Electronic Supplementary Information

Engineering High-Robustness DNA Molecular Circuits by Utilizing Nucleases

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Experimental Section

Materials. All the oligonucleotides used in this work were synthesized by Sangon Co. (Shanghai, China) and their sequences are listed in **Table S1**. The modified oligos were purified by HPLC, while the unmodified oligos were purified by PAGE. APE1, λ exo, Exo III and their corresponding buffers were obtained from NEB (Ipswich, MA). All chemicals were used as received without additional purification. DNase/RNase free deionized water from Tiangen Biotech Co. (Beijing, China) was used in all experiments.

Monitoring the performance of DNA circuits by fluorescence measurement. In a typical fluorescence experiment, the oligos (final concentration 100 nM) as the building blocks of the DNA circuits were annealed from 85 °C to 37 °C within 10 min in 1×Tris/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, and 10 mM magnesium acetate, pH 8.0) for APE1, in 1×ThermoPol buffer (NEB) for λ exo. The modification of buffer was explained in figure legends. The enzymes were introduced. Once inputs (nucleic acids or others) were added, fluorescence was recorded immediately in the FAM channel (ex: 480 nm, em: 510 nm) of a real-time PCR cycler (Rotor-Gene Q, QIAGEN, Germany) at 37 °C, using a gain of 8, with a time interval of 5 s.

Characterization of operational products of DNA circuits by gel electrophoresis. Native PAGE was used to characterizing the products of the λ exo-based circuits. To a 200 μL PCR tube, 100 nM of the as-prepared DNA circuits, 0.56 nM of λ exo, and inputs were mixed well and incubated in the 1×ThermoPol buffer at 37 °C for 1 h. 4 mM EDTA (final concentration) was used to terminate the reaction. The products were loaded on a 20% native PAGE gel (19:1, acrylamide: bisacrylamide). The gel was run at 4 °C at a constant voltage of 160 V and subsequently stained with SYBR Gold. Denaturing PAGE was used to characterizing the products of the APE1-based circuits. To a 200 μL PCR tube, 100 nM of the prepared DNA circuit, inputs, and 0.65 nM of APE1 were mixed well and incubated in the 1×Tris/Mg^{2+} buffer at 37 °C for 2 h. EDTA was also used to terminate the reaction. The solution was heated to 95 °C, then immediately chilled by transferring the samples on ice. The 20% denaturing gel (19:1, acrylamide: bisacrylamide, urea 8.3 M) was pre-run for 30 min. All samples were loaded on the gel,

which was run at room temperature for 2 h at a constant voltage of 160 V and stained with SYBR Gold.

Table S1 Sequence of the oligonucleotides in this work

Figure	Sequence color/Name	Sequence (5'- 3')
1A	Red	ACT(FAM)AGTAG**X**ACTGCTCACAC(BHQ1)
1A	Green 1 (10bp)	GCAGTGCTAC
1A	Green 2 (10bp 1mis)	GCTGTGCTAC
1A	Green 3 (11bp)	AGCAGTGCTAC
1A	Green 4 (11bp 1mis)	AGCTGTGCTAC
1A	Green 5 (12bp)	GAGCAGTGCTAC
1A	Green 6 (12bp 1mis)	GAGCTGTGCTAC
1A	Green 7 (13bp)	GAGCAGTGCTACT
1A	Green 8 (13bp 1mis)	GAGCTGTGCTACT
1A	Green 9 (14bp)	TGAGCAGTGCTACT
1A	Green 10 (14bp 1mis)	TGAGCTGTGCTACT
1A	Green 11 (16bp)	GTGAGCAGTGCTACTA
1A	Green 12 (18bp)	TGTGAGCAGTGCTACTAG
1A	Green 13 (20bp)	GTGTGAGCAGTGCTACTAGT
2A	Red	ACT(FAM)AGTAG**X**ACTGCTCACAC(BHQ1)
2A	Green 1 (region C 15bp)	ATCGCGCTGTCACAGTGCTACTAGT
2A	Green 2 (region C 10bp)	GCTGTCACAGTGCTACTAGT
2A	Green 3 (region C 8bp)	TGTCACAGTGCTACTAGT
2A	Green 4 (region C 7bp)	GTCACAGTGCTACTAGT
2A	Green 5 (region C 6bp)	TCACGGTGCTACTAGT
2A	Green 6 (region C 5bp)	CACAGTGCTACTAGT
2A	Green 7 (region C 4bp)	ACAGTGCTACTAGT
2A	Green 8 (region C 3bp)	CGGTGCTACTAGT
2A	Orange 1 (region C 15bp)	GTGTGAGCAGCTGTGACAGCGCGAT
2A	Orange 2 (region C 10bp)	GTGTGAGCAGCTGTGACAGC
2A	Orange 3 (region C 8bp)	GTGTGAGCAGCTGTGACA
2A	Orange 4 (region C 7bp)	GTGTGAGCAGCTGTGAC
2A	Orange 5 (region C 6bp)	GTGTGAGCAGCCGTGA
2A	Orange 6 (region C 5bp)	GTGTGAGCAGCTGTG
2A	Orange 7 (region C 4bp)	GTGTGAGCAGCTGT
2A	Orange 8 (region C 3bp)	GTGTGAGCAGCCG
2C	Red	ACT(FAM)AGTAG**X**ACTGCTCACAC(BHQ1)
2C	Green 1 (region C 6bp)	CACTACCTTCACCTTTTTTTTTTTTTTTTTGGGGGGTGCTACTAGT
2C	Green 2 (region C 5bp)	CACTACCTTCACCTTTTTTTTTTTTTTTTTGGGGGTGCTACTAGT
2C	Green 3 (region C 3bp)	CACTACCTTCACCTTTTTTTTTTTTTTTTTGGGTGCTACTAGT
2C	Green 4 (region C 0bp)	CACTACCTTCACCTTTTTTTTTTTTTTTTTGCTACTA

2C	Orange 1 (region C 6bp)	GT GTGTGAGCAGCCCCCCTTTTTTTTTTTTTTTCACCATCCACTCTAC
2C	Orange 2 (region C 5bp)	GTGTGAGCAGCCCCCTTTTTTTTTTTTTTTCACCATCCACTCTAC
2C	Orange 3 (region C 3bp)	GTGTGAGCAGCCCTTTTTTTTTTTTTTTCACCATCCACTCTAC
2C	Orange 4 (region C 0bp)	GTGTGAGCAGTTTTTTTTTTTTTTTCACCATCCACTCTAC
2C	Purple (Input)	GTAGAGTGGATGGTGAAGGTGAAGGTAGTG
3A	Red	ACT(FAM)AGTAG**X**ACTGCTCACAC(BHQ1)
3A	Green 1 (ATP aptamer contained strand)	TGCGGAGGAAGGTTGCTACTAGT
3A	Orange 1 (ATP aptamer contained strand)	GTGTGAGCAGACCTGGGGGAGTAT
3A	Green 2 (Thrombin aptamer contained strand)	GGTTGGTGTGGTTGG TGCTACTAGT
3A	Orange 2 (Thrombin aptamer contained strand)	GTGTGAGCAGAGTCCGTGGTAGGGCAGGTTGGGGTGACT
3A	Green 3 (biotinylated strand)	(Biotin)TTTTTTTTTTTTTTTTGCTACTAGT
3A	Orange 3 (biotinylated strand)	GTGTGAGCAGTTTTTTTTTTTTTT(Biotin)
4A	T	(PO_4)CGTGCACGCTGCGACGCGACGATGCTGATCGCTGCAGATCGACTCTCTACTACAGACAGTACATCTGC(BHQ1)
4A	O	GCT(FAM)AGACGCAGATGTACTGTCTGTA
4A	I	GTAGAGAGTCGATCTGCAGCGATCAGCATCGTCGCGTCGCAGCGTGCACG
4B	T	(PO_4)CGTGCACGCTGCGACGCGACGATGCTGATCGCTGCAGATCGACTCTCTACTACAGACAGTACATCTGC(BHQ1)
4B	I1	GCATCGTCGCGTCGCAGCGTGCACG
4B	I2	GTAGAGAGTCGATCTGCAGCGATCA
4B	O	GCT(FAM)AGACGCAGATGTACTGTCTGTA
4C	T	(PO_4)CGTGCACGCTGCGACGCGACGATGCTGATCGCTGCAGATCGACTCTCTACTACAGACAGTACATCTGC(BHQ1)
4C	I1	GCGTCGCAGCGTGCACG
4C	I2	CAGCGATCAGCATCGTC
4C	I3	GTAGAGAGTCGATCTG
4C	O	GCT(FAM)AGACGCAGATGTACTGTCTGTA

4D	T1	(PO$_4$)GCAGCGTGCACGCCTGGCATTAGACGACAGACCGGCTGGGTAGTCAGCGCTACCACTCC
4D	T2	(PO$_4$)TGGGTAGTCAGCGCTACCACTGATCGCTGCAGATCGACTCTCTAC
4D	T3	(PO$_4$)CCACTTGACTACGCGCTGGTTGATCGCTGCAGATCGACTCTCTAC
4D	T4	(PO$_4$)CGTGCACGCTGCGACGCGACGATGCTGATCGCTGCAGATCGACTCTCTACTACAGACAGTACATCTGC(BHQ1)
4D	I1	CAGGCGTGCACGCTGC
4D	I2	GCCGGTCTGTCGTCTAATGC
4D	I3	ACCAGCGCGTAGTCAAGTGG
4D	I4	GCATCGTCGCGTCGCAGCGTGCACG
4D	F1	GTGGTAGCGCTGACTACCCA
4D	F2	GTAGAGAGTCGATCTGCAGCGATCA
4D	O	GCT(FAM)AGACGCAGATGTACTGTCTGTA
S8A	Red 1 (5’ phosphorylated)	(PO$_4$)TCTT(FAM)CACAGACACATA(BHQ1)
S8A	Red 2	TCTT(FAM)CACAGACACATA(BHQ1)
S8A	Green	TATGTGTCTGTGAAGA
S14(A)	Red	CTCATGATCAGAT(BHQ1)CGCGT(FAM)TG
S14(A)	Green 1 (10bp)	CAACGCGATCTTTTTT
S14(A)	Green 2 (11bp)	CAACGCGATCTTTTTTT
S14(A)	Green 3 (12bp)	CAACGCGATCTGTTTTTT
S14(A)	Green 4 (12bp 1mis)	CAACTCGATCTGTTTTTT
S14(A)	Green 5 (13bp)	CAACGCGATCTGATTTTTT
S14(A)	Green 6 (13bp 1mis)	CAACTCGATCTGATTTTTT
S14(A)	Green 7 (14bp)	CAACGCGATCTGATTTTTTT
S14(A)	Green 8 (14bp 1mis)	CAACTCGATCTGATTTTTTT
S14(A)	Green 9 (16bp)	CAACGCGATCTGATCATTTTTT
S14(A)	Green 10 (18bp)	CAACGCGATCTGATCATGTTTTTT
S15(A)	Red	CTCATGATCAGAT(BHQ1)CGCGT(FAM)TG
S15(A)	Green 1 (region C 20bp)	AGCTCATCGCGCTGTCACAGTGATCATGAGTTTTT
S15(A)	Green 2 (region C 15bp)	ATCGCGCTGTCACAGTGATCATGAGTTTTT
S15(A)	Green 3 (region C 10bp)	GCTGTCACAGTGATCATGAGTTTTT
S15(A)	Green 4 (region C 6bp)	TCACAGTGATCATGAGTTTTT
S15(A)	Green 5 (region C 5bp)	CACAGTGATCATGAGTTTTT
S15(A)	Green 6 (region C 3bp)	CAGTGATCATGAGTTTTT
S15(A)	Orange 1 (region C 20bp)	CAACGCGATCCTGTGACAGCGCGATGAGCTTTTTT
S15(A)	Orange 2 (region C 15bp)	CAACGCGATCCTGTGACAGCGCGATTTTTT
S15(A)	Orange 3 (region C 10bp)	CAACGCGATCCTGTGACAGCTTTTT
S15(A)	Orange 4 (region C 6bp)	CAACGCGATCCTGTGATTTTT
S15(A)	Orange 5 (region C 5bp)	CAACGCGATCCTGTGTTTTT
S15(A)	Orange 6 (region C 3bp)	CAACGCGATCCTGTTTTT

S16(A)	Red	CTCATGATCAGAT(BHQ1)CGCGT(FAM)TG
S16(A)	Green (region C 0bp)	CACTACCTTCACCTTTTTTTTTTTTTTTTTTGATCATG AGTTTTT
S16(A)	Orange (region C 0bp)	CAACGCGATCTTTTTTTTTTTTTTTCACCATCCACTC TACTTTTT
S16(A)	Purple (Input)	GTAGAGTGGATGGTGAAGGTGAAGGTAGTGTTTTT

X represents the AP site.

Supplementary Figures

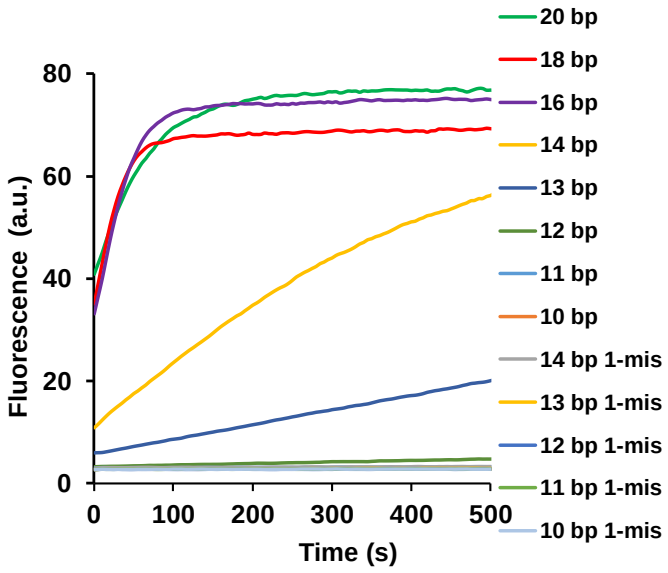


Fig. S1 Fluorescence curves of the hydrolysis reaction of different substrates and APE1. The mismatch is located at the position 3-bp to the 3' end of the AP site. All the substrates are 100 nM, and APE1 is 0.65 nM.

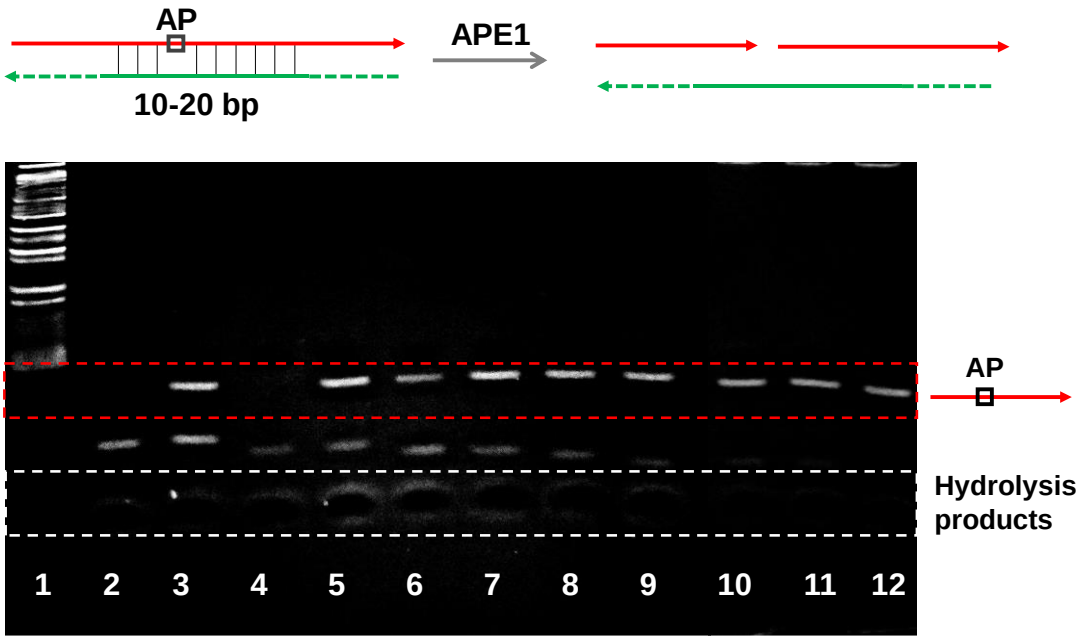


Fig. S2 Denaturing PAGE gel analysis of the APE1-catalyzed hydrolysis products (3 h reaction). Lane 1: 25-bp marker. Lane 2, lane 4, lane 6, lane 8, and lane 11 correspond to the 14-bp, 13-bp, 12-bp, 11-bp, and 10-bp substrates, respectively. Lane 3, lane 5, lane 7, lane 9, and lane 12 are 14-bp, 13-bp, 12-bp, 11-bp, 10-bp substrates with single mismatch (3-bp to the 3' end of the AP site), respectively.

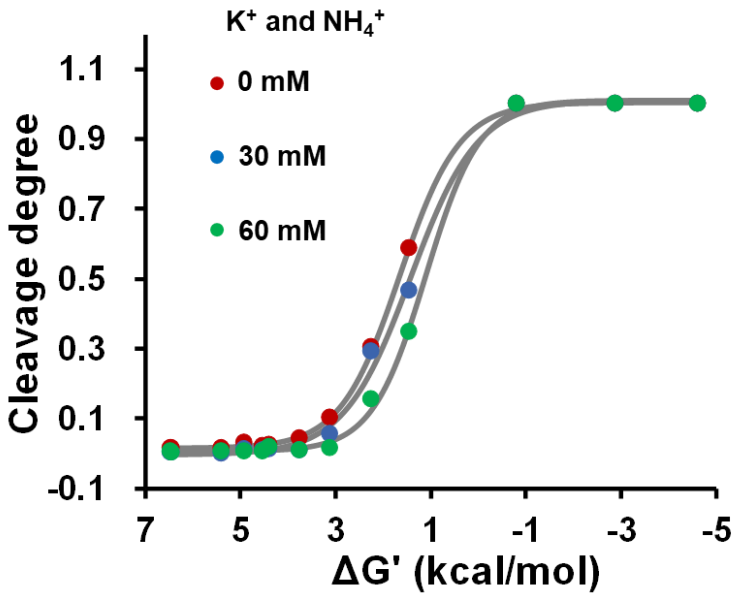


Fig. S3 The relationship of the substrate stability (ΔG') and APE1-catalyzed hydrolysis in different conditions. All the substrates are 100 nM, and APE1 is 0.65 nM.

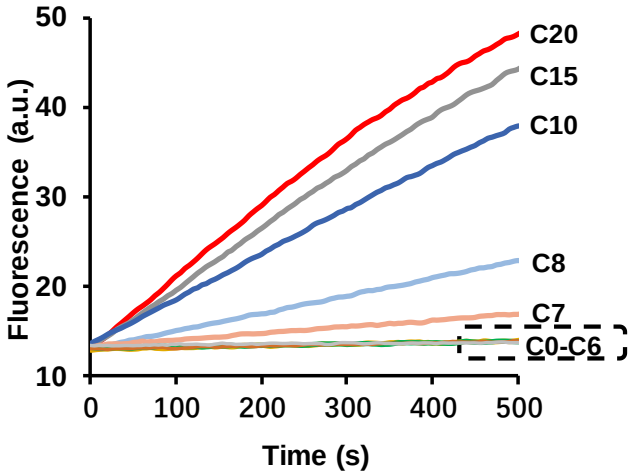
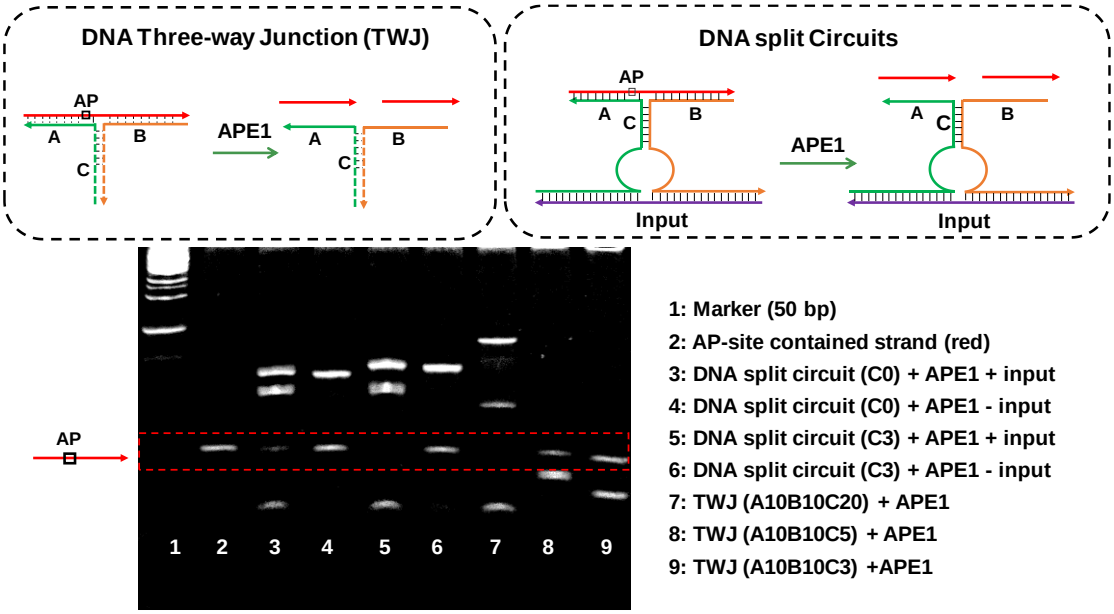


Fig. S4 Fluorescence curves of the reaction of TWJs and APE1. The numbers represent the base-paired number of the association region C. All the oligonucleotides are 100 nM, and APE1 is 0.65 nM.



1: Marker (50 bp)
2: AP-site contained strand (red)
3: DNA split circuit (C0) + APE1 + input
4: DNA split circuit (C0) + APE1 - input
5: DNA split circuit (C3) + APE1 + input
6: DNA split circuit (C3) + APE1 - input
7: TWJ (A10B10C20) + APE1
8: TWJ (A10B10C5) + APE1
9: TWJ (A10B10C3) +APE1

Fig. S5 Denaturing PAGE gel analysis of the hydrolysis products of TWJs and split circuits. Lane 1: 50-bp marker. Lane 2: AP-site contained strand highlighted on the gel (red). Its cleavage is indicative of the hydrolysis. Lane 3-6: the products of DNA split circuits. Lane 7-9: the products of TWJs.

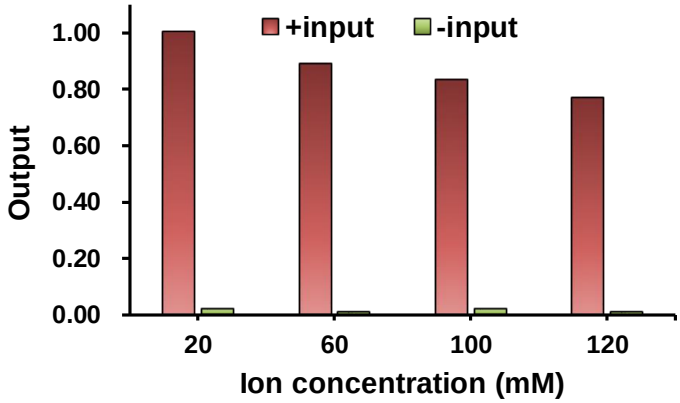


Fig. S6 Salinity tolerance of the split circuit for ssDNA. Ion concentrations represent the additional NH_4Cl and KCl in the Tris buffer. The fluorescence change of the assay containing 20 mM ion is normalized as 1.

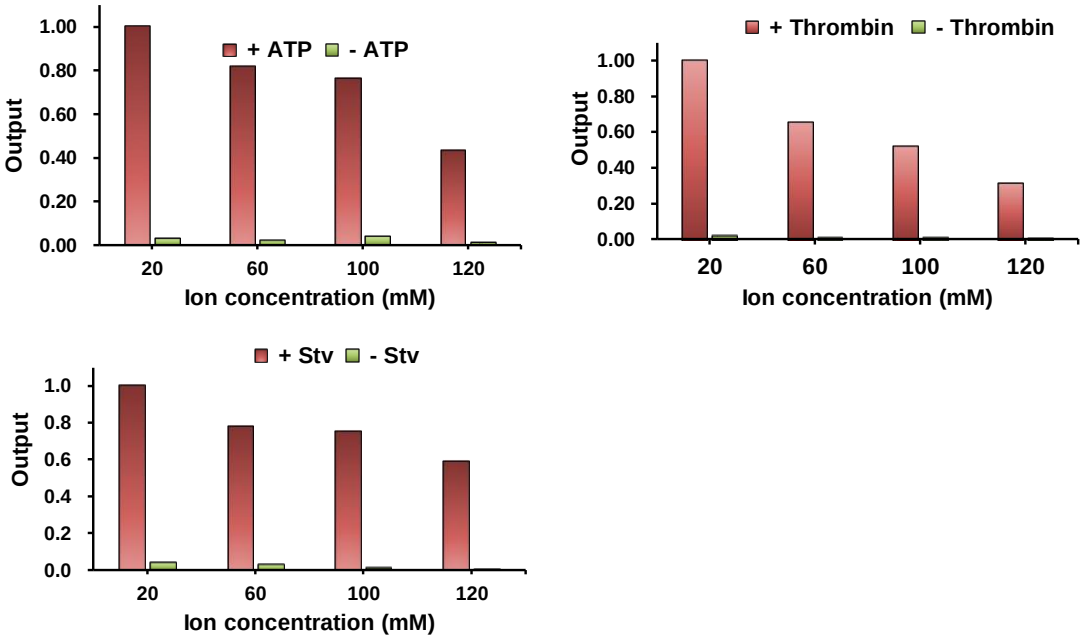


Fig. S7 Salinity tolerance of the split circuits for small molecule and proteins. Ion concentrations represent the additional NH_4Cl and KCl concentrations in the Tris buffer. The fluorescence change of the assay containing 20 mM ion is normalized as 1.

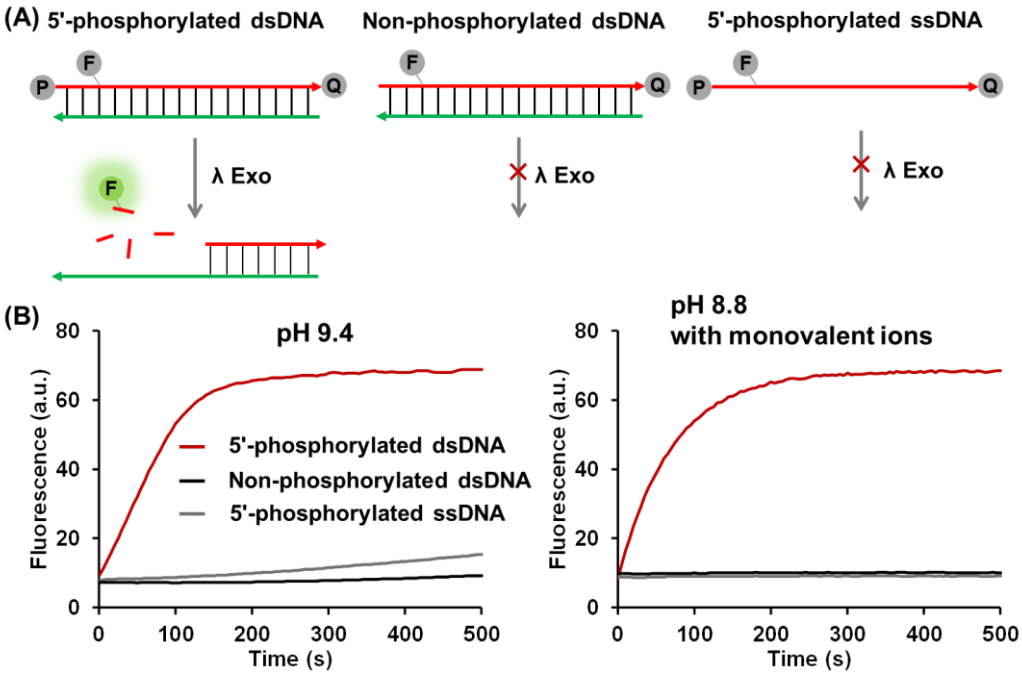


Fig. S8 Substrate preference of λ exo. (A) The Scheme of the fluorescence dequenching assay for validating the substrate preference of λ exo. (B) Fluorescence curves of the enzymatic reaction of different substrates in different conditions. The reactivity of non-phosphorylated dsDNA and 5'-phosphorylated ssDNA were suppressed by using monovalent ions NH_4Cl and KCl whose concentration are both 50 mM, and by lowering pH. All the substrates are 100 nM, and λ exo is 0.56 nM.

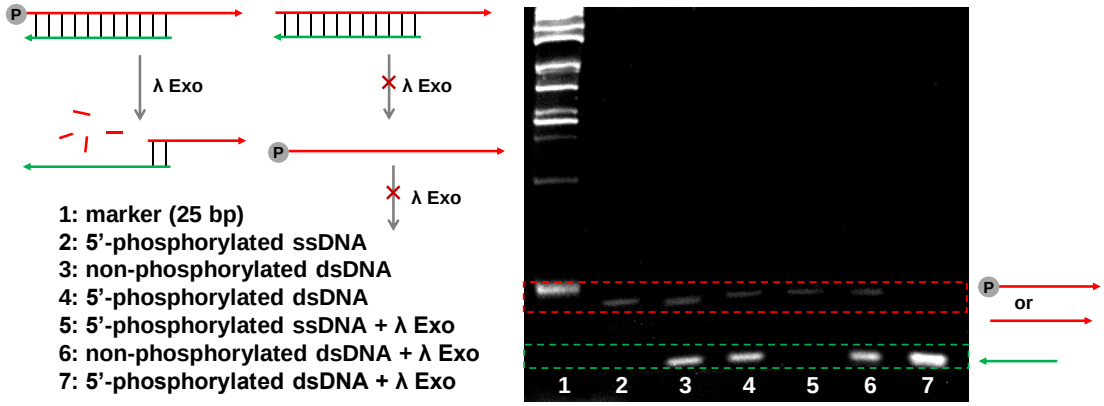


Fig. S9 Denaturing PAGE gel analysis of the digestion of different substrates by λ exo. To discriminate the two strands on the gel, different length was used, the substrates are partially complementary.

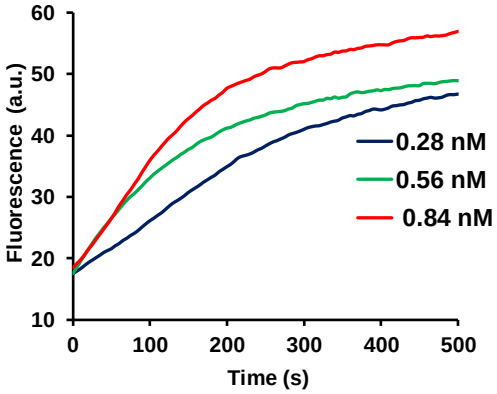


Fig. S10 Tuning the response speed of the one-input linear circuit by λ exo. All the oligonucleotides are 100 nM.

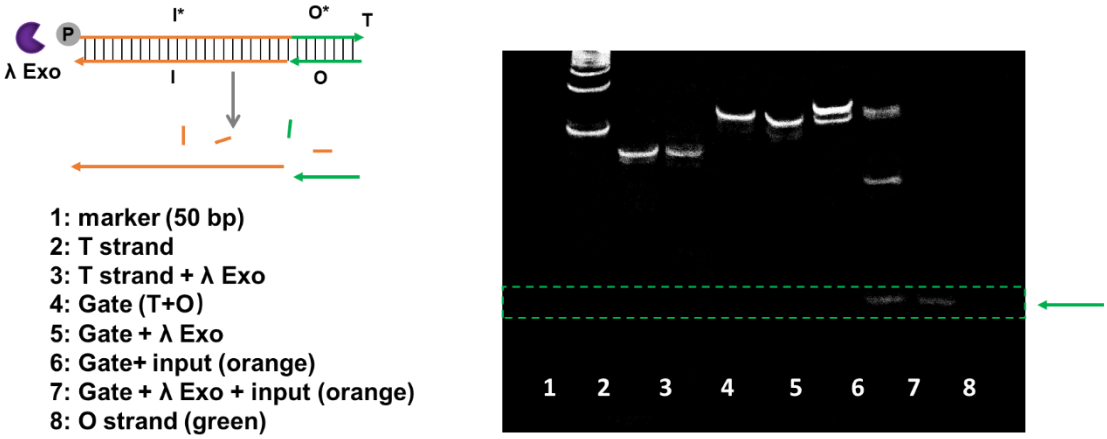


Fig. S11 Native PAGE gel analysis of digestion products of the one-input YES gate. The released output strand is highlighted on the gel.

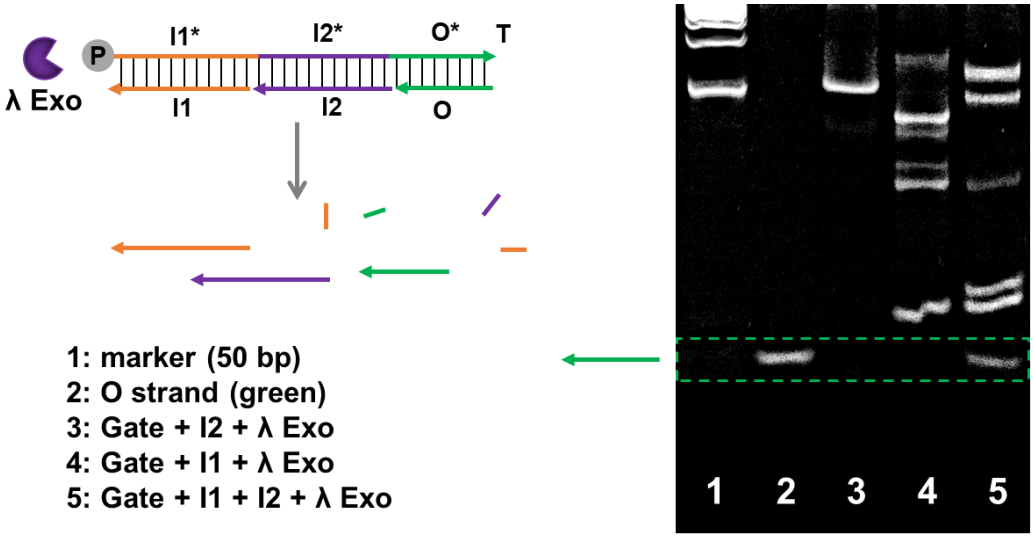


Fig. S12 Native PAGE gel analysis of digestion products of the two-input AND gate. The released output strand is highlighted on the gel.

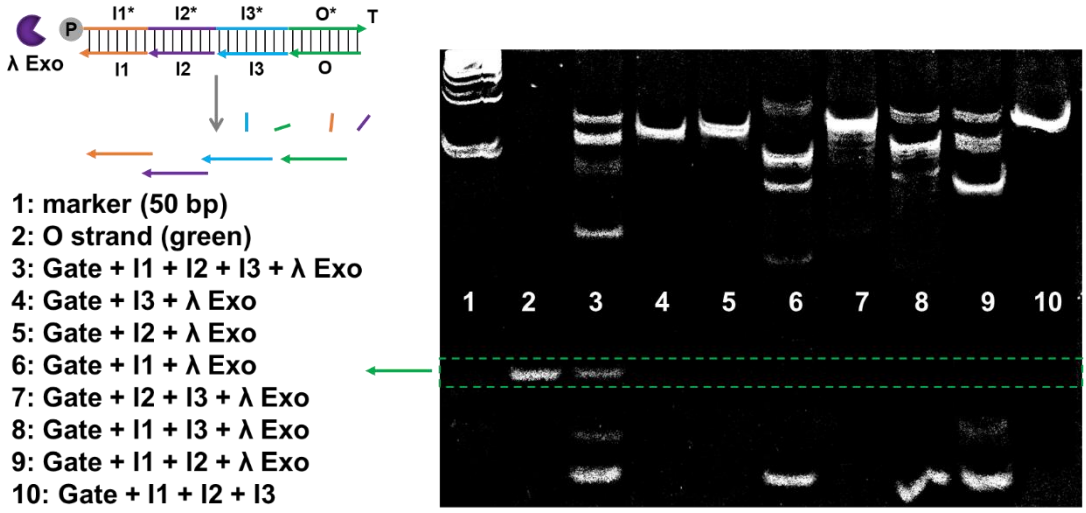


Fig. S13 Native PAGE gel analysis of digestion products of the three-input AND gate. The released output strand is highlighted on the gel.

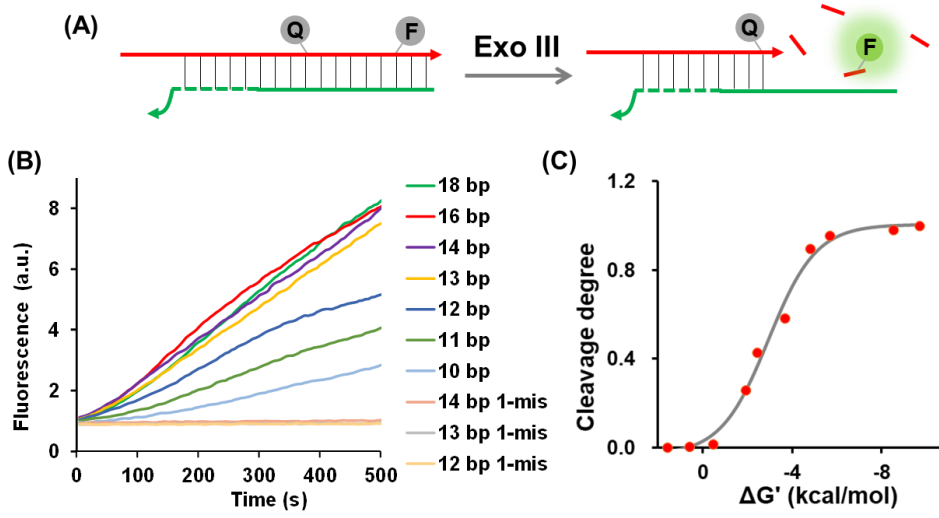


Fig. S14 Exo III digestion is dependent on the substrate stability. (A) Schematic of a

dsDNA digested by Exo III from the blunt 3’ end. The 3’ end overhang is 6-nt to protect the unwanted digestion. (B) Fluorescence curves of dsDNAs with different structures digested by Exo III. (C) Relation of the digestion degree of the dsDNAs by Exo III and the concentration-adjusted $\Delta G^{'}=-RT\ln(c^{\Delta n}\times K_d)$ of the TWJs assembly, where Δn equals to 1. All the substrates are 50 nM, and Exo III is 0.48 nM.

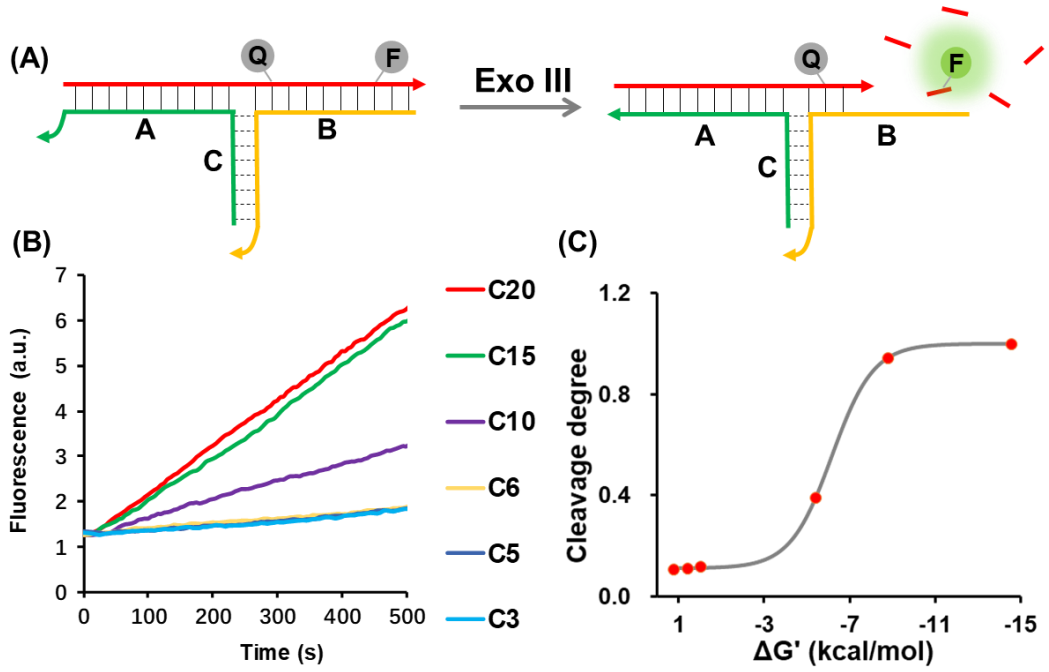


Fig. S15 Interaction of Exo III and DNA three-way junction (TWJ). (A) Schematic of a TWJ digested by Exo III from the blunt 3’ end. The dash lines represent uncertain hybridization state when varying association region C. All of the 3’ end overhangs are 6-nt to protect the unwanted digestion. (B) Fluorescence curves of TWJs with different structures digested by Exo III. The numbers represent the base-paired number of the association region C. (C) Relation of the digestion degree of the TWJs by Exo III and the concentration-adjusted $\Delta G^{'}=-RT\ln(c^{\Delta n}\times K_d)$ of the TWJs assembly, where Δn equals to 2. All the oligonucleotides are 50 nM, and Exo III is 0.48 nM.

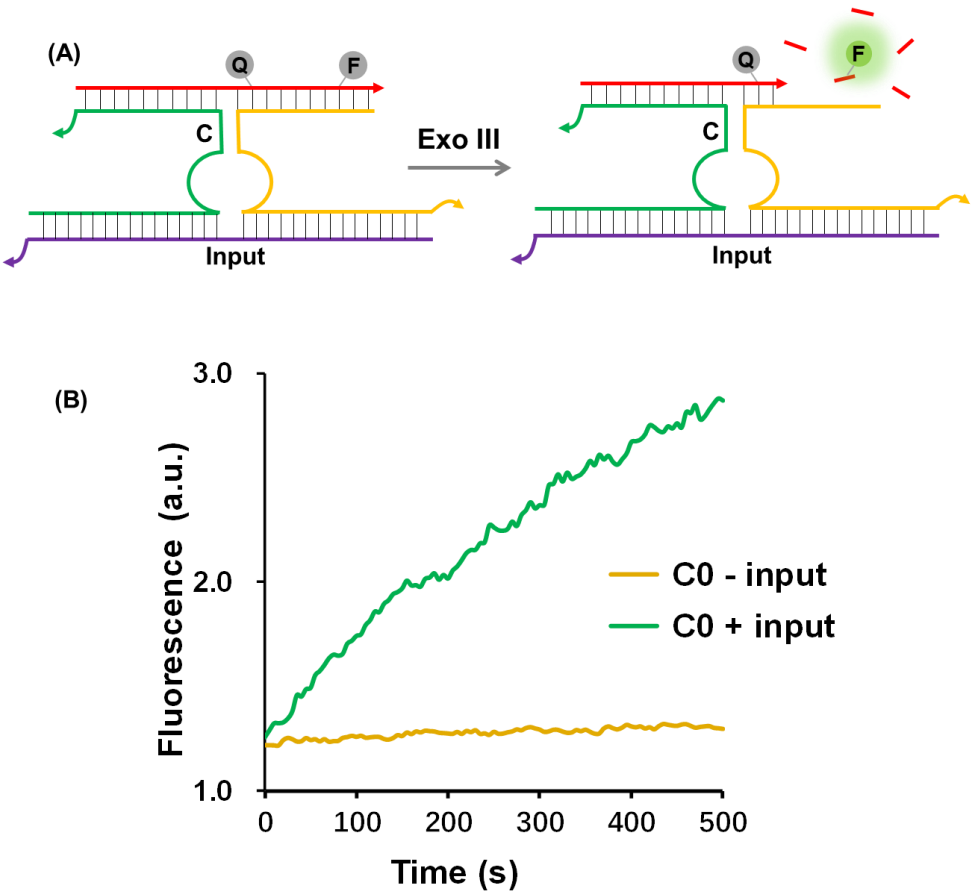


Fig. S16 Engineering DNA split circuits by Exo III. (A) Schematic of the reaction of Exo III and the input-binding split circuit. All of the 3' end overhangs are 6-nt to protect the unwanted digestion. (B) The output response of the split circuit with/without ssDNA input. The base-paired number of the association region C is zero. All the oligonucleotides are 50 nM, and Exo III is 0.48 nM.