Supporting Information

Experimental Section

Materials. All oligonudeotides were synthesized and purified by Sangon Inc. (Shanghai, China) and used without further purification. The substrate was synthesized by TaKaRa Biotechnology Co. (Dalian, China). The used sequences are as follow:

DNAzyme:

5'-SH-TTTTTCA TCTCT TCTCC G*AGCC GGTCG AAATA GTGAG T*-3'

Substrate:

5'-ACTCACTAT (rA) GGAAGAGATG-3'

Auxiliary probe 1 (A1):

5'-<u>TACTC CCCCA GGTGC</u> *ACT CAC TAT TTC GAC CGG CT* -3'

Auxiliary probe 2 (A2):

5'-<u>GCACC TGGGG GAGTA</u> *AGC CGG TCG AAA TAG TGA GT* -3'

rA is the substrate was a single, sessile ribo-adenine. The italic fragments of A1 could hybridize with the italic fragment of A2 and the italic fragment of DNAzyme, while the underline fragments of A1 was complementary to the underline fragment of A2. Hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$, RuHex), 6-mercapto-l-hexanol (MCH), tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. All other chemicals were of analytical grade.

All solutions were prepared with Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ resistivity. DNA buffers contain 100 mmol/L NaCl, 10 μM TCEP and 25 mmol/L

Tris-HCl (pH 7.4). Buffers for targeted Pb^{2+} buffers and electrochemical detections were 50 mmol/L Tris-HCl (pH 7.4) containing 0.5 mol/L NaCl.

Apparatus. All electrochemical detections were carried out using a CHI 660E electrochemical working station (CH Instruments, Shanghai, China) at room temperature. A three-electrode electrochemical cell was used. Gold electrode (2 mm in diameter, CH Instruments, Shanghai, China) was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter electrode and reference electrode, respectively.

DNA sensor preparation. Gold electrodes were polished with aqueous slurries of 0.05 μm α-Al_2O_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 0~1.6V in 0.5 mol/L sulfuric acid until a stable cyclic voltammogram is achieved. The cleaned gold surfaces were immersed in the immobilization buffer containing 0.1 μmol/L DNAzyme for 90 min, and then were passivated with 1.0 mmol/L MCH for 60 min to obtain a well-aligned DNAzyme monolayer. Next, the DNAzyme monolayer-functionalized electrodes were immersed into substrate strand for 2 h at room temperature to achieve DNA sensor. After each modification step, the electrodes should be washed with distilled water to eliminate the physical adsorption.

Detection of Pb^{2+}. Above DNA sensor was immersed into buffer solutions containing different concentrations of Pb^{2+} for 1 h at room temperature, and then incubated into 20 uL of a freshly prepared auxiliary probe solution containing 1 uM

A1 and 1 uM A2 for 120 min to achieve DNA self-assembly.

Electrochemical detection. Before differential pulse voltammetry (DPV) experiments, above self-assembled DNA-modified electrode, as the working electrode, was immersed into the electrochemical cell containing 2 mL of electrochemical buffer solution and 5 mM RuHex for 20 min. The parameter for DPV was set as follow: the potential interval from -0.6 to +0.2 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. Electrochemical impedance (EIS) experiments were performed in the solution containing 5 mmol/L $[Fe(CN)_6]^{3-/4-}$. 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.

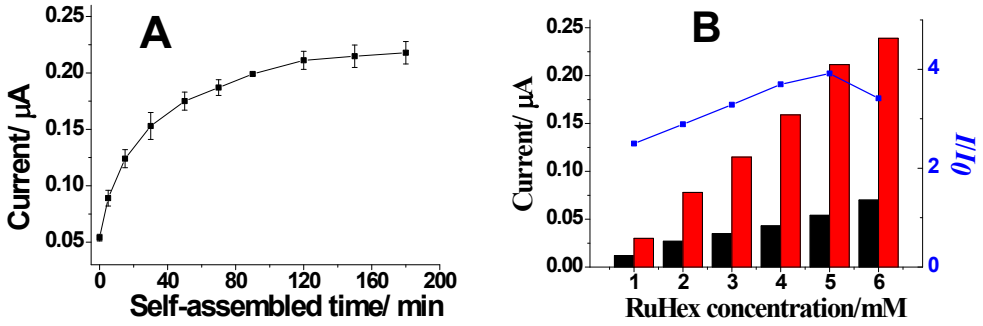
Assay Pb^{2+} in the extraction of Bauhinia championi. 5.0 g of Bauhinia championi was extracted in 20 mL of concentrated HCl via soxhlet extraction for 1 h. 1 mL of extraction was diluted to 1000 mL with Tris-HCl (pH 7.4) consisting of 0.5 mol/L NaCl, which was used to assay using this proposed method.

Results and Discussions

Optimization. In order to achieve better assay results, some conditions are optimized. Firstly, self-assembled time of DNA concatamers is investigated, as shown in Figure S1(A). The peak current increases gradually when the self-assembly time changes from 0 to 120 min. When the self-assembly time extends to 120 min, it is found that peak current increases slightly. Hence, the self-assembled time is set as 120 min. The concentration of RuHex is studied since it also plays an important role in

this method. The result is shown in Figure S1(B). With the enhancement of RuHex concentration, the peak current increases, and background current increases as well. The value of I/I_0 (I and I_0 stand for peak current and background current, respectively) reaches its peak as the concentration of RuHex is 5 mM. Therefore, 120 min of self-assembly time and 5 mM of RuHex are chosen for the following experiment. Figure S1(C) indicates the effects of A1 and A2 concentrations to current intensity. With the enhancement of A1 and A2 concentrations, peak current increases. However, the signal does not increase when the concentrations of A1 and A2 is up to 1.0 μM. Thus, 1.0 μM of A1 and A2 concentrations are chosen.

Figure S1



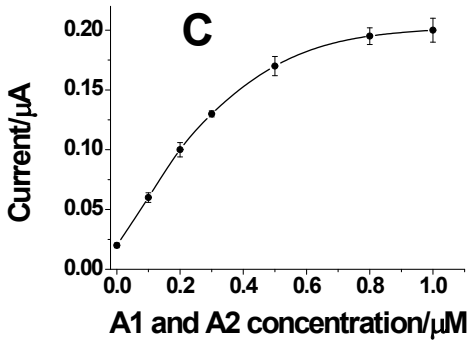


Figure S1 (A) The effect of self-assembly time for the detection of 1 nM Pb^{2+} in RuHex solution. Concentrations of A1 and A2 are 1.0 uM. (B) Optimization of the RuHex concentration for the detection of 1 nM Pb^{2+}. (C) Effects of the concentrations of A1 and A2 for detection of 1 nM target DNA in RuHex solution. Both A1 and A2 were of the same concentration, 0, 0.1, 0.2, 0.3, 0.5, 0.8, and 1.0 μM, respectively. The illustrated error bars stand for the standard deviation of 3 measurements.