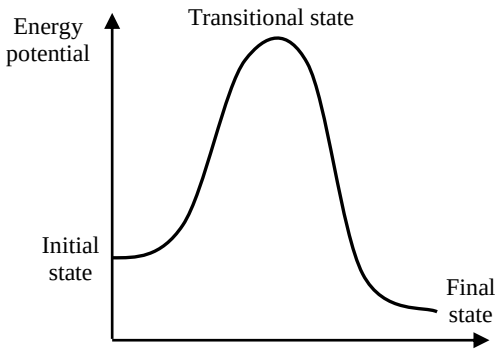
Enzymatic catalysis

The mechanisms of enzymatic catalysis closely reflect the mechanisms of chemical reactions. However, enzymes show an extremely high level of substrate specificity and considerable catalytic efficiency. This is due to active sites that are finely tuned for selective and rapid catalysis of reactions.

In fact, the principle of enzymatic catalysis is to lower the energy barrier of a reaction, thereby increasing the rate at which the products appear.



There are two ways to lower the energy barrier :

- ✓ By destabilizing the initial state ;
- ✓ By stabilizing the transition state.

The functional groups involved in enzymatic catalysis are similar to those involved in chemical catalysis. These groups are :

- ✓ Nucleophiles (electron donors);
- ✓ Electrophiles (affinity for electrons);
- ✓ Acids (proton donors);
- ✓ Bases (proton acceptors).

1. Chemical catalysis :

1.1. Nucleophilic catalysis :

Many protein residues have side chains carrying nucleophilic groups capable of participating in catalysis. This is the case for the hydroxyl group of serine in serine proteases and in alkaline phosphatases and acid phosphatases, the thiol group of cysteine in thiol proteases and in GAP dehydrogenase, etc.

1.2. Electrophilic catalysis :

In electrophilic catalysis, the electrophilic catalyst removes a pair of electrons from the substrate. Electrophilic groups are either the conjugate acids of amino acids, metal ions (Fe, Mn, Cu, Zn, Mo, Co, etc.), or coenzymes. Metalloenzymes are good examples of electrophilic catalysis.

1.3. Acid catalysis :

In general acid catalysis, all species capable of donating protons contribute to accelerating the reaction rate. Reactions in which proton transfer is decisive for the reaction rate are general acid catalysis reactions.

General acid catalysis is a process in which there is a partial transfer of a proton from an acid group (a molecule that can donate one or more H^+ protons), which lowers the free energy of the transition state.

1.4. Basic catalysis :

In general basic catalysis, the catalyst reacts by attacking hydrogen rather than carbon (as in nucleophilic catalysis). Any conjugated base can act as a general basic catalyst.

A reaction can be stimulated by general basic catalysis if the reaction rate is increased by the partial removal of a proton by a base (a molecule that can capture or accept protons).

2. Functional classification of enzymes :

1st class : enzymes that function without coenzymes

For these enzymes, the reaction takes place via the catalytic groups of the protein.

- These may be side chain residues with nucleophilic properties, such as :
  - ✓ Histidine imidazole ;
  - ✓ Aspartate and glutamate carboxylate ;
  - ✓ Amino groups of arginine and lysine ;
  - ✓ Alcohol function of serine and threonine ;
  - ✓ Protein dipoles.
- Some enzymes require the presence of a metal cation (cofactor) coordinated to the protein via atoms such as oxygen, nitrogen, or sulfur (metalloenzymes).

2nd class : enzymes functioning with a dissociable coenzyme

- In this case, the coenzyme acts as a second substrate.
- Certain groups of the protein may be involved.

3rd class : enzymes that contain a prosthetic group

In this case, the reaction involves a metal that changes its valence or spin state (transition metal) or through electronic movement (FMN and FAD).

3. Special characteristics of enzymatic catalysis :

In fact, the tertiary structure of the enzyme generates a large number of factors that contribute to ensuring functional catalytic efficiency. All of these factors together are responsible for the catalytic performance of the enzyme.

The distinctive characteristics of enzymatic catalysis are :

- Enzymatic catalysis is intramolecular catalysis ;
- Enzymatic catalysis is polyfunctional catalysis ;
- The structure of the active site is complementary to the transition state of the substrate ;
- The enzyme creates a specific microenvironment that promotes the reaction ;
- Enzymatic catalysis involves several reaction intermediates.

4. Catalytic mechanisms :

The reason enzymes are powerful catalysts is due to their specificity in binding to the substrate and their optimal arrangement of catalytic groups.

The mechanisms that enzymes use are classified as follows :

- Acid/base catalysis : the enzyme provides the proton acceptor/donor groups.
- Covalent catalysis : the enzyme can combine covalently with the substrate to form an intermediate that allows for a faster reaction.
- Metal-dependent catalysis : the enzyme uses ions as cofactors to stabilize different charges (intermediates), to ionize water molecules and make them more nucleophilic, and/or to hide charges.
- Electrostatic catalysis : the enzyme uses electrostatic forces to align the substrate and stabilize the transition state.
- Catalysis by proximity and orientation : the enzyme fixes the substrate so that the reaction bonds are located near the catalytic site and are oriented in such a way that the transition state is reached very quickly.
- Catalysis by constraint : the enzyme can induce distortion and/or tension at the bond to be cleaved, which destabilizes it and makes it easier to cleave. The enzyme therefore preferentially binds the substrate in the transition state.

4.1. *Acid/base catalysis :*

The ionizable functional groups of the side chains of aminoacyl residues and, if present, prosthetic groups contribute to catalysis by acting as acids or bases. Thus, the unstable intermediate can be stabilized by a proton transfer, leading to the reaction products.

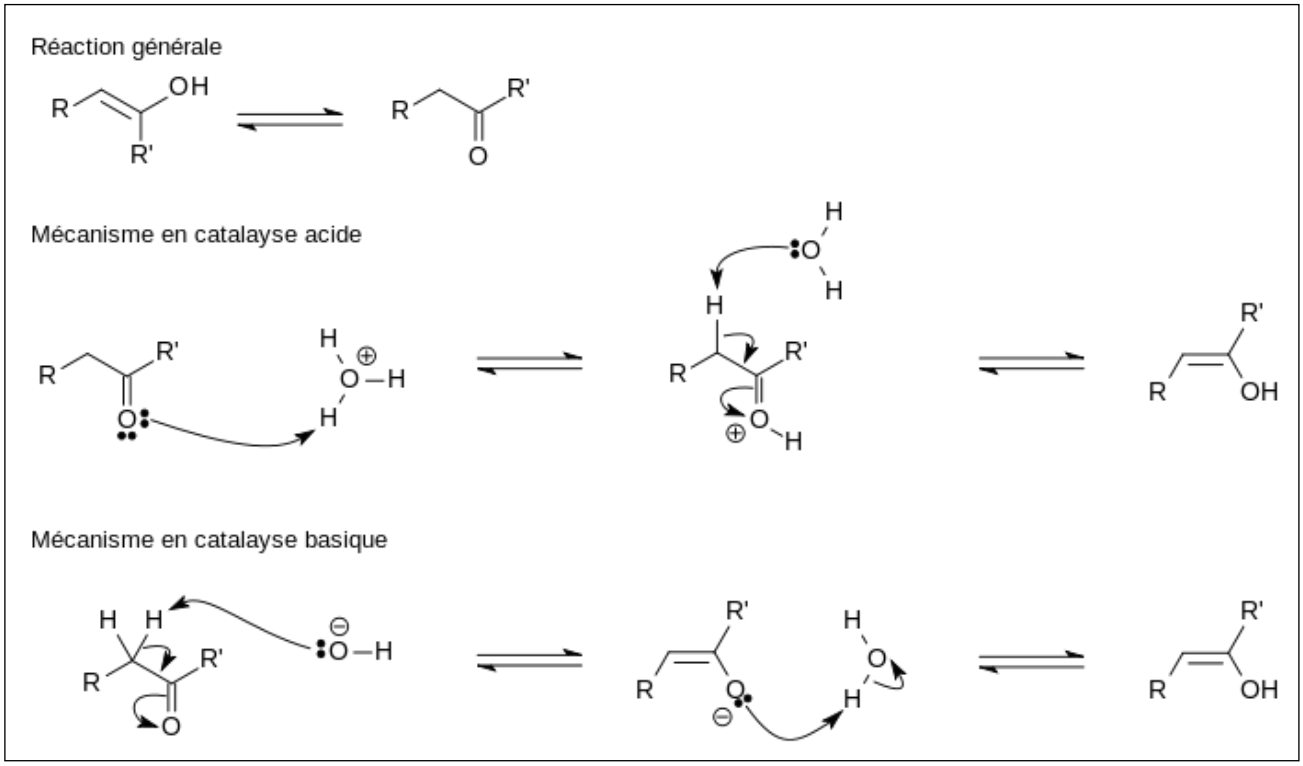
Acid-base catalysis can be either specific or general :

- **Specific catalysis :**

✓ It involves only protons (H_30^+) or ions (OH^-).

✓ The reaction rate is sensitive to changes in proton concentration (pH) ;

✓ But independent of the concentration of other acids (proton donors) or bases (proton acceptors) present in the solution or at the active site (buffer concentration).



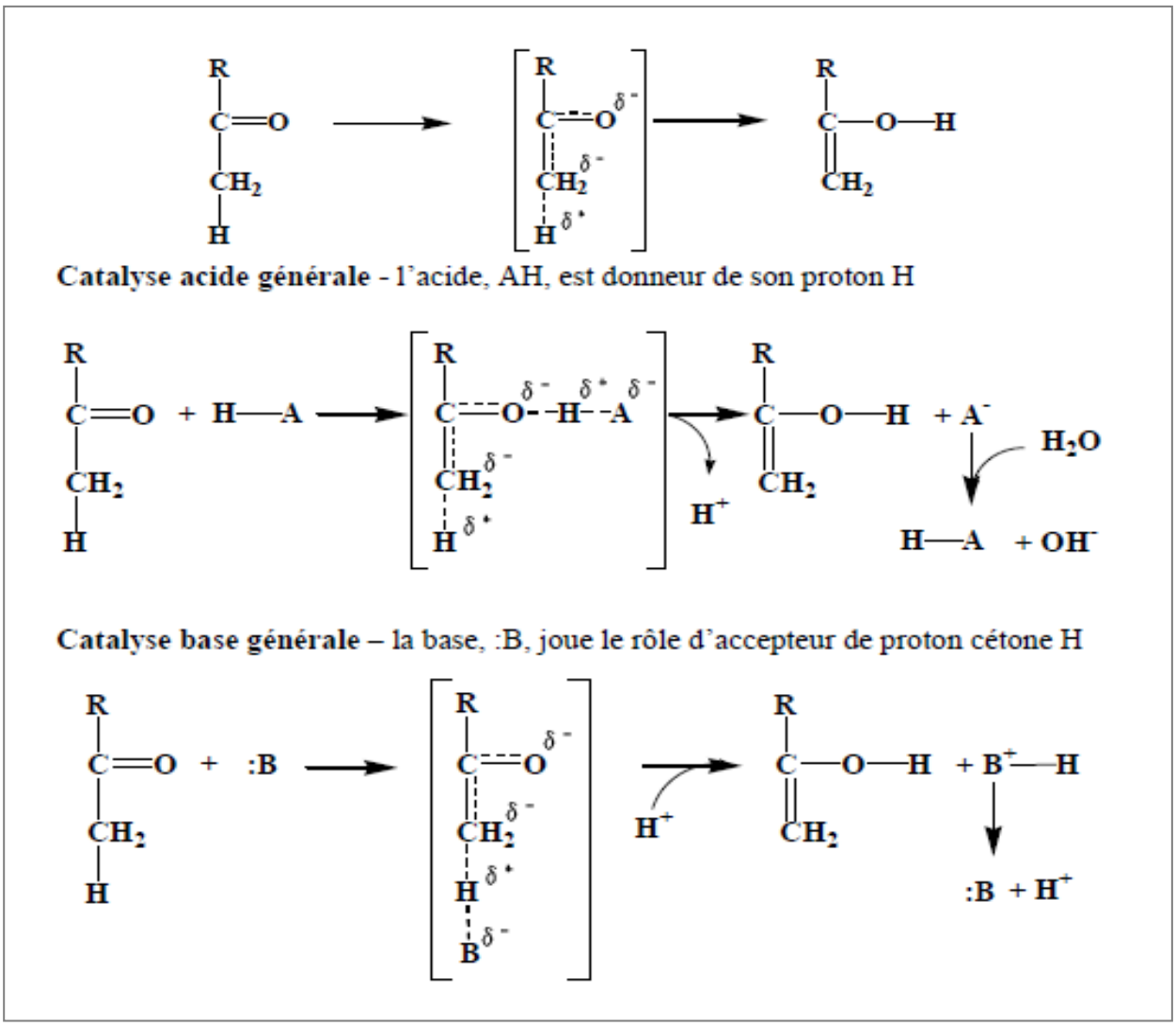
- **General catalysis :**

✓ Corresponds to a transfer of protons from donor and acceptor groups other than water ;

✓ Certain residues in the enzyme's active site can act as proton donors or acceptors (aspartate, glutamate, lysine, arginine, cysteine, serine, histidine, and tyrosine) ;

✓ The rate constant depends on the buffer concentration.

Example : Ketone-enol tautomerism; the transition state [ ] is like an enolate.



4.2. Covalent catalysis :

- ✓ The covalent catalysis process involves the formation of a covalent bond between the enzyme and one or more substrates. The modified enzyme then becomes a reagent.
- ✓ The chemical modification of the enzyme is only temporary. At the end of the reaction, the enzyme returns to its original, unmodified state. It therefore retains its catalytic role.
- ✓ Covalent catalysis is particularly common for enzymes that catalyze group transfers.
- ✓ The enzyme residues involved in covalent catalysis are usually cysteines or serines and sometimes histidines.
- ✓ Side chain groups (such as amine, carboxyl, imidazole, thiol, etc.) can form nucleophilic centers capable of attacking the electrophilic centers of the substrate and forming covalent intermediates (acyl, phosphoryl, or glucosyl, etc.).
- ✓ Once the covalent intermediate is formed, the bond between the enzyme and the transferred group is hydrolyzed.
- ✓ Covalent catalysis often follows a ping-pong mechanism, in which the first substrate is bound, then the corresponding product is released before the second substrate is bound.

4.3. Metal-dependent catalysis :

- ✓ Almost one-third of known enzymes require the presence of metal ions for their catalytic activity.
- ✓ Metalloenzymes are divided into two classes, distinguished by the strength of protein-ion interactions :
  a) Metalloenzymes with strongly bound metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn2+, or Co^{2+} (transition metals).
  b) Enzymes activated by weakly bound metal ions in solution such as Na^{+}, K^{+}, Mg^{2+}, or Ca^{2+} (alkali or alkaline earth metals).

Metal ions participate in catalysis in three main ways :

1) by binding to substrates in order to orient them correctly for the reaction.
2) by participating in redox reactions through reversible changes in the oxidation state of the metal ion.
3) by electrostatically stabilizing or masking negative charges (neutralization).

Example :

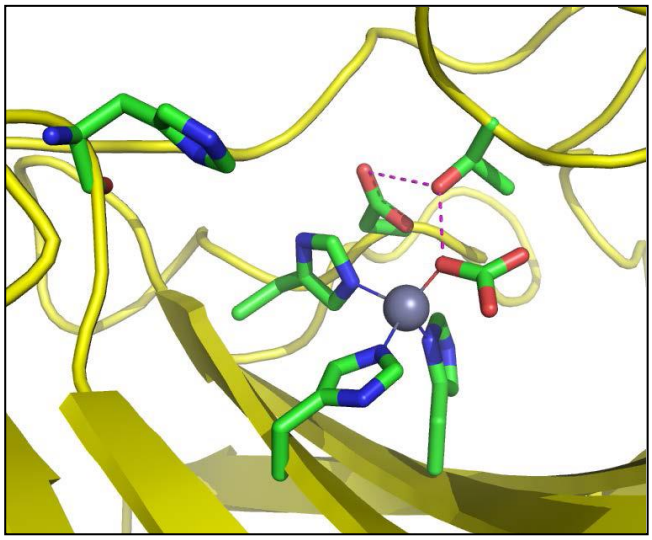
The enzyme carbonic anhydrase is a good example. The enzyme uses zinc to catalyze the reaction :

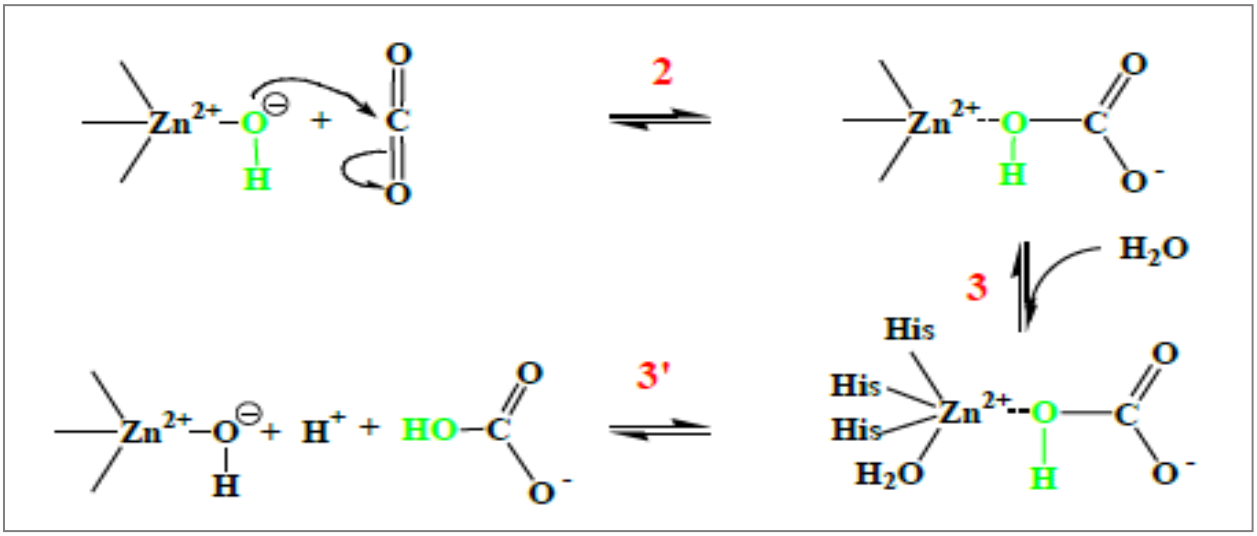
$$\mathbf{CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+}$$ in three steps.

1) Coordination of a zinc molecule by three histidines and a water molecule.

2) Nucleophilic attack by the OH^- of CO_2 and conversion to HCO_3^-

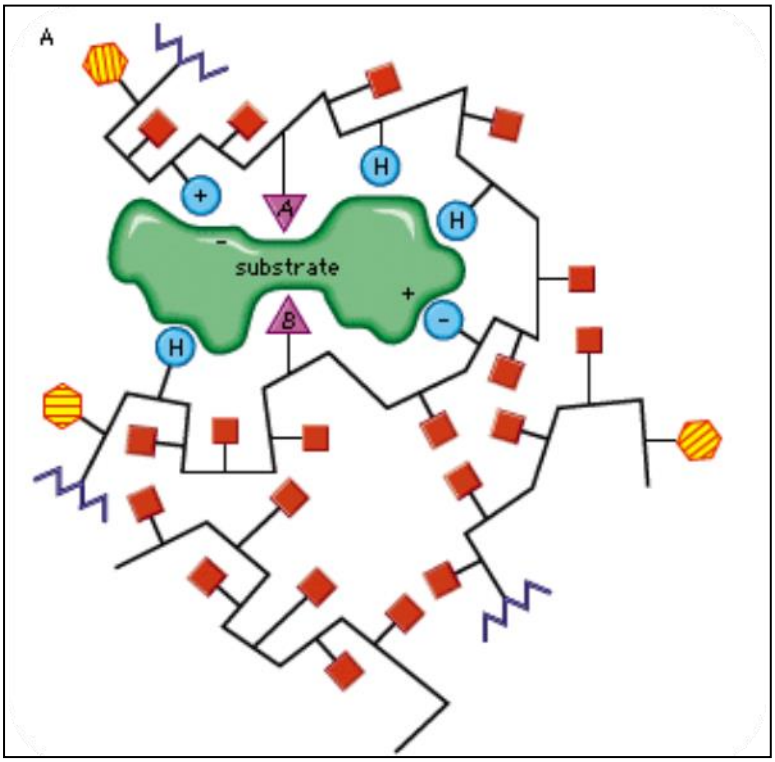
3) Regeneration of the catalytic site by binding another H_2O molecule, possibly before the departure of HCO_3^-, via an intermediate of the five-coordinate zinc complex.





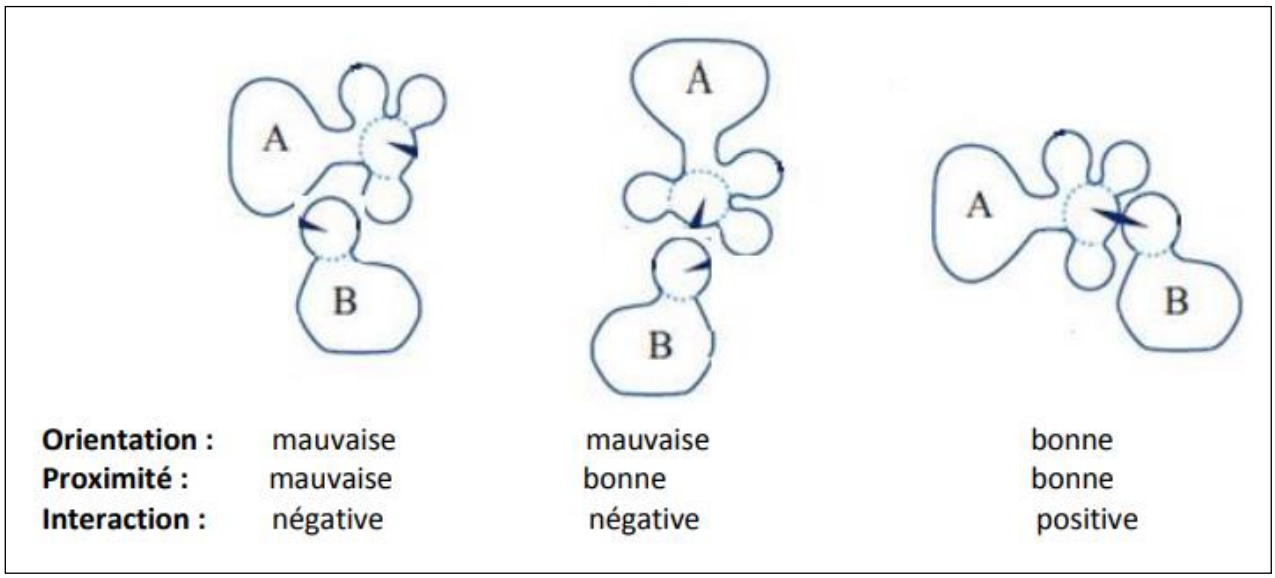
4.4. Electrostatic catalysis :

- ✓ Charge distributions in an active site are arranged to stabilize transition states.
- ✓ Computer simulations indicate that the reduction of the transition state barrier by electrostatic effects is an important component of catalysis.
- ✓ It is believed that the folded enzyme provides a pre-organized polar environment that is already partially oriented to stabilize the transition state.
- ✓ Indeed, the electrostatic fields created by the protein at the active site are complementary to the charge distribution of the substrate in the transition state.



4.5. Catalysis through proximity and orientation effects :

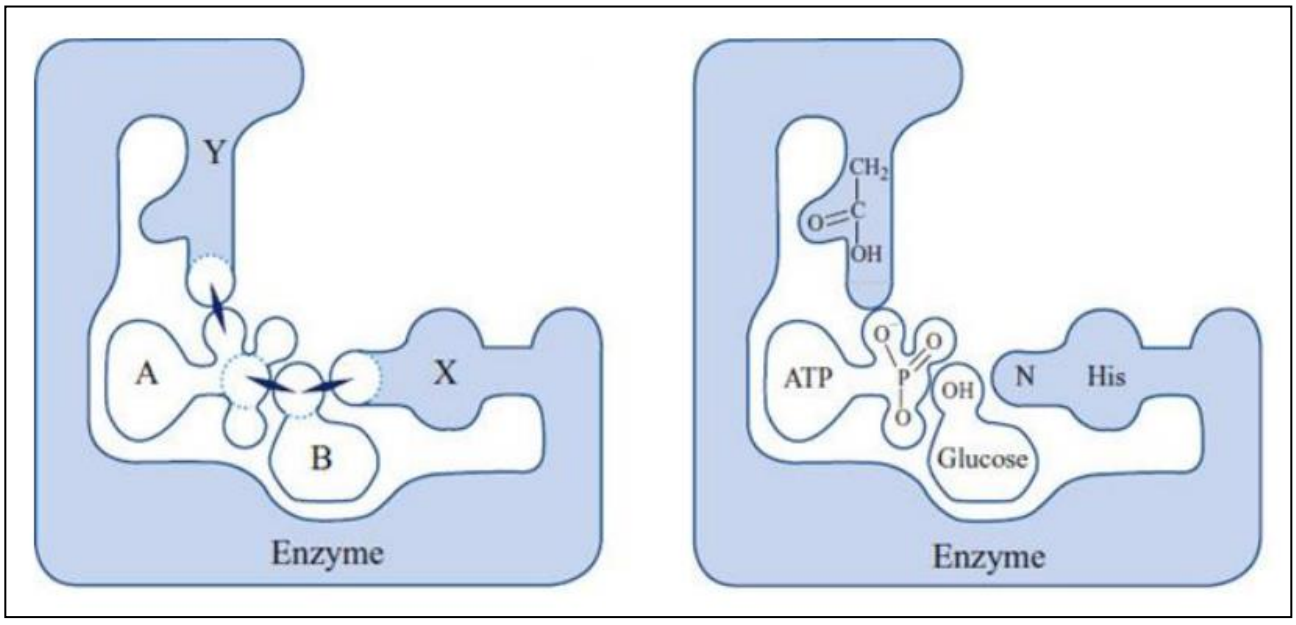
✓ For a reaction to occur, substrate molecules must meet in a suitable arrangement.



✓ Enzymes, thanks to their binding specificity, are able to keep substrate molecules close to each other even in diluted solutions.

✓ Thus, the apparent substrate concentration near the enzyme is higher than the normal concentration.

✓ This environment also orients the substrate molecules in space in an ideal position for them to interact, increasing the reaction rate by at least several thousand times.



4.6. Catalysis by preferential bonding in the transition state : (by constraint effect)

- ✓ One of the most important catalytic mechanisms is based on the notion that the constrained conformation of the reactant more closely resembles the transition state of a reaction than the corresponding unconstrained conformation.
- ✓ Enzymes mechanically force their substrates to take on geometric shapes corresponding to the transition state by binding to binding sites that do not fit properly with undeformed substrates.
- ✓ Enzymes catalyze lytic reactions involving the breaking of covalent bonds and generally bind to their substrate in a conformation that is slightly unfavorable for the bond to be broken. This conformation mimics that of the transition state intermediate, a transient species representing the transition point halfway through the transformation of substrates into products.
- ✓ The resulting stress stretches or twists this target bond, making it weaker and more vulnerable to cleavage.