Supporting Information

A fishnet electrochemical Hg^{2+} sensing strategy based on gold nanopartical-bioconjugate and thymine-Hg^{2+}-thymine coordination chemistry

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Materials and Reagents

HPLC-purified DNA oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd (Shanghai, China). The sequences of these oligomers employed are given below:

Capture DNA: 5′-HS-$(CH_2)_6$-AAAGCGGTTGTGTTCAGTTGC-3′

DNA1: 5′-HS-$(CH_2)_6$-TCTGCTTCTGTTCTCT-3′

DNA2: 5′-HS-$(CH_2)_6$-AGAGTTCAGTTGCTGT-3′

Tris(2-carboxyethyl)phosphinehydrochloride (TCEP), hexaaminerutheium(III) chloride ($[Ru(NH_3)_6]Cl_3$), tris(hydroxymethyl)aminomethane, 6-mercaptohexanol (MCH), and $HAuCl_4 \cdot 3H_2O$ were purchased from J&k Chemical Ltd. All other chemicals were of analytical grade.

DNA buffers involved in this work were composed of 0.1 M NaCl, 1 mM TCEP, and 10 mM tris-HCl (pH 7.4). Target Hg^{2+} buffers contain 0.2 M NaCl and 10 mM tris-HCl (pH 7.4). Buffers for electrochemical measurements were 10 mM tris-HCl (pH 7.4). All solutions were prepared with ultrapure water (18.2 M Ω cm) from a Millipore Milli-Q system (Sartorius, Germany).

Synthesis of Au NPs

The 15±2 nm Au NPs determined by transmission electron microscope (JEOL, Japan) were prepared using the citrate reduction method with some modification.[1] In brief, a 100 mL aqueous solution of 0.01% (w/v) $HAuCl_4$ was added into a round-bottom flask, stirred to boil. Then 3.2 mL 1% trisodium citrate was added rapidly into the boiling solution. The color of the solution became from colorless to wine red after boiling for another 15 min with stirring. After stirring for another 15 min, the solution was cooled down to room temperature. Then it was stored in a refrigerator at 4 ℃ before been used. The concentration (2.3 nM) was calculated by UV-vis spectroscopy based on an extinction coefficient of 4.2×10^8 $M^{-1}\cdot cm^{-1}$ at the wavelength of 520 nm.[2]

Synthesis of single stranded functionalized Au NPs

Two kinds of functionalized Au NPs were synthesized by the well-known

gold-thiol chemistry,[3] which was also beneficial to the stability Au NPs. One is DNA1-functionalized Au NPs. Briefly, Au NPs solution (2.3 nM) was centrifuged at 14,000 rpm for 20 min, and then 80 % supernatant was removed. Then the residual Au NPs solution was incubated with DNA1 with the final concentration of 10 nM Au NPs and 3 μM DNA1 under room temperature for 24 h in darkness. Then the DNA1-AuNPs conjugates were "aged" for another 48 h by gradually addition of 2 M NaCl and 0.2 M Phosphate buffer (PB, pH 7.0) until the solution contained 0.2 M NaCl and 10 mM PB (pH 7.0). Excess reagents were removed by centrifuging at 14000 rpm for 20 min. The red oily precipitate was washed, recentrifuged, and then redispersed in PBS buffer (0.2 M NaCl and 10 mM PB, pH 7.4). For the preparation of DNA2-functionalized Au NPs, the same procedure as described for the preparation of DNA1-functionalized Au NPs was followed. The functionalized Au NPs were stored at 4 ℃, respectively.[4]

Preparation of Capture DNA modified gold electrodes

The substrate gold electrode (2 mm in diameter, CH Instruments) was cleaned using the usual procedure.[5] In brief, the bare gold electrode was firstly dipped in '*Piranha*' solution (98% H_2SO_4: 30%H_2O_2= 3:1 (v/v)) for 20 min to eliminate the adsorbed material and then rinsed with double-distilled water (*Caution! Piranha solution reacts violently with organic materials and should be handled with extreme care!*). After that, the gold electrode was polished carefully to a mirror-like surface with silicon carbide paper and then with 1, 0.3, and 0.05 μm α-alumina power, respectively. Subsequently, the electrode was washed ultrasonically for 5 min in both ethanol and ultrapure water to remove any remaining impurities. Finally, the electrode surface was established by incubating it in 0.5 M H_2SO_4 and scanning the potential between 1.6 V and 0 at the scan rate of 100 mVs^{-1} until a reproducible scan was obtained. After being dried under nitrogen stream, the cleaned electrode was immediately incubated in a solution composed of 1 μM DNA1, 10 mM tris-HCl buffer (pH 7.4) and 1 mM TCEP for 10 h at room temperature. The surface was then passivated with 1 mM MCH for 2 h to reduce nonspecific adsorption of DNA and to

obtain a well aligned DNA monolayer.[6]

Electrochemical measurements

The prepared sensing interface was interacted with the solution containing Hg^{2+} buffers (0.2 M NaCl, 10 mM tris-HCl, pH 7.4, 200 μL) and DNA1-AuNPs (100 μL) at 37℃ for 1 h, and then added DNA2-AuNPs (100 μL) for another 1 h. After the incubation, the electrode was rinsed with the buffer (10 mM tris-HCl, pH 7.4) for 10 min to reduce the physical adsorption. 10 μM $[Ru(NH_3)_6]^{3+}$ was used as electrochemical probe for Differential Pulse Voltammetry (DPV). The impact of the concentrations of DNA-Au NPs, $[Ru(NH_3)_6]^{3+}$ and ionic strength were investigated and the optimal concentrations were selected in subsequent studies

Electrochemical measurements were carried out on an electrochemical workstation(CHI 660C, CH Instruments) at room temperature by using a conventional three-electrode system consisted of a modified gold working electrode, a saturated reference calomel electrode(SCE), and a platinum electrode. 10 mM tris-HCl buffer (pH 7.4) was used as the electrolyte. DPV was performed from 0.05 to -0.45 V with a 50 mV pulse amplitude and 0.05s pulse width to measure peak current of $[Ru(NH_3)_6]^{3+}$. Electrochemical impedance measures were performed in 5mM $Fe(CN)_6^{3-/4-}$ (1:1 mixture) solutions containing 0.1 M KCl. A nitrogen environment was kept in the cell during the whole experiment. The measurement was repeated three times for each concentration of Hg^{2+} ions.

EIS characterization of the sensor

Impedance spectroscopy is a very effective method to describe the properties of the surface-modified electrodes clearly.[7] Thus, electrochemical impedance spectroscopy (EIS) was used to study the gold electrodes modified by a series of experimental procedure employing $Fe(CN)_6^{3-/4-}$as the redox probe in the supporting electrolyst solution. In the Nyquist plots of impedance spectra, a semicircular portion observed at high frequencies relates to a electron-transfer limited process, while the liner section is attributable to the diffusion-limited process. The semicircle diameter could represent the interfacial electron-transfer resistance (Ret).[8]

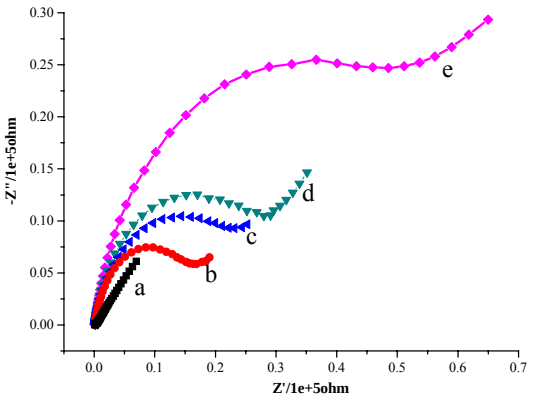

Fig. S1 Nyquist plots (-Zim vs Zre) for EIS measurements in phosphate buffer solution (10 mM, pH 7.4) solution containing 5 mM $Fe(CN)_6^{3-/4-}$ (1:1 mixture) and 0.1 M KCl for (a) bare gold electrode, (b) cDNA and MCH modified the electron, (c) in the presence of Hg^{2+}, (d) in the presence of Hg^{2+} and DNA1-Au NPs, (e) in the presence of Hg^{2+}, DNA1-Au NPs and DNA2-Au NPs. The concentration of Hg^{2+} was 100 nM.

It is showed that the impedance plot for bare gold electrode displays an almost straight line (Ret: 375 Ω, curve a in Fig. S1), as the characteristic of an electrochemical diffusion-limited process of $Fe(CN)_6^{3-/4-}$. The negatively charged phosphate backbone of the capture DNA immobilized on the bare gold electrode electrostatically repels the negatively charged redox probe $Fe(CN)_6^{3-/4-}$ from reaching the gold electrode, and thus inhibits interfacial charge-transfer, leading to an enhanced Ret (16.3 KΩ, curve b), which demonstrates that thiolated capture DNA has been self-assembled successfully on the electrode. In the presence of Hg^{2+}, the Ret is enhanced to a larger value (23.1 KΩ, curve c), owing to the conformational reorganization of the capture DNA from flexible single strands to relatively rigid duplex-like complexes through the interaction of "T-Hg^{2+}-T". When hybridization with DNA1-Au NPs in the presence of Hg^{2+}, the formation of double-stranded DNA between DNA1-Au NPs and capture DNA based on T-Hg^{2+}-T leads to a larger Ret (27.9 KΩ, curve d). After the DNA2-Au NPs are added, alternating interaction of the interface with DNA1-Au NPs and with DNA2-Au NPs results in a DNA-linked nanoparticle superstructure, which greatly inhibits the electron-transfer of the $Fe(CN)_6^{3-/4-}$ on the surface and induces sharply increased Ret (48.5 KΩ, curve e). All these experimental results demonstrate that the sensing interface has been fabricated successfully according to Fig. 1.

References

1. K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.
2. L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian and G. Viswanadham, *Anal. Chem.* , 2000, **72**, 5535.
3. C. A. M. T. A. Taton, R. L. Letsinger, *Science* 2000, **289**, 1757.
4. X. Xue, F. Wang and X. Liu, *J. Am. Chem. Soc.*, 2008, **130**, 3244.
5. D. Han, Y. R. Kim, J. W. Oh, T. H. Kim, R. K. Mahajan, J. S. Kim and H. Kim, *Analyst*, 2009, **134**, 1857.
6. T. M. H. R.Levicky, M.J.Tarlov, S.K.Satija, *J Am Chem Soc*, 1998, **120**, 9787.
7. M. Zayats, Y. Huang, R. Gill, C. A. Ma and I. Willner, *J. Am. Chem. Soc.*, 2006, **128**, 13666.
8. S. Zhang, J. Xia and X. Li, *Anal Chem*, 2008, **80**, 8382.

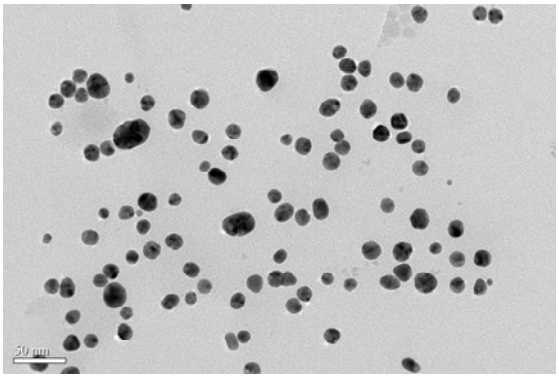

Fig. S2. TEM image of the 15±2 nm Au NPs

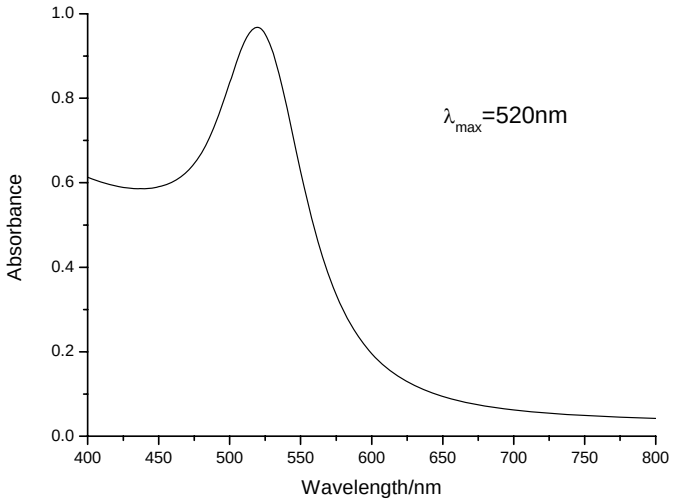

Fig. S3. UV-vis spectra of 15±2 nm Au NPs