Chemistry & Biology, Vol. 11, 1027–1035, August, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.08.004



# "Deoxyribo Nanonucleic Acid": Antiparallel, Parallel, and Unparalleled

**The crystal structure of a single-stranded DNA oligonucleotide has revealed formation of a unique three-dimensional array by continuous antiparallel and parallel pairing between monomers [1]. The array is based on tertiary interactions and represents a second-generation nanotechnological system.**

Looking back at more than a decade of research on the structure and function of the nucleic acids, it is impossible to deny the fact that RNA has stolen the show and that DNA, particularly as far as its structure in the absence of protein is concerned, has led a life in the shadow. RNA can have enzymatic activity and constitutes the structurally and functionally crucial components of a multitude of important biomacromolecular assemblies, including the ribosome, the spliceosome, telomerase, and the signal recognition particle. The crystal structures of the small and large subunits of the ribosome belong to the most stunning achievements furnished by structural biology to date [2, 3]. RNA crystallography has also brought us detailed insights into the structures and catalytic mechanisms of the hairpin [4] and hepatitis delta virus ribozymes [5] and the group I intron [6], among many others, and has yielded numerous conformational motifs (reviewed in [7]). RNA function has also been in the limelight: The discovery of RNA interference and its exploitation in genetics and proteomics as well as for target validation and potentially for therapeutic applications belongs to the most exciting scientific developments of the last decade [8, 9].

So why is it that, even by some in the field, DNA structure is sometimes considered a closed chapter? After all, the structural diversity of DNA has moved beyond the double helical families a long time ago [10]. Moreover, the structural properties of many sequences, such as those featuring only A and T, remain poorly investigated [11]. And there are numerous structures of DNA molecules, some truly surprising and intricate, that demonstrate that DNA is no less sexy than RNA. I will only refer the reader to a small selection of such structures here, namely the Hoogsteen duplex [12], the "Greek key" motif formed by a triplet repeat sequence [13], and recent crystal structures of Holliday junctions [14] and guanine quadruplexes [15]. Those still not convinced should turn to page 1119 of this issue of *Chemistry & Biology*, where Paul J. Paukstelis, Nadrian C. Seeman, and colleagues report the fascinating results of the crystallographic investigation of a single-stranded DNA [1].

The team of researchers has determined the crystal structure at 2.1 Å resolution of the 13-mer d(GGACAGATGGGAG) by single wavelength anomalous dispersion (SAD). Their actual goal was the crystallization of the complex between the 13-mer and its complementary biotinylated DNA strand, tethered to streptavidin. However, similar to previously reported cases, one of the molecules crystallized alone [16]. Unlike many double-stranded DNAs that typically form infinite columns of end-to-end stacked helices with DNA-, water- or metal ion-mediated interactions between columns and layers, the present 13-mer self-assembles into a continuous 3D array through formation of symmetric and asymmetric homo-purine base pairs between strands of antiparallel and parallel orientation (Figure 1). The 13-mer (the 3′-terminal G is disordered in the crystal) snakes through 3D space in a rather complicated manner and engages in numerous noncanonical interactions with symmetry-related strands. Remarkably, only two among the observed six unique base pairs are of the standard Watson-Crick type, and the phosphodiester backbone between the third and the fourth nucleotide assumes a sharp kink (Figure 1). The latter conformational feature also marks the interlayer junction, as the G1-G2-A3 trimer pairs in a parallel fashion with G10-G11-A12 from another monomer in an adjacent layer. The antiparallel domains of the structure form layers that are joined in the third direction by the parallel-stranded helical regions, thus generating a continuous three-dimensional array.

The occurrence of antiparallel and parallel pairing between strands in the same crystal structure is encountered elsewhere too. A recent example is the structure of d(GCGAAAGCT), in which CGAA tetramers form a parallel duplex with homo base pairs ($C:C^+$; G:G, and A:A). The second halves of the same oligonucleotides form antiparallel duplexes with the corresponding regions from two further DNA strands [17]. In addition, three- and four-stranded motifs feature both parallel and antiparallel orientation of strands. However, the presence of a higher number of noncanonical base pairs compared with Watson-Crick pairs in the structure of the 13-mer [1] is unique among DNA crystal structures. The structure of an RNA pseudoknot demonstrated formation of more tertiary hydrogen bonds than Watson-Crick-type hydrogen bonds [18]. But the RNA molecule folds back on itself and therefore does not participate in extensive contacts to neighboring molecules beyond stacking. Neither the crystal lattice of the above DNA structure with mixed parallel and antiparallel pairing nor the one occupied by pseudoknot molecules feature large solvent-filled cavities.

Conversely, the hexagonal lattice of the DNA 13-mer exhibits large channels that run both parallel and perpendicular to the crystallographic 6-fold symmetry axis. The scaffold created by the oligodeoxynucleotide and the resulting cavities of considerable volume are reminiscent of zeolites. It is feasible that these solvent-filled cavities could accommodate relatively large guests. Alternatively, the array formed by the 13-mer DNA could act as a molecular sieve. Although the discovery of this three-dimensional array is entirely fortuitous, its elements can be modified in a rational manner. The authors

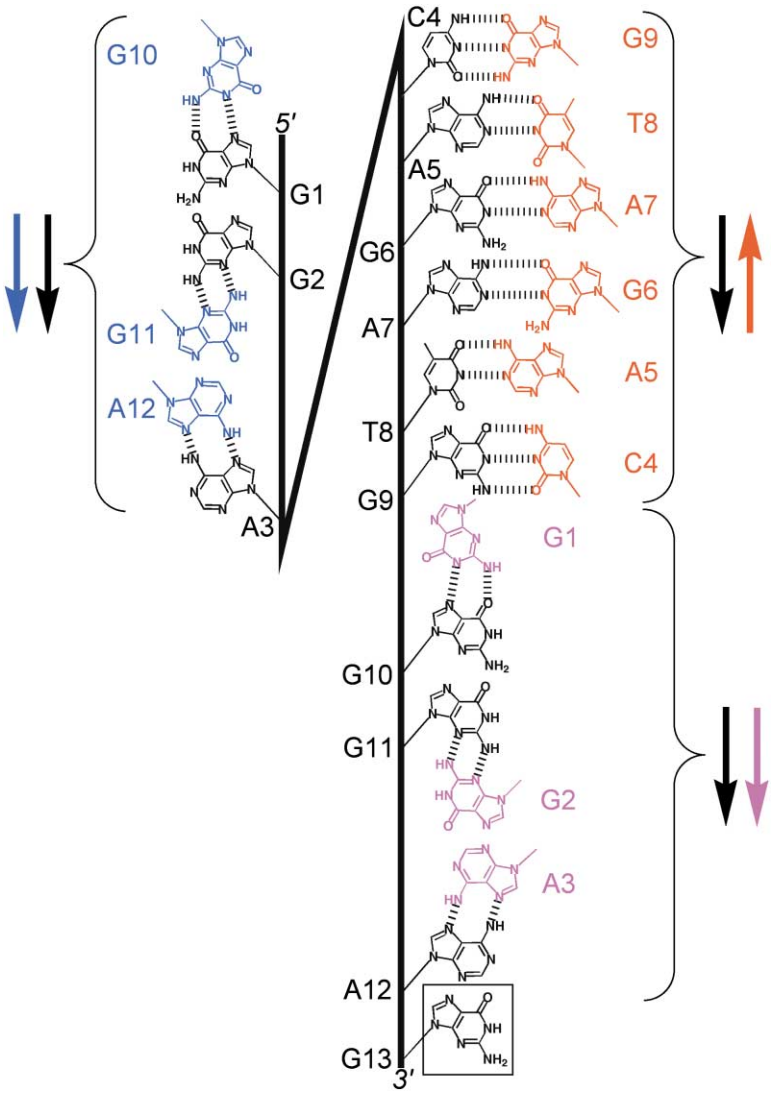


Figure 1. Base-Pairing Interactions and Relative Strand Orientations in the 3D-DNA Array Generated by the 13-mer 5′-GGACAGATGG GAG-3′

Out of six unique base pairs, only two are of the standard Watson-Crick type (C4:G9# and A5:T8#; # denotes a symmetry mate). Symmetry-related molecules are colored differently, hydrogen bonds are dashed lines, and arrows designate the pairing mode between strands (⇵ antiparallel and ⇊ parallel). The zigzag shape of the backbone in the schematic indicates a sharp turn between residues A3 and C4, and the boxed residue G13 is disordered and invisible in the structure.

demonstrate that changing the spacer between the helical (antiparallel strands) region and the interlayer junction (parallel strands) leads to a large expansion of the channels running parallel to the crystallographic 6-fold axis. This indicates that the observed interactions can be used in a predictable way to change the molecular scaffold and create alterations in the supramolecular structure. In the starting structure of the 13-mer, the channels are large enough to accommodate small molecules or oligopeptides and -nucleotides. By comparison, the constructed lattices show expanded channels that are of sufficient size to swallow a protein as large as 45 kDa per unit cell. Interestingly, the 3′-terminal residue protrudes into a channel (the lack of restraints causing its disorder) and thus provides an anchoring point for nucleotide extensions or tethers. Although crystals of DNAs with various cargo molecules attached to the 3′ terminus diffracted quite well, the guest molecules have not been visualized in the structures [1]. This is an indication that the use of molecular scaffolds for structure determination of guest molecules is not straightforward.

DNA is well suited for the generation of nanometer-scale structures. Solid-phase chemistry based on phosphoramidites allows cheap and facile synthesis of relatively long strands with defined sequence, and the material is chemically stable. Base pairing provides predictable interactions and stable cohesion. DNA duplexes are geometrically well characterized and can serve as stiff spacers of defined length. Chemical modification of DNA is easily achieved and allows increased thermodynamic stability and introduction of novel packing motifs and electronic properties (i.e., [19]). Two- and three-dimensional DNA nanostructures based on Watson-Crick base pairing have been assembled using a range of methods to introduce branching [20] including sticky ends [21]. A striking example is the recently designed DNA octahedron based on a very long DNA and several oligodeoxynucleotides where the double-stranded edges are joined by four-way junctions [22]. The work highlighted here [1] now heralds a new generation of DNA nanostructural systems that can be designed based on complex tertiary interactions. Compared with DNA, RNA conformation is likely much harder to tame, although quite a few RNA structural motifs

have now been well characterized (i.e., [23–26]). Another potential drawback is the lower chemical stability of RNA relative to DNA.

One often wonders what structures a single-stranded DNA could adopt. We have hundreds of examples of structures of double-stranded DNAs in the crystal and in solution, but very little is known regarding the conformational variations in a single strand. The work described here provides probably just a glimpse at the potential complexity of DNA structure when noncanonical pairing modes are considered. Although DNA cannot possibly match RNA in terms of structural complexity and numbers of folding motifs, its chemistry offers an ideal basis for the formation of predictable 2D and 3D arrays. The work by Paukstelis and coworkers is an excellent demonstration that DNA "wears many hats" and, used correctly, can serve as an ideal building material for supramolecular assemblies and nanoscale structures.

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**Selected Reading**

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# Pheromone Unwrapping by pH Flip-Flopping

**The Asian elephant utilizes the same sex pheromone as a number of moth species, (*Z*)-7-dodecen-1-yl acetate encapsulated in a serum-derived albumin. The chemical signal is emitted in the urine and received in the mucus of the trunk. The unwrapping of the package is pH mediated.**

The Asian elephant [1], the cabbage loop moth, and many other moth species [2] share a common sex pheromone, (*Z*)-7-dodecen-1-yl acetate (Z7-12Ac), but the packing and processing of this chemical signal is remarkably different in elephants and moths. Female moths advertise their readiness to mate and reproduce by releasing sex pheromones, which are utilized by male moths in long-range odorant-oriented navigation toward females. Sustainable flight and orientation requires a dynamic, sensitive, and selective olfactory system [3–6] to detect specifically pockets of chemical signals that are separated by small clean air spaces in a pheromone plume [7]. To be able to follow the trail, males have only a few milliseconds to reset the olfactory system while navigating through clean air [4, 8]. Three major groups of proteins play pivotal roles in the dynamics, selectivity, and sensitivity of pheromone reception in insects. They are the pheromone receptors (PRs), pheromone binding proteins (PBPs), and pheromone-degrading enzymes (PDEs) [4, 5, 8]. While PBPs serve as liaison between the external environment (air) and the PR, PDEs are essential for inactivation of chemical signal and consequently resetting the receptors [9]. Upon binding pheromones, PBPs transport the chemical signals to their receptors while avoiding premature inactivation by PDEs [4, 5, 8]. Interaction with negatively charged membrane surfaces in the proximity of the pheromone receptors leads to a pH-dependent conformational change in PBPs [10, 11] and delivery of the pheromones to the receptors [4, 5, 8]. Elephants have a much less stringent requirement for the dynamics of pheromone reception. It seems that they do not have a pheromone carrier/protector in the mucus of the trunk. As opposed to the unique helix-rich structures of insect PBPs [12–14], the major odorant binding protein (OBP) in the mucus of the Asian