BE/APh161: Physical Biology of the Cell
Homework 3
Due Date: Wednesday, January 26, 2022

"Champions aren't made in gyms. Champions are made from something they have deep inside them - a desire, a dream, a vision. They have to have the skill, and the will. But the will must be stronger than the skill." - Muhammad Ali

Extra Credit. Provide comments on chap. 5, "Diffusion as Biologys 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% on your score on the homework. However, to turn this in, it needs to be in the form of a referee report (not red ink on the draft) that refers to page numbers for each comment you have. Please submit the pdf of your comments to Rebecca and Rob with subject "Comments on PBOC3 - chap. 5."

1. Phosphorus, Sulfur and the Lives of Cells

In addition to the big ticket chemical elements in cells (carbon, nitrogen, oxygen, hydrogen), other elements come in at lower concentrations, but still with enormous functional importance. Two such elements are phosphorus and sulfur and in this problem, we will try to figure out how much of the cell's dry weight is taken up by these elements and what this implies about the transport of these elements into the cellular interior. A useful vignette to watch in order to do this problem is "Rate-Limiting Hypothesis - Carbon Transport" from the 2021 edition of the course.

(a) Let's begin by trying to estimate the number of phosphorus atoms in a cell. Where do we find phosphorus? There is 10 mM of ATP in a typical bacterium. We all know that in both RNA and DNA, every base carries its own phosphate. Many lipids are phospholipids, with polar heads contain-

ing phosphate atoms as well. Proteins are phosphorylated (see problem 4!!). Don't forget ribosomes. They too are full of phosphorus atoms because they are 2/3 by mass RNA. Given these various facts, estimate the total number of phosphorus atoms in a bacterium. Given a division time of $f \times 10^3$ s, how many phosphate transporters (PitA) are needed to bring all those phosphorus atoms into the cell during that time?

(b) Next, we consider sulfur. Where do we find sulfur atoms in cells? Clearly one of the main amino acids, cysteine, has its known covalent binding properties precisely because of its sulfur atom. The metabolite glutathione has a concentration of 17 mM. Like in the previous part of the problem, in light of these facts, make an estimate of the total number of sulfur atoms in a bacterial cell. Given a division time of $f \times 10^3$ s, how many sulfur transporters (CysUWA) are needed to bring all those sulfur atoms into the cell during that time?

2. DNA replication rates.

Do problem 3.3 of PBoC2. However, as you do this problem, please come at it a few different ways. First, when estimating how much of the full fly genome is shown in the figure, account for the fact that the DNA is compacted by nucleosomes. Second, given that the entire fly genome has been claimed to have ≈ 6000 origins of replication, figure out the mean spacing between such origins and use that estimate as the basis of your own independent estimate of the replication time for the *Drosophila* genome.

3. Estimating the diffusion constant.

In this problem, we are going to use the observed trajectories of diffusing GFP molecules to estimate the diffusion coefficient.

(a) Conventional microscopy to observe individual fluorescent proteins won't work. In this part of the problem, we are going to work out why. During a traditional experiment, the microscope shutter is open during some time interval of order 10s to 100s of milliseconds. By assuming a diffusion constant of 10 $\mu m^2/s$, work out how far the fluorescent protein will move during the time that the shutter is open and compare that distance to the size of the cell itself and comment on how this limits our ability to measure the diffusion

constant. Perform the estimate a second time, this time using the 0.3 ms exposure time shown in Figure 1(A).

(b) Using the trajectories shown in Figure 2 and our simple rule of thumb that $t_{diffusion} = L^2/D$ to estimate the diffusion constant for GFP. Explain your reasoning carefully.

NOTE: to do this problem, the vignettes "Scaling of Diffusion Time," "Diffusion Time by the Numbers" and "Diffusion: Coin Flips" will be helpful.

4. Testing the model of nucleolus scaling.

In class, we discussed the scale and scaling of various cellular structures and processes. In particular, we talked about the scaling of the size of the nucleolus with the size of the nucleus itself in the *C. elegans* embryo. Using a simple model, we derived an expression for the number of molecules that make the nucleolus (in this case FIB-1 molecules) given by

$$M = \left(\frac{N}{V} - \frac{k_{off}}{k_{on}}\right) V, \tag{1}$$

where N is the total number of FIB-1 molecules inside the nucleus, k_{on} is the rate of FIB-1 incorporation into the nucleolus, k_{off} is the rate with which FIB-1 molecules detach from the nucleolus, and V is the nuclear volume.

We explored two types of experiments. First, we discussed an experiment in which the normal course of development leads to the progressive reduction of cell—and nuclear—size. In this case, the FIB-1 concentration within each nucleus remains constant such that we can rewrite Equation 1 as

$$M = (c_{tot} - c_*)\, V, \tag{2}$$

where $c_{tot} = N/V$ is the FIB-1 concentration and $c_* = k_{off}/k_{on}$ is the critical concentration at which the nucleolus forms. A second experiment relied on altering the expression of genes that lead to the formation of *C. elegans* embryos with larger or smaller cells. The assumption is that, in these mutants, the total number of FIB-1 molecules N will not change, but its nuclear concentration will. As a result, we can write Equation 1 for this case of constant number as

$$M = N - c_* V. \tag{3}$$

(a) Read the paper by Weber and Brangwynne (provided on the course website) and, in one short paragraph, explain how they managed to change the size of cells within the embryo and how they ensured that, for all embryo sizes, the total number of FIB-1 molecules remained constant.

The two types of experiments captured by Equations 2 and 3 give us an opportunity to test the predictive power of our model. Specifically, note that Equation 3 predicts that, for the fixed FIB-1 number experiment, the y-intercept of the scaling of the nucleolus with nuclear volume will be given by N while the slope will be $-c_*$.

(b) Write Python code to plot the data (provided on the course website) and perform a manual linear fit to the data in order to estimate the value of N and c_*.

(c) Now, use the parameters inferred in (b) to predict the scaling of nucleolar size versus nucleus volume for the fixed FIB-1 concentration experiment. Specifically, draw a plot where you overlay the experimental data with your theoretical prediction.

5. Protein density on the membrane vs in the cytoplasm

Which is larger, the protein concentration in membranes or in the cytoplasm? Report both of them in units of number per μm^3.

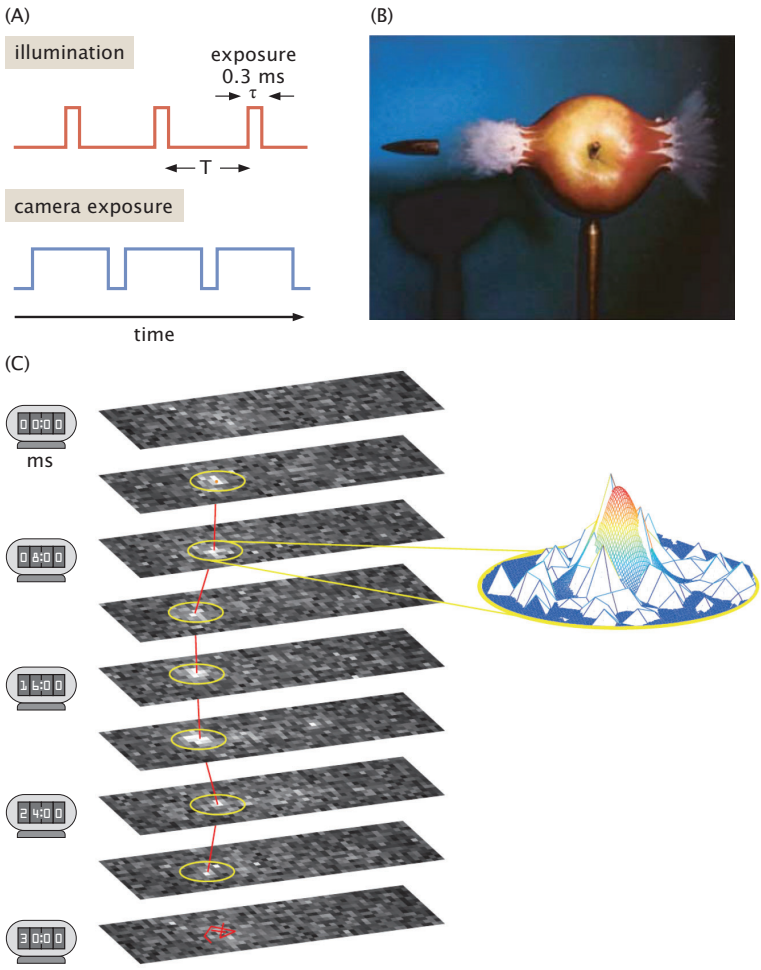

Figure 1: Stroboscopic illumination for high speed photography. (A) Comparison of the pulse of light and the camera exposure times. Brief illumination periods guarantee that the diffusing molecule doesn't move very far. (B) A classic photo from MIT legend Harold Edgerton who pioneered stroboscopic photography for science and fun. (C)

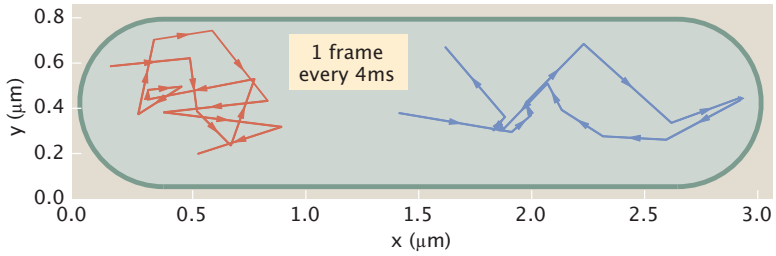

Figure 2: Time series showing positions of diffusing GFP molecules at different times. The red and blue traces correspond to different molecules. The lines are a guide to the eye.