Electronic Supporting Information

Engineering A Unimolecular Multifunctional DNA Probe for Analysis of Hg^{2+} and Ag^{+}

Xiaoxiao He, Zhihe Qing §, Kemin Wang,* Zhen Zou, Hui Shi, Jin Huang

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Biology, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, P. R. Chin. Tel/ Fax: +86 731 88821566; E-mail: kmwang@hnu.cn

§ Joint first author.

Experimental section

Materials and instruments. All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai China). DNA sequences are showed in table S1. All DNA sequences (10 μM) were dissolved in ultrapure water as stock solutions. Silver nitrate and Mercury (Ⅱ) perchlorate trihydrate were obtained from the Dingguo Biological Techlonogy Co.,Ltd (Beijing, China), their stock solutions were prepared by dissolving the desired amount of the materials in ultrapure water, and finally diluted to 50 μM. Water sample was taken from the Xiangjiang River. Other used reagents were purchased from Reagent & Glass Apparatus Corporation of Changsha. All chemical reagents were of analytical reagent grade and were dissolved with ultrapure water without further purification or treatment. The work solution of the oligonucleotide contained 20mM Tris-acetate (pH 7.4) .After prepared, all reagents were stored at 4 ℃ for further use. pH measurements were performed on a 868 pH meter (Orion). Fluorescence values were measured by a Hitachi F-4500 fluorescence spectrofluorometer.

Effect of temperature on the fluorescence emission of the UMDP. The reaction solution (20 mM Tris-acetate ,pH 7.4) containing the DNA probe(10 nM) was placed in a quartz cell, then a few microliters of Hg^{2+} or Ag^{+} stock solution (50 μM) was added to achieve 1 μM concentration while the blank without ions, as the temperature climbed from 10 ℃ to 50 ℃，the fluorescence emission intensity was recorded at 526 nm with 481nm excitation by every 5 ℃.

Hg^{2+} or Ag^{+}-induced fluorescence quenching. The UMDP and the control DNA probe were used. The process was followed: 450 μL of work solution containing 20 mM Tris-acetate (pH 7.4) was added into a quartz cell. Then, 50 μL probe stork solutions (100 nM) were added into the work solution. A few microliters of metal ion stock solution (50 μM) was subsequently added to achieve different ion concentrations and incubated for 3 min. Finally, the fluorescence emission spectra were measured in the emission range 500 nm to 600 nm with excitation of 481 nm. The fluorescence quenching efficiency was determined according to the equation (1):

$$\text{Quenching efficiency} = (F_0\text{-}F)\ /F_0 \times 100\% \qquad (1)$$

where F_0 was the fluorescence intensity when no target ion was added; F was the fluorescence intensity when target ions of corresponding concentration were added to the reaction solution.

Fluorescence recovery test. To further differentiate the two target ions, the fluorescence recovery of the UMDP was tested after C-rich DNA was added. Briefly, the fluorescence of the UMDP incubated with different molar ratios of Ag^{+} to Hg^{2+} ($0Ag^{+}$:$4Hg^{2+}$; $1Ag^{+}$:$3Hg^{2+}$; $2Ag^{+}$:$2Hg^{2+}$; $3Ag^{+}$:$1Hg^{2+}$; $4Ag^{+}$:$0Hg^{2+}$) were first detected. Then, 5μL C-rich DNA sequence stock solution (100 μM) was added into the reaction system and incubated for 5 min, fluorescence measurement was performed and recorded again. The relative fluorescence recovery efficiency was calculated in term of the equation (2):

$$F_r = [\ (F_{\text{C-rich}} - F) / (F_0 - F)\ ] \times\ 100\% \qquad (2)$$

where F_0 was the fluorescence intensity when no target ion was added; F was the fluorescence intensity when target ions of corresponding concentration were added to the reaction solution; $F_{\text{C-rich}}$ was the fluorescence intensity when C-rich DNA (1μM) was added into the reaction solution.

Assay of Hg^{2+} and Ag^{+} in the water samples. The samples taken from Xiangjiang River were filtered using a 0.22 μm membrane to remove the sand and soil, then centrifuged at 12000 rmp for 10 min to remove the small particles. All the water samples were spiked with Hg^{2+} or Ag^{+} at different concentration levels, which were treated on the basis of the presence of possible metal ions in the environmental water, and finally were analyzed using the UMDP.

Table S1 Oligonucleotides used in the present study.

Note	sequence (5'-3')
UMDP	FAM-ACACTGTAAAAAAAAAAAAAA AACACTGTG-(DABCYL)
Control DNA	FAM-ACACTGTAAAAAAAAAAAAAA AACACTGTG
C-rich sequence	CCCCCC CCAAAACCCCCCCC

Fig. S1 Fluorescence emission intensity changes of the UMDP at 526 nm vs temperature after incubation with Hg^{2+} or Ag^{+} ions in the work solution (20 mM Tris-acetate, pH 7.4, 10 nM UMDP).

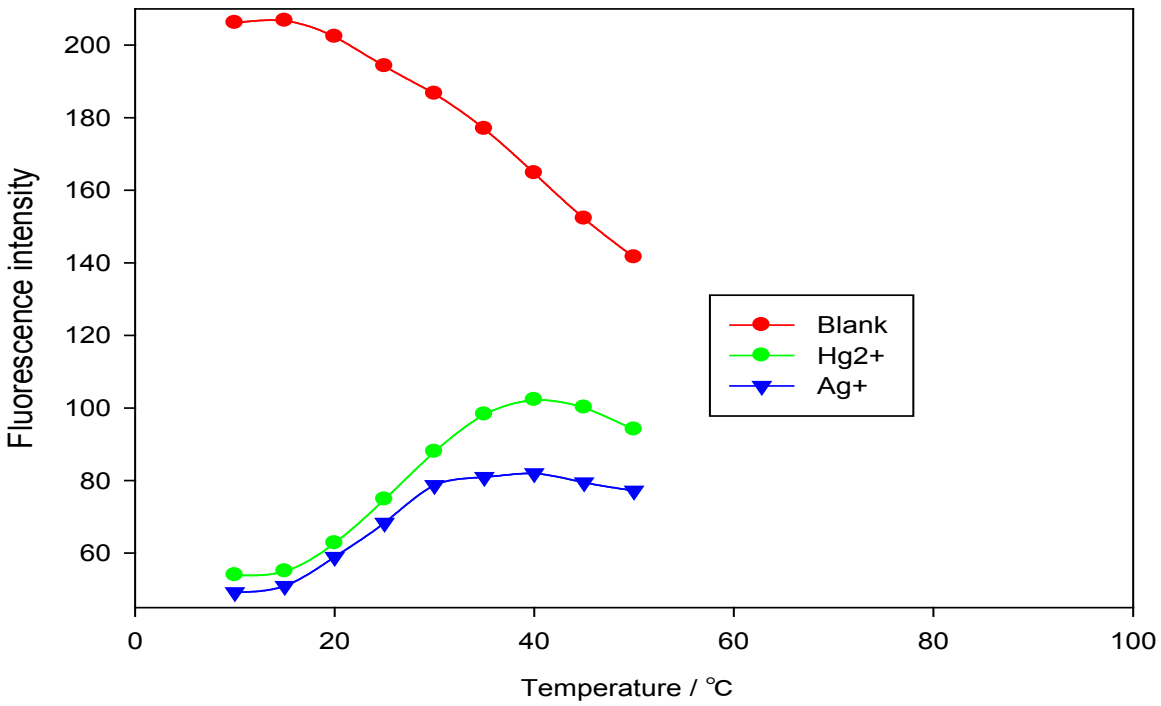

Fig. S2 Fluorescence emission intensity of the UMDP at 526 nm vs NaCl concentration in the work solution (20 mM Tris-acetate, pH 7.4, 10 nM UMDP).

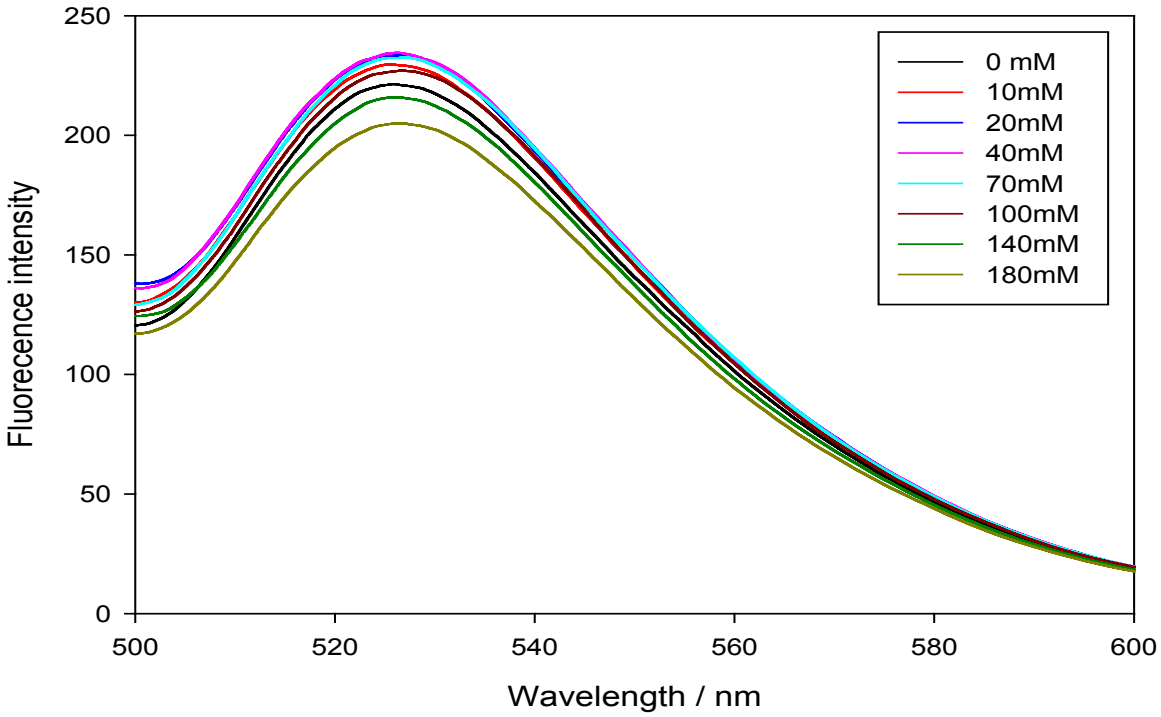

Fig. S3 The fluorescence spectra of the UMDP upon interaction with different concentrations of Hg^{2+} (A) or Ag^{+} (B) in the work solution (20 mM Tris-acetate ,pH 7.4). The arrows indicate the signal changes as increases of target ions concentrations (Hg^{2+}: 0, 0.1, 0.2, 0.3, 0. 4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.2 , 2.0, and 3.0 μM; Ag^{+}: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.2 , and 2.0 μM). Excitation wavelength was fixed at 481 nm.

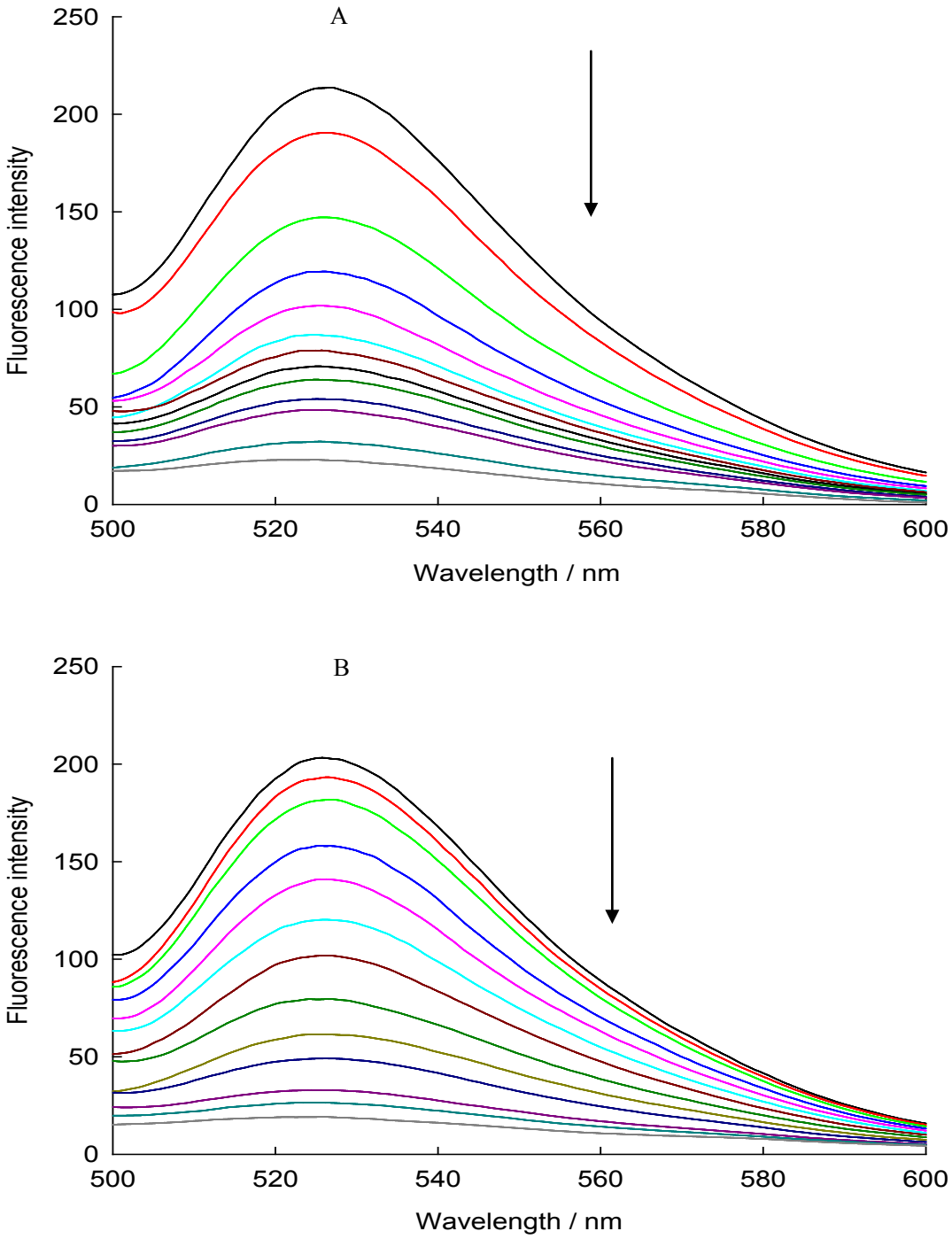

Fig.S4 Calibration curves of quenching efficiency of the UMDP for various concentrations of Hg^{2+} (A) or Ag^{+} (B) ions in the work solution (20 mM Tris-acetate, pH 7.4, 10 nM UMDP).

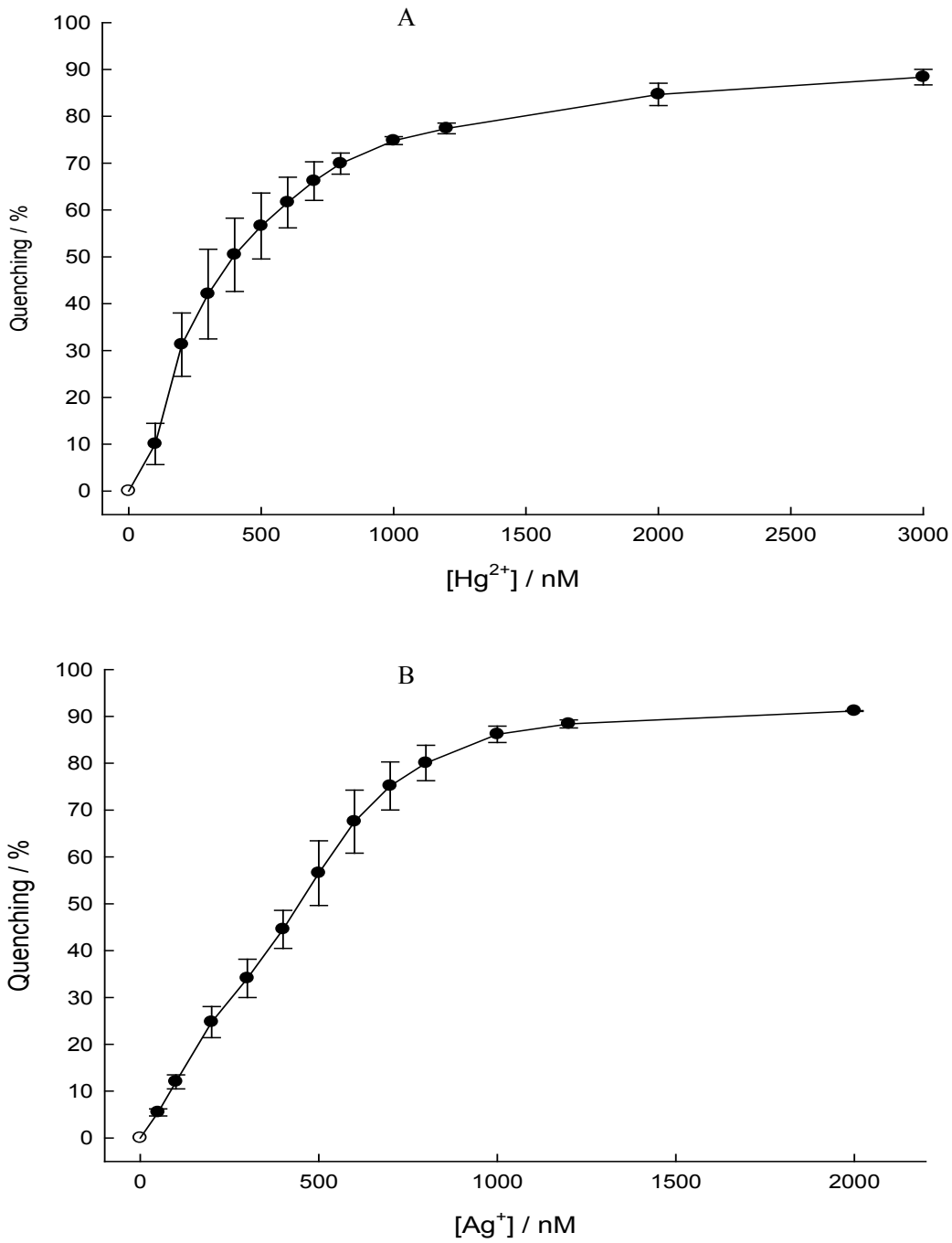

Fig. S5 The fluorescence spectra of the UMDP upon interaction with different concentrations of mixture of Hg^{2+} and Ag^{+} in the work solution (20 mM Tris-acetate ,pH 7.4). The molar ratio of Ag^{+} and Hg^{2+} was fixed as 1:1.