Graduate Topics in Biophysical Chemistry – CH 8990 03
Assignment 4

Due Monday, March 10

Some questions from this assignment have been adapted from those found in Cantor and Schimmel; others were inspired by Gordon Rule at Carnegie Mellon University.

1. A scientist performs a thermal denaturation experiment on a protein. Assuming that the protein is mostly β-strand, sketch the (protein) signal that the scientist would observe if she monitored the transition with:

   a. UV-Vis Spectroscopy

   b. Infrared Spectroscopy

   c. Circular Dichroism Spectroscopy

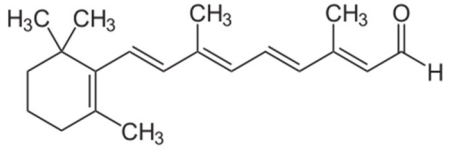
   For each experiment, specify what wavelength (or wavenumber) you chose for your plot and why you chose that wavelength. The magnitude of the transition is not as important as the direction (i.e., whether the signal increases or decreases).

2. van Holde, 9.6

3. How might you use a solvent perturbation of IR spectra to distinguish buried residues from exposed residues in a protein? What would it mean if in pure D_2O the IR absorption frequencies of several buried peptide residues were at a higher frequency than those of exposed residues for a protein with a small fraction of α helix or β sheet?

4. In class, we discussed that the n → π* transition for formaldehyde was disallowed by symmetry considerations. Using the symmetry method discussed in class, show that this transition is disallowed. (Hint: use the table from lecture to determine whether $\langle \pi^* | \vec{\mu} | n \rangle$ is zero.) Why might this transition nevertheless be observed in peptides?

5. Below is the structure for retinal, a chemical involved in vision. Retinal absorbs a photon and undergoes a conformational change, which alters the structure of its bound protein, rhodopsin. As shown, retinal is in its all-trans form (image from Wikipedia).



   The transition dipole moment ($\sqrt{D_{ab}} = \langle \psi_b | \vec{\mu} | \psi_a \rangle$) of retinal is found to be 10 Debye (D) for an absorption band centered at 500 nm. Recall that electric dipoles have the units of charge times distance, and 1 D = 10^{-18} statC cm, where statC is the cgs measure for charge (a statcoulomb; 1 statC corresponds to 3.34×10^{-10} C, and 1 statC = 1 $erg^{1/2}$ $cm^{1/2}$).

a. Show that the units for the Einstein B coefficient are $\left(\frac{\text{erg}}{\text{cm}^3}\text{Hz}^{-1}\right)^{-1}\text{s}^{-1}$.

As discussed in class, the B coefficient is the stimulated absorption/emission rate per unit energy density. The B coefficient is multiplied by a spectral energy density to obtain a rate. The spectral energy density $\rho(\nu)$ has units of energy per volume $\left(\frac{\text{erg}}{\text{cm}^3}\right)$ per frequency (in this case, Hz), so $[\rho(\nu)] = \frac{\text{erg}}{\text{cm}^3}\text{Hz}^{-1}$. When B is multiplied with $\rho(\nu)$, the overall unit is simply a rate, s^{-1}.

b. What is the B coefficient for retinal?

c. Your book gives the equation for the Einstein A coefficient (eq. 8.98), or the rate of *spontaneous* relaxation per energy density. What is this rate for retinal? (If you are consistent with your units, the rate of A is simply s^{-1}.)

d. How would your answer in part (c) change if the transition occurred at NMR frequencies (f = 600 MHz)? Assume D_{ab} remains constant.

e. If the length of retinal is 10 Å, estimate the energy of the first electronic absorption. You can assume that the 6 conjugated π bonds constitute a one-dimensional box, filled by the 12 bonding electrons. According to the Pauli exclusion principle, there will be two electrons per (filled) orbital, so the transition from the highest energy ground state to the lowest energy excited state will **not** involve a change from n = 1 to n = 2. Express your energy in kcal mol^{-1}.

f. How does your answer in part (e) compare to the observed energy at 500 nm (you'll have to convert this to kcal mol^{-1}).

6. Using the CD basis spectra for α helix, β sheet, and random coil (found on the following page), estimate the helical content of the restriction enzyme EcoRI from its spectrum (fig. 10.16 in your book). Be sure to show your work and justify the value you obtain. Hint: This problem is *very* similar to question #2.

Basis CD Spectra
(per-residue molar Δε)

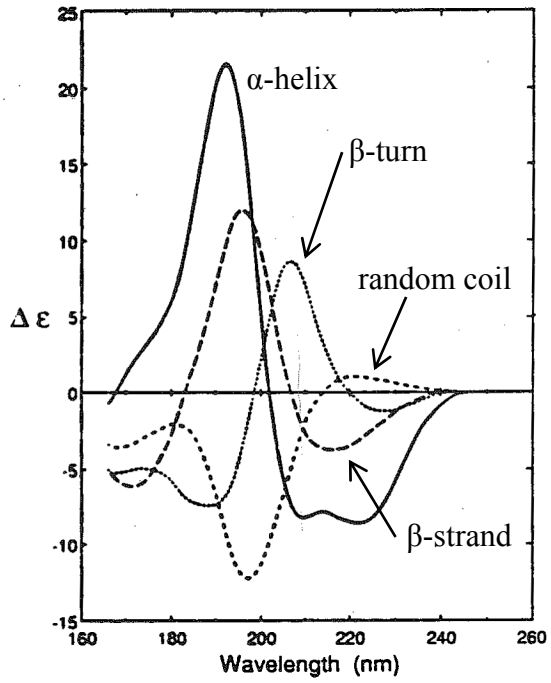


Fig. 2. The CD for various secondary structures: α-helix (——), antiparallel β-sheet (——), β-turn (····), and random coil (— —), redrawn from Ref. 7.

Taken from Johnson, W. C., Jr. (1990) "Protein secondary structure and circular dichroism: A practical guide." *Proteins: Structure, Function, and Genetics*. **7** (3): 205-14.