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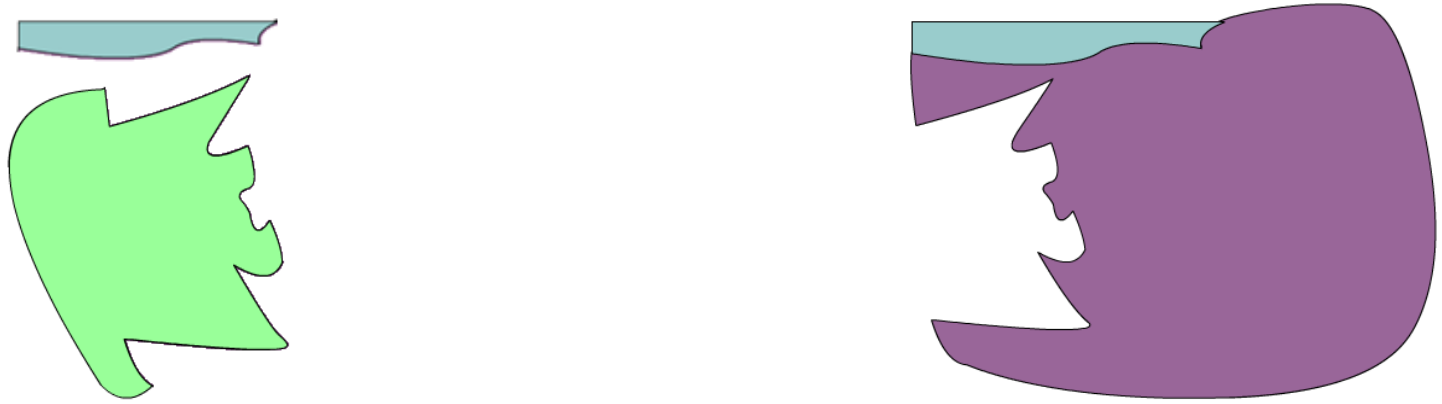
ENZYMES

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 molecule
  - Specificity: the ability of a protein to bind one molecule in preference to other molecules

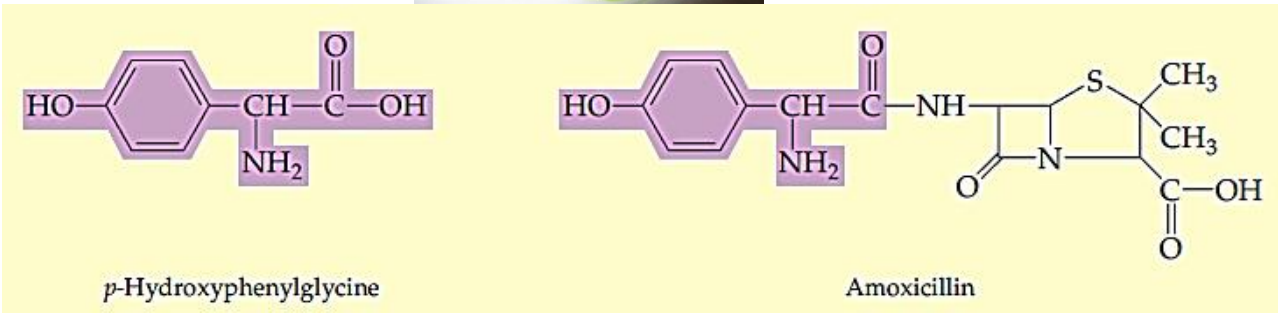


Are enzymes important?

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

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

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



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}
  - Non-enzymatic catalysts (10^2 to 10^4)
  - The actions of enzymes are fine-tuned by regulatory processes
- Examples: catalase (10^8) & carbonic anhydrase (10^7)

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

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

How to express an enzymatic reaction?

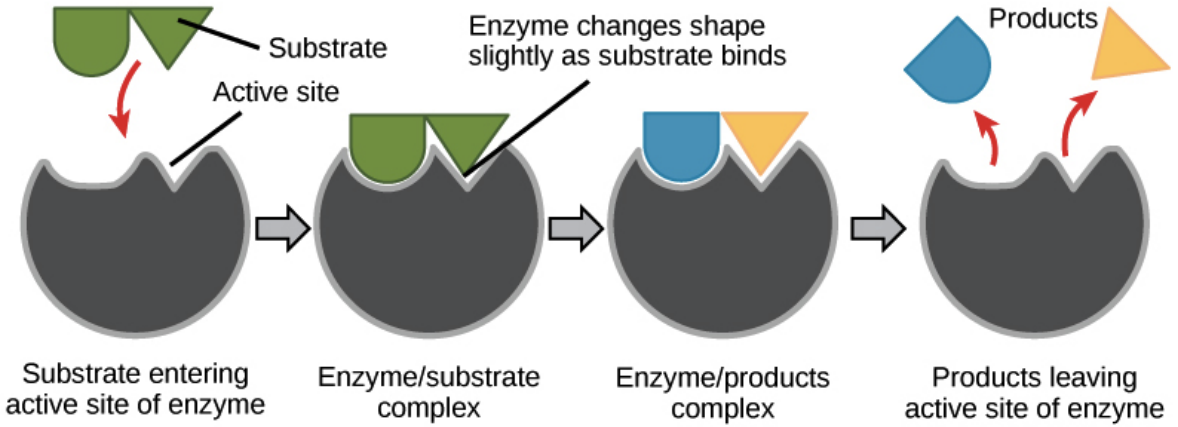
- In enzymatic reactions, reactants are known as substrates
- We can simply express an enzymatic reaction using this formula

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

Or

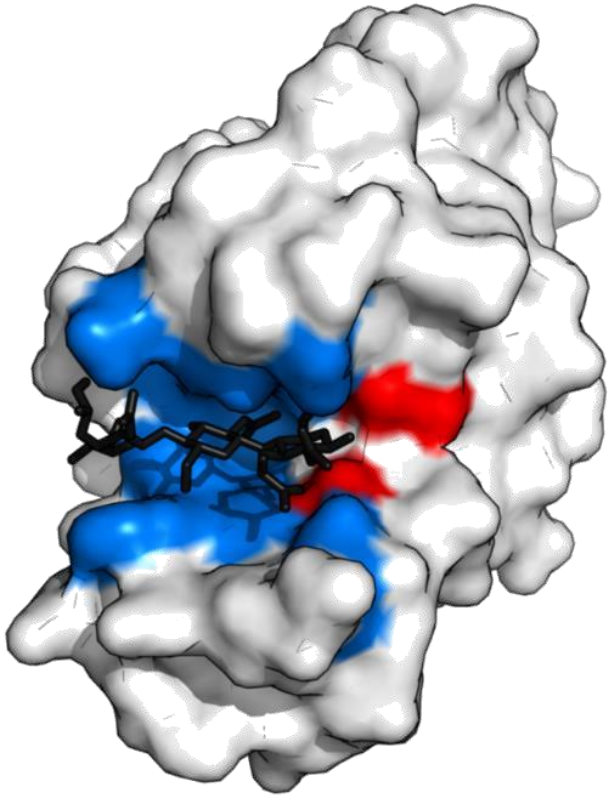
$$E + S \leftrightarrows ES \leftrightarrows E + P$$

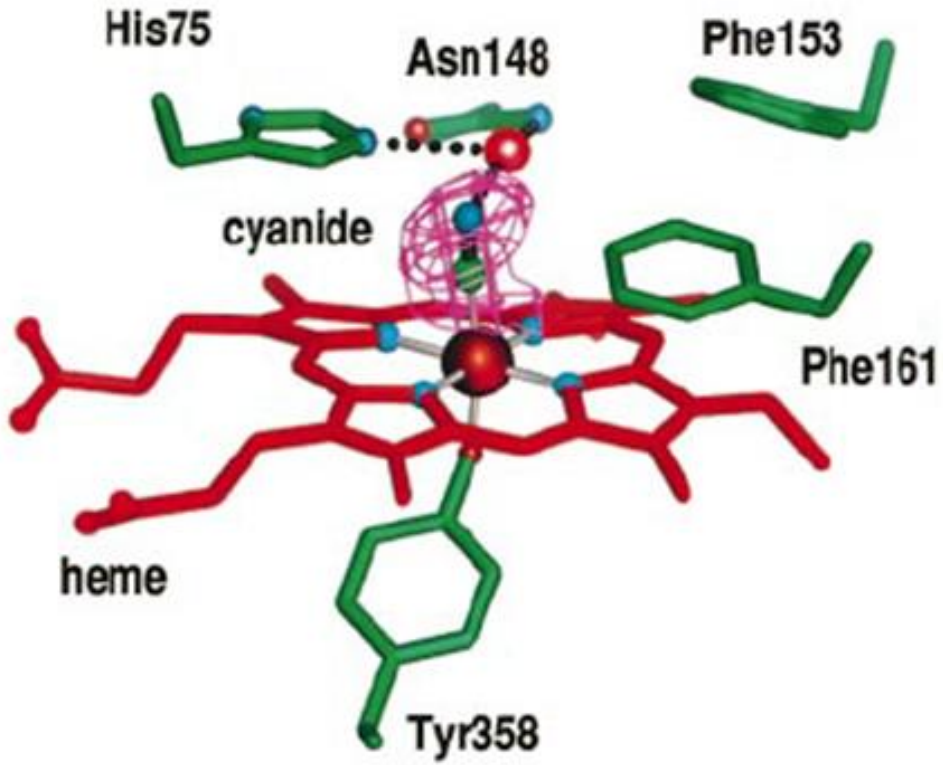
where E is the free enzyme; S is the free substrate, ES is the enzyme-substrate complex; P is the product of the reaction; and EP is the enzyme-product complex before the product is released



Active sites of enzymes

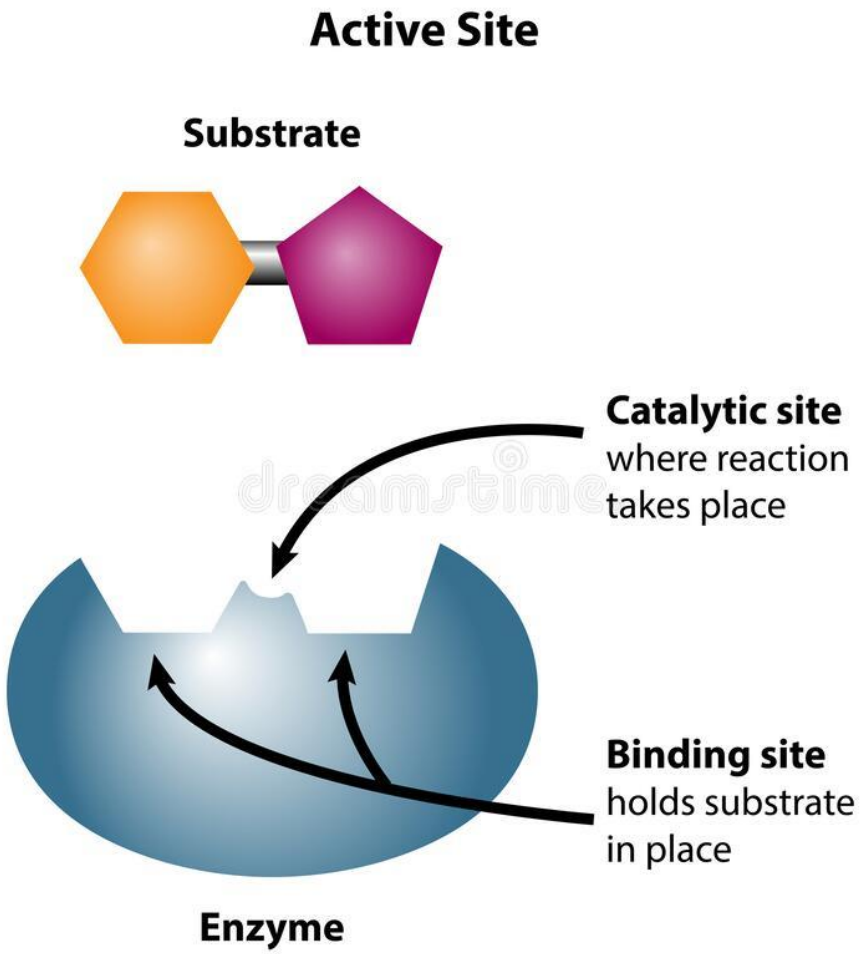
- A specific three-dimensional shape which includes a region where the biochemical reaction takes place
- Contains a specialized amino acid sequence that facilitates the reaction





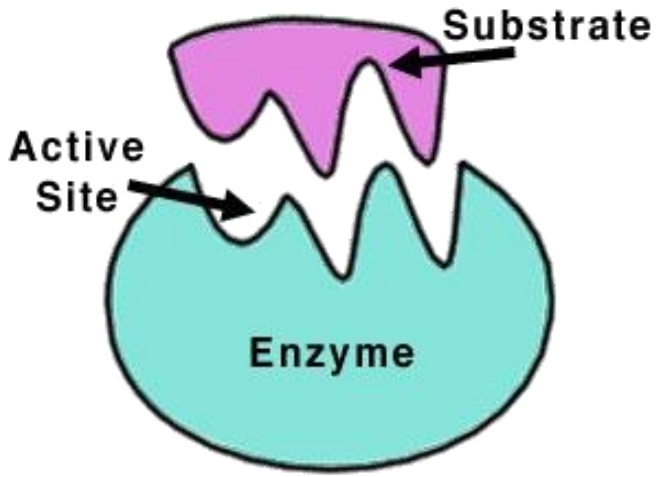
Active sites of enzymes

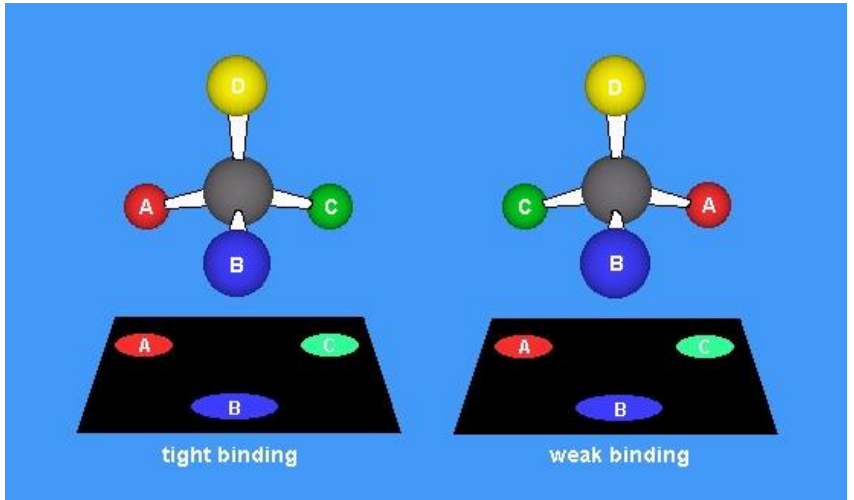
- Within the active site are two sub-sites, the binding site and the catalytic site, The binding & catalytic site may be the same
- Binding site: binds substrate through ionic, H-bonding or other electrostatic forces, or hydrophobic interactions
- Catalytic site: contains the catalytic groups



Features of active site

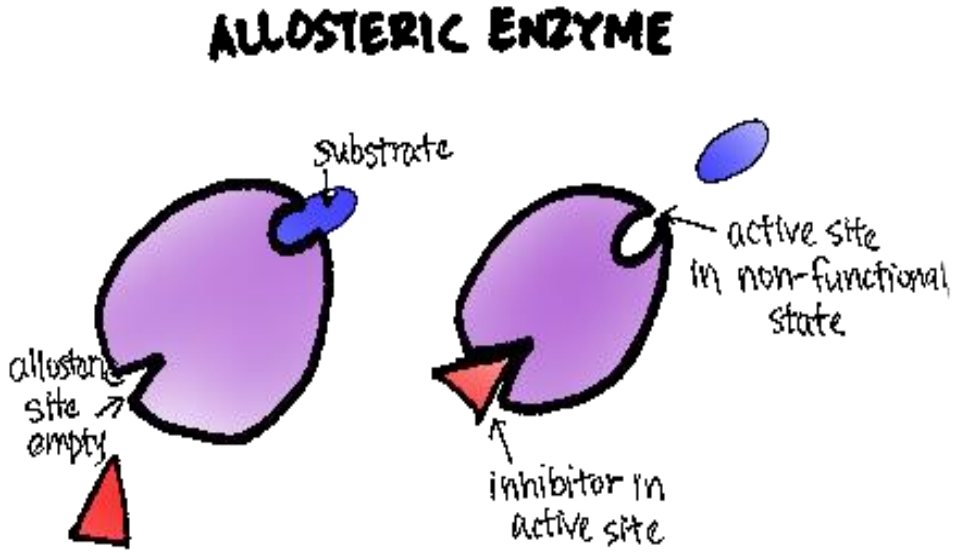
- Active sites; structures that look like canals, clefts or crevices
- Water is usually excluded after binding unless it participates in the reaction
- Substrates are bound to enzymes by multiple weak attractions (electrostatic, hydrogen, van der Waals, & hydrophobic)
- Binding occurs at least at three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

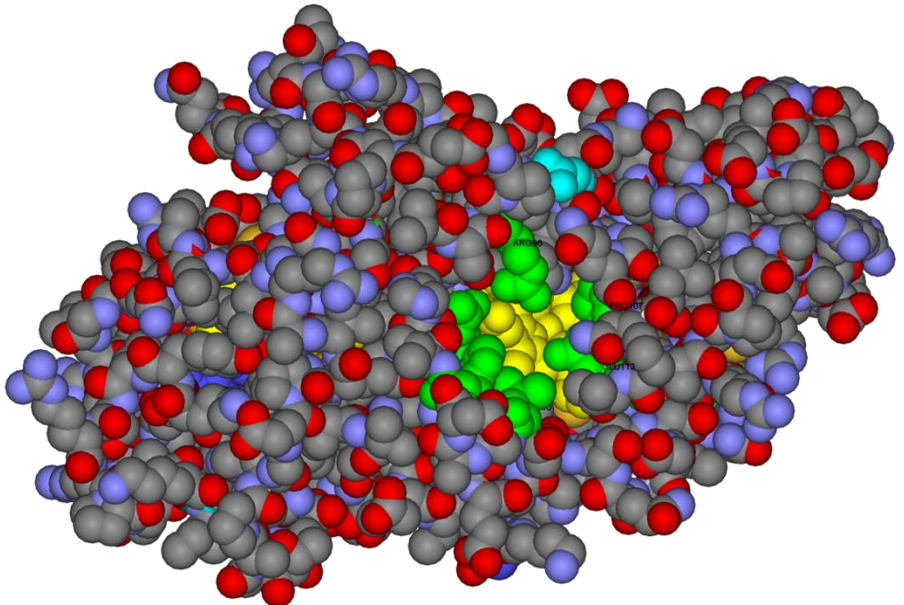




Features of active site

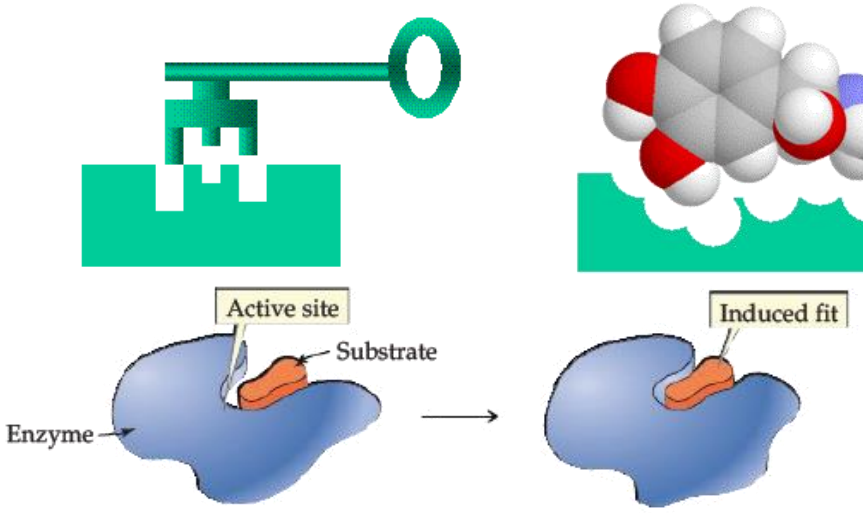
- Forms by groups from different parts of the amino acid sequence usually forming a domain made of multiple secondary structures
- Takes up a relatively small part of the total volume
- The "extra" amino acids help create the three-dimensional active site & in many enzymes, may create regulatory sites

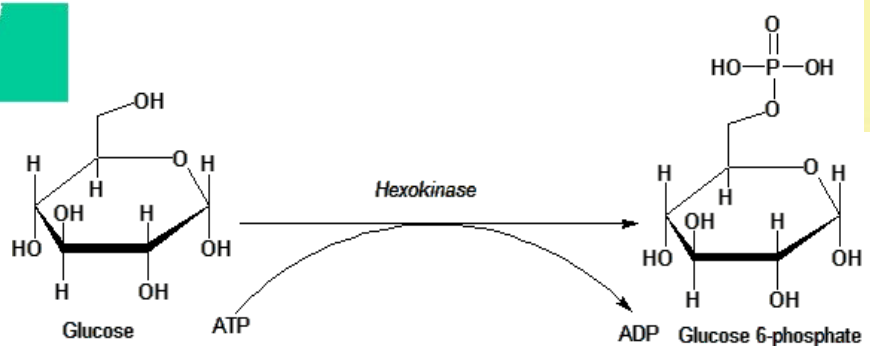




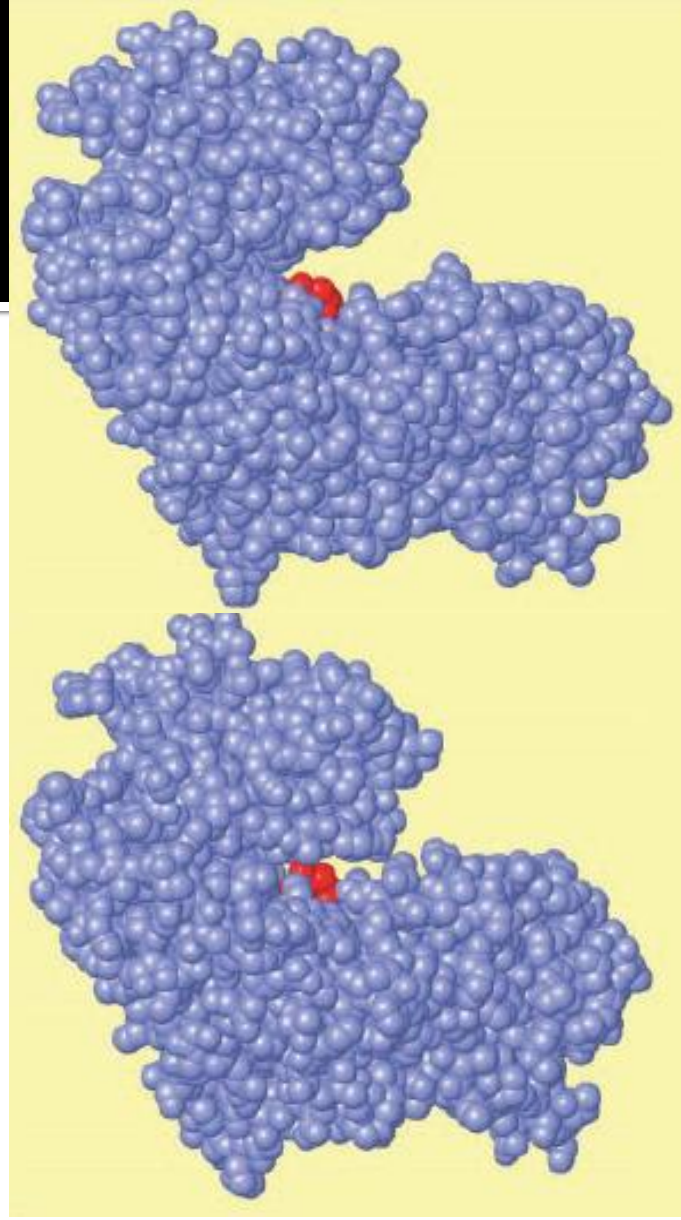
How Do Enzymes Work?

- 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



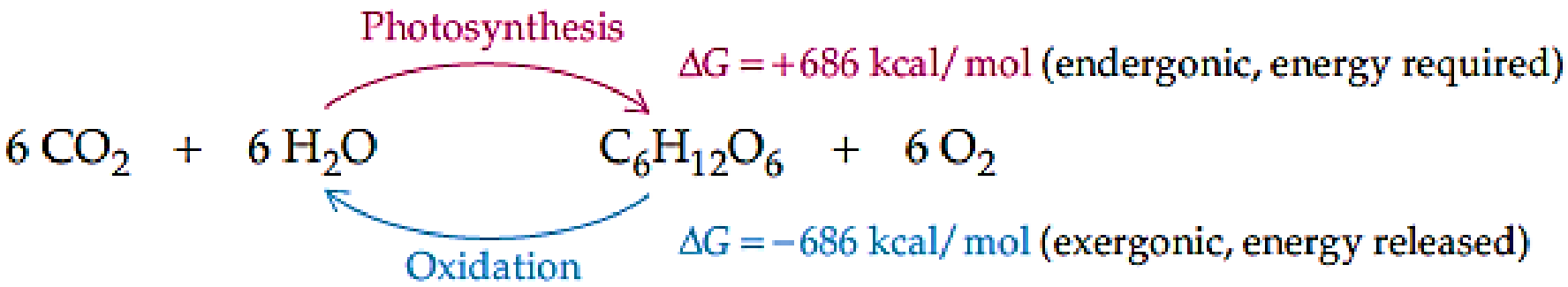


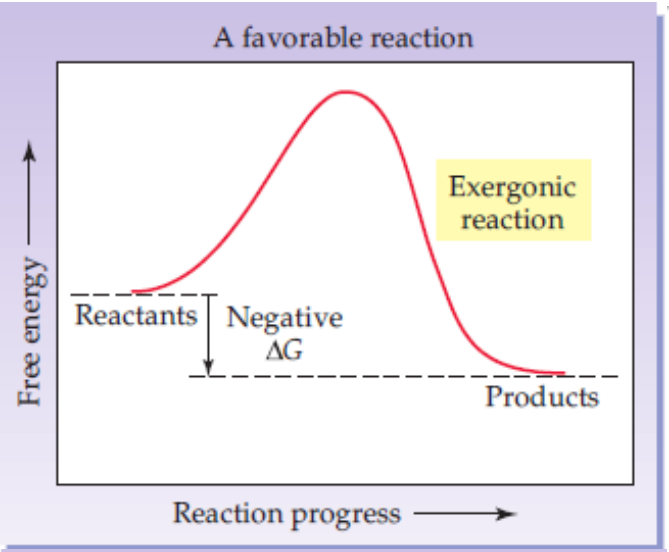
Improving the binding site for ATP & excluding water (might interfere with the reaction)

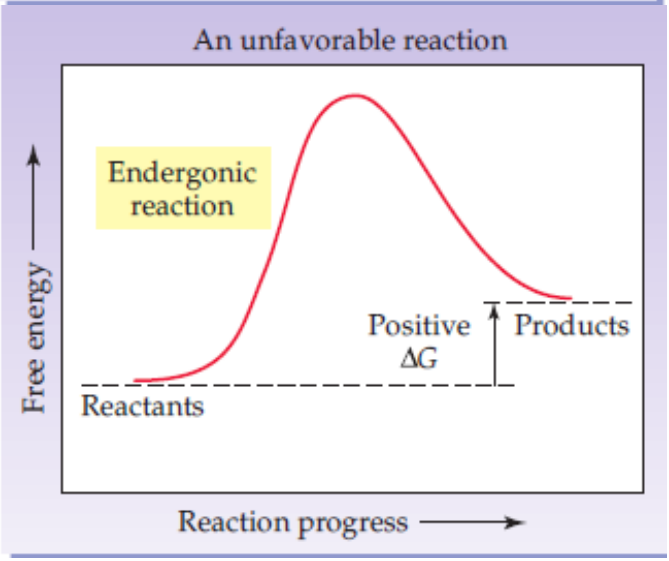


Energy & Biochemical Reactions

- $\Delta G = \Delta H - T\Delta S$
- 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)

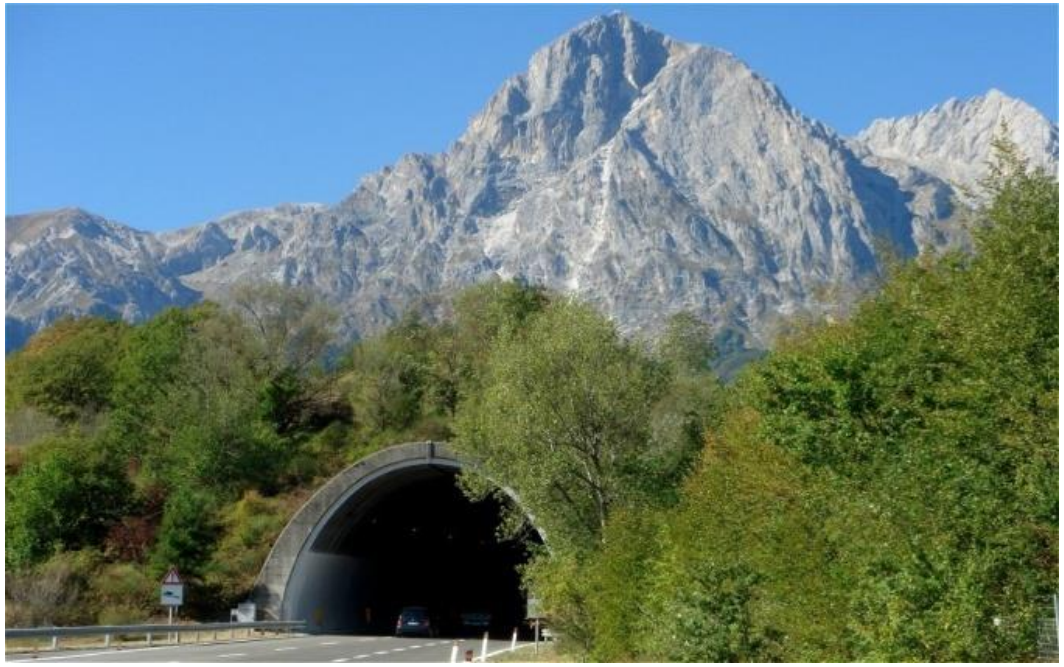


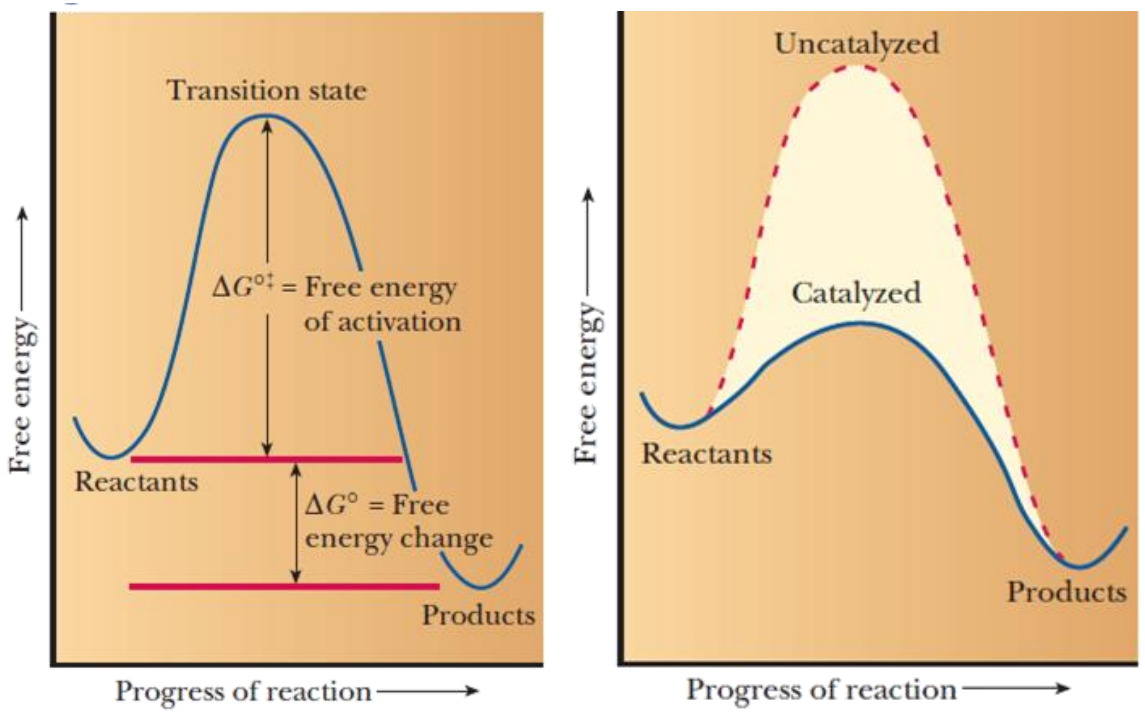




How do enzymes work?

- 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?

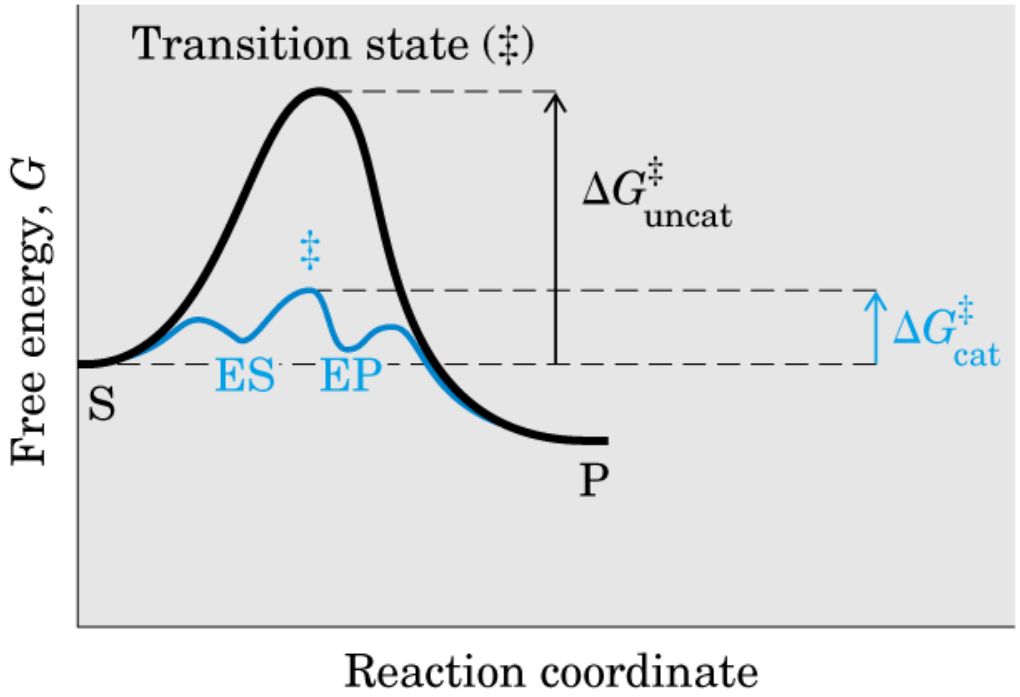


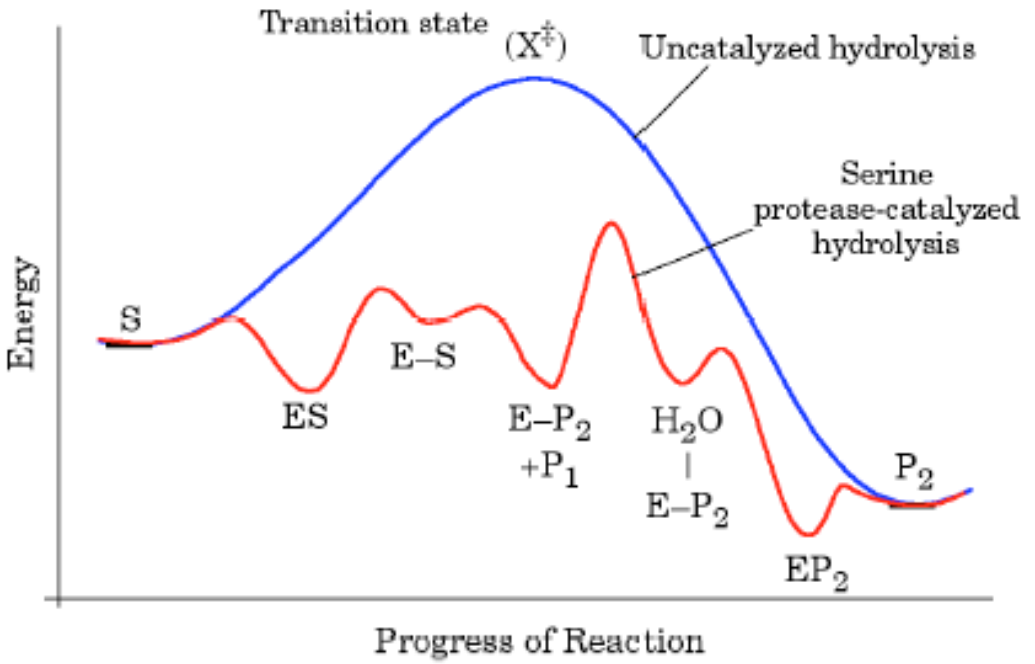


Transition-state complex binds more tightly to the enzyme compared to substrate

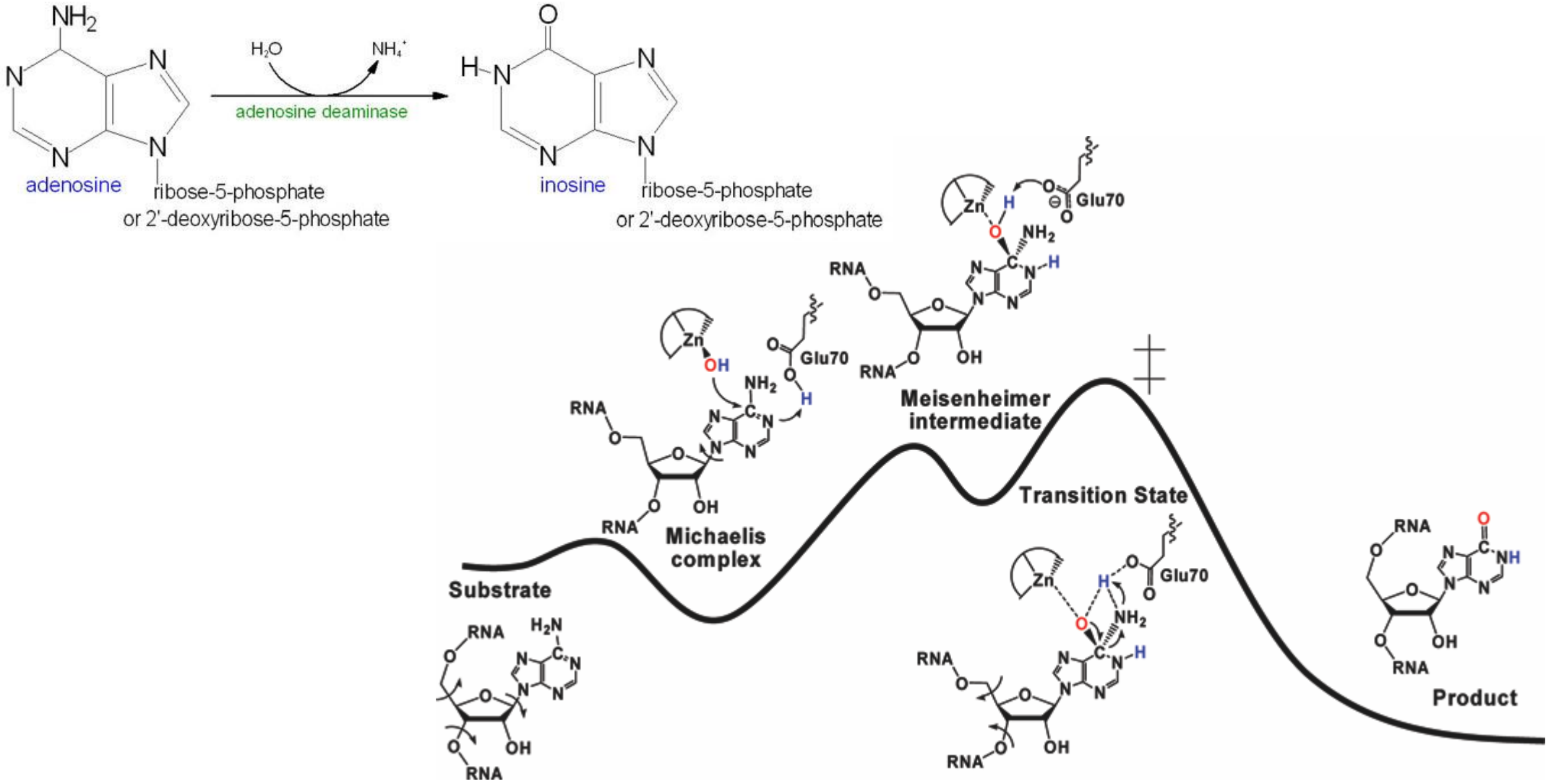
Alternative pathways

- Substrates of enzymatic reactions often undergo several transformations when associated with the enzyme and each form has its own free energy value
- Which one is the activation energy?
- Activation energy & final ΔG calculation



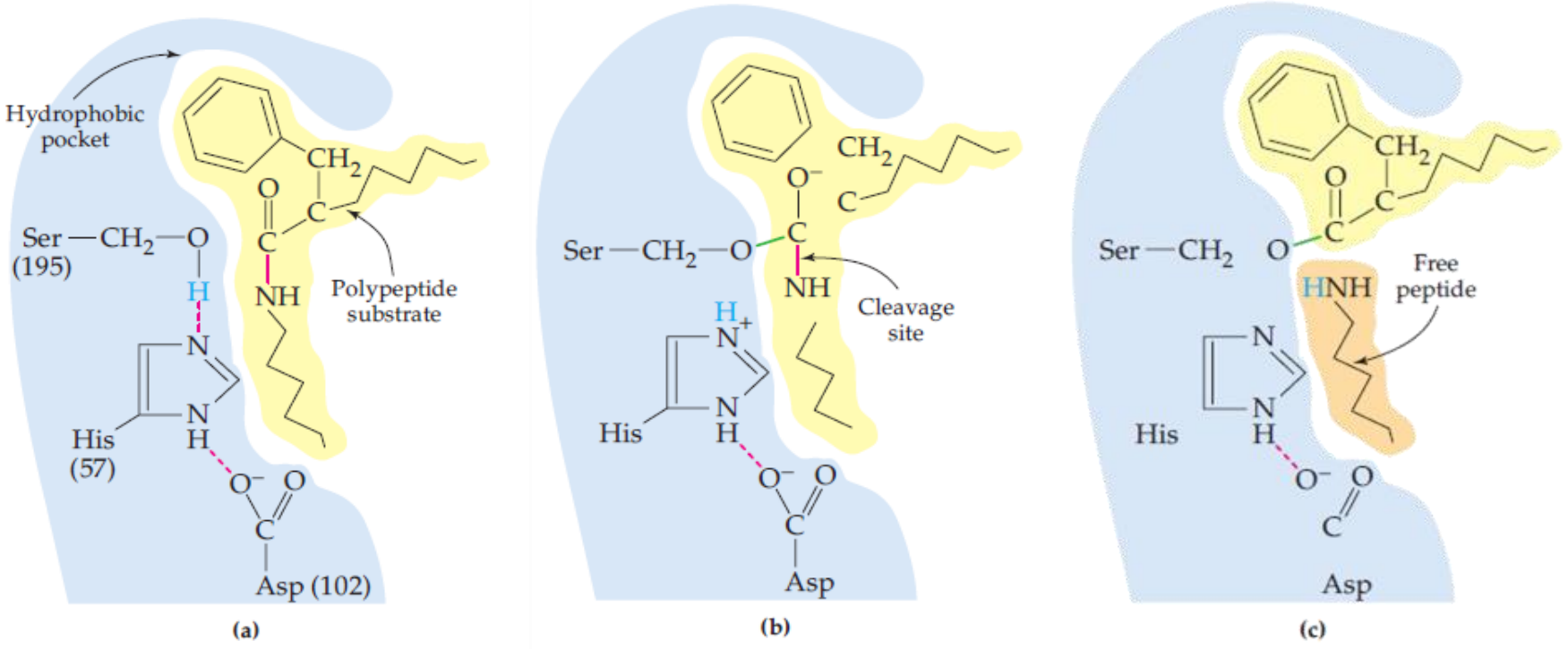


Example: Adenosine Deaminase



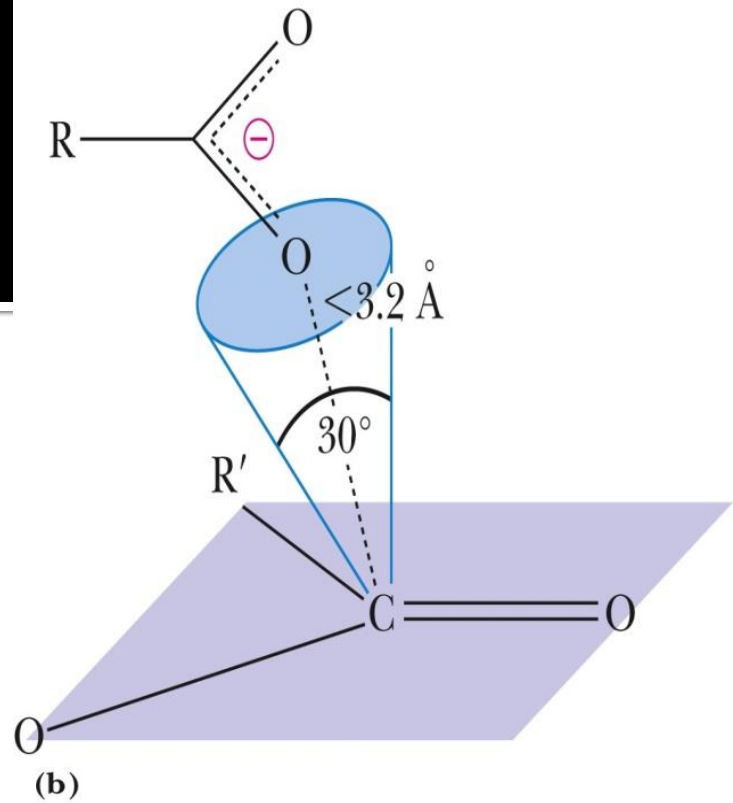
How Do Enzymes Work?

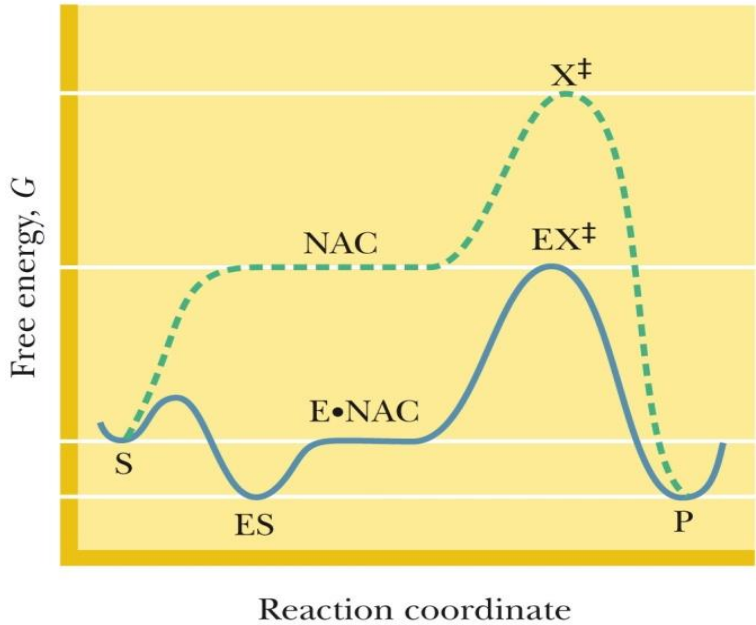
- Proximity effect: Bring substrate(s) and catalytic sites together
- Orientation effect: Hold substrate(s) at the exact distance and in the exact orientation necessary for reaction
- Catalytic effect: Provide acidic, basic, or other types of groups required for catalysis
- Energy effect: Lower the energy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



Catalysis by proximity & orientation

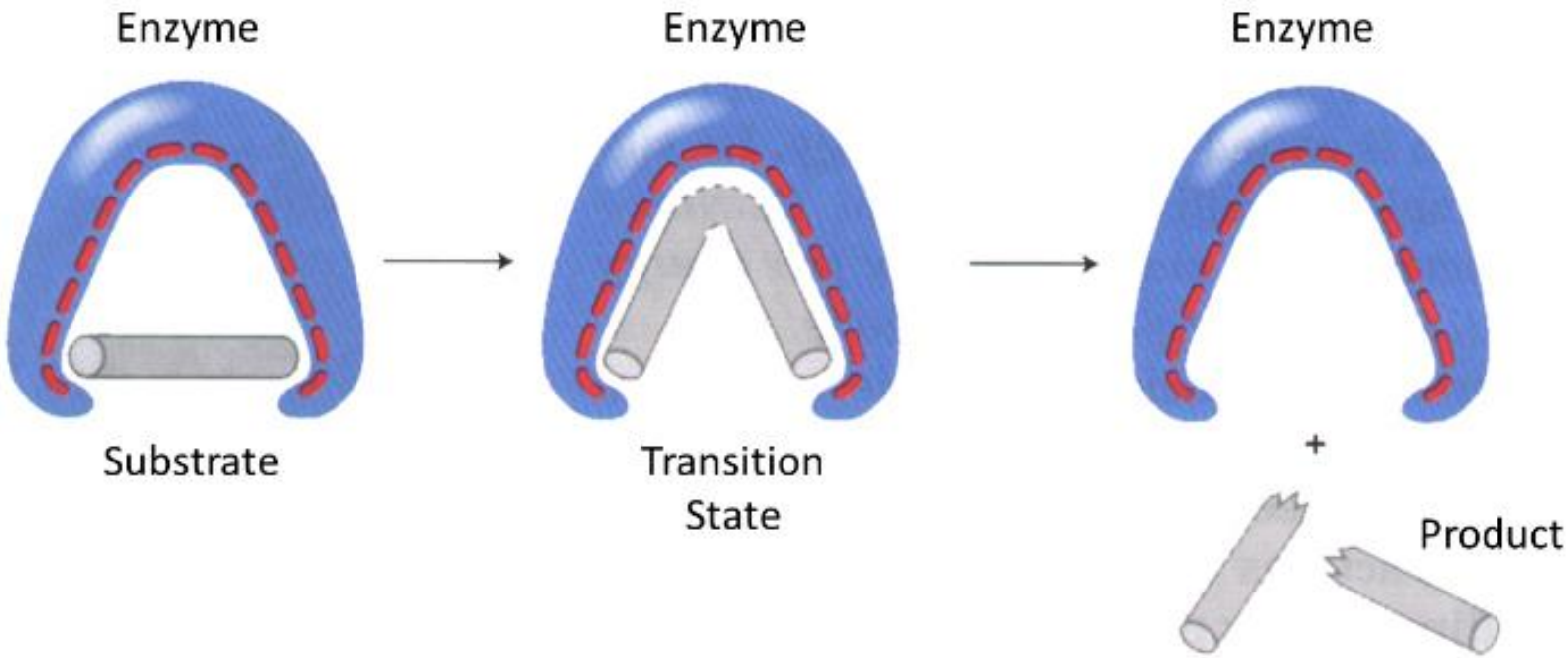
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis
  - Such arrangements have been termed near-attack conformations (NACs)
  - NACs are precursors to reaction transition states





Catalysis by bond strain

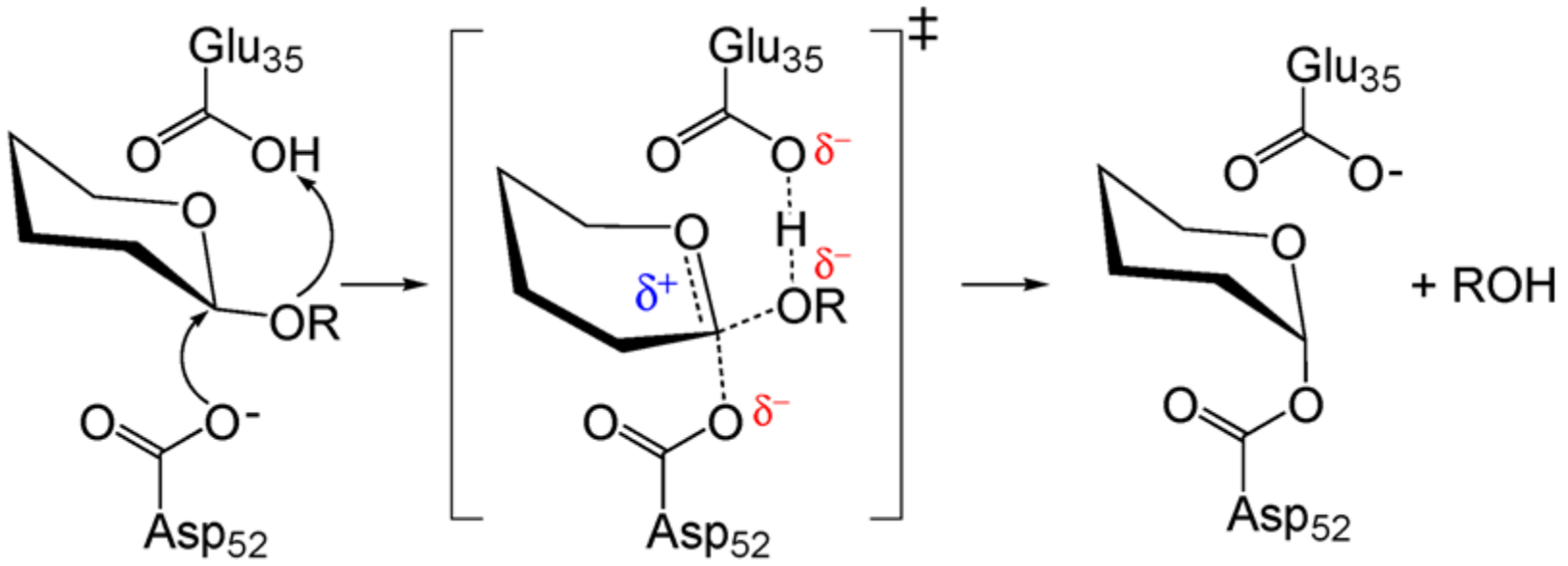
- In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.



Catalysis by bond strain

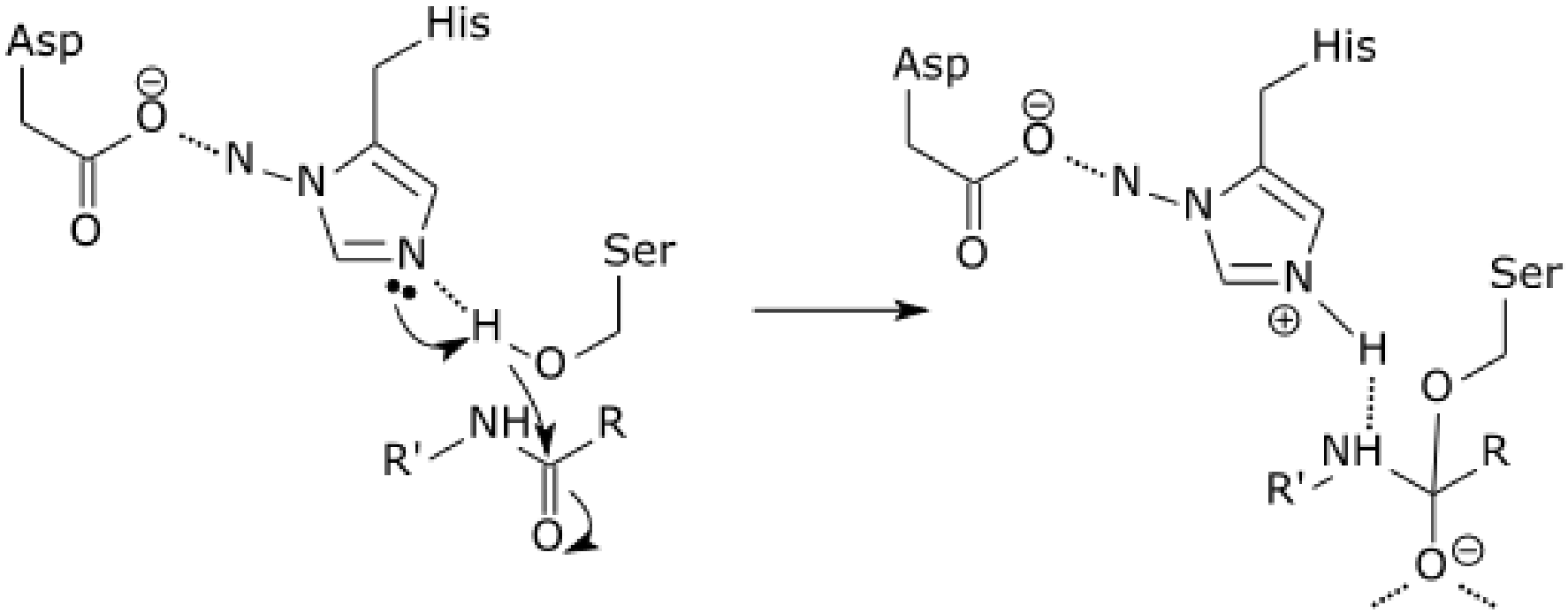
- Example: lysozyme

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



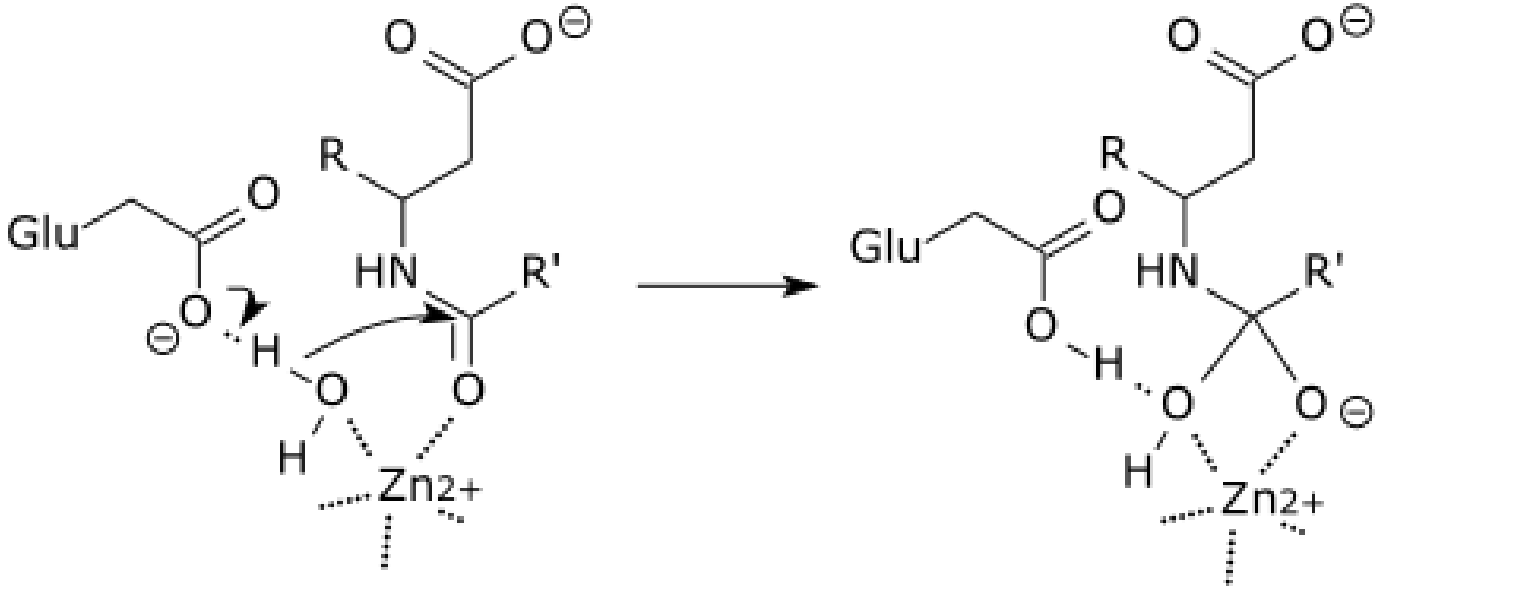
Catalysis involving proton donors (acids) & acceptors (bases)

- The R groups act as donors or acceptors of protons
  - Histidine is an excellent proton donor/acceptor at physiological pH
  - Example: serine proteases



Covalent catalysis

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



Enzyme substrate complex

Tetrahedral intermediate

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

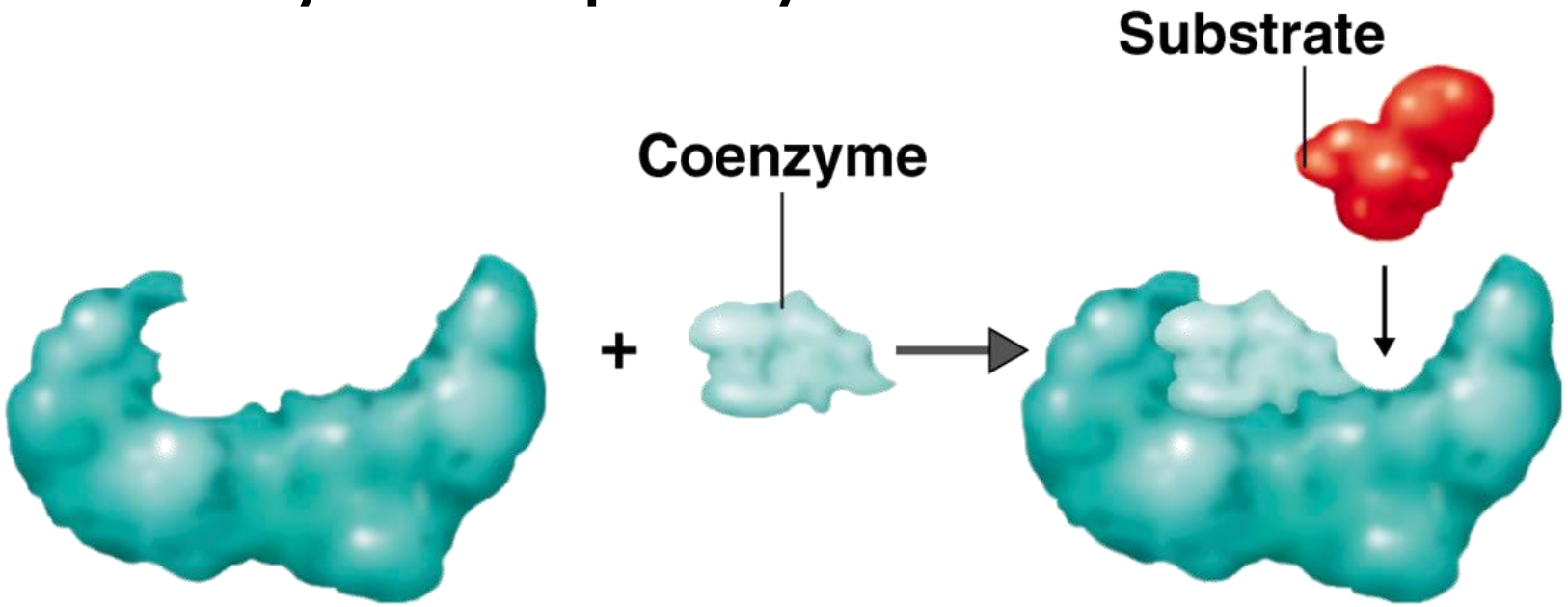
Naming of enzymes; EC numbering
Enzyme Commission number

- 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 (1-7), 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 of the amino acid polypeptide
  - EC 3.4.11.4: cleave off the amino-terminal end from a tripeptide

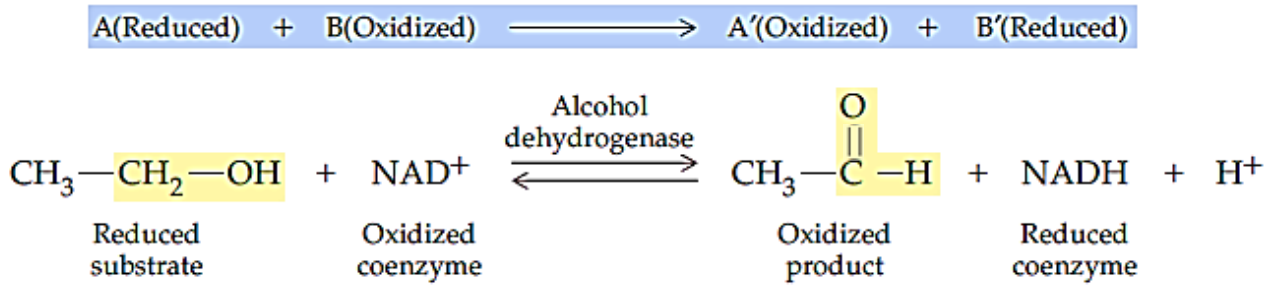
Enzyme Classification (structure)

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

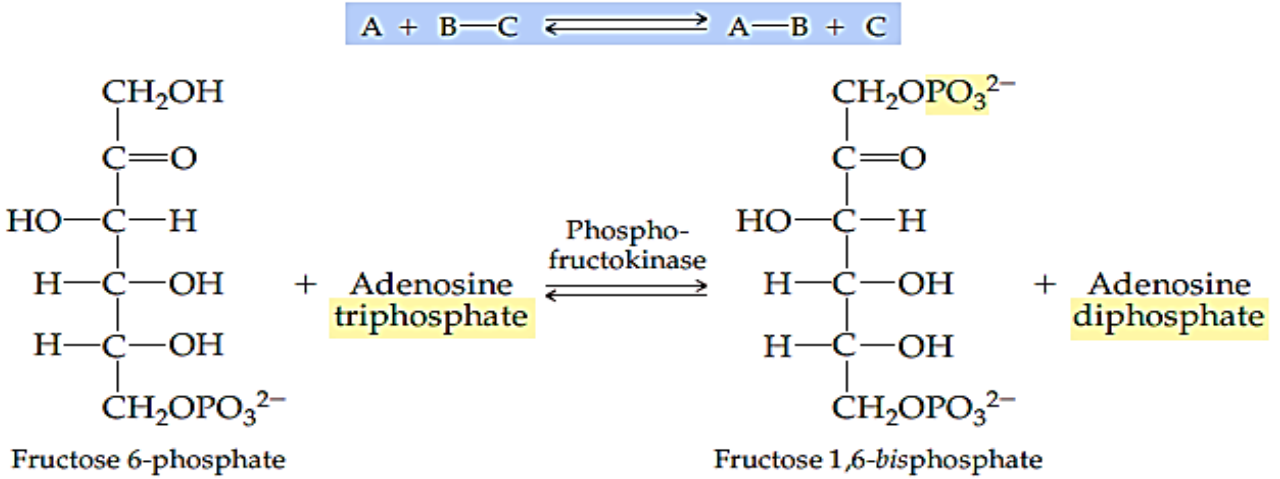


Enzyme Classification (function)

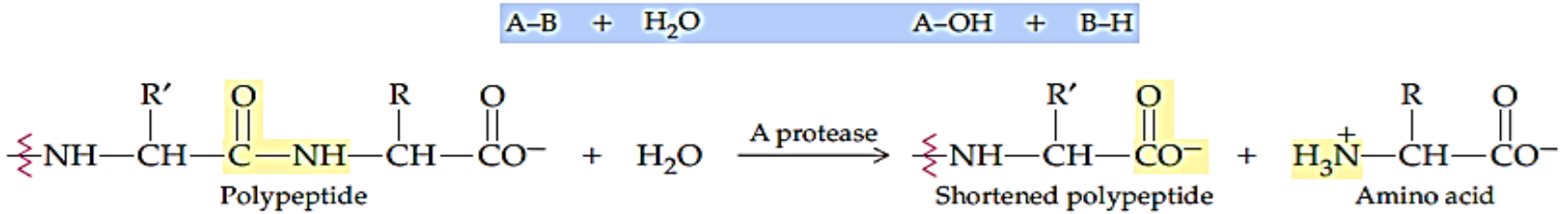
- **Oxidoreductases: addition or removal of O, O_2, H. Require coenzymes (heme)**



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

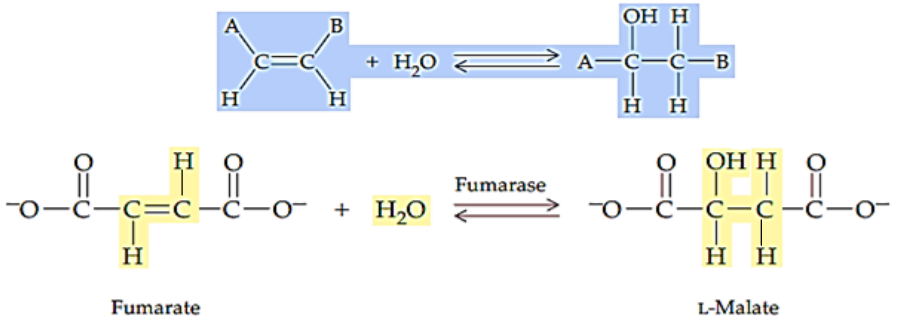


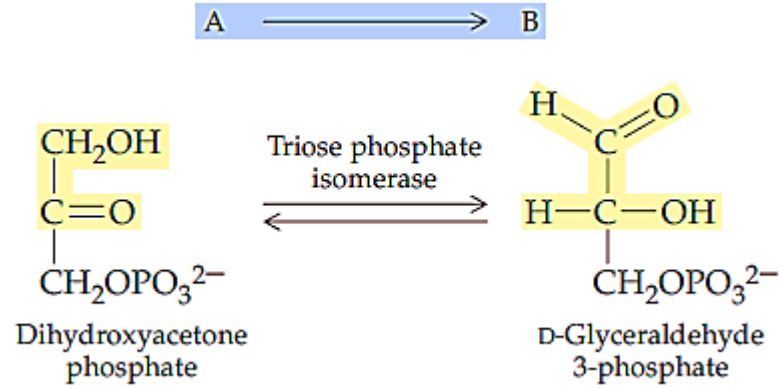
- **Hydrolases: addition of water (carbs. & proteins)**

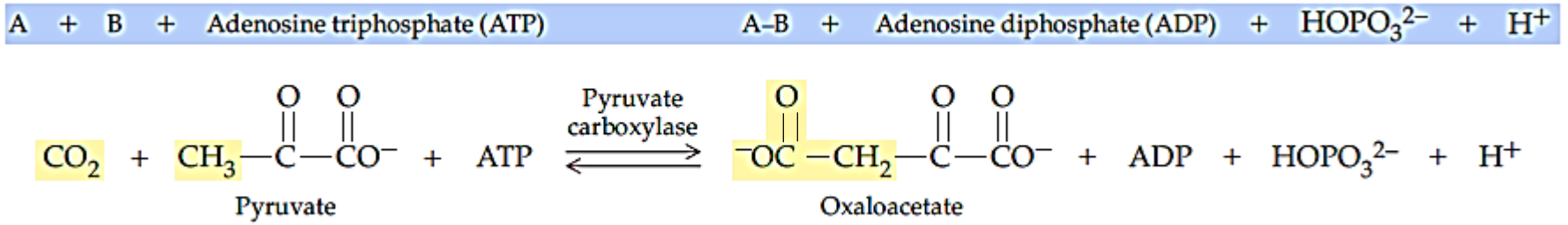


Enzyme Classification (function)

- Lyases: addition of a molecule (H_2O, CO_2, NH_3) to a double bond or reverse (non-hydrolytic)
- Isomerases: one substrate and one product
- Ligases: usually not favorable, so they require a simultaneous hydrolysis reaction
- Translocases: Catalyze the movement of ions or molecules across membranes or their separation within membranes (ATP/ADP translocase)







Oxido-reductases

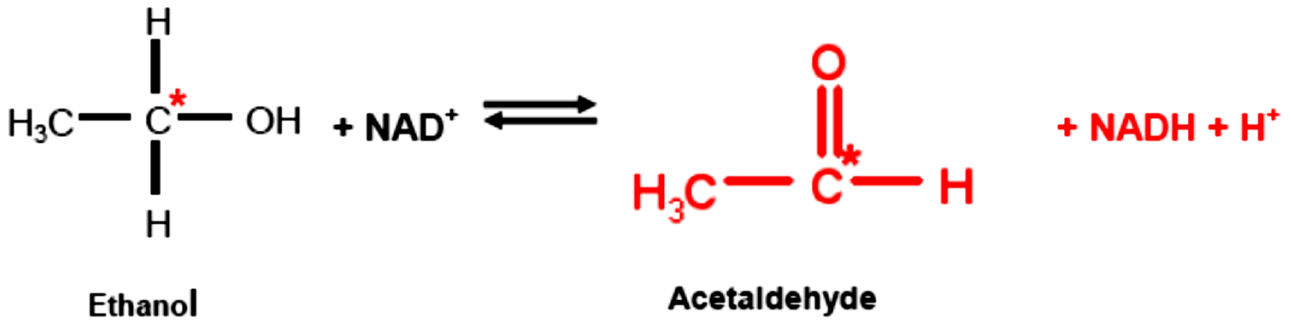
- These enzymes catalyze oxidation & reduction reactions involving the transfer of hydrogen atoms, electrons or oxygen
- This group can be further divided into 4 main classes:
  - ✓ Dehydrogenases
  - ✓ Oxidases
  - ✓ Peroxidases
  - ✓ Oxygenases

Dehydrogenases

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

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

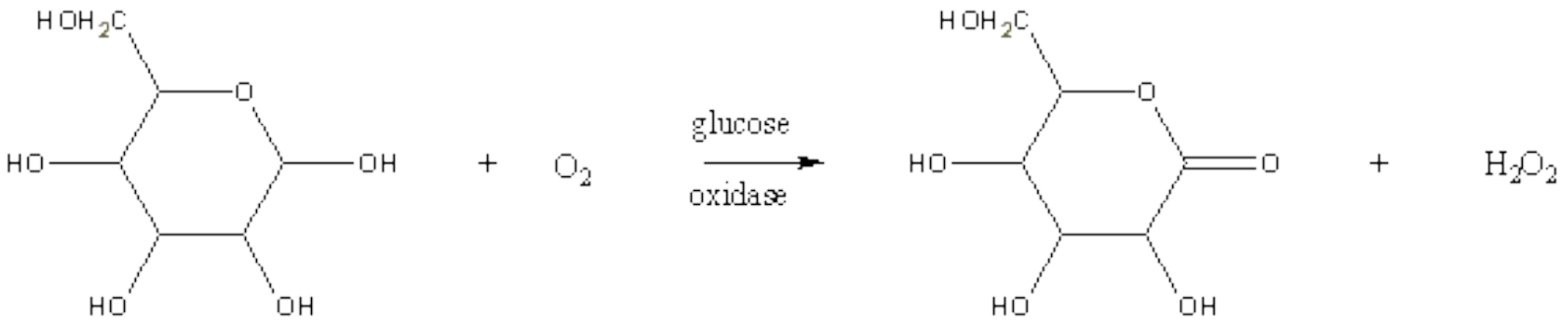
- Alcohol dehydrogenase



Oxidases

- Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product
- Glucose oxidase

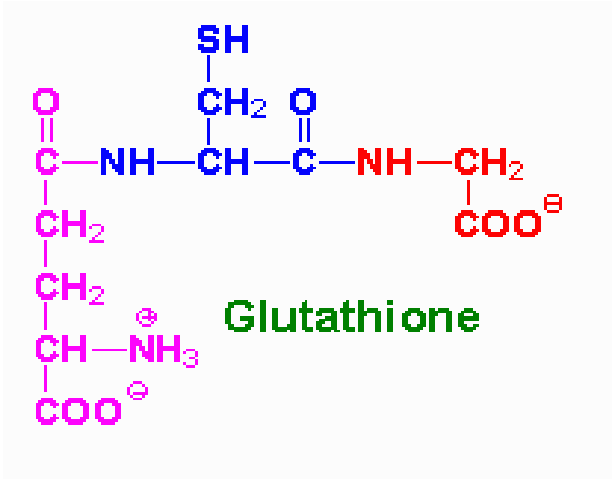
- β-D-glucose + O_2 ⇆ gluconolactone + H_2O_2

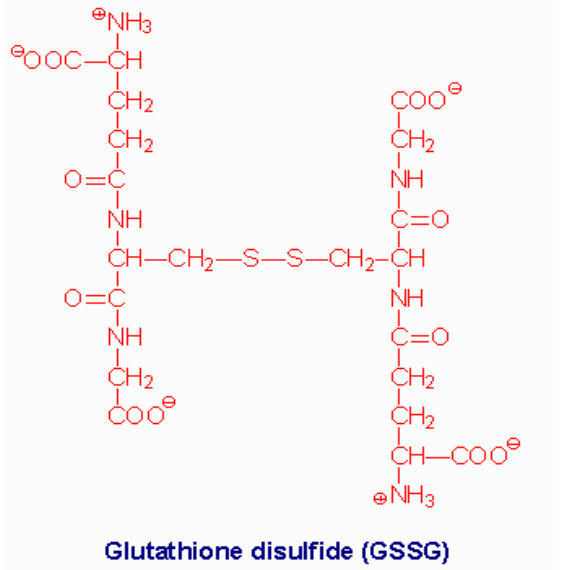


Peroxidases

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

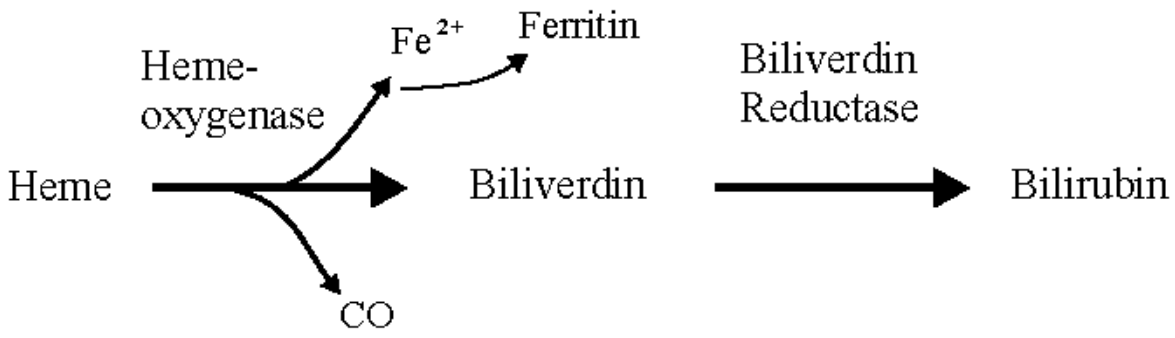
$$2\,GSH + H_2O_2 \leftrightarrows G\text{-}S\text{-}S\text{-}G + 2\,H_2O$$

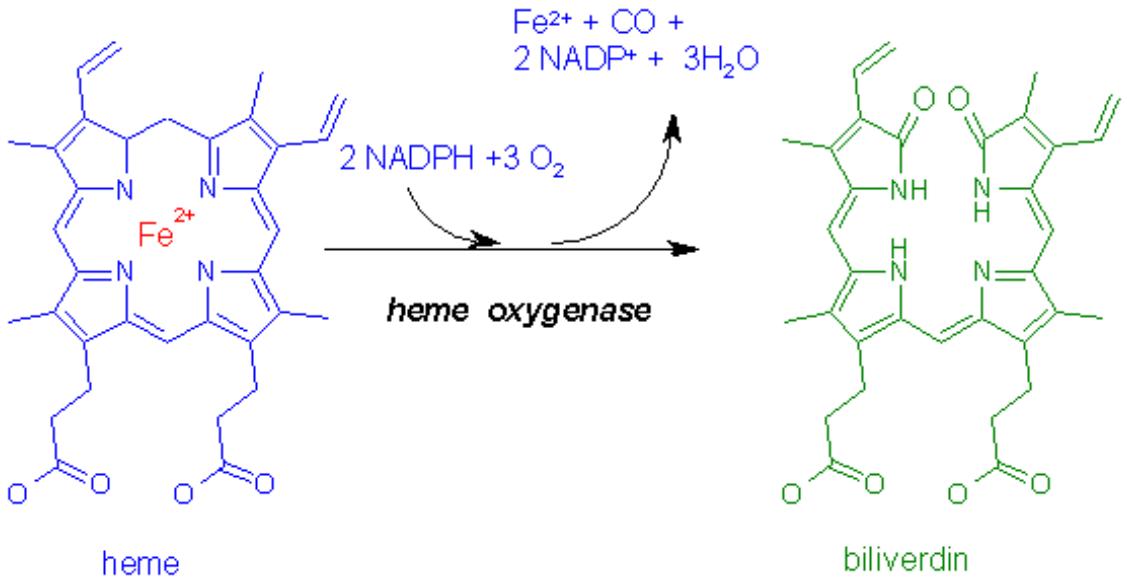




Oxygenases

- Oxygenases catalyze substrate oxidation by molecular O_2
- The reduced product of the reaction in this case is water and not H_2O_2
- 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 (O_2) into the product(s) of the reaction

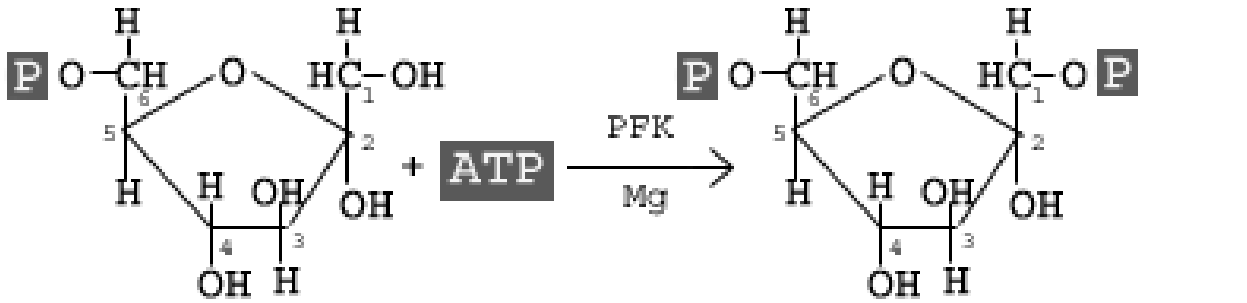




Transferases - Kinases

- 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



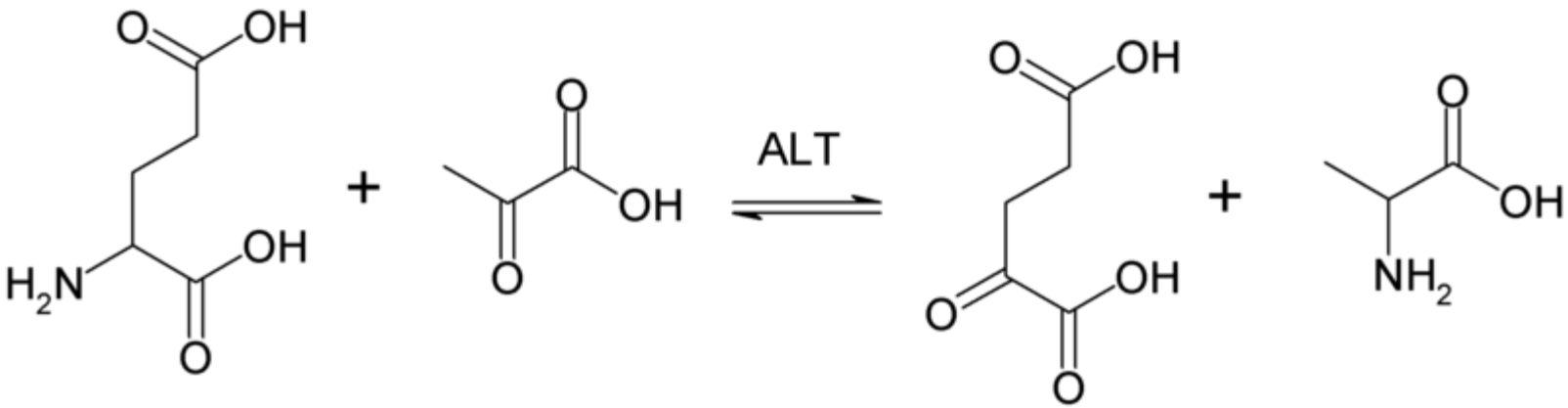
fructose-6-phosphate

fructose-1,6-bisphosphate

Transaminases - Kinases

- 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



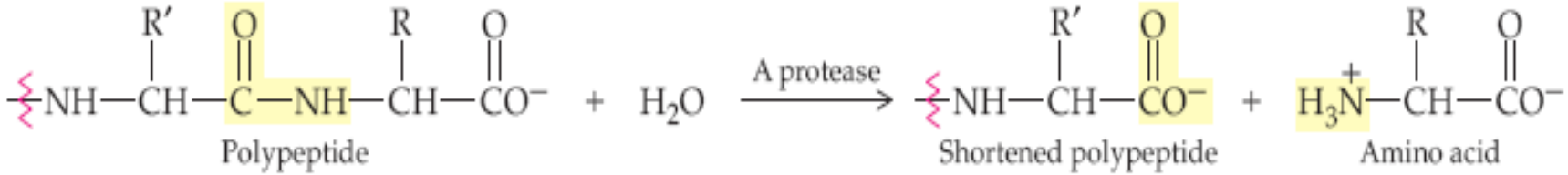
Glutamic acid + Pyruvic acid ⇌ Alpha-ketoglutaric acid + Alanine

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



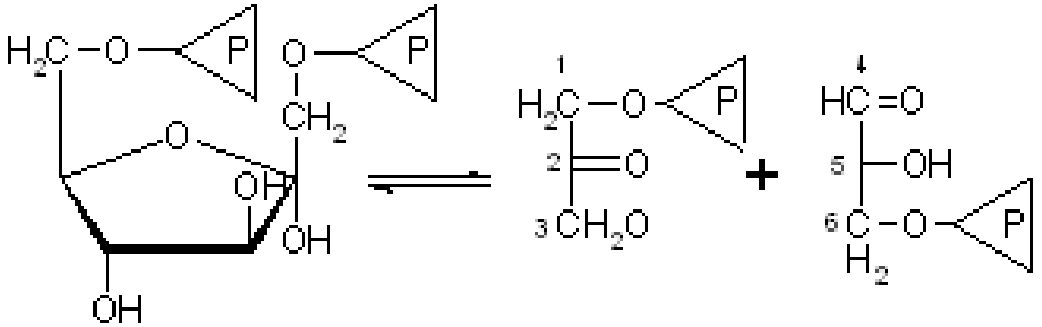
- Trypsin, is quite specific; catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine
- Thrombin, catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only

Lyases

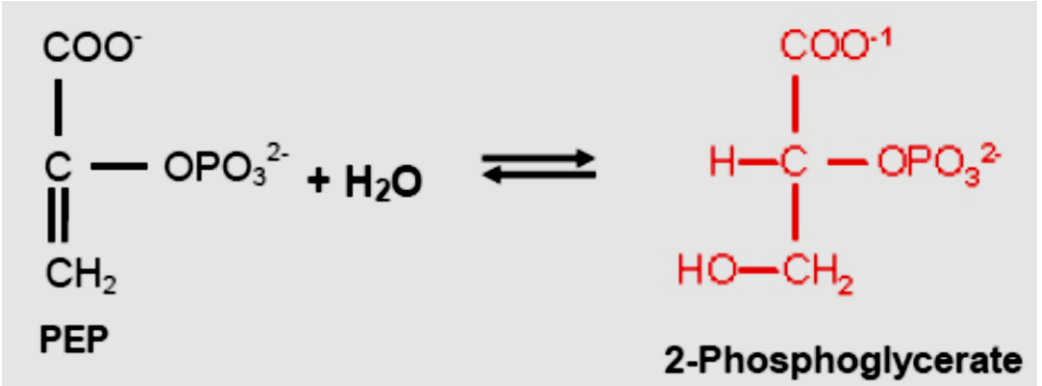
- Catalyze the addition or removal of functional groups from their substrates with the associated formation or removal of double bonds between C-C, C-O and C-N

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

  - F 1,6 bisphosphate ⇆ DHAP + GAP



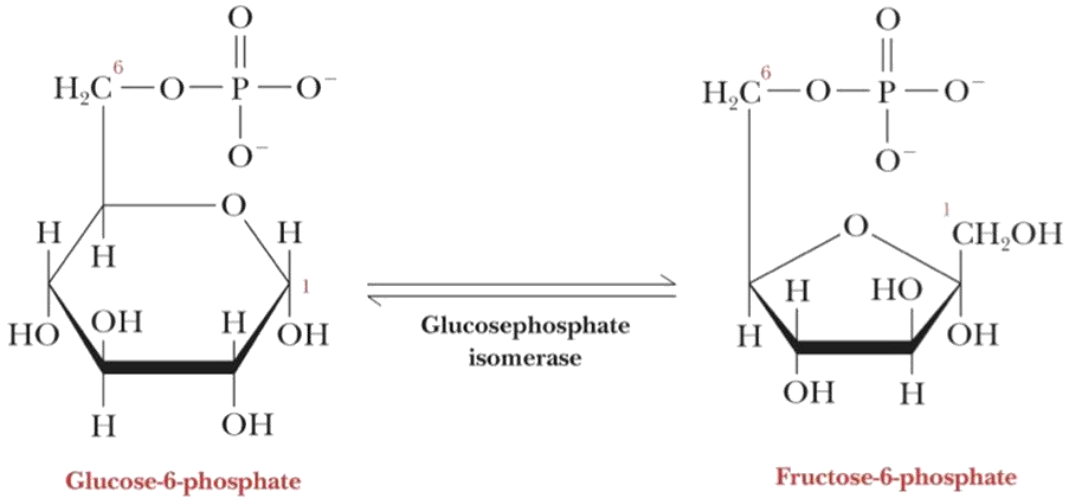
- Enolase; interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation and removal of double bonds



Isomerases - Mutases

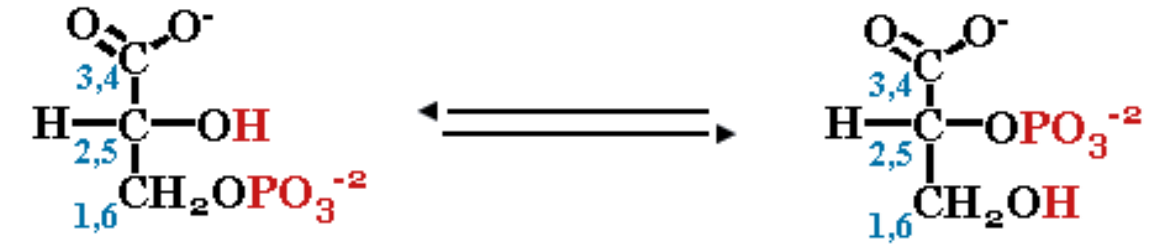
- Catalyze intramolecular rearrangements
- Glucose-6-phosphate isomerase; isomerizes glucose-6-phosphate to fructose-6-phosphate
- Phosphoglycerate mutase; transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate (BPG intermediate)

- 3-P glycerate ⇆ 2 P glycerate



Glucose-6-phosphate

Fructose-6-phosphate



3-phosphoglycerate

2-phosphoglycerate

Ligases - Carboxylases

- 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