Supplementary Material

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

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Name	DNA and RNA sequence (5' to 3')			
Repoter-F	5'FAM-CCACATACATCATATTCCCTCA			
Repoter-Q	TATTGAATGAGGGAATATGATGTATGTGG-3'BHQ1			
Z	TGGAGACGTAGGGTATTGAATGAGGG			
X-6	CCCTCATTCAATACCCTACGTCTCCA			
X-5	CCCTCATTCAATACCCTACGTCTCC			
X-4	CCCTCATTCAATACCCTACGTCTC			
X-3	CCCTCATTCAATACCCTACGTCT			
X-2	CCCTCATTCAATACCCTACGTC			
X-1	CCCTCATTCAATACCCTACGT			
X-0	CCCTCATTCAATACCCTACG			
Y	CCACATACATCATATTCCCTCATTCAATACCCTACG			
Y-s1	CCACATACATCATATTCCCTCATTCAATACCCTAC*G			
Y-s2	CCACATACATCATATTCCCTCATTCAATACCCTA*C*G			
Y-s3	CCACATACATCATATTCCCTCATTCAATACCCT*A*C*G			
Y-s4	CCACATACATCATATTCCCTCATTCAATACCC*T*A*C*G			
Y-s5	CCACATACATCATATTCCCTCATTCAATACC*C*T*A*C*G			
Y-s6	CCACATACATCATATTCCCTCATTCAATAC*C*C*T*A*C*G			
Y-s10	CCACATACATCATATTCCCTCATTCA*A*T*A*C*C*C*T*A*C*G			
Y-s15	CCACATACATCATATTCCCTC*A*T*T*C*A*A*T*A*C*C*C*T*A*C*G			
Y-s20	CCACATACATCATATT*C*C*C*T*C*A*T*T*C*A*A*T*A*C*C*C*T*A *C*G			
Y-remote-s6	CCACATACATCATATTC*C*C*T*C*A*TTCAATACCCTACG			
CHA circuits				
trigger	CTGGCCCCCCTTTCCCTGCACC			
HP1	GTCTACGGTGCAGGGAAAGGGGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCCCCCCTTTCGC			
HP2	TGCCACGTAGGGGCCAGGCTAGGTTGGTGCAGGCTGGCCCCCCCT CCACCTGCACCAACCTAGC			
HP1-L6	GTCTACGGTGCAGGG*A*A*A*G*G*GGGGGGGGCCAGCCTGCACCAAC CTAGCCTGGCCCCCCCTTTCGC			
HP1-L12	GTCTACGGTGCAGGG*A*A*A*G*G*G*G*G*G*G*G*CCAGCCTGCAC			

Table S1. The DNA sequences used in this work.

CAACCTAGCCTGGCCCCCCCTTTCGC

HP1-R2	GTCTACGGTGCAGGGAAAGGGGGGGGGGGCCAGCCTGCACCAACCTAGC			
HP1-R4				
	CTGGCCCCCCC*T*T*T*CGC			
HP1-R6	GTCTACGGTGCAGGGAAAGGGGGGGGGGCCAGCCTGCACCAACCTAGC CTGGCCCCCC*C*C*T*T*T*CGC			
HP1-R12	GTCTACGGTGCAGGGAAAGGGGGGGGGGCCAGCCTGCACCAACCTAGC CTGG*C*C*C*C*C*C*C*T*T*T*CGC			
HP1-R3S6	GTCTACGGTGCAGGGAAAGGGGGGGGGGGCCAGCCTGCACCAACCTAG CTGGCCC*C*C*C*C*TTTCGC			
HP1-R6S6	GTCTACGGTGCAGGGAAAGGGGGGGGGGCCAGCCTGCACCAACCTAGC CTGG*C*C*C*C*C*CCCTTTCGC			
MB	5'FAM-CATGCGAAAGGGGTACGTGGCATG-3'BHQ			
Probabilistic Circuits				
I1	CAAAATCCAAAACCTCTTACCCATCCATTCCACTATACACAC			
I4	GGTGTGTATAGTGGAATGGATG GGT			
I2	CATCCATTCCACTATACACACCACCATCAAATAACTACACAC			
I2-s4	C*A*T*C*CATTCCACTATACACACCACCATCAAATAACTACACAC			
I2-s8	C*A*T*C*C*A*T*T*CCACTATACACACCACCATCAAATAACTACACA C			
I3	CATCCATTCCACTATACACACCTCAATAACATCTCTCTC			
I3-s4	T*C*C*A*TTCCACTATACACACCTCAATAACATCTCTCTC			
I3-s8	T*C*C*A*T*T*C*C*ACTATACACACCTCAATAACATCTCTCTC			
15	CACCATCAAATAACTACACACCAGGTATTCCGACAACACACA			
I6	CTCAATAACATCTCTCCTCCCTAACACAATCACTCTCCTC			
T1	GGTGTGTAGTTATTTGATGGTGGTGTGTGT			
T2	GGAGGAGAGAGATGTTATTGAGGTGTGT			
Rep6-HEX	5'FAM-GAGGAGAGTGATTGTGTTAGGGAGGAG			
Rep6-BHQ	CCTAACAATCACTCTCCTC-3'BHQ			

*: phosphorothioate modification

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ΔG_{37} (kcal·mol ⁻¹)	$\Delta H (kcal \cdot mol^{-1})$	$\Delta S (cal \cdot mol^{-1} \cdot K^{-1})$		
-44.1	-155.6	-359.6		
-39.3	-147.9	-350.3		
-32.7	-138.4	-341.1		
-25.8	-129.4	-334.3		
-18.8	-120.8	-328.8		
	$\Delta G_{37} (\text{kcal·mol}^{-1})$ -44.1 -39.3 -32.7 -25.8 -18.8	$\begin{array}{ccc} \Delta G_{37} (\rm kcal \cdot mol^{-1}) & \Delta H (\rm kcal \cdot mol^{-1}) \\ -44.1 & -155.6 \\ -39.3 & -147.9 \\ -32.7 & -138.4 \\ -25.8 & -129.4 \\ -18.8 & -120.8 \end{array}$		

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

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-modificated 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. [invader] = [reporter]=1000 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.