Membranes

Overview

Membranes → very thin films of molecules that enclose cells, organelles, compartments

Membrane composition

Very different composition in prokaryotes and eukaryotes

Typically composed of lipids and proteins, about 50%/50% by mass

~1 billion lipid molecules / cell

Lipids provide basic structure, while proteins have specific functional roles

Many different kinds of lipids: basic feature is that they are **amphipathic**, i.e., have both hydrophobic and a polar groups

Typical phospholipid consists of

- **head** group consisting of a phosphate group plus a polar group
- glycerol
- hydrocarbon **tail**

Phospholipids can vary in many respects; in particular, length of the tail (14-24 carbons) and number of unsaturated (double) bonds

Most common type of phospholipid in cell membranes is **phosphatidylcholine**

Cholesterols are also ampipathic and found in membranes

Membrane structure

The hydrophobic and polar parts of amphipathic molecules want to phase separate, but cannot because they are covalently bonded

Instead, they can **self-assemble** to form various structures that minimize the exposure of the hydrophobic tails to water

- micelles
- bilayers

- vesicles

Bilayers are about 5nm thick

Structure is determined by the shape of the lipids and also their concentration

Same principle as in soaps that use surfactants

Liposomes are synthetic vesicles that are very important for drug delivery

- active therapeutic can be stored inside the vesicle for controlled release
- form spontaneously when phospholipids added to water
- diameters 25 nm – 1 mm

Thermodynamics of self assembly

Self-assembly occurs at critical concentrations

Consider the self assembly of n monomers M into an assembled micelle structure A:

$$nM \overset{K_{eq}}{\leftrightarrow} A \qquad K_{eq} = \frac{[A]}{[M]^n}$$

Let's say we put some initial concentration of monomers in a solution, and we want to see what fraction are "free" versus in an assembled structure:

$$[M]_0 = [M] + n[A]$$

Substitute in the equilibrium expression:

$$[M]_0 = [M] + nK_{eq}[M]^n$$

We could try to solve this expression for $[M]$, but analytical solutions cannot be found for arbitrary n.

Instead, we'll rewrite it in a simpler fashion. Let's relate K_{eq} to the total concentration $[M]_0$ at which half of the monomers as free:

$$c^* = [M]_0 \quad \text{for which } [M] = \frac{1}{2}[M]_0$$

Substituting into the above equation:

$$c^* = \frac{1}{2}c^* + \frac{nK_{eq}c^{*n}}{2^n}$$

Solving for K_{eq},

$$K_{eq} = \left(\frac{c^*}{2n}\right)\left(\frac{c^*}{2}\right)^{-n}$$

Rewriting the above expression equilibrium expression using c^* instead of K_{eq}:

$$[M]_0 = [M]\left[1 + \left(\frac{2[M]}{c^*}\right)^{n-1}\right]$$

What does this equation predict? Consider the limits for large n values

- For $[M] < \frac{1}{2}c^*$, the second term in brackets is very small. Thus $[M] \approx [M]_0$. In other words, almost all of the monomers are free and very few are associated
- For $[M] > \frac{1}{2}c^*$, the second term in brackets is very large and we can ignore the 1. Thus, almost all of the monomers are associated into large, assembled structures and the fraction of free ones is very small.

Thus, we see assembled structures above the concentration c^* , but not below. For that reason, it is called the **critical micelle concentration.**

Thus self assembled structures are only favorable if there is a critical concentration of monomers available

For bilayers, there is a huge thermodynamic penalty for the exposure of hydrophobic groups to water, which helps maintain these structures

The bilayer as a fluid

Can think of a bilayer as a 2D fluid → lipids and other embedded molecules can move laterally through the bilayer

Kinetics

- diffusion constant for lateral translation $D \sim 10^{-8}$ cm^2/s
- A lipid can diffuse the diameter of a bacterial cell ($2\mu m$) is about a second
- flip-flop of lipids between the two layers is very, very slow ~1 month
- proteins required to facilitate fast flip flop, if needed (**flippases**)

Bilayers are **asymmetric** because flip flop occurs so slowly → monolayers have different compositions

- lipids on the extracellular side of the plasma membrane are generally covalently attached to sugars → **glycolipids**
- asymmetry preserved during transport

Fluidity depends on composition

- longer chain lengths → less fluid
- more double bonds → more fluid
- cholesterol used in animal cells to rigidify bilayers → "fills in the spaces"
- composition constantly **regulated** to obtain desired fluidity in response to temperature, other environmental changes

Lipid rafts

- densely packed subunit of cholesterols, sphingolipies with longer saturated chains
- thicker portion o the bilayer
- float and translate in 2D as a unit in the bilayer
- important in signaling and signal transduction

Membrane proteins

Many proteins are embedded within or associated with the membrane → 30% of genome

- **transmembrane**
- monolayer-associated
- lipid-linked
- protein-attached

These proteins perform critical cellular functions

- **selective transport**
- anchoring cytoskeletal components

- **receptors** for signaling
- **enzymes**

Kinds of transmembrane protein structures found

- Most often **helix bundles**, i.e., groups of alpha helices associated together
- sometimes **beta-barrel**
- oftentimes contain a transmembrane **channel**

Usually **globular** proteins that exist freely inside the cytosol shield their hydrophobic residues by burying them

In contrast, transmembrane proteins must expose hydrophobic residues on their surfaces that make contact with the hydrophobic interior of the bilayer

Very difficult to determine the structure of transmembrane proteins relative to globular ones, but these are among the most common drug targets

However, can usually infer which parts of a transmembrane protein's amino acid sequence are inside the bilayer by looking at regions of high hydrophobicity → **hydropathy** plots

Proteins on the extracellular side of the plasma membrane typically have covalently attached sugars / carbohydrates → glycoproteins

- layer of protection from chemical damage
- sugars adsorb water, gives cell surface slimy appearance
- **recognition** properties
- remember: also **glycolipids**

Plasma membrane is reinforced by the **cell cortex**

- fibrous network of transmembrane anchors and **spectrin** protein fibers
- gives membrane greater strength

Transport through membranes

Diffusive transport through membranes

Most often not through simple diffusion, but facilitated by proteins; here, though, we consider simple diffusion

Consider the flux of a species in direction x:

$$J = -D\left(\frac{dc}{dx}\right)$$

where c in concentration and D the diffusion constant. Flux has units of molecules per area per time.

For spherical solutes we might approximate D using the **Stokes-Einstein** formula:

$$D = \frac{k_B T}{6\pi\mu R}$$

Here, μ is the fluid viscosity and R the radius of the solute. We can relate the radius to molecular weight M:

$$M = \left(\frac{4}{3}\pi R^3\right)\rho$$

Thus

$$D \sim \frac{k_B T}{3\mu}\left(\frac{\rho}{6\pi^2 M}\right)^{\frac{1}{3}}$$

Compare an antibody to a peptide:

$$\begin{aligned}\frac{D_{\text{antibody}}}{D_{\text{peptide}}} &= \left(\frac{M_{\text{peptide}}}{M_{\text{antibody}}}\right)^{\frac{1}{3}} \\ &\approx \left(\frac{10}{150}\right)^{\frac{1}{3}} \\ &= 0.4\end{aligned}$$

Thus a peptide will diffuse through a membrane about 2.5 times as fast as an antibody.

What about the relationship of the species concentrations inside the membrane to that outside?

c phase 1 (outside membrane)

Hc phase 2 (inside bilayer)

Here, H is a **partition coefficient**. It is an equilibrium constant for the partitioning of a species between two phases.

So, we might have a picture like this:

c_1 extracellular space (aqueous)

Hc_1 inside bilayer (oily)

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Hc_2 inside bilayer (oily)

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c_2 intracellular space (aqueous)

If the concentration profile is approximately linear,

$$\begin{aligned} J &= -D\left(\frac{dc}{dx}\right) \\ &\approx -\frac{D(Hc_2 - Hc_1)}{l} \\ &= \frac{DH}{l}(c_1 - c_2) \end{aligned}$$

The combination of constants DH/l is called the **permeability** $\boldsymbol{P}$ and is typically expressed in units of cm/s

- $P_{\text{water}} \sim 10^{-2}\ cm/s$
- $P_{\text{urea}} \sim 10^{-5}\ cm/s$
- $P_{\text{glucose}} \sim 10^{-7}\ cm/s$
- $P_{\text{Na+}} \sim 10^{-12}\ cm/s$
- gases CO_2, N_2, O_2 permeable

Osmotic pressure

Cells need to be able to regulate precisely the concentrations of various solutes inside the plasma membrane (both macromolecular solutes like proteins and small solutes like ions)

Many solutes cannot cross the membrane and thus are "trapped" inside the cell

Cells placed in pure water will burst or **lyse**

- due to **osmosis** → tendency of pure water on the outside of the cell to flow inside, diluting the solutes
- in order to resist inflow of water, an **osmotic pressure** must be applied
- the osmotic pressure needed is proportional to the concentration difference on either side of a cell wall

This effect due to maximizing the entropy in mixtures, by attempting to equalize concentrations

The osmotic pressure of a dilute solution can be shown to follow a law similar to the ideal gas law

$$\Pi = \frac{NRT}{V} = cRT$$

where c is the total molarity of dissolved solutes.

Keep in mind that Π gives the pressure difference between the outside and the inside of the cell.

- pressure outside of the cell = P_{atm}
- pressure inside of the membrane (cytosolic) = $P_{atm} + \Pi$
- pressure the cell wall must exert to prevent cell from swelling = Π

In order for cells not to burst

- extracellular components must exist in some concentrations → buffer
- cells must keep tight control on interior concentrations by **pumping** out ions and other molecules

Passive and active transport mechanisms

Cells use transmembrane proteins to selectively allow passage of molecules either inside or outside of the cell, to maintain the intracellular concentration gradients

- **channels** discriminate on the basis of (small) size and charge → like a coin separator
- **transporters** only allow molecules to pass that fit in a specific binding site, like a trap door that opens when the right key is used

Transporters work like gates that allow the passage of molecules based on binding-recognition and protein conformational changes (**allostery**)

Two kinds of transport

- **passive** – molecules move down concentration gradients
- **active** – transporters pump molecules against a concentration gradient using ATP, the simultaneous pumping of another molecule along a concentration gradient, or light

Channels typically operate through passive transport, but can snap open or closed depending on state of cell

Simple model of transport for uncharged molecules

Imagine a molecule that is being transported to inside a cell according to a passive mechanism, i.e., down a concentration gradient

Even with a large gradient, the molecule will not pass if too large or hydrophilic due to very slow kinetics of passing through the interior hydrophobic region of the bilayer

Transporters can follow a mechanism similar to enzymes

$$S_e \rightarrow \text{extracellular substrate}$$

$$S_i \rightarrow \text{intracellular substrate}$$

$$C \rightarrow \text{transmembrane channel}$$

We can write the kinetics as

$$S_e + C \overset{K_M}{\leftrightarrow} SC \overset{k}{\rightarrow} S_i + C$$

Using the quasi-equilibrium approximation, we arrive at

$$v = \frac{d[S_i]}{dt}$$
$$= \frac{v_{\max}[S]}{K_M + [S]}$$

where

$$v_{\max} = [C]_0 k$$

Thus, there are **saturation kinetics**, that is, a maximum rate that the channel can transport the substrate, regardless of substrate concentration outside the cell. That is, regardless of the concentration gradient.

Example: glucose transporter

Ion transport

Ion concentrations inside the cell are very different than outside. Inside we have

- much lower Na+ concentrations (5-15 mM versus 145 extracellular)
- much higher K+ concentrations (140 mM versus 5 extracellular)
- much less Ca2+
- less Cl-

There is less need for negatively charged small ions inside the cell → most biomolecules, if charged, are negative: phosphates, DNA

Ions cannot pass through membrane because it is too unfavorable free energetically → hydrophobic interior of bilayer

Ions require special treatment because they are charged, and because electrostatic interactions propagate long distances since they decay as r^{-1} versus (r^{-6} for van der Waals)

As one moves across the plasma membrane, there is a change in **electrostatic potential**

- excess of positive charges at the extracellular side
- excess of negative charges on the intracellular side

Thus, it is favorable for positive ions to pass towards the inside of the cell, negative towards the outside

More on this **membrane potential** later

The Na+ K+ pump

The flow of molecules down a concentration gradient is favorable and lowers the free energy → this free energy can be coupled to provide a driving force for unfavorable transport of molecules

Animal cells constantly pump Na+ out of their interiors and pump in K+ using ATP hydrolysis

- 30% or more of total ATP consumption
- sodium exchanged for potassium to prevent charge build-up
- called the **sodium-potassium pump**

The very high concentration of sodium on the exterior of the cell, compared to in the interior, provides a source of high free energy that can be used to drive transport

- couple import of Na+ with export of another molecule using a **coupled transporter**
- an energy source for transport analogous to ATP as an energy source for polymerization

The Ca2+ pump

Calcium ions are kept at very low intracellular concentrations because they can bind to and alter the function of many proteins

Increases in calcium concentrations inside the cells can cause changes to function, by way of modifying the function or activity of proteins (allostery or binding to an active site in an enzyme)

Cells like to use this fact to enable very sensitive responses → by having low concentrations of Ca2+, sudden increases (for example due to opening of a membrane transporter) can promote a large cell response

The Ca2+ pump uses ATP hydrolysis to pump calcium out of the cytosol

H+ pumps

Take the place of Na+ pumps in plants, fungi, and bacteria

Coupled transporters

The large excess of Na+ outside the cell can be used to drive selective transport of other molecules

Several types of **coupled transporters** to simultaneously transport two solutes

- **symport** – brings both solutes in or out at the same time
- **antiport** – brings one solute in, one out
- **uniport** – pumps only one kind of molecule at a time

Membrane potentials in animal cells

The plasma membrane has an electrostatic potential gradient: positive charges build up on the outside of the membrane and negative on the inside

We can describe this behavior using concepts in electricity → in conductors, electrical transport comes from the movement of electrons, but in cells, it comes from the movement of charged ions

Key idea: Many selective channels can open or close in response to changes in the local the membrane potential

Resting potentials

At equilibrium, the asymmetric charge distribution on the intracellular and extracellular sides of the membrane requires an **electrostatic potential**

Electrostatics: the potential energy of a charge with magnitude q in an electric field with potential V is

$$U = qeV$$

If the field varies in space, e.g., across the membrane, then so does the potential energy of that charge:

$$U(x) = qeV(x)$$

We can now appeal to the Boltzmann law to describe the equilibrium probability distribution of the particle's location:

$$\wp(x) \propto e^{-\frac{U(x)}{k_B T}}$$
$$= e^{-\frac{qeV(x)}{k_B T}}$$

We can liken this probability directly to a concentration of ions at a given location

$$c(x) \propto e^{-\frac{qeV(x)}{k_B T}}$$

Now, compare the intracellular to extracellular sides of the plasma membrane

$$\frac{c_i}{c_e} = \exp\left[-\frac{qe(V_i - V_e)}{k_B T}\right]$$
$$= \exp\left[-\frac{qe\Delta V}{k_B T}\right]$$

where ΔV is the difference in the electrostatic potential. Rearranging this equation, we find

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

This important equation is called the **Nernst equation** and the potential difference ΔV the **Nernst potential**. This quantity is the electrochemical potential required to maintain a concentration difference of ions between the intra and extracellular sides of the plasma membrane.

This potential would apply to all charged species simultaneously, if in equilibrium

Actual membrane potentials are estimated to be around -60 mV

Nonequilibrium considerations

Let's calculate the Nernst potential using some typical concentrations for ions

species	exterior concentration (mM)	interior (mM)	$\Delta V = -\frac{k_B T}{qe}\ln\left(\frac{c_i}{c_e}\right)$ (mV)
K+	5	140	-89
Na+	145	10	72
Cl-	110	10	-64

Notice that the calculated membrane potential for K+ and Cl- is very close to the actual value of -60 → these two species are near equilibrium in terms of their concentration profiles on the outside and inside of the membrane

On the other hand, there is far less Na+ on the inside than expected! This means that the concentrations of Na+ are very different from what would be predicted be equilibrium calculations

In fact, animal cells are constantly pumping Na+ outside of the cell → to do so requires sources of high free energy

What would happen if instead the concentration of sodium were such that it gave a membrane potential of -60? Then, we would need

$$c_{i,Na+} = c_{e,Na+} \exp\left[-\frac{e\Delta V}{k_B T}\right] = 289\ mM$$

What would then be the osmotic pressure exerted by all of the ion the solutes?

$$\Pi = RT\Delta c$$

where Δc is the difference in concentration between the interior and exterior, in terms of total concentrations of solutes

$$\Delta c = (140 + 289 + 10) - (5 + 145 + 110)mm$$

$$= 179mM$$

Thus,

$$\Pi = (179mM)RT \approx 450000Pa = 4.4\ atm$$

This is a huge pressure! And we didn't even include all of the macromolecule ions in the interior of the cell in this calculation, which would make it even higher. Therefore, cells *must* pump out sodium in order not to burst

Signaling in neurons

Neurons are able to achieve rapid response to stimuli using the fact that there is a huge driving force for Na+ to enter the cell

Neurons consist of

- **cell body with dendrites (**"receptors")
- **axon** (carrier "wire")
- **nerve terminal** ("connectors" to other neurons)

The central question: how do neurons carry messages with high fidelity (no weakening) long distances?

The answer: **action potentials**

Neurons carry an electrical message using the following sequence of events:

1. N**eurotransmitters** at the dendrites causes a depolarization of the plasma membrane, meaning it shifts the membrane potential at that part of the cell to a less negative value

2. **Voltage-gated Na+ channels** sense this slight change and open up, allowing the rapid, passive inward movement of sodium from the extracellular matrix

3. Eventually, the transmitters will enter an inactivated state when the interior local concentration of Na+ is near its equilibrium value → eventually returning to a closed state, and sodium is pumped out actively using the sodium pump to return the concentrations to usual

4. The local change in the membrane potential then also activated nearby Na+ channels, and a domino-effect cascade ensues, propagating down the length of the axon towards the terminal

5. At the nerve terminal, changes to the membrane potential trigger **voltage-gated Ca2+ channels**, which allow the influx of calcium

6. The influx of calcium triggers intracellular vesicles containing neurotransmitters to fuse with the plasma membrane and release their contents into the extracellular environment → conversion of an electrical (ion) signal to a chemical (binding/recognition) signal

7. The neurotransmitters bind to receptors on an adjacent neuron

Because the electrical signal is converted to a chemical one in between neurons, it enables more complex signal behavior due to binding/recognition:

- **excitatory neurons** can release **excitatory neurotransmitters** that open ion channels and change the membrane potential, continuing the propagation of the signal in the

- **inhibitory neurons** can release **inhibitory neurotransmitters** that bind to channels and prevent them from opening, stopping propagation of the signal

Both kinds of neurotransmitters can be present → integration of both messages is how signals are processed

Complex interactions of signals between many, many neurons is what makes thought possible!

Neurotransmitter receptors are common targets for psychoactive drugs (Ambien, Prozac)