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Supplemental Information

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Supporting Material

The Effect of Basepair Mismatch on DNA Strand Displacement

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SUPPORTING MATERIAL

Oligonucleotide sequences used in this study

Substrate	5'-CA/iCy5/ACCAAAATTGGGACAACACCAGTG/3BioTEG/-3'
Substrate*	5'-CA/iCy5/ATTAAAATTCCGACAACACCAGGT/3BioTEG/-3'

Supplementary Table S1. Substrate strand. The substrate strand sequence is complementary to a region of messenger RNA encoding YFP. We internally labelled the strand with Cy5 to increase photostability(1) and implemented a biotin linker for surface immobilization. The substrate* strand was derived from the substrate and altered to remove secondary structure.

Match	5'-GTCCCAATTTTGGT/BHQ3/-3'
Match*	5'-GTCGGAATTTTAAT/BHQ3/-3'
Mismatch 1	5'-A<u>T</u>CCCAATTTTGGT/BHQ3/-3'
Mismatch 2	5'-G<u>C</u>CCCAATTTTGGT/BHQ3/-3'
Mismatch 3	5'-GT<u>T</u>CCAATTTTGGT/BHQ3/-3'
Mismatch 4	5'-GTC<u>T</u>CAATTTTGGT/BHQ3/-3'
Mismatch 5	5'-GTCC<u>T</u>AATTTTGGT/BHQ3/-3'
Mismatch 6	5'-GTCCC<u>G</u>ATTTTGGT/BHQ3/-3'
Mismatch 7	5'-GTCCCA<u>G</u>TTTTGGT/BHQ3/-3'
Mismatch 8	5'-GTCCCAA<u>C</u>TTTGGT/BHQ3/-3'
Mismatch 9	5'-GTCCCAAT<u>C</u>TTGGT/BHQ3/-3'
Mismatch 10	5'-GTCCCAATT<u>C</u>TGGT/BHQ3/-3'
Mismatch 11	5'-GTCCCAATTT<u>C</u>GGT/BHQ3/-3'
Mismatch 12	5'-GTCCCAATTTT<u>A</u>GT/BHQ3/-3'
Mismatch 13	5'-GTCCCAATTTTG<u>A</u>T/BHQ3/-3'
Mismatch 14	5'-GTCCCAATTTTGG<u>C</u>/BHQ3/-3'

Supplementary Table S2. Incumbent strands. The incumbent strand was labelled with a dark quencher with an absorption spectrum that well overlaps the emission of Cy5. The underlined letter represents the single mismatch. The match* strand was designed to remove secondary structure.

Match	5'-CACTGGTGTTGTCCCAATTTTGGT-3'
Match*	5'-ACCTGGTGTTGTCGGAATTTTAAT-3'
Mismatch 1	5'-CACTGGTGTT<u>A</u>TCCCAATTTTGGT-3'
Mismatch 1*	5'-ACCTGGTGTT<u>A</u>TCGGAATTTTAAT-3'
Mismatch 2	5'-CACTGGTGTTG<u>C</u>CCCAATTTTGGT-3'
Mismatch 3	5'-CACTGGTGTTGT<u>T</u>CCAATTTTGGT-3'
Mismatch 4	5'-CACTGGTGTTGTC<u>T</u>CAATTTTGGT-3'
Mismatch 5	5'-CACTGGTGTTGTCC<u>T</u>AATTTTGGT-3'
Mismatch 6	5'-CACTGGTGTTGTCCC<u>G</u>ATTTTGGT-3'
Mismatch 7	5'-CACTGGTGTTGTCCCA<u>G</u>TTTTGGT-3'
Mismatch 7*	5'-ACCTGGTGTTGTCGGA<u>G</u>TTTTAAT-3'
Mismatch 8	5'-CACTGGTGTTGTCCCAA<u>C</u>TTTGGT-3'
Mismatch 9	5'-CACTGGTGTTGTCCCAAT<u>C</u>TTGGT-3'
Mismatch 10	5'-CACTGGTGTTGTCCCAATT<u>C</u>TGGT-3'
Mismatch 11	5'-CACTGGTGTTGTCCCAATTT<u>C</u>GGT-3'
Mismatch 12	5'-CACTGGTGTTGTCCCAATTTT<u>A</u>GT-3'
Mismatch 13	5'-CACTGGTGTTGTCCCAATTTTG<u>A</u>T-3'
Mismatch 14	5'-CACTGGTGTTGTCCCAATTTTGG<u>C</u>-3'

Supplementary Table S3. Invader strands. The underlined letter represents the single mismatch. The strands marked with an asterisk were designed by removing secondary structure from their corresponding invader strands.

Branch migration as random walk

We put forth a model for strand displacement based on the mean first passage time of a 1D random walk. We begin by assuming that the rate of breaking individual base pairs is much slower than the reverse rate of formation. By this assumption, incumbent strand unzipping and invader strand zipping is almost coincidental, and intermediates states can be specified with one state variable i, which is equal to the number of displaced base pairs. $i = 0$ is the initial state before invasion, and $i = n$ corresponds to complete displacement. We now define a 1D lattice with $n + 1$ sites. Motion at i-th lattice site is performed in single steps at forward and reverse rates, f_i and r_i respectively. This model is equivalent to a random walk with a perfectly reflecting boundary on the left ($i = 0$), and perfectly absorbing boundary on the right ($i = n$).

The mean first passage time from $i = 0$ to $i = n$ is given by(2, 3)

$$\tau = \sum_{i=0}^{n-1} \frac{1}{p_i f_i}, \tag{S1}$$

where p_i is the steady state probability at site i in a partial lattice between 0 and i. Therefore, the inverse of each term, $p_i f_i$, can be interpreted as the effective rate of reaching $i + 1$ from an unspecified previous position. p_i can be expressed with a ratio of forward and reverse rates between two adjacent sites ($\alpha_i = f_{i-1}/r_i$) as

$$p_i = \frac{\alpha_i \alpha_{i-1} ... 1}{1 + \alpha_1 + \alpha_2 \alpha_1 + ... + \alpha_i \alpha_{i-1} ... 1} \tag{S2}$$

Without sequence dependence, branch migration over a matched base pair must be identical in either direction and, therefore, $f_{i-1} = r_i$ or $\alpha_i = 1$. In comparison, $\alpha_i \gg 1$ for the case of a mismatch on the incumbent and $\alpha_i \ll 1$ for the case of a mismatch in the invader. We denote this mismatch-dependent fold-change in α as a, which must be larger than one for an incumbent mismatch and smaller than one for an invader mismatch. We also introduce variation in the forward rate for the first base pair to be displaced with another ratio (f/b). It is thought to be smaller due to slow initiation ($b > 1$)(5). Using these ratios, the MFPT's with an invader mismatch (τ_v) and an incumbent mismatch (τ_c) at position j are given by

$$\tau_v(j) = \frac{1}{f}\left[-\left(\frac{1}{a} - 1\right) j^2 + n\left(\frac{1}{a} - 1\right) j + (b-1)\left(-\left(\frac{1}{a} - 1\right) j + \frac{n}{a}\right) + \frac{n(n+1)}{2}\right], \tag{S3a}$$

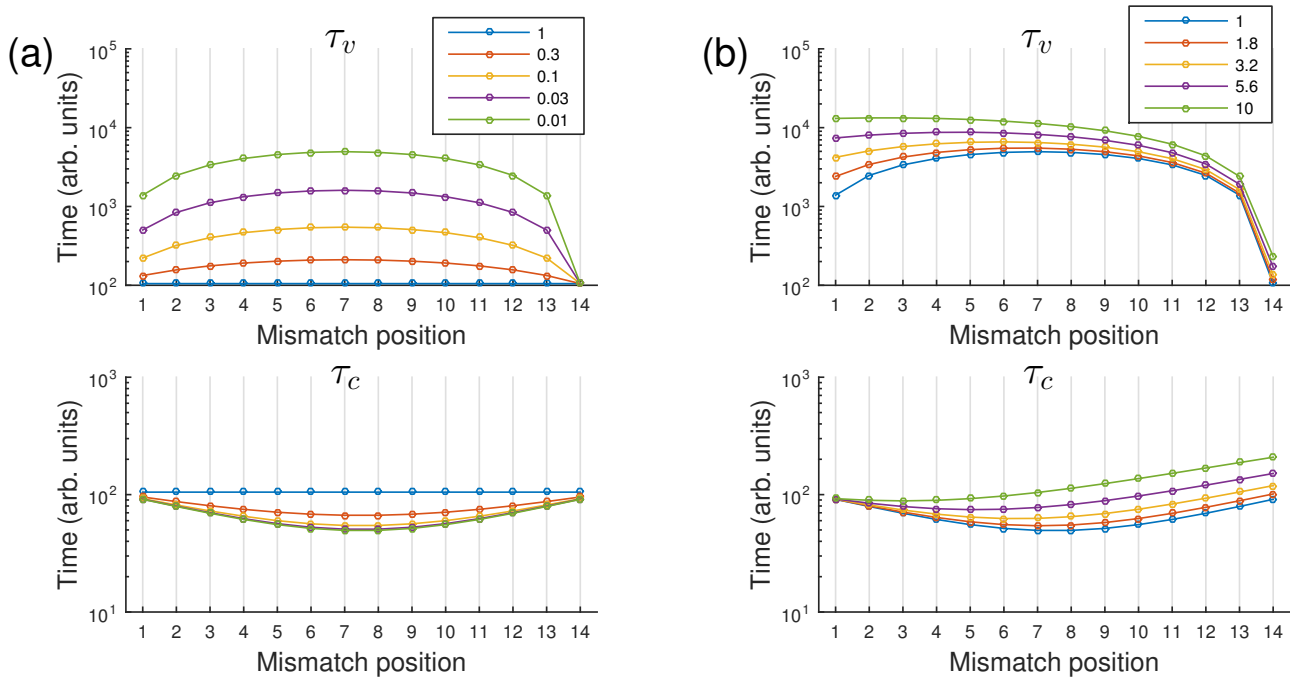
$$\tau_c(j) = \frac{1}{f}\left[(1-a)j^2 - (1-a)(n+1)j + (b-1)((1-a)(j-1) + an) + \frac{n(n+1)}{2}\right], \tag{S3b}$$

respectively. The equations are cast in a form to reveal the dependence of MFPT on mismatch position j. Without slower opening of the first base pair ($b = 1$), MFPT for the invader mismatch is concave down with a center at $n/2$, and MFPT for the incumbent mismatch is concave up with a center at $(n + 1)/2$. Slow opening of the first base pair ($b > 1$) shifts the center towards lower values. As expected, when $a = 1$ and $b = 1$, both MFPT's approach $n^2/2f$.

This 1D model predicts MFPT to be a quadratic function of mismatch position with the slowest displacement near the center position (Fig. S1(a)), which is not consistent with the overall monotonic change we observed with an invader mismatch. Slow initiation of branch migration ($b > 1$) could render the prediction more monotonic (Fig. S1(b)), but it requires an unreasonably large b.

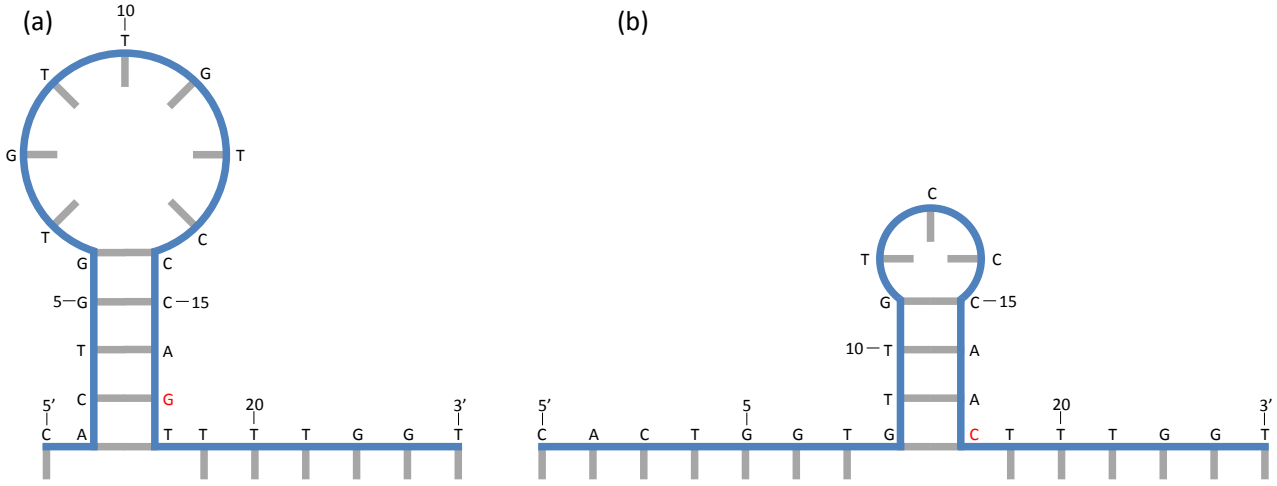
Dissociation kinetics of the invader strand

In effort to instill confidence in our model, we performed a separate experiment involving biotinylated invader strands, Cy3 labelled substrate strands, and Cy5 labelled incumbent strands. The invader strands were immobilized on the surface, and preformed substrate-incumbent duplexes were pumped in and allowed to react. Invader strands were designed to have the same complementary toehold (10nt) adjoined to a tail composed of 14 thymidines to prevent successful strand displacement. Interactions between duplexes and invader strands were recognized as a high FRET state. Lifetimes of high FRET states were recorded and interpreted as dissociation times. Large numbers of these lifetimes were recorded to construct a single exponential probability distribution whose decay rate was determined to be $\sim 0.03\,\mathrm{s}^{-1}$.

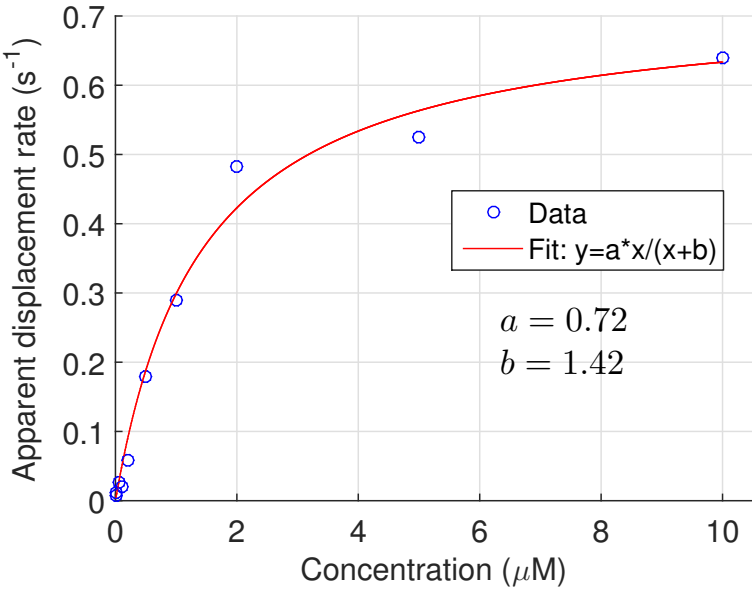


Supplementary Figure S1. Dissociation via branch migration. MFPT's predicted by the 1D lattice model are plotted using Eq. S3. (a) MFPT's with varying a. a characterizes the effect of a mismatch on the forward branch migration rate. A mismatch in the invading strand lengthens MFPT (τ_v), while a mismatch in the incumbent strand shortens it (τ_v). b is fixed to 1. (b) MFPT's with varying b. b represents how slow the first migration step is compared to the rest. As b becomes larger, the center of the curves shifts towards the left, and both τ_v and τ_c become more monotonic as a function of mismatch position. a is fixed to 0.01.

Supplementary Figure S2. Sample video of fluorescence data



Supplementary Figure S3. Putative secondary structures of invader mismatch strands. (a) Mismatch position 7. This is the only conformation predicted by mfold(4) for this structure. The hairpin mostly obstructs the toehold. It is predicted to have a lower free energy than the active form by $\Delta G = -3.61\,\text{kcal/mol}$. (b) Mismatch position 8. This is the predicted conformation by mfold. The toehold is partially obstructed due to the hairpin. It is predicted to have a lower free energy from the active form by $\Delta G = -2.61\,\text{kcal/mol}$.



Supplementary Figure S4. Displacement rate vs. invader concentration. The displacement rate (y) was measured as a function of the concentration of the invader strand (x). The displacement reaction can be modeled by reversible binding (k_+) and unbinding (k_-) steps followed by a unimolecular displacement (r) step. The unbinding rate of the toehold-bound invader was directly measured to be $1/30\,\mathrm{s}^{-1}$, which is much slower than r. In this case, the apparent displacement rate is given by $rx/(x + r/k_+)$. We fit the measured data points (blue hollow circles) using the expression $y = ax/(x + b)$ with two fitting parameters. From a and b, we determine the unimolecular displacement rate (r) and the binding rate (k_+) to be $0.72\,\mathrm{s}^{-1}$ and $0.5\,\mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$, respectively.

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