Introduction

Thousands of chemical reactions occur each instant throughout the body; this coordinated process of chemical change is termed metabolism. Metabolism is a collective term which includes the synthesis (anabolism) and break-down (catabolism) of organic molecules required for cell structure and function and the release of chemical energy used for cell functions. A special class of proteins known as **enzymes**, *a.k.a* biocatalysts, mediates these metabolic reactions. Similar to chemical catalysts, enzymes accelerate the rate at which reactant molecules (here called '**substrates**') are converted to different molecules called **products**. Most of the enzymes are protein molecules, although some RNA molecules called **ribozymes** possess catalytic activity. The number of reactions catalyzed by RNA molecules is very small. Therefore, we shall restrict the term "enzyme" to 'protein catalysts'.

An enzyme comes into contact with the substrates in enzyme-mediated reactions. The substrate becomes bound to the enzyme, forming an enzyme-substrate complex, which breaks down to release products and the enzyme. Thus, at the end of the reaction, the enzyme is free to undergo the same reaction with additional substrate molecules. The overall effect is to accelerate the conversion of substrate into product, with the enzyme acting as a catalyst.

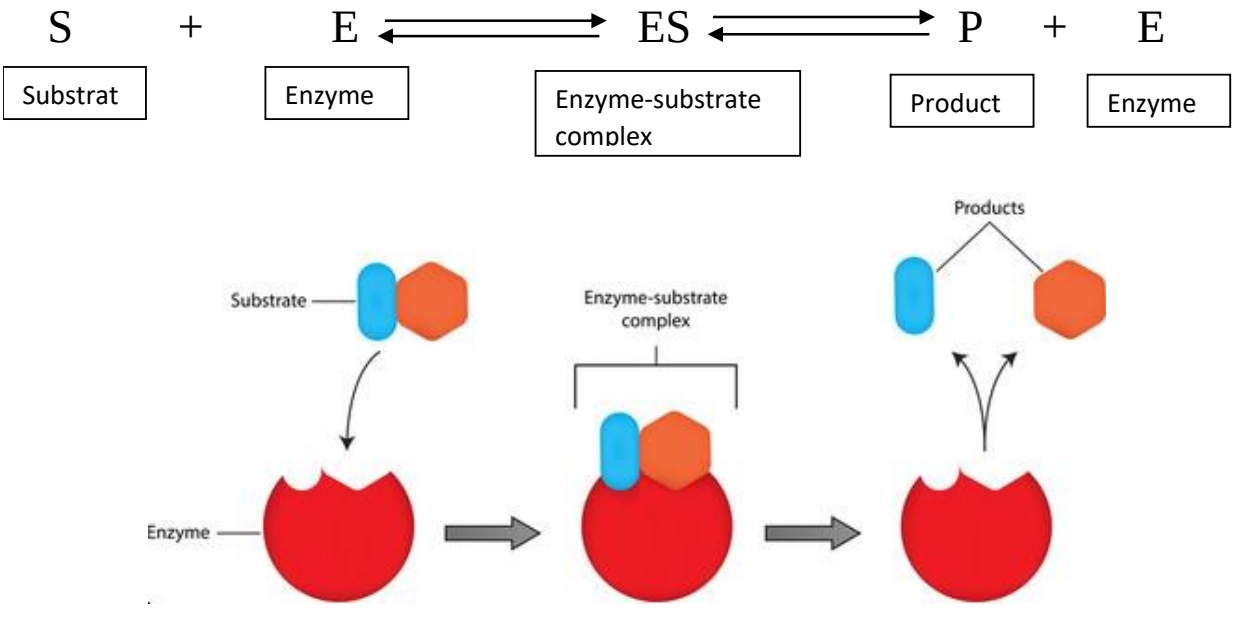

Figure 1. Top: equation of the enzyme catalyzed reaction. The substrate binds to the enzyme to form the enzyme-substrate complex, which then breaks to release the product and the enzyme. Bottom: cartoon illustration of an enzyme catalyzed reaction.

Enzyme active site

The region of the enzyme to which the substrate binds is known as the enzyme's binding site. Only a small portion (around 2–4 amino acids), normally flanked by the binding site is directly involved in catalysis. This small portion is called the catalytic site. The catalytic site and binding site together compose the enzyme's active site. The remaining majority of the enzyme structure serves to maintain the precise orientation and dynamics of the active site. The shape of the enzyme in the region of the active site provides the basis for the enzyme's chemical specificity since the shape of the active site is complementary to the substrate's shape. The amino acids that interact with a substrate at a binding site need not be adjacent to each other along the polypeptide chain since the folding of the protein may bring various segments of the molecule into juxtaposition

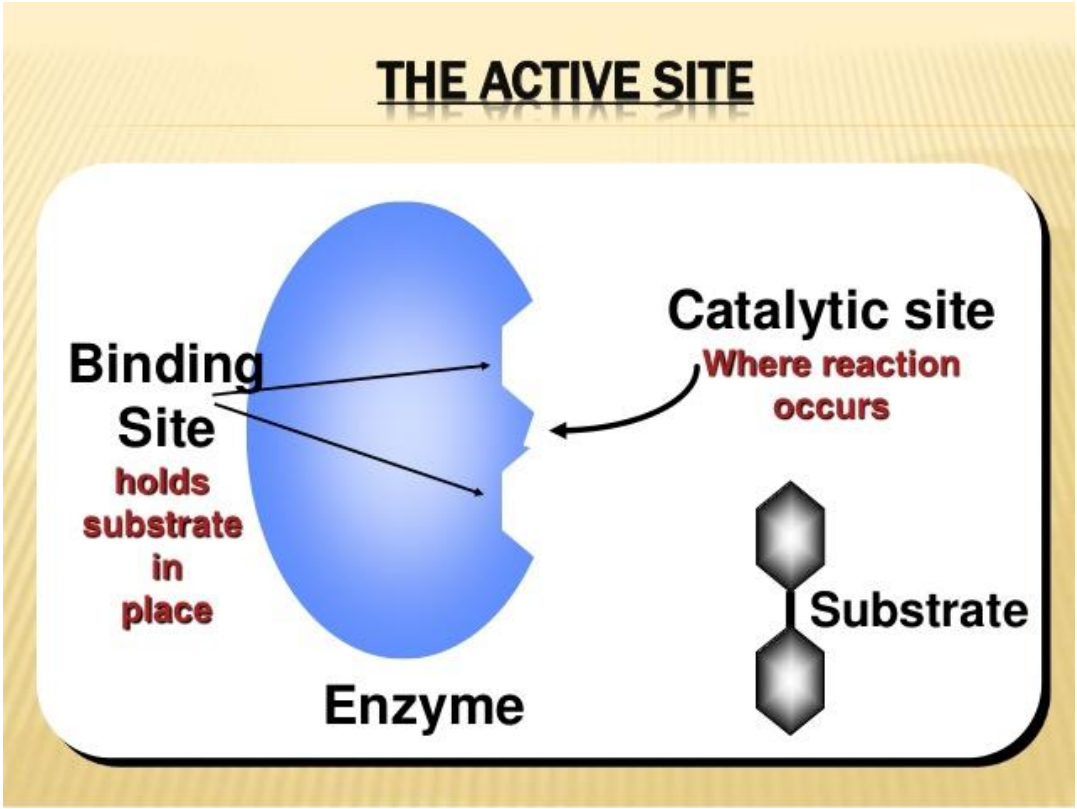

Figure 2. The active site of an enzyme comprises the binding site and the catalytic site. The bulk of the enzyme renders the 3D structure of the enzyme.

Enzymes can either catalyse reactions that build complex molecules from simple units, or breakdown complex substances into simple units. Reactions that build up new, complex molecules from simpler units are known as anabolic reaction whereas reactions those that breakdown complex molecules into simpler units are known as catabolic reaction

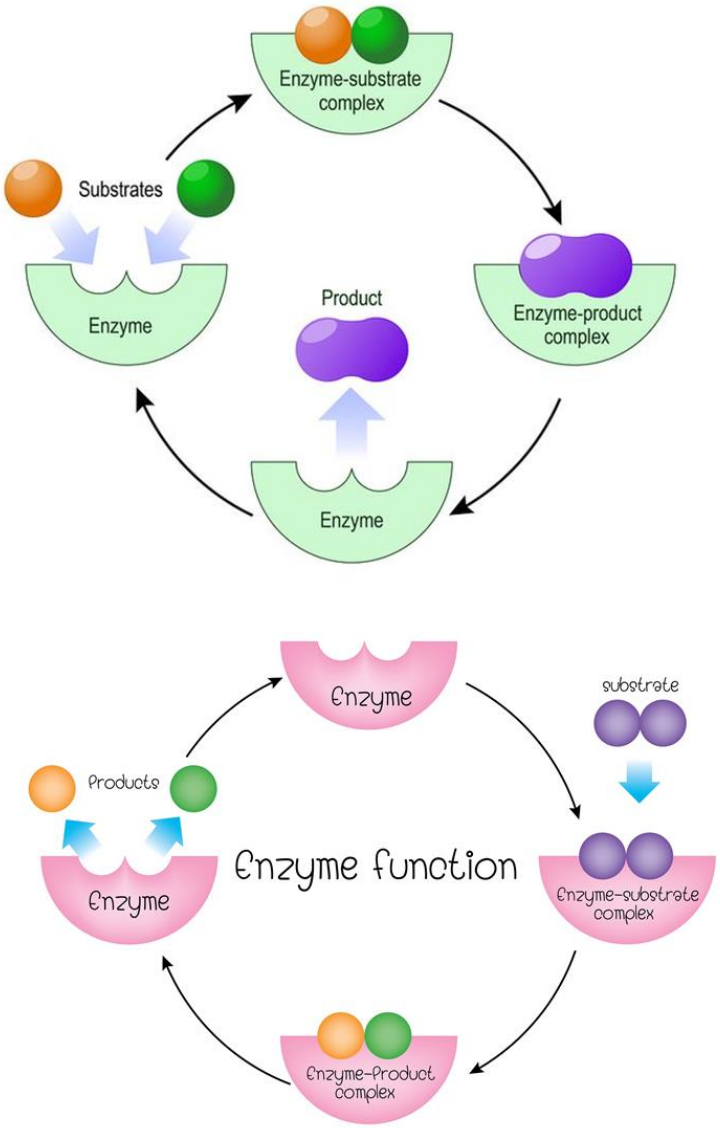

Figure 3. Cartoon illustration of enzyme catalysed anabolic reaction (TOP) and enzyme catalysed catabolic reaction (BOTTOM).

Enzyme-substrate specificity

Proteins with different amino acid sequences have different shapes and therefore differently shaped binding sites, each with its own chemical specificity. The binding between a substrate and an enzyme may be so specific that a binding site can bind only one type of substrate and no other. Such selectivity allows the enzyme to "identify" (by binding) one particular molecule in a solution containing hundreds of different molecules. However, though some binding sites have a chemical specificity that allows them to bind only one type of substrate, others are less specific and thus are able to bind a number of related ligands. Two models to explain the enzyme-substrate specificity exist:

1. The lock and key model
2. The induced fit model.

1. The lock and key model

Proposed by Emil Fischer in 1894, the lock and key model states that the active site and the substrate have specific complementary shapes that fit exactly into one another, much like a key fits into a lock key hole. Only one [type of] very specifically shaped key can open the lock. Other keys may loosely fit, but cannot open the lock. Similarly, only one specific substrate can fit into the active site of the enzyme. Other molecules, despite being similar to the specific substrate cannot exactly fit, and therefore their reactions cannot be catalysed.

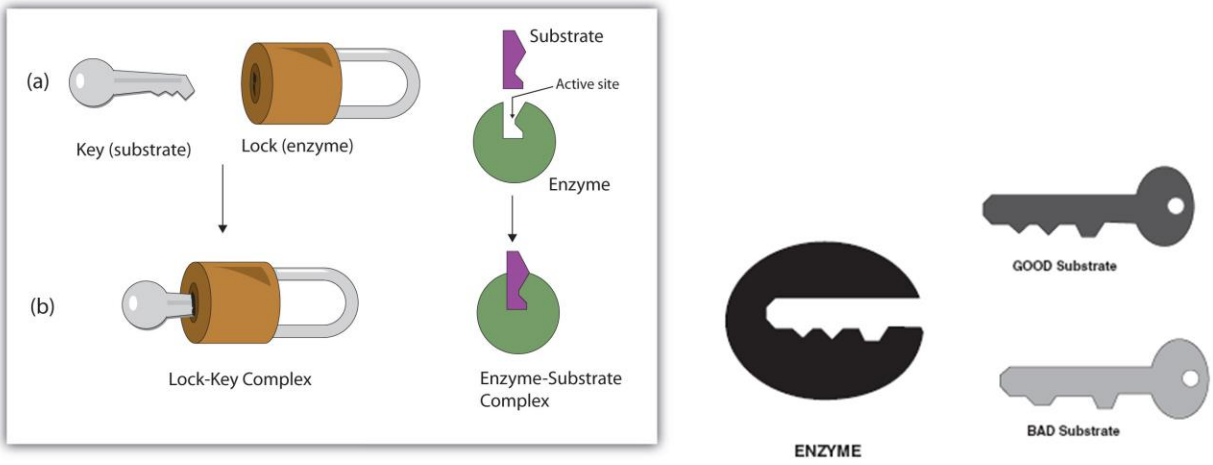

Figure 4. Illustration of the lock and key model of enzyme specificity. Only one specific substrate can fit into the active site of the enzyme, much like only a specific key can fit into and open a lock. Other molecules, despite being similar to the specific substrate cannot exactly fit, and therefore their reactions cannot be catalysed

2. The induced fit model.

In 1958, Daniel Koshland suggested a modification to the lock and key model. Evidence from X-ray crystallography suggests that the active sites of enzymes is not simply a rigid shape: the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function.

The induced fit model is generally accepted as the best current model of enzyme action. The active site still has a distinctive shape, but it is a flexible one. Once the products have left the complex, the enzyme returns to its inactive form until another substrate molecule binds. Induced fit may enhance the fidelity of molecular recognition in the presence of competition via the conformational proofreading mechanism.

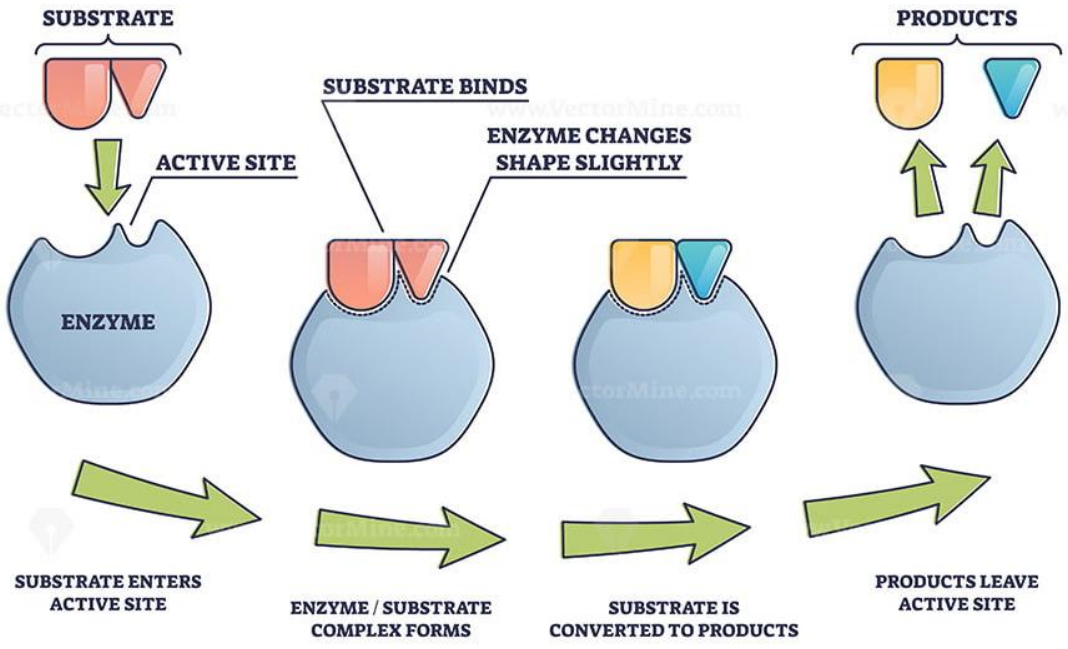

Figure 5. Binding of the substrate modifies the shape of the active site to fit and form the active complex.

Activation energy in enzyme catalysis

In order for a chemical reaction to occur, reactant molecules must acquire enough energy—the **activation energy**—to enter an activated state in which chemical bonds can be broken and formed. To illustrate this, we simply say that the reaction must go over an 'energy hill' called the activation energy before it gets started. It is much like rolling a stone over a hill, as illustrated in the figure below: you need some initial input of energy to get the stone to the top of the hill. Beyond that, the stone will row spontaneously.

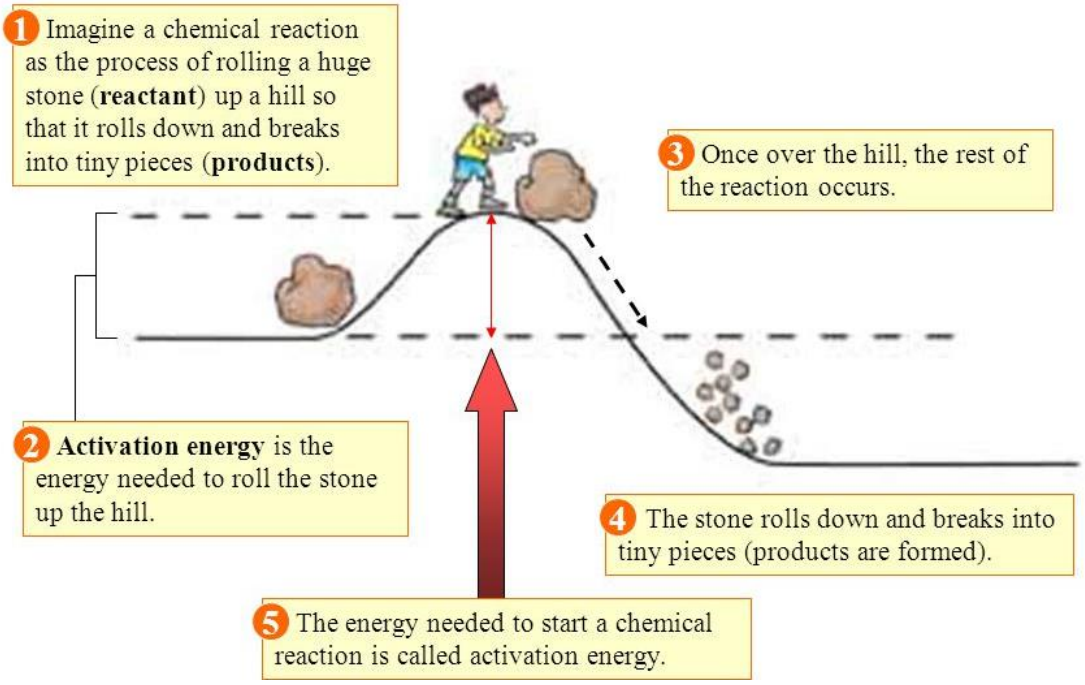

Figure 6. A simplified illustration of activation energy required to start a reaction.

For a reaction to occur, reacting molecules have to collide. Activation energy is obtained when reactants collide with other molecules. Further, the molecules must be in the right orientation and there must be sufficient amount of energy. If the activation energy required for a reaction is large, then the probability of a given reactant molecule acquiring this amount of energy will be small, and the reaction rate will be slow. The higher the activation energy, the slower the rate of a chemical reaction.

The enzyme speeds up the reaction by lowering the activation energy needed for the reaction to start. By providing a surface for the substrate, an enzyme lowers the activation energy of the

reaction. The active site affects the bonds in the substrates, making it easier for them to break. The reacting substances are brought very close together making it easier for new bonds to form between them. Once the reaction is complete, the complex breaks, releasing the products and the enzyme for further catalytic activity. Thus an enzyme lowers the activation energy of a reaction but does not alter the net amount of energy that is added to or released by the reactants in the course of the reaction.

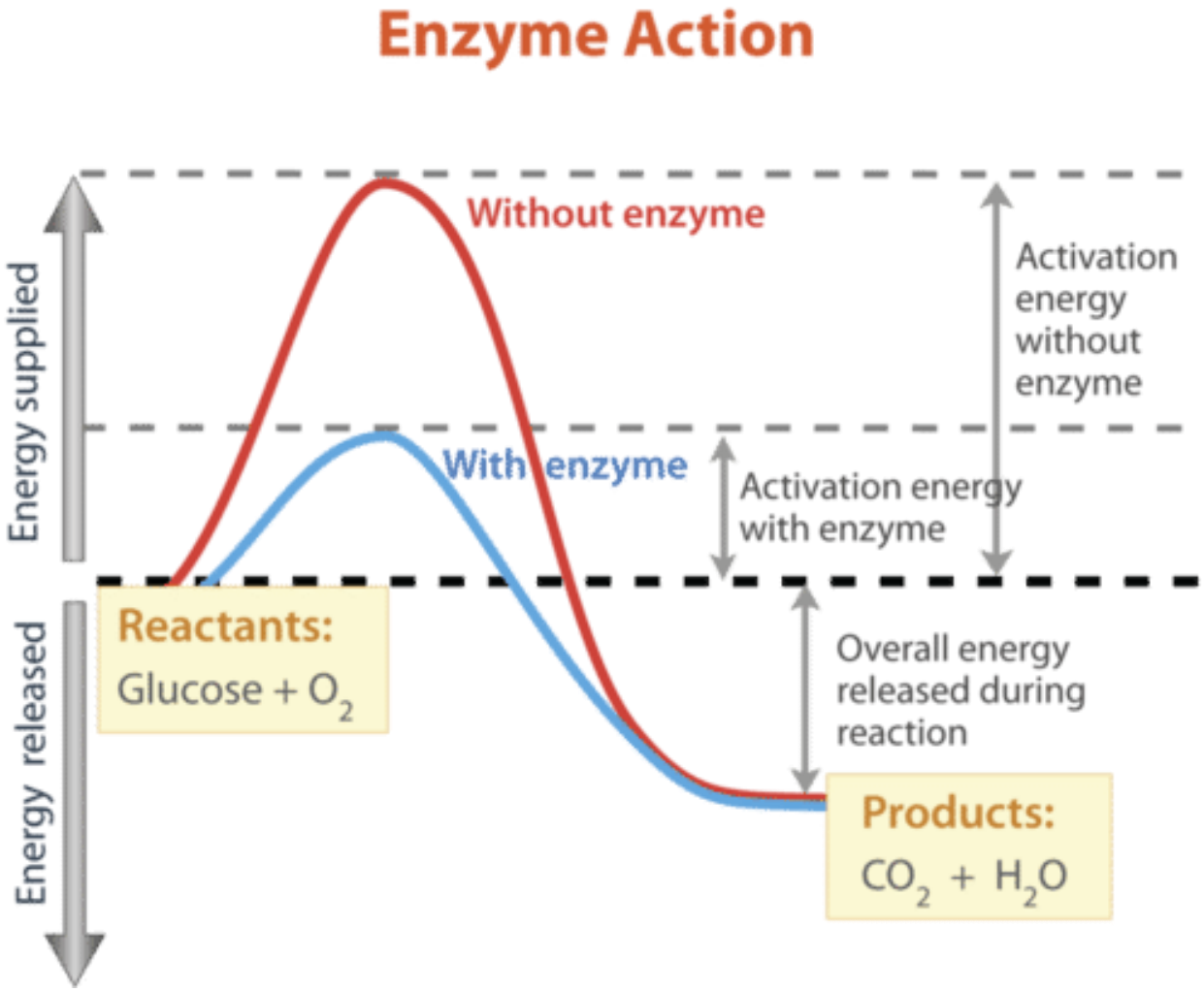

Figure 7. Enzymes speeds up the reaction by lowering the activation energy needed for the reaction to start.

To be clear, enzymes do not alter the position of the chemical equilibrium of the reaction, but only the speed at which it is reached. Also note that enzymes increase the rate of reactions that are otherwise possible. That to say that if a reaction is naturally not possible, an enzyme does not make it possible.

Co-factors and co-enzymes

Many enzymes are inactive in the absence of small amounts of non-protein components bound to them. Such components are generally known as cofactors. If the non-protein components is tightly/permanently bound to the enzyme, it is called a prosthetic group. The binding of a cofactor to an enzyme alters the enzyme's conformation so that it can interact with the substrate. Enzymes that require a cofactor but do not have one bound are called apoenzymes. An enzyme together with the cofactor(s) required for activity is called a holoenzyme.

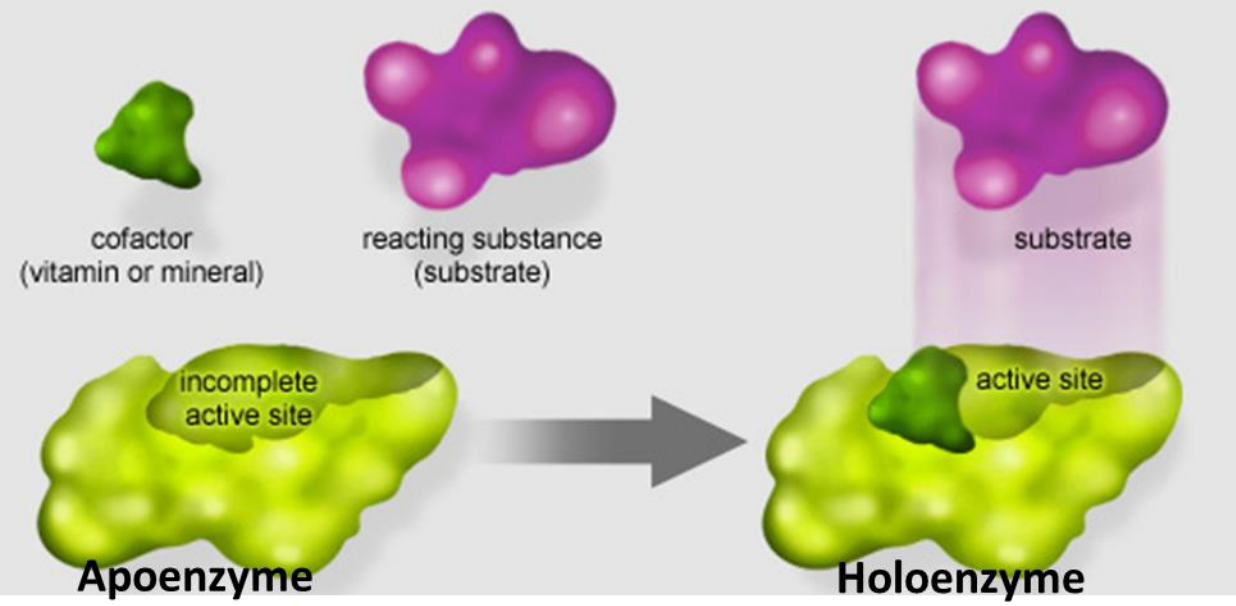

Figure 8. Illustration of the activation of apoenzyme by the binding of a cofactor. Only after activation (converted to holoenzyme) can the enzyme effectively bind substrates.

Cofactors can either be inorganic e.g., metals, such as magnesium, iron, zinc, and copper, or complex organic or metalorganic molecules e.g., flavin and heam. In many cases where the cofactor is an organic molecule, often derived from vitamins, it directly participates act as carrier molecules in the reaction. In such cases, the cofactor is termed a **coenzyme**. What makes a coenzyme different from an ordinary substrate is the fate of the coenzyme. A single coenzyme molecule can be used repeatedly to transfer molecular fragments from one reaction to another. On the other hand, inorganic cofactors serve as biocatalysts that only speed up enzymatic reactions inside a cell

Coenzymes are usually continuously regenerated and their concentrations maintained at a steady level inside the cell. Thus, as with metallic cofactors, only small quantities of coenzymes are necessary to maintain the enzymatic reactions in which they participate.

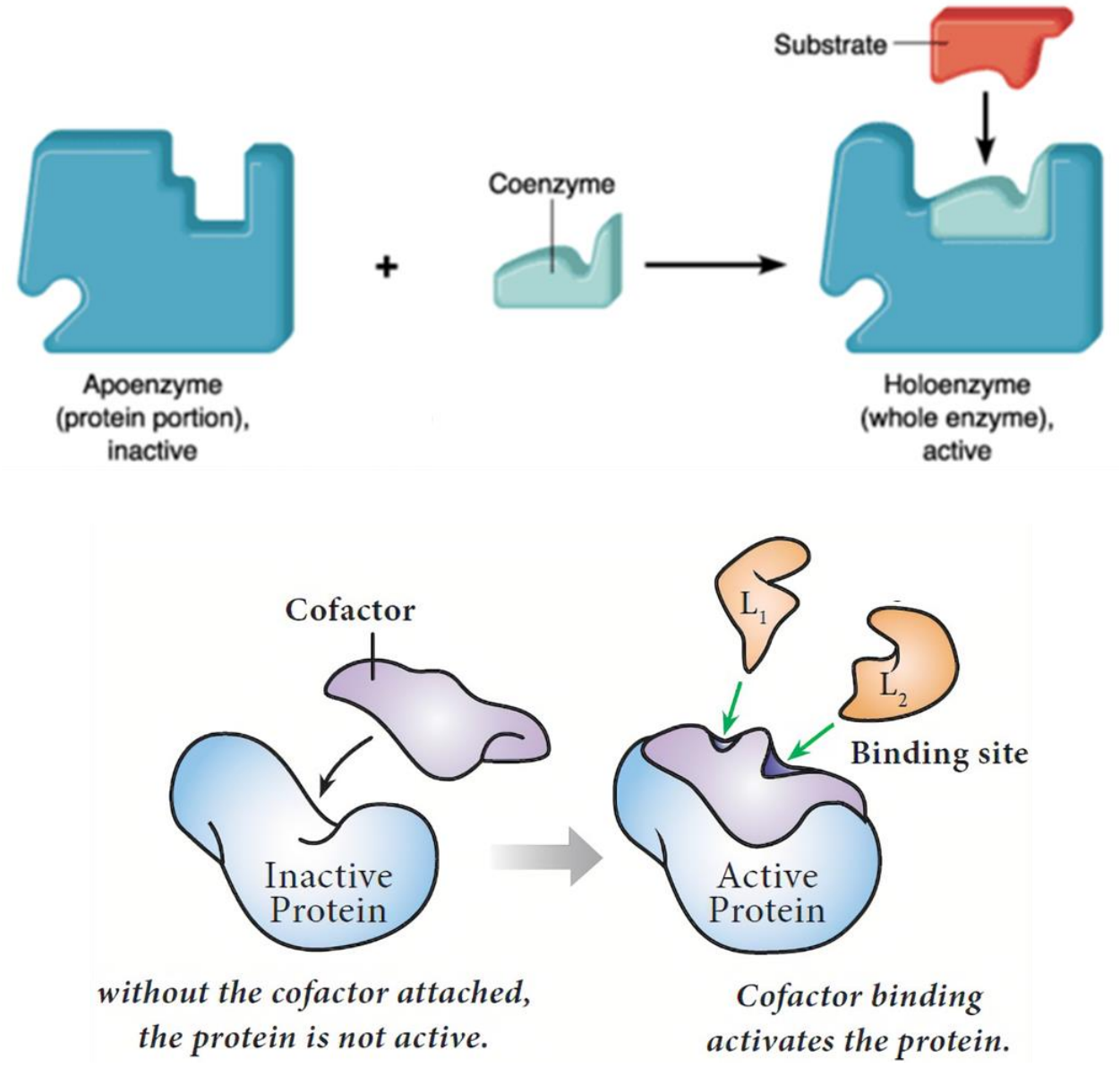

Figure 9. Cofactors can be organic (coenzyme) or inorganic. In either case, the enzyme is inactive before until it is bound by the cofactor.

Factors that affect the rate of enzyme activity

Enzymes are particular about the optimum conditions. Factors affecting enzyme activity include temperature, pH, substrate concentration, enzyme concentration and enzyme inhibition. For purposes of this course, we shall only discuss the effect of enzyme inhibition.

Enzyme inhibition

The substrates' access to the enzyme active site can be hindered in several ways, in what is termed enzyme inhibition. Thus, enzyme reaction rates can be decreased by various types of enzyme inhibitors. Below are some of the types of enzyme inhibition mechanisms:

1. Competitive inhibition

Competitive inhibitors strongly resemble the real substrate of the enzyme. The enzyme active site can [randomly] bind either the real substrate or the inhibitor, but not both at the same time. If the inhibitor, the active site is unavailable for substrate to bind, and thus that particular enzyme does not participate in the reaction. This type of inhibition can be overcome with high substrate concentration.

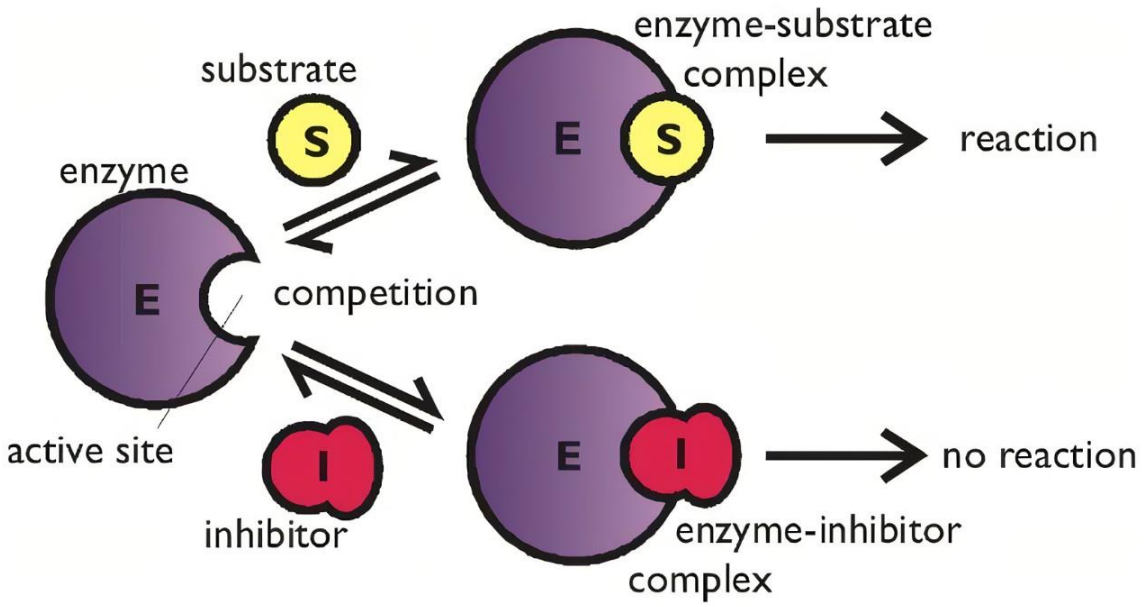

Figure 10. Competitive enzyme inhibition. The inhibitor and substrate cannot bind to the enzyme at the same time.

2. Non-competitive inhibition

A non-competitive inhibitor binds to a site other than where the substrate binds. The site where the inhibitor binds is called the allosteric site. The substrate still binds with its usual affinity. However, the inhibitor reduces the catalytic efficiency of the enzyme. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration.

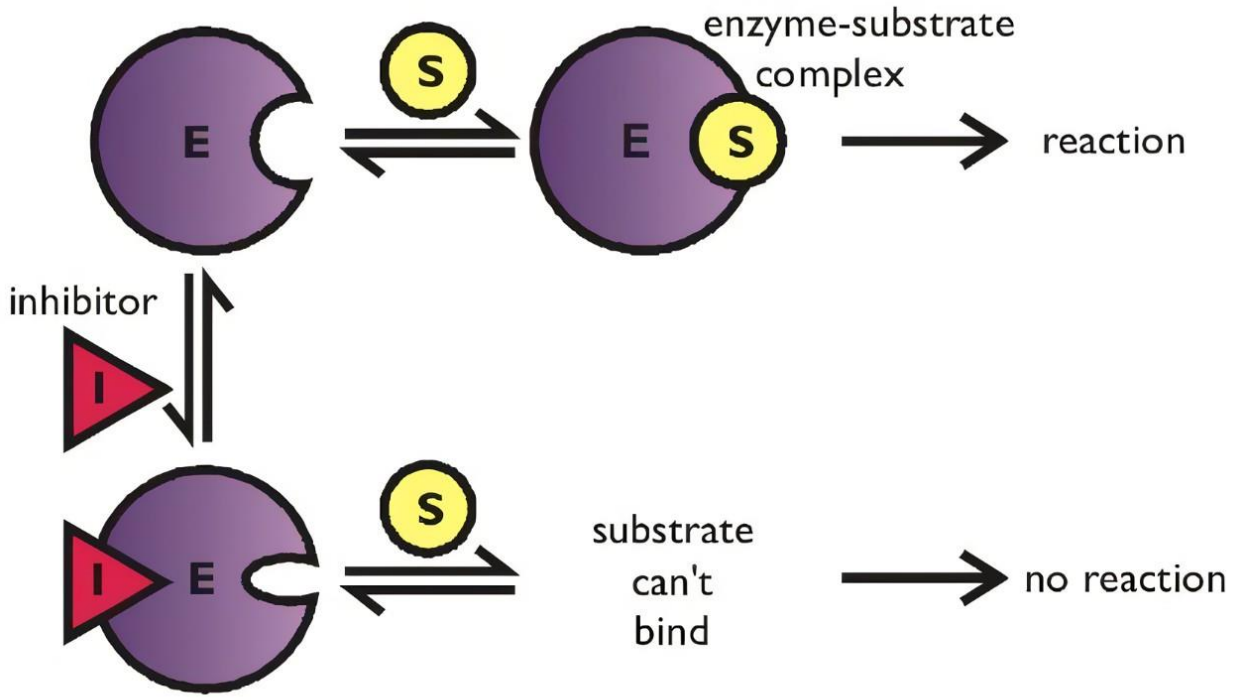

Figure 11. Non-competitive enzyme inhibition. Inhibitor binds to a site other than where the substrate binds

3. Uncompetitive inhibition

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex; hence, these types of inhibitors are most effective at high substrate concentration. Binding of inhibitor does not prevent binding of the substrate, but inactivates the enzyme so that no products are formed. In the presence of the inhibitor, the enzyme-substrate complex is inactive.

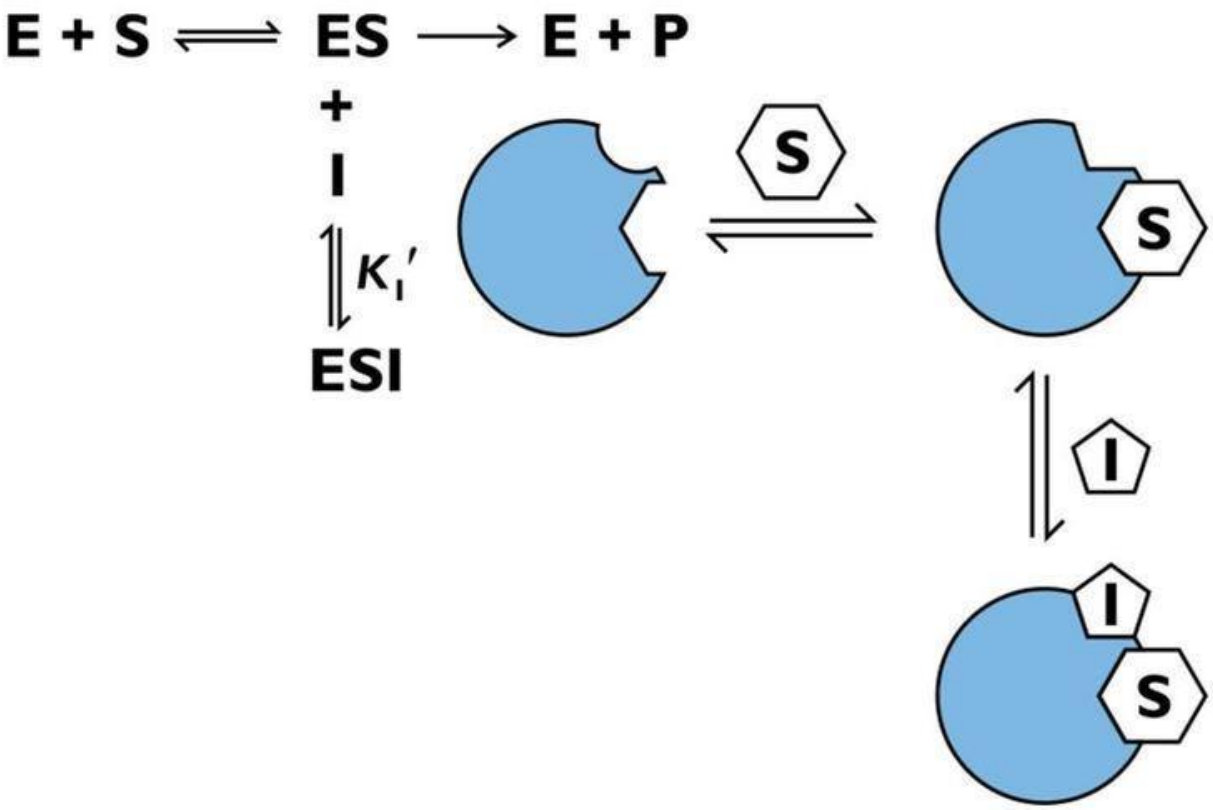

Figure 12. Uncompetitive enzyme inhibition. Inhibitor binds only to the enzyme-substrate complex

4. Mixed inhibition

A mixed inhibitor binds to an allosteric site. The inhibitor may bind to the enzyme whether or not the enzyme has already bound the substrate. The enzyme's function is reduced but not eliminated when bound to the inhibitor.

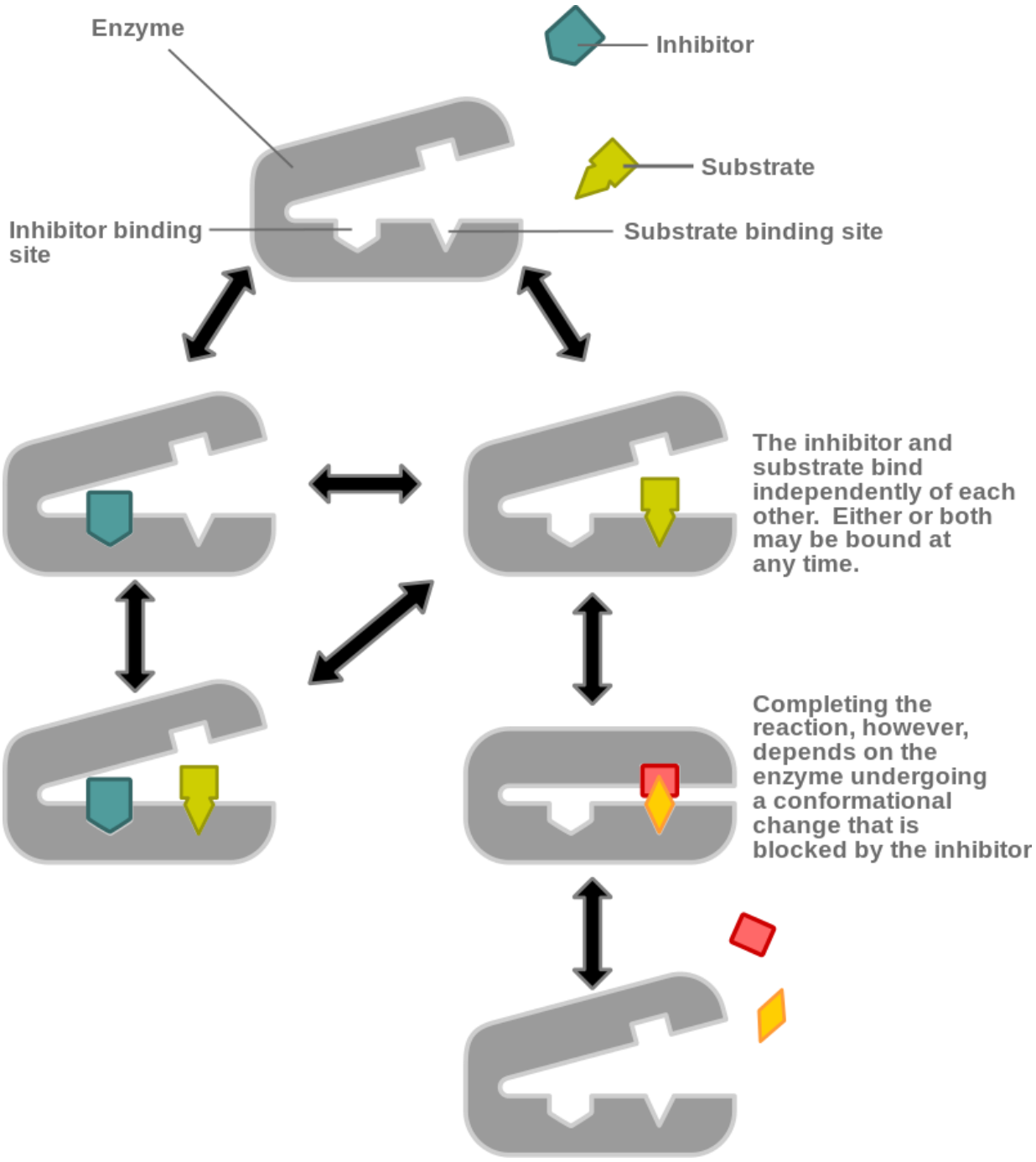

Figure 13. Mixed enzyme inhibition. The inhibitor may bind to the enzyme whether or not the enzyme has already bound the substrate. The presence of the inhibitor prevents the catalysis of the substrate.

5. Irreversible inhibition

While all the above may be reversed, an irreversible inhibitor permanently inactivates the enzyme, usually by forming a covalent bond to the protein. The inhibitor binds to either to the active site, or anywhere close to the active site and prevents the binding of the real substrate. Penicillin is common drug that act in this manner.

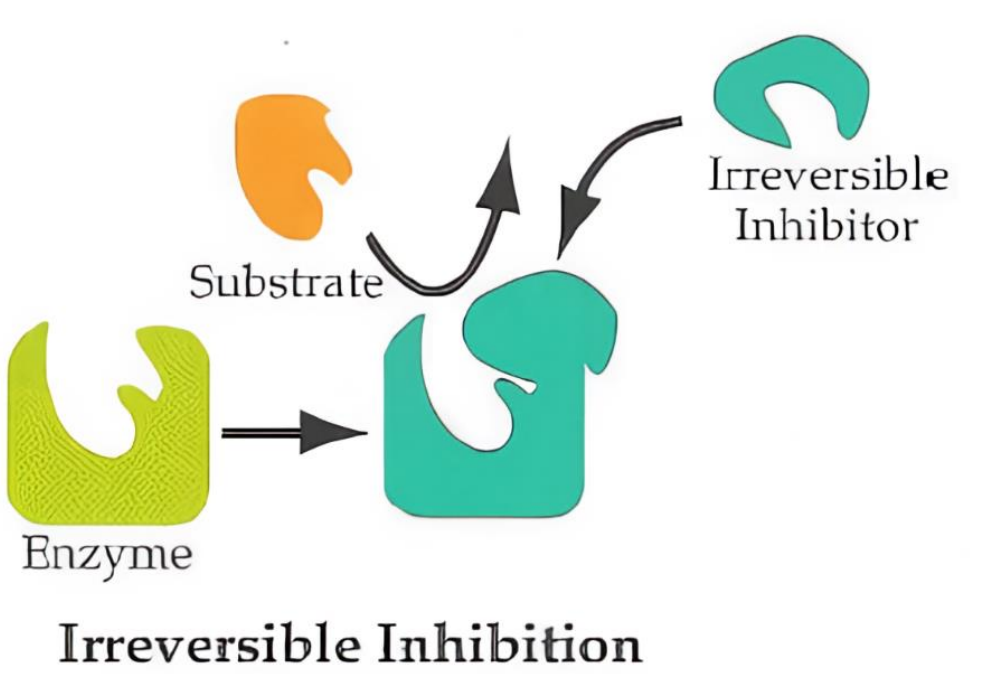

Figure 14. Mixed enzyme inhibition. The inhibitor forms a permeant bond with the enzyme, and prevents the substrate from binding to the active site.

Commercial enzymes

Enzymes are commercially used in a variety of industries such as pharmaceuticals, chemical production, biofuels, food & beverage, and consumer products. In the recent past, industrial uses of enzymes are increasing since they are being used in the production of biofuels and biopolymers.

For more examples of industrial and/or commercial use of enzymes, read the paper by Vinod Kumar and others, titled “Global scenario of industrial enzyme market” available online. You can type/paste the following DOI in the search engine: DOI: 10.13140/2.1.3599.0083