Supporting Information

Amplified detection of DNA by analyte-induced Y-shaped junction probe assembly followed with nicking endonuclease-mediated autocatalytic recycling process

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Experimental section.

Nicking Endonuclease Nt.BbvCI and NEB buffer 4 were purchased from New England Biolabs (Ipswich, MA, USA). DNA oligonucleotides were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of oligonucleotides employed are listed in Table S1. Human serum samples were provided by the Qingdao Center Hospital. All fluorescence measurements were carried out on a F-4500 spectrometer with a scan rate at 1200 nm/min. The excitation wavelength was at 490 nm, and the 24 photomultiplier tube voltage was 700 V. The slits for excitation and emission were set at 5 nm/5 nm. The fluorescence measurements for DNA sensing were performed based on the following procedures. Firstly, each 15 μL of 1 μM MB probe, substrate **1** and **2** in 10 mM tris-HCl solution (pH 7.4, 500 mM NaCl, 1 mM $MgCl_2$) were added into 225 μL 1× NEB buffer 4 (20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 50 mM KAc, 1 mM DTT, pH 7.9) containing 12 U nicking endonuclease Nt.BbvCI. Then 30 uL of 10 mM tris-HCl solution with varying concentrations target DNA was added. After incubation at 37 °C for 30 min, the fluorescence response was recorded. The final concentrations of MB probe, substrate **1** and **2** in the reaction solution (300 μL) were all 50 nM. The fluorescence measurements of general analyte-sensing platform was performed at the same conditions except adding 50 nM subunit **7** to reaction solution.

Table S1. DNA sequences used in the experiments[a]

Name	Sequence (5' to 3')
Subunit 1a	AGGACTGATTCGAGTAGTCCTCTCCCACTGACT
Subunit 1b	GAGGACTGATTCGAGTAGTCCTCTCCCACTGACT
Subunit 1c	AGAGGACTGATTCGAGTAGTCCTCTCCCACTGACT
Subunit 1d	GAGAGGACTGATTCGAGTAGTCCTCTCCCACTGACT
Subunit 2	CGCTGAGGAAAGGAGAGGACT
MB a	FAM-ACTCGAATCAGTCAGTGTCCTCAGCGAGT-Dabcyl
MB b	FAM-CACTCGAATCAGTCAGTGTCCTCAGCGAGTG-Dabcyl
MB c	FAM-CCACTCGAATCAGTCAGTGTCCTCAGCGAGTGG-Dabcyl
MB 2	FAM-CACACGATAAAGTCAGTGTCCTCAGCGTGTG-Dabcyl
Subunit 7	AGAGGACTTGCATGGTATTCTGAAGACGACAAGTCCTCTCCCACTGACT
Target DNA 1	ACTCGAATCAGTC
Single-base mismatched DNA	ACTGGAATCAGTC
Two-base mismatched DNA	ACTGCTATCAGTC
Non-complementary target DNA	ACTTACAATCTAGT
Target DNA 2	TGTCGTCTTCAGAATACCATGCA

[a] The underlined regions in the subunit **2** and the MB identify the recognition site of Nt.BbvCI.

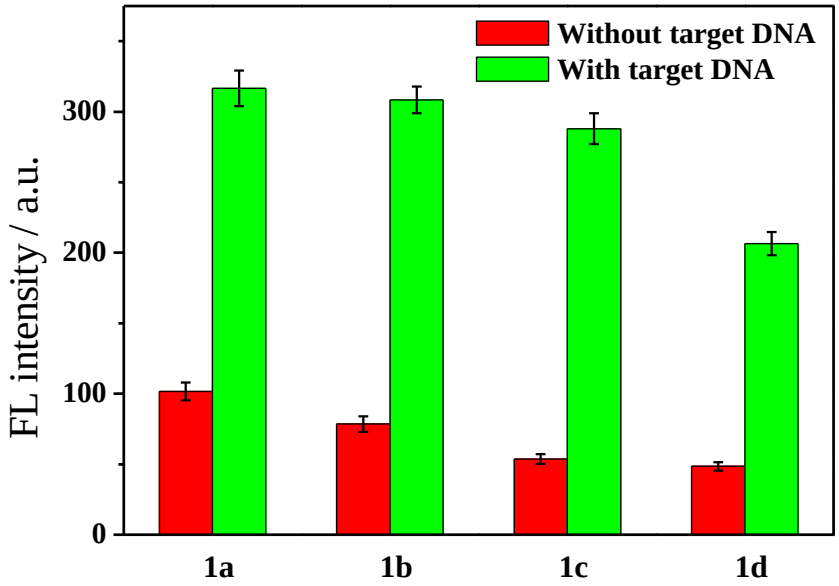

Figure S1. The fluorescence intensity of the autocatalytic system using different subunit **1** that includes various lengths of the hairpin stem in the absence (red bars) or presence (green bars) of 1nM target DNA: 1a, 6 bp of the stem region; 1b, 7 bp of the stem region; 1c, 8 bp of the stem region; 1d, 9 bp of the stem region. The error bars represent the standard deviation of three measurements.

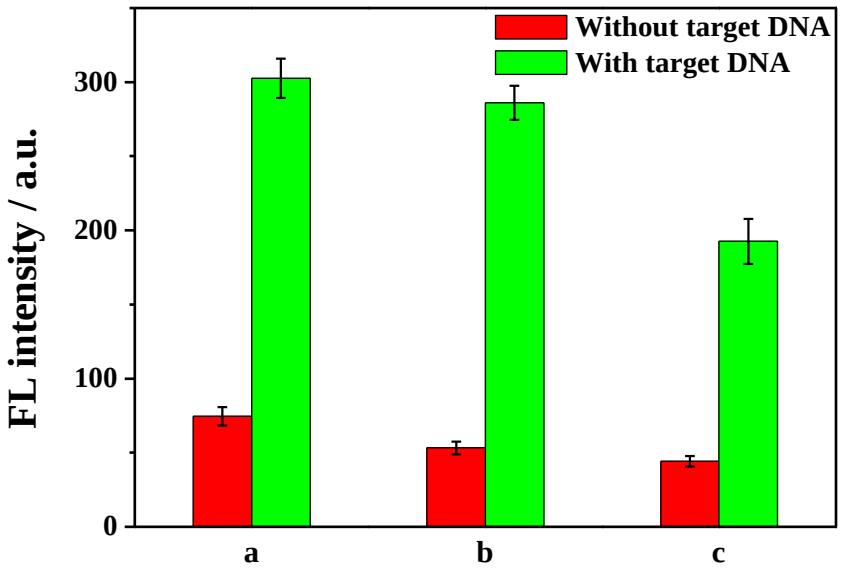

Figure S2. The fluorescence intensity of the autocatalytic system using different MB substrate that includes various lengths of the hairpin stem in the absence (red bars) or presence (green bars) of 1 nM target DNA: a, 5 bp of the stem region; b, 6 bp of the stem region; c, 7 bp of the stem region. The error bars represent the standard deviation of three measurements.

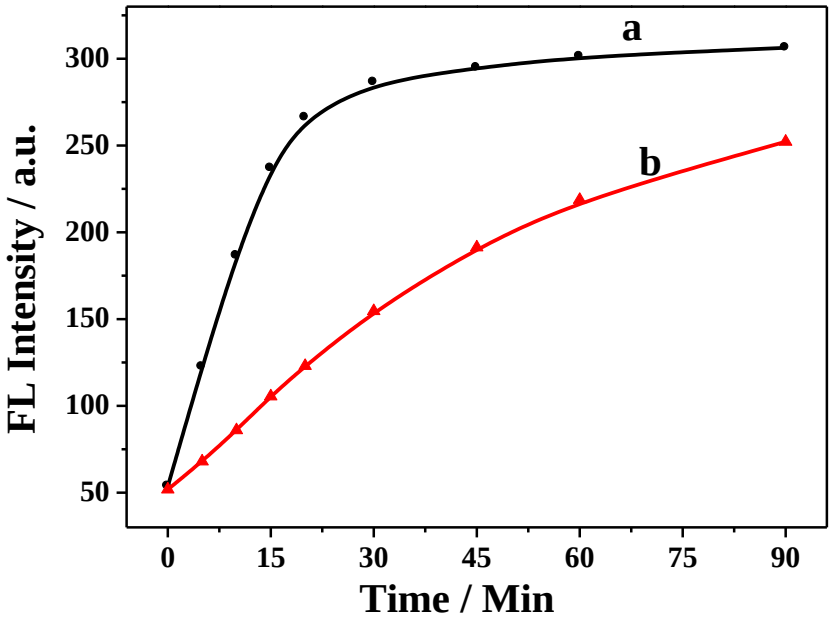

Figure S3. Time responses of fluorescence intensity for autocatalytic sensing system (curve a) and typical nicking endonuclease-mediated recycling strategy (curve b) in the presence of 1 nM target DNA.

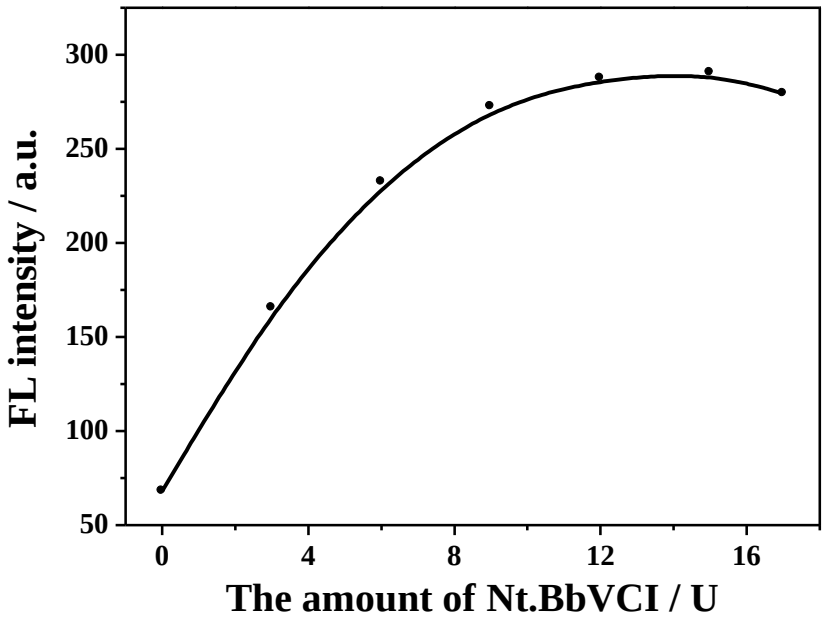

Figure S4. The effect of the nicking endonuclease concentration on the fluorescence response of the sensing system in the presence of 1 nM of target DNA.

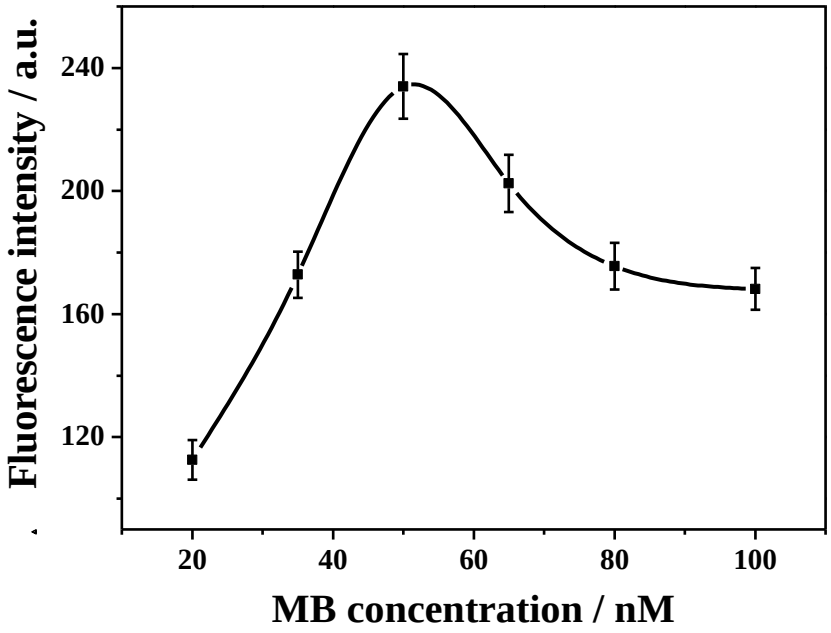

Figure S5.The effect of the MB substrate concentration on the fluorescence intensity change of the sensing system. ΔF was calculated by $F-F_0$, where F and F_0 are the fluorescence intensity of the sensing system at 518 nm in the presence and absence of 1 nM of target DNA, respectively. The error bars represent the standard deviation of three measurements.

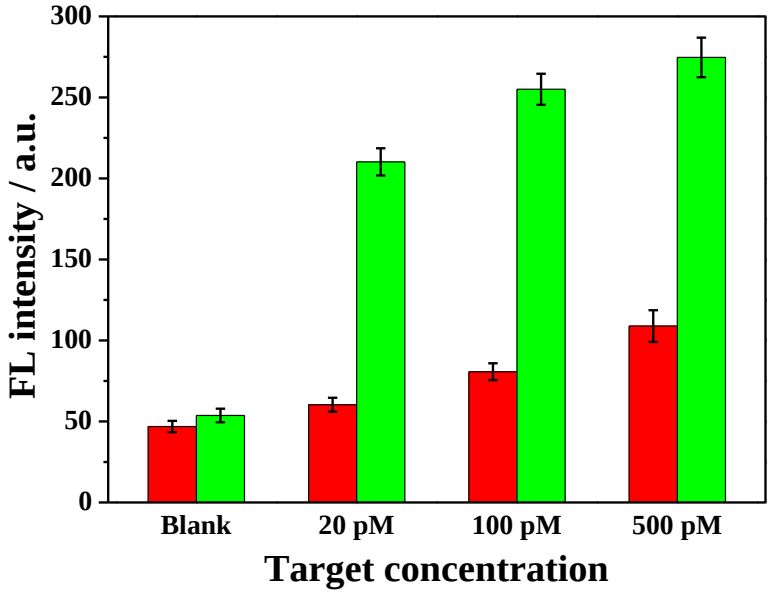

Figure S6. Fluorescence intensity corresponding to autocatalytic recycling strategy (green bars) and typical nicking endonuclease-mediated recycling strategy (red bars) for detection of target DNA. The error bars represent the standard deviation of three measurements.

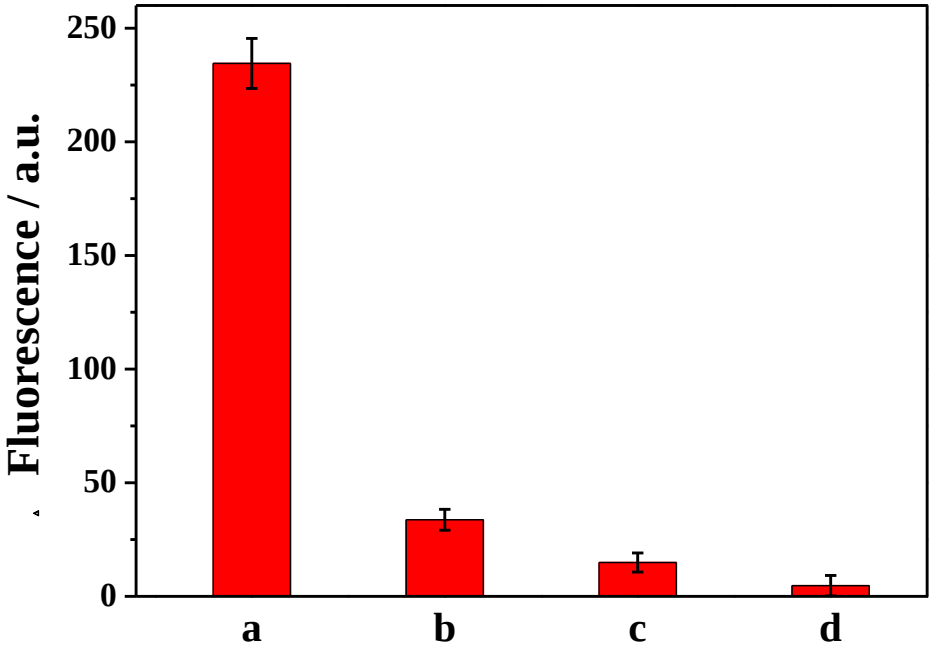

Figure S7. Fluorescence intensity change (ΔF) of the autocatalytic DNA sensor toward four different target DNA sequences with the same concentration of 1 nM: (a) complementary target DNA; (b) single-base mismatched target; (c) two-base mismatched target; (d) non-complementary target DNA. The error bars represent the standard deviation of three measurements. ΔF was calculated by $F-F_0$, where F and F_0 are the fluorescence intensity of the sensing system at 518 nm in the presence and absence of 1 nM of different target DNA, respectively

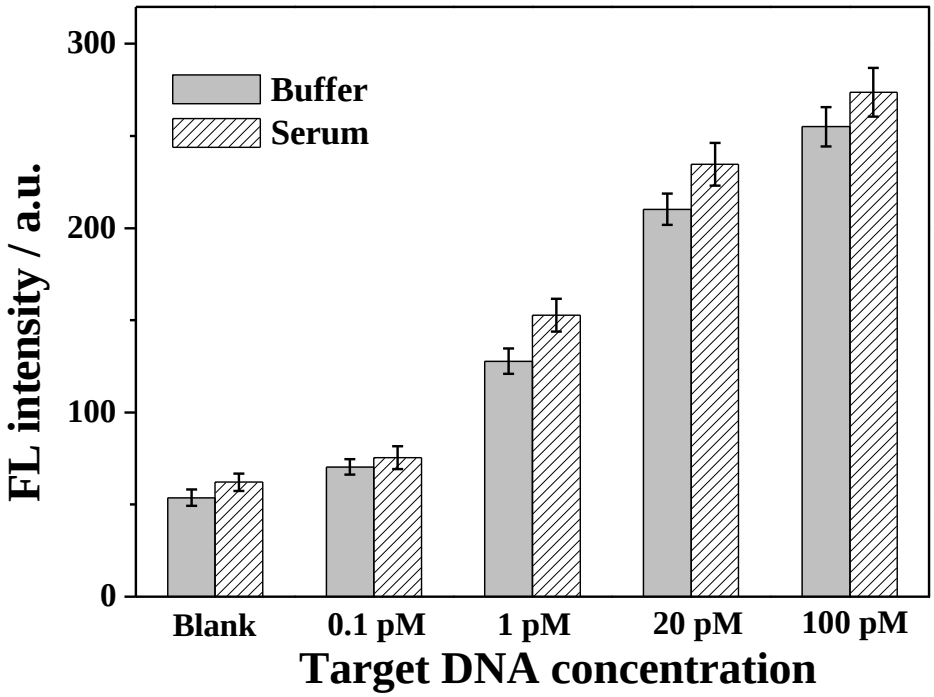

Figure S8. Fluorescence intensity obtained for the fabricated autocatalytic DNA biosensor in buffer and human serum samples spiked with different target DNA concentrations. Human serum was diluted in 1: 10 with 1 × NEB buffer 4. The error bars represent the standard deviation of three measurements.

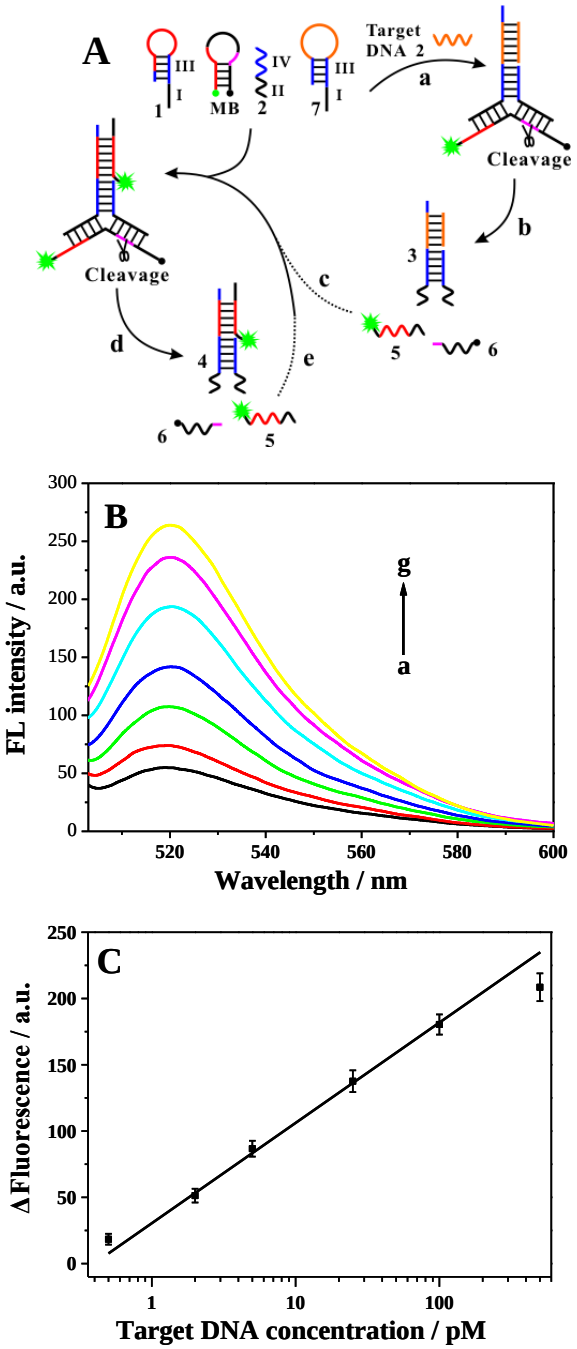

Figure S9. A) Schematic representation of the general analyte-sensing platform: the sensing module process (a–b) initiates the autocatalytic module process (c–e). B) Fluorescence spectra of the general autocatalytic nucleic acid sensing in the presence of different target DNA 2 concentrations: a) 0 pM, b) 0.5 pM, c) 2 pM, d) 5 pM, e) 25 pM, f) 100 pM, g) 500 pM. C) The relationship of the fluorescence signal change at 518 nm with the target concentration. The error bars represent the standard deviation of three measurements.