Crystal Structure of the Self-Complementary 5′-Purine Start Decamer d(GCACGCGTGC) in the A-DNA Conformation. II

Changill Ban and Muttaiya Sundaralingam
Laboratory of Biological Macromolecular Structure, Departments of Chemistry and Biochemistry and The Ohio State Biochemistry Program, The Ohio State University, Columbus, Ohio 43210 USA

ABSTRACT The crystal structure of the alternating 5′-purine start decamer d(GCGCGCGCGC) was found to be in the left-handed Z-DNA conformation. Inasmuch as the A·T base pair is known to resist Z-DNA formation, we substituted A·T base pairs in the dyad-related positions of the decamer duplex. The alternating self-complementary decamer d(GCACGCGTGC) crystallizes in a different hexagonal space group, $P6_122$, with very different unit cell dimensions $a = b = 38.97$ and $c = 77.34$ Å compared with the all-G·C alternating decamer. The A·T-containing decamer has one strand in the asymmetric unit, and because it is isomorphous to some other A-DNA decamers it was considered also to be right-handed. The structure was refined, starting with the atomic coordinates of the A-DNA decamer d(GCGGGCCCGC), by use of 2491 unique reflections out to 1.9-Å resolution. The refinement converged to an R value of 18.6% for a total of 202 nucleotide atoms and 32 water molecules. This research further demonstrates that A·T base pairs not only resist the formation of Z-DNA but can also assist the formation of A-DNA by switching the helix handedness when the oligomer starts with a 5′-purine; also, the length of the inner Z-DNA stretch $(d(CG)_n)$ is reduced from an octamer to a tetramer. It may be noted that these oligonucleotide properties are in crystals and not necessarily in solutions.

INTRODUCTION

It is known that the conformation of alternating self-complementary DNA oligonucleotides $d(XY)_n$ ($n \leq 6$) are influenced by the terminal base type (Quadrifoglio et al., 1984; Jain et al., 1987); 5′-purine start sequences form the right-handed A-DNA, whereas 5′-pyrimidine start sequences form the left-handed Z-DNA. We recently showed an exception to this rule in which the alternating decamer d(GCGCGCGCGC) starting with a 5′-purine crystallized as Z-DNA (Ban et al., 1996). This 5′-purine start structure switches to A-DNA when A and T bases are substituted for the G and C bases as in d(GCACGCGTGC). In the 5′-pyrimidine start alternating Z-DNA structures, A·T base pairs are known to destablize the formation of the left-handed helix (Jovin et al., 1983; Wang et al., 1984) but do not switch handedness. The present structure is the first alternating decamer in the A-DNA conformation.

EXPERIMENTS

Synthesis, crystallization, and data collection

We synthesized the decamer d(GCACGCGTGC) by the phosphotriester method, using an in-house Applied Biosystem DNA synthesizer. We cleaved the decamer from the solid support by using 3 ml of 33% ammonia and deprotected it in the same solution at 55°C for 12 h. The oligonucleotides were then precipitated by ethanol in the presence of 2.5 M ammonium acetate at −25°C. The precipitates were dissolved, and the lyophilized decamer solutions were used for crystallization without further purification.

Bipyramidal crystals grew at room temperature for four days under the following conditions: 1 mM of DNA decamer, 40 mM of sodium cacodylate buffer (pH = 7.0), and 0.5 mM of cobalt hexamine equilibrated against a reservoir of 45% of 2-methyl-2,4-pentanediol. The largest crystal, 0.3 × 0.4 × 0.6 mm, was mounted for data collection. It belonged to the hexagonal space group $P6_122$, with unit cell constants $a = b = 38.97$ and $c = 77.34$ Å. The fact that it was isomorphous to previous A-DNA decamer structures (Ramakrishnan and Sundaralingam, 1993a; Frederick et al., 1989) indicated that it was a right-handed conformation. Three-dimensional 1.9 Å resolution x-ray intensity data were collected on the crystal at room temperature by a Siemens-Nicolet area detector on a four-circle goniometer, with a MaxScience rotating anode source operated at 50 kV and 90 mA and a graphite monochromator to select Cu-Kα radiation. The crystal-to-detector distance was 12 cm. A 180° ϕ-scan at $\chi = 0°$and a 75° ω-scan at $\phi = 90°$, $\chi = 45°$ were performed in 0.25° steps with an exposure time of 60 s/diffraction frame. In all, 1020 frames containing a total of 9346 reflections were collected and processed with XENGEN 2.0 software (Howard, 1990); 2634 reflections were unique with R_{symm} (F) of 3.1% and data completeness of 91%. The structure analysis was carried out with 2491 unique reflections with $F \geq \sigma(F)$ in the resolution range 8.0–1.9 Å.

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Address reprint requests to Dr. Muttaiya Sundaralingam, Departments of Chemistry and Biochemistry, 012 Rightmire Hall, The Ohio State University, 1060 Carmack Road, Columbus, OH 43210-1022. Tel.: 614-292-2999; Fax: 614-292-2524; E-mail: sunda@biot.mps.ohio-state.edu.

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Structure determination and refinement

The atomic coordinates of the isomorphous A-DNA decamer d(GCGGGCCCGC) (Ramakrishnan and Sundaralingam, 1993a) were used as the initial model. A rigid-body refinement using the XPLOR program (Brünger, 1990) with only the data in the resolution range 8.0–2.5 Å gave an R value of 38.1%. The R value dropped to 31.1% after one cycle of positional and isotropic thermal factor refinement. At this point, the correct bases were identified by omit 3Fo-2Fc and Fo-Fc maps, leaving one base at a time. Further refinement using the corrected bases reduced the R value to 25.4%. Additional refinement increasing the resolution from 8.0 to 2.0 Å and simulated annealing at 4000°C resulted in an R value of 20.7%. Using all 2491 observed reflections with $F \geq \sigma(F)$ from 8.0- to 1.9-Å resolution and including 32 water molecules from omit maps (at heights $\geq 3\sigma$ above the mean) dropped the R value to 18.6%. The final model contained 202 nucleotide atoms and 32 water molecules. The crystallographic parameters are listed in Table 1. The atomic coordinates of the decamer will be deposited with the Nucleic Acid Database (Berman et al., 1992).

RESULTS

Overall structure

The decamer duplex possesses the typical features of A-DNA (Fig. 1): a narrow major groove (average phosphorus–phosphorus distance, 8.6 Å) and a broad minor groove (average phosphorus–phosphorus distance, 15.8 Å). The sugar puckerings are C3′-*endo*, with pseudorotation phase angles in the range −1.8–26.7° (average, 15.5°). Superposition of the decamer on fiber A-DNA (Chandrasekaran et al., 1989) gave a rms deviation of 1.1 Å for atomic positions. Inspection of the local helix axes (Ban et al., 1994b) shows that the duplex is bent into the major groove at the fourth and sixth base pair steps by 10°.

The average helical base pair parameters (NEWHEL92, R. E. Dickerson, personal communication) are 10.8 residues/turn with a twist of 33.0°, 2.6-Å rise per residue, 19.8° inclination, −4.2 Å× displacement, −1.2° slide, and 10.7° propeller twist. These values are within the average range of values seen in other A-DNA structures. It is interesting that the helix twist and roll angles show an alternating pattern (Fig. 2). The twists for the py-pu steps are lower than for the pu-py steps; the C(4)pG(5) and C(6)pG(7) steps show a minimum twist of 30.7° and 30.6, respectively, and the G(5)pC(6) step shows a maximum of 35.9°. The roll angles also alternate, but the py-pu steps are higher than the pu-py steps. These trends are consistent with Calladine's (1982) rules. The roll angles are generally negative, with the exception of those for the C(4)pG(5) and C(6)pG(7) steps, which are positive (8.9°). This is consistent with the double bending of the duplex at the fourth and sixth base pair steps. The alternation of the helix twist and roll is dampened at the termini, probably because of end effects and packing. The average backbone torsion angles (Table 2) are also within the ranges observed for other A-DNA crystal structures. All residues of the decamer show the preferred g^-, g^+ conformation for α (P-O5′) and γ (C5′-C4′) torsion angles, except the residue G(7), which has the t, t conformation.

TABLE 1 Crystal and refinement parameters of the A-DNA decamer d(GCACGCGTGC)

Unit cell dimensions	
$a = b$	38.97 (Å)
c	77.34
Space group	$P6_122$
	Hexagonal
Molecule/asymmetric unit cell	Single strand
Resolution range (Å)	8.0–1.9
Number of reflections ($F \geq \sigma(F)$)	
used in refinement	2491
Final R value (%)	18.6
RMS deviation from ideal geometry	
Parameter file used	param_ndb.dna*
Bond lengths (Å)	0.013
Bond angles (°)	3.5
Dihedral angles (°)	32.9
"Improper" angles (°)	3.2
Final model	
Nucleic acid atoms	202
Water molecules	41
Average thermal parameter (Å^2)	
Nucleotide	21
Water molecules	75

*XPLOR parameter file from the Nucleic Acid Database.

Hydration

Of 41 water molecules, 30 are directly hydrogen bonded to the oligonucleotide (Table 3) and the remaining 11 are hydrogen bonded to some of the first coordination sphere waters. Most of the minor groove is blocked by the symmetry-related neighboring molecules, and only four water molecules are hydrogen bonded to it. However, the major groove is highly hydrated by 10 water molecules. All four distal cytosine N4-H atoms not in Watson–Crick base pairing are hydrogen bonded to water; by comparison, the thymine O4 atom is not involved in a similar hydrogen bonding. This may be attributed to the steric interaction from the C5-methyl group. In fact, probably for the same reason 5-methyl cytosine is not hydrated at the distal N4-H (Ramakrishnan and Sundaralingam, 1995). In the pu-py steps the O6 and N6 atoms of the purine nucleotides are bridged by water molecules in the major groove (Fig. 3), a feature not seen in other A-DNA structures. Such water bridges are not found in the py-pu steps. The intrastrand base stacking of a pu-py step favors the water bridges between the strands. However, the significant interstrand base stacking overlap does not favor the water bridges in the py-pu step. The former pu-py step water bridges are also found in the major groove water string of the Z-DNA

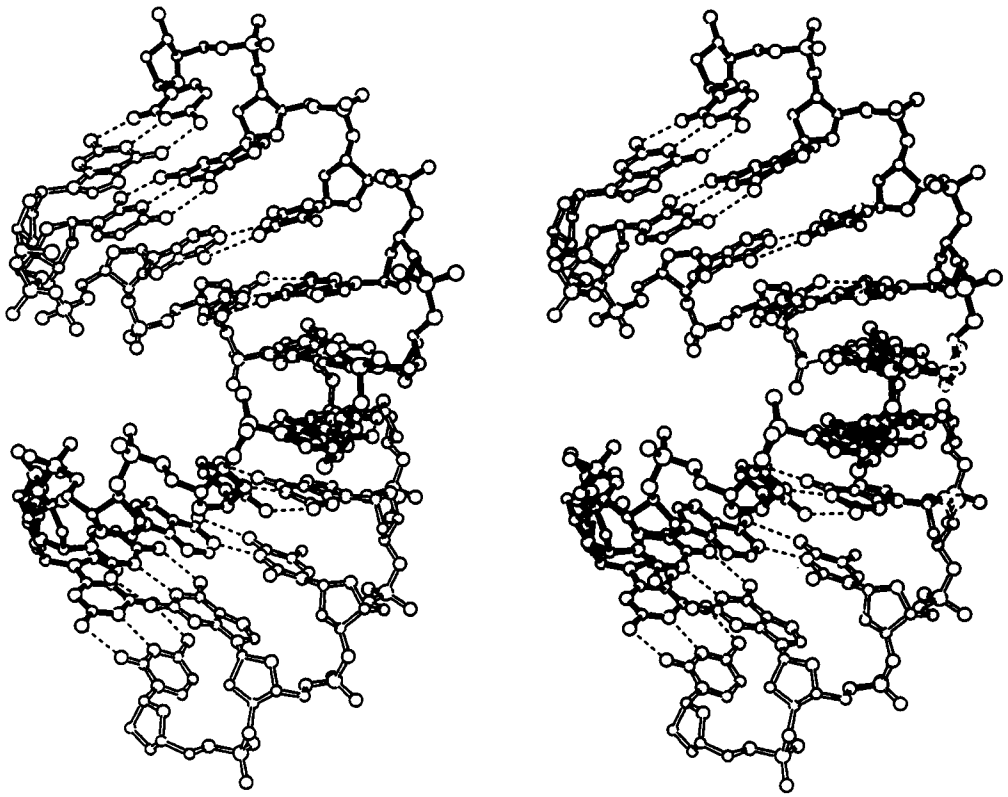


FIGURE 1 Stereo view of the A-DNA d(GCACGCGTGC) decamer duplex viewed normal to the dyad in the plane of the page. The first strand of the duplex is shown as dark bonds, and the dashed lines indicate the Watson–Crick hydrogen bonds of the complementary base pairs.

decamer d(GCGCGCGCGC) (Ban et al., 1996). Thus, the cross-strand water bridges are not specific for the helical handedness.

Packing interaction

All known A-DNA structures show a typical packing pattern in which the terminal base pair of a neighboring molecule abuts the minor groove of the parent molecule (Shakked et al., 1983; Thota et al., 1993). In the hexagonal A-DNA decamer structures d(GCGGGCCCGC) (Ramakrishnan and Sundaralingam, 1993a) and d(ACCGGCCGGT) (Frederick et al., 1989) the N3 and O4′ atoms of the 5′-terminal residues form hydrogen bonds with N2 of G(4) and G(5), respectively, of a symmetry-related duplex. In the present hexagonal decamer, only the sugar-base intermolecular interaction is found; O4′ atom hydrogen bonds (3.1 Å) to the N2 of a symmetry-related G(7) (Fig. 4). This may have caused the *t, t* backbone conformation at the G(7) residue in the present structure (Table 2) and the C(8)/G(8) residues in the other two hexagonal decamers. Although in this structure the fourth C(4)·G(7) base pair is switched to G(4)·C(7), it still is isomorphous to the other hexagonal structures, probably because the intermolecular hydrogen bonding between the first and fourth base pairs is similar (Fig. 4). Thus, this intermolecular hydrogen bonding might be crucial in the hexagonal packing of A-DNA decam-

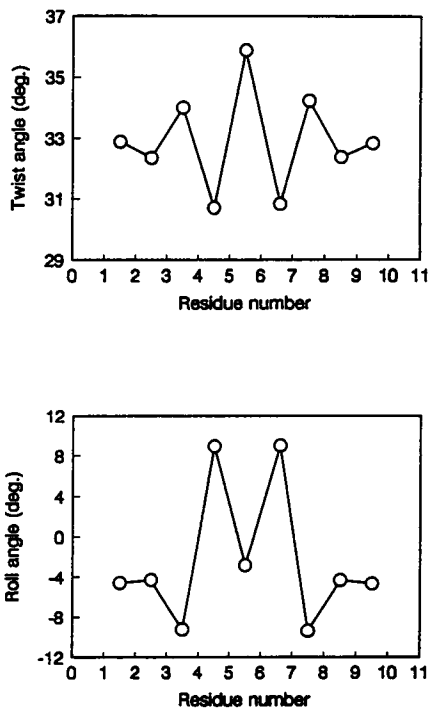


FIGURE 2 Alternation in the helical twist and roll angles of A-DNA d(GCACGCGTGC). The twist angles of the py-pu steps are below the average value, and those of the pu-py steps are above the average value; the roll angles show the reverse trend. Notice that the alternation is high in the center of the molecule and dampened at the termini.

TABLE 2 Backbone torsion angles,* sugar-base glycosyl torsion angles (χ) and pseudorotation phase angles (P) of the A-DNA decamer d(GCACGCGTGC)

Sequence	α	β	γ	δ	ϵ	ζ	χ	P
G(1)	–	–	61°	78°	213°	286°	194°	14°
C(2)	296°	159°	57	82	200	287	204	13
A(3)	294	165	57	82	215	281	207	15
C(4)	287	169	64	77	195	284	204	22
G(5)	286	184	53	88	214	281	197	9
C(6)	281	185	61	80	209	293	199	15
G(7)	**170**	175	**169**	88	211	298	198	−2
T(8)	291	163	58	86	217	277	202	27
G(9)	288	162	63	85	212	282	208	25
C(10)	292	168	54	79	–	–	216	18
Average	268#	170	56#	83	210	285	203	15
RMS	32	9	5	4	7	6	6	8

*The backbone torsion angles, as defined by IUPAC-IUB (1983), are O3′-*P*-α-O5′-β-C5′-γ-C4′-δ-C3′-ϵ-O3′-ζ-P-O5′.
#The α and γ values of G(7) were excluded in the calculation of the average values.

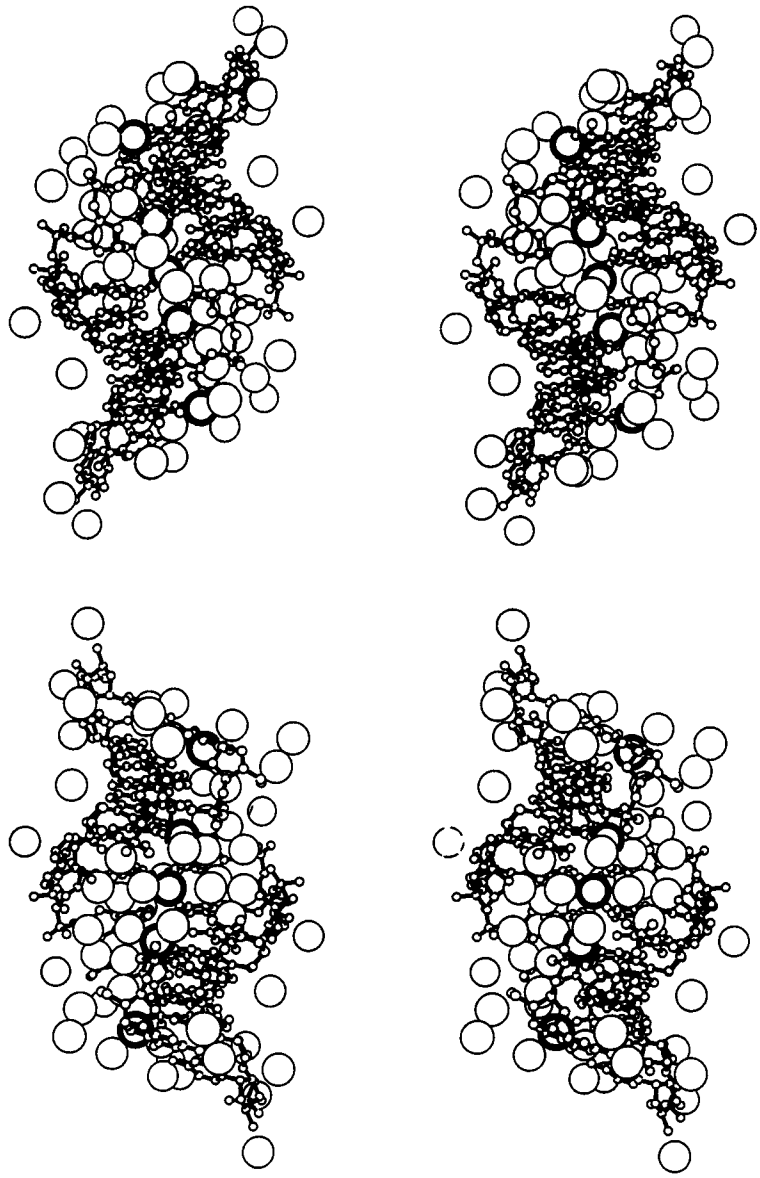
TABLE 3 Hydration of A-DNA d(GCACGCGTGC)

	Major groove sites			Minor groove sites			Backbone sites		
Residue	Atom	Water	Distance (Å)	Atom	Water	Distance (Å)	Atom	Water	Distance (Å)
G(1)	O6	W25	3.4	N2	W12	2.8	O5′	W23	2.6
		W27	3.3		W30	3.4			
C(2)	N4	W29	3.3	O2	W22	2.8	O4′	W39	3.0
A(3)	N6	W36	3.0	N3	W31	3.1	O5′	W44	3.1
							O1P	W20	3.1
								W35	3.3
C(4)	N4	W13	3.1				O3′	W46	3.3
		W36	2.9				O5′	W44	3.3
							O1P	W24	2.7
G(5)	O6	W13	3.3	N2	W12	3.4	O4′	W19	3.1
	N7	W14	2.6				O1P	W37	2.9
C(6)	N4	W45	2.8	O2	W12	2.9	O1P	W16	3.0
								W37	3.1
							O2P	W47	3.0
G(7)	O6	W36	2.6				O4′	W39	3.0
	N7	W11	2.9				O1P	W47	3.2
							O2P	W43	3.0
T(8)							O3′	W18	2.8
							O2P	W41	3.4
G(9)	O6	W27	3.1				O1P	W38	2.8
	N7	W15	2.5					W41	3.2
							O2P	W18	2.7
C(10)	N4	W51	2.8	O2	W30	2.4	O3′	W34	2.6
							O1P	W34	2.6
							O2P	W26	3.3

ers. It may be noted that the A·T base pairs are not involved in the intermolecular interactions.

DISCUSSION

Crystal structures of alternating oligonucleotides starting with a 5′-pyrimidine form Z-DNA even in the presence of A·T base pairs (Wang et al., 1979, 1984; Drew et al., 1980; Fujii et al., 1985; Coll et al., 1988). Results of energy calculations confirm this (Jovin et al., 1983; Dang et al., 1990). We recently showed that the 5′-purine start oligomer $d(GC)_5$ also formed a Z-DNA in crystals (Ban et al., 1996), whereas all other known 5′-purine start oligomers crystallized as A-DNA (Jain et al., 1987; Bingman et al., 1992). We have also observed that shorter self-complementary alternating oligomers, d(GC)n ($n < 5$), with a 5′-purine also form A-DNA (Mooers et al., 1995). Apparently the stretch of the inner $d(CG)_3$ sequence is not of optimal length to form Z-DNA, and the 5′-purine start overrides it. The present decamer d(GCACGCGTGC) also forms A-DNA, maybe because the central d(CG) stretch is a tetramer. Therefore, besides A·T base pairs resisting Z-DNA forma-



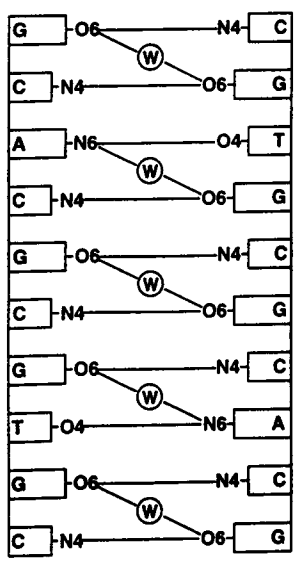


FIGURE 3 Hydration of the decamer d(GCACGCGTGC). All 30 water molecules found in the first shell are shown with a van der Waals radius of 1.4 Å for the water oxygen atoms. (*Top*) Stereo view into the minor groove, showing that the hydration is less here because of the intermolecular interaction. (*Middle*) Stereo view into the major groove, rotated 180° from the top view, showing the increased hydration of the major groove. (*Bottom*) Schematic illustration of the cross-strand water bridges between the O6/N7 atoms of the pu-py step in the A-DNA decamer d(GCACGCGTGC). The water molecules associated with these water bridges are shown as darkly outlined circles in the top and middle figures.

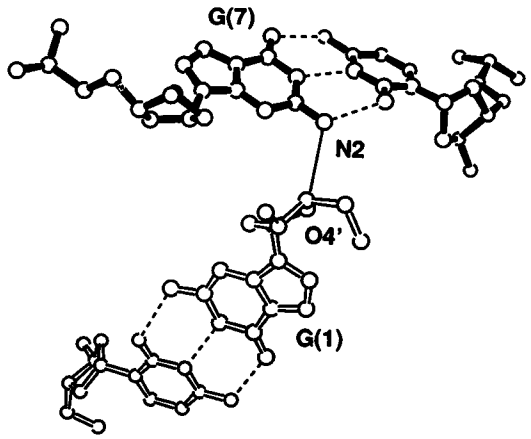


FIGURE 4 The minor groove–backbone interaction in the crystal packing of the A-DNA decamer d(GCACGCGTGC). There is only a single hydrogen bonding between G(7) N2 of the parent molecule and O4′ of a symmetry-related terminal residue (G(1)). This packing pattern is clearly different from the orthorhombic A-DNA decamer packing, where the minor groove base atoms of the symmetry-related molecules are abutting one another and forming the base multiples (Ramakrishnan and Sundaralingam, 1993b; Ban et al., 1994a,b). We therefore refer to these intermolecular interactions as minor groove–backbone packing in hexagonal crystals and as minor groove–base floor packing in orthorhombic crystals.

tion, short central stretches of d(CG) sequences yield only A-DNA and not Z-DNA. This research demonstrates the dynamic nature of DNA in biology and its remarkable dependence on the DNA sequence.

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