.2 to 104% for samples spiked with Hg^{2+} ions and no interference was encountered from these sample matrices. Comparing with the CV-AAS method, the proposed biosensor needs only a low sample volume (10 μL) to determine Hg^{2+} concentration and the procedure is simple and compact.

In summary, an ECL biosensor for Hg^{2+} ions with high sensitivity and good selectivity was developed using a well-designed oligonucleotide for Hg^{2+} identification and probe intercalation. Improved electrochemistry of the ECL species and the increase in probe molecule number resulted in a detection limit of 20 pM, which is lower than that obtained in previous reports for Hg^{2+} sensing. Its simple construction and sample pretreatment, as well as low sample volume makes it suitable for field analysis. With the developed metal–base pairs,[16] this protocol can be easily extended to the analysis of other metal ions.

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