THE UNIVERSITY OF ZAMBIA

PRINCIPLES OF BIOENERGETICS

Dr. Rehana Omar (BSc, MSc, PhD)
School of Medicine
Department of physiological sciences.
rehana@unza.zm

What you should know by the end of todays class

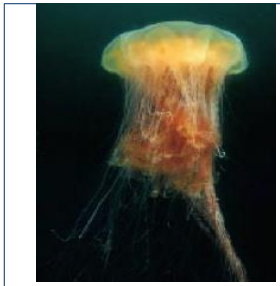
Objectives

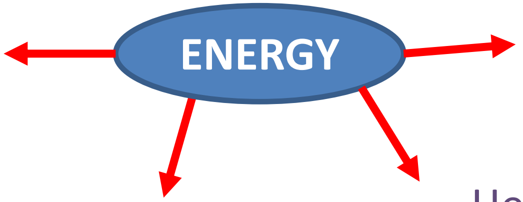
- Chemical thermodynamics
- Standard free energy change of reactions
- Reaction coupling mechanism
- Oxidation-reduction reactions
- Energy-rich compounds
- Enzymes and coenzymes involved in redox reactions

Nelson, D. L & Cox, M. M; ***Lehninger Principles of Biochemistry*** Fifth Edition (2008). W.H. Freeman & Co. Pp 496-515

BIOENERGETICS

Bioenergetics: the quantitative study of energy transduction in living organisms





Production of light e.g. in fireflies, luminescent creatures

Synthesis of macromolecules

Heat and motion

Concentration and electrical gradients

All the energy changes in a living system (cells) follow the **laws of thermodynamics**

There are three quantities of thermodynamics;

Gibb's free energy (Δ*G*): energy available to do work at constant temperature and pressure.

- **–Δ*G*** = an **exergonic** reaction that **releases energy to the surrounding** (**spontaneous** reactions).
- **+ Δ*G*** = an endergonic reaction **takes up energy** from the surrounding (not spontaneous).

Enthalpy (*H*): The heat content of a reacting system. Exothermic systems release heat energy to the surrounding (***-H*** value) while endothermic reactions do the opposite (***+H*** value*).*

Entropy (*S*): An expression of the randomness or disorder of a system. Reactions that proceed to produce disordered (more randomly organized, less complex products) are said to occur with a gain in entropy.

The three quantitative events are related to each other as follows: $\Delta G=\Delta H - T\Delta S$

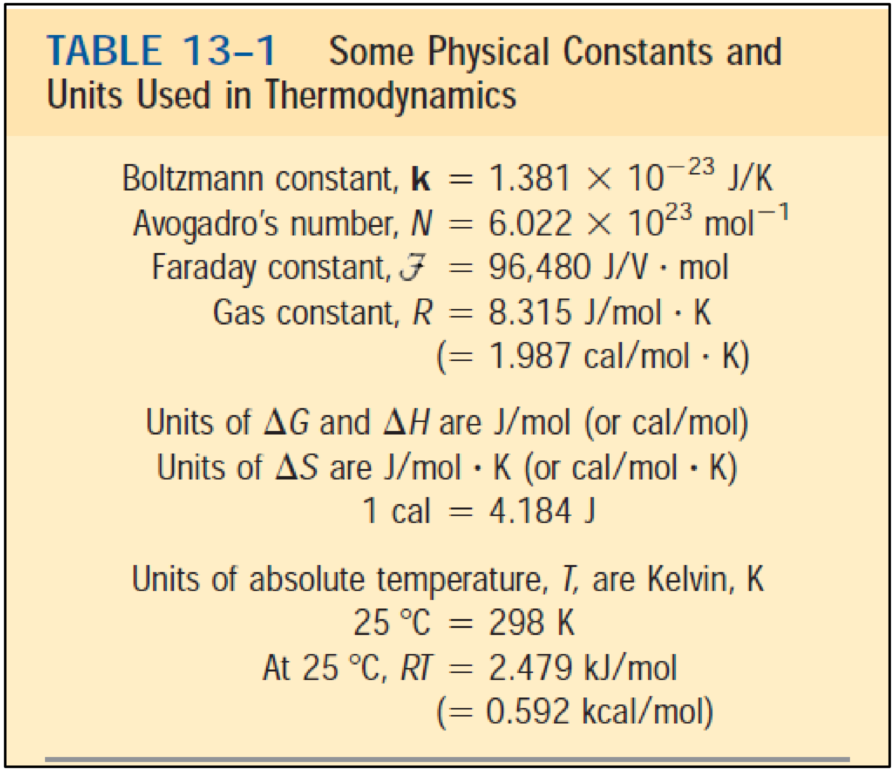


TABLE 13–1 Some Physical Constants and Units Used in Thermodynamics

Boltzmann constant, $\mathbf{k} = 1.381 \times 10^{-23}$ J/K
Avogadro's number, $N = 6.022 \times 10^{23}\ \text{mol}^{-1}$
Faraday constant, $\mathcal{F} = 96{,}480$ J/V · mol
Gas constant, $R = 8.315$ J/mol · K
(= 1.987 cal/mol · K)

Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol · K (or cal/mol · K)
1 cal = 4.184 J

Units of absolute temperature, T, are Kelvin, K
25 °C = 298 K
At 25 °C, RT = 2.479 kJ/mol
(= 0.592 kcal/mol)

Determination of free energy change of a system

How do we calculate the free energy derived from nutritional intake?

The standard free energy change of a system is defined as a reaction happening at initial concentrations of components at 1M of substance or $1atm$ for gasses at $25°, pH\ 7.0$.

Reactions (as in living organism) that are not at standard conditions are calculated as:

$$\Delta G'^{\circ} = -RT \ln K'_{\text{eq}}$$

where $$K_{\text{eq}} = \frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}$$

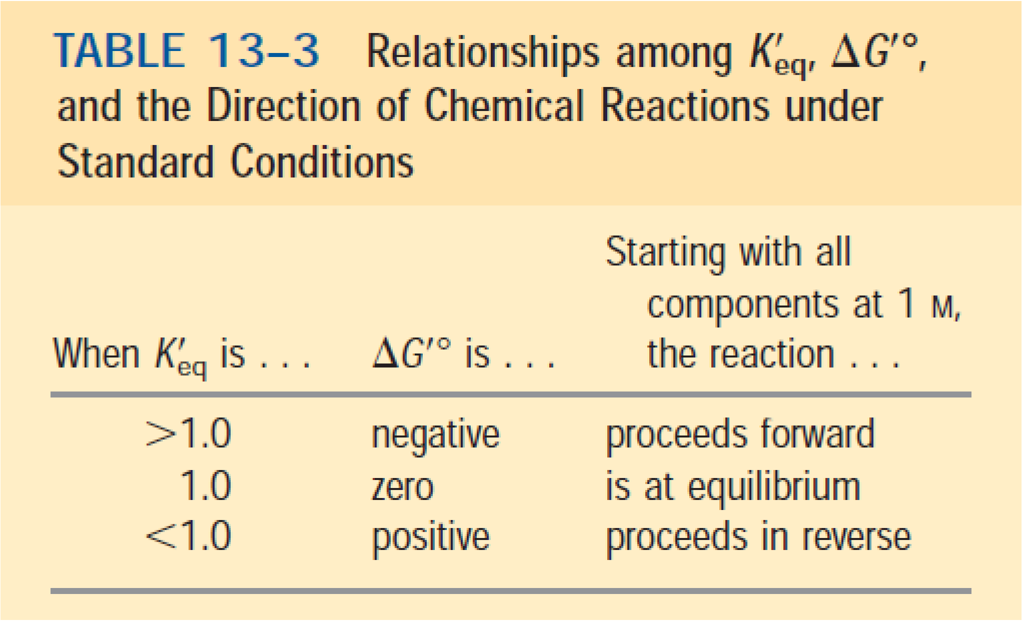


TABLE 13–3 Relationships among K'_{eq}, $\Delta G'^{\circ}$, and the Direction of Chemical Reactions under Standard Conditions

When K'_{eq} is . . .	$\Delta G'^{\circ}$ is . . .	Starting with all components at 1 M, the reaction . . .
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

An example of how to calculate free energy from glucose oxidation

$$\text{Glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$ (catalyzed by phosphoglucomutase)

$$K'_{eq} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose 1-phosphate}]} = \frac{19 \text{ mM}}{1 \text{ mM}} = 19$$

$$\begin{aligned} \Delta G'^{\circ} &= -RT \ln K'_{eq} \\ &= -(8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K})(\ln 19) \\ &= -7.3 \text{ kJ/mol} \end{aligned}$$

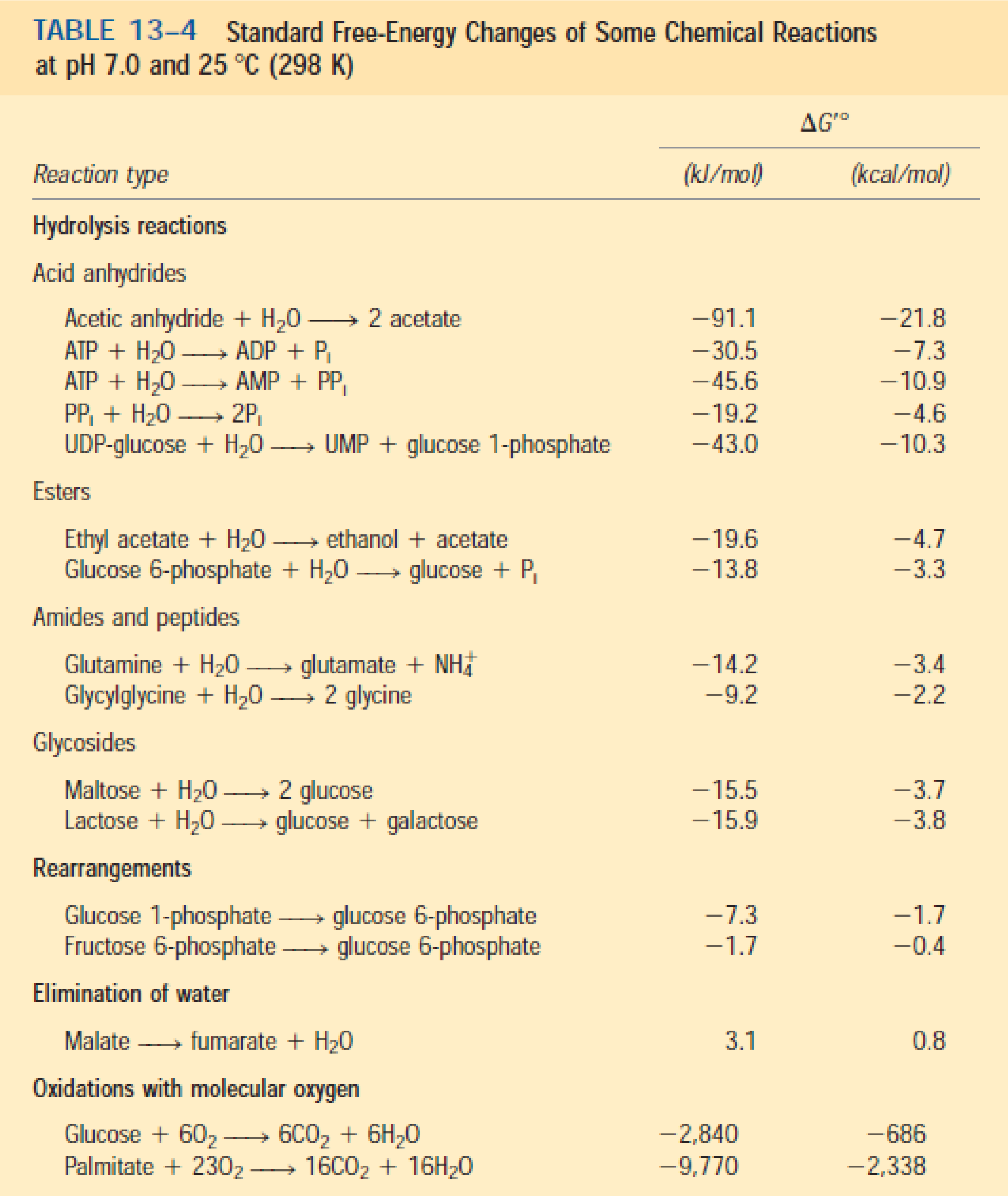


TABLE 13–4 Standard Free-Energy Changes of Some Chemical Reactions at pH 7.0 and 25 °C (298 K)

Reaction type	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G'^{\circ}$ (kcal/mol)
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \longrightarrow$ 2 acetate	−91.1	−21.8
ATP + $H_2O \longrightarrow$ ADP + P_i	−30.5	−7.3
ATP + $H_2O \longrightarrow$ AMP + PP_i	−45.6	−10.9
PP_i + $H_2O \longrightarrow 2P_i$	−19.2	−4.6
UDP-glucose + $H_2O \longrightarrow$ UMP + glucose 1-phosphate	−43.0	−10.3
Esters		
Ethyl acetate + $H_2O \longrightarrow$ ethanol + acetate	−19.6	−4.7
Glucose 6-phosphate + $H_2O \longrightarrow$ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + $H_2O \longrightarrow$ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + $H_2O \longrightarrow$ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + $H_2O \longrightarrow$ 2 glucose	−15.5	−3.7
Lactose + $H_2O \longrightarrow$ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose 1-phosphate $\longrightarrow$ glucose 6-phosphate	−7.3	−1.7
Fructose 6-phosphate $\longrightarrow$ glucose 6-phosphate	−1.7	−0.4
Elimination of water		
Malate $\longrightarrow$ fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \longrightarrow 6CO_2 + 6H_2O$	−2,840	−686
Palmitate + $23O_2 \longrightarrow 16CO_2 + 16H_2O$	−9,770	−2,338

Chemical reactions

- There are many types of reactions of importance that occur;

1. Making/breaking C-C bonds
2. Internal arrangements (isomerization and elimination)
3. Free radical reactions
4. Group transfers
5. Oxidation-reduction reactions.

- Many of these reactions involve compounds rich in electrons aka ***nucleophiles***- these donate electrons. ***Electrophiles*** are electron deficient and seek out electron rich molecules (i.e. these two function together giving and receiving).

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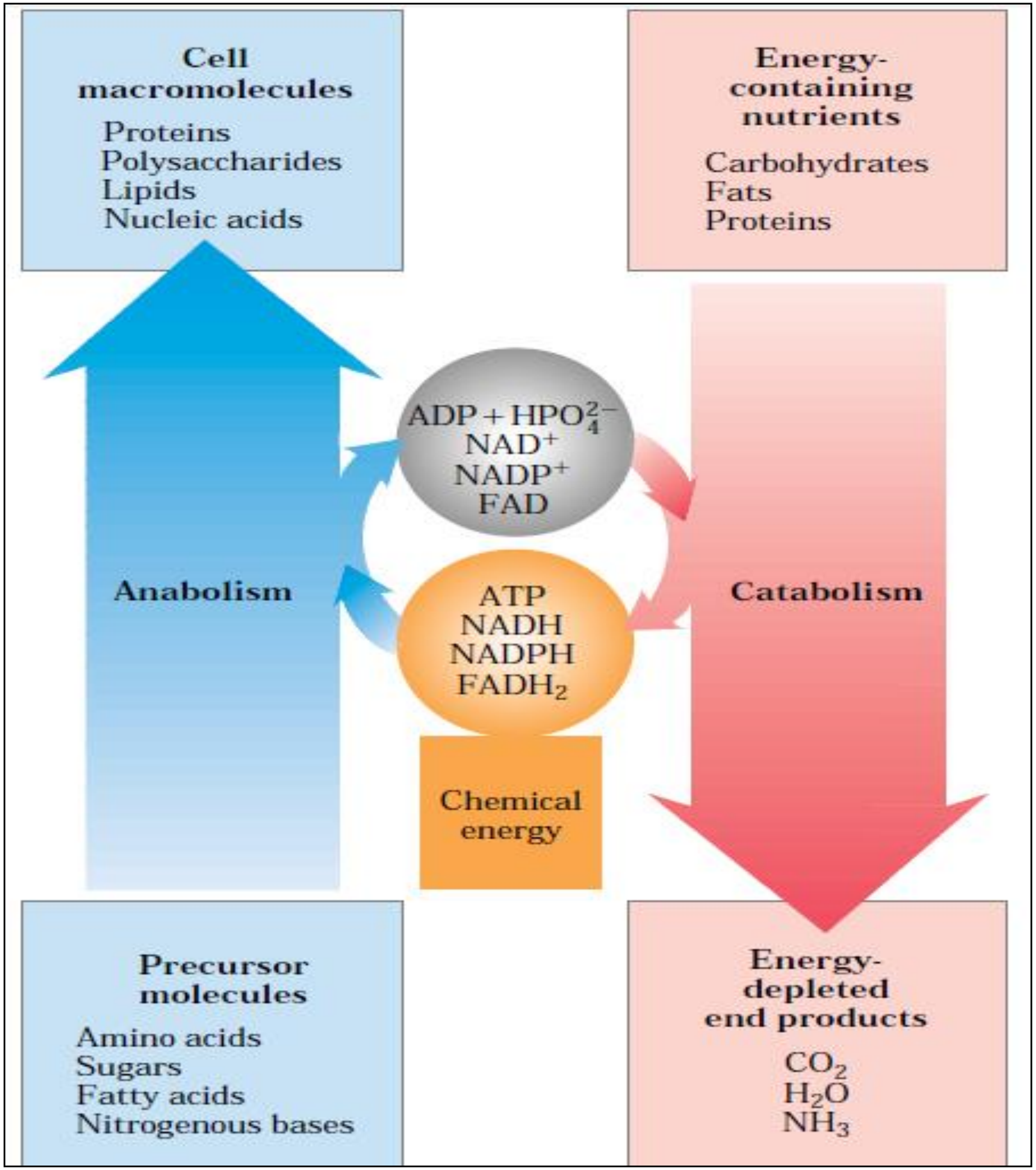

Cell macromolecules

Proteins
Polysaccharides
Lipids
Nucleic acids

Energy-containing nutrients

Carbohydrates
Fats
Proteins

Anabolism

$ADP + HPO_4^{2-}$
NAD^+
$NADP^+$
FAD

ATP
NADH
NADPH
$FADH_2$

Chemical energy

Catabolism

Precursor molecules

Amino acids
Sugars
Fatty acids
Nitrogenous bases

Energy-depleted end products

CO_2
H_2O
NH_3

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Oxidation-Reduction (Redox) Reactions

- This is one of the central features in metabolism.
- Involves transferring an electron from one group to another.
- The group that loses the electron is ***Oxidized.***
- The group that gains this electron is ***Reduced.***

Where do these electrons come from?

- In heterotrophs they are obtained from nutrients (food) that undergo oxidation.
- In photosynthetic organisms, they are obtained from an electron donor that gets excited by UV light.

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Oxidation-Reduction (Redox) Reactions

Where do these electrons go?

- They move through metabolites (reaction intermediates) in a series of redox reactions.
- Eventually they reach electron transport chains and are passed from one carrier to the next carrier which has a higher electron affinity. In this way the flow of electrons is in one direction.
- The movement of electrons down the ETC generates energy.
- The end of the ETC is a something like oxygen which has a high affinity for electrons and thus creates water at the end.

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How electrons create energy

- Lets take glucose.
- It is relatively reduced.
- As it goes through a series of chemical reactions it gets more and more oxidized.
- We can say it loses $2H^+$ and $2e^-$ (i.e. this makes 2 whole hydrogen atoms) during each stage of oxidation.
- Electrons produced will move spontaneously through ETC (spontaneous means exergonic) because the chain always has a biomolecule that has a greater affinity for electrons than the previous biomolecule.
- Spontaneous reactions always release energy.

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How electrons create energy

- BATTERY CELLS EXAMPLE: two types of ions; iron and copper each in their conjugate redox pairs;
- $Fe^{2+} + Cu^{2+} \longrightarrow Fe^{3+} + Cu^{+}$ (e^{-} move from iron to copper).
- As they move they create a force that is equal to the difference in affinities of e by the two entities.
- Larger difference in affinity = greater force created.
- This force is called an **electro motive force**, EMF.
- EMF in turn enables other molecules like enzymes and proteins to function in various cellular processes.

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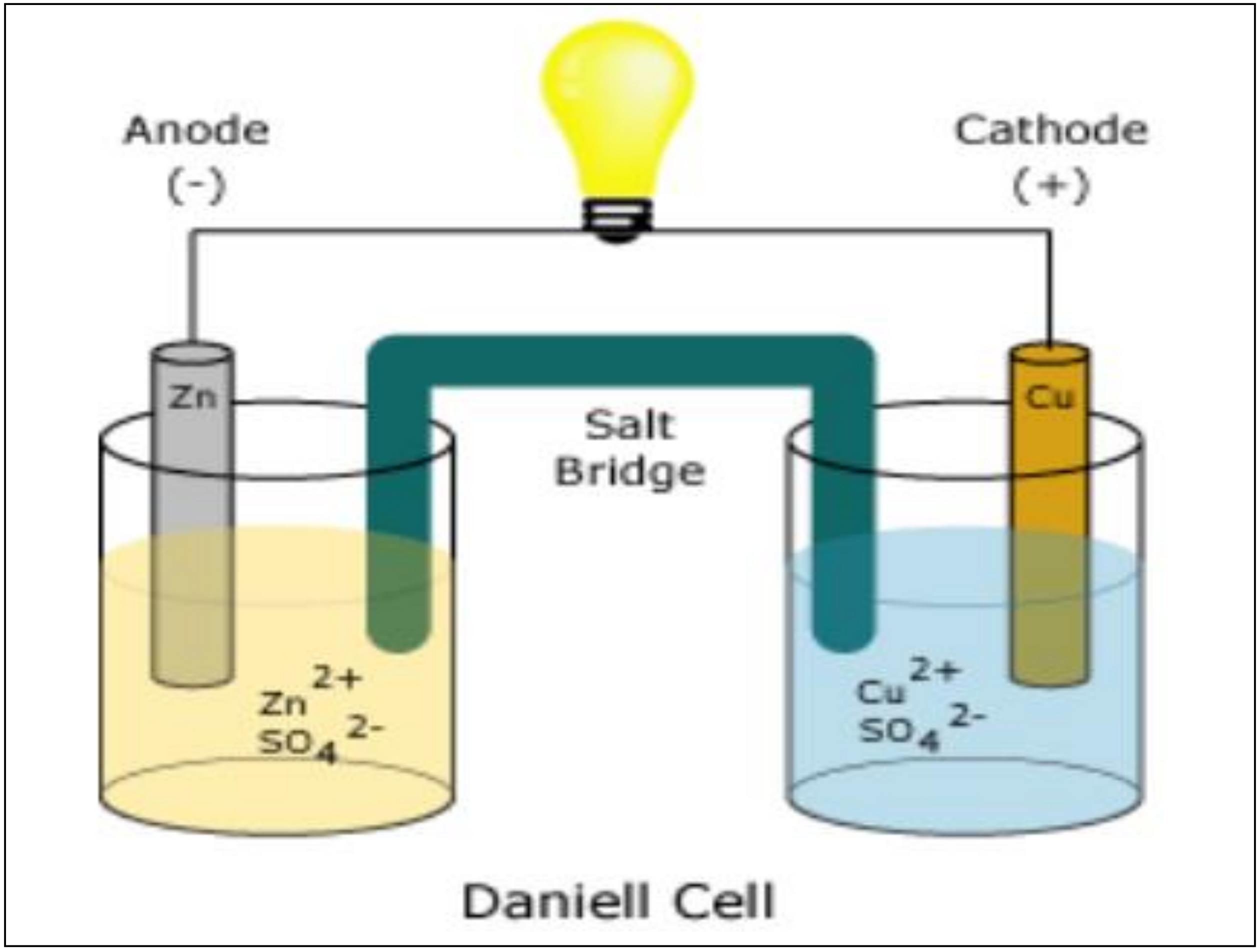
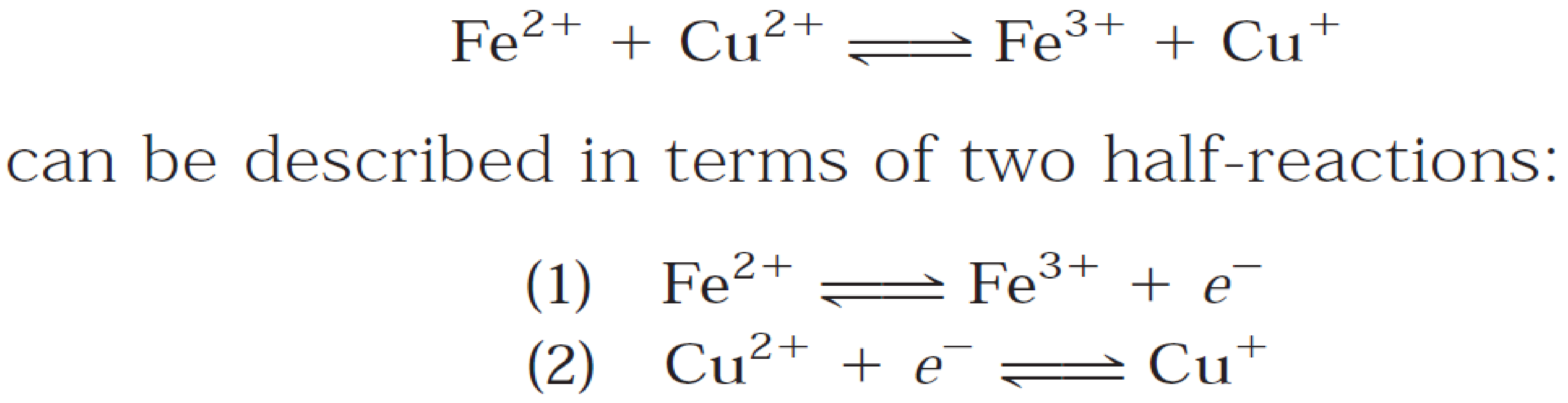
Electron flow creates energy



http://chemistry.about.com

How electrons create energy

- Redox reactions can be written as half equations.
- These reversible half reactions together make the conjugate redox pair.



$$\mathrm{Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}}$$

can be described in terms of two half-reactions:

$$\text{(1)} \quad \mathrm{Fe^{2+} \rightleftharpoons Fe^{3+}} + e^{-}$$

$$\text{(2)} \quad \mathrm{Cu^{2+}} + e^{-} \rightleftharpoons \mathrm{Cu^{+}}$$

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Electron sharing and transfer in compounds

- Electron sharing between compounds is unequal.
- The element that has a higher electronegativity is the one that pulls electrons to itself.
- We can use the order;

H<C<S<N<O to determine where the electrons move to.

- The more electronegative element keeps and claims shared electrons as its own.

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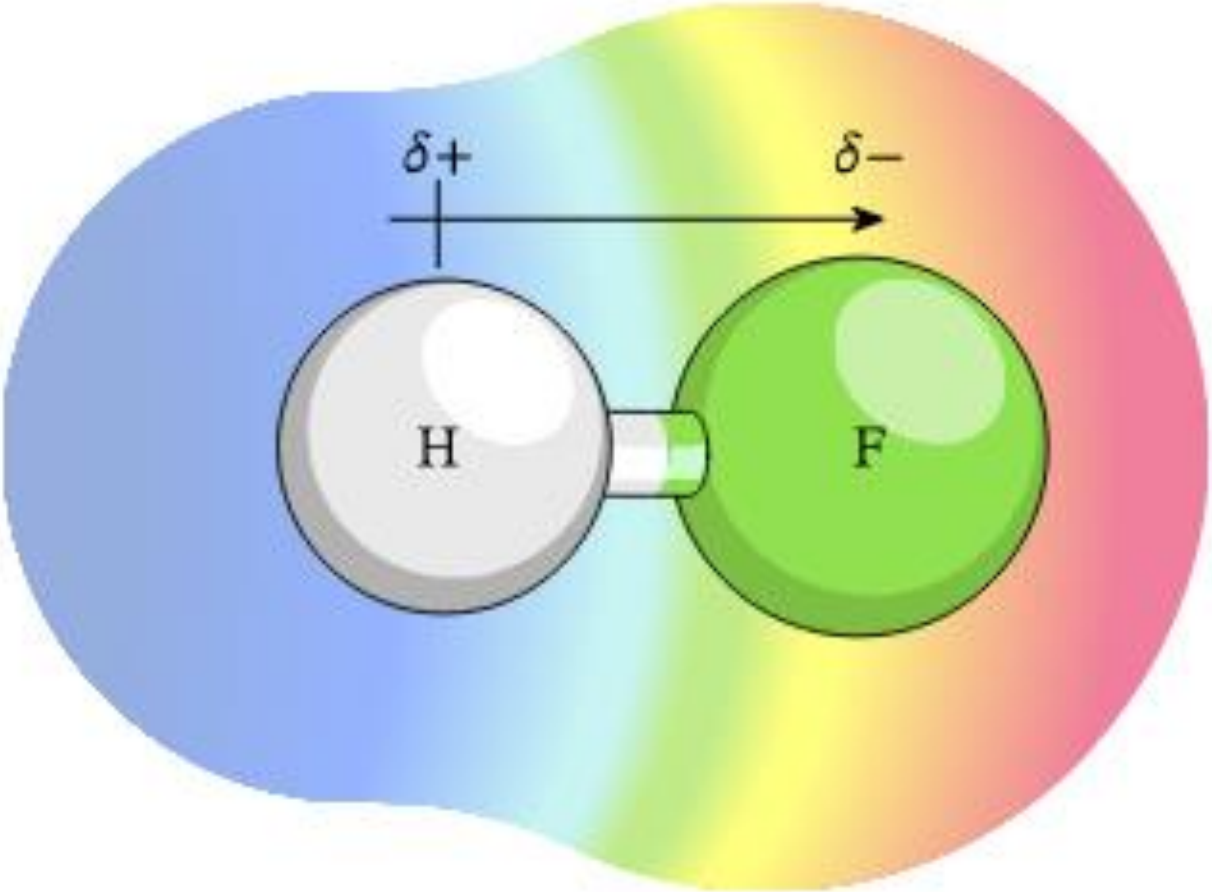
The concept of electronegativity



Electron transfer ways

- Directly in form of electrons; $Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$
- As H atoms; $AH_2 \rightleftharpoons A + 2e^{-} + 2H^{+}$
- As a hydride ion with 2 electrons, (:H⁻).
- Or when oxygen combines to a reactive species and so gets oxidized; $R{-}CH_3 + \frac{1}{2}O_2 \longrightarrow R{-}CH_2{-}OH$

- These types of reactions all occur in cells and generate electron movement which translates into energy for cellular work.

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Reduction Potential

- This is the way in which to measure affinity for electrons between redox conjugate pairs.
- Standard reference used is $H^+ + e^- \longrightarrow \frac{1}{2}H_2$ and is arbitrarily given 0.00v.
- This reaction is a half cell reaction with a standard reduction potential, E° of 0.00.

(Remember that standard refers to 25°C, 1M of reacting species or 1atm for gasses).

- A second cell containing 1M of both oxidized and reduced components of test material is joined by a salt bridge and the voltage created by electron transfer is measured.

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Reduction Potential

- If electrons move from the H cell to the test cell, the voltage is positive.
- Taking electrons from the H cell means the test cell is an oxidizing agent (it gets reduced) and therefore has a stronger affinity for electrons.
- E.g. Oxygen creates a high voltage because the difference in affinities for electrons between oxygen and hydrogen is very high.
- Electrons thus move to oxygen and oxygen is in turn reduced to water.

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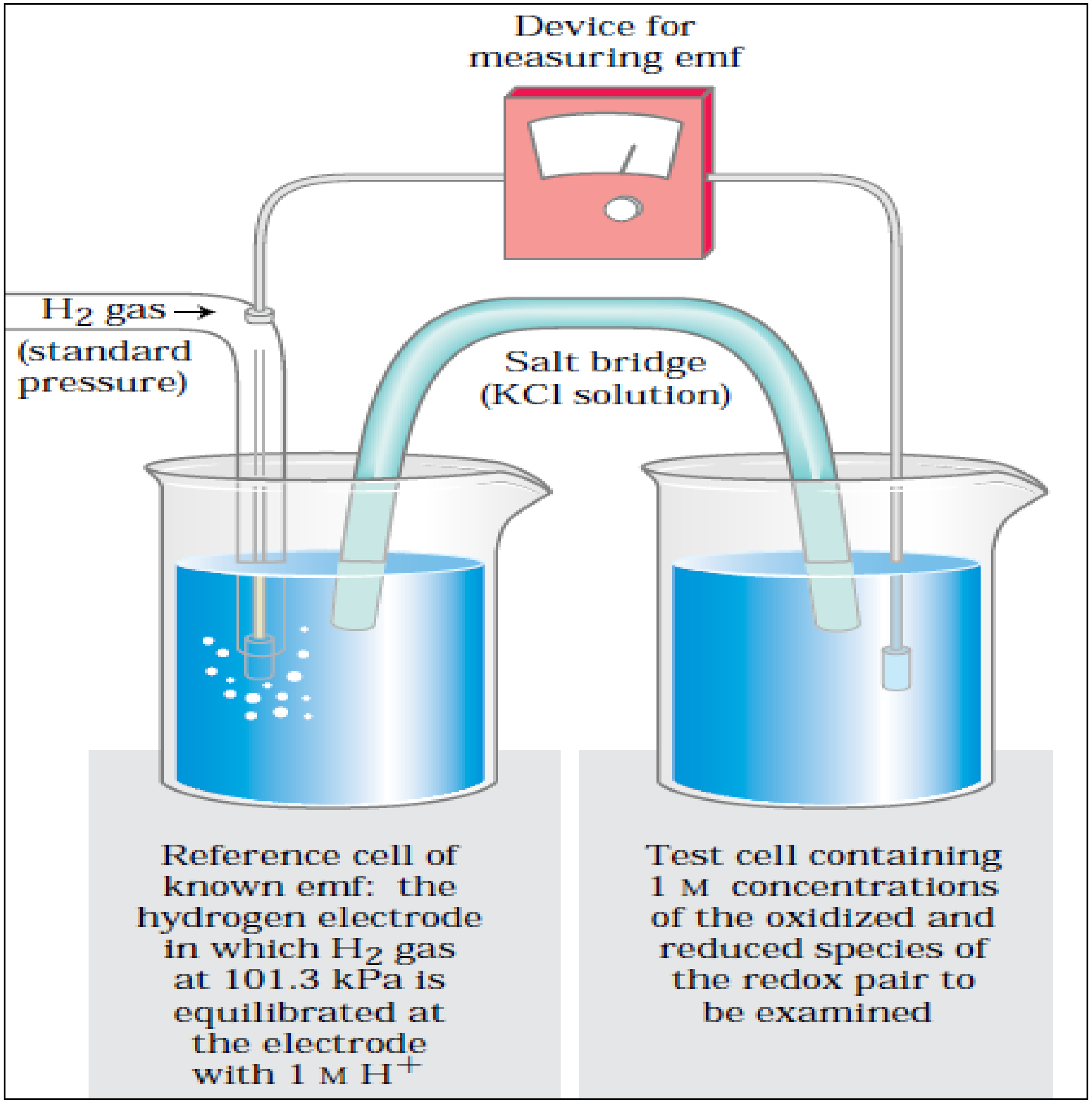
How Reduction Potentials are Measured



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Reduction Potentials

- It is worth noting that in cells at pH 7.0 concentrations wont always be at 1M. They often vary and have reduction potentials calculated based on these concentrations.

- We can now predict the E´° of any two half cells relative to each other and predict the flow of electrons.

- Given two reactions, E´° = E´° of electron acceptor- E´° of electron donor.

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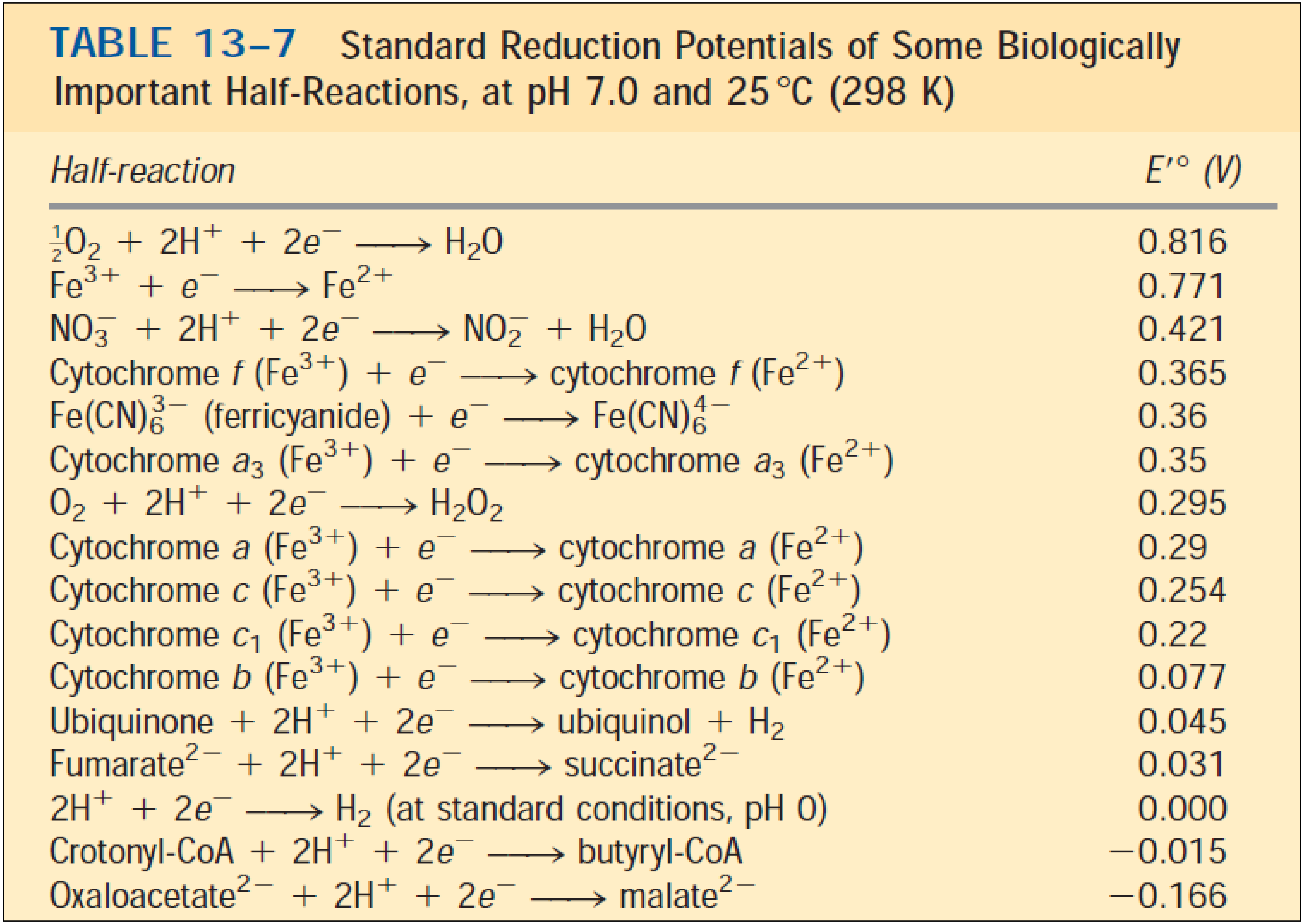

TABLE 13–7 Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25 °C (298 K)

Half-reaction	*E′°* *(V)*
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) $+ e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol $+ H_2$	0.045
Fumarate^{2-} $+ 2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA $+ 2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate^{2-} $+ 2H^+ + 2e^- \longrightarrow$ malate^{2-}	−0.166

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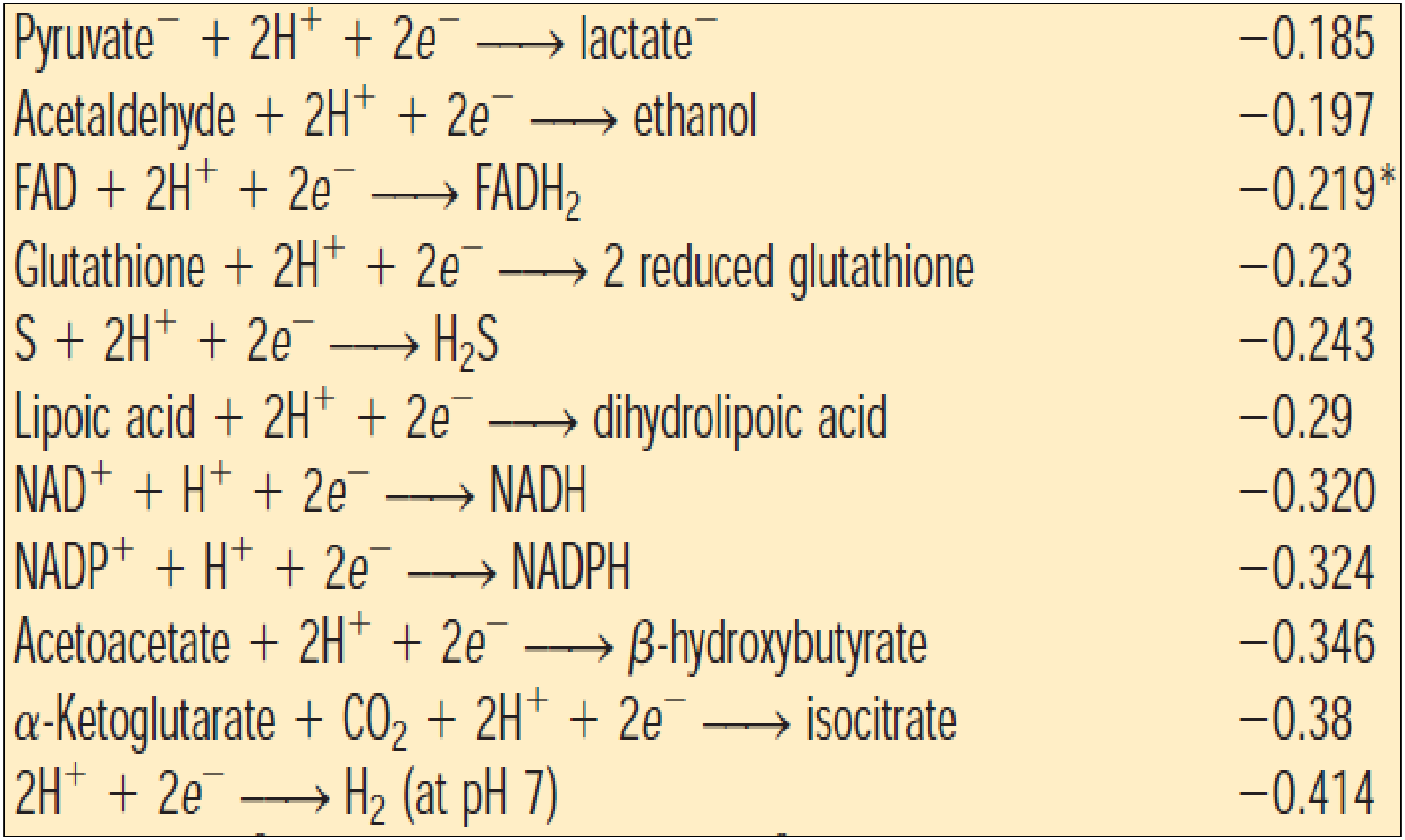
Reduction potentials cont'd



Pyruvate$^-$ + $2H^+$ + $2e^-$ $\longrightarrow$ lactate$^-$	−0.185
Acetaldehyde + $2H^+$ + $2e^-$ $\longrightarrow$ ethanol	−0.197
FAD + $2H^+$ + $2e^-$ $\longrightarrow$ $FADH_2$	−0.219*
Glutathione + $2H^+$ + $2e^-$ $\longrightarrow$ 2 reduced glutathione	−0.23
S + $2H^+$ + $2e^-$ $\longrightarrow$ H_2S	−0.243
Lipoic acid + $2H^+$ + $2e^-$ $\longrightarrow$ dihydrolipoic acid	−0.29
NAD^+ + H^+ + $2e^-$ $\longrightarrow$ NADH	−0.320
$NADP^+$ + H^+ + $2e^-$ $\longrightarrow$ NADPH	−0.324
Acetoacetate + $2H^+$ + $2e^-$ $\longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate + CO_2 + $2H^+$ + $2e^-$ $\longrightarrow$ isocitrate	−0.38
$2H^+$ + $2e^-$ $\longrightarrow$ H_2 (at pH 7)	−0.414

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$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \longrightarrow \text{ethanol} + \text{NAD}^+$$

The relevant half-reactions and their E'° values are:

$$(1)\quad \text{Acetaldehyde} + 2\text{H}^+ + 2e^- \longrightarrow \text{ethanol}$$

$$E'^\circ = -0.197\ \text{V}$$

$$(2)\quad \text{NAD}^+ + 2\text{H}^+ + 2e^- \longrightarrow \text{NADH} + \text{H}^+$$

$$E'^\circ = -0.320\ \text{V}$$

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$$\Delta E'^{\circ} = -0.197\ \text{V} - (-0.320\ \text{V}) = 0.123\ \text{V},$$

We can now calculate **standard free energy** using the equation; ΔG = -nƑ ΔE'°

Where n = number of electrons being transferred
Ƒ = Faraday's constant (96.5Kj/V.mol).

$$\begin{aligned}\Delta G'^{\circ} &= -n\mathcal{F}\,\Delta E'^{\circ} = -2(96.5\ \text{kJ/V}\cdot\text{mol})(0.123\ \text{V})\\ &= -23.7\ \text{kJ/mol}\end{aligned}$$

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How electrons create energy

- Energy generated by electron movement pump protons from one compartment to another and this creates a pH gradient also called a **proton motive force**.
- The pH gradient in mitochondria is what drives the enzyme ***ATP synthetase*** to form ATP from ADP and a phosphate ion in the matrix.

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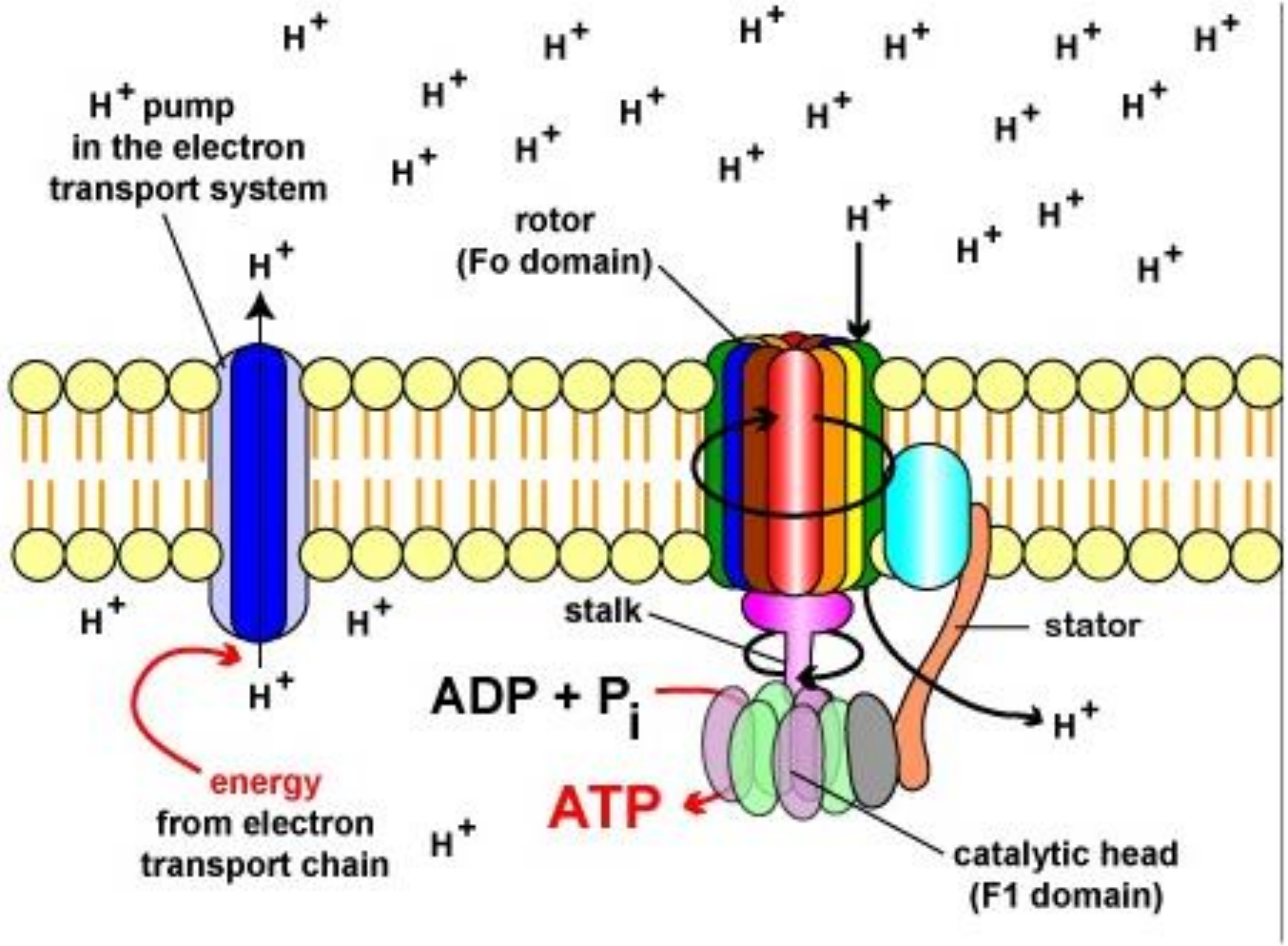
PROTON MOTIVE FORCE



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Electron Carriers in the cell

- Nicotinamide adenine nucleotides (**NAD, NADP**) and flavin nucleotides (FAD, FMN) are water soluble coenzymes.
- NAD and NADP readily move from one enzyme to another.

$$\mathrm{NAD^+} + 2e^- + 2\mathrm{H^+} \longrightarrow \mathrm{NADH} + \mathrm{H^+}$$
$$\mathrm{NADP^+} + 2e^- + 2\mathrm{H^+} \longrightarrow \mathrm{NADPH} + \mathrm{H^+}$$

- **FAD** and **FMN** are more tightly bound to enzymes (called flavoproteins) and thus serve as prosthetic groups.

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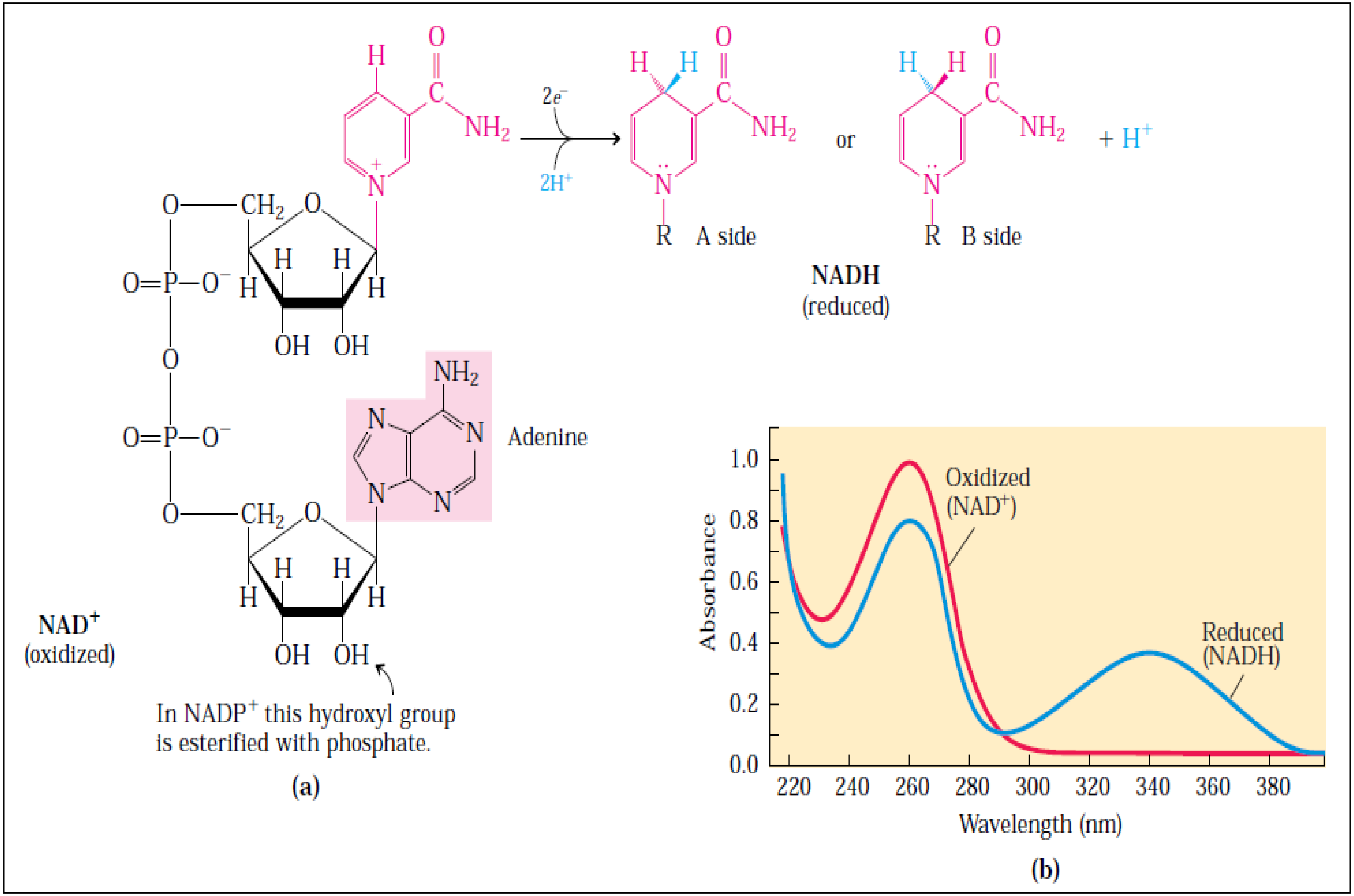

In $NADP^+$ this hydroxyl group is esterified with phosphate.

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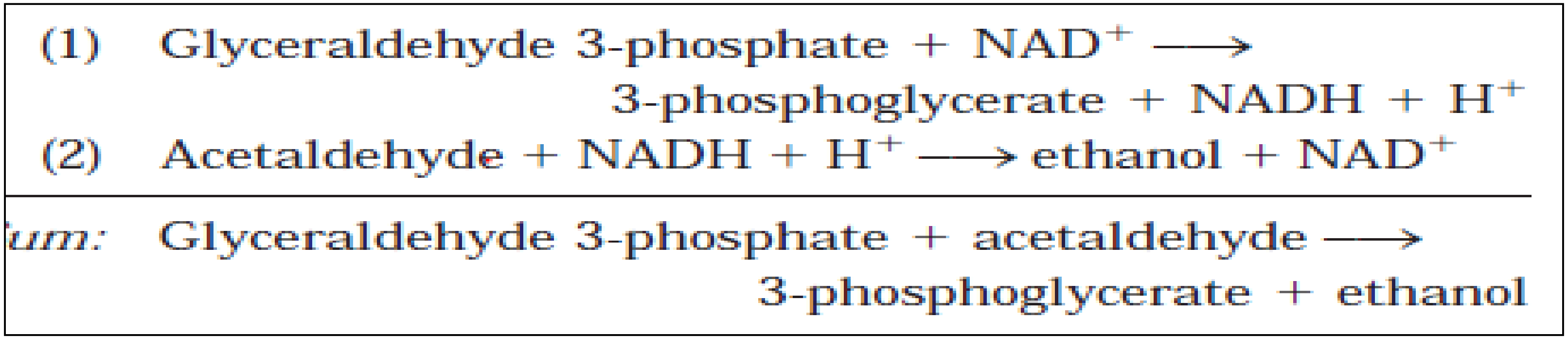
Electron Carriers

- NAD bound loosely to a specific dehydrogenase enzyme will be reduced to NADH and travel to another dehydrogenase enzyme attach and reduce the substrate which this enzyme is working on e.g.



(1) $\text{Glyceraldehyde 3-phosphate} + NAD^+ \longrightarrow \text{3-phosphoglycerate} + NADH + H^+$

(2) $\text{Acetaldehyde} + NADH + H^+ \longrightarrow \text{ethanol} + NAD^+$

um: $\text{Glyceraldehyde 3-phosphate} + \text{acetaldehyde} \longrightarrow \text{3-phosphoglycerate} + \text{ethanol}$

- NAD binds to glyceraldehyde -3-p dehydrogenase
- NADH from 1 binds to alcohol dehydrogenase which reduces acetaldehyde to ethanol.

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Electron Carriers

- Deficiency in vitamin nicotinic acid (niacin) from which the nicotinamide nucleotide carriers are derived leads to dementia, diarrhea and dermatitis (Pellagra).
- The flavin nucleotides are derived from vitamin riboflavin.
- Flavoproteins catalyze redox reactions using these FAD, FMN carries as prosthetic groups. (sometimes covalently bound e.g. to succinate dehydrogenase).
- They require 2 electrons to be fully reduced at which point they absorb light at 360nm wavelength while the partially reduced form absorbs at 450nm.
- Ubiquinone and plastoquinones are another group of lipid soluble electron carriers while a 3rd group are iron-sulfur proteins and cytochromes.

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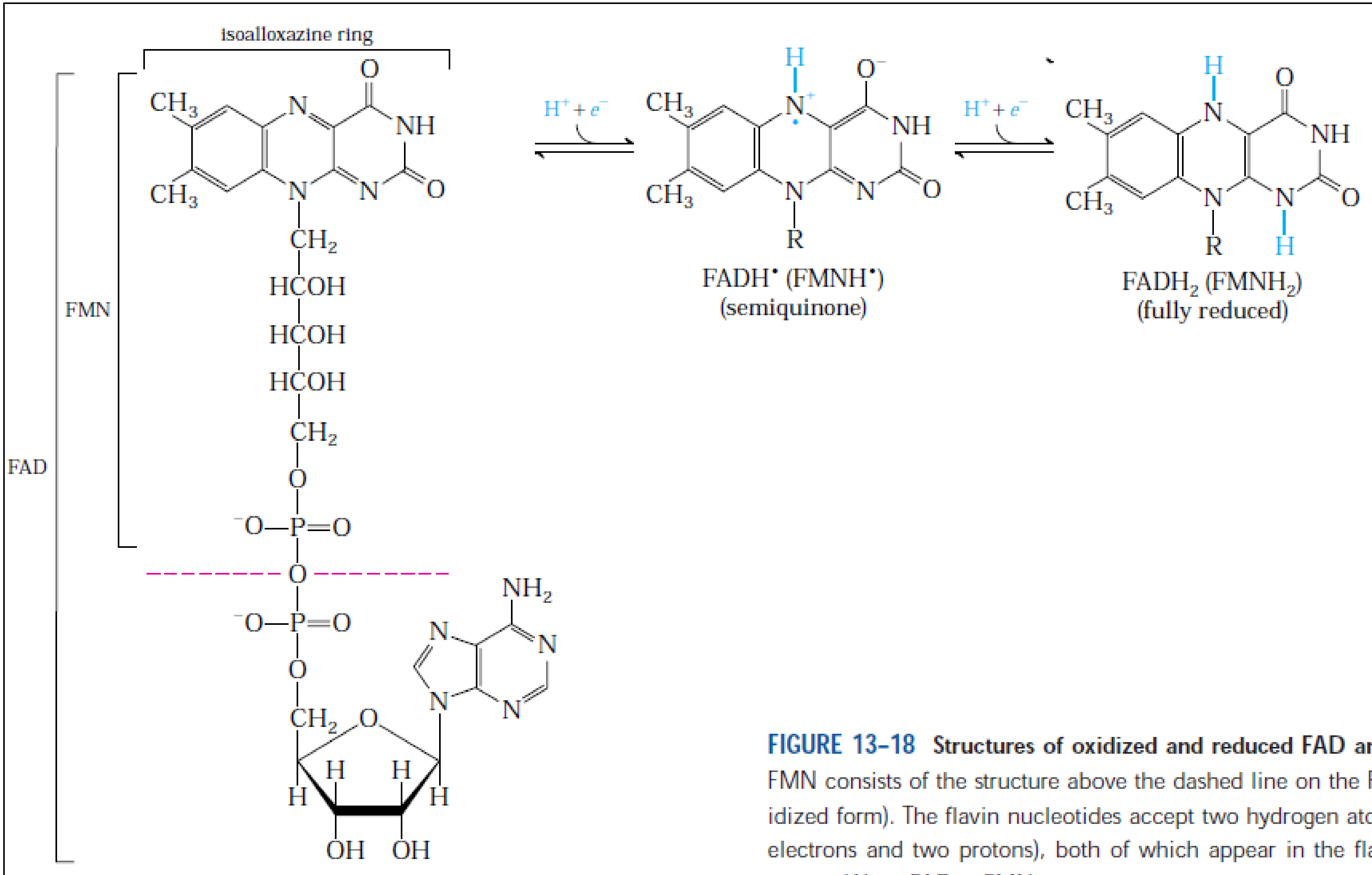

Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

$FADH^\bullet$ ($FMNH^\bullet$) (semiquinone)

$FADH_2$ ($FMNH_2$) (fully reduced)

FIGURE 13–18 Structures of oxidized and reduced FAD and FMN. FMN consists of the structure above the dashed line on the FAD (oxidized form). The flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system. When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable free radical, forms.

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High Energy compounds

- Phosphorylated compounds including ATP and thioesters have large free energies upon hydrolysis
- The basis for this lies in the fact that these high energy compounds are more unstable than their products which upon hydrolysis obtain greater stability.
- ATP hydrolysis breaks the last phosphoanhydride bond thus separating the negatively charged groups.(~30.5kj/mol)
- The released pyrophosphate is stabilized by resonance hybrid formation
- ADP ionizes spontaneously releasing H^+ because products of hydrolysis are below the conc. at equilibrium so hydrolytic reactions are favored to maintain equilibrium.

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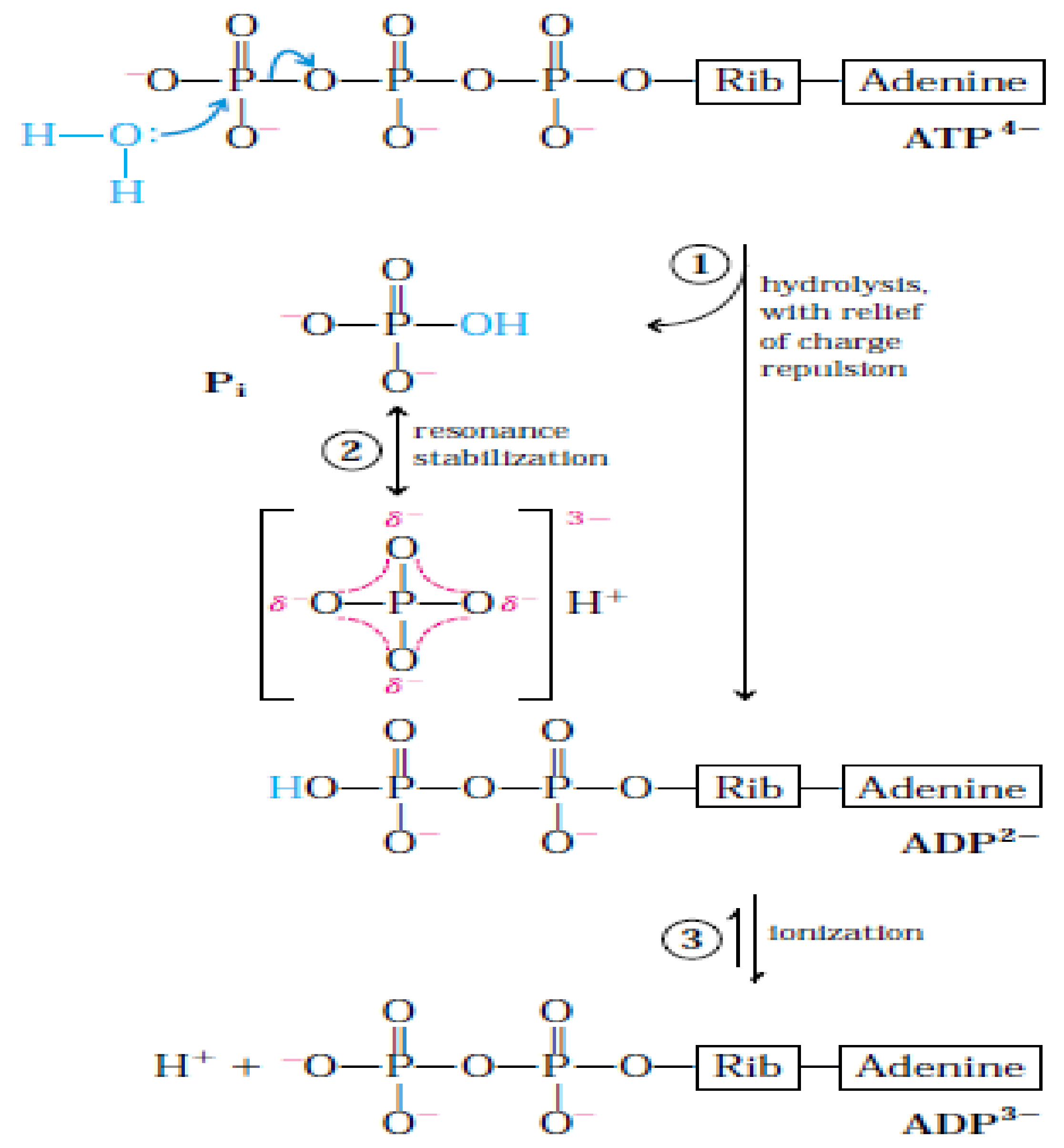

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$

$$\Delta G'^{\circ} = -30.5\ \text{kJ/mol}$$

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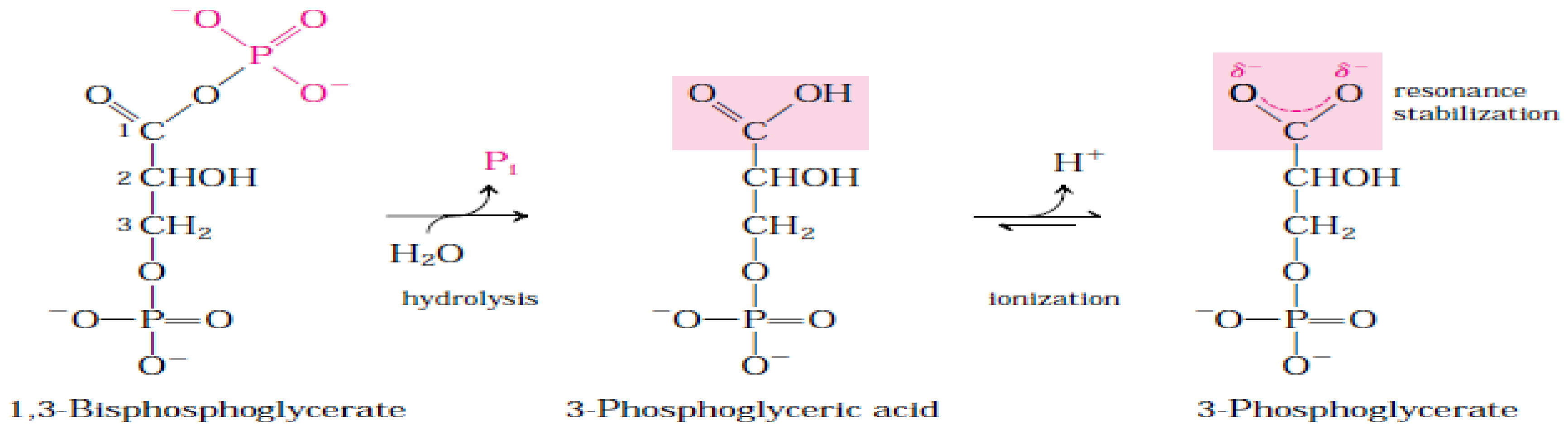
Other examples of high energy molecules



$$\text{1,3-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow \text{3-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$

$$\Delta G'^{\circ} = -49.3 \text{ kJ/mol}$$

- Products are more stabilized than reactants due to immediate dissociation of H ion and formation of resonance bonds

Examples of high energy compounds

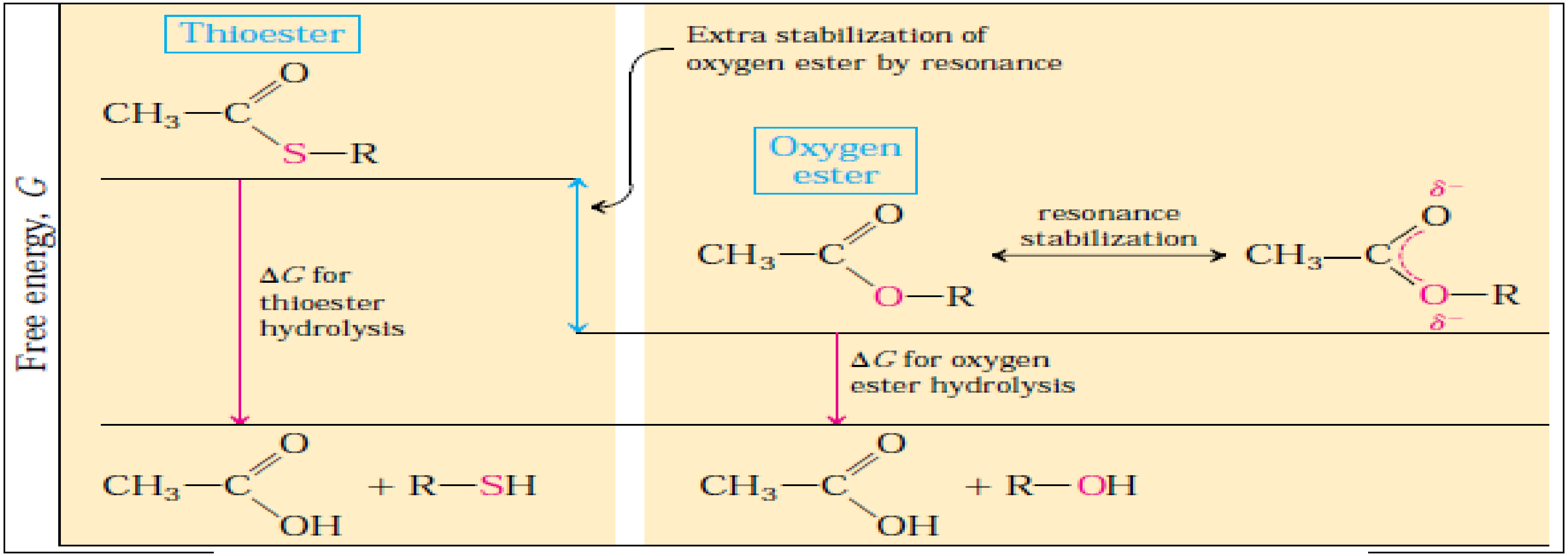


FIGURE 13–7 Free energy of hydrolysis for thioesters and oxygen esters. The *products* of both types of hydrolysis reaction have about the same free-energy content (*G*), but the thioester has a higher free-energy content than the oxygen ester. Orbital overlap between the O and C atoms allows resonance stabilization in oxygen esters; orbital overlap between S and C atoms is poorer and provides little resonance stabilization.