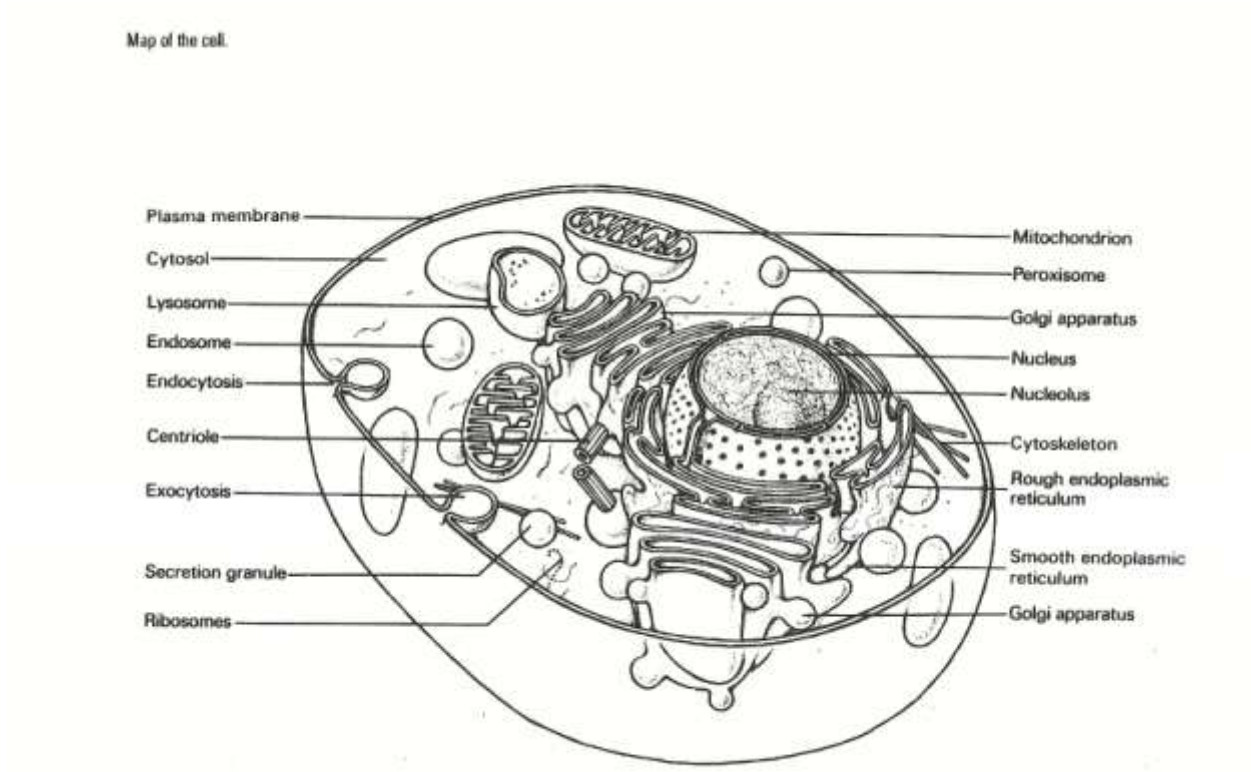
Lipids, membranes and chemiosmosis

The figure below illustrates the interior of a eucaryotic cell such as found in all higher animals.



Its outer limit is the *plasma membrane* and it contains many different types of structures each of which is also highly membranous. The *nucleus* is surrounded by a membranous structure with large holes depicted by large dots through which messenger RNAs (ribonucleic acids), transcribed from genes in the nucleus, are transported to the rough *endoplasmic reticulum* where they are translated into proteins. This is depicted by strings of small dots that represent *ribosomes* sequentially reading the messenger RNAs and translating them into proteins. These proteins are subsequently released from the ribosomes and are transported into the *Golgi apparatus* for post-translational processing. They may subsequently be sequestered in secretion granules, that are small intracellular membranous compartments that can fuse with the plasma membrane in a process called *exocytosis* whereby their contents are released to the exterior of the cell. *Endocytosis* is the reverse of this process and produces *endosomes* that contain molecules from outside the cell. These may be digested in *lysosomes* with which the endosomes fuse. All of these structures are dynamic, membranous structures. The membranes are lipid bilayers with a thickness of 6 to 10 nm (nanometers).

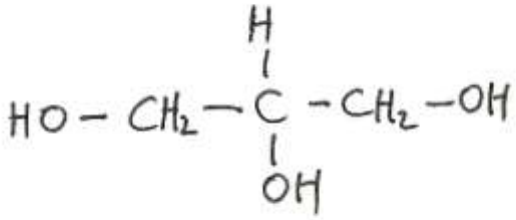
Obviously, the picture in the figure is not a photo-micrograph made with a light microscope because such photo-micrographs are restricted to larger than micron scale, and nanometer scale structures are depicted in the picture. The picture is a deduced representation

made from scores of electron-micrographs made with the electron-microscope. The revolution in electron microscopy techniques in the 1940s through the 1960s made this picture possible. This picture is a static picture as well. In a living cell the membranous structures are in motion and undergo fusions and fissions that constantly change their number, size and shape. Of special interest for us in this text are the *mitochondria*, two of which are shown in the picture (a typical cell may contain hundreds of mitochondria). These are the power plants of the cell in which a variety of processes take place. Before a detailed account of their structure and processes is given, however, a digression into membrane structure is required.

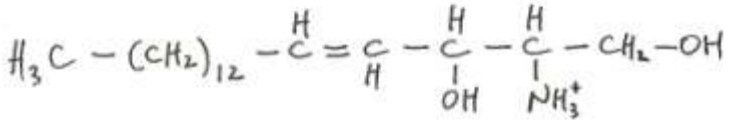
Lipids and membranes

Membranes self-assemble from lipid molecules and proteins. Membranes are highly selective permeability barriers. Except for the processes of exocytosis and endocytosis mentioned above (in fact, both of these processes require the aid of a structural protein, *clathrin*), transport across membranes is mediated by embedded proteins. These proteins confer on the membrane its distinctive features. They serve as pumps, gates, transporters, receptors, energy tranducers, structural elements and enzymes. The ratio of protein to lipid by weight in a membrane can range from 3 to 1 to 1 to 5. In *myelin*, a nerve fiber insulator, the membrane is 18% protein, the plasma membrane is 50% protein and the inner mitochondrial membrane is 75% protein.

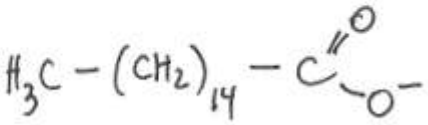
The three major kinds of membrane lipids are *phospholipids*, *glycolipids* and *cholesterol*. Phospholipids are derived from the three carbon alcohol *gycerol*

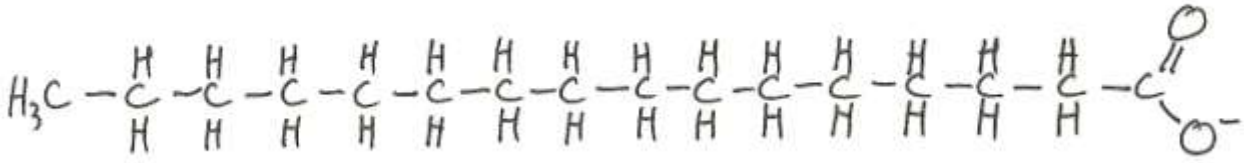


and glycolipids are derived from the more complex alcohol *sphingosine*

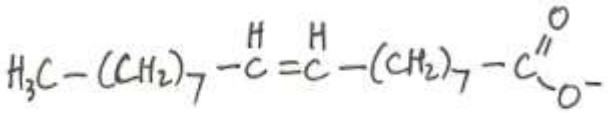


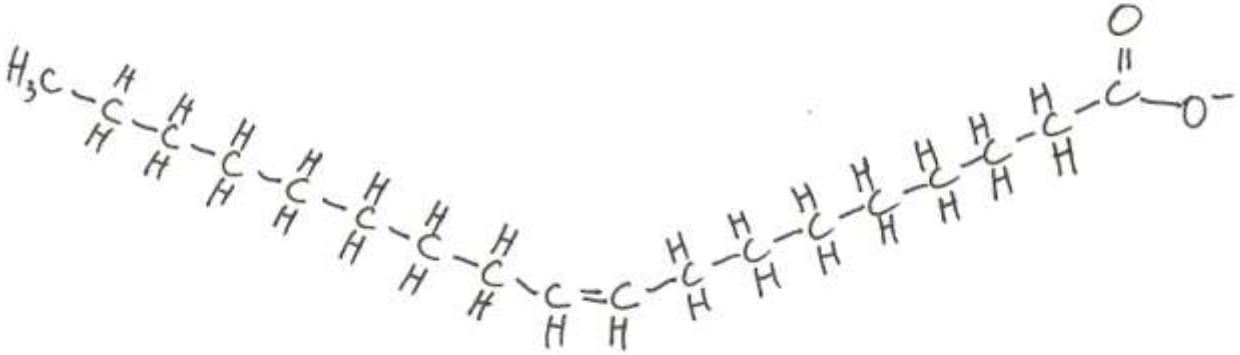
Those derived from glycerol were called *phosphoglycerides* but are now called *glycerophospholipids* and contain two *fatty acid* side chains and a phosphorylated alcohol such as *choline*, *ethanolamine*, *inositol* or *serine*. Typically, the fatty acid chains in phospholipids and glycolipids contain between 14 and 24 carbon atoms. Most common are 16 (*palmitate*)





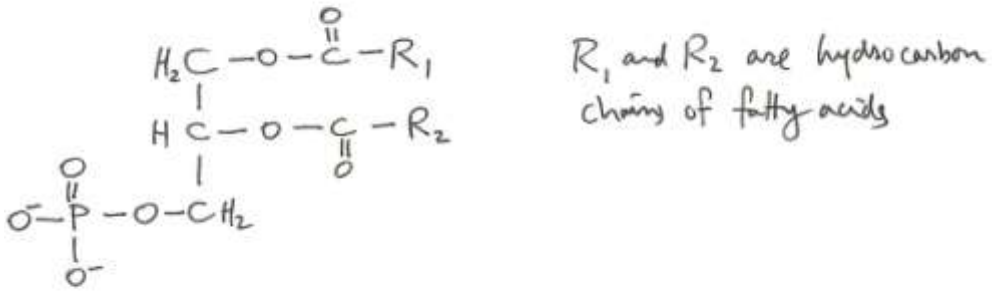
and 18 (*oleate*)



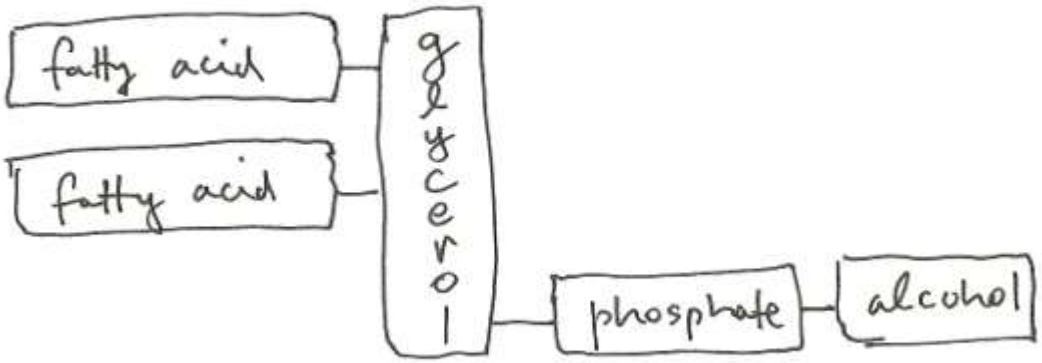


Oleate contains one unsaturated carbon-carbon double bond in the middle of the chain. It is in the *cis* conformation and this is almost always the case with unsaturated biological fatty acid chains. The bend this causes in the fatty acids creates a disruption of regular order in the membrane resulting in a more fluid membrane interior.

In glycerophospholipids, the carboxyl groups of the fatty acids are esterified to the C-1 and C-2 hydroxyl groups of glycerol. In the simplest glycerophospholipid, the C-3 hydroxyl of glycerol is esterified to phosphate, forming *diacylglycerol-3-phosphate*

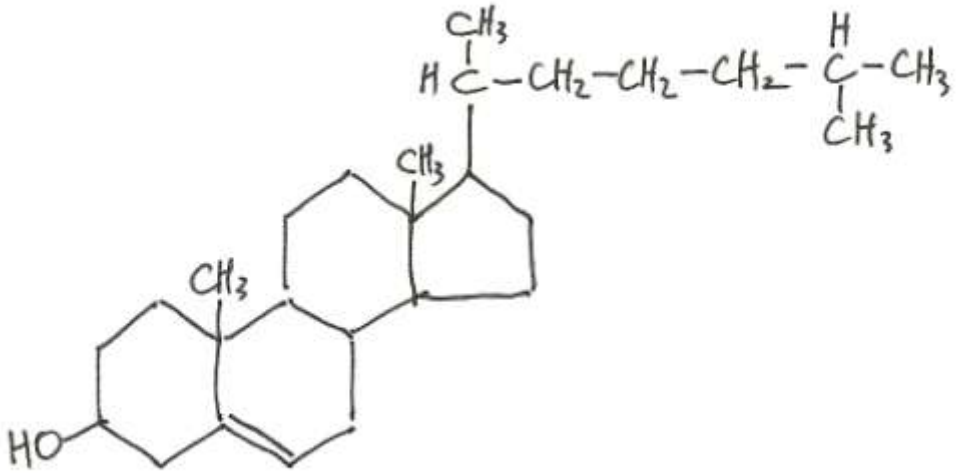


Membranes contain only a small amount of this compound. The major glycerophospholipids are derivatives of this compound in which the phosphate group is esterified to the hydroxly group of one of several alcohols



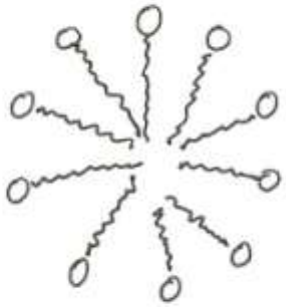
Glycolipids are based on sphingosine. This molecule contains an amino group and already has one fatty acid chain. A second fatty acid carboxly group is linked by an amide bond to this amino group. In *sphingomyelin, phosphoryl choline* is esterified to the hydroxly group of sphingosine. In glycolipids generally, the unit linked to the hydroxly group of sphingosine is a simple sugar or a small sugar polymer. If *glucose* or *galactose* is used then the result is a

cerebroside and if a branched sugar chain with up to seven units is used then the result is a *ganglioside*. Another important neutral lipid in some membranes is cholesterol a *sterol*

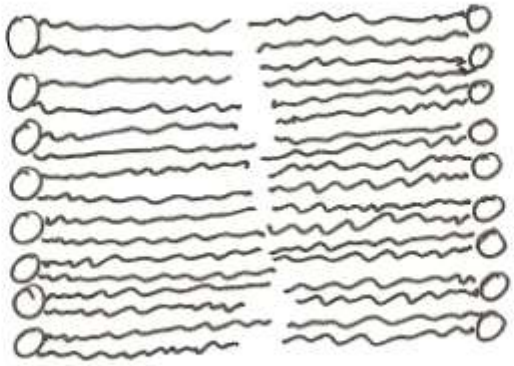


It is found in most eucaryotes but not in procaryotes. The single O that it contains derives from atmospheric O_2. Cholesterol must have evolved after the earth's atmosphere accumulated O_2. Eucaryotic plasma membranes are often rich in cholesterol but the membranes of sub-cellular organelles have less. The evolutionary theory that mitochondria and cholorplasts in eucaryotes arose from captured procaryotic ancestors suggests that their membranes should contain no cholesterol.

Single fatty acids *self-assemble* [link] into micelles

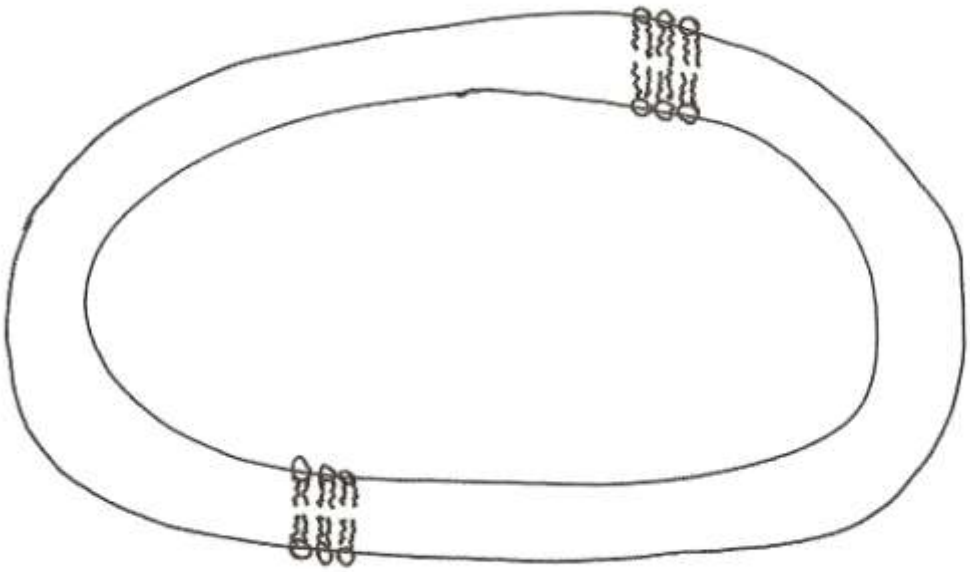


Phospholipids and glycolipids, on the other hand generally form lipid bilayers (also called bimolecular sheets)

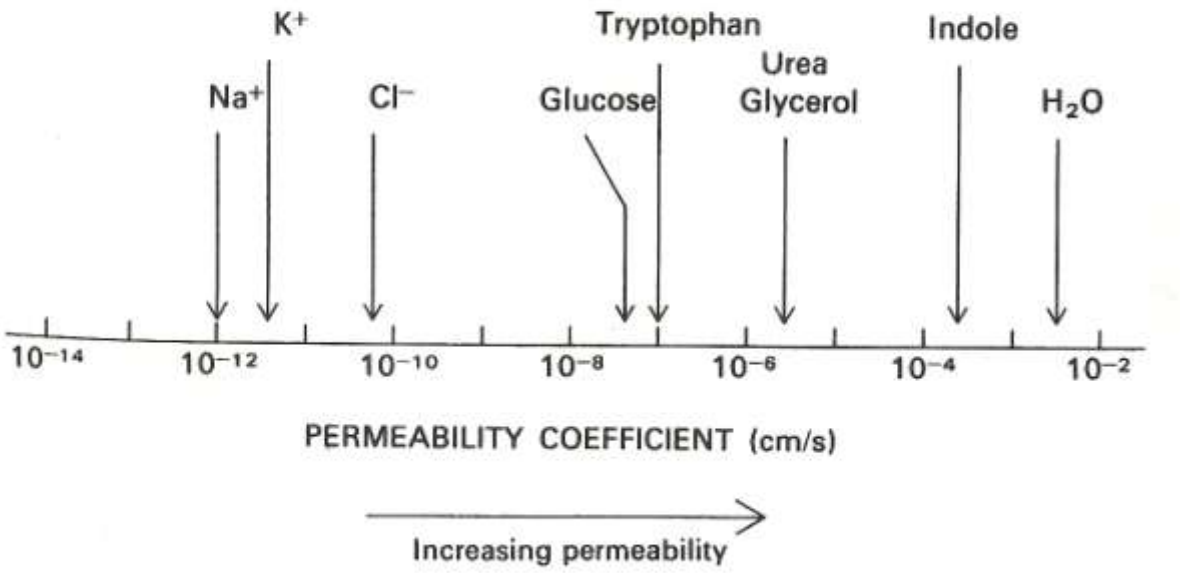


These structures are the result of the *amphipathic* nature of these molecules. That is, they contain both a *hydrophobic* region, the fatty acid chains, and a *hydrophilic* region, the charged phosphate groups and in some cases charged amine groups. The essence of self-assembly has to do with the way water molecules interact with the two regions of the lipid molecules. Water molecules are polar and can hydrogen bond with other molecules. The hydrophobic fatty acid regions of lipids interact more favorably with other hydrophobic molecules then with water and the hydrophilic

regions interact strongly with water. To accommodate both tendencies, fatty acids form micelles in which the hydrophilic carboxyl groups are on the outside in direct contact with water and the hydrophobic fatty acid chains are on the inside interacting with each other. Micelles are usually less than 20 nm in diameter. Phospholipids and glycolipids have two fatty acid side chains and are, therefore, too bulky to readily form micelles without exposing the fatty acids chains to water from the outside. Instead, a bilayer forms in which all of the fatty acids are inside the bilayer, sequestered away from water while the charged hydrophobic ends are on the outside of the bilayer



The bilayers tend to close up making a closed compartment that can have a diameter as large as mm's (millimeter). This is a much more versatile structure than a micelle. Bilayers tend to be highly impermeable to ions and polar molecules, with the conspicuous exception of water molecules. Permeability coefficients are measured in cm/s (centimeters per second). The table below shows the great range of values observed

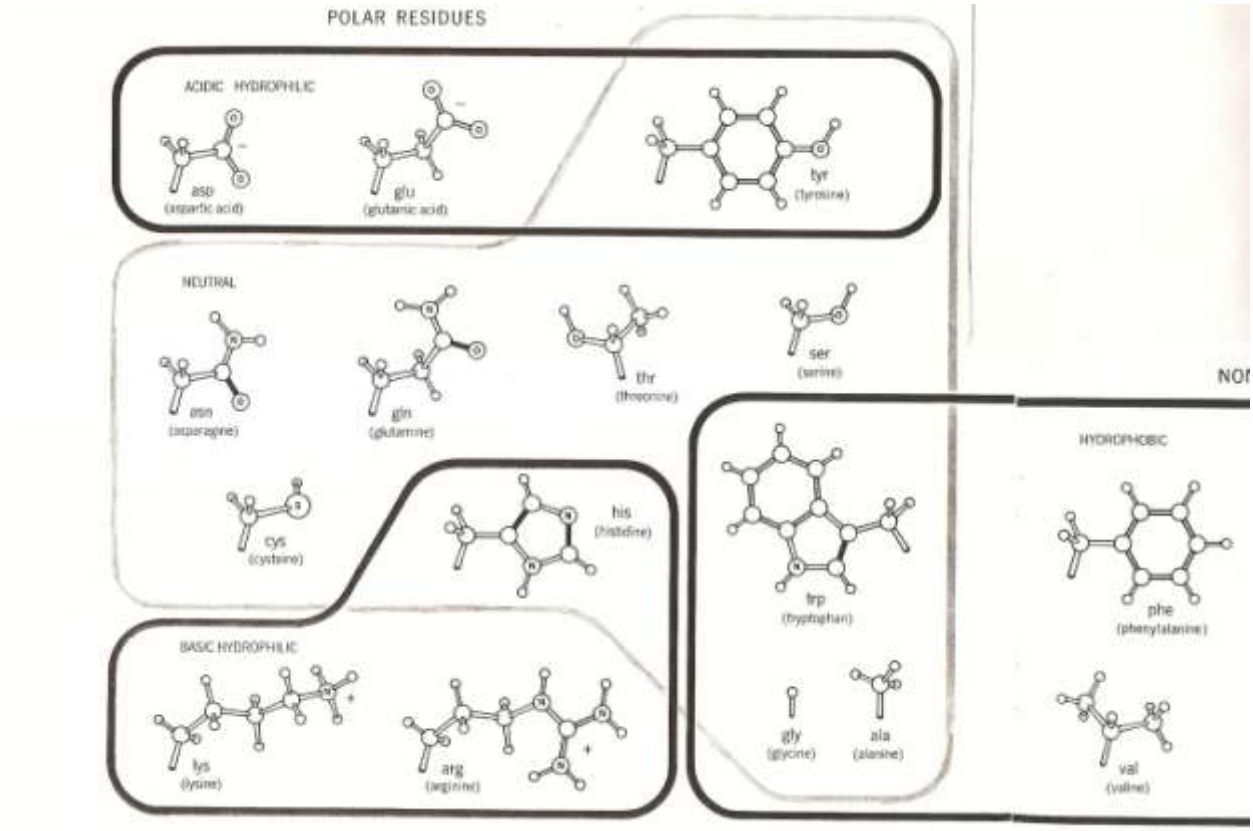


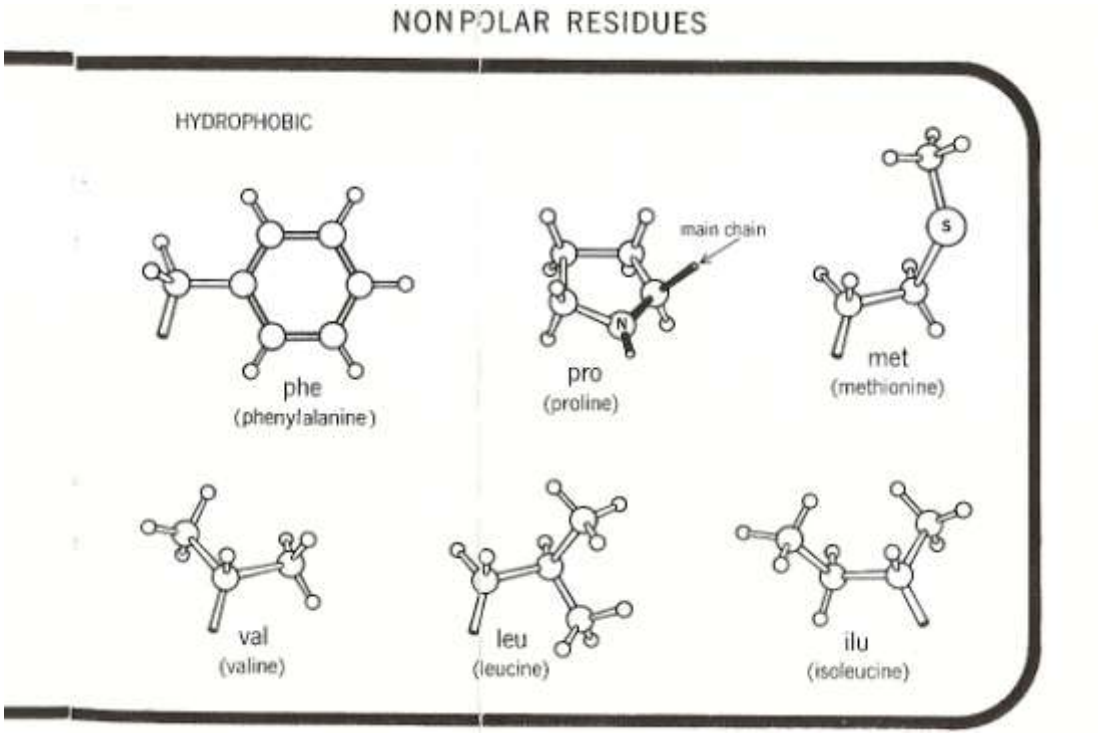
For an ion such as Na^+ (sodium) the incredibly low permeability compared to H_2O results from the solvation shell of water molecules around Na^+ in the aqueous milieu. This shell would have to be removed in order for Na^+ to permeate the bilayer. This is highly unfavorable energetically. The permeability of H_2O is a result of its very small size and its high thermal velocity at physiological temperatures, about 4×10^4 cm/s (this speed is equivalent to 1440 km/hr (kilometer per hour).

When the bilayer closes into a compartment so that there are no edges exposing fatty acid side chains to water, the resulting structure is asymmetric. Sphingomyelin and phosphatidyl choline are preferentially located in the outer layer while phosphatidyl ethanolamine and phosphatidyl serine are preferentially located in the inner layer in the case of red blood cells. This brings up an important fact regarding the nature of the association of lipid molecules in the bilayer. It is not a covalent arrangement. The fatty acid side chains of different lipid molecules interact by hydrophobic forces and van der Waals forces, whereas the charged, hydrophilic ends interact with water molecules by ionic forces and hydrogen bonds.

The individual lipid molecules are free to move laterally within the bilayer with a diffusion constant of about 10^{-8} cm^2/s. Thus, a lipid molecule can diffuse, on the average, a distance of one mm (micron) in one second. This is roughly the size scale of the mitochondria. Nevertheless, a lipid molecule is very unlikely to flip-flop from one layer of the bilayer to the other. It takes a phospholipid 10^9 times longer to flip-flop across the membrane than it does to laterally diffuse 5 nm (which takes 6 microseconds). Thus, the asymmetry of location of lipid species in the bilayer is well preserved in time.

Proteins are associated with lipids in many different ways. Some are peripherally associated with the hydrophilic exterior/interior of the bilayer compartment. These proteins tend to have lots of charged amino acid residues.





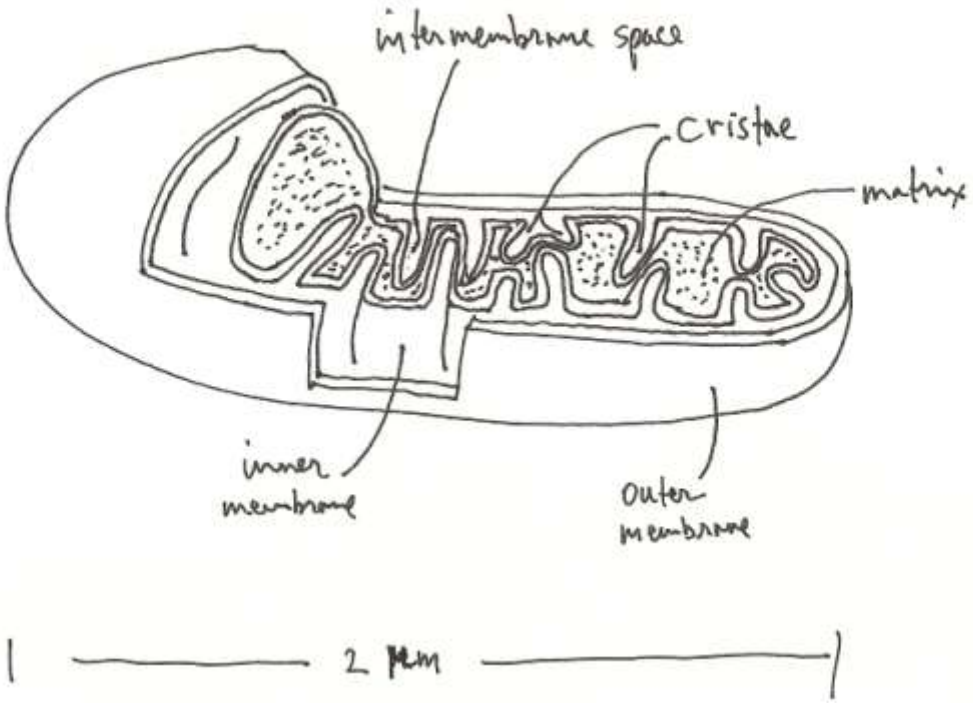
The free energy barrier for a protein to cross the bilayer is even greater than for a lipid molecule to flip-flop across. Other proteins are deeply embedded in the bilayer because of extensive stretches of hydrophobic amino acid residues that make these stretches hydrophobic and lipid-like as well. Some proteins strands traverse back and forth across the bilayer many times. Proteins can laterally diffuse in the membrane almost as well as lipids can but they are generally bigger than lipids and do so more slowly. Other proteins are linked to other molecules by groups that are external to the lipid phase of the membrane and this can greatly reduce their laterally mobility.

Complexes of proteins that are associated with the membrane occur and possess well maintained positions relative to the membrane's interior/exterior orientation, i.e. some of the proteins may be peripheral on the inside or the outside while others are embedded, either crossing the bilayer altogether or situated closer to the exterior or the interior side of the membrane compartment. These elaborate arrangements self-assemble from the proteins that are synthesized elsewhere in the cell (endoplasmic reticulum and Golgi apparatus) and find their way to the correct associations in the correct membrane by diffusion and specific identification labels that may be enzymatically removed once the proteins are in the correct position. Many proteins are facilitated in their association with the membrane by *chaperone* proteins that require the energy of ATP (adenosine triphosphate) to do their jobs.

Cholesterol associates with the fatty acid side chains of the lipids in a bilayer. By disrupting the interactions of saturated fatty acids chains with each other, cholesterol actually promotes fluidity of the lipid interior of the bilayer. However, too much cholesterol can have the reverse effect.

Electron transport chains

Let's look more closely at the mitochondria

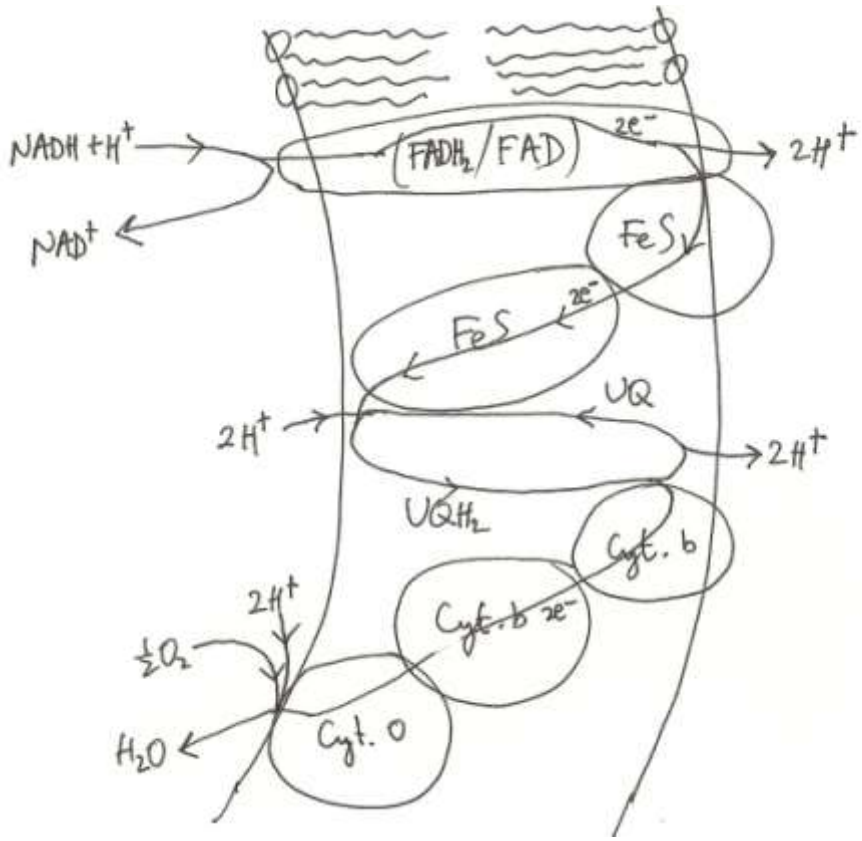


They are the power plants of the cell. They have the shape of a cylinder with a size of roughly 1.0 mm diameter and 2.0 mm length. They have two membranes, a smooth outer membrane and an extensively invaginated inner membrane. The invaginations are called *cristae*. The proteins mediating electron transport and ATP synthesis are bound to the inner membrane. The outer membrane contains the protein *porin*, a non-specific pore that permits the diffusive passage through the membrane of molecules up to 10 kd (kilo-daltons). This includes coenzymes, small peptides and small oligonucleotides. The inner membrane contains ~ 75% proteins and is freely permeable to only O_2, CO_2 and H_2O. It contains proteins for electron transport, ATP synthesis and metabolite transport. These proteins are embedded in the inner membrane lipid interior.

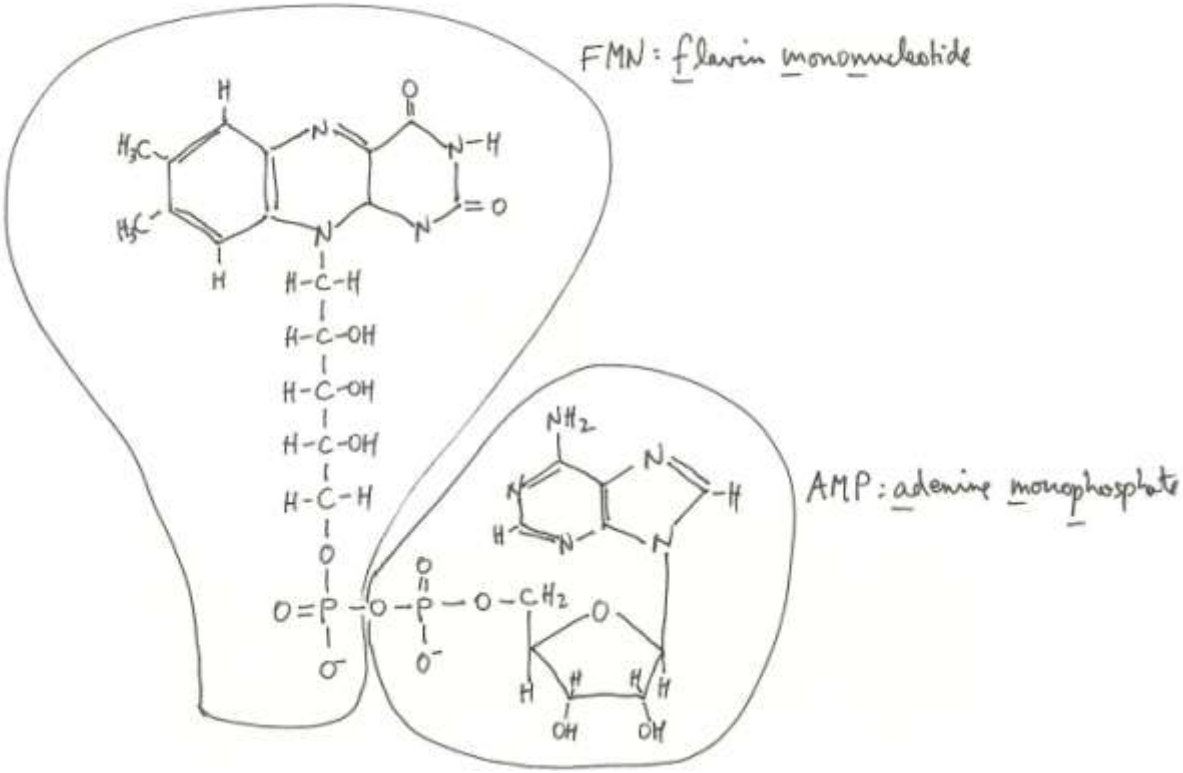
Contained within the compartment surrounded by the inner membrane is a gel-like *matrix* rich in proteins for pyruvate dehydrogenase, the citric acid cycle, fatty acid oxidation and for the mitochondion's genetic machinery, as well as mitochondrial DNA (deoxyribonucleic acid), RNA and *ribosomes*. While the cytoplasm of the cell is an aqueous solution of many substances, the matrix of the mitochondria is so jam packed with proteins and other molecules that there is room left for relatively few water molecules, creating a gel rather than a solution. The evolutionary theory for the origin of mitochondria in eucaryotes as captured procaryotic symbiotes is bolstered by their apparent retention of genetic machinery for the synthesis of some, but not all, of the mitochondrial proteins. This machinery includes ribosomes that are more similar to those of contemporary procaryotes then they are to eucaryotic ribosomes that occur in the rough endoplasmic reticulum of the eucaryotic cell. A similar state of affairs also applies to chloroplasts in plant cells.

Electron transport in bacteria

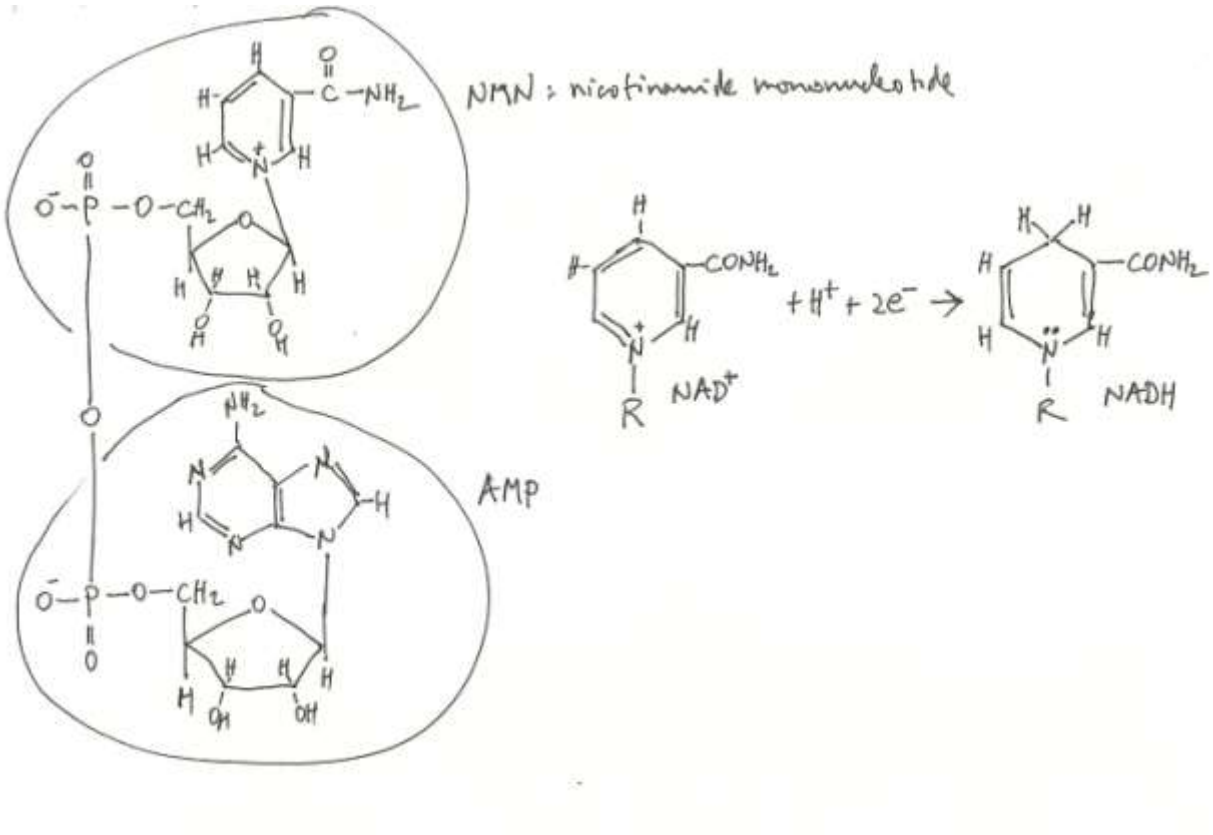
The electron transport chain of bacteria is similar to that of mitochondria but simpler. Thus, we will begin by considering electron transport in bacteria



In the figure the lipid bilayer is depicted by two curved arcs that make clear which side of the bilayer is the inside of the bacterium and which is the outside. Embedded in the bacterial membrane are many replicas of different types of protein complexes. One complex involves proteins labeled with FAD (flavin adenine dinucleotide)

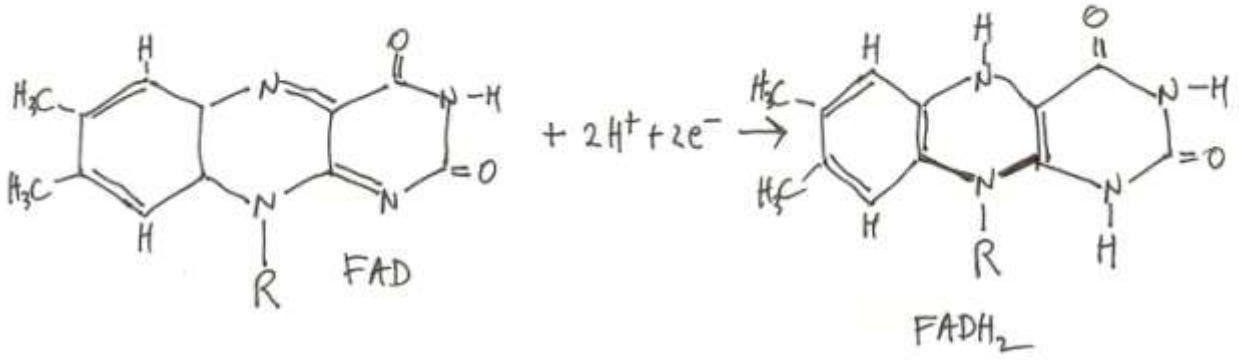


and FeS (iron sulfur protein). These proteins are at the top of the electron transport chain and receive electrons from a universal carrier of metabolically generated reducing equivalents, NADH (nicotinamide adenine dinucleotide)



This reduced molecule is generated by glycolysis and the citric acid cycle, energy metabolism pathways discussed in [*energy metabolism*]. Two electrons are passed from NADH to the FAD binding protein and then to the FeS proteins. These transfers are simple oxidation-reduction reactions. However, some are pure electron transfers while others are transfers of both an electron and a proton.

Indeed, three types of oxidation-reduction reactions occur in biology and all three are exhibited in this intial stage of electron transport. FAD is reduced to $FADH_2$ by a double reduction that involves two electrons and two protons forming the two hydrogen atoms of $FADH_2$ as is depicted in the figure



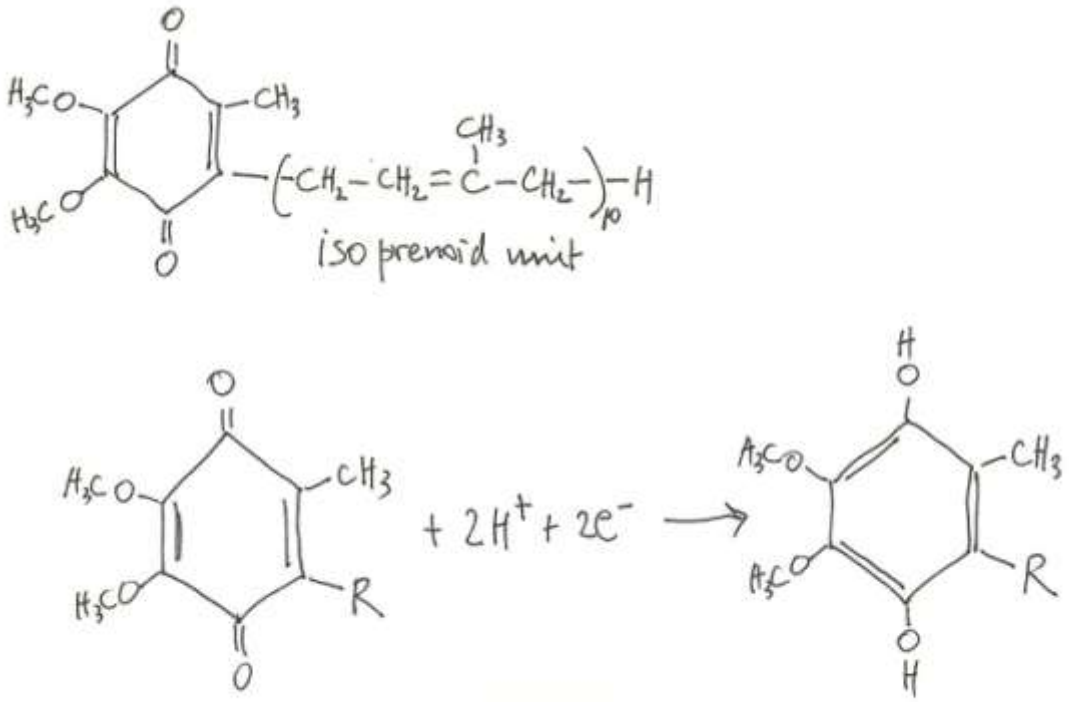
NADH, on the other hand, gives up two electrons and only one proton when it is oxidized by FAD to form NAD^+ and $FADH_2$. The extra proton needed by $FADH_2$ for complete reduction is taken up from a free proton on the inside of the bacterium (actually from a hydronium ion H_3O^+).

$$NADH + H_3^+O + FAD \leftrightarrow NAD^+ + H_2O + FADH_2$$

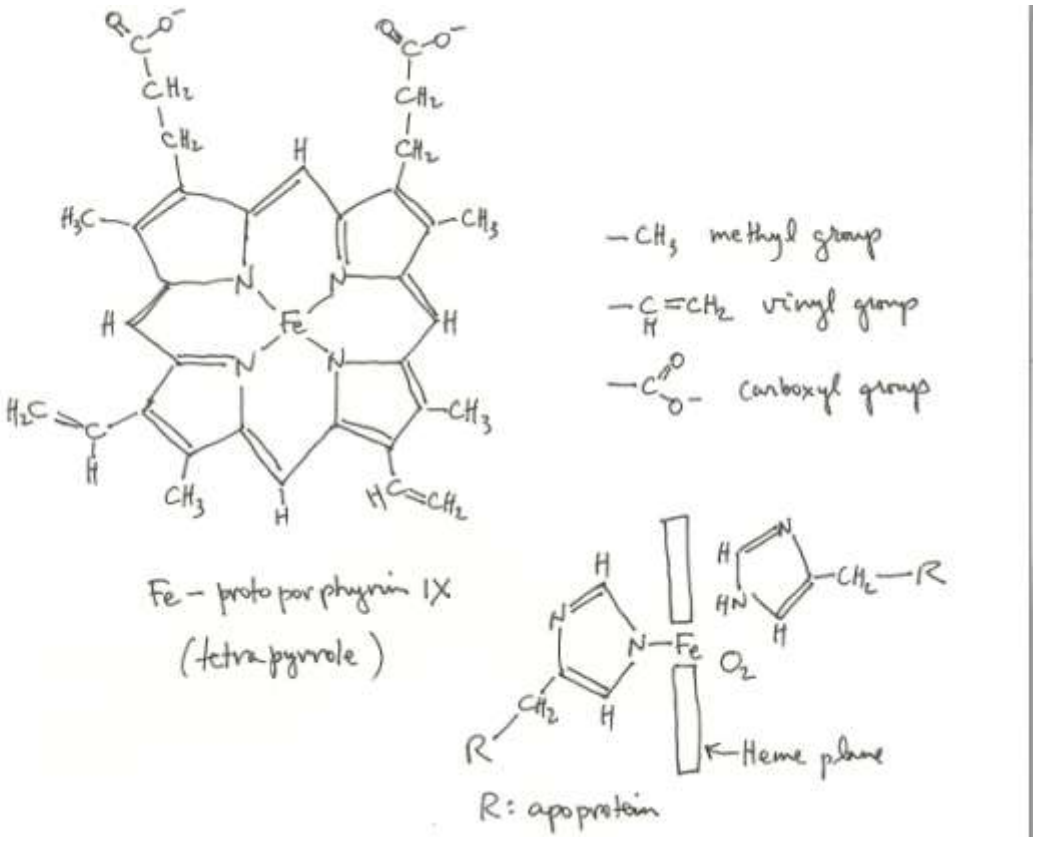
The FeS proteins, on the other hand, engage in pure electron exchanges, i.e. their oxidation-reduction reactions are ferric-ferrous transitions involving the transfer of single electrons. Clearly something must be done about the protons that appear in the first two steps. By positioning the transfer of electrons from $FADH_2$ to FeS adjacent to the exterior surface of the lipid bilayer, the protons can be extruded to the exterior of the bacterium while the electrons are transferred to FeS proteins. Thus, the arrangement of the proteins in the membrane results in a current-current coupling of electron flow to proton flow in which protons are transported across the membrane. This is called *vectorial* chemistry. Since the membrane is impermeable to protons (and to H_3O^+), this creates a transmembrane electrochemical potential made up of a charge imbalance and a pH imbalance. This is the essence of the *chemiosmotic* theory for the energized state of the intact membrane. The two FeS proteins have different affinities for electrons. While their iron-sulfur centers in isolation would have identical electron-transfer potentials, in the context of a surrounding protein they have different potentials because the proteins are different. This permits the sequential and preferential transport of electrons from one FeS protein to the next.

$$Fe^{2+}S-protein1+Fe^{3+}S-protein2 \leftrightarrow Fe^{3+}S-protein1+Fe^{2+}S-protein2$$

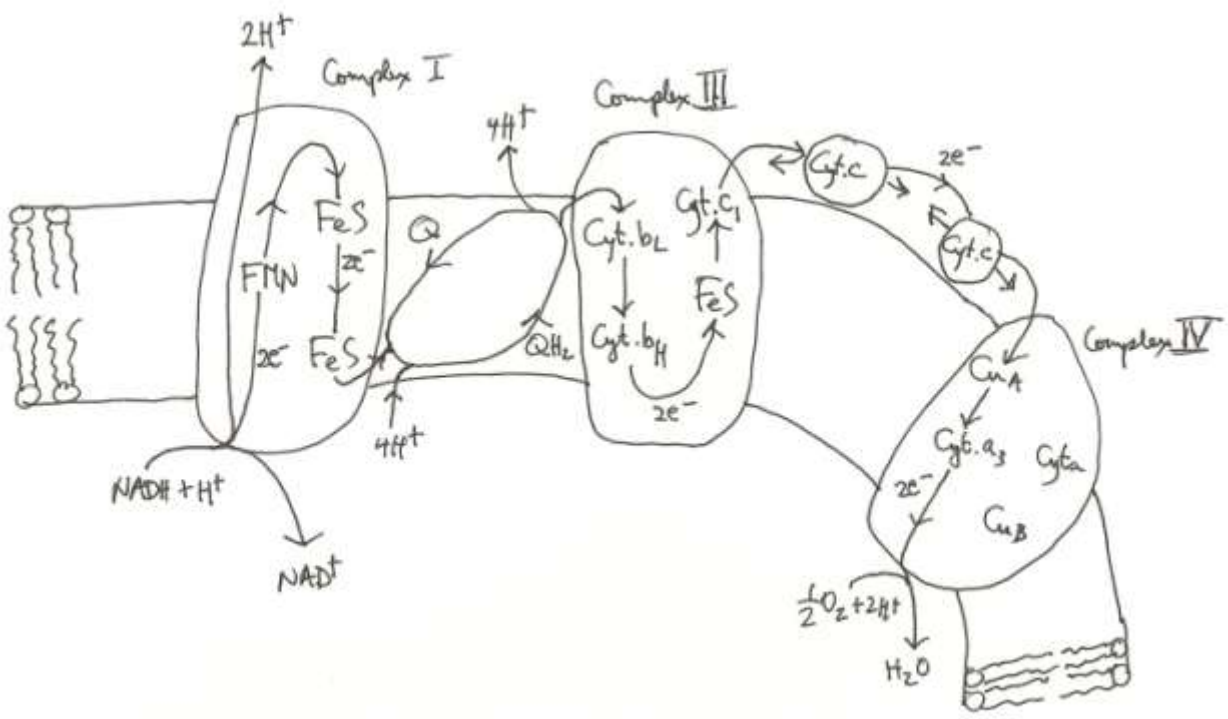
Proceeding down the electron transport chain we see that the second FeS protein gives its electron to a species labeled UQ (ubiquinone)



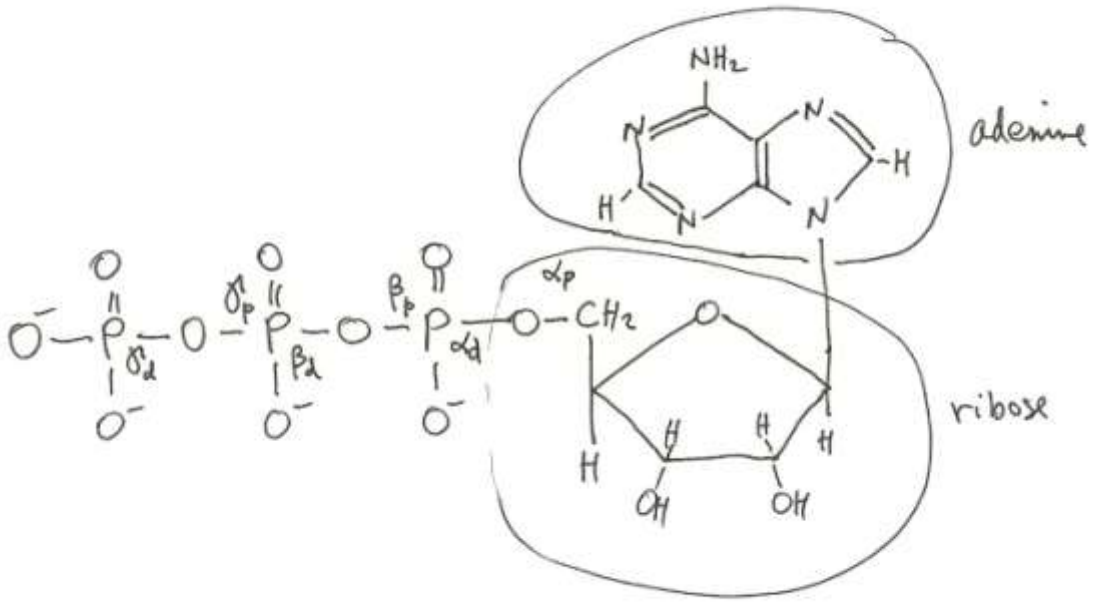
UQ, like FAD is reduced by two electrons (requiring transfers from two FeS proteins) and two protons forming the two hydrogen atoms of UQH_2. It gets its two protons from free protons (H_3^+O) on the inside of the bacterium. UQ rapidly diffuses across the membrane to the outside surface where it reduces a different class of iron containing proteins, the *cytochromes*. These cytochromes contain heme iron



and their elecron-transfer potentials are tuned by the surrounding protein, as in the case of the different FeS proteins. These different cytochromes are labeled with subscripts accordingly. As with the transfer of electrons from $FADH_2$ to FeS proteins, the transfer of electrons from UQH_2 to Cyt_b (cytochrome b) requires release of the protons to the exterior of the bacterium. Once again we have vectorial chemistry and the current-current coupling of electron flow to proton flow. Many replicas of the oriented cytochrome complex occurs in the bacterium's plasma membrane. In liver mitochondria there may be 5000 of these complexes and in heart mitochondria the number is 20,000. They appear to be uniformily distributed so that there is one every 400-500 nm^2 of inner mitochondrial membrane area. The FeS complex and the cytochrome complex are connected by the freely shuttling UQ. The final oxidation step is the oxidation of cytochrome o by desolved molecular oxygen, O_2. The product of this reduction is a molecule of water. In mitochondria the process is somewhat more complicated and cytochrome c connects the UQ-cytochrome c reductase complex to the cytochrome c oxidase complex by free diffusion along the outside surface of the bilayer



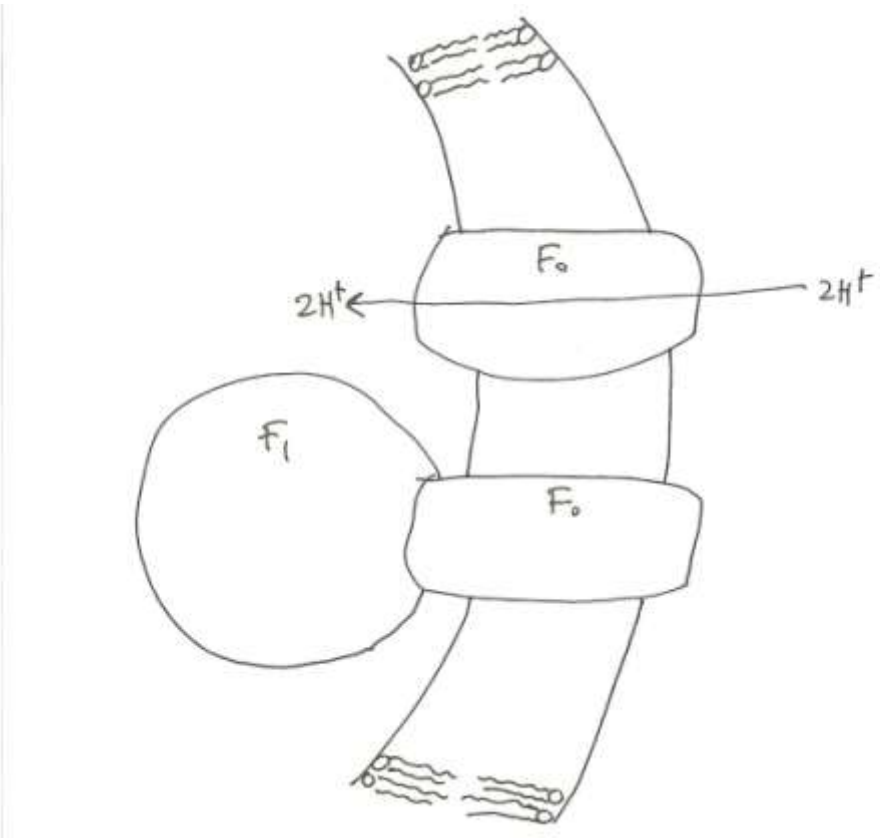
Overall for bacteria, two electrons have traveled through the membrane from NADH to H_2O. They have remained on the inside of the bacterium while going downhill energetically through a series of spontaneous oxidation-reduction reactions. At the same time one proton from NADH and five protons from the interior of the bacterium have also been coupled to the electron current. Two protons have ended up in H_2O on the inside of the bacterium but the other four have been transported vectorially across the membrane to create the energized membrane potential. The energy inherent in the difference of electron-transfer potential between NADH and O_2 has been harvested as membrane energy captured in the disequilibrium of proton concentrations on the inside and outside of the bacterium. This type of energy is readily available for powering various transport processes and other membrane associated mechanisms but it is not suitable for the emense demand for energy for synthetic purposes. This demand is met by the universal energy currency for synthesis, ATP



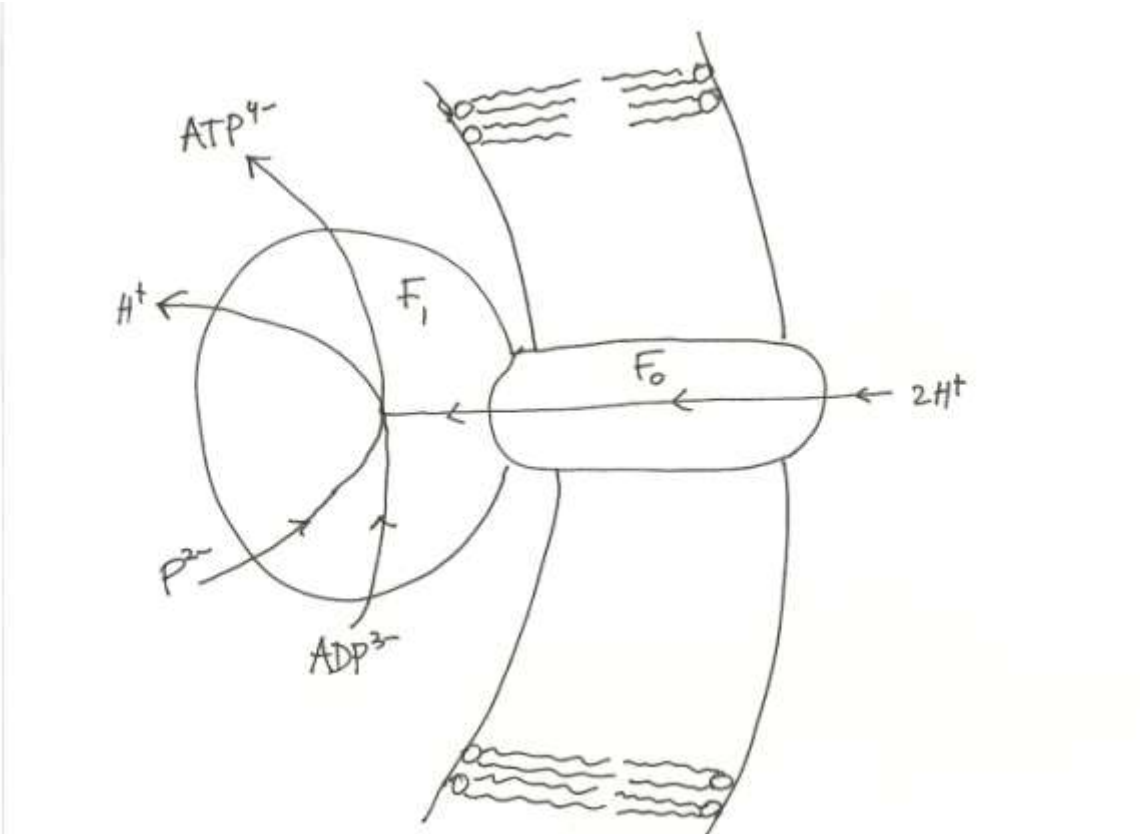
ATP synthesis

ATP has been called the universal energy currency of cells. Indeed, it is the primary source of energy for the synthesis of proteins, polynucleotides, polysaccharides and lipids. It is also essential for a wide variety of allosteric control processes that are triggered by phosphorylations, and dephosphorylations, of susceptible groups on proteins. In addition, ATP plays a central role in the reaction cycles that result in the dynamics of actin and myosin associations in muscle fibers and the dynamics of kinesin and dynein on microtubules. It is the precursor to cyclic-AMP (adenosine monophosphate) that serves as the second messenger in hormone actions. In other processes, GTP (guanodine triphosphate), CTP (cytodine triphosphate) and UTP (uridine triphosphate) may be directly involved and may lose a phosphate. These variants are recharged to triphosphates by ATP. Thus ATP does play many different essential roles although the energized membrane does too. It is the energized membrane that is responsible for the bulk of ATP synthesis (some ATP is generated by glycolysis).

The protein complex in the membrane that generates ATP is called an F-type ATPase. It is a quite large protein complex with one portion, the F_o portion, embedded in the membrane and spanning the entire width of the membrane, and with another portion, the F_1 portion, attached to the F_o portion on the inside of the membrane.



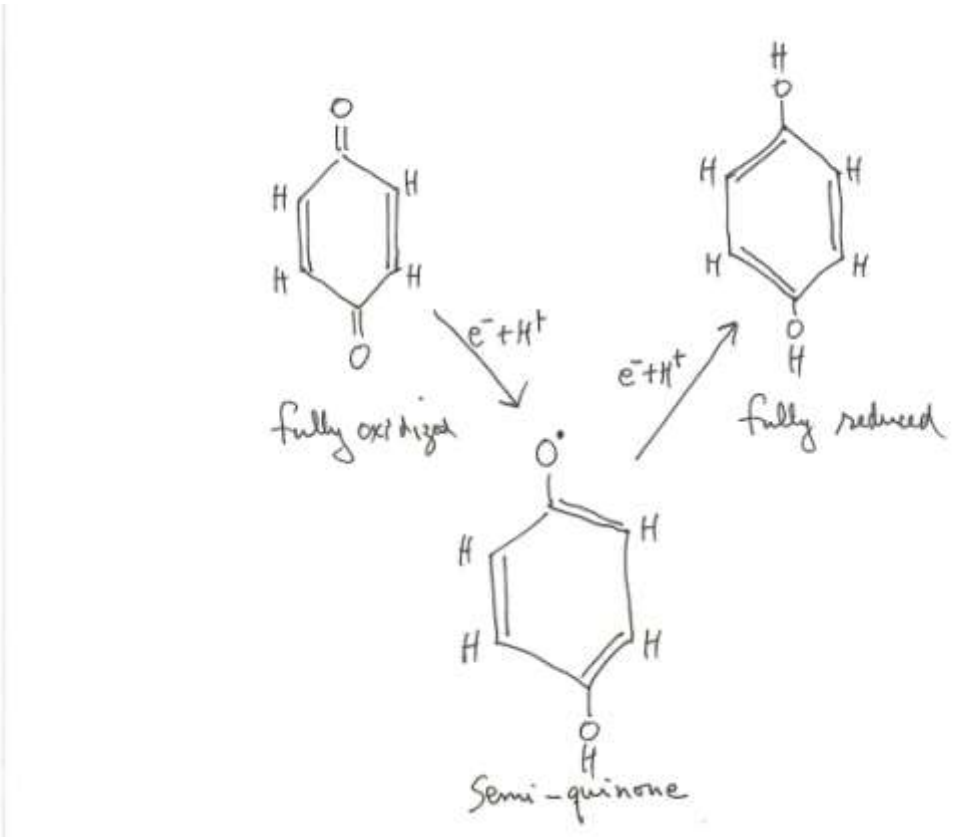
The energized state of the membrane favors transport of protons from the outside to the inside of the bacterium, i.e. this is the direction of a spontaneous decrease in Gibbs free energy. The F_o portion of the ATPase affords a pathway for this translocation of protons and when they arrive at the F_1 portion, a complicated process occurs in which ATP is regenerated from ADP and inorganic phosphate, P



Just as there are many replicas of the FAD-FeS protein complexes and the cytochrome complexes in the bacterial membrane, there are many replicas of the F-type ATPases. This redundancy supports the huge demand for ATP turnover by the cell. Typically, a cell has enough ATP for its energy and other needs for the order of a minute. Thus it must be regenerated from metabolic energy sources. A 185 pound man at rest consumes and regenerates ATP at the rate of ~ 4 mol/hr (this is equivalent to 2 kg/hr) and at 10 times this rate during strenuous activity.

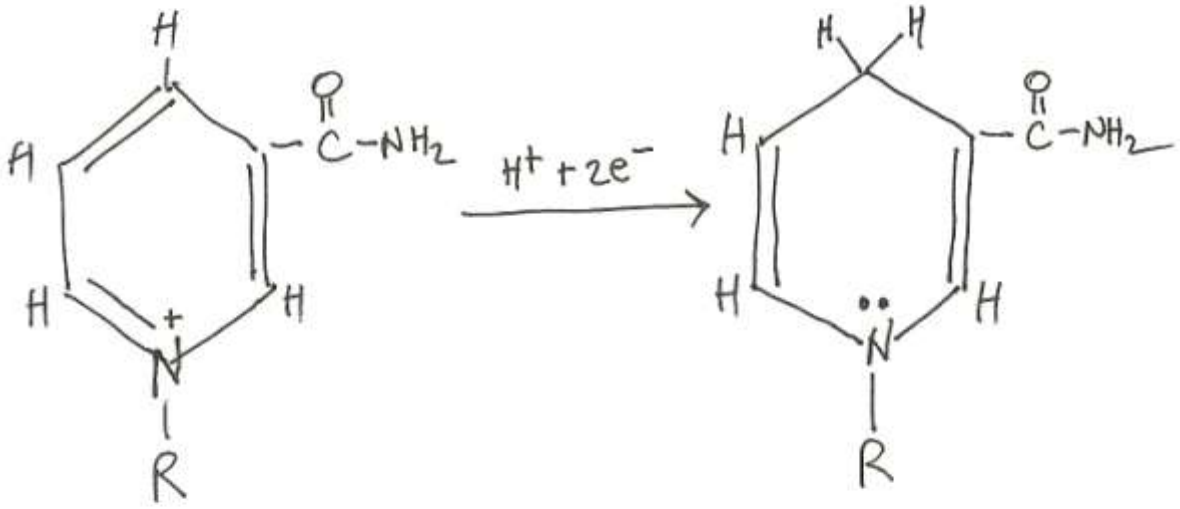
Biochemical thermodynamics

In order to make these energy transactions quantitatively precise, a review of biochemical thermodynamics is needed. Oxidation-reduction (redox) processes always involve pairs of molecules: one that is the oxidized form and one that is the reduced form of some underlying molecular structure. For example, quinone (not to be confused with UQ which has an isoprenoid tail) has the two forms



This redox pair is denoted by (Q/QH_2), an example of the generic form (A_{ox}/A_{red}) for the generic molecular species A. Other examples of redox pairs include the ferri-ferro pair (Fe^{3+}/Fe^{2+}) and the coenzyme NAD: (NAD^+/NADH). The generic event is the transfer of electrons to the oxidized form, thereby reducing it. For (Fe^{3+}/Fe^{2+}) that is all that happens, a pure electron transfer. In (Q/QH_2) a proton accompanies each of two electrons for a complete reduction. The (NAD^+/NADH) redox pair requires two electrons and one extra proton for reduction

$$NAD^+ + 2e^- + H^+ \rightarrow NADH$$



There is always an electron transfer, but there may or may not be a proton transfer too.

Typically, biology occurs at constant temperature, T, and pressure, P. Thus, the Gibbs free energy, G, is the governing thermodynamic potential. The change in G, ΔG, is what is important. The second law of thermodynamics dictates that in a spontaneous process at constant T and P ΔG must be negative. Consider the cytochrome reaction that occurs in mitochondria

$$Cyt_bFe^{2+} + Cyt_cFe^{3+} \leftrightarrow Cyt_bFe^{3+} + Cyt_cFe^{2+}$$

in which an electron is transferred from Cyt_b to Cyt_c. The change in Gibbs free energy is written

$$\Delta G = \Delta G^0 + 2.3RT \log_{10}\left[\frac{[Cyt_bFe^{3+}][Cyt_cFe^{2+}]}{[Cyt_bFe^{2+}][Cyt_cFe^{3+}]}\right]$$

In this expression R is the gas constant given by the product of Avogadro's number (6.0225 x 10^{23} molecules/mol) and Boltzmann's constant k_B (1.381 x 10^{-16} erg/K). This yields R = 8.317 x 10^7 ergs/K-mol = 1.987 cal/K-mol. The conversion identities 1 cal = 4.186 J (Joule) = 4.186 x 10^7 ergs have been used here. The 2.3 (actually 2.3025) comes from the conversion of Naperian logarithms to base 10 logarithms. The expression [M] where M is a particular molecular species denotes the concentration of M in moles per liter.

In *standard state* all reactants are at 1 molar by convention. Thus, at standard state

$$\Delta G = \Delta G^0$$

One mole of electrons has a charge of -1.0 F (Faradays) which is 9.648 x 10^4 C (Coulombs). Thus 1 F = 9.648 x 10^4 J/V = 9.648 x 10^4 x 1/4.186 cal/V = 23.05 Kcal/V where V stands for volts. Common practice is to express redox energetics in terms of electrical potential, i.e. volts instead of in terms of Gibbs free energy. This is done by the identity

$$\Delta E = -\frac{\Delta G}{F}$$

Therefore the cytochrome redox reaction above is described by

$$\Delta E = \Delta E^0 - 2.3\frac{RT}{F}\log_{10}\left[\frac{[Cyt_b Fe^{3+}][Cyt_c Fe^{2+}]}{[Cyt_b Fe^{2+}][Cyt_c Fe^{3+}]}\right]$$

Since electrons are negatively charged, a spontaneous change is one in which ΔE increases. The quantity ΔE^0 is obtained from a table of redox potentials.

Half reaction as a reduction	$E^{\circ\prime}$ at pH 7 (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
Cyt a_3-$Fe^{3+} + e^- \rightarrow$ Cyt a_3-Fe^{2+}	0.55
$SO_4^{2-} + 2H^+ + 2e^- \rightarrow SO_3^{2-} + H_2O$	0.48
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.42
$\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow H_2O_2$	0.30
Cyt a-$Fe^{3+} + e^- \rightarrow$ Cyt a-Fe^{2+}	0.29
Cyt c-$Fe^{3+} + e^- \rightarrow$ Cyt c-Fe^{2+}	0.22
Cu^{2+} + e- $\rightarrow Cu^+$	0.15
Cyt b-Fe^{3+} +$e^- \rightarrow$ Cyt b-Fe^{2+}	0.12
Ubiquinone + $2H^+ + 2e^- \rightarrow$ Ubiquinone-H_2	0.10
Fumarate + $2H^+ + 2e^- \rightarrow$ Succinate	0.030
Pyruvate + $NH_3 + 2H^+ + 2e^- \rightarrow$ Alanine	-0.13
Acetaldehyde + $2H^+ + 2e^- \rightarrow$ Ethanol	-0.163
Oxalacetate + $2H^+ + 2e^- \rightarrow$ Malate	-0.175
Pyruvate + $2H^+ + 2e^- \rightarrow$ Lactate	-0.190
Riboflavin + $2H^+ + 2e^- \rightarrow$ Riboflavin-H_2	-0.200
FAD + $2H^+ + 2e^- \rightarrow FADH_2$	-0.219
S + $2H^+ + 2e^- \rightarrow H_2S$	-0.23
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$	-0.320
$NADP^+ + 2H^+ + 2e^- \rightarrow NADPH + H^+$	-0.320
Pyruvate + $CO_2 + 2H^+ + 2e^- \rightarrow$ Malate	-0.33
Uric acid + $2H^+ + 2e^- \rightarrow$ Xanthine	-0.36
Acetyl-S-CoA + $2H^+ + 2e^- \rightarrow$ Acetaldehyde + CoA	-0.41
$CO_2 + 2H^+ + 2e^- \rightarrow$ Formate	-0.420
$H^+ + e^- \rightarrow \frac{1}{2}H_2$	-0.420
Ferredoxin-$Fe^{3+} + e^- \rightarrow$ Ferrodoxin-Fe^{2+}	-0.420
3-Phosphoglycerate + $2H^+ + 2e^- \rightarrow$ Glyceraldehyde-3-phosphate + H_2O	-0.55
Acetate + $2H^+ + 2e^- \rightarrow$ Acetaldehyde	-0.60
Succinate + $CO_2 + 2H^+ + 2e^- \rightarrow$ α-Ketoglutarate + H_2O	-0.67
Acetate + $CO_2 + 2H^+ + 2e^- \rightarrow$ Pyruvate	-0.70

We find from the table that

E^0 = 0.22 V for ($Cyt_cFe^{3+}/Cyt_cFe^{2+}$)
E^0 = 0.12 V for ($Cyt_bFe^{3+}/Cyt_bFe^{2+}$)

Therefore, $\Delta E^0 = E^0_{final} - E^0_{initial}$ where final and initial are determined by the reduced member of the pair. Thus, in this example, Cyt_cFe^{2+} is the reduced product so that E^0_{final} = 0.22 V. Therefore, ΔE^0 = 0.22 V - 0.12 V = 0.10 V. This is a potential, not an energy. When multiplied by -F, an energy is obtained: ΔG^0 = - 9648 J. This means that **in standard state** Cyt_b gives an electron to Cyt_c.

A pH dependent redox reaction is the oxidation of ubiquinone by ferri-cyanide

$$2H_2O + UQH_2 + 2Fe^{3+}cyanide \leftrightarrow UQ + 2Fe^{2+}cyanide + 2H_3^+O$$

The redox potential change for this process is given by

$$\Delta E = \Delta E^0 - 2.3\frac{RT}{2F}\log_{10}\left[\frac{[UQ][Fe^{2+}cyanide]^2[H_3^+O]^2}{[UQH_2][Fe^{3+}cyanide]^2[H_2O]^2}\right]$$
$$= \Delta E^0 + 2.3\frac{RT}{F}\,pH - 2.3\frac{RT}{2F}\log_{10}\left[\frac{[UQ][Fe^{2+}cyanide]^2}{[UQH_2][Fe^{3+}cyanide]^2}\right]$$

Several points need to be emphasized about this expression. Firstly, the 2F in the first equality reflects the fact that 2 electrons are transferred in this reaction. Secondly, since pH = - $\log_{10}[H_3^+O]$ and $[H_3^+O]$ is squared in the first line of the formula, the pH term in the second line is multiplied by a factor of RT/F, not RT/2F. Thirdly, the $[H_2O]$ factors have been omitted in the second line since the convention for the E^0 values is that $[H_2O]$ is at standard state, i.e. has the value 1, the true value of nearly 55.55 molar being built into the table entries automatically. At T = 300K, a good approximation for 2.3 RT/F is 0.06 V or 60 mV (millivolt). Thus, each pH unit is worth 60 mV. If protons are released during a reaction, then at pH 7, ΔE is larger by 0.42 V per mole of protons. If protons are consummed, then ΔE is reduced.

G^0 for H_2 is zero by convention. In the redox pair (H^+/H_2) H_2 is on the product side and H^+ is on the reactant side. Therefore, at pH 7, E^0 for (H^+/H_2) is - 0.42 V. Since pH 7 occurs frequently, the convention is to write $E^{0'}$ = - 0.42 V. The prime on this redox potential signifies that pH 7 applies. As an example consider the reduction of pyruvate to lactate by NADH

$$NADH + H_3^+O + pyruvate \leftrightarrow NAD^+ + H_2O + lactate$$

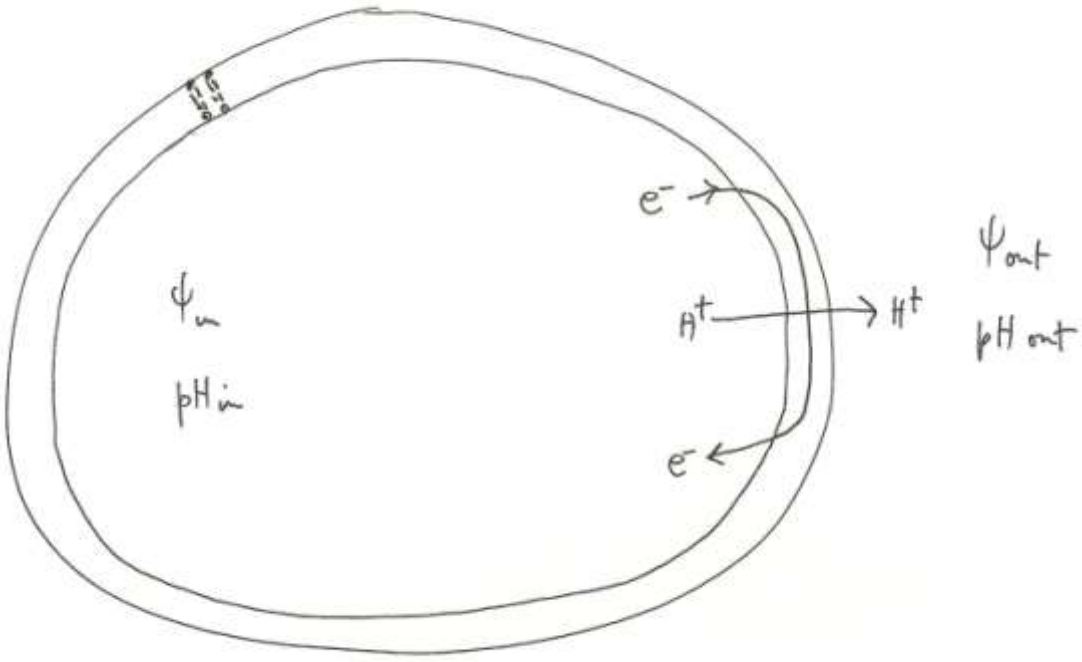
The redox potential change for this process is given by

$$\Delta E = \Delta E^0 - 2.3\frac{RT}{2F}\log_{10}\left[\frac{[NAD^+][H_2O][lactate]}{[NADH][H_3^+O][pyruvate]}\right]$$
$$= \Delta E^0 - 2.3\frac{RT}{2F}\,pH - 2.3\frac{RT}{2F}\log_{10}\left[\frac{[NAD^+][lactate]}{[NADH][pyruvate]}\right]$$
$$= \Delta E^{0'} - 2.3\frac{RT}{2F}\log_{10}\left[\frac{[NAD^+][lactate]}{[NADH][pyruvate]}\right]$$

wherein $\Delta E^{0'}$ is determined from the table for pH 7, and the standard state for water is built into these values $\Delta E^{0'}$ = - 0.19V - (- 0.32V) = 0.13V. Again the factors of 2 represent the participation of 2 electrons in the reduction. Since only one external proton is required because NADH provides two electrons and one proton, the pH term retains the 2 in the denominator.

Membrane potentials

Consider the schematic diagram representing the current-current coupling of electron flow to proton translocation across the membrane



This figure captures the essence of what happens in bacteria and in mitochondria. The electrical potential inside and outside the membrane compartment is denoted by ψ_{in} and ψ_{out} respectively. Define $\Delta\psi$ by $\Delta\psi = \psi_{out} - \psi_{in}$ and ΔpH by $\Delta pH = pH_{out} - pH_{in}$. Clearly, the result of active

electron transport is $\Delta\psi > 0$ and ΔpH < 0. The Gibbs free energy per particle is called the chemical potential, μ. Therefore,

$$\Delta\mu = \mu_{out} - \mu_{in} = |e|\Delta\psi + k_B T \ln\left(\frac{[H^+_{out}]}{[H^+_{in}]}\right)$$

For a mole of protons, this expression needs to be multiplied by Avogadro's number, N_A, which yields

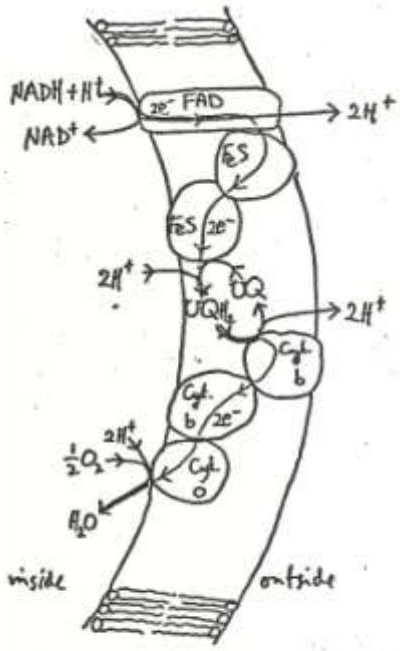
$$N_A\Delta\mu = F\Delta\psi - 2.3RT\Delta pH$$

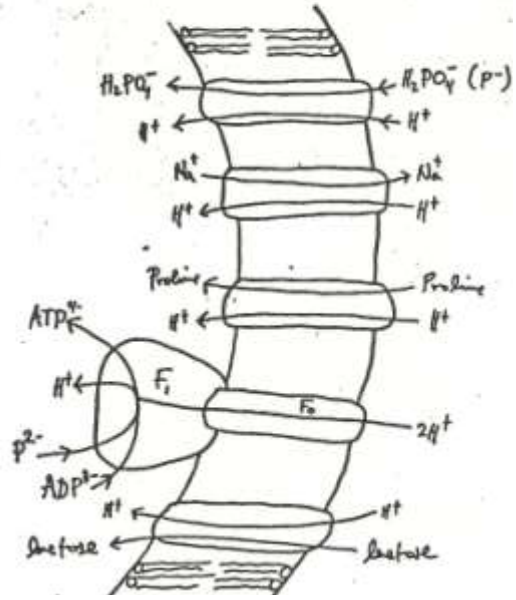
The left hand side is defined to be FΔp where Δp is called the *protonmotive force*. Clearly,

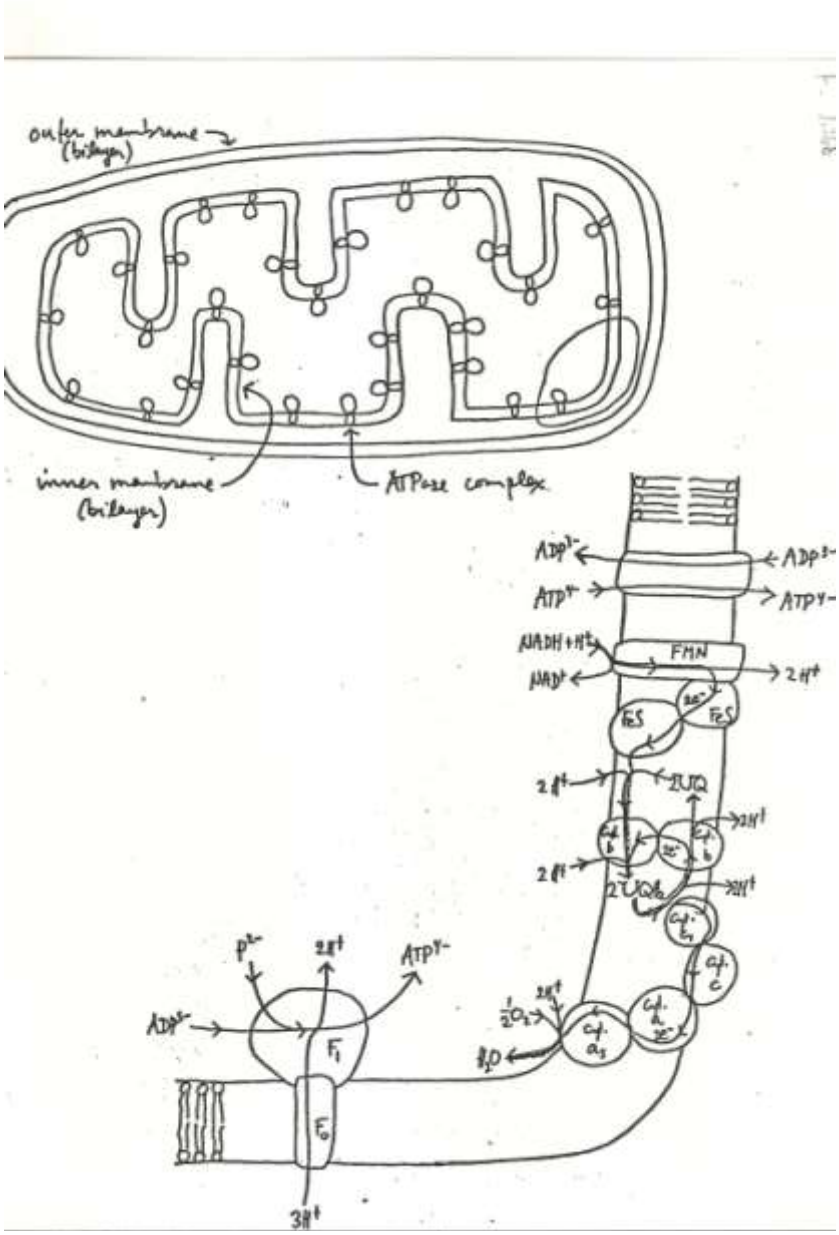
$$\Delta p = \Delta\psi - 2.3\frac{RT}{F}\Delta pH$$

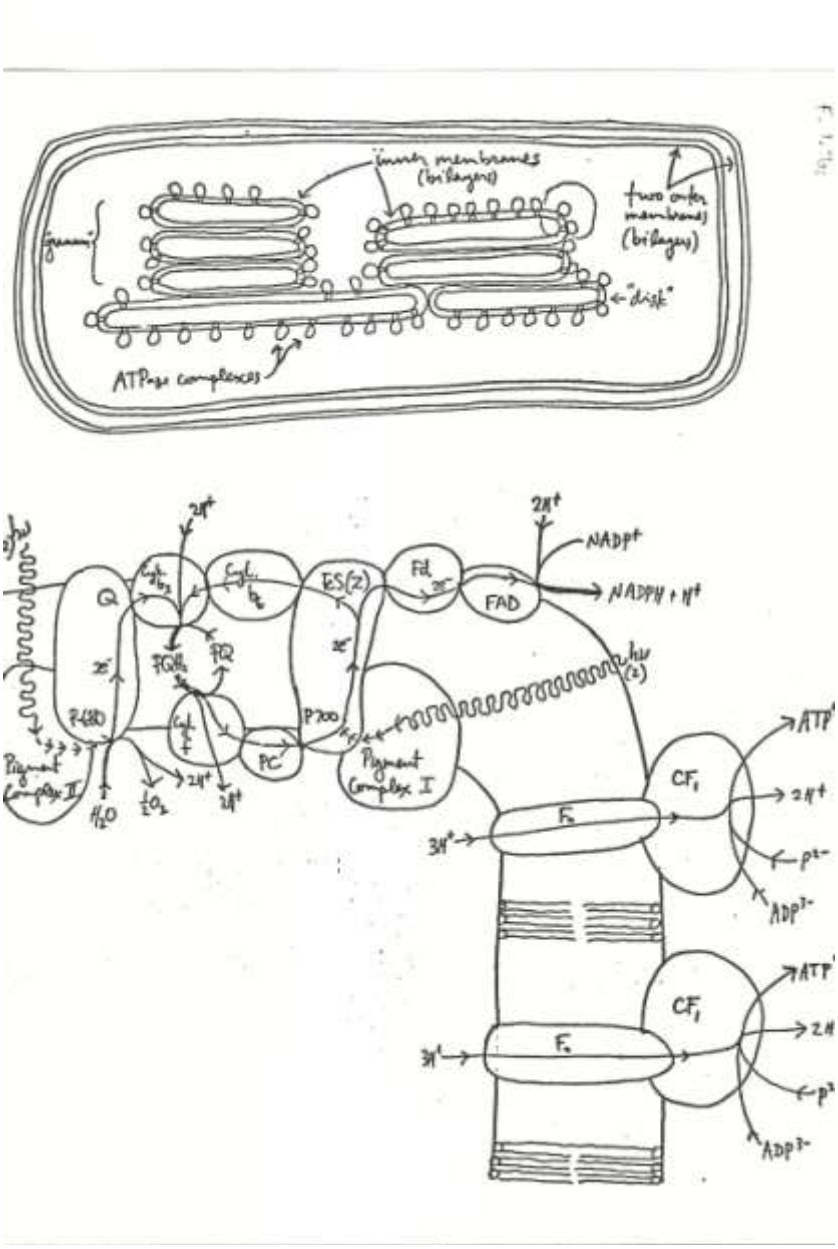
This terminology is misleading since Δp is not a force but an electrical potential. For the figure above, Δp > 0. Since protons have positive charge, they will spontaneously move towards more negative electrical potentials, the opposite of electrons. The tendency to do so is partly electrical, $\Delta\psi$, and partly *osmotic* or *chemical*, $-2.3\frac{RT}{F}\Delta pH$. Peter Mitchell, the primary proponent of this point of view early on, termed this *chemiosmosis*.

How big are Δp, $\Delta\psi$, and ΔpH for bacteria, mitochondria and chloroplasts?


NADH + H+
NAD+
2e- FAD
2H+
FeS
FeS 2e-
2H+
UQ
UQH2
2H+
Cyt. b
Cyt. b 2e-
2H+
½O2
Cyt. o
H2O
inside
outside


H2PO4-
H2PO4- (P-)
H+
H+
Na+
Na+
H+
H+
Proline
Proline
ATP4-
H+
H+
F1
Fo
2H+
P2-
ADP3-
H+
H+
Lactose
Lactose


outer membrane
(bilayer)
inner membrane
(bilayer)
ATPase complex
ADP^{3-}
ATP^{4-}
NADH + H^+
NAD^+
FMN
$2H^+$
FeS
Fe_2S
2e⁻
2UQ
2UQH₂
P^{2-}
$2H^+$
ATP^{4-}
ADP^{3-}
F_1
F_0
$3H^+$
$\frac{1}{2}O_2$
H_2O
cyt. a_3
cyt. c



Note that bacteria and mitochondria inner membranes have equivalent topologies with respect to inside and outside whereas the chloroplast inner membranes are inside-out relative to the other two cases. Representative values during active metabolism are

Membrane	$\Delta p(mV)$	$\Delta\psi(mV)$	ΔpH
bacteria	190	70	-2
mitochondria	224	140	-1.4
chloroplasts	-210	~0	3.5

In chloroplasts, the result $\Delta\psi \sim 0$ is caused by their high permeability to Cl^- anions that leak across the membrane to kill $\Delta\psi$. Since the grana are inside-out and have a relatively small

interior volume, it is possible to create a larger ΔpH than bacteria or mitochondria do that compensates the $\Delta\psi \sim 0$.

These are steady state results, not equilibria. The energy required to move a proton through an electrochemical potential Δp of approximately 200 mV is 1.6 x 10^{-19} x 0.2 V = 3.2 x 10^{-20} J = 3.2 x 10^{-13} ergs. This is about 8 k_BT at T = 300 K. This energy must be supplied by the electron transport chain energy.

The decomposition of Δp into $\Delta\psi$ and $-2.3\frac{RT}{F}\Delta pH$ permits experimental tests of chemiosmosis that selectively check these two terms. Two distinct K^+ transport systems are ideally suited for this purpose, *valinomycin* and *nigericin*. Valinomycin, a dodecapeptide with hydrophobic, lipophilic amino acid residues, binds K^+ and can diffuse across the lipid membrane. If a membrane $\Delta\psi$ is created by electron transport, then K^+ will achieve an equilibrium distribution across the mebrane with respect to this $\Delta\psi$ in which $\mu_{K^+ in} = \mu_{K^+ out}$. Equilibrium with respect to $\Delta\psi$ is given by

$$F\psi_{out} + RT\ln[K^+_{out}] = F\psi_{in} + RT\ln[K^+_{in}]$$

This is equivalent to

$$\frac{[K^+_{in}]}{[K^+_{out}]} = \exp\left[\frac{F\Delta\psi}{RT}\right]$$

Since valinomycin transport has no H^+ dependence, there is no dependence on the ΔpH term in Δp. The ionophore, nigericin, however, is a membrane associated polypeptide antiporter of K^+ and H^+.

$$K^+_{in} + H^+_{out} \leftrightarrow K^+_{out} + H^+_{in}$$

With steady state values of $\Delta\psi$ and $-2.3\frac{RT}{F}\Delta pH$ imposed by electron transport the overall chemical potential change associated with this exchange can be written

$$-|e|\Delta\psi + 2.3k_BT\Delta pH + |e|\Delta\psi + k_BT\ln\left(\frac{[K^+_{out}]}{[K^+_{in}]}\right) = 0$$

in which the first two terms refer to protons going from outside to inside and the last two terms refer to potassium ions going from inside to outside. As long as electron transport maintains ΔpH < 0 and nigericin is available for reverse flow of H^+, K^+ ions will be transported outwardly until an equilibrium for K^+ ions is reached given by the expression above or its equivalent

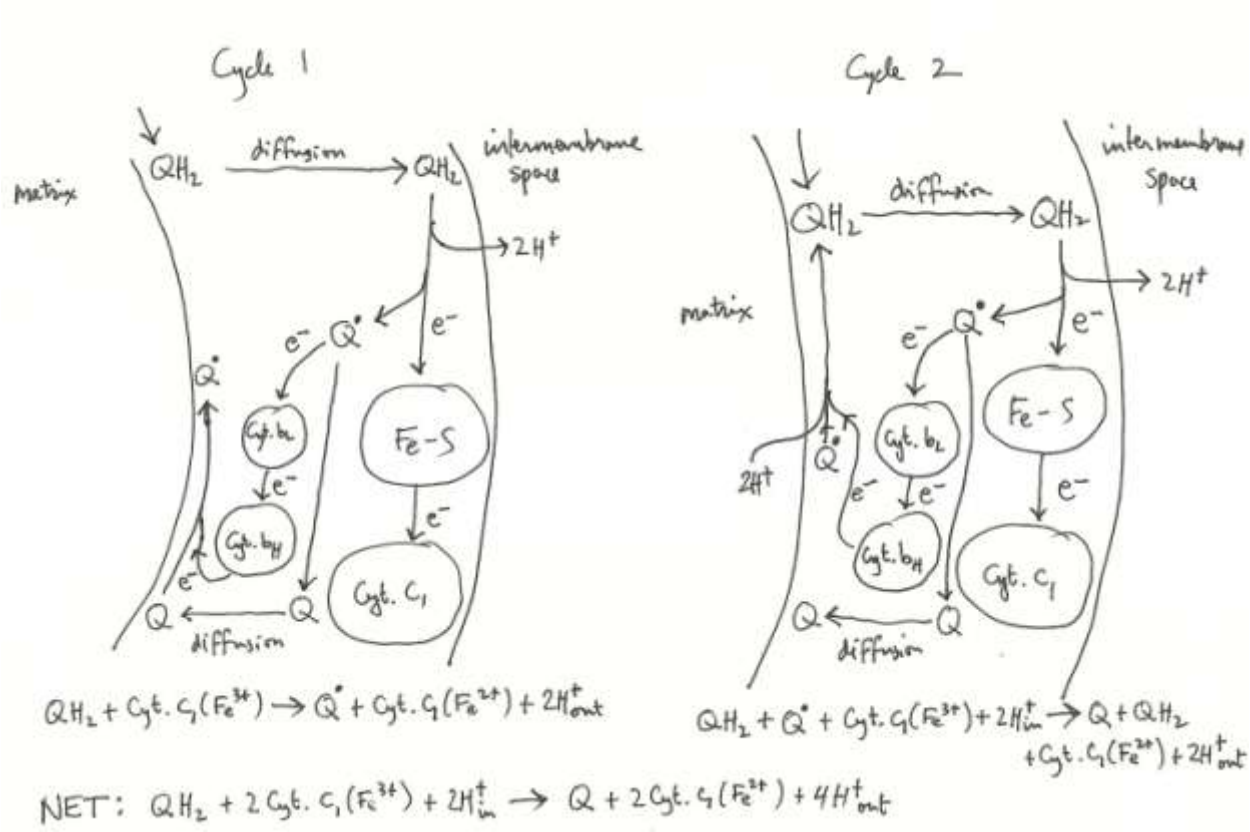
$$\ln\left(\frac{[K^+_{out}]}{[K^+_{in}]}\right) = -2.3\Delta pH$$

Note that in the $\Delta\psi$ case, $\frac{[K^+_{in}]}{[K^+_{out}]} > 1$ and in the ΔpH case $\frac{[K^+_{out}]}{[K^+_{in}]} > 1$. With these two processes, the two contributions to Δp can be separately measured.

Mitchell cycle for mitochondria

Peter Mitchell revived and championed the idea of chemiosmosis during the the 1960s and 1970s. At first he had little support from others but eventually prevailed and was awarded the Nobel prize in chemistry for his work in 1978. The problem he confronted was how electron transport was coupled to ATP synthesis. This was a problem dating back to the 1940s. The difficulty in finding a solution was that biochemists persisted in looking for chemical intermediates such as are found in glycolysis. The techniques for looking for chemical intermediates required breaking apart the bacterial or mitochondrial membranes. Since the mechanism of coupling requires an intact membrane in order to maintain the chemiosmotic electrochemical transmembrane potential, these methods failed to provide a solution. Mitchell's recognition of the importance of the coupling of electron current to proton current and subsequent coupling of proton current to phosphorylation was the key to finding a solution.

In bacteria, the quinone (UQ) cycle translocates 2 H^+'s for each pair of electrons transported down the electron transport chain. In mitochondria, it appears that 4 H^+'s are translocated instead. Mitchell proposed a mechanism, called the Mitchell cycle, for this that is now amply justified experimentally. In mitochondria, the quinone species is called CoQ (coenzyme Q) and is virtually identical with the UQ of bacteria and the redox pair is ($CoQ/CoQH_2$). However, in the Mitchell cycle, a semiquinone plays a role. This species carries an electron on one quinone oxygen atom and has a negative charge. It is denoted by $CoQ^{\bullet}$. The mitchell cycle operates in two stages called cycle 1 and cycle 2



In cycle 1, two FeS proteins of the upper portion of the electron transport chain together with 2 protons from the matrix side of the inner mitochondrial membrane reduce CoQ to $CoQH_2$ on the matrix side of the membrane. This $CoQH_2$ freely diffuses across the membrane to the intermembrane space side. It releases 2 protons to the outside and reduces a special FeS protein with one electron leaving the semiquinone $CoQ^\bullet$. The FeS protein reduces Cyt_{c1}. The semiquinone transfers its one electron to Cyt_{bL} and becomes the oxidized quinone CoQ. Cyt_{bL} reduces Cyt_{bH}. The CoQ freely diffuses back across the membrane to the matrix side. The arrangement of the cytochromes Cyt_{bL} and Cyt_{bH} in the membrane interior is such that when CoQ gets back to the matrix side, it is partially reduced to the semiquinone $CoQ^\bullet$ by Cyt_{bH}. Thus the charged species $CoQ^\bullet$ does not freely diffuse across the membrane but is regenerated when the neutral species CoQ has done so. This concludes cycle 1 with one $CoQH_2$ having been converted into $CoQ^\bullet$, one Cyt_{c1} having been reduced and two protons having been translocated across the membrane. In cycle 2, another $CoQH_2$ generated from two FeS proteins in the upper portion of the electron transport chain along with two protons from the matrix diffuses across the membrane to the intermembrane space side. Again, it releases two protons to the outside and reduces one Cyt_{c1} through the intermediation of a special FeS protein, creating a semiquinone. This semiquinone in turn reduces Cyt_{bL} which reduces Cyt_{bH} and yields a fully oxidized CoQ that diffuses freely across the mebrane back to the matrix side. The $CoQ^\bullet$ produced in cycle 1 is now reduced by the reduced Cyt_{bH} just created in cycle 2 and this requires two protons from the matrix side. The result is regeneration of $CoQH_2$. Thus, the overall net result is that one $CoQH_2$ is converted into one CoQ, two oxidized Cyt_{c1}'s are reduced and two matrix protons are taken

up. The two protons from $CoQH_2$ that are originally from the matrix and the two protons from the matrix during cycle 2 are extruded to the outside for a total of four protons extruded for two electrons transported to two Cyt_{c1}'s. The results in a more efficient harvesting of electron energy as proton energy then occurs in the simple quinone cycle used by bacteria in which only two protons are translocated for two electrons.

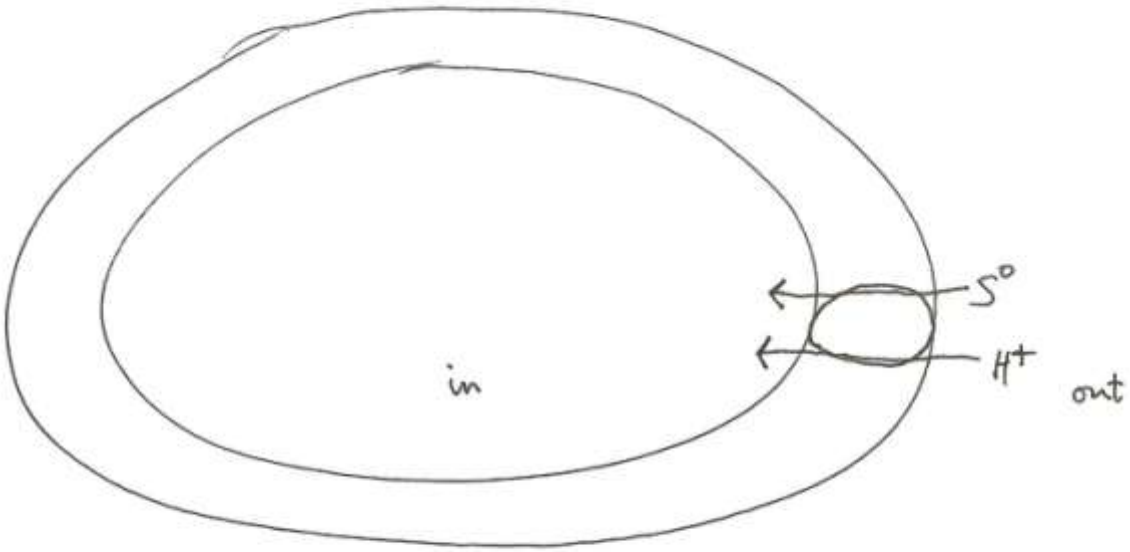
Transporters

The efflux of protons from the membrane compartment that results from current-current coupling of electron flow and proton translocation builds up the chemiosmotic membrane proton potential

$$\Delta p = \Delta \psi - 2.3\frac{RT}{F}\Delta pH$$

This potential provides an impetus for protons to re-enter the compartment. To do so, however, requires a functional pathway. Many such pathways exist and are created by specific proteins embedded in the membrane. All sorts of current-current couplings of proton re-entry with other solute species' fluxes across the membrane occur. Each requires a specific protein transporter. In the the following examples, the notation $\Delta A = A_{out} - A_{in}$ will be used where A stands for any molecule. It will be assumed that metabolism is at steady state and that the proton potential across the membrane is maintained constant. The coupled solute will come to equilibrium with respect to the nonequilibrium steady state value of Δp.

The figure below shows an example of obligate co-transport of a neutral solute species, S^0, into the membrane compartment



Such a transporter is called a symporter. At equilibrium for the solute species

$$\Delta\mu_{S^0} + |e|\Delta p = 0$$

The chemical potential change for a neutral species is given by

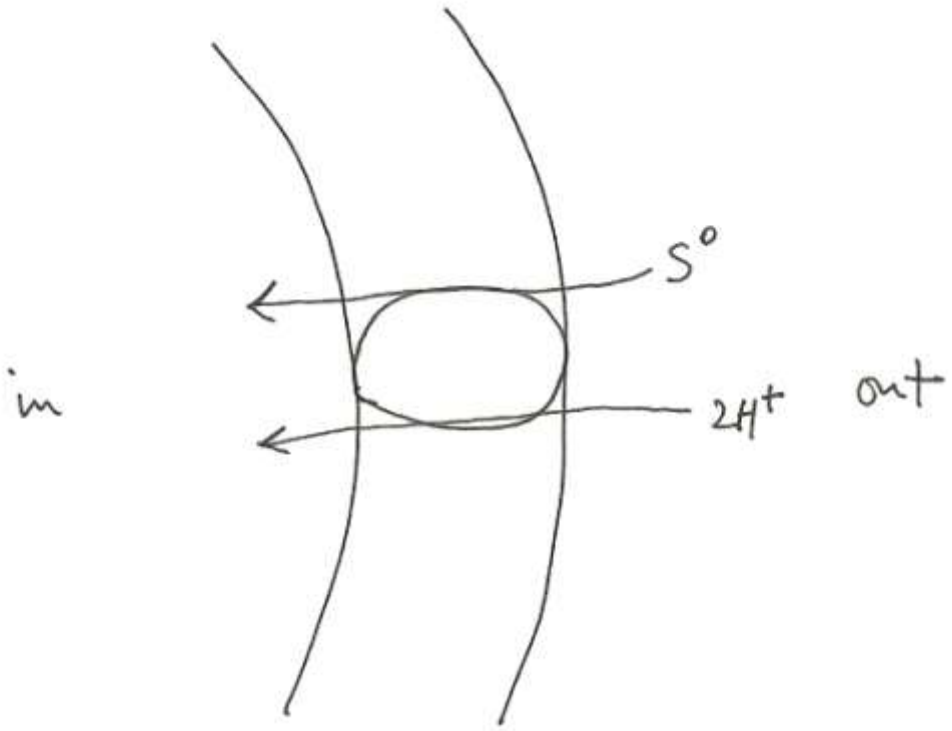
$$\Delta\mu_{S^0} = k_B T \ln\left[\frac{[S^0_{out}]}{[S^0_{in}]}\right]$$

Together with the expression for Δp, equilibrium implies

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^0_{out}]}{[S^0_{in}]}\right] = -\Delta p = -\Delta\psi + 2.3\frac{RT}{F}\Delta pH$$

Clearly, a positive membrane potential, i.e. Δp > 0 causes the neutral solute species to become partitioned across the membrane so that more is inside the compartment than is outside.

In the figure below

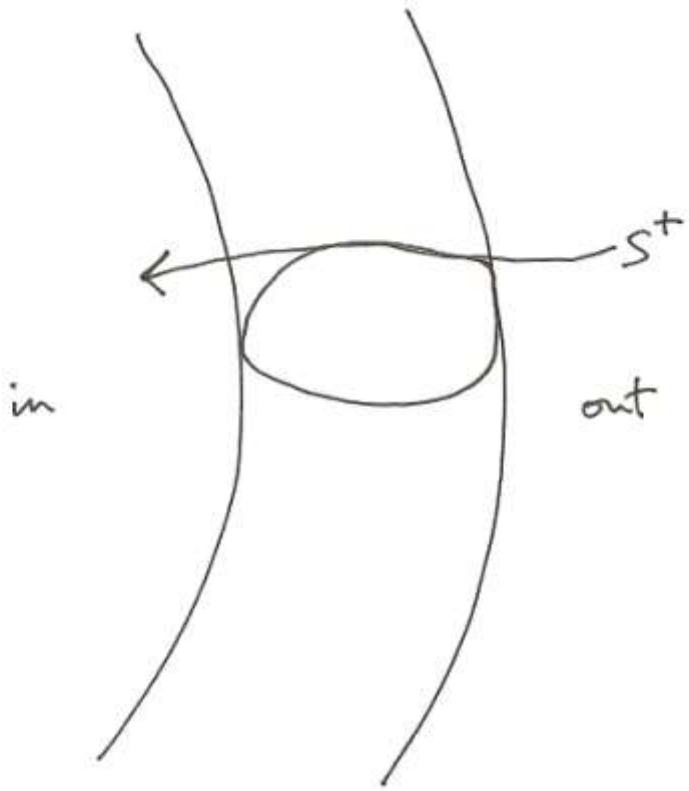


a symporter that couples the re-entry of two protons to a neutral solute species is depicted. This produces the equilibrium equation for the solute given by

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^0_{out}]}{[S^0_{in}]}\right] = -2\Delta p = -2\left(\Delta\psi + 2.3\frac{RT}{F}\Delta pH\right)$$

Clearly, a greater ratio of solute inside to outside is created when two protons are coupled to the transport of one molecule of solute than is the case for one proton coupling. Notice that ratio of solute concentrations outside to inside for a two proton symporter is the square of the ratio for a one proton symporter.

There also exist transporters that are not coupled to obligate proton re-entry but that couple directly to the electrical potential portion of Δp, $\Delta\psi$. This requires that the transported species be charged. For a cation, S^+, transport to the inside takes place



In this case the equilibrium is given by

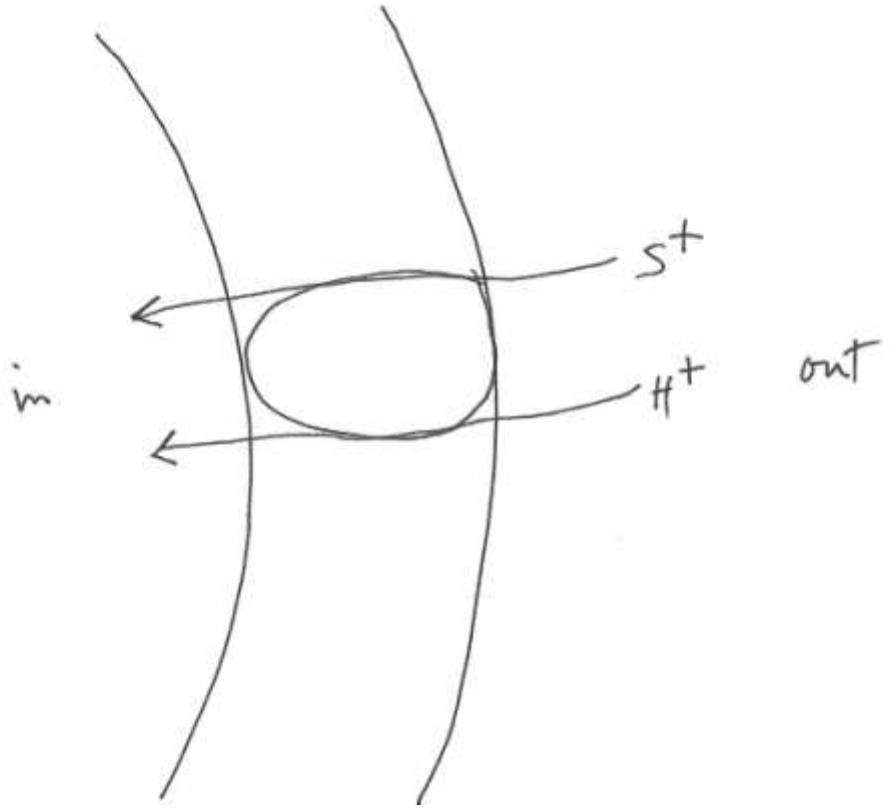
$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^+_{out}]}{[S^+_{in}]}\right] = -\Delta\psi$$

because the chemical potential for the cation is given by

$$\Delta\mu_{S^+} = |e|\Delta\psi + k_B T \ln\left[\frac{[S^+_{out}]}{[S^+_{in}]}\right]$$

and the electrical potential, $\Delta\psi$, is maintained constant. Clearly, a positive membrane electrical potential, i.e. $\Delta\psi > 0$, will result in the influx of the cation.

Now consider a cationic symporter.

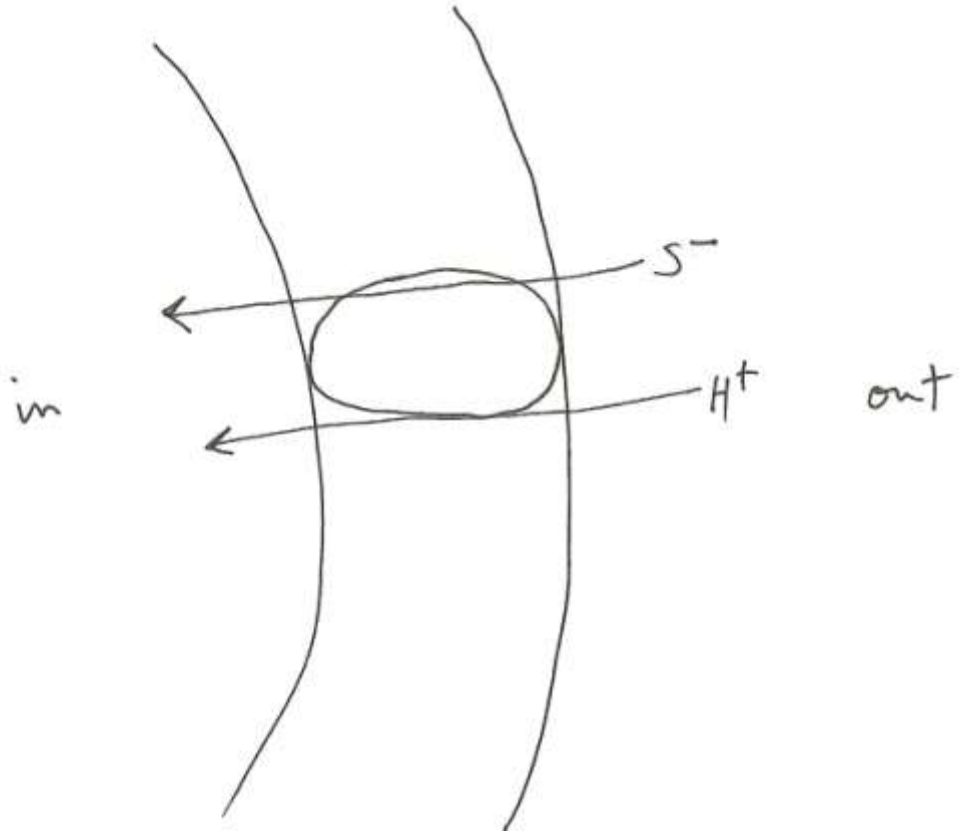


The equilibrium condition becomes

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^+_{out}]}{[S^+_{in}]}\right] = -\left(2\Delta\psi - 2.3\frac{RT}{F}\Delta pH\right)$$

Especially notice the factor of 2 in the electrical potential term caused by two positive charges going inside.

Somewhat more subtle is the symport of an anion, S^-.



The chemical potential for the anion is given by

$$\Delta\mu_{S^-} = -|e|\Delta\psi + k_B T \ln\left[\frac{[S^-_{out}]}{[S^-_{in}]}\right]$$

The condition for equilibrium for the anion is

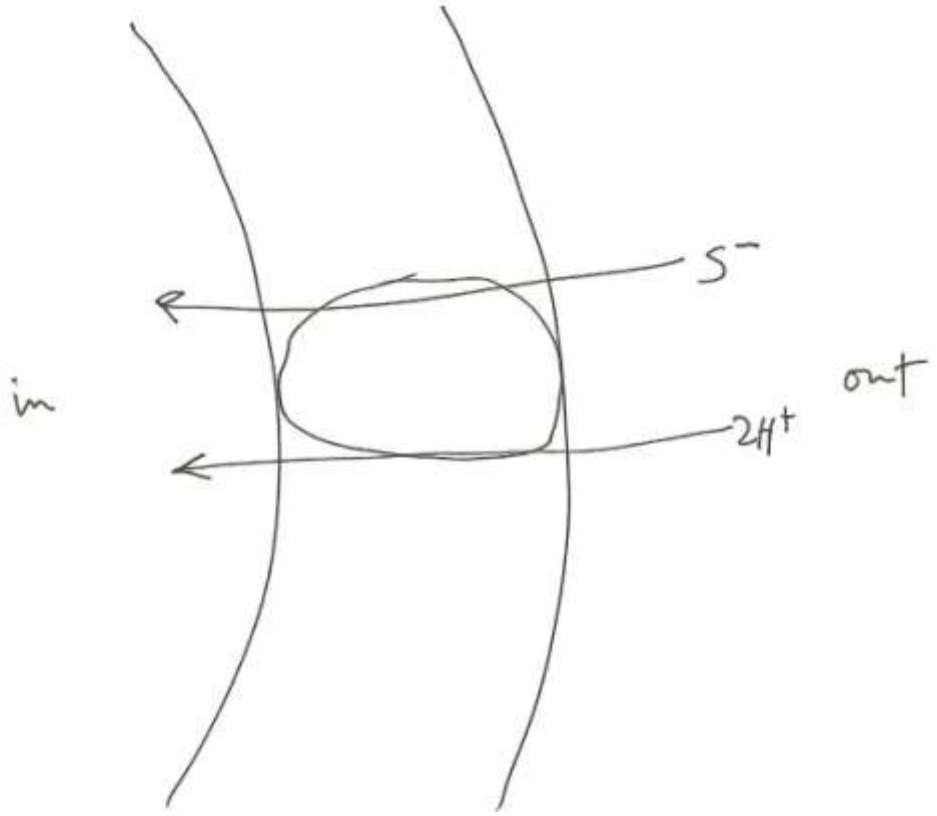
$$\Delta\mu_{S^-} + |e|\Delta p = 0$$

which implies

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^-_{out}]}{[S^-_{in}]}\right] = 2.3\frac{RT}{F}\Delta pH$$

Since this symporter does not transport net charge the electrical potential portion of Δp does not contribute and only the pH difference does.

An anion symporter requiring two protons

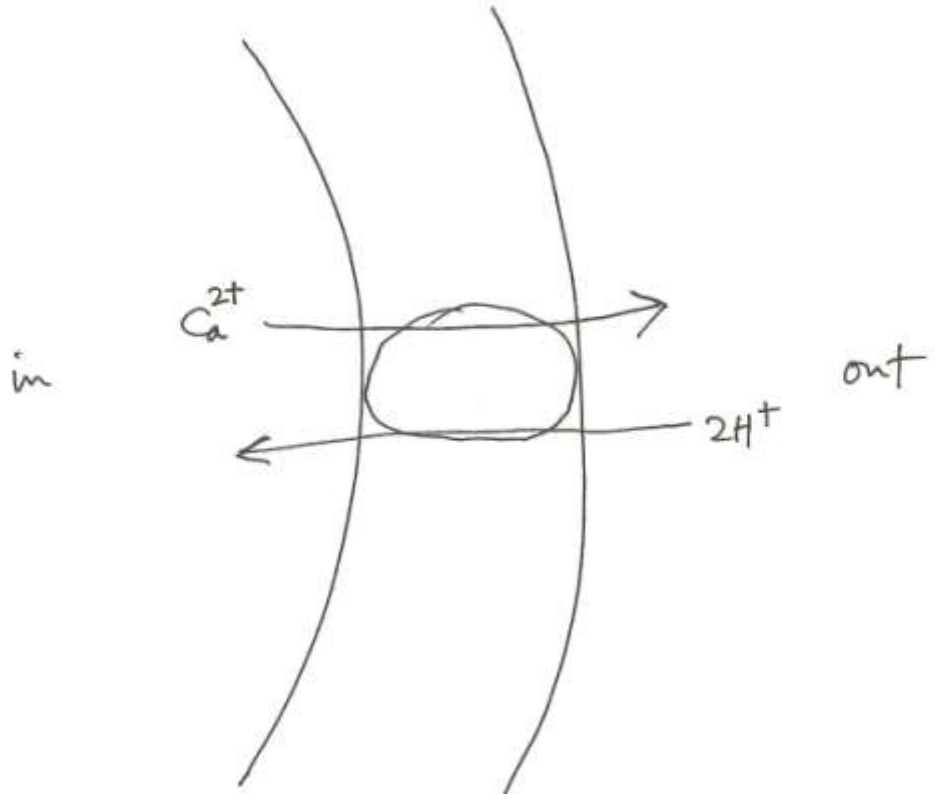


can be shown to lead to the equilibrium condition for the anion

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[S^-_{out}]}{[S^-_{in}]}\right] = -\Delta\psi + 2\times 2.3\frac{RT}{F}\Delta pH$$

This there is a partial cancellation of the electrical potential terms and a doubling of the pH term.

There also exist protein transporters that move ions in the opposite direction to the proton re-entry. These are called antiporters. Calcium is translocated by a calcium antiporter that is coupled to two proton re-entry



The process may be represented by the vectorial reaction

$$Ca_{in}^{2+} + 2H_{out}^{+} \leftrightarrow Ca_{out}^{2+} + 2H_{in}^{+}$$

The equilibrium condition can be written as

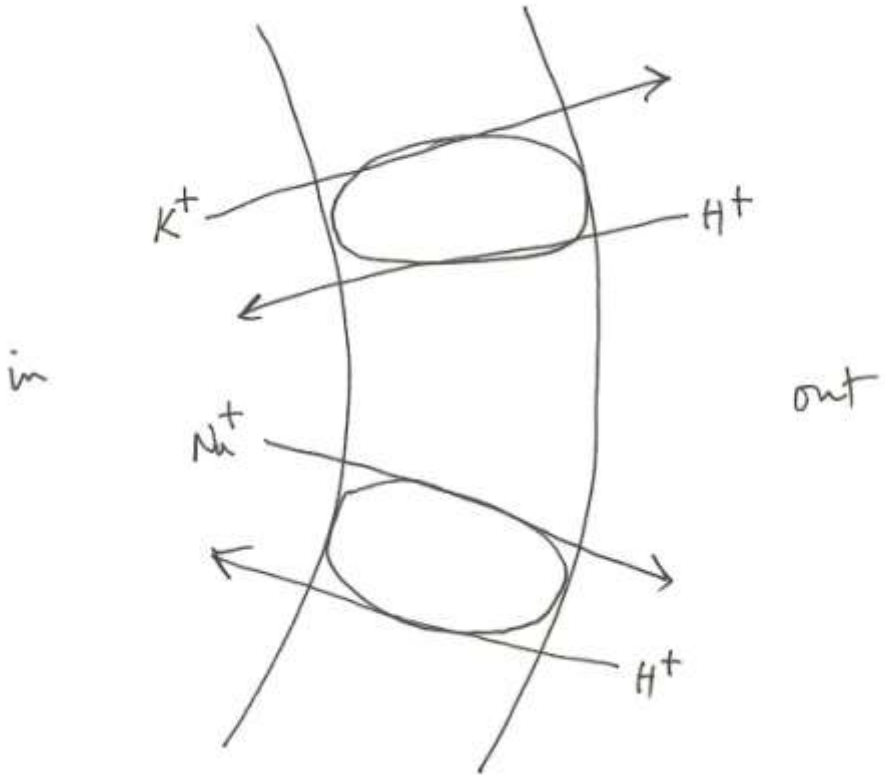
$$k_B T \ln[Ca_{in}^{2+}] + 2|e|\psi_{in} - 2\times 2.3 pH_{out} + 2|e|\psi_{out} =$$
$$k_B T \ln[Ca_{out}^{2+}] + 2|e|\psi_{out} - 2\times 2.3 pH_{in} + 2|e|\psi_{in}$$

This can be rewritten as

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[Ca_{out}^{2+}]}{[Ca_{in}^{2+}]}\right] = -2\times 2.3\frac{RT}{F}\Delta pH$$

Clearly, a lower pH on the outside compared to the inside will help drive calcium ions outside.

Similar cationic antiporters exist for potassium, K^+, and for sodium, Na^+



Each of these antiporters couples just one proton to the cation flux. The equilibrium result for sodium is

$$2.3\frac{RT}{F}\log_{10}\left[\frac{[Na^{+}_{out}]}{[Na^{+}_{in}]}\right] = -2.3\frac{RT}{F}\Delta pH$$

Replacing Na with K gives the comparable result for potassium.

As an example, consider a cation symporter using one proton in a bacterium membrane with $\Delta\psi$ = 70 mV and ΔpH = -2. Exponentiation of the equilibrium result above for this case yields

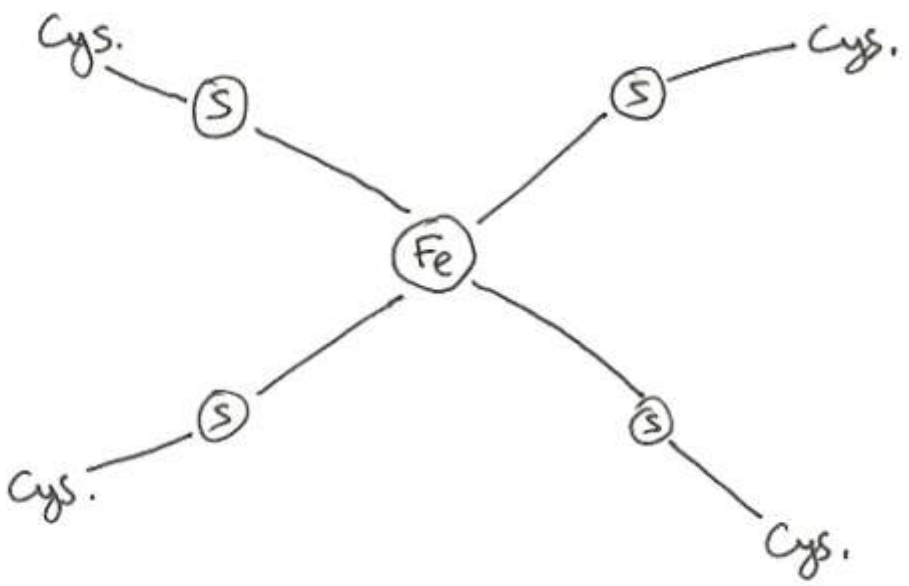
$$\frac{[S^{+}_{out}]}{[S^{+}_{in}]} = \exp\left[-\frac{F}{RT}\left(2\Delta\psi - 2.3\frac{RT}{F}\Delta pH\right)\right] =$$
$$\exp\left[-5.14\right] = 5.8\times10^{-3}$$

Thus, the concentration of S^{+} on the inside is 171 times that on the outside.

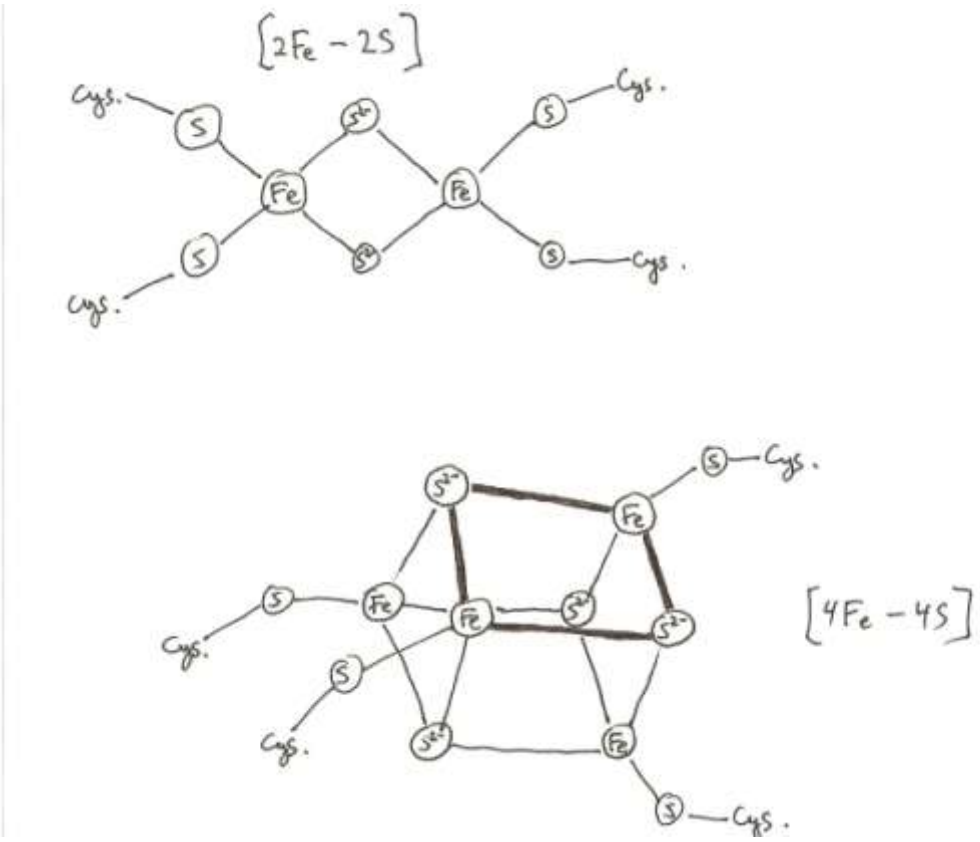
Each of the examples described above occurs for some molecular species. A great many transport systems exist in the membrane and they are responsible for the traffic of metabolites into and out of the membrane compartment. The energy for these processes is supplied by the chemiosmotic membrane potential.

Electron transport details

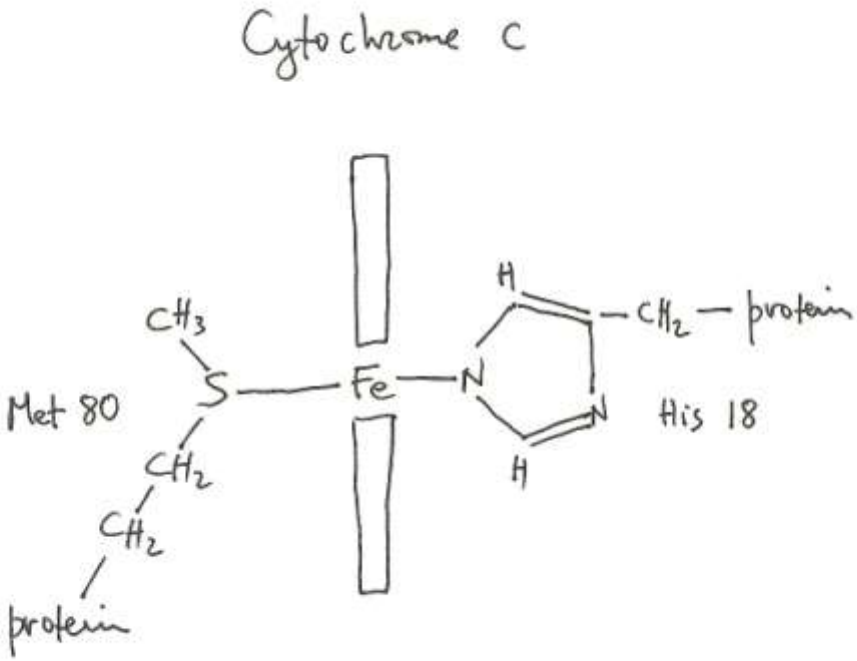
It was mentioned earlier that Cyt_c takes electrons from Cyt_{c1} to the cytochrome oxidase complex where the electrons ultimately reduce oxygen to water. Cyt_c does its job by freely diffusing along the outer surface of the mitochondrial inner mebrane. Cyt_c is a highly conserved protein that has a conformation that has remained constant for over a billion years. Cyt_c from any eucaryotic species will react *in vitro* with cytochrome oxidase from any other species. The amino acid sequences for over eighty Cyt_c species have been determined. 26 of the 104 amino acid residues have been invariant for over 1.5 billion years. Cytochrome oxidase complex contains copper atoms. These copper atoms are an integral part of the reaction center where oxygen is reduced. The active site involves a complex of copper with sulfur and iron and the reaction takes about one msec. The FeS proteins (also called ISPs for iron sulfur proteins) come in several varieties that basically differ in the number of iron atoms involved in the iron sulfur complex. In bacteria only, the simplest case occurs [Fe]



In [Fe] a single iron atom is coordinated by four sulfur atoms that are on cysteine residues of the protein. In mitochondria, [2Fe-2S] and [4Fe-4S] complexes occur as well



In these cases either 2 or 4 iron atoms and free sulfur atoms form a nano-crystal coordinated to the protein by cysteine sulfur atoms. In each of these three cases, four cysteine residues are involved. These are very primitive structures and resemble the crystal structure of the mineral pyrite (triolite). The other iron atoms in the electron transport chains are in cytochromes where they occur as heme-Fe



Heme-Fe not only occurs in cytochromes but also in the hemoglobins and myoglobins of blood. In the latter cases, heme-Fe serves as a carrier of O_2 molecules whereas in the cytochromes it is the active site for redox exchanges of electrons.