بسم الله الرحمن الرحيم

BioChemistry | FINAL 10

Enzymes pt.2

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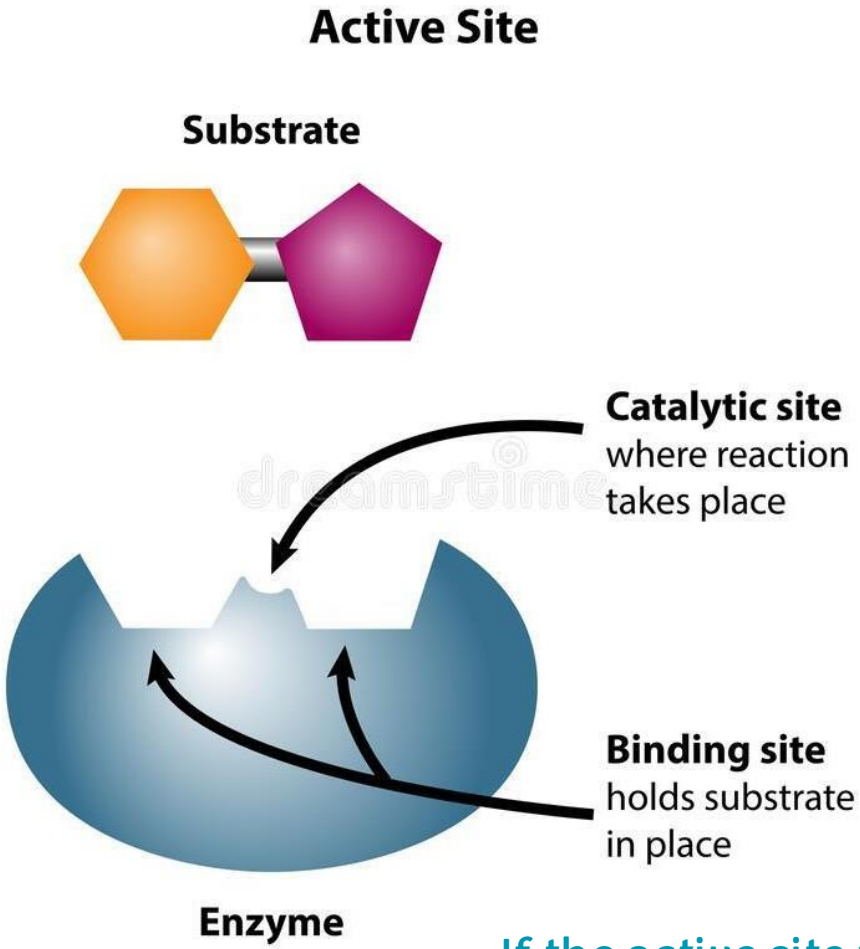
Previous lecture

The active site is a three dimensional space and it has specialized amino acids sequence and they are polar by nature ,the active site is like a canal or pocket ,the active site is small with regard to the whole enzyme , the binding between active site and the substrate is noncovalent , any material which bind to active site by covalent bond should be poison , toxin , drugs .

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

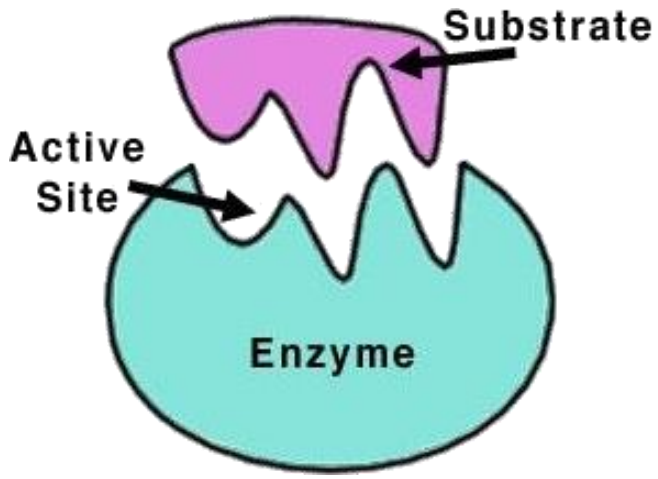
- Binding site: binds substrate through ionic, H-bonding or other electrostatic forces, or hydrophobic interactions
- Catalytic site: contains the catalytic groups

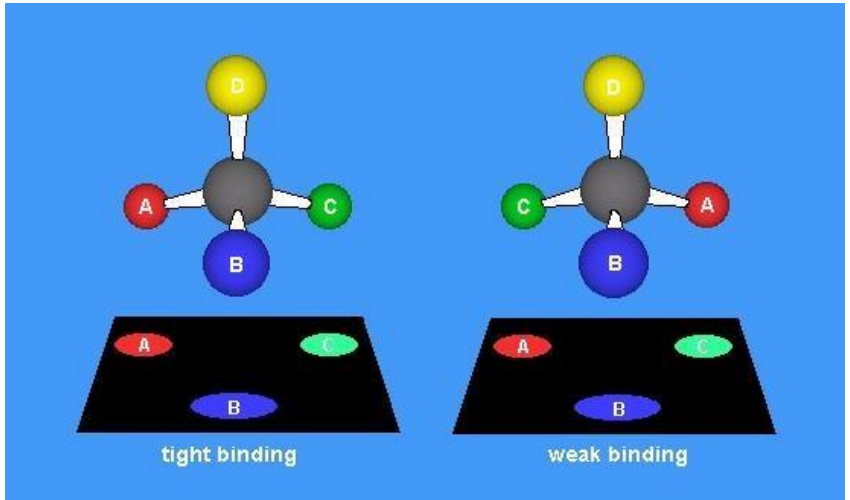


If the active site was small it will do both functions the binding and the catalytic one

Features of active site

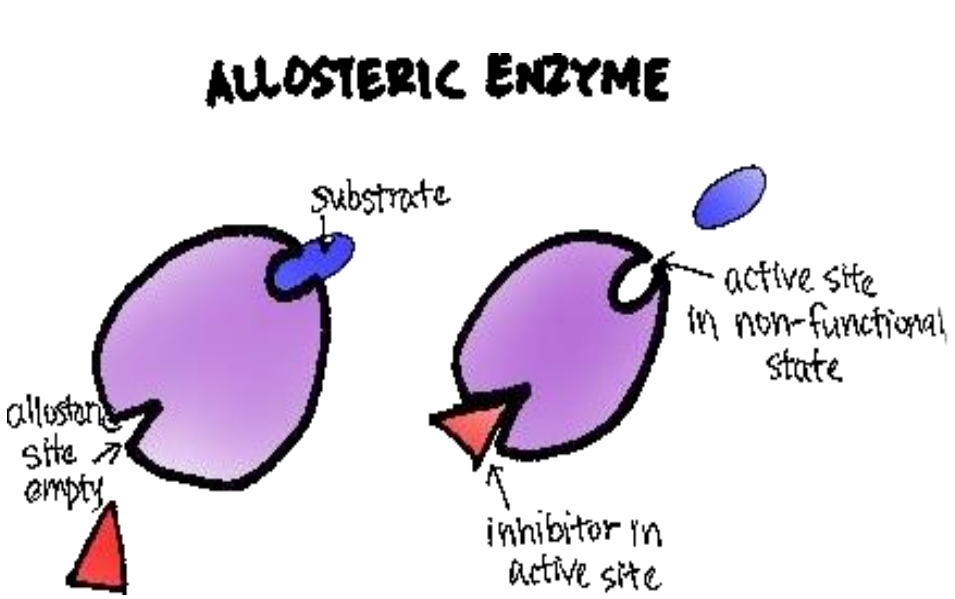
- Active sites; structures that look like canals, clefts or crevices
- Water is usually excluded after binding unless it participates in the reaction
- Substrates are bound to enzymes by multiple weak attractions (electrostatic, hydrogen, van der Waals, & hydrophobic)
- Binding occurs at least at three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

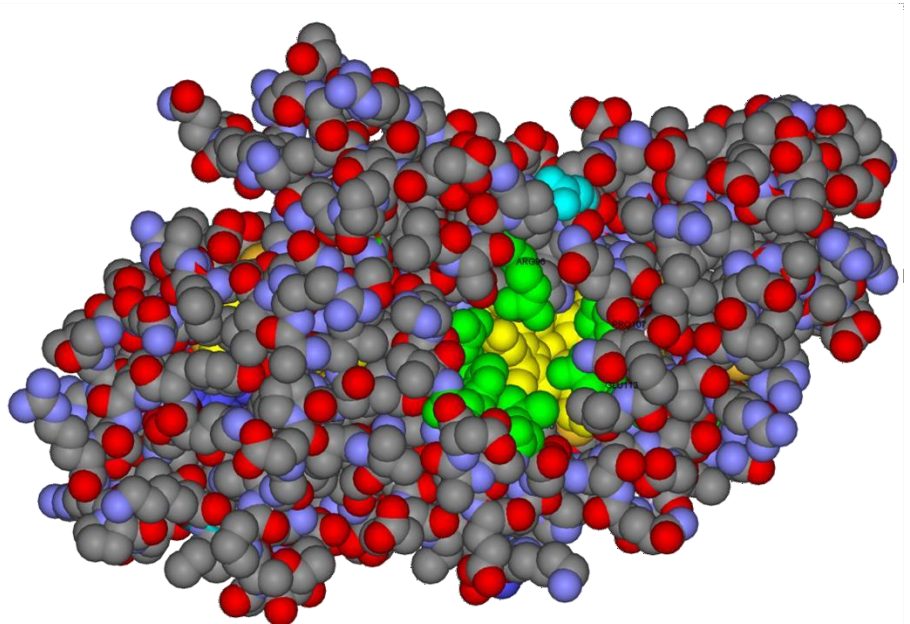




Features of active site

- 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

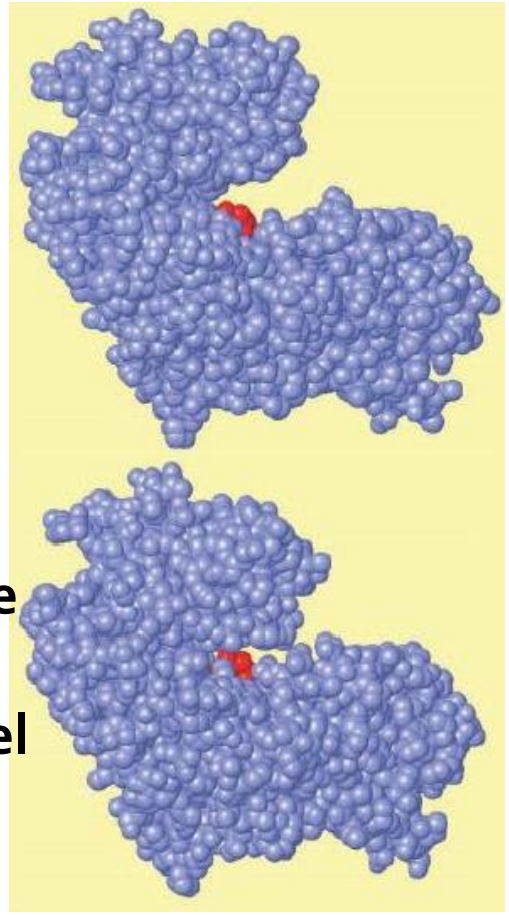




How Do Enzymes Work?

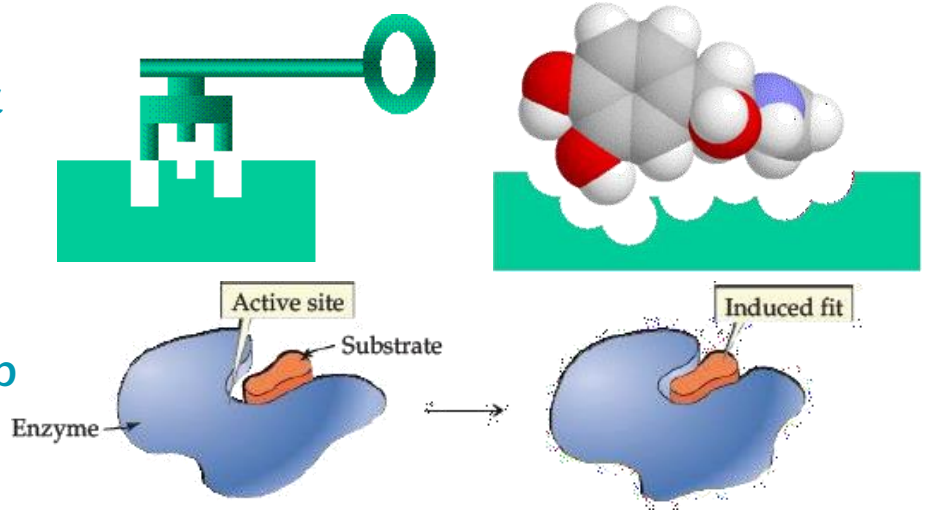
The older(lock-and-key) theory not valid any more because of two reasons the first one that the proteins are dynamic with huge number of conformations with little differences so could look be changed !! the second reason that : experimentally we have found that enzymes could work on more than one substrate as an example of that hexokinase which add phosphate group to glucose ,fructose and mannose .

- 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

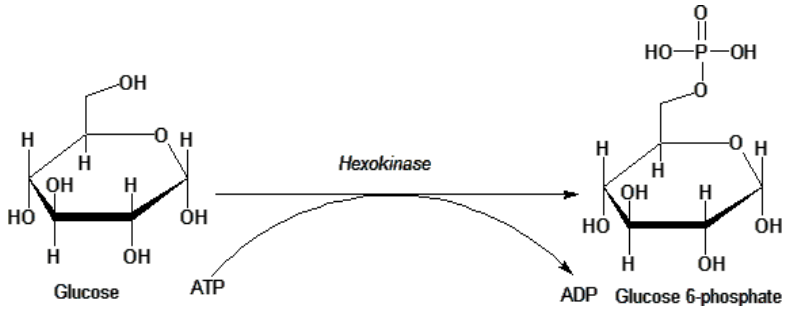


the more realistic model induced indicates that the 100% fit is induced (the 100% fit wasn't there from the start),but that doesn't mean that there wasn't any fit and complementary

This is hexokinase before and after binding to glucose (red) with regard to induce fit model.



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



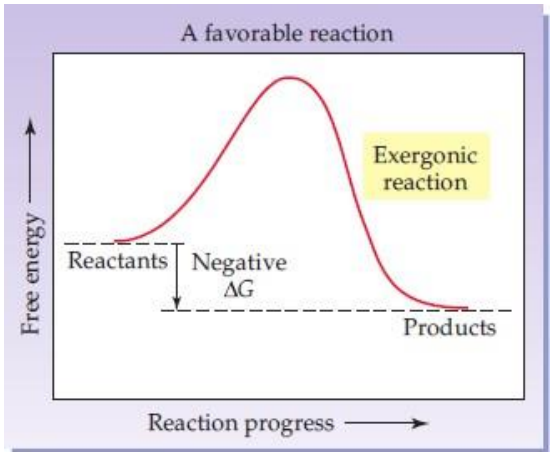
Energy & Biochemical Reactions

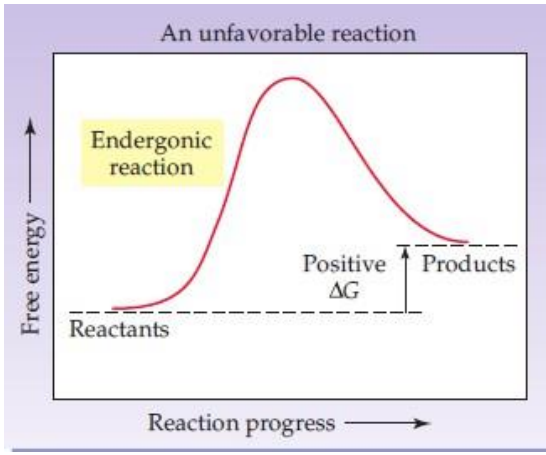
- $\Delta G = \Delta H - T\Delta S$
- Spontaneous vs. non-spontaneous, favorable vs. non-favorable, exergonic vs. endergonic, exothermic vs. endothermic, switch of signs
- ΔG, ΔG°
- Biochemical pathways; storage (endergonic) & release (exergonic)
- Kinetics (rate) vs. Thermodynamics (favorability)

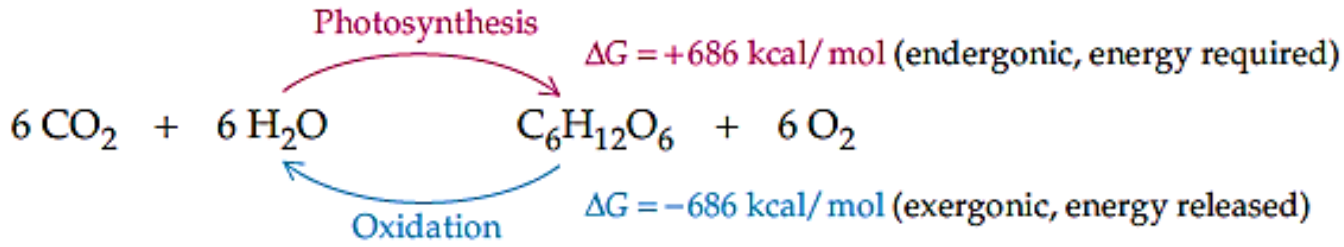
ΔG Is the thing that determine if the reaction feasible and not the other factors , the positive means that it is non-favorable reaction a negative means it is favorable reaction

ΔH the total energy change of the system

ΔS the change in how molecules are arranged and interact with each other







Why reactions happen ?

To achieve situation of higher stability ,but as you see above in **endergonic** reaction it goes from lower energy to higher energy which doesn't make sense and illogical, here to increase the amount of energy we use ATP. But with regard to **exergonic** chart it is very logical and biochemically spontaneous, although they are not because of the activation energy which without it all exergonic reactions would have happened before ages.

All reactions in the world follow the energy equation, but what does it mean: $\Delta G = \Delta H - T\Delta S$
At first, ΔH is the enthalpy change, and it represents the total difference of energy between the reactants and the products (can be calculated by considering the bond energies for example, in a reaction involving glucose and water, the O-H bonds are important to account for). While ΔG, the Gibbs free energy, represents the final balance of energy that tells us if the reaction can occur spontaneously or not. The term ΔS (entropy) expresses the difference in the organization of molecules. For example, water in the states of gas, liquid, and solid shows different entropies, since gas is more disordered than liquid, and liquid is more disordered than solid. In this way, Gibbs free energy combines both heat changes and entropy changes to determine the spontaneity of a reaction.

Biologically you can study reaction from two point of views thermodynamically or kinetically , thermodynamically you study the starting and ending states of reactions, kinetically you are studying what are between the start and end point and this related to enzymology (the speed ,the intermediates, transition states)

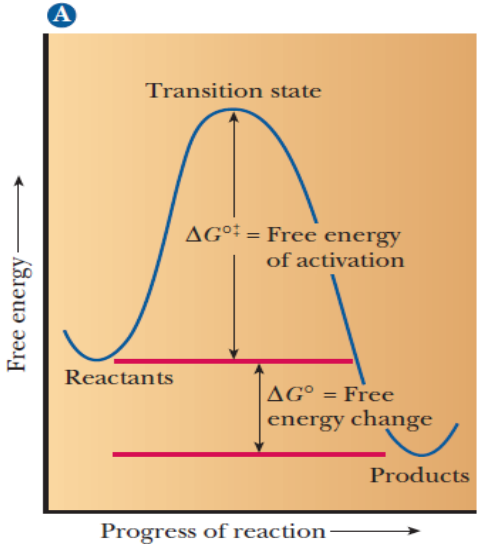
How do enzymes work?

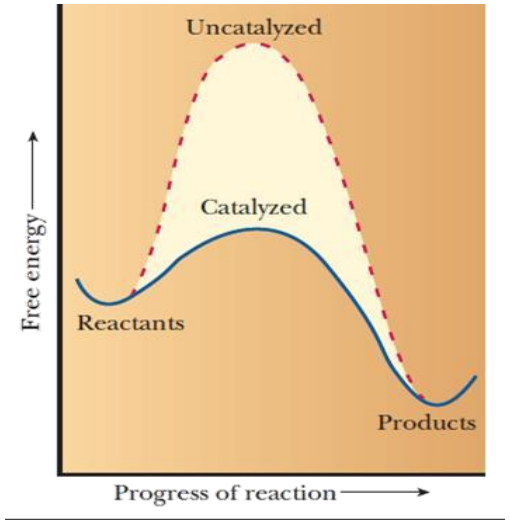
- Enzymes speed up reactions, but have no relation to equilibrium or favorability
  - **What enzyme can do ?**

It lowers the activation energy which has nothing to do to stored energy in reactant and product so enzymes doesn't affect the equation above

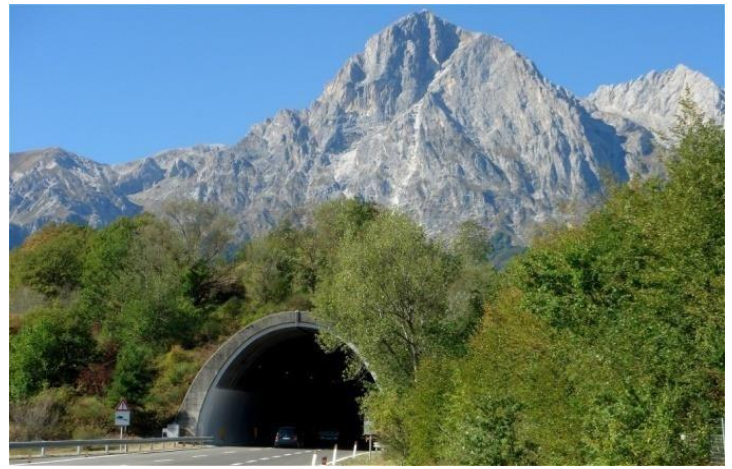
- What is an activation energy ($\Delta G^{\circ\ddagger}$) concept?
- Specificity varies (stereoisomers), however, there is none non- specific
- Spontaneous vs. rate!
- What is the transition state?

Some enzymes when working on the substrate has more than one intermediate, but all the time there is only one transition state and one activation energy, from definition you can understand more (the energy needed to convert reactant to nonstable state called transition state)





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

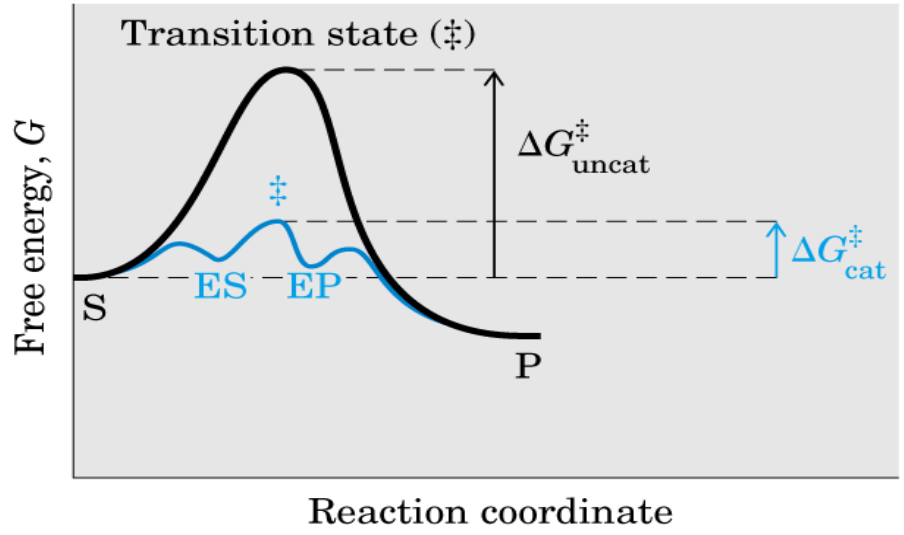


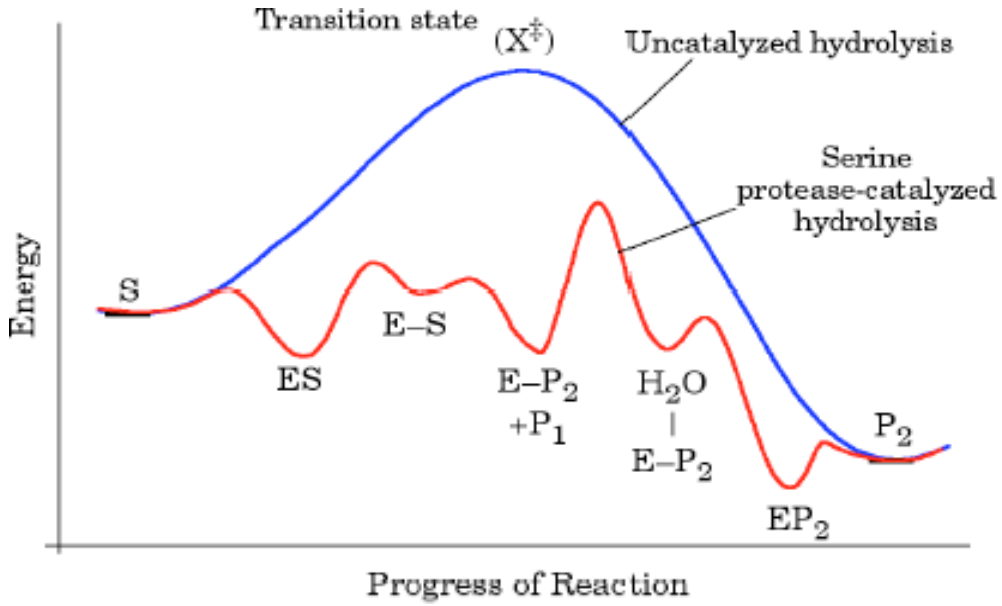
Alternative pathways

- Substrates of enzymatic reactions often undergo several transformations when associated with the enzyme and each form has its own free energy value

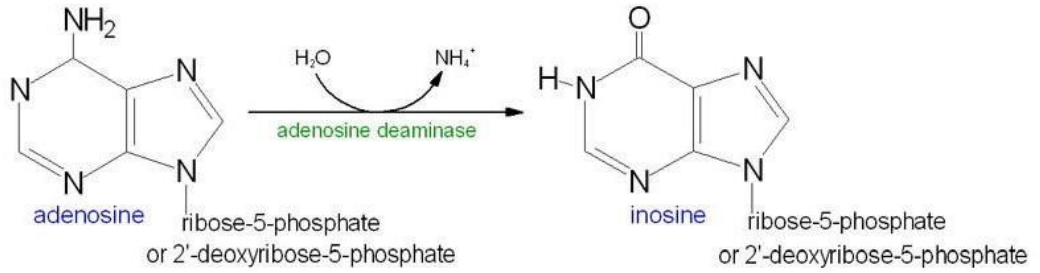
- Which one is the activation energy?

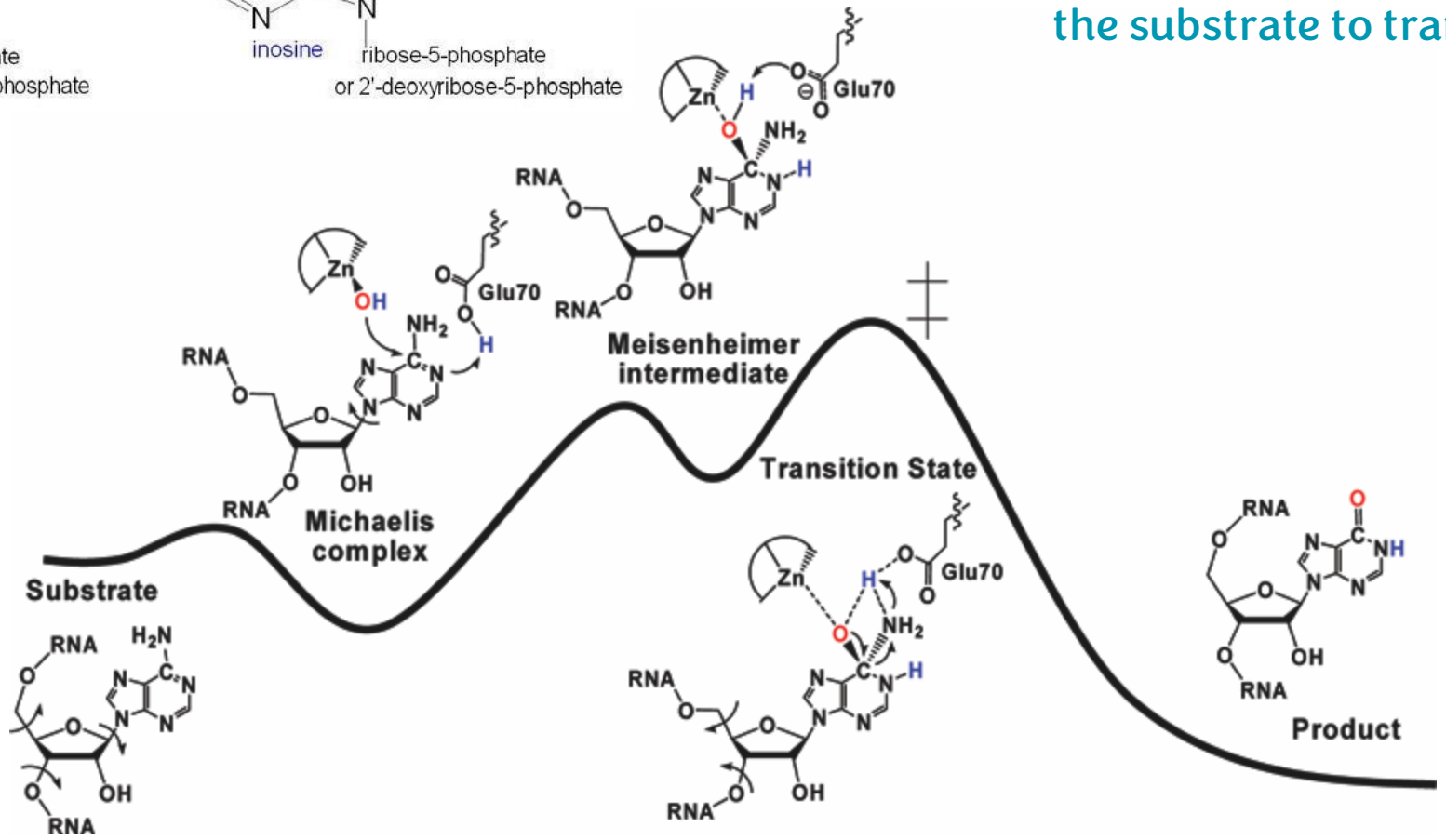
- Activation energy & final ΔG calculation





Example: Adenosine Deaminase





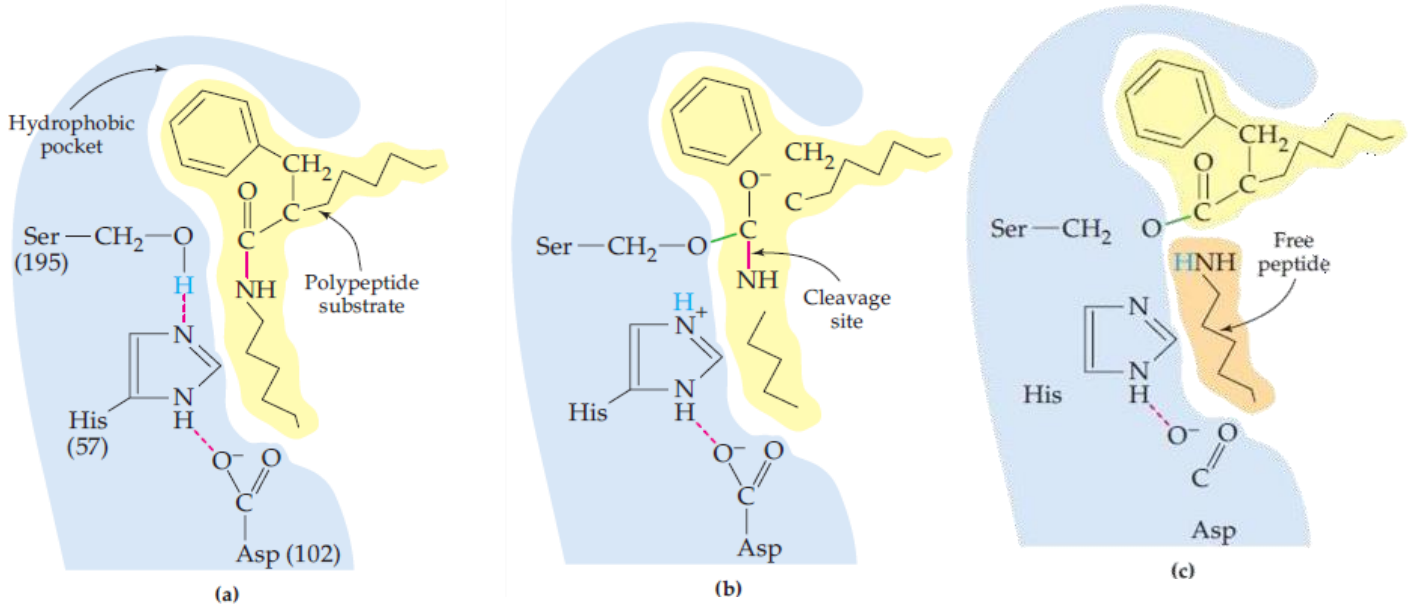
The top with (++) represent the transition state and the activation energy is the energy required to convert the substrate to transitions

How Do Enzymes Work?

How enzymes work from a mechanism point of view.

- **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.** By R groups of amino acids in active site
- **Energy effect: Lower the energy barrier by inducing strain in bonds in the substrate molecule**

Enzymes lower the time required for the rxn to happen, by bringing the substrates together and in the right orientation instead of relying on the random collisions to react.

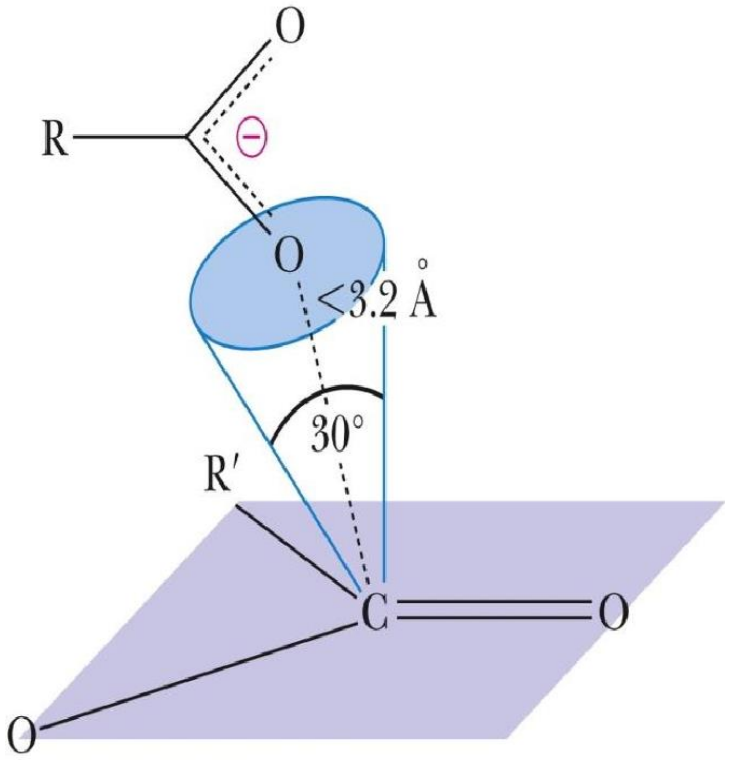


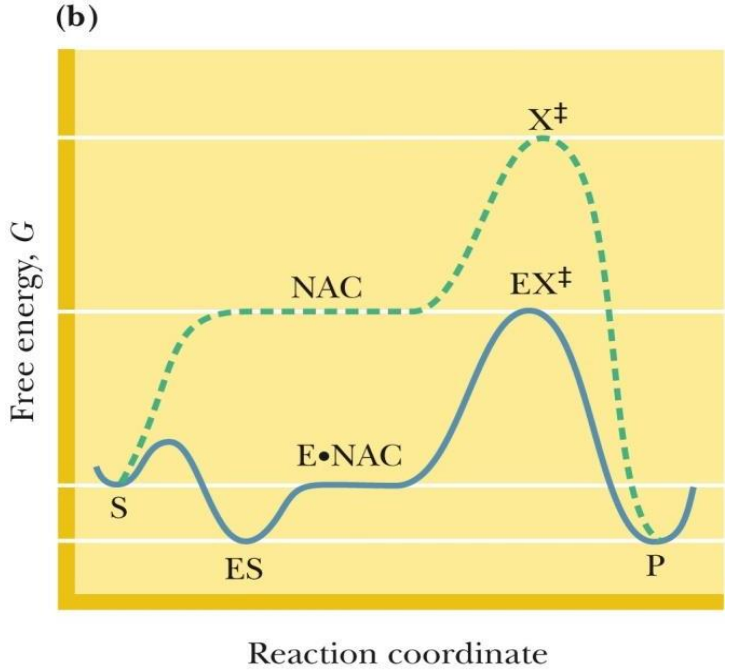
The first 3 mechanisms are used by all enzymes.

Catalysis by bond strain isn't used by all enzymes.

Catalysis by proximity & orientation

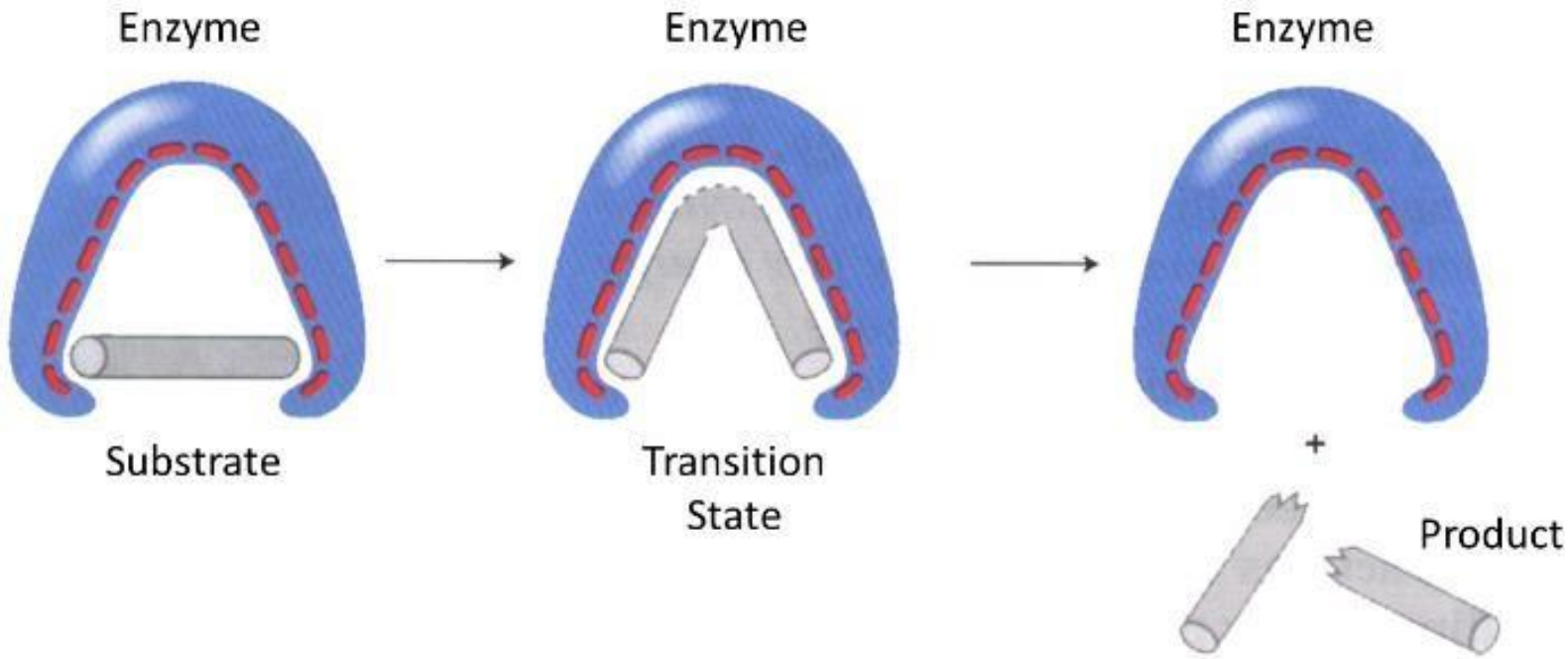
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis
  - Such arrangements have been termed near-attack conformations (NACs)
  - NACs are precursors to reaction transition states





Catalysis by bond strain

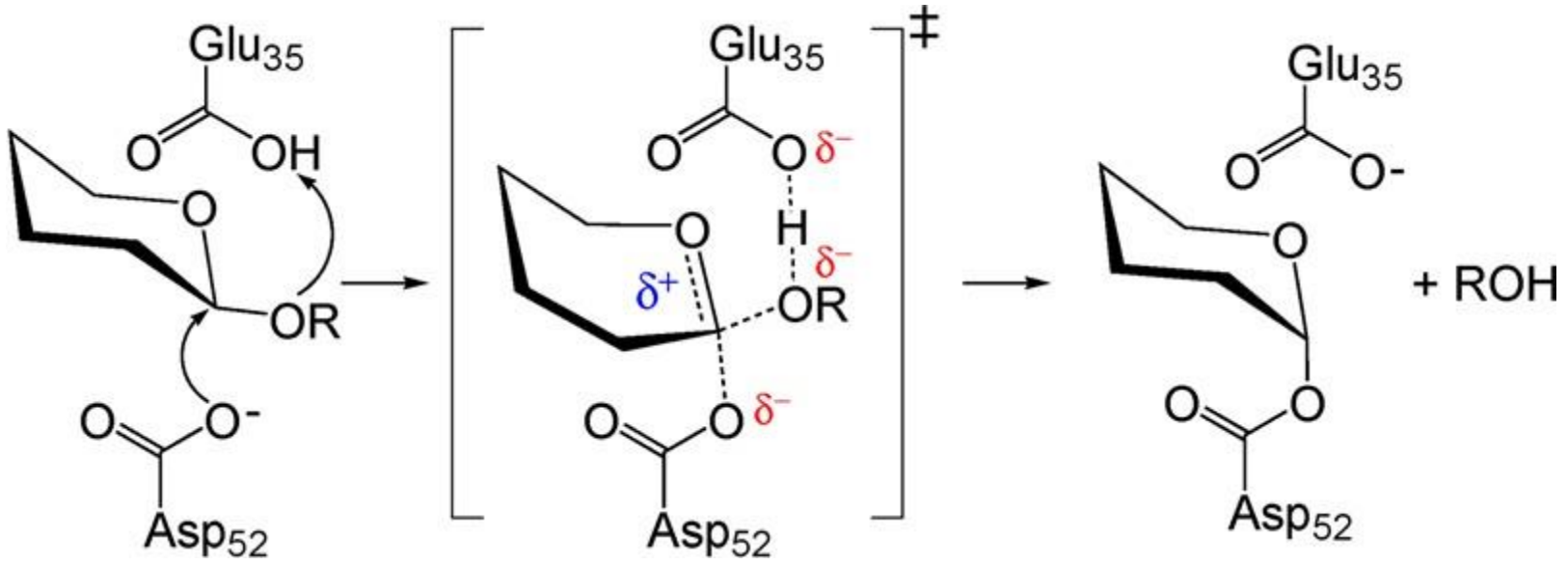
- In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.



Catalysis by bond strain

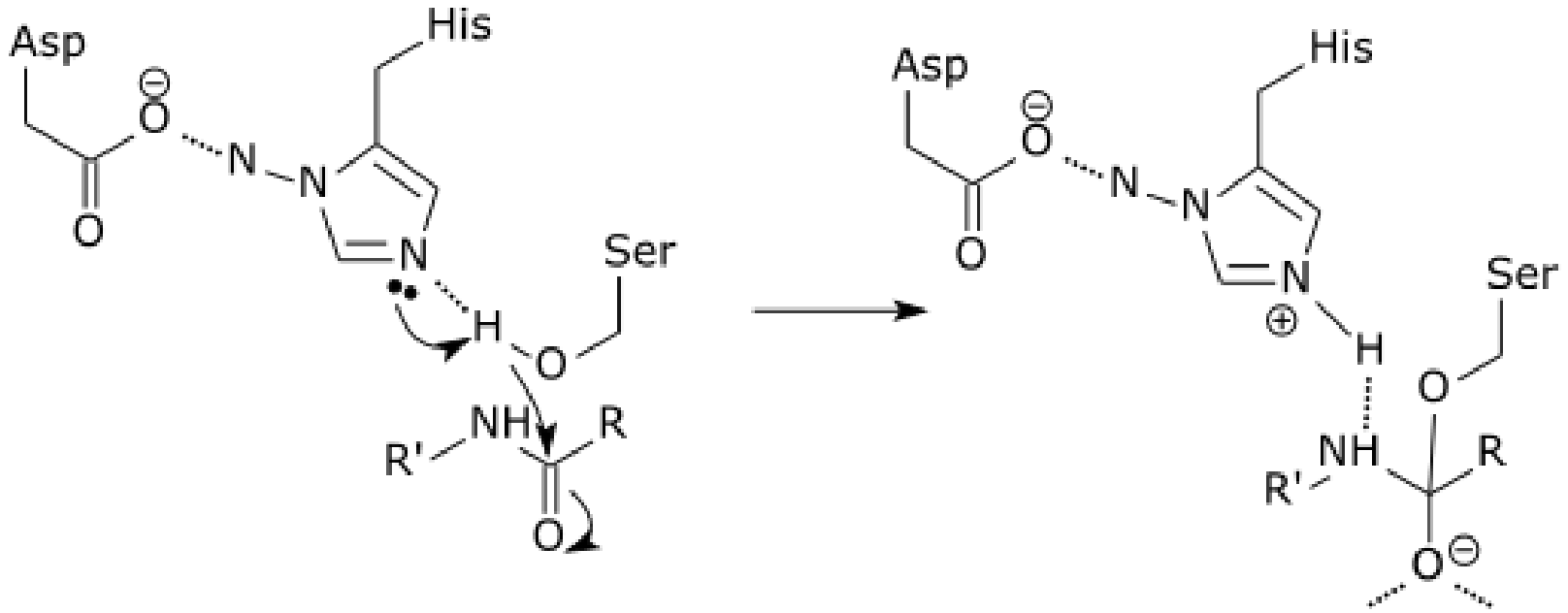
- Example: lysozyme

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)

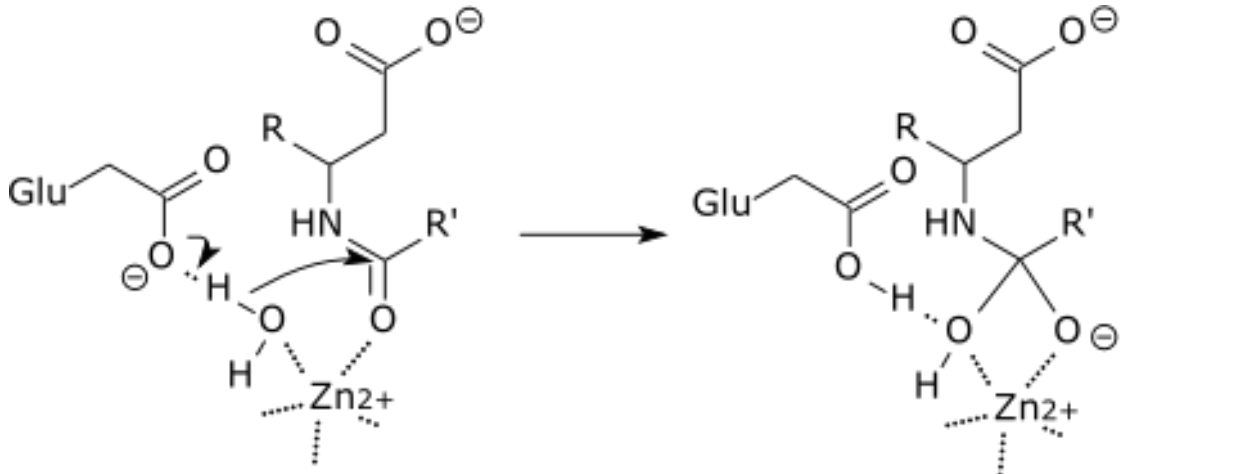
- The R groups act as donors or acceptors of protons
  - Histidine is an excellent proton donor/acceptor at physiological pH
  - Example: serine proteases



Involves negatively or positively charged R groups of amino acids in the active sites

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

The initial binding of a material to an enzyme cannot be covalent, but the substrate can form a temporary covalent bond with the active site during the reaction.

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
  Or the material the enzyme synthesizes followed by synthase

Most common way

- For example; ATPase is an enzyme that breaks down ATP, whereas ATP synthase is an enzyme that synthesizes ATP
  The action (type of reaction) of the enzyme is dropped from the if the enzyme breaks down materials like ATPase.
- Some enzymes have common names that provide little information about the reactions that they catalyze

  Common (Historical) names were given to enzymes before a proper understanding of their actions and stuck with them. They don't provide info about their actions.

- 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

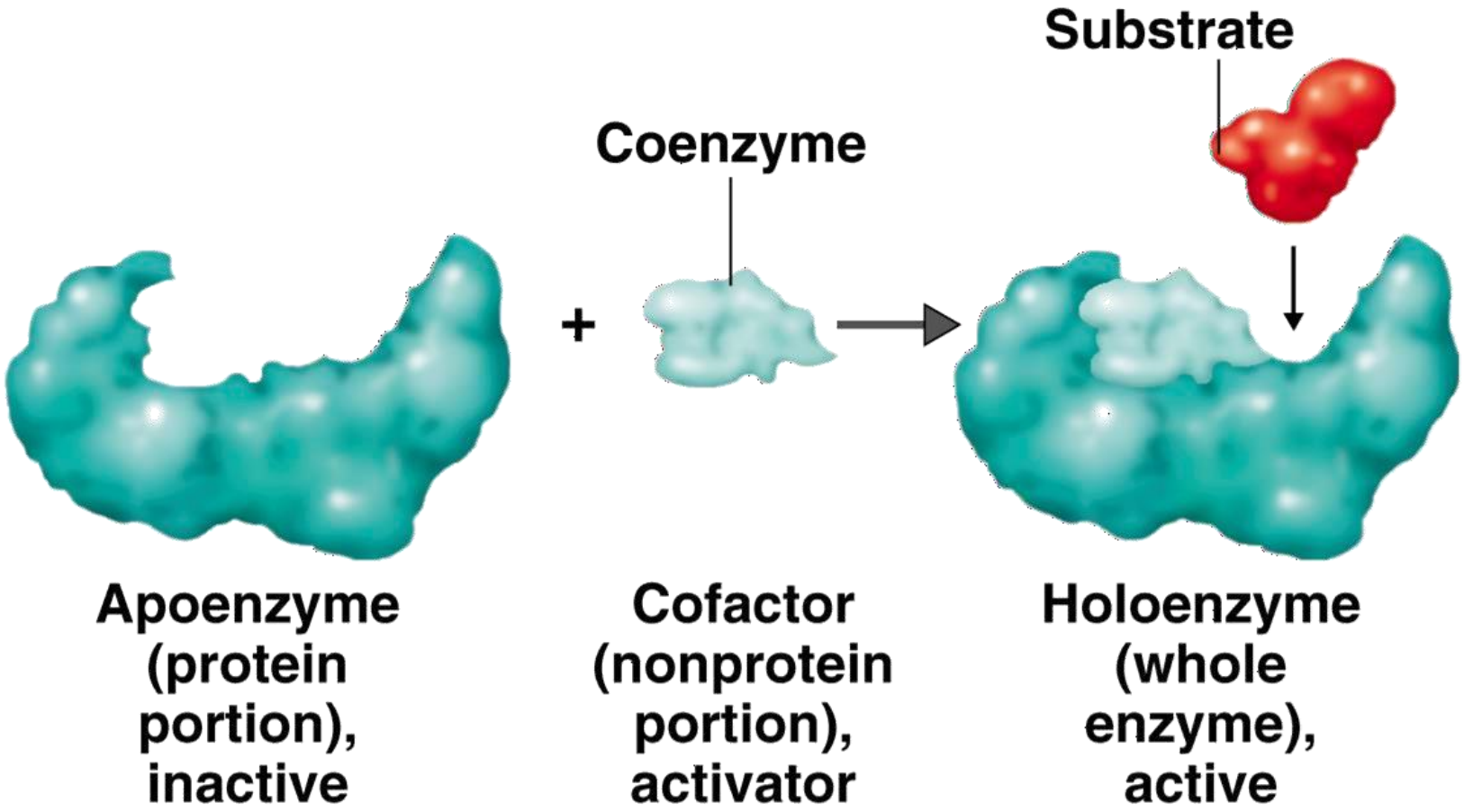
- Numbering format:
  - EC followed by four numbers separated by periods
  - Major class (1-7), Minor class, subclass, further sub-classification

- 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

The professor only requires us to know what the major class is when given EC number of an enzyme

Enzyme Classification (structure)

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



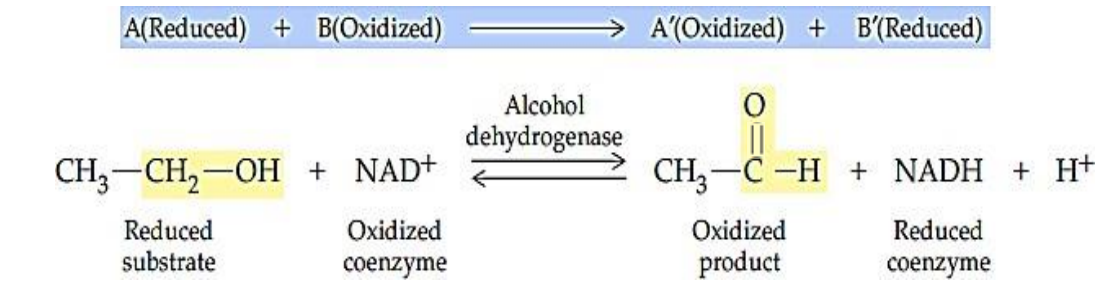
Simple: enzymes that don't require a non-protein material to function

Complex: do require. (In fact the cofactor is the part the does the job, without it the rxn doesn't occur).

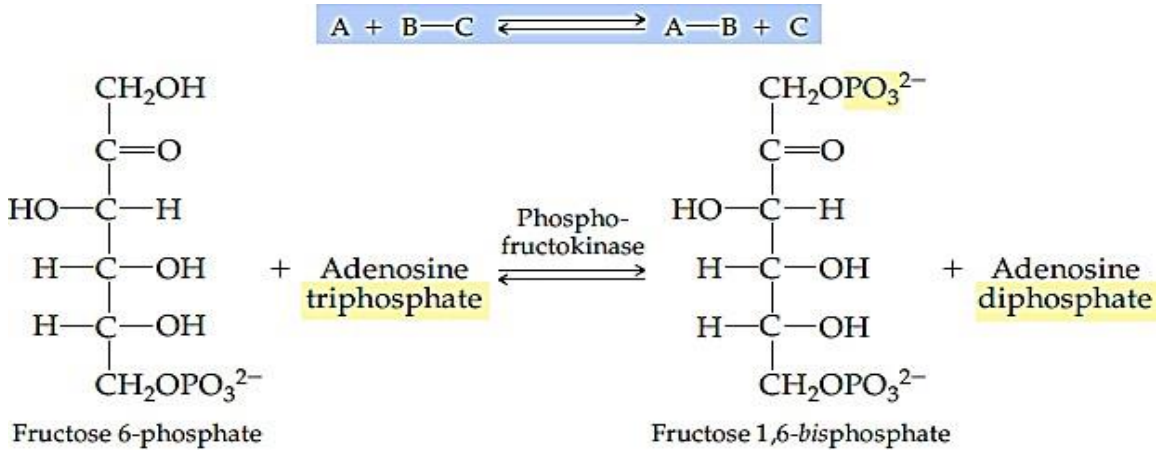
Complex enzymes can be apo(not bound to the cofactor)or holo (bound)

Enzyme Classification (function)

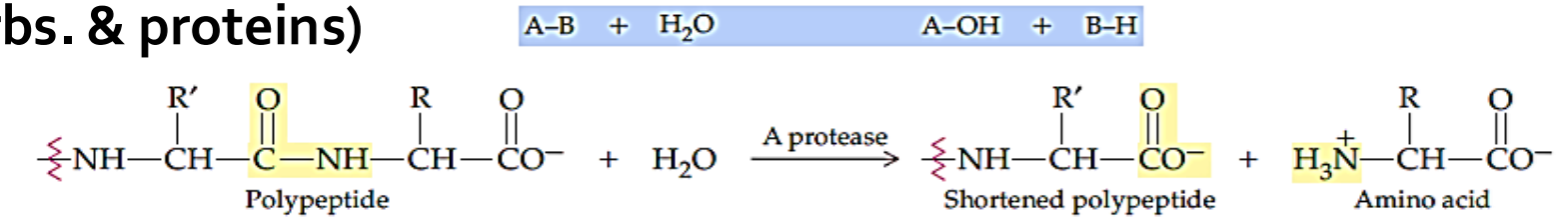
1. **Oxidoreductases:** addition or removal of O, O_2, H. Require coenzymes (heme)



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



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



Oxidoreductases: The reaction has to have at least 2 reactants and 2 products. One reactant gets oxidised the other reduced

Transferases There has to be at least 2 reactants and 2 products. Removing the group from one and adding it to another.Not adding a group from the solution

Hydrolases split molecules by adding water.

Enzyme Classification (function)

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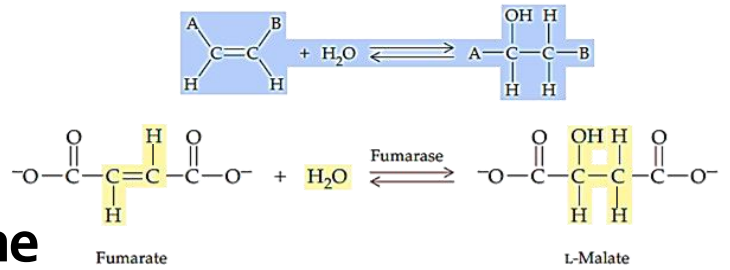
- **Lyases: addition of a molecule (H_2O, CO_2, NH_3) to a double bond or reverse.**



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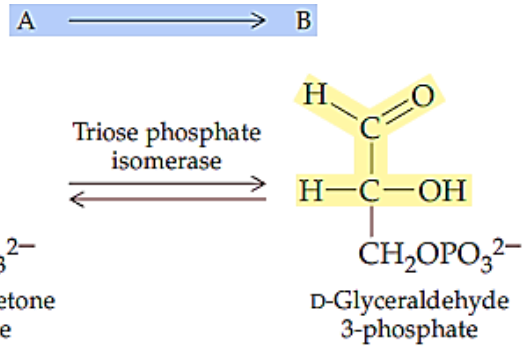
- **Isomerases: one substrate and one product.** That are isomers of each other.



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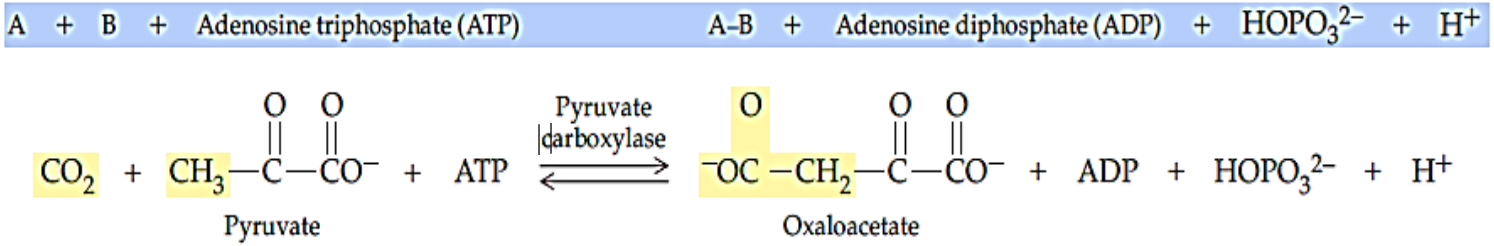
- **Ligases: usually not favorable, so they require a simultaneous hydrolysis reaction.** They make molecules larger; that's why they require a source of energy like ATP



Lyases catalyse the addition or removal of a group breaking or forming a double bond in the process. (hallmark)

Lyases with water: the molecule doesn't split, water is added or removed.

Hydrolases: Water splits the molecule.

The 7th major class is : Translocases
They catalyze the translocation of a substrate from one place to another.
Usually present in membranes (plasma, mitochondria, etc) .

Example: ATP-ADP Translocase in mitochondria : Exchanges ATP (moves it out to cytosol) for ADP (moves it into matrix).
Works on a 1:1 ratio: for each ATP exported, one ADP is imported.
from inside of mitochondria to the cytosol. That's why their ratio is fixed.

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: **4 minor classes**
  - ✓ Dehydrogenases
  - ✓ Oxidases
  - ✓ Peroxidases
  - ✓ Oxygenases

Reducing agent:
The substance that gets oxidised

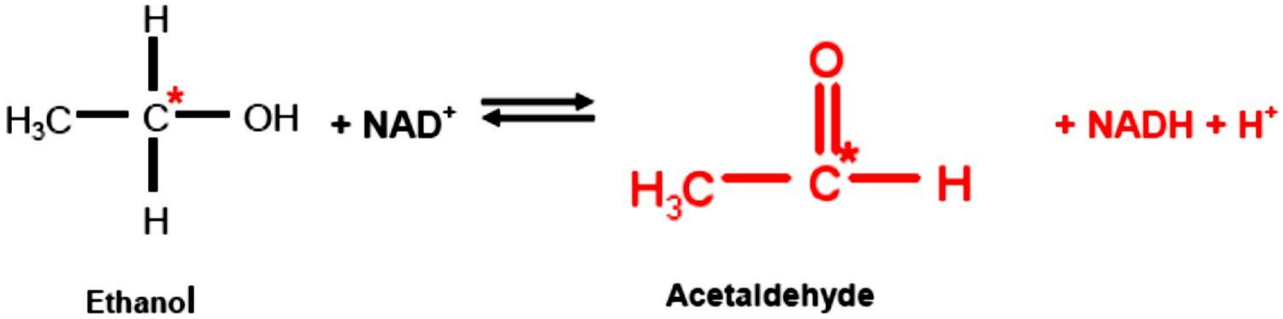
Oxidizing agent:
The substance that gets reduced

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



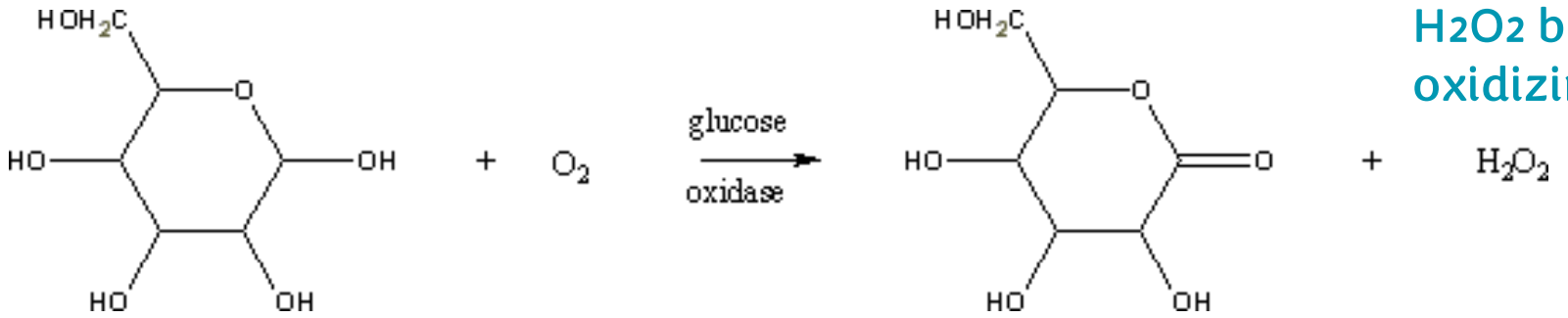
All of them unless otherwise specified, use NAD+ as an oxidizing agent.

Succinate dehydrogenase uses FAD instead.
It has to be specified.

When given a name of an enzyme you should be able to know the product and the electron acceptor.

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**



O2 is the oxidising agent.

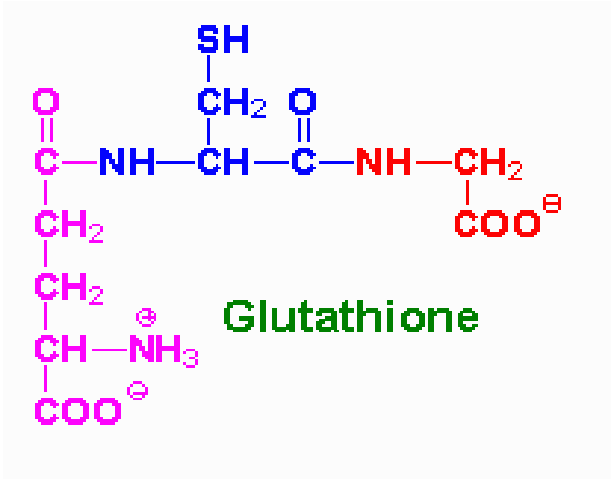
Not always H2O2 is produced.

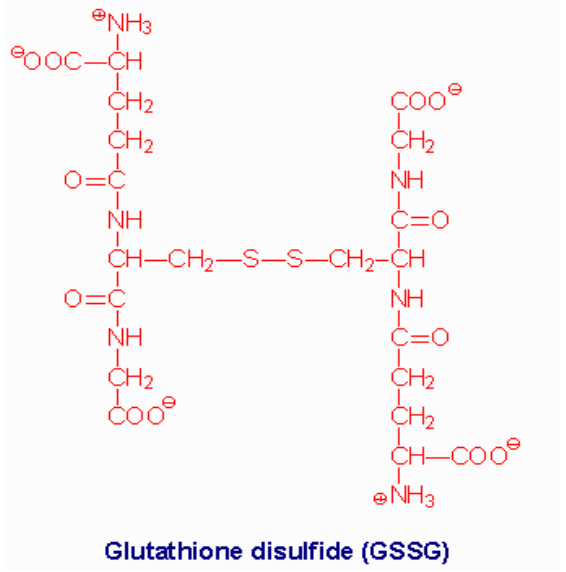
H2O2 is toxic. Peroxidases are enzymes that detoxify H2O2 by using it as an oxidizing agent.

Peroxidases

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

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



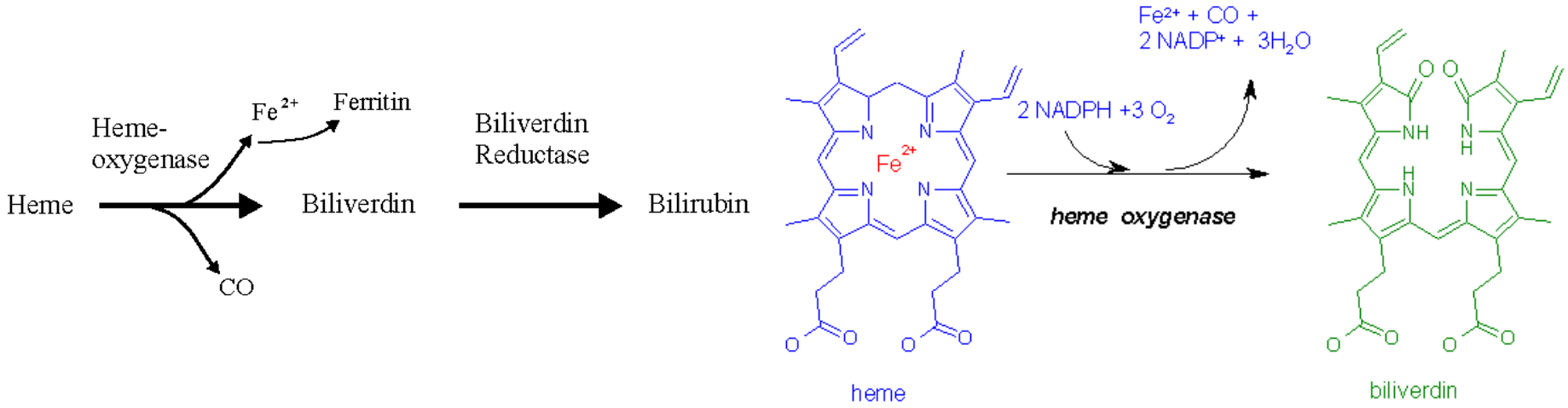


H2O2 is the oxidising agent.

You can see that it got reduced by receiving 2 H

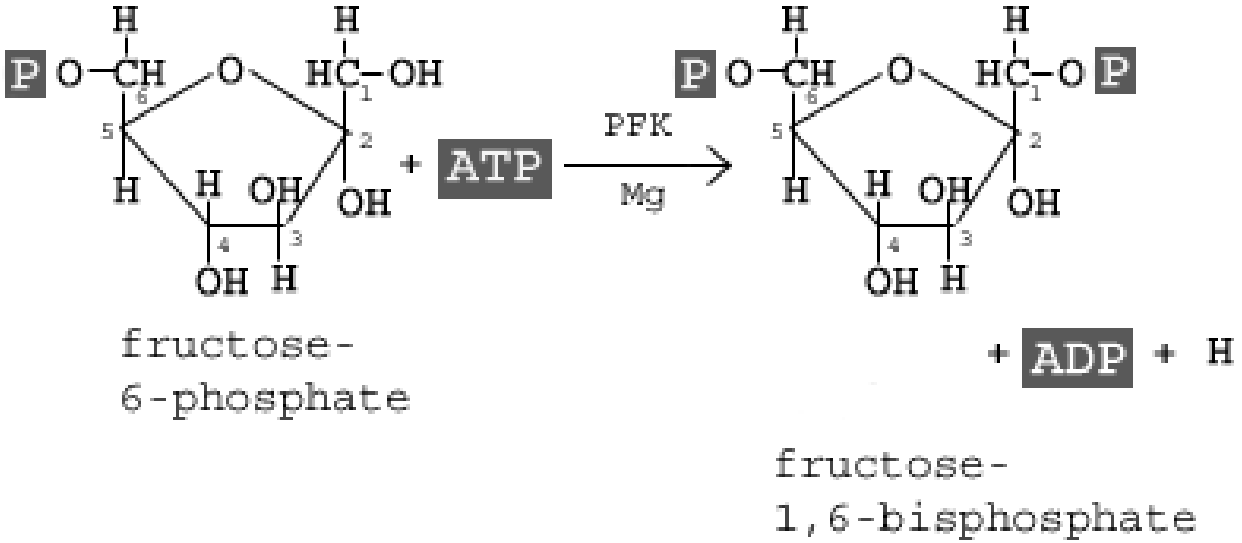
Oxygenases

- Oxygenases catalyze substrate oxidation by molecular O_2. Inserting oxygen into the substrate.
- 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



fructose-6-phosphate

fructose-1,6-bisphosphate

They include(minor classes):

Kinases:
Catalyze the transfer of a phosphate group from one molecule to another.
The doner is ATP unless otherwise specified.

Transaminases (aminotransferases)

Aminotransferases (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**

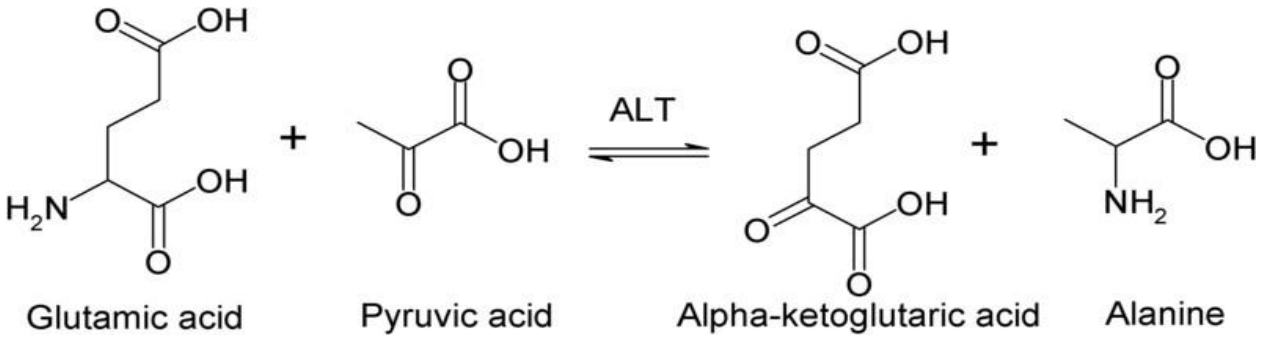
Amino group significance:
Source of nitrogen in our bodies

Transamination is how non-essential aa are synthesized

MEMORIZE:
Amino acid-Corresponding keto acid

Glutamaic AcidALpha keto glutarate
Alanine-pyruvic acid (product of glycolysis0
Aspartic Acid- oxaloacetate (krybb's cycle)

Examples: ALT (Alanine Transaminase) and AST (Aspartate Transaminase). Important for liver function



Remove NH2 from amino acid → Replace it with =O → Amino acid becomes ketoacid

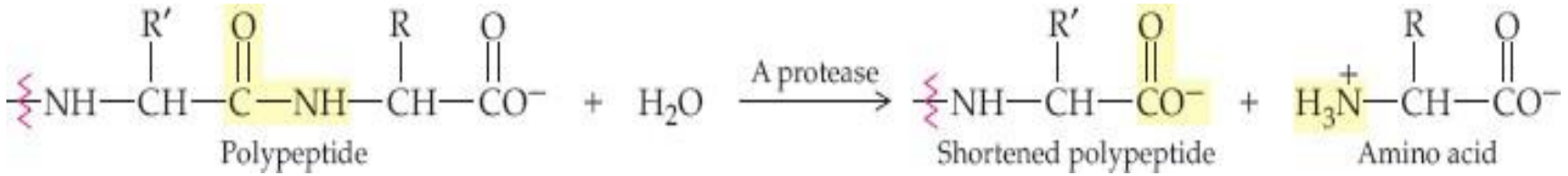
=O in the reacting keto acid → Replaced by NH2 → Ketoacid becomes amino acid

Hydrolases

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

Proteases

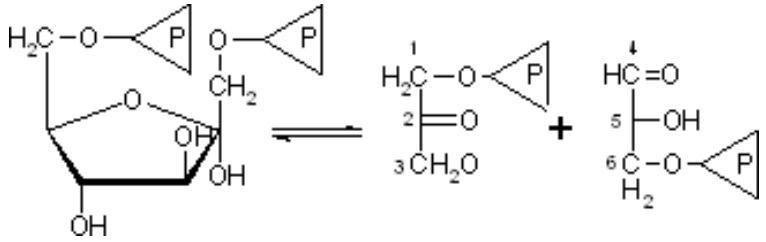
- These enzymes catalyze proteolysis, the hydrolysis of a peptide bond within proteins
- Proteolytic enzymes differ in their degree of substrate specificity



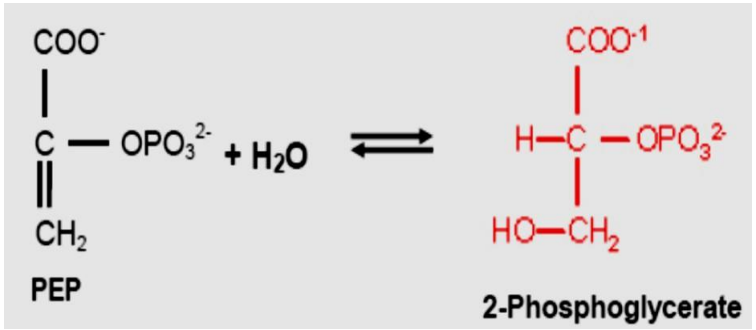
- Trypsin, is quite specific; catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine
- Thrombin, catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only

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. Might split the substrate but water isnt involoved in splitting.
- Aldolase; breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate
  - F 1,6 bisphosphate ⇆ DHAP + GAP



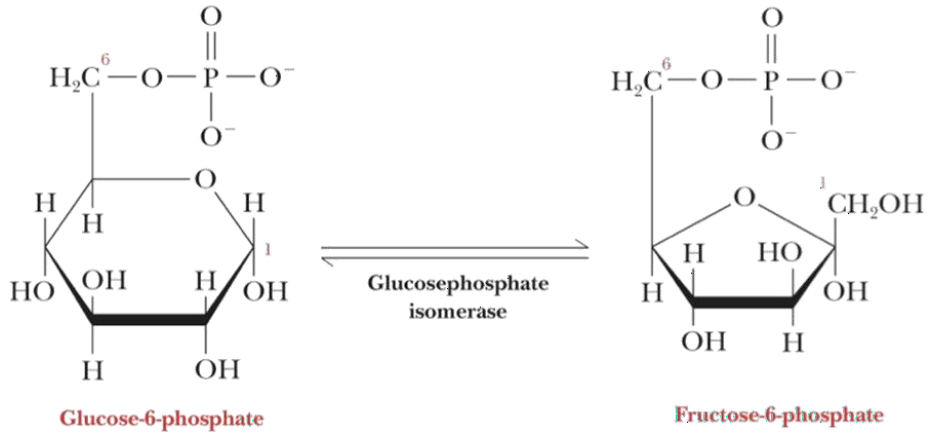
- Enolase; interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation and removal of double bonds

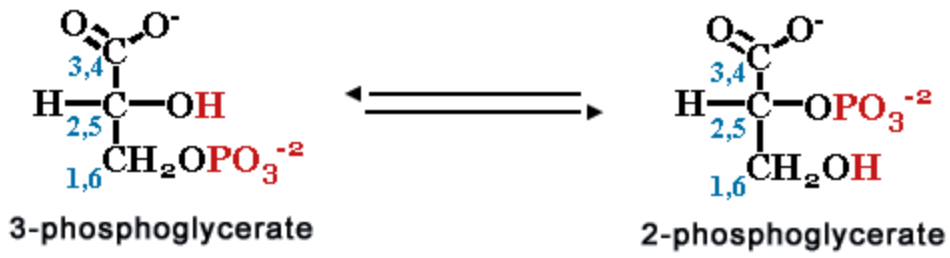


Isomerases

Mutase means you are dealing with an isomerase.

- 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



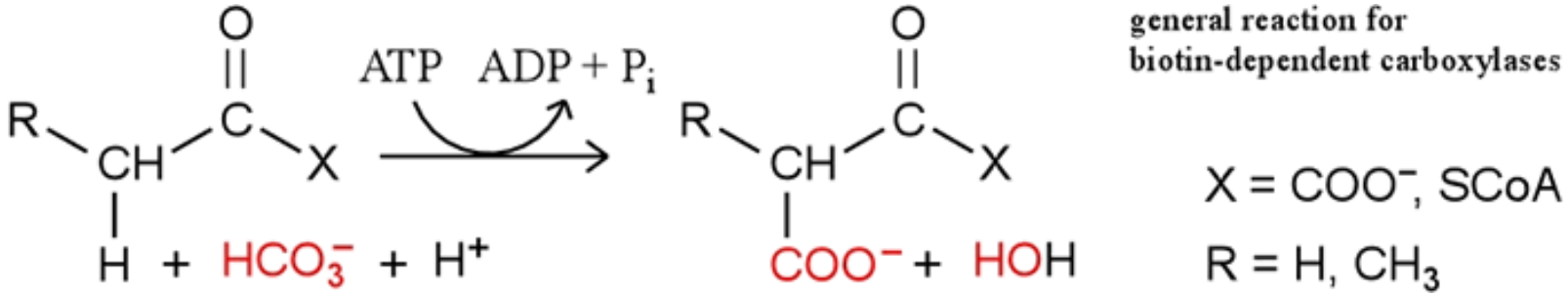


Ligases

- 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. **Because we are building, adding more carbons.**
- Pyruvate carboxylase

Carboxylases are ligases while decarboxylases are lyases

- **Pyruvate + HCO_3^- + ATP ⇆ Oxaloacetate + ADP + Pi**



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			Additional slides were added
V1 → V2			

رسالة من الفريق العلمي: