Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

An allosteric DNA switch-mediated catalytic DNA circuit for ratiometric and sensitive nucleic acid detection

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Table S1. The used nucleic acid sequences in the experiment

Name	Sequence from 5' to 3'
A	Cy5-GCCTGACGTATAAAGTGAGGTTGATGG
B	CACCTGCCTGACCCTCACTTTATACGTCAGGCT-BHQ- GTCCTTTCTCCTTGCGAATC
C	GATTCGCAAGGAGAAAGGAC-Cy3-TTAGGTG
D	CCATCGATTCGCGTCCTTTCTCCTTGCGAATCCCTCACTTTATACGTCAGGC
Target	CCATCAACCTCACTTTATACGTCAGGC
D2	CCATCGATTCGCGTCCTTTCTCCTTGCGAATCTTCTGTCCCGCGTACTGAAT
1MT-3	CCTTCAACCTCACTTTATACGTCAGGC
1MT-7	CCATCATCCTCACTTTATACGTCAGGC
1MT-13	CCATCAACCTCATTTTATACGTCAGGC
1MT-22	CCATCAACCTCACTTTATACGACAGGC
2MT	CCATCAACATCACTTTAGACGTCAGGC

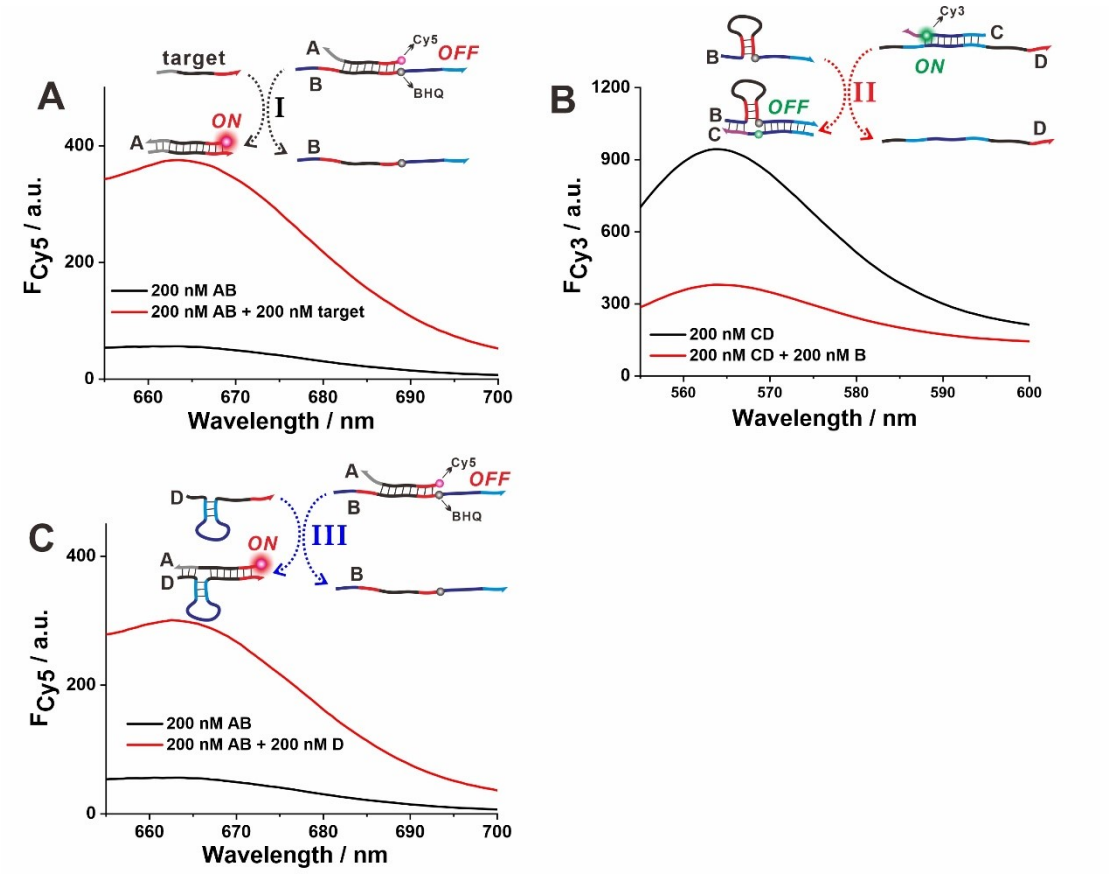

Figure S1. Fluorescence experiments investigating each separate step (I to III) of the proposed allosteric DNA switch-mediated catalytic DNA circuit strategy. (A) Fluorescence responses of Cy5 for probe AB and its mixing with target DNA. (B) Fluorescence responses of Cy3 for probe CD and its mixing with strand B. (C) Fluorescence responses of Cy5 for probe AB and its mixing with strand D. Inset in Figure S1 A to C shows the corresponding working mechanism for each step.

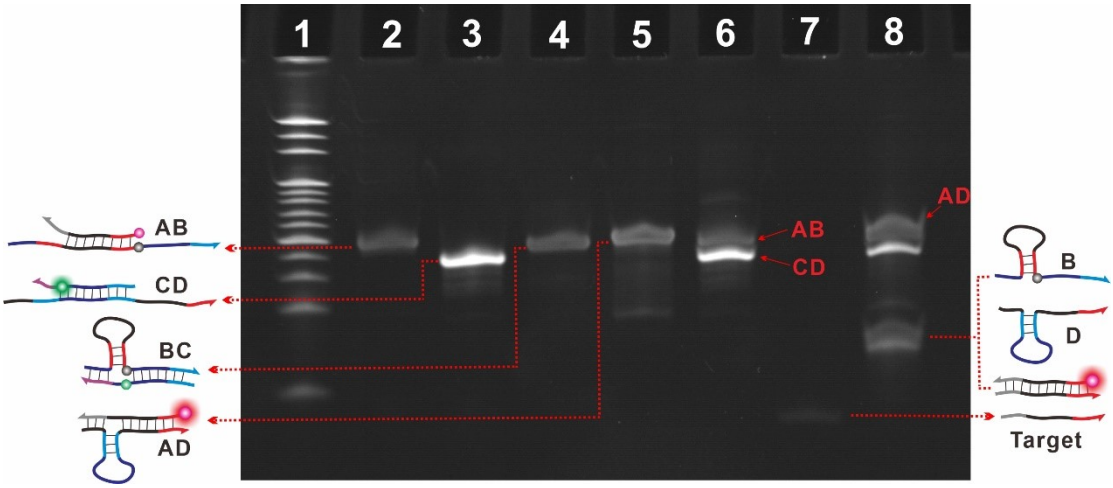

Figure S2. Gel electrophoresis characterizations for the allosteric DNA switch mediated catalytic DNA circuit reaction strategy: lane 1, 20 bp DNA ladder; lane 2, hybrid AB; lane 3, hybrid CD; lane 4, hybrid BC; lane 5, hybrid AD; lane 6, hybrids AB and CD; lane 7, target DNA; lane 8, hybrids AB and CD in the presence of target DNA. The DNA concentrations used in the lanes were 200 nM except the target DNA concentration of 10 nM in lane 8.

Table S2. Detection performance comparison between current sensing system and those reported enzyme-free fluorescence methods.

Ref.	Detection limit	Detection range	Strategy
[1]	0.7 nM	2.0 nM – 40 nM	Hybridization chain reaction and fluorescence resonance energy transfer
[2]	10 pM	10 pM – 1 nM	Bimolecular beacons-based enzyme-free strategy
[3]	10 pM	10 pM – 400 nM	Target-catalyzed dynamic assembly and pyrene excimer
[4]	220 fM	500 fM – 10 nM	Entropy-driven strand displacement and DNAzyme
[5]	7.0 pM	10 pM – 1nM	Entropy-driven target recycling and hybridization chain reaction
[6]	13 pM	0.1 nM – 50 nM	Helper probes-based strand displacement and G-quadruplex
[7]	1 pM	1 pM – 250 pM	Hybridization-triggered DNAzyme cascade
[8]	56 pM	500 pM – 20 nM	Catalyzed hairpin assembly
[9]	1.9 pM	10 pM – 1 μM	Cascade toehold-mediated strand displacement
[10]	0.58 pM	0.5 pM – 3 pM	Helper probes-assisted target recycling
[11]	5 pM	5 pM –10 nM	Entropy beacon
[12]	72 pM	0.5 nM – 50nM	Catalyzed hairpin assembly
This work	0.1 pM	0.1 pM – 100 nM	Allosteric DNA switch-medicated reciprocal strand displacement amplification

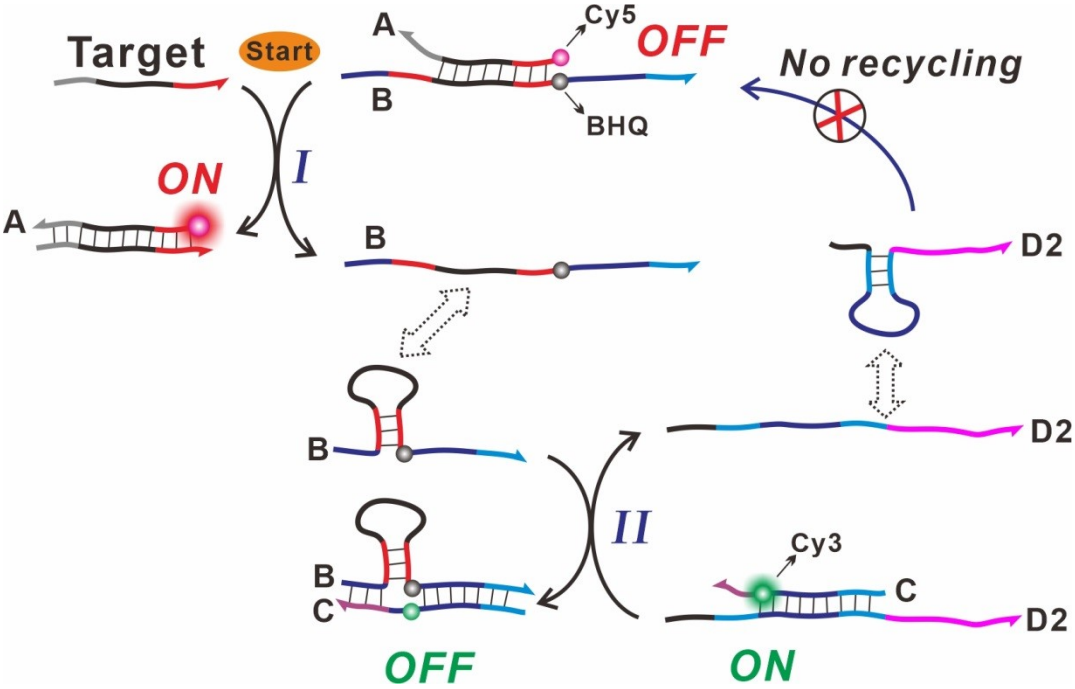

Figure S3. Schematic illustration of allosteric DNA switch-mediated strand displacement strategy. In this case, the strand D2 was used as the substitute of strand D. The released D2 from hybrid probe CD2 could not further interact with probe AB via toehold-mediated strand displacement reaction for recycling amplification. It thus could be denoted in current study as an allosteric DNA switch-mediated strand displacement strategy and used for the performance comparison with the developed allosteric DNA switch-mediated catalytic DNA circuit strategy.

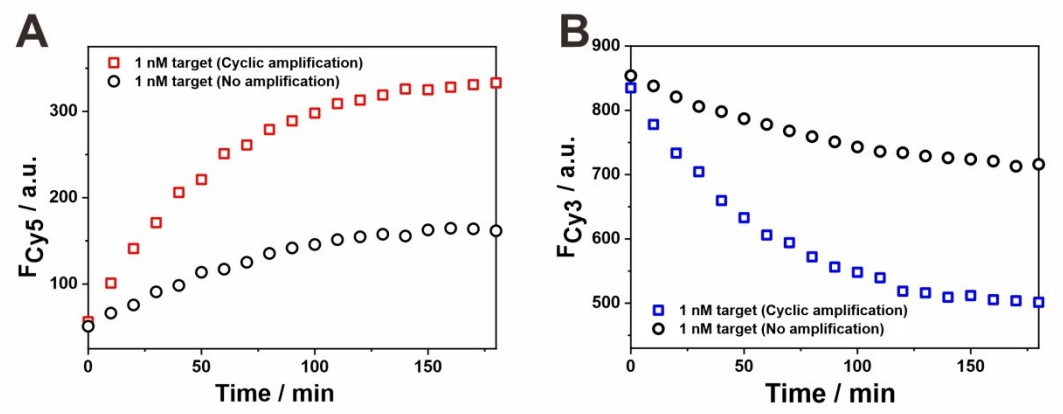

Figure S4. Time dependence of fluorescence responses of Cy5 (A) and Cy3 (B) toward 1 nM target DNA by using the allosteric DNA switch-mediated catalytic DNA circuit strategy (red or blue squares) and the allosteric DNA switch-mediated strand displacement strategy (black circle).

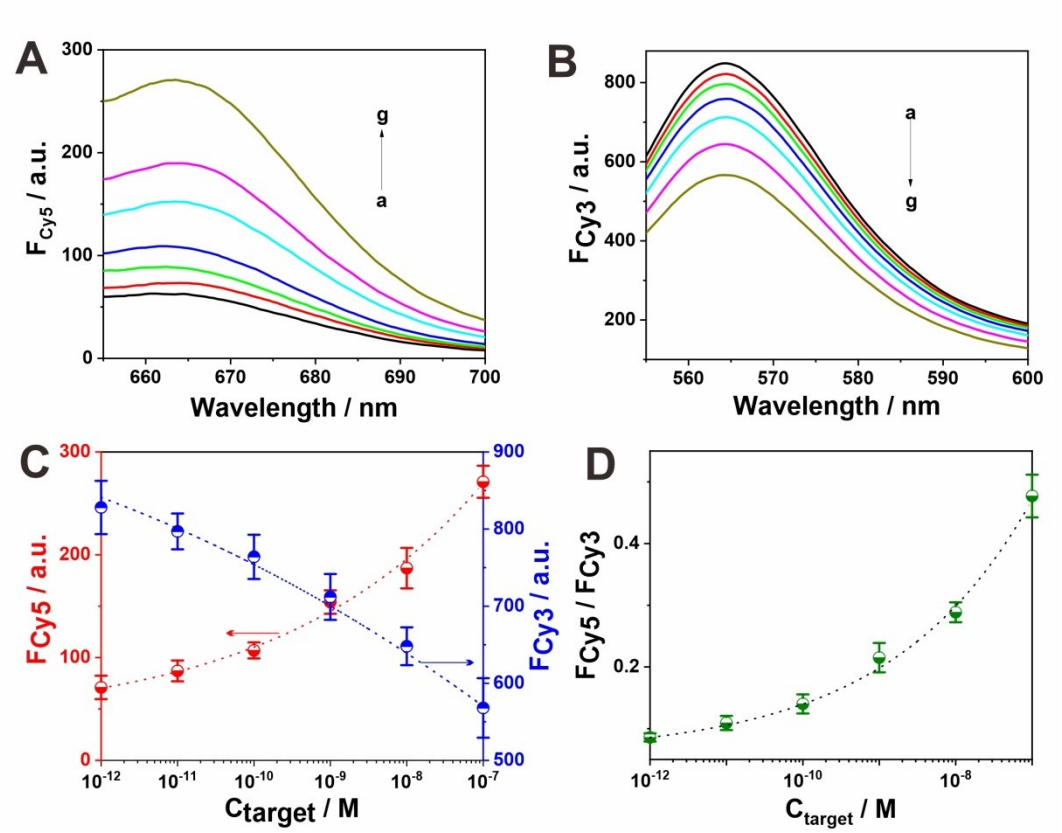

Figure S5. Fluorescence spectra of Cy5 (A) and Cy3 (B) for the sensing system fabricated by the allosteric DNA switch-mediated strand displacement strategy toward different concentrations of target DNA. The concentrations for the curves a to g were 0, 1, 10, 100 pM, 1, 10, 100 nM, respectively. (C) The calibration curves for fluorescence intensities of Cy5 and Cy3 versus target DNA concentration. (D) The relationship of the fluorescence ratios of Cy5 to Cy3 with different target DNA concentrations.

Table S3. Recovery experiments of the sensing system toward spiked target DNA in diluted serum

Samples	Added (pM)	Detected (pM)	Recovery
1	5.00×10^{1}	$(4.14 \pm 0.59)\times10^{1}$	83%
2	5.00×10^{2}	$(5.38 \pm 0.47)\times10^{2}$	107%
3	5.00×10^{3}	$(5.99 \pm 0.42)\times10^{3}$	119%

The results were based on three repetitive experiments.

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