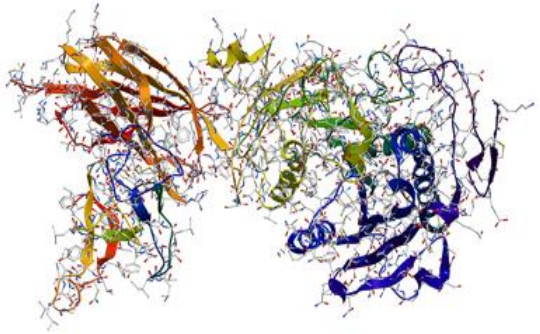


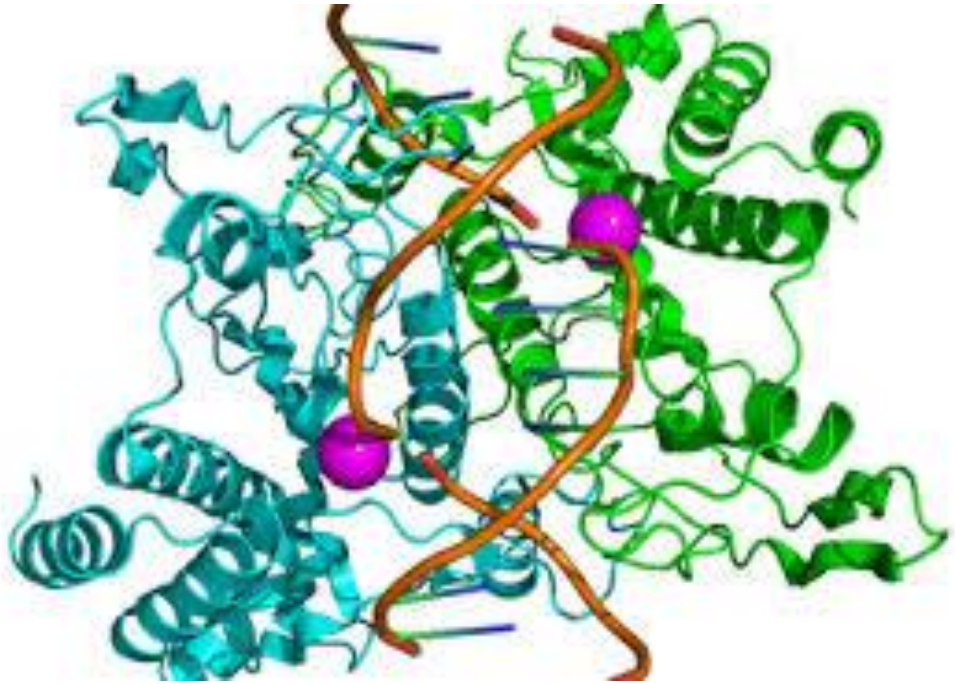
ENZYMES

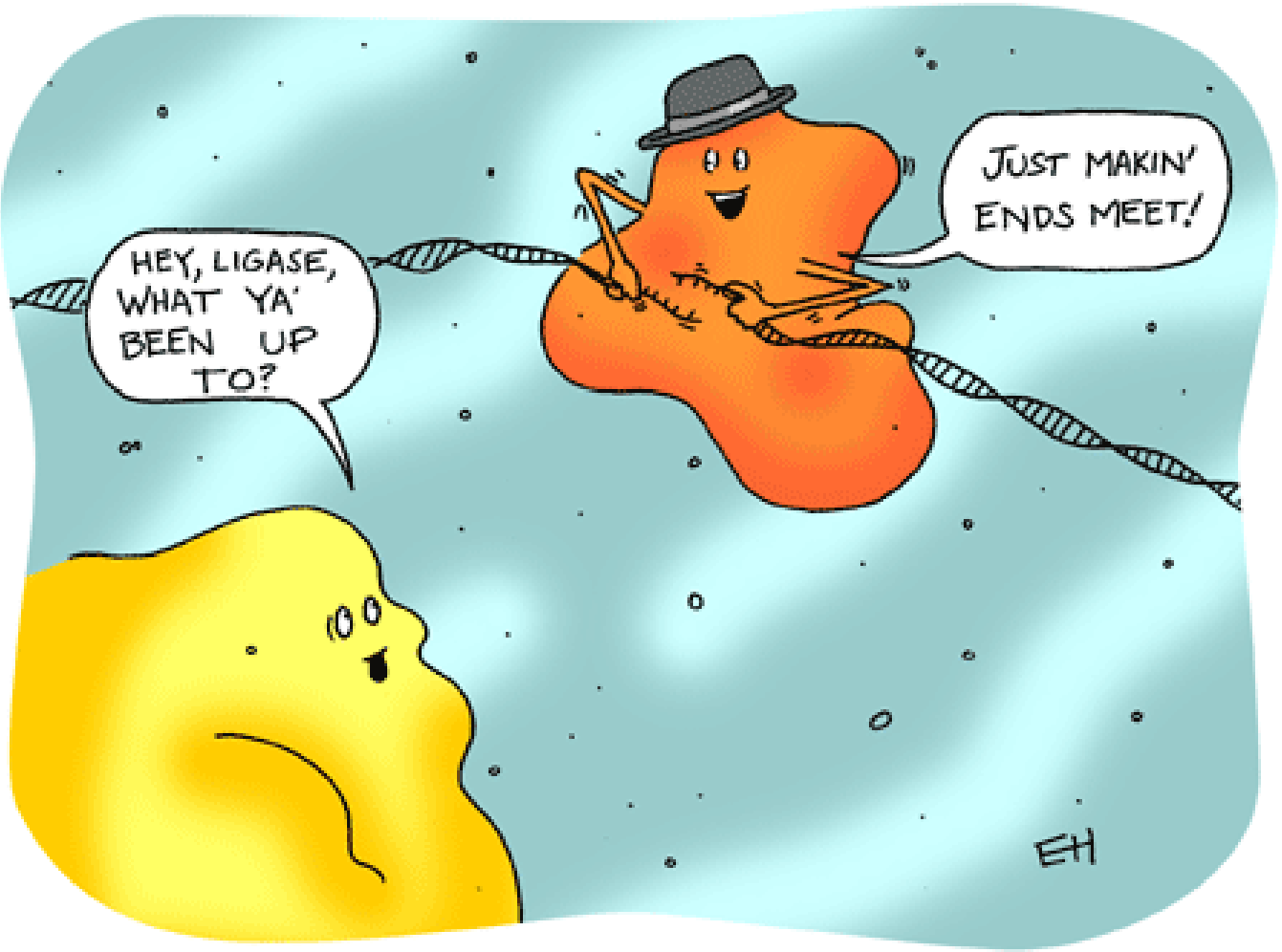
1st year MBBS

DR BELA INAYAT
BIOCHEMISTRY DEPTT
KGMC


بسم الله الرحمن الرحيم

WHAT DOES ENZYME DO ???

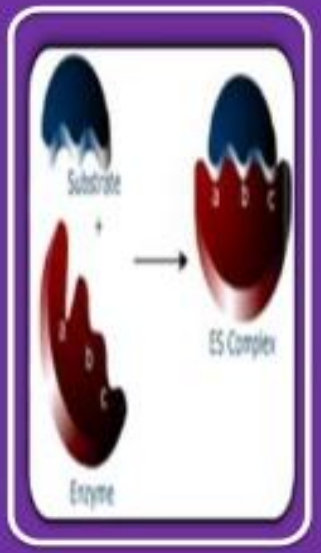



HEY, LIGASE, WHAT YA' BEEN UP TO?
JUST MAKIN' ENDS MEET!
EH

Learning objectives

- Definition of enzymes
- Structure and properties of enzymes
- Mechanism of action of enzymes
- Classification of enzymes
- Enzyme catalysis

Definition of enzymes



Enzymes

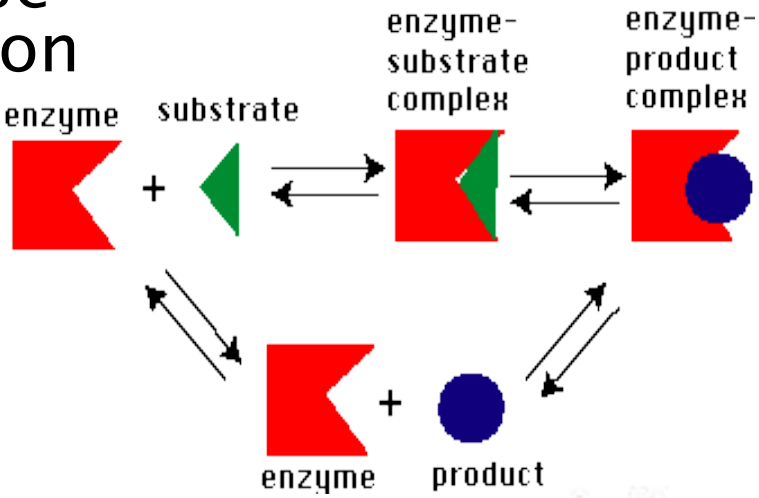
- Enzymes are proteins functioning as catalysts that speed up reactions by lowering the activation energy.
- The enzyme catalysts regulate the structure and function of cells and organisms.

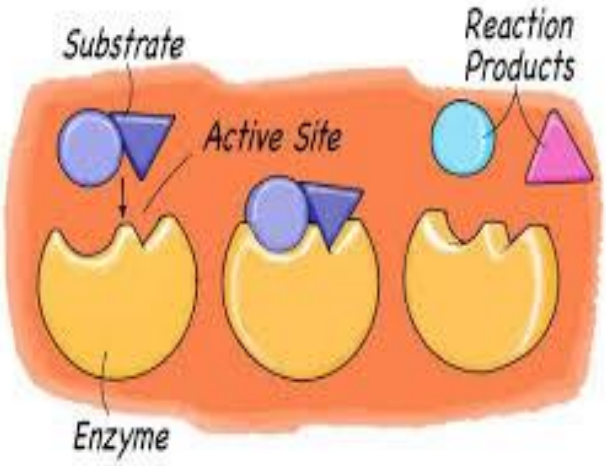
Definitions

- Catalyst
- substance that increase the velocity of chemical reaction without itself undergoing any change.

- Substrate
- substance on which enzymes act .

- Product
- substance produced by chemical reaction.
- Active site
- are on enzymes where substrate binds.

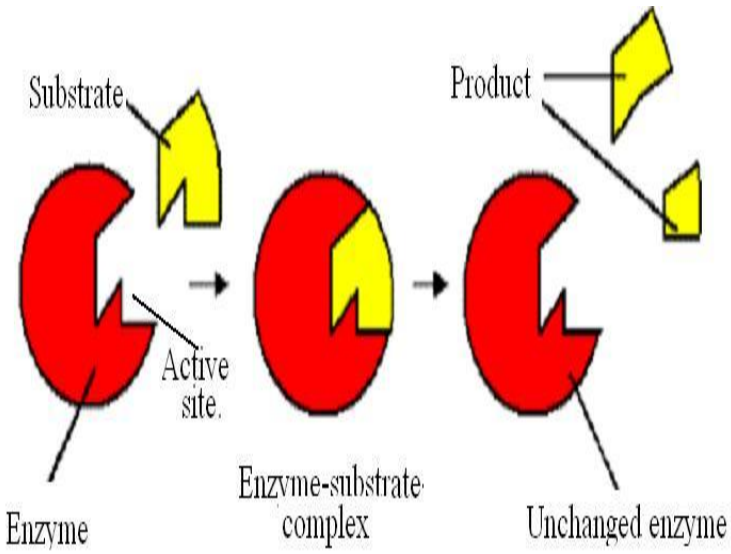




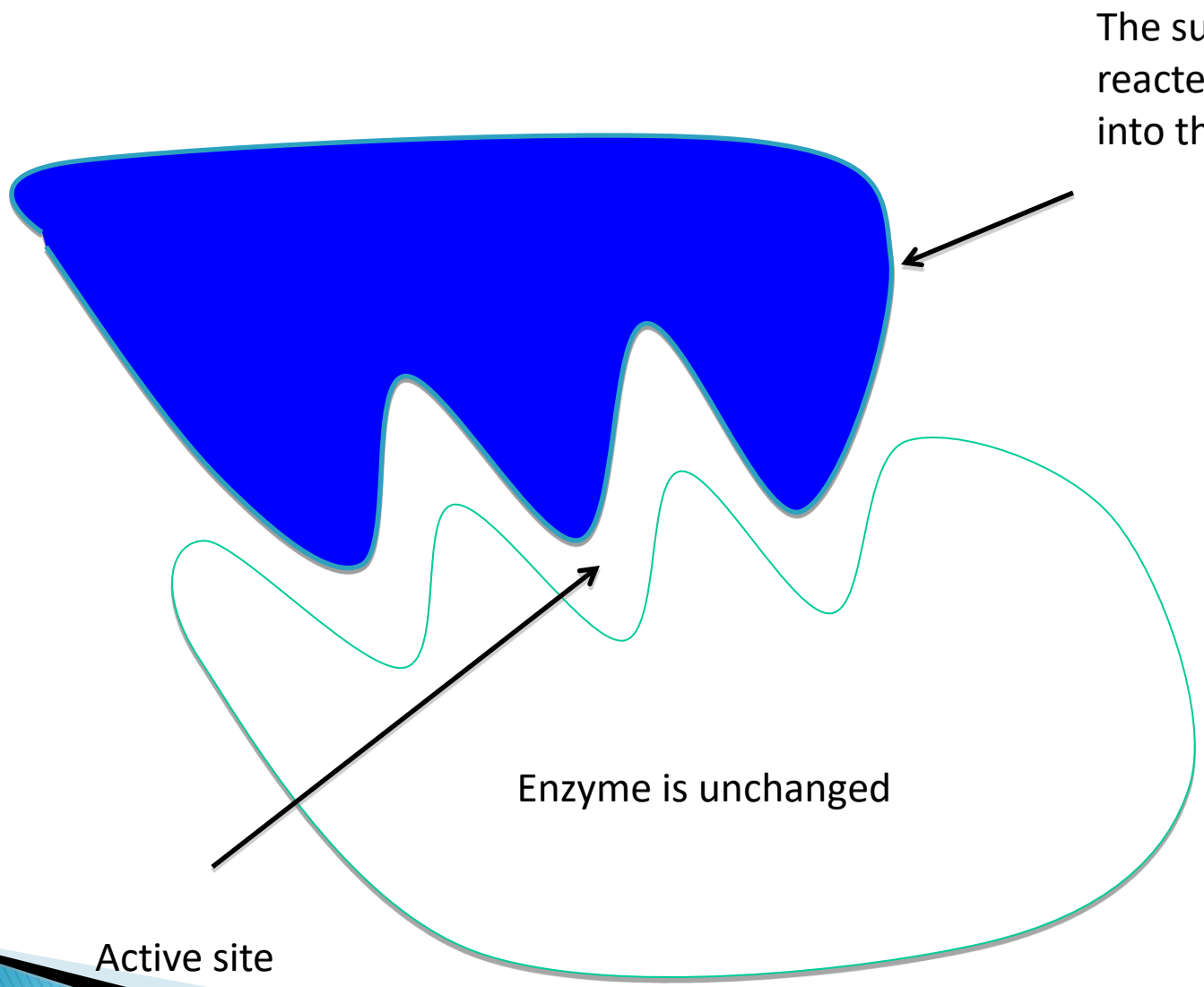
INTRODUCTION

- Enzymes are important group of biomolecules synthesized by the living cells.
- Enzymes only accelerate the rate of chemical reaction,but do not initiate them,chemical reactions can take place without enzymes,but then reactions will be extremely slow.

- Actions of most enzymes are under strict regulation in a variety of ways.
- Enzymes that catalyze the conversion of one or more compounds (substrates) into one or more different compounds (products) enhance the rates of the corresponding non-catalyzed reaction by factors of atleast 10(6).
- Like all catalysts enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.



Basic Enzyme Diagram



Properties of enzymes

- Each enzyme has its own tertiary stucture and specific conformation.
- Functional unit is holoenzyme , made up of
- Apoenzyme(protein part),
- coenzyme (non protein part)

- Active enzyme protein part non-protein part

The Parts of an Enzyme

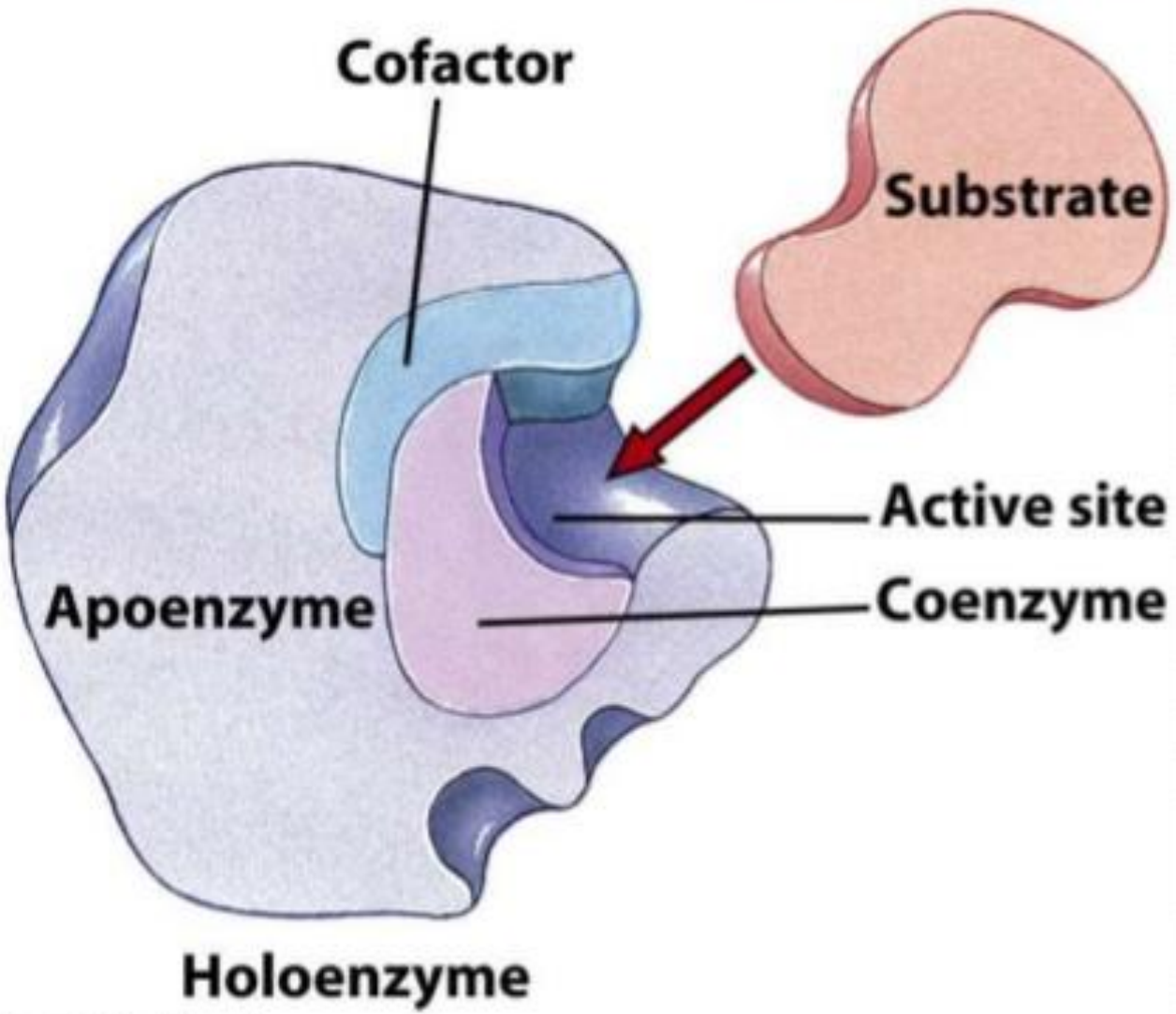


Figure 5-6 Microbiology, 6/e

- Highly efficient
- Extremely selective catalysts
- Enzymes are specific both for the type of reaction catalyzed and for a single substrate or a small set of closely related substrates.
- Enzymes are also stereospecific----------typically catalyze reaction of only one sterioisomer of a given compound,e.g D but not L sugars,L but not D amino acids.

Enzyme structure

- Enzymes are **proteins**
- They have a **globular** shape
- A complex 3-D structure

Human pancreatic amylase

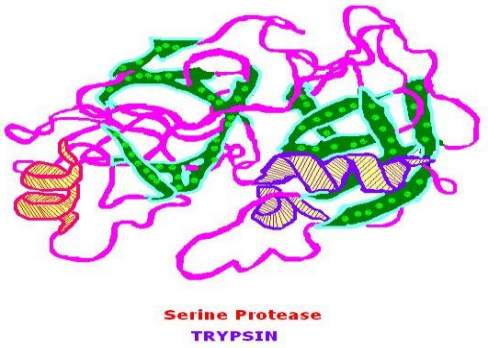
© Dr. Anjuman Begum

PROTEIN NATURE OF ENZYMES

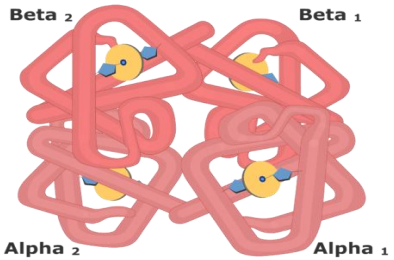
- In general all enzymes are protein in nature with large molecular weight,except ribozymes that are RNA molecules with enzymatic activity.Ribozymes play a key role in RNA splicing,transfer RNA synthesis etc.

- Some enzymes are SIMPLE PROTEINS while others are CONJUGATED PROTEINS.

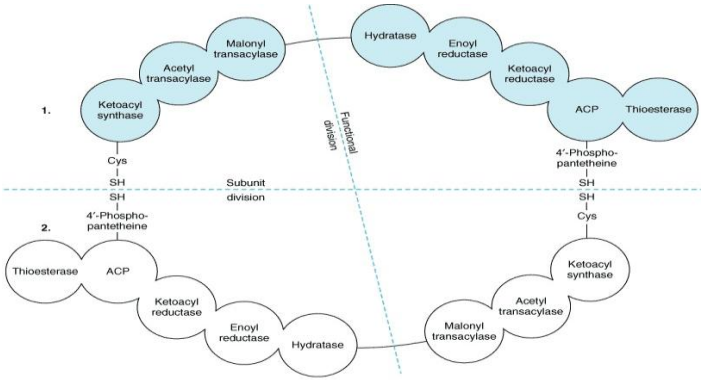
- Enzymes with only one polypeptide chain are called "MONOMERIC enzymes"



- Those with more than one polypeptide chain are called "OLIGOMERIC enzymes"



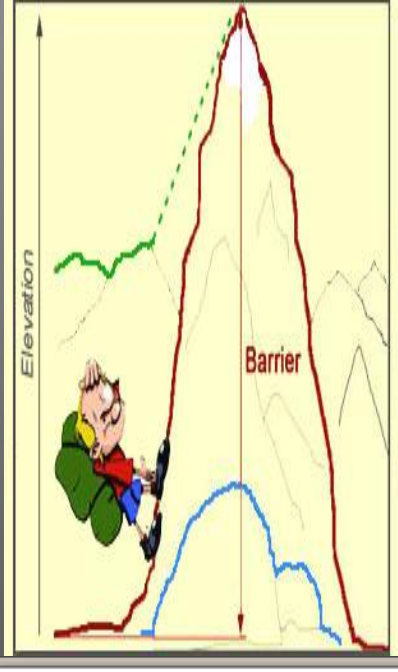
- Multienzyme complex when many different enzyme catalysing reaction sites are located at different site of the same macro molecule.

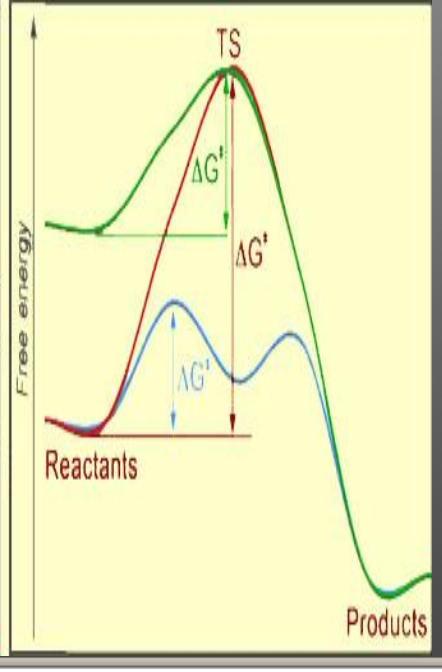


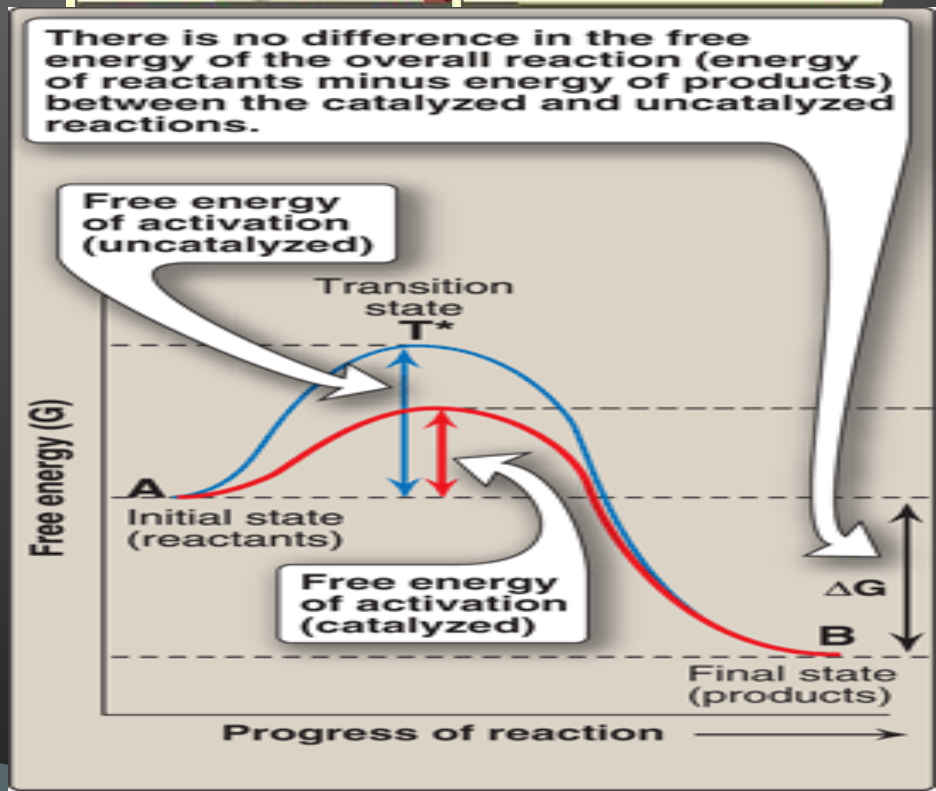
Mechanism of enzyme action

How enzymes work?

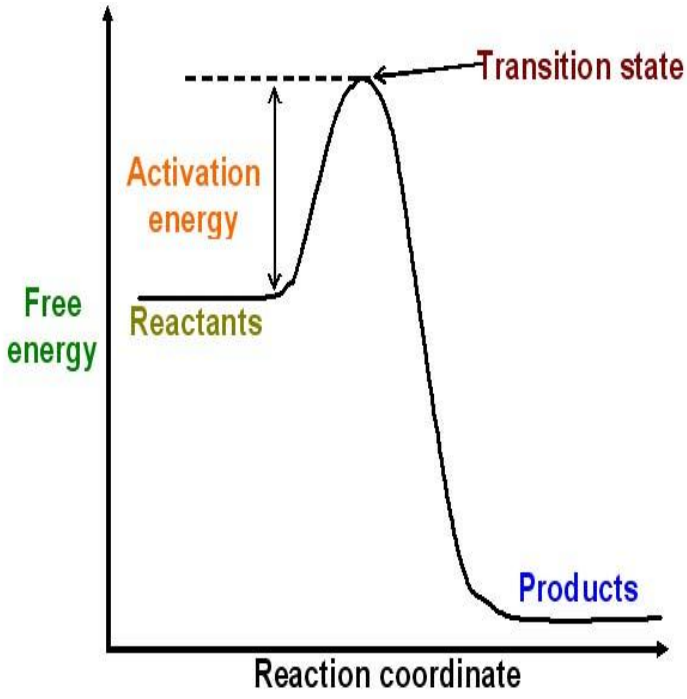
- All chemical reactions have energy barrier separating reactants and products.
- This barrier is the energy difference between reactants and high energy intermediate which occurs during the formation of product.
- This is called **the free energy of activation**







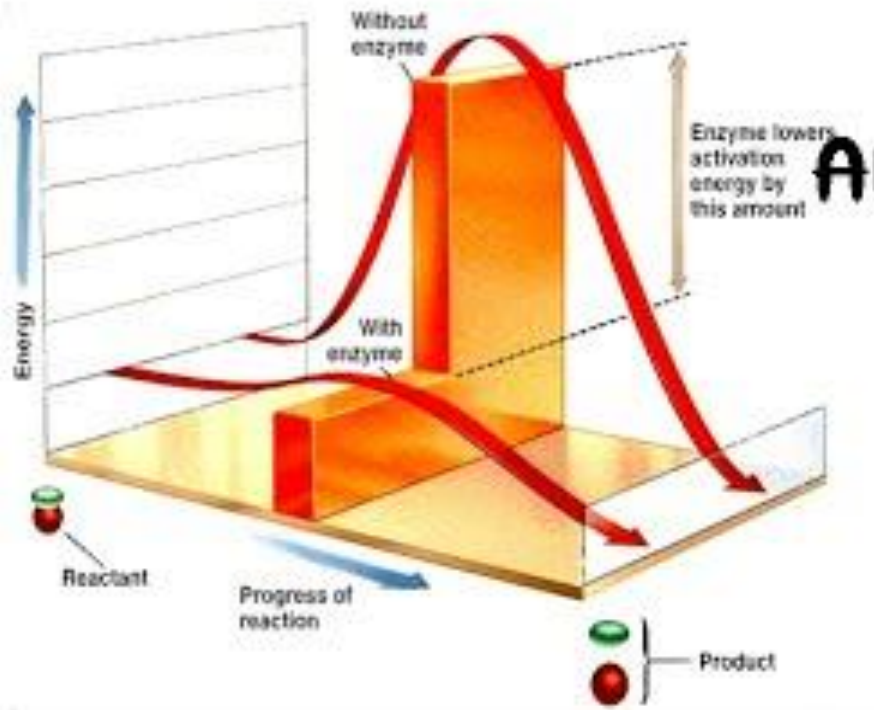
- **Chemical reactions**
- Chemical reactions need an initial input of energy ***THE ACTIVATION ENERGY***



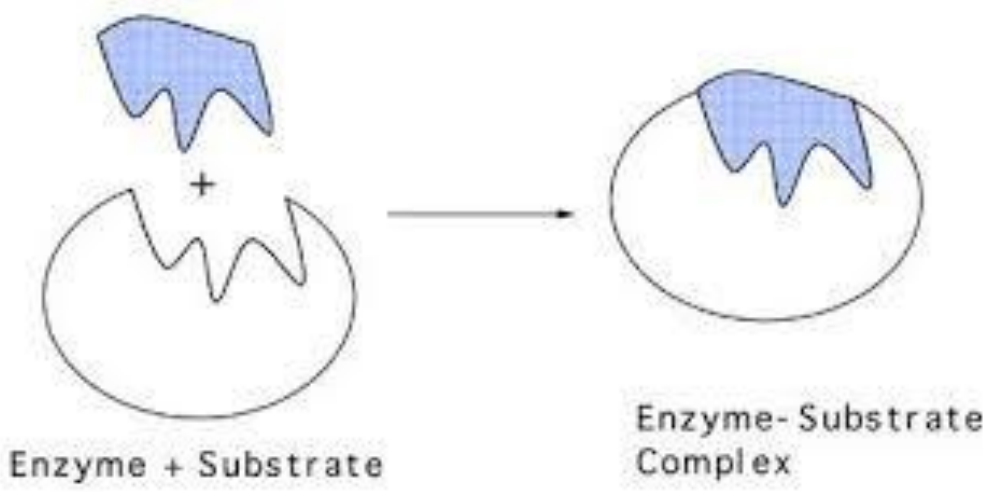
- During this part of the reaction the molecules are said to be in a ***transition state.*** At this stage energy of activation is at its peak.

- The activation energy for the forward reaction is the amount of FREE ENERGY that must be added to go from the ENERGY level of the reactants to the ENERGY level of the TRANSITION STATE……. Once a reactant molecule absorbs enough ENERGY to reach the transition state,it can proceed through the remainder of the reaction.

ACTIVATION ENERGY AND ENZYMES



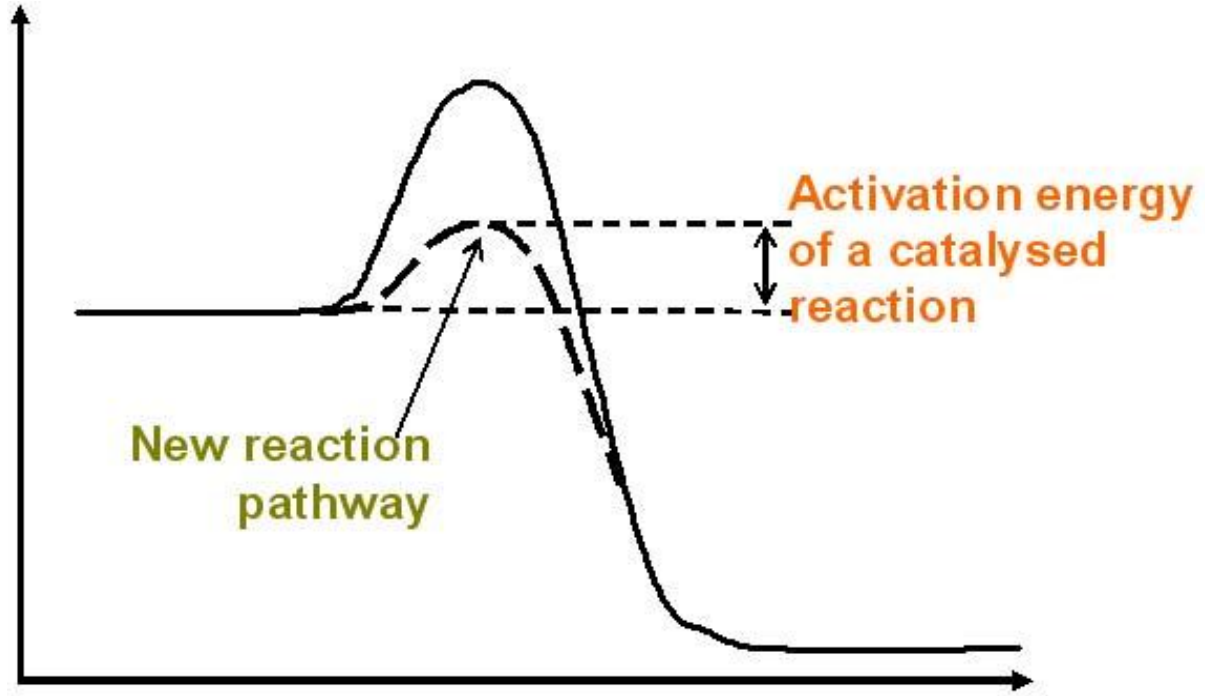
- Enzymes are powerful catalysts and accelerate reactions millions of time by reducing the energy of activation.
- Requirement for catalysis is formation of enzyme-substrate complex.
- $E + S \rightleftharpoons ES \rightarrow E + P$



Making reactions go faster

- Increasing the temperature make molecules move faster
- Biological systems are very sensitive to temperature changes.
- Enzymes can increase the rate of reactions without increasing the temperature.
- They do this by lowering the activation energy.
- They create **a new reaction pathway** "a short cut"

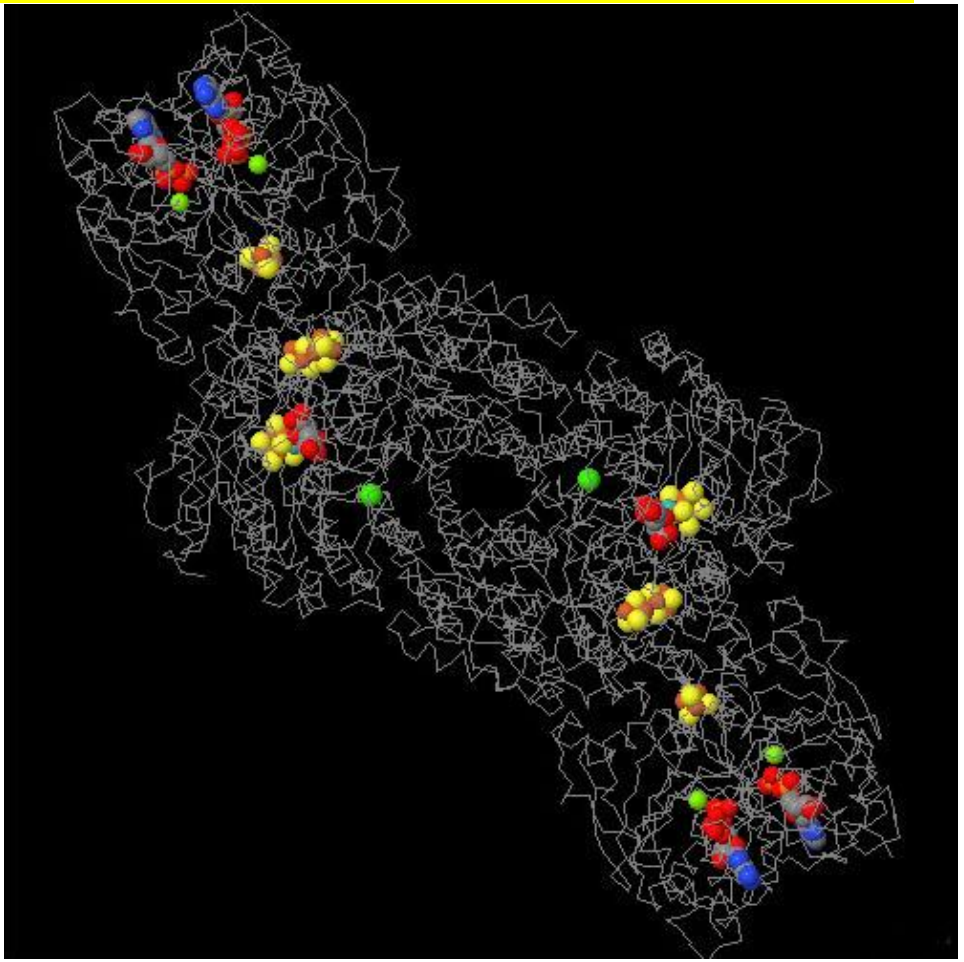
An enzyme controlled pathway



- Enzyme controlled reactions proceed 108 to 1011 times faster than corresponding non-enzymic reactions.

Co enzymes

- An additional non-protein molecule that is needed by some enzymes to help the reaction
- Tightly bound cofactors are called prosthetic groups
- Cofactors that are bound and released easily are called coenzymes
- Many vitamins are coenzymes



Nitrogenase enzyme with Fe, Mo and ADP cofactors

Jmol from a RCSB PDB file © 2007 Steve Cook

H.SCHINDELIN, C.KISKER, J.L.SCHLESSMAN, J.B.HOWARD, D.C.REES
STRUCTURE OF ADP X ALF4(-)-STABILIZED NITROGENASE COMPLEX AND ITS

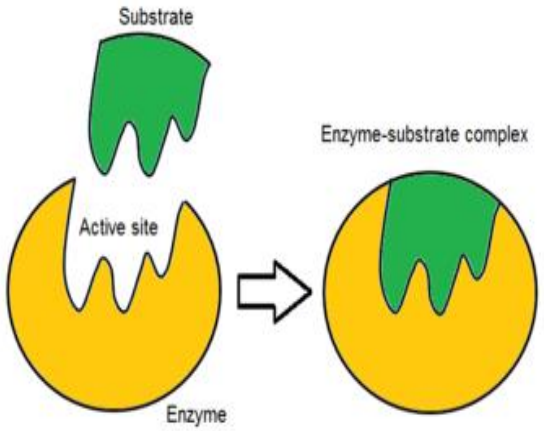
IMPLICATIONS FOR SIGNAL TRANSDUCTION; NATURE 387:370 (1997)

- Co enzymes are regarded as co- substrate or second-substrate
- Coenzymes undergo alteration during the enzymatic reaction, which are later regenerated, while substrate is converted to product.
- Participate in various reactions involving transfer of atoms or groups like hydrogen, keto ,amino, acyl, methyl carbon dioxide etc.
- Specificity of enzyme is mostly dependent on the apoenzyme and not on co enzyme.

ACTIVE SITE

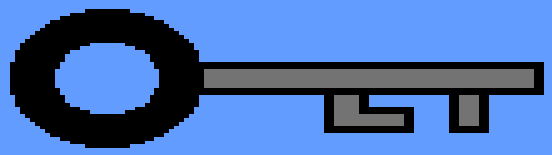
- In the early 20th century it was observed that presence of substrates renders enzymes more resistant to the denaturing effects of elevated temperature

- Emil Fischer proposed that enzymes and substrates interact to form ENZYME-SUBSTRATE Complex (ES) whose thermal stability is far better than the enzyme itself----------understanding chemical nature and kinetic behaviour

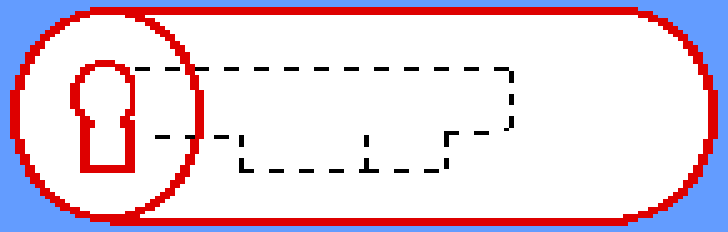


- Fischer reasoned that high specificity with which enzyme recogonises and makes complex with substrate is just like lock and key.
- This enzymatic lock is referred to as ACTIVE SITE
- In most enzymes the active site takes the form of a cleft or pocket on the enzyme's surface

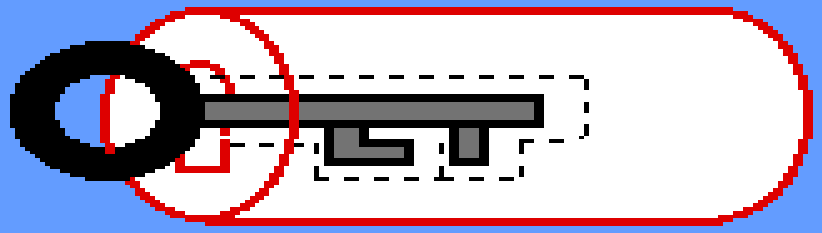
Lock and Key Analogy



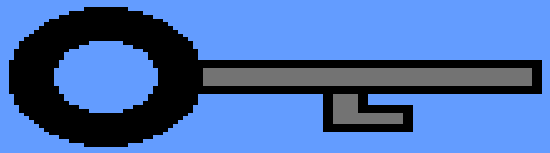
key = substrate



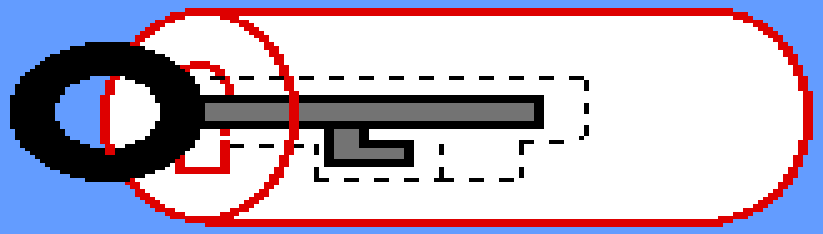
lock = enzyme



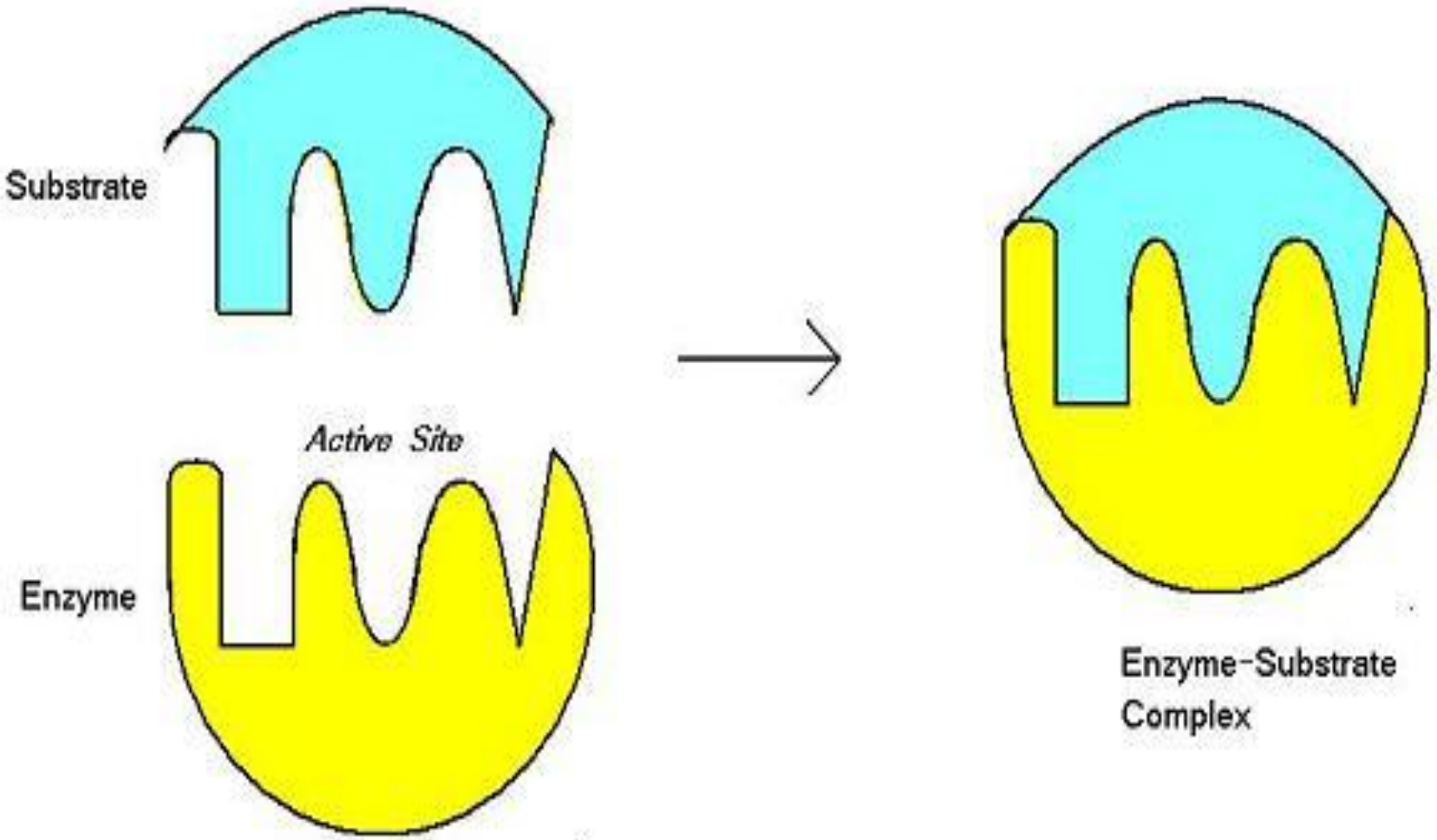
correct fit,
will react



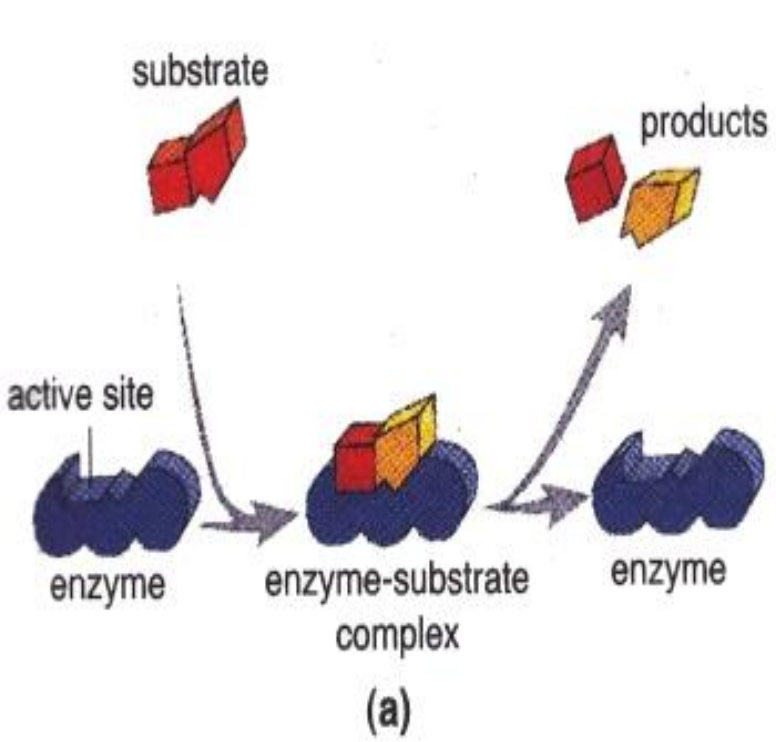
incorrect substrate

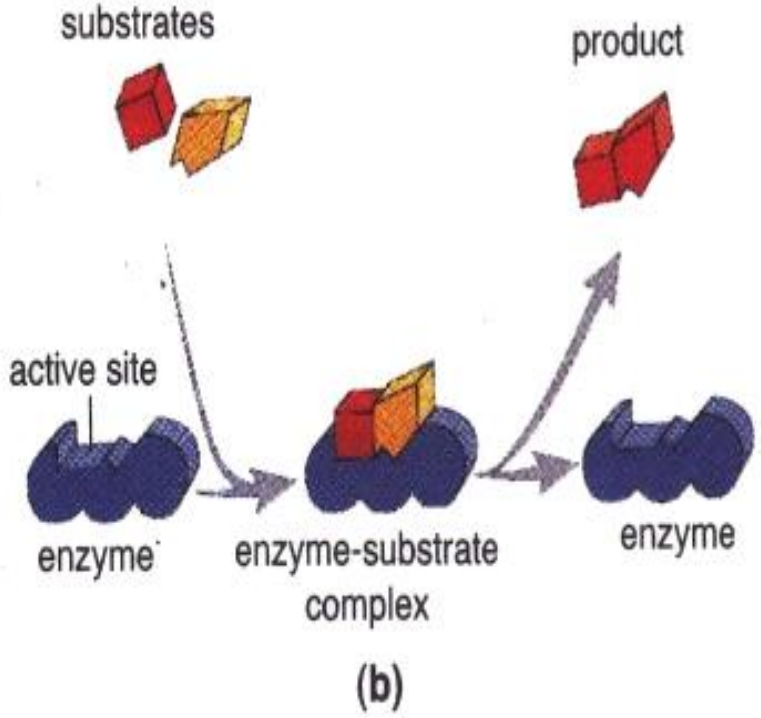


no reaction

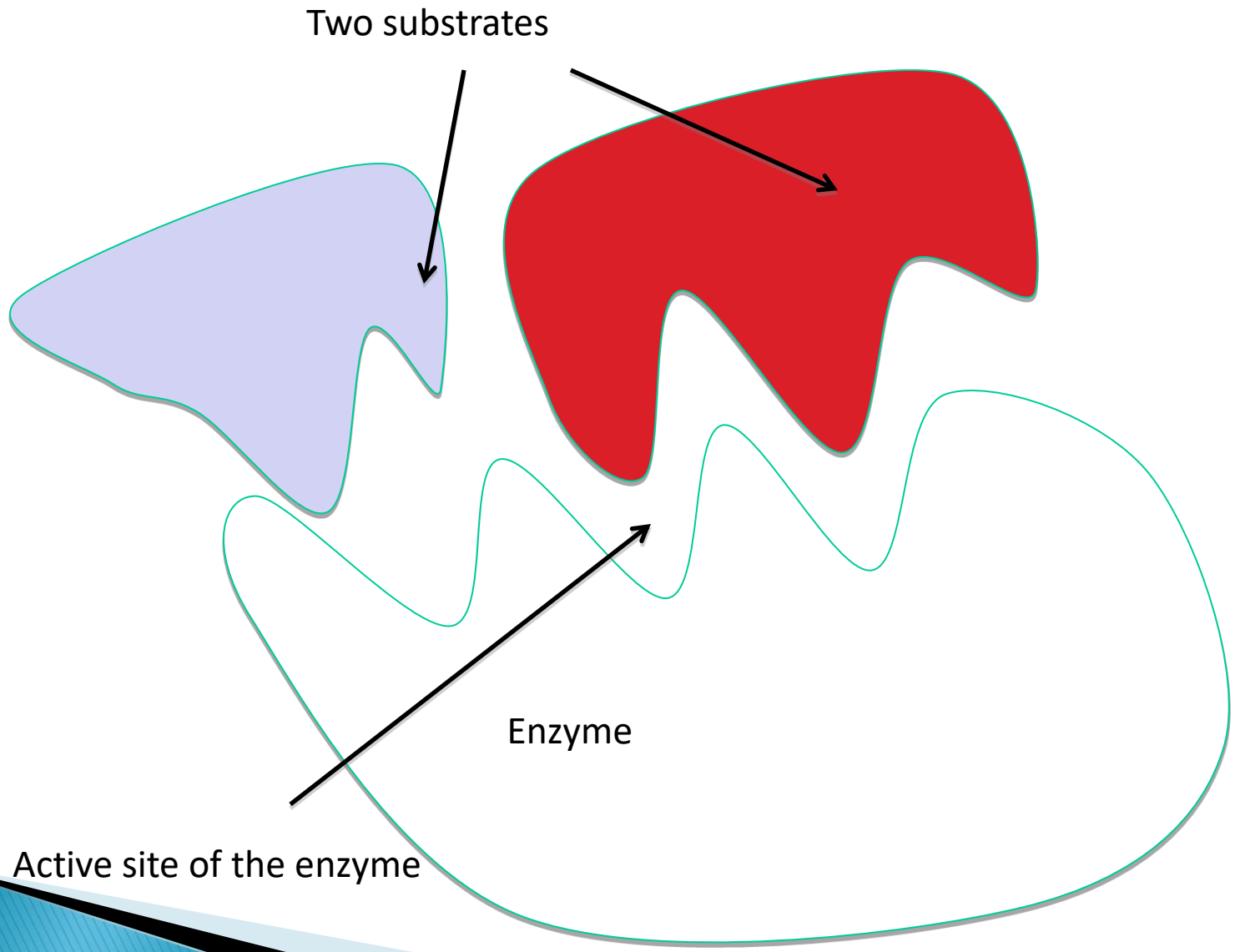


Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

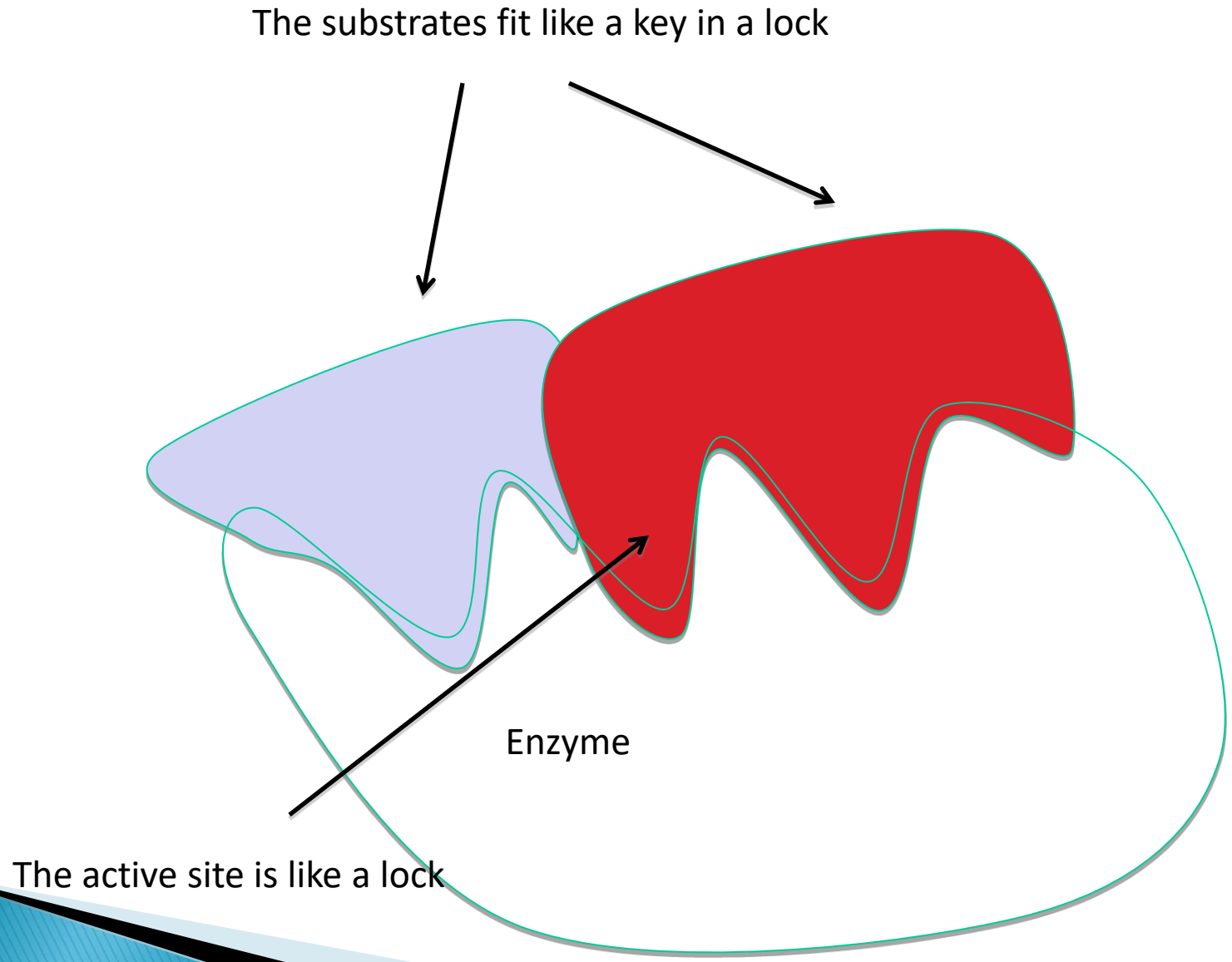

substrate
products
active site
enzyme
enzyme-substrate complex
enzyme
(a)


substrates
product
active site
enzyme
enzyme-substrate complex
enzyme
(b)

Lock and Key Model

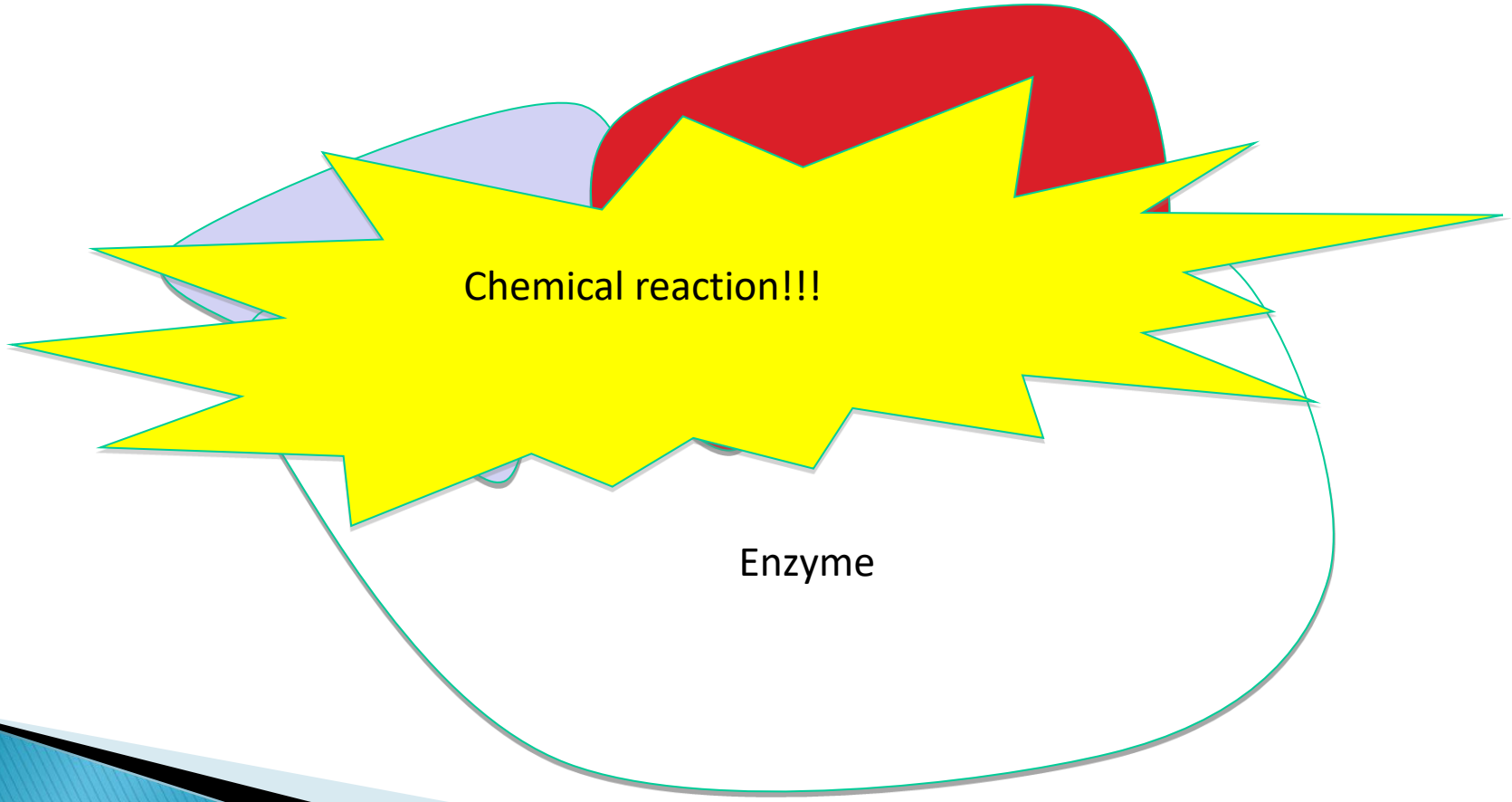


Lock and Key Model

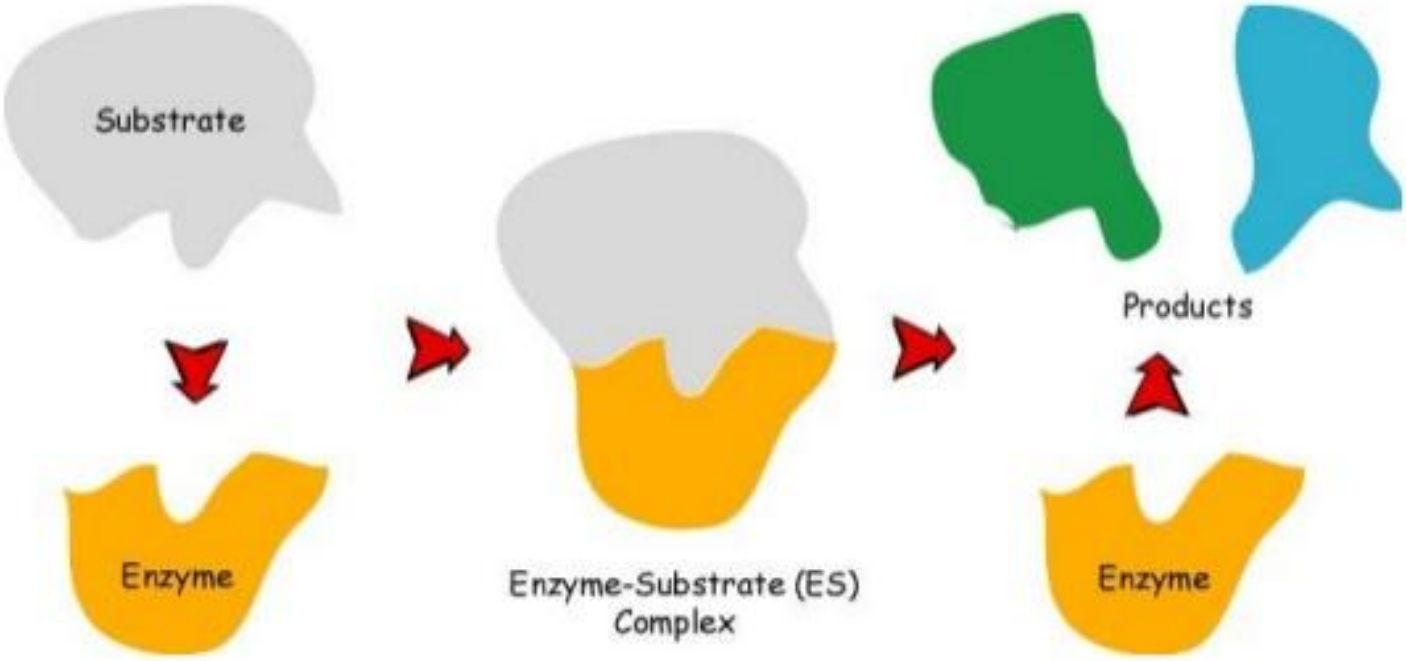


Lock and Key Model

The activation energy for these substrates to bind together has been lowered by the enzyme.

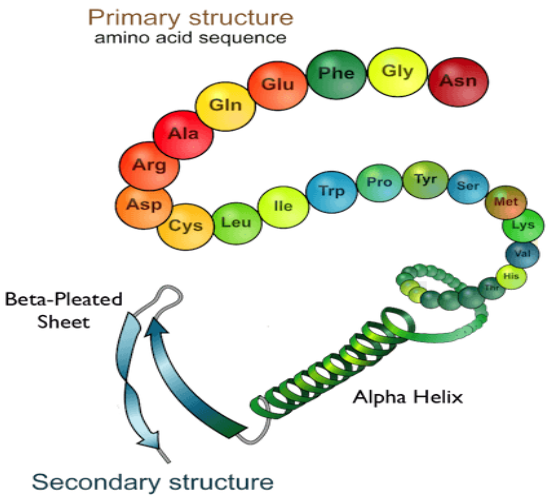


Lock and Key


Substrate
Enzyme
Enzyme-Substrate (ES) Complex
Products
Enzyme

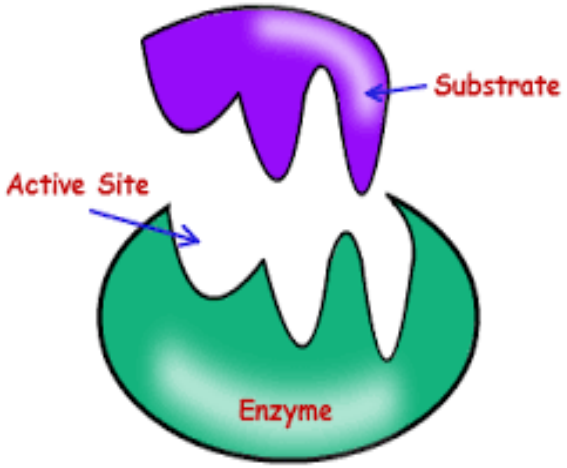
Features of Active site

- Active site is due to tertiary structure of protein.
- Made up of amino acids (known as catalytic residue),which are far from each other in primary structure.
- Regarded as clefts or crevices or pockets occupying a small region.



Flexible to promote the specific substrate binding.

- Posseses a substrate binding site and a catalytic site(for catalysis of specific reaction).
- Co enzymes are present as a part of catalytic site.
- The substrate binds at the active site by weak noncovalent bonds.



- The active site is much more than simply a binding site for substrates
- It provides a 3 dimensional environment that both shields substrates from Solvents and Facilitates Catalysis
- Binds co-factors or prosthetic groups needed for catalysis

- Within active site substrates are alligned in close proximity and optimal orientation to the co-factors and prosthetic groups responsible for catalysis and their chemical transformation into PRODUCTS.
- Products have a different shape from the substrate
- Once formed, they are released from the active site
- Leaving it free to become attached to another substrate

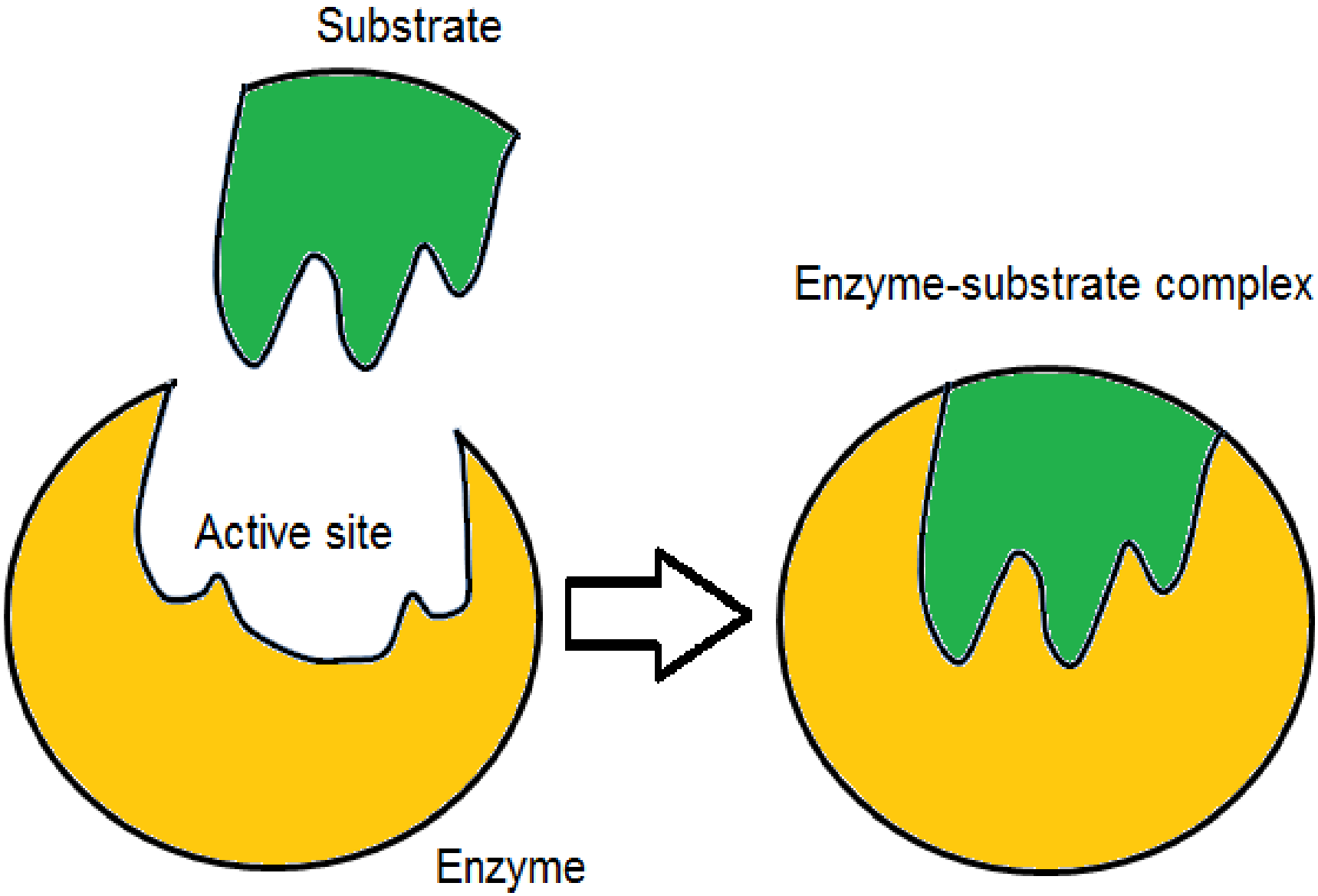
INDUCED FIT MODEL

- Fischer's lock & key model showed exquisite specificity for enzyme-substrate interactions
- But because of the rigidity of the active site it failed to account for the dynamic changes accompanying catalysis.
- To overcome this drawback, Daniel Koshland introduced INDUCED FIT MODEL.

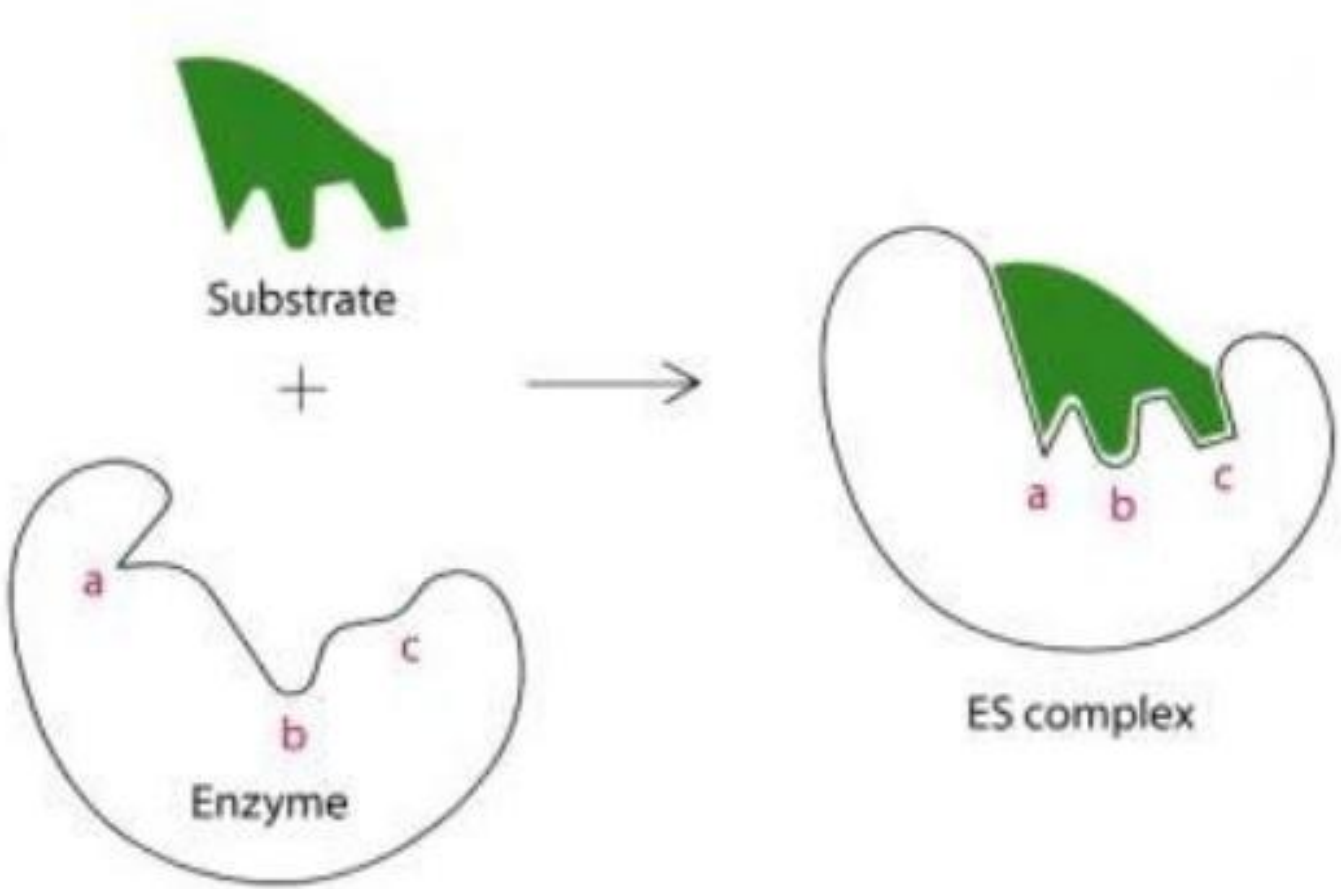

Remembering
Daniel E. Koshland Jr.
1920–2007

- Daniel proposed that when substrate approaches and binds to the enzyme they induce a conformational change which is analogous to placing a hand (SUBSTRATE) into a glove (ENZYME)

- The enzyme brings about reciprocal changes in the substrates,harnessing the energy of binding to facilitate the transformation of SUBSTRATES into PRODUCTS


Substrate
Enzyme-substrate complex
Active site
Enzyme

Induced Fit



THE IUB CLASSIFICATION OF ENZYMES

- For identification of enzymes , International Union of Biochemistry(IUB) adopted a nomenclature system based on chemical reaction type and reaction mechanism.
- Enzymes are grouped into six classes
- Each enzyme has a code number (EC No) comprising four figures (digits) separated by points

The four digits characterize

- CLASS
- SUB-CLASS
- SUB-SUB-CLASS
- SERIAL NUMBER of a particular ENZYME

The IUB name of HEXOKINASE is ATP:D-hexokinase 6-phosphotransferase EC 2.7.1.1

- Class 2 (transferase)
- Subclass 7 (transfer of phosphate gp)
- Sub-Sub-Class 1 (alcohol function as the phosphate acceptor)
- The fourth digit denotes the enzyme hexokinases.

Hexose-6 indicates that the transfer of phosphate from ATP to hydroxyl group on carbon no 6 of hexose.

Definition

An enzyme is a protein or RNA produced by living cells, which is highly specific and highly catalytic to its substrates.

Enzymes are a very important type of macromolecular biological catalysts.

Classification

CLASSIFICATION

- The SIX main classes of enzymes (O-T-H-L-I-L)are
- 1. OXIDOREDUCTASE: enzymes involved in oxidation and reduction of their substrates.
- Example:
- Lactate dehydrogenase,glucose 6 phosphate dehydrogenase.
- Cofactors and coenzymes NAD,NADH2 FAD,FADH2

2. TRANSFERASES: catalyze the transfer of a particular group from one substrate to another.(glycosyl,methyl,and phosphoryl moieties)

These catalyze transfers of
groups like phosphate or amines.

- Example:
- L-alanine + a-ketoglutarate -→
  pyruvate + L-glutamate
- Kinases are transferases:
  they transfer a phosphate from ATP to something else

- 3. HYDROLASES: catalyze hydrolytic cleavage of C–C,
- C–O, C–N, and other bonds.

- Example: pyrophosphatase
- Pyrophosphate + H_2O –→ 2 Phosphate

- 4. LYASES:
- Catalyze cleavage of C–C, C–O, C–N, and other bonds by atom elimination leaving behind a double bond.
- Non-hydrolytic, nonoxidative elimination (or addition) reactions
- Addition across a double bond or reverse
- Example: pyruvate decarboxylase
- More typical lyases add across C=C

- 5. ISOMERASES:

Catalyze geometric or structural changes within a molecule.

glucose-6-P -→ fructose-6-P

- Subcategory :

- Mutases: shift a single functional group from one carbon to another (phosphoglucomutase)

- 6. LIGASES:

  Catalyze the joining together of two substrates
- Example:
- RNA synthetase
- DNA ligases
- glutamine synthetase

MECHANISM OF CATALYSIS

- Enzymes use various combinations of general mechanisms to achieve catalysis.

Mechanism of enzyme catalysis

The formation of an enzyme-substrate complex (ES) is very crucial for the catalysis to occur and for the product formation. Enzyme catalysed reaction proceeds 10^6 to 10^{12} times faster than a non-catalysed reaction. This is mainly due to four processes-

1. **Acid-base catalysis**
2. **Substrate strain**
3. **Covalent catalysis**
4. **Proximity catalysis**

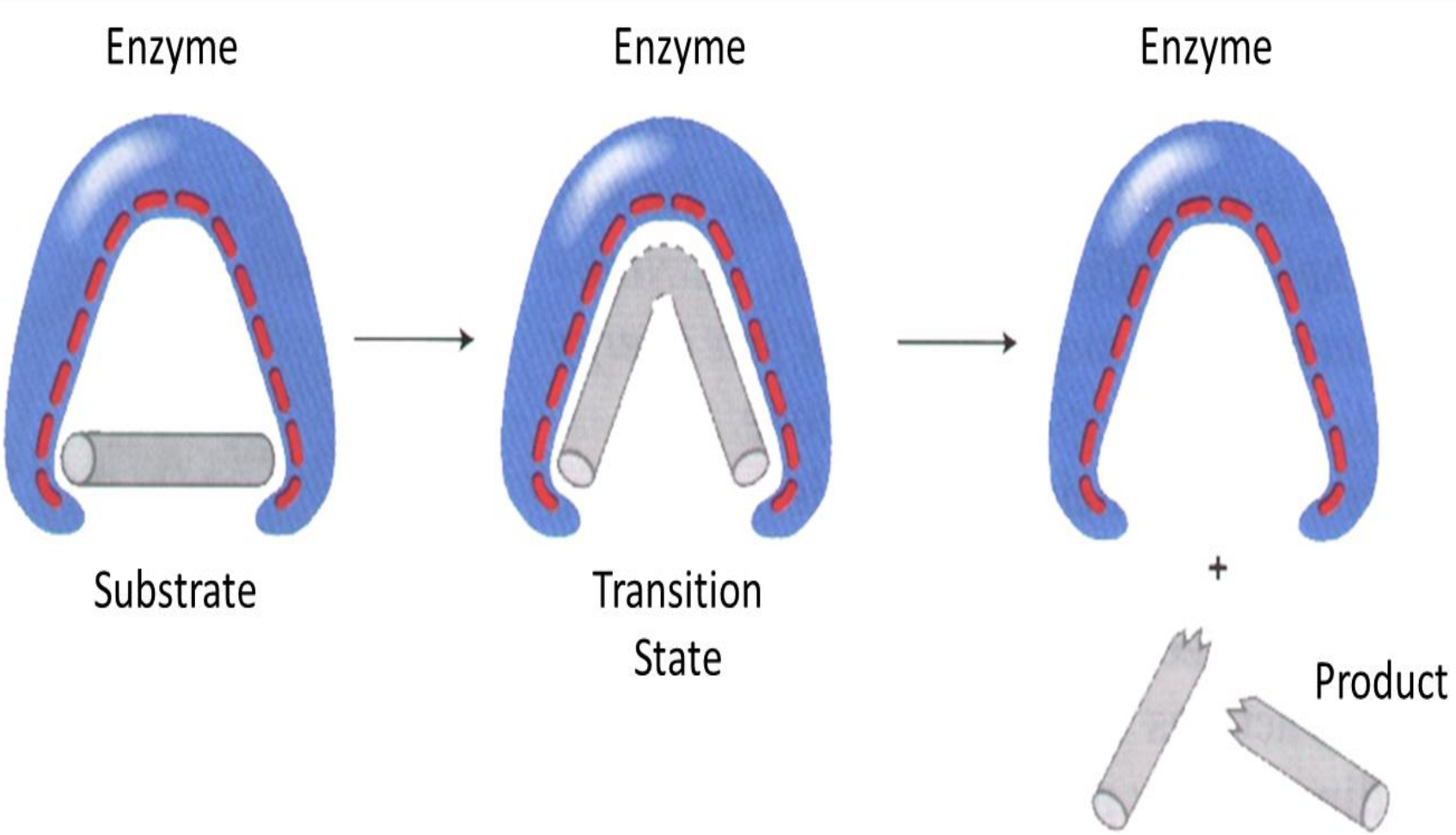
1. ACID-BASE CATALYSIS:

- The ionizable functional groups of aminoacyl side chains and of prosthetic groups(where present) contribute to catalysis by acting as acids or bases. Acid-base catalysis can either be SPECIFIC or GENERAL.

- In specific acid or specific base catalysis, the rate of reaction will be sensitive to changes in the concentration of protons but independent of concentration of other acids(proton donors) or bases(proton acceptors) present in solution.

Reactions of whose rates are responsive to all acids or bases present are termed as general acid or general base catalysis.

- 2. CATALYSIS BY STRAIN:
- Enzymes that catalyze -lytic reactions that involve breaking a covalent bond, typically bind their substrates in a conformation slightly unfavorable for the bond that will undergo cleavage.
- The resulting strain distorts or stretches the targeted bond, weakening it and making it more vulnerable to cleavage.


Enzyme
Enzyme
Enzyme
Substrate
Transition State
+
Product

3. COVALENT CATALYSIS:

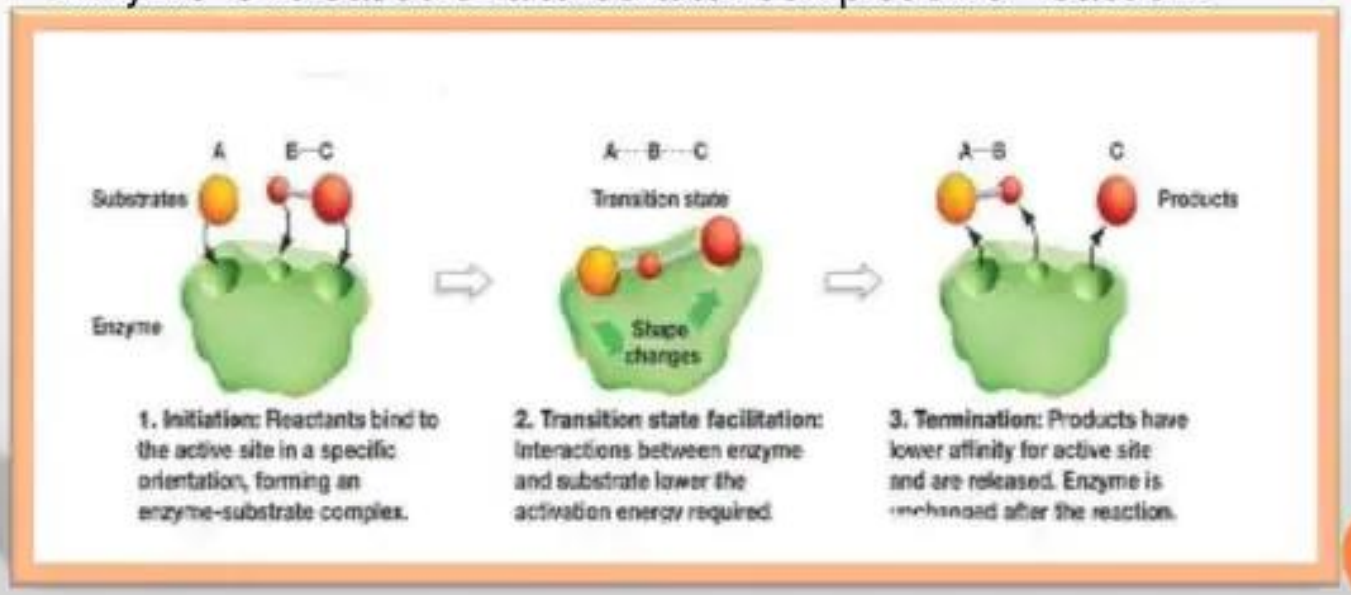
- The process of covalent catalysis involves the formation of a covalent bond between the enzyme and one or more substrates.

- Covalent catalysis introduces a new reaction pathway whose activation energy is low and therefore is faster than the reaction pathway in homogenous solution.

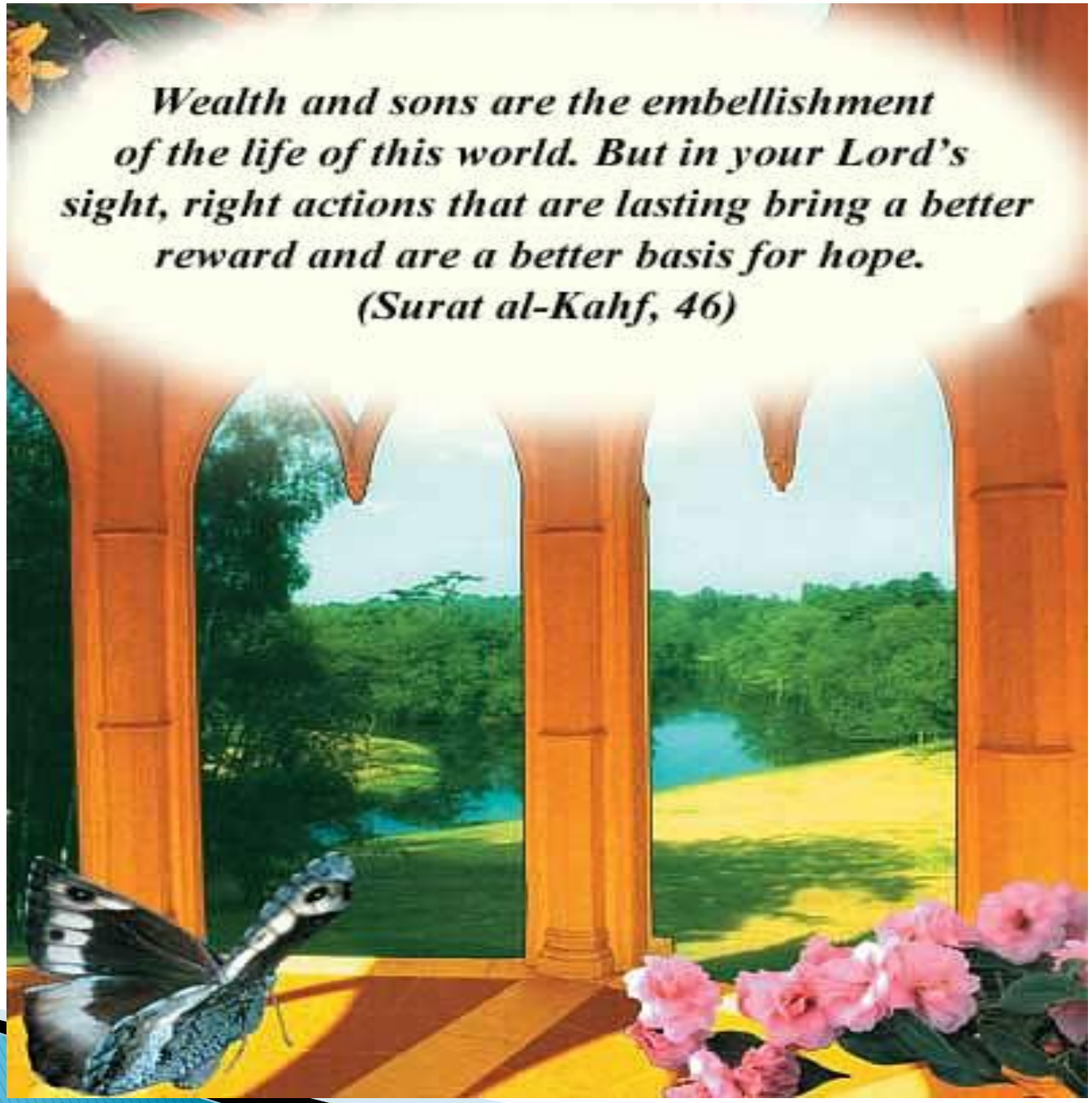
- The chemical modification of enzyme is transient and on completion of the chemical reaction the enzyme returns back to its original unmodified state. Enzyme role is purely catalytic, this type is common for group transfer reactions.

COVALENT CATALYSIS

- Enzymes form covalent linkages with substrate forming transient enzyme-substrate complex with very low activation energy.
- Enzyme is released unaltered after completion of reaction.



- 4. CATALYSIS BY PROXIMITY:
- For molecules to react they must come within bond forming distance of one another. The higher their concentration the more frequently they will encounter one another, and the greater will be the rate of their reaction.


Wealth and sons are the embellishment
of the life of this world. But in your Lord's
sight, right actions that are lasting bring a better
reward and are a better basis for hope.
(Surat al-Kahf, 46)


thank
you