

MitoSeminar II:

Some calculations in bioenergetics

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Respiratory chain and oxidative phosphorylation: Summary

- * **Transfer of electrons** via electron carriers (*respiratory chain*) to oxygen in the inner membrane
- * **Proton pumping** from the matrix to the cytosolic site of the inner mitochondrial membrane (proton gradient).
Protonmotive force = pH gradient + membrane potential
- * Protons flow through *ATP synthase* and power the **synthesis of ATP.**

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Stoichiometry of oxidative phosphorylation

- **If F_0 complex has 10 c subunits: one complete turn means flow of 10 protons.**
- **The F_1 part has three ATP-producing sites: one complete turn of the shaft means synthesis of three ATP from ADP + Pi**
- **At least 3 H^+ have to be pumped out of the matrix for production of 1 ATP**
- **One more H^+ is consumed for import of phosphate**
- **Transport of 2 electrons from NADH to oxygen (complexes I, III, IV) pumps 10 protons, from $FADH_2$ to oxygen (complexes III, IV) 6 protons .**
- **Oxidation of 1 NADH produces 2.5 ATP**
- **Oxidation of 1 $FADH_2$ produces 1.5 ATP**

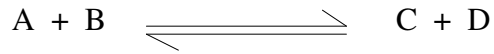
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$$\Delta G = \Delta H - T \Delta S$$

The chemical reaction can occur only if the ΔG is negative.
It means: when the products have less free energy than the reactants have.

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ΔG and chemical equilibrium



$$K_{eq} = \frac{[C_{eq}][D_{eq}]}{[A_{eq}][B_{eq}]}$$

$$\Delta G = -RT \ln K_{eq} + RT \ln \frac{[C][D]}{[A][B]}$$



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Standard free enthalpy

$$\Delta G^{o'} = -RT \ln K_{eq}'$$

1.0M, 25 °C pH 7.0

R ... universal gas constant 8.3143 J mol⁻¹K⁻¹

T ... absolute temperature in Kelvins (298.15 K = 25°C)

$$\Delta G = -RT \ln K_{eq} + RT \ln \frac{[C][D]}{[A][B]}$$

$$\Delta G = \Delta G^{o'} + RT \ln \frac{[C][D]}{[A][B]}$$

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Table 1.1 Relationship of the equilibrium constant (K_{eq}) of a reaction* to the $\Delta G^{0'}$ value of that reaction

Approximate $\Delta G^{0'}$ (kJ mol ⁻¹)	K'_{eq}
+17.1	0.001
+11.4	0.01
+5.7	0.1
0	1.0
-5.7	10.0
-11.4	100
-17.1	1000

*For a reaction $A + B \leftrightarrow C + D$, the equilibrium constant is the molar concentration of $C \times D$ divided by that of $A \times B$.

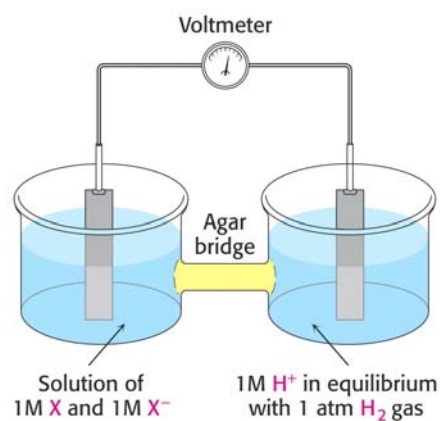
$$\left(K_{eq} = \frac{[C][D]}{[A][B]} \right); K'_{eq} \text{ is the } K_{eq} \text{ at pH 7.0.}$$

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Redox potential



Difference in affinities to electrons between two redox couples, in volts.



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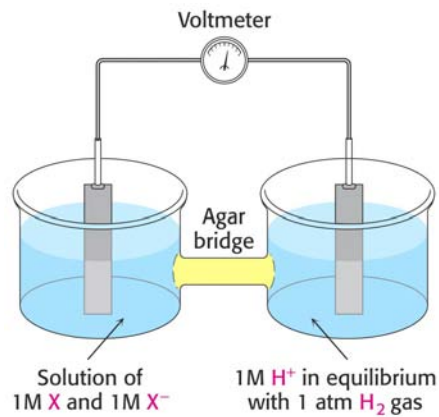
Redox potential

E° ...standard redox potential, 1.0 M, pH 0

$E^{\circ\prime}$... standard redox potential, 1.0 M, pH 7

Reference hydrogen electrode:

$E^{\circ} = 0.0 \text{ V}$ $E^{\circ\prime} = -0.42 \text{ V}$



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Table 12-1. Some redox potentials of special interest in mammalian oxidation systems.

System	E'_{\circ} Volts
H ⁺ /H ₂	-0.42
NAD ⁺ /NADH	-0.32
Lipoate; ox/red	-0.29
Acetoacetate/3-hydroxybutyrate	-0.27
Pyruvate/lactate	-0.19
Oxaloacetate/malate	-0.17
Fumarate/succinate	+0.03
Cytochrome <i>b</i> ; Fe ³⁺ /Fe ²⁺	+0.08
Ubiquinone; ox/red	+0.10
Cytochrome <i>c</i> ₁ ; Fe ³⁺ /Fe ²⁺	+0.22
Cytochrome <i>a</i> ; Fe ³⁺ /Fe ²⁺	+0.29
Oxygen/water	+0.82

(from Harper's Illustrated Biochemistry, 27th edition, McGraw-Hill Co. 2006)

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Relationship between free enthalpy and redox potential

$$\Delta G = -nF \Delta E$$

n ... number of transferred electrons

F ... Faraday constant = 96 485 C /mol

C (Coulomb) = J/ V

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The Nernst equation

Tells voltage of galvanic cell, or redox potential, for various concentrations of components.

$$\Delta G = \Delta G^{\circ'} + RT \ln \frac{[C][D]}{[A][B]} + \Delta G = -nF \Delta E$$

$$\Delta E = \Delta E^{\circ'} + \frac{RT}{nF} \ln \frac{[OX]}{[RED]}$$

Walther Hermann Nernst (1864-1941): Nobel Prize 1920

Equation also called Nernst-Peters

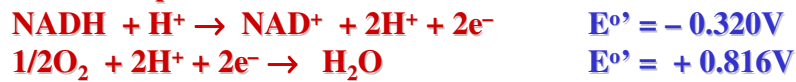
(Peters applied Nernst equation for redox processes)

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Example I: Transfer of electrons from NADH to oxygen:



Redox couples:



For the whole reaction:

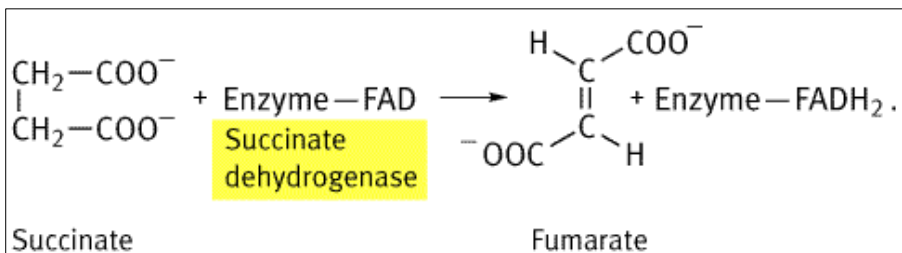
$$\Delta E^{\circ'} = 0.816\text{V} - (-0.320\text{V}) = 1.136\text{V}$$

$$\Delta G^{\circ'} = -nF \Delta E^{\circ'}$$

$$\Delta G^{\circ'} = -2(96.5 \text{ kJ V}^{-1} \text{ mol}^{-1})(1.136\text{V}) = \underline{-219.25 \text{ kJ mol}^{-1}}$$

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Example II: Succinate dehydrogenase



Redox couples:



$$\Delta G^{\circ'} = -2(96.5 \text{ kJ V}^{-1} \text{ mol}^{-1})(-0.15\text{V}) = \underline{+28.95 \text{ kJ mol}^{-1}}$$

Reaction cannot proceed ???

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Example II: Succinate dehydrogenase

Redox couples:



But, if fumarate is consumed in the next reaction, and FAD reoxidized, the actual ratios succinate/fumarate and FAD/FADH₂ would not be 1:1

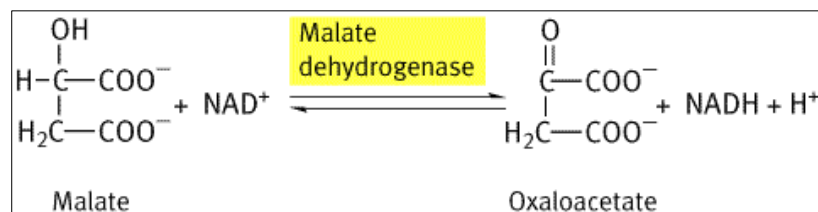
If succinate : fumarate is 500:1, redox potential of the system using the Nernst equation would be:

$$\Delta E = \Delta E^{\circ'} + \frac{RT}{nF} \ln \frac{[\text{OX}]}{[\text{RED}]} = 0.03 + (-0.08) = - 0.05 \text{ V}$$

And for FAD/FADH₂ 500:1 $\Delta E = -0.12 + 0.08 = - 0.04 \text{ V}$

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Likewise malate dehydrogenase reaction:

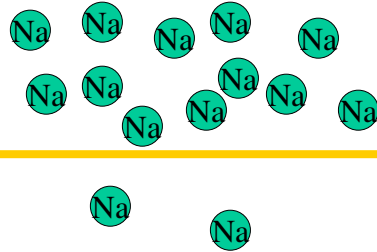


Redox couples:



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Ionic gradient on a membrane can also do work



$$c(\text{Na}^+)_1 = 120 \text{ mmol/l}$$

$$c(\text{Na}^+)_2 = 12 \text{ mmol/l}$$

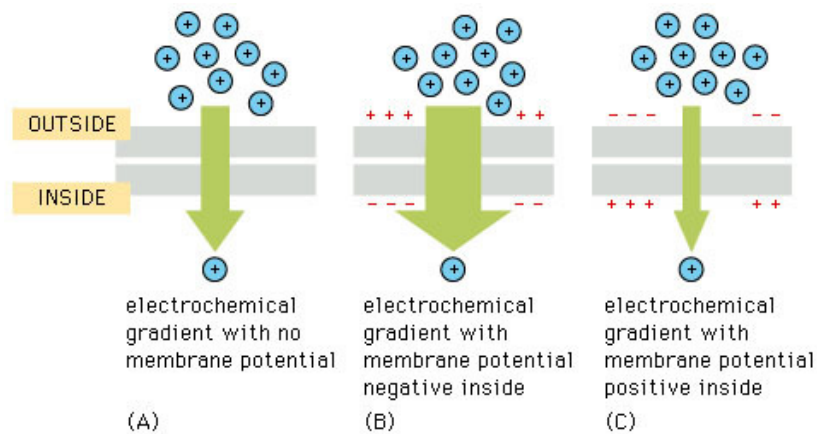
$$\Delta G = -RT \ln \frac{c_1}{c_2}$$

$$= -(8.3143 \times 298.15) \times \ln 10 =$$

$$= -5.7 \text{ kJ/mol}$$

Equation is valid if $c_1 > c_2$ (diffusion to c_2); for 37 °C the coefficient is -5.9

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Protonmotive force

$$\Delta p = \Delta \text{pH} + \Delta \psi$$

Difference in concentration of protons
- about 1 pH unit
...from Nernst equation is 60 mV

Electric potential on the inner membrane.
Must be measured, value e.g. 160 mV

$$\Delta p = 60 \text{ mV} + 160 \text{ mV} = 220 \text{ mV}$$

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Efficiency of mitochondrial production of ATP

Oxidation of 1 mole of NADH leads to pumping of 10 mol protons and production of 2.5 mol ATP, protonmotive force is 220 mV

Oxidation of 1 mol NADH: $\Delta G^{\circ} = -219.25 \text{ kJ/mol}$

Protonmotive force $\Delta p = 220 \text{ mV}$ corresponds to:

$$\Delta G = \Delta_{\mu} \text{H}^+ = -F \cdot \Delta p = -21.2 \text{ kJ/mol}$$

$\Delta_{\mu} \text{H}^+$
is electrochemical proton gradient

For pumping 10 mol protons: $\Delta G = 10 \times (21.2) = 212 \text{ kJ}$

...Most of the energy from oxidation is converted to proton gradient

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For pumping 10 mol protons: $\Delta G = 10 \times (21.2) = 212 \text{ kJ}$

For 1 ATP is needed: $\Delta G^{\circ} = 30.5 \text{ kJ/mol}$,

In the cell really (excess of ATP): $\Delta G' = \text{asi } 50 \text{ kJ/mol}$

4 protons do work 84.9 kJ/mol, which is certainly enough for 1 ATP

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Oxidation of 1 mol NADH gives theoretically energy for making 4.4 mol ATP, practically 2.5 mol is produced ... efficiency cca 57 % (for standard conditions cca 35 %)

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