Electronic Supplementary Information

Half adder and half subtractor logic gates based on nicking enzymes

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Reagents and Materials

Nicking enzyme Nt.BbvCI, Nb.BtsI and CutSmart buffer were purchased from New England Biolabs Inc. All Oligonucleotides (HPLC grade) and 10× TAE buffer were obtained from Sangon Biological Engineering Technology Co. Ltd., and all detailed sequence information is shown in Table S1. All chemicals used were of analytical reagent without further purification. All solutions were prepared using ultrapure water (18.2 MΩ/cm) obtained from a Milli-Q purification system.

Table S1. DNA sequences used in this investigation.

Strands	**Sequences**
ZG	CATCTGTTCCTTGCAGTTGCTGAGGTTCATCACTGCTTGTTTTCAAGCAGTGATGAA
YG	CATCCTAAGTTGTGTAGCAGTGAGGTCCTCAGCAGACTTTTGTCTGCTGAGGACC
a	CATTGTTCATTGTATTGCTGAGGT
c*a*	GTGGACCTCAGCAATACAATGAACAATG

ac	CATTGTTCATTGTATTGCTGAGGTCCAC
acm	CATTGTTCATTGTATTGCTGAGGTCCACCACTGCTTG
m*c*	CAAGCAGTGGTGGA
d	CATCCTAAGTTGTGTAGCAGTGAGG
f*d*	GGACCTCACTGCTACACAACTTAGGATG
df	CATCCTAAGTTGTGTAGCAGTGAGGTCC
dfn	CATCCTAAGTTGTGTAGCAGTGAGGTCCTCAGCAGAC
n*f*	GTCTGCTGAGGAC
b	CTATCCATCTAACTATCCATCCTCAGCT
b*	AGCTGAGGATGGATAGTTAGATGGATAG
bs-b	CTATCCATCTAACTATCCATCCTCATAT
bs-bg	CTATCCATCTAACTATCCATCCTCATATCACTGCTTCAAT
g*b_2*	ATTGAAGCAGTGATATGAG
e_1	GTGTAGAGTTGAATGCTGAGGT
e*	CACCACCTCAGCATTCAACTCTACAC
e	GTGTAGAGTTGAATGCTGAGGTGGTG
eh	GTGTAGAGTTGAATGCTGAGGTGGTGCACTGCAATTTC
h*e_2*	GAAATTGCAGTGCACCA
b-0	ACTATCCATCTAACTATCCATCCTCAGC
b*-0	GCTGAGGATGGATAGTTAGATGGATAGT
BT-0	ACTATCCATCTAACTATCCATCCTCAGCCACTGCTTTTTTAAGCAGTGGC
b_1-0	ACTATCCATCTAACTATCCATCC

b-1	ACTATCCATCTAACTATCCATCCTCAGCT
b-2	ACTATCCATCTAACTATCCATCCTCAGCTT
b*-1	AGCTGAGGATGGATAGTTAGATGGATAGT
b*-2	AAGCTGAGGATGGATAGTTAGATGGATAGT

Apparatus

The measurement of DNA sample concentration was conducted using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc. USA), and the absorption intensities were measured at 260 nm. Gel images were obtained using a Gel Doc XR+ system, Bio-RAD (USA). All spectrofluorometric measurements were recorded with a real-time PCR machine(Agilent, G8830A) equipped with a 96-well fluorescent plate reader.

Results and Discussion

We first tested the persistence of the two nicking enzymes at 37°C. In fact, we tried the experiment of enzyme cutting substrates at different temperatures. Our experimental results showed that at 37°C, the cutting rate of the two enzymes was the closest, and the substrate after cutting met our requirements for subsequent reactions (see Fig. S2). We made Nt.BbvCI cut substrate ZF first. The reaction time was 60 minutes, and then substrate YG was directly added to the reaction system for another 60 minutes. The reaction process is shown in Fig. S1A. We also used substrates YF and ZG to perform a similar test on Nb.BtsI. Finally, we observed that the degree of cutting was consistent with each enzyme nicking a substrate separately. The experimental PAGE results are shown in lane 1 to lane 10 of Fig. S1D. These results showed that the results from two substrates being cut by two enzymes were consistent with each of those of two substrates cutting separately. Thus, they both met our persistence requirements. This ensured that the nicking enzymes chosen did not play a single role in our reaction system during the subsequent construction of logic gates, but rather could simultaneously change multiple substrates.

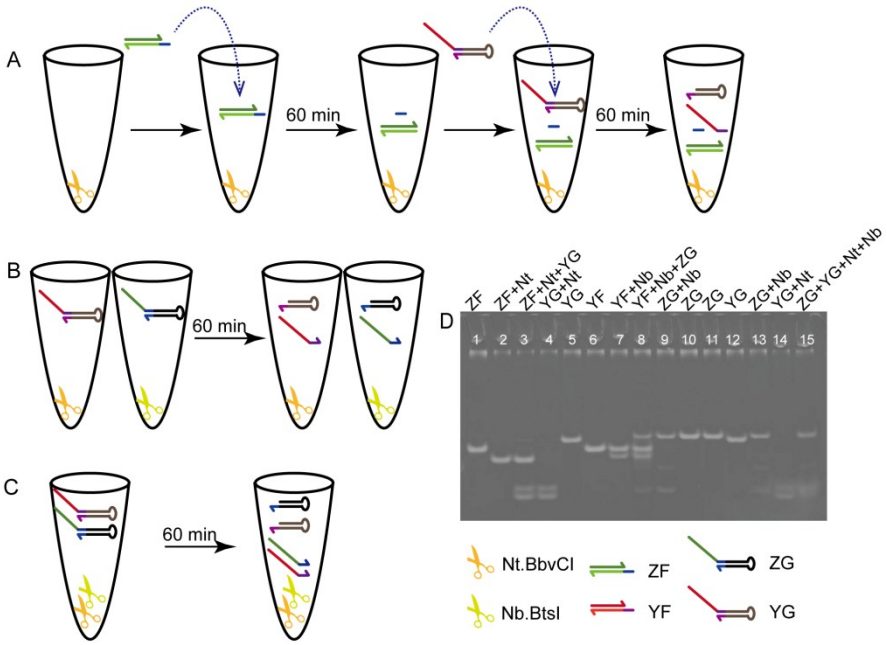

Fig. S1. (A) The experimental procedure for continuous substrate cutting with nicking enzymes. (B) Schematic diagram of Nt.BbvCI cutting substrate YG and Nb.BtsI cutting substrate ZG separately. (C) Schematic diagram of Nt.BbvCI and Nb.BtsI cutting substrate ZG and YG together. (D) Native PAGE for performance testing of the persistence and compatibility of these nicking enzymes. Lane 1: ZF; Lane 2: ZF and Nt; Lane 3: ZF, Nt and YG; Lane 4: YG and Nt; Lane 5: YG; Lane 6: YF; Lane 7: YF and Nb; Lane 8: YF, Nb and ZG; Lane 9: ZG and Nb; Lane 10: ZG; Lane 11: ZG; Lane 12: YG; Lane 13: ZG and Nb; Lane 14: YG and Nt; Lane 15: ZG, YG, Nt and Nb. All reaction mixtures were allowed to react at 37°C for 3 h. Also include the name of the substrate used in the experiment.

We then tested the compatibility of our selected nicking enzymes. Due to the limitations of logical function, the sequence of substrates inevitably will be somewhat similar. Under these conditions, the compatibility between the two nicking enzymes was a key challenge that needed to be overcome. We first cut substrates YG and ZG with both Nt.BbvCI and Nb.BtsI (Fig. S1B). The two enzymes were then mixed to cut the mixture of YG and ZG (Fig. S1C). We then compared the experimental PAGE results of these two cutting schemes (lane 11 to lane 15 in Fig. S1D). These results showed that the compatibility of the two enzymes was up to the standard anticipated and demonstrated that they could work in one reaction system at the same time. This set the stage for nicking to act as a two-way input signal for a logic gate. This also made it possible to build a logical operating system based on a nicking enzyme platform.

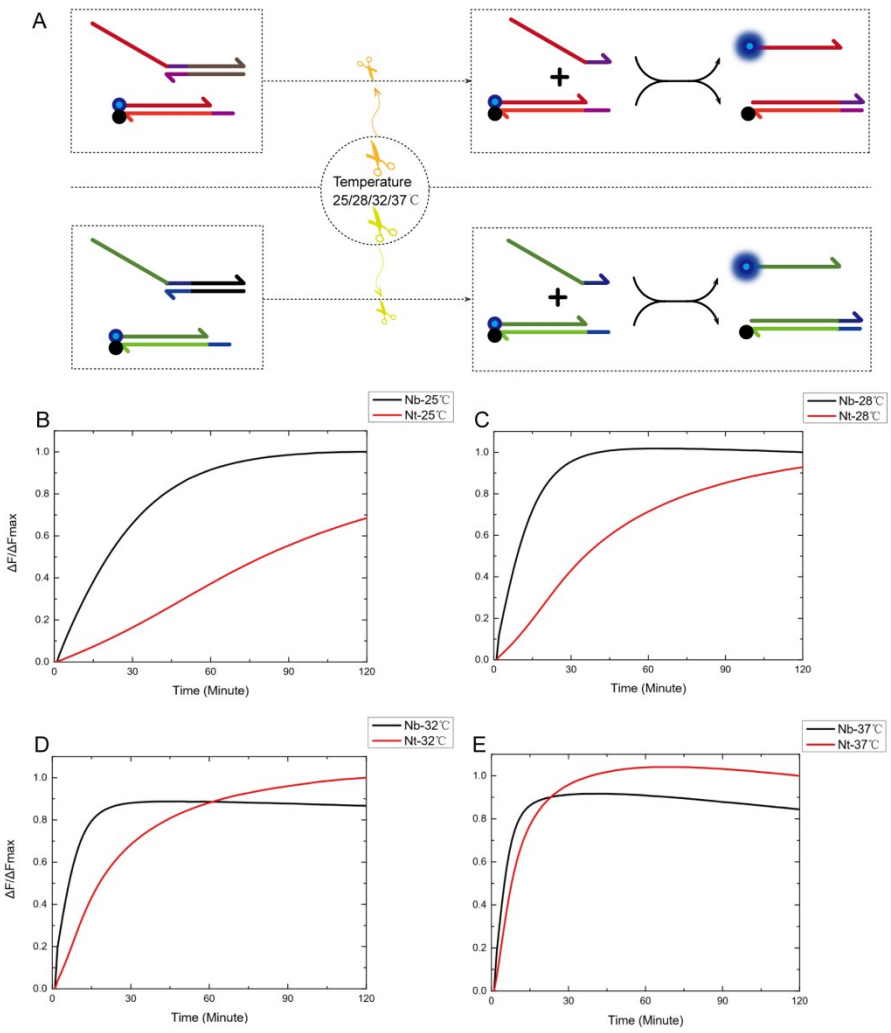

Fig. S2. (A) Schematic diagram of the experimental reaction process. (B) Kinetic characterization of the cutting efficiency of nicking enzymes at 25°C. (C) Kinetic characterization of the cutting efficiency of nicking enzymes at 28°C. (D) Kinetic characterization of the cutting efficiency of nicking enzymes at 32°C. (E) Kinetic characterization of the cutting efficiency of nicking enzymes at 37°C.

To determine the efficiency of Nt.BbvCI and Nb.BtsI at different temperatures, we designed the following experiment. We mixed a solution of the substrates YF and YT and the other two substrates ZF and ZT in two test tubes, respectively. When substrate YT and ZT were cut, a chain displacement reaction occurred with substrate YF and ZF, respectively, and the fluorescence intensity increased. We then added the nicking enzymes Nt.BbvCI and Nb.BtsI to the two test tubes at different temperatures, respectively. The rate of increase in fluorescence intensity was then measured. Fig. S2A is a schematic diagram of the experimental reaction process. Enzyme Nt.BbvCI cuts substrate YT, and then the cut YT will undergo chain displacement reaction with YF, resulting in a fluorescence intensity increase. Enzyme Nb.BtsI will cut substrate ZT, and then the cut ZT will undergo chain replacement reaction with ZF. Two enzymes were added to two test tubes at the same time. Results from different temperatures are shown in Fig. S2B-S2E. These

results show that the efficiency of the two enzymes was closest at 37°C.

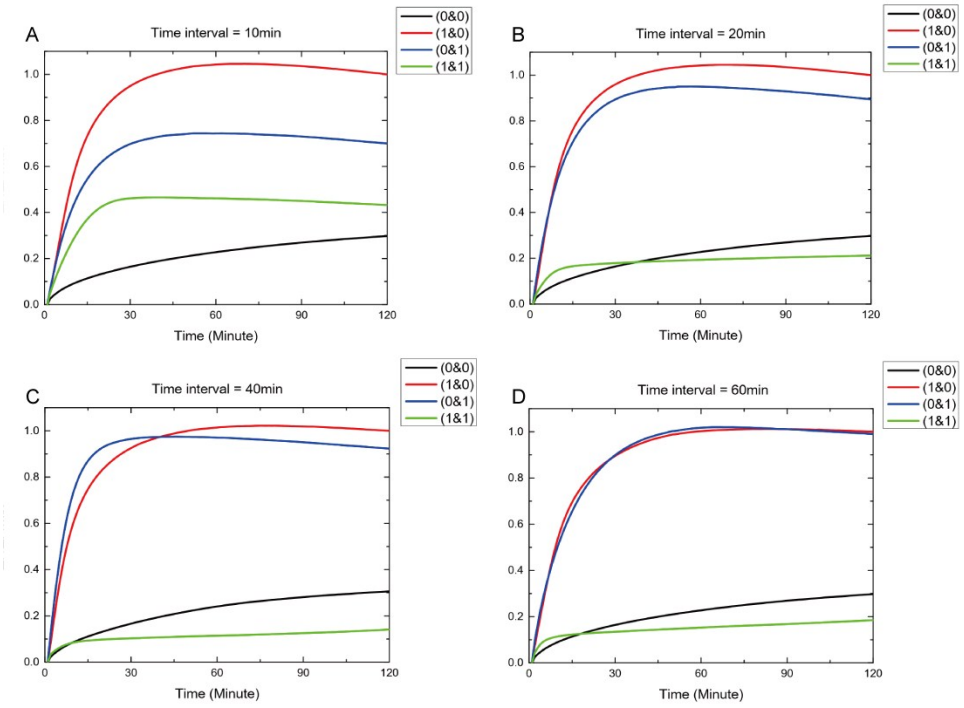

Fig. S3. (A) Kinetic characterization of condition over a 10 min time interval. (B) Kinetic characterization of condition when the time interval was 20 min. (C) Kinetic characterization of condition when the time interval was 40 min. (D) Kinetic characterization of condition when the time interval was 60 min.

Fig. S3 focuses on how we chose the appropriate time interval for incubation. We set the time interval from 10–60 minutes. When the time interval was 10 min, there was no obvious threshold limit for the XOR logical operation, which was not a usable result. When the time interval was 20 min, the XOR logical operation already demonstrated a clear threshold limit. At a time interval of 40 min, the thresholds for XOR logical operations become clearer. When the time interval is 60 min, the results for the XOR logical operation were the most optimal and were chosen for further use.

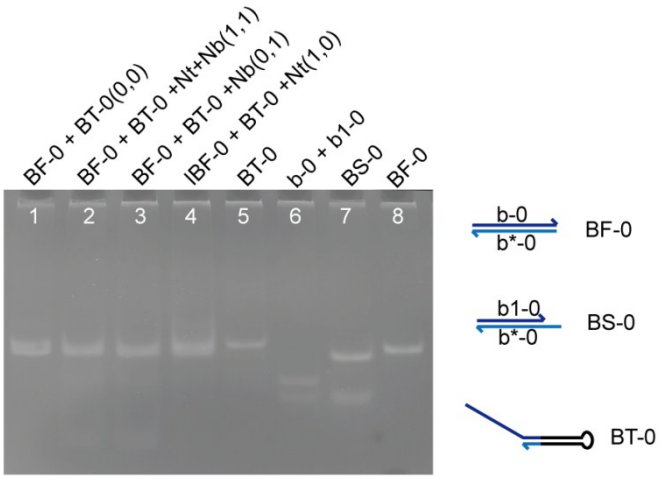

Fig. S4. Native PAGE for the first version of the AND logic gate. Lane 1: BF-0 and BT-0; Lane 2: BF-0, BT-0, Nt and Nb; Lane 3: BF-0, BT-0 and Nb; Lane 4: BF-0, BT-0 and Nt; Lane 5: BT-0; Lane 6: strand b-0 and strand b_1-0; Lane 7: BS-0; Lane 8: BF-0.

When designing an AND logic gate, we encountered some problems due to DNA sequence. As shown in Fig. S4, there was little difference between an input of 1 & 1 and an input of 0 & 1. There also was no difference when the input was 1 & 0 and when the input was 0 & 0. These results indicated that Nt.BbvCI as input 1 didn't work. Therefore, we examined the sequence of substrate BF-0. The recognition site for enzyme Nt.BbvCI was exposed on one end of BF-0. We hypothesized that in this reaction system, the recognition site for enzyme Nt.BbvCI had failed. Therefore, we add encapsulation to the recognition site on BF-0. We tried BF-1, which encapsulated one pair of bases, and BF-2, which encapsulated two pairs of bases (Fig. S5), and results show that the failure of enzyme Nt.BbvCI was effectively solved through additional encapsulation.

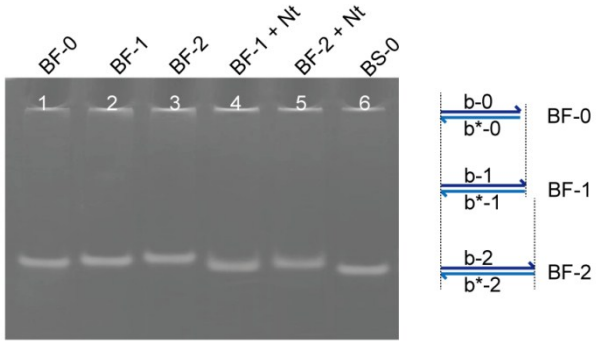

Fig. S5. Native PAGE for the modification of substrate BF. Lane 1: BF-0; Lane 2: BF-1; Lane 3: BF-2; Lane 4: BF-1 and Nt; Lane 5: BF-2 and Nt; Lane 6: BS-0.

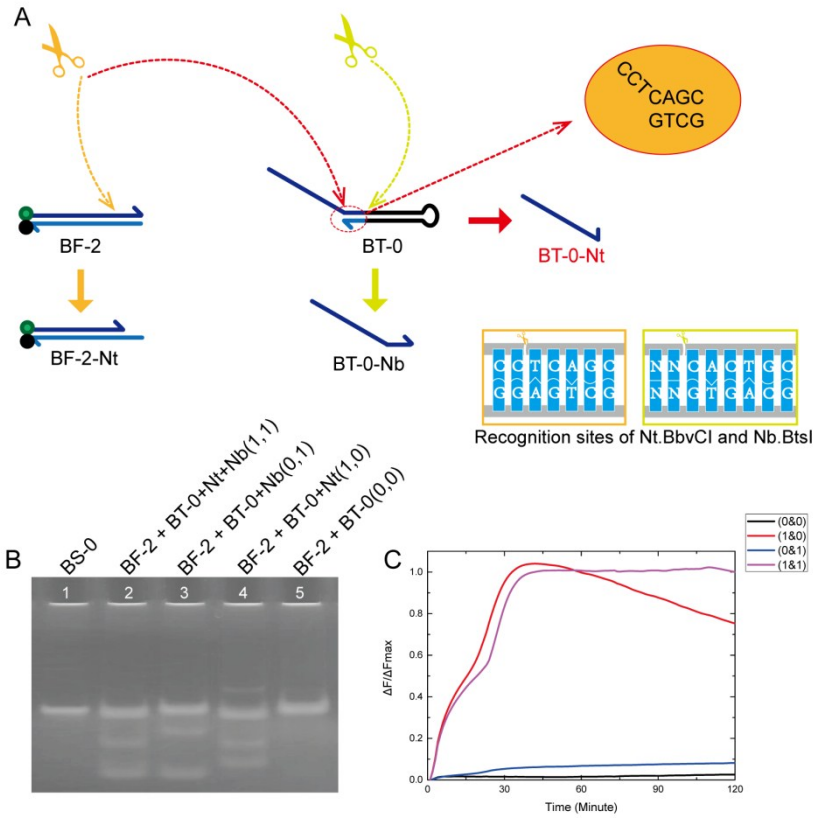

Fig. S6. (A) Schematic of the sequence problem of the substrate BT-0. The substrate BT-0 can be catalyzed by two enzymes, producing different products, namely, BT-0-Nb and BT-0-Nt. The bottom right hand corner is the recognition sites of Nt.BbvCI and Nb.BtsI. (B) Native PAGE for the second version of the AND logic gate. Lane 1: BS-0; Lane 2: BF-2, BT-0, Nt and Nb; Lane 3: BF-2, BT-0 and Nb; Lane 4: BF-2, BT-0 and Nt; Lane 5: BF-2 and BT-0. (C) Kinetic characterization of the second version of the AND logic gate.

However, we encountered a new problem in subsequent design. As shown in Fig. S6A, the overlapping sequence of BT-0 and BF-2 was exactly recognition site for Nt.BbvCI, although the recognition site on substrate BT-0 was incomplete. Unfortunately, there was still a lot of substrate BT-0 that was cut by the Nt.BbvCI, which was undesirable. The results of gel electrophoresis are shown in Fig. S6B. The substrate BT-0 behaved abnormally and was indeed nicked by Nt.BbvCI. Kinetic characterizations are shown in Fig. S6C. Our design supposes it is reasonable to have a reaction without any enzyme (0 & 0) and a reaction with only Nb.BtsI (0 & 1). In the first half of the reaction (1 & 1) in which both Nt.BbvCI and Nb.BtsI were involved, there were two stages of rapid rise rate, because the rate of the chain replacement reaction was jointly affected by the efficiency of the substrates catalyzed by the two nicking enzymes. To make matters more complicated, the substrate BT-0 was catalyzed by both enzymes at the same time, and the two catalytic products BT-0-Nt and BT-0-Nb were also involved in the subsequent reaction. Reactions involving only Nt.BbvCI (1 & 0) were the most problematic. As the substrate BT-0 was catalyzed by Nt.BbvCI, the curve of the first half was similar to that of (1 & 1). As the catalytic product BT-

0-Nt was not driven by toehold in the subsequent chain replacement reaction, the reaction was carried out in reverse and only slowly reached equilibrium.

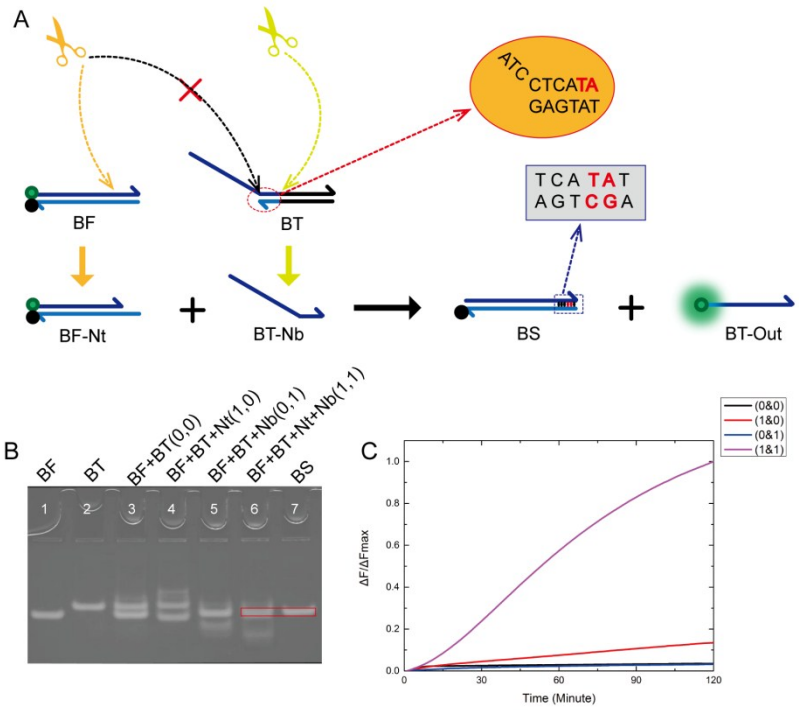

Fig. S7. (A) Schematic diagram of substrate BT sequence modification. The substrate BT was only catalyzed by Nb.BtsI to produce BT-Nb. The substitution reaction produced BS, the right base of which was not completely complementary (shown in red). (B) Native PAGE for the final version of the AND logic gate. Lane 1: BF; Lane 2: BT; Lane 3: BF and BT; Lane 4: BF, BT and Nt; Lane 5: BF, BT and Nb; Lane 6: BF, BT, Nt and Nb. (C) Kinetic characterization of the final version of the AND logic gate.

Thus, we modified the sequence of substrate BT. As shown in Fig. S7A, substrate BT was not catalyzed by Nt.BbvCI, and the changed sequence is marked in red. Such changes improved the signal leak, but at a cost. BT-Nb and BF-Nt were not completely complementary, leading to a significant reduction in the rate of chain replacement reactions. The right side of the chain replacement product BS was not completely complementary. From Fig. S7C it can be seen that the reaction involving Nt.BbvCI alone had a normal curve. The curve of the reaction in which both Nt.BbvCI and Nb.BtsI were involved became smooth and slow, leading to a logic system that was more stable.

References

1. X. D. Zheng, J. Yang, C. J. Zhou, C. Zhang, Q. Zhang, X. P. Wei, Nucleic Acids Res., 2019, **47**, 1097-1109.

2. X. D. Lin, Y. Q. Lin, J. K. Deng, Y. L. Lyu, P. C. Qian, Y. F. Li, S. Wang, Chem. Sci.,2018, **9**, 1774-1781.