Enzymes I
Characteristics and classification

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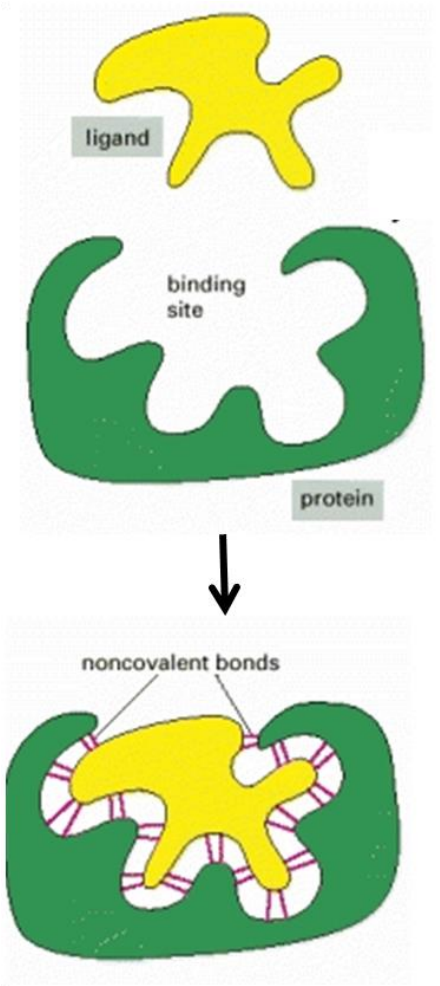
General properties of proteins

- 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.

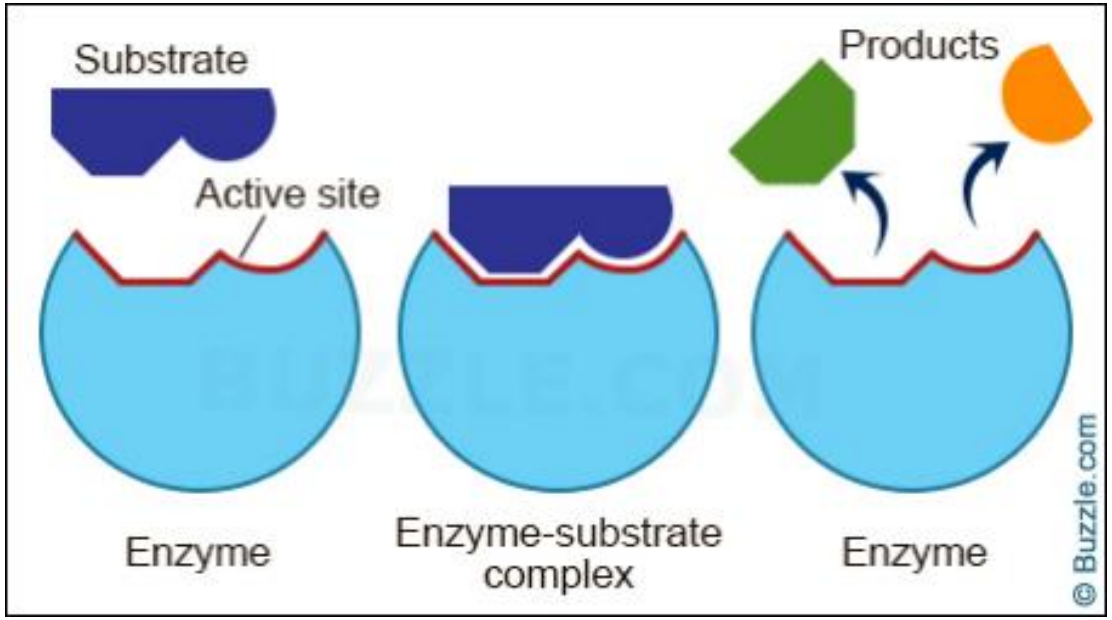


- Two properties of a protein characterize its interaction with ligands:
  - Affinity: the strength of binding between a protein and other molecules.
  - Specificity: the ability of a protein to bind one molecule in preference to other molecules.

What are enzymes?

- Enzymes: Specialized **proteins** that accelerate (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 completion of the reaction, they revert to their original structure.

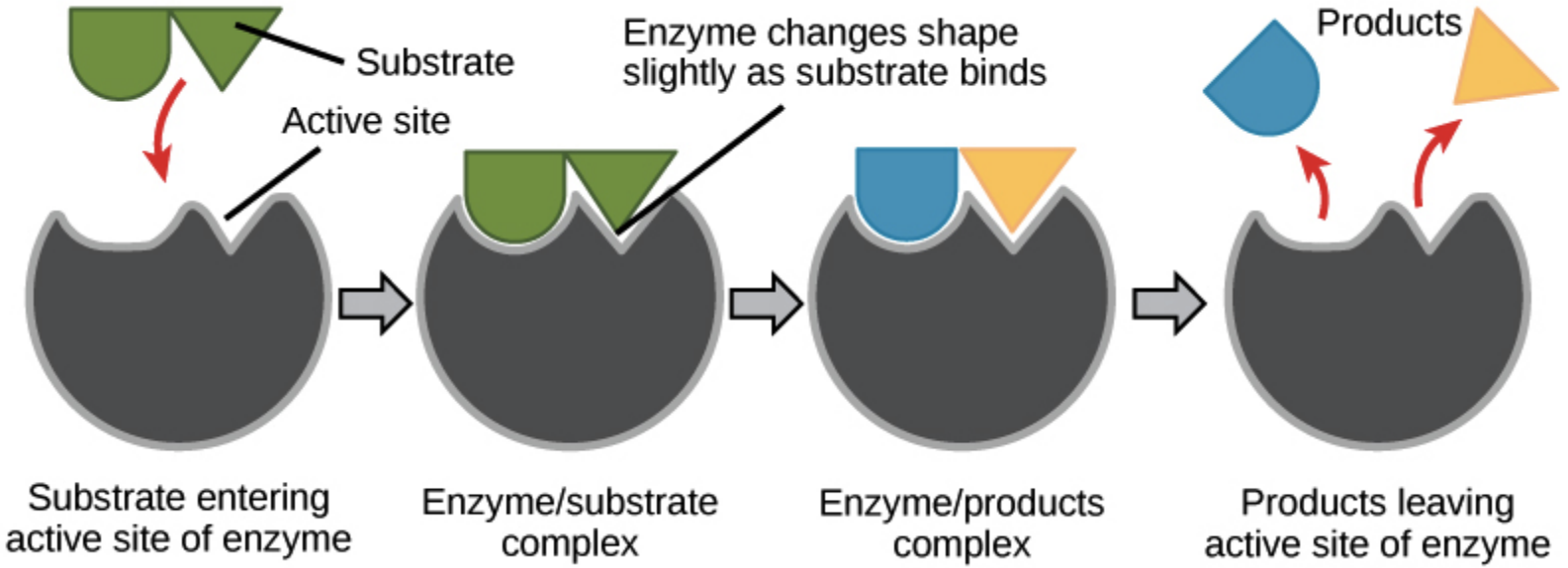


How can we express an enzymatic reaction?

- Simple expression of enzymatic reaction:

$E + S \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$ For further simplicity: $E + S \leftrightarrows ES \leftrightarrows E + P$

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)

Do not memorize

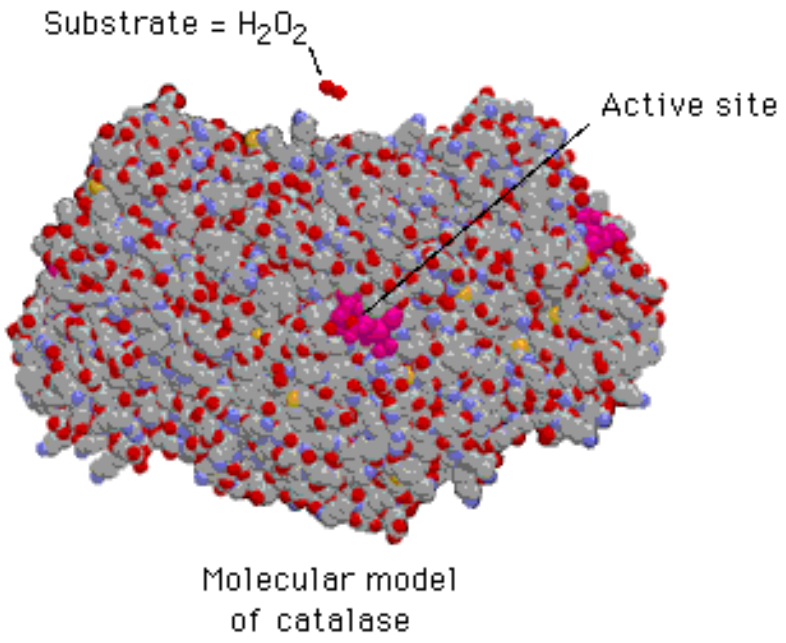
  - Carbonic anhydrase: $CO_2 + H_2O \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$
    - One enzyme molecule hydrates 10^6 molecules of CO_2 per second (versus 1 per 10 seconds for uncatalyzed reactions)
  - Catalase: $2\,H_2O_2 \xleftrightarrow{\text{Catalase}} 2\,H_2O + O_2\,(g)$

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

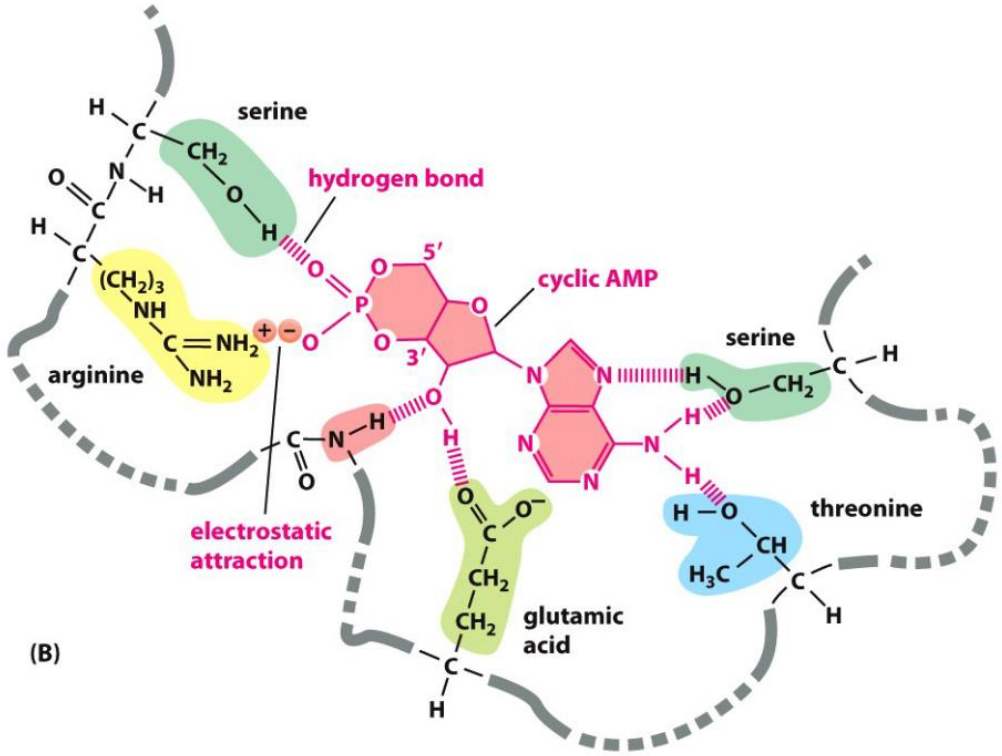
Do not memorize

Where does the reaction occur?

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

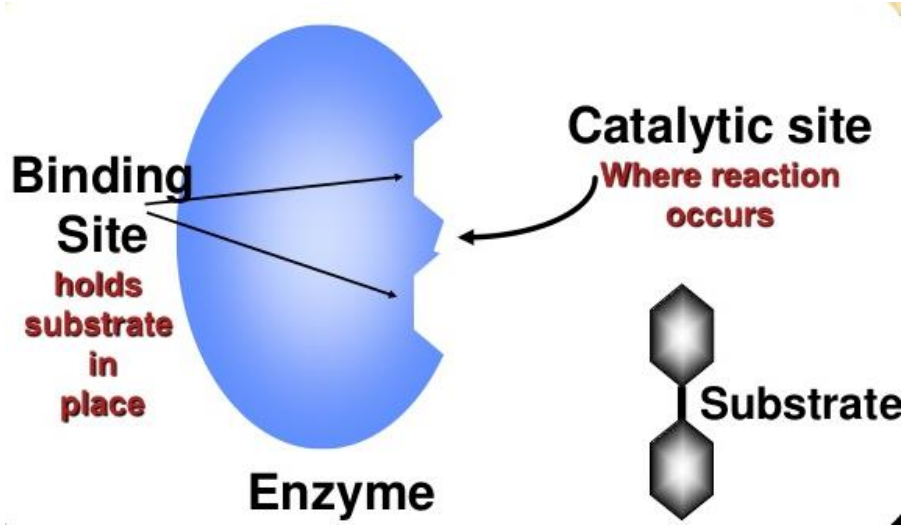


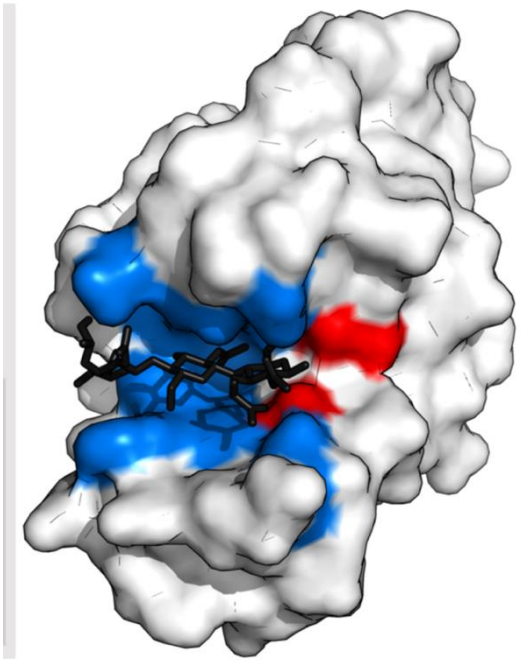
- The active site contains a specialized amino acid sequence that facilitates the reaction.



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.



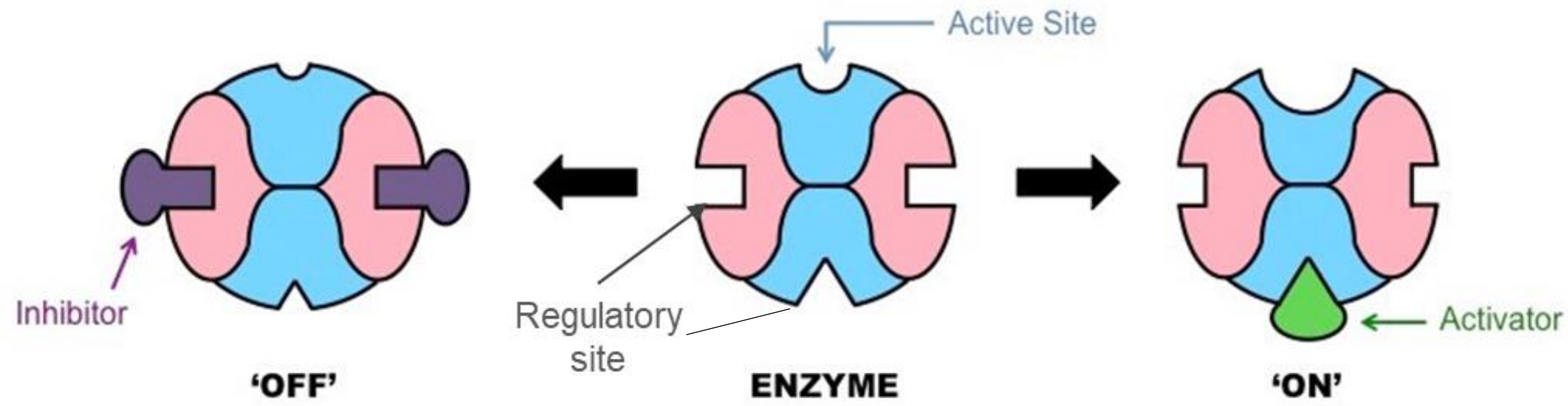


ACTIVE SITE

BINDING SITES	CATALYTIC SITE
Bind and orient substrate(s)	Reduce chemical activation energy

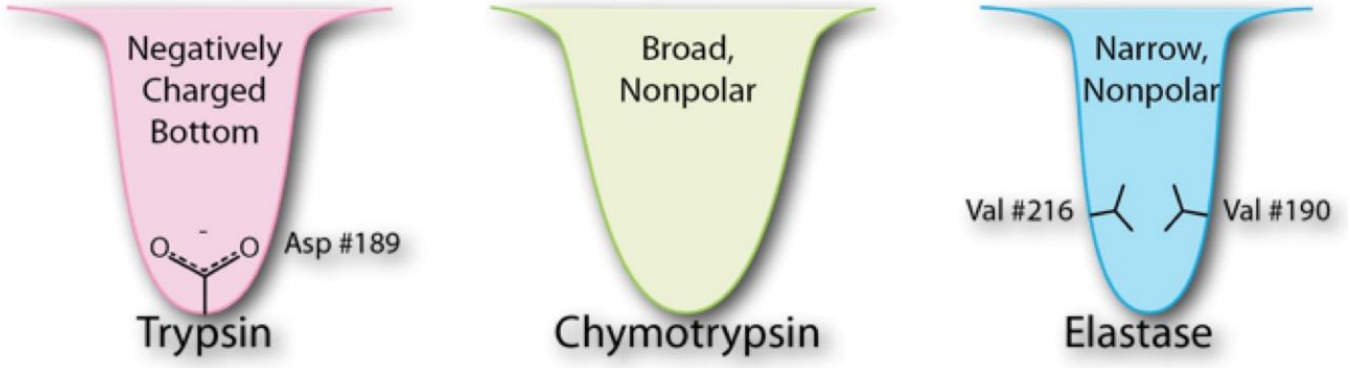
A regulatory site(s) can also be present.

- Binding of a substrate into the active site can be regulated by a *regulatory site* that is bound by a regulator (activator or inhibitor) of either the catalytic activity of the enzyme or the binding of the substrate.



Binding specificity

- The specificity of enzymes is due to their precise interaction of active sites with their substrates.



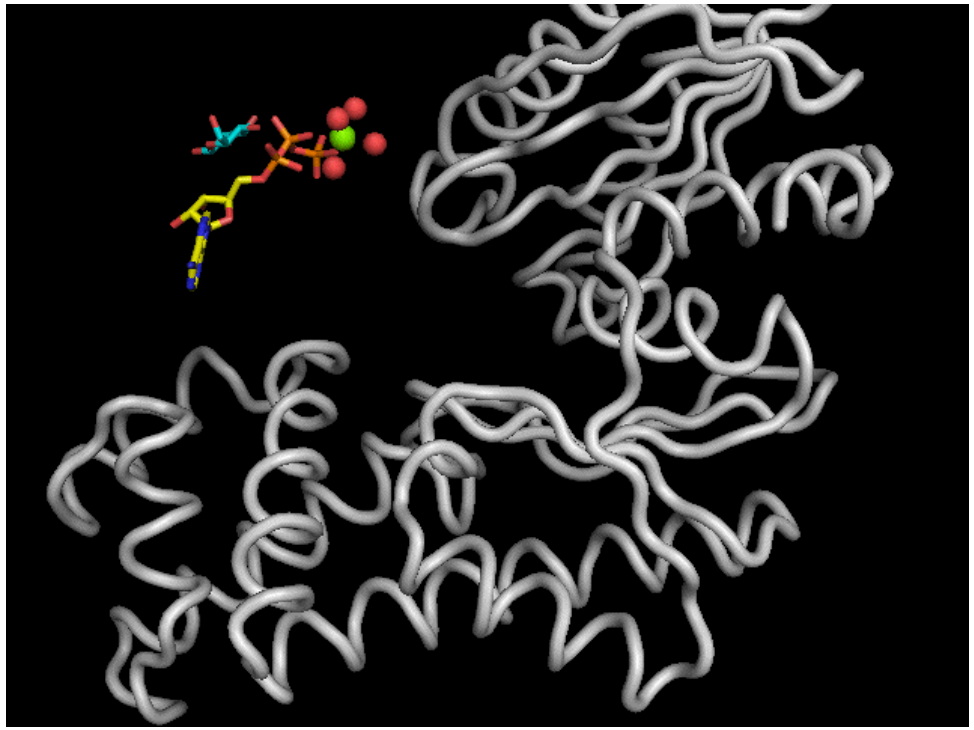
Lysine
Arginine

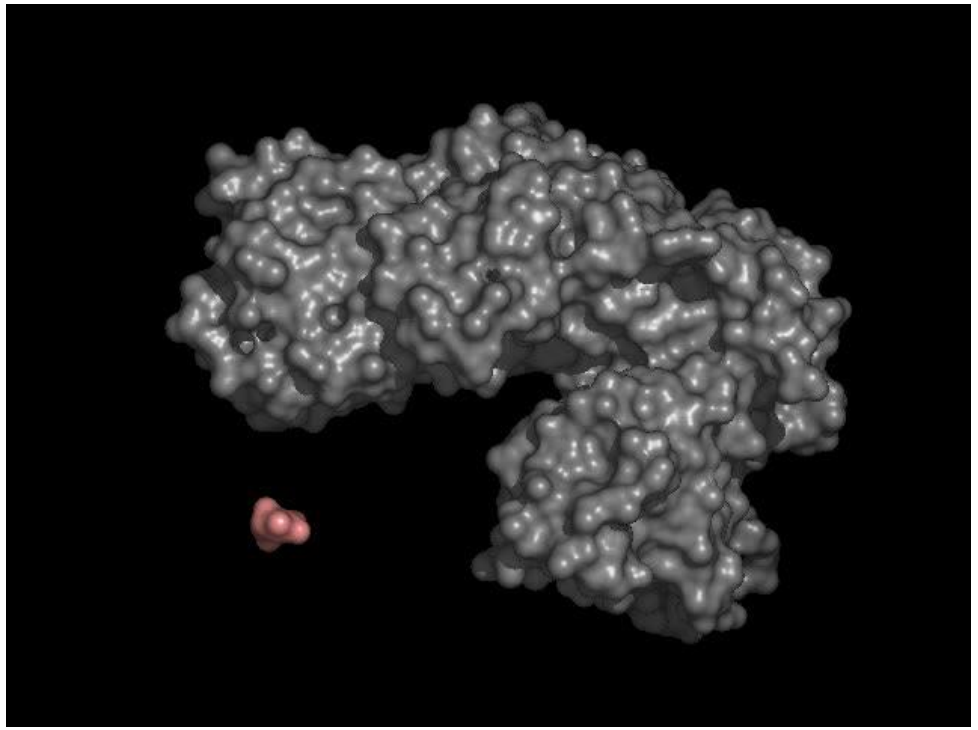
Phenylalanine
Tryptophan
Tyrosine

Glycine
Alanine
Valine

Features of active site 1

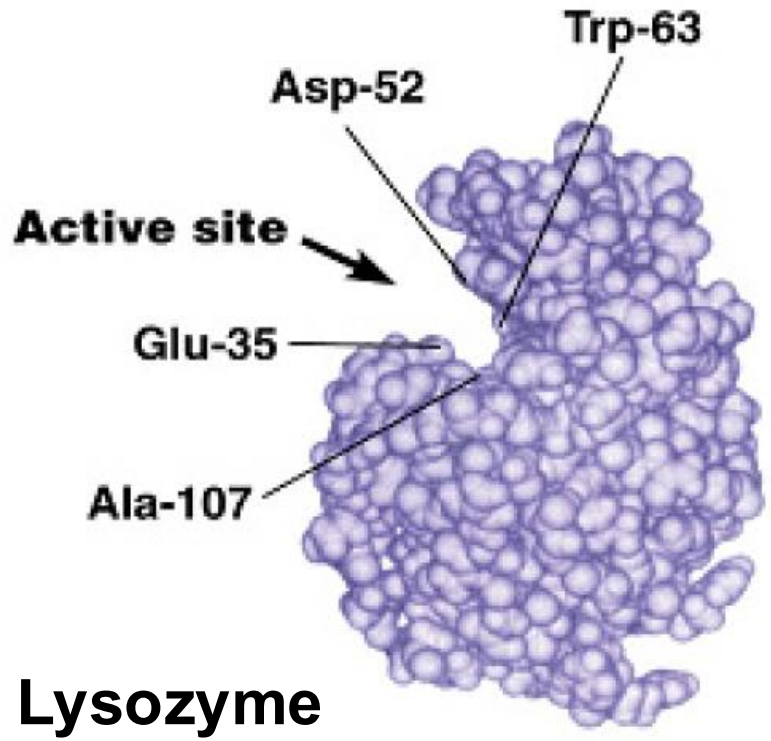
- It is internal relative to the enzyme and looks like a canal





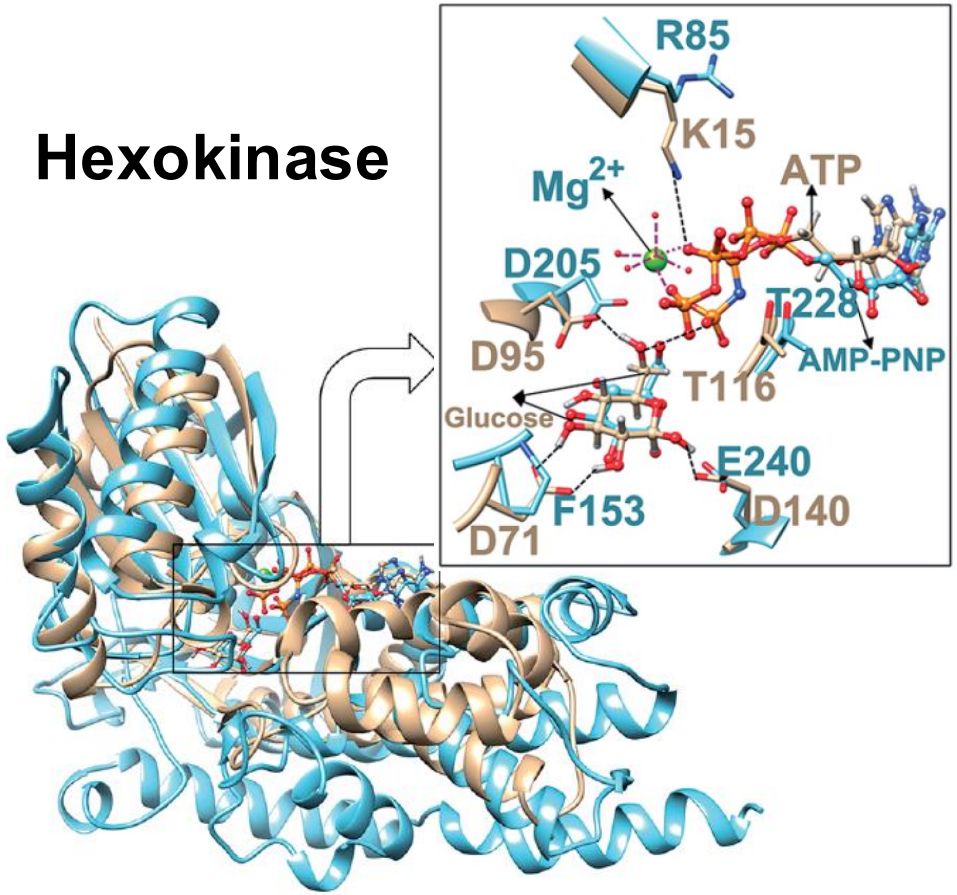
Features of active site 2

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



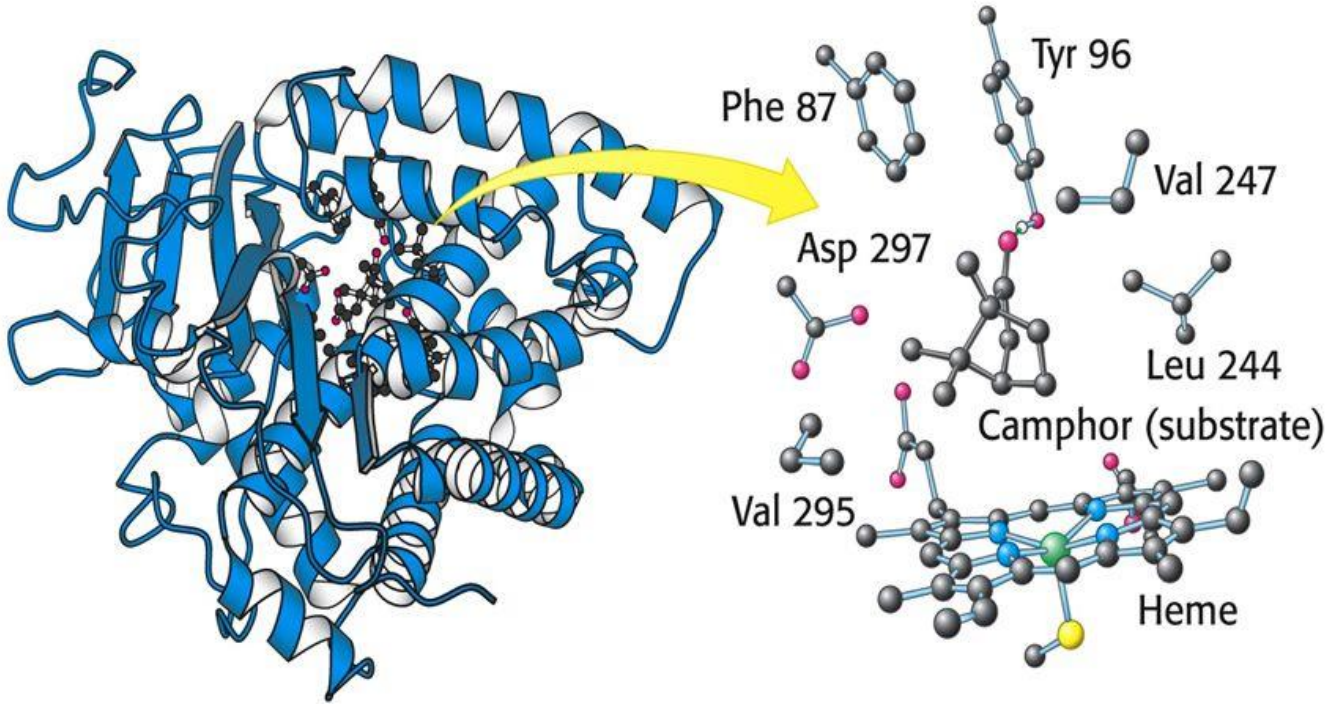
Lysozyme

Hexokinase



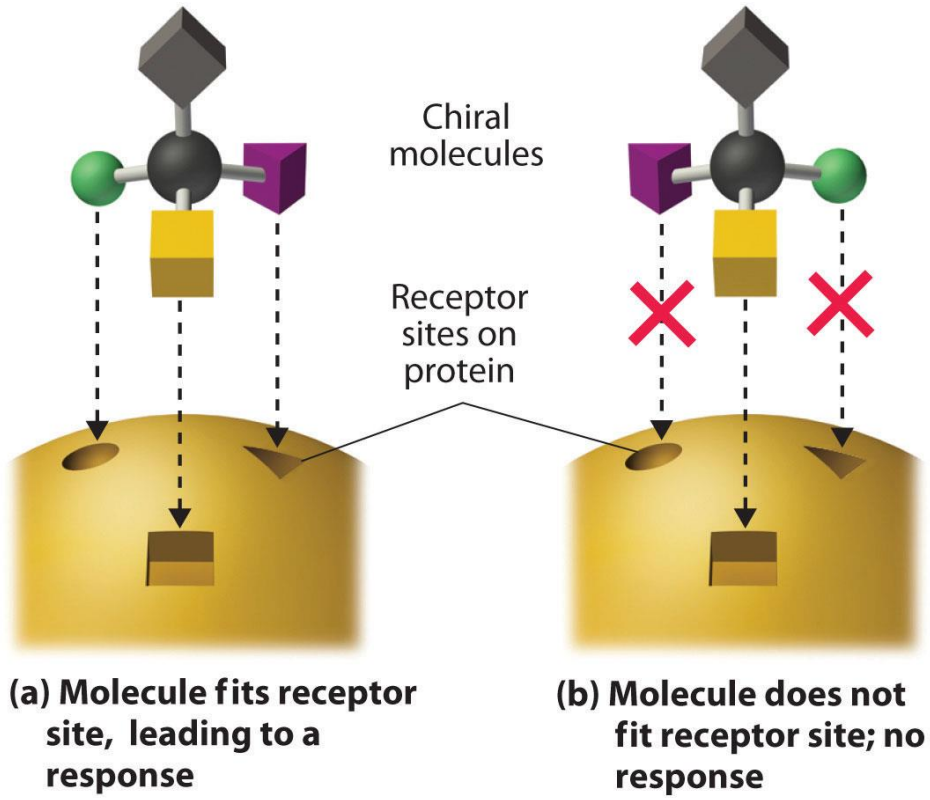
Features of active site 3

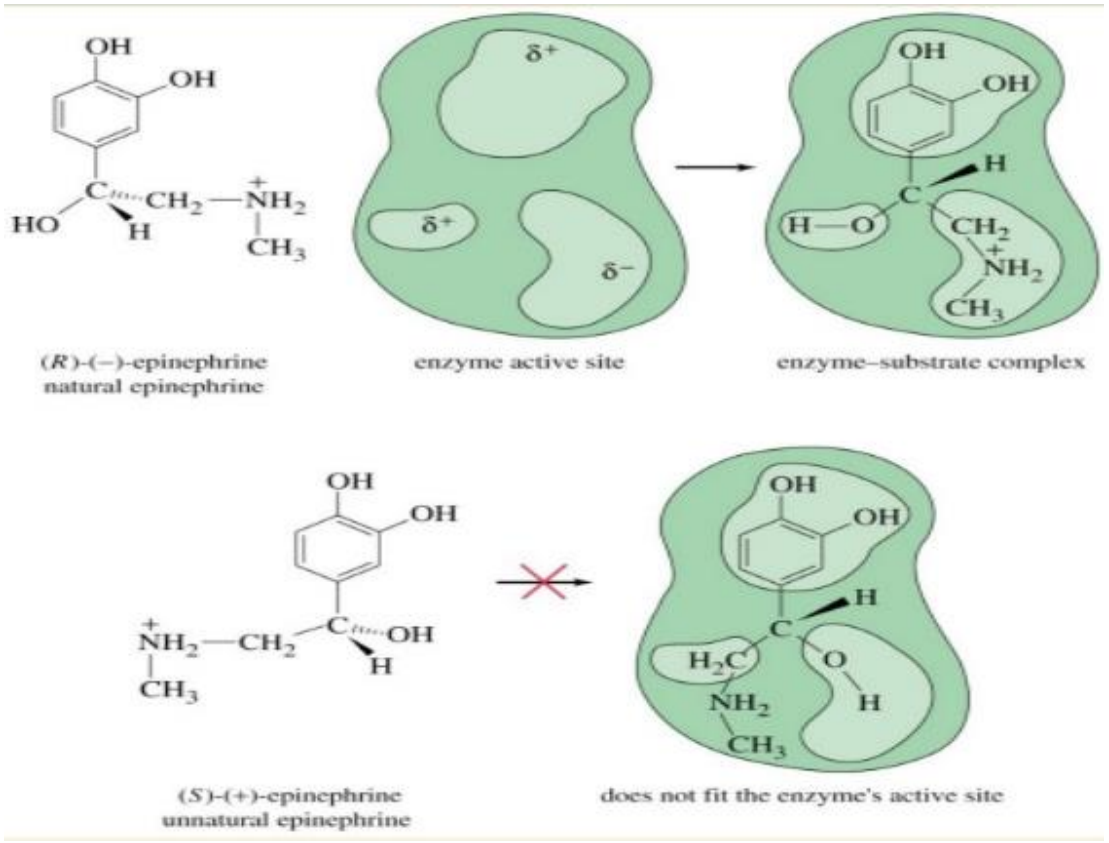
- The amino acid residues can be nonpolar and polar.
  - Water is usually excluded unless it is part of the reaction.
- Substrates bind to enzymes by multiple weak attractions.



Features of active site 4

- Binding of substrates to active sites occurs at minimally three points.
  - Chirality determines specificity.

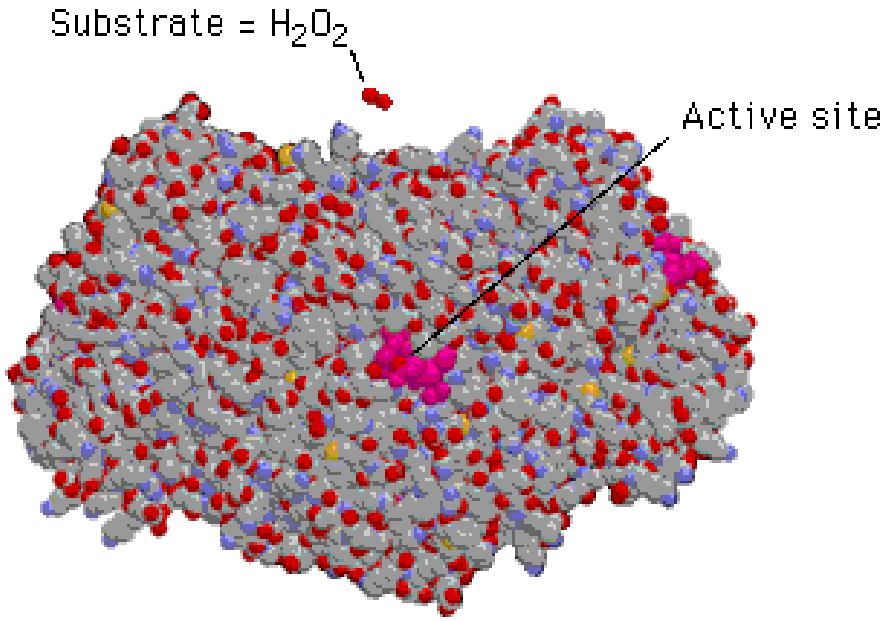




Features of active site 5

- 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.



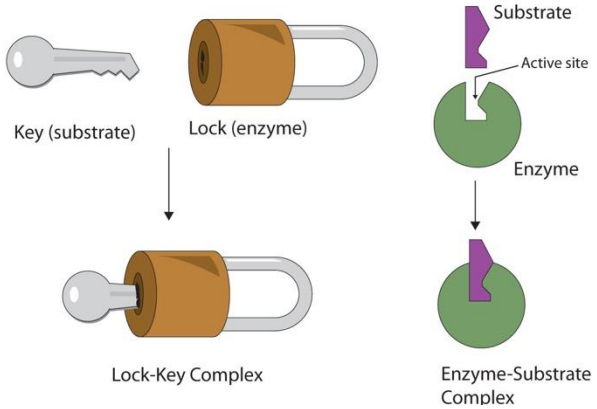
Molecular model of catalase

How do substrates fit into the active site of enzymes?

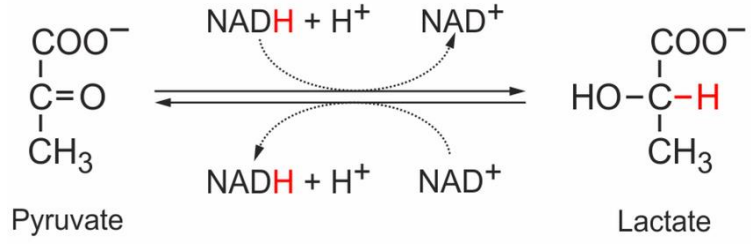
The two models

Lock-and-key model

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

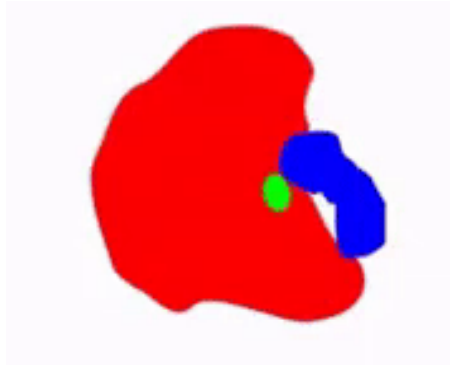


- Lactate dehydrogenase

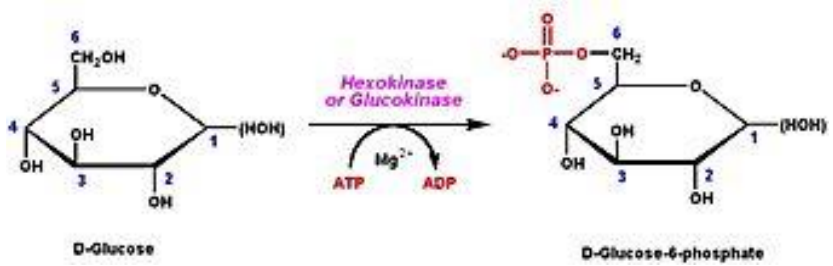


Induced fit model *(more favored)*

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



- Hexokinase



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 to occur.

Free energy (G) and reaction types

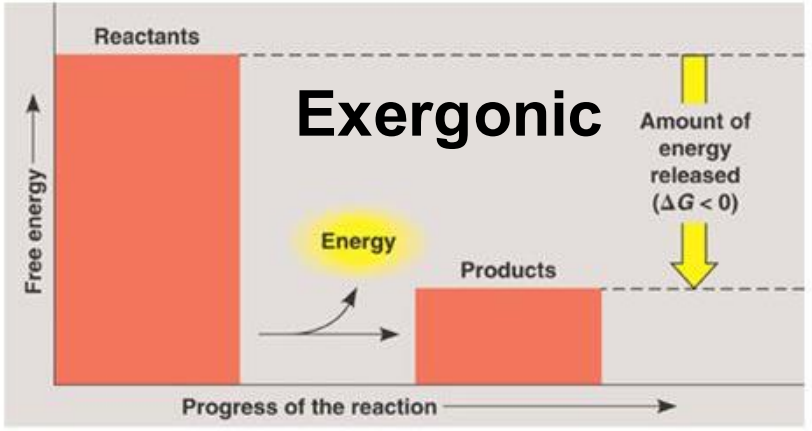
- The difference between the free energy values between reactants and products (free-energy change ΔG):

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

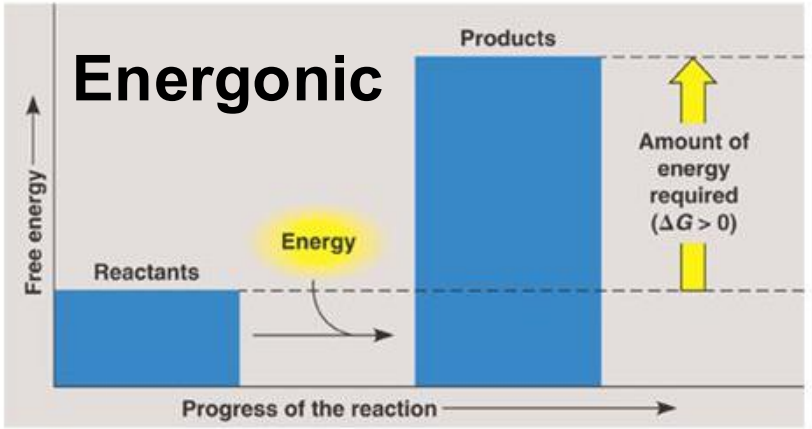
- Reactions are exergonic, endergonic, or at equilibrium.

*Bond Energies, *D*, (kJ/mol)	
C—H	414
C—C	348
C—N	293
C—O	351

Do not memorize



(a) Exergonic reaction: energy released



(b) Endergonic reaction: energy required

What does it mean?

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

- If ΔG is negative, $G_{products}$ is less than $G_{reactants}$, energy is not needed to drive the reaction, but is released, making the forward reaction (from left to right) spontaneous (the reaction is called exergonic).
- If ΔG is positive, $G_{products}$ is more than $G_{reactants}$, 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.

	Exergonic	Endergonic
What happens to the energy?	Released	Absorbed
ΔG	Negative	Positive
Energy of reactants	Higher than products	Lower than products
Input of energy	Not required	Required
Spontaneity	Spontaneous/favorable	Unspontaneous/unfavorable
Exothermic or endothermic	Exothermic	Endothermic

Spontaneous vs. instantaneous

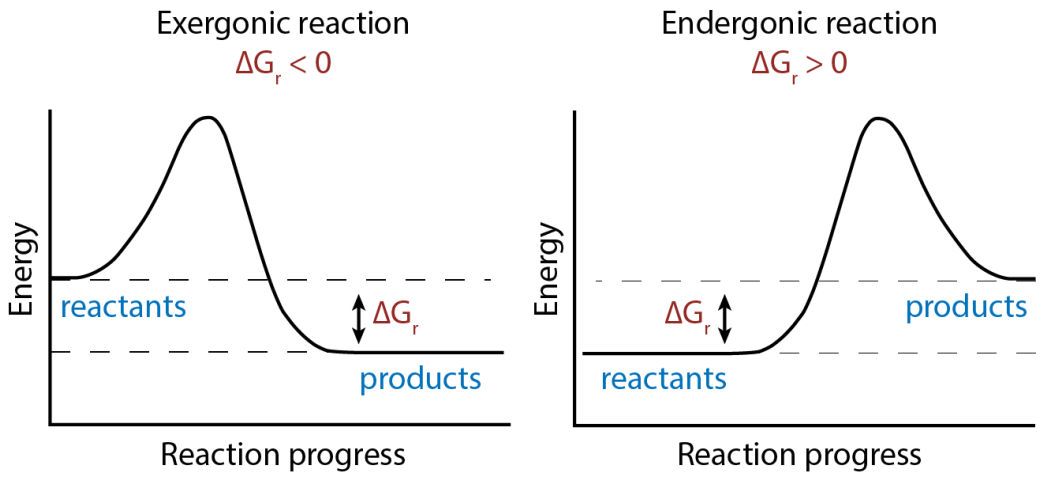
- Spontaneous means naturally occurring, and instantaneous means occurring right away.
  - The term spontaneous in chemistry has nothing to do with how fast or slow a reaction or process takes place. It concerns the direction in which a reaction or process *can* change, given a set of starting conditions.
- The simplest examples are phase changes, such as water going between liquid and solid. For water, we can write:

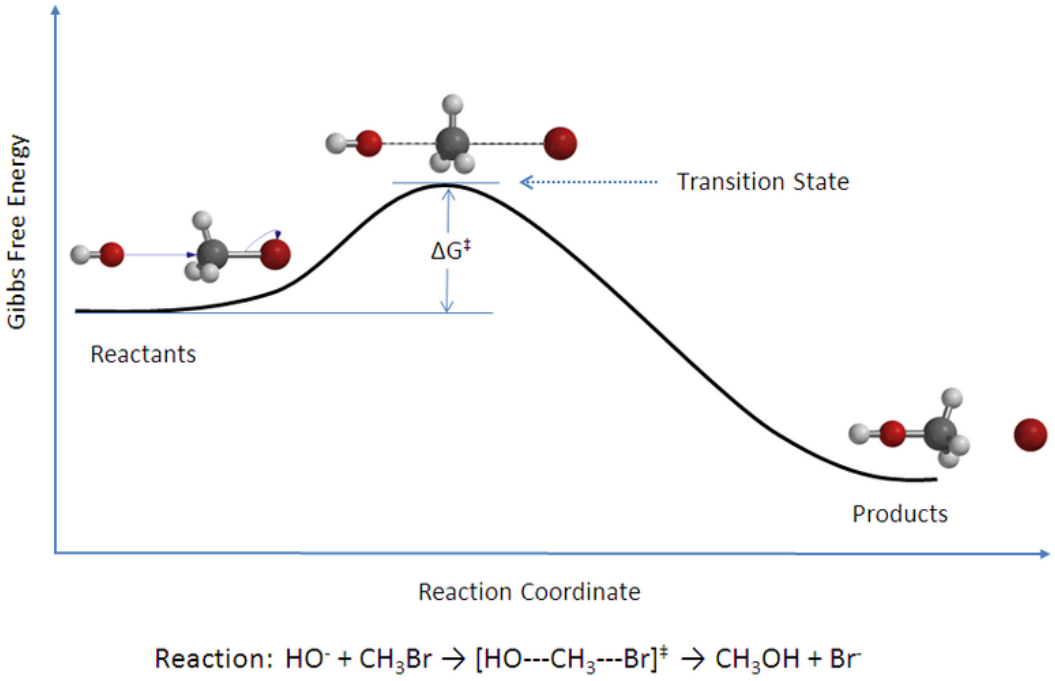
$$H_2O_{liquid} \rightleftarrows H_2O_{solid}$$

- Below 0°C, the equilibrium lies to the right, so we would say the left-to-right process is spontaneous, whereas the right-to-left process is non-spontaneous.
- Above 0°C, the right-to-left process is spontaneous, whereas the left-to-right process is non-spontaneous.
- At the phase transition temperature, 0°C, left and right are equally favored, so the reaction is at equilibrium.

Transition state

- Any reaction goes through a transition state molecule (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.
  - The configuration of the transition state molecule is most unstable and has the highest activation energy.

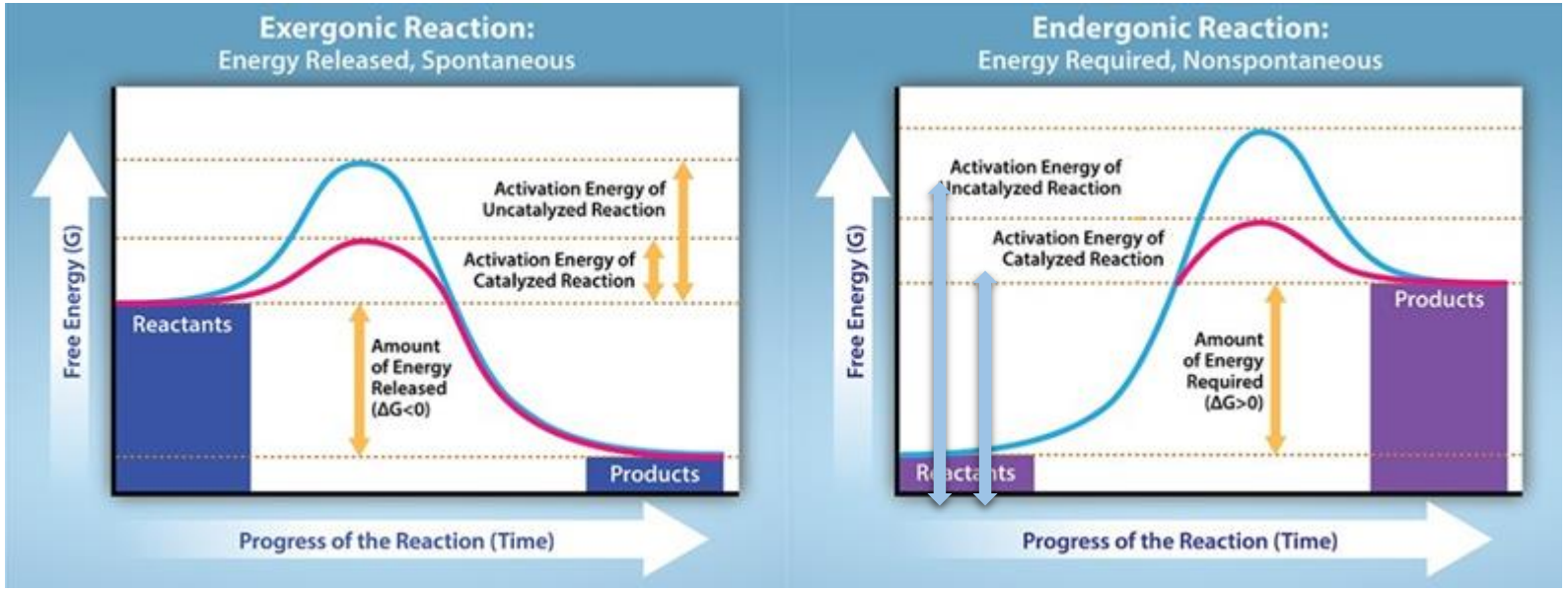


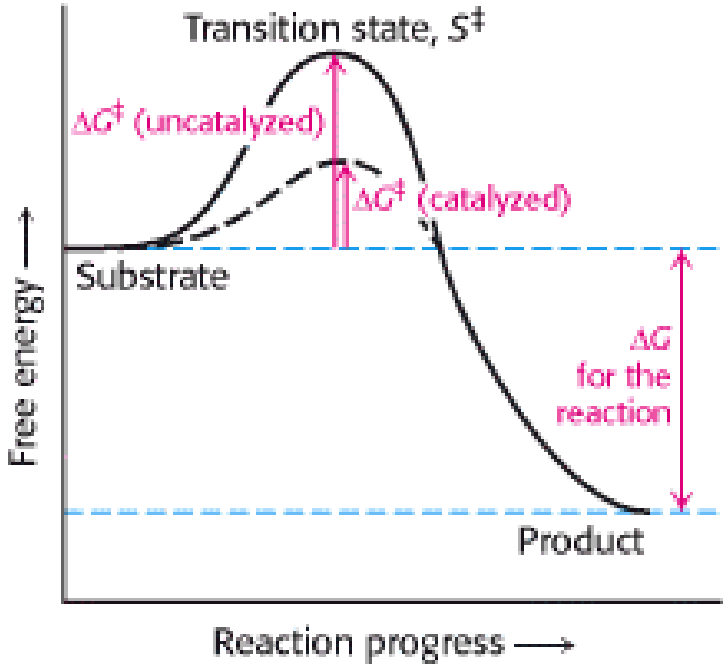


The free energy of the transition state is higher than that of the substrate or the product.

What do enzymes do?

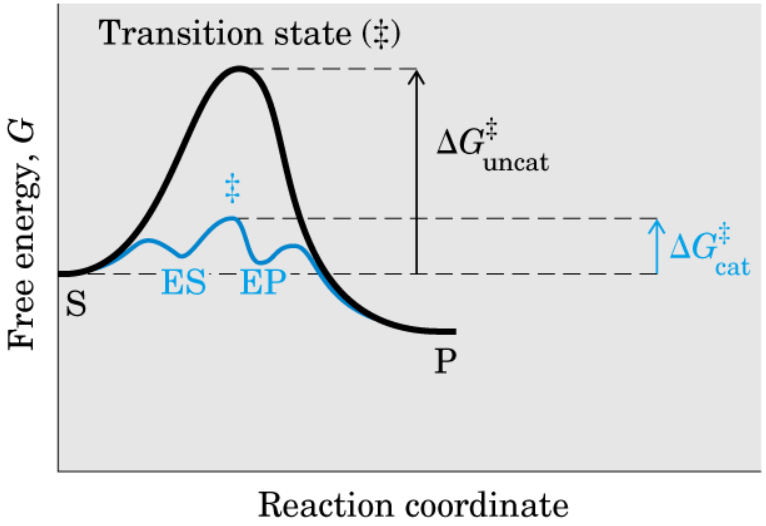
- Enzymes lower the activation energy of the transition state or, in other words, enzymes facilitate the formation of a transition state at a lower energy.

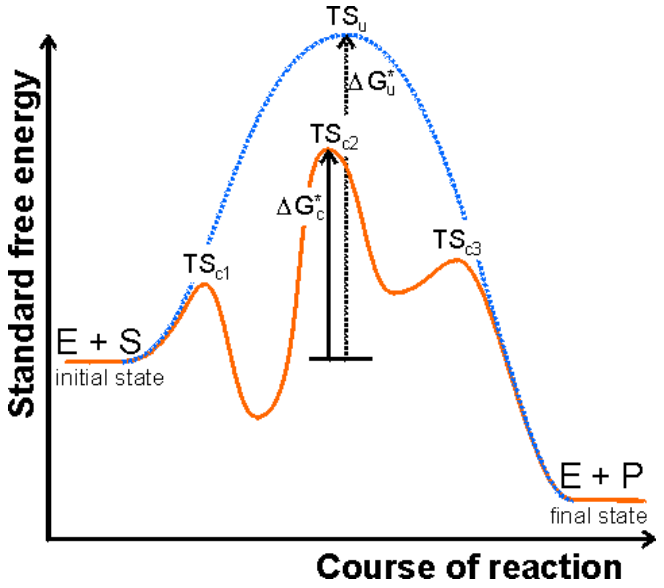




Alternative pathways

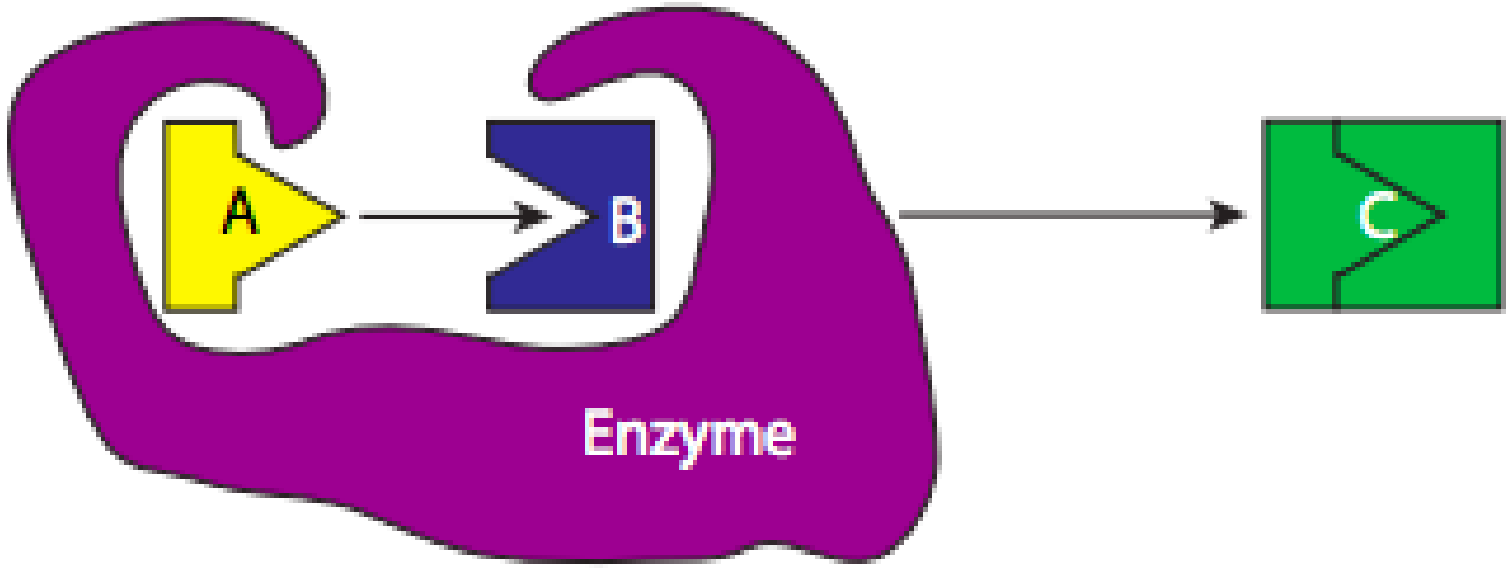
- Substrates often undergo several transformations when associated with the enzyme and each form has its own free energy value.
- The activation energy corresponds to the intermediate with the highest free energy.
- The energy of activation does not enter into the final ΔG calculation for a reaction.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





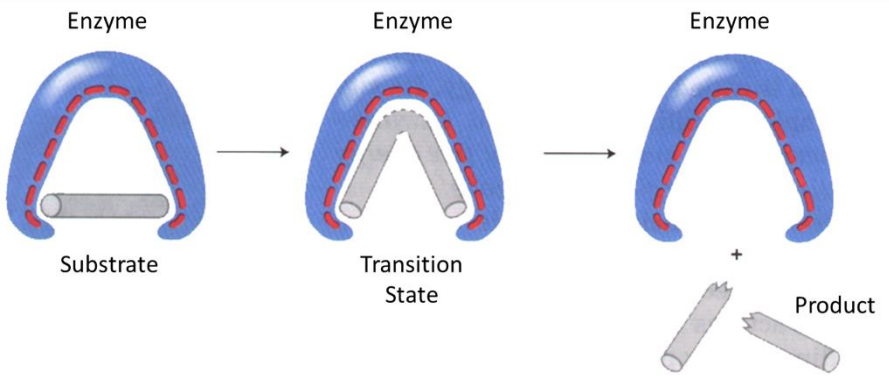
How do enzymes catalyze reactions?

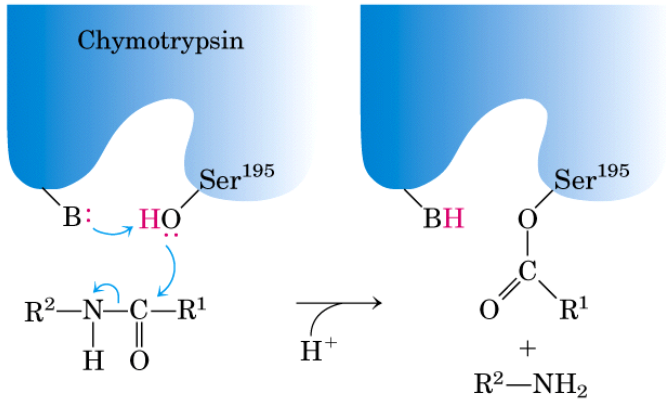
- Enzymes place substrates proximal to each other.
- Enzymes orient the substrates inside the active site in the best possible fit.



Catalytic strategies

- Enzymes use different strategies to catalyze reactions such as:
  - Straining the bonds within the substrates, making them easier to break
  - Driving acid/base reactions, usually involving a proton donor/acceptor such as histidine, serine, etc.
  - Covalent catalysis, where the substrate is covalently linked to an amino acid side chain at the active site of the enzyme during the course of the reaction.
  - Metal-Ion Catalysis, where enzymes contain metal ions to drive catalysis.
  - Cofactor Catalysis, where a cofactor is needed for an enzyme to form a covalent bond with the substrate during the course of the reaction.

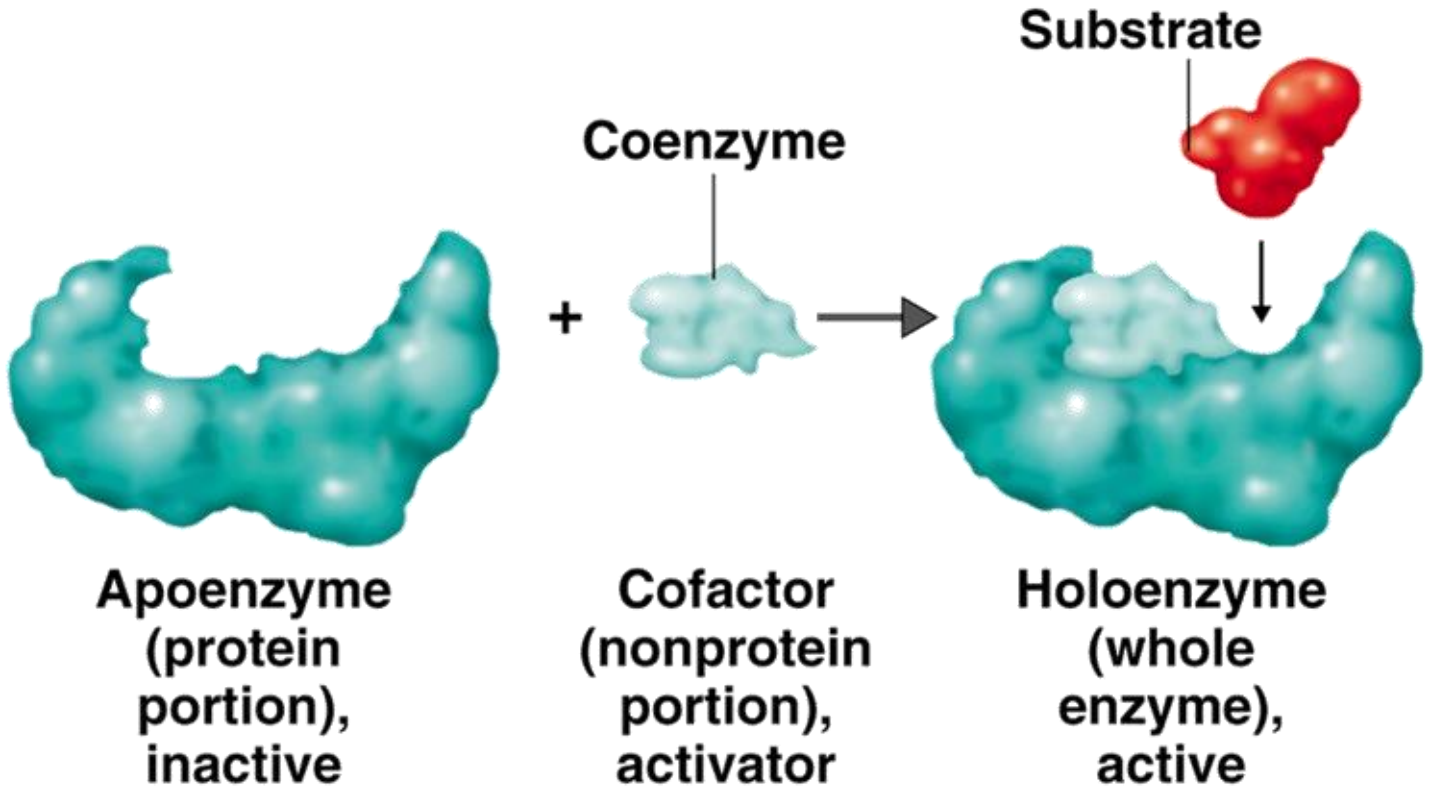




Classification of enzymes

Enzyme Classification (structure)

- Simple vs. complex (conjugated)
- Holoenzyme (catalytically active) vs. apoenzyme (inactive)

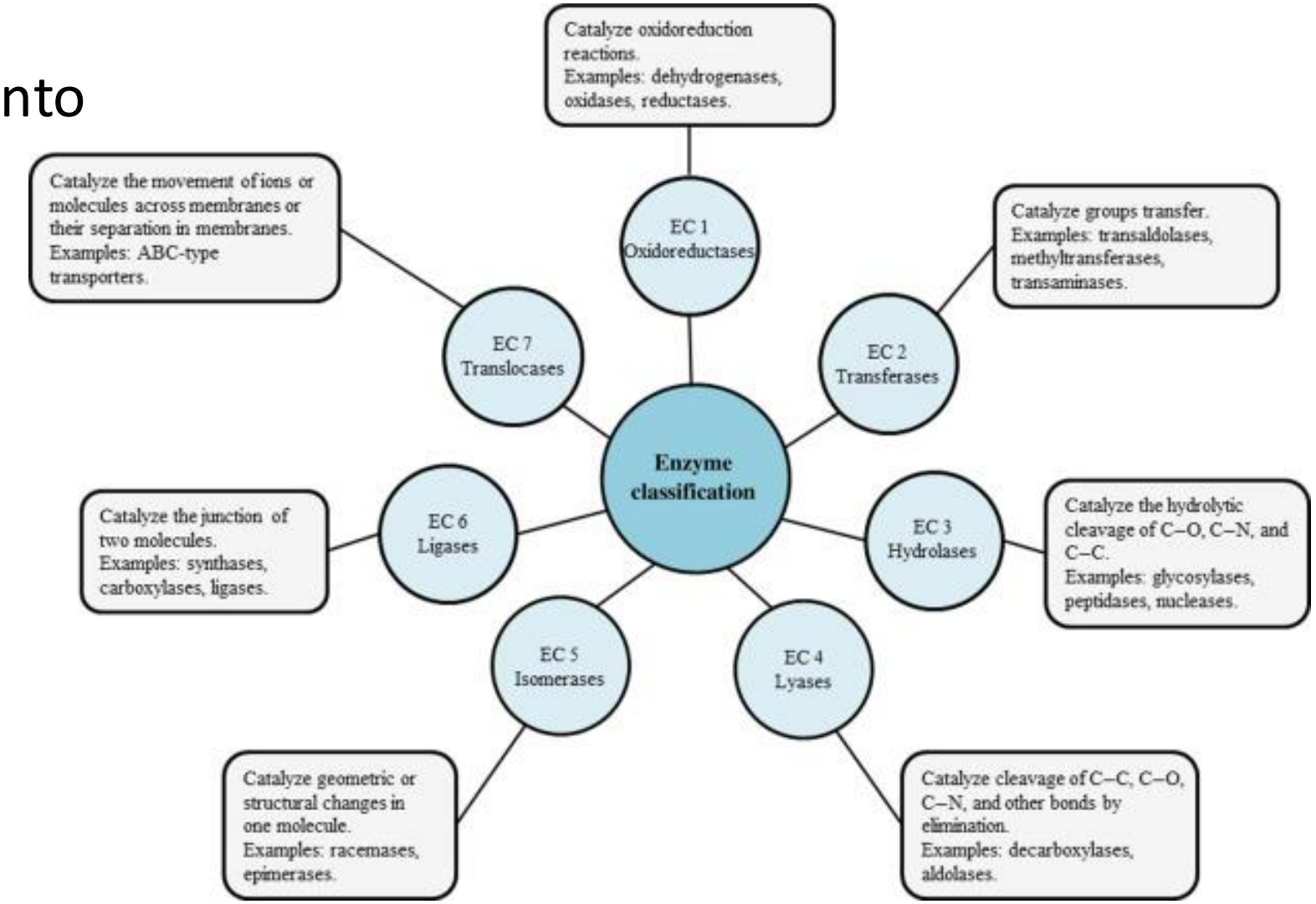


Naming of enzymes

- 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

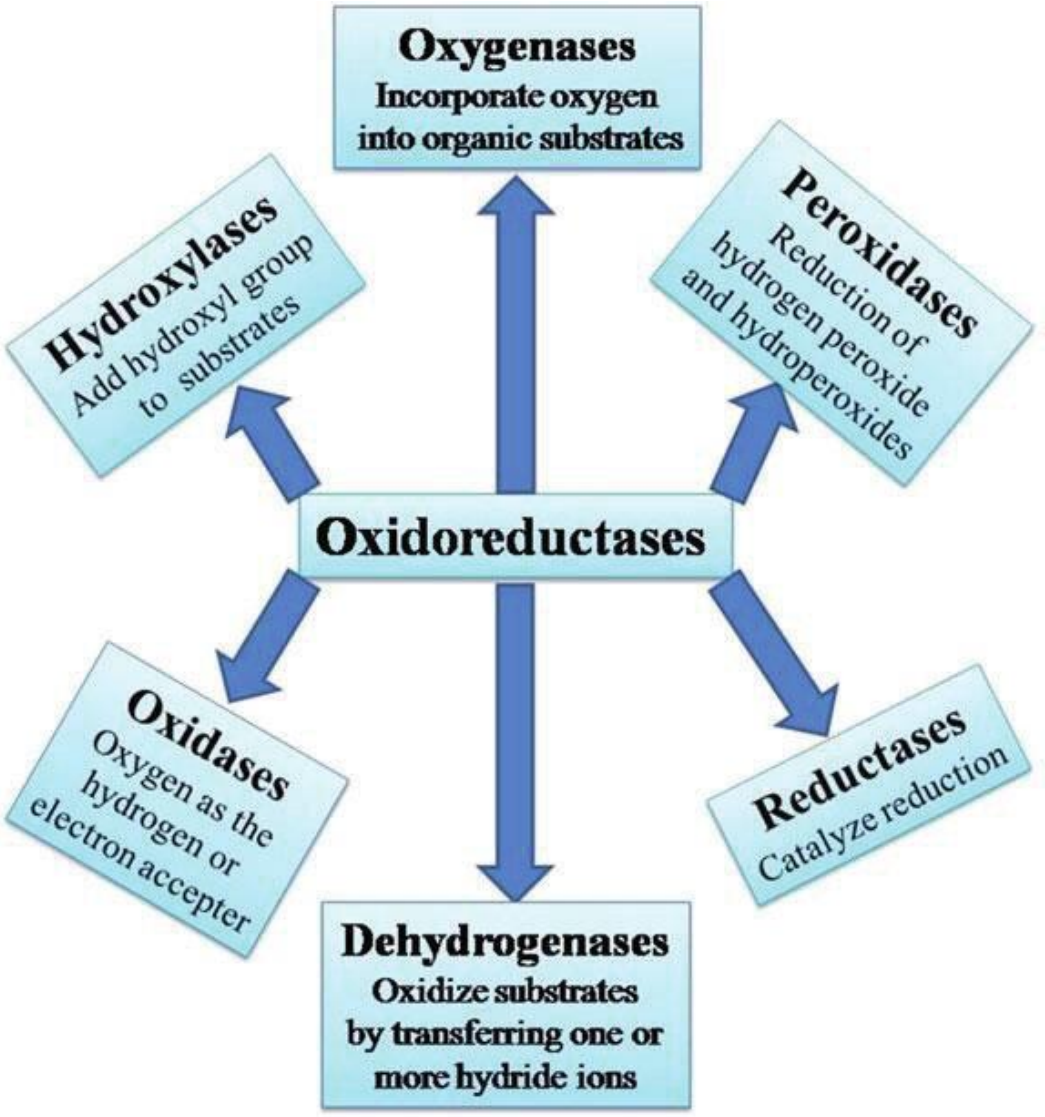
Enzyme classes according to function

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

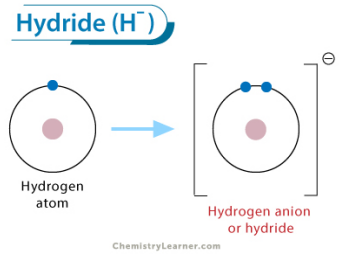


1. Oxidoreductases

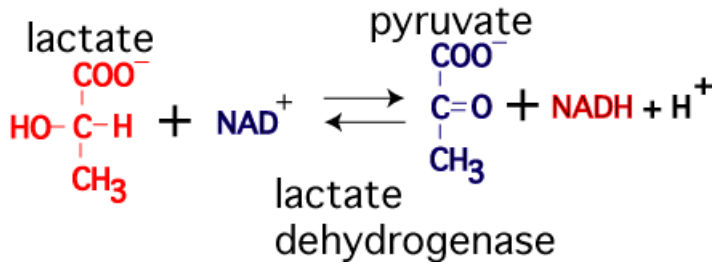
- They catalyze oxidation/reduction reactions involving the transfer of hydrogen atoms or electrons.
- They can be divided into 4 main classes:
  - Dehydrogenases
  - Oxidases
  - Peroxidases
  - Oxygenases
  - Reductases
  - Hydroxylases



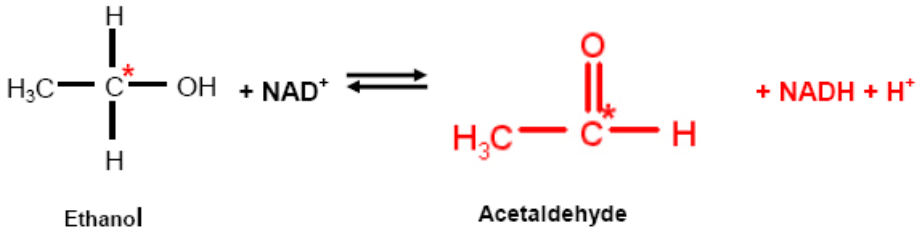
1a. Dehydrogenases



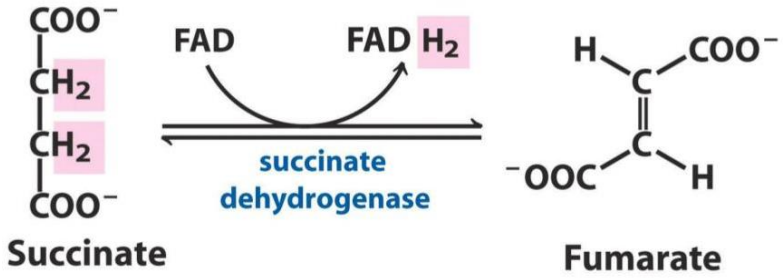
- Dehydrogenases transfer electrons in the form of hydride ions (H^-) or hydrogen atoms using an electron-transferring coenzyme, such as NAD^+/NADH or $FADH_2$.
- Lactate dehydrogenase:



- Alcohol dehydrogenase:



- Succinate dehydrogenase

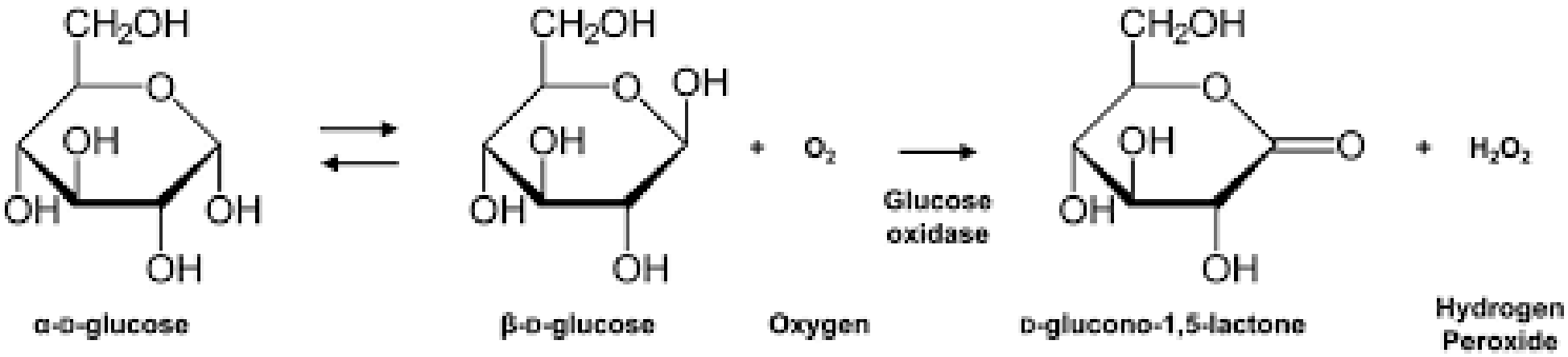


Do not memorize the reactions but understand them

1b. Oxidases

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

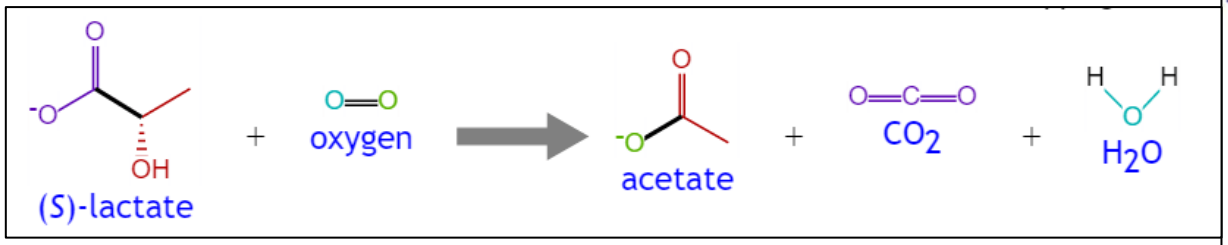
$$\text{Glucose} + O_2 \leftrightarrows \text{Gluconolactone} + H_2O_2$$

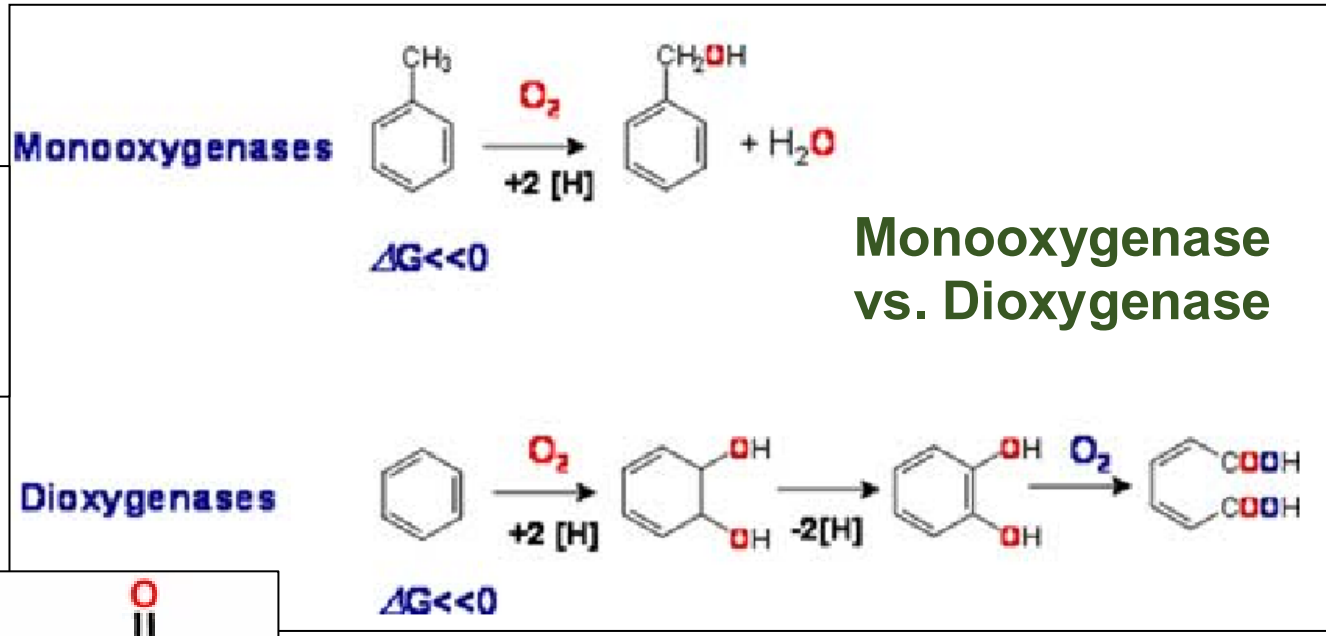


1c. Oxygenases

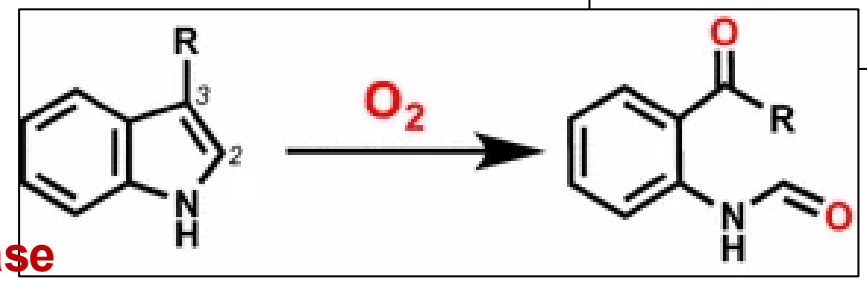
- Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate.
  - The reduced product is water, not H_2O_2.

Lactate-2-monooxygenase





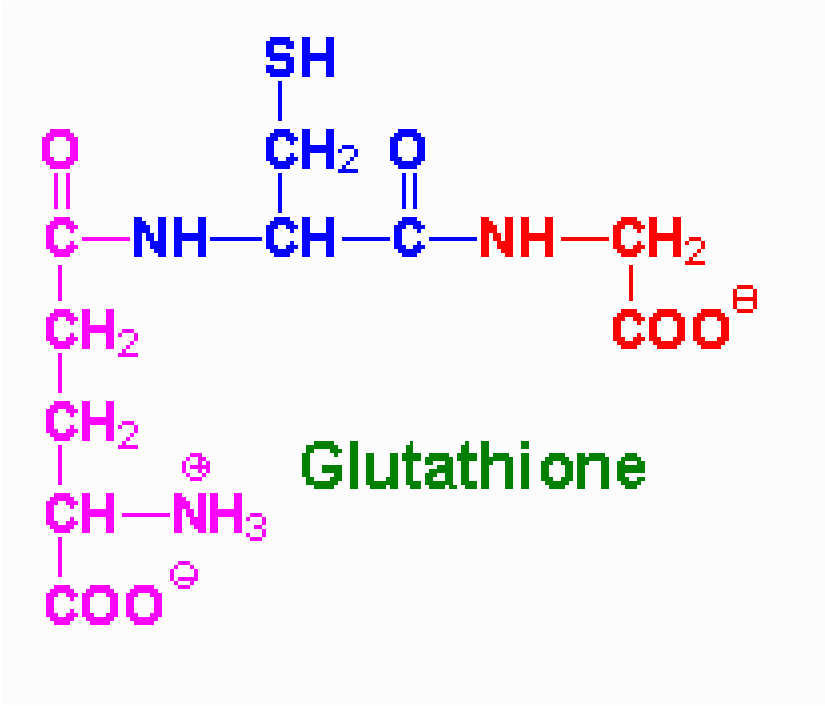
Monooxygenase vs. Dioxygenase

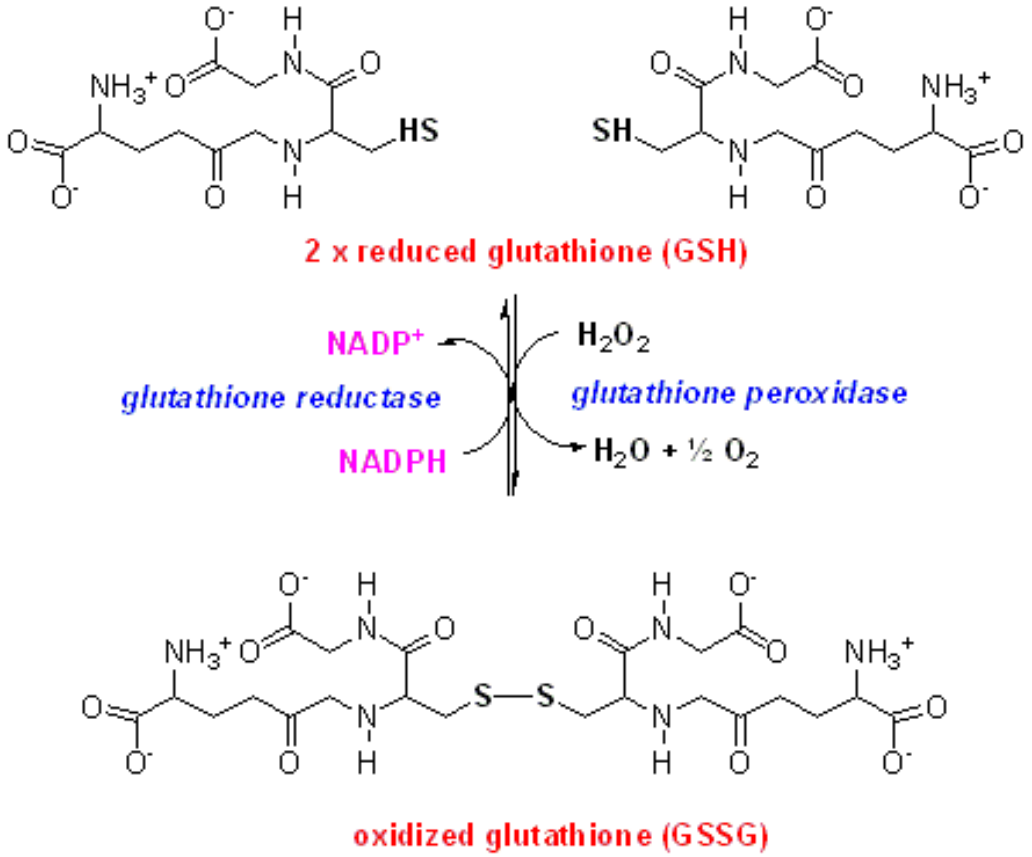


Tryptophan 2,3-dioxygenase

1d. Peroxidases

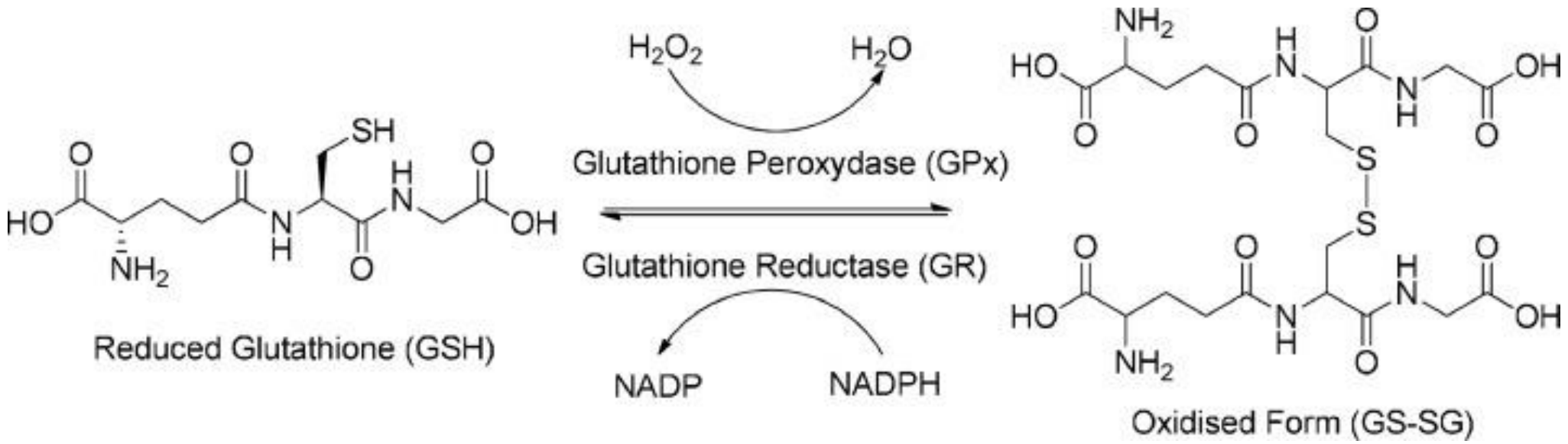
- Peroxidases catalyze oxidation of a substrate by hydrogen peroxide.
- Example: oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide:





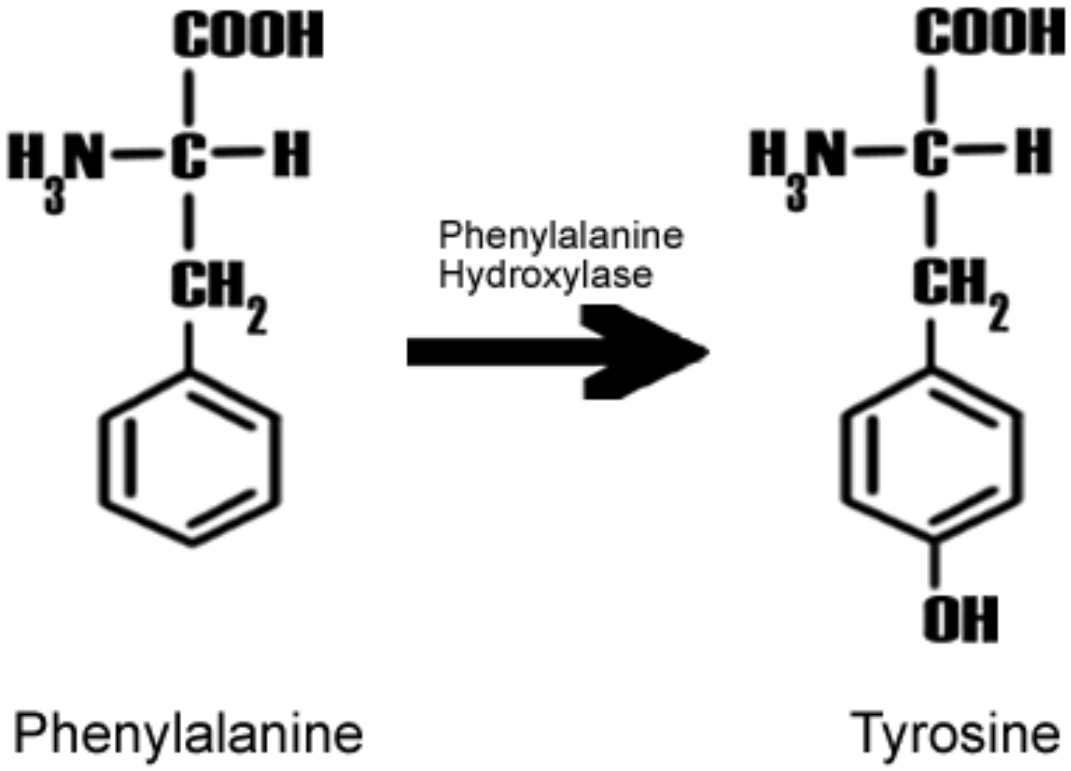
1f. Reductases

- These catalyze the reduction of substrates.
  - Example: glutathione reductase



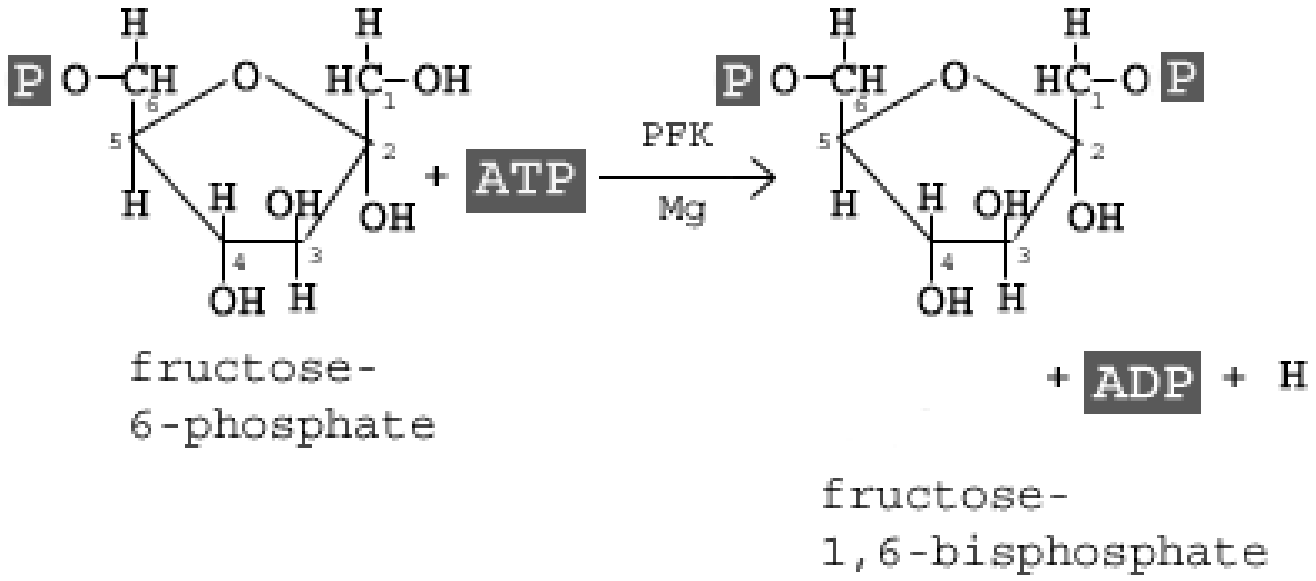
1e. Hydroxylases

- Hydroxylases add hydroxyl groups to a substrate.



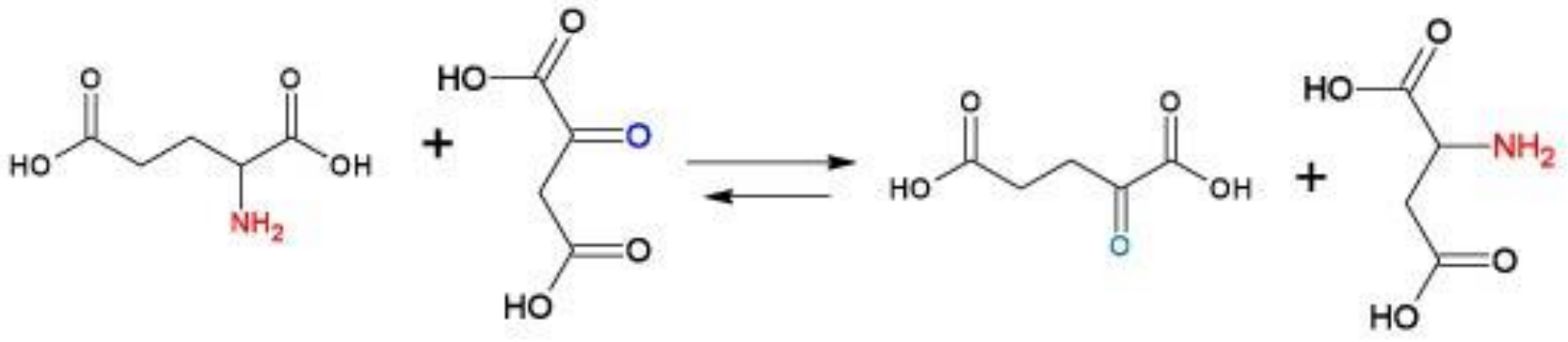
2. Transferases

- 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

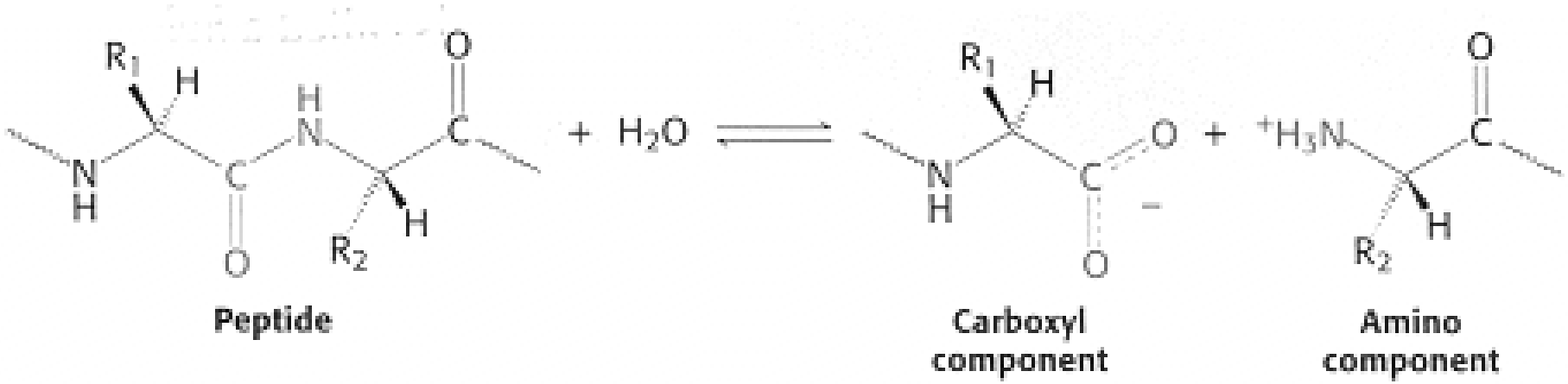
- A transaminase transfers an amino functional group from one amino acid to a keto acid, converting the amino acid to a keto acid and the keto acid to an amino acid.
  - Interconversion of certain amino acids.
  - Aspartate transaminase:



Glutamate Oxaloacetate α-Ketoglutarate Aspartate

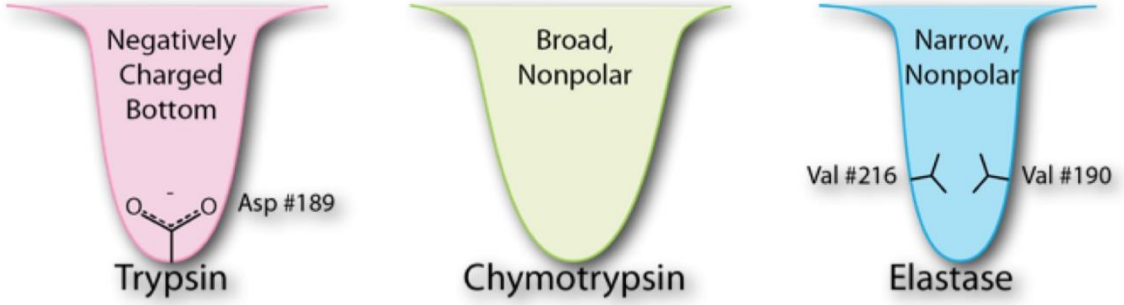
3. Hydrolases

- 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.



Lysine
Arginine

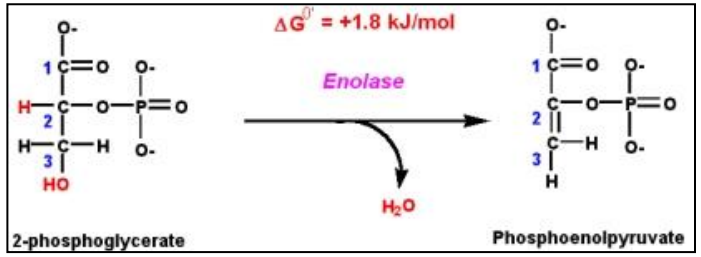
Phenylalanine
Tryptophan
Tyrosine

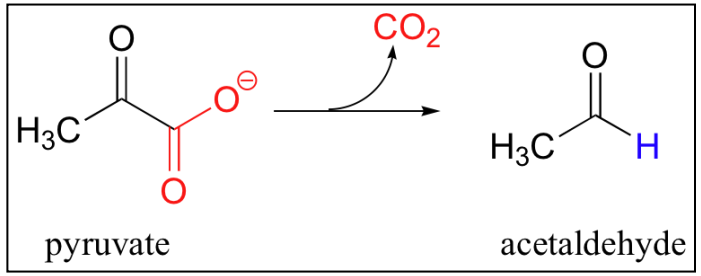
Glycine
Alanine
Valine

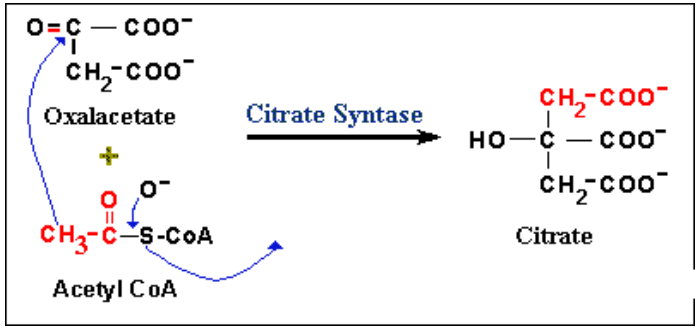
4. Lyases

- Lyases either cleave C-C, C-O, C-N, and other bonds by means other than hydrolysis or oxidation, leaving double bonds or rings, or add 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 molecule to a double bond or formation of a new carbon-carbon bond.
  - Example: citrate synthase



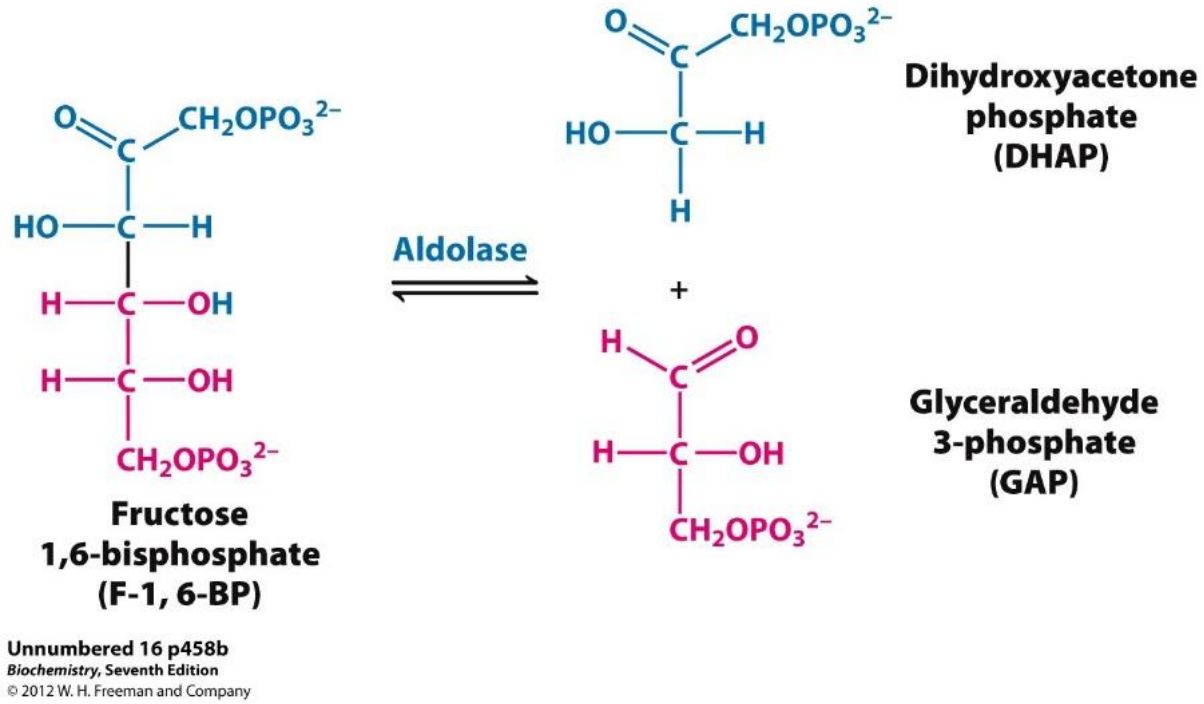




*("en" = **ene** for the C=C and "ol" is for alcoh**ol***

Example: aldolase

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



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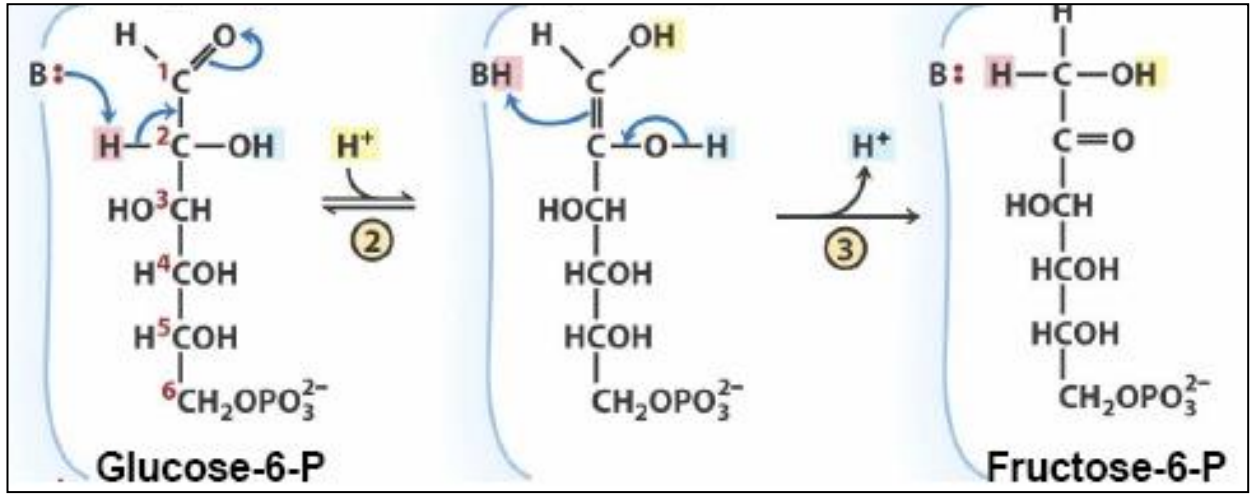
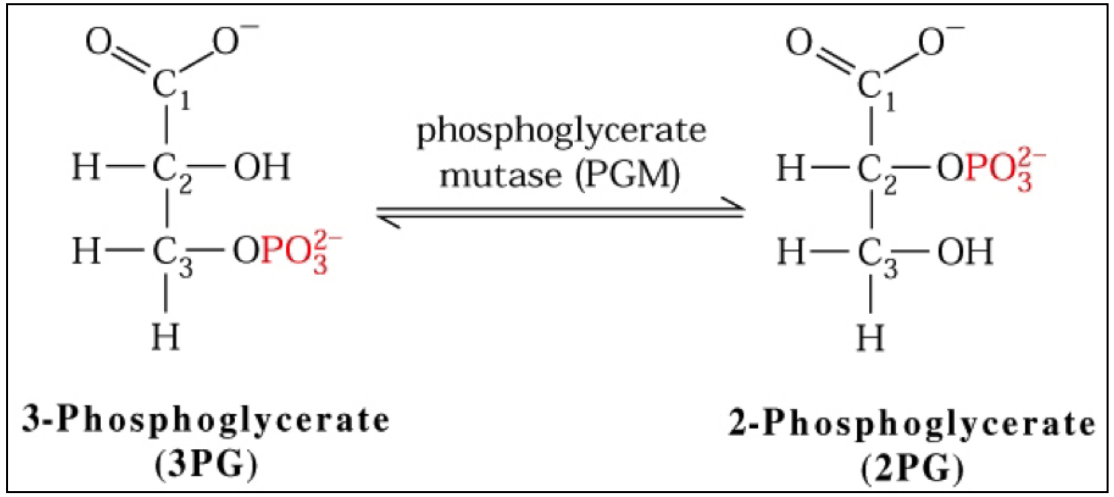
5. Isomerases

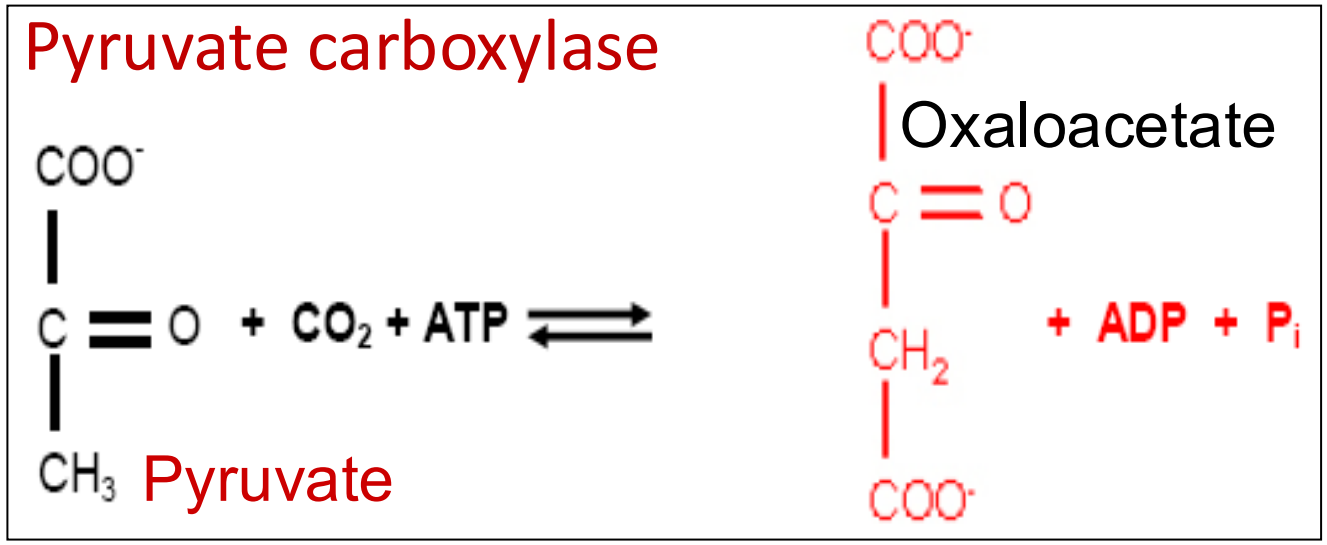
- These enzymes catalyze intramolecular rearrangements.
- Enzymes that rearrange the bond structure of a compound are called isomerases, whereas enzymes that catalyze the movement of phosphate from one atom to another are called mutases.
  - Phosphoglucoisomerase isomerizes glucose-6-phosphate to fructose-6-phosphate.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

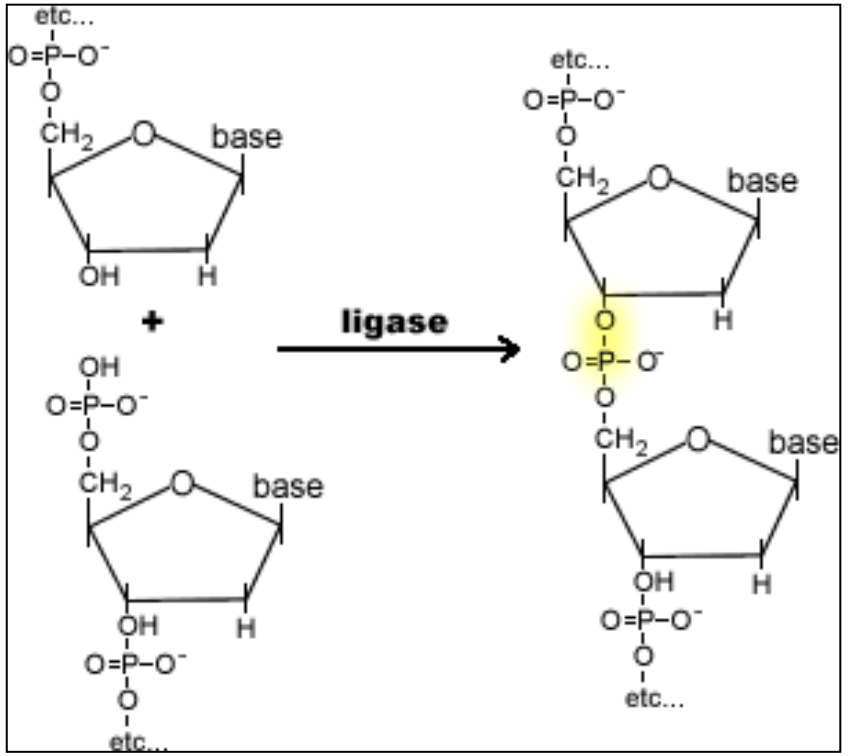




6. Ligases

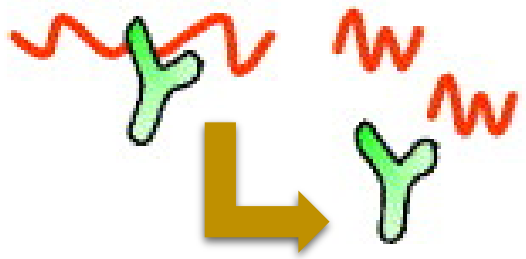
- Ligases join C-C, C-O, C-N, C-S, and C-halogen bonds.
- The reaction is usually accompanied by the consumption of a high-energy compound such as ATP and other nucleoside triphosphates.
- Synthetases derive the energy from the cleavage of high-energy phosphate bonds.
  - Synthases use a different source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





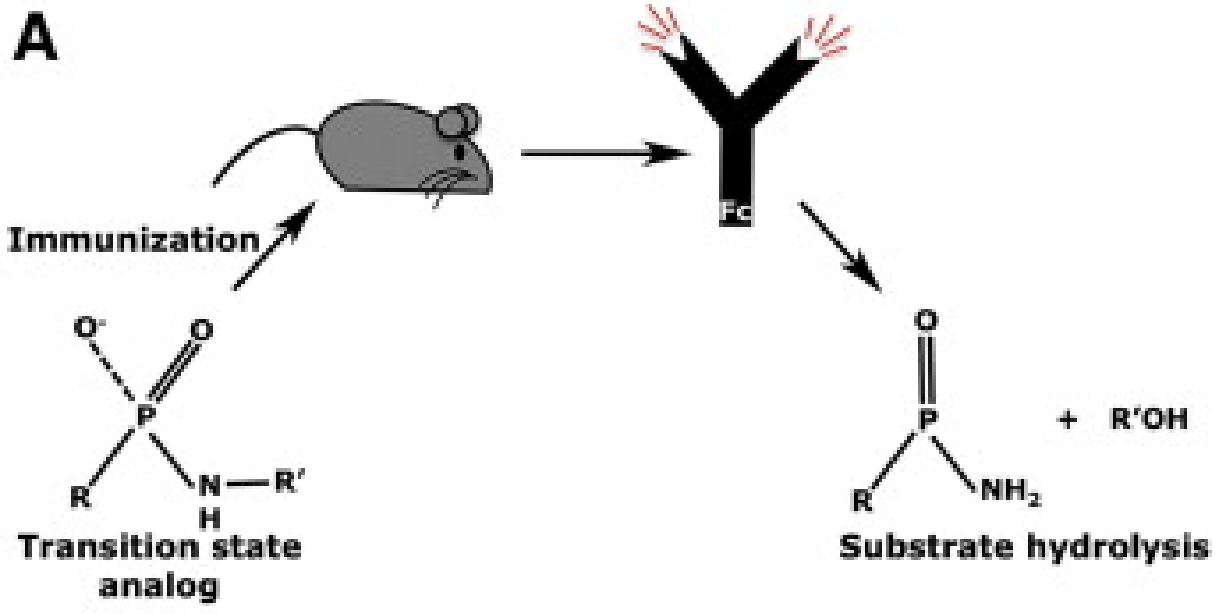
Some things are just different

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 and are called ribozymes.
- Some ribozymes are ribunucleoproteins made of both a protein and RNA, but catalysis is performed by the RNA part.
  - Examples include RNA splicing and protein synthesis in ribosomes.