Supplementary Information

Enzyme-assisted waste-to-reactant transformation to engineer renewable DNA circuits

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S1. Materials and Methods

S1.1. Materials

DNA oligonucleotides (Table S1) and 4S GelRed used in this study were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The 6-FAM/Dabcyl-modified oligonucleotides were purified through high-performance liquid chromatography (HPLC), while ULTRAPAGE for non-modified oligonucleotides. The nicking enzyme Nt.BbvCI was purchased from New England Biolabs Inc. Other chemicals used were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All the oligonucleotides were dissolved in buffer CS (50 mM KAc, 2 mM Tris-Ac, 10 mM $Mg(Ac)_2$, pH = 7.9 at 25 ℃) and stored at 4 ℃. The reaction buffer is buffer CS-BSA (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 100 ug/ml BSA, pH = 7.9 at 25 ℃). Ultrapure water (18.2 MΩ·cm) (Millipore simplicity, USA) was used in all experiments. All the oligonucleotides were quantified by measuring the absorbance at 260 nm using NanoDrop 2000 Spectrophotometer (Thermo Fisher, USA).

S1.2. DNA Sequence Design

With the help of NUPACK software,[1] we designed and analyzed the sequence to reduce secondary structures and interactions.

S1.3. Preparation of the substrate

The triple-stranded DNA reactant-substrate S_R was prepared by mixing L, P_1 and P_2 strands in a molar ratio of 1 : 1.2 : 1.2, then heating up to 95 ℃ for 10 min and slowly cooling down to 25 ℃. 12% non-denaturing polyacrylamide gel was made by mixing 21 mL 40% acrylamide/bis (19 : 1), 1.4 mL 50×TAE, 420 uL APS, 42 ul TEMED in 47 mL ultrapure water and continuously stirring for 7 min. After loading the sample pre-mixed with 33% glycerol per lane, gel was run at 240 V for 6 hours using Hoefer standard vertical electrophoresis unit (Hoefer, Inc., San Francisco, CA, USA) with a PS300-B power supply at the freezer to prevent the temperature from elevating. Stained by GelRed dye, the desired substrate bands were able to be seen under UV light and cut from the gel into pieces, then soaked in buffer CS at 4 ℃ for two days to ensure sufficient extraction of the DNA. Finally, the supernatants were separated with gel pieces and re-quantified using NanoDrop 2000 Spectrophotometer.

S1.4. Fluorescence measurements

The fluorescence kinetics were performed on a F-7000 fluorometer (Hitachi). With the use of 6-FAM fluorophore, the excitation/emission were set at 497/520 nm for kinetic characterization. Experiments were carried out at 37 ℃ in CS-BSA buffer. Taking the reaction in Figure 1c as a typical example of experimental operation. First, in a 200 ul reaction volume, samples were prepared by mixing 50 nM S_R, 100 nM F, 0 or 50 U/ml Nt.BbvCI and 0 or 50 nM C together. Second, samples were shaken and centrifuged into uniform solution and then transferred to the microcell of the fluorometer. Finally, the fluorescence kinetic was monitored by tracking the value at 10 s intervals.

S1.5. Non-denaturing gel electrophoresis

Samples were run on 12% non-denaturing gel at 120 V for 1 h at room temperature with the electrophoresis tank Tanon VE 180. Gel photos were taken with the Gel Image System Tanon 1600.

S2. Supplementary Table

Name	Sequences (5' to 3')
L	CGAACTGACCAATAGCTGAGGAGAAGTAGTGCAAGTCGTGGATGTGTG
P_1	CGACTTGCACTACTTCTCC
P_2	TCAGCTATTGGTCAGTTCG
F	GACTTGCACTACT/i6FAMdT/CTCCTCAGCTA/iDabcyldT/TGGTCAGTTC
F_{mis}	GACTTGCACTACT/i6FAMdT/CTCCACAGCTA/iDabcyldT/TGGTCAGTTC
C-6/6	CATCCACGACTTGCACTAC
C-7/6	ACATCCACGACTTGCACTAC
C-8/6	TACATCCACGACTTGCACTAC
C-9/6	ATACATCCACGACTTGCACTAC
C-10/6	GATACATCCACGACTTGCACTAC
C-11/6	CGATACATCCACGACTTGCACTAC
C-10/4	GATACATCCACGACTTGCACTACTT
C-10/5	GATACATCCACGACTTGCACTACT
C-10/7	GATACATCCACGACTTGCACTA
C-10/8	GATACATCCACGACTTGCACT
F′	GACTTGCACTACTTCTCCTCAGCTATTGGTCAGTTC
F_{mis}′	GACTTGCACTACTTCTCCACAGCTATTGGTCAGTTC
C′	TTTTTTTTGATACATCCACGACTTGCACTA

Table S1. The DNA oligonucleotide sequences used in the experiments.

S3. Supplementary Figure

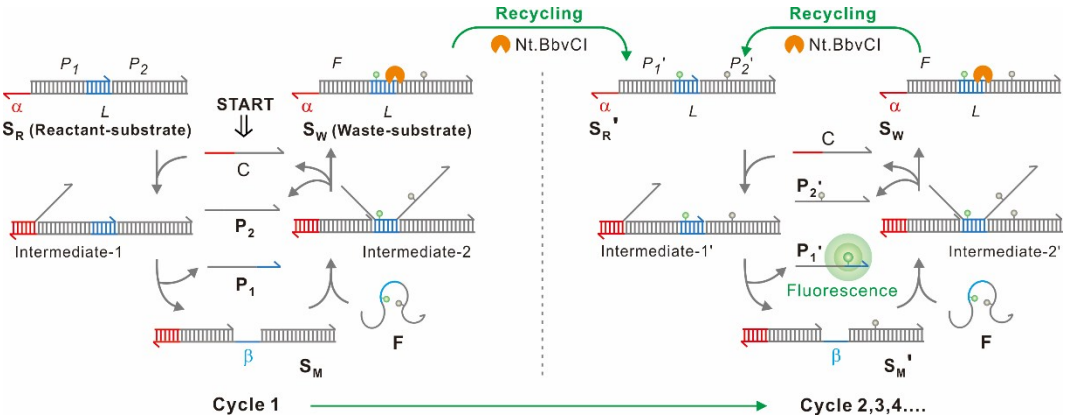

Fig. S1 Detailed illustration of the proposed renewable DNA circuit monitored by dual-labelled fuel F. In the conventional entropy-driven catalytic DNA circuit, every reactant-substrate S_R could only be used once, that is, one cycle per S_R (Cycle 1). But with the introduction of Nt.BbvCI, S_W would be recycled and transformed into active reactant S_R', then proceeds in a cycle-to-cycle manner until the fuel F was exhausted (Cycle 2,3,4…). S_R' was the same as S_R in sequence, and they were only different in whether labelling or not.

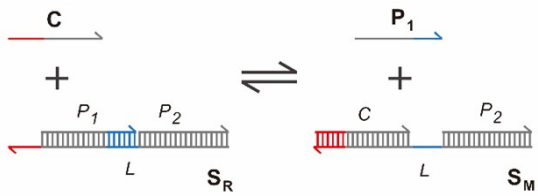

Fig. S2 Schematic of toehold-exchange reaction between catalyst C and protector strand P_1 with corresponding substrates (i.e., S_R and S_M).

References

1. J. N. Zadeh, C. D. Steenberg, J. S. Bois, B. R. Wolfe, M. B. Pierce, A. R. Khan, R. M. Dirks and N. A. Pierce, *J. Comput. Chem.*, 2011, **32**, 170-173.