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 MgCl₂, 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

\[ F = \frac{(F_s - F_R)}{(F_{SR} - F_R)} \]

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

$$I + SR \xrightleftharpoons{k} SI + R$$

(2)

According to the experiment, we had

$$c_1 = 2c_{SR} = 2c$$

(3)

where $c_1$ and $c_{SR}$ were the initial concentrations of strand I and duplex SR, respectively.

Then the product R generation rate was expressed as

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

(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.

$$\frac{[R]}{c} = 1 - \frac{1}{2e^{-k,c} - 1}$$

(5)

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

$$F = 1 - \frac{[R]}{c} = \frac{1}{2e^{k,c} - 1}$$

(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.

$$\ln k + E_a / RT - \ln A = 0$$

(7)

where $A$ denoted the preexponential factor.
Table S1 Sequences of oligonucleotides used in this work

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>TET-GATACAGACAGCAGTTGGCCTTCTTATA-TAMRA</td>
</tr>
</tbody>
</table>

Spacer length (0-5 nt) and toehold length (8, 10, 12, 14 nt)

| S0-8 | TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCA |
| S1-8 | TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCA |
| S2-8 | TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCA |
| S3-8 | TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCA |
| I0-8 | TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-a| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-b| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-c| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-d| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-e| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-f| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-g| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| I0-8-h| TGCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA   |
| S0-10| TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCAAC |
| S1-10| TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCAAC |
| S2-10| TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCAAC |
| S3-10| TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCAAC |
| S4-10| TATAAGAAGGCCAACTGCTGTCTGTATCATCAGAGCAAC |
| I0-10| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-a| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-b| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-c| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-d| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-e| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
| I0-10-f| GTTGTCTCAGTGACAGCACAGCAGTTGGCCTTCTTATA |
Different spacer sequences (Figure S4)

S4-14  TATAAGAAGGCAACTGCTGCTGCTATCTT777ACTGAGCAACG
        TATAAGAAGGCAACTGCTGCTGCTATCTT777ACTGAGCAAC

S5-14  TCGA
        TCGA

I0-14  TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-a TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-b TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-c TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-d TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-e TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-f TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-g TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-h TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-i TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-j TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-k TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-l TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-m TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA
I0-14-n TCGAGTTGCTCAGTGATACAGACGCAGTTGCGCTTTCTTATA

Different toehold sequences (Figure S3)

S0-10  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC
S2-10-TT  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC
(S2-10)  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC

S2-10-CC  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC
S2-10-CT  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC
S2-10-TC  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC

Different spacers sequences (Figure S4)

S0-10A  TATAAGAAGGCAACTGCTGCTGCTATCCTTACTGAGCAAC
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 NUPACK²

<table>
<thead>
<tr>
<th>Temperature (℃)</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.98</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>0.96</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>0.92</td>
<td>0.99</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>0.83</td>
<td>0.97</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>0.66</td>
<td>0.93</td>
<td>0.98</td>
<td>1</td>
</tr>
</tbody>
</table>
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
