Biochemistry 503 – Fall 2003 - Exam II

Sign Pledge Here:
Questions are 10 points unless noted otherwise.

1. 15 points

(a) Write the Beer-Lambert equation with the appropriate units? If you know the amino acid sequence of a protein, how would you estimate the protein concentration from absorbance at 280 nm?

(b) What is the definition of fluorescence quantum yield? Which natural amino acids can be used for fluorescence spectroscopy, rank them in order of fluorescence sensitivity?

(c) Proteins show CD signals at far-UV (190-250 nm) and near-UV (> 250 nm) regions of the electromagnetic spectrum. Explain what each region detects? Draw one example CD spectrum corresponding to the type of information that may be obtained at each of these two regions?
2. (a) What is the definition and unit of NMR chemical shift? What are the ranges of detection for the NMR signals of the backbone amide protons at 500 MHz and 800 MHz spectrometers?

(b) What are the two kinds of coupling interactions between protons that are used for NMR measurements? List two examples of NMR experiments that allow you to detect these couplings. What range of distances can be measured by each coupling procedure?
3. (a) Give one example of a 2D NMR spectrum that detects a heteronuclear interaction? What range of information may be obtained from this spectrum?

(b) Give one example of a 3D NMR spectrum? What range of information may be obtained from this spectrum?
4. (a) Give two examples of non-covalent bonds that stabilize protein structures? In each case, how is the bond energy related to the distance between two interacting groups?

(b) How is free energy related to enthalpy and entropy? Write the equation with appropriate units.
5. (a) Peptidyl prolyl isomerases assist with the isomerization of which bond in proteins? Draw both possible isomers?

(b) Draw a schematic to show how GroEL-GroES complex assists folding of a polypeptide?
6. (a) Explain how NMR spectroscopy can be used to detect the dynamics of hydrogen bonds in a protein?

(b) Using 15N-labeled form of a given protein what kinds of NMR measurements allow estimation of rates and amplitudes of the motions in the protein backbone?
7. Binding of heavy atoms for isomorphous replacement changes the average observed diffraction intensity by the fraction \( \Delta I/I \), where

\[
\Delta I/I \sim \left( \frac{2N_h}{N_p} \right)^{1/2} \left( \frac{f_h}{f_p} \right)
\]

- \( N_h = \) number of heavy atoms
- \( N_p = \) number of protein atoms \( \sim 7*N_{\text{residues}} \)
- \( f_h = \) number of electrons in the heavy atom
- \( f_p = \) avg. number of electrons per protein atom \( \sim 7 \)

(a) For a 500 residue protein with one bound Pb atom (Z=82), what is the fractional change in intensity \( \Delta I/I \) ?

(b) Pb has 82 electrons; Nb has 41 electrons. What yields the better (larger) signal: one Pb atom or two Nb atoms?

(c) You have collected data from two crystals of a 700 residue protein; one is a wildtype (native) crystal and the other is a Ag (Z=47) isomorphous derivative. From 20-4Å, the \( R_{\text{sym}} (R_{\text{merge}}) \) of each dataset is 0.05

From 20-3.5Å, the \( R_{\text{sym}} (R_{\text{merge}}) \) of each dataset is 0.10

From 20-3.0Å, the \( R_{\text{sym}} (R_{\text{merge}}) \) of each dataset is 0.20

Over what resolution range (or ranges) would you expect to be able to detect the isomorphous difference from the bound Ag? Please justify your answer.
8. You have solved two crystal structures of a G-protein coupled receptor (GPCR), one in the absence and one in the presence of a potent agonist (ligand that binds with high affinity). In the absence of agonist, the atoms in the residues lining the binding pocket have an average B factor of 80Å². In the presence of agonist, the average B factor of these atoms drops to 30Å².

a. What is your interpretation of this result?

b. What is the difference in rms amplitude of movement between these two sets of atoms in the two structures?
9. A typical potential function for molecular dynamics looks like:

\[ V(r) = \sum \frac{1}{2} k_b (b - b_o)^2 + \sum \frac{1}{2} k_\theta (\theta - \theta_o)^2 + \sum k_\phi [1 + \cos(n\phi - \delta)] \]

\[ + \sum \left[ \left( \frac{A}{r} \right)^{12} - \left( \frac{C}{r} \right)^6 \right] + \frac{q_i q_j}{Dr} \]

(a) Briefly explain the physical meaning of each of these terms.

(b) How is this potential function used in a molecular dynamics calculation?

(c) What is a typical time-step for a molecular dynamics simulation, and why is it used?