Biochemistry

4. Bio-Energetics

4.1) Introduction

Prof. Dr. Klaus Heese

Bio-Energetics:

the quantitative study of energy transductions in living cells and the physical-chemical nature underlying these processes.

Several Forms of Energy in Biological Systems

- Kinetic energy (including heat or thermal energy, the energy of the motions of molecules

- Mechanical Energy (e.g. changes in lengths of cytoskeletal filaments generates forces that push or pull on membranes and organelles) ---> *see lecture about neuron-energy*

- Potential energy (chemical potential energy (stored in the bonds connecting atoms in molecules), concentration gradient (across membranes), electric potential (across plasma membranes)).


Cells Need Energy

- ----> to generate and maintain its highly ordered structure (biosynthesis of macromolecules).

- ----> to generate motion (mechanical work). ---> *see lecture about neuron-energy*

- ----> to generate concentration and electrical gradients across cell membranes (active transport).

- ----> to generate heat and light.

Cells have to use chemical energy

- Living cells are generally held at constant temperature and pressure: chemical energy (free (Gibbs) energy, ΔG) has to be used by living organisms.
- Biological energy transformation obey the two basic laws of thermodynamics.
- The free energy concept of thermodynamic is more important to biochemists than to chemists

A typical **(bio-) chemical reaction** can be described as:

$$aA + bB + cC + \ldots \longrightarrow zZ + yY + xX + \ldots$$
$$\longleftarrow$$
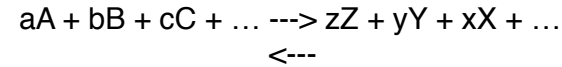
With the equilibration constant:

$$K_{eq} = \frac{[X]^x [Y]^y [Z]^z}{[A]^a [B]^b [C]^c}$$

$= k_f/k_r$; where k_f or k_r are the rate constants for the forward and reverse reactions, respectively

Thermodynamic quantities describe energy changes occurring in a chemical reaction

- $\Delta G = G_{products} - G_{reactants} = \Delta H - T\Delta S =$
  (Gibbs Free Energy [J/mol]; Enthalpy [KJ/mol], Entropy [J/mol K])
  $\Delta G'^o + RT \ln Q$ (Q = [products]/[reactants])
- $\Delta G'^o = -RT \ln K'_{eq}$ (K'_{eq} : equilibrium constant)
- The actual free energy change (ΔG) determines whether a reaction occurs spontaneously.
- The standard free energy change in biochemistry ($\Delta G'^o$) is a constant (measured under a standard set of conditions).
- ΔG for a reaction can be larger, smaller, or the same as $\Delta G'^o$, depending on the concentrations of the reactants and products.

- *If ΔG <0,* the forward reaction (from left to right as usually written) will tend to occur spontaneously.

- *If ΔG >0,* the reverse reaction will tend to occur.

- *If ΔG =0,* both forward and reverse reactions occur at equal rates; the reaction is at equilibrium.

2

- The ΔG and ΔG° values are **additive** when reactions are **coupled**, thus a thermodynamically unfavorable reaction can be driven by a favorable one.

- The overall K_{eq} is multiplicative (the product of two), although ΔG° is additive (the algebraic sum of two).

- *Note: the rate of a chemical reaction has nothing to do with its ΔG or ΔG°, but is determined by its activation energy ($\Delta G^{\ddagger}$)! (see ---> enzymes as catalysts to lower E_A)*

- In an exothermic reaction, the products contain less bond energy than the reactants, the liberated energy is usually converted to heat (the energy of molecular motions), and ΔH is negative.

- in an endothermic reaction, the products contain more bond energy than the reactants, heat is absorbed, and ΔH is positive.

- the combined effects of the changes in the enthalpy and entropy determine if ΔG for a reaction is positive or negative. An exothermic reaction ($\Delta H<0$) in which entropy increases ($\Delta S>0$) occurs spontaneously ($\Delta G<0$). An endothermic reaction ($\Delta H>0$) will occur spontaneously if ΔS increases enough so that the $T\Delta S$ term can overcome the positive ΔH.

- many biological reactions lead to an increase in order and thus a decrease in entropy ($\Delta S<0$).

The ΔG and ΔG° values are **additive** when reactions are **coupled**:
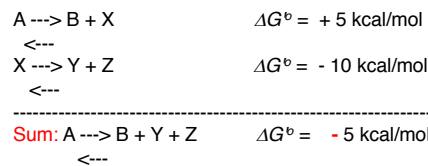
$\Delta G^{\prime o}$ of a reaction can be calculated from its K_{eq}

$$\Delta G^{\circ} = -2.3RT \log K_{eq} = -1362 \log K_{eq}$$

(under standard conditions)

$$K_{eq} = 10^{-(\Delta G^{\circ}/2.3RT)}$$

An unfavorable chemical reaction can proceed if it is coupled with an energetically favorable reaction:

A ---> B + X $\Delta G^{\circ} = +5$ kcal/mol
 <---
X ---> Y + Z $\Delta G^{\circ} = -10$ kcal/mol
 <---

Sum: A ---> B + Y + Z $\Delta G^{\circ} = -5$ kcal/mol
     <---

ATP is the universal 'currency' for biological energy

- This was first perceived by Fritz Lipmann and Herman Kalckar in 1941 when studying glycolysis.
- Hydrolysis of the two phosphoanhydride bonds in ATP generates *more stable products* releasing large amount of free energy
- ($\Delta G^{\prime o}$ is about -30.5 kJ/mol; ΔG_p **in cells is -50 to -65 kJ/mol**).

- The ATP molecule is kinetically stable at pH 7 and enzyme catalysis is needed for its hydrolysis.
- ATP actually exists as a sum of various species in cells (e.g., ATP^{4-} and $MgATP^{2-}$).

Hydrolysis of ATP releases Substantial Free Energy and Drives Many Cellular Processes

($\Delta G'^o$ is about -30.5 kJ/mol; **ΔG_p in cells is -50 to -65 kJ/mol**).

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Phosphoanhydride bonds

Phosphoester bond

Adenosine triphosphate (ATP)

Adenine

Ribose

$$ATP + H_2O \longrightarrow ADP + P_i + H^+$$

$$ATP + H_2O \longrightarrow AMP + PP_i + H^+$$

$$ADP + H_2O \longrightarrow AMP + P_i + H^+$$

$\Delta G'^o$ is about 7.3 kcal/mol for the hydrolysis of one bond and about 3x more than $\Delta G'^o$ for:

Glycerol 3-phosphate

Example: ATP provides energy usually through group transfer (protein could also be such acceptors)

(a) Written as a one-step reaction

Glutamate

Gln synthetase

Glutamine

See: Neuron-Glia interaction !

Enzyme-bound glutamyl phosphate

(b) Actual two-step reaction

Three positions on ATP for attack by the nucleophile $R^{18}O$

Not phosphate

Nucleophilic attacks

|		
ADP	AMP	PP$_i$
Phosphoryl transfer	Pyrophosphoryl transfer	Adenylyl transfer
(a)	(b)	(c)

ATP usually provides energy by group transfer of phosphoryl groups , not phosphate groups; forming covalent intermediates), not by simple hydrolysis.
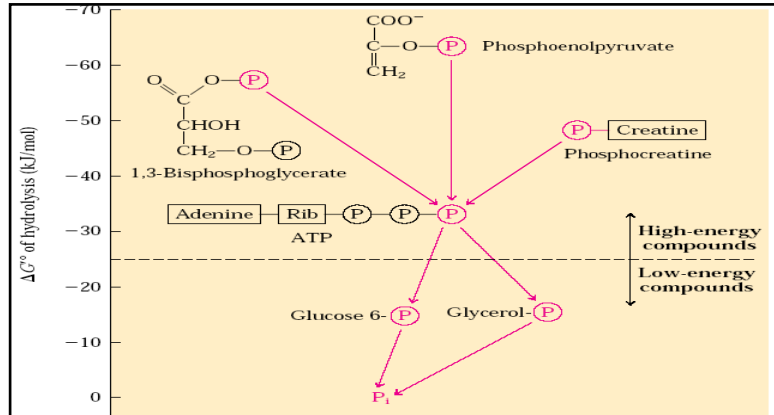
ATP has an intermediate phosphoryl group transfer potential, thus ADP can accept and ATP can donate phosphoryl groups (*forming the ATP-ADP cycle and acting as an energy currency*)

$$Phosphocreatine^{2-} + H_2O \longrightarrow creatine + P_i^{2-}$$
$$\Delta G'^\circ = -43.0 \text{ kJ/mol}$$
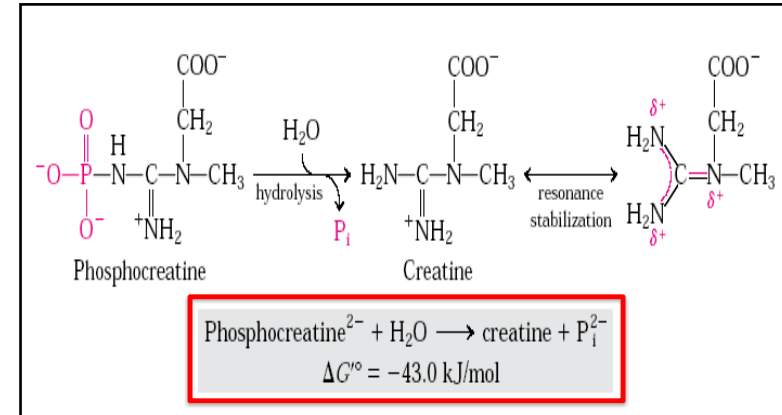
ATP is not a long-term storage form of free energy in living cells, but phosphocreatine is one such phosphoryl reservoir, or so-called phosphagen. Therefore, PC can transfer a P_i on ADP to form ATP.

Redox Reaction: a brief reminder

e.g.:
Oxidation: $2 Fe^{2+} \longrightarrow 2 Fe^{3+} + 2 e^-$ and Reduction: $2 e^- \frac{1}{2} O_2 \longrightarrow O^{2-}$
redox: $2 Fe^{2+} + \frac{1}{2} O_2 \longrightarrow 2 Fe^{3+} + O^{2-}$

Fe^{2+} is oxidized and O_2 is reduced = redox reaction; O_2 oxygen is an electron acceptor in many redox reactions in aerobic cells.

Many biologically important oxidation and reduction reactions involve the removal or the addition of hydrogen atoms (protons plus electrons) rather than the transfer of isolated electrons on their own. The oxidation of succinate to fumarate, which also occurs in mitochondria, is an example. Protons are soluble in aqueous (H_2O) solution as H_3O^+, but electrons are not and must be transferred directly from one atom or molecule to another without a water-dissolved intermediate. In this type of oxidation reaction, electrons often are transferred to small electron-carrying molecules, sometime referred to as coenzymes. The most common of these electron carriers are NAD^+ (nicotinamide adenine dinucleotide), which is reduced to NADH, and FAD (flavin adenine dinucleotide), which is reduced to $FADH_2$. the reduced forms of these coenzymes can transfer protons and electrons to other molecules, thereby reducing them.

a brief reminder

Redox Reaction: e.g.:
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The readiness with which an atom or a molecule *gains* an electron is its **reduction potential E**. the tendency to *lose* electrons, the **oxidation potential**, has the same magnitude but opposite sign as the reduction potential for the reverse reaction. Reduction potentials are measured in volts (V) from an arbitrary zero point set at the reduction potential of the following half-reaction under standard conditions (25 ºC, 1 atm, and reactants at 1 M):

reduction
$H^+ + e^- \xleftrightarrow{} \frac{1}{2} H_2$
oxidation
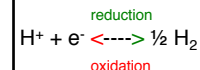
The value of E for a molecule or an atom under standard conditions is its standard reduction potential E'_0. A molecule or ion with a positive E'_0 has a higher affinity for electrons than the H^+ ion does under standard conditions. Conversely, a molecule or ion with a negative E'_0 has a lower affinity for electrons than the H^+ ion does under standard conditions. Like the $\Delta G^{0'}$, standard reduction potentials may differ somewhat from those found under the conditions in a cell because the concentrations of reactants in a cell are not 1M.

a brief reminder

Redox Reaction: e.g.:
Oxidation: $2\ Fe^{2+} \longrightarrow 2\ Fe^{3+} + 2\ e^-$ and Reduction: $2\ e^- \ ½\ O_2 \longrightarrow O^{2-}$
redox: $2\ Fe^{2+} + ½\ O_2 \longrightarrow 2\ Fe^{3+} + O^{2-}$

In redox reactions, electrons move spontaneously toward atoms or molecules having more positive reduction potentials. In other words, a compound having a more negative reduction potential can transfer electron to (i.e., reduce) a compound with a more positive reduction potential. In this type of reaction, the change in electric potential ΔE is the sum of the reduction and oxidation potentials for the two half-reactions. The ΔE for a redox reaction is related to the change in free energy ΔG by the following expression:

ΔG (cal/mol or J/mol) $= -nF\Delta E = -n$ (23,064 cal V^{-1} mol^{-1}) ΔE (volts),
(Gibbs Free Energy)

Where n is the number of electrons transferred. Note that a redox reaction with a positive ΔE value will have a negative ΔG and thus will tend to proceed from left to right.

Reduction potentials (E)
measure affinity for electrons

- Standard reduction potential (E'^o) of each oxidant is measured by connecting a (*test*) half-cell having the oxidized and reduced species of the redox pair each at 1 M, or 1 atm for gases, *pH 7* to a (*reference*) half-cell having 1 M H$^+$ and 1 atm H$_2$, whose E'^o is arbitrarily assigned as 0.00 V.
- By convention, the redox pair having a higher tendency to acquire electrons is given a positive value of E'^o.

ΔG can be calculated via reduction potential ΔE

- ΔG^o of a redox reaction can be calculated from the ΔE (= E of the electron donor − E of the electron acceptor):

$E = E^0 + RT/nF\ \ln Q$; Q = [electron acceptor]/[electron donor]

- $\Delta G = -nF\Delta E$ or $\Delta G^{r0} = -nF\Delta E^{r0}$

n = number of electrons transferred. Note that a redox reaction with a positive ΔE value will have a negative ΔG and thus will tend to proceed from left to right.

Measuring the standard reduction potential (E'^o)
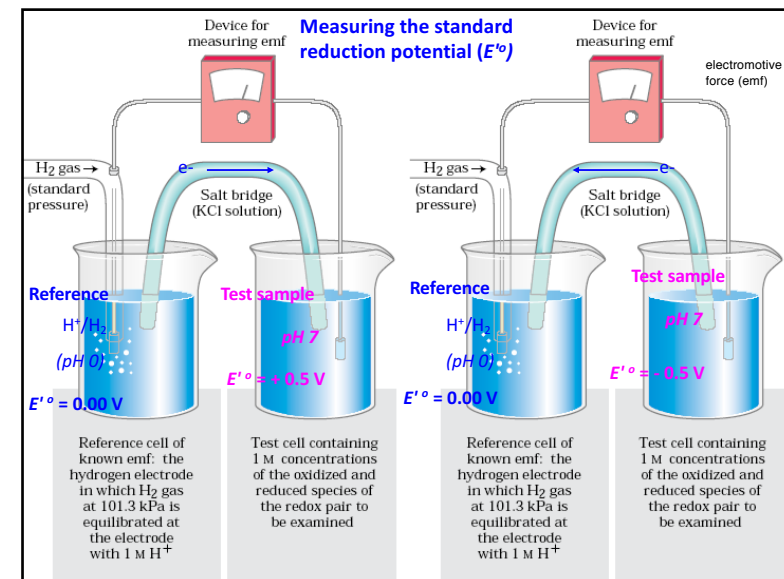
ΔG can be calculated via ΔE

- The actual reduction potential (E) depends on electrons transferred per molecule (n), temperature (T), ratio of [electron acceptor]/[electron donor]:

$E = E^0 + RT/nF \ln Q$; Q = [electron acceptor]/[electron donor]

ΔG can be calculated via ΔE

- ΔG^o of a redox reaction can be calculated from the ΔE (= E of the electron donor − E of the electron acceptor):

$E = E^0 + RT/nF \ln Q$; Q = [electron acceptor]/[electron donor]

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Electron transfer via oxidation-reduction RedOx - reactions generates biological energy

- When electrons flow from a low affinity carrier (e.g., glucose) to a high affinity carrier (e.g., to O_2 to form H_2O), an electromotive force (emf) will be generated (with energy released and work done).
- Energy transducers (*proteins*) are needed.
- Oxidation of energy-rich biological fuels *often* means dehydrogenation (catalyzed by *dehydrogenases*) from carbons having various oxidation states.
- In the living cells, electrons are transferred directly as electrons (between metal ions), as hydrogen atoms ($H^+ + e^-$), or as a hydride ion ($:H^-$ or $H^+ + 2e^-$).

Example: Conversion of Succinate to Fumarate
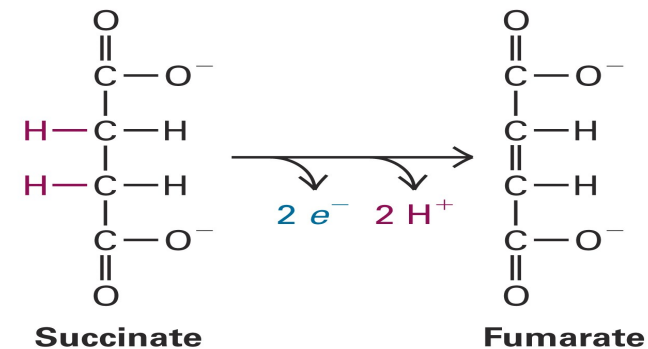
In this oxidation reaction (which occurs in mitochondria as part of the citric acid cycle) succinate loses two electrons and two protons. These are transferred to FAD, reducing it to $FADH_2$.

The Electron Carrying co-enzymes NAD⁺ and FAD

(a)

Oxidized: NAD⁺   Reduced: NADH

Nicotinamide

Ribose — 2P — Adenosine (NAD⁺)

Ribose — 2P — Adenosine (NADH)

$NAD^+ + H^+ + 2\,e^- \rightleftharpoons NADH$

(b)

Oxidized: FAD   Reduced: FADH₂

Flavin

Ribitol — 2P — Adenosine

$FAD + 2\,H^+ + 2\,e^- \rightleftharpoons FADH_2$

(a) NAD⁺ (nicotinamide adenine dinucleotide) is reduced to NADH by addition of two electrons and one proton simultaneously. In many biological redox reactions (e.g., succinate to fumarate), a pair of hydrogen atoms (two protons and two electrons) are removed from a molecule. One of the protons and both electrons are transferred to NAD⁺; the other proton is released into solution. (b) FAD (flavine adenine dinucleotide) is reduced to FADH₂ by addition of two electrons and two protons. In this two-step reaction addition of one electron together with one proton first generates a short-lived semiquinone interemediae (not shown), which then accepts a second electron and protons.
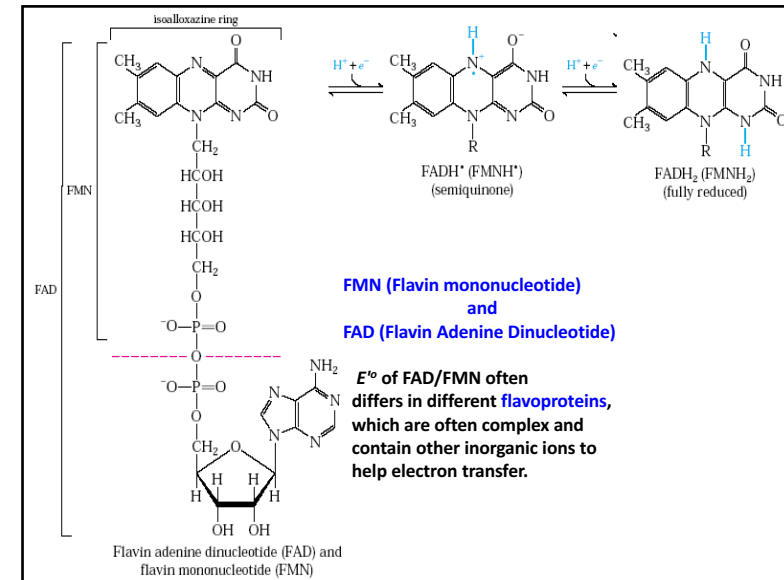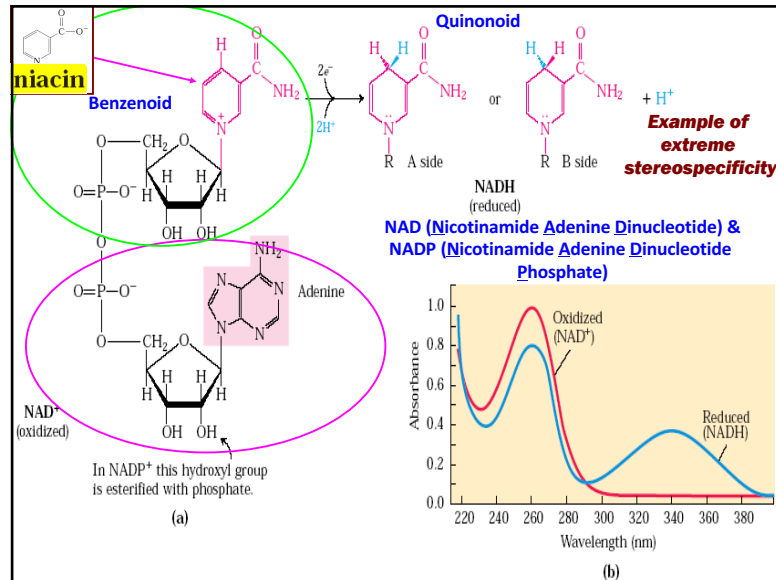
A few universal carriers collect electrons from the stepwise oxidation of various substrates

- Cellular oxidation of a nutrient occurs via stepwise reactions (pathways) for efficient energy transduction.
- NAD⁺, NADP⁺, FAD, and FMN are universal reversible electron carriers (as coenzymes of various enzymes).
- NAD and NADP are dinucleotides able to accept/donate a hydride ion (with $2e^-$) for each round of reduction/oxidation.
- *NAD (as NAD⁺) usually acts in oxidations and NADP (as NADPH) in reductions.*

- In each specific NAD- or NADP-containing dehydrogenase, the hydride ion is added/taken stereospecifically from one side (A or B) of the nicotinamide ring (*example of extreme stereospecificity*).

- FAD or FMN is able to accept/donate one or two electrons (as hydrogen atom), with absorption maximum for the oxidized and reduced forms being 570 nm and 450 nm respectively (*they also act in such light receptor proteins as cryptochromes and photolyases*).

- NAD and NADP can easily diffuse out of the enzymes, but FMN and FAD are tightly bound to the enzymes (thus being called *prosthetic groups*, and the complex proteins being called *flavoproteins*).

- NADH and FADH₂ will be further oxidized via the respiratory chain for ATP production.

- ADP is commonly present in all these universal electron carriers (as well as in Coenzyme A and ATP).

niacin
Benzenoid
Quinonoid
Example of extreme stereospecificity

A side   B side

NADH (reduced)

NAD (Nicotinamide Adenine Dinucleotide) & NADP (Nicotinamide Adenine Dinucleotide Phosphate)

$+ H^+$

2e^-
2H$^+$

Oxidized (NAD$^+$)
Reduced (NADH)

NAD$^+$ (oxidized)

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

Adenine

(a)

Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
220 240 260 280 300 320 340 360 380
Wavelength (nm)
(b)

isoalloxazine ring

FMN
FAD

FADH$^•$ (FMNH$^•$) (semiquinone)

FADH$_2$ (FMNH$_2$) (fully reduced)

$H^+ + e^-$

FMN (Flavin mononucleotide) and FAD (Flavin Adenine Dinucleotide)

E'^o of FAD/FMN often differs in different flavoproteins, which are often complex and contain other inorganic ions to help electron transfer.

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

Key concepts

- The change in free energy ΔG is the most useful measure for predicting the direction of chemical reactions in biological systems. Chemical reactions tend to proceed in the directions for which ΔG is negative.
- Directly or indirectly, light energy captured by photosynthesis in plants and photosynthetic bacteria is the ultimate source of chemical energy for almost all cells.
- The chemical free energy change $\Delta G^{\prime o}$ equals -2.3 RT log K_{eq}. Thus the value of $\Delta G^{\prime o}$ can be calculated from the experimentally determined concentrations of reactants and products at equilibrium.
- A chemical reaction having a positive ΔG can proceed if it is coupled with a reaction having a negative ΔG of larger magnitude.
- Many otherwise energetically unfavorable cellular processes are driven by hydrolysis of phosphoanhydride bonds in ATP.
- An oxidation reaction (loss of electrons) is always coupled with a reduction reaction (gain of electrons).
- Biological oxidation and reduction reactions often are coupled by electron-carrying co-enzymes such as NAD$^+$ and FAD.
- Oxidation-reduction reactions with a positive ΔE have a negative ΔG and thus tend to proceed spontaneously.

Summary

- Bio-Energy is chemical energy, studied in terms of free energy and free energy changes (ΔG).
- ATP acts as the free energy carrier (currency) in cells.
- Bio-Energy is mainly produced via stepwise electron flow (redox reactions) through a series of electron carriers having increasing levels of reduction potential (E).
- Electrons released from the oxidation of nutrient fuels are initially channeled to a few universal electron carriers (including NADH and FADH$_2$).