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Supporting Information

Time-Resolved Fluorescent Detection of Hg^{2+} in Complex Environment by Conjugating Magnetic Nanoparticles with a Triple-helix Molecular Switch

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Experimental Section:

Materials and Apparatus. Oligonucleotides used in this study were synthesized by Takara Biotechnology Co., Ltd. (Dalian, China), and the sequences of all oligonucleotides (P1-P4) are listed in Table S1. We obtained the stock solution of the oligonucleotides in highly pure water (sterile Minipore water, 18.3 MΩ), the DNA concentrations were estimated by UV absorption using published sequence-dependent absorption coefficients. [1] Streptavidin-coated iron oxide nanoparticles of 30 nm were obtained from Ocean Nanotech. All work solutions were prepared with MOPS buffer (pH 6.0, 20 mM, 5 mM $NaNO_3$, 5 mM $Mg(NO_3)_2$).

UV-Vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Hitachi U-3010 UV/Vis spectrophotometer (Kyoto, Japan). Both steady-stated fluorescence and time-resolved fluorescence were measured on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ) with an accessory of temperature controller. Fluorescence emission spectra were collected

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using a bandwidth of 5 nm and 0.2 × 1.0 cm^2 quartz cuvettes containing 500 μL of solution. pH was measured by model 868 pH meter (Orion).

Conjugation of Capture Probes on Magnetic Nanoparticles. In order to conjugate complementary sequence of MSO (P4, as listed in Table S1) on the MNPs, the streptavidin-coated iron oxide nanoparticles were dispersed at 0.1 mg/mL in 100 mM phosphate-buffered saline (PBS), pH 7.4. An excess amount of biotin-labeled P4 was then added. The mixtures were vortexed at room temperature for 1 h followed by washing 3 times with PBS buffer using centrifugation at 14000 rpm to remove any P4 that did not conjugate to the MNPs. The conjugates were dispersed in PBS and stored at 4 °C.

Kinetics Studies. To study the kinetics and time-dependence of the interactions of THMS and subsequent Hg^{2+}, the excimer fluorescence intensity of P2 at 458 nm was recorded. The excimer fluorescence of 500 μL P2 (100 nM) was monitored for a few minutes. Then, MSO was added to the MOPS buffer and the final concentration was 120 nM, excimer fluorescence was measured at room temperature. After confirming that there was no change of fluorescence with time, P4, Hg^{2+} were added and the final concentration was 120 nM and 2.5 μM, respectively, and the level of fluorescence was then recorded with time.

Performance of Hg^{2+} Detection. The THMS complex was formed in the MOPS buffer by adding a few μL of P3 to 0.5 mL of 100 nM P2 in a centrifuge tube, the addition was limited to 25 μL so that the volume change was insignificant. For Hg^{2+} detection, 400 μL of the THMS formed by P2 and P3 was mixed with MNPs-

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conjugated P4 at room temperature. A few μL of Hg^{2+} solution of different concentrations was then added to the mixture solution, then incubated at room temperature for 20 min. The tube was placed on a magnetic separator to extract the magnetic nanoparticles, and the fluorescence was recorded. For performance of Hg^{2+} measurement in cell media, 10 μL of 1.0 M *N*-ethylmaleimide as thiol-blocking reagent was added to avoid the formation of thiol-Hg^{2+} complex. Then, the pH was adjusted to 6.0. The subsequent steps were the same as described above.

Time-resolved Fluorescent Detection of Hg^{2+} in Human Urine. To apply the time-resolved fluorescent measurement, Hg^{2+} in human urine was detected. The urine sample was voluntarily provided by healthy people and stored at 4 °C, the thiols compounds were also separated in advance by *N*-ethylmaleimide and the pH was adjusted to 6.0. Before fluorescence detection, the samples were 10-fold diluted with the buffer solution and spiked with 0, 1.0, 1.5, and 2.0 μM Hg^{2+}, respectively.

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Table S1. Oligonucleotides Used in This Work*

Entry	Sequence(5'-3')
MSO1 for Hg^{2+}	CTCTCT**TTGTTCTTCTTGTTGT**TCTCTC
MSO2 for Hg^{2+}	CTCTCTCT**TTGTTCTTCTTGTTGT**TCTCTCTC
MSO3 for Hg^{2+}	CTCTCTCTC**TTGTTCTTCTTGTTGT**CTCTCTCTC
P1	Py-TTTTTGAACAATGGAACATTTTTTT-Py
P2	Py-GAAGAGAGAGAGAGCTCTTC-Py
P3	CTCTCTCTTGTTCTTCTTGTTGTCTCTCTC
P4	biotin- AAAAAATCTTCTTGTTGTTCTT
P5	TAMAR-CTCTCTTTGTTCTTCTTGTTGTAGAGAG- Dacybl

*The boldface type is the mercury specific DNA sequences, the underlined sequence indicates the bases that form a triplex. * Py represents the fluorophore pyrene.

Table S2. Comparison of optical sensors for mercury detection.

Methods	Detection media	Detection limit	Signal transduction	Ref.
FRET/MSO	buffer solution	40 nM	steady-stated fluorescence	11a
Sybr Green/MSO	buffer solution	1.3 nM	steady-stated fluorescence	11b
Hydrogels/MSO	buffer solution	10 nM	steady-stated fluorescence	11c
Gold Nanorods/MSO	buffer solution	0.15 nM	circular dichroism	12a
AuNP/MSO	buffer solution	40 nM	colorimetry	12b
Luminol /MSO	buffer solution	50 pM	chemiluminescence	12c
MNP/THMS	human urine	10 nM	time-resolved fluorescence	this work

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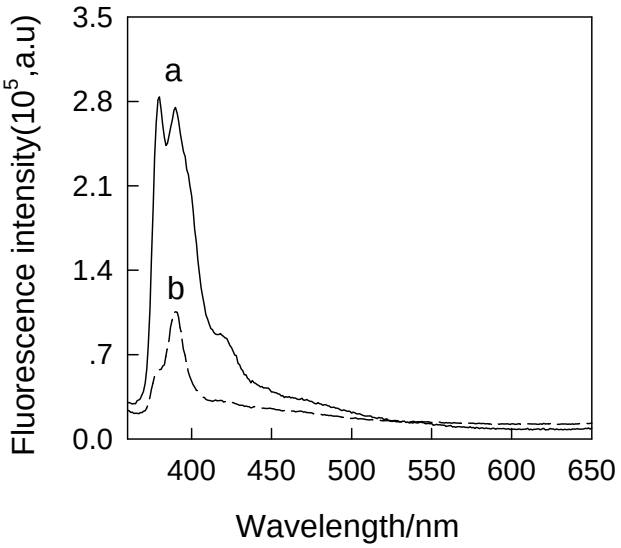

Figure S1. Fluorescence emission spectra of P1 (100 nM) before (a) and after (b) addition of 5.0 μM Hg^{2+} in the MOPS buffer solution at 20 °C. λ_{ex}=340 nm.

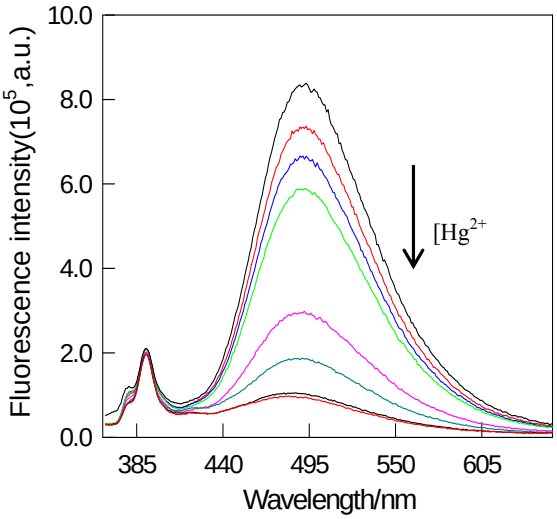

Figure S2. Fluorescence emission spectra of 100 nM P2 in the presence of increasing amounts of Hg^{2+} in the MOPS buffer. The arrow indicates the signal changes as increasing in Hg^{2+} concentrations (0, 0.1, 0.5, 1.0, 5.0, 10.0, 30.0 and 50.0 μM Hg^{2+}). λex=340 nm.

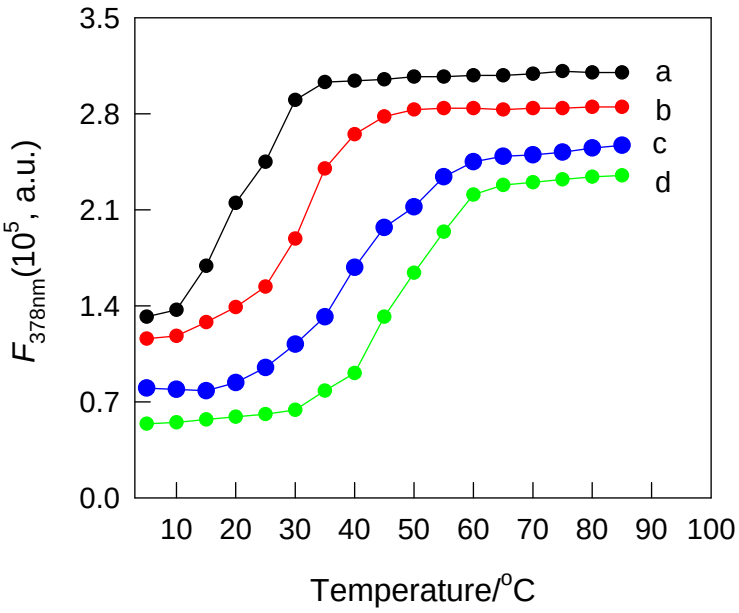

Figure S3. Fluorescence intensity as a function of temperature for P1 with different concentrations of Hg^{2+} (from trace a to trace d: 0.2, 0.5, 2.0, and 5.0 μM). λ_{ex}=340 nm

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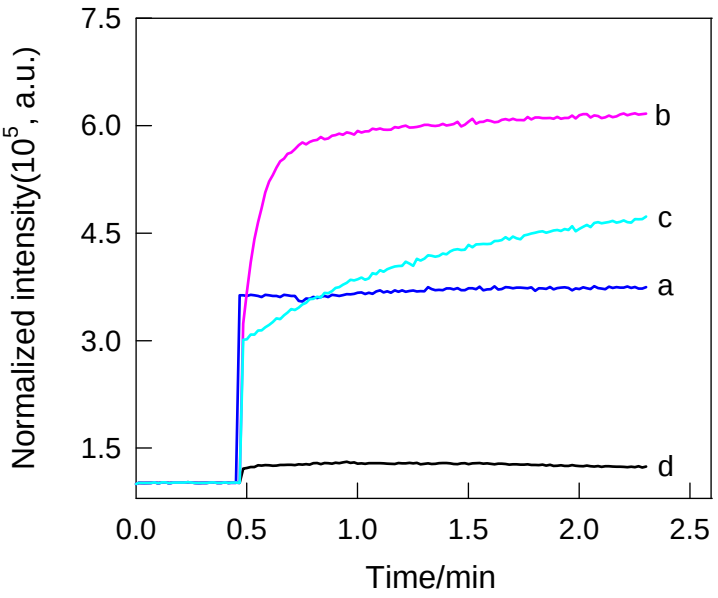

Figure S4. Kinetic responses of the THMSs with different stem lengths (from trace a to trace d: stem lengths of MSOs were 6, 7, 8 and 9 bases) toward of 2.5 μM Hg^{2+} in MOPS buffer solution after magnetic separation at 20°C. For comparison, the excimer fluorescence intensity of the THMS before addition of Hg^{2+} was normalized to 1.0. Fluorescence emission was recorded at 458 nm with an excitation wavelength of 340 nm.

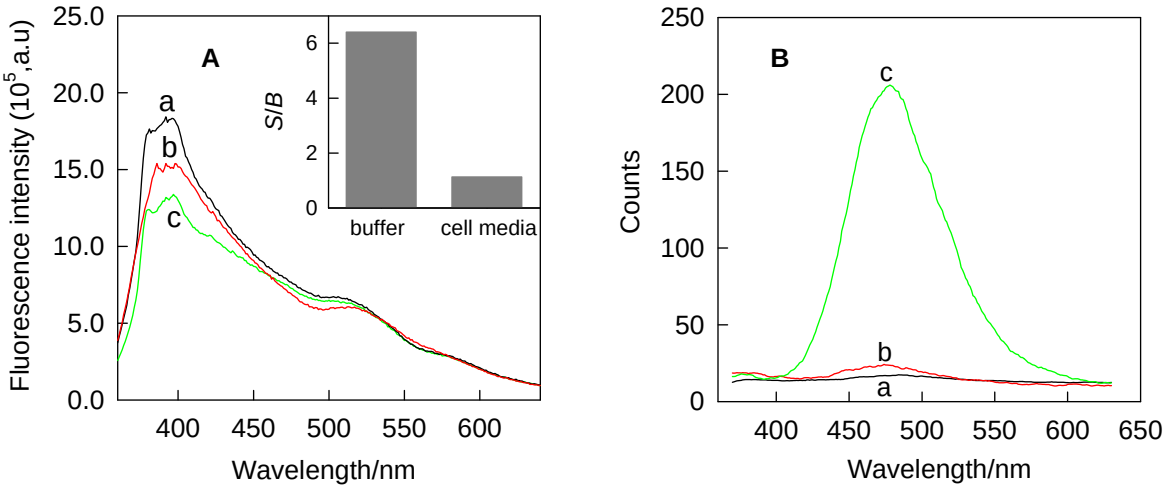

Figure S5. (A) Steady-state fluorescence spectra of cell media (a), the THMS in cell media (b), and (b) + 2.4 μM Hg^{2+} in cell media (c). Inset: patterns of S/B for different reaction media resulting from the spectral detection at 458 nm. (B) TRES of the cell media (a), the THMS in cell media (b), and (b) + 2.4 μM Hg^{2+} in cell media (c) with delay time window of 40 ns. λ_{ex}=340 nm.

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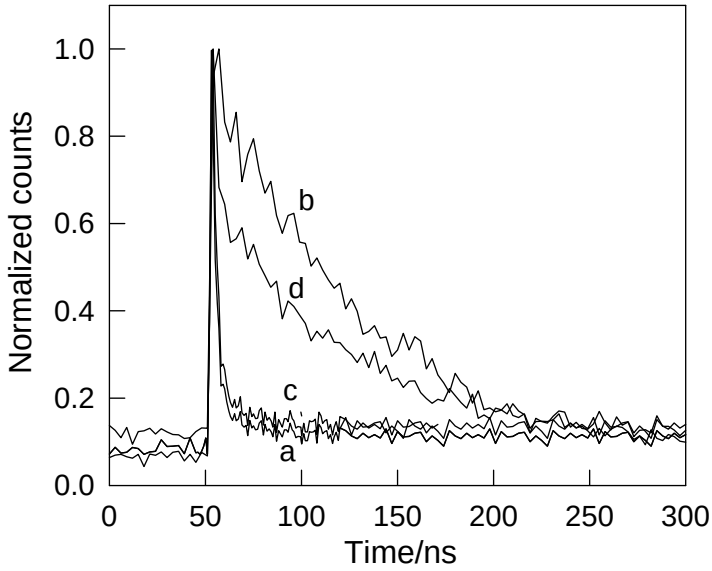

Figure S6. Fluorescence decay measurements of cell medium(a), (a) + 100 nM P2 (b), (b) + 120 nM P3+ 120 nM P4 (c), (c) + 1. 4 μM Hg^{2+}(d). λex=340 nm..