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Electrochemiluminescent detection of Hg(II) by exploiting the differences in the adsorption of free T-rich oligomers and Hg(II) loaded T-rich oligomers on silica nanoparticles doped with Ru(bpy)$_3$$^{2+}$

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Abstract The detection scheme for Hg(II) ions described here is based on the finding that nanoparticles (NPs) prepared from silica and chitosan, and doped with the ruthenium(II) bipyridyl complex [Ru(bpy)$_3$$^{2+}$] display large differences in their affinity for T-rich oligomers and Hg(II)-loaded T-rich oligomers. The effect was exploited to design a sensitive electrochemiluminescence (ECL) based assay for Hg(II). In the absence of Hg(II), the oligomers (with 40 bases) strongly bind to the surface of the NPs. Such oligomer-loaded NPs do not bind to a glass carbon electrode (GCE) modified with carbon nanotubes (CNT) in a nafion host matrix. If, however, Hg(II) is present, it will cause the formation of Hg(II)-loaded double-stranded oligomers via T-Hg-T duplexes which are not easily adsorbed on the doped silica NPs. Such NPs are readily assembled on the GCE and this will result in strong ECL. The increase in ECL intensity is a viable parameter for detection of Hg(II) with a detection limit as low as 0.3 pM. Such as sensitivity has not been accomplished in previously reported ECL based methods for detecting Hg(II). In our perception, the method based on differential adsorption presented here is not limited to the detection of Hg(II) but represents a method of wider scope in that other analytes may be detected by this method if respective oligomer probes are available.

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Introduction

Mercury ions, the most stable form of inorganic mercury, are highly toxic environmental pollutants and can cause serious human health problems, even at a low concentration level [1]. Therefore, it is highly desirable to develop sensitive methods for the detection of Hg(II). Indeed, many traditional methods including atomic absorption spectroscopy (AAS) [2], cold vapor atomic fluorescence spectrometry (CV-AFS) [3], and inductively coupled plasma-mass spectroscopy (ICP-MS) [4], etc., have been successfully used to detect Hg(II) in different samples. Although showing high sensitivity, these methods generally involve extensive sample pretreatment processes and bulky instrumentation. These significantly limit their applications in routine monitoring.

The coordinative interaction between Hg(II) and bisthymine demonstrated by Ono and Togashi [5] has attracted significant interest and been widely applied in sensing and molecular nano-architectures. Previous work [6, 7] demonstrated Hg(II) can specifically bind to such a DNA to form a T-Hg(II)-T complex. A large number of sensors were designed for Hg^{2+} based on T-Hg^{2+}-T coordination chemistry [8–11]. Up to now, many signal readout methods such as electrochemiluminescence [12–14], fluorescence [15–17], colorimetric [18–20] and electrochemical [21–25] method, etc., have been designed to sensitively detect Hg^{2+}. Of these methods, the ECL signal readout method is promising since it owns the simple equipment, high detection sensitivity,

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versatility and robustness. Furthermore, the application of ECL biosensor for Hg^{2+} combines the advantages of the selectivity of the T-rich DNA probe recognition Hg^{2+} reactions and the high sensitivity of ECL technique. For example, Yin et al. developed an ECL sensor for Hg^{2+} by immobilizing T-rich DNA probe on electrode and using the DNA/Ru(phen)$_3$$^{2+}$ (phen = phenanthroline) conjugate as multiple ECL labels [26]. This ECL sensor not only showed higher selectivity but also presented a 20 pM detection limit for Hg^{2+}. Thereafter, Xu's group reported a label-free supersandwich ECL method for the detecting sub-nanomolar Hg^{2+} by immobilizing T-rich capture DNA probe on electrode [27]. Although more ECL signal molecules were bound to electrode by intercalation in DNA, no further improvement on detection limit was achieved because of the interstitial space from ECL signal reporter to electrode surface. In addition, Ma et al. developed an extremely sensitive ECL sensor for Hg^{2+} and a 2.4 pM detection limit for Hg^{2+} was obtained [28]. This method required labeling Ru(bpy)$_3$$^{2+}$/G4 PAMAM dendrimer conjugates on T-rich DNA probe and further immobilizing this ECL active DNA probes on electrode. However, all of these reported ECL sensors need to either label or immobilize T-rich DNA probe procedures. These operations not only were complicated, time-consuming and expensive, but also affected the performances of the DNA probe binding to Hg^{2+}. Therefore, it is desirable to develop a simple, label-free, immobilization-free ECL method for detecting Hg(II).

Herein, the two differential adsorption effects of the doped silica NPs with T-rich oligomers (DNA) or Hg(II)-loaded T-rich oligomers (DNA/Hg) and with Nafion/CNT modified GCE were explored to design a ECL scheme for Hg. Compared to the previously ECL schemes for Hg, on one hand, the scheme is simple and rapid because the DNA probe was label-free, immobilization-free and the reaction of DNA probe with Hg was proceeded in homogeneous solution. On the other hand, due to the pre-concentrating effect of ECL active NPs on modified GCE, the method is also sensitive. The increase in ECL intensity was proportion to the Hg(II) ion concentration. Based on this ECL signal difference, the ECL method was successfully used to detect Hg(II) in simple sample.

Experimental

Materials and reagents

Cyclohexane, ammonia solution (25–28 wt %), acetone, silver nitrate and ethanol were purchased from Xian Chemical Reagent Factory (http://weinan0827.11467. com); n-hexanol was provided by Tianjin Chemical Reagent Factory. Tetraethoxysilane (TEOS, 99 %), chitosan, Tris-(2,2′-bipyridyl)-dichlororuthenium (II) hexahydrate, Triton X-100 and Nafion 117 (~5 % in a mixture of lower aliphatic alcohols and water) were purchased from Sigma-Aldrich (http://www.sigmaaldrich.com). The sequence of mercury ion aptamer (DNA) was 5′-GTT GTT CTT CCT TTG TTT CCC CTT TCT TTG GTT GTT CTT C-3′ and was obtained from Shenggong Bioengineering Ltd. (Shanghai, China, http://www.sangon.com). Phosphate buffer (10 mM) was prepared by mixing an appropriate content of 200 mM Na$_2$HPO$_4$ and 200 mM NaH$_2$PO$_4$. The composition of DNA stock solution buffer was 10 mM phosphate buffer (pH 7.4). The work solution of the oligonucleotide was obtained by diluting the stock solution with a 10 mM phosphate buffer (pH 7.4). Ammonium persulphate (APS), 29:1 acrylamide/bis-acrylamide 30 % gel stock solution, 5 × TBE (Tris-borate-EDTA) buffer, and N,N,N',N'-tetramethylethylenediamine (TEMED) were provided by Shenggong Bioengineering Ltd. (Shanghai, China, http://www.sangon.com). Formaldehyde solution was obtained from Tianli Chemical Reagent Factory (http://tjdongli05315.11467.com). Sodium hydroxide and acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd (http://www.reagent.com.cn). Mercury(II) nitrate (Hg(NO$_3$)$_2$) was purchased from Shanghai Reagent Factory (China). Other metal ion solutions were prepared from nitrate salts. In addition to these, all other reagents were of analytical grade, and the aqueous solutions were prepared with ultrapure water (Milli-Q water, 18.2 MΩ).

Apparatus

Cyclic voltammetric measurements were recorded by using a CHI420b electrochemical workstation (Shanghai Chenhua CHI Instruments Inc., China, http://www.chinstr.com). All electrochemical measurements were performed with a conventional three-electrode system consisting of modified glassy carbon working electrode, Pt wire counter electrode and Ag/AgCl (saturated KCl) reference electrode. The ECL signals were conducted by a chemiluminescence analyzer (IFFM-D, Xi'an Remax Electronic Science and Technology Co., Ltd. China, http://remax.testmart.cn). Transmission Electron Microscopy (TEM) image of the SRCNPs was carried out by using a JEM-2100 (Japan, http://www.jeol.co.jp/en). A JASCO J-815 spectrometer (Applied Photophysics Ltd., England) was utilized to record CD spectra of the solution consisting of 1 μM DNA in the absence or presence of 1 mM Hg^{2+} in the wavelength range from 320 to 220 nm at room temperature. A multiposition magnetic stirrer (IKA, Germany, http://www.ika.cn) and high-speed centrifuge (5804 R, Germany, http://www.eppendorf.cn) were used for stirring the synthesis of SRCNPs and separating nanoparticles, respectively. Atomic fluorescence measurements were performed on an atomic fluorescence spectrometer (AFS-

2202E) (Beijing, China). Gel electrophoresis experiments were carried out by using a gel electrophoresis apparatus (Beijing, China, http://www.ly.com.cn).

Synthesis of silica nanoparticles doped with Ru(bpy)$_3^{2+}$ and chitosan

Silica nanoparticles doped with Ru(bpy)$_3^{2+}$ and chitosan (SRCNPs) were synthesized according to previously published water-in-oil (W/O) microemulsion method with minor modifications [29]. A mixture of 1.80 mL of Triton X-100, 1.80 mL of 1-hexanol, and 7.50 mL of cyclohexane was vigorously stirred in a multiposition magnetic stirrer. Then, 300 μL of distilled water was added to form a transparent W/O microemulsion system. Next, 100 μL of 0.1 % chitosan in 1.0 % v/v acetic acid was added to the microemulsion. After vigorous shaking of the mixture for 30 min to promote a uniform mixing, 50 μL 0.01 M Ru(bpy)$_3^{2+}$ aqueous solution was added into the mixture. Afterward, the mixed solution was stirred for 1 h, followed by adding 34 μL 0.1 M NaOH into the system to obtain neutral ambient conditions. Next, 90 μL of tetraethylorthosilicate (TEOS) was added gradually under stirring into the solution, followed by the addition of 60 μL of NH$_4$OH to initiate the polymerization reaction. The resulting mixture was allowed to proceed for 24 h with magnetic stirring to form the SRCNPs. To separate SRCNPs from the microemulsion, acetone was added into the synthesized solution. After washing with ethanol and water three times, respectively, the obtained SRCNPs were centrifuged at 2057 rcf for 10 min to eliminate bigger particles from the solution. Next, the supernatant obtained was further centrifuged at 7200 rcf to get the resulting NPs. The prepared SRCNPs were dispersed into 2 mL of ultrapure water by vortex and stored in the 4 °C refrigerator before use.

Preparation of modified GCE

The GCE with 3 mm in diameter was treated with alumina slurry (0.3 and 0.05 μm Al$_2$O$_3$) to obtain a mirror smoothness, followed by sonication in ethanol and ultrapure water in turn for 5 min. Coating the well-polished electrode with a Nafion/CNT film was achieved by dropping 10 μL of Nafion/CNT solution onto the surface of the pretreated GCE and evaporated at ambient temperature.

Polyacrylamide gel electrophoresis

To obtain a 15 % hydrogel, 5 mL 30 % gel solution (29:1), 1 mL TBE buffer (5×), 100 μL APS (10 %), 10 μL TEMED, and 3.89 mL deionized water were mixed. The gel was allowed to polymerize for 3 h at ambient temperature and then soaked in 1 × TBE buffer (pH 8.0) for use. 10 μL of each sample was mixed with 2 μL of loading buffer (6×) and

loaded into 15 % native polyacrylamide gel electrophoresis (PAGE). The PAGE was carried out in 1 × TBE buffer at a constant voltage of 120 V for about 52 min at room temperature. After silver staining for 15 min, the gels were imaged with a gel imaging system.

Procedures for Hg^{2+} detection

Prior to the experiment, all the DNA oligonucleotides were heated to 90 °C for 5 min in dry block heater and then allowed to cool slowly to room temperature. 30 μL aliquot of 10 nM DNA was mixed uniformly with 30 μL various levels of concentration of target Hg(II) solution in 10 mM phosphate buffer (pH 7.4) by vortex, which was incubated for 30 min. Next, 60 μL of the synthesized SRCNPs solution was added into 60 μL of the mixture solution and carried out for 35 min. Subsequently, the modified GCE was dipped into the abovementioned reaction solution, followed by thoroughly rinsing for the follow-up ECL characterization.

ECL measurement procedure

The resulting electrode was inserted in phosphate buffer (pH 7.4) containing 5 μmol·L^{-1} TPA. The ECL responses related to Hg(II) concentrations were recorded when the scan potential was applied from 0.5 to 1.3 V with a scan rate of 100 mV·s^{-1}. The voltage of the pMT was set at −800 V in the process of detection.

Results and discussion

The differential adsorption of silica nanoparticles doped with Ru(bpy)$_3^{2+}$ and chitosan for free T-rich oligomers and Hg(II) loaded T-rich oligomers

The formation of T–Hg–T duplex complex was firstly measured by CD spectra and the result was shown in Fig. S1 of the Supporting Information (SI). Secondly, the morphology and optical characteristics of the synthesized SRCNPs were characterized by TEM, UV–vis and fluorescence spectra methods (as shown in Fig. S2 and Fig. S3 of SI). Lastly, the interaction of DNA probes or DNA/Hg complexes with SRCNPs was studied using gel-electrophoresis. As can be seen in the experimental results (shown in Fig. 1), the bands in lanes 1 and 2 corresponded to DNA probes and the mixture of DNA probes with SRCNPs, respectively. Compared with the lane 1, there was no band appeared in lane 2. This result indicated that most of DNA probes were adsorbed onto the surface of SRCNPs through the super-molecular interaction between chitosan in SRCNPs and DNA probe bases. In contrast, the bands in lanes 3 and 4 corresponded to the DNA/Hg complex and the mixture of this duplex complex with SRCNPs, respectively. The
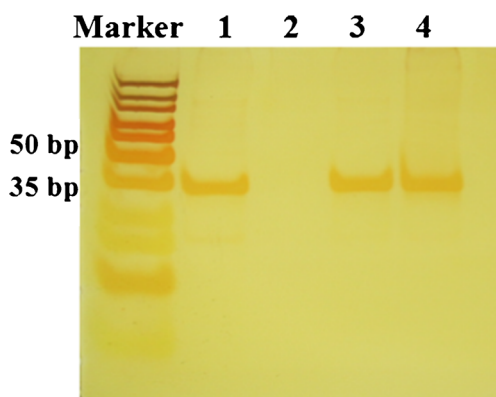
Fig. 1 PAGE analysis of the recognition process after the introduction of DNA or DNA/Hg duplex into SRCNPs solution. 1:DNA; 2:DNA+ SRCNPs; 3:DNA+Hg^{2+}; 4:DNA+Hg^{2+}+SRCNPs. DNA concentration: 2 μM, Hg(II) concentration: 200 μM

Scheme 1 Representation of the interaction of ssDNA probes or ssDNA/ Hg complexes with SRCNPs

bands in lane 3 and lane 4 appeared at the same position. This showed that the SRCNPs did not change the gel electrophoresis behavior of DNA/Hg complex and obviously suggested that the DNA/Hg complexes were not adsorbed on the surface of SRCNPs. All of these results mentioned above indicated that there was large adsorption difference for DNA probes from DNA/Hg complexes on SRCNPs surface.

These phenomena can be explained as followings: the ssDNA probes contained un-paired bases, so they easily interacted with the amino and hydroxy groups of SRCNPs to form SRCNPs/DNA nanocomposites via hydrogen bond, electrostatic, and hydrophobic. On the contrary, upon addition of Hg(II) to ssDNA solution, it reacted with ssDNA to form very stable double-strand T-Hg-T complex. DNA/Hg duplex interacted with SRCNPs through exposed backbone phosphates of dsDNA and the amino and hydroxy groups of SRCNPs. In comparison with un-paired bases of ssDNA, this intermolecular force was much weaker, resulting in most of SRCNPs existing in their free state.

So the possible interaction procedure of ssDNA probes or ssDNA/Hg complexes with SRCNPs can be described with the scheme 1.

The differential adsorption of two different silica nanoparticles doped with Ru(bpy)$_3^{2+}$ and chitosan at modified GCE

In our test, after ssDNA or ssDNA/Hg(II) interacted with suitable amount of SRCNPs, the modified GCE was dipped into this solution to achieve the adsorption of SRCNPs on electrode surface. Thereafter, the resulting electrode was characterized by ECL in phosphate buffer (pH 7.4) containing 5 μmol·L^{-1} TPA. The interaction of two different nanoparticles (SRCNPs and SRCNPs/DNA) with modified GCE was explored by ECL (as shown in Fig. 2).

As can be seen in Fig. 2a, a weak ECL signal was observed. This result indicated that very little SRCNPs were adsorbed on
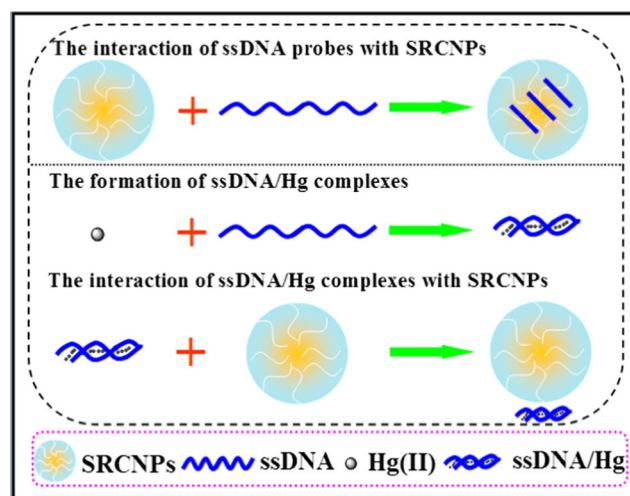
the modified electrode. In contrast, in the same experimental procedure but 10 nM ssDNA probes firstly reacted with Hg(II) ions to form the ssDNA/Hg complexes, the resulting electrode presented much stronger ECL signal (as shown in Fig. 2b). These results suggested that, in this case, there were lots of SRCNPs adsorbed on the modified electrode surface.

This can be explained by the multi-negative charge of DNA which renders the SRCNPs/DNA nanocomposites also negatively charged. Thus, the electrostatic repulsive force between Nafion film and SRCNPs/DNA nanocomposites reduced the adsorption of nanocomposites onto the surface of modified electrode and produced the weak ECL signal. On the contrary, upon addition of Hg(II) to ssDNA solution, the free state SRCNPs were easily adsorbed on the surface of modified GCE [30], causing the increase of ECL signal.
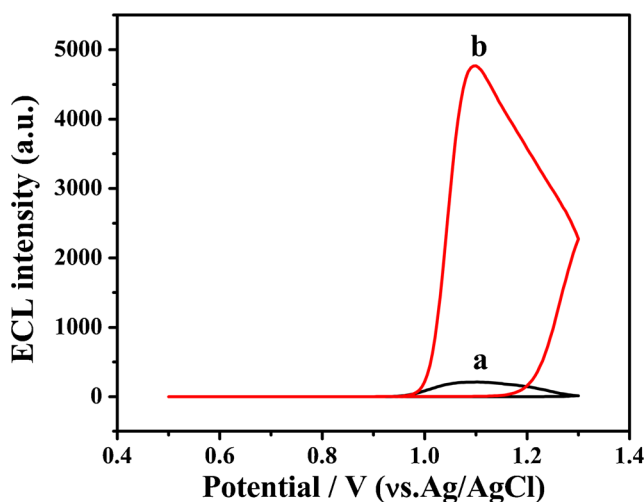

Fig. 2 ECL response performance of different modified electrodes: (a) the modified electrode with the mixture of SRCNPs and ssDNA probes, (b) the modified electrode with the mixture of SRCNPs and ssDNA/Hg complexes

On the basis of this great difference in ECL signals in the absence and presence of Hg(II), we can develop a label-free, immobilization-free ECL sensing scheme for the detection of mercury ion. Scheme 2 described here the sensing mechanism for mercury ion.

Optimization of method

The following parameters were optimized: (a) Concentration of SRCNPs; (b) Effect of incubation time of ssDNA and Hg(II); (c) Effect of the interaction time between SRCNPs and ssDNA probes or ssDNA/Hg complexes; (d) The assembled time of the mixture solution of ssDNA probe or ssDNA/Hg^{2+} duplex with SRCNPs on the modified GCE; (e) Effect of the concentration of chitosan. Respective data and Figures were given in Fig. S4, Fig. S5, Fig. S6, Fig. S7 and Fig. S8 of SI. The following experimental conditions were found to give best results: (a) An SRCNPs concentration of 0.56 mg·mL^{-1}; (b) Incubation time (between ssDNA and Hg(II)) of 30 min; (c) Interaction time (between SRCNPs and ssDNA probes or ssDNA/Hg complexes) of 35 min; (d) The assembled time of 45 min; (e) An chitosan concentration of 0.1 %.

Analytical performances

Under optimal assay conditions, the relationship between the ECL intensity and the concentration of target Hg(II) was investigated. It was found that the ECL difference ($\triangle I_{ECL}$ = I - I$_0$) of the two events (before and after the DNA interacted with Hg(II)) was linear with the concentration of Hg(II) in the range from 1.0 to 9.0 and 10 to 90 pM (Fig. 3a). The $\triangle I_{ECL}$-C calibration curve was built to realize quantitative determination for Hg(II). The relationships between $\triangle I_{ECL}$ and concentration of Hg(II) were shown in Table 1. A detection limit of 0.3 pM (S/N=3) for Hg(II) was obtained. The analytical properties of our method were
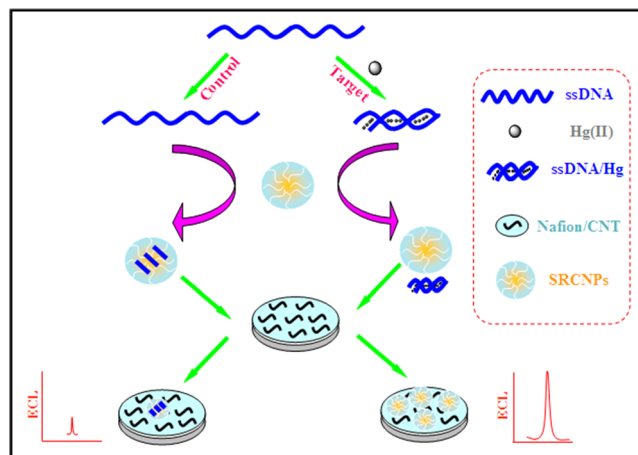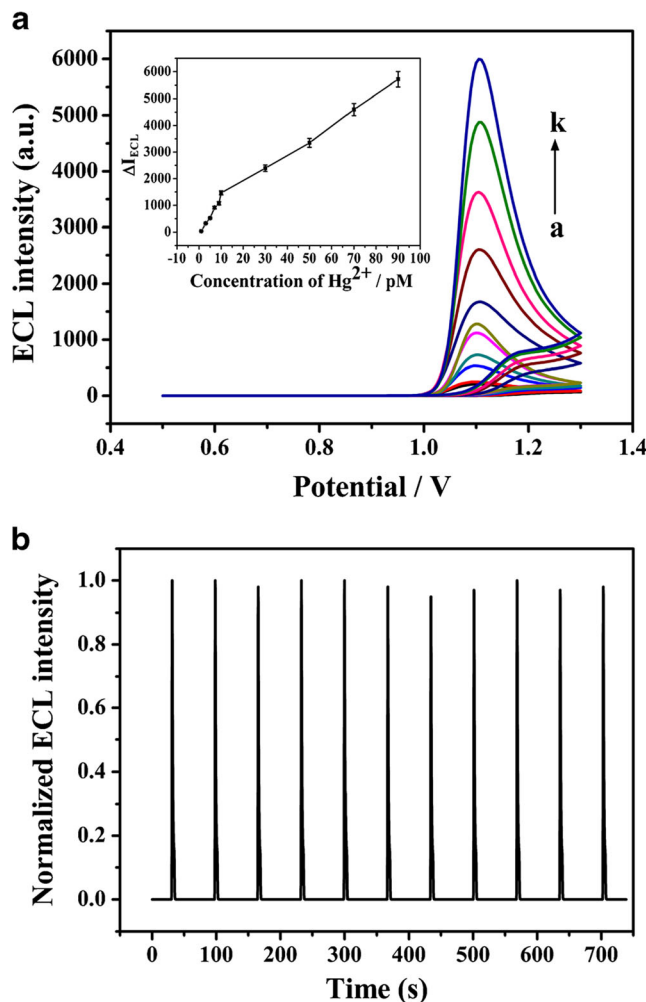

Scheme 2 The ECL sensing scheme

Fig. 3 **a** ECL responses of the modified electrode to 10 nM DNA probe in the presence of different concentration of target Hg(II): (**a**) 0 pM, (**b**) 1.0 pM, (**c**) 3.0 pM, (**d**) 5.0 pM, (**e**) 7.0 pM, (**f**) 9.0 pM, (**g**) 10 pM, (**h**) 30 pM, (**i**) 50 pM, (**j**) 70 pM, (**k**) 90 pM in phosphate buffer (pH 7.4) containing 5 μmol·L^{-1} TPA. Scan rate: 100 mV·s^{-1}. The resulting calibration plot of insert was $\triangle I_{ECL}$-C. **b** Successively cyclic ECL curves of SRCNPs modified GCE in phosphate buffer (pH 7.4) containing 5 μmol·L^{-1} TPA

compared with previously reported other methods (Table S1). Apparently, the detection limit of our method was comparable with those of other Hg detection methods.

Moreover, for testing the stabilization of this ECL sensing method, eleven ECL measurements of the SRCNPs modified GCE upon continuous cyclic scans showed constant signals (Fig. 3b). The results clearly indicated that the approach possessed good stability.

Table 1 Linear equations between $\triangle I_{ECL}$ and concentration of Hg(II)

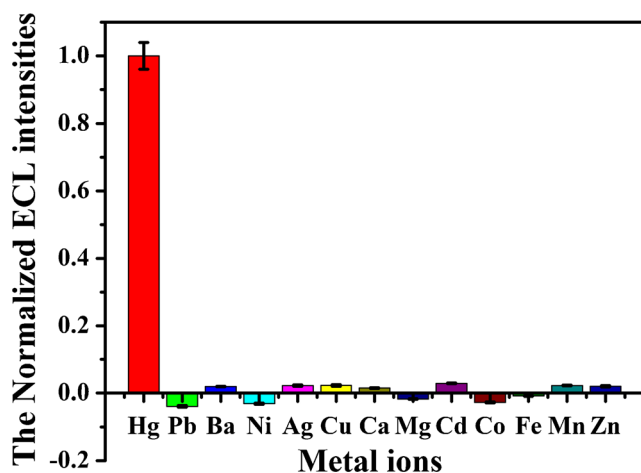| concn range of Hg^{2+} /pM	Linear equation	R
1.0 to 9.0	$\triangle I_{ECL}$ = −90.20 + 132.71 C	0.9910
10 to 90	$\triangle I_{ECL}$ = 827.28 + 53.53 C	0.9975

Fig. 4 The selectivity of ECL sensing scheme for Hg(II) in the presence of other metal ions (the concentrations of Hg(II) was 5 pM, other metal ions was 500 pM)

Finally, to assess the selectivity of the presented method for Hg(II) detection, a mixed solution (5.0 pM Hg(II) coexisted with 500 pM other metal ions including Pb^{2+}, Ba^{2+}, Ni^{2+}, Ag^{+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, respectively) were tested using the same process as described before. It was worthwhile to notice that very little potential interference from other metal ions was observed, which showed the method displayed good selectivity against other relevant metal ions (Fig. 4). These results indicate that this approach can be applied to real environmental samples.

Determination of Hg(II) in water samples

To further evaluate the practical application of the method in actual samples analysis, freshwater sample (tap water) was used to analyze. Firstly, the tap water sample was collected after discharging tap water for about 20 min and boiled for 5 min to remove chlorine [18]. Secondly, the sample collected was filtered through a 0.22 μm membrane (Millipore) beforehand to remove impurities. These samples spiked with Hg^{2+}, with concentration of 0 and 80.0 pM, were detected and made a comparison between the method and AFS (atomic fluorescence spectrometer). The results deriving from the average of three determinations were shown in Table 2. Based on the

Table 2 Detection of Hg(II) in water sample using the method and AFS

Hg^{2+} (pM)

Sample	Added	ECL method mean[a] ± SD[b]	AFS mean[a] ± SD[b]	Recovery(%)
Tap water 1	0	3.1 ± 0.4	N.d.	
Tap water 2	80.0	81.7 ± 2.1	81.2 ± 2.0	98

[a] Mean of three measurements. [b] SD: standard deviation. N.d.: not detectable

comparison, the results obtained by the method showed good agreement with the found values measured by AFS.

Conclusion

In summary, by taking advantage of differential adsorption effect and the T-rich DNA probe recognition Hg^{2+} as the model system, we developed a label-free, immobilization-free and sensitive ECL scheme to detect Hg(II). This scheme does not involve any chemical labeling and immobilization of DNA probe and therefore is much more convenient and low-cost. In addition, this scheme presented here also may in principle pave the way for detecting other analytes. However, the ECL method can be used to detect targets in simple sample, which may not be suitable for the determination of complex samples.

Compliance with ethical standards The authors declare that they have no competing interests.

References

1. Nolan E, Lippard S (2008) Tools and tactics for the optical detection of mercuric ion. Chem Rev 163:3443–3480
2. Kunkel R, Manahan S (1973) Atomic absorption analysis of strong heavy metal chelating agents in water and waste water. Anal Chem 45:1465–1468
3. Geng W, Nakajima T, Takanashi H, Ohki A (2008) Determination of mercury in ash and soil samples by oxygen flask combustion method-Cold vapor atomic fluorescence spectrometry (CVAFS). Hazard Mater 154:325–330
4. Bings N, Bogaerts A, Broekaert J (2006) Atomic spectroscopy. Anal Chem 78:3917–3946
5. Ono A, Togashi H (2004) Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions. Angew Chem Int Ed 43:4300–4302
6. Miyake Y, Togashi H, Tashiro M, Yamaguchi H, Oda S, Kudo M, Tanaka Y, Kondo Y, Sawa R, Fujimoto T, Machinami T, Ono A (2006) Mercury[II]-mediated formation of thymine-Hg[II]-thymine base pairs in DNA duplexes. J Am Chem Soc 128:2172–2173
7. Zhang J, Tang Y, Lv J, Fang S, Tang D (2015) Glucometer-based quantitative determination of Hg(II) using gold particle encapsulated invertase and strong thymine-Hg(II)-thymine interaction for signal amplification. Microchim Acta 182:1153–1159
8. Lin Z, Li X, Kraatz H (2011) Impedimetric immobilized DNA-based sensor for simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+}. Anal Chem 83:6896–6901
9. Zhang J, Huang W, Xie W, Wen T, Luo H, Li N (2012) Highly sensitive, selective, and rapid fluorescence Hg^{2+} sensor based on DNA duplexes of poly (dT) and graphene oxide. Analyst 137:3300–3305
10. Huang D, Niu C, Wang X, Lv X, Zeng G (2013) "Turn-On" fluorescent sensor for Hg^{2+} based on single-stranded DNA

functionalized Mn:CdS/ZnS quantum dots and gold nanoparticles by time-gated mode. Anal Chem 85:1164–1170

11. Gan X, Zhao H, Chen S, Quan X (2015) Electrochemical DNA sensor for specific detection of picomolar Hg(II) based on exonuclease III-assisted recycling signal amplification. Analyst 140:2029–2036

12. Yu Y, Lu C, Zhang M (2015) Gold Nanoclusters@ $Ru(bpy)_3^{2+}$-layered double hydroxide ultrathin film as a cathodic electrochemiluminescence resonance energy transfer probe. Anal Chem 87:8026–8032

13. Lin X, Luo F, Zheng L, Gao G, Chi Y (2015) Fast, sensitive, and selective ion-triggered disassembly and release based on tris (bipyridine) ruthenium (II)-functionalized metal-organic frameworks. Anal Chem 87:4864–4870

14. Zhang M, Ge L, Ge S, Yan M, Yu J, Huang J, Liu S (2013) Three-dimensional paper-based electrochemiluminescence device for simultaneous detection of Pb^{2+} and Hg^{2+} based on potential-control technique. Biosens Bioelectron 41:544–550

15. Cui X, Zhu L, Wu J, Hou Y, Wang P, Wang Z, Yang M (2015) A fluorescent biosensor based on carbon dots-labeled oligodeoxyribonucleotide and graphene oxide for mercury (II) detection. Biosens Bioelectron 63:506–512

16. Chen G, Jin Y, Wang L, Deng J, Zhang C (2011) Gold nanorods-based FRET assay for ultrasensitive detection of Hg^{2+}. Chem Commun 47:12500–12502

17. Zhang Y, Tang L, Yang F, Sun Z, Zhang G (2015) Highly sensitive DNA-based fluorometric mercury(II) bioassay based on graphene oxide and exonuclease III-assisted signal amplification. Microchim Acta 182:1535–1541

18. Wang H, Wang Y, Jin J, Yang R (2008) Gold nanoparticle-based colorimetric and "Turn-On" fluorescent probe for mercury(II) ions in aqueous solution. Anal Chem 80:9021–9028

19. Li T, Dong S, Wang E (2009) Label-free colorimetric detection of aqueous mercury ion (Hg^{2+}) using Hg^{2+}-modulated G-quadruplex-based DNAzymes. Anal Chem 81:2144–2149

20. Zhu Y, Cai Y, Zhu Y, Zheng L, Ding J, Quan Y, Wang L, Qi B (2015) Highly sensitive colorimetric sensor for Hg^{2+} detection based on cationic polymer/DNA interaction. Biosens Bioelectron 69:174–178

21. Zhu Z, Su Y, Li J, Li D, Zhang J, Song S, Zhao Y, Li G, Fan C (2009) Highly sensitive electrochemical sensor for mercury(II) ions by using a mercury-specific oligonucleotide probe and gold nanoparticle-based amplification. Anal Chem 81:7660–7666

22. Tang J, Huang Y, Zhang C, Liu H, Tang D (2016) DNA-based electrochemical determination of mercury(II) by exploiting the catalytic formation of gold amalgam and of silver nanoparticles. Microchim Acta 1–8

23. Mor-Piperberg G, Tel-Vered R, Elbaz J, Willner I (2010) Nanoengineered electrically contacted enzymes on DNA scaffolds: functional assemblies for the selective analysis of Hg^{2+} ions. J Am Chem Soc 132:6878–6879

24. Xuan F, Luo X, Hsing I (2013) Conformation-dependent exonuclease III activity mediated by metal ions reshuffling on thymine-rich DNA duplexes for an ultrasensitive electrochemical method for Hg^{2+} detection. Anal Chem 85:4586–4593

25. Yang L, Zhang C, Jiang H, Li G, Wang J, Wang E (2014) Insertion approach: bolstering the reproducibility of electrochemical signal amplification via DNA superstructures. Anal Chem 86:4657–4662

26. Tang C, Zhao Y, He X, Yin X (2010) A "turn-on" electrochemiluminescent biosensor for detecting Hg^{2+} at femtomole level based on the intercalation of $Ru(phen)_3^{2+}$ into ds-DNA. Chem Commun 46:9022–9024

27. Yuan T, Liu Z, Hu L, Zhang L, Xu G (2011) Label-free supersandwich electrochemiluminescence assay for detection of sub-nanomolar Hg^{2+}. Chem Commun 47:11951–11953

28. Ma F, Zhang Y, Qi H, Gao Q, Zhang C, Miao W (2012) Ultrasensitive electrogenerated chemiluminescence biosensor for the determination of mercury ion incorporating G4 PAMAM dendrimer and Hg(II)-specific oligonucleotide. Biosens Bioelectron 32:37–42

29. Wu M, Qian G, Xu J, Chen H (2012) Sensitive electrochemiluminescence detection of c-Myc mRNA in breast cancer cells on a wireless bipolar electrode. Anal Chem 84:5407–5414

30. Dang J, Guo Z, Zheng X (2014) Label-free sensitive electrogenerated chemiluminescence aptasensing based on Chitosan/$Ru(bpy)_3^{2+}$/Silica nanoparticles modified electrode. Anal Chem 86:8943–8950