BE/APh 161: Physical Biology of the Cell, Winter 2025
Homework #3
Due at the start of lecture, 2:30 PM, January 30, 2025.

In this homework, we will explore ligand-receptor binding in depth, using many of the skills from statistical mechanics we learned last week. It may seem a bit redundant, but this is a great model system to hone your statistical mechanical skills. You will also gain a much deeper insight into the deceptively simple, but ubiquitous, ligand-receptor binding.

Before we get into that, though, I wanted to give another problem practicing mathematizing cartoons and drawing insights from the results. We will play with the Polach-Widom experiment we mentioned in the first lecture.

Problem 3.1 (The Polach-Widom experiment and excess enzyme, 30 pts)**.**
This problem is inspired by Chapter 8 of Helmut Schiessel's book Biophysics for Beginners, 2nd Ed.

In eukaryotic cells, DNA is wrapped around histones and packaged into chromosomes. The transcription machinery is sterically occluded from accessing the DNA when it is wrapped around the histone. The DNA "breathes" on the histone, becoming unwound on occasion. The more time the DNA is unwrapped, the easier it is for the transcription machinery to engage and for the gene associated with the segment of DNA to be expressed. So, a quantitative understanding of the dynamics of DNA-histone interactions is valuable to learn about regulation of gene expression.

To address this question, Polach and Widom (*J. Mol. Biol.*, **254**, 130, 1995) devised a now-classic experiment, depicted in Fig. 1. They purified histone-DNA complexes where the DNA sequence contains a recognition sequence for a restriction enzyme. The restriction enzyme cuts the DNA at the restriction site. They can then measure the number of cut fragments over time to learn about the dynamics of unwinding. In this problem, we will work out the chemical kinetics to see how we can interpret the experiment. The ultimate goal is to figure out the probability that the DNA is unwound from the histone.

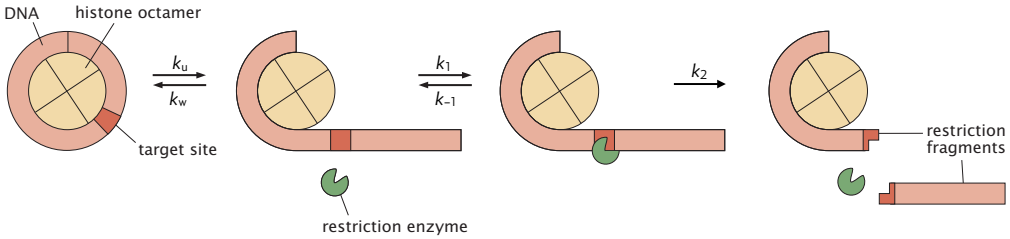

Figure 1: Schematic of the chemical reactions of the Polach-Widom experiment. DNA wrapped around a nucleosome reversibly unwinds exposing a target site whose sequence is the recognition sequence for a restriction enzyme. The restriction enzyme then binds reversibly to the target site. Bound restriction enzyme can then irreversibly cleave the DNA. Figure adapted from Fig. 10.22 of Phillips, Kondev, Theriot, and Garcia, *Physical Biology of the Cell, 2nd Ed.*, 2012, which was itself adapted from Polach and Widom, *J. Mol. Biol.*, **254**, 130, 1995.

a) As will become clear as we work out this problem, Polach and Widom needed a measurement of the rate of cleavage for bare DNA in the absence of a histone. The reaction scheme for this scenario is the same as in Fig. 1, except without the first step. We can write it in text as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}. \tag{3.1}$$

Here, E denotes the restriction enzyme, S the restriction site, and P is the cut fragment. Not surprisingly, this is the reaction scheme for Michaelis-Menten kinetics. However, the typical approximation (the quasi-steady state approximation) used to derive the familiar Michaelis-Menten expressions for the rate of an enzyme-catalyzed reaction does *not* hold. But not to worry! Polach and Widom set up their experiment such that the total concentration of restriction enzyme was much greater than the total concentration of cleavage sites on the DNA, $c_\mathrm{E}^0 \gg c_\mathrm{S}^0$. As a result, we can make a different simplification, which is that $c_\mathrm{E} \approx c_\mathrm{E}^0$, a constant. So that with this approximation, the dynamics may be written as a linear system of equations, which can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} c_\mathrm{S} \\ c_\mathrm{ES} \end{pmatrix} = \mathsf{A} \cdot \begin{pmatrix} c_\mathrm{S} \\ c_\mathrm{ES} \end{pmatrix}. \tag{3.2}$$

Write down the matrix A.

b) Show that if

$$r_\mathrm{bare} \equiv \frac{k_1 k_2 c_\mathrm{E}^0}{(k_1 c_\mathrm{E}^0 + k_{-1} + k_2)^2} \ll 1, \tag{3.3}$$

the slowest time scale of the dynamics is $1/k_{\text{bare}}$, such that $c_{\text{P}}(t) \approx c_{\text{S}}^0(1 - \text{e}^{-k_{\text{bare}}t})$, assuming we start with no cleaved product. Be sure to write an expression for k_{bare}.

c) Now consider the full reaction scheme in Fig. 1. Denote by c_{W} the concentration of wound cut sites, represented by the leftmost image in the figure. We will make a rapid steady state approximation for the winding/unwinding reaction such that the dynamics of that reaction are much faster that those of the others. Let f be the fraction of sites that are available for cleavage. Note that f is the key quantity of interest. We want to know how much of the time a segment of DNA is free of the histone. Show that $f = k_u/(k_u + k_w)$.

d) Show that the dynamics may be written as

$$\frac{\text{d}}{\text{d}t}\begin{pmatrix} c_{\text{W}} + c_{\text{S}} \\ c_{\text{ES}} \end{pmatrix} = \mathsf{B} \cdot \begin{pmatrix} c_{\text{W}} + c_{\text{S}} \\ c_{\text{ES}} \end{pmatrix}. \tag{3.4}$$

Be sure to write down an expression for B. You should include f in your expressions; that is, do not write it out at $k_u/(k_u + k_w)$.

e) Show that if

$$r_{\text{hist}} \equiv\ll \frac{k_1 k_2 c_{\text{E}}^0 f}{(k_1 c_{\text{E}}^0 f + k_{-1} + k_2)^2} \ll 1, \tag{3.5}$$

that, analogously to part (b), $c_{\text{P}}(t) \approx (c_{\text{W}}^0 + c_{\text{S}}^0)(1 - \text{e}^{-k_{\text{hist}}t})$. Be sure to write an expression for k_{hist}. You can use previously derived results if they are useful.

f) Show that if $k_1 c_{\text{E}}^0/(k_{-1} + k_2) \ll 1$, the $f = k_{\text{hist}}/k_{\text{bare}}$. This means that Polach and Widom could measure the production of cleaved product modeled as a simple exponential for bare DNA and also in the presence of histones, and from those measurements they could work out the fraction of time the DNA is detached from a histone.

g) *Not graded.* There were a lot of assumptions that led to the handy, experimentally very useful result that $f = k_{\text{hist}}/k_{\text{bare}}$. These do, in fact, hold! You can read the analysis in Prinsen and Schiessel, *Biochimie*, **92**, 1722, 2010, where they investigate measured parameter values and verify that the assumptions hold.

Problem 3.2 (Ligand-receptor binding and small numbers of molecules, 40 pts)**.**
In this problem, we will explore the effect of having small number of ligands and receptors in a small volume, as is often the case in cells. Imagine we have a cell with volume V_{cell} that contains L total ligands and R total receptors. (Of course here we mean copies of specific ligand-receptor pair; cells have lots of ligands and receptors of different type.) The receptors and ligands are all free to move about in the cell. Each receptor can bind a single ligand. Let n be the number of receptors that are bound to ligands.

a) Compute the expected number of bound receptors, n, as a function of L, R, and $W \equiv K_\mathrm{d} V_\mathrm{cell}$. In doing the calculation, assume that R and L are large, which enables you to use

$$K_\mathrm{d} = \frac{c_L c_R}{c_{LR}}. \tag{3.6}$$

b) W is a dimensionless number. What is its physical meaning?

c) When L and R are not large, just knowing the expected number of bound receptors is not enough to fully understand what the molecules are doing in our system. We therefore would like to know $P(n)$, the probability mass function of n. I.e., $P(n)$ is the probability that there are n bound receptors at equilibrium. Show that

$$P(n) = \frac{\left[W^n\, n!(R-n)!(L-n)!\right]^{-1}}{\displaystyle\sum_{n=0}^{\min(R,L)} \left[W^n\, n!(R-n)!(L-n)!\right]^{-1}}. \tag{3.7}$$

d) Plot $P(n)$ for various values of L, R, and W. Comment on what you see, especially for small L and R. By "small," I mean between 1 and 100. (Are there ligands and receptors with these sorts of copy numbers in cells?) Think carefully about how to represent your plot so that you can highlight the important physical consequences of your analysis. Be sure to discuss your plots. *Hint:* It will be difficult to compute the statistical weights and the partition function. Work with logarithms of the statistical weights when you can. If you are using Python, `scipy.special.gammaln()` and `scipy.misc.logsumexp()` might be useful functions.

e) The coefficient of variation is the ratio of the standard deviation of a distribution to its mean. Plot the coefficient of variation of $P(n)$ for $W = 1000$, R going from 1 to 10^5, and $L = 2R$. What does this say about variability in number of of species? When can you just use your result from part (a), and when should be think more carefully about the full distribution?

Problem 3.3 (Cooperative ligand-receptor binding, 30 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

$$\mathrm{LR} \rightleftharpoons \mathrm{L} + \mathrm{R}, \quad K_{\mathrm{d},1} \tag{3.8}$$

$$\mathrm{RL} \rightleftharpoons \mathrm{R} + \mathrm{L}, \quad K_{\mathrm{d},2} \tag{3.9}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{L} + \mathrm{RL}, \quad K_{\mathrm{d},3} \tag{3.10}$$

$$\mathrm{LRL} \rightleftharpoons \mathrm{LR} + \mathrm{L}, \quad K_{\mathrm{d},4}. \tag{3.11}$$

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_{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_{LRL}) in terms of the ligand concentration c_{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_{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.

$$\mathrm{LRL} \rightleftharpoons \mathrm{R} + \mathrm{L} + \mathrm{L}, \tag{3.12}$$

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_{LRL}. Compare this result to your results in parts (c) and (d).

f) **Hill functions** are commonly used to describe cooperative binding. A Hill function for binding of n ligands to a receptor is of the form.

$$p_{RL_n} = \frac{c_L^n}{K^n + c_L^n} \tag{3.13}$$

What does the analysis in this problem say about using Hill functions to describe cooperative binding?