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# Highly Sensitive Electrochemical Sensor for Mercury(II) Ions by Using a Mercury-Specific Oligonucleotide Probe and Gold Nanoparticle-Based Amplification

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**We report a highly sensitive electrochemical sensor for the detection of $Hg^{2+}$ ions in aqueous solution by using a thymine (T)-rich, mercury-specific oligonucleotide (MSO) probe and gold nanoparticles (Au NPs)-based signal amplification. The MSO probe contains seven thymine bases at both ends and a "mute" spacer in the middle, which, in the presence of $Hg^{2+}$, forms a hairpin structure via the $Hg^{2+}$-mediated coordination of T−$Hg^{2+}$−T base pairs. The thiolated MSO probe is immobilized on Au electrodes to capture free $Hg^{2+}$ in aqueous media, and the MSO-bound $Hg^{2+}$ can be electrochemically reduced to $Hg^{+}$, which provides a readout signal for quantitative detection of $Hg^{2+}$. This direct immobilization strategy leads to a detection limit of 1 $\mu$M. In order to improve the sensitivity, MSO probe-modified Au NPs are employed to amplify the electrochemical signals. Au NPs are comodified with the MSO probe and a linking probe that is complementary to a capture DNA probe immobilized on gold electrodes. We demonstrated that this Au NPs-based sensing strategy brings about an amplification factor of more than 3 orders of magnitude, leading to a limit of detection of 0.5 nM (100 ppt), which satisfactorily meets the sensitivity requirement of U.S. Environmental Protection Agency (EPA). This Au NPs-based $Hg^{2+}$ sensor also exhibits excellent selectivity over a spectrum of interference metal ions. Considering the high sensitivity and selectivity of this sensor, as well as the cost-effective and portable features of electrochemical techniques, we expect this Au NPs amplified electrochemical sensor will be a promising candidate for field detection of environmentally toxic mercury.**

Mercury ions, the most stable form of inorganic mercury, are highly toxic environmental pollutants and have serious medical effects.[1] For example, microbial biomethylation of mercuric ions ($Hg^{2+}$) yields methyl mercury that accumulates in bodies through the food chain, which is known to cause brain damage and other chronic diseases.[2] Therefore, it is highly desirable to develop sensitive methods for the detection of $Hg^{2+}$. Indeed, there have been numerous reports on optical $Hg^{2+}$ detection by using $Hg^{2+}$-sensitive fluorophores or chromophores.[3−5] However, most of these fluorophores or chromophore-based $Hg^{2+}$ sensors only work in organic media, which cannot be directly used to detect mercury contaminants in aqueous media.

Recently, the coordinate interaction between $Hg^{2+}$ and bis-thymine has attracted significant interest.[6,7] In detail, T−T mismatches in DNA duplexes selectively and strongly capture $Hg^{2+}$ (binding constant higher than A−T), and the metal-mediated T−Hg−T forms stable DNA duplexes (as shown in the inset of Scheme 1). A T-rich mercury-specific oligonucleotide (MSO) probe (5′-TTCTTTCTTCCCCTTGTTTGTT-3′), which includes two T-rich domains spaced by a "mute" oligonucleotide sequence spacer, was designed to selectively bind with $Hg^{2+}$. In the presence of $Hg^{2+}$, the complex of $Hg^{2+}$ with thymines yields a stable hairpin structure,[8] based on which several novel $Hg^{2+}$ detection assays in aqueous media have been developed.[8−23] For example, a fluorescence resonance energy transfer (FRET) sensor for $Hg^{2+}$ was designed by using a MSO probe labeled with fluorophore/quencher units at either end.[8] MSO-modified gold nanoparticles (Au NPs) were also employed as a colori-

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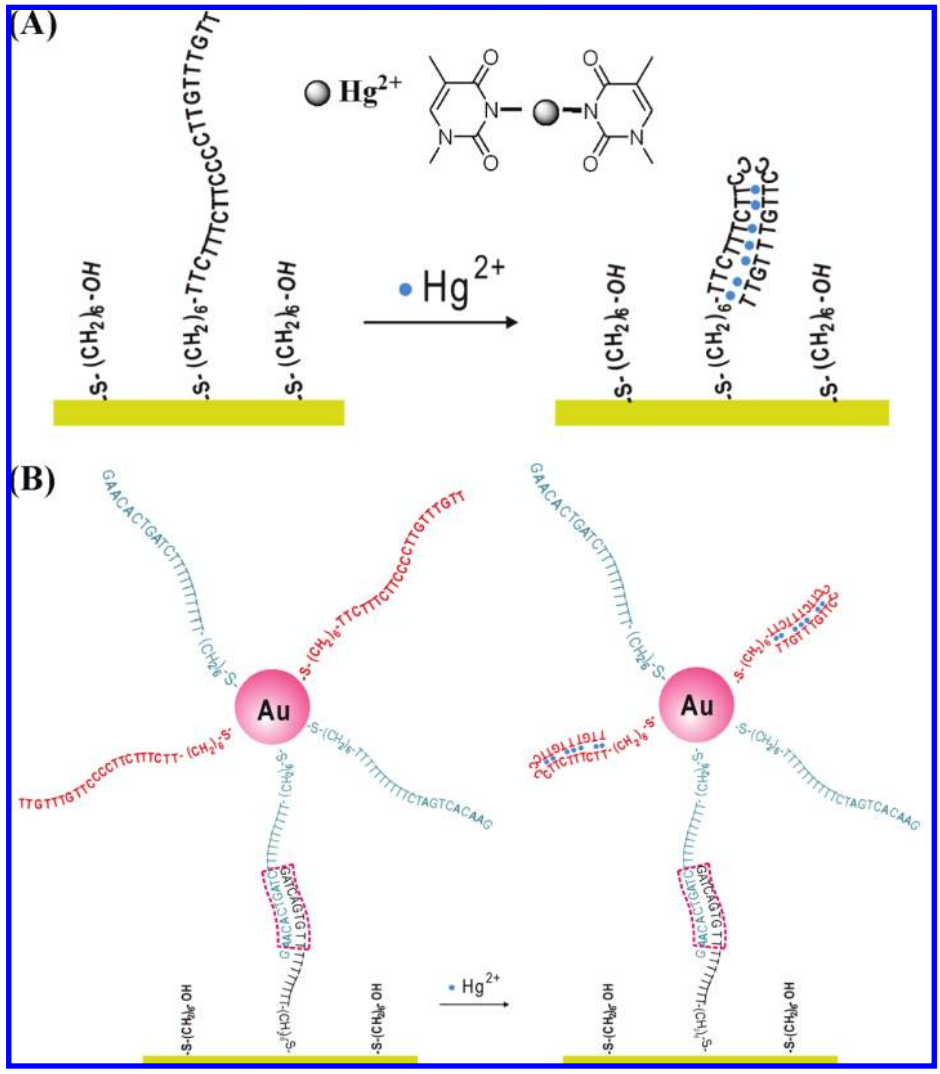
**Scheme 1. (A) Directly Immobilized MSO Probe and (B) Au NPs Mediated Immobilized MSO Probe**




metric probe for $Hg^{2+}$, which relied on the $Hg^{2+}$-induced aggregation of Au NPs and resulting red to blue color change.[12,16,20] This visual probe was recently coupled with a power-free and portable microfluidic device for the rapid detection of $Hg^{2+}$ with naked eyes.[24] In addition, conjugating polymers[10,13] and DNAzymes[15,17] were also exploited to couple with this MSO probe for $Hg^{2+}$ detection. However, most of these methods rely on optical techniques, such as colorimetry,[9,12,14,25] fluorescence[8,11,17] and fluorescence polarization.[26]

In the present study, we describe an electrochemical sensor for $Hg^{2+}$ detection by using the MSO probe. Compared with an optical instrument, electrochemical devices are impressively cost-effective and miniaturizable.[27−31] The MSO probe is immobilized on Au electrode surfaces to capture $Hg^{2+}$ in aqueous solution, and the electrochemical reduction of surface confined $Hg^{2+}$ provides a readout signal for the quantitative detection of $Hg^{2+}$. We also demonstrated that the sensitivity of this $Hg^{2+}$ sensor could be significantly improved with Au NPs-based signal amplification, which leads to an amplification factor of more than 3 orders of magnitude. This amplified electrochemi-


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**Table 1. Sequences of Oligonucleotides Employed in This Work**

| | sequences |
|---|---|
| MSO probe | 5′ HS-TTCTTTCTTCCCCTTGTTTGTT-3′ |
| capture probe | 5′-GATCAGTGTTTTTTTTTTT-SH 3′ |
| linking probe | 5′-GAACACTGATCTTTTTTTTTT-SH 3′ |

cal $Hg^{2+}$ sensor can detect down to 100 ppt (0.5 nM) of $Hg^{2+}$, which satisfactorily meets the requirement of the U.S. Environmental Protection Agency (EPA) for acceptable $Hg^{2+}$ level in drinking water (10 nM, 2 ppb).

## EXPERIMENTAL SECTION

**Materials.** Oligonucleotides were synthesized by Takara Biotechnology Co. (Dalian, China) and purified by HPLC. The sequences are listed in Table 1. $HAuCl_4$, mercaptohexanol (MCH), hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Aldrich and used as received. $Hg^{2+}$ stock solution (0.1 M) was prepared by dissolving $Hg(NO_3)_2$ with 0.5% $HNO_3$. The pH of the Hg stock solution was adjust to 6.0, to prevent the formation of HgO particles. The Hg stock solution was diluted to desired concentration with 50 mM 4-(2-hydroxyerhyl)piperazine-1-erhanesulfonic acid (HEPES) buffer (pH 7.4) containing 0.1 M $NaNO_3$. $MgCl_2$, $CaCl_2$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Cr(NO_3)_2$, $Zn(NO_3)_2$, $AgNO_3$, and all other reagents were of analytical grade. All solutions were prepared with Milli-Q water (18.2 MΩ $cm^{-1}$) from a Millipore system.

**Direct Immobilization of the MSO Probe on Au Electrode.** Gold disk electrodes (2 mm in diameter) were first polished on microcloth (Buehler) with Gamma micropolish deagglomerated alumina suspension (0.05 μm). Residual alumina powder was removed by sonicating in ethanol and water for 5 min, respectively. Then the electrodes were electrochemically cleaned to remove any remaining impurities. After dried with nitrogen, the electrodes were ready for DNA immobilization.

The thiolated MSO probe was immobilized on electrodes via the Au−S chemistry. In brief, the Au electrodes were incubated in 0.2 μM of the MSO probe solution (immobilization buffer containing 10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl, pH 7.4) for 2 h. The DNA-modified electrodes were further incubated in 1 mM MCH for 20 min to obtain well-aligned DNA monolayers.

**Preparation of MSO Probe Modified Au NPs.** Au NPs with an average diameter of 12 ± 1 nm were prepared with the citrate reduction method.[32] Briefly, Au NPs were prepared by boiling an aqueous solution of 1 mM $HAuCl_4$ (100 mL) under rapid stirring and adding 10 mL of sodium citrate (38 mM). After over 10 min of boiling, the solution was allowed to be cooled to room temperature and filtered through a 0.8 μm membrane. Concentration of the as-prepared Au NPs was determined by UV−vis spectroscopy using the Lambert−Beer's law (molar extinction coefficient of 12 nm Au NPs is $2.7 \times 10^8$ $M^{-1}$ $cm^{-1}$ at $\lambda_{520}$).[33]

The as-prepared Au NPs were further comodified with the thiolated MSO probe and the linking probe. Briefly, 200 μL of Au NPs (50 nM) was incubated with a mixture of MSO probe (6 μL, 100 μM) and linking probe (4 μL, 100 μM) for 16 h at room temperature. The mixture was slowly brought up to a final salt concentration of 0.1 M NaCl and 10 mM phosphate (pH 7.4) and allowed to age for 40 h. Centrifugation was performed at 14 000 rpm for 40 min in order to remove excessive DNA. The precipitate was washed with 0.1 M NaCl, 10 mM phosphate buffer (pH 7.4) solution, recentrifuged, and finally dispersed in 10 mM phosphate buffer (pH 7.4) containing 0.3 M NaCl for further use.

**Immobilization of the MSO Probe Modified Au NPs on Electrode.** This MSO probe modified Au NPs were further linked to Au electrodes via the hybridization between the linking probe and a capture probe that was immobilized on Au electrodes. Of note, the capture probe contained a recognition part for the linking probe (as detailed in Scheme 1B). In brief, the function of the capture probe (the black sequence in Scheme 1B) and linking probe (the blue sequence in Scheme 1B) is to immobilize the MSO-modified Au NPs on electrodes via the DNA hybridization of the recognition part (addressed by the red frame in Scheme 1B).

Modification of the capture probe on Au electrodes followed the procedure as in the direct immobilization method of the MSO probe. The capture probe modified Au electrodes were then incubated with Au NPs modified with the MSO and the linking probe for 2 h. Thus the prepared electrodes were extensively rinsed, dried with $N_2$, and ready for further $Hg^{2+}$ detection assays.

**Electrochemical Assay for $Hg^{2+}$ Detection.** Electrochemical detection of $Hg^{2+}$ was performed by first incubating the MSO probe modified electrodes with different concentrations of $Hg^{2+}$ for 1 h. Then the modified electrodes were carefully washed with buffer (50 mM HEPES, 0.1 M $NaNO_3$) and subjected to subsequent electrochemical measurements.

Electrochemical experiments were carried out with a CHI 620 electrochemical workstation (CH Instruments Inc., Austin). A conventional three-electrode cell, consisting of a modified Au working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode (saturated with 3 M NaCl), was used for the electrochemical measurement. HEPES buffer (0.05 M, pH 7.4) containing 0.1 M $NaNO_3$ was used as the electrolyte. The electrochemical reduction of $Hg^{2+}$ to $Hg^+$ was recorded by a cathodic scan of square-wave voltammetry (SWV). SWV measurements were taken at a frequency ($f$) of 15 Hz with pulse amplitude ($E_{sw}$) of 25 mV and step increment ($\Delta E_s$) of 4 mV. The initial potential ($E_i$) of SWV was 0.7 V (vs Ag/AgCl) (0.6 V for the Au NPs amplified strategy) and final potential ($E_f$) was 0.3 V (vs Ag/AgCl) (0.1 V for the Au NPs amplified strategy). The surface coverage of capture probe on Au electrode was calculated according to the classical Tarlov's protocol.[34−36] Briefly, an electroactive complex, $[Ru(NH_3)_6]^{3+}$,



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served as the signaling molecule, which bound to anionic phosphate of DNA strands in a stoichiometric approach. The amount of adsorbed $[Ru(NH_3)_6]^{3+}$ could be chronocoulometrically calculated with a pulse period of 250 ms and a pulse width of 700 mV. The electrolyte for chronocoulometric measurement was 10 mM HEPES buffer (pH 7.4) containing 50 $\mu$M $[Ru(NH_3)_6]^{3+}$.

**Microgravimetric and Scanning Electronic Microscopy (SEM) Measurements.** Microgravimetric measurements was performed with a quartz crystal microbalance with dissipation (QCM-D) (D300, Q-Sense, Gothenburg, Sweden) to measure $Hg^{2+}$ binding at MSO modified gold surfaces. QCM-D is a novel QCM technique, which simultaneously monitors frequency ($F$) and dissipation ($D$) changes at surfaces.[37] $F$ indicates the mass changes occurring at surfaces while $D$ suggests conformational variations of surface-confined species. An AT-cut piezoelectric quartz crystal disk (14 mm × 0.3 mm, active area of 1 $cm^2$) coated with an Au layer (100 nm thick) was used as the substrate. In QCM experiments, the gold substrates were extensively cleaned as previously reported[36] and modified as described above. The scanning electron microscopy (SEM) image was conducted on a LEO 1530 VP microscope (Germany) to characterize the accumulated Au NPs at surfaces.

## RESULTS AND DISCUSSION

The MSO probe was immobilized on Au electrodes, which brought free $Hg^{2+}$ ions in aqueous media to electrode surfaces. We proposed two modes of immobilization. In the first mode, this MSO probe is directly immobilized on Au electrode via an Au−S bond (Scheme 1A). The surface coverage of MSO probe was calculated to be $8 \times 10^{11}$ molecule/$cm^2$ based on the chronocoulometric measurement. This MSO probe modified electrode was incubated with $Hg^{2+}$ for 1 h to bring $Hg^{2+}$ to the electrode surface. The incubation time was experimental optimized from the reduction current of surface confined $Hg^{2+}$ (Figure S1 in the Supporting Information), which reached a maximum after 0.5 h incubation (In order to accumulate sufficient $Hg^{2+}$ on the electrode surfaces, the incubation time was set as 1 h in the present study). Then the electrode was extensively rinsed to remove physically adsorbed $Hg^{2+}$ and was ready for further electrochemical experiments in the $Hg^{2+}$-free electrolyte.

The successful association of $Hg^{2+}$ to the modified electrodes and in turn conformational change of the T-rich probe was monitored via QCM-D. QCM-D is a relatively new and powerful tool for monitoring mass and viscoelastic changes on surfaces.[38] In addition to the traditional mass change information, it also provides dissipation ($D$) change information.[39] $D$ is defined as

$$D = E_{lost}/2\pi E_{stored}$$

where $E_{lost}$ is the energy dissipated during each oscillation cycle, and $E_{stored}$ is the total energy of the system. Once $Hg^{2+}$ was

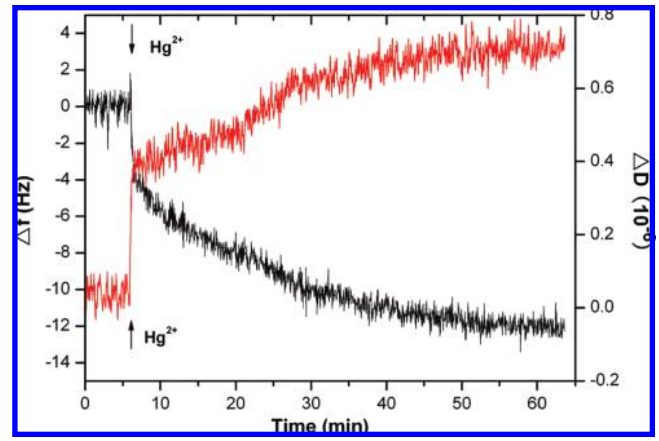


**Figure 1.** QCM-D sensorgrams for the directly immobilized MSO probe modified electrode upon reacting with $10^{-4}$ M $Hg^{2+}$. Red and black lines show the dissipation ($\Delta D$) and frequency ($\Delta f$) changes, respectively.

brought to the QCM electrode surface by the immobilized MSO probe, the loading of $Hg^{2+}$ altered the conformation of MSO at the surface, resulting in increased energy dissipation ($D$). Figure 1A shows the QCM-D sensorgrams for the directly immobilized MSO probe modified electrodes in the presence of $10^{-4}$ M $Hg^{2+}$. Clearly, upon the addition of $Hg^{2+}$, an immediate decrease in frequency (~12 Hz) along with an increase in dissipation (~0.7) was observed. Mass changes on the quartz surface were related to changes in frequency of the oscillating crystal according to the Sauerbrey equation and calculated to be 30 ng/$cm^2$. Even in the theoretical saturation state (7 $Hg^{2+}$ ions for each immobilized probe), only 1.8 ng/$cm^2$ of $Hg^{2+}$ could be bound to the electrode surface according to the surface coverage of the MSO probe ($8 \times 10^{11}$ molecule/$cm^2$). Thus, the frequency change reflects not only the mass change of the surface attached $Hg^{2+}$ but also the adsorbed water coupled with $Hg^{2+}$ binding, which is usually involved in QCM measurements.[39] The concomitantly increased dissipation change suggested that the $Hg^{2+}$ were indeed confined on the surface.[39] In order to preclude the possibility that $Hg^{2+}$ electrostatically bound on MSO and produced the QCM-D signal, we also challenged this MSO modified electrode in a mixture of nine interference ions: $Ca^{2+}$ and $Mg^{2+}$ (1 mM, each), $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Mn^{2+}$, and $Co^{2+}$ (1 $\mu$M, each) (Figure S2 in the Supporting Information). As compared with the significant frequency drop (~12 Hz) and $\Delta D$ increase (~0.7) of $Hg^{2+}$, the nine interference ions only brought a small frequency drop (~1.5 Hz) and $\Delta D$ increase (~0.2), suggesting that the electrostatic binding of metal ions to the phosphate bone of MSO contribute minimally to the QCM-D signal.

The reduction of surface-confined $Hg^{2+}$ was examined electrochemically. The resulting reduction current intensity was used for the quantitative detection of $Hg^{2+}$. Square-wave voltammogram was used to record the voltammetric response of the surface confined $Hg^{2+}$ (the advantage of SWV over cyclic voltammogram and differential pulse voltammogram (DPV) is shown in Figure S3 in the Supporting Information). Figure 2 depicts the square-wave voltammograms corresponding to the directly immobilized MSO modified electrodes upon incubating with various concentrations of $Hg^{2+}$ (curves a−e, $Hg^{2+}$ concentration varies from 0 to 0.1 mM). Clearly a reduction peak at


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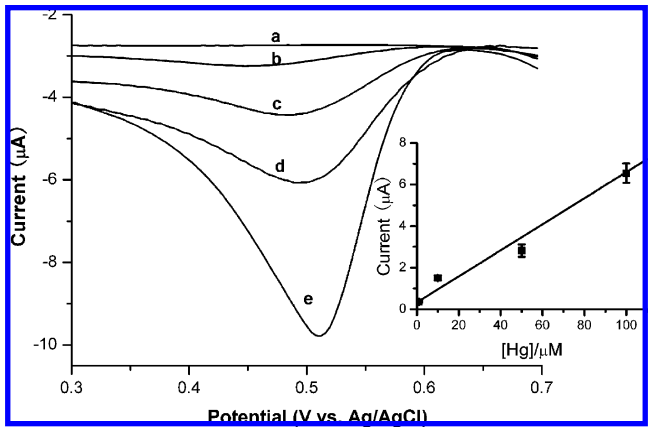


**Figure 2.** Square-wave voltammograms (SWV) corresponding to the detection of different concentrations of $Hg^{2+}$ by the directly immobilized MSO probe through the electrochemical reduction of $Hg^{2+}$: (a) 0, (b) $1 \times 10^{-6}$, (c) $1 \times 10^{-5}$, (d) $5 \times 10^{-5}$, and (e) $1 \times 10^{-4}$ M. The inset is the derived current intensity upon analyzing different concentration of $Hg^{2+}$. In all experiments, the T-rich probe modified electrode was incubated with $Hg^{2+}$ for 1 h. Then the modified electrode was carefully rinsed, and the electrochemical measurements were carried out in 0.05 M HEPES buffer (pH 7.4) containing 0.1 M $NaNO_3$ as the electrolyte. $f = 15$ Hz, $\Delta E_s = 4$ mV, $E_{sw} = 25$ mV, $E_i = 0.7$ V (vs Ag/AgCl), and $E_f = 0.3$ V (vs Ag/AgCl).

~0.5 V (vs Ag/AgCl) was observed, which was the reduction potential of surface confined $Hg^{2+}$ (from $Hg^{2+}$ to $Hg^{+}$).[40] As the concentrations of $Hg^{2+}$ were increased, the resulting reduction currents in the SWV were intensified. Of note, the background noise (i.e., $Hg^{2+}$-free) is completely blank in this potential window. The resulting reduction current intensity was depicted in the inset of Figure 2, leading to a limit of detection of 1 $\mu$M (signal-to-background ratio larger than 3).

The sensitivity of this electrochemical $Hg^{2+}$ assay could be significantly improved by using an Au NPs-amplified strategy (Scheme 1B). Au NPs with the diameter around 10 nm could accommodate 50−100 DNA strands on their surface. Previous reports have demonstrated that the high curvature of 12 nm Au NPs enabled the loading of single stranded DNA with a 100 times higher density than on flat Au surface.[41−43] In our previous study, this Au NPs amplified strategy was successfully used in a sandwich type DNA detection assay and a detection limit as low as 10 fM was achieved.[44,45] In addition to increased surface loading, the high curvature of Au NPs could also reduce the steric hindrance for the folding of the MSO probe.[41] Herein in the Au NPs-based strategy, three types thiolated DNA probe were used. These included the MSO probe (red sequence), linking probe (blue sequence), and capture probe (black sequence) that contained a recognition part with the linking probe. The MSO probe was first coimmobilized on Au NPs with the linking probe. Then the MSO probe modified Au NPs were brought to the proximity of Au electrodes via the hybridization between the linking probe and the capture probe that was immobilized on electrodes. The molecular ratio of MSO probe and linking probe for Au NPs modification was optimized to be 2:3. Of note, the exact value of the MSO surface coverage could not be calculated by the Tarlov's protocol due to the coexistence of multiple types of DNA probes on the surface.

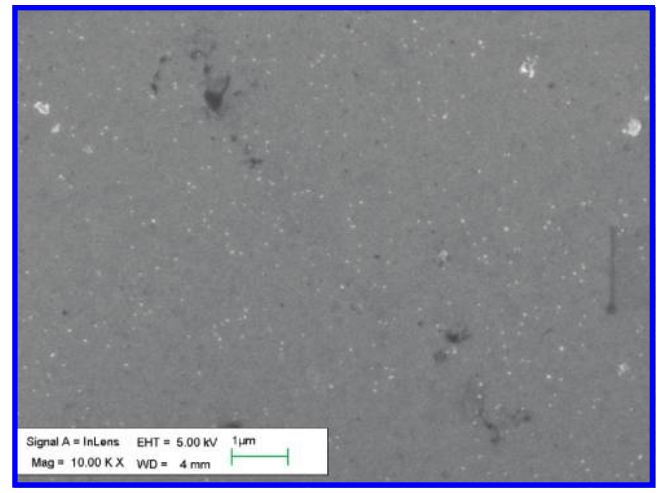


**Figure 3.** SEM image of the surface immobilized Au NPs through the hybridization between the linking DNA probe and capture DNA probe.

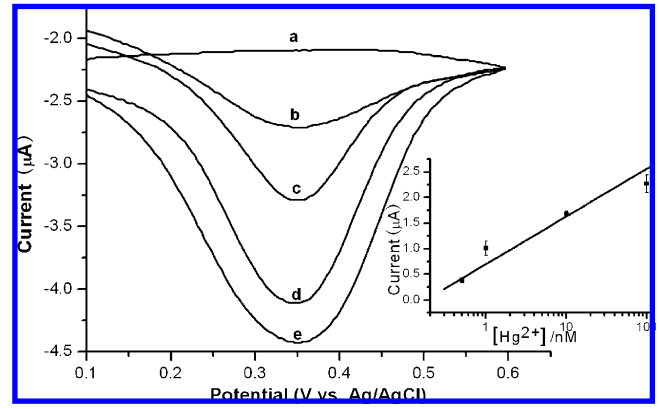


**Figure 4.** SWV corresponding to the detection of different concentrations of $Hg^{2+}$ by the Au NPs amplified strategy through the electrochemical reduction of $Hg^{2+}$: (a) 0, (b) $5 \times 10^{-10}$, (c) $1 \times 10^{-9}$, (d) $1 \times 10^{-8}$, and (e) $1 \times 10^{-7}$ M. The inset is the derived current intensity upon analyzing different concentrations of $Hg^{2+}$. The experimental conditions are detailed in the caption of Figure 2.

The successful accumulation of DNA-modified Au NPs on electrodes was confirmed by SEM measurement. Figure 3 shows the SEM image of Au NPs on electrodes. Individual Au NPs (white dots) were clearly observed. The $Hg^{2+}$ assay based on the Au NPs amplified strategy was also interrogated with SWV. Figure 4 depicts the SWVs corresponding to the Au NPs modified electrode in the presence of varied concentrations of $Hg^{2+}$ (curves a−e, $Hg^{2+}$ concentration varies from 0 to 100 nM). The resulting reduction current intensity is depicted in the inset of Figure 4. Clearly, with the amplification of Au NPs, as low as 0.5 nM of $Hg^{2+}$ could be electrochemically detected with a signal-to-background ratio larger than 3. Thus, this method enabled the detection of $Hg^{2+}$ with a limit of detection that corresponds to 0.5 nM (100 ppt). In addition, the reduction potential of $Hg^{2+}$

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**Table 2. Comparison of the Sensitivity of Different MSO-Based $Hg^{2+}$ and Electrochemical $Hg^{2+}$ Sensor**

| MSO based $Hg^{2+}$ sensor | limit of detection (nM) | reference |
|---|---|---|
| fluorometric assay based on the fluorophore and quencher modified MSO beacon | 40 | 8 |
| fluorometric assay based on the allosteric DNAzyme catalytic beacons | 2.4 | 17 |
| fluorometric assay based on polythiophene | 42 | 10 |
| fluorescence polarization method | 1 | 23 |
| fluorometric assay based on Au NPs quenched fluorophore modified MSO | 40 | 14 |
| colorimetric assay based on the aggregation of thiolated MSO modified Au NPs | 100 | 12 |
| colorimetric assay based on the salt-induced aggregation of nonmodified MSO stabilized Au NPs | 10 | 23 |
| colorimetric assay based on the $Hg^{2+}$ stimulated DNA machine | 10 | 9 |
| colorimetric assay based on the $Hg^{2+}$ modulated G-quadruplex DNAzyme | 50 | 22 |
| **electrochemical $Hg^{2+}$ sensor** | **limit of detection (nM)** | **reference** |
| square wave anodic stripping voltammetry on iridium ultramicroelectrode | 0.5 | 27 |
| anodic stripping voltammetry on crown ether modified glassy carbon electrode | 10 | 28 |
| anodic stripping voltammetry on polythiophene modified sonogel−carbon electrode | 7 | 29 |
| anodic stripping voltammetry on chitosan modified carbon paste electrode | 628 | 31 |
| square wave voltammetry on Au NPs amplified MSO based sensor | 0.5 | present study |

shifted to ~0.35 V (vs Ag/AgCl) by using this Au NPs amplification. As compared with the 0.5 V (vs Ag/AgCl) of $Hg^{2+}$ reduction potential in Figure 2, this 150 mV lower in potential was possibly attributed to the different stability of the reduced form of $Hg^{2+}$ (i.e., $Hg^{+}$) on Au electrode and Au NPs. A previous study has demonstrated that mercury atoms tend to preferentially adsorb on Au NPs surface and form core/shell nanostructure (instead of formation amalgam).[46] We propose that Au NPs might also stabilize $Hg^{+}$, thus decrease the reduction potential.

We also ran a control experiment by using Au NPs modified with only the linking probe, which eliminated the coordination effect of MSO with $Hg^{2+}$ and simply involved the electrostatic attraction of DNA probes and Au NPs with $Hg^{2+}$. In this situation, only 0.5 mM of $Hg^{2+}$ could be detected (data not show), which clearly highlighted the importance of using the MSO probe, that is, the $Hg^{2+}$-based coordination with thymine. Table 2 summarizes the sensitivity reported for several MSO-based optical $Hg^{2+}$ ions sensors as well as electrochemical $Hg^{2+}$ ions sensors. The present Au NPs amplification strategy reveals a superior sensitivity as compared to optical protocols of MSO-based and other electrochemical $Hg^{2+}$ sensors (only a sensor employing iridium ultramicroelectrodes and anodic stripping possesses comparable sensitivity[27]).

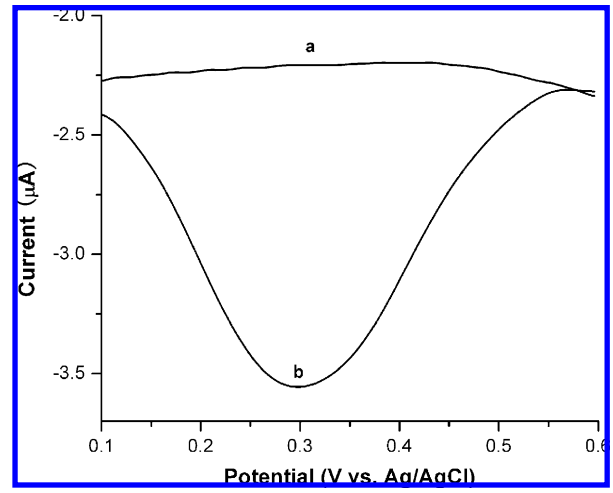


**Figure 5.** SWV corresponding to detection of (a) 0 and (b) $1 \times 10^{-7}$ M of $Hg^{2+}$ in a mixture of metal ions containing $Ca^{2+}$ and $Mg^{2+}$ (1 mM, each), $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Mn^{2+}$, and $Co^{2+}$ (1 $\mu$M, each) by the Au NPs amplified strategy.

This Au NPs-amplified electrochemical $Hg^{2+}$ sensor was also highly selective. Figure 5 depicts the electrochemical response of $Hg^{2+}$ in a mixture of nine different interference metal ions. Figure 5a was the SWV of the Au NPs modified electrode upon treatment with a mixture of another nine different metal ions (1 mM of $Ca^{2+}$ and $Mg^{2+}$, 1 $\mu$M of $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Mn^{2+}$, and $Co^{2+}$). Importantly, all other metal ions were electrochemically inert in this potential window. In contrast, upon addition of 100 nM $Hg^{2+}$ to the mixture of metal ions, a $Hg^{2+}$ reduction peak at 0.30 V (vs Ag/AgCl) was clearly observed (Figure 5b). Compared with the $Hg^{2+}$ reduction potential in pure buffer matrix (0.35 V vs Ag/AgCl, Figure 4), we speculated that this 50 mV negative shift in reduction potential was possibly attributed to that the interference ions electrostatic bound on the phosphate bones of MSO that facilitated electron-transfer of $Hg^{2+}$ to $Hg^{+}$.[47] In a few recent publications,[48,49] Ono and Dong reported that $Ag^{+}$ could be intercalated into the cytosine−cytosine (C−C) base pairs. In our design, the MSO probe also contains a four cytosine base in the middle as a "mute" spacer. Although this short four cytosine could not form a stable base-pair in the presence of $Ag^{+}$,[8] in order to check the selectivity of the present sensor over $Ag^{+}$, we still challenged the sensor in a solution containing $Ag^{+}$ and found that essentially no Ag reduction peak was observed (Ag reduction potential is between 0.1 and 0.2 V vs AgCl) (Figure S4 in the



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Supporting Information).[50] $Ag^+$ was not included in the other nine interference ions and is based on the consideration to avoid the formation of insoluble AgCl complexes (in this case, no free $Ag^+$ remains and subsequently no $Ag^+$ reduction signal is obtained, suggesting false negative signal). All these results imply that this electrochemical sensor is a highly selective for $Hg^{2+}$ ions and only relies on the selective binding of $Hg^{2+}$ ions with a T−T pair.

## CONCLUSIONS

In summary, we have demonstrated a highly sensitive electrochemical sensor for the detection of $Hg^{2+}$ in aqueous media. With the introduction of Au NPs amplification, as low as 0.5 nM (100 ppt) $Hg^{2+}$ can be electrochemically detected. The sensitivity of this electrochemical sensor compares favorably with that of several recently reported state-of-the-art fluorescence methods.[17,26] Importantly, electrochemical devices are cost-effective and field portable, which is a significant advantage compared with relatively bulky and expensive spectroscopy. High-throughput electrochemical $Hg^{2+}$ detection is also possible by using screen-printed electrode arrays. In addition, this method avoids the labeling of the MSO probe with fluorophore tags, which significantly reduces detection cost. Considering the high sensitivity and selectivity of this sensor, as well as the cost-effective and portable features of electrochemical technique, we expect this Au NPs-amplified electrochemical sensor will be a promising candidate for field detection of environmentally toxic mercury.

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## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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