Assignment #3: Architecture and Energetics of Globular Proteins

Readings: B&T, Ch. 3,4,5,12.  Remember – Hour exam Feb 25!!
Research papers: Read along with RASMOL!
  Accompanying RASMOL file: Lyswt.pdb
* Blaber et al. (1993) Biochemistry 32: 11363 (Burying polar groups inside proteins)
  Accompanying RASMOL files: Lysa98s.pdb, Lysv149t.pdb

Even reading the best references, you will not get a good feeling for structures without working diligently with your molecular models. You MUST do the following exercises with them during spring break and be bring your models to class Feb 27 for a workout!

1. Build 10 prototype L-amino acids. These should be "generic," in that you need not put explicit R-groups. BUT, you should mark the proper R-group bond on the alpha carbon with, for instance a short green straw. Don't put on the alpha-H. IMPORTANT!! -- Make sure that you use the correct trigonal N and C atoms for the peptide bonds, as described in the kit instructions. Now, read your assignments well, until you think that you understand the structures of α-helices and β-strands, "out of the book."

2. Using these 10 amino acids, build an α-helix (approx 2-3 turns), N-to-C running upwards. Do this first totally by hand, i.e., just by twiddling the bonds and looking at pictures in textbooks. Then, after you cannot take any more frustration (but at least 45 minutes!), do it by going through, residue-by-residue, setting phi-psi angles to -60° (by eye). Don't forget to start putting in H-bonds (with white straws) after getting the 4th residue on! Otherwise, the model will droop (unless you happen to live in a zero-gravity environment).

   Notice the positions of R-groups in the 1st and 8th residue, the 2nd and 9th, etc. What's going on? This is not easy; it should take you at least two hours to master the exercises up to here. If you've got this far in less time, then you are approaching the problem frivolously.

3. Make two β-strands of 4 or 5 residues each. Check your phi-psi angles. From these strands, put in H-bonds to make an antiparallel 2-strand ribbon. What do you notice about the pattern of H-bonds and of R-groups? Did the structure form pleats?

4. With your ribbon, explicitly build in a cysteine on each strand, anywhere you like, and try to form a cross-strand disulfide bond (checking, of course, the correct straw sizes to use for C-S and S-S bonds!). What do you notice?

5. With your two strands, make a parallel ribbon. Are you having fun putting in the H-bonds? Alter this parallel ribbon so that every second residue is a D-amino acid. What happens??

6. See what happens when you explicitly build a proline into the first position of a helix. Then try to put it into the fourth position.
Problems

Assignment #3 (due Mar. 14)

1. Here are some spectroscopic melting-curve data for two versions of the β-adrenergic receptor phosphatase (βARF), the wild-type, and a mutant A25S. When fully melted, the protein's absorbance at 222nm approximately doubles. Analyze these raw data, and report for the two proteins:
   a. $T_m$ values
   b. $\Delta H^\circ$
   c. $\Delta S^\circ$
   d. $\Delta G^\circ$ at 25°C.

e. How much free energy is associated with the A-->S mutation? What else would you like to know before you tried to interpret this $\Delta \Delta G^\circ$ seriously?

<table>
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<th>T°C</th>
<th>A222, WT</th>
<th>A222, A25S</th>
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<tr>
<td>5</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
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f. What is the standard-state $\Delta G^\circ$ for each of these proteins at T=15°C?

2. A recent paper described the engineering thermostability into a globular protein (a protease). By mutating only 8 amino acid residues, they were able to raise the “melting temperature” from 45°C to over 90°C! There were three classes of mutations: (1) changing two residues to Cys to create a disulfide bond in the protein interior, (2) several Gly --> Gln mutations on the protein surface, and (3) several Ala-->Pro mutations, also on the protein surface.

The authors achieved their goal by “rational design,” by which they mean that they were guided by a single principle in choosing these particular mutations to stabilize the enzyme. What was this principle, and describe why these mutations worked.

3. Consider a pair of wild-type/mutant melting curves as above. Suppose further that for these sister-proteins, the standard-state entropy of folding is the same, $\Delta S^\circ$. Then, prove that the difference in $\Delta G^\circ$ between mutant and wild-type measured at any temperature, $\Delta \Delta G^\circ$, is simply given in terms of the shift in $T_m$, $\Delta T_m=T_m^{mut} - T_m^{wt}$.

$$\Delta \Delta G^\circ = \Delta S^\circ \Delta T_m$$
4. A single protein molecule is covalently attached to a flat inert surface through the amino group of a lysine residue located approximately in the middle of the amino acid sequence of the protein. The polymer is uncharged and hydrophilic. Experimental tests determine that the surface-attached protein is more resistant to denaturation than is the same protein when it is free in solution. In 2-3 sentences provide a simple, plausible explanation for this effect.

5. Problem on burying and hydrophobic cores. Suppose a spherical protein of total volume $V$ has $N$ residues, and suppose the average surface area per residue is $s_o$ and the average volume per residue is $v_o$.

Find $N$ such that the number of residues at the surface = the number of buried residues filling the volume.

Answer: $N \sim 288\pi v_o^2/s_o^3$

(Hint: $s_o \sim 50 \text{ Å}^2$. You should be able to figure out $v_o$ from your knowledge of chemistry.)

Look at a few proteins and decide whether this estimate is reasonable or nuts!