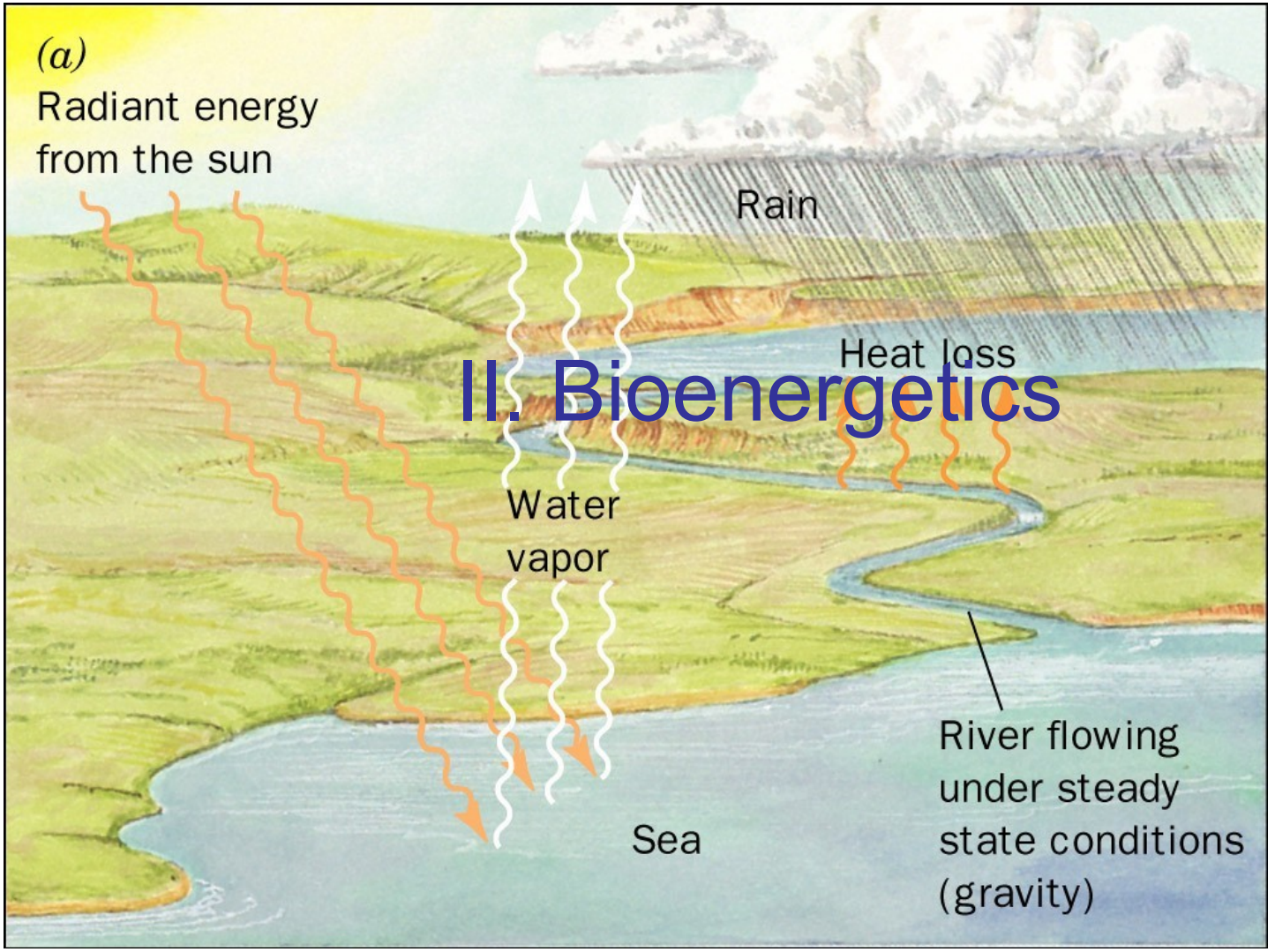
II. Bioenergetics



Bioenergetics

Bioenergetics is the quantitative study of energy relationships and energy conversion in biological systems.

Biological energy transformations obey the laws of thermodynamics.

1. You can't win (1st Law) – For any physical or chemical change, the total amount of energy in the universe remains constant.

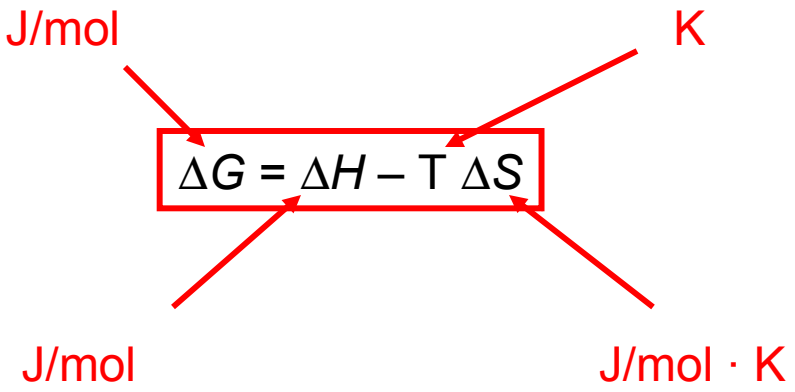
2. You even can't break even (2nd Law) – In all natural processes, the entropy of the universe increases.

Bioenergetics

Gibbs free energy, *G* – amount of energy capable of doing work

Enthalpy, *H* – the heat content of the reacting system

Entropy, *S* – quantitative expression for the randomness or disorder in a system.



Bioenergetics

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

A process tend to occur spontaneously only if ΔG is negative

For any chemical reaction ΔG is a function of the standard free-energy change ΔG^{0}

$$\Delta G = \Delta G^{0} + R\,T\,ln\,\frac{[C]^{c}\,[D]^{d}}{[A]^{a}\,[B]^{b}}$$

Bioenergetics

Some physical constants and units

Boltzmann constant, **k** $= 1.381 \times 10^{-23}$ J/K

Avogadro's number, $N = 6.022 \times 10^{23}$ 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)

Bioenergetics

Changes of free-energy under standard conditions: ΔG^0

298 K = 25 °C

101.3 kPa = 1 atm

$c_{(\text{reactants and products})}$ = 1 M

ie. ΔG^0 is the free energy change between the standard state and the equilibrium state

ΔG^0 is directly related to the equilibrium constant K_{eq}

$$\Delta G^0 = - R\,T\,\ln K_{eq} \qquad K_{eq} = \frac{[\text{Products}]}{[\text{Reactants}]} = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

Bioenergetics

For biological systems, we typically use transformed standard constants:

$$\Delta G'^{0} \text{ and } K'_{eq}$$

Since biological systems typically maintain a steady state, the concentrations of H_2O, H^+, and/or Mg^{2+} can be assumed to be invariant

and are incorporated into the constants $\Delta G'^{0}$, K'_{eq}.

$$\Delta G'^{0} = - R\ T\ ln\ K'_{eq}$$

Free Energy of a Reaction

$$\Delta G'^{0} = - R\ T\ ln\ K'_{eq}$$

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G'^{\circ}$ (kcal/mol)*
10^3	−17.1	−4.1
10^2	−11.4	−2.7
10^1	−5.7	−1.4
1	0.0	0.0
10^{-1}	5.7	1.4
10^{-2}	11.4	2.7
10^{-3}	17.1	4.1
10^{-4}	22.8	5.5
10^{-5}	28.5	6.8
10^{-6}	34.2	8.2

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

For every 10 fold change in K_{eq}, ΔG changes by 5.7 kJ/mol

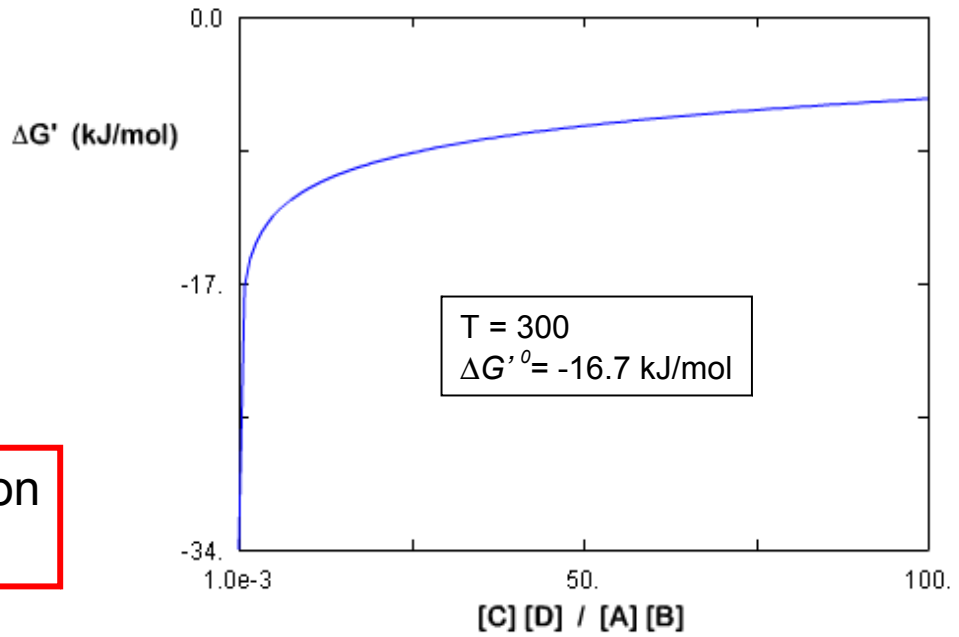
Actual Free-Energy Changes

The actual free-energy changes depend on reactant and product concentrations.

Each chemical reaction has a characteristic standard free-energy change ($\Delta G'^{0}$). It is a constant!

$\Delta G'$ is a function of (reactant/product) concentration and the temperature.

$$\Delta G' = \Delta G'^{0} + R\,T\,ln\,\frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$



The criterion for spontaneity of a reaction Is $\Delta G'$, *not* $\Delta G'^{0}$

Sequential Reactions – Energy Coupling

Sequential reactions sharing a common intermediate have their own standard free-energy change. The standard free-energy values of a sequential reaction are additive.

$A \rightarrow B$	$\Delta G_1^{\prime 0}$
$B \rightarrow C$	$\Delta G_2^{\prime 0}$
$A \rightarrow C$	$\Delta G_1^{\prime 0} + \Delta G_2^{\prime 0}$

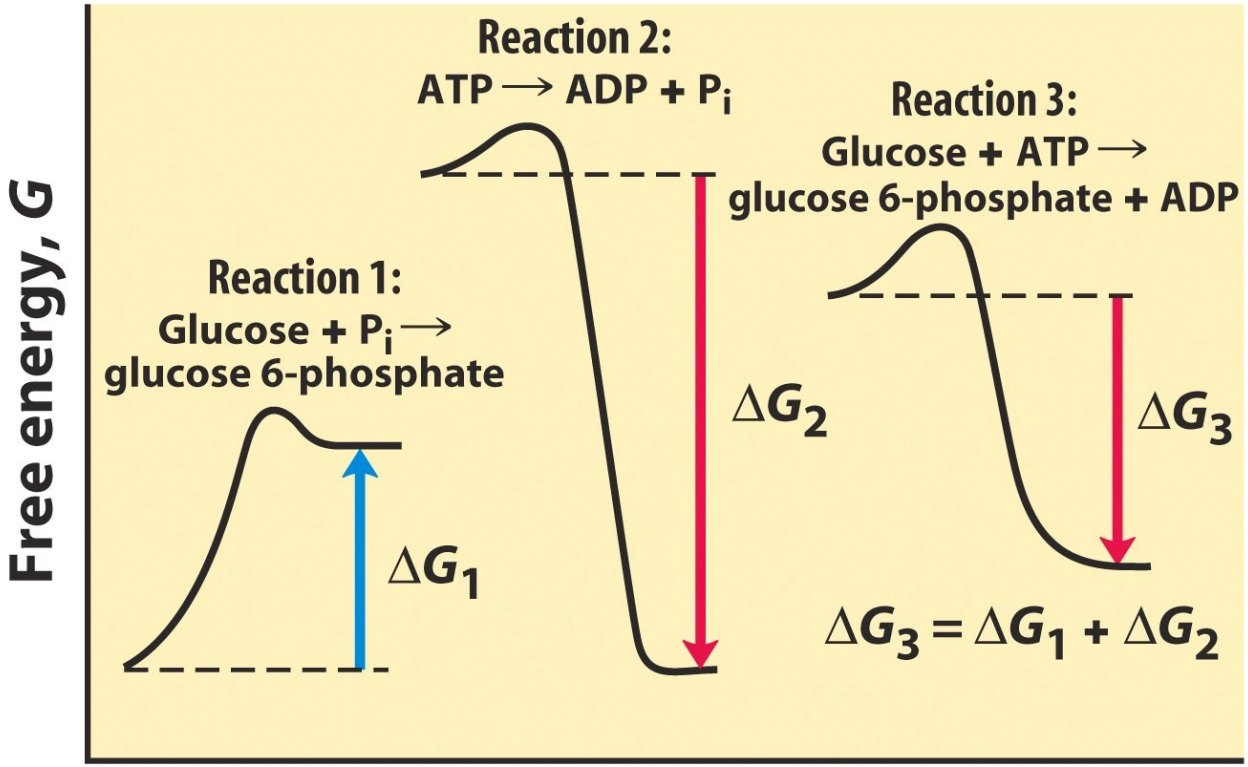
$$\Delta G^{\prime 0}_{total} = \Delta G_1^{\prime 0} + \Delta G_2^{\prime 0}$$

Energy coupling is valid means for understanding the energetics of :

1) elementary steps in an overall reaction and
2) for multiple steps in a metabolic pathway

Sequential Reactions
Energy Coupling

$\Delta G_2'^0$ = -30.5 kJ/mol



$\Delta G_1'^0$ = 13.8 kJ/mol

$\Delta G_3'^0$ = -16.7 kJ/mol

Free-Energy Change ATP Hydrolysis

Standard free energy of ATP hydrolysis is -30.5 kJ/mol.
But what about the actual free energy of ATP hydrolysis in the cell?

Example: Human erythrocytes

c(ATP) = 2.25 mM
c(ADP) = 0.25 mM
c(P_i) = 1.65 mM

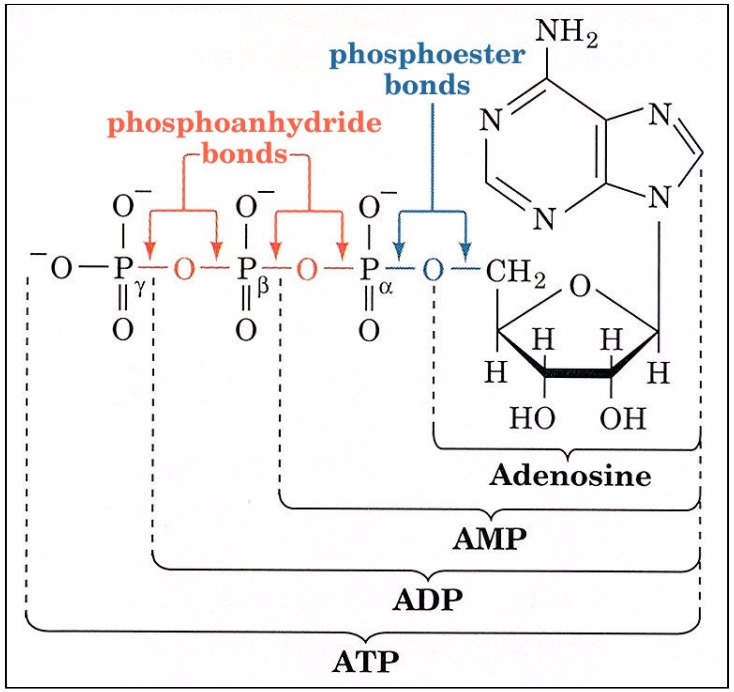
$$\Delta G'_p = \Delta G'^{0} + R\,T\,ln\,\frac{[ADP]\,[P_i]}{[ATP]}$$

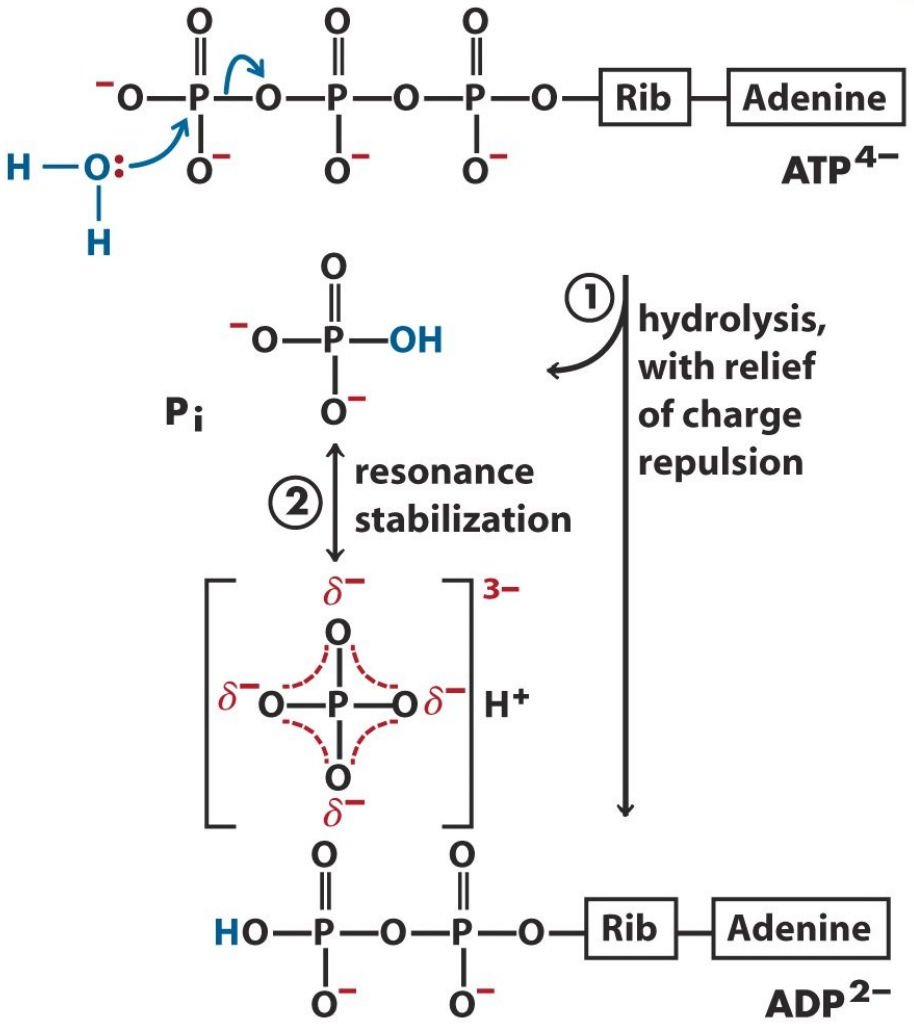
$$\Delta G_p = -30.5\text{kJ/mol} + 8.315\ \text{J/mol}\cdot\text{K} \cdot 298\ \text{K} \cdot \ln 1.8 \times 10^{-4}$$
$$= -30.5\ \text{kJ/mol} - 21\ \text{kJ/mol}$$
$$= -51.5\ \text{kJ/mol}$$

ΔG_p is called the phosphorylation potential

Chemical Basis for "High Energy" Compounds

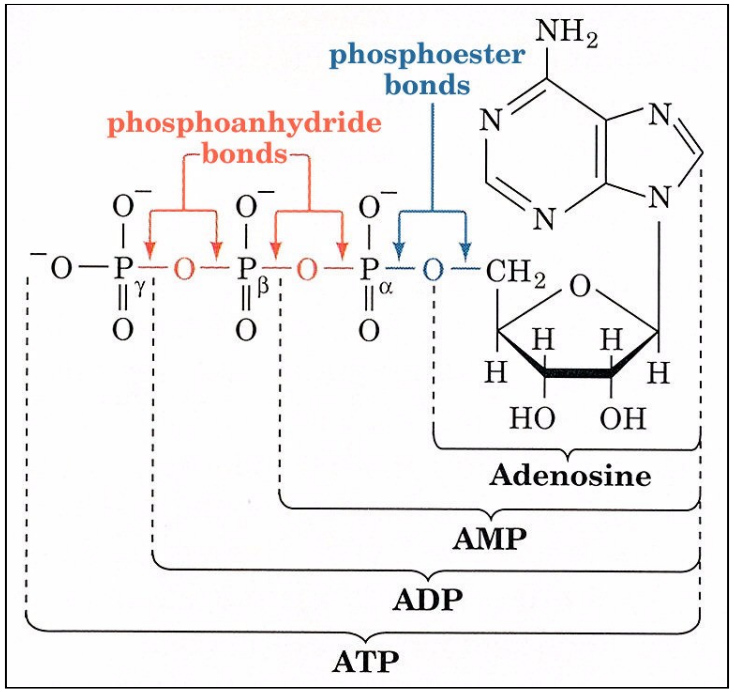
ATP - Hydrolysis

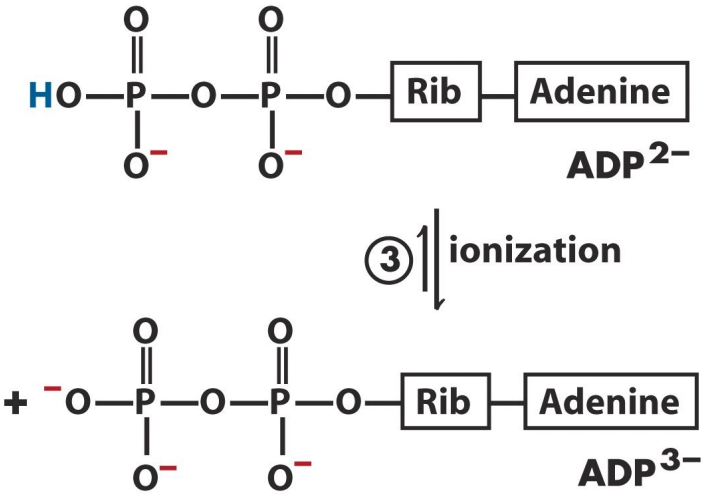




Chemical Basis for "High Energy" Compounds

ATP - Hydrolysis



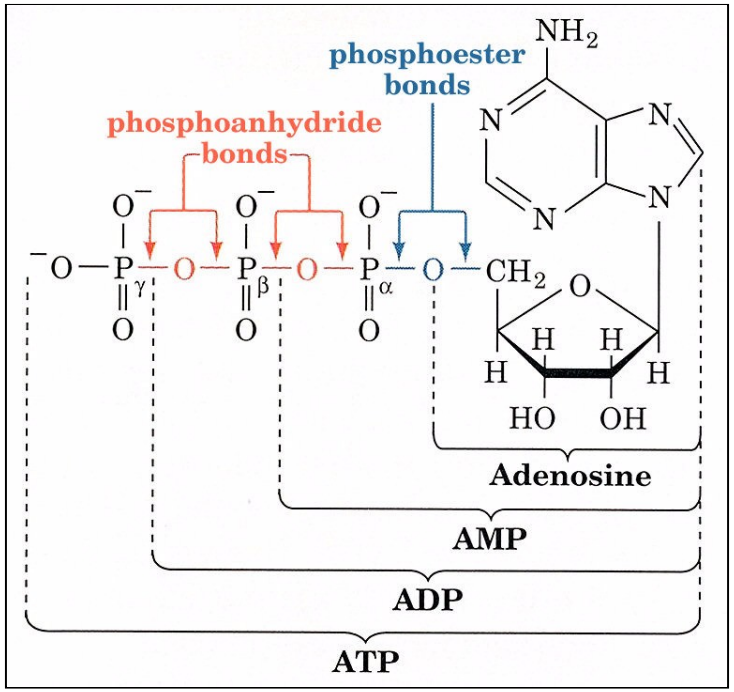


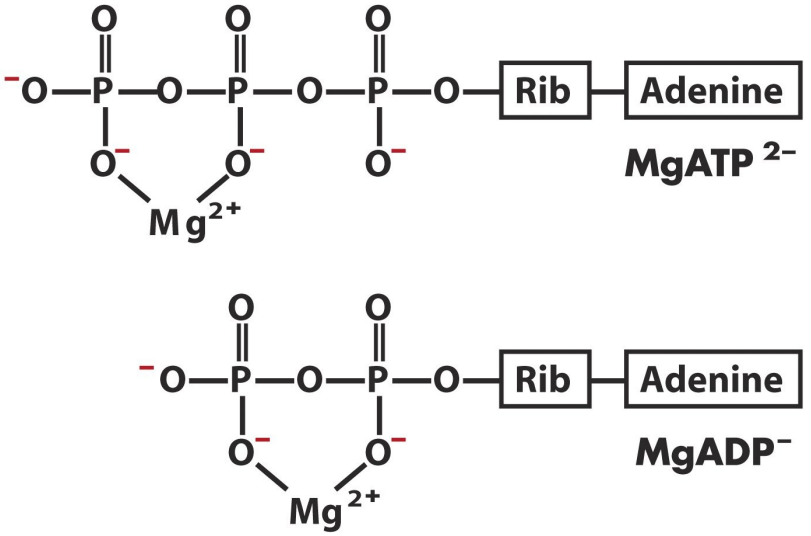
$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$

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

Chemical Basis for "High Energy" Compounds

ATP - Hydrolysis





Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups.
ie. electrostatic shielding

“High energy” bonds

"High energy" bonds can be represented by the "~" symbol.

~P represents a phosphate group with a large negative ΔG of hydrolysis.

Compounds with “high energy bonds” are said to have high group transfer potential.

Potentially, 2 ~P bonds can be cleaved, as 2 phosphates are released by hydrolysis from ATP.

$AMP{\sim}P{\sim}P \rightarrow AMP{\sim}P + P_i$ ($ATP \rightarrow ADP + P_i$)

$AMP{\sim}P \rightarrow AMP + P_i$ ($ADP \rightarrow AMP + P_i$)

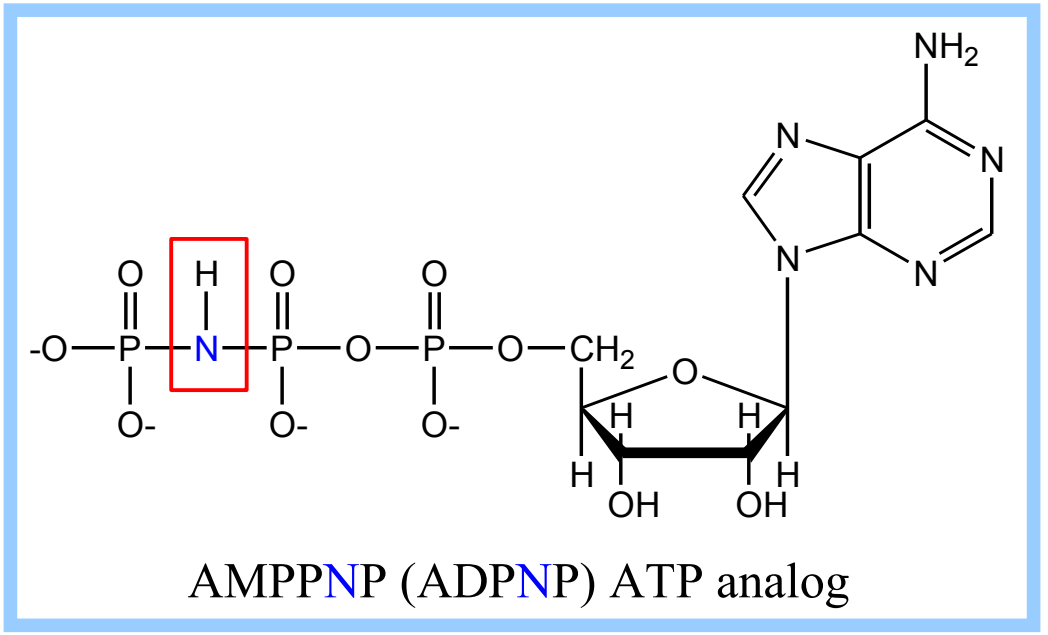
Alternatively:

$AMP{\sim}P{\sim}P \rightarrow AMP + P{\sim}P$ ($ATP \rightarrow AMP + PP_i$)

$P{\sim}P \rightarrow 2\,P_i$ ($PP_i \rightarrow 2P_i$)

Nucleotide Analogs

Artificial ATP analogs have been designed that are resistant to cleavage of the terminal phosphate by hydrolysis.



AMPPNP (ADPNP) ATP analog

Example: AMPPNP.

These analogs have been used to study the dependence of coupled reactions on ATP hydrolysis.

Note: they have made it possible to crystallize an enzyme that catalyzes ATP hydrolysis with an ATP analog at the active site.

Inorganic polyphosphate

Many organisms store energy as inorganic polyphosphate, a chain of many phosphate residues linked by phosphoanhydride bonds:

P~P~P~P~P...

- Hydrolysis of P_i residues may be coupled to energy-dependent reactions.

Depending on the organism or cell type, inorganic polyphosphate may have additional functions.

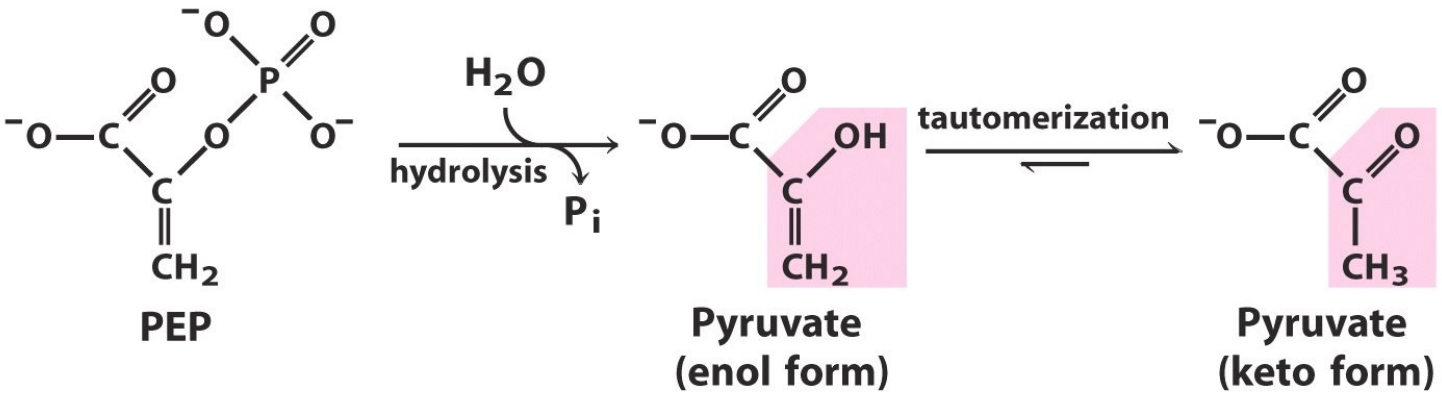
- Example: reservoir for P_i, a chelator of metal ions, a buffer or a regulator.

In prokaryotes, polyphosphate kinase-1 (PPK1) catalyzes the addition of phosphate to polyphosphate:

$$\text{ATP} + \text{polyP}_n \xrightleftharpoons{Mg^{2+}} \text{ADP} + \text{polyP}_{n+1} \qquad \Delta G'^{0} = -20 \text{ kJ/mol}$$

Chemical Basis for "High Energy" Compounds

Phosphoenolpyruvate (PEP) - Hydrolysis

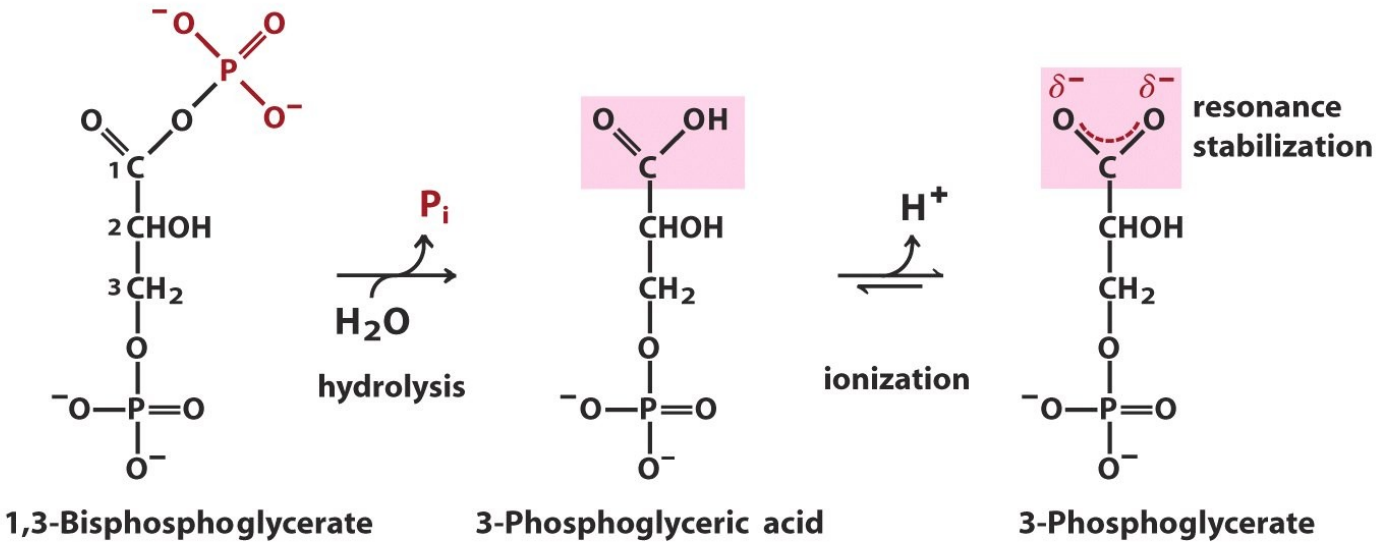


$$PEP^{3-} + H_2O \longrightarrow pyruvate^{-} + P_i^{2-}$$

$$\Delta G'^{\circ} = -61.9\ kJ/mol$$

Chemical Basis for "High Energy" Compounds

1,3-Bisphosphoglycerate - Hydrolysis

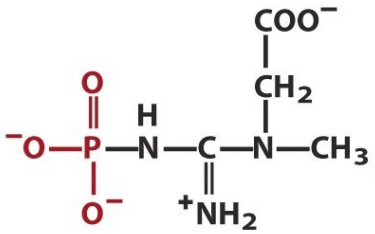


$$\text{1,3-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow \text{3-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$

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

Chemical Basis for "High Energy" Compounds

Phosphocreatine - Hydrolysis



Phosphocreatine

$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$
$$\Delta G'^{\circ} = -43.0\ \text{kJ/mol}$$

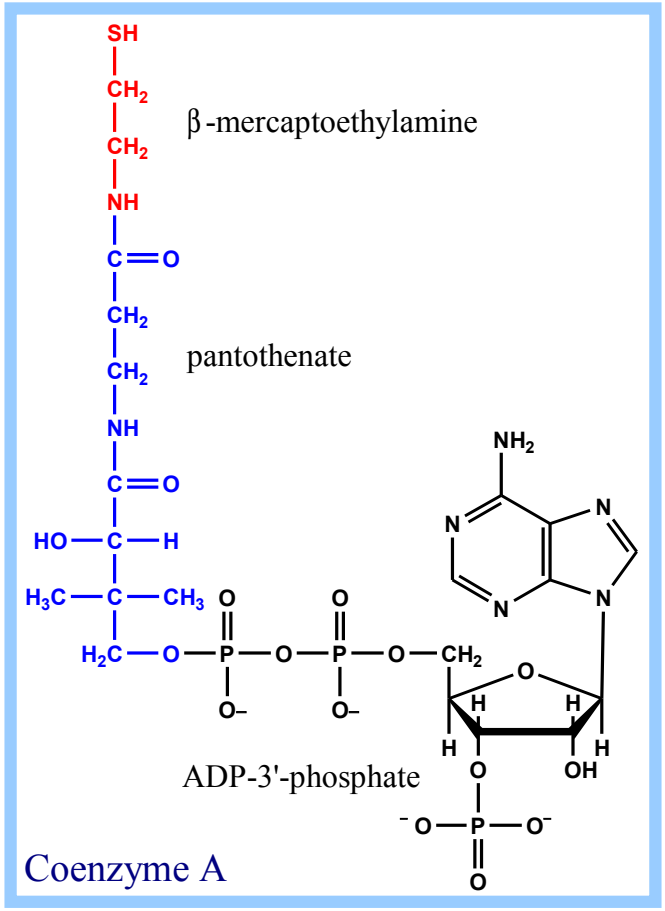
Chemical Basis for "High Energy" Compounds

Thioesters: Acetyl-CoA hydrolysis

Coenzyme A includes

β-mercaptoethylamine linked to the B vitamin pantothenate, which is linked to ppAp

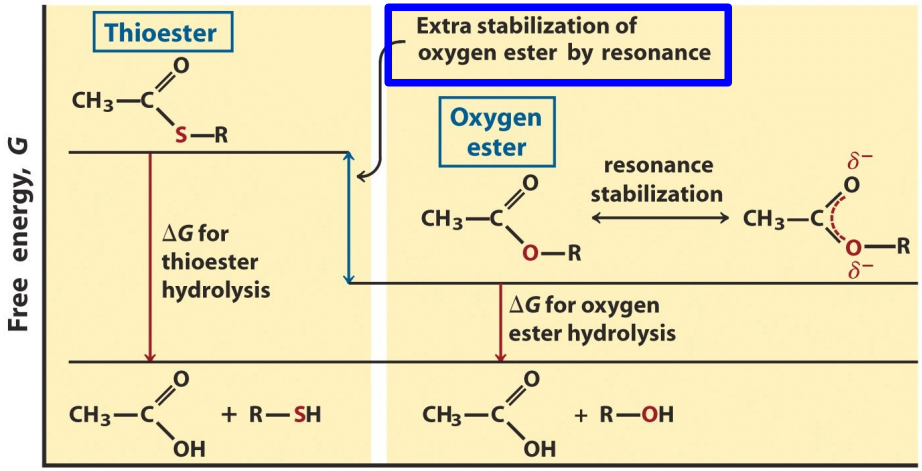
The functional group is the thiol (SH) of β-mercaptoethylamine.



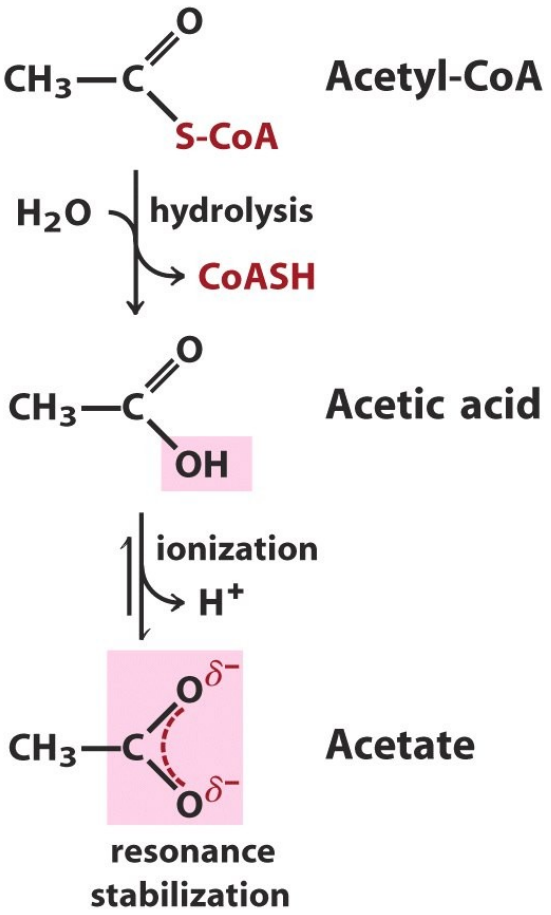
Why does binding to CoA result in "activation" of the respective component?

Chemical Basis for "High Energy" Compounds

Thioesters: Acetyl-CoA hydrolysis



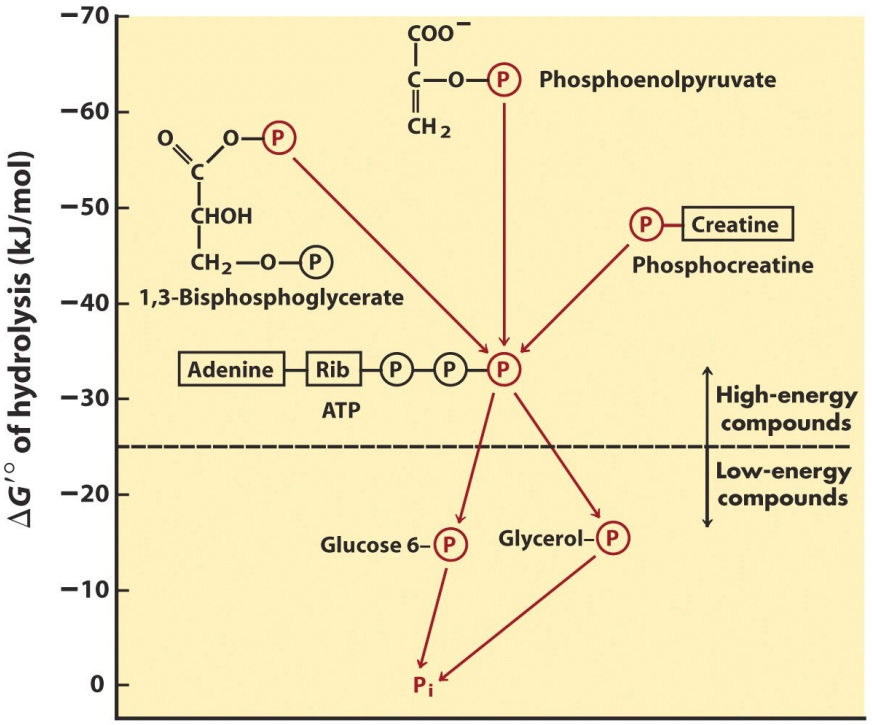
(Oxygen) esters are stabilized by resonance structures not available to thioesters



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4\ \text{kJ/mol}$$

Energy Ranking

	$\Delta G'^\circ$	
	(kJ/mol)	*(kcal/mol)*
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate ($\rightarrow$ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP ($\rightarrow$ AMP + P_i)	−32.8	−7.8
ATP ($\rightarrow$ ADP + P_i)	−30.5	−7.3
ATP ($\rightarrow$ AMP + PP_i)	−45.6	−10.9
AMP ($\rightarrow$ adenosine + P_i)	−14.2	−3.4
PP_i ($\rightarrow$ 2P_i)	−19.2	−4.0
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5



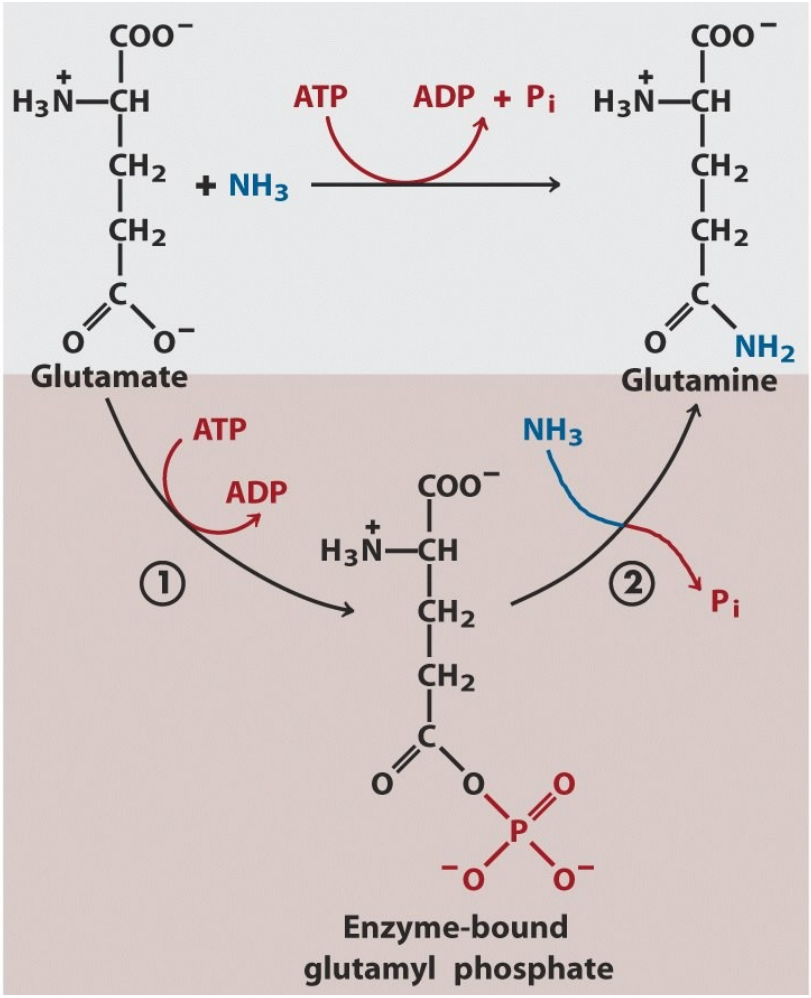
ATP has special roles in energy coupling & P_i transfer.

ΔG of phosphate hydrolysis from ATP is intermediate among examples below. ATP can thus act as a P_i donor, & ATP can be synthesized by P_i transfer, e.g., from PEP.

Group Transfer

ATP provides energy not by simple hydrolysis.
It is provided by group transfers.

A reaction usually written as a one-step reaction may actually involve two steps.



A phosphoryl group is transferred from ATP to a substrate (here glutamate), then the phosphoryl group is displaced by a reactant (here NH_3) resulting in the release of P_i.

Why is that important?

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Role of "high energy" bonds:

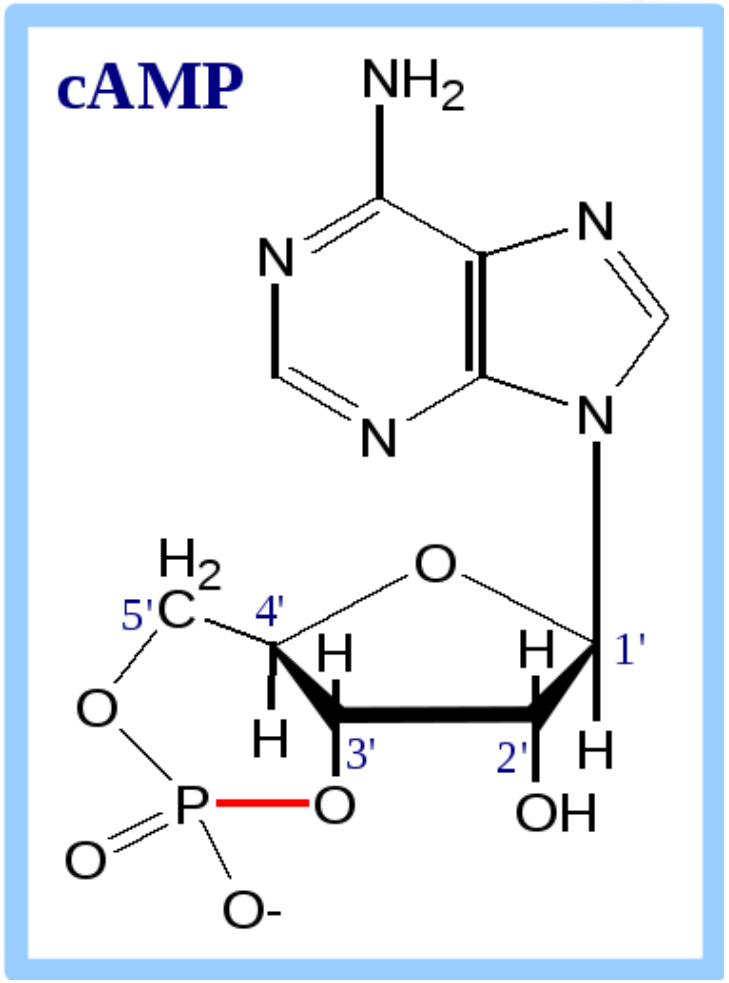
- **Energy transfer or storage**
  ATP, PP_i, polyphosphate, phosphocreatine
- **Group transfer**
  ATP, Coenzyme A
- **Transient signal**
  cyclic AMP

Transient signals (*eg. cA*

cAMP (3', 5'-cylic AMP) is sterically constrained by having a phosphate with ester linkages to 2 hydroxyls of the same ribose.

Hydrolysis of one of these linkages (in red), converting cAMP to 5'-AMP is highly spontaneous.

The lability of cAMP to hydrolysis makes it an excellent transient signal.



Why doesn't ATP undergo spontaneous hydrolysis?

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Thermodynamics

"High-energy" bond hydrolysis is energetically favorable / spontaneous reaction.

Kinetics

While energetics are favorable, the large activation energy barrier associated with the hydrolysis of many "high energy" bonds is very slow in the absence of an enzyme catalyst (referred to as kinetic stability)

Kinetic stability is essential feature of "energy storage" molecules

- Rapid ATP hydrolysis in the absence of a catalyst would render ATP useless as an energy storage molecule as it would fall apart before use
- Allows for ATP hydrolysis only when reaction is coupled to a useful cellular reaction

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FIAT LUX

Yet another class of reactions!

Redox reactions!

Oxidation & Reduction

Principles of electrochemistry:

When describing electron transfers, the oxidation and reduction halves of the reaction can be considered separately.

Overall Reaction

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

Half Reactions

Oxidation of ferrous ion (loss of an electron):

$$Fe^{2+}\text{(reduced)} \rightleftharpoons Fe^{3+}\text{(oxidized)} + e^{-}$$

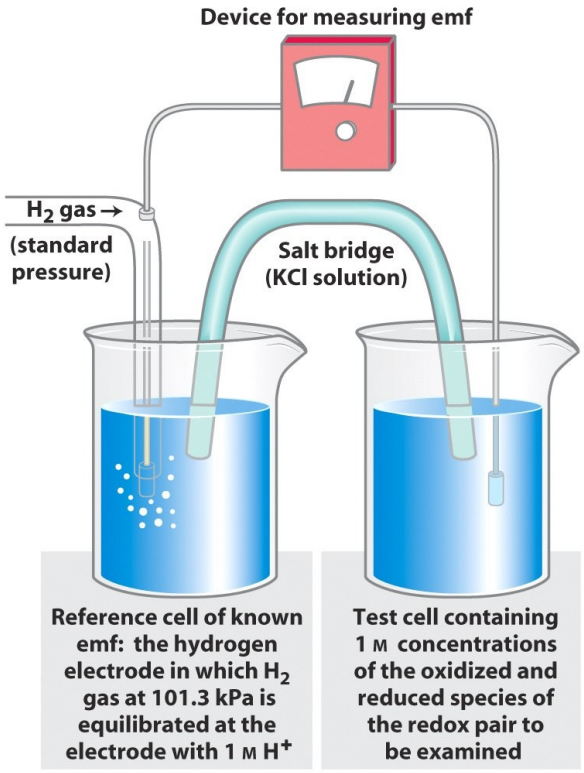
Reduction of cupric ion (addition of an electron):

$$Cu^{2+}\text{(oxidized)} + e^{-} \rightleftharpoons Cu^{+}\text{(reduced)}$$

Reduction potential measures affinity for electrons

The standard reduction potential, E^0 for any given redox pair is referenced on the half-reaction:

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



The reduction potential of a half-cell depends on concentrations / activities of the chemical species present

$$E = E^0 - \frac{R\,T}{n\,F} \ln \frac{[\text{el. acceptor}]}{[\text{el. donor}]}$$

For T = 298 K

$$E = E^0 - \frac{0.026\ \text{V}}{n} \ln \frac{[\text{el. acceptor}]}{[\text{el. donor}]}$$

Standard Reaction Potentials and Free-Energy Change

The flow of electrons make energy available:

The free-energy change of a redox reaction.

$\Delta G = - n F \Delta E$ or $\Delta G'^{0} = - n F \Delta E'^{0}$

Example:
Acetaldehyde + NADH + $H^+ \rightarrow$ Ethanol + NAD^+ $\Delta E'^{0} = 0.123$ V

$\Delta G'^{0} = - n F \Delta E'^{0} = -2$ (96.5 kJ/V · mol)(0.123 v) = -23.7 kJ/mol

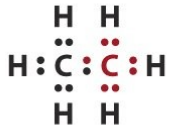
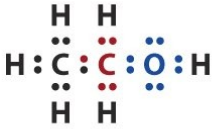
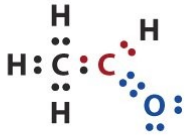
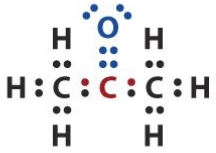
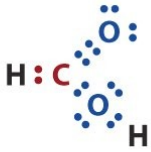
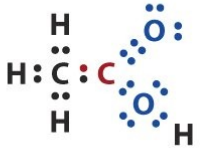
Standard Reduction Potentials

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
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−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 + $2H^+ + 2e^- \longrightarrow \beta$-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

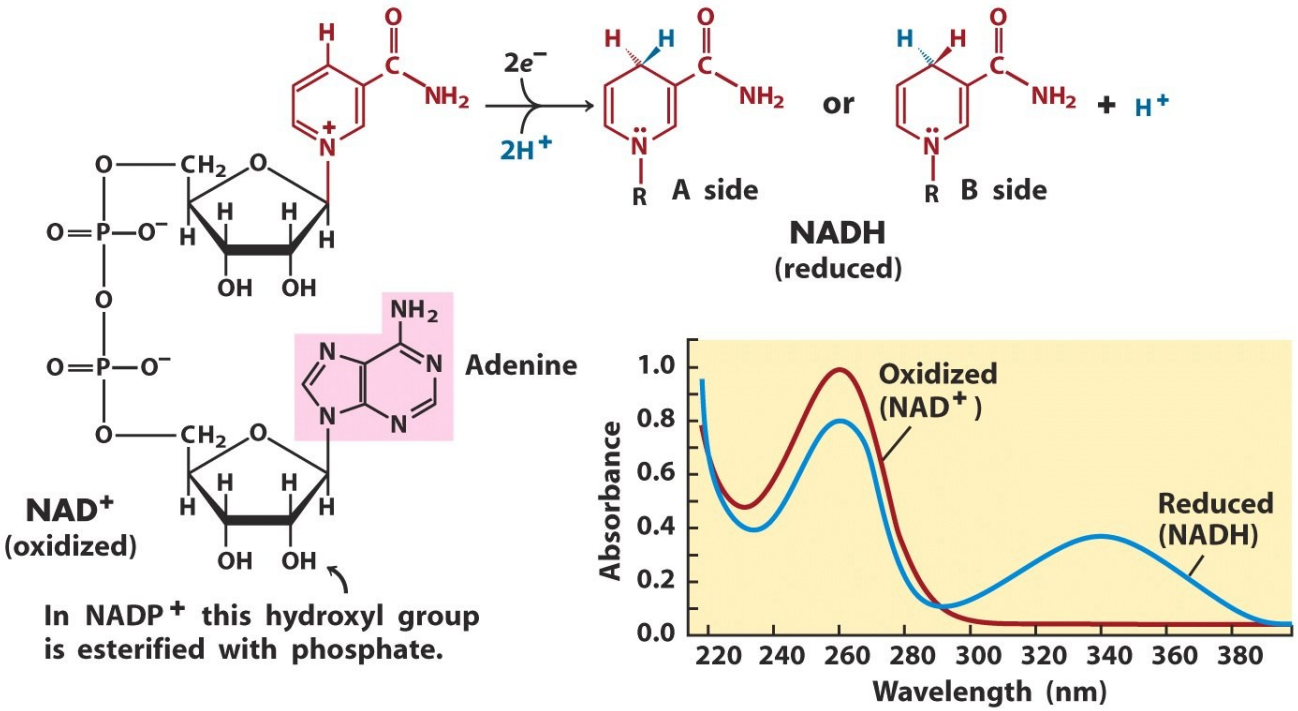
pH = 7.0
T=25 °C

The electrochemical potential for oxidation half reactions (reverse of written reactions) has the same magnitude and opposite sign

Oxidation States of Carbon

Methane		8
Ethane (alkane)		7
Ethene (alkene)		6
Ethanol (alcohol)		5
Acetylene (alkyne)		5
Formaldehyde		4
Acetaldehyde (aldehyde)		3
Acetone (ketone)		2
Formic acid (carboxylic acid)		2
Carbon monoxide		2
Acetic acid (carboxylic acid)		1
Carbon dioxide		0

Some Coenzymes and Proteins Serve as Electron Carriers



NAD+, Nicotinamide Adenine Dinucleotide, is an electron acceptor in catabolic pathways.

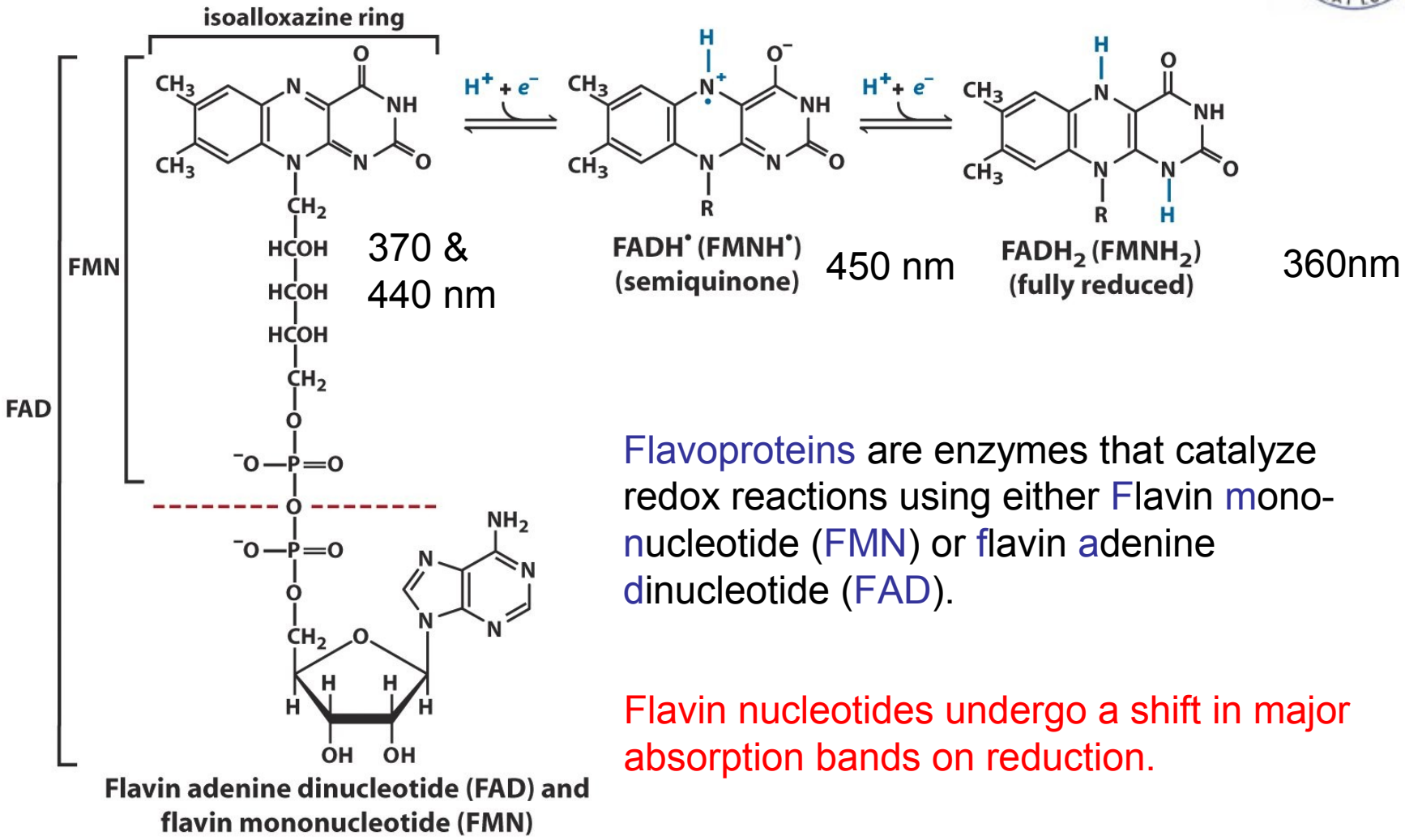
Optical test?

NADP+/NADPH is similar except for Pi.

NADPH is e- donor in synthetic pathways.

The nicotinamide ring is derived from the vitamin niacin.

Flavoproteins and Flavin Nucleotides



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

Flavoproteins are enzymes that catalyze redox reactions using either Flavin mono-nucleotide (FMN) or flavin adenine dinucleotide (FAD).

Flavin nucleotides undergo a shift in major absorption bands on reduction.