
بسم الله الرحمن الرحيم
رب اشرح لي صدري ويسر لي أمري
صدق الله العظيم

Enzymes

Presented By

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Objectives:

- **Concept for enzymes.**
- **Mechanism of enzyme action.**
- **Factors affect rate of enzyme action.**
- **Enzyme specificity.**
- **Enzyme kinetics (Km & Vmax).**
- **Enzyme inhibition.**
- **Regulation of enzyme activity.**
- **Clinical uses of enzymes in diagnosis and prognosis of different diseases.**
- **Classes of enzymes.**
- **Coenzymes.**

Catalytic Proteins 1: Enzymes

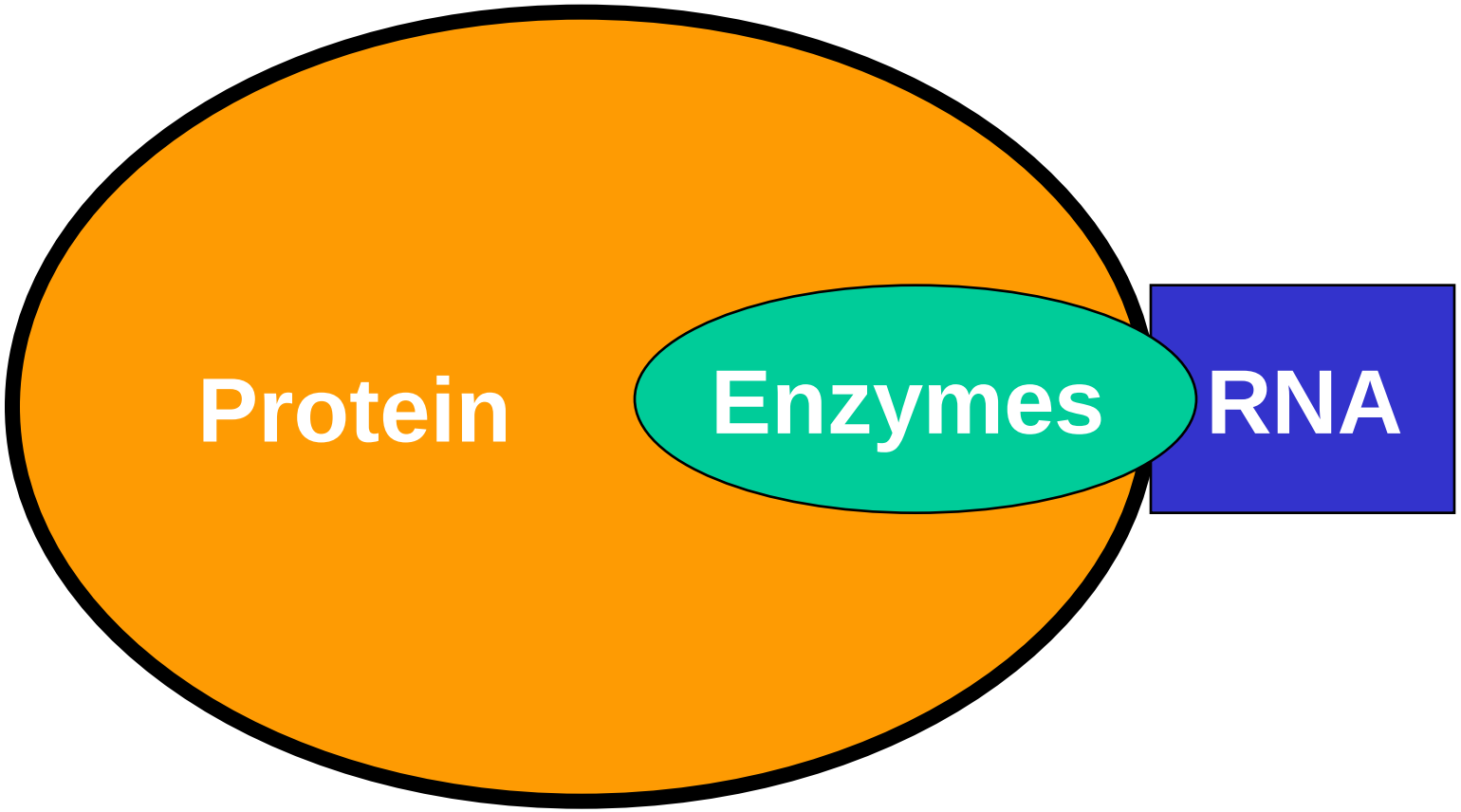
Enzymes: "The biological catalysts"

- They are organic thermo-labile catalysts that **increase** the chemical reaction without change.
- They accelerate the rate of chemical reaction **without being consumed in the reaction**.

Chemical Nature of Enzymes

- All enzymes are **protein** in nature except ribozymes (RNA in nature).

What is the difference between an enzyme and a protein?



- All enzymes are proteins except some RNAs
- not all proteins are enzymes

Chemical Nature of Enzymes

- **Protein enzymes** are classified into **2 types:**
- **1- Simple Protein enzymes:** They are formed of protein only.

2- Complex (conjugated) Protein : They are formed of protein part and non protein part.

2- Complex (conjugated) Protein :

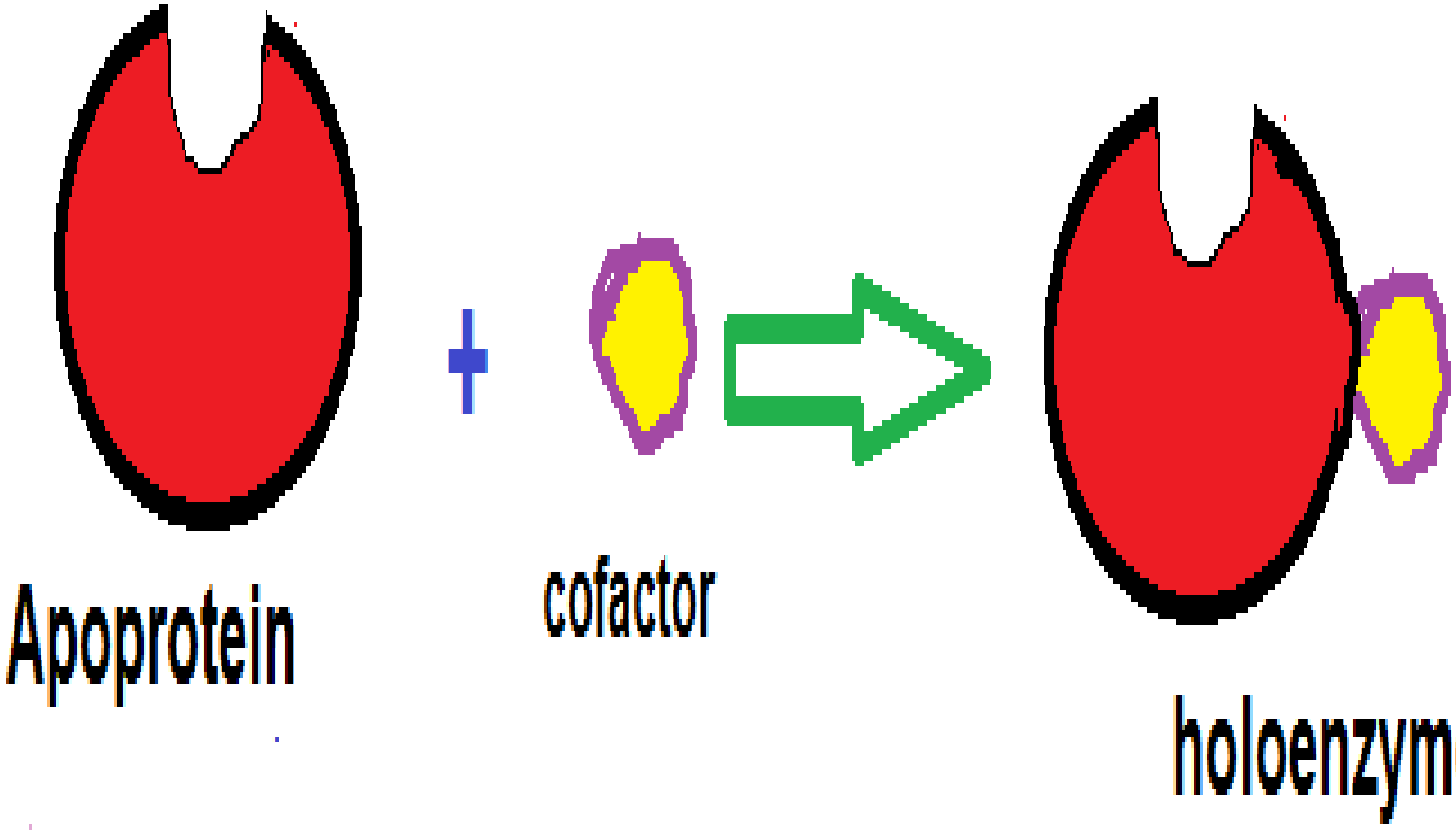
enzymes formed of **two parts:**

1) **Protein part:** called **apoenzyme**

2) **Non- protein:** called **cofactor**

- The **whole enzyme** is called **holoenzyme.**

NATURE OF ENZYME



The cofactor may be **coenzyme or prosthetic group**

- **Coenzyme: Is organic, thermo-labile , loosely attached to enzyme.**
- They are mainly **vitamin B derivatives e.g. FAD, NAD.**
- **Prosthetic group: Is inorganic, thermo-stable, firmly attached to enzyme.**
- They are usually **metal ions e.g. Ca, Zn**

Enzymes vocabulary

substrate

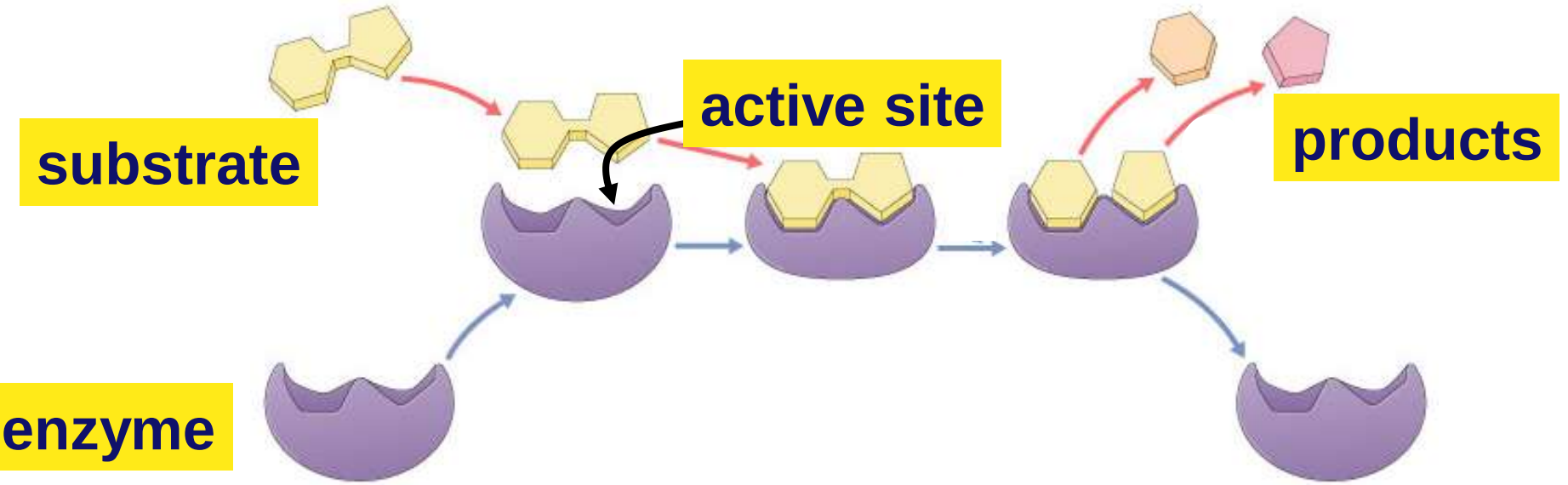
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association

product

- end result of reaction

active site

- enzyme's catalytic site; substrate fits into active site

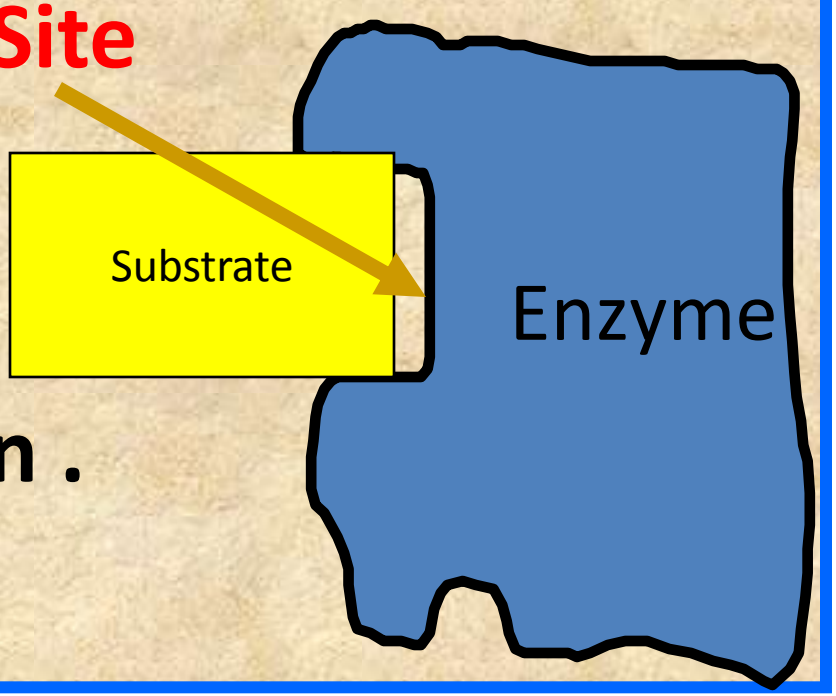


ACTIVE SITE(CATALYTIC SITE)

- A **restricted region** of an **enzyme** molecule which **binds** to the **substrate.**
- **It is formed from**

Amino acids sequences

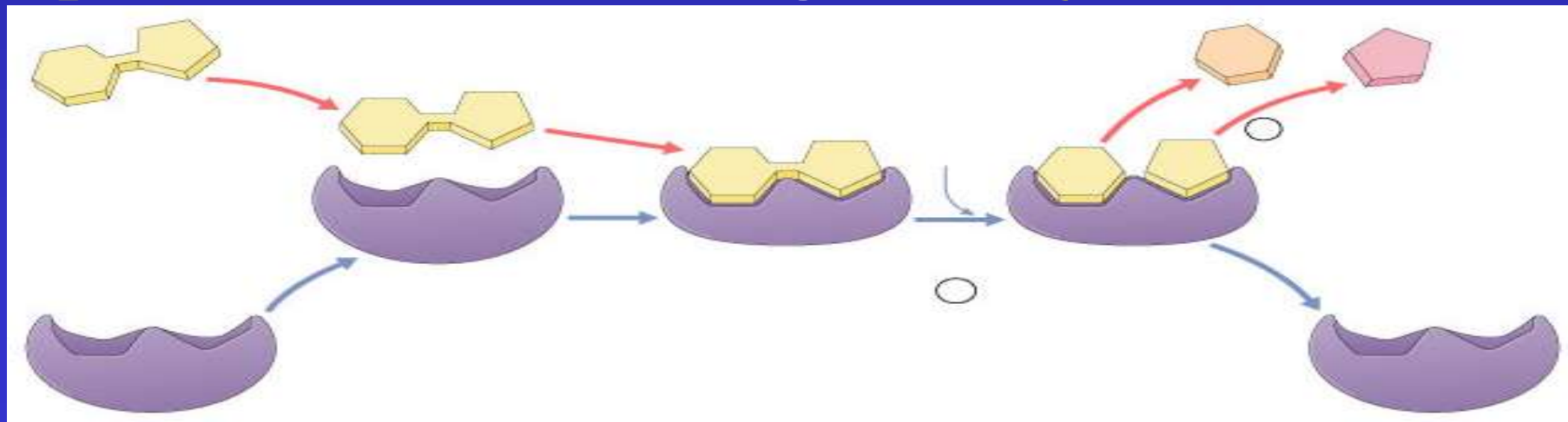
in the polypeptide chain .



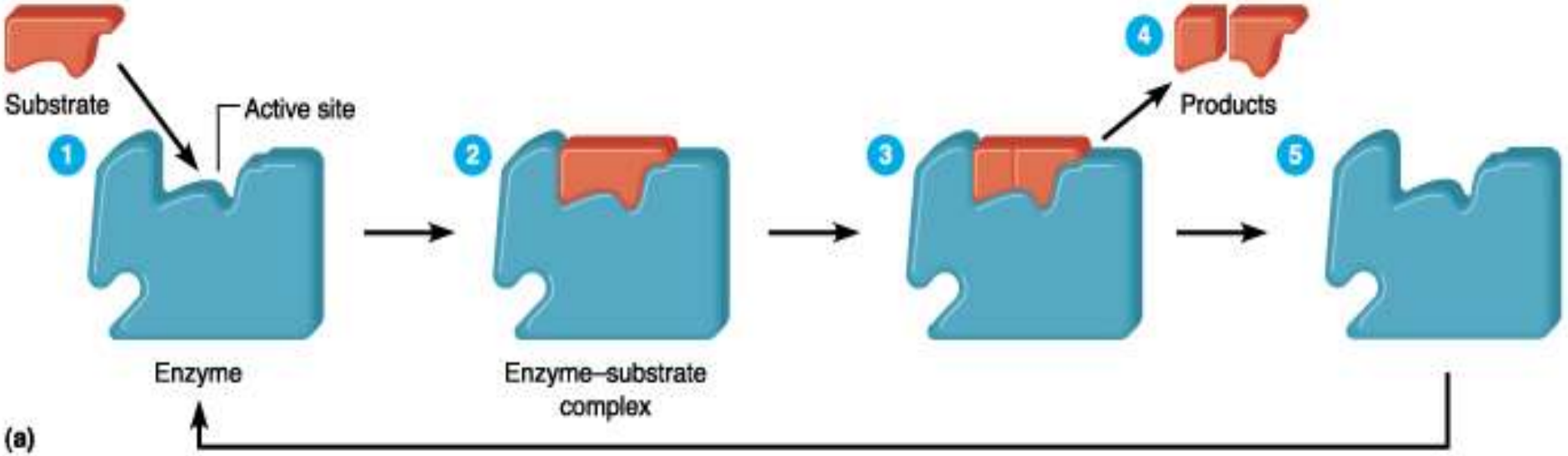
Mechanism of enzyme action

1- The **substrate (S)** binds to the **enzyme (E)** at its active catalytic site to form activated intermediate **enzyme substrate complex (ES).**

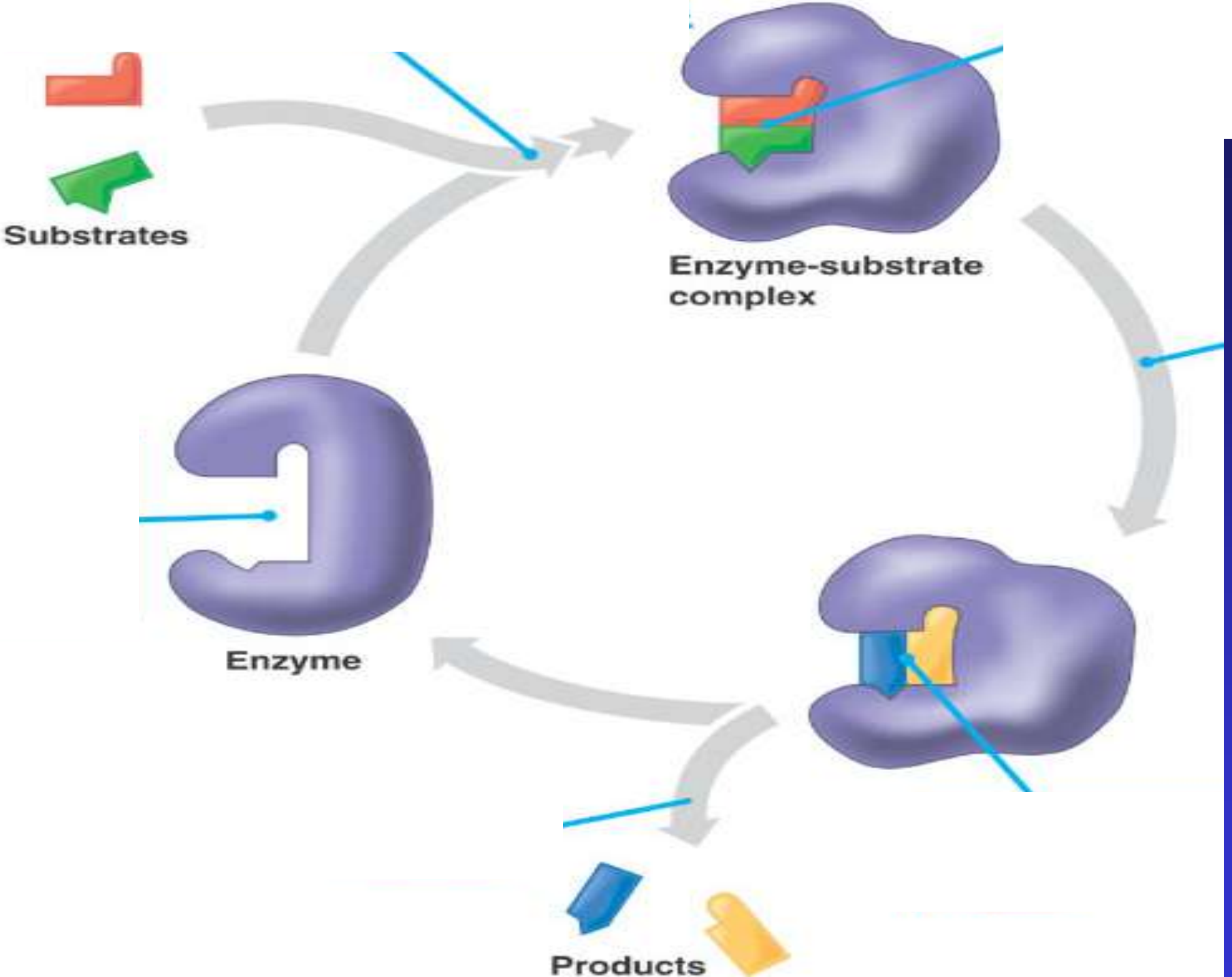
2- The activated complex (ES) **cleaved** to the **products (P)** and the original **enzyme (E)**

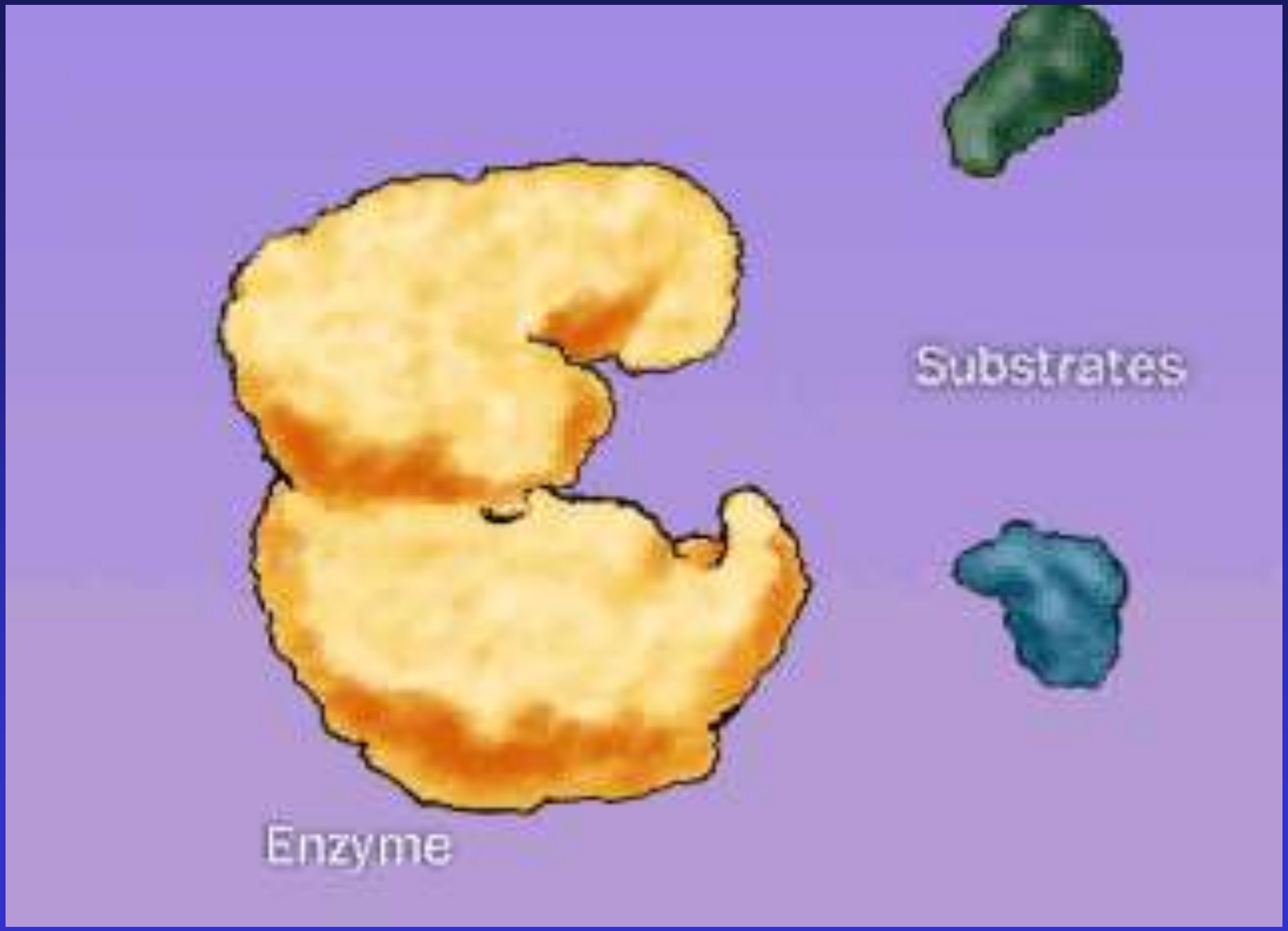


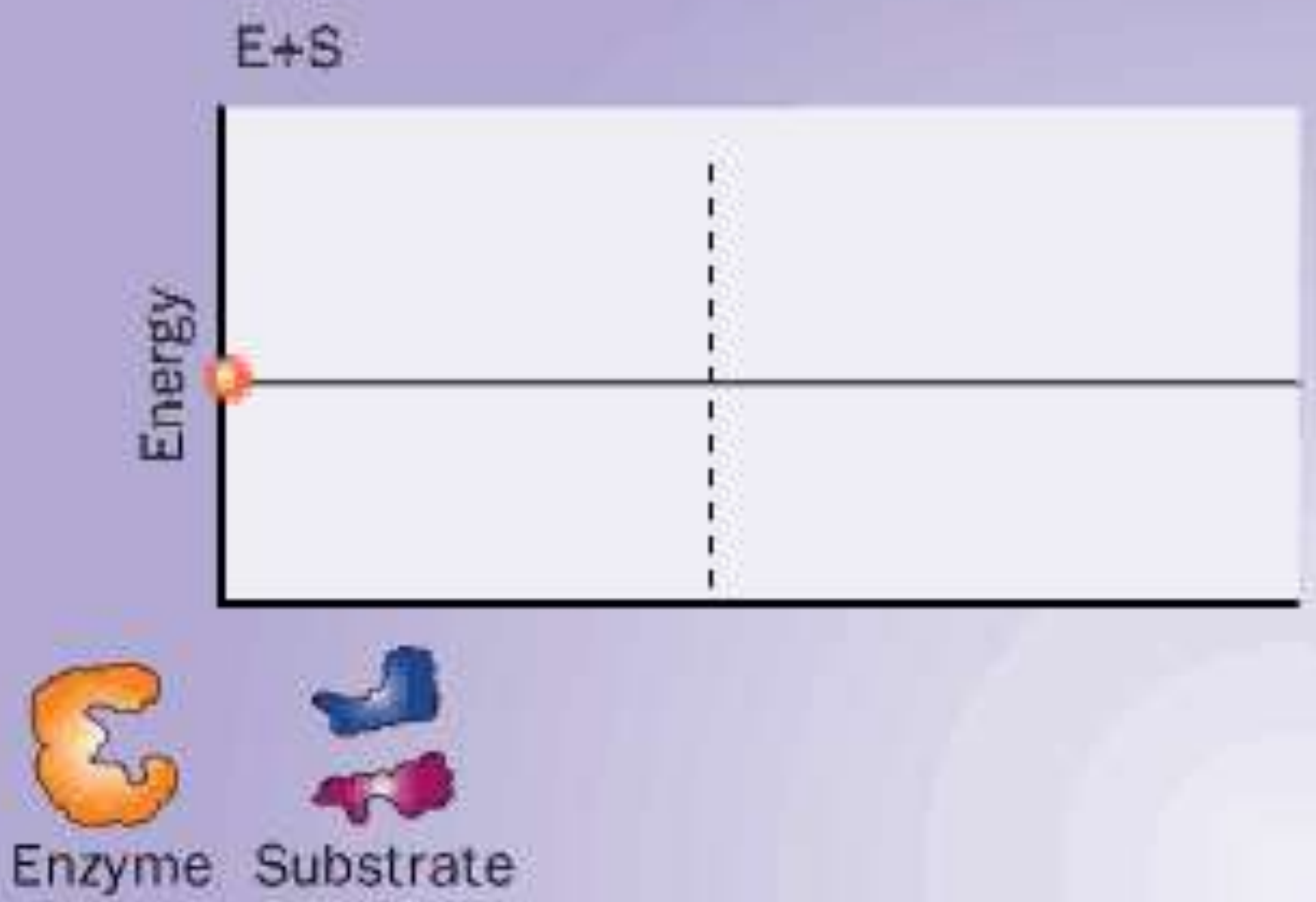
Enzymatic reaction steps



1. Substrate approaches active site
2. Enzyme-substrate complex forms
3. Substrate transformed into products
4. Products released
5. Enzyme recycled


Substrates
Enzyme-substrate complex
Enzyme
Products


Substrates
Enzyme


E+S
Energy
Enzyme
Substrate

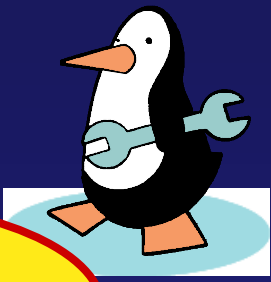
In **conjugated protein enzymes**, the **coenzyme** acts as an **acceptor** for **one of the products** helping the **cleavage** of the enzyme substrate complex.

Theories of enzyme substrate binding (Two theories)

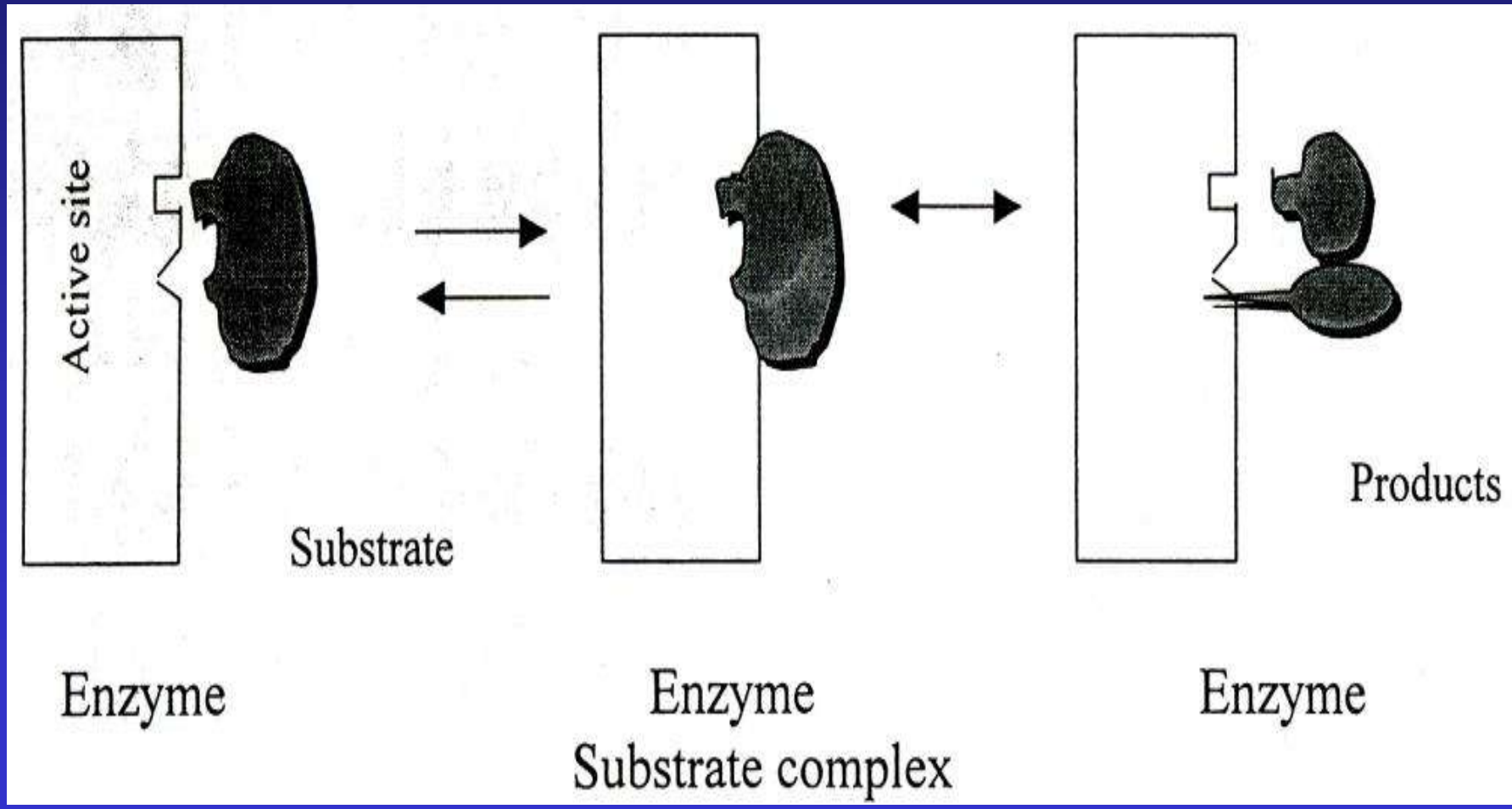
1- Lock and key theory: "key fits into lock"

- The **catalytic site** of the enzyme has a shape that is **complementary (fit)** to the shape of the **substrate**.
- ***The substrate fits in this catalytic site in a similar way to lock and key. The key will only fits its own lock .***

Key and lock model

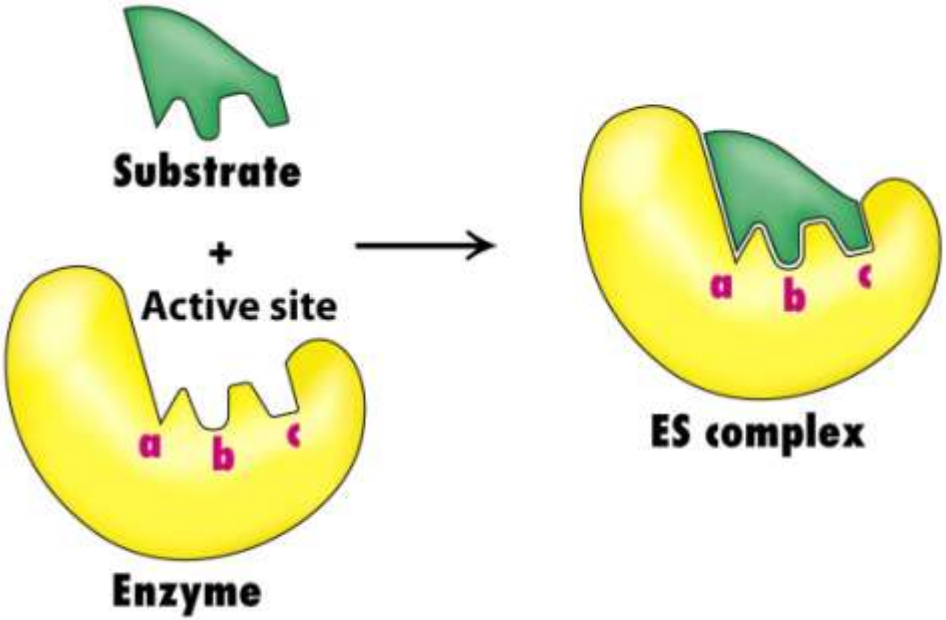


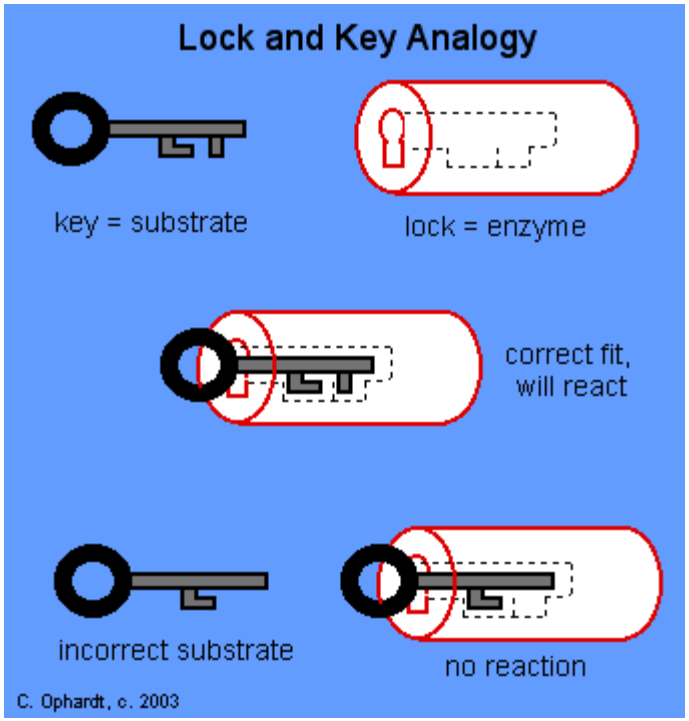
Size
doesn't matter...
Shape matters!



Lock and Key model

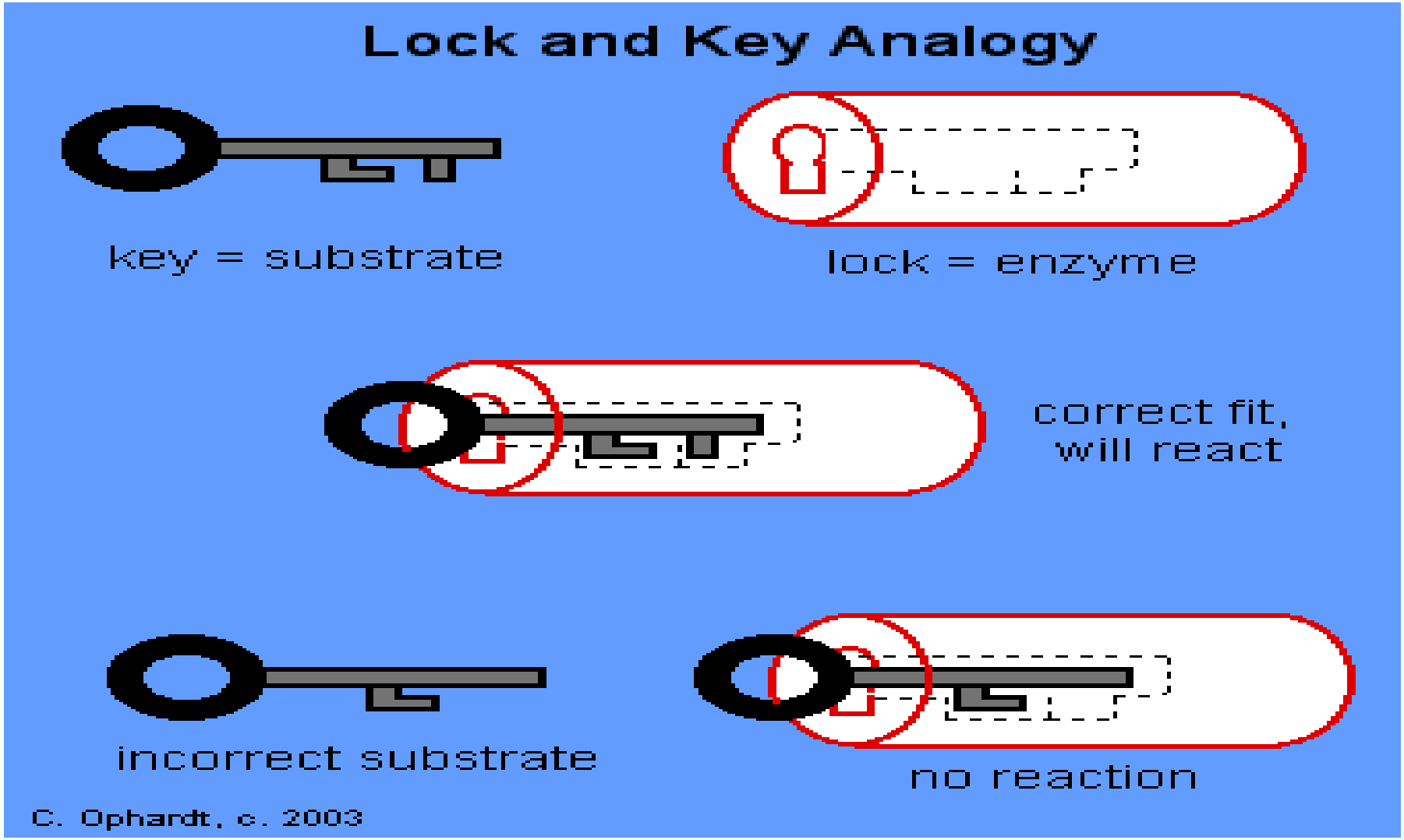
Proposed by Fischer in 1894





In this model, the active sites of the unbound enzyme is complementary in shape to the substrate

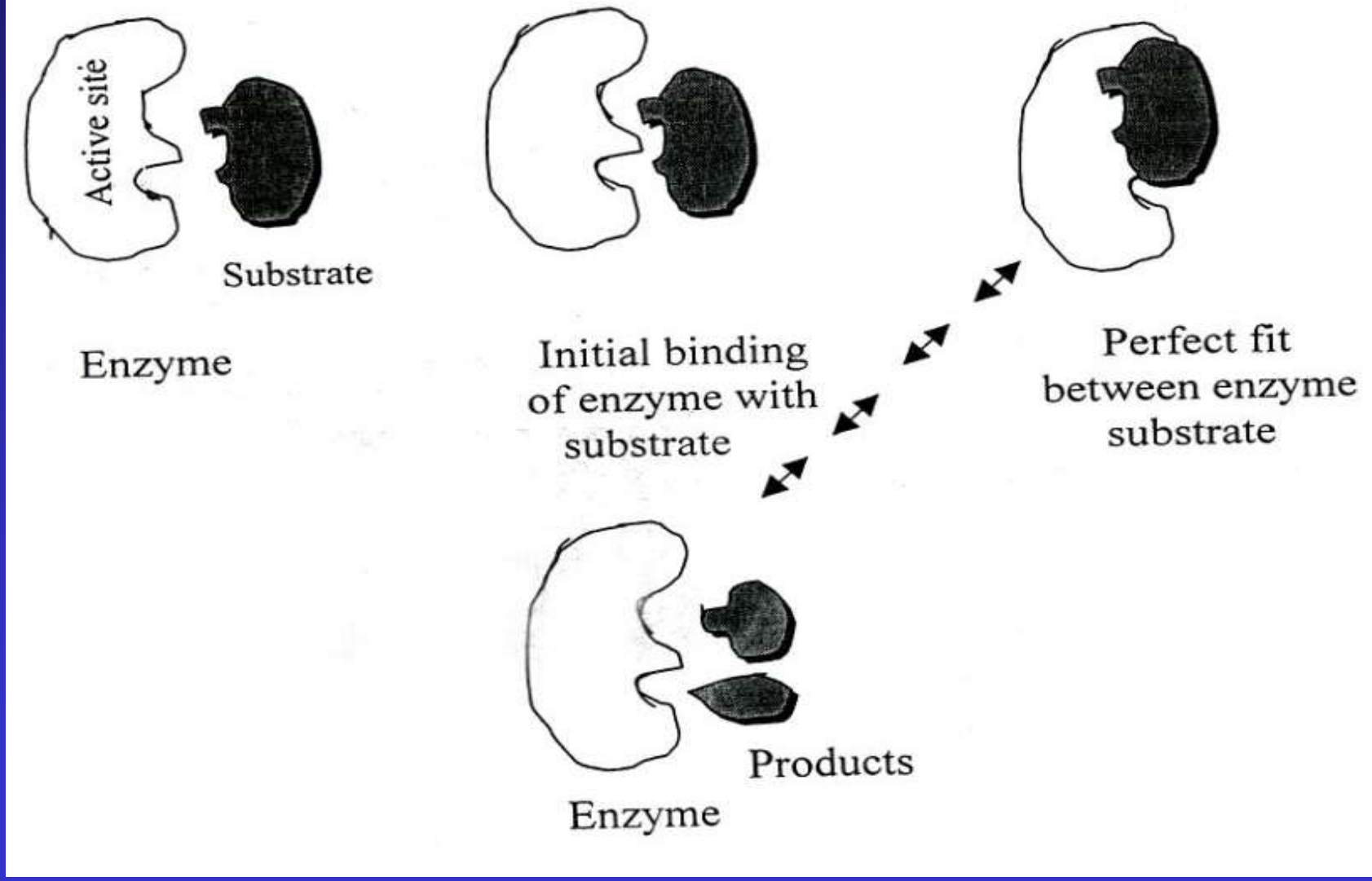
KEY AND LOOK


Lock and Key Analogy
key = substrate
lock = enzyme
correct fit,
will react
incorrect substrate
no reaction
C. Ophardt, c. 2003

2- Induced fit theory: -

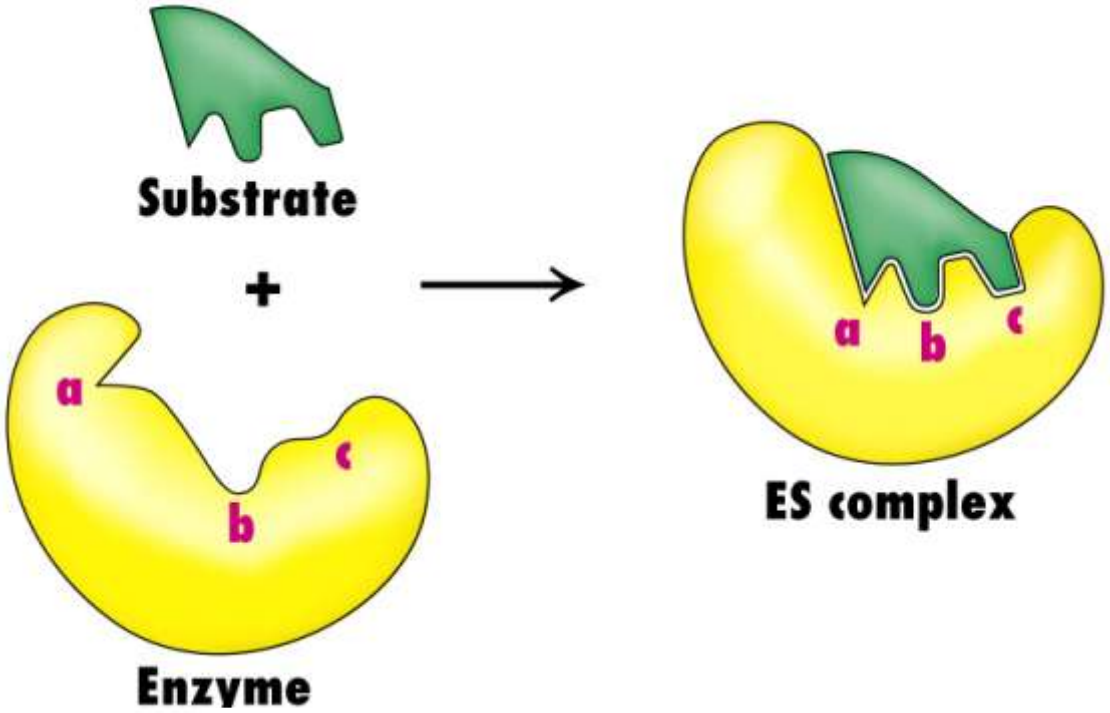
- *It is a more **flexible model**, where the catalytic site is **not fully formed.***
- The **catalytic site** of the enzyme is **not complementary** to the substrate.
- **Binding** of the substrate to the enzyme **induces changes** in the **shape** of the catalytic site **making it more fit** for substrate.

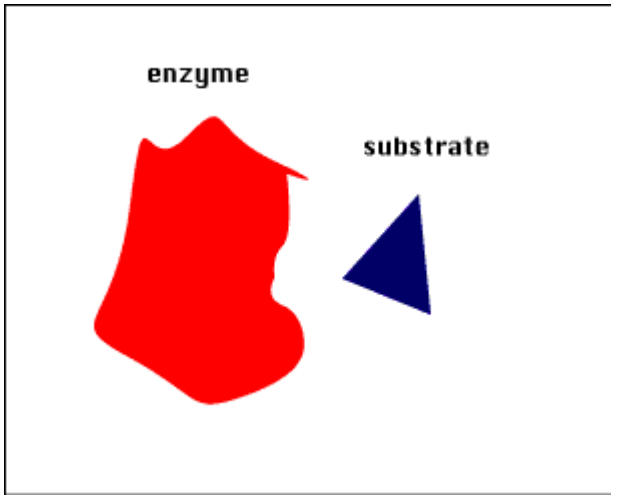
Induced- fit model



Induced-fit model

Proposed by Koshland in 1958



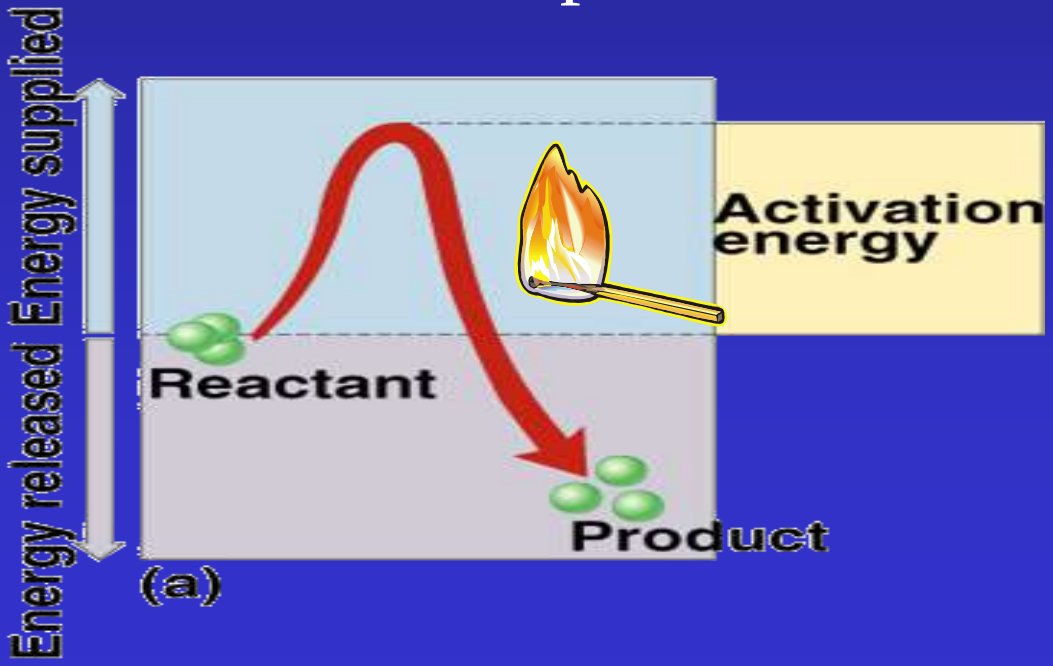


In this model, the enzyme changes shape on substrate binding

Enzyme action

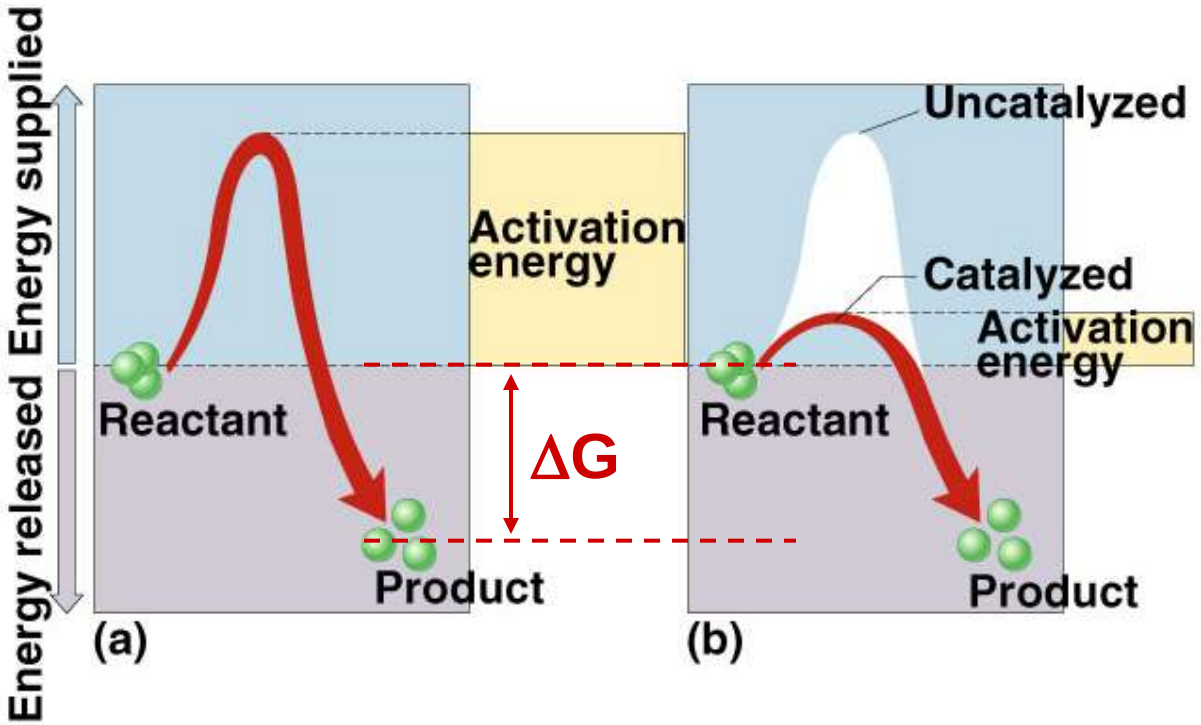
1- Enzymes **increase the rate** of reaction by **decreasing the activation energy** of reaction.

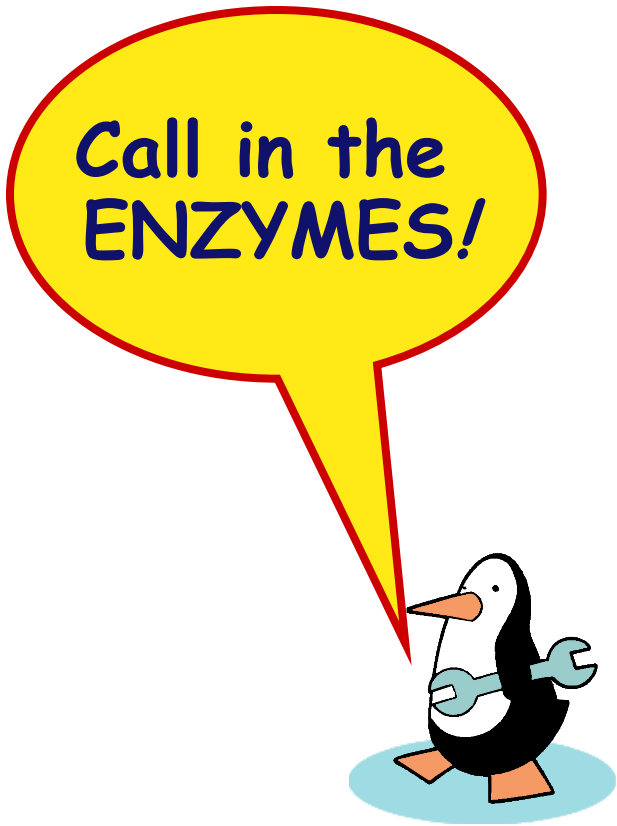
2- The **activation energy** is the energy barrier between reactants and products.



- **So what's a cell got to do to reduce activation energy?**
  - **get help! … chemical help…**

ENZYMES

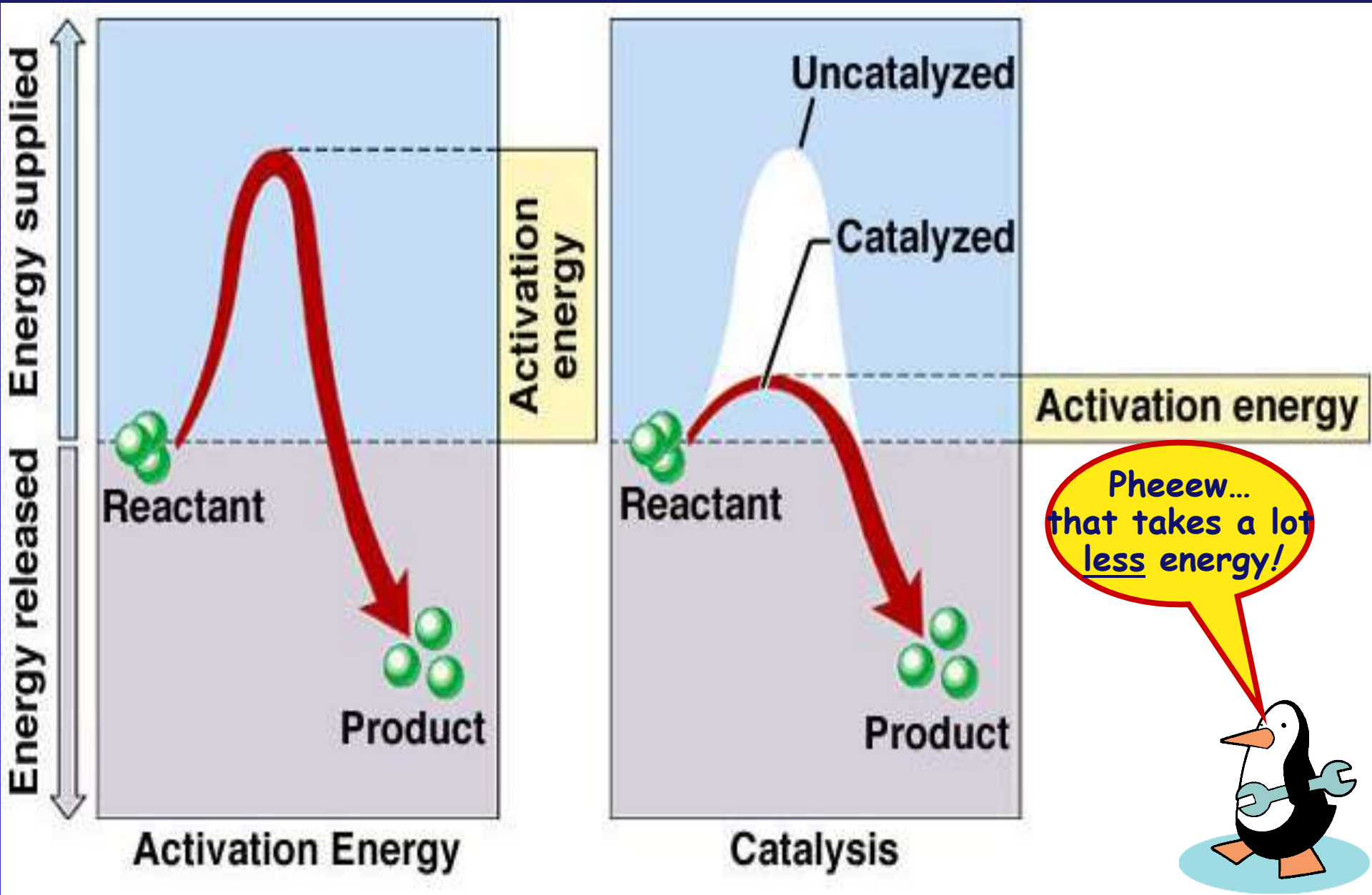


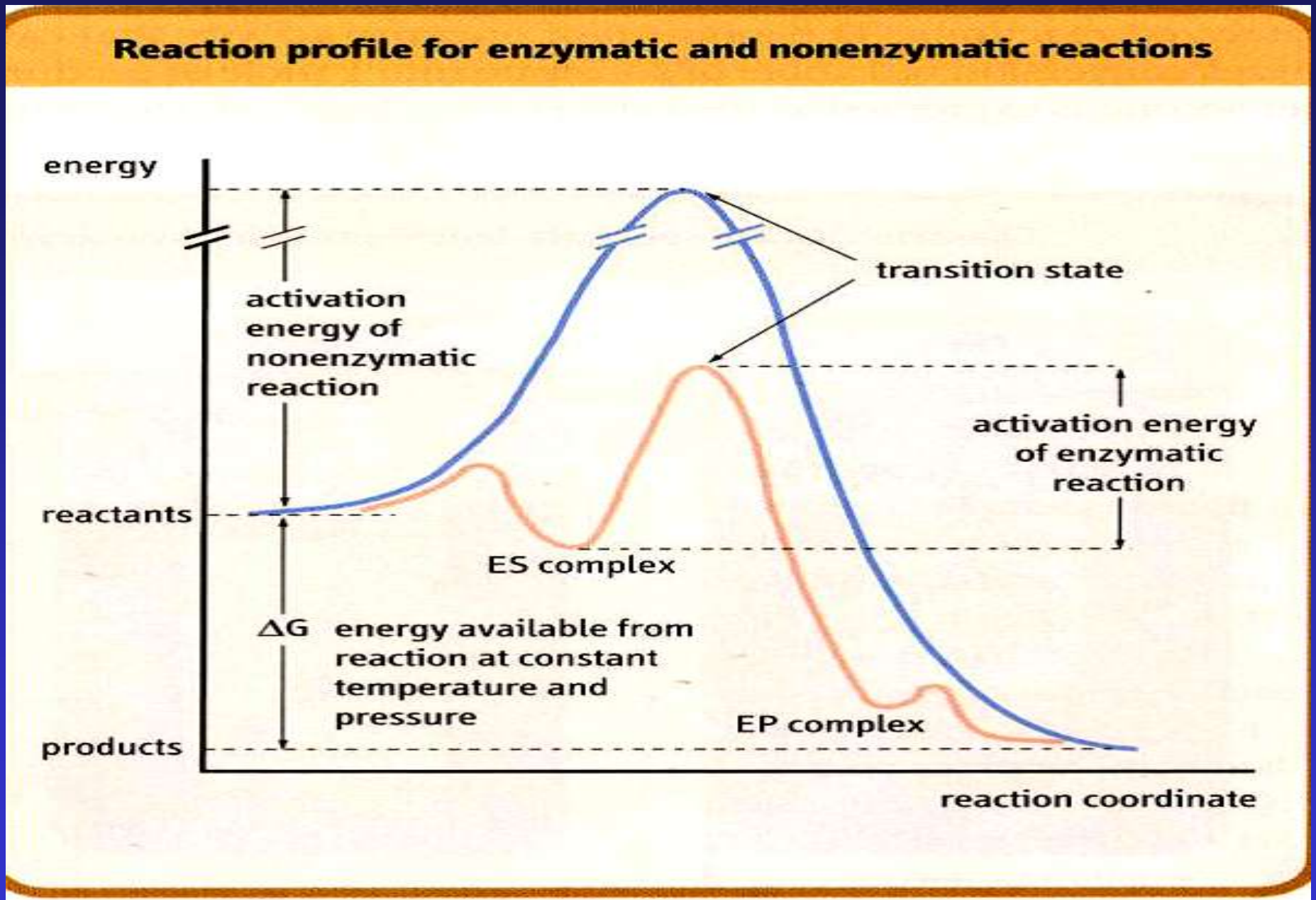


Enzymes increase the rate of reaction by:

- It **decreases** the energy needed for activation **(activation energy).**
- It **decreases** the **energy barrier** between reactants and end products.

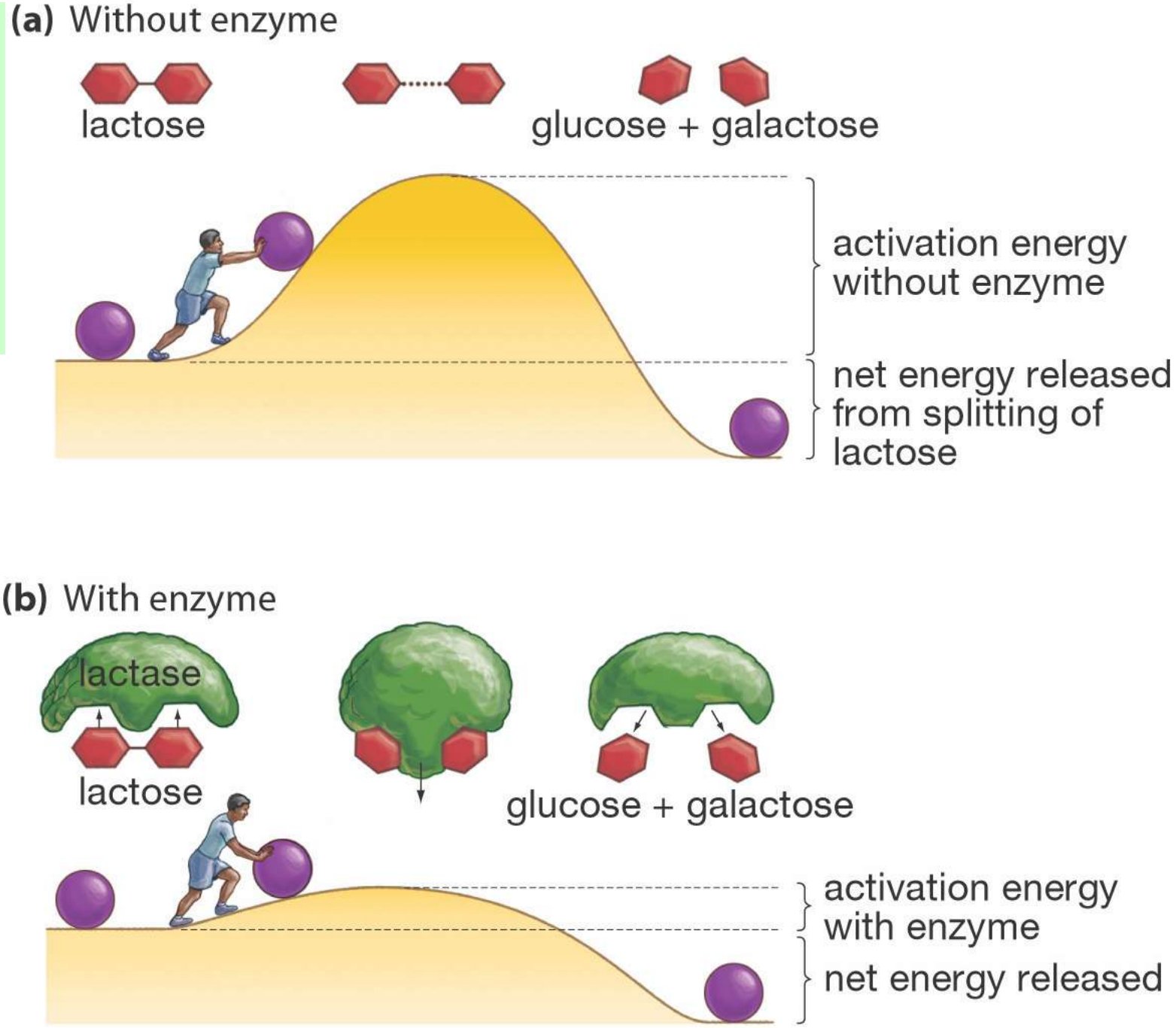
Catalysts Work by Reducing the Activation Energy of a Reaction

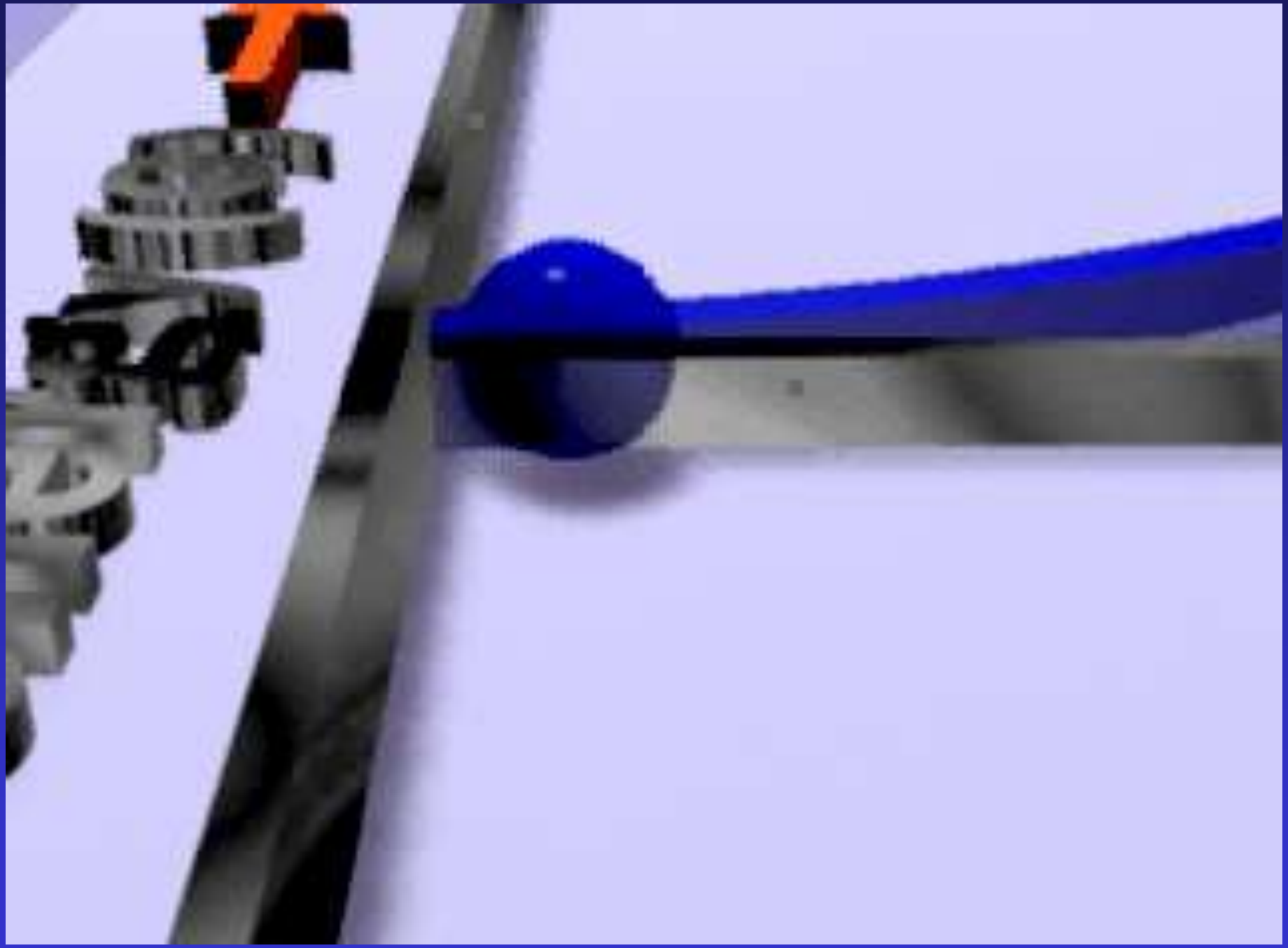




Reaction profile for enzymatic and non-enzymatic reactions

Enzymes Lower a Reaction's Activation Energy



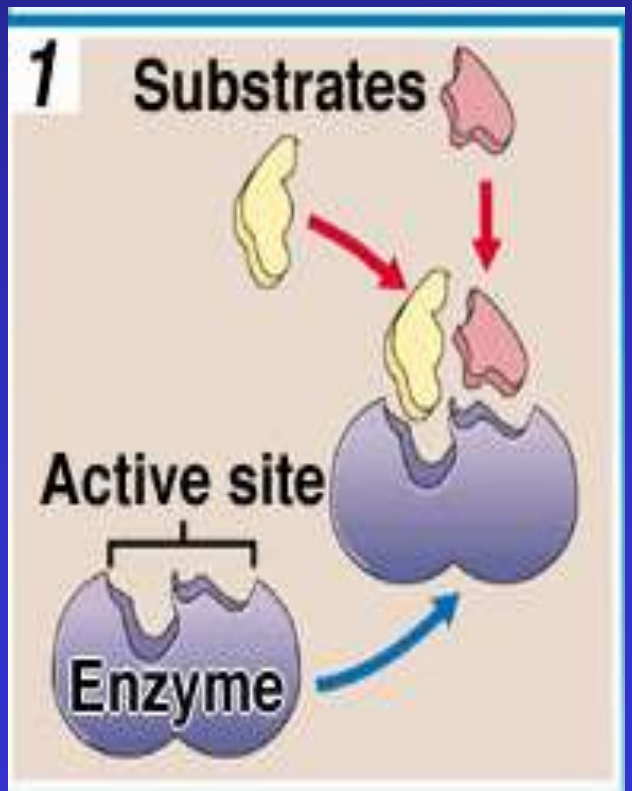


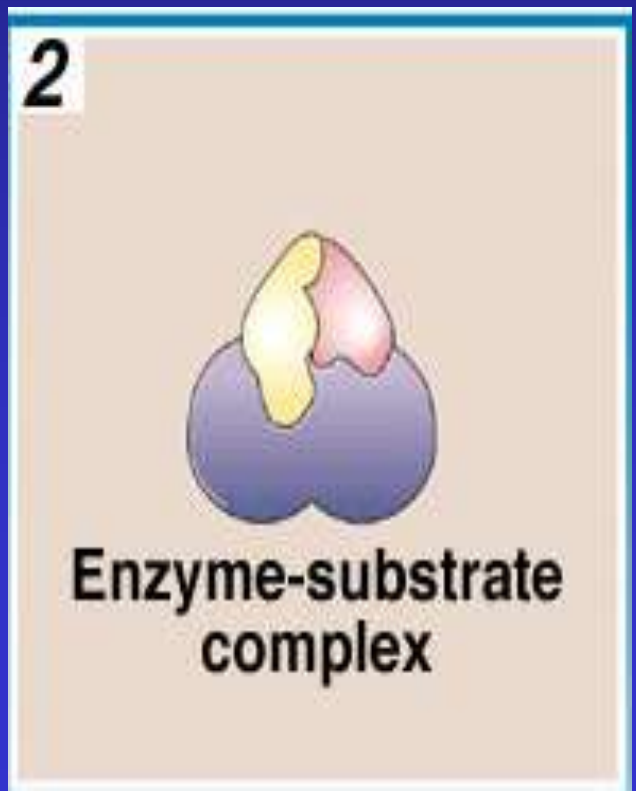
Mechanism of Enzyme Activity

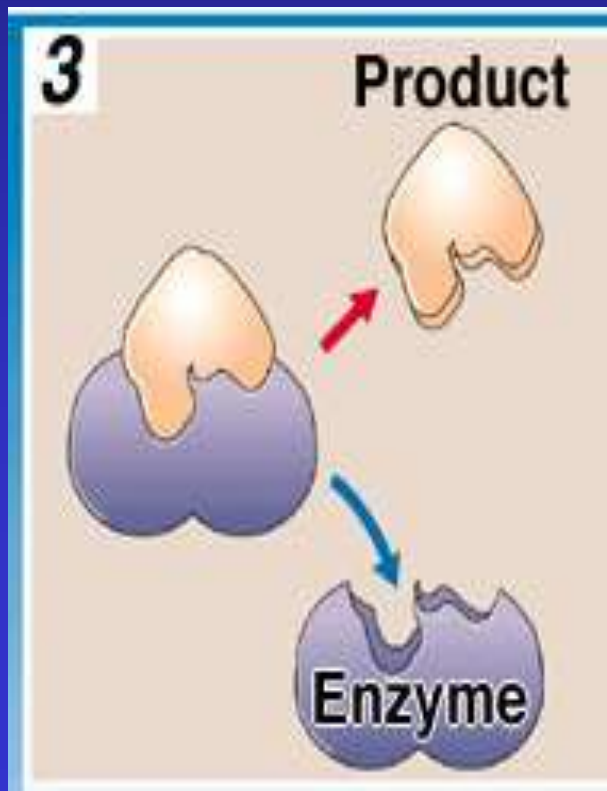
A substrate(s) fits into a binding site on the enzyme.

The enzyme lowers the energy required to reach the transition state.

The product no longer fits the binding site and is released.







Enzyme Nomenclature

• The word **enzyme** is formed from two Greek words: **en** means **inside** and **zyme**, which means **yeast** i.e., the word enzyme means **inside yeast**.

• **There are many methods for naming enzymes:**

1- The **old trivial name** as **pepsin** and **trypsin.**

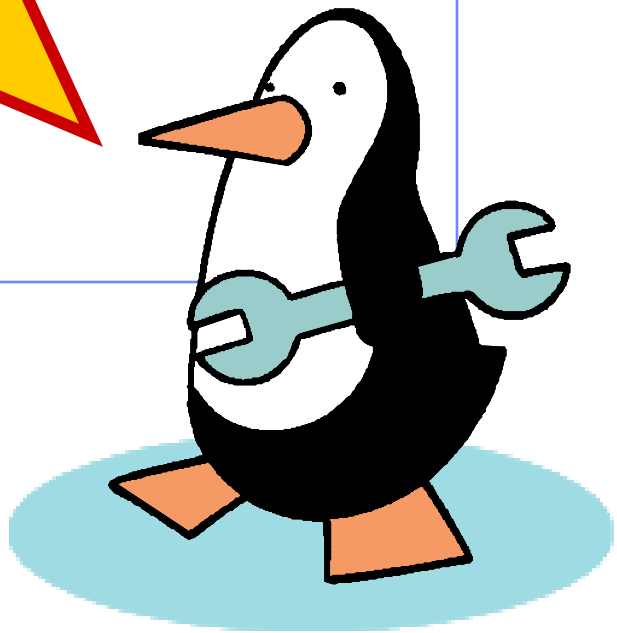
2- The name of **substrate and the suffix – ase** added to it as **lactase** acting on lactose and **sucrase** acting on sucrose.

3- Two words, one for the **substrate** and the other for the **type of reaction** e.g. **succinate dehydrogenase, pyruvate decarboxylase** and **glutamine synthetase**.

- **Enzyme Code (EC):** Each enzyme has a numerical code which is formed of **four digits** separated by dots:
  - The **first digit** denotes the **class (reaction type) of the enzyme.**
  - The **second digit** denotes the **functional group** upon which the enzyme acts.
  - The **third digit** denotes the **coenzyme.**
  - The **fourth digit** denotes the **substrate.**

For example **1.1.1.1 enzyme**, **1 means oxido-reductase**, **1.1** means that the functional group is **hydroxyl group (-OH), 1.1.1** means **NAD** is the coenzyme and **1.1.1.1** means **alcohol**. So, 1.1.1.1 means **alcohol dehydrogenase** enzyme.

Got any Questions *?!*



Activity

- **1- Prosthetic group is :**

A) organic.

B) thermostable.

C) losely attached to the enzyme

D) called apoenzyme.

- **2- The catalytic activity of an enzyme is restricted to its small portion called**

(A) Active site

(B) Passive site

(C) Allosteric site

(D) All Choices are correct

Activity

- **3-An activated enzyme made of polypeptide chain and a co-factor is**

  (A) Coenzyme
  (B) Substrate
  (C) Apoenzyme
  (D) Holoenzyme

3- Enzymes are largely …… in their chemical nature.

(A) Lipids
(B) Steroids
(C) Protein
(D) All A, B and C

Activity

- **5-The "lock and key" model of enzyme action illustrates that a particular enzyme molecule**

(A) forms a permanent enzyme-substrate complex

(B) may be destroyed and resynthesized several times

(C) interacts with a specific type of substrate molecule which is complementary to its shape

(D) reacts at identical rates under all conditions

- 1- Discuss :

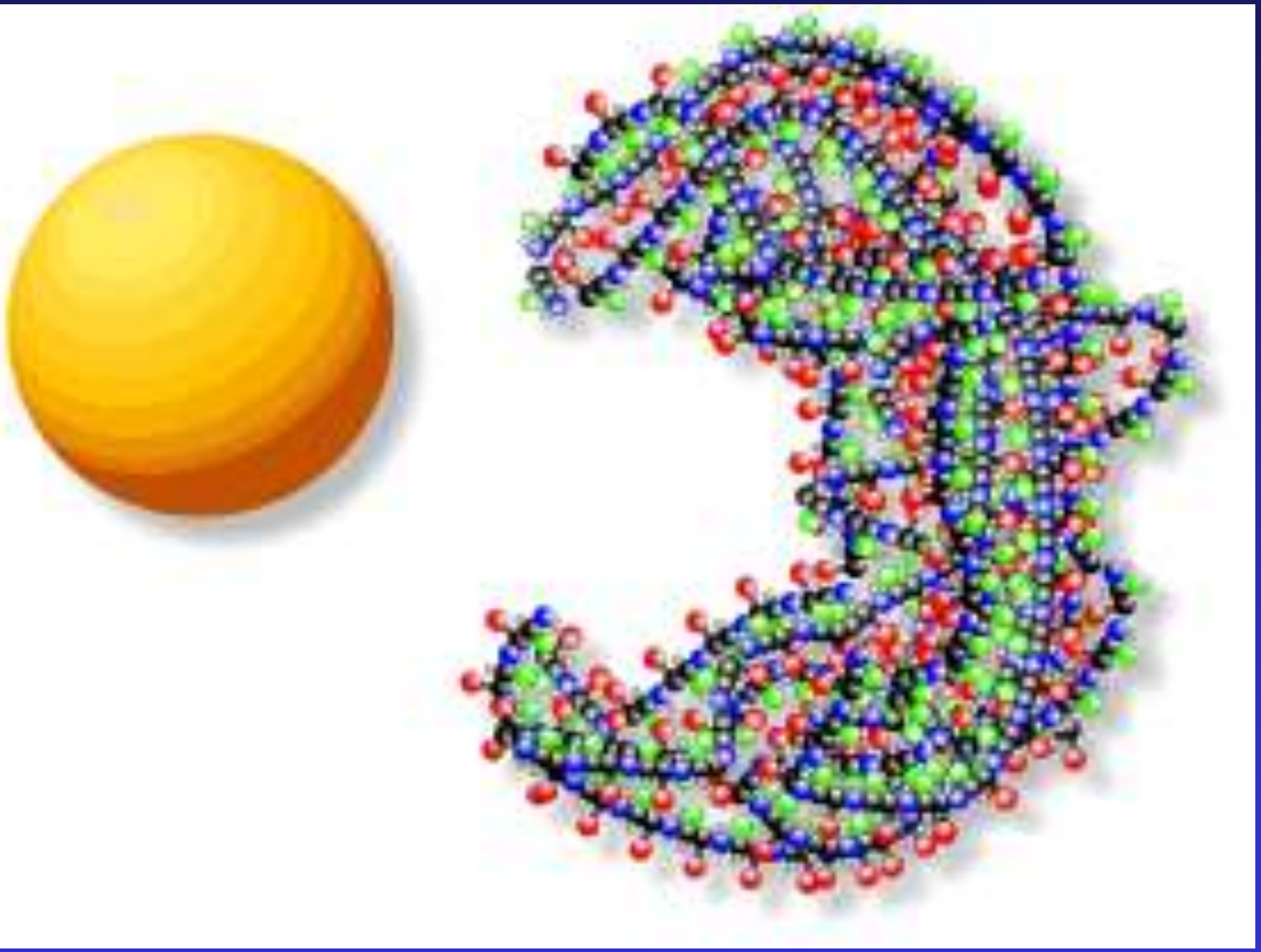
  (A) Theories of enzyme action

  (B) Enzyme code

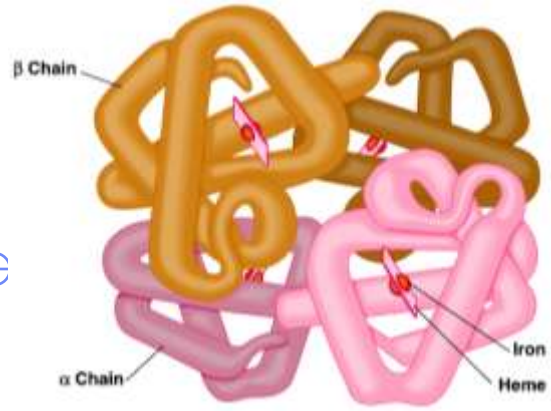
- 2- Give an account on the chemical nature of the enzymes.

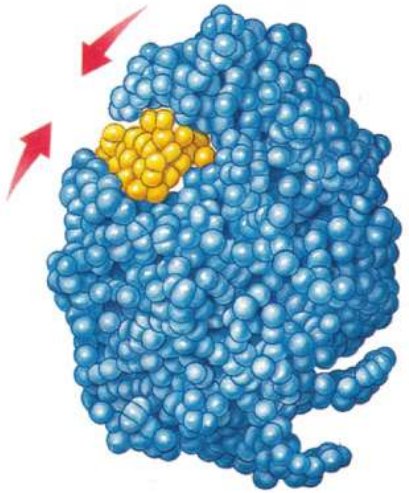
- 3- In a table ,give 3 differences between:

  Coenzyme & prosthetic group.

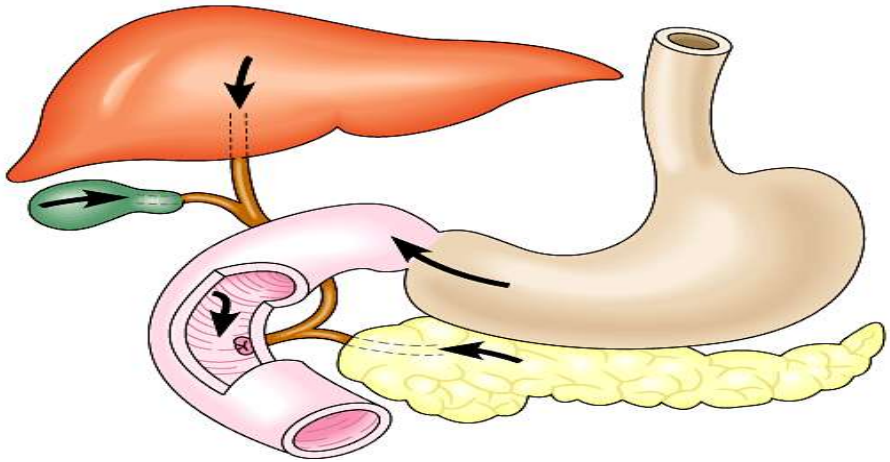


Thank you





Factors Affect Rate of Enzyme Action



Factors Affect Rate of Enzyme Action

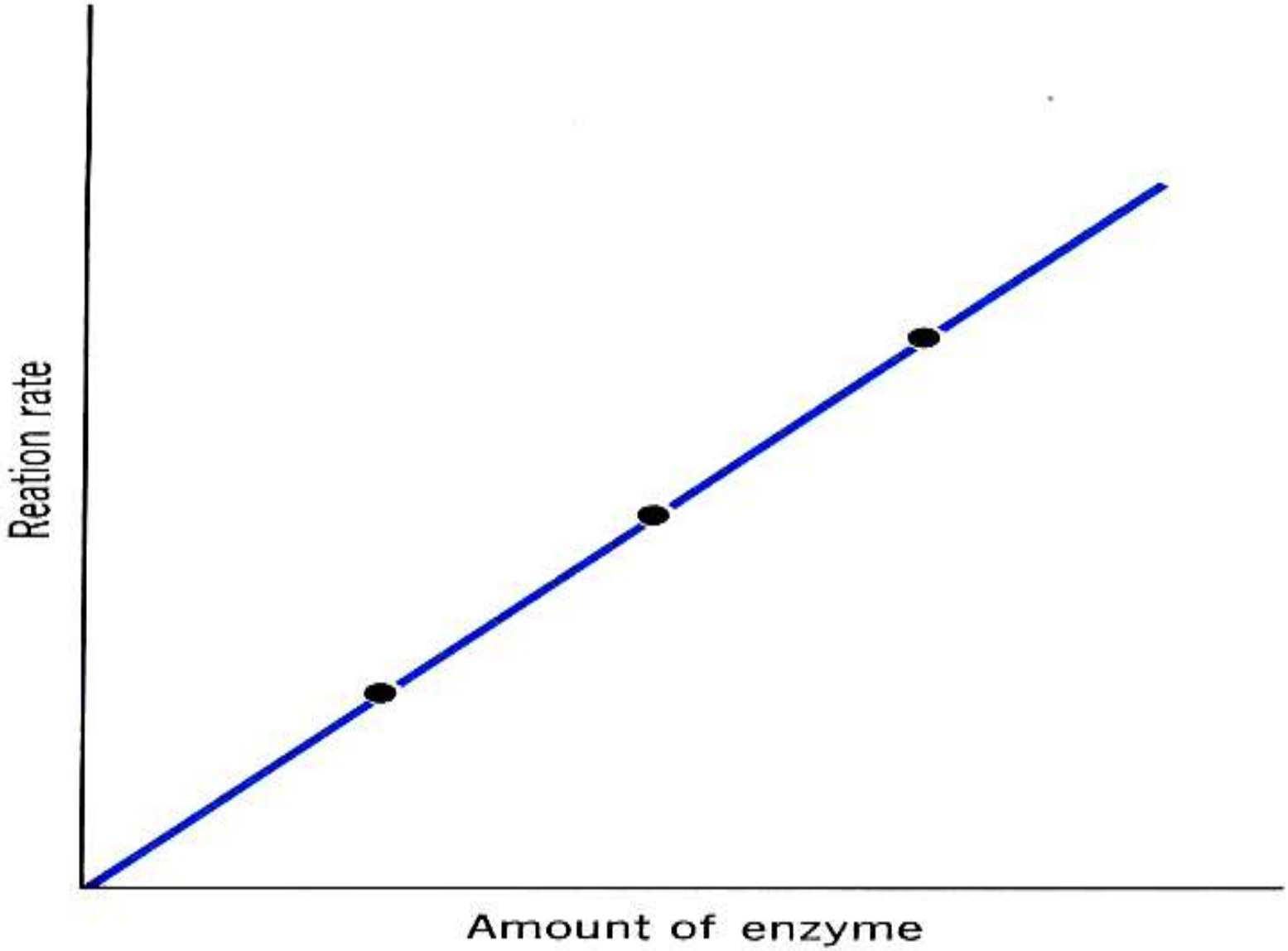
- Enzyme concentration
- Substrate concentration
- Temperature
- pH
- Concentration of coenzymes
- Concentration of ion activators
- Time
- Inhibitors

catalase

Factors affecting the rate of enzyme action

1- Effect of enzyme concentration

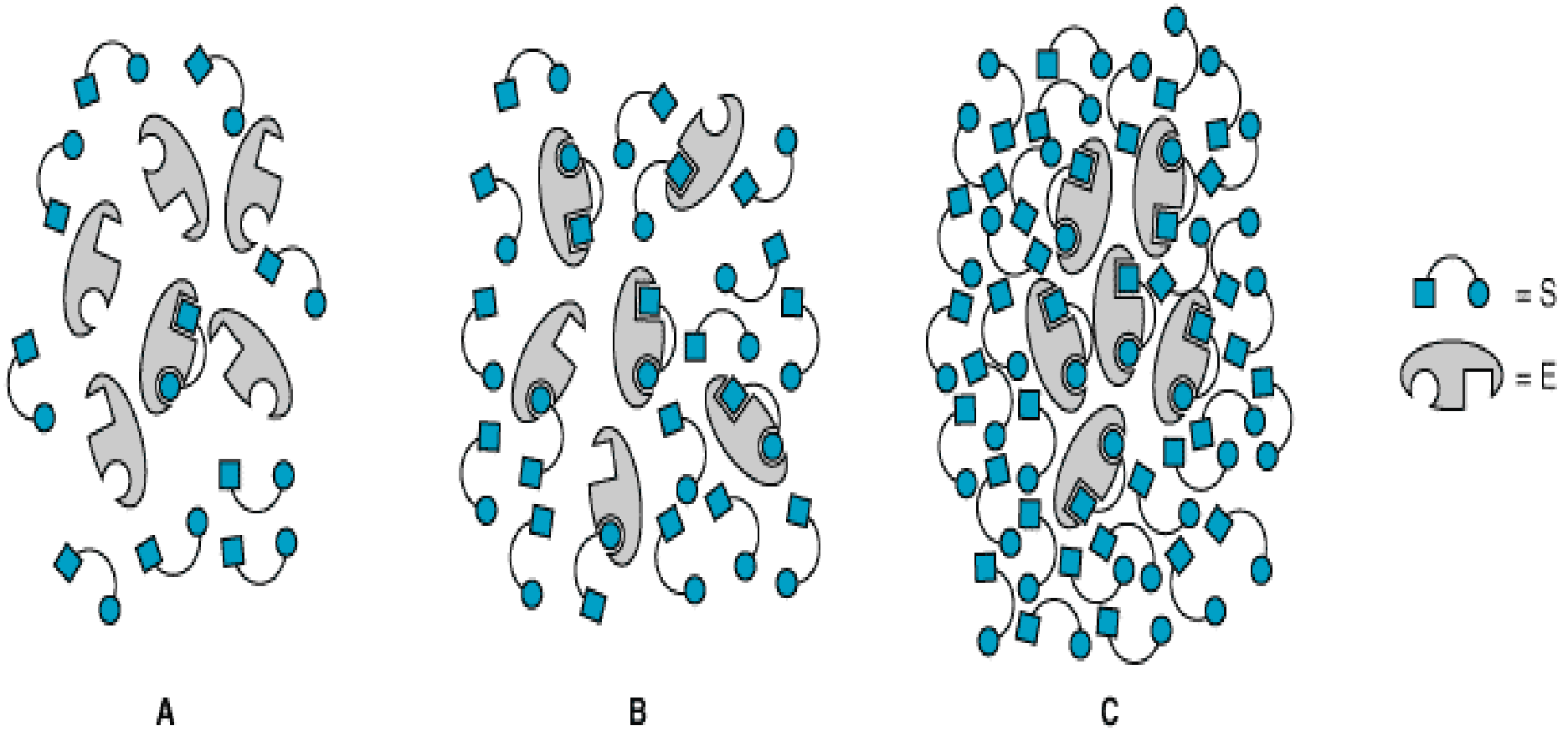
The rate of enzyme action is **directly proportional** to the concentration of enzyme **provided** that there are sufficient supply of substrate & constant conditions.


Reation rate
Amount of enzyme

2- Effect of substrate concentration

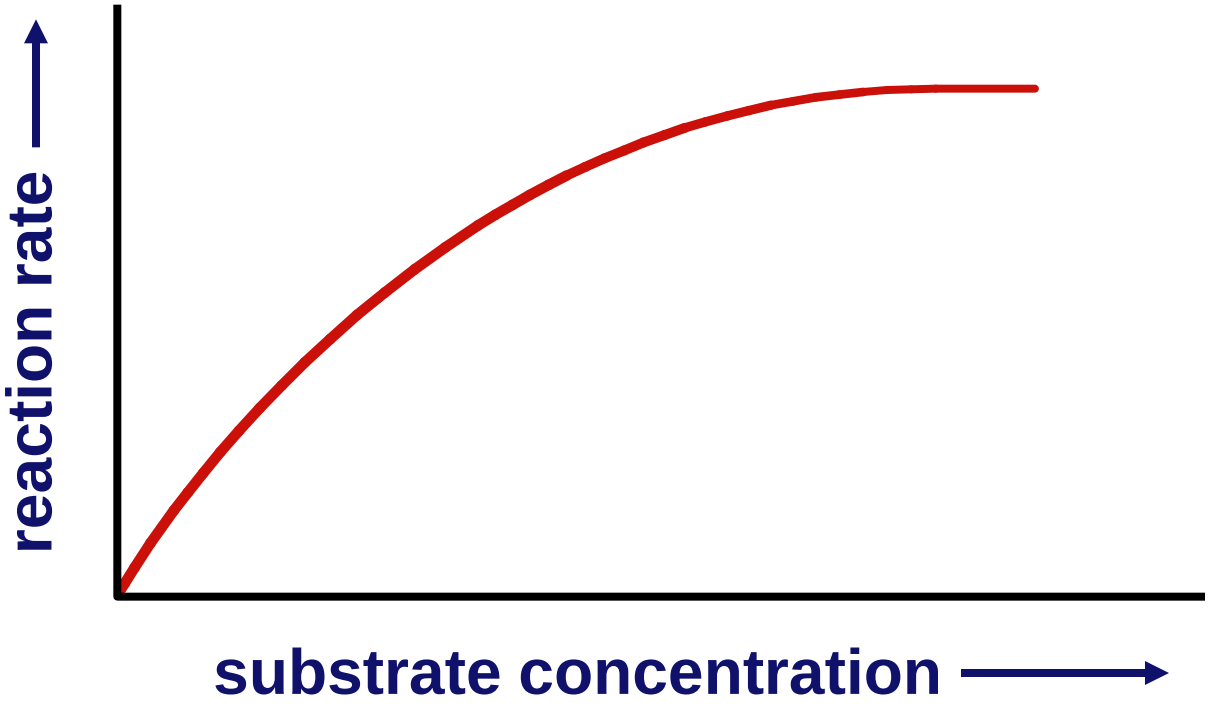
-The rate of reaction **increases** as the substrate concentration increases up to certain point at which the reaction rate is maximal **(Vmax.)**

At Vmax, the enzyme is **completely saturated** with the substrate any increase in substrate concentration doesn't affect the reaction rate.

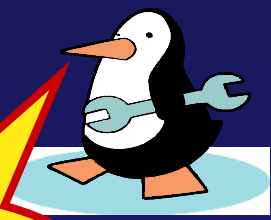


Source: Murray RK, Granner DK, Rodwell VW: *Harper's Illustrated Biochemistry*, 27th Edition: http://www.accessmedicine.com

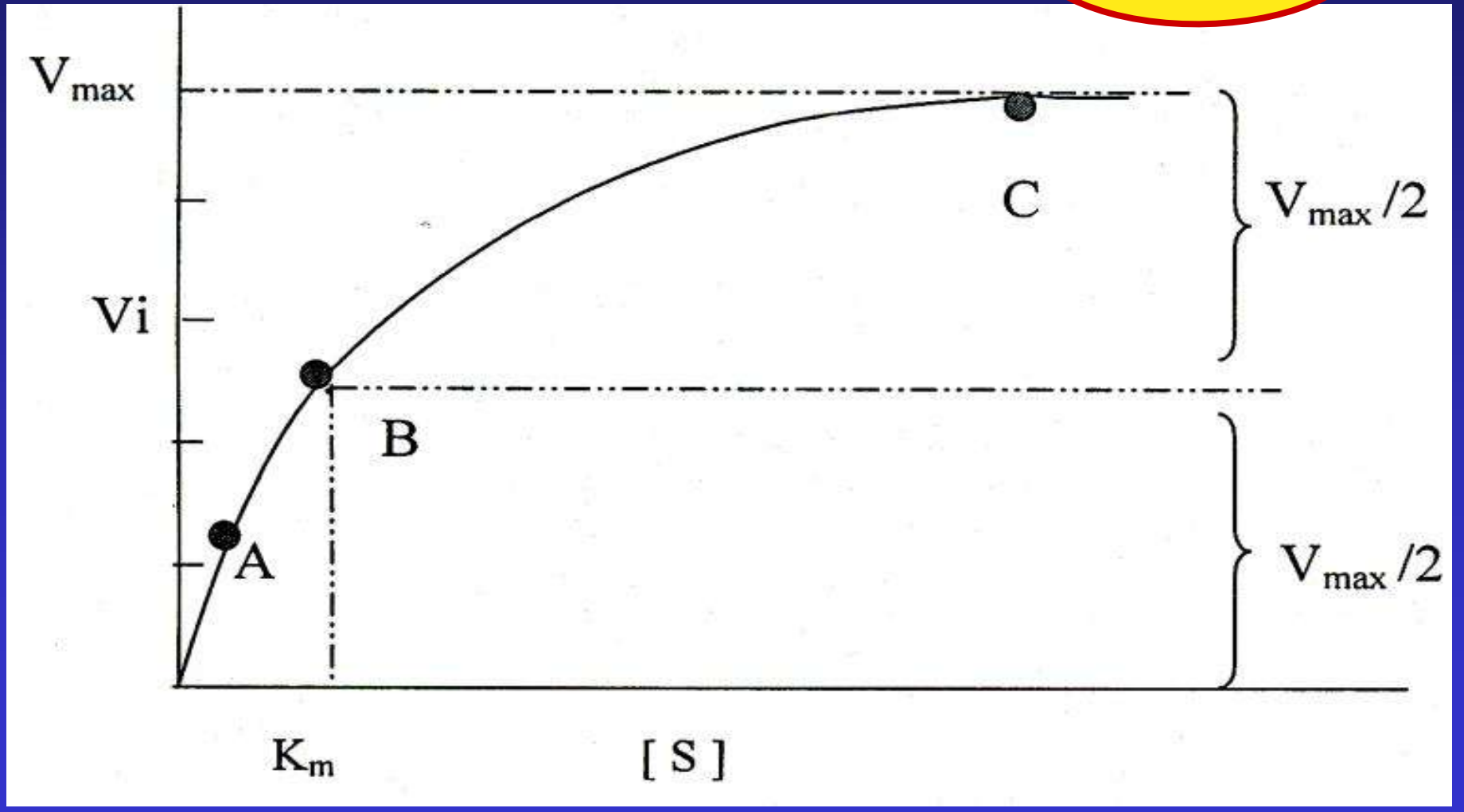
Substrate concentration



Effect of substrate concentration on the velocity of an enzyme- catalyzed reaction



What's happening here?!

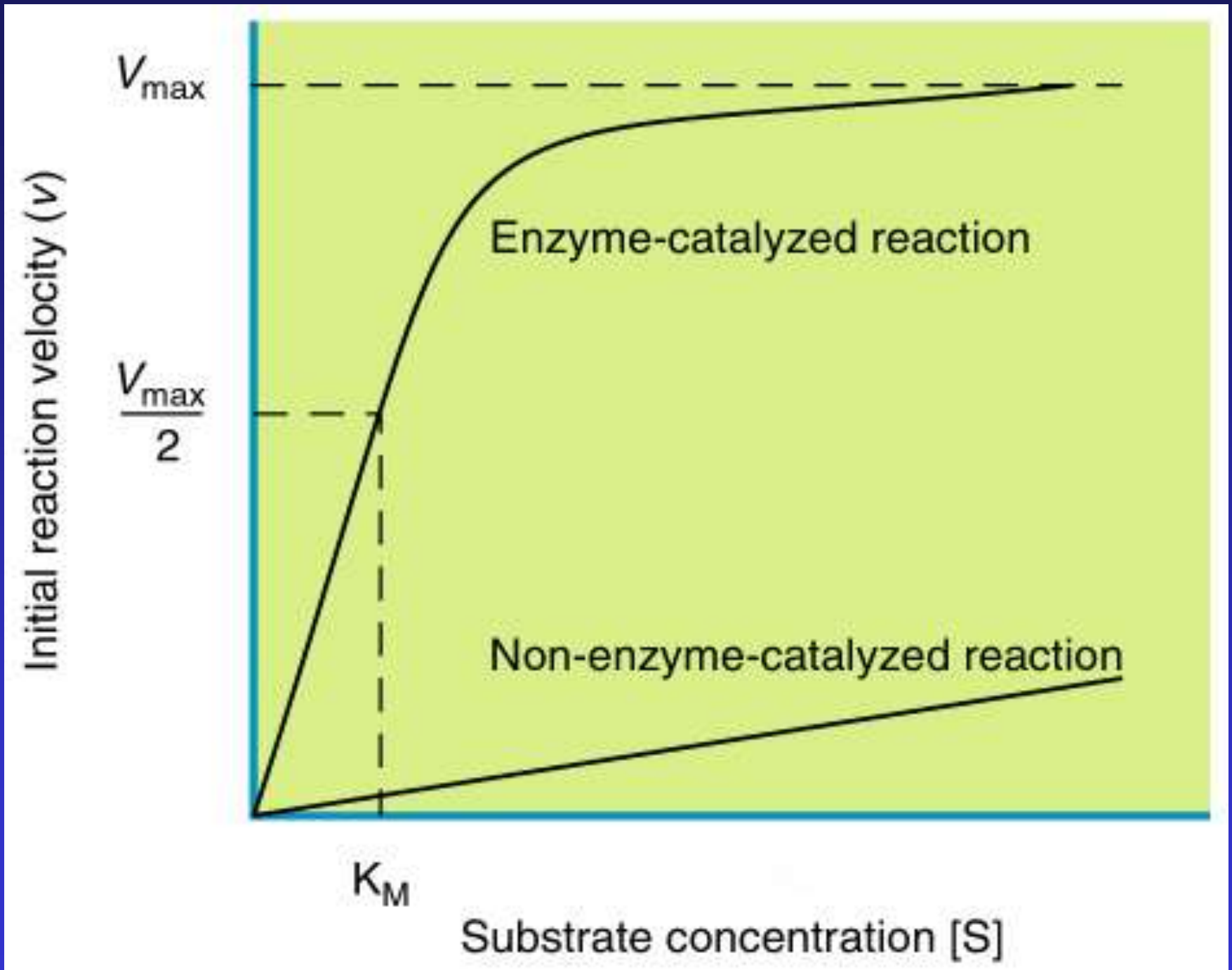


Michaelis constant (Km)

- It is the substrate concentration that produces **half maximum** velocity of enzyme

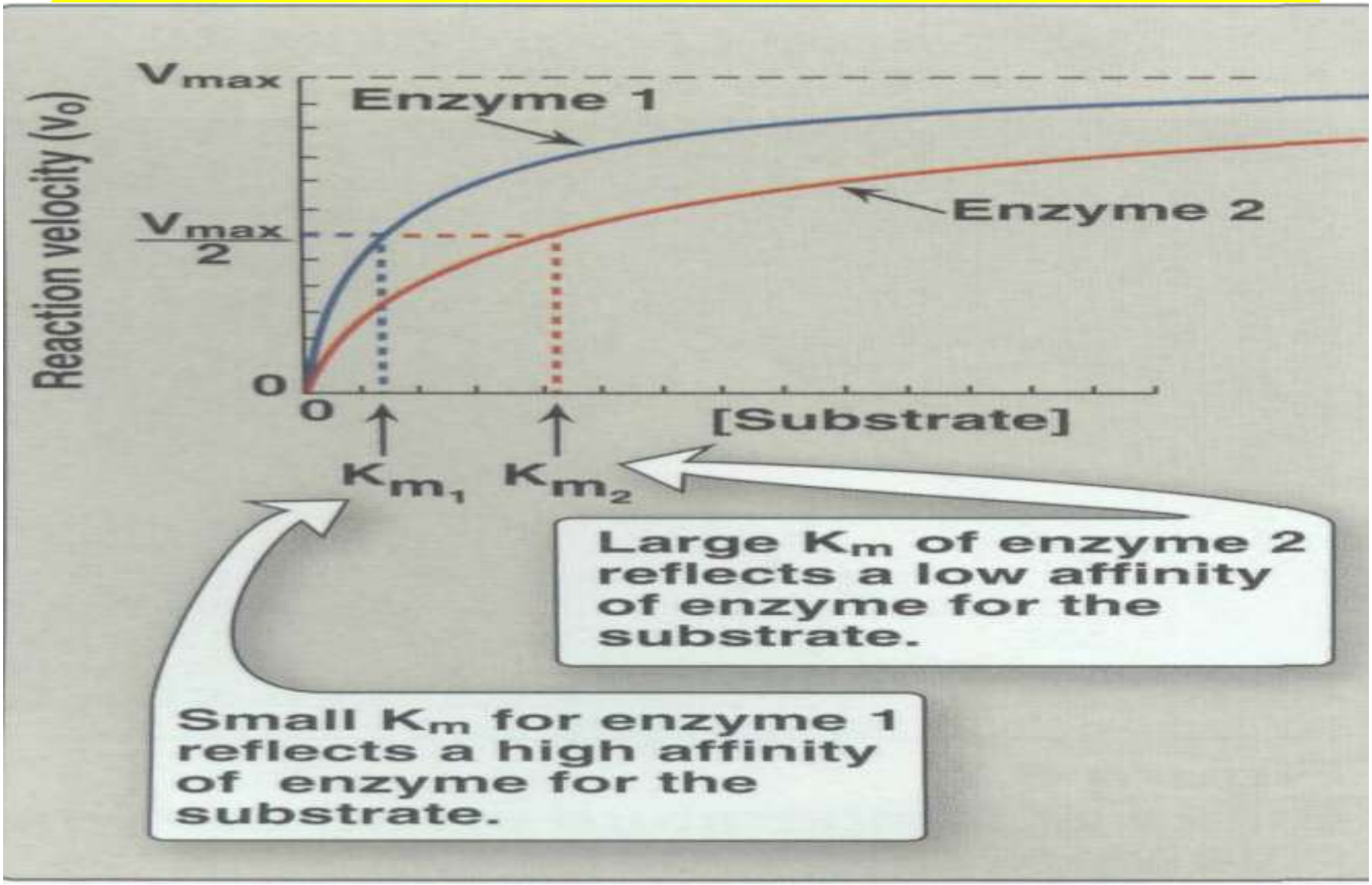
- **<u>Enzymes with low Km:</u>** have **high affinity** to the substrate i.e. they **act at maximal velocity at low substrate concentration**
- E.g. **Hexokinase** acts on glucose at **low concentration (fasting state)**

- **Enzymes with high Km**: they have **low affinity** to the substrate i.e. they act at maximal velocity at **high substrate concentration**
- E.g. **Glucokinase** enzyme acts on glucose at **high concentration (fed state)**


V_{max}
$\frac{V_{max}}{2}$
Initial reaction velocity (v)
Enzyme-catalyzed reaction
Non-enzyme-catalyzed reaction
K_M
Substrate concentration [S]

CONCENTRATION OF SUBSTRATE

Km

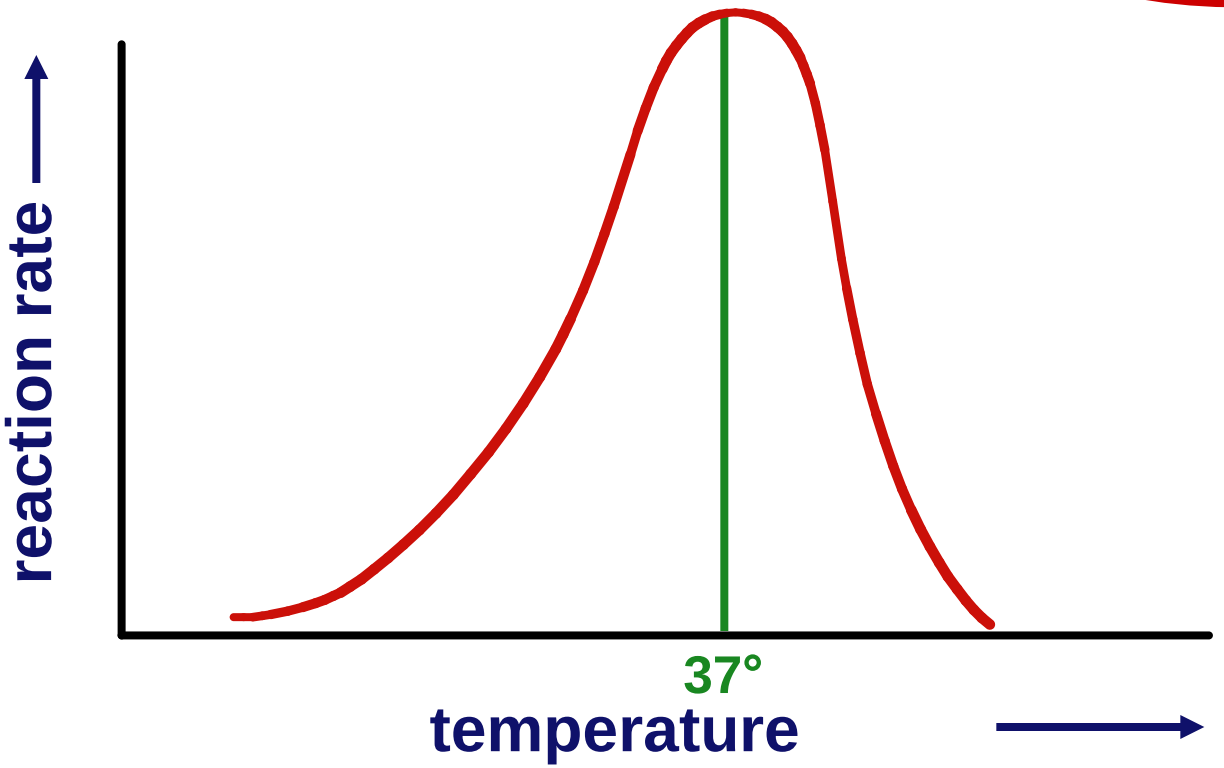


3- Effects of temperature

- Rate of reaction **increases gradually** with the rise in temperature until reach a **maximum** at a certain temperature, called **optimum temperature**

- The optimum temperature is **37-40 C** in humans

Temperature



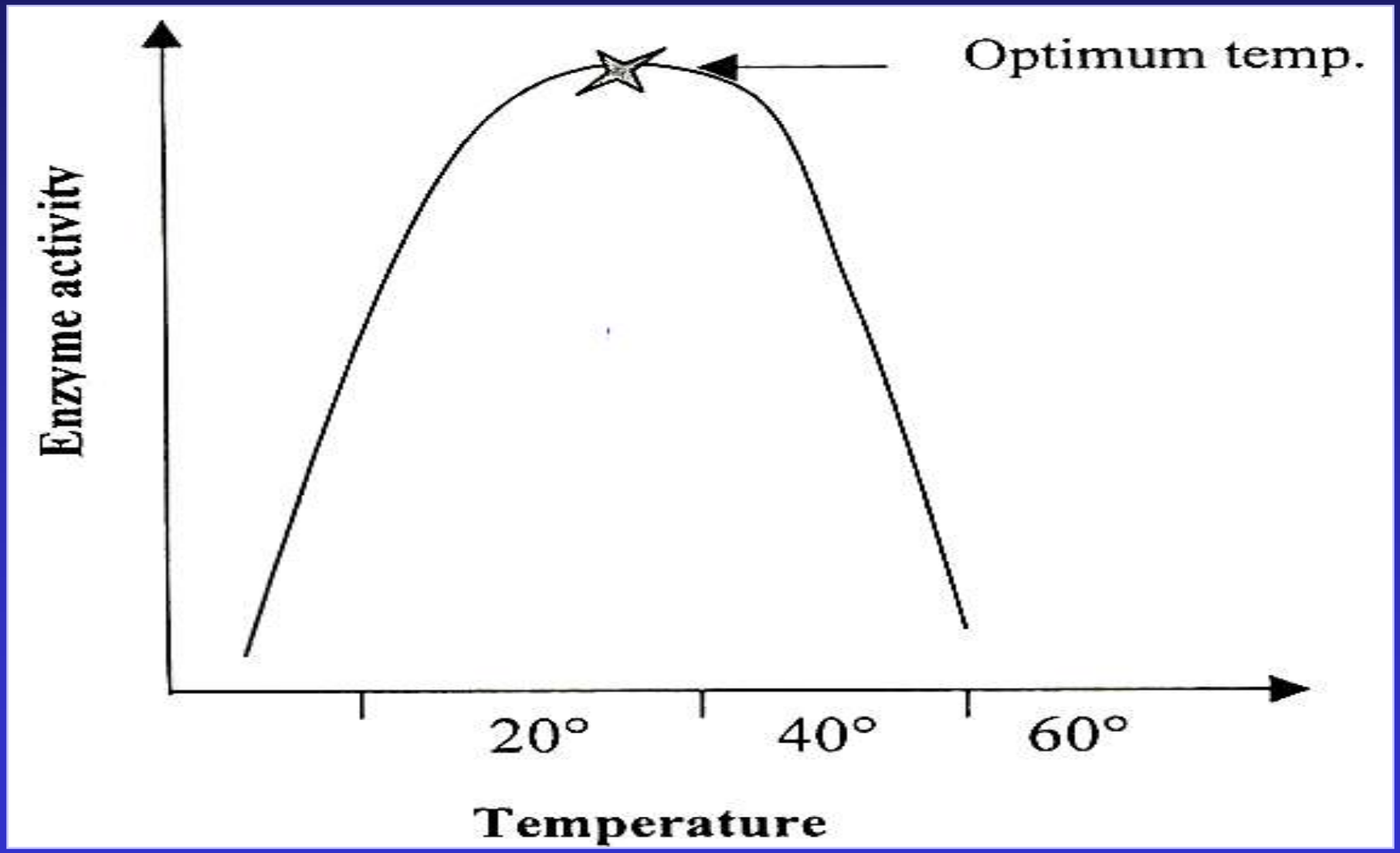
The effect of temperature on reaction rate is due to:

1- Increase of temperature **increase the initial energy** of substrate and thus **decrease the activation energy**

2- **Increase of collision** of molecules: more molecules become in the bond forming or bond breaking distance.

After the optimum temperature, the rate of reaction **decrease** due to **denaturation of the enzyme (60-65 C).**

Effect of temperature on the velocity of an enzyme-catalyzed reaction .



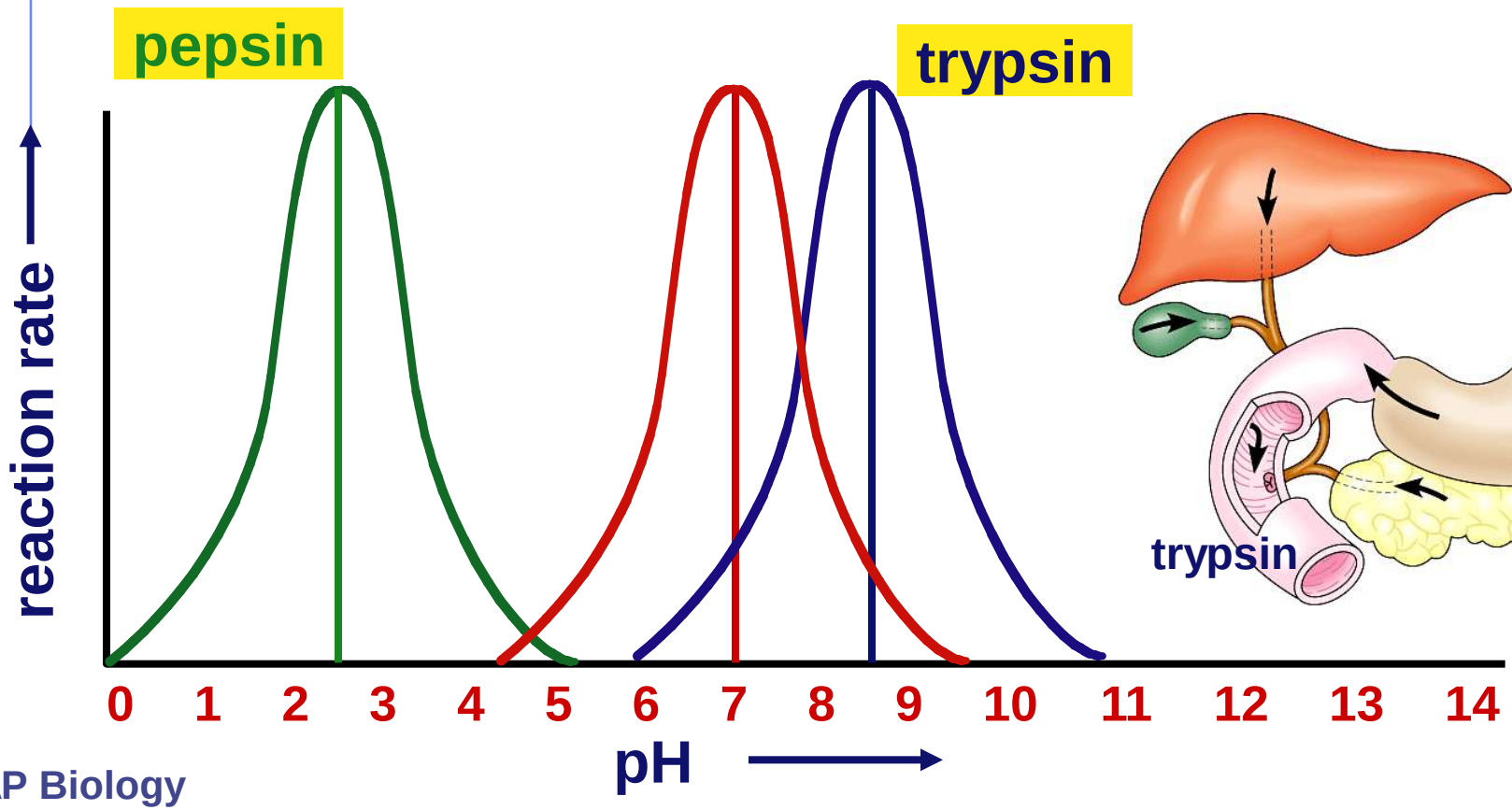
4- Effect of PH

- Each enzyme has an **optimum PH** at which its **activity is maximal**

- E.g. Optimum PH of **pepsin = 1.5 - 2**
- Optimum PH of **pancreatic lipase = 7.5 - 8**
- Optimum PH of **salivary amylase = 6.8**

pH


What's happening here?!


pepsin
trypsin
reaction rate
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH

pepsin
trypsin

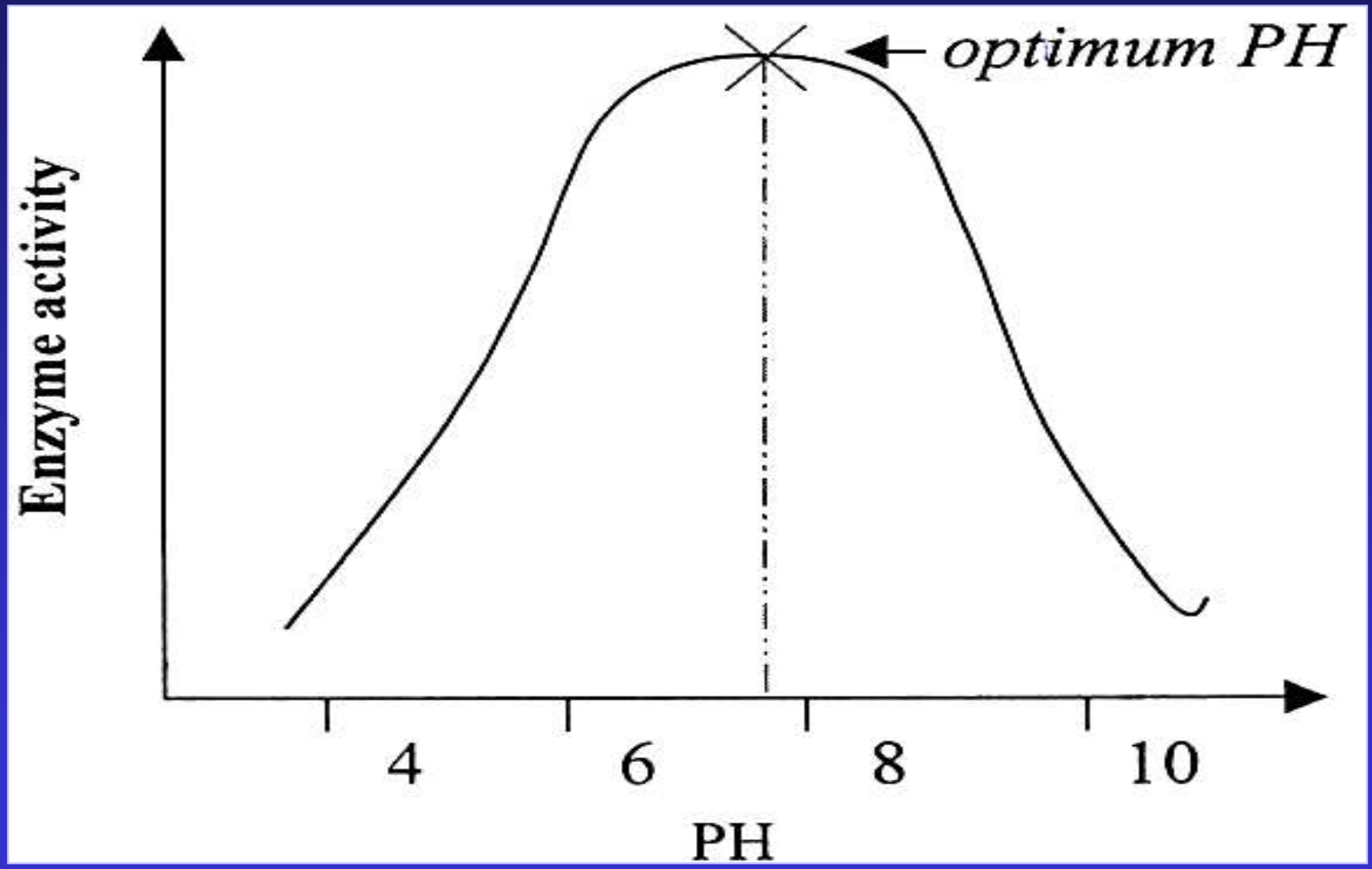
Change of PH **above or below optimum PH**

decrease rate of enzyme action due to:

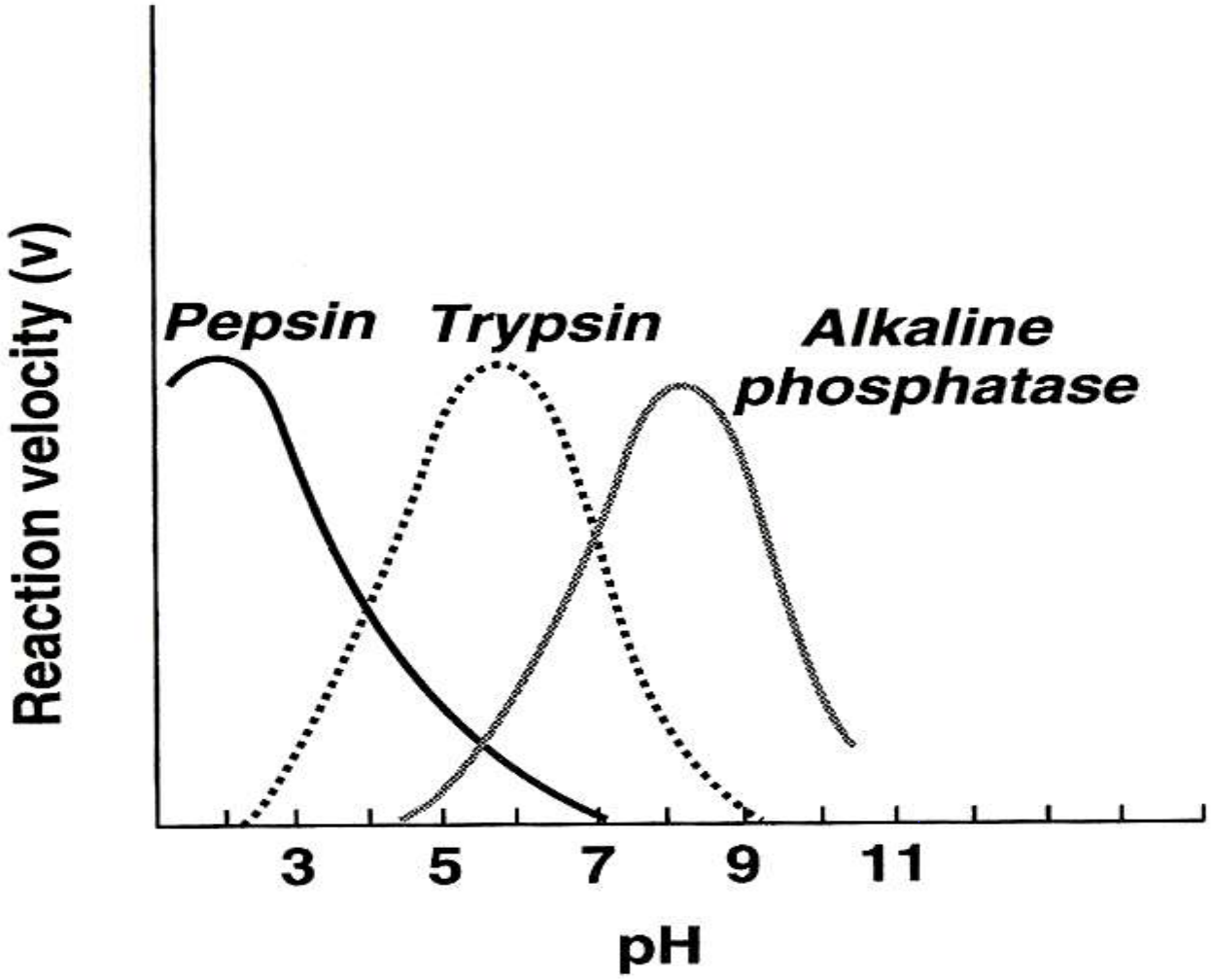
1- The enzyme activity depends on the **ionization state** of both enzyme and substrate which is affected by PH.

2- Marked change in PH will cause **denaturation** of enzyme.

Effect of pH on enzyme activity



Effect of pH values on different enzymes



5- Concentration of coenzymes: In the conjugated enzymes that need coenzymes, the **increase** in the **coenzyme concentration** will **increase** the reaction rate.

6- Concentration of ion activators: The **increase** in metal ion activator **increase** the reaction rate

Enzymes are activated by ions:

1- **Chloride** ion activate **salivary amylase**

2- **Calcium ion** activate **thromobokinase** enzyme

7- Effect of time:

- In an enzymatic reaction, **the rate of reaction is decreased by time.**
- This is **due to:**

1- The **decrease in substrate** concentration.

2- The accumulation of the **end products**.

3- The **change in PH** than optimum PH.

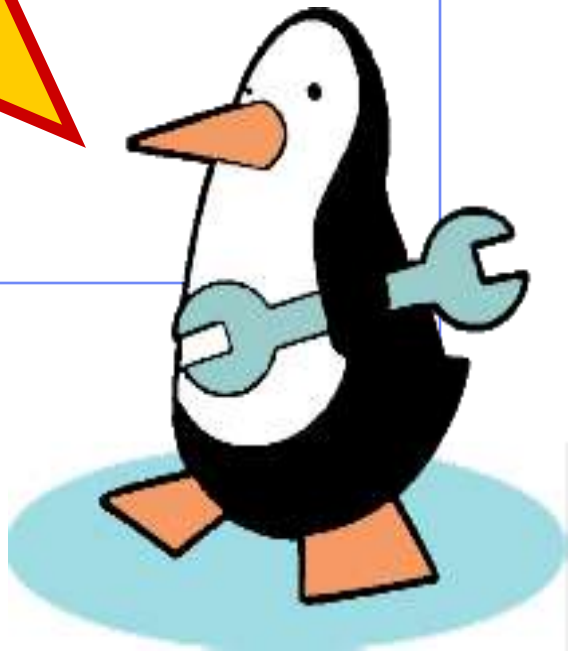
8- Presence of enzymes inhibitor: presence of enzyme **inhibitor decreases** or stops the enzyme activity.

Enzyme inhibitors may be:

1- Competitive inhibitors.

2- Non competitive inhibitors.

Don’t be inhibited!
Ask Questions!



Activity

- 1- High Km enzyme:

  A) has high affinity to its substrate.

  B) Need high conc. of its substrate to reach its Vmax .

  C) has high max. velocity .

  D) like hexokinase.

- 2- Optimum pH:

  A) it is the same for all enzymes

  B) it is acidic for pepsin enzyme.

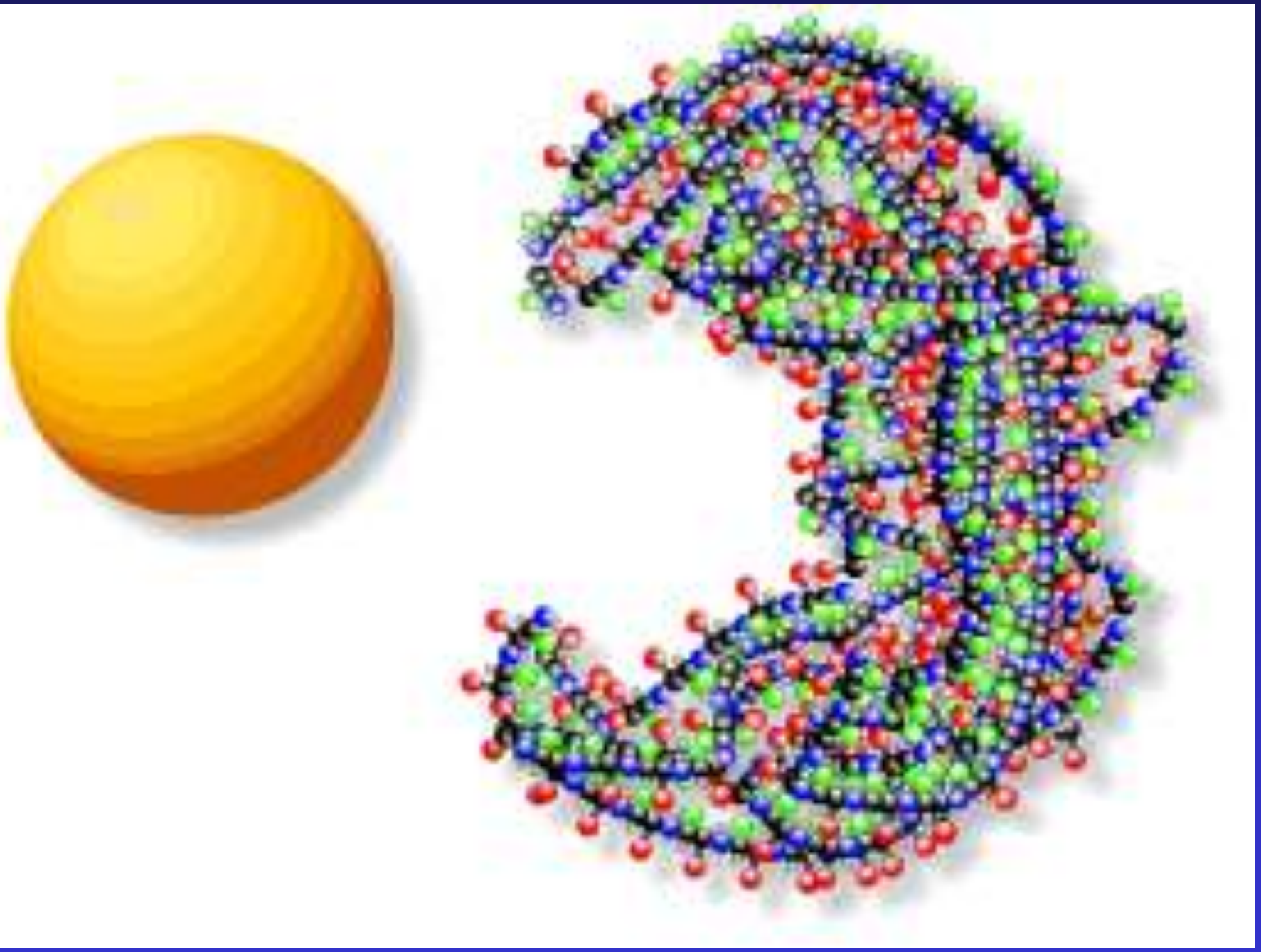
  C) at which the enzyme act at lowest rate

  D) the enzyme is stable under its marked changes

- 1- Enumerate the factors affecting enzyme action and discuss one of them .

- 2- Discuss the effect of substrate conc. on enzyme action.

- 3- Optimum temp. & optimum pH (def. of each & explain how can they affect the enzyme action .



Thank you