Supporting Information

"Molecular beacon"-directed fluorescence of Hoechst dyes for visual detection of Hg (II) and biothiols and its application for a logic gate

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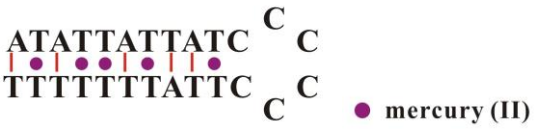
Experimental Section

Reagents and materials. The oligonucleotides used in this study were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) with the following sequences: **ATprobe**: 5'-ATATTATTATCCCCCCTTATTTTTTT-3'; **PMprobe**: 5'-ATATTATTATCCCCCCATAATAATAT-3'. The following metal salts: $Mg(NO_3)_2$, $Cu(NO_3)_2$, $Mn(Ac)_2$, $Zn(Ac)_2$, $Cr(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Co(Ac)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Hg(Ac)_2$, $Ca(Ac)_2$, $CrCl_3$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $NaNO_3$ and KNO_3 were reagent-grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Cysteine and other 19 amino acids were purchased from Sigma-Aldrich (St. Louis, MO). 10×$NaNO_3$-MOPS buffer (500 mM $NaNO_3$ and 200 mM 3-(4-morpholinyl)-1-propanesulfonic acid, pH 7.0) was prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. All chemicals used in this work were of analytical reagent and obtained from commercial sources and directly used without additional purification. All buffers were prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system.

Instrumentation. Fluorescence was measured in a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) using a black 384 well microplate (Fluotrac 200, Greiner, Germany). Photographs were taken with a digital camera. A circular dichroism spectrometer (Chirascan, Applied Photophysics Limited, UK) equipped with a temperature controller and controlled by a PC was used for all circular dichroism measurements at 20 °C. A 1mL quartz cell of 1 cm path length was used.

Data analysis. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was employed to perform the data processing. Each sample was repeated in duplicate, and data were averaged. The DNA structures used in this study were predicted by the popular structure-prediction program Mfold (http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form).

Table S1 Structures predicted by Mfold for **ATprobe** and **PMprobe** and Hg^{2+}-directed **ATprobe** formation of a step-loop hairpin

ATprobe	5'-ATATTATTATCCCCCCTTATTTTTTT-3' dG = 1.47 dH = -14.20 dS = -50.52 T_m = 7.9 ℃ AT--------------| T AT A TA T TTTTTTTATTCCCCCC^ T 20 10 Note: The structure was predicted by Mfold
PMprobe	5'-ATATTATTATCCCCCCATAATAATAT-3' dG = -3.41 dH = -64.50 dS = -196.97 T_m = 54.3 ℃ 10 | CC ATATTATTAT C TATAATAATA C ^ CC 20 Note: The structure was predicted by Mfold
ATprobe+mercury(II)	 Note: Hg^{2+}-directed formation of a step-loop hairpin was proposed in this work

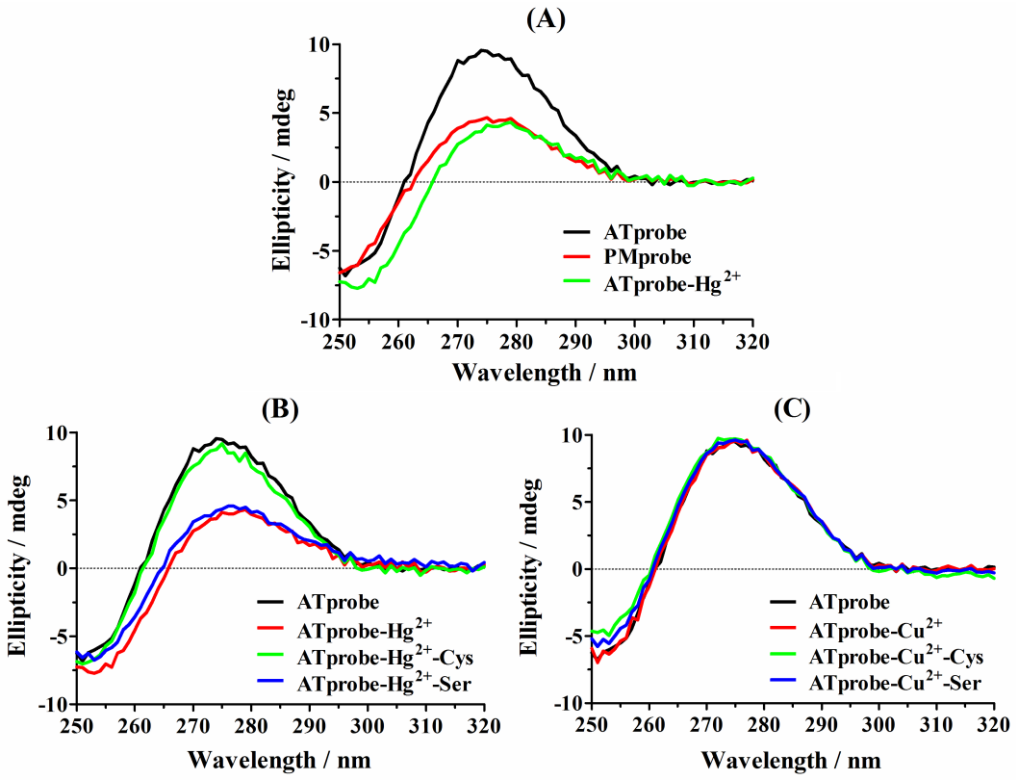


Fig. S1 (A) Circular dichroism (CD) spectra of 2 μM **ATprobe** in the solution treated with none (black line) and 5 μM Hg^{2+} (green line), additionally, a perfectly matched DNA hairpin **PMprobe** acted as a control (red line). (B) Investigation of CD spectra of 2 μM **ATprobe** toward 5 μM Hg^{2+} (red line) and following 10 μM Cys (green line) (Ser as a control, blue line), respectively. (C) A control CD experiment of **ATprobe** toward 5 μM Cu^{2+} and following 10 μM Cys or Ser, respectively.

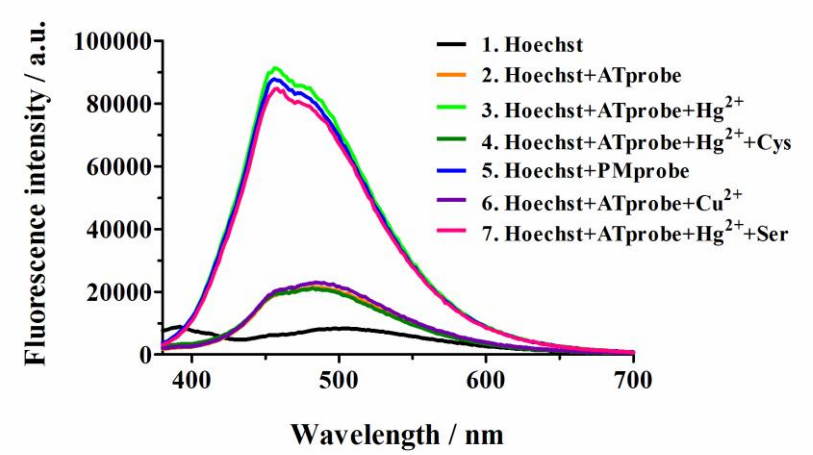


Fig. S2 Fluorescent response of 0.01 mg/mL Hoechst in solution upon the addition of 2) 2 μM **ATprobe**, 3) 2 μM **ATprobe** + 5 μM Hg^{2+}, 4) 2 μM **ATprobe** + 5 μM Hg^{2+} + 10 μM Cys, 5) 2 μM **PMprobe**, 6) 2 μM **ATprobe** + 5 μM Cu^{2+} and 7) 2 μM **ATprobe** + 5 μM Hg^{2+} + 10 μM Ser excited at 360 nm, respectively. The picture was taken under the 365 nm handheld UV lamp excitation using a digital camera.

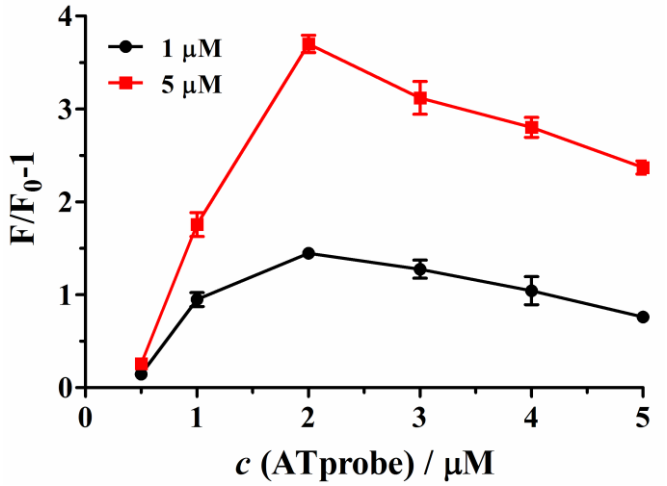


Fig. S3 Optimization of the optimal stoichiometric ratio of **ATprobe** and Hoechst for sensing Hg^{2+}. Best discrimination ability between 1 μM and 5 μM Hg^{2+} can be obtained when 2μM **ATprobe** and 0.01 mg/mL Hoechst dye are used.

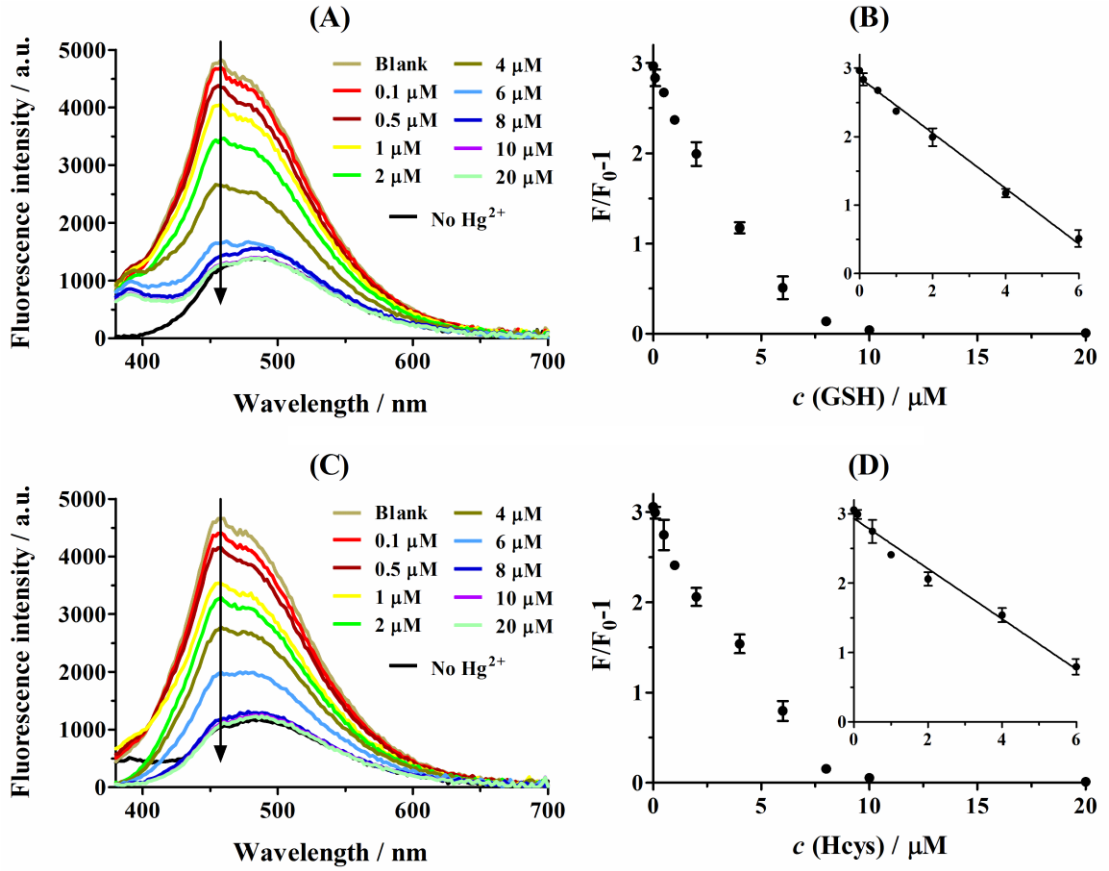


Fig. S4 The fluorescence emission spectra are depicted for various (A) GSH and (C) Hcys concentrations of 0, 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 μM with the excitation wavelength at 360 nm. Plot of the fluorescence ratios (F/F_0-1) vs. the increasing concentrations of (B) GSH and (D) Hcys of the same data. Insets: magnification of the plot of the fluorescence ratios (F/F_0-1) corresponding to the concentration in the range 0-6.0 μM.

Table S2. Determination of Hg^{2+} in real samples

Sample	*Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Tap water	Not found	1.30	1.32	101.53
		2.50	2.53	101.20
$River water	Not found	1.30	1.35	103.85
		2.50	2.45	98.00

*This was verified using our reported graphene-based fluorescence biosensor (data not shown) (Reference: M. Zhang, B. C. Yin, W. Tan and B. C. Ye, *Biosens. Bioelectron.*, 2011, 26, 3260-3265);

$The water sample was collected from the Qin Chun River in the campus of East China University of Science and Technology. The sample was first filtered through a 0.22 μM membrane to remove soil and other particles, and then tested by the proposed method.

Table S3. Determination of Cysteine in real samples

Sample	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Urine (50%)	Not found	6.00	5.70	95.00
		7.00	7.24	103.43
*Serum (2%)	Not found	3.00	2.80	93.33
		4.50	4.67	103.78
		5.50	5.72	104.00
		7.50	7.46	99.47

*High concentration of serum is unsuitable for the presented sensor to follow the spiked amount of Cysteine.