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Electronic Supplementary Information (ESI)

for

An ultrasensitive electrochemical “turn-on” label-free biosensor for Hg^{2+} with AuNP-functionalized reporter DNA as a signal amplifier

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1. Experimental section

Reagents and Apparatus: DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., LTD. (Dalian, China). The sequences of three oligonucleotides employed are as follows:

5'-SH-(CH2)6-ACCGTGTTTGCCTTTGACCTC-3' (DNA **1**);

5'-GCTGTCGGATGAGGAGGTCTTTGGCTTTCACGGT-3' (DNA **2**);

5'-SH-(CH2)6-CTCATCCGACAGC-3' (DNA **3**).

Methylene blue (MB, Shanghai Reagents, Shanghai, China), 6-mercaptohexanol (MCH, 98%, Sigma–Aldrich,) and all other chemicals were of analytical reagent grade and used without further purification or treatment. All metal ion solutions were prepared from nitrate salts. DNA buffers involved in this work were composed of 200 mM NaCl and 10 mM PBS (pH 7.4). A buffer containing 0.1M PBS (pH 7.4) and 2 M NaCl was used for preparation of DNA-AuNPs. For regeneration of sensing surface, 1 M NaOH was used. All solutions were prepared with Milli-Q water (resistance >18 MΩ•cm) from a Millipore system. All electrochemical measurements were carried out at room temperature in a laboratory-made measuring cell using a CHI 760B electrochemical workstation (Shanghai Chenhua Equipments, China). The three electrode system used is consisted of a gold working electrode (2 mm diameter), a saturated calomel reference electrode, and a platinum foil auxiliary electrode. UV-visible absorption spectra were recorded on a MultiSpec-1501 UV/vis spectrophotometer (Shimadzu, Japan). Centrifugation was performed on a sigma-3k30 centrifuge (Sigma, Germany).

Synthesis of AuNPs: AuNPs of ~13 nm in diameter were prepared according to the literature method.[1] In brief, 100 mL of 1.0 mM $HAuCl_4$ solution was heated to reflux under vigorous stirring; the rapidly addition of 10 mL of 38.8 mM sodium citrate solution into the boiling solution accompanied with a color change from pale yellow to wine-red. After boiling for 10 min under stirring, the solution was continuously stirred for an additional 15 min to cool down. Then it was filtered through a 0.32-μM membrane filter and stored in a refrigerator at 4 °C before being used. The concentration of AuNPs was estimated by UV/vis spectroscopy based on an extinction coefficient of 2.7×10^{8} M^{-1} •cm^{-1} at λ =520 nm for 13-nm particles.[2]

Preparation of AuNPs functionalized reporter DNA: The AuNPs functionalized reporter DNA were freshly prepared by addition of thiolated DNA (**3**) (3 μM, 100 μL) in sterilized water into 1 mL AuNPs aqueous solution. After standing at 4 °C for 16 h, the DNA-AuNPs conjugates were "aged" by gradually addition of 2 M NaCl and 0.1 M PBS buffer every 8 h until the solution contained 0.3 M NaCl and 10 mM PBS. Then the solution was centrifuged for 25 min at 4 °C (14000 rpm) with the supernatant being removed. The red oily precipitate was washed with 10 mM PBS, recentrifuged, and then redispersed in 10 mM PBS buffer, stored at 4 °C.[3]

Fabrication of sensing interface: The gold electrode was polished with a 0.05-μm alumina powder and soaked in an ultrasonic bath successively with ultrapure water, absolute alcohol, and ultrapure water for 5 min each. Then, the gold electrode was dipped in piranha solution (H2SO4/H2O2, 7:3 by volume) for 20 min. Finally, the electrode was rinsed with ultrapure water and dried under nitrogen stream. The cleaned electrode was interacted with thiolated DNA (1) (1 μM) for 12 h at 4 °C. Afterward, the modified electrode was dipped in MCH (1 mM) for 20 min to

block the nonspecific sites and obtain an optimal oligonucleotide orientation,[4] and then, rinsed with 10 mM PBS for several minutes to obtain the sensing interface.

Electrochemical measurements: The prepared sensing interface was interacted with DNA **2** (2 μM) and Hg^{2+} at 37 °C for 1 h and then interacted with DNA-AuNPs, or the mixture of prehybridized DNA **2** with AuNPs functionalized reporter DNA and Hg^{2+} at 37 °C for 1 h. After incubation, the electrode was rinsed with 10 mM PBS to reduce the physical adsorption. The electrode was immersed in 10 mM PBS containing 25 μM MB for 15 min at room temperature and washed with 10 mM buffer under stirring for appropriate time. Differential pulse voltammetry (DPV) was performed from 0 to -0.5 V with a 50 mV pulse amplitude and 5 ms pulse width to measure the reduction peak current of MB. Electrochemical impedance measurements were performed in 5 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl at a scan rate of 100 mV/s. All potentials were referenced to the SCE reference electrode.

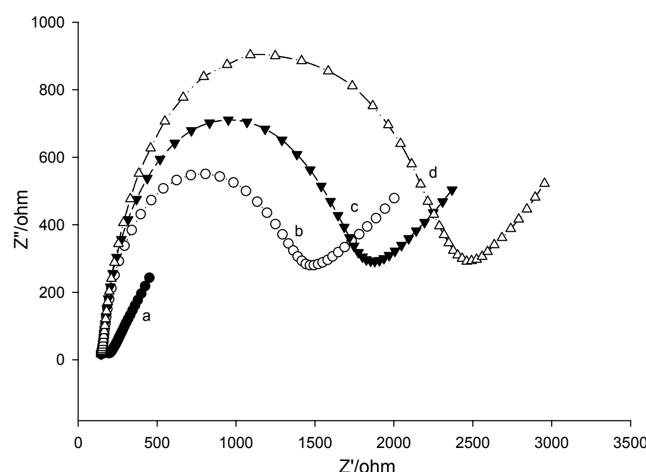

Fig. S1 Nyquist plots corresponding to the gold electrode. (a) The bare electrode, (b) after immobilization of capture probe, (c) in the presence of Hg^{2+} (100 nM) without AuNPs functionalized reporter DNA s, (d) in the presence of Hg^{2+} (100 nM) and AuNPs functionalized reporter DNA

2. Impedimetric characterization of the sensor

The assembly of capture probe on gold electrode, the formation of "T-Hg^{2+}-T" induced double-stranded DNA, and the hybridization of linker and DNA-AuNPs could be examined by faradic impedance spectroscopy.[5] Figure S1 depicts a Nyquist plot of impedance for the stepwise modification process with gold electrode. In the impedance spectra, the semicircle portion at higher frequencies corresponds to the electron-transfer-limited process, and the linear portion seen at lower frequencies to the diffusion process. The increase in the diameter of the semicircle reflects the increase in the interfacial charge-transfer resistance (Rct).[6] The value of Rct was 49.6 Ω for the bare gold electrode, revealing a very small semicircle domain. After immobilization of capture probe, the value of Rct increased to 1347.5 Ω. The increase in Rct was ascribed to the immobilization of negatively charged DNA probes on the electrode surface resulting in a negatively charged interface that electrostatically repels the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$ and inhibits interfacial charge-transfer.[7] In the presence of Hg^{2+} and the linker, the

value of Rct increased to 1734.3 Ω owing to the formation of double-stranded DNA through the interaction of "T-Hg^{2+}-T". After hybridization with DNA-AuNPs, the value of Rct further increased to 2316.5 Ω. Here the AuNPs are no longer good conductors for $[Fe(CN)_6]^{3-/4-}$ but instead are negatively charged species. The surface of AuNPs has been covered with a large amount of ssDNA and interfered electron transfer on its surface.[8]

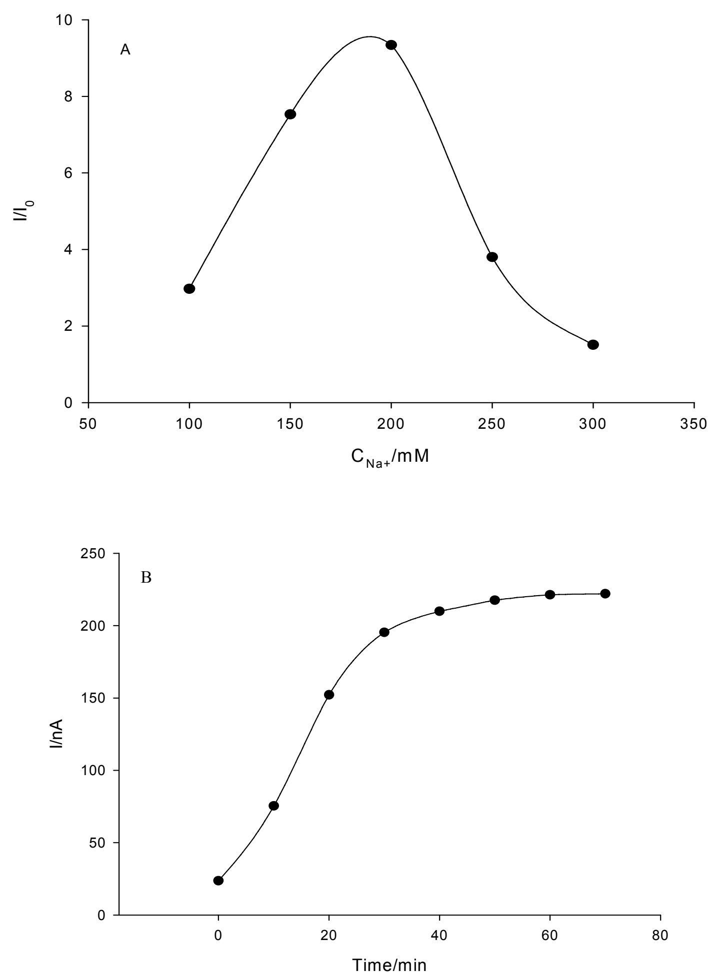

Fig. S2 Influence of (A) the Na^+ concentration, and (B) the incubation time to the response of the sensor. The descriptors I_0 and I are the peak current of the sensor in the absence and presence of 100 nM of Hg^{2+}.

3. Effects of ionic strength and incubation time

With the design strategy finalized, the performance of the developed electrochemical biosensor is still strongly influenced by the assay conditions such as ionic strength of the solutions and incubation time for the hybridization reaction. In order to obtain an optimal assay condition, the effects of Na^+ concentration and incubation time were investigated.

It is well known that the ionic strength strongly affects the melting temperature (T_m) of double-stranded DNA, hence the performance of this "T-Hg^{2+}-T" sensor. The Na^+ concentration was investigated by differential pulse voltammetry (DPV). Figure S2A shows the response of the sensor recorded with different concentrations of NaCl. It is clear that the signal-to-background

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ratio increases significantly when the concentration of NaCl changes from 100 to 200 mM, but decreases with a higher NaCl concentration. The results showed that the ionic strength in solution could affect the hybridization interaction between the capture probe and linker probe, consequently the amount of the AuNPs functionalized reporter DNA and the amount of adsorbed MB molecules which provided electrochemical signal. The increase of NaCl concentration could enhance the hybridization interaction of capture and linker probes induced by "T-Hg^{2+}-T" interaction. However, at too high NaCl concentration, the background increased substantially even though there were "T-T" mismatches. Therefore, the optimal concentration of NaCl was selected as 200 mM in subsequent studies.

In the presence of Hg^{2+}, the linking probe together with reporter DNA–AuNPs hybridizes to capture probe through the strong "T-Hg^{2+}-T" bonding, with more MB molecules absorbed and an increase of peak current. Our preliminary experimental results showed that a long incubation time could cause a visible increase of peak current. Therefore, the effect of incubation time on the Hg^{2+}-induced signal changes was further investigated by recording differential pulse voltammetry (DPV) with different incubation time and the results are shown in Figure S2B. One observes that the response increased rapidly with the increase of incubation time and tended to stabilize after more than 60 min. Considering the fact that too long incubation time might result in non-specific adsorption of the DNA-AuNPs to the gold electrode and increase the background current, 60 min was selected as the optimum incubation time for the following studies.

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