Electronic supplementary 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 was purchased 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'）
A11	ACCACCACAAATAAAATAAAACAAAATAAACAAGAGAATAGAGAGAGA GAAGAGAGATCCCCCCCCCCCCCCCCCCC
A12	TTGTTTATTTTGTTTTATTTTATTTTCACCCTACCACCCACACCC
A13	ATCTCTCTTCTCTCTCTCTATTCTCCTCCTTTCCCTTCCTTCCCG
A21	GTCAATGTAAAATAATAAGAAAGAAAGAAAAGAGGTGGTGTGTGTGTG TGTATAGTAGCCCCCCCCCCCCCCCCCCC
A22	TCTTTTCTTTCTTTCTTATTATTTTCCTTCTTCTTCCTTCTTCTT
A23	ACACACAACACACACACAAACTACTATACACACACACACACCACC
B1	TATAGTAGTTTGTGTGTGTGTTGTGTGTCGGGAAGGAAGGGAAAGGAG GAGAATAG
B2	ACACACAACACACACACAAACCCCCCCCCCCCCCCCCCC
B3	CCCCCCCCCCCCCCCCCCCCCTCCTTTCCCTTCCTTCCCG
R11	TCACCCTACCACCCACACCC
R12	GGGTGTGGGTGGTAGGGTGAAAATAAAA
R21	CCTTCTTCTTCCTTCTTCTT
R22	AAGAAGAAGGAAGAAGAAGGAAAATAAT
IA	ATCTCTCTTCTCTCTCTCTATTCTCTTGTTTATTTTGTTTTATTTTATTTG TGGTGGT
IB	CTACTATACACACACACACCACCTCTTTTCTTTCTTTCTTATTATTTT ACATTGAC
CY5-R22	CY5-AAGAAGAAGGAAGAAGAAGGAAAATAAT
R21-BHQ-3	CCTTCTTCTTCCTTCTTCTT-BHQ-3
ROX-R12	ROX-GGGTGTGGGTGGTAGGGTGAAAATAAAA
R11-BHQ-2	TCACCCTACCACCCACACCC-BHQ-2
B3*-BHQ-1	CCCCCCCCCCCCCCCCCCCCCTCCTTTCCCTTCCTTCCCG-BHQ-1
BHQ-1-B2*	BHQ-1-ACACACAACACACACACAAACCCCCCCCCCCCCCCCCCC
B1-FAM	TATAGTAGTTTGTGTGTGTGTTGTGTGT(-FAM） CGGGAAGGAAGGGAAAGGAGGAGAATAG

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

Experimental Section

DNA powder was centrifuged and dissolved with PBS buffer solution (pH=7.4, c (NaCl) =800 mM).

Native polyacrylamide gel electrophoresis (native-PAGE)

Prepare 10 mL of 12.0% native polyacrylamide gel in room temperature with 4 mL of 30% acrylamide (Acryl/Bis solution (29:1), 30% (w/v)), 4 mL ultrapure water (18.25 MΩ), 2 mL 5x TBE，5 μL TEMED，50 μL of 10% ammonium persulfate and put it on the gel plate for 45 min standing to form 12.0% 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 1μL of 100X SYBR green I, and then the mixture was added into the gel for electrophoresis. A 12.0% 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 120 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.

Fluorescence spectra measurement

To prepare the Gate A1, Gate A2 and Gate B, A11 strand was mixed with A12 strand and A13 strand in assay buffer. And A21 strand was mixed with A22 strand and A23 strand in assay buffer. B1 strand was mixed with B2 strand and B3 strand in assay buffer. Then, the mixtures were annealed by heating to 95℃ for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the logic circuits. The prepared Gate A1, Gate A2 and Gate B were used as the platform directly for Fluorescence assays. The final concentration of 40 nM Gate A1, 40 nM Gate A2, 20 nM Gate B, 40 nM Reporter 1, 40 nM Reporter 2, 80 nM IA, 80 nM IB.

The outputs of the cascade circuits without redundant module are mainly the fluorescence produced by FAM and the outputs of the cascade circuits with redundant module are mainly the fluorescence produced by ROX, CY5 and FAM. The emission spectra of FAM, ROX and CY5 were collected at 517 nm with the excitation wavelength of 495 nm, 604 nm with the excitation wavelength of 595 nm and 663 nm with the excitation wavelength of 645 nm, respectively. The slit widths for the excitation and emission were 2 nm. FluoroMax-4 fluorescence spectrometer was used for the detection.

1. The native-PAGE results of interaction between logic gates

To verify the stability of the logic gate in the cascade circuit, the logic gates are reacted with each other in different combinations. The native-PAGE result (Figure S1) shows that they do not react and interfere with each other, confirming that the logic gates have high stability, and thus there is no influence on circuit computing.

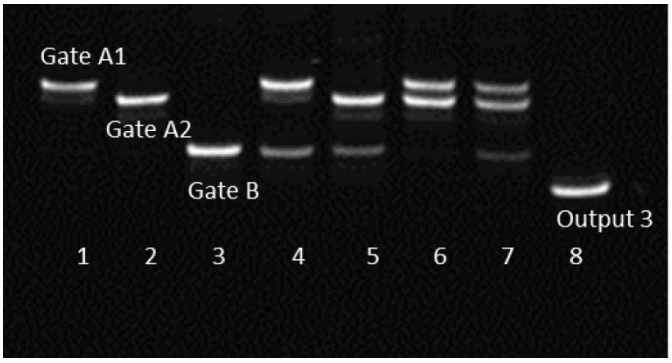

Figure S1. The 12% native-PAGE results of the markers and the reactions of Gates. Lane 1-8: Gate A1, Gate A2, Gate B, Gate A1+Gate B, Gate A2+Gate B, Gate A1+Gate A2, Gate A1+Gate A2+Gate B, Output 3.

2. The native-PAGE results of interaction between logic gates and Reporter.

To verify that the logic gate and the reporter do not interact with each other, the logic gates are reacted with corresponding reporter. The native-PAGE result (Figure S3) shows that they do not react and interfere with each other, confirming that the logic units in the cascade circuits can co-exist.

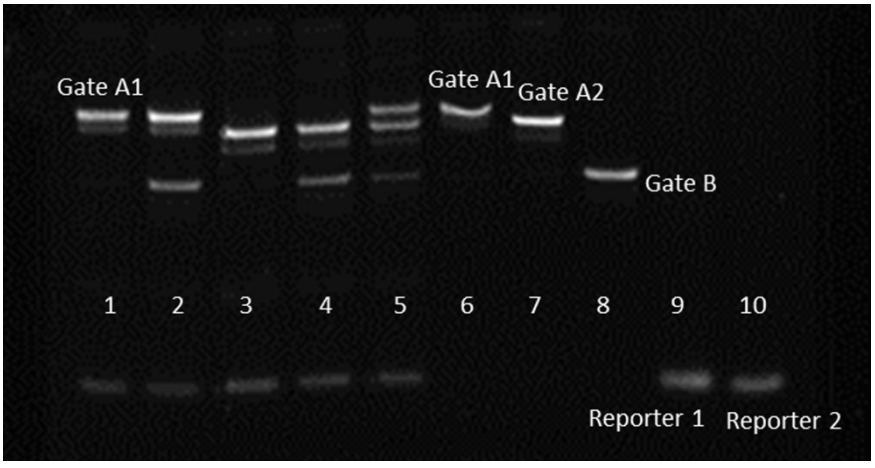

Figure S2. The 12% native-PAGE results of the markers and the reactions of Gates and Reporter. Lane 1-10: Gate A1+Reporter 1, Gate A1+Reporter 1+Gate B, Gate A2+Reporter 2, Gate A2+Reporter 2+Gate B, Gate A1+Reporter 1+ Gate A2+Reporter 2+Gate B, Gate A1, Gate A2, Gate B, Reporter 1, Reporter 2.

3. The schematic illustration of the molecular cascade logic circuit without redundant module.

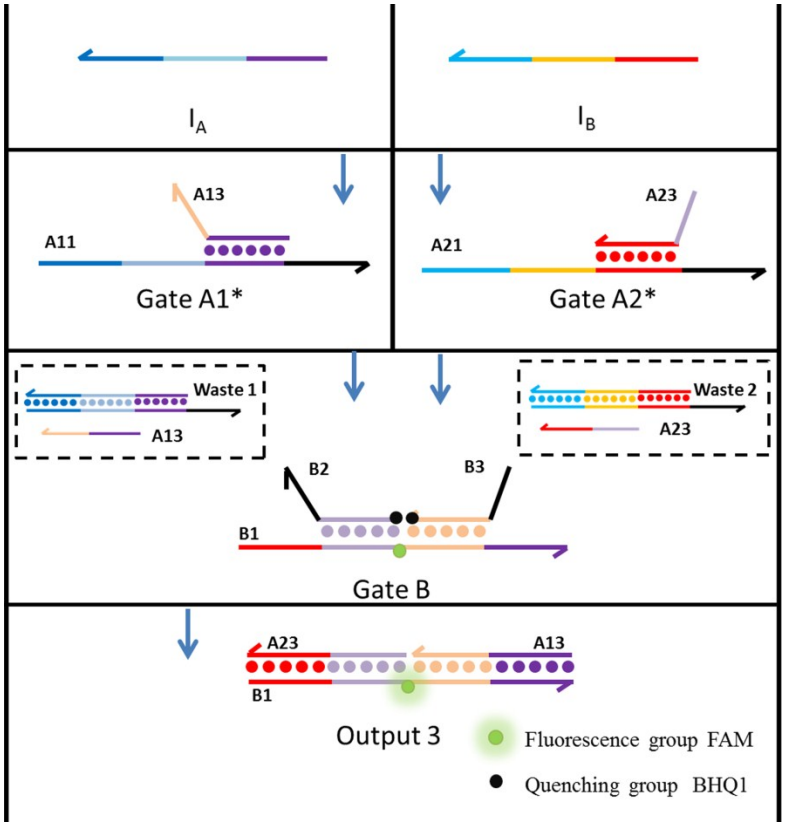

Figure S3 The schematic illustration of the molecular cascade logic circuit without redundant module.

4. The operating result of each stage of cascade circuit without redundant module

To verify whether the reaction result of each stage of the cascade circuit without redundant modules conforms to the schematic diagram, native-PAGE tests were conducted. The result shows that only introducing IA and IB simultaneously, the circuit would obtain output 3.

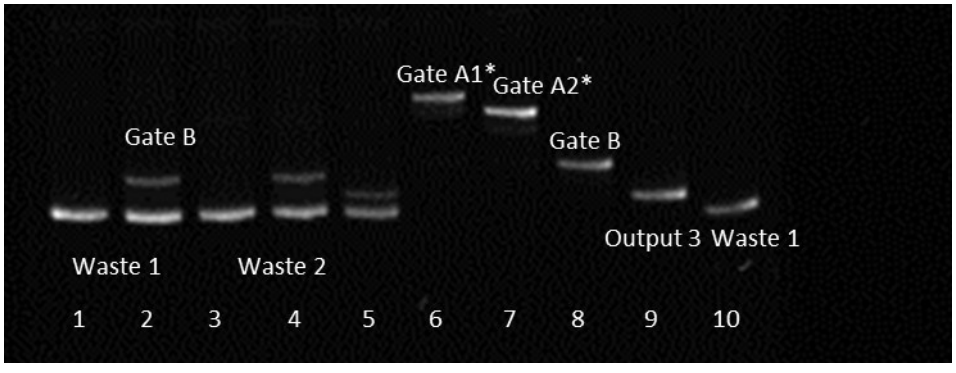

Figure S4 The 12% native-PAGE results of the markers and step-by-step reaction results of the cascade circuit without redundant module. Lane 1-10: Gate A1*+IA, Gate A1*+IA+Gate B, Gate A2*+IB, Gate A2*+IB+Gate B, Gate A1*+IA+ Gate A2*+IB+Gate B, Gate A1*, Gate A2*, Gate B, Output 3, Waste 1.

5. The operating result of each stage of cascade circuit with redundant module

To verify whether the reaction result of each stage of the cascade circuit with redundant modules conforms to the schematic diagram, native-PAGE tests and fluorescence experiment were conducted. The result shows that only introducing IA and IB simultaneously, the circuit would obtain output 1/2/3(Figure S5A lane 7), and a strong signal of FAM (Figure S5B).

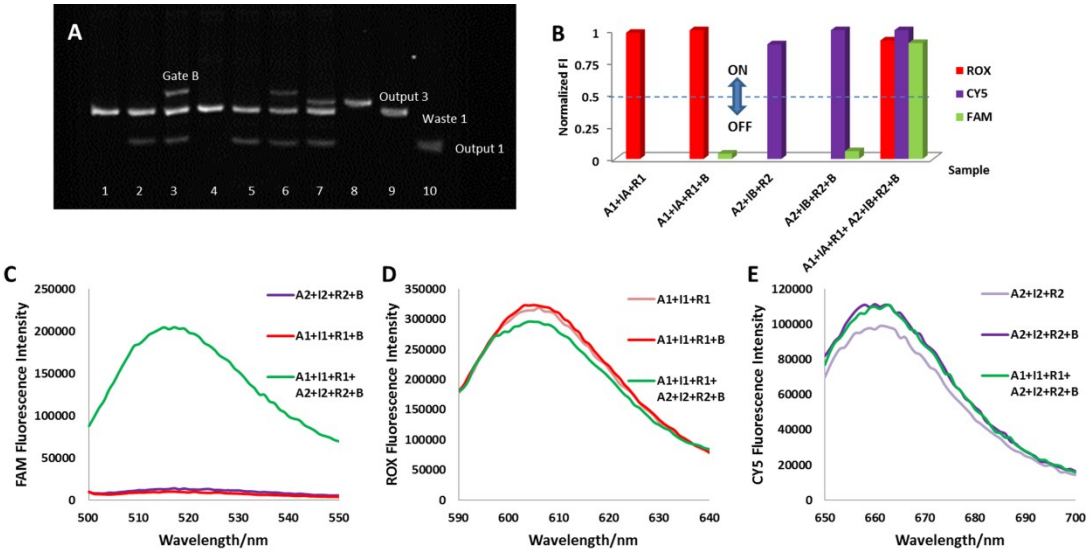

Figure S5 (A). The 12% native-PAGE results of the markers and step-by-step reaction results of the cascade circuit with redundant module. Lane 1-10: Gate A1+IA, Gate A1+IA+Reporter 1, Gate A1+IA+Reporter 1+Gate B, Gate A2+IB, Gate A2+IB+ Reporter 2, Gate A2+IB+Reporter 2+Gate B, Gate A1+IA+Reporter 1+Gate A2+IB+Reporter 2+Gate B, Output 3, Waste 1, Output 1. (B). The normalized fluorescence responses of step-by-step reaction results of the cascade circuit with redundant module. The original FAM(C), ROX (D) and CY5 (E) fluorescence responses of step-by-step reaction results of the cascade circuit with redundant module.

6. The position of Output 1/2, Waste 1/2, Reporter 1/2 in native-PAGE

The result shows that Output 1/2, Waste 1/2, Reporter 1/2 bands were on the same horizontal line due to its same molecular weight.

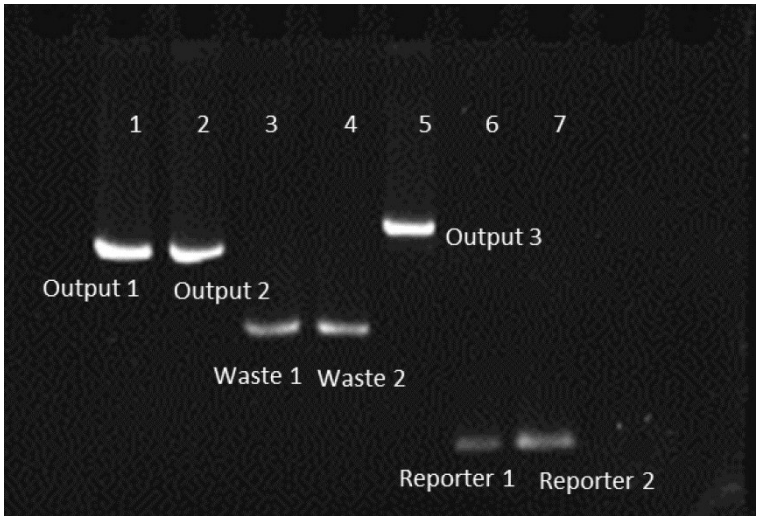

Figure S6 The 12% native-PAGE results of markers. Lane 1-7: Output 1, Output 2, Waste 1, Waste 2, Output 3, Reporter 1, Reporter 2.

7. The original fluorescence curves response of the cascade circuits without redundant module.

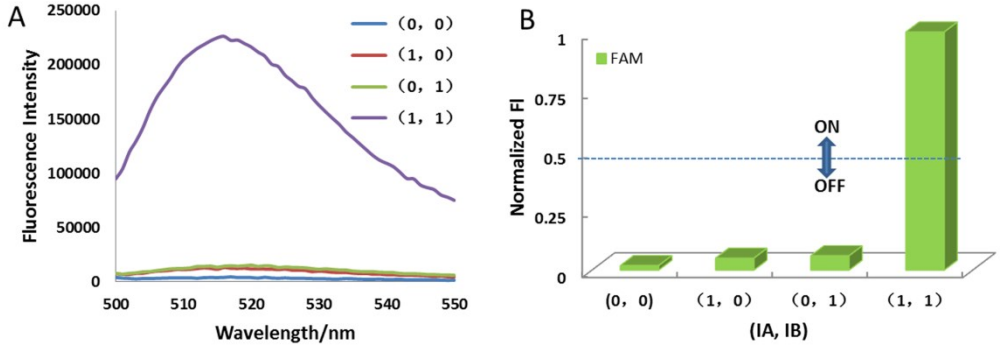

Figure S7. (A) The original FAM fluorescence curves responses of the cascade logic circuit without redundant module. (B) The normalized fluorescence responses of the cascade logic circuit without redundant module.

8. The original fluorescence curves response of the cascade circuits with redundant module.

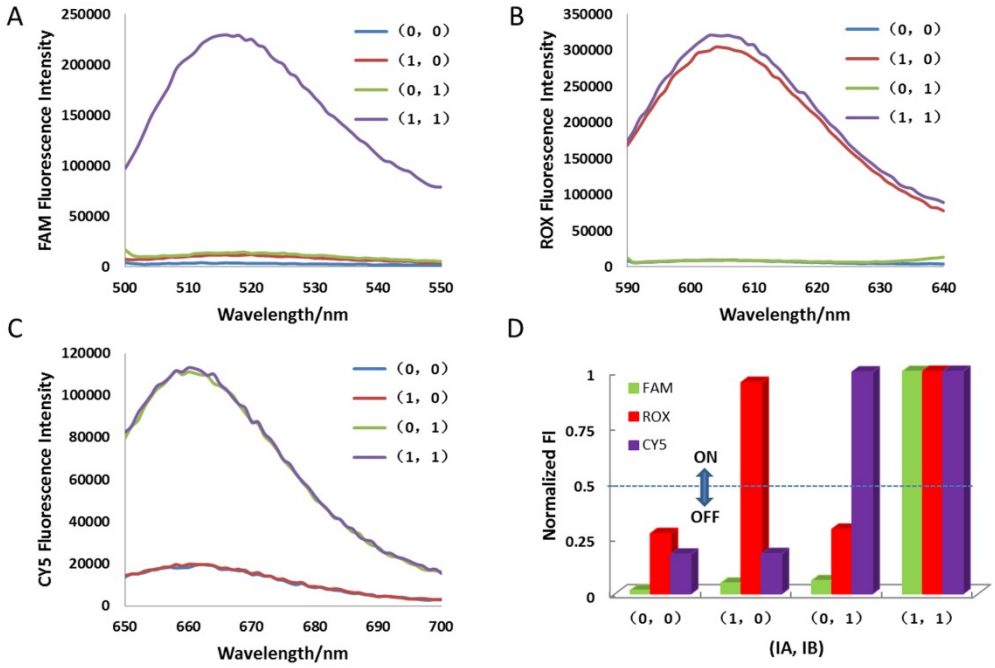

Figure S8. The original FAM (A), ROX (B), CY5(C) fluorescence curves responses of the cascade logic circuit with redundant module. (D) The normalized fluorescence responses of the cascade logic circuit with redundant module.