Free Energy, Enzymes and Metabolism

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eLearning in Studienbereichen – MNF – Universität Potsdam

Content

I. Free Energy and Biological Reactions

Quiz 2
Thermodynamics 2
Energy 2
Changing the state of a system with heat and work 3
Heat 3
Work 3
Energy is conserved 3
Enthalpy 4
Hess's law of heat summation 4
Standard enthalpy of reaction 5
Entropy 5
Free energy 6
Chemical equilibrium 7
Standard free energy 7
Concentration of reactants 7
Coupled reactions 8
ATP 9
ATP is the universal currency of free energy in cells 9
Generation of ATP 10
Further Readings 10
References 10
OER and OCW 10

II. Metabolism

Quiz 11
Metabolic pathways 11
Catabolism and anabolism 11
Some pathways have both anabolic and catabolic features 12
Localization in cells 13
Catalysis 13
Enzymes 13
Activation energy 14
Pathway throughput is controlled by enzymes 15
Control of enzyme activity 15
Allosteric control 15
Covalent modifications 16
Isoenzymes (isozymes) 17
Proteolytic activation 17
FAD 18
Further Readings 18
References 18
OER and OCW 18

III. What is life?

Quiz 19
'Alive' 19
Cells and Organisms 19
Viruses 19
Living systems 19
Phototrophs and chemotrophs 20
Further readings 20
References 20
OER and OCW 20

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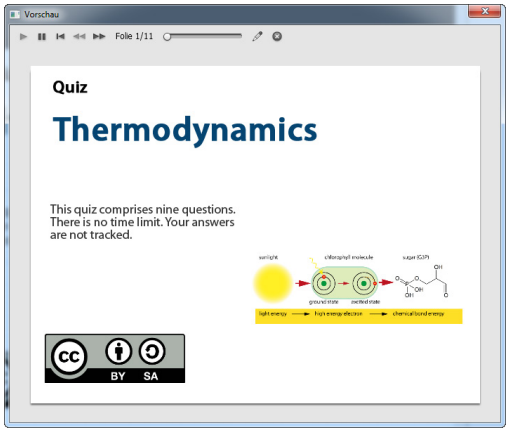
I. Free Energy and Biological Reactions

The laws of thermodynamics govern the behaviour of biochemical systems. Free energy is the indicator of spontaneity, which brings together the concept of enthalpy and entropy.

Enzymes accelerate the attainment of thermodynamic equilibria. Living organisms continuously ingest high-enthalpy, low-entropy nutrients, which they convert to low-enthalpy, high-entropy waste products.

Quiz

Start this chapter with the quiz to test for your mastery. You may reattempt the quiz later to check your progress.



Quiz 1. Thermodynamics. Click to play - HTML5 or Flash (opens new window in your standard web browser).

Thermodynamics

Thermo is heat, and dynamics is the motion of heat. The term thermodynamics refers to the quantitative description of the relationships between the different forms of energy and of chemical equilibria. Thermodynamics is entirely empirical.

Thermodynamics is built on laws (see below). **These laws are universally valid, they cannot be circumvented.**

The laws of thermodynamics govern the behaviour of biochemical systems and apply to all physical and biological processes. They determine the conditions under which specific processes can or cannot take place.

Knowledge of changes in thermodynamic quantities, such as ΔH or ΔS, allows predicting the equilibrium positions in reactions and whether under given circumstances a reaction can or cannot take place.

Energy

All organisms require energy. The ways in which living things obtain and utilize energy is a major theme of biology.

Energy appears in different types such as:

1. Kinetic energy
2. Heat energy (a manifestation of the kinetic energy associated with the random motion of molecules)
3. Potential energy (e.g. development of kinetic energy associated with the motion of an object as it falls from the top of a tower; or, within chemical systems, the potential energy of a mixture of gasoline and oxygen, which may react to form carbon dioxide and water and release heat)
4. Thermal energy
5. Chemical energy

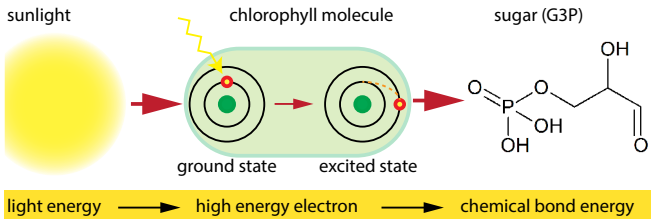


Figure 1. Energy can be converted from one form to another. The process of photosynthesis is an example of the conversion of light energy (electromagnetic energy) into chemical bond energy.

Any form of energy can be transformed into another form (Figure 1), but the total energy always remains the same (first law of thermodynamics, see below).

Definition: **Energy** is the **ability to perform work**. The SI unit of work is **joule (J)**. One J is the work done, or energy expended, by a force of one Newton moving an object one meter along the direction of the force (= 1 Nm).

Changing the state of a system with heat and work

Systems can be **open** (mass and energy can transfer between the system and the surroundings), **closed** (energy can transfer between the system and the surroundings), or **isolated** (neither mass nor energy can transfer between the system and the surroundings).

A living cell represents an "open" thermodynamic system (Figure 2). A study of folding of a biological polymer in aqueous solution represents a "closed" system if no mass and heat is exchanged with the surroundings.

The state of a system is defined by macroscopic properties such as p, T, V, n, and m. Changes in the state of a system are produced by interactions with the environment through heat and work, which are two different modes of energy transfer.

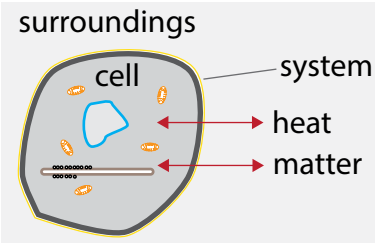


Figure 2. Living cells and organisms are open thermodynamic systems. The "system" is the object being studied, e.g., a cell. "Surroundings" is everything else in the universe.

Heat

Heat is energy transferred due to temperature differences only. Heating is transfer of energy from a hotter body to a colder one. It occurs spontaneously whenever a suitable physical pathway exists between the bodies. Heat energy is carried into or out of the system in the form of transfers in the microscopic thermal motions of particles.

Zeroth law of thermodynamics (based on observation): If two bodies are in contact, no further observable changes take place; thermal equilibrium prevails.

Two systems which are individually in thermal equilibrium with a third are in thermal equilibrium with each other; all three systems have the same value of the property called **temperature**.

Work

Any other means for changing the energy of a system is called work. Work refers to forms of energy transfer which can be accounted for in terms of changes in the macroscopic physical variables of the system.

Examples include:

Pressure-volume work: Work is done on the surroundings, when a compressible substance, e.g. a gas (the system), exerts a force on the surroundings via a piston, which moves through some distance.

Mechanical work: Work is done on a weight that is elevated to a certain height. Work (Nm) = force (N) × distance moved in direction of force (m)

Electrical work: Electrical work is the work done on a charged particle by an electric field.

We define work as being **positive** when the system does work on the surroundings (energy leaves the system). If work is done on the system (energy added to the system), the work is **negative.**

Sometimes this is given with opposite sign, the equations change accordingly.

Energy is conserved

The **first law of thermodynamics** is a mathematical statement of the **law of conservation of energy**.

Energy can be neither created nor destroyed. Energy can only be transferred

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from one form of energy to another. In other words, the energy content of the universe (but not the energy content of the system you look at) is constant.

$$\Delta U = U_{final} - U_{initial} = Q - W$$

U is energy, *Q* represents the heat absorbed *by* the system *from* the surroundings, and *W* is the work done *by* the system *on* the surroundings.

U can be changed only by the flow of energy as heat or by work.

Processes in which the system releases heat, which by convention are assigned a negative *Q*, are known as **exothermic processes**.

Processes in which the system gains heat (positive *Q*) are known as **endothermic processes** (Figure 3).

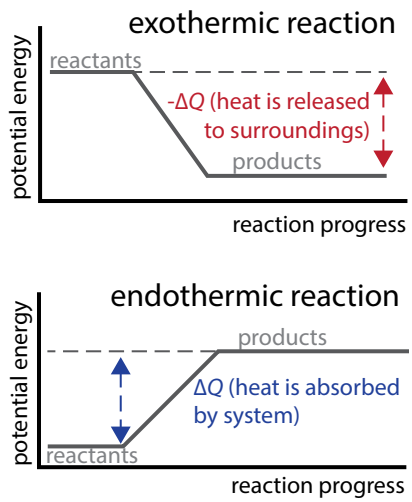


Figure 3. Energy profiles for chemical reactions.

Enthalpy

Enthalpy *H* is the heat content of a system at constant pressure. The enthalpy is defined to be the sum of the internal energy *U* plus the product of the pressure *P* and volume *V*:

$$H = U + P * V$$

H is the enthalpy (in joules), *U* is the internal energy (in joules), *P* is the pressure of the system, (in pascals), and *V* is the volume (in cubic meters).

Chemists tend to use the enthalpy *H* more often than the internal energy *U* in **reactions at constant pressure** (**Δ*P* = 0**).

The enthalpy can be used to calculate the heat transfer taking place in a closed system under constant pressure. Since biochemical reactions usually are conducted at constant pressure, enthalpy *H* is a quantity with which to describe biological systems.

The difference between the enthalpy *H* and the internal energy *U* is often not significant because if the pressure-volume (*PΔV*) work is negligible, *U* and *H* are the same.

$$\Delta H = \Delta U + P\Delta V$$

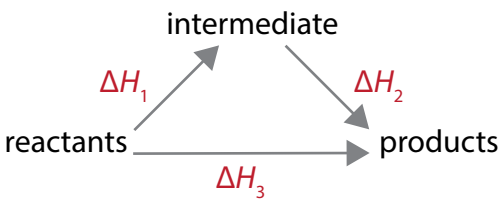
The volume changes in most biochemical processes are *negligible*, so

$$\Delta H = \Delta U$$

Under constant pressure, a condition typical of most biochemical processes, the enthalpy change between the initial and final states of a process, Δ*H*, is the easily measured heat that it generates or absorbs. Thus, enthalpy changes can be obtained directly from measurements of heat absorption at constant pressure.

Hess's law of heat summation

Hess's law states in any series of reactions that start with the same reactants and end with the same products, the net change in energy must be the same.



That means the enthalpy change, Δ*H*, of the combined reaction is equal to the sum of all the individual enthalpy changes. Whenever two chemical equations can be added to give a net reaction, their enthalpy changes may also be added

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to give the enthalpy change of the net reaction.

$$\Delta H_1 + \Delta H_2 = \Delta H_3$$

If this were not true, it would be possible to think up a series of reactions in which energy would be created but which would end up with the same substances the reactions started with. Thus, Hess' law is understood as an expression of the principle of conservation of energy.

Hess's law allows obtaining ΔH values for reactions which cannot be carried out experimentally.

For example, we wish to determine the enthalpy change resulting from the **oxidation of 1 g of glucose to CO_2 and H_2O by muscle tissue**. A direct measurement in the muscle would be extremely difficult given the numerous metabolic reactions that interfere with our enthalpy measurement. However, we can measure glucose's enthalpy of combustion in a constant pressure calorimeter rather than a muscle, and still obtain the same value. This value is indepentend of the mechanisms (i.e., metabolic pathway) through which the muscle converts glucose to CO_2 and H_2O, as long as these substances actually are the final metabolic products.

Standard enthalpy of reaction

In order to make the data useful for scientific and engineering applications, there is a general agreement to report and tabulate enthalpy changes for a mole of reaction at a standard temperature and pressure.

Such quantities are called the standard enthalpy of reaction.

Example: The **standard enthalpy for the combustion of methane** is 890.4 kJ per mole:

ΔH_0 = - 890.4 kJ/mol (1 mol or 22.4 l of gas, 298 K, 1 atm)

Entropy

Entropy is a measure of the amount of disorder or degree of randomness in a system.

Entropy is one of the factors that determine the free energy of the system (see later). The concept of entropy is central to the **second law of thermodynamics**, which deals with physical and chemical processes and whether they occur spontaneously.

The second law is essentially different from the first law; the two principles are independent and cannot in any sense be deduced from one another.

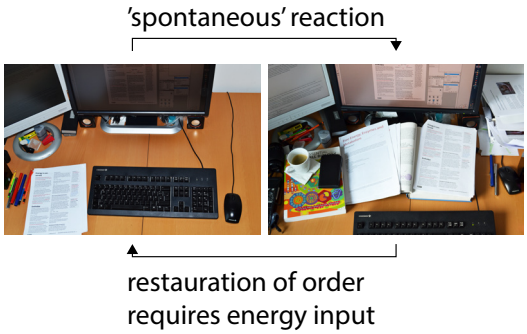

Figure 4. The restauration of order does not occur spontaneously. Human intervention will release enough heat to compensate for the reordering of the items on the desk.

The **entropy** is symbolized by S, and is practically defined by the equation

$$\Delta S \geq \frac{Q}{T}$$

where Q is the amount of heat received by the system and T is the absolute temperature. The SI unit of entropy is Joule per Kelvin ($J\,K^{-1}$).

For any constant energy process (ΔU = 0), a **spontaneous process** is characterized by

$$\Delta S > 0$$

Since the energy of the universe is constant (energy can neither be created nor destroyed), any spontaneous process must cause the entropy of the universe to increase:

$$\Delta S_{system} + \Delta S_{surrounding} = \Delta S_{universe} > 0$$

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This is the usual expression for the second law of thermodynamics. The entropy of the universe tends towards a maximum. The universe is moving towards chaos, randomness and disorder. A system can only be ordered at the expense of disordering its surroundings (to an even greater extent) by the application of energy to the system.

A system will spontaneously adopt its most probable arrangement, the one in which entropy is a maximum. The entropy of the thermodynamic system is a measure of how far the equalization has progressed.

Living organisms, which are particularly well ordered, achieve this order at the expense of disordering the nutrients they consume. Thus, eating is as much a way of acquiring order as it is of gaining energy (see below).

An **example of an entropy-driven process** is the **transport of O_2 and CO_2** between the lungs and the tissues by blood. O_2 and CO_2 tend to maintain a uniform concentration throughout the body (i.e., the highest level of disorder).

In the lungs, where the concentration of O_2 is higher than that in the venous blood passing through them, more O_2 enters the blood than leaves it. On the other hand, in the tissues, where the O_2 concentration is lower than that in the arterial blood, there is net diffusion of O_2 from the blood to the tissues.

The reverse situation holds for CO_2 transport since the CO_2 concentration is low in the lungs but high in the tissues.

For more details on the laws of thermodynamics see:

UC Davis ChemWiki (2014) Laws of thermodynamics

BBC Radio 4 (2004) The second law of thermodynamics (45 min audio)

Free energy

The concept of free energy was formulated by J.W. Gibbs in 1878. Free energy is the **indicator of spontaneity**, which **brings together the concept of enthalpy and entropy**.

The spontaneity of a process (e.g. a chemical reaction in a cell) cannot be predicted from knowledge of the system's entropy change alone. This is because exothermic processes (ΔH is negative) may be spontaneous even though they are characterized by $\Delta S < 0$.

On the other hand, the existence of entropy means that many spontaneous chemical reactions need not proceed with an evolution of heat (Table 1).

We need a function that predicts whether or not a given process is spontaneous. The **Gibbs free energy** combines the concepts of enthalpy and entropy, and the relevant equation is:

$$G = U + P * V - T * S = H - T * S$$

U is the internal energy (in *J*), *P* is pressure, *V* is volume, *T* is temperature (in Kelvin), *S* is entropy (*J* per *K*), *H* is enthalpy (in *J*).

Table 1. Relationship betwee ΔH and ΔS.

	- ΔH	+ ΔH
+ ΔS	always spontaneous	high T: spontaneous low T: non-spontaneous
- ΔS	low T: spontaneous high T: non-spontaneous	never spontaneous

Spontaneous processes, that is, those, with **negative ΔG** values, are said to be **exergonic**. The free energy lost during the exergonic reaction (as equilibrium is approached) is either transformed into heat or used to increase the amount of entropy.

A reaction can occur spontaneously only if ΔG is negative.

Processes that are not spontaneous, those with **positive ΔG** values, are termed **endergonic**.

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For more details on Gibbs free energy see:

Khanacademy (2014) Gibbs free energy and spontaneity

UC Davis ChemWiki (2014) Free energy

Chemical equilibrium

Chemical reactions will generally proceed until they reach a state of equilibrium. At this point, the rates of the forward and reverse reactions are equal, and there is no further net change in the concentrations of the reactants or products.

Processes at equilibrium, those in which the forward and backward reactions are exactly balanced, are characterized by

$$\Delta G = \Delta H - T\Delta S = Q_P - T\Delta S = 0$$

Standard free energy

The standard free energy makes it possible to compare the energetics of different reactions. ΔG depends on the concentrations of the molecules in the reaction mixture.

In contrast, the standard free energy, ΔG_0, is independent of concentration. It is based on the assumptions of ideal conditions where the concentrations of all reactants are set to 1 mole/liter.

Concentration of reactants

If a mixture is not at equilibrium the liberation of the excess free energy is the "driving force" for the composition of the mixture to change until equilibrium is reached.

The ΔG of a reaction depends only on the free energy of the products (the final stage) minus the free energy of the reactants (the initial stage). The ΔG of a reaction is independent of the path (or molecular mechanism) of the transformation.

Let's look at the following reaction:

$$A + B \leftrightarrow C + D$$

A and B react and form C and D. Thus, when we mix together the reactants A and B, C and D will form. However, when we take the pure C and D, they also will react to some extent and form A and B.

$$K = \frac{[C][D]}{[A][B]}$$

By convention, the products form the numerator. The equilibrium constant K depends on temperature, volume, pressure, and concentrations.

The relationship between the concentration and the free energy of the substances is given by

$$\Delta G = \Delta G^0 + RT * ln \frac{[C][D]}{[A][B]}$$

R is the universal gas constant. T (temperature) is given in Kelvin (K).

The expression for the free energy change of a reaction consists of two parts:

- A **constant term** (standard free-energy change), ΔG_0, whose value depends only on the reaction taking place (gives the energy when concentration of all reactants under standard conditions is 1 mol/l), and
- a **variable term** that depends on the concentration of the reactants and the products, and the temperature.

Whether the ΔG for a reaction is larger, smaller, or the same as ΔG^0 depends on the concentrations of the reactions and products.

At equilibrium, the rate at which reactants turn into products is equal to the rate with which products turn into reactants. The concentration of the reactants and the concentration of the products do not change with time. It will appear as if the reaction has stopped.

The ratio of the reactants and the products equals a constant, the reaction's

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equilibrium constant, ***K***. The equilibrium is attained when free energy is at its minimum value.

The equilibrium expression describes the situation at the point at which the concentration effect just balances the push given to the reaction by ΔG_0, so that $\Delta G = 0$ and there is no net change of free energy to drive the reaction in either direction.

If $\Delta G = 0$, the equation above becomes:

$$\Delta G^0 = -R \cdot T \cdot ln K_{eq} = -R \cdot T \cdot \ln \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}}$$

This equation shows that the equilibrium constant is directly proportional to ΔG_0. The equilibrium depends on the intrinsic character of the molecules, as expressed in the value of ΔG_0.

The more energetically favourable the reaction, the more product will accumulate if the reaction proceeds to equilibrium.

The concept of free energy change also applies to interactions where one molecule binds to another by means of noncovalent bonds. Two molecules will bind to each other if the ΔG_0 of the interaction is negative. The equilibrium constant indicates the strength of molecular interactions.

Coupled reactions

A convenient feature of thermodynamic calculations is that if two or more chemical equations are summed, ΔG for the resulting overall equation is just the sum of the ΔG's for the individual equations. Thus, for sequential reactions, the changes in free energy are additive.

The additivity of free energy changes allows an endergonic reaction to be driven by an exergonic reaction under the proper conditions.

In cells, endergonic biochemical reactions are driven by ATP (Figure 5).

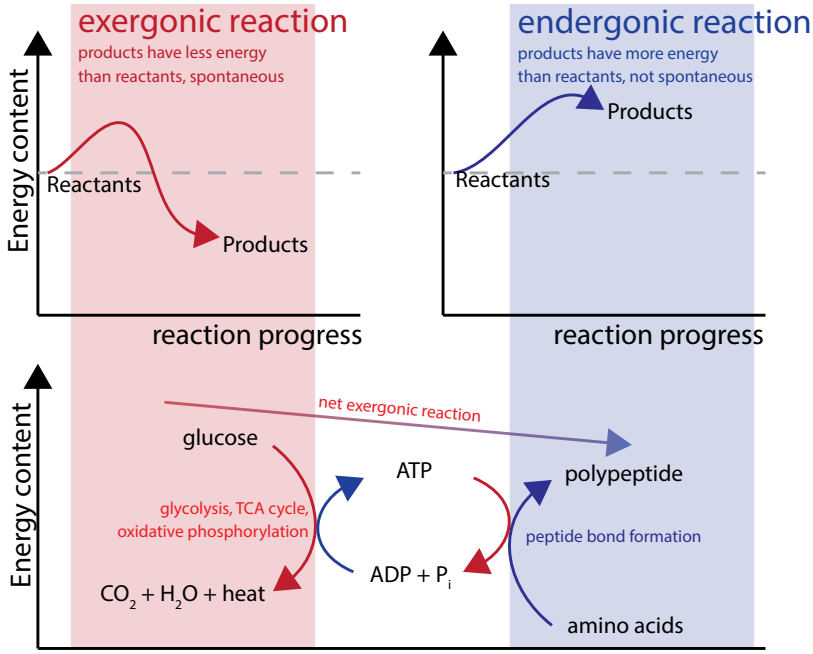

Figure 5. Coupled reactions. The laws ot thermodynamics dictate that a reaction will not proceed spontaneously unless the products of the reaction have lower energy than the reactants. A reaction where the products have higher energy than the reactions (endergonic reaction) can only proceed when there is an input of energy. Exergonic reactions like burning of glucose (glycolysis, TCA cycle, and oxidative phosphorylation) drives ATP synthesis. The ATP molecules are used to power endergonic reactions like protein synthesis.

This phenomenon is the thermodynamic basis for the operation of metabolic pathways, since most of these reaction sequences are comprised of endergonic as well as exergonic reactions. Many enzymes catalyze energetically unfavourable reactions by coupling them with energetically favourable ones.

Consider the following two-step reaction process:

$$A + B \leftrightarrow C + D; \Delta G_1 \geq 0$$
$$D + E \leftrightarrow F + G; \Delta G_2 < 0$$
$$A + B + E \leftrightarrow C + F + G; \Delta G_3 = \Delta G_1 + \Delta G_2 < 0$$

If $\Delta G_1 \geq 0$, reaction 1 will not occur spontaneously. However, if ΔG_2 is sufficiently exergonic so that $\Delta G_1 + \Delta G_2 < 0$, reaction 2 will convert *D* to the products. Reaction 1 will operate in the forward direction to replenish the equilibrium concentration of *D*.

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Thus, the highly exergonic reaction 2 drives the endergonic reaction 1. The two reactions are said to be coupled through their intermediate, *D*.

That these coupled reactions proceed spontaneously can also be seen by summing reactions 1 and 2 to yield the overall reaction 3. As long as the overall pathway is exergonic, it will operate in the forward direction.

In biological systems, endergonic reactions and exergonic reactions are usually coupled via ATP.

During the hydrolysis of ATP the phosphoryl group of ATP is transferred to a hydroxyl ion from water with a value of $\Delta G^{o\prime} = -30.5$ kJ/mol. The Gibbs energy decrease ($-\Delta G^0$) upon hydrolysis is called the **group transfer potential**.

$$ATP + H_2O \leftrightarrow ADP + P_i$$

$$\Delta G^{0'} = -30.5 \frac{kJ}{mol}$$

$$ATP + H_2O \leftrightarrow AMP + PP_i$$

$$\Delta G^{0'} = -45.6 \frac{kJ}{mol}$$

ATP

Of central importance to the energy metabolism of all cells is the **adenylate system** which consists of adenosine triphosphate (**ATP**), adenosine diphosphate (**ADP**), and adenosine monophosphate (**AMP**) together with inorganic phosphate (P_i), pyrophosphate (PP_i), and magnesium ions.

ATP occurs in all known life forms. ATP is a nucleotide consisting of an adenine, a ribose, and a triphosphate unit. Three phosphoryl groups are sequentially linked to adenosine via a phosphoester bond followed by two phosphoanhydride bonds.

Adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are similarly constituted as ATP but with only two and one phosphoryl groups, respectively. The phosphoryl groups are referred to as the α, β, and γ phosphates.

ATP is a thermodynamically unstable molecule with respect to hydrolysis to either ADP and AMP. The hydrolysis of ATP is highly exergonic and used to drive numerous endergonic biochemical processes.

An average person at rest consumes and regenerates ATP at a rate of ~ 3 mol (1.5 kg) h^{-1} and as much as an order of magnitude faster during strenuous activity.

Phosphoryl-transfer reactions are of enormous metabolic significance. Some of the most important reactions of this type involve the synthesis and hydrolysis of ATP. Note that ATP has an intermediate phosphate group-transfer potential.

For example, **phosphoenol pyruvate** can spontaneously transfer a phosphoryl group to ADP to form ATP, which can, in turn, spontaneously transfer a phosphoryl group to glucose and other compounds (Table 2).

A further property of ATP is that is has a **high kinetic stability to hydrolysis**, because phosphoanhydride bonds have unusually large free energies of activation.

ATP is reasonably stable under physiological conditions but is readily hydrolyzed in enzymatically mediated reactions.

Table 2. Group transfer potential of selected compounds.

Compound	$\Delta G^{o\prime}$ (kJ mol^{-1})
Phosphoenol pyruvate	– 61.9
ATP / ADP + P_i	– 30.5
Glucose-6-phosphate	– 13.8
Glycerol-3-phosphate	– 9.2

ATP is the universal currency of free energy in cells

The free energy of ATP hydrolysis, a

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highly exergonic process, is harnessed to drive many otherwise endergonic biological processes. ATP acts as a **free-energy donor** in most energy-requiring processes such as motion, active transport, or biosynthesis (Figure 6).

The exergonic consumption of ATP drives endergonic processes such as protein and nucleic acid biosynthesis. Therefore, ATP forms a universal free energy "currency".

The hydrolysis of ATP in a coupled reaction changes the equilibrium ratio of products to reactants by a very large factor. For example, the formation of glucose-6-phosphate, the initial step in the metabolism of glucose, is an energonic reaction and requires ~14 kJ mol^{-1}. It is driven by the exergonic hydrolysis of ATP (- 31 kJ mol^{-1}). The coupled reaction is exergonic (- 17 kJ mol^{-1}).

ATP can play its role as energy transmitter because the ATP-generating system of cells maintains the [ATP]/[ADP][P_i] ratio (phosphorylation state ratio) at a high level, typically of the order of 500 M^{-1}.

Generation of ATP

The three main pathways used to generate energy in eukaryotic organisms are

- **Glycolysis, citric acid cycle and oxidative phosphorylation**: The overall process of oxidising glucose to carbon dioxide can produce up to 36 molecules ATP from one molecule of glucose).
- **β oxidation** is the process by which fatty acids, in the form of fatty acyl-CoA molecules, are broken down in mitochondria (or glyoxysomes in plants) to generate acetyl-CoA, the entry molecule for the citric Acid cycle.
- In plants, ATP is also produced during **photosynthesis**.

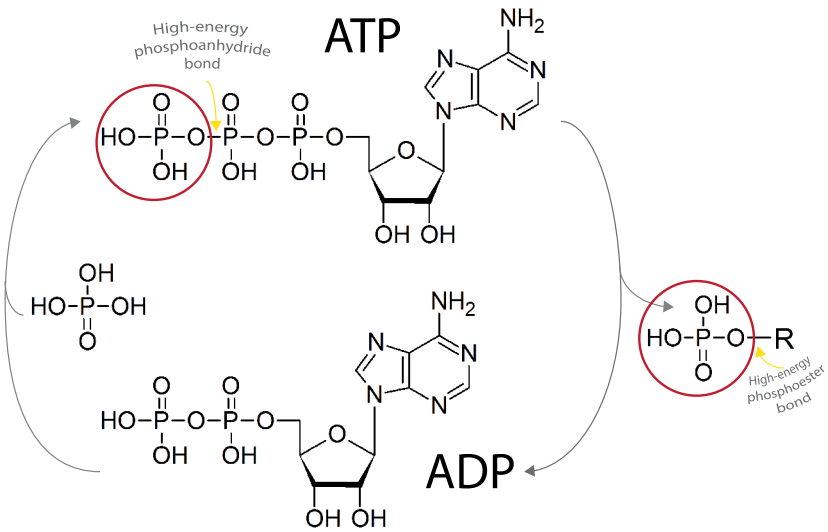

Figure 6. Phosphate transfer from ATP to a target molecule R. R includes metabolites, proteins, ribonucleosides and deoxyribonucleosides.

Further Readings

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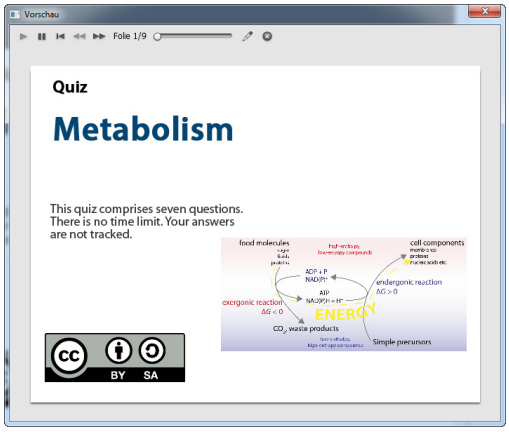
II. Metabolism

Metabolism is the overall process through which living systems acquire and utilize the free energy they need to carry out their various functions. Enzymes function to lower the activation energy of a reaction.

Quiz

Start this chapter with the quiz to test for your mastery. You may reattempt the quiz later to check your progress.



Quiz 1. Metabolism. Click to play - HTML5 or Flash (opens new window in your standard web browser).

Metabolic pathways

Organisms are not at equilibrium. They require a continuous influx of free energy to maintain order. Organisms maintain their non-equilibrium status by coupling the exergonic reactions of nutrient oxidation to the endergonic processes required to maintain the living state (such as the performance of mechanical work, the active transport of molecules against concentration gradients, and the biosynthesis of complex molecules).

Metabolism is the overall process through which living systems acquire and utilize the free energy they need to carry out their various functions.

Metabolic pathways are series of consecutive enzymatic reactions that produce specific products. These pathways are interdependent, and their activity is coordinated by sensitive means of communication in which **allosteric enzymes** are predominant.

Catabolism and anabolism

The reaction pathways that comprise metabolism are often divided into two categories: Those involved in degradation (catabolism) and those involved in biosynthesis (anabolism).

In **catabolic pathways** or degradation, complex metabolites (nutrients and cell constituents) are exergonically broken down to simpler products so as to salvage their components and/or to generate energy.

Large molecules are broken down to basic building blocks. For example, protein degradation produces amino acids, starch and glycogen degradation results in the production of glucose (Table 1).

Table 1. Examples for catabolic pathways. Large molecules such as polysaccharides, lipids, nucleic acids, and proteins are broken down into smaller units.

Catabolic pathway	Purpose
Glycolysis (oxygen available)	Degradation of glucose, generation of ATP and NADH and pyruvate which is fed into the tricarboxylic acid (TCA) cycle
Glycolysis (anaerobic conditions)	Degradation of glucose, generation of ATP and compounds such as ethanol and carbon dioxide (yeast cells) or lactic acid (muscle cells)
ß-oxidation	Degradation of fatty acids, production of acetyl-CoA which can be fed into the TCA cycle
Aerobic respirations	ATP production via pyruvate decarboxylation, TCA cycle, and oxidative phosphorylation

The free energy (ΔG) released during these processes is conserved by the synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and

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phosphate, or by the reduction of coenzyme $NADP^+$ to NADPH.

ATP and NADPH are the major free energy sources for anabolic pathways. These useful forms of energy are produced in photosynthesis or catabolism and are employed to generate complex structures from simple ones, or energy-rich states from energy-poor ones.

Those reactions that require energy – such as the synthesis of glucose, fats, protein or DNA – are **anabolic reactions**. In anabolism or biosynthesis, biomolecules are synthesized from simpler components (Table 2).

Anabolism starts with simple precursors such as glucose for the formation of complex polysaccharides, intermediates of the tricarboxylic acid (TCA) cycle for amino acid biosynthesis, or acyl groups for fatty acid synthesis.

The useful forms of energy that are produced in catabolism (ATP and NADPH) are employed in anabolism to generate complex structures from simple ones, or energy-rich states from energy-poor ones.

Thus, anabolic and catabolic processes are coupled together through the universal free energy currency, ATP (Figure 1).

Many basic metabolic pathways are nearly identical from organism to organism, suggesting that all known life forms are descended from a single ancestor.

Some pathways have both anabolic and catabolic features

For example, one major task of the **TCA-cycle** is the generation of reducing power (NADH) (Table 3). The TCA cycle oxidises acetyls and four pairs of electrons are tansferred (three to NAD^+ and one to FAD).

The NADH and $FADH_2$ is used in oxidative phosphorylation for ATP production. The carbon of the acetyls is released as CO_2. This is the CO_2 that we breathe out.

Table 2. Examples for anabolic pathways. Anabolic pathways use simple components to generate complex structures. Some anabolic pathways such as the fatty acid biosynthesis are the reverse of catabolic pathways (but many are not).

Anabolic pathway	Purpose
Amino acid and protein biosynthesis	Amino acids are synthesized in different pathways and used for protein biosynthesis in a process termed translation
Nucleotide and nucleic acid biosynthesis	Purine and pyrimidine ribonucleotides are synthesiized in series of reactions and used for nucleic acids (including tRNA, mRNA, rRNA, and DNA)
Fatty acid biosynthesis	Fatty acids for membranes and energy storage, synthesis occurs through condensation of C2 units, the reverse of the ß-oxidation process
Cholesterol biosynthesis	Cholesterol for membranes and as precursor of steroid hormones, synthesis occurs through the fusion of isoprene (C5) units

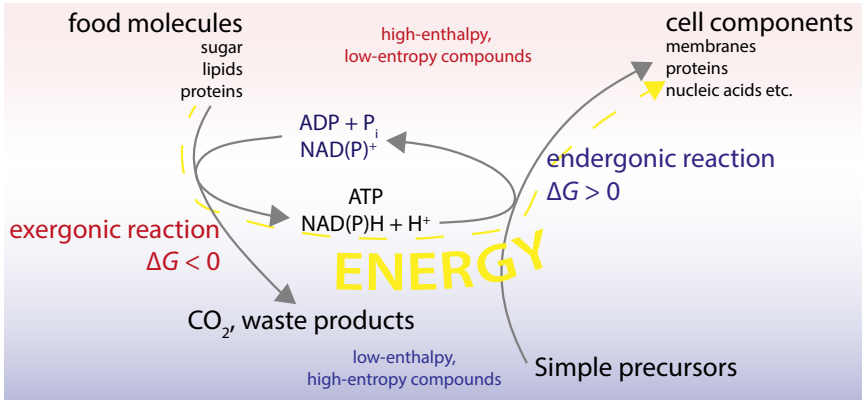

Figure 1. Catabolism, energy carriers, and anabolism. ATP and NADH serve as energy carriers that link the breakdown of food and biosynthesis of cellular compounds.

On the other hand, the TCA-cycle provides carbon precursors for amino acid biosynthesis and many additional anabolic processes.

In line with that finding, the TCA-cycle does not only operate as a cycle but has different flux modes.

For more details on the TCA cycle see:

Khanacademy (2014) Cellular respiration

Wikibooks (2014) Principles of Biochemistry/ Krebs cycle or Citric acid cycle

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Table 3. Examples for metabolic pathways with both anabolic and catabolic properties.

Metabolic pathway	Purpose
Tricarboxylic acid (TCA) cycle [or citric acid cycle or Krebs cycle]	Catabolism: Oxidation of acetyls and provision of NADH for oxidative phosphorylation and ATP production Anabolism: Provision of precursors for amino acids and many other molecules
Oxidative pentose phosphate pathway (OPPP)	Catabolism: alternative pathway to glycolysis Anabolism: Generation of NADPH for anabolic processes and pentoses for synthesis of nucleic acids

Localization in cells

Metabolic pathways are localized in specific cell compartments.

For example, glycolysis takes place in the cytoplasm, the TCA cycle in mitochondria, and fatty acid degradation in mitochondria and peroxisomes.

Gluconeogenesis, the synthesis of glucose from noncarbohydrate precursors, requires enzymes localized in the cytoplasm, mitochondria, and membrane of the endoplasmic reticulum. F

atty acid synthesis takes place in the cytoplasm of adipose tissues (fat cells) and other cells.

Cooperating enzymes of a metabolic pathway can be organized into macromolecular complexes. This allows efficient transfer of biosynthetic intermediates (substrate channelling or **metabolic channeling**).

For more details on metabolism see:

Scitable by Nature Education (2014) Metabolism is the complete set of biochemical reactions within a cell

Khanacademy and Stanford Medicine (2014) Basics of metabolism

Catalysis

A large negative value of ΔG does not ensure a chemical reaction will proceed at a measurable rate. This depends on the detailed mechanism of the reaction, which is independent of ΔG.

Only with the introduction of the proper **enzymes** will the hydrolysis of these molecules proceed at a reasonable pace. A catalyst, which by definition is unchanged by a reaction, cannot affect the ΔG of a reaction.

Consequently, an enzyme can only accelerate the attainment of thermodynamic equilibrium; it cannot promote a reaction that has a positive ΔG.

Enzymes

Biological reactions are highly specific. Only reactions that lie on metabolic pathways take place at significant rates despite the many other thermodynamically favourable reactions that are also possible.

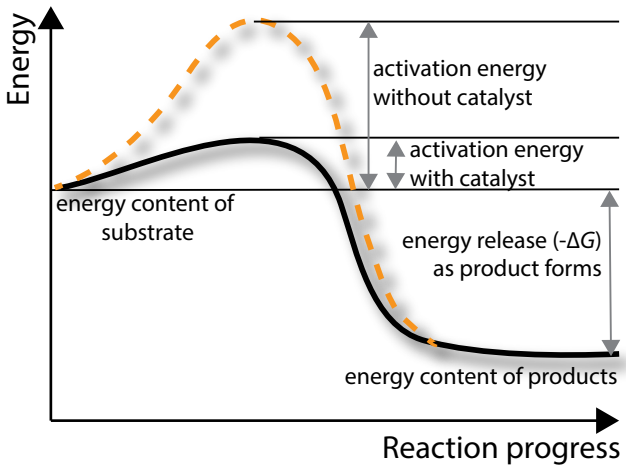

Figure 2. Energy profile of a chemical reaction.

Thermodynamics does not indicate the rates at which possible processes actually occur.

For example, although thermodynamics tell us that glucose and oxygen react with the release of energy, it does not

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indicate that this mixture is indefinitely stable at room temperature in the absence of the appropriate enzymes.

Here the enzymes are extremely important. Enzymes are **chemical catalysts** with particular properties:

1. **Higher reaction rates**: The rates of enzymatically catalyzed reactions are typically factors of 10^6 to 10^{12} greater than those of the corresponding uncatalyzed reactions.
2. **Milder reaction conditions**: Enzymatically catalyzed reactions occur under mild conditions (temperatures below 100°C, atmospheric pressure, and nearly neutral pH).
3. **Greater reaction specificity**: Enzymes have a vastly greater degree of specificity with respect to the identities of both their substrates (reactants) and their products than do chemical catalysts. Enzymatic reactions rarely have side products.
4. **Capacity for regulation**: The catalytic activities of many enzymes vary in response to the concentrations of compounds other than their substrates. The mechanisms of these regulatory processes include allosteric control, covalent modification of enzymes, and variation of the amounts of enzymes synthesized.

However, enzymes cannot alter the laws of thermodynamics and consequently cannot alter the equilibrium of a chemical reaction. This inability means that an enzyme accelerates the forward and reverse reactions by precisely the same factor.

Enzymes accelerate the attainment of equilibria but do not shift their positions. The equilibrium position is a function only of the free energy difference (ΔG) between reactants and products.

Activation energy

The free energy difference between reactants and products accounts for the equilibrium of the reaction, but enzymes accelerate how quickly this equilibrium is attained. How can we explain the rate enhancement in terms of thermodynamics?

A chemical reaction goes through a **transition state** that has a higher free energy than does either the substrate and the product. Even energetically favourable reactions require activation energy to get them started (Figure 2).

The transition state is the most seldom state because it is the one with the highest free energy. The difference in free energy between the transition state and the substrate is called the activation energy.

The energy of activation does not enter into the final ΔG calculation for the reaction, because the energy input required to reach the transition state is returned when the transition state forms the product.

Enzymes function to lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state (Figure 3).

Substrates are bound to enzymes by multiple weak interactions such as electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions. Free energy is released by the formation of a large number of weak interactions between an enzyme and its substrate.

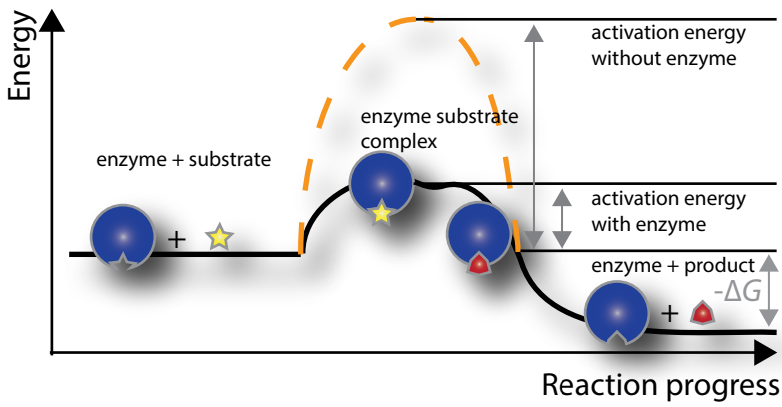

Figure 3. Enzymes lower the activation energy.

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The full complement of such interactions is formed only when the substrate is in the transition state. The energy released by the interactions lowers the activation energy.

Pathway throughput is controlled by enzymes

The transition state is unstable. The forward and backward reactions will be accelerated by the same factor by an enzyme. ΔG of the reaction determines formation of either the substrate or the product.

A subset of biological reactions is far from equilibrium. This situation arises from a buildup of reactants because there is insufficient enzyme activity to equilibrate the reaction.

Changes in substrate concentrations therefore have relatively little effect on the rate of the reaction; the enzyme is essentially saturated. Only changes in the activity of the enzyme, through allosteric interactions, for example, can significantly alter this rate.

An enzyme can therefore be analogous to a dam on a river. It controls substrate flux (rate of flow) by varying its activity (allosterically or by other means), much as a dam controls the flow of a river by varying the opening of its flood gates.

The **rate of an enzyme reaction** (V) increases until a maximum value (V_{max}) is reached (Figure 4). At this point all substrate-binding sites are occupied. Turnover numbers between less than 1 and more than 500,000 substrate molecules per second have been measured.

The **Michaelis constant**, K_M, is the concentration of substrate at which half the active sites are filled. K_M provides a measure of the substrate concentration for significant catalysis to take place.

A low value of K_M indicates that a substrate binds tightly to the enzyme, a large value corresponds to weak binding. For many enzymes, K_M provides an approximation of substrate concentration in a cell.

K_M values between less than 1 μM and more than 10 mM substrate concentration have been measured.

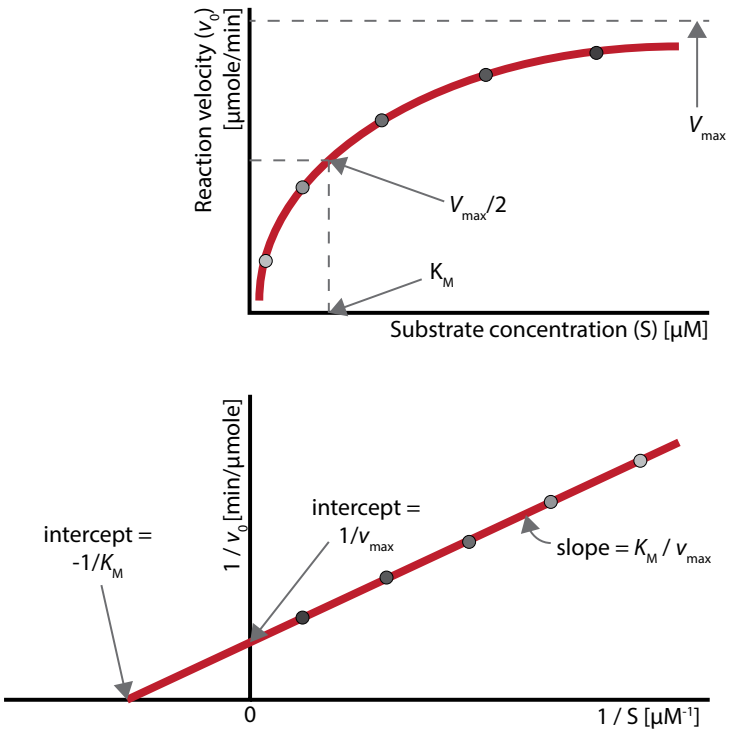

Figure 4. Michaelis-Menten kinetics. TOP: The maximal reaction velocity (v_{max}) is approached asymptotically. The Michaelis Menten constant (K_M) is the substrate concentration at which velocity is $v_{max}/2$. BOTTOM: The Lineweaver-Burk plot is a double-reciprocal plot of the Michaelis-Menten equation. Plotting the data in this way allows v_{max} and K_M to be calculated.

Control of enzyme activity

Substrates, products, inhibitors, toxins, and other metabolites and small molecules increase or decrease enzyme activity.

Allosteric control

Allosteric regulation is the regulation of an enzyme or protein by binding an effector molecule at the protein's allosteric site (that is, a site other than the protein's active site, see below). Effectors that en-

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hance the protein's activity are referred to as **allosteric activators**, whereas those that decrease the protein's activity are called **allosteric inhibitors**.

Enzyme activity is influenced by a large number of factors. Environmental conditions, such as **pH**, **temperature**, or **salt concentration** may change the three dimensional shape of an enzyme, altering its rate of activity and/or its ability to bind the substrate.

The catalytic activities of many enzymes vary in response to the concentrations of compounds other than their substrates. Any substance that is bound by a protein (e.g., an ion, a metabolite, another protein, or a nucleic acid) is referred to as a **ligand** for that protein (Latin *ligare*, to bind).

Proteins bind to ligands by weak chemical interactions (hydrogen bonds, electrostatic attractions, van der Waals attractions, and hydrophobic effect). The region of a protein that associates with a ligand is known as **binding site**.

The binding site of allosteric regulators (regulatory small compounds) is different from the active site that binds the substrate. Both sites "communicate" via **conformational changes**.

Binding of a ligand (e.g. a metabolite) stabilizes a specific conformation which is associated with a specific level of activity. Thus, enzymatic activity may be regulated by allosteric alteration of substrate-binding affinity (Figure 5).

Covalent modifications

The activity of a protein (i.e. its conformation) can also be controlled by covalent modifications. A very common reversible covalent modification is **phosphorylation**.

The activity of many proteins is altered by the covalent attachment of a phosphoryl group (Figure 6). ATP serves as the phosphoryl donor. The phosphorylation is catalysed by protein kinases, the removal of phosphoryl groups by hydrolysis is catalysed by protein phosphatases.

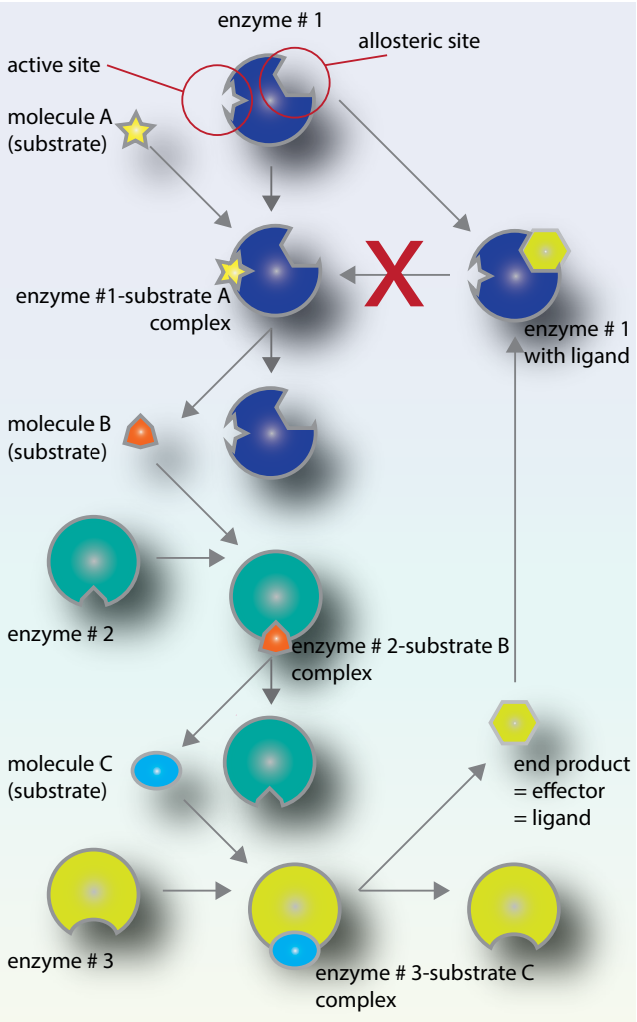

Figure 5. Feedback inhibition by an allosteric effector regulates the flow through metabolic pathways. Binding of an allosteric inhibitor at one site of enzyme # 1 causes the protein to shift to a conformation in which the active site has a lower substrate-binding affinity. The end product of a biosynthetic pathway often acts as as an inhibitor of the enzyme that catalyzes the first step.

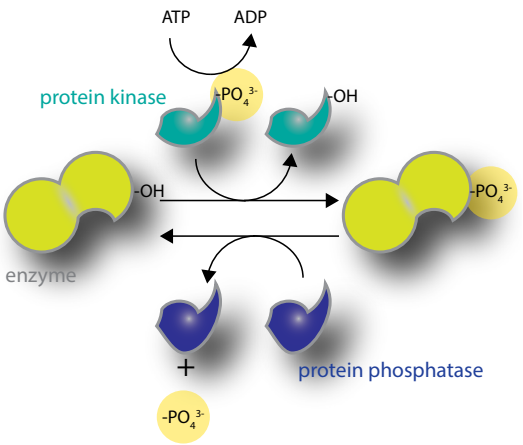

Figure 6. Regulation of enzyme activity by a protein kinase and protein phosphatase.

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Isoenzymes (isozymes)

Multiple forms of enzymes (isozymes) allow varying regulation of the same reaction at distinct locations or times. Isozymes catalyse the same reaction but differ in enzymatic and regulatory properties.

Isozymes allow fine-tuning of metabolism to meet the requirements of a tissue or developmental stage.

Proteolytic activation

Proenzymes are inactive precursor and are not catalytically active. Proenzymes (or **zymogens**) are irreversibly activated by hydrolysis of one or a few peptide bonds.

Proenzymes are often labelled with the suffix 'ogen', e.g. pepsinogen and pepsin (pepsin degrades food proteins into peptides in the stomach) (Table 5). Cleavage of peptide bond(s) activates the zymogen by conformational changes.

Table 5. Zymogens or proenzymes, examples.

Zymogen	Function
Pepsinogen	Inactive precursor of pepsin, a digestive enzyme in the stomach.
Trypsinogen	Inactive precuror of trypsin, a digestive enzyme in the pancreas.
Fibrinogen	Inactive presursor of fibrin that is involved in blood-clotting.

Cofactors

Cofactors may be metal ions or organic molecules. Some cofactors are required for the catalytic activity of the enzyme and the enzyme cannot perform alone.

The term **holoenzyme** refers to the catalytically active enzyme that consists of both the **apoenzyme** (enzyme without cofactor) and the cofactor (Figure 7).

Prosthetic groups refer to tightly bound coenzymes. For example, the heme prosthetic group of hemoglobin is tightly bound through extensive hydrophobic and hydrogen bonding.

Coenzymes are small organic molecules but not made of amino acids. Table 6 gives examples. They are loosely bound, released in the same way as substrates and products, and chemically changed by the enzymatic reactions in which they participate.

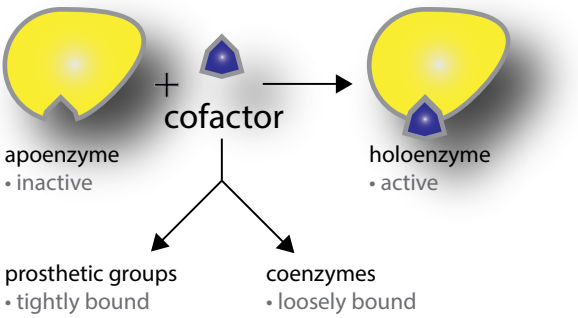

Figure 7. Cofactors comprise prosthetic groups and coenzymes.

Table 6. Common coenzymes.

Coenzyme	Reaction mediated
Adenosine trisphosphate (ATP), see page 9	Energy transport
Biotin	Carboxylation, e.g. in synthesis of fatty acids
Coenzyme B12 (cobalamine)	Alkylation, e.g. in amino acid metabolism
Coenzyme A	Acyl transfer, e.g. entry in TCA cycle
Coenzyme Q (ubiquinone)	Electron transport
Flavin coenzymes (FAD), see page 18	Oxidation - reduction
Nicotinamide coenzymes (NAD^+ and $NADP^+$), see page 17	Oxidation - reduction
Pyridoxal phosphate	Amino group transfer (transamination reactions) and other reactions
Tetrahydrofolate	One-carbon group transfer, e.g. in amino acid metabolism
Thiamine pyrophosphate	Oxidative decarboxylation, e.g. of pyruvate to form acetyl-coenzyme A

NAD^+ and $NADP^+$

Nicotinamide adenine dinucleotide (shortly NAD^+) is an important coenzyme. NADH is a major electron (or hydrogen) carrier in the oxidation of fuel molecules. Cells use NAD^+ to accept or donate electrons in redox reactions. During this

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reactions the reductant is oxidised and NAD^+ reduced to NADH (Figure 8).

The phosphorylated form of NAD^+/NADH is $NADP^+$/NADPH. NADH and NADPH are metabolically not interchangeable. They differ only by a phosphate group.

NADH is used to synthesize ATP in oxidative phosphorylation. NADPH is used for reductive biosynthesis. Thus, the two related coenzymes have similar chemistry, but perform different roles in metabolism.

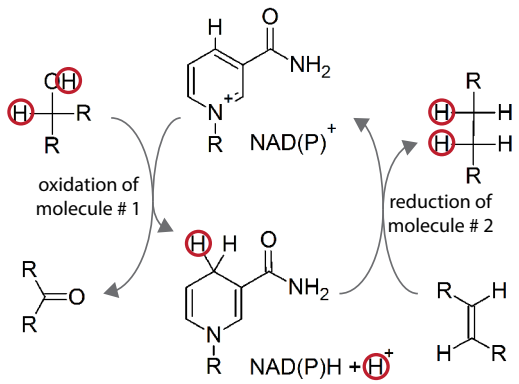

Figure 8. NAD(P)H is an important carrier of electrons. In the oxidation of a substrate, the nicotinamide ring of $NAD(P)^+$ accepts a hydrogen ion and two electrons. The reduced form is called NAD(P)H.

FAD

The other major electron (hydrogen) carrier is **flavin adenine dinucleotide** (FAD). FAD, like NAD^+, can accept two electrons. FAD, unlike NAD^+, takes up two protons.

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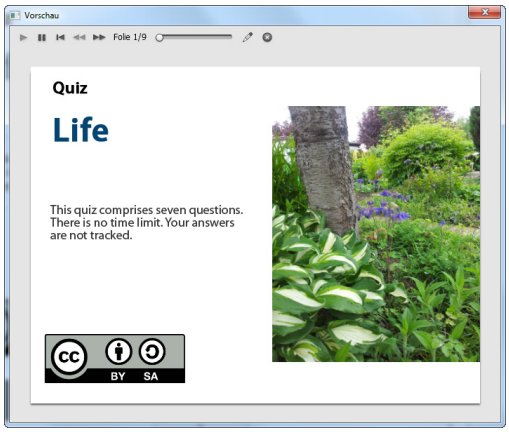
III. What is life?

Quiz

Start this chapter with the quiz to test for your mastery. You may reattempt the quiz later to check your progress.



Quiz 1. Metabolism. Click to play - HTML5 or Flash (opens new window in your standard web browser).

'Alive'

Scientists have spent considerable time pondering this problem. According to Koshland (2002), something could be considered '**alive**' if it meets the following conditions:

(1) Living things must have a program to make copies of themselves from generation to generation. In living systems, the program of life is encoded in DNA.

(2) Life adapts and evolves in step with external changes in the environment through mutation and natural selection.

(3) Organisms have compartmentalized structures – cells and their various organelles.

(4) Living things have the ability to take energy from their environment and change it from one form to another.

(5) Organisms have regeneration systems that replace parts of themselves that are subject to wear and tear.

(6) Living creatures respond to environmental stimuli.

(7) Organisms are able to maintain numerous metabolic reactions even in a single instance in time.

Cells and Organisms

Life is based on morphological units known as cells. A cellular membrane and other parts are common to all cells, from organisms as different as bacteria and human beings. Cells with different functions often have different shapes.

There are two major classifications of cells:

- The **eukaryotes**, which have a membrane-enclosed nucleus encapsulating their DNA (deoxyribonucleic acid), and
- the **prokaryotes**, which lack this organelle.

The eukaryote-prokaryote concept is fundamentally cytological, and only secondarily phylogenetic.

Molecular characterizations reveal that the evolutionary differences among eubacteria, archaebacteria, and eukaryotes are of a more profound nature than those that distinguish traditional kingdoms, such as animals and plants, from one another (Woese et al., 1990).

Viruses

Viruses are not made of cells. Viruses are essentially strands of DNA or RNA protected by a protein shell. They cannot reproduce on their own. Instead, viruses require cells of living beings to reproduce.

Thus, some scientists define viruses as **complex organic molecules**.

Living systems

Living organisms are **open thermody-**

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namic systems. An organism is an open system, because there is an exchange of energy and an exchange of matter. There is a permanent interaction of an organism with its surroundings.

Because living organisms are open systems they can never be at equilibrium. They continuously **ingest high-enthalpy, low-entropy nutrients**, which they convert to low-enthalpy, **high-entropy waste products**.

The free energy resulting from this process is used to do work and to produce the high degree of organization characteristic of life. If this process is interrupted, the organism ultimately reaches equilibrium, which for living things is synonymous with death.

Living things must maintain a **nonequilibrium state** for several reasons.

- Only a nonequilibrium process can *perform work*.
- The complex regulatory functions characteristic of life require a nonequilibrium state because a process at equilibrium cannot be directed.
- The complex cellular and molecular systems that conduct biological processes can be maintained only in the nonequilibrium state.

Living systems are inherently unstable. Their regeneration requires the continuous influx of free energy. Living things are characterized by being in a **steady state**. By this it is meant that all flows in the system are constant so that the system does not change with time.

Phototrophs and chemotrophs

The multitude of interactions between organisms and the environment defines ecosystems. The dynamics of an ecosystem is mainly driven by two processes:

- The circular flow of nutrients and
- the flux of energy from the sun to plants.

All the other organisms such as animals and fungi require the carbohydrates and organic compounds produced by plants. Thus, the steady state of the biosphere is maintained by the sun.

Phototrophs (plants, some protists, and certain bacteria) acquire free energy from the sun through photosynthesis, a process in which light energy powers the endergonic reaction of CO_2 and H_2O to form carbohydrates and O_2.

Photosynthetic organisms use the energy of sunlight to convert simple energy-poor molecules into more complex energy-rich molecules that serve as fuels. Photosynthetic organisms transform light energy into chemical energy for the vast majority of organisms, human beings included.

Chemotrophs obtain their free energy by oxidizing organic compounds (carbohydrates, lipids, proteins) obtained from other organisms, ultimately phototrophs.

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