Membrane Permeability

A. Permeability If you take a pure solution of phospholipids and shake it hard in a test tube, or alternatively, place the lipids in an organic solvent and allow the latter to evaporate, you can produce artificial vesicles with a pure lipid bilayer, called liposomes. The dimensions of the liposomes depend on the way in which they are made. Liposomes have been used to test the permeability of lipid bilayers to various compounds. For example, to test their permeability to Na\(^+\), the liposomes are placed in a solution of Na\(^+\) for a certain amount of time, isolated by, for example, gel filtration, and then assayed for how much Na\(^+\) has been able to pass through to the interior of the liposome. The flux (the amount of Na\(^+\) entering per time) is given by:

\[
J \text{ (in moles/cm}\,^2\text{s}) = -D^* \frac{dc}{dx}
\]

This can be approximated to

\[
J = -\frac{D}{d} * (\text{conc}_{\text{out}} - \text{conc}_{\text{in}})
\]

where D is the diffusion coefficient, \(dc/dx\) is the gradient between the inside and outside of the liposome and d is the thickness of the bilayer. D/d is also known as the permeability coefficient, P, and is a measure of how fast a molecule diffuses, in cm/s. Thus, the equation can be rewritten as

\[
J = P \Delta c
\]

Water can cross the bilayer with P = 10\(^{-3}\); although this is faster than ions, it is still fairly slow due to the fact that the concentration of water is much lower inside the bilayer than on the outside and inside of the liposome (on the order of mM vs 55 M.) In general, the lower the concentration of a given molecule inside the bilayer, the harder it is for that molecule to get across. In other words, the permeability of a compound is a reflection of how soluble that compound is in lipid. Some sample permeability coefficients (column 1) illustrate this point. (See also Alberts p. 509, Fig. 11-2).

<table>
<thead>
<tr>
<th>Substance</th>
<th>P, cm/sec</th>
<th>Pd=D(_{\text{app}}) (cm(^2)/s)</th>
<th>(D_{\text{H2O}})</th>
<th>(\beta = D_{\text{app}} / D_{\text{H2O}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>10(^{-3})</td>
<td>5 x 10(^{-10})</td>
<td>2 x 10(^{-5})</td>
<td>2.5 x 10(^{-5})</td>
</tr>
<tr>
<td>glucose</td>
<td>10(^{-8})</td>
<td>5 x 10(^{-15})</td>
<td>5 x 10(^{-6})</td>
<td>5 x 10(^{-9})</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>10(^{-10})</td>
<td>5 x 10(^{-17})</td>
<td>5 x 10(^{-5})</td>
<td>5 x 10(^{-12})</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>10(^{-14})</td>
<td>5 x 10(^{-21})</td>
<td>5 x 10(^{-5})</td>
<td>5 x 10(^{-16})</td>
</tr>
</tbody>
</table>

Since we estimate that the thickness of the lipid bilayer is 5 nm, we can calculate the diffusion coefficient, D\(_{\text{app}}\), of these molecules across the liposome (2\(^{\text{nd}}\) column). If we then compare this number with \(D_{\text{H2O}}\), the diffusion coefficient in water, (column 3) we can see the difference in solubility of a chemical species in lipids vs. in water (column 4).

Thus, charged species have almost no chance of getting across the membrane by diffusion. The lipid bilayer is a nearly perfect insulator - only water and gases can get through.
So how does a cell transport ions across its membranes? In order for anything else to get in or out, carriers are required.

Imagine a liposome loaded with 100 mM KCl, which is now placed in a solution of 5mM KCl and 190 mM sucrose (to maintain osmotic balance). If the K+ ions on the outside solution is labelled, will we find any inside the liposomes over time?

As we might predict from the behaviour of Na+ and Cl-, the permeability to K+ is also extremely low, and so we expect very little to traverse the lipid bilayer. However, if the drug valinomycin is added to the solution, a net efflux of K+ is observed, and eventually isotopic equilibrium is achieved.

Valinomycin is an ionophore of fungal origin. The structure of valinomycin is shown in V&V p. 518, Fig. 18-7 and 18-8. It has a cyclic arrangement wherein 6 carbonyl atoms point towards the interior of the molecule, and can coordinate a K+ ion (but not the smaller Na+ ion). Its hydrophobic exterior makes it soluble in the lipid bilayer. Thus, it can selectively transport K+ ions across the membrane.

Initially, there was a balance of charge inside and outside the liposome. However, in the presence of valinomycin, a membrane potential will be established, because K will be transported out of the liposome down its concentration gradient while the liposome remains essentially impermeable to Cl- ions.

The chemical potential of this can be represented as:

\[
\mu_i = \mu_i^\circ + RT \ln K_i + zF \psi_i \\
\mu_o = \mu_o^\circ + RT \ln K_o + zF \psi_o
\]

where  
F = Faraday’s constant = -23kcal/V. mol 
\(z\) = the charge of the ion (no units)

\[
\Delta G = \Sigma \gamma_i (\mu_i - \mu_i^\circ) + RT \ln (K_o / K_i) + zF (\psi_o - \psi_i)
\]

Since the standard state values, \(\mu_o^\circ \) and \(\mu_i^\circ \) are the same,

\[
\Delta G = RT \ln (K_o / K_i) + zF (\psi_o - \psi_i)
\]

At equilibrium, \(\Delta G = 0\). Rearrangement of the terms gives us the Nernst equation (see also MBOC p. 526):
\[(\psi_o - \psi_i) = -\frac{RT}{zF} \ln \left(\frac{K_o}{K_i}\right) = -\frac{RT}{zF} \ln K_{eq}\]

(\(K_{eq} = \frac{K_o}{K_i}\) since the movement is from the inside to outside, i.e. \(K_i \rightarrow K_o\)). Thus, we can see that the membrane potential is proportional to the concentration of ions outside vs. inside the cell or membrane.

Returning to our initial situation of the liposome, we can calculate the membrane potential as:

\[(\psi_o - \psi_i) = -\frac{RT}{zF} \ln \left(\frac{K_o}{K_i}\right) = -60\text{mV} \log \left(\frac{5}{100}\right) = 78\text{mV}\]

that is to say, if the inside is zero, then outside is +78 mV. K\(^+\) ions contributes significantly to the membrane potential of cells. As a consequence of the high internal K\(^+\) concentration of all cells (about 150 mM), the membrane potential is positive (negative on the inside).

How many molecules can actually create a membrane potential? We can calculate this by first imagining the cell membrane as a capacitor, with a capacitance of 1\(\mu\)F/cm. For a liposome of 1\(\mu\)m diameter, only \(1 \times 10^4\) molecules need to leak out to create a membrane potential. This number therefore does not significantly alter the internal vs. external ion concentration. (see MBOC p. 527, Fig. 11-19).

**B. Membrane proteins.**

So how do ions and other substance pass through the membrane? Many transmembrane proteins exist, that form an aqueous channel in the lipid bilayer. How does a protein cross the membrane, if membranes are nonpolar and peptides are polar? It turns out that almost all protein stretches that cross the membrane are alpha-helical. These alpha-helical regions are composed of amino acids with nonpolar side chains, and it is common practice to predict that a long stretch of hydrophobic residues in a protein sequence is likely to represent a transmembrane domain. Kyte-Doolittle plots, which plot the relative hydrophobicity of a given residue versus its number in the amino acid sequence, can give a first-glance view of likely transmembrane domains; for example, for the 260 amino acid bacteriorhodopsin, seven 20-30 amino acid stretches were found (see plot in MBOC p. 488, Fig. 10-16). Using the known parameters of alpha helices, the total of 20-30 amino acids can be converted to a helical rise of 3 nm, which corresponds to the length of the hydrophobic part of the lipid bilayer. In the next lecture, we will talk about 2 specific kinds of transmembrane proteins.