Supporting Information

Differential Stabilities and Sequence-Dependent Base Pair Opening Dynamics of Watson-Crick Base Pairs with 5-Hydroxymethylcytosine, 5-Formylcytosine, or 5-Carboxylcytosine

Marta W. Szulik[1*], Pradeep S. Pallan[2], Boguslaw Nocek[3], Markus Voehler[1], Surajit Banerjee[4], Sonja C. Brooks[5], Andrzej Joachimiak[3], Martin Egli[2], Brandt F. Eichman[5], and Michael P. Stone[1*]

[1]Department of Chemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt Ingram Cancer Center, and Center for Structural Biology, Vanderbilt University, Nashville, TN 37235

[2]Department of Biochemistry, Vanderbilt Institute of Chemical Biology, and Center for Structural Biology, School of Medicine, Vanderbilt University, Nashville. TN 37232

[3]Bioscience Division, Argonne National Laboratory, Argonne, IL 60439

[4]Northeastern Collaborative Access Team and Department of Chemistry and Chemical Biology, Cornell University, Argonne National Laboratory, Argonne, IL 60439

[5]Department of Biological Sciences, Vanderbilt Institute of Chemical Biology, and Center for Structural Biology, Vanderbilt University, Nashville, TN 37235

To whom correspondence should be addressed:
michael.p.stone@vanderbilt.edu

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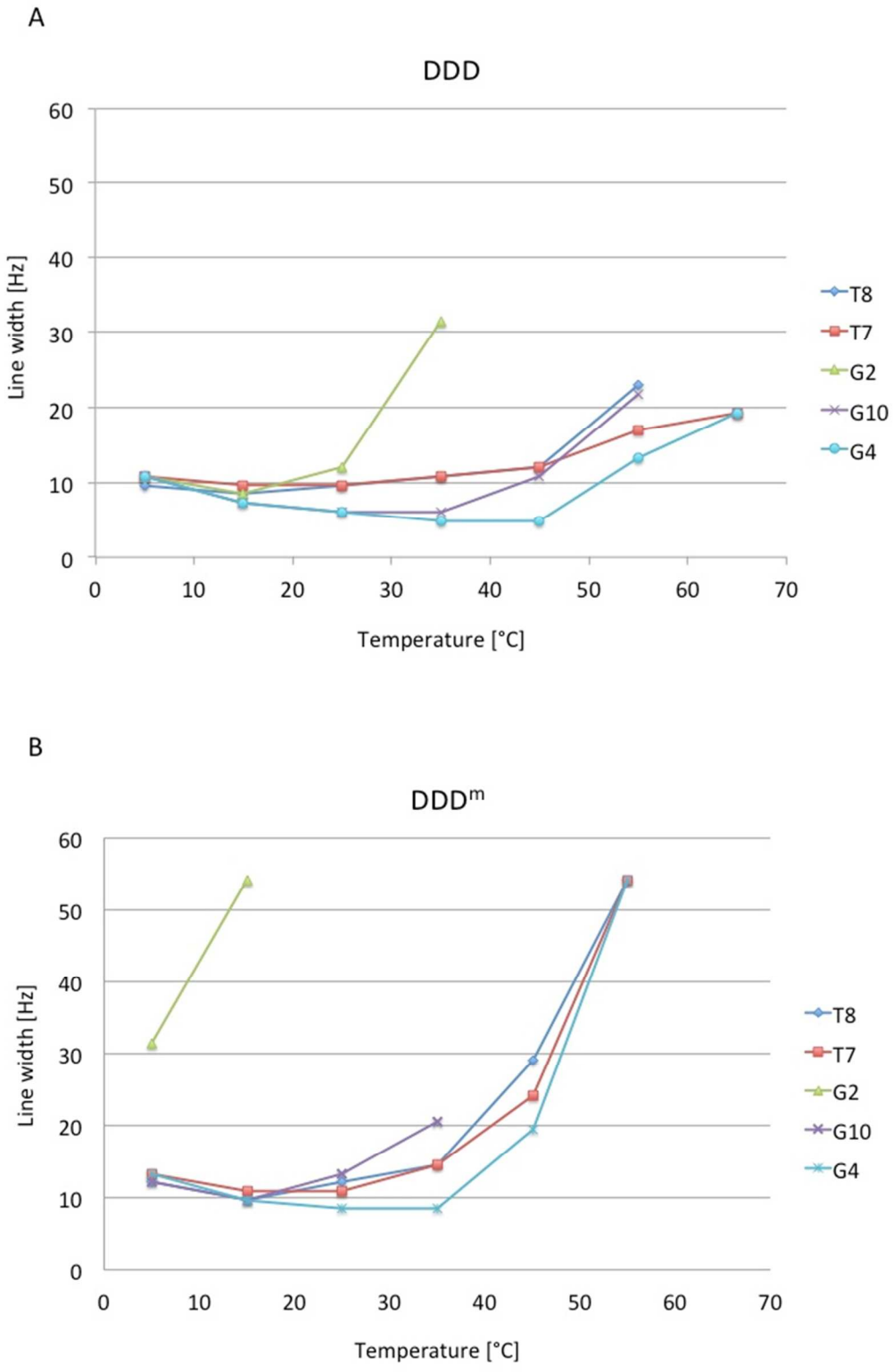
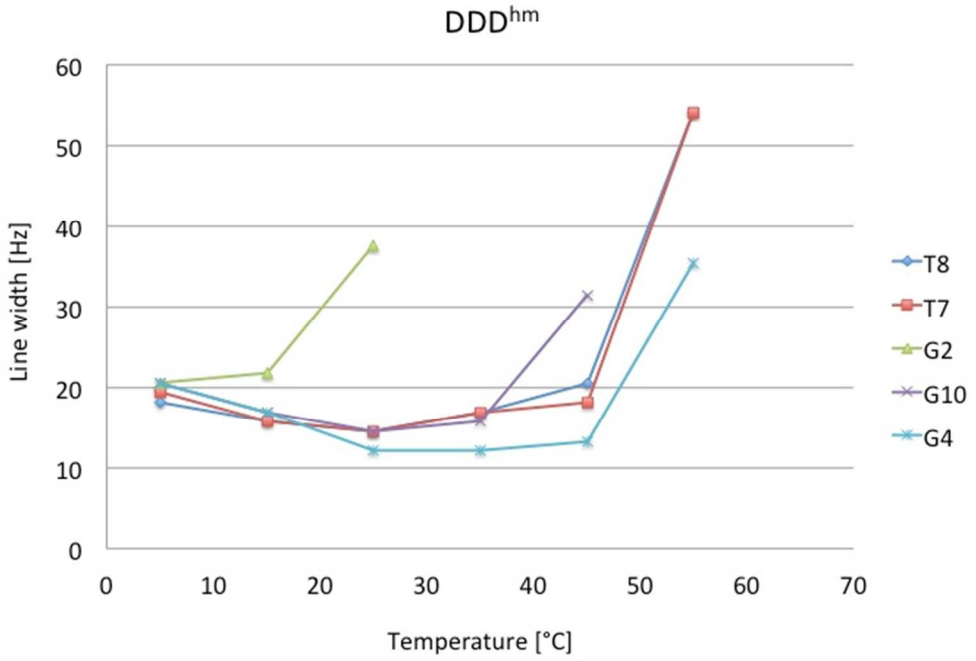
Table S1. Crystallization conditions.

Condition	DDD^{hm}	DDD^{f}	DDD^{ca}
pH	7.0	6.0	6.0
Buffer	40 mM Na Cacodylate	40 mM Na Cacodylate	40 mM Na Cacodylate
Salts	80 mM NaCl, 20 mM $MgCl_2$	80 mM NaCl	80 mM $SrCl_2$
Additives	12 mM spermine 4HCl	12 mM spermine 4HCl	12 mM spermine 4HCl
2-methyl-2,4-pentanediol (MPD)	10 % (v/v)	10 % (v/v)	10 % (v/v)

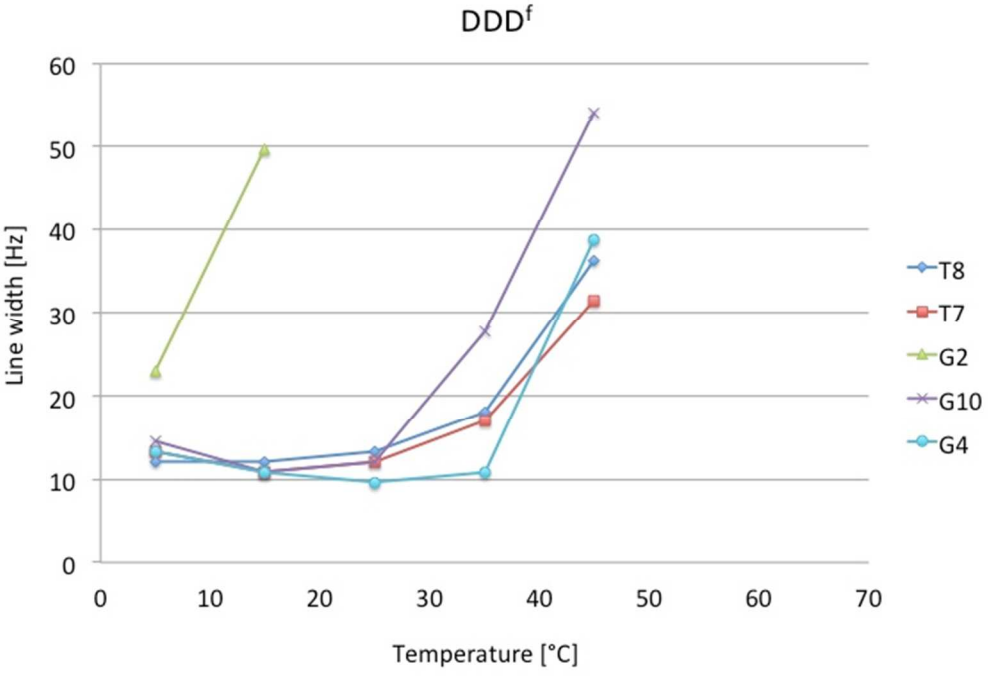
Figure S1. Temperature dependence of line widths of the imino proton resonances of the (A) DDD, (B) DDD^{m}, (C) DDD^{hm}, (D) DDD^{f} and (E) DDD^{ca} duplexes.



C


DDD^hm
Line width [Hz]
Temperature [°C]
0
10
20
30
40
50
60
70
T8
T7
G2
G10
G4

D


DDD^f
Line width [Hz]
Temperature [°C]
0
10
20
30
40
50
60
70
T8
T7
G2
G10
G4

E

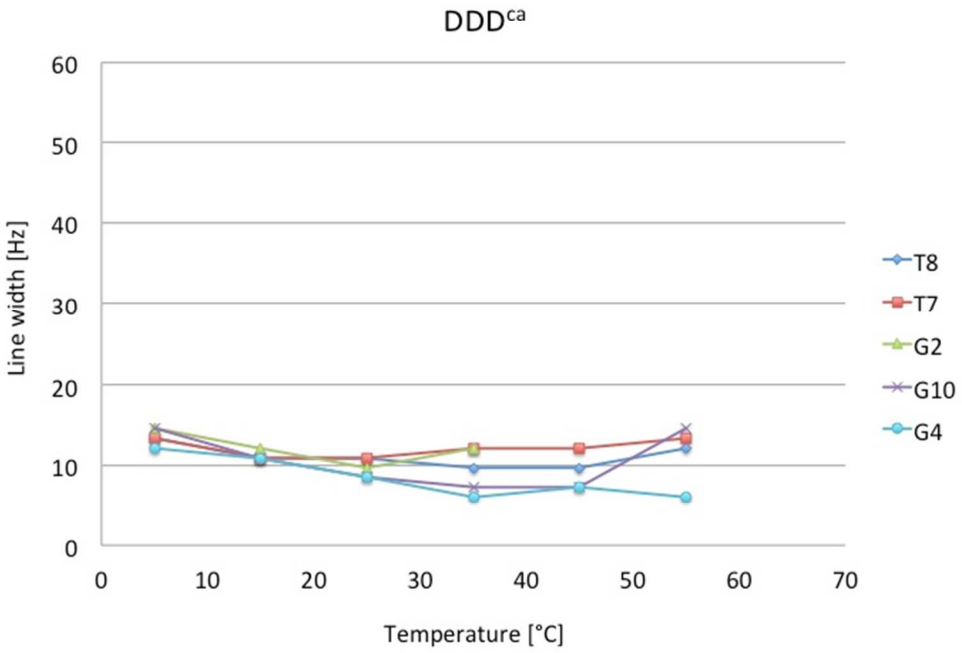

DDDca
60
50
40
30
20
10
0
Line width [Hz]
0
10
20
30
40
50
60
70
Temperature [°C]
T8
T7
G2
G10
G4

Figure S2. Modification site of the DDD^{hm} displaying dual conformers of the 5hmC modified base and its interactions with surrounding waters and residues.

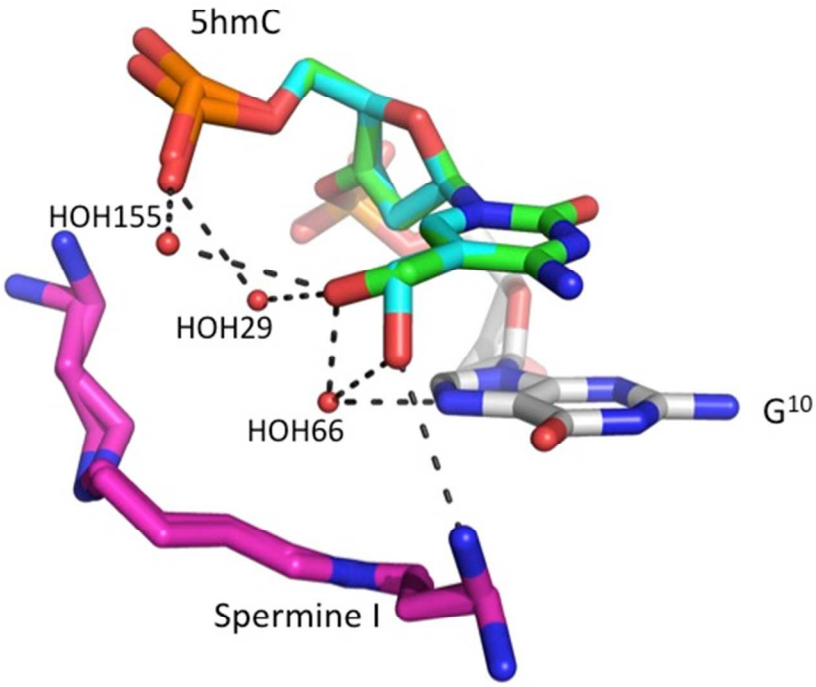


Figure S3. Modification site of the DDD^{hm} displaying interactions between the modified 5hmC and 3'-flanking G^{22} through water molecules.

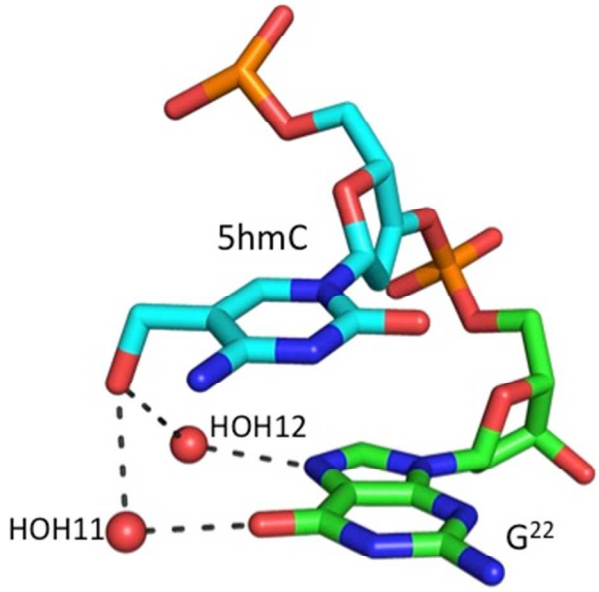


Figure S4. Expanded plots from the aromatic-anomeric region of the NOESY spectra, depicting sequential NOE connectivities of (A) DDD, (B) DDD^{m}, (C) DDD^{hm}, (D) DDD^{f} and (E) DDD^{ca} duplexes. Data were collected at 900 MHz.