APh161: Physical Biology of the Cell
Homework 4
Due Date: Thursday, February 2, 2006

"My regard for the inventor of the harp is not made less by knowing that the instrument was very crudely constructed and still more crudely played. Rather, I admire (the inventor) more than I do the hundreds of craftsmen who in ensuing centuries have brought this art to the highest perfection." Galileo Galilei

Reading: Chap. 21 of "Physical Biology of the Cell" chap. 8 of ECB.

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. The Genetic Switch Revisited.

In class we worked out the dynamics of a genetic switch using a model of repressor binding in which the Hill coefficient $n = 2$. In this problem, you will work out this model in more depth for several different choices of the Hill coefficient.

(a) Consider the case done in class for $n = 2$. Begin by deriving the probability of repressor binding assuming that the repressor is a dimer and show that p_{bound} is of the Hill form. Then derive the differential equations for the protein concentrations like I did in class and write everything in dimensionless form in terms of the two dimensionless protein concentrations, x and y. Next, show that in steady state (as we did in class) that

$$x = \frac{\alpha(1+x^2)^2}{(1+x^2)^2 + \alpha^2}. \tag{2}$$

Now plot the two sides of the equation and examine their intersection for three choices of $\alpha = 1, 2, 3$. Explain the significance of your graphical result with respect to the number of solutions. Basically, you are telling the same story I told in class, but rather than solving the problem analytically you are solving it graphically. In addition, make vector field plots that show the fixed points for these same different values of α and use these plots as a second way of explaining the dynamics.

(b) Repeat the derivation given in class but now for the case where the Hill coefficient is $n = 1$ and show that there is only one solution (i.e. there is no switching behavior). Make sure to discuss, comment and observe. I want to hear you reason about this problem, not just show the one particular result that is asked of you.

(c) Now consider the case $n = 4$. Work out the equation that is equivalent to that you derived in part (a). Find the solutions for x for representative choices of α and comment on the switching behavior in this case. Make sure you also comment on the significance of the choice of $n = 4$ itself.

Extra Credit: a) For the case $n = 2$ solve the problem numerically for several different initial concentrations of protein $x(0) = x_0$ and $y(0) = y_0$. This is something Matlab can do wonderfully and will permit you to plot the concentrations of x and y as a function of time. (b) Examine the literature and find some particular values for the parameters k, K_d and μ that show up in our model. Comment on what this implies about the parameter α used in our models.