Lecture 19

Bioenergetics

Bioenergetics

- Bioenergetics describes the energy transformation process in living systems
- Living systems are complex and ordered structures, improbable from the thermodynamic point of view, to keep the order they exchange matter and energy with surrounding
- Need energy for this, convert:
- Sunlight $\longrightarrow$ chemical energy

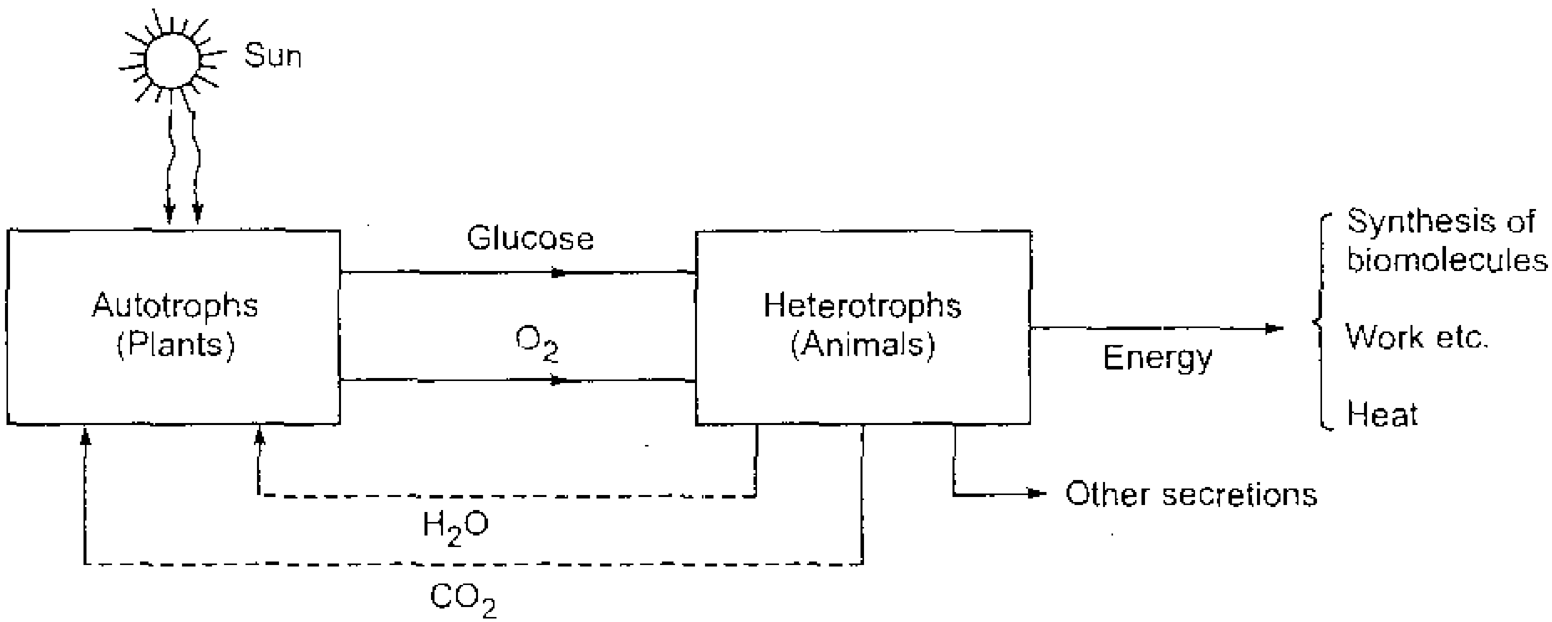
Mechanical work : muscle contraction

Active transport in membrane and nerve impulses

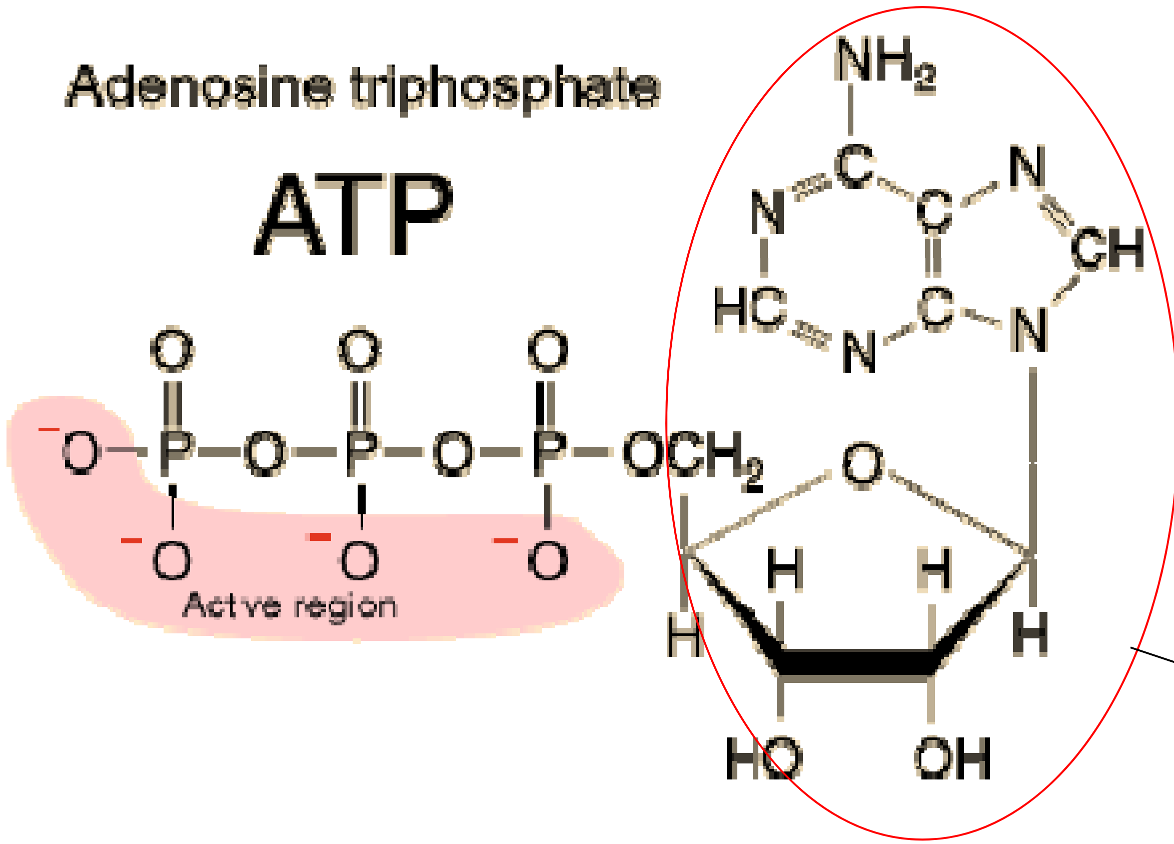
Synthesis of other biomolecules

Mechanisms to capture energy

- Each cell employs a special mechanism to capture energy
- On the basis of this mechanism cells are classified:
- **Autotrophic cells** – get energy directly from sunlight through the process called:
- **Photosynthesis** – produce glucose, energy-rich molecule from water, carbon dioxide and light



- **Heterotrophic cells** – use ready-made energy-rich glucose, carbohydrates, fat and proteins through the process called
- **Cellular respiration** – use oxygen from the atmosphere, oxidize the fuel molecules, produce energy and give carbon dioxide to the atmosphere



ATP converts to ADP - the energy is released

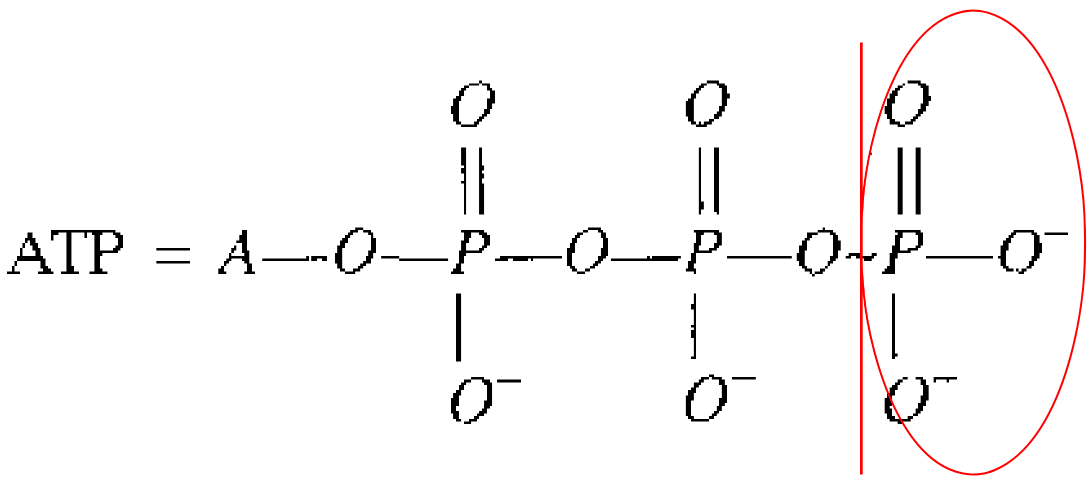
7.3 kcal/mol = 30.6 kJ/mol.

Adenosine residue A

Cells do not have a high temperature or high voltage and do not change volume to produce work. They produce energy at constant P and V in many-steps chemical processes and store energy very efficiently in ATP molecule, and use it when energy is needed to drive all biochemical reactions.

ATP plays two important roles in biochemical reactions: 1. Energy balance ; 2. Stoichiometric catalysis

- 1. Energetics:



The last (terminal) phosphate group binds to the other molecule and phosphorylates it, the energy is released:

For example if water binds to ATP at 25C pH=7:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{P}_i \qquad \Delta G^{\circ} = -30.6\,\mathrm{kJ/mole}$$

$$P_i = PO_4^{3-}$$

or 7.3 kcal/mol

ΔG is a state function and depends on the initial and final step and not on the path. Total ΔG of reaction can be calculated by steps:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{P}_i \qquad \Delta G^\circ = -30.6\mathrm{kJ/mole}$$

$$\mathrm{Glucose} + P_i \rightarrow \mathrm{Glucose} - 6 - P + H_2O \qquad \Delta G^\circ = 13.8\mathrm{kJ/mole}$$

$$\mathrm{Glucose} + \mathrm{ATP} \rightarrow \mathrm{Glucose} - 6 - \mathrm{P} + \mathrm{ADP} \qquad \Delta G^\circ = -16.8\mathrm{kJ/mole}$$

Coupling of two reactions is necessary for the energy consuming reaction to occur. This reaction is catalyzed by enzyme hexokinaze. ATP hydrolysis and glucose phosphorylation occur simultaneously.

The amount of ADP and ATP in the cell is maintained at constant ratio. ADP binds Pi and converts into ATP when light is absorbed in photo synthesis (or in cellular respiration),

The reaction is multi-step, overall:

$$\mathrm{ADP} + \mathrm{P}_i \xrightarrow{h\nu} \mathrm{ATP} + \mathrm{H_2O} \qquad \Delta G^\circ = 30.6\mathrm{kJ/mole}$$

2. Stoichiometry

Determines both the rate of biochemical reactions and concentration of reactants

$$\text{Glucose} + P_i \rightarrow \text{Glucose} - 6 - P + H_2O$$

$$\boxed{\Delta G^\circ = 13.8\text{kJ/mole}}$$

$$\Delta G^\circ = -2.3RT \log K$$

$$K = \frac{[\text{Glucose} - 6 - \text{P}]}{[\text{Glucose}][\text{P}_i]} = 10^{-3}$$

K~10^{-3} for R=8.31 J/molK, T=298K,
Pi should be too high

Considering parallel reaction:

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose} - 6 - \text{P} + \text{ADP}$$

$$\boxed{\Delta G^\circ = -16.8\text{kJ/mole}}$$

Now we get K~10^3, the relative yield is determined by ATP/ADP ratio

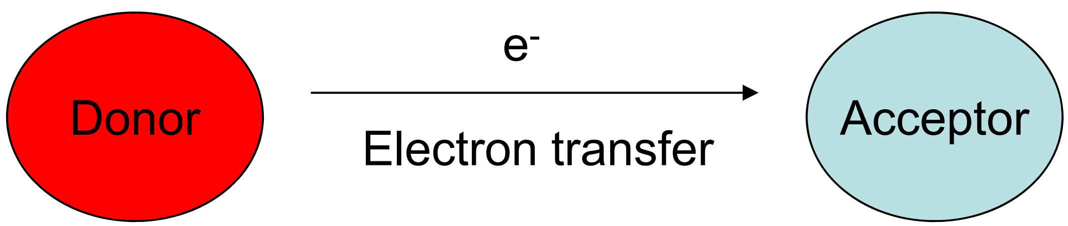
$$\frac{[\text{Glucose} - 6 - \text{P}]}{[\text{Glucose}]} = \text{K}\frac{[\text{ATP}]}{[\text{ADP}]}$$

ATP/ADP is maintained by photosynthesis and cellular respiration

Red-Ox reactions

- Many biochemical reactions are
- multi-step oxidation-reduction reactions
- enzyme-catalyzed

- Red-Ox reaction is reaction between donor and acceptor molecules:
-



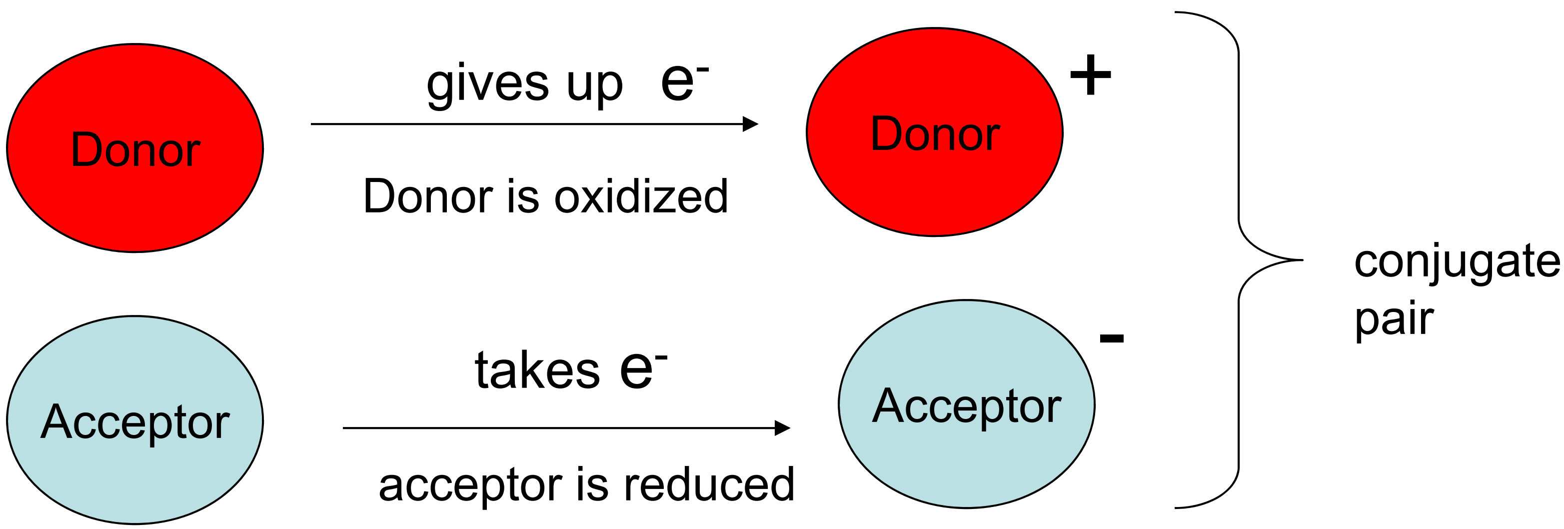
Reductant or reducing agent, gives up e

Oxidant or oxidizing agent, accepts e

Sometimes H is transferred (H^+ together with electron, $H=H^+ + e^-$)
In this case donor molecule loses a H atom, and acceptor acquires
this is called dehydrogenation or hydrogenation)

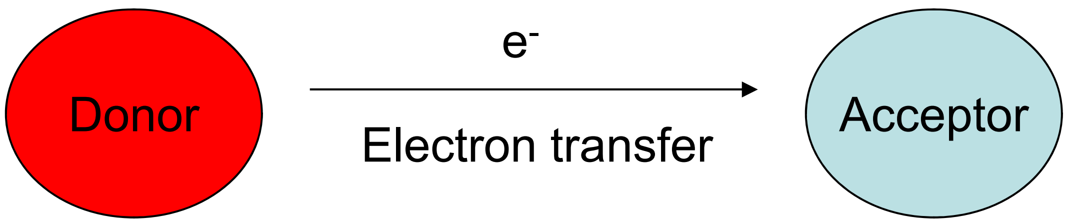
Red-Ox potential

- Oxidation and reduction of molecules goes together:
- Donor is oxidized - acceptor is reduced



Free energy in eV ΔG ~ Corresponding potential difference is called Red-Ox potential, $\Delta\phi$

- Electron moves from negative to positive (form lower to higher red-ox potential)



Red-Ox potential

$$\Delta\phi = \phi_f - \phi_i$$

$$\phi = \phi_0 + \frac{RT}{F}\ln\frac{[A]}{[D]}$$

Red-Ox potential for e transfer

$\Delta\phi$ is positive – process spontaneous, energy is released

$\Delta\phi$ is negative – process requires energy

$\Delta\phi_0$ Standard potential, measured at 25C, pH=7, C=1M

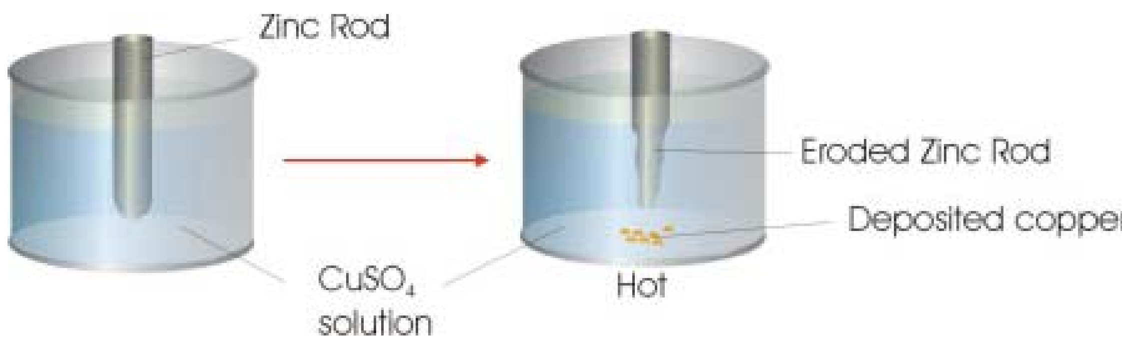
$$\Delta G = -nF\Delta\phi$$

n-moles of electrons transferred

Faraday's constant $F = N_A e$
F=96485 Coulomb/mole

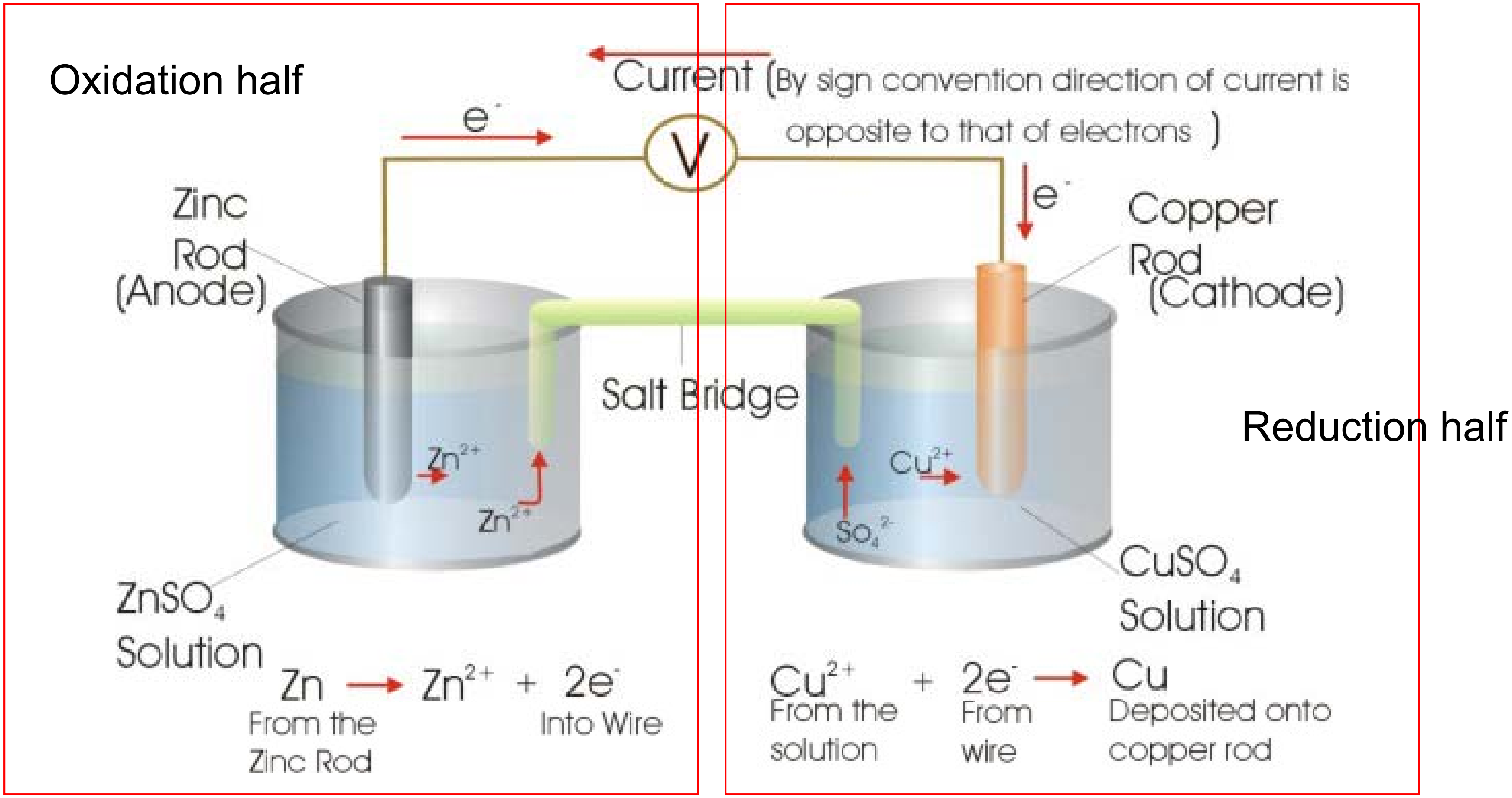
Electrochemical reactions

- If we take a zinc rod and place it in a container filled with copper sulphate solution heat will be produced. This happens due a spontaneous red-ox reaction:
- Zn(Solid) + CuSO4(Aqueous) $\longrightarrow$ ZnSO4(Aqueous) + Cu (Solid) deposited
- As the reaction would proceed the zinc rod would get eroded, copper particles would get deposited and solution would become warm



- It would be useful to be able to convert this chemical energy to electrical energy instead of heat energy. This is done by an electrochemical cell.

Electrochemical or Galvanic or Voltaic Cells



An Electrochemical Cell is a device used to convert chemical energy (produced in a red-ox reaction) into electrical energy – electromotif (emf) of the cell or potential difference between electrodes.

$$\varepsilon = -\frac{1}{nF}\Delta G$$

Construction of an Electrochemical Cell

- Red-Ox reaction:
- $Zn(Solid) + CuSO_4(Aqueous) \longrightarrow ZnSO_4(Aqueous) + Cu\ (Solid)$

- The ionic form of the reaction is:
- $Zn + Cu^{2+} \longrightarrow Zn^{2+} + Cu$

- This reaction can be split into the following two half reactions:

- 1. Oxidation half reaction
- $Zn \longrightarrow Zn^{2+} + 2e^-$

- 2. Reduction half reaction
- $Cu^{2+} + 2e^- \longrightarrow Cu$

Electrochemical Cells

Consists of two conductors (called electrodes) each immersed in a suitable electrolyte solution.

For electricity to flow:
The electrodes must be connected externally by means of a (metal) conductor.

The two electrolyte solutions should be in contact to permit movement of ions from one to the other.

Cathode is electrode at which reduction occurs: $Cu^{2+} + 2e^- \longrightarrow Cu$
.
Anode is electrode at which oxidation occurs: $Zn \longrightarrow Zn^{2+} + 2e^-$.

Energy of electrochemical cell

$$\varepsilon = -\frac{1}{nF}\Delta G$$ (electromotive force, emf)

Is the potential difference between electrodes and can be measured by voltmeter or potentiometer

Standard Hydrogen Electrode (SHE) is used, for which potential=0, by definition, when platinum electrode immersed into the solution, where $[H^+]$=1M is in equilibrium with H_2 gas at P=1Atm

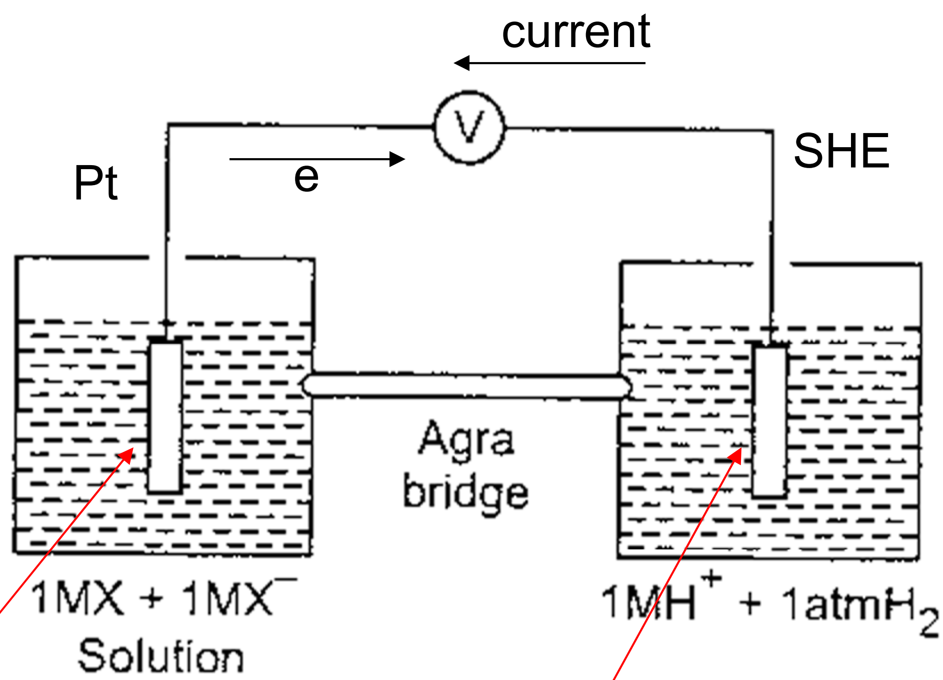
Potential of any other electrode can be measured when connected to SHE

Measuring Red-Ox potential

Red-ox couple:

$$X^{-} \rightarrow X + e^{-}$$

reductant oxidant



e flow from sample half-cell (Pt) to SHE (st H electrode)

reactions:

$$X^{-} \rightarrow X + e^{-}$$

$$H^{+} + e^{-} \rightarrow \frac{1}{2}H_2$$

V is measured experimentally for the sample electrode, as V(SHE)=0

Red-Ox couples in biological systems

- Living cells contain specific biochemical red-ox couples and enzymes which catalyze red-ox reactions. These reactions occur in photosynthesis and cellular respiration

- Red-ox couples:
- Nicotine amide adenine dinucleotide – NAD and NADP (NAD-phosphate), NAD^+ and $NADP^+$, they are reduced to NADH, NADPH
- Flavin nucleotides: FMN and FAD $\longrightarrow$ FMNH2 and FADH2

$$\left.\begin{aligned} &\mathrm{NAD^+ + (2H^+ + 2e^-) \rightarrow NADH + H^+} \\ &\mathrm{NADP^+ + (2H^+ + 2e^-) \rightarrow NADPH + H^+} \end{aligned}\right\}$$

Standard red-ox potential = -0.32V

Oxidation of NADH by oxygen in cellular respiration.

Net energy transfer:

CR- use oxygen from the atmosphere, oxidize the fuel molecules, produce energy and give carbon dioxide to the atmosphere

$$\mathrm{NADH} \xrightarrow{\text{oxidation}} \mathrm{NAD}^+ + \mathrm{H}^+ + 2e^- \qquad +0.32\mathrm{V} \times 2 = 0.64\mathrm{V}$$

$$2\mathrm{H}^+ + \frac{1}{2}\mathrm{O}_2 + 2e^- \xrightarrow{\text{reduction}} \mathrm{H}_2\mathrm{O} \qquad +0.82\mathrm{V} \times 2 = 1.64\mathrm{V}$$

$$\boxed{\mathrm{NADH} + \mathrm{H}^+ + \frac{1}{2}\mathrm{O}_2 \rightarrow \mathrm{NAD}^+ + \mathrm{H}_2\mathrm{O} \qquad +0.64\mathrm{V} + 1.64\mathrm{V} = 2.28\mathrm{V}}$$

$NADH$-NAD^+ standard potential +0.32 V

O_2-H_2O standard potential +0.82 V

Total energy released, 2.28 eV per molecule NADH

Red-Ox reactions in Photosynthesis:

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

Photosynthesis produces carbohydrates and oxygen from CO2 and water

$$2H_2O \quad \text{oxidation} \quad \rightarrow O_2 + 4H^+ + 4e^- \qquad -0.82V \times 4 = -3.28$$

reduction

$$CO_2 + 4H^+ + 4e^- \rightarrow CH_2O + H_2O \qquad -0.4V \times 4 = -1.6V$$

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

-3.28V

-1.6V=-4.88V

H_2O-O_2 standard potential -0.4 V

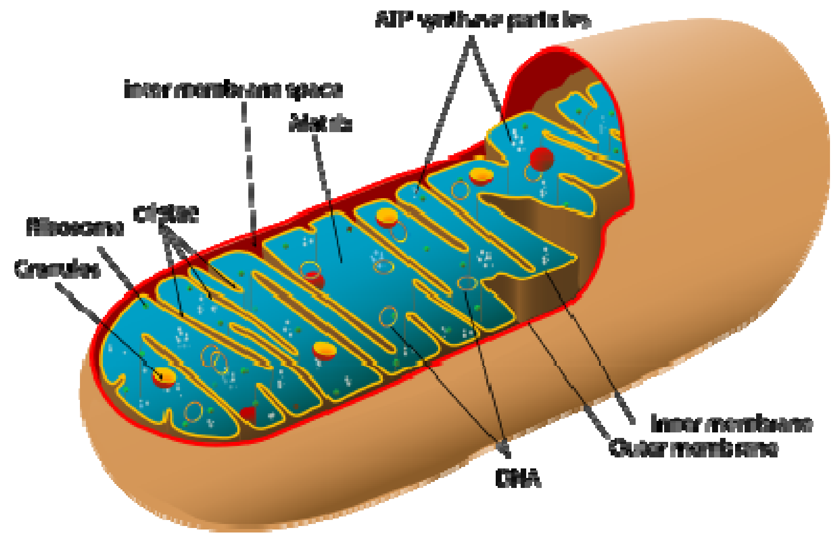
CO_2-CH_2O standard potential -0.82 V

(electron has to go against the potential)

Total energy is required, 4.88 eV to produce one molecule CH2O and O2 from H2O

Mitochondria and lysosome

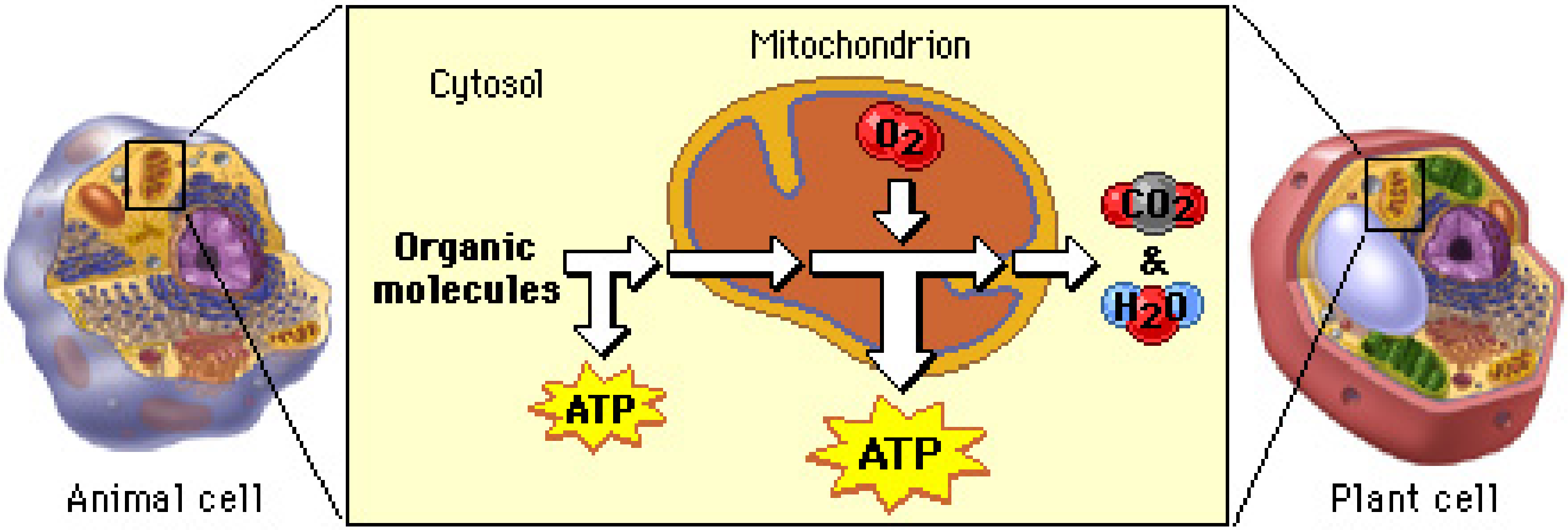
- Heterotropic cells oxidize complex food molecule (carbohydrates, fats, proteins) use oxygen from atmosphere and release carbon dioxide - this is called ***cellular respiration***
- Respiration occurs in both plants and animals, carried out in
- **mitochondria:**
- energy factories of the cells.
- Energy rich molecule adenosine triphosphate (ATP) is produced in the mitochondria using energy stored in food.
- ATP converts to ADP (adenosine diphosphate) and supplies energy to other processes in cell
-



Mitochodrion

Cellular Respiration

- Cellular respiration is the process by which the chemical energy of "food" molecules is released and partially captured in the form of ATP. Carbohydrates, glucose, fats, and proteins are used as fuels in cellular respiration.



- Energetics of cellular respiration:

- Oxidation of energy rich food molecules (carbohydrates, fats, proteins) into simple energy-poor molecules (CO_2 and H_2O) - the energy is released

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Glucose oxidizes $\longrightarrow$ 670kcal (2820 kJ)

$\downarrow$

This energy is used to synthesize 36 moles of ATP (36 x 31=1116 kJ, 42% of the energy released is used)

This is multi-step process, involve enzymes

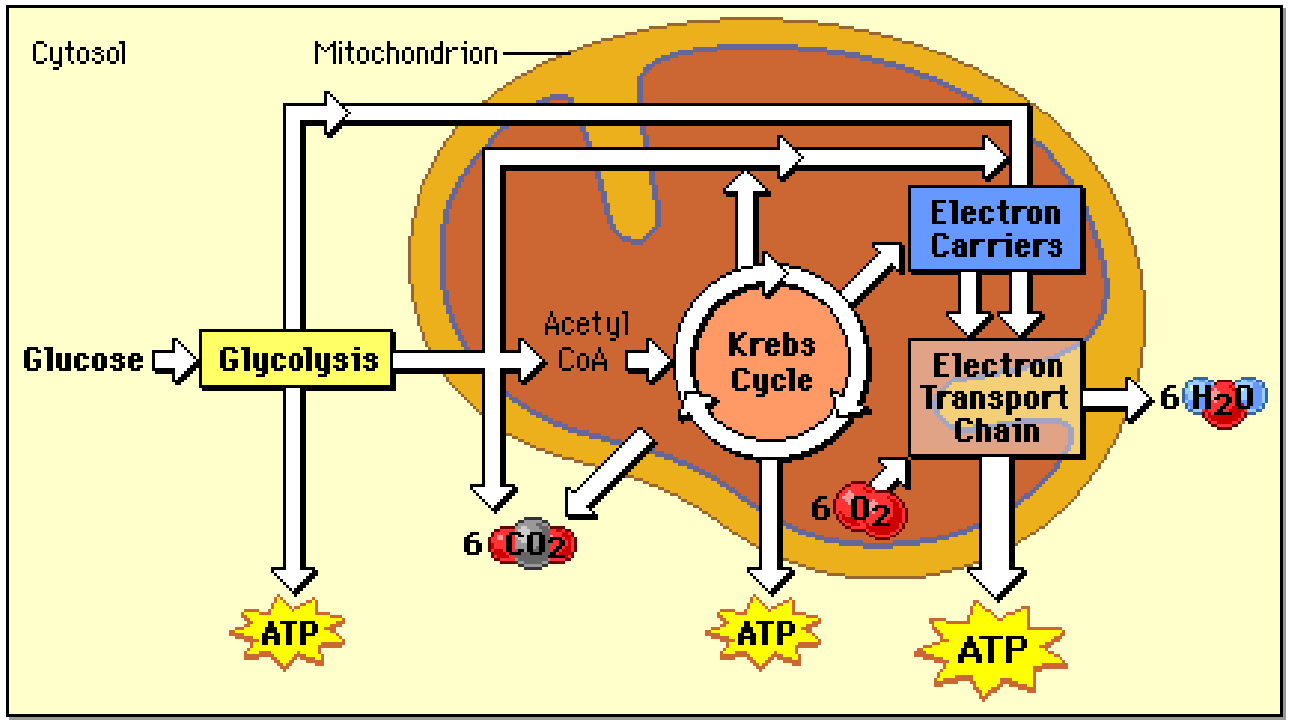
Cellular Respiration

- cellular respiration: three metabolic processes: glycolysis, the Krebs cycle, and oxidative phosphorylation. Each of these occurs in a specific region of the cell.

•1. Glycolysis occurs in the cytosol.

•2. The Krebs cycle takes place in the matrix of the mitochondria.

•3. Oxidative phosphorylation via the electon transport chain is carried out on the inner mitochondrial membrane.



•In the absence of oxygen, respiration consists of two metabolic pathways: glycolysis and fermentation. Both of these occur in the cytosol.

Glycolysis

- 6-carbon glucose, is broken down into two molecules of a 3-carbon molecule called pyruvate. This change is accompanied by a net gain of 2 ATP molecules and 2 NADH molecules.

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2CH_3COCOOH + 2ATP$$

glucose Pyruvic acid (pyruvate)

ΔG=197 kJ/mol released
62 kJ is required to synthesize 2 moles of ATP

30% efficiency

Kreb’s Cycle

- The Krebs cycle occurs in the mitochondrial matrix and generates a pool of chemical energy (ATP, NADH, and FADH2) from the oxidation of pyruvate, the end product of glycolysis.
- Pyruvate is transported into the mitochondria and degrades further to carbon dioxide and water in Kreb’s cycle, chemical energy is released and captured in the form of NADH, FADH2, reduced flavins and ATP.

1-st step						**2-st step**
glucose	→	hydrolysis	→	pyruvic acid		degrade further to CO_2 and H_2O in a cycle of reactions
protein	→	hydrolysis	→	amino acids	→	
fats	→	hydrolysis	→	fatty acids	→	

Cellular respiration

The first stage – hydrolysis of sugars -glycolysis

Second stage – further degradation to water and carbon dioxide,

energy is used to produce ATP molecules and reduced nucleotides

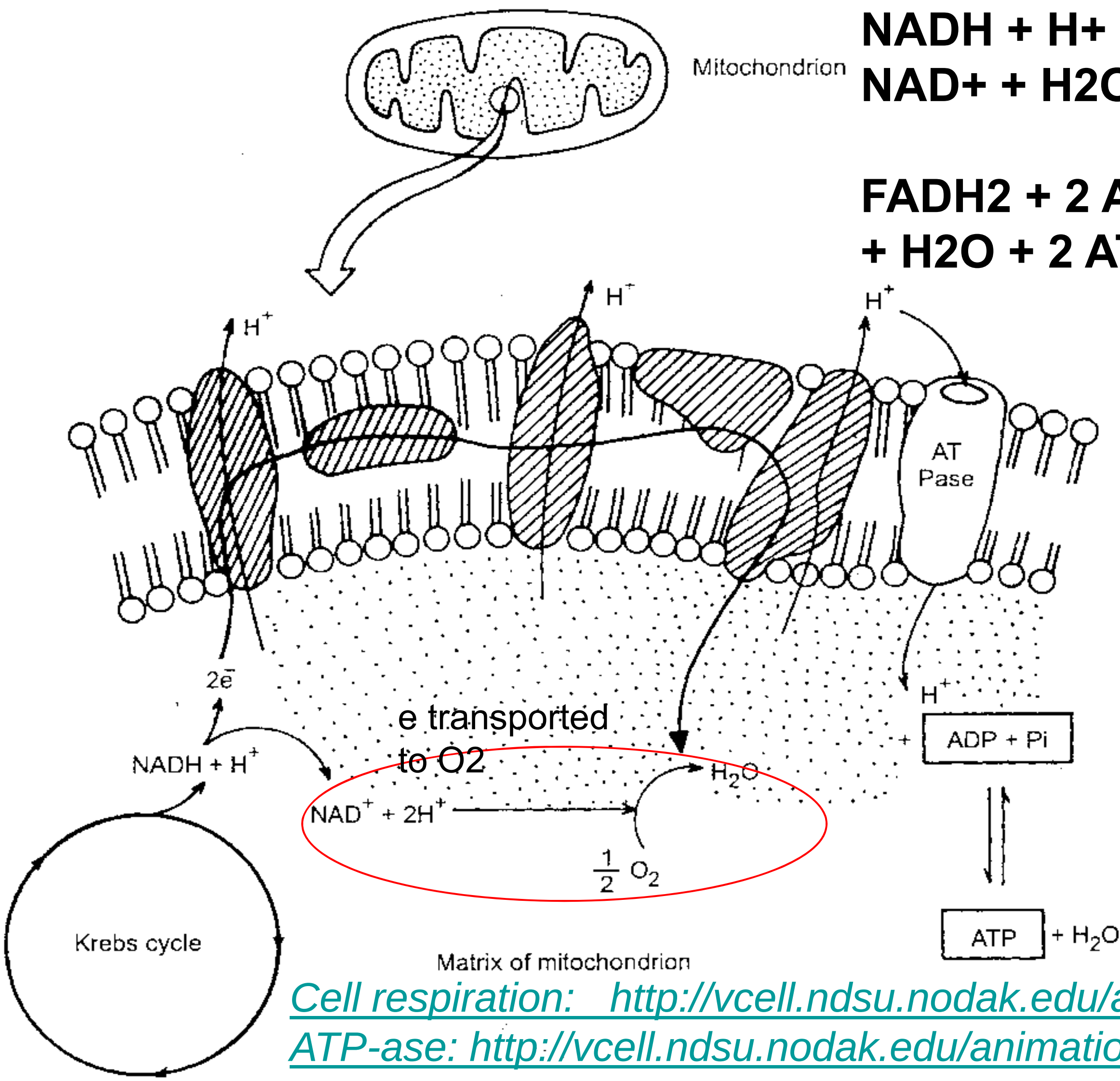
Kreb’s cycle

Third stage – respiratory chain –sequence of reactions where reduced nucleotides are oxidized –oxidation of NADH and phosphorylation of ADP into ATP is called oxidative phosphorylation

Respiration and oxidative phosphorylation. Electron transport chain

NADH + H+ + 3 ADP + 3 Pi + 1/2 O2 → NAD+ + H2O + 3 ATP

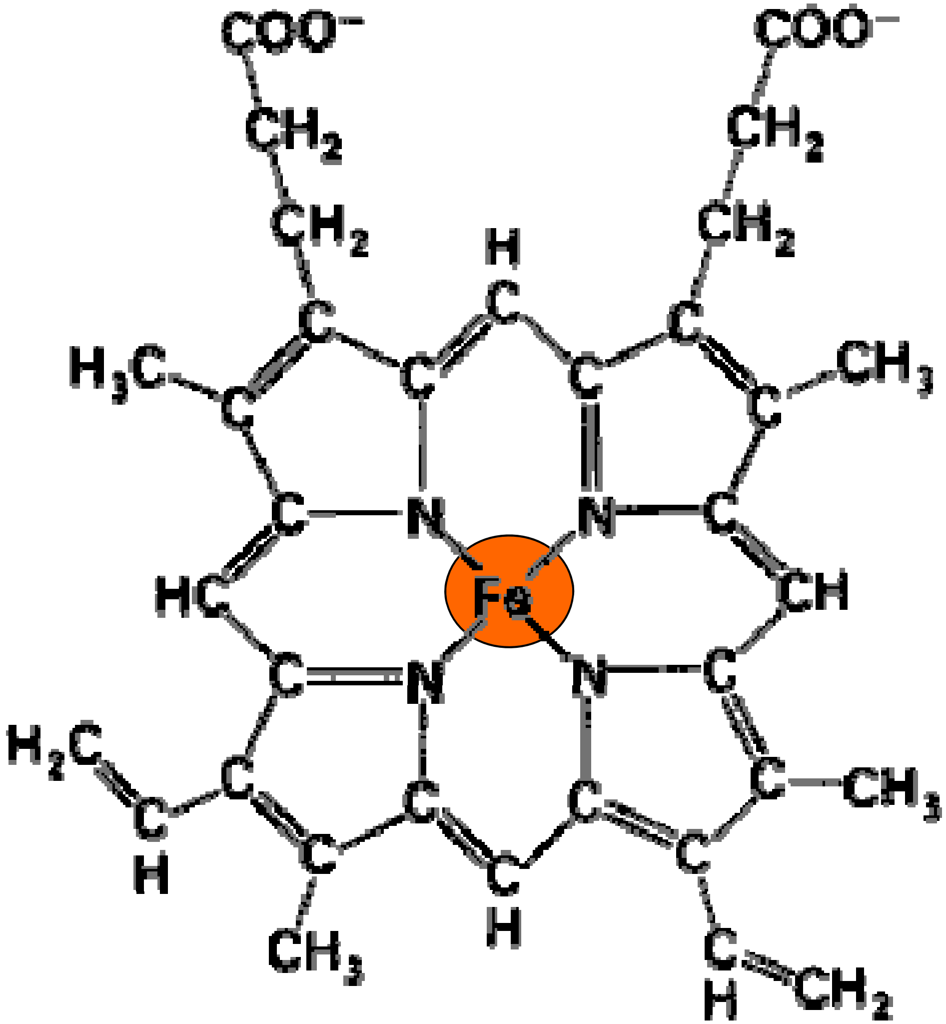
FADH2 + 2 ADP + 2 Pi + 1/2 O2 → FAD+ + H2O + 2 ATP



Enzymes effectively act as carriers and transport electrons from one molecule to another – respiratory electron transport chain

Cell respiration: http://vcell.ndsu.nodak.edu/animations/etc/movie-flash.htm

ATP-ase: http://vcell.ndsu.nodak.edu/animations/atpgradient/movie-flash.htm



Heme
(Fe-protoporphyrin IX)

Important protein - Cytochrome contains Heme group

Fe^{2+}

$\downarrow\uparrow$ Easily accepts or donates e

Fe^{3+}

passes e to other molecules until it is used to reduce oxygen to water

- All cells are able to synthesize ATP via the process of glycolysis. In many cells, if oxygen is not present, pyruvate is metabolized in a process called fermentation.
- Fermentation complements glycolysis and makes it possible for ATP to be continually produced in the absence of oxygen. By oxidizing the NADH produced in glycolysis, fermentation regenerates NAD+, which can take part in glycolysis once again to produce more ATP.

- The chemical energy stored in glucose generates far more ATP in aerobic respiration than in respiration without oxygen (glycolysis and fermentation).
- Each molecule of glucose can generate 36-38 molecules of ATP in aerobic respiration but only 2 ATP molecules in respiration without oxygen (through glycolysis and fermentation).

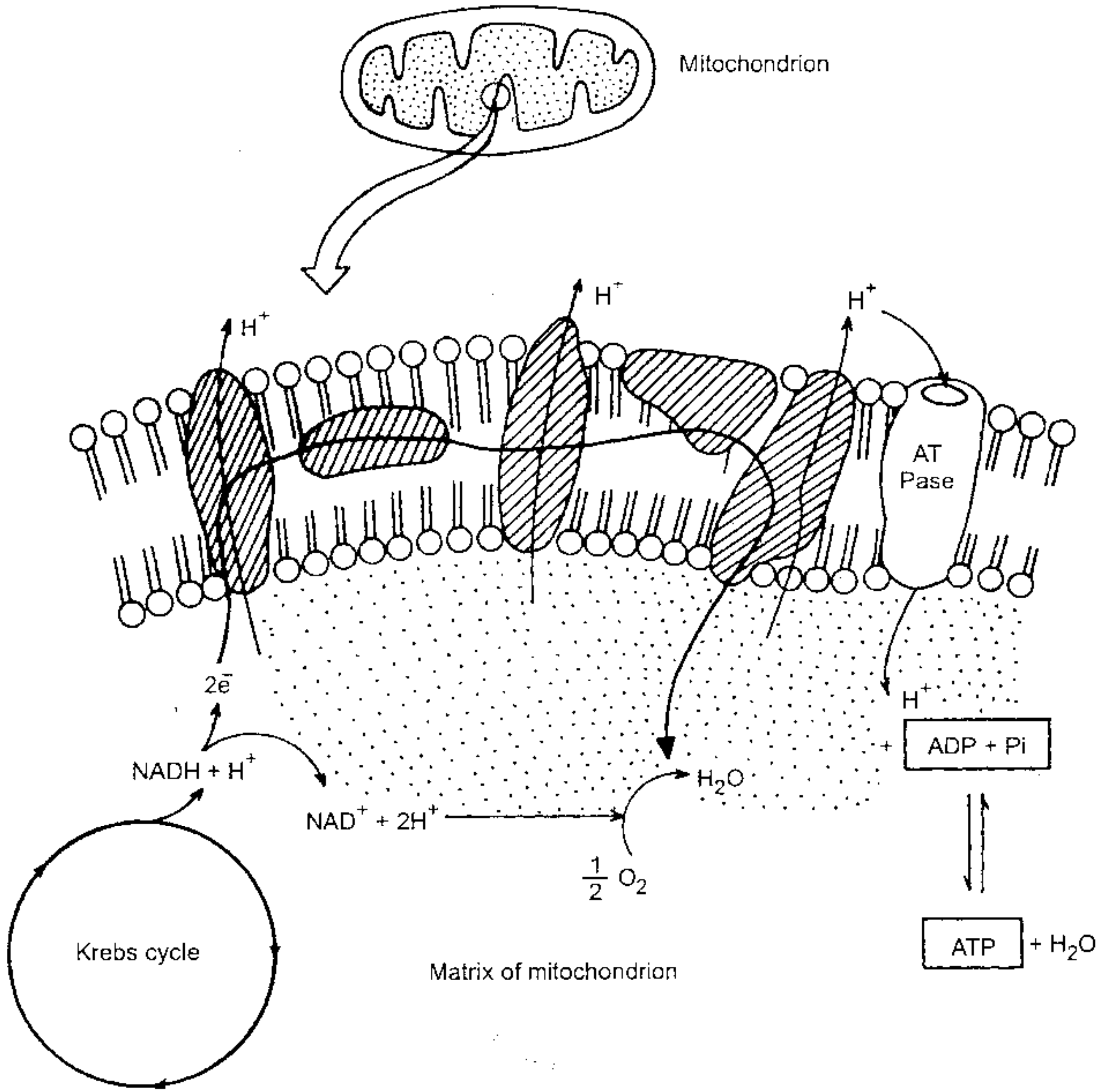
Chemiosmotic theory

- Cell respiration – goal to synthesize ATP
- ATP provides the energy
- How enzymes synthesizing ATP couple with respiratory chain and use energy released during electron transfer?

↓

- Chemiosmotic theory, Mitchel 1961:

- There is no direct coupling between respiratory electron transport chain and phosphorylation
- The coupling occurs through the membrane of mitochondria - electron transfer is coupled with proton transfer



Energy obtained through the transfer of electrons is used to pump protons from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane called ΔΨ. This electrochemical proton gradient allows ATP synthase (ATP-ase) to use the flow of H+ through the enzyme back into the matrix to generate ATP from ADP and inorganic phosphate Pi.

(NADH coenxyme Q reductase; accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them further to multiple complexes which use the electrons and hydrogen ions to reduce molecular oxygen to water.

- Every time electrons move through the membrane they move protons along with them. 6 protons move during oxidation of 1 molecule NADH, as electron pair moves up and down in membrane 3 times, membrane becomes polarized (more protons outside). This generates proton current back inside the membrane, and is linked to synthesis of ATP
- Total electrochemical potential (force pulling protons back) is called proton motive force:

$$\Delta p = \Delta\phi + \frac{RT}{F}\ln\frac{[H^+]_0}{[H^+]_i} \qquad \Delta p = \Delta\phi - \frac{2.3RT}{F}\Delta pH$$

$$\Delta p = 140 - 60(-1.4) = 224 mV. \qquad \Delta p = \Delta\phi - 60\Delta pH$$

140 mV

Proton current helps to transport ADP and Pi inside and transport ATP outside the membrane

coupling

- Electron transport chains are the source of energy for all known forms of life. They are red-ox reactions that transfer electrons from an electron donor to an electron acceptor. The transfer of electrons is coupled to the translocation of protons across a membrane, producing a proton gradient. The proton gradient is used to produce useful work.
- The coupling of thermodynamically favorable to thermodynamically unfavorable biochemical reactions by biological macromolecules is an example of an **emergent property** – a property that could not have been predicted, even given full knowledge of the primitive geochemical systems from which these macromolecules evolved.
- It is an open question whether such emergent properties evolve only by chance, or whether they *necessarily* evolve in any large biogeochemical system, given the underlying laws of physics.