بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 12

Enzymes pt.2

Written by : Raghad Farraj
Hala Marei
Shahd Thamer

Reviewed by : Jana Sawafta
Rand Alkhateeb

Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

بسم الله الرحمٰن الرحيم

Classification of Enzymes

There are always exceptions but we are going to focus on the general outline of enzymes

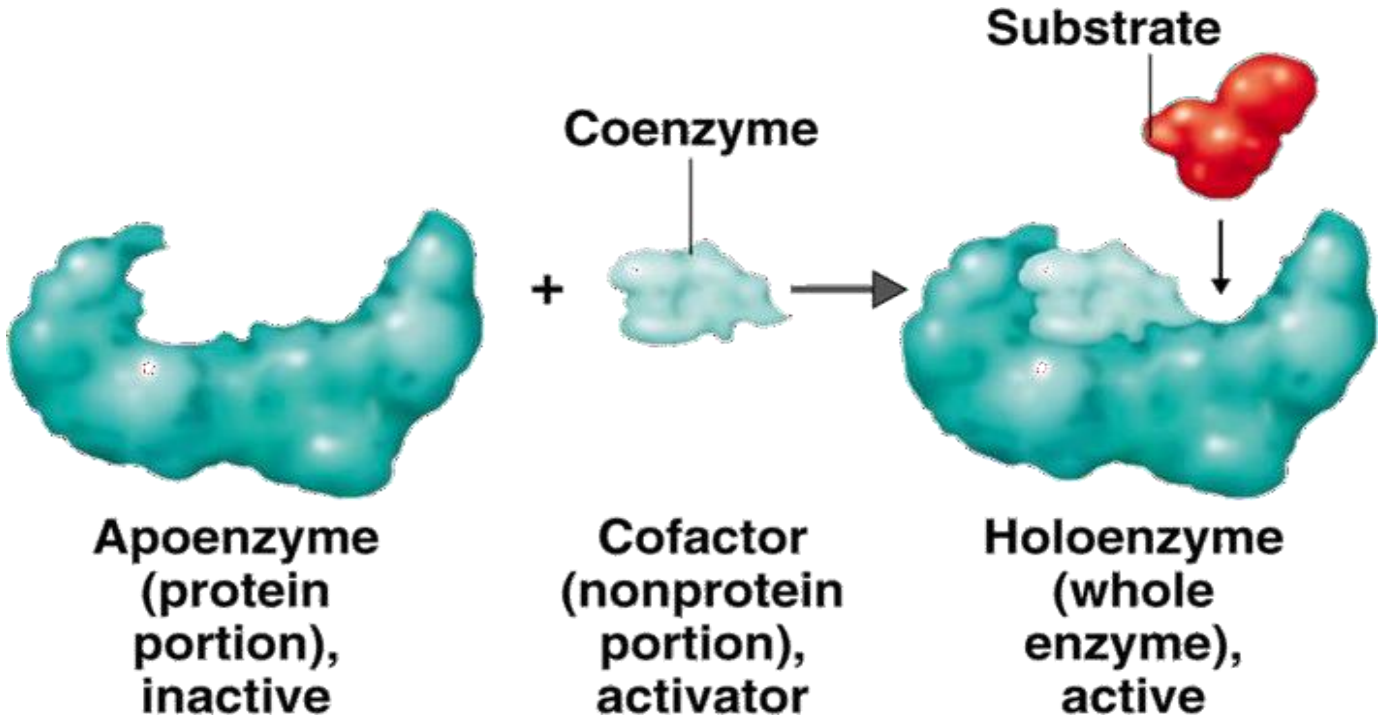
Recall: Enzymes catalyze reactions and have two main features: **affinity** and **specificity**, allowing them to bind particular molecules. **Specificity is determined by the enzyme's primary structure (amino acid sequence)**, which determines the arrangement of amino acids in the active site. The substrate, activator, or inhibitor binds to specific amino acids within the enzyme.

Enzyme Classification (structure)

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

Apoprotein (apoenzyme): the protein part alone, made only of amino acids. It may consist of one or more polypeptide chains.
Holoprotein (holoenzyme): the complete functional protein, consisting of the protein part plus a non-protein component such as a metal ion or organic molecule.

Later, we are going to talk about **cofactors** that are non-protein molecules that bind to enzymes, and help them catalyze reactions



Naming of enzymes

- In general, enzymes end with the suffix (-ase).

  Similarly, many sugars end in **"-ose"** (eg: glucose)

- 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.

  Names usually indicate the substrate or reaction
  eg: **kinase** (transfers phosphate), **esterase** (breaks ester bonds), and **saccharidase** (breaks carbohydrates)

  Early enzymes had common names:

- Some enzymes have common names
  - Examples: the proteolytic enzyme trypsin

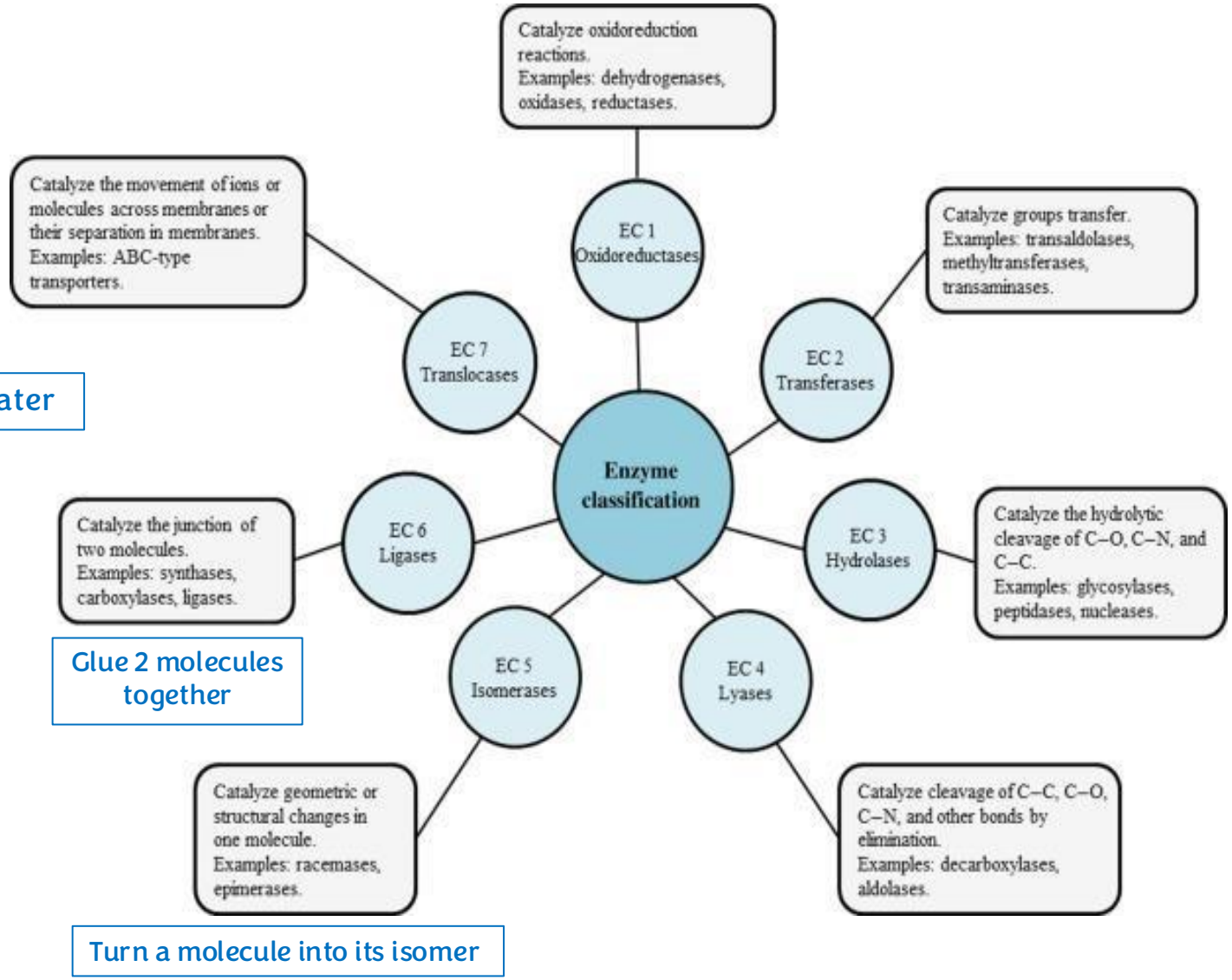
  and chymotrypsin

Trypsin: cleaves peptide bonds after **Lys or Arg.**
Chymotrypsin: cleaves after **Phe, Tyr, or Trp.**

Enzyme classes according to function

Depending on reactions they can carry out

- Enzymes are classified into six major groups:
  - Oxidoreductases
  - Transferases
  - Hydrolases — uses water to break bonds
  - Lyases — break or form bonds without use of water
  - Isomerases
  - Ligases



Glue 2 molecules together

Turn a molecule into its isomer

Everything the doctor mentioned explaining the slide is already included **in the diagram**

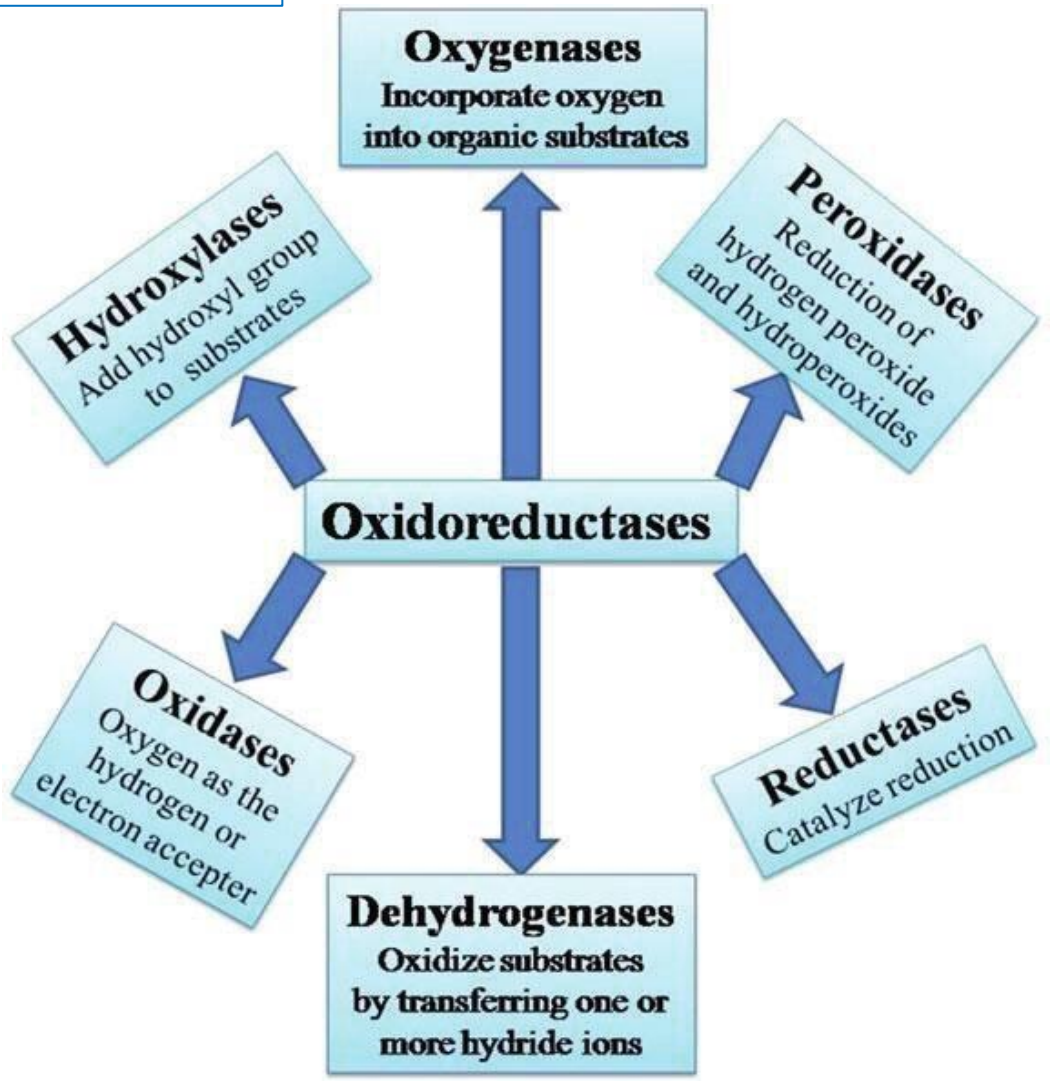
Scientists later realized that **each enzyme class contains different types**, so enzymes are named in such way: **EC 1.x.x.x**.
We won't go into such details.

1. Oxidoreductases

Largest group of enzymes and they have many functions

- 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
  - Reductases
  - Hydroxylases

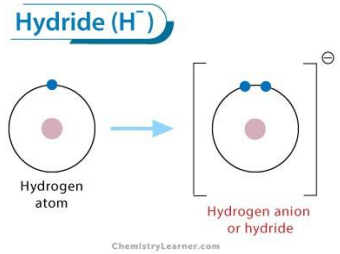
It is preferred that you memorize reactions because we're going to have them later on in our metabolism course, but as of now the doctor said he's not going to ask you this enzyme does what reaction.
He going to bring reactions that you never saw before and tell you this is the action of which enzyme, or the other way round, all from your understanding ☺



1a. Dehydrogenases

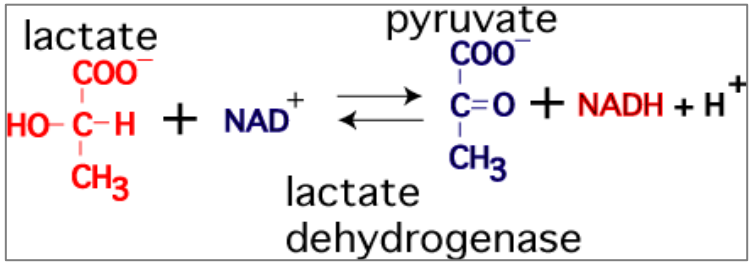
De: remove, hydrogenase: protons

Dehydrogenases can catalyse both the forward and the reverse reaction



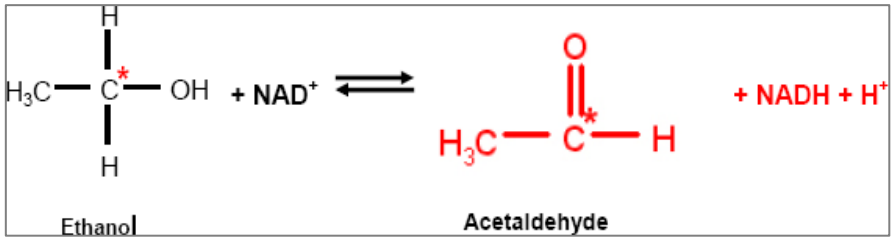
- Dehydrogenases transfer electrons in the form of hydride ions (H^-) or hydrogen atoms using an electron-transferring coenzyme, such as NAD^+/NADH or $FADH_2$.
  - Lactate dehydrogenase:

    Lactate oxidized to pyruvate



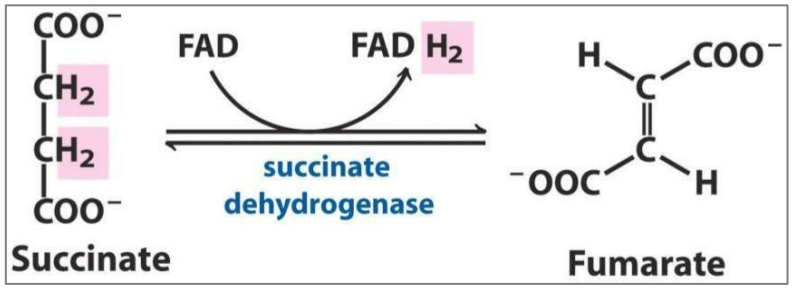
  - Alcohol dehydrogenase:

    Alcohol oxidized to aldehyde



  - Succinate dehydrogenase:

    Succinate oxidized to Fumarate (2 hydrogens involved)



In general, if you see NAD^+, FAD, or **FMN** involved in a reaction, it is likely a **dehydrogenase**

Transfers electrons from one molecule to other, one becomes oxidised and the other reduced)

Hydride ion (H^-)
1 proton + 2 electrons

NAD^+/FAD = oxidized form
→ accepts hydrogen
$NADH/FADH_2$ = reduced form
→ can donate hydrogen

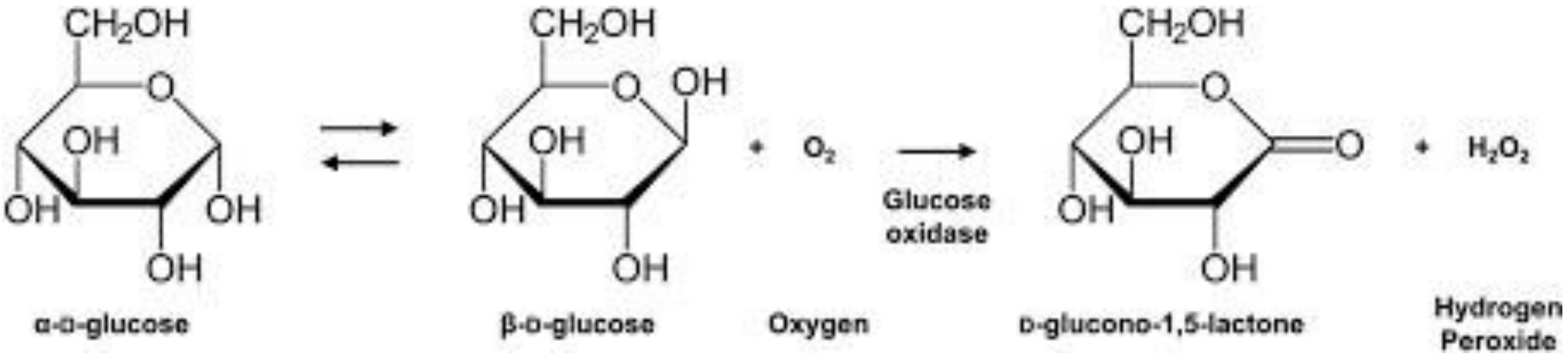
Do not memorize the reactions but understand them

1b. Oxidases

If O_2 is the reactant and is reduced to H_2O_2, the enzyme is an oxidase

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

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

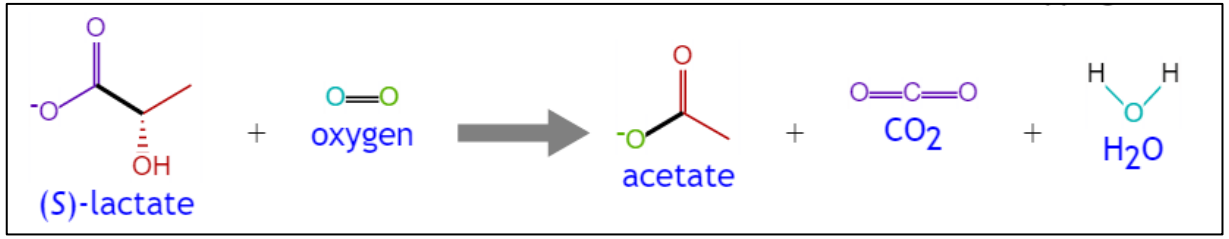


1c. Oxygenases

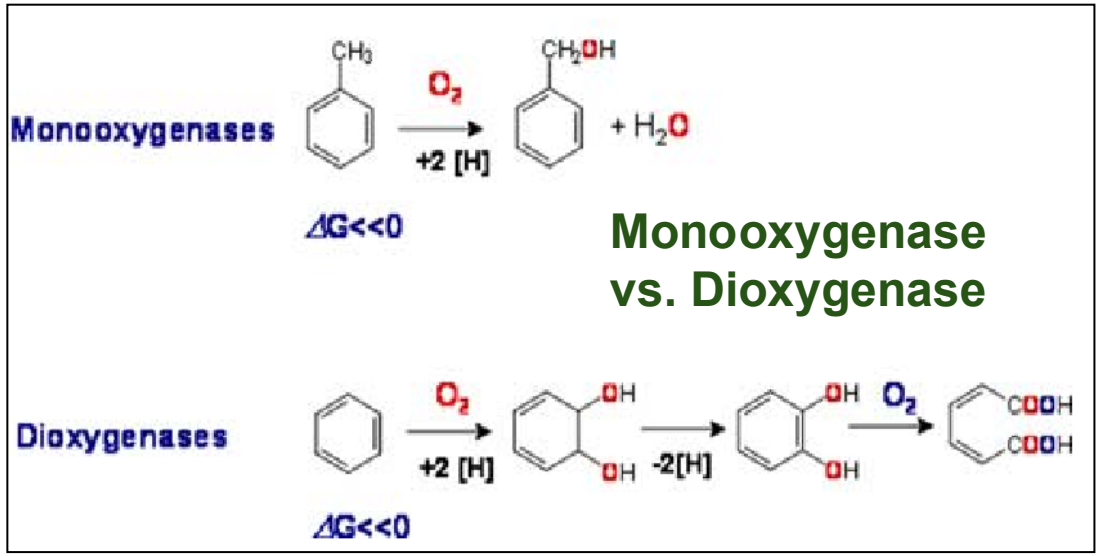
Oxygenases add oxygen from O_2 directly to the substrate

- Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate.
  - The reduced product is water, not H_2O_2.

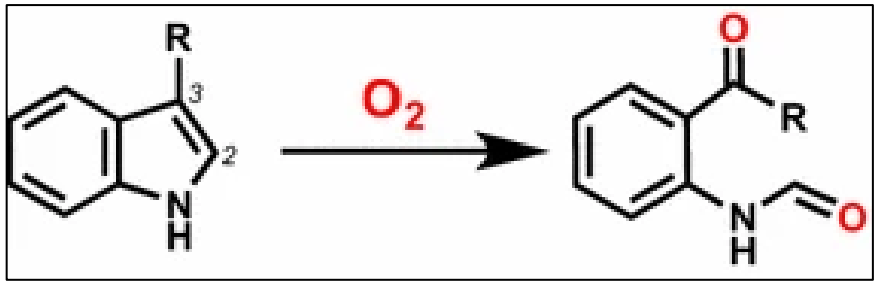
Lactate-2-monooxygenase



1 oxygen is transferred to the lactate to make acetate (mono)



Tryptophan 2,3-dioxygenase



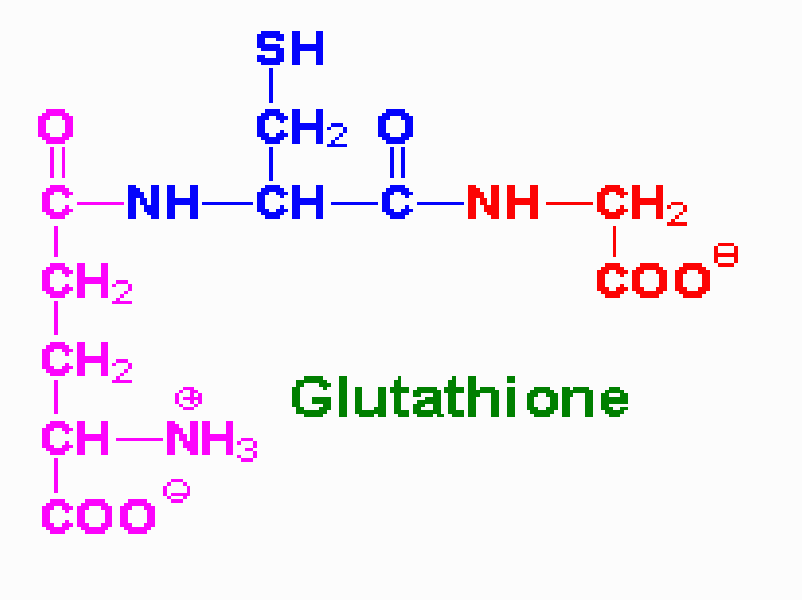
2 oxygens transferred to the same substrate (di)

Oxidases and Oxygenases catalyze oxidation of the substrate, differently:
Oxidase: substrate loses electrons → O_2 accepts them → H_2O_2 forms
Oxygenase: substrate is oxidized by having **oxygen incorporated into it,** forming either monooxygenase (one oxygen) or dioxygnase (two oxygens on the same substrate) and no H_2O_2 in the products, perhaps **water**

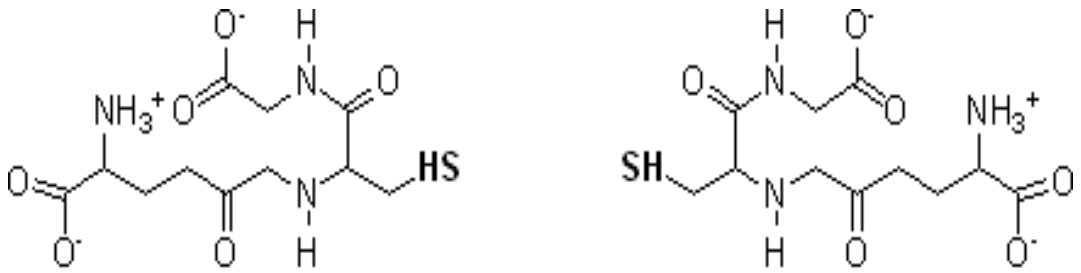
1d. Peroxidases

We remove the hydrogen peroxide and transferred into water (unlike oxidase where hydrogen peroxide is in the products)

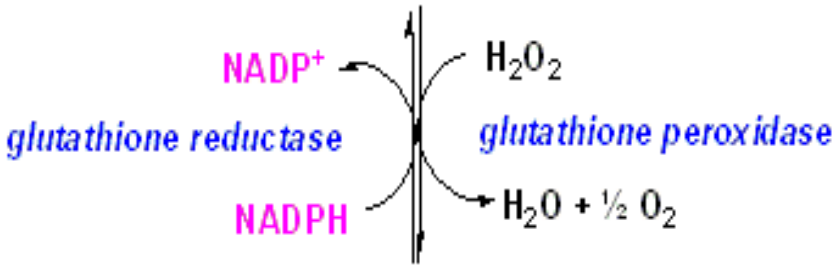
- Peroxidases catalyze oxidation of a substrate by hydrogen peroxide.
- Example: oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide:

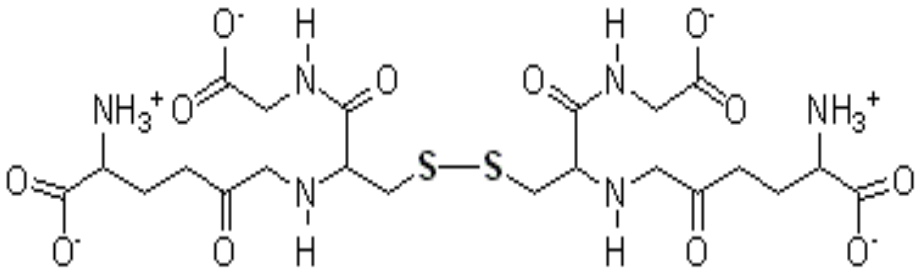


A tripeptide that contain gamma-glutamate (the website bonds is on the gamma, carbon) cystine and Glycine , this removes hydrogen peroxide from cells because it is dangerous and toxic and it's a active oxygen species, and it becomes oxidized glutathione (diglutathione, in the next slide)



2 x reduced glutathione (GSH)





oxidized glutathione (GSSG)

This glutathione must get back to its reduced form to be recycled so the second use it again in metabolism
Whenever the metabolism happens, there will be a production for glutathione

So that pharmacies and IRBs and what else are working on antioxidants which is simply substances like glutes that helps the body to get rid of the oxidizing agents to tighten the skin and make it more fresh

The formation of disulfide bond

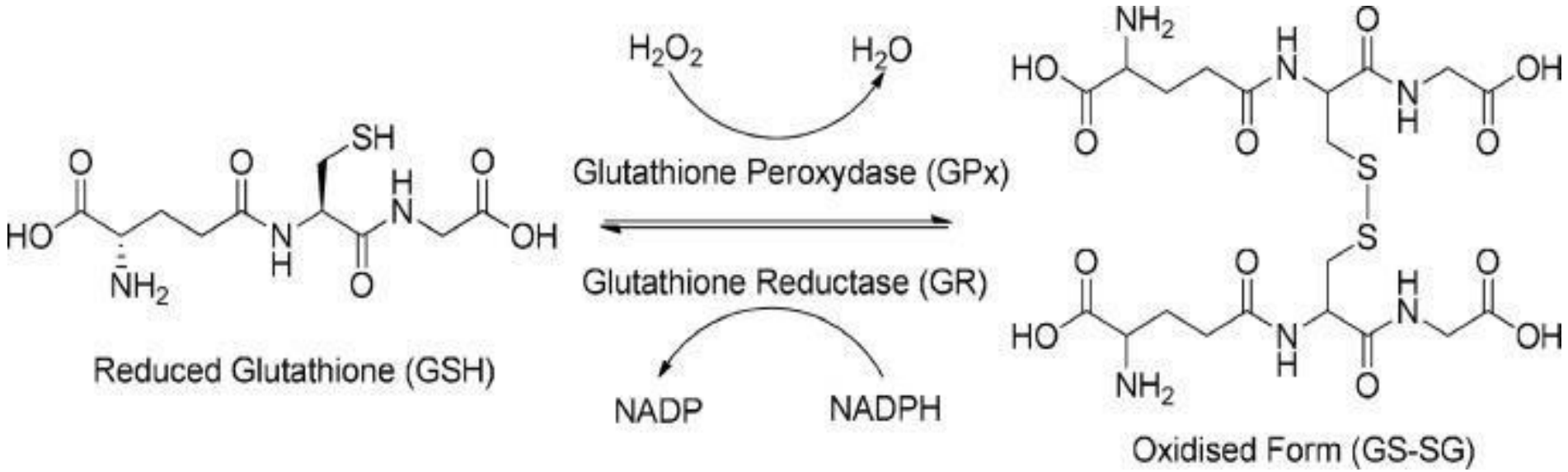
The difference between NAD and NADP
NADP has phosphate and the other one doesn't.
NADP is used in cells in anabolism in building of large molecules, NAD to breakdown molecules.
Both of them does the same function oxidation and reduction.
The NADP is a part of a previous reaction while NAD is not.
The purpose of the phosphate is to determine the enzyme that can work on the molecule. As a function, the are totally the same.

1f. Reductases

- These catalyze the reduction of substrates.
  - Example: glutathione reductase

And something must have oxidation

We talked about the glutathione we said it must turn back to its reduced form and this reduction is responsible for that

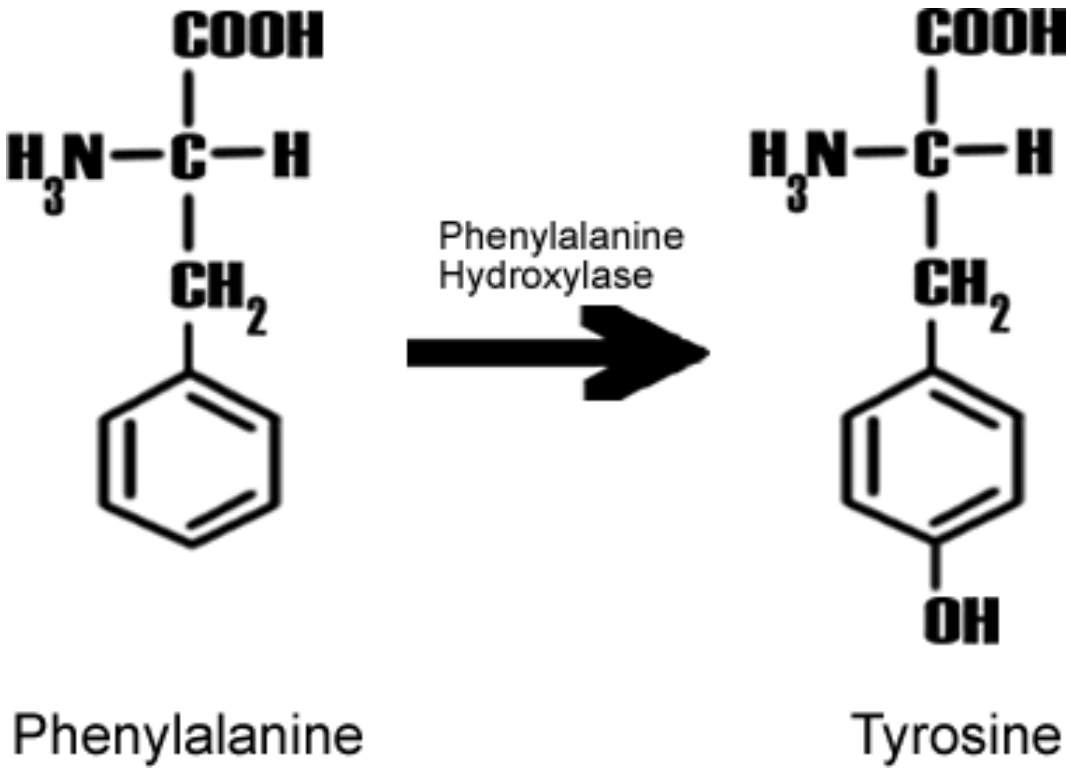


Why not de hydrogenase? Actually, reductase is usually major molecule like glutathione and a small.thing like NADH than undergoes oxidation what is really important is to have a substrate to undergo reduction

1e. Hydroxylases

- Hydroxylases add hydroxyl groups to a substrate. From minor group

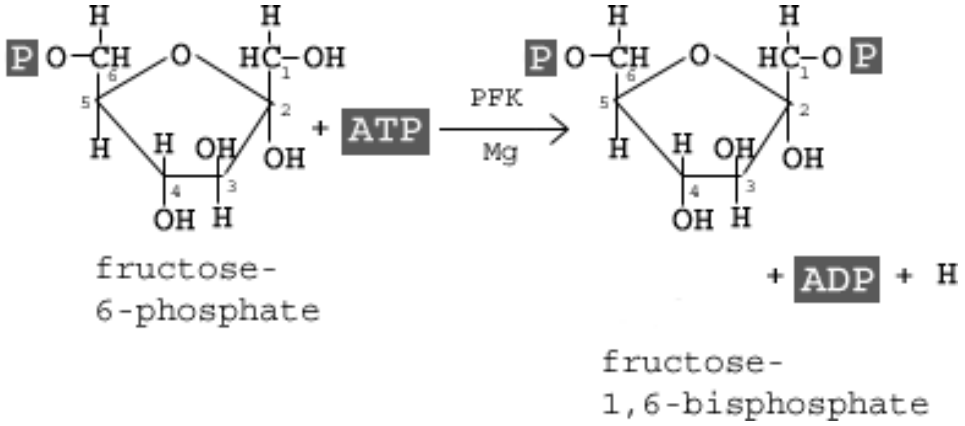
And of course, there must be another subject that undergoes reduction



Phenylalanine Tyrosine

2. Transferases

- These enzymes transfer a functional group (something that contains C, N, P, or S) from one substrate to an acceptor molecule.
- Example: Kinases (major enzymes that transferred group is a phosphate from ATP to the substrate) ATP is a high energy molecule because we have the energy stored in the bones between the phosphate groups, the cell may break this bond for energy and the substrate becomes energy rich. This is the first reaction in glycolysis (glucose into fructose-6-phosphate by hexokinase) and then:
  - Phosphofructokinase catalyzes the transfer of phosphate from ATP to fructose-6-phosphate: take the phosphorylated fructose and give it another phosphate



fructose-6-phosphate

fructose-1,6-bisphosphate

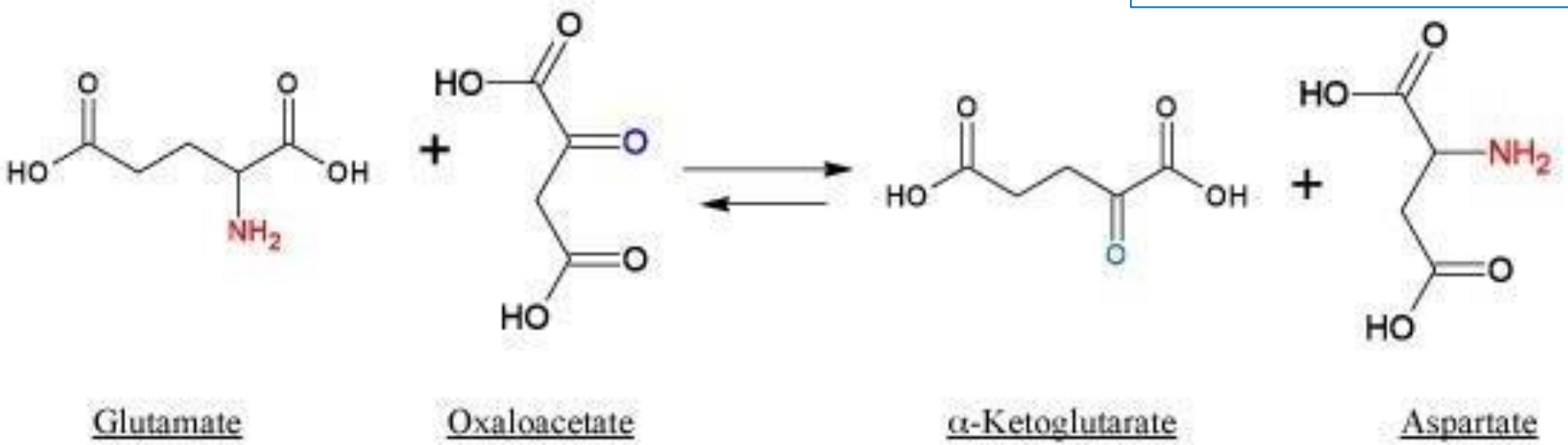
The di means that we added the groups together , while bis means that we added two groups on both sides

Another example: transaminases

- A transaminase transfers an amino functional group from one amino acid to a keto acid cause it contains carboxyl group and ketone group, converting the amino acid to a keto acid and the keto acid to an amino acid.
  - Interconversion of certain amino acids.
  - Aspartate transaminase:

When we want to examine the blood test for the enzymes of the liver, we examine aspartate (AST) high difference of it indicate that we have liver damage like cirrhosis or viral infection, hepatitis c.

gluta = 5 carbons



Glutamate Oxaloacetate α-Ketoglutarate Aspartate

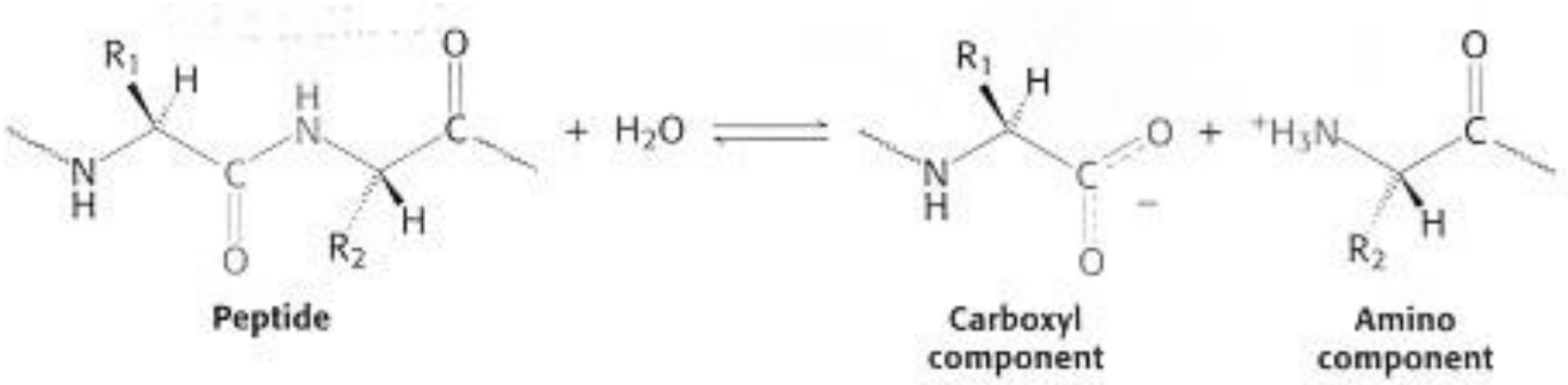
Those are the triaminzse, they're present in the liver and kidney

They are existed in the cribs cycle

3. Hydrolases

Remember when we one talked about proteases like trybsine , kinotrypsine and anasthase they catalyze different reaction in strategies that we talked about (covalent catalysis) and break the bond (for more details check the previous lecture)

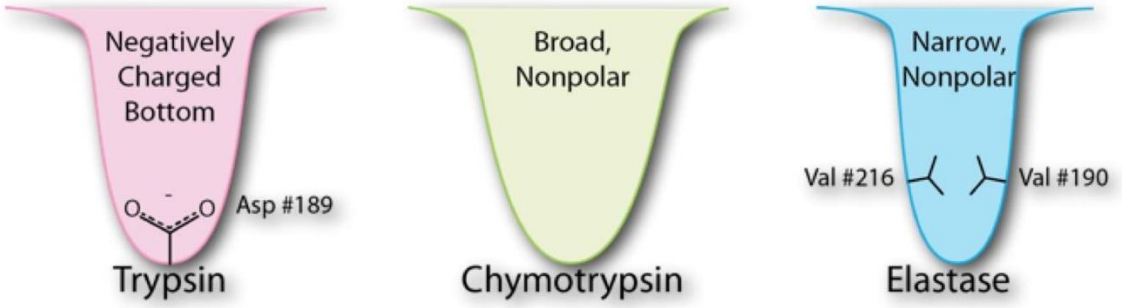
- They catalyze cleavage reactions using water across the bond in the form of OH^- and H^+ to being broken or the fragment condensations.
  - Proteases, esterases, lipases, glycosidases, and phosphatases are **hydrolases** named depending on the type of bond cleaved.
    - Example: proteases
      -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



Specific examples: digestive enzymes

- 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.

Saccharidase , lipase the same thing they break up bonds using water, esterase it breaks the ester bonds using water



Lysine
Arginine

Phenylalanine
Tryptophan
Tyrosine

Glycine
Alanine
Valine

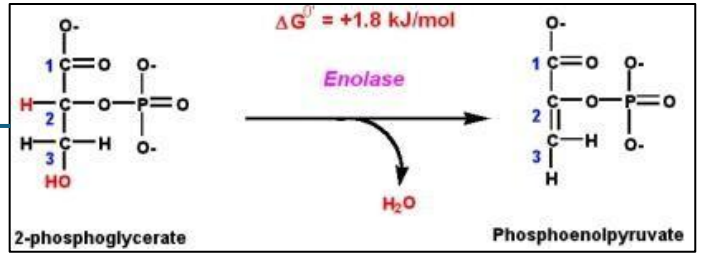
4. Lyases

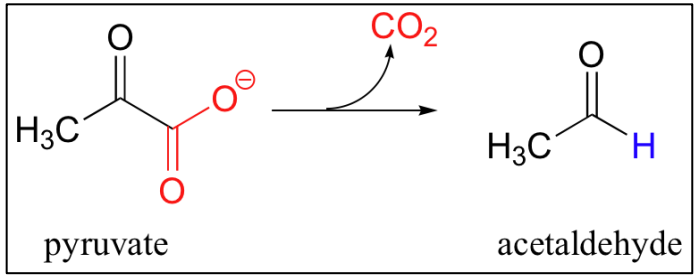
- Lyases either cleave C-C, C-O, C-N, and other bonds by means other than hydrolysis or oxidation, leaving double bonds or rings, or add groups to
- double bonds **without hydrolysis**.

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

- 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 molecule to a double bond or formation of a new carbon-carbon bond.
  - Example: citrate synthase

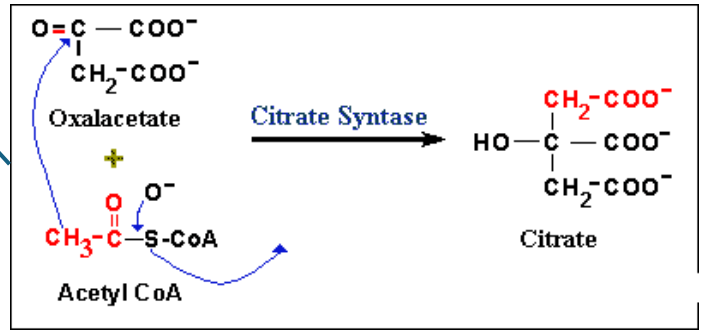
Phosphoenol pyruvate has phosphate, When phosphoenol pyruvate converts into pyruvate the phosphate group is transferred from the phospholenol pyruvate to ADP (Which converts to ATP)-> kinase enzyme (transfer reaction, transferase). This pyruvate is broken up by NAD+ (oxidizing agent, gets reduced to NADH) and the pyruvate is oxidized to acetyl coenzyme A-> dehydrogenase enzyme (key: the conversion of NAD+ into NADH)





This acetyl coenzyme A(2 carbon) merges with oxaloacetate(4 carbon) =citrate(6 carbon) and that's why it's called citric acid cycle. Enzyme involved is synthase (citrate synthase)

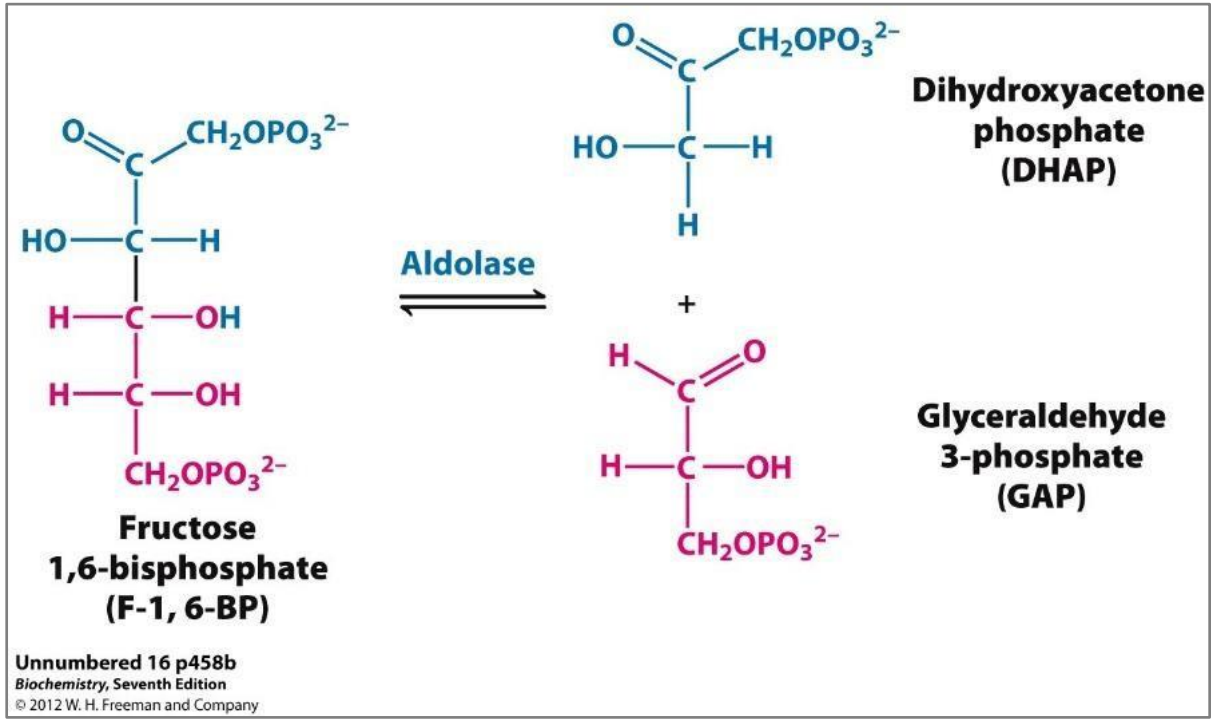
Citrate is converted to isocitrate and then to alpha keto glutarate (by removing CO2, became a 6 carbon molecule) which is decarboxylation reaction.
And if we decided to transfer the functional amino group in aspartate to alpha keto glutamate (the aspartate converts to oxaloacetate) and will become a glutamate by transaminase enzyme



Example: aldolase

Lyase (Broke the c-c bond without using water)

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

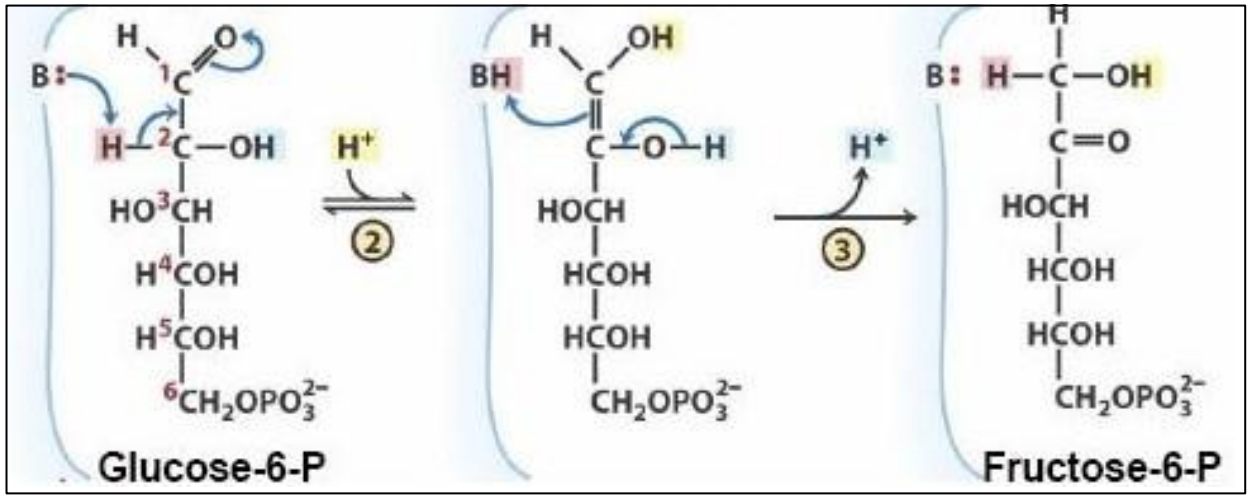


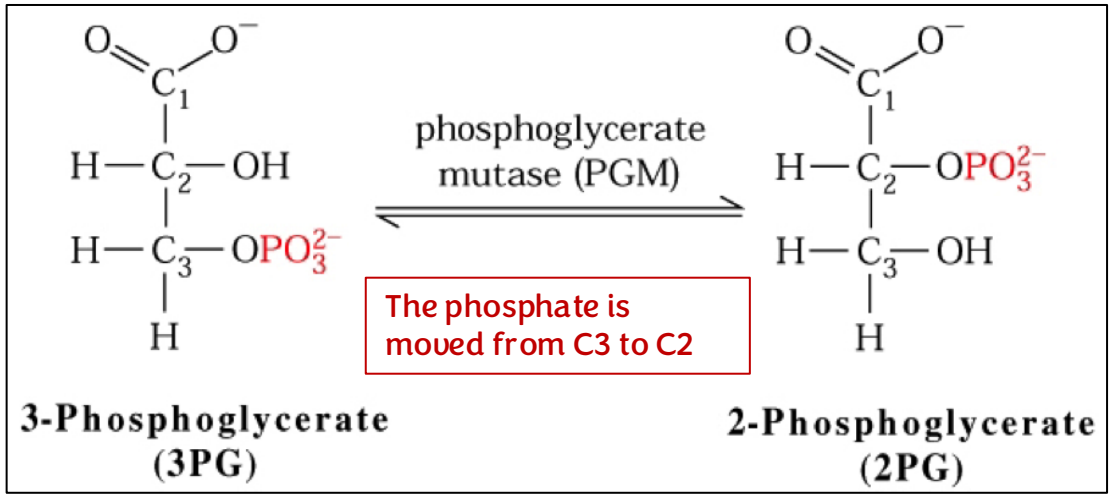
5. Isomerases

For instance, in glycolysis ;glucose initially converted to glucose-6-phosphate (by kinase; a type of transferase) and which is converted to fructose-6-phosphate by isomerase

- 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:

Another reaction derived by isomerase is : dihydroxyacetone phosphate (DHAP) to D-glyceraldehyde 3-phosphate





The phosphate is moved from C3 to C2

When we add DNA fragment to another we use ligase

6. Ligases

Like gluing two molecules(Smaller molecule to larger one)

Lyase joins two groups WITHOUT using energy (ATP)

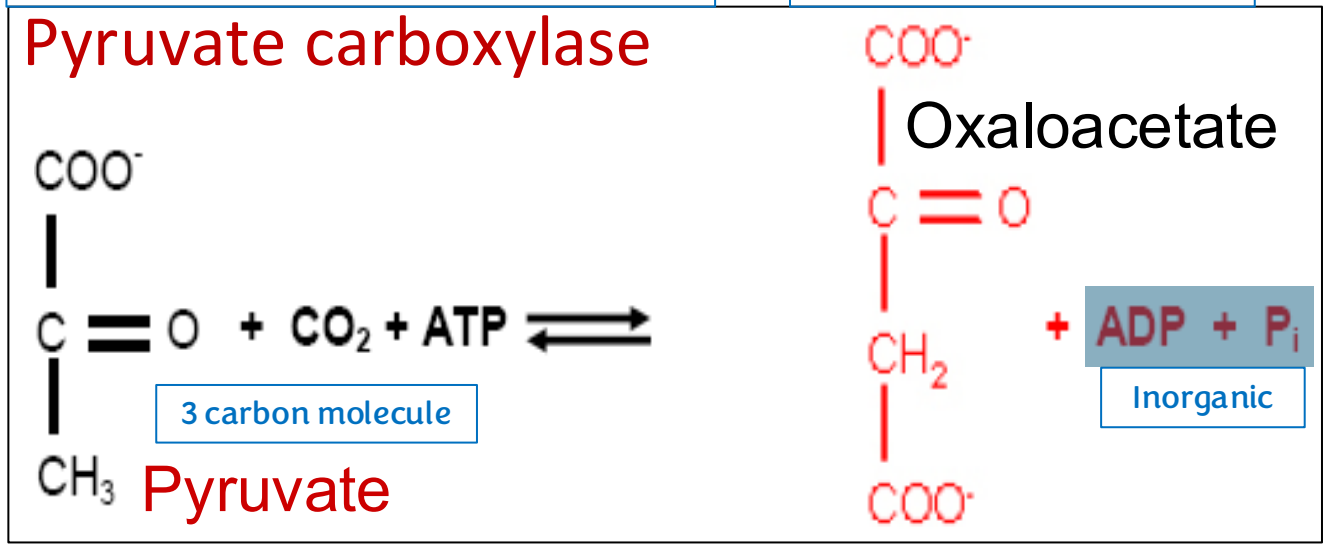
- Ligases join C-C, C-O, C-N, C-S, and C-halogen bonds.

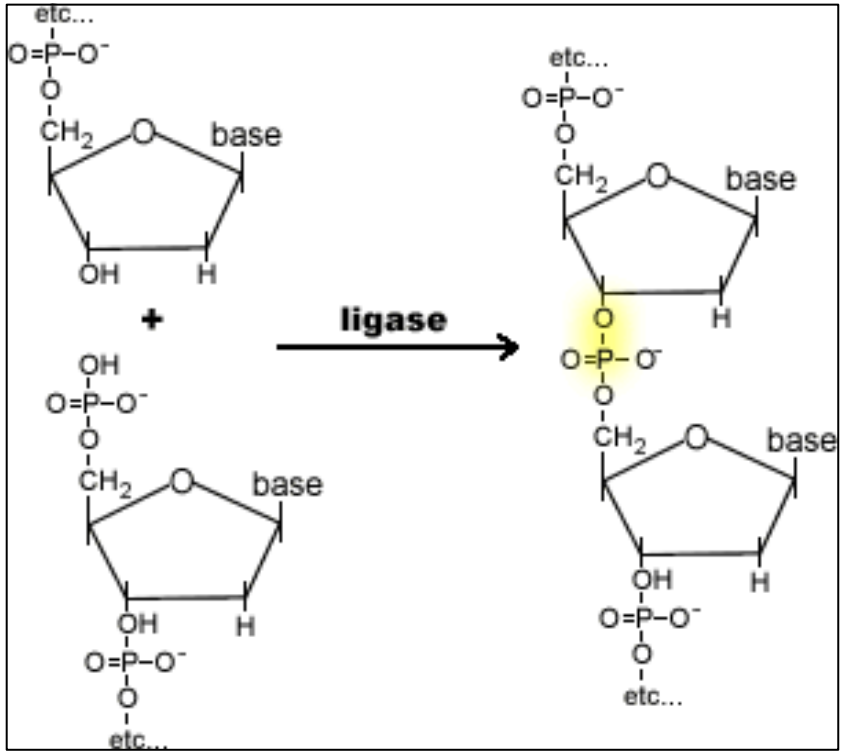
Using energy ATP to form larger molecules

- 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.
  - Synthases use a different source of energy.

Note: transferase uses ATP too, but the phosphate is transferred to other molecule. Here the phosphate group is free and we used the energy resulted from breaking the bond.

Phosphate is a product here with ADP; which is a crucial point to distinguish ligase.





Some things are just different

Abzymes

We don't have them in our bodies but scientists created them, how?

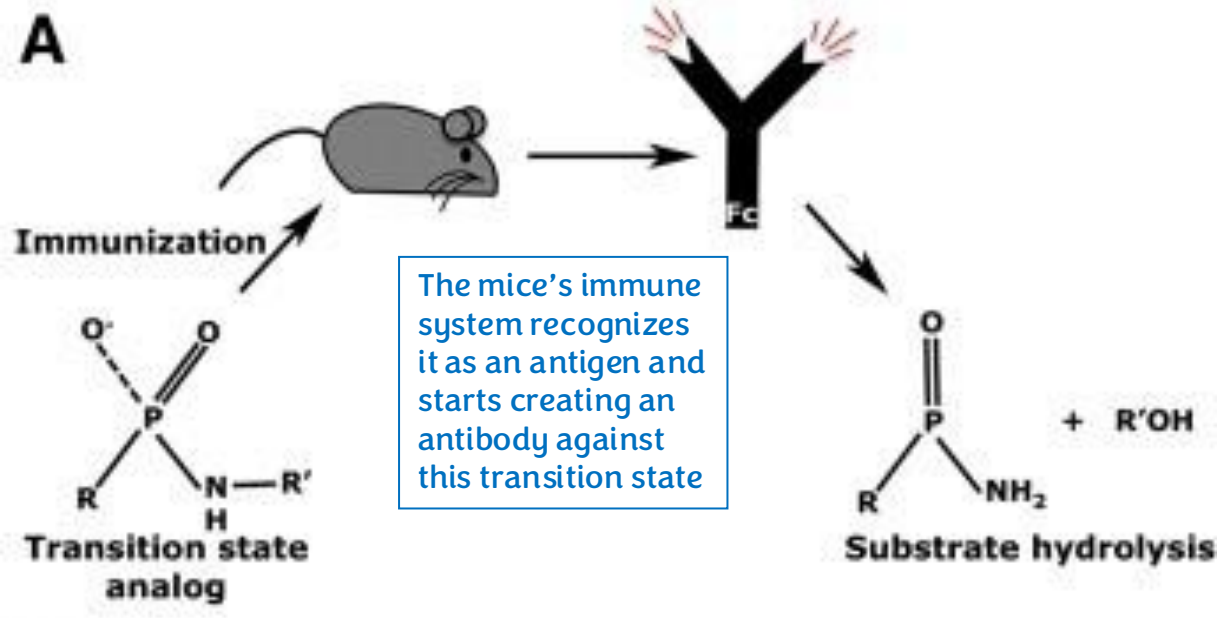
These antibodies are isolated and then injected in humans. Antibody binds to the substrate (drug) as if this substrate is transition state and the binding is very strong then transformed to product. It works SOMETIMES (because the structure of the drug is not identical or close to transition state always)

High affinity binding with substrate like in transition state which makes the antibody work as enzyme

- Abzymes are antibodies acting as enzymes.
- They are produced against transition-state analogs.
- How? A host animal is injected with a transition-state analogue. The animal makes antibodies against it (binding with high affinity at specific binding points mimicking an enzyme's active site surrounding a transition state.

Recall transition state is a high energy unstable molecule between the reactants and products. The interaction between the enzyme and the transition state is very strong and requires energy.

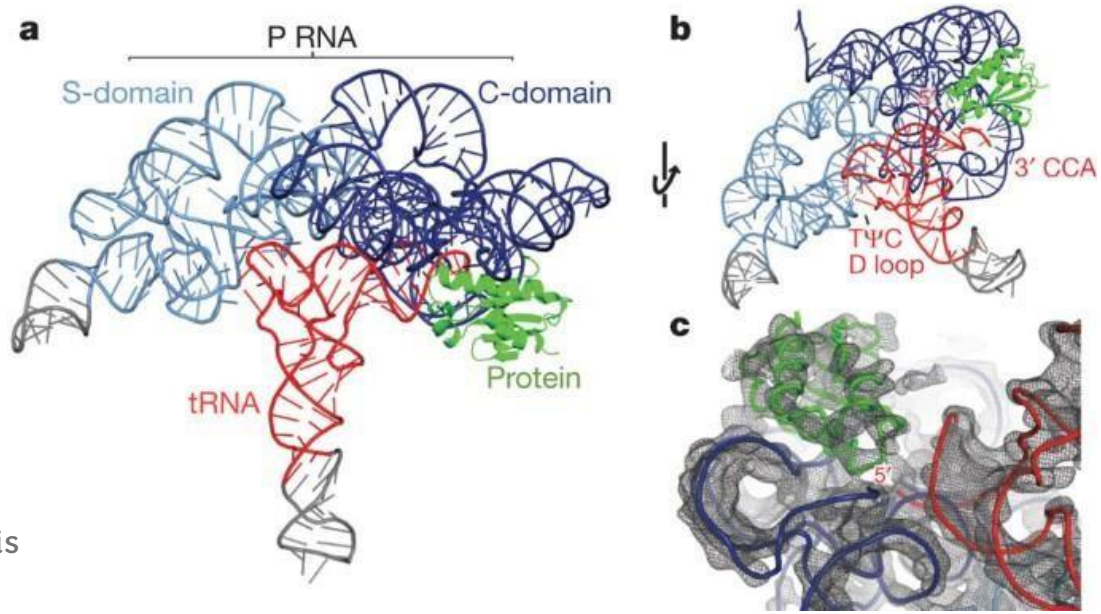
Abzymes with activity similar to cocaine esterase, which degrades cocaine, have been developed against analogs of its transition-state complex. Monthly injections are used to <u>treat addicted individuals</u> by destroying cocaine in the blood and, thereby, decreasing their dependence on cocaine.



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 discovered that RNA could contain genetic material like in viruses and function as an enzyme they came to the conclusion that life starts with RNA



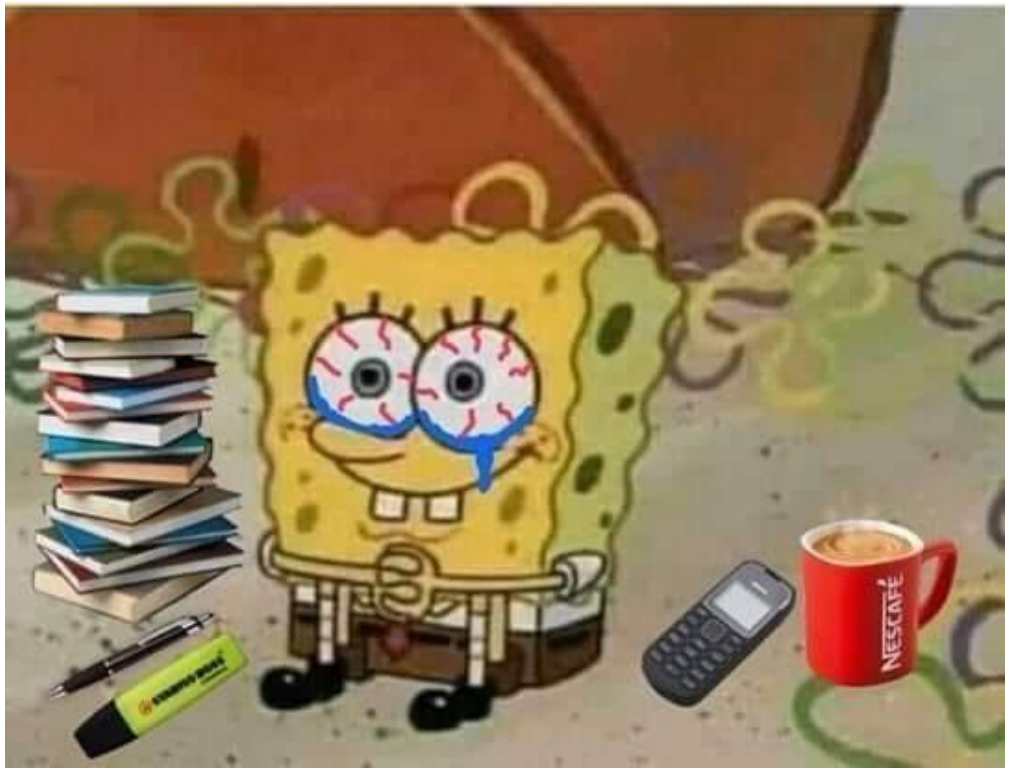
Ribosomes are protein factories, they bind to mRNA and with two tRNA (which will hold the amino acid sequence) , ribosomes bind two amino acids together by peptidyl transferase (elongation of peptide or amino acid chain which is the key concept of protein synthesis)

This is done by peptidyl transferase and RNA inside the ribosome not the ribosome itself (work as enzymes) -> Ribozymes

Recall RNA splicing from Biology 101 ;extracting introns and binding exons together(to produce a mature RNA) this process is done by RNA molecules

Further explanation; splicing is done by spliceosomes (Ribozymes) or the RNA sequence itself! Which is called self-splicing and the RNA works as a ribozyme

Test yourself



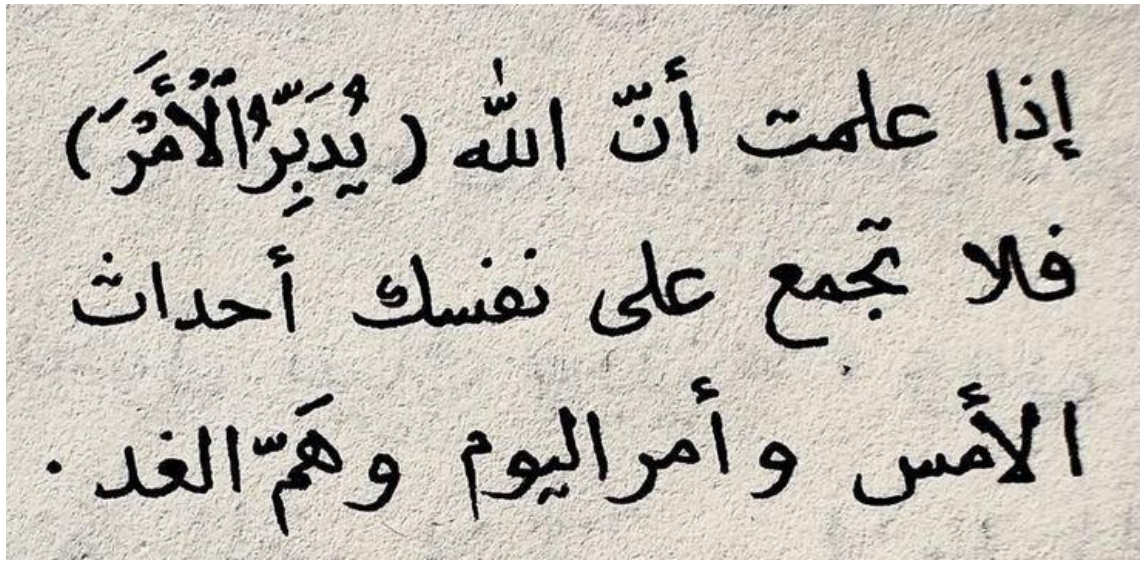
Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
https://youtu.be/4Men02UoT_A?si=CjCWyNkMGPfzLJFM

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)



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			
V1 → V2			