Wrinkled DNA

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ABSTRACT

The B form of poly d(GC):poly d(GC) in orthorhombic microcrystallites in oriented fibers has a secondary structure in which a dinucleotide is the repeated motif rather than a mononucleotide as in standard, smooth B DNA. One set of nucleotides (probably GpC) has the same conformations as the smooth form but the alternate (CpG) nucleotides have a different conformation at C3'-O3'. This leads to a distinctive change in the orientation of the phosphate groups. Similar perturbations can be detected in other poly d(PuPy):poly d(PuPy) DNAs such as poly d(IC):poly d(IC) and poly d(AT):poly d(AT) in their D forms which have tetragonal crystal environments. This suggests that such perturbations are intrinsic to all stretches of duplex DNA where purines and pyrimidines alternate and may play a role in the detection and exploitation of such sequences by regulatory proteins.

INTRODUCTION

Polynucleotide duplexes in fibers are quite polymorphic (1): for example, poly d(AT):poly d(AT) has been observed in at least 3 forms (A,B,D); so also has poly d(GC):poly d(GC) with its A, B and Z forms (2). Only the Z allomorph of poly d(GC):poly d(GC) is known to reflect its primary structure in its secondary structure by having dramatically different conformations in alternate nucleotides (2,3). On the other hand, the fiber patterns of the A and B allomorphs all resemble quite closely those obtained with calf thymus DNA which have been interpreted adequately in terms of secondary structures with a repeated mononucleotide motif.

There is direct X-ray evidence that the B forms of poly d(GC):poly d(GC) and poly d(AC):poly d(GT) have structural units with projected lengths which are 0.68 nm rather than 0.34 nm. This comes from the observation of meridional reflexions on the 5th layer lines of X-ray patterns (such as Fig. 1a) from oriented but poorly crystalline fibers. In more crystalline, orthorhombic B forms of poly d(GC):poly d(GC), the diagnostic (005) reflexion in the X-ray

Figure 1: X-ray fiber diffraction patterns of: (a) poorly crystalline B poly d(GC):poly d(GC) showing a 0.68 nm meridional reflexion on the 5th layer line; (b) polycrystalline poly d(GC):poly d(GC); (c) pseudo-hexagonal D poly d(AAT):poly d(ATT) showing a 0.30 nm meridional reflexion on the 8th layer line; (d) tetragonal D poly d(IC):poly d(IC).

fiber diffraction pattern (Fig. 1b) is absent, but this could be because of packing symmetry. It is interesting, however, that the unit cell of this form has different dimensions ($\underline{a}$ = 3.79 nm, $\underline{b}$ = 3.61 nm, $\underline{c}$ = 3.36 nm) from that of B form calf thymus DNA (where $\underline{a}$ = 3.08 nm, $\underline{b}$ = 2.24 nm, $\underline{c}$ = 3.37 nm) and contains 4 molecules rather than 2. This implies that the B allomorph of poly d(GC):poly d(GC) is distinguishable from that of general or typical DNA.

To determine if alternate nucleotides in the B form of poly d(GC):poly d(GC) are distinctly different requires a detailed X-ray

analysis to decide whether molecules that are 5-fold helices of dinucleotides are significantly superior to molecules that are 10-fold helices of mononucleotides. We have undertaken such an analysis and find that this is indeed the case. In poly d(GC):poly d(GC) the GpC nucleotides conformationally are essentially identical to those in standard B DNA but the CpG nucleotides are different in a subtle way which leads to a regular perturbation on the surface of the poly d(GC):poly d(GC) helices. They therefore can be considered to have a wrinkled version of the regular B DNA structure.

If such wrinkles are intrinsic to stretches of DNA with purines and pyrimidines alternating in the base sequence, we would expect them to persist in DNAs with different compositions and in other forms such as D. In this connection it is interesting that the 8-fold D helices of poly d(AAT):poly d(ATT) pack in a pseudo-hexagonal crystal form (4) (with unit cell dimensions $\underline{a} = \underline{b} = 1.98$ nm, $\underline{c} = 2.41$ nm, $\gamma = 120^\circ$) (Fig. 1c) whereas the D helices of poly d(AT):poly d(AT) (5,6) and poly d(IC):poly d(IC) (7) pack differently in apparently isomorphous tetragonal crystal forms ($\underline{a} = \underline{b} = 1.69$ nm, $\underline{c} = 2.42$ nm, $\gamma = 90^\circ$) (Fig. 1d). Therefore, we have asked whether the D forms of poly d(IC):poly d(IC) and poly d(AT):poly d(AT) are also wrinkled and better described as 4-fold helices of dinucleotides rather than 8-fold helices of mononucleotides. This question has been answered affirmatively also, suggesting that wrinkles are a persistent feature of DNA duplexes with alternating purine/pyrimidine sequences.

There have been earlier speculations (e.g. 8,9) that the PypPu and PupPy nucleotides in such DNAs might be rather different. Klug *et al* (8), inspired by the results of the structure analysis of the oligonucleotide d(AT)₂ (10), suggested an "alternating B" structure in which the Py and Pu nucleosides would have differently puckered furanose rings. This would, however, produce morphological changes much greater than we observe. We have found that the differences between alternate nucleotides are rather more subtle. This is what has prompted us to describe our structures as "wrinkled" versions of certain well-known, "smooth" allomorphs. They are, however, in the general meaning of the term, "alternating B" structures.

Also it has been claimed from studies of B-like oligonucleotide duplexes in single crystals (e.g. 11) that there are some local differences in nucleotide conformations which are sequence-specific. Since short, pseudo-helical, oligonucleotide duplexes are obviously susceptible to "end effects" (12) and crystal packing forces, it is of no little interest to determine

whether or to what extent the perturbations observed in their structures persist in polymers.

EXPERIMENTAL TECHNIQUE

The best molecular and crystal models of each form considered were obtained by the method of Linked Atom Least Squares (13,14). In this LALS method, the main molecular variables were the 6 conformation angles associated with the backbone of each nucleotide, and the glycosylic angle describing the orientation of each base with respect to its attached deoxyribose. Additional molecular variables were the conformation angles of the furanose rings and their endocyclic bond angles which were allowed to vary in a stiffly elastic fashion. Packing variables were the positional and orientation parameters appropriate for each crystal space group. X-ray variables were a scale factor (K) for the observed structure amplitudes and an isotropic attenuation factor (B) for the calculated amplitudes.

The variables were adjusted so as to minimize (in least-squares fashion) the expression

$$\Omega = \sum \omega_m \Delta F_m^2 + \sum k_i \Delta d_i^2 + \sum \lambda_h G_h$$

$$= \underline{X} + \underline{C} + \underline{L}, \text{ say.}$$

X involves the differences between the observed X-ray amplitudes and those calculated from the model structure. C involves both the close non-bonded interatomic distances, d_j , which are driven towards some acceptably large value ${}_0d_j$, and the conformation and bond angles which are elastically restrained. L involves constraints G_h which become zero when residue connectivity and furanose ring closure have been achieved.

Competing molecular and crystal structures can be assessed for significant differences by using Ω , X or C as statistics in Hamilton's test (15,16).

Details of this and other strategies which are now quite commonplace in the application of LALS to fiber diffraction analyses have been discussed many times before (e.g. 16,17) and will not be described further.

POLY D(GC):POLY D(GC)

These duplexes pack in orthorhombic ($P2_12_12_1$) array (Fig. 2a) in such a way that the four molecules in each unit cell are at the corners of a rhombus of side 1.90 nm and acute angle rather accurately 72° . Although this arrangement resembles that of calf thymus B DNA both in symmetry and in intermolecular spacings, it turns out not to be able to accommodate smooth (10_1) B DNA helices (Figs. 3c,d) without there being many overshoot

interatomic contacts between neighbouring molecules. Consequently, when Ω is minimized for such a model, reduction of steric compression is achieved only at the expense of a much increased value (0.46) for the X-ray discrepancy index $R = \sum |\Delta F_m| / \sum o F_m$.

By contrast a 5_1 model (Fig. 3a,b) (Table 2) with a dinucleotide motif has no unacceptable steric compression and $R = 0.34$. This good fit between observed and model amplitudes (Table 1) can be shown by Hamilton's test (15)

Figure 3: Wrinkled B DNA: (a) diad axis projection; (b) helix axis projection. Smooth B DNA: (c) diad axis projection; (d) helix axis projection. Wrinkled D DNA: (e) diad axis projection; (f) helix axis projection. Smooth D DNA: (g) diad axis projection; (h) helix axis projection.

TABLE 1: Structure amplitudes: observed (A) and calculated (B) for wrinkled B poly d(GC):poly d(GC). M denotes a meridional reflexion and N a systematic absence. For reflexions too weak to be measured, the estimated threshold value and the calculated value are given in parentheses in (A) and (B) respectively. Such reflexions were included in the refinement only when the calculated amplitudes had values greater than the threshold.

H K L=0	1	2	3	4	5	6	7	8	9	10
0 0	M	M	M	M	M	M	M	M	M	M
1 0										
0 1	M	415	531	(197)	(228)	(254)	(280)	(306)	(331)	(357)
1 1	M	806	1478	(236)	(268)	287	(331)	(362)	(391)	(421)
2 0	1736	(217)	(248)	(286)	(323)	(360)				
0 2	2097	(227)	(258)	(296)	(332)	(368)	(404)	836	(476)	1131
2 1	1917									
1 2	M	(248)	1764	1789	487	382	799	(484)	877	(836)
2 2	(295)	(306)								
3 0	M	(318)	1376	1327	476	854	368	(636)		
0 3										
3 1	M	(333)								
1 3	M	(348)	1243	(406)	463	1247	(625)	(867)	1891	1213
3 2	M									
2 3	417	(360)	846	1234	925	1796	(867)	(810)	2871	2167
4 0	(408)	(417)	(439)	(471)	(505)	(550)	(582)	(636)	(661)	
4 1	1852									
0 4	1016	(438)	(480)	871	810	(588)	(612)	(666)	(703)	2404
1 4	3									
3 3	M	(453)	(474)	(505)	(543)	1790	(628)	(671)	1796	(786)
4 2	889	(470)	(491)	347	(568)	(586)	828	(666)	(736)	
2 4	626	(486)	(508)	(536)	(572)	(613)	(666)	(701)	(748)	
5 0	M	(521)	(540)	(569)	(605)	(646)	(690)	(736)		
4 3	61	937	(532)	(551)						
0 5	M	(548)	(567)	743	964	2439	1915	1779	2229	
1 5										
5 2	M	(566)	448	(612)	(647)	1886	(732)	(778)	(828)	
2 5	(581)	(587)	(605)	434		(706)	1777	(786)	(848)	
4 4	(600)	(606)	(624)	(651)		(728)	(770)	(817)	(867)	
5 3	M	437	462	(662)		(727)	(781)	(828)	980	
6 0	817									
3 5	1	(632)	(637)	(655)	370		(786)	1621	(848)	(887)
0 6										
6 1	335	(670)	(686)	1322		(786)	(829)	903	(929)	
5 4										
4 5	1364	862	845	848		1236	788	(801)	731	
6 2	(707)	(713)	(729)	(764)		(826)	(870)	(918)	(970)	
3 6										
7 1	M	719	698	(787)		2081	(802)	(900)	842	
5 5										
7 2										
6 4										
0 7	2877	(778)	742	1181		(868)	1027	798	(1034)	
1 7										
4 6	(788)		(806)	(830)		(900)	(844)	(883)		
2 7										
7 3	1529		699	736		(823)	(867)			
3 7										
6 5	912		(866)	907		(888)	(1002)			
5 6										
6 6	(864)		(874)	814		(886)	(1010)			
7 4	M		(888)	(908)		(977)	(1021)			
8 2	(878)		(897)	(920)						
4 7										
0 8										
1 8	713		(920)	2344		(1011)	1484			
8 3										
2 8	(822)		(841)							
8 8	(834)		(853)	1981		(1044)	1195			
7 7	M		(874)	(897)		707	(1108)			
8 0										
8 4	(871)		(890)			(1080)				
9 2										
4 8	(1002)		926			901				
7 8										
0 9										
8 7										
1 9										
8 9	911		963			(1141)				
9 9	(1090)		(1078)							

H K L=0	1	2	3	4	5	6	7	8	9	10
0 0	(8248)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(899)
1 0										
0 1	(0)	413	533	(7)	(282)	(180)	(617)	(203)	(171)	(80)
1 1	(0)	329	677	(6)	(484)	280	(48)	(906)	(841)	(14)
2 0	1287	(119)	(464)	(425)	(48)	(48)				
0 2	1961	(184)	(690)	(879)	(86)	(7)	(996)	348	(198)	990
2 1	1853									
1 2	(0)	(283)	2124	1141	271	286	807	(886)	196	(844)
2 2	(588)	(118)								
3 0	(0)	(69)	651	1080	399	272	363	(170)		
0 3										
3 1	(0)	(422)								
1 3	(0)	(395)	1619	(48)	873	862	(387)	(960)	1836	1482
3 2	(0)									
2 3	959	(450)	299	895	818	290	(891)	(482)	1304	1482
4 0	(390)	(184)	(88)	(237)	(74)	(262)	(504)	(184)	(825)	
4 1	1363									
0 4	1074	(420)	(170)	496	290	(841)	(417)	(906)	(1809)	1816
1 4	3									
3 3	(0)	(862)	(124)	(30)	(688)	2398	(29)	(1082)	2598	(73)
4 2	801	(86)	(96)	848	(187)	(438)	987	(348)	(908)	
2 4	790	(45)	(84)	(791)	(136)	(814)	(818)	(400)	(828)	
5 0	(0)	(83)	(128)	(0)	(208)	(669)	(36)	(28)		
4 3										
5 1	788	(128)	(190)							
3 4										
0 5	(0)	(361)	(418)	433	202	1087	1190	1082	2088	
1 5										
5 2	(0)	(368)	418	(26)	(317)	972	(36)	(1233)	(1289)	
2 5	(188)	(228)	(102)	863		(315)	1876	(119)	(746)	
4 4	(21)	(86)	(83)	(487)		(283)	(128)	(331)	(880)	
5 3	(0)	428	325	(18)		(274)	(63)	(147)	818	
6 0	821									
3 5										
6 1	(204)	(663)	(631)	289		(1087)	1367	(861)	(908)	
0 6										
6 2	39	(803)	(878)	1687		(1381)	(886)	727	(770)	
5 4										
4 5	1837	798	693	874		1822	952	(486)	943	
2 6						(140)	(911)	(123)	(218)	
6 3	(77)	(89)	(187)	(407)						
3 6										
7 0										
7 1	(0)	743	296	(10)		1287	(61)	(812)	752	
5 5										
7 2										
6 4										
0 7	2702	(781)	748	685		(1348)	1248	840	(871)	
1 7										
4 6	(114)		(296)	(304)		(844)	(863)	(236)		
2 7										
7 3	1589		191	641		(718)	(481)			
3 7										
6 5	1080		(888)	984		(878)	(1088)			
5 6										
6 6	(1362)		(884)	913		(388)	(1000)			
7 4	(0)		(888)	(24)		(808)	(28)			
8 2	(388)		(378)	(1143)						
4 7										
0 8										
1 8	448		(1078)	1787		(886)	422			
8 3										
2 8	(971)		(867)							
8 8										
7 7	(224)		(886)	1961		(886)	867			
8 9	(0)		(841)	(20)		989	(22)			
9 0										
8 4	(20)		(1486)			(772)				
8 2										
4 8	(273)		917			410				
7 8										
0 9										
8 7										
1 9										
8 9	1138		1072			(888)				
9 9	(280)		(883)							

Table 2: Cartesian and cylindrical polar coordinates of atoms in a dinucleotide in poly d(GC):poly d(GC). The coordinates of the diadically related dinucleotide in the antiparallel chain are given by r , $-\phi$, $-Z$. Successive dinucleotides can be generated by adding multiples of 72° to ϕ and the same multiple of 0.672 nm to Z .

GROUP	ATOM	X(NM)	Y(NM)	Z(NM)	R(NM)	PHI(DEG)
PHOSPHATE	P	-0.3569	0.9569	0.3017	0.9227	112.76
	O1	-0.3975	0.9811	0.3394	1.0595	112.95
	O2	-0.4549	0.7855	0.2114	0.9086	120.94
	O3	-0.3179	0.7499	0.4195	0.8145	112.98
	O5	-0.2168	0.9570	0.2262	0.9937	104.94
SUGAR	C1	-0.0446	0.5502	0.1402	0.5920	94.63
	C2	-0.0907	0.6575	0.0482	0.6837	57.86
	C3	0.0126	0.7807	0.0874	0.7608	89.05
	C4	-0.0045	0.7511	0.2380	0.7512	90.34
	C5	-0.0949	0.6253	0.3620	0.8686	86.33
	O4	-0.0453	0.6141	0.2989	0.6157	94.22
	H1	0.0604	0.5270	0.1259	0.5305	83.47
	H12	-0.0714	0.6288	-0.0614	0.6338	86.47
	H22	-0.1909	0.6940	0.0650	0.7157	105.38
	H3	-0.0085	0.6625	0.0548	0.6626	90.57
	H4	0.0534	0.7542	0.2953	0.7690	82.94
	H15	-0.0438	0.9516	0.3036	0.9526	92.63
	H25	-0.1184	0.8261	0.4043	0.8345	98.15
CYTOSINE	N1	-0.1250	0.4249	0.1340	0.4429	106.39
	C2	-0.0590	0.3040	0.1501	0.3095	100.80
	O2	0.0643	0.3055	0.1687	0.3152	78.11
	N3	-0.1294	0.1886	0.1448	0.2257	124.46
	C4	-0.2617	0.1912	0.1245	0.3241	143.85
	H4	-0.3269	0.0760	0.1201	0.3356	166.91
	C5	-0.3331	0.3125	0.1075	0.4268	135.95
	C6	-0.2938	0.4296	0.1132	0.5021	121.17
	H14	-0.2772	-0.0180	0.1319	0.2774	-177.94
	H54	-0.4298	0.0751	0.1051	0.4323	170.00
	H5	-0.4319	0.3175	0.0924	0.5360	143.68
	H6	-0.3054	0.5179	0.1020	0.6012	120.53
PHOSPHATE	P	0.2569	0.9411	0.0596	0.9901	72.89
	O1	0.2530	0.8704	0.2006	0.9194	71.39
	O2	0.2162	0.9568	-0.0223	0.9809	77.27
	O3	0.1467	0.7272	0.0541	0.7418	78.59
	O5	0.3621	0.7677	-0.0114	0.8575	63.54
SUGAR	C1	0.3426	0.4442	-0.1956	0.5610	52.36
	C2	0.3719	0.5829	-0.2520	0.6914	57.46
	C3	0.5100	0.6078	-0.1912	0.7334	50.00
	C4	0.4870	0.5530	-0.0503	0.7369	48.63
	C5	0.4424	0.6541	0.0534	0.7857	55.53
	O4	0.3252	0.4407	-0.0639	0.5919	48.11
	H1	0.4021	0.3700	-0.2488	0.5465	42.62
	H12	0.3903	0.5790	-0.3605	0.6919	56.35
	H22	0.3009	0.6552	-0.2118	0.7210	65.33
	H3	0.5381	0.7129	-0.1852	0.8932	52.96
	H4	0.5774	0.5029	-0.0195	0.7657	41.05
	H15	0.5328	0.6923	0.1116	0.8627	52.43
	H25	0.3700	0.6079	0.1205	0.7116	58.67
GUANINE	N1	0.0116	0.0578	-0.1686	0.0590	78.64
	C2	0.1493	0.0574	-0.1574	0.1599	21.02
	N2	0.2050	-0.0616	-0.1362	0.2150	-16.56
	N3	0.2227	0.1673	-0.1857	0.2733	36.79
	C4	0.1487	0.2786	-0.1882	0.3158	61.90
	C5	0.0122	0.2986	-0.2007	0.2989	87.59
	C6	-0.0674	0.1715	-0.1908	0.1843	111.44
	O6	-0.1894	0.1604	-0.1951	0.2482	139.74
	H7	-0.0225	0.4211	-0.2022	0.4219	93.46
	C8	0.0863	0.4963	-0.2229	0.4942	79.71
	H9	0.1986	0.4063	-0.2020	0.4522	63.94
	N9	-0.0355	-0.0300	-0.1802	0.0465	-139.82
	H12	0.3053	-0.0688	-0.1272	0.3129	-12.70
	H22	0.1491	-0.1436	-0.1294	0.2070	-43.53
	H6	0.0946	0.5852	-0.2353	0.5928	80.82

to be superior to the fit provided by the best 10_1 model at the 99.5% level of significance.

A rather different 5_1 model which mimicked the "alternating B" model of Klug *et al* (8) in assigning C2'-endo furanose rings to all C and C3'-endo rings to all G was also refined. We found that in our system such a model is sterically compressed and has $R = 0.55$, the highest X-ray discrepancy index of any model we have refined. Not surprisingly Hamilton's test shows that this model can be rejected with greater than 99.5% confidence.

In passing it should be emphasized that we have been discussing right-handed helices. Left-handed (10_9) ten-fold helices are just as unacceptable ($R = 0.49$) as right-handed (10_1) ten-fold helices. Left-handed (5_4) five-fold helices of dinucleotides are rather better ($R = 0.41$) but can be rejected with 99.5% confidence (15) in favor of the right-handed (5_1) helices for which $R = 0.34$.

POLY D(IC):POLY D(IC)

We found that these duplexes pack in a very symmetrical tetragonal ($P4_122$) array ($a = b = 1.7$ nm, $c = 2.45$ nm) with the molecules oriented so that molecular diads are parallel with the unit cell edges (Fig. 2b). Specifically it is the diads in the middle of the paired CpI segments which do

Table 3: Comparison of distances (in nm) between some O and N atoms in neighbouring, diadically-related bases in wrinkled and smooth forms of B DNA.

Distance	GpC		CpG	
	Wrinkled	Smooth	Wrinkled	Smooth
Major groove				
N4...N4	0.75	0.66	0.28	0.35
O6...O6	0.28	0.29	0.51	0.47
N7...N7	0.69	0.70	0.95	0.90
Minor groove				
(G)N3...(G)N3	0.63	0.67	0.47	0.45
N2...N2	0.42	0.43	0.30	0.31
O2...O2	0.70	0.73	0.70	0.73

this. Consequently the interleaving diads of the paired IpC segments are parallel to the base diagonals of the tetragonal unit cell. Smooth (8₁) D DNA helices (Fig. 3g,h) are sterically compressed in such a packing arrangement and do not fit the X-ray amplitudes as well (R = 0.32) as wrinkled (4₁) helices (Fig. 3e,f) (Table 5), analogous to the B form of

Table 4: Structure amplitudes: Observed (A) and calculated (B) for wrinkled D poly d(IC):poly d(IC). Notations and explanations are the same as in Table 1.

A										
H	K	L=0	1	2	3	4	5	6	7	8
0	0	M	M	M	M	M	M	M	M	M
1	0	328	162	141	339	199	122	190		
1	1	(122)	81	180	230	316	149	219	1011	462 321
2	0	(138)	305	235	(214)	489	(296)	201		
2	1	180	341	229	443	547	813	672	408	(338) (288)
2	2	217	(208)	186	(284)	382	(306)	(367)	(367)	
3	0	198	233	(283)	(296)	(321)	(345)	(373)		
3	1	985	246	(297)	704	448	284	(367)		
3	2	328	(328)	301	401	332	284	(429)		
4	0	437	(368)	(377)	(364)	(414)	(439)	(466)		
4	1	(377)	299	177	(406)			340		
3	3	(388)	(393)	243	298			(463)		
4	2	661		(428)	(442)					
4	3	869		342	341					
5	1	434		(541)	(510)					
5	2	364								
4	4	(548)								
5	3	402								
6	0									
6	1	423								
6	2	(631)								

B										
H	K	L=0	1	2	3	4	5	6	7	8
0	0	(1003)	(0)	(0)	(0)	(80)	(0)	(0)	(0)	(0)
1	0	407	108	119	280	164	187	178		
1	1	(38)	219	245	238	311	39	130	885	288 421
2	0	(134)	305	242	(79)	398	(18)	498		
2	1	142	256	103	369	825	326	795	616	(190) (181)
2	2	186	(196)	250	(14)	422	(11)	(77)	(64)	
3	0	242	174	(8)	(18)	(478)	(96)	(98)		
3	1	847	100	(201)	607	444	306	(91)		
3	2	182	(180)	120	284	272	183	(218)		
4	0	284	(17)	(86)	(8)	(180)	(70)	(21)		
4	1	(286)	172	182	(361)			294		
3	3	(136)	(40)	94	244			(26)		
4	2	702		(57)	(42)					
4	3									
5	0	297		381	408					
5	1	820		(209)	(204)					
5	2	239								
4	4	(170)								
5	3	327								
6	0									
6	1	314								
6	2	(94)								

Table 5: Cartesian and cylindrical polar coordinates of atoms in a dinucleotide in poly d(IC):poly d(IC). The coordinates of the diadically related dinucleotide in the antiparallel chain are given by r , $-\phi$, $-Z$. Successive dinucleotides can be generated by adding multiples of 90° to ϕ and the same multiple of 0.6125 nm to Z .

GROUP	ATOM	X(NM)	Y(NM)	Z(NM)	R(NM)	PHI(DEG)
PHOSPHATE	P	-0.5773	0.6141	0.5235	0.8488	133.23
	O1	-0.6290	0.7258	0.6077	0.9804	136.31
	O2	-0.6683	0.5632	0.4205	0.8740	138.88
	O3	-0.5325	0.4836	0.6287	0.7851	137.17
	O5	-0.4376	0.6560	0.4557	0.7886	123.71
SUGAR	C1	-0.1794	0.4931	0.3244	0.4873	111.80
	C2	-0.2138	0.5804	0.2478	0.6185	110.22
	C3	-0.1594	0.6826	0.3476	0.7010	103.15
	C4	-0.1886	0.6243	0.4824	0.6822	107.65
	C5	-0.3247	0.6811	0.5496	0.7545	115.49
	O4	-0.2112	0.4812	0.4958	0.5233	113.70
	H1	-0.0731	0.4316	0.3133	0.4377	88.82
	H12	-0.1857	0.5880	0.1932	0.6068	104.87
	H22	-0.3219	0.5819	0.2392	0.6738	118.33
	H3	-0.2104	0.7778	0.3327	0.8057	105.14
	H4	-0.1135	0.6354	0.3530	0.6489	100.31
	H15	-0.3131	0.7825	0.3539	0.8484	111.66
	H25	-0.3425	0.6357	0.6431	0.7221	118.31
CYTOSINE	N1	-0.2588	0.3344	0.2619	0.4228	127.74
	C2	-0.1894	0.2180	0.2498	0.2898	130.38
	O2	-0.0650	0.2180	0.2578	0.2278	166.84
	N3	-0.2829	0.1087	0.2108	0.2817	157.31
	C4	-0.3636	0.1127	0.2035	0.4084	184.01
	N4	-0.4577	0.0636	0.1850	0.4577	179.56
	C5	-0.4658	0.2315	0.2351	0.5211	183.62
	C6	-0.3845	0.3396	0.2748	0.5305	138.28
	H14	-0.4064	-0.0752	0.1423	0.4140	-168.27
	H24	-0.3575	0.0036	0.1887	0.3575	179.51
THYMINE	N1	-0.3865	0.2346	0.2306	0.6133	157.51
	N6	-0.4418	0.4245	0.2384	0.6127	136.14
	C2	0.0544	0.6226	0.2569	0.8643	86.22
	O1	0.1122	0.8884	0.4172	0.8935	82.78
	O2	-0.0388	0.5058	0.2178	0.5066	82.48
SUGAR	O3	-0.0176	0.6887	0.3381	0.6850	91.47
	O5	0.1721	0.7682	0.2026	0.7882	77.38
	C1	0.2181	0.4819	0.0073	0.5223	85.25
	C2	0.2744	0.6108	-0.0524	0.6835	83.01
	C3	0.3834	0.6276	0.0420	0.7407	57.52
THYMINE	C4	0.3348	0.5814	0.1705	0.6786	60.48
	C5	0.2876	0.7080	0.2531	0.7542	67.89
	O4	0.2260	0.4935	0.1479	0.5482	85.86
	N1	0.2869	0.3578	-0.0241	0.4870	84.77
	H12	0.3105	0.5945	-0.1540	0.6707	82.42
	H22	0.2048	0.6535	-0.0382	0.7231	73.35
	H3	0.4248	0.7380	0.0484	0.8463	58.87
	H4	0.4074	0.5326	0.2947	0.6795	52.59
	H15	0.3676	0.7818	0.2714	0.8637	64.81
	H25	0.2614	0.6709	0.3622	0.7200	68.71
	N1	-0.1167	0.1328	-0.1338	0.1768	131.31
	C2	0.0160	0.1204	-0.1072	0.1215	82.43
	N3	0.0843	0.2184	-0.0711	0.2570	86.65
	C4	0.0281	0.3356	-0.0628	0.3367	85.21
	C5	-0.0819	0.3932	-0.0870	0.3739	106.13
	C6	-0.1857	0.2511	-0.1857	0.3147	127.07
	O6	-0.3055	0.0532	-0.1834	0.3889	140.72
	H7	-0.1335	0.4824	-0.0673	0.5102	105.18
	C8	-0.0167	0.5458	-0.0317	0.5460	81.75
	N9	0.0770	0.4853	-0.0688	0.4818	80.41
	H1	-0.1860	0.0500	-0.1606	0.1734	163.23
	H2	0.0527	0.0235	-0.1180	0.0638	87.48
	H6	-0.0035	0.4425	-0.0100	0.6425	86.31

poly d(GC):poly d(GC), which do not give rise to unacceptable non-bonded interatomic distances and lead to $R = 0.26$ (Table 4).

The discriminating power of these diffraction data is evident in the fact that the best left-handed (4_3) model has $R = 0.34$ but can be rejected with 99.5% confidence. Similarly, models with conformations at C4'-C5' restrained to be trans rather than gauche plus were found to be significantly inferior whether right-handed or left-handed: $R(4_1) = 0.34$, with rejection at the 99.5% confidence level; $R(4_3) = 0.35$, with rejection again at the 99.5% confidence level.

It is possible even to reject (with 99.5% confidence) an alternative $P4_{122}$ crystal model containing molecules with the optimum conformation but oriented 45° differently with respect to the c -axis. Not only is $R = 0.34$ significantly greater, there is also an obvious steric difficulty in short 0...0 distances (0.23 nm) between phosphate groups of neighbouring molecules. POLY D(AT):POLY D(AT)

The D-forms of poly d(IC):poly d(IC) and poly d(AT):poly d(AT) are apparently isomorphous. For both crystal structures, c (respectively 2.45 nm and 2.42 nm) and $a = b$ (respectively 1.71 nm and 1.69 nm) are quite similar. Nevertheless there are substantial differences in the intensities of some of their X-ray reflexions (Tables 4a and 6a), most notably in (211), (313),

Table 6: Structure amplitudes: observed (A) and calculated (B) for wrinkled D poly d(AT):poly d(AT). Notations and explanations are the same as in Table 1.

A												B											
H	K	L=0	1	2	3	4	5	6	7	8	9	H	K	L=0	1	2	3	4	5	6	7	8	9
0	0											0	0	(1088)	(0)	(0)	(0)	(38)	(0)	(0)	(0)	(48)	(0)
1	0	360	360	(98)	284	188	188	298	1123	(201)		1	0	436	108	(180)	188	183	46	128	808	(207)	
1	1	(108)	(119)	284	183	397	192	284	861			1	1	(82)	(188)	238	286	428	143	337	707		703
2	0	181	360	298		518	238	518				2	0	71	338	298		428	78	887			(83)
2	1	180	218	208	368	853	841	842	844		(342)	2	1	38	237	128	321	638	888	878	818		
2	2	230	234	248	281	(288)	(287)	330	(337)			2	2	338	248	118	88	(482)	(88)	182	(428)		
3	0	518	288	(248)	483	823			(324)			3	0	128	247	(80)	172	511		(98)			
3	1	381	283	(288)	517			700				3	1	884	188	(218)	808			373			
3	2	444	348	(284)	327	823	(348)					3	2	228	212	(308)	418	884	(188)				
4	0	418	338	402	(342)	(380)						4	0	227	148	271	(10)	(182)					
4	1	347	(330)	(338)								4	1	424	(87)	(208)							
3	3	838										3	3	380									
4	2	548										4	2	828									
5	0											5	0										
5	1	847										5	1	881									
5	2	(448)										5	2	(348)									

(114), (304) and (206). Perhaps, therefore, we should not have been surprised to find that a poly d(AT):poly d(AT) isomorphous with the best P4₁22 poly d(IC):poly d(IC) model had serious steric flaws involving contacts between thymine methyl groups and charged phosphate oxygen atoms of neighbouring molecules. It turned out that the best way to relieve this compression was to change the molecular orientations by 45° to provide the alternate P4₁22 arrangement (Fig. 2c). Not only does this abolish the unacceptable (0.23 nm) O...Me contacts, but it results also in a better fit with the intensities of the "anomalous" reflexions. Consequently the X-ray index of discrepancy falls from R = 0.34 to R = 0.30 (Table 6). The refined molecular model of poly d(AT):poly d(AT) is essentially isomorphous (Tables 5 and 7) with that of poly d(IC):poly d(IC) but, as we discussed, the crystal structures are only pseudo-isomorphous although they have the same symmetry and very similar unit cell dimensions. What we have just visualized is a potent effect of the methyl groups of thymine which in this instance have contrived to produce a 90° change in the mutual orientation of neighbouring DNA double helices.

TECHNICAL COMMENT

It is of no little importance that these results are obtained with X-ray diffraction data derived from polymers. Heretofore sequence-specific structural details usually have been extrapolated from studies of oligomer single crystals with the implicit assumption that direct conclusions would not be available from fiber diffraction analyses of long polymers. It should be

Table 7: Cartesian and cylindrical polar coordinates of atoms in a dinucleotide in poly d(AT):poly d(AT). The coordinates of the diadically related dinucleotide in the antiparallel chain are given by r , $-\phi$, $-Z$. Successive dinucleotides can be generated by adding multiples of 90° to ϕ and the same multiple of 0.604 nm to Z .

GROUP	ATOM	X(NM)	Y(NM)	Z(NM)	R(NM)	PHI(DEG)
PHOSPHATE	P	-0.5532	0.6520	0.5357	0.8551	130.31
	O1	-0.6494	0.6047	0.4348	0.8874	137.04
	O2	-0.5940	0.7715	0.6127	0.9737	127.59
	O3	-0.5164	0.5335	0.6362	0.7425	134.07
	O5	-0.4110	0.6789	0.4662	0.7936	121.19
SUGAR	C1	-0.1706	0.4487	0.3209	0.4800	110.82
	C2	-0.2051	0.5741	0.2421	0.5056	105.66
	C3	-0.1448	0.6761	0.3388	0.5865	102.10
	C4	-0.1766	0.6205	0.4779	0.6451	105.89
	C5	-0.2930	0.6895	0.5491	0.7495	113.15
	O4	-0.2057	0.4882	0.4946	0.5924	113.19
	H1	-0.0638	0.4285	0.3121	0.4332	98.47
	H12	-0.3129	0.5951	0.2364	0.6679	117.87
	H22	-0.1512	0.5777	0.1474	0.3871	104.57
	H3	-0.1951	0.7706	0.3249	0.7351	104.21
	H4	-0.0876	0.6266	0.5406	0.6327	97.96
	H15	-0.2532	0.7855	0.5701	0.8343	106.82
	H5	-0.3117	0.5335	0.5431	0.7061	116.20
THYMINE	N1	-0.2452	0.3277	0.2764	0.4093	126.81
	C2	-0.1711	0.2157	0.2473	0.2793	128.42
	O2	-0.0496	0.2124	0.2506	0.2181	103.14
	H3	-0.2435	0.1055	0.2654	0.3655	156.59
	C4	-0.3807	0.0963	0.1325	0.3932	165.52
	O4	-0.4345	-0.0061	0.1550	0.4345	-179.19
	C5	-0.4498	0.2208	0.2253	0.5011	153.86
	H5	-0.5253	0.2215	0.2128	0.5389	159.71
	C6	-0.3818	0.3294	0.2653	0.5042	139.21
	H3	-0.1917	0.6229	0.1847	0.1930	173.20
	H6	-0.4317	0.4131	0.2875	0.3975	136.26

GROUP	ATOM	X(NM)	Y(NM)	Z(NM)	R(NM)	PHI(DEG)
PHOSPHATE	P	0.0574	0.8147	0.2524	0.8167	85.97
	O1	-0.0360	0.8726	0.1545	0.8734	92.36
	O2	0.1007	0.9049	0.3613	0.9104	83.85
	O3	-0.0041	0.6811	0.3146	0.6811	90.35
	O5	0.1864	0.7368	0.1762	0.7794	76.17
SUGAR	C1	0.2615	0.4722	0.0108	0.5398	61.02
	C2	0.3243	0.5964	-0.0503	0.5788	61.46
	C3	0.4315	0.6134	0.0555	0.7500	54.87
	C4	0.3603	0.5953	0.1895	0.6307	56.56
	C5	0.2988	0.7113	0.2543	0.7715	67.21
	O4	0.2655	0.4880	0.1518	0.5338	61.35
	H1	0.3189	0.3843	-0.0185	0.4994	50.31
	H12	0.2267	0.6819	-0.0480	0.7286	69.37
	H22	0.3715	0.5754	-0.1463	0.6850	87.15
	H3	0.4678	0.7161	0.0527	0.8553	56.85
	H4	0.4230	0.5417	0.2594	0.6910	51.62
	H15	0.3733	0.7906	0.2604	0.8743	64.72
	H5	0.2553	0.6860	0.3549	0.7355	68.65
ADENINE	N1	-0.0921	0.1285	-0.1337	0.1581	125.62
	C2	0.0369	0.1171	-0.1023	0.1227	72.92
	H3	0.1221	0.2104	-0.0658	0.2432	58.88
	C4	0.0682	0.3312	-0.0624	0.3370	79.36
	C5	-0.0683	0.3295	-0.0918	0.3649	100.78
	C6	-0.1493	0.2502	-0.1295	0.2914	120.83
	H6	-0.2791	0.2624	-0.1610	0.3631	136.76
	H7	-0.0940	0.4941	0.0773	0.5030	100.77
	C8	0.0200	0.5438	-0.0402	0.5442	87.90
	H8	0.1195	0.4511	-0.0290	0.4867	75.16
	H2	0.0742	0.0944	0.1071	0.0781	18.62
	H16	-0.3320	0.1817	-0.1872	0.3794	151.31
	H26	-0.3230	0.2522	-0.1584	0.4779	132.92
	H8	0.0336	0.6411	-0.0216	0.6420	87.00

understood however, that the polydinucleotide structures we considered for poly d(GC):poly d(GC) have only 18 net degrees of freedom with the constrained linked-atom description. There are, however, 184 X-ray reflexions (96 above threshold and 88 below) in the field. These contribute to the statistic $\chi^{1/2}$ which was used to make the significance statements on the basis of Hamilton's test which also takes into account the number of degrees of freedom in a model or between models. It should be mentioned also that the contact term C in the expression minimized in the least-squares refinement has 203 unique terms -- additional data if you like.

For poly d(IC):poly d(IC) the number of unique X-ray data is $57 + 36 = 93$ and of contact data 135. For poly d(AT):poly d(AT) the numbers are $52 + 28 = 80$ and 139 respectively. In both structures the number of degrees of freedom in the constrained linked-atom description of the structure is 16. Here again the significance statements were made on the basis of the values of $\chi^{1/2}$ for different models even although more robust assertions could have been made on the basis of $\Omega^{1/2}$ in every case.

MORPHOLOGY

When the diad axis projections of the wrinkled and smooth forms of both B and D DNA are compared (Figs. 3a,c and 3e,g), one notices how grossly

Figure 4: Detailed views of dinucleoside phosphate duplex fragments in smooth and wrinkled B DNA. CpG in (a) smooth B DNA has a quite different phosphate orientation from that in (b) wrinkled B DNA. By contrast, GpC in (c) smooth and (d) wrinkled B DNA have quite similar shapes. The small filled circles show the positions of the helix axes.

similar the wrinkled and regular versions are. When, however, the helix axis projections are compared (Figs. 3b,d and 3f,h), one cannot but be struck by the differences evident on the molecular peripheries. These make it immediately obvious why the crystal packings of the smooth and wrinkled allomorphs are quite distinct.

When "close-ups" of the different structures are compared (Figs. 4 and 5) another interesting feature emerges. The general shapes of the PypPy phosphodiester (which of course are the same as those of PypPu in the smooth allomorphs) are conserved in the wrinkled allomorphs but the PypPu shapes are distinctly different. This is most noticeable in the orientations of the phosphate groups. In smooth B DNA, the normals to the O1-P-O2 planes make an angle of 49° with the helix axis: in wrinkled B DNA, the angle for the GpC phosphates is similar (39°), but for the CpG phosphates it is 75° . The

Figure 5: Detailed views of dinucleoside phosphate duplex fragments in smooth and wrinkled D DNA. CpI in (a) smooth D DNA has a somewhat different phosphate orientation from that in (b) wrinkled D DNA. By contrast, IpC in (c) smooth and (d) wrinkled D DNA have quite similar shapes. The small filled circles show the positions of the helix axes.

corresponding angle in smooth D DNA, is 47° like those for IpC (49°) and ApT (47°) in wrinkled D DNA, but different from the angles for CpI (57°) and TpA (56°).

CONFORMATIONS

It is interesting that the wrinkles in poly d(PuPy):poly d(PuPy) DNAs are characterized by a change in the shape of one nucleotide (PypPu) rather than by equal and opposite changes in both nucleotides from the "average" shape observed in the smooth allomorphs. This is underscored by the values of particular conformation angles (Table 8). In wrinkled D DNA, the root-mean-squared difference in the main conformation angles between PypPu and PupPy is $\langle(\Delta\theta)^2\rangle^{1/2} = 15.7^\circ$, whereas between PupPy in smooth and wrinkled forms, the value is only 10.3° . For wrinkled B DNA, the differences (36.0° and 9.5°)

TABLE 8: The values of the molecular, crystal packing and X-ray parameters for wrinkled B poly d(GC):poly d(GC), wrinkled D poly d(IC):poly d(IC) and wrinkled D poly d(AT):poly d(AT). Estimated standard deviations are given in parentheses.

Parameters	GpC	CpG	IpC	CpI	ApT	TpA
Backbone						
Conformations ($^{\circ}$)						
$\theta(C4'-C3'-O3'-P)$	-156(4)	-85(4)	-152(5)	-128(5)	-157(5)	-133(7)
$\theta(C3'-O3'-P-O5')$	-158(5)	-169(4)	-154(5)	-141(5)	-145(6)	-153(5)
$\theta(O3'-P-O5'-C5')$	-30(5)	-66(5)	-51(6)	-76(5)	-45(6)	-72(6)
$\theta(P-O5'-C5'-C4')$	126(4)	145(3)	140(4)	142(4)	142(5)	143(4)
$\theta(O5'-C5'-C4'-C3')$	47(4)	22(3)	61(5)	68(4)	55(5)	69(5)
$\theta(C5'-C4'-C3'-O3')$	143(2)	148(2)	146(2)	148(2)	142(3)	154(3)
Glycosylic						
Conformations ($^{\circ}$)						
$\theta(C2'-C1'-N1-C2)$	143(3)	-	129(3)	-	128(4)	-
$\theta(C2'-C1'-N9-C4)$	-	164(3)	-	138(3)	-	138(4)
Furanose						
Conformations ($^{\circ}$)						
$\theta(C4'-O4'-C1'-C2')$	-35(2)	-16(3)	-28(2)	-28(2)	-36(3)	-17(4)
$\theta(O4'-C1'-C2'-C3')$	53(2)	37(2)	41(2)	43(2)	45(3)	37(3)
$\theta(C1'-C2'-C3'-C4')$	-48(2)	-42(2)	-40(2)	-42(2)	-38(3)	-43(3)
$\theta(C2'-C3'-C4'-O4')$	30(2)	34(2)	25(2)	28(2)	19(3)	35(3)
$\theta(C3'-C4'-O4'-C1')$	3(3)	-12(3)	2(3)	0(3)	10(4)	-11(4)
Furanose Endocyclic						
Bond Angles ($^{\circ}$)						
$\tau(C4'-O4'-C1')$	105(1)	108(1)	109(1)	109(1)	109(2)	110(2)
$\tau(O4'-C1'-C2')$	105(1)	107(1)	105(1)	105(1)	104(2)	108(2)
$\tau(C1'-C2'-C3')$	95(1)	100(1)	99(1)	98(1)	97(2)	96(2)
$\tau(C2'-C3'-C4')$	100(1)	100(2)	104(1)	103(1)	105(2)	105(2)
$\tau(C3'-C4'-O4')$	106(2)	107(2)	105(2)	104(2)	104(3)	101(3)
Dihedral Angle between						
Planes in a Base-pair ($^{\circ}$)	-14(1)		-17(2)		-21(2)	
Crystal Packing						
$\mu(^{\circ})$	0.0		0.0		45.0	
u/a	0.1727(20)		0.0		0.0	
v/b	0.25		0.0		0.0	
w/c	0.1678(14)		0.0		0.0	
X-ray Scale (K) and	2.36(18)		2.38(24)		1.60(14)	
Attenuation (B) (nm^{-2})	-0.12(6)		-0.07(6)		-0.18 (5)	

are much more pronounced partly because the conformations of alternate nucleotides are not merely different quantitatively but at one bond (C3'-O3') are different qualitatively: in PupPy, (C3'-O3') is trans but in PypPu it is gauche minus. Interestingly in a B-DNA-like oligomer duplex d(CGCGAATTCGCG)₂ (11), the nucleotides with this conformation are the second GpC and its quasi-diadically-related mate on the complementary strand. In the oligomer the gauche minus C3'-O3' conformations are coupled with trans O3'-P conformations. Elsewhere in the oligomer these conformations usually are gauche minus. In poly d(GC):poly d(GC) all O3'-P conformations are trans.

Our assertion that PypPu (rather than PupPy) in wrinkled allomorphs has a shape different from that in the smooth versions is strongly supported by the X-ray analysis of poly d(IC):poly d(IC) where an inversion of the base sequence results in a (better than 99.5%) significant increase in the discrepancy index (R becomes 0.30). In addition, the preferred sequence assigns a larger value ($\Delta = 10^\circ$) to the glycosylic torsion angle of the purine nucleoside as anticipated from mononucleotide structures (18). Our X-ray data for poly d(GC):poly d(GC) and for poly d(AT):poly d(AT) do not discriminate significantly between the sequence isomers but there are marked differences (respectively 21° and 10°) between the glycosylic conformations of alternate residues and we have used these to assign sequences. In these cases also it turns out that PypPu contains the "anomalous" conformations.

BASE STACKING

Naturally one compares the base stacks (Fig. 4 and 5) to try to discover the source of the wrinkles. In smooth and wrinkled B DNA, GpC stacks are scarcely distinguishable as one would expect from the similarity of conformations in the connecting bonds. Somewhat surprisingly, the CpG stacks are also quite similar despite the changes in the torsion angles of the connecting links. The same is true of the stacks in smooth and wrinkled D DNA. What are different are the precise positions of the bases with respect to the helix axes. These are such that the minor groove is more than 0.1 nm deeper in the wrinkled D form.

It would appear, therefore, that the base-base interactions are fairly conservative. What would be different would be the interactions with cations or water molecules within the grooves of the duplexes.

IMPLICATIONS

We have shown explicitly only that the B form of poly d(GC):poly d(GC) and the D forms of poly d(IC):poly d(IC) and poly d(AT):poly d(AT) are wrinkled

similarly. For the purpose of the following discussions we will assume that the B forms of poly d(AT):poly d(AT), poly d(AC):poly d(GT) and other poly d(PuPy):poly d(PuPy) DNA sequences are wrinkled in the same way as poly d(GC):poly d(GC).

It follows that we would expect hydrolytic cleavage of poly d(AT):poly d(AT) by, say, DNAase I to occur preferentially at the P-03' bonds in the ApT sequences, which we have now shown to have the same conformations as "smooth" DNA and not in the TpA sequences which have another shape. This is precisely what has been observed (19,8).

We would expect also that the two nuclear magnetic resonances of phosphorus atoms in alternating purine/pyrimidine DNAs would be indeed more different (20) than those calculated from smooth B DNA.

Also, it has been known for some time (21) that poly d(AT):poly d(AT), in presumably a B-like form, binds the lac repressor of E. coli nearly 10^3 times more strongly than a DNA of general sequence. It is not unlikely that common sequence elements between poly d(AT):poly d(AT) and the lac operator are responsible for this because, in part, a third of the bases are the same on each side of the pseudo diad axis of the operator region but, in addition, in three-quarters of the sequence there are at least purines or pyrimidines even when there are not As or Ts. It is therefore likely that the operator also has wrinkled secondary structural features in common with poly d(AT):poly d(AT). This could have a profound effect on the repressor/operator binding energy not only because of the altered phosphate orientations but also because the functional groups on the bases would have altered spatial relationships with one another throughout the major and minor grooves. For example, in wrinkled B poly d(AT):poly d(AT), distances between neighbouring, diadically-related O and N atoms would be changed by up to 0.09 nm (Table 3) from those which would prevail in a smooth B DNA.

All in all it is evident that the subtle but profound differences between smooth and wrinkled DNAs have important implications for recognition, binding and reactivity.

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