BE/APh161: Physical Biology of the Cell
Homework 6
Due Date: Wednesday, February 18, 2015

"You can't depend on your eyes when your imagination is out of focus."
- Mark Twain

This problem set is in a way a "midterm" review of everything we have done so far in the course. My aim is to get you to review all the different topics we have covered and to bring them together as a full toolkit for examining important and exciting biological problems. The first few problems revisit the subject of gene regulation. The third problem invites you to reconsider the Monod-Wyman-Changeux model of allostery for ion channels and the final problem flexes your muscles in thinking about diffusion. Useful reading can be found in chaps. 5 and 13 of PBoC2.

1. Transcriptional Activation.

Consider the process of activation of transcription. We are going to think about a bacterial promoter. The states and weights for this promoter are shown in Figure 19.9 of PBOC2. Explain the states and weights for each state and also comment on the rate of transcription that you expect from each of those states. Work out the fold-change and plot it as a function of the number of activators, essentially reproducing Figure 19.29A.

2. Induction.

In class we discussed the "induction" process whereby a chemical (such as IPTG) binds to a transcription factor and changes its state of activity. In this problem, you are going to work out the details for yourself for a repressor molecule that has a single binding site for inducer. When inducer is bound, the repressor can no longer bind DNA at all. Your goal is to compute the fold-change in gene expression as

$$\text{fold-change} = \frac{p_{bound}(P, inducer)}{p_{bound}(P, inducer = 0)}. \tag{1}$$

Specifically, you want to compute the activity as a function of the inducer concentration. Increasing inducer favors the inactive state of the repressor. Imagine your parameters such that the active state of repressor binds DNA with an energy of -15 k_BT. Consider that the K_d of the inducer for binding to the repressor is 100 nM for the active state and 1 nM for the inactive state. Finally, consider the case in which the active state has an energy $-3k_BT$ lower than the inactive state. (You can mess with these parameters to see how the system behaves for other values). Make a plot of the fold-change as a function of inducer concentration using log scale on the x-axis and comment on they key features of your graph.

3. Toxins and ion channels.

Imagine an ion channel that is gated by the binding of an extracellular ligand. This ion channel has two binding sites for the ligand.

(a) Write the states and weights for this channel - it can be in either the closed state with energy ϵ_{closed} or the open state with energy ϵ_{open}. The ligand binds the closed state with energy ϵ_b^{closed} and the open state with energy ϵ_b^{open} With the states and weights in hand, work out an expression for the open probability.

(b) Many toxins alter the ability of ion channels to function. Here we imagine a toxin that inhibits gating simply by binding to the same site as the gating ligand. In this part of the problem identify all of the possible states of the channel and obtain an expression for the open probability as a function of the concentration of both the gating ligand and the toxin concentration. What is the intuition about how the presence of the toxin inhibitor alters the probability that the channel will open?

4. Fluorescence Recovery After Photobleaching and Diffusion.

In class I introduced the experimental method known as FRAP (Fluorescence Recovery After Photobleaching). This technique is founded upon an annoying feature of fluorescent molecules, namely, that if you shine light on them for too long they stop giving off light. As often happens, people figured out how to turn this annoyance into something useful. In particular,

FRAP is often used to learn about the way that different parts of cells are in diffusive contact.

In this problem, I want you to carry out a full derivation of the concentration as a function of position and time after photobleaching a cell of radius 25 microns with a "hole" of radius 2 microns. (Looking at the treatment of the one-dimensional version of this problem in chap. 13 of PBoC will be helpful. Also, this part of the problem is effectively problem 13.4 of PBoC2.) For simplicity, ignore the presence of a nucleus, think of the cell as a perfect circle and imagine the photobleached region as a circle at the center of the circular cell.

(a) Consider an initial concentration c_0 of the fluorescent molecule of interest which is uniformly distributed throughout the cell. How many molecules of the fluorescent molecule are there - write an equation that gives this number?

(b) Before doing any calculations, explain what the final concentration c_∞ will be after infinite time, when the system has returned to equilibrium. You may assume that once a molecule has been photobleached it is effectively dead and can be forgotten.

(c) Your goal now is to compute the recovery curve. What this means is that you need to work out how many fluorescent molecules are in the photobleached region as a function of time. Make graphs for the case where the photobleached region is centered about the origin. Make sure when you make your plots you use reasonable values for the diffusion constant - justify your choice.

(d) One of the uses of the FRAP technique is to determine the diffusion constant of various molecules within the cytoplasm of cells. Discuss how that might work on the basis of the derivation you have given here.

To do this problem you will need the table of zeros of the first derivative of $J_0(x)$ given in the file attached to the homework. Make sure you explain exactly what you are doing and what your results mean. Also, I want you to plot the results for the recovery curve for different number of terms kept in the Bessel series. Use just enough terms in the Bessel series such that

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your answer has 5% accuracy in the region of interest, namely, the FRAPed region, and tell us how many terms you used.

Here is some stuff that will come in handy when thinking about this problem. First, you should try to obtain this equation

$$\frac{d^2\rho}{dz^2} + \frac{1}{z}\frac{d\rho}{dz} + \rho = 0. \tag{2}$$

The only solution to this equation that does not diverge for $z \to 0$ is the zero order Bessel function $J_0(z)$. Next, the boundary conditions at the edge of the cell will lead to a condition of the form

$$J_0'(kR) = 0. \tag{3}$$

Interestingly, the roots of J_0' are just the roots of J_1 because of the identity $J_0'(z) = -J_1$. The full solution you are looking for will emerge as (make sure you demonstrate this clearly and convincingly)

$$c(r,t) = a_0 + \sum_{i=1}^{\infty} a_i e^{-DK_i^2 t} J_0(K_i r). \tag{4}$$

We can determine the coefficients a_i using the initial condition $c(r, 0)$. Another identity that will prove useful when doing the calculation of the coefficients is: $\int z J_0(z)\, dz = z J_1(z)$.