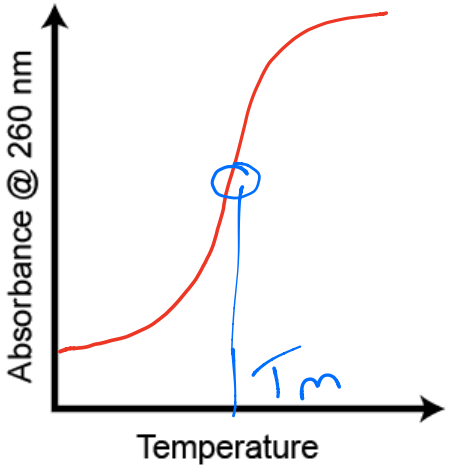
DNA Folding/Unfolding

1. DNA and RNA strongly absorb UV light. What part of these molecules is primarily responsible for this absorbance? Why did you choose this region? The nitrogenous bases absorb UV light. This region contains a nice conjugated pi system – hopefully you remember from Organic Chem that pi bonds absorb UV and conjugated pi systems absorb this light strongly and in the near UV. DNA absorbs maximally at 260 nm.

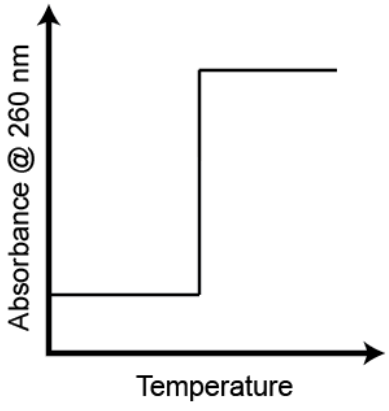
   The phosphate backbone also absorbs UV light, but at much lower frequencies.

2. The hyperchromic effect is used to study DNA melting. This is a general phenomenon that explains the difference in absorbance between folded and unfolded DNA and RNA molecules.
   a. Which absorbs light more strongly: folded or unfolded nucleic acids? Why did you pick this? Don't spend a lot of time on this in class, but be thinking about explanations behind physical observations that we make. Unfolded. The best answer is that it's due to the electronic interaction between the pi systems in the stacked bases. Another possible answer that you may have come up with deals with surface area – the free bases have more surface are to absorb the light while the confined bases have smaller surface for interaction with photons.
   b. Based on your answer above, predict what a DNA melting curve would look like. Melting curves monitor the unfolding of DNA as the temperature is increased.



   c. Mark your diagram with the melting temperature – this is the temp when 50% of the DNA is unfolded.
   d. Check out figure 24-22 in your book. Does the shape of the curve agree with your prediction?

   e. The sigmoidal shape ("S-shape") of this curve is very meaningful. What does this curve shape tell us about the melting of DNA? It means that the unflolding is **cooperative**. It takes an initial "spark" to get the DNA to begin unfolding, but once it gets going, the rest unfolds much easier. Understanding cooperativity is important! You should be able to explain what it means and why it's relevant.
   f. What would a step-shaped melting curve mean? It would mean that all the DNA in a helix melts apart (separates into individual strands) at the same temperature. So below the melting temp, the DNA is 100% folded and above the melting temp the DNA is 100% denatured.

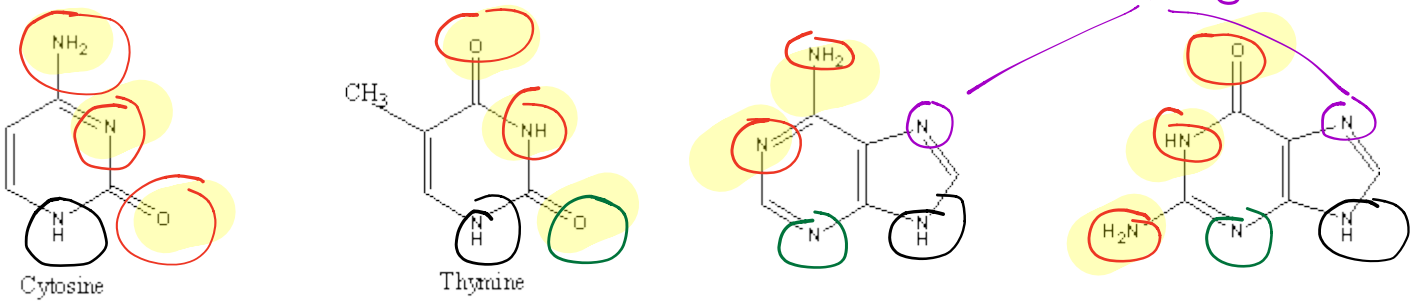


This exercise is going to help you understand the difference between the hydrophobic effect and pi stacking in DNA and consider the thermodynamic forces that stabilize DNA.

H-bonding

3. Below are the structures of each of the four bases in DNA.
   a. Identify all parts of the Watson and Crick face that will H-bond with water.
   b. Identify all parts of the Watson and Crick face that will H-bond with another base when DNA folds.
   c. Is there a difference between the number of H-bonds that will be present in folded and unfolded DNA? Not really – each N and O on the Watson and Crick face (the side of the base that base pairs in DNA) starts off with a H-bond with water and ends up with an H-bond with the complementary base after the helix forms.
   d. Based on your answers, do you think that H-bonding is an important part of the energy that helps DNA fold? Yes and no – it is important to ensure that DNA pairs up with the proper complementary sequence, but it is not important when we consider the energy that stabilized the DNA double helix.

major groove



Black = attached to deoxyribose

Green = minor groove... available to H-bond with H_2O in B-DNA

Pi Stacking

4. Summarize the hydrophobic effect. What are the two steps that need to be considered to understand the enthalpy/entropy relationship? Why is water such an important part of the phenomenon?

Water molecules are highly ordered around non-polar molecules. When these non-polar molecules aggregate, the waters are shed into bulk solvent ($\Delta S >>> 0$). This is the primary driving force (energetic reason) behind hydrophic molecules aggregating in water.

5. Complete the structures of dAMP and dTMP.

   a. On the structures above, identify all regions that will become shielded from solvent during DNA folding.

b. Are the regions that you identified above hydrophobic? No, as we saw above, these regions H-bond with water!

c. Can water interact with these regions using any of the common intermolecular forces? If so, which? H-bonding

d. How would the solvent reorganization be different than what we discovered in the hydrophobic effect? Yes – water can interact with the bases normally (through H-bonds and dipole-dipole interactions) with the nitrogenous bases so they do no form such ordered structures. Consequently, the solvent reorganization is much less significant.

e. Do you expect the process of DNA folding to be driven primarily by entropy or enthalpy? Explain your choice. It's primarily an enthalpy term. The small, favorable solvent reorganization entropy is cancelled out by the order created when the helix forms (on a simple level – two molecules become one; on a more complex level – the individual chains of polynucleic acids have a lot of degrees of freedom (they can flop about fairly unconstrained) – these degrees of freedom are lost when the helix forms and the torsion angles are restricted.

f. Based on your answers to the above questions, is DNA folding endothermic or exothermic?

g. Recalling that heat is a product in an exothermic reaction and a reactant for an endothermic process, can you use Le Chatelier's Principle to explain why DNA melts as the temperature increases.

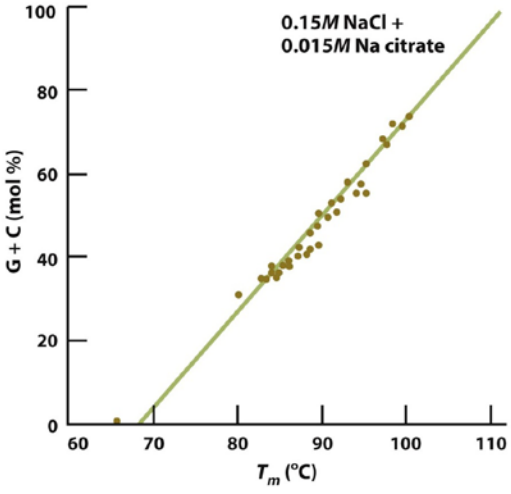
Unfolded ⇌ folded + heat

Heat is a produce for exothermic reactions. Increasing the temperature increases the "concentration" of heat. Based on LeChatelier's Principle, this should shift the reaction to reactants. This would suggest that increased temperature leads to a higher concentration of unfolded DNA. Yep, this makes sense. Science works.

A:T vs. G:C in DNA Stability

6. Summarize what this figure tells us about DNA stability. Increasing the GC content in DNA increases the melting temperature and therefore its stability.

7. As we figured out above, H-bonds are not particularly important in the energy that stabilizes DNA/RNA structures. Based on this, propose a reason that increasing the G-C content of DNA increases the melting temperature. Pi stacking is the most important energy that stabilizes DNA structure. Since H-bonds don't significantly contribute, GC base pairs MUST stack more favorably than AT base pairs.



8. Check out Table 24-2 from your book. Does this data support your proposed reason? If not, use the information in this table to propose a reason that GC content stabilizes DNA structure. Indeed, the table shows GC containing stacks are more favorable (more negative ΔG)