HG to class: This final homework is for extra credit. You don’t have to solve it, and I’ll only take it into account if it improves your grade. This homework will be worth 200% of a regular assignment. As a result, you will note that this homework is longer and harder than usual. Make sure to explain all your reasoning and calculations carefully. You will be evaluated on both having the right results, and on the soundness of your arguments.

1. Open reading frames in random DNA
   Do problem 4.7 of PBoC.

2. The Bleach-Chase Method

   In class we talked about the molecular census. Synthesis of new proteins is not the only kinetic process that governs how many proteins will be found in a given cell. An additional kinetic process of great importance is the decay of the proteins. In a recent paper by Eden et al. (see the course website for this paper), the rate of protein decay was measured using a technique called “bleach-chase”.

   (a) Read the paper and write a one paragraph summary of what the paper is about, the essence of the method and the results. Note also that on all of the derivations that follow in this problem, you will be graded not only on having the right equations but also upon the clarity and logic of your presentation. Your job is to use the mathematics to explain how this method works and to explain precisely what is measured and how it is analyzed. This problem is an example of how we can take something right out of today’s most very recent research results (paper from last year!) and turn it into a little story that you could explain to your scientifically-minded friends. Further, the question of how to actually go about measuring protein degradation rates is very hard and this paper shows us a very interesting answer to that question.

   (b) To make the degradation process accessible, these cells have a fluorescent protein fused to some protein of interest whose degradation rate is the subject of enquiry. (NOTE: one
of the things that they worried about and we should worry about too as readers is that maybe the act of tethering a big fluorescent protein to the protein of interest would alter its degradation rate, the very quantity we are trying to measure.) In the absence of any photobleaching, the evolution of the total number of fluorescent proteins, \( N_f \), follows the simple dynamical equation

\[
\frac{dN_f}{dt} = \beta - \alpha N_f,
\]  

(1)

which acknowledges a rate of protein production \( \beta \) and a degradation rate \( \alpha \). Explain what this equation means and solve it as a function of time assuming that the initial number of proteins is zero. You can solve it analytically or numerically using Matlab. If you solve it numerically, plot it for a reasonable choice of parameters such as the Bicoid production and degradation rate. What is the steady-state value of the number of fluorescent proteins per cell? What is the characteristic time scale to reach this steady state in terms of parameters \( \alpha \) and \( \beta \)?

(c) The problem of trying to infer the degradation rate from just looking at the amount of fluorescent protein is, however, that its dynamics is dictated not only by the degradation rate, but also by the production rate. The question is: can we create an experimental condition where we measure a quantity that is solely determined by the degradation rate? The experimental idea is that we have two populations of identical cells. What this means is that these populations have the same genomes, have been subjected to the same growth conditions and environmental stimuli. In practice, what this really means is that the average fluorescence intensity of our protein of interest in the two populations is the same. At a certain instant in time, we then photobleach one of the two populations so that their fluorescent intensity is now reduced relative to its initial value and relative to the value in the unphotobleached population. We now have two populations. First, we have the number of unbleached molecules, \( N_u \). Second we have the number of bleached molecules, \( N_p \), with a conservation law which is that the total number of proteins of our species of interest is given by \( N_u + N_p \).

A similar equation to Equation 1 describes the dynamics of the unphotobleached molecules in the cells that have been subjected to photobleaching, with the number that are unphotobleached given by \( N_u \) and described by the dynamical equation

\[
\frac{dN_u}{dt} = \beta - \alpha N_u.
\]  

(2)

On the other hand, the number of photobleached proteins are subject to a different dynamical evolution described by the equation

\[
\frac{dN_p}{dt} = -\alpha N_p,
\]  

(3)

since all that happens to them over time is that they degrade. Explain why there are two populations of proteins within the photobleached cells and why these are the right equations.

(d) Notice that while the evolution of the unbleached population still depends on both the production and degradation rates the time evolution of the bleached species is only dependent
on the degradation rate. As a result, if we could track the amount of unbleached molecules as a function of time we would have direct access to \( \alpha \). The trick used by the authors in the paper is to evaluate the difference in the number of fluorescent proteins in the two populations. An absolutely critical assumption then is that

\[
\frac{dN_f}{dt} = \frac{dN_u}{dt} + \frac{dN_p}{dt} \tag{4}
\]

The point is that over time after photobleaching, the photobleached cells will become more fluorescent again as new fluorescent proteins are synthesized.

The key idea here is then to plot the difference between the intensity of the cells that were not disturbed by photobleaching and those that were. In particular, show the simple result that

\[
\frac{d(N_f - N_u)}{dt} = -\alpha(N_f - N_u). \tag{5}
\]

Note that this quantity is directly experimentally accessible since it calls on us to measure the level of fluorescence in the two populations and to examine the difference between them. Further, note that the dynamics depends only upon the one parameter that we are trying to measure. Integrate this equation by solving it analytically or numerically in Matlab, and show how the result can be used to determine the constant \( \alpha \) that characterizes the dynamics of protein decay. Explain what you would actually plot if you were making the measurements and how that would yield the parameter \( \alpha \).

(e) In the paper, they discuss both degradation and dilution. Explain the distinction between these two ideas. Comment on which you think is dominant in bacteria based on what you learned from this paper.

3 A minimal genetic switch

In class, we introduced how genetic switches can be constructed using two repressors that repress each other’s gene expression. Here, we consider a simpler regulatory architecture that can also result in a genetic switch. Specifically, we will model the self-activation of an activator molecule. For this problem, you might find it useful, to review the phase portrait concept we described in class for the case of mRNA production and degradation, as well as the phase diagram of the logistic equation you had to draw in Problem 2 of Homework 4.

Figure 1(A) presents a regulatory architecture, where an activator activates its own production. Figure 1(B) shows the states and weights for our model. In this problem, we will ignore mRNA and associate the rate indicated in the figure with the rate of protein production. Here, a promoter has two activator binding sites in its vicinity. In the absence of activators, or in the presence of only one activator, the rate of protein production is \( r_0 \). When both activators are bound, the rate is \( r \). The activators bind with a dissociation constant \( K_d \) and interact with a cooperativity factor \( \omega = e^{-\beta\varepsilon_{int}} \), where \( \varepsilon_{int} \) is the interaction energy between activators. \( A \) is the concentration of activator.
(a) Write down an equation describing the temporal evolution of the number of activators. Consider the rate of production stemming from the model shown in Figure 1, as well as a rate of protein degradation $\gamma$.

(b) Plot the phase diagram for this equation in order to find how many equilibria the system can support. Namely, plot the rates of production and degradation as a function of the activator concentration. Use $K_d = 5 \text{ nM}$, $\gamma = 0.1/\text{min}$, $r_0 = 0.01 \text{ nM/min}$, and $r = 0.5 \text{ nM/min}$. Make plots for $\omega = 1$ and $\omega = 10$.

(c) Draw vectors indicating the direction of the concentration change under your plots as we did in class for the mRNA production and degradation case, and as you explored in the homework in the context of the logistic equation. How many equilibrium points do you find? Indicate whether these points correspond to stable or unstable equilibria. You can review the concept of phase portraits by reading “Computational Exploration: Growth Curves and the Logistic Equation” on page 103 of PBoC2, paying special attention to vectors drawn on the lower part of Figure 3.10.

(d) Solve the equation you derived in (a) for different initial conditions, and plot all of them on the same graph. Choose initial conditions that help illustrate how the system can converge to different levels of activator in steady state.

Figure 1: A simple autoactivation switch model. (A) Cartoon of the autoactivation switch. (B) States, weights and rates for the autoactivating genetic switch model.
4 Making the fly wing

In this problem, we carry out estimates about the number of cells that make the fly wing. Remember to describe your calculations in detail.

(a) Estimate the number of cells in the adult fly wing by using only a picture of a wing (which you can just get from Google). Read about the measurements of the cells in the fly wing performed by Abouchar et al. (2014) and comment on how accurate your estimate was.

(b) At three hours into development, there are about six cells that will make the future wing. Read Garcia-Bellido et al. (1979), Scientific American 241:102, paying close attention to the figure titles “Clues to Growth” on page 105. Calculate how many division cycles (generations) were necessary to go from the six-cell stage to the cells that make an adult wing and estimate the time between mitosis.