BE/APh 161: Physical Biology of the Cell, Winter 2019
Homework #5
Due at the start of lecture, 2:30 PM, February 13.

Problem 5.1 (Hill functions and the word cooperativity (25 pts))**.**
An activating **Hill function** is of the form

$$f(x) = \frac{(x/k)^n}{1 + (x/k)^n}, \tag{5.1}$$

where the parameter n is called the **Hill coefficient**. A repressive Hill function is

$$f(x) = \frac{1}{1 + (x/k)^n}. \tag{5.2}$$

Hill functions are often used to phenomenologically model systems we have been treating by using the machinery of statistical mechanics to mathematize cartoons of molecular interactions.

The word "cooperativity" is typically used in two different senses in molecular biology. In one meaning, "cooperativity" is the value of a phenomenological Hill coefficient. Another use is related to the added energy of binding a second ligand after a first is bound.

Consider cooperative binding of two repressors. Specifically, let J be the extra energy beyond the energy of the repressor-DNA interaction that is involved in the binding of the second repressor. Using the statistical thermodynamical approach as we have in class, write down an expression for fold change under the weak promoter approximation. In what limit is this expression equivalent to the phenomenological Hill function, (5.2) with $n = 2$? What is the value of k in equation (5.2) in terms of the values used in the expression derived from states and weights using statistical thermodynamics? Based on this analysis, how are the two different definitions of cooperativity related, if at all?

Problem 5.2 (Allosteric induction, 35 pts)**.**
In our discussion of genetic switches, we described how we could "flip the switch" by introducing an inducer. For the Gardner, et al. switch, one of these inducers was IPTG. IPTG works by binding a repressor and thereby weakening its affinity for its operator. We describe a repressor bound to inducer IPTG as inactive. As a result of its repressor being incapacitated, the gene gets expressed.

In this problem, inspired by this paper, we will investigate allosteric induction of LacI by IPTG. IPTG binds the LacI in *E. coli* to induce production of β-galactosidase. LacI is present as a dimer, and we define by R to be the number of LacI dimers in a cell. LacI can exist in an active state and an inactive state. We define $\Delta E^{\text{A}}_{\text{rd}}$ to be

the energy of binding of active repressor to the operator minus that of the active repressor binding nonspecifically. We define $\Delta E_{\text{rd}}^{\text{I}}$ similarly for the inactive repressor. Each LacI dimer can bind zero, one, or two IPTG molecules. There is no change in binding energy between the first and second IPTG binding events. Let K_{A} and K_{I} respectively be the dissociation constant for an active and inactive LacI dimer binding IPTG.

a) Recall how we defined fold change as a function of the number of repressors, R, in a cell. If P_{b} is the probability that the polymerase is bound to its promoter, then

$$\text{fold change}(R) = \frac{P_{\text{b}}(R)}{P_{\text{b}}(R=0)}. \tag{5.3}$$

Let c be the concentration of IPTG. Show that we can write the fold change as a logistic function,

$$\text{fold change} = \frac{1}{1+\mathrm{e}^{-\beta F}}, \tag{5.4}$$

with Bohr parameter

$$F = \Delta E_{\text{rd}}^{\text{A}} - k_B T \ln \frac{R}{N_{\text{NS}}} + k_B T \ln\left(1+\mathrm{e}^{-\beta F_{\text{MWC}}}\right), \tag{5.5}$$

where

$$F_{\text{MWC}} = \Delta E_{\text{AI}} + 2 k_B T \ln \frac{1+c/K_{\text{A}}}{1+c/K_{\text{I}}}. \tag{5.6}$$

Here we have introduced one new parameter, ΔE_{AI}, which is the energy difference between the inactive and active states of the LacI dimer; $\Delta E_{\text{AI}} = E_{\text{I}} - E_{\text{A}}$. We have also made a weak promoter appoximation and have assumed that active repressor binds to the operator much more strongly than inactive repressor. *Hint*: Look at the image on the paper website. In the equation on that page, $P_{\text{A}}(c)$ is the probability that a given LacI dimer is active as a function of the IPTG concentration, c. It might be useful to derive $P_{\text{A}}(c)$ separately, and then use that in an expression for the fold change that you derive as if you know $P_{\text{A}}(c)$.

b) Comment on the physical meaning of equations (5.5) and (5.6). In other words, how does this equation tell us how the respective molecules contribute to regulation?

c) In Fig. 1, you can see some examples of properties of the induction curves. Importantly, we will focus on saturation, dynamic range, and leakiness (we will not work with the effective Hill coefficient or $[\text{EC}]_{50}$ in this exercise). They

describe how responsive a cell is to induction. Describe in words what these terms mean. Then, make plots of each of these properties as a function of repressor copy number. When you make this plot, use the parameters the authors measured for one of their operators of interest.

$\beta\Delta E_{\mathrm{AI}}$	4.5
$\beta\Delta E_{\mathrm{rd}}^{\mathrm{A}}$	-13.9
K_{A}	139 µM
K_{I}	0.53 µM

You can approximate N_{NS} as the total number of base pairs in the *E. coli* genome, 4.6 million. Note that you should vary R on a logarithmic scale.

Comment on the curves you plot.

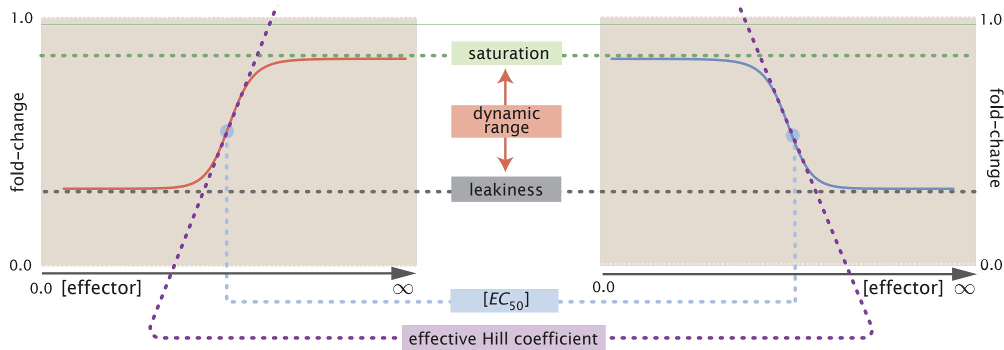

Figure 1: Characterization of effector curves. The left curve is for an inducer, and the right for corepression. We consider the former in this problem. This figure is from Razo, et al., 2017, http://dx.doi.org/10.1101/111013, and available under a CC-BY 4.0 license.

Problem 5.3 (A simplified repressilator, 40 pts)**.**
In this problem, we study a synthetic genetic circuit developed by Michael Elowitz and Stan Leibler called the repressilator. It is described in Elowitz and Leibler, *Nature*, **403**, 335–338, 2000. The circuit consists of three genes, lacI, tetR, and cI, that repress each other in a cyclic fashion. Another gene with a tet-repressible promoter was fused to green fluorescent protein (GFP) for a readout. So, if tetR has low copy numbers, we will see a large GFP signal and vice versa. A diagram of the repressive interactions of the genes is shown in Figure 2. So notation does not get cumbersome, we will refer to lacI as “1”, tetR as “2”, and cI as “3”. The copy number of protein i per cell is p_i.

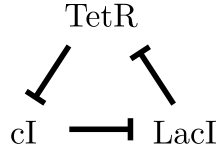

Figure 2: Schematic of the repressilator described in Elowitz and Leibler, *Nature*, **403**, 335–338, 2000.

a) Give an intuitive explanation as to why the repressilator system can give rise to oscillatory gene expression.

b) Write down a system of ODEs describing the time evolution of the p_i's. Ignore mRNA dynamics. That is, write down ODEs similar to those we wrote in class for the synthetic genetic switch. For the purposes of this problem, assume that the fold change in expression of a repressed gene is given by a Hill function with Hill coefficient n.

c) Nondimensionalize these equations. As a simplifying assumption, take all phenomenological coefficients of each protein to be the same. I.e., they all have the same degradation rate, they all have the same basal production rate, etc. Your result should be of the form

$$\frac{\mathrm{d}p_1}{\mathrm{d}t} = -p_1 + \frac{\alpha}{1+p_3^n} \tag{5.7}$$

$$\frac{\mathrm{d}p_2}{\mathrm{d}t} = -p_2 + \frac{\alpha}{1+p_1^n} \tag{5.8}$$

$$\frac{\mathrm{d}p_3}{\mathrm{d}t} = -p_3 + \frac{\alpha}{1+p_2^n}, \tag{5.9}$$

all variables and parameters are dimensionless.

d) Show that this system has a unique fixed point.

e) Use linear stability analysis to show derive the stability properties of the fixed point. Specifically, show that

$$\text{the fixed point is} \begin{cases} \text{stable} & \text{for all } \alpha \text{ if } n \leq 2 \\ \text{stable} & \text{if } n > 2 \text{ and } \alpha < \frac{n}{2}\left(\frac{n}{2}-1\right)^{-\frac{n+1}{n}} \\ \text{unstable} & \text{if } n > 2 \text{ and } \alpha > \frac{n}{2}\left(\frac{n}{2}-1\right)^{-\frac{n+1}{n}}. \end{cases} \tag{5.10}$$

Is the instability oscillatory? (Remember that a nonzero imaginary part of an eigenvalue gives oscillations in the dynamics.) What can you say about the role

of cooperativity in the repressilator system? *Hint*: In doing the linear stability analysis, it will help you to recall that there are three cube roots of unity.

$$\sqrt[3]{1} = \left\{1, -\frac{1}{2}\left(1 + i\sqrt{3}\right), -\frac{1}{2}\left(1 - i\sqrt{3}\right)\right\}. \tag{5.11}$$

f) Solve the repressilator system numerically for $n = 3$ and $\alpha = 3$, $\alpha = 10$, and $\alpha = 100$. Plot and comment on your results.

g) (*5 pts extra credit*) If you are feeling ambitious, build an interactive plot with sliders where you can adjust n and α and look at the response of the repressilator.