Electronic Supplementary Information (ESI) for Metallo-Toeholds: Controlling DNA strand displacement driven by Hg(II) ions

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Experimental Section

Reagents and Chemicals.

The domain sequences used in this study were synthesized by Shanghai Sangon Biotechnology (Shanghai, China). The DNA sequences are presented in Table S0. Tris(hydroxymethyl)aminomethane (Tris), anhydrous sodium acetate (NaOAc), magnesium acetate tetrahydrate [$Mg(OAc)_2$], and potassium nitrate (KNO_3) were purchased from Sinapharm Chemical Reagent (Shanghai, China). Mercury(II) nitrate [$Hg(NO_3)_2$] was obtained from Wanshan Mineral Products (Tongren, Guizhou, China). All salts were of analytical grade and used without further purification. The oligomer samples were dissolved in a buffer consisting of 50 mM Tris-HOAc, 100 mM NaOAc, 0.005 M $Mg(OAc)_2$, and 0.03 M KNO_3 at pH 7.4.[1] The concentrations of oligonucleotide solutions were quantified by measuring the UV absorbance at 260 nm. Unless otherwise stated, all other reagents were of analytical reagent grade and used without further purification or treatment. Ultrapure water (Milli-Q plus, Millipore, Bedford, MA) was used throughout. The DNA complex required prior annealing treatment. All annealing processes were performed using a dry bath (ABSON, USA). The samples were heated at 95 °C, equilibrated for 15 min at this temperature, then slowly cooled to 20 °C at a constant rate over the course of 2 h.

Instrumentation

Spectofluorimetry. Spectofluorimetry studies were performed using a Fluorolog-3

spectrofluorophotometer (Jobin Yvon S.A.S, France) with excitation at 588 nm and emission at 607 nm (optimal signal for ROX fluorophore). In all spectrofluorimetry experiments, the total reaction volume was 200 μL. Both the excitation and emission slit widths were set to 3.0 nm. The kinetics experiments displayed in Figs. 1 and S3, as well as other experiments not shown, were performed with an integration time of 3 s for every 30-s time-point. The typical fluorescence spectra displayed in Figs. 2 and S2 were recorded from 595 to 800 nm. Prior to each experiment, all cuvettes were cleaned strictly: each cuvette was washed three times in ultrapure water, three times in EtOH, and then was flushed with a constant stream of dry N_2 gas to avoid water condensation.

Native polyacrylamide gel electrophoresis (PAGE). A 12% acrylamide (19:1 acrylamide:bis) native polyacrylamide gel was run to prepare Figs. 3 and S4. The acrylamide solution was diluted from 40% stock acrylamide; 6× glycerol was employed as the loading buffer. Gels were run at 120 V for 60 min at 25 °C.

Table S0. Domain sequences tested in this study. Bold domain sequences represent the metallo-toehold; bases in italics formed T:T mismatched base pairs that allowed insertion of a Hg^{2+} ion. Sequences were designed to possess minimal secondary structure and crosstalk.[2]

Name	Complete sequence	Length (nt)
Message oligomer	5′-CCACATACATCATATT CCCTCATTCAATACCCTACG-3′	36
Input oligomer	5′-CCCTCATTCAATA CCCTACG***T*****C*****T*****AGC**-3′	26
Complementary oligomer of target complex	5′-**GCT*****T*****G*****T***CGTAGGGT ATTGAATGAGGG-3′	26
Fluorophore-labeled oligomer of reporter	5′-TATTGAATGAGGGAA TATGATGTATGTGG-(ROX)-3′	29
Quencher-labeled oligomer of reporter	5′-(BHQ-2)-CCACATACAT CATATTCCCTCA-3′	22

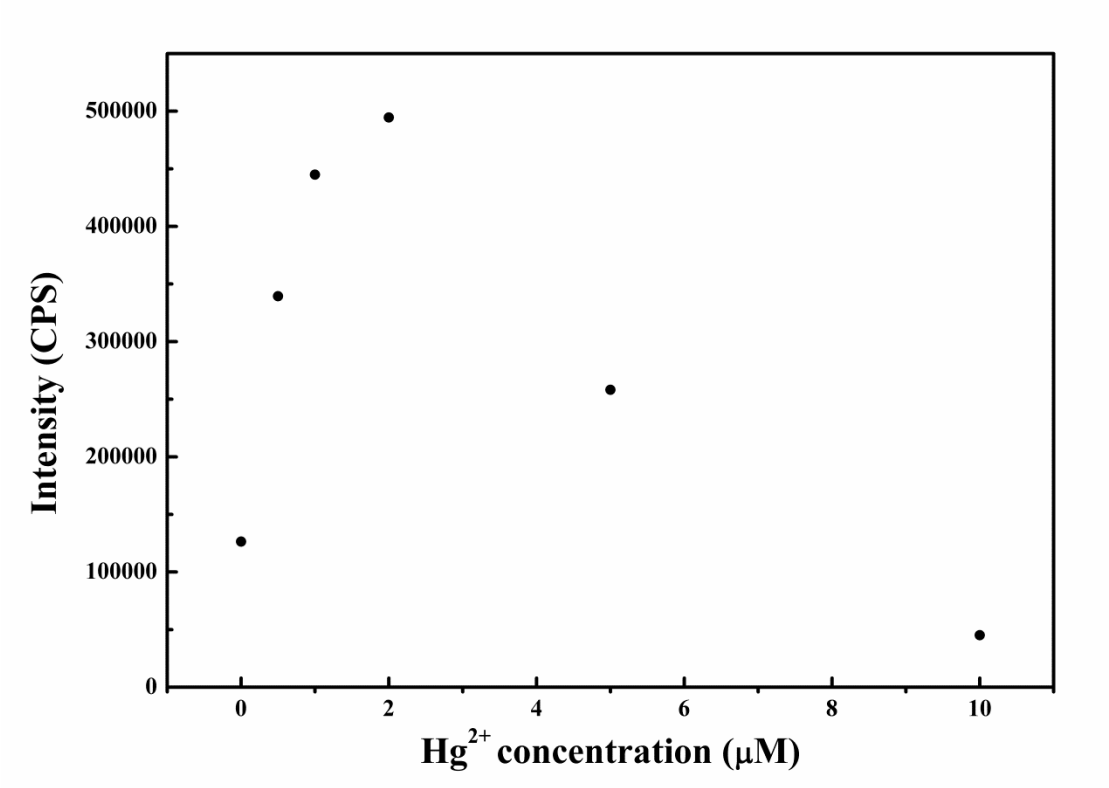

Fig. S1. Fluorescence emission intensity at 607 nm of the reaction in response to Hg^{2+} ions after 24 h, plotted with respect to the Hg^{2+} concentration. Initial concentrations: [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM.

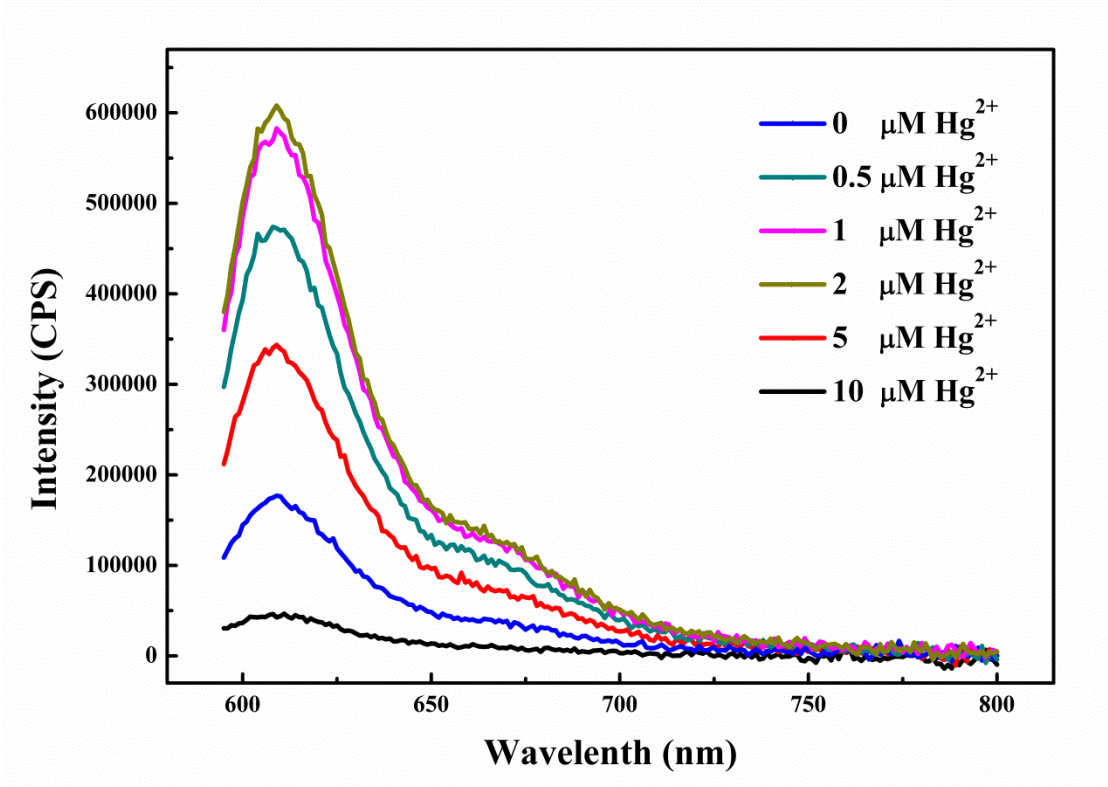

Fig. S2. Fluorescence spectra of the reaction in response to Hg^{2+} ions after 48 h. Initial concentrations: [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM.

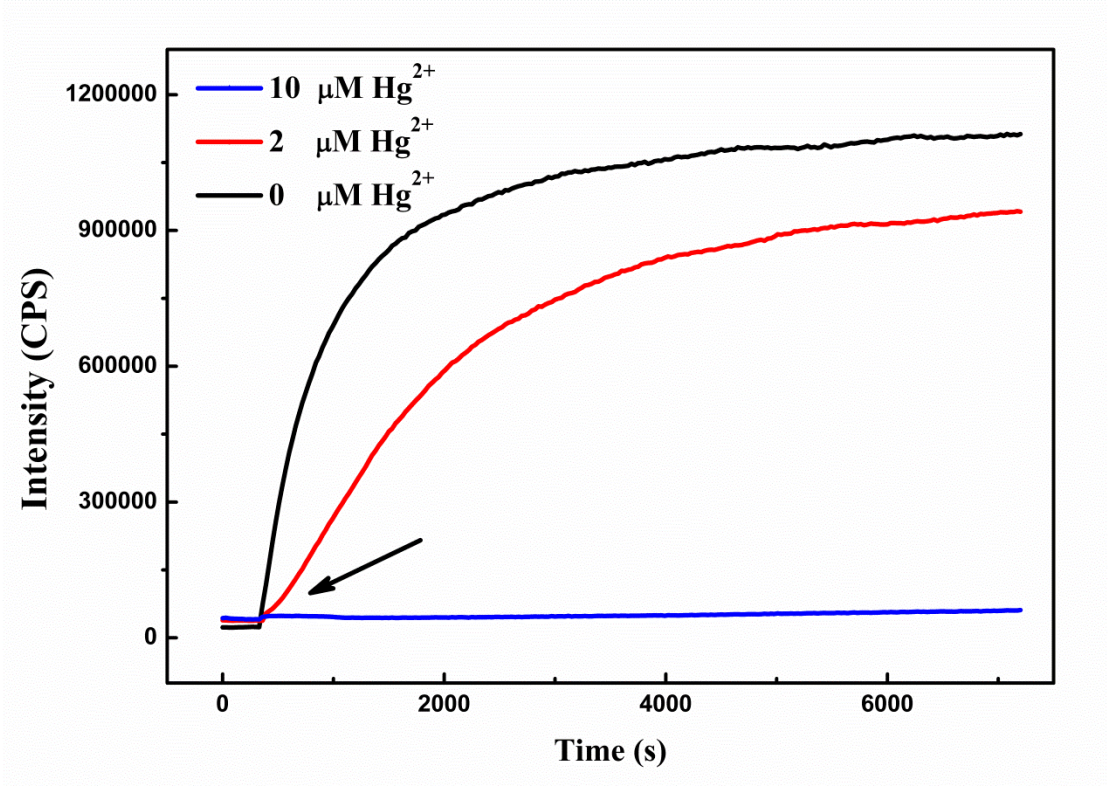

Fig. S3. Influence of Hg^{2+} ion concentration on the standard matched toehold strand displacement kinetics. Each measurement featured two distinct steps: (i) the cell was filled with buffer containing DNA-mix and fluorescence reporter complex; (ii) input-oligomer and Hg^{2+} ions were introduced into the cell to begin the DNA strand displacement process. The transition between each regime is marked by an arrow. Initial concentrations in step (ii): [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM.

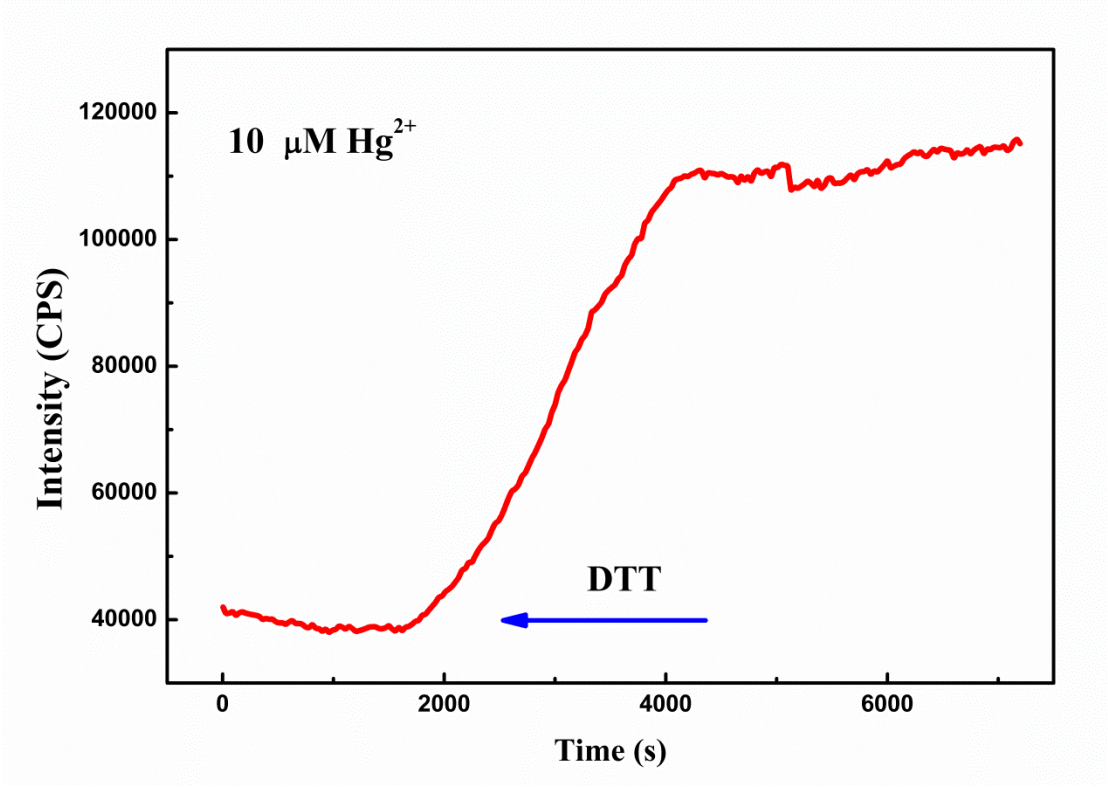

Fig. S4. Real-time fluorescence measurements of DNA-mix and 10 μM $Hg(NO_3)_2$ at 25 °C upon addition of DTT. This experiment featured two distinct steps: (i) the cell was filled with 200 μL buffer containing DNA-mix and 10 μM $Hg(NO_3)_2$; (i) DTT was introduced into the cell. The transition between the two regimes is marked by an arrow. Initial concentrations in step (ii): [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM; [DTT] = 10 μM.

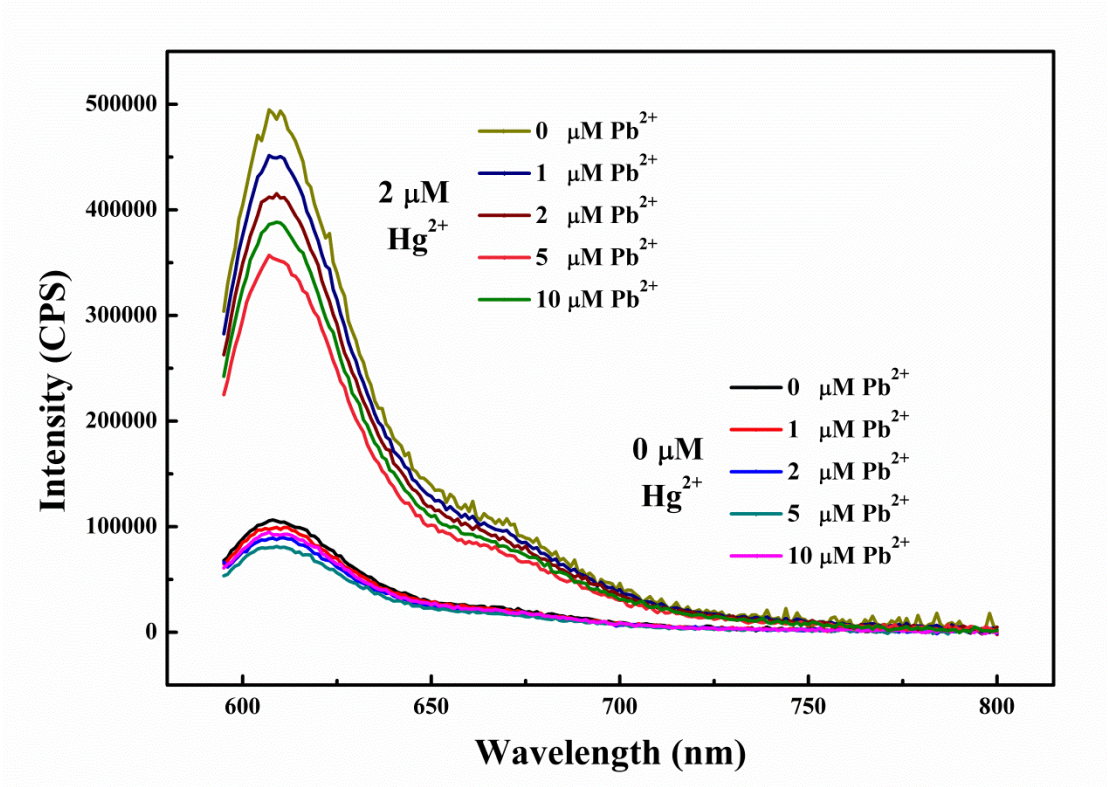

Fig. S5. Fluorescence spectra of the reaction in response to Pb^{2+} ions after 24 h. Initial concentrations: [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM.

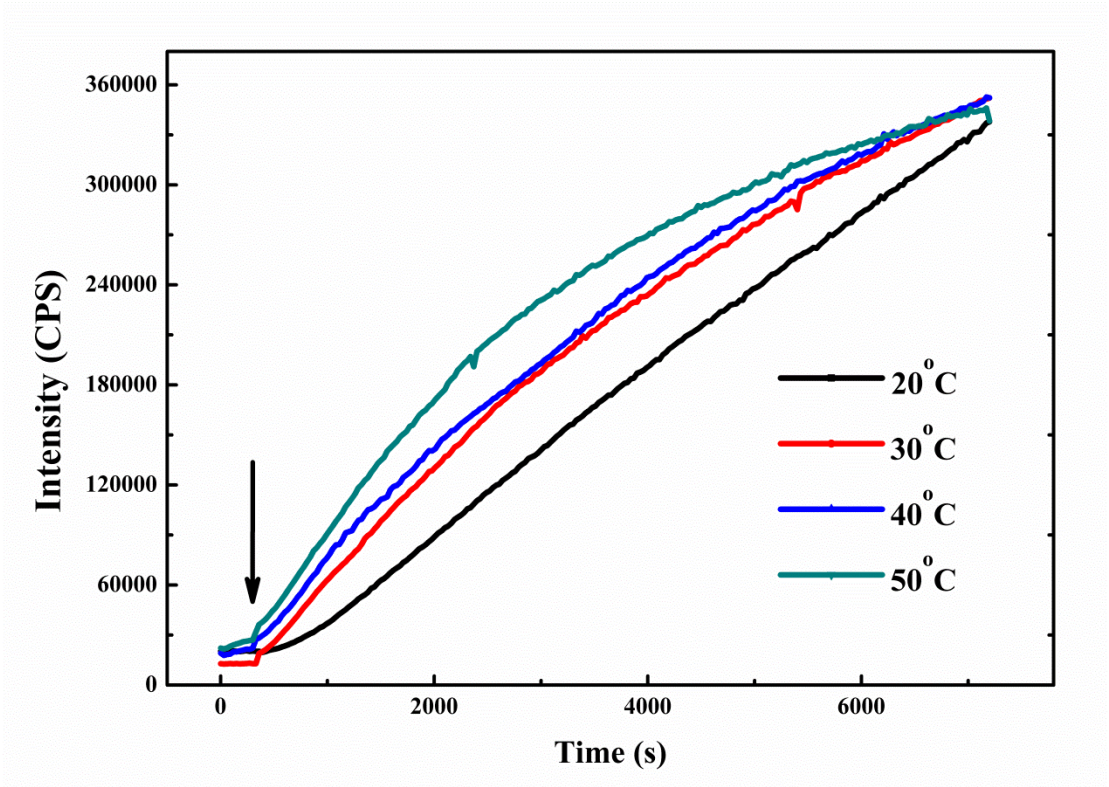

Fig. S6. Influence of temperature on the metallo-toeholds strand displacement kinetics. Each measurement featured two distinct steps: (i) the cell was filled with buffer containing DNA-mix and fluorescence reporter complex; (ii) input-oligomer and Hg^{2+} ions were introduced into the cell to begin the DNA strand displacement process. The transition between each regime is marked by an arrow. Initial concentrations in step (ii): [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM. [Hg^{2+}] = 2 μM.

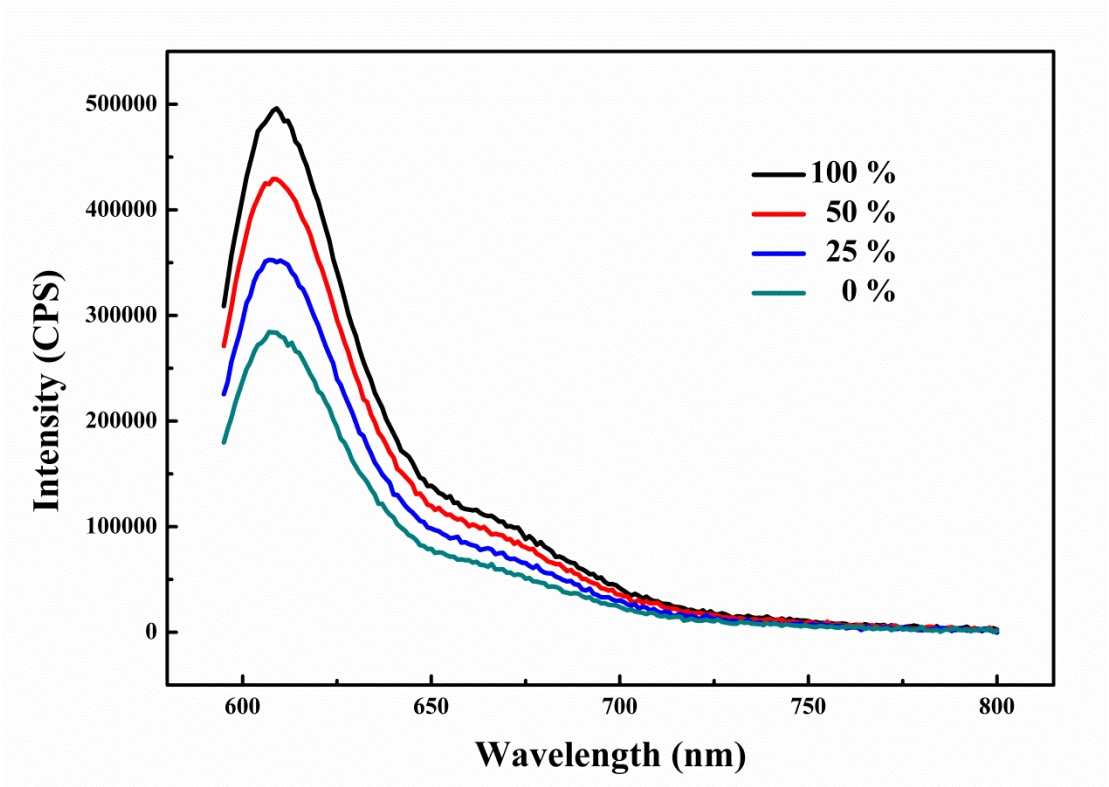

Fig. S7. Fluorescence spectra of the reaction in response to ionic strength after 24 h. Initial concentrations: [target complex] = [fluorescence reporter complex] = 100 nM; [input-oligomer] = 120 nM; [Hg^{2+}] = 2 μM.

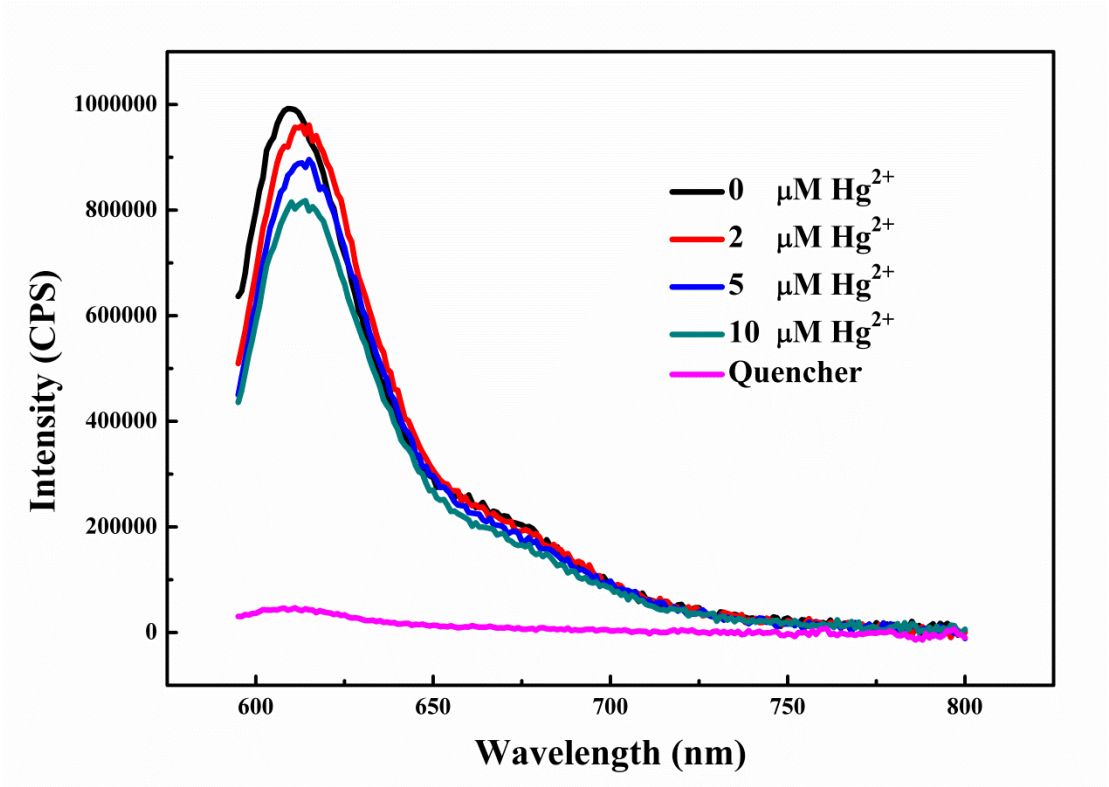

Fig. S8. Fluorescence spectra of fluorophore-labeled oligomer in the presence of Hg(II) ions and in the absence of the quencher-labeled oligomer. Initial concentrations: [fluorophore-labeled oligomer] = 100 nM.

References

1. N. Lu, C. Y. Shao and Z. X. Deng, *Analyst*, 2009, **134**, 1822-1825.
2. P. P. Xu, F. J. Huang and H. J. Liang, *Biosens. Bioelectron.* 2013, **41**, 505-510.