

## Electronic Supplementary Information

Naked-eye colorimetric sensor for Hg<sup>2+</sup> monitoring with cascade signal amplification based on target-induced conjunction of split DNAzyme

## Experimental Section

### Chemicals and materials

HAuCl<sub>4</sub>·3H<sub>2</sub>O, trisodium citrate, Tween-20, mercury nitrate, bovine serum albumin (BSA), tris-(hydroxymethyl)aminomethane (Tris), and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, Mo). Other reagents and chemicals were of analytical grade and used without purification. All solution was prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA).

All DNA oligonucleotides were HPLC-purified and purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed as follows:

binding DNA: 5'-TAGCTTCAGTTCTCTCGTCAGAT-3'

I

split catalytic DNAzyme A: 5'-ATCTGTCGAGTG-GTTACACCCATGT-TCGTCA-3'

II

split catalytic DNAzyme B: 5'-TCTTGC-AGCGATTAAC-AACTGATGCTA-3'

E

substrate DNA: 5'-CTGGTCTGGTGCAGCACTGGTATGACGATrAGGCAAGATCATAAGTAGGTACGTCA-3'

D

C

blocking DNA: 5'-CAGACTACTACTTATGACACATAC TACCAGTGCTGCACCAGACCAG-3'

hairpin DNA:

I

5'-AACTGATGCTATGACGTACCTACTTATGATCTTGCTAGCATCAGTTGTTACACCCATGTTCGTCA-3'

DNA probe 1 (AuNPs-1): 5'-SH-AAAAAAGTATGTGTCATA-3'

DNA probe 2 (AuNPs-2): 5'-AGTAGTAGTCTGAAAAAA-SH-3'

### Preparation of gold nanoparticles (AuNPs)

AuNPs with an average diameter of 20 nm were synthesized as following. In a 300 mL triangle flask, 150 mL of 0.01% HAuCl<sub>4</sub> in doubly distilled water were brought to boil with vigorous stirring, followed by the addition of 6 mL of 1% trisodium citrate. The solution turned deep blue within 15 s,

and the final color changed to wine-red within about 80 s. Boiling was pursued for an additional 10 min; the heating source was removed, and the colloid solution was stirred for another 20 min. The resulting AuNPs solution was stored in dark bottles at 4°C and used to prepare the AuNPs-DNA conjugate.

### **Preparation of AuNPs-DNA conjugate**

Conjugation reactions were carried out by adding 120 µL of thiolated DNA (DNA probe 1 or DNA probe 2, 1.0 OD) to 880 µL of the 10-fold concentrated AuNPs solution. After standing at 4°C for 24 h, the solution was subjected to “aging” by the addition of NaCl up to a concentration of 150 mM, and a certain quantity of 1% sodium dodecyl sulfate (SDS) was added to reach a final concentration of 0.01%. The solution was allowed to stand for another 24 h at 4°C, and the excess of reagents was removed by centrifugation for 20 min at 12000 rpm. The supernatant was discarded, and the red pellets were resuspended in 1 mL of buffer (pH 7.5) containing 20 mM Na<sub>3</sub>PO<sub>4</sub>, 5% BSA, 0.25% Tween-20, and 10% sucrose. The resulting AuNPs-DNA probe 1/probe 2 conjugate (AuNPs-1/AuNPs-2) solution was stored at 4°C before further use.

### **Hg<sup>2+</sup> assay procedure**

The caged substrate (partial DNA duplex between substrate DNA and blocking DNA) was first prepared by mixing substrate DNA and blocking DNA at a final concentration of 0.6 µM in 20 mM Tris-Ac buffer (pH 7.4, 150 mM NaAc, 20 mM MgAc<sub>2</sub>), heating the mixture to 95°C for 10 min and gradually cooled to 25°C at a constant rate of 1°C/min.

Various concentrations of Hg<sup>2+</sup> was then incubated with the mixture of binding DNA (0.2 µM), split catalytic DNAzyme A (0.4 µM), split catalytic DNAzyme B (0.2 µM), hairpin DNA (0.3 µM), and the caged substrate (0.6 µM) at room temperature for 120 min. Finally, 100 µL of the above reaction mixture was added into 500 µL of the prepared AuNPs-DNA conjugate solution containing AuNPs-1 and AuNPs-2. After thorough shaking and incubating for about 30 min at room temperature, the color change of the solution and the corresponding UV-vis absorption spectra were recorded.

To evaluate the specificity of the assay, other metal ions (Pb<sup>2+</sup>, Cu<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Sn<sup>2+</sup>, Cr<sup>3+</sup>, Co<sup>2+</sup>, and Mn<sup>2+</sup>) at 100 nM were tested in the same way.

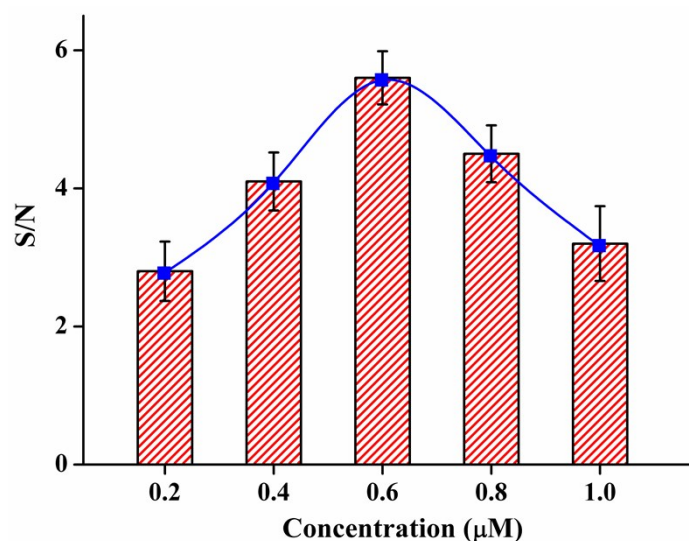
For real sample analysis, river water was collected from the Pear River (Guangzhou, China), tap water was obtained from our laboratory, lake water was taken from South China Botanical Garden, and pond water was collected from our institute. Those water samples were filtered through a 0.2 µm membrane to remove the insoluble impurities. The river water samples were spiked with different concentrations of Hg<sup>2+</sup> and diluted 5 times with the reaction buffer for recovery studies. Other procedures were the same as described above. The other three water samples (tap water, lake water, and pond water) were analyzed separately using the colorimetric sensor and the standard AFS (atomic fluorescence spectrometer).

## Instrumentation

UV-vis absorption spectra of AuNPs were recorded on a TU-1902 UV-vis spectrophotometer (Persee, China). A Nano ZS/Mastersizer 2000E system (Malvern Instruments Ltd., Malvern, UK) was used to conduct all DLS measurements under the following conditions: temperature 25°C, detector angle 90°, incident laser wavelength 633 nm. Each particle size was the average of three repeated measurements. A JEM-3010 transmission electron microscope (Hitachi, Japan) was used to characterize the morphology of AuNPs.

## Optimization of assay conditions

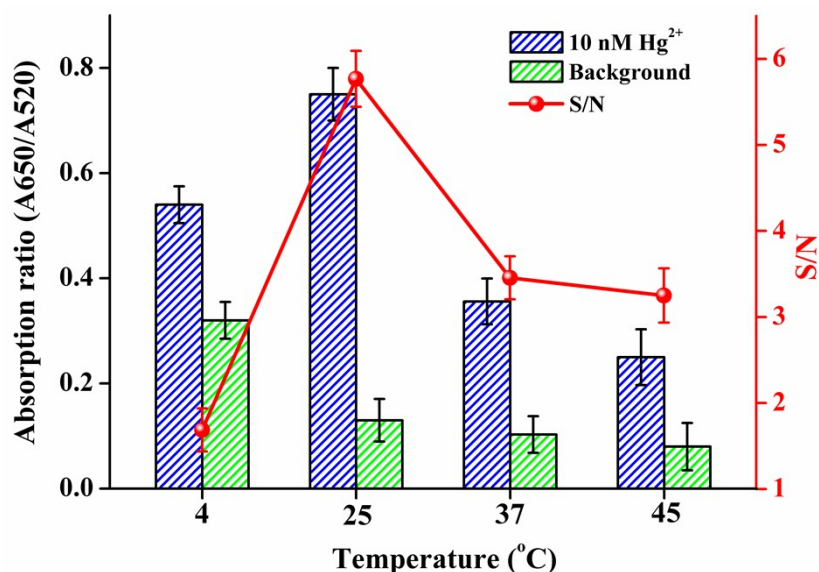
In current study, the concentration of DNA substrate plays an important role in the sensing process. As shown in Fig. S1, the maximum signal-to-noise (S/N) ratio was obtained with the concentration of DNA substrate at 0.6  $\mu\text{M}$ . A higher concentration could generate a high background signal. While a lower concentration could affect the efficiency of the sensor to detect the target  $\text{Hg}^{2+}$ , thus yielding a weak response signal. Therefore, the concentration of DNA substrate was selected as 0.6  $\mu\text{M}$  for target detection.



**Fig. S1** Effect of the concentration of DNA substrate on the performance of the colorimetric sensor. Binding DNA: 0.2  $\mu\text{M}$ , split catalytic DNAzyme A: 0.4  $\mu\text{M}$ , split catalytic DNAzyme B: 0.2  $\mu\text{M}$ , hairpin DNA: 0.3  $\mu\text{M}$ . The experiments were performed at room temperature ( $\sim 25^\circ\text{C}$ ). The error bars represent the standard deviation of three independent measurements.

The effect of reaction temperature on the response of the colorimetric sensor was also investigated by detecting 10 nM  $\text{Hg}^{2+}$  at different temperatures (4  $^\circ\text{C}$ , 25  $^\circ\text{C}$ , 37  $^\circ\text{C}$ , and 45  $^\circ\text{C}$ ). As shown in Fig. S2, the maximum absorption ratio ( $A_{650}/A_{520}$ ) of the solution containing 10 nM  $\text{Hg}^{2+}$  (blue histogram) was obtained at 25  $^\circ\text{C}$ . At high temperature (37  $^\circ\text{C}$  and 45  $^\circ\text{C}$ ), the DNAzyme nearly

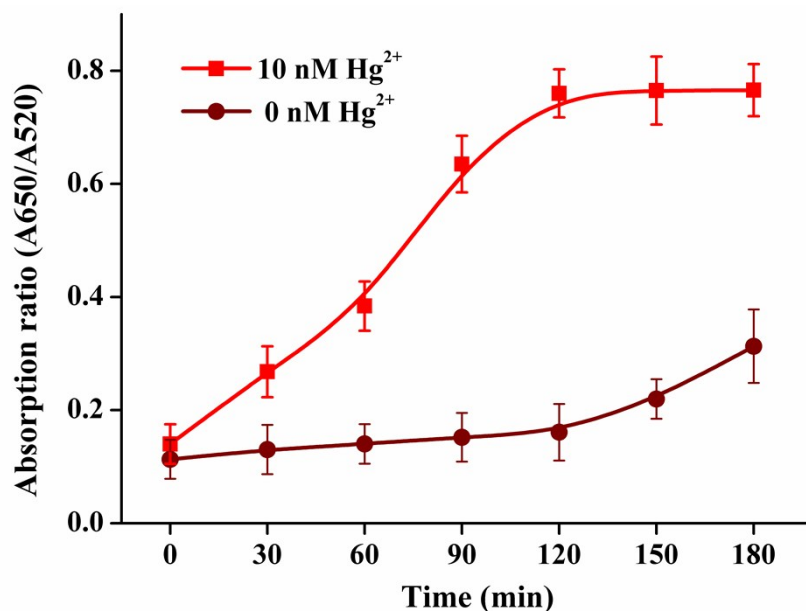
lost all of its activity because the cooperative assembly of the supramolecular DNAzyme *via* nucleic acid hybridization was unstable at this temperature. On the other hand, the background signal (green histogram) increased at low temperature (4 °C) as the split catalytic DNAzymes A and B may bind to the blocked substrate to form an active DNAzyme structure even in the absence of target  $\text{Hg}^{2+}$ . In order to get the best S/N level (red line in Fig. S3), 25 °C was considered to be the optimum reaction temperature.



**Fig. S2** Effect of the reaction temperature on the performance of the colorimetric sensor. The histograms represent the absorbance ratio of the solution with 10 nM  $\text{Hg}^{2+}$  (blue) and without  $\text{Hg}^{2+}$  (green). The red line represents the S/N ratio. The error bars represent the standard deviation of three independent measurements.

The incubation time of DNAzyme is another important parameter affecting the analytical performance of the designed biosensor. As shown in Fig. S3, the absorption ratio (A650/A520) of the solution containing 10 nM  $\text{Hg}^{2+}$  increased accordingly with the extension of the incubation time and reached a plateau after 120 min, which indicated the completion of the cyclic cleavage reaction (red

line). However, the background signal (in the absence of  $\text{Hg}^{2+}$ ) maintained its increase along with increasing the incubation time (brown line). To obtain the best signal-to-background level and the maximal signal amplification efficiency, 120 min was selected as the optimum incubation time.



**Fig. S3** Effect of the incubation time of DNAzyme on the performance of the colorimetric sensor. Time course of colorimetric response recorded in the absence (brown line) or in the presence of 10 nM  $\text{Hg}^{2+}$  (red line). The experiments were performed at room temperature ( $\sim 25$  °C). The error bars represent the standard deviation of three independent measurements.

**Table S1** Recovery experiments of  $\text{Hg}^{2+}$  determination in river water samples.

Sample	Added (nM)	Found (mean <sup>a</sup> ± SD <sup>b</sup> ) (nM)	Recovery (%)
River water 1	0.1	0.88 ± 0.05	88
River water 2	1	1.02 ± 0.12	102
River water 3	10	9.8 ± 0.68	98

River water 4	25	26.5 ± 1.84	106
River water 5	50	48.6 ± 3.62	97.2

<sup>a</sup>Mean of three determinations. <sup>b</sup>SD, standard deviation.

**Table S2** Determination of Hg<sup>2+</sup> (pM) in Real Samples Using the Proposed Method and AFS.

Sample	AFS <sup>a</sup>	Proposed method <sup>b</sup>	Relative error (Re) <sup>c</sup> (%)
tap water	82.5 ± 5.6	86.5 ± 5.2	4.8
lake water	145.6 ± 6.8	137.4 ± 8.3	-5.6
pond water	963.8 ± 12.4	985.6 ± 15.6	2.3

<sup>a</sup>Each sample was analyzed using the standard AFS (atomic fluorescence spectrometer), and all values were obtained as an average of three repetitive determinations ± standard deviation (mean ± SD).

<sup>b</sup>Each sample was analyzed using our proposed colorimetric sensor, and all values were obtained as an average of three repetitive determinations ± standard deviation (mean ± SD).

<sup>c</sup>Our proposed method vs. AFS.