1 A Feeling for the Numbers in Biology, Round Two
Now that you are well versed in biological estimation, start preparing your second and final estimate. The format of the presentation will again be five minutes. **This time, you can work in groups of two if you want to.** Write a short paragraph describing the estimate you’re interested in, and the approach you will follow. This second estimate could consist of a further elaboration of the first estimate you did, or could be completely new. For inspiration, you can look at the various vignettes in Cell Biology by the Numbers, the “Estimates” sections in PBoC, Guesstimation, etc.

Remember that you’re supposed to learn something from your calculations. As a result, always compare your result to some expectation in order to put it in context of the biological phenomenon you’re addressing. As shown in class for our calculation of the time it takes to replicate the bacterial genome, even estimates that are “wrong” can teach us something. Also, keep in mind that an estimate is not just about looking things up. Finally, remember that you’re streetfighting: you don’t need to be reporting numbers to various significant digits.

Send this paragraph as an email to Hernan and Simon by the homework due date.

**Note to class:** You can complement these problems by reading the paper “A First Exposure to Statistical Mechanics for Life Scientists: Applications to Binding” on the course website.

2 Ion channels and statistical mechanics
In this problem, we will derive a mathematical description of the current passing through a voltage-gated ion channel. To model this channel, we assume that it can exist in an open or closed configuration as shown in Figure 1(A). The thermal fluctuations in the cell result in the channel switching between these states over time as presented in Figure 1(B). Figure 1(C) shows how these fluctuations in channel state can be directly read out from the
current flowing through the channel.

(a) Use the statistical mechanics protocol to calculate the probability of the channel being in the open state, $p_{\text{open}}$. Assume that the open state has an energy $\varepsilon_{\text{open}}$, and that the energy of the closed state is $\varepsilon_{\text{closed}}$.

(b) Plot $p_{\text{open}}$ as a function of $\Delta \varepsilon = \varepsilon_{\text{open}} - \varepsilon_{\text{closed}}$. Explain what happens in the limits $\varepsilon_{\text{open}} \ll \varepsilon_{\text{closed}}$ and $\varepsilon_{\text{open}} \gg \varepsilon_{\text{closed}}$. What significance does $\Delta \varepsilon = 0$ have for $p_{\text{open}}$?

In a simple model of a voltage-gated ion channel, $\Delta \varepsilon = q(V^* - V)$. Here, $V$ is the voltage applied to the membrane and $q$ is the effective gating charge, which describes the movement of charges along the membrane as the channel configuration changes. You can learn more about this model in section 17.3.1 of PBoC2.

(c) What is the significance of $V^*$? Namely, what happens to the probability of being open when $V = V^*$?

(d) On the website, you will find measurements of $p_{\text{open}}$ vs. $V$ for a sodium-gated ion channel. Make a plot where you overlay this data with our model prediction in order to estimate $V^*$ and the gating charge $q$. Report the gating charge in units of the charge of the electron.

![Figure 1: Current through an ion channels. (A) The ion channel can exist in a closed or open configuration, (B) fluctuating in time between these two states. (C) The current flowing through the channel is directly related to the state of the channel.](image)

3 Ligand-receptor and the lattice models of solutions
In class, we used statistical mechanics to calculate the probability of a repressor binding to the promoter. Here, the reservoir for the repressor was the non-specific genomic DNA. In this problem, we extend these calculations to consider a ligand molecules in solution that can bind to a receptor. In this case, the reservoir for the ligand is the solution. Note that this problem will required many derivations. Make sure to explain each step you take carefully.
Figure 2 shows how the statistical mechanics protocol is applied to the problem of a ligand binding to its receptor. We assign zero energy to the state without ligand bound. The ligand binding energy is given by $\varepsilon_b$. In addition, there is a cost $\mu$ of transferring a ligand from the solution to the receptor.

We begin by calculating $\mu$, which is defined in terms of the free energy of $L$ ligands in solution, $F(L)$, as $\mu = F(L) - F(L-1)$. If we assume that every ligand in solution has an energy $\varepsilon_{sol}$, then the free energy is given by

$$F(L) = L\varepsilon_{sol} - TS,$$

where $S$ is the entropy and $T$ the absolute temperature. To calculate the entropy term, we can use the formula $S = k_B \ln W(L)$, where $W(L)$ is the number of configuration the $L$ ligands can adopt in the solution. In order to obtain $W(L)$ we invoke a a so-called lattice model of the solution. Here, we divide the solution into $\Omega$ boxes of volume $v$ as shown in Figure 3.

(a) Calculate the number of configurations $W(L, \Omega)$ by using the lattice model from Figure 3 and a similar reasoning to that we used in class to calculate the number of configuration repressors could adopt in their DNA non-specific reservoir.

(b) Show that $\mu = \varepsilon_{sol} - k_B T \ln \left( \frac{\Omega}{L} \right)$. Further, show that the probability of a receptor being occupied is given by

$$p_{\text{bound}} = \frac{(L/\Omega) e^{-\beta \Delta \varepsilon}}{1 + (L/\Omega) e^{-\beta \Delta \varepsilon}},$$

where $\Delta \varepsilon = \varepsilon_b - \varepsilon_{sol}$.

(c) It is sometimes easier to deal with the concentration of ligands $[L]$ rather than with the number of ligand molecules $L$. The ligand concentration can be written as $[L] = L/V$, where $V$ is the volume of the solution. Note that, in our lattice model, $V = \Omega v$. Show that $p_{\text{bound}}$ can be rewritten as

$$p_{\text{bound}} = \frac{[L]/[L]_0 e^{-\beta \Delta \varepsilon}}{1 + [L]/[L]_0 e^{-\beta \Delta \varepsilon}},$$

where $[L]_0 = 1/v$, which is called the standard biochemical concentration. What is $[L]_0$ in molars if $v = 1$ nm$^3$?

(d) Since $k_B T$ is an energy, we can define our binding energies in units of $k_B T$. Plot three curves of $p_{\text{bound}}$ vs. $[L]$ for $[L]_0 = 0.6$ M and for $\Delta \varepsilon$ equal to -7.5, -10, and -12.5 $k_B T$ on a log-log plot. You might want to use the command “logspace” in order to define the range of values of $[L]$ you want to plot. What features of the curve (e.g., slope, position) does the binding energy $\Delta \varepsilon$ control?

4 Binding energies vs. dissociation constants
\[
\begin{array}{ccc}
\text{STATE} & \text{ENERGY} & \text{BOLTZMANN} \\
\includegraphics[width=0.2\textwidth]{state1} & 0 & 1 \\
\includegraphics[width=0.2\textwidth]{state2} & \epsilon_b - \mu & e^{\beta(\epsilon_b - \mu)}
\end{array}
\]

Figure 2: The statistical mechanics protocol for the ligand receptor problem.

Figure 3: Lattice model of a solution of ligands. The lattice is comprised of \( \Omega \) boxes of volume \( v \), with \( L \) ligands.

You might be more familiar with the dissociation constant \( K_d \) rather than with binding energies. We can represent the binding reaction in the biochemical notation as

\[
L + R \rightleftharpoons L - R,
\]

where \([L]\) is the concentration of free ligands, \([R]\) is the concentration of free receptors, and \([L - R]\) is the concentration of ligand-receptor complexes. Here, we have also introduced the dissociation constant which is defined as

\[
K_d = \frac{[L][R]}{[L - R]}.
\]

(a) Calculate \( p_{\text{bound}} \) in this biochemical notation by noting the \( p_{\text{bound}} \) is the fraction of bound receptors

\[
p_{\text{bound}} = \text{fraction of bound receptors} = \frac{\text{number of bound receptors}}{\text{number of all receptors}} = \frac{[L - R]}{[R] + [L - R]].
\]

Specifically, using the definition of the dissociation constant in Equation 4, show that \( p_{\text{bound}} \) can be written as

\[
p_{\text{bound}} = \frac{[L]/K_d}{1 + [L]/K_d}.
\]
(b) Compare Equations 3 and 7 to show that

\[ K_d = \frac{1}{v} e^{\beta \Delta \varepsilon} \]  \hspace{1cm} (8)

and use this formula to calculate the \( K_d \) values in Molars corresponding to the choices of energy for the plots in the previous problem.