Methods in Biophysical Chemistry – CH 8613
Assignment 3

Due Friday, September 30

1. Consider a four-residue protein segment in the middle of a long coil region. Thus four-residue segment undergoes a helix-coil transition.

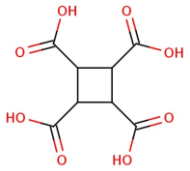
   a. Write the sum of statistical weights (Z) for this four-residue region in terms of s and σ. (Rembmber that because σ is small, the weight of any state with σ^2, σ^3, etc. will be essentially zero.) (10 points)

   b. Write an expression for the average number of helical residues in the peptide (ν). (5 points)

   c. Show that the expression you derived in part (b) is equivalent to the following expression: (5 points)

$$\nu = \frac{s}{Z}\left(\frac{\partial Z}{\partial s}\right)$$

2. In this question, we will examine how statistical mechanics can be used to model binding to a small molecule. Consider cyclobutane 1,2,3,4-tetracarboxylic acid:



Similar to our treatment of helix-coil theory, we can write a simple model for enumerating the binding modes of this molecule. In this model, we write a 0 if no proton is bound, and a 1 if a proton is bound. Then, the following diagrams all represent states where one proton is bound:

$$\begin{pmatrix}1 & 0\\ 0 & 0\end{pmatrix} \qquad \begin{pmatrix}0 & 0\\ 1 & 0\end{pmatrix} \qquad \begin{pmatrix}0 & 1\\ 0 & 0\end{pmatrix} \qquad \begin{pmatrix}0 & 0\\ 0 & 1\end{pmatrix}$$

If we set use the state with no protons bound as our reference state (with a statistical weight of 1), we can calculate the statistical weights of the other states. Recall that a statistical weight is simply a ratio of concentrations. If all sites are independent and identical with an acid association constant of *K*, calculating relative concentrations is straightforward:

$$H^+ + A^- \rightarrow HA \qquad K = \frac{[HA]}{[H][A]} = \frac{\begin{bmatrix}1 & 0\\ 0 & 0\end{bmatrix}}{[H]\begin{bmatrix}0 & 0\\ 0 & 0\end{bmatrix}}$$

Thus, the statistical weight for binding at the top left site is simply:

$$w = \frac{\begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}}{\begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix}} = K[H] = S$$

In this expression, we see that the weight of each state is simply related to the acid association constant and the proton concentration, and for convenience we've defined this product as *S*.

a. Following the procedure outlined above, enumerate all of the possible states of binding and give their statistical weights in a table. For now, you should assume that all sites are equivalent (with the same *K*) and independent (binding at one site does not influence binding at another). (Hint: you will have to start with the fully deprotonated molecule and then use equilibrium expressions to calculate weights for one proton bound, then two protons bound, etc.) (5 points)

This molecule has four ionizable carboxylic acid groups in close proximity; thus we'd expect them to interact, effectively shifting the pK_A. For example, if two neighboring sites are both bound, we would expect this to be favorable, since there is an energetic penalty for having two negative charges close to one another. We can model this with a *nearest neighbor interaction parameter* τ, which favors states where two neighboring sites are bound. This parameter is a part of our model; under normal circumstances we would have to fit it using experimental data. Thus, proton binding equilibrium constants are modified depending on how many unfavorable interactions are removed. For example:

$$\begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} + H^+ \rightarrow \begin{bmatrix} 1 & 1 \\ 0 & 0 \end{bmatrix} \text{ or } \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} + H^+ \rightarrow \begin{bmatrix} 1 & 0 \\ 1 & 0 \end{bmatrix} \quad K_{eq} = \tau K$$

where *K* is the single-site equilibrium constant. Here, binding an additional protein removes one unfavorable charge-charge interaction, so the normal binding *K* is modified by a factor of τ.

Another case is the initial binding. In this situation, *two* unfavorable nearest-neighbor interactions are eliminated upon binding:

$$\begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix} + H^+ \rightarrow \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \quad K_{eq} = \tau^2 K$$

Finally, we'll assume that the 1, 3 and 2, 4 deprotonated states do not interact. In other words, there are no benefits to removing unfavorable interactions in the following reaction, since all of the unfavorable interactions have already been removed:

$$\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} + H^+ \rightarrow \begin{bmatrix} 1 & 0 \\ 1 & 1 \end{bmatrix} \quad K_{eq} = K$$

b. Using the framework sketched out above, modify your original the statistical weights with the interaction parameter τ. As you did before, write the complete table of states, including the weight and number of protons bound. When you have adjusted the weights, write the partition function for this system. (*Hint:* Don't just write weights in a table – think about the reactions themselves and appropriate K_{eq} values are given our model above.) (10 points)

c. What is the equilibrium constant for the following reaction? (5 points)

$$\begin{bmatrix}1 & 0\\ 0 & 0\end{bmatrix} + 3H^{+} \rightarrow \begin{bmatrix}1 & 1\\ 1 & 1\end{bmatrix}$$

d. Write an expression for the fraction of sites bound in this molecule, using our model. The fraction of sites bound is given as the weighted average number of sites bound (ν) divided by the total number of sites (4). You may either write this expression directly using the weighted average, or you can use the technique from above (where $S = K[H]$ and $\nu = \frac{S}{Z}\left(\frac{\partial Z}{\partial S}\right)_{\tau}$). (5 points)

e. Write a single expression for the fractional population of molecules existing in the 1,3 or 2,4 states as a function of [H^{+}] (e.g. they can be either $\begin{bmatrix}1 & 0\\ 0 & 1\end{bmatrix}$or $\begin{bmatrix}1 & 0\\ 0 & 1\end{bmatrix}$). (3 points)

f. Because $\Delta\bar{G}^{0} = -RT\ln K_{eq}$, the framework above can also be viewed energetically. For example, in case where the nearest-neighbor interaction occurs, one could write:

$$\Delta\bar{G}^{0} = -RT\ln K_{eq}$$
$$\Delta\bar{G}^{0}_{tot} = -RT\ln \tau K$$
$$\Delta\bar{G}^{0}_{tot} = -RT\ln K - RT\ln \tau$$
$$\Delta\bar{G}^{0}_{tot} = \Delta\bar{G}^{0}_{single} + \Delta\bar{G}^{0}_{interaction}$$

Thus, one can imagine τ as corresponding to an interaction energy benefit (or penalty, if $\tau < 1$). In the system above, do you expect that τ will be greater than or less than one and why? (2 points)

g. Assume that $K = 10^{4}$ (this corresponds to a single-site pK_A of 4). Submit a plot for the fraction of protons bound ($\nu/4$) vs. pH (*not* simply [H]) for the following values of τ: 0.01, 0.1, 1.0, 10, 100. Plot your functions at pH intervals of 0.1 units ranging from pH 1 to pH 14. All curves should be on the same plot and clearly labeled for comparison. You may use Excel, but keep in mind that many of Excel's default options are not suitable for scientific analysis (e.g. no axis labels, and grey text/lines). (10 points)

3. van Holde, Question 4.6 (10 points)
4. van Holde, Question 8.2 (10 points)
5. van Holde, Question 8.6 (15 points)