Electronic Supplementary Material (ESI) for Analytical Methods

Supporting information

G-quadruplex fluorescence quenching ability: a simple and efficient strategy to design a single-labeled DNA probe

Xiaohai Yang, Ying Zhu, Pei Liu, Leiliang He, Qingzhao Li, Qing Wang, Kemin Wang*, Jin Huang, Jianbo Liu

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, P. R. China.

Tel/ Fax: +86 731 88821566; E-mail: kmwang@hnu.edu.cn

Experimental Section

1.1 Materials and instruments

All oligonucleotides with different sequences (Table S1) were synthesized by Takara Biotechnology Co. (Dalian, China) and purified by HPLC. 3-(N-morpholino) propanesulfonic acid (MOPS) was purchased from Sigma-Aldrich, Inc. (Shanghai, China). $Hg(NO_3)_2$, $Mn(NO_3)_2$, $Ni(NO_3)_2$, $Ca(NO_3)_2$, $CdSO_4$, $ZnSO_4$, $Cu(NO_3)_2$, $Pb(NO_3)_2$, $Mg(NO_3)_2$, $CoSO_4$, $FeSO_4$, $Fe_2(SO_4)_3$, $NaNO_3$, KNO_3 were analytical grade. Hg^{2+} was prepared by dissolving some $Hg(NO_3)_2$ with 0.5% HNO_3 as the stock solution, and HNO_3 was added to adjust the solution to pH 6.0 to prevent the formation of HgO particles. All work solutions were 10 mM MOPS (100 mM $NaNO_3$, 20mM KNO_3 pH=7.0). All solutions were prepared with Milli-Q water (18.2 MΩ·cm) from a Millipore system.

Fluorescence measurements were performed on a Hitachi F4500 fluorescence spectrophotometer.

1.2 Performance of Hg^{2+} detection

First, the mercury assay solution was prepared. 50 nM probe MP3 were added to pH=7.0 10 mM MOPS buffer solution containing 100 mM $NaNO_3$ and 20mM KNO_3.

This solution was heated at 90 ^{o}C for 10 min to remove aggregates and gradually cooled to room temperature. Then, the solutions were allowed to incubate for 1h at room temperature, allowing the folding of probe MP3 into the G-quadruplex structure.

Second, 500 μL of the assay solution prepared above was transferred to a cuvette. The cuvette was placed in the fluorescence spectrophotometer at room temperature to record the fluorescence emission spectra. After the initial reading, 1 μL of concentrated Hg^{2+} solution was added. After vortexing, the fluorescence emission spectra were recorded by fluorescence spectrophotometer again. The selectivity of probe MP3 was confirmed by adding other metal ions instead of Hg^{2+} in a similar way.

1.3 Real sample detection

A real water sample was collected from the Xiang River (Changsha, China) and filtered through 0.22 μm membrane before testing. 250 μL of the river water was mixed with 50 μL of concentrated Hg^{2+} solution, then 200 μL concentrated assay buffer solution was added to the above prepared solution. The final volume of 500 μL containing 50 nM probe MP3, Hg^{2+} of a certain concentration and 10 mM MOPS (100 mM $NaNO_3$, 20 mM KNO_3, pH=7.0), the dilution factor was 2.0. After incubate for 1h at room temperature, the fluorescence response was monitored by the fluorescence spectrophotometer.

1.4 Performance of cysteine detection

First, the cysteine assay solution was prepared. In 500 μL of the mercury assay solution, 1 μL of concentrated Hg^{2+} solution was added, and the final concentration of Hg^{2+} was 1 μM. After vortexing, the fluorescence emission spectra were recorded by the fluorescence spectrophotometer. Then, 1 μL of concentrated cysteine (Cys) solution was added, after vortexing, the cuvette was put back into the fluorescence spectrophotometer to record spectra again.

1.5 Sensitivity and selectivity of the Cys-sensing system

As a sulfur-containing amino acid, Cys can be used as a strong binder of Hg^{2+}. When Cys is added into the Hg^{2+} assay solution, the T-Hg^{2+}-T base pairs may be disrupted, and the fluorescence increased. Therefore, the Hg^{2+} detection method may be further explored as a Cys detection method. To study the feasibility of this method for Cys quantitation, the fluorescence of the assay solution as a function of Cys concentration was measured. As shown in Fig. S4, the fluorescence increased with the addition of Cys at concentration range from 0.1-2.0 μM, with detection limit of 85 nM.

To determine the selectivity of the Cys-sensing system, the effects of other amino acids were investigated. As shown in Fig.S5, in the absence of Cys, none of the tested amino acids caused a significant increase in fluorescence ratio. These results suggested an excellent selectivity for Cys over other amino acids.

Figure Legend:

Table S1 Conformation of Sequence

Name	Sequence (5'-3')
MP0	FAM-TTG TTT GTT CCC CTT CTT TCT T
MP1	FAM-TTG TTT GTT CCC CTT CTT TCT T***G***
MP2	FAM-TTG TTT GTT CCC CTT CTT TCT T***GG TTG GTG TGG TTG G***
MP3	FAM-TTT GTT TGT TCC CCT TCT TTC TT***G GTT GGT GTG GTT GG***
MP4	FAM-TTT TGT TTG TTC CCC TTC TTT CTT ***GGT TGG TGT GGT TGG***
MP5	FAM-TTT TTG TTT GTT CCC CTT CTT TCT T***GG TTG GTG TGG TTG G***
MP6	FAM-TTG TTT GTT CCC CTT CTT TCT TT***G GTT GGT GTG GTT GG***

Table S2 Recoveries of Hg^{2+} (n= 4)

Samples	Hg^{2+} (nM)		Recovery (%)
	Added	Recovered	
River water	50.0	47.9±0.7	95.8±1.4
	100.0	105.7±5.2	105.7±5.2
	500.0	467.0±16.4	93.4±3.3

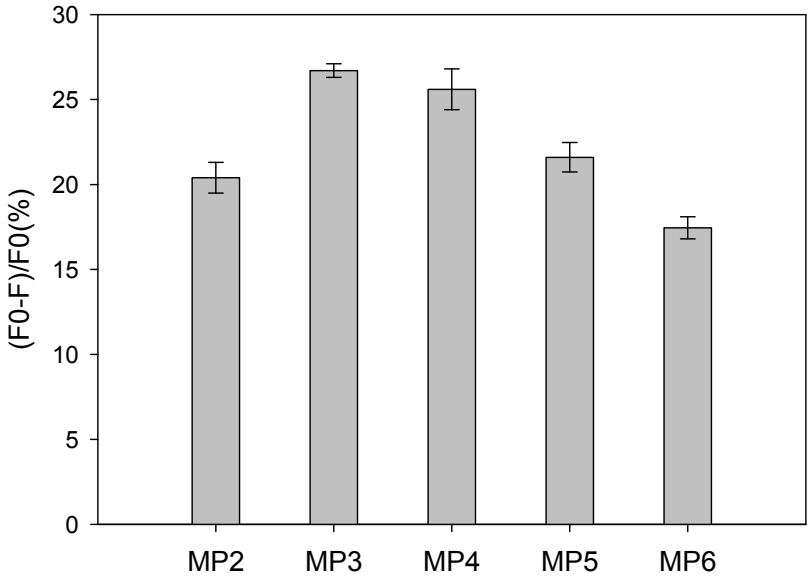

Fig.S1 Sequence optimization for Hg^{2+} assay. Hg^{2+} concentration is 0.5 μM.

Probe: 50 nM, $\lambda_{ex/em}$ = 488/526 nm, slit width: 10 nm

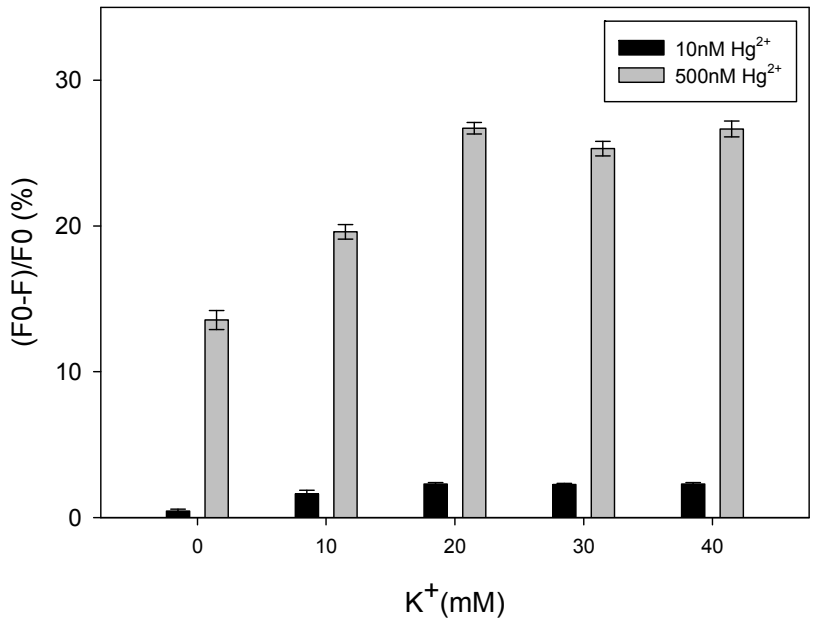

Fig.S2 The K^+ concentration optimization. The fluorescence ratio of Hg^{2+}-sensing system after addition of 10 nM Hg^{2+} in the different K^+ concentration buffer, from 0 to 40 mM (black bar); and addition of 500 nM Hg^{2+} in the different K^+ concentration buffer, from 0 to 40 mM (gray bar). Probe: 50 nM MP3, $\lambda_{ex/em}$ = 488/526 nm, slit width: 10 nm.

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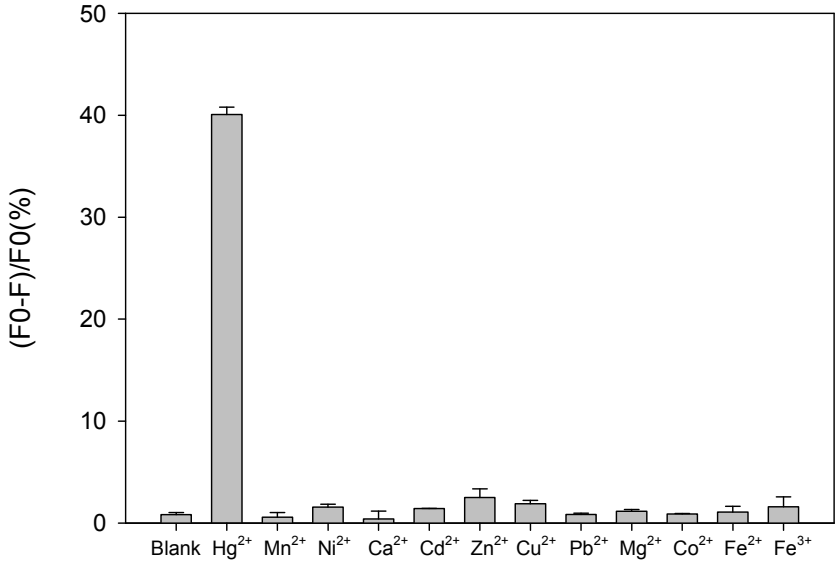

Fig.S3 Selectivity of the Hg^{2+} assay. All metal ions were tested at 1μM. Probe: 50 nM MP3, $\lambda_{ex/em}$ = 488/526 nm, slit width: 10 nm.

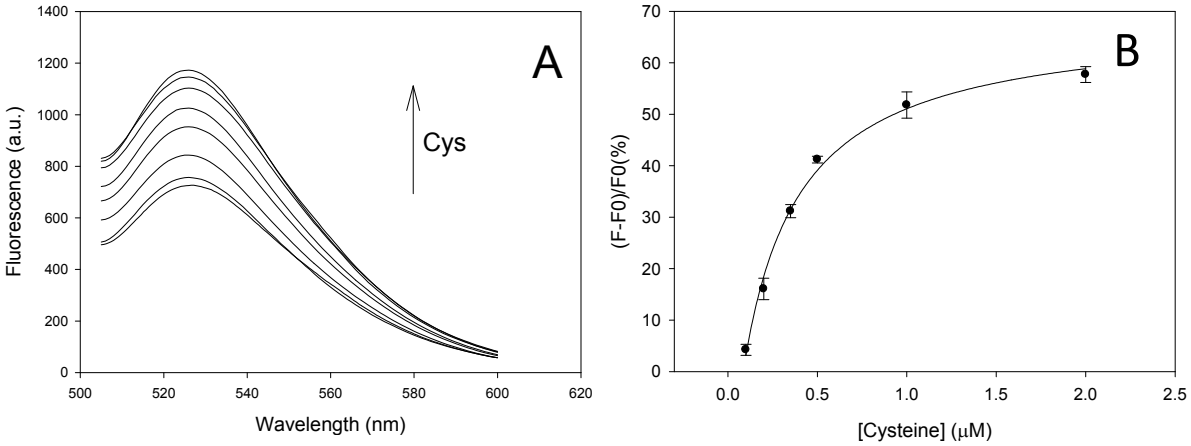

Fig.S4 (A) Fluorescence emission spectra in the presence of varying Cys concentrations. The concentrations of Cys are (arrow direction): 0, 0.10, 0.20, 0.35, 0.50, 1.0 and 2.0 μM. (B) Calibration curve of fluorescent Cys assay. Probe: 50 nM MP3, $\lambda_{ex/em}$ = 488/526 nm, slit width: 10 nm.

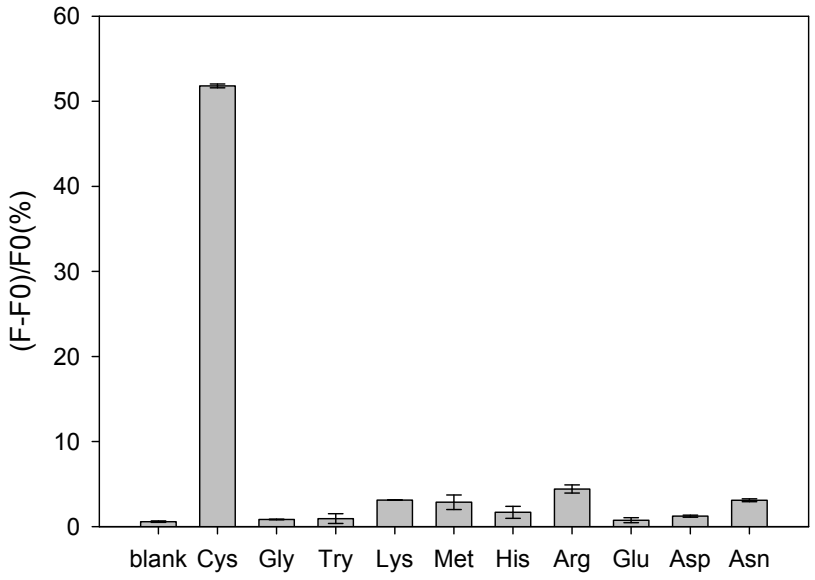

Fig.S5 Selectivity of the Cys assay. All amino acids were tested at 1μM. Probe: 50 nM MP3, $\lambda_{ex/em}$ = 488/526 nm, slit width: 10 nm.