

Supplementary Material

Improving Dynamic Control of Toehold-Mediated Strand Displacement Reactions through Phosphorothioate Modifications

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Table S1. The DNA sequences used in this work.

[illegible]

	CAACCTAGCCTGGCCCCCCCCCTTTCGC
HP1-R2	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCCCCCCCCTT*T*CGC
HP1-R4	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCCCCCCC*T*T*T*CGC
HP1-R6	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCCCC*C*C*T*T*T*CGC
HP1-R12	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGG*C*C*C*C*C*C*C*T*T*T*CGC
HP1-R3S6	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCC*C*C*C*C*C*TTTCGC
HP1-R6S6	GTCTACGGTGCAGGGAAAGGGGGGGGCCAGCCTGCACCAACCTAGC CTGG*C*C*C*C*C*CCCTTTCGC
MB	5'FAM-CATGCGAAAGGGGTACGTGGCATG-3'BHQ

Probabilistic Circuits

I1	CAAAATCCAAAACCTCTTACCCATCCATTCCACTATACACAC
I4	GGTGTGTATAGTGAATGGATG GGT
I2	CATCCATTCCACTATACACACCACCATCAAATAACTACACAC
I2-s4	C*A*T*C*CATTCCACTATACACACCACCATCAAATAACTACACAC
I2-s8	C*A*T*C*C*A*T*T*CCACTATACACACCACCATCAAATAACTACACA C
I3	CATCCATTCCACTATACACACCTCAATAACATCTCTCTCCTC
I3-s4	T*C*C*A*TTCCACTATACACACCTCAATAACATCTCTCTCCTC
I3-s8	T*C*C*A*T*T*C*C*ACTATACACACCTCAATAACATCTCTCTCCTC
I5	CACCATCAAATAACTACACACCAGGTATTCCGACAACACACA
I6	CTCAATAACATCTCTCTCCTCCCTAACACAATCACTCTCCTC
T1	GGTGTGTAGTTATTTGATGGTGGTGTGT
T2	GGAGGAGAGAGATGTTATTGAGGTGTGT
Rep6-HEX	5'FAM-GAGGAGAGTGATTGTGTTAGGGAGGAG
Rep6-BHQ	CCTAACACAATCACTCTCCTC-3'BHQ

*: phosphorothioate modification

Table S2. The thermodynamic parameters of DNA duplexes with varying numbers of phosphorothioate modifications.

Name	ΔG_{37} (kcal·mol ⁻¹)	ΔH (kcal·mol ⁻¹)	ΔS (cal·mol ⁻¹ ·K ⁻¹)
Y / Z	-44.1	-155.6	-359.6
Y-s5 / Z	-39.3	-147.9	-350.3
Y-s10 / Z	-32.7	-138.4	-341.1
Y-s15 / Z	-25.8	-129.4	-334.3
Y-s20 / Z	-18.8	-120.8	-328.8

We calculated the thermodynamic parameters using the method described by John et. al.[1].

[1] S. John, Proc. Natl. Acad. Sci. U.S.A. 1998, 55, 1460–1465.

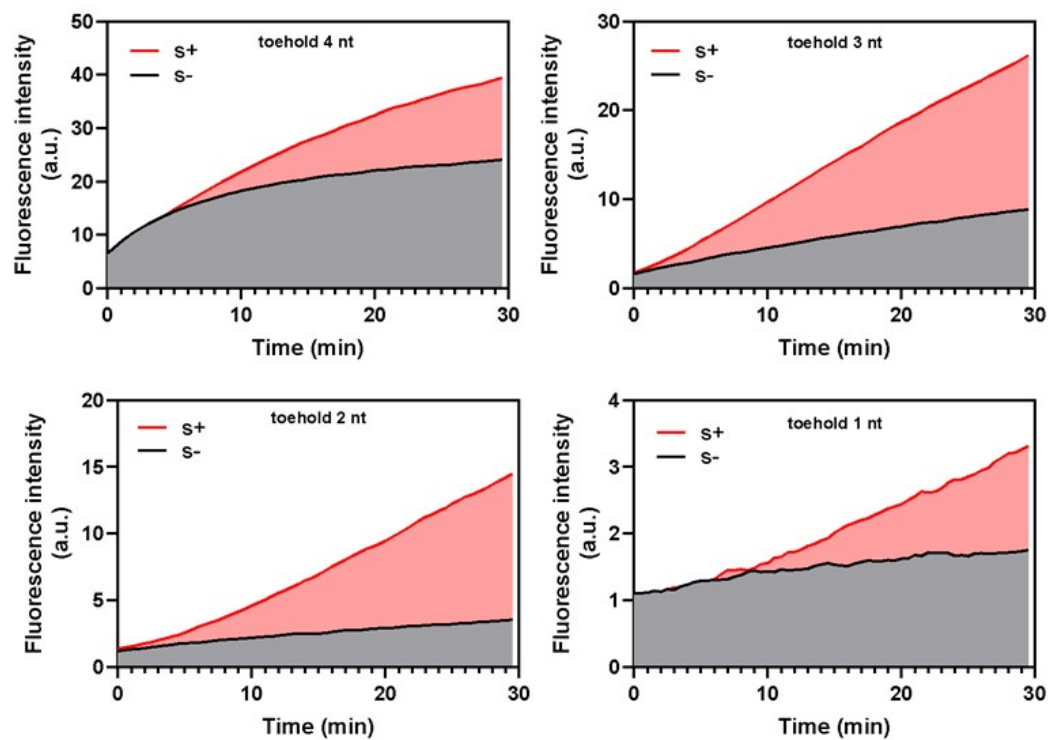


Figure S1. Fluorescence schematic diagram of strand displacement reactions with phosphorothioate modification (S+) versus non-modification (S-) under various toehold lengths (0, 1, 2, 3, 4 nt). $[X]=100$ nM, $[Y]=100$ nM, $[Z]=100$ nM, and $[Reporter]=100$ nM for all tests.

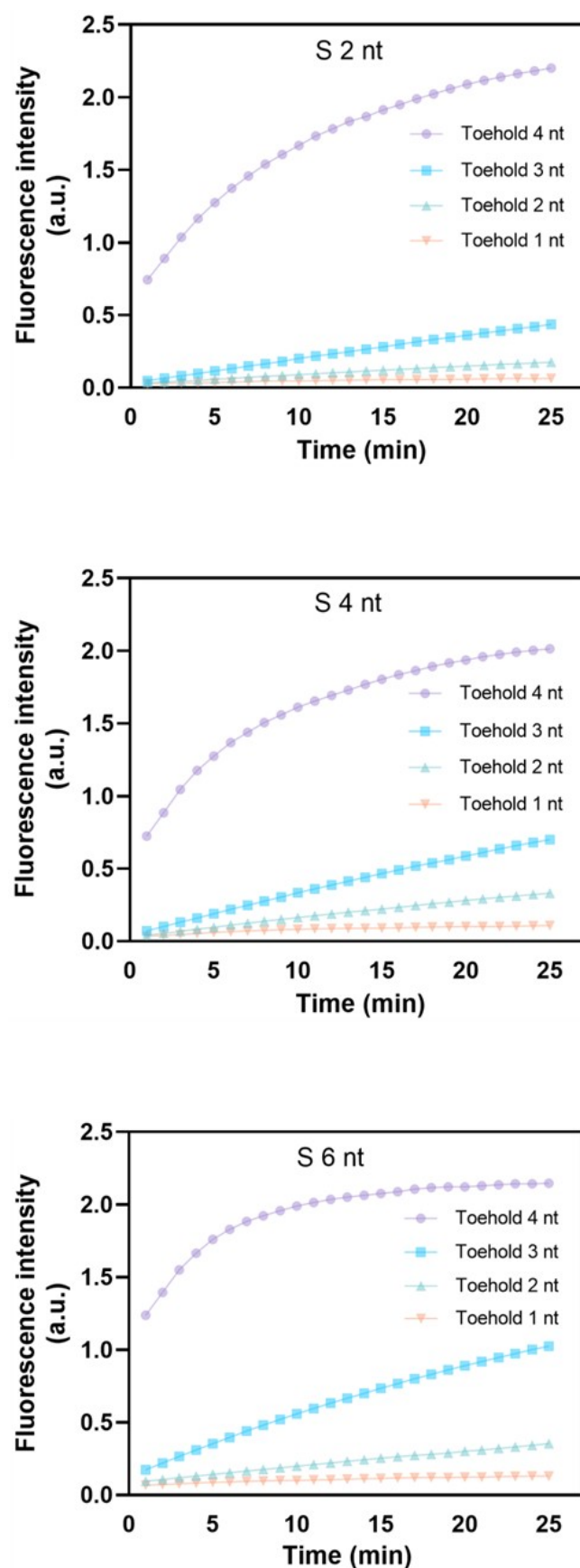


Figure S2. Fluorescence schematic diagram of strand displacement reactions with various phosphorothioate modification (2, 4, 6 nt) under various toehold lengths (0, 1, 2, 3, 4 nt). $[X]=100$ nM, $[Y]=100$ nM, $[Z]=100$ nM, and $[Reporter]=100$ nM for all tests.

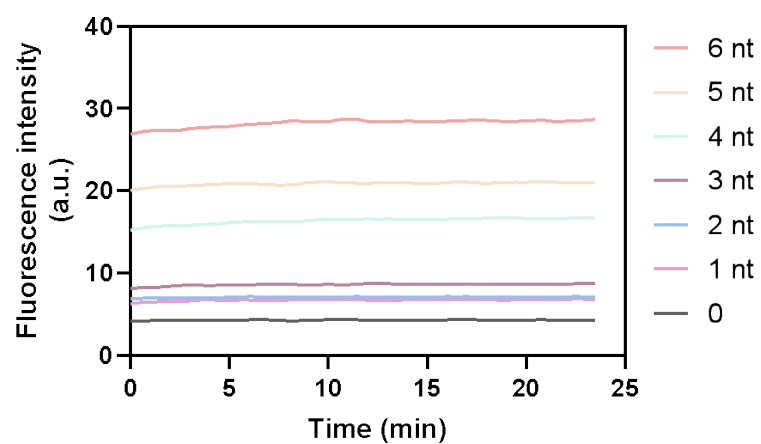


Figure S3. Fluorescence schematic diagram of strand displacement reactions with Toehold 6 nt under various phosphorothioate-modified bases (0, 1, 2, 3, 4, 5, 6 nt).

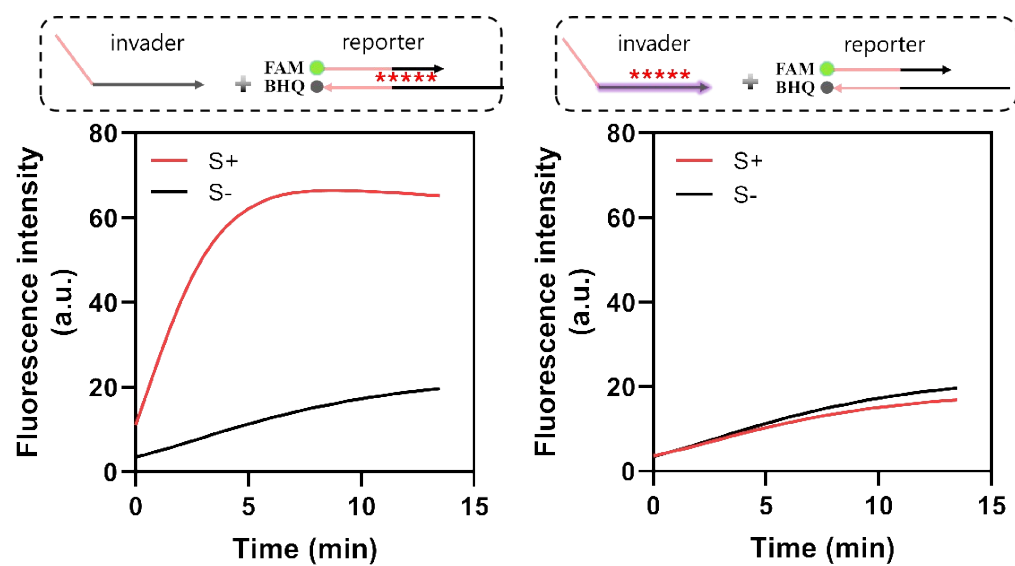


Figure S4. The impact of phosphorothioate modification on the invader strand or the displaced strand on the strand displacement reaction. $[\text{invader}] = [\text{reporter}] = 1000 \text{ nM}$.

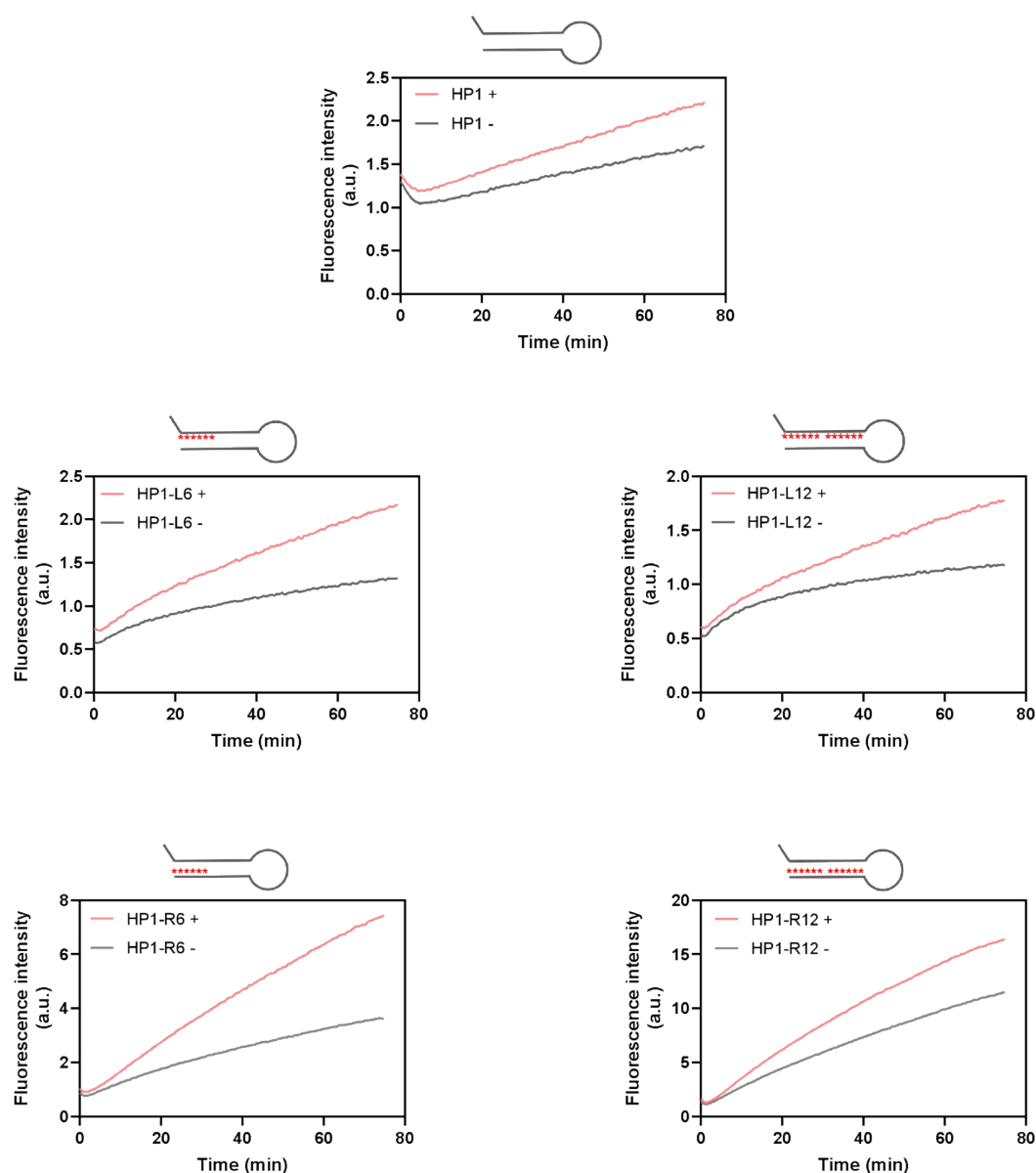


Figure S5. Fluorescence schematic diagram of CHA reaction under phosphorothioate modification of HP1 with varying numbers and positions (HP1, HP1-L6, HP1-L12, HP1-R6, HP1-R12). [HP1] = 200 nM, [HP2] = 200 nM, [MB] = 200 nM, and [Trigger] = 20 nM.

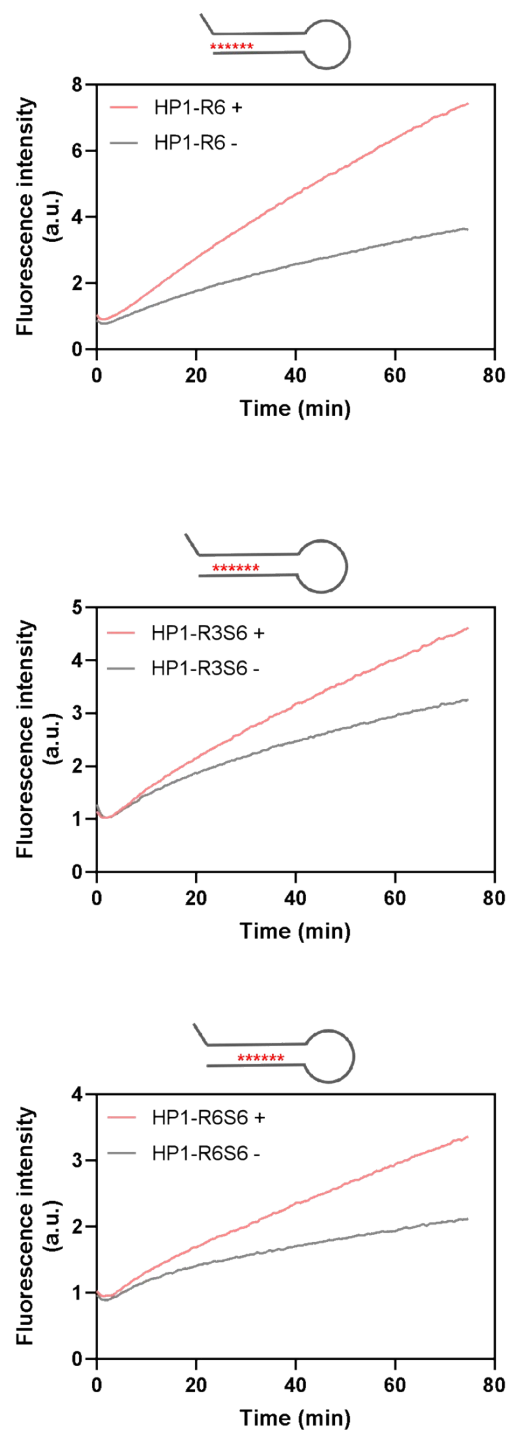


Figure S6. Fluorescence schematic diagram of CHA reaction under phosphorothioate modification of HP1 with varying positions (HP1-R6, HP1-R3S6, HP1-R6S6). [HP1] = 200 nM, [HP2] = 200 nM, [MB] = 200 nM, and [Trigger] = 20 nM.

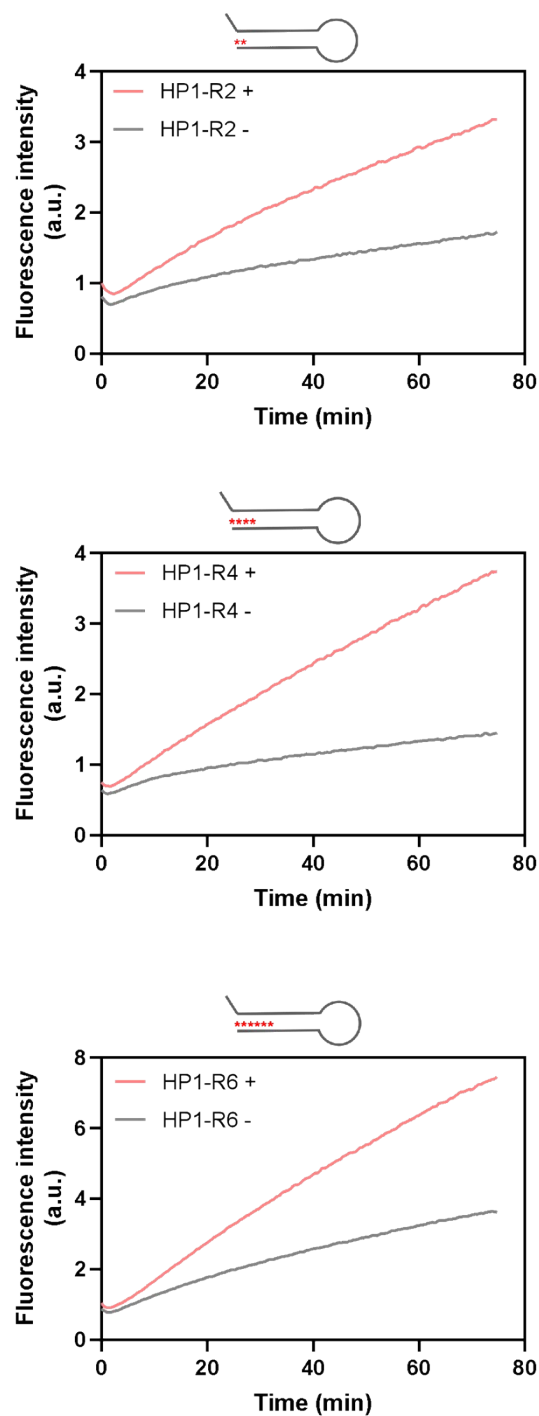


Figure S7. Fluorescence schematic diagram of CHA reaction under phosphorothioate modification of HP1 with varying numbers (HP1-R2, HP1-R4, HP1-R6). [HP1] = 200 nM, [HP2] = 200 nM, [MB] = 200 nM, and [Trigger] = 20 nM.