Supporting Information

Heterochiral DNA Strand-Displacement Based on Chimeric D/L-Oligonucleotides

Brian E. Young and Jonathan T. Sczepanski*

Department of Chemistry, Texas A&M University, College Station, Texas 77843

S1. Supplementary Text.

Materials and Methods.

General. Oligonucleotides were either purchased from Integrated DNA Technologies (Coralville, IA) or prepared by solid-phase synthesis on an Expedite 8909 DNA/RNA synthesizer. Synthesizer reagents, D-nucleoside phosphoramidites, and Cyanine 3 phosphoramidites were purchased from Glen Research (Sterling, Va). L-nucleoside phosphoramidites were purchased from ChemGenes (Wilmington, Ma). Black Hole Quencher 2 CPG resins were purchased from LGC Biosearch Technologies (Petaluma, CA). All other reagents were purchased from Sigma Aldrich (St. Louis, MO).

Sequence Design. All DNA sequences for complexes D/L-A_1 and D/L-A_2 (Figure 1a,b) were rationally designed, with the D-DNA sequence domains (t and 1) being complementary to the 5′ end of human micro-RNA-155 and the L-DNA sequence domain (2 and 3) being complementary to the reporter complex L-R (Figure 1c). The 2′-O-methyl (2′OMe) ribonucleotide versions of D/L-A_1 and D/L-A_2 (M-D/L-A_1 and M-D/L-A_2, respectively; Figure S4) were prepared by replacing all D-DNA nucleotides with the same 2′OMe ribonucleotides and truncating the branch migration domain (domain 1) to maintain approximately the same T_m as the corresponding all D-DNA D/L-complexes (Figure S1). Reporter (L-R) was based on a design described previously.[1] All DNA melting temperatures were approximated using the IDT OligoAnalyzer tool, utilizing the nearest neighbor approximation.[2]

Oligonucleotide Purification. Unmodified D-oligonucleotides were purchased from IDT, while all pure L-oligonucleotides and chimeric D/L-oligonucleotides were synthesized in house by standard procedures. All single-stranded oligonucleotides were purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE, (19:1 acrylamide:bisacrylamide). Purified material was excised from the gel and eluted overnight at 23 °C in a buffer consisting of 200 mM NaCl, 10 mM EDTA, and 10 mM Tris pH 7.6. The solution was filtered to remove gel fragments, and the eluted material was precipitated with ethanol. In order to form complexes D/L-A_1 and D/L-A_2, the corresponding DNAs (Figure S1 and Table S1) were annealed in a reaction mixture containing the appropriate amount of each strand (see below), 300 mM NaCl, 1 mM EDTA, 10 mM Tris (pH 7.6) and were heated to 90 °C for 3 minutes then cooled slowly to room temperature over 1 hour. For D/L-A_1, 50 μM C1 was annealed to 75 μM C2. For D/L-A_2, 50 μM L-OUT was annealed to 75 μM C3 and C4. The corresponding 2′OMe versions of each complex were prepared identically (Figure S4 and Table S1) Annealed complexes were purified by 10% native PAGE (19:1 acrylamide:bisacrylamide). For L-R, 10 μM L-F was annealed to 11 μM L-Q and the complex was used without further purification.

Monitoring of heterochiral strand-displacement reactions by spectrofluorimetry. Each strand displacement reaction was monitored using a GloMax Discover multi-well plate reader from Promega Corp

(Madison, WI). All reaction mixtures contained either 150 nM or 1 μM of the indicated DNA and RNA input, respectively, 300 nM L-R, 300 mM NaCl, 1 mM EDTA, and 10 mM Tris (pH 7.6) and were carried out at 37 °C. Reactions were prepared to a final volume of 30 μL, transferred to a 384 well microplate, and initiated by the addition of 100 nM chimeric complex. Fluorescence was monitored with excitation/emission wavelengths at 520 nm/580-640 nm (bandpass filter: Cy3), and normalized to the activated reporter complex (L-R) representing the maximum achievable signal using the following equation:[3]

$$F_n = \frac{F - F_0}{F_c - F_0}$$

Where F_n is the normalized fluorescence intensity, F is the measured fluorescence, F_0 is the quenched fluorescence, and F_c is the control fluorescence at each time a measurement was taken.

Monitoring of heterochiral strand-displacement reactions in cell culture medium (DMEM). All reaction mixtures contained either 0 or 100 nM of the indicated chimeric complex (D/L-A_1, M-D/L-A_1, D/L-A_2 or M-D/L-A_2) and 300 nM reporter complex (L-R) and were incubated at 37 °C for the indicated times in Dulbecco's Modified Eagle Media (DMEM) containing 10% FBS. After 6 hours, 1 μM of input was added to the reaction. For experiments in serum-supplemented medium, a 2′OMe ribonucleotide version of the input (M-D-IN) was used to avoid nuclease degradation. Aliquots were taken at the indicated times and fluorescence monitored using a GloMax Discover multi-well plate reader from Promega Corp (Madison, WI). Fluorescence was monitored with excitation/emission wavelengths at 520 nm/580-640 nm (bandpass filter: Cy3), and normalized as described above.

Kinetic Analysis of Heterochiral inversion. We determined the rate constant for each of these heterochiral strand-displacement reactions as previously described.[4-6] Briefly, reaction mixtures containing 150 nM DNA input (D-IN), 300 nM L-R, 300 mM NaCl, 10 mM TRIS (pH 7.6) and 1 mM EDTA were initiated by the addition of 100 nM complex D/L-A_1 or D/L-A_2 at 37 °C and monitored by spectrofluorimetry as above. Under these conditions, the reporter kinetics did not limit the reaction rate. We fit our data using an equation derived from the second order rate law with respect to D-IN and the chimeric complexes (D/L-A_1 and D/L-A_2). Due to the stability of the waste duplexes, all reverse reactions were considered negligible with respect to the determined rate constants. After fitting rate constants were calculated to be 1.5×10^4 $M^{-1}s^{-1}$ and 2.9×10^4 $M^{-1}s^{-1}$ for Reactions A and B respectively (Figure S6).

S2. Supplementary Figures.

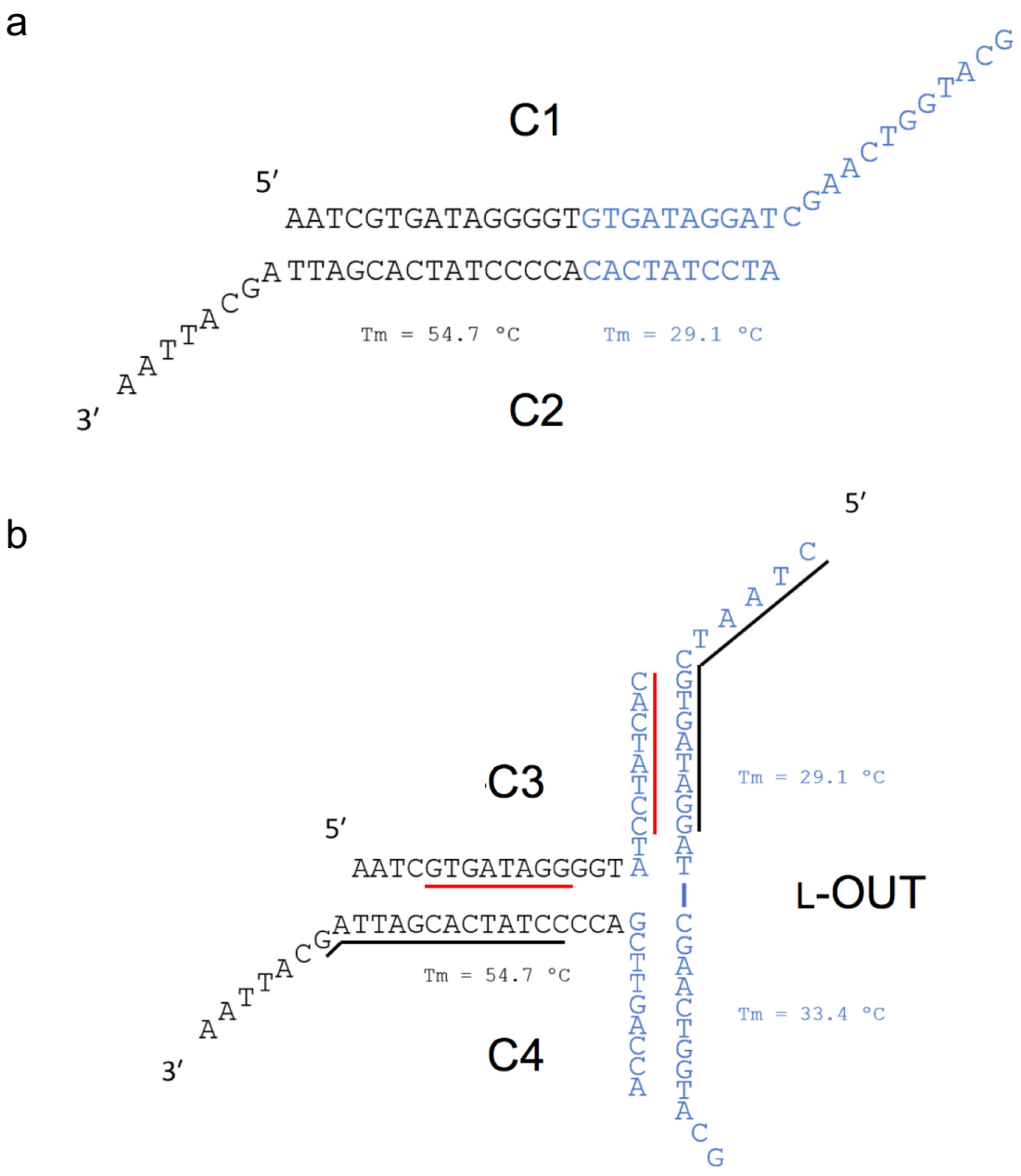

Figure S1. Sequences, predicted secondary structures, and domain melting temperatures (T_m) of chimeric complexes D/L-A_1 (a) and D/L-A_2 (b) used in these experiments. T_m values of individual domains were approximated using the OligoAnalyzer tool available on the IDT website, which employs a nearest-neighbor model.[2] Underlined regions in complex D/L-A_2 (b) are complementary to each other, yet do not hybridize during assembly of the three-way junction because they have opposite stereochemistry (D versus L). However, this sequence complementarity precluded the preparation of an all D-DNA version of this complex.

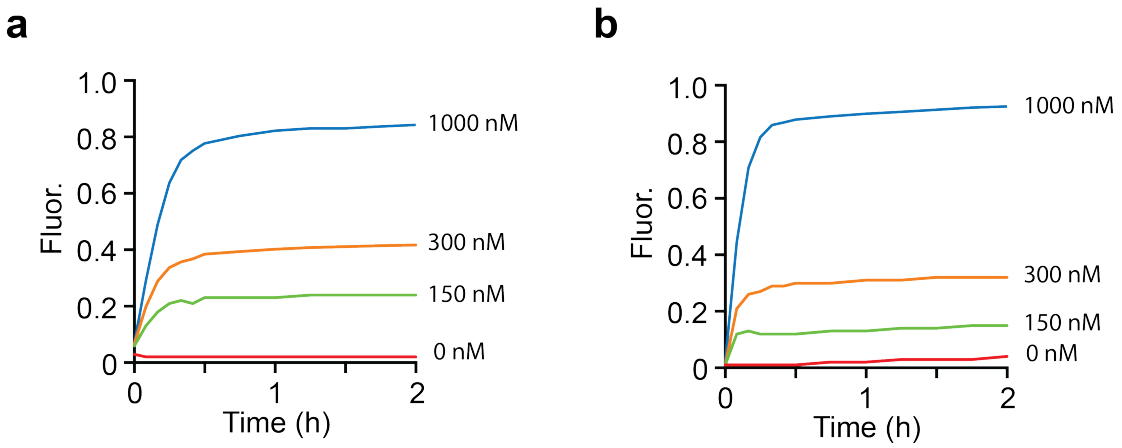

Figure S2. Fluorescent monitoring (Cy3) of heterochiral circuits using different concentrations of D-IN_{RNA}. All reaction mixtures containing 100 nM of either D/L-A_1 (a) or D/L-A_2 (b), 300 nM L-R, 300 mM NaCl, and 10 mM TRIS (pH 7.6) were carried out at 37 °C. Reactions were initiated with the indicated concentration of RNA input (0, 150, 300, or 1000 nM).

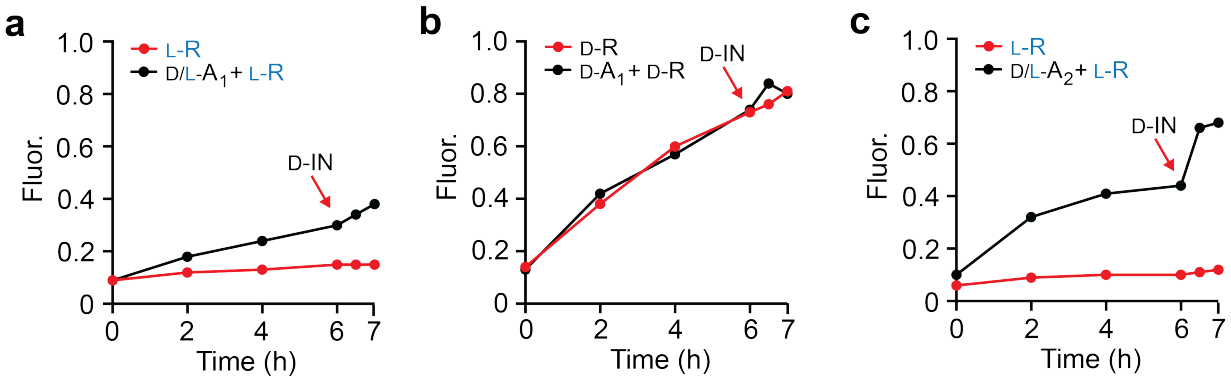

Figure S3. Operation of reactions A and B in DMEM supplemented with 10% FBS. The concentration of circuit components was identical to those described in Figure 1 (main text) and the reactions were carried out at 37 °C. D-IN (1 μM) was added after 6 hours. The circuit in panel b is the all D-DNA version of the circuit in panel a (i.e. Reaction A).

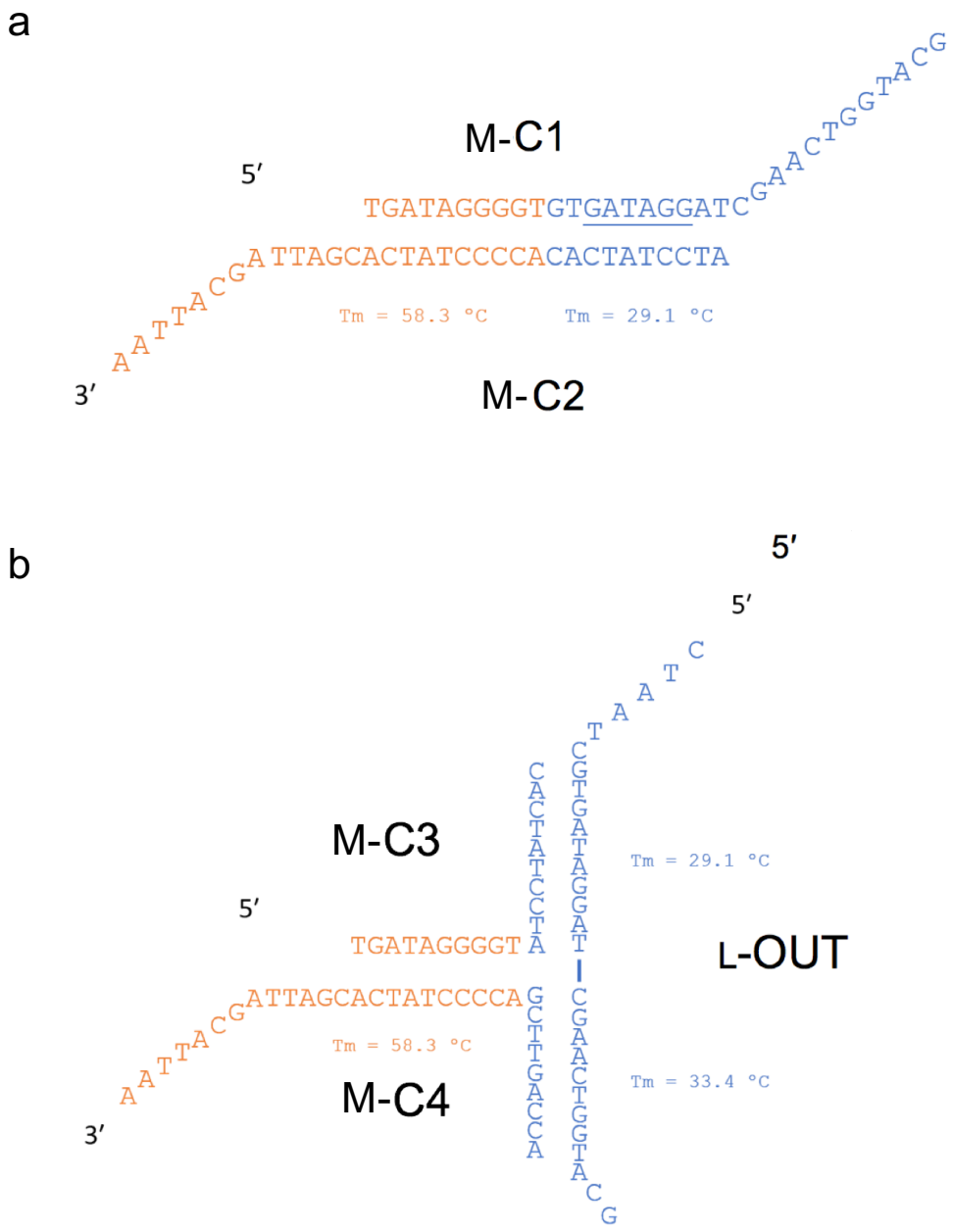

Figure S4. Sequences, predicted secondary structures, and domain melting temperatures (T_m) of chimeric complexes M-D/L-A_1 (a) and M-D/L-A_2 (b) used in these experiments. D-2′OMe-modified domains (orange) were shortened to maintain the same approximate T_m as their corresponding D-DNA domains (Figure S1). T_m values of individual D-DNA domains were approximated using the OligoAnalyzer tool available on the IDT website, which employs a nearest-neighbor model.[2] T_m values for the 2′OMe-modified domains were approximated using the corresponding 2′OMe/RNA duplex.

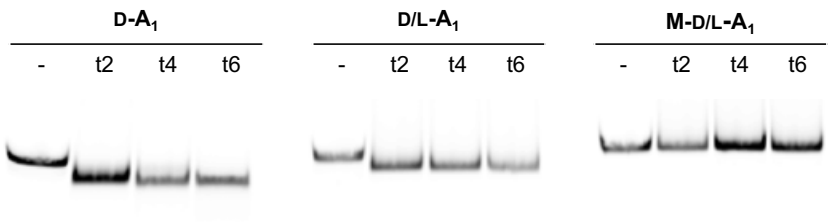

Figure S5. Chimeric duplexes D/L-A_1 and D-A_1 (the all D-DNA version of D/L-A_1) are degraded in DMEM supplemented with 10% FBS, whereas M-D/L-A_1 (the D-2′OMe-modified version of D/L-A_1) is stable under the same conditions. For these experiments, complexes D/L-A_1, D-A_1, and M-D/L-A_1 were prepared with a Cy5-labled strand (C2_Cy5, D2_Cy5, and M-C2_Cy5, respectively, for visualization) (Table S1). Each complex (100 nM) was incubated in DMEM containing 10% FBS at 37 °C for the indicated times (0, 2, 4, or 6 hours). Aliquots were analyzed by 10% native PAGE and visualized by Cy5 fluorescence.

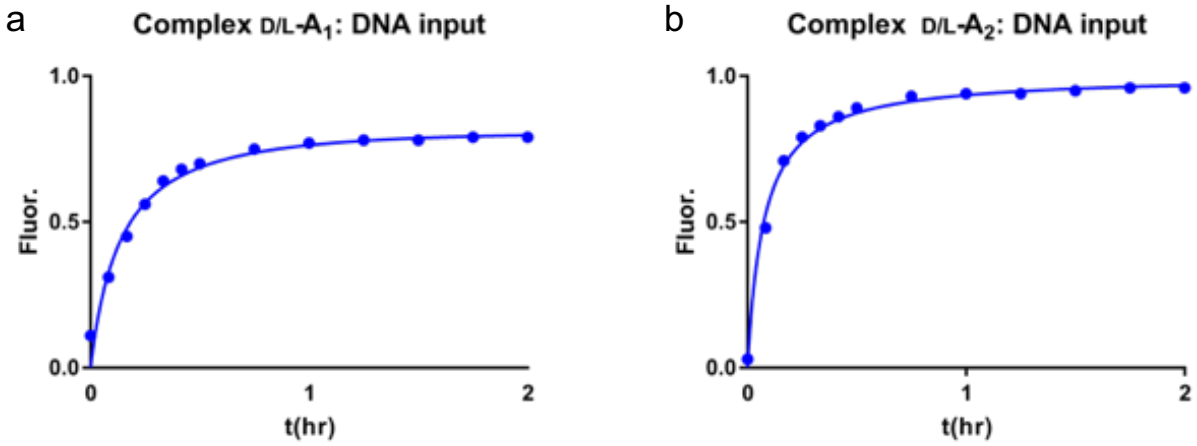

Figure S6. Kinetic characterization of heterochiral strand-displacement reactions. All reactions were performed as described in the methods sections. Experimental data (dotted line) was fit to a second-order rate model (solid line) as described in the methods section. Here, 1 fluorescent (Fluor.) unit corresponds to complete consumption of reporter L-R (100 nM) in the reaction. Rate constants were calculated to be 1.5×10^4 $M^{-1}s^{-1}$ and 2.9×10^4 $M^{-1}s^{-1}$ for Reactions A (a) and B (b), respectively.

S3. Supplementary Tables.

Table S1. Names, sequences, and chirality of all oligonucleotides used in this work. D-DNA (black), D-RNA (red), L-DNA (blue) and 2′OMe (orange) are indicated by color. Cy3 = cyanine 3 dye; Cy5 = cyanine 5 dye; 3BHQ2 = Black Hole Quencher 2.

Sequence Name	Sequence Identity 5′→3′	Oligomer Stereochemistry
D-IN	TTAATGCTAATCGTGATAGGGGA	D
D-IN_s	AATTACGAAATCGTGATAGGGGA	D
D-IN_{RNA}	UUAAUGCUAAUCGUGAUAGGGGU	D
L-R_F	Cy3_GGCGTACCAGTTCGATCCTATCAC	L
L-R_Q	GGATCGAACTGGTACGCC/3BHQ2/	L
C1	AATCGTGATAGGGGTGTGATAGGATCGAACTGGTACG	D/L
D1	AATCGTGATAGGGGTGTGATAGGATCGAACTGGTACG	D
M-C1	TGATAGGGGTGTGATAGGATCGAACTGGTACG	D/L
C2	ATCCTATCACACCCCTATCACGATTAGCATTAA	D/L
C2_Cy5	Cy5_ATCCTATCACACCCCTATCACGATTAGCATTAA	D
D2	ATCCTATCACACCCCTATCACGATTAGCATTAA	D
D2_Cy5	Cy5_ATCCTATCACACCCCTATCACGATTAGCATTAA	D
M-C2	ATCCTATCACACCCCTATCACGATTAGCATTAA	D/L
M-C2_Cy5	Cy5_ATCCTATCACACCCCTATCACGATTAGCATTAA	D/L
C3	AATCGTGATAGGGGTATCCTATCAC	D/L
M-C3	TGATAGGGGTATCCTATCAC	D/L
C4	ACCAGTTCGACCCCTATCACGATTAGCATTAA	D/L
M-C4	ACCAGTTCGACCCCTATCACGATTAGCATTAA	D/L
L-OUT	CTAATCGTGATAGGATCGAACTGGTACG	L

1. Kabza, A. M.; Young, B. E.; Sczepanski, J. T., Heterochiral DNA Strand-Displacement Circuits. *J. Am. Chem. Soc.* **2017,** *139*, 17715-17718.
2. SantaLucia, J.; Allawi, H. T.; Seneviratne A. P., Improved Nearest-Neighbor Parameters for Predicting DNA Duplex Stability. *Biochemistry* **1996,** *35*, 3555-3562.
3. Yurke, B.; Mills, A. P., Using DNA to Power Nanostructures. *Genet. Program. Evol. Mach.* **2003,** *4*, 111-122.
4. Olson, X.; Kotani, S.; Yurke, B.; Graugnard, E.; Hughes, W. L., Kinetics of DNA Strand Displacement Systems with Locked Nucleic Acids. *J. Phys. Chem. B* **2017,** *121*, 2594-2602.
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6. Srinivas, N.; Ouldridge, T. E.; Sulc, P.; Schaeffer, J. M.; Yurke, B.; Louis, A. A.; Doye, J. P.; Winfree, E., On the biophysics and kinetics of toehold-mediated DNA strand displacement. *Nucleic Acids Res.* **2013,** *41*, 10641-58.