



Enzymes I

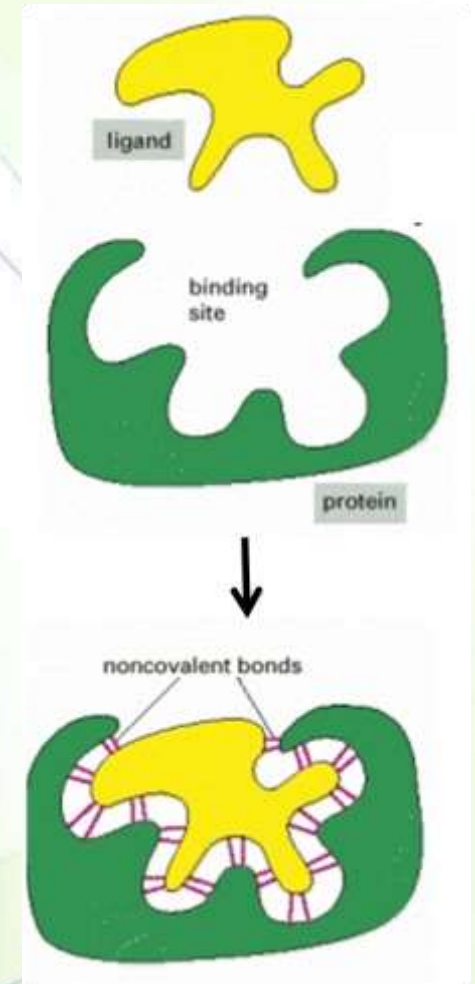
Characteristics and classification

Summer semester, 2023

General properties of proteins



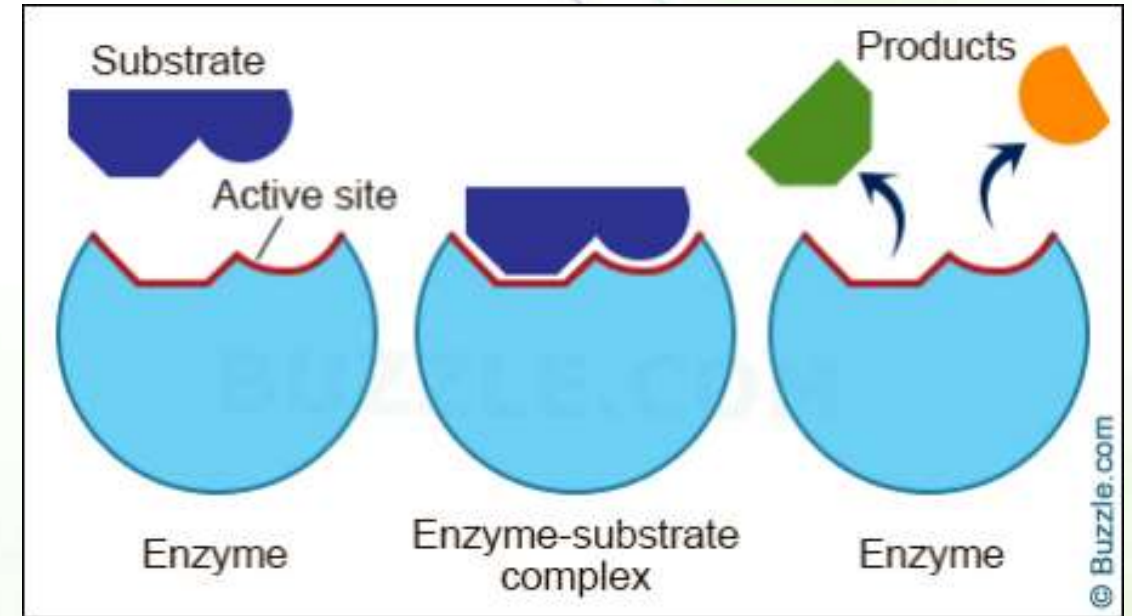
- The function of proteins depends on their ability to bind other molecules (ligands).
 - **Ligand:** a substance that forms a complex with a biomolecule, usually via non-covalent interactions, to serve a biological purpose.
- Two properties of a protein characterize its interaction with ligands:
 - **Affinity:** the strength of binding between a protein and other molecules.
 - **Specificity:** the ability of a protein to bind one molecule in preference to other molecules.



What are enzymes?



- Enzymes: Specialized **proteins** that conduct (catalyze) chemical reactions under biological conditions.
 - Exception: ribozymes (*to be discussed*)
 - In enzymatic reactions, reactants are known as substrates.
- Most enzymes have very specific functions in converting specific **substrates** to **products**.
- Enzymes are catalysts.
 - They exist in small amounts relative to the reactants.
 - They increase the rate of a reaction.
 - At the end of the reaction, they do not change.



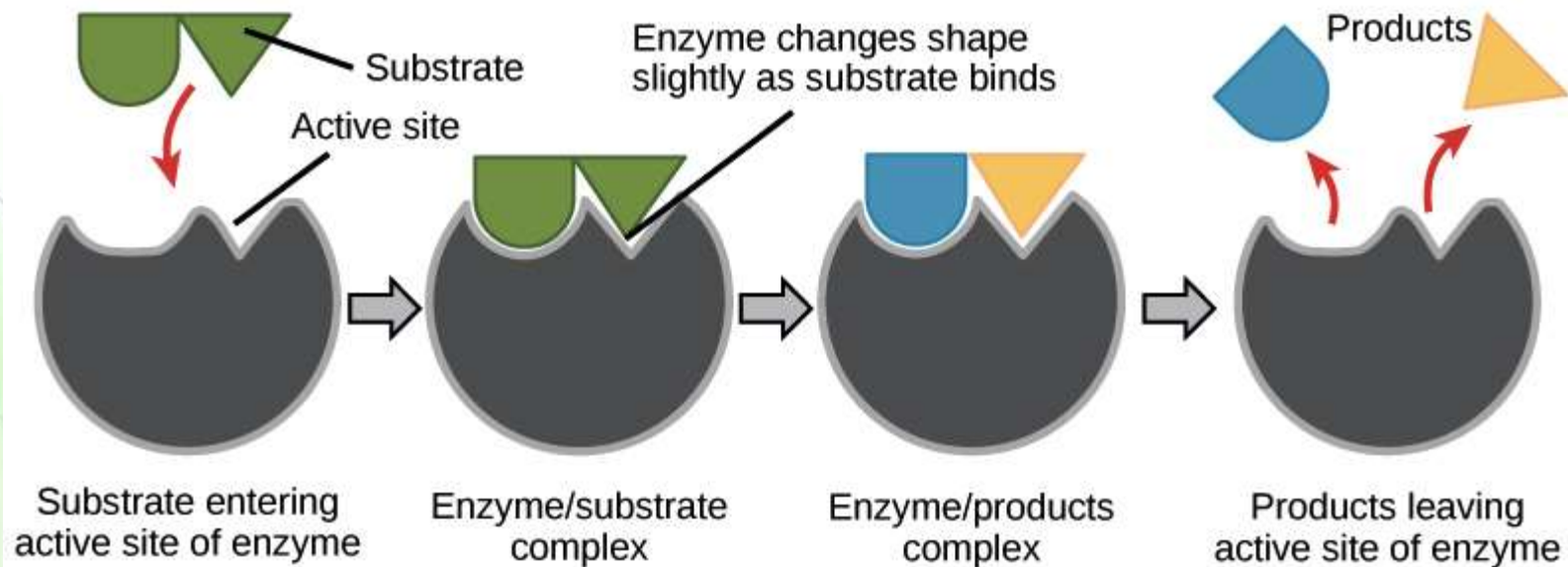
How can we express an enzymatic reaction?



- Simple expression of enzymatic reaction:



E = free enzyme; S = free substrate, ES = enzyme-substrate complex; P = product of the reaction; and EP = enzyme-product complex before the product is released



What do enzymes do?



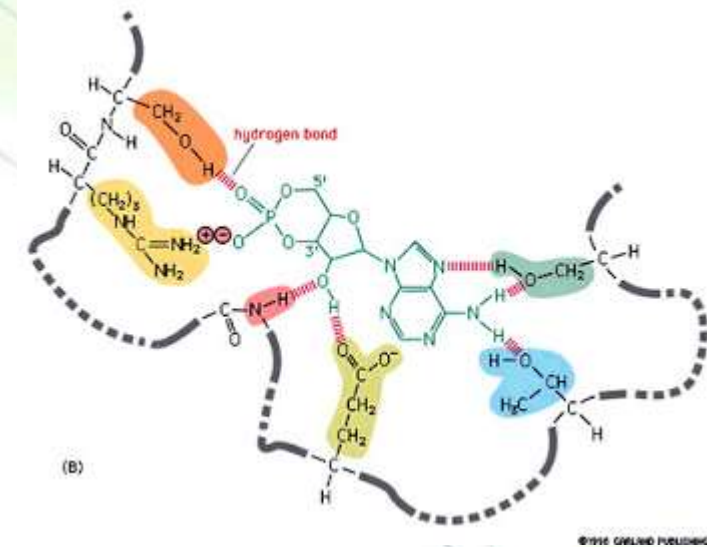
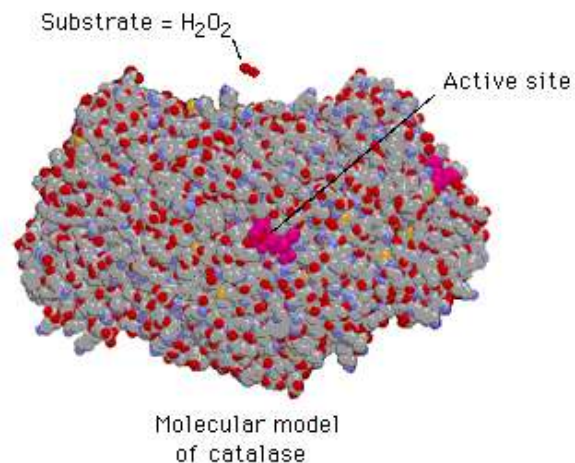
- Enzymes accelerate reactions (usually within a range of 10^6 to 10^{14}).
 - Example: Catalase (10^8) & carbonic anhydrase (10^6)
 - Carbonic anhydrase: $\text{CO}_2 + \text{H}_2\text{O} \xrightleftharpoons{\text{Carbonic anhydrase}} \text{H}_2\text{CO}_3$
 - One enzyme molecule hydrates 10^6 molecules of CO_2 per second (versus 1 per 10 seconds for uncatalyzed reactions)
 - Catalase: $2 \text{H}_2\text{O}_2 \xrightleftharpoons{\text{Catalase}} 2 \text{H}_2\text{O} + \text{O}_2 (\text{g})$

Reaction Conditions	Relative Rate
No catalyst	1
Platinum surface	2.77×10^4
Catalase	6.51×10^8

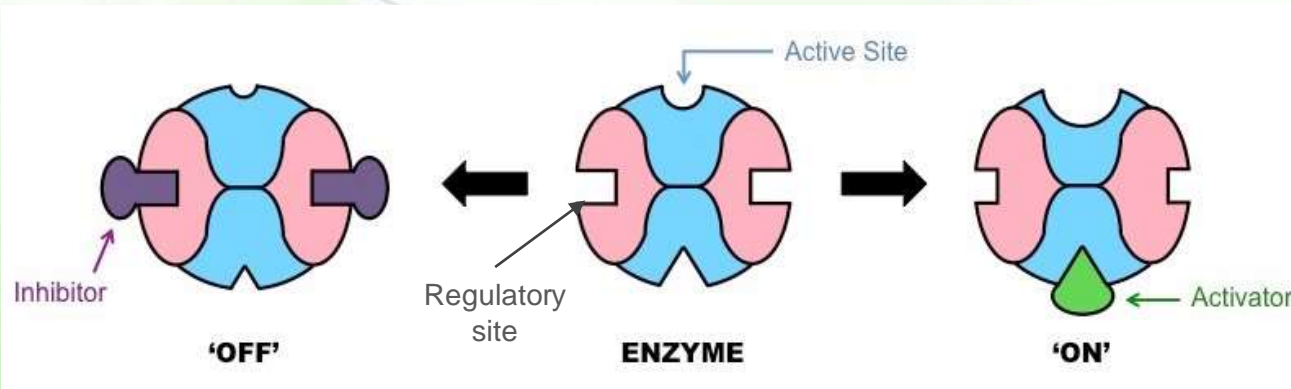
Where does the reaction occur?



- Each enzyme has a specific three-dimensional shape called **the active site** (a region where the biochemical reaction takes place).



The active site contains a specialized amino acid sequence that facilitates the reaction.

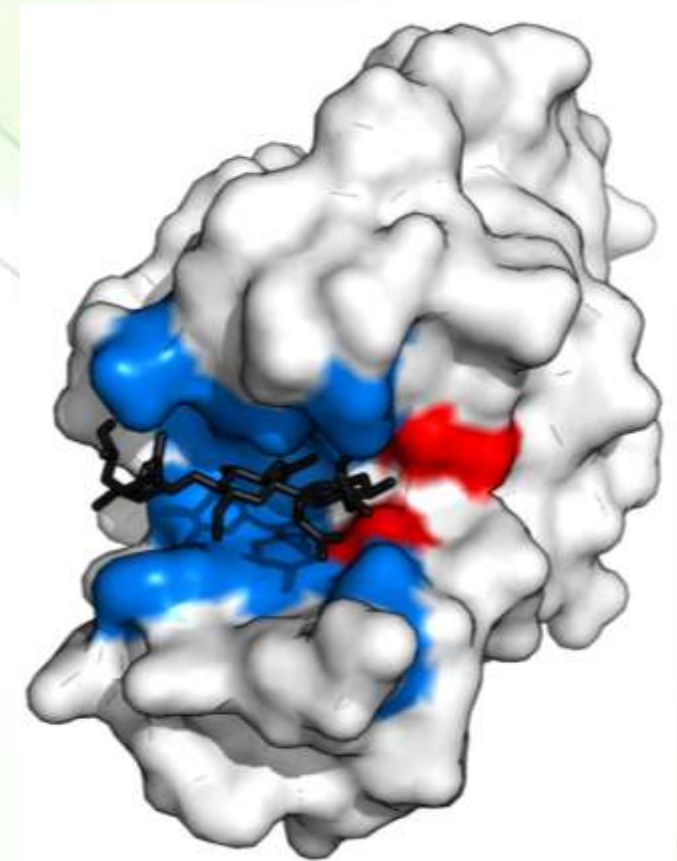
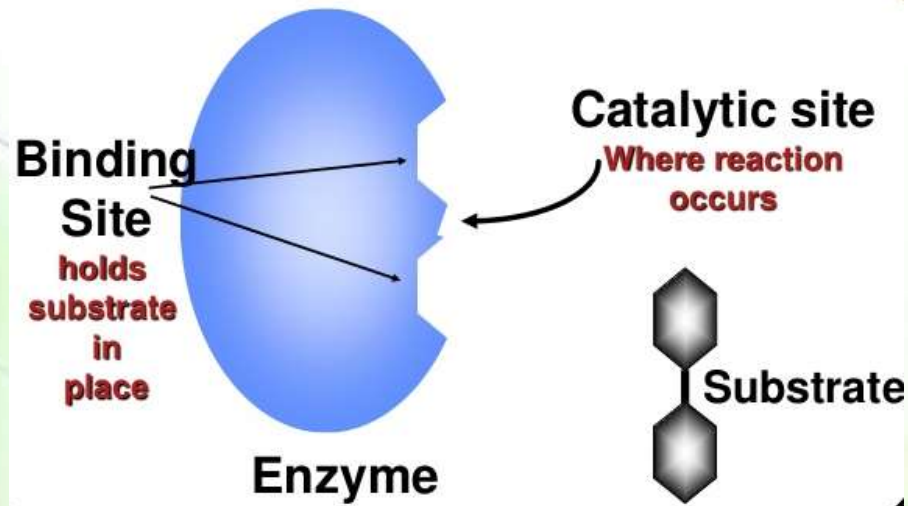


Binding of a substrate into the active site can be regulated by a **regulatory site**.

Catalytic and binding groups



- Within the active site are two sub-sites: the binding and catalytic sites.
- The catalytic site contains amino acid residues (catalytic group) that carry out the actual reaction.
- In some enzymes, the binding and catalytic sites are the same.



ACTIVE SITE

BINDING SITES

Bind and orient substrate(s)

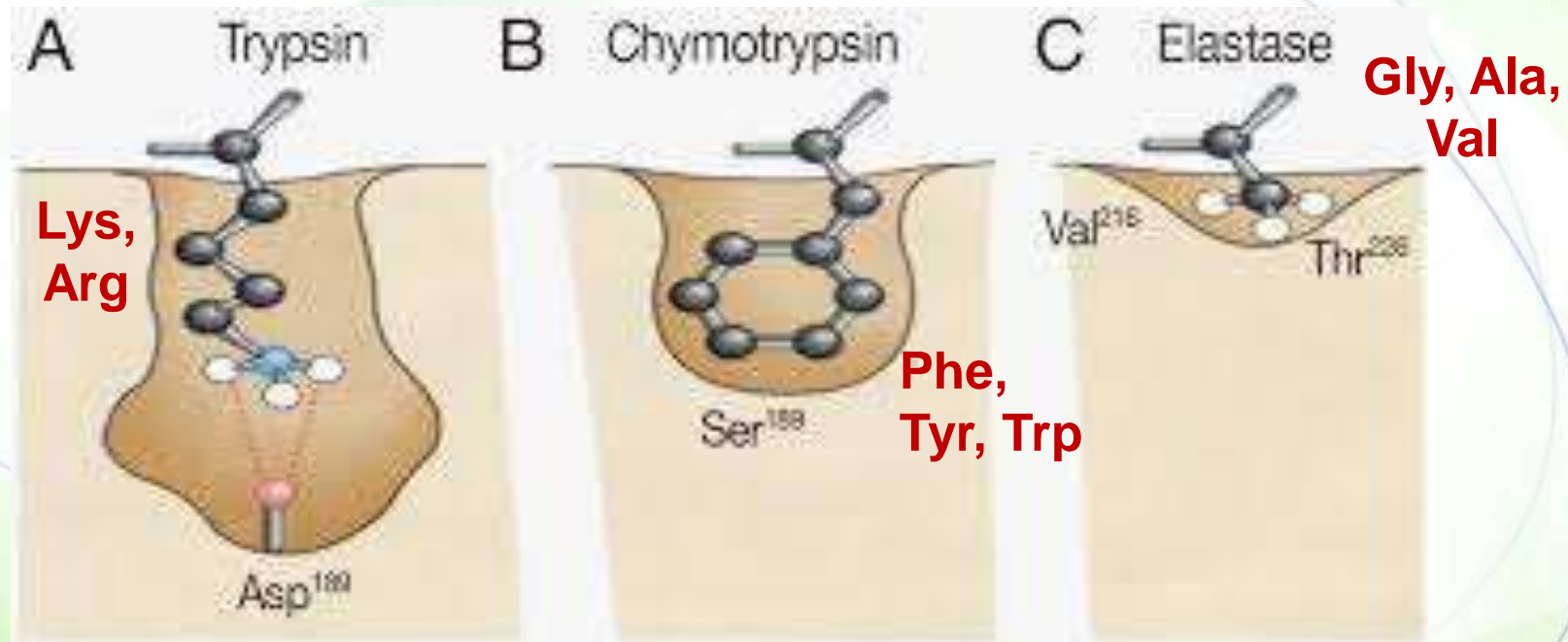
CATALYTIC SITE

Reduce chemical activation energy

Binding specificity



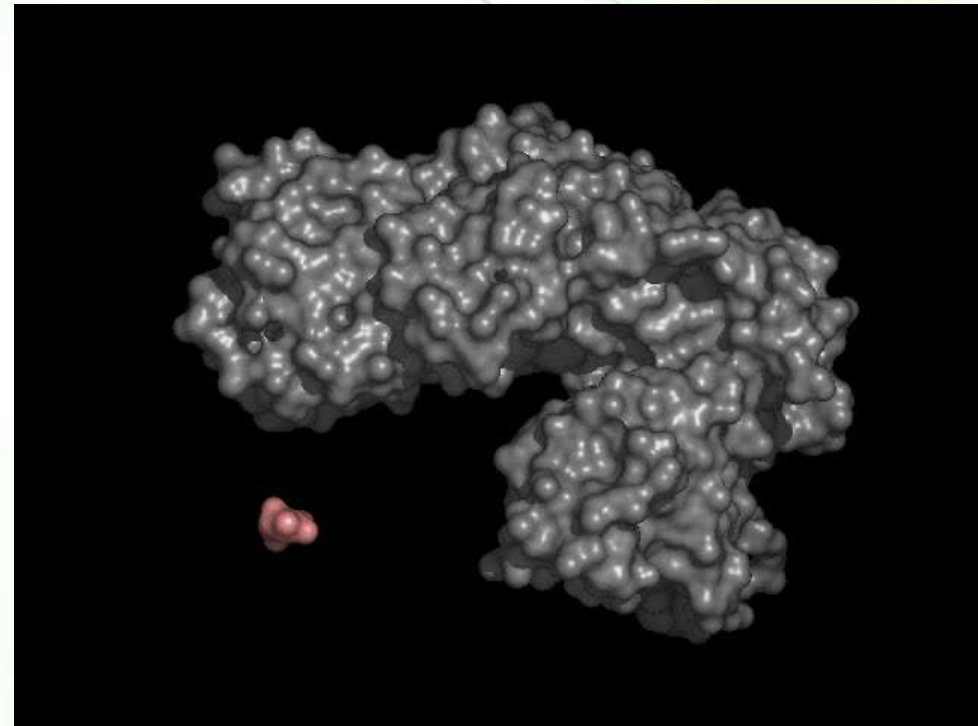
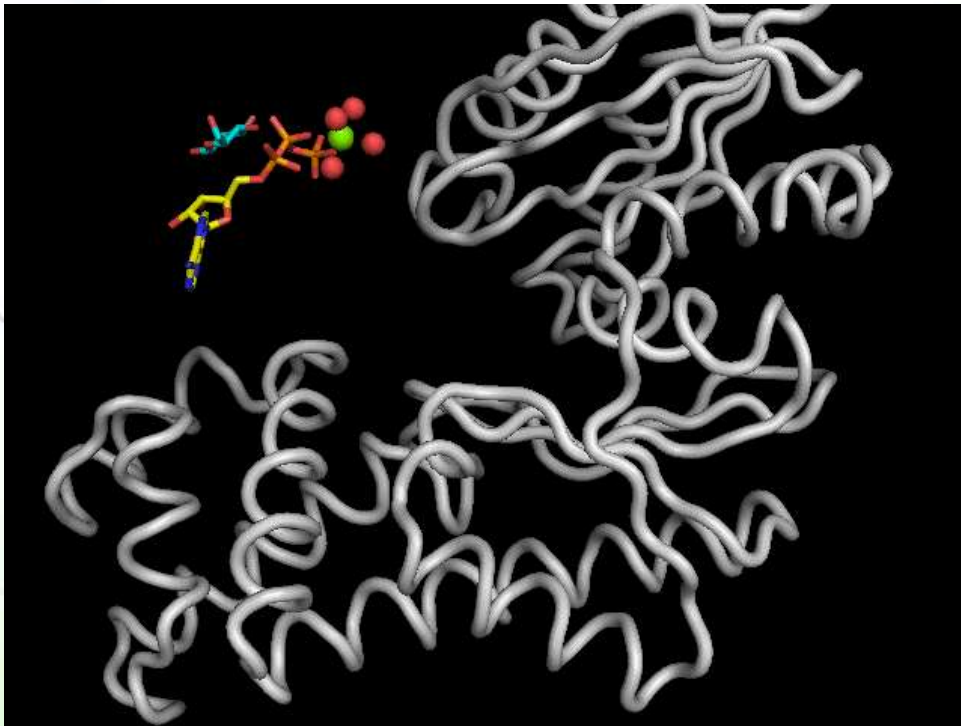
- The specificity and selectivity of enzymes is due to their precise interaction of active sites to their substrates and the degree of compatibility for this interaction.



Features of active site 1



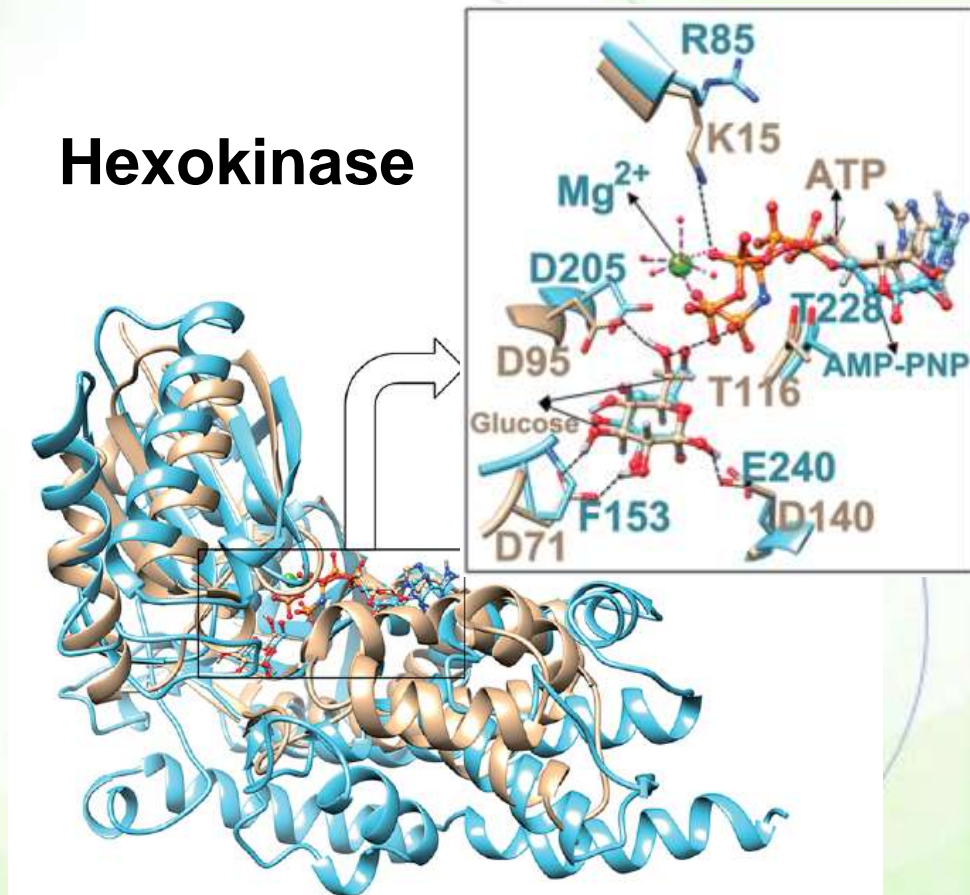
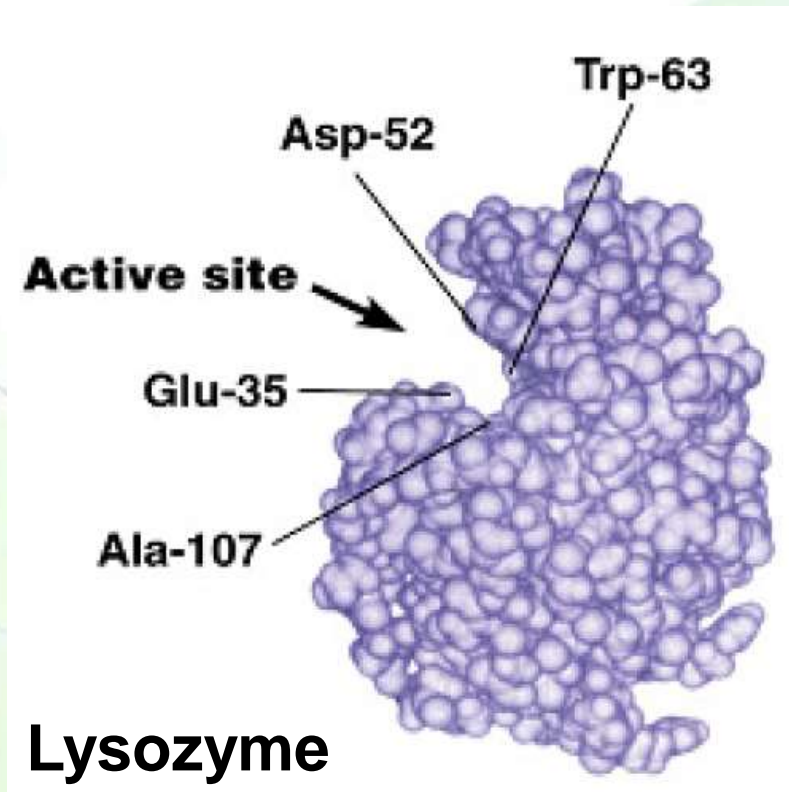
- It is internal relative to the enzyme and looks like a canal



Features of active site 2



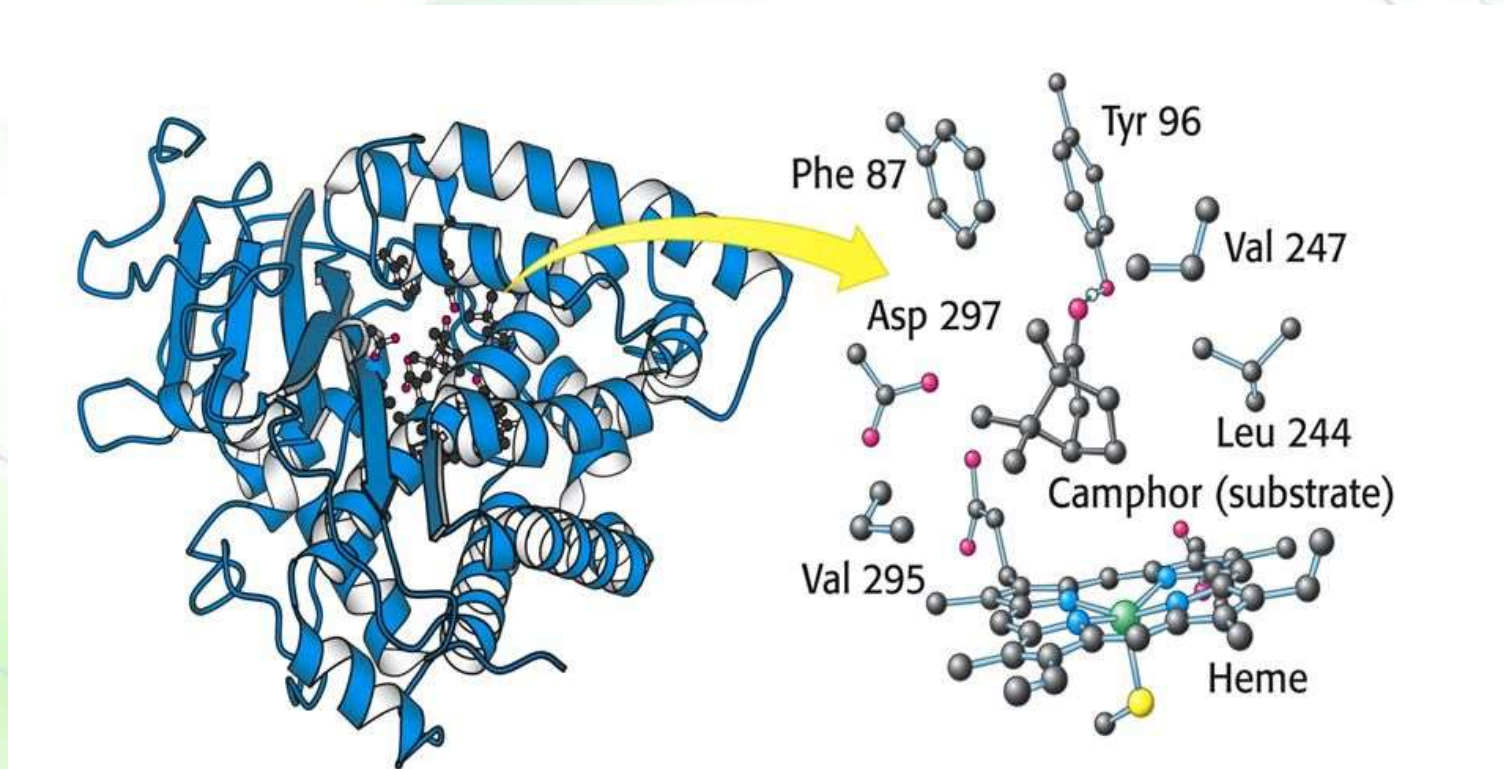
- It is a three-dimensional pocket formed by amino acid groups that come from different parts of the primary structure usually forming a domain.



Features of active site 3



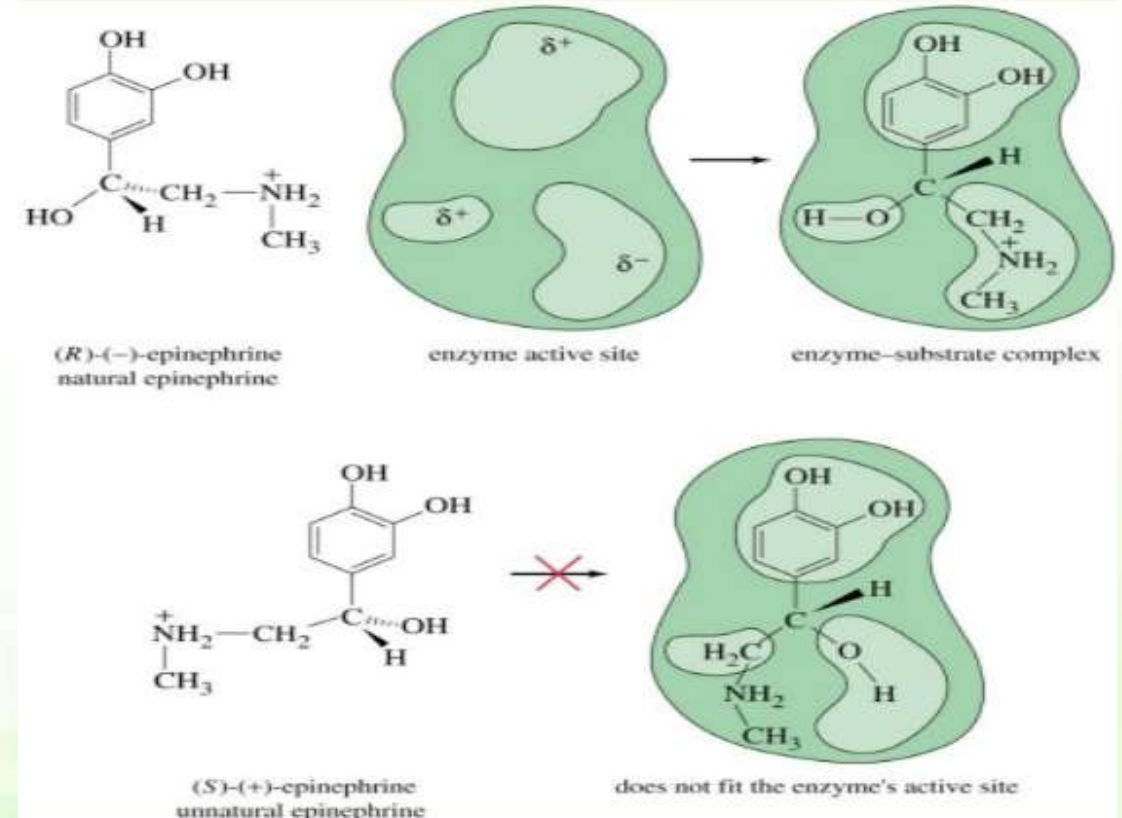
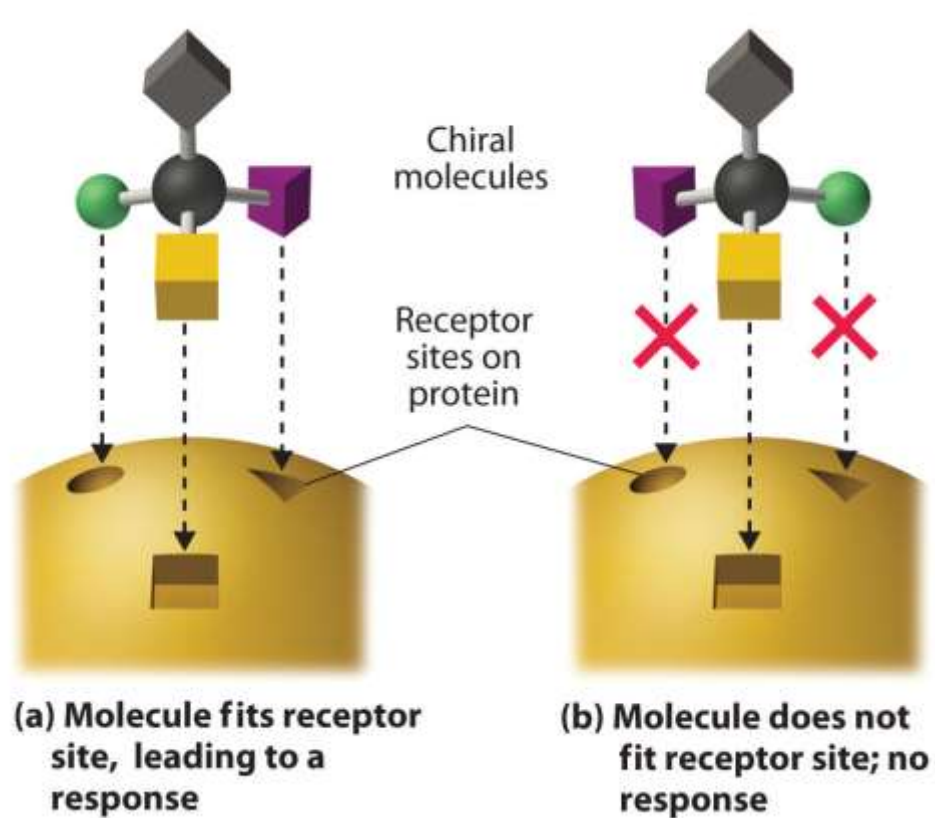
- The amino acid residues can be nonpolar and polar.
 - Water is usually excluded unless it is part of the reaction.
- Substrates bind to enzymes by multiple weak attractions.



Features of active site 4



- Binding of substrates to active sites occurs at, at least, three points.
 - Chirality is important, hence specificity.

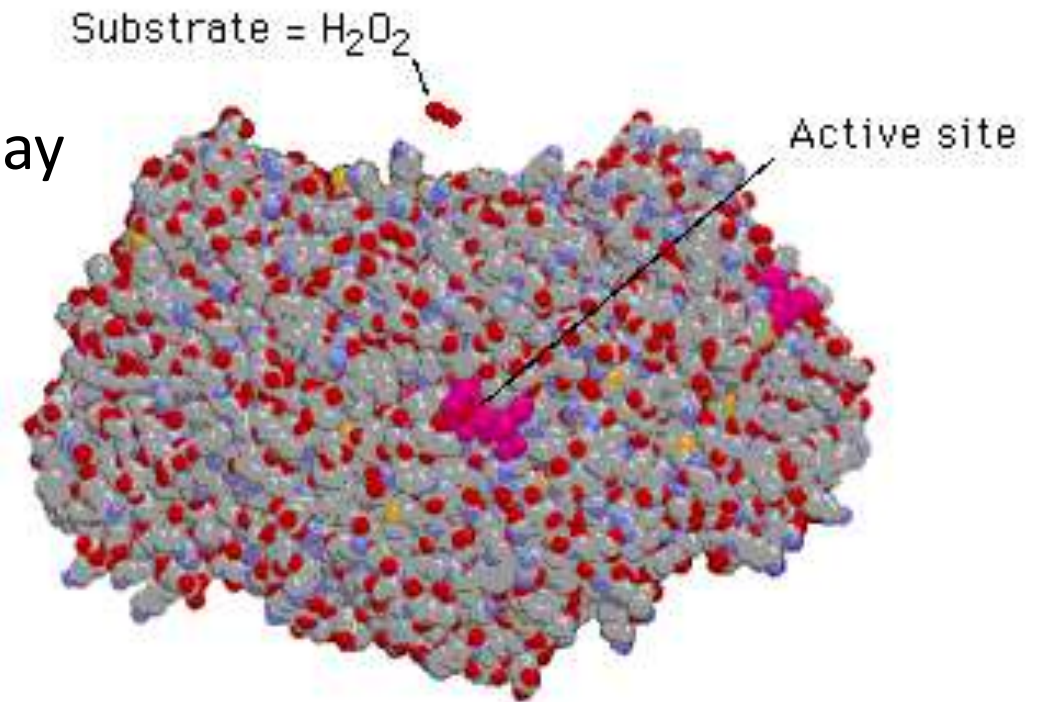


Features of active site 5



- It is small relative to the total structure of an enzyme.
- The “extra” amino acids create the 3D active site.

The remaining amino acids may make up regulatory sites.



Molecular model
of catalase

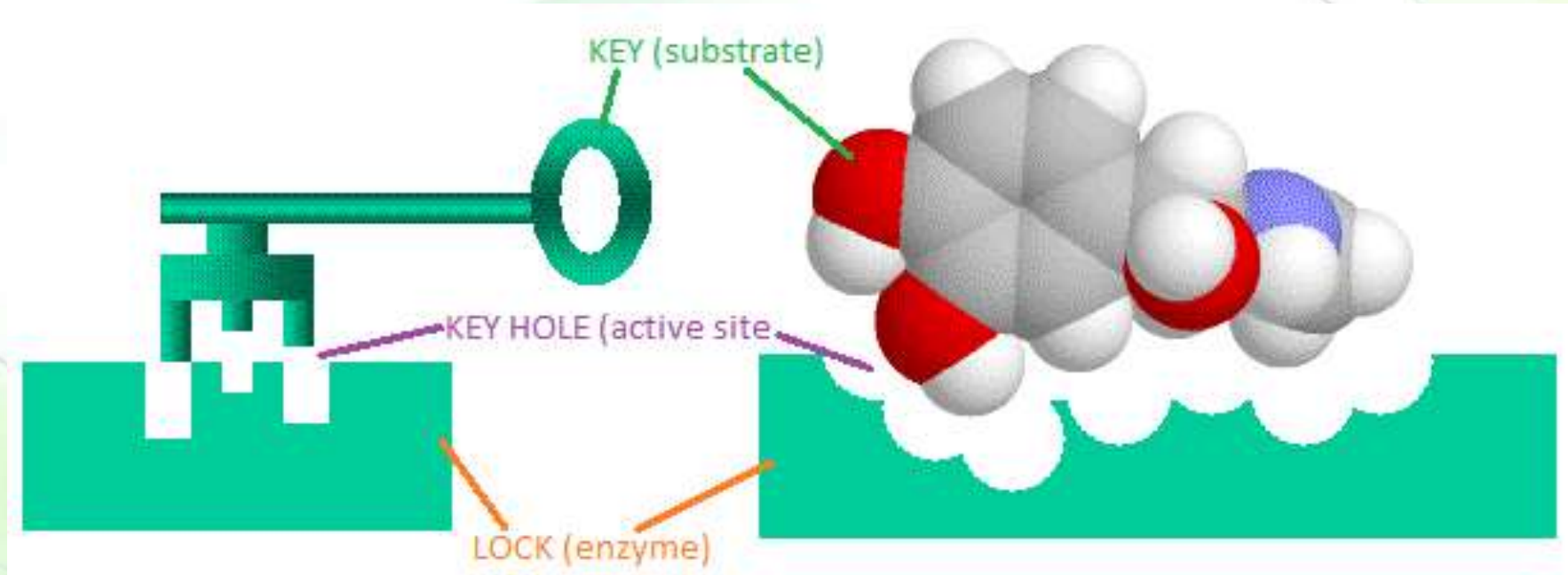


How do substrates fit into the active site of enzymes?

Lock-and-key model (old)



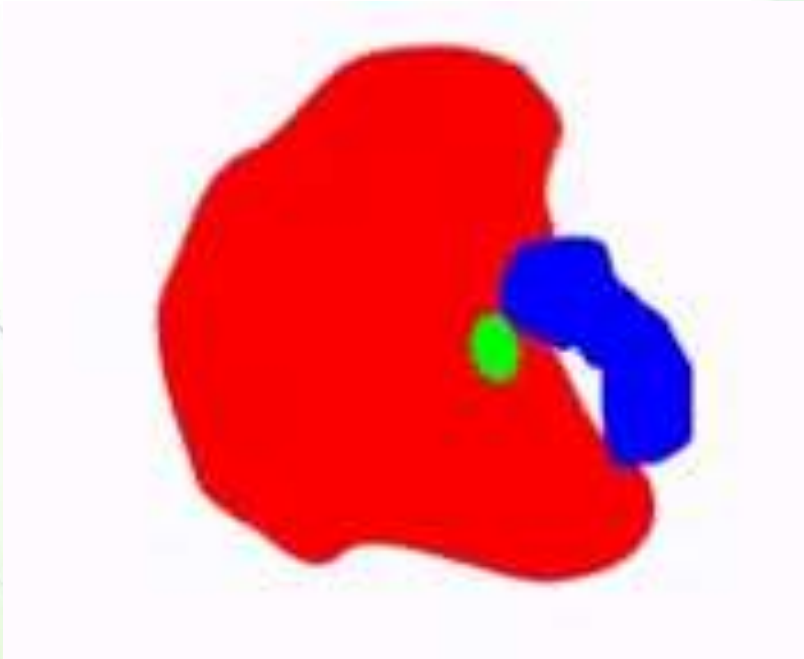
- Here, the substrate fits directly into the active site.



Induced fit model



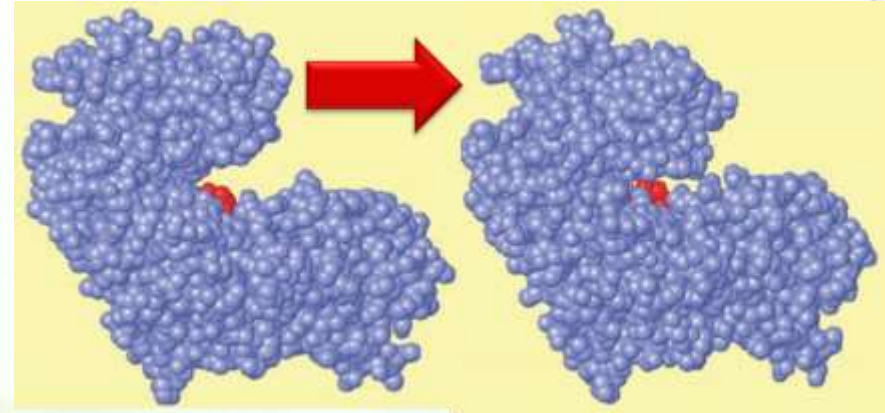
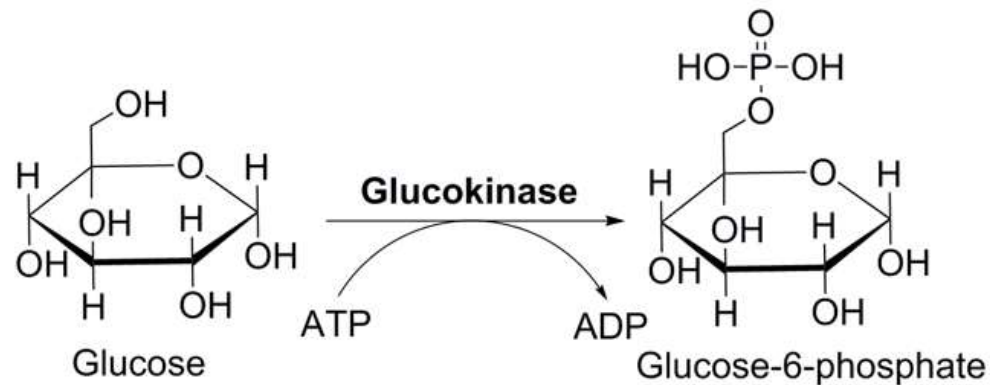
- Enzymes are flexible and active sites can be modified by binding of substrate.



Glucokinase is the king of both!



- Glucose binds the active site of glucokinase as a lock and key resulting in the induced fit.
- The induced fit changes the conformation of the whole enzyme closing the cleft, and improving the binding for ATP, the other substrate.
- Thus, the multiple interactions between the substrate and the enzyme in the catalytic site serve both for substrate recognition and for initiating the next stage of the reaction.



How do enzymes accelerate reactions?

Types of energy



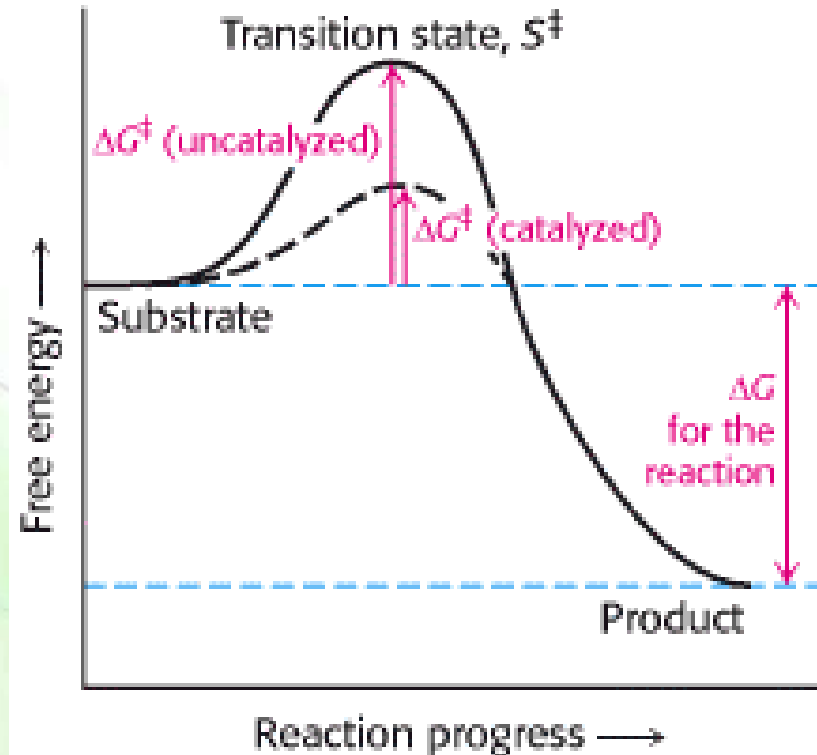
- There are two forms of energy
 - potential - capacity to do work (stored)
 - kinetic - energy of motion
- Potential energy is more important in the study of biological or chemical systems.
- Molecules have their own potential energy stored in the bonds connecting atoms in molecules.
 - It is known as free energy or G (for Josiah Gibbs).
 - It is the energy that is available for reactions.

Free energy (G)



- The difference between the free energy values between reactants and products (free-energy change ΔG):

$$\Delta G = G_{\text{products}} - G_{\text{reactants}}$$



ΔG accounts for the equilibrium of the reaction and enzymes accelerate how quickly this equilibrium is reached.

What does it mean?



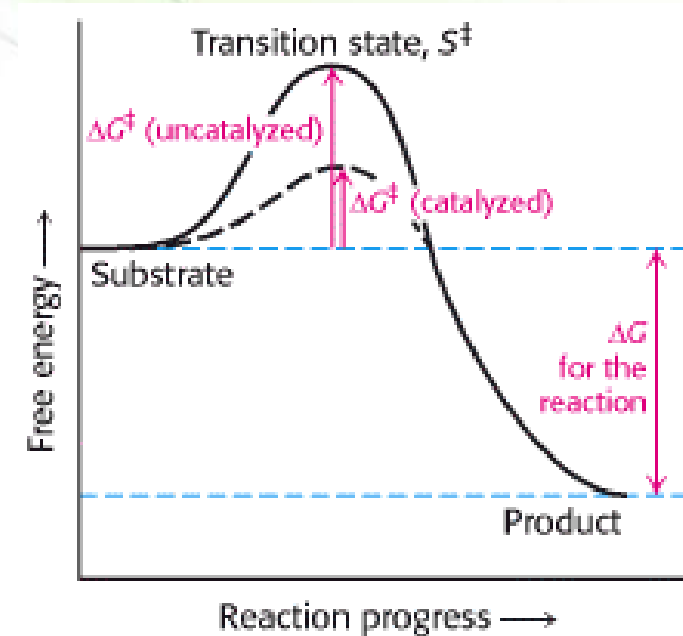
$$\Delta G = G_{\text{products}} - G_{\text{reactants}}$$

- If ΔG is negative, G_{products} is **less** than $G_{\text{reactants}}$, energy is not needed to drive the reaction, but released, making the forward reaction (from left to right) spontaneous (the reaction is called exergonic).
- If ΔG is positive, G_{products} is **more** than $G_{\text{reactants}}$, an input of energy is needed, making the reaction not spontaneous (the reaction is called endergonic).
 - The reverse reaction is exergonic and, thus, spontaneous.
- If ΔG is zero, both forward and reverse reactions occur at equal rates; the reaction is at equilibrium.

What do enzymes do?



- Any enzymatic reaction goes through a transition state (ES) that has a higher free energy than does either S or P.
- The difference in free energy of the transition state and the substrate is called the activation energy.
- Enzymes lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state at a lower energy.

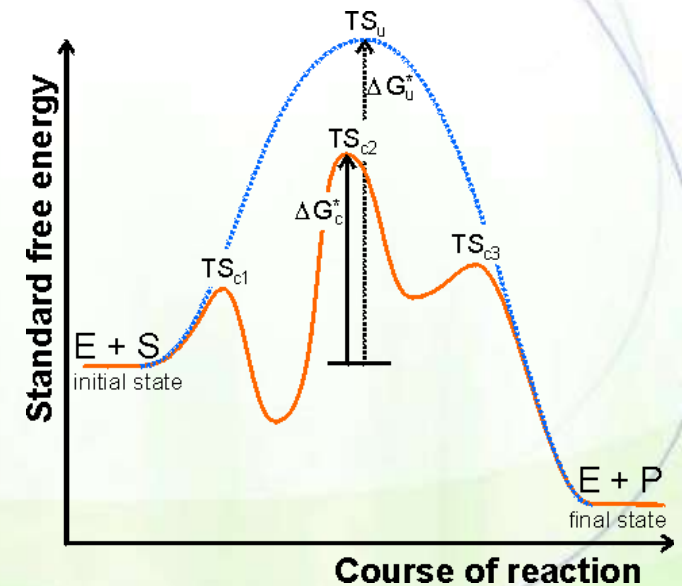
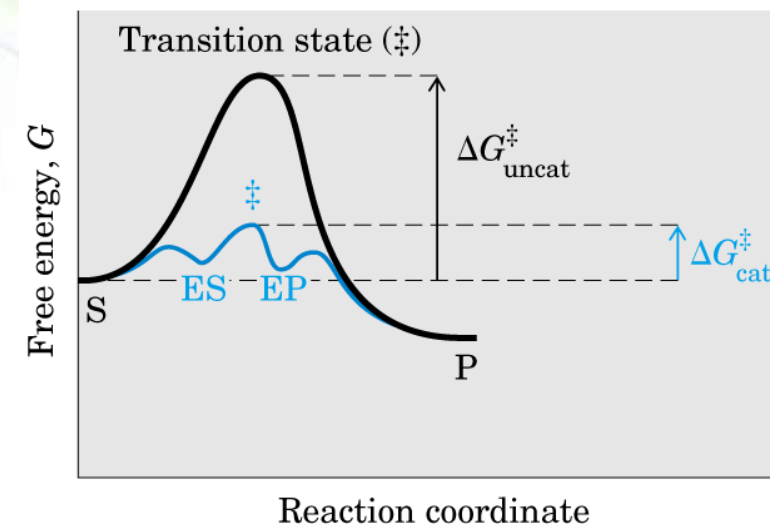


- **At the highest energy level, the substrate configuration is most unstable and is most tightly bound to the enzyme.**
- **The bonds are maximally strained.**

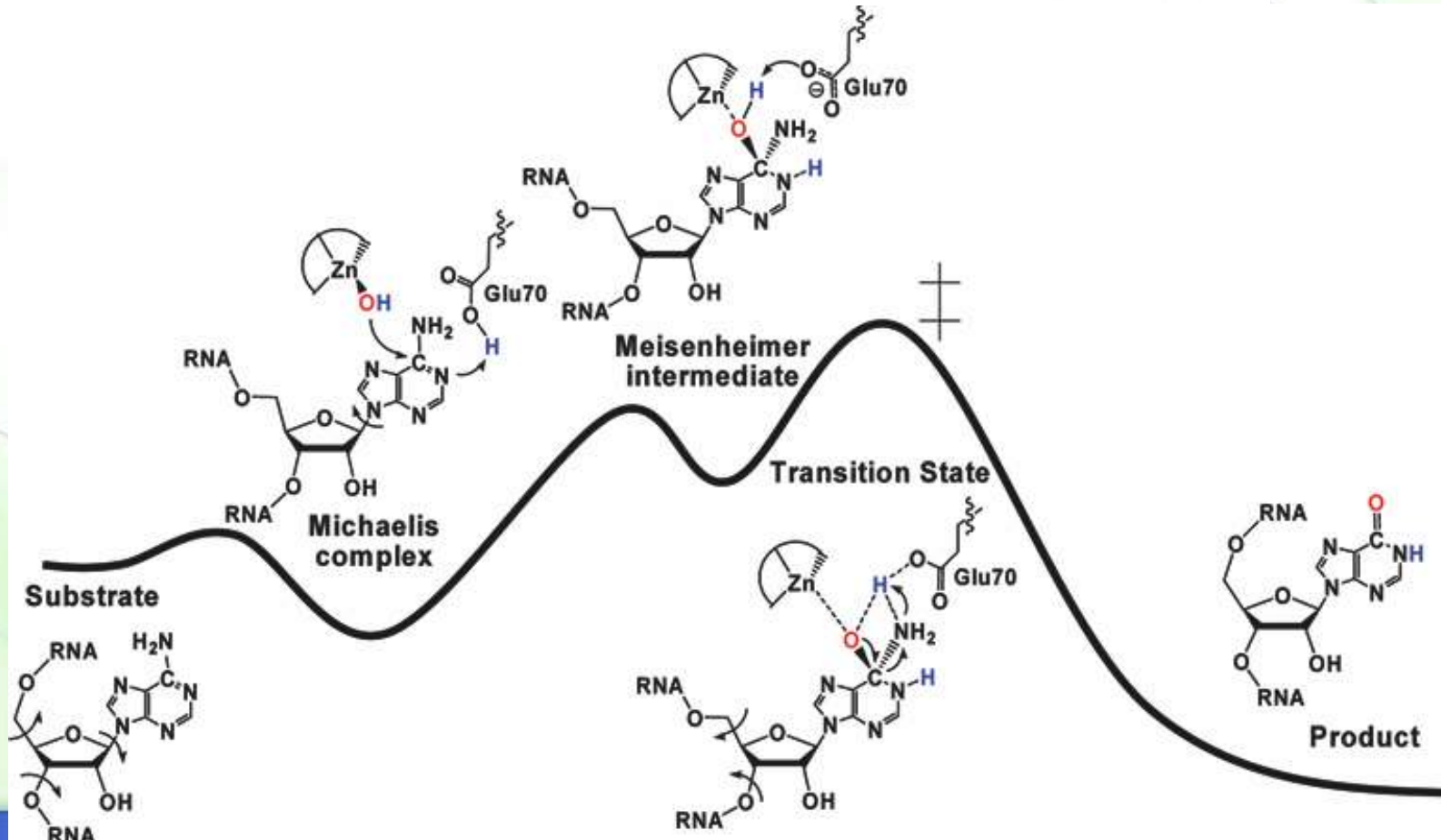
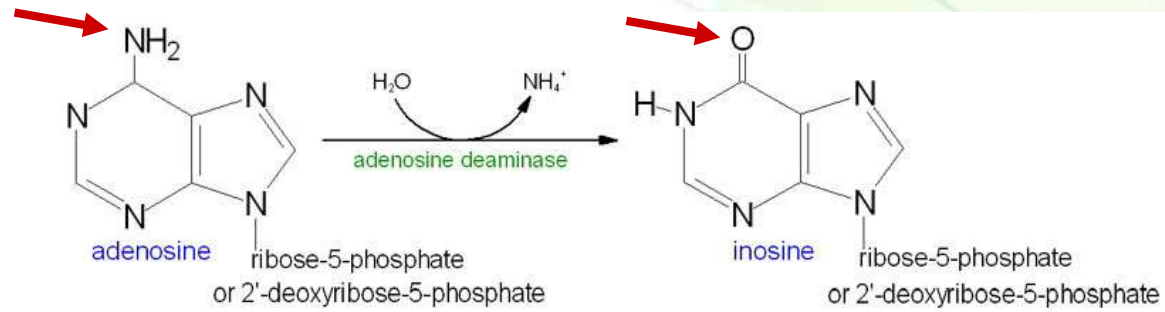
Alternative pathways



- Substrates often undergo several transformations when associated with the enzyme and each form has its own free energy value.
- The activation energy corresponds to the complex with the highest energy.
- The energy of activation does not enter into the final ΔG calculation for a reaction.



Example: Adenosine Deaminase



How do enzymes catalyze reactions?



- Proximity of substrates together
- Orientation of the active site to fit the substrate in the best fit possible
- Changing the energy within bonds allowing the breakup and formation of bonds
- Catalysis is the end-result.

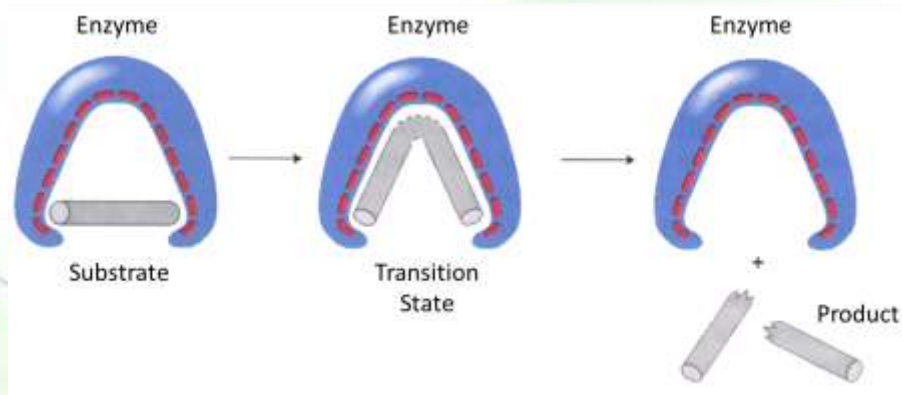
- Examples of possible mechanisms to do so:
 - Catalysis by bond strain
 - Catalysis involves acid/base reactions
 - Covalent catalysis

Enzymes may use a combination of these mechanisms.

Catalysis by bond strain

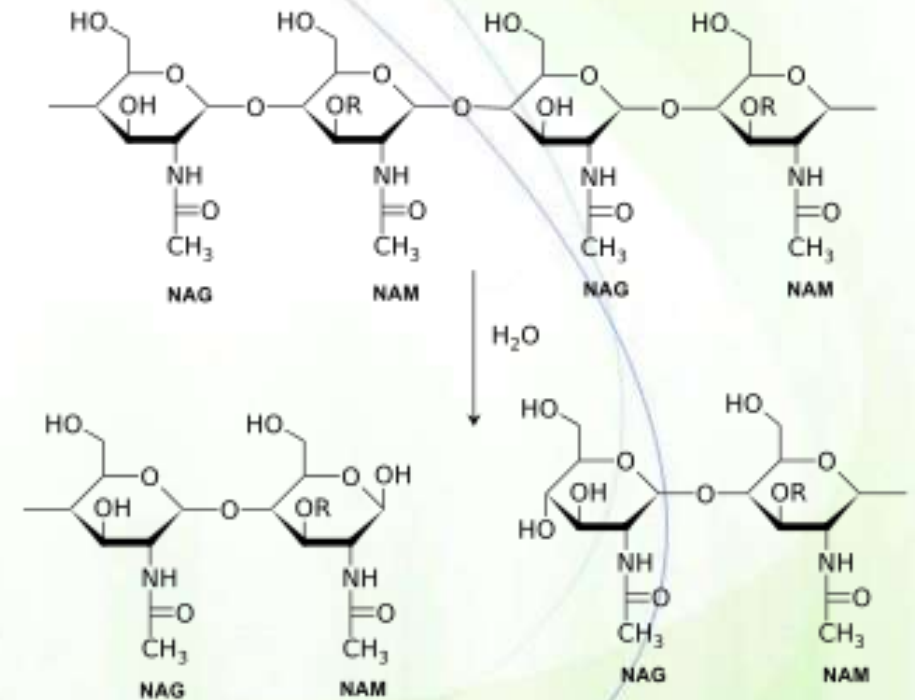


- The induced structural rearrangements produce strained substrate bonds, which more easily attain the transition state. The new conformation makes the substrate bonds “vulnerable”.



Example: lysozyme

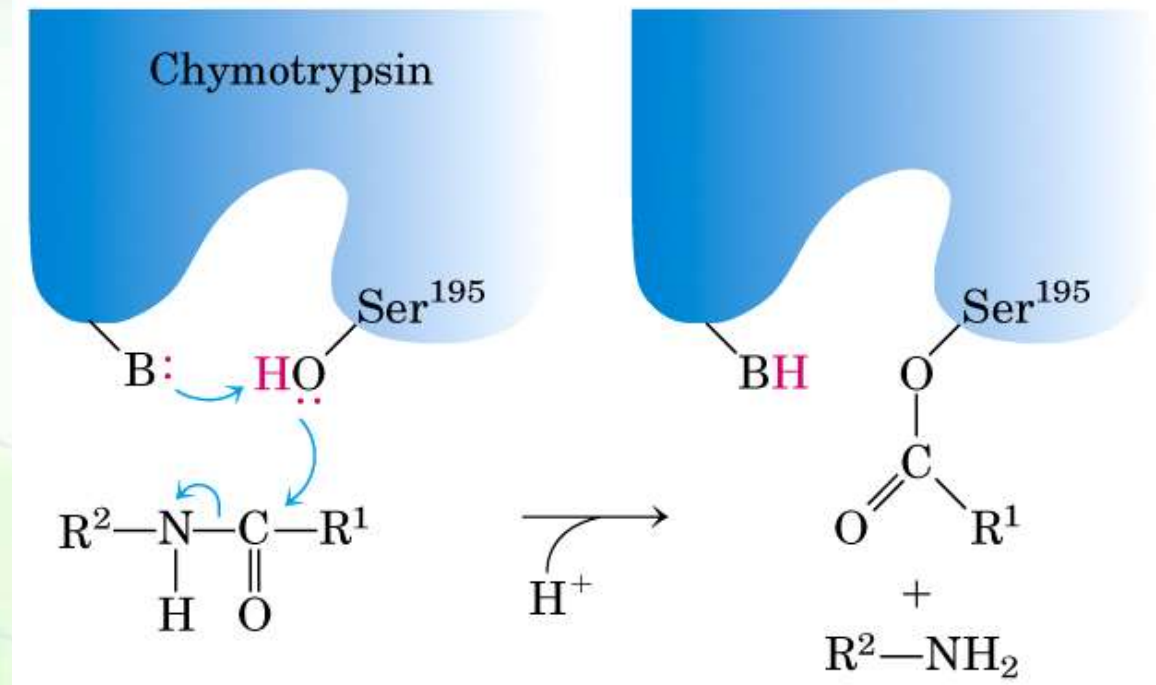
The substrate is distorted from the typical 'chair' hexose ring into the 'sofa' conformation, which is similar in shape to the transition state.



Catalysis involves acid/base reactions



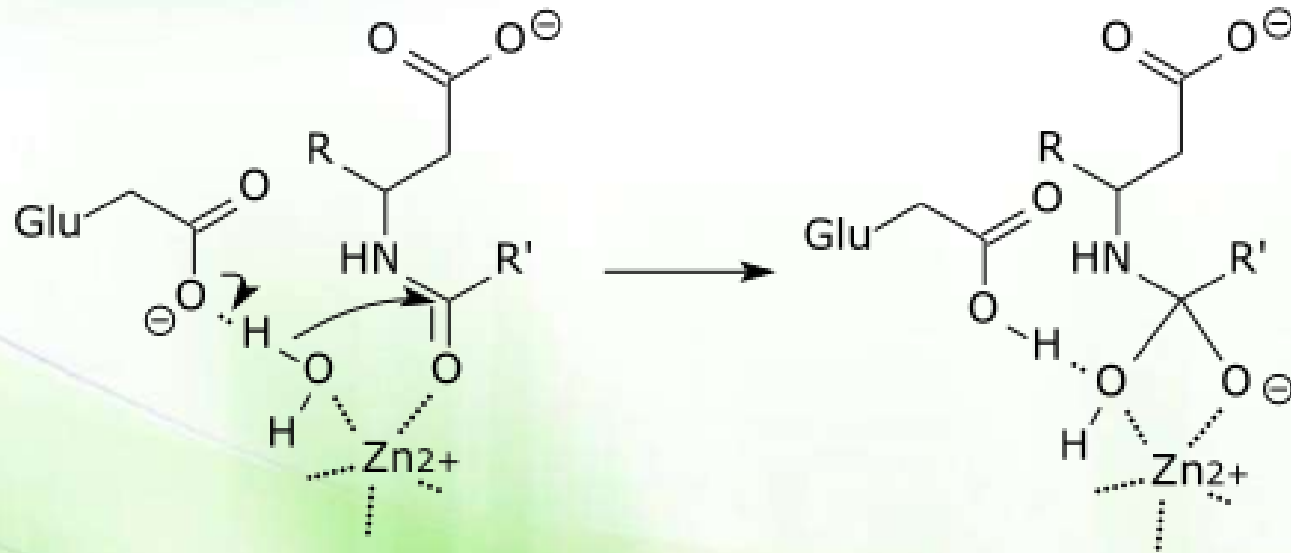
- The R groups of amino acids act as donors (acids) or acceptors (bases) of protons.
 - Histidine at physiological pH
 - Serine in serine proteases



Covalent catalysis



- A covalent intermediate forms between the enzyme or coenzyme and the substrate.
- Examples of this mechanism is proteolysis by serine proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase).



Enzyme substrate complex

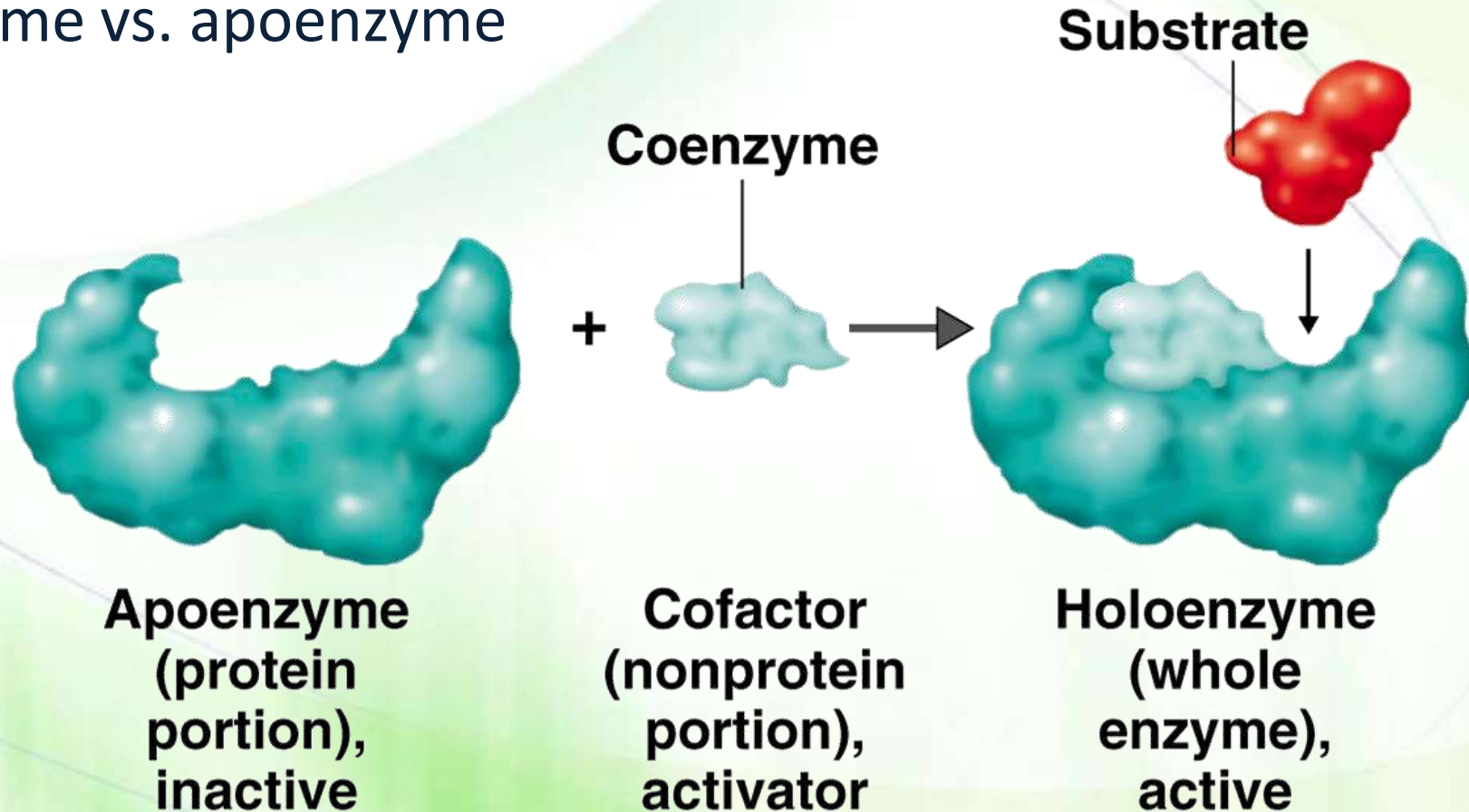
Tetrahedral intermediate

Classification of enzymes

Enzyme Classification (structure)



- Simple vs. complex (conjugated)
- Holoenzyme vs. apoenzyme



Naming of enzymes



- In general, enzymes end with the suffix (-ase).
- Most other enzymes are named for their substrates and for the type of reactions they catalyze, with the suffix “ase” added.
 - ATPase breaks down ATP.
 - ATP synthase synthesizes ATP.
- Some enzymes have common names
 - Examples: the proteolytic enzyme trypsin.



Enzyme classes according to function



- Enzymes are classified into six major groups:
 - Oxidoreductases
 - Transferases
 - Hydrolases
 - Lyases
 - Isomerases
 - Ligases

1. Oxidoreductases

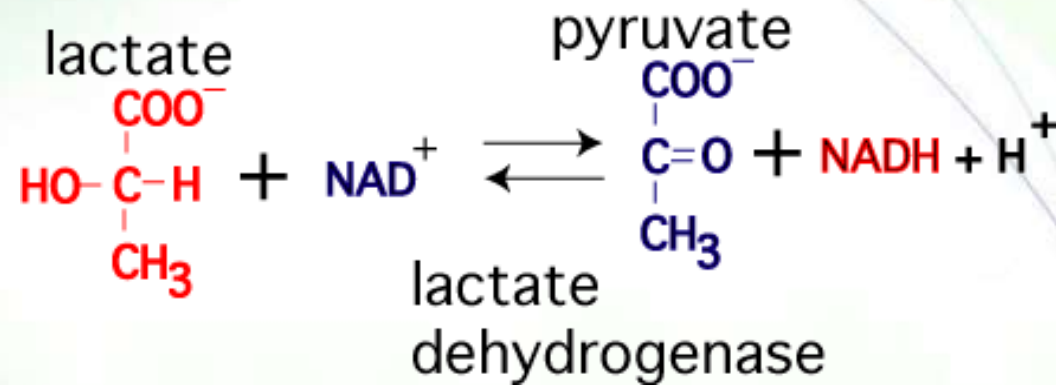


- They catalyze oxidation/reduction reactions involving the transfer of hydrogen atoms or electrons.
- They can be divided into 4 main classes:
 - Dehydrogenases
 - Oxidases
 - Peroxidases
 - Oxygenases

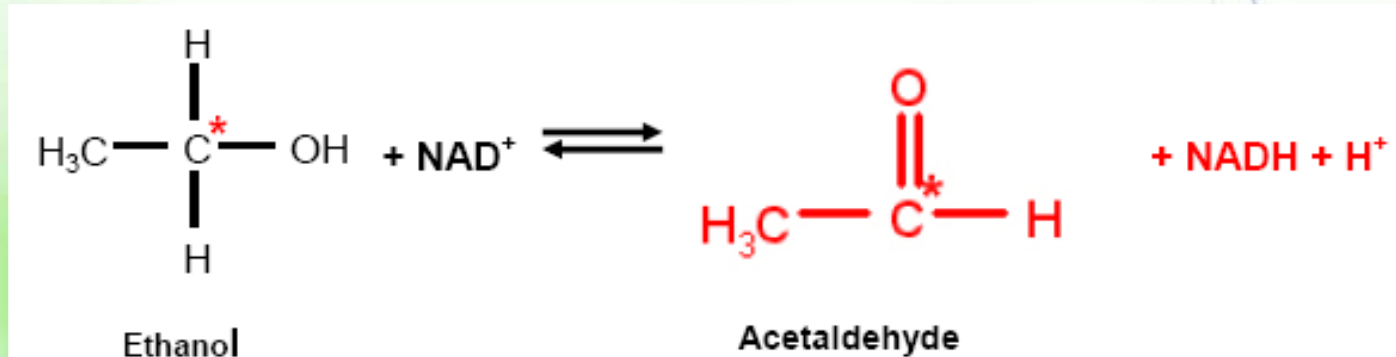
1a. Dehydrogenases



- Dehydrogenases transfer electrons in the form of hydride ions (H) or hydrogen atoms using an electron-transferring coenzyme, such as NAD⁺/NADH or FADH₂.
- Lactate dehydrogenase:



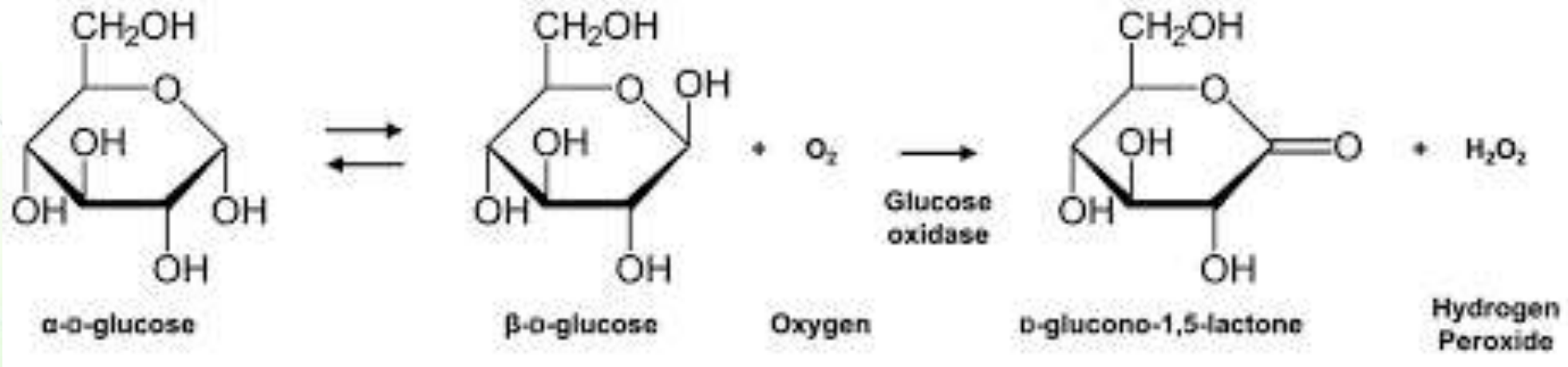
- Alcohol dehydrogenase:



1b. Oxidases



- Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product.
- Glucose oxidase catalyzes this reaction:

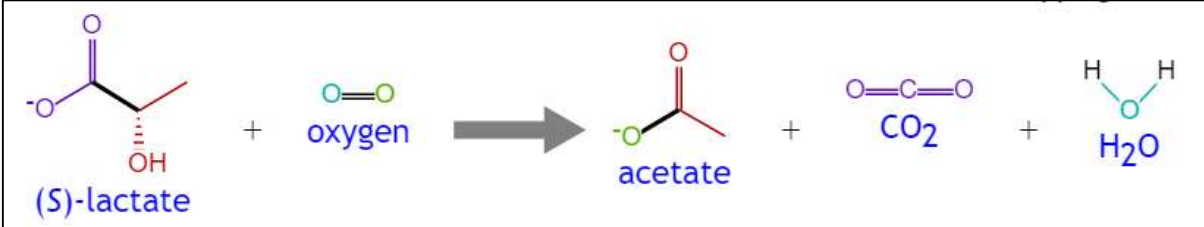


1c. Oxygenases

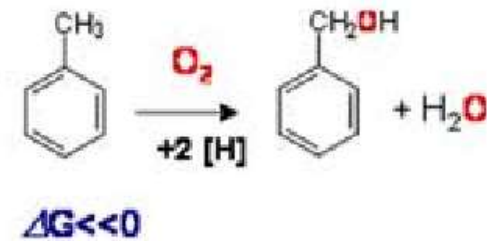


- Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate.
 - The reduced product is water, not H_2O_2 .

Lactate-2-monoxygenase

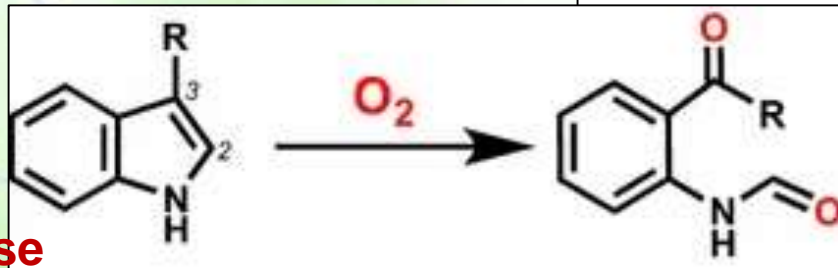
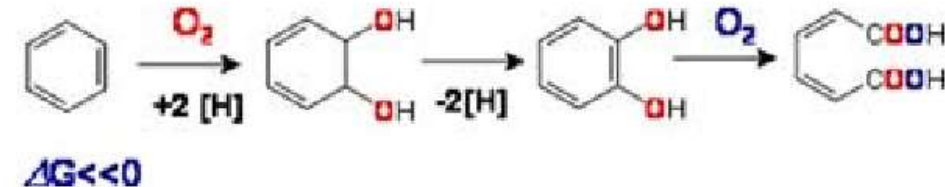


Monoxygenases



**Monoxygenase
vs.
Dioxygenase**

Dioxygenases

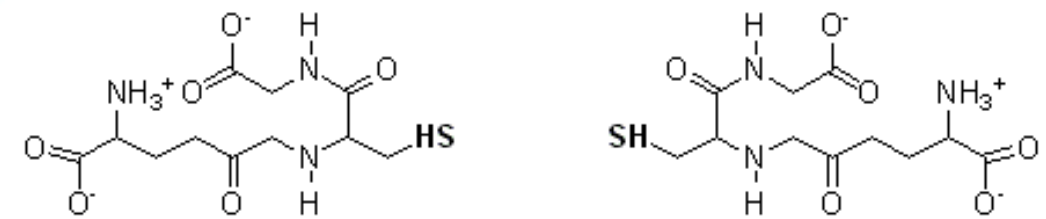
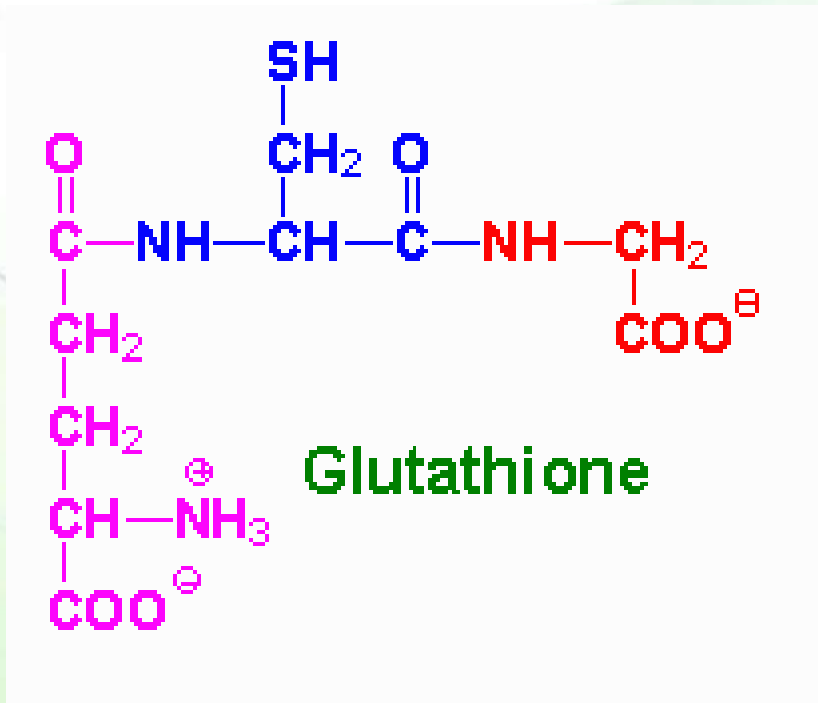


Tryptophan 2,3-dioxygenase

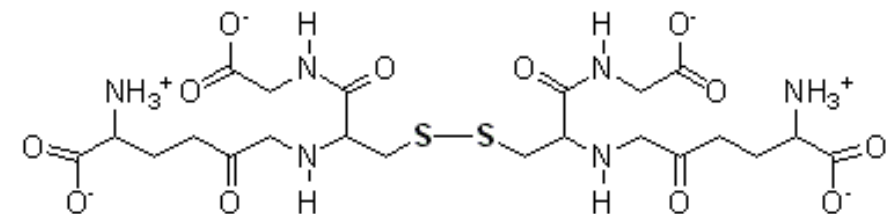
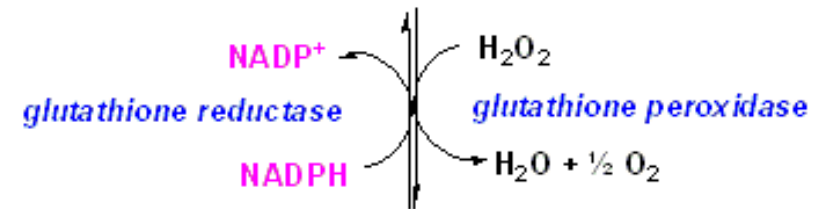
1d. Peroxidases



- Peroxidases catalyze oxidation of a substrate by hydrogen peroxide.
- Example: oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide:



2 x reduced glutathione (GSH)

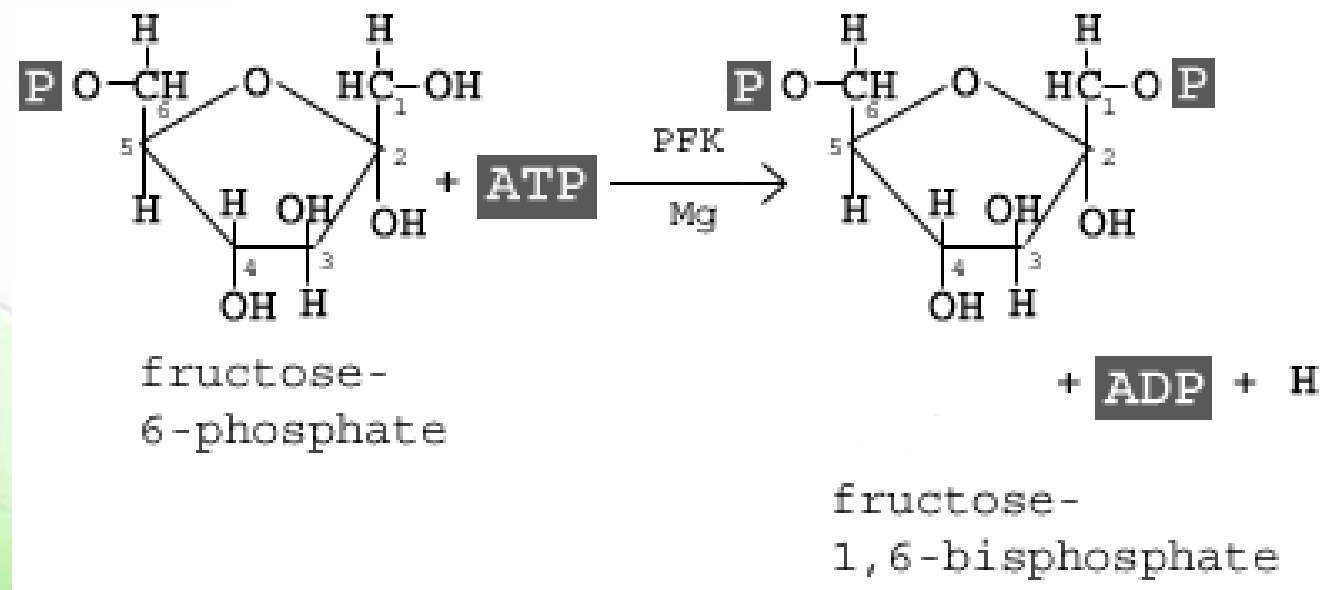


oxidized glutathione (GSSG)

2. Transferases



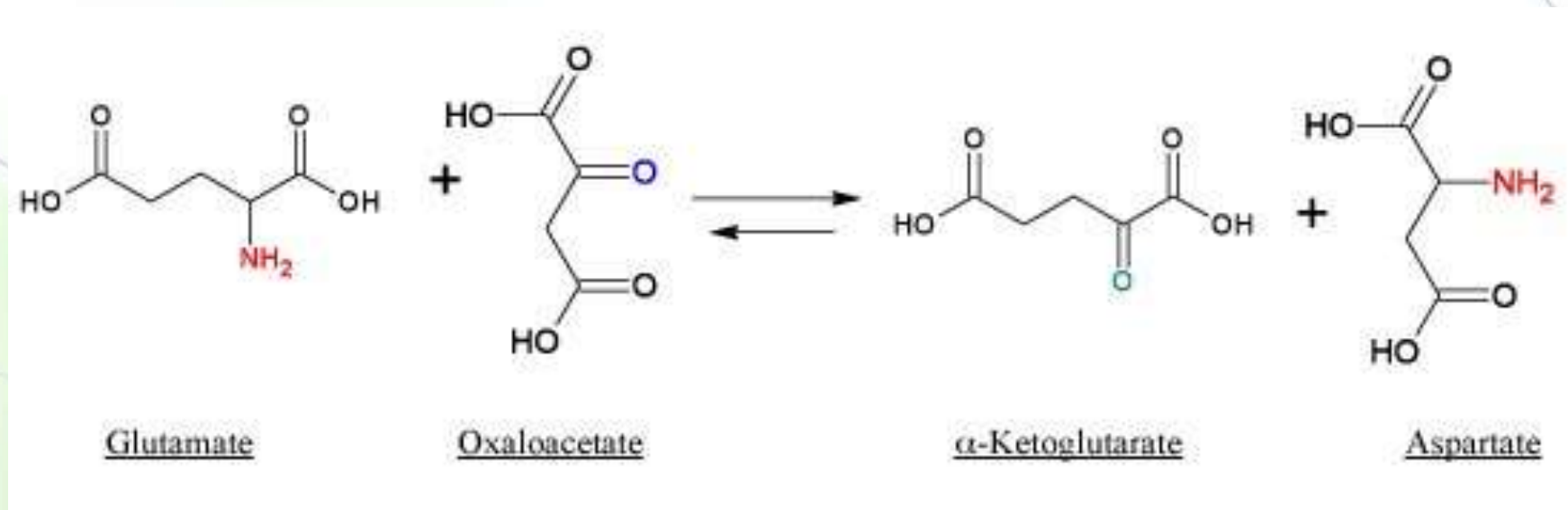
- These enzymes transfer a functional group (C, N, P, or S) from one substrate to an acceptor molecule.
- Example: Kinases (the transferred group is a phosphate)
 - Phosphofructokinase catalyzes the transfer of phosphate from ATP to fructose-6-phosphate:



Another example: transaminases



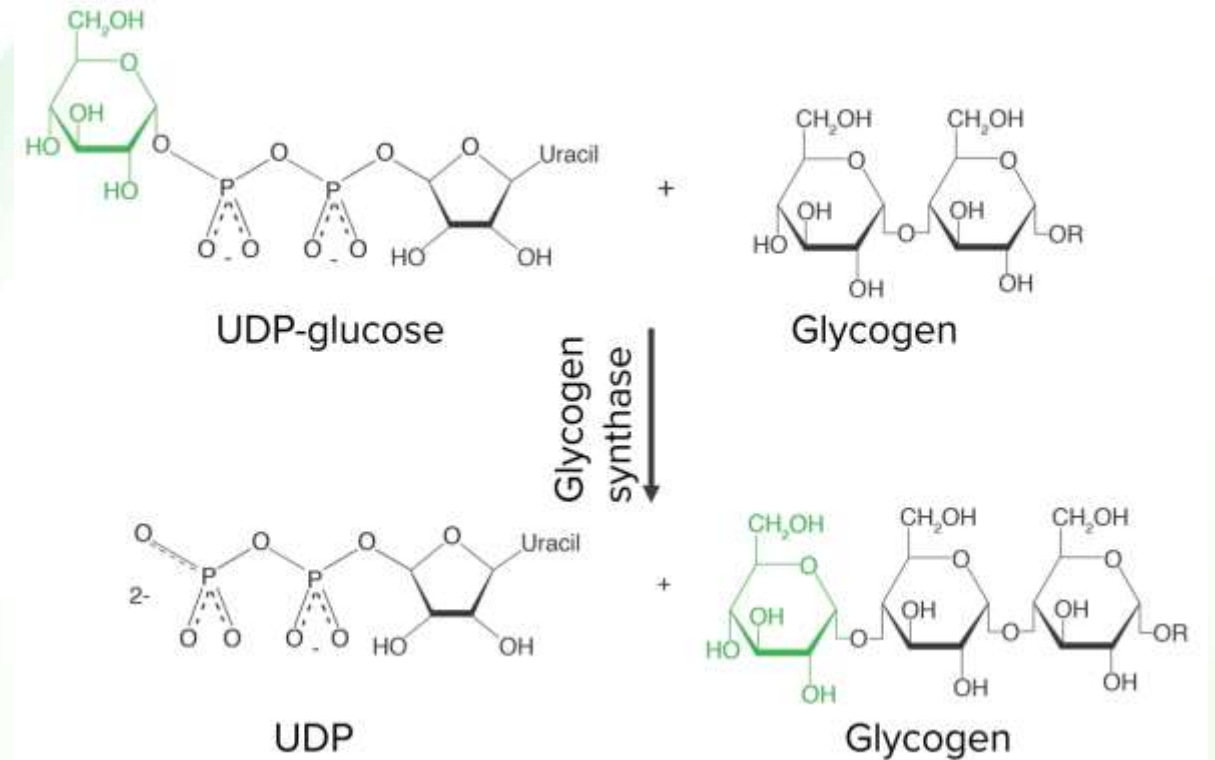
- A transaminase transfers an amino functional group from one amino acid to a keto acid, converting the amino acid to a keto acid and the keto acid to an amino acid.
 - Interconversion of certain amino acids.
 - Aspartate transaminase:



A third example: synthases



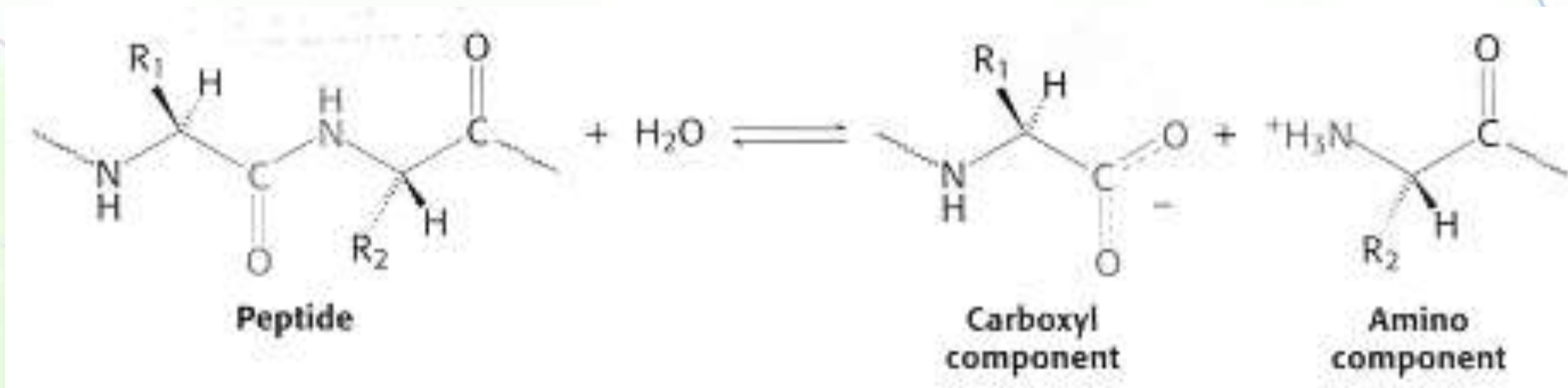
- When the synthesized compound is physiologically important, the transferase may be called a synthase.
- For example, glycogen synthase, whose systematic name is UDP-glucose-glycogen glycosyltransferase, transfers a glucosyl residue from uridine diphosphate (UDP)-glucose to the end of a glycogen molecule elongating glycogen by one glucose residue.



3. Hydrolases



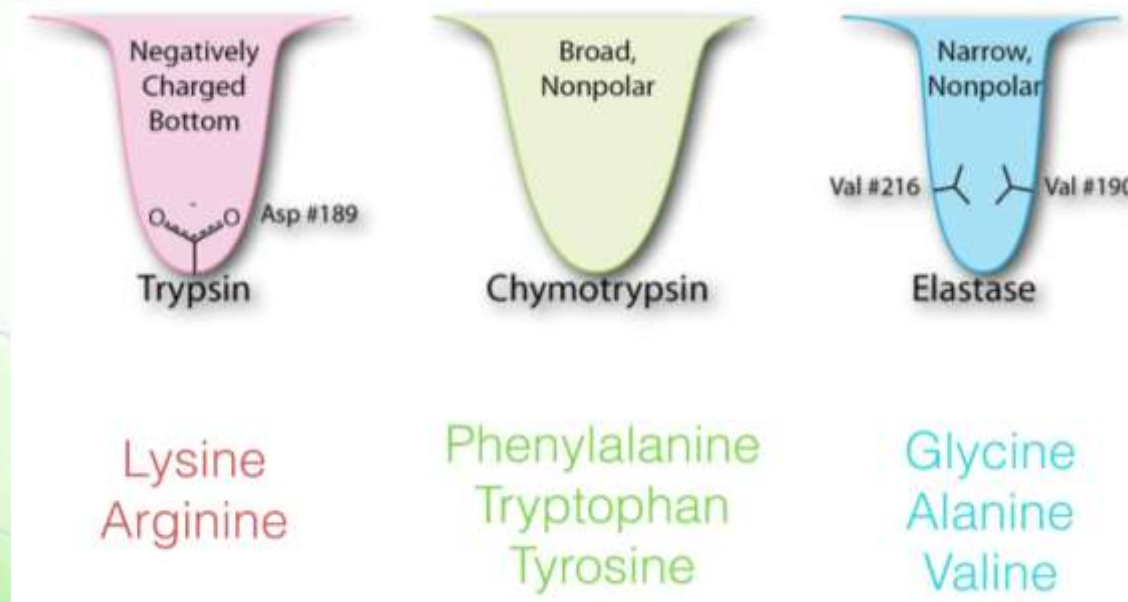
- They catalyze cleavage reactions using water across the bond in the form of OH^- and H^+ to being broken or the fragment condensations.
- Proteases, esterases, lipases, glycosidases, and phosphatases are hydrolases named depending on the type of bond cleaved.
- Example: proteases
 - A class of hydrolytic enzymes is proteases that catalyze proteolysis, the hydrolysis of a peptide bond within proteins.



Specific examples: digestive enzymes



- Proteolytic enzymes differ in their degree of substrate specificity.
- Trypsin breaks up peptide bonds on the carboxyl side of only **Lys and Arg**.
- Chymotrypsin hydrolyzes peptide bonds involving **bulky aromatic amino acids**.
- Elastase hydrolyzes peptide bonds involving small, uncharged groups such as **Ala, Val, or Gly**.

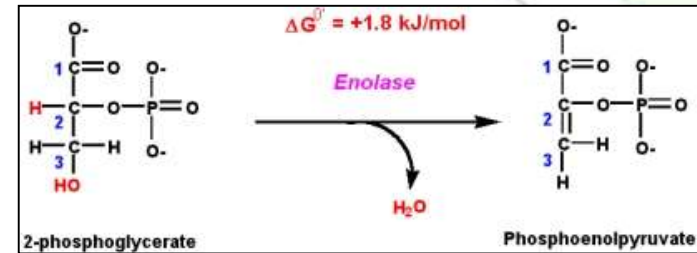


4. Lyases

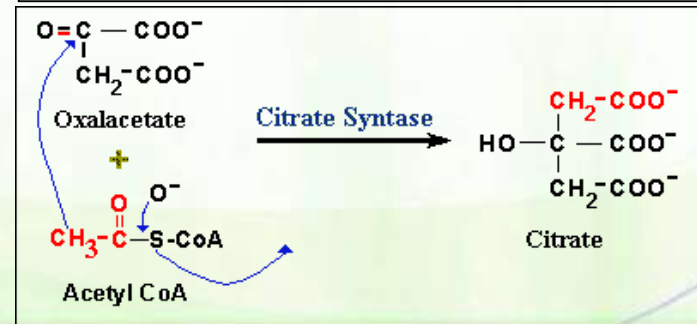
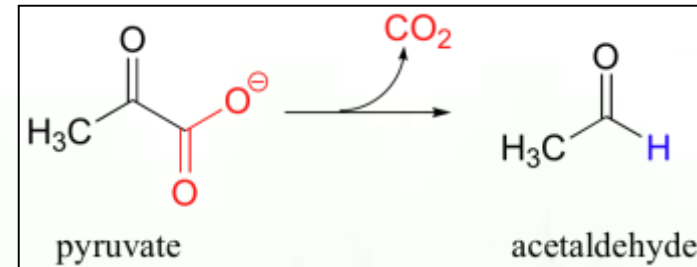


- Lyases cleave C-C, C-O, C-N, and other bonds by means other than hydrolysis or oxidation, leaving double bonds or rings, or conversely adding groups to double bonds without hydrolysis.

- Dehydrases:** Removal of H_2O from the substrate to give a double bond
 - Example: enolase
- Decarboxylases:** Replacement of a carboxyl group by a hydrogen
 - Example: pyruvate decarboxylase
- Synthases:** Addition of a small molecule to a double bond or when the direction of the reaction favors the formation of a carbon-carbon bond
 - Example: citrate synthase



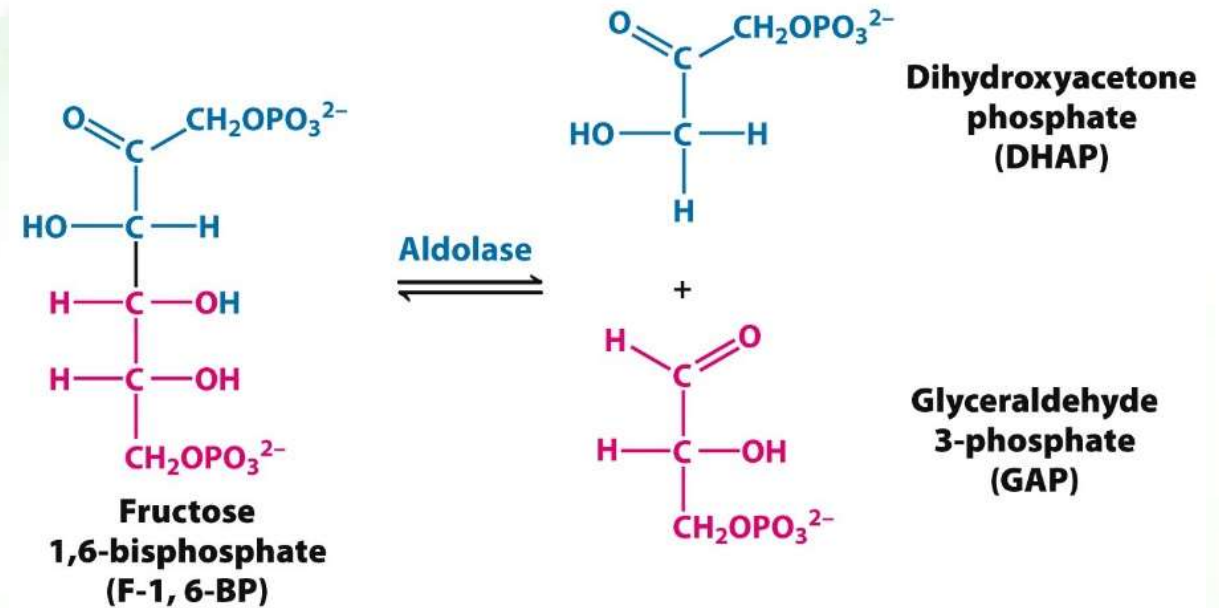
("en" = **ene** for the C=C and "ol" is for alcohol)



Example: aldolase



- Aldolase breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate.

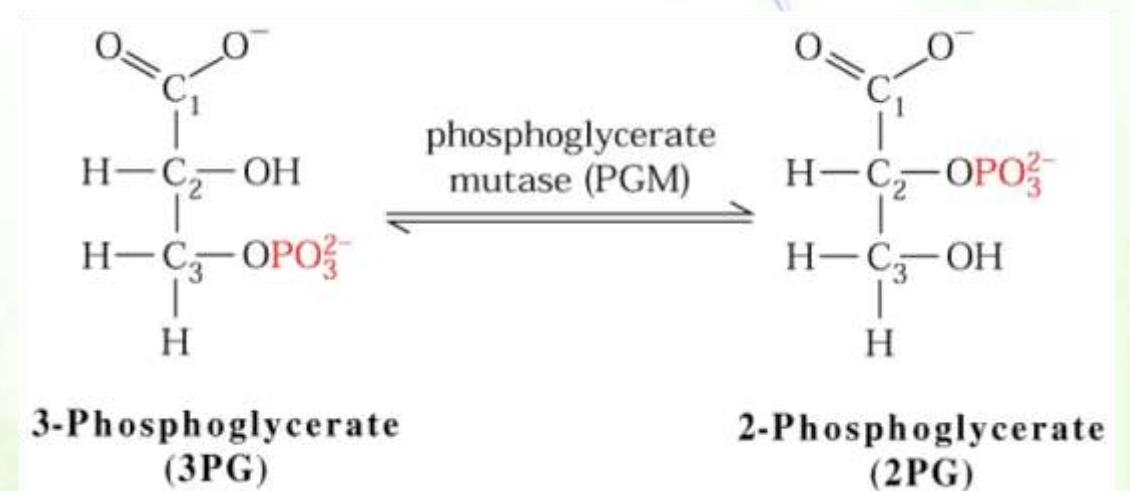
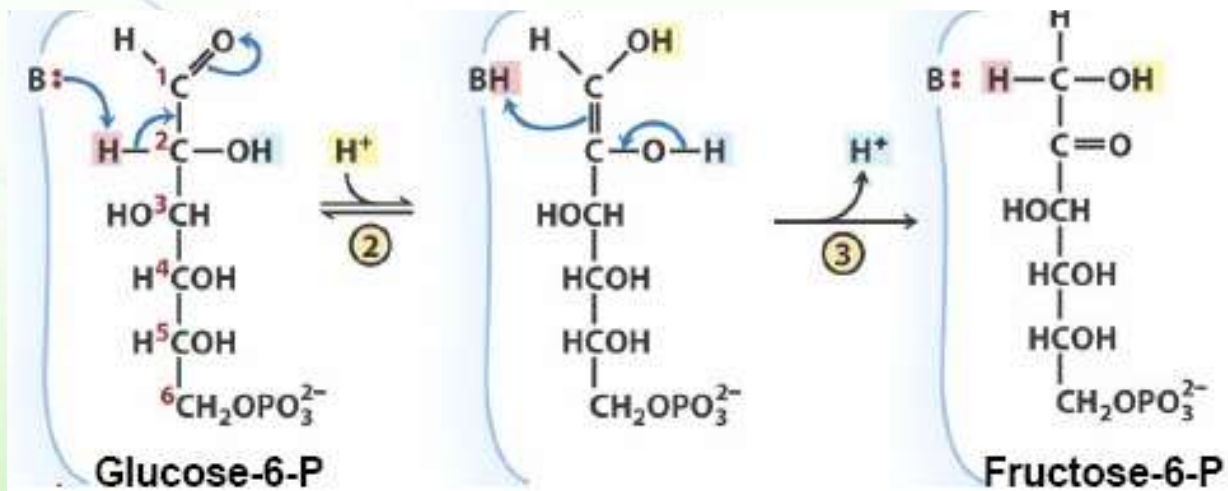


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5. Isomerases



- These enzymes catalyze intramolecular rearrangements.
- Enzymes that rearrange the bond structure of a compound are called isomerases, whereas enzymes that catalyze the movement of phosphate from one atom to another are called mutases.
 - Phosphoglucoisomerase isomerizes glucose-6-phosphate to fructose-6-phosphate.
 - Phosphoglycerate mutase transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate:

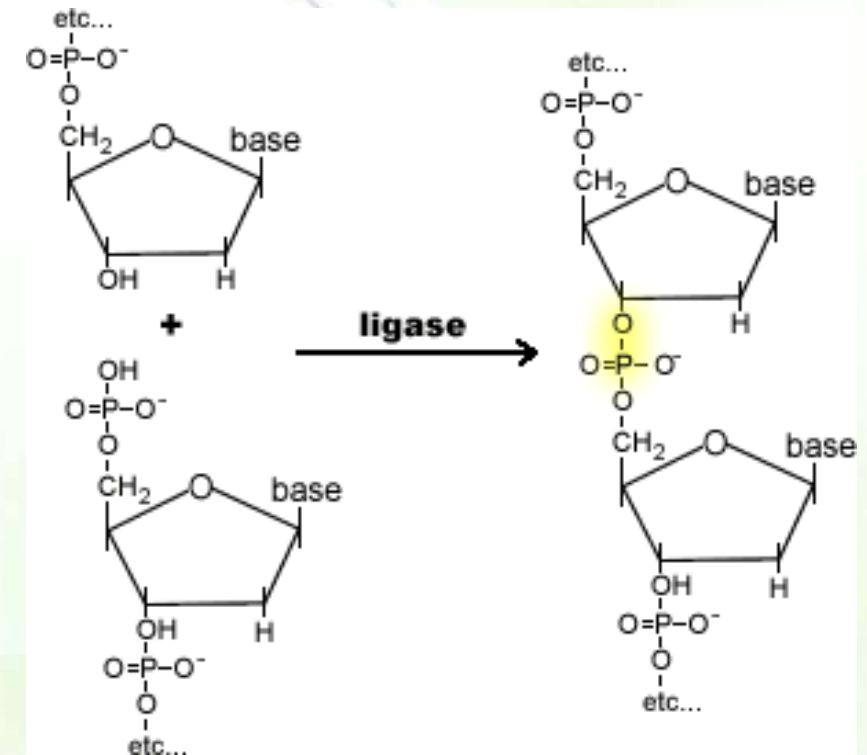
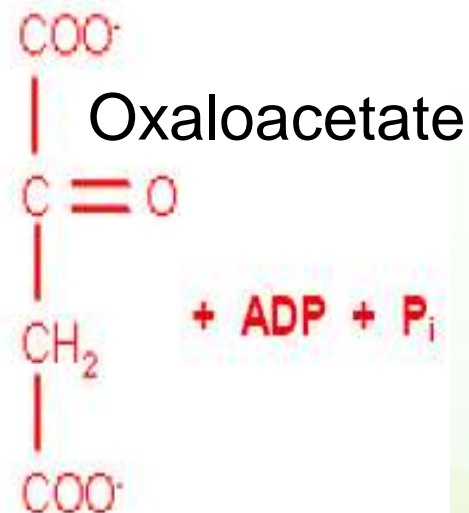
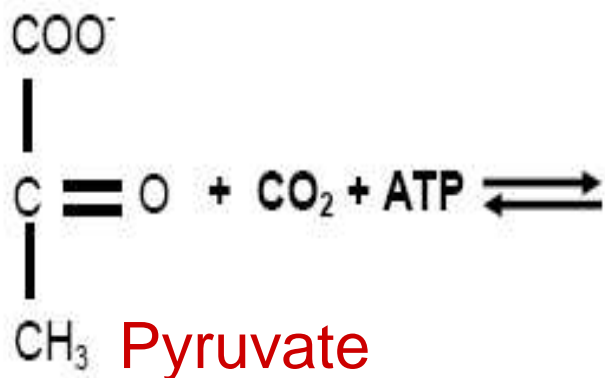


6. Ligases



- Ligases join C-C, C-O, C-N, C-S, and C-halogen bonds.
- The reaction is usually accompanied by the consumption of a high-energy compound such as ATP and other nucleoside triphosphates.
- Synthetases derive the energy from the cleavage of high-energy phosphate bonds.
 - Synthases use a different source of energy.

Pyruvate carboxylase



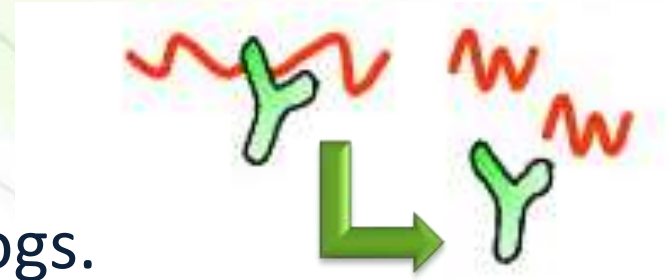


Somethings are just different

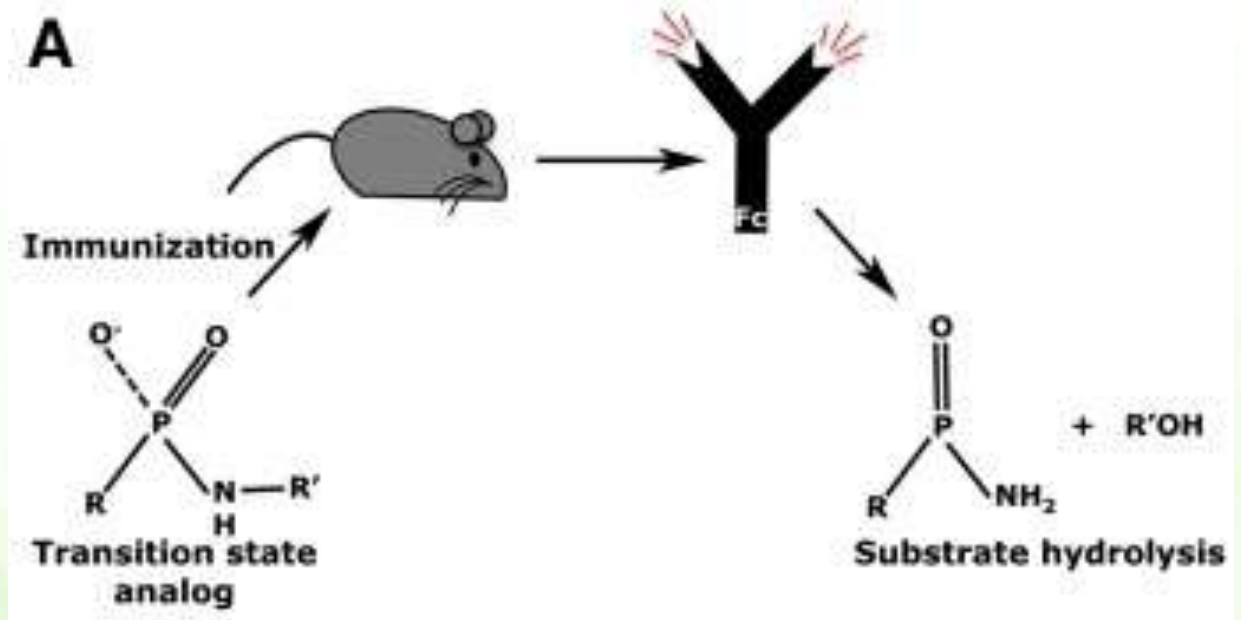
Abzymes



- Abzymes are antibodies acting as enzymes.
- They are produced against transition-state analogs.
- How? A host animal is injected with a transition-state analogue. The animal makes antibodies against it (binding with high affinity at specific binding points mimicking an enzyme's active site surrounding a transition state).



Abzymes with activity similar to cocaine esterase, which degrades cocaine, have been developed against analogs of its transition-state complex. Monthly injections are used to treat addicted individuals by destroying cocaine in the blood and, thereby, decreasing their dependence on cocaine.



Ribozymes



- Most enzymes are proteins, but RNA molecules can act as enzymes, too.
- Ribozymes are enzymes made of both protein and RNA.
- For some, catalysis is performed by RNA.
 - Examples include those involved in RNA splicing and protein synthesis in ribosomes.
- The catalytic efficiency of RNAs is less than that of protein enzymes, but can be enhanced and stabilized by the presence of protein subunits.

