Supplementary information

A catalytic DNA circuit-programmed and enzyme-powered autonomous DNA machine for nucleic acid detection

Shuang Liu, Chen Xin, Xiaoxiao Yu, Zhenbo Ding and Shufeng Liu*

Key Laboratory of Optic-Electric Sensing and Analytical Chemistry for Life Science, 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; Tel@Fax: 86-532-8402-2681.

Table of contents

Experimental sections

Reagents. All the oligonucleotides were HPLC-purified and synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China) with the base sequences listed in Table S1. Nt.BbvCI and Klenow Fragment (3'-5' exo-) were purchased from New England Biolabs (Ipswich, MA, USA). Deoxyribonucleoside-5'-triphosphate (dNTPs) mixture and fetal calf serum were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Other chemicals were obtained from Shanghai Chemical Reagents (Shanghai, China) and used without further purification. All solutions were prepared with deionized water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) with a resistivity of 18.2 MΩ cm.

Three-strand duplex DNA probe. Three-strand duplex DNA probe (TP) was prepared by annealing the same concentration (2 μM) of template DNA strand (TS), assistant DNA strand (AS) and protector DNA strand (PS) in 20 mM Tris-HCl buffer (100 mM NaCl, 5mM $MgCl_2$, pH 7.4). The mixture was firstly heated to 90 ^oC and then cooled gradually to room temperature over 2 h. The obtained three-strand duplex DNA probe was stored at 4 ^oC before use.

Target DNA detection. The target DNA detection was conducted by mixing 200 nM three-strand duplex DNA probe (TP), 40 nM fuel strand (FS), 6U Nt.BbvCI, 2 U Klenow, 200 μM dNTPs and different concentrations of target DNA in 20 mM Tris-HCl buffer (100 mM NaCl, 5 mM $MgCl_2$, pH 7.4) at 37 ^oC for 80 min. Other experimental conditions have been indicated in the text. Then, the fluorescence responses were recorded. The above procedures were also employed for target DNA detection in the case of no enzymes.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis experiments were carried out by using a 17.5% polyacrylamide hydrogel 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. The gel images were captured by using a FR-980 gel imager (Shanghai, China).

Table S1. The used nucleic acid sequences in the experiment

Name	Sequence from 5’ to 3’
Template strand (TS)	AGGAG/iDabcyldT/GAAATGGTGGAGCTGAGGGGAGTCT TCCAGTGTGATGA
Protector strand (PS)	CACTGGAAGACTCCCCTTTTTTTTTT
Assistant strand (AS)	CAGCTCCACCATTTC-6-FAM
Fuel strand (FS)	CACTGGAAGACTCCCCTCAGCTCCACCATTTC
Target DNA (TD)	TCATCACACTGGAAGACTCTTT
One-base mismatched sequence (1MT)	TCATCACACTGGAAGAATCTTT
Two-bases mismatched sequence (2MT)	TCATCACACTGGAAGGATCTTT
Non-complementary target (NC)	GACGTCAGACTTCCTGCGATTT

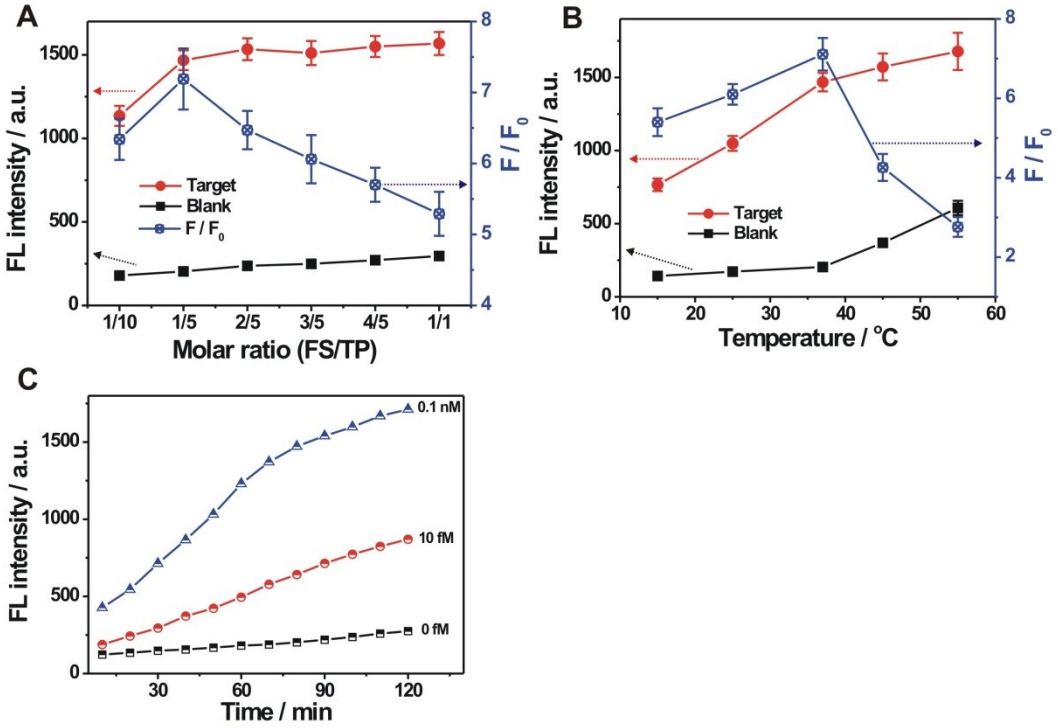

Figure S1. (A) Molar ratio optimization of FS with TP. The fluorescence values toward 0.1 nM target DNA, background values and signal to background ratio (F/F_0, F and F_0 represents the corresponding fluorescence and background values) were obtained at different molar ratios of FS/TP (1/10, 1/5, 2/5, 3/5, 4/5, 1/1; the concentration of TP was set at 200 nM). (B) Optimization of reaction temperature. The fluorescence values toward 0.1 nM target DNA, background values and signal to background ratio (F/F0) were obtained at different temperatures (15, 25, 37, 45 and 55 ^{o}C). (C) Fluorescence responses at 0, 10 fM, and 0.1 nM target DNA versus time.

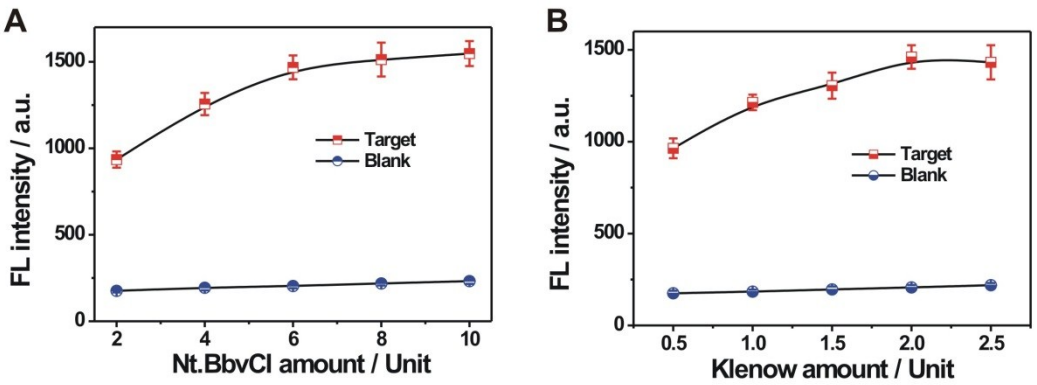

Figure S2. (A) Optimization of Nt.BbvCI amount. (B) Optimization of Klenow amount.

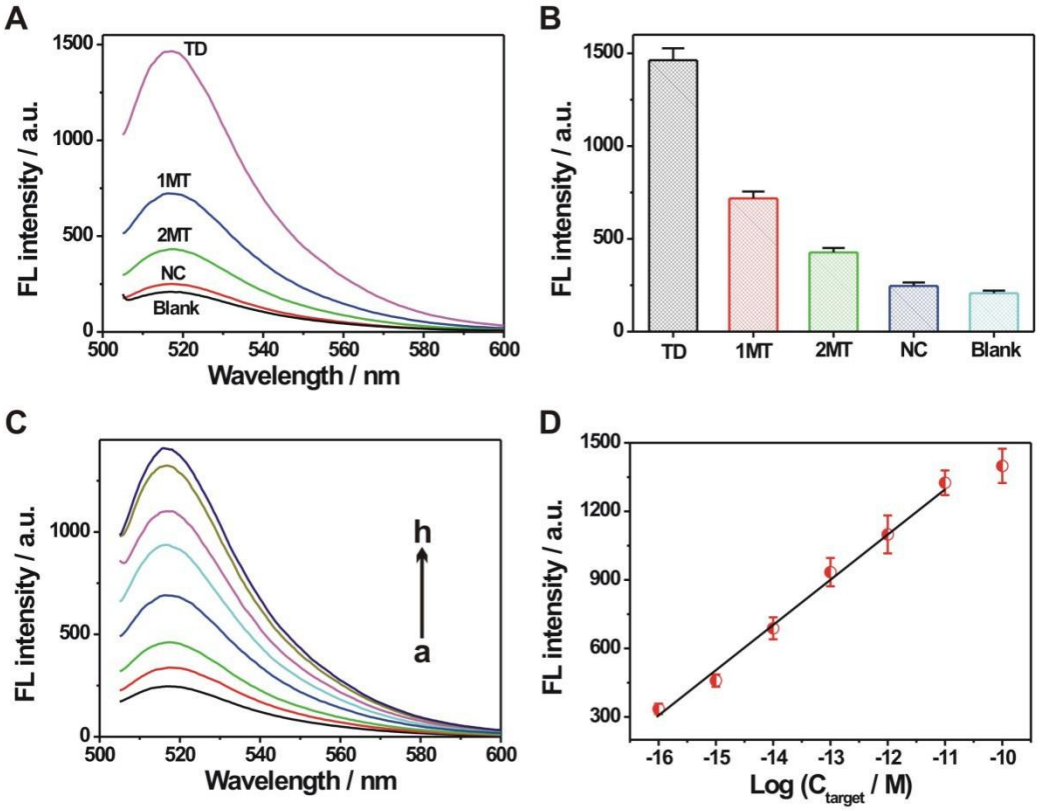

Figure S3. (A) Corresponding fluorescence spectra at various DNA sequences including non-complementary (NC), two-base mismatched (2MT), single-base mismatched (1MT), and complementary target DNA (TD). All the sequences have the same concentrations (0.1 nM). (B) Bar charts of the fluorescence intensities at different DNA sequences. (C) Fluorescence responses of the sensing system toward different concentrations of target DNA spiked into the 10% diluted fetal bovine serum (FBS): (a) 0 M, (b) 0.1 fM, (c) 1 fM, (d) 10 fM, (e) 0.1 pM, (f) 1 pM, (g) 10 pM, (h) 0.1 nM. (D) Calibration curve between the fluorescence intensity and the logarithm value of the target DNA concentration. Error bars (standard deviations) for each point were obtained based on three independent measurements.

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

Ref.	Linear range	Detection limit	Strategy
[1]	0.5 nM -50 nM	91 pM	Isothermal exponential amplification and thioflavin T
[2]	0.05 nM-150 nM	6.2 pM	Dual-cyclical nucleic acid strand-displacement polymerization
[3]	1 fM-100 fM	0.75 fM	Exonuclease III-induced isothermal amplification
[4]	0.01 nM-500 nM	10 pM	Template-dependent extension
[5]	None	10 fM	Fok I/DNA Machine and DNAzyme
[6]	1 fM-0.1 nM	1 fM	Target recycling and cascade circular exponential amplification
[7]	10 pM-0.15μM	10 pM	Palindromic molecular beacon-based intramolecular strand-displacement amplification
[8]	10 pM-1 μM	1.9 pM	Cascade toehold-mediated strand displacement
[9]	50 fM-5 nM	15.6 fM	Entropy-driven catalysis and catalytic hairpin assembly
[10]	0.1 pM-200 nM	16 fM	Target self-amplification-based DNA machine
This work	0.1 fM-10 pM	0.01 fM	Catalytic DNA circuit-programmed and enzyme-powered DNA machine

Table S3. Recovery experiments of target DNA in diluted serum using the proposed method

Samples	Added (fM)	Detected (fM)	Recovery
1	5	4.47 ± 0.35	89%
2	50	47.1 ± 0.39	94%
4	500	539 ± 29	108%

The results were based on three repetitive experiments.

References

[1] H. Zhao, J. Dong, F. Zhou and B. Li, *Microchim. Acta,* 2015, **182**, 2495.

[2] J. Xu, Z. S. Wu, W. Shen, H. Xu, H. Li and L. Jia, *Biosens. Bioelectron.,* 2015, **73**, 19.

[3] Q. Xue, Y. Lv, Y. Zhang, S. Xu, R. Li, Q. Yue, H. Li, L. Wang, X. Gu, S. Zhang and J. Liu, *Biosens. Bioelectron.,* 2014, **61**, 351.

[4] H. Zhou, S. J. Xie, S. B. Zhang, G. L. Shen, R. Q. Yu and Z. S. Wu, *Chem. Commun.,* 2013, **49**, 2448.

[5] Y. Weizmann, Z. Cheglakov and I. Willner, *J. Am. Chem. Soc.,* 2008, **130**, 17224.

[6] X. Sun, L. Wang, M. Zhao, C. Zhao and S. Liu, *Chem. Commun.*, 2016, **52**, 11108.

[7] H. Li, Y. Tang, W. Zhao, Z. Wu, S. Wang and R. Yu, *Anal. Chim. Acta*, 2019, **1065**, 98.

[8] Q. Li, Z. Liu, D. Zhou, J. Pan, C. Liu and J. Chen, *Analyst*, 2019, **144**, 2173.

[9] E. Xiong, D. Zhen and L. Jiang, *Chem. Commun.*, 2018, **54**, 12594.

[10] H. Xu, Y. Jiang, D. Liu, K. Liu, Y. Zhang, S. Yu, Z. Shen, Z. S. Wu, *Anal. Chim. Acta*, 2018, **1011**, 86.