Introduction to Nucleic Acids:
Forces That Stabilize Nucleic Acid Double Helices

Before we begin describing the structural features of the oligonucleotides and double helices, a few remarks about the forces that govern base-base interactions are required. Two different interactions exist: a) those in the plane of the bases (horizontal); most commonly hydrogen bonds; b) those perpendicular to the base planes or "base stacking" effects; these are stabilized by London dispersion forces and the hydrophobic effect.

1) Hydrogen bonds.

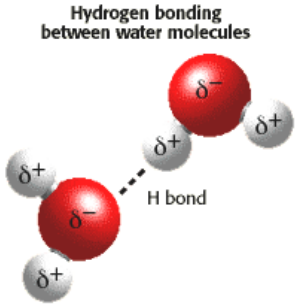


Figure 14

Hydrogen bonds are electrostatic in character. In general a hydrogen bond

X - H ··· Y

is formed if a hydrogen atom connects two atoms of higher electronegativity. Since these bonds are electrostatic, their strength depends on the partial charges located on the component atoms in the bond. The interaction between two water molecules, probably the most common hydrogen bonding interaction on the planet looks like this (Figure 14):

Under the influence of a hydrogen bond, the H becomes more electropositive and X,Y becoming more negative. This affect increases the affinity of X,Y for H and strengthens the interaction. If Y is the oxygen of the -OH group, the hydrogen attached to it is more positive and hence becomes a better donor.

Table 2 Comparison of Some Energy Values in Covalent and Hydrogen Bonds

Bond type	Bond length Å	Bond energy kcal/mole	Energy required for lengthening by 0.1 Å kcal/mole
Covalent			
C–C	1.54 ± 0.02	83.1	3.25
C–H (in ethane)	1.09 ± 0.02[a]	98.8[b]	3.60[c]
Hydrogen bond			
O–H · · · O	2.75 ± 0.2[d] (O · · · O distance)	3 to 6[c]	0.1[b]

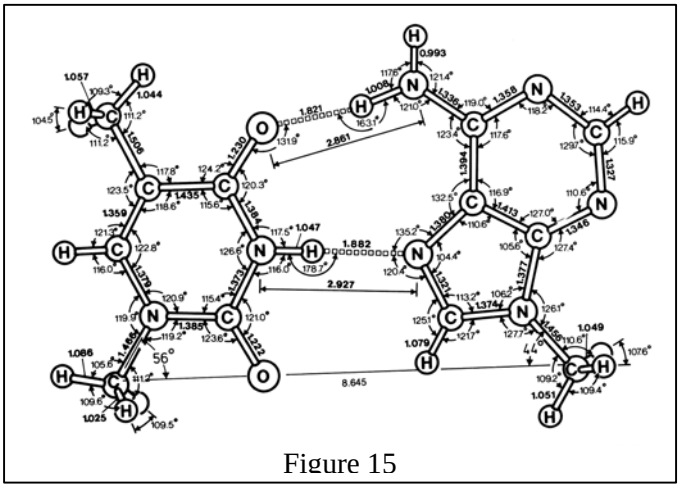


Figure 15

As you should already know, the strength of the an H bond is 20-30X weaker than a covalent bond. This weakness is reflected both in the bond's greater length and is relatively weak directionality **(TABLE 2)**.This weakness should not be mistaken for insignificance!!!! Moreover, the "slop" in directionality is not limitless. In fact, the tolerable distortion level in the angle of a hydrogen bond measured between the vector of the bond and the angle of the X - H bond is less than 20°. That is to say that the most favorable hydrogen bond angles are 180° **(FIGURE 15)**. Some bonds in the figure are distorted 27° and hence is weaker than the others distorted less than 2°.

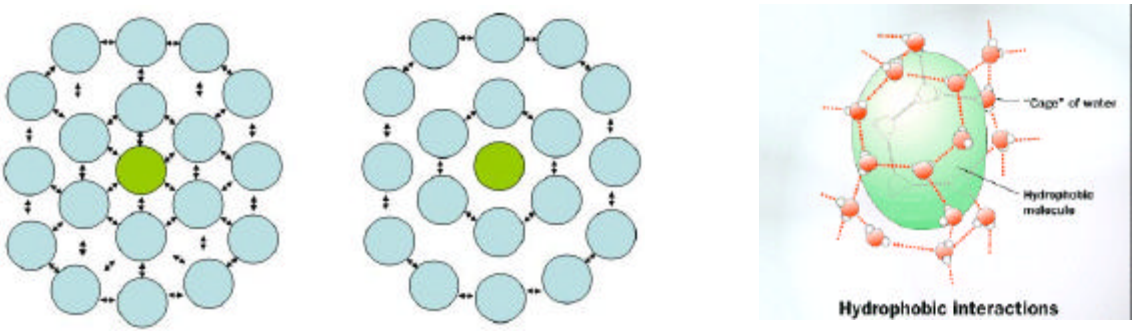
2) Base Stacking.

Bases in solution pile up like coins in a roll. In aqueous solution, the bases in a single stranded oligonucleotide are stacked such that the base planes are separated by their van der Waals distance, 3.4 ? , parallel to one another. Base stacking is the least understood but, undoubtedly most important force stabilizing helices.

Stacking is a diffusion controlled, additive, and stabilized by weak forces. The enthalpies associated with base stacking are favorable, while the entropy associated with the stacking of the bases is strongly unfavorable. The stacking reaction is overall favorable, however, since the entropy and enthalpy of the solvent are both strongly favorable. Stacking is made-up of two separate forces: hydrophobic effect and London dispersion forces.

2a. Hydrophobic interactions.

If a hydrophobic base is dissolved in water, the water molecules cluster around it in an order fashion. This is caused by the fact that they cannot form H-bonds with the non-polar base and adopt an ordered "clathrate" structure to maximize H-bonding with itself. This ordering is a very unfavorable entropy change for the water. Burying this hydrophobic base in the stack, releases this water and results in an overall entropy gain for water (Figure 16).



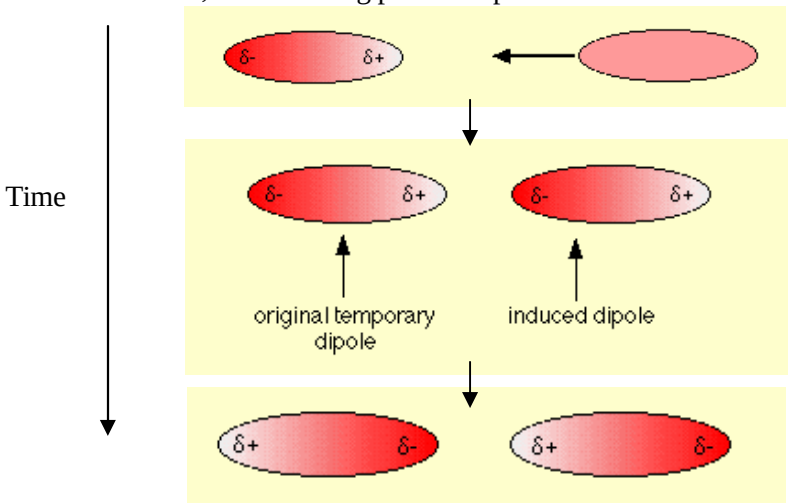
A **B**

Figure 16

The importance of the hydrophobic effect in helix formation is seen by considering the effect on the energetics of solvent interactions upon folding the non-polar bases into the helical structure. Folding the polar backbone atoms, into a regular structure has slightly unfavorable ? H and slightly unfavorable TΔS. The effect of burying the non-polar side chains is dramatic, the T? S of the solvent is incredibly favorable, and the ΔH is also favorable (owing to the now completely satisfied H-bonding potential of the solvent).

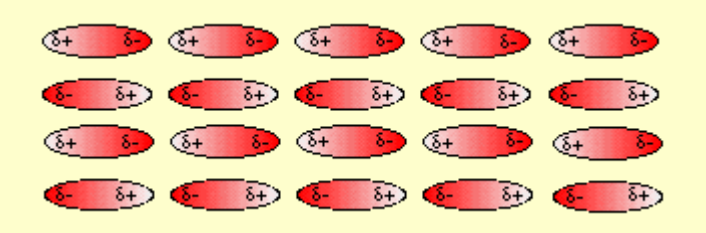
2b. London Dispersion forces.

The bases stack upon one another at their van der Waals distance. It is at this distance where two molecules have an attraction for one another. This attraction, termed a van der Waals interactions is a gravitational forces. At too close a distance the electron of the two approaching molecules overlap, causing repulsion. At any given instant, the electronic charge distribution within atomic groups is asymmetric due to electron fluctuation, Therefore, dipoles created in on group of atoms polarize the electronic system of the the neighboring atoms or molecules, thus inducing parallel dipoles that attract each other.



These forces are additive and are extremely distance dependent, falling off with the sixth power of distance (r^6).

There is no reason why this induced dipolar interaction has to be restricted to two molecules. As long as the molecules are close together this synchronized movement of the electrons can occur over huge numbers of molecules.



Since London dispersion attraction depends on formation of induced dipoles, the polarizable p electron cloud of the aromatic bases is extremely important. Therefore, stacking requires aromaticity of the bases-nonaromatic bases do not display stacking interactions. The strength of stacking interactions depends on how polarizable the p electron cloud of a base is. This in turn depends on the e- withdrawing or donating potential of the base substituents. These determine the primary basis for base stacking which is the electron structure of the bases. Since all bases have different substituents, the stacking potential of the bases are all different. Additionally, the electronic structure of a base can be modified by chemical modification-e.g., alkylation, halogenation.

Table 3 Total Stacking Energies [kcal/mole dimer] for the Ten Possible Dimers in B-DNA Type Arrangement Obtained by Quantum Chemical Calculations[a] [From (542)]

Stacked dimers	Stacking energies [kcal/mole dimer]
↑C·G / G·C↓	−14.59
↑C·G / A·T↓ ↑T·A / G·C↓	−10.51
↑C·G / T·A↓ ↑A·T / G·C↓	− 9.81
↑G·C / C·G↓	− 9.69
↑G·C / G·C↓ ↑C·G / C·G↓	− 8.26
↑T·A / A·T↓	− 6.57
↑G·C / T·A↓ ↑A·T / C·G↓	− 6.57
↑G·C / T·A↓ ↑T·A / C·G↓	− 6.78
↑A·T / A·T↓ ↑T·A / T·A↓	− 5.37
↑A·T / T·A↓	− 3.82

[a] Arrows designate direction of sugar phosphate chain and point from $C_{3'}$ of one sugar unit to $C_{3'}$ of the next, both carbons attached to the same phosphodiester link.

Stacking is BOTH base composition and base sequence dependent. In general, the stacking interactions of base paired nucleotide dimers containing G+C base pairs are more stable than those containing A+T base pairs **(TABLE 3)**.

Another generalization that can be made is that

3'pyrimidine-purine5'
5'purine-pyrimidine3'

is more stable than

3'purine-pyrimidine5'
5'pyrimidine-purine3'

For example $(5'G\text{-}C3')_2$ is 5kcal/mol more stable than $(5'C\text{-}G3')_2$. The origin of this sequence-energy correlation is seen in **FIGURE 17**. In the alternating purine,pyrimidine sequences, overlap between adjacent base pairs in a stack is much greater in B-DNA the pyrimidine, purine alternation, note the polar groups placed over the center of the p electron cloud, than the purine-purine stack.

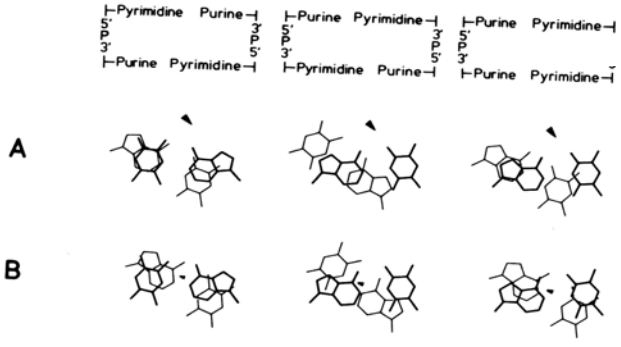


Figure 17 Base stacking overlaps in A- and B-type double helices, illustrated from top to bottom for A-, B-, and D-DNA. A–T base-pairs are stacked in sequences T–A on A–T, A–T on T–A, and A–T on A–T. The T–A on T–A stacking is comparable to A–T on A–T, with top and bottom reversed. Because views are perpendicular to base-pairs and not along the helix axes, the latter project as lines, indicated by arrows pointing from upper to lower base-pair. Note that in A- and D-DNA, arrows point in opposite directions because in the former the tilt is positive and negative in the latter. The arrow in B-DNA is smallest because the tilt is only slightly negative. For C-DNA the stacking patterns are between B- and D-DNA. Redrawn from (845).

Helical Formation

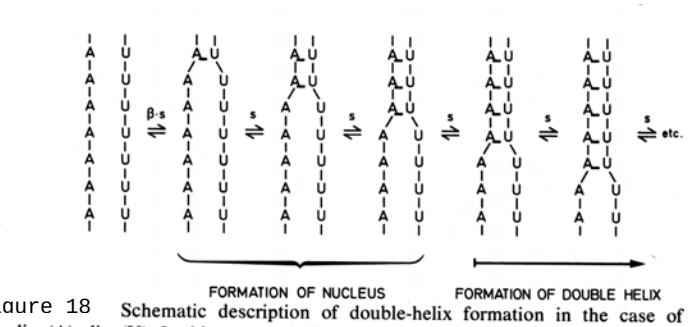


Figure 18 Schematic description of double-helix formation in the case of oligo(A)·oligo(U). In this system, helix growth parameter s is about 10 at 0°C and 1 at the melting temperature. Nucleation parameter β, 10^{-3} liters/mole, diminishes stability constant $K = \beta \cdot s$ of primary base-pair formation but does not influence formation of additional, stacked base-pairs which form cooperatively with $K = s$ according to a *linear Ising model*. In constrast to the *isodesmic model* for base stacking (Figure 6-9), where each step is independent of the other, in the *cooperative process* described by the Ising model, base-pair formation and stacking are influenced by the next neighbors, except for the very first base–base association.

Let us now turn to how both base stacking and H-bond formation are involved in helix formation. The association of double stranded polynucleotide helices is a cooperative process. One can think of helix formation as analogous to closing a zipper. The first step is association of the a single base pair with a stability constant expressed as the product of a nucleation parameter, b, which essentially represents the unfavorable entropy of bringing together two ends of separate chains and a chain growth parameter, *s*, which represents the favorable aspects of hydrogen bonding and hydrophobic interactions **(FIGURE 18)**.

The addition of a second stacked base pair is not influenced by the nucleation parameter because the proximity effect is taken care of in the first association. This is the basis of the cooperativity of helix formation, **base pair formation and stacking are influenced by the nearest neighbors, except for the first base pair formed**. Because of the overall unfavorability of the nucleation constant, ß, the energy of helix formation is unfavorable until after about three base pairs have formed. From then on, growth of the double helix is spontaneous, due mainly to the geometrical constraints of the sugar phosphate backbone, as implied by the sterochemistry of the nucleotide unit, whose preferred configurations is preset to form a double helix. Also, the summation of these weak forces, over a number of nucleotides provide cooperative energy. This is illustrated schematically in **FIGURE 19** and quantitatively expressed by K, the helical stability constant.

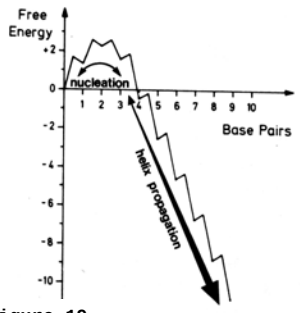


Figure 19 After the unfavorable positive free energy contribution in the nucleation process is overcome, the free energy for additional steps becomes negative and the helix grows spontaneously. Relative total free energy (ΔG) of helix formation in arbitrary units is plotted as a function of the number of consecutive, stacked base-pairs assembled into a helical array. From (544).

$$K = (ß*s) * (n*s)$$

where ß<1 (~10^{-3} *l* M^{-1}), s= 10 @ 0°C and 1 @ T_m and n=number of base pairs

Helical Breakdown/Denaturation (Melting)

The denaturation of a double helix is also cooperative, for much the same reasons as the formation is. In unzipping a helix, a bulge is formed due to input of energy. These bases are unable to H-bond with solvent and the solvent is order around the aromatic ring. In order to relieve this unfavorable situation, a nucleus of stacked single-stranded polynucleotide is formed which has favorable van der Waals interactions and has reduced hydrophobic surface in contact with the solvent. Then on the same ideas for unzipping apply as for zipping