DNA Structure, Chemistry

1. Query: Are A and B forms of DNA plectonemic? What is the difference between paranemic and plectonemic helices.

Yes, DNA is a plectonemic double helix. The strands are inter-wound. All forms of DNA A, B and Z are plectonemic. Imagine a segment of DNA double helix. Hold the ends (5' and 3') of one of the strands. Let your friend hold the ends of the other strand. If the two of you pull the strands, they will not come apart (but be entangled).

DNA is not paranemic. Paranemic is the opposite of plectonemic. If you place two right handed helices side by side you can separate them by pulling them apart. Such a double helix would be paranemic. Imagine two springs. If you slide one into the other you have a paranemic double helix. If you slide the spring out, you have separated the two helices. If you performed the same pulling experiment as above, you can separate the two springs (or helices). One slides away from the other (the reverse of what you did to get the double helix).

The handedness is not important for paranemic or plectonemic. You can have a right or left handed plectonemic helix. The same holds for paranemic.

Z form DNA is a left handed plectonemic double helix

2. Query: Could you clarify why agarose and polyacrylamide are used for separating DNA molecules within different size ranges?

Larger DNA (RNA) molecules have longer reorientation intervals compared to smaller ones. This is regardless of whether you use agarose or polyacrylamide for electrophoresis matrix. Under the voltage gradient, smaller molecules will migrate towards the positive electrode (DNA and RNA are negatively charged polymers) with higher velocity.
Since, agarose has larger pore size compared to polyacrylamide, small DNA molecules can get through them more easily than in polyacrylamide. In other words, the difference between the reorientation times of a molecule that is 50 bp and one that is 52 base pairs will be negligible in agarose. However it will still be significant in polyacrylamide. Hence agarose is preferred for separating molecules in the size range from a few hundred base pairs to several thousand base pairs. Polyacrylamide is useful for separating DNA molecules in the range of a few base pairs to a couple of hundred base pairs.

And regardless of agarose or polyacrylamide, the concentration of the gel matrix will determine the reorientation time. The higher the percentage of agarose (or acrylamide), the greater is the barrier to movement and longer the reorientation time. Lower percentage gels are use for separation of larger DNA molecules; higher percentage gels are used for separation of smaller DNA molecules.

3. Query: **Why is the DNA stability in aqueous buffer higher than that in ethanol solution**?

Let us heat two samples of the same DNA (same concentration), one in aqueous solution and the other in 10% ethanol solution. We find that the sample in ethanol melts (strands separate) at a lower Tm (melting temperature), as measured by the increase in absorbance of UV light at 260nm wavelength.

Explanation: Hydrophobic/stacking interactions between base pairs contribute more to stability of the DNA structure than hydrogen bonding interactions between the bases.

Water is a hydrogen bonding solvent. When the strands begin to separate in water solution, water will form hydrogen bonds with the free bases. That is water competes with complementary bases for hydrogen bonding. However, there is little or no competition here with regards to hydrophobic interactions. With competition for hydrogen bonding (and not for hydrophobic interactions), DNA melts at a certain temperature T1.

Ethanol is a weak hydrogen bonding molecule but the ethyl group can take part in hydrophobic interactions. Thus, when the strands separate, bases have to compete with ethanol for hydrophobic interaction, but there is little competition for hydrogen bonding. DNA under these conditions melts at a temperature T2, which is lower than T1.

Conclusions: When hydrophobic interactions are interfered with (as in the ethanol solution) DNA becomes less stable (has a lower melting temperature Tm) than when hydrogen bonding interactions are interfered with (as in the aqueous solution). Therefore hydrophobic interactions contribute more to the stability of the structure than hydrogen bonding!

[The final stability of the DNA structure derives form (a) hydrophobic/stacking interactions of base pairs plus (b) hydrogen bonding interactions from complementary base pairs. The experiment we did tells us that when you compete the hydrophobic interactions (with ethanol), the DNA structure gets destabilized more easily than when you compete the hydrogen bonding interactions (with water). Therefore hydrophobic interactions provide more stability than hydrogen bonding interactions.]

4. Query: Why is it easier to discriminate a base pair in B form DNA by contacting it through t he major groove than through the minor groove?

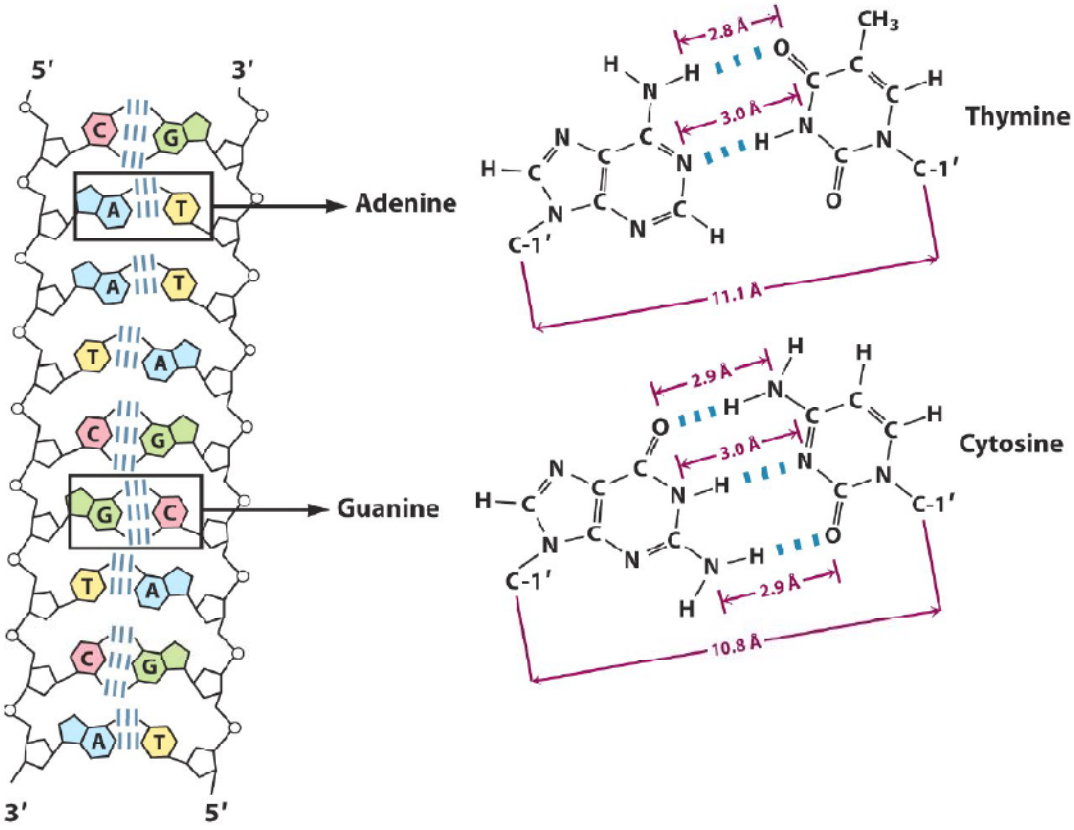
Discrimination means being able to tell one base pair apart from the other three; for example, A-T from G-C, T-A and C-G

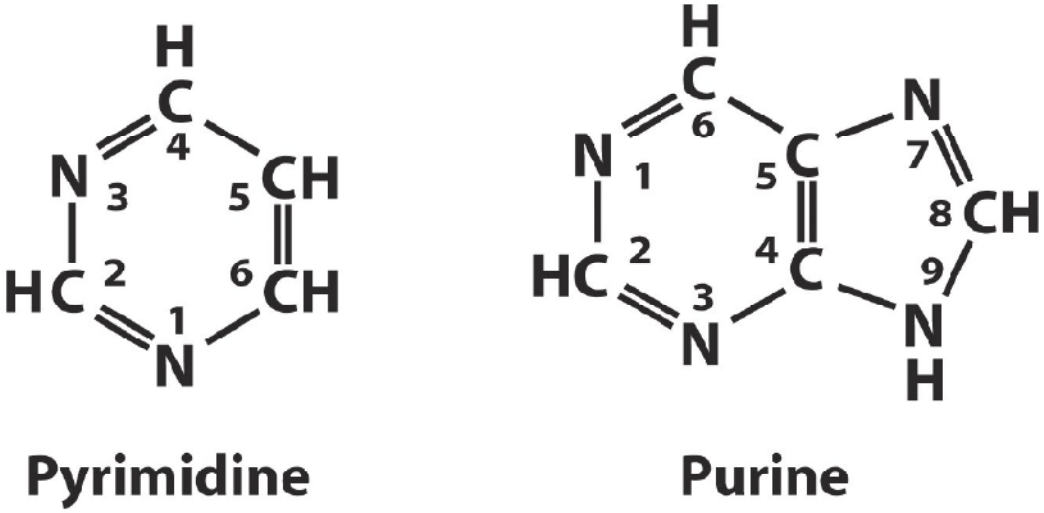
First, look at the numbering of purines and pyrimidines. Remember where you start and which way you go. For pyrimidines, you start with the nitrogen 1 which is linked to the dexoyribise (C-1) and go clockwise. With purines, you start with the nitrogen at the left top corner (the way the ring is drawn in the diagram here, and you go counterclockwise (1-6). For the five membered ring on the right you start at the top right corner nitrogen (N7), and go clockwise to nitrogen at the bottom (N9), which is linked to the deoxyribose (C-1).

Now look at the A-T and G-C base pairs drawn in the top diagram showing their hydrogen bonded states in B form DNA. In the diagram, when you look at the base pairs from the bottom side you are viewing the minor groove. Remember the rule: you can see the C1-N glycosidic

bonds extending towards the helix axis to form an acute angle. When you look at the base pairs from the opposite side (top in the diagram), you are viewing the major groove.

To make things easier you may want to number the atoms in the base pairs according to the rule we described above.





You can see the following atoms or groups of Adenine and Thymine in the major groove:

Adenine: N7; the amino group on C6. **Thymine**: the carbonyl oxygen at C4; the methyl group at C5. All of these groups are good for base pair recognition: Nitrogen, oxygen and amino groups by hydrogen bonding, the methyl group by hydrophobic/Van der Waals contact.

For the A-T pair, you can see the following groups from the minor groove side:

Adenine: N3. **Thymine**: the carbonyl oxygen at C2

Similarly for the G-C pair, the important contact positions in the major groove are;

Guanine: N7; the carbonyl oxygen at C6; **Cytosine**: the amino group at C4.

For this base pair, the minor groove side reveals:

Guanine: the amino group at C2; N3. **Cytosine**: the carbonyl oxygen at C2

[You should at least know that for **purines**, N7 and the substituent group at C6 are disposed in the major groove; N3 and the substituent at C2 (the amino group of Guanine) are located in the minor grove). For **pyrimidines**, the substituent at C4 and the methyl group at C5 (for thymine) are positioned in the major groove. The carbonyl oxygen at C2 is present in the minor groove.]

You can already see that there are more contact points (and hence more discrimination possible) from the major groove side.

You may do the following exercise. Copy the A-T and G-C base pairs on to a transparent sheet. Flip these and make another copy on a second transparent sheet. You have now the T-A and G-C base pairs as well. You can make rectangular cutouts of all four base pairs. You can place the GC base pair over the A-T bp by overlaying the purine and pyrimidine positions perfectly. You will immediately see how the contact points in the major and minor grooves are positioned differently. Now place the T-A pair over and adjust the positions as best as you can. There is a little play here because the C1 to C1 distance is slightly smaller 10.8Å for G-C (or C-G) pair compared to 11.1Å for A-T (or T-A) pair (see Figure above). But this is small enough to be not too complicated. And finally you can place the C-G pair over the T-A pair.

You will see how the contact points line up for each of the base pairs in the major and minor groove sides. There are more distinct hydrogen bond donor and acceptor possibilities (with additional discrimination trough the C5 methyl group of thymine in the major groove side compared to the minor groove side.

[When you overlap the base pairs, try to get the C1 (ribose)-N glycosidic bonds aligned, and then get the bases to overlap.]