**Due Sunday September 29, 2019**   52 pts   Total time required: ~ 95 min.

1. (10 pts, 20 min) CSP (cold shock protein) is produced in bacteria after a sudden drop in temperature. The wild-type protein contains an Ile at position 18 in the chain. You can view the structure of the wild-type and mutant proteins on the Jmol page associated with this problem set. Three different mutant proteins are studied, with the Ile replaced by Phe, Val, or Ala. The thermodynamic parameters for unfolding of the wild-type protein are: $\Delta H° = +200$ kJ/mol, $\Delta S° = +600$ J/mol-deg. The denaturation curves for all four proteins are shown on the right.
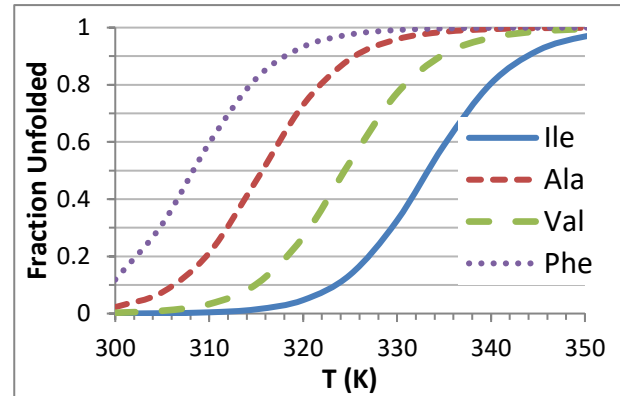
  i) Estimate the $\Delta S°$ for unfolding of all three mutant proteins (Ala, Val, Phe) using the data (entropy change due to transfer to water) given in lecture 8 and the $\Delta S°$ observed for the wild-type protein. The units on the y-axis of the plot are J/mol-deg (4 pts). You can assume the entropy for the transfer of the sidechains to water is:

       Ile = -2.5 J/mol-K
       Val = -1.5 J/mol-K
       Phe = -2.5 J/mol-K
   Ala = -0.5 J/mol-K

  ii) Using your estimate from *part i)* calculate the $\Delta H°$ for the unfolding for all three mutant proteins [Hint: No van't Hoff analysis is required, remember that $\Delta H° = T_M \Delta S°$]. Use the following values for T_M: Ile=333.3, Ala=315.6, Val=324.5, Phe=308.3 (3 pts)

  iii) Explain the differences in enthalpy and entropy between the wild-type and all three mutants in terms of the structural differences. (3 pts)

2. (6 points, 10 min) The Jmol page for this question contains structures for the complex between two different Fv fragments (A & B) bound to the same dinitrophenyl ligand (amino-DNP). The chemical structure of this hapten is shown on the right. In answering the following questions you should focus on the two nitro groups (NO_2) as well as the aromatic ring on the DNP, the ethylamine group ($-CH_2-CH_2-NH_3^+$) is not important for binding.

amino-DNP
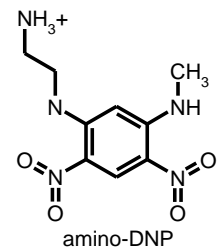
  i) Give the residue name and number (position in sequence) of the tryptophan residues that contact the hapten, also indicate whether they are on the heavy or light chain. (2 pts)

  ii) What are the **two** energetically favorable interactions between these Trp residues and the hapten that would stabilize its bound state? (2 pts)

  iii) Antibody A binds the hapten more tightly than B, why? [Hint: Are there any additional interactions between the hapten and A versus B?] (2 pts).

3. (10 pts, 20 min)

  i) You are using equilibrium dialysis to measure the binding constant of DNP to both F_v fragments (A and B). The concentration of the F_V inside the dialysis bag is 5 µM (µM=10^{-6} M). Use the "dry-lab" associated with this problem to obtain the amount of ligand in the dialysis bag. From these data obtain the fractional saturation for both proteins (4 pts).

  ii) Use these values to plot a binding curve (Y versus [L]). Estimate the value of the K_D from your binding curve. You will need these values to fit the binding curve using the Solver routine in Excel. You can download an Excel sheet that is set up for Solver:

  http://www.andrew.cmu.edu/user/rule/bc_oli/Pset/PS04/binding_Solver_DNP.xlsx (2 pts). Additional information on using Solver is available on the Jmol page for this problem set.

    Submit your binding curves and provide your estimates of K_D from your binding curve and the best fit values using solver.

  iii) Calculate the standard energy of binding for each K_D value (T=300K). Based on the K_D values and the $\Delta G°$ values, which Fv fragment binds the DNP with higher affinity, Fv-A or Fv-B? (2 pts)

iv) Relate the difference in binding energy to the difference in the interaction of the two Fv fragments and the bound DNP ligand (2 pts).

4. (12 pts, 20 min) This problem investigates the effects of changing the dissociation constants (i.e. ligand-protein binding energy) on the degree of cooperativity for a protein that binds two identical ligands. You should download the EXCEL spreadsheet for this problem:

www.andrew.cmu.edu/user/rule/bc_oli/Pset/PS05/bindingcurve_sim.xlsx.

	K^{μ}_{D1}	K^{μ}_{D2}	n_h	K_D	f_{ML}
A	5	20			
B	10	10			
C	50	2			
D	200	0.5			
E	1000	0.1			

Change the values of K_{D1} and K_{D2} (cells F2 and G2 on spreadsheet, highlighted yellow) to the values shown in the table on the right to determine the empty cells in the table:
   a) n_h (Hill coefficient),
   b) K_D
   c) fraction of the system in the (ML) form at Y = 0.5 (f_{ML}).

You should get n_h and K_D from the Hill plot, f_{ml} is the cell in the table highlighted green. Before you begin, think whether you expect the binding to be cooperative or non-cooperative, based on the differences between the two K_D values. Note that the units are µM for the binding curve and log(M) for the Hill plot. The K_D values entered for the various questions are in µM, i.e. entering 10 in cell F2 is really 10 µM.

i) Is the Hill coefficient consistent with the values of the individual dissociation constants, K_{D1} and K_{D2} for each pair of values? Justify your answer by comparing the binding constants to the Hill coefficient for the five proteins? (2 pts)

ii) Sketch the Hill plot, on the same graph, for all five of the above conditions. What is the difference between the five Hill plots? The slope or the x-intercept? (4 pts)

iii) How does the observed K_D, as determined from the Hill plot, change for the different values of K_{D1} and K_{D2} (2 pts)?

iv) How does the fraction of the system in the (ML) form at Y=0.5 change as the cooperativity changes? Which of these five scenarios (A-E) more closely represents infinite positive cooperativity? Justify your statement based on the Hill coefficient (2 pts).

v) How does the shape of the binding curve change for each set of values? What is a more reliable measure of the cooperativity? The shape of the binding curve (hyperbolic for non-coop, sigmoidal for positively cooperative, or flattened hyperbolic for neg-cooperativity) or the Hill coefficient (2 pts)?

5. (14 pts, 25 min) Fetal hemoglobin, although also tetramer, is composed of different chains than adult hemoglobin which give it different oxygen binding characteristics. The fractional saturation of fetal hemoglobin at a number of different oxygen concentrations is given in the table on the right. The units of oxygen are µM. One data point is missing, at [O_2]= 0.5 uM, which you will obtain from optical absorption.

[O_2] (µM)	Y
0.2	0.008
0.5	
1.0	0.510
2	0.890
5	0.990

The absorption spectra for deoxy (blue, dotted) and oxy-(red, solid) hemoglobin are shown on the right, along with the molar extinction coefficients of the deoxy (Y=0) and oxy form (Y=1) of hemoglobin at 684 nm (ε = 2261.5 $M^{-1}cm^{-1}$ (deoxy) and 274.4 $M^{-1}cm^{-1}$ (oxy)) and 796 nm (ε=795 $M^{-1}cm^{-1}$). You can assume the concentration of hemoglobin in solution is 1 x 10^{-4}M.

i) Calculate the expected absorption of the solution of fetal hemoglobin at 684 nm for both the oxy and deoxy forms, this
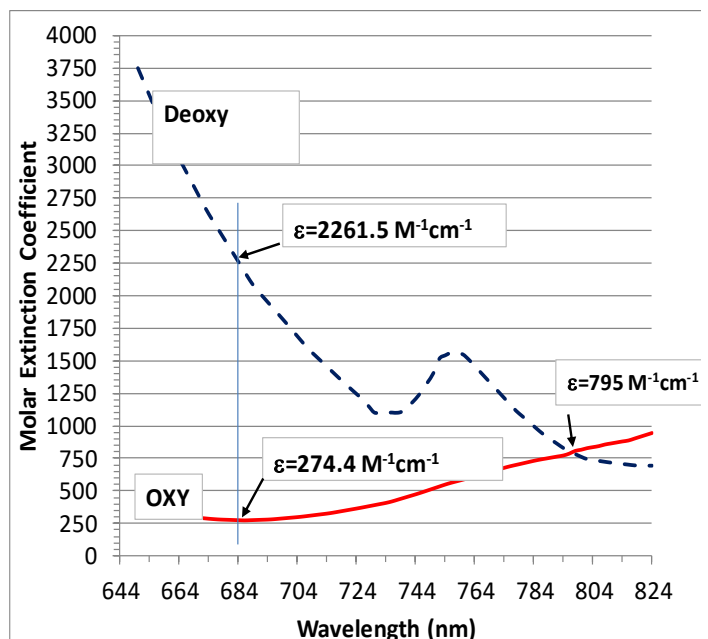
gives you A_{ML} and A_M, respectively (2 pts).

ii) The absorbance of a solution with $[O_2]=0.5$ μM was 0.201. What is the fractional saturation at this oxygen concentration? (2 pts)

iii) Using the values of Y in the above table, plot a Hill plot. Convert the oxygen concentration to molar before doing your plot. Determine the observed K_D value and the Hill coefficient from your data (4 pts).

iv) Using the same graph, sketch the central portion of a Hill plot for normal adult hemoglobin (K_D = 10 μM, n_h =3) (2 pts).

v) Compare your data (K_D and n_h) for normal hemoglobin to that obtained for fetal hemoglobin.   What are the significant differences and similarities? (2 pts)

vi) Why are these differences and similarities in K_D and n_h important for the *biological* function of fetal hemoglobin? (2 pts)