MCB137L/237L: Physical Biology of the Cell
Spring 2025
Homework 10
(Due 4/08/25 at 2:00pm)

Hernan G. Garcia

1 Bacterial foraging

Bacteria use swimming to seek out food. Imagine that the bacterium is in a region of low food concentration. For the bacterium to profit from swimming to a region with more food, it has to reach there before diffusion of food molecules makes the concentrations in the two regions the same. Here we find the smallest distance that a bacterium needs to swim so it can outrun diffusion.

(**a**) Make a plot in which you sketch the distance traveled by a bacterium swimming at a constant velocity v as a function of time t, and the distance over which a food molecule will diffuse in that same time. Indicate on the plot the smallest time and the smallest distance that the bacterium needs to swim to outrun diffusion. You don't need to use Python here, just make the plot by hand and show the two curves schematically.

(**b**) Calculate these minimum times and distances for an *E. coli* swimming at a speed of $30\,\mu\text{m/s}$. The diffusion constant of a typical food molecule is roughly $500\,\mu\text{m}^2/\text{s}$.

(**c**) Estimate the number of ATP molecules the bacterium must consume (hydrolyze) per second in order to travel at this speed, assuming that all of the energy usage goes into overcoming fluid drag. The drag force felt by the bacterium is given by

$$F = 6\pi\eta R v, \tag{1}$$

where R is the typical size of an *E. coli*, η is the viscosity (we can assume it's swimming in water) and v is the speed of the bacterium. The power necessary to move the bacterium at a speed v against this viscous drag is

$$P = Fv. \tag{2}$$

The amount of energy released from one ATP molecule is approximately $20\,k_{\text{B}}T$. Note that the bacterial flagellar motor is actually powered by a proton gradient and this estimate focuses on the ATP equivalents associated with overcoming fluid drag.

2 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. For example, you can look at 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. Alternatively, you can look at Wikipedia or at a textbook of your choosing.

Let's work out the diffusion-limited rate with which the complex made out of aminoacid-tRNA, EF-Tu, and GTP arrive to an active ribosome. To make this possible, you will have to estimate the diffusion constant and size of this complex. You can use what you learned about these molecules from the sources 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 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.

3 What Living Organisms Must Fight

In class we talked about how systems will tend towards the state of maximum entropy. In this problem, you are going to flesh out the details of the calculations leading to the graphs we showed in class and will provide your own graphs.

(a) Equilibrium with respect to mass transport. Consider a system partitioned equally into two parts, each of which contains Ω lattice sites. We want to write the total entropy as $S_{tot}(L) = S_L(L) + S_R(L_{tot} - L)$. Show that these contributions to the entropy can be written as

$$S_L(L) = k_B \log \frac{\Omega^L}{L!} \tag{3}$$

for the left side and

$$S_R(L_{tot} - L) = k_B \log \frac{\Omega^{L_{tot}-L}}{(L_{tot} - L)!} \tag{4}$$

for the right side. Using the Stirling approximation, derive the expression

$$S_{tot}(L) = -k_B L_{tot} \left[\frac{L}{L_{tot}} \log \frac{L}{L_{tot}} + \left(1 - \frac{L}{L_{tot}}\right) \log \left(1 - \frac{L}{L_{tot}}\right) + \left(\log \frac{L_{tot}}{\Omega} - 1\right) \right] \tag{5}$$

for the total entropy. Plot the entropy of the left part, the right part and the total entropy as a function of the number of ligands in the left side of the container which can run from

$L = 0$ to $L = L_{tot}$. To make this plot, you will need to assume a certain number of lattice sites. Imagine a container with $\Omega = 10^9$ lattice sites. If each such lattice site has a volume of 1 nm^3, then the total volume of each side is 1 μm^3.

(b) We next consider the case in which the partition between the two sides is mobile. In this case, we are interested in how the entropy on the left side and the right side play against each other, conspiring to give a total entropy of the form

$$S_{tot}(x) = S_L(x) + S_R(x), \tag{6}$$

where x is the label used to characterize the position of the interface. As usual, the entropy is given by the Boltzmann formula which in this case takes the form

$$S_L(x) = k_B \log W_L(x) \tag{7}$$

and

$$S_R(x) = k_B \log W_R(x). \tag{8}$$

To make progress, we now need to reckon the number of states as a function of the position x of the partition. When the partition is at the midpoint, each of the subcompartments has a volume V. The volume swept out by the motion of the partition by a distance x is xA, where A is the cross-sectional area of that partition. As a result, show that the number of lattice sites added or subtracted due to the motion of the partition is xA/v where v is the volume corresponding to a single lattice site, leading to the results

$$W_L(x) = \frac{(\frac{V+xA}{v})^{L_L}}{L_L!}, \tag{9}$$

and

$$W_R(x) = \frac{(\frac{V-xA}{v})^{L_R}}{L_R!}. \tag{10}$$

Use these results to show that

$$S_{tot}(x) = k_B L_L \log \frac{V+xA}{v} - k_B \log L_L! + k_B L_R \log \frac{V-xA}{v} - k_B \log L_R!, \tag{11}$$

and make a plot of the resulting entropy of the two sides and the total entropy as a function of the position of the partition x.

4 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. You can revisit FRAP by reading "Experiments Behind the Facts: Measuring Diffusive Dynamics" on page 513 of PBoC or by referring to the primary literature. 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 1A. Here, we will solve for the fluorescence recovery dynamics by discretizing *E. coli* into small boxes as shown in Figure 1B.

(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 1C.

(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 1D.

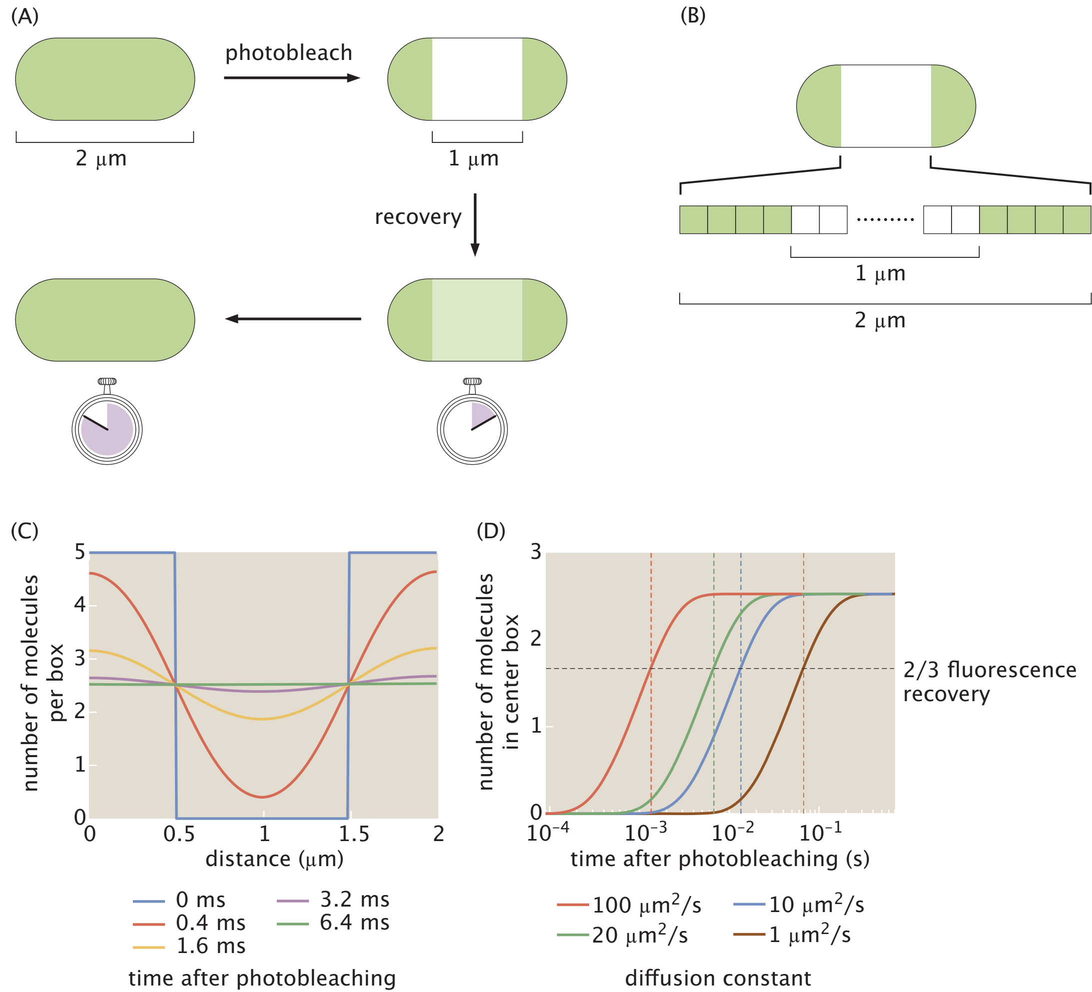

Figure 1: Simulating a bacterial FRAP experiment. (A) The center 1 μm of a 2 μm bacterium expressing GFP is bleached. The time course of fluorescent recovery within the bleached region is recorded. (B) Simulation of the FRAP process by considering the bacterium as a one-dimensional array of boxes containing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

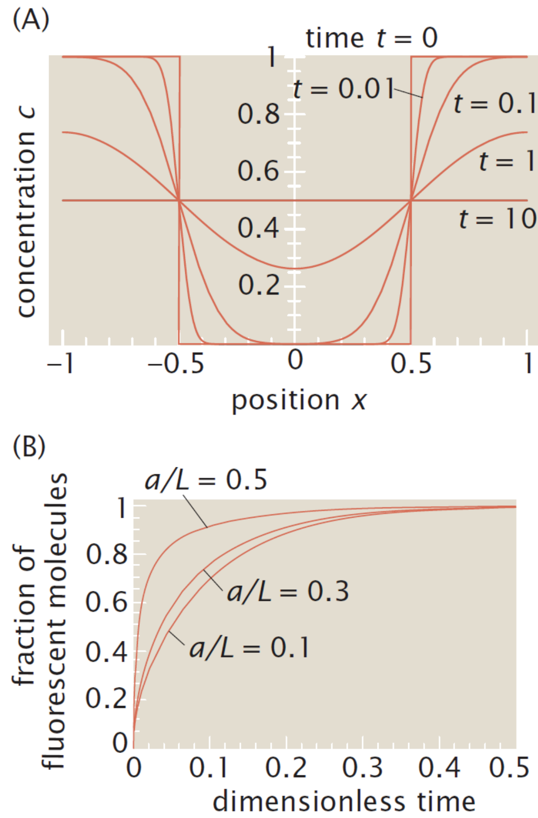

Figure 13.17: One-dimensional model of FRAP. (A) Concentration profile for different times after photobleaching. The bleached region is half the size of the confining region, $2L$.
(B) Fluorescence recovery as a function of time for different sizes of bleached regions. Recovery is fastest when the bleached region is half the size of the confining region. In both graphs, time is measured in units of L^2/D and length in units of L.

Figure 2: Figure 13.17 from PBoC.