BE/APh 161: Physical Biology of the Cell, Winter 2018
Homework #5
Due at the start of lecture, 2:30 PM, February 7.

Problem 5.1 (The different meanings of the word cooperativity (25 pts))**.**
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. 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 (States and weights for a repressor in a genetic switch, 25 pts)**.**
In lecture, we considered a genetic switch where repressor 1 represses expression of repressor 2 and repressor 2 represses expression of repressor 1. We assumed each repressor had the same physical constants with respect to binding to operators. Considering one of the repressors binding to its operon, we treated the case where either zero, one, or two repressors could be bound to an operator. There was no cooperativity; each binding event to an operator has the same ΔE_{rd}. We wrote that the fold change in gene expression due to this repression is

$$f_2(R) = \frac{1}{(1 + KR_1)^2}, \tag{5.2}$$

where R_1 is the copy number of repressor 1. Derive this result. In particular, write down an expression for K.

Problem 5.3 (How to make a genetic switch, 25 pts)**.**
Consider a genetic circuit as in problem 5.2 in which protein 1 represses expression of protein 2 and protein 2 represses expression of protein 1. The repression mechanism for each is "simple repression," as we defined in lecture to be the case where a single repressor binds to an operator. Show that this system cannot function as a genetic switch.

Problem 5.4 (Allosteric induction, 25 pts)**.**
In our discussion of genetic switches, we described how we could "flip the switch" by introducing an inducer. For the Gernder, et al. switch, one of these inducers was IPTG. IPTG works by binding a repressor and thereby weakening its affinity for its operator. As a result of its repressor being incapacitated, the gene gets expressed.

IPTG binds the Lac repressor (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 ΔE_{RA} to be the energy of binding of active repressor to the operator minus that of binding non-specifically. In the notation we have been using in class,

$$\Delta E_{\text{RA}} = E^{\text{S}}_{\text{RA}} - E^{\text{NS}}_{\text{RA}}. \tag{5.3}$$

We define ΔE_{RI} 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.

In this problem, inspired by this paper, we will investigate allosteric induction.

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

$$\text{fold change}(R) = \frac{p_{\text{bound}}(R)}{p_{\text{bound}}(R=0)}. \tag{5.4}$$

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

$$\text{fold change} = \frac{1}{1+\text{e}^{-\beta F}}, \tag{5.5}$$

with Bohr parameter

$$F = \Delta E_{RA} + k_B T \ln\left(1+\text{e}^{-\beta F_{\text{MWC}}}\right) - k_B T \ln \frac{R}{N_{\text{NS}}}, \tag{5.6}$$

where

$$F_{\text{MWC}} = \Delta E_{\text{AI}} + 2k_B T \ln \frac{1+c/K_A}{1+c/K_I}. \tag{5.7}$$

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, $p_A(c)$ is the probability that a given LacI dimer is active as a function of the IPTG concentration, c. It might be useful to derive $p_A(c)$ separately, and then use that in an expression for the fold change that you derive as if you know $p_A(c)$.

b) Comment on the physical meaning of equations (5.6) and (5.7). 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 $[EC]_{50}$ 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.

$\beta\,\Delta E_{RA}$	-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.

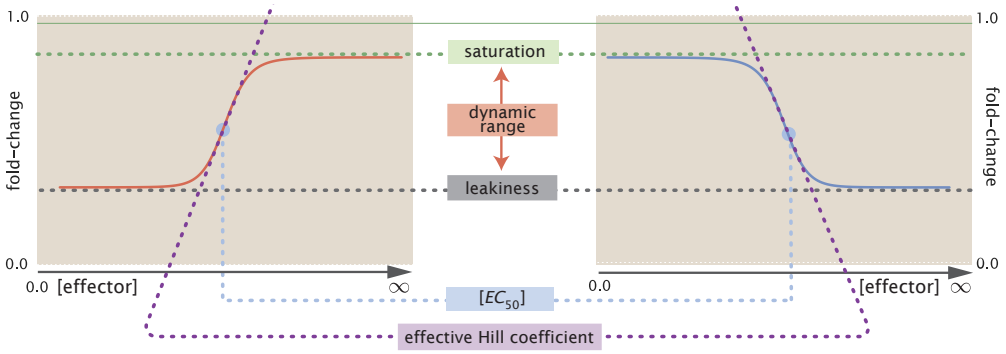

Figure 1: Characterization of effector curves. The left curve is for an inducer, and the right for corepression. We consider the former in this problem. This figure is from Razo, et al., 2017, http://dx.doi.org/10.1101/111013, and available under a CC-BY 4.0 license. It was created by our very own Griffin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