Insights into Watson-Crick/Hoogsteen Breathing Dynamics and Damage Repair from the Solution Structure and Dynamic Ensemble of DNA Duplexes containing m^1A

Bharathwaj Sathyamoorthy[1,2], Honglue Shi[2], Huiqing Zhou[1], Yi Xue[1,2], Atul Rangadurai[1], Dawn K. Merriman[2], and Hashim M. Al-Hashimi[1,2,*]

Supplementary Information

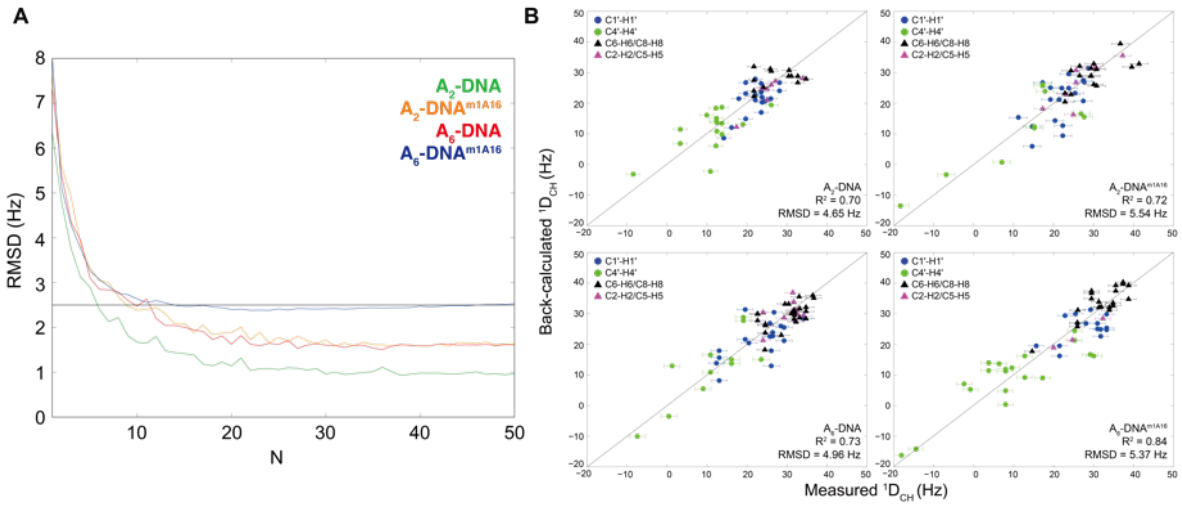

Figure S1. (A) Comparison of measured and predicted RDCs (normalized RMSD) as a function of ensemble size (N) used in SAS. (B) Cross-validation of ensembles to assess the agreement between RDC-selected ensembles with the subset of RDCs (25%) that was not included in SAS. While we observe some deterioration in the cross-validation RDC RMSD (4.6-5.6 Hz) and R^2 = 0.70-0.84 relative to the SAS ensemble (RMSD = 2.4-2.5 Hz and R^2 = 0.95-0.96) is, the quality of cross-validation analysis is considerably better than the full MD trajectory (RDC RMSD = 6.8-8.5 Hz and R^2 = 0.61-0.64).

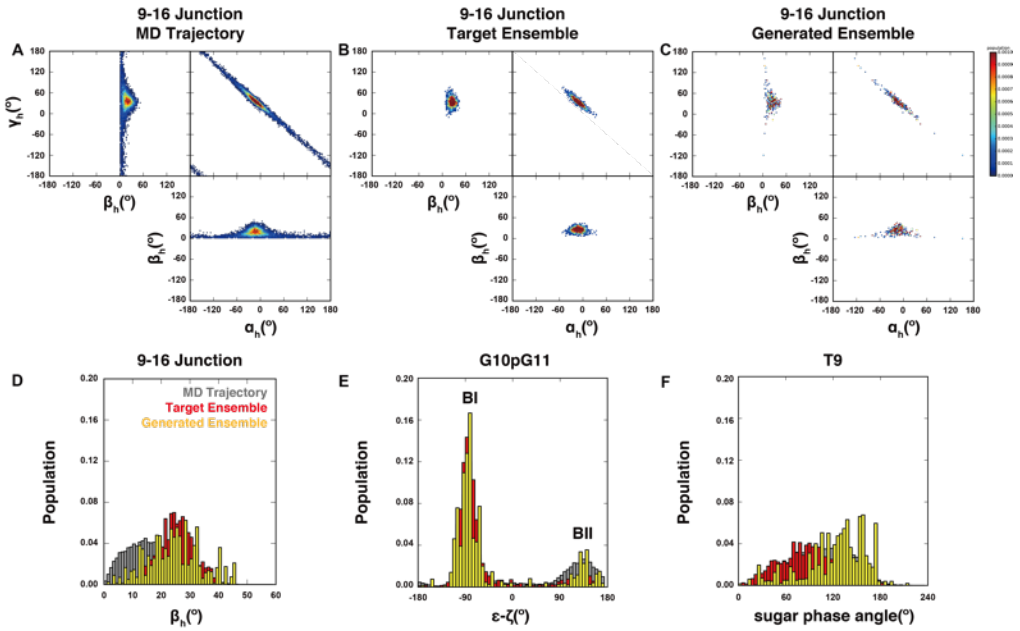

Figure S2. Simulations testing ability of RDCs to define features of the DNA ensemble. Comparison of inter-helical kink angles for target and RDC-generated ensembles for target ensembles 1 (A-D), 2 (E) and ensemble 3 (F) (see methods). Shown are 3D inter-helical (α_h, β_h and γ_h) distributions at the A16–T9 junction for (A) MD pool, (B) target ensemble 1, and (C) RDC-generated ensemble. (D) 1D comparison of kink angle (β_h) distributions at the A16–T9 junction; (E) ε-ζ at G10pG11; and (F) sugar phase angle at T9.

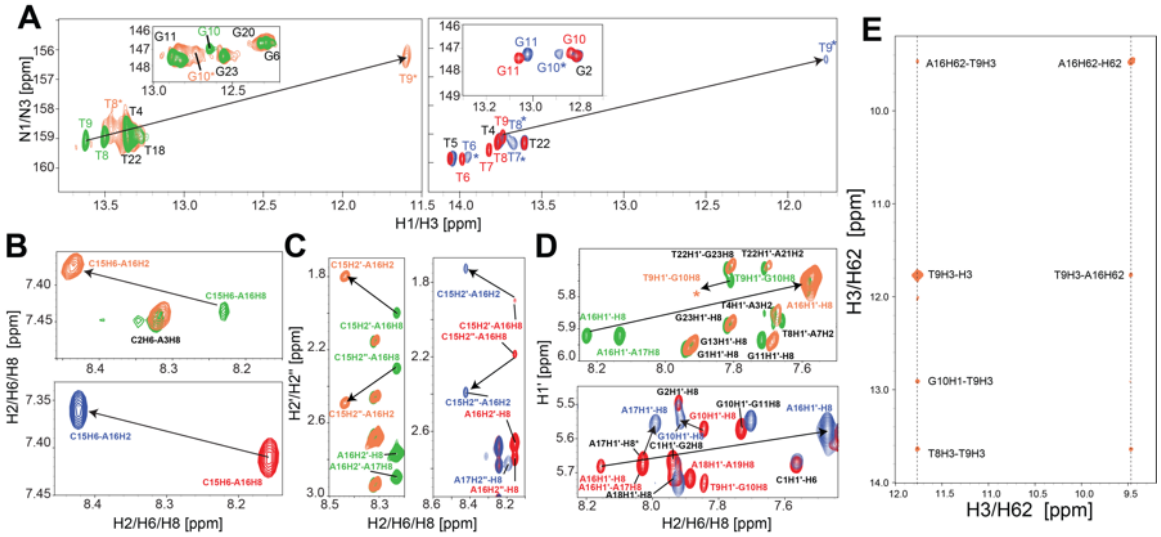

Figure S3. (A) 2D [^{15}N, ^{1}H] imino SOFAST-HMQC spectra (1) acquired at 25 ºC (A_2-DNA, A_6-DNA, A_6-DNA^{m1A16}) and 5 ºC (A_2-DNA^{m1A16}) displaying all base paired imino resonances, except the terminal G–C bps. T9-N3/H3 up-field shifted resonance depicting the formation of a non-canonical HG bp with m^{1}A16. (B-E) 2D [^{1}H, ^{1}H] NOESY spectra acquired at 25 ºC (A-C) and at 9 ºC (D) confirming the formation of m^{1}A16•T9 HG bp. (B, C) Change in the sequential NOEs between C15-H6/H2'/H2''–A16-H8 to C15-H6/H2'/H2''–A16-H2 and (D) increase in intensity of the A16-H1'–A16-H8 intra-residue NOE identifies the formation of a *syn* m^{1}A16 base. (E) T9-H3–A16-H8 and T9-H3–A-H62 connectivity observed in A_2-DNA^{m1A16} (2). Sequential imino NOEs between T9-H3 with T8-H3 and G10-H1 indicate stable formation of a duplex.

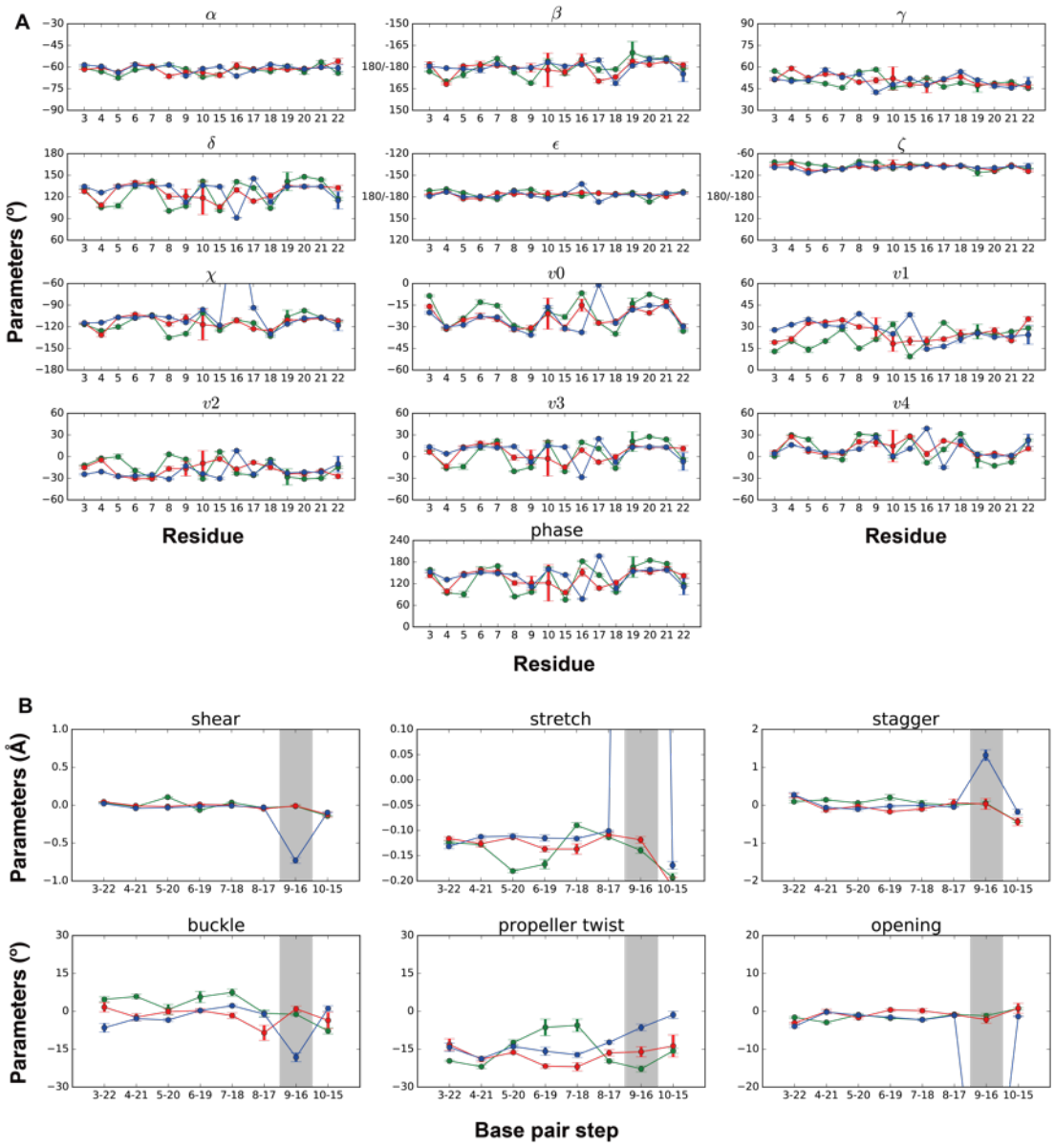

Figure S4. Local perturbations induced by m^{1}A in the solution NMR structures. (A, B) Shown are average and standard deviations for (A) local torsion and sugar phase angles and (B) base pair parameters for A_2-DNA (green), A_6-DNA (red) and A_6-DNA^{m1A16}(blue), respectively. Base pair step parameters involving the T9•m^{1}A16 HG bp shaded in grey are omitted due to ill definition of the reference coordinate by 3DNA.

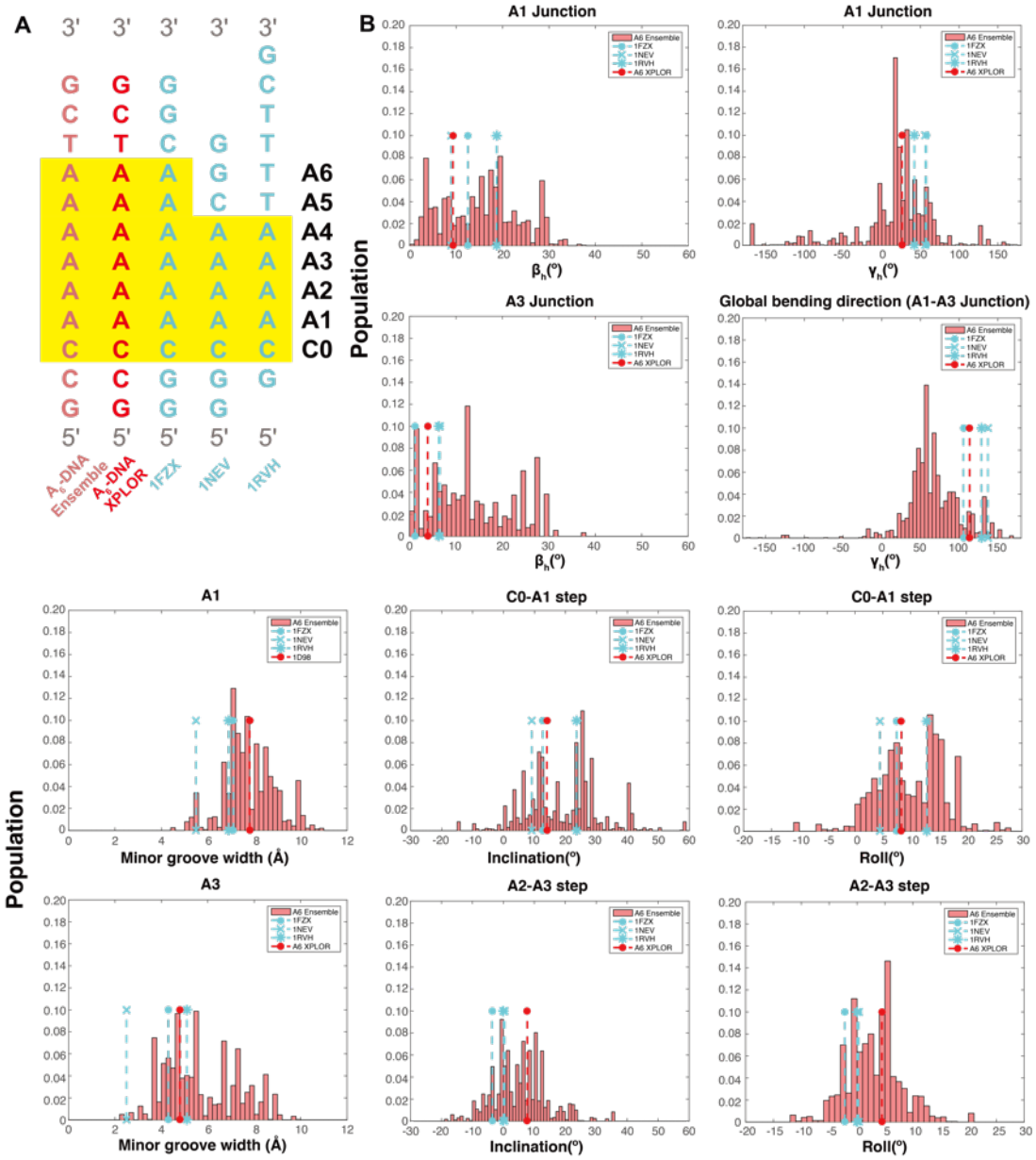

Figure S5. A_6-DNA structure and ensemble reproduce structural features of A-tract containing duplexes. (A) A_6-DNA ensembles (pink), A_6-DNA XPLOR structures (red) and prior A-tract solution structures (cyan) marked by PDBID used in this analysis. All the sequences are aligned with the 5'-CAA junctions with residues numbered from C0 to A6. (B) Comparison of local and global structural parameters.

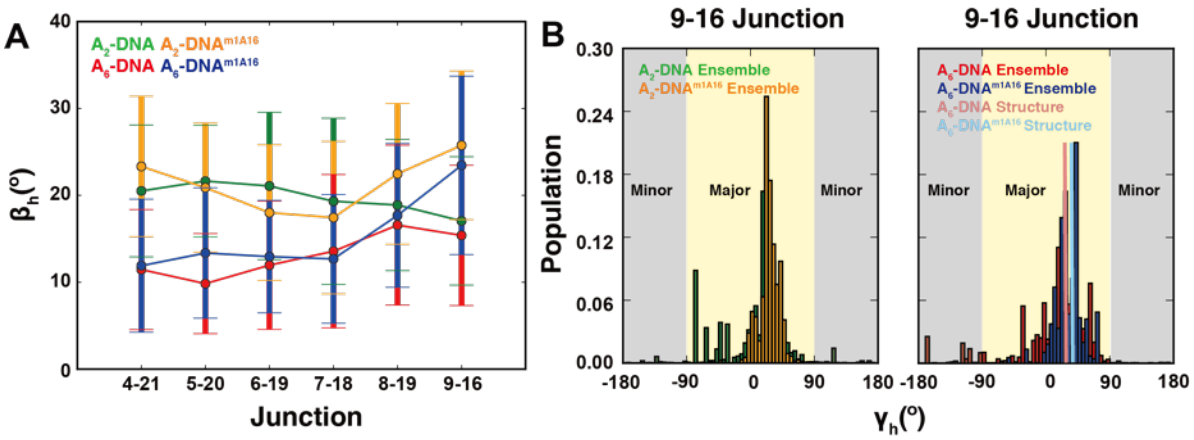

Figure S6. (A) Average and standard deviations for local kink angle (β_h) in RDC-selected ensembles as a function of junction position. (B) Local kinking directions (major versus minor groove, given by γ_h) at the A16–T9 bp in RDC-selected ensembles and XPLOR structures.

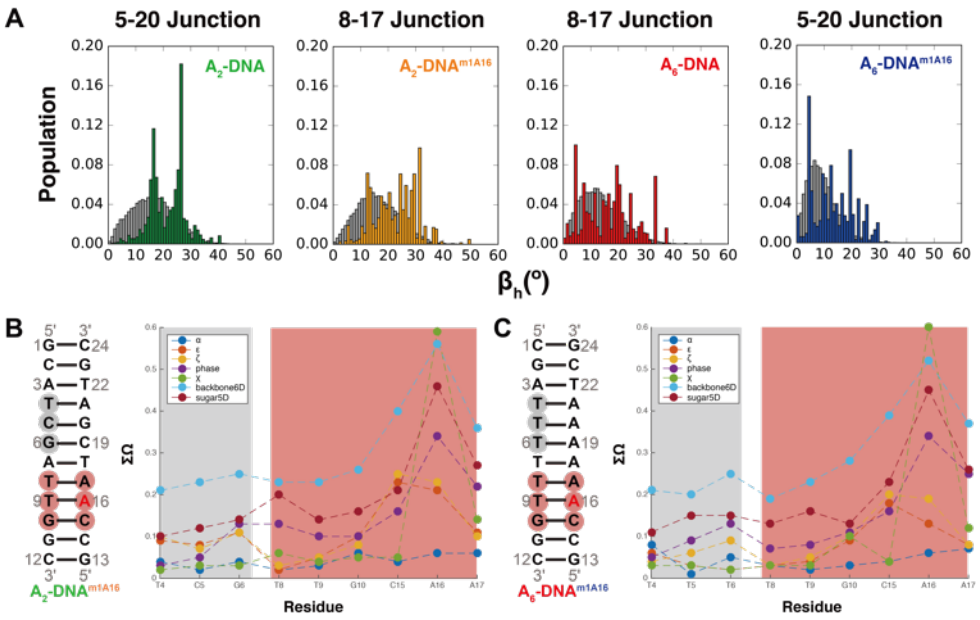

Figure S7. (A) Comparison of RDC-selected (in color) and MD (in gray) ensembles. Representative examples of local kink angle (β_h) distribution at specific base pair junctions. (B, C) Analysis of similarity and differences between the dynamic ensembles of A_2- and A_6-DNA with and without m^1A modification with REsemble (3). ΣΩ quantifies the difference between two distributions (or multiple distributions in multi-dimensional analysis) and ranges between 1 and 0 for maximum difference and similarity, respectively. Shown are ΣΩ values for 1D torsion angles (α, ε, ζ, χ), sugar phase angles, 5D sugar torsion angles (ν_0-ν_4) and 6D backbone torsion angles (α, β, γ, δ, ε, ζ) between ensembles of DNA with and without m^1A to assess the local perturbations in m^1AT HG bp and its flanking bps (in red). ΣΩ values for local parameters at sites (in gray) > 2 bps away from m^1A site defines differences expected purely due to uncertainty ($\Sigma\Omega_{control}$) and larger ΣΩ values are considered statistically significant. The largest deviations are in χ (ΣΩ in 0.6; $\Sigma\Omega_{control}$ = 0.0), ε and ζ (ΣΩ in 0.0~0.3; $\Sigma\Omega_{control}$ = 0.1), sugar phase angle (ΣΩ in 0.1~0.3; $\Sigma\Omega_{control}$ = 0.1), 6D-backbone torsion angle (ΣΩ in 0.2~0.5; $\Sigma\Omega_{control}$ = 0.2) and 5D-sugar torsion angle (ΣΩ in 0.1~0.5; $\Sigma\Omega_{control}$ = 0.15) of the residues in and flanking m^1A•T HG bp.

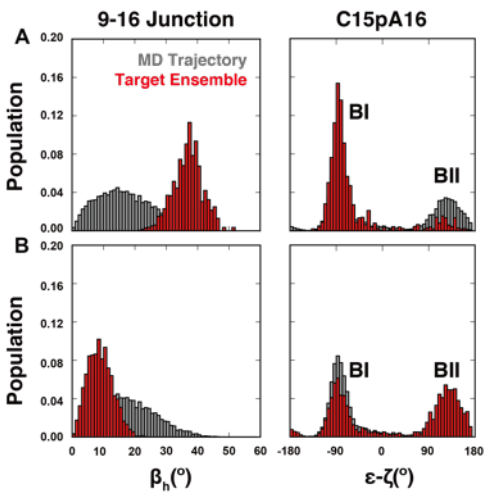

Figure S8. (A, B) Comparison of inter-helical kink angle and BI/BII distributions for the MD ensemble and a target ensemble from MD selected pool to have average kink angles (A) β_h = 37° and (B) β_h = 9° at the T9-A16 bp junction from A_2-DNA MD pool (average β_h = 17°).

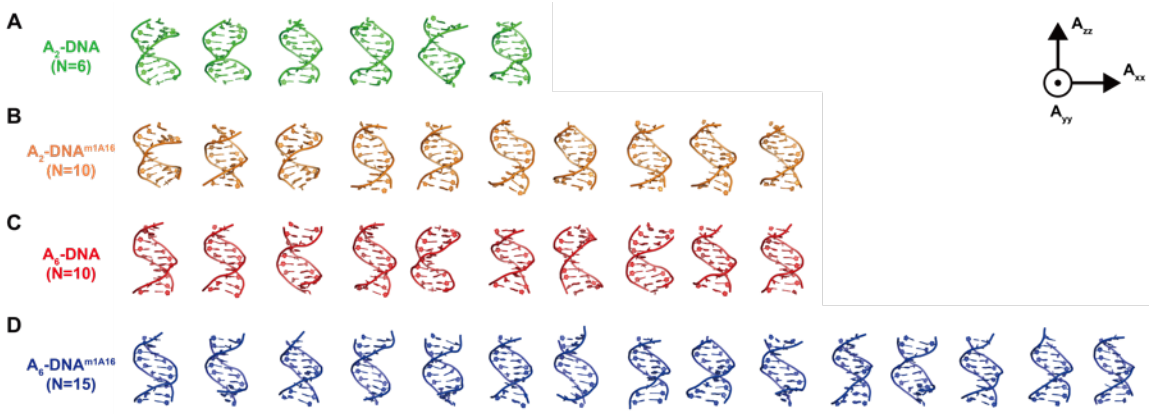

Figure S9. Principal axis of alignment tensor for each conformer in the ensembles of (A) A_2-DNA (green) (B) A_2-DNA^{m1A16} (orange) (C) A_6-DNA (red) (D) A_6-DNA^{m1A16} (blue), respectively.

Table S1: Structure statistics for A_2-DNA, A_2-DNA^{m1A16}, A_6-DNA and A_6-DNA^{m1A16}

	A_2-DNA	A_2-DNA^{m1A16}	A_6-DNA	A_6-DNA^{m1A16}
Dihedral angle restraints	164	164	164	164
H-bond restraints	72	72	72	72
Base-pair planarity restraints	26	26	26	26
NOE restraints				
Total	284	255	251	261
Intra-residue	125	106	144	145
Inter-residue	159	142	107	109
m^1A methyl group	0	7	0	7
RDC restraints	57	52	67	58
Dihedral angle violations (> 6º)	0	0	0	0
NOE violations (> 0.40 Å)	0	0	0	0
RDC RMSD (Hz)	1.8	2.1	1.8	1.9
Heavy-atom RMSD range[a] (Å)	0.08-0.32	0.16-0.53	0.22-0.39	0.05-0.25
ADIT[b] and Molprobity[c] Validation				
Close contacts	None			
RMSD for covalent bonds	0.009 Å			
RMSD for covalent angles	1.4º			
Clashscore, all atoms	1.32	7.86	6.57	1.31
Bad bonds	0/539	0/539	0/537	0/537
Bad angles	0/826	0/826	0/822	0/822
PDB ID	5UZD	--	5UZF	5UZI
BMRB ID	30253	--	30254	30255

a – Heavy-atom RMSD calculated with respect to mean structure, excluding terminal end bps
b – ADIT NMR: http://deposit.pdb.org/validate/
c – MOLPROBITY http://molprobity.biochem.duke.edu/

Table S2: Chemical shifts and perturbations upon N1-methylation of A16 in A_6- and A_2-DNA constructs.

Chemical shifts of A_6-DNA^{m1A16}

A_6-DNA^{m1A16}	C1'	H1'	C2'	H2'	H2"	C4'	H4'	P	C6/C8	H6/ H8	N1/C2/ N3/C5	H1/H2/ H3/H5	C7	H7
C1	87.9	5.66	39.9	2.32	1.79	88.3	4.01		143.1	7.56	99.8	5.85		
G2	84.0	5.49	40.0	2.78	2.68	87.4	4.29	-0.39	138.2	7.92	147.3	12.81		
A3	85.0	6.30	41.3	2.96	2.67	87.8	4.48	-0.45	141.5	8.24	155.0	7.83		
T4	85.0	5.94	39.1	2.59	2.04	85.4	4.21	-0.85	138.4	7.15	159.2	13.76	14.0	1.33
T5	85.3	6.16	39.0	2.64	2.23	85.9	4.23	-0.77	140.2	7.42	159.8	14.04	14.4	1.51
T6	85.2	6.16	39.0	2.65	2.23	86.0	4.21	-0.84	140.3	7.43	159.8	13.95	14.4	1.58
T7	85.1	6.11	39.0	2.65	2.18	86.0	4.22	-0.70	140.2	7.44	159.3	13.68	14.4	1.57
T8	85.1	6.07	38.9	2.57	2.16	86.1	4.21	-0.67	140.4	7.45	159.1	13.70	14.5	1.63
T9	85.1	5.74	39.4	2.15	1.57	84.8	3.98	-0.80	140.0	7.15	156.4	11.77	14.7	1.66
G10	84.3	5.54	39.4	2.72		87.5	4.32	-0.65	138.8	7.91	147.2	12.89		
G11	84.8	5.94	41.2	2.67	2.49	86.9	4.33	-0.37	137.5	7.70	147.2	13.02		
C12	86.5	6.14	41.6	2.14		86.6	3.99	-0.41	143.2	7.41	98.4	5.39		
G13	85.5	5.97	40.5	2.74	2.64	89.0	4.21		138.8	7.95				
C14	86.7	5.98	40.0	2.40	2.11	86.1	4.22	-0.66	142.8	7.46	98.7	5.35		
C15	86.0	5.78	39.3	2.39	1.72	86.2	4.12	-0.55	143.4	7.35	98.2	5.54		
m1A16	88.1	5.58	39.1	2.25	2.02	86.4	4.02		147.1	7.48	149.8	8.42		
A17	84.6	5.54	41.1	2.76		87.2	4.34		141.9	8.19	154.1	7.11		
A18	84.4	5.72	41.2	2.76	2.48	87.2	4.34	-0.64	140.9	7.98	153.6	6.89		
A19	84.3	5.80	41.6	2.85	2.45	87.1	4.37	-0.72	140.9	7.92	153.4	6.88		
A20	84.1	5.87	41.9	2.88	2.48	86.9	4.41	-0.76	140.8	7.88	153.3	6.96		
A21	84.7	6.05	41.9	2.86	2.42	86.8	4.42	-0.68	141.0	7.94	154.5	7.60		
T22	84.9	5.88	39.5	2.41	1.92	85.1	4.17	-0.76	138.5	7.02	159.3	13.61	13.9	1.16
C23	86.4	5.70	39.5	2.35	1.96	85.5	4.10	-0.57	143.8	7.41	98.8	5.57		
G24	84.7	6.14	41.9	2.59	2.34	88.1	4.16	-0.21	139.3	7.91				

Chemical shifts are referenced to 50 µM DSS and 85% H_3PO_4 for ^{1}H and ^{31}P (see methods).

Chemical shifts perturbations upon single m^1A incorporation in A_6-DNA

Measured as the difference in chemical shift observed for A_6-DNA^{m1A16} and A_6-DNA (positive and negative values indicate down-/up-field shifts).

Perturbation	C1'	C2'	C4'	C6/C8	N1/C2/N3/C5	C7	P	H1'	H2'	H2"	H4'	H6/H8	H1/H2/H3/H5	H7
C1	0.0	0.0	0.0	0.0	0.0			-0.01	0.00	-0.01	0.00	0.00	-0.01	
G2	0.0	0.0	0.0	0.0	0.0		0.00	0.00	0.00	-0.01	0.00	0.00	0.00	
A3	0.0	0.0	0.0	0.0	0.0		0.00	0.00	-0.01	-0.01	0.00	0.00	0.00	
T4	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.00	-0.01	0.01	0.00	0.00	-0.01	0.00
T5	0.1	0.0	0.0	0.0	0.0	0.0	0.01	-0.01	-0.01	0.00	-0.01	0.00	-0.02	0.00
T6	0.0	0.0	0.0	0.0	-0.1	0.0	0.00	-0.01	0.01	0.01	0.00	0.00	-0.04	0.01
T7	0.0	0.0	0.0	0.0	-0.2	0.0	0.03	-0.02	-0.02	-0.02	-0.01	0.01	-0.15	0.01
T8	-0.1	0.0	0.3	0.2	-0.2	0.0	0.03	0.01	-0.02	0.05	0.02	0.03	-0.07	-0.02
T9	0.1	0.4	-0.5	0.4	-2.7	0.1	-0.10	0.00	-0.21	-0.40	-0.10	-0.07	-1.97	-0.02
G10	0.0	-0.8	-0.2	0.3	0.1		-0.17	-0.03	0.05		0.00	0.07	0.05	
G11	0.1	0.1	0.0	0.0	-0.2		-0.15	0.00	-0.02	-0.02	-0.01	-0.03	-0.04	
C12	0.0	0.0	0.0	0.0	0.0		0.00	-0.01	0.00		0.00	0.00	-0.03	
G13	0.2	0.1	-0.1	0.0				0.00	-0.01	0.02	0.00	0.00		
C14	0.0	0.3	0.0	0.0	-0.2		-0.04	-0.04	-0.03	0.03	0.01	0.02	-0.02	
C15	-0.3	0.0	0.5	0.2	-0.7		-0.01	0.54	0.20	-0.18	0.11	-0.06	-0.08	
A16	3.6	-1.0	-0.7	5.6	-4.5			-0.10	-0.50	-0.65	-0.30	-0.67	1.24	
A17	0.6	0.3	0.1	1.0	0.5			-0.12	0.04		0.00	0.16	0.16	
A18	0.2	-0.1	0.1	0.0	0.1		0.01	0.01	-0.01	0.02	-0.01	0.05	0.02	
A19	0.1	-0.1	0.0	0.1	0.0		0.02	0.01	-0.01	0.01	0.01	0.04	0.00	
A20	0.0	-0.1	0.0	0.0	0.0		0.02	0.00	0.00	0.02	0.00	0.02	0.00	
A21	0.0	0.0	0.0	0.0	0.0		0.02	-0.01	0.00	-0.01	0.00	0.00	-0.01	
T22	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-0.01	-0.01	0.00	-0.01	0.00	0.00	0.00
C23	0.0	0.0	0.0	0.0	0.0		0.00	-0.01	-0.01	-0.01	0.00	0.00	-0.01	
G24	0.0	0.0	0.0	0.0			0.00	-0.01	0.00	-0.01	0.00	0.00		

Chemical shifts of A_2-DNA^{m1A16}

A_2-DNA m1A16	C1'	H1'	C2'	H2'	H2"	C4'	H4'	P	C6/C8	H6/H8	N1/C2/ N3/C5	H1/H2/ H3/H5	C7	H7#
G1	85.4	5.96	40.4	2.73	2.62	89.0	4.20		138.8	7.93				
C2	86.3	5.64	39.7	2.46	2.15	85.9	4.19	-0.69	142.8	7.44	98.5	5.42		
A3	85.2	6.27	40.8	2.94	2.68	87.7	4.41	-0.34	141.8	8.32	154.5	7.68		
T4	85.0	5.84	39.1	2.39	1.97	85.1	4.14	-0.86	138.3	7.11	159.0	13.50	14.2	1.37
C5	86.4	5.53	39.6	2.31	1.92	85.4	4.05	-0.62	143.5	7.34	98.7	5.49		
G6	83.9	5.61	40.5	2.76	2.64	87.4	4.31	-0.46	138.2	7.83	146.9	12.48		
A7	85.0	6.16	41.6	2.87	2.56	87.4	4.41	-0.71	141.2	8.09	154.6	7.67		
T8	85.1	5.86	39.2	2.46	1.94	85.5	4.15	-0.83	138.3	7.11	**158.7**	**13.46**	14.1	1.33
T9	85.4	5.75	39.5	2.15	1.59	85.1	3.98	-0.97	139.7	7.12	**156.3**	**11.60**	14.6	1.56
G10	84.2	5.54	39.5	2.70		87.2	4.30	-0.71	138.7	7.88	147.4	12.86		
G11	84.8	5.94	41.1	2.67	2.49	86.8	4.34	-0.43	137.5	7.69	147.4	13.02		
C12	86.5	6.15	41.6	2.14		86.6	4.00	-0.47	143.2	7.40	98.4	5.38		
G13	85.2	5.95	40.1	2.75	2.58	89.1	4.20		138.7	7.92				
C14	86.6	5.97	39.9	2.39	2.10	86.0	4.21	-0.72	142.8	7.44	98.7	5.33		
C15	86.1	5.84	39.3	2.49	1.79	86.2	4.15	-0.59	143.4	7.37	98.1	5.52		
m1A16	88.3	5.74				86.5	4.14		147.1	7.58	149.8	8.44		
A17	84.7	6.11				86.9	4.42		140.9	8.11	154.5	7.58		
T18	85.4	5.79				85.4	4.14	-0.89	138.5	7.11	159.0	13.50	14.2	1.33
C19	86.5	5.60	39.9	2.32	1.89	85.5	4.05	-0.64	143.5	7.34	98.7	5.48		
G20	83.9	5.57	40.5	2.75	2.64	87.2	4.30	-0.54	138.2	7.83	146.9	12.51		
A21	85.0	6.16	41.4	2.86	2.56	87.3	4.40	-0.65	141.1	8.12	154.7	7.70		
T22	84.9	5.70	39.5	2.31	1.89	85.0	4.08	-0.82	138.0	7.01	159.0	13.50	14.2	1.34
G23	84.5	5.88	40.5	2.66	2.55	86.9	4.31	-0.63	138.2	7.81	147.7	12.74		
C24	86.6	6.15	41.6	2.14		86.7	4.02	-0.37	143.3	7.43	98.5	5.42		

Chemical shifts of T8/T9 N3/H3 were measured at 5 ºC as they were exchange broadened at 25 ºC.

Chemical shifts perturbations upon single m^1A incorporation in A_2-DNA

Measured as the difference in chemical shift observed for A_2-DNA^{m1A16} and A_2-DNA (positive and negative values indicate down-/up-field shifts).

Perturbation	C1'	C2'	C4'	C6/C8	N1/C2/N3/C5	C7	P	H1'	H2'	H2"	H4'	H6/H8	H1/H2/H3/H5	H7
G1	0.1	0.1	-0.1	0.0				0.00	-0.01	0.02	0.00	0.00		
C2	0.0	0.0	-0.1	0.0	-0.1		-0.08	-0.01	0.00	-0.01	-0.01	0.00	-0.01	
A3	0.0	0.0	0.0	0.0	0.0		-0.08	-0.01	-0.01	-0.01	-0.01	0.00	0.00	
T4	0.0	0.0	0.0	0.0	0.1	0.0	-0.08	-0.01	0.00	0.00	0.00	0.00	0.00	0.00
C5	0.0	0.0	-0.1	0.0	-0.1		-0.09	0.00	-0.01	0.00	0.00	0.00	-0.02	
G6	0.0	0.0	0.2	0.0	0.0		-0.09	0.00	-0.01	-0.01	0.00	-0.01	-0.01	
A7	0.0	0.0	-0.1	0.0	0.0		-0.04	-0.01	-0.02	-0.03	-0.01	-0.02	0.02	
T8	0.0	0.1	0.3	0.0	-0.4	0.1	-0.06	0.00	0.00	0.03	0.02	0.02	-0.17	0.06
T9	0.1	0.4	-0.4	0.2	-2.9	0.1	-0.19	0.01	-0.20	-0.39	-0.09	-0.09	-2.15	0.01
G10	0.1	-0.7	0.1	0.2	0.2		-0.22	-0.04	0.01		-0.02	0.08	0.05	
G11	0.0	0.1	0.0	0.0	-0.1		-0.19	0.01	-0.01	-0.02	-0.01	-0.02	-0.04	
C12	0.0	0.0	0.0	0.0	0.0		-0.08	0.00	0.00		0.00	0.00	-0.03	
G13	0.0	0.0	0.0	0.0				-0.01	-0.01	-0.01	-0.01	0.00		
C14	-0.1	0.2	0.0	0.0	-0.2		-0.11	-0.05	-0.04	0.00	0.00	0.01	-0.03	
C15	-0.3	-0.1	0.7	0.2	-0.8		-0.06	0.51	0.18	-0.20	0.09	-0.05	-0.08	
A16	3.6		-1.2	5.6	-4.1			-0.19			-0.25	-0.65	1.25	
A17	0.0		-0.1	-0.1	0.0			-0.02			-0.01	-0.02	0.00	
T18	0.4		0.2	0.3	-0.1	0.1	-0.11	0.02			0.02	0.10	0.03	0.06
C19	0.1	0.1	-0.2	0.0	0.0		-0.06	0.01	0.00	-0.02	0.00	0.03	0.02	
G20	0.0	0.0	0.0	0.0	0.0		-0.10	0.00	0.00	0.00	0.00	0.00	-0.01	
A21	0.1	0.0	-0.1	0.0	0.0		-0.09	-0.01	-0.01	-0.01	-0.01	0.00	0.00	
T22	0.0	0.0	0.0	0.0	0.0	0.0	-0.08	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00
G23	0.0	0.0	0.0	0.0	0.0		-0.08	-0.01	0.00	-0.01	-0.01	0.00	0.00	
C24	0.0	0.0	0.0	0.0	0.0		-0.08	-0.01	-0.01		-0.01	0.00	-0.01	

Table S3: 3-bond scalar couplings (in Hz) measured between H1' and H2'/H2" from 2D [^{1}H, ^{1}H] DQF-COSY spectra for A_6- and A_6-DNA^{m1A16} constructs. Empty cells denote lack of measurement due to resonance overlap.

Residue	H1'-H2"		H1'-H2'	
	A_6-DNA	A_6-DNA^{m1A16}	A_6-DNA	A_6-DNA^{m1A16}
C1	8.1	8.0	6.3	6.3
G2			6.3	6.6
A3	8.7	8.6	6.7	6.8
T4		9.0	6.6	6.5
T5			6.6	
T6				7.2
T7	8.7		6.4	
T8	8.7	8.9	6.3	6.9
T9		7.6		7.3
G10			7.6	7.6
G11	8.4	8.2	6.6	6.6
C12			7.5	7.8
G13	8.4	8.2	6.6	7.0
C14	8.1	8.4	6.3	6.3
C15		9.2	6.7	6.5
A16		8.0	6.6	
A17			6.6	
A18			7.2	
A19	9.1	9.7	6.6	7.1
A20	9.3	9.5	6.6	6.7
A21	8.7	8.7	6.3	6.5
T22	8.4	8.7	6.6	6.7
C23	9.1	8.3		6.3
G24	6.9	6.8	7.8	7.8

Table S4. Robustness of local kinking angles (β_h) with various ensemble size (N).

		4-21		5-20		6-19		7-18		8-17		9-16	
Size	RMSD	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std
A_2-DNA													
10	1.8	22.1	7.4	23.2	5.9	21.2	9.6	18.8	8.7	19.7	7.1	**19.1**	**8.1**
20	1.23	20.1	7.6	22.7	7.1	22.7	7.9	21.2	9.4	20.5	7.9	**20.1**	**9.2**
30	1.01	22.2	8.3	22.6	7.8	22.4	9.5	22.5	10.4	20.3	9.2	**18.5**	**10.1**
50	0.97	20.9	8.2	22.8	7.8	22.8	9.3	22.5	9.9	21.6	7.9	**19.6**	**9.1**
80	1.11	20.7	8.3	22.9	7.5	23.0	9.2	21.4	10.5	20.0	9.0	**18.8**	**9.8**
100	1.2	21.0	7.9	22.5	7.5	22.5	9.1	21.0	10.1	20.3	8.6	**18.7**	**9.4**
150	1.41	20.9	8.1	22.0	7.4	22.2	9.0	20.9	9.9	19.7	8.8	**18.7**	**9.2**
200	1.58	21.3	7.7	21.9	7.5	22.2	8.5	20.6	9.6	19.6	8.8	**18.3**	**9.1**
300	1.86	21.6	7.6	21.6	7.5	21.7	8.3	20.3	9.3	19.8	8.6	**18.4**	**9.1**
400	2.08	21.5	7.9	21.5	7.4	21.8	8.3	20.2	9.1	19.7	8.6	**18.4**	**9.0**
500	2.25	21.3	7.8	21.2	7.5	21.4	8.3	19.8	9.1	19.4	8.5	**18.3**	**9.2**
800	2.66	21.3	8.2	21.1	7.6	21.3	8.3	19.3	8.9	18.9	8.5	**17.9**	**8.9**
1000	2.88	21.1	8.2	20.9	7.6	21.1	8.1	19	8.6	18.4	8.4	**17.8**	**9.0**
1500	3.29	21	8.2	20.6	7.7	20.6	8.1	18.3	8.4	17.9	8.4	**17.5**	**8.9**
2000	3.62	20.9	8.2	20.5	7.6	20.4	8.0	18	8.3	17.5	8.4	**17.5**	**8.9**
A_2-DNA^{m1A16}													
10	2.43	21.3	7.0	22.1	5.8	19.2	10.3	17.5	12.5	21.8	7.1	**24.9**	**8.3**
20	1.69	23.8	7.1	21.2	6.5	17.6	6.4	16.8	8.7	22.3	7.4	**26.3**	**8**
30	1.73	20.9	8.3	20.7	8.9	20.2	7	19.1	9.3	22.3	9.2	**25.4**	**9.9**
50	1.61	22.3	7.8	21.2	8	18.8	8.1	18.7	9.4	22.7	8.5	**26**	**8.3**
80	1.73	22.2	8.3	20.9	8.4	19.5	8.7	19.4	9.6	22.7	9.3	**25.6**	**8.9**
100	1.82	22.1	8.6	21	8.3	19.6	8.5	19.2	9.7	22.5	8.9	**25.7**	**8.3**
150	2	22.1	8.1	20.7	8.2	19.6	8.5	18.7	9.4	22.2	8.8	**25.5**	**8.7**
200	2.17	21.8	8.3	20.8	8.2	19.6	8.2	18.4	9.1	21.8	9	**25.2**	**8.7**
300	2.44	21.4	8.1	20.5	7.9	19.4	8.1	18.3	9	21.8	8.5	**24.8**	**8.7**
400	2.66	21	8.1	20.5	8.1	19.2	8.3	18.1	8.8	21.3	8.4	**24.5**	**8.7**
500	2.85	21.2	7.9	20.6	8	19.2	8.1	18.1	8.7	21.3	8.2	**24.5**	**8.7**
800	3.27	21	7.9	20.5	7.9	19.2	8.2	17.8	8.6	20.9	8.3	**24.2**	**8.6**
1000	3.5	21	8	20.4	7.9	19.1	8.2	17.6	8.6	20.6	8.1	**24.1**	**8.5**
1500	3.95	20.7	7.9	20.3	7.9	19	8.2	17.3	8.4	20.1	8.2	**23.9**	**8.5**
2000	4.32	20.6	7.9	20	7.8	18.7	8.2	17.2	8.4	19.8	8.2	**23.7**	**8.6**

A_6-DNA													
10	2.45	9.6	6.8	8.5	7.9	11.4	8.6	12.1	9.1	15.1	9.6	**14.1**	**7.7**
20	1.64	10.9	7.2	9.7	5.8	12.2	5.7	12.7	7.4	15.5	8.9	**16.4**	**8.8**
30	1.62	11.2	7.1	10.3	6.3	11.9	7.8	13.5	10.1	18	9.3	**16.6**	**7.3**
50	1.64	11.7	7.1	11.1	6.9	12.1	7.2	13.5	8.4	17.5	8.3	**16.3**	**8.5**
80	1.78	11.4	7.4	10.8	7.4	11.8	7.2	13.1	8.3	17.4	9.4	**16.5**	**8.3**
100	1.85	11.4	7	10.9	6.7	12	7.2	12.8	8.1	17.1	9.1	**16.2**	**8.8**
150	2.09	11.5	7.3	10.9	7	12.3	7.5	13.1	8.1	16.7	8.6	**15.9**	**8.8**
200	2.28	11.8	7.4	11.1	7.4	12.5	7.7	13.6	8	16.8	8.4	**16**	**8.9**
300	2.59	11.9	7	11.5	7.2	12.6	7.7	13.6	7.8	16.6	8.6	**15.9**	**9**
400	2.84	11.8	7	11.4	7.1	12.4	7.3	13.2	7.7	16.1	8.8	**15.9**	**8.9**
500	3.06	11.6	7	11.3	7.2	12.4	7.4	13.1	7.6	15.8	8.6	**16**	**8.8**
800	3.55	11.4	6.8	11	6.9	12	7.1	12.8	7.4	15.5	8.4	**15.6**	**8.7**
1000	3.81	11.2	6.6	10.8	6.8	11.9	6.9	12.7	7.2	15.3	8.3	**15.5**	**8.5**
1500	4.29	10.9	6.6	10.6	6.7	11.6	6.8	12.3	7.1	14.8	8.1	**15.2**	**8.5**
2000	4.67	10.9	6.6	10.4	6.5	11.3	6.6	12	6.9	14.5	8	**15.2**	**8.4**
A_6-DNA^{m1A16}													
10	2.92	13.2	9.3	14.7	7.3	14.1	6.3	11.8	5.8	17.6	8.6	**22.7**	**10.3**
20	2.46	12.6	8.4	14.3	7.8	14	6.3	13.7	8.8	18	9.4	**23.8**	**10.8**
30	2.46	11.1	7.6	12.4	7.8	12.1	6	11.6	7.5	17.7	9.1	**23.6**	**10.4**
50	2.53	11.9	7.3	13.2	7	13.4	6.5	13.5	6.8	17.9	8.2	**23.9**	**10**
80	2.72	12.2	7.3	13.7	7.7	13.4	7.5	13	7.1	17.3	7.6	**23.1**	**9.6**
100	2.81	12.1	7.6	13.1	7.4	12.6	7.1	12.4	6.9	17.4	7.4	**22.9**	**9.5**
150	3.04	12.3	7.1	12.7	7.1	12.2	6.7	12.6	6.6	17.1	7.6	**23.1**	**9.3**
200	3.22	12.1	6.7	12.2	6.9	11.8	6.6	12.3	6.7	16.7	7.6	**22.5**	**9**
300	3.49	12	6.7	11.9	6.7	11.5	6.3	11.9	6.5	16.4	7.6	**22.4**	**8.8**
400	3.71	11.8	6.6	11.5	6.6	11.1	6.2	11.6	6.3	16.1	7.5	**22.2**	**8.7**
500	3.9	11.5	6.5	11.3	6.5	10.9	5.9	11.2	6.1	16	7.3	**21.9**	**8.7**
800	4.31	11.4	6.6	10.9	6.3	10.8	5.9	11.2	6.1	15.9	7.2	**21.7**	**8.8**
1000	4.53	11.2	6.4	10.8	6.3	10.8	5.8	11	6.1	15.7	7.1	**21.7**	**8.7**
1500	4.95	10.8	6.2	10.5	6.3	10.7	5.8	10.6	6	15.4	7	**21.6**	**8.7**
2000	5.28	10.6	6	10.2	6	10.4	5.6	10.2	5.9	15.1	7	**21.3**	**8.7**
10	2.92	13.2	9.3	14.7	7.3	14.1	6.3	11.8	5.8	17.6	8.6	**22.7**	**10.3**

Table S5. Robustness of local kinking angles (β_h) with various RDC data sets.

Type	Construct	Global Bending (º)	Local Kinking Angles β_h (º)					
			4-21	5-20	6-19	7-18	8-17	9-16
Ensemble (no terminal RDCs)	A_2-DNA	11 ± 5	21 ± 8	22 ± 6	21 ± 8	19 ± 10	19 ± 8	**17 ± 7**
	A_2-DNA^{m1A16}	15 ± 8	23 ± 8	21 ± 7	18 ± 8	17 ± 9	22 ± 8	**26 ± 9**
	A_6-DNA	14 ± 8	11 ± 7	10 ± 6	12 ± 7	14 ± 9	17 ± 9	**15 ± 8**
	A_6-DNA^{m1A16}	22 ± 10	12 ± 8	13 ± 8	13 ± 6	13 ± 7	18 ± 8	**23 ± 10**
Ensemble (no 2bp terminal RDCs)	A_2-DNA	11 ± 5	21 ± 8	22 ± 7	21 ± 9	19 ± 9	19 ± 8	**18 ± 8**
	A_2-DNA^{m1A16}	15 ± 7	24 ± 8	20 ± 7	18 ± 7	17 ± 9	21 ± 9	**25 ± 9**
	A_6-DNA	15 ± 8	10 ± 7	9 ± 6	10 ± 7	11 ± 8	15 ± 8	**15 ± 8**
	A_6-DNA^{m1A16}	22 ± 11	11 ± 6	13 ± 7	12 ± 7	12 ± 7	18 ± 8	**24 ± 11**

XPLOR parameters used for m1A residue (nucleic.top modified to include the following)

```
RESIdue A1M
 GROUP
  ATOM P    TYPE=XP     CHARge=1.20    END
  ATOM O1P  TYPE=XO2    CHARge=-0.40   END
  ATOM O2P  TYPE=XO2    CHARge=-0.40   END
  ATOM O5'  TYPE=XOS    CHARge=-0.36   END
 GROUP
  ATOM C5'  TYPE=XC2    CHARge=-0.070  END!"
  ATOM H5'  TYPE=XH     CHARge=0.035   END!"
  ATOM H5'' TYPE=XH     CHARge=0.035   END!"
 GROUP
  ATOM C4'  TYPE=XCH    CHARge=0.065   END! "
  ATOM H4'  TYPE=XH     CHARge=0.035   END!"
  ATOM O4'  TYPE=XOS    CHARge=-0.30   END
  ATOM C1'  TYPE=XCH    CHARge=0.165   END!"
  ATOM H1'  TYPE=XH     CHARge=0.035   END!"
 GROUP
  ATOM N9   TYPE=XNS    CHARge=-0.19   END
  ATOM C4   TYPE=XCB    CHARge=0.19    EXCL=( N1 )  END
 GROUP
  ATOM N3   TYPE=XNC    CHARge=-0.26   EXCL=( C6 )  END
  ATOM C2   TYPE=XCE    CHARge=0.225   EXCL=( C5 )  END !"
  ATOM H2   TYPE=XH     CHARge=0.035   END!"
 GROUP ! N1Me is added here
  ATOM N1   TYPE=XNC    CHARge=0.685   END
  ATOM C6   TYPE=XCA    CHARge=0.28    END
  ATOM C1   TYPE=XC3    CHARge=-0.070  END!"
  ATOM H11  TYPE=XH     CHARge=0.035   END!"
  ATOM H12  TYPE=XH     CHARge=0.035   END!"
  ATOM H13  TYPE=XH     CHARge=0.035   END!"
GROUP
  ATOM N6   TYPE=XN2    CHARGE=-0.42   END!
  ATOM H61  TYPE=XH2    CHARge=0.21    END!
  ATOM H62  TYPE=XH2    CHARge=0.21    END!
 GROUP
  ATOM C5   TYPE=XCB    CHARge=0.02    END
  ATOM N7   TYPE=XNB    CHARge=-0.25   END
  ATOM C8   TYPE=XCE    CHARge=0.195   END!"
  ATOM H8   TYPE=XH     CHARge=0.035   END!"
```

```
GROUP
 ATOM C2'  TYPE=XCH    CHARge=0.115   END!"
 ATOM H2'' TYPE=XH     CHARge=0.035   END!"
 ATOM O2'  TYPE=XOH    CHARge=-0.40   END
 ATOM H2'  TYPE=XHO    CHARge=0.25    END
GROUP
 ATOM C3'  TYPE=XCH    CHARge=-0.035  END!"
 ATOM H3'  TYPE=XH     CHARge=0.035   END!"
GROUP
 ATOM O3'  TYPE=XOS    CHARge=-0.36   END

 BOND P O1P    BOND P O2P    BOND P O5'
 BOND O5' C5'   BOND C5' C4'   BOND C4' O4'    BOND C4' C3'  BOND O4' C1'
 BOND C1' N9    BOND C1' C2'   BOND N9 C4     BOND N9 C8    BOND C4 N3
 BOND C4 C5     BOND N3 C2     BOND C2 N1     BOND N1 C6    BOND C6 N6
 BOND N6 H61    BOND N6 H62    BOND C6 C5     BOND C5 N7    BOND N7 C8
 BOND C2' C3'   BOND C2' O2'   BOND O2' H2'    BOND C3' O3'  {* BOND O3'+P *}
 BOND C1' H1'   BOND C2' H2''  BOND C3' H3'    BOND C4' H4'  BOND C5' H5'
 BOND C5'  H5'' BOND C8 H8     BOND C2 H2
 ! For N1Me
 BOND N1  C1   BOND C1  H11   BOND C1  H12   BOND C1  H13
{* DIHE -O3' P O5' C5'   DIHE -O3' P O5' C5'  *}
 DIHE P O5' C5' C4'    DIHE O5' C5' C4' O4'    DIHE O5' C5' C4' C3'
 {* DIHE C4' C3' O3' +P   DIHE C3' O3' +P +O5'    DIHE C3' O3' +P  +O5'  *}
 DIHE O4' C1' N9 C4
 ! N O T E:  SUGAR RING TERMS SET UP AS W. OLSON DOES IT
 DIHE O4'  C1'  C2'  C3' ! O-C-C-C, twofold term
 DIHE O4'  C1'  C2'  C3'
 DIHE C1'  C2'  C3'  C4'
 DIHE C2'  C3'  C4'  O4' ! O-C-C-C, twofold term
 DIHE C2'  C3'  C4'  O4'
 DIHE C3'  C4'  O4'  C1'
 DIHE C4'  O4'  C1'  C2'
 ! AND THE SPECIAL GAUCHE TERMS
 DIHE C5'  C4'  C3'  O3'
 DIHE O4'  C4'  C3'  O3'
 DIHE O4'  C1'  C2'  O2'
 DIHE C1'  C2'  C3'  O3'
 DIHE C4'  C3'  C2'  O2'
 DIHE O3'  C3'  C2'  O2'
 DIHE C3'  C2'  O2'  H2'
```

```
! SO THE ALLHYDROGEN TERMS
DIHE O4'  C4'  C3'  H3'
DIHE O4'  C1'  C2'  H2''
DIHE C4'  O4'  C1'  H1'
DIHE C1'  O4'  C4'  H4'
DIHE C3'  C4'  C5'  H5'
DIHE C3'  C4'  C5'  H5''
! For N1Me
DIHE C6   N1   C1   H11
DIHE C6   N1   C1   H12
DIHE C6   N1   C1   H13
IMPRoper H11 H12 N1 H13  ! copied from thy methyl
IMPRoper C1   C2   C6   N1
! Dihedrals to keep the two purine rings parallel:
impr C8   C4   C5   N1     impr C8   C5   C4   C2
impr N3   C4   C5   N7     impr C6   C5   C4   N9

! The ring-spanning impropers have been left out.
!IMPR C5'  O4'  C3'  C4'    IMPR O3'  C2'  C4'  C3'    IMPR N9   C2'  O4'  C1'
{* chiral impropers included for DG and SA *}
improper H1'  C2'  O4'  N9
improper H2'' C3'  C1'  O2'
improper H3'  C4'  C2'  O3'
improper H4'  C5'  C3'  O4'
{* chiral improper included for H5'/H5'' definition, according to *}
{* Wijmenga, Mooren and Hilbers in NMR of nucl. acids, (Ed. Roberts) *}
improper H5'  O5'  H5'' C4'    !C5'

IMPR C1'  C4   C8   N9
IMPR N9   C4   C5   N7     IMPR C4   C5   N7   C8     IMPR C5   N7   C8   N9
IMPR N7   C8   N9   C4     IMPR C8   N9   C4   C5     IMPR N6   N1   C5   C6
IMPR H62  C6   H61  N6     IMPR C4   N3   C2   N1     IMPR N3   C2   N1   C6
IMPR C2   N1   C6   C5     IMPR N1   C6   C5   C4     IMPR C6   C5   C4   N3
IMPR C5   C4   N3   C2     IMPR H8   N7   N9   C8
IMPR H2   N1   N3   C2
!IMPR C2'  C3'  C1'  O2'

! this first improper is insufficient to keep the N2 group coplanar,
! so I'm adding more.  JJK 3/10/04
! Changed signs to match Discover? params, JJK 3/16/04
! Changed them back to match IUPAC (Eur J Biochem 131,9, fig 2) JJK 7/19/04
```

```
IMPRoper C5 C6 N6 H61
IMPRoper N1 C6 N6 H62
IMPRoper C5 C6 H61 H62
IMPRoper N1 C6 H62 H61
DONO H61  N6
DONO H62  N6
DONO H2'  O2'

ACCE N3 " "
ACCE N1 " "
ACCE N7 " "
ACCE O1P  P
ACCE O2P  P
ACCE O2' " "
ACCE O3' " "
ACCE O4' " "
ACCE O5' " "

{* IC -O3' P    O5'  C5'   1.6001  101.45  -39.25  119.00   1.4401 *}
{* IC -O3'  O5' *P   O1P    1.6001  101.45 -115.82  109.74   1.4802 *}
{* IC -O3'  O5' *P   O2P    1.6001  101.45  115.90  109.80   1.4801 *}
IC  P   O5'  C5'  C4'    1.5996  119.00 -151.39  110.04   1.5160
IC O5'  C5'  C4'  C3'    1.4401  108.83 -179.85  116.10   1.5284
IC C5'  C4'  C3'  O3'    1.5160  116.10   76.70  115.12   1.4212
{* IC C4'  C3'  O3'  +P    1.5284  111.92  159.13  119.05   1.6001 *}
{* IC C3'  O3'  +P   +O5'   1.4212  119.05  -98.86  101.45   1.5996 *}
IC O4'  C3'  *C4' C5'    1.4572  104.06 -120.04  116.10   1.5160
IC C2'  C4'  *C3' O3'    1.5284  100.16 -124.08  115.12   1.4212
IC C4'  C3'  C2'  C1'    1.5284  100.16   39.58  102.04   1.5251
IC C3'  C2'  C1'  N9     1.5284  101.97  144.39  113.71   1.4896
IC O4'  C1'  N9   C4     1.5251  113.71  -96.00  125.97   1.3703
IC C1'  C4   *N9  C8     1.4896  125.97 -179.94  105.00   1.3768
IC C4   N9   C8   N7     1.3703  105.00   -0.07  113.93   1.2970
IC C8   N9   C4   C5     1.3768  105.00    0.06  106.60   1.3650
IC N9   C5   *C4  N3     1.3703  106.60 -179.93  126.69   1.3486
IC C5   C4   N3   C2     1.3650  126.69   -0.04  111.18   1.3130
IC C4   N3   C2   N1     1.3486  111.18   -0.02  128.64   1.3399
IC N3   C2   N1   C6     1.3130  128.64    0.06  118.95   1.3456
IC C5   N1   *C6  N6     1.4034  117.43 -179.96  119.06   1.3410
IC N1   C6   N6   H61    1.3456  119.06  179.96  120.00   1.0100
IC H61  C6   *N6  H62    1.0100  120.00  180.00  120.00   1.0100
```

```
 IC C1'  C3'  *C2' O2'    1.5284  102.04 -114.67  110.81   1.4212 !INFERENCE
 IC H2'  O2'  C2'  C3'    0.9600  114.97  148.63  111.92   1.5284 !GUESS

 ! the all hydrogen part (NOT TOO CAREFULLY DONE /LN)
 IC O4'  C2'  *C1' H1'    0.0     0.0     -115.0  0.0      0.0
 IC C1'  C3'  *C2' H2''   0.0     0.0      115.0  0.0      0.0
 IC C2'  C4'  *C3' H3'    0.0     0.0      115.0  0.0      0.0
 IC C3'  O4'  *C4' H4'    0.0     0.0     -115.0  0.0      0.0
 IC C4'  O5'  *C5' H5'    0.0     0.0     -115.0  0.0      0.0
 IC C4'  O5'  *C5' H5''   0.0     0.0      115.0  0.0      0.0
 ! THE BASE:
 IC N9   N7   *C8   H8    0.0     0.0      180.0  0.0      0.0
 IC N1   N3   *C2   H2    0.0     0.0      180.0  0.0      0.0

END {* A1M *}
```

References

1. Farjon, J., Boisbouvier, J., Schanda, P., Pardi, A., Simorre, J.-P. and Brutscher, B. (2009) Longitudinal-Relaxation-Enhanced NMR Experiments for the Study of Nucleic Acids in Solution. *J. Am. Chem. Soc.*, **131**, 8571-8577.
2. Yang, H., Zhan, Y., Fenn, D., Chi, L.M. and Lam, S.L. (2008) Effect of 1-methyladenine on double-helical DNA structures. *FEBS Lett.*, **582**, 1629-1633.
3. Yang, S., Salmon, L. and Al-Hashimi, H.M. (2014) Measuring similarity between dynamic ensembles of biomolecules. *Nat. Meth.*, **11**, 552-554.