A left-handed supramolecular assembly around a right-handed screw axis in the crystal structure of homo-DNA†

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Duplexes of homo-DNA, a hexose analogue of DNA and autonomous pairing system, exhibit unusual conformational features, and in the crystal structure create a unique double-helical supramolecular motif whose main characteristic is a handedness that is opposite to that of the underlying crystallographic symmetry.

In addition to the well known double-helical conformations DNA can adopt three-,[1] four-[2] and even five-stranded species.[3] Base pairing is not limited to the Watson–Crick type but comprises a host of other hydrogen bonding motifs between bases, among them purine:purine and pyrimidine:pyrimidine pairs (reviewed recently in ref. 4).[5] This conformational versatility and the predictability of base-pairing interactions and the lengths and orientations of duplexes formed render DNA an excellent building material for nano-scale supramolecular assemblies.[6] These include constructions of complex topologies (reviewed in ref. 6),[7] several nanomechanical devices,[8] and continuous three-dimensional lattices with greatly enhanced solvent channels suitable for accommodating protein guests.[9]

We recently determined the crystal structure of the antiparallel duplex formed by a DNA homolog ((4′ → 6′)-linked oligo(2′,3′-dideoxy-β-D-glucopyranosyl)nucleotide or homo-DNA; Fig. 1).[10,11] Final coordinates and structure factors are available from the Protein Data Bank, http://www.rcsb.org (PDB ID 2H9S). The octamer dd(CGAATTCG) adopts an irregular, weakly twisted conformation with an average distance of 3.8 Å (rise) between adjacent base-pair planes (Fig. 1B). The hexose-phosphate backbones are strongly inclined relative to the base-pair axes. This leads to stacking between bases from opposite strands (inter-strand stacking) and a virtual absence of intra-strand stacking (the type

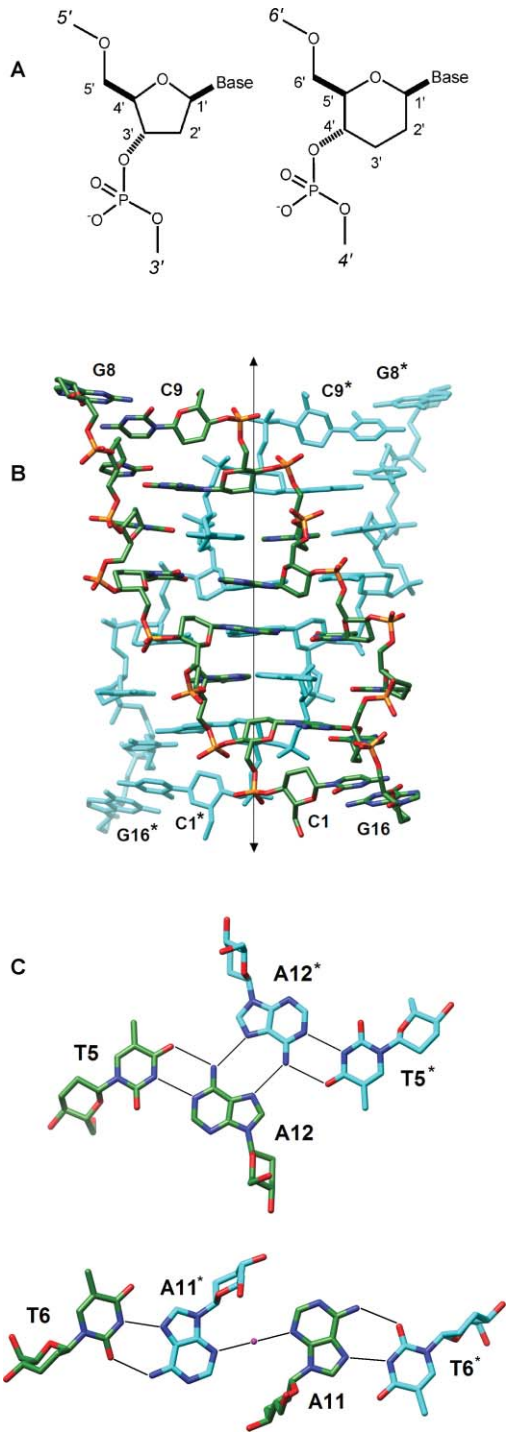

Fig. 1 (A) Configuration and linkage of DNA (left) and homo-DNA (right). (B) Conformation of the homo-DNA duplex $[dd(CGAATTCG)]_2$ in the crystal (colored by atom) and generation of a dimer around a crystallographic dyad, involving base-swapping and (C) formation of base tetrads and reverse-Hoogsteen pairs (viewed along the crystallographic dyad). Residues of octamer strands are numbered 1 to 8 and 9 to 16, asterisks in panels B and C designate symmetry-related nucleotides, hydrogen bonds are drawn with thin solid lines, and a water molecule in panel C is drawn as a pink sphere.

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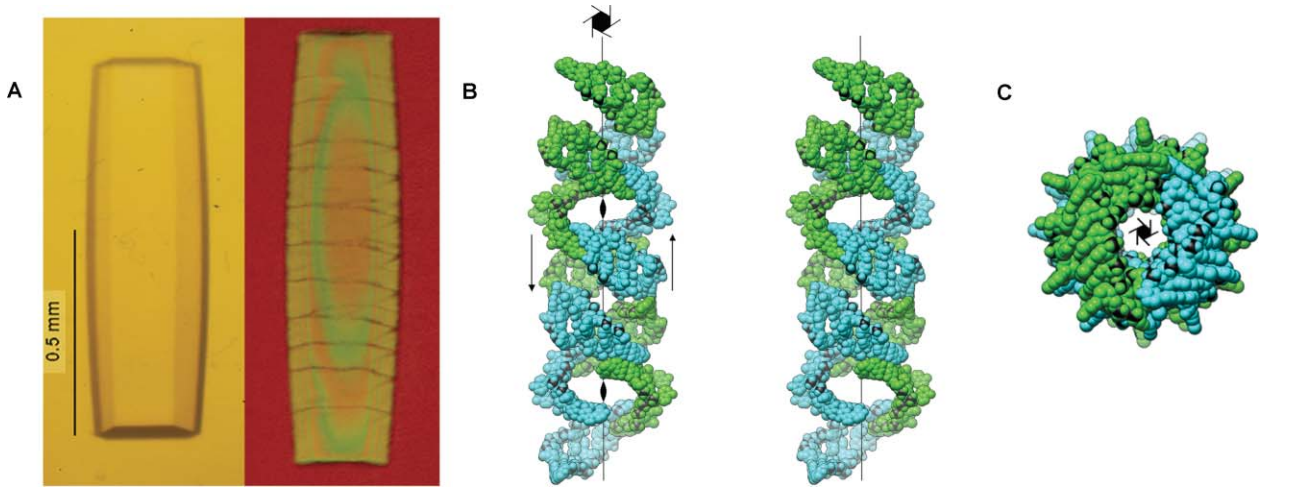

Fig. 2 (A) Native homo-DNA crystal (left) and a crystal displaying a left-handed helical fracture (right). (B) Stereo diagram of the *left-handed* double-stranded superhelix formed around *right-handed* sixfold screw axes (6_1). (C) The superhelix viewed along the screw axis. Green and cyan duplexes are symmetry-related through local dyads (two of which are shown in the figure). Green duplexes among themselves and cyan duplexes among themselves are related *via* the sixfold screw axis.

predominantly found in B-form DNA). In the crystal lattice duplexes dimerize *via* base swapping and formation of reverse-Hoogsteen A:A and A:T base pairs (Fig. 1B, C).

Crystals of homo-DNA that are dehydrated slightly or were soaked in solutions of heavy atom salts in attempts to identify derivatives for crystallographic phasing often exhibited a continuous left-handed hairline fracture (Fig. 2A). The crystals belong to the enantiomorphic space group pair $P6_122/P6_522$ whereby the former contains right-handed (6_1) and the latter contains left-handed (6_5) sixfold screw axes. In view of the left-handed fracture exhibited by crystals, $P6_522$ was considered to be the more likely space group (*nb*, it is impossible to differentiate between enantiomorphic space group pairs without phasing information). Surprisingly, after multi-wavelength anomalous dispersion (MAD) phasing using a single phosphoroselenoate derivative the space group turned out to be $P6_122$.[10] The opposite helical senses of the hairline fracture and the sixfold screw axes in that space group constituted a puzzling contradiction.

An inspection of the packing interactions in homo-DNA crystals reveals the existence of a left-handed double-stranded superhelix around the right-handed sixfold screw axes (Fig. 2B). Pairs of homo-DNA duplexes (cyan and green, Fig. 2B) from opposite strands are related *via* local crystallographic dyads that are spaced by *c*/12 (11.15 Å) along the screw axis. Therefore, the two strands of the superhelix run in opposite directions. The superhelix describes two full turns over the length of the crystallographic *c*-axis (133.85 Å; Fig. 2B) and has a diameter of *ca.* 40 Å (Fig. 2C). In the individual strands of the superhelix homo-DNA octamer duplexes are arranged head-to-head (Fig. 3A) and tail-to-tail (Fig. 3B). At one end (head; base pair C1:G16), guanines from C1:G16 pairs interact in the minor groove under formation of two N2–H···N3 hydrogen bonds. The overlap between duplexes at that site is further stabilized by hydrogen bonds between terminal 4′-hydroxyl groups (G16) and exocyclic carbonyl oxygens O2 (C15; Fig. 3A). At the other end (tail; base pair G8:C9), G8:C9 pairs from a base quartet, whereby Gs and Cs from overlapping duplexes interact *via* (C)N4–H···O6(G) and

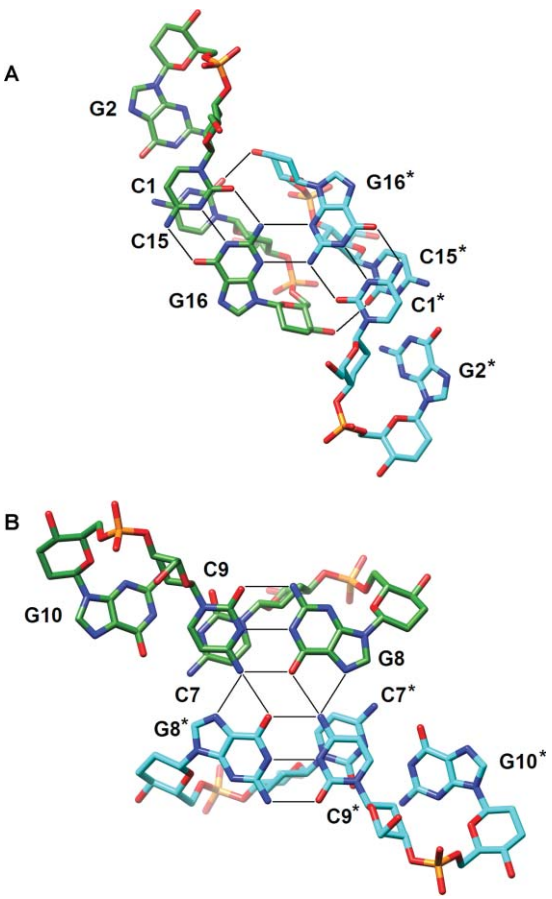

Fig. 3 Hydrogen bonding interactions between individual homo-DNA duplexes in the left-handed superhelix (viewed approximately along the crystallographic dyad). (A) Head-to-head arrangement mediated by minor groove interactions between two C1:G16 base pairs. (B) Tail-to-tail arrangement mediated by major groove interactions between two G8:C9 base pairs.

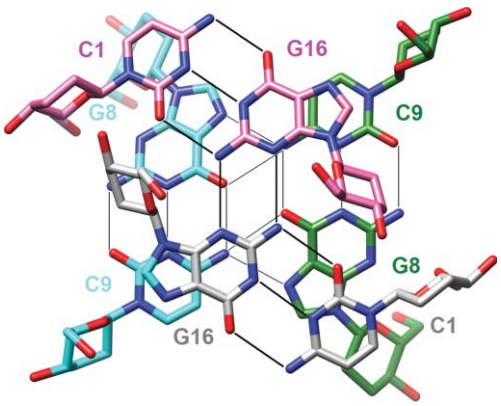

Fig. 4 Hydrogen bonding and stacking interactions within layers extending perpendicularly to the direction of left-handed superhelices. The G:C base pairs belong to four different duplexes and the upper and lower base tetrads are identical to those depicted in Fig. 3A and B, respectively. The view is approximately along the crystallographic dyad.

(C)N4–H···N7(G) hydrogen bonds. This arrangement generates a van der Waals contact between O6 carbonyl oxygens from symmetry-related guanines (Fig. 3B).

Infinite stacks of duplexes along a particular direction (*i.e.* ref. 12, 13) or formation of superhelices (*i.e.* of the right-handed type as described in ref. 14, 15) are packing motifs that are not uncommon in crystals of oligonucleotides. However, individual molecules in such supramolecular assemblies typically engage in base-stacking interactions at the seams. If the supramolecular assembly is helical it is often a single-stranded helix (a continuous left-handed superhelix composed of double-stranded DNA is also found in nucleosome core particles[16]). More importantly, the helical sense of the supramolecular structure is the same as that of the symmetry element by which it is generated (*i.e.* a right-handed superhelix around a right-handed sixfold screw axis; see Fig. 4B of ref. 14). Interestingly, the crystal structure in space group $P3_212$ of a 2′,3′-dideoxy-1′,5′-anhydro-D-arabino-hexitol nucleic acid (HNA) duplex features a right-handed superhelix around the left-handed threefold screws axis (although this appears not to have been noted by the authors).[17] Thus, to our knowledge the unusual opposite helicality of the double-stranded superhelix and crystallographic symmetry appears to have gone unnoticed to date.

How is the left-handed superhelix embedded into the homo-DNA crystal lattice? Each duplex in the superhelix participates in a dimer of dimers, involving base swapping (duplexes are related *via* a crystallographic dyad; Fig. 1B). The resulting cross-shaped tetraplexes form layers through stacking (Fig. 4) that extend perpendicularly to the sixfold screw axes. The interactions between individual layers involve exclusively hydrogen bonding (Fig. 3) and may overall be weaker than those within layers that comprise hydrogen bonding and stacking (Fig. 1B, 4). Thus, the analysis of the lattice interactions supports the view that the left-handed hairline fracture observed with homo-DNA crystals is a macroscopic manifestation of a microscopic feature, namely the left-handed superhelix. Dehydration of crystals or interactions with metal ions as a result of heavy atom soaks may distort or disrupt the suprahelical assembly along sixfold screw axes and this likely leads to shearing and separation of horizontal layers.

The substitution of the DNA 2′-deoxyribose by 2′,3′-dideoxy-glucopyranose in the backbone of homo-DNA not only leads to a drastically different duplex structure (Fig. 1) but in addition triggers changes at the supramolecular level. The most spectacular among the supramolecular motifs is a left-handed double-stranded helix with a right-handed symmetry (Fig. 2). Other unique features include the tight crossover of duplexes with associated base swapping and the prevalence of base tetrads (Fig. 3, 4) for building the homo-DNA crystal lattice.

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Notes and references

1 G. Felsenfeld, D. R. Davies and A. Rich, *J. Am. Chem. Soc.*, 1957, **79**, 2023–2024; H. E. Moser and P. B. Dervan, *Science*, 1987, **238**, 645–650; P. A. Beal and P. B. Dervan, *Science*, 1991, **251**, 1360–1363; R. W. Roberts and D. M. Crothers, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 9397–9401; M. D. Frank-Kamenetskii and S. M. Mirkin, *Annu. Rev. Biochem.*, 1995, **64**, 65–95; D. Praseuth, A. L. Guieysse and C. Hélène, *Biochim. Biophys. Acta*, 1999, **1489**, 181–206; S. Rhee, Z.-J. Han, K. Liu, H. T. Miles and D. R. Davies, *Biochemistry*, 1999, **38**, 16810–16815.

2 M. Gellert, M. N. Lipsett and D. R. Davies, *Proc. Natl. Acad. Sci. U. S. A.*, 1962, **48**, 2013–2018; D. Sen and W. Gilbert, *Nature*, 1988, **334**, 364–366; W. I. Sundquist and A. Klug, *Nature*, 1989, **342**, 825–829; J. R. Williamson, M. K. Raghuraman and T. R. Cech, *Cell*, 1989, **59**, 871–880; D. Sen and W. Gilbert, *Methods Enzymol.*, 1992, **211**, 191–199; G. Laughlan, A. I. H. Murchie, D. G. Norman, M. H. Moore, P. C. Moody, D. M. J. Lilley and B. Luisi, *Science*, 1994, **265**, 520–524; G. N. Parkinson, M. P. H. Lee and S. Neidle, *Nature*, 2002, **417**, 876–880; R. Holliday, *Genet. Res.*, 1964, **5**, 282–304; D. M. J. Lilley, *Q. Rev. Biochem.*, 2000, **33**, 109–159; B. F. Eichman, M. Ortiz-Lombardia, J. Aymami, M. Coll and P. S. Ho, *J. Mol. Biol.*, 2002, **320**, 1037–1051; K. Gehring, J.-L. Leroy and M. Guéron, *Nature*, 1993, **363**, 561–565; L. Chen, L. Cai, X. Zhang and A. Rich, *Biochemistry*, 1994, **33**, 13540–13546; I. Berger, M. Egli and A. Rich, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 12116–12121; J. Kondo, W. Adachi, S. Umeda, T. Sunami and A. Takenaka, *Nucleic Acids Res.*, 2004, **32**, 2541–2549.

3 J. C. Chaput and C. Switzer, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 10614–10619.

4 M. Egli, *Curr. Opin. Chem. Biol.*, 2004, **8**, 580–591.

5 W. Saenger, *Principles of Nucleic Acid Structure*, Springer Verlag Inc., New York, 1984.

6 N. C. Seeman, *Nature*, 2003, **421**, 427–433.

7 W. M. Shieh, J. D. Quispe and G. F. Joyce, *Nature*, 2004, **427**, 618–621.

8 C. Mao, W. Sun, Z. Shen and N. C. Seeman, *Nature*, 1999, **397**, 144–146; B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel and J. L. Newmann, *Nature*, 2000, **406**, 605–608; H. Yan, X. Zhang, Z. Shen and N. C. Seeman, *Nature*, 2002, **415**, 62–65.

9 P. J. Paukstelis, J. Nowakowski, J. J. Birktoft and N. C. Seeman, *Chem. Biol.*, 2004, **10**, 1119–1126; N. Valls, C. Gouyette, I Udón and J. A. Subirana, *J. Am. Chem. Soc.*, 2004, **126**, 7812–7816.

10 M. Egli, P. S. Pallan, R. Pattanayek, C. J. Wilds, P. Lubini, G. Minasov, M. Dobler, C. J. Leumann and A. Eschenmoser, *J. Am. Chem. Soc.*, 2006, **128**, 10847–10856.

11 M. Egli, P. Lubini and P. S. Pallan, *Chem. Soc. Rev.*, 2007, **36**, 31–45.

12 A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel and A. Rich, *Nature*, 1979, **282**, 680–686.

13 M. Egli, S. Portmann and N. Usman, *Biochemistry*, 1996, **35**, 8489–8494.

14 S. Portmann, N. Usman and M. Egli, *Biochemistry*, 1995, **34**, 7569–7575.

15 Y. Xiong and M. Sundaralingam, *RNA*, 2000, **6**, 1316–1324; K. Shi, R. Biswas, S. N. Mitra and M. Sundaralingam, *J. Mol. Biol.*, 2000, **299**, 113–122.

16 K. Luger, A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond, *Nature*, 1997, **389**, 251–260.

17 R. Declercq, A. V. Aerschot, R. J. Read, P. Herdewijn and L. V. Meervelt, *J. Am. Chem. Soc.*, 2002, **124**, 928–933.