Electronic Supplementary Information (ESI)

A hybridization-triggered DNAzyme cascade assay for enzyme-free amplified fluorescence detection of nucleic acid

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Experimental Section

1. Reagents and Materials.

Oligonucleotides used in this study (Table S1) were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China), and purified by reverse-phase high performance liquid chromatography (HPLC). The concentrations of all work solutions, including Tris, PBS and HEPES were 50 mM (100 mM NaCl, pH 7.4). The pH measurements were carried out using an Orion 3 Star pH meter (Thermo Fisher, USA). DEPC-treated ultrapure water (18 MΩ.cm, Milli-Q, Millipore) was used to prepare all aqueous solutions. RPMI 1640 cell medium was purchased from Clontech (Mountain View, CA, USA). Fetal bovine serum was purchased from Hyclone (Logan, UT, USA). All other chemical reagents, in analytical grade, were purchased from Shanghai Chemical Reagents (Shanghai) and used without further purification. All the tips and tubes were purchased from Shanghai Sangon Biotechnology Co (Shanghai, China) and used after sterilizing.

Table S1. Oligonucleotides used in this study. [a]

Oligonucleotides	Sequence (5'-3')

8-17 DNAzyme	ATCATCTTCTCCGAGCCGGTCGAAATACCAGTTGAT
Mutant DNAzyme	T
	ATCATCTTCTCCG**G**GCCGGTCGAAATACCAGTTGAT
	T
Substrate strand	CAGTTACATTCT(FAM)CTATrAGGAAGATGAT-BHQ-1
Perfectly match DNA	AATCAACTGGGAGAATGTAACTG
One mismatch DNA	AATCAACTGG**T**AGAATGTAACTG
Two mismatch DNA	AAT**G**AA**G**TGGGAGAATGTAACTG
Three mismatch DNA	AAT**G**AACTGGGA**C**AAT**C**TAACTG
Random DNA	CCTTTTCAAAACTTACTATATTC
Mis SD-joint 1	ATCAT**T**TTCTCCGAGCCGGTCGAAATACCAGTTGAT
Mis SD-joint 2	T
Mis D-arm 1	AT**T**ATCTTCTCCGAGCCGGTCGAAATACCAGTTGAT
Mis D-arm 2	T
Mis T1	ATCATCTTCTCCGAGCCGGTCGAAATA**T**CAGTTGAT
Mis T2	T
Target DNA 1	ATCATCTTCTCCGAGCCGGTCGAAATACCA**T**TTGAT
DNAzyme 1	T
Target DNA 2	AATCAACTG**T**GAGAATGTAACTG
DNAzyme 2	AATCAA**T**TGGGAGAATGTAACTG
Target DNA 3	TCGTCTTGCAGAGAATGTAACTG
DNAzyme 3	ATCATCTTCTCCGAGCCGGTCGAAATATGCAAGACG
	A
	TTATGTACGCGAGAATGTAACTG
	ATCATCTTCTCCGAGCGGTCGAAATAGCGTACATAA
	ATAGTTGACGGAGAATGTAACTG
	ATCATCTTCTCCGAGCCGGTCGAAATACGTCAACTA
	T

[a] The underlined letters in **bold** represent the mismatch bases, rA denotes adenine

ribonucleotide.

2. Fluorescence measurement

All fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan) equipped with an aqueous thermostat (Amersham). The excitation wavelength was set at 488 nm (slit 5 nm), and the emission spectra was collected from 505 nm to 650 nm (slit 5 nm). Both excitation and emission spectra were set at the PMT voltage of 700 V. The fluorescence intensities at 520 nm were collected to study the performances of the proposed detection assay.

3. Procedure for conditions optimizing

3.1. Effect of buffer

100 nM DNAzyme and 100 nM substrate strands were incubated in three different buffers including PBS, Tris-HCl, HEPES (50 mM buffer, 100 mM NaCl, 500 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. Then 50 nM target DNA was added into the above solution and then incubated for another 4 h at 37 °C. Finally, the fluorescence emission intensities at 520 nm were collected.

3.2. The concentration ratio of substrate to DNAzyme

Different concentrations of substrate strands were incubated with 100 nM 8-17 DNAzyme in buffer (50 mM Tris-HCl, 100 mM NaCl, 500 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. Then 50 nM target DNA was added into the above solution and then incubated for another 4 h at 37 °C. Finally, the fluorescence emission intensities at 520 nm were collected.

3.3. Zn^{2+} concentration

200 nM substrate strands and 100 nM 8-17 DNAzyme were incubated with different concentrations of Zn^{2+} in buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.4) for 30 min at 37 °C to make full reaction. Then 50 nM target DNA was added into the above solution and then incubated for another 4 h at 37 °C. Finally, the fluorescence emission intensities at 520 nm were collected.

4. Gel Electrophoresis Experiments

Polyacrylamide gel (15%) was employed to verify the assembly of the S-D complex in the absence and presence of target DNA. A mixture solution containing 1 μM DNAzyme, 2 μM substrate were incubated in 50mM Tris-HCl for 30 min at 37 °C to make full reaction. Aliquots of solution were incubated with 1 μM target DNA at 37 °C for 4h. 15% denaturing polyacrylamide gel was also employed to verify the cleavage reaction in the absence and presence of target DNA.

5. Procedure for target DNA detection

200 nM substrate strands and 100 nM 8-17 DNAzyme were incubated in buffer (50 mM Tris-HCl, 100 mM NaCl, 300 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. Then different concentrations of targets DNA were added into the above solution and incubated for another 4 h at 37 °C. Finally, the fluorescence emission intensities at 520 nm were collected.

6. Kinetic studies of HTDC assay

6.1 Procedure for reaction time optimization

To obtain an optimal reaction time, 200 nM substrate strands and 100 nM 8-17 DNAzyme were incubated in buffer (50 mM Tris-HCl, 100 mM NaCl, 300 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. The mixture was then transferred to a cuvette. After that, 10 nM target DNA was added into above solution and then real-time monitoring of target DNA was immediately started at 37 °C, which was recorded for 6000 s with time intervals of 2 s at $\lambda_{ex}/\lambda_{em}$ = 488/520. As a control, the other solution without target DNA was also investigated according to the above procedure.

6.2 Detection procedure for target DNA

For the working range study, 200 nM substrate strands and 100 nM 8-17 DNAzyme were incubated in buffer (50 mM Tris-HCl, 100 mM NaCl, 300 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. The mixture was then transferred to a cuvette. After that, different concentrations of target DNA were added into the above solution and real-time monitoring of target DNA were immediately started at 37 °C, which were recorded for 6000 s with time intervals of 5 s at $\lambda_{ex}/\lambda_{em}$ = 488/520.

7. Study for selectivity

200 nM substrate strands and 100 nM 8-17 DNAzyme were incubated in buffer (50 mM Tris-HCl, 100 mM NaCl, 300 μM Zn^{2+}, pH 7.4) for 30 min at 37 °C to make full reaction. Then perfectly match DNA(100 nM) and its analogue ((100 nM)) (one mismatch DNA, two mismatch DNA, three mismatch DNA and random DNA) were added into the above solution and then incubated for another 4 h at 37 °C. Finally, the fluorescence emission intensities at 520 nm were collected.

8. Detection of target DNA in complex samples

RPMI 1640 cell medium supplemented with 15% fetal bovine serum was generally considered as a model for preparation of complex biological system. With the purpose of evaluating the generality of the proposed assay, different amounts of target DNA were spiked into the complex fluids. According to the detection procedures above-mentioned, the fluorescence recoveries were finally calculated.

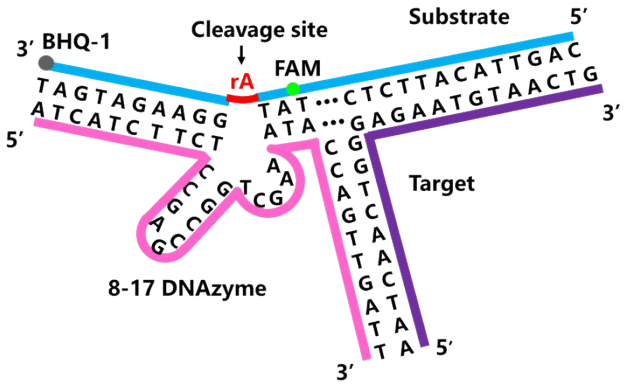

Fig S1. DNA sequences of the active DNAzymes in the presence of target DNA.

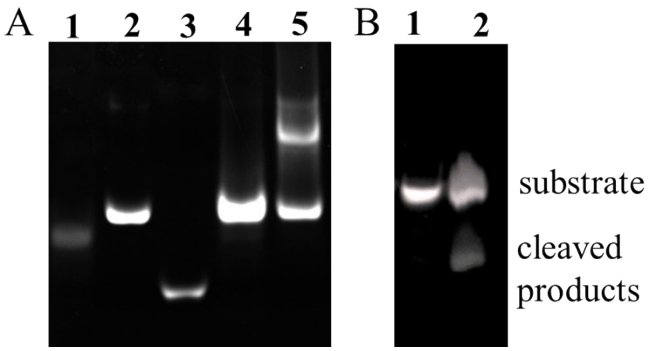

Fig S2. Gel electrophoresis of the occurrence of HTDC. (A) The assembly of substrate, DNAzyme and target DNA were validated by 15% polyacrylamide gel. Lane 1: substrate (2 μM); Lane 2: DNAzyme (1 μM); Lane 3: target DNA (1 μM); Lane 4: substrate + DNAzyme; Lane 5: substrate + DNAzyme +target DNA. (B) The cleavage of the substrate was validated by 15% denaturing polyacrylamide gel in the presence of the target DNA (1 μM) (lane 2) and absence of target DNA with only cofactor Zn^{2+} (3 mM) (lane 1).

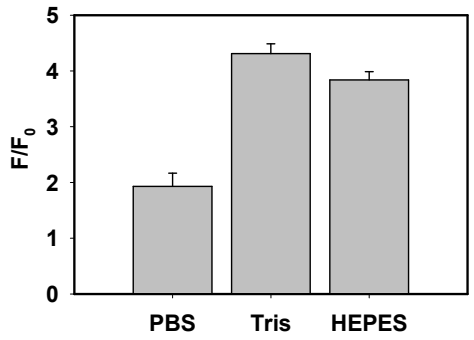

Fig S3. The effect of buffers on nucleic acid detection. Error bars represent the standard deviations from three independent experiments. The concentrations of substrate, DNAzyme and target were fixed at 100 nM, 100 nM and 50 nM, respectively.

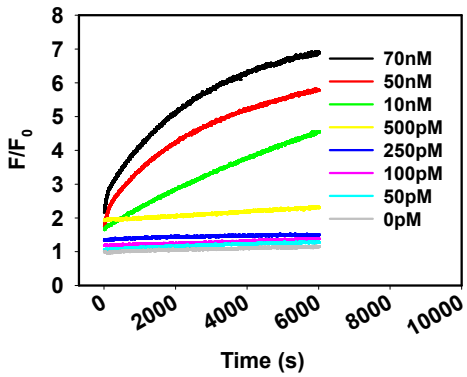

Fig S4. Fluorescence increases of the hybridization-triggered DNAzyme cascade assay over time with different targets concentrations (from bottom to top: 0 pM, 50 pM, 100 pM, 250 pM, 500 pM, 5 nM, 10 nM, 50 nM, 70 nM). The concentrations of substrate and DNAzyme were fixed at 200 nM, and 100 nM, respectively.

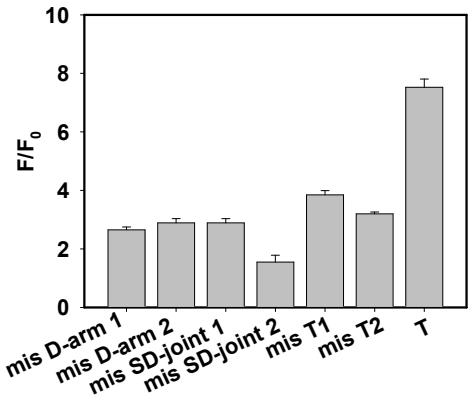

Fig S5. One base mismatch control experiment. Six main mismatch positions: two were on the different positions of the DNAzyme arm binding with target DNA, namely mismatch DNAzyme-arm 1 (mis D-arm 1) (100 nM) and mismatch DNAzyme-arm 2 (mis D-arm 2) (100 nM); two were on the different joints of the substrate and DNAzyme, namely mismatch joint of S-D complex 1 (mis SD-joint 1) (100 nM) and mismatch joint of S-D complex 2 (mis SD-joint 2) (100 nM); two were on the different positions of target, namely mismatch target 1 (mis T1) (100 nM) and mismatch target 2 (mis T2) (100 nM), which have the same mismatch position corresponding to the mis D-arm 1 and mis D-arm 2. The concentrations of substrate and DNAzyme were fixed at 200 nM and 100 nM, respectively.

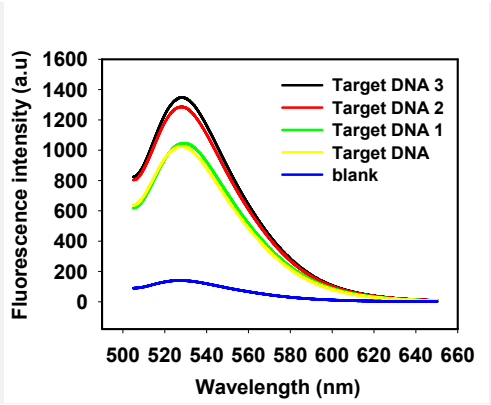

Fig S6. The versatile verification of the HTDC assay. Three different targets, including target DNA 1 (100 nM), target DNA 2 (100 nM) and target DNA 3 (100 nM), were chosen. The concentrations of substrate and DNAzyme target were fixed at 200 nM and 100 nM, respectively.