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Dynamic monitoring of an enzymatically driven dissipative toehold-mediated strand displacement reaction

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Materials and reagents

All oligonucleotides were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China) and employed without further purification. The details of DNA sequences were shown in **Table S1**. Lambda Exonuclease was obtained from New England Biolabs (Ipswich, MA, USA). The regents used for gel analysis: Acrylamide/bis-acrylamide gel stock solution, ethidium bromide (EB), N, N, N, N-tetramethylethylenediamine (TEMED), Ammonium persulfate, 20 bp DNA ladder, 6×DNA loading buffer and DEPC-treated deionized water were all obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). Tris-HCl buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM $MgCl_2$, pH 8.3) was used for strand displacement reactions. The deionized water with a resistance of 18 MΩ cm was used throughout the experiment.

Construction and fluorescence analysis of two dissipative toehold-mediated strand displacement systems

All oligonucleotides were suspended to a final concentration of 10 μM in 1×TE Buffer (pH=8.0, 10 mM Tris-HCl, 1 mM EDTA). They were heated to 90 °C for 5 min and then stored directly at 4°C.

System 1: Enzymatically-driven dissipative strand displacement reaction

Specific to this system, a DNA fuel strand is used here as the fuel and the Lambda exonuclease as the fuel-consuming unit. To form the toehold containing duplex, strands S1 and S1-complement (S2) were annealed in Tris-HCl buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM $MgCl_2$, pH 8.3) at a final concentration of 50 nM. The DNA samples were annealed from 90 °C to room temperature slowly to achieve a fully hybridization. Then, 0.3 μL Lambda exonuclease (5 U/μL) and 1 μL fuel chain S3 (10 μM) were added into the above solution. The change of fluorescence intensity was recorded continuously with time. For the cycled operation of dissipative strand displacement reaction, 1 μL of fuel chain S3 (10 μM) was further added into the reaction system as soon as the fluorescence response drops close to the starting value.

System 2：Self-amplification system

For the self-amplification system, strands of incumbent, target, and output were mixed in 1:1:1 ratio in Tris-HCl buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM $MgCl_2$, pH 8.3) at a final concentration of 250 nM (sequences in Supplementary Table S1). The mixture was heated to 90°C in a water bath for 10 minutes and then cooled

slowly to room temperature. The fluorescence intensity was recorded for 10 min after the addition of 3 μL (10 μM) capture chain.

After the measurement, 0.3 μL Lambda exonuclease (5 U/μL) and 1 μL fuel chain (10 μM) were added into the reaction solution to continually track the change of fluorescence response. While the fluorescence response basically reached a plateau, 1 μL of fuel chain (10 μM) was further added in the reaction solution. The above operations were repeated to complete multiple continuous fluorescence detections.

Polyacrylamide gel electrophoresis characterization

The 12% polyacrylamide gel electrophoresis analysis was performed on a Bio-rad gel electrophoresis analyzer in 1 × TAE buffer (40 mM Tris, 20 mM CH_3COOH, 2 mM EDTA and 12.5 mM $MgCl_2$) at 180 V for 3 min and then at 135 V for 60 min. After that, gels were stained in deionized water containing ethidium bromide (EB) and imaged by a FR-980A analysis system (Shanghai Furi Science & Technology Co., Ltd., Shanghai, China).

Table S1 DNA sequences used in the experiment

Name	Sequences (5'-3')
S1(DNA output)	TAGTATCAACAACAGTAACAT/FAM
S1(RNA output)	UAGUAUCAAGAACAGUAACAU/FAM
S2	BHQ_2/ATGTTACTGTTGTTGATACTACTTTTAG
S3-3	PO_4/AAGTAGTATCAACAACAGTAACAT
S3-4	PO_4/AAAGTAGTATCAACAACAGTAACAT
S3-5	PO_4/AAAAGTAGTATCAACAACAGTAACAT
S3-6	PO_4/TAAAAGTAGTATCAACAACAGTAACAT
S3-7	PO_4/CTAAAAGTAGTATCAACAACAGTAACAT
Output	FAM/GTGGGAGTTGGAGTAGAGTG
Incumbent	GTGGAATAGATCCTGATAGCGAGAC
Target	TTGCTAGGTCTCGCTATCAGGATCTATTCCACCACTCTACTC CAACTCCCAC/BHQ_2
Capture	GTGGGAGTTGGAGTAGAGTGGTGGAATAGATCCTGATAG CGAGAC
Fuel	PO_4/ATAGATCCTGATAGCGAGACCTAGCAA

The strands of output, incumbent, target, capture and fuel were used for the operation of a self-amplification DNA reaction network. The number after S3 indicated the hybridized base numbers for toehold recognition.

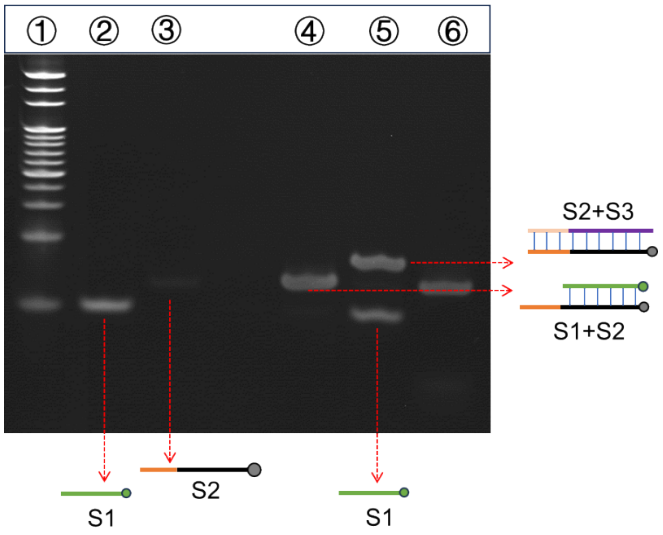

Fig. S1 Gel electrophoresis images. The corresponding DNA sequences were DNA marker (lane 1), S1 (lane 2), S2 (lane 3), the mixture of S1 and S2 (lane 4), the mixture of S1, S2 and S3 (lane 5), and the mixture of S1, S2, S3 and Lambda exonuclease (lane 6). The DNA marker consists of 10 fragments between 20 and 200 bp in multiples of 20 bp and additional fragments at 300, 400, and 500 bp.

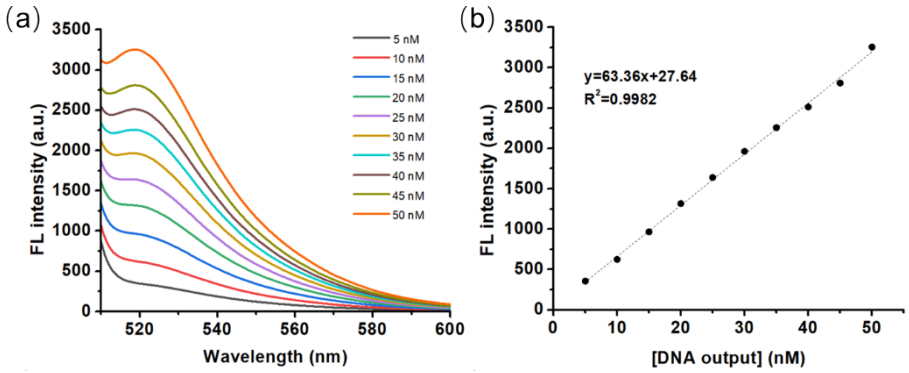

Fig.S2 (a) Fluorescence spectrum at different DNA output concentrations. (b) Calibration relationship for fluorescence intensity with DNA output concentration.

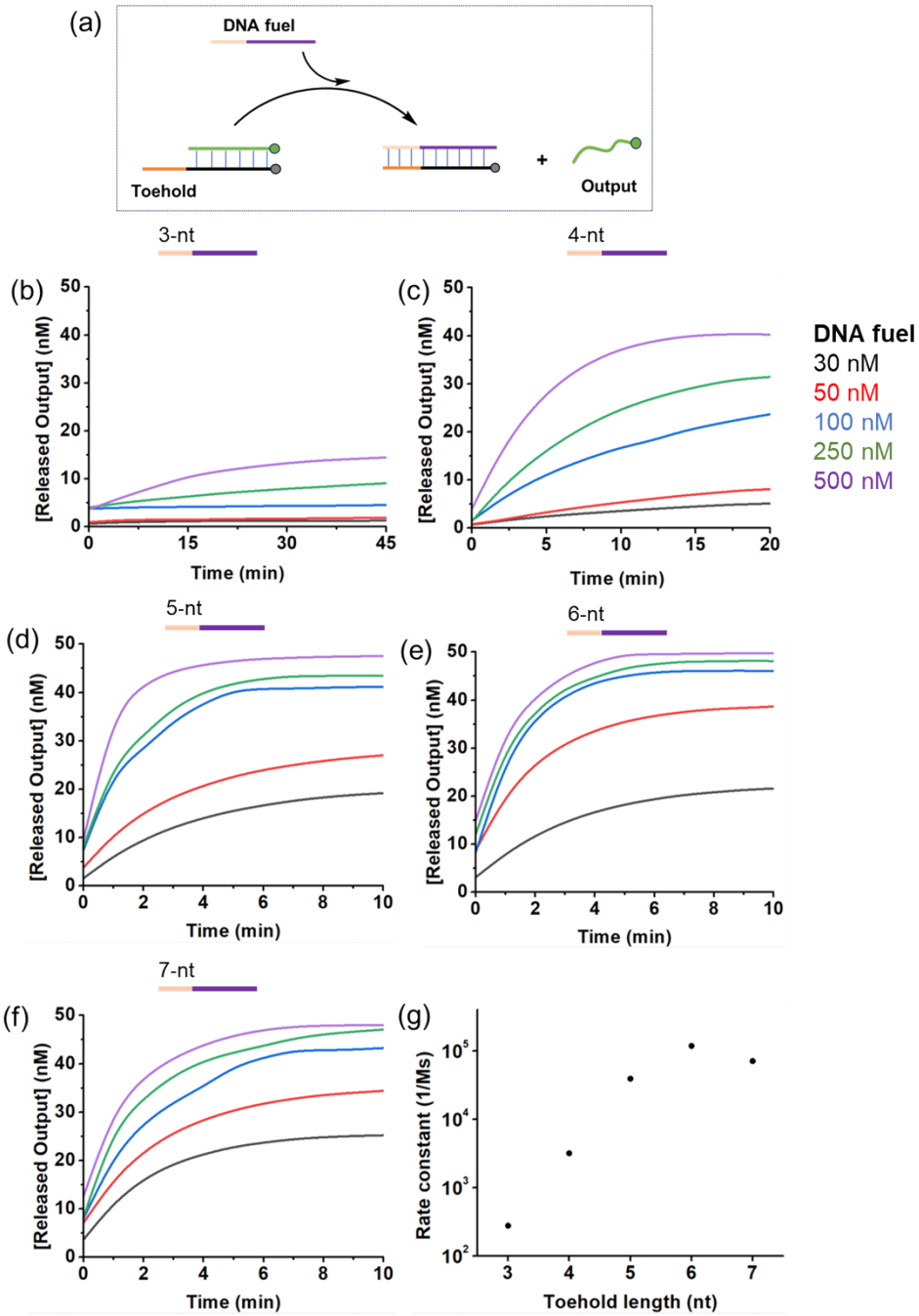

Fig. S3 (a) Scheme of conventional toehold-mediated strand displacement reaction. (b-f) Time trajectory of the displacement induced by a DNA fuel strand was measured for a series of fuel strands differing in the length of the toehold part from 3 to 7 nts. The fluorescence signal was reported as [Released Output] (nM), which was converted by the calibration curve of fluorescence intensity versus output strand concentration (Fig.S2). (g) Obtained second order rate constants for the strand displacement with a DNA invader depending on the toehold length.

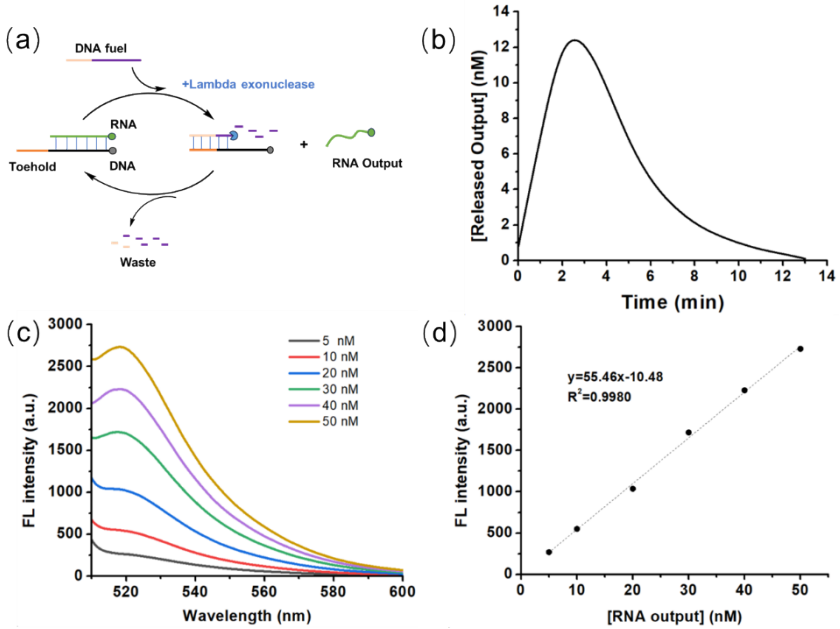

Fig.S4 (a) Scheme for dissipative strand displacement reaction of DNA/RNA hybrid. (b) Time trajectory of the displacement response of the dissipative system with RNA as the output strand. The fluorescence signal is reported as [Released Output], indicating the transiently released RNA strand. The concentrations of RNA/DNA complex, fuel strand and Lambda exonuclease in the system were 50 nM, 100 nM and 15 U/mL, respectively. (c) Fluorescence spectrum at different RNA output concentration. (d) Calibration relationship for fluorescence intensity with RNA output concentration.

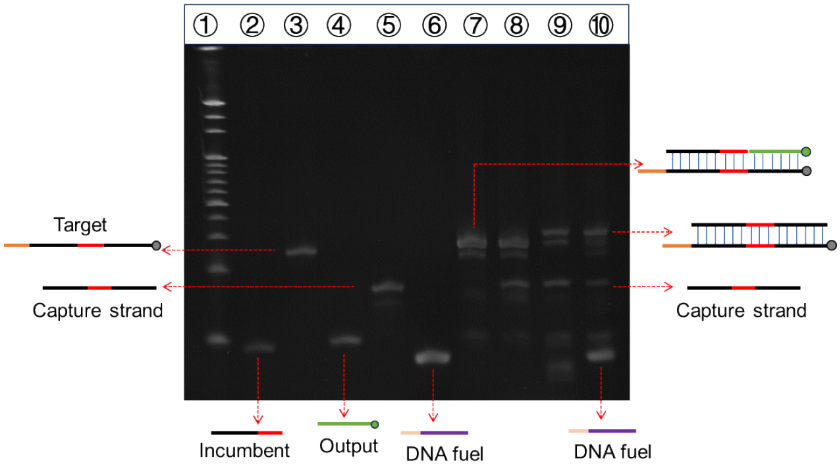

Fig. S5 Gel electrophoresis images. Lane 1, DNA Marker (20 bp); Lane 2, incumbent; Lane 3, target; Lane 4, output; Lane 5, capture strand; Lane 6, DNA fuel; Lane 7, the mixture of incumbent, target and output; Lane 8, addition of capture into the mixture of Incumbent, Target and Output; Lane 9, addition of capture, DNA fuel and Lambda exonuclease into the mixture of incumbent, target and output; Lane 10, addition of capture and DNA fuel into the mixture of incumbent, target and output. The DNA marker consists of 10 fragments between 20 and 200 bp in multiples of 20 bp and additional fragments at 300, 400, and 500 bp.

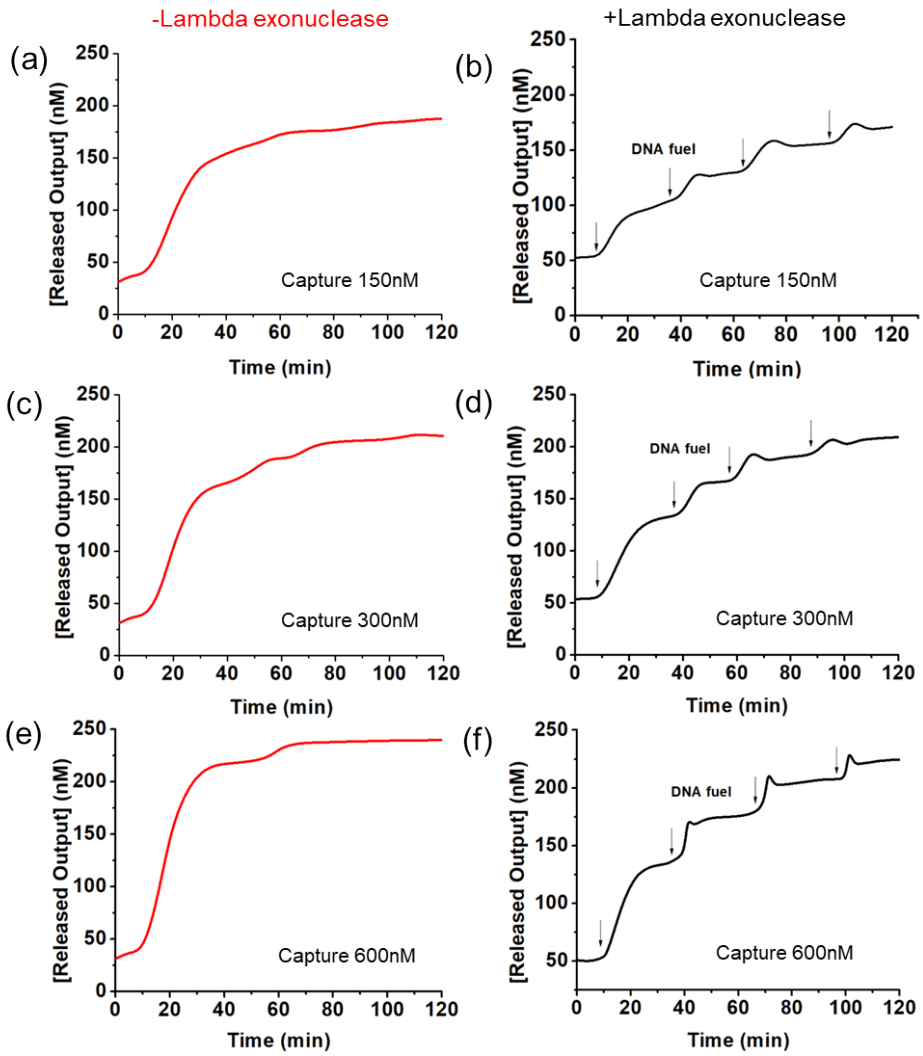

Fig. S6 Time trajectory of the output strand release upon addition of different amounts of capture strands (150 nM, 300 nM and 600 nM) in the absence or presence of Lambda exonuclease. The amplification reaction solution contains 250 nM target, incumbent and output strand, respectively.