

Supplementary Material

A dumbbell double nicked duplex dodecamer DNA with PEG₆ tether

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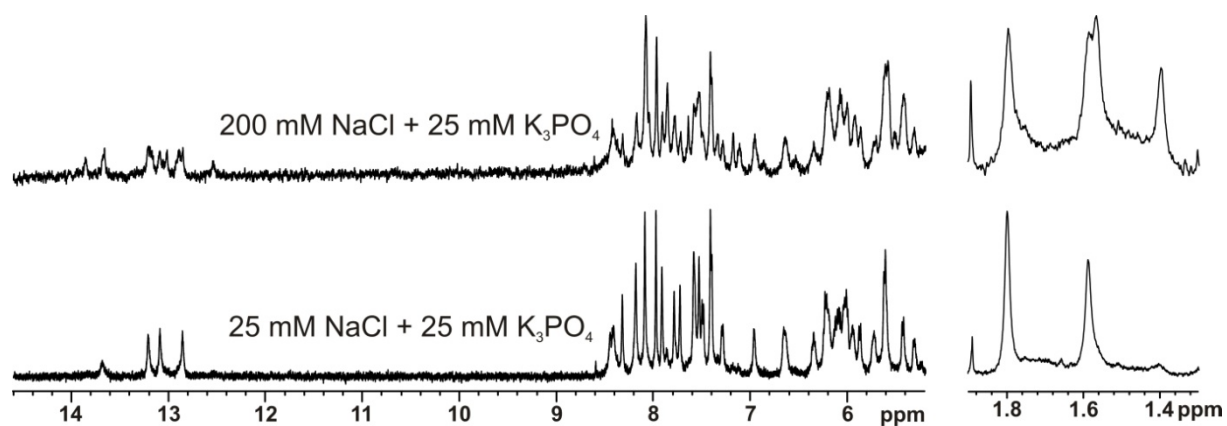


Fig. 1S The ionic strength dependence of equilibrium at low DNA hairpin concentration (0.25 mM) at 10° C.

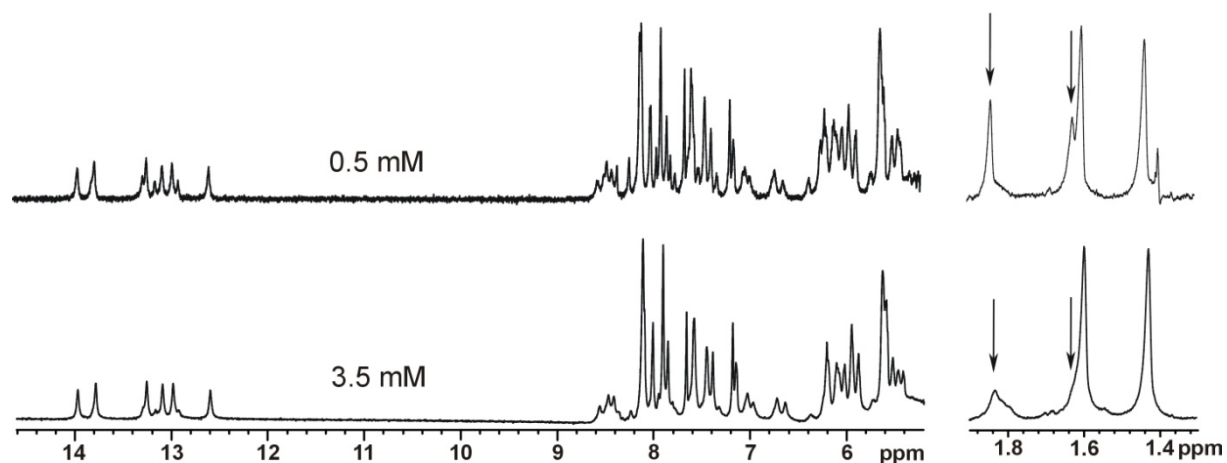


Fig. 2S. Hairpin concentration dependence of hairpin – dumbbell equilibrium in a 25/25 mM $K_3PO_4/NaCl$ water buffer at 0° C. On the right side of the spectra the thymidine resonances are displayed. The arrows indicate the thymidine resonances assigned to hairpin motif.

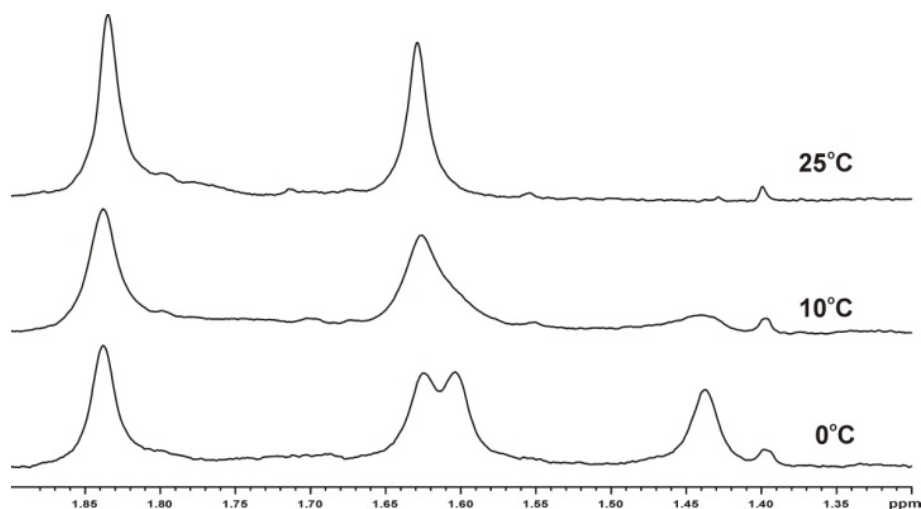


Fig. 3S The temperature dependence of the hairpin – dumbbell equilibrium in 0.35 mM H_2O solution and 25/25 mM $K_3PO_4/NaCl$ buffer, pH 6.1 observed on thymidine methyl groups.

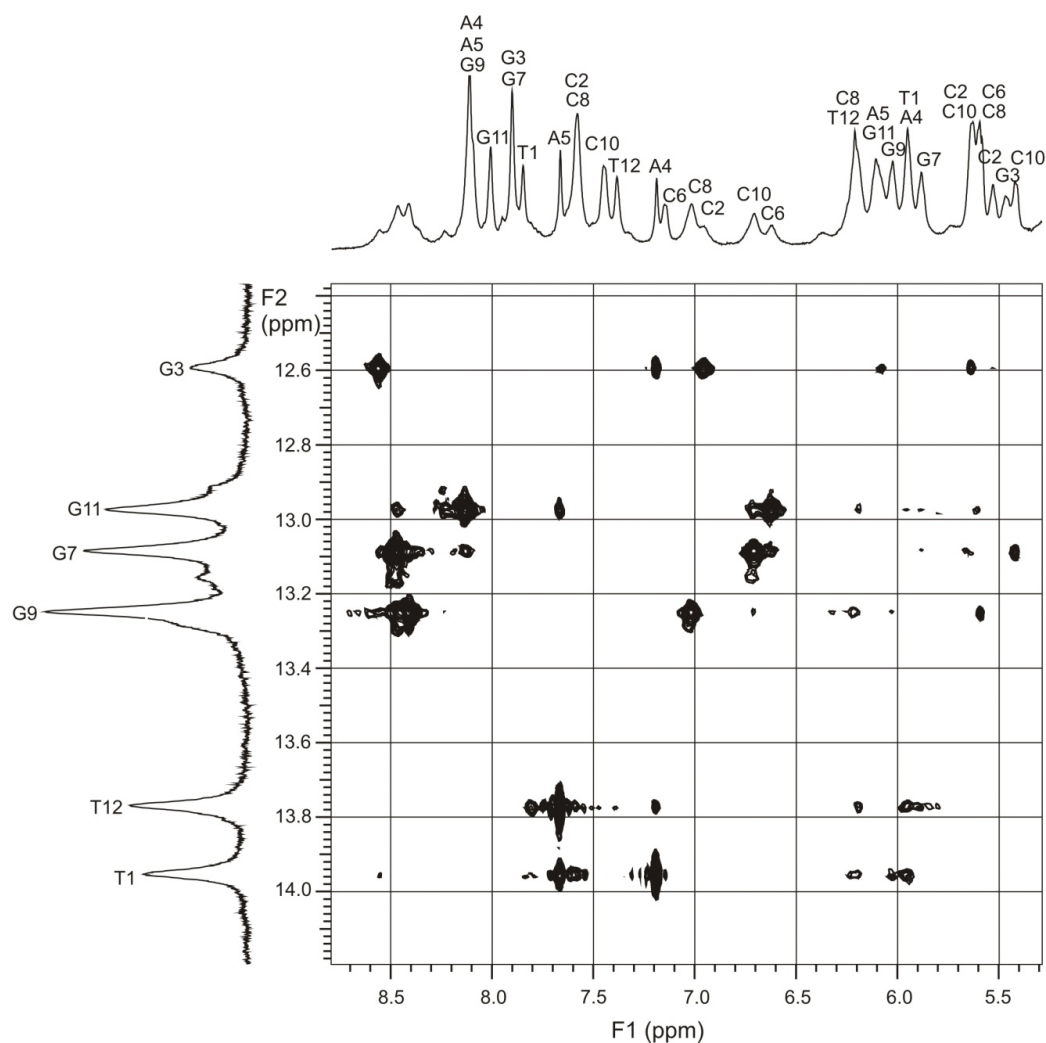


Fig. 4S. Imine region of NOESY spectrum of dumbbell DNA at 0°C, 3.5 mM in monomer, pH 6.1, in 25/25 mM K₃PO₄/NaCl buffer.

Table 1S. Hairpin ¹H chemical shifts in H₂O/D₂O (9:1) at 25° C, pH 6.0, in 25/25 mM K₃PO₄/ NaCl buffer.

Base	H1'	H2'	H2''	H3'	H4'	H5', H5''	H6/8	H2/5/Me	NH2	NH
T1	6.14	na	2.43	na	na	na	7.55	1.83	-	b
C2	6.04	na	2.24	na	na	na	7.52	5.90	b	-
G3	5.41	na	2.40	4.88	4.18	3.92, 3.97	7.74	-	-	b
A4	5.34	na	2.62	4.94	4.04	na	8.17	na	-	-
A5	6.20	2.83	2.86	5.06	4.46	4.09, 4.16	8.32	na	-	-
C6	5.64	1.97	2.34	4.84	4.20	na	7.30	5.34	6.57; 8.19	-
G7	5.96	2.64	2.73	5.02	4.39	4.09, 4.15	7.92	-	-	13.10
C8	6.26	2.26	2.48	4.87	4.22	na	7.59	5.64	6.90; 8.40	-
G9	6.02	2.79	2.86	4.98	4.35	3.90, 4.01	8.10	-	-	13.22
C10	5.76	2.11	2.44	4.89	4.23	na	7.41	5.45	6.59; 8.45	-
G11	6.09	2.70	2.82	5.05	4.43	4.11, 4.18	7.97	-	-	12.87
T12	6.36	2.43	2.50	4.93	4.29	3.98, 4.05	7.43	1.63	-	b

- a) Data obtained in H₂O buffer, pH 6.0, 2.5 mg, c = 0.35 mM, 25°C.
Buffer solution contains 25 mM NaCl, 25 mM K₃PO₄·H₂O and 10% of D₂O.
TSPA was added as internal reference.
- b) Broad signal not observed at 25°C.

Table 2S. Dumbbell ¹H chemical shifts in 3.5 mM H₂O solution in a 25/25 mM NaCl/K₃PO₄ buffer at 0°C, pH=6 from NOESY spectrum.

Base	H1'	H2'	H2''	H3'	H4'	H5', H5''	H6/8	H2/5/Me	NH2	NH
T1	5,95	2,13	2,55	4,71	4,18	3,91	7,85	1,60	-	13,95
C2	5,53	2,00	2,36	4,84	4,14	no	7,57	5,63	6,95; 8,56	-
G3	5,47	2,69	2,76	4,99	4,32	4,01	7,90	-	-	12,59
A4	5,94	2,69	2,90	5,07	4,17	na	8,11	7,19	-	-
A5	6,08	2,56	2,84	na	4,44	na	8,10	7,66	-	-
C6	5,61	1,82	2,27	4,80	4,12	na	7,15	5,18	6,62; 8,13	-
G7	5,88	2,61	2,69	4,98	4,36	na	7,90	-	-	13,09
C8	6,21	2,26	2,46	4,85	4,20	na	7,58	5,59	7,02; 8,41	-
G9	6,03	2,81	2,86	4,98	4,35	3,88	8,11	-	-	13,25
C10	5,64	2,22	2,46	4,89	4,24	na	7,45	5,42	6,71; 8,47	-
G11	6,10	2,73	2,88	5,06	4,46	4,19	8,01	-	-	12,97
T12	6,19	2,36	2,42	4,96	4,26	na	7,38	1,44	-	13,77

Data obtained in H₂O buffer, 25 mM NaCl, 25 mM K₃PO₄·H₂O, 10 vol % D₂O pH 6.0, c = 3.5 mM, 0° C. Chemical shifts given vs internal TSPA.

Table 3S. Some chosen helical parameters In computed dumbbell structure .

Interstrand base pair	x-displacement dx (Å)	y-displacement dy (Å)	inclination η (°)	tip θ (°)	buckle κ (°)	propeller twist Ω (°)	opening σ (°)
G9 - C8	-1.40±0.24	1.22±0.22	5.43±1.91	-12.78±1.72	19.61±6.17	1.80±7.09	3.62±2.65
C10 - G7	-1.67±0.21	1.03±0.17	5.03±2.38	-12.53±1.85	15.48±4.36	-15.76±6.25	-4.33±1.28
G11 - C6	-1.61±0.22	0.66±0.19	-0.65±2.06	-3.43±1.01	7.37±3.24	-28.64±3.80	0.07±1.75
T12 - A5	-1.30±0.17	0.16±0.14	-5.01±1.77	-0.96±1.62	-17.99±2.51	-14.02±4.56	8.85±1.07
T1 - A4	-1.02±0.17	0.34±0.15	-6.42±1.57	-11.48±1.33	-3.65±1.52	13.04±3.36	4.19±1.41
C2 - G3	-1.30±0.16	-0.13±0.14	-7.48±1.82	0.39±0.89	-10.18±1.48	16.53±2.33	-0.24±1.17
G3 - C2	-1.30±0.17	0.08±0.15	-7.26±1.86	0.17±1.47	9.35±3.81	15.00±4.85	0.25±1.81
A4 - T1	-1.02±0.15	-0.38±0.14	-6.43±1.78	11.79±1.47	3.37±1.81	15.11±4.83	4.41±1.73
A5 - T12	-1.31±0.17	-0.22±0.16	-4.99±1.65	1.34±1.31	19.06±1.36	-12.67±1.75	8.34±1.17
C6 - G11	-1.62±0.17	-0.77±0.17	-0.03±1.65	4.25±0.94	-8.41±2.55	-29.27±3.14	-0.62±1.90
G7 - C10	-1.68±0.20	-1.12±0.20	5.37±1.75	13.00±1.18	-12.41±3.55	-16.87±6.01	-4.20±1.42
C8 - G9	-1.33±0.22	-1.33±0.25	6.17±1.80	13.23±1.26	-15.67±5.77	4.72±3.93	6.06±2.96
mean	-1.38±0.16	-0.04±0.14	-1.35±1.61	0.25±0.73	0.50±1.12	-4.25±1.22	2.20±0.49
B-DNA*	-0.71	0.0	-5.91	0.0	0.0	3.72	-4.11
A-DNA*	-5.43	0.0	19.09	0.0	0.0	13.7	-4.61

*canonical forms

Table 4S. Differences between computed dumbbell parameters and canonical form B-DNA

Interstrand base pair	x-displacement dx (Å)	y-displacement dy (Å)	inclination η (°)	tip θ (°)	buckle κ (°)	propeller twist Ω (°)	opening σ (°)
G9 - C8	0,69	-1,22	-11,34	12,78	-19,61	1,92	-7,73
C10 - G7	0,96	-1,03	-10,94	12,53	-15,48	19,48	0,22
G11 - C6	0,23	-0,70	-0,71	3,68	-6,88	24,39	2,13
T12 - A5	0,59	-0,16	-0,90	0,96	17,99	17,74	-12,96
T1 - A4	0,31	-0,34	0,51	11,48	3,65	-9,32	-8,30
C2 - G3	0,59	0,13	1,57	-0,39	10,18	-12,81	-3,87
G3 - C2	0,59	-0,08	1,35	-0,17	-9,35	-11,28	-4,36
A4 - T1	0,31	0,38	0,52	-11,79	-3,37	-11,39	-8,52
A5 - T12	0,60	0,22	-0,92	-1,34	-19,06	16,39	-12,45
C6 - G11	0,91	0,77	-5,88	-4,25	8,41	32,99	-3,49
G7 - C10	0,97	1,12	-11,28	-13,00	12,41	20,59	0,09
C8 - G9	0,62	1,33	-12,08	-13,23	15,67	-1,00	-10,17

Table 5S. Some chosen helical parameters in computed dumbbell structure

Sequence base pair	shift Dx (Å)	slide Dy (Å)	rise Dz (Å)	tilt τ (°)	roll ρ (°)	twist Ω (°)
G9 - C10	-0.44±0.16	-0.88±0.19	3.56±0.22	5.03±2.06	6.05±4.21	29.34±1.47
C10 - G11	0.17±0.20	-1.25±0.25	3.41±0.17	-2.90±1.07	28.59±4.13	28.52±2.52
G11 - T12	0.44±0.08	-1.69±0.17	3.85±0.14	-0.09±1.09	7.68±3.06	31.73±1.02
T12 - T1	0.74±0.11	-0.64±0.10	3.38±0.04	4.20±0.40	-23.54±2.81	36.43±1.42
T1 - C2	-0.26±0.13	-1.91±0.07	3.39±0.08	-0.75±0.59	22.49±2.26	22.77±1.08
C2 - G3	0.01±0.17	-0.70±0.17	2.81±0.11	0.33±1.58	-0.08±4.37	36.32±2.06
G3 - A4	0.25±0.12	-1.86±0.18	3.38±0.12	0.33±1.05	21.66±3.53	22.33±1.12
A4 - A5	-0.72±0.11	-0.68±0.13	3.38±0.06	-4.15±0.70	-24.04±1.65	36.28±1.01
A5 - C6	-0.46±0.09	-1.78±0.13	3.90±0.11	0.19±0.47	8.01±1.33	32.38±0.73
C6 - G7	-0.17±0.17	-1.15±0.26	3.33±0.10	1.21±0.84	28.12±2.22	27.42±2.18
G7 - C8	0.59±0.15	-0.84±0.18	3.57±0.17	-4.72±1.41	6.53±2.75	28.74±1.07
mean	0.01±0.03	-1.22±0.06	3.45±0.03	-0.12±0.25	7.41±0.64	30.21±0.51
B-DNA*	0.0	0.0	3.38	0.0	0.0	36.0

* canonical form

Table 6S. Differences between computed dumbbell parameters and canonical form B-DNA

Sequence base pair	shift Dx (Å)	slide Dy (Å)	rise Dz (Å)	tilt τ (°)	roll ρ (°)	twist Ω (°)
G9 - C10	0,44	0,88	-0,18	-5,03	-6,05	6,66
C10 - G11	-0,17	1,25	-0,03	2,90	-28,59	7,48
G11 - T12	-0,44	1,69	-0,47	0,09	-7,68	4,27
T12 - T1	-0,74	0,64	0,00	-4,20	23,54	-0,43
T1 - C2	0,26	1,91	-0,01	0,75	-22,49	13,23
C2 - G3	-0,01	0,70	0,57	-0,33	0,08	-0,32
G3 - A4	-0,25	1,86	0,00	-0,33	-21,66	13,67
A4 - A5	0,72	0,68	0,00	4,15	24,04	-0,28
A5 - C6	0,46	1,78	-0,52	-0,19	-8,01	3,62
C6 - G7	0,17	1,15	0,05	-1,21	-28,12	8,58
G7 - C8	-0,59	0,84	-0,19	4,72	-6,53	7,26

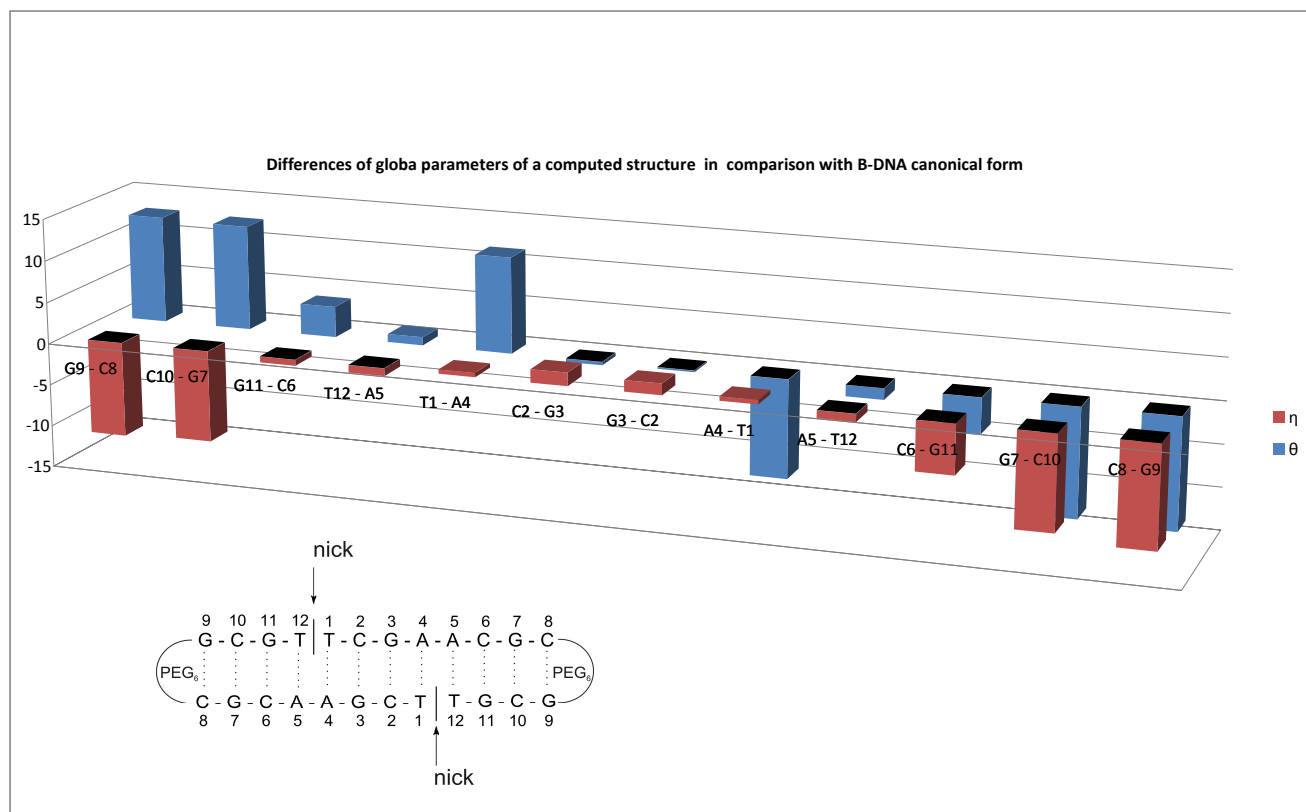


Fig. 5S Graphical presentation of differences of some global parameters in computed structure from simulating annealing in comparison with canonical B-DNA form.

Table 7S. Selected helical parameters in the Molecular Dynamics calculated dumbbell structure (no constraints)

base pair	x-displacement dx (Å)	y-displacement dy (Å)	inclination η (°)	tip θ (°)	buckle κ (°)	propeller twist Ω (°)	opening (σ) (°)
G9 - C8	-1,41±0,28	0,13±0,18	6,50±2,07	-0,63±2,34	-10,55±4,95	1,14±5,05	1,50±1,01
C10 - G7	-1,54±0,23	0,07±0,19	5,06±2,64	-2,24±2,93	-0,98±4,97	-9,04±4,35	-0,16±1,22
G11 - C6	-1,39±0,25	0,15±0,17	4,36±2,67	1,42±3,16	-4,46±3,76	-14,02±7,80	1,97±1,08
T12 - A5	-1,53±0,25	-0,12±0,22	3,91±3,10	0,66±2,76	-9,19±4,42	-21,22±3,26	1,96±2,04
T1 - A4	-1,41±0,26	-0,38±0,36	8,56±5,43	1,36±3,61	-7,78±6,01	-16,69±6,36	3,88±1,27
C2 - G3	-1,33±0,26	-0,17±0,24	12,42±7,21	-2,43±1,35	-7,76±3,02	-0,67±3,52	1,69±0,69
G3 - C2	-1,30±0,21	0,14±0,14	12,00±6,44	3,62±1,53	4,53±3,27	-2,22±4,37	1,09±1,40
A4 - T1	-1,43±0,23	0,39±0,27	8,46±5,16	0,36±2,42	7,18±4,56	-15,49±5,28	4,60±2,61
A5 - T12	-1,58±0,21	0,10±0,11	3,85±3,08	0,95±2,67	9,35±3,45	-17,80±5,13	1,71±0,71
C6 - G11	-1,56±0,18	-0,14±0,08	4,34±3,20	-0,62±2,62	4,06±4,14	-13,86±6,46	1,24±1,15
G7 - C10	-1,57±0,17	-0,08±0,19	5,20±2,51	2,24±1,50	0,97±6,55	-7,49±5,80	0,03±1,59
C8 - G9	-1,52±0,16	-0,12±0,16	6,29±1,88	1,06±1,97	11,34±6,11	-0,37±4,95	1,20±1,06
average	-1,46±0,20	0,00±0,10	6,75±3,13	0,48±1,03	-0,27±1,16	-9,81±0,95	1,73±0,39
B-DNA*	-0,71	0,00	-5,91	0,00	0,00	3,72	-4,11
A-DNA*	-5,43	0,00	19,09	0,00	0,00	13,70	-4,61

*canonical forms

Table 8S. Differences in values of selected helical parameters in the calculated dumbbell structure (no constraints) and canonical B-DNA; In brackets are shown discrepancies between dx differences in both dumbbell structures.

base pair	x- displacement dx (Å)	y- displacement dy (Å)	inclination η (°)	tip θ (°)	buckle κ (°)	propeller twist Ω (°)	opening σ (°)
G9 - C8	0,70 (0,01)	-0,13	-12,41	0,63	10,55	2,58	-5,61
C10 - G7	0,83 (-0,13)	-0,07	-10,97	2,24	0,98	12,76	-3,95
G11 - C6	-0,07 (-0,30)	-0,15	2,39	-0,94	4,19	4,21	-0,24
T12 - A5	0,82 (0,23)	0,12	-9,82	-0,66	9,19	24,94	-6,07
T1 - A4	0,70 (0,39)	0,38	-14,47	-1,36	7,78	20,41	-7,99
C2 - G3	0,62 (0,03)	0,17	-18,33	2,43	7,76	4,39	-5,80
G3 - C2	0,59 (0,00)	-0,14	-17,91	-3,62	-4,53	5,94	-5,20
A4 - T1	0,72 (0,41)	-0,39	-14,37	-0,36	-7,18	19,21	-8,71
A5 - T12	0,87 (0,27)	-0,10	-9,76	-0,95	-9,35	21,52	-5,82
C6 - G11	0,85 (-0,06)	0,14	-10,25	0,62	-4,06	17,58	-5,35
G7 - C10	0,86 (-0,11)	0,08	-11,11	-2,24	-0,97	11,21	-4,14
C8 - G9	0,81 (0,19)	0,12	-12,20	-1,06	-11,34	4,09	-5,31