Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information:

Designing logic gates based on 3-Way DNAzyme complex

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S1 Materials

All DNA strands were purchased from Sangon Biotech Co., Ltd (Shanghai, China). DNA strands without RNA modification were purified by polyacrylamide gel electrophoresis (PAGE). DNA strands with RNA modification were purified by high-performance liquid chromatography (HPLC). The base sequences of all DNA strands are shown in S1. The simulation results with NUPACK are shown in S2. DNA strands were diluted in 1×TAE as a stock solution and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA), and absorption intensities were recorded at 260 nm. Other chemicals are analytical grade and were not used for further purification.

S2 Methods

S2.1 Native polyacrylamide gel electrophoresis

The native PAGE was performed on the Mini-PROTEAN Tetra System (Bio-Rad Laboratories, USA) with 8% native polyacrylamide gel in 1× TAE/Mg2+ buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA2Na, and 12.5 mM Mg(OAc)2, pH 8.0) at 70 V for 2 h at 4℃. The gel was then imaged in 1 × Stains All nucleic acid gel stain solution for about 30 min.

S2.2 DNA Logic gate preparation

All DNA logic gates were formed through two processes, in 1× TAE/Mg2+ buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA2Na, and 12.5 mM Mg(OAc)2, pH 8.0). First, The mixture was annealed at the reaction conditions of 95°C for 4 min, 65°C for 30 min, 50°C for 30 min, 37°C for 30 min, 22°C for 30 min, and finally preserved at 4°C In the second step, the substrates were added into the annealed

mixture and incubated at a constant temperature of 25°C overnight. There was no purification in the experiment. All the chemicals and materials used can be found in the Extended Supplemental Information.

S2.3 Fluorescent signal detection

Measurement of fluorescence was done using a real-time PCR machine (Agilent, G8830A) equipped with an 8 × 12-well fluorescence plate reader. Fluorescent reactions were performed in 1×TAE/Mg2 + buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA2Na, and 12.5 mM Mg(OAc)2, pH 8.0) and in a typical 20-ul reaction volume at 25°C. The fluorescence intensity was measured twice at the initial environmental values followed by measurements every three minutes. Relevant test results were counted and normalized. In each reaction system, the fluorescence intensity of each scanning point was subtracted from the fluorescence intensity of the initial environment to obtain the change in fluorescence intensity. The final measurement result was calculated by dividing the observed change in fluorescence intensity by the maximum increase in fluorescence.

S3 Inhibit gate

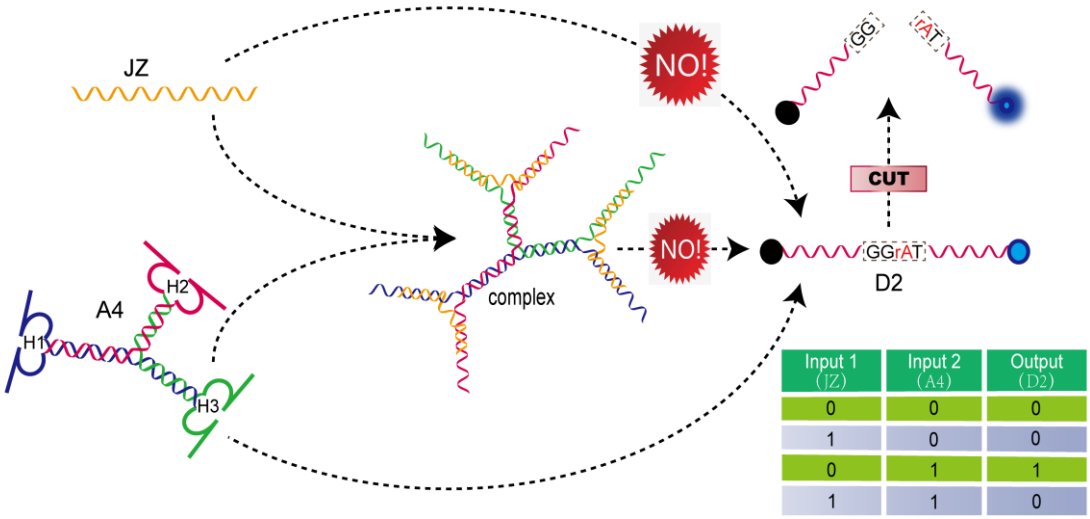

Fig S1 Inhibit gate system principle scheme and Truth Table.

To enrich the 3-way DNAzyme complex adaptability, we try to explore the Inhibit gate logic system, our design is shown in Fig S1. The Inhibit gate logic system consists of A4 which formed in the early AND gate logic system, ssDNA named JZ, and ssDNA named D2 which modified with a fluorescent group (FAM) and a quenching group (BHQ) . We regarded JZ as Input1, A4 as Input2, and D2 as Output. (1) When there is no input in the system (the input of 0 & 0), the output signals D2 remain in a relatively static state; its fluorescent groups and quenching groups suppress each other, and the overall output signal is 0. (2) When only JZ is input (the input of 1 & 0), JZ and D2 don't react, D2 continues in the initial state, and the overall output signal of the system is 0. (3) When only A4 is input (the input of 0 & 1), A4 is a 3-way DNAzyme complex that has DNAzyme H2 that specifically recognizes D2, so D2 can be identified and cut, and the overall output signal of the system is 1. (4) When both JZ and A4 are added (the input of 1 & 1), JZ and A4 react involving strand displacement with each other. The DNAzyme recognition domain and conservative domain of A4 will be blocked by JZ, and A4 will lose the function of DNAzyme H2. Thus, A4 do not recognize and cut D2, the overall output signal is 0.

As in the previous experiment, we again adopted the method of fluorescence intensity

measurement to conduct real-time monitoring of the Inhibit gate logic system, as shown in Figure S2. When only A is input, the fluorescence curve has an obvious upward trend, indicating that the fluorescence intensity has been significantly enhanced, and thus the output signal is 1. In other cases, the fluorescence curve hardly changes and the fluorescence intensity basically remains unchanged, thus the output signal is 0.

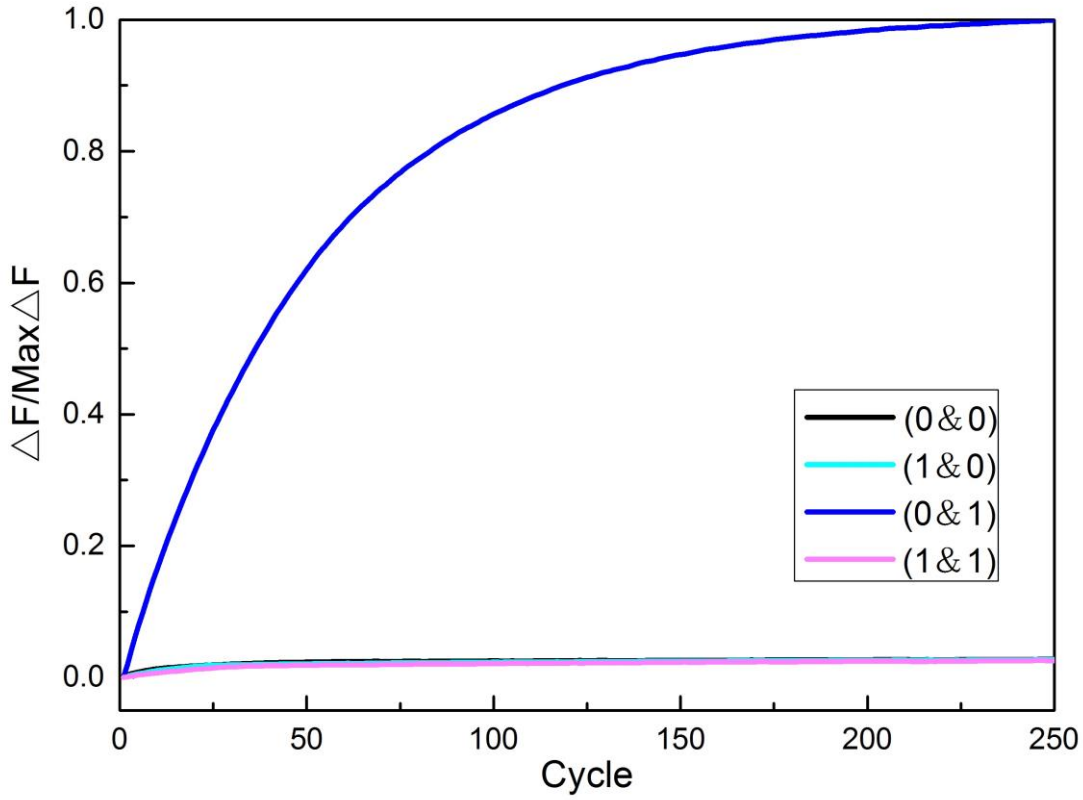

Fig S2 Fluorescence real-time monitoring of the Inhibit gate system.

S4 Simulation results using NUPACK software

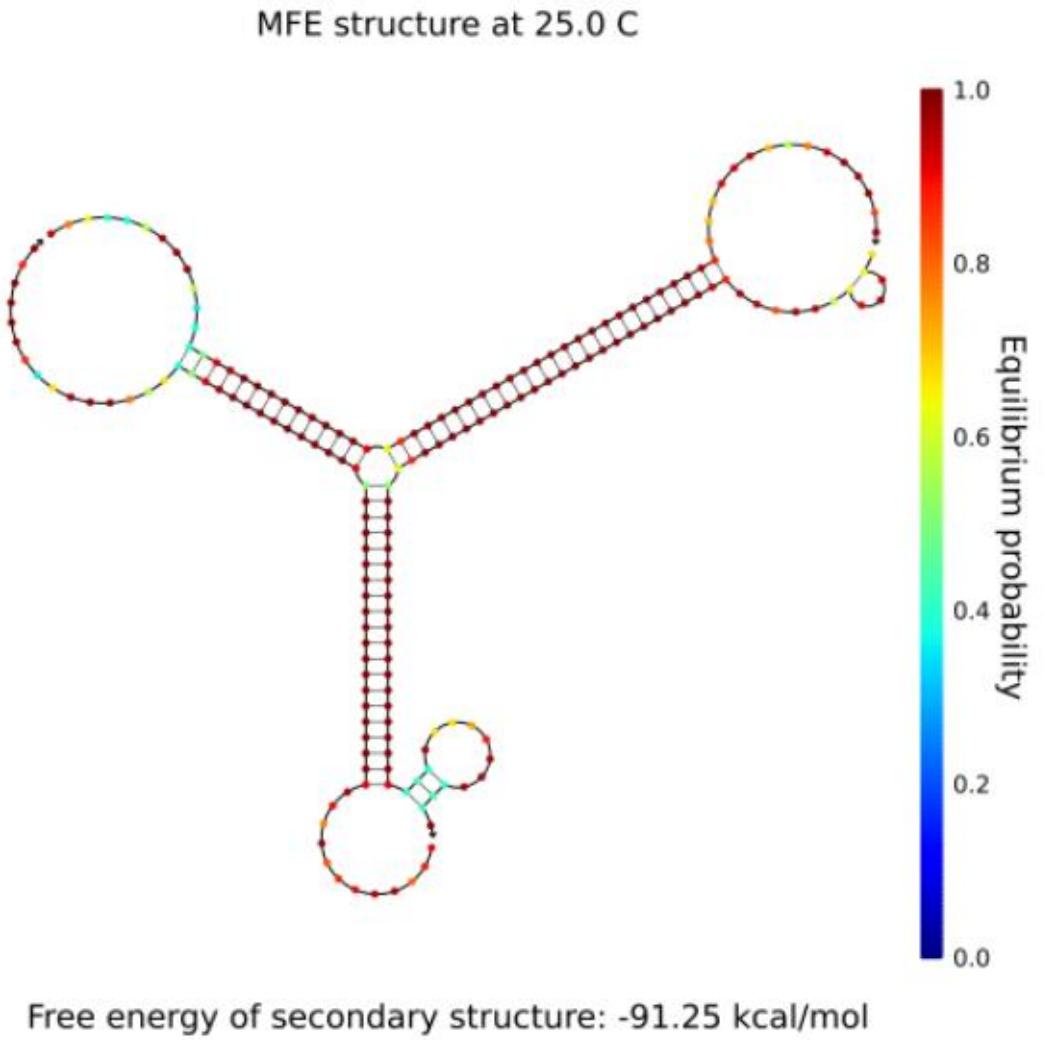

Fig S3 The simulation results of 3-Way DNAzyme complex which in the AND gate logic system.

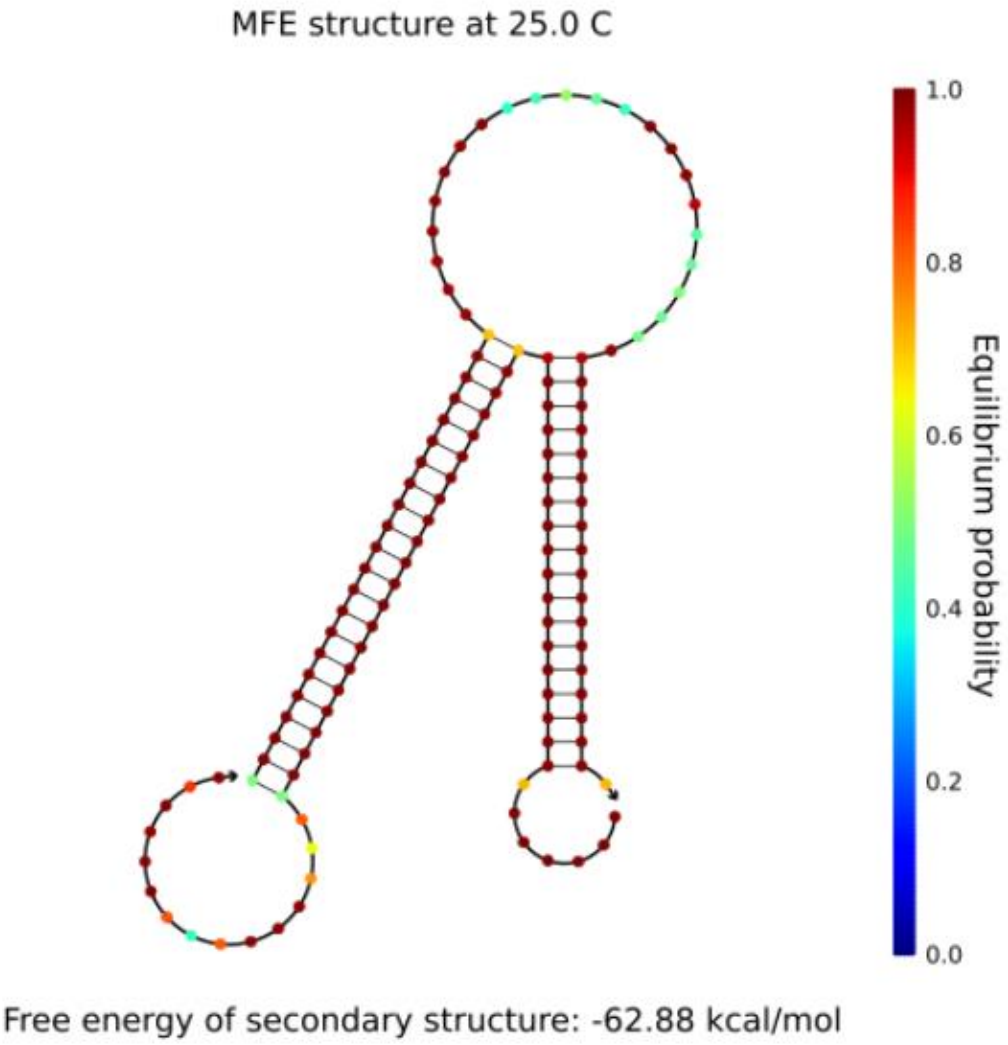

Fig S4 The simulation results of F1 which in the feedforward catalytic logic circuit.

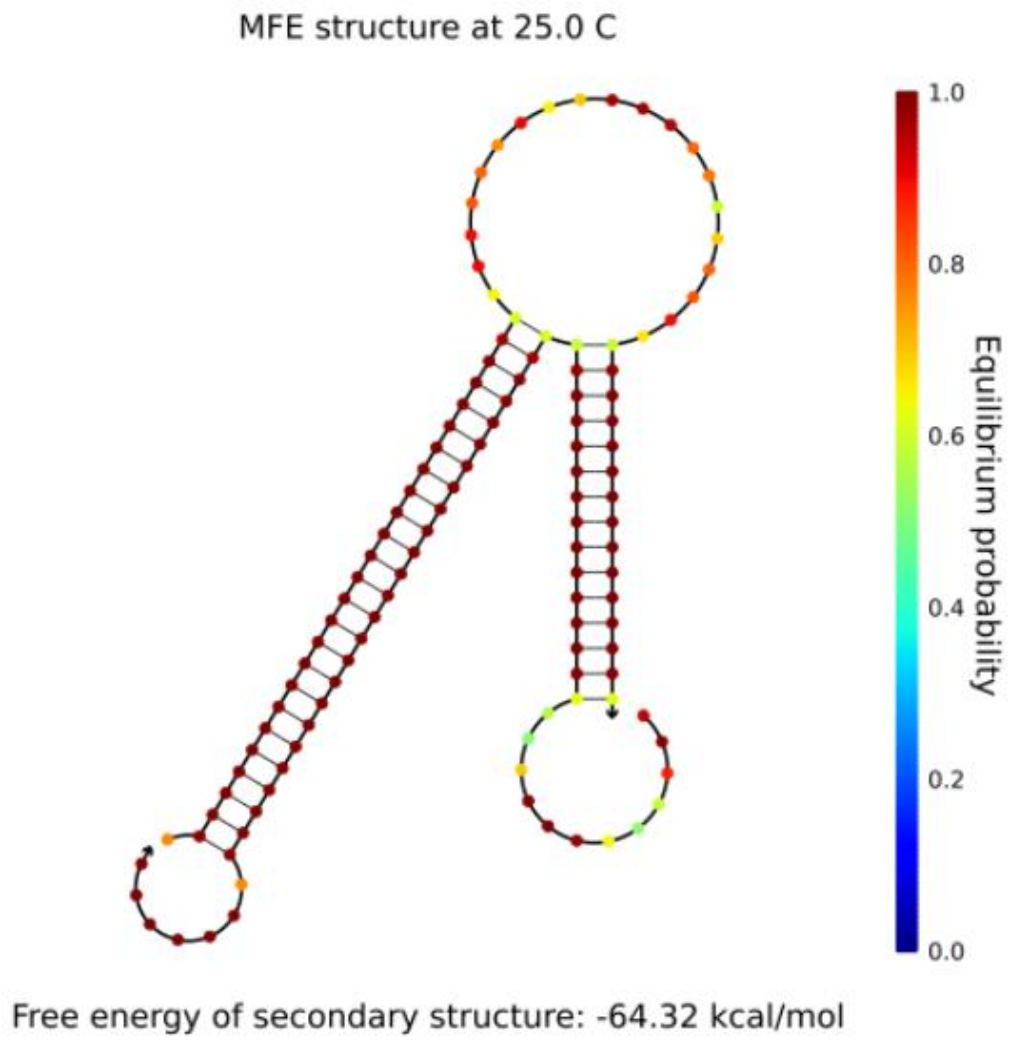

Fig S5 The simulation results of F2 which in the feedforward catalytic logic circuit**.**

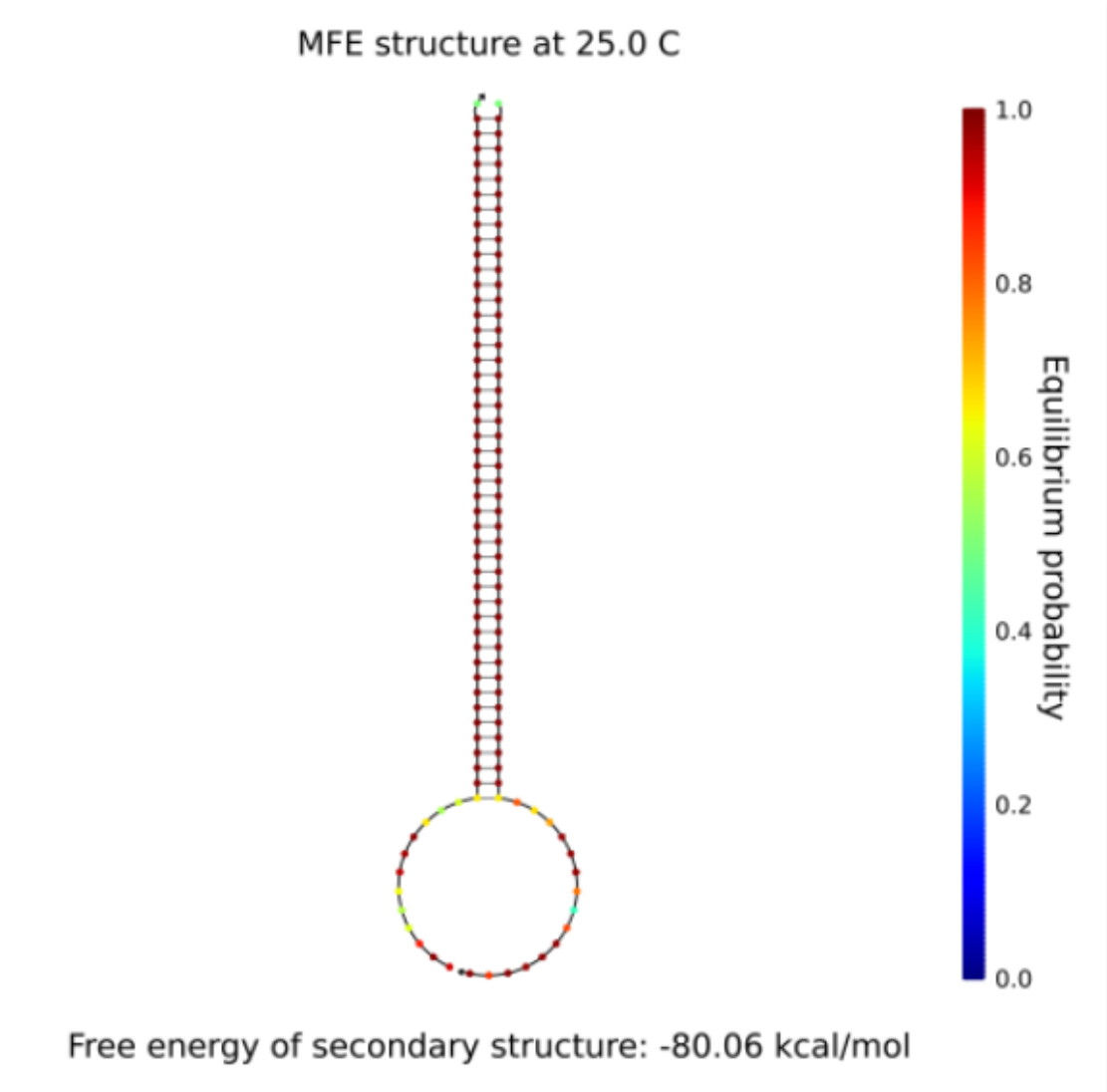

Fig S6 The simulation results of F4 which in positive feedback catalytic logic circuit.

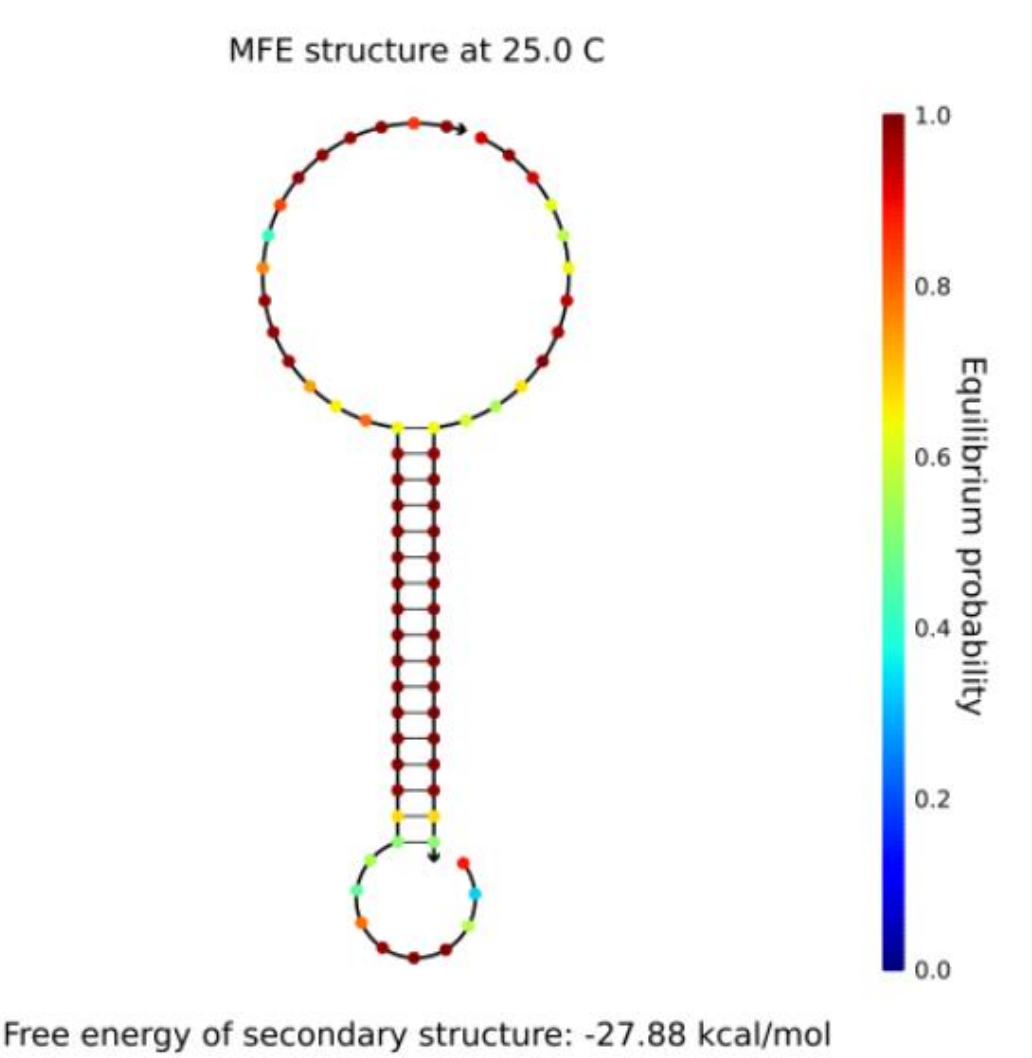

Fig S7 The simulation results of B1 which is used in half subtractor.

S5 DNA sequences in this study

Table S1 DNA sequences in this study

Name	Sequences (from 5′ to 3′)	Length(n.t.)
L1	TGGTACAGCGATCCGTTCCATAAGCTAGCACACA ACGCTAGCTTATCACCCATGTTACTCT	61
L2	GATATCAGCGATATAAGCTAGCGTTGAACACAC ACTGATTGATCGTGTGTTCACCCATGTCTTAGA	66
T	TGGTACAGCGATAACACACGATCAATCAGTGTGT GTTTGTGCTAGCTTATGGAACGGCACCCATGTCT TAGA	72
A1	CTCTTCAGCGATCCGTTCCATAAGCTAGCACACA ACGCTAGCTTATCACCCATGTTACTCT	61
A2	TGGTACAGCGATAACACACGATCAATCAGTGTGT GTTTGTGCTAGCTTATGGAACGGCACCCATGTTA GTGA	72
A3	GATATCAGCGATATAAGCTAGCGTTGAACACAC ACTGATTGATCGTGTGTTCACCCATGTCTTAGA	66
FA	AGTTGGTCTCACGATGCTACTGGTGTCTGCCTTC CACGAGTCTTGCTCACCCATGTTACTCT	62
FA*	AGCAAGACTCGTGGAAGGCAGATTTTTTCTAAGT /rA/GGTACCATTTTCACCAGTAGCATCGTGAGA	65
FB	GATATCAGCGATAGCAAGACTCGTGGAAGGCAG ACACCAGTAGCATCGTGAGACCAACT	60
FB*	TCTCACGATGCTACTGGTGTCTGCCTTTTTTCACT AT/rA/GGAAGAGTTTCCACGAGTCTTGCT	62
J1	ACATGGGTGATAAGCTAGCGTTGTGTCACCCATG TTACTCT	41
J2	GATATCAGCGATACACAACGCTAGCTTAT	29

JZ	ACATGGGTGATCGCTAAAAAA	21
D1	TCTAAGT/rA/GGTACCA	15
D2	AGAGTAT/rA/GGATATC	15
D3	TCACTAT/rA/GGAAGAG	15