BE/APh161: Physical Biology of the Cell
Homework 5
Due Date: Wednesday, February 14, 2024

"Thinking, analyzing, inventing are not anomalous acts; they are the normal respiration of the intelligence." - Jorge Luis Borges

1. Setting up the fly body plan.

One of the most important ideas for how positional information arises in multicellular organisms is the idea of a morphogen gradient (another serious contender is a Turing pattern). In this problem we will use a steady-state solution to the reaction-diffusion equation for Bicoid to understand how the exponential profile shown in Figure 1 is set up. Stated simply, the development of the Bicoid gradient can be thought of as resulting from a competition between the diffusion of Bicoid protein that is synthesized at the anterior end of the embryo (the mother deposits localized *bcd* mRNA there as shown in Figure 2) and the degradation of this protein while it is diffusing around.

(A) Give a brief description (a paragraph or less) of the Bicoid gradient in *Drosophila* and how it is relevant to fly development. Further, to get a feeling for the Bicoid gradient, redraw the Bicoid profile shown in Figure 1 in terms of the absolute number of Bicoid proteins per nucleus. You can make the drawing by hand or plot some approximate curve using Python. To make this estimate, you will need to use the information about nuclear sizes in nuclear cycle 14 provided in Figure 4C of Gregor2007a (provided on the course website).

(B) Make a derivation of the reaction-diffusion equation and use it to justify the form

$$\frac{\partial Bcd(x,t)}{\partial t} = D\frac{\partial^2 Bcd(x,t)}{\partial x^2} - \frac{Bcd(x,t)}{\tau}. \tag{1}$$

Make sure you explain carefully where all of these terms come from. To do so, begin the usual way by considering a one-dimensional concentration profile and by finding the rate of change of number of Bicoid molecules in the box at position x by considering the flux into $(J_m(x - \Delta x/2))$ and out of $((J_m(x + \Delta x/2))$ the box using arguments like those made in class. However, you need to generalize that treatment by accounting for the fact that

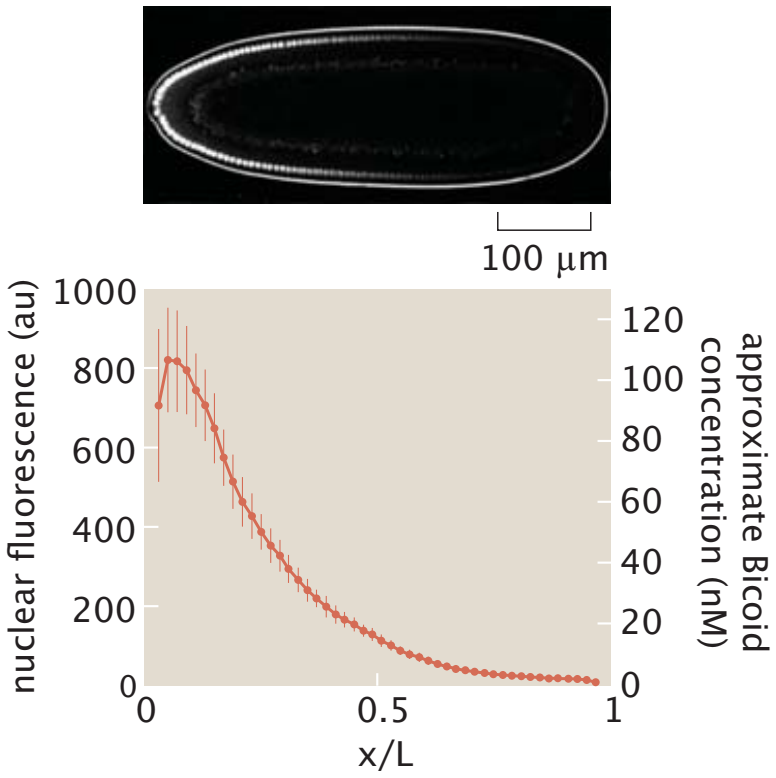

Figure 1: The Bicoid morphogen. The Bicoid activator is distributed in an exponential gradient. (Adapted from F. Liu *et al.*, Proc Natl Acad Sci USA 110:6724 2013.)

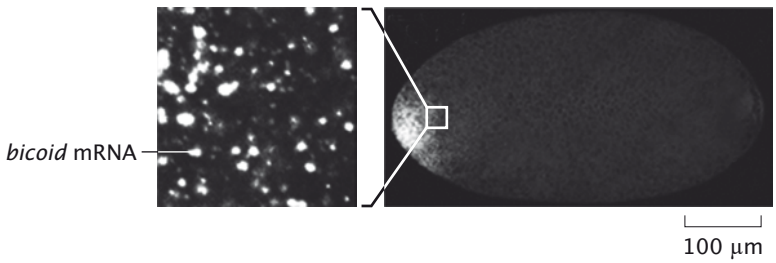

Figure 2: *bicoid* mRNA distribution. Using single molecule mRNA FISH, the localization of individual *bicoid* mRNA molecules at the anterior end of the embryo can be revealed. (Adapted from Petkova et al. (2014), *Current Biology* 24:1283.)

a Bicoid molecule has the probability $r\Delta t$ of degrading in time interval Δt, where $r \approx 1/\tau$, where τ is the degradation time.

(C) Now solve this equation in steady-state by finding the general solution subject to the boundary condition that $J(0,t) = j_0$ and $J(L,t) = 0$. Make sure you explain what these boundary conditions mean relative to the biology of the problem. Suggest approximations that can be made to simplify the result, specifically, can you exploit the fact that the embryo is much larger than the decay length to simplify the solution?

(D) Describe the observed concentration profile of Bicoid along the anterior-posterior axis of the fly mathematically. What is the functional form? Experimentally, Thomas Gregor has found that the Bcd profile is an exponential of the form $Bcd(x) = Bcd_0 e^{-x/\lambda}$ where x is the position along the embryo, Bcd_0 is the Bicoid concentration at $x = 0$ and λ is the decay constant of the gradient. Does that experimental profile jibe with your solution?

(E) The paper by Drocco *et al.* uses a photoactivatable fluorescent protein to measure the lifetime of the Bicoid protein. Read the paper (available on the course website) and explain the technique in one paragraph. You might find it useful to draw a schematic plot such as shown in Figure 1f of the paper.

(F) What is the value of the decay constant λ for the gradient shown in Figure 1? To estimate this magnitude, you can just fit "by eye" by plotting your solution for different values of Bcd_0 and λ. Now, compare the measured λ value with that you can predict by plugging in realistic values of D, τ into your solution. To make this possible, read the papers by Abu-Arish *et al.* and Drocco *et al.*, provided on the course website.

(G) One of the most important and interesting ideas to come out of the idea of positional information contained in morphogen gradients was the so-called French flag model which we will explore here. This model posits that the Bicoid concentration dictates the position of the cephalic furrow. As seen in Figure 3, the idea of the model is that boundaries in the embryo are determined by threshold values of the morphogen. The idea of the model is that if the gene dosage gets changed, as seen in the mutant profile, the boundary will still occur at the same value of the morphogen. That hypothesis is enough to determine the shift in boundary position with gene dosage.

To test this model, we will analyze several experiments (Nusslein-Vohlhard and Driever and Liu *et al.*) where they measured cephalic furrow position as a function of different dosages of the *bicoid* gene in embryos. As seen above, an exponential gradient of Bicoid is described by

$$Bcd(x, \lambda, \alpha, Bcd_0) = Bcd_0\, \alpha\, e^{-x/\lambda}, \tag{2}$$

where x is the position along the embryo, Bcd_0 is the Bicoid concentration at $x = 0$, λ is the decay constant of the gradient and α is the Bicoid dosage, with $\alpha = 1$ corresponding to the wild-type. Work out a model for the position of the cephalic furrow x_{new} as a function of the gene dosage α, the morphogen gradient decay length λ and the position of the wild-type cephalic furrow, x_{CF}.

(H) Note that, given a measured $x_{CF} \approx 32\%$ of the embryo length, your model has no free parameters. Compare the prediction from your model with the data for x_{new} vs. α obtained by Nusslein-Vohlhard, and by Driever and Liu *et al.*. Comment on how well your prediction matches the data that is provided with the homework. What could be going on?

2. Diffusive speed limits: It's not just a good idea, it's the law

In order for a chemical reaction to take place, the reactants must be at the same place at the same time. A very interesting calculation explores the way in which diffusion can control the on rate for reactions. Imagine some reaction in which A and B come together to form the complex AB. To simplify the problem, we are going to imagine B as a sphere of radius a that is fixed at the origin of our coordinate system. Further, we are going to imagine that very far away the concentration of A is held at c_0. What I really mean by this is that $lim_{r\to\infty} c(r) = c_0$, where $c(r)$ is the concentration of reactant A as a function of distance from the origin. Our goal is to compute the so-called "diffusion-limited on rate" for the reaction. We begin by working out the steady-state solution to the diffusion equation with the boundary condition that $c(a) = 0$, which corresponds to the physical statement that the sphere is a "perfect absorber". What this really means is that every time a molecule of A arrives at the sphere, the reaction occurs. (Note that this tells us that the diffusion-limited on rate is the fastest that a reaction could

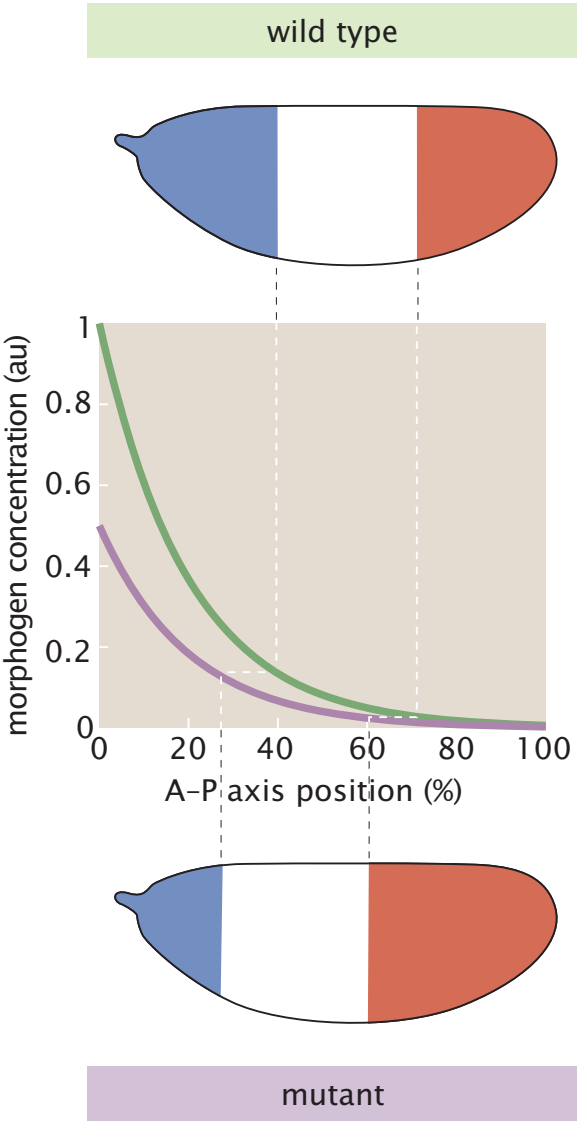

Figure 3: Concept of the French flag model. The green profile shows the wild-type morphogen concentration and the purple profile shows that of a mutant with half the wild-type gene dosage.

occur. It could be true that after the molecule arrives, it has to wait for some favorable orientation to occur, for example, which would make the rate of the reaction even slower).

(a) Solve the diffusion equation

$$\frac{\partial c(\mathbf{r},t)}{\partial t} = D\nabla^2 c(\mathbf{r},t) \tag{3}$$

in steady state and find the concentration profile $c(r)$ as a function of c_0 and a. Explain why we can write the concentration only as a function of the *scalar* r as opposed to the vector $\mathbf{r}$.

(b) Use that result to compute the diffusive flux $J(a)$ at the surface of the sphere. Here you need to invoke Fick's law relating flux and concentration, but acknowledging that you are working in spherical coordinates.

(c) Use the result of part (b) to write an equation for dn/dt, the rate at which A molecules arrive at the sphere and thus the rate of production of AB. The function $n(t)$ simply tells me how many molecules have arrived at the "perfect absorber" during the time between $t = 0$ and the time t.

(d) Now, use the result of part (c) to write an equation of the form

$$\frac{dn}{dt} = k_{on}c_0, \tag{4}$$

and hence write an expression for k_{on}. This is the so-called Smoluchowski rate.

(e) Find a numerical value for this diffusion limited on rate, k_{on}. Justify the units it has and provide an actual numerical value by estimating the relevant parameters that determine k_{on}.

3. What Living Organisms Must Fight.

In the vignette on the "calculus of equilibrium" 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 in that

vignette 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

$$S_L(L) = k_B \log \frac{\Omega^L}{L!} \tag{5}$$

for the left side and

$$S_R(L_{tot} - L) = k_B \log \frac{\Omega^{L_{tot}-L}}{(L_{tot} - L)!} \tag{6}$$

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

$$S_{tot}(L) = -k_B L_{tot} \left[\frac{L}{L_{tot}} \ln \frac{L}{L_{tot}} + \left(1 - \frac{L}{L_{tot}}\right) \ln \left(1 - \frac{L}{L_{tot}}\right) - \left(\ln \frac{L_{tot}}{\Omega} - 1\right) \right] \tag{7}$$

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 on each side of the partition 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

$$S_{tot}(x) = S_L(x) + S_R(x), \tag{8}$$

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

$$S_L(x) = k_B \log W_L(x) \tag{9}$$

and

$$S_R(x) = k_B \log W_R(x). \tag{10}$$

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 states added or subtracted due to the motion of the partition is xA/v, leading to the results

$$W_L(x) = \frac{(\frac{V+xA}{v})^{L_L}}{L_L!}, \tag{11}$$

and

$$W_R(x) = \frac{(\frac{V-xA}{v})^{L_R}}{L_R!}. \tag{12}$$

Use these results to show that

$$S_{tot}(x) = k_B L_L \log \frac{V+xA}{v} - k_B \log L_L! + k_B \log \frac{V-xA}{v} - k_B \log L_R!, \tag{13}$$

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. Interpret the equilibrium state as the state of maximum entropy and how this is equivalent to equality of pressure.