Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Modulating the DNA strand-displacement kinetics with the one-sided remote toehold design for differentiation of single-base mismatched DNA

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Materials and reagents

Tris (hydroxymethyl)-aminomethane of molecular grade was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade and obtained from Beijing Chemical Works (Beijing, China). Wahaha® purified water was used in this work. All oligonucleotides were listed in Table S1 and synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China) without further purification. All oligonucleotide stock solutions were Tris-HCl buffer (20 mM Tris, 5 mM $MgCl_2$, 300 mM NaCl, pH=7.6) and stored in dark at 4 °C.

General procedures for fluorescence measurements

Fluorescence emission at 539 nm was measured with a F-7000 spectrofluorometer (Hitachi, Japan) with excitation at 522 nm. A water-bath circulator was used to maintain the reaction at a controlled temperature as indicated.

Double-stranded probes were prepared by mixing the complementary strands at 1:1 ratio in Tris-HCl buffer, heated at 95 °C for 5 min and cooled to room temperature for 2h. In a typical experiment, appropriate amount of double-stranded probes was added to Tris-HCl buffer to achieve 10 nM final concentration with a total volume of 1.0 mL sample solution. Then, 5 μM invader strand with 4 μL was added and mixed quickly within 30 s, and the time-dependent fluorescence of the sample was recorded every 2 s to obtain the corresponding kinetic curves (Figure S1-S4).

The discrimination factor (DF)

The discrimination factor, as we previously developed,[1] is calculated by the initial reaction rate ratio between the complementary and single-base mismatched strands. In practical calculation for convenience, the reaction rate ratio was replaced by the ratio of the fluorescence change rate (dF/dt) at initial reaction stage (30-90 s).

The reaction rate constant and activation energy

The time-dependent fluorescence intensities were normalized by Equation 1

$$F = (F_S - F_R) \Big/ (F_{SR} - F_R) \quad (1)$$

where F_S, F_R and F_{SR} denoted the fluorescence intensity of each sample, solution with 10 nM Strand R and solution with 10 nM SR duplex, respectively. The following

assumptions were made for the kinetic analysis.

Assumption 1. The reaction was a bimolecular reaction. We presume that the one-sided remote toehold-mediated strand-displacement reaction was a bimolecular reaction, like the standard toehold-mediated strand-displacement reaction.

Assumption 2. The reaction was irreversible. The equilibrium constant for the reaction was greater than 10^9 at 25 °C as calculated with NUPACK.[2]

Then, the reaction system can be modeled as

$$\mathrm{I} + \mathrm{SR} \underset{}{\overset{k}{\rightleftharpoons}} \mathrm{SI} + \mathrm{R} \tag{2}$$

According to the experiment, we had

$$c_{\mathrm{I}} = 2c_{\mathrm{SR}} = 2c \tag{3}$$

where c_{I} and c_{SR} were the initial concentrations of strand I and duplex SR, respectively.

Then the product R generation rate was expressed as

$$\frac{d[R]}{dt} = k_s[I][SR] = k_s(c - [R])(2c - [R]) \tag{4}$$

where k denoted the rate constant of reaction (2).

By solving Eqs. (4), the strand-displacement fraction can be described as the following equation.

$$\frac{[R]}{c} = 1 - \frac{1}{2e^{-k_s ct} - 1} \tag{5}$$

Therefore, the normalized fluorescence was a function of reaction rate constant.

$$F = 1 - \frac{[R]}{c} = \frac{1}{2e^{kct} - 1} \tag{6}$$

from which the reaction rate constant k can be obtained.

According to Arrhenius equation (7), the activation energy can be given by linear fitting of the reaction rate constants at different temperatures.

$$\ln k + E_a / \mathrm{R}T - \ln A = 0 \tag{7}$$

where A denoted the preexponential factor.

Table S1 Sequences of oligonucleotides used in this work

Name	Sequences (5' to 3')
R	TET-GATACAGACAGCAGTTGGCCTTCTTATA-TAMRA
Spacer length (0-5 nt) and toehold length (8, 10, 12, 14 nt)	
S0-8	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCA
S1-8	TATAAGAAGGCCAACTGCTGTCTGTATC*T*ACTGAGCA
S2-8	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCA
S3-8	TATAAGAAGGCCAACTGCTGTCTGTATC*TTT*ACTGAGCA
I0-8	TGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-a	TGCTCAGCGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-b	TGCTCACTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-c	TGCTCCGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-d	TGCTGAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-e	TGCCCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-f	TGGTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-g	TCCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-8-h	CGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
S0-10	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCAAC
S1-10	TATAAGAAGGCCAACTGCTGTCTGTATC*T*ACTGAGCAAC
S2-10	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCAAC
S3-10	TATAAGAAGGCCAACTGCTGTCTGTATC*TTT*ACTGAGCAAC
S4-10	TATAAGAAGGCCAACTGCTGTCTGTATC*TTTT*ACTGAGCAAC
I0-10	GTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-a	GTTGCTCAGCGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-b	GTTGCTCACTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-c	GTTGCTCCGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-d	GTTGCTGAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-e	GTTGCCCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-f	GTTGGTCAGTGATACAGACAGCAGTTGGCCTTCTTATA

I0-10-g	GTTCCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-h	GTCGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-i	GCTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-10-j	CTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
S0-12	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCAACTC
S2-12	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCAACTC
S3-12	TATAAGAAGGCCAACTGCTGTCTGTATC*TTT*ACTGAGCAACTC
S4-12	TATAAGAAGGCCAACTGCTGTCTGTATC*TTTT*ACTGAGCAACTC
S5-12	TATAAGAAGGCCAACTGCTGTCTGTATC*TTTTT*ACTGAGCAACTC
I0-12	GAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-a	GAGTTGCTCAGCGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-b	GAGTTGCTCACTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-c	GAGTTGCTCCGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-d	GAGTTGCTGAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-e	GAGTTGCCCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-f	GAGTTGGTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-g	GAGTTCCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-h	GAGTCGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-i	GAGCTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-j	GACTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-k	GCGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-12-l	CAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
S0-14	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCAACTCGA
S2-14	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCAACTCGA
S3-14	TATAAGAAGGCCAACTGCTGTCTGTATC*TTT*ACTGAGCAACTCGA

S4-14	TATAAGAAGGCCAACTGCTGTCTGTATC*TTTT*ACTGAGCAACTCGA
S5-14	TATAAGAAGGCCAACTGCTGTCTGTATC*TTTTT*ACTGAGCAACTCGA
I0-14	TCGAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-a	TCGAGTTGCTCAGCGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-b	TCGAGTTGCTCACTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-c	TCGAGTTGCTCCGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-d	TCGAGTTGCTGAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-e	TCGAGTTGCCCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-f	TCGAGTTGGTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-g	TCGAGTTCCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-h	TCGAGTCGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-i	TCGAGCTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-j	TCGACTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-k	TCGCGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-l	TCCAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-m	TGGAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
I0-14-n	CCGAGTTGCTCAGTGATACAGACAGCAGTTGGCCTTCTTATA
Different spacer sequences (Figure S4)	
S0-10	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCAAC
S2-10-TT (S2-10)	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCAAC
S2-10-CC	TATAAGAAGGCCAACTGCTGTCTGTATC*CC*ACTGAGCAAC
S2-10-CT	TATAAGAAGGCCAACTGCTGTCTGTATC*CT*ACTGAGCAAC
S2-10-TC	TATAAGAAGGCCAACTGCTGTCTGTATC*TC*ACTGAGCAAC
Different toehold sequences (Figure S3)	
S0-10A	TATAAGAAGGCCAACTGCTGTCTGTATCACTGAGCAAC

(S0-10)	
S2-10A (S2-10)	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*ACTGAGCAAC
S0-10B	TATAAGAAGGCCAACTGCTGTCTGTATCTTACTCGCTG
S2-10B	TATAAGAAGGCCAACTGCTGTCTGTATC*TT*TTACTCGCTG
I0-10B	CAGCGAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-a	CAGCGAGTACGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-b	CAGCGAGTCAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-c	CAGCGAGCAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-d	CAGCGACTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-e	CAGCGCGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-f	CAGCCAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-g	CAGGGAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-h	CACCGAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-i	CCGCGAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
I0-10B-j	GAGCGAGTAAGATACAGACAGCAGTTGGCCTTCTTATA
Standard toehold and symmetrical remote toehold (Figure S5)	
I2-10	GTTGCTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-a	GTTGCTCAGC*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-b	GTTGCTCACT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-c	GTTGCTCCGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-d	GTTGCTGAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-e	GTTGCCCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-f	GTTGGTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-g	GTTCCTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-h	GTCGCTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-i	GCTGCTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA
I2-10-j	CTTGCTCAGT*TT*GATACAGACAGCAGTTGGCCTTCTTATA

The first numbers after the names of oligonucleotide represent the spacer length (nt),

and the second numbers represent the toehold length (nt) of corresponding system. The italic letters denote the spacer sequences of the S strands. The underlined letters denote the toehold of the S and I strands. The framed letters represent the mismatched sites.

Table S2 The percent hybridization of 8 nt, 10 nt, 12 nt and 14 nt toeholds as a function of temperature calculated with NUPACK[2]

Toehold lengths (nt) / Temperature (℃)	8	10	12	14
15	0.98	1	1	1
20	0.96	1	1	1
25	0.92	0.99	1	1
30	0.83	0.97	0.99	1
35	0.66	0.93	0.98	1

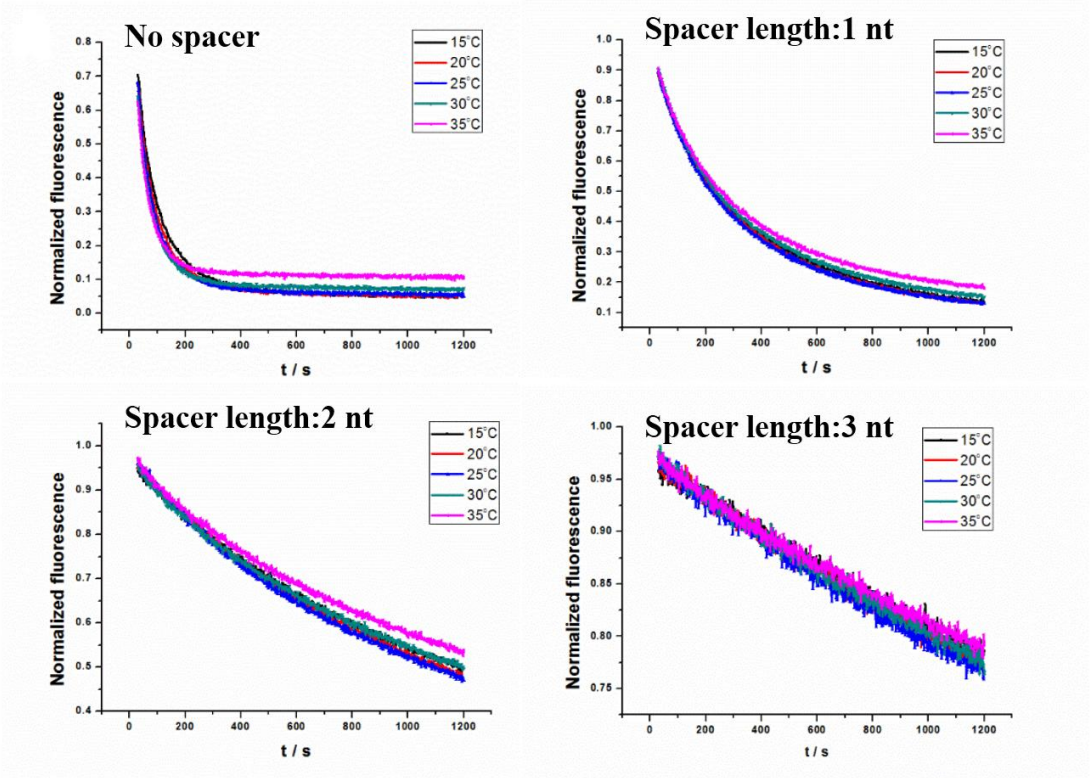

Figure S1. The kinetic curves of one-sided remote toehold-mediated reactions with different spacer lengths and temperatures in the 8-nt toehold design.

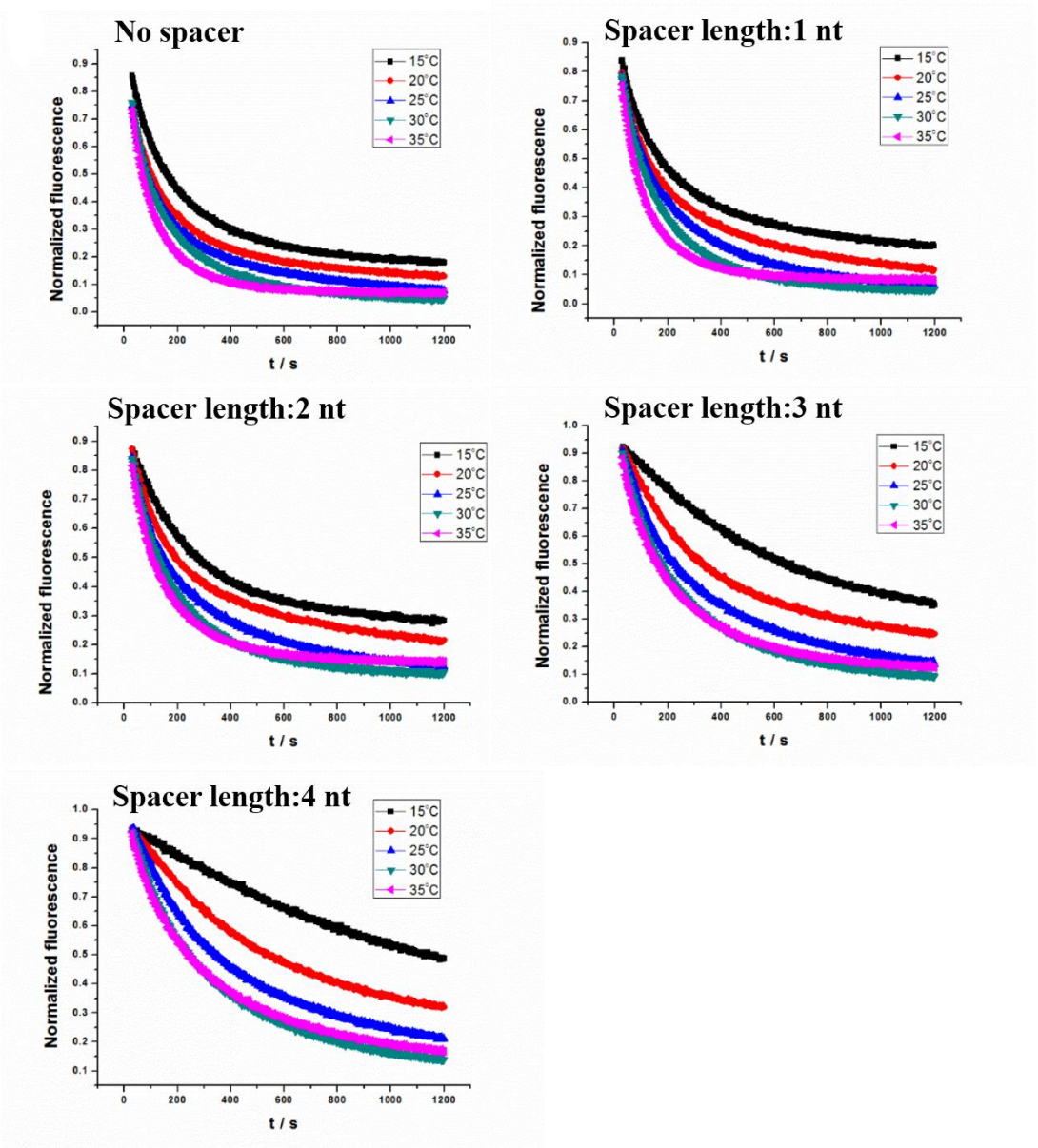

Figure S2. The kinetic curves of one-sided remote toehold-mediated reactions with different spacer lengths and temperatures in the 10-nt toehold design.

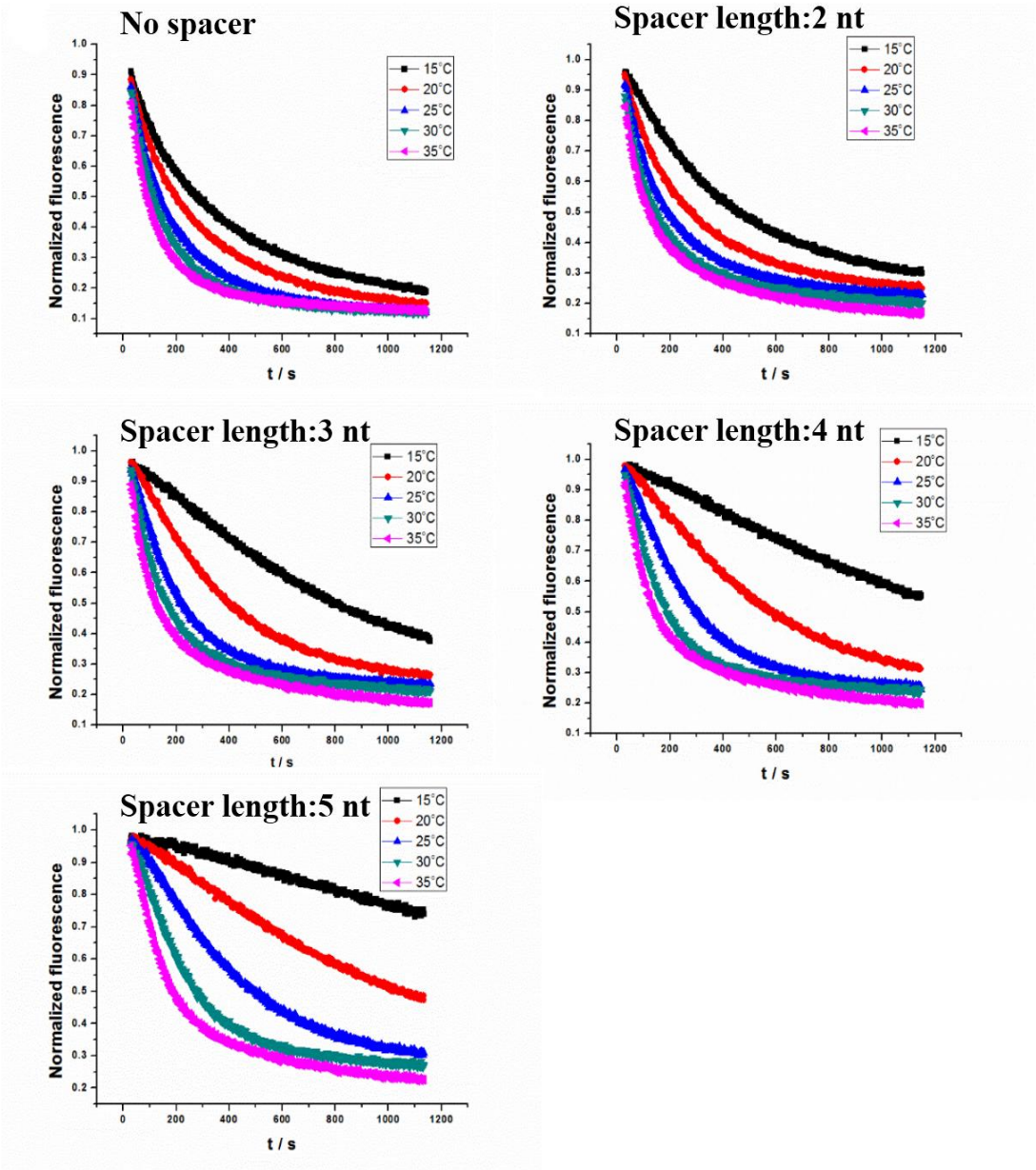

Figure S3. The kinetic curves of one-sided remote toehold-mediated reactions with different spacer lengths and temperatures in the 12-nt toehold design.

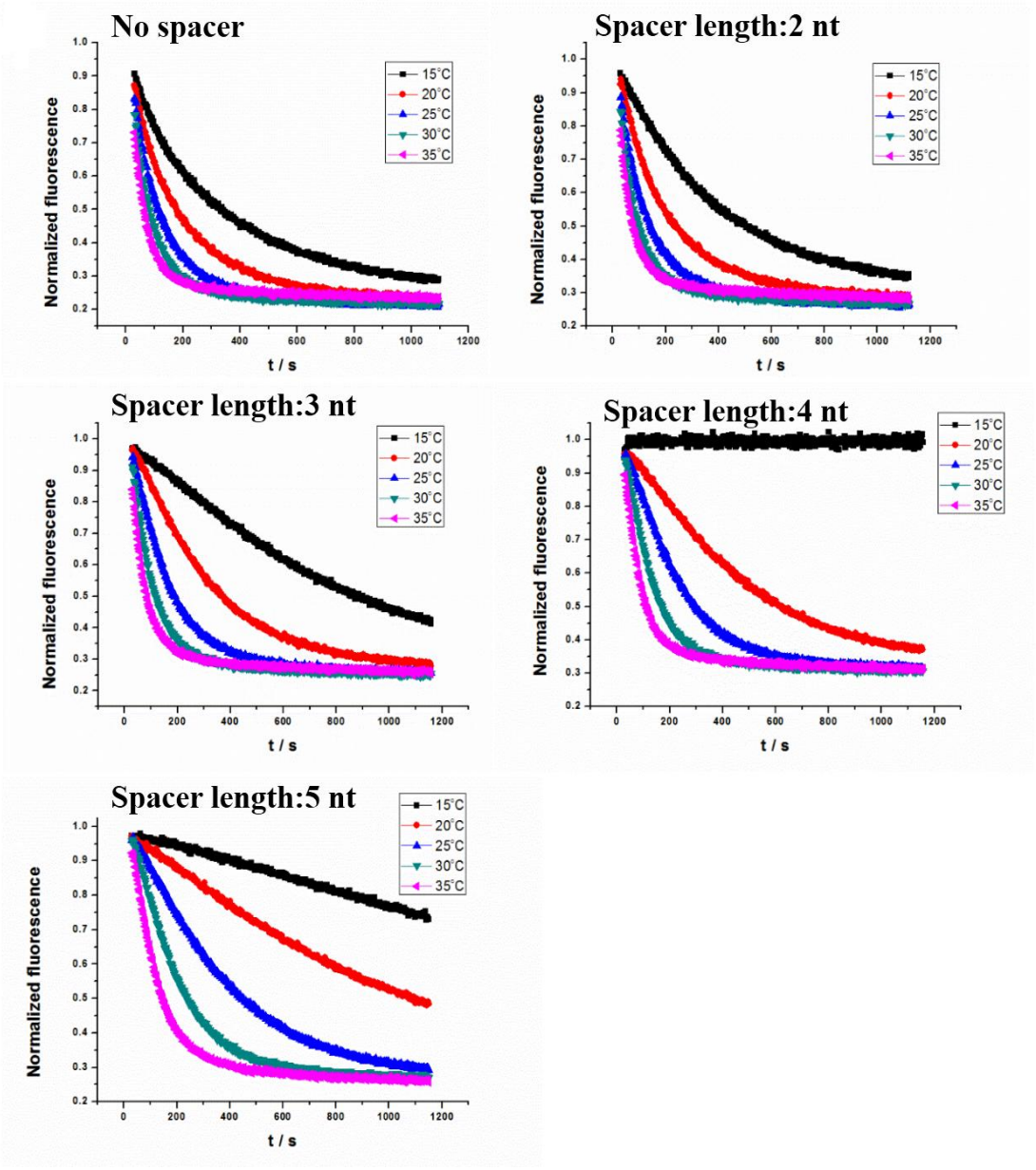

Figure S4. The kinetic curves of one-sided remote toehold-mediated reactions with different spacer lengths and temperatures in the 14-nt toehold design.

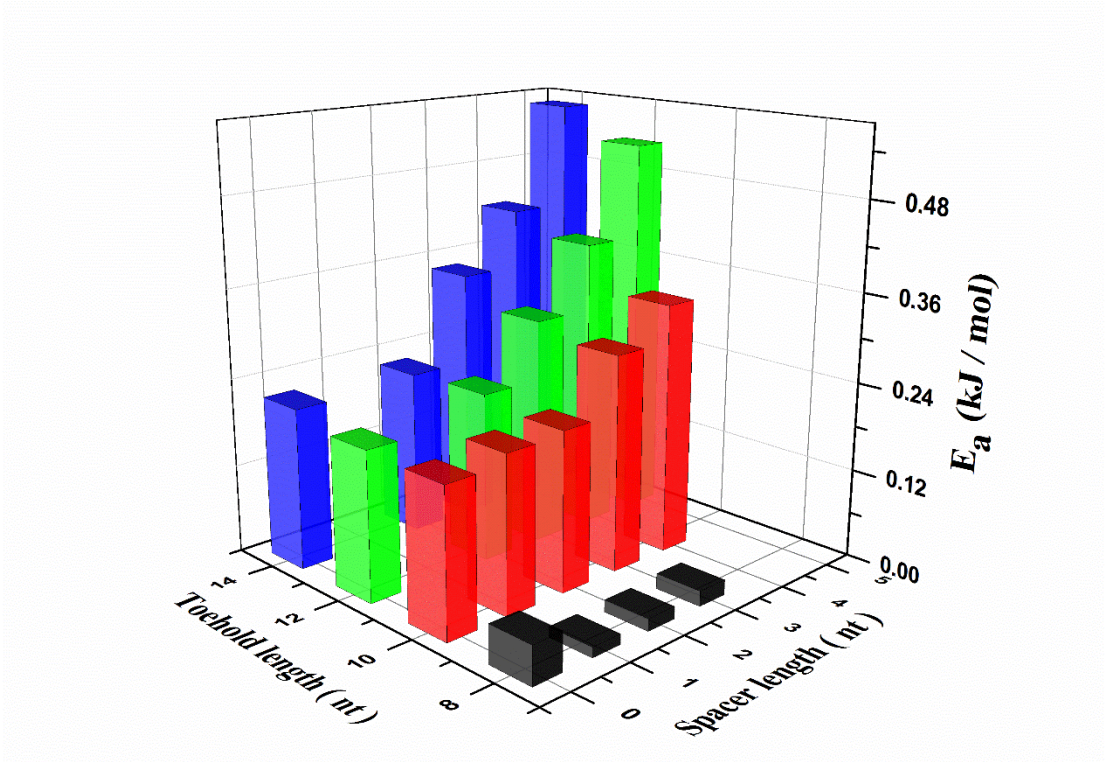

Figure S5. The activation energy as a function of the toehold length and spacer length.

Generally, the activation energies of the above strand-displacement reactions increased with the longer toehold and spacer length (a few data points were not involved). The activation energies of reactions with 8 nt toehold are small for sure regardless the poor fitting of Eq. (6).

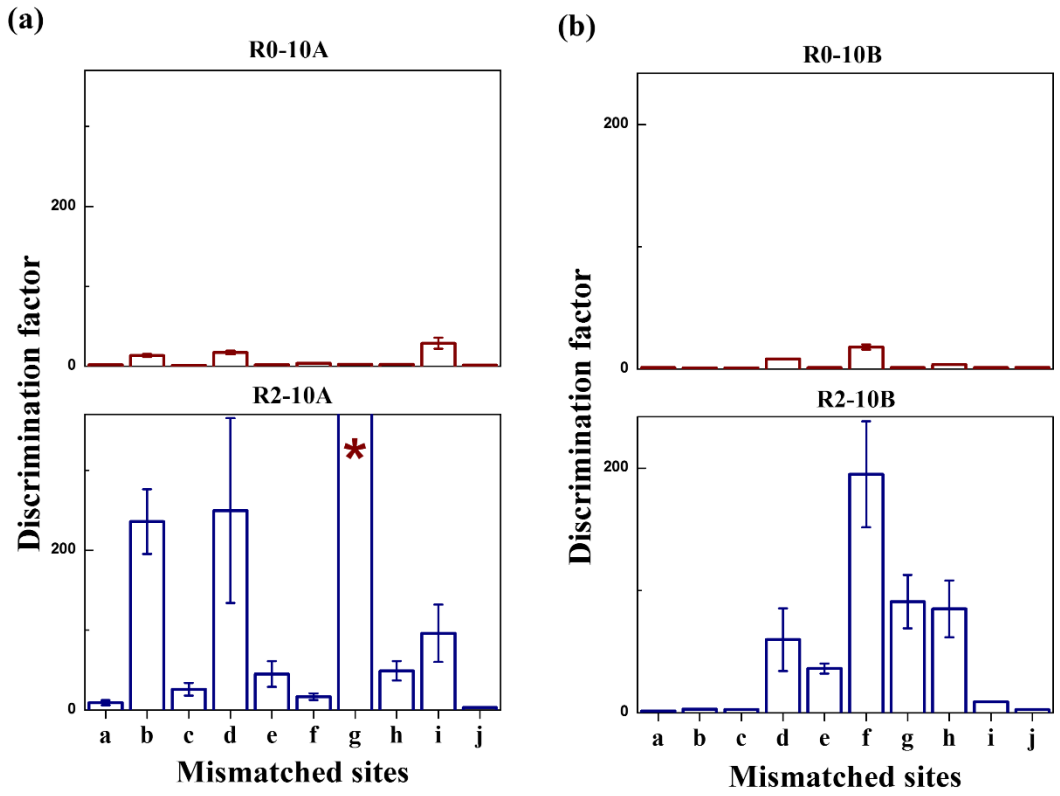

Figure S6. The influence of the toehold sequence on the discrimination factor of different mismatched sites. The mismatched site on the toehold domain started from the site "a" which is located next to the spacer domain. *: the discrimination factor at this site is 771.

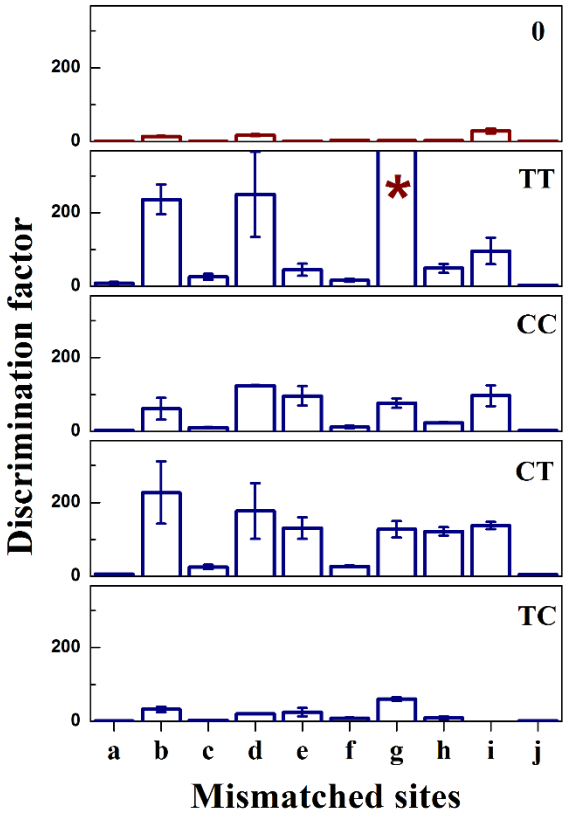

Figure S7. The influence of the spacer sequence on the discrimination factor of different mismatched sites. The mismatched site on the toehold domain started from the site “a” which is located next to the spacer domain. *: the discrimination factor at this site is 771.

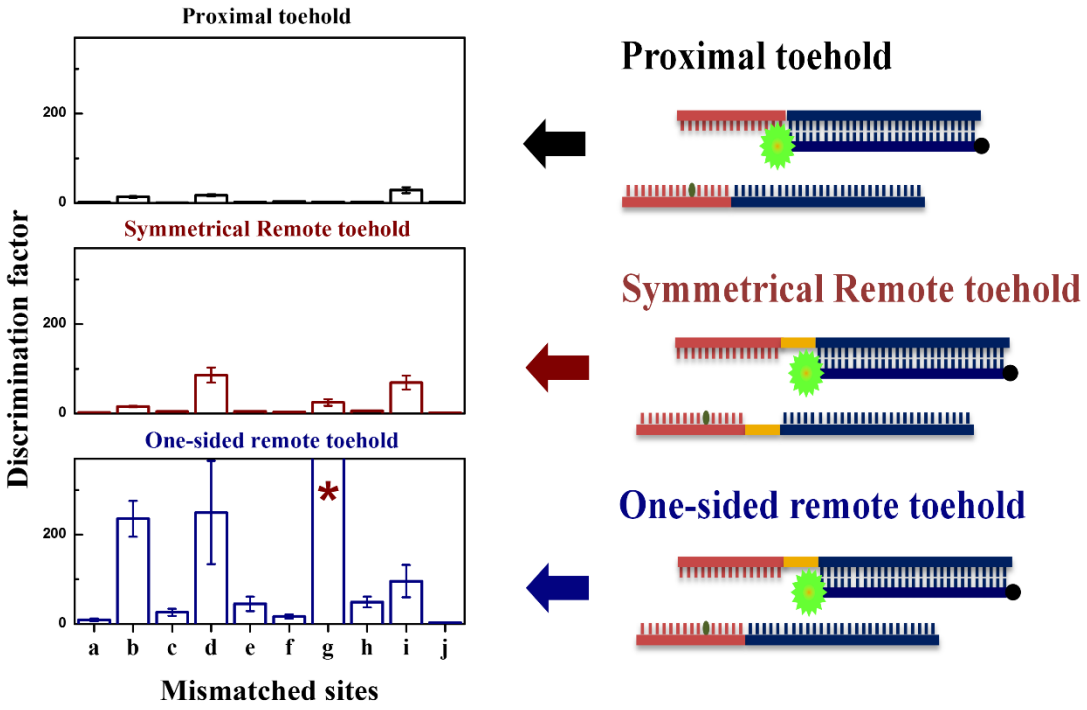

Figure S8. The influence of the strand sequence on the discrimination factor of different mismatched sites. The mismatched site on the toehold domain started from the site "a" which is located next to the spacer domain. *: the discrimination factor at this site is 771.

References

1 C. Li, Y. Li, X. Xu, X. Wang, Y. Chen, X. Yang, F. Liu and N. Li, *Biosens. Bioelectron.*, 2014, **60**, 57–63.

2 R. M. Dirks, J. S. Bois, J. M. Schaeffer, E. Winfree and N. A. Pierce, *SIAM Rev.*, 2007, **49**, 65–68.