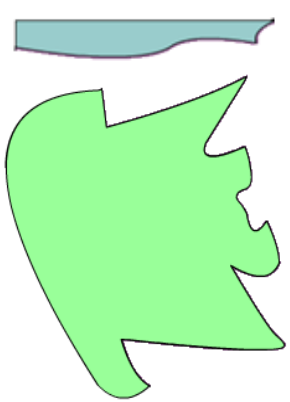
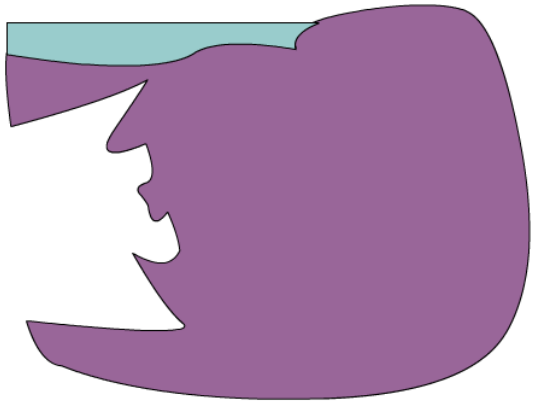
Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

ENZYMES

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





The Biological Catalysts; Enzymes

- What are enzymes? (specialized proteins, small amounts, acceleration, no change). Ribozymes are the exception
- Enzymes are the most efficient catalysts known
  - usually in the range of 10^6 to 10^{14} (up to 10^{20}).
  - Non-enzymatic catalysts (10^2 to 10^4). Collision theory
- The actions of enzymes are fine-tuned by regulatory processes
- Examples: catalase (10^8) & carbonic anhydrase (10^7)

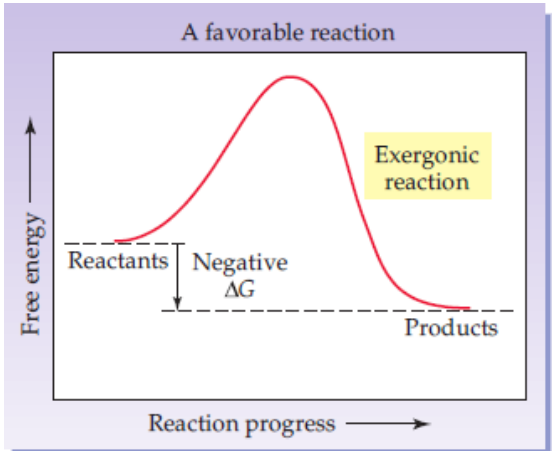
$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2\,(g)$$

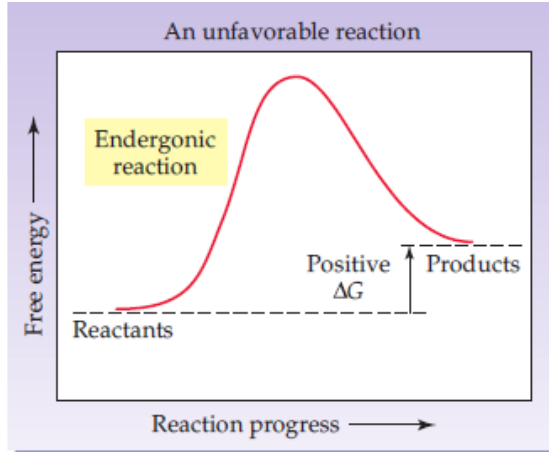
$$CO_2 + H_2O \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

Reaction Conditions	Activation Free Energy kJmol^{-1}	Activation Free Energy 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

Energy & Biochemical Reactions

- $\Delta G = \Delta H - T\Delta S$
- Spontaneous vs. non-spontaneous, favorable vs. non-favorable, exergonic vs. endergonic, exothermic vs. endothermic, switch of signs
- ΔG, $\Delta G°$
- Biochemical pathways; storage (endergonic) & release (exergonic)
- Kinetics (rate) vs. Thermodynamics (favorability)





Photosynthesis

$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$ $\Delta G = +686$ kcal/mol (endergonic, energy required)

Oxidation

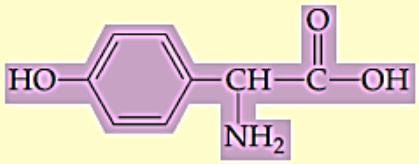
$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$ $\Delta G = -686$ kcal/mol (exergonic, energy released)

Are enzymes important?

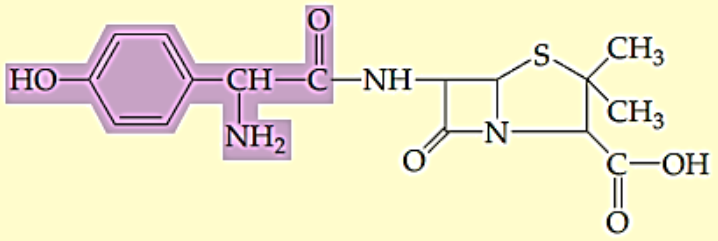
- **In the human body, almost every metabolic process involve the use of enzymes**

Sucrose (table sugar), yeast enzyme breaks sucrose into its two smaller sugar

crushed leaves are exposed to the oxygen in air, a polyphenoloxidase breaks up polyphenols into tannins which impart the darker color and characteristic flavors



p-Hydroxyphenylglycine



Amoxicillin

How to express an enzymatic reaction?

- In enzymatic reactions, reactants are known as substrates
- We can simply express an enzymatic reaction using this formula

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

Or

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

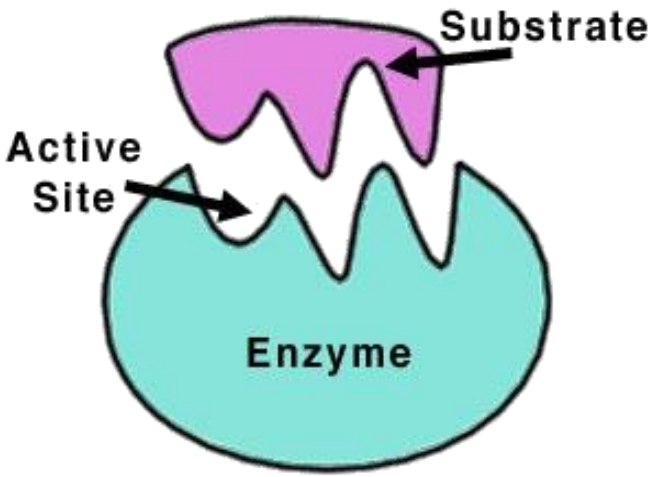
where E is the free enzyme; S is the free substrate, ES is the enzyme-substrate complex; P is the product of the reaction; and EP is the enzyme-product complex before the product is released

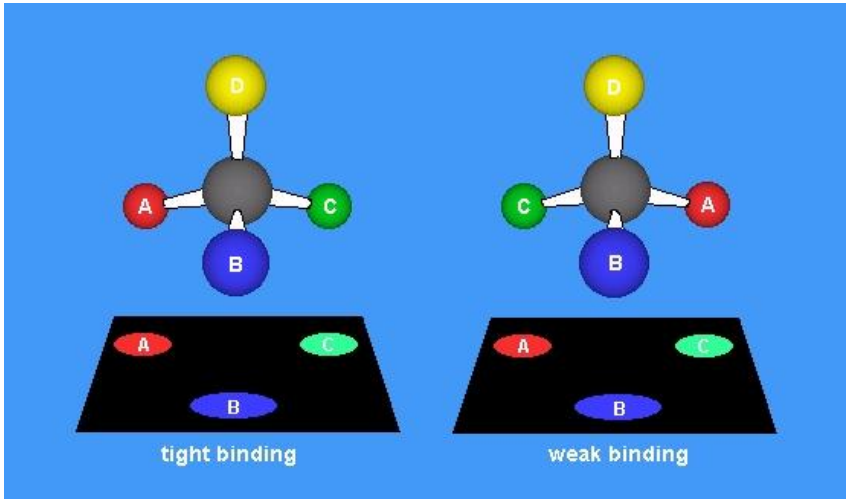
Active sites of enzymes

- A specific three-dimensional shape which includes a region where the biochemical reaction takes place
- Contains a specialized amino acid sequence that facilitates the reaction
- Within the active site are two sub-sites, the binding site and the catalytic site, The binding & catalytic site may be the same
- Binding site: binds substrate through ionic, H-bonding or other electrostatic forces
- Catalytic site: contains the catalytic groups

Features of active site

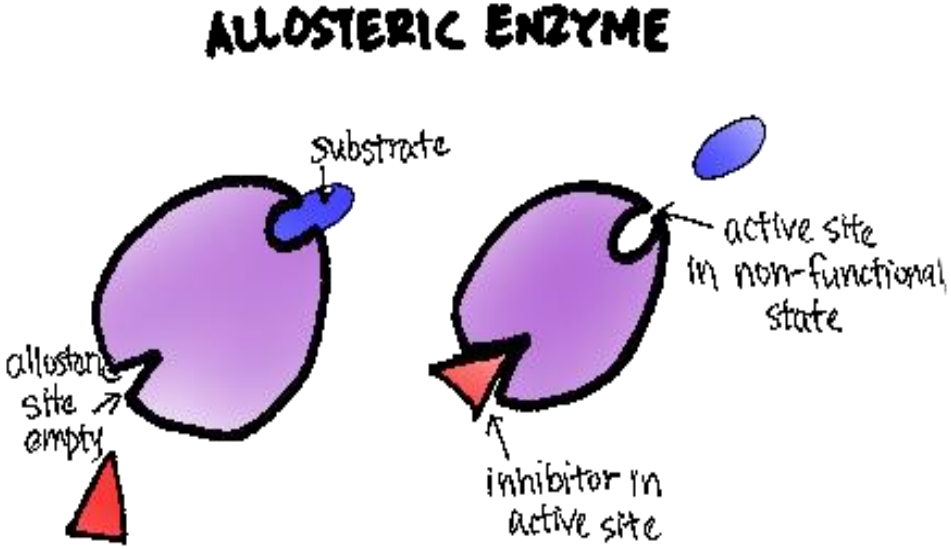
- Active sites; structures that look like canals, clefts or crevices
- Water is usually excluded after binding unless it participates in the reaction
- Substrates are bound to enzymes by multiple weak attractions (electrostatic, hydrogen, van der Waals, & hydrophobic)
- Binding occurs at least at three points (chirality)

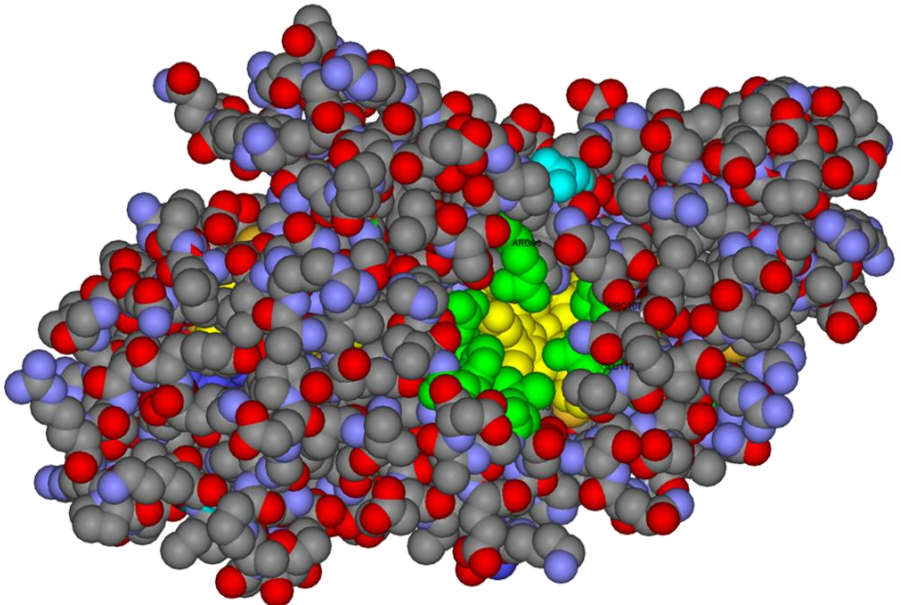




Features of active site

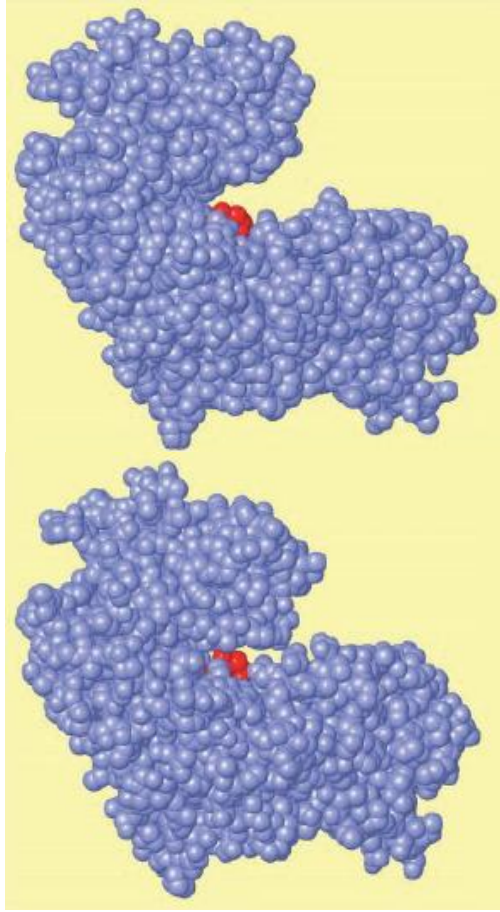
- Forms by groups from different parts of the amino acid sequence usually forming a domain made of multiple secondary structures
- Takes up a relatively small part of the total volume
- The "extra" amino acids help create the three-dimensional active site & in many enzymes, may create regulatory sites

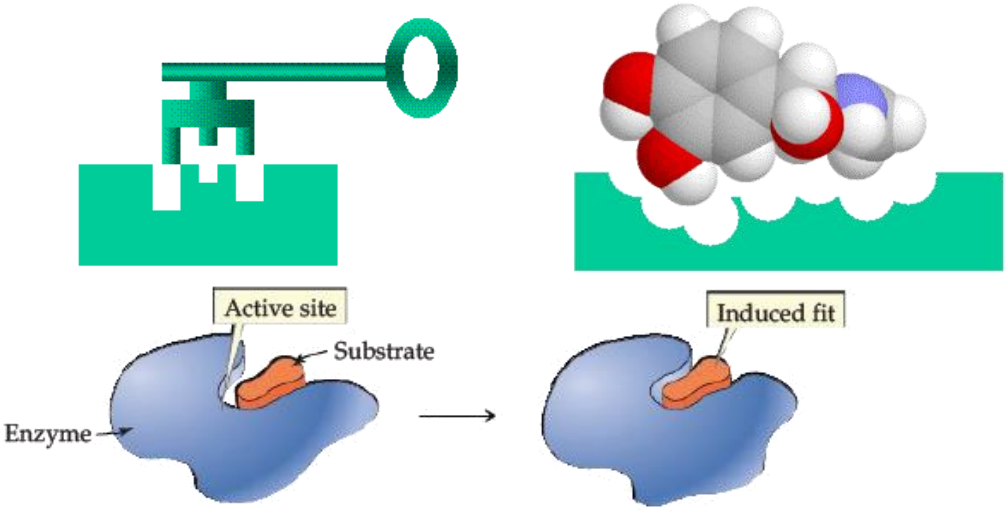




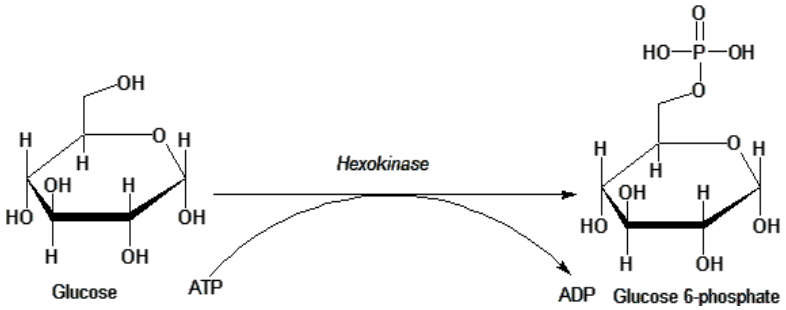
How Do Enzymes Work?

- Binding leads to formation of transition-state
- Usually, substrate binds by non-covalent interactions to the active site
- The catalyzed reaction takes place at the active site, usually in several steps
- Two models, lock-and-key vs. induced-fit model
- Glucose and hexokinase, phosphorylation



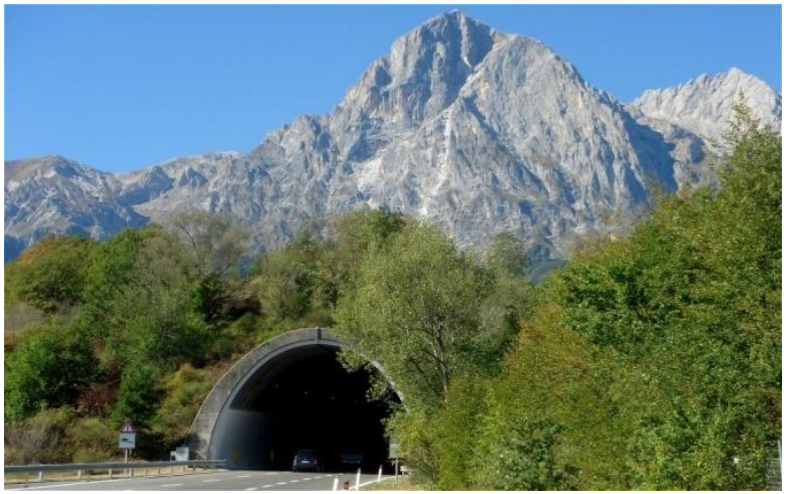


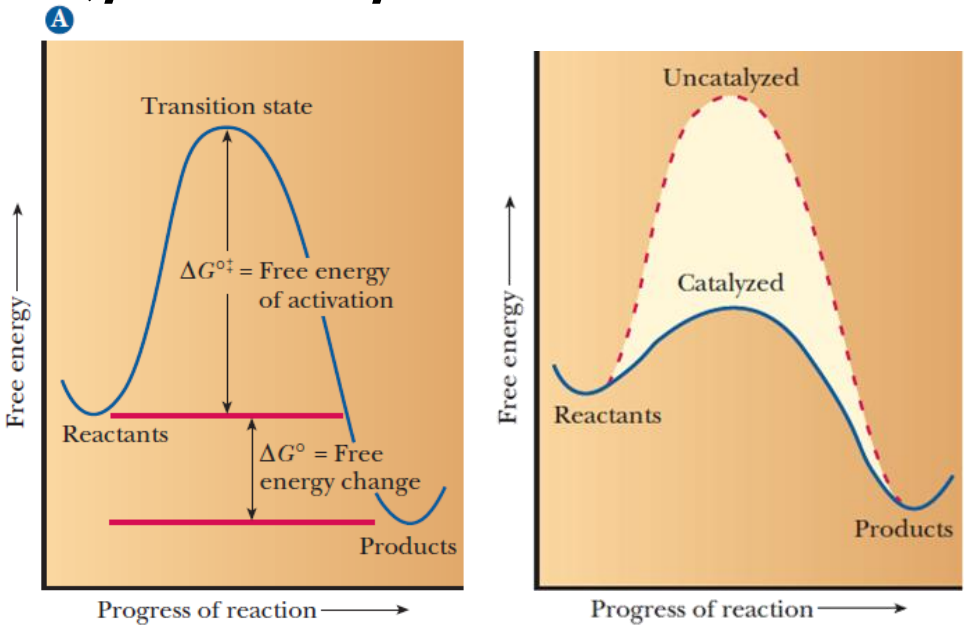
Improving the binding site for ATP & excluding water (might interfere with the reaction)



How do enzymes work?

- Enzymes speed up reactions, but have no relation to equilibrium or favorability
- What is an activation energy ($\Delta G^{\circ\ddagger}$) concept?
- Specificity varies (stereoisomers), however, there is none non-specific
- Spontaneous vs. rate!
- What is the transition state?

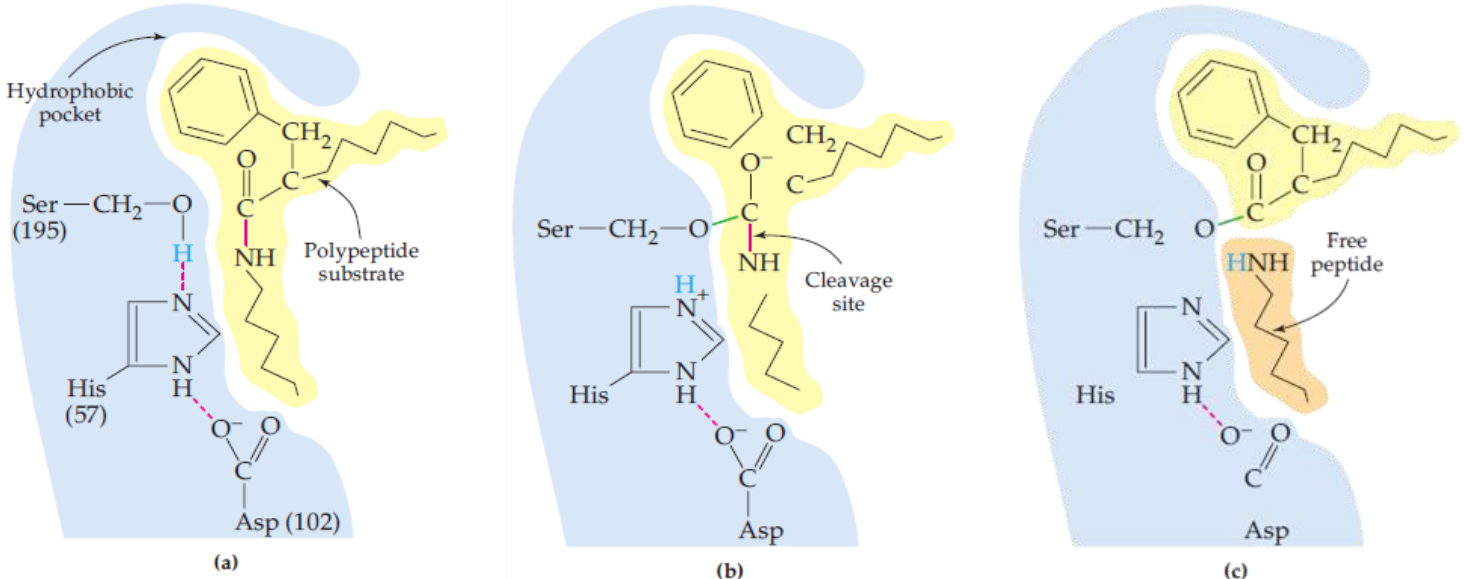




Transition-state complex binds more tightly to the enzyme compared to substrate

How Do Enzymes Work?

- Proximity effect: Bring substrate(s) and catalytic sites together
- Orientation effect: Hold substrate(s) at the exact distance and in the exact orientation necessary for reaction
- Catalytic effect: Provide acidic, basic, or other types of groups required for catalysis
- Energy effect: Lower the energy barrier by inducing strain in bonds in the substrate molecule



Naming of enzymes

- In general, enzymes end with the suffix (-ase)
- Most enzymes are named for their substrates and for the type of reactions they catalyze, with the suffix "ase" added
- For example; ATPase is an enzyme that breaks down ATP, whereas ATP synthase is an enzyme that synthesizes ATP
- Some enzymes have common names that provide little information about the reactions that they catalyze
- Examples include the proteolytic enzyme trypsin

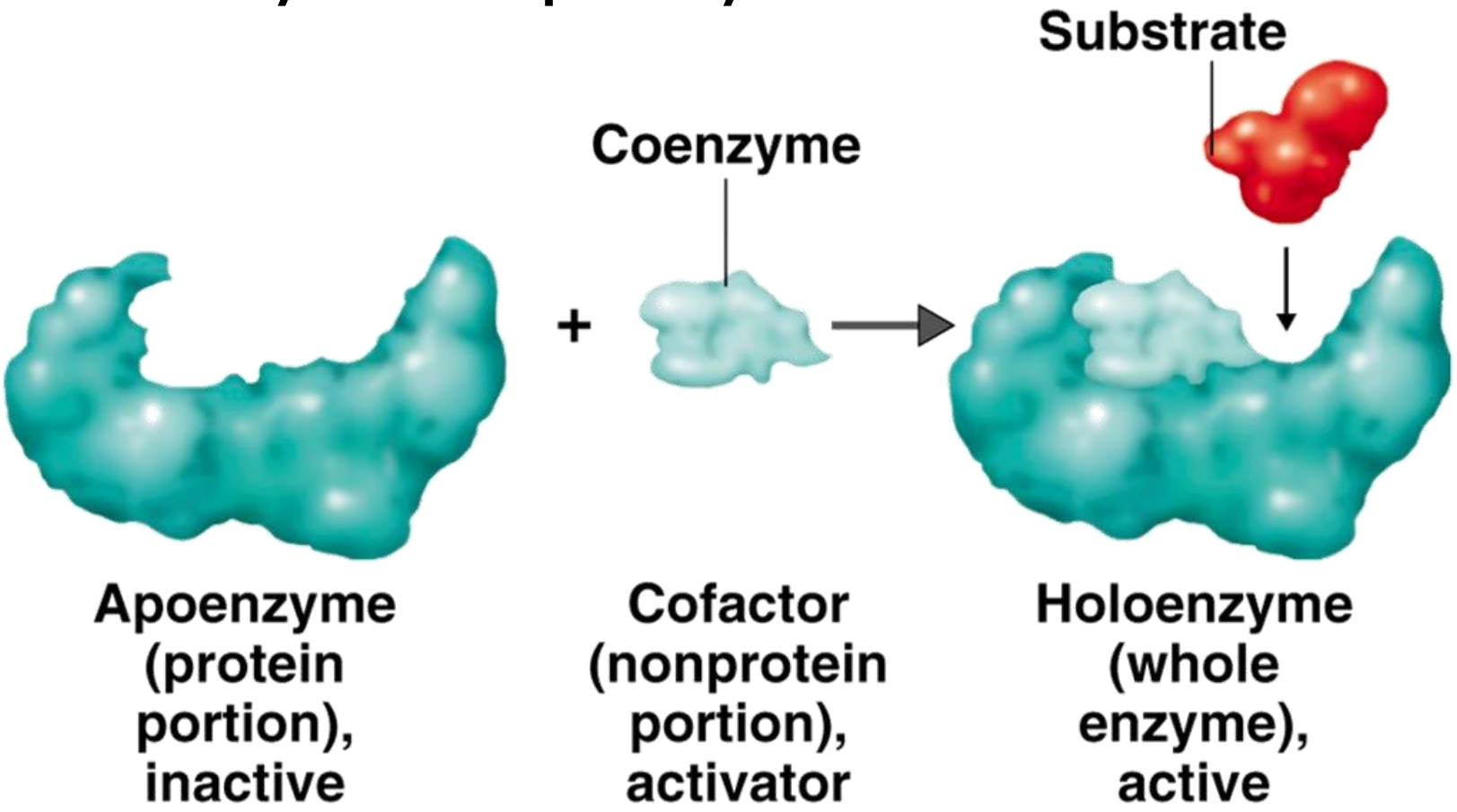
Naming of enzymes; EC numbering Enzyme Commission number

- A numerical classification scheme for enzymes, based on the chemical reactions they catalyze
- Strictly speaking, EC numbers do not specify enzymes, but enzyme-catalyzed reactions
- Numbering format:
  - EC followed by four numbers separated by periods
  - Major class (1-6), Minor class, subclass, further subclassification

- For example: tripeptide aminopeptidases "EC 3.4.11.4"
  - EC 3: hydrolases
  - EC 3.4: hydrolases that act on peptide bonds
  - EC 3.4.11: hydrolases that cleave off the amino-terminal amino acid polypeptide
  - EC 3.4.11.4: cleave off the amino-terminal end from a tripeptide

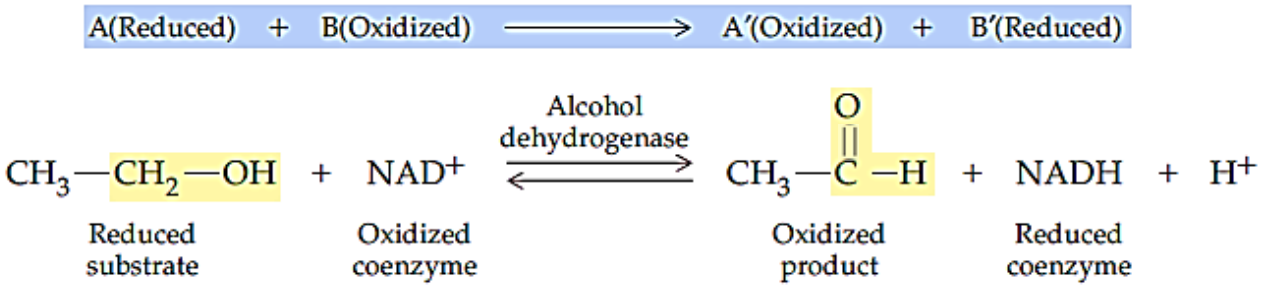
Enzyme Classification (structure)

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

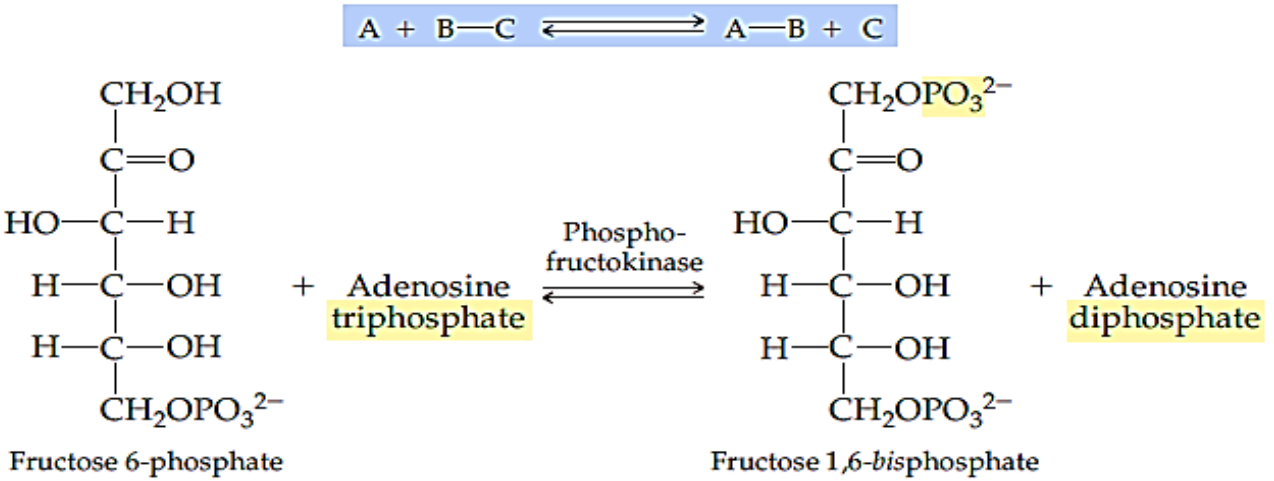


Enzyme Classification (function)

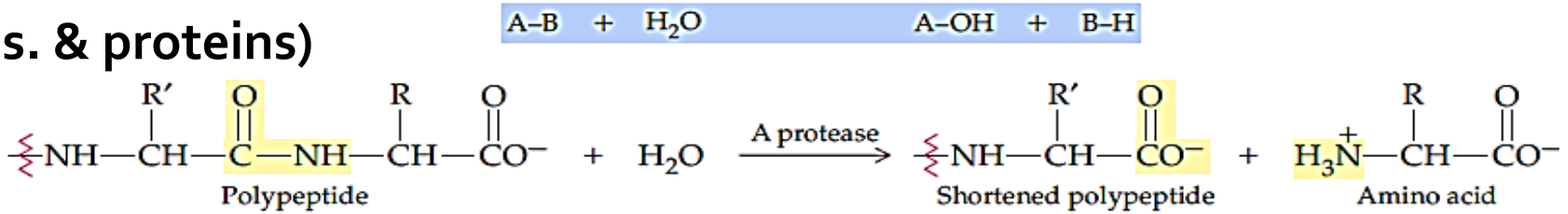
- **Oxidoreductases: addition or removal of O, O_2, H. Require coenzymes (heme)**



- **Transferases*:* transfer of a group from one molecule to another**

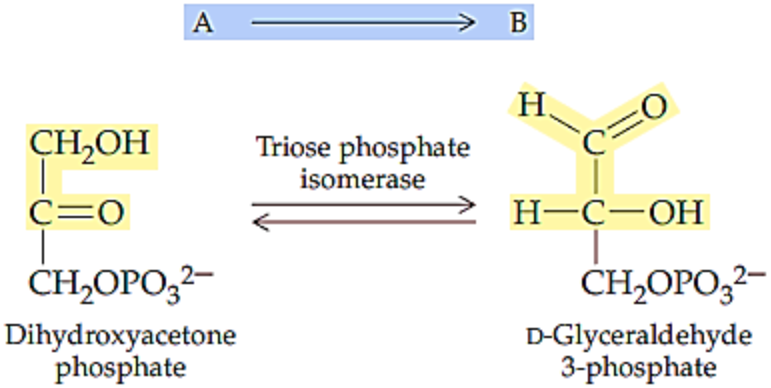


- **Hydrolases: addition of water (carbs. & proteins)**

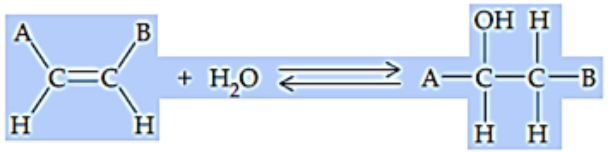


Enzyme Classification (function)

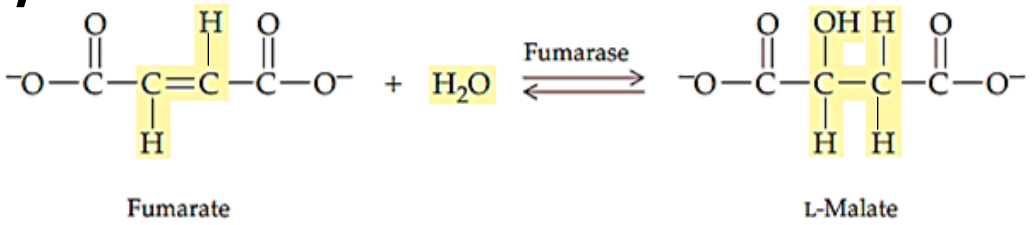
- **Isomerases: one substrate and one product**

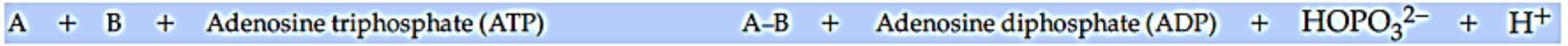


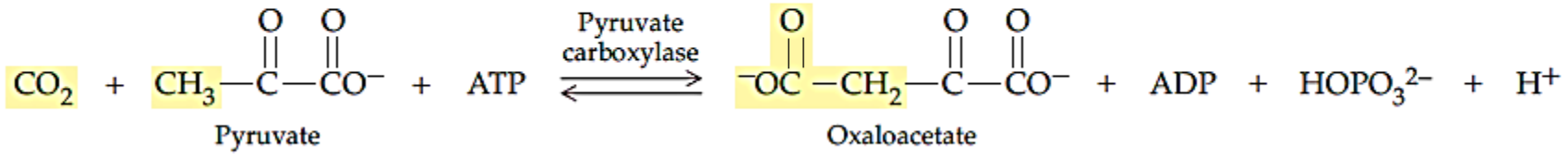
- **Lyases: addition of a molecule (H_2O, CO_2, NH_3) to a double bond or reverse**



- **Ligases: usually not favorable, so they require a simultaneous hydrolysis reaction**







Oxidoreductases

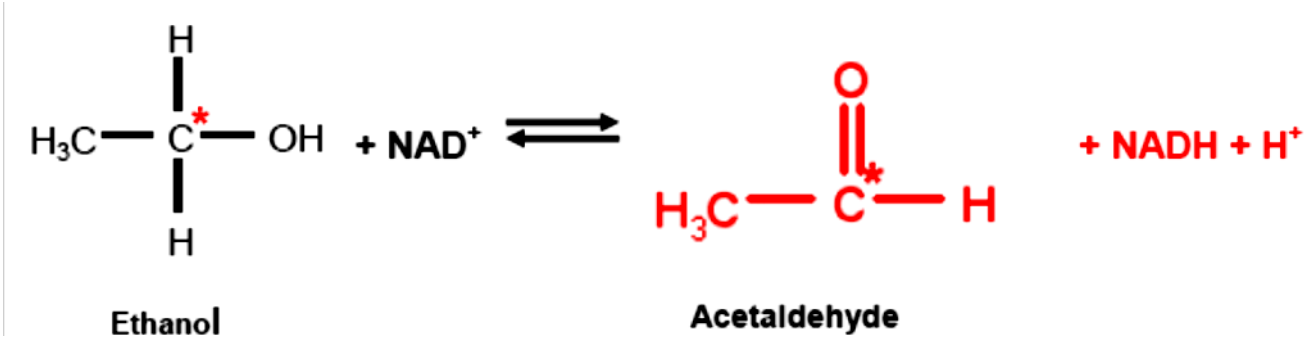
- These enzymes catalyze oxidation & reduction reactions involving the transfer of hydrogen atoms, electrons or oxygen
- This group can be further divided into 4 main classes:
  - ✓ Dehydrogenases
  - ✓ Oxidases
  - ✓ Peroxidases
  - ✓ Oxygenases

Dehydrogenases

- Dehydrogenases catalyze hydrogen transfer from the substrate to a molecule known as nicotinamide adenine dinucleotide (NAD+)
- Lactate dehydrogenase

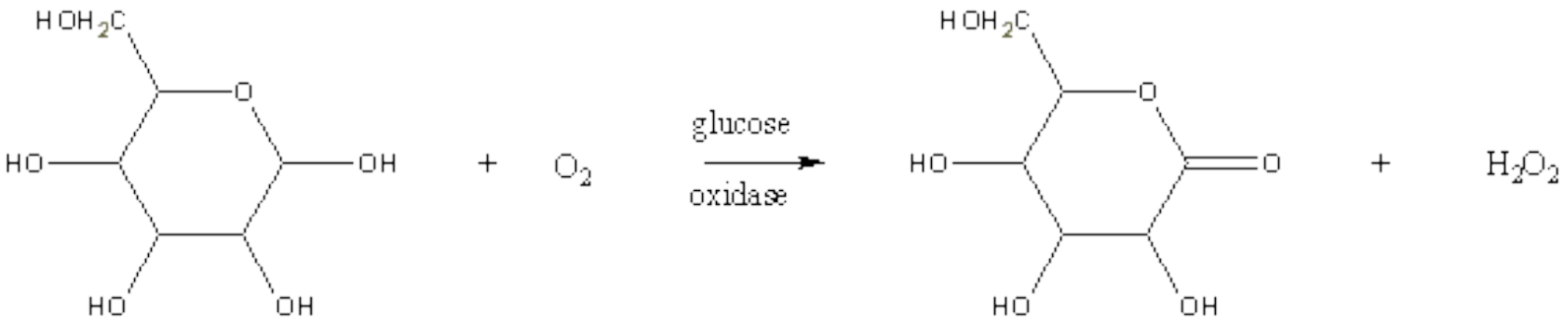
$$\text{Lactate} + NAD^+ \leftrightarrows \text{Pyruvate} + NADH + H^+$$

- Alcohol dehydrogenase



Oxidases

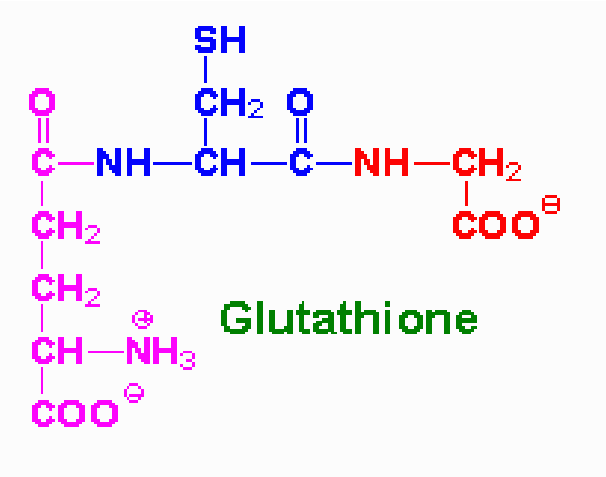
- Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product
- Glucose oxidase
  - β-D-glucose + O_2 ⇆ gluconolactone + H_2O_2

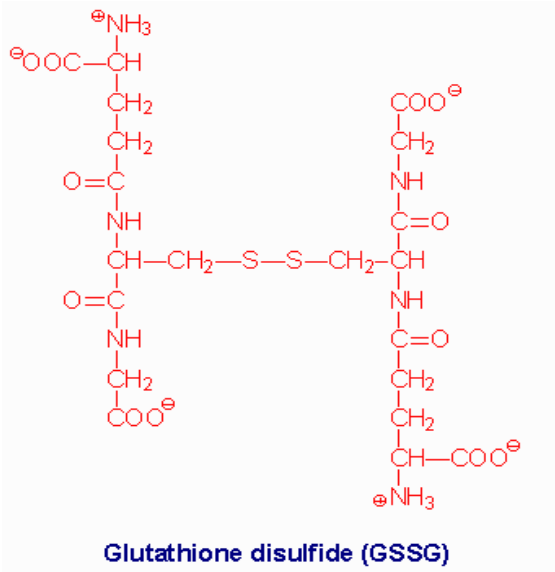


Peroxidases

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

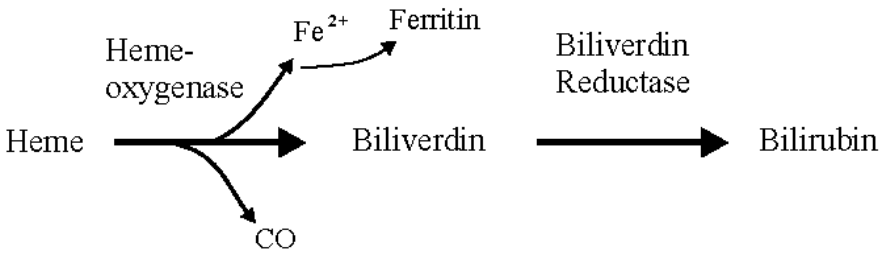
$$2\,GSH + H_2O_2 \leftrightarrows G\text{-}S\text{-}S\text{-}G + 2\,H_2O$$

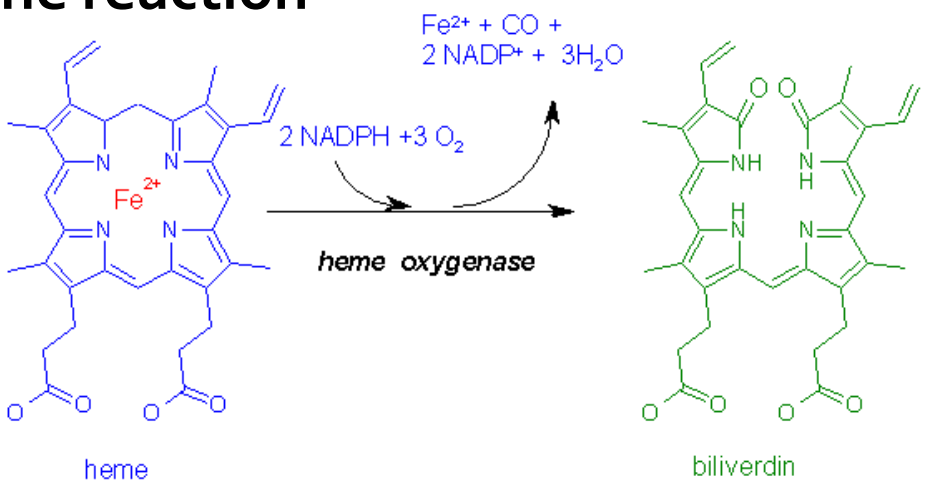




Oxygenases

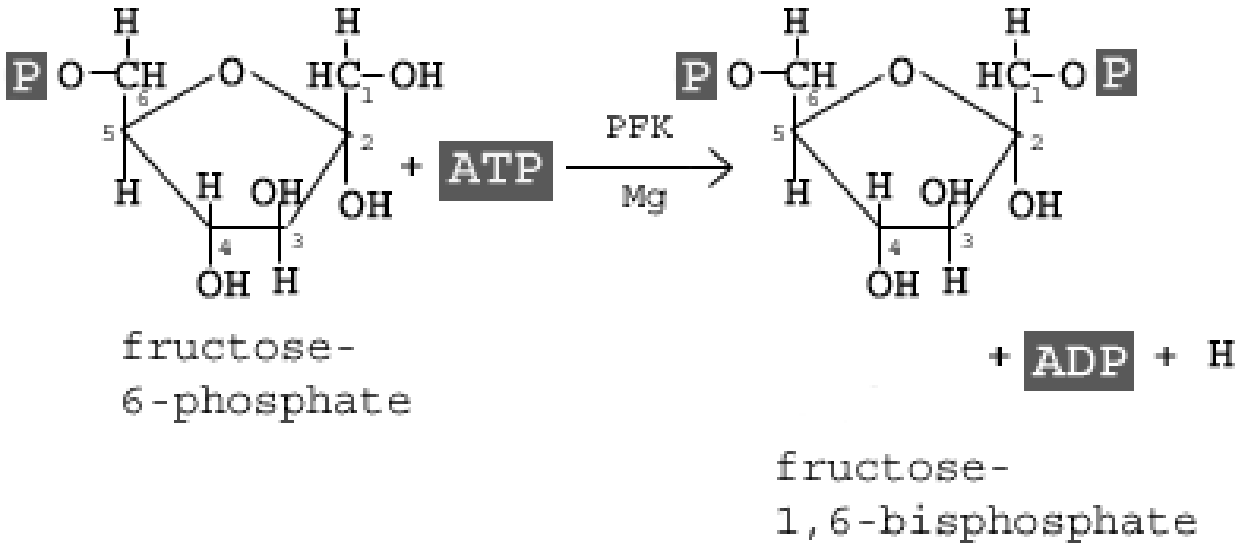
- Oxygenases catalyze substrate oxidation by molecular O_2
- The reduced product of the reaction in this case is water and not hydrogen peroxide
- There are two types of oxygenases:
- Monooxygenases; transfer one oxygen atom to the substrate, and reduce the other oxygen atom to water
- Dioxygenases, incorporate both atoms of molecular oxygen (O2) into the product(s) of the reaction





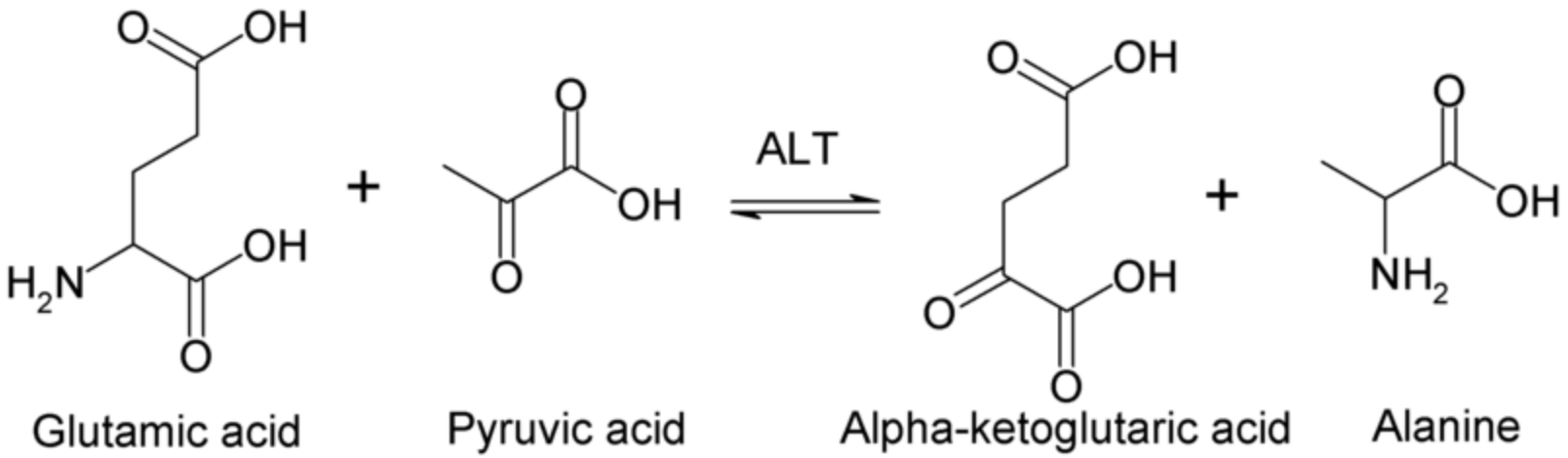
Transferases

- These enzymes transfer a functional group (C, N, P or S) from one substrate to an acceptor molecule
- Phosphofructokinase; catalyzes transfer of phosphate from ATP to fructose-6-phosphate:
  - Fructose 6-P + ATP ↔ F 1,6 bisphosphate + ADP



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
- This allows for the interconversion of certain amino acids

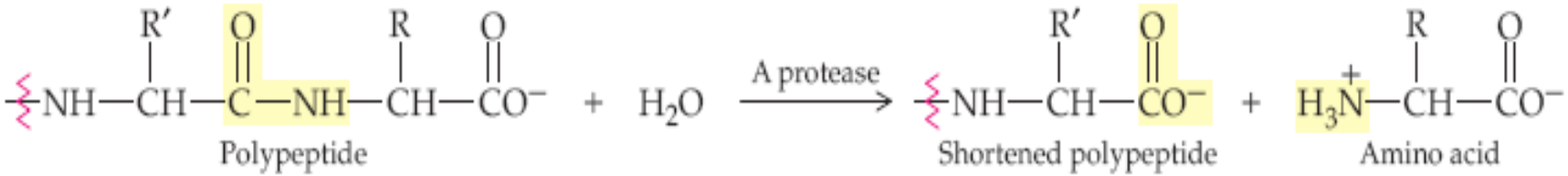


Hydrolases

- These enzymes catalyze cleavage reactions while using water across the bond being broken
- Peptidases, esterases, lipases, glycosidases, phosphatases are all examples of hydrolases named depending on the type of bond cleaved

Proteases

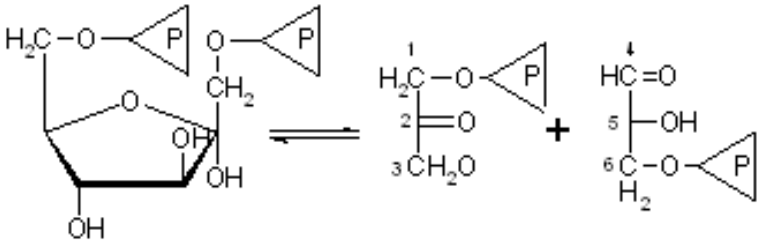
- These enzymes catalyze proteolysis, the hydrolysis of a peptide bond within proteins
- Proteolytic enzymes differ in their degree of substrate specificity



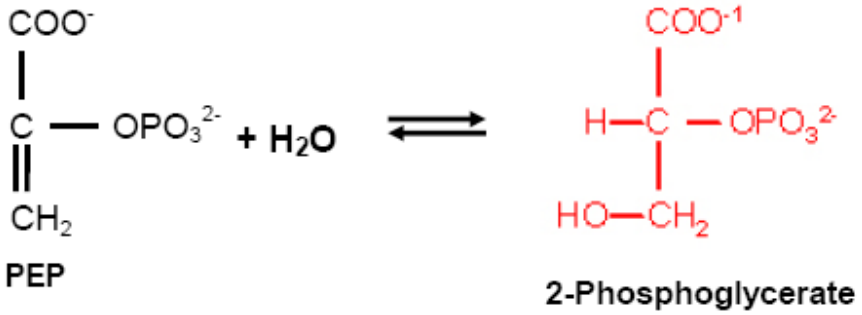
- Trypsin, is quite specific; catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine
- Thrombin, catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only

Lyases

- Catalyze the addition or removal of functional groups from their substrates with the associated formation or removal of double bonds between C-C, C-O and C-N
- Aldolase; breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate
  - F 1,6 bisphosphate ⇆ DHAP + GAP

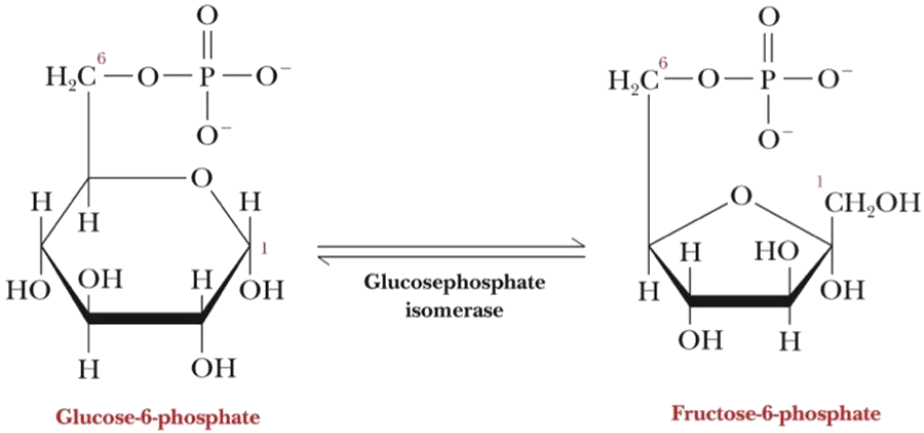


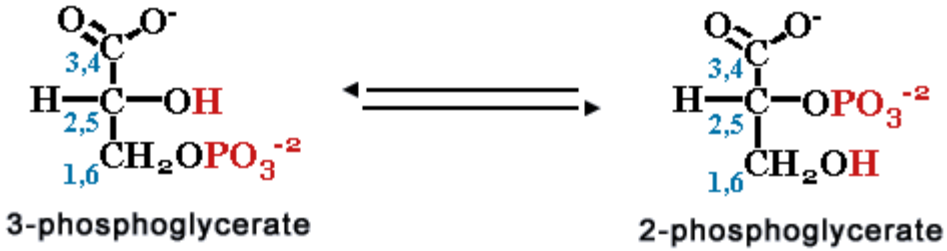
- Enolase; interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation and removal of double bonds



Isomerases

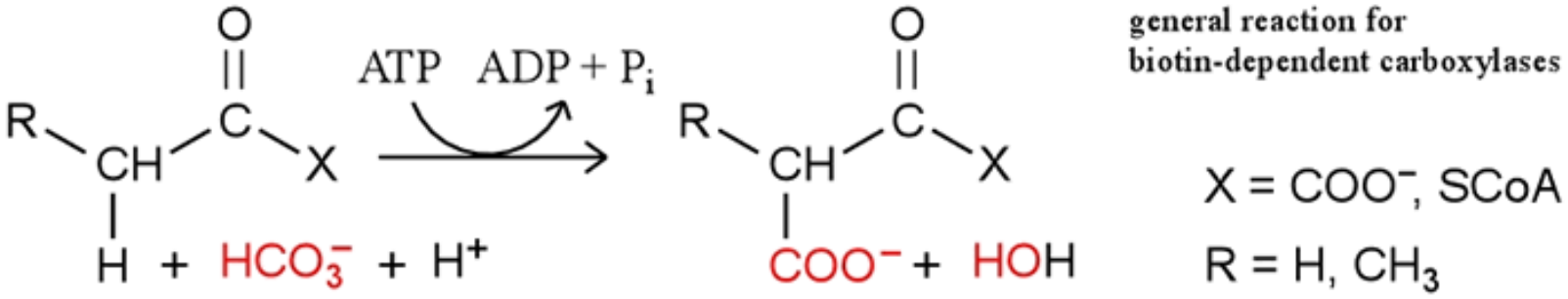
- Catalyze intramolecular rearrangements
- Glucose-6-phosphate isomerase; isomerizes glucose-6-phosphate to fructose-6-phosphate
- Phosphoglycerate mutase; transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate (BPG intermediate)
- 3-P glycerate ⇆ 2 P glycerate





Ligases

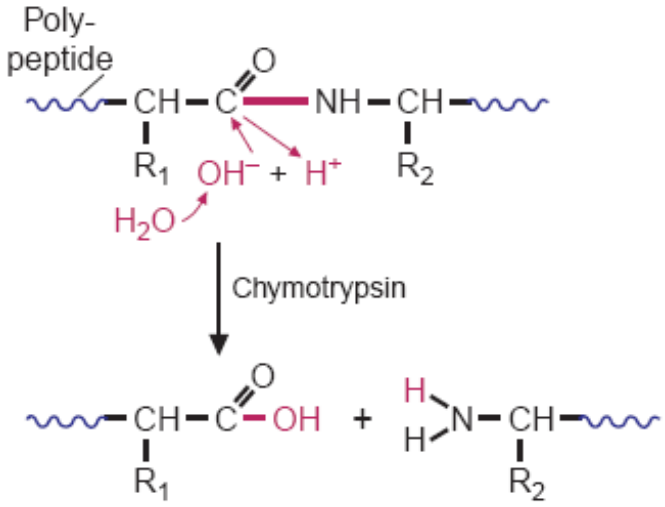
- Ligases join C-C, C-O, C-N, C-S and C-halogen bonds
- The reaction is usually accompanied by the consumption of a high energy compound such as ATP
- Pyruvate carboxylase
  - Pyruvate + HCO_3^- + ATP ⇆ Oxaloacetate + ADP + Pi



CATALYTIC MECHANISM OF CHYMOTRYPSIN

Mechanism of Action

Chymotrypsin



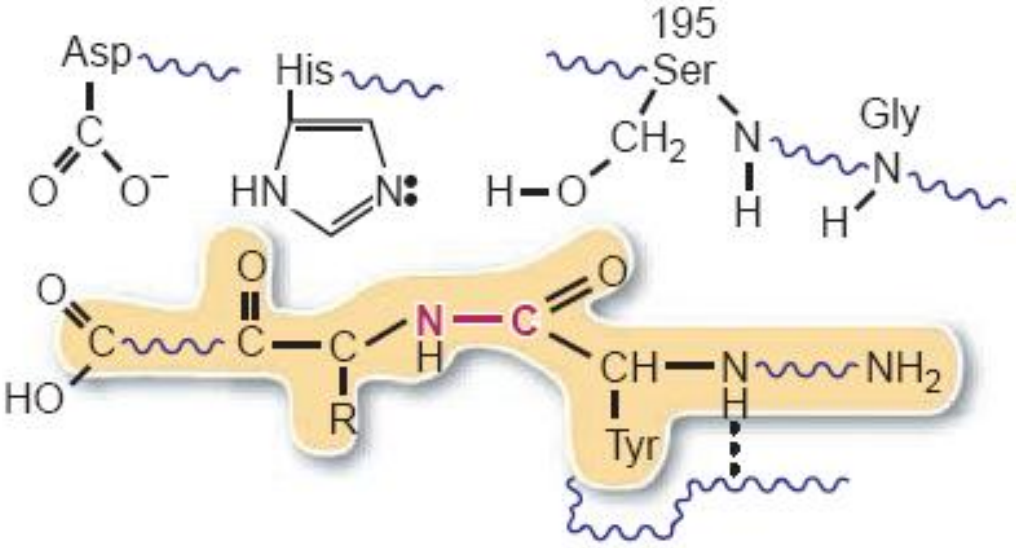
- A digestive enzyme
- A member of serine protease superfamily
  - use serine in the active site to form a covalent intermediate during proteolysis
- The bond that is cleaved is called the *scissile bond*
- In the absence of chymotrypsin: rate is very slow
  - Too few OH^- molecules in H_2O with enough energy to form the transition-state complex
  - & too few OH^- molecules colliding with the substrate at the right orientation

The catalyzed reaction

- Oxyanion intermediate is formed by using the hydroxyl group of a serine residue for the attack instead of a free hydroxyl anion
- The rate is faster because Functional groups in the enzyme active site:
  - Activate the attacking hydroxyl group
  - Stabilize the oxyanion transition-state complexes
  - Form a covalent intermediate
  - & destabilize the leaving group
- The reaction takes place in two stages:
  - Cleavage & formation of intermediate
  - Hydrolysis of intermediate to release protein substrate

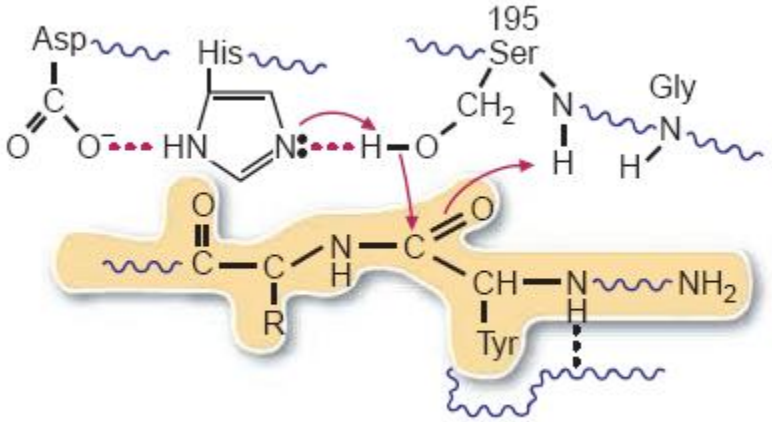
The catalyzed reaction… Cont.

- **Hydrolysis: carbonyl side of Phe, Tyr, or Trp**
- **Recognition site consists of a hydrophobic binding pocket**
- **Glycines hold the substrate rigidly**
- **Moving serine 195 into attacking position provides specificity through orientation**
- *Proximity & orientation*



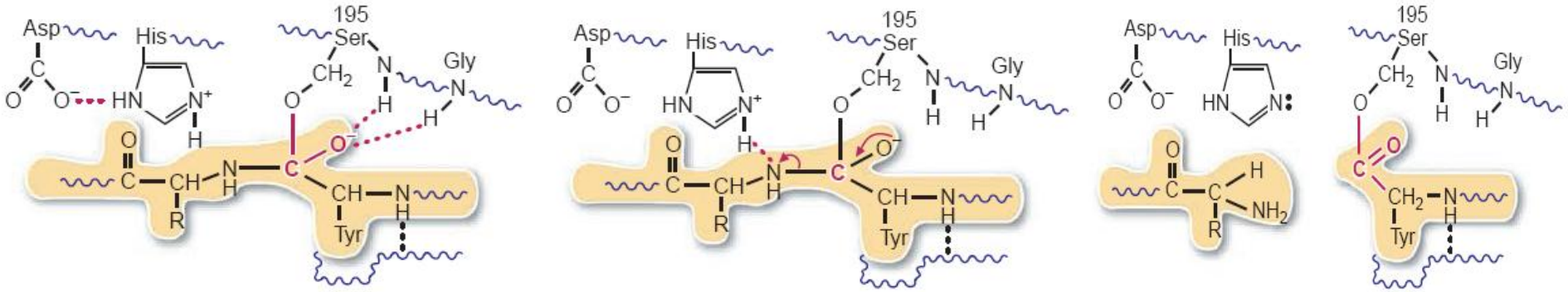
The catalyzed reaction… Cont.

- **The *catalytic triad: Asp-His-Ser***
- **Nucleophilic catalysis:**
  - Serine hydroxyl group attacks the carbonyl carbon
- **Aspartate & histidine cooperate in converting the OH into a better nucleophilic attacking group**
  - Histidine acts as a base (*acid–base catalysis*)
  - Necessary because the "normal" pK for the serine hydroxyl group is very high



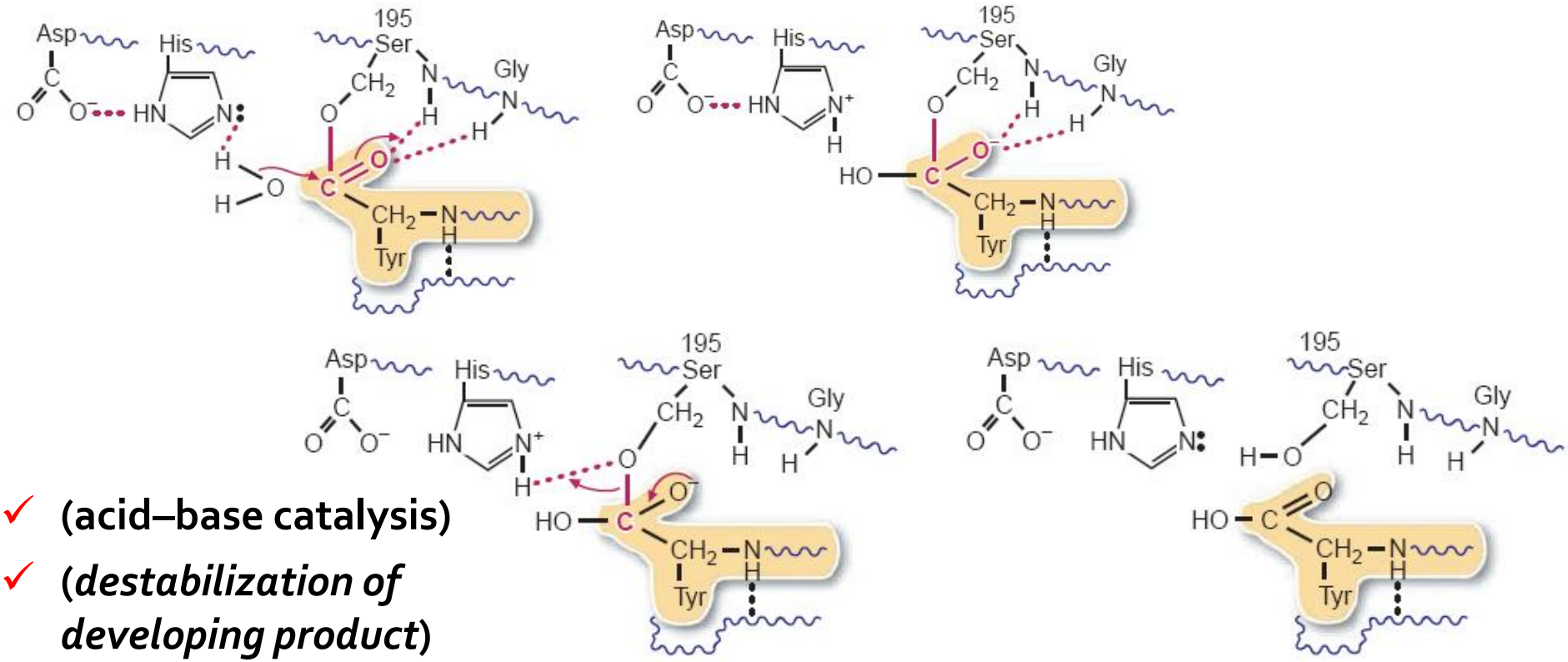
The catalyzed reaction… Cont.

- **Oxyanion tetrahedral transition state complex formed & stabilized by H-bonds**
- **Stabilization of the transition-state complex lowers its energy level & increases the number of molecules that reach this energy level**
- **Serine forms a full covalent bond with carbon & peptide bond cleaved**
- *Covalent catalysis*: **often involves serine or cysteine residues**



The catalyzed reaction… Cont.

- Histidine activates water to form an OH^- for a nucleophilic attack (a second oxyanion transition-state complex)
- Finally, histidine adds the proton back to serine
- Product dissociates



✓ (acid–base catalysis)

✓ (*destabilization of developing product*)