1-Introduction

Bacterial metabolism refers to the complete set of biochemical and physiological reactions that occur within bacterial cells. It can be divided into two main pathways:

- One pathway involves the breakdown of substrates to release energy.
- The other pathway involves the synthesis of new molecules required for building bacterial structures, a process that consumes energy generated by degradative reactions.

In general, metabolism is defined as the collection of chemical reactions that involve the molecules present in living cells. Its role is to meet two essential needs:

1. The synthesis of molecules necessary for vital cellular functions (matter conservation).
2. The production of energy required to carry out these vital processes (energy conservation).

Thus, metabolism encompasses all chemical transformations—both biosynthetic and degradative—that support the formation of bacterial components and ensure their proper functioning.

- Every chemical reaction in bacterial metabolism is catalyzed by specific enzymes.
- Bacterial metabolic traits serve as key biochemical markers that are essential for bacterial identification and taxonomy.

The study of metabolism provides several important applications:

- **Isolation:** defined as inoculation carried out for the purpose of separating microorganisms to obtain distinct colonies. This step is necessary to obtain pure cultures, which are indispensable for bacterial study and identification.
- **Determining nutritional requirements and metabolic pathways:** understanding how bacteria degrade substrates or synthesize end products or intermediates allows their conventional identification.

Bacterial metabolism can therefore be divided into two complementary processes:

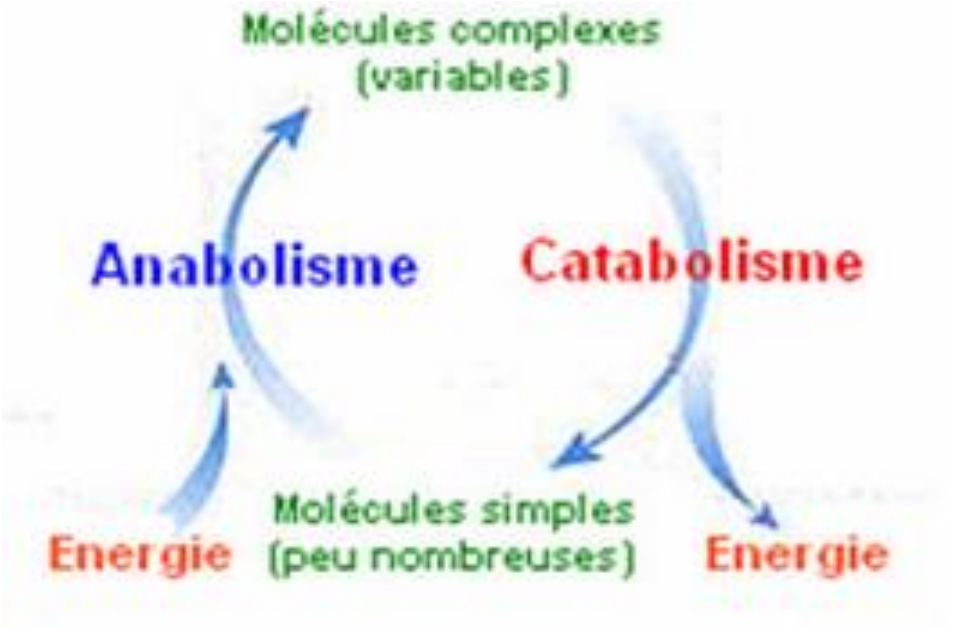
1. **Anabolism:** the set of reactions that synthesize complex molecules from simpler ones, requiring an input of energy.

2. **Catabolism:** the set of reactions that release energy by breaking down complex molecules into simpler forms.

In summary:

Metabolism = Catabolism + Anabolism

It is essential to view metabolism as a whole: the energy released through catabolism is utilized to drive anabolic processes.



Chapter I: Energy metabolism of microorganisms

1-Energy sources and trophic types

Microorganisms exhibit extraordinary metabolic diversity, especially in how they obtain energy and carbon. This diversity forms the basis for classifying them into different trophic types. The combination of energy source (light or chemical compounds) and carbon source (CO_2 or organic molecules) allows us to distinguish several major groups (Preisser, 2007).

1-1- Phototrophs

These microorganisms capture light energy and convert it into chemical energy.
They often use pigments such as chlorophylls or bacteriochlorophylls.

Phototrophs can be divided into:

- Photoautotrophs → use light as an energy source and CO_2 as the sole carbon source (e.g., cyanobacteria).
- Photoheterotrophs → use light for energy but require organic compounds as carbon sources (e.g., purple non-sulfur bacteria) (Hülsen et al., 2022).

1-2- Chemotrophs

Instead of light, they rely on the oxidation of chemical compounds to generate energy.

Two main subgroups exist:

- Chemoorganotrophs → obtain energy by oxidizing organic compounds such as carbohydrates, lipids, or proteins (most bacteria and fungi).
- Chemolithotrophs → obtain energy from inorganic compounds, such as hydrogen, ammonia, nitrite, ferrous iron, or reduced sulfur compounds. These organisms play crucial roles in biogeochemical cycles like nitrogen and sulfur cycling (Pierson, 2001).

In addition to the energy source, microorganisms are also classified by their carbon source.

1-3- Autotrophs → use CO_2 as their principal carbon source; many are either photoautotrophs (light + CO_2) or chemoautotrophs (inorganic compounds + CO_2).

1-4- Heterotrophs → require organic carbon (sugars, lipids, amino acids) from the environment; they include many bacteria and almost all fungi (Clarke, 2013).

1-5- Mixotrophs

Some microorganisms show metabolic flexibility, meaning they can switch between different modes depending on environmental conditions.

For example, certain purple bacteria can grow as photoautotrophs in the light but shift to chemoorganotrophic growth in the dark.

The classification of microorganisms according to energy and carbon sources highlights their remarkable adaptability. This metabolic diversity not only ensures survival in varied environments, from sunlit waters to deep-sea vents, but also makes microorganisms essential players in ecological processes and industrial applications (Moroz et al., 2019).

Table 1. The combination of different trophic types

Energy source	**Electron (reducing) source**	**Carbon source**	**Trophic type**
Light / Photo-	Organic compounds	Organic	**Photoorganoheterotroph**
Light / Photo-	Organic compounds	CO_2 (inorganic)	**Photoorganoautotroph**
Light / Photo-	Inorganic (e.g. mineral)	Organic	**Photolithoheterotroph**
Light / Photo-	Inorganic	CO_2	**Photolithoautotroph**
Chemical / Chemo-	Organic	Organic	**Chemoorganoheterotroph**
Chemical / Chemo-	Organic	CO_2	**Chemoorganoautotroph**
Chemical / Chemo-	Inorganic	Organic	**Chemolithoheterotroph**
Chemical / Chemo-	Inorganic	CO_2	**Chemolithoautotroph**

2-Final electron acceptor and types of respiration

Respiration in microorganisms is the process by which electrons are transferred from a donor compound to a terminal acceptor, allowing energy to be conserved in the form of ATP. The identity of the final electron acceptor is what distinguishes the different types of respiration.

2-1-Aerobic respiration

In this pathway, oxygen (O_2) serves as the terminal electron acceptor. Substrates are completely oxidized, and oxygen is reduced to water. This is the most efficient respiratory process in terms of ATP production and is widely used by many bacteria in oxygen-rich environments (Csuros, 1999).

2-2-Anaerobic respiration

In the absence of oxygen, microorganisms may use other inorganic molecules as electron acceptors. Nitrate (NO_3^-), sulfate (SO_4^{2-}), carbon dioxide (CO_2), or ferric iron (Fe^{3+}) are common examples. These acceptors are reduced to products such as nitrite, hydrogen sulfide, methane, or ferrous iron, depending on the organism. Although less efficient than aerobic respiration, this process supports growth in oxygen-limited or oxygen-free environments (Csuros, 1999).

2-3-Fermentation

Unlike respiration, fermentation does not involve an electron transport chain. Instead, an organic compound acts as the final electron acceptor. For example, pyruvate may be reduced to lactate or ethanol. Fermentation yields little energy compared to respiration, but it allows survival when no external acceptor is available.

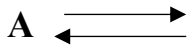
The type of respiration reflects the adaptability of microorganisms to their surroundings. Aerobic respiration is the most energy-yielding, anaerobic respiration enables survival in environments without oxygen, and fermentation provides a backup strategy under strict anaerobic conditions (Csuros, 1999).

This section will be further developed in the upcoming courses.

3-Enzymes and chemical reactions

A chemical reaction is a process in which a reactant (A) undergoes a transformation that results in the formation of a product (B).

In reactions that require the presence of an enzyme, the reactant (A) acted upon by the enzyme is referred to as the substrate. Through this interaction, the substrate is converted into a product (B).



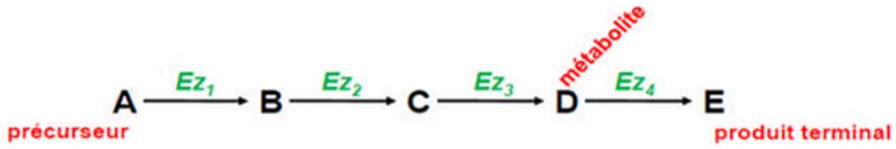
Reactant **Product**

In biology, most reactions occur in the presence of enzymes. These molecules, which are generally proteins, act as biological catalysts that significantly increase the speed of chemical reactions in a highly specific manner. Enzymes display both substrate specificity, each enzyme

acts only on a particular class of substrates, and reaction specificity, each enzyme catalyzes only one type of chemical transformation (Yoo et al., 2017).

Note: The name of an enzyme usually reflects the type of reaction it catalyzes, followed by the suffix -ase (for example: transferase, hydroxylase).

In cellular metabolism, chemical reactions do not occur in isolation but are organized into sequences of interconnected steps. Such an ordered series of reactions is referred to as a metabolic pathway (Romalde et al., 2019).



There are different types of metabolic pathways:

- **Catabolic pathways**, which generate energy by breaking down complex molecules.
- **Anabolic pathways**, which consume energy to build complex molecules.
- **Amphibolic pathways**, which can function in both directions, serving roles in both energy production and biosynthesis.

Each intermediate in a metabolic pathway (such as B, C, or D) is referred to as a **metabolite**. A pathway typically begins with a **precursor molecule** (A) and ends with a **final product** (E) (Romalde et al., 2019).

The activity and concentration of enzymes within a pathway are carefully regulated according to cellular needs, often by the presence of substrates or metabolic intermediates. By activating or inhibiting key enzymes, the cell can accelerate or slow down an entire pathway.

- All enzymes are proteins.
- Enzymatic proteins act as catalysts: even at very low concentrations, they increase the rate of chemical reactions without altering the final outcome. Importantly, the enzyme itself remains unchanged at the end of the reaction.
- Each enzyme is specific to a given reaction, meaning it always catalyzes the same transformation on the same class of substrates.
- Enzymatic proteins are synthesized by living organisms, and this synthesis is genetically determined. Their presence in the genome has been preserved through evolution because

the reactions they catalyze are essential for the survival and functioning of the organism (Svendsen, 2003).

Notes

Substrate:

- A molecule that enters a reaction to be transformed through the catalytic action of an enzyme.
- Any molecule that undergoes permanent modification during an enzymatic reaction is referred to as a substrate.
- Often, an enzyme does not act on a single molecule but rather on a whole class of structurally related substrates (Robinson, 2015).

Product:

- A molecule that appears during a reaction catalyzed by an enzyme.
- The new molecule that results from this transformation is called the product.

Cofactor:

A chemical component that is essential for an enzymatic reaction to occur. It may function to:

- transport or complete a substrate,
- accept a product,
- or contribute to the structural integrity of the enzyme.

Cofactors can be simple ions, such as zinc in carbonic anhydrase, or small inorganic molecules commonly found in biological systems, such as water. Some cofactors are more complex organic molecules synthesized by the cell; these are referred to as coenzymes (Robinson, 2015).

Coenzyme:

- A biological molecule that functions as a necessary cofactor in enzymatic catalysis.
- Coenzymes participate directly in the chemical transformation, often serving as carriers of electrons, atoms, or functional groups between substrates.
- Coenzymes are biological molecules, meaning that their natural synthesis can only be carried out by living cells. When the genetic information of a species does not encode the capacity to synthesize a given coenzyme, the whole molecule, or part of it, must be supplied through external sources, usually the diet. Such essential dietary components are known as vitamins. Coenzymes are therefore cofactors, and their presence is indispensable for enzymatic catalysis.

- When coenzymes are bound to enzymes through weak interactions, such as electrostatic forces, the binding is renewed during every catalytic cycle. The energy involved in the enzyme–coenzyme interaction is of the same magnitude as that of the enzyme–substrate interaction. In this case, the concentration of coenzymes in the medium must be comparable to that of the substrate; this condition is described as stoichiometric. These coenzymes are referred to as free coenzymes because they dissociate from the enzyme after each catalyzed reaction.

Note: An electrostatic bond is a type of chemical interaction based on the attraction between opposite charges, in contrast to a covalent bond.

- In contrast, when coenzymes are attached to enzymes by strong covalent bonds, their concentration is directly tied to that of the enzyme itself, and thus remains very low. This condition is described as catalytic. These are known as bound coenzymes, because they remain permanently associated with the enzyme and do not dissociate during the reaction (Robinson, 2015).

3-1-Enzyme families

The official enzyme nomenclature is divided into six main classes; each further subdivided into subclasses. The classification code specifies:

1. The type of reaction (class)
2. The type of substrate function being metabolized (subclass)
3. The type of acceptor involved
4. The serial number within the subclass.

a. Oxidoreductases

- These enzymes catalyze redox reactions, which are chemical processes involving the transfer of electrons.
- The species that gains electrons is called the oxidant, while the species that loses electrons is called the reductant.
- Oxidoreductases usually act in association with redox coenzymes such as NAD^+ or FAD.
- General reaction scheme:

$$A^- + B \rightarrow A + B^-$$

Here, *A* is the **reductant** (electron donor) and *B* is the **oxidant** (electron acceptor)

b. Transferases

- Transferases are enzymes that catalyze the transfer of a functional group (for example, a phosphate, methyl, or ethyl group) from one molecule, called the **donor**, to another, called the **acceptor**.
- General reaction scheme:

$$A–X + B \rightarrow A + B–X$$

In this case, *A* is the donor and *B* is the acceptor (Guiraud, 1999).

- Kinase = Phosphotransférases avec transfert d'un ATP vers une autre molécule

D - hexose 6 - phosphotransférase = hexokinase

ATP + D-hexose ----------> ADP + D hexose 6 – phosphate

D - Fructose 6 - phosphate 1 - phosphotransférase = phospho - fructokinase

ATP + D - Fructose 6 - phosphate ----------> ADP + D - Fructose 1,6 - bis phosphate

c. Hydrolases

Hydrolases are enzymes that catalyze the hydrolysis of molecules, in which a chemical bond is broken through the addition of water. Their general reaction can be written as:

$$\textbf{R-R' + } H_2O \rightleftharpoons \textbf{R-OH + R'-H}$$

glucanohydrolase = cellulase

Glucose(n) --------> Glucose(n-1) + D-glucose

Example:

- **Glucanohydrolases (cellulases):**

$$\textbf{Glucose(n)} \rightarrow \textbf{Glucose(n}-1\textbf{)+D-glucose}$$

Subclasses of hydrolases include:

- **Esterases**, which hydrolyze ester bonds (R–CO–O–R')

- **Peptidases**, which hydrolyze peptide bonds (AA_1–CO–NH–AA_2)
- **Glycosidases**, which hydrolyze glycosidic bonds in oligo- or polysaccharides ($sugar_1$–O–$sugar_2$)
- **Phosphatases**, which hydrolyze phosphate-containing compounds, e.g.: (exemple : ATP + H2O ⇌ ADP + P) (Robinson, 2015).

d. Lyases

- Lyases are enzymes that catalyze the breaking of chemical bonds without hydrolysis or oxidation, often resulting in the formation of a double bond or a new ring structure. They can also catalyze the reverse reaction, adding groups to double bonds (Robinson, 2015).
- *Example:* The enzyme adenylate cyclase catalyzes the conversion of ATP into cyclic AMP (cAMP) (Guiraud, 1999).

ATP → AMPc + PPi

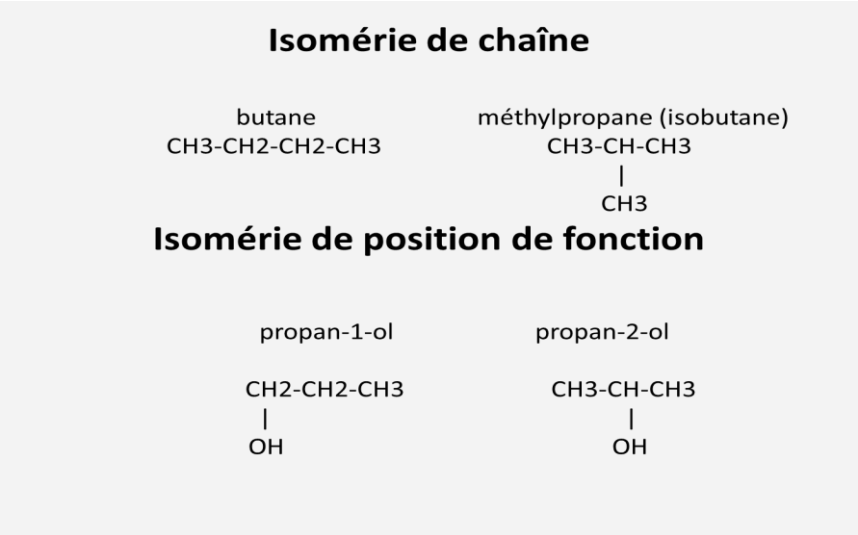
e. Isomerases

Isomerases catalyze intramolecular rearrangements, converting a molecule into one of its isomers. This often involves the shifting of functional groups within the molecule.

General reaction:

A → B, where *B* is an isomer of *A*.

Note: **Isomerism** refers to the situation in which two molecules have the same molecular formula but different structural or stereochemical arrangements. These isomers can display distinct physical, chemical, and biological properties (Robinson, 2015).



f. Ligases

Ligases are enzymes that catalyze the formation of a covalent bond between two molecules, a process that is generally coupled with the hydrolysis of a high-energy bond (such as ATP). This coupling provides the necessary energy for bond formation (Robinson, 2015).

Example: Pyruvate carboxylase, which catalyzes the following reaction:

ATP + Pyr + CO_2 + H_2O -------> ADP + Pi + oxaloacétate

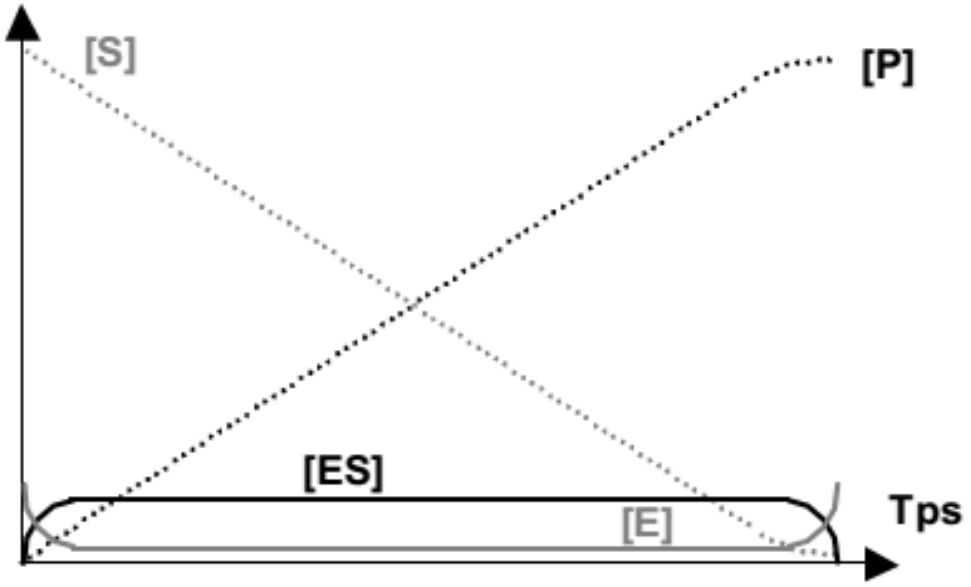
3-2-Kinetics of substrate degradation

The kinetics of substrate degradation refers to the study of how quickly a substrate is consumed during an enzymatic reaction and how this rate depends on factors such as substrate concentration, enzyme availability, and environmental conditions. In enzymatic catalysis, the relationship between the reaction rate and substrate concentration is typically described by the Michaelis–Menten model (Stein, 2011).

- At low substrate concentrations, the reaction rate increases almost linearly with the amount of substrate available, since most enzyme active sites are free to bind.
- As the substrate concentration rises, more active sites become occupied, and the rate of reaction begins to level off.
- At saturation, when nearly all enzyme active sites are occupied, the reaction reaches its maximum velocity, known as Vmax. At this point, increasing substrate concentration further does not significantly affect the rate.

A key parameter in this model is the Michaelis constant (Km), which represents the substrate concentration at which the reaction rate is half of Vmax. It provides an indication of the enzyme's affinity for its substrate: a low Km reflects a high affinity, while a high Km indicates weaker binding (Stein, 2011).

This kinetic behavior is fundamental for understanding how enzymes regulate metabolic fluxes and how cells adapt their metabolic activity to varying environmental conditions.



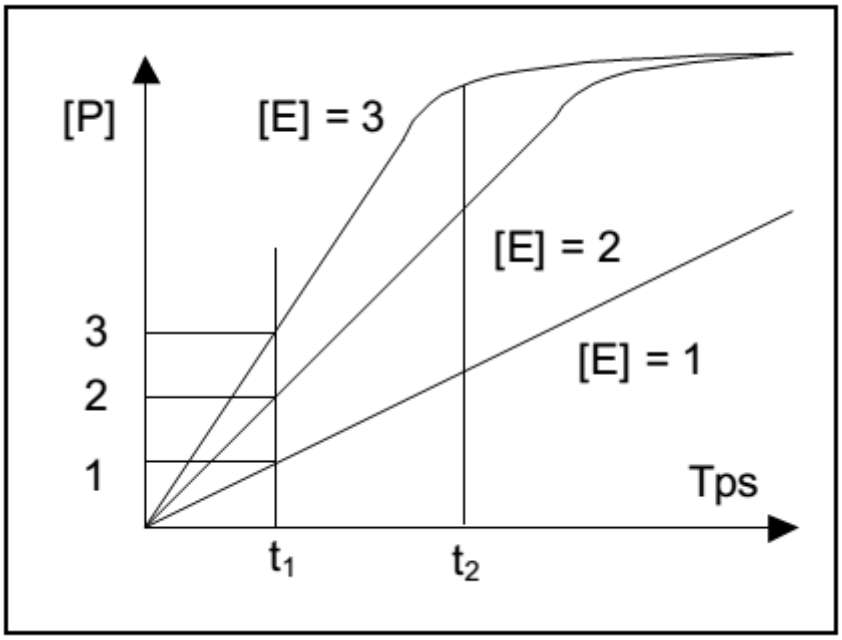
3-3-Influence of concentration

The graph illustrates the effect of enzyme concentration [E] on the formation of the reaction product [P] over time (Tps). Three curves are shown, corresponding to different enzyme concentrations: [E]=1[E], [E]=2[E], and [E]=3[E].

- At the beginning of the reaction, the rate of product formation is directly proportional to the enzyme concentration. This is evident at time t1, where higher enzyme concentrations produce a greater quantity of product.
- As time progresses, the slope of the curves decreases, reflecting the gradual depletion of substrate. Eventually, the reaction reaches a plateau, where no additional product is formed because the substrate has been consumed or the system has reached equilibrium.
- At time t2, it is clear that reactions with higher enzyme concentrations reach the maximum product level faster than those with lower enzyme concentrations.

- However, the final amount of product formed is ultimately limited by the initial substrate concentration, not by the enzyme concentration. Increasing [E] accelerates the reaction but does not increase the maximum yield.

The figure demonstrates that enzyme concentration affects the rate of reaction but not the total amount of product that can be generated (Abedi et al., 2011).



3-4-Enzymatic inhibition

Any molecule that alters the rate of an enzymatic reaction is referred to as an effector:

- Effectors that increase enzymatic activity are called activators.
- Those that decrease enzymatic activity are called inhibitors.
- Some molecules can act as either activators or inhibitors depending on the conditions.

An enzyme inhibitor is a ligand that is not transformed by the enzyme but modifies its behavior.

Various types of inhibition have been described (Rosa and Quesada, 2018).

A ligand is a molecule that binds reversibly to a target macromolecule, such as a protein or nucleic acid, usually with a functional role, for example in structural stabilization, catalysis, modulation of enzymatic activity, or signal transmission.

Studying the effects of inhibitors is important because it allows us to:

- refine our understanding of the catalytic mechanism of an enzymatic reaction,
- gain deeper insight into the specificity of an enzyme,
- and obtain physical and chemical information about the active site.

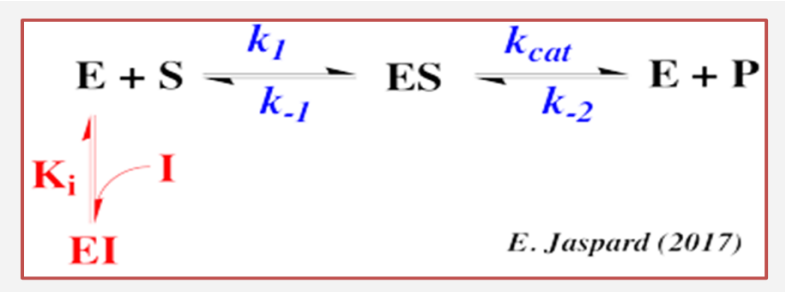
In this context, we will consider three main types of enzyme inhibition: competitive inhibition, non-competitive inhibition, and uncompetitive inhibition (Rosa and Quesada, 2018).

a. Competitive inhibition

This is a mechanism in which the binding of the inhibitor prevents the binding of the substrate. In other words, the enzyme cannot bind both the substrate (ES complex) and the inhibitor (EI complex) at the same time; the two binding events are therefore mutually exclusive (exclusive binding) (Rosa and Quesada, 2018).

Reaction scheme:

- The substrate (S) competes with the inhibitor (I) for the same active site of the enzyme (E).
- When the substrate binds, the enzyme-substrate complex (ES) is formed, which can then be converted into product (P).
- When the inhibitor binds, the enzyme-inhibitor complex (EI) is formed, preventing the substrate from binding and thus blocking product formation.

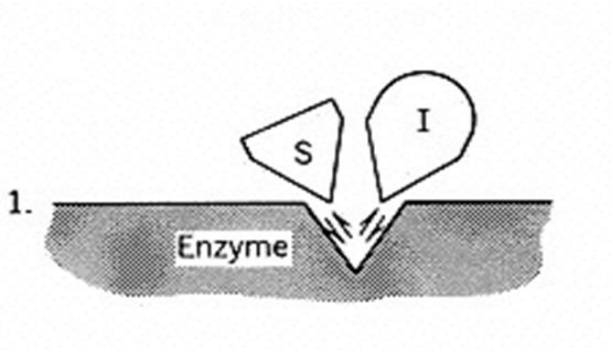


✓ **The substrate and the inhibitor share the same binding site** (this is the most classical model):

Model 1. They compete directly because they have a structural similarity.

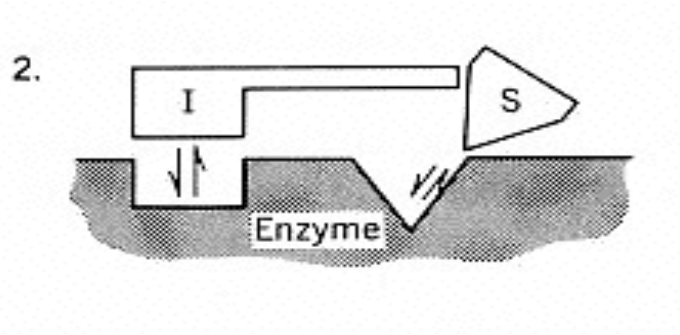
In this model, the inhibitor mimics the structure of the substrate and binds to the same active site on the enzyme. As a result, only one of them—either the substrate (S) or the inhibitor (I),

can occupy the site at a given time. This structural analogy explains the competitive relationship between the substrate and the inhibitor (Strelow et al., 2012).

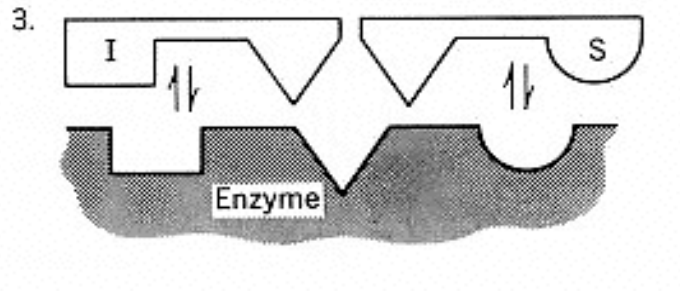


✓ **The binding sites of the substrate and the inhibitor are distinct:**

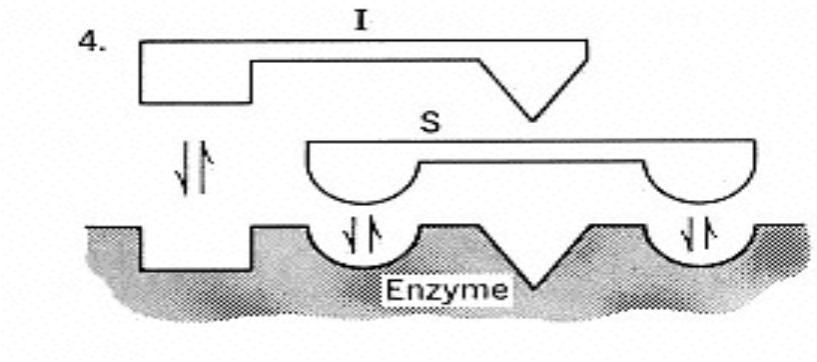
- **Model 2:** The inhibitor binds to a different site on the enzyme, but its presence creates steric hindrance. This spatial obstruction prevents the substrate from accessing its binding site, thereby blocking the reaction (Strelow et al., 2012).



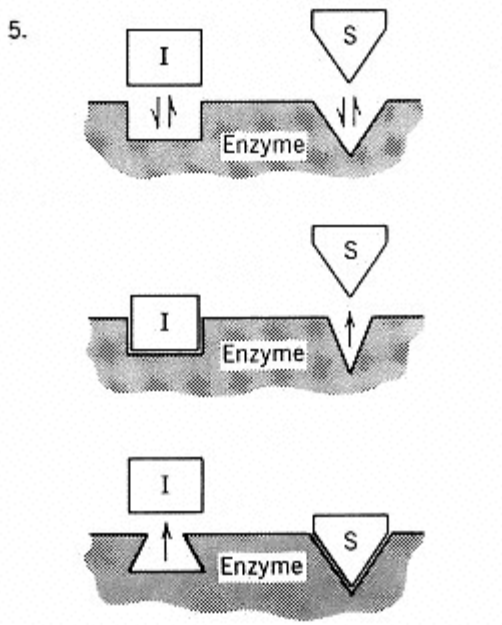
- **Model 3:** Both the substrate and the inhibitor share a common chemical group that interacts with the enzyme. This group binds to a third binding site on the enzyme, resulting in competition between the substrate and the inhibitor, even though their main binding sites are different (Strelow et al., 2012).



Model 4: The binding sites of the substrate and the inhibitor partially overlap. In this situation, both molecules cannot bind simultaneously, as the inhibitor occupies or interferes with part of the substrate's binding region (Strelow et al., 2012).



Model 5: The binding of the inhibitor induces a conformational change in the enzyme, which either distorts or hides the substrate's active site. Conversely, substrate binding can also alter the conformation and reduce inhibitor access. This type of regulation is frequently observed in multimeric enzymes with allosteric regulation (Strelow et al., 2012).

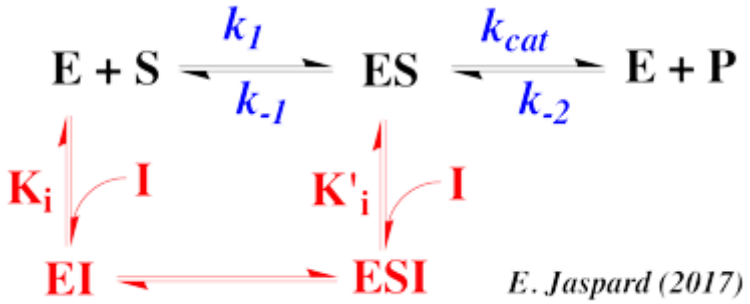


b. Non-competitive inhibition

A classical non-competitive inhibitor does not interfere with the binding of the substrate, and vice versa. This is because the binding sites of the substrate and the inhibitor are distinct.

As a result, the inhibitor can bind either to the free enzyme (E) or to the enzyme–substrate complex (ES). Similarly, the substrate can attach both to the free enzyme (E) and to the enzyme–inhibitor complex (EI).

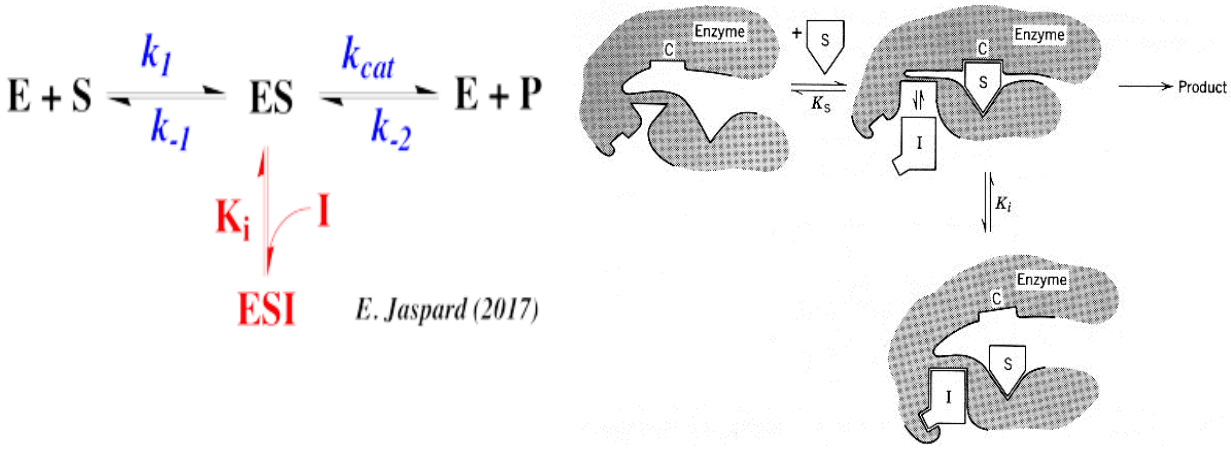
Unlike competitive inhibitors, non-competitive inhibitors do not share structural similarity with the substrate. Instead, they reduce the enzyme's catalytic efficiency regardless of whether the substrate is bound or not (Strelow et al., 2012).



c. Uncompetitive inhibition

This type of inhibition is often referred to as inhibition through trapping of the intermediate complex. The name reflects the mechanism more accurately: the enzyme must first bind to the substrate to form the enzyme–substrate complex (ES), and only then can the inhibitor attach.

In other words, the inhibitor does not bind to the free enzyme but specifically targets the ES complex, preventing it from proceeding to product formation. This results in a reduction of both the maximum reaction rate (Vmax) and the apparent affinity constant (Km) in enzyme kinetics (Whiteley, 2000).

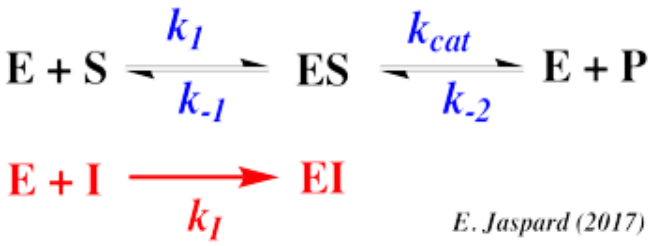


d. Inactivation: irreversible inhibition

An inhibitor is said to act irreversibly when it establishes a covalent bond with the enzyme. In such cases, the enzyme is permanently modified and can no longer carry out its catalytic function.

This type of molecule is called an inactivator, as it essentially destroys the enzymatic activity rather than simply regulating it. Unlike reversible inhibitors, which can dissociate from the

enzyme, irreversible inhibitors permanently block the enzyme and often require new enzyme synthesis to restore activity (Whiteley, 2000).



e. Substrate inhibition

This type of inhibition occurs when the substrate itself becomes inhibitory at very high concentrations. In such situations, instead of increasing the reaction rate, an excess of substrate can actually reduce enzymatic activity.

This phenomenon generally happens when the active site of the enzyme is relatively large and contains multiple subsites, each capable of binding to a part of the substrate molecule. When substrate concentration becomes excessive, additional molecules may bind to inappropriate subsites, disrupting the normal catalytic process and thereby lowering enzyme efficiency (Strelow et al., 2012).