Suggested problems from the end of chapter 9: 2,4,5,7,8,9,10,12,13,15,19,20,21.

Lipid structures

Addition of small amounts of lipids to an aqueous solution results in formation of a monolayer at the air-solution interface.

Further addition of lipids results in the formation of micelles, with the polar groups pointing towards the solution and the hydrophobic tails packed together to exclude water.
Micelle formation by detergents

- Detergents are reagents with both hydrophobic and hydrophilic moieties.
- Detergents can solubilize hydrophobic molecules in aqueous solutions by interfering with the hydrophobic interactions that predominate in micelle formation. Detergents also can solubilize membranes by inserting within the membranes and interfering with the hydrophobic packing interactions that hold membranes together.
- Nevertheless, detergents also can form micelles on their own due to interactions among their hydrophobic groups. The concentration at which a molecule forms micelles is the Critical Micelle Concentration (CMC).

<table>
<thead>
<tr>
<th>Structure</th>
<th>M&lt;sub&gt;r&lt;/sub&gt;</th>
<th>CMC</th>
<th>Micelle M&lt;sub&gt;r&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>625</td>
<td>0.24 mM</td>
<td>90-95,000</td>
</tr>
<tr>
<td>Octylglucoside</td>
<td>292</td>
<td>25 mM</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;ES (Dodecyl octaethylene ether)</td>
<td>530</td>
<td>0.071 mM</td>
<td></td>
</tr>
</tbody>
</table>

Lipid Bilayers

Why do bilayers form?

Spheroid shells result from the tapered van der waal “envelops” of the fatty acids. The diameter of micelle depends on the length of the fatty acids tails. As the volume of the micelle expands, water accumulates inside. This is not a stable arrangement and the micelle collapses. As the micelle collapses into ellipsoid shapes, water is maintained in some spaces but not others.
The lipids that make up biological membranes typically have two extended fatty acid moieties. The van der waal "cylinder" associated with these lipids results in the formation of extended micelles. These can be described as lipid bilayers.

An electron micrograph of a liposome. Liposomes can form when glycerophospholipids or sphingomyelins are allowed to associate after being heated in an aqueous solution. The vesicle is filled with solution, and a lipid bilayer forms a membrane around the solution.
Lateral diffusion is very fast, an estimated 1 \( \mu \text{meter/s} \) within a bacterial cell membrane. Rotations about the C-C bonds of the carbon tails are rapid, and the viscosity of the solution near the non-polar tails is very low. In contrast, the viscosity increases near the polar groups due to their limited mobility. The outer areas just outwards from the polar groups are surrounded by water molecules and have a high viscosity in comparison to the viscosity of the solution near the hydrophobic tails.

The lipid tails interdigitate and fill gaps in the solution. These gaps are created by tails of different lengths and kinked tails that result from the inclusion of double bonds in the hydrocarbon chains.

See lipid bilayer worksheet.
The fluid mosaic lipid bilayer model of biological membranes

S.J. Singer and G.L. Nicholson proposed the fluid mosaic model of biological membranes in 1972. The model argued that the lipid bilayer is essentially a solvent for proteins whose function required them to exist in membranes. Two classes of proteins were proposed:
1. Integral membrane proteins are associated with the hydrophobic portions of the membrane. A detergent was required to dissociate the proteins from the membrane.
2. Peripheral membrane proteins are associated with the polar groups of the lipids, most likely through electrostatic interactions. These proteins can be removed from the membrane without disrupting membrane structure. A high salt treatment normally removes these proteins from the membranes.

This model was superseded by the membrane raft model.

Lateral diffusion of proteins within membranes

The photobleaching recovery approach:
• Label an integral membrane protein with a fluorophore.
• Photobleach a small segment of the membrane.
• Measure the time required to recover the full signal. The signal should increase as a function of the migration of unbleached protein into the assayed membrane segment.
Integral membrane proteins are asymmetrically oriented amphiphiles

Amphiphiles are molecules that contain hydrophobic as well as hydrophilic moieties. Integral membrane proteins have hydrophobic regions that are imbedded in the membrane. The polar groups of the integral membrane proteins are exposed to the cytosol or extracellular aqueous solutions. Modified groups occur almost exclusively on the extracellular side of the membrane.

This illustrates the different parts of human erythrocyte glycoporin A, a channel forming molecule in red blood cells.

Glycoproteins

Glycoproteins contain carbohydrate residues covalently linked to the amino acids side chains. There are two main groups of linkages:

1. O-linked saccharides are linked to the hydroxyl groups of threonine, serine, or hydroxyserine.

![Diagram of glycoprotein structures]
2. N-linked saccharides are bound to the amide nitrogen of asparagine.

There are normally two N-acetylg glucosamine linked to a branched manose structure. In addition, there are more complex saccharide structures linked to the mannose residues: high mannose, complex, and hybrid.
Glycoproteins function in protecting the blood of arctic and antarctic fish from freezing in temperatures as low as -1.9°C. The saccharides are O-linked to threonine residues. The threonine residues are a part of an Ala-Ala-Thr sequence that can be repeated up to 50 times. The mechanism is not clear, but the repeated sugar units may bind the ends of ice crystals and prevent further crystal growth. These residues do not seem to interfere with nucleation of water crystals.

Glycoproteins - role of sugar residues in protein degradation

• Sialic acid residues are added to proteins via N-linked saccharides. Slowly, the sialic acid residues are cleaved by enzymes bound to arterial walls.
• This results in exposure of the galactose residues, which can then be bound tightly by a specific receptor in the liver.
• The receptor-bound glycoprotein is endocytosed and degraded by liver lysosomes.

*How would this degradation pathway be advantageous to the organism?
Proteoglycans

Proteoglycans are proteins with serine- O-linked glycosaminoglycans

<table>
<thead>
<tr>
<th>Hyaluronic acid</th>
<th>Chondroitin-4-sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Hyaluronic acid" /></td>
<td><img src="image" alt="Chondroitin-4-sulfate" /></td>
</tr>
</tbody>
</table>

Proteoglycans

- Proteoglycans are found in extracellular spaces, where the saccharide moieties increase the viscosity of the solution.
- Hyaluronic acid solutions have a high viscosity when put under low shear stress (an object under shear stress has equal and opposite forces applied across opposite faces of the object). Under these conditions hyaluronic acid molecules assumes an unordered, tangled confirmation. This results in a high solution viscosity.
- Under high shear stress conditions, hyaluronic acid molecules assume an ordered confirmation that decreases solution viscosity.
- This behavior is characteristic of solutions with high elasticity. As such, these solutions are useful in bathing joints that experience routine high and low shear stresses, such as knees, ankles, and shoulders. The fluids are called synovial fluids. Hyaluronic acid also is found in the vitreous humoral fluid of the eye.
Proteoglycans
Proteoglycans are found in cartilage, which also contains collagen.
To the right is an electron micrograph of hyaluronic acid structure from cartilage. The central strand is the hyaluronic acid chain, and the projections on the side are proteins called core protein.
The core protein has, in addition to O-linked hyaluronic acid, N-linked oligosaccharides.
On the basis of such electron microscopy, proteoglycans structures within cartilage have been proposed (below).

Integral membrane proteins
The trans-membrane domains SOMETIMES can be predicted on the basis of primary amino acid sequence. One can calculate the additive hydropathy of a continuous chain, or the free energy of hydration of the first residue in a putative $\alpha$ helical structure.
The stability of individual amino acids in membranes can be calculated

The graphs show relative stabilization energies as a function of location in the membrane for
(a) Arg, Asp, Glu, Lys, Asn, Gln, and Pro;
(b) Ala, Gly, Ile, Leu, Met, Phe, and Val;
(c) His, Tyr, and Trp

Integral membrane proteins

• Bacteriorhodopsin is an integral membrane protein whose function is to maintain a proton gradient across a membrane. The H⁺ gradient is used to produce ATP.

• Such bacterial membranes contain purple patches of 75% bacteriorhodopsin and 25% lipids. The protein is arranged in a 2 dimensional crystal. This arrangement was used by R. Henderson and N. Unwin to solve the structure by measuring the x-ray diffraction pattern of the two-dimensional crystal.

• The protein contains 7 α helices of about 25 residues each. Each helix is almost perpendicular to the membrane plane.

• The helices are hydrophobic, whereas the connecting loops are hydrophilic.
Integral membrane proteins - structure of bacteriorhodopsin

• Note the 7 transmembrane helices. The helices are hydrophobic, whereas the connecting loops are hydrophilic.
• The structure is shown together with a ball and stick model of retinal.
Retinal is the light absorbing group that becomes covalently linked to Lysine 216 of rhodopsin in organisms capable of vision.
• During absorption of light, 11-cis-retinal is converted to 11-trans-retinal, which cannot absorb visible light. This results, eventually, in transmission of light signal and "seeing."
• 11-trans-retinal isomerase converts the trans form to cis, permitting renewed sensing of light.

Integral membrane proteins

• The photosynthetic reaction center of purple bacteria is a three subunit enzyme of ~300 residues each.
• Together the complex binds 4 chlorophyll molecules, 4 other chromophores, and an iron atom.
• The protein complex contains 11 transmembrane α helices that form a cylinder 45 Å long.
• The three subunits are in blue, pink, and brown.
• The light absorbing groups are in yellow.
• Cytochrome C protein is bound to the complex at the extracellular face.
• The membrane is modeled on the basis of hydrophobic group exposure to the outside of the protein complex.
Integral membrane proteins

The purple bacteria photosynthetic reaction center structure. Nitrogen is in blue, oxygen in red, sulfur in yellow, and carbon in white. The section of the complex that is thought to be imbedded in the membrane is hydrophobic, exposing a very small amount of oxygen and nitrogen atoms.

Integral membrane proteins - porins

• The porin group of proteins are channel-forming proteins in gram-negative bacteria. Porins permit small hydrophilic solutes to enter the bacteria.

• Mitochondria and chloroplasts also contain porins that serve in the same capacity. Bacterial porins are monomers or trimers of identical subunits.

• Each 30 to 50 kDa subunits makes an antiparallel β barrel about 55Å long and 7Å wide.

• The trimer is encircled by a 27Å long hydrophobic stretch.

• The channel is "lined" with hydrophilic groups.
Integral membrane proteins - porins

Bacterial porin trimer, top view.

Integral membrane proteins - porins

Hydrophobicity of the membrane-exposed region of the porin trimer. Carbon atoms are in yellow or white, oxygen atoms are red, and nitrogen atoms are blue.
Lipid modifications of proteins

• The C₁₅ farnesyl groups or C₂₀ geranylgeranyl groups can be linked to a cysteine sulfur. Proteins become modified via specific enzymatic reactions. A common sequence for lipid modification is cys-X-X-Y, where Y can be alanine, methionine, serine, or leucine. Following the modification, the X-X-Y tail is cleaved (see Figure 9.23).

• Palmitoylation modification also occurs at a cysteine sulfur. The modification can be reversed via palmitoyl thioesterase and the modified protein is usually found at the cytoplasmic face of the membrane.

• Myristoylation is a form of lipid modification via an amide bond to the \( \alpha \) carbon of an N terminus glycine residue.

• In comparison to an integral membrane protein, what is the expected diffusion coefficient of a lipid-modified peripheral protein? On the basis of your answer, speculate why prenylation, myristoylation, and palmitoylation modifications occur on proteins that often are involved in signal transduction.

Modes of transport: simple diffusion, facilitated diffusion, and active transport

• The flipase enzymes catalyze the exchange of lipids form the cytoplasmic face to the extracellular face of the membrane. This is a form of facilitated diffusion because the concentration of lipids on the cytoplasmic side is higher, so the transfer occurs down an electrochemical gradient.

• Other enzymes can catalyze the transfer of specific lipids across a bilayer against an electrochemical gradient. This form of translocation is called active transport, and ATP or other sources of energy can be used to carry out this reaction.

• A third form of transport across membrane is simple diffusion. Here the molecules move across the membrane down an electrochemical gradient.

See transport across membranes worksheet
Flux of solutes across membranes

The flux, $J$, of solute $A$ is the rate of transport in a defined area. It is defined by

$$J_A = D_A \frac{d[A]}{dx}$$

$D_A$ is the diffusion coefficient of $A$. $\frac{d[A]}{dx}$ is the change in concentration of $A$ per distance unit.

This means that a substance diffuses in the direction that eliminates its concentration gradient, at a rate proportional to the magnitude of the gradient (this is Fick’s first law of diffusion).

The permeability coefficient, $P$, for a solute $A$, is defined by $P_A = \frac{D_A}{x}$.

The permeability coefficient $P_A$ is the tendency of solute $A$ to penetrate the non-polar membrane core. This also is sometimes called the partition coefficient, which describes the ratio of the solubility in a non-polar solvent to the solubility in a polar solvent (here, solubility in the internal portion of the membrane divided by the solubility in water).

For a membrane with a thickness of $x$, the above flux equation simplifies to

$$J_A = \frac{D_A}{x} ([A]_{out} - [A]_{in}) = P_A ([A]_{out} - [A]_{in})$$

If you plot the flux versus the concentration difference across the membrane, and obtain a straight line, what variable would you be able to calculate?

Facilitated transport

Sometimes the flux of solutes across membranes does not vary linearly with the concentration differences across the membrane. In this case there must be a system that transports these solutes across the membrane.

An example is glucose transport across the erythrocyte membrane.

This is characterized by:

• Speed and specificity
• Saturation kinetics
• Susceptibility to competitive inhibition
• Susceptibility to chemical inhibition

1. Speed and specificity. The ability of a solute to cross the membrane faster than would be predicted by the partition coefficient. This must be specific to the solute. For example, glucose vs. mannose.

2. Saturation kinetics. The plot of solute concentration vs. flux must be saturable.

3. Inhibition by Competition. A molecule can compete with the solute for the transporting channel. For example, 6-benzyl-D-galactose.

4. Inhibition by modification. A protein-specific chemical reagent can inhibit the transporting channel. For example, treatment of erythrocytes with iodoacetate inactivates the facilitated diffusion of glucose.
Facilitated transport

Schematic diagram showing a possible mechanism for the facilitated transport of glucose. Note that there is at least 2 conformational changes (binding-competent to dissociating).

Active transport

Uniport

Symport

Antiport
Active transport - the sodium-potassium pump

The Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pumps sodium out of the cell and potassium into the cell. The stoichiometry is 3Na\textsuperscript{+}(in) + 2K\textsuperscript{+}(out) + ATP + H\textsubscript{2}O ⇔ 3Na\textsuperscript{+}(out) + 2K\textsuperscript{+}(in) + ADP + P\textsubscript{i}

The proposed mechanism has 4 functional states for the protein. A key step is a Na\textsuperscript{+} dependent phosphorylation of the protein pump. Why is this advantageous? Note that there are 2 conformational changes during each cycle, and these are essential if this mechanism is correct.

Transport across membranes (1)

1. The free energy of a solute varies with its concentration and is given by

$$\bar{G}_A - \bar{G}_A^o = RT \ln[A]$$

where $\bar{G}_A$ is the chemical potential of A, or the free energy of A per mole, and $\bar{G}_A^o$ is the free energy of A in its standard state.

2. If there is a difference in the concentrations of A on either side of the membrane, then there is a chemical potential difference across the membrane, which is given by

$$\Delta \bar{G}_A = \bar{G}_A(\text{in}) - \bar{G}_A(\text{out}) = RT \ln \left( \frac{[A]_{\text{in}}}{[A]_{\text{out}}} \right)$$

for the equilibrium A (out) ⇔ A (in)

which describes the import reaction.
Transport across membranes (2)

\[ \Delta \bar{G}_A = \bar{G}_A(\text{in}) - \bar{G}_A(\text{out}) = R T \ln \left( \frac{[A]_{\text{in}}}{[A]_{\text{out}}} \right) \]

for the equilibrium of the IMPORT reaction \( A (\text{out}) \leftrightarrow A (\text{in}) \)

Would the chemical potential difference be greater or less than 0 if \([A]\) was greater on the outside the membrane? Would transport into the cell be a favorable reaction?

Would the chemical potential difference be greater or less than 0 if \([A]\) was lower on the outside the membrane? Would transport into the cell (import) be a favorable reaction?

Transport across membranes (3)

In addition to the chemical potential difference across membranes, there also is a charge difference across membranes because ions are maintained at different concentrations across membranes. So the chemical potential equation must be amended to reflect the ionic charge difference if \( A \) is ionic. For the import reaction \( A (\text{out}) \leftrightarrow A (\text{in}) \)

\[ \Delta \Psi = \Psi(\text{in}) - \Psi(\text{out}) \]

Where \( \Psi \) is the charge at each side of the membrane, and \( \Delta \Psi \) is the membrane potential.

If \( A \) is ionic, the chemical potential across the membrane must include the charge difference. The resulting \( \bar{G}_A \) is called the **electrochemical potential** of \( A \).

The electrochemical potential, which is the same as the free energy of the IMPORT reaction, is given by

\[ \Delta \bar{G}_A = R T \ln \left( \frac{[A]_{\text{in}}}{[A]_{\text{out}}} \right) + Z_A F \Delta \Psi \]

Where \( Z_A \) is the ionic charge of \( A \).

\( F \) is the faraday constant, which is the charge of a mole of electrons, and is 96494 C \( \cdot \) mol\(^{-1}\). 1V = 1 J \( \cdot \) C\(^{-1}\).

\( \Delta \Psi \) can be measured directly, and values of -100mV (inside negative) are common.