Lecture Outline

1. Membrane protein structures
2. Ion channels
Polypeptides are unstable in membranes

Davson & Danielli model

secondary structures

- alpha helix
- beta sheet

multi-TM Helices

Beta barrels
Freeze-fracture EM reveals integral membrane proteins

Moor and Mühlethaler, 1963
A gold-mine of highly ordered membrane protein

- Stoeckenius & Rowen (1967) isolated purple membranes from H. Halobium archaea (> 4.3 M salt conc.)
- Osterhelt & Stoeckenius (1970) identified a single molecular species with MW 26 kDa formed the purple membrane. They showed that it was responsible for the color due to the binding of retinal, bound via a Schiff base linkage to lysine 216.
- First membrane protein structure in 1975 by Henderson & Unwin by EM diffraction

Deisenhofer, …, Huber & Michel (1985) solved the first x-ray crystal structure of a membrane protein, the photosynthetic reaction centre of Rhodopseudomonas viridis at 3 Å resolution

Detergent solubilization was critical

**Figure 1.** The reaction center from the photosynthetic bacterium *Rhodopseudomonas viridis.* The electron transfer cofactors are depicted as structures comprised of red spheres. The cytochromes supply electrons to a bacteriochlorophyll dimer called the "special pair" that absorbs light and reduces a bacteriopheophytin intermediate. There are four proteins, the "L" and "M" subunits (dark and light blue) whose alpha-helices span the membrane bilayer, the "H" subunit (yellow) on the side of the reaction center that accepts the electrons released by absorption of photons, and the cytochrome subunit that ligates the electron donor hemes (gold).
Membrane proteins structures are challenging

http://blanco.biomol.uci.edu/mpstruc/
Structure determination of membrane proteins

- X-ray Crystallography
  - In detergent
  - Major challenge to find crystal conditions for non-anisotropic diffraction
  - Lipidic cubic phase

- 2-dimensional electron diffraction
  - High resolution for samples prone to 2d crystallization

- Single particle reconstruction by cryo-electron microscopy
  - Any samples, best if ordered and homogeneous

https://doi.org/10.1016/j.sbi.2015.07.009

Complex I

γ-secretase

Ryanodine receptor
Roles of membrane proteins
Overcoming the physical barrier of the membrane
Mechanisms of membrane transport

- Passive transport
- Active transport

- Channel-mediated diffusion
- Carrier-mediated transport

- Concentration gradient

Lipid bilayer
The lipid bilayer separates concentration gradients and charge

![Lipid bilayer diagram]

\[ C = \frac{\varepsilon A}{d} = \frac{k\varepsilon_0 A}{d} \]

<table>
<thead>
<tr>
<th>ion conc. (mM)</th>
<th>sea water</th>
<th><em>E. coli</em></th>
<th><em>S. cerevisiae</em></th>
<th>mammalian cell (heart or RBC)</th>
<th>blood plasma</th>
<th>BNID</th>
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<tbody>
<tr>
<td>K(^+)</td>
<td>(\approx10)</td>
<td>30-300</td>
<td>300</td>
<td>100</td>
<td>4</td>
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<tr>
<td>Na(^+)</td>
<td>(\approx500)</td>
<td>10</td>
<td>30</td>
<td>10</td>
<td>100-200</td>
<td>104050</td>
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<tr>
<td>Mg(^{2+})</td>
<td>(\approx50)</td>
<td>30-100 (bound); 0.01-1 (free)</td>
<td>50</td>
<td>10 (bound) 0.5 (free)</td>
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<tr>
<td>Ca(^{2+})</td>
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<td>3 (bound); 100 nM (free)</td>
<td>2 (bound)</td>
<td>10-100 nM (free)</td>
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<td>Cl(^-)</td>
<td>(\approx500)</td>
<td>10–200 media dependent</td>
<td>5-100</td>
<td>100</td>
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<td>105409, 110744</td>
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</table>

Table 1: Ionic concentrations in sea water, a bacterial and yeast cell, inside a mammalian cell and in the blood. Concentrations are all in units of mM. Values are rounded to one significant digit. Unless otherwise noted, concentration is total including both free and bound ions. Note that concentrations can change by more than an order of magnitude depending on cell type and physiological and environmental conditions such as the medium osmolarity or external pH. Na\(^+\) concentrations are especially hard to measure due to trapping and sticking of ions to cells. Most Mg\(^{2+}\) ions are bound to ATP and other cellular components. More BNIDs used to construct table: 104083, 107487, 110745, 110754.
The equilibrium potential

\[ V = \frac{RT}{zF} \ln \frac{C_o}{C_i} \]

**where**

- \( V \) = the equilibrium potential in volts (internal potential minus external potential)
- \( C_o \) and \( C_i \) = outside and inside concentrations of the ion, respectively
- \( R \) = the gas constant (2 cal mol\(^{-1}\) K\(^{-1}\))
- \( T \) = the absolute temperature (K)
- \( F \) = Faraday’s constant (2.3 \times 10^4 cal V\(^{-1}\) mol\(^{-1}\))
- \( z \) = the valence (charge) of the ion
- \( \ln \) = logarithm to the base e
## The equilibrium potential

<table>
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<tr>
<th>compartment</th>
<th>potential difference (mV)</th>
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<tr>
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<td>-40 to -80</td>
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<td>104084</td>
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<td>-120 (-170 pmf)</td>
<td>101103</td>
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<tr>
<td>rat liver mitochondria, high fat diet</td>
<td>-140 (-150 pmf)</td>
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<td>E. coli fermentive growth on glucose</td>
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<td>E. coli aerobic growth on glycerol</td>
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<tr>
<td>S. aureus growth on aerobic rich media</td>
<td>-130 (-210 pmf)</td>
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<tr>
<td>alga <em>Nitella</em></td>
<td>-140</td>
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Table 1: Electric potential difference over a range of biological membranes. Negative values indicate that the outer compartment is more positive than the inner compartment. pmf is the total proton motive force that includes the effect of pH. When the pH of the media changes the electric potential of single celled organisms tends to change such that the pmf remains in the range -100 to -200 mV.
Ion channels are resistors

**Basic components of Hodgkin–Huxley-type models.** Hodgkin–Huxley type models represent the biophysical characteristic of cell membranes. The lipid bilayer is represented as a capacitance ($C_m$). Voltage-gated and leak ion channels are represented by nonlinear ($g_n$) and linear ($g_L$) conductances, respectively. The electrochemical gradients driving the flow of ions are represented by batteries ($E$), and ion pumps and exchangers are represented by current sources ($I_p$).

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**Figure 1. Current-voltage ($I$-$V$) relationship.**

This figure shows a hypothetical current-voltage ($I$-$V$) relationship using Equation 1. Experimentally, such an $I$-$V$ plot can be obtained by changing the membrane potential to the desired values ranging from $-200$ to $+100$ mV and measuring the resulting current values at each voltage. Ohm's law ($V = IR$) states that the voltage difference across the plasma membrane is equal to the product of membrane current and membrane resistance (Equation 1). In this hypothetical example, it is assumed that the resting membrane potential is zero ($V_{rest} = 0$ mV). For the purpose of $I$-$V$ relationships, Equation 1 may be rearranged to obtain Equation 2: $I = V/R$. If current is plotted as a function of the membrane potential, the result is a current voltage ($I$-$V$) plot such as the one shown in this figure. Note that when the membrane potential is at the resting membrane potential (i.e., $V = V_{rest} = 0$ mV), the current is zero. The slope of this plot is the inverse of resistance (slope = 1/R). In this hypothetical example, the slope reveals a membrane resistance of 1 megaOhms ($R = 1 \text{ M}\Omega$). Moreover, in this example, the slope is constant at all membrane potential values. The inverse of resistance is conductance ($G$), which is a measure of the ease with which ions can permeate the membrane.

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**Ohm's Law**

$V = I \cdot R$

**Capacitance**

$Q = C_m \cdot V_m$

$$\frac{dQ}{dt} = I(t) = C_m \frac{dV_m}{dt}$$

**Sum of**

$\sum I_x = N \cdot P_o \cdot \gamma_x (V_m - E_x)$

$\gamma_{\text{Na}^+}, \gamma_{\text{K}^+}, \gamma_{\text{Cl}^-} \text{ etc...}$
First single-channel recording - gramicidin
The action potential

(A) Resting membrane potential

(B) Rising phase of action potential

(C) Falling phase of action potential
The problem of selectivity

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<tr>
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<th>Group 1</th>
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</table>

Sizes of atoms and their ions in pm
Solving the K+ selectivity problem

Armstrong, 2003

Zhou et al., 2001
Solving the Na+ selectivity problem
Chloride permeability is generally non-specific
Mechanisms of gating

Figure 1: Different mechanisms of ion channel gating. The green channel is gated by a transmembrane voltage. The blue channels are gated by ligands that bind the protein and induce a conformational change. The red channel is gated by mechanical forces.