Biology 5357: Chemistry & Physics of Biomolecules - Membranes & Membrane Proteins

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Lecture 3:
Membrane proteins: ion permeation & passive transport

Hille, B., Armstrong, C., MacKinnon, R. (1999). **Ion channels: From idea to reality** Nature Medicine 5(10), 1105-1109. https://dx.doi.org/10.1038/13415

The wide world of membrane proteins.

- intercellular joinings (e.g. cadherins)
- enzymatic activity (e.g. proteases)
- transport - active & passive
- cell-cell recognition
- achoring & attachment
- signal transduction (e.g. GPCRs)

In addition, there can be regulatory proteins that interact with these proteins, and these may be transmembrane or they may be peripheral, interacting with either surface of the membrane.

Mechanisms of membrane transport.

- Free diffusion - passive diffusion directly through the membrane. Preferable pathway for small non-polar molecules.
- Channels - A protein provides an open pathway where ions can a substance can passively diffuse down its concentration gradient
- Transport
    - passive transport - a protein binds a substrate and through conformational change provides access to the other side of the membrane. The driving force is the concentration gradient.
    - facilitated transport - active, uphill movement of one species that is linked to the downhill movement of another substrate.
    - active transport - uphill movement linked to ATP hydrolysis

All of the concentration gradients are ultimately linked to ATP pumps.

The membrane is an electrostatic barrier. As discussed previously, the membrane hydrocarbon provides a low dielectric slab that is approximately 30 Å thick and from the

perspective of a protein, it extends in practically infinite directions ... at least in a cell. The Born equation provides the calculation of the free energy cost to transfer an ion from the high dielectric water to low dielectric core of the protein, however, this considers a continuum dielectric with no structural details.

To calculate the electrostatic free energy in a system with a more complex dielectric structure, one can solve the Poisson-Boltzman equation and obtain the electrostatic field, and electrostatic component to the free energy across the structure. The figures show the free energy cost for a K+ ion traversing across the 30 Å membrane slab of dielectric 2, yielding a cost of 40 kcal/mole at the center of the membrane. Making a channel across the membrane with diameter of 3 Å and dielectric of 80, large enough to allow a K+ ion to pass, reduces the electrostatic energy to less than 20 kcal/mole .... but this is still too costly for ion diffusion!

The reason for this is the reaction field. While the ion itself can be solvated by water in the pore, when a charge is near a dielectric boundary it sets up a net field on that boundary that opposes the passing charge. One can understand this if you draw the way that water would rearrange around a K+ ion. Once it hits the low dielectric boundary, the molecules can no longer reorient and so there is a net positive field at this surface. Thus, a narrow high dielectric pore would appear to have a layer of positive charges at the surface and the ion would still be unfavorable in this channel in the membrane.

Thus, we need proteins that span the membrane to offer further electrostatic stabilization to enable diffusion limited passage of ions across the membrane.

The nature of ion channels.

- continuous pores when open
- the driving force is the concentration gradient
- variety of permeation rates, with the fastest being diffusion limited
- can be extremely selective
- control of permeation is determined by gating via conformational changes.

Ion channels are resistors. The low dielectric membrane is considered a capacitor, capable of separating charge. Ion permeation through the channels can be modeled as an electrical current, with the ion channels having a defined resistance. If the channels are freely open, they follow Ohm's law.

Ion permeation defines the equilibrium potential of a cell. The selectivity of certain ion channels allows for a cell to establish and equilibrium membrane potential. If the membrane is selectively permeable to one type of ion, and there is a concentration gradient of that ion across the membrane, then the ion will at first be driven to diffuse down its concentration gradient. When that first ion passes across the membrane, it carries a positive charge and

leaves a negative charge which establishes a potential difference across the membrane and the electrical field opposes further diffusion down the concentration gradient. Thus, an electrical and chemical equilibrium will be established where no net flux occurs. The potential that will be established is described by the Nernst equation. It is often referred to as the Nernst potential, equilibrium potential and sometimes the reversal potential in electrophysiological studies. If there are several ions that are permeable, then this equilibrium is described by the Goldman-Hodgkin-Katz equation.

Cellular resting potentials are often described by K+ permeation. K+ channels are highly selective and often open at resting potential providing a K+ selective leak that is used to set the resting membrane potential. In reality, these potentials depend entirely on the types of ion channels that are permeating ions at rest, and the concentration gradient that has been established by other pumps and transporters. Different cells have different ionic gradients and so one can predict the different resting potentials if K+ channels were responsible for the leak currents. Try calculating the different equilibrium potentials for the different cell types if K+ or Na+ channels were responsible for setting the resting potential.

In addition to the electrical potential gradient, there is also proton motive force (pmf) which adds in the contribution from the H+ gradient across the membrane.

Ionic currents. Typically, ion channels have single channel currents around a pA, which means that the channels are passing a million ions per second. This is around the diffusion limited rate and these single-channel currents can be measured directly by electrophysiological techniques.

Gramcidin is the first ion channel that was ever studied, and is an ionophoric antibiotic peptide that can spontaneously partition into the membrane. The two halves of the channel come together and dimerize to form a continuous pore across the membrane which conducts Na+ ions. Single-channel conductances can be measured in a black lipid membrane electrophysiological setup.

Whole-cell currents can be measured in a similar approach called patch-clamping, where an electrode is used to provide electrical access to the membrane. The total cellular current is described by the following equation:

$I = N * g * Po * (Vm-E)$

- N - number of channels in the membrane
- g - single channel conductance. Conductance = 1/resistance.
- Po - probability of opening, form gating behavior

- (Vm - E) - the electrochemical driving force. How far away is the membrane potential from the equilibrium potential?

The different types of current vs. time profiles are shown and can offer a complex description of the changes in permeation of the membrane over time. This is critically important for physiology.

Gating. Deviations of the I-V curve from the linear Ohmic relationship usually arrives by gating. This is from changes in the conformation of the protein in response to voltage or other factors which modulate the probability of conductance.

Activation. Ion channels can be activated by voltage, binding of ligands, membrane tension, osmotic gradients - ionic concentration, temperature, and more ... These are the proteins that are sensing practically all of the stimuli from the outside world.

Voltage-dependent activation is critical to excitable cells that are involved in muscle contraction and propagation of neuronal signals. They include K+ channels, Na+ channels and Ca2+ channels. The have 6 transmembrane segments, with the last two forming the pore domain. The fourth transmembrane helix contains lysines (and some arginines) at every 3 residues along the helix - raising mysteries within the field of how such a protein could stably exist within the membrane. The answer comes from water accessbility and stabilization by other partner charges within the protein. However, this segment ends up being very sensitive to the potential across the membrane and thus the conformational change links channel opening to membrane potential.

Inactivation. Channels will also undergo conformational changes that reduce the conductance, and this is known as inactivation. This is like a timed shutdown device. There are different mechanisms of how this happens, but it is often a conformational change that has a higher probability of transitioning from the active state.

Closed - Open - Inactive. Most ion channels follow these three states in their reaction scheme. However, there may be many sub-states within each of these states. These states, and the probability of transitioning between these states can be measured from electrophysiology with exquisite detail. For voltage-gated channels, different voltage protocols can be delivered to the membrane to measure the output current.

Rectification. Many ion channels demonstrate some sort of rectification behavior, meaning that the current in one direction is different from the current in the other direction. This results in an intrinsic non-linearity in the I-V curve, and is due to asymmetry of the ion channel itself. In some K+ channels, extreme rectification like a diode, has been achieved through intracellular blockade of blocking cations.

The action potential. When there are several different types of voltage-gated ion channels are expressed in the membrane, they set up a situation where the cell can be excitable meaning that it can form an action potential, which is a defined membrane potential wave-form that leads to downstream action or signaling. You only need two ion channels to do this, although typically many are involved to tune the action potential.

Consider the case where you have a K+ leak channel that is open at resting membrane potential, e.g. -90 mV, and a voltage-gated K+ channel and Na+ channel. The intracellular and extracellular concentration gradients are as described for the cardiac myocytes (e.g. high K+ in, low K+ out, high Na+ out, low Na+ in). With the K+ leak channels open, then potential is at the equilibrium potential for the K+ gradient. With a stimulus current (usually offered by a hyperpolarization activated channel like HCN), the membrane potential becomes more positive and starts to activate Na+ channels. Once these open, the membrane potential quickly goes to a potential in between the K+ and Na+ equilibrium potential ... and directly to the Na+ Nernst potential of K+ channels close. Then, this leads to the slower activation of voltage gated K+ channels, which restores the membrane potential back to the K+ equilibrium. It is the coupling between activation and inactivation that defines this behavior, and this is critically important to physiological process.

Selectivity. The action potential relies on K+ and Na+ selectivity, otherwise, there would be no change in the equilibrium potentials. It turns out that K+ channels are far more selective than Na+ channels: 1 Na+ passes for every 10,000 K+. For Na+ channels, about 1 K+ passes for every 100 Na+.

Selectivity can be easily achieved by size constraints. If you examine the ionic radii, Na+ is smaller than K+, so how does a K+ channel exclude a smaller ion but let through the larger one? A second question is how does this happen while maintaining diffusion limited conductance? The structure of the K+ channel selectivity filter shows how these problems are solved. The ions permeate along a single-filed path of 5-6 sites where the carbonyl groups in the peptide backbone mimic a water hydration structure. This is not necessarily the exact hydration structure that is obtained in bulk, but it is one that is optimized for K+ binding over Na+ solvation. Na+ interacts with water more strongly, and it a different hydration structure, and is not preferentially solvated in the selectivity filter structure. By lining up a series of these sites, the channel can yield high selectivity. In addition, since multiple ions can occupy the filter at a time, the ions will push each other through as if connected like a train. This is called the knock-on, knock-off mechanism, and allows for high ion throughput with high affinity binding.

For Na+ channels, the pores are actually large enough to accomodate both ions - large molecules even. However, Na+ is the preferential ion to pass. The reason for this also comes down to water. In order for a K+ ion to pass, all of the waters must be removed and the cost

for full dehydration is too unfavorable. However, a Na+ ion can keep a single water molecule and thus the dehydration cost is less. Still, every now and then a K+ ion will pass. Thus the intermediate selectivity.

Anion channels also exist but do not typically encounter problems of selectivity because the main biological anion is chloride. In plants, its nitrate (NO3-). So, typically, channels like the CLC family will pass all of these anions. However, recently, a new type of anion channel was discovered that permeates fluoride with high selectivity. The mechanisms by which this happens, or how chloride ions permeate in CLCs, is still uncertain.

Porin channels will pass through both cations and anions and are unselective or partially selective. Bottom line, the larger the pore, the less selective it will be. The main purpose of the pores in this case is to increase the amount of water in the membrane and reduce the reaction field, so the involvement of ion interactions with the protein is reduced.

Aquaporins - these are not ion channels and are instead highly selective water channels. They are mainly responsible for much of the osmotic effects that we discussed earlier, and aquaporins are widely expressed. They are extremely selective for water over the hydronium ion. However, one main question has been how can a water channel exist within passing H+ through a Grotthus mechanism, where the proton can hop along a water pathway. Recently, high resolution crystal strucutres of these proteins have been obtained a sub-angstrom resolution showing that the mechanism is much like the K+ selectivity filter, and rearrangement of the water structures presents this type of proton leakage.