MCB137L/237L: Physical Biology of the Cell
Spring 2020
Homework 5: Pattern Formation and Biological Dynamics
(Due 2/24/22 at 11:00am)

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1 Analytical solution to the diffusion equation

In class, we derived the diffusion equation in 1D given by

$$\frac{\partial c(x,t)}{\partial t} = D\frac{\partial^2 c(x,t)}{\partial x^2}, \tag{1}$$

where $c(x,t)$ is concentration of molecules, and D is the diffusion constant. Further, during the discussion section, we solved this equation numerically by "spreading the butter" for an initial condition corresponding to having N_0 molecules centered at $x = 0$.

(a) The analytical solution to the diffusion equation under the inital conditions described above is given by

$$c(x,t) = \frac{N_0}{\sqrt{4\pi D t}} e^{-\frac{x^2}{4Dt}}. \tag{2}$$

Show that this is indeed a solution of the diffusion equation. To make this possible, plug in the proposed $c(x,t)$ above into the diffusion equation, do the derivatives on each side and show that, indeed, $\frac{\partial c(x,t)}{\partial t}$ is equal to $D\frac{\partial^2 c(x,t)}{\partial x^2}$.

Remember what you learned in calculus about the product of derivatives and the chain rule! Given a function $f(x,y)$, you can think of the partial derivative $\frac{\partial}{\partial x}$ as a measure of the derivative as we walk along the x-direction as shown in Figure 1. Operationally, taking a partial derivative is like taking a regular derivative: you just treat all other variables as constants. For example, let's define a function of x and y

$$f(x,y) = ax^2y^3. \tag{3}$$

Now, we take the partial derivative with respect to x

$$\frac{\partial f}{\partial x} = ay^3 \frac{\partial}{\partial x}\left(x^2\right). \tag{4}$$

Note that we just thought of ay^3 as constants and took them out of the derivative. As a result, we get

$$\frac{\partial f}{\partial x} = ay^3 2x. \tag{5}$$

Similarly,

$$\frac{\partial f}{\partial y} = ax^2 3y^2. \tag{6}$$

For more information on the partial derivative, please refer to "The Math Behind the Models: the Partial Derivative" on page 212 of PBoC.

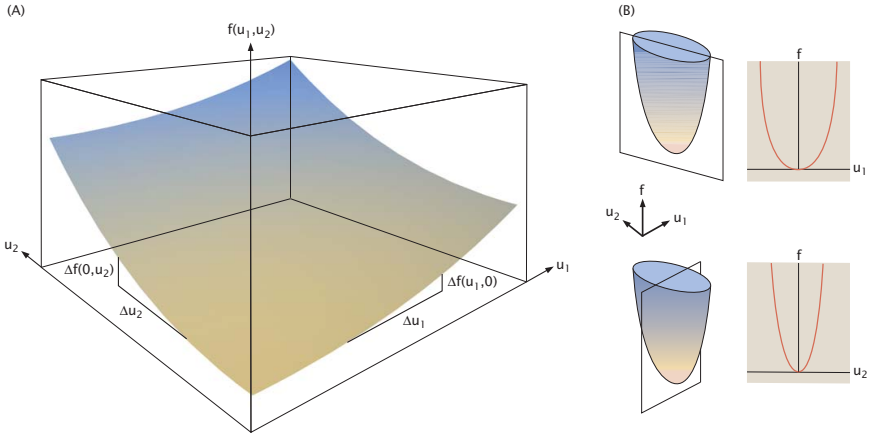

Figure 1: Illustration of the concept of a partial derivative. (A) The plot shows the function $f(u_1, u_2)$ which depends upon the variables u_1 and u_2. If u_2 is held fixed, the surface is reduced to a curve and the partial derivative is nothing more than the ordinary derivative familiar from calculus, but on this particular curve. (B) Planar cuts through the function $f(u_1, u_2)$.

(b) Now, let's plot this analytical solution. Specifically, plot the concentration profile (i.e., concentration vs. position) for 0.01 ms, 0.1 ms, 1 ms, 5 ms and 10 ms in a single figure. Note that we are not asking you to plot the $t = 0$ time point because Python won't necessarily know how to deal with the fact that, while the term $\frac{N_0}{\sqrt{4\pi Dt}}$ approaches infinity as $t \to 0$, the term $e^{-\frac{x^2}{4Dt}}$ approaches 0 for the same limit. Use a typical diffusion constant for a protein in the cell of $D = 10\ \mu\text{m}^2/\text{s}$. You'll have to make reasonable choices for the model parameter N_0. Think hard about the range of x-values over which to plot this distribution. To define this range of x-values to plot, you can use the "$numpy.arange(x_{min}, x_{max}, step)$" operation as we did in class. You can also use the "$numpy.linspace(x_{min}, x_{max}, N_{points})$" command, which you can look up in the Python help. You might note that your concentration peaks beyond N_0! This is because you're plotting $c(x, t)$, the concentration in an infinitesimal box of size dx. This means that the integral $\int_{-\infty}^{+\infty} c(x, t)dx = N_0$, indicating that the total amount of molecules is N_0. We will discuss this subtlety in class.

(c) Finally, we will check that our simulation makes sense by estimating the diffusion constant from the plots you've made. How long does it take for the distribution to spread to about

0.5 μm? Is this consistent with the diffusion constant you used for your simulation? Note that we're not after an exact result for D, but instead are performing a sanity check to see whether our results make sense.

2. Creating morphogen gradients.

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 2 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 3) and the degradation of this protein while it is diffusing around.

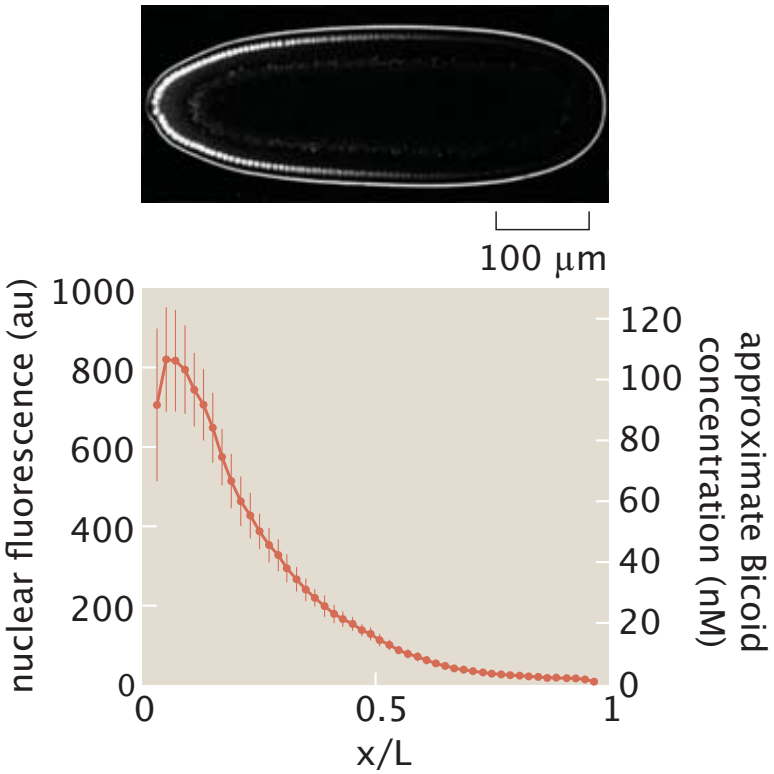

Figure 2: The Bicoid morphogen. The Bicoid activator is distributed in an exponential gradient. (Adapted from F. Liu *et al.*, Proc Natl Acad Sci USA 110:6724 2013.)

(a) Give a brief description (a paragraph or less) of the Bicoid gradient in *Drosophila* and how it is relevant to fly development.

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

$$\frac{\partial Bcd(x,t)}{\partial t} = D\frac{\partial^2 Bcd(x,t)}{\partial x^2} - \frac{Bcd(x,t)}{\tau}. \tag{7}$$

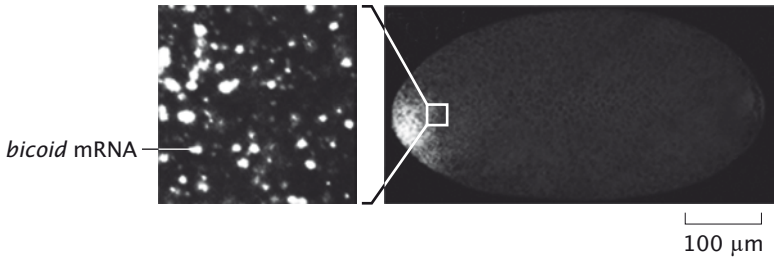

Figure 3: *bicoid* mRNA distribution. Using single molecule mRNA FISH, the localization of individual *bicoid* mRNA molecules at the anterior end of the embryo can be revealed. (Adapted from Petkova et al. (2014), *Current Biology* 24:1283.)

Make sure you explain carefully where all of these terms come from. To do so, you can build on the derivation of the diffusion equation we did in class based on particules jumping between adjacent boxes. Specifically, begin with the usual way by considering a one-dimensional concentration profile and by finding the rate of change of the number of Bicoid molecules in the box at position x by considering the flux into $(kN(x-a))$ and out of $(kN(x+a))$ the box, with a being the size fo the box and k the rate of jumping of a particle, using arguments like those made in class. However, you need to generalize that treatment by accounting for the fact that a Bicoid molecule has the probability $r\Delta t$ of degrading in time interval Δt, where $r \approx 1/\tau$ with τ being the degradation time.

(c) Now, show that $Bcd(x,t) = Bcd_0 e^{-x/\lambda}$, with λ being a decay length and Bcd_0 being the Bicoid concentration at $x = 0$, is a solution of the reaction-diffusion equation 7 in steady-state. How is λ determined by the model parameters D and τ? EXTRA CREDIT: Solve this equation in steady-state by finding the general solution subject to the boundary condition that $J(0,t) = j_0$ and $J(L,t) = 0$. Remember that you can use Fick's law to relate the flux to a change in Bicoid concentration over time. 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) 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.

(e) What is the value of the decay constant λ for the gradient shown in Figure 2? 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.

3. The French flag model

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 4, the idea of the model is that boundaries in the embryo are determined by threshold values of the morphogen. The model predicts that, if the gene dosage of the morphogen 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.

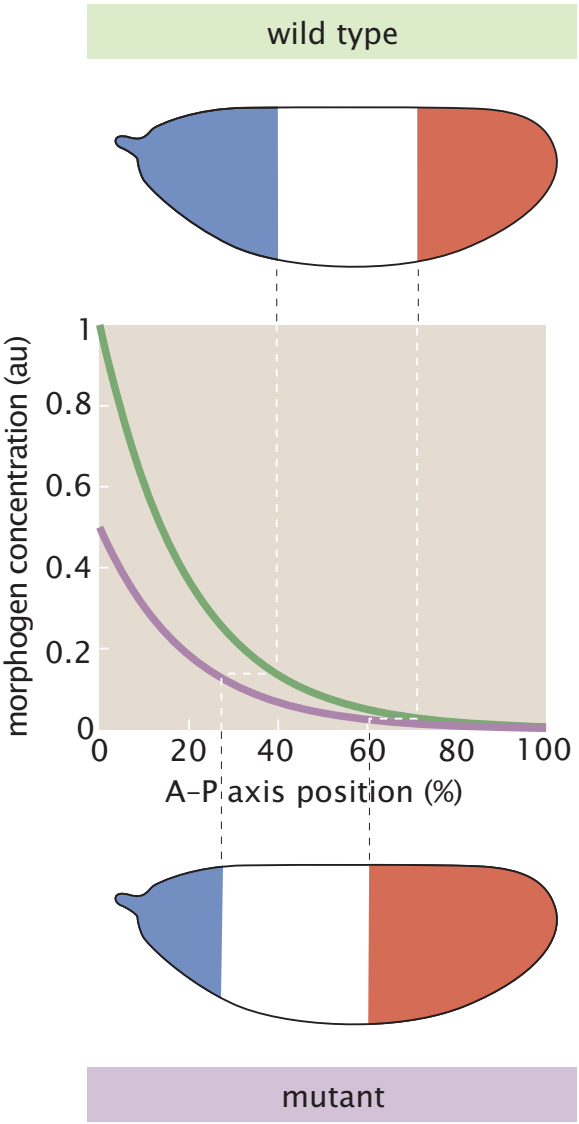

Figure 4: Concept of the French flag model.

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. An exponential gradient of Bicoid is described by

$$Bcd(x, \lambda, \alpha, Bcd_0) = Bcd_0\, \alpha\, e^{-x/\lambda}, \tag{8}$$

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.

(a) Work out a model that predicts 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}.

(b) 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.* (provided on the course website). Comment on how well your prediction matches the data that is provided with the homework. What could be going on?

4. 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

$$N_{\rm K}(t + \Delta t) = N_{\rm K}(t) - (\lambda \Delta t) N_{\rm K}(t). \tag{9}$$

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

$$N_{\rm K}(0) = N_{\rm K}(t) + N_{\rm Ar}(t), \tag{10}$$

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 9 and 10 to construct differential equations to find the relationship between $N_{\mathrm{K}}(t)$, $N_{\mathrm{Ar}}(t)$, and t.

(a) Using equations 9 and 10 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 10 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 from Bailey's paper 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 5) 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 5: 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, $\frac{^{40}\text{K}}{\text{K}_{\text{total}}} \approx 1.2 \times 10^{-4}$. Additionally, use the decay constant $\lambda \approx 5.8 \times 10^{-11}$ yr^{-1}.

Retinal conformations

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

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

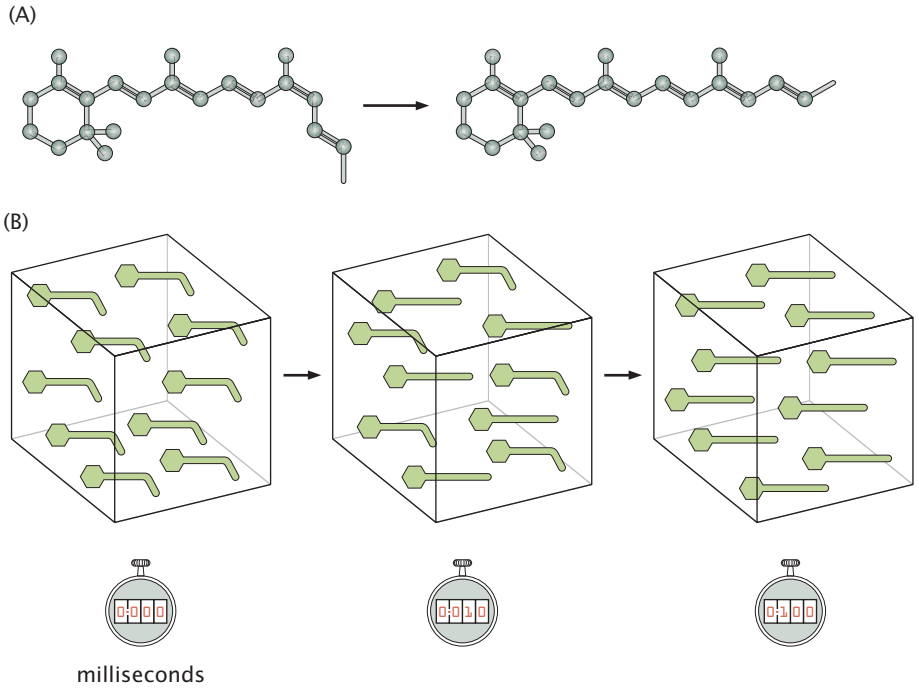

Figure 6: Different views of the isomerization process. (A) Schematic of an isomerization process where species A is decaying into species B. In this case, we use the two forms of retinal to characterize the process. (B) Schematic of the change in the populations of the two species over time.

Reactions of the form

$$\mathrm{A} \rightarrow \mathrm{B}. \tag{11}$$

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 6. 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.