APh105c
Homework 4
Due Date: Friday, April 28, 2006

"Their drills are bloodless battles and their battles are bloody drills." - Josephus

Reading: Read chaps. 22 and 23 of Dill and Bromberg.

1. Crowding and Binding

In this problem you will work out the way in which crowding agents can *enhance* the likelihood that a ligand will bind to its receptor. For concreteness, consider a box with N lattice sites (this is our toy model of the solution). In this box there is a receptor, L ligands which target that molecule and C crowding molecules which do nothing more than occupy space and jiggle around (but, they have a big effect on the equilibrium of the receptor with its ligands and that is the moral of the problem).

(a) For the case in which there are no crowding agents, work out the probability that the receptor will be occupied by a ligand (in class we called this p_{bound}) as a function of the number of ligands. Write your result in terms of the difference in the energy of the ligands when they are in solution and when they are bound to the receptor. To determine the number of lattice sites use $N = \Omega_{cell}/\Omega_{protein}$ to get an estimate by taking Ω_{cell} as the volume of a typical cell like *E. coli* and $\Omega_{protein}$ as the characteristic volume of a typical protein. Explain why this is a reasonable way for us to set up our lattice model. For a characteristic energy difference like $10k_BT$, make a plot of the binding probability as a function of the number of ligands. Make sure you state any approximations that you make and justify them. I would like to see you derive an expression that is valid for the dilute limit ($L << N$) using

$$\frac{N!}{(N-L)!} \approx N^L \tag{1}$$

and for the more general case as well - then plot both cases and compare

them to show how the approximate result breaks down once L is comparable to N. Also, rewrite your expression for p_{bound} in terms of the *concentration* of molecules - to do this you will have to take ratios like N/L and divide top and bottom by Ω_{cell} so that you have concentration units.

(b) Now consider the case in which there are C crowding molecules present in the solution. These molecules can't bind to the receptor, but what they can do is take up space (and hence change the entropy of the L ligands). Compute p_{bound} again, but now in the presence of the crowding agents. Note that you need to be careful that the total number of ligands and crowding molecules doesn't exceed the number of sites N on our lattice which is the model of solution. Make sure that you plot your results for L running from 0 all the way to $N - C$. Make sure that your plot shows a number of different choices for C. What I want you to observe is that as C increases, p_{bound} begins to deviate from the ideal solution limit. Please see the papers about macromolecular crowding that are associated with this homework.

2. Isothermal Atmosphere.

In class I worked out how the chemical potential changes for the case in which there is a spatially varying field of force. Repeat that derivation for the special case of molecules in a gravitational potential and work out an expression for the density as a function of height. Then, in the spirit of the "feeling for numbers" work out the density in Pasadena and on top of Mt. Everest. Compare those densities.

2. Equilibrium Constants.

Equilibrium constants are one of the key interpretive tools in molecular biology and biochemistry. In this problem, we revisit some of the conceptual foundations of equilibrium constants.

a) In class I swiftly derived the law of mass action on the basis of minimization of the Gibbs free energy. Repeat that derivation here making sure to attend to all details and explaining all of your steps and logic.

b) We begin by examining a kinetic interpretation of equilibrium constants

and the associated question of whether or not the barrier which separates the two states of interest should play any role in the equilibrium constant (we already know the answer is no, but we will demonstrate it). Consider the simple reaction A + B $\rightleftharpoons$ C. In this case, we know that the equilibrium constant is given by

$$K = \frac{[C]}{[A][B]}. \tag{2}$$

However, we can also express this equilibrium constant in terms of the rate constants for the reaction. In particular, note that the reaction has a kinetic equation

$$\frac{d[C]}{dt} = k_{on}[A][B] - k_{off}[C]. \tag{3}$$

At equilibrium, the left hand side of this reaction is zero and hence we have

$$K = \frac{k_{on}}{k_{off}} = \frac{[C]}{[A][B]}. \tag{4}$$

Sketch an energy landscape with two wells and a barrier between them. Call the free energy when A and B are separate E_1 and that when they are associated E_2. To make a transition from 1 to 2 requires going over a barrier of height E_b. Put all of this in your sketch. Transition state theory tells us that the rate of a reaction is of the form

$$\text{rate} = \nu_0 exp(-E/kT), \tag{5}$$

where E is the height of the barrier and ν_0 is an "attempt frequency". Compute the ratio k_{on}/k_{off} and show that the barrier height drops out of the problem (as it should).

c) For weak acids, we introduced the notion of a dissociation constant defined as

$$K = \frac{[H^+][A^-]}{[HA]}, \tag{6}$$

with an associated definition of the pK via pK=-log K. Hang meat on the statement made in class that the pK is the value of the pH for which the dissociation reaction has gone half way to completion. In particular, derive the Henderson-Hasselbalch equation and use this equation to prove the relation

between pK and pH.

d) The ideas described above are useful in thinking about the charge state of biopolymers such as DNA and proteins as a function of pH. If x refers to the fraction of the molecules that are ionized, show using our relation between pH and pK that

$$x = \frac{10^{pH-pK}}{1 + 10^{pH-pK}} \tag{7}$$

and use this to characterize the charge on nucleotides (with a pK of roughly 1.) and aspartic acid (pK = 3.9) and glutamic acid (pK=4.2). In particular, plot the fraction dissociated as a function of pH and comment on the charge state at pH 7.