بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 25

Enzymes (pt.2)

﴿ وَإِن تَتَوَلَّوْا۟ يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوٓا۟ أَمْثَٰلَكُم ﴾

اللهم استعملنا لنصرة دينك

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APPROVED
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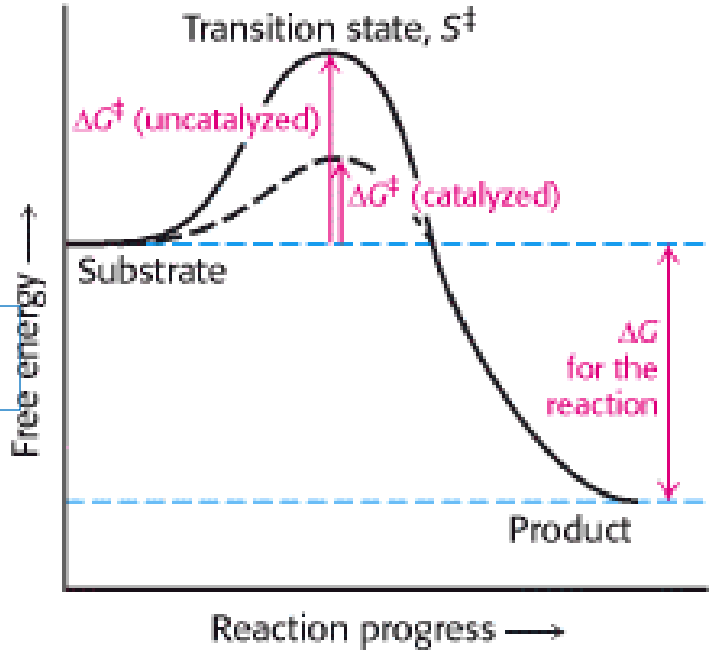
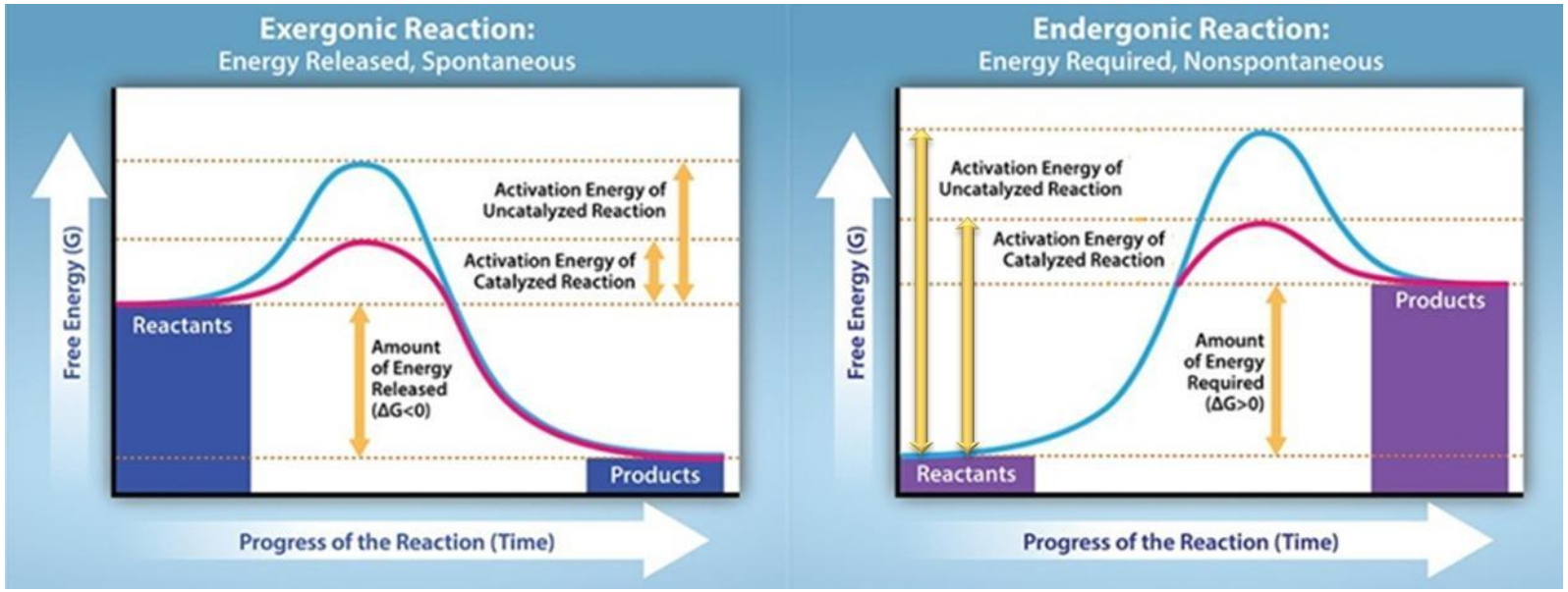
What do enzymes do?

Activation energy is the push needed to start a reaction.

- Enzymes lower the activation energy of the transition state or, in other words, enzymes facilitate the formation of a transition state at a lower energy.
  - *Still, the free energy of the transition state in still higher than those of the substrate or the product.*

Regardless of whether the reaction is exergonic or endergonic.

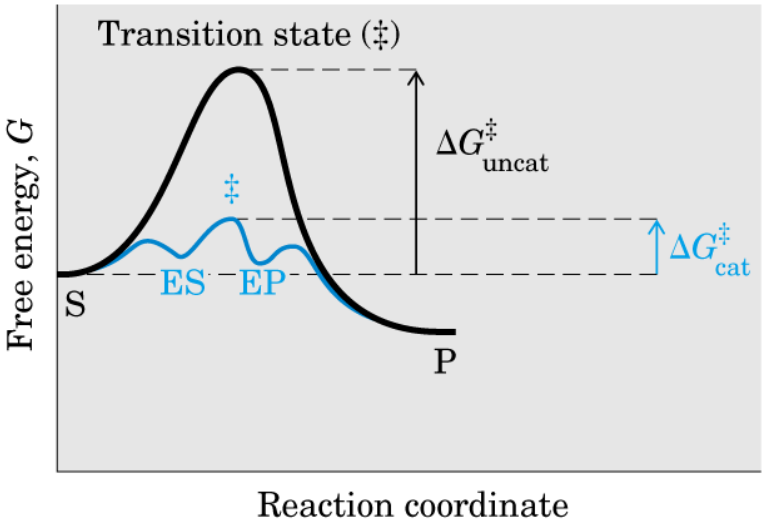


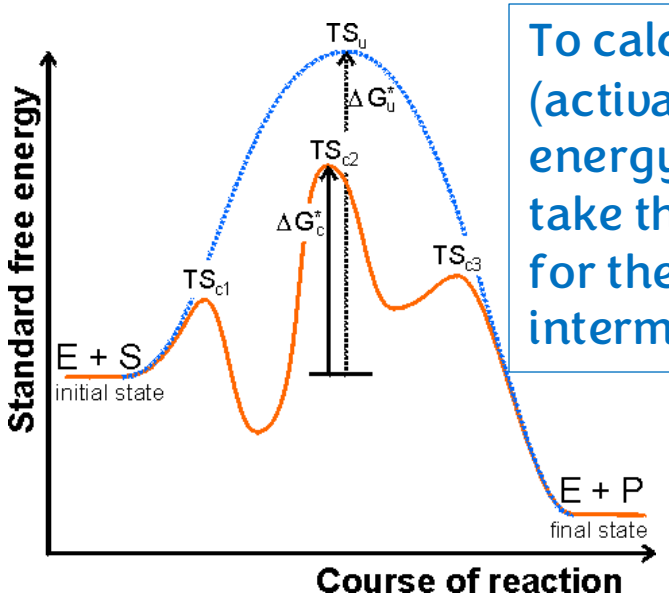


ΔG (the difference between product and reactant energies) is not affected .
What changes is the activation energy (the difference between transition state energy and reactant energy).

Alternative pathways

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





To calculate the (activation energy)here, we take the highest G_t for these different intermediates.

How do enzymes catalyze reactions?

And what they do to reduce activation energy?

- Enzymes place substrates proximal to each other.
- Enzyme orient the substrates inside the active site in the best possible fit.

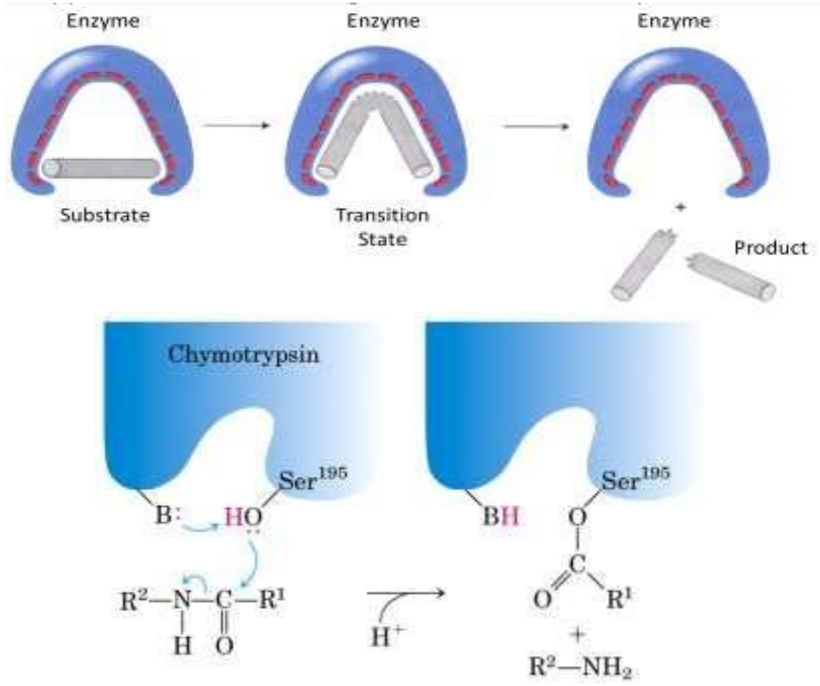
In the right angle

To ensure the formation of non covalent interactions

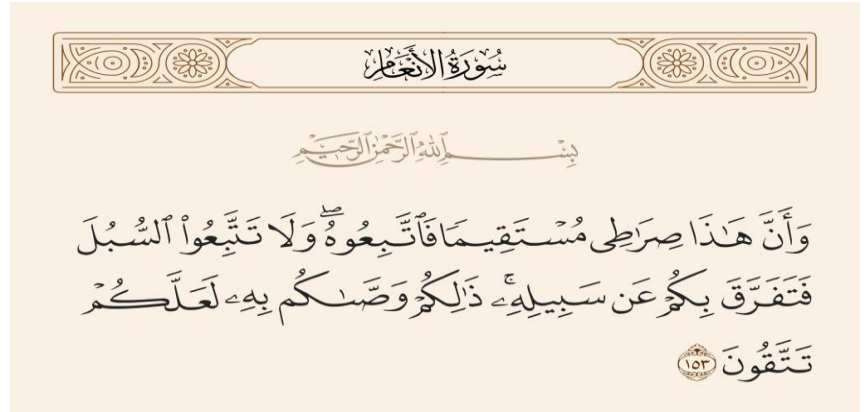
- Enzymes use one or a combination of strategies to catalyze reactions such as:
  - Straining the bonds within the substrates making them vulnerable (weaker) and easier to break.
  - Driving acid/base reactions
    - Proton donor/acceptor
      - Histidine, serine, etc.
  - Forming bonds.

We predict the active site to have amino acids that might be protonated or deprotonated.
Examples: trypsin & chymotrypsin

It is easy for histidine to be protonated or deprotonated at physiological pH."

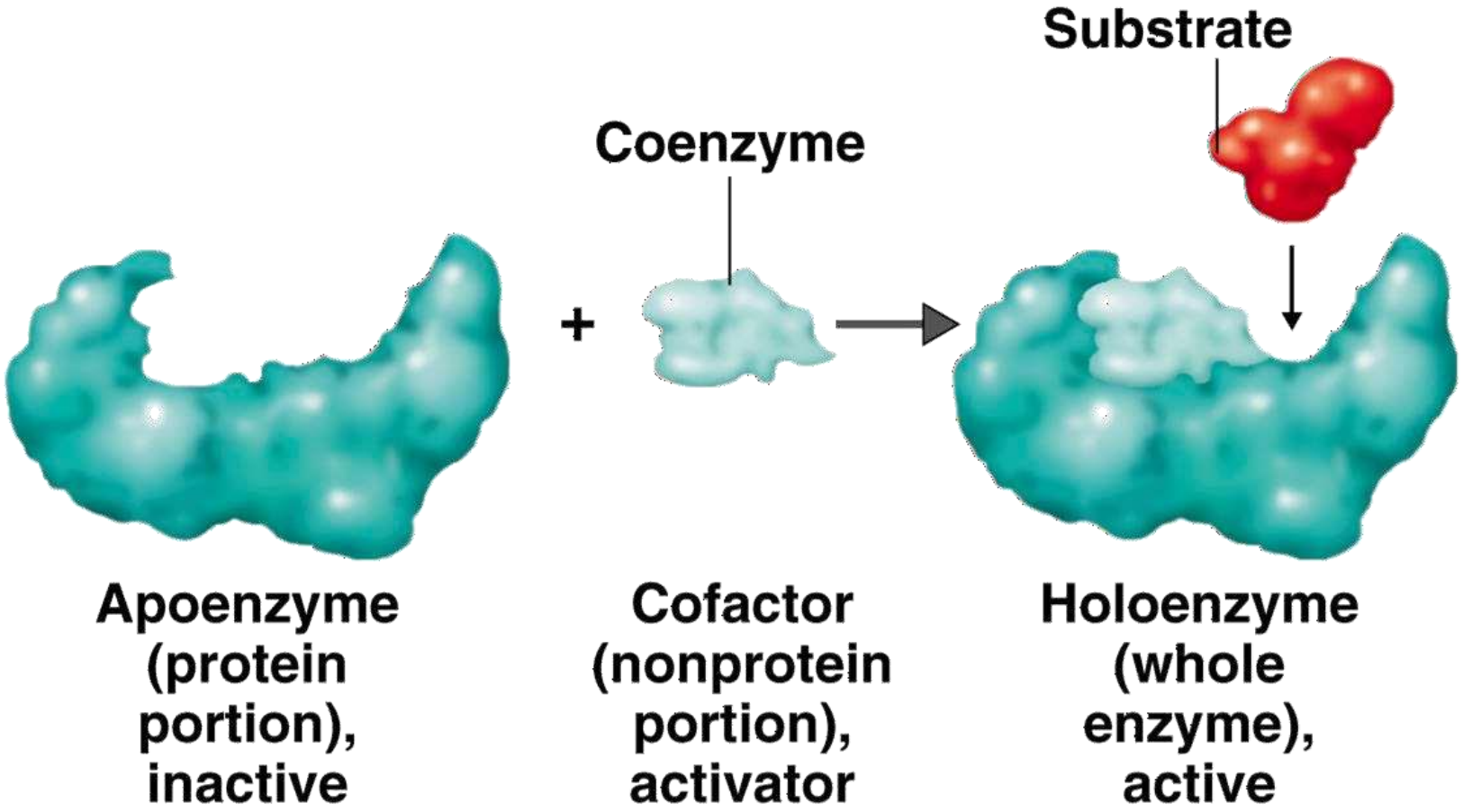


Classification of enzymes



Enzyme Classification (structure)

- Simple vs. complex (conjugated)
- Holoenzyme (catalytically active) vs. apoenzyme (inactive)



Naming of enzymes

-Ose => sugar.
-Ase => enzyme.

- In general, enzymes end with the suffix (-ase).
- Most other enzymes are named for their substrates and for the type of reactions they catalyze, with the suffix “ase” added.
  - ATPase breaks down ATP.
  - ATP synthase synthesizes ATP.
- Some enzymes have common names
  - Examples: the proteolytic enzyme trypsin.

Enzyme classes according to function

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

1. Oxidoreductases

The largest class of enzymes

- They catalyze oxidation/reduction reactions involving the transfer of hydrogen atoms or electrons.
- They can be divided into 4 main classes:
  - Dehydrogenases
  - Oxidases
  - Peroxidases
  - Oxygenases

1a. Dehydrogenases

They remove hydrogen from substrate.

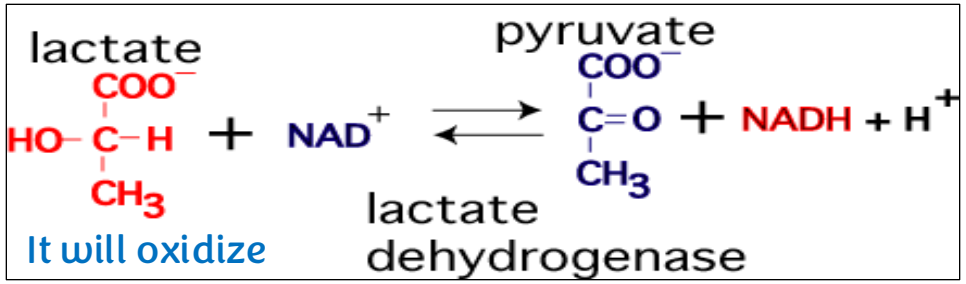
Hydrogen with extra ion .

- Dehydrogenases transfer electrons in the form of hydride ions (H^-) or hydrogen atoms using an electron-transferring coenzyme, such as NAD^+/NADH or $FADH_2$.

NAD+/ FAD : oxidized form .
NADH / FADH2 : reduced form.

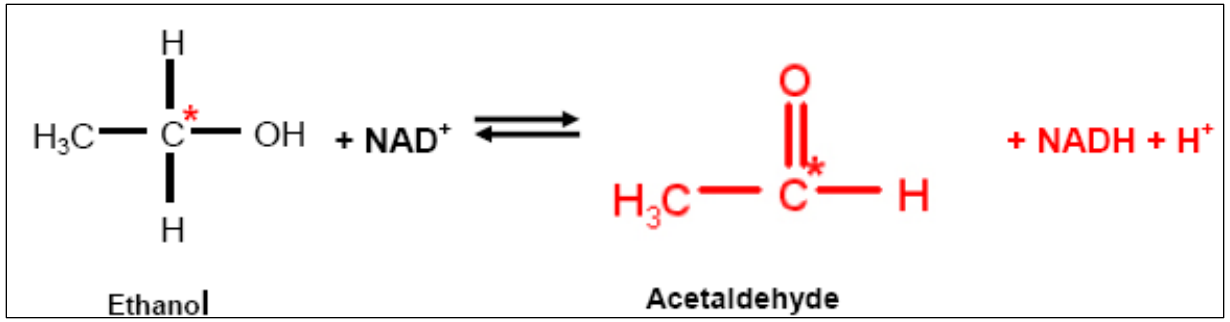
Dehydrogenase can catalyze both the forward and reverse reactions .

- Lactate dehydrogenase:



Do not memorize the reactions but understand them

- Alcohol dehydrogenase:



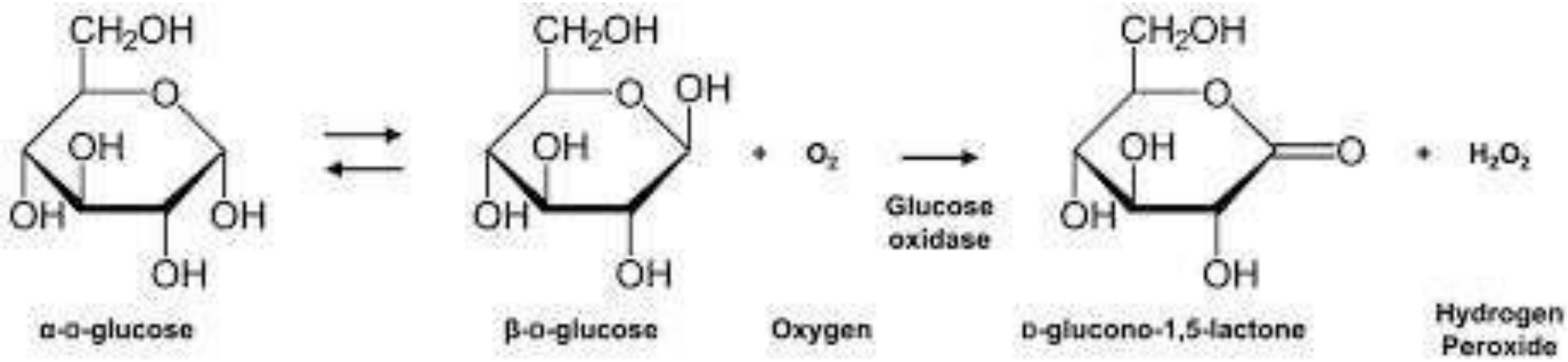
We recognize this enzyme by its association with FAD and NAD.

1b. Oxidases

Drive oxidation and reduction rxn , but oxygen considered as a substrate .

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

$$\text{Glucose} + O_2 \rightleftarrows \text{Gluconolactone} + H_2O_2$$

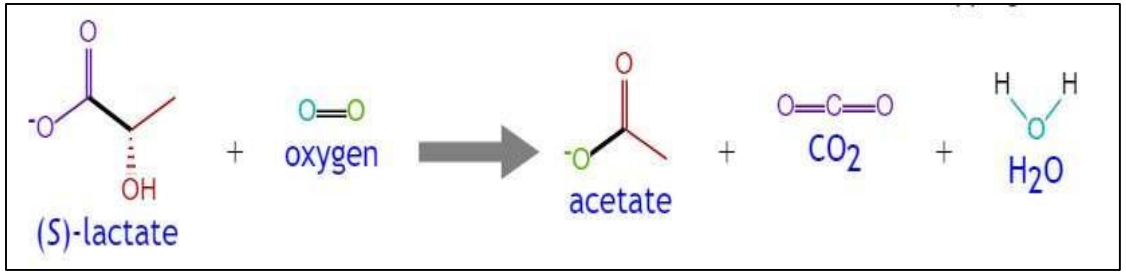


Oxygen is in the reactants.
Peroxide is in the products.

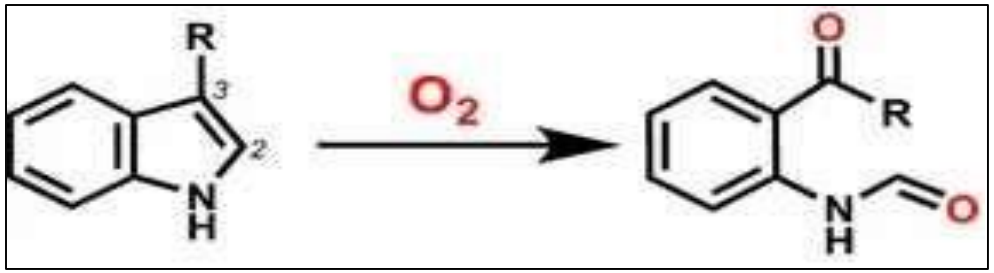
1c. Oxygenases

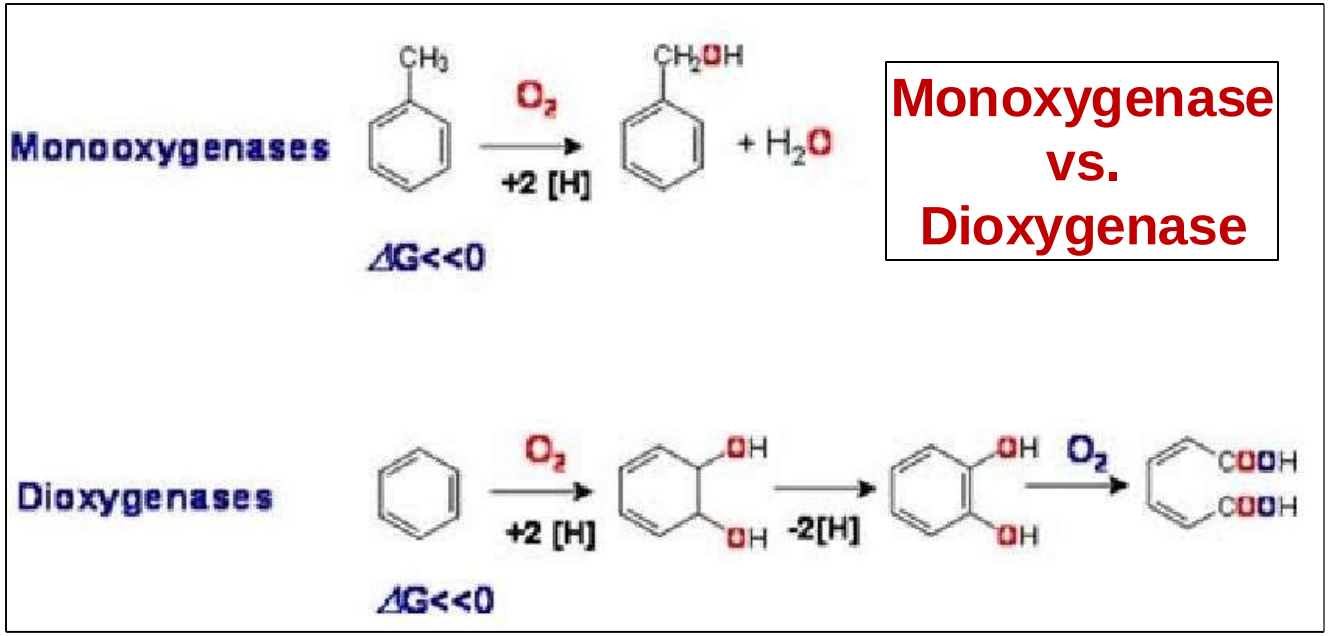
- Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate. Either one atom of oxygen or both atoms.
  - The reduced product is water, not H_2O_2.

Lactate-2-monooxygenase



Tryptophan 2,3-dioxygenase



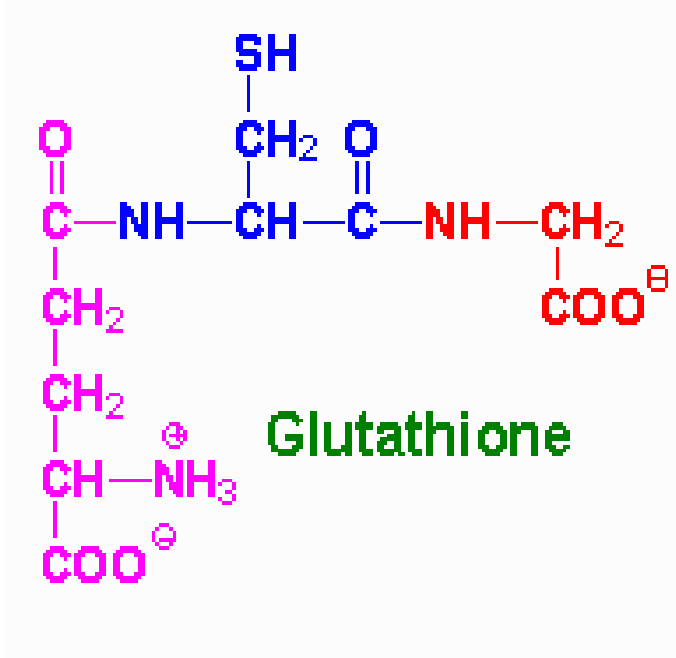


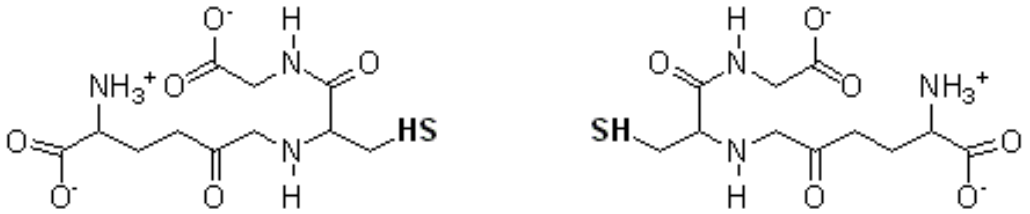
Monoxygenase vs. Dioxygenase

Adding one or two atoms of oxygen to the substrate.

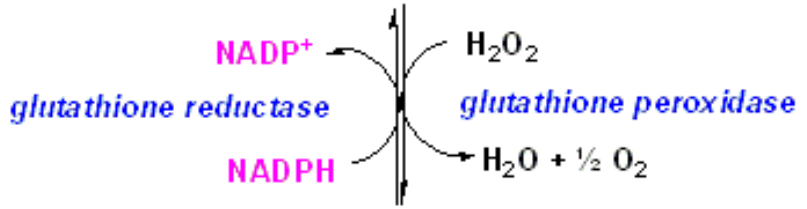
1d. Peroxidases

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

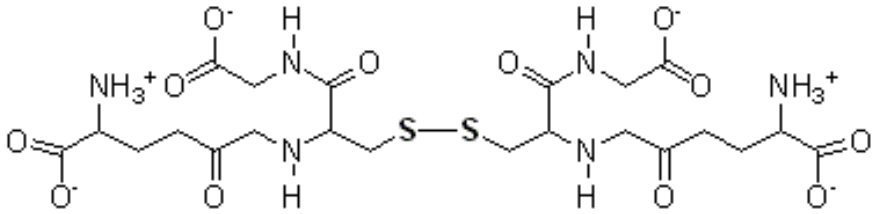




2 x reduced glutathione (GSH)



Reduction of hydrogen peroxide to water.

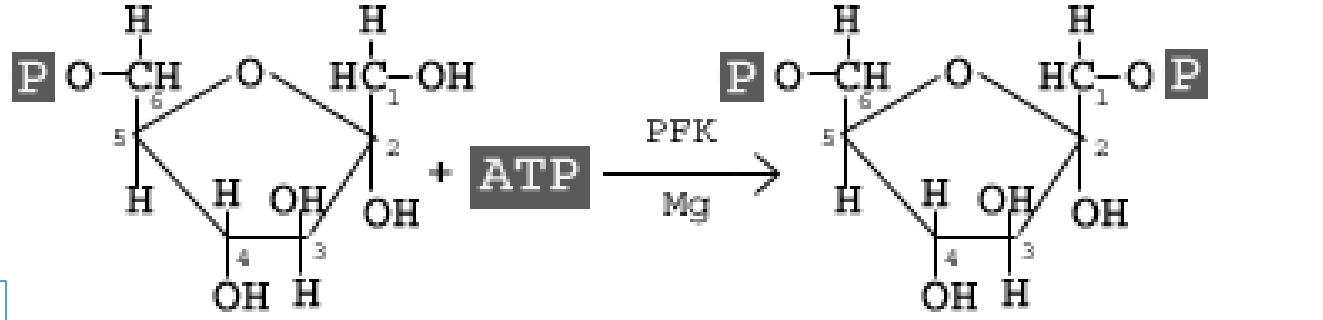


oxidized glutathione (GSSG)

Hydrogen peroxide (H_2O_2) serves as a substrate

2. Transferases

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



fructose-6-phosphate

fructose-1,6-bisphosphate

Change the functional group of the molecule.

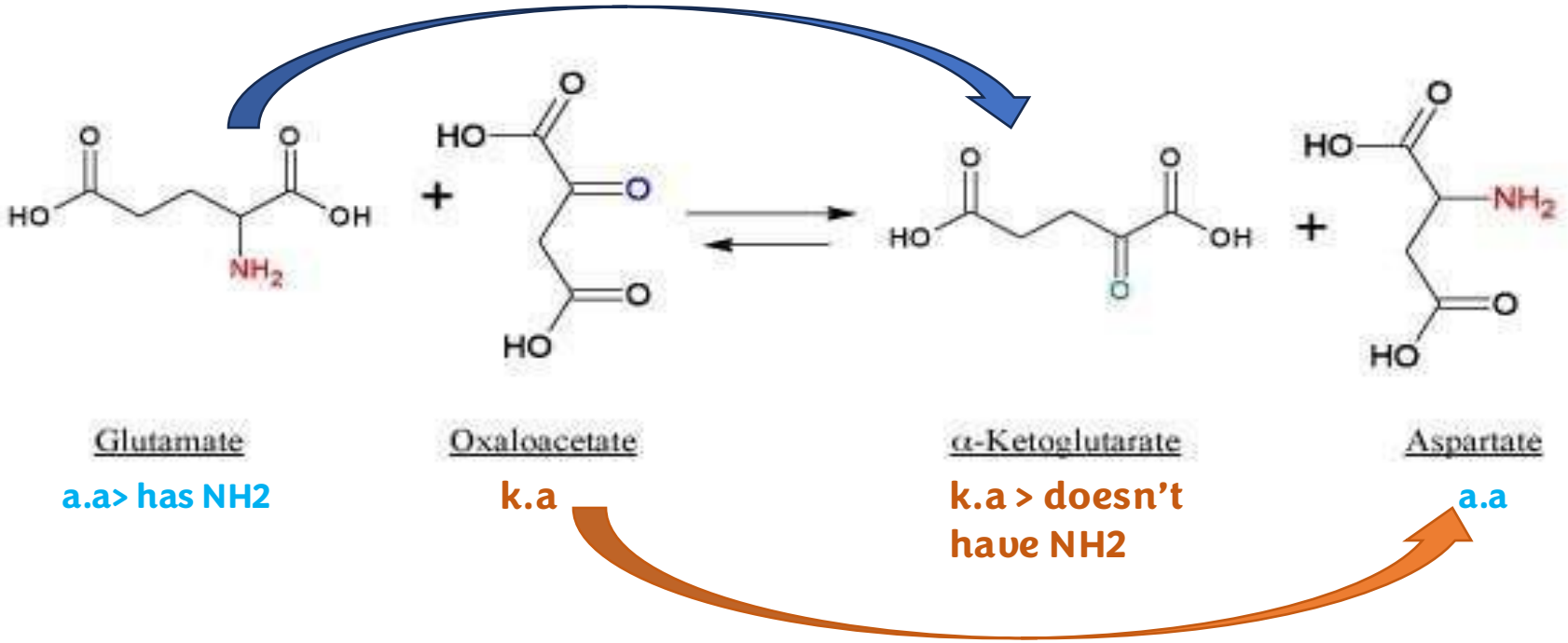
To distinguish phosphokinases , we note the addition of a phosphate group (functional group) to the substrate.

Another example: **transaminases**

Reversible reaction, same enzyme can move the reaction forward and backward

- 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.
  - Aspartate transaminase:

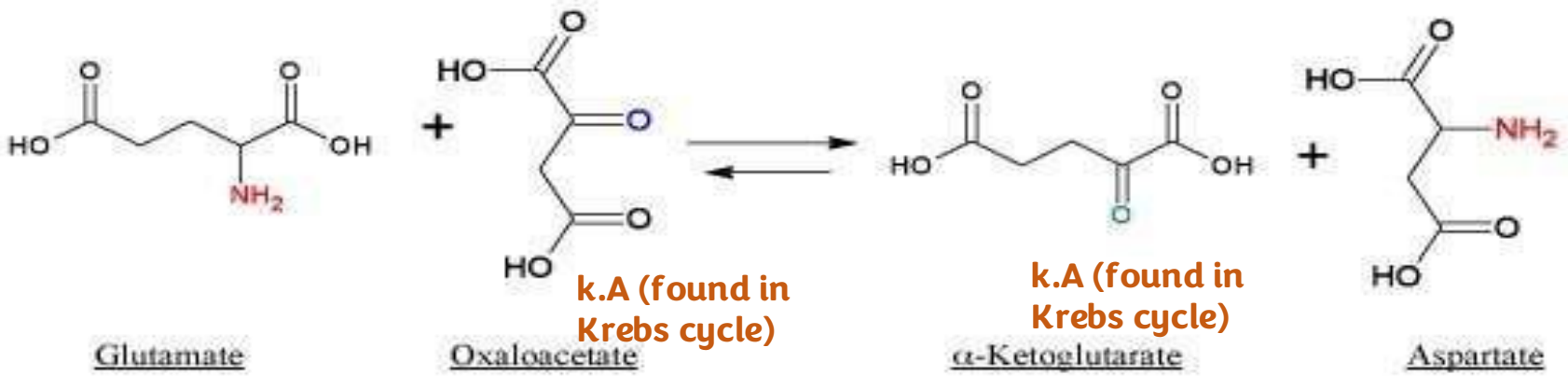
Amino acid – NH2 = Keto acid
Keto acid + NH2 = Amino acid



Transaminases most probably follow the ***lock and key model***, why?
Bcz if one of the two reactants bonded to the active site and **changed its shape** , the other one wouldn't be able to interact with the active site.
Here, the **order** of substrate binding **isn't important** as it is in glucokinase
(refer to the previous lecture)

Another 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. **Based on the body's needs**
  - Aspartate transaminase:



k.A (found in Krebs cycle)

k.A (found in Krebs cycle)

Could amino acids be used as sources of energy? Yes, if the amino acids were converted to any of the Krebs cycle intermediate to make energy (ATP)

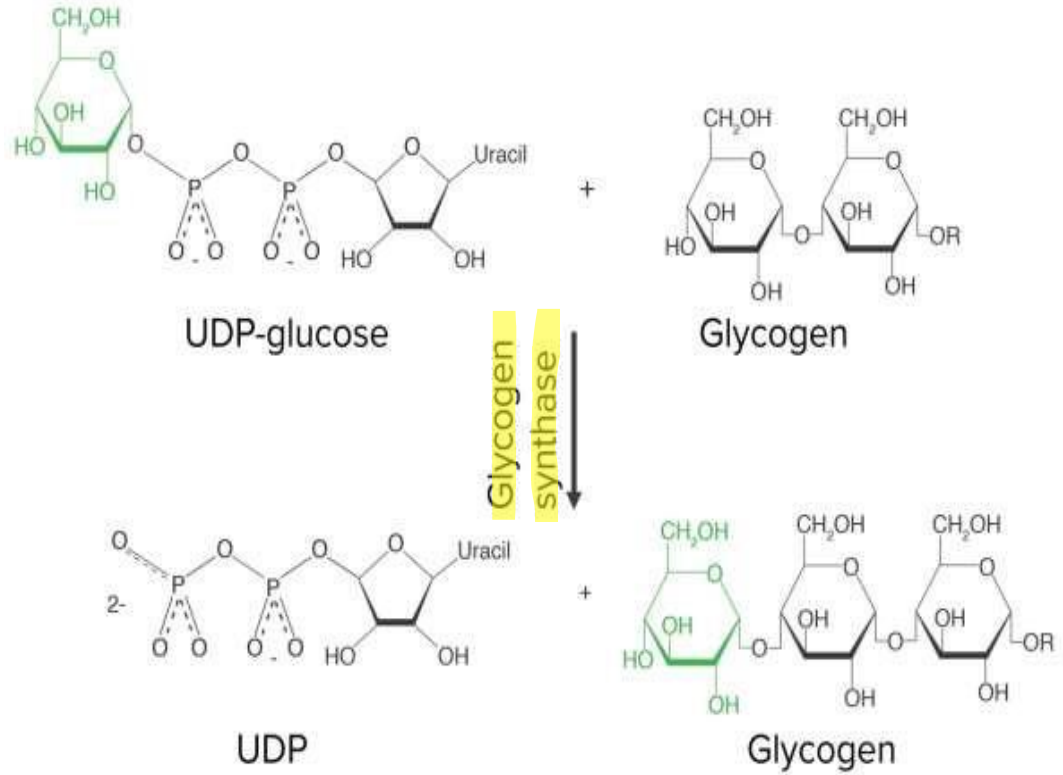
Removal of amine group from one molecule, and addition to another

A third example: **synthases**

To produce compounds which are not physiologically important, we use synthases classified as lyases

- When the synthesized **compound is physiologically important**, the **transferase** may be called a **synthase**.
- For example, *glycogen synthase*, whose systematic name is

  UDP- glucose-glycogen glycosyltransferase, transfers a glucosyl residue from uridine diphosphate (UDP)-glucose to the end of a glycogen molecule elongating glycogen by one glucose residue.

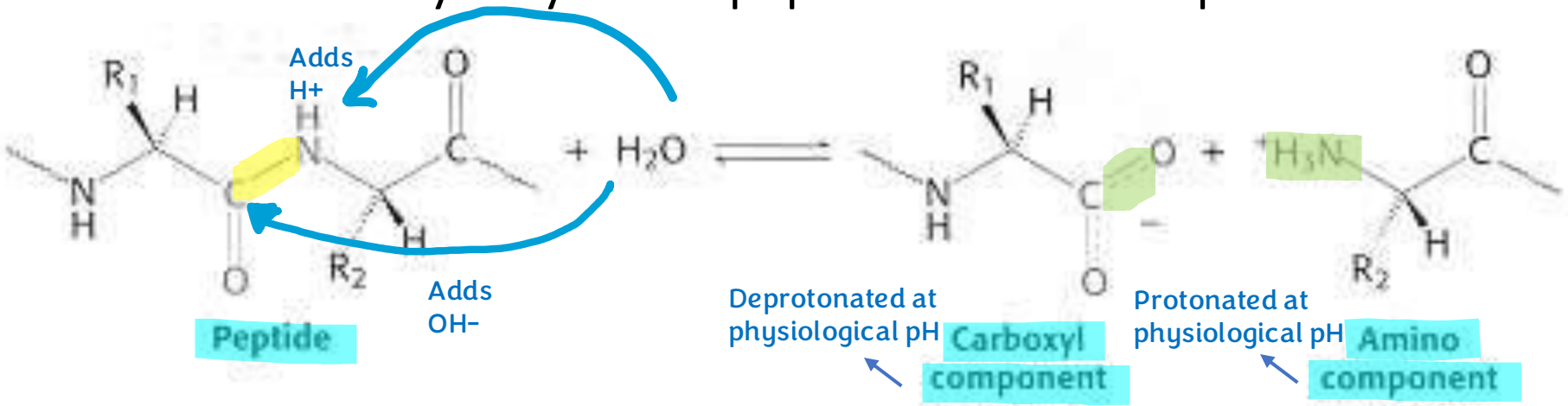


Transfers a molecule/functional group and produces physiologically significant molecules.

3. Hydrolases

They use water to break up covalent bonds

- They catalyze **cleavage** reactions using water across the bond in the form of OH^- and H^+ to being broken or the fragment condensations.
  - **Proteases**, **esterases**, **lipases**, **glycosidases**, and **phosphatases** are hydrolases named depending on *the type of bond* cleaved.
    - Example: proteases
      - A class of hydrolytic enzymes is proteases that catalyze proteolysis, the hydrolysis of a peptide bond within proteins.



Peptidase/Proteases > break peptide bonds
Succaridase/glycosidase > breaks sugars (glycosidic)
Lipase > breaks lipids
Esterase > breaks ester bonds
Phosphatase > removes PO_4^{-3} group
**water is needed for all of them.

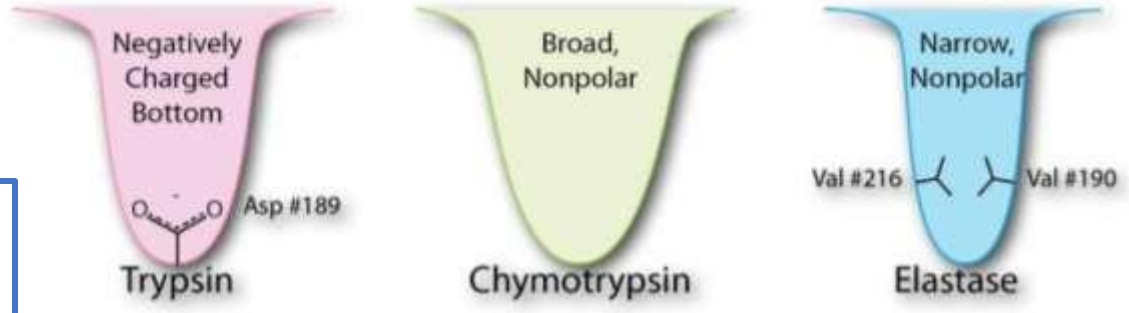
H_2O is a substrate, results in a broken bond/2 molecules

Specific examples: **digestive enzymes**

Refer to the previous lecture.

- Proteolytic enzymes differ in their degree of substrate specificity.
- Trypsin breaks up peptide bonds on the carboxyl side of only Lys and Arg.
- Chymotrypsin hydrolyzes peptide bonds involving bulky aromatic amino acids.
- Elastase hydrolyzes peptide bonds involving small, uncharged groups such as Ala, Val, or Gly.

Use water to break bonds, between peptides\proteins
Each with its own specialty



Lysine
Arginine

Phenylalanine
Tryptophan
Tyrosine

Glycine
Alanine
Valine

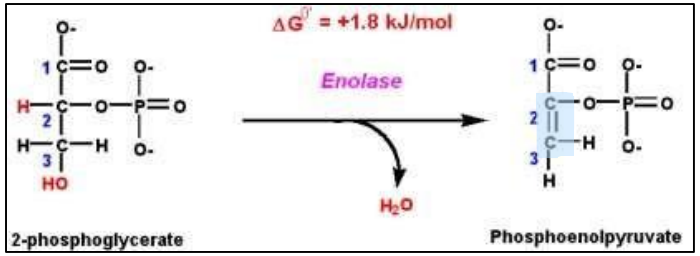
4. Lyases

A diverse class of enzymes
Their reactions differ from each other

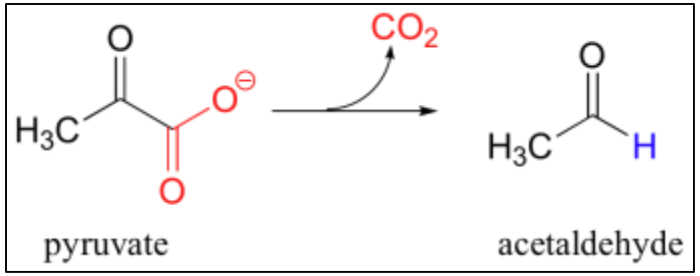
- Lyases **cleave** C-C, C-O, C-N, and **other bonds** by means other than hydrolysis or oxidation, leaving double bonds or rings, or conversely adding groups to double bonds without hydrolysis.

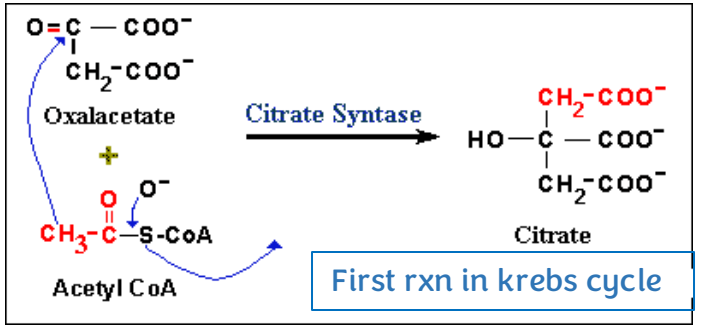
Remove hydrogen

- **Dehydrases**: **Removal** of H_2O from the substrate to give a double bond
  - Example: enolase
- **Decarboxylases**: **Replacement** of a carboxyl group by a hydrogen
  - Example: pyruvate decarboxylase
- **Synthases**: **Addition** of a small molecule to a double bond or when the direction of the reaction favors the formation of a carbon-carbon bond
  - Example: citrate synthase



*("en" = **ene** for the C=C and "ol" is for alcoh**ol**)*

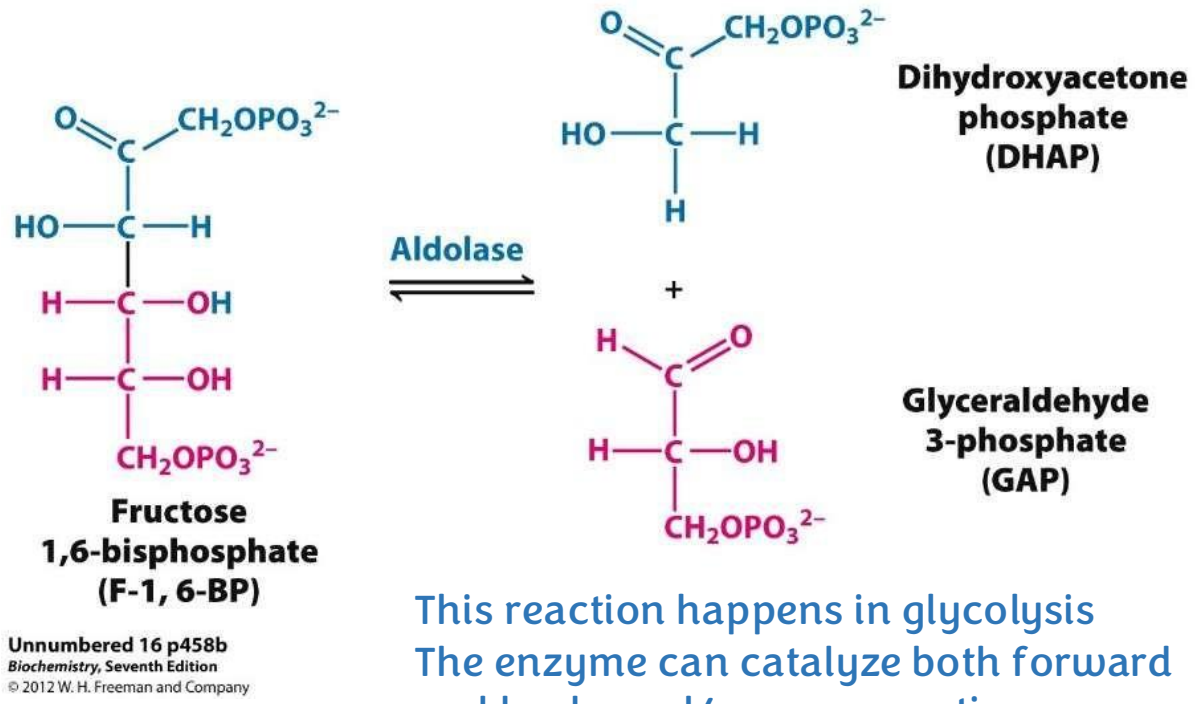




Broken bond, no usage of water, no NAD+/FAD, no isomers, no O2/H2O2 (rxn doesn't follow any of the 5 other classes)

Example: aldolase

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



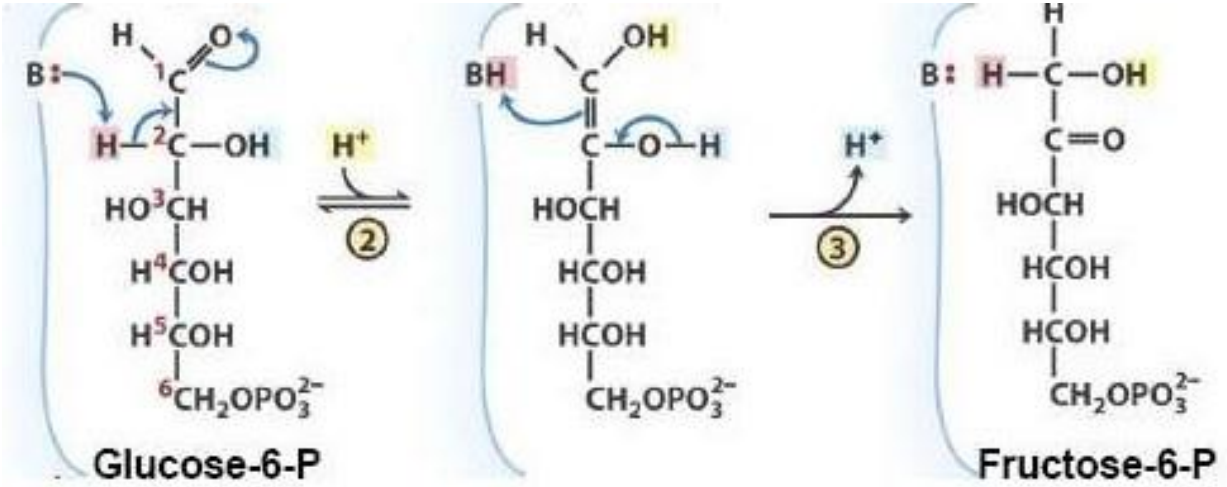
This reaction happens in glycolysis
The enzyme can catalyze both forward and backward/reverse reaction

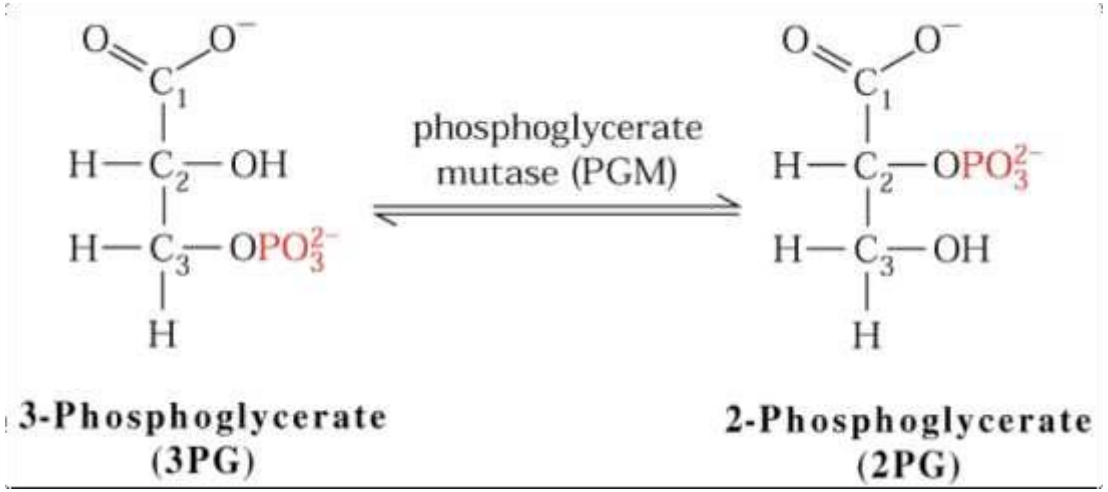
5. Isomerases

Convert an isomer into another
Doesn't require energy usually

Isomerization\rearrangement of bonds\atoms in the same molecule OR changing the place of a P group

- These enzymes catalyze **intramolecular** rearrangements.
- Enzymes that **rearrange** the bond structure of a compound are called **isomerases**, whereas enzymes that *catalyze* the movement of phosphate from one atom to another are called *mutases*.
  - Phosphoglucoisomerase **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:





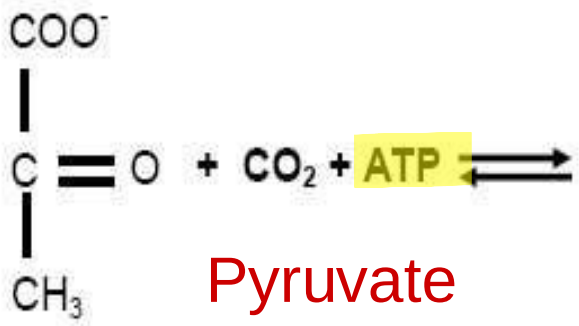
Same molecule--different isomers

6. Ligases

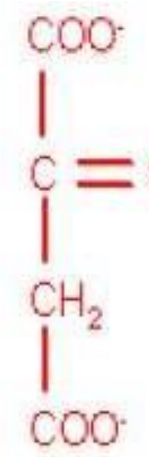
- Connect two molecules together,
- Use energy derived from ATP >> ATP is a substrate, ADP is a product
- Ligases are discussed further in molecular biology.

- 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.
- **Synthetases** derive the energy from the cleavage of high-energy phosphate bonds(ATP).
  - **Synthases** use a different source of energy**(bond energy).**

Pyruvate carboxylase

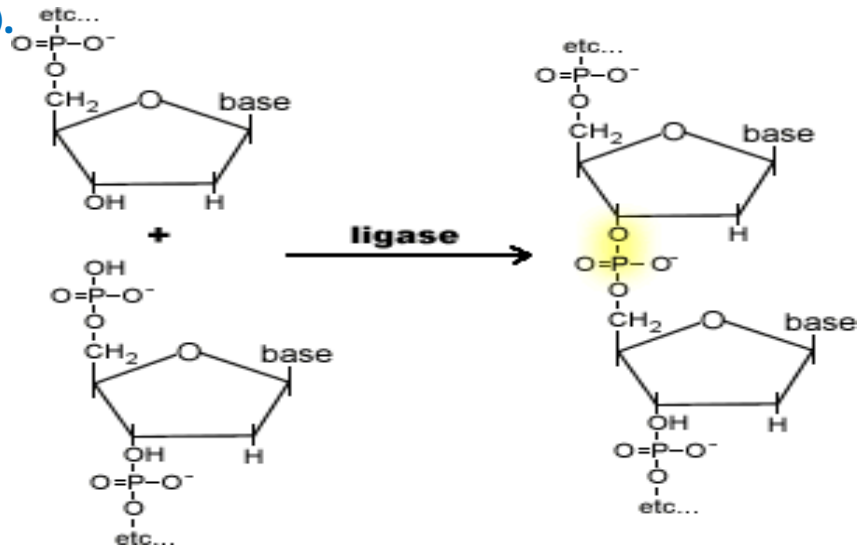


Pyruvate



Oxaloacetate

+ ADP + P_i



Connect molecules/2 subs 1 prod/energy is used(ATP)

**note:

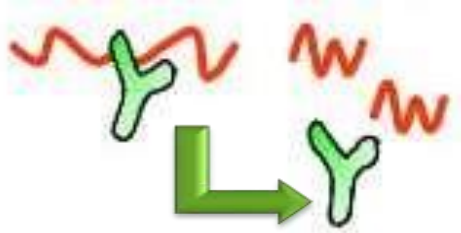
kinases (transferases)> use ATP to transfer the phosphate group to another molecule (results in ADP alone, and P becomes a part of the substrate)

ligases > use ATP to acquire energy (results in ADP and a free P group) [no phosphorylation occurs]

Somethings are just different

لَا إِلَهَ إِلَّا أَنْتَ سُبْحَانَكَ إِنِّي كُنْتُ مِنَ الظَّالِمِينَ

Abzymes

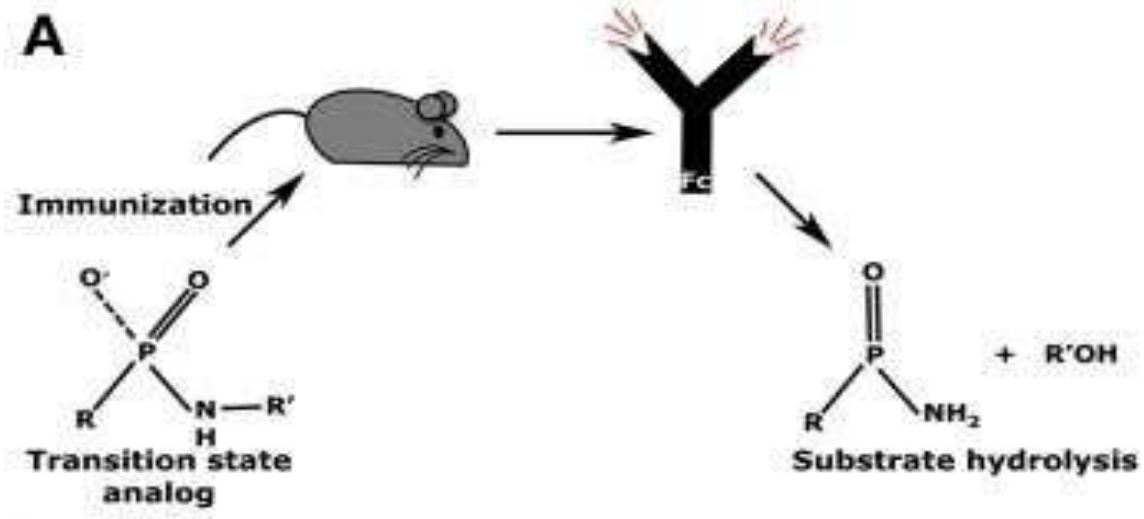


- Abzymes are antibodies\immunoglobulins acting as enzymes\that can catalyze reactions.

  Why? Bcz the **binding** of the antibody with the transition state molecule looks exactly like the binding of the active site of the enzyme with the transition state molecule
- They are produced against transition-state analogs(synthetic TS molecule).
- How? A host animal is injected with a transition-state analogue. The animal makes antibodies against it (binding with high affinity at highly specific binding points mimicking an enzyme's active site surrounding a transition state.

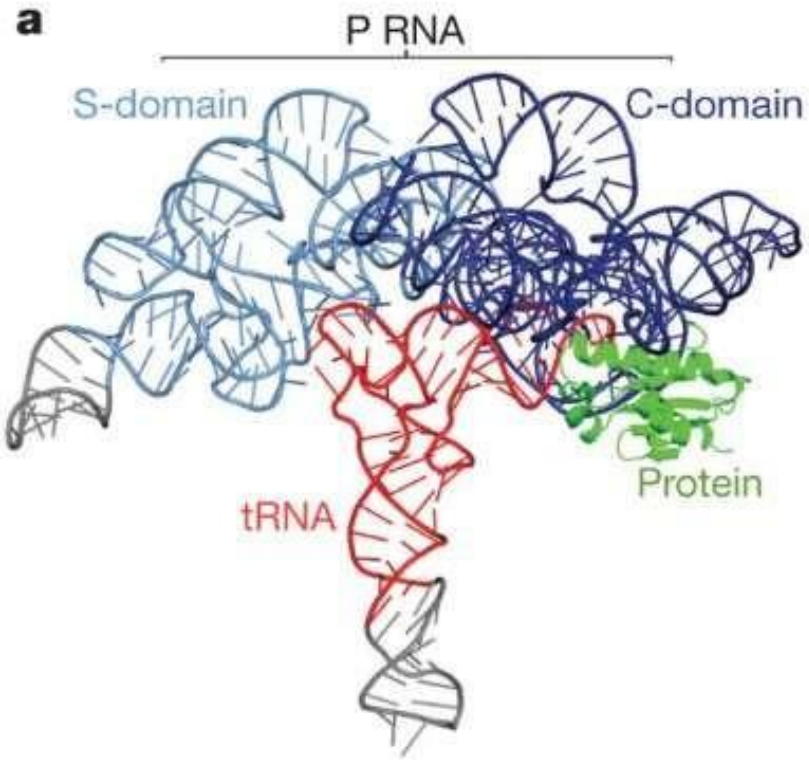
Abzymes with activity similar to cocaine esterase, which degrades cocaine, have been developed against analogs of its transition-state complex*(so they're made to treat cocaine addicts).* ***Monthly injections are used to treat addicted individuals by destroying cocaine in the blood and, thereby, decreasing their dependence on cocaine.***

Cocaine is transformed inside the body by cocaine esterase.



Ribozymes

- Most enzymes are proteins, but RNA molecules can act as enzymes and are called ribozymes.
- Some ribozymes are **ribunucleoproteins** made of both a protein and RNA, but catalysis is performed by the RNA part.
  - Examples include RNA splicing and protein synthesis in ribosomes.



When scientists found out about this, they deduced that RNA is the base of life> bcz it can function both as a genetic material and as an enzyme, which is contrary to proteins and DNA (solved evolution)

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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	3	To calculate ΔG…	Activation energy
V2 → V3	18	The second blue arrow was mistakenly placed.	

Additional Resources Used:

1. Mark’s Biochemistry for medical students pg(309-310)(330-335)
2. YouTube video #1
3. YouTube Video #2
4. Webpages
5. Anything else…

رسالة من الفريق العلمي:

"اللهُم ارحم أمواتنا وأموات المسلمين واجعل أرواحهم من الأرواح المُنعمة المُبشرة بالخلود في جنة الفردوس يارب العالمين".