BE/APh 161: Physical Biology of the Cell, Winter 2016
Homework #4
Due at the start of lecture, 1PM, February 1, 2014.

Problem 4.1 (Calibrating an optical trap (based on problem 6.2 of *PBoC2*, 20 pts)**.**
In lecture, we talked about using an optical trap to hold a bead in place and exert a force to unfold an RNA hairpin. I mentioned in that lecture that we can know how much force the trap is exerting on the bead. In this problem, we will explore how we might calibrate the trap to enable determination of force. Specifically, we know the center of the trap, since we can aim the optics, and we know the center of the bead, since we can measure it through imaging. Knowing these two things, we would like to compute the force.

We define the energy of the bead in the trap as $E(x)$, where x is the displacement of the bead from the center of the trap. The force exerted by the trap on the bead, is

$$f = -\frac{\partial E}{\partial x}. \tag{4.1}$$

In general, $E(x)$ can be a complicated function of x, but if x is small, we might appoximate $E(x)$ as its truncated Taylor series expansion about $x = 0$, where the $E(x)$ is minimal.

$$E(x) = E_0 + \frac{kx^2}{2} + \mathcal{O}(x^3), \tag{4.2}$$

where k is called the trap stiffness and is formally given by

$$k = \left.\frac{\mathrm{d}^2 E}{\mathrm{d}x^2}\right|_{x=0}. \tag{4.3}$$

So, the force is $f = -kx$ for small displacements. Thus, if we know k, we can determine the force from the center of the trap and the position of the bead, the difference of which gives x.

To calibrate the trap, we will let a bead sit in the trap and rattle around due to thermal motion. We will take many many images to get a set of values for x. Show that if we average the square of these displacements, we can get an estimate for the trap stiffness using

$$k = \frac{k_B T}{\langle x^2 \rangle}. \tag{4.4}$$

Hint: Define $p(x)$ as the probability that the bead shows displacement x at equilibrium. Then,

$$\langle x^2 \rangle = \sum_x x^2 p(x) = \int \frac{\mathrm{d}x}{m(x)}\, x^2 p(x), \tag{4.5}$$

where $m(x)$ is the density of states. This is necessary to convert the sum into an integral. We will assume that $m(x) = m_0$, a constant, meaning that the density of states is uniform in x. You might also want to look up Gaussian integrals to help you evaluate the definite integral you will encounter.

Problem 4.2 (Pulling DNA using a two-state model, 35 pts)**.**
Later in the class, we will delve into polymer physics. In this problem, we will dip our toe in those waters using a two-state model.

Recall from our discussion in lecture on January 20 about the Liphardt, et al. paper that the early portion of the force extension curve involved pulling the DNA tethers until they were taught. We will develop a model to describe pulling a single segment of double-stranded DNA. We model the DNA polymer as a 1-D random walk, as described on page 341 of *PBoC2*. It consists of N segments, each of length a. Each segment can either point right or left. We stretch the DNA segment by holding the ends and pulling with a total force f. We take each segment to be independent of the others. We will ignore end effects and consider the end segments to be the same as all others.

a) Write a states and weights diagram for a single segment of the polymer. *Hint*: Remember our discussion in lecture about deriving the statistical weights for generic thermodynamic potentials. The force should enter into your weights.

b) From your states and weights diagram, derive the probability that a given segment points to the right.

c) We want to find the probability that the end-to-end distance of a DNA segment under a force f is L. It is easier to note that $L = (2N_r - N)a$, where N_r is the number of segments that point to the right, and then find the probability of observing N_r. Write an expression for $P(N_r)$. Your expression from part (b) will be useful, and the binomial theorem may be useful as well.

d) Show that

$$\langle L \rangle = \frac{1}{Z} \frac{\partial Z}{\partial(\beta f)}, \tag{4.6}$$

where Z is the partition function that appears in the denominator of $P(N_r)$ that you derived in part (c). Compute $\langle L \rangle$.

e) Compute the magnitude of the fluctuations in L. I.e., compute the variance $\sigma_L^2 = \langle L^2 \rangle - \langle L \rangle^2$. How does the ratio $\sigma_L / \langle L \rangle$ depend on N?

Problem 4.3 (Dimoglobin and carbon monoxide, inspired by problem 7.5 of *PBoC2*, 25 pts)**.**
In chapter 7 of *PBoC2*, we read about dimoglobin, a toy model for hemoglobin in which there are two binding sites for oxygen. In this problem we will use this toy model to look at the competition for binding sites between oxygen and the toxin carbon monoxide (CO).

a) Derive an expression for the probability that dimoglobin is saturated with oxygen. You should assume that binding a second oxygen molecule is cooperative with extra binding energy J_{O} and that the binding of a second CO molecule is cooperative with extra binding energy J_{C}. You can also assume that binding a CO molecule when an O_2 is already bound (and vice versa) is not cooperative. Your answer should be in terms of the partial pressures of oxygen and carbon monoxide, their respective dissociation constants, and J_{O} and J_{C}.

b) The dissociation constant for CO for hemoglobin is 240 times that of O_2. Assume this is the case for dimoglobin as well. The dissociation constant for oxygen is $K_{\mathrm{d,O_2}} = 26$ mmHg. Plot the probability that dimoglobin is saturated with oxygen as a function of the partial pressure of CO for various values of J_{O} and J_{C}. Assume that this experiment is done in air contaminated with carbon monoxide. Comment on what you see in the plots.

Problem 4.4 (Measuring copy numbers, based on problem 2.7 of *PBoC2*, 20 pts + 10 extra credit)**.** We will soon be studying gene expression in lecture, in the readings, and also in this homework. Experimentally, measurement of gene expression can be a tricky business. Expression is often read out by fluorescent intensity of a fluorescently tagged gene of interest. This is useful for computing fold change of expression, but it is not immediately obvious how to compute total numbers of expressed proteins.

A clever method to compute absolute numbers of fluorescent proteins was worked out in Rosenfeld, et al., *Science*, **307**, 1962–1965, 2005. They noted than in a colony of bacterial cells, the average square fluorescent intensity difference between two daughter cells is related to the total fluorescent intensity of the mother cell by

$$\langle (I_1 - I_2)^2 \rangle = \alpha I_{\text{tot}}. \tag{4.7}$$

Here, α is the fluorescent intensity of a single protein such that $I_1 = \alpha N_1$, where N_1 is the number of fluorescent proteins in daughter cell 1. The total fluorescent intensity of the mother cell immediately before division is $I_{\text{tot}} = I_1 + I_2$.

a) Derive equation (4.7). *Hint*: Think about the probability distribution that describes the inheritance of fluorescent proteins for each daughter cell.

b) Comment on what Figure 1 says about the validity and applicability of equation (4.7).

c) (*10 points extra credit*) Download the data set here and compute an estimate for α. (This is fabricated and is not the data set in Fig. 1.)

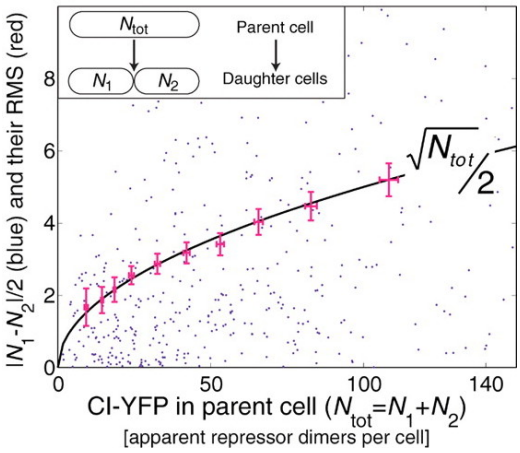

Figure 1: Figure modified from Rosenfeld, et al., *Science*, **307**, 1962–1965, 2005. The authors inferred values of N_1 and N_2 using a value of α obtained from performing a curve fit using equation (4.7). "RMS" in the y-axis label refers to "root mean square." In other words, the y-axis is $\sqrt{\langle (N_1 - N_2)^2 \rangle}/2$.