Assignment 8
Biophys 4322/5322

Tyler Shendruk

April 13, 2012

1 Problem 1) Phillips 14.2

The effect of crowding on the chemical potential of a molecular species in solution can be captured by the equation

$$\mu = \mu_0 + k_\mathrm{B}T \ln\left(\frac{c\gamma}{c_0}\right), \tag{1}$$

where the subscripts zero are for a reference state. The effective concentration is given by $c\gamma$, where c is the actual concentration that is present in the solution and γ is called the activity coefficient. The simple lattice model of proteins in solution used repeatedly in the chapter implies a corresponding model for the activity coefficient. Work out this activity coefficient and compare your formula with the experimental results shown in Figure 14.21.

Let's use a lattice model of Ω boxes of volume ν like Phillips did on pg. 520 to find the partition function because once we know the partition function, we know the free energy and once we know the free energy, we know the chemical potential. But unlike Phillips we only have one species (with N constituents - which by the way means that the true concentration is $c = N/\Omega\nu$) so the partition function is simpler

$$Z = \frac{\Omega!}{(\Omega-N)!N!}e^{-\beta N\epsilon}, \tag{2}$$

where ϵ is the energy of interaction between the solute and the solvent. The free energy is

$$G(N) = -k_\mathrm{B}T\ln Z \tag{3}$$

and the chemical potential is the energy cost to add one more molecule *i.e.*

$$\begin{aligned}
\mu = G(N) - G(N-1) &= -k_\mathrm{B}T\ln\left(\frac{\Omega!}{(\Omega-N)!N!}e^{-\beta N\epsilon}\right) + k_\mathrm{B}T\ln\left(\frac{\Omega!}{(\Omega-(N-1))!\,(N-1)!}e^{-\beta(N-1)\epsilon}\right)\\
&= N\epsilon - (N-1)\,\epsilon - k_\mathrm{B}T\ln\left(\frac{\Omega!}{(\Omega-N)!N!}\right) + k_\mathrm{B}T\ln\left(\frac{\Omega!}{(\Omega-(N-1))!\,(N-1)!}\right)\\
&= \epsilon + k_\mathrm{B}T\ln\left(\frac{\Omega!}{(\Omega-(N-1))!\,(N-1)!}\frac{(\Omega-N)!N!}{\Omega!}\right)\\
&= \epsilon + k_\mathrm{B}T\ln\left(\frac{(\Omega-N)!N!}{(\Omega-(N-1))!\,(N-1)!}\right)\\
&= \epsilon + k_\mathrm{B}T\ln\left(\frac{N}{\Omega-N}\right).
\end{aligned} \tag{4}$$

Comparing Eq. (4) to the form we are looking for, we identify

$$\mu_0 = \epsilon \tag{5}$$

$$c = \frac{N}{\Omega\nu} \tag{6}$$

$$\frac{\gamma}{c_0} = \frac{\nu}{1-N/\Omega}. \tag{7}$$

It makes sense in that last one if $c_0 = \nu^{-1}$ which would make

$$\gamma = \left[1 - \frac{N}{\Omega}\right]^{-1}. \tag{8}$$

Plot it.

2 Problem 2) Phillips 14.3

Use the approximate formula for the pressure of a gas of hard spheres to extract an effective hard-sphere radius for hemoglobin from the data given in the textbook. How does this effective radius compare with the dimensions of the molecule obtained by X-ray scattering?

The equation given in Phillips is

$$p = k_{\mathrm{B}} T [H] \left(1 + x + 0.625 x^2 + 0.287 x^3 + 0.11 x^4\right) \tag{9}$$

where $x = 4[H]v$ and v is the volume of the hard sphere. To match with the data we need the osmotic pressure in units of mmHg and the concentration should be in grams/litre: $c_H = M[H]$ where hemoglobin's mass is $M = 68kDa = 68 \times 1.66 \times 10^{21} g = 1.13 \times 10^{-19} g$. So then x becomes

$$\begin{aligned} x = 4[H]v = \frac{4c_H}{M} v &= \frac{3.54 \times 10^{19}}{g} c_H v \\ &= \frac{3.54 \times 10^{19}}{g} c_H \left(v \frac{10^{-24} L}{nm^3}\right) \\ &= 3.54 \times 10^{-5} c_H v \end{aligned} \tag{10}$$

where now c_H is in g/L and v is in nm^3 and the osmotic pressure in these units is

$$p = 0.25\,[\mathrm{mmHg}] \times c_H \left(1 + x + 0.625 x^2 + 0.287 x^3 + 0.11 x^4\right). \tag{11}$$

A good fit to the data should give

$$v = 112 \mathrm{nm}^3, \tag{12}$$

which means that the radius of hemoglobin is

$$R = \left(\frac{3}{4\pi} v\right)^{1/3} = 2.9 \mathrm{nm}. \tag{13}$$

This is exactly what the caption to the figure in the book gives.

3 Problem 3) Phillips 14.4

Compute the depletion force between a sphere of radius R and a planar surface in the presence of smaller spheres of radius r.

This was mostly done in the textbook except the textbook did two colloids and you are to do a colloid and a wall. Just follow pg. 526-528. I'll sketch it very quickly:

- Variables:
  - r radius of smaller molecules
  - R radius of large molecule
  - L length of the box
  - N number of small molecules

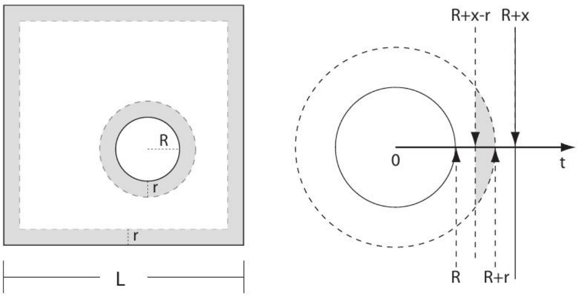

Figure 1: 2D representation of the excluded volumes in Eq. (15).

- Free energy

$$G_{ex} = Nk_{\mathrm{B}}T\frac{V_{ex}}{V_{box}}. \tag{14}$$

- $V_{box} = L^3$.
- The excluded volume is

$$V_{ex} = \underbrace{\underbrace{L^3}_{\text{V of box}} - \underbrace{(L-2r)^3}_{\text{V not taken up by small sphere}}}_{\text{V of shell around walls}} + \underbrace{\frac{3\pi}{3}(R+r)^3}_{\text{effective V of big sphere}} + V_{\text{overlap}}. \tag{15}$$

- The overlap volume is the volume of the cap *i.e.* the difference between a spherical cone and a regular cone (just like for two colloids):

$$V_{\text{s cone}} = \frac{2\pi}{3}(R+r)^3(1-\cos\theta) = \frac{2\pi}{3}(R+r)^3(R+r-[R+x-r]) \tag{16}$$

$$V_{\text{r cone}} = \frac{\pi}{3}\left[(R+r)^2 - (R+x-r)^2\right](R+x-r) \tag{17}$$

$$\begin{aligned} V_{\text{overlap}} &= V_{\text{s cone}} - V_{\text{r cone}} \\ &= \frac{\pi}{3}\left[2(R+r)^3 - 3(R+r)^2(R-r) + 3(R+r)^2 x + (R-r+x)^3\right], \end{aligned} \tag{18}$$

where x is the distance between the sphere and the wall.

- The force is

$$\begin{aligned} F &= -\frac{\partial G_{ex}}{\partial x} = -\frac{Nk_{\mathrm{B}}T}{V_{box}}\frac{\partial V_{ex}}{\partial x} \\ &= -nk_{\mathrm{B}}T\frac{\partial V_{ex}}{\partial x} = -nk_{\mathrm{B}}T\frac{\partial V_{\text{overlap}}}{\partial x} \\ &= -\pi nk_{\mathrm{B}}T\left[(R+r)^2 - (R-r+x)^2\right]. \end{aligned} \tag{19}$$

4 Problem 4) Phillips 14.5

Repeat that derivation for the free energy of exclusion as a function of the osmotic pressure of the depletant molecules. Then estimate the force between two beads whose radii 1μm in a concentrated protein solution, when the concentration is equal to the value characteristic of an E. coli cell. Use $r = 3$nm for the typical radius of a protein.

The quickest way to derive this is through the partition function on a lattice *i.e.* think about the deletion free energy in terms of an osmotic effect (which it is). The partition function is

$$Z = \frac{\Omega!}{N!\,(\Omega - N)!} e^{-\beta N \epsilon}. \tag{20}$$

If a second object is close to the an original object in the solvent than the second reduces the number of states the first object can take *i.e.* if they are a distance D apart then

$$\begin{aligned} G(D) &= k_{\mathrm{B}} T \ln (Z(\Omega)) - k_{\mathrm{B}} T \ln (Z(\Omega_D)) \\ &= k_{\mathrm{B}} T \ln \left(\frac{Z(\Omega)}{Z(\Omega_D)} \right) = k_{\mathrm{B}} T \ln \left(\frac{\frac{\Omega!}{N!(\Omega-N)!} e^{-\beta N \epsilon}}{\frac{\Omega_D!}{N!(\Omega_D-N)!} e^{-\beta N \epsilon}} \right) \\ &= k_{\mathrm{B}} T \ln \left(\frac{\frac{\Omega!}{(\Omega-N)!}}{\frac{\Omega_D!}{(\Omega_D-N)!}} \right) \approx k_{\mathrm{B}} T \ln \left(\frac{\Omega^N}{\Omega^N(D)} \right) \\ &= -N k_{\mathrm{B}} T \ln \left(\frac{\Omega_D}{\Omega} \right). \end{aligned} \tag{21}$$

We don't actually know Ω or Ω_D but we do suspect that it is directly proportional to the volume available like

$$\Omega \propto \frac{V}{\nu} \tag{22}$$

where ν is the volume of a lattice cell and V is the total volume of the system. When two objects are a distance D apart the number of states is reduced because the available volume is reduced:

$$\Omega_D = \propto \frac{V - V_{ex}}{\nu}. \tag{23}$$

So then the free energy is

$$\begin{aligned} G(D) &= -N k_{\mathrm{B}} T \ln \left(\frac{\Omega_D}{\Omega} \right) = -N k_{\mathrm{B}} T \ln \left(\frac{V - V_{ex}}{V} \right) \\ &= -N k_{\mathrm{B}} T \ln \left(\frac{\Omega_D}{\Omega} \right) = -N k_{\mathrm{B}} T \ln \left(1 - \frac{V_{ex}}{V} \right) \\ &\approx N k_{\mathrm{B}} T \ln \frac{V_{ex}}{V} = \Pi_0 V_{ex}. \end{aligned} \tag{24}$$

The approximation is good if $V_{ex} \ll V$ which seems reasonable in most cases and we've recognized the osmotic pressure of the small molecules $\Pi_0 \equiv N k_{\mathrm{B}} T / V$.

The textbook found V_{ex} and the force between two spheres to be

$$F = \frac{\partial G}{\partial D} = \Pi_0 \pi \left[(R + r)^2 - \frac{D^2}{4} \right] \tag{25}$$

when $2R < D < 2(R + r)$.

We're interested in an estimate so let's say $r \ll R$ (so we can drop higher order r terms) and $D \sim 2R + r$ so that

$$\begin{aligned} F &= \Pi_0 \pi \left[(R + r)^2 - \frac{D^2}{4} \right] = \Pi_0 \pi \left[R^2 + 2Rr + r^2 - \frac{4R^2 + 2Rr + r^2}{4} \right] \\ &\approx \Pi_0 \pi \left[2Rr - \frac{Rr}{2} \right] = \frac{3\pi}{4} \Pi_0 R r. \end{aligned} \tag{26}$$

Say the concentration is 3mM then $\Pi_0 = 3 mol/m^3 \times 2.5 \times 10^3 J/mol \approx 7.5 \times 10^3 \mathrm{Pa}$ and so the force is

$$\begin{aligned} F &= \frac{3\pi}{4} \Pi_0 R r = \frac{3\pi}{4} 7.5 \times 10^3 \frac{\mathrm{N}}{\mathrm{m}^2} \times 10^{-6} \mathrm{m} \times 3 \times 10^{-9} \mathrm{m} \\ &\approx 53 \times 10^{-12} \mathrm{N} = 53 \mathrm{pN}. \end{aligned} \tag{27}$$

5 Problem 5) Phillips 14.7

(Calculate the diffusion coefficient by considering the possible trajectories of the tracer particles and their probabilities. Express your answer in terms of the diffusion coefficient D_0 of the tracer particles in the absence of crowders, the volume fraction of the crowders ϕ, and the ratio of the tracer and crowder sizes $r = b/a$.

The tracer wants to do jumps of size b. It goes to the right with probability p_+, to the left with probability p_- or stays put with probability p_0. There is no symmetry breaking therefore the probability of going right or left is equal $p_+ = p_- = p_\pm$. The textbook already covered the case when there is only one species:

$$p_\pm = \frac{1}{2}\left(1-\phi\right)$$

but this is **not true** when the sizes are different. Now the tracer particle of size b occupies $r = b/a$ sites (of size a) so it needs the r sites beside it to be open. The probability of this is the product of the probability that each is empty (which are all independent of each other of course) *i.e.*

$$p_\pm = \prod^{r} \frac{1}{2}\left(1-\phi\right) = \frac{1}{2}\left(1-\phi\right)^r. \tag{28}$$

The probability of staying put is 100% minus the probability of going left or right:

$$p_0 = 1 - p_+ - p_- = 1 - 2p_\pm = 1 - \left(1-\phi\right)^r. \tag{29}$$

With all the probabilities in hand, we can find the mean square displacement for N steps:

$$\begin{aligned}\left\langle x^2\right\rangle &= N\left\langle x_1^2\right\rangle \\ &= N\left(b^2 p_+ + b^2 p_- + 0\times p_0\right) = N\left(2b^2 p_\pm\right) \\ &= Nb^2\left(1-\phi\right)^r,\end{aligned} \tag{30}$$

which we could write

$$\left\langle x^2\right\rangle = 2Dt \tag{31}$$
$$= 2D_0\left(1-\phi\right)^r t \tag{32}$$
$$\tag{33}$$

if we define

$$D = D_0\left(1-\phi\right)^r \tag{34}$$
$$D_0 = \frac{b^2}{2\tau}, \tag{35}$$

where τ is the time scale of a single jump.

5.1 Part b)

Plot $\ln\left(D/D_0\right)$ as a function of the volume fraction for different values of r. How well does this model explain the data shown in Figure 14.20(A)?

From **Part a)**

$$D = D_0\left(1-\phi\right)^r$$
$$\frac{D}{D_0} = \left(1-\phi\right)^r$$
$$\ln\left(\frac{D}{D_0}\right) = r\ln\left(1-\phi\right) \tag{36}$$
$$\ln\left(\frac{D}{D_0}\right) \approx r\phi. \tag{37}$$

Plot it. Even if you don't make the approximation at the end (because we're interested in **crowded** environments) this form pretty clearly doesn't capture the curvature seen in Phillips Fig. 14.20(a) and so underestimates the effect of crowding.