# Nucleic Acid Hydration

DNA hydration
DNA processing

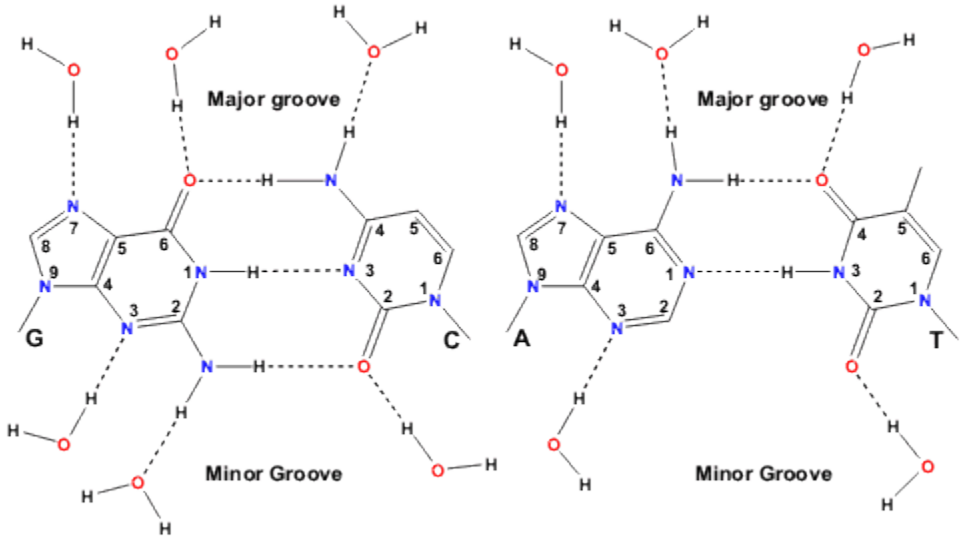
Nucleic acid hydration is crucially important for their conformation and utility [1093], as noted by Watson and Crick [828]. The strength of these aqueous interactions is far greater than those for proteins due to their highly ionic character [542b]. The DNA double helix can take up a number of conformations (for example, right handed A-DNA pitch 28.2 Å 11 bp, B-DNA pitch 34 Å 10 bp, C-DNA pitch 31Å 9.33 bp, D-DNA pitch 24.2 Å 8 bp and the left handed Z-DNA pitch 43Å 12 bp) with differing hydration. The predominant natural DNA, B-DNA, has a wide and deep major groove and a narrow and deep minor groove and requires the greatest hydration.

### DNA hydration

B-DNA needs about 30%, by weight, water to maintain its native conformation in the crystalline state. Partial dehydration converts it to A-DNA (with a narrower and deeper major groove and very wide but shallow minor groove). The transition for this transformation occurs at about 20 water molecules per base pair, with its midpoint at about 15 water molecules per base pair [1343]. The B-DNA possesses a spanning water network, and it is the loss of its continuity [1343] together with the competition between hydration and direct cation coupling to the free oxygen atoms in the phosphate groups [1394] that gives rise to the transition to A-DNA. This dehydration-induced structural transition decreases the free energy required for A-DNA deformation and twisting, which is usefully employed by encouraging supercoiling but eventually leads to denaturation [441]. Further dehydration results in the least hydrated D-DNA (favored by excess counter-ions that shield the DNA phosphate charges), which has a very narrow minor groove with a string of alternating water and counter-ions distributed along its edge [816].

Hydration is greater and more strongly held around the phosphate groups that run along the inner edges of the major grooves. The water molecules are not permanently situated however, due to the rather diffuse electron distribution of the phosphate groups. Hydration is more ordered and more persistent around the bases with their more directional hydrogen-bonding ability and restricted space. Water molecules are held relatively strongly with residence times for the first hydration shell being about 0.5 - 1 ns. Because of the regular structure of DNA, hydrating water is held in a cooperative manner along the double helix in both the major and minor grooves. The cooperative nature of this hydration aids both the zipping (annealing) and unzipping (unwinding) of the double helix. Water motion within the grooves is slowed down compared with the bulk water, with the greatest reduction within the more restricting minor groove [930]. On melting, about four water molecules per base pair are released in spite of extra hydration sites being released by the previously hydrogen-bonded base pairing [707], thus confirming the importance of this cooperative nature of the water binding within the grooves.

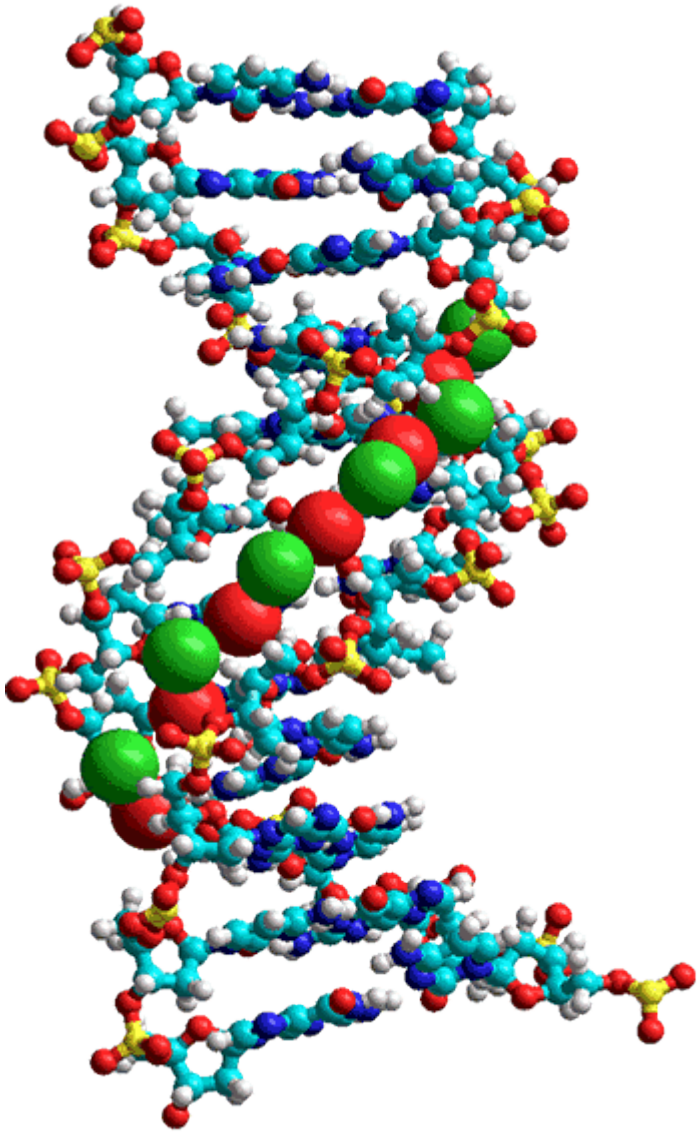
Nucleic acids have a number of groups that can hydrogen bond to water, with RNA having a greater extent of hydration than DNA due to its extra oxygen atoms (that is, ribose O2') and unpaired base sites. These extra hydroxyl groups also create additional hydration in duplex RNA as they provide a scaffold for the minor groove hydration network [708].




In DNA, the bases are involved in hydrogen-bonded pairing, close to the 0.28 nm bond length found between hydrogen-bonded water molecules in liquid water. The aqueous environment causes a slight lengthening (~1%) of the DNA hydrogen bonds, and weakens them significantly (~50%) [1867]. All these groups, except for the hydrogen-bonded ring nitrogen atoms (pyrimidine N3 and purine N1) are capable of one further hydrogen-bonding link to water within the major or minor grooves in B-DNA. A molecular dynamics simulation indicated that both grooves were equally hydrated with hydration roughly $C_{N4}/G_{N2}/T_{O2} > A_{N6}/C_{O2}/G_{O6} > A_{N3}/G_{N3}/G_{N7}/T_{O4} >> A_{N7}$ [1249].

Thus, in B-DNA, guanine will hydrogen bond to a water molecule from both the minor groove 2-amino- and major groove 6-keto-groups with further single hydration on the free ring nitrogen atoms (minor groove N3 and major groove N7). Cytosine will hydrogen bond to a water molecule from both the major groove 4-amino- and minor groove 2-keto-groups. Adenine will hydrogen bond to a water molecule from the major groove 6-amino-group with further single hydration on the free ring nitrogen atoms (minor groove N3 and major groove N7). Thymine (and uracil, if base-paired in RNA) will hydrogen bond to a water molecule from both the minor groove 2-keto- and major groove 4-keto-groups. Phosphate hydration in the major groove is thermodynamically stronger but exchanges faster. There are six (from crystal structures,

[143]) or seven (from molecular dynamics, [144]) hydration sites per phosphate[a], not including hydration of the linking oxygen atoms to the deoxyribose or ribose residues. The deoxyribose oxygen atoms (O3' phosphoester, ring O4' and O5' phosphoester) all hydrogen bond to one water molecule whereas the free 2'-OH in ribose is much more capable of hydration and may hold on to about 2.5 water molecules.[b] The total for all these hydrations, in a G ≡C duplex, would be about 26-27 but about 14 of these water molecules are shared. There are a number of ways in which these water molecules can be arranged with B-DNA possessing 22 possible primary hydration sites per base pair in a G ≡C duplex but only occupying 19 of them [144]. The DNA structure depends on how these sites are occupied; water providing the zip, holding the two strands together. It should be noted that about 2% of the hydrating water molecule sites may be transiently replaced by cations.

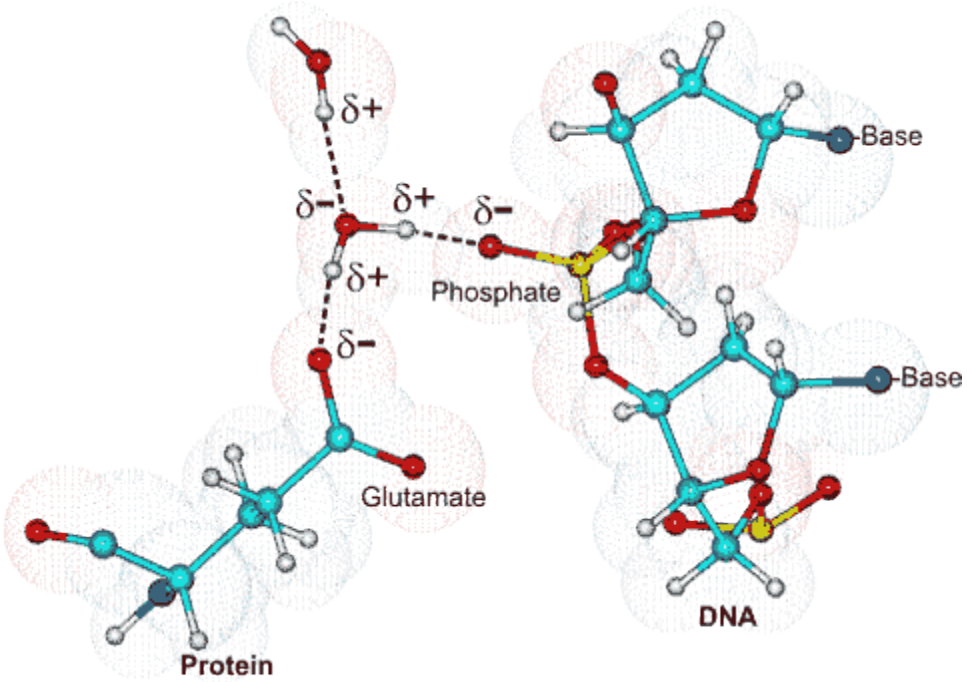


The hydration of the B-DNA minor groove is dependent on the DNA sequence with water-bridge lifetimes varying from 1 to 300 ps [1767], depending on the sequence; usually involving single water molecules connecting the strands but connection via pairs of water molecules, with varying interchange between these forms, may allow greater structural flexibility in the DNA and/or interaction with specific proteins [1605]. There is a spine of hydration running down the bottom of the B-DNA minor groove, particularly where there is the A=T duplex [145] (see right, where the water oxygen atoms are shown large green and red, where the red atoms are of the primary hydration water and the green atoms are of the secondary hydration water, [1136]), which is important in stabilizing it [146]. Thus, A=T duplex sequences favor water binding in the minor groove and also protein binding there driven by the large entropy release on this low entropy water's release [1136]. Water molecules hydrogen-bond by donating two hydrogen bonds, so bridging between thymine 2-keto(s) and/ or adenine ring N3(s) in sequential opposite strands (that is, not paired bases). This water has been called cross-strand bridging water (CSBW) and appears to be important for charge transfer (hole transport) between separated guanine bases down the DNA duplex [1221]).CSBW water is fully hydrogen bonded by accepting two further hydrogen-bonds from secondary hydration water, so fixing the primary hydration water more

firmly in place such that they exchange slower (0.9 ns) than any other water hydrating the DNA. The primary hydration may occur regularly down the minor groove connecting the strands but any cooperative effect is through the secondary hydration. The minor groove has a complex hydration pattern including water hexagons from the initial spine of hydration (above) through secondary hydration out to the 4th aqueous shell [797]. This hydration is more strongly held than in the G $\equiv$C duplex giving rise to greater apparent hydration (about 44 water molecules per A=T duplex base pair [179]). The A=T base pairing produces the narrower minor groove and more pronounced spine of hydration, whereas the G $\equiv$C base pairing produces a wider minor groove with more extensive primary hydration, due in part to the 50% greater hydration sites. Such solvent interactions are key to the hydration environment, and hence its recognition [1565], around the nucleic acids and directly contributes to the DNA conformation; B-DNA possessing higher phosphate hydration, less exposed sugar residues and smaller hydrophobic surface, is stabilized at high water activity whereas A-DNA, with its shared inter-phosphate water bridges, is more stable at low water activity. Thus, if the relative humidity is kept constant, there will tend to be a transformation from B-DNA to A-DNA with increasing temperature [179]. The much greater loss in primary hydration of G $\equiv$C base pairs (compared with A=T base pairs) on changing from B-DNA to A-DNA is clearly responsible for the tendency of G $\equiv$C base pairs rather than A=T base pairs to form the A-DNA conformation. In contrast to B-DNA, A-DNA possesses a hollow core down its axis where water can form a hydrogen bonded structure linking to the bases from the side of the major groove (as shown above) [553]. Clearly any disruption of this core structure may lead to the A-DNA $\longrightarrow$ B-DNA transition.

**DNA processing**

The processing of the genetic information within DNA is facilitated by highly discriminatory and strong protein binding. It has been shown that the interfacial water molecules can serve as 'hydration fingerprints' of a given DNA sequence [889]. The major driving force for the specificity is the entropy increase due to the release of bound water molecules (estimated at 3.6 kJ $mol^{-1}$ for minor groove water and 2.3 kJ $mol^{-1}$ for major groove water, both at 300 K [1096]),[c] with the DNA sequence determining the hydration pattern in the major and minor grooves (see above). Less perfect (that is, weaker) binding involves mainly secondary hydration water loss and so would allow sliding of the protein along the DNA [1176], facilitated by the remaining primary hydration water molecules [889]. For example, about 110 water molecules are released on binding of the restriction endonuclease *Eco*RI to its site GAATTC leaving an essentially dry interface and firmly bound complex (with binding constant ~10,000 times that for nonspecific binding), whereas changing just one base out of the recognition sequence leaves those water molecules mostly unaffected and only little different from *Eco*RI non-specifically binding to DNA [1176b]. Thus, the key to the formation of specific links between proteins and DNA is that the interfacial water molecules allow the protein facile movement along the binding cleft whilst retaining contact information [1443]. Final binding makes use of both direct and water-mediated hydrogen bonds; for example, the restriction endonuclease MspI makes specific contacts with all eight bases in the four base pair recognition sequence (5'-CCGG-3' and complementary 3'-GGCC-5'), by six direct and five water-mediated hydrogen bonds and thirteen water-mediated links to the phosphates [1444].




Protein sliding along the DNA is assisted by uniform complementary electrostatic interactions between the positive protein and negative DNA as moderated by the intervening water, whereby the protein follows the helical pathway of the groove rather than jumping between the major groove and the more negative minor groove [1176c]. Where negative charges exist on the protein that create unfavorable binding electrostatics, the similar charges may be screened, as shown right. It is important that a balance of positive and negative charges exist to ensure that the binding is generally not too strong, so avoiding excessive binding friction except where required.

It has been (independently) proposed that the separation of DNA double helices is enabled by the formation of clathrate-like water structuring using its screening dipoles [1222], an idea that ties this basic life process to the ES $\rightleftharpoons$ CS equilibrium and its icosahedral water clusters.

Highly structured water molecules, with lengthy residence times, have been found to be essential for the structural dynamics and function of ribozymes [1106] (catalytically competent highly structured noncoding RNAs) where water communicates structural rearrangements in an analogous manner to its action around many proteins.

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**Footnotes**

Both methods have drawbacks. X-ray crystallography gives better resolution at lower hydration but the lower hydration will change the structure. Another disadvantage is its inability to define the orientation of water molecules. The results of molecular dynamics calculations are highly dependent on the methodology and force field used.

The ribose residue is a furan possessing a flexible (five-membered) ring with low energy barriers between a number of conformations. This encourages localized weak hydrogen bonding around its free 2'-hydroxyl group (for more details of this see the polysaccharide hydration section).

The entropy 3.6 kJ $mol^{-1}$ for minor groove water may be compared with that of ice (6.0 kJ $mol^{-1}$ 0 °C), indicating that the minor groove water tends towards being ice-like.

Source: http://www1.lsbu.ac.uk/water/nucleic.html