Supporting Information

An autocatalytic DNA machine by autonomous target recycling and cascade circular exponential amplification for the one-pot, isothermal and ultrasensitive nucleic acid detection

Xinya Sun, Li Wang, Mingsha Zhao, Changzhi Zhao and Shufeng Liu*

Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, 53 Zhengzhou Road, Qingdao 266042, China.

* Corresponding author. *E-mail:sliu@qust.edu.cn*

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Experimental Section.

Materials and chemicals. Bst 2.0 DNA polymerase, nicking endonuclease Nb.BbvCI, dNTPs and CutSmart buffer were purchased from New England Biolabs (Ipswich, MA, USA). Acrylamide/bisacrylamide 39:1 40% gel stock solution, N,N,N',N'tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and ethidium bromide (EB) were purchased from Yantai Science and Biotechnology Co., Ltd. (Yantai, China). Fetal bovine serum was obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). Healthy human serum samples were kindly provided by the Qingdao Center Hospital (Qingdao, China). The HPLC-purified oligonucleotide sequences were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and listed in Tab. S1. All other chemicals were obtained from Shanghai Chemical Reagents (Shanghai, China) and used without further purification.

Target DNA detection. Before DNA detection, all hairpin DNA samples were firstly heated to 90 °C for 5 min and then allowed to cool to 37 °C for 1h. The target DNA recogniton and cascade circular exponential amplification reaction was carried out in 100 μL 1×CutSmart buffer (pH 7.9, 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/ml BSA) containing 100 nM HP, 100 nM MB, 20 U nicking endonuclease Nb.BbvCI, 10 U Bst 2.0 DNA polymerase, 200 μM dNTPs and varying concentrations of the target DNA. The reaction mixture was incubated at 37 °C for 90 min, and then the fluorescence responses were recorded.

Polyacrylamide gel electrophoresis. To prepare the hydrogel, 3.5 mL 40% gel solution (39:1), 160 μL 50×TAE Buffer, 80 μL 10% APS, 4 μl TEMED and 4256 μL deionized water were mixed. This mixture contained a final gel percentage of 17.5%. The polyacrylamide gel electrophoresis (PAGE) was carried out in 1×TAE buffer at a constant voltage of 180 V for 3 min, and then at a constant voltage of 135V for about 90 min at room temperature, and then stained in EB dye solution.

Instrument. All fluorescence measurements were carried out on a F-2700 spectrometer with a scan rate at 1500 nm/min. The excitation wavelength was set to 490 nm and the 24 photomultiplier voltage was 700V. The slits for excitation and emission were set at 5 nm / 5 nm.

Tab. S1 Sequence of synthesized oligonucleotide probes for target DNA detection[a]

Name	Sequence (5' to 3')
HP1	CGTTCTATAGTGCTGAGGAAAGGACAGGCACAA ACACGCACTATAGAACGTTT
HP2	CGTTCTATAGTGCTTTTTAAAGGACAGGCACAA ACACGCACTATAGAACGTTT
HP3	CGTTCTATAGTGCTGAGGAAATCAGTTATATGGATGATGTGGTATGCACTATAGAACGTTT
MB1	Dabcyl-AGTACCTCTTGCTGA GGT ACT/6-FAM/CGTTCTAT
MB2	Dabcyl-AGTACCTGT TTTTCAGGTACT/6-FAM/CGTTCTAT
TD	GTG CGT GTT TGT GCC TGT CCT GGG
1MT	GTGCGTCTTTGTGCCTGTCCTGGG
2MT	GTGCGTCATTGTGCCTGTCCTGGG
NC	ATAGCAACGTCCTACACCTCGAAC
HBV DNA	ATACCACATCATCCATATAACTGAAAGCCA

[a]HP denotes hairpin DNA probe. MB represents molecular beacon. 1MT, 2MT, and NC denote single-base mismatched DNA, two-bases mismatched DNA, and non-complementary DNA, respectively. The underlined letters in 1MT and 2MT indicate the mismatched base. The HBV DNA represents the target DNA related to the hepatitis B virus (HBV). The HP3 is designed toward the detection of HBV DNA.

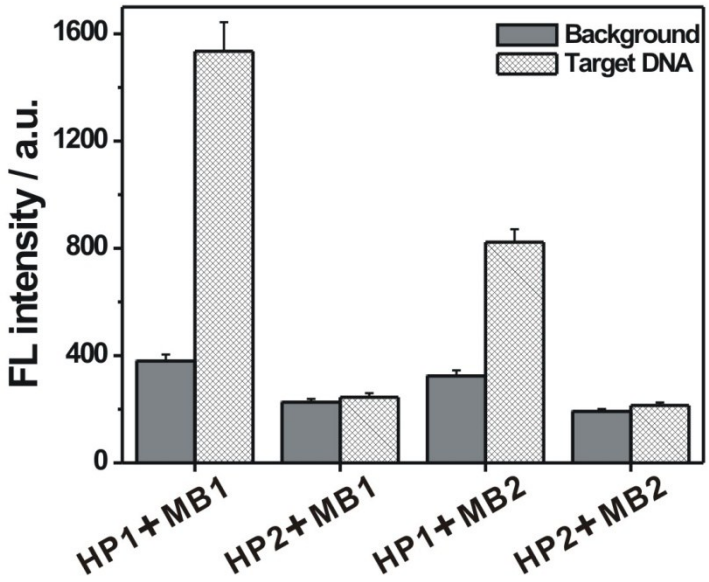

Fig.S1. The bar chart of the fluorescence responses obtained by using different HPs (HP1 or HP2) and MBs (MB1 or MB2) toward the blank and 10 pM target DNA. The difference for the HP2 and MB2 from the HP1 and MB1 is that they contain no recognition site of the nicking endonuclease in the respective loop region.

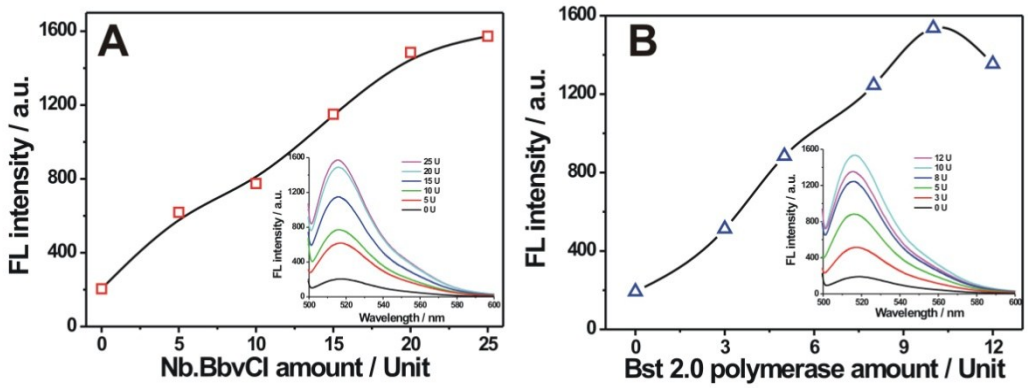

Fig.S2. (A) The effect of nicking endonuclease Nb.BbvCI amount on the fluorescence response of the sensing system in the presence of 10 pM target DNA. Different Nb.BbvCI amounts including 0, 5, 10, 15, 20 and 25 Unit were used. (B) The effect of Bst 2.0 DNA polymerase amount on the fluorescence response of the sensing system in the presence of 10 pM target DNA. Different poymerase amounts including 0, 3, 5, 8, 10 and 12 Unit were used. The inset shows the corresponding fluorescence spectra.

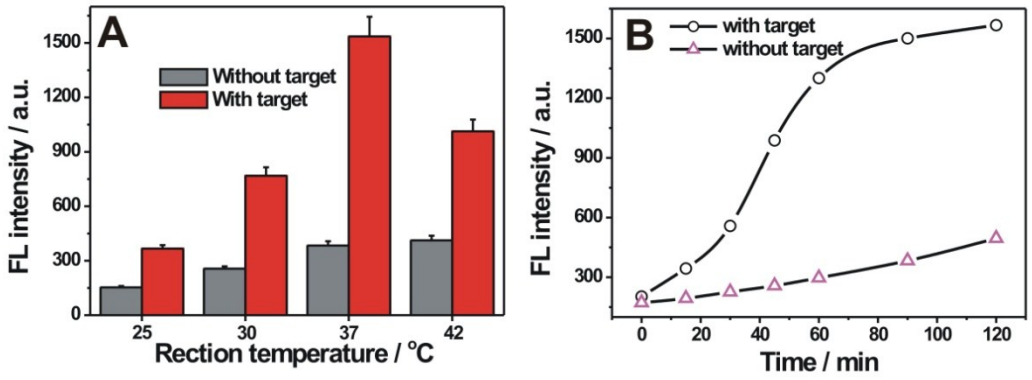

Fig.S3. (A) The effect of the reaction temperature on the fluorescence response of the sensing system toward blank and 10 pM target DNA. (B) The fluorescence response of the TR-CEA strategy with time in the presence and absence of 10 pM target DNA, respectively.

Tab.S2 Comparison of detection performance for target DNA by ours and those reported fluorescence methods

Method	Detection limit	Strategy	Ref.
Fluorescence	10 pM	Autonomous Ligation DNAzyme Machinery	[1]
Fluorescence	1 pM	Catabolic DNAzyme-mediated process	[2]
Fluorescence	91 pM	Isothermal exponential amplification and thioflavin T	[3]
Fluorescence	10 fM	Assembled DNAzyme wires	[4]
Fluorescence	20 fM	Exo III-aided cascade target recycling and DNAzyme	[5]
Fluorescence	6.2 pM	Dual-cyclical nucleic acid strand-displacement polymerization	[6]
Fluorescence	10 pM	Zn^{2+}-ligation DNAzyme-driven DNAzyme cascade	[7]
Fluorescence	80 pM	Catalytic hairpin assembly and SYBR Green I	[8]
Fluorescence	0.75fM	Exonuclease III-induced isothermal amplification	[9]
Fluorescence	10 pM	Template-dependent extension	[10]
Fluorescence	10 fM	Fok I/DNA Machine and DNAzyme	[11]
Fluorescence	1 fM	Target recycling and cascade circular exponential amplification	This work

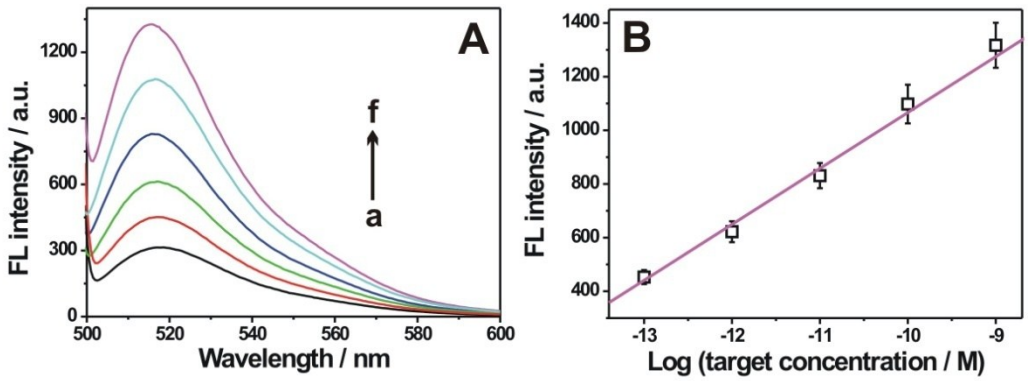

Fig.S4. (A) Fluorescence responses corresponding to the analysis of different concentrations of target DNA by using MB2 as the substitute of MB1. The concentrations of target DNA for the curves from (a) to (f) are: (a) 0 M, (b) 0.1 pM, (c) 1 pM, (d) 10 pM, (e) 0.1 nM, and (f) 1 nM. (B) The linear relationship between the fluorescence intensity and the logarithm value of the target DNA concentration. Error bars represent standard deviations of measurements (n = 3). Herein, the target DNA detection is operated by the target recycling and unidirectional isothermal exponential amplification strategy.

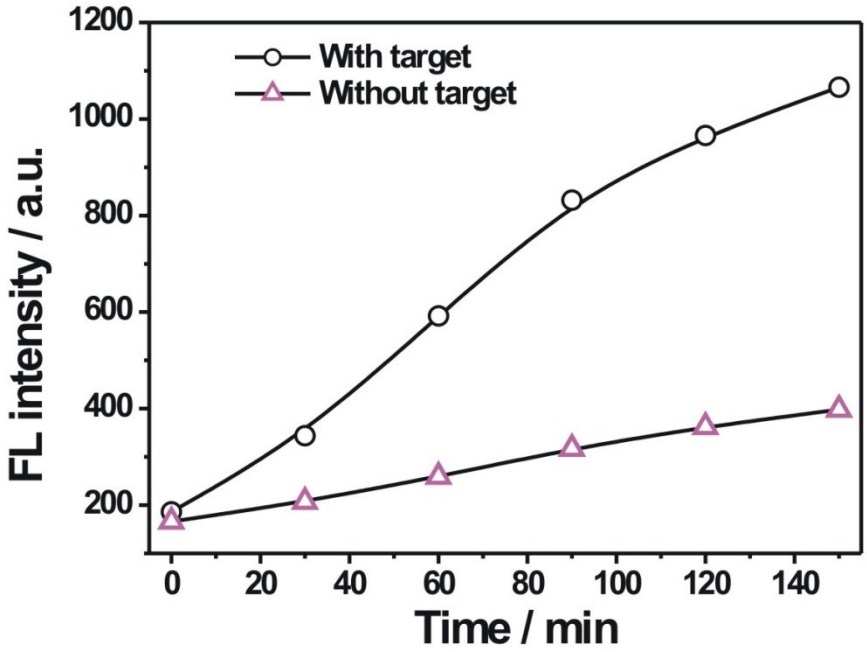

Fig.S5. The time response of the sensing system fabricated by the target recycling and unidirectional isothermal exponential amplification strategy in the presence and absence of 10 pM target DNA, respectively. The MB2 was used as the substitute of MB1.

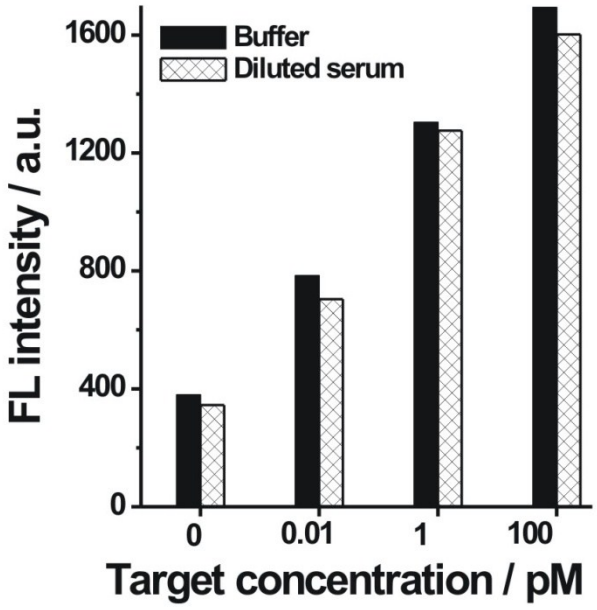

Fig.S6. Fluorescence intensity obtained for the developed sensing system by the TR-CEA strategy in buffer and 10% fetal bovine serum (10 fold diluted) spiked with different DNA concentrations.

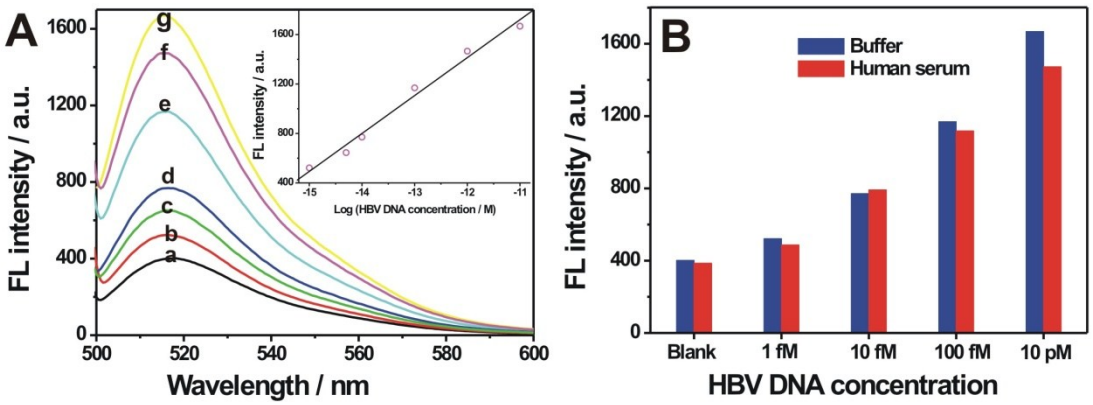

Fig.S7. (A) The detection performance of the fabricated sensing system by the TR-CEA strategy toward the target DNA sequence of HBV. The HBV DNA concentrations for the curves from (a) to (g) are: (a) 0 M, (b) 1 fM, (c) 5 fM, (d) 10 fM, (e) 100 fM, (f) 1 pM, and (g) 10 pM. The inset shows the linear relationship between the fluorescence intensity and the logarithm value of the HBV DNA concentration. (B) The fluorescence response comparison for the sensing sytem toward different concentrations of HBV DNA spiked in buffer and 10% healthy human serum. The HP3 is used as the substitute of the HP1 and contains the binding sequence for HBV DNA in the loop region.

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