MCB137L/237L: Physical Biology of the Cell
Spring 2020
Homework 5: Diffusion as the null model of biological dynamics, Part II
(Due 3/12/20 at 3:30pm)

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"We are not students of some subject matter, but students of problems. And problems may cut right across the boundaries of any subject matter or discipline" - Karl Popper

1 Analytical solution to the diffusion equation

In class, we derived the diffusion equation in 1D given by

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

where $c(x,t)$ is concentration of molecules, and D is the diffusion constant. Further, during the discussion section, we solved this equation numerically by "spreading the butter" for an initial condition corresponding to having N_0 molecules centered at $x = 0$.

(a) The analytical solution to the diffusion equation under the inital conditions described above is given by

$$c(x,t) = \frac{N_0}{\sqrt{4\pi D t}} e^{-\frac{x^2}{4Dt}}. \tag{2}$$

Show that this is indeed a solution of the diffusion equation. To make this possible, plug in the proposed $c(x,t)$ above into the diffusion equation, do the derivatives on each side and show that, indeed, $\frac{\partial c(x,t)}{\partial t}$ is equal to $D\frac{\partial^2 c(x,t)}{\partial x^2}$.

Remember what you learned in calculus about the product of derivatives and the chain rule! Given a function $f(x,y)$, you can think of the partial derivative $\frac{\partial}{\partial x}$ as a measure of the derivative as we walk along the x-direction as shown in Figure 1. Operationally, taking a partial derivative is like taking a regular derivative: you just treat all other variables as constants. For example, let's define a function of x and y

$$f(x,y) = ax^2y^3. \tag{3}$$

Now, we take the partial derivative with respect to x

$$\frac{\partial f}{\partial x} = ay^3 \frac{\partial}{\partial x}\left(x^2\right). \tag{4}$$

Note that we just thought of ay^3 as constants and took them out of the derivative. As a result, we get

$$\frac{\partial f}{\partial x} = ay^3 2x. \tag{5}$$

Similarly,

$$\frac{\partial f}{\partial y} = ax^2 3y^2. \tag{6}$$

For more information on the partial derivative, please refer to "The Math Behind the Models: the Partial Derivative" on page 212 of PBoC.

(b) Now, let's plot this analytical solution. Specifically, plot the concentration profile (i.e., concentration vs. position) for 0.01 ms, 0.1 ms, 1 ms, 5 ms and 10 ms in a single figure. Note that we are not asking you to plot the $t = 0$ time point because Python won't necessarily know how to deal with the fact that, while the term $\frac{N_0}{\sqrt{4\pi Dt}}$ approaches infinity as $t \to 0$, the term $e^{-\frac{x^2}{4Dt}}$ approaches 0 for the same limit. Use a typical diffusion constant for a protein in the cell of $D = 10$ μm^2/s. You'll have to make reasonable choices for the model parameter N_0. Think hard about the range of x-values over which to plot this distribution. To define this range of x-values to plot, you can use the "$numpy.arange(x_{min}, x_{max}, step)$" operation as we did in class. You can also use the "$numpy.linspace(x_{min}, x_{max}, N_{points})$" command, which you can look up in the Python help. You might note that your concentration peaks beyond N_0! This is because you're plotting $c(x,t)$, the concentration in an infinitesimal box of size dx. This means that the integral $\int_{-\infty}^{+\infty} c(x,t)dx = N_0$, indicating that the total amount of molecules is N_0. We will discuss this subtlety in class.

(c) Finally, we will check that our simulation makes sense by estimating the diffusion constant from the plots you've made. How long does it take for the distribution to spread to about 0.5 μm? Is this consistent with the diffusion constant you used for your simulation? Note that we're not after an exact result for D, but instead are performing a sanity check to see whether our results make sense.

2 Measuring diffusion constants using FRAP

In class, we briefly introduced Fluorescence Recovery After Photobleaching (FRAP) as a means to measure diffusion constants in living cells. Revisit FRAP by reading "Experiments Behind the Facts: Measuring Diffusive Dynamics" on page 513 of PBoC. In this problem we will simulate a FRAP experiment in *E. coli*. Specifically, we will consider a one-dimensional *E. coli* cell with a uniform distribution of fluorescent proteins. The cell is 2 μm in length. At time $t = 0$, a window of a width of 1 μm centered around the middle of the cell is bleached as shown in Figure 2A. Here, we will solve for the fluorescence recovery dynamics by discretizing *E. coli* into small boxes as shown in Figure 2B.

(a) Modify the code we wrote together in class in order to simulate the initial conditions imposed by bleaching. Explain your choice for the number of boxes you will use to simulate

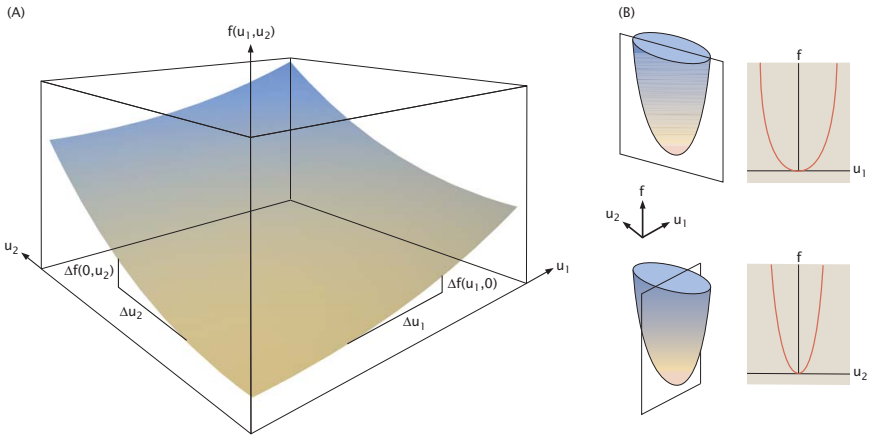

Figure 1: Illustration of the concept of a partial derivative. (A) The plot shows the function $f(u_1, u_2)$ which depends upon the variables u_1 and u_2. If u_2 is held fixed, the surface is reduced to a curve and the partial derivative is nothing more than the ordinary derivative familiar from calculus, but on this particular curve. (B) Planar cuts through the function $f(u_1, u_2)$.

E. coli. Using a typical diffusion constant for a protein ($D = 10\ \mu\text{m}^2/\text{s}$), make a series of plots that show fluorescence as a function of position along the cell for different time points. Specifically, start by plotting the first and last time points of your simulation. Make sure that, for this final time point, the molecules have reached a uniform distribution and explain why this has to be the final outcome of the experiment. Then, plot three more time points that illustrate the dynamics of the fluorescence recovery on top of these initial and final curves. Your plot should look similar to that shown in Figure 2C.

(b) Estimate the recovery time as the time it takes for the fluorescence in the center of the bleached region to reach 2/3 of its maximum value. To make this possible, perform simulations for $D = 2\ \mu\text{m}^2/\text{s}$, $D = 10\ \mu\text{m}^2/\text{s}$ and for $D = 20\ \mu\text{m}^2/\text{s}$ and plot recovery time as a function of D as shown in Figure 2D.

3 The biological consequences of diffusion limited rates

In class, we introduced the diffusive speed limit as a fundamental constraint of biological reactions such as enzyme action. Here, we further explore the biological consequences of this speed limit. Specifically, we estimate the maximum rate of translation.

Learn about the complex of aminoacid-tRNA, EF-Tu, and GTP that binds to an active ribosome. You can look at, for example, the section "Large Protein Movements Can Be Generated From Small Ones" on page 179 of Alberts *et al.* (5th edition), and their Figure 3-74, and section "Elongation Factors Drive Translation Forward and Improve its Accuracy" on page 377.

Let's work out the rate for a complex of aminoacid-tRNA, EF-Tu, and GTP binding to an active ribosome. That is, make an estimate of the size, diffusion constant, and diffusion-limited rate of the arrival of an aminoacid-tRNA+EF-Tu+GTP complex to the ribosome. To make this possible, you can use of what you learned about these molecules from the sources

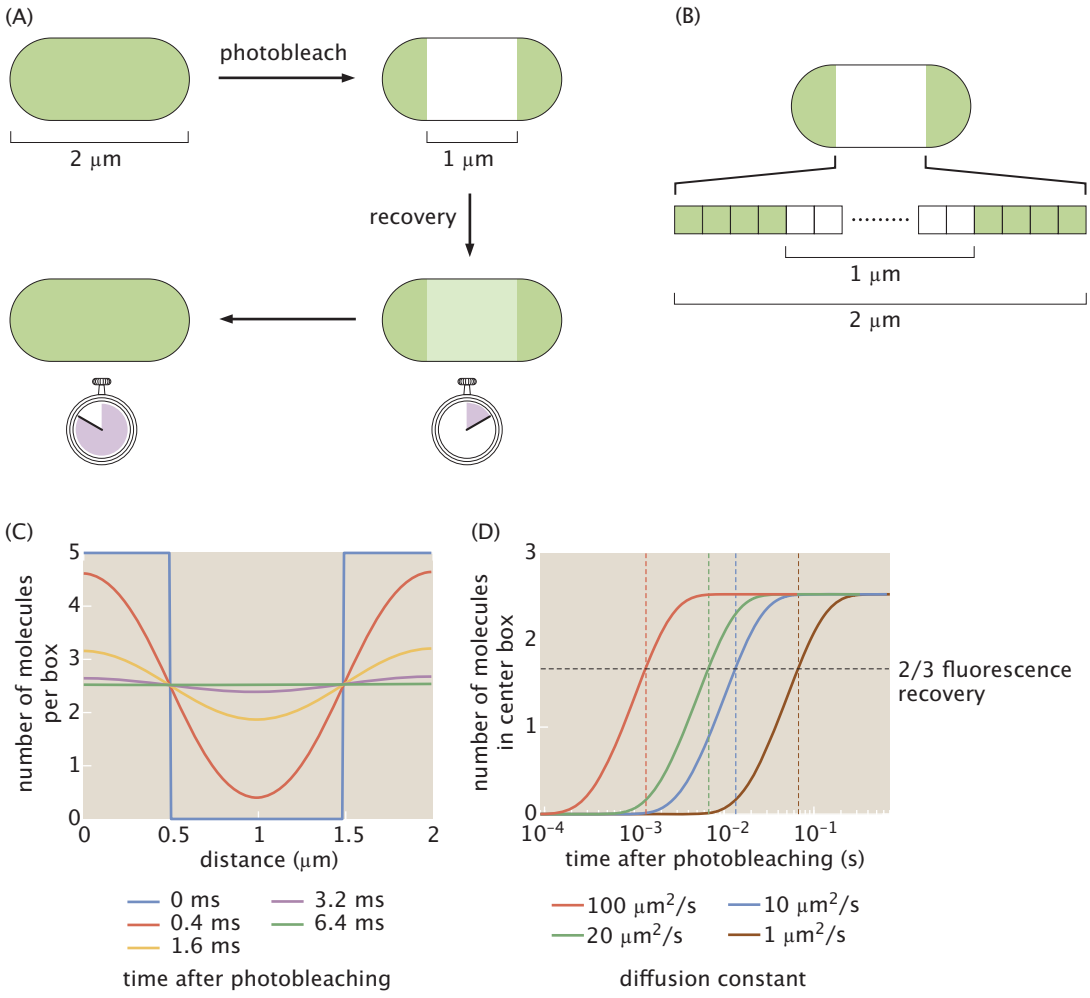

Figure 2: Simulating a bacterial FRAP experiment. (A) The center 1 μm of a 2 μm bacterium expressing GFP is bleached. The time course of fluorescent recovery within the bleached region is recorded. (B) Simulation of the FRAP process by considering the bacterium as a one-dimensional array of boxes containing a given number of GFP molecules. At each time step, every molecule jumps to the right or left with equal probability, except for the boxes at each of the ends of the cell. (C) Simulated number of GFP molecules as a function of position along the bacterium for different time points. (D) The time for the center box to recover its fluorescent content can be used to determine the diffusion constant.

suggested above or refer to BioNumbers. Compare your rate to the known translation rate. Of course, you will have to make some assumptions about c_o, the overall concentration of tRNA molecules in the cell. Find some typical concentrations on by looking at Dong *et al.* (1996), which is provided on the course website. If you want to learn more about the consequences of this speed limit on bacterial growth, see the Klumpp *et al.* paper also provided on the website.

4 The length scale of morphogen gradients

Later in the course, we are going to introduce the important and fascinating topic of reaction-diffusion equations as a window onto the process of pattern formation. One of the outcomes of the careful analysis we will do there is the existence of solutions to the equations describing morphogen dynamics that lead to morphogen gradients.

In this problem, we exploit the skills we have been working out on scaling analysis to figure out how the length scale of the morphogen depends upon key molecular parameters. In particular, we will think about the formation of the gradient of the Bicoid activator along the anterior-posterior axis of the embryo shown in Figure 3. This protein gradient is formed as a result of the translation of *bicoid* mRNA, which is provided by the mother and localized at the anterior end of the embryo as shown in Figure 4. *bicoid* mRNA is stable throughout this stage of development.

As the mRNA gets translated at a rate r, the resulting Bicoid molecules diffuse through the embryo at a rate D, and are also subject to degradation with a decay rate γ. These processes lead to the creation of the exponential-like concentration gradient of Bicoid throughout the embryo shown in Figure 3. In this problem, we exploit the skills we have been working out on scaling analysis to figure out how the length scale of the morphogen gradient depends upon the key molecular parameters mediating its creation.

Let's begin by building some intuition for what we mean when we talk about the length scale of a morphogen gradient. Let's assume that the Bicoid gradient can be described by the concentration profile given by

$$Bicoid(x) = Bicoid_0\, e^{-x/\lambda}, \tag{7}$$

where x is the position along the embryo measured with respect to the embryo length of approximately $L = 480$ μm, $Bicoid_0$ is the concentration of the morphogen at $x = 0$, and λ is the length scale of the gradient. Note that we're ignoring the small decay in Bicoid concentration toward the anterior end of the embryo.

(a) What is the meaning of λ? Specifically, what is the relative decrease in Bicoid concentration with respect to $Bicoid_0$ when $x = \lambda$? Use your result to estimate the value of λ from the data shown in Figure 3 both as a fraction of the embryo length and in absolute units (in μm).

(b) Let's imitate the types of scaling analyses we have performed in class to find an expression for the length scale λ in terms of the model parameters r, D and γ. First, we could posit that the length scale is given by

$$\lambda = r^\alpha D^\beta \gamma^\delta. \tag{8}$$

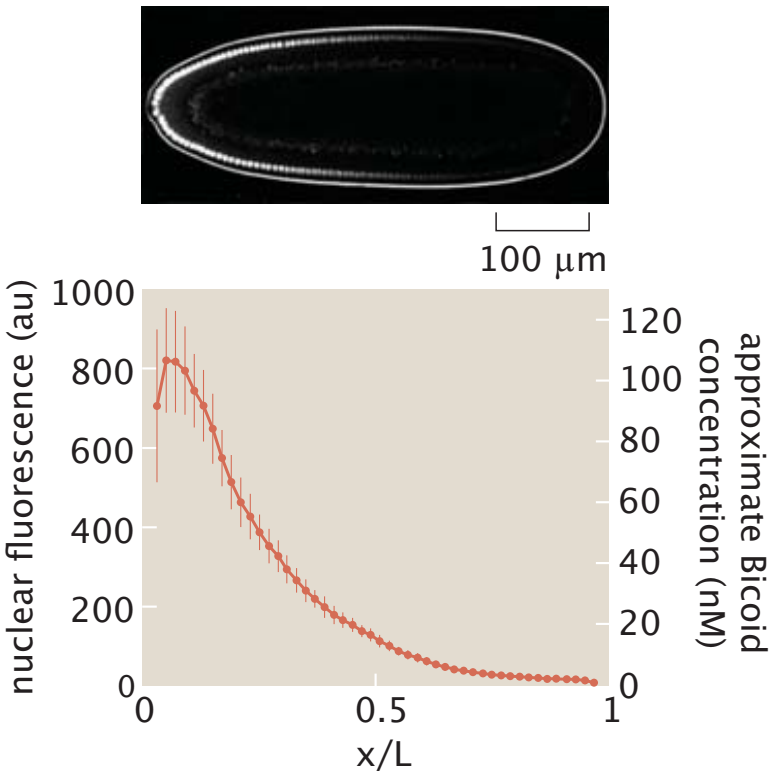

Figure 3: The Bicoid morphogen. The Bicoid activator is distributed in an exponential gradient. (Adapted from F. Liu *et al.*, Proc Natl Acad Sci USA 110:6724 2013.)

Note that, since r and γ have units of inverse time, it will not be possible to determine the exponents α and δ uniquely. As a result, before we launch on dimensional analysis, we need to use physical intuition to further constrain our calculation. Note that λ is a measure of how far each Bicoid molecule gets due to diffusion (with diffusion constant D) before it is degraded (with a degradation rate γ). As a result, how far a molecule goes is independent of the rate with which molecules are produced such that $\alpha = 0$ and our expression reduces to

$$\lambda = D^{\beta}\gamma^{\delta}. \tag{9}$$

Now, use dimensional analysis to find the numerical values of the exponents β and γ. Make sure to explain the units of each of the molecular parameters.

(c) Given a typical diffusion constant for proteins of $D = 10\ \mu m^2/s$ and a degradation time $\gamma = 1/50\ \text{min}^{-1}$, estimate the length scale of the Bicoid morphogen in the fly embryo and compare it to your measurements from (a).

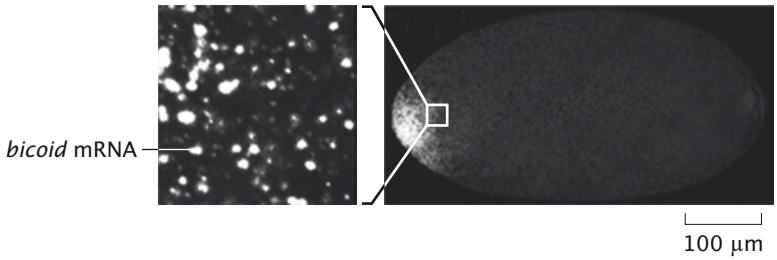

Figure 4: *bicoid* mRNA distribution. Using single molecule mRNA FISH, the localization of individual *bicoid* mRNA molecules at the anterior end of the embryo can be revealed. (Adapted from Petkova et al. (2014), *Current Biology* 24:1283.)