BIOE 459/559: Cell Engineering
Membrane Permeability

References:

Water Movement Through Lipid Bilayers, Pores and Plasma Membranes. Theory and Reality, Alan Finkelstein, 1987
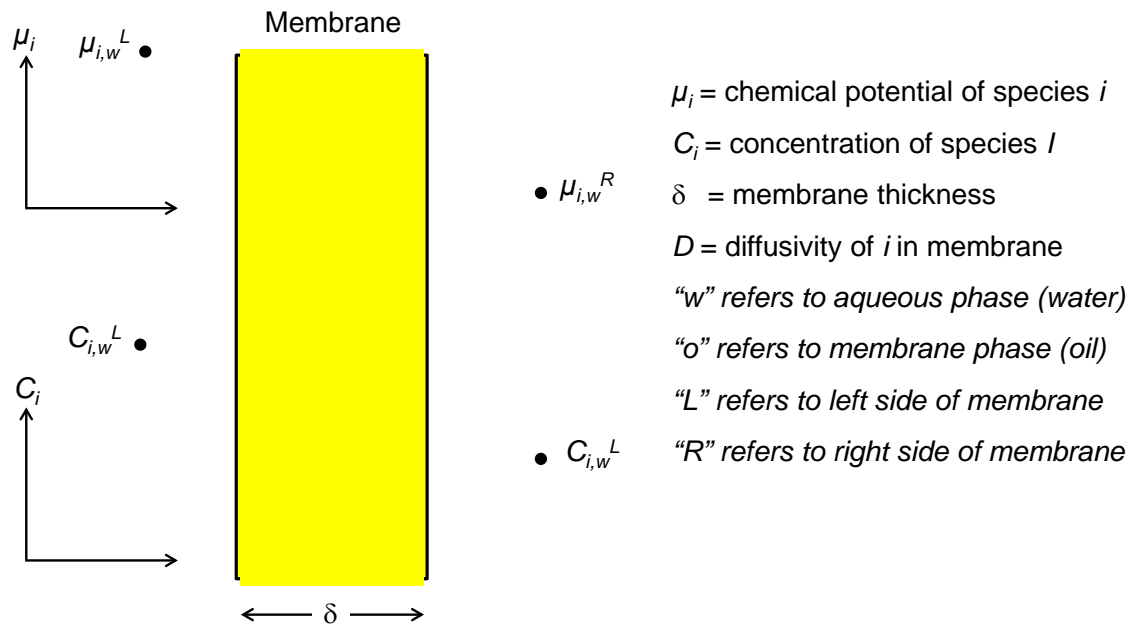*Membrane Permeability, 100 Years Since Ernest Overton*, Eds. DW Deamer, A Kleinzeller, DM Fambrough, 1999

Solubility-Diffusion Model of Membrane Transport:

The solubility-diffusion modeling approach breaks the transport of a molecule across the cell membrane into 3 steps: (1) movement from the hydrophilic aqueous environment outside the membrane to the hydrophobic environment within the membrane bilayer, (2) diffusion from one side of the membrane to the other, and (3) movement from the hydrophobic environment within the membrane to hydrophilic aqueous environment outside the membrane. We will make the following assumptions:
- Dilute, ideal solutions
- No pressure or temperature differences across membrane
- Chemical equilibrium at membrane edges
- Steady-state diffusion across membrane
- Well-mixed aqueous solutions on either side of membrane

Sketch the concentration and chemical potential profiles between the left and right sides of the cell membrane on the figure below.

μ_i = chemical potential of species i

C_i = concentration of species I

δ = membrane thickness

D = diffusivity of i in membrane

"w" refers to aqueous phase (water)

"o" refers to membrane phase (oil)

"L" refers to left side of membrane

"R" refers to right side of membrane

The molar flux of species i across the cell membrane, J_i, can be described using Fick's law:

$$J_i = \tag{1}$$

The partition coefficient K is the equilibrium constant between i in the aqueous phase and i in the membrane phase:

$$K = \frac{C_{i,o}}{C_{i,w}} \tag{2}$$

Assuming each edge of the membrane is in chemical equilibrium, the equation for the partition coefficient (Eq. 2) can be combined with the diffusion equation (Eq. 1), yielding:

$$J_i = \hspace{4cm} (3)$$

The membrane permeability coefficient for species i is defined as

$$P_i = \frac{DK}{\delta} \hspace{4cm} (4)$$

Plugging this definition into Eq. 3 results in

$$J_i = \hspace{4cm} (5)$$

Sketch the concentration and chemical potential profiles between the left and right sides of the cell membrane on the figure below (left) using the following values of K: 0.25, 0.5 and 1. Sketch a plot of the permeability as a function of K in the figure below (right).
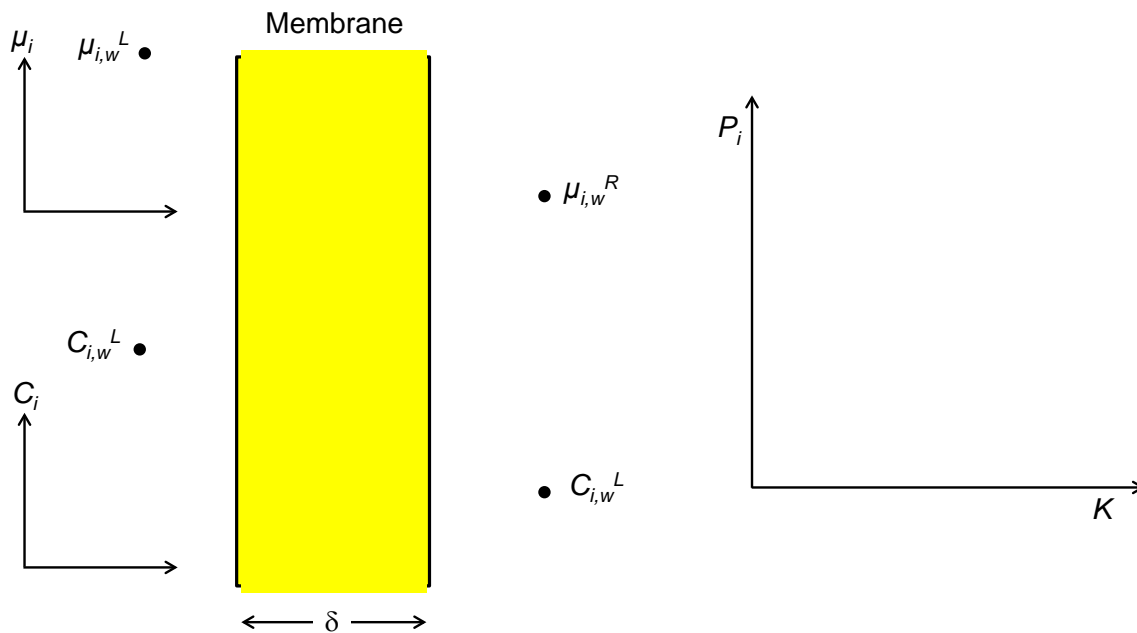
At equilibrium, the concentration of i is the same on both sides of the membrane. Sketch a plot of the equilibrium concentration and chemical potential profiles on the figure below.
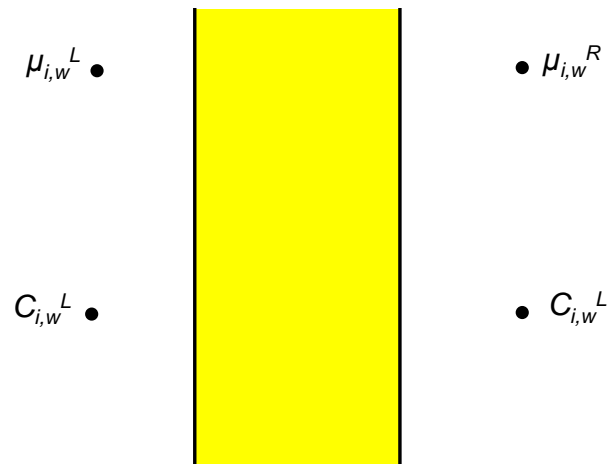
<u>Water Transport through the Membrane Bilayer</u>

The solubility-diffusion modeling approach can be applied to water transport across the cell membrane, yielding the following for the molar flux of water,

$$J_w = P_w(C_w^L - C_w^R) \tag{6}$$

where P_w is known as the filtration permeability coefficient, and C_w is the water concentration.

The original approach for modeling water transport across the cell membrane considered an osmotic pressure driving force (rather than a concentration driving force). With this approach, the volumetric flux J_w^* is assumed to be proportional to the osmotic pressure driving force:

$$J_w^* = -L_p(\pi^L - \pi^R) \tag{7}$$

where L_p is the hydraulic permeability coefficient. These two ways for modeling water transport can be reconciled as follows. The osmotic pressure for an ideal and dilute solution can be expressed as

$$\pi = RTC_s = RT(C - C_w) \tag{8}$$

where C_s is the total solute concentration, C is the total molar concentration (including both solutes and water). Substituting Eq. 8 into Eq. 7 and assuming the total molar concentration is constant yields
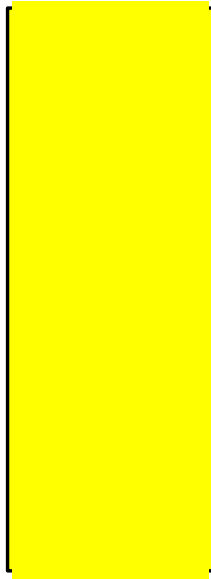
$$J_w^* = L_pRT(C_w^L - C_w^R) \tag{9}$$

To convert the volumetric flux J_w^* to the molar flux J_w we can use the molar volume of water v_w

$$J_w = \frac{J_w^*}{v_w} = \frac{L_pRT}{v_w}(C_w^L - C_w^R) \tag{10}$$

Therefore, $P_w =$

In the figure below, there is a concentration gradient driving flow of water from left to right. Sketch a plot of the water concentration and solute concentration profiles.

Water Transport through Membrane Pores

Many cell types have been found to express aquaporins, membrane pores that allow passage of water through the cell membrane. To model this process, we will assume that water can enter the pore, but all solutes are excluded. Draw the solutes and water molecules on the figure below, where the right side of the membrane has a higher solute concentration than the left.
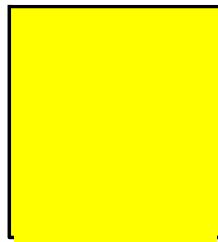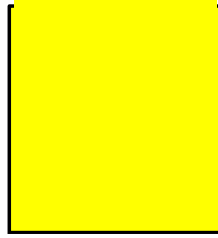
Because solute molecules cannot enter the pore, no concentration gradient exists inside the pore. Nonetheless, water will still flow to the right towards the side of the membrane with the higher solute concentration. What is the driving force for movement of water inside the pore?

We will assume chemical equilibrium between the aqueous solution at the pore edge and the pure water just inside the pore. At the pore edge, the chemical potential is (assuming an ideal solution)

$$\mu_{w,a} = \mu_w^0(P_0, T) + v_w(P_a - P_0) + RT \ln x_{w,a} \tag{11}$$

where P_a is the pressure in the aqueous solution at the pore edge, P_0 is the pressure at the reference state and $x_{w,a}$ is the mole fraction of water in the aqueous solution. Just inside the pore the chemical potential is

$$\mu_{w,p} = \mu_w^0(P_0, T) + v_w(P_p - P_0) + RT \ln x_{w,p} = \mu_w^0(P_0, T) + v_w(P_p - P_0) \tag{12}$$

where P_p is the pressure just inside the pore and $x_{w,p} = 1$ is the mole fraction of water inside the pore. Equating these chemical potentials yields

$$P_a - P_p = -\frac{RT}{v_w} \ln x_{w,a} \tag{13}$$

This equation can be applied at the left and right sides of the cell membrane, allowing us to determine the pressure difference driving water flow through the pore. Assuming that the pressure in the aqueous solution (outside the pore) is the same on both sides of the membrane (a good assumption for mammalian cells), we get

$$P_p^L - P_p^R = \frac{RT}{v_w} \left(\ln x_{w,a}^L - \ln x_{w,a}^R \right) \tag{14}$$

If we assume a dilute solution, we can use a linear approximation for the natural logs, which yields

$$P_p^L - P_p^R = \frac{RT}{v_w} \left(\frac{C_{w,a}^L}{C} - \frac{C_{w,a}^R}{C} \right) \tag{15}$$

where C is the total molar concentration. For a dilute aqueous solution $C = 1/v_w$, hence

$$P_p^L - P_p^R = RT\left(C_{w,a}^L - C_{w,a}^R\right) \tag{16}$$

This equation tells us that a difference in water concentration at the pore edges leads to a pressure gradient within the pore. Sketch a plot of the chemical potential, water concentration and pressure profiles on the figure below.

$\mu_{w,a}^L \, \bullet$

$\bullet \, \mu_{w,a}^R$

$C_{w,a}^L \, \bullet$

$\bullet \, C_{w,a}^R$

$P_a^L \, \bullet$

$\bullet \, P_a^R$
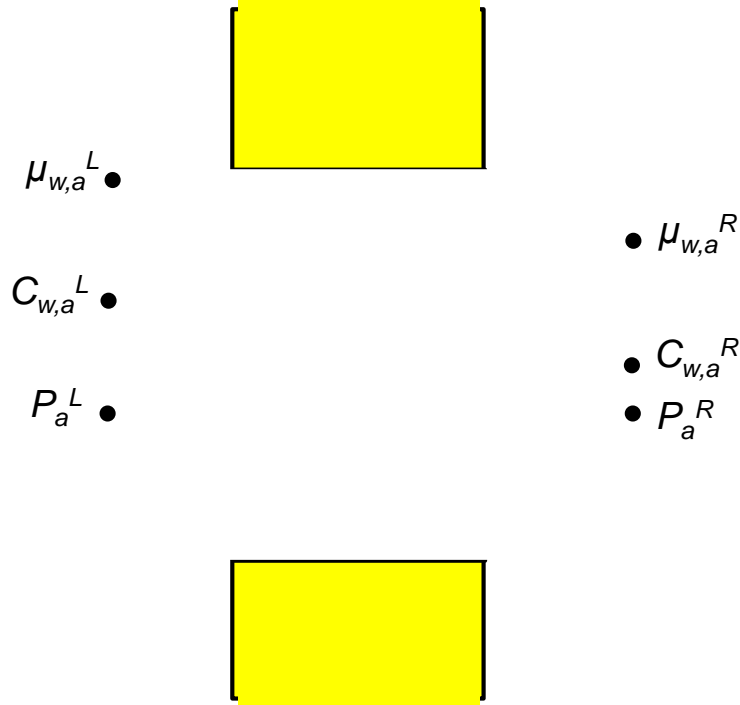
The pressure gradient drives flow of water through the pore by two different mechanisms: bulk flow and diffusion. Bulk flow can be modeled using the Hagen-Poiseuille equation for laminar flow through a cylindrical tube. The molar flux of water through the pore due to bulk flow is

$$J_{bf} = \frac{Q}{v_w \pi r^2} = \frac{\left(P_p^L - P_p^R\right) r^2}{8\eta\delta v_w} \tag{17}$$

where Q is the volumetric flow rate through the pore, r is the pore radius, η is the viscosity of water and δ is the pore length. The molar flux of water due to diffusion is

$$J_d = -\frac{D_w}{RT} C_w \frac{d\mu_w}{dx} = -\frac{D_w}{RT} C_w v_w \frac{dP_p}{dx} = \frac{D_w}{RT}\left(\frac{P_p^L - P_p^R}{\delta}\right) \tag{18}$$

where D_w is the self-diffusion coefficient of water. The total molar flux of water through the pore is equal to the sum of fluxes due to bulk flow and diffusion

$$J_{w,p} = J_{bf} + J_d = \left(\frac{P_p^L - P_p^R}{\delta}\right)\left(\frac{r^2}{8\eta v_w} + \frac{D_w}{RT}\right) \tag{19}$$

Substituting Eq. 16 into this equation yields

$$J_{w,p} = \left(C_{w,a}^L - C_{w,a}^R\right)\left(\frac{r^2 RT}{8\eta v_w \delta} + \frac{D_w}{\delta}\right) \tag{20}$$

To get the flux of water molecules across the entire cell membrane, J_w, we multiply $J_{w,p}$ by the total pore cross sectional area, $n\pi r^2$ (where n is the total number of pores in the cell membrane) and then divide by the total area of the cell membrane, A.

$$J_w = \frac{J_{w,p}(n\pi r^2)}{A} = P_w(C_w^L - C_w^R) \tag{21}$$

The equation above shows that in the case of water transport through pores, the filtration permeability coefficient P_w is equal to

$$P_w = \left(\frac{r^2 RT}{8\eta v_w \delta} + \frac{D_w}{\delta}\right)\left(\frac{n\pi r^2}{A}\right) \tag{22}$$

Simultaneous Water Transport through Pores and the Bilayer

If we want to consider water transport through pores and water transport through the membrane bilayer simultaneously, we need to include both mechanisms in our expression for the molar flux of water

$$J_w = \left[\frac{DK}{\delta} + \left(\frac{r^2 RT}{8\eta v_w \delta} + \frac{D_w}{\delta}\right)\left(\frac{n\pi r^2}{A}\right)\right](C_w^L - C_w^R) \tag{23}$$

In this case the filtration permeability coefficient would be

$$P_w = \frac{DK}{\delta} + \left(\frac{r^2 RT}{8\eta v_w \delta} + \frac{D_w}{\delta}\right)\left(\frac{n\pi r^2}{A}\right) \tag{24}$$

## Measurement of Cell Membrane Water Permeability

The water permeability is typically determined by fitting a membrane transport model to measurements of the change in cell volume after exposure to hypo- or hypertonic solutions. If water is the only substance that crosses the cell membrane (which occurs if all solutes can be considered impermeable), then the change in cell volume can be described by

$$\frac{dV_{cell}}{dt} = -L_p A\left(\pi^e - \pi^i\right) \tag{25}$$

where the superscripts "i" and "e" refer to the intracellular and extracellular solutions, respectively. Assuming ideal and dilute solutions, the above equation can be written

$$\frac{dV_{cell}}{dt} = L_p ART\left(C_s^i - C_s^e\right) \tag{26}$$

Sketch a plot of cell volume as a function of time after exposure to hypotonic and hypertonic solutions.
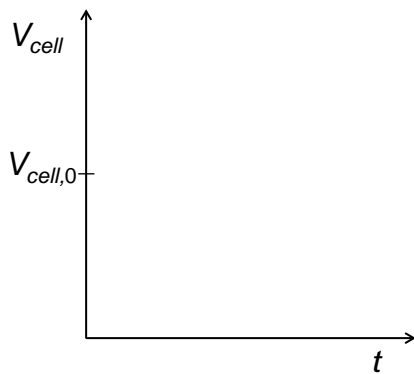
To solve Eq. 26, we need to find an expression for how the cell volume and the intracellular solute concentration are related. The cell volume V_{cell} is typically divided into two compartments, the osmotically inactive volume, V_b, and the cell water volume V_w. The osmotically inactive volume consists of intracellular macromolecules, lipids and other solutes. The following picture shows a cell under isotonic conditions, where the solute concentration is $C_{s,0} = 0.3$ osmoles/L.

$V_{cell,0} =$

moles of intracellular solute =
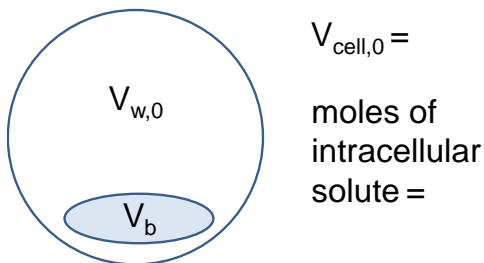
If the cell membrane is not permeable to solutes, then the total moles of intracellular solute remain constant. This leads to the Boyle-van't Hoff expression, which relates the intracellular solute concentration to the cell volume:

$$C_s^i = \frac{C_{s,0}\left(V_{cell,0} - V_b\right)}{V_{cell} - V_b} \tag{27}$$

Substituting this equation into Eq. 26 yields

$$\frac{dV_{cell}}{dt} = L_p ART\left(\frac{C_{s,0}\left(V_{cell,0} - V_b\right)}{V_{cell} - V_b} - C_s^e\right) \tag{28}$$

In experiments to determine the membrane permeability, the extracellular concentration is changed and the resulting change in cell volume is measured. The cell volume data is then fit with Eq. 28 to determine the hydraulic permeability coefficient L_p. Equation 28 contains several cell-specific parameters, including the cell surface area A, the isotonic cell volume $V_{cell,0}$, the osmotically inactive volume V_b and the hydraulic permeability coefficient L_p. Before fitting Eq. 28 to cell volume data to determine the permeability, it is first necessary to determine the values of the other cell specific parameters (i.e., A, $V_{cell,0}$ and V_b). The isotonic cell volume is simply determined by measuring the cell volume under isotonic conditions, and the cell surface area is commonly assumed to be equal to the area of a sphere with the isotonic volume. To determine V_b, the equilibrium cell volume is measured in several different hypo- and hypertonic solutions and the resulting data are fit with the Boyle-van't Hoff equation:

$$V_{cell} = V_b + \frac{C_{s,0}}{C_s^e}\left(V_{cell,0} - V_b\right) \tag{29}$$

Sketch the equilibrium cell volume on the following plot
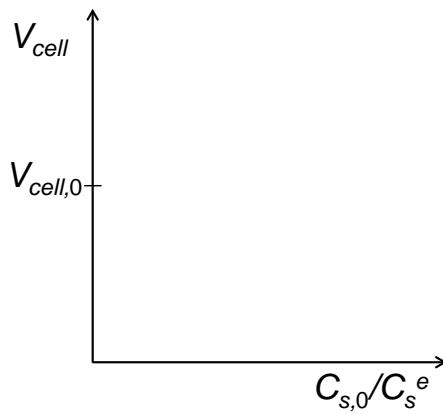
Membrane Transport of Water and a Single Permeating Solute

When water and a permeating solute (e.g., glycerol) can both cross the cell membrane, the membrane transport model is a bit more complicated. In such a case, the total cell volume (V_{cell}) consists of the volume occupied by water (V_w), the volume occupied by permeating solute (V_p) and the osmotically inactive volume (V_b). The rate at which the water volume changes is

$$\frac{dV_w}{dt} = L_p ART \left(C_s^i - C_s^e \right) = L_p ART \left(\frac{C_{s,0} \left(V_{cell,0} - V_b \right) + n_p^i}{V_w} - C_s^e \right) \tag{30}$$

where $n_p{}^i$ is the moles of intracellular permeating solute. The rate at which the moles of intracellular permeating solute changes is

$$\frac{dn_p}{dt} = P_p A \left(C_p^e - C_p^i \right) = \tag{31}$$

Equations 30 and 31 are coupled, in the sense that it is impossible to solve one without also solving the other. This is the case because both differential equations contain the dependent variables V_w and $n_p{}^i$. After solving Eqs. 30 and 31, the total cell volume can be computed as follows

$$V_{cell} = V_w + v_p n_p^i + V_b \tag{32}$$

Where v_p is the molar volume of the permeating solute. In experiments to determine the membrane permeability to water and permeating solute, cells are exposed to solution containing permeating solute and the resulting change in cell volume is measured. The cell volume data is then fit with Eqs. 30-31 to determine the hydraulic permeability coefficient L_p and the permeability of the permeating solute P_p. As with experiments for measuring the water permeability, it is first necessary to determine $V_{cell,0}$, V_b and A from equilibrium cell volume measurements.

Imagine a cell that is initially in isotonic saline solution, and then exposed to a hypertonic solution containing permeating solute at $t = 0$. Assuming that transport of both water and permeating solute occurs by diffusion through the membrane bilayer, sketch a plot of the concentration profiles C_w and C_p just after the cells are exposed to the hypertonic solution. To the right, draw a plot of the expected change in cell volume with time, assuming that water transport is faster than transport of permeating solute.