Supporting Information

Addressable activated cascaded DNA sequential logic circuit model for processing identical input molecules

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Materials

All oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed in Table S1.

SYBR Green I purchase from Thermo Fisher Scientific. Ammonium persulfate, TEMED, 5x TBE, 6x loading buffer were purchased from Shanghai Sangon.

DNA Sequence

Table S1. Sequences of oligonucleotides used in the present work.

Name	Sequences（from 5′ to 3′）
Input 1	ACCCACACCACCACCCTTCTTCCCTTTCTCCTTTCTTCTCACCTTTCTTTCCCTCCTCCCTCTCTC
First input0/Second input0	CTTCTTCCCTTTCTCCTTTCTTCT-BHQ1CACCTTTCTTTCCCTCCTCCCTCTCTC CCACCACAACACCAC
Third input 0	CTTCTTCCCTTTCTCCTTTCTTCT-BHQ2CACCTTTCTTTCCCTCCTCCCTCTCTC CCACCACAACACCAC
IS-B	CTTCTTCCCTTTCTCCTGTTTGTTCTTTCTTCT-BHQ1CACCTTTCTTTCGTTTATTTG CCTCCTCCCTCTCTC
IS-C	CTTCTTCCCTTTCTCTTTATTATTCTTTCTTCT-BHQ1CACCTTTCTTTCGTTTTATTT CCTCCTCCCTCTCTC
IS-D(Single FL)	CTTCTTCCCTTTCTCATACCCACACTTTCTTCT-BHQ1CACCTTTCTTTCACACTACAT CCTCCTCCCTCTCTC
IS-D(Multi FL)	CTTCTTCCCTTTCTCATACCCACACTTTCTTCT-BHQ2CACCTTTCTTTCACACTACAT CCTCCTCCCTCTCTC
Blocker B2	AACAAACAGACCCACACCACCACC
Blocker B1	CCACCACAACACCACCAAATAAAC
Blocker C2	AATAATAAAACCCACACCACCACC
Blocker C1	CCACCACAACACCACAAATAAAAC
Coder 2	GGTGGTGGTGTGGGTGAGAAAGGGAAGAAG
Coder 1	GAGAGAGGGAGGAGGGTGGTGTTGTGGTGG
Co-Recorder	GTGGTGTTGTGGTGGGAGAGAGGGAGGAGGGAAAGAAAGGT-FAMGAGAAGAAAGGAGAAAGGGAAGAAGGGTGGTGGTGTGGGT
Co-Recorder(Multi FL)	GTGGTGTTGTGGTGGGAGAGAGGGAGGAGGGAAAGAAAGGT-HEXGAGAAGAAAGGAGAAAGGGAAGAAGGGTGGTGGTGTGGGT
Co-Recorder(Multi FL)	GTGGTGTTGTGGTGGGAGAGAGGGAGGAGGGAAAGAAAGGT-ROXGAGAAGAAAGGAGAAAGGGAAGAAGGGTGGTGGTGTGGGT
Coder 2-A	GGTGGTGGTGTGGGTGAGAAAGGGAAGAAG AAAAAAAAAAAAAAAAAAAAAAAAAAAA
Coder 1-A	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAGAGAGGGAGGAGG GTGGTGTTGTGGTGG
Input 0-C	CCCCCCCCCCCTTCTTCCCTTTCTCCTTTCTTCTCACCTTTCTTTCCCTCCTCCCTCTCTCCCACCACAACACCACCCCCCCCCC

The DNA sequences were marked with colors in the corresponding areas in Figure 1/3

Experimental Section

All oligonucleotides were dissolved in PBS buffer (800 mM NaCl, C($PO4^{3-}$)=10 mM, pH=7.4) and diluted to a concentration of 100 μM.

Design scheme of trigger

As shown in Figure S1, Gate B* is a four-strand complex with double-loop hairpin structure consisting of DNA co-Recorder, IS-**C**, Blocker B1 and Blocker B2. The DNA Recorder is used to record input information and generate the corresponding output molecules. The two domains owned by Blocker have different functions. 1. Domain 1* and 5* are used to enclose the toeholds area at both ends of the Co-recorder and making the trigger in inactive. 2. Domain 6* and 7 * Contains address information, which can be used as a toehold for strand replacement reaction to active the trigger. AS-B is a three-strand structure consisting of IS-B, Coder 1 and Coder 2 while domain 1/5 and 6/7 were linked on it.

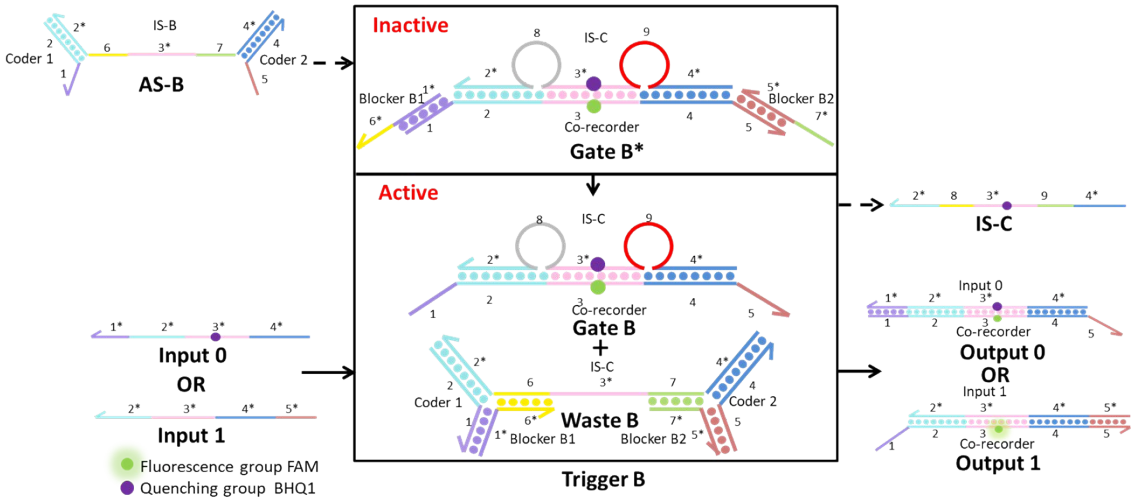

Figure S1. Schematic illustration of the trigger logic circuit. The solid arrow indicates the direction in which the input/output information is movement in the system, and the dotted arrow indicates the direction in which the state AS is transmitted.

The AS from the upstream level circuit first addressing the blocker of the inactive trigger and then completes the activation of the circuit through combinatorial displacement reaction. Then remove Blocker B1 and Blocker B2 from Gate B* through combinatorial displacement reaction, thereby completing the activation of trigger. The toeholds at both ends of Gate B are recovered to combine with the input to generate a double-strand output structure with storage information function. Respectively, Output 1 is generated by DNA Input 1 and Co-recorder through 3-end strand displacement reaction, while output 0 is generated by DNA Input 0 and Co-recorder through 5-end strand displacement reaction. In addition, when the trigger in its active state stores the input signal, it simultaneously releases IS-C as a key component of New AS which can activate downstream unit.

To verify the effectiveness of the trigger logic circuit, we firstly used fluorescence spectrometry to monitor its operation. FAM fluorophore was marked in the middle of DNA co-recorder and BHQ1 was marked in the middle of IS-C. The result of relative fluorescence intensity higher than the threshold 0.5 is defined as 1, otherwise as 0. After the trigger activated by AS, putting in Input 1 initiated quick strand displacement with Gate, thereby keeping quenching group of DNA Recorder away; the

fluorescence intensity obviously increased in a few dozens of seconds (See Figure S2), indicating recording of input information 1. When Input 0 was put in, strand displacement also took place, but the quenching group on DNA Input 0 kept the fluorescence intensity at a low level, indicating input information as 0. Secondly, native polyacrylamide gel electrophoresis (native-PAGE) was conducted to verify the molecular structure changes. As shown in Figure S3, the lane 7-8 experiment results prove that AS was completely consumed in activating the trigger. Concurrently, after Input 0 and Input 1 were put in separately, DNA bands corresponding to Output 0 and Output 1 could be easily observed in the electrophoresis. When any input is put in the inactive trigger, no band of output structure could be observed. The electrophoresis and the fluorescence results support each other and proved that the trigger operates as designed.

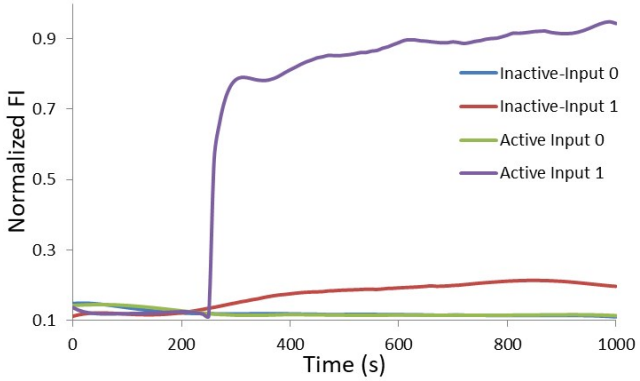

Figure S2. The fluorescent kinetic curve of the trigger system.

Native polyacrylamide gel electrophoresis (native-PAGE)

Prepare 10 mL of 9.0% native polyacrylamide gel in room temperature with 3 mL of 30% acrylamide (Acryl/Bis solution (29:1), 30% (w/v)), 5 mL ultrapure water (18.25 MΩ), 2 mL 5x TBE，5 μL TEMED，50 μL of 10% ammonium persulfate and mixed them. The mixture was poured into the gel plate for 45min standing to form 9.0% polyacrylamide gel.

Different mixtures of the DNA solution were incubated for at room temperature; the concentration of each oligonucleotide was 1 μM. 10 μL of each sample was mixed with 2 μL of 6x loading buffer, and then the mixture was added into the gel for electrophoresis. A 9.0% native polyacrylamide gel was prepared using 1x TBE buffer (89 mM Tris base, 89 mM Boric acid, 2 mM EDTA, pH=8.3). The NATIVE-PAGE was carried out in 0.1x TBE buffer at a constant voltage of 100 V for about 80 min at room temperature (using Bio-Rad Mini-Protean Tetra Electrophoresis System). The gel was scanned by Tanon-2500 automatic digital gel image analysis system.

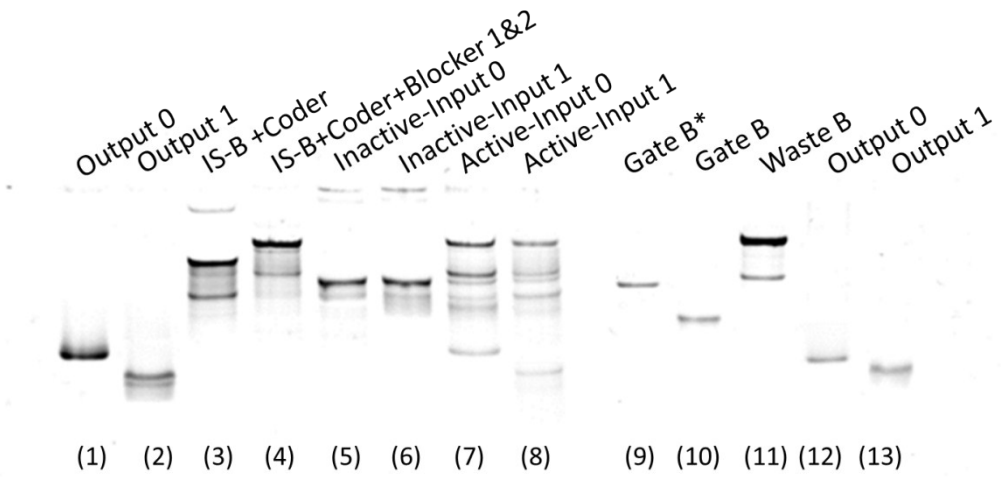

Figure S3. The 9% native-PAGE results of trigger.

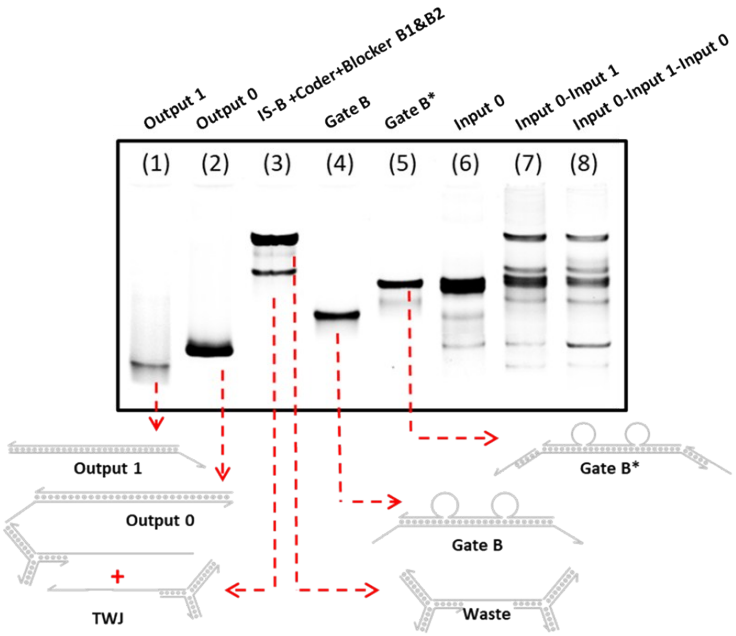

Figure S4. The 9% native-PAGE results of register. Lane 1-lane 5 is marker, lane 6-lane 8 is the step of three bit input of (0,1,0) input to register system. The gel electrophoresis result (100 V, 80 min, 9% native page) of one typical input condition (0, 1, 0) verified the molecular structural change when Input 0 and Input 1 were input to the system in three steps. This results clearly showed that the corresponding bands of output 0 band can be observed after the input of Input 0 and the bands corresponding to Gate B* and Gate C* became shallow at the same time, both proving the occurrence of recording. When Input 1 was added, the band related to Output 1 could also be observed. The gel electrophoresis results fully corresponded with the fluorescence results and had double confirmed the consistency between the running and design of the register.

Fluorescence spectra measurement

Trigger logic circuit.

To prepare the Gate*, Recorder, Signal B, Blocker 1 and Blocker 2 was mixed assay buffer (5 uM). The mixtures were annealed by heating to 95 °C for 10 min and slowly cooled down to 75°C. Then; the mixtures were annealed by keeping to 75 °C for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the trigger.
To prepare the AS, Signal A, Coder1 and Coder2 was mixed assay buffer(5 uM).
Added in the test tube 3 uL annealed AS(5 uM), and 3 uL Gate*(5 uM), as well as PBS buffer solution (492 uL, PH=7.4, c (NaCl) =800 mM) to form the Trigger, the concentration of AS and Gate* was 30 nM. It was placed in room temperature for 7000 s before adding 2 uL Input solution(5 uM). The fluorescence detection was started for 250 s before adding Input solution. The concentration of Input was 20 nM, that of Gate*, AS was 30 nM.

Comparative experiment
Added in the test tube 3 uL annealed Gate*(5 uM), as well as PBS buffer solution (495 uL, PH=7.4, c (NaCl) =800 mM), the concentration of Gate* was 30 nM. The fluorescence detection was started for 250 s before adding 2 uL Input solution (5 uM). The concentration of Input was 20 nM, that of Gate* was 30 nM.

Register sequential logic circuit.

To prepare the Gate A, Co-Recorder strand was mixed with IS-B strand in assay buffer. The mixtures were annealed by heating to 95 °C for 10 min and slowly cooled down to 75°C. Then, the mixtures were annealed by keeping to 75 °C for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the register.

To prepare the Gate B*, Co-Recorder, IS-C, AddressB1 and AddressB2 was mixed assay buffer. The mixtures were annealed by heating to 95 °C for 10 min and slowly cooled down to 75°C. Then; the mixtures were annealed by keeping to 75 °C for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the register.

To prepare the Gate C*, Co-Recorder, IS-D, AddressC1 and AddressC2 was mixed assay buffer. The mixtures were annealed by heating to 95 °C for 10 min and slowly cooled down to 75 °C. Then; the mixtures were annealed by keeping to 75 °C for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the register.

To prepare the Coder, Coder 1 and Coder 2 was mixed assay buffer.

Added in the test tube some annealed Gate (2 uL 5 uM GateA, 3 uL 5 uM GateB* and GateC*), as well as PBS buffer solution (PH=7.4, c (NaCl) =800 mM), the concentration of Gate A was 20 nM, the concentration of Gate B* and Gate C* was 30 nM. When the Input was added to test solution, fluorescence detection was carried out.

The concentration of first Input and GateA was 20 nM, the concentration of the second Input, the third Input, the first Coder, the second Coder, Gate B* and Gate C* was 30 nM.

Firstly, after the first 200 s, joined the first Input (2 uL, 5 uM). Secondly, after waiting for 2000 s, added the first Coder (3 uL, 5 uM). Thirdly, after waiting for 4000 s, added the second Input(3 uL, 5 uM). Fourth, after waiting for 2000 s, added the second Coder(3 uL, 5 uM). Lastly, after waiting for 4000 s, add the third Input(3 uL, 5 uM).

The outputs of the Trigger and the Register were mainly the fluorescence produced by FAM. The emission spectrum of FAM was collected at 515 nm with the excitation wavelength of 493 nm, respectively. The slit widths for the excitation and emission were 1 nm. FluoroMax-4 fluorescence spectrometer was used for the detection.

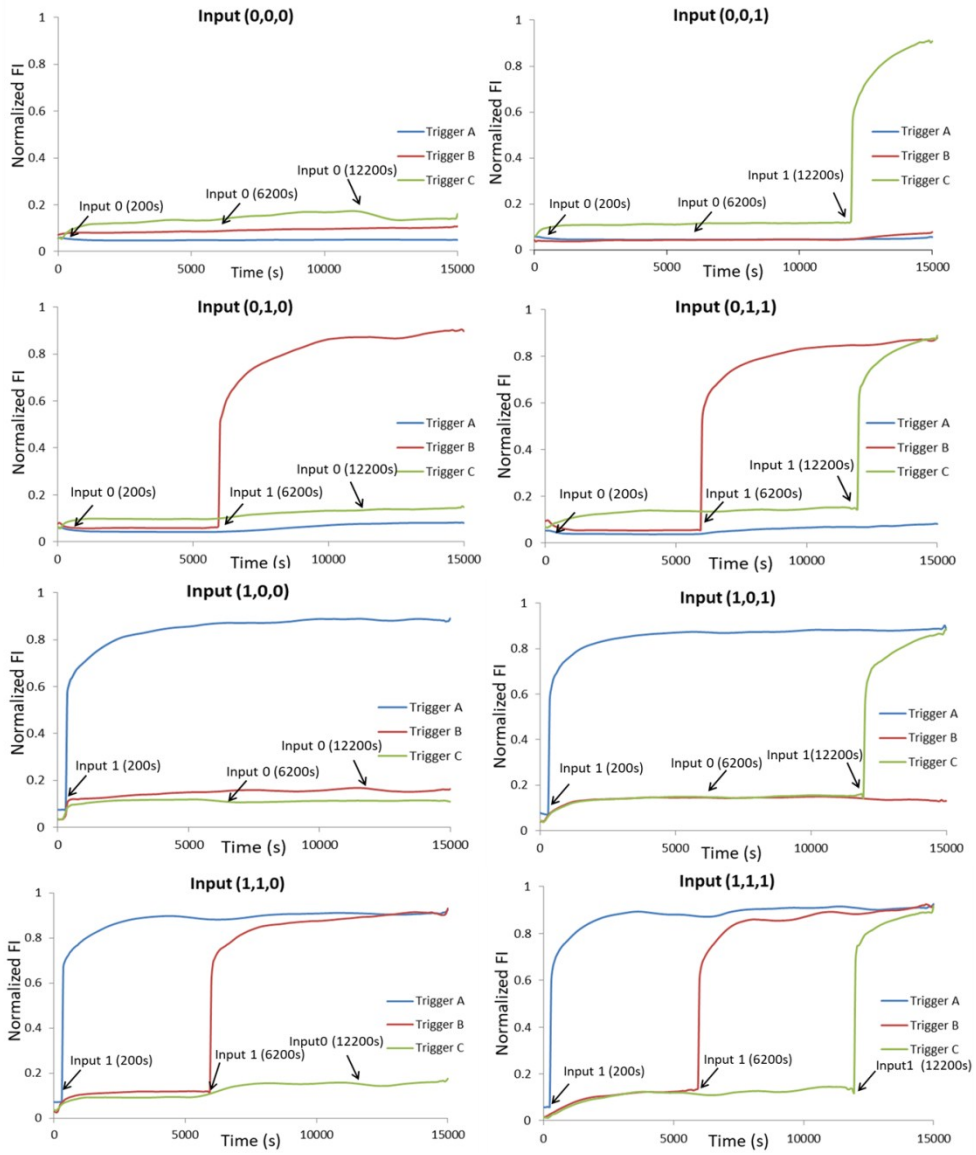

Figure S5.The three type of fluorescent kinetic curve for the register system. The sequences of the three-bit input are (0,0,0), (0,0,1) , (0,1,0) , (0,1,1) , (1,0,0) , (1,0,1) , (1,1,0) and (1,1,1) .

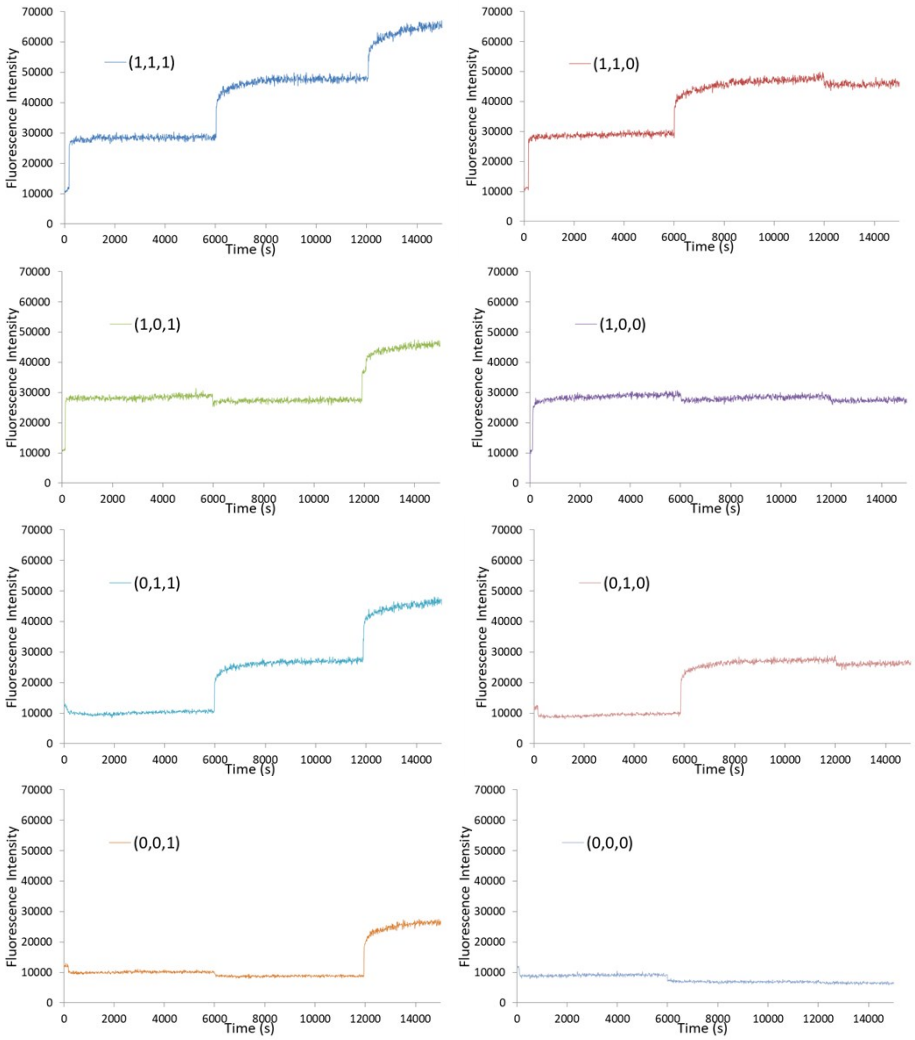

Figure S6. The fluorescent kinetic curve of the register system. The sequences of the three-bit input are (0,0,0), (0,0,1) , (0,1,0) , (0,1,1) , (1,0,0) , (1,0,1) , (1,1,0) and (1,1,1) .

The calculation of molecular commonality rate (MCR).

$$MCR = \frac{The\ number\ of\ standard\ DNA\ molecular\ parts}{The\ number\ of\ all\ DNA\ molecular\ parts} * 100\%$$

Standard DNA molecular parts the register logic circuit:

Table S2. Sequences of Standard DNA molecular parts the register logic circuit.

Input 1	ACCCACACCACCACCCTTCTTCCCTTTCTCCTTTCTTCTCACCTTTCTTTCCCTCCTCCCTCTCTC
input0	CTTCTTCCCTTTCTCCTTTCTTCCACCTTTCTTTCCCTCCTCCCTCTCTC CCACCACAACACCAC
Coder 2	GGTGGTGGTGTGGGTGAGAAAGGGAAGAAG
Coder 1	GAGAGAGGGAGGAGGGTGGTGTTGTGGTGG
Co-Recorder	GTGGTGTTGTGGTGGGAGAGAGGGAGGAGGGAAAGAAAGGGAGAAGAAAGGAGAAAGGGAAGA AGGGTGGTGGTGTGGGT

$The\ number\ of\ standard\ DNA\ molecular\ parts = 5$

All DNA molecular part of the register logic circuit:

Table S3. Sequences of all DNA molecular parts the register logic circuit.

Input 1	ACCCACACCACCACCCTTCTTCCCTTTCTCCTTTCTTCTCACCTTTCTTTCCCTCCTCCCTCTCTC
input0	CTTCTTCCCTTTCTCCTTTCTTCCACCTTTCTTTCCCTCCTCCCTCTCTC CCACCACAACACCAC
IS-B	CTTCTTCCCTTTCTCCTGTTTGTTCTTTCTTCCACCTTTCTTTCGTTTATTTG CCTCCTCCCTCTCTC
IS-C	CTTCTTCCCTTTCTCTTTATTATTCTTTCTTCCACCTTTCTTTCGTTTTATTT CCTCCTCCCTCTCTC
IS-D	CTTCTTCCCTTTCTCATACCCACACTTTCTTCCACCTTTCTTTCACACTACAT CCTCCTCCCTCTCTC
Blocker B2	AACAAACAGACCCACACCACCACC
Blocker B1	CCACCACAACACCACCAAATAAAC
Blocker C2	AATAATAAAACCCACACCACCACC
Blocker C1	CCACCACAACACCACAAATAAAAC
Coder 2	GGTGGTGGTGTGGGTGAGAAAGGGAAGAAG
Coder 1	GAGAGAGGGAGGAGGGTGGTGTTGTGGTGG
Co-Recorder	GTGGTGTTGTGGTGGGAGAGAGGGAGGAGGGAAAGAAAGGGAGAAGAAAGGAGAAAGGGAAGA AGGGTGGTGGTGTGGGT

$The\ number\ of\ standard\ DNA\ molecular\ parts = 12$

The register's MCR $= \frac{5}{12} * 100\% = 41.7\%$

Optimization of annealing temperature

Annealing temperature is able to affect the formation of Gate A Gate B * Gate C *. In order to explore the effect of annealing temperature on the system, the following experiments were conducted. The solution was heated to 95 °C, annealing for 10 min at 95°C , 85°C，75°C，65°C，55°C respectively, and then slowly cooled down to room temperature. At last, the Input 0 with BHQ1 modification was

used to test the formation of the annealed structure. If Gate A, Gate B* and Gate C were formed completely, when Input 0 introduced, the fluorescence intensity would not change. The smaller the decrease of fluorescence was, the more stable the structure was. From the experiment results we can find that the annealing temperature is able to influence the formation of the system.

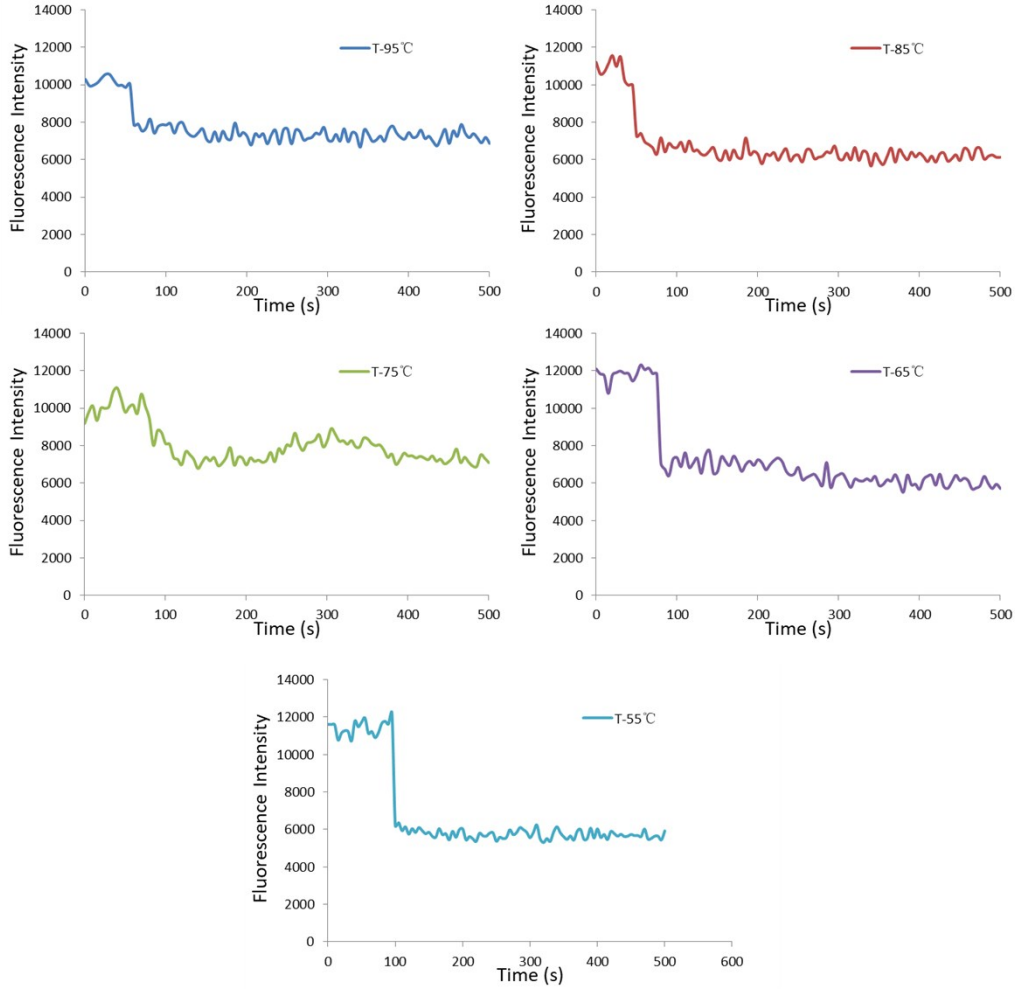

Figure S7: The optimization of register by changing the annealing temperature.

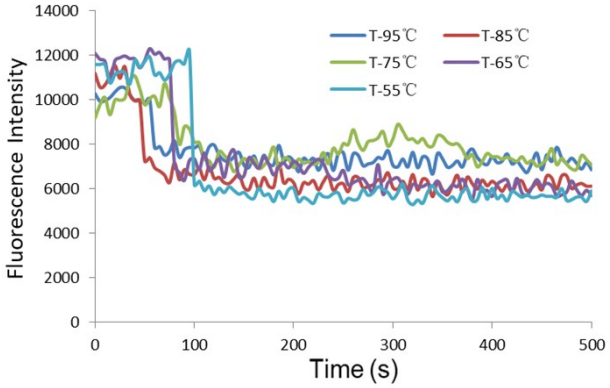

Figure S8: The optimization of register by changing the annealing temperature.

The suitable annealing temperature was 75°C. But why can we get this conclusion? We explored based the structure of Gate A Gate B * Gate C *. If you look at the structure of Gate A as a double-stranded DNA (Figure S3).

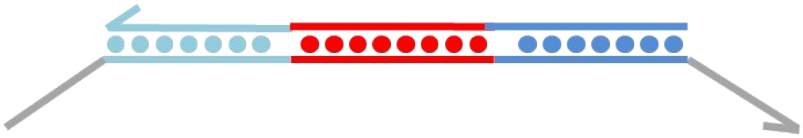

Figure S9: The structure of ds-DNA.

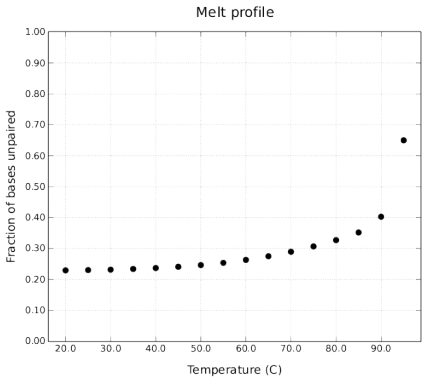

Figure S10: The Melt profile model of ds-DNA..

Theoretically, the theoretical melt temperature of Gate A was about 95 °C and the computing method by nupack. [1-3] However, in our opinion, the above DNA structure was considered as a complex of three double strands instead a long straight chain.

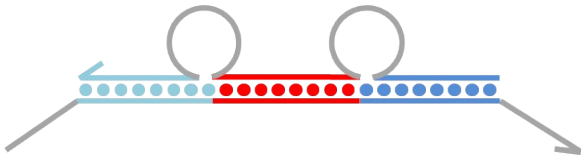

Figure S11: The structure of Gate A.

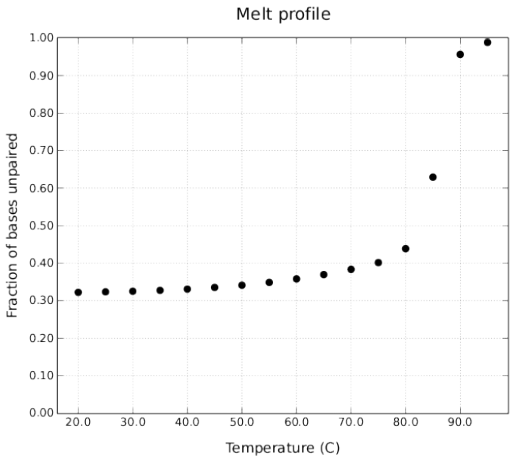

Figure S12: The Melt profile model of Gate A.

The theoretical melting temperature of the structure of the Tm was about 85°C, Annealing temperature = Tm-10, thus the optimum annealing temperature was at about 75°C. After the AS is

inputted in trigger system,

Exploring the time of the activation process.

Activation time for trigger is able to affect the running of register. In order to explore the effect of activation time on the system, the following experiments were conducted. The reaction time of AS with inactive-trigger was set to 0 s, 500 s, 1000 s, 2000 s, 4000 s and 6000 s respectively. We used input1 molecule to test the efficiency of active process and the experimental results were shown on Figure S11.

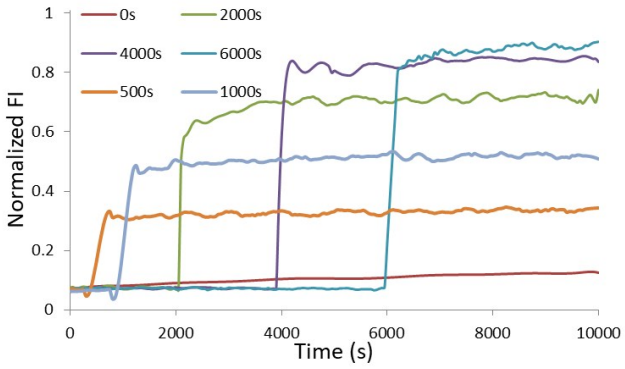

Figure S13. The effect of reaction time for activation efficiency.

We tried to use chemical reaction kinetics to explain this slower process. As shown as Figure S12, the activation reaction is a two-step series reaction. In this reaction, step (2) is rate-determining step and its rate constant is 1/10 of DNA strand displacement (DSD) reaction[4]. Therefore, the reaction requires longer reaction time than the DSD.

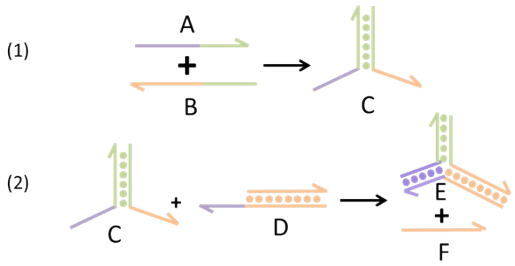

Figure S14. The simple scheme of activation reaction.

Reference

1. R. M. Dirks, J. S. Bois, J. M. Schaeffer, E. Winfree, and N. A. Pierce. Thermodynamic analysis of interacting nucleic acid strands. *SIAM Rev*, 49:65-88, 2007.

2. R. M. Dirks and N. A. Pierce. An algorithm for computing nucleic acid base-pairing probabilities including pseudoknots. *J Comput Chem*, 25:1295-1304, 2004.

3. R. M. Dirks and N. A. Pierce. A partition function algorithm for nucleic acid secondary structure including pseudoknots. *J Comput Chem*, 24:1664-1677, 2003.

4. Chen, X. Expanding the rule set of DNA circuitry with associative toehold activation. J. Am. Chem. Soc. 2012, 134, 263-271.