BE 25 Winter 2024
Homework #8
Due at 9 AM PST, March 7, 2024

Problem 8.1 (Donnan potentials in red blood cells, 10 pts)**.**
This problem is based on problem 7.18 of Tinoco, et. al. The typical Mg^{2+} concentration in blood plasma is 2.2 mM. If the potential across the membrane of a red blood cell is -90 mV (the inside of the RBC is more negative than the outside), what is the magnesium ion concentration inside a red blood cell? *Hint:* Can you neglect the osmotic pressure?

Problem 8.2 (Using osmotic pressure to determine molecular weight, 20 pts)**.**
Charles Tanford was an important protein biophysical chemist. (He is an interesting character worth reading about. I enjoyed his book *Ben Franklin Stilled the Waves*.) In the 1967 he published a series of papers on denatured proteins along with Savo Lapanje. In one set of experiments, they used an osmometer to measure the osmotic pressure of a solution containing a protein of unknown molecular weight. A schematic of an osmometer is shown below.

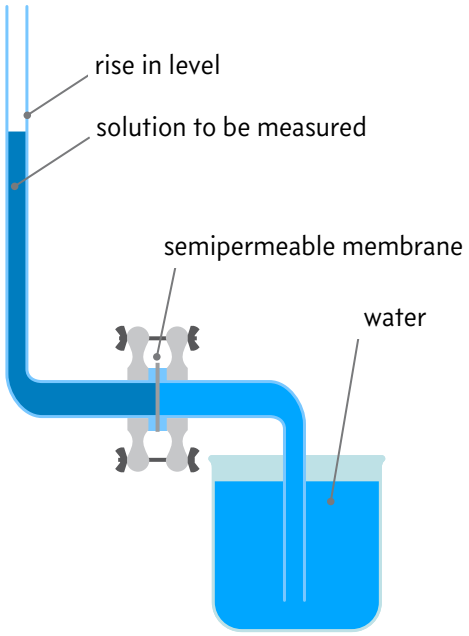

Figure 1: A schematic of an osmometer, adapted from an image by Benedikt Seidl, licensed under a CC BY-SA 3.0 license. The change in height of a solution of interest after adding solute is used to determine the osmotic pressure across the semipermeable membrane.

To prepare a solution, they added a known mass of protein to a known volume of solvent. They did this for several mass concentrations, and then used a plot like the one shown below from their paper (Lapanje and Tanford, *J. Am. Chem. Soc.*, **89**,

5030–5033, 1967) to obtain the molar mass. Note that the variable C on the y-axis of the plot below is a *mass* concentration, given in units of grams per liter.

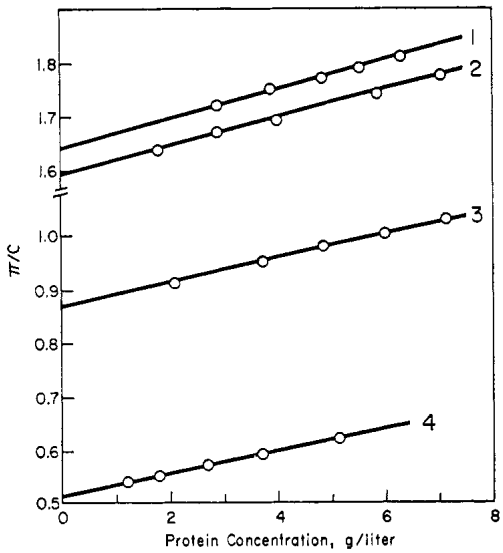

Figure 1. Representative experimental data. The units for osmotic pressure are centimeters of solvent (density 1.14 g/cc). The value of RT in the units employed (at 25°) is 2.20×10^4. Curves 1 and 2 represent duplicate runs, starting with different stock solutions, for ribonuclease. These were the only two duplicate runs which showed such a large difference in the intercept. Curves 3 and 4 are representative runs for chymotrypsinogen and aldolase.

a) Sketch how the curves would look for ideal (dilute solutions). Do the experimental curves shown in the above figure deviate from ideality? If so, why do you think they deviate?

b) Considering curves 2, 3, and 4, which are respectively for ribonuclease, chymotrypsin (actually its inactive precursor), and aldolase, estimate the molecular weights of the respective proteins.

Problem 8.3 (Random walk with drift, 35 pts)**.**
Consider a one-dimensional random walk where the probability of stepping rightward is θ. If $\theta \neq 1/2$, the random walk is said to be *biased.* Consider a random walk of N steps of length ℓ, each of which takes time τ.

a) Show that the mean displacement $\langle x \rangle = Nb\ell$, where $b = 2\theta - 1$ is the directional bias of the talk ($-1 \leq b \leq 1$).

b) Show that the variance of the displacement $\left\langle (x - \langle x \rangle)^2 \right\rangle = N\left(1 - b^2\right)\ell^2$.

c) The amount of time spent on a biased random walk is $t = N\tau$. Define diffusion

coefficient

$$D = \frac{\ell^2(1-b^2)}{2\tau} \tag{8.1}$$

and velocity

$$v = \frac{b\ell}{\tau}. \tag{8.2}$$

Then, show that the probability distribution for the displacement is

$$P(x;t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left[-\frac{(x-vt)^2}{4Dt}\right]. \tag{8.3}$$

d) Consider a collection of particles undergoing this random walk. Define the concentration of these particles to be $c(x,t) = n_0 P(x,t)$. Show that $c(x,t)$ so defined is a solution to the diffusion-advection equation you derived in the previous problem.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x}. \tag{8.4}$$

In solving this problem, you have worked out that the biased random walk has a drift velocity of $v = b\ell/\tau$. Thus, bias in the walk functions like a weak flow is driving transport more into one direction.

Problem 8.4 (Rate constants, 35 pts)**.**
In the first half of the course, we did not talk about how rate constants for chemical reactions are determined. In this problem, we will work out how rate constants might vary with temperature and other properties of a system and its reactants.

a) We will be discussing the Arrhenius rate law, which is phenomenological. Nonetheless, Arrhenius took inspiration from a *thermodynamic* relation,

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{1}{k_B T^2}\sum_i \nu_i h_i^0, \tag{8.5}$$

where K is a chemical equilibrium constant for a reaction. This relation is called the **van't Hoff equation**, not to be confused with the van't Hoff formula we saw in lecture describing osmotic pressure. Derive this equation. *Hints:* Use the fact that $\mu_i^0 = h_i^0 - T s_i^0$, since μ_i^0 is the per-particle Gibbs free energy of solute i, and the Gibbs free energy is a Legendre transform of the enthalpy. Also, there is an additional assumption involved this equation. What is it?

b) Taking inspiration from the van't Hoff equation, Arrhenius figured that analogously to the equilibrium constant, the *rate* constant k of a reaction might have a similar dependence on an energy. He wrote

$$\left(\frac{\partial \ln(k/k_0)}{\partial T}\right)_p = \frac{E_a}{k_B T^2}, \tag{8.6}$$

where E_a is referred to as an **activation energy** and k_0 imparts units on the rate constant. Show that the above relation leads to which is known as **Arrhenius's equation**,

$$k = A\mathrm{e}^{-E_a/k_B T}, \tag{8.7}$$

a phenomenological but highly effective prescription for rate constants. Activation energies are positive, as can be worked out from **transition state theory**, which is beyond the scope of this course. The larger the activation energy, the smaller the rate constant k.

c) The parameter A is named the **preexponential factor**, but is also called the **frequency factor**. While the former name is a descriptive mathematical name for the parameter A, the latter is a descriptive physical name. The idea is that the prefactor describes how often molecules can come into contact with each other in solution and the exponential term describes how often those contacts result in a chemical reaction. We will now work out an expression for A.

To have a concrete model in mind, we will consider a protein and a ligand binding to each other. We put ourselves in the position of the center of the protein, which is embedded in a sea of solvent and ligand. The ligands may come into proximity of the protein via diffusion. Let c_{L}^{∞} be the concentration of ligand far from the protein. This is the bulk concentration of ligand in the whole solution. Because the protein is reactive, ligand will be locally depleted near the protein surface, so there will be a diffusive flux of ligand toward the protein. This is described by the diffusion equation,

$$\frac{\partial c_{\mathrm{L}}}{\partial t} = D\nabla^2 c_{\mathrm{L}}. \tag{8.8}$$

Here, D is the diffusion coefficient of ligand *relative* to protein, so it has a value of $D = D_{\text{protein}} + D_{\text{ligand}}$.

i) Assuming the ligand reacts as soon as it make contact with the surface of the protein, which happens at a radial distance r_0 from the center of the protein, such that $c_{\mathrm{L}}(r = r0) = 0$ solve for the steady state radial concentration profile of ligand, $c_{\mathrm{L}}(r)$. Here, r is the radial distance from the origin. We are assuming the protein is spherical. *Hint:* The Laplace operator ∇^2 in spherical polar coordinates is defined according to

$$\nabla^2 f = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial f}{\partial r}\right) + \frac{1}{r^2\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial f}{\partial\theta}\right)$$

$$+ \frac{1}{r^2 \sin^2 \theta} \frac{\partial^2 f}{\partial \phi^2}, \tag{8.9}$$

where θ is the polar angle and ϕ is the azimuthal angle.

ii) From your expression for $c_{\mathrm{L}}(r)$, write an expression for the radial flux of ligand, $j(r)$.

iii) Write an expression for the total number of ligands impinging on the protein per unit time.

iv) You have just computed the number of collisions with ligands per protein per unit time. Your expression should be in terms of r_0, D, and c_{L}^{∞}. Multiplying that expression by the protein concentration c_{P} will give the total number of collisions per unit time. From this, write down the frequency factor A.

d) Reactions that have very low activation energy proceed almost immediately upon collisions. Such reactions are termed **diffusion limited**. They are limited in their speed by how quickly diffusion can deliver the reactants to each other for reaction. Write down an expression for the diffusion limited rate constant for protein-ligand binding in terms of r_0, D_{protein} and D_{ligand}.