SUPPORTING INFORMATION

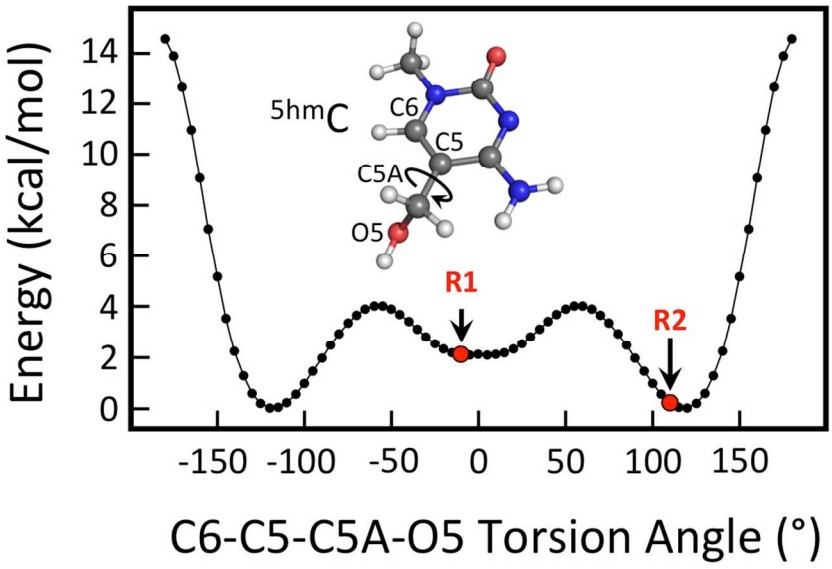
Supplementary Table 1: Crystallographic parameters and refinement statistics for $G^{5hm}CC$ and $G^{5m}CC$ core Holliday junction structures.

	$G^{5hm}CC$ Core Junction	**$G^{5m}CC$ Core Junction**
Crystallographic Parameters		
PDB Entry	5DSB	5DSA
Wavelength (Å)	1.5418	1.5418
Resolution range (Å)[1]	30.67 - 1.50 (1.55 - 1.50)	30.59 - 1.69 (1.75 - 1.69)
Space group	C2	C2
Unit cell dimensions		
a (Å)	66.07	65.97
b (Å)	24.77	24.54
c (Å)	38.06	37.99
β (deg)	111.82	111.98
Total reflections	366673	260245
Unique reflections[1]	9344 (863)	6158 (511)
Multiplicity	6.7 (2.2)	6.0 (2.1)
Completeness (%)[1]	98 (91)	93.82 (88)
Mean I/σ(I) [1]	23.47 (2.04)	51.00 (3.781)
Wilson B-factor	19.65	20.53
R_{merge}[1]	0.029 (0.418)	0.068 (0.245)
R_{pim}[1]	0.036 (0.478)	0.023 (0.173)
R_{meas}[1]	0.041 (0.592)	0.072 (0.302)
$CC_{1/2}$	0.999	0.952
CC*	1	0.988
Refinement Statistics		
Reflections used for R-free	4.99%	4.85%
R_{cryst}[1, 2]	0.2506 (0.4023)	0.2480 (0.3408)
R_{free}[1. 2]	0.2883 (0.4302)	0.2775 (0.3648)
Number non-hydrogen atoms	507	495
RMSD for bond lengths (Å)	0.011	0.009
RMSD for bond angles (deg)	1.28	1.19
Average B-factor	25.33	27.31
Macromolecules	24.37	26.42
Solvent	29.70	31.56

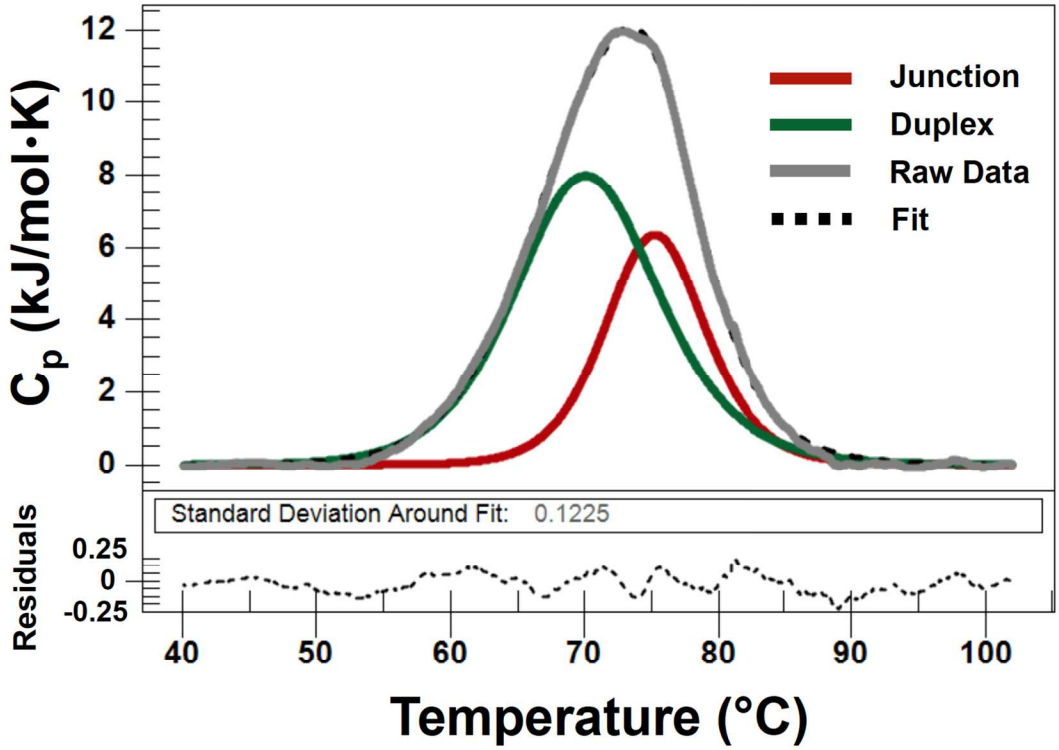
[1] *Values for the highest-resolution shell are given in parentheses*

[2] *Values for R_{cryst} and R_{free} for the current structures are within 1 standard deviation of published B-type duplex and junction DNA structures* (Hays, *et al*., 2005, *Proc. Natl. Acad. Sci.,* **102**: 7157-7162.

Supplemental Figure 1



Supplemental Figure 1: Energies for rotational isomers (rotamers) of an isolated 5-hydroxymethylcytosine (^{5hm}C, inset). Quantum mechanical energies at the MP2 level were calculated as the hydroxymethyl substituent is rotated around the C5-C5A bond (the C6-C5-C5A-O5 torsion angle labeled along the horizontal axis). Torsion angles for rotamers observed in the crystal DNA junction structure are shown as red circles. The dominant R1 rotamer (-12.0°) falls into a calculated local energy minimum, while the minor R2 rotamer (111.4°) falls in a global energy minimum well that is close to the lowest energy rotation (at 119°).



Supplemental Figure 2: Representative DSC melting profile showing the melting data (gray) is fit with a two-component analysis indicating the presence of both junction (red) and duplex (green) DNA. A two-state scaled model was used, which incorporated a weighting term to account for differences in concentration between the duplex and junction populations. The composite fit (black dashes) modeling both junction and duplex melting events shows good agreement with the raw data (standard deviation around fit = 0.1225), and the residuals plot (bottom panel) is randomly distributed around zero. In contrast, fitting the raw data with a single peak resulted in larger and less random distribution of residuals and a 0.3469 standard deviation around the fit, indicating the melting event is indeed best modeled with a two-component fit.