Methods in Biophysical Chemistry – CH 8613
Assignment 5

Due Friday, October 28

1. A scientist is measuring changes to a protein's structure as drug is added into solution. Using CD spectroscopy, he believes that, instead of monitoring the complete spectrum of $\Delta\varepsilon$ at 100 different wavelengths, he can monitor the spectrum at five different wavelengths. During the experiment, three different concentrations of drug are measured. The resulting data matrix (A) has the following form:

$$\begin{bmatrix} \theta_{11} & \theta_{12} & \theta_{13} \\ \theta_{21} & \theta_{22} & \theta_{23} \\ \theta_{31} & \theta_{32} & \theta_{33} \\ \theta_{41} & \theta_{42} & \theta_{43} \\ \theta_{51} & \theta_{52} & \theta_{53} \end{bmatrix}$$

In this nomenclature, θ_{ij} corresponds to the molar ellipticity at frequency *i* for titration point *j*.

   a. If the scientist uses SVD to decompose this data matrix into U_{nm}, s_{nn}, and V^T_{nn} matrices, find the value of θ_{23} in terms of elements of U_{nm}, s_{nn}, and V^T_{nn}. Note that you do *not* need to multiply all three matrices to show this. (Hint: multiply two out of three matrices, then multiply the appropriate terms to determine θ_{23}). (5 points)

   b. In terms of the physical meanings of the U_{nm}, s_{nn}, and V^T_{nn}, explain each term in your product from part (a). (5 points)

   c. After doing the SVD, the S matrix is found to be:

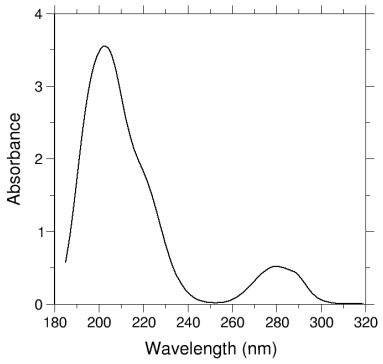
$$\begin{bmatrix} 58.3 & 0 & 0 \\ 0 & 35.1 & 0 \\ 0 & 0 & 0.01 \end{bmatrix}$$

   How many different species are present during the titration? Why? (5 points)

2. van Holde, question 11.6. You do not need to report an uncertainty on your result. The cgs unit for viscosity is the Poise (P), where 1 P = 1 g cm^{-1} s^{-1}. The volume you obtain should make sense, and it may help if you convert your volume to a spherical radius to see if you obtain something "protein-sized." *Hint:* The first value in the table, with units, is 0.30×10^4 K P^{-1}. (10 points)

3. A scientist is preparing to do fluorescence studies on a protein containing a single, buried Trp residue. She wants to estimate the expected fluorescence lifetime of the Trp. Using ProtParam, she determines that the extinction coefficient at 280 nm for her protein is 9970 M^{-1} cm^{-1}.

   Measuring the absorption of her protein sample in a 1 cm UV cell, she obtains the following result. The spectrum is available on the course website (`hw05-spec.dat`)



   a. Calculate the Einstein A coefficient for this tryptophan. To do this problem, you should think about equations 8.98, 8.102, 8.115 and 8.116 in your text. You will need to estimate an integral, namely:

$$\int_{band} \frac{\varepsilon(\upsilon)}{\upsilon} d\upsilon$$

   You are welcome to solve this integral any way you like, but I'd recommend using a Riemann sum. Given the digitized data, you can approximate the area as a sum of rectangular areas with a width *dv* and a height $\frac{\varepsilon(\upsilon)}{\upsilon}$ (use Excel). Remember to convert from absorbance to ε and to convert from wavelength to frequency, and pay close attention to units! *Your value for the A coefficient should be reasonable given the discussion in class.* (7 points)

   b. What is the intrinsic fluorescence lifetime (τ_A) for the Trp in part (a)? If ϕ_f for this Trp is found to be 0.2, what is τ_{obs}? (3 points)

4. Denaturation of immunoglobulin proteins typically results in an increase in observed quantum yield for its Trp residues. For most other proteins, a decrease in quantum yield is observed.

   a. Briefly explain why a decrease in quantum yield would be expected for protein denaturation. (5 points)

   b. Why do you think the opposite effect is observed in immunoglobulin proteins? (Hint: take a look at the Trp residues of some typical immunoglobulin proteins, PDB IDs 1CLY or 1IGT.) (5 points)

5. Dansyl chloride is a fluorophore that reacts with primary amines to fluorescently label proteins. A biochemist is interested in studying a conformational change in a protein that contains a strong chromophore. To start, he measures the lifetime of the dansyl group by using a very short pulse of light is used to excite fluorescence and then measuring decay. The intensity vs. time after excitation is given below.

Fluorescence Intensity (counts)	**Time (ns)**
1.1×10^4	0
4.9×10^3	5
2.3×10^3	10
1.3×10^3	15
5.6×10^2	20

a. What is the lifetime of this fluorophore (τ_{obs})? (7 points)

b. Under the conditions above, the quantum yield was determined to be 0.75. What is the intrinsic rate constant (k) for radiative fluorescence decay (τ_A)? (3 points)

c. Prior calibration in this system indicated that a 25 Å separation between dansyl and the protein's chromophore led to an efficiency of fluorescence energy transfer of 50%. In this system, the observed fluorescence lifetime of the dansyl group is observed to be 5.8 ns. What is the distance between the dansyl group and the chromophore when the dansyl group is attached to the protein protein? (5 points)

6. The binding of a ligand quenches the fluorescence of a protein. The following data were acquired:

$[L_{tot}]$ (μM)	**F_0/F**
0.0	1.01
0.5	2.31
1.0	3.50
2.0	5.95
3.0	8.50
5.0	13.47

Given that the protein concentration is 0.25 μM, what is the association constant (K_A) of the ligand-protein interaction? As this is a rough estimate, you do not need to report an uncertainty on your result. (10 points)