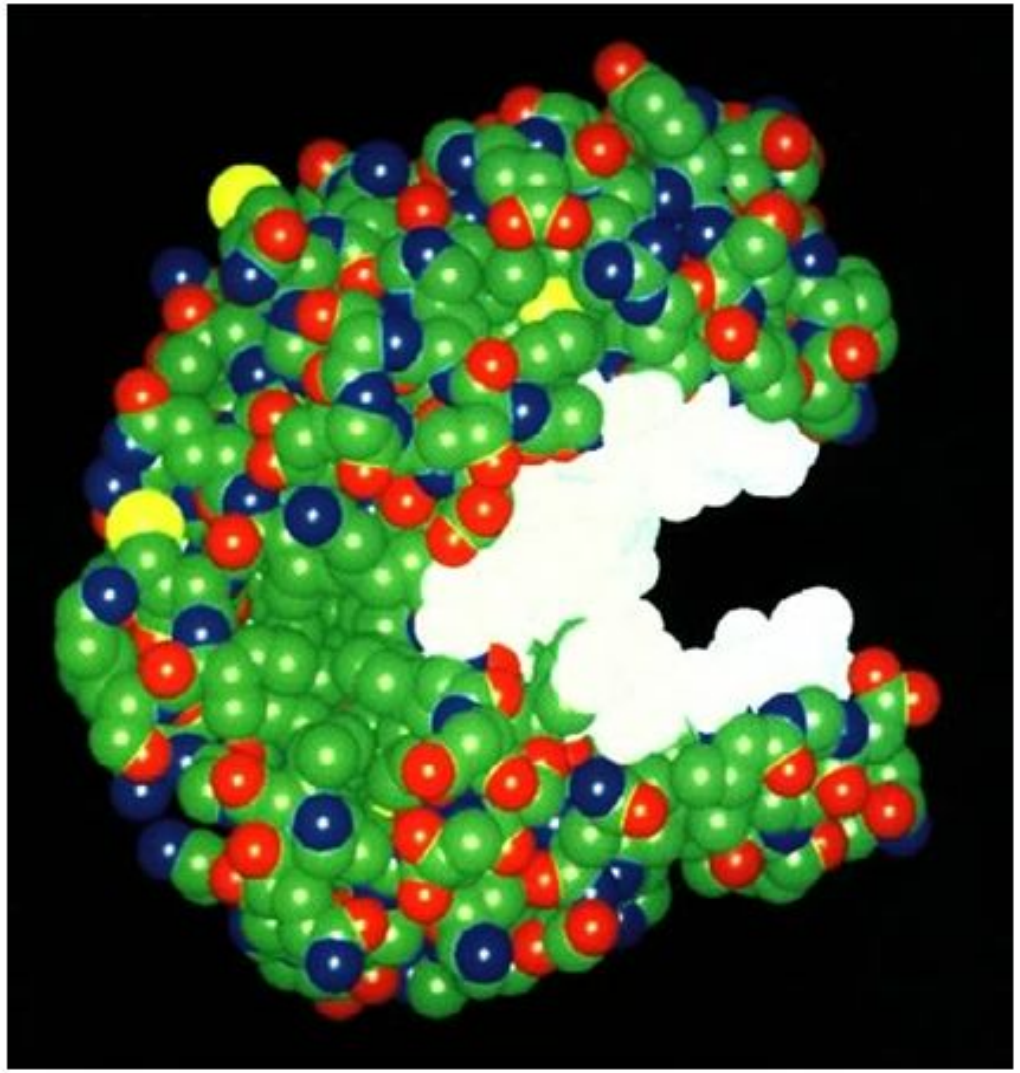
Unit3

ENZYMES, PHOTOSYNTHESIS, METABOLISM AND BIOENERGETICS

- Enzymes

WHAT ARE ENZYMES

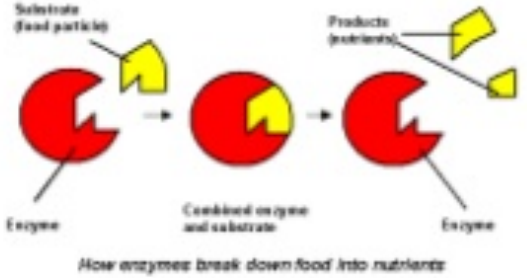
- An enzyme is a catalyst in cellular reactions.
- DEFINE *catalyst*
- Enzymes are protein or nucleic acids with (tertiary and quaternary structure).



PROPERTIES OF ENZYMES

1. Speeds up chemical reaction

2. Required in small amounts



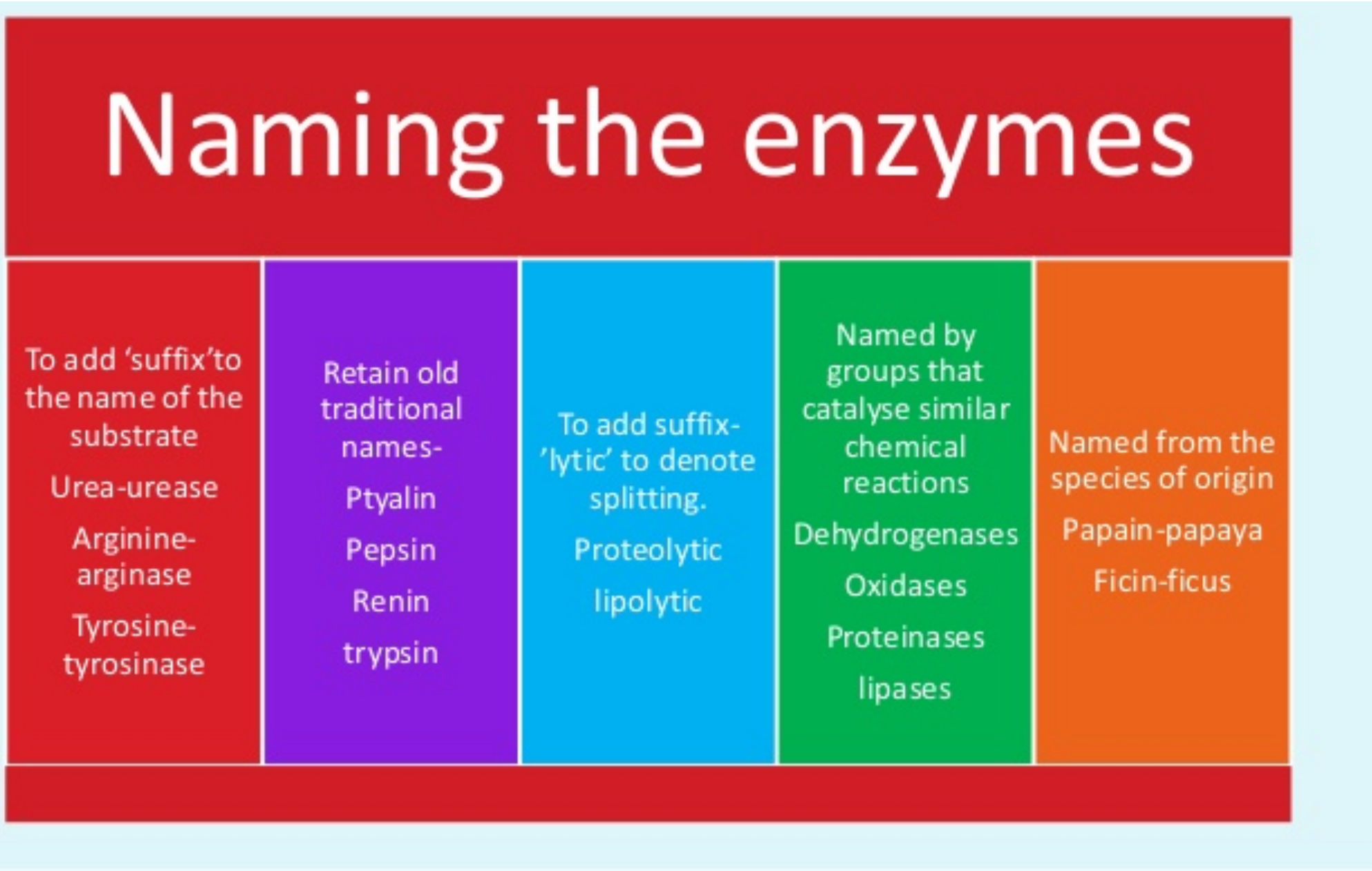
How enzymes break down food into nutrients

3. Specific in action

4. Affected by temperature

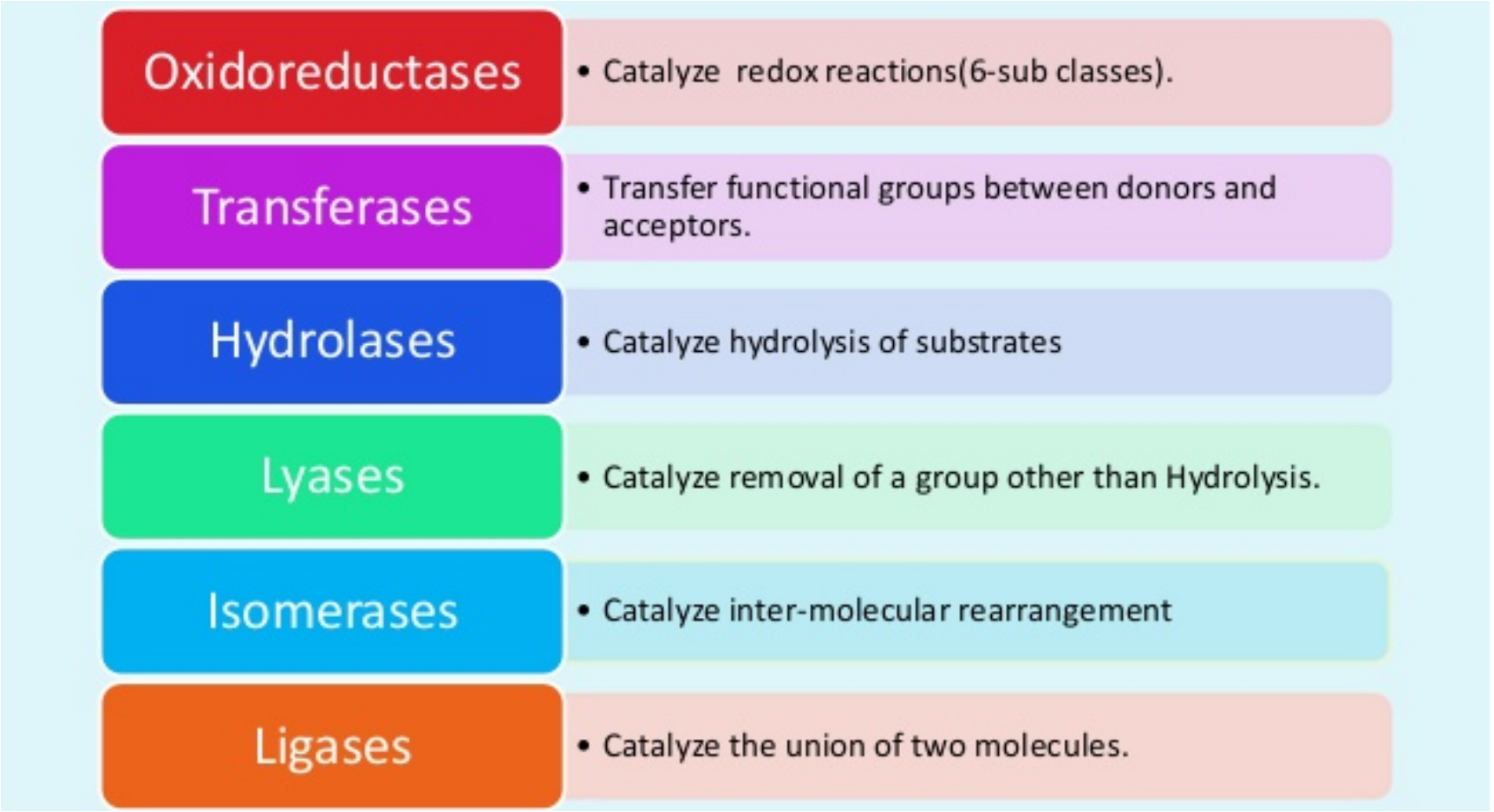
5. Affected by pH

ENZYME NOMENCLATURE



CLASSIFICATION OF ENZYME

To prevent any ambiguity, The Enzyme Commission of the IUBMB (International Union of Biochemistry and Molecular Biology) has adopted a classification system where the enzymes were divided into 6 classes



SIGNIFICANCE OF ENZYME

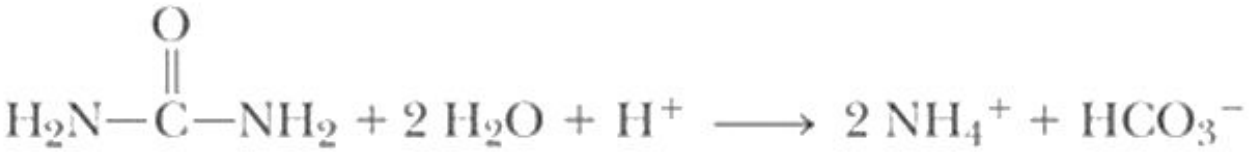
Enzymes are characterized by three distinctive features

- **Catalytic power**
- **Specificity**
- **Thermodynamics of enzymatic reactions**

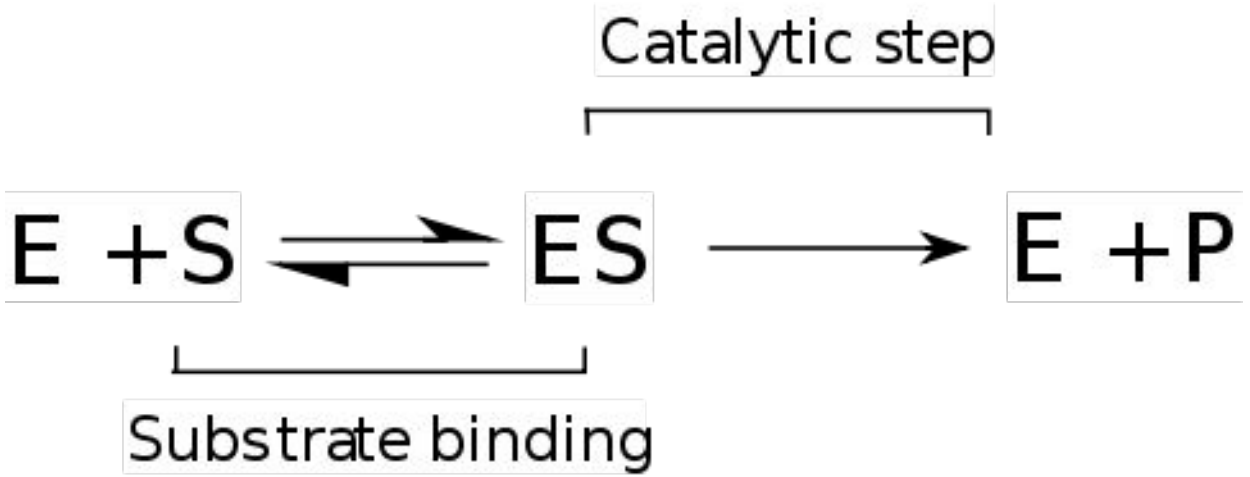
CATALYTIC POWER (REACTION RATE)

- Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates!

- Urease is a good example:
  - Catalyzed rate: $3x10^{4}$/sec
  - Uncatalyzed rate: $3x10^{-10}$/sec
  - Ratio is $1x10^{14}$ (catalytic power)

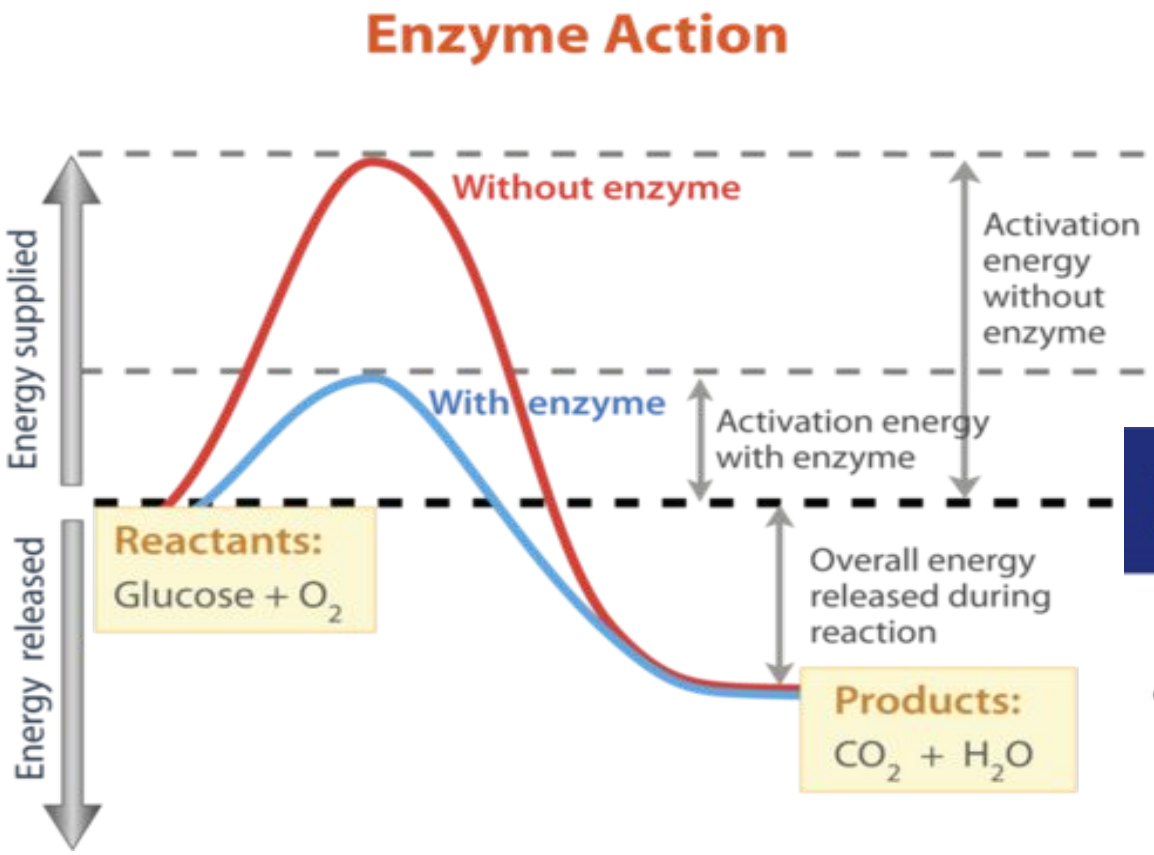


CATALYTIC POWER (REACTION RATE)



- Conversion of reactants to products proceeds with the formation of at least one high energy transition state which imposes an "energy barrier" for the reactants.
- To surmount this barrier the reactants acquire a critical energy called activation energy and form an energy rich transition state before forming products.
- Activation energy can be defined as the minimum energy required for initiating a chemical reaction.

Thermodynamics of Enzyme activity

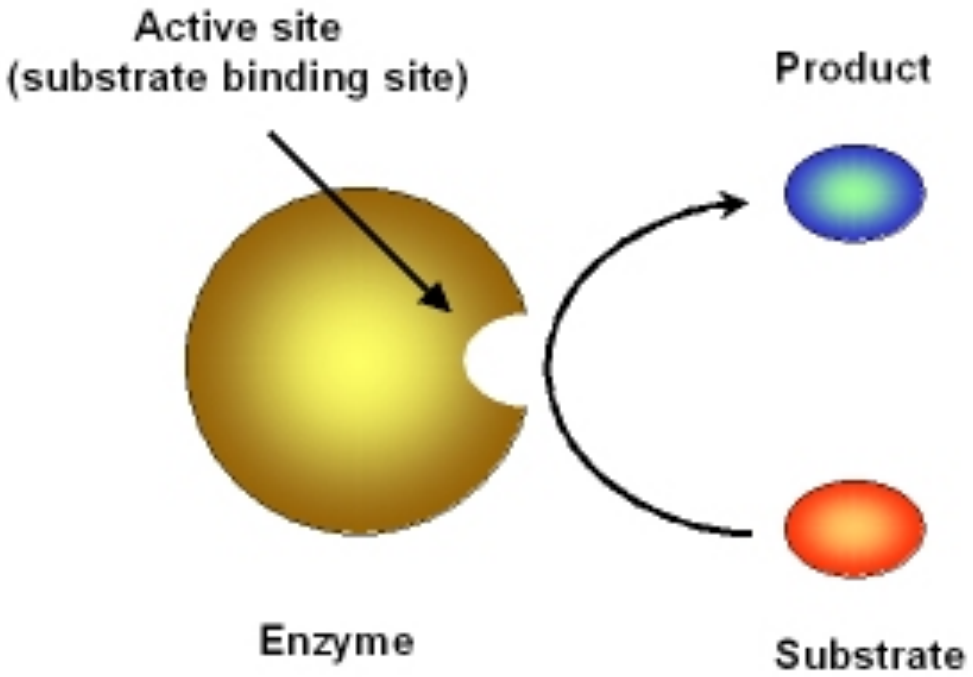


Lowering Activation Energy

- Enzymes lower activation energy in four ways:
  - Bringing substrates closer together
  - Orienting substrates in positions that favor reaction
  - Inducing the fit between a substrate and the enzyme's **active site** (**induced-fit model**)
  - Shutting out water molecules

SPECIFICTY OF ENZYMES

- Enzymes have active sites where the substrates binds.
- Substrates bind to the group residues at the active site with the help of covalent, ionic, hydrogen, hydrophobic and Vander Waals forces.



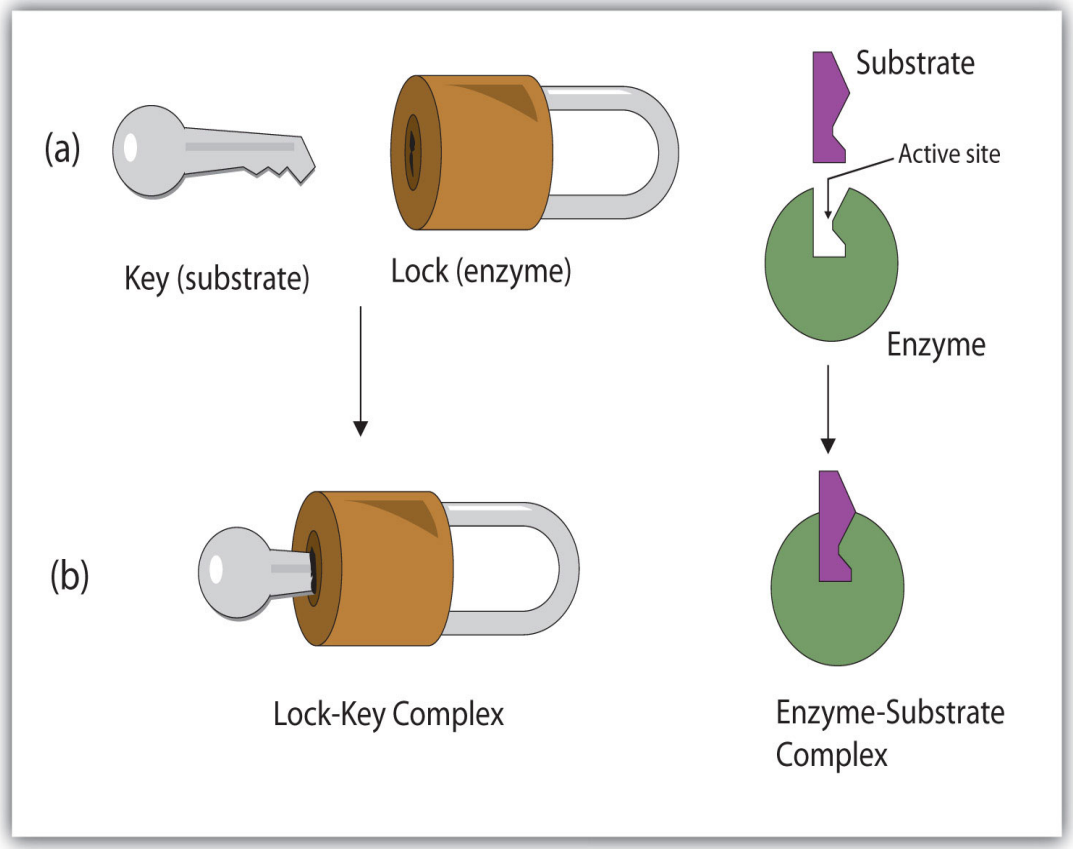
TYPES OF SPECIFICTY

Enzymes are highly specific and catalyze only one type of reaction.

1. **Absolute specificity**: The enzyme is specific for one substrate e.g.; urease acts only on urea; glucose oxidase oxidizes only glucose but not other monosaccharides.
2. **Relative specificity**: The enzyme acts on *a group of closely related substrates*: pancreatic lipase hydrolyzes alpha ester bonds in triglycerides irrespective of the nature of fatty acid attached. (bond specificity).
3. **Group specificity**: Most proteolytic enzymes show group specificity, for example; trypsin hydrolyzes peptide bonds provided only by arginine and lysine. (bond and group specificity).
4. **Stereospecificity:** Human enzymes are specific for L-amino acids and D-monosaccharides.

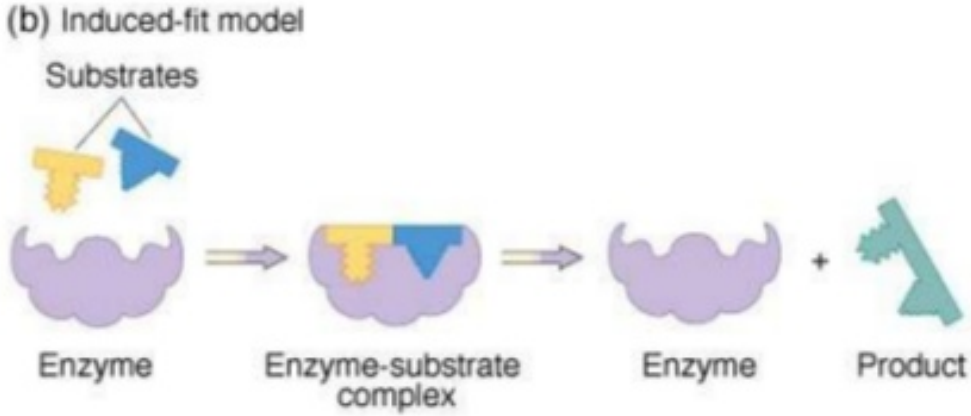
HYPOTHESIS FOR ENZYME SPECIFICTY

- **LOCK AND KEY HYPOTHESIS**



- **INDUCED FIT HYPOTHESIS**

- In this model;



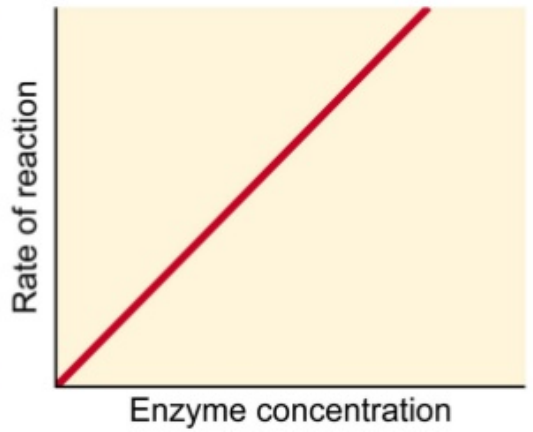
- The reagents don't fit exactly into the enzymes active site. But because enzymes are globular proteins with flexible shapes, the enzyme changes its active site shape to accommodate the reagents

FACTORS AFFECTING ENZYME ACTIVITY

1. ENZYME CONCENTRATION

- at low enzyme concentration there is **great competition** for the active sites and the rate of reaction is **low**.
- As the enzyme concentration **increases**, there are **more active** sites and the reaction can proceed at a **faster** rate.
- Eventually, increasing the enzyme concentration *beyond a certain point* has no effect because the substrate concentration becomes the *limiting* factor.

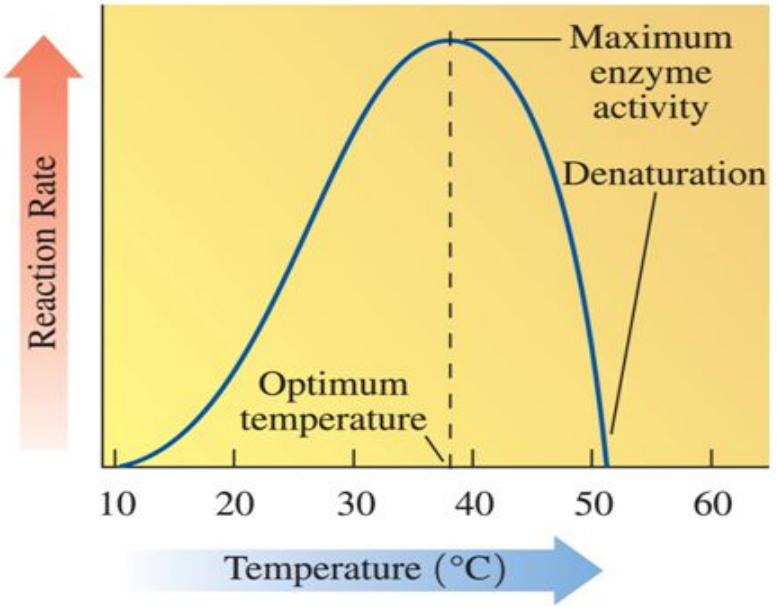
Reaction rate $\propto$ **[E]**



2. TEMPERATURE

Enzymes

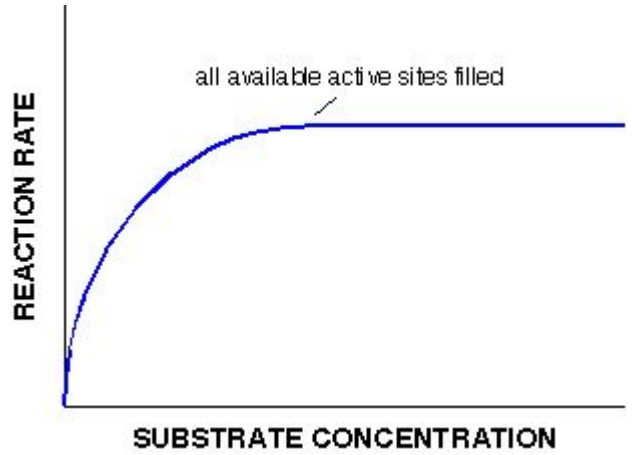
- are most active at an **optimum temperature** (usually 37 °C in humans)
- show little activity at low temperatures.
- lose activity at temperatures above 50 °C as denaturation occurs with loss of catalytic activity



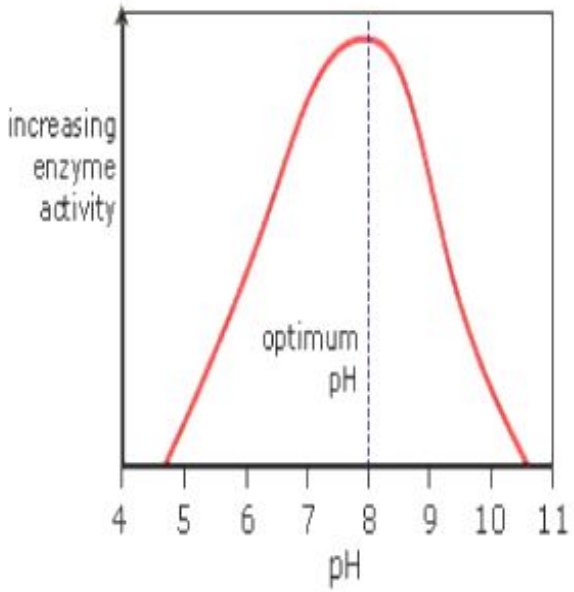
FACTORS AFFECTING ENZYME ACTIVITY

3. SUBSTRATE CONCENTRATION

- at a low substrate concentration there are many active sites that are not occupied. This means that the reaction rate is low.
- When more substrate molecules are added, more enzyme-substrate complexes can be formed.
- As there are more active sites, and the rate of reaction increases.
- Eventually, increasing the substrate concentration yet further will have no effect. The active sites will be saturated so no more enzyme-substrate complexes can be formed.

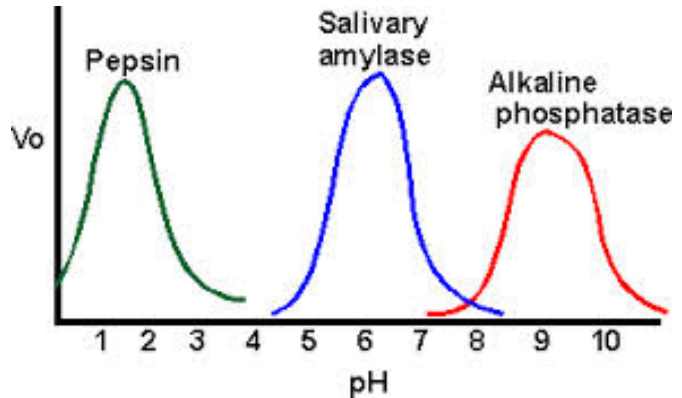


4. pH



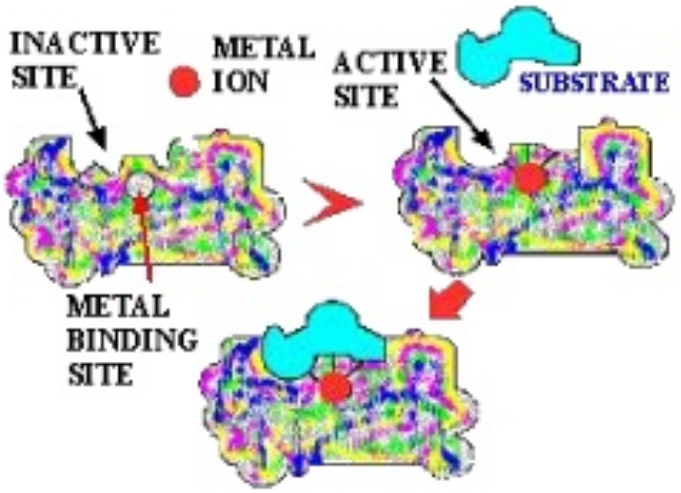
- Hydrogen ion concentration also have an influence on enzyme activity.
- For most enzymes, the effective pH range is 4.0-9.0.
- Beyond these limits, denaturation of enzymes take place.
- Optimum pH for pepsin is 2.0 and for trypsin 8.0

Each enzyme works at a particular pH



FACTORS AFFECTING ENZYME ACTIVITY

5. COFACTORS



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- Cofactors are substances that are essential to the catalytic activity of some enzymes.
- Cofactors may alter the shape of enzymes slightly to make the active sites functional or to complete the reactive site.
- Enzyme cofactors include coenzymes (organic molecules) or activating ions (eg. Na+, K+..)
- Vitamins are often coenzymes (eg. Vit B1, Vit B6…)

FACTORS AFFECTING ENZYME ACTIVITY

6. INHIBITORS

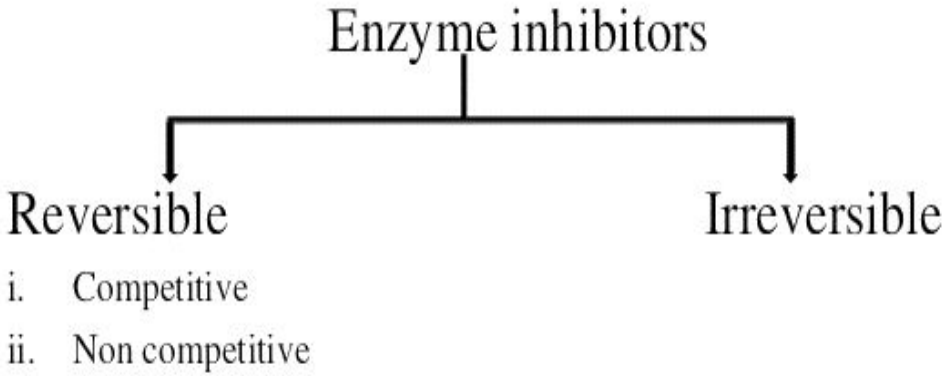
-The catalytic activity of enzymes, in addition to substrate concentration, is affected by the type and concentration of inhibitors, i.e. compounds which decrease the rate of catalysis, and activators, which have the opposite effect.

Inhibitors

- are molecules that cause a loss of catalytic activity.
- prevent substrates from fitting into the active sites.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

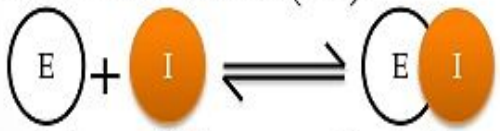
$$E + I \rightleftharpoons EI \not\longrightarrow \text{no P}$$



FACTORS AFFECTING ENZYME ACTIVITY

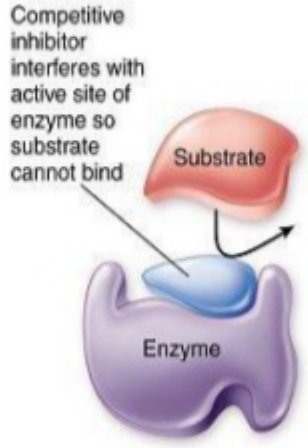
1) Reversible inhibitor:

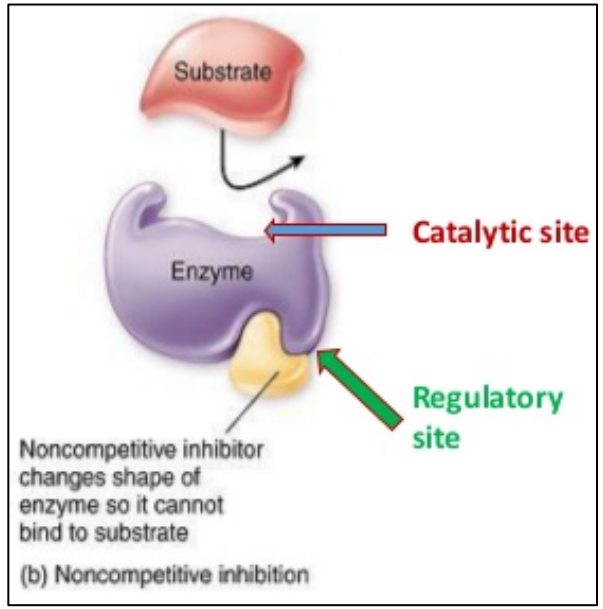
- Inhibitor binds to Enzyme reversibly through non covalent interactions.
- An Equilibrium is established between the free inhibitor & EI Complex and is defined by an equilibrium constant (Ki).

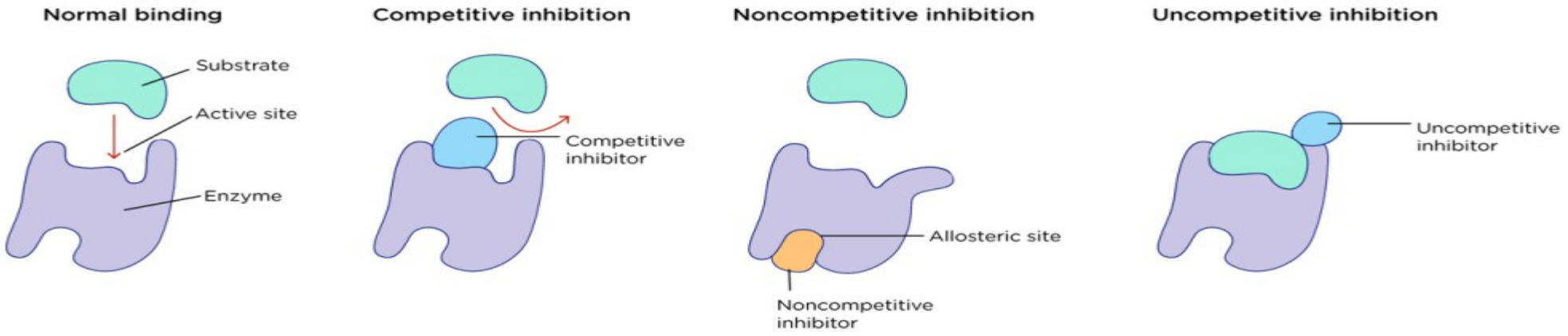


- The activity of Enzyme Is fully restored on removing the Inhibitor by dialysis.

Isoleucine Binds to regulatory site on Threonine Dehydratase & functions as non-competitive inhibitor





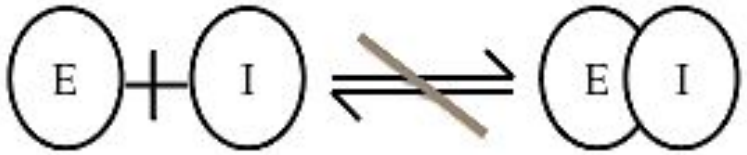


Jack Westin

FACTORS AFFECTING ENZYME ACTIVITY

2) Irreversible inhibitor:

- Inhibitor binds at or near the active site of the enzyme irreversibly, usually by covalent bonds, so it can't dissociate from the enzyme.
- Irreversible inhibitors combine with the functional groups of the amino acids in the active site, irreversibly.
- Irreversible inhibitors occupy or destroy the active sites of the enzyme permanently and decrease the reaction rate.
- Enzyme activity is not regained on dialysis.



MECHANISM OF ENZYME ACTION

Covalent Catalysis:

A group on the enzyme becomes covalently modified during reaction, e.g. by forming a covalent bond to the substrate during the reaction.

General Acid-Base Catalysis:

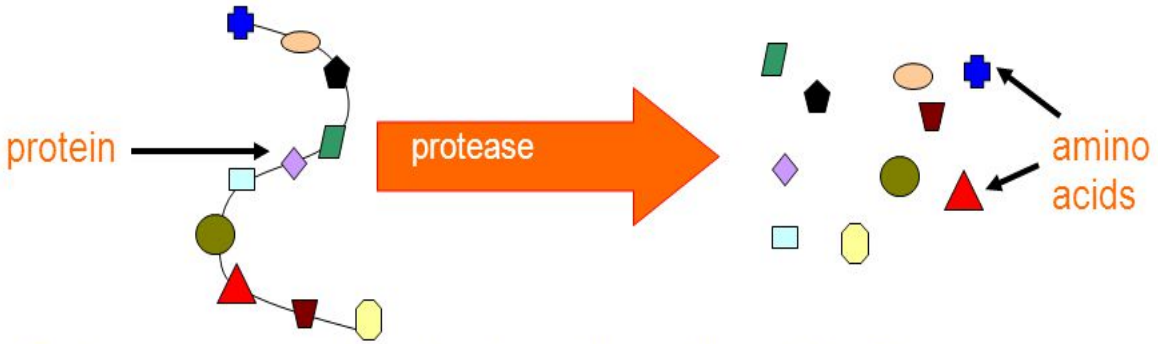
A group on the enzyme acts as an acid or base: it removes a proton from or donates a proton to the substrate during the reaction.

Metal Ion Catalysis:

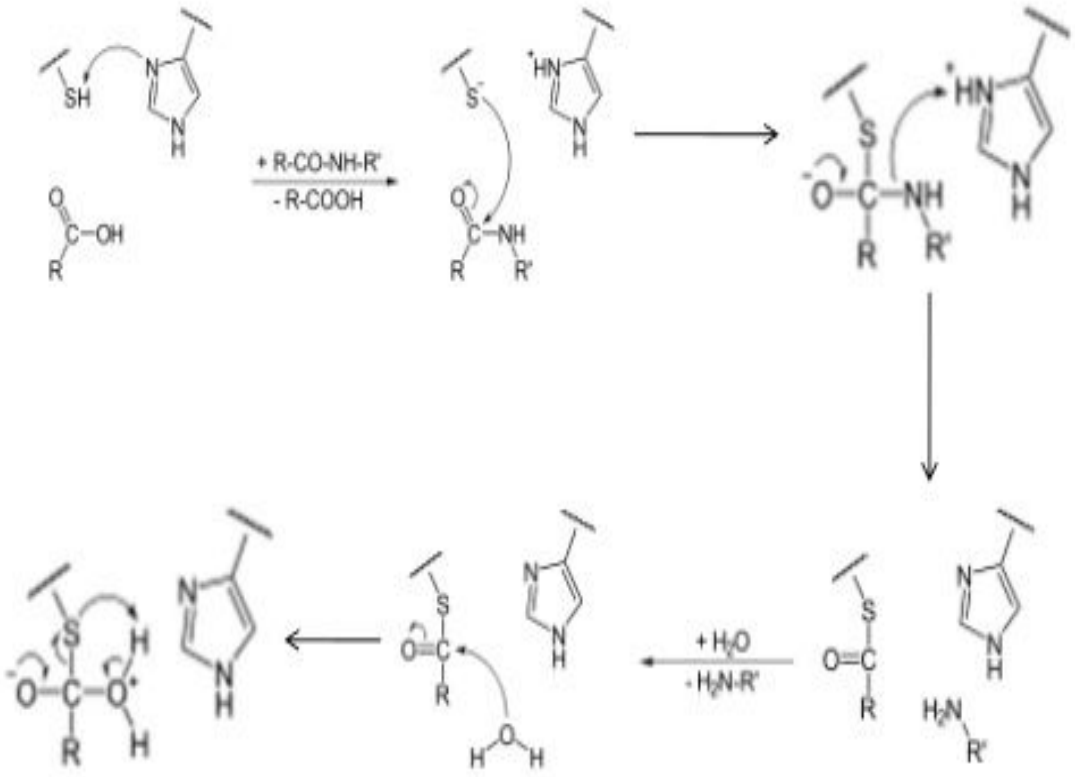
A metal ion is used by the enzyme to facilitate a chemical rearrangement or binding step.

EXAMPLES

Proteases are enzymes that catalyze the hydrolytic cleavage of peptide bonds in proteins. They break down proteins and peptides in food before they are absorbed.



Protease enzymes work best under *acidic* conditions (pH < 7).

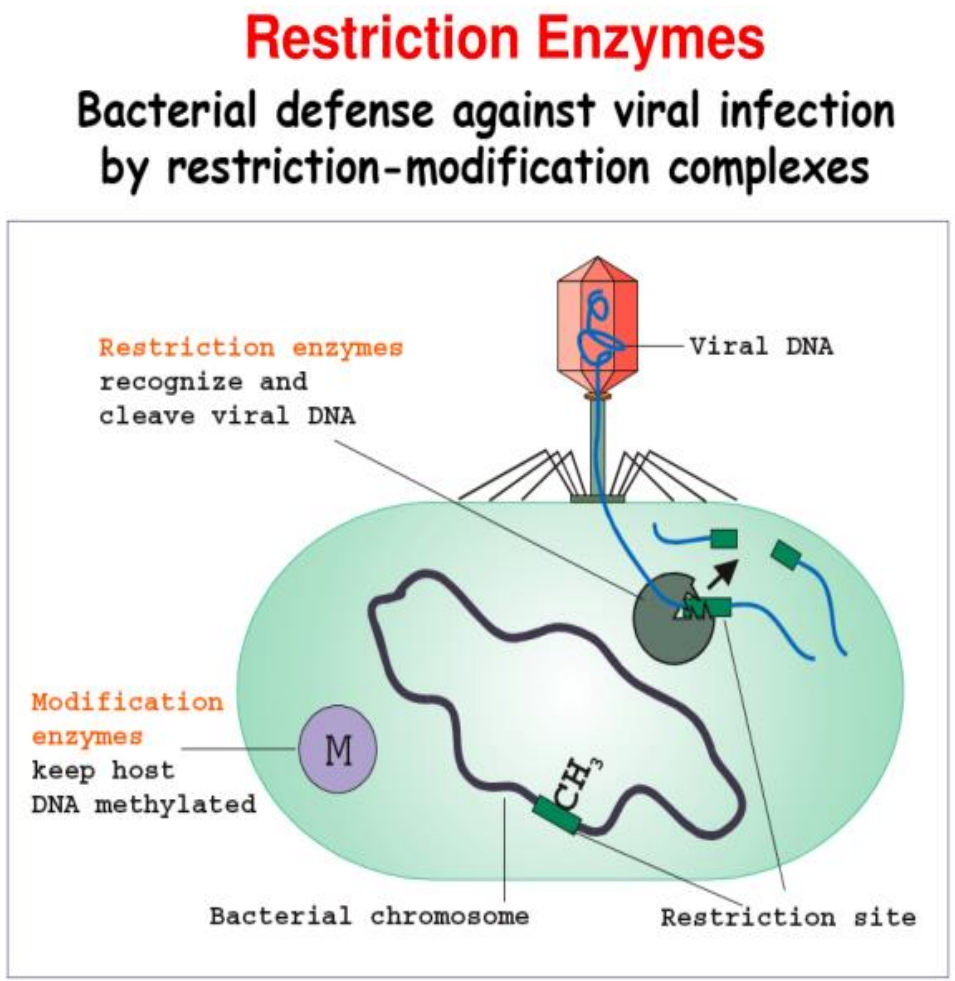


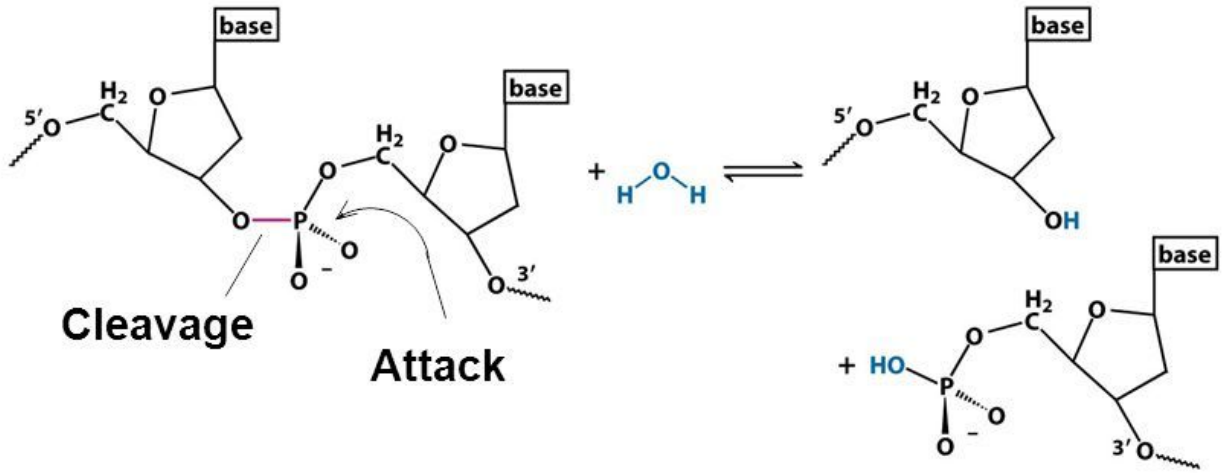
Mechanism of action of **Papain:**

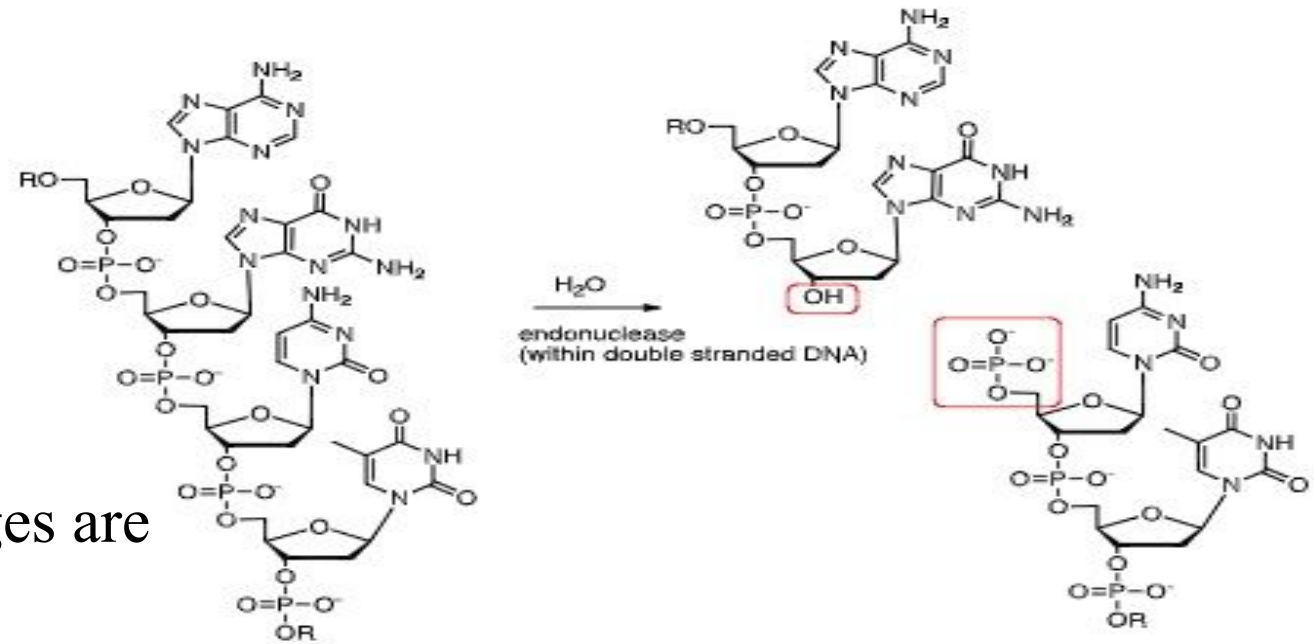
1. Deprotonation of thiol in cysteine by basic histidine - Acid hydrolysis

2. Nucleophilic attack by deprotonated cysteine on substrate carbonyl atom

EXAMPLES

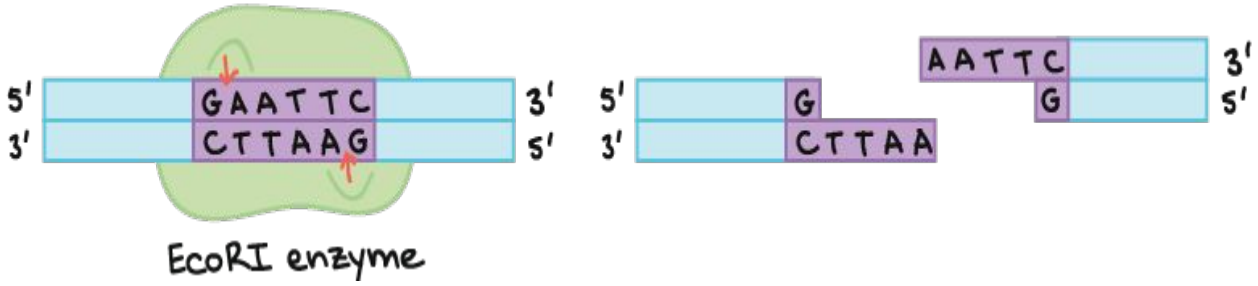


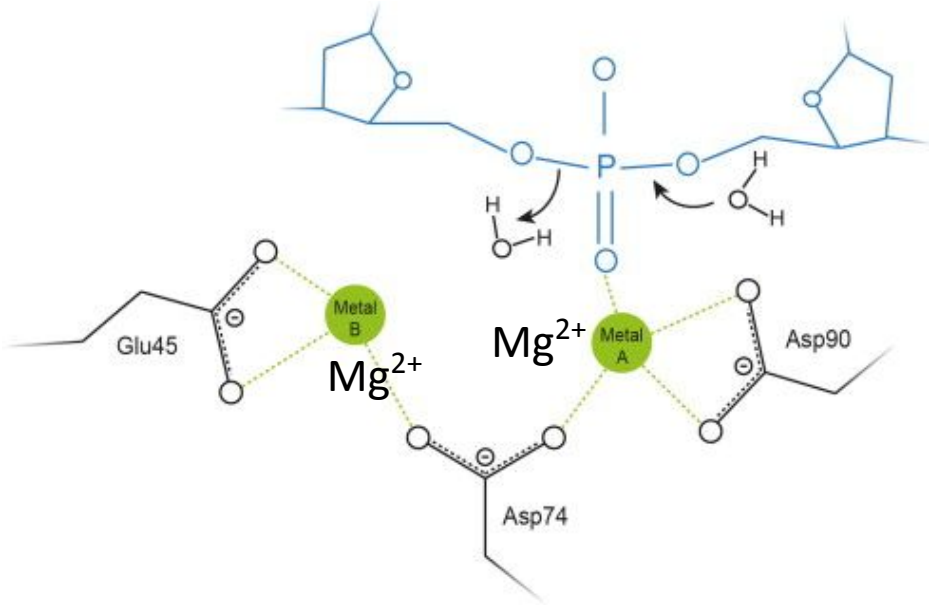




Enzymes are very specific w.r.t

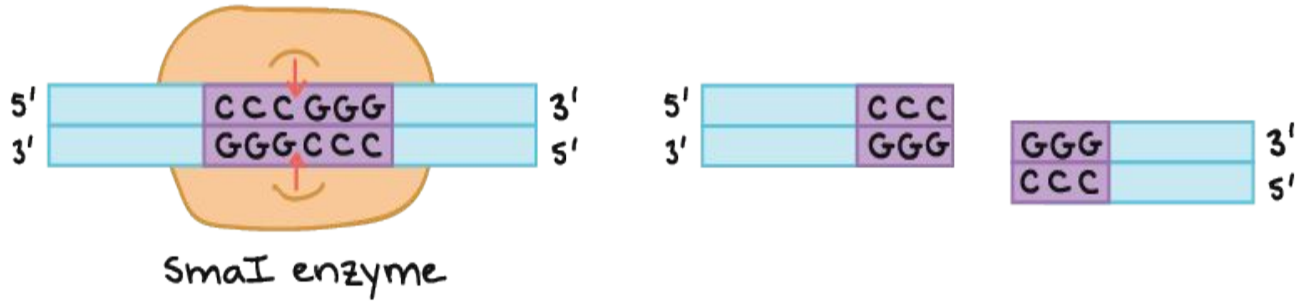
- Recognition sequences so that miscleavages are minimized.
- Host DNA should not be degraded.





Source	Restriction enzyme	Recognition site
Escherichia coli	EcoRI	5′ G↓AATTC 3′ 3′ CTTAA↑G 5′
Bacillus amyloliquefaciens	BamHI	5′ G↓GATCC 3′ 3′ CCTAG↑G 5′
Haemophilus influenzae	HindIII	5′ A↓AGCTT 3′ 3′ TTCGA↑A 5′

The symbols ↑ and ↓ indicate where the DNA is cut.



SUMMARY

- What are enzymes
- Naming and classification of enzymes
- Significance of enzymes
- Factors affecting enzyme activity
- Mechanism of enzyme action
- Examples of some enzymes