MCB137L/237L: Physical Biology of the Cell
Spring 2022
Homework 4: Diffusion as the null model of biological dynamics
(Due 2/17/22 at 11:00am)

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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

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 and I am not giving you the whole thing. 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 points (out of 100) on your score on the homework. Remember that, to turn this in, you either need to provide us with a red ink comments throughout the PDF, or a referee report that refers to page numbers for each comment you have. Just saying that you liked the chapter is not enough! Please submit the PDF of your comments to Hernan by the homework due date.

1. DNA Synthesis Over Your Lifetime

Estimate the total length of DNA your body will produce over your lifetime. To make this estimate, you can first figure out which cells are the most numerous in your body by reading Sender *et al.*, Cell 164:337 (2016), which is provided on the course website. Then, find out how often these cells get renewed.

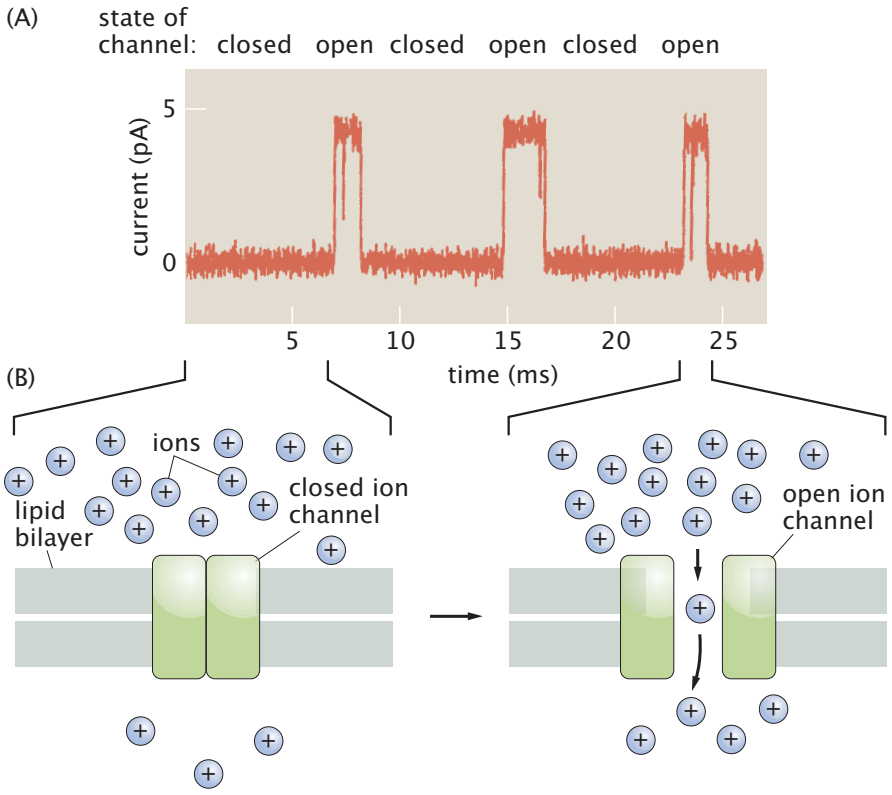
2. Ion channel currents

Figure 1A shows a single-channel recording of the current passing through a voltage-gated sodium channel. The data reveal that the channel transitions between open and closed states as shown in Figure 1B. When in the open state, Na^+ ions can flow from one side of the membrane to the other, resulting in a current across the membrane.

Given that ions have a typical diffusion constant of 1000 $\mu m^2/s$, given the difference between the sodium intracellular and extracellular concentrations shown in Figure 1C, and using a

rough guess for the radius of an ion channel, estimate the current that flows through the ion channel when in the open state.

Recall that the charge of one monovalent ion is 1.6×10^{-19} C (Coulomb), and that 1 A = 1 C/s (Ampere = Coulomb/second). Compare your estimate to the current measured in Figure 1A.



(C)

$D_{small\ ions}$ = 1000 μm²/s

ion species	intracellular concentration (mM)	extracellular concentration (mM)
K^+	155	4
Na^+	12	145
Ca^{2+}	10^{-4}	1.5
Cl^-	4	120

Figure 1: Electrical current flowing through an ion channel. (A) Current flowing through a single voltage-gated sodium channel. (B) The channel recording reveals transitions through an open and a closed state. (C) The concentration gradient of Na^+ ions across the membrane can be used to estimate the current when the channel is open. (A, adapted from B. U. Keller et al., *J. Gen. Physiol.* 88:1, 1986; B, adapted from B. Hille, Ion Channels of Excitable Membranes. Sinauer Associates, 2001)

3. 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 *E. coli*. Using a typical diffusion constant for a protein ($D = 10$ μm^2/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 $D = 2$ μm^2/s, $D = 10$ μm^2/s and for $D = 20$ μm^2/s and plot recovery time as a function of D as shown in Figure 2D.

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.

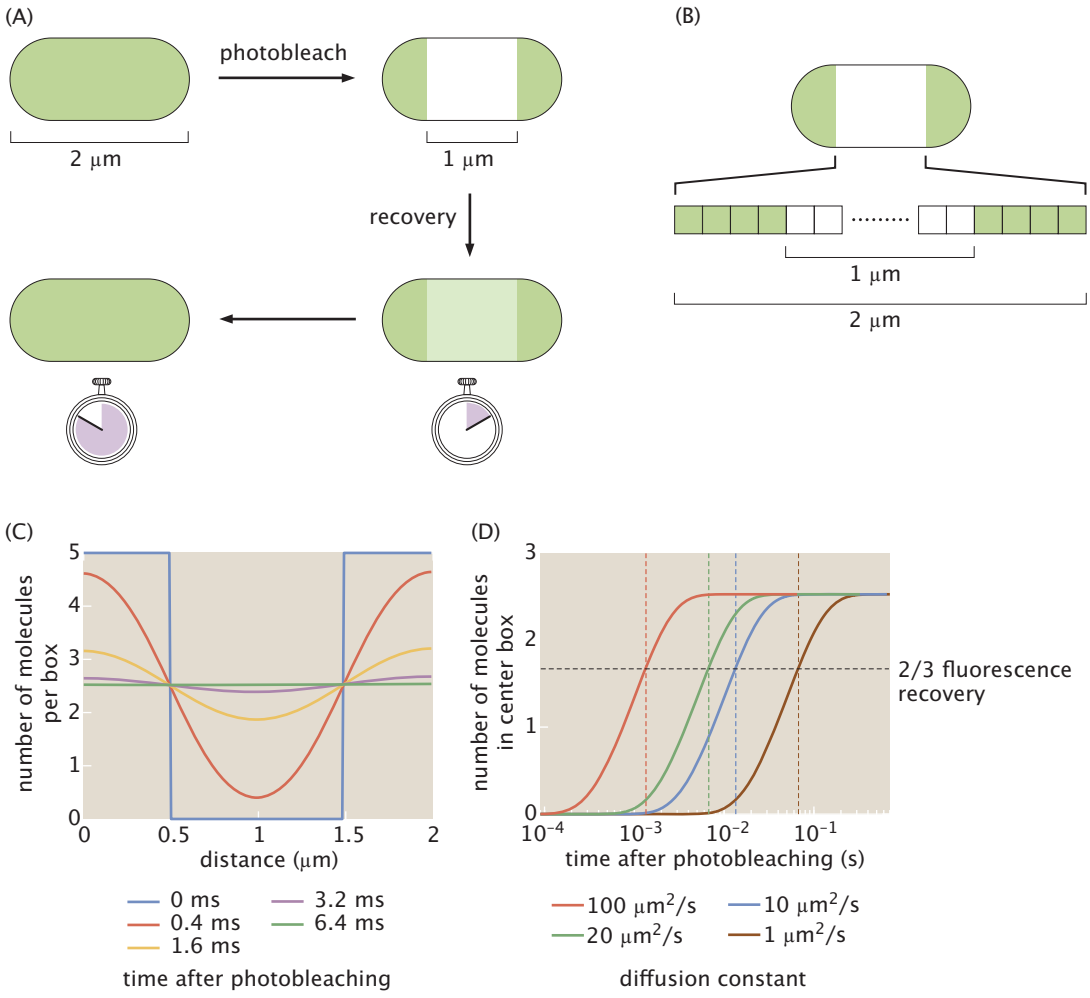


Figure 2: Simulating a bacterial FRAP experiment. (A) The center 1 μm of a 2 μm bacterium expressing GFP is bleached. The time course of fluorescent recovery within the bleached region is recorded. (B) Simulation of the FRAP process by considering the bacterium as a one-dimensional array of boxes containing 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.

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

$$Bicoid(x) = Bicoid_0\, e^{-x/\lambda}, \tag{1}$$

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

$$\lambda = r^\alpha D^\beta \gamma^\delta. \tag{2}$$

Note that, since r, D and γ have units of inverse time, it will not be possible to determine the exponents α, β 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

$$\lambda = D^\beta \gamma^\delta. \tag{3}$$

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 $\gamma = 1/50$ min^{-1}, estimate the length scale of the Bicoid morphogen in the fly embryo and compare it to your measurements from (a).

5. Diffusion on a microtubule

Read the great paper by Helenius *et al.* (provided on the course website) dissecting the mechanism of microtubule depolymerization by the kinesin MCAK. Here, they show how the MCAK molecular motor diffuses along the microtubule towards both ends, triggering the depolymerization of a few tubulin dimers before falling off the microtubule.

(a) In their Figure 2b, they show the mean squared displacement of MCAK $\langle x^2 \rangle$ as a function of time t. Remember that, using dimensional analysis, we concluded that $\langle x^2 \rangle = Dt$, where D is the diffusion constant (there's a difference of a factor of two between our expression and the one used by Helenius *et al.*, but we can ignore that for now). Fit the data in the figure (provided on the course website) "by eye" in order to determine the value of D. To

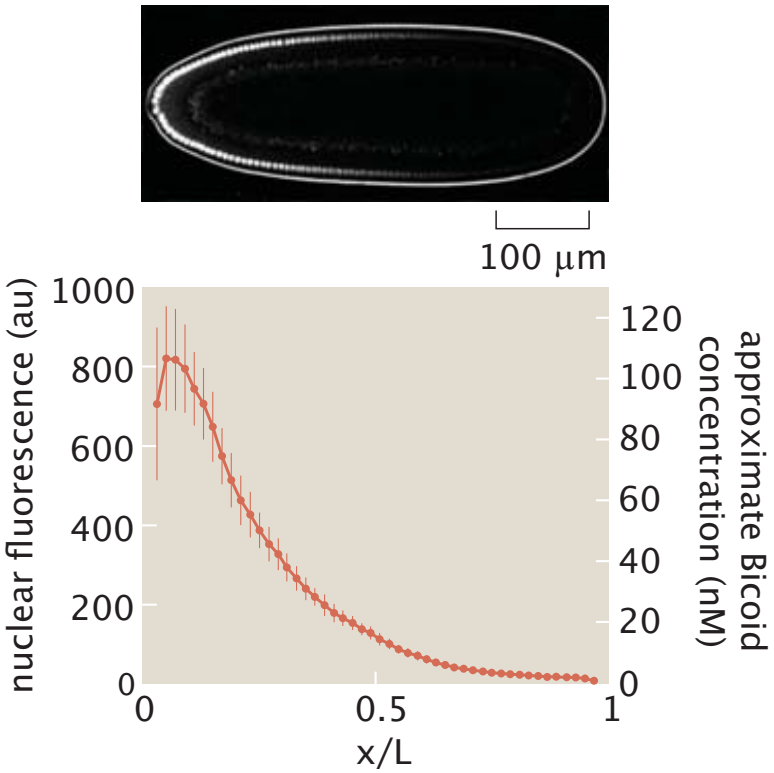


Figure 3: The Bicoid morphogen. The Bicoid activator is distributed in an exponential gradient. (Adapted from F. Liu *et al.*, Proc Natl Acad Sci USA 110:6724 2013.)

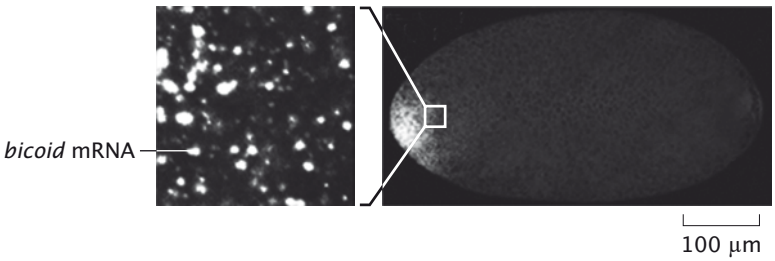


Figure 4: *bicoid* mRNA distribution. Using single molecule mRNA FISH, the localization of individual *bicoid* mRNA molecules at the anterior end of the embryo can be revealed. (Adapted from Petkova et al. (2014), *Current Biology* 24:1283.)

make this possible, plot the expected relation between $\langle x^2 \rangle$ and t for different values of D and decide which value of D better recapitulates the data. EXTRA CREDIT: Write a chi^2 minimization program to determine the diffusion constant. Make sure to plot the chi^2 as a function of D.

(b) In their Figure 3, they argue that a diffusive mechanism can be faster than one of directed motion on short length scales. Explain how this assertion is supported by the plot shown in their Figure 3b, and reproduce the plot in Python.