

Supporting information

**A Binding-induced Sutured Toehold Activation
for Controllable DNA Strand Displacement Reactions**

Jing Zhu^a, Lei Wang^b and Wei Jiang^{*a}

^a Key Laboratory for Colloid and Interface Chemistry of Education Ministry,
School of Chemistry and Chemical Engineering, Shandong University, 250100 Jinan,
P.R. China

^b School of Pharmaceutical Sciences, Shandong University, 250012 Jinan, P.R.
China

Corresponding author: Tel: +86 531 88363888; fax: +86 531 88564464.

E-mail: wjiang@sdu.edu.cn

Experimental Section

Materials and Apparatus

Oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China), and their sequences and modifications were listed in Table S1 and Table S2. Thermodynamic parameters and secondary structures of all oligonucleotides were calculated using bioinformatics software (<http://www.bioinfo.rpi.edu-/applications/>). Standard mercury in 2-5% nitric acid was purchased from J&K Scientific Ltd. (Beijing, China). Adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), and uridine 5'-triphosphate (UTP) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Reagents for the native polyacrylamide gel electrophoresis (PAGE), including 40% acrylamide mix solution, ammonium persulfate, and 1,2-bis (dimethylamino)-ethane (TEMED) were purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). All other reagents were of analytical grade and used as received. The ultrapure water which was obtained from a Millipore Milli-Q water purification system (>18.25 M Ω) was used to prepare all of the solutions.

All fluorescence measurements were performed on a Hitachi F-7000 spectrofluorophotometer (Hitachi, Japan). The instrument was operated under the following parameters: $\lambda_{\text{ex}} = 495$ nm (bandpass 5 nm), $\lambda_{\text{em}} = 529$ nm (bandpass 5 nm), PMT detector voltage = 700 V.

Probe Preparation for the Binding-induced Sutured Toehold-mediated Strand Displacement

For preparation of DNA report sequence (RS) for Hg²⁺-induced sutured toehold-initiated strand displacement, 10 μ L of 10 μ M FAM-labeled TB was mixed with 10 μ L of 50 μ M BHQ1-labeled B* in 80 μ L of T-Mg buffer (10 mM Tris-HCl, 5 mM MgCl₂, pH 8.0) with a final concentration of 1 μ M. The resulting solution was annealed by heating at 90 °C for 5 min and then slowly cooled down to 25 °C over 2 h. The Hg²⁺ binding sequence (BS) with a nonbinding state at a concentration of 5 μ M was also annealed in T-Mg buffer (10 mM Tris-HCl, 5 mM MgCl₂, pH 8.0). Similarly,

the solution was heated to 90 °C for 5 min and then cooled down to 25 °C slowly in a period of 2 h.

DNA report sequence (RS-ATP) for ATP-induced sutured toehold-initiated strand displacement was prepared at a final concentration of 1 μM by mixing 10 μL of 10 μM FAM-labeled TB-ATP with 10 μL of 50 μM BHQ1-labeled B*-ATP in 80 μL of TMNa buffer (10 mM Tris-HCl, 10 mM MgCl_2 , 50 mM NaCl, 1 mM DTT, pH 7.9). The mixture was heated to 90 °C for 5 min and then cooled down to 25 °C slowly in a period of 2 h. The ATP binding sequence (BS-ATP) with nonbinding state at a concentration of 1.5 μM was also annealed in TMNa buffer (10 mM Tris-HCl, 10 mM MgCl_2 , 50 mM NaCl, 1 mM DTT, pH 7.9). Similarly, the resulting solution was heated to 90 °C for 5 min and then cooled down to 25 °C slowly in a period of 2 h.

Fluorescent Monitoring of Sutured Toehold-mediated Strand Displacement Reaction

The Hg^{2+} -induced conformation change of the BS and the sutured toehold-mediated strand displacement process were performed at 37 °C for 3 h in 100 μL T-Mg buffer containing 500 nM BS, 100 nM DNA RS and Hg^{2+} at different concentrations. The fluorescence intensity of the mixture solution was measured on a spectrofluorophotometer with the excitation wavelength at 495 nm and the emission wavelength at 529 nm. To monitor the kinetic process of sutured toehold-mediated strand displacement reaction, we measured the fluorescent tendency of the mixture solution from 5 min to 4 h with varied concentrations of Hg^{2+} . Similarly, The ATP-induced conformation change of the BS-ATP and the sutured toehold-mediated strand displacement process was carried out at 37 °C for 10 min in 100 μL TMNa buffer containing 150 nM BS-ATP, 100 nM DNA RS-ATP and ATP at different concentrations. The fluorescence intensity of the resulting solution was measured on a spectrofluorophotometer with the excitation wavelength at 495 nm and the emission wavelength at 529 nm. To monitor the kinetic process of sutured toehold-mediated strand displacement reaction, we measured the fluorescent tendency of the mixture solution from 2 min to 10 min with varied concentrations of ATP.

Native Polyacrylamide Gel Electrophoresis Experiments

In a typical experiment, 12% native polyacrylamide gel electrophoresis (PAGE) experiments were carried out in $1 \times$ TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0) for 2 h. After separation, PAGE gels containing DNA were stained using ethidium bromide, and imaged by a Biorad ChemiDoc XRS (BIO-RAD, America).

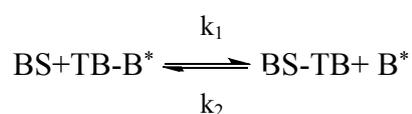
Text S1

Fluorescence Data Processing

Duplex TB-B* was formed by the annealing of a FAM fluorophore (F) labeled TB and a BHQ1 quencher (Q) labeled B* which was denoted as RS. When the Hg²⁺ triggered the conformation change of the BS, the subsequent strand displacement between the BS in binding-activated state and RS was promoted, leading to the formation of the duplex strand BS-TB and release of the B* which made the fluorescence of FAM recovered. The strand displacement kinetics could therefore be monitored by measuring the fluorescence intensity of the strand BS-TB as a function of time. The fluorescence signals are normalized using the equation

$$F_n = \frac{F - F_{TB-B^*}}{F_{BS-TB} - F_{TB-B^*}} \quad (1)$$

where F is the fluorescence intensity of each sample, F_{TB-B^*} is the fluorescence intensity of strand TB-B*, and F_{BS-TB} is the fluorescence intensity of BS-TB duplex. F_{TB-B^*} was measured before the beginning of each run as the ingredients are successively added. F_{BS-TB} was measured after the equilibrium of the strand displacement reaction. It was presumed that the sutured toehold-mediated strand displacement based on hairpin reconfiguration was same as the typical strand displacement as a biomolecular reaction,¹⁻³



According to the literature,¹ when the concentration of TB-B* was equal to the initial concentration of the BS, the back reaction of Equation 1 could be neglected, and then the displacement fraction was given as a function of time by

$$F_n = \frac{[BS - TB]}{[TB - B^*]_0} = \frac{k_1[TB - B^*]_0 t}{1 + k_1[TB - B^*]_0 t}$$

where the k_1 was the rate constant of the strand displacement reaction. Therefore, we could obtain the k_1 by applying the time-dependent normalized fluorescence intensity plots. When the initial concentration of BS (500 nM) was in large excess over the TB-B* (100 nM), then the displacement fraction was given as a function of time by

$$F_n = \frac{[BS - TB]}{[TB - B^*]_0} = 1 - e^{-k_1[TB - B^*]_0 t}$$

Then, we could obtain the k_1 by applying the time-dependent normalized fluorescence intensity plots.

Table S1 Sequences of Oligonucleotides Used in the Hg²⁺-induced sutured toehold-mediated strand displacement reaction ^a

name	sequence(5'-3')
BS ₁	ATT GTC ACT GTC CGT CGA GTT TTG TTT GTT GTC TCG ACC TTC TTT CTT ATT TCC TGT ATG CC
BS ₂	ATT GTC ACT GTC CGT CGA GTTG TTG TTT GTT GTC TCG ACC TTC TTT CTTC ATT TCC TGT ATG CC
BS ₃	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG CC
BS ₄	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GGTC TCG ACCC TTC TCTT CTTC ATT TCC TGT ATG CC
BS ₅	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT TGTGTC TCG ACCTCT TTC TCTT CTTC ATT TCC TGT ATG CC
BS ₃₋₉	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG C
BS ₃₋₁₀	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG CC
BS ₃₋₁₁	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG CCT
BS ₃₋₁₂	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG CCT A
BS ₃₋₁₄	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT TCC TGT ATG CCT ACC
BS-NT	ATT GTC ACT GTC CGT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT T
BS-NB	GT CGA GTTG TTG TTGT GTT GTC TCG ACC TTC TCTT CTTC ATT T
TB-9	GC ATA CAG GCT CGA CGG ACA GTG ACA AT-6-FAM
TB-10	GGC ATA CAG GCT CGA CGG ACA GTG ACA AT-6-FAM

TB-11 *A GGC ATA CAG GCT CGA CGG ACA GTG ACA AT-6-FAM*

TB-12 *TA GGC ATA CAG GCT CGA CGG ACA GTG ACA AT-6-FAM*

TB-14 *GGTA GGC ATA CAG GCT CGA CGG ACA GTG ACA AT-6-FAM*

B* BHQ1-ATT GTC ACT GTC CGT CGA G

^aThe sequence in red in the binding sequence (BS) is the Hg²⁺ recognition sequence. The toehold domains were highlighted in purple and italic in all of the BS and TB. The sequence in green in BS, TB and B* were branch migration domains.

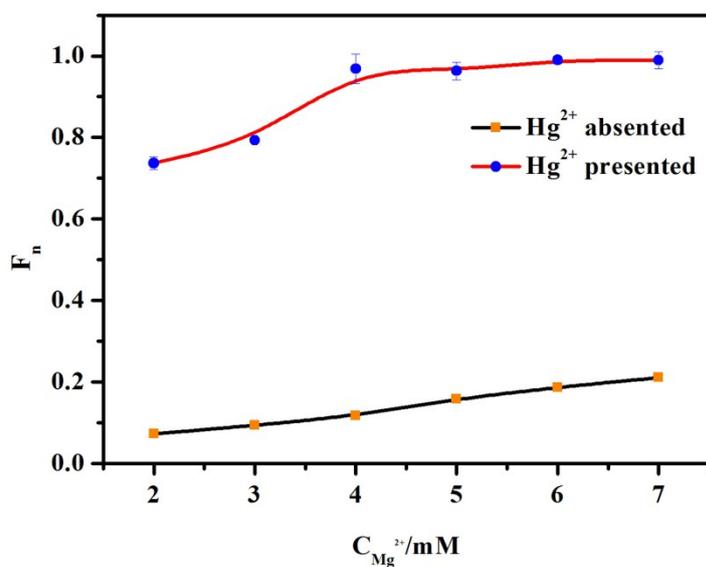


Figure S1. The effect of ionic strength for the fluorescence response of the Hg²⁺-induced sutured toehold strand displacement. The error bars represent the standard deviation of three measurements. Following the increase of ionic strength, the signal showed a negligible change in the absence of Hg²⁺. However, the fluorescence intensity gradually increased and became almost leveled off at the concentration of 4 mM of Mg²⁺.

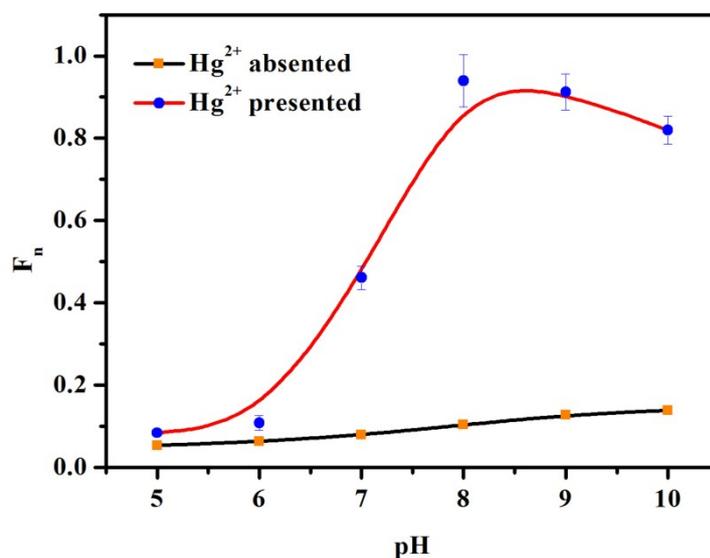


Figure S2. The fluorescence response on the different pH values of reaction buffer. The error bars represent the standard deviation of three measurements. With the increase of the pH value from 5.0 to 8.0, the formation of T-Hg²⁺-T was enhanced because the hydrogen ion was free with the insertion of Hg²⁺ into the T-T pair.⁴The fluorescence intensity decreased gradually when the value was greater than 8.0. Such a result may be caused by the formation of Hg²⁺-related precipitation in the excessive pH value.

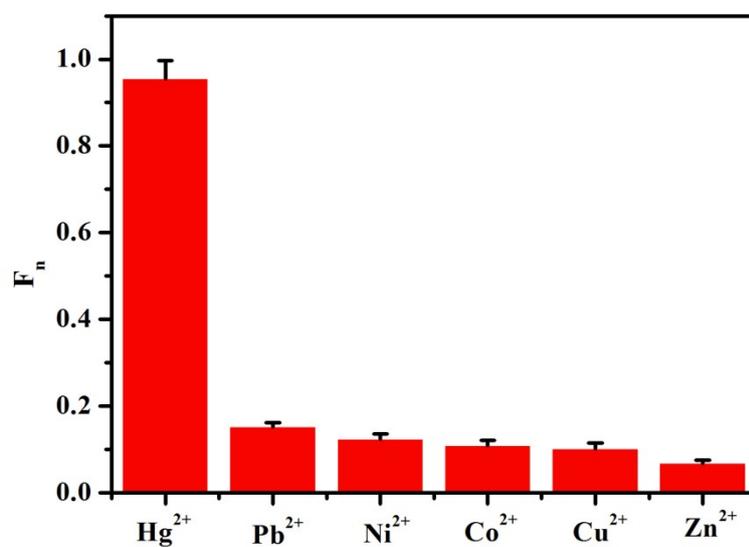


Figure S3. The selectivity of Hg²⁺-induced sutured toehold activation strategy. The error bars represent the standard deviation of three measurements. The concentration was 10 μ M for Hg²⁺ and 1 mM for other metal ions.

Table S2 Sequences of Oligonucleotides Used in the ATP-induced sutured toehold-mediated strand displacement reaction ^b

name	sequence (5'-3')
BS-ATP	ATT GTC ACT GTA AAC CTC CGT ACC TGG GGG AGT ATT GCG GAG GAA GGT AC TGG TGT ATG G
TB-ATP	C CAT ACA CCCG GAG GTT TAC AGT GAC AAT-FAM
B*-ATP	BHQ1-ATT GTC ACT GTA AAC CTC CG

^bThe sequence in blue was the aptamer for ATP binding. The toehold domains in BS-ATP and TB-ATP were highlighted in purple and italic. The sequence in green in BS-ATP, TB-ATP and B*-ATP were branch migration domains.

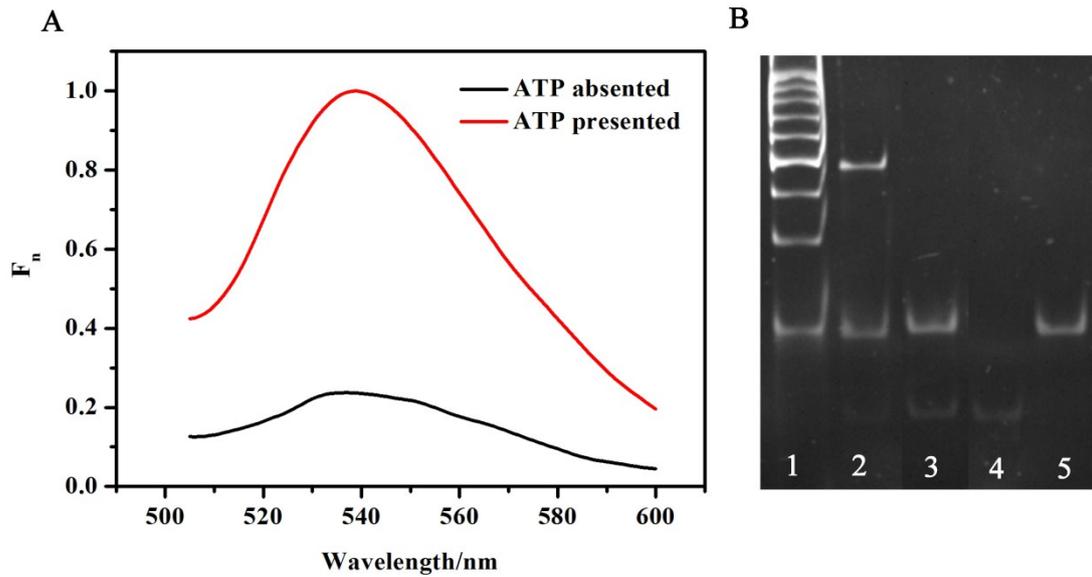


Figure S4. Verification of the ATP-induced sutured toehold activation strategy. (A) Fluorescence experiment. The curve in red was measured after adding RS-ATP (100 nM) and ATP (75 μ M) into BS-ATP (150 nM) at 5 min. The curve in black was measured after adding RS-ATP (100 nM) into BS-ATP (150 nM) at 5 min. (B) The native PAGE experiment. Lane 1: ladder size marker. Lane 2: 150 nM BS-ATP + 100 nM RS + 75 μ M ATP. Lane 3: 150 nM BS-ATP + 100 nM RS + 0 μ M ATP. Lane 4: 100 nM RS-ATP which was formed by prehybridizing 100 nM FAM labelled TB-ATP and 500 nM BHQ-1 labelled B*-ATP). Lane 5: 150 nM BS-ATP.

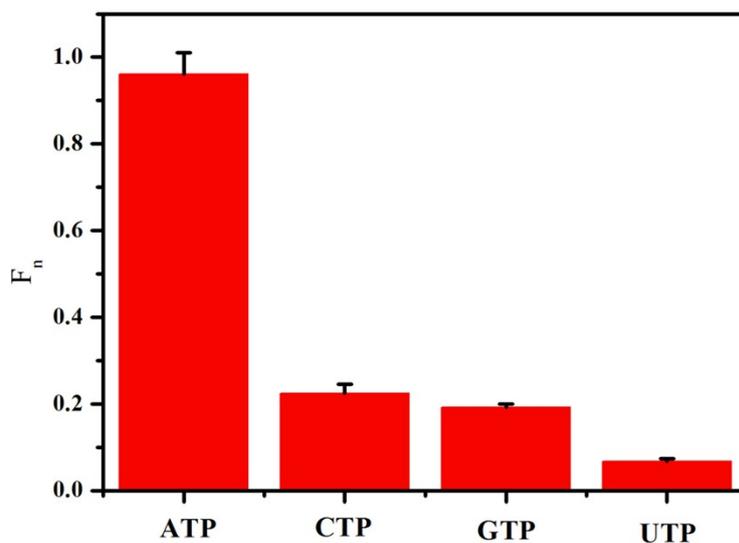


Figure S5. The selectivity of ATP-induced sutured toehold activation strategy. The error bars represent the standard deviation of three measurements. The concentration was 75 μM for ATP. The concentrations for CTP, GTP and UTP were 100 μM , respectively.

Reference

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