Graduate Topics in Biophysical Chemistry – CH 8990 03
Assignment 5

Due Monday, March 31

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, show that:

$$\theta_{12} = s_1 U_{11} V_{12}^T + s_2 U_{12} V_{22}^T + s_3 U_{13} V_{32}^T$$

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_{12}.

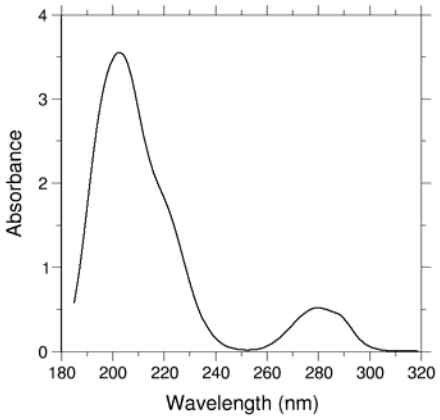
b. After doing the SVD, the S matrix is found to be:

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

How many different species are present during the titration? Why?

2. 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.*

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. 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."

4. van Holde, question 11.8. Here again, you should pay close attention to units!

5. 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.

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.)

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	0.87
0.5	1.20
1.0	1.36
2.0	1.65
3.0	1.93
5.0	2.62

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.