Electronic Supplementary Information

Hairpin DNA-fueled dynamic self-assembly of three-arm DNA branched junctions consisting of active DNAzyme structures for enzyme-free ultrasensitive detection of nucleic acids

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Experimental part:

Chemicals

Oligonucleotides used in this study were synthesized by Shanghai Sangon Biotechnology Company, Ltd. (China). The sequence information of all oligonucleotides is listed in Table S1. The DNA stock solutions of 10 μM were prepared by dissolving oligonucleotides in 30 mM HEPES buffer (pH 7.8). RPMI 1640 cell medium was obtained from Shanghai Sangon Biotechnology Company, Ltd. Fetal bovine serum (FBS) was obtained from Zhejiang Tianhang Biological Technology Co., Ltd. The chemical reagents with analytical grade were obtained from Alfa Aesar, and were used without further purification. The deionized water was obtained via the Nanopure Infinity ultrapure water system.

Gel electrophoresis analysis

Different mixtures of target DNA with probes A, B and C were incubated for 3 h at 20 °C. The concentration of each oligonucleotide was 500 nM. 10 μL of each sample was added into the 15% native polyacrylamide gel. The electrophoresis experiment was performed at 70 V for 2 h in 1× TBE buffer. After that, the gel was soaked in a 1× SYBR Gold solution for 20 min. Finally, the images were obtained on the Tanon-2500R gel imaging system (Shanghai, China) by the illumination with ultraviolet (UV) light.

Real-time monitoring of the hairpin DNA-fueled self-assembly

Probes were heated to 95 °C for 5 min and then allowed to cool to room temperature for at least 2 h before use. A mixture of 200 μL of three hairpin probes, target DNA and fluorescence self-quenching substrate was added into a quartz cuvette, which was then placed in the fluorescence spectrophotometer and incubated at 20 °C. The fluorescence intensity of solution was recorded every several minutes by a F7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with an aqueous thermostat accurate to 0.1 °C. The excitation wavelength was set at 488 nm, Ex and Em slits were both set at 5.0 nm with a PMT voltage of 700 V. Fluorescence emission spectra

were then obtained with a 0.2 cm × 1 cm quartz cuvette containing 200 μL of solution. The concentration of each oligonucleotide was 500 nM. The control experiment was the same as the above procedure, except for the absence of the target DNA.

Detection of target DNA in buffer solution and the complex sample

The detection experiments were performed in 200 μL of 30 mM HEPES buffer (300 mM KCl, and 10 mM $MgCl_2$, pH 7.8) consisting of 500 nM probe A, 500 nM probe B, and 500 nM probe C at 20 °C. The target DNA was added into the detection system to catalyze the assembly reaction. Subsequently, 1 mM substrate (S) was introduced to the resulting solution and incubated for 2 h. Finally, the fluorescence measurements of resulted solutions were carried out by fluorescence spectrophotometer. To test the capability of the proposed strategy for detecting the target DNA from complex biological samples, the dulbecco's-modified eagles medium (DMEM) containing 10% fetal bovine serum was used as a model complex biological sample. This cell medium was first spiked with different concentrations of target DNA. Then, the detection process using the developed strategy was performed, and the recovery was calculated.

Table S1. Sequences of oligonucleotides used in the study

Probe	DNA sequence (5'-3')
probe A	GATAT CAGCG ATCTT ATTGG AAGTC AGTGT GGAAA ATCTC TAGCA TCCAA TCACA ACTGC TAGAG ATTTT CCACA AAGCA CCCAT GTTAC TCT
probe B	GATAT CAGCG ATCTT TGTGG AAAAT CTCTA GCAGT TGTGA TTGGA TCCAC ACTGA CTTCC AATCA CAACT GCTAG AAGCA CCCAT GTTAC TCT
probe C	GATAT CAGCG ATCTT CTAGC AGTTG TGATT GGAAG TCAGT GTGGA TGCTA GAGAT TTTCC ACACT GACTT CCAAT AAGCA CCCAT GTTAC TCT
target DNA	GCTAG AGATT TTCCA CACTG ACT
1-mismatched DNA	GCTAG AGATT GTCCA CACTG ACT
2-mismatched DNA	GCTAG AGATT GTCCT CACTG ACT
deleted DNA	GCTAG AGATT TCCAC ACTG A CT
inserted DNA	GCTAG AGATT TATCC ACACT GACT
random DNA	AAAAA GGAAA GGGGG GACTC ACTAT A
substrate	BHQ1-AGAGT ATrAGG ATATC-FAM
probe D	GATAT CAGCG ATCTT AAAAT CTCTA GC
Probe E	AGTCA GTGTG GAAGC ACCCA TGTTA CTCT

Table S2. Recovery tests for target DNA in the cell culture samples

sample	**added (nM)**	**detected (nM)**	**recovery (%)**
1	1	$1.12^{a} \pm 0.23^{b}$	112.04
2	10	$9.45^{a} \pm 0.57^{b}$	94.50
3	50	$49.19^{a} \pm 4.27^{b}$	98.38
4	100	$103.27^{a} \pm 7.60^{b}$	103.27
5	200	$213.42^{a} \pm 10.72^{b}$	106.71

[a] The average value of three measurements. [b] Standard deviation.

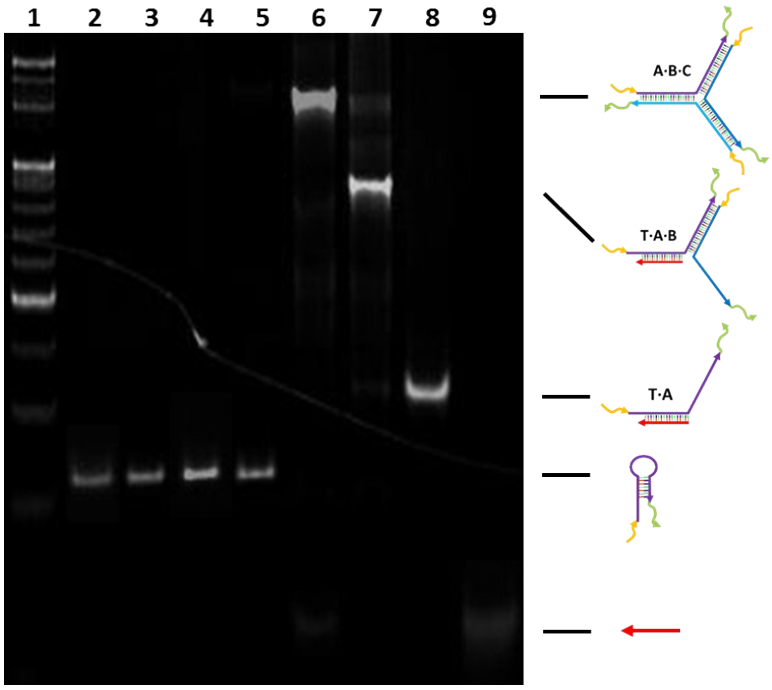

Fig. S1 Gel electrophoresis demonstrating the hairpin DNA-fueled self-assembly of three-arm branched nucleic acid depicted in Scheme 1.

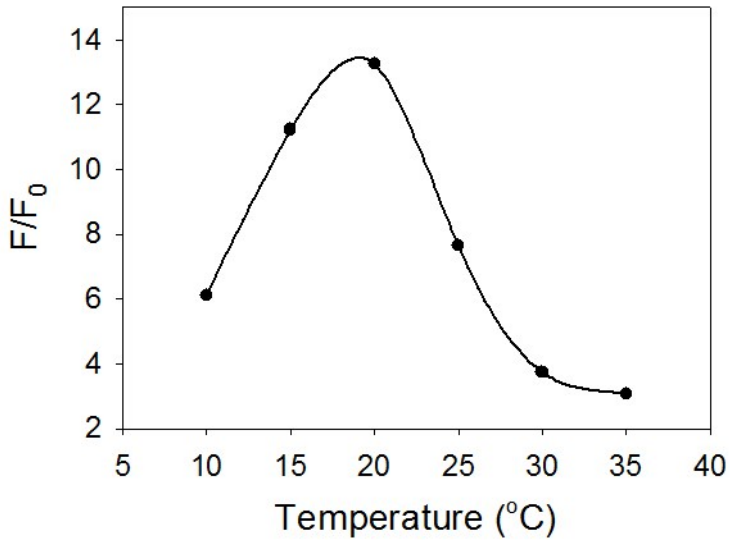

Fig. S2 The relationship between the fluorescence intensity ratio (F/F_0) and temperature. F is the fluorescence intensity of FAM in the presence of target DNA, and F_0 is the fluorescence intensity of FAM in the absence of target DNA. λ_{ex} = 488 nm, λ_{em} = 526 nm.

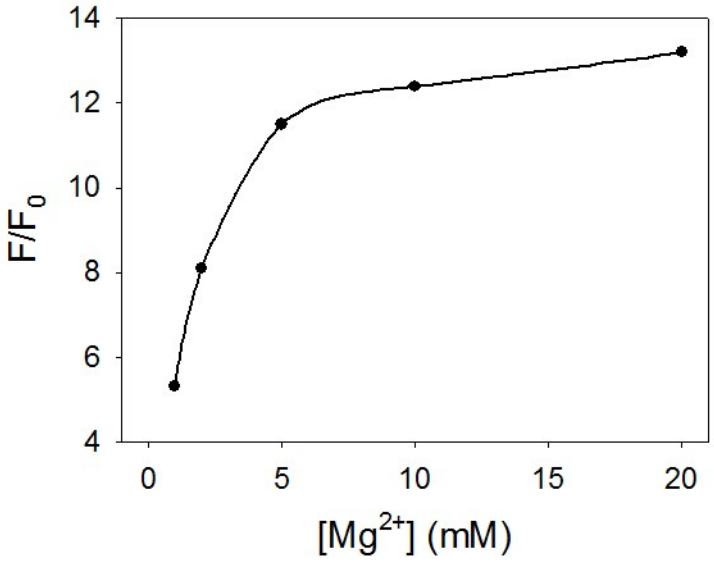

Fig. S3 The relationship between the fluorescence intensity ratio (F/F_0) and the concentration of Mg^{2+} ions. F is the fluorescence intensity of FAM in the presence of target DNA, and F_0 is the fluorescence intensity of FAM in the absence of target DNA. λ_{ex} = 488 nm, λ_{em} = 526 nm.

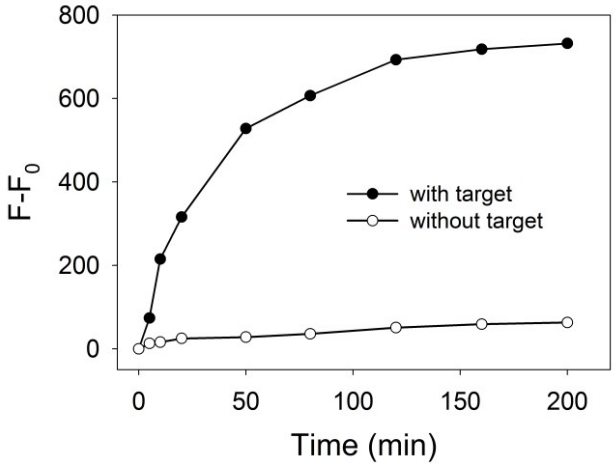

Fig. S4 Real-time monitoring of the fluorescence intensity in the presence and absence of target DNA (λ_{ex} = 488 nm, λ_{em} = 526 nm). F is the fluorescence intensity at the corresponding time point, and F_0 is the fluorescence intensity at the start of the assembly reaction.