DNA Kinetics Competition Strategy of Hybridization Chain Reaction for Molecular Information Processing Circuits Construction

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Supporting Information

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/II purchase from Thermo Fisher Scientific. Ammonium persulfate、TEMED,5x TBE,6x loading buffer purchase from Shanghai Sangon

DNA Sequence

Table S1. Sequences of oligonucleotides used in the present work

Name	Sequences（from5' to 3'）
x	GCTTCTCTCTTCTCTTCC
y	GTGGTGGTGGAAGAGAAGTCCGCCTGTCAATTATCTTCTCTTCC
z	TGAGAGGAGGAAGAGAAGAACCAACCACCACCAACTTCTCTTCC
α	CTTCTCTTCCACCACCACACT-FAM-CAACAGGAAGAGAAGAGAGAAGC
β	CTTCTCTTCCTCCTCTCATT-ROX-CTCTCTGGAAGAGAAGATAATTGA
γ	CTTCTCTTCCAATATAATCGCGGCCAGGAAGAGAAGTTGGTGGT
blocker1	BHQ1-AGTGTGGTGGTGGAAGAG
blocker2	BHQ2-GAATGAGAGGAGGAAGAG

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

Experimental Section

Experimental Details.

DNA powder was centrifuged and dissolved with PBS buffer solution (PH=7.4, c(NaCl)=300mM)

Took some DNA solution and diluted it to specified concentration before annealing. Firstly, heated it to 95℃ holding for 5min, reduced the temperature to 60℃ at the speed of 5℃/min, and then quickly reduced the temperature to restore the supposed state of DNA.

Took some solution α and prepared it into input a. Took some β and blocker1 and prepared them into Input b. Took some γ and blocker2 and prepared them into Input c.

Added in the test tube some annealed x, y and z, as well as PBS buffer solution (PH=7.4, c(NaCl)=300 mM) to form the key cylinder of keypad, the concentration of x, y and z was 50 nM.

Added input a, input b and input c in the "lock cylinder" as per specified sequence; every time when a solution was added, it was placed in 25℃ room temperature for 25min before adding another solution. When all the input was added, added test solution; 20min later, fluorescence detection was carried out. The concentration of x, y, z was 50 nM, that of α, β and γ was 20 nM, and that of blocker1 and blocker2 was 50 nM.

Fluorescence Detection

The outputs of the logic circuit are mainly the fluorescence produced by ROX and FAM. And the fluorescence intensity is decided by the DNA concentration. Hitachi F7000 fluorescence spectrometer was used for the detection.

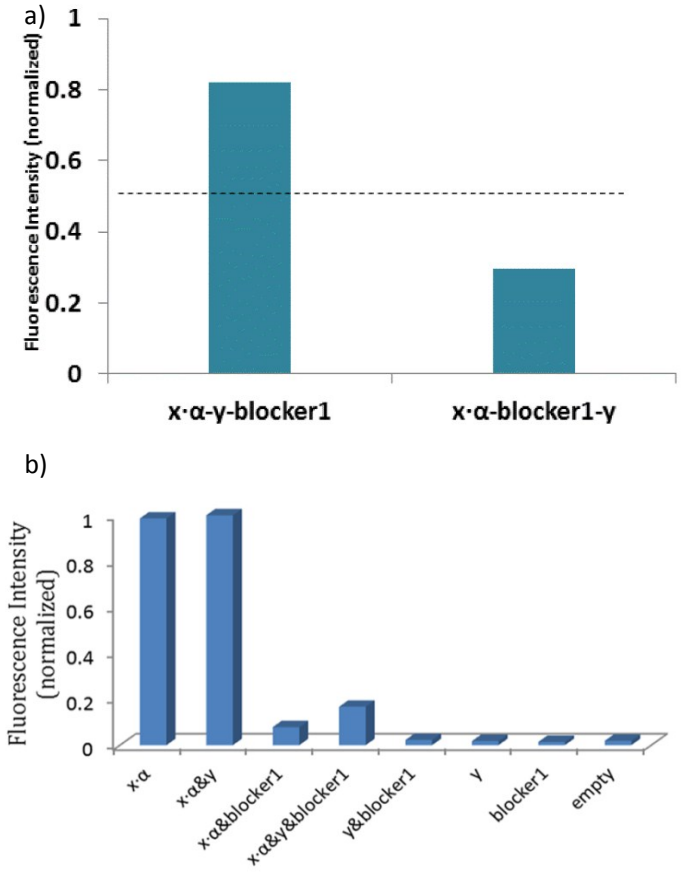

Figure S1: a) Normalized fluorescence intensities of different sequence addition of γ/blocker1; b) Fluorescent result of all inputs combinations of the priority encoder.

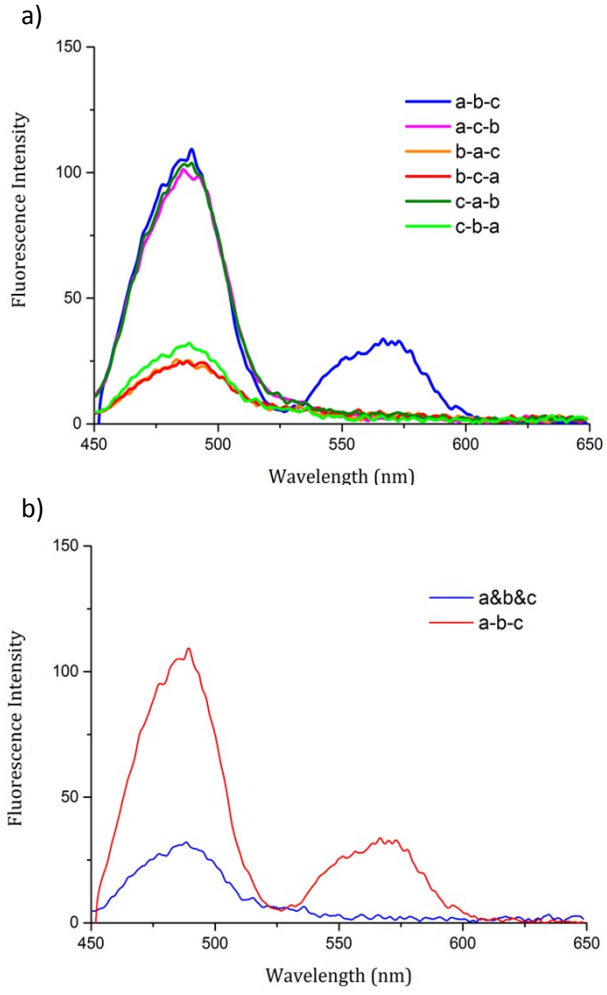

Figure S2: a) Result analysis of synchronous fluorescence detection in room temperature;b） Result analysis of synchronous fluorescence detection; The blue line represents the input of a-b-c in sequence while the red line stands for the input of a, b, c simultaneously.

Native polyacrylamide gel electrophoresis (native-PAGE).

Preparation of Native polyacrylamide gel

Prepare 10 ml of 13.5% native polyacrylamide gel in room temperature with 4.5 ml of 30% acrylamide (Acryl/Bis solution (29:1), 30% (w/v)), 3.5ml ultrapure water (18.25 MΩ), 2ml 5x TBE， 5ul TEMED， 50 ul of 10% ammonium persulfate and put it on the gel plate for 20 min standing to form 13.5% polyacrylamide gel.

Different mixtures of the DNA solution were incubated for 1 h 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 13.5% native polyacrylamide gel was prepared using 1 x TBE buffer (89 mM Tris base, 89 mM Boric acid, 2 mM EDTA, pH 8.3). The NATIVE-PAGE was carried out in 0.1 x TBE buffer at a constant voltage of 80 V for about 1h 50min at room temperature(using Tanon VE-180B electrophoresis tank and DYY-8C Electrophoresis System).The staining in diluted SYBR I and SYBR II solution. The gel was scanned by a Gel Doc XR+ system (Bio-Rad).

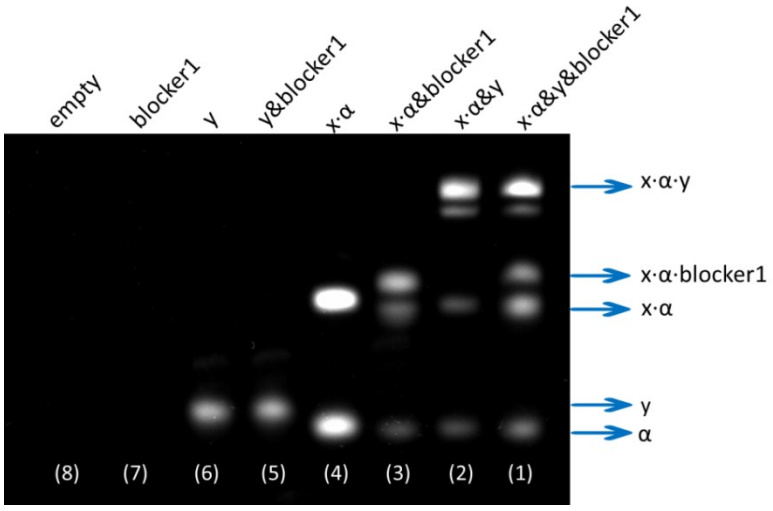

Figure S3 : Native-PAGE results of all combination of the first priority encoder inputs. The arrow in the left points to the DNA sequence of corresponding strap of this line. All the DNA concentration in native-PAGE is 1μM.

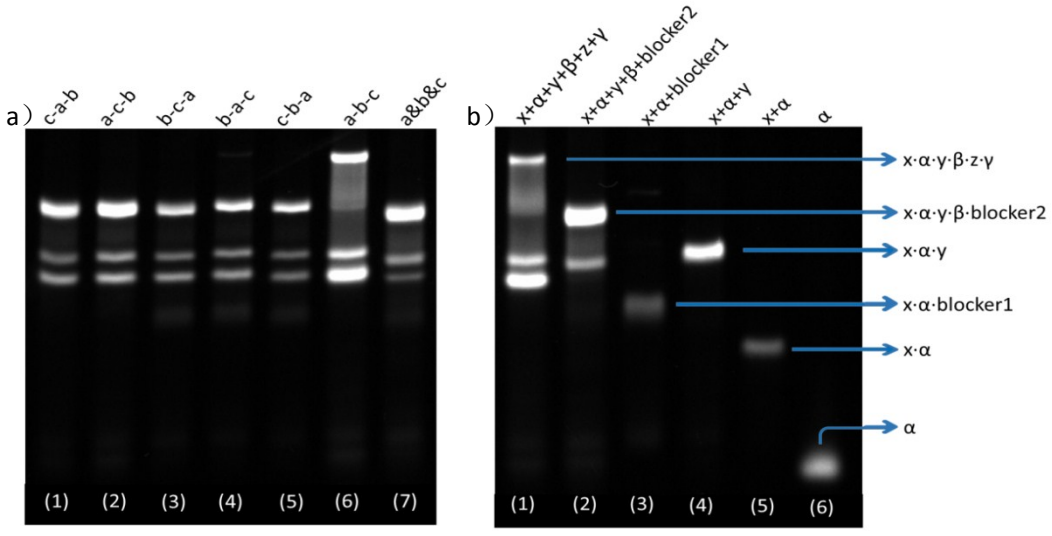

Figure S4 : a) In the top is the input sequences of input a, b and c. The strap in Channel 7 is the result of concurrent input of a, b and c. b) In the top is the adding sequences of marker DNA. The DNA sequences corresponding to straps in each channel are in the right. α, β and γ concentration are 1μM and other DNA concentration are 2μM.

Step	State			Input：abc					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	1	0	0	0	0	0	0
2	0	0	1	0	1	1	0	0	1	0
3	0	0	0	0	0	1	1	1	1	1
4	0	0	0	0	0	1	0	1	1	1

Step	State			Input：acb					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	1	0	0	0	0	0	0
2	0	0	1	0	0	0	1	1	1	0
3	0	0	1	0	1	1	1	1	1	0
4	0	0	1	0	0	1	1	0	1	0

Step	State			Input：bac					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	0	1	1	0	0	0	0
2	1	1	1	1	1	1	0	0	0	1
3	0	1	1	0	1	0	1	1	0	1
4	0	0	1	0	0	0	1	0	0	0

Step	State			Input：bca					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	0	1	1	0	0	0	0
2	1	1	1	0	1	1	1	1	0	1
3	1	0	1	1	0	1	1	0	0	0
4	0	0	1	0	0	0	1	0	0	0

Step	State			Input：cab					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	0	0	0	1	1	0	0
2	1	1	1	1	0	0	1	1	0	0
3	0	0	1	0	1	1	1	1	1	0
4	0	0	1	0	0	1	1	0	1	0

Step	State			Input：cba					Output State	
	x	y	z	α	β	blocker1	γ	blocker2	FAM	ROX
1	1	1	1	0	0	0	1	1	0	0
2	1	1	1	0	1	1	1	1	0	0
3	1	0	1	1	0	1	1	0	0	0
4	0	0	1	0	0	0	1	0	0	0

Figure S5 : State transition table under different sequences inputs.