

Electronic Supplementary Information

**Ultrasensitive visual detection of Hg²⁺ ions via the Tyndall
Effect of gold nanoparticles**

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Experimental Section

Materials and Apparatus

Hydrogen tetrachloroaurate (III) ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) and sodium citrate were purchased from Sigma-Aldrich. Polysorbate-20 (Tween-20) was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Mercury nitrate ($\text{Hg}(\text{NO}_3)_2$) and other metal salts were the products of Xilong Chemical Co., Ltd. (Guangzhou, China). All other chemicals were of analytical grade and used as received without further purification. The used buffer is 10 mM phosphate-buffered saline (pH 7) that contains 100 mM NaCl. Unless stated otherwise, all stock and buffer solutions were prepared with deionized water (with a specific resistivity $\geq 18.2 \text{ M}\Omega \cdot \text{cm}$) that was produced by an ultrapure water system (UPS-II-20L) of Chengdu Yuechun Technology Co., Ltd. (Chengdu, China). The real water samples were collected from a pond (that had been contaminated with mercury) near a factory. Hydrophilic polycarbonate nanoporous membranes (Whatman, $\sim 50 \text{ nm}$ in average pore size) were purchased from GE Healthcare Life Sciences.

Gold nanoparticles (GNPs) were synthesized with a domestic microwave oven. Optical characterization of dispersed and aggregated GNPs was performed on a UV-Vis spectrometer (Cary 50, Varian, USA). Their morphology characterization was carried out using a transmission electron microscope (TEM, JEM-2100F, JEOL, Japan) and a scanning electron microscope (SEM, SU 5000, Hitachi, Japan). The particle sizes (diameters) of the aggregated GNPs were determined with dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK). The refractive index

of GNPs and Hg-Au alloys were measured with an Abei refractometer (2WAJ) that was from Shanghai CSOIF Co., Ltd. The Tyndall Effect (TE) signals were produced by using a 635-nm red laser pointer pen (5 mW) as a handheld light source bought from Deli Group Co., Ltd. (Ningbo, China). Images of all colorimetric results were recorded by using a smartphone (Huawei Mate 10).

Preparation and modification of GNPs

The colloidal GNPs were synthesized by a microwave-assisted sodium citrate reduction method.¹ Briefly, for the synthesis of GNPs with ~21 nm in average diameter, 1 mL of a 1 wt % (w/v) H₂AuCl₄·3H₂O solution and 2 mL of a 1 wt % (w/v) sodium citrate solution were rapidly mixed with 97 mL of water, followed by heating in a home-use microwave oven at high power (800 W) for 8 min. After the resulting GNP solution was allowed to cool to room temperature (25 °C), it was diluted with water to the original total volume of 100 mL and was then stored at 4 °C for further use. The final GNP concentration was estimated to be ~8.8 nM.

SPR-based method for Hg²⁺ detection

For the common surface plasmon resonance (SPR) signalling method, 200 μL of the buffer, 200 μL of Hg²⁺ sample, and 400 μL of a red GNP solution (8.8 nM) were mixed successively at room temperature (25°C). The Hg²⁺-caused aggregation of GNPs resulted in the color change in the reaction solution from red to purple or blue allowed for the qualitative analysis of Hg²⁺ with the naked eye. The color change

degree positively relied on the Hg^{2+} level. UV-Vis spectrum of each reaction mixture was also measured for further quantitative analysis.

TE-based method for Hg^{2+} detection

For the TE signalling method, 200 μL of the buffer, 200 μL of Hg^{2+} sample, and 400 μL of buffer containing 0.88 nM Tween-20-coated GNPs were mixed successively and were incubated for 45 min at room temperature (25°C). The red laser pointer pen was used to provide the visual TE signal positively depending on the Hg^{2+} concentration. For further quantitative analysis, each TE image was captured by using a smartphone and in turn, its signal intensity, i.e., the average gray (AG) value, was measured using the grayscale option in the Analyze function of the ImageJ processing software. The ΔAG value for each Hg^{2+} sample was defined as: $\Delta\text{AG} = \text{AG}_{\text{Hg}^{2+}} - \text{AG}_{\text{blank}}$, where $\text{AG}_{\text{Hg}^{2+}}$ and AG_{blank} were obtained from the Hg^{2+} sample and a blank sample (i.e., a buffer sample without the analyte ion), respectively. Specificity experiments were performed in the same manner but using K^+ , Na^+ , Ag^+ , Mg^{2+} , Mn^{2+} , Pb^{2+} , Zn^{2+} , Ni^{2+} , Ca^{2+} , Cd^{2+} , Fe^{3+} , Cr^{3+} or Al^{3+} instead of Hg^{2+} .

Reference

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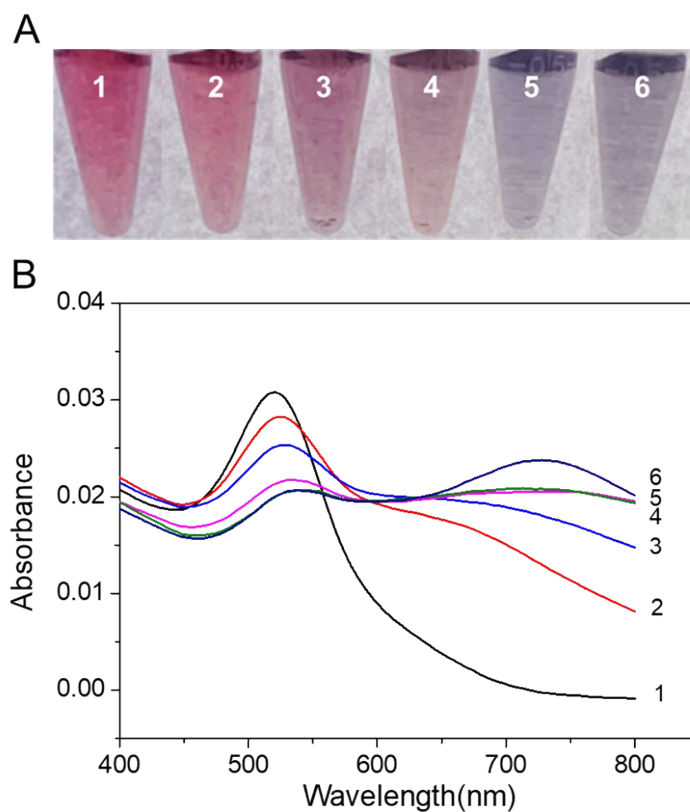


Fig. S1 (A) Colorimetric results obtained from the mixing of 8.8 nM GNPs and NaCl solutions in water with different concentrations at room temperature (~ 25 °C): (1) 0, (2) 40, (3) 60, (4) 80, (5) 100 and (6) 120 mM. (B) UV-Vis spectra measured from these mixture solutions shown in (A). The results suggest that the NaCl solutions with a level ≥ 100 mM could lead to a complete GNPs' aggregation.

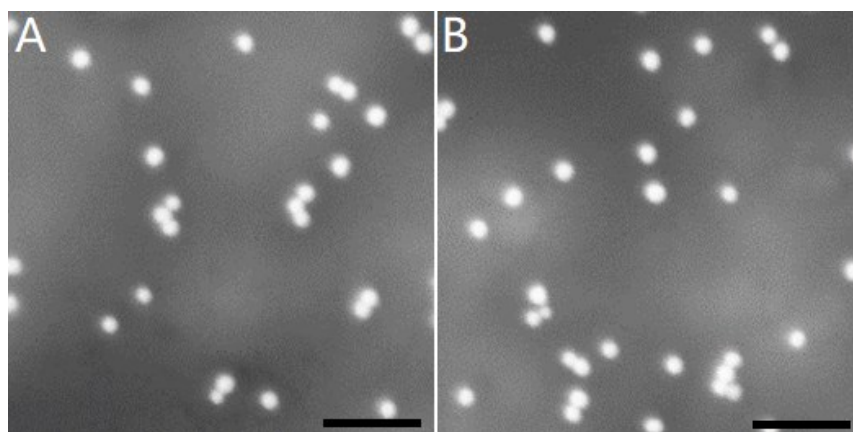


Fig. S2 Scanning electron microscope images obtained from the GNPs with (A) and without (B) Tween-20 modification. The scale bar is 100 nm.

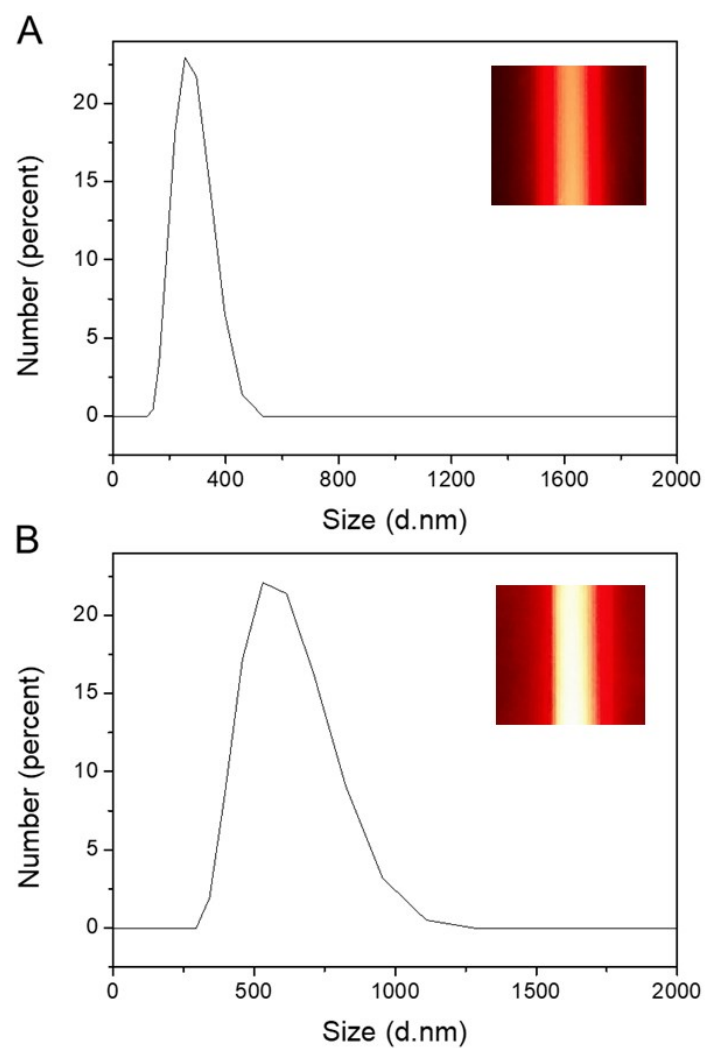


Fig. S3 Hydrodynamic sizes of the GNP aggregates produced in the assays of 200 and 1 μM Hg^{2+} samples. Insets show the corresponding TE images. The results indicate that the TE signals positively rely on the particle sizes of the GNP aggregates.

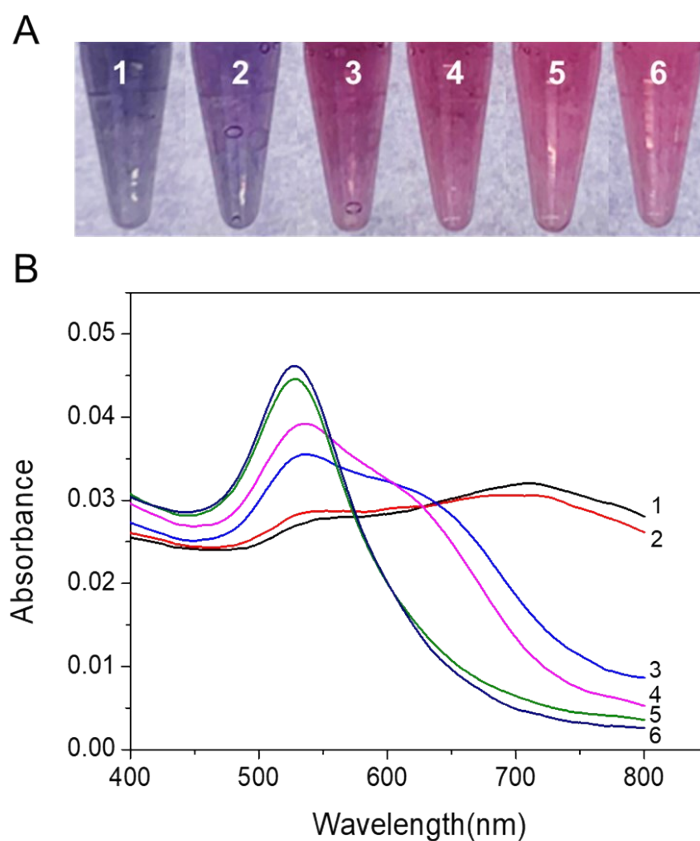


Fig. S4 (A) Colorimetric results obtained from the modification of GNPs (in buffer containing 100 mM NaCl) with Tween-20 molecules with different volume ratios (v/v): (1) 1:1800, (2) 1:1600, (3) 1:1400, (4) 1:1200, (5) 1:1000, and (6) 1:800. (B) UV-Vis spectra recorded from the mixture solutions shown in (A). The results suggest that the Tween-20 concentration with a volume ratio \leq 1:1600 could make the GNPs them stably disperse in the high-ionic-strength condition, which was then recommended for all the following experiments.

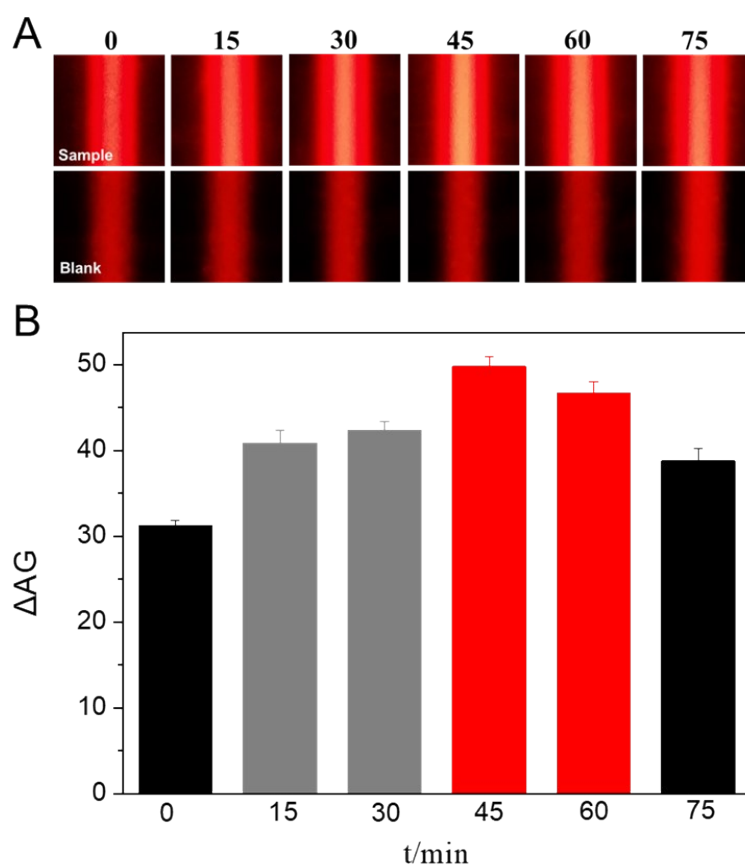


Fig. S5 (A) TE results obtained from the reactions of Tween-20-modified GNPs and Hg^{2+} samples ($1 \mu\text{M}$) or blank samples (buffer without the target ion) with different incubation time (min). (B) The AG changes (ΔAG) between the TE images recorded from the Hg^{2+} and blank samples shown in (A). The results suggest that 45 min should be chosen as the optimal incubation time as it produced the highest ΔAG value. Each error bar represents a standard deviation across three replicate experiments.

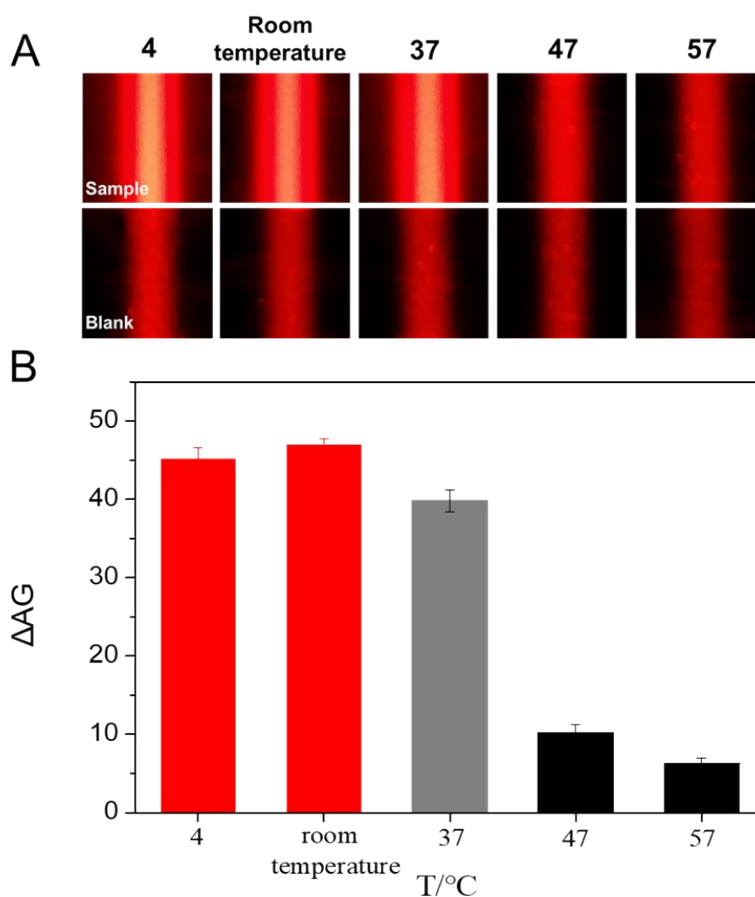


Fig. S6 (A) TE results obtained from the reactions of Tween-20-modified GNPs and Hg^{2+} samples ($1 \mu\text{M}$) or blank samples (buffer without the target ion) at different incubation temperatures. (B) The AG changes (ΔAG) between the TE images recorded from the Hg^{2+} and blank samples shown in (A). The results suggest that room temperature ($\sim 25 \text{ }^\circ\text{C}$) should be chosen as the optimal incubation temperature as it produced the highest ΔAG value. Each error bar represents a standard deviation across three replicate experiments.

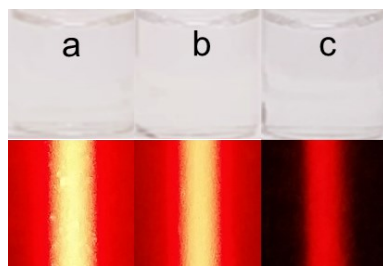


Fig. S7 Colorimetric (top) and TE (bottom) results obtained from the analysis of different Hg²⁺ Samples with the new TEA method: (a) 10 μM, (b) 250 μM, and (c) 1.25 mM.

Table S1 Comparison of the new Hg²⁺ assay with some previous colorimetric ones

with nanoprobe

Colorimetric probe	Signalling method	Quantifying method	Portability	Linear concentration range	Limit of detection	Ref.
Reduced graphene oxide-polyethylenimine - Pd Nanohybrids	Chromogenic reaction	UV-Vis spectrum	No	1 nM - 5000 nM	1 nM	1
Molybdenum(IV) selenide nanosheets	Chromogenic reaction	UV-Vis spectrum	No	25 nM - 2.5 μM	3.5 nM	2
Au@AgPt	SPR	UV-Vis spectrum	No	1 μM - 1 mM	1 μM	3
AuNP@β-cyclodextrin	SPR	UV-Vis spectrum	No	0.4 μM - 15 μM	147 nM	4
Silver nanoprisms	SPR	UV-Vis spectrum	No	3 nM - 500 nM	3 nM	5
GNP	SPR	UV-Vis spectrum	No	1 nM - 1000 nM	2.9 nM	6
GNP	SPR	UV-Vis spectrum	No	25 nM - 750 nM	50 nM	7
GNP	SPR	UV-Vis spectrum	No	15.4 nM - 92.3 nM	10 nM	8
GNP	SPR	UV-Vis spectrum	No	9.7 nM - 0.62 μM	1.1 nM	9
GNP	SPR	UV-Vis spectrum	No	8.76 nM - 127 μM	8.76 nM	10
GNP	SPR	UV-Vis spectrum	No	0.01 - 1.5 μM	7.7 nM	11
GNP	Tyndall Effect	Smartphone	Yes	1.9 - 62.5 nM/ 62.5 nM - 1 μM	0.13 nM	This work

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Table S2 Recovery of Hg²⁺ in several polluted pond water samples

Sample	Found ^a (nM)	Added (nM)	Total ^b (nM)	Recovery (%)	RSD ^c (%; <i>n</i> = 6)
1	41.2	50	93.8	105.1	1.86
2	41.5	50	90.2	97.4	2.63
3	87.9	100	192.3	104.4	2.17

^aThe original Hg²⁺ concentrations in the samples using atomic absorption spectroscopy.

^bThe total Hg²⁺ concentrations in the samples using the proposed method and the regression equation: $y = 66.17853x - 96.8376$ ($R = 0.9925$).

^cRSD, relative standard deviations.