BE/APh161: Physical Biology of the Cell
Homework 4
Due Date: Wednesday, February 10, 2021

"We must travel in the direction of our fear." - John Berryman

Extra Credit. Provide comments on chap. 5, "Diffusion as Biology's Null Hypothesis for Dynamics' of the upcoming third edition of *Physical Biology of the Cell*. Note that this is an unfinished draft of the chapter. Figure placements are not necessarily correct and there are still a number of internal discussions amongst the author team about how to finish things off. We are especially interested in mistakes, flaws in logic, confusing figures, unclear discussions, etc., but are happy to entertain comments at all scales. This extra credit will constitute an additional 15% on your score on the homework.

1. Fluorescence Recovery After Photobleaching by Pencil and Paper and by Computation.

NOTE: relevant vignettes to watch are those in section 4.1.

In this problem, we are going to consider a "one-dimensional" cell. Of course, this sounds contrived, but really we are saying that the fluorescence only depends upon a single coordinate. We will consider the long axis of bacterial cells as the region to be photobleached. So, we will think of a region of length $2L = 4\ \mu m$ that initially has uniform fluorescence. We then photobleach (i.e. destroy the fluorescence) between $-a$ and a, with $a = 0.5\ \mu m$. Consider the concentration in the unbleached region to be $c_0 = 1\ \mu M$, and let the diffusing molecules have a diffusion coefficient of 10 $\mu m^2/s$. For each section below, we will use a different approach to working out the dynamics of the recovery process.

(a) FRAP by coin flips. In this part of the problem, you are going to write a simulation code that takes random walkers that start either in the region $-L$ to $-a$ or a to L and flip coins and let them jiggle around. For each such walker, the only rule you will need is that if on a given flip they try to leave the region from $-L$ to L, you will reflect them off the walls. The goal is to do 100s of such simulations and then plot the concentration as a function of position for different time points. After one time step, almost all of the

walkers will be in the unbleached regions. But over time, more and more molecules will have ventured into the photobleached region. Your goal is to get the full profile of the independently diffusing molecules. Make plots of the concentration as a function of the number of steps. If the lattice parameter you use is $d = 40\ nm$, this will mean that you have 100 such lattice points. You can reconcile your simulation time step, the lattice parameter and the diffusion coefficient through the relation $D = d^2/\tau$, where τ is the time step.

(b) FRAP by math. For this part of the problem, I am going to explicitly walk you through the steps and your job is to really carefully demonstrate that everything works and holds together, showing all of the steps. To compute the recovery curves, we first solve the diffusion equation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{1}$$

for the concentration of fluorescent molecules $c(x,t)$, with the initial concentration after photobleaching given by

$$c(x,0) = \begin{cases} c_0 & \text{for } -L \text{ to } -a \\ 0 & \text{for } -a \text{ to } a \\ c_0 & \text{for } a \text{ to } L. \end{cases} \tag{2}$$

We also impose the boundary condition $\partial c/\partial x = 0$ for $x = -L$ and $x = L$, which says that the flux of fluorescent molecules vanishes at the boundaries of the one-dimensional cell (no material flows in or out). This mimics the real-life situation with fluorescent proteins confined to the volume of the cell, to the cell membrane, or to some other subcellular structure.

To solve the diffusion equation with the prescribed initial and boundary conditions, we begin by expanding the concentration profile $c(x,t)$ in terms of cosine functions using "Fourier series,"

$$c(x,t) = A_0(t) + \sum_{n=1}^{\infty} A_n(t)\cos\left(\frac{x}{L}n\pi\right). \tag{3}$$

This expansion guarantees that the boundary conditions are met, namely each of the functions $A_n(t)\cos(xn\pi/L)$ has vanishing first derivatives with respect to x at $x = \pm L$. Furthermore, since the initial concentration profile takes the same values for positive and negative x, it is readily expanded in

cosine functions since the concentration profile is symmetric about the origin. The solution of the diffusion equation now boils down to finding the functions $A_n(t)$ such that both the diffusion equation and the initial condition are satisfied.

To proceed, we substitute the series expansion of $c(x,t)$ into the diffusion equation. This yields

$$\frac{\partial A_0}{\partial t} + \sum_{n=1}^{\infty} \frac{\partial A_n(t)}{\partial t} \cos\left(\frac{x}{L} n\pi\right) = D \sum_{n=1}^{\infty} \left[-A_n(t) \frac{n^2\pi^2}{L^2}\right] \cos\left(\frac{x}{L} n\pi\right), \qquad (4)$$

which, due to the orthogonality property of the cosine functions for different n (see Equation 8 below), turns into a set of independent differential equations,

$$\begin{array}{l} \frac{\partial A_0}{\partial t} = 0 \\ \frac{\partial A_n}{\partial t} = -\frac{Dn^2\pi^2}{L^2} A_n(t) \quad (n \geq 1) \end{array} \qquad (5)$$

Show that the solution to each one of these (infinite in number) equations is an exponential function

$$A_n(t) = A_n(0) \mathrm{e}^{-(Dn^2\pi^2/L^2)t}, \qquad (6)$$

which when substituted into Equation 3 gives

$$c(x,t) = A_0(0) + \sum_{n=1}^{\infty} A_n(0) \mathrm{e}^{-(Dn^2\pi^2/L^2)t} \cos\left(\frac{x}{L} n\pi\right). \qquad (7)$$

Make sure you demonstrate this. The final piece of the puzzle is the determination of the constants $A_n(0)$.

To compute the initial amplitudes of the cosine functions, we resort to the orthogonality property of these functions, namely,

$$\int_{-L}^{L} \cos\left(\frac{x}{L} n\pi\right) \cos\left(\frac{x}{L} m\pi\right) \mathrm{d}x = L\delta_{n,m}. \qquad (8)$$

In particular, multiply both sides of Equation 7 by $\cos(m\pi x/L)$ for different values of m, and then integrate over x to derive the equations

$$\begin{array}{c} A_0(0) = \frac{1}{2L} \int_{-L}^{L} c(x,0) \mathrm{d}x \\ A_n(0) = \frac{1}{L} \int_{-L}^{L} c(x,0) \cos\left(\frac{x}{L} n\pi\right) \mathrm{d}x \quad (n \geq 1) \end{array} \qquad (9)$$

for the initial amplitudes. Substitute the initial concentration profile, $c(x,0)$, into these equations, and perform the integrals, to show that

$$\begin{aligned} A_0(0) &= c_0 \tfrac{L-a}{L} \\ A_n(0) &= -2c_0 \tfrac{\sin(n\pi a/L)}{n\pi} \quad (n \geq 1) \end{aligned} \tag{10}$$

Put these results back into the derived formula for $c(x,t)$, Equation 7 and show that the solution for the concentration profile as a function of time is given by

$$c(x,t) = c_0 \left[1 - \frac{a}{L} - \sum_{n=1}^{\infty} \frac{2\sin(n\pi a/L)}{n\pi} \mathrm{e}^{-(Dn^2\pi^2/L^2)t} \cos\left(\frac{x}{L} n\pi\right) \right]. \tag{11}$$

Make a plot of your resulting concentration profile as a function of time for several different times. Also, make sure you illustrate how your result depends upon how many terms you keep in the series. Obviously, you can't do an infinite number of terms. Note that at long times, such that t is much greater than L^2/D, which is the diffusion time for a box of length L, the concentration profile tends to a constant value equal to $c_\infty = c_0(1 - a/L)$. This can be understood in a very simple way. Namely, at long times, we expect diffusion to make the concentration profile uniform over the $2L$ interval. Show that the fact that the number of fluorescent molecules does not change in time leads to the equation

$$c_\infty(2L) = c_0[2(L-a)], \tag{12}$$

which gives the computed value of the concentration at long times.

(c) FRAP by chemical master equation. In the vignette entitled "Diffusion: Master Equation" I wrote down the evolution equation

$$p(x,t+\Delta t) = p(x,t)+(k\Delta t)p(x-a,t)+(k\Delta t)p(x+a,t)-(k\Delta t)p(x,t)-(k\Delta t)p(x,t). \tag{13}$$

In that vignette, I argued that the equation as written is the basis of a very nice way to numerically investigate diffusion problems. Here you will consider a 4 μm long cell that is discretized into 100 boxes. As you did in the previous two parts of the problem, you are going to integrate the chemical master equation by starting with the initially bleached profile and then plotting the concentration as a function of time.

2. The Mutual Repression Switch Revisited.

In three vignettes, I sketched the concept of the mutual repression circuit. It is one thing to watch someone else do something, and quite another to do it for ourselves. In this problem, we are going to build on the foundations laid there to make a comparison between the analytic work that we did and a direct numerical solution to the protein dynamics. But further, the aim of this problem is for you to do in detail (rather than watch) every step of the analysis that I did in my vignettes.

(a) Draw a schematic of the regulatory circuit and explain all the features of that circuit.

(b) Write down the states, weights and rates and use them to write the two coupled differential equations for the dynamics of this circuit. Explain your steps as though you are talking to a beginner.

(c) Rewrite those equations in dimensionless form and make sure you explain the units of all quantities that have dimensions and how by combining them, you render the equations dimensionless.

(d) Use the differential equation integration code you developed earlier in the course to integrate these two coupled equations (use the dimensionless form) and plot $u(t)$ and $v(t)$ for several choices of initial conditions and show that the system has switch-like behavior.

(e) Repeat what I did in the vignette on fixed points to show that for $\alpha >> 1$ that the fixed points are $u_* = \alpha$ and $v_* = 1/\alpha$ and compare those analytic results to the fixed points you find in your numerical solutions.

(f) Repeat the linear stability analysis I did in the vignette, but explaining it in your own words and being sure to do all of the relevant Taylor expansions, with explanation. Solve the resulting linear differential equations and describe how you know if the solution is stable or unstable.

(g) In the real experimental situation of the Collins *et al.* paper from 2000, how do they flip the switch?