Enzymes I

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Summer semester, 2017-2018

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

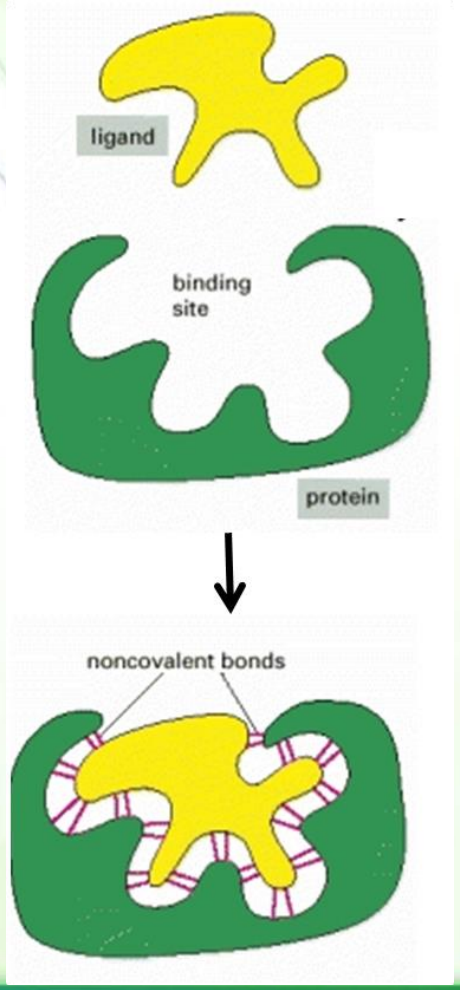
General properties of proteins

- 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 without being consumed in the process.

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

- In general, EP is not considered

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

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)

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2\,(g)$$

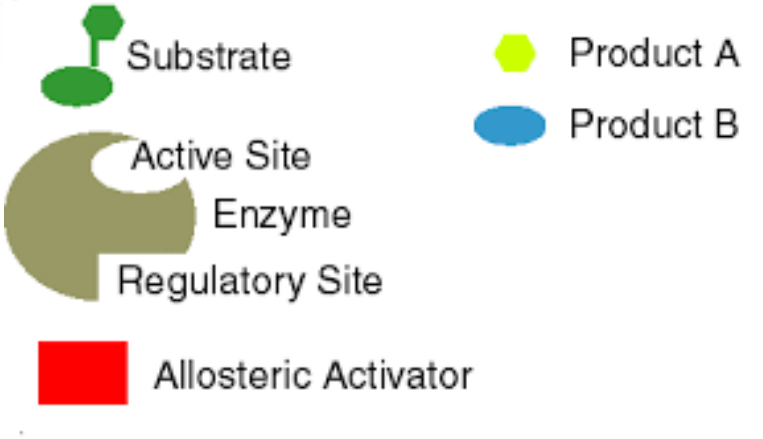
$$CO_2 + H_2O \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

  - Carbonic anhydrase (one of the fastest enzymes known)
    - One enzyme molecule hydrates 10^7 molecules of CO2 per second (versus 10^2 to 10^4 for uncatalyzed reactions)

Reaction Conditions	Activation Free Energy		Relative Rate
	kJmol^{-1}	kcal mol^{-1}	
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

Active sites of enzymes

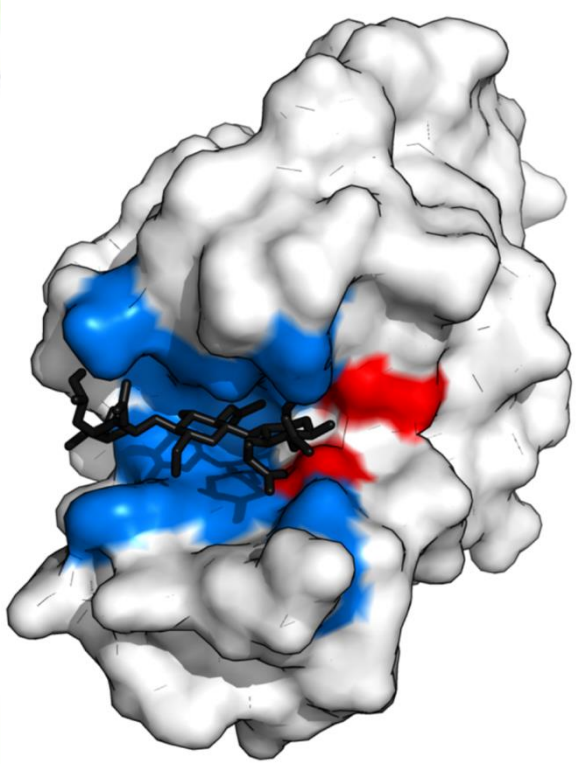
- Each enzyme has a specific three-dimensional shape that includes the ***active site*** (a region where the biochemical reaction takes place).



- **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.

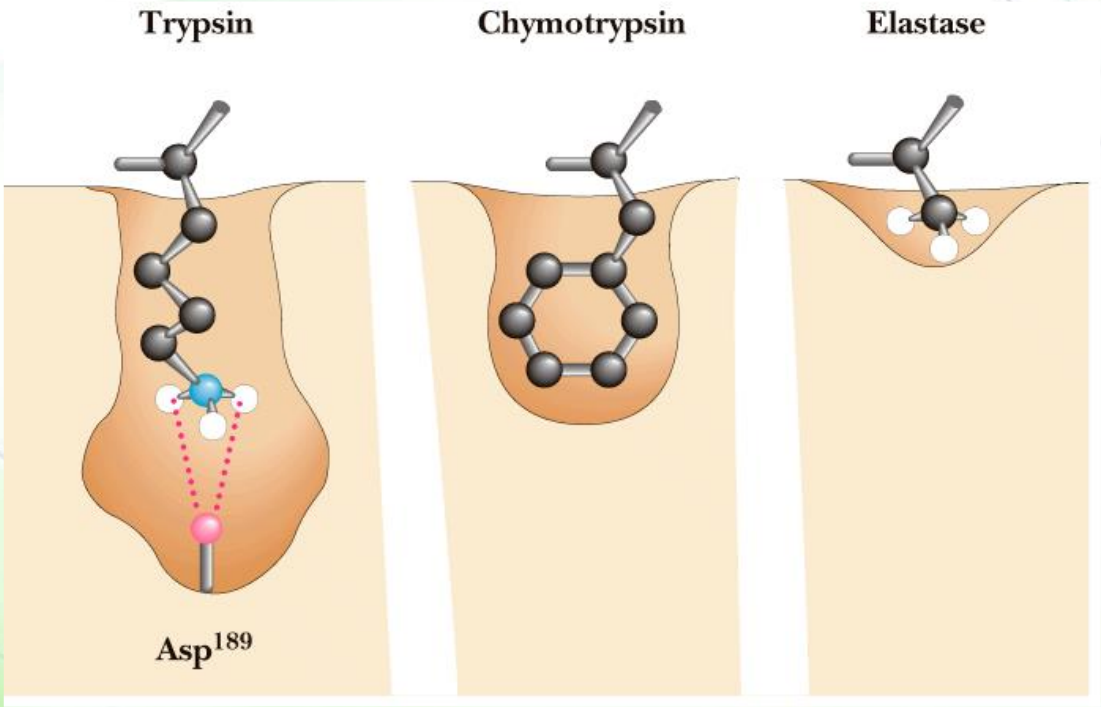


ACTIVE SITE

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

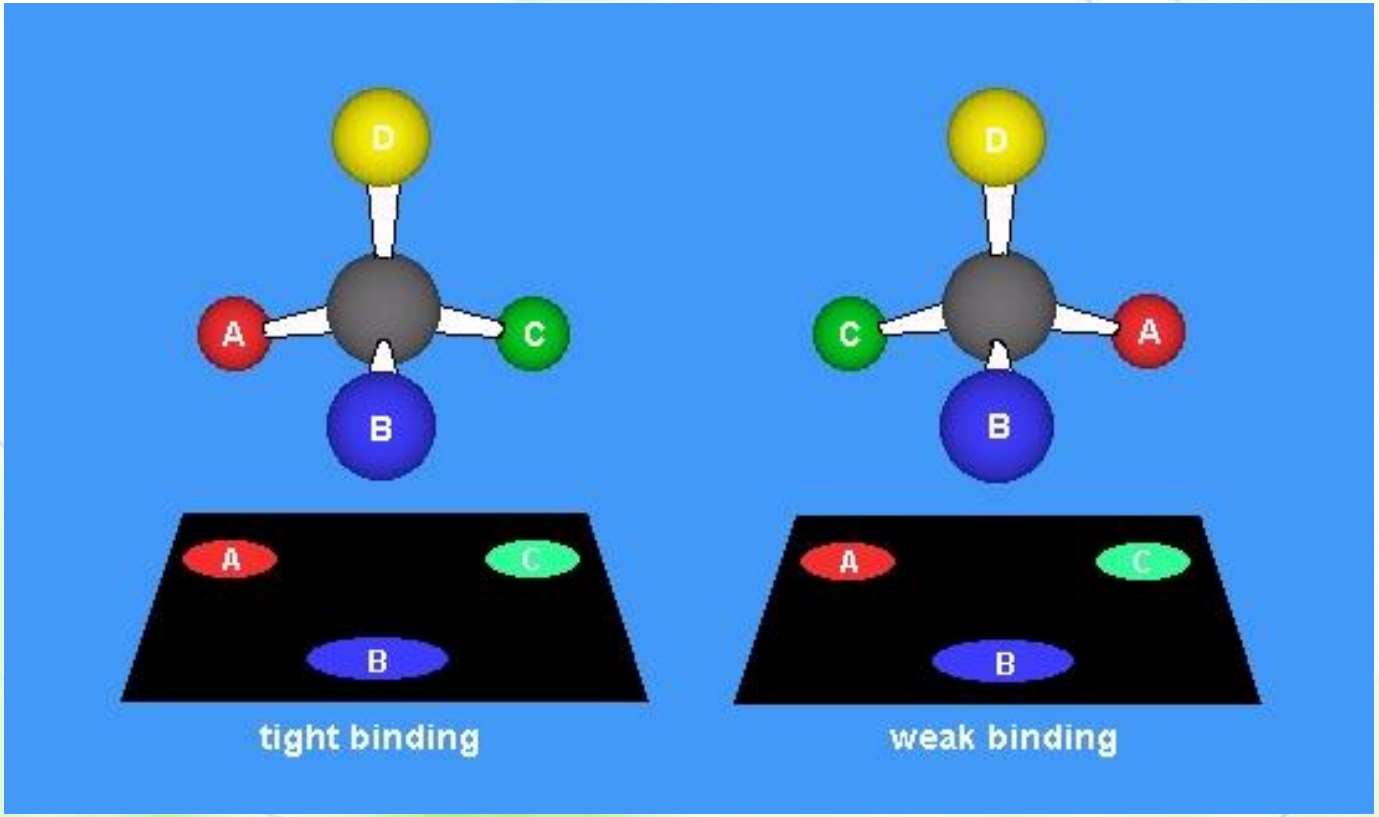
Binding specificity

- The specificity and selectivity of enzymes is due to their precise interaction to substrate promoting the formation of the ***transition states***.



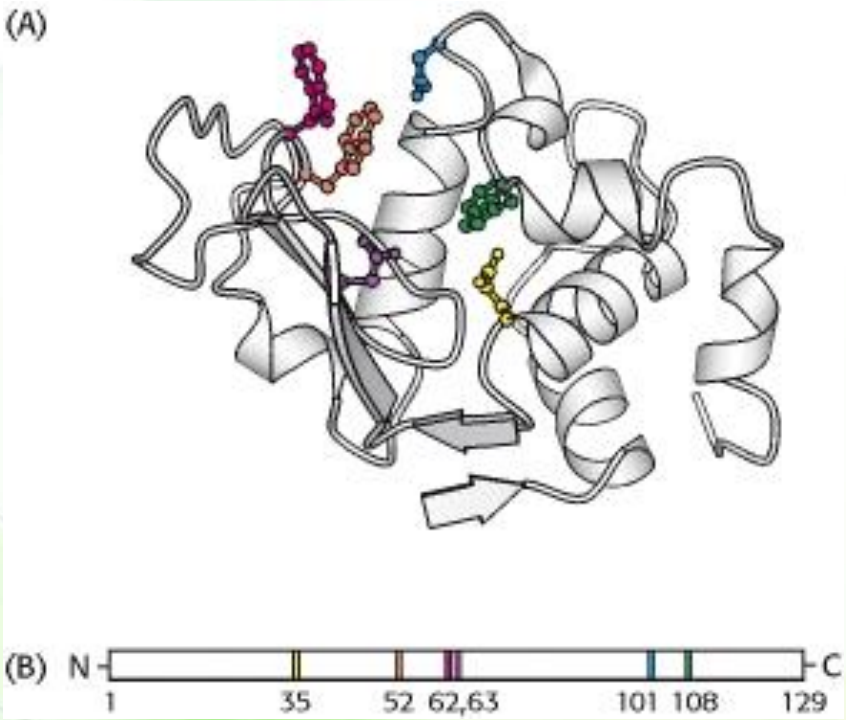
Features of active site 1

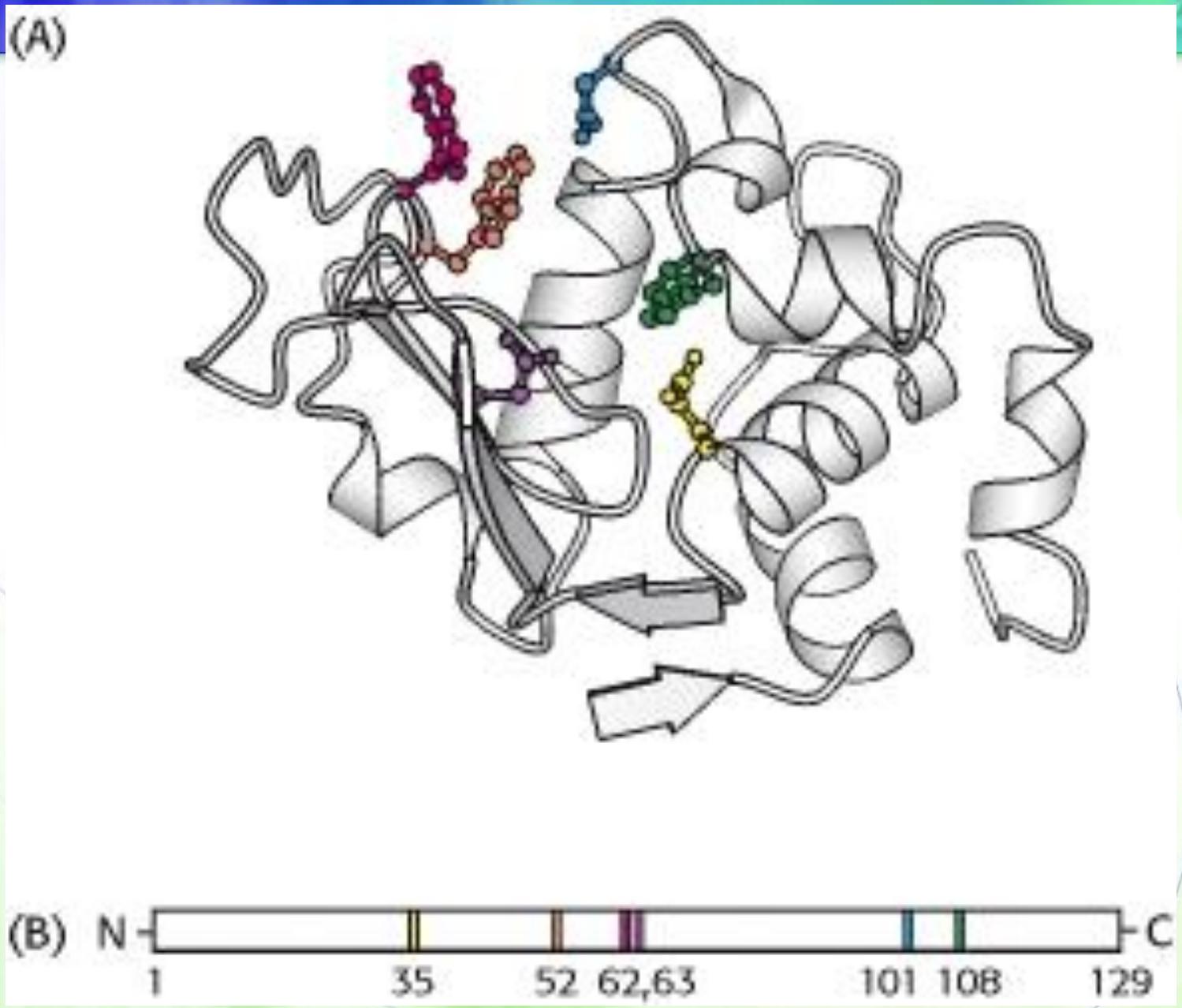
- Binding occurs at least three points.
  - This illustrates the importance of chirality.



Features of active site 2

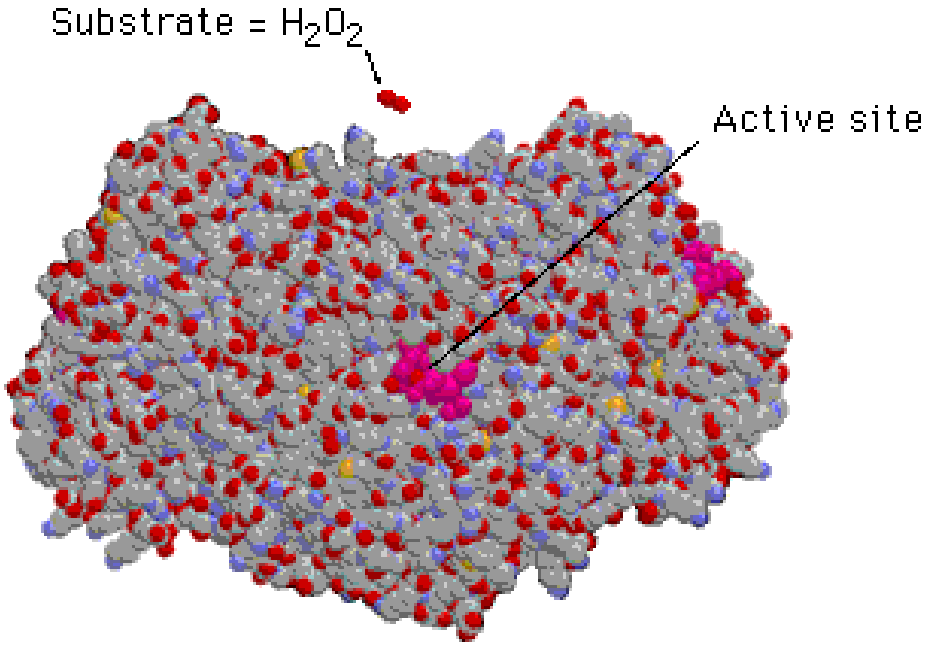
- It is a three-dimensional pocket or cleft formed by groups that come from different parts of the primary structure usually forming a domain.




(A)
(B)
N
C
1
35
52
62,63
101
108
129

Features of active site 3

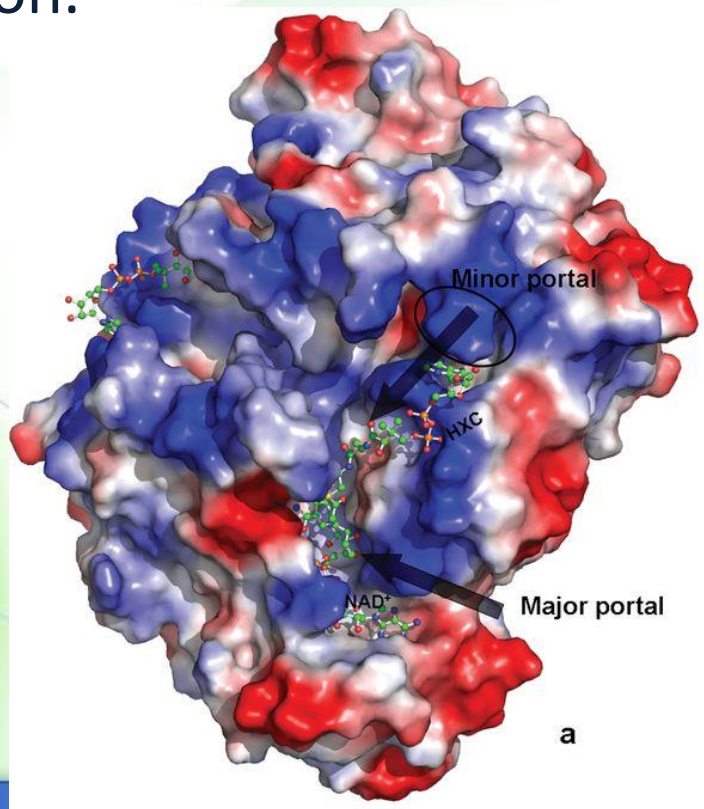
- It takes up a relatively small part of the total volume of an enzyme.
- The “extra” amino acids help create the three-dimensional active site.
- **In many proteins, the remaining amino acids may make up regulatory sites.**

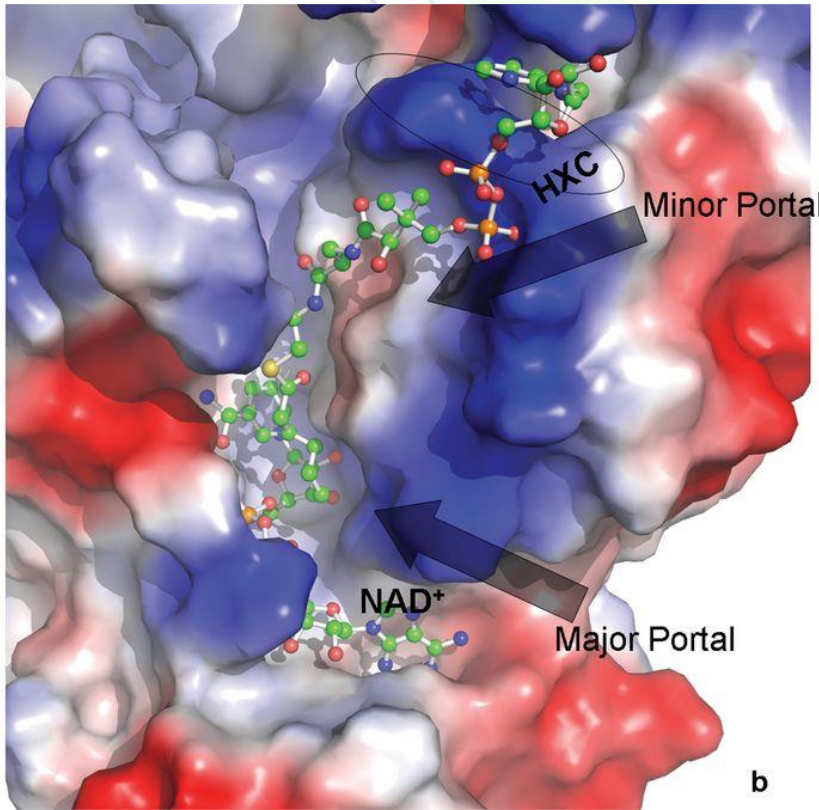


Molecular model of catalase

Features of active site 4

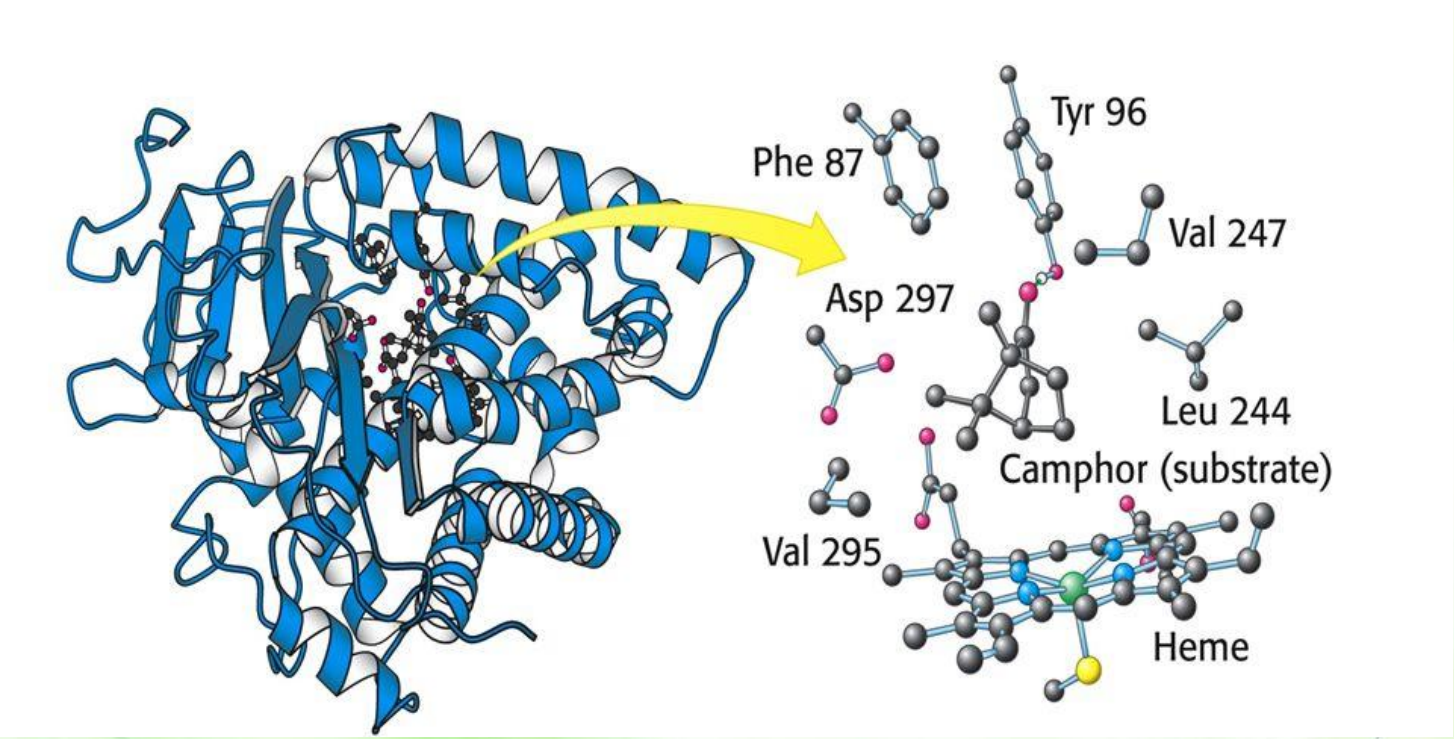
- It is structure that looks like a canal, cleft, or crevice.
- The cleft usually contains nonpolar amino acids, but it may also contain polar residues.
- Water is usually excluded unless it participates in the reaction.





Features of active site 5

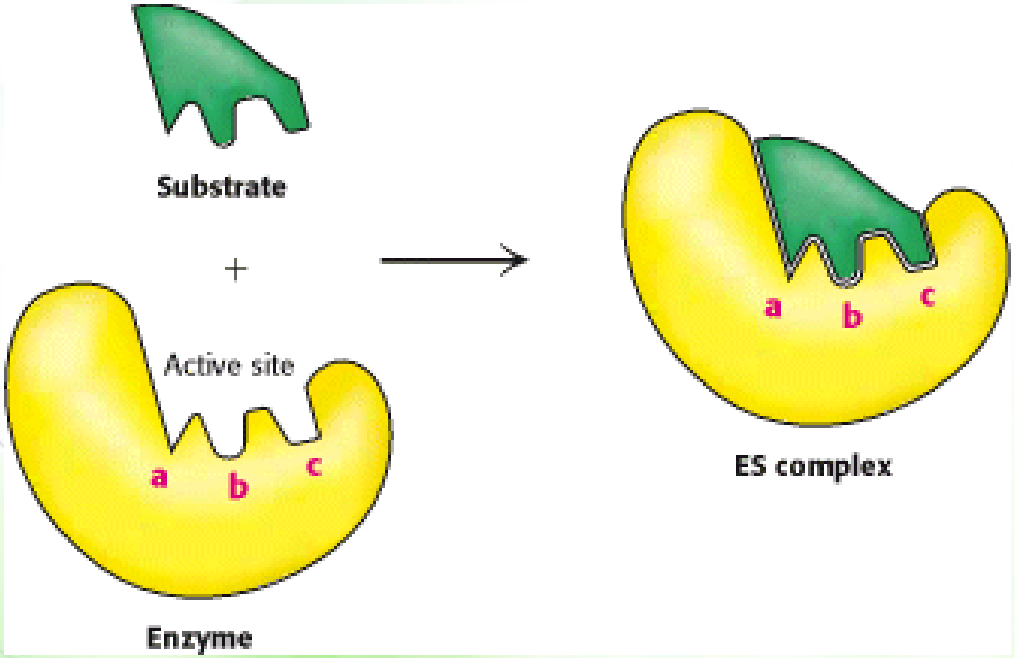
- Substrates are bound to enzymes by multiple weak attractions including electrostatic interactions.



How do substrates fit into the active site of enzymes?

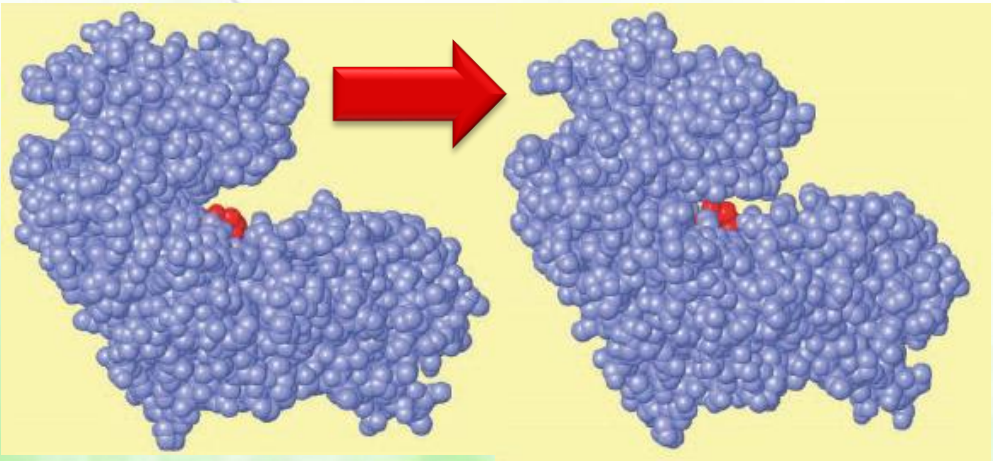
Lock-and-key model (old)

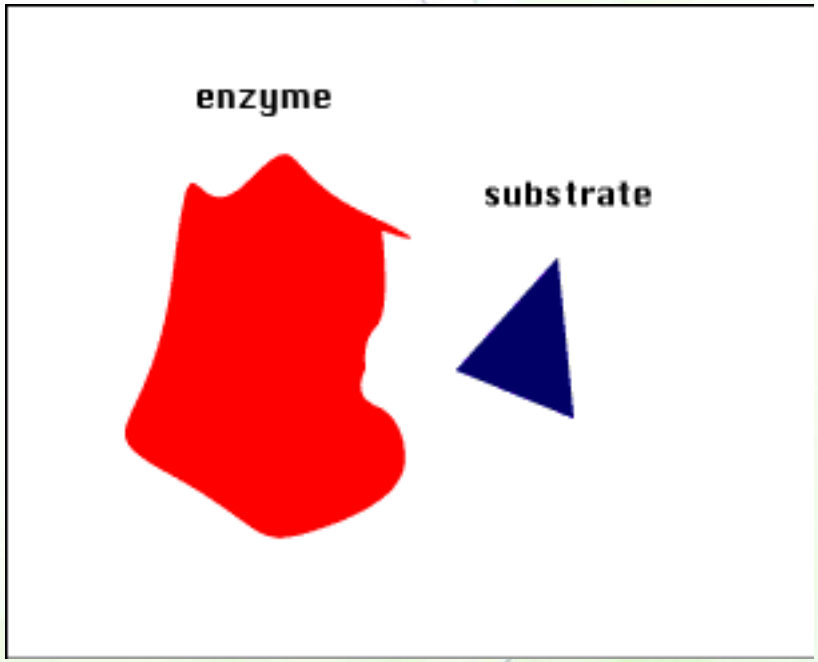
- The first is known as lock-and-key model where the substrate fits directly into the active site.
- Not really!!



Induced fit model

- The induced fit model indicates that enzymes are flexible and that the shapes of the active sites can be modified by the 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.

Free energy (G)

- All molecules have energy within them.
- This is known as free energy or *G* (for Josiah Gibbs).
- It indicates the energy of a system that is available for useful work.
- In any given reaction (reactants $\rightarrow$ products), the difference between the free energy values between reactants and products (free-energy change ΔG) is

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

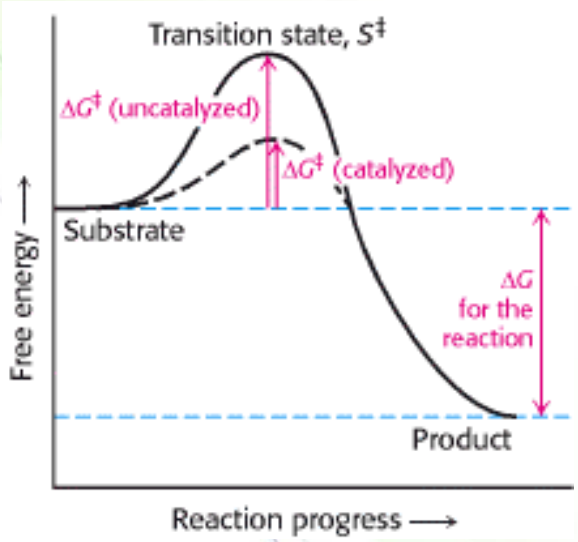
What does it mean?

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

- 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?

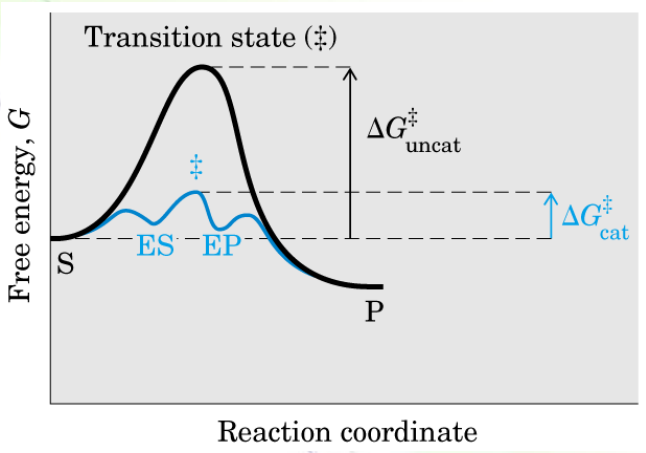
- The free-energy difference between reactants and products accounts for the equilibrium of the reaction and enzymes accelerate how quickly this equilibrium is reached.
- Any enzymatic reaction whether endergonic or exergonic goes through a transition state (ES) that has a higher free energy than does either S or P.
- The difference in free energy of the transition state and the substrate is called the ***activation energy***.
- Enzymes function to lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state at a lower energy.

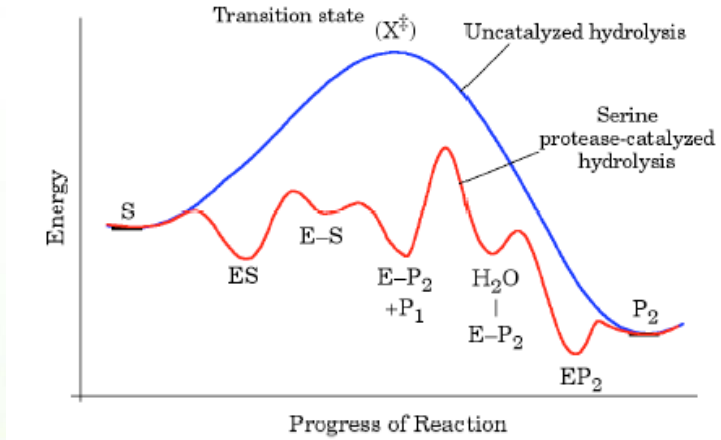


- **At the highest energy level, the substrate configuration is most unstable and is most tightly bound to the enzyme.**
- **The bonds or the electronic configuration are maximally strained.**

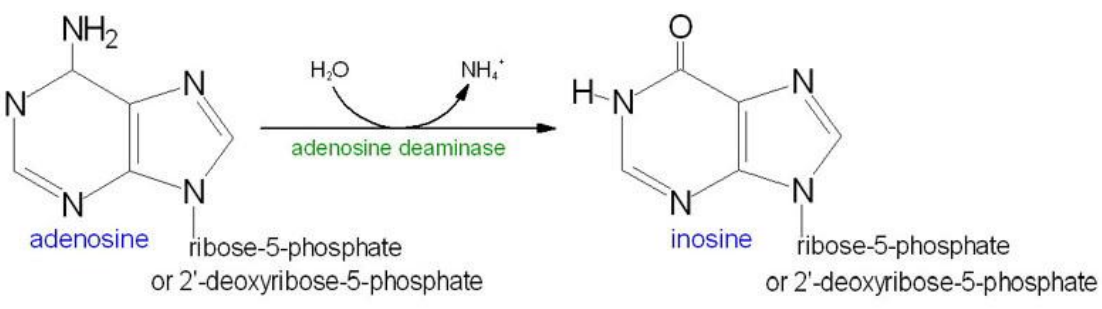
Alternative pathways

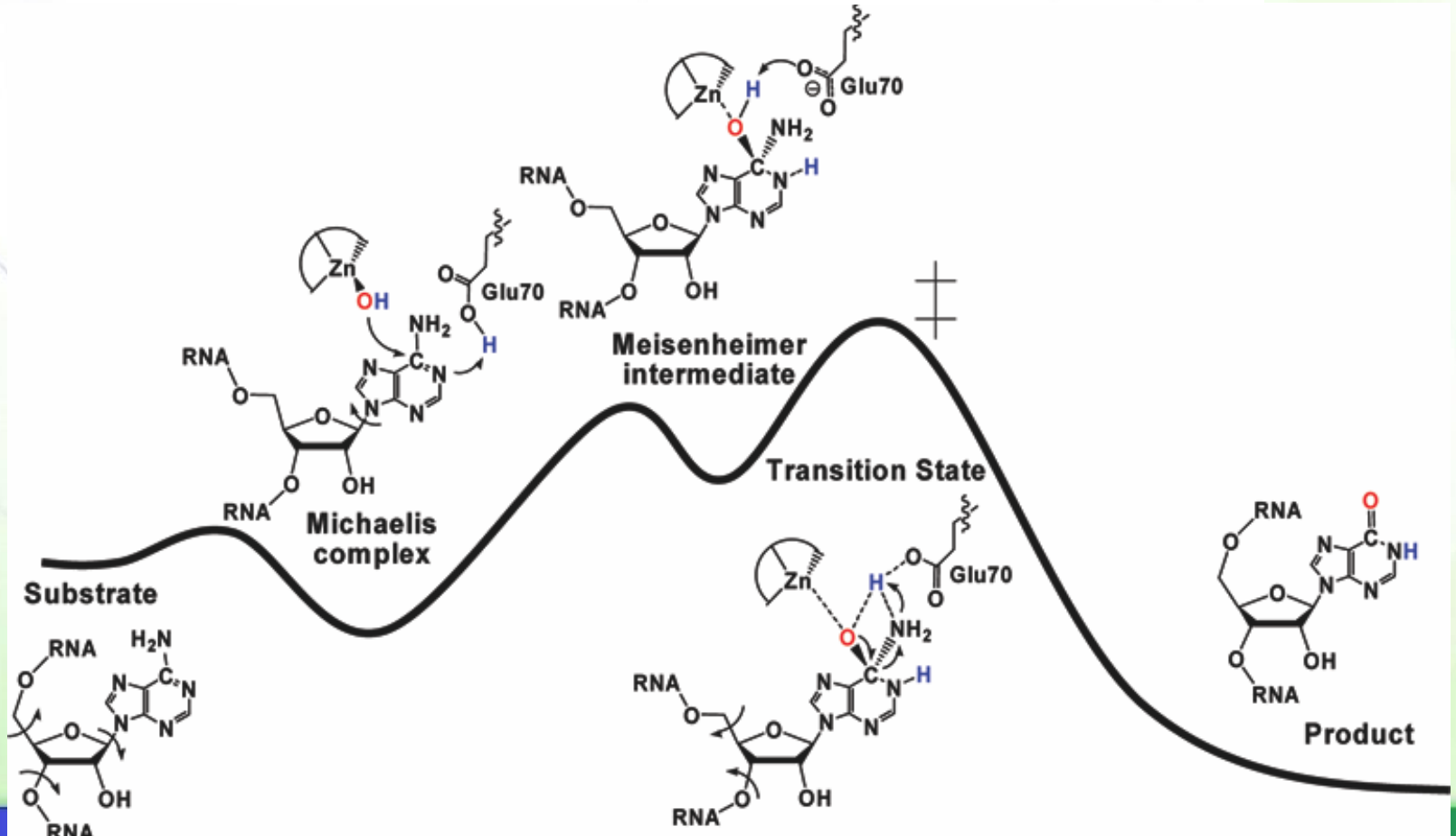
- Substrates of enzymatic reactions often undergo several transformations when associated with the enzyme and each form has its own free energy value.
- The activation energy corresponds to the complex with the highest energy.
- The energy of activation does not enter into the final ΔG calculation for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





Example: *Adenosine Deaminase*



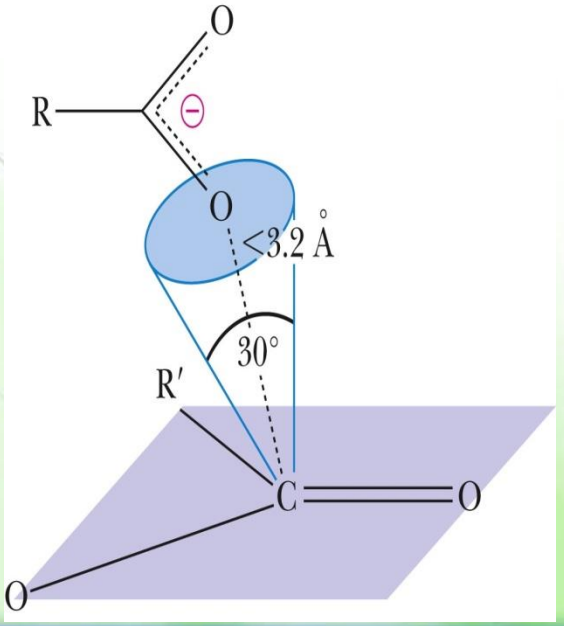


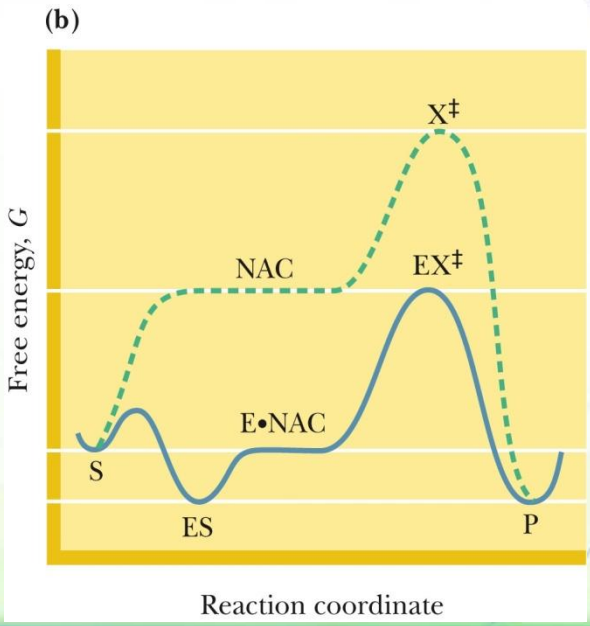
Enzyme-substrate interactions

- After binding takes place, one or more mechanisms of catalysis generates transition- state complexes and reaction products.
- The possible mechanisms of catalysis are four.

Catalysis by proximity and orientation

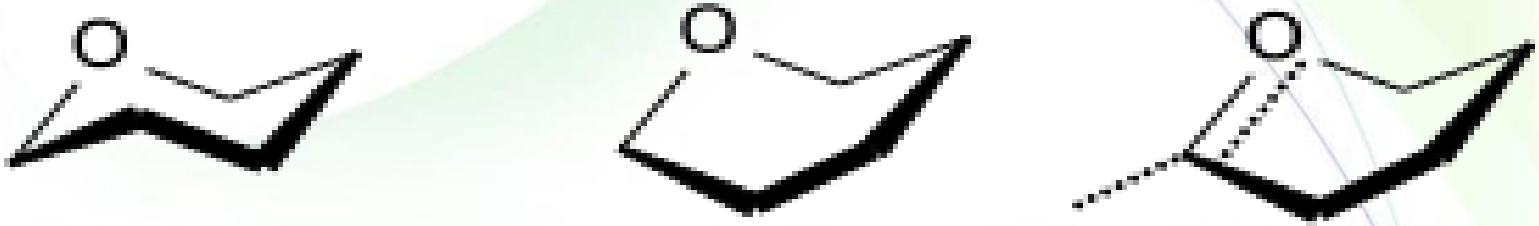
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis.
  - Such arrangements are termed near-attack conformations (NACs).
  - NACs are precursors to reaction transition states.





Catalysis by bond strain

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

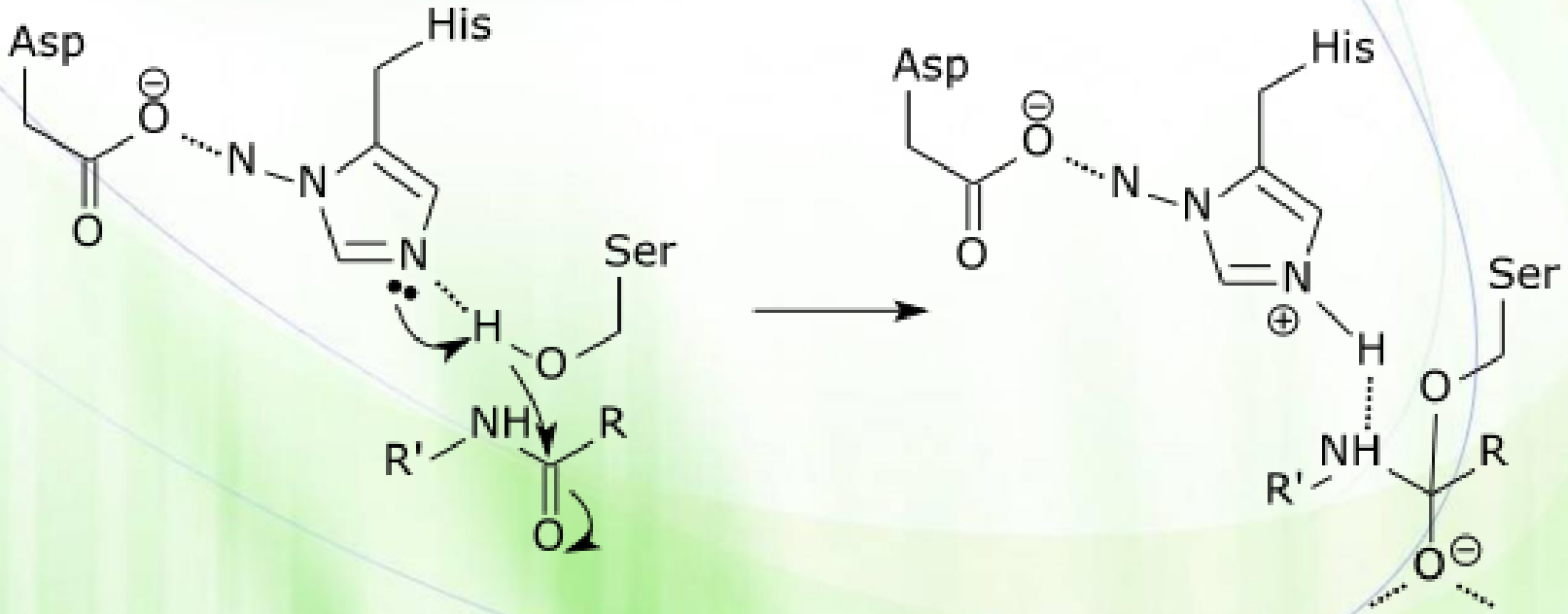


- Example: lysozyme

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.

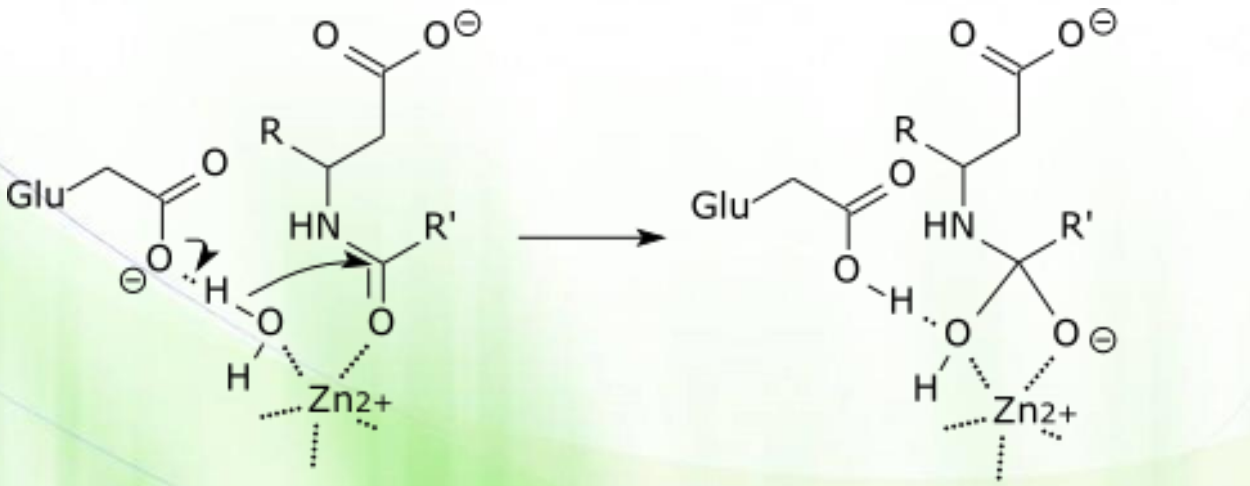
Catalysis involves acid/base reactions

- 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

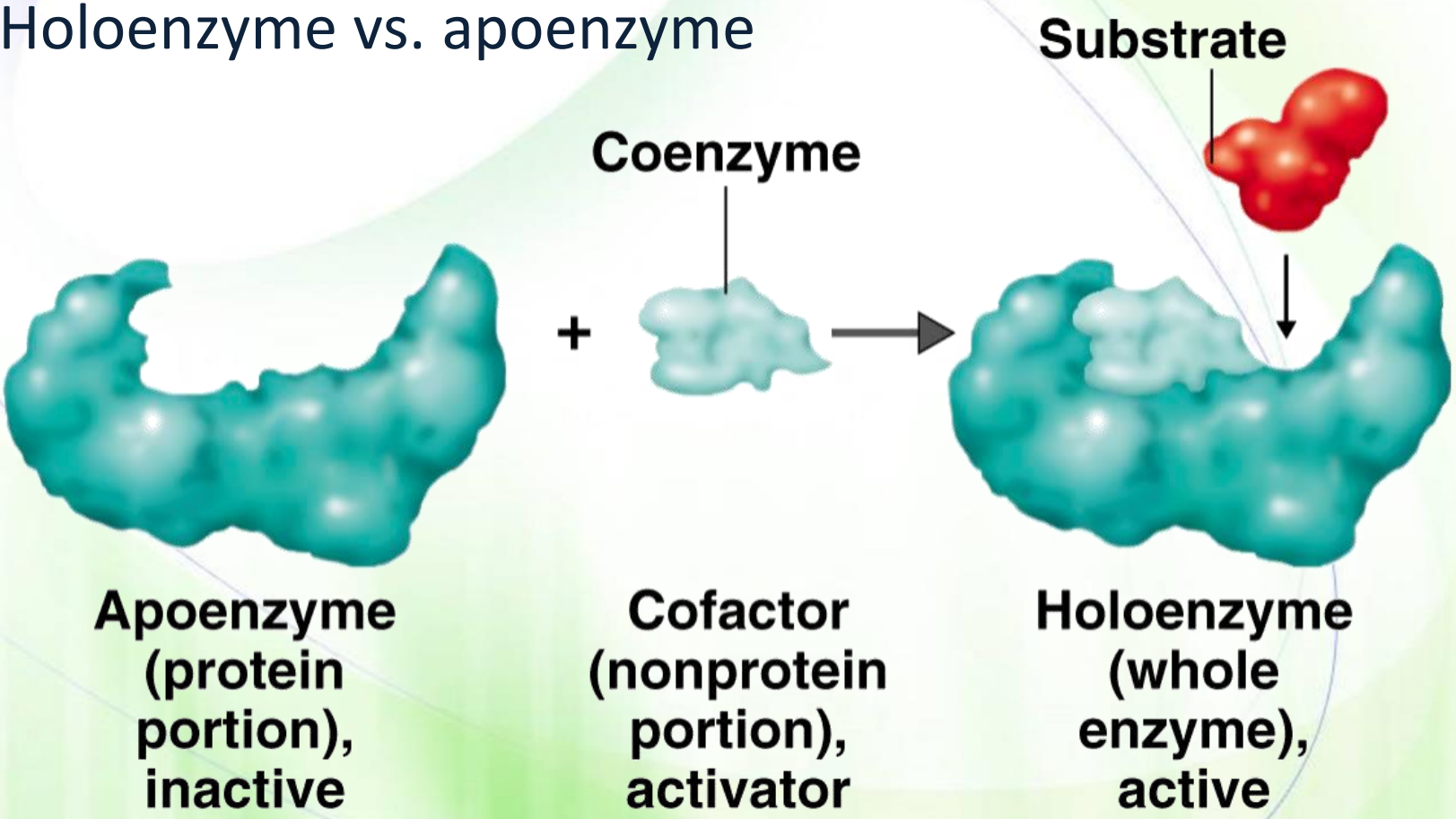
- A covalent intermediate forms between the enzyme or coenzyme and the substrate.
  - Examples of this mechanism is proteolysis by serine proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase).



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.
  - An ATPase is an enzyme that breaks down ATP, whereas ATP synthase is an enzyme that synthesizes ATP.
- Some enzymes have common names
  - Examples: the proteolytic enzyme trypsin.

Enzyme classes

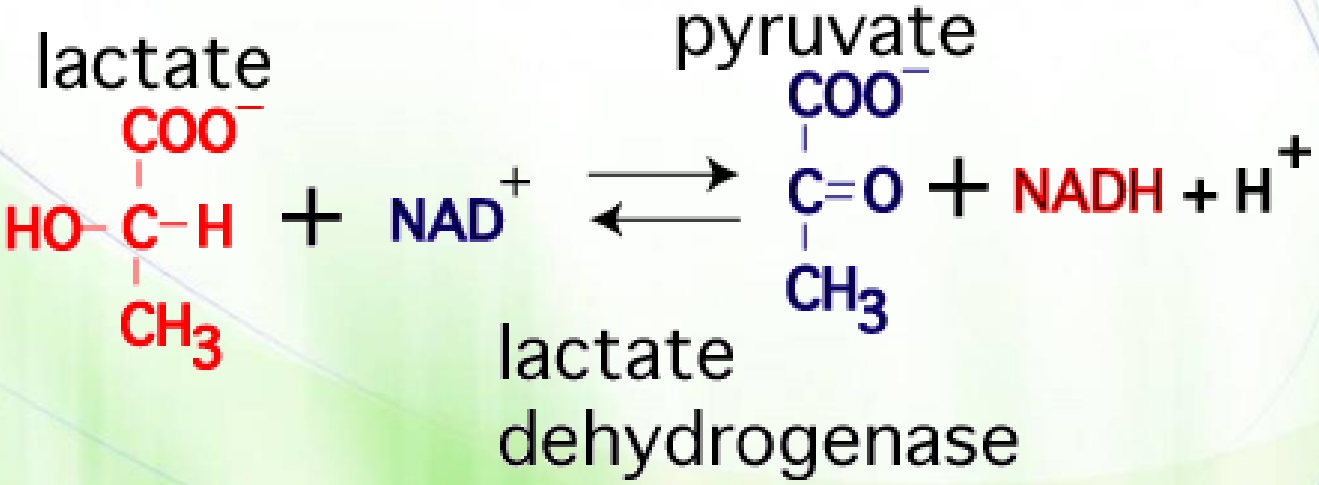
- Enzymes are classified into six major groups:
  - Oxidoreductases
  - Transferases
  - Hydrolases
  - Lyases
  - Isomerases
  - Ligases

1. Oxidoreductases

- These enzymes catalyze oxidation and reduction reactions involving the transfer of hydrogen atoms or electrons.
- This group can be further divided into 4 main classes:
  - Dehydrogenases
  - Oxidases
  - Peroxidases
  - Oxygenases

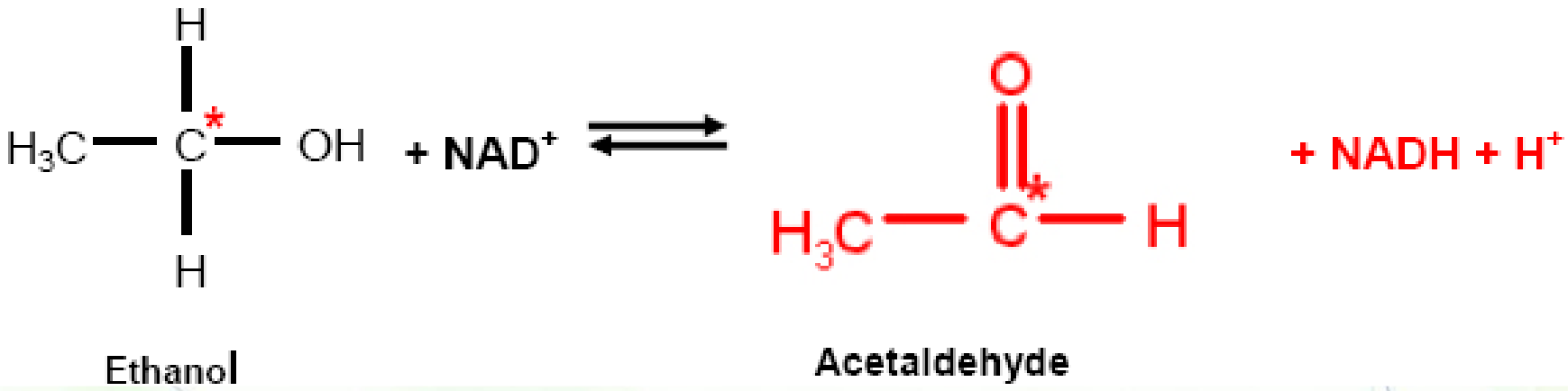
1a. Dehydrogenases

- Dehydrogenases catalyze hydrogen transfer from the substrate to a molecule known as nicotinamide adenine dinucleotide (NAD+)
- An example of this is lactate dehydrogenase, which catalyzes the following reaction:



Example: alcohol dehydrogenase

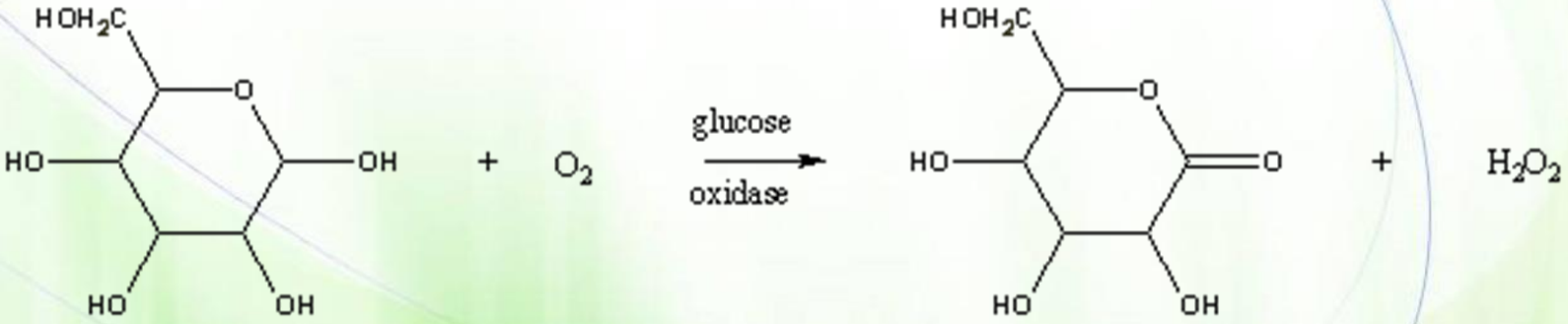
- Another example is alcohol dehydrogenase.



1b. Oxidases

- Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product.
- An example is glucose oxidase, which catalyzes the following reaction:

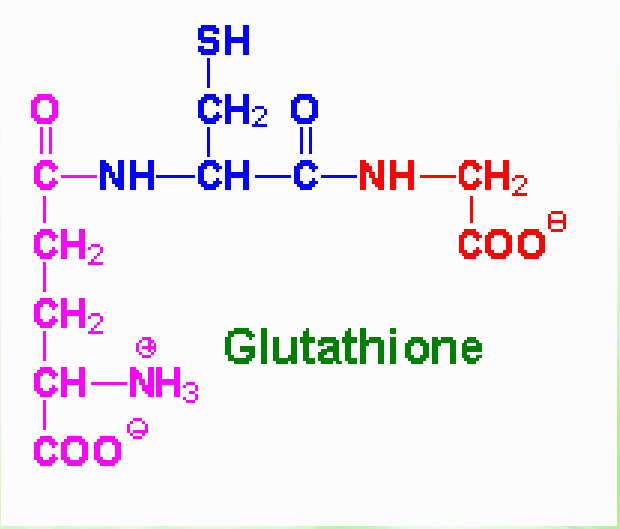
$$\beta\text{-D-glucose} + O_2 \leftrightarrows \text{gluconolactone} + H_2O_2$$

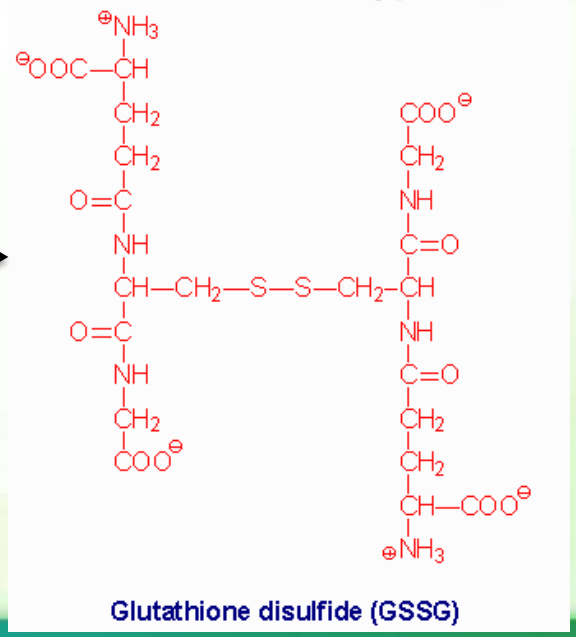


1c. Peroxidases

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

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





1d. Oxygenases

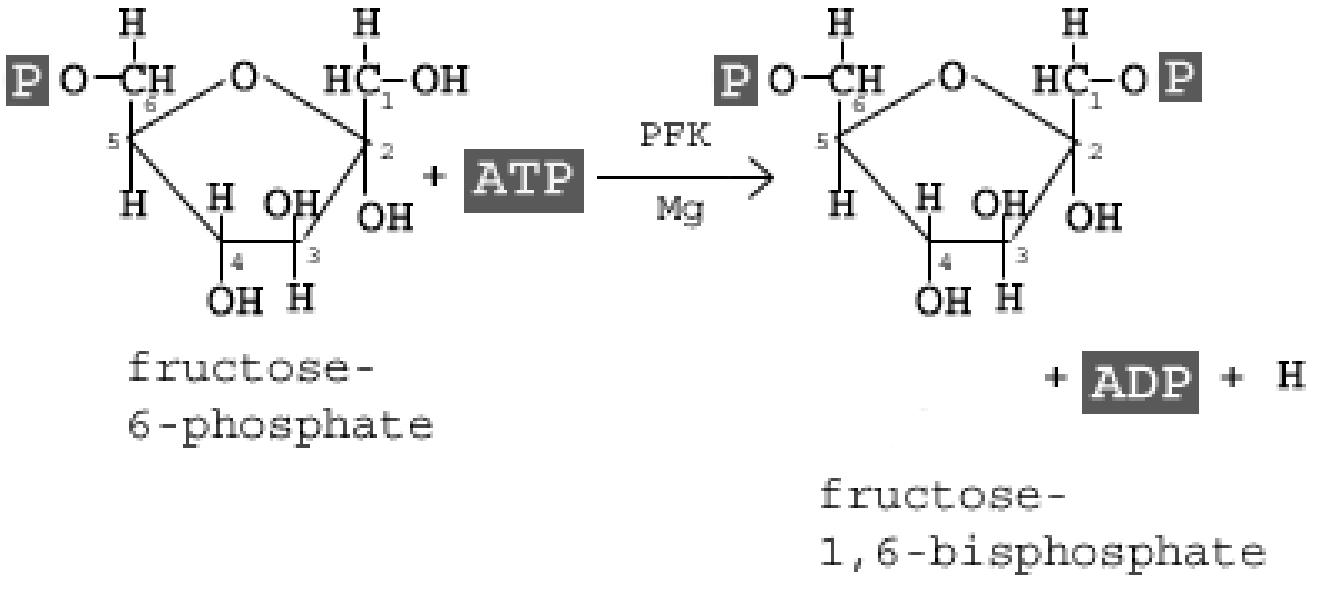
- Oxygenases catalyze substrate oxidation by molecular oxygen.
- The reduced product of the reaction in this case is water and not hydrogen peroxide.
- An example is the oxidation of lactate to acetate catalyzed by lactate-2-monooxygenase.

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

2. Transferases

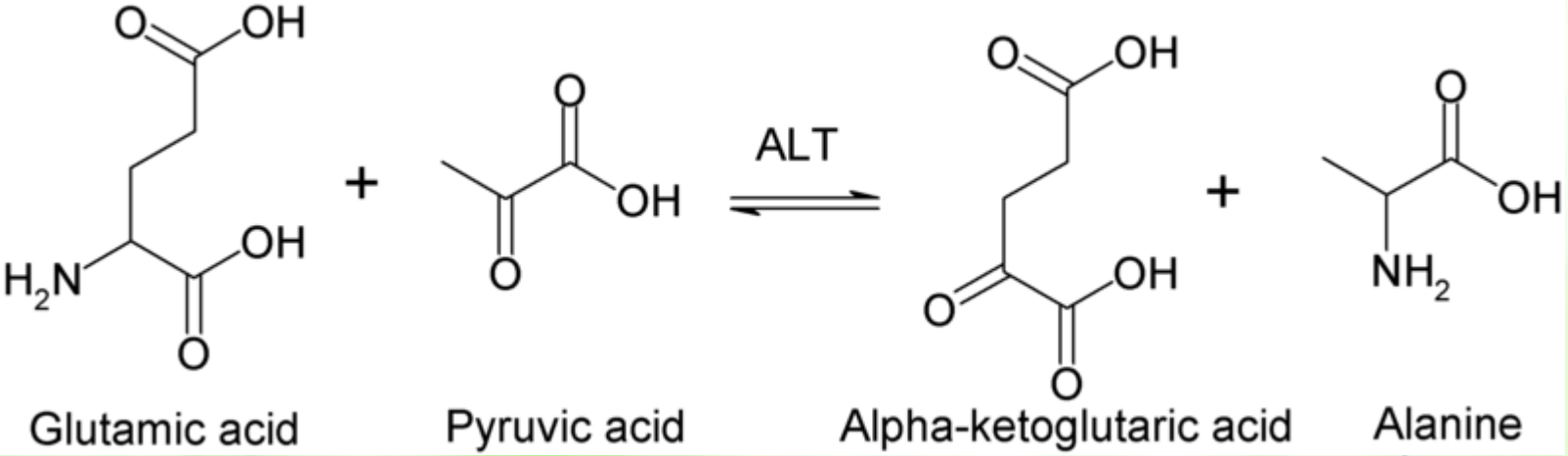
- These enzymes transfer a functional group (C, N, P or S) from one substrate to an acceptor molecule.
- An example is Phosphofructokinase, which catalyzes transfer of phosphate from ATP to fructose-6-phosphate:

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.

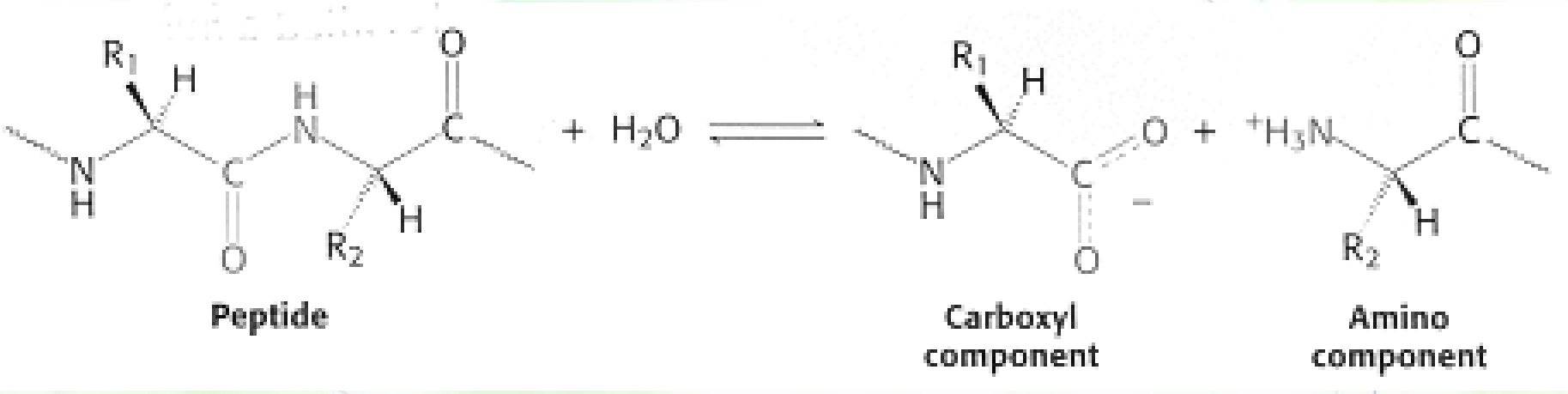


3. Hydrolases

- These enzymes catalyze cleavage reactions while 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

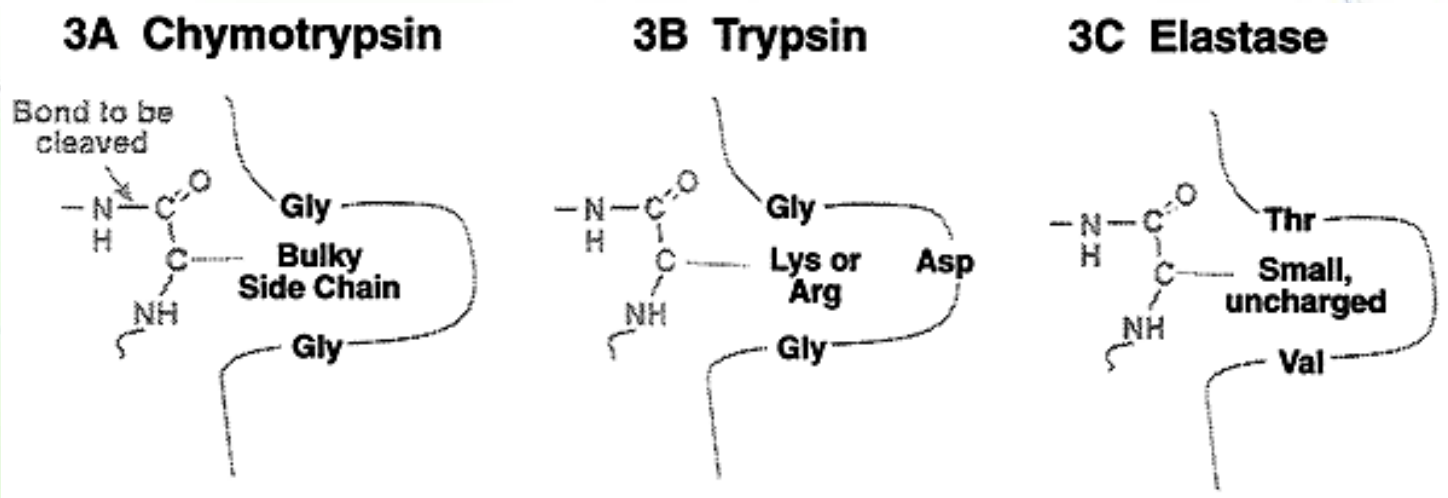
- A class of hydrolytic enzymes is proteases.
- These enzymes catalyze proteolysis, the hydrolysis of a peptide bond within proteins.



- Proteolytic enzymes differ in their degree of substrate specificity.

Specific exmaples

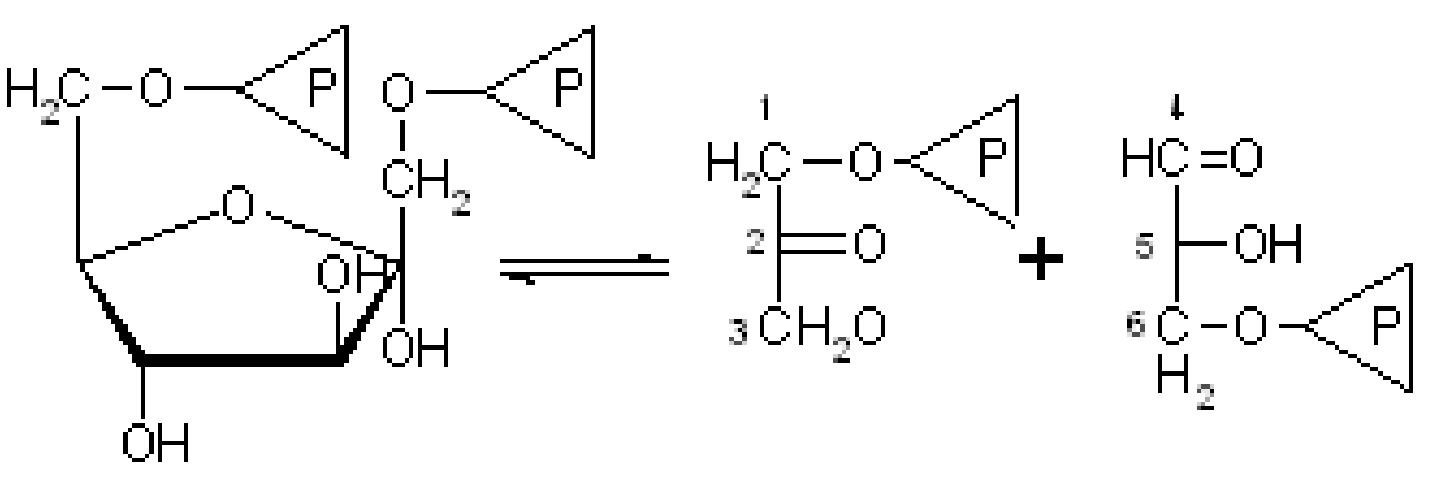
- Trypsin, a digestive enzyme, is quite specific and catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine residues.
- Thrombin, an enzyme that participates in blood clotting, catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only.



4. Lyases

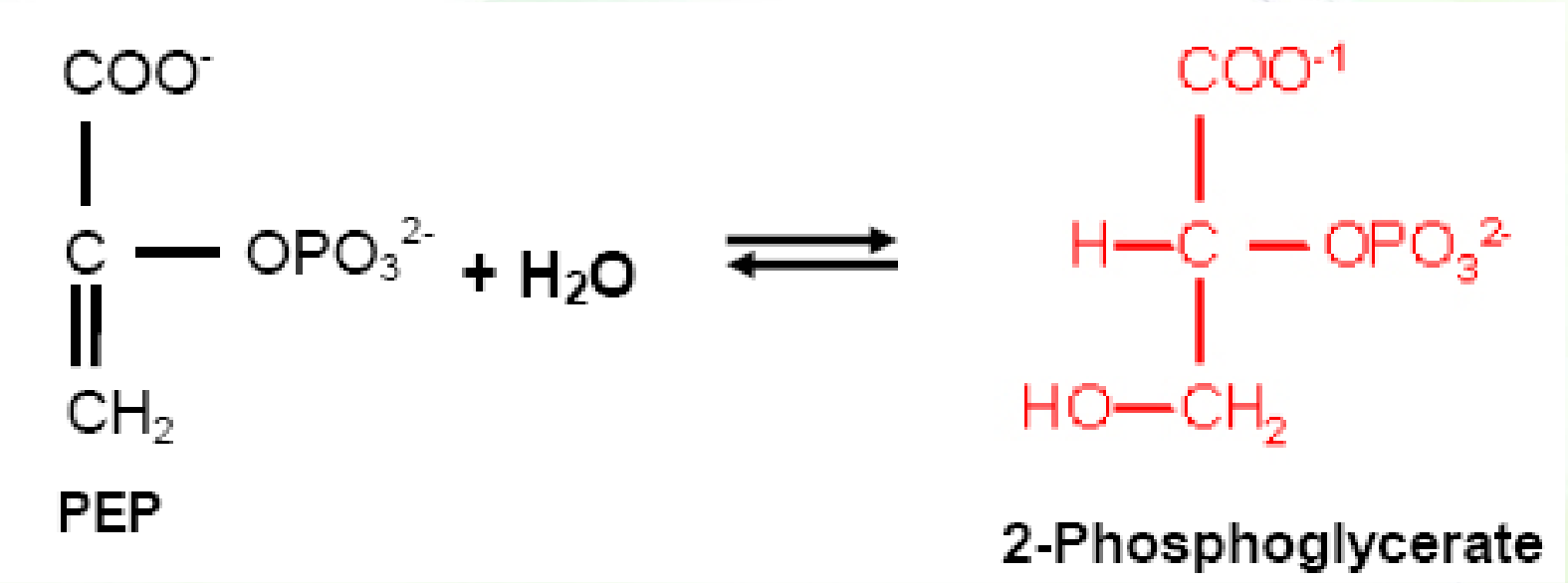
- These enzymes remove groups from substrates with the associated formation or removal of double bonds between C-C, C-O and C-N without hydrolysis.
- An example is aldolase, which breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate.

F 1,6 bisphosphate ⇆ DHAP + GAP



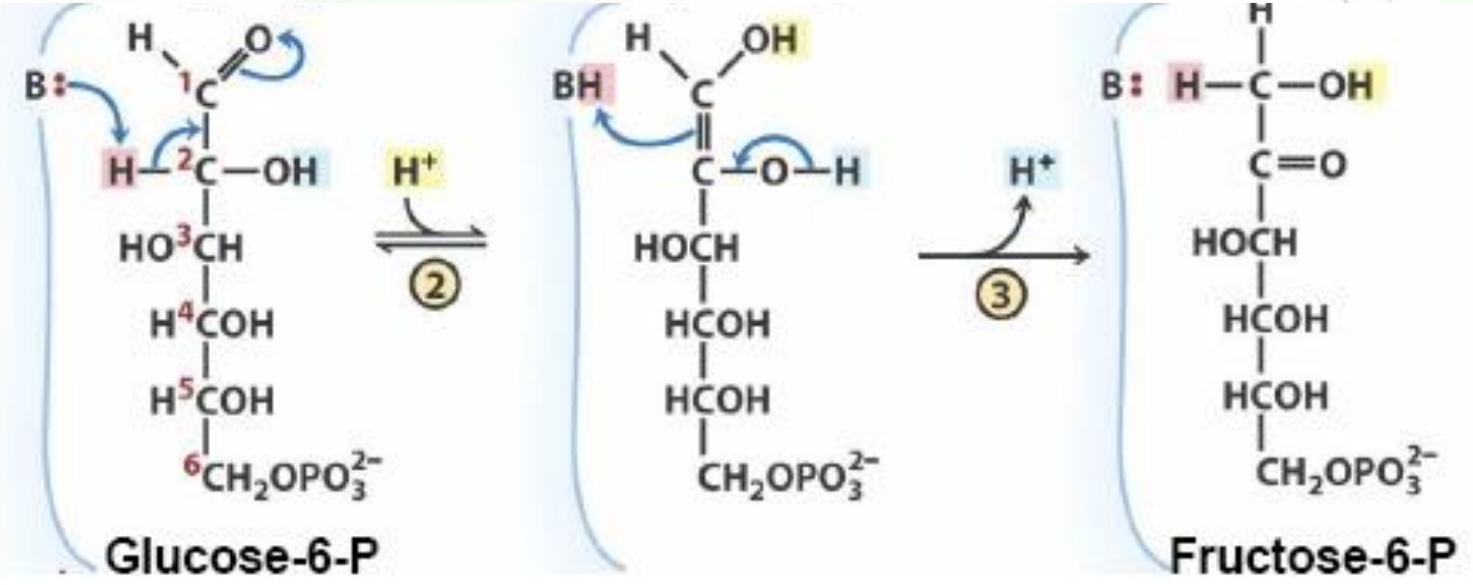
Example: enolase

- Another example is enolase, which interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation and removal of double bonds.



5. Isomerases

- These enzymes catalyze intramolecular rearrangements.
- An example is phosphoglucoisomerase, which isomerizes glucose-6-phosphate to fructose-6-phosphate.



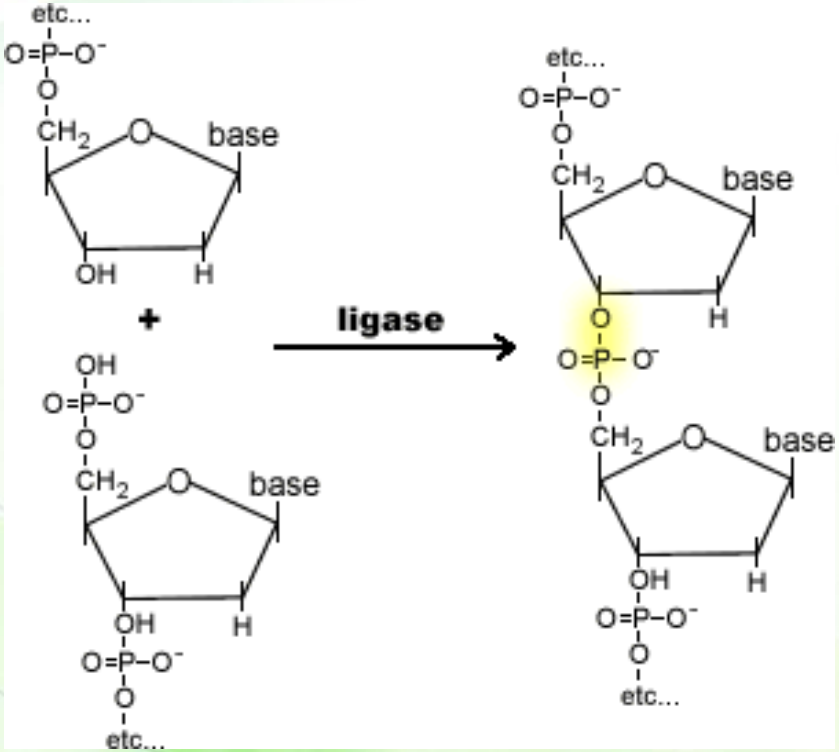
Example: posphoglycerate mutase

- Another example is posphoglycerate mutase, which 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

- An example of this type of enzyme is pyruvate carboxylase, which catalyzes the following reaction:

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