1 Behind the Torch of Life

1.1 Bioenergetics and Thermodynamics

Bioenergetics is the quantitative study of energy transductions. Of particular interest is the second law of thermodynamics.

Definition 1.1 (**The second law of thermodynamics**). *The Second Law of Thermodynamics states that the total entropy of an isolated system can never decrease over time. Isolated systems spontaneously evolve toward thermodynamic equilibrium, the sate with the* ***maximum entropy****.*

In a **chemical reaction** entropy increases when the products of the reaction are less complex and more disordered than its substrates. Therefore many biochemical reactions seam to contradict the second law as they "produce order"
To compensate the produced order by cells in their growth and division **free energy** is taken from the environment (organisms are not an isolated system) in the form of nutrients or solar light and exchanged for heat and entropy.

Definition 1.2 (**Enthalpy, H**). *Enthalpy (H) is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and DH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of* ΔH*.*

Definition 1.3 (**Entropy, S**). *Entropy is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.*

Definition 1.4 (**Free energy (G)**). *It represents the energy available to do biological work, such as muscle contraction, active transport, and biosynthesis. The change in Gibbs free energy (*ΔG*) for a reaction is given by:*

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

In biochemistry, Gibbs free energy (G) determines whether a metabolic reaction "can occur spontaneously" (but may still be unlikely because of TS) in living systems. A process is favorable if it is **Exergonic Reaction**, $\Delta G < 0$.
An important property is that variations in delta G are **additive**:

$$\Delta G_{\text{total}} = \Delta G_1 + \Delta G_2$$

This property lets us **make unfavorable reaction favorable when coupling them to a highly favorable reaction**. This is explored by various biological pathways.

Remark 1.5 (**Standard transformed constants**). Physical constants based on this biochemical standard state are called **standard transformed constants** and are written with a prime (such as $\Delta G'^{\circ}$ and K'_{eq}) to distinguish them from the untransformed constants used by chemists and physicists.

1.1.1 Equilibrium

By the second law of Thermodynamics, a reaction continues until equilibrium, the maximal entropy is reached. This is described by the **equilibrium constant** (K) that quantifies the ratio of **product over reactant** concentrations at equilibrium. It is defined for a general reaction:

$$aA + bB \rightleftharpoons cC + dD$$

$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

$$\Delta G^\circ = -RT \ln K$$

Remark 1.6 (**steady state**). In biological process the equilibrium is practically never reached. Nevertheless the system reaches a steady state, where the the concentrations stays constant thanks to a net flow equal to zero.

1.1.1.1 Reaction Quotient

The **Reaction Quotient** is similar to the equilibrium constant, but it uses the actual, observed concentrations of reactants and products, rather than the equilibrium concentrations. Q is defined as:

$$Q = \frac{[C]^c_{\text{obs}}[D]^d_{\text{obs}}}{[A]^a_{\text{obs}}[B]^b_{\text{obs}}} \tag{1}$$

$$\Delta G = \Delta G^o + RT \ln Q \tag{2}$$

1.1.1.2 Mass Action Ratio (Q/K)

The **Mass Action Ratio** is the ratio of the reaction quotient (Q) to the equilibrium constant (K).The mass action ratio helps us understand **where a reaction is going**:

$$\Delta G = RT \ln \frac{Q}{K} \tag{3}$$

- If $Q/K < 1$, then $\Delta G < 0$, and the reaction will proceed in the forward direction to reach equilibrium.
- If $Q/K > 1$, then $\Delta G > 0$, and the reaction will proceed in the reverse direction to reach equilibrium.
- If $Q/K = 1$, then $\Delta G = 0$, and the reaction is at equilibrium.

1.1.1.3 Henderson-Hasselbach

Since in a biological context the environment is buffered at near-constant pH, the Henderson-Hasselbach equation is generally applicable to determine the ratio of the different protonation states of a compound.

$$\text{pH} = \text{pKa} + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

1.2 Back to OCI

The reactions that do occur in cells represent a toolbox that evolution has used to construct metabolic pathways that circumvent the "impossible" reactions. Most of the reactions in living cells fall into one of five categories:

- reactions that make or break carbon–carbon bonds
- internal rearrangements, isomerizations, and eliminations
- free-radical reactions
- group transfers
- oxidation-reductions

Remark 1.7 (**Covalent Bond**). A covalent bond consists of a shared pair of electrons, and the bond can be broken in two general ways. In **homolytic cleavage**, each atom leaves the bond as a radical, carrying one unpaired electron. In **heterolytic cleavage** which is more common, one atom retains both bonding electrons.

Remark 1.8 (**Nucleophiles and Electrophiles**). Nucleophiles (functional groups rich in and capable of donating electrons) and electrophiles (electron-deficient functional groups that seek electrons).

1.2.0.1 Reactions that make or break carbon–carbon bonds

Heterolytic cleavage of C-C bonds yealds a carboanion and a carbocation. Conversely, the formation of a C-C bond involves the combination of a nucleophilic carboanion and an electrophilic carbocation. *Note, that carboanions and carbocations are generally so unstable that their formation as reaction intermediates can be energetically impossible even with the help of en enzyme.*

Therefore this reactions need assistance by functional groups containing electromotive atoms (O and N). This can alter the electronic structure of adjacent carbon atoms (**carbonyl-groups**, withdrawing electrons), stabilizing and facilitation the formation of carboanion and cation intermediates. *This can be further enhanced by the presence of metal ions sutch as Mg2+ for example.*

Aldol condensation is comman way to creat C-C bonds, i.e. the aldolase reaction which converts six-carbon compounds to three-carbon compounds in glycolysis is an aldol condenstation in reverse.

In a **Claisen condensation**, the carbanion is stabilized by the carbonyl of an adjacent thioester; an example is the synthesis of citrate in the citric acid cycle. Sometimes imine or certain cofactors play the role as the "electron-withdrawer".

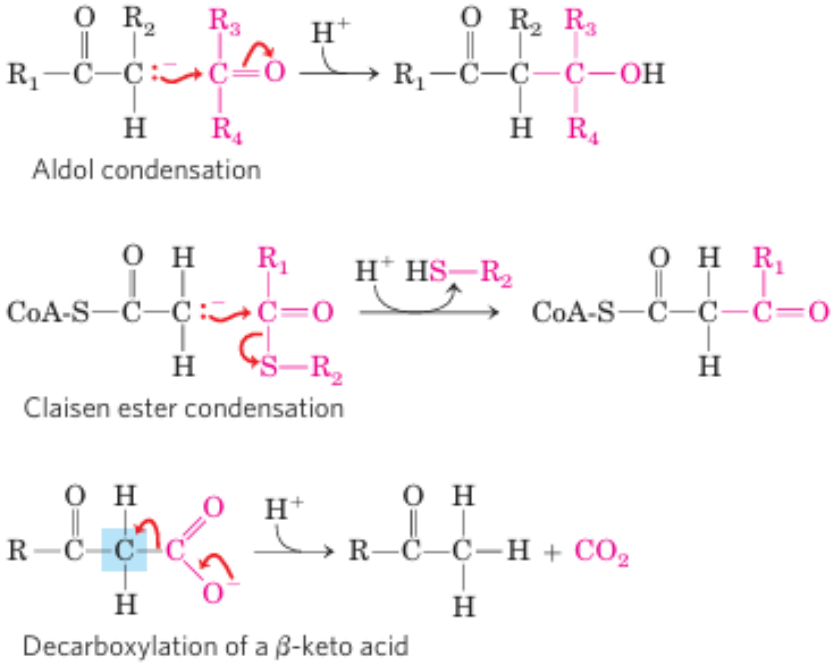


Figure 1: make or break carbon–carbon bonds

1.2.0.2 Internal rearrangements, Isomerizations, and Eliminations

In this type of reactions **electrons are redistributed altering the bonding framework** without changing the overall oxidation state of the molecule. For example different groups undergo oxidation-reduction leading to **cis-trans rearrangements** or shifting the **position of double bonds** , i.e. formation of fructose 6-phosphate form glucose 6-phosphate in glycolysis. Here C1 is reduced and C2 is oxidized.

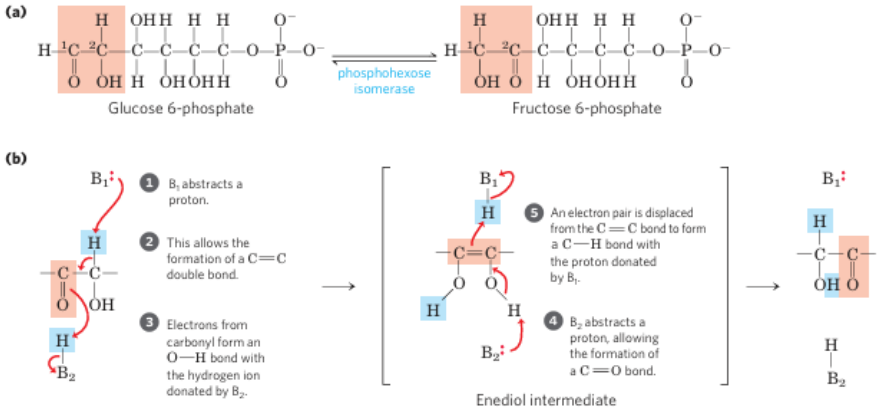


Figure 2: Isomerization and elimination reactions

En example for an **elimination** reaction is the loss of water from an alcohol resulting in a double C=C bond. *Similar reaction can result from eliminations in amines.*

1.2.0.3 Free-Radical Reactions

The homolytic cleavage of covalent bonds generate free radicals. These radicals can then trigger other rations.

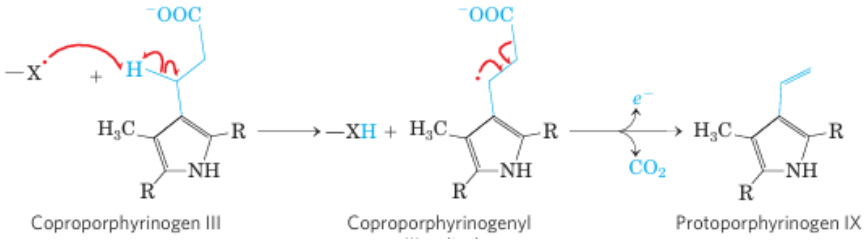


Figure 3: A free radical–initiated decarboxylation reaction

1.2.0.4 Group Transfer Reactions

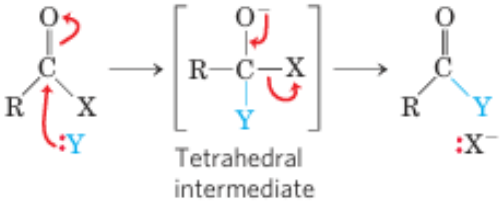
The transfer of acyl, glycosyl, and phosphoryl groups from one nucleophile to another is common in living cells. Acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an acyl group to form a tetrahedral intermediate.

A general idea in metabolism is to attach a good leaving group to a metabolic intermediate to trigger subsequent reactions. Since nucleophilic substitutions is made more favorable by the attachment of a phophoryl group to an otherwise poor leaving group such as -OH.

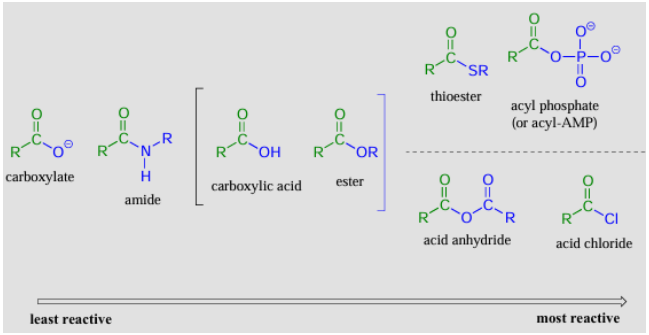
Remark 1.9 (**Good leaving group**). Recall that **weaker bases are better leaving groups**. One has to look how could the leaving group stabilize / balance the negative charge: **Inorganic orthophosphate** (the ionized form of H_3PO_4 at neutral pH, a mixture of $H_2PO_4^-$ and HPO_4^{2-}, commonly abbreviated as P_i) and inorganic pyrophosphate ($P_2O_7^{4-}$, abbreviated as PP_i); **esters and anhydrides of phosphoric acid** and **thiols** are also good leaving groups.

1.2.0.5 Oxidation-Reduction Reactions

Carbon atoms can only exist in five oxidation states, depending on their binding partners. Note that **carbon is less electronegative than all atomes it is bound to, except hydrogen**. Thus all atoms that bind



(a) Nucleophilic substitution



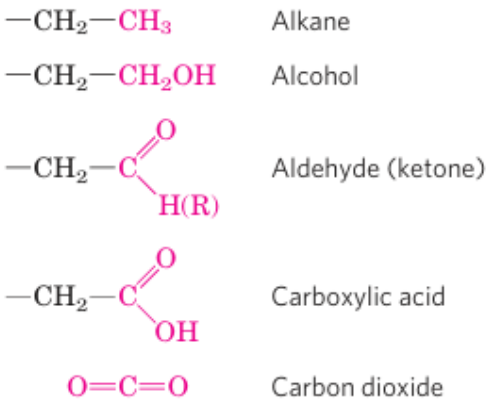
(b) Rlative reactivity of carboxylic acid derivatives

Figure 4: Group Transfer Reactions

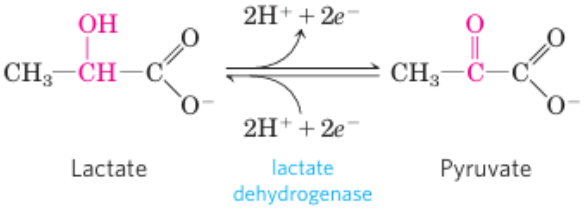
to carbon oxidaize it except hydrogen and therefore removing a hydrogen and replacing that bond with any other atom (including carbon) is synonymous with oxydation. Recall that every oxidation must be linked to a reduction. Note that, **Oxydations generally release energy** (camp fires where wood is oxidized).

Often in biological oxidations, a compound loses two electrons and two hydrogens (2 hydrogen atoms), these reactions are called **dehydrogenations** catalized by **Dehydrogenase**.

Sometimes in biological oxidations a carbon becomes covalently bounded to a oxygen. The corresponding enzymes are called **Oxidase** and if the oxygen atom is derived from molecular oxygen they are called **Oxygenase**.



(a) The 5 oxidation levels of carbons



(b) lactate dehydrogenase

Figure 5: Oxidation-Reduction Reactions

1.3 Phosphoryl and ATP fun

In a phosphate transfer reaction, a phosphate group is transferred from a phosphate group donor molecule to a phosphate group acceptor molecule.

1.3.0.1 Posphate groups

Recall some important properties of phosporus from organic chemistry:

- Phosphates are **excellent leaving groups** in biological organic reactions, which can be seen for example in the hydrolysis of ATP.
- Phosphoric acid (H3PO4) is triprotonic, meaning that it has three acidic protons availble to donate with pKa values of 1, 6.5, 13, respectivly.
- Phosphorus can break the octet rule because it is in the third row of the periodic table and thus has **d orbitals** available for bonding.
- The phosphate group is really tetrahedral, the **negative charges are delocalized** over the non-bridging oxygens, and there is some degree of protonation at physiological pH (with the exeption of the phosphate di-ester group.)

Phosphate transfer enzymes generally contain a **Mg2+ ion bound in the active site** in a position where it can interact with non-binding phophate oxygens on the substrate. This magnesium ion pulls the electron density away from the phosphorus atom, making it more electophilc.

A phosphate transfer reaction can be thought of as a SN2 reaction at a carbon center. Recall that the phosphorus can form a **"5-bond" tranistion state**.

1.3.0.2 The phosphate enzymes

Definition 1.10 (**Kinases**). *Kinase (from Greek kinein, "to move") is an enzyme that catalyzes the transfer of phosphate groups from high-energy donor molecules, such as ATP, to specific substrates, a process known as phosphorylation.*

Definition 1.11 (**Phosphatases**). *Phosphatase is an enzyme that removes phosphate groups from proteins or other molecules, a process known as dephosphorylation, which often regulates cellular activity.*

Remark 1.12 (**Reactions catalyzed by kinases an phosphatases are <u>not</u> the reverse of one another**). Kinases irreversible transfer phopshate groups from ATP (or sometimes other nucleoside triphosphates) to various organic acceptors compounds, while phophatases tranfer phosphate from organic compunds to water, releasing it as inorganic phosphate: this are hydrolysis reactions. Kinase reactions involve an inherently "uphill" step (phosphorylation of alcohols for example) being paid with an inherently "downhill" step (cleavage of an anhydride bond in ATP). Phosphatase reactions, on the other hand, are thermodynamically "downhill", and while they require an enzyme to speed them up, they do not involve "spending" energy the way kinases do.

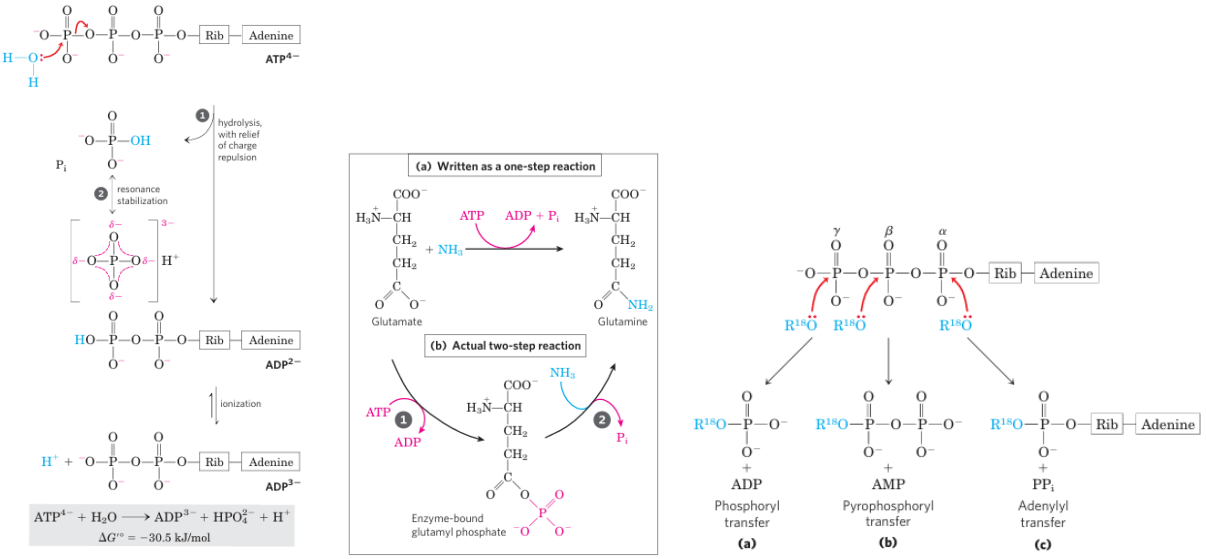
1.3.0.3 ATP

ATP (Adenosine Triphosphate) is the the **energy currency** of the cell and links catabolism and anabolism. ATP is a high energy compund which can be seen when considering hydrolysis of ATP (highly exergonic), since:

- Hydrolysis relieves electrostatic repulsion between the negatively charged phosphates. One way to picture this is a**coil springing open, releasing potential energy**
- Inorganic phosphate can be stabilized by resonance hybride.
- ADP-2 can ionize
- The products are better solvated than the reactants.

Note: that ATP-cleaving reaction are exothermic, but also have a high energy barrier, making it them very slow unless catalyzed by an enzyme. This helps us gain a tight control over the reactions in our metabolic pathways.

ATP provide energy by transferring its phosphate group and not by mere hydrolysis. Nevertheless often we say that a given reaction is coupled to ATP hydrolysis which provides the energy required for the reaction to happen. Note that **ATP hydrolysis per se only provides heat.**
In many reactions **ATP is used as a phosphate donor** to a substrate that, once phosphorylated, acquires an higher free energy.

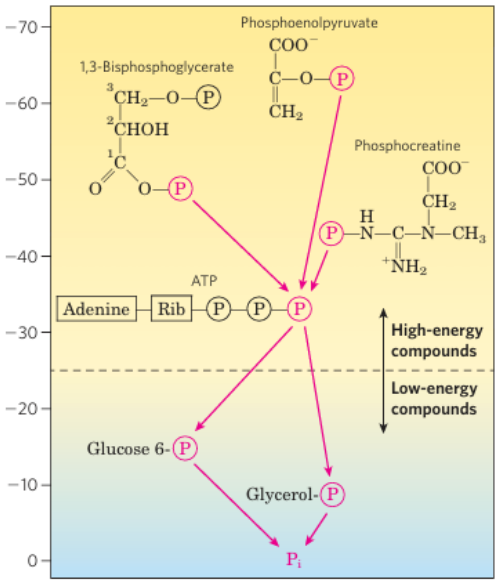


(a) ATP hydrolysis (b) ATP provides energy (c) ATP attack

Figure 6: ATP

Note: To maintain its high group transfer potential, ATP concentration must be held far above the equilibrium concentration by energy-yielding reactions of catabolism.
Moreover, inorganic polyphosphate, present in all cells, may serve as a reservoir of phosphoryl groups with high group transfer potential.

To produce ATP we need higher energy compounds. Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphoryl group transfer potential. Thioesters also have high free energies of hydrolysis.



(a) High energy phosphorylated compounds

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

(b) Standard free energies of Hydrolysis

Figure 7: Hydrolysis of Phosphate compounds

Remark 1.13 (**Arsenate Poisoning**). A toxic condition caused by exposure to arsenate (AsO_4^{3-}),in which arsenate disrupts the cellular metabolism by **mimicking inorganic phosphate**. Arsenate can uncouple oxidative phosphorylation by substituting for inorganic phosphate oxidative pathways, ATP synthesis, leading to decreased ATP production and cellular toxicity.
For example, in the presence of arsenate, the product of glyceraldehyde 3-phosphate dehydrogenase is 1-arseno-3-phosphoglycerate, which nonenzymatically decomposes to 3-phosphoglycerate and arsenate; this substrate for the phosphoglycerate kinase is therefore bypassed, which leads in **no net glycolytic synthesis of ATP**.

1.4 Biological Oxidation-Reduction Reactions

Since we need high energy compounds to produce ATP. We have to ask us but how do we produce these Hi-NRG (NRG = energy)? **-The flow of electrons can do it!**

Definition 1.14 (**Electromotive force (emf)**). *Electrons flow from a reducing agent to an oxidizing agent due to their different electron affinities. This difference in affinities is called the electromotive force. Note that the reducing agent undergoes oxidation and the oxidizing agent undergoes reduction.*

Living cells have an biological "circuit", with a relatively reduced compound such as glucose as the source of electrons. As glucose is enzymatically oxidized, the released electrons flow spontaneously through a series of electron-carrier intermediates to another chemical species, such as O2. This **electron flow is exergonic**, because O2 has a higher affinity for electrons than the electron-carrier intermediates. This is exploited by the **ATP synthase** in the inner mitochondrial membrane that uses the proton-motive force to do chemical work.

1.4.0.1 Dehydrogenation = Oxidation

Dehydroganation corresponds to oxidation, since the carbon is less electronegative tha all atoms it is bound to except hydrogen. *Note that not all oxidation-reduction reactions involve carbon, i.e conversion from nitrogen to ammonia.*

There are different ways that electrons can be transferred: Directely as electrons, as hydrogen atoms, as a hydrogen ion, or through direct combination with oxygen. Since all of this 4 types occur biologically, the term **Reducing Equivalent** is used.

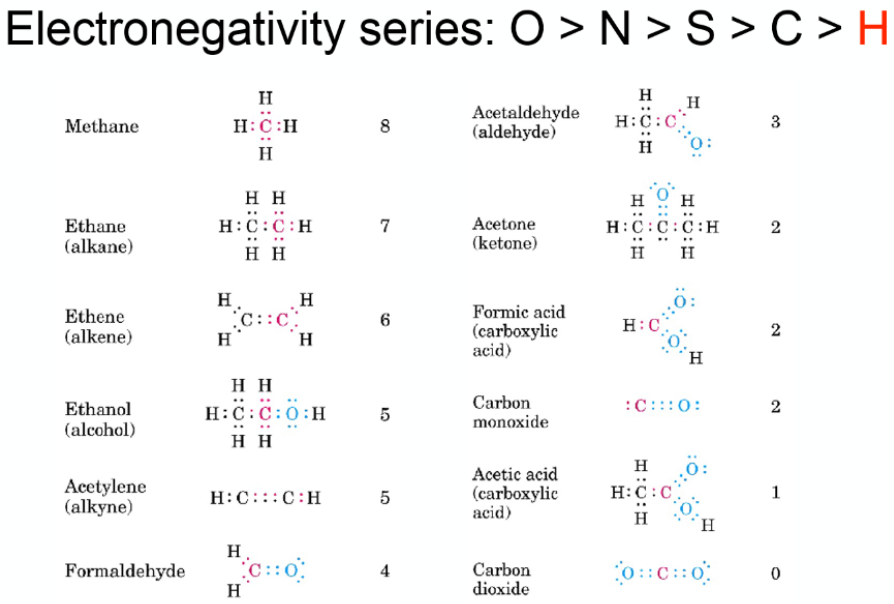


Figure 8: Oxidation levels of a carbon compound in the biosphere

1.5 Electron Carriers

There are a multitude of enzymes that catalyze oxidation reactions from a variety of substrates, but most electrons end up in a **small set of univalve electron carriers**, such as NAD+, FAD, and Q (ubiquionel).

Definition 1.15 (**Electron Carriers**). *Electron Carrier are Molecules that can accept and donate electrons, facilitating the transfer of energy in redox reactions (e.g., NAD+, FAD).*

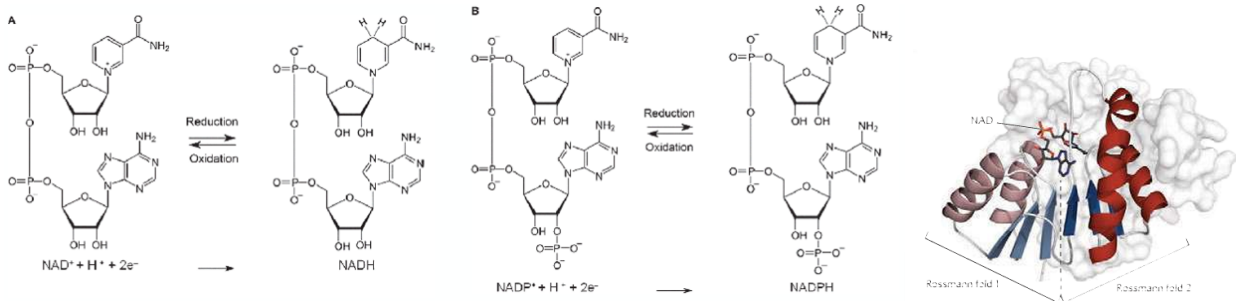
1.5.0.1 NADH and NADPH

These **watersolubable** coenzymes (*NAD^+ (Nicotinamide Adenine Dinucleotide) and $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate)*) can undergo **reversible reduction of the necotinamide ring**, as a substrate undergoes oxidation (dehydrogenation) giving up **2 hydrogen atoms**.

- NAD+ and NADP+ take **2 electrons and 1 proton** while the second proton is released into solution.

In many cells and tissues, the ratio of NAD+ (oxidized) to NADH (reduced) is high, favoring hydride transfer from a substrate to NAD+ to form NADH. By contrast, NADPH is generally present at a higher concentration than NADP+, favoring hydride transfer from NADPH to a substrate.

This reflects the specialized metabolic roles of the two coenzymes: **NAD+ generally functions in oxidations—usually as part of a catabolic reaction; NADPH is the usual coenzyme in reductions—nearly always as part of an anabolic reaction**.



(a) NAD (a) and NADP (b)

(b) Rossmann fold

Figure 9: NADH and NADPH

Remark 1.16 (**Dietary Deficiency of Niacin (Vitamin B3) cause Pellagra**). NAD and NADP are derived from the Niacin (Vitamin B3)), which is synthesized from tryptophan. Humans do generally not sysnthesize sufficient quantities of nacine, and this is especially so for individuals with diests low in tryptophan (diets based on maize for example). This leads to the disease pellagra.
But note since NAD+ is can oxidize many thousands of molecules of glucose, since it the reduction always can be reversed. It is not necessarily to consume a lot of the vitamin, in contrary to glucose for example.

1.5.0.2 FAD and FNM

FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide) are coenzymes that are used in oxidation-reduction reactions by **Flavoprotein**. They are **tightly bound to their enzymes**, in contrast to NAD and NADP. Moreover flavin nucleotides can also only carry one electron and one proton taking on the partially reduced form.

- FAD and FNM take **1 or 2 electrons and 1or 2 protons** and remain tighly linked to the enzyme.

The ability to take on the partially reduced form is crucial for FAD and FMN, since they are **key elements in electron transport reactions** (e.g. **ETC Complex I and II**), where electrons are passed one at a time.

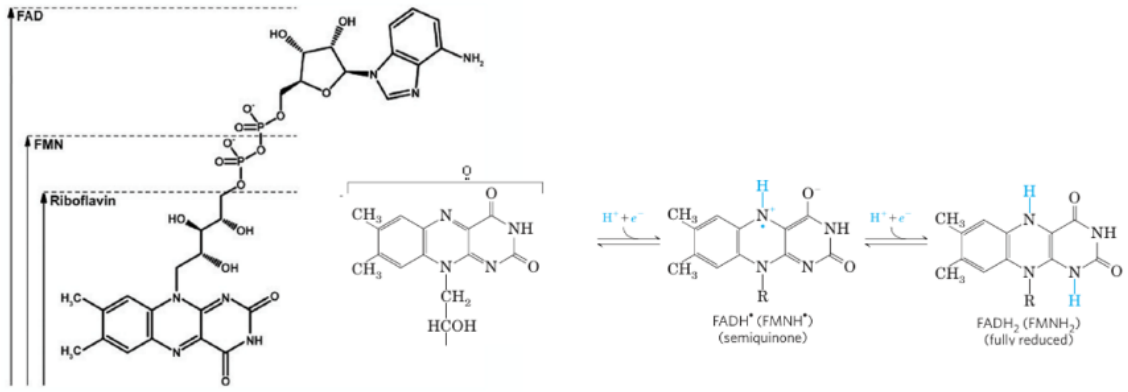


Figure 10: Flavin Nucleotides

These Flavin nucleotides are derived from the **vitamin riboflavin** (B2)

1.5.0.3 Ubiquinone, Q

Ubiquinone (Coenzyme Q) is a **lipid-soluble** electron carrier in the electron transport chain, transferring electrons between complex I/II and complex III. Ubiquinone exists in oxidized (Q), semiquinone (Q^-), and reduced (QH_2) forms.

- Coenzyme Q takes **1 electron** or **2 electrons and 2 protons** and freely diffuses through the mitochondrial membrane.

2 Metabolism

Metabolism is the sum of all biochemical reactions in a living organism. The metabolism can be divided into two main pathways: catabolism and anabolism.

2.1 Catabolism <=> Anabolism

Catabolism from greek meaning "**breaking down**" (Kata refers to down and bolë means to throw) is the process of breaking down complex molecules into simpler ones, **releasing/ producing energy in the form of ATP**. Examples include glycolysis, the Krebs cycle, and oxidative phosphorylation, which break down glucose and fatty acids to produce ATP. *Note catabolic pathways are mostly* ***converging***

In contrast, **Anabolism** from Greek meaning "**building up**" (Ana means up or again) is the synthesis of complex molecules from simpler ones, **requiring energy**. This process is essential for growth, repair, and maintenance of cells. Examples include protein synthesis, DNA replication, and lipid biosynthesis. *Note anabolic pathways are mostly* ***diverging***

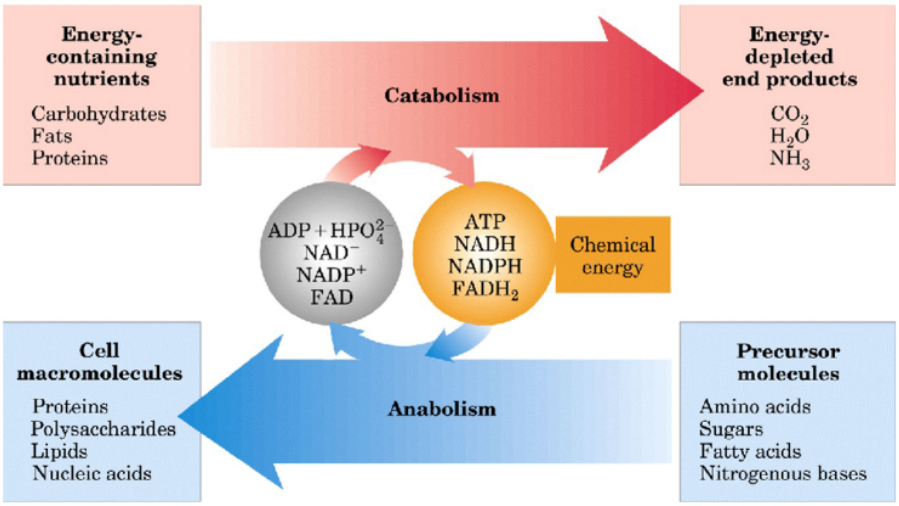


Figure 11: Catabolism <=> Anabolism

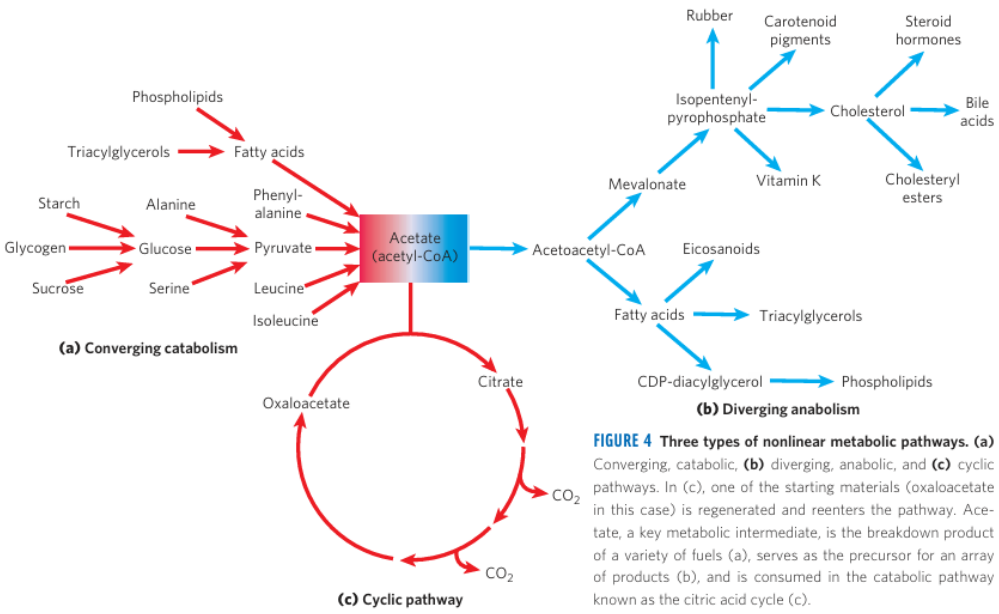


FIGURE 4 **Three types of nonlinear metabolic pathways. (a)** Converging, catabolic, **(b)** diverging, anabolic, and **(c)** cyclic pathways. In (c), one of the starting materials (oxaloacetate in this case) is regenerated and reenters the pathway. Acetate, a key metabolic intermediate, is the breakdown product of a variety of fuels (a), serves as the precursor for an array of products (b), and is consumed in the catabolic pathway known as the citric acid cycle (c).

Figure 12: Three types of nonlinear metabolic pathways