BE/APh161: Physical Biology of the Cell
Homework 5
Due Date: Wednesday, February 9, 2022

"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

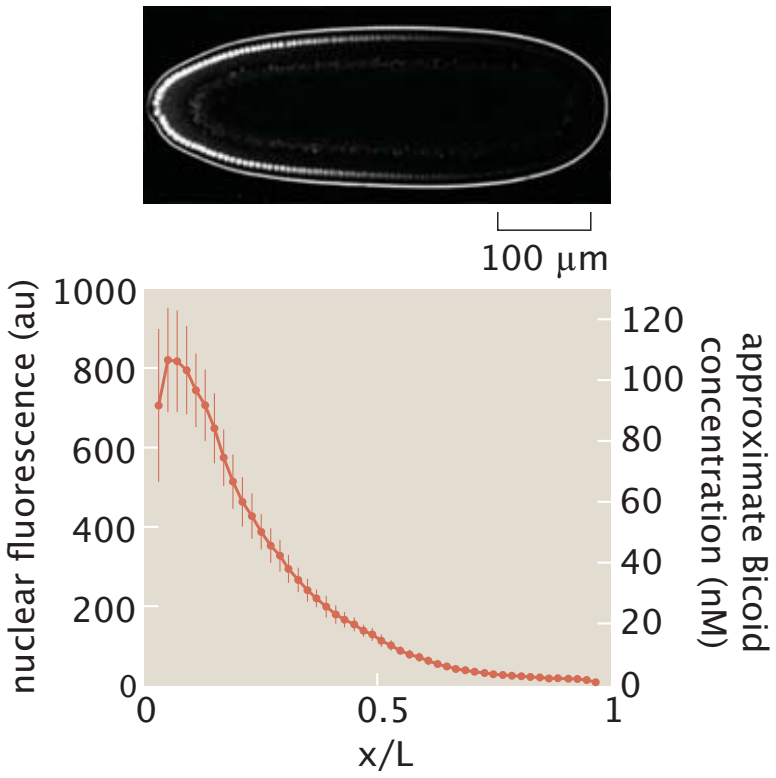

Figure 1: The Bicoid morphogen. The Bicoid activator is distributed in an exponential gradient. (Adapted from F. Liu *et al.*, Proc Natl Acad Sci USA 110:6724 2013.)

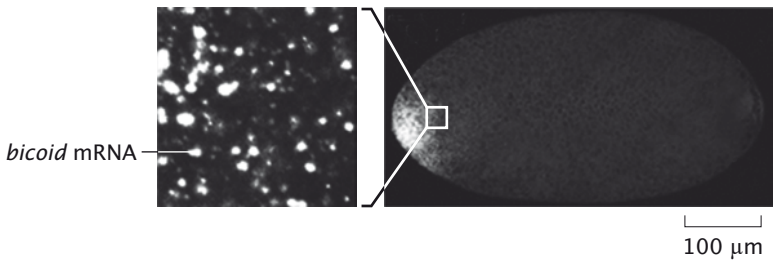

Figure 2: *bicoid* mRNA distribution. Using single molecule mRNA FISH, the localization of individual *bicoid* mRNA molecules at the anterior end of the embryo can be revealed. (Adapted from Petkova et al. (2014), *Current Biology* 24:1283.)

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 $Bcd(x) = Bcd_0 e^{-x/\lambda}$, does that 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

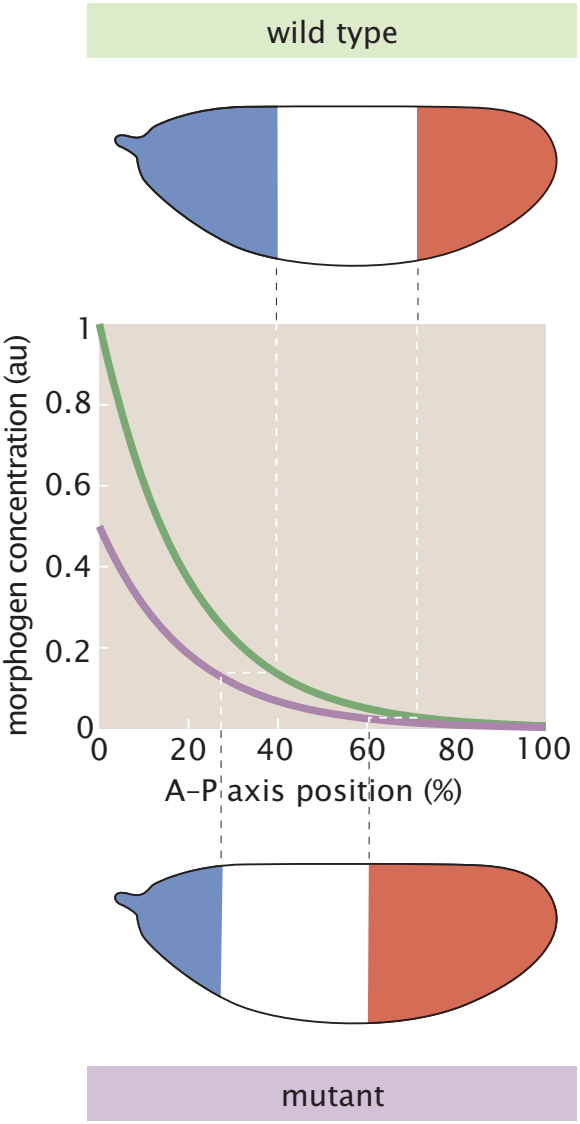

Figure 3: Concept of the French flag model.

and Driever and Liu *et al.*) where they measured cephalic furrow position as a function of different dosages of the *bicoid* gene in embryos. 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. 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

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

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

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

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

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

and

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

Use these results to show that

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

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.

3. Dynamics of A $\rightarrow$ B reactions.

One of the most interesting topics in science is how we have learned to probe deep time. Surprisingly, DNA sequence has permitted us to explore deep time in the biological setting. Of course, biology and the dynamics of the Earth are not independent phenomena and the point of the rest of this problem is to better understand the details of how scientists figure out how old the Earth is as well as how old various fossil-bearing strata are. To that end, we will first consider a simple model of the radioactive decay process for potassium-argon dating methods, recognizing that there are many other dating methods that complement the one considered here.

Potassium-Argon dating

Potassium-argon dating is based upon the decay of ^{40}K into ^{40}Ar. To a first approximation, this method can be thought of as a simple stopwatch in which at t = 0 (i.e. when the rocks crystallize), the amount of ^{40}Ar is zero, since it is presumed that all of the inert argon has escaped. We can write an equation for the number of potassium nuclei at time $t + \Delta t$ as

$$N_{\mathrm{K}}(t + \Delta t) = N_{\mathrm{K}}(t) - (\lambda \Delta t) N_{\mathrm{K}}(t). \tag{12}$$

Stated simply, this means that in every small time increment Δt, every nucleus has a probability $\lambda \Delta t$ of decaying, where λ is the decay rate of ^{40}K into ^{40}Ar. We also employ the important constraint that the number of total nuclei in the system must remain constant, so that

$$N_{\mathrm{K}}(0) = N_{\mathrm{K}}(t) + N_{\mathrm{Ar}}(t), \tag{13}$$

where $N_{\mathrm{K}}(0)$ is the number of ^{40}K nuclei present when the rock is formed, $N_{\mathrm{K}}(t)$ is the number of ^{40}K nuclei present in the rock at time t, and $N_{\mathrm{Ar}}(t)$ is likewise the number of ^{40}Ar nuclei present in the rock at time t. In this part of the problem you will use equations 12 and 13 to construct differential equations to find the relationship between $N_{\mathrm{K}}(t)$, $N_{\mathrm{Ar}}(t)$, and t.

(A) Using equations 12 and 13 as a guide, write differential equations for $N_{\mathrm{K}}(t)$ and $N_{\mathrm{Ar}}(t)$. How do these two expressions relate to one another?

(B) Next, we note that the solution for a linear differential equation of the form $\frac{dx}{dt} = kx$ is given by $x(t) = x(0)e^{kt}$. Use this result to solve for $N_{\mathrm{K}}(t)$.

(C) Use the constraint encapsulated by equation 13 to write an equation for the lifetime of the rock, t, in terms of the ratio $\frac{N_{\mathrm{Ar}}}{N_{\mathrm{K}}}$.

Age of the Galapagos Islands

The potassium-argon dating method described above has been used in several contexts central to some of the most important evolutionary questions in biology. As we go from West to East in the Galapagos Archipelago, the ages of the islands increase, with Santa Cruz older than Isabella, for example. But how are these numbers known and what evidence substantiates these claims when naturalist guides make them? In a beautiful article from Science Magazine in 1976 (Science, New Series, Vol. 192, No. 4238 (Apr. 30, 1976), pp. 465-467), Kimberly Bailey tells us of her efforts to determine the ages of the islands of Santa Cruz, San Cristobal and Espanola. We will now use her data to find out the K-Ar ages of several of these islands ourselves.

(D) Read Bailey's short paper and give a brief synopsis (1 paragraph) of her approach and findings.

(E) Use the results from Sample H70-130 and JD1088 of Table 1 to determine ages for Santa Cruz Island and Santa Fe Island. To do this, you will need to navigate a few subtleties. First, note that the amount of Argon is presented in moles, and so you can use those numbers directly. To determine the number of moles of ^{40}K, you will need to use the weight percentage that is K_2O and use that in combination with the mass of the sample to figure out how much K is present. Note that not all of the potassium in the sample will be the isotope ^{40}K, so you will need to use the ratio of ^{40}K to total potassium, $\frac{^{40}\mathrm{K}}{\mathrm{K_{total}}} \approx 1.2 \times 10^{-4}$. Additionally, use the decay constant $\lambda \approx 5.8 \times 10^{-11}$ yr^{-1}.

Determining Lucy's age

In 1974, a fossil of *Australopithecus afarensis* (shown in Figure 4) was

discovered in Ethiopia. This specimen, which was dubbed "Lucy," marks an important step in understanding human evolution because at the time of its discovery, it was the earliest known species to show evidence of bipedal locomotion. Because Lucy was found in an area that was rich in volcanic rock, potassium-argon dating was an ideal method for determining Lucy's age (Aronsen 1977).

Unfortunately for us, real-world K-Ar dating data are generally not neatly presented in the form of N_{Ar} and N_{K}. Instead, geologists will measure a concentration of ^{40}Ar in mol/g and a weight percent of K_2O. These data must be used to identify the number of ^{40}Ar and ^{40}K nuclei in the sample. In this part of the problem, we will look at such measurements from an actual paleontological specimen as reported in Aronsen (1977) in order to determine its age.

Figure 4: The remains of Lucy, a specimen of *Australopithecus afarensis*.

(F) Using the table of ^{40}Ar and K_2O measurements below (Aronsen 1977), obtain an estimate for Lucy's age. Be sure to explain the steps you take to

obtain your answer. Since each sample is taken from the area in which Lucy was found, we expect each sample to give you roughly the same answer; you will need to take the mean of the ages of each sample to obtain an estimate for Lucy's age.

Assume that each sample has a total mass of 1 g. Also, note that not all of the potassium in the sample will be the isotope ^{40}K, so you will need to use the ratio of ^{40}K to total potassium, $\frac{^{40}\mathrm{K}}{\mathrm{K_{total}}} \approx 1.2 \times 10^{-4}$. Additionally, use the decay constant $\lambda \approx 5.8 \times 10^{-11}$ yr^{-1}.

Table 1. Outcome of measurements of potassium and argon for dating the rocks in the vicinity of Lucy.

Sample Number	$^{40}\mathrm{Ar} \times 10^{-12}$ mol/g	wt.%$\mathrm{K_2O}$
1	2.91	0.657
2	3.18	0.755
3	3.08	0.680

Reactions of the form

$$\mathrm{A} \rightarrow \mathrm{B}. \tag{14}$$

are ubiquitous in the natural world. Thus far, we examined these equations in the context of radioactive decay, a phenomena central to biology because it provides a way of understanding biological evolution. Part of the intention of this problem is to illustrate the broad reach of these reactions in problems ranging from the dating of incredibly important fossils such as the famed Lucy to the molecules of vision.

(G) Apply the results from your analysis of radioactive dating to now write an equation for the decay of 13-*cis*-retinal to all-*trans*-retinal, as is illustrated in Figure 5. The half-life of this reaction is $\tau = 2\,\mathrm{s}$. Make sure you write down a formal relationship between the rate constants to use in your rate equation and the half-life of the reaction.

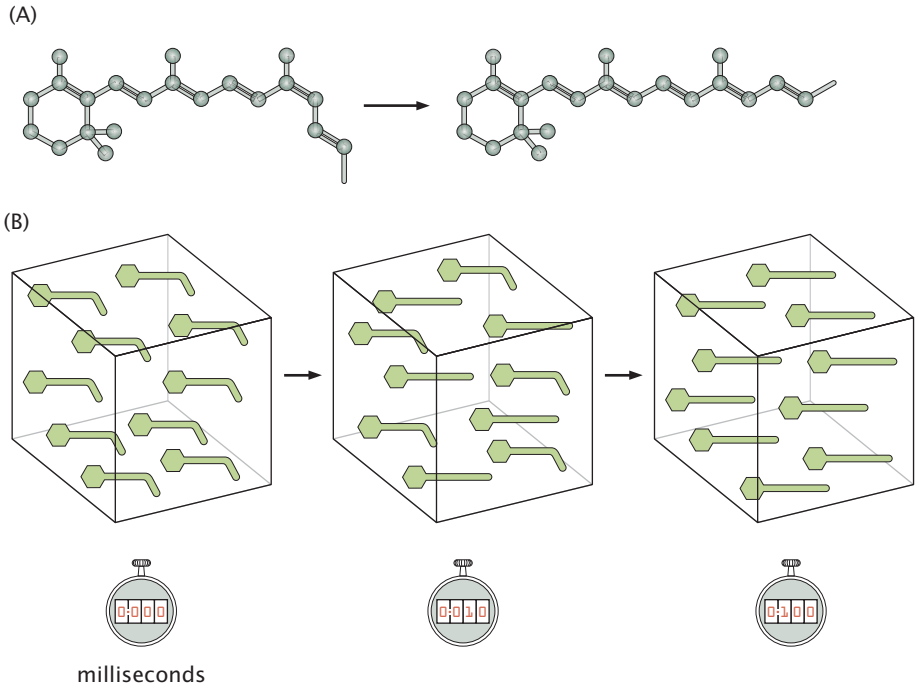

Figure 5: Different views of the isomerization process. (A) Schematic of an isomerization process where species A is decaying into species B. In this case, we use the two forms of retinal to characterize the process. (B) Schematic of the change in the populations of the two species over time.