Enzymes And Its Properties

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- A vast multitude of chemical reactions occur in living organisms
- It is these reactions that keep the organism going
- These reactions would occur at extremely low velocities in the absence of catalysts

- Common catalysts used in non-living systems are:
- Acids
- Alkalis
- Metals
- These are not suitable for living organisms because of their:
- Toxicity
- Lack of specificity

- Biological catalysts should be:
- Safe (non-toxic)
- Specific (generally catalyzing only one reaction)
- Capable of adjusting their catalytic activity

- All these properties are present in enzymes

Enzymes were first discovered in yeast (enzyme means '*in yeast*')

They were later found in other living organisms as well

They could catalyze reactions outside the living organisms also

Chemically, all enzymes were found to be proteins

Definition

Enzymes are protein catalysts that catalyse chemical reactions in biological systems .

But this definition is not entirely correct

Some RNA molecules (ribozymes) have been found to catalyze some reactions

- The reactant on which the enzyme acts is known as the substrate of the enzyme
- The enzyme converts the substrate into a product or products
- Substrate ---------- Product

Enzyme specificity

- If an enzyme catalyses a number of reactions, it will be impossible to regulate individual reactions .
- However, the enzymes are highly specific .
- Generally, one enzymes catalyses only one reaction.
- This is of crucial importance for regulation of metabolic pathways

Enzyme specificity may have the following orders:

- Group specificity
- Substrate specificity
- Stereo-specificity

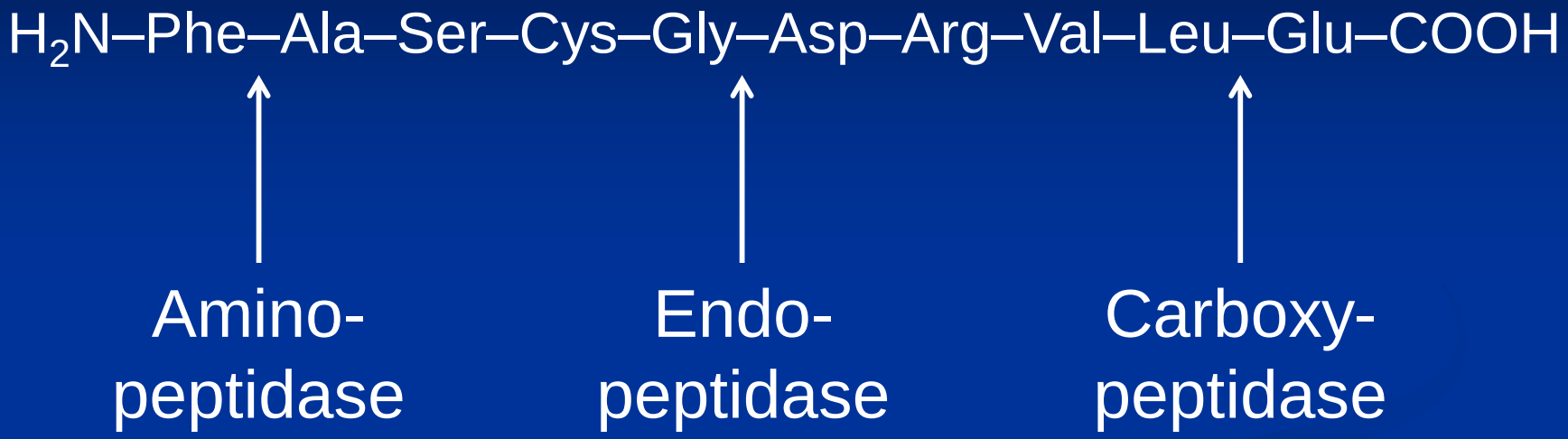
Group specificity

- Enzyme is specific for a bond but not for the actual substrate.
- Group-specific or bond-specific enzymes are commonly present in digestive secretions
- For example, pepsin is specific for peptide bond but not for any protein .
- Thus, a large variety of dietary proteins can be digested by the same enzyme .
- Trypsin, chymotrypsin, nucleases, lipases and glycosidases are other examples

Some group-specific enzymes have a slightly higher degree of specificity

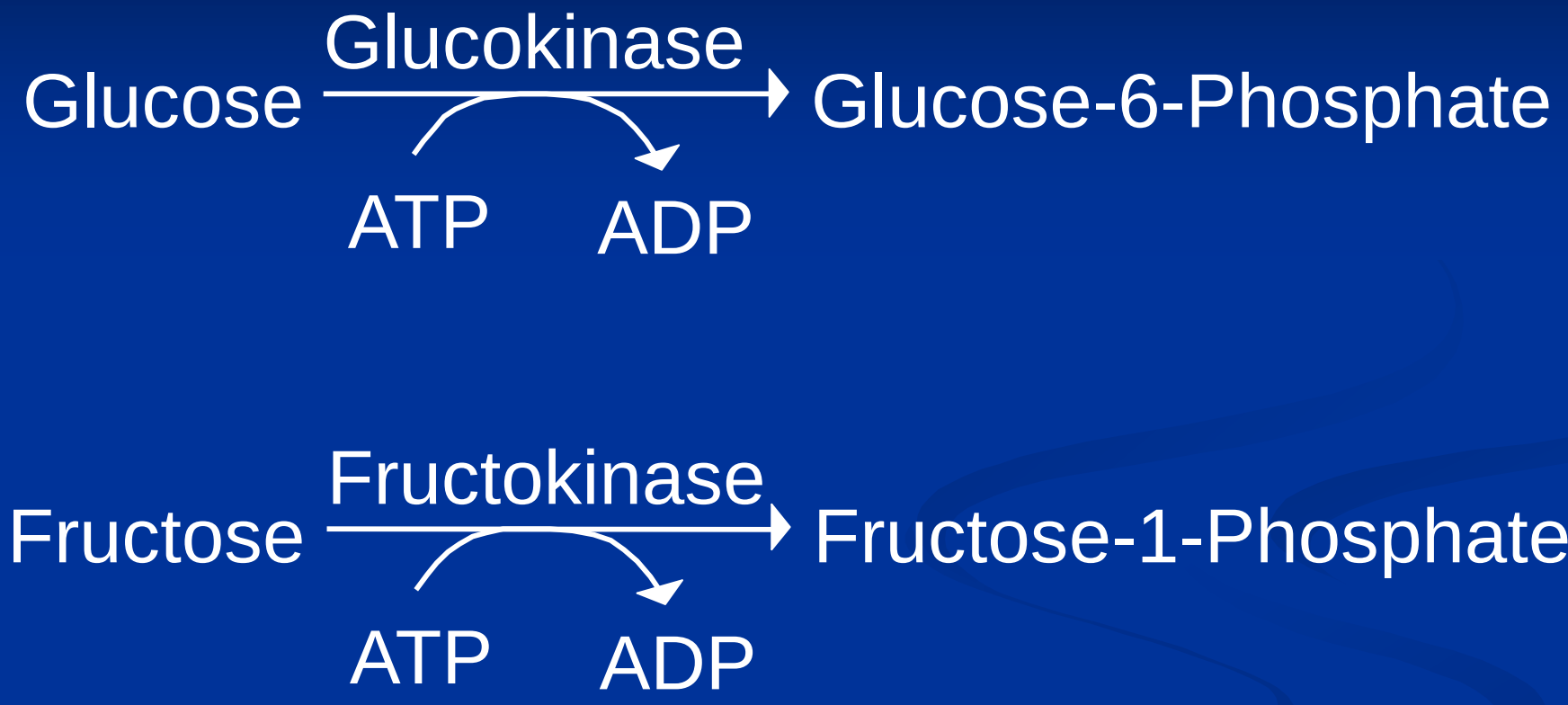
- For example, aminopeptidase hydrolyses only N-terminal peptide bond

- Carboxypeptidase hydrolyses only the C-terminal peptide bond.
- Endopeptidases hydrolyse the internal peptide bonds only


H2N–Phe–Ala–Ser–Cys–Gly–Asp–Arg–Val–Leu–Glu–COOH
Amino-peptidase
Endo-peptidase
Carboxy-peptidase

- **Substrate specificity**

- Most enzymes are specific for a chemical bond/group as well as the substrate
- For example, glucokinase and fructokinase are substrate-specific enzymes.
- They transfer a phosphate group from ATP to one specific substrate


Glucose
Glucokinase
Glucose-6-Phosphate
ATP
ADP
Fructose
Fructokinase
Fructose-1-Phosphate
ATP
ADP

Stereo-specificity

- Many biomolecules exhibit stereo-isomerism.
- Examples are carbohydrates and amino acids Enzymes acting on these are specific for one stereo-isomer

Mammalian enzymes acting on carbo-hydrates are generally specific for D-isomers .

- Those acting on amino acids are generally specific for L-isomers.
- Exceptions are racemases which inter-convert the D- and L-isomers

Coenzymes and cofactors

- Some enzymes require a non-protein substance for their catalytic activity .

- If the non-protein substance is organic, it is known as a coenzyme.

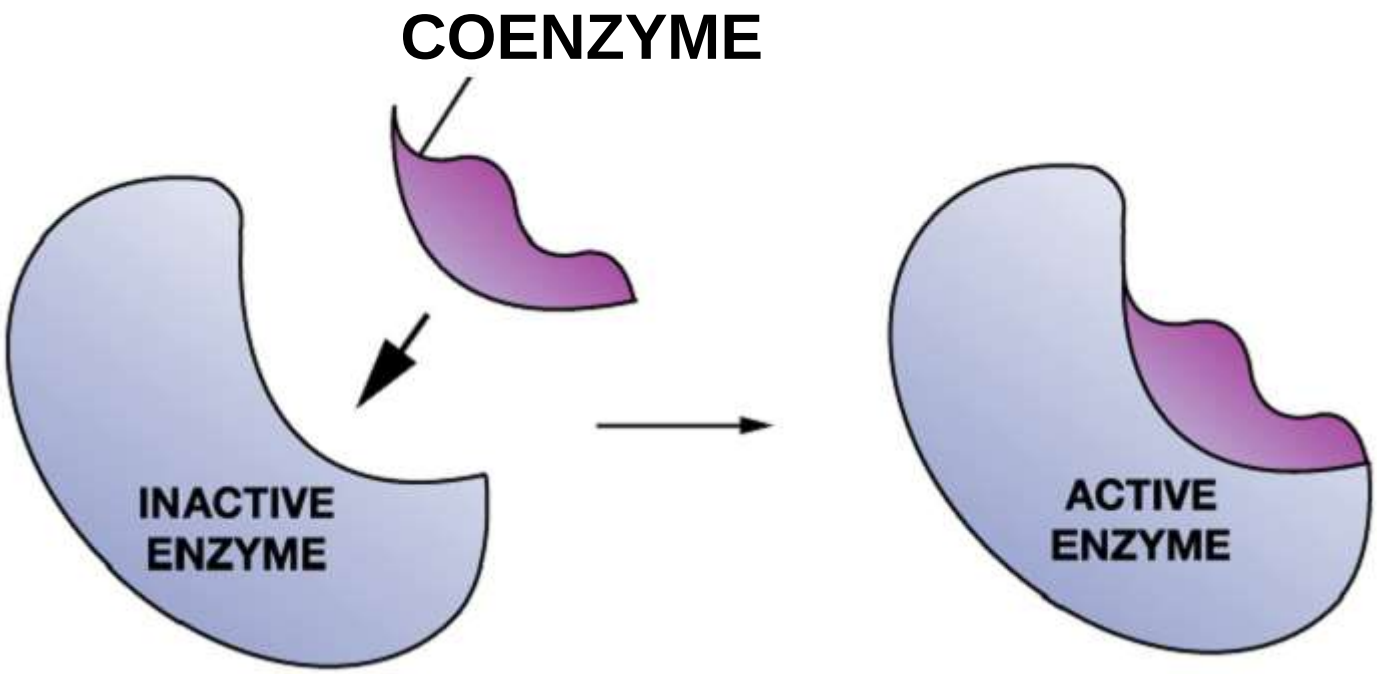
- If the non-protein substance is inorganic, it is known as a cofactor

The coenzyme or the cofactor may be:

An integral part of the enzyme or Its presence may be required during the reaction

- The protein portion of an enzyme that requires a coenzyme is called apoenzyme
- Apoenzyme combines with coenzyme to form the active holoenzyme.

Apoenzyme + Coenzyme ⟶ Holoenzyme


COENZYME
INACTIVE ENZYME
ACTIVE ENZYME
APOENZYME
HOLOENZYME

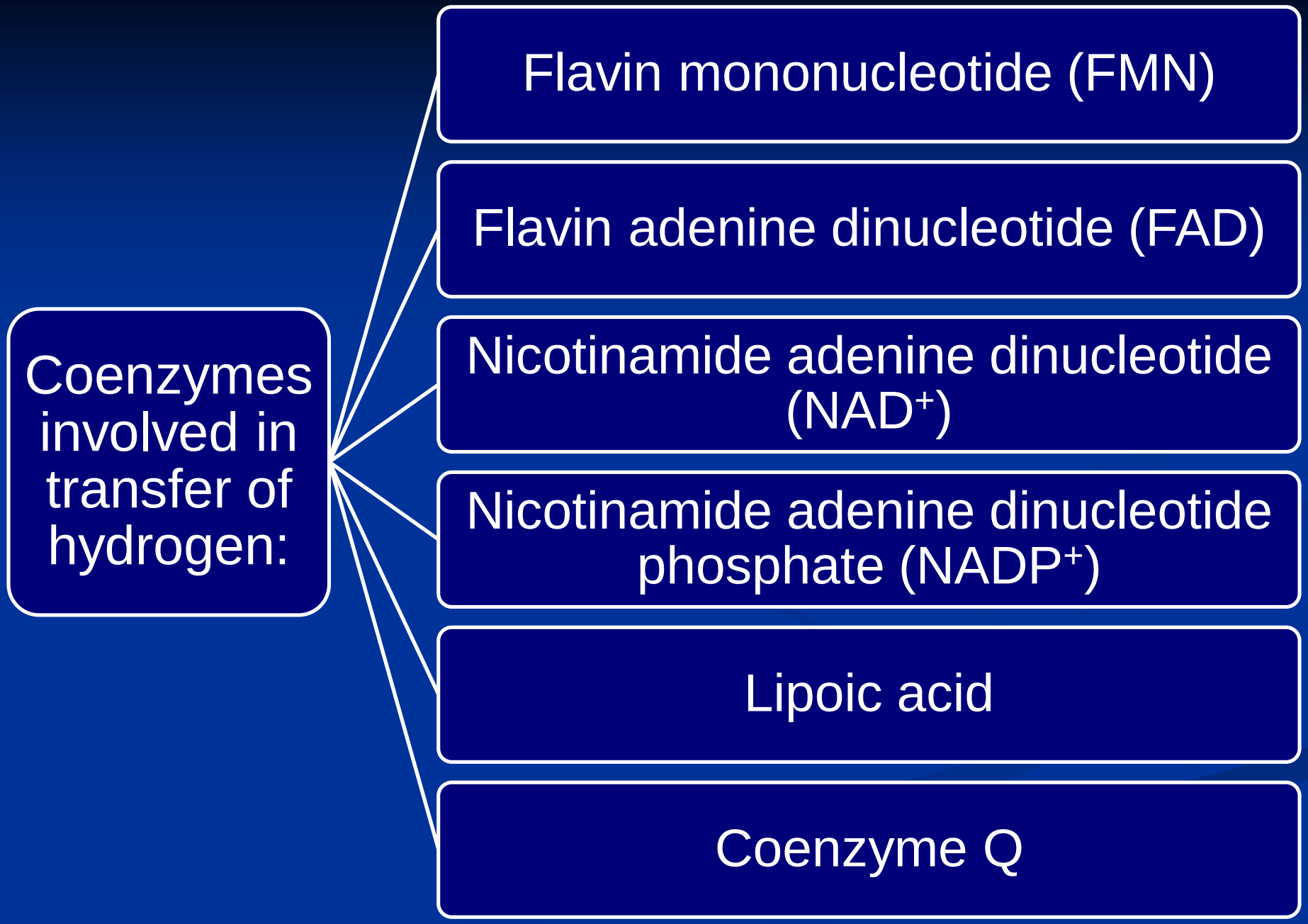
- The coenzymes generally contain vitamins of B-complex family.
- Some act as coenzymes by them- selves e.g. biotin.
- Others are converted into coenzymes

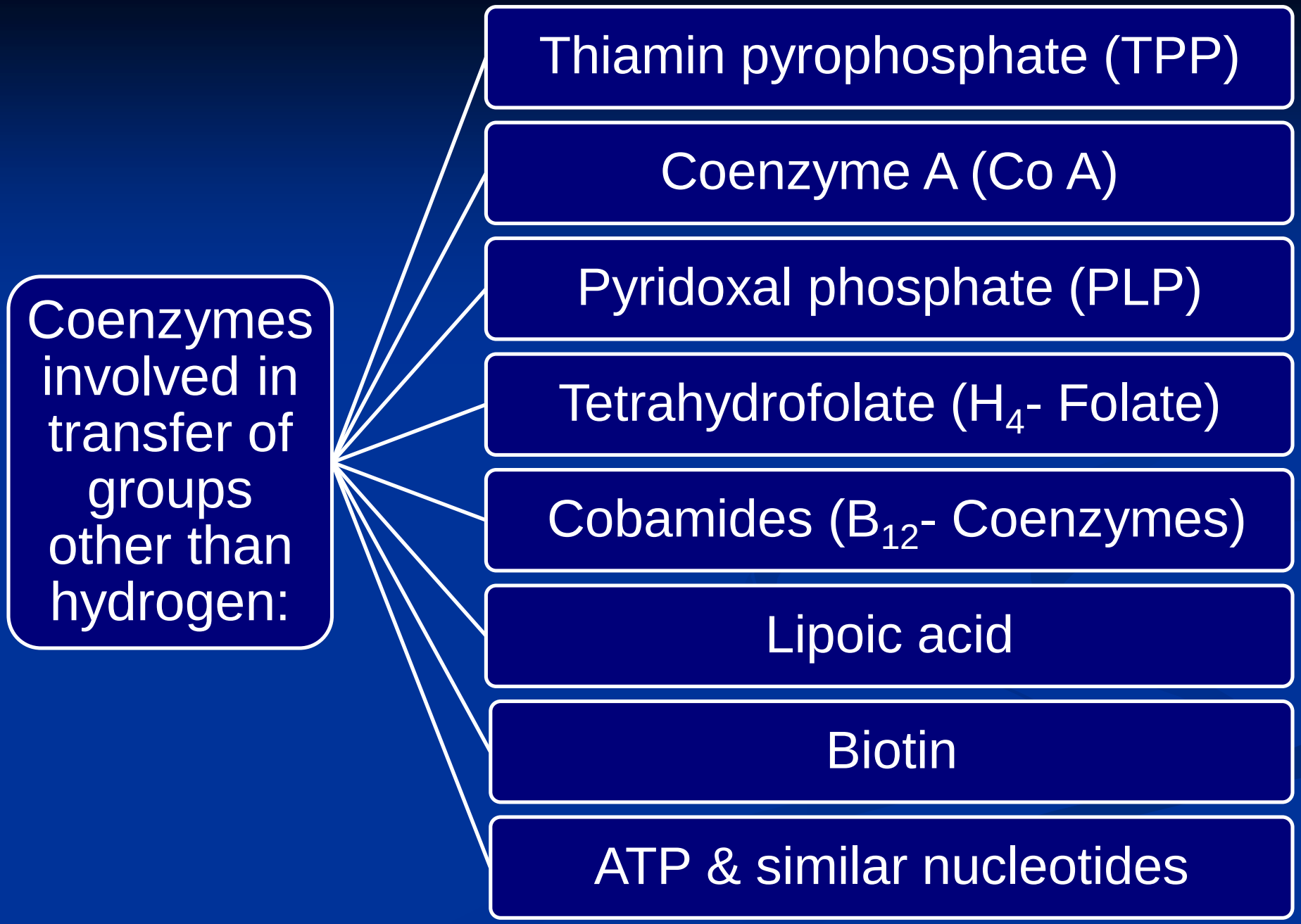
B-Complex vitamins that are converted into coenzymes are:

- Thiamin
- Riboflavin
- Pantothenic acid
- Pyridoxine
- Folic acid
- Vitamin B_{12}

- Coenzymes are generally required in group transfer reactions such as:
- Oxidation-reduction
- Transamination
- Phosphorylation

- Coenzymes can be divided into two groups:
- Coenzymes involved in transfer of hydrogen.
- Coenzymes involved in transfer of groups other than hydrogen


Coenzymes involved in transfer of hydrogen:
Flavin mononucleotide (FMN)
Flavin adenine dinucleotide (FAD)
Nicotinamide adenine dinucleotide (NAD^+)
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)
Lipoic acid
Coenzyme Q

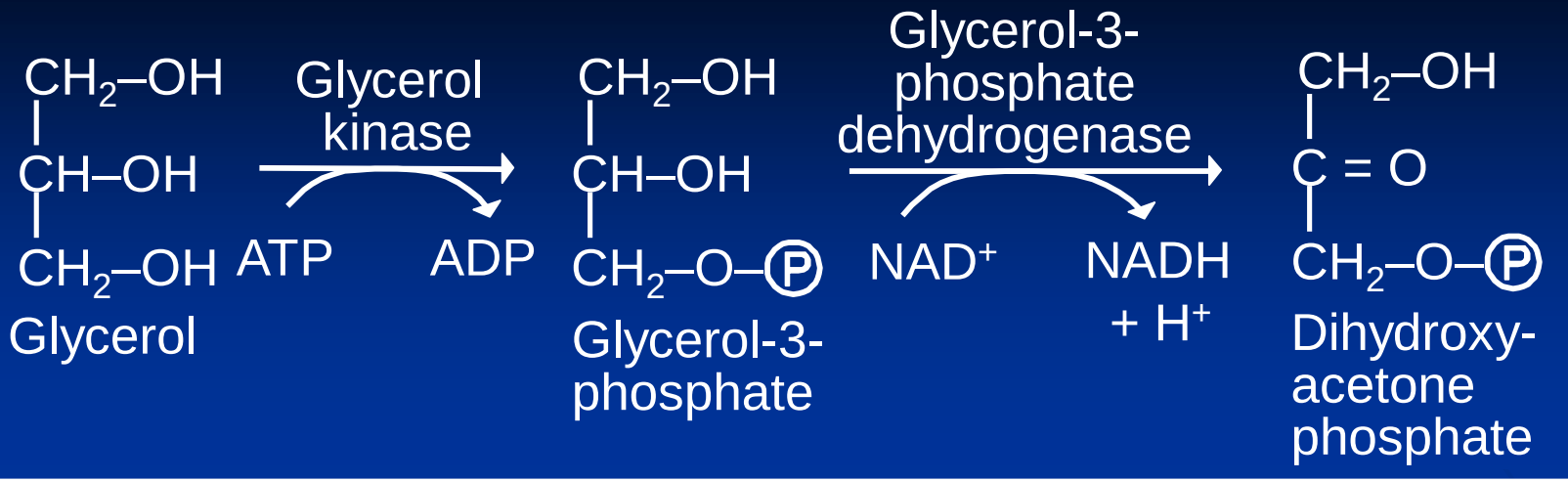

Coenzymes involved in transfer of groups other than hydrogen:
Thiamin pyrophosphate (TPP)
Coenzyme A (Co A)
Pyridoxal phosphate (PLP)
Tetrahydrofolate (H_4- Folate)
Cobamides (B_{12}- Coenzymes)
Lipoic acid
Biotin
ATP & similar nucleotides

Role of coenzymes

The enzyme acts upon its substrate, and converts it into a product.

Coenzyme acts as a co-substrate or a second substrate in the group transfer reactions.

The coenzyme either donates or accepts the group that is being transferred



In the first reaction, the coenzyme ATP acts as a second substrate and donates a phosphate group

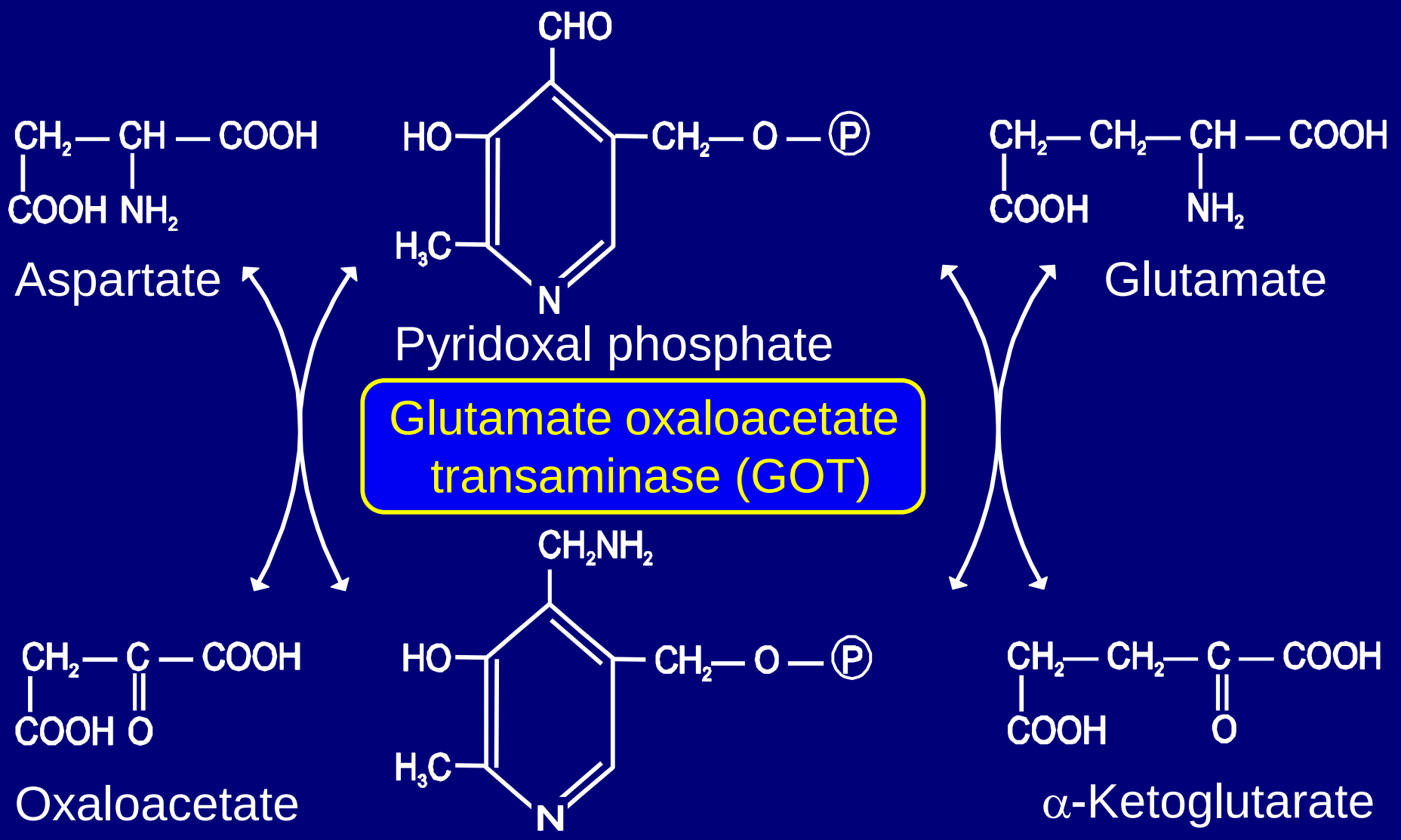
In the second reaction, the coenzyme NAD^+ acts a second substrate and accepts the hydrogen atoms

The chemical change in the coenzyme is opposite to that in the substrate

Some coenzymes accept a chemical group from one substrate and donate it to another

Thus, they act only as carriers, and regain their original form at the end of the reaction

Pyridoxal phosphate, for example, acts as a carrier of amino group in transamination


CHO
HO
CH2 — O — Ⓟ
H3C
N
Pyridoxal phosphate
CH2 — CH — COOH
COOH NH2
Aspartate
CH2 — CH2 — CH — COOH
COOH NH2
Glutamate
Glutamate oxaloacetate transaminase (GOT)
CH2NH2
HO
CH2 — O — Ⓟ
H3C
N
Pyridoxamine phosphate
CH2 — C — COOH
COOH O
Oxaloacetate
CH2 — CH2 — C — COOH
COOH O
α-Ketoglutarate

- Pyridoxal phosphate first accepts the amino group from aspartate, and is converted into pyridoxamine phosphate.
- Pyridoxamine phosphate then transfers the amino group to α-ketoglutarate, and is converted into pyridoxal phosphate.
- In the coupled reaction, aspartate is converted into oxaloacetate, and α-ketoglutarate is converted into glutamate

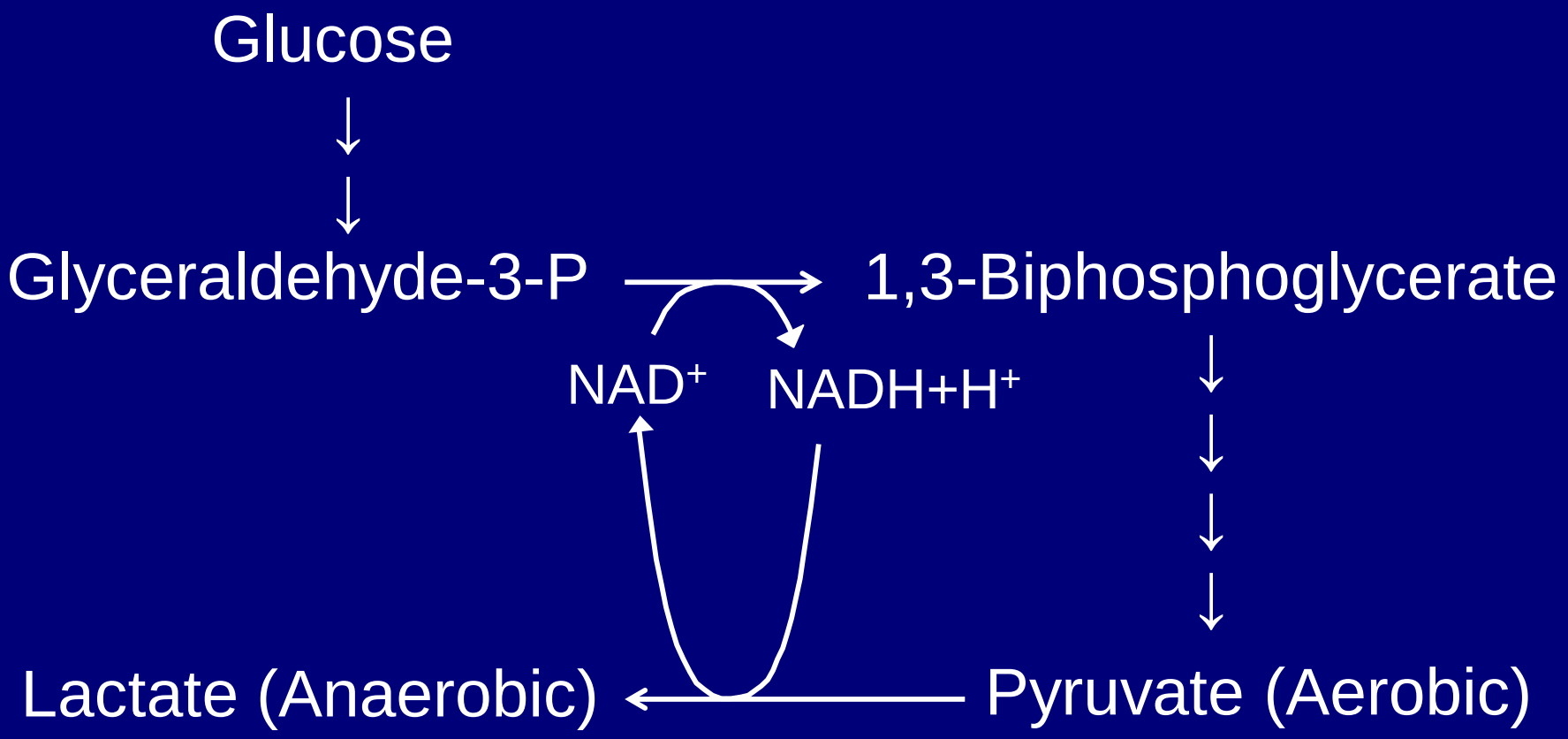
- The coenzyme goes back to its original form at the end of the reaction.
- Though pyridoxal phosphate is a reactant, the reaction is usually shown as:
- Aspartate + α-Ketoglutarate
  Oxaloacetate + Glutamate

- Sometimes, the change in the coenzyme is more important than that in the substrate.
- In glycolysis, glucose is oxidized to pyruvate, and NAD^+ is reduced in one reaction.
- Reduced NAD^+ transfers its hydrogen atoms to oxygen, and NAD^+ is regenerated.

If the conditions are anaerobic, NAD^+ cannot be regenerated due to lack of oxygen.

One more reaction occurs in which pyruvate is reduced to lactate, and NADH is oxidized to NAD^+.

Here, regeneration of NAD^+ is more important for continuation of glycolysis


Glucose
Glyceraldehyde-3-P
1,3-Biphosphoglycerate
NAD+
NADH+H+
Lactate (Anaerobic)
Pyruvate (Aerobic)

Enzyme nomenclature and classification

The nomenclature of enzymes has undergone many changes over the years

The names given to enzymes in the beginning were vague and uninformative

Some of the early names are pepsin, ptylin, zymase etc

These names give no information about the reaction catalyzed by the enzyme

- Later on, a slightly more informative nomenclature was adopted.
- Suffix -ase was added to the name of the substrate e.g. lipase, protease etc.
- Still the type of reaction catalyzed by the enzyme remained unclear

- Nomenclature was modified further, to include the name of the substrate followed by the type of reaction ending with –ase.
- This resulted in names like lactate dehydro-genase, pyruvate carboxylase, glutamate decarboxylase etc
- Even these names do not give complete information, for example whether a coenzyme is required or a byproduct is formed

- International Union of Biochemistry (IUB) formed an Enzyme Commission to make the names of enzymes informative and unambiguous,
- The enzyme commission proposed a method of nomenclature and classification of enzymes which is applicable to all living organisms

According to IUB system:

- The enzymes have been divided into six classes (numbered 1 - 6)
- Each class is divided into subclasses
- Subclasses are divided into subsubclasses
- Subsubclasses are divided into individual enzymes

Nomenclature

The name of the enzyme has two parts.

The first part includes the name(s) of the substrate(s) including cosubstrate (coenzyme).

The second part includes the type of reaction ending with -ase .

If any additional information is to be given, it is put in parenthesis at the end

For example, the enzyme having the trivial name glutamate dehydrogenase catalyzes the following reaction:

L-Glutamate + $NAD(P)^+$ + $H_2O \rightarrow \alpha$-Ketoglutarate + $NAD(P)H + H^+ + NH_3$

According to IUB system, this enzyme is known as L-Glutamate: NAD(P) oxido-reductase (deaminating)

The IUB name shows that:

This enzyme acts on L-glutamate

NAD^+ or $NADP^+$ is required as a co-substrate

Type of reaction is oxidoreduction i.e. L-glutamate is oxidised and the co-substrate is reduced

The amino group of L-glutamate is released as ammonia

Moreover, each enzyme has been given a code number consisting of four digits:

First digit shows the number of the class.

Second digit shows the number of the subclass .

Third digit shows the number of the subsubclass.

Fourth digit shows the number of the enzyme

The code number of L-glutamate: NAD(P) oxidoreductase (deaminating) is EC 1.4.1.3

This shows that is it the third enzyme of subsubclass 1 of subclass 4 of class 1

EC is the acronym for Enzyme Commission

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The enzymes are divided into six classes in IUB classification:

Oxidoreductases
Transferases
Hydrolases
Lyases
Isomerases
Ligases

Oxidoreductases-

These are the enzymes that catalyze oxidation-reduction reactions

One of the substrates is oxidised and the other is reduced

Different subclasses act on different chemical groups

Groups undergoing the reaction include
–CH=CH–, >CH–OH, >C=O, >CH–NH_2 etc

Examples of oxidoreductases are:

- Glutamate dehydrogenase
- Lactate dehydrogenase
- Malate dehydrogenase
- Glycerol-3-phosphate dehydrogenase

Transferases

- Transferases transfer a group other than hydrogen from one substrate to another
- Such groups include methyl group, amino group, phosphate group, acyl group, glycosyl group etc

Examples of transferases are:

- Hexokinase
- Glucokinase
- Glutamate oxaloacetate transaminase
- Ornithine carbamoyl transferase

Hydrolases

- Hydrolases hydrolyse bonds such as peptide, ester, glycosidic bonds etc
- They are commonly found in the digestive secretions and lysosomes
- They hydrolyse carbohydrates, lipids, proteins etc

Examples of hydrolases are:

Amylase

Lipase

Pepsin

Ribonuclease

Lyases

- Lyases remove chemical groups from substrates by mechanisms other than hydrolysis
- The groups removed may be water, amino group, carboxyl group etc

Examples of lyases are:

- Aldolase
- Enolase
- Fumarase

Isomerases

- Isomerases catalyse inter-conversion of isomers of compound
- include aldose-ketose isomers, stereo-isomers etc

Examples of isomerases are:

- Alanine racemase
- Triose phosphate isomerase
- Phosphohexose isomerase
- Ribose-5-phosphate ketoisomerase

Ligases

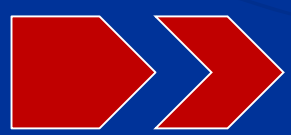
- These enzymes ligate or bind two substrates together
- Binding occurs by a covalent bond
- A source of energy is required e.g. a high-energy phosphate

Examples of ligases are:

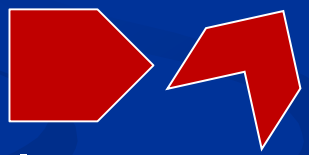
- Glutamine synthetase
- Squalene synthetase
- Acetyl CoA carboxylase

Mechanism of action of enzymes

- At temperatures above absolute zero (–273°C), molecules are in constant motion because of their kinetic energy
- A chemical reaction occurs when molecules of reactants collide with each other in the correct orientation (kinetic theory of reaction)



Correct orientation



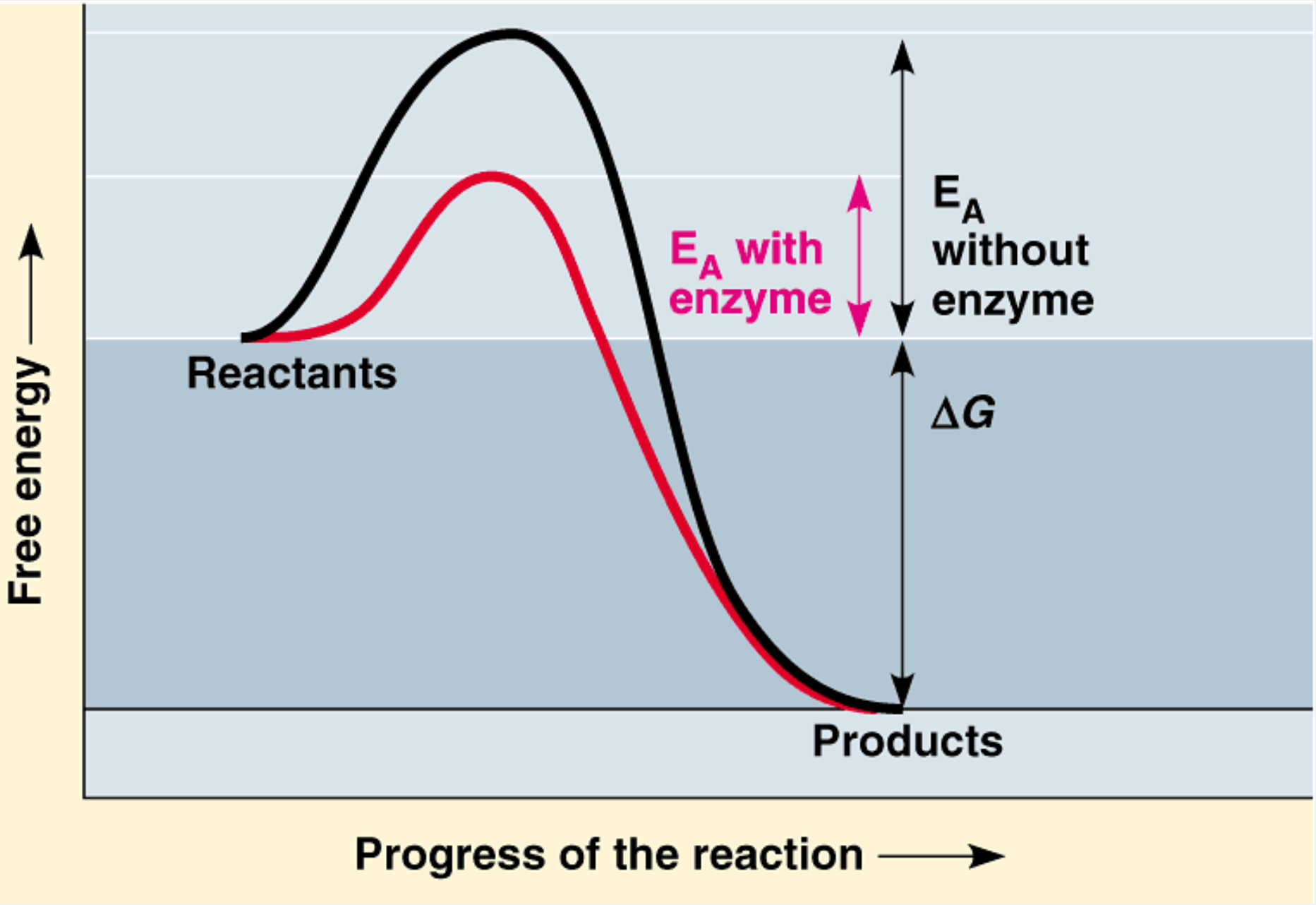
Incorrect orientation

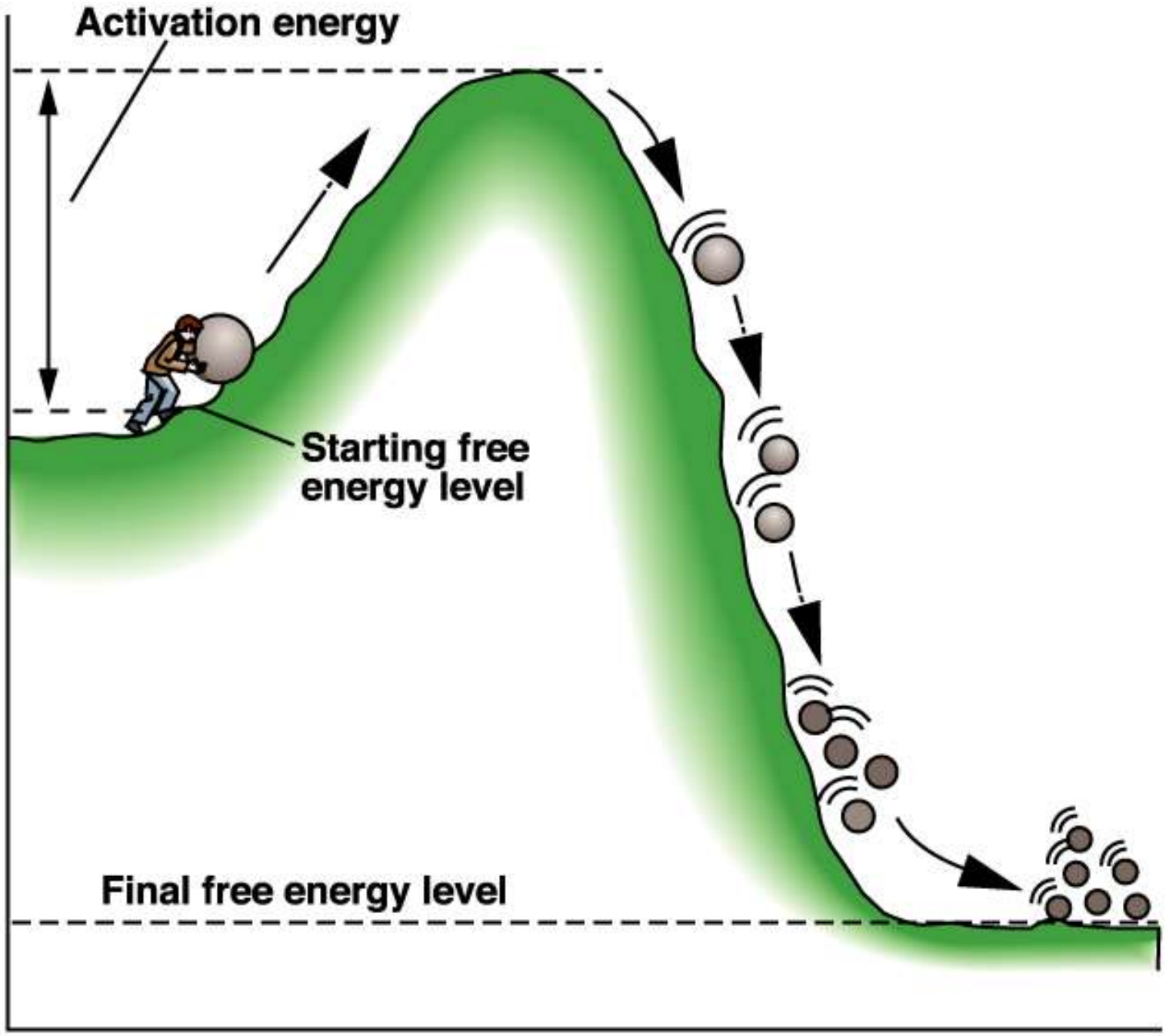
- The greater the frequency of collisions between the reactant molecules, the greater will be the rate of reaction
- The frequency of collisions can be increased by raising the temperature

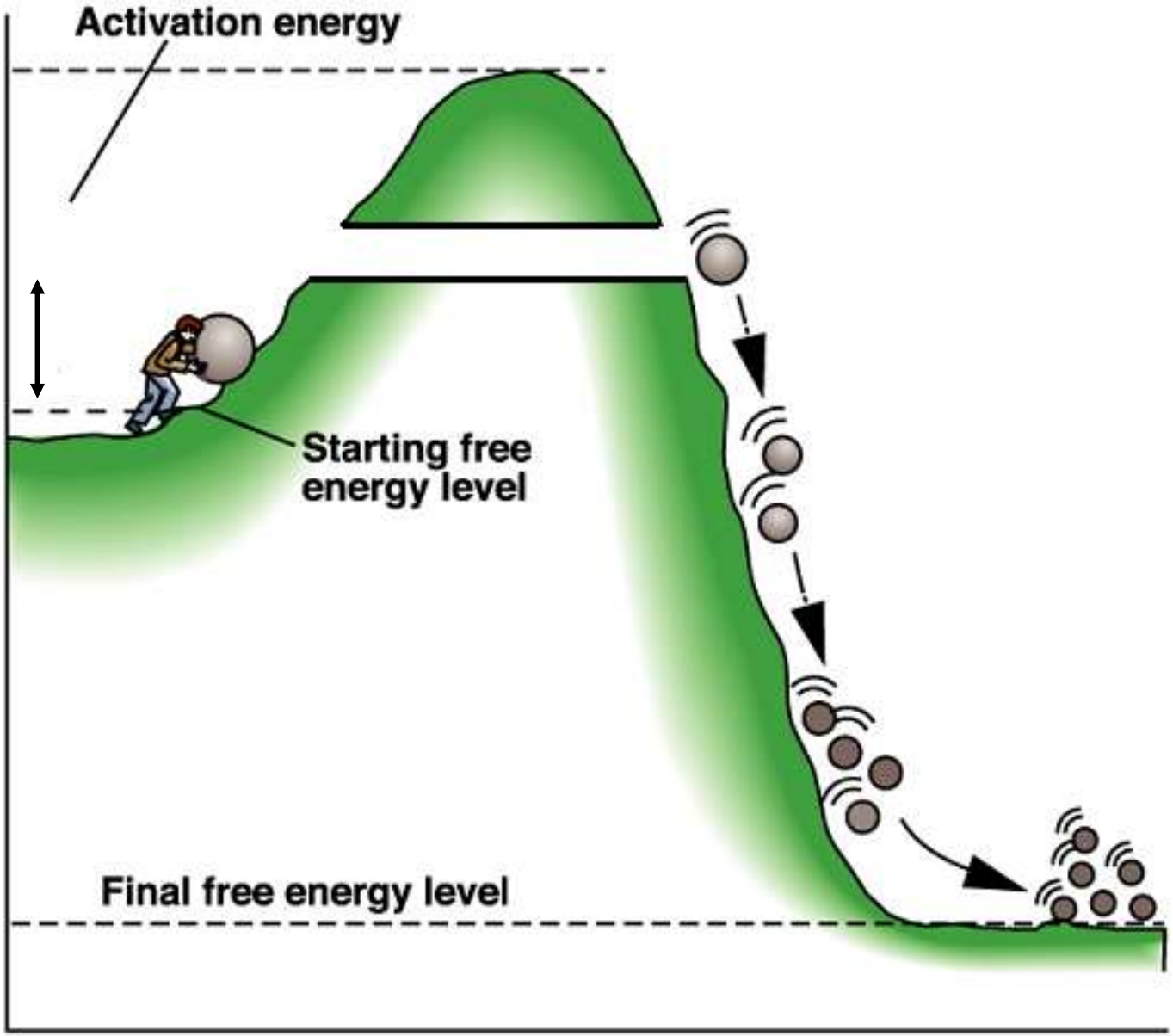
A rise in temperature would increase:

- Molecular motion
- Frequency of collisions
- Rate of reaction
- Energy level of reactants has to be raised to a critical level for the reaction to occur
- Energy input required to reach the critical level is known as the energy of activation

- The option of raising temperature is not available in living organisms
- In living organisms, the enzymes provide an alternate pathway for the reaction
- Enzymes lower the energy of activation

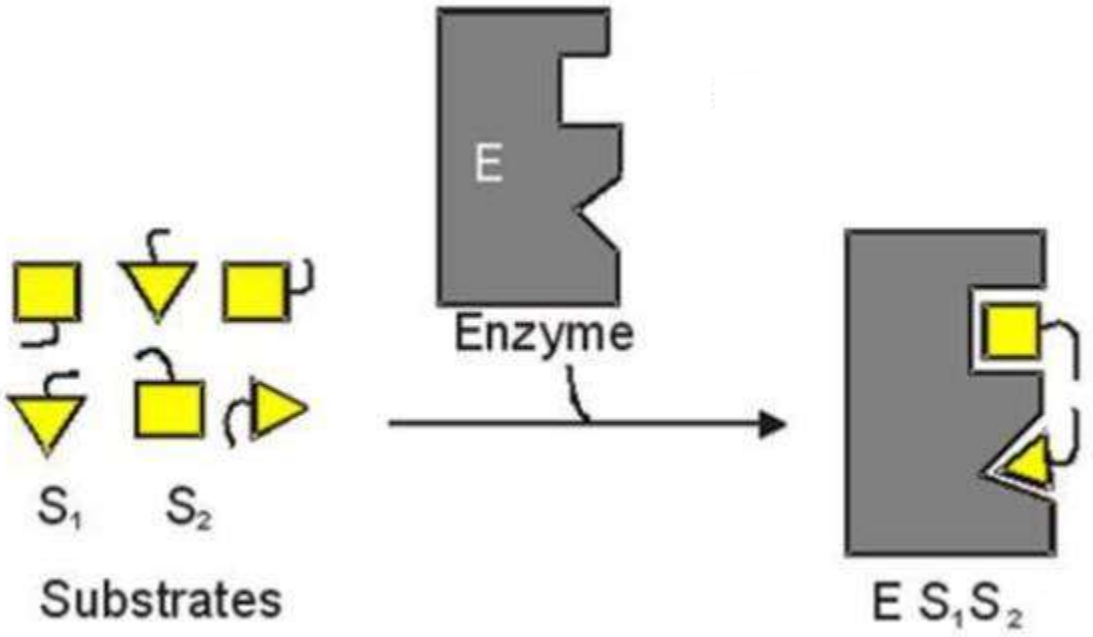

Free energy
Reactants
E_A with enzyme
E_A without enzyme
ΔG
Products
Progress of the reaction


Activation energy
Starting free energy level
Final free energy level


Activation energy
Starting free energy level
Final free energy level

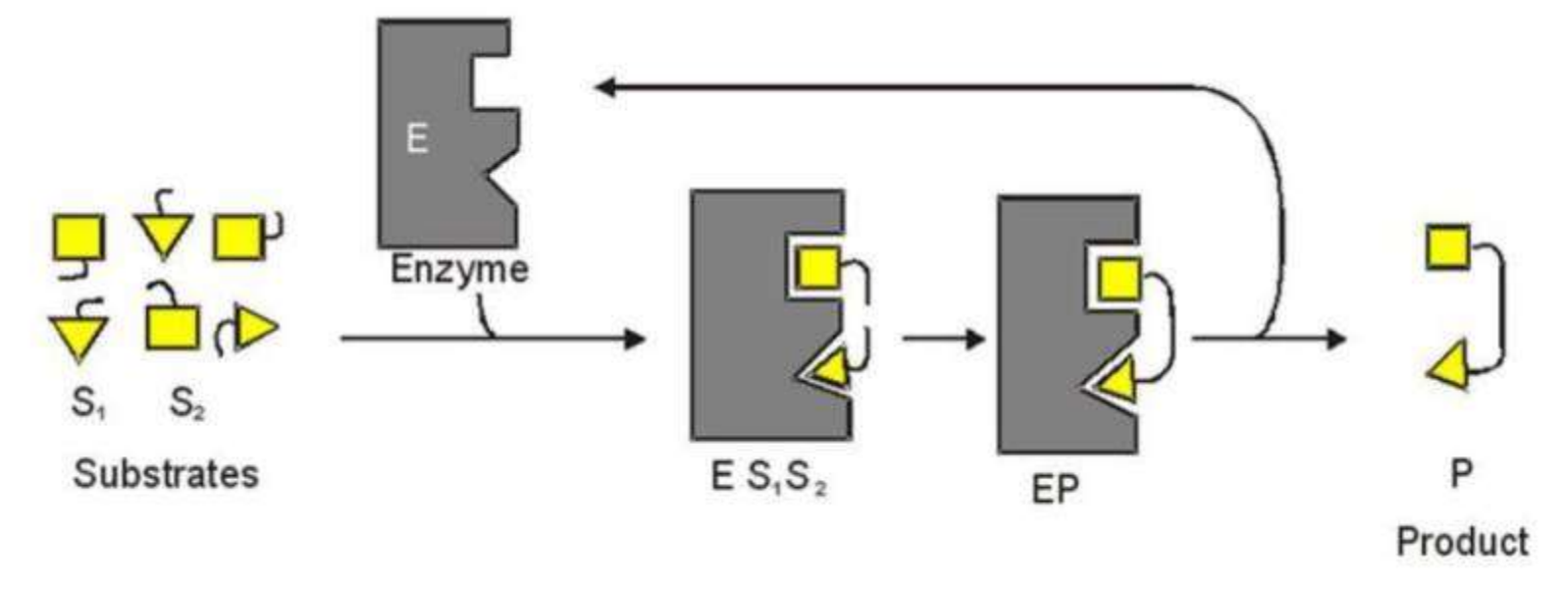
Enzyme-substrate interaction

- The enzyme molecules are much larger than their substrates
- An enzyme possesses a specific binding site for its substrate(s)
- This site is known as substrate site (active site)

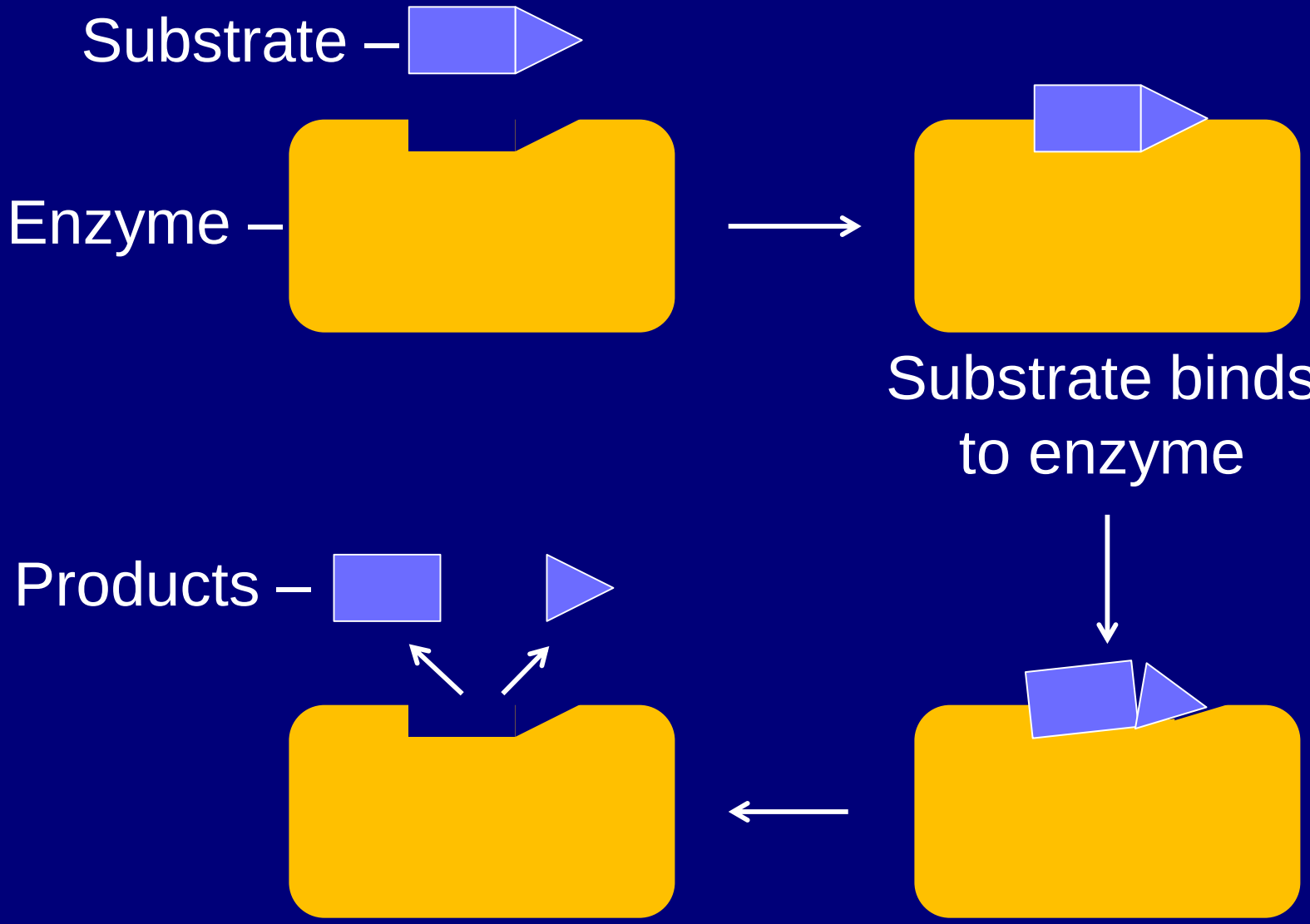


The substrate binds to the substrate site forming enzyme-substrate (ES) complex

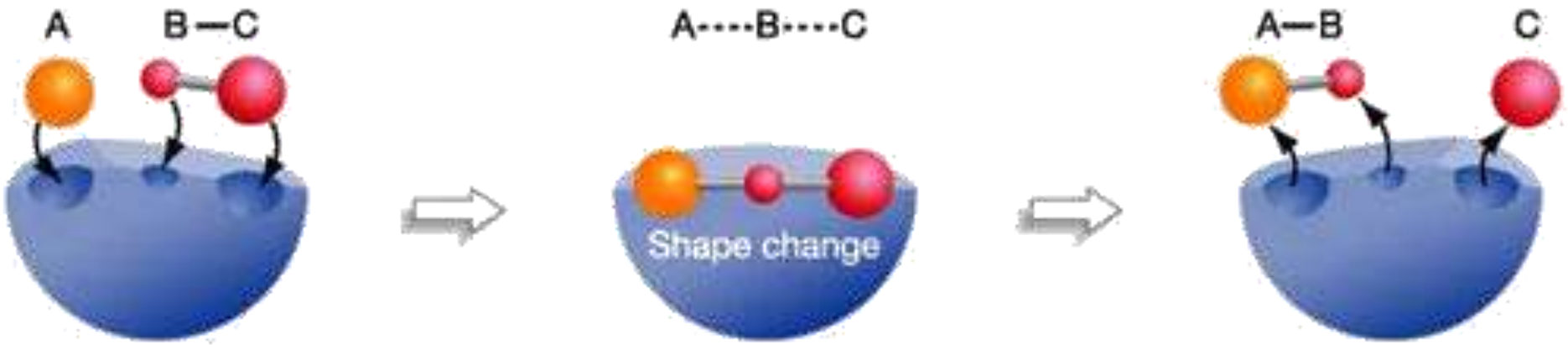
The binding may bring two substrates in close proximity (bond-forming distance) in the correct orientation so that a bond is formed between the two



- The binding of a substrate to the enzyme many induce a strain in the substrate
- As a result, a bond is broken in the substrate
- The substrate is split into two or more products which are released


Substrate –
Enzyme –
Substrate binds to enzyme
A strain occurs in the substrate; a bond is broken
Substrate splits into products which are released
Products –

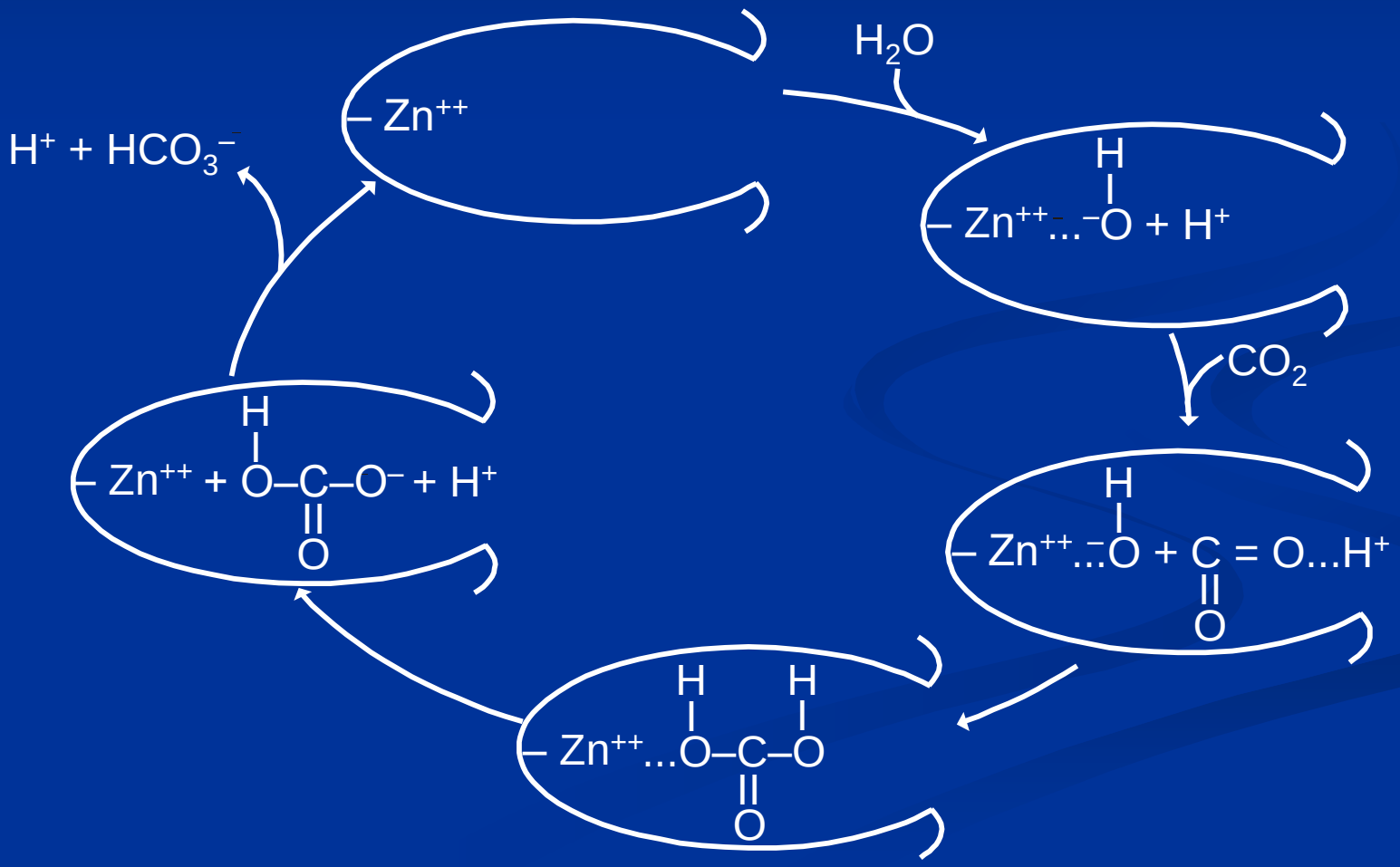
On binding of two substrates to the enzyme, a chemical group may be transferred from one substrate to another



The catalytic action of the enzyme may be exerted by:

- Cofactors
- Coenzymes
- Some amino acid residues in the substrate site

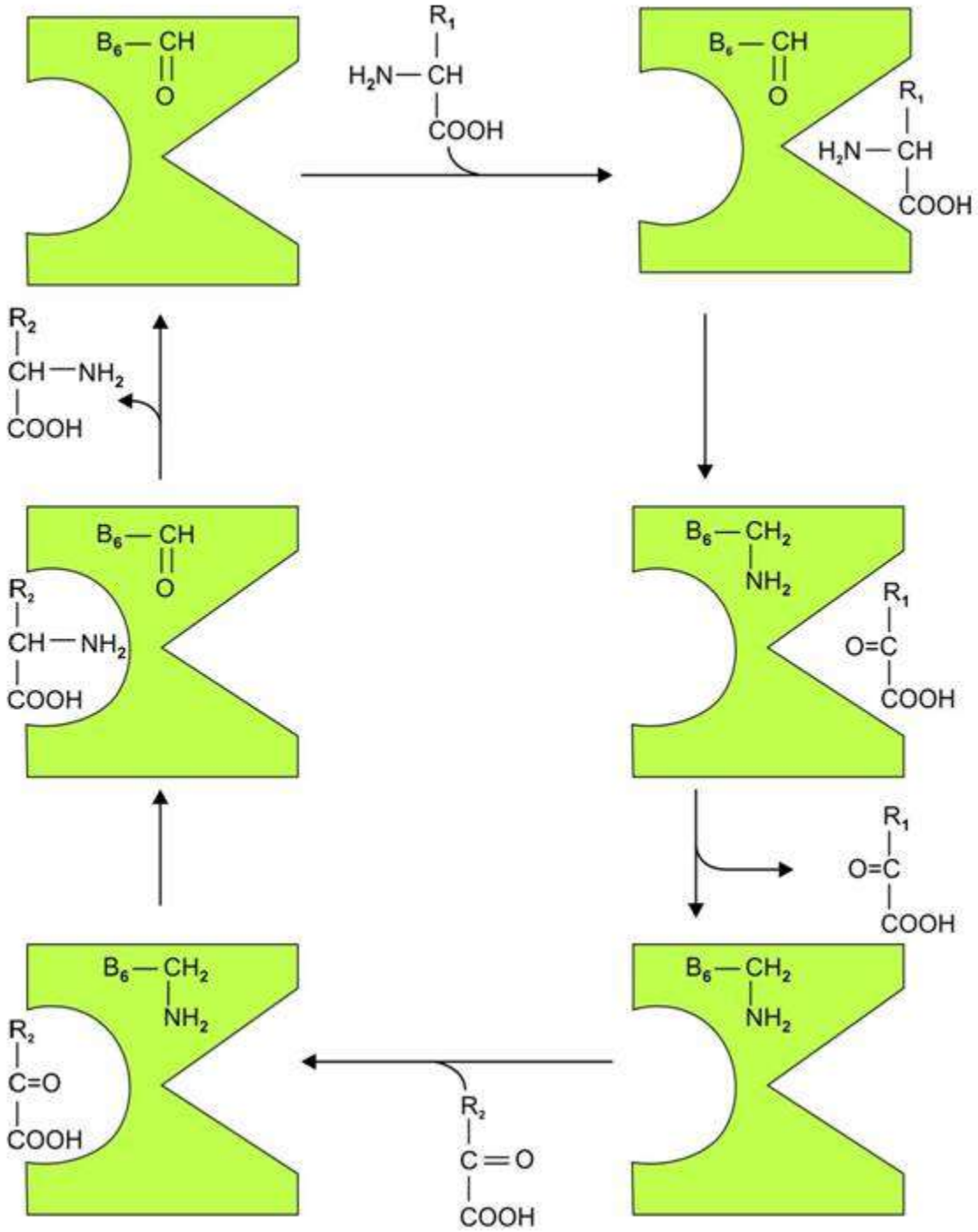
In the reaction catalysed by carbonic anhydrase, the cofactor (zinc) catalyses the reaction

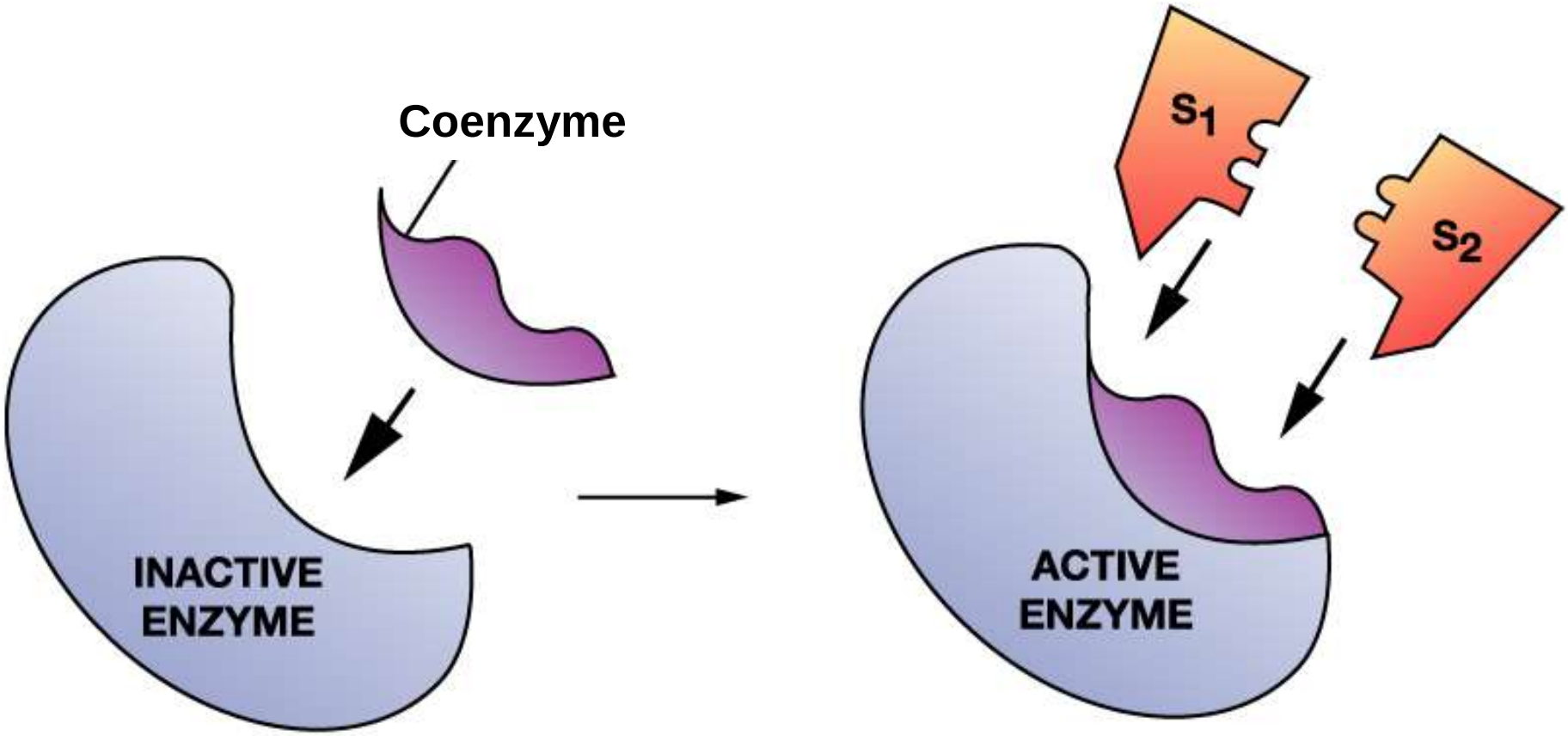


In transamination reactions, the coenzyme is involved in catalysis

The coenzyme (pyridoxal phosphate) is present at the substrate site

It accepts an amino group from an amino acid, and then donates it to a keto acid

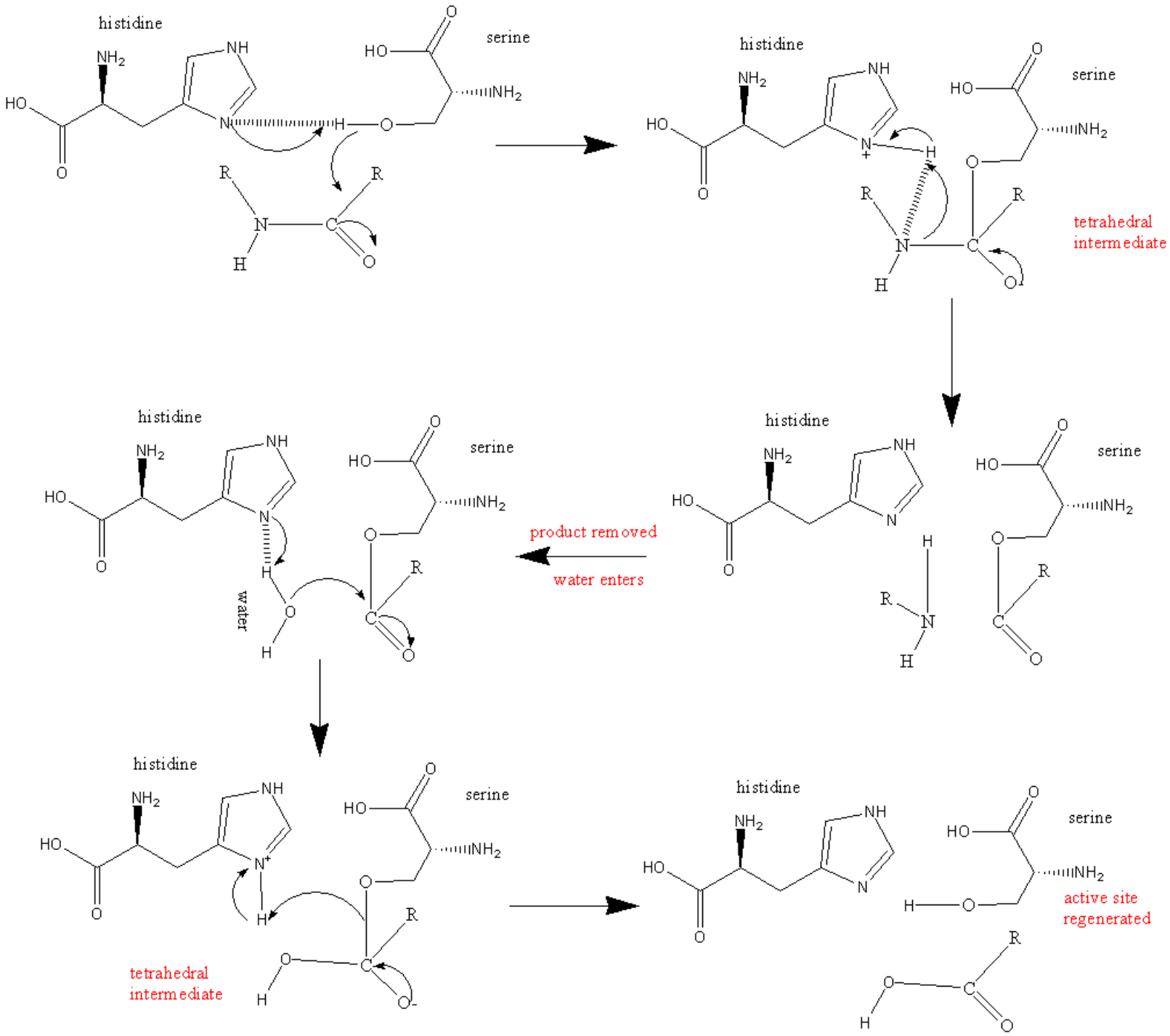

B6 — CH
O
R1
H2N — CH
COOH
B6 — CH
O
R1
H2N — CH
COOH
R2
CH — NH2
COOH
B6 — CH2
NH2
R1
O=C
COOH
B6 — CH
O
R2
CH — NH2
COOH
R1
O=C
COOH
B6 — CH2
NH2
B6 — CH2
NH2
R2
C=O
COOH
R2
C=O
COOH


Coenzyme
INACTIVE ENZYME
S1
S2
ACTIVE ENZYME

Amino acids participating in catalysis are serine, histidine, cysteine, aspartate etc

In serine proteases, a serine residue at the active site catalyses proteolysis

Examples of serine proteases are trypsin, chymotrypsin, thrombin etc


histidine
serine
tetrahedral intermediate
product removed
water enters
water
tetrahedral intermediate
active site regenerated

Models of enzyme conformation

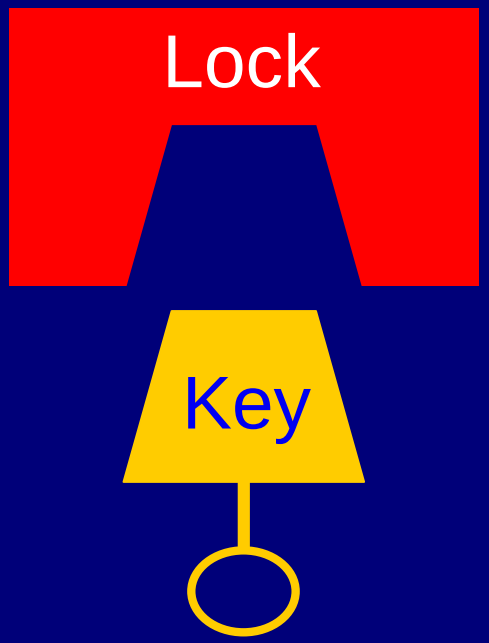
The first model was proposed by Emil Fischer

Also known as rigid template model

A different model was later proposed by Koshland

Also known as induced fit model

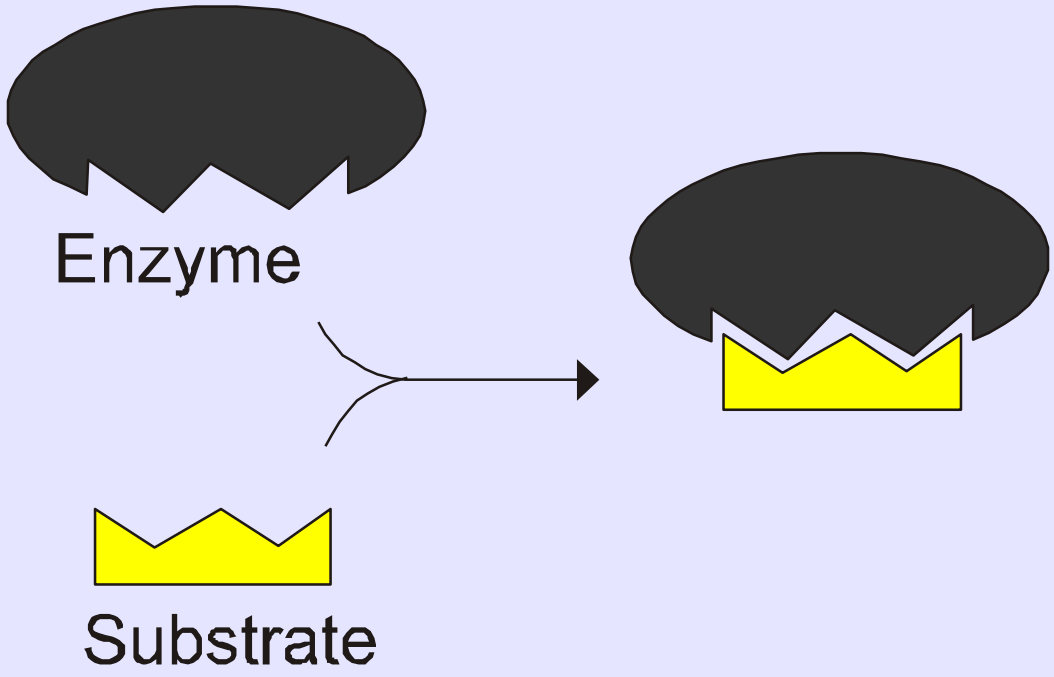
Fischer's model



Conformation of enzymes very rigid

Lock and key type of complementarity between substrate and enzyme

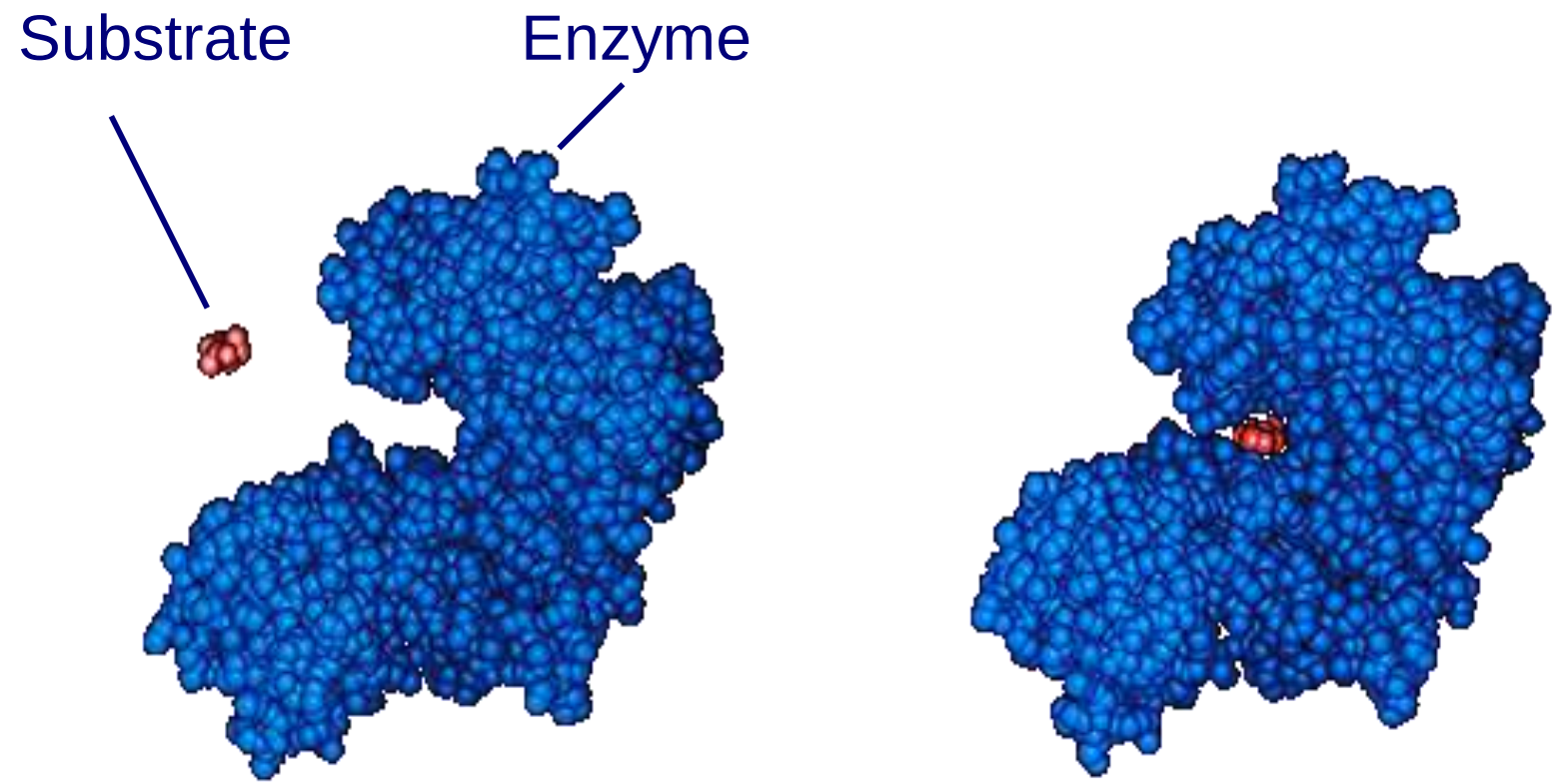
Complementarity responsible for specificity of enzymes



Fischer's model

Fischer’s model did not agree with certain experimental findings obtained later

Conformation of enzyme was found to change when it combined with its substrate



Before substrate binding

After substrate binding

Koshland's model conforms to known findings

In the absence of substrate, complementarity between enzyme and substrate is not apparent

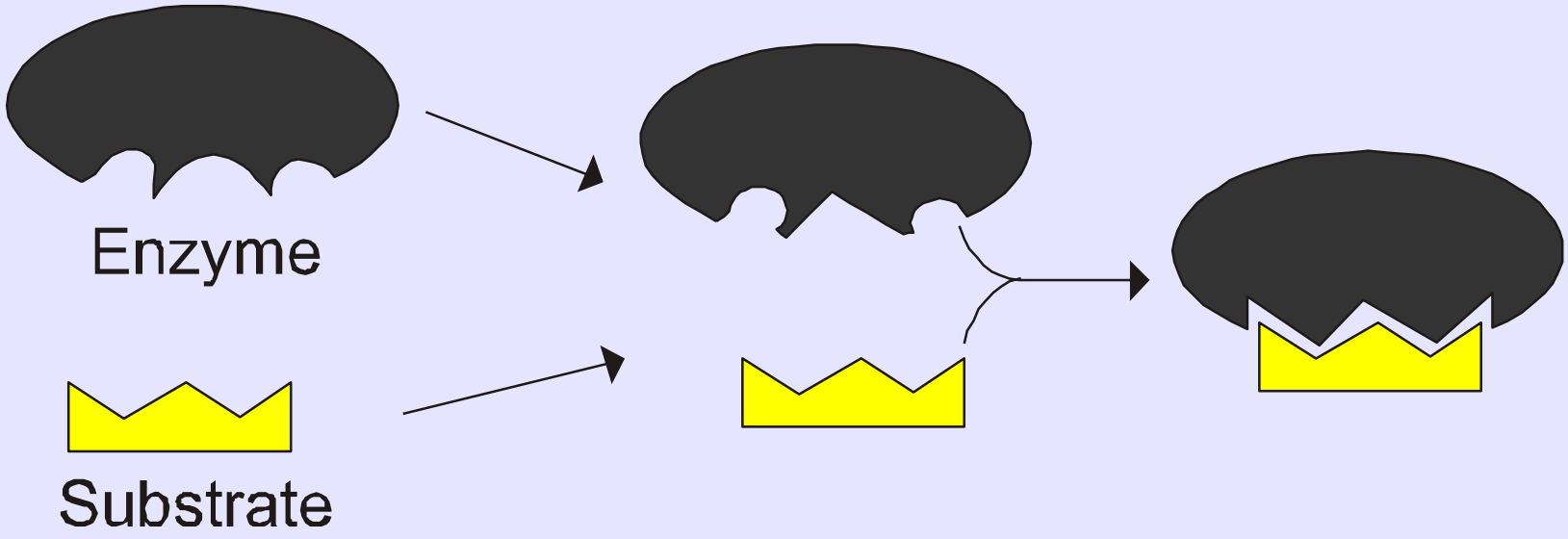
Approach of substrate induces change in conformation of the enzyme

The substrate site becomes complementary to the substrate

Change in conformation of the enzyme produces ‘induced fit’

The substrate binds to the enzyme, and is converted into the product

Release of the product restores the enzyme to its original conformation



Koshland’s model

THANKS