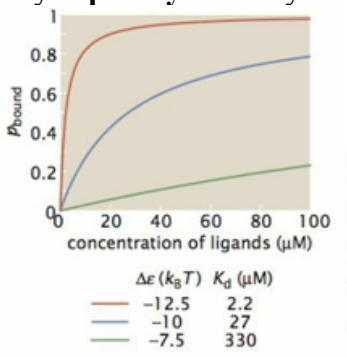
PHYS3511-Biological Physics

Fall 2018, Assignment #6 Due Wednesday November 14, 2018

Read Chapter 6: section 6.1 page 237-248; Section 6.2.2 and 6.2.3 page 262 to 267; Section 6.4.1 and 6.4.2 page 270 to 273. The information on these sections may be used in multiple-choice questions in **quiz 2** and the **final exam**.

Exercise 1) In Figure below, pick at least **two points** from each of the **three** curves, and verify **explicitly** that they are consistent with the $\Delta\epsilon, K_d, c_0$ and the equation for P_{bound}.



function of ligand concentration. The figure shows the average number of ligands bound as a function of the number of ligands in solution. The plot shows curves for three choices of Δε: −7.5, −10, and −12.5 k_BT, and a standard state $c_0 = 0.6$ M. The binding energies are also translated into the language of equilibrium dissociation constants.

$$p_{\text{bound}} = \frac{(c/c_0)e^{-\beta\Delta\varepsilon}}{1+(c/c_0)e^{-\beta\Delta\varepsilon}}.$$

Begin by calculating the reference concentration, c_0. On page 244, the concentration (number of particles, or mass, per unit volume) is calculate by $\frac{N}{\Omega V_{box}}$, where N is the number of particles, Ω is the total number of lattices, which follows that ΩV_{box} is the total volume. I emphasized in class that the value of V_{box} is arbitrary. On page 244, the authors of the textbook state that a reasonable value is 1 nm^3. If this were the case then the reference should be the value when 1 particle is in a lattice of volume, $V_{box} = 1nm^3$. The **reference concentration** is $c_0 = \frac{1}{1nm^3 \times 10^{-27}\frac{m^3}{nm^3}} = 1 \times 10^{27} m^{-3}$. We would like to convert this to molar concentration (M), $1M = 1\frac{mol}{L}$, with $1L = 10^{-3}m^3$, $1mol = 6.023 \times 10^{23}$, which gives $c_0 = 1 \times 10^{27}\frac{1}{m^3 \times 10^3 \frac{L}{m^3}}\frac{1}{6.023\times10^{23} mol^{-1}} = 1.6\frac{mol}{L}$=1.6M.

Note that this is different than the $c_0 = 0.6M$, used in the textbook. This is the value the textbook claim to in the equation 6.19. In all calculations we will use $c_0 = 0.6M$.

$$P_{bound} = \frac{\left(\frac{c}{c_0}\right)e^{-\beta\Delta\varepsilon}}{1+\left(\frac{c}{c_0}\right)e^{-\beta\Delta\varepsilon}}, \Delta\varepsilon = \varepsilon_b - \varepsilon_{sol}.$$

From equation 6.111,

$$P_{bound} = \frac{[L]/K_d}{1+[L]/K_d}, \Delta\varepsilon = \varepsilon_b - \varepsilon_{sol}, where\ [L] = c$$

$$\frac{[L]}{K_d} = \left(\frac{c}{c_0}\right)e^{-\beta\Delta\varepsilon} \rightarrow K_d = c_0 e^{\beta\Delta\varepsilon} = \frac{e^{\beta\Delta\varepsilon}}{v}, where\ c_0 = \frac{1}{v} = 0.6M.$$

Solving

$$\frac{\Delta\varepsilon}{k_BT} = ln\left(\frac{K_d}{c_0}\right)$$

In the figure for $K_d = 2.2\mu M = 2.2 \times 10^{-6} M$

$$\frac{\Delta\varepsilon}{k_B T} = ln\left(\frac{2.2 \times 10^{-6} M}{0.6M}\right) = -12.5.$$

In the figure for $K_d = 27\mu M = 27 \times 10^{-6} M$

$$\frac{\Delta\varepsilon}{k_B T} = ln\left(\frac{27 \times 10^{-6} M}{0.6M}\right) = -10.$$

In the figure for $K_d = 330\mu M = 330 \times 10^{-6} M$

$$\frac{\Delta\varepsilon}{k_B T} = ln\left(\frac{330 \times 10^{-6} M}{0.6M}\right) = -7.5.$$

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 7.5$, select $c = 20\mu M = 2 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{7.5}}{1 + \left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{7.5}} = 0.057$$

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 7.5$, select $c = 80\mu M = 8 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{7.5}}{1 + \left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{7.5}} = 0.19$$

These two values match the green plot above.

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 10$, select $c = 20\mu M = 2 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{10}}{1 + \left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{10}} = 0.42$$

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 10$, select $c = 80\mu M = 8 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{10}}{1 + \left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{10}} = 0.75$$

These two values match the blue plot above.

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 12.5$, select $c = 20\mu M = 2 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{12.5}}{1 + \left(\frac{2 \times 10^{-5} M}{0.6M}\right) e^{12.5}} = 0.11$$

For $-\beta\Delta\varepsilon = -\frac{\Delta\varepsilon}{k_B T} = 12.5$, select $c = 80\mu M = 8 \times 10^{-5} M$:

$$P_{bound} = \frac{\left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{12.5}}{1 + \left(\frac{8 \times 10^{-5} M}{0.6M}\right) e^{12.5}} = 0.972$$

These two values match the red plot above.

Exercise 2) Chapter 6, Problem 6.3

A) This would simply be equation 6.18,

$$P_{bound} = \frac{(c/c_0)e^{-\beta(\Delta\varepsilon)}}{1 + (c/c_0)e^{-\beta(\Delta\varepsilon)}}, where\ c = ligand\ concentration,$$

c_0 = 0.6 M being the reference concentration used in exercise 1, $\Delta\varepsilon = \varepsilon_b - \varepsilon_{sol}$. In this case, the ligand will be RNA polymerase binding to non-specific DNA sites, with energy, ε_{pd}^{NS}, and to (specific) lac P1 and T7A1 promoter on DNA, with energy, ε_{pd}^{S}. For detail look at equation 6.31 and read page 247 and 248.

B) We have *in vitro* (test tube) experiments data of **dissociation constant**, K_d, of RNA polymerase, $\Delta\varepsilon = \varepsilon_{pd}^{S/NS} - \varepsilon_{sol,p}$, with $\varepsilon_{sol,p}$ being the energy of RNA polymerase in solution, and ε_{pd}^{NS} and ε_{pd}^{S} being the binding energy of RNA polymerase to non-specific (NS) and specific (S) DNA site, respectively.

For non-specific sites, $K_d = 10\mu M$. Now use the result of exercise 1)

$$K_d = c_0 e^{\beta\Delta\varepsilon} \rightarrow \frac{\Delta\varepsilon}{k_B T} = ln\left(\frac{K_d}{c_0}\right) = ln\left(\frac{10^{-5}M}{0.6M}\right) = -11,$$

$$\Delta\varepsilon^{NS} = \varepsilon_{pd}^{NS} - \varepsilon_{sol,p} = -11 k_B T$$

For <u>specific RNA polymerase binding to lac P1 promoter</u> $K_d = 550nM$

$$\frac{\Delta\varepsilon_{lacP1}}{k_B T} = ln\left(\frac{K_d}{c_0}\right) = ln\left(\frac{5.5 \times 10^{-7}M}{0.6M}\right) = -13.9,$$

$$\Delta\varepsilon_{lacP1} = \varepsilon_{pd}^{S,lacP1} - \varepsilon_{sol,p} = -13.9 k_B T$$

Hence the *in vivo* binding energy

$$\Delta\varepsilon_{PD}^{lacP1} = \varepsilon_{pd}^{S,lacP1} - \varepsilon_{pd}^{NS} = \Delta\varepsilon_{lacP1} - \Delta\varepsilon^{NS} = -2.9 k_B T$$

For <u>specific RNA polymerase binding to T7 promoter $K_d = 3nM$</u>

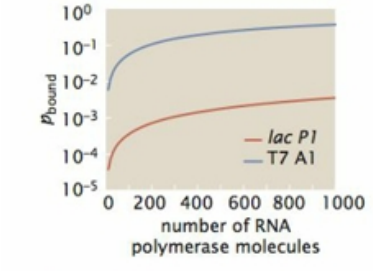
$$\frac{\Delta\varepsilon_{T7}}{k_B T} = ln\left(\frac{K_d}{c_0}\right) = ln\left(\frac{3 \times 10^{-9}M}{0.6M}\right) = -19.11,$$

$$\Delta\varepsilon_{T7} = \varepsilon_{pd}^{S,T7} - \varepsilon_{sol,p} = -19.11 k_B T$$

Hence the *in vivo* binding energy

$$\Delta\varepsilon_{PD}^{S,T7} = \varepsilon_{pd}^{S,T7} - \varepsilon_{pd}^{NS} = \Delta\varepsilon_{T7} - \Delta\varepsilon^{NS} = -8.11 k_B T$$

Exercise 3) Combine the plot below with your results of $\Delta\epsilon_{pd}$ from exercise 2, and the relation of P_{bound}. To estimate the values of N_{NS} for both curves.



occupancy as a function of the number of RNA polymerase molecules. p_{bound} is computed using values for the specific and nonspecific binding of RNA polymerase obtained *in vitro* and corresponding to the *lac* promoter, and the A1 promoter from the phage T7.

$$p_{bound} = \frac{1}{1 + \frac{N_{NS}}{P} e^{\beta\Delta\varepsilon_{pd}}},$$

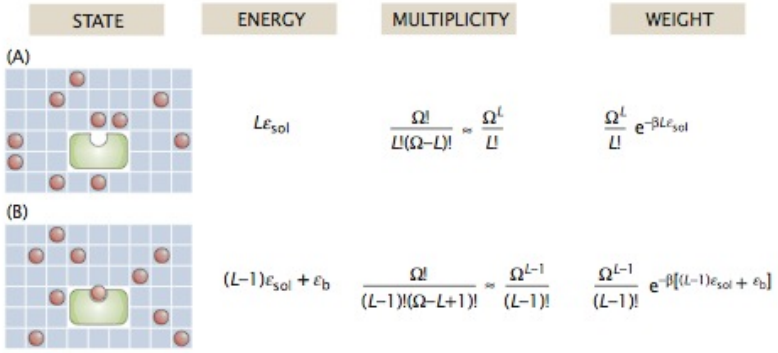
Use equation 6.23, $P_{bound} = \frac{1}{1+\frac{N_{NS}}{P}e^{\beta\Delta\varepsilon_{pd}}}$.

For **lac P1**, for number of RNA polymerase, with $\beta\Delta\varepsilon_{PD}^{lacP1} = -2.9$, P = 300, $P_{bound} \sim 10^{-3} \rightarrow 1000 = 1 + \frac{N_{NS}}{300} e^{-2.9} \rightarrow N_{NS} = 5.4 \times 10^6$. This is very close to the E. Coli DNA length of $\sim 5 \times 10^6 bp$. Of course this is based on an approximate value that I read from the plot.

For **T7 A1**, for number of RNA polymerase, with $\beta\Delta\varepsilon_{PD}^{T7} = -8.11$, P = 100, $P_{bound} \sim 10^{-1} \rightarrow 10 = 1 + \frac{N_{NS}}{100} e^{-8.11} \rightarrow N_{NS} = 5.9 \times 10^6$. This is on the large side, since phage T7 genome size is about 2×10^5. Most likely this is because most in vivo (living) experiments are performed in E. Coli, in this case the DNA gene of the T7 Phage may have been spliced onto the E. Coli DNA.

Important points for final exam: 1) students should understand why we can us the same values for NS, and different values for S (lacP1 and T7); 2) students should understand why some values are *in vitro*, while others are *in vivo*.

Exercise 4) Chapter 6, Problem 6.5



The left figure shows the binding model when the ligands are **indistinguishable**. For example, if all ligands are on the lattice (no binding), the multiplicity of L ligands on Ω lattice sites is

$$multiplicity = \frac{\Omega!}{L!\,(\Omega - L)!}$$

The L! is needed to correct for the overcounting. On the other hands if the ligands are **distinguishable** then the multiplicity of arranging L ligands on Ω lattice sites is

$$multiplicity = \Omega(\Omega - 1)(\Omega - 2)\cdots(\Omega - \mathrm{L} + 1) = \frac{\Omega!}{(\Omega - L)!}.$$

For the case with one bound ligand and $L - 1$ligands on the Ω lattice sites, the multiplicity is

$$multiplicity = \Omega(\Omega - 1)(\Omega - 2)\cdots(\Omega - \mathrm{L} + 1)(\Omega - \mathrm{L} + 2) = \frac{\Omega!}{(\Omega - L + 1)!}$$

Using the above the partition function:

$$Z = \frac{\Omega!}{(\Omega - L)!} e^{-\beta L\varepsilon_{sol}} + \frac{\Omega!}{(\Omega - L + 1)!} e^{-\beta(L-1)\varepsilon_{sol}} e^{-\beta\varepsilon_b}$$

The probability of a bound ligand is

$$P_{bound} = \frac{\frac{\Omega!}{(\Omega-L+1)!}e^{-\beta(L-1)\varepsilon_{sol}}e^{-\beta\varepsilon_b}}{\frac{\Omega!}{(\Omega-L)!}e^{-\beta L\varepsilon_{sol}} + \frac{\Omega!}{(\Omega-L+1)!}e^{-\beta(L-1)\varepsilon_{sol}}e^{-\beta\varepsilon_b}}$$

If we divide by the numerator and denominator by

$$\frac{\Omega!}{(\Omega-L)!}e^{-\beta L\varepsilon_{sol}}$$

We obtain

$$P_{bound} = \frac{\frac{(\Omega-L)!}{\Omega!}e^{\beta L\varepsilon_{sol}}\frac{\Omega!}{(\Omega-L+1)!}e^{-\beta(L-1)\varepsilon_{sol}}e^{-\beta\varepsilon_b}}{1+\frac{(\Omega-L)!}{\Omega!}e^{\beta L\varepsilon_{sol}}\frac{\Omega!}{(\Omega-L+1)!}e^{-\beta(L-1)\varepsilon_{sol}}e^{-\beta\varepsilon_b}}$$

Using

$$\frac{(\Omega-L)!}{\Omega!}\frac{\Omega!}{(\Omega-L+1)!}e^{\beta L\varepsilon_{sol}} \times e^{-\beta(L-1)\varepsilon_{sol}}e^{-\beta\varepsilon_b} = \frac{1}{(\Omega-L)}e^{-\beta(\varepsilon_b-\varepsilon_{sol})},$$

and $\Delta\varepsilon = \varepsilon_b - \varepsilon_{sol}$, and assume dilute condition $\Omega - L \sim \Omega$, we obtain $P_{bound} = \frac{(1/\Omega)e^{-\beta\Delta\varepsilon}}{1+(1/\Omega)e^{-\beta\Delta\varepsilon}} = \frac{\left(\frac{L}{V_{box}}\right)\left(\frac{L}{V_{box}}\right)^{-1}\frac{1}{\Omega}e^{-\beta\Delta\varepsilon}}{1+\left(\frac{L}{V_{box}}\right)\left(\frac{L}{V_{box}}\right)^{-1}\frac{1}{\Omega}e^{-\beta\Delta\varepsilon}}, P_{bound} = \frac{\left(\frac{L}{\Omega V_{box}}\right)\left(\frac{L}{V_{box}}\right)^{-1}e^{-\beta\Delta\varepsilon}}{1+\left(\frac{L}{\Omega V_{box}}\right)\left(\frac{L}{V_{box}}\right)^{-1}e^{-\beta\Delta\varepsilon}}.$

Now note that in the textbook V_{box} is the volume of 1 lattice, hence ΩV_{box} is the total volume of the system, and $c = \frac{L}{\Omega V_{box}}$ is the concentration of the ligand. If we define $c_0 = \frac{L}{V_{box}}$ is the reference volume, we obtain

$$P_{bound} = \frac{\left(\frac{c}{c_0}\right)e^{-\beta\Delta\varepsilon}}{1+\left(\frac{c}{c_0}\right)e^{-\beta\Delta\varepsilon}}.$$

Note that defining $c_0 = \frac{L}{V_{box}}$ as a reference concentration is valid since the value of V_{box} is completely arbitrary.

NOTE: P_{bound} is a thermodynamics quantity. That the result of P_{bound} is the same regardless of whether the ligands are **distinguishable** or **indistinguishable**, means that **thermodynamics measurements** cannot be used to detect quantum properties, such as the **indistinguishability** of **identical particles** (ligands). The exception is liquid helium 4, which does have some measurable quantum thermodynamics properties.

Exercise 5) Chapter 6, Problem 6.9

We will use equation 6.92 for the osmotic pressure, $P = n_s k_B T$, where $n_s = \frac{N_s}{V}$ is the number concentration of the solute.

For proteins, the table on the next page gives $N_s = 2.6 \times 10^6$, with E. Coli volume $V = 1\mu m^3 = 1 \times 10^{-18} m^3$. Note that it is good practice to work in SI unit. This gives, $n_s = \frac{N_s}{V} = 2.6 \times 10^{24} m^{-3}$. P= $n_s k_B T = 2.6 \times 10^{24} m^{-3}(1.381 \times 10^{-23} J \cdot K^{-1})(300K) = 1 \times 10^5 N \cdot m^{-2} = 1 \times 10^5 Pa = 1atm.$

Table 2.1: Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., Physiology of the Bacterial Cell, Sinauer Associates, 1990 and M. Schaechter et al., Microbe, ASM Press, 2006.)

Substance	% of total dry weight	Number of molecules
Macromolecules		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide (outer membrane)	3.4	1.2×10^6
DNA	3.1	2
Murein (cell wall)	2.5	1
Glycogen (sugar storage)	2.5	4,360
Total macromolecules	**96.1**	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	**3.9**	

• 6.3 Polymerase binding to the promoter revisited

The probability of promoter occupancy can be computed using both statistical mechanics and thermodynamics (that is, using equilibrium constants). These two perspectives were already exploited for simple ligand–receptor binding in Sections 6.1.1 and 6.4.1.

(a) Write an expression for the probability of finding RNA polymerase bound to the promoter as a function of the equilibrium constants for specific and nonspecific binding.

(b) *In vitro*, the dissociation constant of RNA polymerase binding to nonspecific DNA is approximately 10 μM and the dissociation constants of RNA polymerase to the *lac P1* and T7A1 promoters are 550 nM and 3 nM, respectively. Use these constants and the results from (a) to estimate the *in vivo* binding energies of RNA polymerase to *lac P1* and T7A1 promoters.

• 6.5 Distinguishable ligands

Derive the probability that a receptor is occupied by a ligand using a model that treats the L ligands in solution as distinguishable particles. Show that the expression is the same as obtained in the text (Equation 6.19), where the ligands were treated as indistinguishable.

• 6.9 Osmotic pressure of a cell

In Section 6.2.3, we derived the van't Hoff formula for the osmotic pressure and performed an estimate of the osmotic pressure experienced by a bacterium as a result of its impermeability to inorganic ions. Examine the contribution to the osmotic pressure of a bacterium coming from the presence of proteins within the cell.

How does this compare with the contribution to the osmotic pressure from inorganic ions described in the section? See Table 2.1 and Figure 2.4 for the relevant data.