# Thermodynamics of Ion and Electron Transport across Biological Membranes

# Thermodynamics of Ion Transport

- The cell membrane may be regarded as a barrier that slows down the transfer of material into or out of the cell.
- Here we focus on the transport of ions across biological membranes.
- We begin by developing some general ideas about solutions of electrolytes.
- Then we describe the thermodynamics of ion transport mediated by special membrane-spanning proteins.
- We will also see how electron transfer reactions during the later stages of aerobic metabolism of glucose couple to the movement of protons across biological membranes and contribute to the synthesis of ATP.

# Ions in solution

- Electrolyte solutions exhibit non-ideal behavior even at very low concentrations because the solute particles, the ions, do not move independently of one another.
- There are long-range Coulombic interactions between the ions in a solution.
- Since they exhibit non-ideal behaviour, we take the activities of ions into consideration.
- Once we know the activity of the species J, we can write its chemical potential by using:

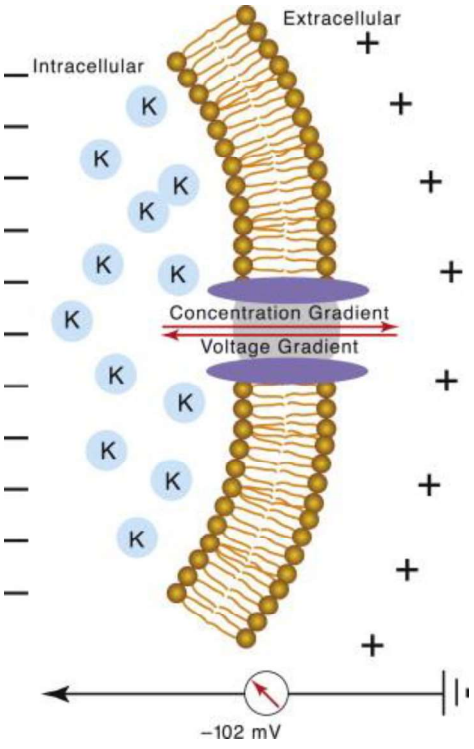
$$\mu_J = \mu_J^{\ominus} + RT \ln a_J$$

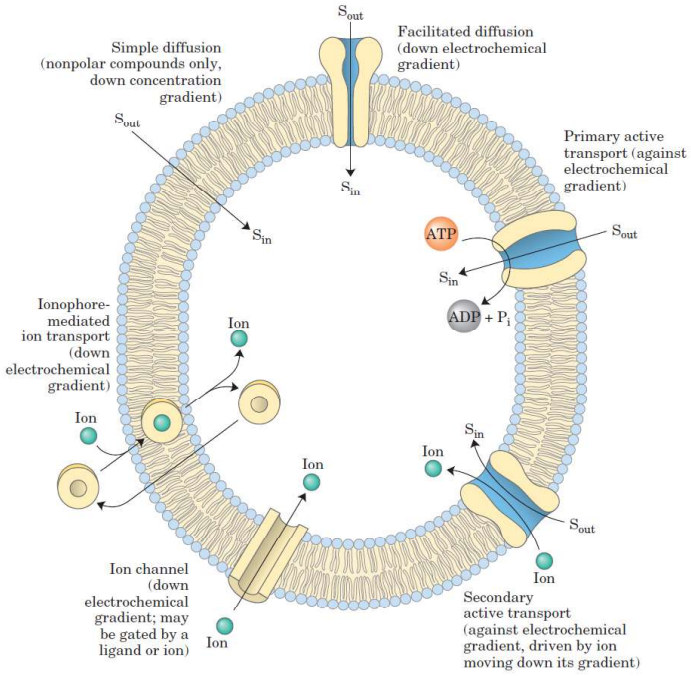
- The activity, $a$J, is a kind of effective concentration and is related to concentrations by multiplication bv an activity coefficient, γJ.

$$a_J = \gamma_J b_J$$

- where bj is the molality

# Passive and active transport of ions across biological membranes

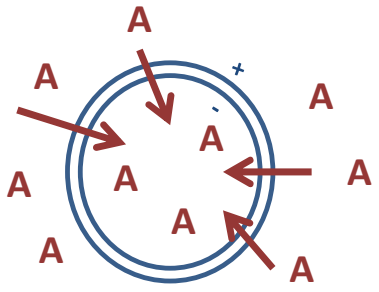


- The thermodynamic tendency to transport a species A through a biological cell membrane is partially determined by an activity gradient across the membrane, which results in a difference in molar Gibbs energy between the inside and the outside of the cell:

$$\Delta G_m = G_{m,in} - G_{m,out} = RT \ln \frac{a_{in}}{a_{out}}$$

- The equation implies that transport into the cell of either neutral or charged species is thermodynamically favorable if $a_{in} < a_{out}$ or, if we set the activity coefficients to 1, if $[A]_{in} < [A]_{out}$.



- However, an ion also needs to cross a membrane potential difference $\Delta\phi = \phi_{in} - \phi_{out}$ that arises from differences in Coulomb repulsions on each side of the bilayer.
- This potential difference is measured in volts (V, where 1 V = 1 J $C^{-1}$).
- We show in the following *Derivation* that the Gibbs energy of transfer of an ion of charge number *z* across a potential difference $\Delta\phi$ adds a term $zF\Delta\phi$ to the equation that formulates the thermodynamic tendency to transport a species A through a biological cell membrane:

$$\Delta G_m = RT \ln \frac{[A]_{in}}{[A]_{out}} + zF\Delta\phi$$

- where F is **Faraday's constant** (F = 96.485 kC $mol^{-1}$), the magnitude of electric charge per mole of electrons and z is the charge number or ions.

- Estimate the equilibrium membrane potential of a cell at 298 K by using the fact that the concentration of $K^+$ inside the cell is about 20 times that on the outside.
- Repeat the calculation, this time using the fact that the concentration of $Na^+$ outside the cell is about 10 times that on the inside.

- Because the cell is at equilibrium, set $\Delta G_m = 0$.
- z = +1 for both K and Na.

$$\Delta G_m = RT \ln \{[A]_{in} / [A]_{out}\} + zF\Delta\phi = 0$$
$$zF\Delta\phi = - RT \ln \{[A]_{in} / [A]_{out}\}$$

$$\Delta\phi = -\frac{RT}{zF} \ln \frac{[A]_{in}}{[A]_{out}}$$

- When $[K]_{in}/[K]_{out} = 20$

$$\Delta\phi = -\frac{(8.3145 \text{ J K}^{-1} \text{ mol}^{-1}) \times (298 \text{ K})}{9.648 \times 10^4 \text{ C mol}^{-1}} \ln 20$$
$$= -7.69 \times 10^{-2} \text{ V} = -76.9 \text{ mV}$$

The negative sign denotes that the inside has the lower potential.

- When $[Na]_{in}/[Na]_{out} = 0.10$

$$\Delta\phi = -\frac{(8.3145 \text{ J K}^{-1} \text{ mol}^{-1}) \times (298 \text{ K})}{9.648 \times 10^4 \text{ C mol}^{-1}} \ln 0.10$$
$$= 5.91 \times 10^{-2} \text{ V} = 59.1 \text{ mV}$$

The positive sign denotes that the outside has the lower potential.

- **Self-exercise:** Is the transport of Na ions across a cell membrane spontaneous when $[Na]_{in}/[Na]_{out} = 0.10$ and $\Delta\phi = 50$ mV?

$$\Delta G_m = RT \ln \frac{[A]_{in}}{[A]_{out}} + zF\Delta\phi$$

- Above equation implies that there is a tendency, called **passive transport**, for a species to move down concentration and membrane potential gradients.
- In **active transport**, a species moves against these gradients and the process is driven by its coupling to the exergonic hydrolysis of ATP.
- That is, when the sum of *RT*ln([A]in/[A]out) and *zF*Δϕ is positive, the overall Gibbs energy of transport can be made negative (and the process becomes spontaneous) by a large and negative Gibbs energy of ATP hydrolysis. It follows that the overall Gibbs energy of transport into a cell may be written as:

$$\Delta G_m = RT \ln \frac{[A]_{in}}{[A]_{out}} + zF\Delta\phi + \Delta_r G^{ATP}$$

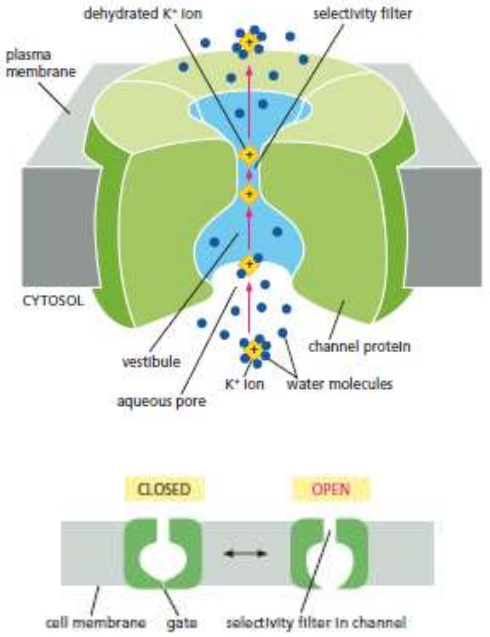
- where $\Delta_r G^{ATP}$ is the Gibbs energy of hydrolysis of ATP at specific concentrations of ATP, ADP, Pi, and hydronium ion.

# Ion channels and ion pumps

- The transport of ions into or out of a cell needs to be mediated (that is, involve other species) because charged species do not partition well into the hydrophobic environment of the membrane.
- There are two mechanisms for ion transport: by an **ion pump** or transport through a **channel**.

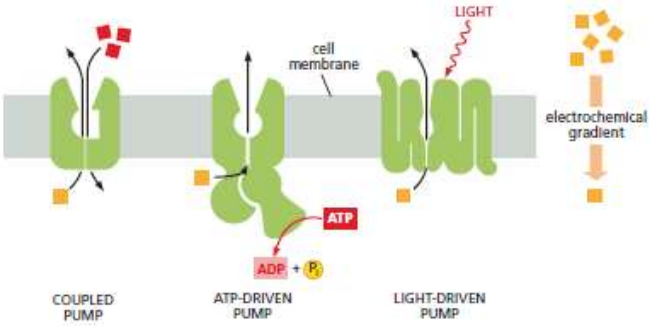
# Channels

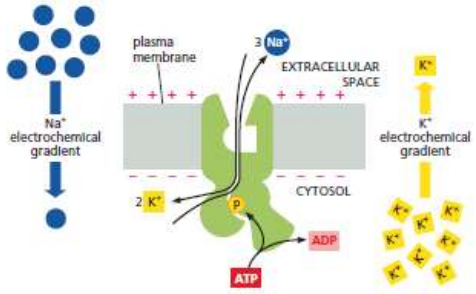
- Ion channel is a protein that creates a hydrophilic pore through which the ion can pass. It permits the movement of specific ions down a membrane potential gradient.
- They are highly selective, so there is a channel protein for $Ca^{+2}$, another for $Cl^{-}$, and so on.
- In a *voltage-gated channel*, the opening of the gate is triggered by a membrane potential, and in a *ligand-gated channel* the binding of an *effector* molecule to a specific receptor site on the channel initiates ion transport.



# Ion Pumps

- Ions such as H, Na, K, and Ca2 are often transported actively across membranes by integral proteins called **ion pumps**.
- Ion pumps are molecular machines that work by adopting conformations that are permeable to one type of ion but not others, depending on the state of phosphorylation of the protein.
- Because protein phosphorylation requires dephosphorylation of ATP, the conformational change that opens or closes the pump is endergonic and requires the use of energy stored during metabolism.




$Na^+$ - $K^+$ pump is an ATP driven pump that keeps the $Na^+$ concentration in the cytosol about 10–30 times lower than in the extracellular fluid and the $K^+$ concentration about 10–30 times higher.

# CASE STUDY: Action potentials

- A striking example of the importance of ion channels is their role in the propagation of impulses by neurons, the fundamental units of the nervous system. Here we give a thermodynamic description of the process.
- The cell membrane of a neuron is more permeable to $K^+$ ions than to either $Na^+$ or $Cl^-$ ions. The key to the mechanism of action of a nerve cell is its use of $Na^+$ and $K^+$ channels to move ions across the membrane, modulating its potential.
- For example, the concentration of $K^+$ inside an inactive nerve cell is about 20 times that on the outside, whereas the concentration of $Na^+$ outside the cell is about 10 times that on the inside.
- The difference in concentrations of ions results in a transmembrane potential difference of about -62 mV. This potential difference is also called the **resting potential** of the cell membrane.

- To estimate the resting potential, we need to understand that the cell is never at equilibrium.
- Ions continually cross the membrane, which is more permeable to some ions than others.
- To take into account membrane permeability, we use the **Goldman equation** to calculate the resting potential:

$$\Delta\phi = \frac{RT}{F} \ln\left(\frac{\sum_i P_i[M_i^+]_{out} + \sum_j P_j[X_j^-]_{in}}{\sum_i P_i[M_i^+]_{in} + \sum_j P_j[X_j^-]_{out}}\right)$$

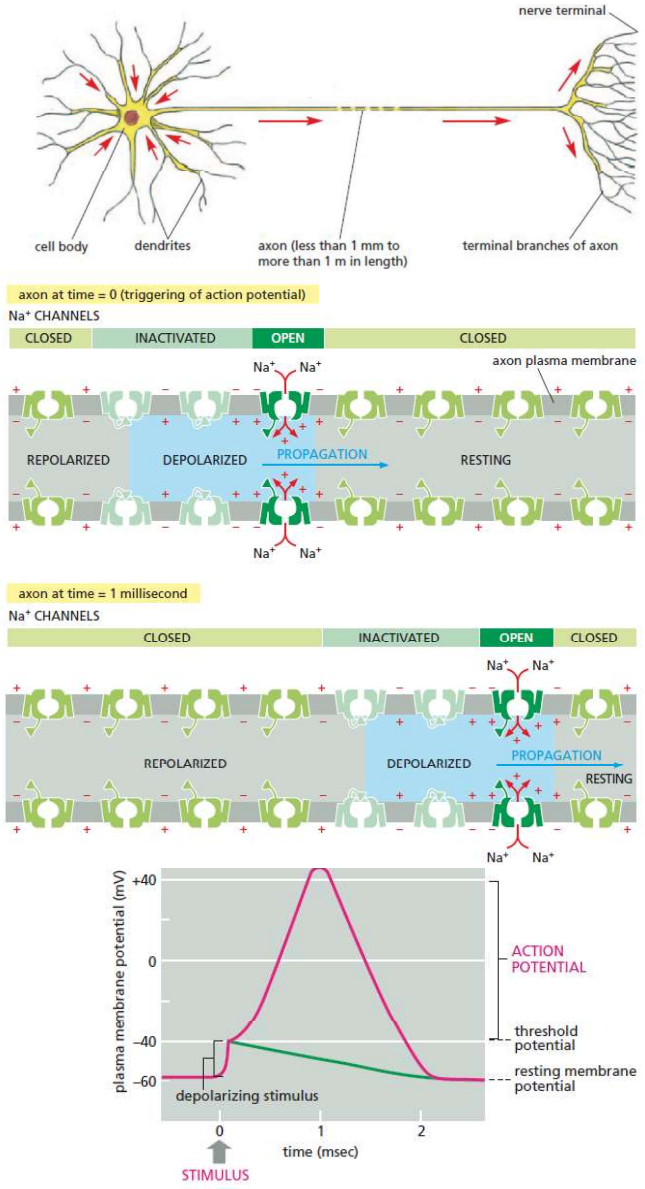
- where $P_i$ and $P_j$ are the relative permeabilities, respectively, for the cation $M_i^+$ and the anion $X_j^-$ and the sum is over all ions.

- For example, taking the permeabilities of the K, Na, and Cl ions as $P_{K+} = 1.0$, $P_{Na+} = 0.04$, and $P_{Cl-} = 0.45$, respectively, the temperature as 298 K, and the concentrations as $[K]_{in} = 400$ mmol $L^{-1}$, $[Na]_{in} = 50$ mmol $L^{-1}$, $[Cl]_{in} = 50$ mmol $L^{-1}$, $[K]_{out} = 20$ mmol $L^{-1}$, $[Na]_{out} = 500$ mmol $L^{-1}$, and $[Cl]_{out} = 560$ mmol $L^{-1}$ :

$$\Delta\phi = \frac{(8.3145\ \text{J K}^{-1}\ \text{mol}^{-1}) \times (298\ \text{K})}{9.648 \times 10^4\ \text{J mol}^{-1}} \times \ln\left(\frac{(1.0 \times 20) + (0.04 \times 500) + (0.45 \times 50)}{(1.0 \times 400) + (0.04 \times 50) + (0.45 \times 560)}\right)$$
$$= -6.0 \times 10^{-2}\ \text{V} = -60\ \text{mV}$$

We see that the Goldman equation leads to an estimate that agrees well with the experimental value of -62 mV.

- The transmembrane potential difference plays a particularly interesting role in the transmission of nerve impulses. Upon receiving an impulse, which is called an **action potential**, a site in the nerve cell membrane becomes transiently permeable to $Na^+$ and the transmembrane potential changes.
- To propagate along a nerve cell, the action potential must change the transmembrane potential by at least 20 mV to values that are less negative than -40 mV.
- Propagation occurs when an action potential at one site of the membrane triggers an action potential at an adjacent site, with sites behind the moving action potential relaxing back to the resting potential.



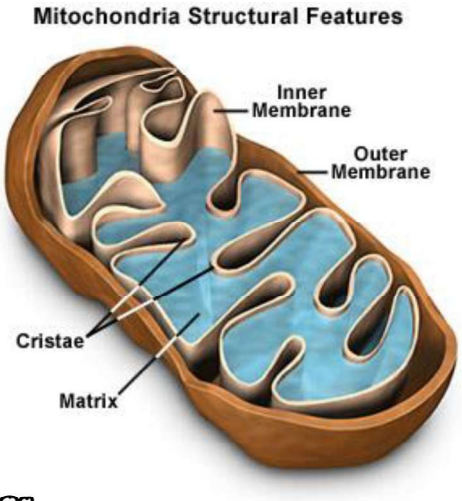
# Thermodynamics of Transport of Electrons across Membranes

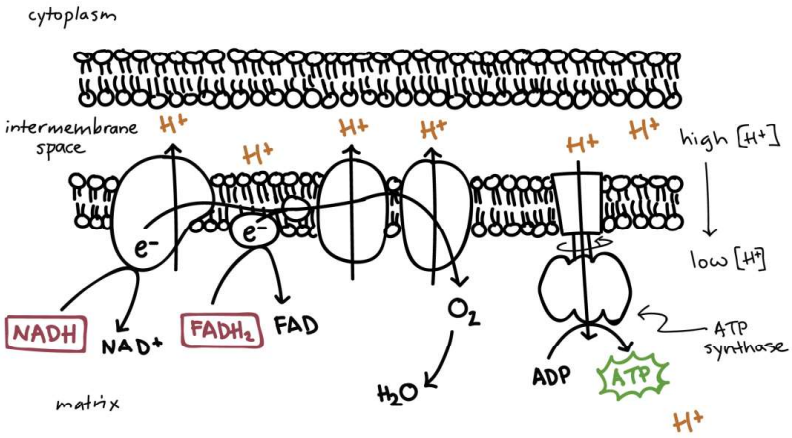
- Electron transfer between protein-bound cofactors or between proteins plays a role in a number of biological processes, such as the oxidative breakdown of foods, photosynthesis, nitrogen fixation, the reduction of atmospheric $N_2$ to $NH_3$ by certain microorganisms, and the mechanisms of action of oxidoreductases, which are enzymes that catalyze redox reactions.
- Here, we will the redox reactions associated with the aerobic oxidation of glucose.

$$C_6H_{12}O_6(s) + 6\ O_2(g) \underset{\text{Photosynthesis}}{\overset{\text{Aerobic oxidation}}{\rightleftharpoons}} 6\ CO_2(g) + 6\ H_2O(l)$$

# Electron Transport Chain

- The centrally important processes of biochemistry include the electrochemical reactions between proteins in the mitochondrion of the cell, for they are responsible for delivering the electrons extracted from glucose to water.



- Some organic co-factors and metal centers in proteins act as electron transfer agents in a number of biological processes; we need to be able to predict which species is reduced or oxidized in a redox reaction.
- A redox reaction is the outcome of the loss of electrons from one species and their gain by another species.
- Any redox reaction may be expressed as the difference of two reduction **half reactions**.

Reduction of $Cu^{2+}$: $Cu^{2+}(aq) + 2\ e^- \longrightarrow Cu(s)$
Reduction of $Zn^{2+}$: $Zn^{2+}(aq) + 2\ e^- \longrightarrow Zn(s)$
Difference: $Cu^{2+}(aq) + Zn(s) \longrightarrow Cu(s) + Zn^{2+}(aq)$

- Express the oxidation of nicotinamide adenine dinucleotide (NADH), which participates in aerobic metabolism, to $NAD^+$ by oxygen, when the latter is reduced to $H_2O_2$, in aqueous solution as the difference of two reduction half reactions.
- The overall reaction is

$$NADH(aq) + O_2(g) + H^+(aq) \rightarrow NAD^+(aq) + H_2O_2(aq)$$

$$O_2(g) + 2\ H^+(aq) + 2\ e^- \longrightarrow H_2O_2(aq)$$

$$NAD^+(aq) + H^+(aq) + 2\ e^- \longrightarrow NADH(aq)$$

# Standard Potential

- Standard potential is a measure of the ability to accept or donate electrons.
- A couple with a low standard potential has a thermodynamic tendency to reduce a couple with a high standard potential.
- More briefly: *low reduces high* and, equivalently, *high oxidizes low.*
- *Electrons move from low standard potential to high standard potential.*
- *If standard potential of a molecule is low (negative) it is a good electron donor.*
- *If standard potential of a molecule is high (positive) it is a good electron acceptor.*

$$E^{\oplus}\ (NADH) = -0.32\ V$$

$$E^{\oplus}\ (O_2) = 0.82\ V$$

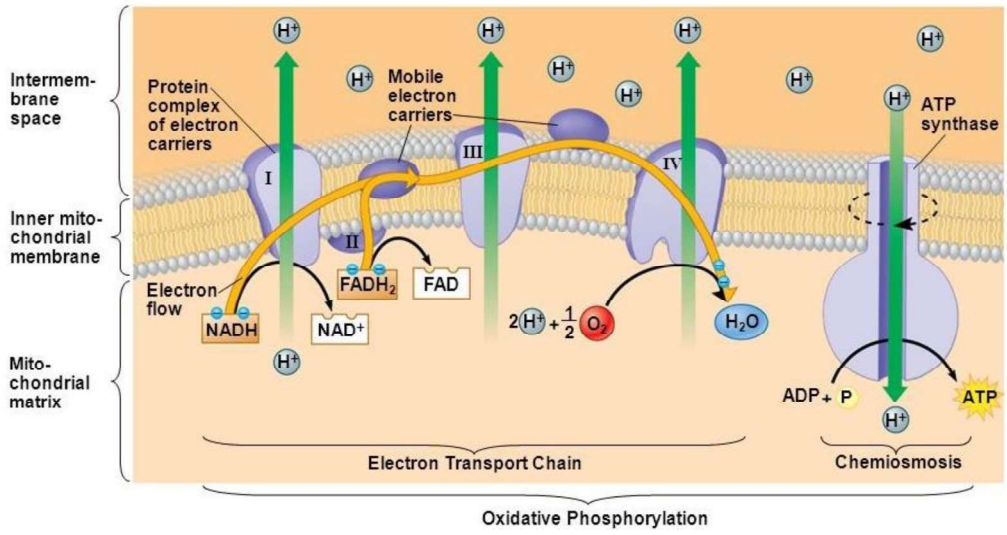
- The half-reactions for the oxidation of glucose and the reduction of $O_2$ are:

$$C_6H_{12}O_6(s) + 6\ H_2O(l) \longrightarrow 6\ CO_2(g) + 24\ H^+(aq) + 24\ e^-$$
$$6\ O_2(g) + 24\ H^+(aq) + 24\ e^- \longrightarrow 12\ H_2O(l)$$

- We see that the exergonic oxidation of one $C_6H_{12}O_6$ molecule requires the transfer of 24 electrons to six $O_2$ molecules.
- However, the electrons do not flow directly from glucose to $O_2$. In biological cells, glucose is oxidized to $CO_2$ by $NAD^+$ and FAD during glycolysis and the citric acid cycle:

$$C_6H_{12}O_6(s) + 10\ NAD^+ + 2\ FAD + 4\ ADP + 4\ P_i + 2\ H_2O \longrightarrow$$
$$6\ CO_2 + 10\ NADH + 2\ FADH_2 + 4\ ATP + 6\ H^+$$

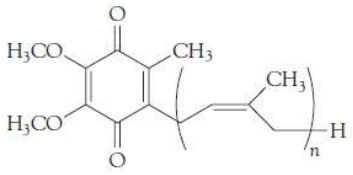
- In the **electron transport chain**, electrons from the powerful reducing agents NADH and FADH2 pass through four membrane-bound protein complexes and two mobile electron carriers before reducing $O_2$ to $H_2O$.
- We shall see that the electron transfer reactions drive the synthesis of ATP at three of the membrane protein complexes.



- The respiratory chain begins in complex I (NADH-Q oxidoreductase), where NADH is oxidized by coenzyme Q (Q) in a two-electron reaction:

$$H^+ + NADH + Q \xrightarrow{\text{Complex I}} NAD^+ + QH_2$$

$$E^{\ominus} = +0.42\ \text{V},\ \Delta_r G^{\ominus} = -81\ \text{kJ mol}^{-1}$$



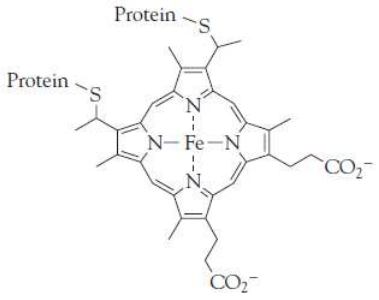

Coenzyme Q, Q

- Additional Q molecules are reduced by FADH2 in complex II (succinate-Q reductase):

$$FADH_2 + Q \xrightarrow{\text{Complex II}} FAD + QH_2 \qquad E^{\oplus} = +0.015\ \text{V},\ \Delta_r G^{\ominus} = -2.9\ \text{kJ mol}^{-1}$$

- Reduced Q migrates to complex III (Q-cytochrome *c* oxidoreductase), which catalyzes the reduction of the protein cytochrome *c* (Cyt *c*).
- Cytochrome *c* contains the heme *c* group, the central iron ion of which can exist in oxidation states 3 and 2.
- The net reaction catalyzed by complex III is

$$QH_2 + 2\ Fe^{3+}(\text{Cyt } c) \xrightarrow{\text{Complex III}} Q + 2\ Fe^{2+}(\text{Cyt } c) + 2\ H^+$$
$$E^{\oplus} = +0.15\ \text{V},\ \Delta_r G^{\oplus} = -30\ \text{kJ mol}^{-1}$$




5 Heme *c*

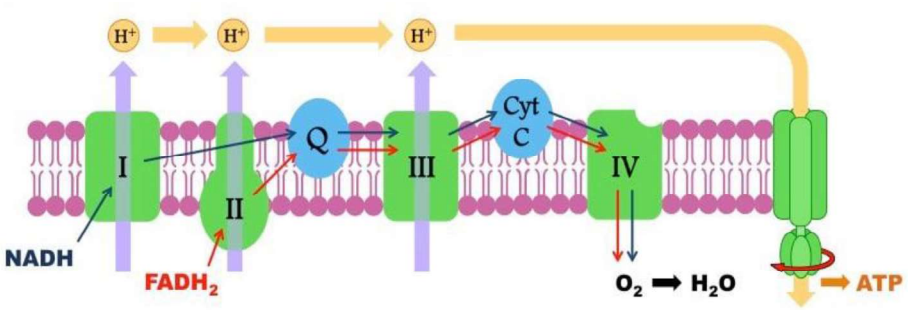
- Reduced cytochrome *c* carries electrons from complex III to complex IV (cytochrome *c* oxidase), where $O_2$ is reduced to $H_2O$:

$$2\ Fe^{2+}(Cyt\ c) + 2\ H^+ + \tfrac{1}{2}\ O_2 \xrightarrow{\text{Complex IV}} 2\ Fe^{3+}(Cyt\ c) + H_2O$$
$$E^{\oplus} = +0.815\ V,\ \Delta_r G^{\oplus} = -109\ kJ\ mol^{-1}$$

Oxygen has the highest (most positive) standard reduction potential which means that it is most likely to accept electrons from other carriers. That is precisely why it is found at the end of the ETC.

- The reactions that occur in complexes I, III, and IV are sufficiently exergonic to create a high $H^+$ concentration in the intermembrane space.
- Flow of H+ ions down their electrochemical gradient drive the synthesis of ATP in the process called **oxidative phosphorylation**:

$$ADP + P_i + H^+ \longrightarrow ATP \qquad \Delta_r G^{\oplus} = +30 \text{ kJ mol}^{-1}$$

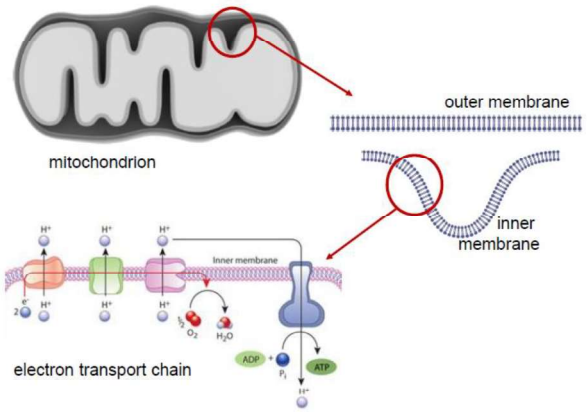


- We have seen in previous class that the phosphorylation of ADP to ATP can be coupled to the exergonic dephosphorylation of other molecules.
- Indeed, this is the mechanism by which ATP is synthesized during glycolysis and the citric acid cycle.

**Substrate level phosphorylation**

- However, **oxidative phosphorylation** operates by a different mechanism.

- The protein complexes associated with the electron transport chain span the inner membrane, and phosphorylation takes place in the intermembrane space.
- The Gibbs energy of the reactions in complexes I, III, and IV is first used to do the work of moving protons across the mitochondrial membrane.
- The complexes are oriented asymmetrically in the inner membrane so that the protons abstracted from one side of the membrane can be deposited on the other side.
- For example, the oxidation of NADH by Q in complex I is coupled to the transfer of four protons across the membrane.
- The coupling of electron transfer and proton pumping in complexes III and IV contribute further to a gradient of proton concentration across the membrane. Then the enzyme H-ATPase uses the energy stored in the proton gradient to phosphorylate ADP to ATP.
- Experiments show that 11 molecules of ATP are made for every three molecules of NADH and one molecule of $FADH_2$ that are oxidized by the respiratory chain.
- The ATP is then hydrolyzed on demand to perform useful biochemical work throughout the cell.



# Chemiosmotic Theory

- The **chemiosmotic theory** proposed by Peter Mitchell explains how H-ATPases use the energy stored in a transmembrane proton gradient to synthesize ATP from ADP.
- Thus, the ATPase uses the enery available from the movement of $H^+$ gradient across inner membrane of mitochondrion to mictochondrion matrix to phopshorylate ADP to ATP.
- We can calculate the gibbs energy available for phosphorylation of ADP to ATP by:

$$\Delta G_m = RT \ln \frac{[H^+]_{in}}{[H^+]_{out}} + F\Delta\phi$$

- where $\Delta\phi = \phi_{in} - \phi_{out}$ is the membrane potential difference and we have used $z = 1$.

- After using ln $[H^+]$ = (ln 10) log [H] and substituting $\Delta pH = pH_{in} - pH_{out} = \log [H^+]_{in} - \log [H^+]_{out}$, it follows that:

$$\Delta G_m = F\Delta\phi - (RT \ln 10)\Delta pH$$

- In the mitochondrion, ΔpH ≈ 1.4 and Δϕ ≈ 0.14 V, so it follows from the above equation that Δ*G*m = 21.5 kJ $mol^{-1}$.
- Because 31 kJ $mol^{-1}$ is needed for phosphorylation, we conclude that at least 2 mol $H^+$ (and probably more) must flow through the membrane for the phosphorylation of 1 mol ADP.