Supporting Information:

Reversible Molecular Switching of Molecular Beacon: Controlling DNA Hybridization Kinetics and Thermodynamics Using Mercury(II) Ions

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Experimental Section

General Procedures. All of the molecular beacons and oligonucleotide targets were puchased from AuGCT biotechnology Co., Beijing, China. They were disolved in DNA water as stock solution and the concentration was identified according to UV absorption at 260 nm (Hitachi U-3010 Spectrophotometer). Stock solution of Mg^{2+} and Hg^{2+} were prepared from analytical grade chloride salts and were dissolved in deionized water and work solution were obtained by series diluting the stock solutions with deionized water. All other reagents and solvents were obtained from Aldrich or Sigma, analytical reagent grade.

Hg^{2+}-induced fluorescence quenching. Fluorescence measurements were carried out on a

Hitachi F-4500 fluorescence spectrofluoremeter, excited at 490 nm and detected at 500-600 nm. In a tiypical experiments, 250uL 0.1 M Tris/HCl buffer solutions (5mM MgCl2, pH 7.5) with 100nM **1** were parallel prepared. After adding different volume of Hg^{2+}, solution was diluted to 500uL and then the fluorescence was measured.

Fluorescence recovery by cDNA. 250uL 0.1M Tris/HCl buffer solutions (5mM MgCl2, pH 7.5) with 100nM **1** and certain concentration of Hg^{2+} were parallel prepared. After adding different volume of **2** or **3** , solution was diluted to 500uL and then incubate for 1 h at room temperature before measuring.

Melting temperature (T_m) measurements. Solutions with different concentration of Hg^{2+} were parallel prepared as previous method. Quartz cuvette was placed in the fluorescence spectrofluoremeter and the temperature was controlled by PolyScience 9112 refrigerating/heating circulators. Stabilized for 3min at every 5 centigrade form 10°C to 80°C, the fluorescence was detected.

Kinetics experiment. Fluorescence of 500uL solution, containing 0.05 M Tris/HCl buffer (pH 7.5), 2.5 mM Mg^{2+} and 50 nM **1,** was monitered by spectrofluoremeter. Several uL of Hg^{2+} was adding to the aliquot and fluorescence decreased quickly. For addtion volume was very small, the total volume change will be insignificant. Then, adding a few uL of **2**, **3,** DNase I or SSB (resist nuclease cleavage experiment and SSB interfere experiment), fluorescence will recover at different rate and show kinetics character differences.

Spectroscopic data

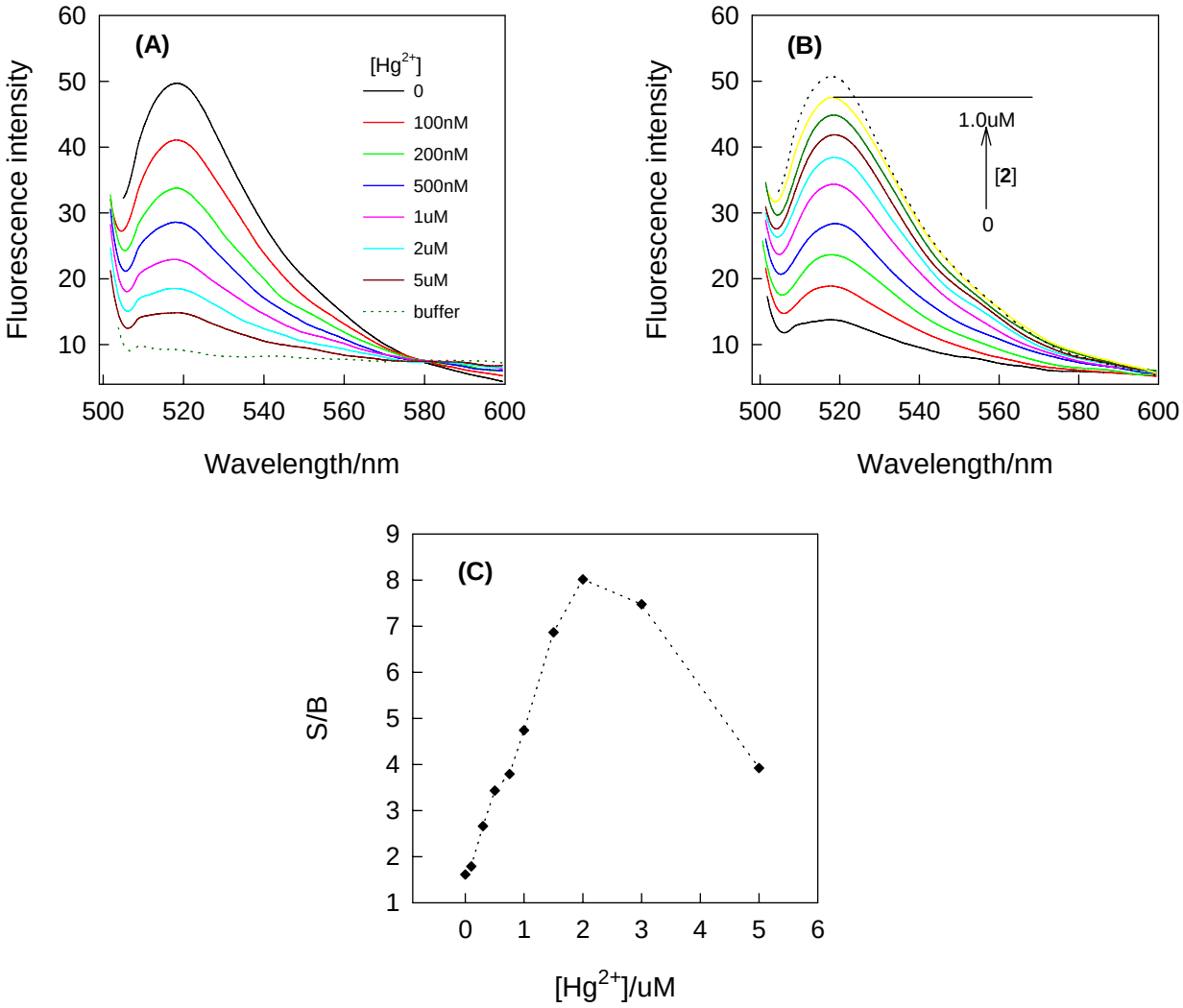

Figure S1. Effect of Hg^{2+} on the fluorescence response of 1 to 2:

(**A**) Effect of different concentrations of Hg^{2+} ion on the fluorescence emission spectra of **1**. [**1**] = 50nM, λ_{ex} = 480nm.

(**B**) Fluorescence restoration of Hg-**1** by different concentrations of target **2**. The arrows indicate the signal changes as increases in the target concentrations (0, 0.01, 0.03, 0.05, 0.075, 0.1, 0.2, 0.5, and 1.0μM). The dashed line corresponds to the fluorescence emission spectrum of **1** without Hg^{2+} and target DNA.

(**C**) Effect of Hg^{2+} concentration on the signal-to-background ratio (S/B) of **1** and **2**. S/B = $(F_{hybrid}-F_{buff})/(F_{free}-F_{buff})$, where F_{buff}, F_{free}, and F_{hybrid} are the measuring signals of buffer solution, Hg-**1** without target, and probe-target hybrid, respectively. The result shows that

increases in the metal ion concentration leads to low background signal, and thus the high S/B. But when the Hg^{2+} is over 3.0μM, the S/B will deteriorate because efficient hybridization between the target and the probe cannot occur under such conditions.

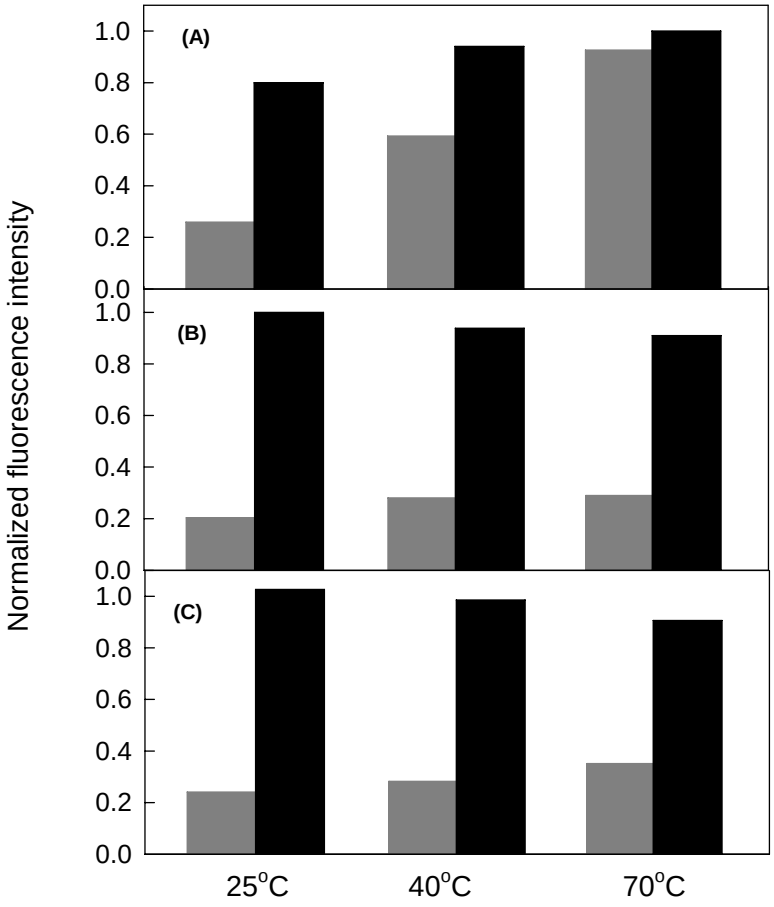

Figure S2. Effect of Hg^{2+} on the thermostability of 1 and its ability to hybridize with 2.

(**A**) Fluorescence signal changes of **4** upon hybridization with **2** at different temperatures.

(**B**) Fluorescence signal changes of **1** + 1.0μM Hg^{2+} upon hybridization with **2** at different temperatures.

(**C**) Fluorescence signal changes of **1** + 5.0μM Hg^{2+} upon hybridization with **2** at different temperatures.

Gray bars are the fluorescence intensity of the probes in the absence of **2**, black bars are the fluorescence intensity of the probes in the presence of 150 nM **2**. For comparison, the

fluorescence intensity was normalized to the highest intensity observed at each case. It shows that under high temperature, **4** is ineffective for DNA hybridization assay due to high background- signal, while in the presence of Hg^{2+}- **1** could hybridize with **2**.

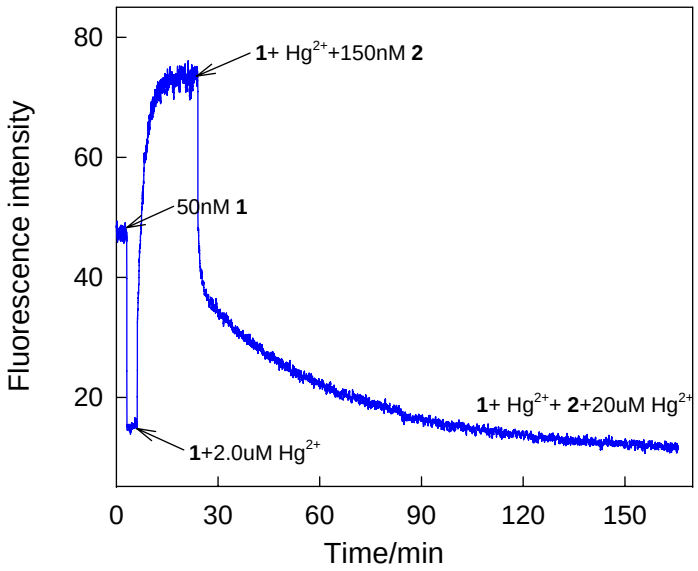

Figure S3 Hg^{2+} modulates DNA binding reversibility.

Fluorescence quenching of the mixtures of (**1** + **2**) upon addition of 20.0 μM Hg^{2+}. For each spectrum, we distinguish four steps: (1) the cell is filled with 500μL Tris/HCl buffer containing 50nM **1**; (2) Hg^{2+} was introduced in the cell; (3) a 3-fold excess of target was added; (4) excess of Hg^{2+} was added to the duplex complex. The transition between each regime is marked with an arrow.