Chapter 8

Protein: Feeling the Groove of DNA

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B′-form DNA differs from standard B-form DNA by a more narrow minor groove, which is filled in the B′-form by a highly ordered and geometric array of water molecules. A-tracts (sequences of contiguous $^{5'}$ApA$^{3'}$ and/or ApT steps without TpA steps or C·G base pairs) have the greatest propensity to adopt the B′-form. We show that binding of a protein (P22 c2 repressor N-terminal domain) to DNA induces the B to B′ transition of the central non-contacted region of the operator, regardless of the DNA sequence. P22R NTD provides an experimental system for studying B′-DNA of any sequence, even those with C·G base pairs. One can determine how hydration and distributions of ions of B′-DNA change with sequence, while holding DNA conformation fixed. Here we describe three-dimensional structures of P22R NTD-DNA complexes containing $^{5'}$ApT$^{3'}$, TpA, GpC or CpG at the center of the non-contacted region of the DNA operator. All these dinucleotide steps are in the B′-form, with a narrow minor grove and a spine of hydration. Many features of the water interactions within the minor groove are conserved for the sequences ApT, TpA and GpC. The primary water molecules, at the base of the hydration motif, are highly restrained in translation and rotation. These water molecules are in discrete sites, interacting exclusively with hydrogen bond acceptors in optimally spaced binding pockets formed by DNA functional groups on the floor and walls of the minor groove. The water

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interactions at the CpG step differ fundamentally from those of ApT, TpA or GpC. At the CpG step, the primary water molecule interacts with a mixture of hydrogen bond donors and acceptors at closely spaced and overlapping binding sites. The translational and rotational restraints on the primary water molecules are released by the 2-amino groups of CpG step, breaking the entire hydration motif. The work presented in this chapter suggests that stability of DNA complexes with minor groove ligands is modulated in a structurally comprehensible way by the entropy of release of water molecules from the minor groove.

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Introduction

Double-stranded DNA can adopt a variety of conformations (*1*) including linear and bent, and A, B and B′ [reviewed by Hud (*2*)] and Z [reviewed by Rich (*3*)]. The propensity to adopt a given conformation is influenced by sequence and environment.

B-DNA

Rosalind Franklin collected two distinct diffraction patterns from DNA fibers, which she reversibly inter-converted by changing the level of hydration. Without knowledge of the 3D structures, she called the highly hydrated form the “B structure” and the less hydrated form the “A Structure” (*4*). We now know that the vast majority of genomic DNA, with sequences containing a mix of A·T and C·G base pairs, is found in the B-form. In B-DNA, the bases stack like pennies, with their normals in line with the helical axis (Figure 1A). The phosphodiester backbones are on the outside of the duplex where they form the walls of two grooves. The edges of the base pairs provide the floors of the grooves. In B-form DNA, the major groove is deep and wide. The minor groove is deep and narrow, but there is sufficient space there to allow two strings of water molecules to combine to form a “ribbon of hydration” along the floor of the groove (*5*). B-form DNA is characterized by significant frequency of cations in the major groove, adjacent to the N-7 and the O-6 atoms of guanines (*6*).

B′-DNA

The B′-form of DNA (*7*), shown in Figure 1B, is a variant of B-form DNA. It was first described by Arnott (*8*), and has been shown by Tullius and coworkers (*9–11*) to form spontaneously in A-tracts, which contain contiguous $^{5'}$ApA$^{3'}$ and/or ApT steps. TpA steps provide a barrier to formation of the B′-form. That barrier can be overcome by environment: the B′-form can be induced by proteins (*12*, *13*), crystal lattice effects (*14*) and some minor groove binders (*15*). C·G base pairs appear to provide the largest barrier to the transition from B- to B′-form. Only in complexes of certain proteins (*12*, *13*) are C·G base pairs observed in B′-form.

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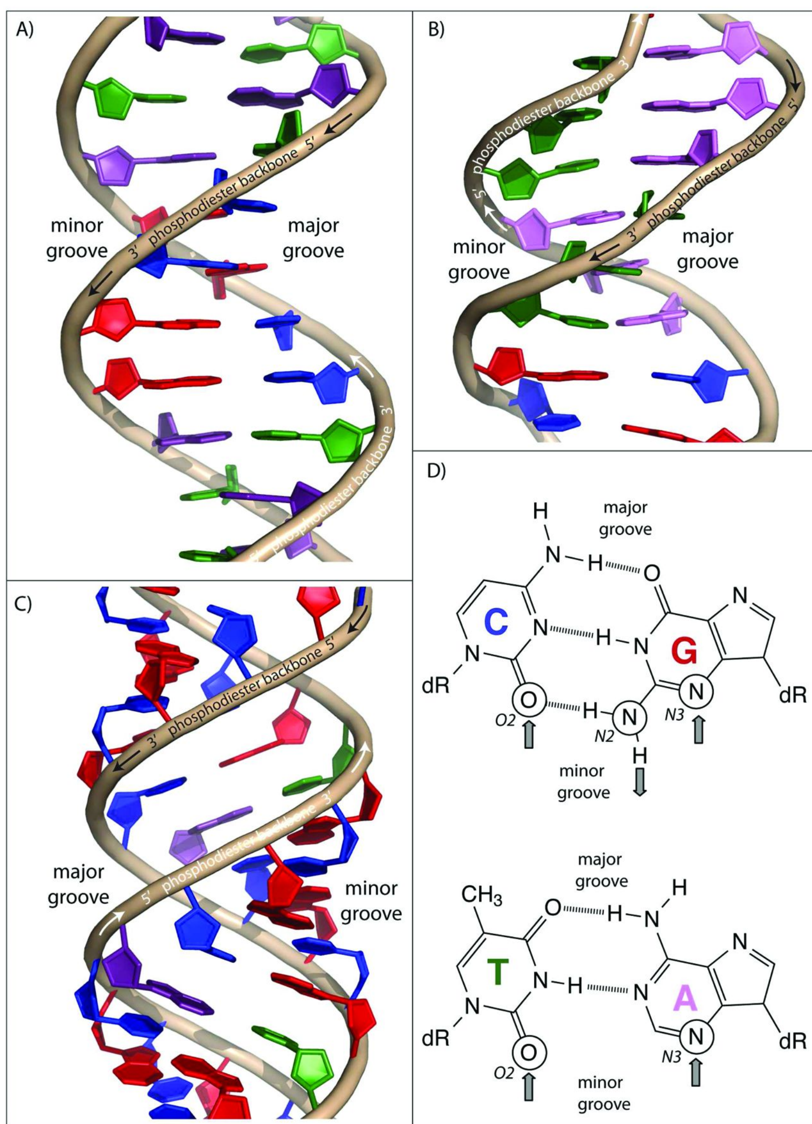

Figure 1. A) B-form DNA from idealized fiber coordinates. B) B′ DNA from the central region of the single crystal structure of [d(CGCAAATTTGCG)]$_2$. (PDB entry 1S2R) C) A-form DNA from idealized fiber coordinates. D) Watson-Crick base pairs with the major and minor grooves indicated, along with the hydrogen bonding functionalities on the floor of the minor groove. The up arrows indicate hydrogen bond acceptors, and the down arrows in indicated hydrogen bond donors.

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The B′-form differs from standard B-form DNA by (i) a narrow minor groove (*9*, *16*), (ii) a one-layer thick sheet of water molecules containing a zig-zag spine of hydration (*17*, *18*) as a foundation for higher-level minor groove assemblies (*19–21*), (iii) a propensity to bend DNA when appropriately phased (*22–24*), (iv) monovalent cations within the minor groove and a depletion of cations from the major groove (*20*, *25–28*), (v) negative propeller twisting of base pairs (*29*, *30*), (vi) unusual rigidity (*31*, *32*), and (vii) a 'pre-melting' thermal transition that is distinct from duplex melting (*33–35*). The B′-form helical axis is thought to be linear (*36*, *37*). Axial bends arise at the junctions between B′-form and flanking B-form DNA (*2*). The hydration within the B-form minor groove appears to be monolithic and cooperative, with an extended and interdependent hydration assembly (*13*).

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A-DNA

DNA is converted from B-form to A-form by partial dehydration, or by addition of a 2′-hydroxyl group. Duplex RNA is found exclusively in A-form. DNA base composition and sequence is an important influence of the B/A balance. GC rich DNA most readily converts from B- to A-form. In the A-form, the bases are pushed away from the helical axis and are inclined relative to the penny-like bases of B-form DNA (Figure 1C). The DNA is compressed along the helical axis. The phosphodiester backbones are pulled together across the major groove, which is very deep and very narrow. In comparison with B-DNA, the major grove of A-DNA contains greater populations of cations, which are more extensively localized (*38*).

Protein Recognition and Deformation of DNA

The binding of proteins to specific sequences of DNA is a basic requirement for biological transactions ranging from gene expression, to DNA restriction and modification, to regulating chromatin structure. Proteins use both direct and indirect readout to read DNA sequence. In direct readout, DNA bases make contacts with protein sidechains and backbone atoms. Direct readout can employ hydrogen bonding (*39*) and/or shape complementarity (*12*).

In indirect readout, DNA sequence is sensed at a distance by its propensity to assume or to resist various altered conformations. The propensity for DNA to alter its state is modulated by sequence. Proteins exploit sequence-dependent DNA conformational polymorphism and deformability in recognizing sequence. Previously the role of DNA conformation and deformability in protein recognition was put in a quantitative framework by Olson and Zhurkin (*40*). Williams has described how DNA electrostatic potential and charged species can alter DNA conformation, especially minor groove width (*41*). Koudelka and Carlson (*42*) and Crothers and Zhakked (*43*) experimentally determined relationships between DNA flexibility and protein affinity. Honig (*44*) has proposed that electrostatic potential and minor groove width are recognized by proteins.

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We have determined a series of high-resolution crystal structures to help understand direct and indirect readout of DNA sequence by helix-turn-helix proteins. The results indicate a mechanism of sequence recognition in which the affinity of a given protein for DNA is attenuated by the sequence-dependence of the free energy required to induce a specific type of DNA deformation (*13*, *14*). DNA sequence can be 'read' by the ease with which it can be converted from the B- to the B′-form. Structures of complexes of the P22 c2 repressor N-terminal domain (P22R NTD) with DNA operators (*13*, *14*) confirm that direct readout of helix-turn-helix proteins involves interactions of recognition helices with the floor of the major groove (Figure 2). P22R NTD directly reads DNA sequence primarily by shape complementarity rather than hydrogen bonding interactions. Shape complementarity between the DNA and the P22R NTD recognition helix is consistent with effects of mutations on P22R NTD - DNA complex stability (*40*, *41*).

A Molecular-Level Model of Indirect Readout

Solution experiments demonstrated P22R NTD indirectly reads the sequence of the non-contacted bases at the center of its operator (*45–48*). These non-contacted bases are indicated in Figure 2. The sequence of the central non-contacted bases modulates the affinity of P22R NTD for DNA. The 3D structures suggest that P22R NTD induces a transition of the non-contacted region from the B-form to the B′-form (*12*, *13*). In the P22R NTD – DNA complex, regardless of the sequence, the minor groove of the non-contacted region is narrow, and is hydrated by an zig-zag spine of hydration. P22R NTD clearly induces a B- to B′-form transition of the non-contacted region of the DNA, no matter the sequence. The differential effects of DNA sequence on the barrier to this transition allow the protein to sense the non-contacted DNA sequence.

Exploring the Sequence Landscape of B′-Form DNA

P22R NTD provides an experiment system for studying the B′ form of DNA for any sequence. Within the central region of the DNA operator (Figure 2), binding by P22R NTD will force any sequence, even sequences containing C·G base pairs, into B′-form. The DNA conformation is held fixed by the protein, allowing one to characterize the hydration and cation interactions of a variety of sequences in a conformationally well-controlled system. We have determined x-ray structures of the P22R NTD in complexes with a series of synthetic operators. These complexes are the same except that they contain ApT, TpA, GpC or CpG at the center of the non-contacted region of the DNA operator (indicated by the highlighted base pairs in Figure 2). DNAs containing these sequences vary in their affinity for P22R NTD.

Nonetheless, they all contain B′-form DNA, with a narrow minor grove and a spine of hydration. The similarities in the groove widths and minor groove hydration in this series are observable in Figures 3 and 4. Although the sequences

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differ, the water interactions within the minor groove are essentially conserved for ApT, TpA and GpC, which are the three highest affinity sequences.

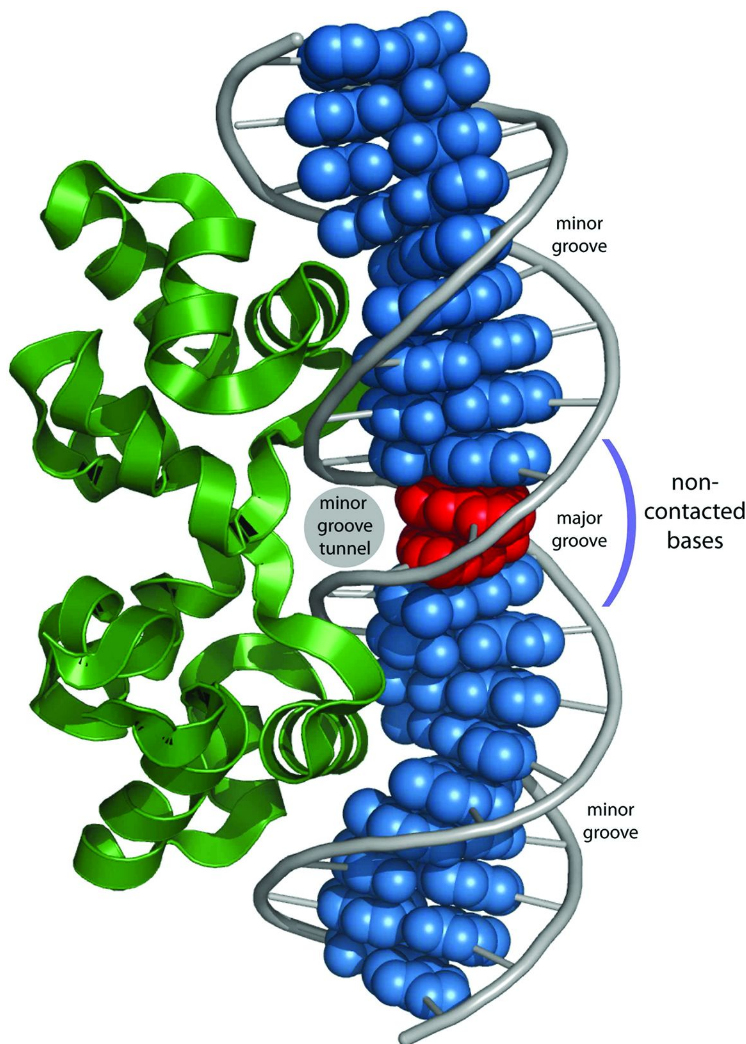

Figure 2. P22R NTD-DNA Complexes. The P22R NTD (ribbon) binds as a dimer to a 20 base pair consensus sequence, making direct contacts with the major groove of the outer bases of the DNA operator. The protein does not contact the central four base pairs (indicated), but forces the DNA into the B'-form, reading the sequence indirectly. The central sequence has a narrow minor groove and a spine of hydration. The dimer interface is located above the minor groove creating a tunnel with the non-contacted region (indicated by the grey circle). The two central DNA bases (shaded) have been varied in the series of structures described here as shown in Figures 3 and 4. The DNA bases are rendered as spheres, and the DNA backbone is rendered as a tube.

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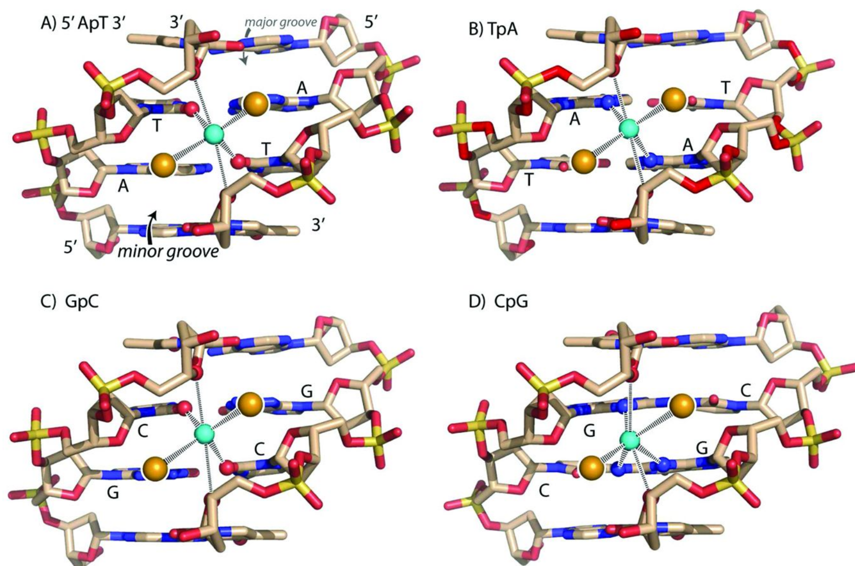

Figure 3. Water Coordination in the B′-form Minor Groove. The primary water of the spine of hydration, interacts with the floor of the minor groove, is hexa-coordinated (indicated by dashed lines) by two base functional groups, by two O4′ atoms of deoxyriboses and by two additional water molecules in the secondary layer. The coordination by the O4′ atoms and the secondary layer water molecules are independent of sequence. The base atoms coordinatingthe waters and their relative dispositions vary with the DNA sequence. Shown are the coordination at an A) ApT step; B) TpA step; C) GpC step; D) CpG step. The coordination geometries are conserved at ApT, TpA and GpC but not CpG.

ApT steps stabilize B′-form DNA. At an ApT step within B′-DNA, the primary water molecule, at the base of the zig-zag spine of hydration, is highly constrained to a discrete site, forming four hydrogen bonds with the DNA (Figures 3A and 4A) in a well-defined pocket. The DNA uses the O2 atoms of adjacent, cross-strand T's, and two cross-strand O4′ ribose atoms to form the binding pocket for this water molecule (the numbering scheme for the atoms on the floor of the minor groove is indicated in Figure 1D). All four atoms of the DNA are hydrogen bond donors, restraining not only the translation but the rotation of this water molecule. This water molecule cannot freely rotate because it contributes two hydrogens simultaneously to four hydrogen bond acceptors of the DNA (*13*). ApA steps, which also stabilize B′-form DNA, forms the same four-HB acceptor pocket as ApT (*19*), except that one of the O2 atoms is replaced by the N3 of an A. The combined data suggest that hydrogen bonds between O2(T) and water are stronger than hydrogen bonds between N3(A) and water.

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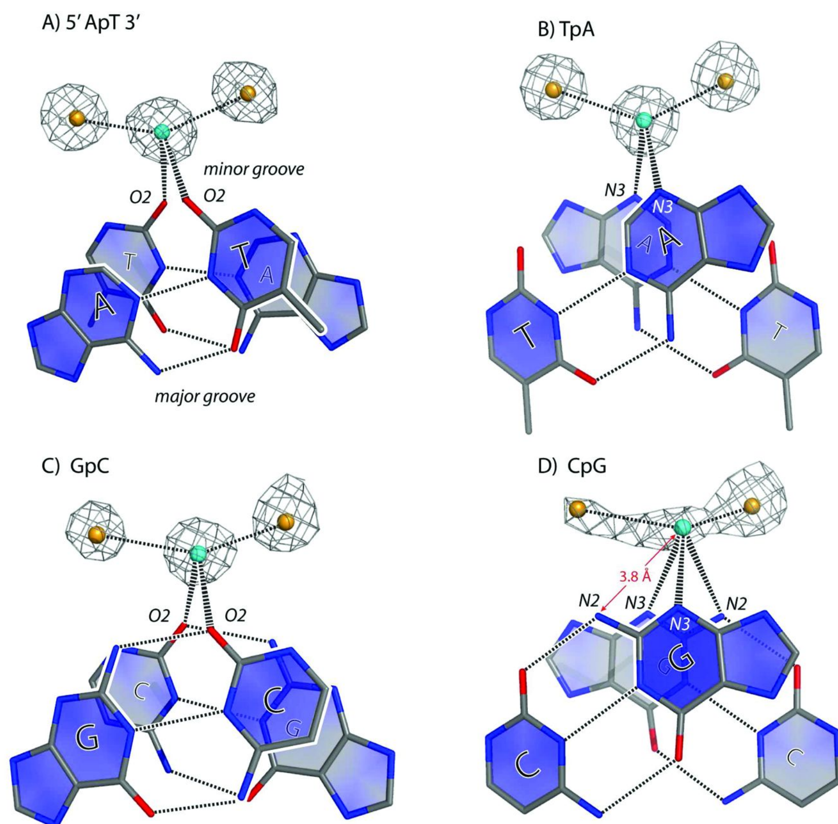

Figure 4. Water Localization in the Minor Groove Tunnel. Electron density (2Fo-Fc, gray mesh) surrounding the primary and secondary water molecules within the B′-form minor groove. Water molecules are highly localized, and are contained within spherical electron density in the A) the ApT step; (B) the TpA step; and the (C) the GpC step. However, the electron density at the D) CpG step shows elongated tube of electron density consistent with delocalization - multiple overlapping positions - for these water molecules. The primary water molecule at the CpG step occupies alternate positions by shifting from one 2-amino group to another. The maps were constructed from single crystal x-ray diffraction data of the P22R NTD-DNA complexes. All structures refined at a resolutions better than 1.8 Å and the 2Fo-Fc maps are contoured at 1 σ. Refinement statistics are R-cryst/R-free; ApT, 18.4/21.4; TpA, 20.4/22.5; GpC, 18.9/22.8; CpG, 18.7/23.6

When DNA is forced into the B′ form, this elaborate hydrogen-bonding scheme can be accomplished by other sequences. An TpA step is considered a B′-breaker in free DNA. However when forced by the protein into the B′-form, the TpA step conserves the hydrogen bonds with the primary water molecule, replacing both O2’s of the T’s with N3’s of A’s (Figures 3B and 4B).

Similarly at the GpC step, the hydrogen bonds of the primary water molecule are conserved in B′-form DNA, by replacing the O2’s of T’s with the O2’s of C’s

(Figures 3C and 4C). In each case the water molecule is fixed in position and rotation, by simultaneous interactions with four hydrogen bond with acceptors of the DNA (including two O4′ atoms of the deoxyribose). Thus the hydrogen bonding pattern in the minor groove is highly degenerate. The degree of localization of these water molecules can be inferred as high by the well-formed spheres of electron electron density, as shown in the maps in Figures 4A-C.

The hydration at CpG, the weakest P22R NTD binder of the four sites examined, differs fundamentally from in ApT, TpA or GpC. CpG contains two adjacent amino groups on the floor of the minor groove (Figure 3D and 4D). These amino groups extend the binding region, delocalizing the primary water molecule along the minor groove. The delocalizing effects of the 2-amino groups are clearly observable in the electron density map (Figure 4D) and in Koudelka's chemical footprinting results (*13*). The primary water molecule teeter-totters between the two 2-amino groups, forming hydrogen bonds with one 2-amino group, or with the other, but never with both. This water molecule is not fixed at a specific site as in the other three dinucleotides.

At the CpG step, the primary water molecule interacts with a mixture of hydrogen bond acceptors (N3 and O4′) and acceptors (2-amino groups). Therefore the rotational restraints on this water molecule are released at the CpG step. The delocalization of the primary water molecule disturbs the entire hydration assembly, with water molecules in the secondary layer shifting and rotating in concert with those in the primary layer. Since the hydration structure of B′-DNA is globally interdependent, these 2-amino-groups alter patterns of hydration at proximal sites.

Feeling the Minor Groove

The minor groove is a natural binding cleft that has been exploited by nature and in biotechnology. Natural antibiotics such as distamycin (*49*, *50*), and proteins such as TATA box binding protein (*51*) target the minor groove. Synthetic polyamide hairpins can be programmed to bind in the minor groove of DNA to any sequence (*52*). Guanine destabilizes DNA complexes of many minor groove ligands. It was clear from inspection of DNA-ligand models (*49*) and crystal structures (*53*) that guanine, in the form of the 2-amino group, can afford a steric impediment to binding. The 2-amino group of guanine extends further out from the floor of minor groove than any other functional group of DNA. Our recent work, as described here, suggests another effect of the 2-amino group that could be of equal or even greater thermodynamic importance. The free energy gain from release of water molecules from the minor groove can be tuned by their constraints within the groove. Release of translationally and rotationally constrained water molecules of an A-tract would afford significantly greater entropic gain than release of the translationally and rotationally unconstrained waters associated with CpG steps. Therefor the entropy of association of water molecules within the minor groove, and the entropy of release of those waters to bulk upon ligand or protein binding, appears to be an important element of DNA recognition.

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