**BE/APh 161: Physical Biology of the Cell, Winter 2014**
**Homework #3**
Due at the start of lecture, 1PM, January 29, 2014.

**Problem 3.1** (Comments on *Cell Biology by the Numbers* part 3, 10 pts)**.**
We continue in our reading in *CBBTN*. This time, please read chapter 2, pages 74–150, and send comments about two vignettes. Remember to email your answers to me and the TAs and indicate whether you would like to be anonymous when I send the comments to the book's authors. Also, please either send your responses as text in an email or as a PDF. Do not send MS Word documents.

**Problem 3.2** (Ligand-receptor binding: practical calculations, loosely based on problem 6.4 of *PBoC2*, 12 pts)**.**
In lecture, we considered simple ligand-receptor binding. We considered a single receptor with many ligands and found that the equilibrium probability of a receptor having a ligand bound to it is

$$p_{\text{bound}} = \frac{1}{1 + K_{\text{d}}/c_{\text{L}}}, \tag{3.1}$$

where $c_{\text{L}}$ is the concentration of free ligand and $K_{\text{d}}$ is the dissociation constant, expressed in the same units as $c_{\text{L}}$.

a) Show that the expression given in equation (3.1) holds even if we have many receptors. For concreteness of notation, assume that the the *total* receptor concentration (including both bound and unbound) is $c^0_{\text{R}}$, with $c^0_{\text{L}}$ being similarly defined for ligands. You may take the law of mass action,

$$K_{\text{d}} = \frac{c_{\text{L}} c_{\text{R}}}{c_{\text{LR}}}, \tag{3.2}$$

as given, though it may be derived from statistical mechanics.

b) As I mentioned in lecture, it is not always easy to measure $c_{\text{L}}$. This is especially true when we do binding experiments with purified proteins in a test tube, where we know $c^0_{\text{L}}$ and $c^0_{\text{R}}$. Derive an expression for $p_{\text{bound}}$ as a function of the total ligand concentration $c^0_{\text{L}}$, the total receptor concentration, $c^0_{\text{R}}$, and the dissociation constant, $K_{\text{d}}$.

c) Show that in the limit of $c^0_{\text{L}} \gg c^0_{\text{R}}$,

$$p_{\text{bound}} \approx \frac{1}{1 + K_d/c^0_{\text{L}}}. \tag{3.3}$$

**Problem 3.3** (Cooperative ligand-receptor binding, 25 pts)**.**
We continue to explore ligand-receptor binding in this problem. We consider the case where we have a receptor that has two distinct binding pockets for ligands. We will refer to the binding pockets as the left and right binding pockets. Each binding pocket can bind a single ligand, and the receptor may have either zero, one, or two ligands bound at each time. We call the compound where the left binding pocket is bound LR, the compound where the right is bound RL, and the compound where both are bound LRL. So, written as chemical reactions with dissociation constants, we have

$$\text{LR} \rightleftharpoons \text{L} + \text{R}, \quad K_{\text{d},1} \tag{3.4}$$

$$\mathrm{RL} \rightleftharpoons \mathrm{R} + \mathrm{L}, \quad K_{\mathrm{d},2} \tag{3.5}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{L} + \mathrm{RL}, \quad K_{\mathrm{d},3} \tag{3.6}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{LR} + \mathrm{L}, \quad K_{\mathrm{d},4}. \tag{3.7}$$

a) Show that by the law of mass action, $K_{\mathrm{d},4} = K_{\mathrm{d},2}K_{\mathrm{d},3}/K_{\mathrm{d},1}$.

b) Consider a single receptor in a solution of ligands with concentration $c_{\mathrm{L}}$. Write down a states and weights table.

c) Use your states and weights table to derive an expression for the probability that both binding pockets are occupied by ligands ($p_{\mathrm{LRL}}$) in terms of the ligand concentration $c_{\mathrm{L}}$ and $K_{\mathrm{d},1}$, $K_{\mathrm{d},2}$, and $K_{\mathrm{d},3}$. Be sure to explicitly write how the dissociation constants depend on the energies of the respective states.

d) Assume $K_{\mathrm{d},1} = K_{\mathrm{d},2} \equiv K_{\mathrm{d}}$. Plot $p_{\mathrm{LRL}}$ vs. $c_{\mathrm{L}}/K_{\mathrm{d}}$ for various values of $K_{\mathrm{d},3}/K_{\mathrm{d}}$. If $K_{\mathrm{d},3} < K_{\mathrm{d}}$, the binding is said to be cooperative, meaning that binding a second ligand is stronger once the first ligand is bound. Use your plot to comment on the effect of cooperativity in this example.

e) Assume now that only a single chemical reaction is allowed.

$$\mathrm{LRL} \rightleftharpoons \mathrm{R} + \mathrm{L} + \mathrm{L}, \tag{3.8}$$

with an equilibrium constant we will call $K$. This means that the receptor may have only zero or two ligands bound to it. Write the states and weights diagram and derive an expression for $p_{\mathrm{LRL}}$. Compare this result to your results in parts (c) and (d). What does this say about using Hill functions to describe cooperative binding?

**Problem 3.4** (Pulling DNA using a two-state model, 20 pts)**.**
Later in the class, we will delve into polymer physics. In this problem, we will tap our toe in those waters using a two-state model.

Recall from our discussion in lecture 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 left end and pulling on the right end with 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.

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

$$\langle L \rangle = \frac{1}{Z} \frac{\partial Z}{\partial \beta f}, \tag{3.9}$$

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 $N$?