Electronic Supplementary Information

Simultaneous setection of mercury (II) and silver (I) ions with picomolar sensitivity†

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EXPERIMENTAL SECTION

Materials. The template-1 (5'-ATC TTG TCA CTG CGC ATC CTC AGC TTT ACT GTC CTT GGA ACA CCC-3'), the template-2 (5'-GGA TCG TCA TTC ACT TAC CTC AGC CCC TGT TCC AAG GAC AGT TTT-3'), the QF probe-1 (5'-*FAM*-ATC TTG TCA CTG CGC-*Eclipse-3'*), the QF probe-2 (5'-*TAMRA*-GGA TCG TCA TTC ACT-*Eclipse-3'*), the deoxynucleotide solution mixture (dNTPs) and the DNA ladder marker were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The nicking enzymes of Nb.BbvCI, Nb.BtsI and Nt.AlwI, the polymerase of Klenow fragment (3'→5' exo -), NEB buffer 2, NEB buffer 4, and bovine serum albumin (BSA) were obtained from New England BioLabs (Beverly, MA, USA). $Hg(ClO_4)_2$ and $AgNO_3$ were purchased Strem Chemicals (Newburyport, MA, USA) and Acros Organics (Geel, Belgium), respectively. All other chemical reagents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Bifunctional SDA Reaction. The reaction mixtures were prepared separately as part A and part B.

Part A consisted of the template, dNTPs, and NEB buffer 2. Part B consisted of Nb.BbvCI nicking enzyme, polymerase, and target metal ions. The reaction mixtures were first incubated at 95 °C for 3 min to denature template-1 and template-2. Then part B was added into part A after being cooled to 37 °C. To generate the triggers, the reaction of part A with part B was performed in 10 μL of solution containing 25 nM template-1, 25 nM template-2, 250 μM dNTPs, 1× NEB buffer 4 (20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9), 3 U Nb.BbvCI, 1 U polymerase, and target metal ions at 37 °C for 60 min.

NESA Reaction and Fluorescence Measurement. After SDA reaction, 10 μL of triggers were incubated with a solution containing 0.125 μL of 100 μM QF probe-1 (or 0.3 μL of 100 μM QF probe-2), 5 μL of 10× NEB buffer 2 (10 mM Tris-HCl, 50mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) at 37 °C for 15 min. Then 0.5 μL of 100× BSA (10 mg/mL) and 0.5 μL of 10 U/μL Nb.BtsI (or 1.0 μL of 10 U/μL Nt.AlwI) were added into the reaction solution, followed by incubation at 37 ºC for 45 min with a final volume of 50 μL. At last, the resultant reaction solution was subjected to the fluorescence measurement by a Hitachi F-4500 fluorometer (Tokyo, Japan) equipped with a xenon lamp as the excitation source. The spectra of FAM from QF probe-1 and TAMRA from QF probe-2 were recorded with the excitation wavelength of 496 nm and 558 nm, respectively. The excitation and emission slits were set for 5.0 nm and 5.0 nm, respectively. The fluorescence intensity at the emission wavelength of 520 nm and 582 nm was used for quantitative analysis of Hg^{2+} and Ag^{+}, respectively.

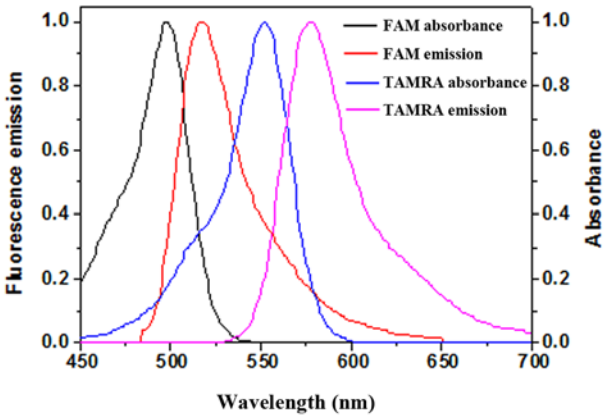

Fig. S1 Normalized absorption and emission spectra of FAM and TAMRA. Black line, absorption spectrum of FAM; red line, emission spectrum of FAM; blue, absorption spectrum of TAMRA; magenta line, emission spectrum of TAMRA.

Optimization of Experimental Condition. To ensure the good performance of the proposed method, we optimized the amount of Nb.BtsI and Nt.AlwI experimentally. Even though the high fluorescence signal can be obtained at high amount of nicking enzymes, the background signal increases correspondingly.[1] To optimize the amount of Nb.BtsI, we examined the variance of F/F_0 value with the amount of Nb.BtsI, where F and F_0 are the fluorescence intensity at the emission wavelength of 520 nm in the presence and in the absence of Hg^{2+}, respectively. As shown in Fig. S2A, the F/F_0 value increases with the increase of Nb.BtsI from 0. 1 U to 5 U, followed by the decrease beyond the amount of 5 U. Thus, 5 U Nb.BtsI was used in the subsequent research. To optimize the amount of Nt.AlwI, we investigated the variance of F/F_0 value with the amount of Nt.AlwI, where F and F_0 are the fluorescence intensity at the emission wavelength of 582 nm in the presence and in the absence of Ag^{+}, respectively. As shown in Fig. S2B, the F/F_0 value increases with the increase of Nt.AlwI from 1 U to 10 U, followed by the decrease beyond the amount of 10 U. Thus, 10 U Nt.AlwI was used in the subsequent research.

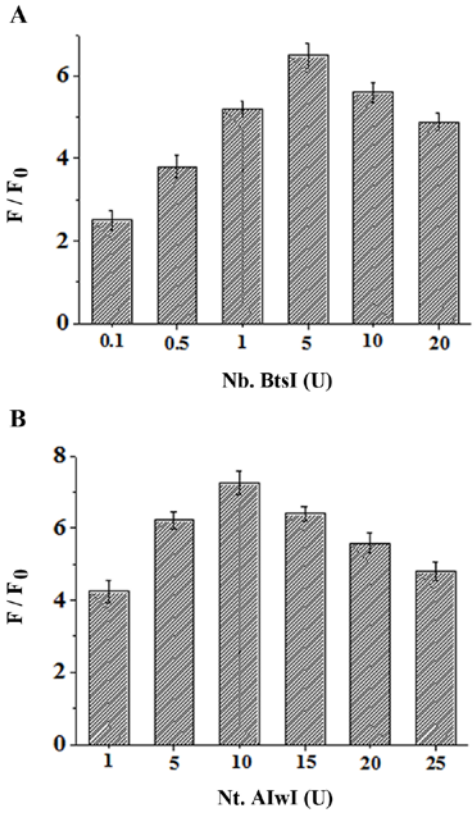

Fig. S2 (A) Variance of the F/F_0 value with the amount of Nb.BtsI. F and F_0 are the fluorescence intensity at the emission wavelength of 520 nm in the presence and in the absence of 1.25 nM Hg^{2+}, respectively. (B) Variance of the F/F_0 value with the amount of Nt.AlwI. F and F_0 are the fluorescence intensity at the emission wavelength of 582 nm in the presence and in the absence of 10 nM Ag^{+}, respectively. The pH value of reaction system is 7.9. Error bars show the standard deviation of three experiments.

Simultaneous detection of Hg^{2+} and Ag^{+}. In order to investigate the cross-talk between Hg^{2+} and Ag^{+}, we prepared total thirty-six solutions with different concentrations of two metal ions (Table S1), and measured their fluorescence intensities in response to both QF probe-1 and QF probe-2. A two-dimensional contour plot is obtained (Fig. S3, the colors represent the fluorescence intensity of 520 nm or 582 nm). As shown in Fig. S3A, the fluorescence intensity of 520 nm enhances with

the increase of Hg^{2+} concentration, but remains unchanged with the increase of Ag^{+} concentration. Moreover, the line of Hg^{2+} ions in 520 nm read-out is parallel to the Ag^{+} axis, indicating the read-out of 520 nm corresponds to only Hg^{2+} ion.[2] As shown in Fig. S3B, the fluorescence intensity of 582 nm enhances with the increase of Ag^{+} concentration, but remains unchanged with the increase of Hg^{2+} concentration. Moreover, the line of Ag^{+} ions in 582 nm read-out is parallel to the Hg^{2+} axis, indicating that the read-out of 582 nm corresponds to only Ag^{+} ion.[2] These results clearly demonstrate that the proposed method can be used for simultaneous detection of Hg^{2+} and Ag^{+} without cross-talk.

Table S1. Thirty-six solutions with different concentrations of Hg^{2+} and Ag^{+} for cross-talk analysis.

Ag^{+} Hg^{2+} → ↓	0pM	2 pM	10 pM	50 pM	250 pM	1250 pM
0 pM	1	2	3	4	5	6
16 pM	7	8	9	10	11	12
80 pM	13	14	15	16	17	18
400 pM	19	20	21	22	23	24
2000 pM	25	26	27	28	29	30
10000 pM	31	32	33	34	35	36

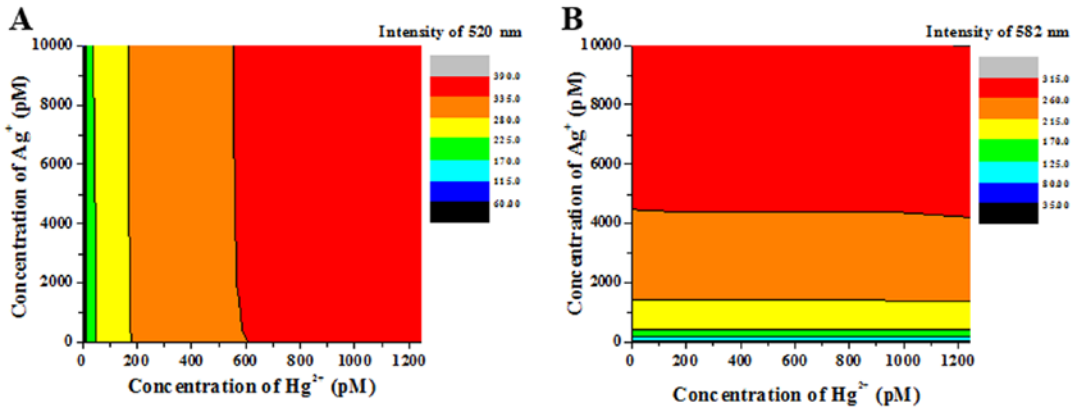

Fig. S3 Study of cross-talk between Hg^{2+} and Ag^{+} in solutions with different ion concentrations. The fluorescence intensities of 520 nm (A) and 582 nm (B) were plotted versus Hg^{2+} and Ag^{+} concentration in a two-dimensional contour plot. The intensity information is color coded.

Real water sample analysis. In order to validate the feasibility of the proposed method for practical applications, we tested spiked tap water samples with the addition of Hg^{2+} or Ag^{+} at two different concentrations of 0.5 nM and 1 nM. As shown in Table S2, the analytical recoveries are measured to be in the range from 93 % to 106 %, and the values of relative standard deviation (RSD) are calculated to be less than 6 %. These results indicated that the proposed method holds a great promise for real water sample analysis with great accuracy and reliability.

Table S2. Analytical recovery of Hg^{2+} and Ag^{+} added to the tap water samples (n = 3)

Ions	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
	0	0	-	-
Hg^{2+}	0.5	0.49	98	4.25
	1	1.06	106	4.90
	0	0	-	-
Ag^{+}	0.5	0.52	104	5.12
	1	0.93	93	3.60

REFERENCE

(1) B. C. Yin, Y. Q. Liu, B. C. Ye, *J. Am. Chem. Soc.*, 2012, **134**, 5064.

(2) L. L. del Mercato, A. Z. Abbasi, M. Ochs, W. J. Parak, *ACS Nano*, 2011, **5**, 9668.