Supporting Information

A Versatile Molecular Beacon-like Probe for Multiplexed Detection Based on Fluorescence Polarization and Its Application for Resettable 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: **P_1**: 5'-FAM-$(CH_2)_6$-CCCTAACCCTAACCCTAACCCT-3'; **P_2**: 5'-TATAATAAATTTTAAATAT-$(CH_2)_6$-FAM-3'. The following metal salts: $AgNO_3$, $Mg(NO_3)_2$, $Cu(NO_3)_2$, $Mn(Ac)_2$, $Zn(Ac)_2$, $Cr(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Co(Ac)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Hg(Ac)_2$, $Ca(Ac)_2$, $CrCl_3$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $NaNO_3$ 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). Ethylenedaminetetraaceticacid (EDTA) disodium salt dehydrate was purchased from Pharmacia Biotech Inc. (Uppsala, Sweden). 10× HEPES buffer (100 mM HEPES, 1 M $NaNO_3$, pH 7.4) 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). Fluorescence polarization was measured in a fluorescence microplate reader (BioTek Instruments, Winooski, VT, USA) with an excitation wavelength at 485 nm and an emission wavelength at 528 nm using a black 384-well microplate (Fluotrac 200, Greiner, Germany).

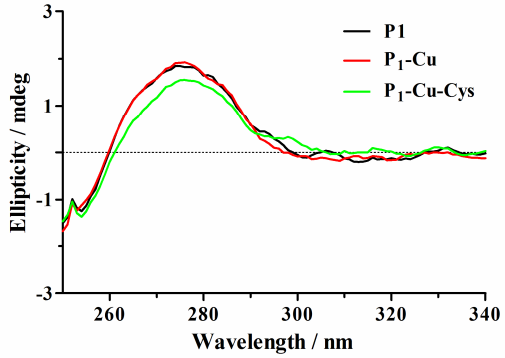

Fig. S1 Circular dichroism (CD) spectra of 500 nM **P_1** in the solution treated with (A) none (black line), 5 μM Cu^{2+} (red line), 5 μM Cu^{2+} and following 10 μM Cys (green line).

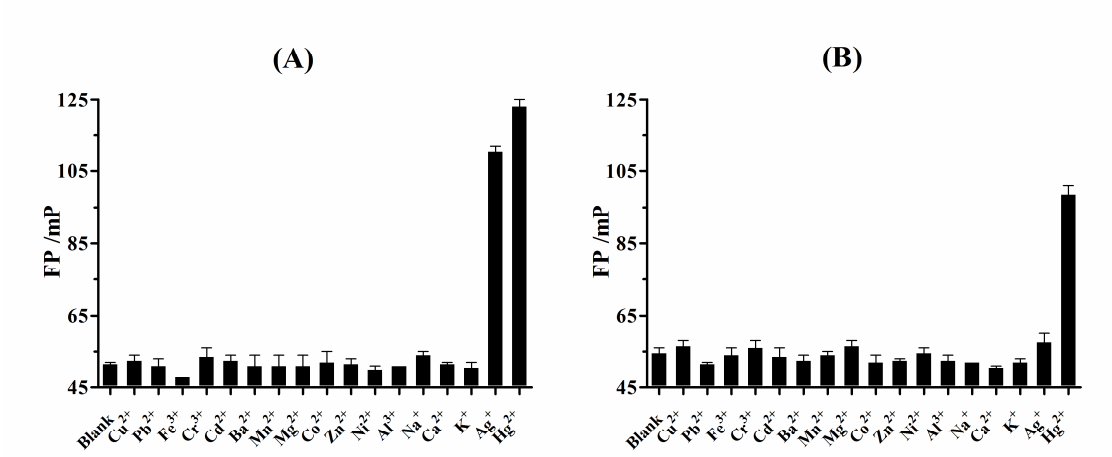

Fig. S2 Selectivity analysis for Ag^{+} and Hg^{2+} deteciton. Bars represent the FP value of (A) 100 nM $\mathbf{P_1}$ and (B) 100 nM $\mathbf{P_2}$ toward the presence of 2 μM Ag^{+} and 5 μM other metal ions including Cu^{2+}, Pb^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Ba^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+}, Na^{+}, Ca^{2+}, K^{+} and Hg^{2+}.

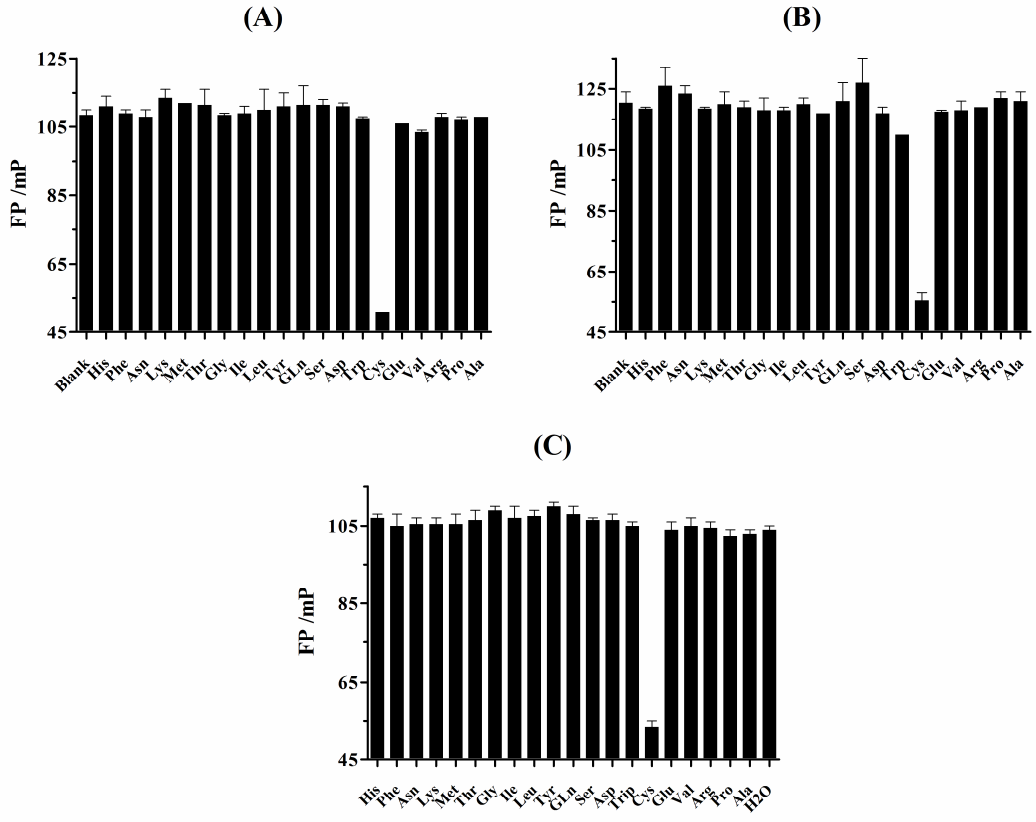

Fig. S3 Selectivity analysis for cysteine (Cys) deteciton. Bars represent the FP value of 100 nM $\mathbf{P_1}$ solution containing (A) 2 μM Ag^{+} and (B) 10 μM Hg^{2+} toward the presence of 10 μM Cys and other 19 amino acids, respectively. (C) Bars represent the FP value of 100 nM $\mathbf{P_2}$ solution containing 10 μM Hg^{2+} toward the presence of 10 μM Cys and other 19 amino acids, respectively.

Table S1 Comparison of the different probes (P_1 and P_2) for metal ion sensing

Probe	Metal ion	Fitting equation
P_1	Ag^+	Boltzmann sigmoidal equation (the fitting range from 0-2 μM): $Y = 44.29 + 68.11/[1 + \exp(0.49 - X)/0.24]$, R^2=0.996; Linear equation (the fitting range from 0-1 μM): $Y = 56.01X + 50.84$, R^2=0.991; where Y is the FP value and X is the concentration of Ag^+
	Hg^{2+}	Two phase association equation (the fitting range from 0-60 μM): $Y = 47.76 + 27.66\ [1 - \exp(-1.18X)] + 67.26\ [1 - \exp(-0.1X)]$, R^2=0.995; Linear equation (the fitting range from 0-1 μM): $Y = 27.76X + 48.37$, R^2=0.952; where Y is the FP value and X is the concentration of Hg^{2+}
P_2	Hg^{2+}	Boltzmann sigmoidal equation (the fitting range from 0-2 μM): $Y = 42.23 + 85.38/[1 + \exp(0.46 - X)/0.21$; R^2=0.993; Linear equation (the fitting range from 0-1 μM): $Y = 80.39X + 48.94$, R^2=0.991; where Y is the FP value and X is the concentration of Hg^{2+}

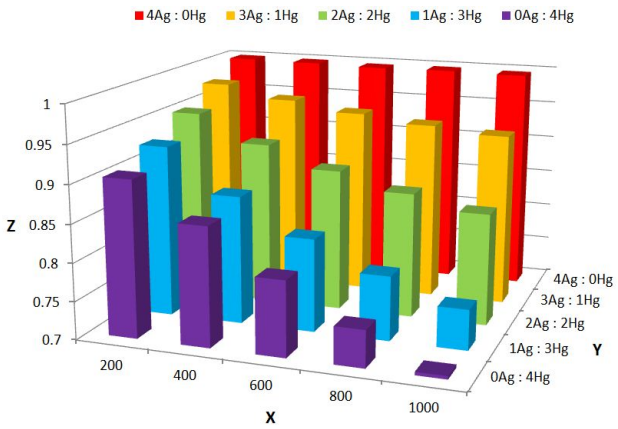

Fig. S4 Differentiation of Ag^+ and Hg^{2+} by relative fluorescence polarization decrease efficiency upon the sample treated with EDTA (50 μM). x-axis: total ions concentration (nM); y-axis: the concentration proportions of Ag^+ and Hg^{2+}; z-axis: the fluorescence polarization decrease efficiency.

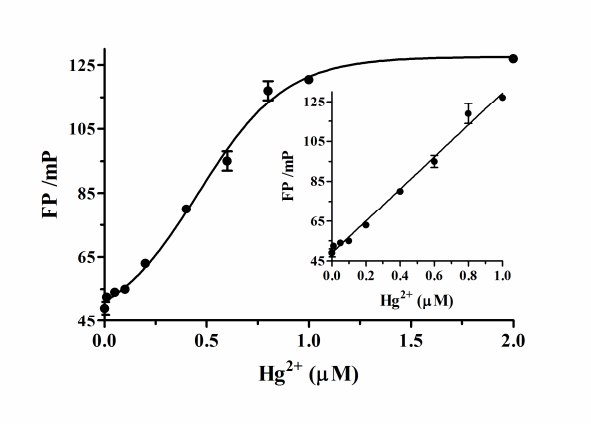

Fig. S5 Plot of FP value of 100 nM $\mathbf{P_2}$ probe corresponding to the Hg^{2+} concentration in the range 0–2 μM with a Boltzmann sigmoidal equation: $Y = 42.23 + 85.38/[1 + \exp(0.46 - X)/0.21]$, where Y is the FP value and X is the concentration of Ag^{+} (regression coefficient R^2=0.993). Inset: magnification of the plot of FP value of 100 nM $\mathbf{P_2}$ probe corresponding to the Hg^{2+} concentration in the range 0–1 μM with a linear equation: $Y = 80.39X + 48.94$, R^2=0.991. Using the $\mathbf{P_2}$ probe, Hg^{2+} could be detected at concentrations as low as 0.01 μM based on three times signal-to-noise level of the blank sample (3σ).

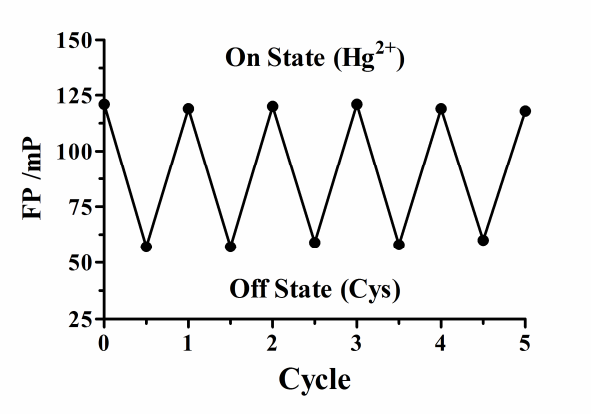

Fig. S6 Reversible switching of the logic systems consisting of the MB-like P_1 probe (100 nM) between the on and off states through the alternating addition of 10 μM Hg^{2+} and 10 μM Cys.