Biophysical Chemistry – CH 4404 01
Assignment 6

Due Friday, October 18 at 5:00 pm

Please complete the answers to this assignment on a separate page (or pages), showing your work and sources (if you referred elsewhere for constants, enthalpies, etc.).

1. In class we related the folding free energy to the fraction of protein that is folded.

$$N \leftrightarrow U, \text{ where } f = \frac{1}{1+K}$$

   We also discussed in class several weeks ago how $\Delta\bar{G}^0$ (and thus K) for chemical reactions depends on temperature. To a first approximation, $\Delta\bar{G}^0$ vs. temperature can be determined using $\Delta\bar{G}^0(T) - \Delta\bar{G}^0(T_{ref}) = -(T - T_{ref})\Delta\bar{S}^0(T_{ref})$ if the entropy is known at a reference temperature. Alternatively, the Van't Hoff Equation can be used if only the enthalpy is known. Both of these expressions assume enthalpy and entropy are constant over a small range of T. In Assignment 4, we examined the consequences if that assumption didn't hold.

   For this assignment, we will simplify matters and assume that $\Delta\bar{H}^0$ and $\Delta\bar{S}^0$ are constant *vs.* T.

   (a) Derive an expression for the fraction of folded protein *vs.* temperature given $\Delta\bar{G}^0(298\ K) = 5$ kcal mol^{-1} and $\Delta\bar{H}^0 = 168$ kcal mol^{-1}. (4 points)

   (b) Using Excel (or any other plotting program) plot your expression for *f* from (a) vs. temperature for temperatures from 20 ^{o}C to 90 ^{o}C. On your plot, indicate the position where $\Delta\bar{G}^0$ is zero. Where is $\Delta\bar{G}$ zero? (4 points)

   (c) Thermophiles are organisms that live in very hot environments, such as volcanic vents under the ocean or in hot springs. When examined, many of the proteins in thermophilic organisms have similar stabilities at room temperature to those in their "normal" mesophilic counterparts. Assume your results from (a) and (b) are from a mesophilic bacteria that that thrives under normal temperatures. What can you say about the enthalpy of unfolding for proteins in its thermophilic counterpart? (2 points)

2. It is not always possible to do equilibrium dialysis on a system. Sometimes measuring the concentrations is not straightforward, or the binding may be sufficiently weak or tight that it may not be detectable. In this problem, we will consider the case where one can still measure $\bar{\nu}$, but where one only knows $[L]_{total}$ and cannot measure $[L]_{free}$ directly. The reaction of interest is single-site binding:

$$P \cdot L \leftrightarrow P + L$$

(a) Given *K* and initial values of $[P]_{total}$ and $[L]_{total}$, set up an equilibrium table to determine general expressions for the concentrations of [PL], [P], and [L] at equilibrium. You will have to solve a quadratic equation. (4 points)

(b) If $[P]_{total}$ is 5 μM and *K* is 5×10^{-7}, make a plot of $[L]_{total}$ vs. $\bar{\nu}$. Your values for $[L]_{total}$ should range from 0.1 μM to 10 μM in increments of 0.1. On the same plot, plot [L] vs. $\bar{\nu}$. (3 points)

(c) Repeat part (b) for $[P]_{total}$ of 0.1 μM. What happens to the difference between the $[L]_{total}$ vs. [L] curves? If you could not use equilibrium dialysis, would you want your $[P]_{total}$ to be greater than, less than, or approximately equal to the *K*? Why? Note that for this problem K can be thought of as $5 \times 10^{-7} \text{M} = 0.5$ μM. (3 points)

3. A (protein) transcription factor binds to a short strand of DNA at two overlapping sites. Let $^{\circ}DNA^{\circ}$ represent the DNA with no transcription factor bound at either site A or B. Then, $^{\bullet}DNA^{\circ}$ and $^{\circ}DNA^{\bullet}$ represent the DNA when sites A and B are occupied. Note that $^{\bullet}DNA^{\bullet}$ is not possible, because the sites overlap. The binding reaction at each site is:

$$P + {}^{\circ}DNA^{\circ} \rightleftarrows {}^{\bullet}DNA^{\circ}, \; K_{eq} = K_A$$
$$P + {}^{\circ}DNA^{\circ} \rightleftarrows {}^{\circ}DNA^{\bullet}, \; K_{eq} = K_B$$

(a) Using the methods outlined in class, write an expression for the degree of binding to the DNA. In the vocabulary used in class, the protein is the "ligand" for our purposes. Then, write expressions for the concentration of [$^{\circ}DNA^{\circ}$], [$^{\bullet}DNA^{\circ}$] and [$^{\circ}DNA^{\bullet}$] in terms of K_A, K_B, the free protein concentration [P], and the total DNA concentration $[DNA_{tot}]$. (6 points)

(b) You have determined $K_A = 4 \times 10^3$, and $K_B = 8 \times 10^4$. Assume you have a total DNA concentration of 50 μM. On the same plot, graph [$^{\circ}DNA^{\circ}$], [$^{\bullet}DNA^{\circ}$], and [$^{\circ}DNA^{\bullet}$] as a function of the free protein concentration [P]. To make your plot sufficiently smooth, plot [P] from 0 to 1 mM in increments of 5 μM. (4 points)

4. A scientist is using equilibrium dialysis to measure zinc binding to a protein. Using atomic absorption spectroscopy, the scientist is able to measure the total concentration of zinc at equilibrium inside and outside of the bag:

Data point	Protein (μM)	Zinc Inside (μM)	Zinc Outside (μM)
1	20	20.1	5.1
2	20	74.8	29.3
3	20	125.9	65.6
4	20	173.9	110.5
5	20	200.2	131.9

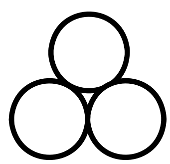
(a) Using the data above, determine the equilibrium constant for binding as well as the number of total binding sites, assuming each site is equivalent and independent. Submit a copy of your Scatchard plot. (4 points)

(b) The above data were collected at 25 ^{0}C; the scientist repeated the experiment at 15 ^{0}C and got the following results:

Data point	Protein (μM)	Zinc Inside (μM)	Zinc Outside (μM)
1	20	20.0	2.9
2	20	75.5	22.2
3	20	124.8	58.1
4	20	171.7	101.8
5	20	199.2	126.5

Estimate $\Delta\bar{H}^0$ and $\Delta\bar{S}^0$ of binding assuming that both are constant as a function of temperature. (6 points)

5. A homotrimeric protein is a multimeric protein consisting of three copies of an identical protein subunit. For many proteins, it is reasonable that the homotrimer will be symmetric, with each subunit arranged around a C_3 symmetry axis:



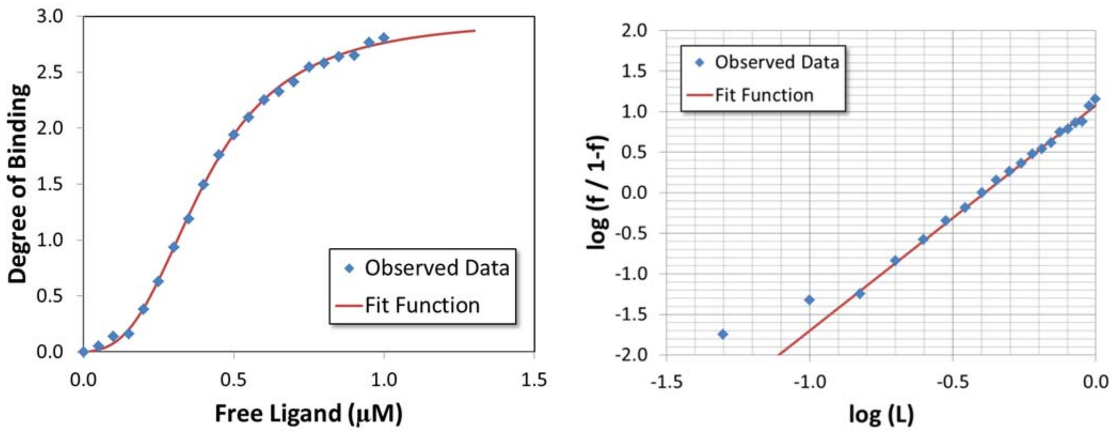
An example of a homotrimeric protein in biology is Tumor Necrosis Factor α (TNF-α). TNF-α is a protein involved in immune response, and it can trigger cell death in response to catastrophic events (e.g. cancer and bacterial infection). It can also trigger inflammation, and thus there are times when it is useful to interfere with TNF-α. Using drugs that bind TNF-α, it is possible to prevent it from binding to receptors on the cell surface.

A reasonable model for TNF-α binding is a nearest-neighbor binding model, with an allosteric term τ and a single (identical) site binding constant K. Unlike the linear chain model we discussed in class, every subunit has two nearest neighbors. Rather than a linear chain, this binding can be envisioned as a circular loop.

(a) There are a total of eight states for binding to this system. List these states and give the statistical weight for each in terms of K and τ. (8 points)

(b) Write an expression ν, the degree of binding as a function of τ and S = K[L]. (4 points)

(c) You have developed a small-molecule that binds to TNF-α. You perform a series of binding experiments to determine $\bar{\nu}$ vs. [L]. Two representations of the data are below. Using the model from part (a) and (b), you optimize the parameters and find that, for the best fit, K is 8.3×10^4 and τ is 30.



Estimate the Hill coefficient from this data and comment how it compares to the maximally cooperative value (all-or-none) expected for a three-site system. Do you think the model (the "Fit Function" in the left-hand plot) is a good one? (3 points)