Electronic Supplementary Material (ESI) for Analytical Methods.
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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:

DNA	Sequence
DNAzyme	5'-SH-TTTTTCATCTCTTCTCC G*AGCC GGTCG AAATA GTGAG T*-3'
Substrate	5'-ACTCACTAT (rA) GGAAGAGATG-3'
Auxiliary probe 1 (A1)	5'-TACTC CCCCA GGTGC *ACT CAC TAT TTC GAC CGG CT* -3'
Auxiliary probe 2 (A2)	5'-GCACC TGGGG GAGTA *AGC CGG TCG AAA TAG TGA GT* -3'

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. To immobilization buffer solution, Tris-acetate (TAE, 25 mM, pH 7.4) containing 10 mmol/L NaCl, 10 μM TCEP was used. TAE (25 mM, pH 7.4) containing 100 mmol/L NaCl and 10 $MgCl_2$ was used as hybridization buffer solution,. Solutions containing metal ion were prepared by dissolving nitrates into TAE (pH 7.4).

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.

Preparation of DNA sensor. Gold electrodes (the diameter of 3mm) were polished with aqueous slurries of 1.0, 0.3, 0.05 μm α-Al_2O_3 powders on the polishing microcloth, respectively, to obtain a mirror-like surface. After rinsed with water, the electrodes were electrochemically cleaned in 0.5 M sulfuric acid by consecutive cyclic voltammetry in the potential range of 0~1.6V until a stable curve was achieved. The cleaned gold surfaces were immersed in the immobilization buffer containing 0.05 μmol/L DNAzyme for 90 min, and then were treated with 1.0 mmol/L MCH for 30 min, which is able to minimize nonspecific binding, maximize the efficiency of hybridization of capture and target probes, and reach a well-aligned DNAzyme monolayer. Next, this DNAzyme monolayer-functionalized electrodes were immersed into substrate strand solution for 2 h at room temperature to achieve DNA-substrate electrodes. It is noting that the electrodes should be washed with distilled water to eliminate the physical adsorption after each modification step.

Cleavage of Pb^{2+} and HCR reaction. For cleavage of Pb^{2+}, above mentioned DNA-substrate electrodes were immersed into buffer solutions containing different concentrations of Pb^{2+} fully for 1 h at room temperature, and then rinsed using distilled water carefully. After cleavage, the electrodes were incubated into 20 uL of a freshly prepared auxiliary probe solution containing 1 uM A1 and 1 uM A2 for 120

min to perform DNA self-assembly.

Electrochemical detection. In the electrochemical assay, three-electrode system was used, that is, above DNA-modified electrode was used as the working electrode, platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively. Electrodes were immersed were performed in the solution containing 5 mmol/L $[Fe(CN)_6]^{3-/4-}$ for EIS experiments. 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. To calculate the DNA surface coverage on the gold electrode, 5.0 μM $[Ru(NH_3)_6]^{3+}$ as a cationic indicator was used. At first, the surface concentration (Γ_{Ru}) of $[Ru(NH_3)_6]^{3+}$ on the gold electrode was calculated by the formula as follow[1], [2]:

$$\Gamma_{Ru}=Q/n\mathrm{F}\mathrm{A}$$

where Q is the charge obtained by integrating the reduction peak area of surface-bound $[Ru(NH_3)_6]^{3+}$, n is the number of electrons involved in the redox reaction, F is Faraday's constant, and A is the electrode area. And then the DNA surface coverage (Γ_{DNA}) on the gold electrode was achieved using the following equation:

$$\Gamma_{DNA}=\Gamma_{Ru}(z/m)\mathrm{N_A}$$

where z is the valence of the redox cation (+3) and m is the number of nucleotides in the DNA.

The preparation of *Bauhinia championi* extraction. 5.0 g of *Bauhinia championi* was extracted in 20 mL of concentrated HCl via soxhlet extraction for 1 h.

1 After filtration to remove insoluble substance, 1 mL of extraction was diluted to 1000

2 mL with Tris-HCl (pH 7.4) consisting of 0.5 mol/L NaCl.

3 Results and Discussion

4 In order to obtain better assay results, the concentrations and the self-assembled

5 time of A1 and A2 are investigated. Figure S1A indicates that R_{ct} changes upon

6 different concentrations of A1 and A2. With the enhancement of A1 and A2

7 concentrations, R_{ct} increases gradually. However, the signal reaches maximum when

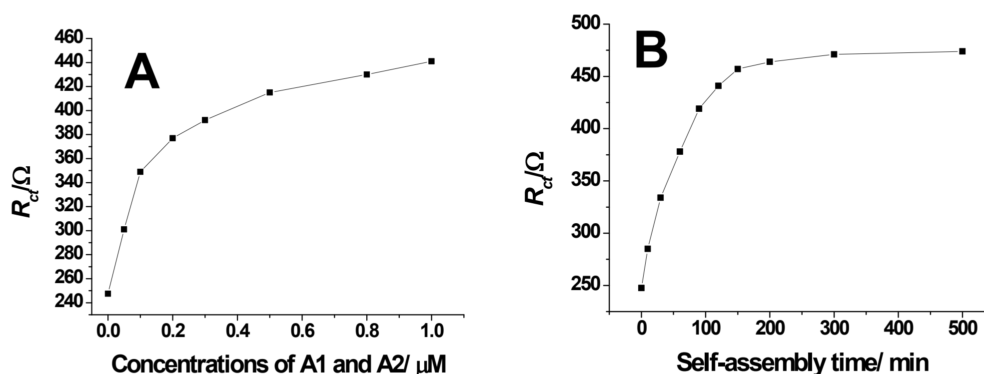
8 the concentrations of A1 and A2 are up to 1.0 μM. Thus, 1.0 μM of A1 and A2 are

9 chosen. At 1.0 μM of A1 and A2, and then the self-assembled time is examined. From

10 Fig. S2B, it is found that when the self-assembly time extends to 120 min, R_{ct}

11 increases slightly, even approaches the plateau. Therefore, the self-assembled time is

12 set as 120 min.



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14 **Figure S1** (A) Effect of the concentrations of A1 and A2 to R_{ct} value. (B)

15 Optimization of self-assembly time in the 1.0 μM of A1 and A2.

16 Here, this sensor has been used to assay Pb^{2+} in the river samples. The river

17 samples from Minjiang River were let stand overnight, and filtered by a filter

membrane (0.2 μm) to remove insoluble substance. By the detection, there is no Pb^{2+} detected. The reason may be that the content of Pb^{2+} in the river is lower than the lowest detectable concentration of this sensor. And then we spiked known amounts of Pb^{2+} into the river sample, shown in Table 1. It is found that there are satisfactory recoveries (93.6~103%) achieved.

Table 1 Assay of Pb^{2+} in river samples

Sample number	Added (nM)	Found (nM)	Recover (%)
	1.0	1.03	103
1	2.0	1.95	97.5
	5.0	4.86	97.2
	1.0	0.94	94
2	2.0	2.04	102
	5.0	4.68	93.6

Reference

[1] H.-Z. Yu, C.-Y. Luo, C. G. Sankar and D. Sen, *Anal. Chem.*, 2003, **75**, 3902–3907

[2] A. K. H. Cheng, B. Ge and H.-Z. Yu, *Anal. Chem.*, 2007, **79**, 5158–5164