BE/APh 161: Physical Biology of the Cell, Winter 2016
Homework #5
Due at the start of lecture, 1PM, February 17, 2016.

Problem 5.1 (The different meanings of the word cooperativity (10 pts))**.**
In class we talked about the fact that the word "cooperativity" is typically used in two different senses in molecular biology. In one meaning, "cooperativity" is the value of a Hill coefficient. Another use is related to the added energy of binding a second ligand after a first is bound. We have been calling this energy J.

Consider cooperative binding of two repressors. A phenomenological Hill function for the fold change in gene expression as a function of the total repressor concentration r is

$$\text{fold change} = \frac{k^2}{k^2 + r^2}. \tag{5.1}$$

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, (5.1)? What is the value of k in equation (5.1) 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 (Transcriptional machinery in eukaryotes (40 pts))**.**
This is essentially problem 19.6 of PBoC2.
In the thermodynamic models of gene regulation we have discussed, the RNA polymerase is treated as a single molecular species. While this might be a reasonable assumption for transcription in prokaryotes, in eukaryotes tens of different molecules need to come together in order to form the transcriptional machinery. In this problem we will consider a simplified "eukaryotic" model for transcription where a functioning polymerase is made out of two different subunits, X and Y, that come together at the promoter. This is in a similar spirit as our analysis of "dimoglobin."

a) Calculate the probability of finding the complex XY bound to the promoter in the case where unit X binds to DNA and unit Y binds to X. In what limit can this be reduced to an effective one-molecule problem such as in the bacterial case?

b) Calculate the fold-change in gene expression for simple repression using transcriptional machinery such as that proposed in (a). Remember, by "simple repression" we mean that a single repressor binds to the promotor region thereby precluding binding of any of the polymerase components. Explore the weak promoter assumption in order to reduce the expression to that corresponding to the bacterial case.

c) Repeat part (b) for the case where an activator can contact Y.

d) Repeat (a), (b), and (c) for a case where Y binds to a site on the DNA that is near the X-binding site, and there is an interaction energy between X and Y.

Problem 5.3 (A simplified repressilator, 50 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 1. 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.

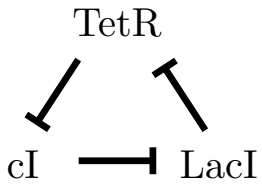

Figure 1: Schematic of the repressilator described in Elowitz and Leibler, *Nature*, **403**, 335–338, 2000.

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

$$\frac{\mathrm{d}p_1}{\mathrm{d}t} = -p_1 + \frac{\beta}{1+p_3^n} \tag{5.2}$$

$$\frac{\mathrm{d}p_2}{\mathrm{d}t} = -p_2 + \frac{\beta}{1+p_1^n} \tag{5.3}$$

$$\frac{\mathrm{d}p_3}{\mathrm{d}t} = -p_3 + \frac{\beta}{1+p_2^n}, \tag{5.4}$$

where p_i now has a constant absorbed into it.

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

$$\text{the fixed point is } \begin{cases} \text{stable} & \text{for all } \beta \text{ if } n \leq 2 \\ \text{stable} & \text{if } n > 2 \text{ and } \beta < \dfrac{n}{2}\left(\dfrac{n}{2}-1\right)^{-\frac{n+1}{n}} \\ \text{unstable} & \text{if } n > 2 \text{ and } \beta > \dfrac{n}{2}\left(\dfrac{n}{2}-1\right)^{-\frac{n+1}{n}}. \end{cases} \tag{5.5}$$

From this result, 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.

$$\sqrt[3]{1} = \left\{1, -\frac{1}{2}\left(1 + i\sqrt{3}\right), -\frac{1}{2}\left(1 - i\sqrt{3}\right)\right\}. \tag{5.6}$$

f) Solve the repressilator system numerically for $n = 3$ and $\beta = 3$, $\beta = 10$, and $\beta = 100$. 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 β and look at the response of the repressilator. You can send the code for the interactive plot to the course instructors via email.