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Supporting Information for

How the Fluorescent Labels Affect the Kinetics of Toehold-Mediated DNA Strand Displacement Reaction?

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Supplementary text

S1. Design of DNA sequence and the preparation of materials.

All the DNA oligonucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). NUPACK[1] software and the three-letter-code (A, C, and T) rule[2] were obeyed to design the sequence of DNA oligonucleotides to avoid undesired secondary structures. The DNA oligonucleotides labelled with fluorophores dye or quencher were purified using high-performance liquid chromatography (HPLC), while ULTRAPAGE for non-modified oligonucleotides. 4S Gelblue was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). The buffer condition was 1× Tris-Acetate-EDTA-Mg^{2+} (1× TAE/Mg^{2+}) buffer (40 mM Tris-Acetate, pH balanced to 8.4, with 1 mM EDTA, purchased as 50x stock from Sangon Biotechnology Co., Ltd., and 12.5mM $MgCl_2$ was added). Ultrapure water (18.2 MΩ·cm) (Millipore Co., USA) was used in all the experiments.

S2. Preparation and purification of DNA duplexes.

All oligonucleotides were first suspended in 1× Tris-Acetate-EDTA-Mg^{2+} (1× TAE/Mg^{2+}) buffer and stored at 4 °C at 100 μM. The concentration of each strand was calculated as c = OD260/e × 10^6, where the extinction coefficient of them were provided by Sangon Biotechnology Co., Ltd. (Shanghai, China). The concentrations of DNA samples were quantified by measuring the absorbance at 260 nm using Cary 300UV-Vis spectrophotometer, and then concentrated to 5 μM. DNA duplexes were prepared by annealing two complementary DNA strands together (at the ratio 1:1.2) at 50 μM concentration. Then the samples were kept at 95 °C for 5 min and slowly cooled down to 20 °C at the rate of 1 °C/min. After that, we utilized 12% non-denaturing PAGE purification to remove the extra single-strand oligonucleotides and undesired structures[3]. Equation e = e(top strand)+e(bottom strand)-3200N_{AT} -2000N_{GC} was used to calculate the extinction coefficients of substrate-signal duplex, where N_{AT} and N_{GC} are the number of AT pairs and GC pairs in the double-stranded domain, respectively[4]. The purified DNA samples were stored at 4 °C for further use.

S3. Fluorescence kinetic measurement.

The fluorescence spectrophotometer (F-7000, Hitachi) was used to measure the fluorescence kinetics of all the DNA strand displacement reactions at 25 °C. The excitation/emission was set at 492 nm/520 nm for kinetics characterization with the use of 6-FAM fluorophore and 643 nm/667 nm for Cy5 fluorophore. The slits of excitation and emission were both 5 nm. All the experiments

were run in Tris-acetate-EDTA buffer containing 12.5 mM Mg^{2+} (1× TAE/Mg^{2+}). Three parallel experiments were performed for each system.

S4. UV melting experiments.

The UV melting experiments were conducted using Cary 300UV-Vis spectrophotometer. The absorbance at 260 nm was recorded and the heating rate is 1 °C/min. Melting profiles of the buffer absorbance were subtracted from melting curves of DNA samples. All the measurements were made in 1× Tris-Acetate-EDTA-Mg^{2+} (1× TAE/Mg^{2+}) buffer. The concentration of DNA duplex samples was 1 μM. Three parallel experiments were performed for each system.

S5. Melting temperature (T_m).

Our experiments demonstrated that the modification of the signal strand using fluorophore dye or quencher slows down the TMSDR kinetics, and that of the toehold domain on the substrate strand speeds up the TMSDR kinetics. To illustrate how these modifications of signal or substrate strand using fluorescence labels affect the TMSD kinetics, we measured the melting temperatures of reactant duplexes (substrate-signal, S/Sig_1) and product duplexes (substrate-invader, S/I). Melting temperatures can be used as a measure of the stabilities of these duplexes. Figure S6 shows an example of the UV melting profile of the unmodified signal-substrate duplex. We used logistic function to fit the scatter graph and derive the value of T_m. All the melting temperatures of reactant duplexes and product duplexes were measured by three times to derive an average value and its standard deviation.

S6. The catalytic DNA circuits.

The duplex of signal (Sig_1) and substrate strand is used to construct catalytic circuit based on the principle of TMSDR. The schemes for DNA circuits are displayed in Figure S11A and S11B. The fluorophore dye or quencher are attached on the 5’ end of signal strand (Figure S11A) or the 3’ end of substrate strand (Figure S11B). We found that the effects of labels on kinetics of catalytic circuits are similar to the situation of one-step TMSDR (Figure 1 and 2 in the main text). That is, the modifications on the 3’ end of toehold domain of substrate strand accelerate the circuit kinetics and that on 5’ end of incumbent signal strand slow down the kinetic process of TMSDR.

S6. The fluorescent labels being modified in the middle position of signal-substrate duplex.

The fluorophore dyes or quenchers can also be modified in the middle position of signal-substrate duplex. Herein, we examined the kinetics of TMSDR with the 13th base (thymine) of the

Sig1 strand being modified with fluorophore dye (Figure S3). The recorded kinetic curves indicated that modifications at the middle position of the Sig1 strand can significantly speed up the strand displacement reaction. The melting temperatures of these signal-substrate duplexes suggest that the introduction of modifications at the middle position of the Sig1 strand destabilizes the signal-substrate duplex (Figure S7), which is similar to the case of introducing mismatch at the middle of the duplex[5].

S7. Calculation of thermodynamic parameters.

The thermodynamic parameters were obtained from melting profiles[6]. The peak height maxima, $(d\theta/dT)_{max}$, is obtained from the derivative melting curves. The parameter θ is the fraction of melted base pairs which is defined using $\theta = (A - A_{min})/(A_{max} - A_{min})$, where A, A_{min} and A_{max} are the sample absorbance, the maximum value of sample absorbance and the minimum value of sample absorbance, respectively. The transition enthalpies, the entropies, and free energies, were calculated using established formulas[6]. $\Delta H^0_{vh} = -(2+2n)RT_m^2(\frac{d\theta}{dT})_{max}$, $\Delta S^0_{vh} = \frac{\Delta H^0_{vh}}{T_m} - Rln(\frac{C_t}{2n})^{n-1}$, $\Delta G^0_T = \Delta H^0_{vh} - T\Delta S^0_{vh}$, where R is the ideal gas constant, C_t is the concentration of duplex sample and n is the molecularity of the melting reaction. For the measurement of melting temperature, the value of n is 2. The free energy change caused by the attachment of fluorescent label is calculated using $\Delta\Delta G^0_T = (\Delta G^0_T)_{labelled\ duplex} - (\Delta G^0_T)_{no\ label}$. In all calculations, we chose $T = 25°C$, since all the experiments presented in this work are peformed at this temperature.

Supplementary figures

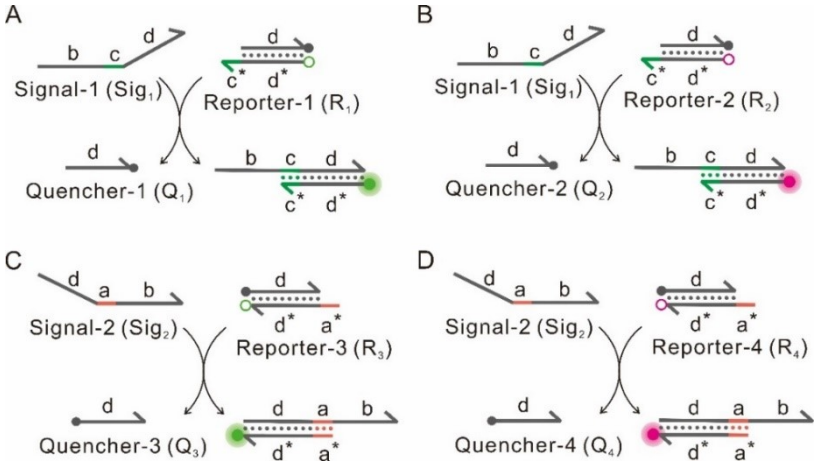

Figure S1. The schematic diagram of the reporter systems. Diagrams A, B, C, and D represent the reporter systems of R_1, R_2, R_3 and R_4, respectively. Reporter systems R_1 and R_3 contain 6-FAM and Dabcyl, which are modified at the opposite ends of reporter strand and quencher strand, while the other two reporter systems R_2 and R_4 are prepared using Cy5 and BHQ2.

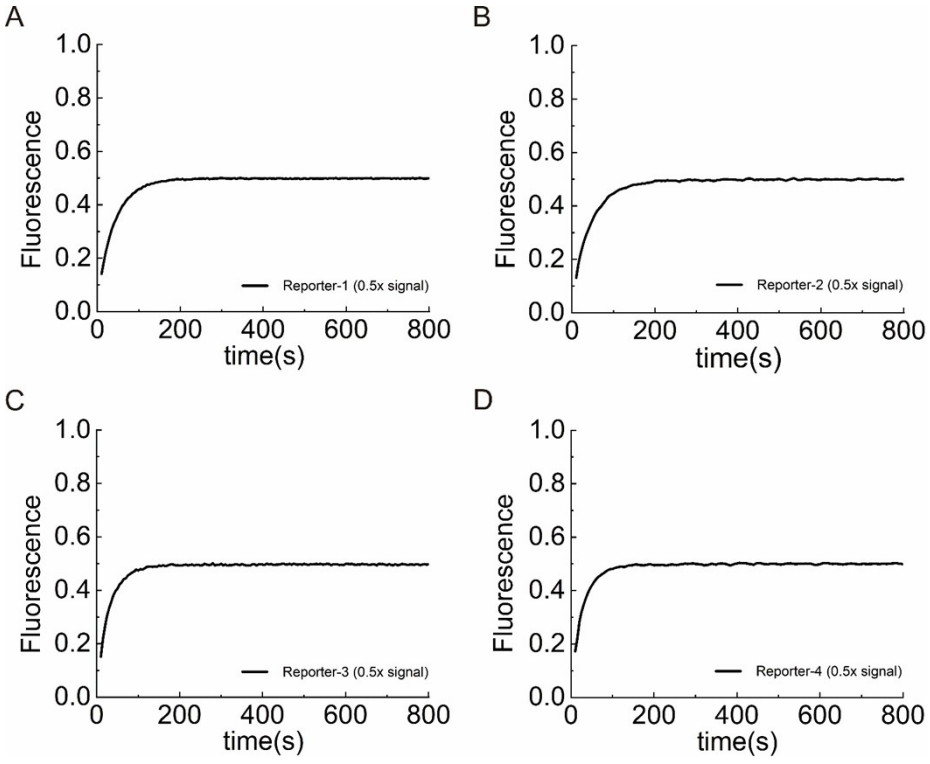

Figure S2. Real-time fluorescent kinetics of the reporter systems, triggered with the signal strand. A, B, C, and D represent the reporter system of R_1, R_2, R_3 and R_4, respectively. In these experiments, $[R_1] = [R_2] = [R_3] = [R_4] = 10$ nM, $[Sig_1] = 5$ nM, $[Sig_2] = 5$ nM. All the experiments were run at 25 °C in Tris-acetate EDTA buffer containing 12.5 mM Mg^{2+} for 800 s (1× TAE/Mg^{2+}). The rate constants of these reporter systems are $k_1 = 4.40\times10^6$ $M^{-1}s^{-1}$, $k_2 = 3.95\times10^6$ $M^{-1}s^{-1}$, $k_3 = 6.73\times10^6$ $M^{-1}s^{-1}$, $k_4 = 7.30\times10^6$ $M^{-1}s^{-1}$, respectively.

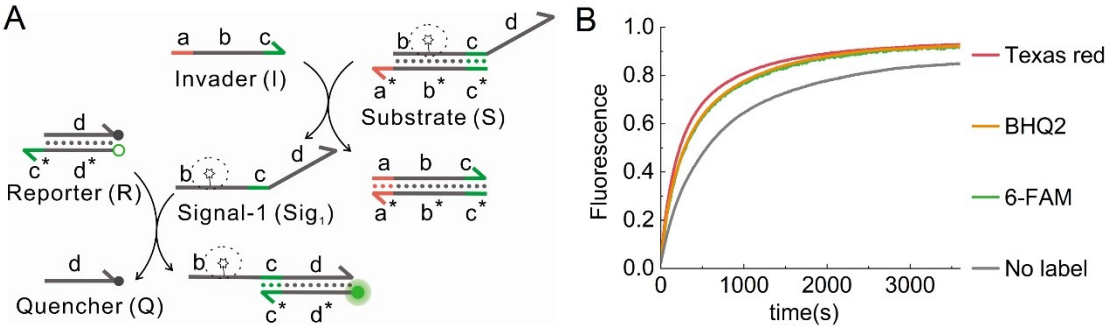

Figure S3. (A) Schematic diagram of DNA circuit based on TMSDR. The signal strand (Sig_1) is tagged with fluorescent label, which is modified at the 13th base (thymine). (B) Real-time fluorescent kinetics of the circuit displayed in (A). All the experiments are performed at 25°C, [Invader] = 5 nM, [Substrate-signal] = 10 nM, [Reporter] = 20 nM.

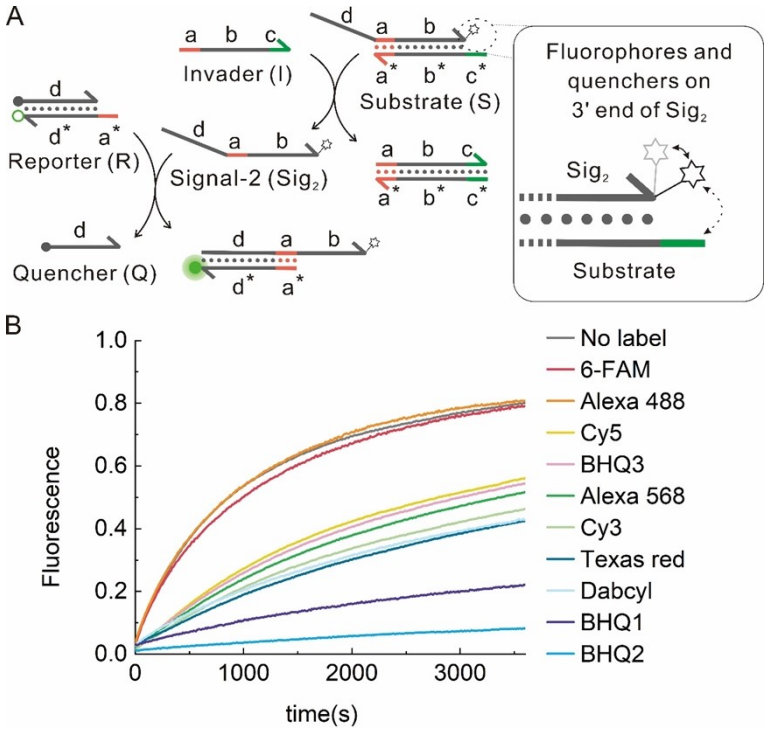

Figure S4. (A) Schematic diagram of DNA circuit based on TMSDR, in which the fluorophore dye or quencher is modified onto the 3' end of the Sig_2 strand. The length of toehold (domain c* in green) is 5-nt. (B) Real-time fluorescent kinetics of the circuit for substrate being modified with varied fluorescent labels. All the experiments were performed at 25°C, [Invader] = 10 nM, [Substrate-signal] = 10 nM, [Reporter] = 20 nM.

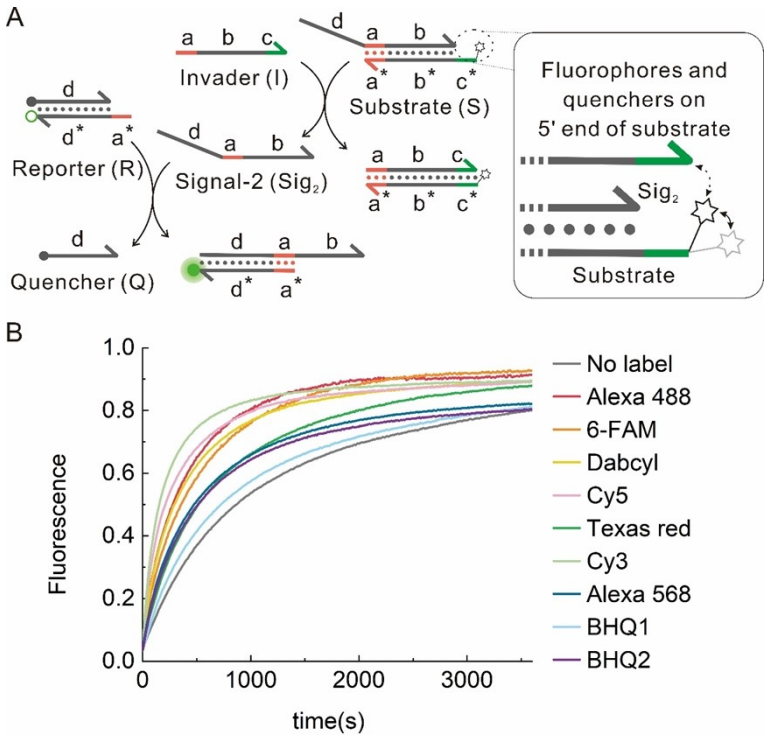

Figure S5. (A) Schematic diagram of DNA circuit based on TMSDR, in which the fluorophore dye or quencher is modified onto the 5' end of toehold domain in the substrate strand. The length of toehold (domain c* in green) is 5-nt. (B) Real-time fluorescent kinetics of the circuit for substrate being modified with varied fluorescent labels. All the experiments were performed at 25°C, [Invader] = 10 nM, [Substrate-signal] = 10 nM, [Reporter] = 20 nM.

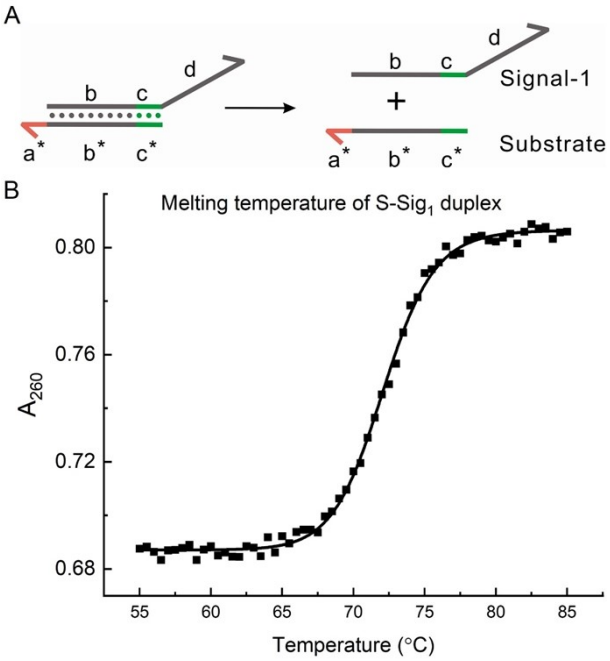

Figure S6. (A) Schematic diagram of melting process of the signal-substrate duplex. (B) UV melting profile of signal-substrate duplex with no modification. [Substrate-signal] = 1 μM.

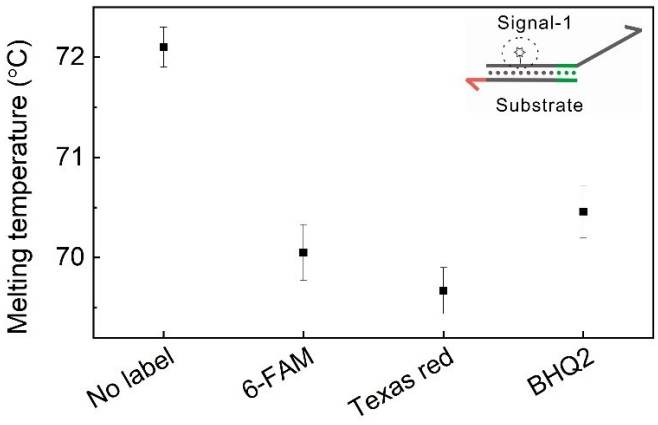

Figure S7. The measured melting temperature for the signal-substrate duplex with different labels of fluorophore dyes or quenchers. The fluorescent label is modified to the 13th base (thymine) of the signal strand. Error bars indicate the standard deviation calculated based on three parallel experiments. The concentrations of DNA duplexes are 1 μM.

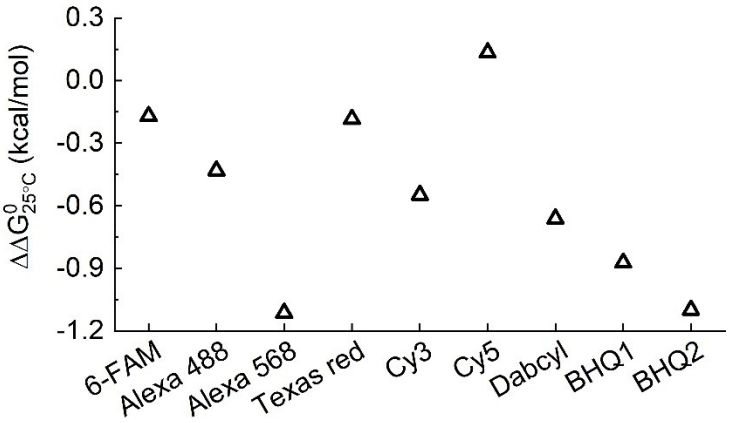

Figure S8. The change of free energies for the duplexes in Figure 4A. The labels are modified at the 5' end of signal strand of Sig_1-substrate duplex.

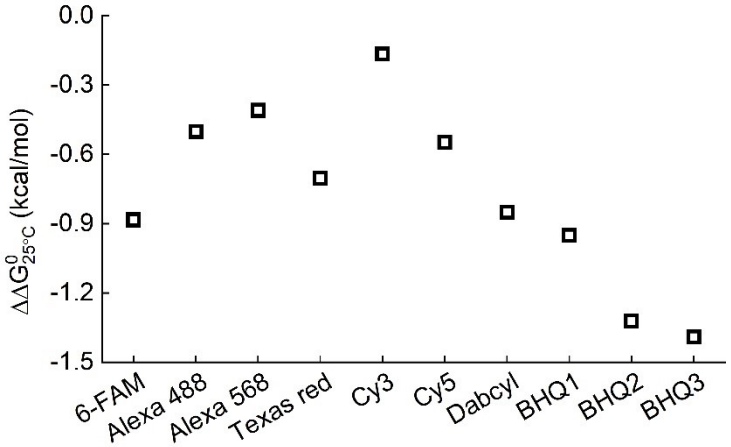

Figure S9. The change of free energies for the duplexes in Figure 4B. The labels are modified at the 3' end of substrate strand of Sig_1-substrate duplex.

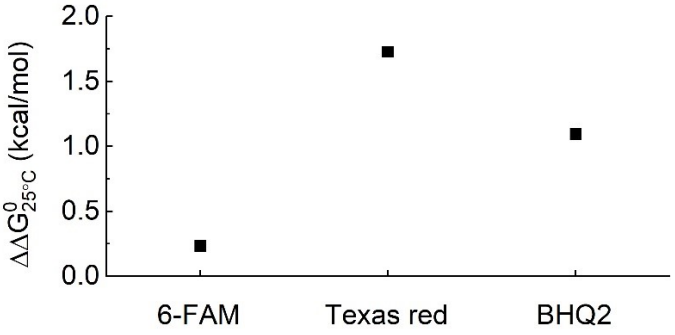

Figure S10. The change of free energies for the duplexes in Figure S7. The labels are modified at the 13th position of the signal strand.

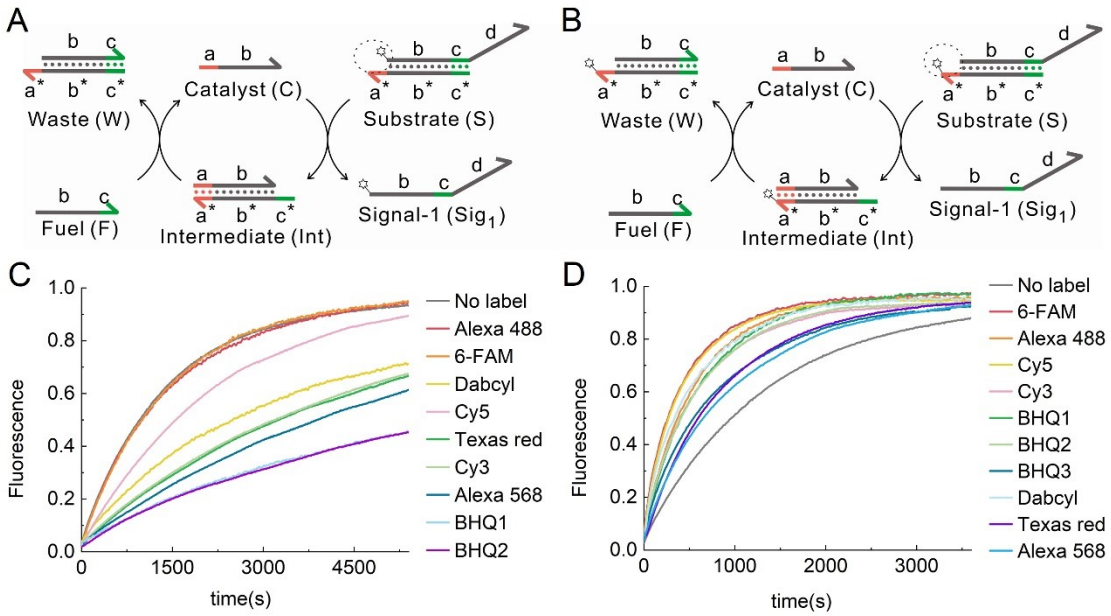

Figure S11. Schematic diagram of catalytic DNA circuits based on the principle of TMSDR. (A) The signal strand (Sig_1) is tagged with fluorescent label at its 5' end. (B) The substrate strand (S) is tagged with fluorescent label at its 3' end. (C) Real-time fluorescent kinetics of the circuit displayed in (A). (D) Real-time fluorescent kinetics of the circuit displayed in (B). All experiments were performed at 25°C, [Catalyst] = 5 nM, [Substrate-signal] = 10 nM, [Fuel] = 100 nM, [Reporter] = 20 nM.

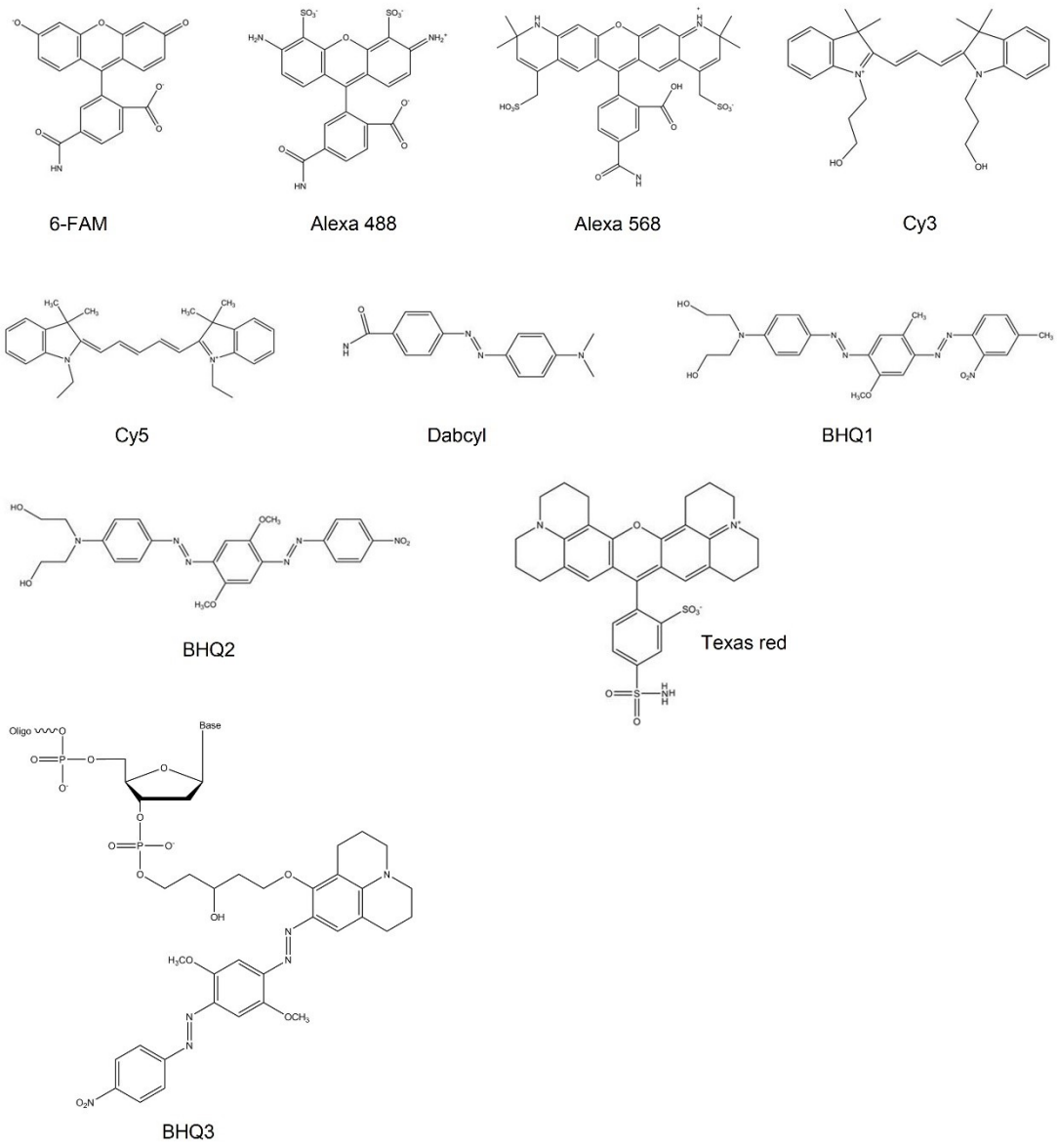

Figure S12. The structures of fluorophore dyes and quenchers. Note that the quencher group BHQ3 can only be modified at the 3’ end of DNA strand.

Supplementary tables

Table S1. Strand sequences used in systems with fluorescent labels at the 3' toehold domain.

Strand name	Sequence (From 5' - 3')
Reporter-1	6-FAM-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-1	ATACCAATCCACACTTCCA-Dabcyl
Reporter-2	Cy5-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-2	ATACCAATCCACACTTCCA-BHQ2
Invader	ATCACCTCACCAACACATACACCACTCAT
Signal-1	CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
6-FAM-Signal-1	6-FAM-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Alexa 488-Signal-1	Alexa 488-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Alexa 568-Signal-1	Alexa 568-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Texas red-Signal-1	Texas red-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Cy3-Signal-1	Cy3-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Cy5-Signal-1	Cy5-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Dabcyl-Signal-1	Dabcyl-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
BHQ1-Signal-1	BHQ1-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
BHQ2-Signal-1	BHQ2-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Substrate-1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Substrate-1-6-FAM	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-6-FAM
Substrate-1-Alexa 488	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Alexa 488
Substrate-1-Alexa 568	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Alexa 568
Substrate-1-Texas red	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Texas red
Substrate-1-Cy3	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Cy3
Substrate-1-Cy5	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Cy5

Substrate-1-Dabcyl	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Dabcyl
Substrate-1-BHQ1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ1
Substrate-1-BHQ2	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ2
Substrate-1-BHQ3	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ3
Invader*	ACATACTCACCAACACATACACCACTCAT
Invader**	CTCACCTCACCAACACATACACCACTCAT
Substrate*	ATGAGTGGTGTATGTGTTGGTGAGTATGT
Substrate**	ATGAGTGGTGTATGTGTTGGTGAGGTGAG

Table S2. Strand sequences used in systems with fluorescent labels at the 5' toehold domain.

Strand name	**Sequence (From 5' - 3')**
Reporter-3	GAGGTGATTGGAAGTGTGGATTGGT-6-FAM
Quencher-3	Dabcyl-ACCAATCCACACTTCCAAT
Reporter-4	GAGGTGATTGGAAGTGTGGATTGGT-Cy5
Quencher-4	BHQ2-ACCAATCCACACTTCCAAT
Invader	ATCACCTCACCAACACATACACCACTCAT
Signal-2	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA
Signal-2-6-FAM	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-6-FAM
Signal-2-Alexa 488	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Alexa 488
Signal-2-Alexa 568	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Alexa 568
Signal-2-Texas red	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Texas red
Signal-2-Cy3	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Cy3
Signal-2-Cy5	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Cy5
Signal-2-Dabcyl	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-Dabcyl
Signal-2-BHQ1	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-BHQ1
Signal-2-BHQ2	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-BHQ2

Signal-2-BHQ3	ACCAATCCACACTTCCAATCACCTCACCAACACATACACCA-BHQ3
Substrate-1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT
6-FAM-Substrate-1	6-FAM-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Alexa 488-Substrate-1	Alexa 488-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Alexa 568-Substrate-1	Alexa 568-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Texas red-Substrate-1	Texas red-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Cy3-Substrate-1	Cy3-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Cy5-Substrate-1	Cy5-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Dabcyl-Substrate-1	Dabcyl-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
BHQ1-Substrate-1	BHQ1-ATGAGTGGTGTATGTGTTGGTGAGGTGAT
BHQ2-Substrate-1	BHQ2-ATGAGTGGTGTATGTGTTGGTGAGGTGAT

Table S3. Strand sequences used in catalytic systems with fluorescent labels at the 3' toehold domain.

Strand name	**Sequence (From 5' - 3')**
Reporter-1	6-FAM-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-1	ATACCAATCCACACTTCCA-Dabcyl
Reporter-2	Cy5-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-2	ATACCAATCCACACTTCCA-BHQ2
Catalyst	ATCACCTCACCAACACATACACCA
Signal-1	CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
6-FAM-Signal-1	6-FAM-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Alexa 488-Signal-1	Alexa 488-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Alexa 568-Signal-1	Alexa 568-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Texas red-Signal-1	Texas red-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA

Cy3-Signal-1	Cy3-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Cy5-Signal-1	Cy5-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Dabcyl-Signal-1	Dabcyl-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
BHQ1-Signal-1	BHQ1-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
BHQ2-Signal-1	BHQ2-CTCACCAACACATACACCACTCATACCAATCCACACTTCCA
Substrate-1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT
Substrate-1-6-FAM	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-6-FAM
Substrate-1-Alexa 488	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Alexa 488
Substrate-1-Alexa 568	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Alexa 568
Substrate-1-Texas red	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Texas red
Substrate-1-Cy3	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Cy3
Substrate-1-Cy5	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Cy5
Substrate-1-Dabcyl	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-Dabcyl
Substrate-1-BHQ1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ1
Substrate-1-BHQ2	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ2
Substrate-1-BHQ3	ATGAGTGGTGTATGTGTTGGTGAGGTGAT-BHQ3
Fuel	CTCACCAACACATACACCACTCAT

Table S4. Strand sequences used in systems with labels at the middle position of signal strand.

Strand name	Sequence (From 5’ - 3’)
Reporter-1	6-FAM-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-1	ATACCAATCCACACTTCCA-Dabcyl
Reporter-2	Cy5-TGGAAGTGTGGATTGGTATGAGTGG
Quencher-2	ATACCAATCCACACTTCCA-BHQ2
Invader	ATCACCTCACCAACACATACACCACTCAT
Signal-1	CTCACCAACACATACACCACTCATACCAATCCACACT

	TCCA
internal 6-FAM -Signal-1	CTCACCAACACA/int6-FAM dT/ACACCACTCATACCAA TCCACACTTCCA
internal Texas red-Signal-1	CTCACCAACACA/intTexasred dT/ACACCACTCATACCA ATCCACACTTCCA
internal BHQ2-Signal-1	CTCACCAACACA/intBHQ2 dT/ACACCACTCATACCAAT CCACACTTCCA
Substrate-1	ATGAGTGGTGTATGTGTTGGTGAGGTGAT

Reference

(1) Zadeh, J. N.; Steenberg, C. D.; Bois, J. S.; Wolfe, B. R.; Pierce, M. B.; Khan, A. R.; Dirks, R. M.; Pierce, N. A. *Journal of computational chemistry* **2011**, *32*, 170.

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