SUPPORTING INFORMATION

Differential effects of strand asymmetry on the energetics and structural flexibility of DNA internal loops

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Figure S1. Alignments of the strands for the smFRET constructs. The constructs for the bending experiments are 0 x 0 (a), 3 x 3 (b), 2 x 4 (c), 1 x 5 (d), and 0 x 6 (e). The constructs for the fraying experiments are 6 x 6 (f) and 0 x 12 (g).

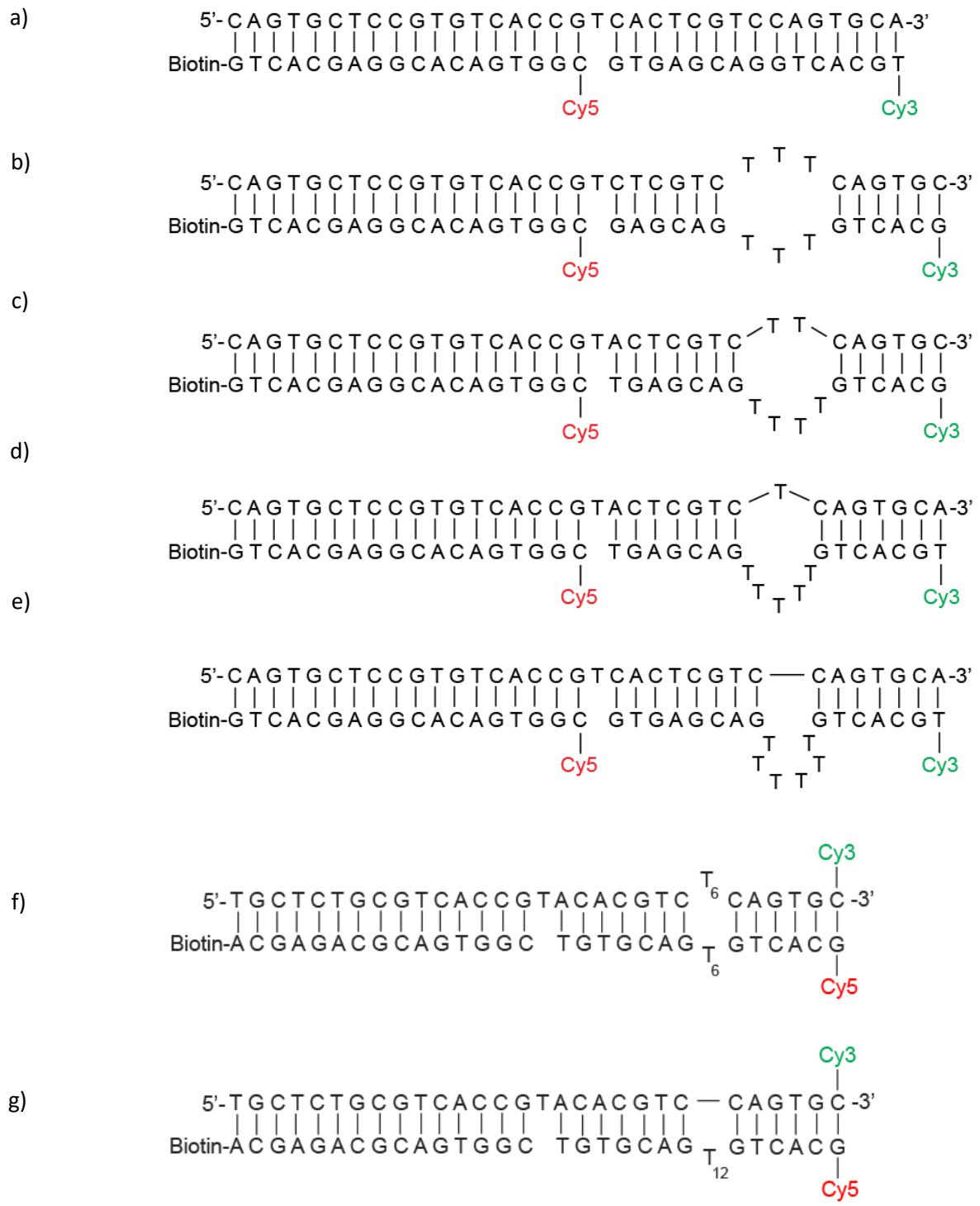

Figure S2. Optical melting analysis of reference duplex DNA (0 x 0). Inverse melting temperature (T_M^{-1}) versus total strand concentration (ln[Ct/4]) for the reference duplex in the absence (●) and presence (○) of 12% glucose. The solid lines represent best-fit lines from which thermodynamic parameters (*ΔS*, *ΔH*, and *ΔG*) in Table S1 were extracted using Eq. 2.

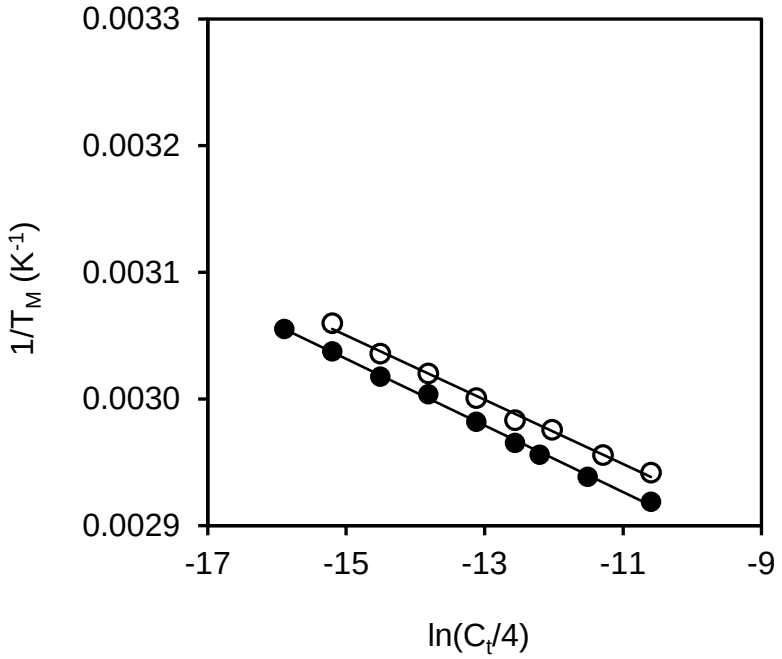

Table S1. Thermodynamics for DNA duplex (0 x 0) at 1 M NaCl, pH 7 in absence and presence of 12% glucose.

	T_m^{-1} vs. log C_t parameters				Curve fit parameters			
Glucose	$-\Delta H^o$ (kcal/mol)	$-\Delta S^o$ (eu)	$-\Delta G^o_{37}$ (kcal/mol)	T_m (°C)	$-\Delta H^o$ (kcal/mol)	$-\Delta S^o$ (eu)	$-\Delta G^o_{37}$ (kcal/mol)	T_m (°C)
-	95.7 ± 2.6	259.0 ± 7.4	15.32 ± 0.04	67.3	95.4 ± 4.0	259.1 ± 12.1	15.02 ± 0.20	66.0
+	91.3 ± 2.1	247.6 ± 11.2	14.49 ± 0.13	66.6	100.3 ± 3.7	275.3 ± 11.4	14.96 ± 0.41	66.7
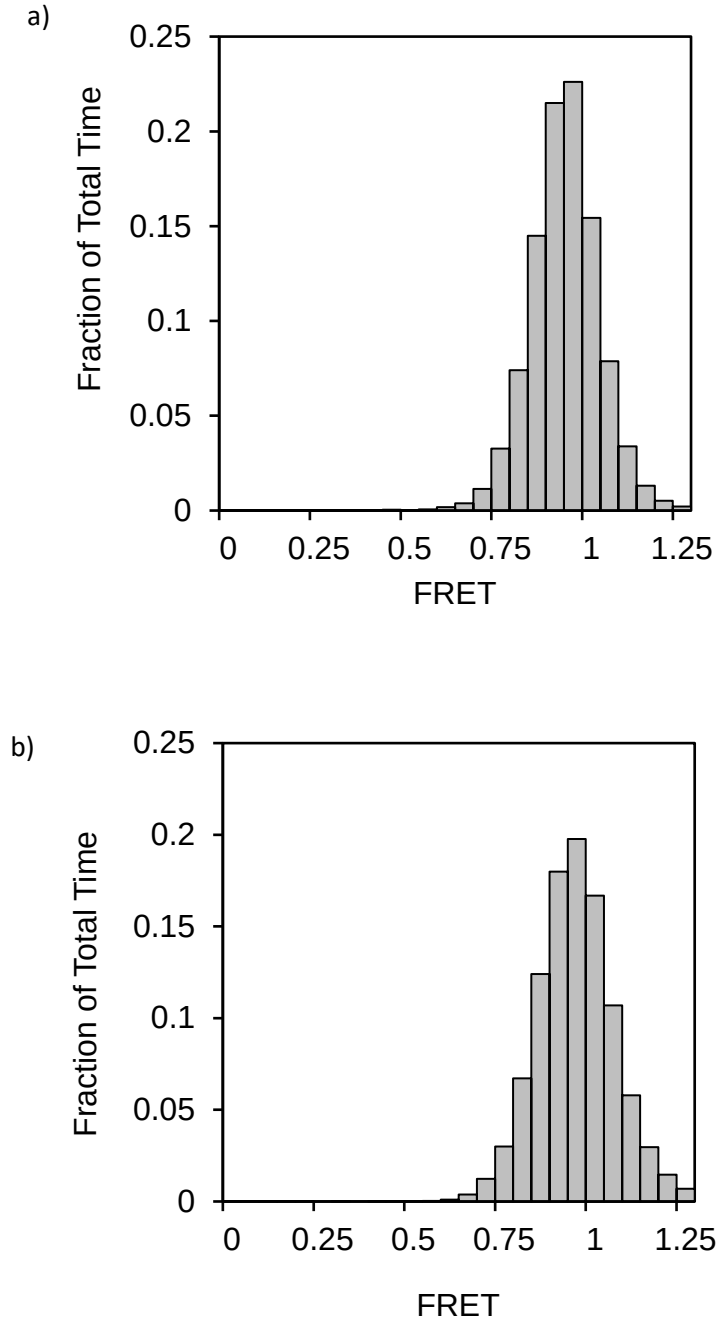
Figure S3. Single-molecule FRET histograms for N_{total} = 12 with terminally positioned Cy3 and Cy5 dyes to detect helical openings at 1 M NaCl. Schematics for the constructs are shown in Figure S1 f) and g). Normalized histograms are shown for the internal loop constructs 0 x 12, N_{mol} = 267 (a) and 6 x 6, N_{mol} = 210 (b).