Supporting Information

Experimental Section

Materials. All oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China) and used without further purification. The used sequences are as follow:

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (from 5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture DNA</td>
<td>GCT TCT GTT CTC TGTC-SH</td>
</tr>
<tr>
<td>linker DNA</td>
<td>SH-AAAAAAAAAA GTC TGT GTT CTG TTG C</td>
</tr>
<tr>
<td>Auxiliary DNA 1(A1)</td>
<td>TAC TCC CCC AGG TGC GCA ACA GAA CAC AGA C</td>
</tr>
<tr>
<td>Auxiliary DNA 2(A2)</td>
<td>GCA CCT GGG GGA GTA GTC TGT GTT CTG TTG C</td>
</tr>
</tbody>
</table>

Capture DNA can hybrid with one part of linker DNA-modified AuNPs (in underlined) in the presence of Hg$^{2+}$. Additionally, A1 (in italic) also is complementary with linker DNA (in underlined) and A2 (in underlined).

Hexammineruthenium(III) chloride ([Ru(NH$_3$)$_6$]$^{3+}$, RuHex), 6-mercaptohexanol (MCH), Tris(2-carboxyethyl) phosphinehydrochloride (TCEP), and HAuCl$_4$ were purchased from Sigma, USA. All chemicals were of analytical grade.

All solutions were prepared with Milli-Q water (Milli-Q, Millipore, 18.2-MΩ resistivity). Buffer A for the immobilization of DNA on the electrode contained 25 mM Tris-HCl (pH 7.4) and 10 μM TCEP. Buffers B for the hybridization of DNA contained 10 mM Tris-HCl (pH 7.4) and 0.3 M NaCl. Buffer C for electrochemical detections contained 200 mM Tris-HCl (pH 7.4), 0.5 mM RuHex and 1 M NaCl.

Preparation of AuNPs. 13 nm of AuNPs were prepared by the citrate-mediated reduction of HAuCl$_4$. 45 mL of deionized water and 1 mL of HAuCl$_4$ (20mg/mL) were mixed into a two-necked flask. When above mixture was heated to reflux, 5ml
of trisodium citrate solution (38.8mM) was quickly added, resulting in a change in solution color from pale yellow to deep red. The solution was heated under reflux for 10 min and then allowed to cool to room temperature for the following experiment.

**Preparation of DNA-AuNP conjugates.** DNA-AuNPs were prepared by the literature with little modification\textsuperscript{43}. Briefly, the solution containing 9 \( \mu \)L of linker DNA (100 \( \mu \)M) and 1 \( \mu \)L of double distilled water was mixed with 1 \( \mu \)L of 10 mM TCEP for 1 h at room temperature to cleave the disulfide bond. Next, above treated DNA solution was pipette into 500 \( \mu \)L of as-prepared Au NPs in a NaOH-treated tube, and kept at room temperature overnight. Subsequently, NaCl was added into the solution, and the final concentration of NaCl was 0.1 M. After 24 h, the solution was centrifuged, and the nanoparticles should be at the bottom of the tubes were cleaned using Buffer B to remove unbound linker DNA. Finally, linker DNA-functionalized AuNPs were dispersed in 500 \( \mu \)L of Buffer B for the following experiment.

**Preparation of the sensors and sensing Hg\textsuperscript{2+}.** Gold electrodes (2 mm in diameter, CH Instruments, Shanghai, China) were polished with aqueous slurries of 0.05 \( \mu \)m \( \alpha \)-Al\textsubscript{2}O\textsubscript{3} powders on the polishing microcloth, and rinsed with water, respectively. They were then electrochemically cleaned by consecutive cyclic voltammetry in the potential range of 0~1.6 V in 0.5 M sulfuric acid until stable cyclic voltammograms were achieved. And then the cleaned gold surfaces were immersed into the immobilization buffer containing 0.1 \( \mu \)M capture DNA for 90 min, and then passivated with 1.0 mM MCH for 60 min to obtain a well-aligned DNA monolayer. Next, the capture DNA-immobilized electrodes were immersed into
DNA-AuNP solution containing different concentrations of Hg$^{2+}$ for 2 h at room temperature to achieve the bio-bar-code-attached electrode. For the HCR reaction, above bio-bar-code-attached electrode was immersed into Buffer B containing 1 uM A1 and 1 uM A2, and self-assemble for 1 hour. In order to avoid the nonspecific adsorption, the electrodes should be washed with distilled water after each modification step.

**Electrochemical assay.** For electrochemical detection, here, a three-electrode electrochemical cell was used. All electrochemical detections were carried out using a CHI 660E electrochemical working station (CH Instruments, Shanghai, China) at room temperature. Above self-assembled DNA-modified electrode, platinum wire and Ag/AgCl (saturated with KCl) were used as the working electrode, the counter electrode and reference electrode, respectively. In the differential pulse voltammetry (DPV) experiments, three electrodes were immersed into the electrochemical cell containing 2 mL of detection buffer solution and 5 mM RuHex for 20 min. The parameter for DPV was set as follow: the potential interval from -0.4 to 0 V vs. Ag/AgCl, modulation amplitude 0.05 V, pulse width 0.06 s, and sample width 0.02 s. The current intensity at about -0.23 V was used for quantification. In addition, electrochemical impedance experiments were performed in the solution containing 5 mM [Fe(CN)$_6$]$^{3-}/^{4-}$. The biased potential was 0.214 V (vs. Ag/AgCl) and the amplitude was 5.0 mV, and the electrochemical impedance spectra were recorded in the frequency range of 10 kHz to 1 Hz.

**Assay Hg$^{2+}$ in the extraction of Bauhinia championi.** 2.64 g of Bauhinia
championi was mixed with 20 mL of concentrated HCl, and extracted via soxhlet extraction for 1 h. 1 mL of extraction was diluted to 1000 mL with Buffer B, which was used to assay using this proposed method.

**Results and Discussions**

**Characterization of AuNP.** Firstly, we character the as-prepared AuNPs. By transmission electron microscope (TEM, as shown in Fig. S1), it is found that AuNPs have uniform sizes with well-monodispersed and well-resolved, and the diameters of AuNPs were about 20±3.5 nm, displaying that the AuNPs are prepared successfully.

**Optimization of the Experimental Conditions.** For the purpose of better assay results, some experimental conditions have been optimized. Firstly, the concentration of capture DNA has been optimized, as shown in Fig. S2(A). The current intensity increases with the increasing concentration of capture DNA till 0.5 uM. However, higher concentration of capture DNA leads to the current intensity decreases. It may be attributed that too high density of capture DNA would negatively influence the hybridization of followed by DNA-functionalized AuNP. Hence, 0.5 uM of capture DNA is chosen. And then, the self-assembly time of A1 and A2 is investigated, as shown in Fig. S2(B). The DPV current intensity enhances gradually with the increasing of time. Once the self-assembly time exceeds 80 min, it is observed that current intensity increases slightly. Fig. S2(C) indicates the effects of A1 and A2 concentrations to current intensity. It is found that the current signal reaches the maximum when the concentrations of A1 and A2 are up to 0.7 uM. Thus,
the self-assembly time of 80 min and the concentrations of 0.7 uM of A1 and A2 are chosen. Additional, we carry out a control experiment: A1, A2 and RuHex are mixed together, and then interacts with AuNPs, which is different from our proposed method that after the HCR process on AuNP RuHex is introduced. The electrode is immersed into Buffer C for the detection. As shown in Fig. 2D, the current of the control experiment (curve a) is weaker than that of previous method (curve b). It may be attributed that the ion exchange equilibrium between RuHex electrostatic adsorbed on the electrode and other cations (such as NaCl) in detection solution would occur, resulting in the decreasing of RuHex on the electrode and the DPV response declined, which is reverse to our proposed method. Therefore, we do not choose the control experiment for the detection process.

**Figure S1**

![TEM of as-prepared AuNPs](image_url)

**Figure S1** TEM of as-prepared AuNPs
Fig. S2 The peak current responses of sensor under different conditions in RuHex solution. (A) Different concentration of capture DNA. (B) Different self-assembly times. (C) Different concentrations of A1 and A2. (D) Different detection process: a. after the HCR process on AuNP, RuHex is introduced; b. A1, A2 and RuHex are mixed together, and then interacts with AuNPs