Supporting Information

1. Experimental Sections

1.1 Materials and Chemicals

DNA oligonucleotides were synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

Padlock probe:

5'-P-*GTG GTT GG*T TTG CAT TTC AGT TTA CGG TTT AGC ATT TCG CAA TTT TC*G GTT GGT*-3'

Aptamer:

5'-CGG TTG GTG TGG TTG G-3'

Primer 1:

5'-CCA ACC ACA CCA ACC G-3'

Primer 2:

5'-GCA TTT CAG TTT ACG-3'

5'-phosphorylated linear padlock probe contains 15 nucleotides (nt), complementary to primer1 (in italic), and the aptamer was complementary DNA of Primer 1, which could bind with thrombin. Escherichia coli (*E. coli*) DNA ligase set (including Escherichia coli DNA ligase, 10×Escherichia coli DNA ligase buffer, and 10×BSA (0.05%)) were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). The deoxynucleotide solution mixture (dNTPs), Bst DNA polymerase large fragment, and their corresponding buffer were purchased from New England Biolabs (NEB). SYBR Green I was purchased from Xiamen Biovision Biotechnology Co. Ltd. (Xiamen, China). All other chemicals were of analytical reagent grade and obtained from Sigma Chemical Co., USA. In this experiment, double distilled water (Milli-Q,

Millipore, resistance 18.2-MΩ) was used throughout the experiments. DNA buffer solutions were prepared by dissolving DNA into 0.1 M pH 7.4 phosphate buffer solutions (PBS) containing 0.3 M NaCl.

1.2 Ligation and RCA and HRCA Reaction

Different concentrations of thrombin were mixed with aptamer probe (5 nM) by equal volume for 30 min. Then, the same volume of Primer 1 (5 nM) was introduced into above solution for incubation at 37 ^{o}C for 30 min with the vibration. The ligation reaction was performed in the ligation buffer solution containing 0.5 nM padlock probe, 6 U *E. coli* DNA ligase, 0.05% BSA and 0.167mM nicotinamide adenosine dinucleotides (NAD), which was incubated at 37 ^{o}C for 60 min[1]. For RCA reaction, the product of the ligation was incubated with a RCA reaction solution containing: 1×phi29 buffer phi29 DNApolymerase (10 units/μL), 0.6 mM dNTP at 37 ◦C for 60 min. While for HRCA reaction, the product of the ligation was added to a HRCA reaction solution containing 1×polymerase buffer, 5 nM Primer 2, 0.6 mM dNTP and 6.4 U Bst DNA polymerase at 37 ^{o}C for 60 min. These amplification reactions were terminated at 95 °C for 10 min. HRCA products were analyzed by 2% agarose gel electrophoresis containing 8 M urea and screened with a standard silver-staining method.

1.3 Fluorescence Detection

Above HRCA product and 4 μL SYBR Green I were mixed and incubation at room temperature for 15 min. The fluorescence measurements were performed at room temperature on a Varian Cary Eclipse Fluorescence Spectrophotometer except specific indication. The emission spectra were collected from 505 to 595 nm with the excitation wavelength of 480 nm. Both the excitation and emission slit widths were set to 10.0 nm. The fluorescence intensity at 518 nm is used to quantitative analysis.

2. Optimized of the reaction conditions

In order to initiate HRCA reaction well, some reaction conditions are optimized.

dNTP plays an important role in strand extension. As shown in Figure S1(A), with the increment of dNTP concentration, the fluorescent intensity enhances gradually, and finally reaches a constant after 0.6 mM, indicating that SG dye embeds into HRCA production completely in the presence of 0.6 mM dNTP. Thus, the concentration of dNTP is 0.6 mM.

For the concentration of Bst DNA polymerase, it is found that 6.4 U of Bst DNA polymerase brings in a maximal fluorescent intensity in the range of 1.6~8U (shown in Figure S1(B)). Hence, the concentration of Bst DNA polymerase is set as 6.4 U.

The prime 2 is another important factor for the efficiency of HRCA. When the concentration of prime 2 exceeds 5 nM, the fluorescent intensity enhances very slowly, showing a maximum amplification efficiency of HRCA is achieved at the concentration of 5 nM. In this study, 5 nM prime 2 is used.

A long HRCA reaction time is expected to generate more copies of the circular template for better signal amplification. The time-dependent fluorescence is monitored the HRCA reaction. At the beginning, the fluorescent intensity boosts up gradually, showing that the HRCA production is generated continuously. While the fluorescent intensity trends to a constant value at 60 min, indicating the saturation of HRCA production. Therefore, 60 min is chosen as the optimum time for HRCA reaction.

References

1. Y. Q. Cheng, X. Zhang, Z. P. Li, X. X. Jiao, Y. C. Wang, *Angew. Chem. Int. Ed.* **2009**, *48*, 3268.

Figure S1

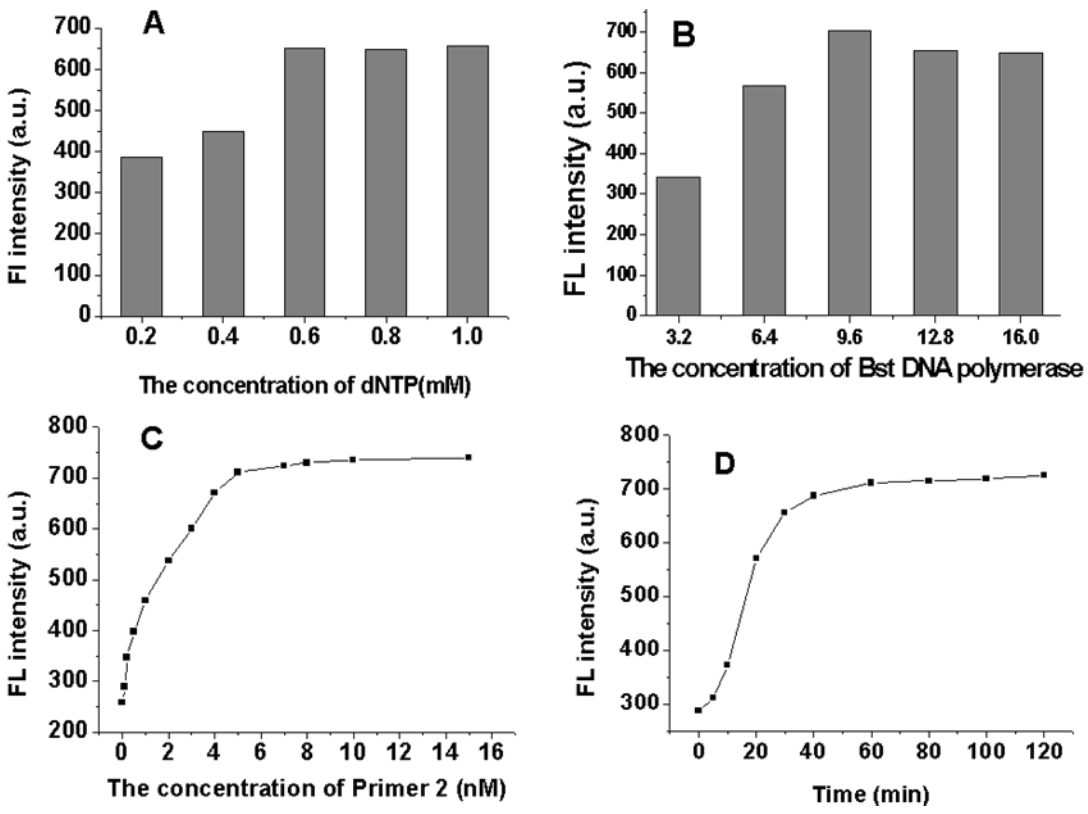

Figure S1 A) Fluorescence intensity of this aptameric sensor upon the concentration of dNTP; B) Fluorescence intensity of this HRCA method upon the concentration of Bst DNA polymerase; C) Fluorescence intensity of this method upon the

90 concentration of Primer 2; D) Time-dependent fluorescence changes in HRCA
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