# Module 9 / Hybridization of DNA/RNA Segments



Describe the criteria for determining the most stable structure between two DNA molecules, two RNA molecules, between a DNA and a RNA molecule.

Describe the hydrogen bonding that exists between complimentary base pairs in DNA and RNA.

## Hybridization of DNA and RNA

On the previous page you determined that the most stable complimentary base pairing takes place between A and T with two hydrogen bonds and between G and C with three hydrogen bonds in DNA. Combining that finding with the backbone information that described DNA as containing A, T, G, and C as the possible bases and A, U, G, and C as the possible bases in RNA, the complimentary base pairing in RNA would include A with U (examine the difference in structure between T and U) with two hydrogen bonds and G with C as described in DNA. In each case these comlimentary base pairings included a purine hydrogen bonded to a pyrimidine. This means that the distance between the attachment sites for the sugar in all the base pairs are identical giving uniform dimensions to the distance between the two backbones along the length of two strands that are hydrogen bonded together.

Because T and U are identical in the hydrogen bonding that each makes with A, this means that a backbone (strand) of DNA can hydrogen bond with another strand of DNA but also with a strand of RNA. The ability of the strands of DNA and RNA to hydrogen bond with each other either as homodimers (DNA-DNA, RNA-RNA) or as heterodimers (DNA-RNA) is referred to as hybridization.

As a general rule, hybridization will take place to maximize the number of hydrogen bonds that can be formed between two polynucleotide strands. However, another structural limitation is placed on the formation of the hydrogen bonds between two lengths of polynucleotide strands: the strands must be anti-parallel to each other. This means that the since the backbne has a 5' end and a 3' end, two strands hydridized to each other must have one strand oriented 5' to 3' and the complimentary strand 3' to 5' as illustrated below.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5'- | G- | C- | A- | U- | A- | G- | -3' |
| 3'- | C- | G- | U- | A- | U- | C- | -5' |

This short duplex structure is anti-parallel and has 15 hydrogen bonds holding the two strands together. As written this is a duplex of two strands of RNA. The figure below shows the same structure as a duplex of DNA.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5'- | dG- | dC- | dA- | dT- | dA- | dG- | -3' |
| 3'- | dC- | dG- | dT- | dA- | dT- | dC- | -5' |

Can you generate a hybrid structure using the top 5' to 3' DNA strand from the figure above and create the appropriate RNA strand hybridized to it?

did I get this

The examples thus far imply hybridization between two separate strands of polynucleotides. Is it possible to have hybridization occur within a single strand of DNA or RNA? If so, how would you illustrate that intramolecular structure?

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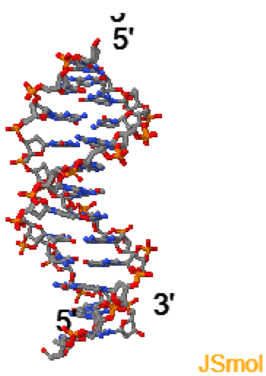
## Stability of DNA - Base Stacking and Hydrogen Bonds

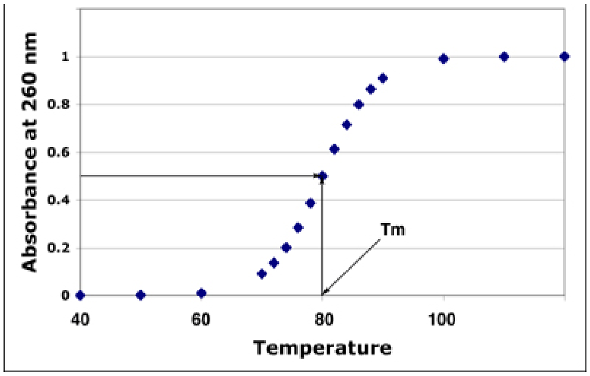
The stability of double stranded DNA is due to two factors, hydrogen bonds and base stacking. Hydrogen bonds provide an attractive force between the strands while base stacking (van der Waals) stabilizes the helical structure. The Jmol below shows the stacking of the bases and illustrates the hydrogen bonding pattern for a GC basepair. The stability of the DNA is to some extent determined by the number of hydrogen bonds holding the two strands together. Thus DNA with more GC base pairs is likely to be more stable than one with more AT base pairs.

DNA Structure (B-helix)




Experimentally it is possible to measure this stability by following the melting of DNA with increasing temperature. Below is a graph of the melting of DNA that coincides with an increase in the ultraviolet (UV) absorbance of the DNA at 260 nm. The increase in absorbance is referred to as the hyperchromic effect and is a measure of the breaking of the hydrogen bonds between the bases and the separation of the two strands.

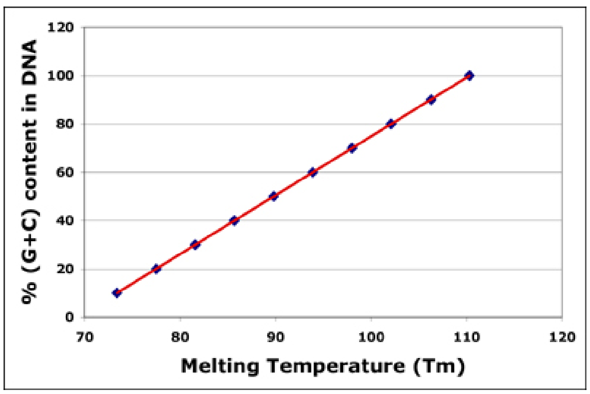
Measurement of Melting Temperature (Tm) of DNA




The melting temperatre (Tm) for a piece of DNA is the temperature at which 50% is no longer hybridized.

The midpoint of the transition from the double stranded to the single stranded form of the DNA is called the melting temperature (Tm) for the DNA. In most organisms the Tm of the chromosomal DNA ranges from 85-100 degrees C. It is possible to determine the composition of the DNA experimentally from its Tm because the Tm of DNA is directly proportional to the GC content of the DNA as graphically illustrated below.

Measuring the composition of DNA




The melting temperature is directly proportional to the composition of the DNA. This is also a measure of the hydrogen bonding content of the DNA.

While the melting temperature does not tell us anything about the proteins that are coded by the DNA, it does tell us something about the tolerance of the organism in which the DNA is found. For example, thermophilic bacteria (those that survive at extremely high temperatures) have DNA with very high Tm values so that their DNA does not melt at their normal environmental temperature. As we will see later, this also has implications for how the same proteins can be made in two different organisms using DNA with vastly different compositions.

In summary, for the hybridization of two strands of DNA and RNA

1. the chains are antiparallel
2. the two chains are held together by hydrogen bonding between bases
3. the stability of the DNA is directly proportional to the number of hydrogen bonds between chains
4. hydrogen bonding between strands of DNA and RNA follow the pattern
   - A hydrogen bonded through two bonds to T in DNA
   - A hydrogen bonded through two bonds to U in RNA
   - G hydrogen bonded through three bonds to C in both DNA and RNA