Problem Set No. 4

Due: Monday, 11/18/10 at the start of class

Objective: To understand the thermodynamic and kinetic laws governing membrane structure, transport, and function.

Review problems

You should pay special attention to these questions after reading. Note that the answers are given in the back of the book. Formulate your answers fully first and then check them. This can be a significant aid in your understanding of the material, and similar questions may be asked on the final. You do not need to provide written answers in the solutions you hand in.

- ECB 11-8
- ECB 11-9
- ECB 11-11
- ECB 12-9
- ECB 12-10
- ECB 12-11
- ECB 12-14

Problem 1

Consider the model of a random walk discussed in class,

$$\langle r_n^2 \rangle = nl^2 \quad \left(= \frac{t}{\tau} l^2 \right)$$

We can also use this basic approach to estimate the size of an unstructured long polymer like DNA. In this case, we model DNA as the *path* taken by a random walk. Instead of the walk taking a random step every small unit of time τ, we can think of the DNA strand as taking a new random direction (step) every m base pairs. The number of base pairs we have to traverse before DNA can take a random new direction is related to its flexibility, and is roughly 300 base pairs. The length of a DNA molecule is about 3.4 Å per base pair and the length of a rigid unit $3.4m$ Å. With this analogy, compute the root-mean-squared end-to-end distance, $\sqrt{\langle r^2 \rangle}$, of an unstructured DNA strand that is 50 million base pairs long. This number roughly corresponds to the human chromosome 19. Compare this to the length of the chromosome when condensed for cell division, $\sim 2\ \mu m$.

Problem 2

Glucose transport across the plasma membrane into the intracellular space has been identified as the limiting step in the delivery of this energy source to muscle cells. So-called GLUT membrane transporter proteins bind to and facilitate transport of glucose into the cell. In particular, impairment of this transport mechanism is strongly implicated in patients with type 2 diabetes and modern research efforts are attempting to understand how such impairment occurs [Shepherd and Kahn, New England Journal of Medicine 341, 248 (1999)].

a) Consider a simple facilitated model of glucose transport. Let extracellular and intracellular concentrations of glucose be given by $[G]_e$ and $[G]_i$, respectively. Let the total concentration of GLUT proteins in the plasma membrane be given by $[P]_0$. The mechanism of glucose transport might be given by

$$G_e + P \overset{K_M}{\leftrightarrow} GP \overset{k_2}{\rightarrow} G_i + P \qquad K_M = \frac{[G_e][P]}{[GP]}$$

At the same time intracellular glucose is consumed at a rate proportional to its concentration,

$$G_i \overset{k_3}{\rightarrow} \text{products}$$

Making rapid equilibrium assumptions as necessary, find the steady-state concentration $[G_i]$ as a function of $[G_e]$, $[P]_0$, and the rate coefficients and equilibrium constants defined above. Show that it can be put into the following form,

$$[G_i]_{SS} = \frac{c_{\max}[G_e]}{[G_e] + K_{M,\text{app}}}$$

b) This simple model neglects important known effects of the small protein insulin, thought to modulate glucose transport through the GLUT proteins. Diabetic patients develop a resistance to insulin's effects. One potential mechanism for the manner in which insulin interacts with transport is that its binding to GLUT is required. The mechanism above might be modified as,

$$P + I_e \overset{K_I}{\leftrightarrow} PI \qquad K_I = \frac{[P][I]}{[PI]}$$

$$G_e + PI \overset{K_M}{\leftrightarrow} GPI \overset{k_2}{\rightarrow} G_i + PI \qquad K_M = \frac{[G_e][PI]}{[GPI]}$$

where I_e is extracellular insulin. Show that $[G_i]_{SS}$ can also be put into the form above, but with potentially different expressions for $c_{\max}$ and $K_{M,\text{app}}$. (Consumption is also present here.)

c) An alternative mechanism for insulin is that it increases the concentration of GLUT in the membrane, for example, by regulating GLUT's expression and transport. An approximate relationship for this mechanism might be $[P]_0 \propto [I_e]$. Experiments have measured the steady-state concentration of glucose in muscle cells as a function of extracellular insulin concentration [Gottesman et al., J. Clinical Investigation 70, 1310 (1982)]. These reveal that $[G_i]_{SS}$ always increases linearly with $[I_e]$. According to your models in (a) and (b), then, which of these two mechanisms is more likely? Be sure to justify your answer.

Problem 3

You are an engineer at a pharmaceutical company designing a synthetic vesicle to be used as a carrier for delivering a therapeutic of interest to cells. The idea is that the vesicle will be loaded with the drug (water-solubilized) and injected into the bloodstream. You need to make sure that the vesicle will not rupture when stored in a buffer solution that has a total solute concentration of c_B. You have two variables that you can play with, the concentration of the drug inside the vesicles c_D (in moles per volume) and their size, given by radius r. Recall the osmotic pressure equation, $\Pi = RT\Delta c$, where Δc has units of moles per volume.

a) For the vesicles to remain intact, all of the pressures at the bilayer must balance. That is, the difference in pressure between the vesicle interior and exterior (buffer) must be equal to pressure exerted by the bilayer that resists swelling the vesicle. This latter pressure can be shown to be given by,

$$P = \frac{2\gamma}{r}$$

where γ is the surface tension of the bilayer. Find an expression for the minimum concentration of drug c_D that must be loaded into the vesicle to prevent lysis, as a function of its radius r. Are small or large vesicles more stable?

b) For delivery purposes, it is desirable that each vesicle contain a total of 10^{-18} moles of the therapeutic. If the size-dependent concentraton of therapeutic used is what you found in part (a), how big should your vesicles be? Quote your answer as the radius in nm. Assume typical values of $c_B \approx 100\ nM$, $T = 300\ K$, and $\gamma \approx 70\ mN/m^2$.

Problem 4

The Nernst equation describes the relationship between *equilibrium* concentrations of ionic species partitioned across a membrane with potential drop ΔV. That is, the concentrations of species if they could freely move between the interior and the exterior of the membrane.

$$\Delta V = -\frac{k_B T}{qe} \ln\left(\frac{c_i}{c_e}\right)$$

$$\approx -(60\ mV) \log_{10}\left(\frac{c_i}{c_e}\right) \quad \text{at } T = 300\ K \text{ for +1 charged ions}$$

$$\approx +(60\ mV) \log_{10}\left(\frac{c_i}{c_e}\right) \quad \text{at } T = 300\ K \text{ for -1 charged ions}$$

Consider a cell to be filled with four ionic species: Na+, K+, Cl- and negatively charged macromolecules like DNA and proteins. We will assume that the macromolecules are the only species that cannot cross the plasma membrane, while the small ions can by way of transmembrane channels. The total concentration of these macromolecules, in units of electron charge, is $c_{\text{i,macro}} = 125\ mM$. Outside of the cell, in the extracellular space, no macromolecules are present and the concentrations of the three small ions are approximately

$$c_{e,Na^+} = 145\ mM \qquad c_{e,K^+} = 5\ mM \qquad c_{e,Cl^-} = 150\ mM$$

Note that there is a net charge of zero in the extracellular space ($145 + 5 - 150 = 0$). We will ignore any other ions.

a) The net charge in the intracellular space must also be zero. If the intracellular concentrations also all obey the equilibrium Nernst relation for the same membrane potential, what will the potential ΔV be? Hint: write an equation demanding the net intracellular charge be zero, and then use the Nernst equation in it.

b) If the macromolecule concentration were zero, what would the membrane potential be? What would the concentration differences across the cell membrane look like at equilibrium?

c) In neurons, voltage-gated Na+ channels allow the rapid influx of sodium ions in response to the action potential, which rapidly changes the actual membrane potential from -60 mV to about 40 mV. If the exterior concentrations of the three ion species above remain the same, what would be the equilibrium intracellular concentration of K+ at this potential according to the Nernst relation? Compare this concentration of K+ to the average actual concentration of 140 mM. Would there therefore be a driving force for the influx or efflux of K+ from the cell, at this membrane potential ?

Problem 5

In class we discussed unfacilitated diffusive transport through the plasma membrane, arriving at an equation for the flux of a species (moles/area/time) given by

$$J = P(c_e - c_i)$$

where J gives the flux into the cell, P is the permeability of the species of interest, and c_e and c_i are extra- and intracellular concentrations of the species, respectively.

a) Show that, for a spherical cell of radius r_c, the concentration of a species inside the cell with time follows the relationship

$$\frac{dc_i}{dt} = \frac{3P}{r_c}(c_e - c_i)$$

Assume the interior of the cell is well mixed. Hint: consider the change in the total amount of the species inside the cell with time, which relates to cell volume and intracellular concentration.

b) Assume the extracellular concentration is kept constant. We can then define a new variable, $\Delta c \equiv c_e - c_i$ and rewrite the differential equation as

$$\frac{d\Delta c}{dt} = -\frac{3P}{r_c}\Delta c$$

Solve this differential equation to obtain Δc as a function of time, given an initial value of Δc_0 at $t = 0$. What is the behavior of Δc as $t \to \infty$?

c) For a spherical cell 10 μm in diameter, how long would it take the concentration of sodium ions inside the cell to reach 50% of the concentration outside of the cell, if all membrane transport processes were purely diffusive (i.e., no channels or transporters)? The extracellular concentration of sodium is 145 nM and its permeability is 10^{-12} cm/s.