Molecular composition of enzymes Characteristics and mechanisms of enzyme-catalyzed reactions

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General lecture plan:

- **Biological and non-biological catalysis**
- **Stages of enzymatic catalysis**
- **Change in free energy during an enzymatic reaction**
- **Structure and classification of enzymes**
- **Examples of enzymatic cofactors**
- **Metalloenzymes**
- **Active site of an enzyme**
- **Mechanism of enzymatic catalysis**
  General strategies of enzyme catalysis
- **Models of enzymatic activity**
- **Covalent catalysis**
- **Acid catalysis and base catalysis**
- **Nomenclature**

Enzymes (from the Latin “fermentatio” – “fermentation”) or enzymes (from the Greek “enzyme” – in yeast) are proteins or low-molecular polyribonucleotides (ribozymes) that have catalytic activity

Biological and non-biological catalysis

Enzymes and inorganic catalysts, despite their different natures, share a number of common properties:

1) The ability to catalyze only thermodynamically feasible processes.
2) Accelerate both forward and reverse reactions without shifting the equilibrium in one direction or the other.
3) The ability to form highly reactive intermediates with reactants.
4) Both are not consumed during the reaction.

However, there are also significant differences between them:

1) Enzyme activity is much higher than that of inorganic catalysts (on average, 10 times higher).
2) Enzymes are more specific to the substrates and types of reactions they catalyze.
3) As a rule, relatively moderate conditions (body temperature, neutral pH, normal atmospheric pressure) are optimal for enzyme activity. Inorganic catalysts, on the other hand, often require more aggressive conditions (high temperature, pressure, acidic or alkaline environments).
4) Enzyme activity can be regulated by various mechanisms (allosteric regulation, covalent modification, etc.), unlike the activity of inorganic catalysts.
5) The rate of an enzymatic reaction directly depends on the amount of enzyme present.
6) Due to the protein nature of most enzymes, their activity depends on temperature, pH, and other factors.

Enzymes accelerate reactions that occur spontaneously: $\Delta G_{chem.}$ p<0

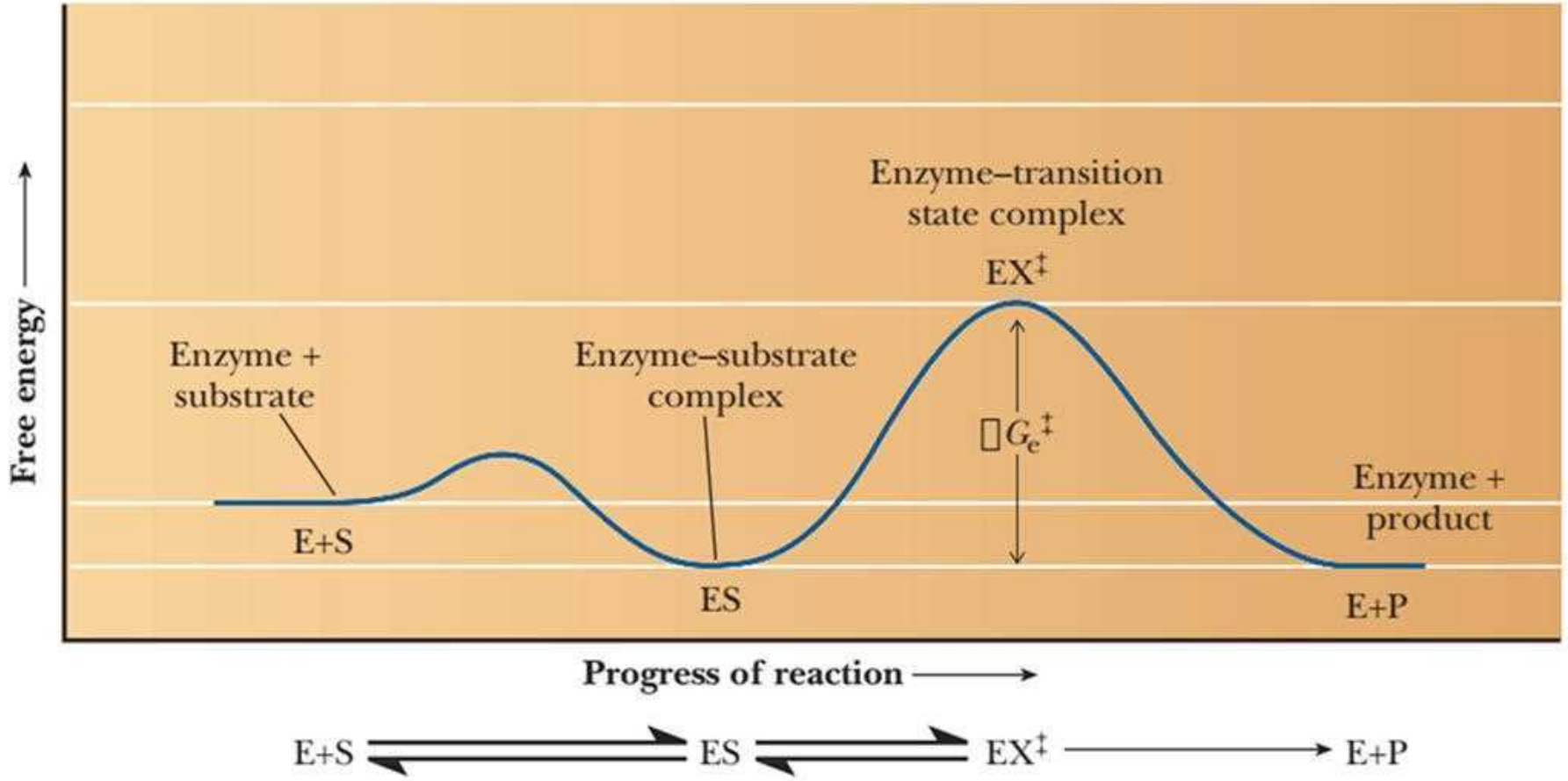
In chemistry, ΔG (change in Gibbs free energy) is a measure of a reaction's spontaneity at constant temperature and pressure. A negative ΔG indicates a spontaneous reaction that can proceed without external energy input, while a positive ΔG signifies a non-spontaneous reaction that requires energy to occur. ΔG is calculated using the equation

$$\Delta G=\Delta H-T\backslash\Delta S$$

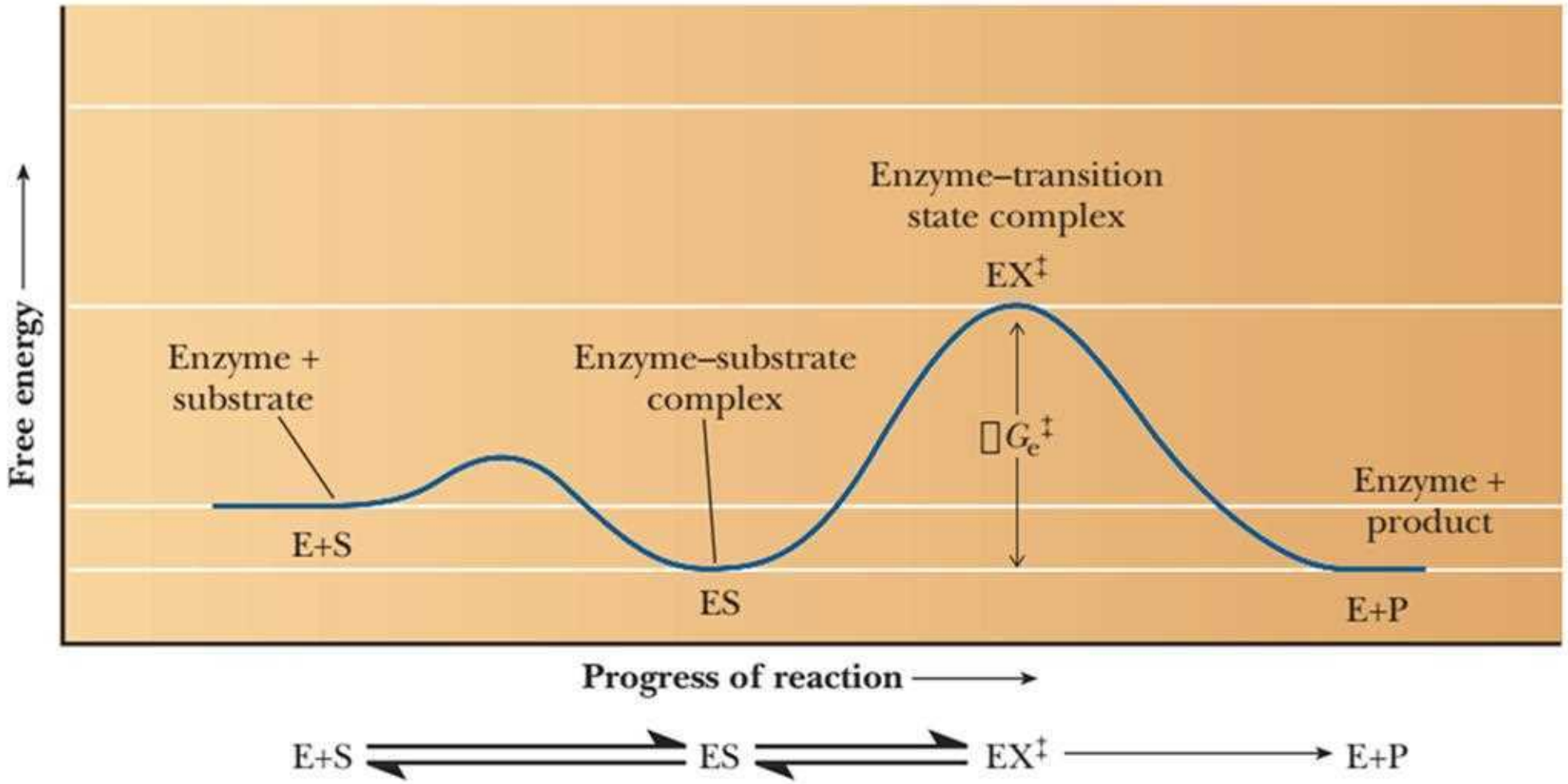
ΔH is the change in enthalpy, T is the temperature in Kelvin, and ΔS is the change in entropy (measure of disorder).

Stages of enzymatic catalysis

1) Interaction between the enzyme's active site (E) and substrate (S).
2) Formation of the enzyme-substrate complex (ES) due to the similar spatial structure of the enzyme's active site and substrate.
3) Formation of the enzyme-substrate complex (ES*) activated by the redistribution of electron density in the substrate's chemical bonds under the influence of the enzyme's active site functional groups.
4) Formation of new chemical bonds in the product molecule (P) and formation of the enzyme + product complex (EP).
5) Release of the reaction product (P) from the enzyme's active site into the surrounding environment.



Change in free energy during an enzymatic reaction



The interaction of the substrate (S) with the formation of the product (P) proceeds through the formation of a transition state, which has a higher free energy.

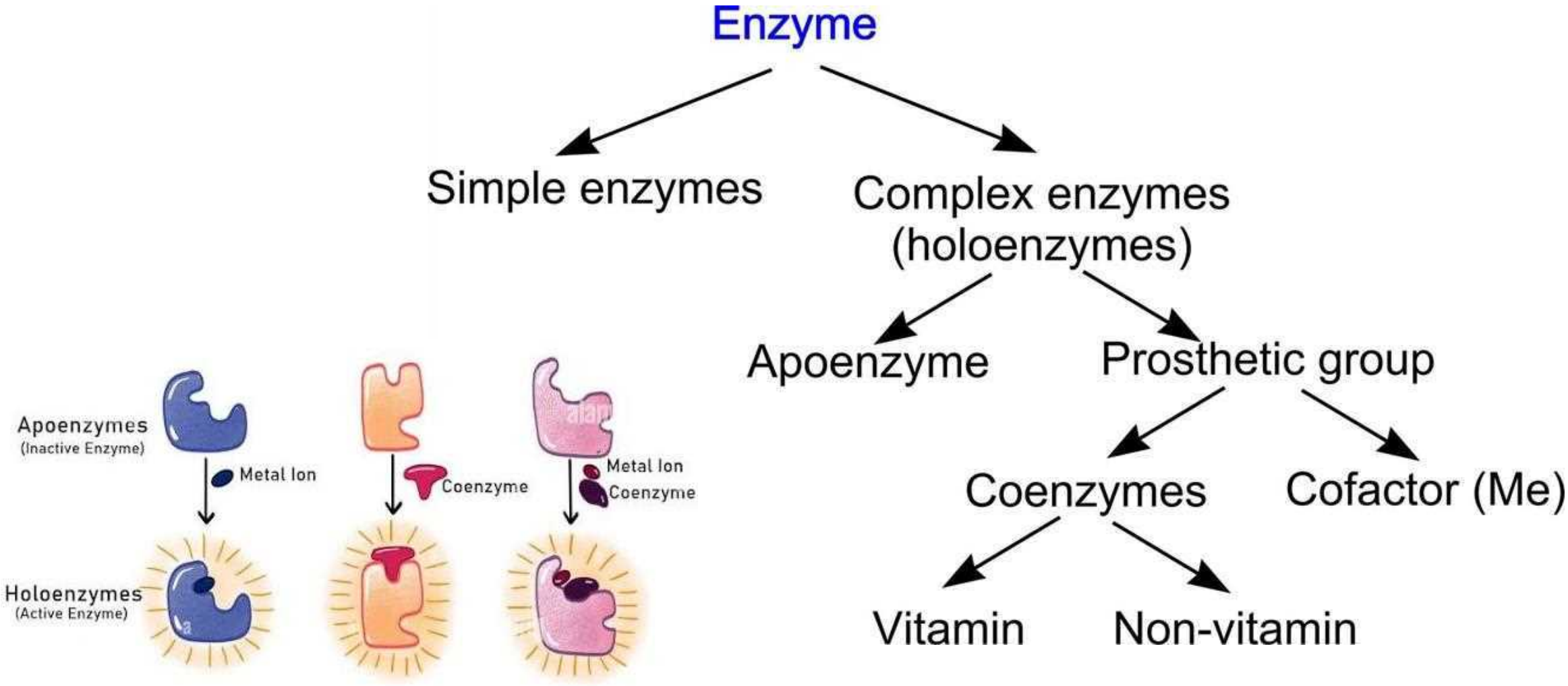
The difference in free energy between the transition state energy and the substrate energy is called the activation free energy (ΔGact). The higher this value, the slower the reaction.

The free energy barrier consists of two parts: the first is *associated with the process of substrate binding by the enzyme*, and the second is *associated with the chemical reaction step itself*.
Each half-reaction is characterized by a specific change in free energy (ΔG), which is required to complete the chemical transformation.

Important

- Enzymes cannot violate the laws of thermodynamics.
- Enzymes cannot carry out reactions that are thermodynamically unfavorable under given conditions.
- Enzyme activity depends on factors such as temperature and pH due to their protein nature, as well as substrate concentration and the presence of activators or inhibitors.

Structure and classification of enzymes

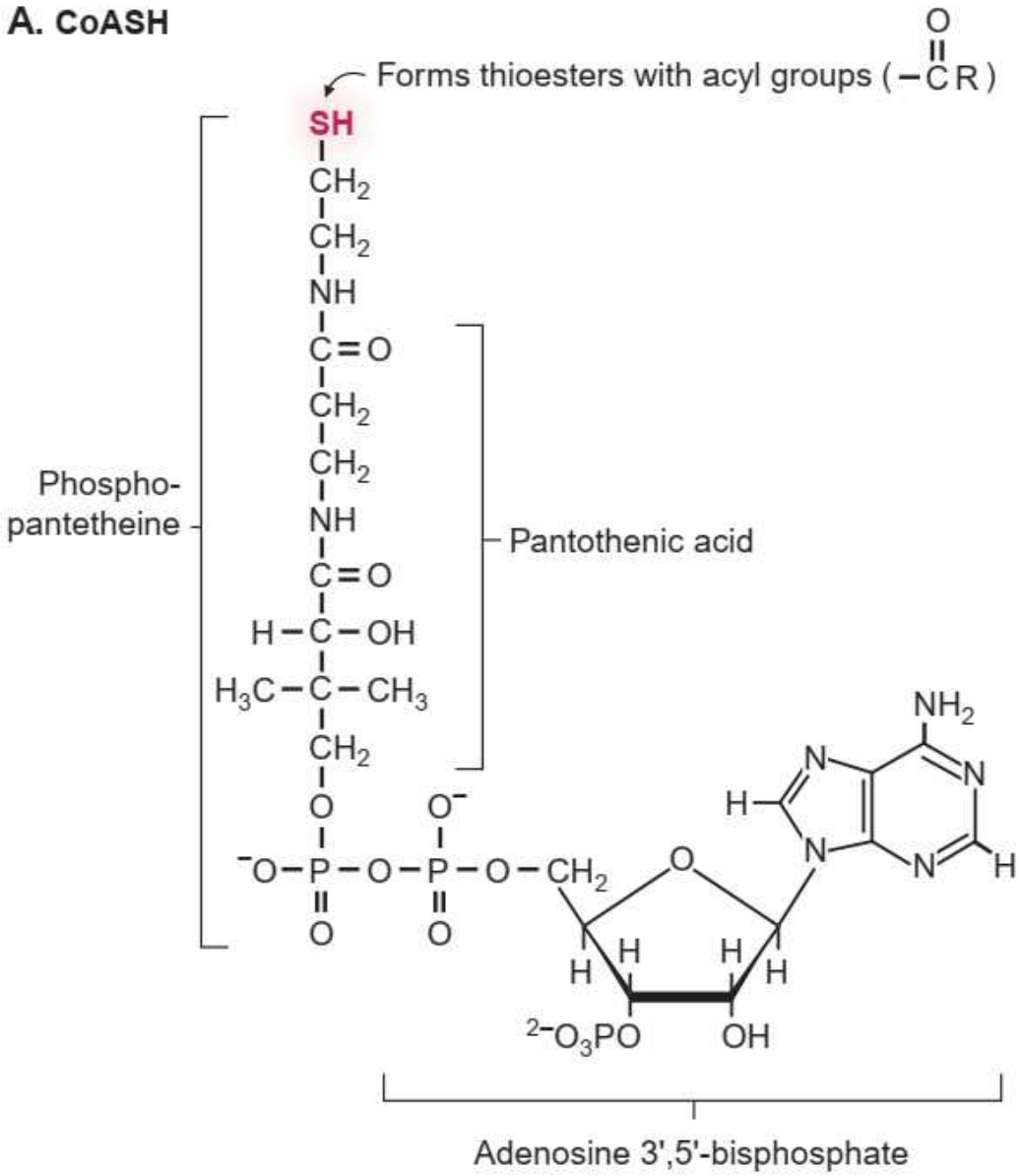


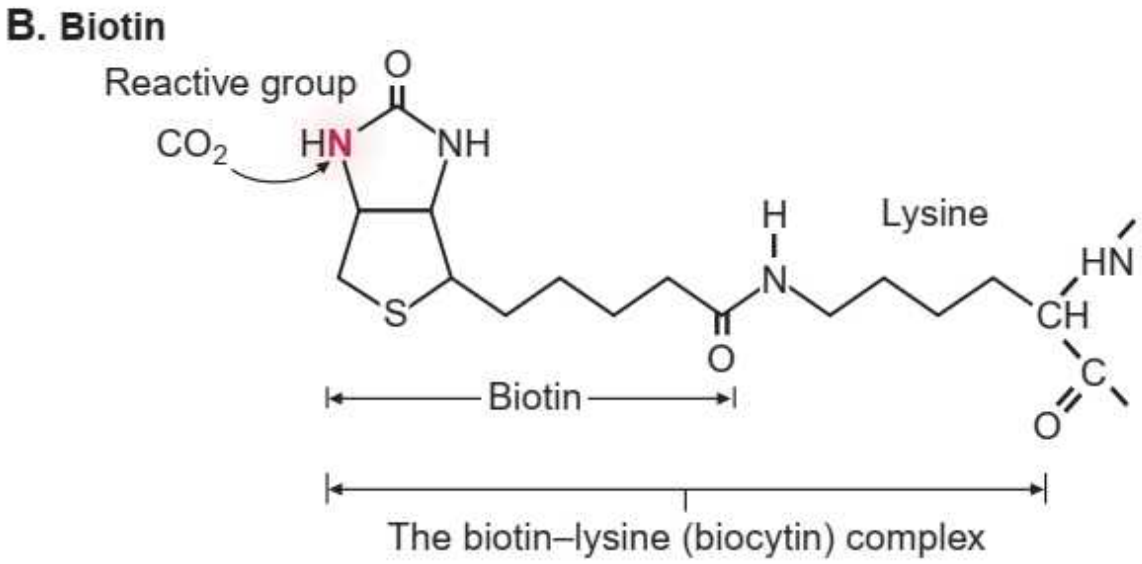
The prosthetic group and apoenzyme do not perform catalysis independently

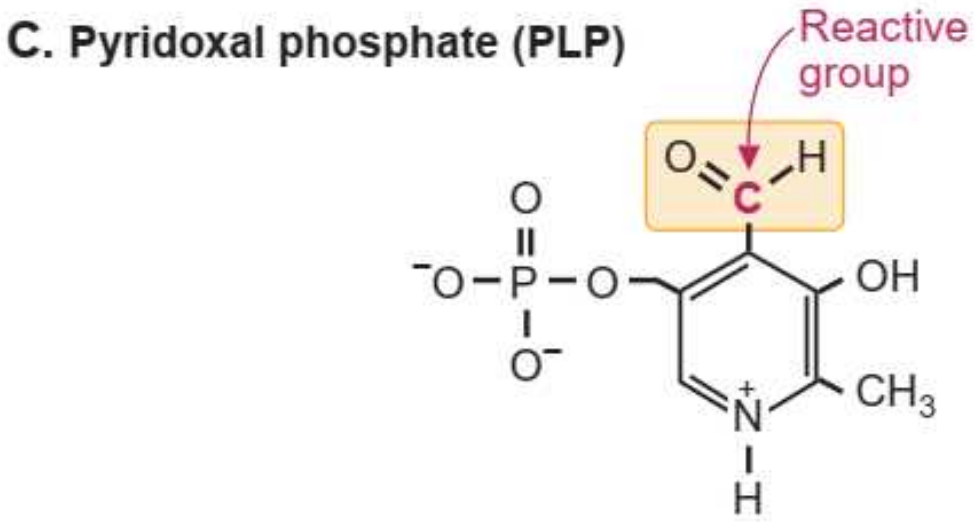
Examples of cofactors – vitamin derivatives

Cofactor	Role	Precursor vitamin
NAD^+, $NADP^+$	Hydrogen (electron) transfer	Nicotinic acid – vitamin PP
FAD	Transfer of hydrogen atoms and electrons	Riboflavin is a vitamin B_2
Coenzyme A	Activation and transfer of acyl groups	Pantothenic acid
Biotin	Binding CO_2	Biotin
Pyridoxal phosphate	Transfer of amino groups	Pyridoxine–vitamin B6
Tetrahydrofolic acid (THFA)	One-carbon transfer	Folic acid

Examples of cofactors – vitamin derivatives







Metalloenzymes

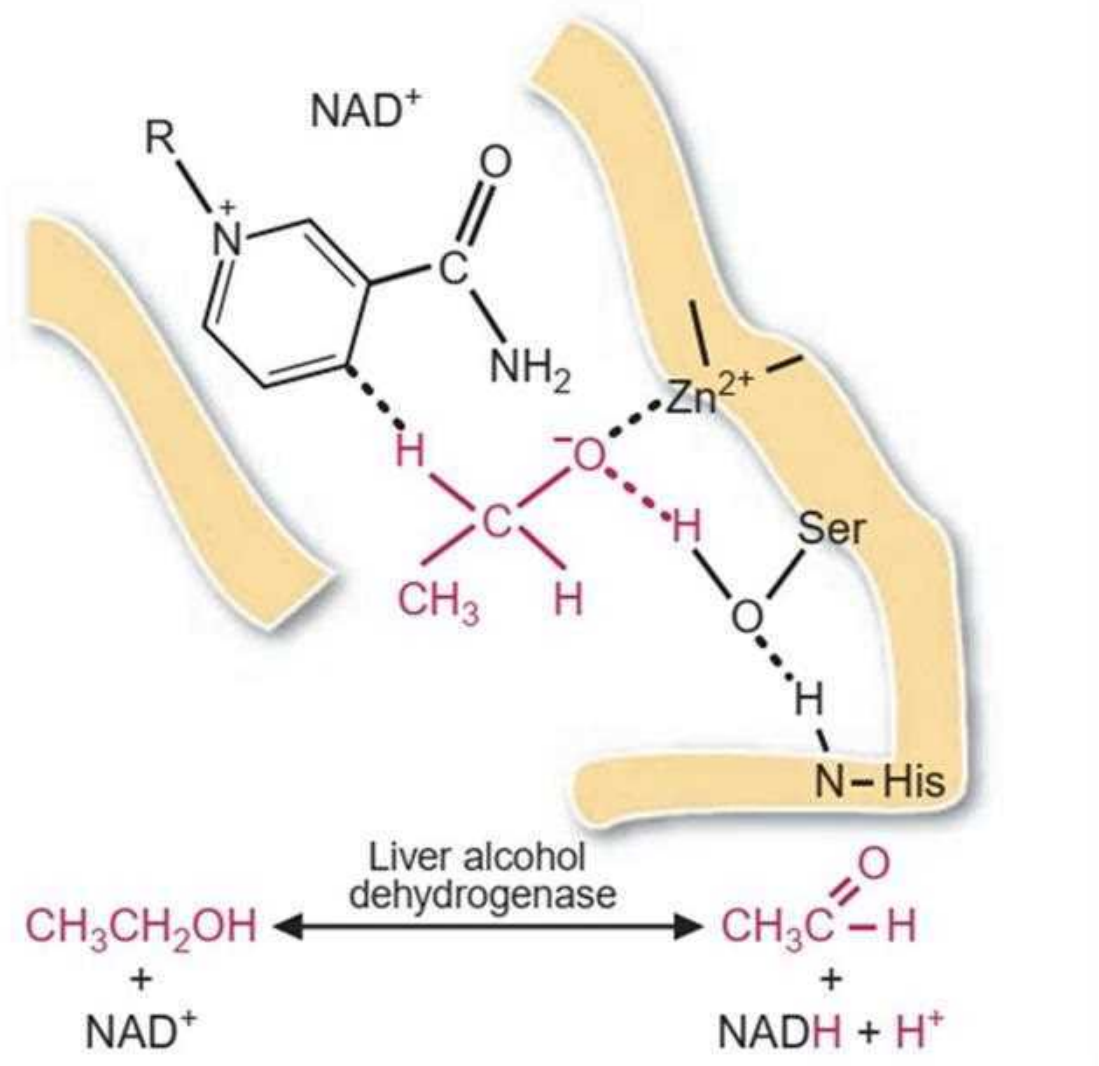
Examples of metalloenzymes

Enzyme	Metal
Cytochromes, catalase, peroxidase, tryptophan oxidase	Fe
Ascorbate oxidase, tyrosinase, phenoloxidase	Cu
Xanthine oxidase, nitrate reductase, aldehyde oxidase	Mo
Some peptidases	Co
Amylase, lipase	Ca
Carbonic anhydrase, lactate dehydrogenase, uricase, carboxypeptidase	Zn
Pyruvate carboxylase, phosphatases, phosphoglucokinase	Mg
Arginase, phosphoglucomutase, cholinesterase	Mn

Functions of Metals in Metalloenzymes

1) *Reaction initiation*. The metal is part of the enzyme's active site. For example: tyrosinase (2 Cu^{2+}), monoamine oxidase (4 Cu^{2+}), ceruloplasmin (8 Cu^{2+}).
1) *Stabilization of the structure*. When located outside the active site. For example: transketolase (Ca^{2+}).
2) *Substrate binding*. The metal is located in the active site (in the substrate-binding zone). For example: hexokinase (Mg^{2+}).
3) *Substrate binding and catalysis*. For example: pyruvate kinase (Mg^{2+}, K^{+}), arginase (Mn^{2+}), alkaline phosphatase (2 Zn^{2+}).

Metalloenzymes



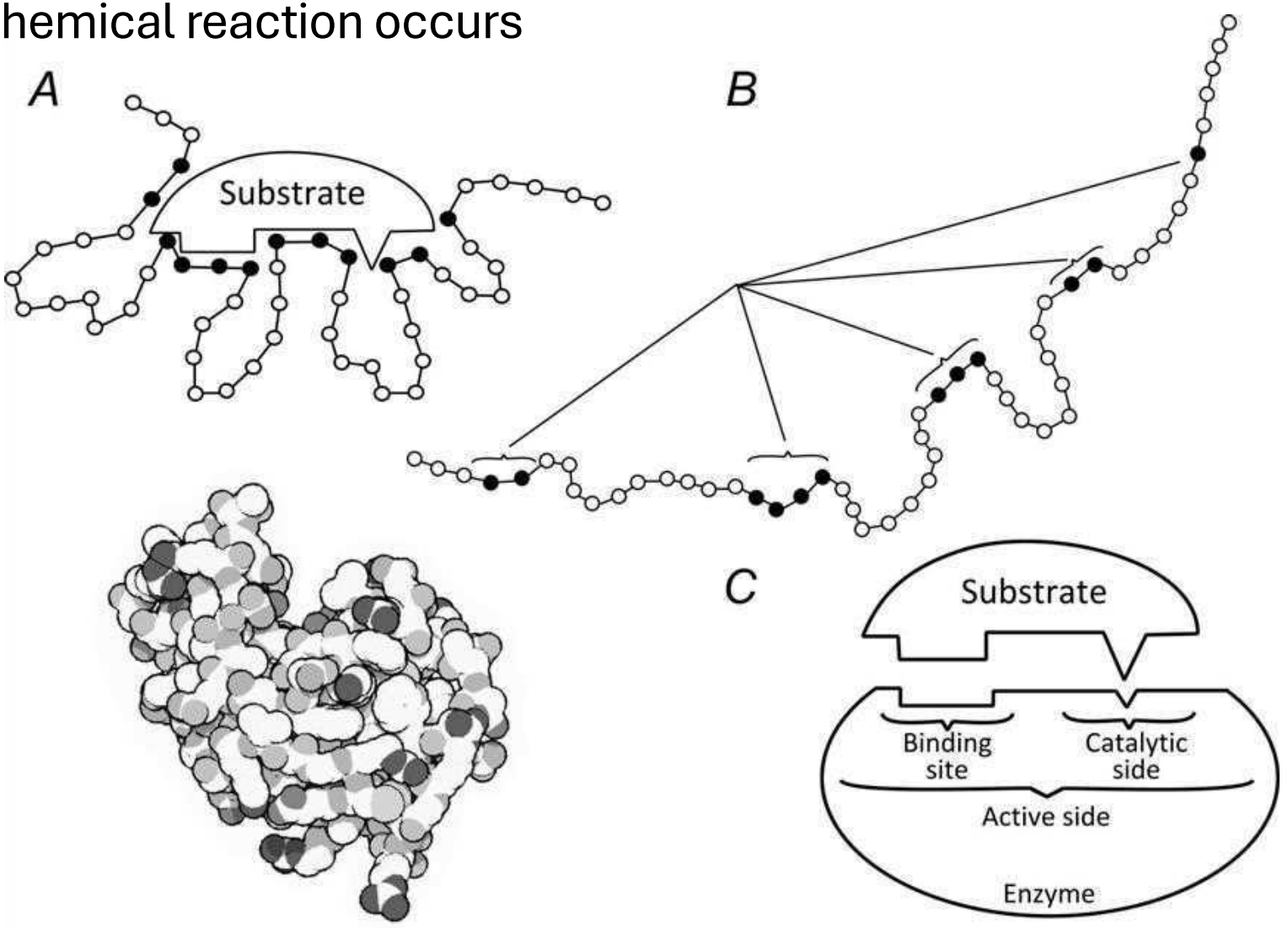
Structure of the active site of alcohol dehydrogenase

- Many complex enzymes can contain both organic and inorganic (metal) cofactors. For example, Zn^{2+}-containing NAD^+-dependent alcohol dehydrogenase
- Cofactors can also act as structural components of a protein without being involved in catalysis

The **active center (or active site) of an enzyme** is the area where the substrate is bound and the catalyzed chemical reaction occurs

- Active site formed by the juxtaposition of several amino acid residues (3 to 12), which may be located either adjacent to each other or in different sections of the chain.

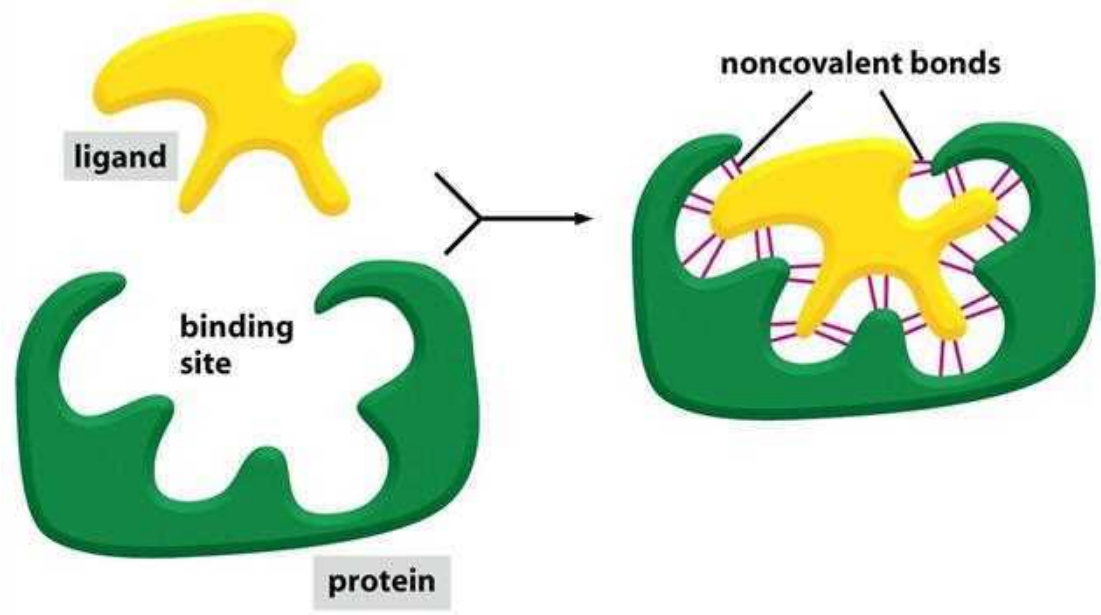
- In the active side of enzymes, the most common AA residues are those with a polar radical or positive/negative charge, such as Ser, His, Arg, Cys, Asp, Glu, and Tyr, which can act as both proton donors and acceptors.



The active center of the 129-amino acid residue lysozyme enzyme is formed by the convergence of 5 amino acid residues at positions 35, 52, 62, 63, and 101 in the chain

Active site of an enzyme

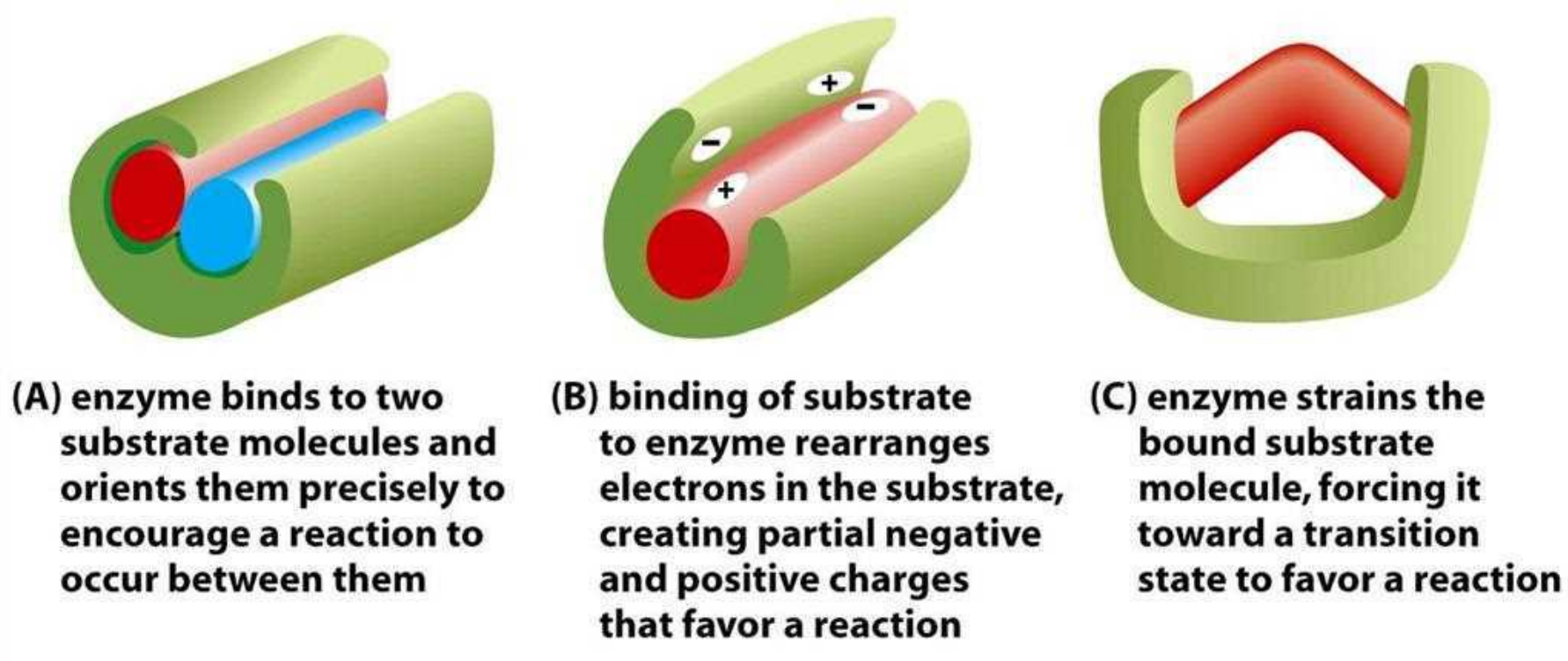
- The active site is characterized by a complex three-dimensional structure, which resembles a narrow depression or crevice. Water molecules generally cannot penetrate this region (unless they are involved in the reaction itself), as hydration shells reduce the electrostatic attraction of the substrate to the enzyme;
- orients substrates so that the functional groups involved in the reaction are close together, providing a proximity effect;
- can change its activity under the influence of various factors affecting its structure.



Mechanism of enzymatic catalysis

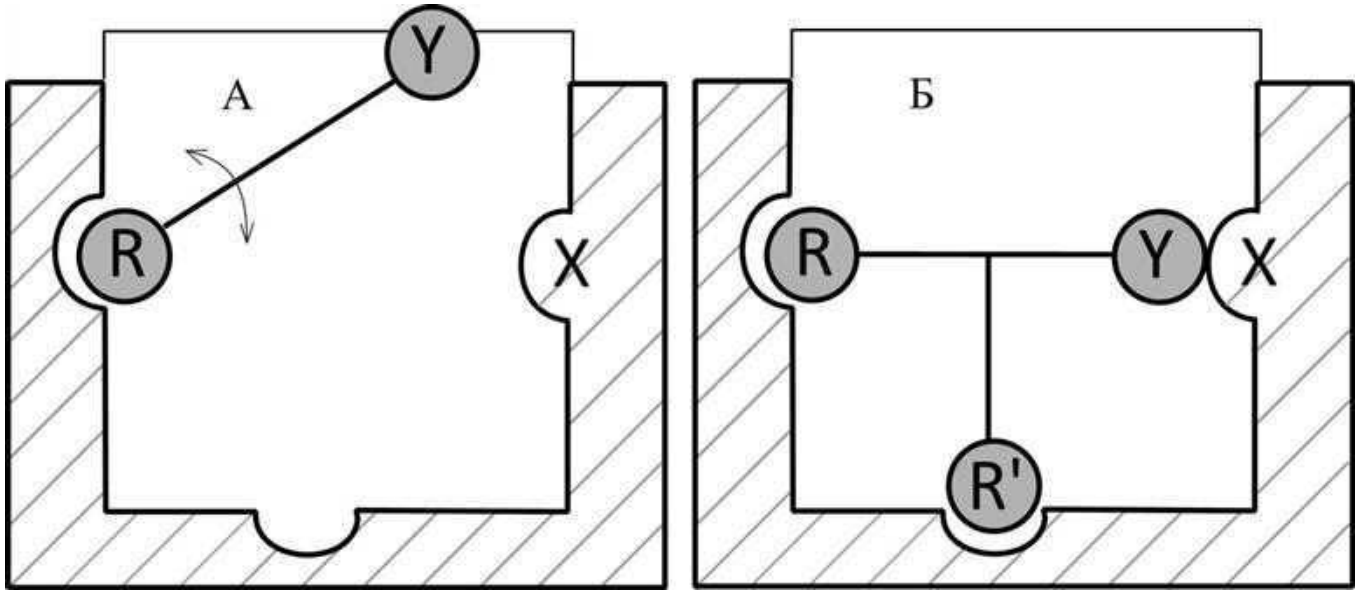
General strategies of enzyme catalysis

- Holding substrates together in a precise alignment.
- Charge stabilization of reaction intermediates
- Applying forces that distort bonds in the substrate to increase the rate of a particular reaction.



General strategies of enzyme catalysis

If the substrate is only anchored at *two points* in the enzyme's active site, this binding is insufficient for reliable fixation.
At least three contact points are required for proper orientation and binding of the substrate in the active site.

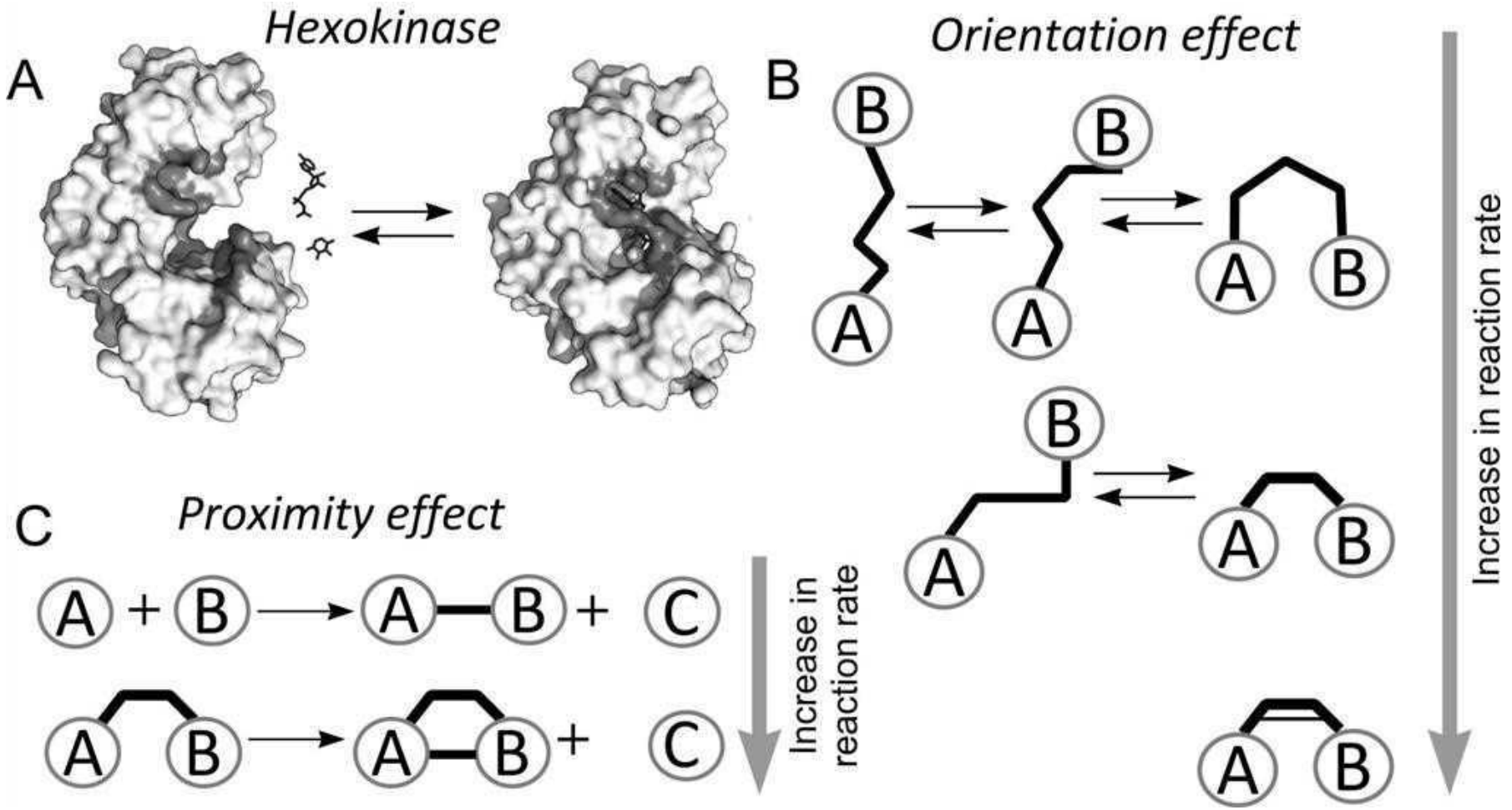


Substrate binding variants with a) two contact points and b) three contact points

Enzyme-substrate complexes are formed through:

- covalent bonds;
- hydrophobic interactions between the apolar regions of the substrate and the dehydrated regions of the globule's surface layer;
- electrostatic interactions between charged groups of the substrate and the protein portion of the enzyme;
- hydrogen bonding.

General strategies of enzyme catalysis: Proximity and orientation effect

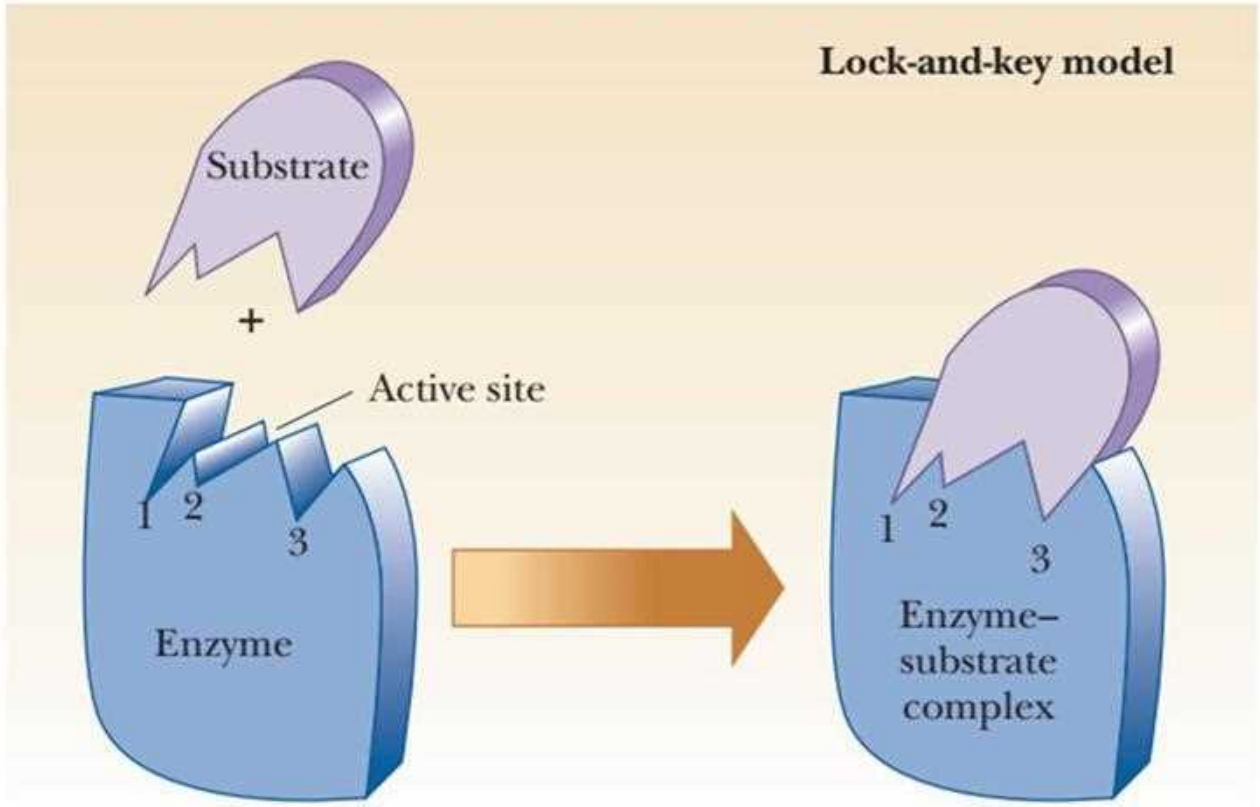


During the formation of the enzyme-substrate complex, the modified regions of the substrate molecule are positioned in the most favorable positions relative to the catalytic group of the enzyme's active site. This proximity and orientation effect is analogous to an effective increase in the concentration of reactants and imparts an intramolecular character to the reaction, with a significant increase in rate by 3–4 orders of magnitude.

Models of enzymatic activity

E. Fisher’s “lock-and-key” model (1894) - one of the earliest models of enzymatic activity, assumes a perfect match between the substrate and the active site of the enzyme

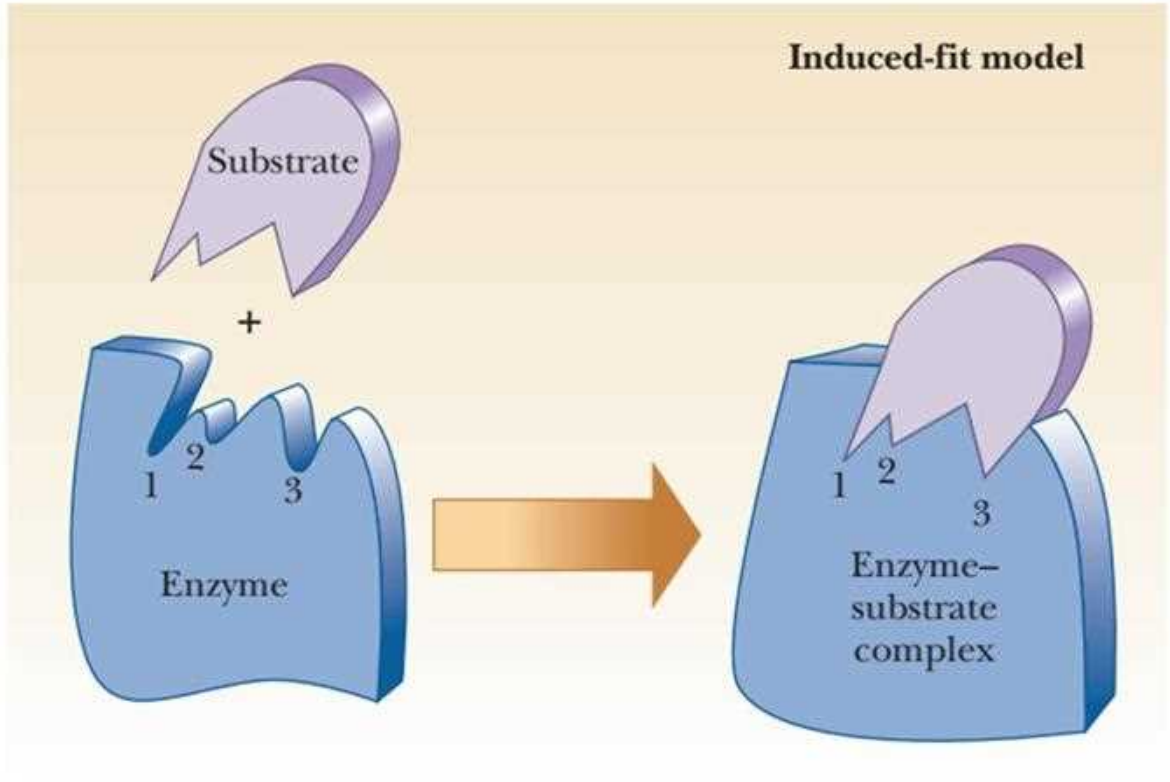
The main drawback of this model is that the substrate-enzyme complex is so tightly bound that it enters an energy minimum, from which escape to the reaction products is impossible. Therefore, the "lock-and-key" model more accurately describes the action of enzyme inhibitors, which bind tightly to the active site and block it.



A In the lock-and-key model, the shape of the substrate and the conformation of the active site are complementary to one another.

Models of enzymatic activity

The “hand-glove” model or induced-fit model of D. Koshland (1958) suggests that the enzyme structure is not fixed but can undergo conformational changes during interaction with the substrate.

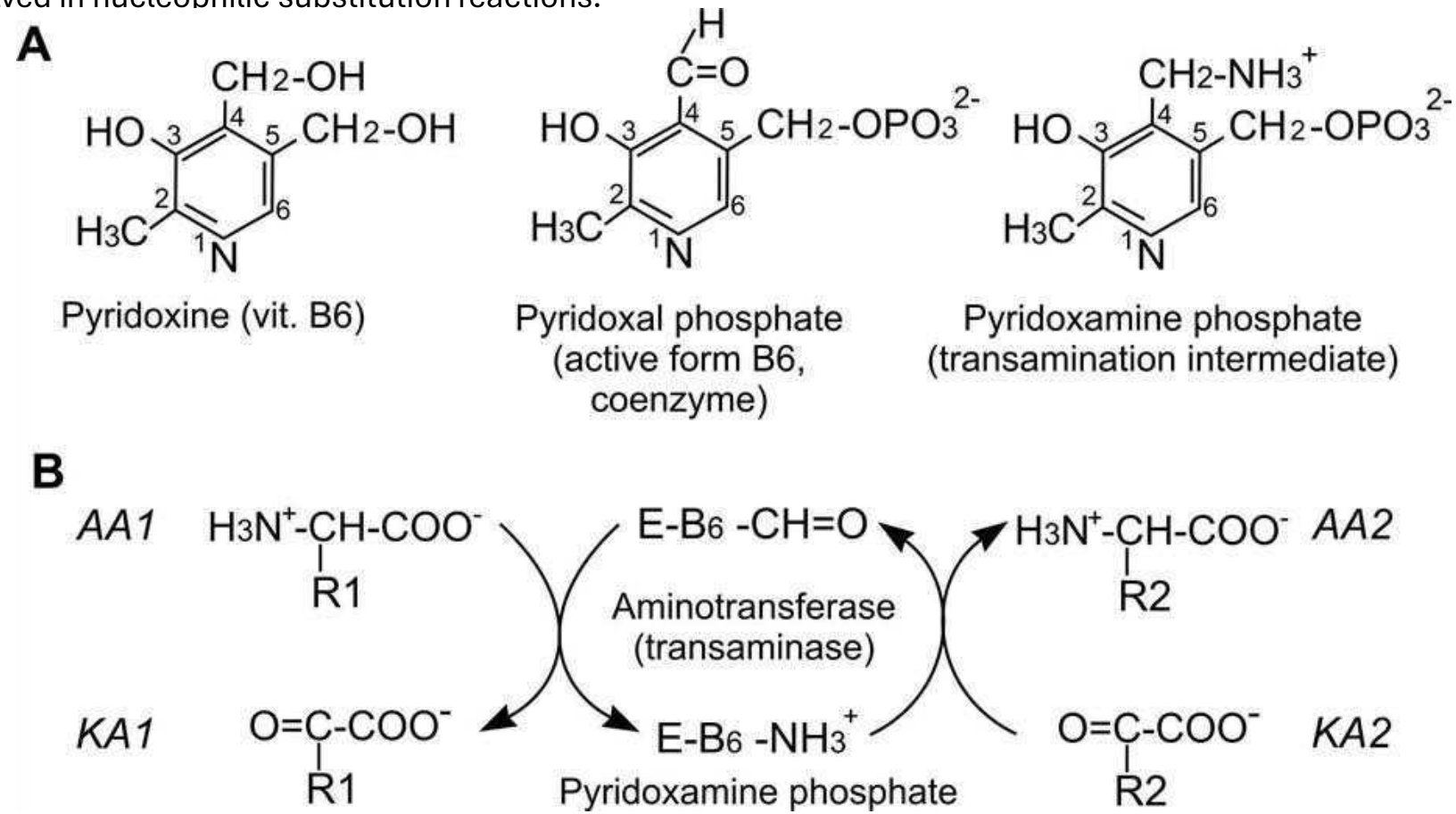


B In the induced-fit model, the enzyme undergoes a conformational change upon binding to substrate. The shape of the active site becomes complementary to the shape of the substrate only after the substrate binds to the enzyme.

Covalent catalysis

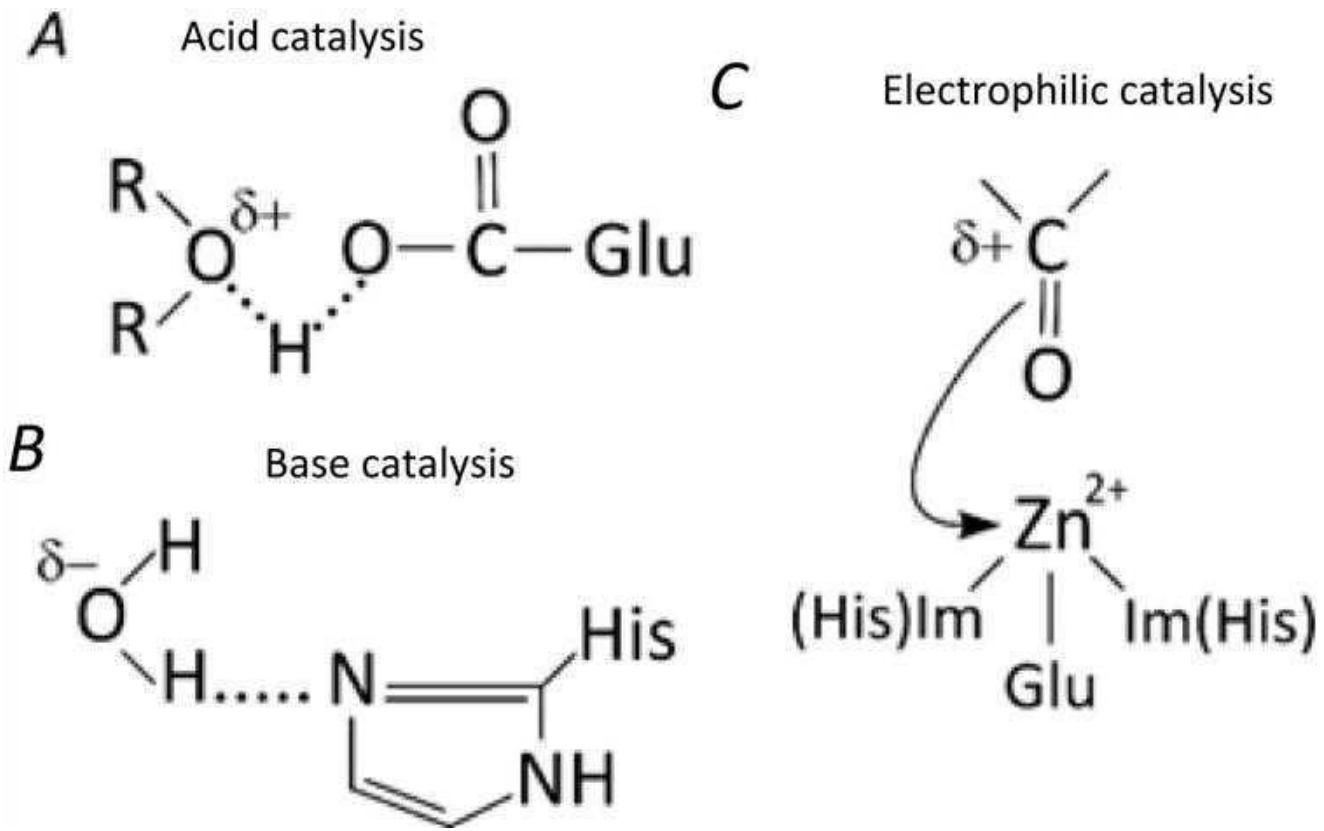
The principle of covalent catalysis is the formation of intermediate and short-lived bonds between substrate molecules and the catalytic groups of the enzyme.

Another name for this type of catalysis is nucleophilic catalysis, as the key role in its implementation is played by the basic groups and cofactors involved in nucleophilic substitution reactions.



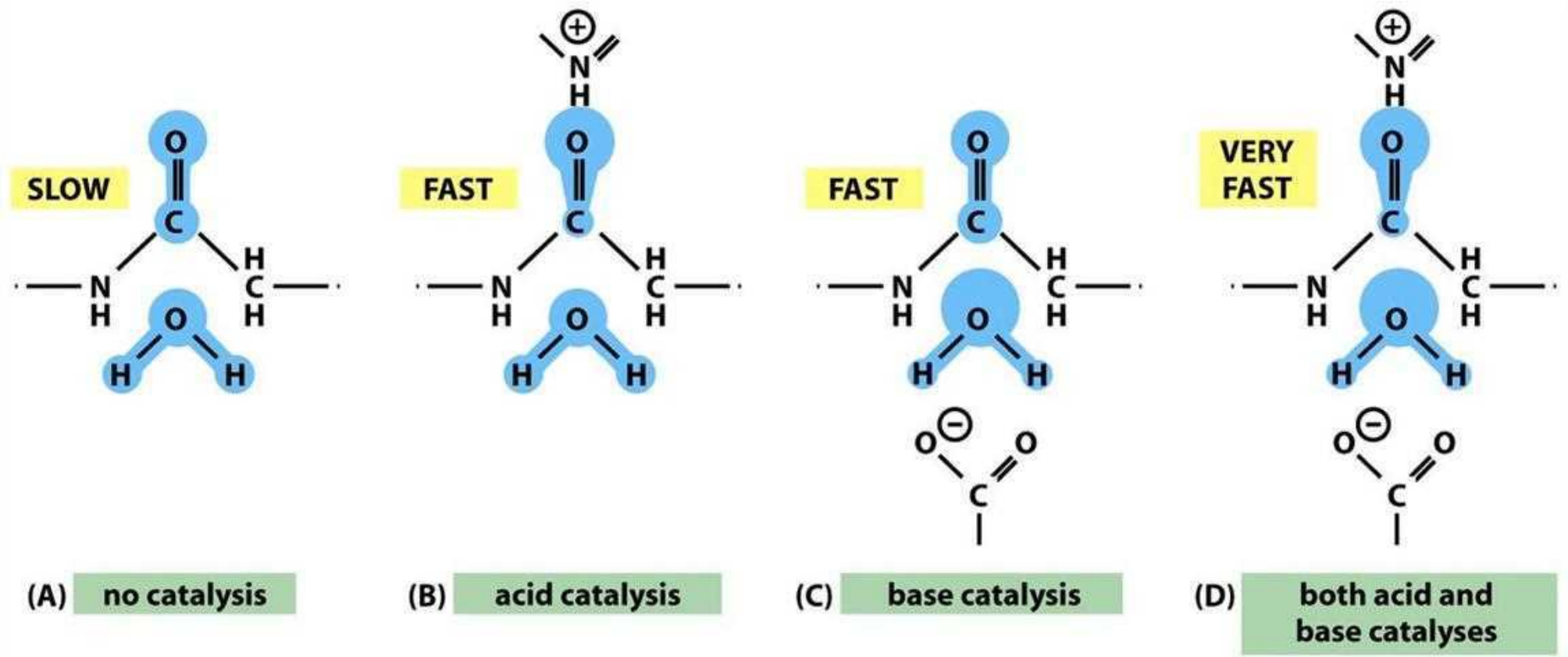
For example, transamination reactions. The reaction proceeds in a "ping-pong" pattern. The coenzyme of aminotransferases is pyridoxal phosphate (the active form of vitamin B6), in which one hydroxymethyl group (at C-4) of vitamin B6 is oxidized to an aldehyde, and the other (at C-5) is phosphorylated.

Acid catalysis and base catalysis



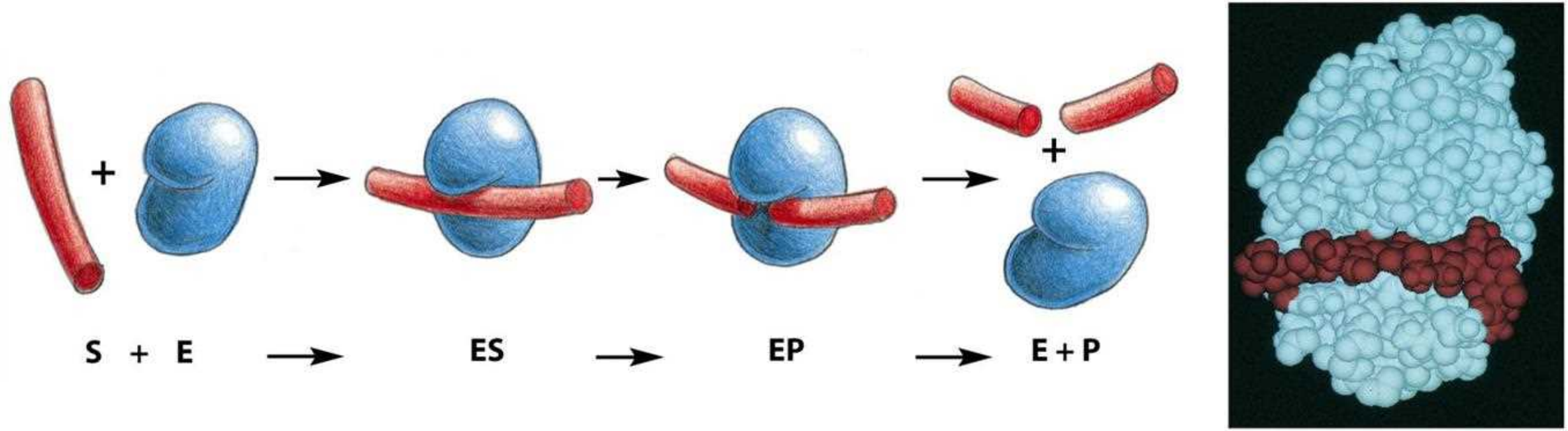
Some amino acids can exist in various charged states depending on their environmental conditions. This can affect both protein structure and enzyme activity. Their active sites contain a significant number of electrophilic groups (with free orbitals) and nucleophilic groups (Figure C, with free electrons), such as imidazoles, carboxyls, sulfhydryls, hydroxyls, and amino groups. These groups act as proton donors (H+) (Figure A) and acceptors (Figure B) (of acids and bases), participating in the formation of the intermediate enzyme-substrate complex.

Acid catalysis and base catalysis



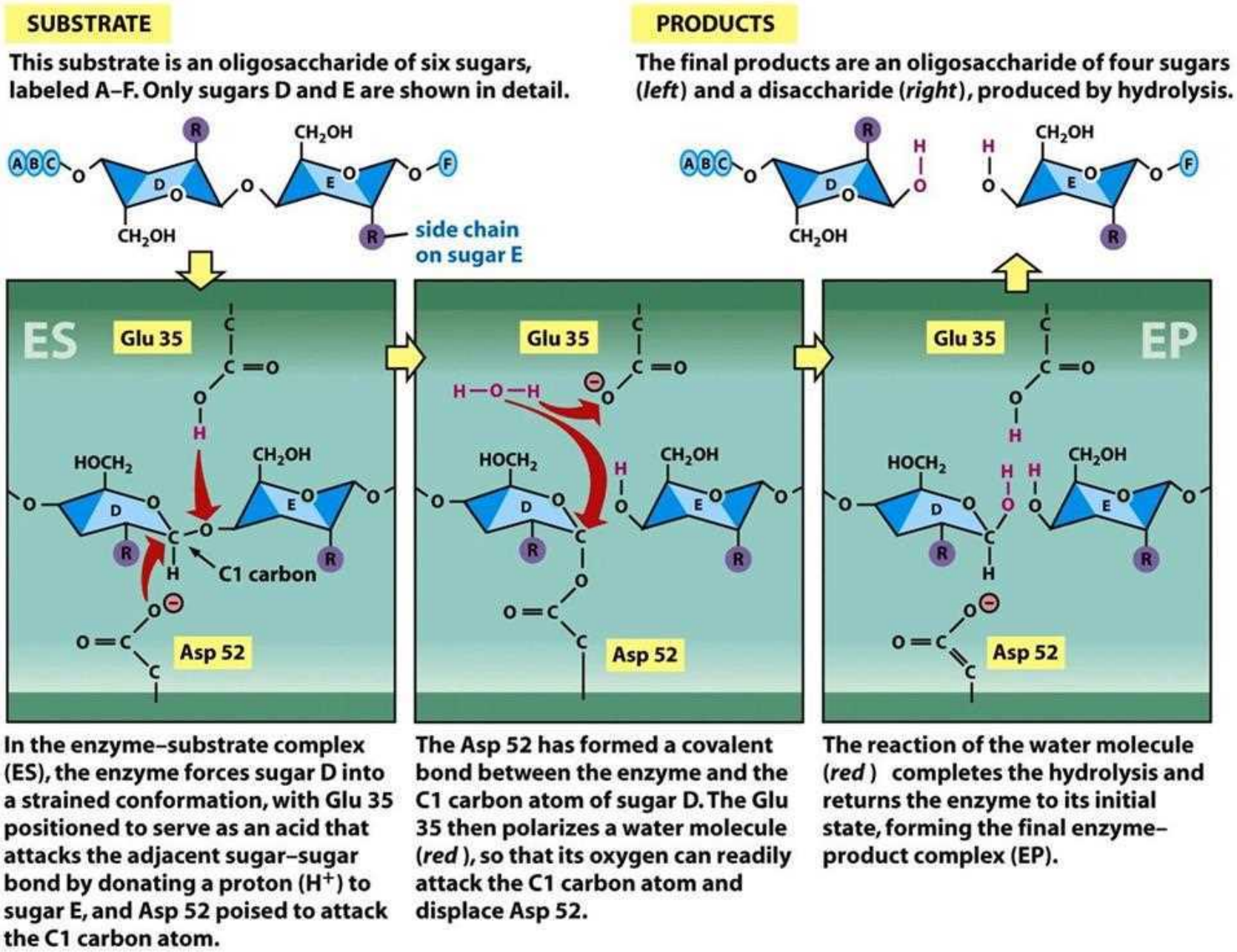
Hydrolysis reaction (A)The start of the uncatalyzed reactions show electron distribution in the water and carbonyl blonds. (B) An acid likes to donate a proton(H+) to other atoms. By pairing with the carbonyl oxygen an acid causesel ectrons to move away from the carbonyl carbon making this atom much more attractive to the electronegative oxygen of an attacking water molecule. (C) A base likes to take up H+. By pairing with a hydrogen of the attacking water molecule, a base causes electrons to move toward the water oxygen, making it a better attacking group for the carbonyl carbon. (D) By having appropriately positioned atoms on its surface, an enzyme can perform both acid catalysis and base catalysis at the same time.

Acid catalysis and base catalysis



(A) The enzyme lysozyme (E) catalyzes the cutting of a polysaccharide chain, which is its substrate (S). The enzyme first binds to the chain to form an enzyme-substrate complex (ES) and then catalyzes the cleavage of a specific covalent bond in the backbone of the polysaccharide, forming an enzyme-product complex (EP) that rapidly dissociate. Release of the severed chain (the products P) leaves the enzyme free to act on another substrate molecule. (B) A space-filling model of the lysozyme molecule bound to a short length of polysaccharide chain before cleavage.

Acid catalysis and base catalysis



Nomenclature

Oxidoreductase subclasses

I Class.

Oxidoreductases catalyze oxidation-dehydrogenation under aerobic and anaerobic conditions:

- Reductases are anaerobic.
- Oxidases are aerobic.

Oxidoreductase coenzymes:

1. Nicotinamide (NAD, NADP).
2. Flavin (FMN, FAD).
3. Metalloporphyrin (hemes a, b, c, d).
4. Quinone coenzymes (ubiquinone).
5. Peptide (glutathione).
6. Lipoic acid.

Subclass	*Type of catalyzed redox reaction*	*Example*
With oxygen		
Oxidases	Transfer of hydrogen atoms to oxygen	$RH_2 + \frac{1}{2}O_2 \xrightarrow[oxidases]{} R + H_2O$
Monoxidases	Insertion of 1 oxygen atom into the substrate	*monooxygenase or hydroxylase:* $RH + \frac{1}{2}O_2 \xrightarrow[hydroxylase]{} ROH$
Dioxygenases	Insertion of 2 oxygen atoms into the substrate	$RH_2 + O_2 \xrightarrow[dioxygenases]{} HO - R - OH$
Peroxidases	Oxidation with H_2O_2 participation	$RSH + H_2O_2 \xrightarrow[peroxidases]{} RS - SR + H_2O$
Without oxygen		
Dehydrogenases (DH)	Dehydrogenation reaction	$R–CH_2–CH_2–R + FAD \leftrightarrow$ $R–CH=CH–R + FADH_2$
Reductases	Reduction of the π–bond involving a hydrogen atom	$RCH=CH–R' + 2H \xrightarrow[reductase]{} RCH_2–CH_2R$
Cytochromes (cit)	Electron transfer	$Cytochrome\ (Fe^{3+})\ c + Cytochrome(Fe^{2+})\ c_1 \rightarrow Cytochrome\ (Fe^{2+})\ c + Cytochrome\ (Fe^{3+})c_1$

Nomenclature

Class II. Transferases transfer atomic groups and molecular residues.
Molecular groups transferred by transferases:
amino-, sulfo-, glycosyl-, phospho-, aldehyde, ketone residues, and one-carbon fragments.

Subclasses of transferases

Subclass	*Transferable group*	*Example*
Aminotransferases or transaminases	Amino group	$RNH_2 + R'H \xleftrightarrow[aminotransferases]{} RH + R'NH_2$
Methyltransferases	Methyl	$RCH_3 + R'H \xleftrightarrow[methyltransferases]{} RH + R'CH_3$
Kinases (phosphotransferases)	Phosphoric acid residue from ATP	$ROH + АТФ \xrightarrow[kinases]{} ROPO_3^{2-} + АДФ$

Transferase coenzymes:

1. Pyridoxine (pyridoxal phosphate, pyridoxamine phosphate).
2. Pantothenic (CoA, dephospho-CoA, 4-phosphopantothenate).
3. Nucleotide (UDP-glucose, CDP-choline).
4. Folic (tetrahydrofolic acid, THF).
5. Cobamide (methylcobalamin).

Nomenclature

Class III Hydrolases. Enzymes that catalyze the decomposition of organic compounds and the cleavage of various bonds (e.g., C-O, C-N, and O-P) in the presence of water.

This class is divided into 11 subclasses.

Main subclasses of hydrolases

Subclass	*Bond type*	*Example*
Peptidases	*Peptide*	$\boldsymbol{protein + H_2O \xrightarrow{peptidase} peptides}$
Glycosidases	*Glycoside*	$\boldsymbol{ROR' + HOH \xrightarrow{glucoside} RH + R'OH}$ $\boldsymbol{maltose + H_2O \xrightarrow{maltase} 2\ glucoses}$
Lipases	*Ester in lipids*	$\boldsymbol{RCOOR' + HOH \xrightarrow{lipase} RCOOH + R'OH}$
Esterases	*Ester*	$\boldsymbol{RCOOR' + HOH \xrightarrow{esterase} RCOOH + R'OH}$
Nuclease	*Phosphoester in NA*	
Phosphatases	*Phosphoester*	$\mathrm{R{-}OPO_3^{2-}} + \boldsymbol{HOH \leftrightarrow ROH + HPO_3^{2-}}$ $\mathrm{Glu{-}6{-}p} + \boldsymbol{H_2O \xrightarrow{glu\text{–}6\text{–}p\text{–}phosphatase} Glu + H_3PO_4}$

Nomenclature

IV Class Lyases accelerate the nonhydrolytic cleavage of groups of atoms (most often CO_2, H_2O, NH_3, H_2S, etc.) with the formation of a double bond or the addition of groups of atoms to a double bond without the participation of a macroerg . This results in the release of CO_2, H_2O, and NH_3.

Subclasses of lyases

Subclass	*Type of catalyzed reaction*	*Example*
Dehydratases	Removal of H_2O from a substrate	$R–CH_2–CHOH–R' \xrightarrow{dehydratase} R–CH=CH–R' + \boldsymbol{HOH}$
Decarboxylases	Removal of CO_2 from a substrate	$\boldsymbol{RCOOH} \xrightarrow{decarboxylase} \boldsymbol{RH + CO_2}$
Deaminases	Removal of NH_3 from a substrate	$R–CH_2–CH_2–NH_2 \xleftrightarrow{deaminase} \boldsymbol{NH_3} + R–CH=CH_2$
Hydratases	Addition of H2O to a substrate	$R–CH=CH–R' + HOH \leftrightarrow R–CH_2–CHOH–R'$ $\boldsymbol{fumarate} + \boldsymbol{H_2O} \xrightarrow{fumarase}$ L–malate
Phosphorylases	Phosphorylation	$ROR + HPO_4^{2-} \xleftrightarrow{phosphorylase} R–OPO_3^{2-} + ROH$ $glycogen + HPO_4^{2-} \xleftrightarrow[glycogen]{phosphorylase} R–OPO_3^{2-} + ROH$
Synthases	Joining two molecules without the participation of high-energy molecules (macroerg)	$\boldsymbol{A + B \leftrightarrow AB}$ $\boldsymbol{AcCoA + oxaloacetate} \xrightarrow{citrate\ synthase} citrate$

Nomenclature

Class Ⅴ. Isomerases catalyze the interconversion of isomers: AB ↔ BA. This can occur through the intramolecular transfer of hydrogen atoms, phosphate or acyl groups, changes in the spatial arrangement of atoms, displacement of double bonds, etc.

Subclasses of isomerases

Subclass	*Reaction*	*Example*
Isomerases	Interconversion of aldoses into ketoses "glucose-6-phosphate	glucose–6–phosphate $\xleftrightarrow{phosphoglucoisomerase}$ fructose–6–phosphate
Mutases	Transfer of groups within the molecule	glucose–6–phosphate $\xleftrightarrow{phosphoglucomutase}$ glucose–1–phosphate
Epimerases	Interconversion of epimers	ribulose–5–phosphate $\xleftrightarrow{phosphoribuloseepimerase}$ glucose–1–phosphate
Racemases	Interconversion of D and L isomers	L–AA $\xleftrightarrow{racemase}$ D–AA

Nomenclature

Class Ⅵ Synthetases

Enzymes that catalyze the synthesis of substances using ATP energy are called ligases (or synthetases).

The synthetase class is divided into 6 subclasses depending on the type of bond formed, for example:

1. Enzymes that form C–O bonds (amino–acyl–tRNA synthetases).
2. Enzymes that form C–S bonds (acetyl–CoA synthetase).
3. Enzymes that form C–N bonds (asparagine synthetase, glutamine synthetase).
4. Enzymes that form C–C bonds (pyruvate carboxylase), etc.

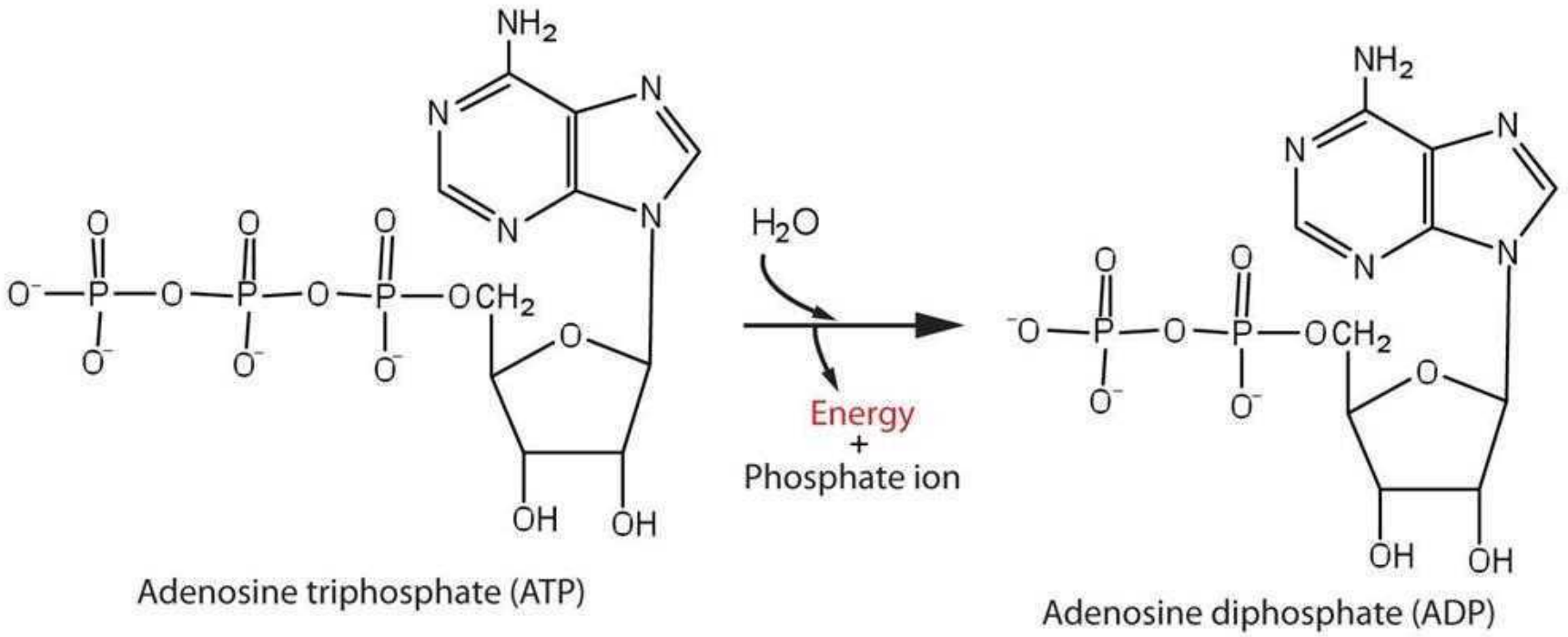
acetyl–CoA synthetase

$$ATP + acetate + co - A \rightarrow AMP + pyrophosphate + acetyl - coA$$

Nomenclature

Class VII Translocases

Class VII translocases, catalyze the transfer of ions or molecules across membranes. Some of these enzymes function by *hydrolyzing ATP—adenosine triphosphatases (ATPases)—*and are currently classified as class III "Hydrolases" (EC 3.6.3.X). For example, K+/Na+-ATPase, Ca2+-ATPase, and H+/K+-ATPase catalyze the hydrolysis of ATP to ADP and organic phosphate (P), releasing energy that is expended on the transport of K+ and Na+, or Ca2+, or H+ and K+ ions, respectively, across the membrane against the concentration gradient of these ions.

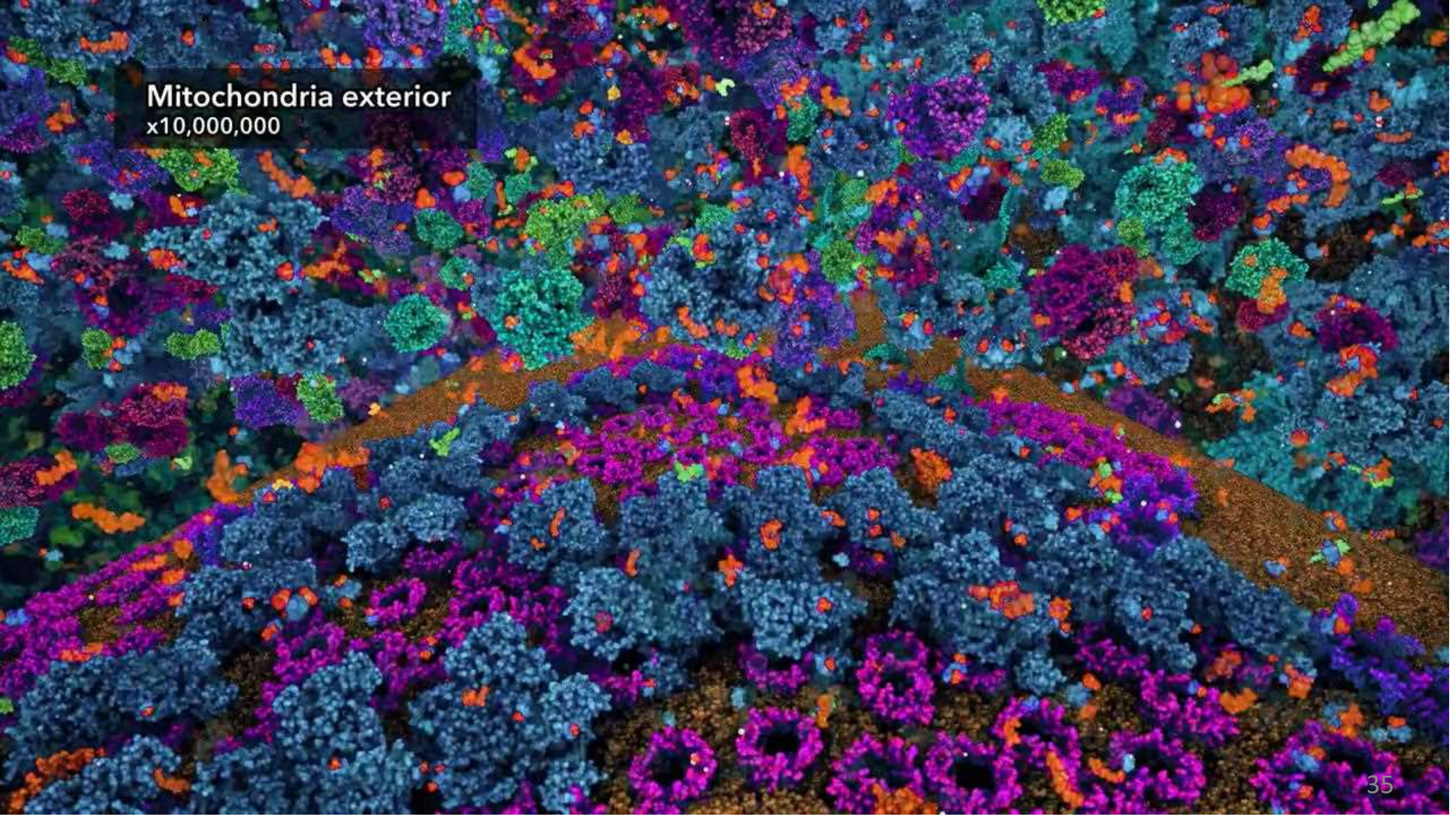


Nomenclature

ENZYME	REACTION CATALYZED
Hydrolases	general term for enzymes that catalyze a hydrolytic cleavage reaction; *nucleases* and *proteases* are more specific names for subclasses of these enzymes.
Nucleases	break down nucleic acids by hydrolyzing bonds between nucleotides.
Proteases	break down proteins by hydrolyzing bonds between amino acids.
Synthases	synthesize molecules in anabolic reactions by condensing two smaller molecules together.
Isomerases	catalyze the rearrangement of bonds within a single molecule.
Polymerases	catalyze polymerization reactions such as the synthesis of DNA and RNA.
Kinases	catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins.
Phosphatases	catalyze the hydrolytic removal of a phosphate group from a molecule.
Oxido-Reductases	general name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often more specifically named either *oxidases, reductases,* or *dehydrogenases.*
ATPases	hydrolyze ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function, for example, motor proteins such as *myosin* and membrane transport proteins such as the *sodium–potassium pump.*

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin and lysozyme that were discovered and named before the convention became generally accepted at the end of the nineteenth century. The common name of an enzyme usually indicates the substrate and the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

Thank you for your attention!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