Physical Biology of the Cell

Chapter 17

Biological Electricity

Ion Channels and Nerve Impulses
pH and the Charge on DNA and Proteins

\[
H_2O \rightleftharpoons H^+ + OH^- \quad K_{eq} = 10^{-14}
\]
\[
[H^+] = [OH^-] = 10^{-7} \text{ M}
\]
\[
pH = -\log[H^+] = 7 \text{ in pure water, } \sim 7.2 \text{ in most cells.}
\]

The charged state of a macromolecule depends on the solution pH.
Assume that molecule MH can release a single \( H^+ \) to solution

\[
HM \rightleftharpoons H^+ + M^- \\
K_d = \frac{[H^+][M^-]}{[HM]}
\]
\[
pK = -\log K_d = -\log[H^+] - \log[M^-] + \log[HM]
\]
\[
pH = pK + \log \frac{[M^-]}{[HM]} \text{ Handelson – Hasselbach Equation}
\]

half of MH is dissociated \([M^-] = [HM] \) when \( pH = pK \)

- pK of DNA is 1.0
- At normal pH, phosphates on DNA backbone are fully dissociated.
- DNA is strong acid.
- Different amino acid side chains in different states of dissociation.
Electrostatics in Water

The force between two charged particles

$$F = \frac{1}{4\pi \varepsilon_0 D} \frac{q_1 q_2}{r^2} \quad \frac{1}{4\pi \varepsilon_0} = 9 \times 10^9 \text{ Nm}^2/\text{C}^2 \quad \text{and } D=1 \text{ for vacuum.}$$

- Electrostatic forces diminished in polar solvents like water, because negative charges of the dipoles orient towards the positive charge and \textit{vice versa}.
- Dielectric constant \( D = 80 \) for water.
- Therefore, electrostatic interactions are 80 fold weaker in water compared to vacuum.
- In the presence of metal salts, this screening effect is even more pronounced.
Electrostatics in Salty Solutions

• At cellular pH=7.2, phospholipid head groups of the lipid bilayer are negatively charged.
• Positive counterions in solution will surround the membrane and neutralize the total charge on the molecule.
• Negative ions will stay away from the bilayer surface.
• Competition between electrostatic energy minimization and entropy minimization (structural organization).
• At large distances, membrane potential will diminish and ions will be evenly distributed.
• What is the characteristic decay length of the concentration gradient?
Consider ions that have a charge $\pm ze$

\[ c_+ = c_- = c_\infty \]

Charge per area of a membrane is \( \sigma \left( \frac{\text{coulomb}}{m^2} \right) \)

Ionic concentrations will only vary in perpendicular direction to the plane of the membrane.

Electrostatic energy of positive and negative ions under the membrane potential \( V(x) \) will be

\[ U = \pm zeV(x) \]

The distribution of ions from the Boltzmann Equation:

\[ c_+ = c_\infty e^{-zeV(x)/kT} \]

\[ c_- = c_\infty e^{zeV(x)/kT} \]
Gauss’ Law

Charges on the membrane can be modeled as thin sheet of fixed negative charges

Surface charge density is $\sigma_q \left( \frac{\text{coulomb}}{m^2} \right)$

The electric field $\vec{E}(x)$ above the sheet is negative.

Everything is constant in $y$ and $z$.

$\vec{E}_{\text{surface}} = \frac{\sigma_q}{D\varepsilon_0}$

Electric field decays by distance due to the existence of the net positive charge density $\rho_q(x)$

$\vec{E} \left( x + \frac{1}{2} dx \right) - \vec{E} \left( x - \frac{1}{2} dx \right) = \frac{\rho_q(x)dx}{D\varepsilon_0}$

$\frac{d\vec{E}}{dx} = \frac{\rho_q(x)}{D\varepsilon_0}$ (Gauss’ Law)

since $\vec{E} = -\frac{dV}{dx}$, \( \frac{d^2V}{dx^2} = -\frac{\rho_q(x)}{D\varepsilon_0} \) (Poisson Equation)
We need to find out the electric potential $V(x)$

The total charge density at position $x$:

$$\rho(x) = zec_+(x) - zec_-(x)$$

The Poisson Equation:

$$\frac{d^2V}{dx^2} = -\frac{\rho(x)}{D\varepsilon_0}$$

$$\frac{d^2V}{dx^2} = \frac{zec_\infty}{D\varepsilon_0} \left( e^{zeV(x)/kT} - e^{-zeV(x)/kT} \right)$$

$$e^{zeV(x)/kT} = 1 + zeV(x)/kT \quad e^{-zeV(x)/kT} = 1 - zeV(x)/kT \quad \text{(Linearization when } V(x) \text{ is small)}$$

$$\frac{d^2V}{dx^2} = \frac{2z^2e^2c_\infty}{D\varepsilon_0} V(x) \quad V(x) = Ae^{-x/\lambda_D} + Be^{x/\lambda_D}$$

$$\lambda_D = \sqrt{\frac{D\varepsilon_0 kT}{2z^2e^2c_\infty}} \quad \text{Debye Screening Length}$$

Since the potential far from the membrane is zero, $B = 0$.

Electric field at the surface of the membrane: $E_x(0) = \frac{\sigma}{D\varepsilon_0} \quad \text{Gauss' Law}$

$$E_x(x) = \frac{-dV(x)}{dx} \quad \text{and therefore} \quad A = \frac{\sigma\lambda_D}{D\varepsilon_0}$$

$$V(x) = \frac{\sigma\lambda_D}{D\varepsilon_0} e^{-x/\lambda_D} \quad \text{and} \quad \rho(x) = -\frac{\sigma}{\lambda_D} e^{-x/\lambda_D}$$

For a charged protein at physiological salt concentration $c_\infty = 200 \text{ mM,} \quad \lambda_D = 0.7 \text{ nm.}$

The charge on the protein will not be felt beyond this distance.
Macromolecular Binding

• The Poisson-Boltzmann Equation shows us that charged macromolecules will not feel one another in cytoplasm until they are nearby.
• Once they are nearby, the detailed surface pattern of positive and negative residues on a protein can be felt by its neighbor.
• Although many different types of macromolecules wander in cytoplasm, only those with precisely matching shapes and charge distributions will bind together.
• Proteins interact with each other at specific surfaces (binding sites), overall shape and charge of these proteins are less important.
• Single noncovalent interaction is not strong enough to form a stable interaction, because their magnitude is on the order of a kT in cytoplasm.
Membranes are constituted by lipids, which are amphiphilic molecules having a hydrophobic tail and a polar head group.

- Hydrophobic effect forces lipid molecules to self-assemble, by hiding their tail domains and exposing their head groups.
- Depending on the shape and the number of head groups per head of lipid molecules, they form either a bilayer or a micelle, through free energy minimization.

Within the bilayer, individual lipid molecules can move by:

1. Lateral diffusion
2. Flipping from one side to another. This slow process can be sped up by flippases.
Cells are Surrounded by a Lipid Bilayer

(A) Fluid Mosaic Model: Two dimensional fluid of phospholipids studded with membrane proteins.

(B) Phospholipids with different chemical character.
- Cholesterol can self-associate to form sub-domains (lipid rafts).
- Membrane proteins form oligomers and clusters, distort the thickness and composition of the bilayer.

(C) Lipid bilayer interacts with other proteins and sugars.
- Complex sugars are attached to phosphate head groups at the extracellular domains.
- Cytoskeleton interacts with cytosolic portions of certain transmembrane proteins.
• Plasma membrane is a lipid bilayer.
• Membrane bound proteins are synthesized at endoplasmic reticulum.
• Nuclear envelope consist of two bilayers with a thin space between them. Nuclear pore complex regulates exchange of material between cytoplasm and nucleus.
• E. coli consists of two bilayers separated by a rigid cell wall.
Permeability of a Lipid Bilayer and Ion Channels

Permeability \( P \) is defined as:

\[
    \text{Flux of particles: } j = P(c_{\text{in}} - c_{\text{out}})
\]

- Water has a fairly high permeability across a lipid bilayer.
- Water concentrations can be equilibrated across the membrane (consistent with our derivation of osmotic pressure)
- Permeability of glucose is four order of magnitudes lower.
- Ions are almost impermeable across the membrane, which acts like a charged capacitor in cases where there is significant concentration difference inside and outside the cell.
- Biological membranes are decorated with ion transporting channels that allow specific metal ions to be transported across the membrane (ion channels)
- Ion channels are gated to open only when a certain signal is received (mechanical, ligand-binding, or voltage).
- Ion channel opening/closing plays numerous roles in cell physiology.
Biological Electricity

- Cells create and manipulate transmembrane gradient of positive (Na\(^+\), K\(^+\), Ca\(^{2+}\)) and negative (Cl\(^-\)) ions.
- To generate a concentration gradient, cells pump ions in and out by consuming ATP energy.
- Ions are only allowed to pass the membrane through predefined conduits.
- Potential difference across the membrane can be disrupted by opening specific ion channels through chemical, mechanical or electrical stimuli.
- This disturbance can propagate along the cell membrane at a very high speed (~10-100 m/sec).

<table>
<thead>
<tr>
<th>Ion species</th>
<th>Intracellular concentration (mM)</th>
<th>Extracellular concentration (mM)</th>
<th>Nernst potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>155</td>
<td>4</td>
<td>-98</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>12</td>
<td>145</td>
<td>67</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>10(^{-4})</td>
<td>1.5</td>
<td>130</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>4</td>
<td>120</td>
<td>-90</td>
</tr>
</tbody>
</table>
Assume that the membrane is uncharged.

Assume that the cell has a higher KCl concentration than the exterior $c_2 > c_1$.

Cell membrane is slightly permeable to K$^+$ ions and nonpermeable to Cl$^-$ ions.

K$^+$ ions face a dilemma:
- increase entropy by balancing the concentrations on both sides.
- electrostatic attractions of Cl$^-$ ions.

Only a few K$^+$ ions will pass to other side and they will not go far.

They will deplete a thin layer just inside and outside the membrane.

Between C-D, positive charges accumulate because they face the interior of the cell which is negatively charged due to the escape of positive ions.

Between A-B, negatively charged ions accumulate because they face positively charged exterior.

The corresponding electrostatic potential is created by the charge distribution.
Electrostatic energy of a unit charge $e$ when placed inside of a cell with potential $V_{in}$

$U = zeV_{in}$

If ions are separated into two regions with corresponding potentials $V_{in}$ and $V_{out}$

\[
\frac{c_1}{c_2} = e^{-zeV_1/kT} \cdot \frac{e^{-zeV_2/kT}}{e^{-zeV_1/kT}}
\]

\[
V_2 - V_1 = \frac{kT}{ze} \ln \frac{c_1}{c_2} \quad \text{(Nernst Potential)}
\]

\[
\frac{kT}{e} = 25 \text{ mV}
\]

Nernst equation says that, in equilibrium, the electrochemical potential of any permeant ion species must be the same.
**Donnan Equilibrium**

*In living cells, there are several important ion species: \(Na^+, K^+, Cl^-\)*

Cells are also full of nucleic acids and proteins carrying net negative charge.

*Impermeant macromolecules carry approximately 125 mM of excess electrons.*

*Small ions will cross the membrane to reduce the total free energy of the cell.*

If we assume the exterior ion concentrations are

\[ [Na^+] = 140 \text{ mM} \quad [K^+] = 10 \text{ mM} \quad [Cl^-] = 150 \text{ mM} \quad \text{(neutral)} \]

and inside the cell is neutral too: \( c_{in, Na^+} + c_{in, K^+} - c_{in, Cl^-} - 125 \text{ mM} = 0 \)

Nernst Equation states that:

\[
\Delta V_{\text{membrane}} = -\frac{kT}{e} \ln \frac{c_{in, Na^+}}{c_{out, Na^+}} = -\frac{kT}{e} \ln \frac{c_{in, K^+}}{c_{out, K^+}} = -\frac{kT}{e} \ln \frac{c_{in, Cl^-}}{c_{out, Cl^-}}
\]

\[
\frac{c_{in, Na^+}}{c_{out, Na^+}} = \frac{c_{in, K^+}}{c_{out, K^+}} = \frac{c_{out, Cl^-}}{c_{in, Cl^-}} \quad \text{(Gibbs - Donnan Relation)}
\]

Solution of this equation is

\[ \Delta V_{\text{membrane}} = -10 \text{ mV} \quad c_{in, Na^+} = 210 \text{ mM} \quad c_{in, K^+} = 15 \text{ mM} \quad c_{in, Cl^-} = 100 \text{ mM} \]

Cells can maintain a permanent electrostatic potential without spending any energy.

All available ions share in the job of neutralizing the negative charge of macromolecules.
### Cellular Ion Concentrations

<table>
<thead>
<tr>
<th>Ion species</th>
<th>Intracellular concentration (mM)</th>
<th>Extracellular concentration (mM)</th>
<th>Nernst potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>155</td>
<td>4</td>
<td>-98</td>
</tr>
<tr>
<td>Na⁺</td>
<td>12</td>
<td>145</td>
<td>67</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>10⁻⁴</td>
<td>1.5</td>
<td>130</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>4</td>
<td>120</td>
<td>-90</td>
</tr>
</tbody>
</table>

**Table 17.1** Ion concentrations and the Nernst potential for small ions within the cell. The numbers are typical of mammalian skeletal muscle cells, which have a resting potential of $V_{\text{mem}} = -90 \text{ mV}$. (Data

*Cells are far away from the Donnan Equilibrium.*

Resting potential of the skeletal muscle cell is -90 mV, indicating that interior of the cell is at a potential which is 90 mV lower than the exterior.

Membrane potential is comparable to $kT/e$, meaning that thermal forces play a role in charge distribution.

If the Nernst potential of an ion is close to -90 mV, it means that concentration differences are balanced out by the membrane potential.

*Nernst potential of K⁺ and Cl⁻ ions are near the transmembrane potential,*

*whereas for Na⁺ and Ca²⁺ ions it is of the wrong sign.*

High relative concentration of sodium and calcium outside the cell is maintained against both an unfavorable chemical gradient and unfavorable electrical potential.

Cells need to constantly pump out sodium and chloride across their membranes using metabolic energy.

The excess positive charge inside is balanced by negative charges on DNA and proteins.
Ion Pumps: Sodium-Potassium ATPase Channel

- Na-K ATPase channel uses ATP energy to pump out three Na\(^+\) ions and import two K\(^+\) ions:
  1) To generate transmembrane potential beyond Donnan Equilibrium
  2) Partially offset loss of electric charge from the exported sodium.

Energy required to run the pump through the cycle:

1. To pump one sodium ion out

\[
\Delta G = \Delta G_{\text{conc}} + \Delta G_{\text{pot}} = eV_{\text{Na}}^{\text{Nernst}} - e\Delta V_{\text{membrane}} = e(67 - (-90))mV = 157\text{ meV}
\]

2. To import one potassium ion:

\[
\Delta G = \Delta G_{\text{conc}} + \Delta G_{\text{pot}} = -eV_{\text{K}}^{\text{Nernst}} + e\Delta V_{\text{membrane}} = e(98 + (-90))mV = 8\text{ meV}
\]

Both process require energy.

Total energy of three sodium ions out and two potassium ions in:

\[
\Delta G = 3 \times 157\text{ meV} + 2 \times 8\text{ meV} = 487\text{ meV} \approx 19kT
\]

Na-K ATPase channel is nearly 100% efficient that one ATP hydrolysis (\(\Delta G = -20\text{ kT}\)) is used per cycle.

This is for a good reason because 60% of the metabolic energy of sedentary humans is used by this machine.
Voltage-Gated Ion Channels

The channel is composed of a spring and an electric dipole.

In the open state, the spring is in its equilibrium length

In the closed state, the spring is compressed by a distance $\Delta x$ with

$$\text{The corresponding conformational energy of the closed state is } \varepsilon_c = \frac{1}{2} k_s (\Delta x)^2 > 0$$

In the open state, the dipole is oriented in opposite direction to the electric field of the membrane.

$$\text{The corresponding electrical energy } U = \vec{p} \cdot \vec{E} = -pE = -el \frac{V_{mem}}{d} \quad \text{and } V_{mem} < 0, \quad \text{hence } U > 0$$

In the closed state, the dipole is oriented perpendicular to the electric field of the membrane. $U = 0$.

$$p_{open} = \frac{e^{-\beta \varepsilon_{open}}}{e^{-\beta \varepsilon_{open}} + e^{-\beta \varepsilon_{closed}}}$$

without membrane voltage:

$$p_{open} = \frac{1}{1 + e^{-\beta \varepsilon_c}}$$

with negative membrane potential

$$p_{open} = \frac{e^{\beta el V_{mem}/d}}{e^{\beta el V_{mem}/d} + e^{-\beta \varepsilon_c}} = \frac{1}{1 + e^{-\beta (\varepsilon_c + el V_{mem}/d)}}$$
Cells that express voltage-gated sodium and potassium channels are termed excitable cells: neurons, muscle cells, eggs, sperms…

Like all cells, excitable cells maintain a membrane potential which is negative in the cell interior.

The membrane is more permeable to $K^+$ and $Cl^-$ ions to $Na^+$ and $Ca^{2+}$.

Therefore, transmembrane potential is equal to the equilibrium potential of potassium and chloride.

Changes in ion permeability through the membrane may result in changes in transmembrane potential.

When voltage-gated sodium channels open, sodium enters the cell, reducing the magnitude of the membrane potential, termed *depolarization*.

When voltage-gated potassium channels open, potassium ions exit the cell, increasing the magnitude of the membrane potential, termed *hyperpolarization*.

MUSCLE CONTRACTION: