Enzymes I

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Resources

- Mark's Basic Medical Biochemistry

Other resources

- NCBI Bookshelf:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books

The Medical Biochemistry Page:
http://web.indstate.edu/thcme/mwking/home.html

- Biochemistry, Garret and Grishan, Second Ed.: http://web.virginia.edu/Heidi/home.htm

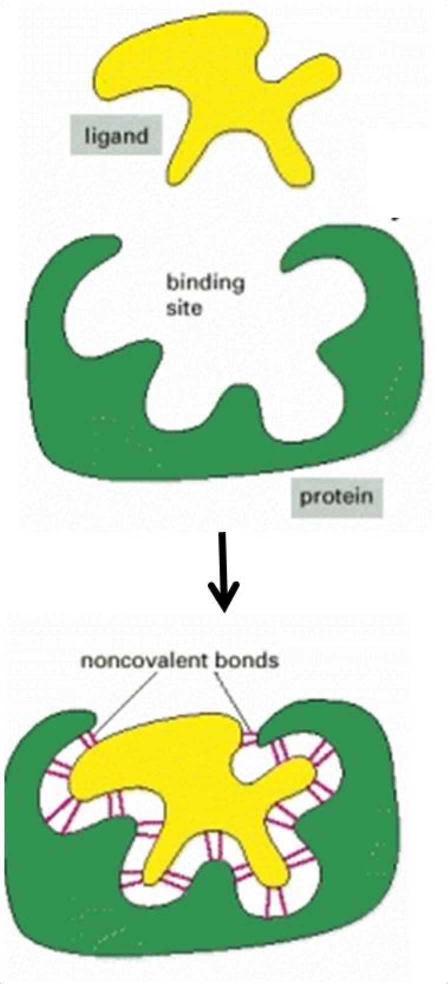
General properties of proteins

- The function of nearly all proteins depends on their ability to bind other molecules (ligands).

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 are able to conduct (catalyze) chemical reactions under biological conditions.
  - Exception: ribozymes
- Most enzymes have very specific functions converting specific substrates to the corresponding products.
- Enzymes are catalysts
  - They are usually used in small amounts relative to the reactants.
  - They modify and increase the rate of a reaction
  - At the end of the reaction, they undergone no change

How to express an enzymatic reaction?

- In enzymatic reactions: reactants = substrates
- Simple expression of enzymatic reaction:

$$E + S \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$$

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

- For simplicity:

$$E + S \leftrightarrows ES \leftrightarrows E + P$$

What do enzymes do?

- Enzymes accelerate reactions (range of 10^6 to 10^{14} (to 10^{20}).
  - Example:
    - Catalase (10^8) & carbonic anhydrase (10^7)

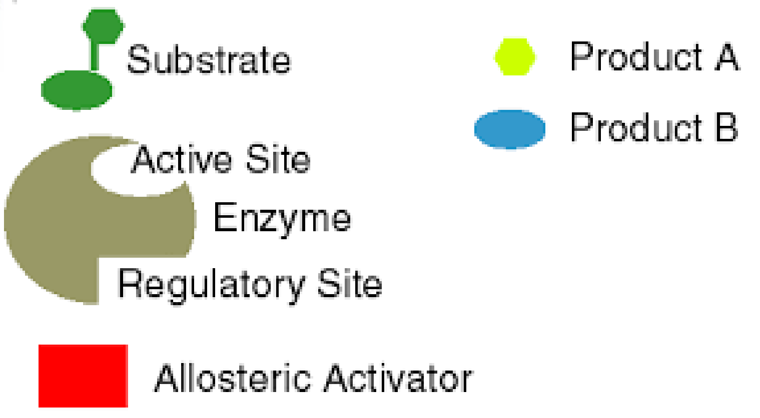
$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2(g) \qquad CO_2 + H_2O \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

  - Carbonic anhydrase
    - One enzyme molecule hydrates 10^7 molecules of CO_2 per second (versus 10^2 to 10^4 for uncatalyzed reactions)

	Activation Free Energy		
Reaction Conditions	**kJmol^{-1}**	**kcal mol^{-1}**	**Relative Rate**
No catalyst	75.2	18.0	1
Platinum surface	48.9	11.7	2.77×10^4
Catalase	23.0	5.5	6.51×10^8

Where does the reaction occur?

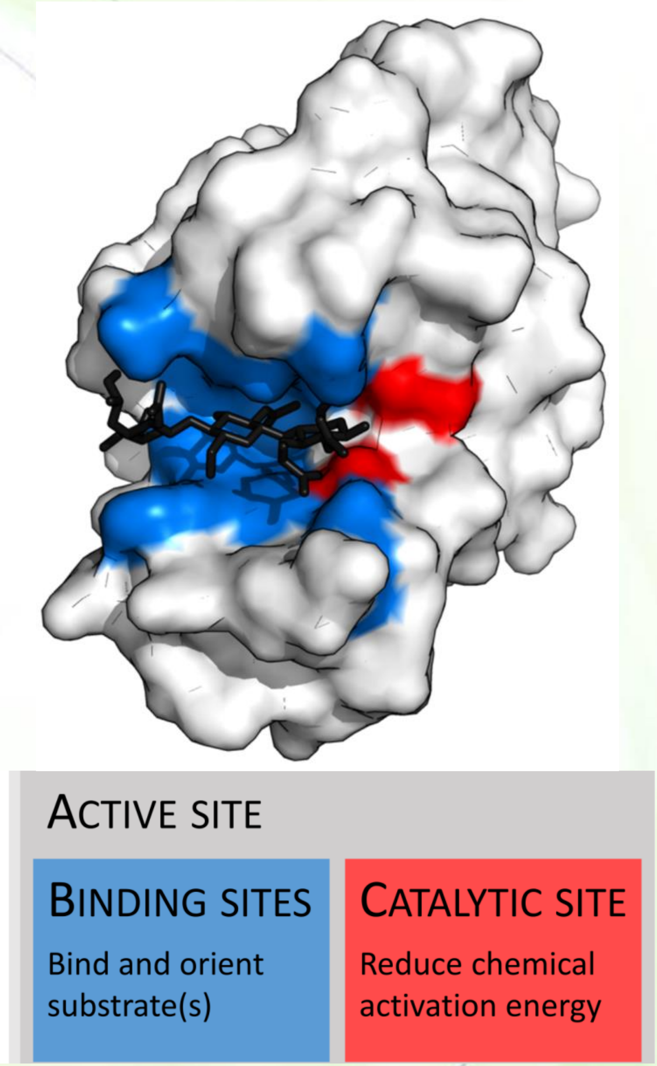
- Each enzyme has a specific three-dimensional shape that includes the ***active site*** (a region where the biochemical reaction takes place).



- **The active site contains a specialized amino acid sequence that facilitates the reaction.**
- **If a substance fits into the active site and binds to the enzyme, it is said to have affinity for the active site.**
- **Binding of a substrate into the active site can be regulated by a *regulatory site*.**

Catalytic group

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



ACTIVE SITE

BINDING SITES

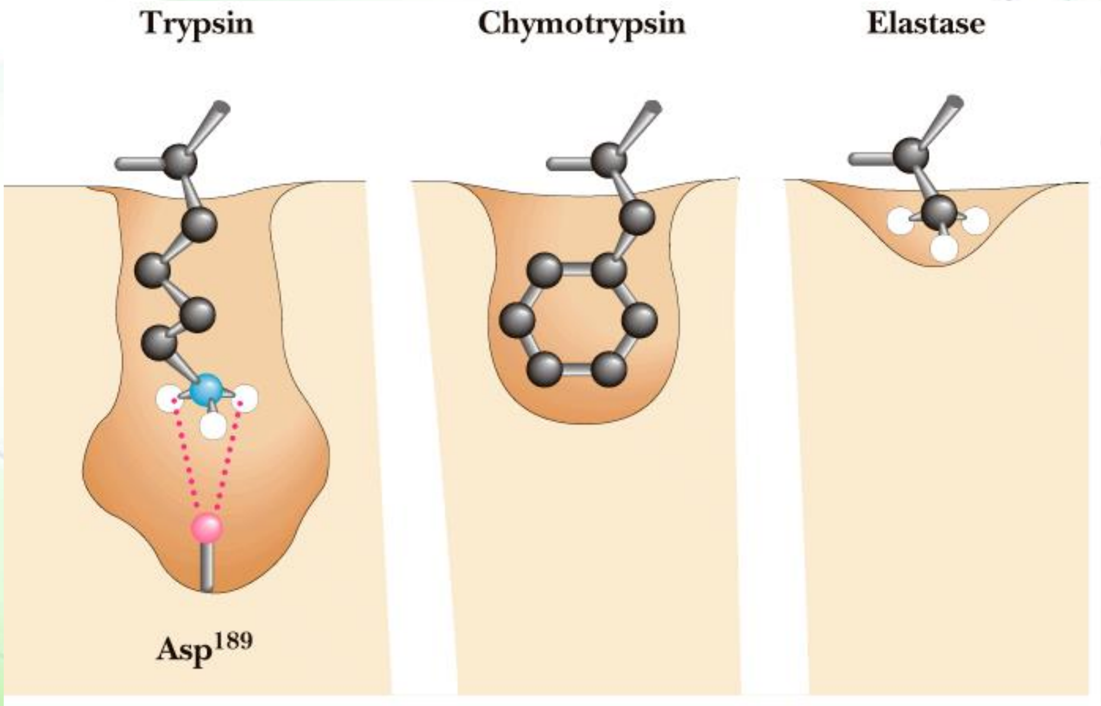
Bind and orient substrate(s)

CATALYTIC SITE

Reduce chemical activation energy

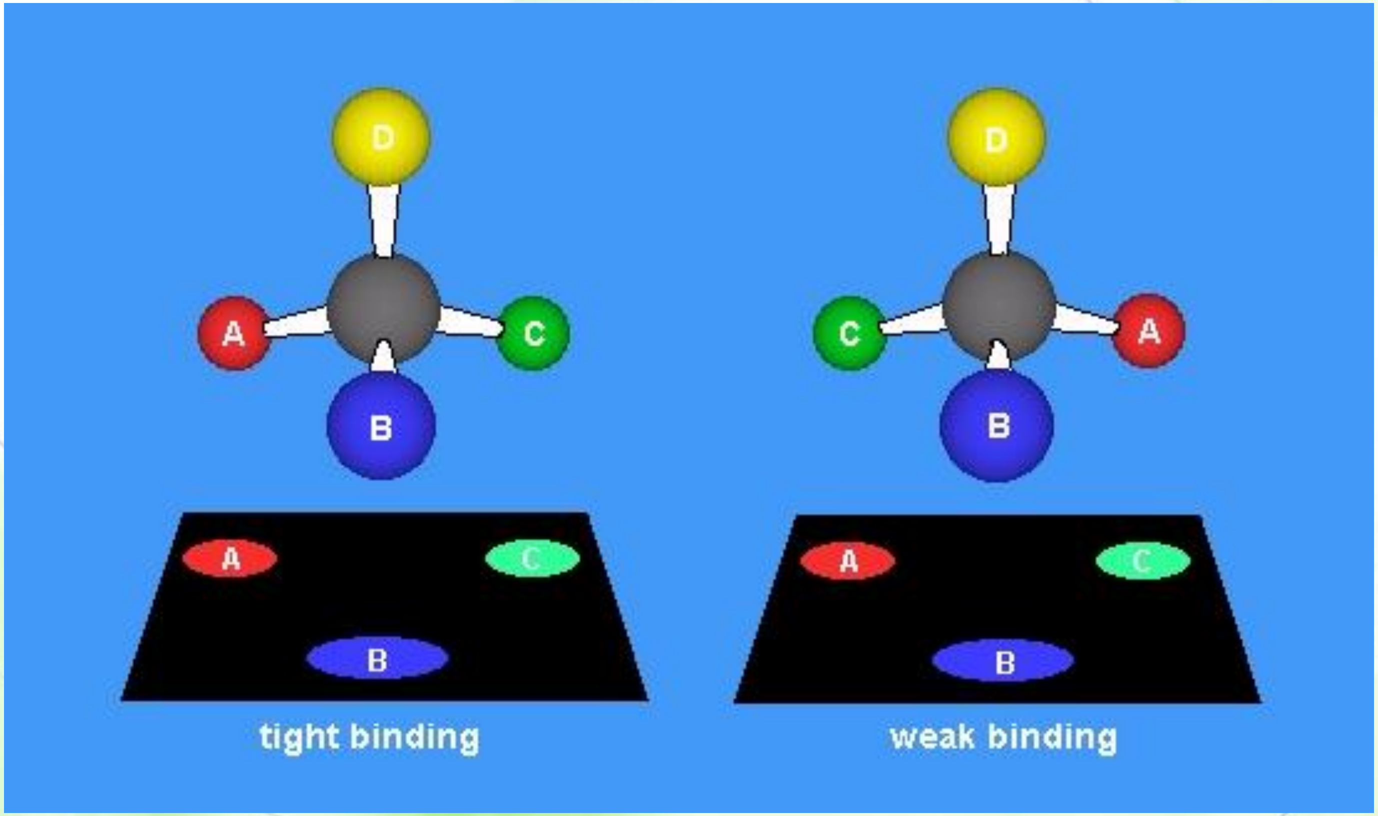
Binding specificity

- 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.



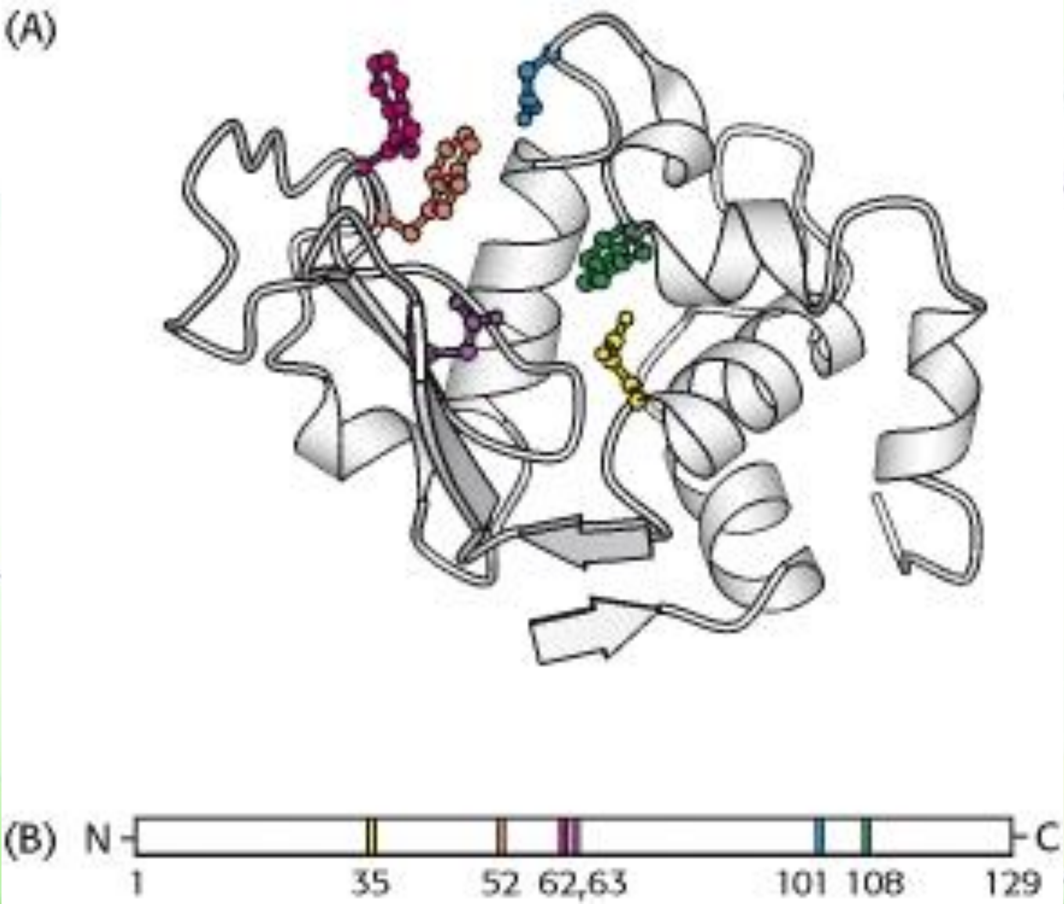
Features of active site 1

- Binding occurs at least three points.
  - Chirality is important.



Features of active site 2

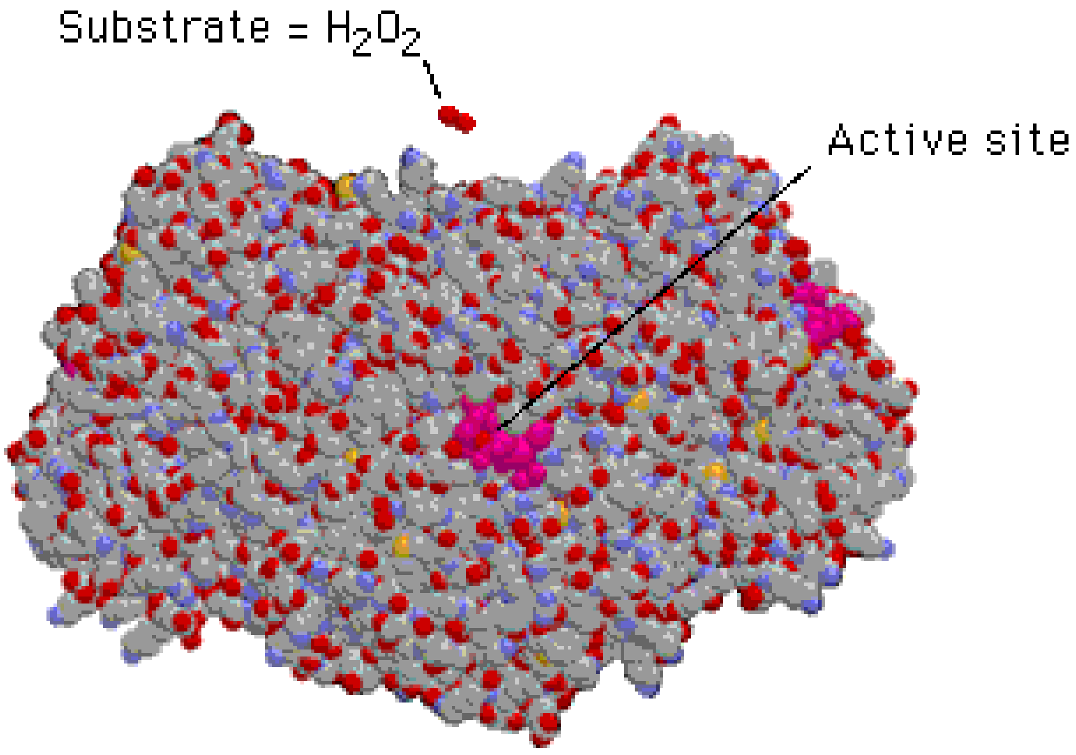
- It is a three-dimensional pocket or cleft formed by groups that come from different parts of the primary structure usually forming a domain.



Features of active site 3

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

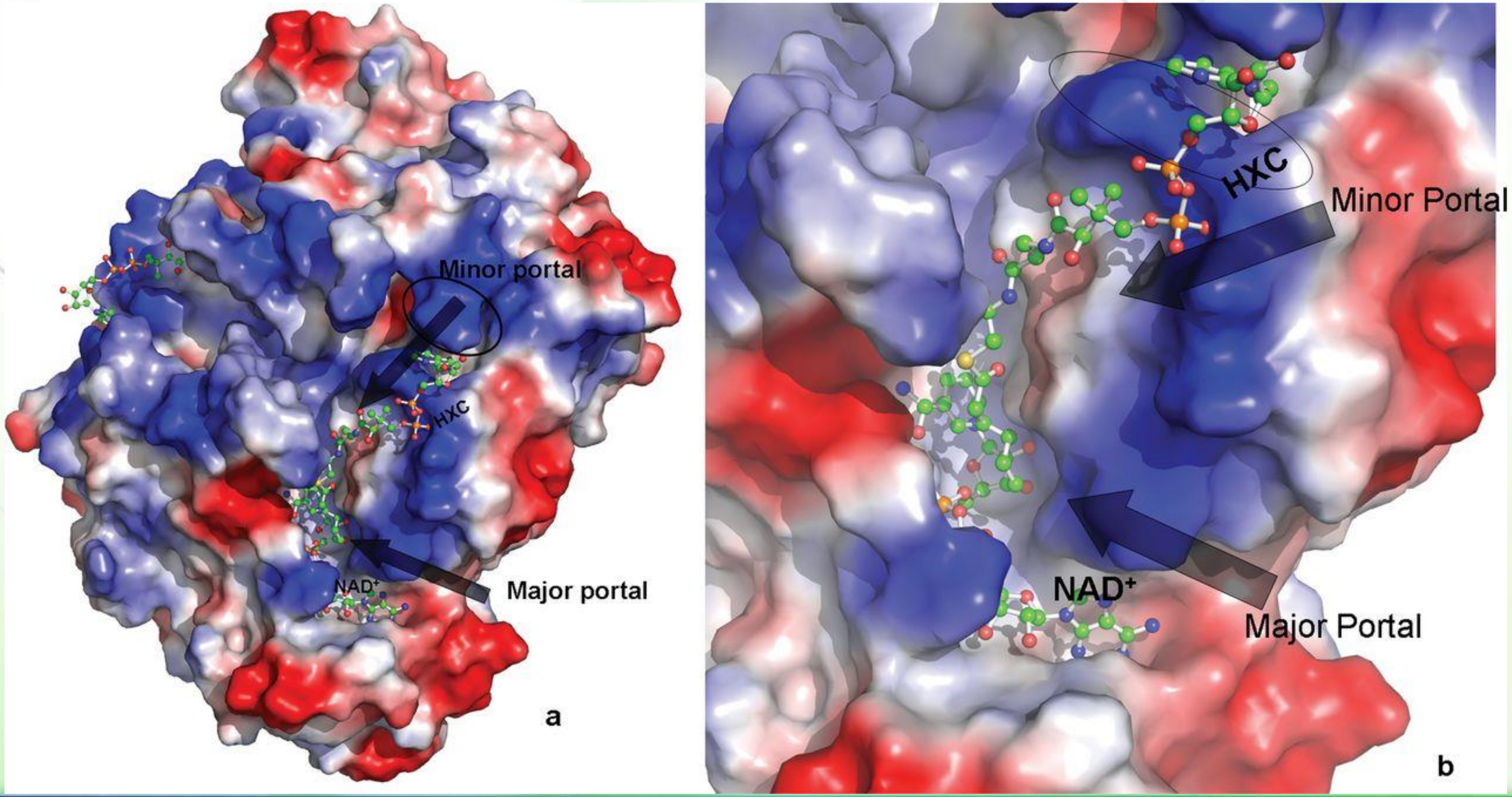
- **In many proteins, the remaining amino acids may make up regulatory sites.**



Molecular model
of catalase

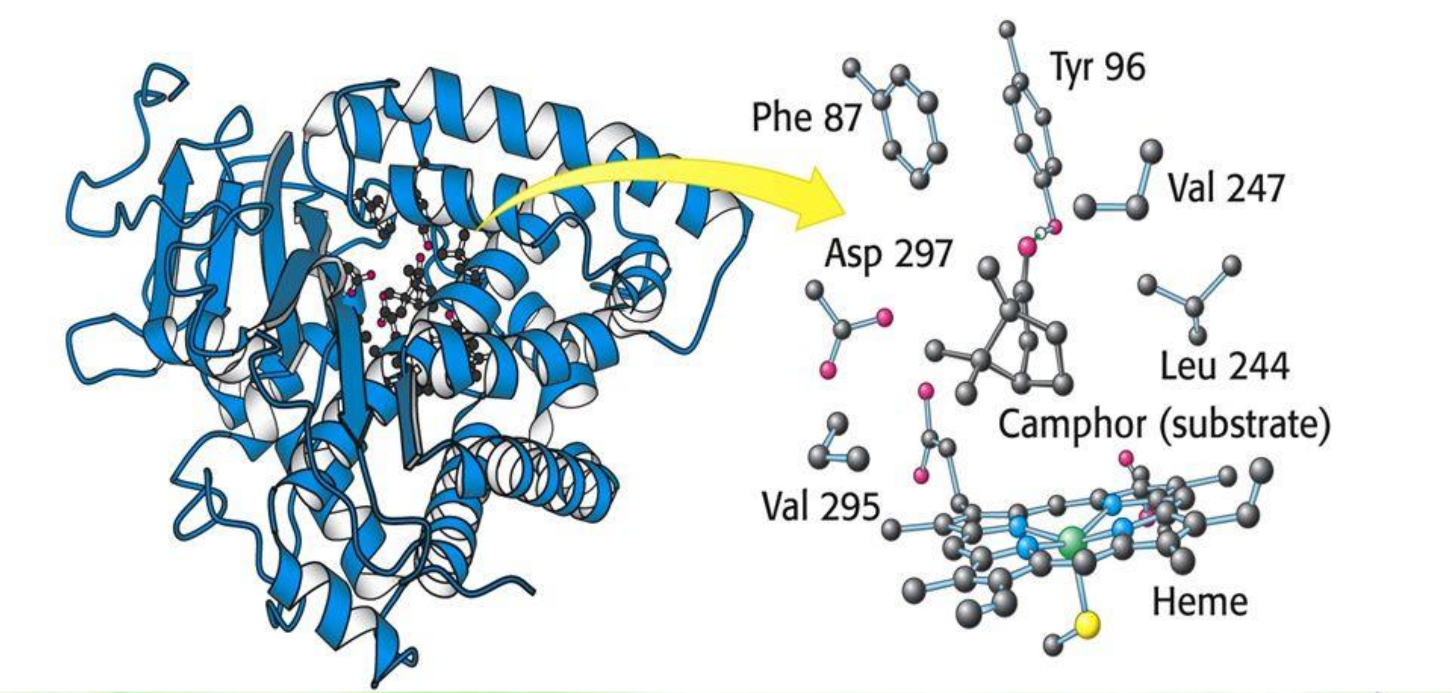
Features of active site 4

- It looks like a canal, cleft, or crevice.
- It contains nonpolar as well as polar residues.
- Water is usually excluded unless it is part of the reaction.



Features of active site 5

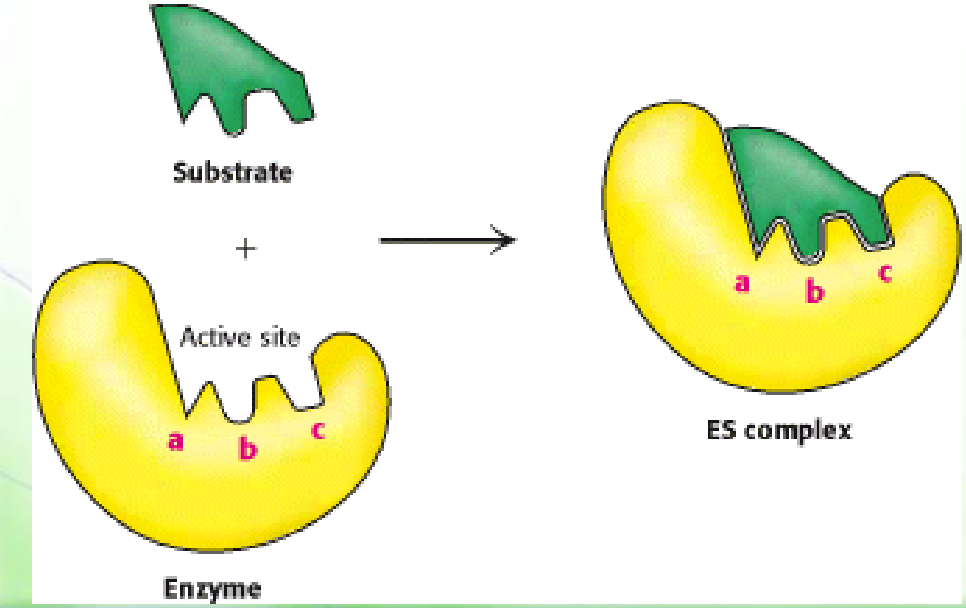
- Substrates bind to enzymes by multiple weak attractions.



How do substrates fit into the active site of enzymes?

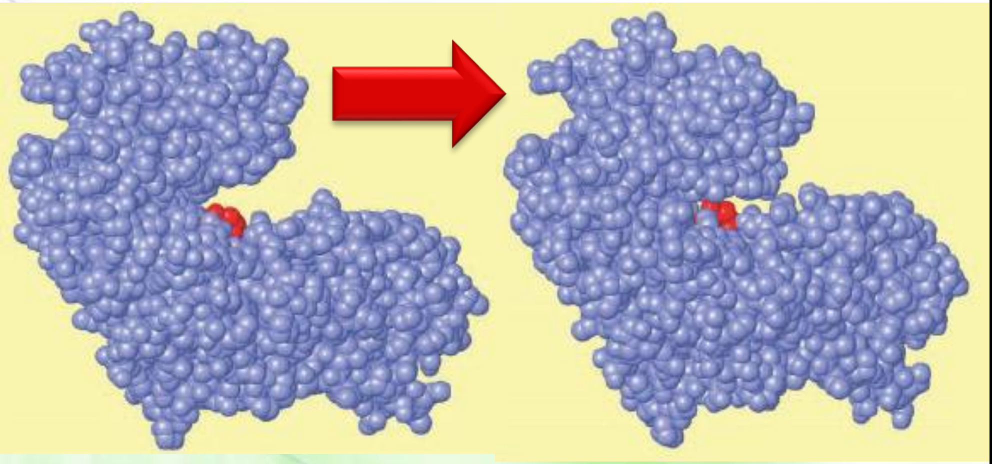
Lock-and-key model (old)

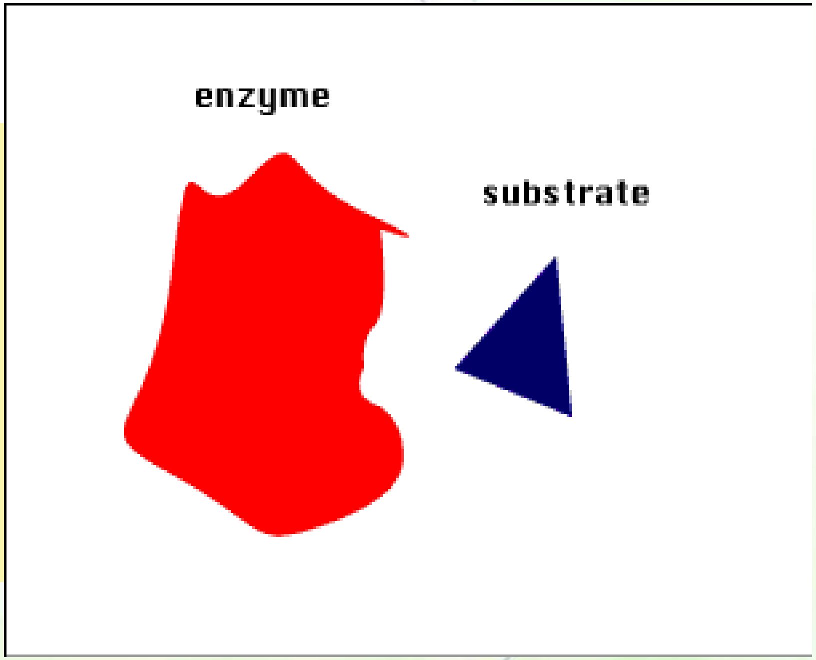
- Here, the substrate fits directly into the active site.
- Not really!!
  - Proteins are dynamic in nature.
  - Some enzymes can bind different substrates.
  - Some enzymes catalyze multi-substrate reactions.



Induced fit model

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





How do enzymes accelerate reactions?

Types of energy

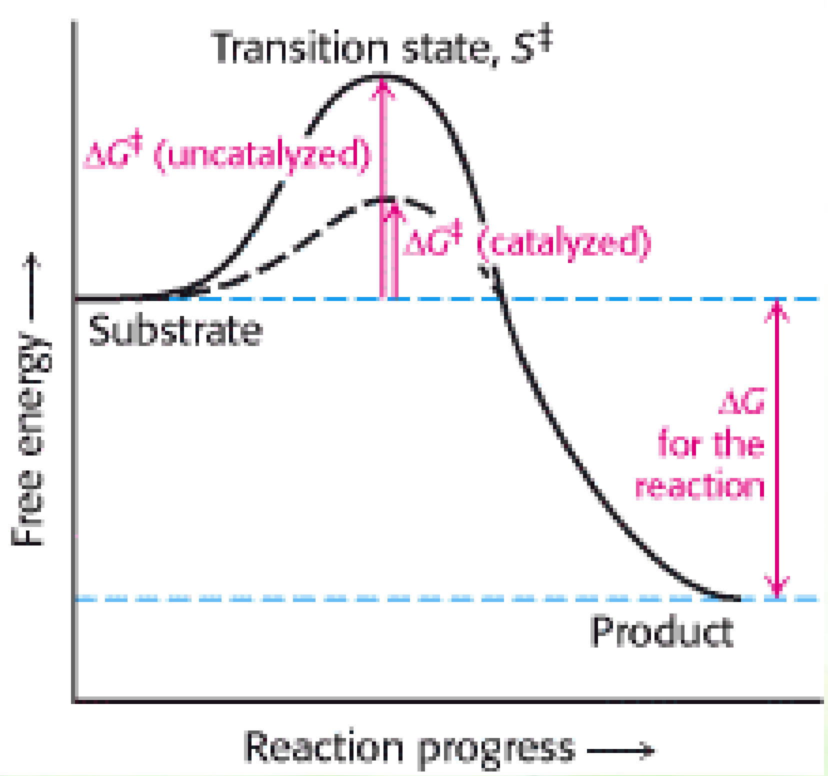
- There are two forms of energy
  - potential - capacity to do work (stored)
  - kinetic - energy of motion

- 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):

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



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

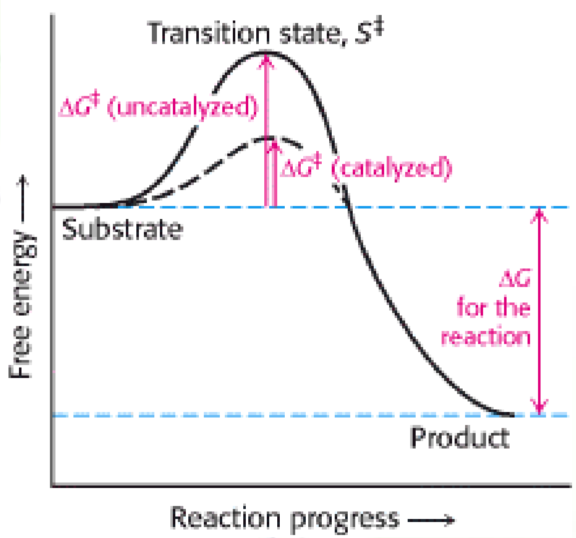
What does it mean?

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

- If ΔG is negative, $G_{products}$ is less than $G_{reactants}$, 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 $G_{reactants}$, 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?

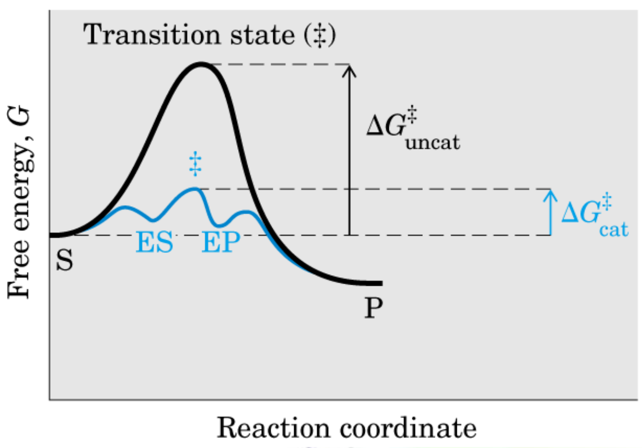
- Any enzymatic reaction goes through a transition state (ES) that has a higher free energy than does either S or P.
- The difference in free energy of the transition state and the substrate is called the ***activation energy***.
- Enzymes lower the activation energy, or, in other words, enzymes facilitate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

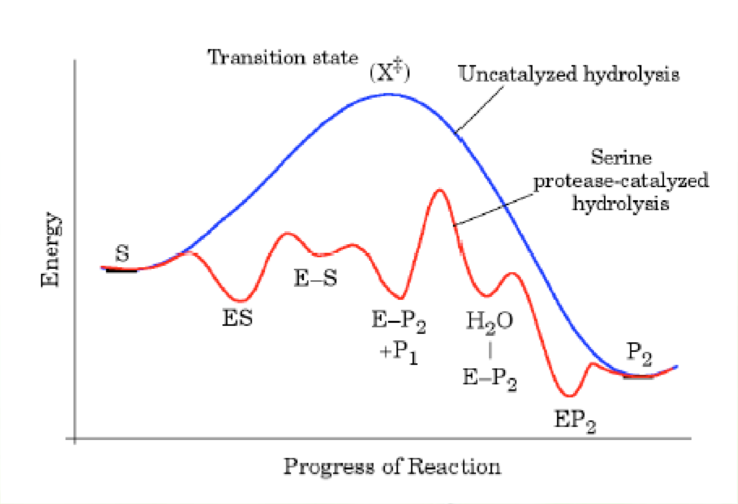


- **At the highest energy level, the substrate configuration is most unstable and is most tightly bound to the enzyme.**
- **The bonds or the electronic configuration are maximally strained.**

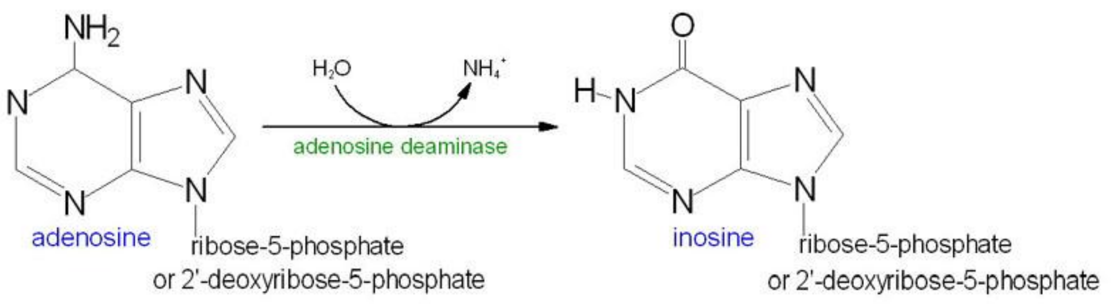
Alternative pathways

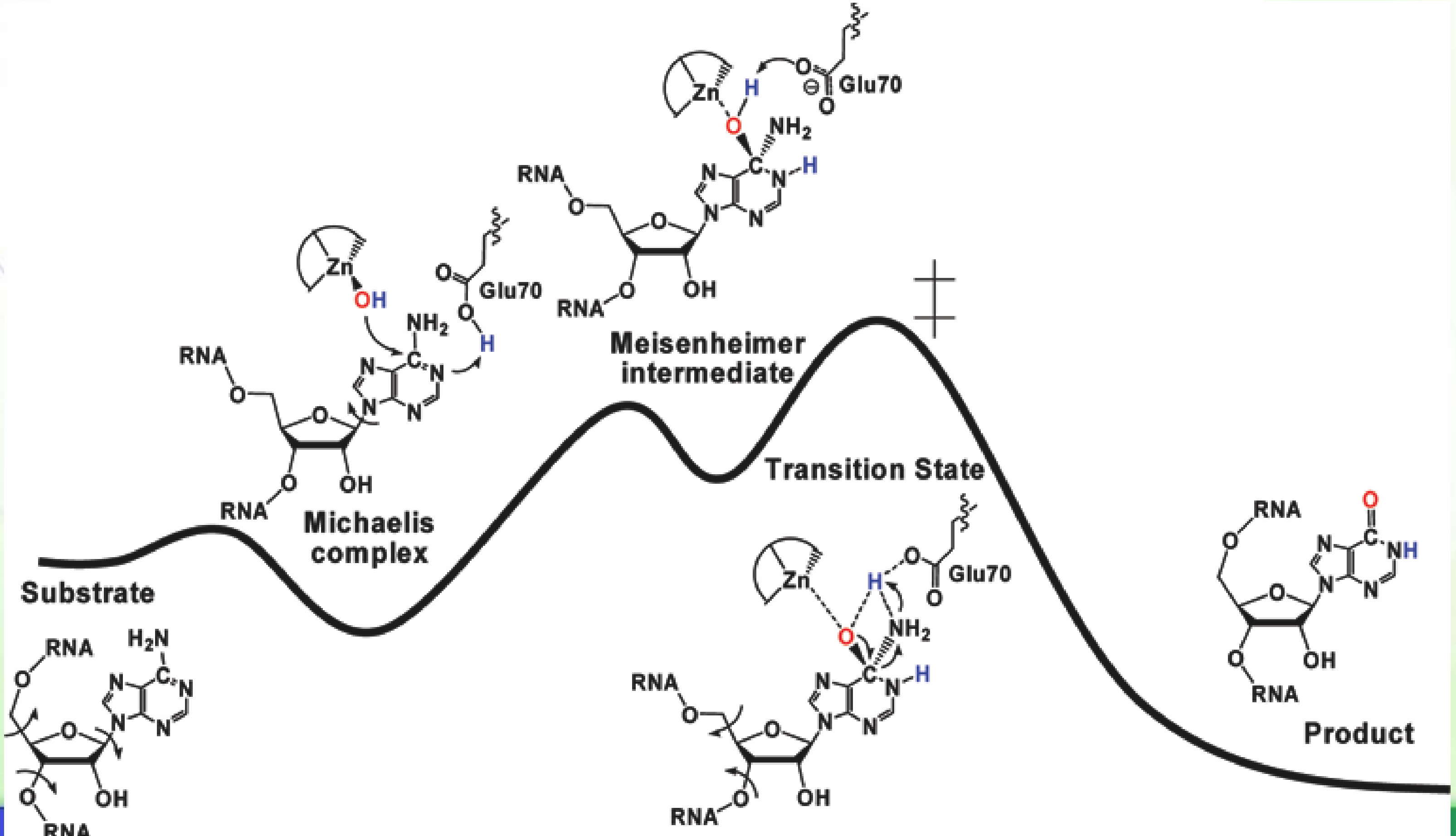
- Substrates often undergo several transformations when associated with the enzyme and each form has its own free energy value.
- The activation energy corresponds to the complex with the highest energy.
- The energy of activation does not enter into the final ΔG calculation for a reaction.





Example: *Adenosine Deaminase*





How do enzymes catalyze reactions?

1. Proximity of substrates together
2. Orientation of the active site to fit the substrate in the best fit possible
3. Changing the energy within bonds allowing the break up and formation of bonds
4. Catalysis is the end result.

Examples of possible mechanisms to do so:

1. Catalysis by bond strain
2. Catalysis involves acid/base reactions
3. Covalent catalysis

Catalysis by bond strain

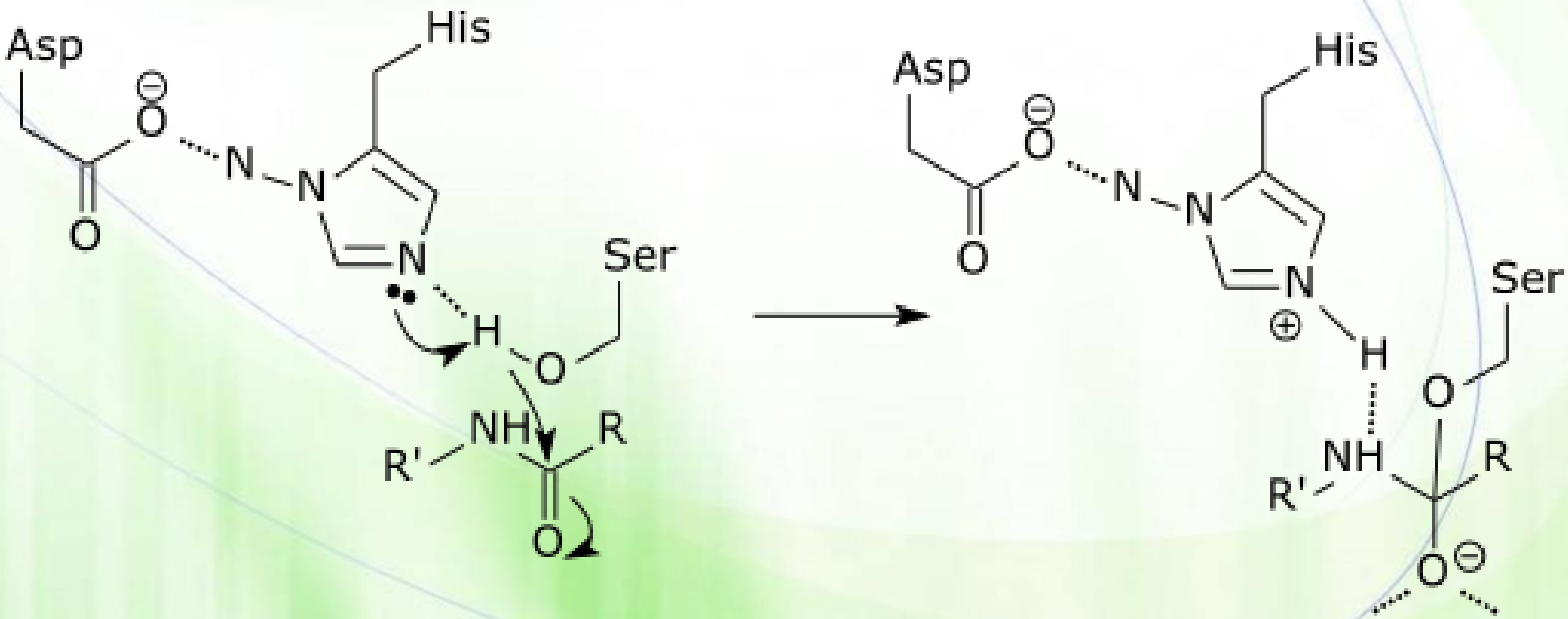
- In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.

- Example: lysozyme

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

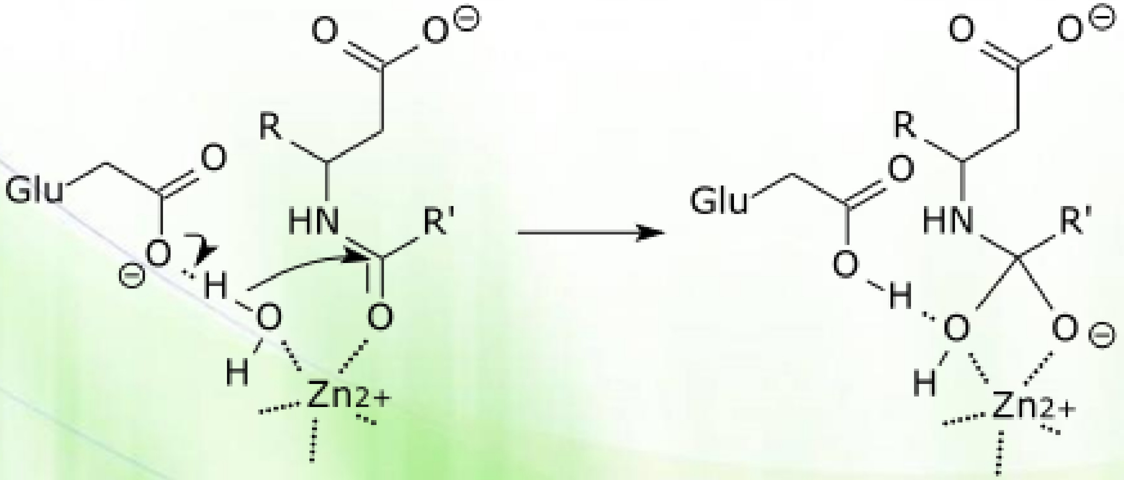
Catalysis involves acid/base reactions

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



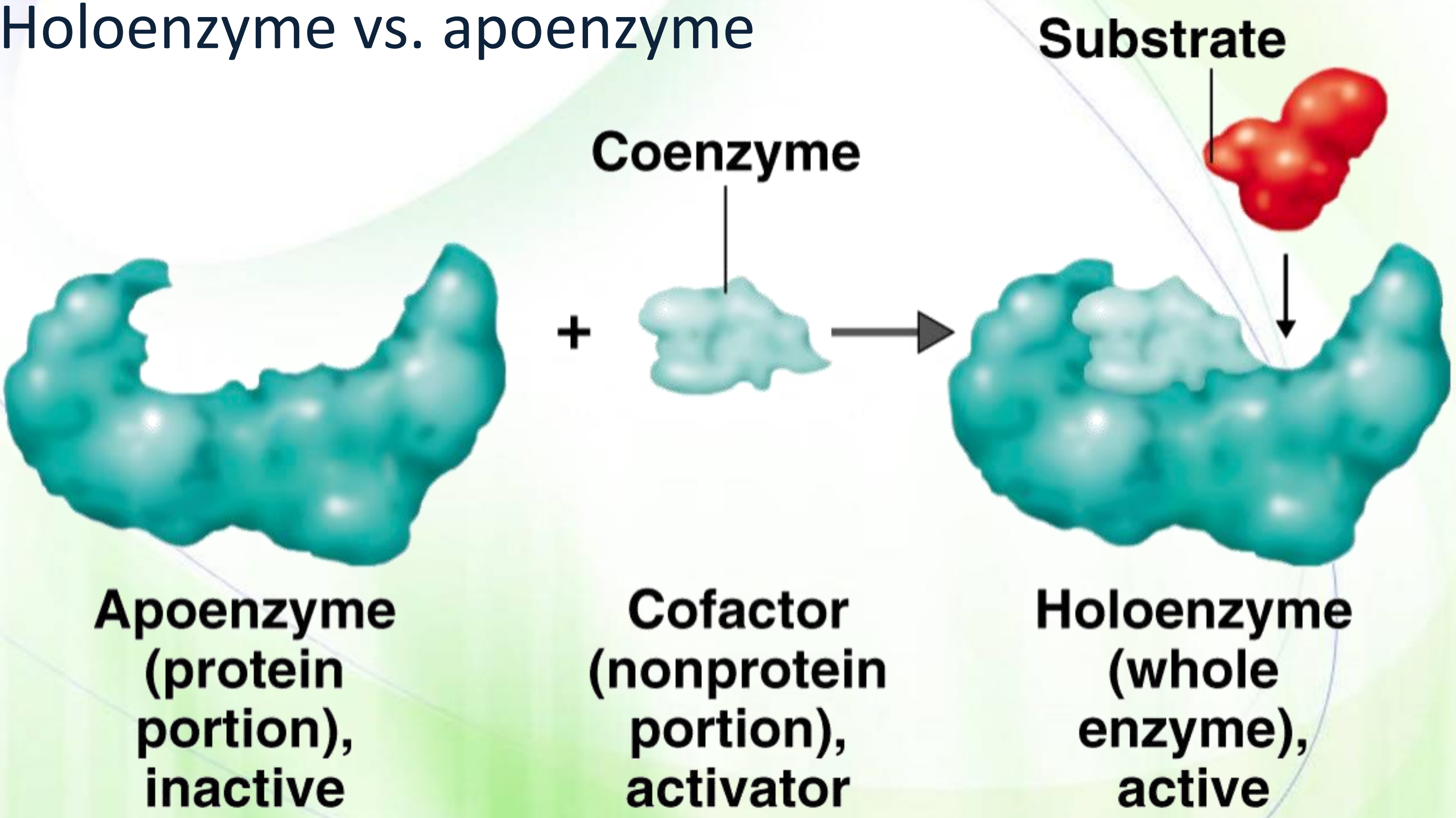
Enzyme substrate complex

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

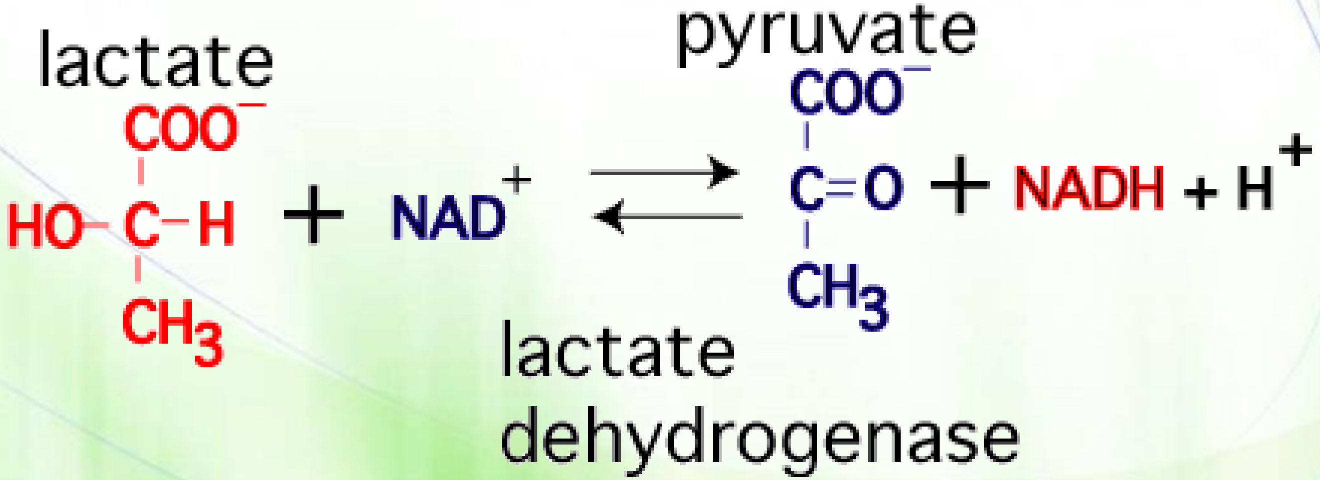
- Enzymes are classified into six major groups:
  - Oxidoreductases
  - Transferases
  - Hydrolases
  - Lyases
  - Isomerases
  - Ligases

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

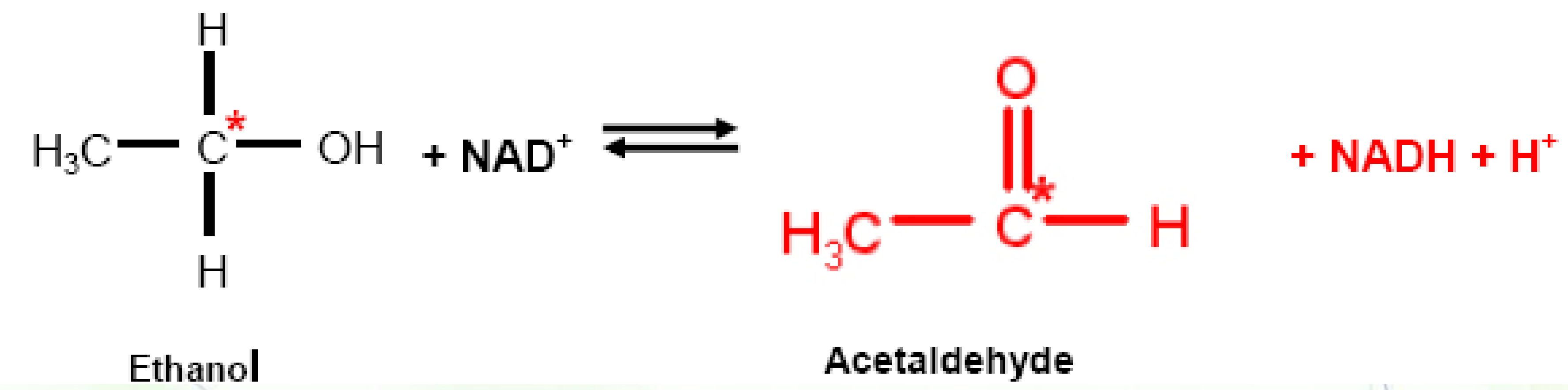
1a. Dehydrogenases

- Dehydrogenases transfer electrons in the form of hydride ions (H) or hydrogen atoms using an electron-transferring coenzyme, such as NAD/NADH or FADH2.
- An example of this is lactate dehydrogenase, which catalyzes the following reaction:



Example: alcohol dehydrogenase

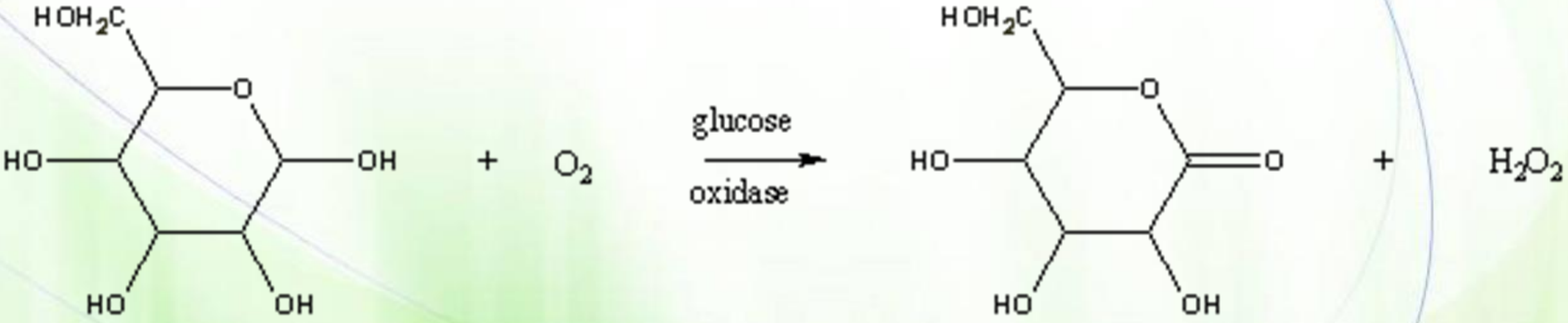
- Another example is alcohol dehydrogenase.



1b. Oxidases

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

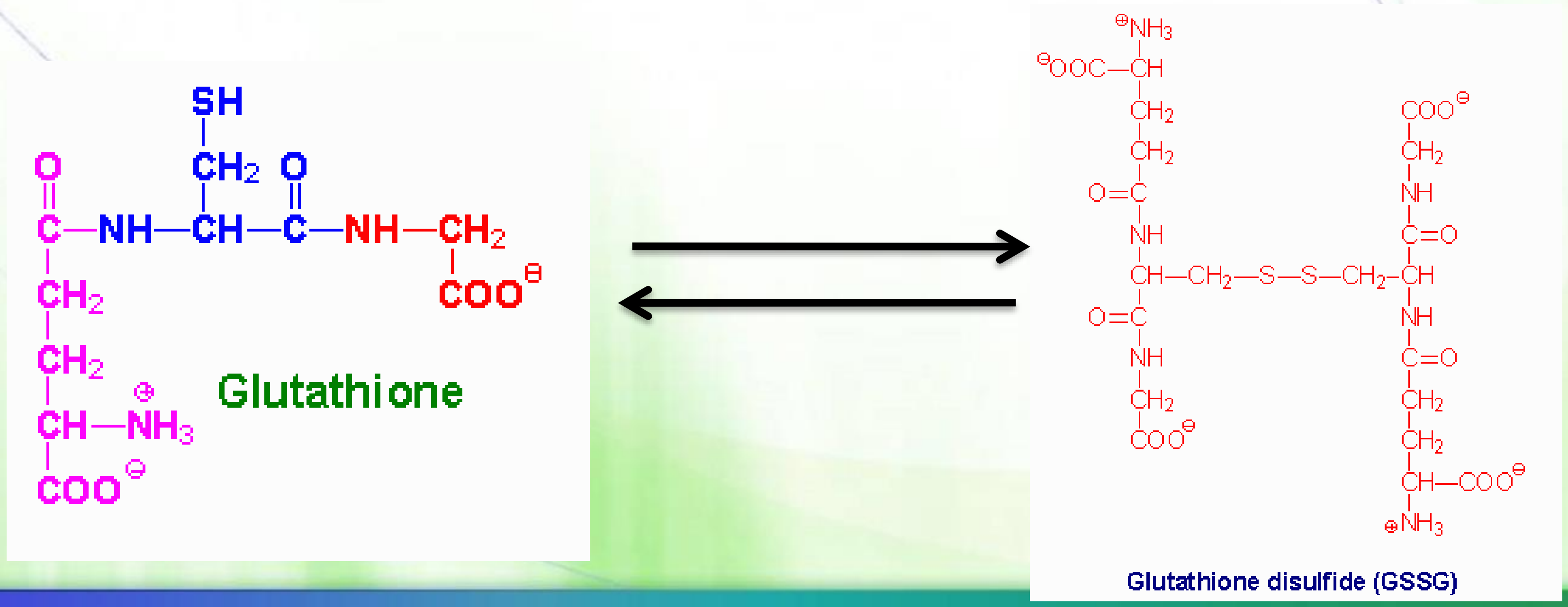
β-D-glucose + $O_2 \leftrightarrows$ gluconolactone + H_2O_2



1c. Peroxidases

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

$$2\ \mathrm{GSH} + \mathrm{H_2O_2} \leftrightarrows \mathrm{G\text{-}S\text{-}S\text{-}G} + 2\ \mathrm{H_2O}$$

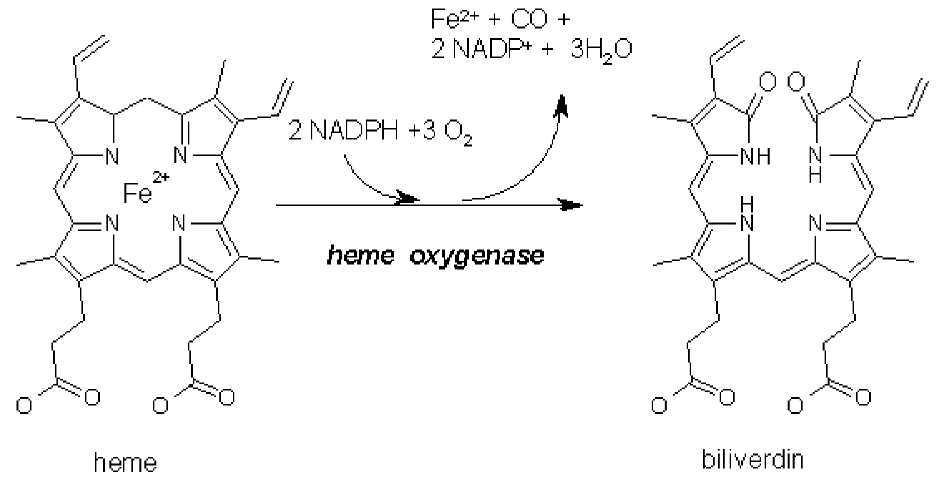


1d. Oxygenases

- Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate.
- The reduced product is water, not H_2O_2.
- Monoxygenase vs. dioxygenase
- Example 1: oxidation of lactate to acetate by lactate-2-monooxygenase.

$$CH_3\text{-}CH(OH)\text{-}COOH + O_2 \leftrightarrows CH_3COOH + CO_2 + H_2O$$

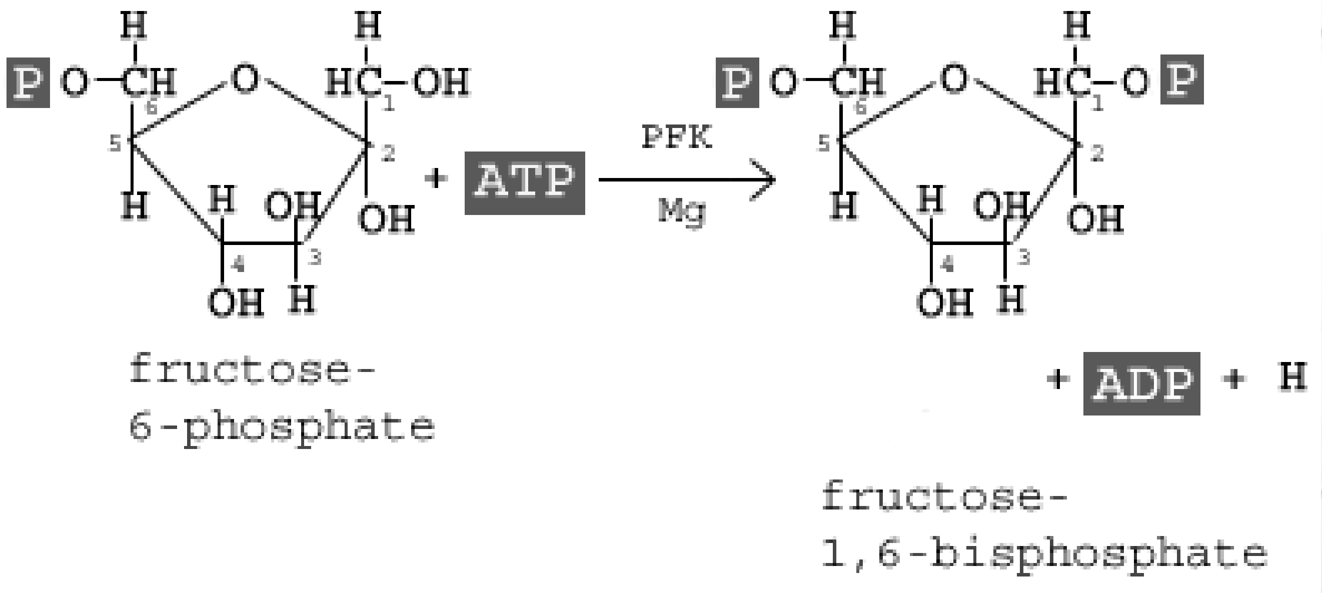
- Example 2: heme dioxygenase



2. Transferases

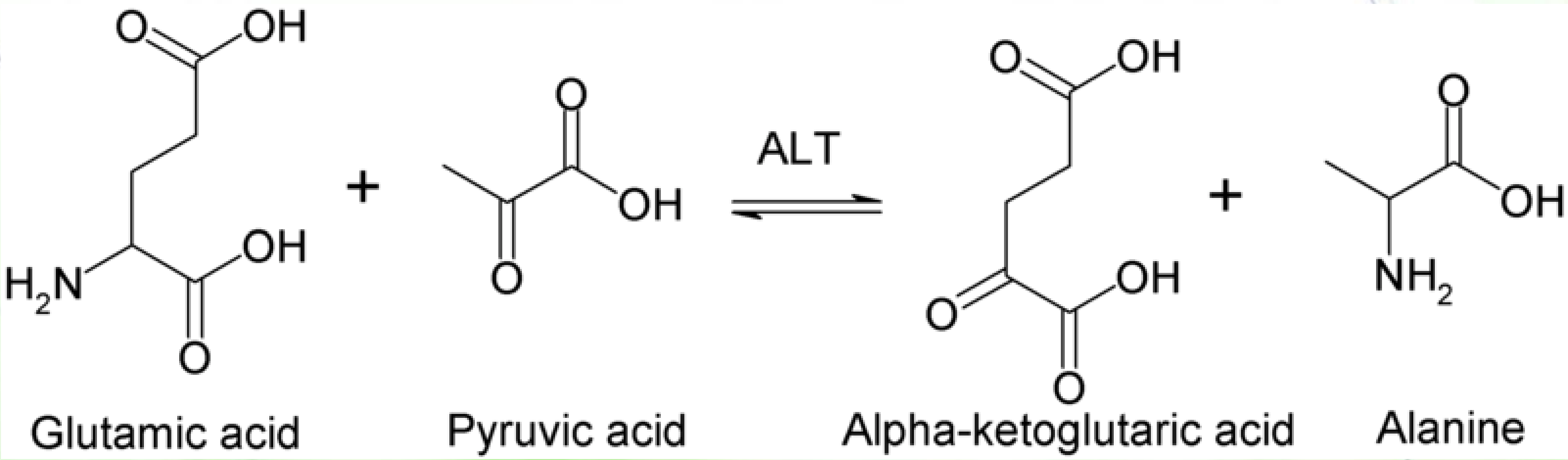
- These enzymes transfer a functional group (C, N, P or S) from one substrate to an acceptor molecule.
- Example: Kinases (the transferred group is a phosphate)
  - Phosphofructokinase catalyzes transfer of phosphate from ATP to fructose-6-phosphate:

Fructose 6-P + ATP ↔ F 1,6 bisphosphate + ADP



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.

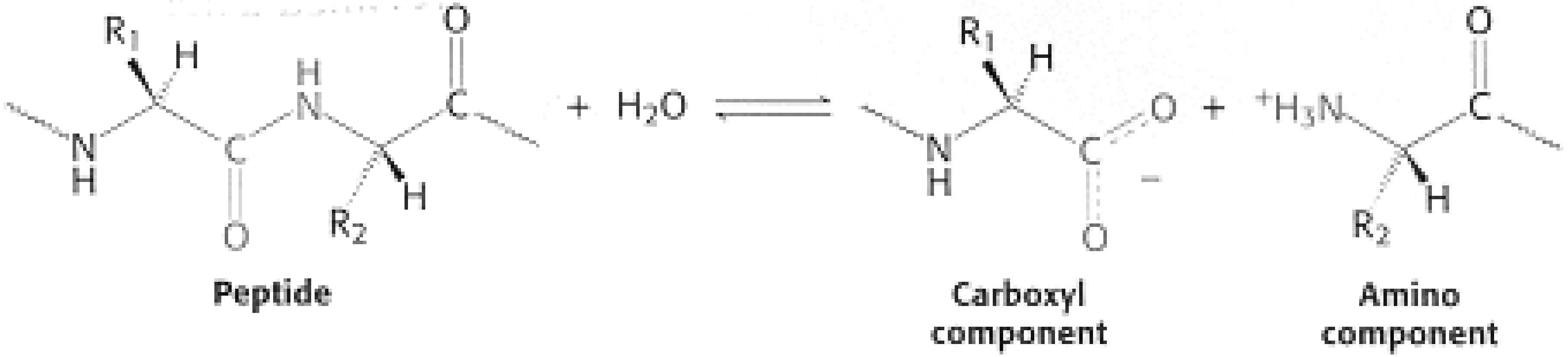


3. Hydrolases

- They catalyze cleavage reactions using water across the bond being broken or the fragment condensations.
  - Peptidases, esterases, lipases, glycosidases, phosphatases are all examples of hydrolases named depending on the type of bond cleaved .

Example: proteases

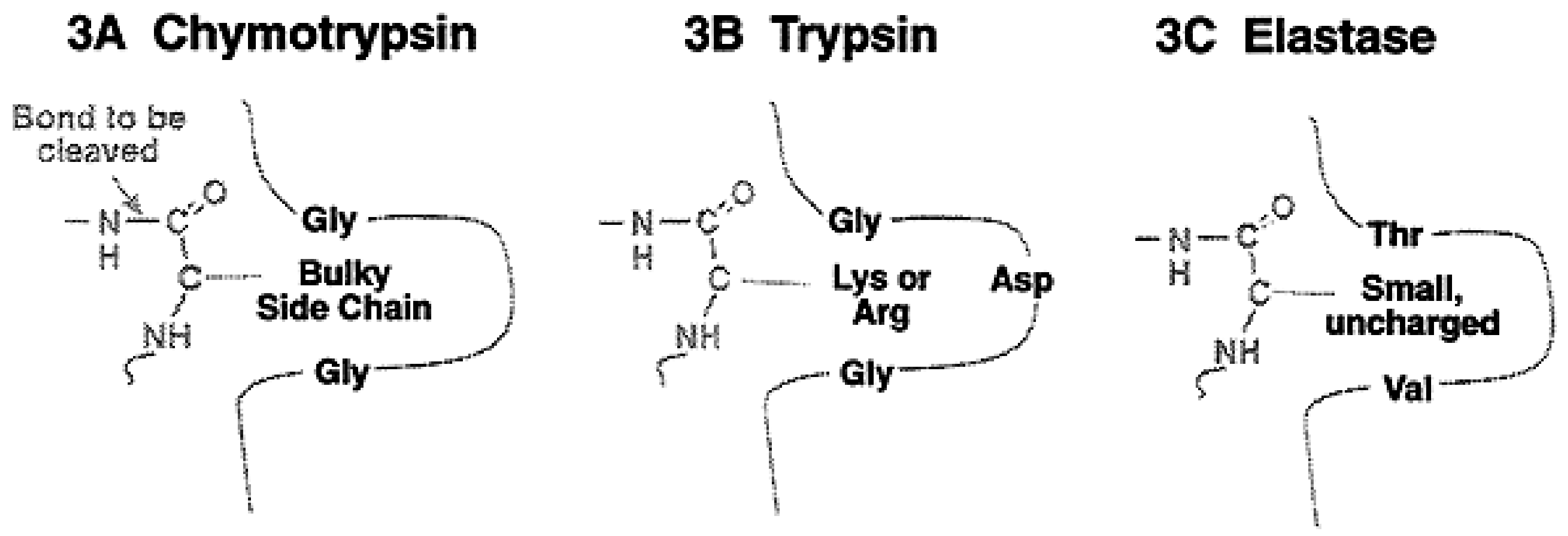
- A class of hydrolytic enzymes is proteases.
- These enzymes catalyze proteolysis, the hydrolysis of a peptide bond within proteins.



- Proteolytic enzymes differ in their degree of substrate specificity.

Specific exmaples

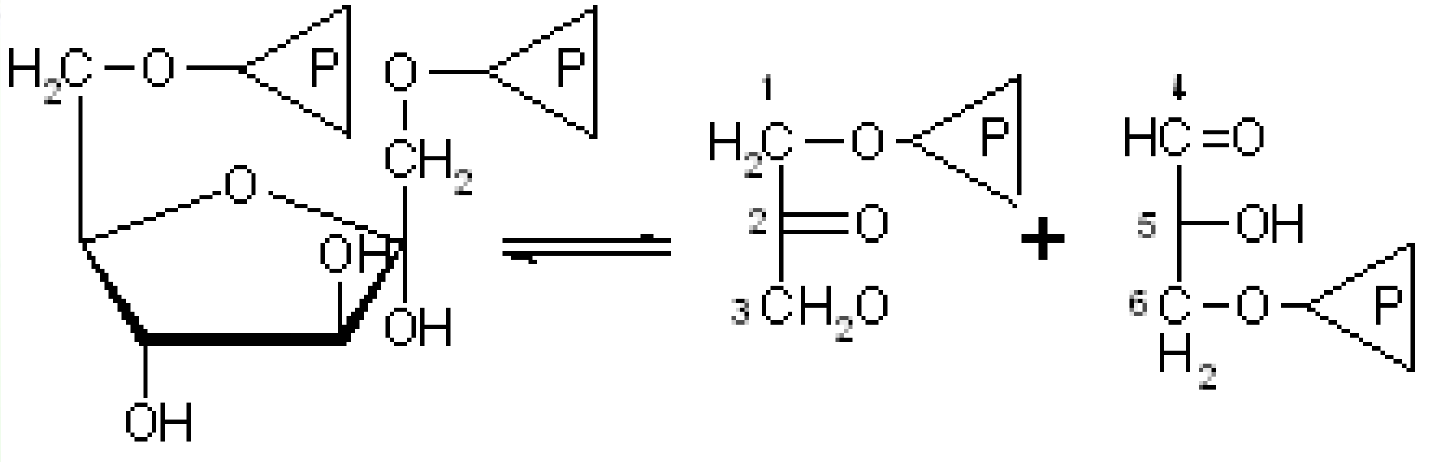
- Trypsin, a digestive enzyme, breaks up peptide bonds only on the carboxyl side of lys and arg residues.
- Thrombin participates in blood clotting by catalyzing the hydrolysis of Arg-Gly bonds.



4. Lyases

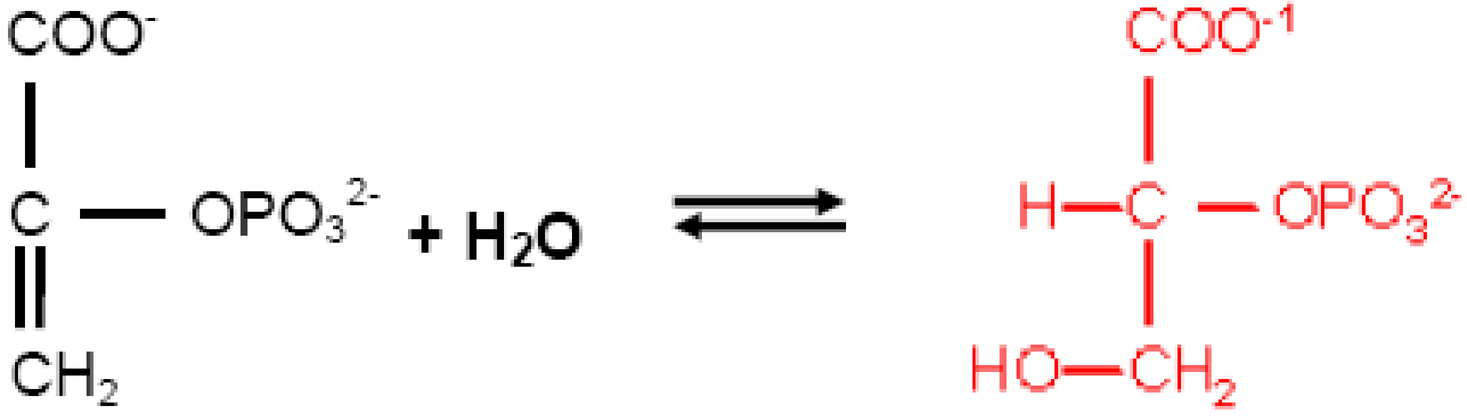
- These enzymes remove groups from substrates with the associated formation or removal of double bonds between C-C, C-O and C-N without hydrolysis.
- Example: aldolase breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate.

F 1,6 bisphosphate ⇆ DHAP + GAP



Example: enolase

- Enolase interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation or removal of double bonds.

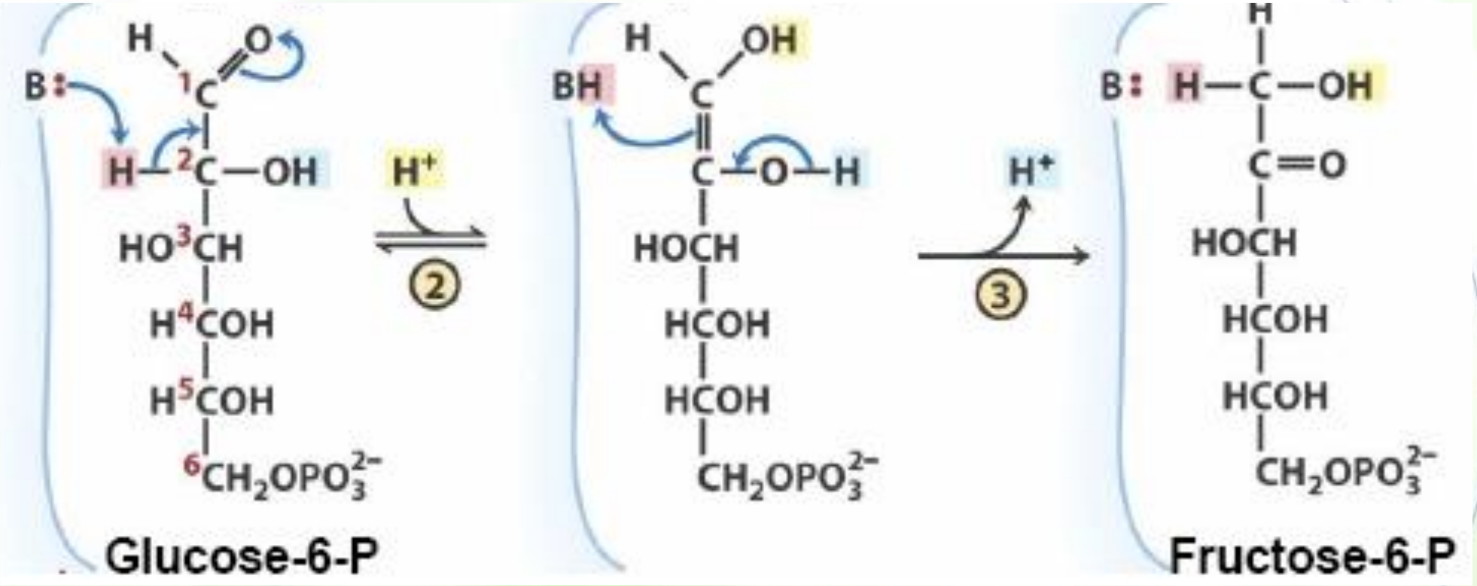


PEP

2-Phosphoglycerate

5. Isomerases

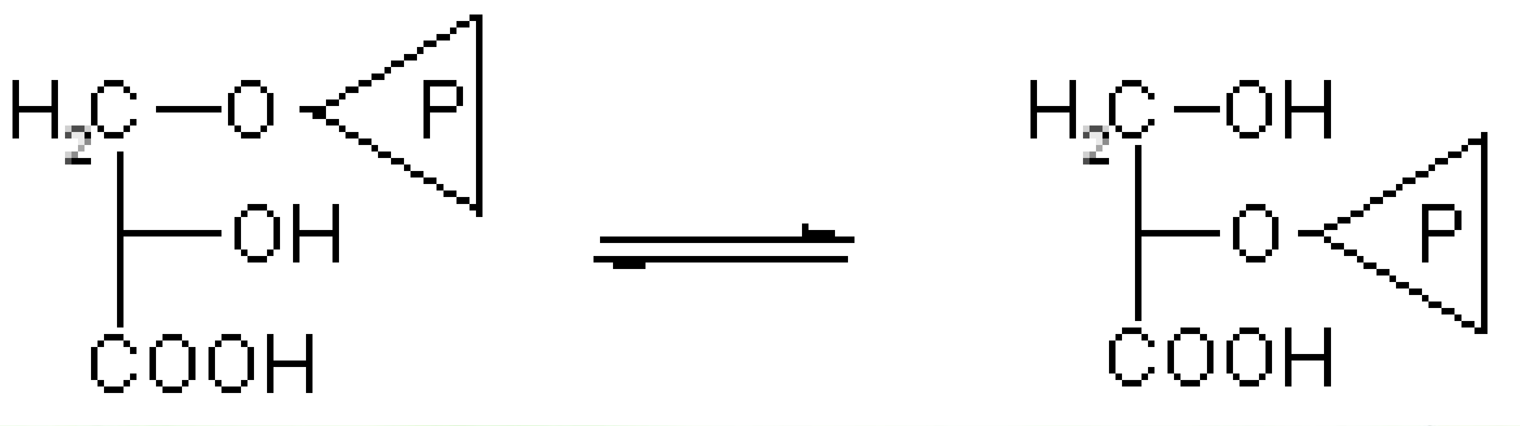
- These enzymes catalyze intramolecular rearrangements.
- Phosphoglucoisomerase isomerizes glucose-6-phosphate to fructose-6-phosphate.



Example: posphoglycerate mutase

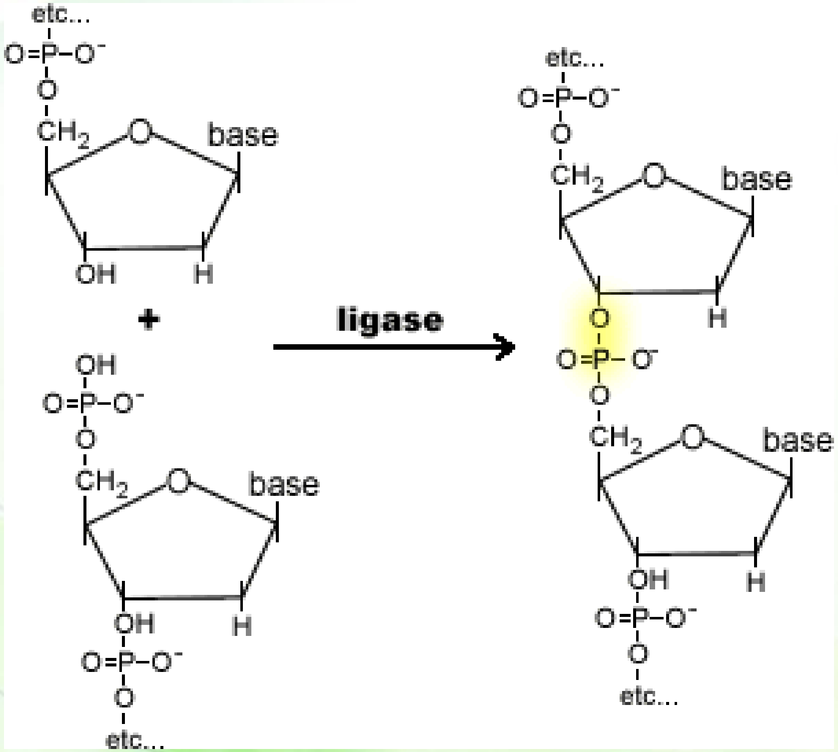
- Posphoglycerate mutase transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate:

3-P glycerate ⇆ 2 P 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.



Example: pyruvate carboxylase

- Example: pyruvate carboxylase:

  pyruvate + HCO_3^- + ATP ⇆ Oxaloacetate + ADP + Pi