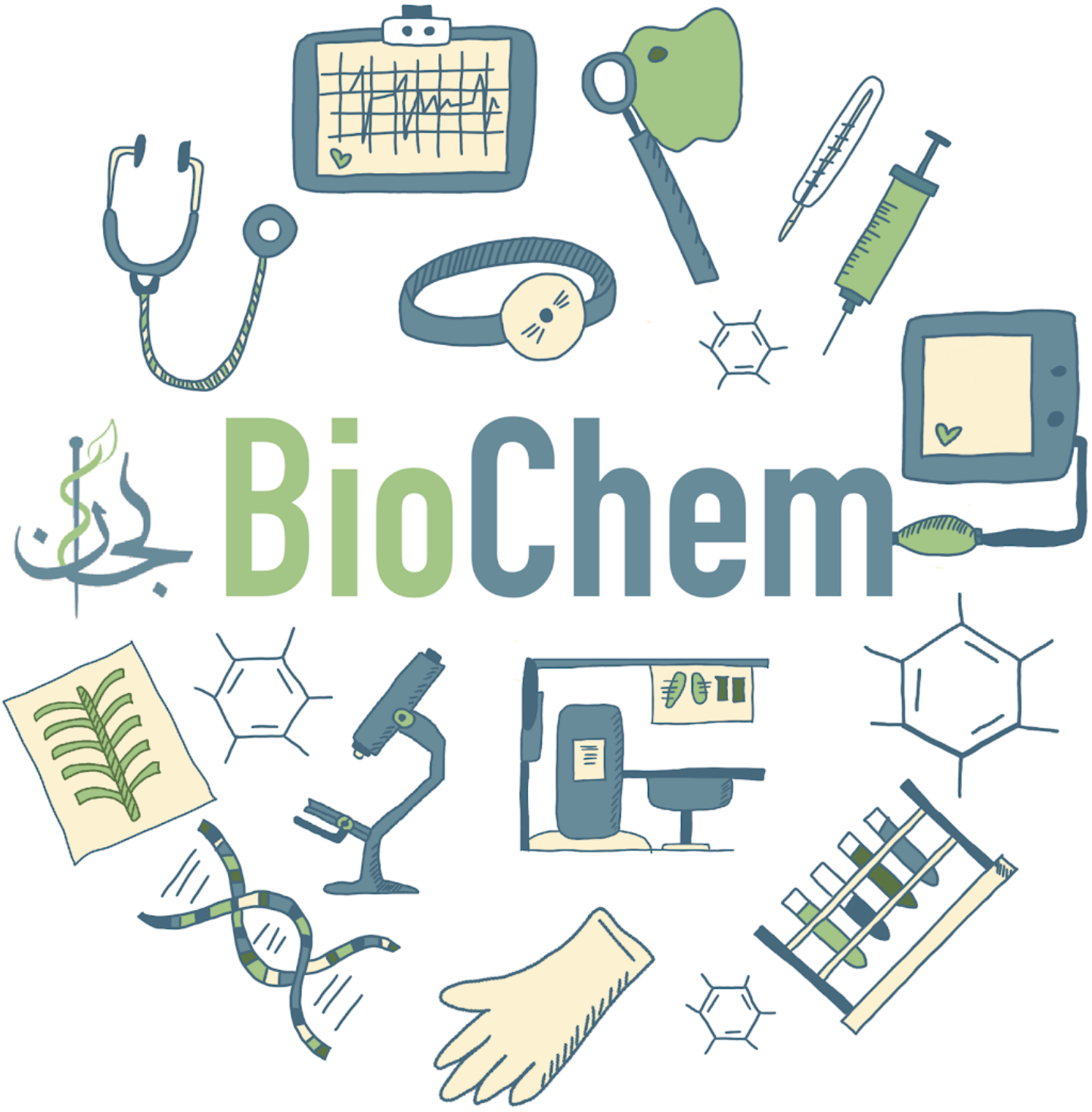

BioChem

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Doctor:

For us to understand how chemical reactions occur, first let's ask this question: "why do chemical reactions occur?

-Student 1: to produce specific outcomes, products or to produce energy.
Doctor: wrong! These are reactions' outcomes, always remember: you -as human being- understand, but nothing in your body understands, and this is our question: **why chemical reactions occur?**

-Student 2: **to transform from a situation that is less stable to another situation that is more stable.**
Doctor: great!! It is always about stability, to achieve a situation of higher stability, to go from a lower stable state into a higher stable state.

What is the relationship between *energy and stability*?
-Student 1: if something has more energy, it has less stability.
Doctor: great!! always, it is an **inverse relationship**, the more the energy is, the less the stability will be and vice versa.

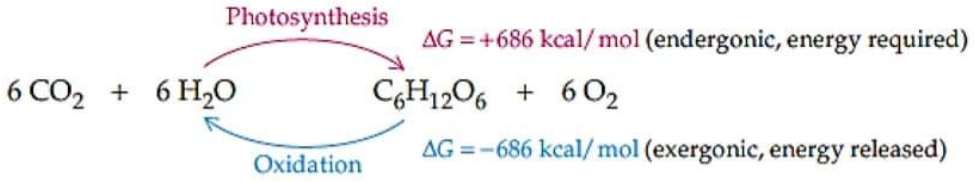
What are the types of energy when you are looking at the chemical reactions?
They are two types:
-Student 1: **kinetic energy & potential energy.**
Doctor: right, kinetic energy means (الطاقة الحركية) & potential energy means (الطاقة الكامنة).

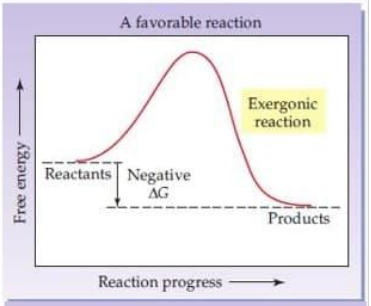
"Potential energy is the stored energy, for any material to undergo a chemical reaction, it should have a stored energy inside it, and we call that a potential energy."

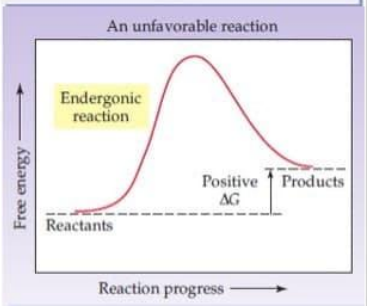
How to calculate this potential energy?
By the total energy inside the bonds in these materials, (how much energy that we will get after breaking down all bonds in this material), we calculate this amount of energy, and then we will go to the product and calculate the amount of energy by using the same method, and we will know the difference between these two values (the starting point & the ending point) / (the reactant & the product) and this we expressed as "***the difference in potential energies -the difference in the bond energies between the reactants and the products- ΔG".***

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)







when we want to go from a high scale of potential energy to a low scale of potential energy, that means **"this reaction would go to a more stable state"** or this reaction is favorable, so when the potential energy of the reactant is higher than the product one we call this reaction favorable because it releases energy (exergonic - **releasing energy reaction**), in biochemistry "wise" we call it "spontaneous", and it is a biochemical term ***not a literal term, we will treat this issue later on***.

On the other hand, if we are going from a low potential energy scale to a high potential energy scale, What we call this reaction?
We call it (endergonic - **absorbing energy reaction**) and in biochemical terms "non-spontaneous" ,and we can call it "unfavorable".

Any mathematical equation is an expression of what is there, we shouldn't memorize the mathematical equation without the full understanding, because this mathematical equation expresses the theory, so ΔH means :

$\Delta H = \Delta G + T\Delta S$

So it has two factors:

1. ΔG which means the difference between the bonds energies of reactants and products
2. ΔS how molecules are arranged around each other.

-So it takes in account the bond energies ΔG between molecules like water molecules which have bond energies inside oxygen and hydrogen, so the oxygen along with the hydrogen have a bond energy, and **this bond and bond energy can not be changed when the state of water is changed from the liquid to the gaseous state -for example-, actually, what is changed is the arrangement of molecules around each other and the distances between them** which is expressed by ΔS that *measures the randomness in the molecule (how is the order of molecules around each other).*

-As a summary ΔH accounts for all energies or the total energy in the system (the energy inside the bonds & the energy in the system due to the arrangement).

-So ΔG is the total energy minus the randomness.

How do we calculate"Delta Δ" ???

Always (Final - Initial) or (end - start).

When we are talking about favorable reactions, the products have less amount of energy than the reactants, so ΔG carries a sign of negative - / and for the unfavorable reactions the sign is positive + .

Pay attention !!! It is the most important piece in this sheet !!!

"We are talking about starting and ending point of reactions, there is a potential energy stored inside the reactant and there is a stored energy inside the product also, what happens between the reactant and the product is the pathway that the reaction would follow or how reactants are gonna be converted into products, so when you looking for any biochemical reaction, you can look to it from two different views: either **you look at the starting and ending view** and it is a prediction science (will this reaction occur or not? / is this reaction spontaneous or not?) and that is what we call the *thermodynamic science* (it is concerned with the potential energy, **we don't study anything between the starting and ending point** and we will talk about it in the next semester), **while in the enzymology we are studying what happens between A & B** (the pathway / how many steps in this reaction? / what is the transition state of the material? / what is the activation energy of the material) **without taking into account what is A and what is B** (and we call it the kinetic science)."

A classical Q in the biochemistry exams: We started an uncatalyzed reaction between two materials, the reaction had a ΔG of -10 Kj/mol, in another experiment we added the enzyme that is needed for this reaction, what is the change that will happen to the ΔG value ???

It will not be changed, because these are two different concepts, ΔG is concerned with the final and initial points while the enzymes are concerned with the pathway itself, there is nothing to do with ΔG.

It is something that seems like when you want to reach somewhere, actually the distance between these two places is constant whatever the pathway that you follow is, **the main purpose of enzymes is making the reactions follow the shortest pathway .**

What do we mean by ΔG^0 ??
It means ΔG in the standard conditions:

1. Temperature 25^0 degrees.
2. The pressure of 1 ATM.
3. The concentration of 1 mol
4. The acidity of 7 pH.

Now we have finished answering the Q : What do enzymes do from a model point of view, now we will talk from an energy point of view….

So, what enzymes do in the aspect of energy???
They decrease the ***activation energy*** (the energy that is needed to reach the transition state, it converts the stable reactants into an unstable structure).
Transition state is the intermediate state between the reactants and products, which is a very unstable structure with a high level of energy.

In some reactions, we can go into many intermediates, we can't call all of them transition states, **we call one of them a transition state**, and others are called intermediates of these reactions.

From this transition state point, either **this state will come back as reactants** or **it will become products.**

So enzymes decrease the transition state like when you climb a mountain, but you can make the situation easier when you follow the straight street which is in a lower level.

So why do reactions happen? *to get a more stable product.*
What do enzymes do? *decrease the energy of the transition state.*

Now how do the reactions happen in the solutions??

There is a theory that explains the reaction which is called the *"Random Collision Theory"*, this theory tell us that molecules will keep randomly moving in the solution till they meet each other, and when they meet each other they collide, and this collision will produce energy, if that energy is enough to pass by that barrier -which we call it the activation energy-, then the reaction will proceed.

"Sometimes the collision is not successful, it doesn't produce an enough amount of energy to pass by the transition state, so reaction will not be going on and this is why reactions don't occur by themselves, regardless they are spontaneous or not"*; and this is the "silly" question that will be asked in the biochemistry:*

Do spontaneous reactions occur spontaneously? very important !!!

We say spontaneous according to the sign of ΔG which has nothing to do in between the start and end point, **so fast or slow reaction has nothing to do with spontaneous reaction.**

Spontaneous reactions won't occure spontaneously, because at the beginning we need a push up of energy (activation energy) to reach the transition state or to convert the stable reactants into unstable transition state.

What is the wisdom that lies behind the need of activation energy, so no reaction will take place unless we provide an activation energy?

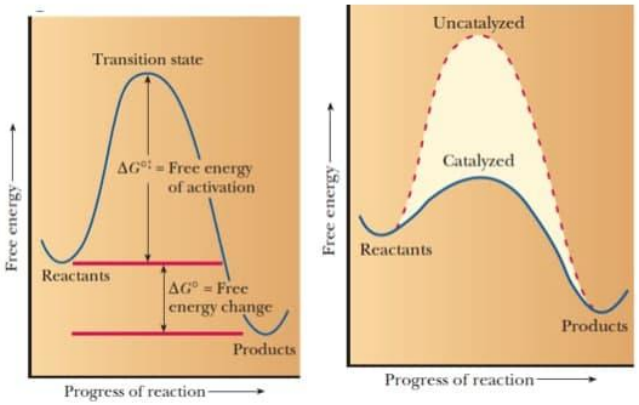
Because if the spontaneous reactions happen spontaneously without providing any activation energy, reactions will happen all the time and we will be in the product state all the time, then we will end with no reactions because we will always have products, the reactants finished a long time ago.

So the smart method to control the occurrence of spontaneous and nonspontaneous reactions is enzymes, if there are materials (reactants) but with no enzymes, the reaction will not be turned on, because there is no reaction to occur spontaneously, if materials are there and enzymes also are there, the reaction will move.

Your body will secrete a certain enzyme in a certain moment for a certain action and then it will stop.

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

The activation energy has a sign of two positive signs on top of each other ΔG^{0}++, like in the photo above.

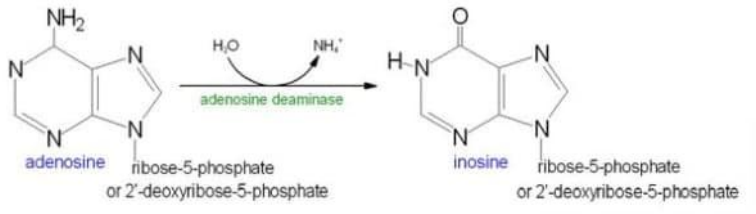
When you look at uncatalyzed reactions compared to catalyzed reactions, how can you <u>differentiate the two plots from each other</u>??

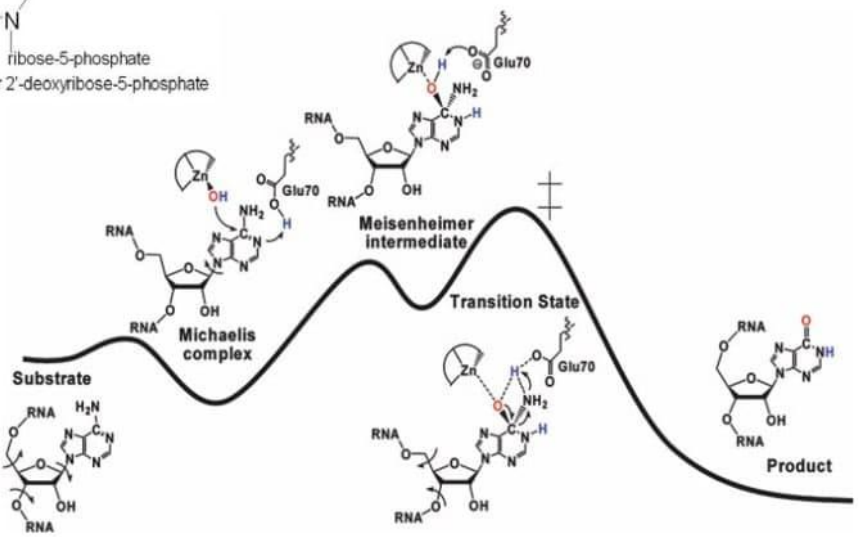
The activation energy would be lower than the catalyzed reaction and this is what enzymes do**.**

Some reactions have many intermediates, which one is the transition state and which one should carry the sign of activation energy? Not all of them are in the transition state, only one activation energy and one transition state we will have.

The answer is *"the one with the highest potential energy"*, we can calculate it according to the difference between the reactants and the highest scale that you have achieved for these intermediates then you will calculate the ΔG^{0}++ which is corresponded to the activation energy.

Example: Adenosine Deaminase





Adenosine Deaminase is an enzyme which converts adenosine into inosine (notice the diagram above), it takes out the amine group from this structure and whenever the amine group is removed, it will be replaced by an oxygen, actually it is replaced by double bond oxygen.

Adenosine is the nitrogenous base which is present in the RNA (doctor said RNA, he didn't mention the DNA, but we all know this fact), so how can we cleave it off? by replace the amine group by a double bond oxygen and this reaction follows a pathway that contains many intermediates (3 intermediates, according to the photo, the last one is the transition state because it has the highest potential energy) and then it will come to the products , this reaction is favorable (the products have less energy than the reactants).

Now we will talk from a mechanism point of view:

How do enzymes work? How do they decrease the activation energy?

let's begin with this story:

If you want to meet your friends at JU for the first time, without any previous knowledge and no specific place is defined to meet in, you may not meet them, if you define the school of medicine it is much easier, but still you may not meet them, you can define a specific room and specific chair and then you will meet them.

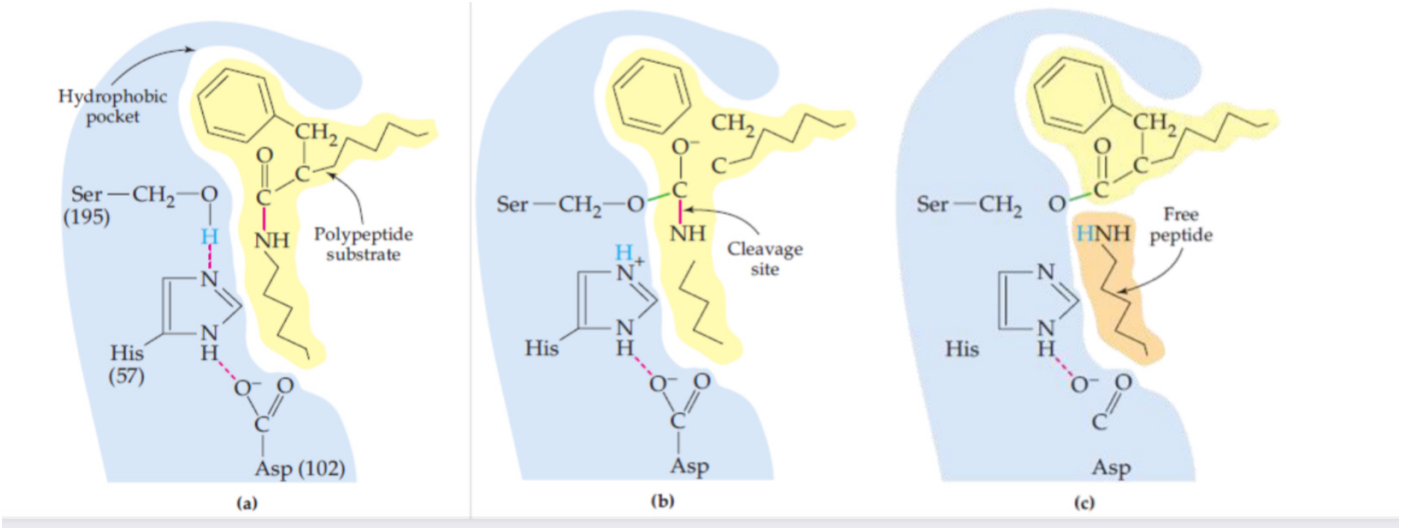
Same thing according to the enzymes "they have a proximity effect" to bring the substrates close to each other.

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.
Again!
How do reactions occur?
According to the random collisions theory, the molecules are randomly moving in the solution and then they meet each other to produce a successful collision, what do enzymes do? They have a very high affinity to a certain chemical structure, this chemical structure doesn't need to keep moving in the solution randomly because of the high affinity ,they will come in bind with each other, and they will start reaction over there, this is **the proximity effect**, after the initial binding, there will be **an orientation effect**, which means that the active site will change its shape to better fit of the substrate, where the substrate will rotates inside the active site where the hydrophobic amino acids will attach themselves in a hydrophobic pockets, nonpolar with nonpolar, polar with polar, negatively charged with positively charged to have a maximum closeness of the substrate to the active site, nearest place for the action to occur.
After they become very close to each other due to the proximity and orientation effect, all enzymes have the proximity and orientation effect in their active sites, and they make the substrate very close to the side chains of the amino acids which are in the active site, accordingly, this is the nearest-attack conformation of the active site (NAC), so the active site will change its shape and it will has the NAC, NAC is always a precursor for the transition state, because when two materials come very close to each other, they will bind with each other and this will introduce a reaction that will reach to the transition state.

Energy effect: Lower the energy barrier by inducing strain in bonds in the substrate molecule.
Let's try to explain the definition:
-**After** the substrate is in the best orientation **binding** will start **from outside (**the amino acids that exist on the active site would start binding with the substrate) and this will **weaken** the bonds inside the substrate itself.
-When the enzyme starts to make bonds with the substrate => it means that the energy (the solidarity between the atoms in the substrate) will change , and they will be breaking down the bonds and forming them so that what we call **The energy effect.**
-Catalytic effect: Provide acidic, basic, or other types of groups required for catalysis
-Proximity,orientation and changing in energy will end up **by** producing materials and this **catalysis** and we call it **catalytic effect.**
-**Catalysis**: is the end product of the reaction.

Let’s see this example:

-We have the active site (the blue region) of the **chymotrypsin**: the enzyme which breaks down the proteins (the peptide bond between the amino acids) inside the intestine , it is secreted in the duodenum.
-It will break down the protein at a certain point after **aromatic amino acids** **(**phenylalanine->tyrosine->tryptophan**)**



- **Let’s explain more about it:**

Initial binding -> the side chain of phenylalanine (benzene ring) which is nonpolar **fits** itself inside the **hydrophobic pocket** -> we get the best orientation-> it becomes very close to the catalytic amino acids (that are **triad** here :1-serine 2-histidine 3-aspartic acid)

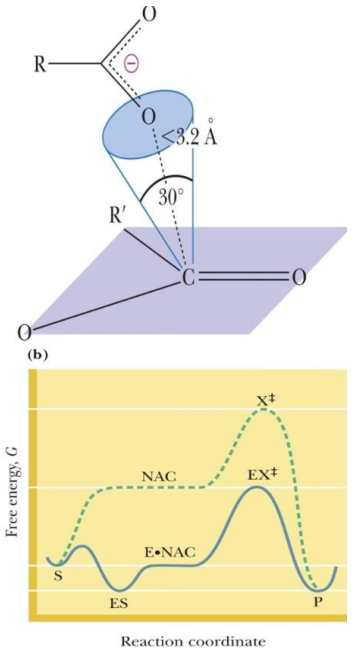
- **How will the reaction happen?**

The aspartic acid (102) attacks the **histidine**(57) and takes out the hydrogen atom from it
-At the other side of the **histidine** it takes out a proton from the **serine** so the **serine** will **attack** the carbon that is in the substrate and **bound** to it , so the carbon now has 5 bonds (unstable condition which means that it won’t work like this) so the **weakest bond** which is with the nitrogen (the amide bond) will get broken.
At the end: everything will go back as it was.

☐ **There are some strategies that can be applied on all the enzymes and some of them are applied on certain enzymes** such as:

1. **Catalysis by proximity and orientation** (applied on all the enzymes):

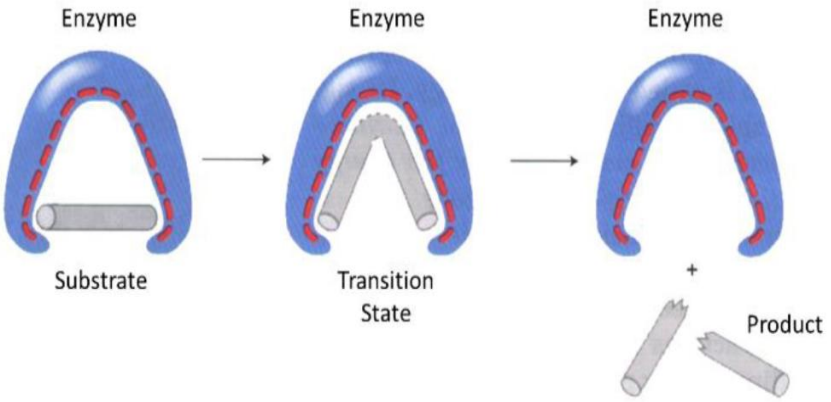
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis
  - Such arrangements have been termed near-attack conformations (NACs)
  - NACs are precursors to reaction transition states



2.Catalysis by bond strain:(adopted by some of the enzymes)

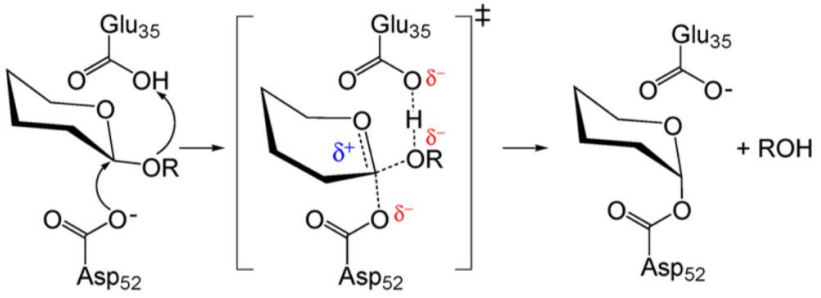
In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.

We have the conformation of the substrate as the picture below 👇 so the active site changes its shape & **causes strain** on the bond and **affects** the angle of the bond so it will be **broken.**



Example of this strategy: Lysozyme

It breaks down **polysaccharides** (are consist of monosaccharides) by causing strain on the bond between each monosaccharide, converting them from the chair conformation (the left photo)——> to the sofa conformation (where it can be broken easier & is similar in shape to the transition state) so we will get separated monosaccharides.

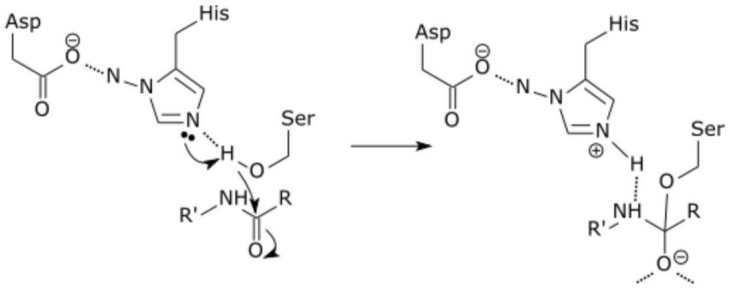


3.Ionic mechanism:(the acid-base catalysis)

As the name implies , we must have proton **donor & acceptor**

It is adopted by serine proteases as we talked about the chymotrypsin:

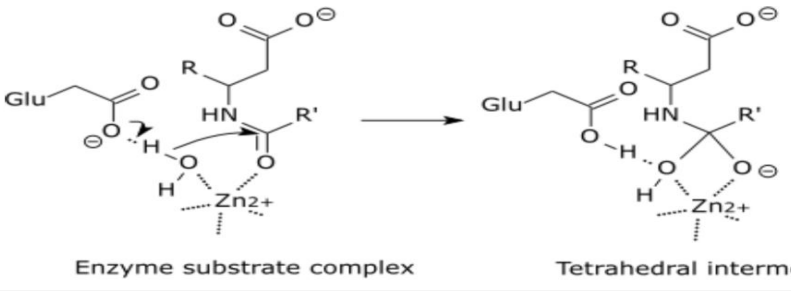
Aspartic acid (negatively charged) affects-> **serine**



4. Covalent catalysis:

As we said in the last lecture: **initially** the bond between the active site and the enzyme is **weak ,** but **during the reaction** there might be formation of covalent bond

-Examples of this mechanism is proteolysis by serine proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase).



- ☐ **Each enzyme might contain more than one type of catalytic** and it depends on types of amino acids which are present in the side chain inside the active site

Naming 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 numbering)**

-Most scientific method

-Each enzyme has a certain number that composed of **4 digits**

-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

First digit refers to Major class (1-7),**2nd digit:**Minor class, **3rd:**further subclassification, **4th :**the enzyme itself

Note: you are required to memorize the major classes of enzymes **in order.**

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

☐ **Enzymes classifications:**

(According to the structure):

-Simple vs. complex (conjugated)

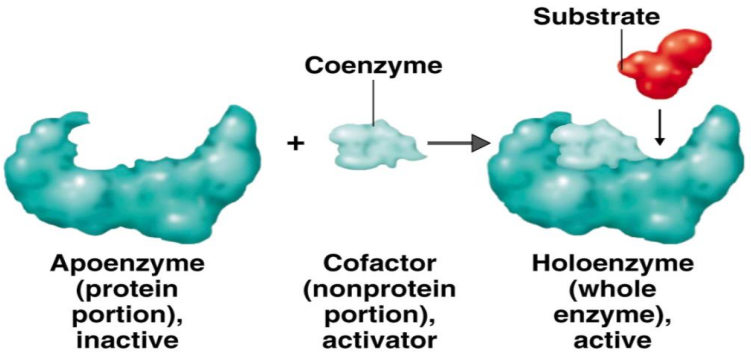
Simple:do their reaction depending only on the amino acid sequence

Complex: need another **non-proteinaceous** material to bind to them and make them active which we call this type of enzymes **coenzymes.**

-Holoenzyme vs. apoenzyme

Holoenzyme:if the coenzyme bound to the enzyme

Apoenzyme : no coenzyme bound to the enzyme



(According to the function):

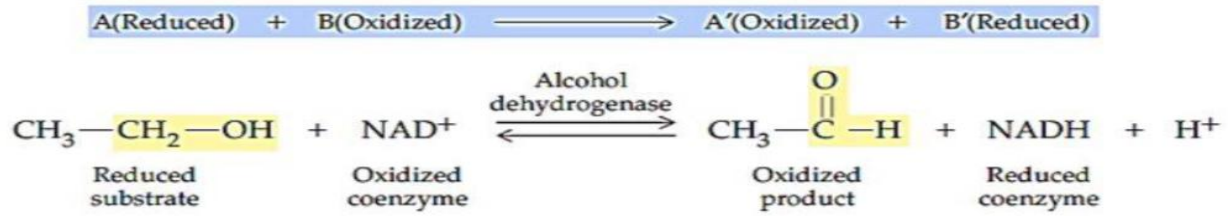
Classified into 7 major classes:

1. **Oxidoreductases:** catalyze the oxidation and reduction reactions, addition or removal of O, O2, H & Require coenzymes(heme).

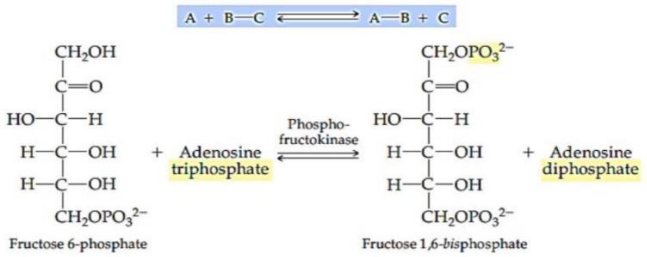
   Reduction: gain of electrons.

   Oxidation: loss of electrons.

They occur simultaneously, any oxidation should be accompanied by reduction, so oxidoreductase reactions should have at least 2 reactants and 2 products.

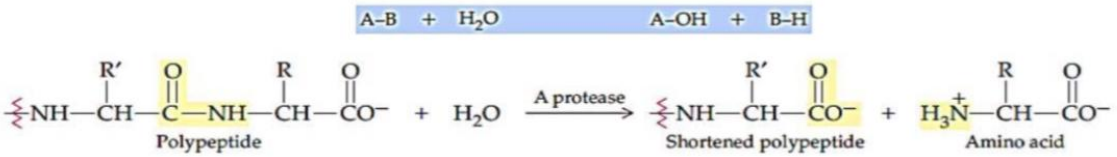


2. **Transferases**: catalyze the transfer of a functional group from molecule to another, so there should be at least 2 reactants and 2 products in the reaction.



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

Catalyze it to break down the substrate into different entities by adding water.

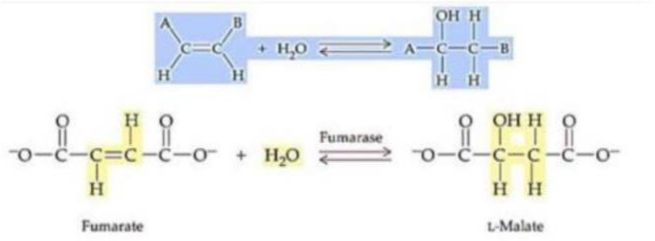


4.Lyases: addition of a molecule (H2O, CO2, NH3 to a double bond or reverse(non-hydrolytic).

-Catalyze the addition or removal of functional groups, but the reaction is coupled to formation or breakdown of a double bond.

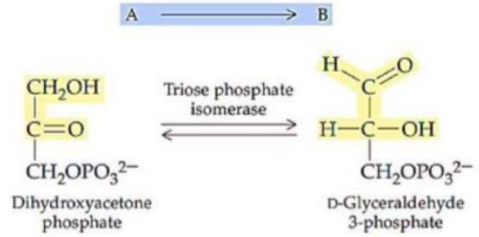
Lyases : breakdown of the double bond.

Hydrolases: break down the whole molecule.



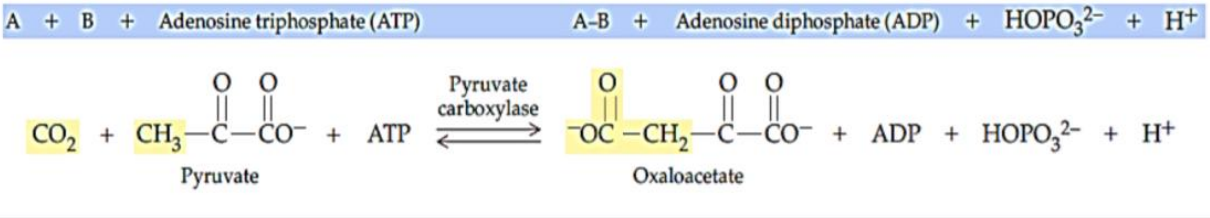
5.Isomerases: catalyze the conversion of one substrate into another.

-One substrate and one product.



6.Ligases: usually not favorable, so they require a simultaneous hydrolysis reaction.

-Catalyze building up materials (which requires energy)



7.Translocases: Catalyze the movement of ions or molecules across membranes or their separation within membranes (ATP/ADP translocase) like the inner membrane of the mitochondria, (catalyze the translocation of materials from one environment to another).

Let's move to the minor classification:

- ☐ **Oxidoreductases**

-These enzymes catalyze oxidation & reduction reactions involving the transfer of hydrogen atoms, electrons or oxygen

-This group can be further divided into **4 main classes**:

1) Dehydrogenases 2) Oxidases 3)Peroxidases 4)Oxygenases

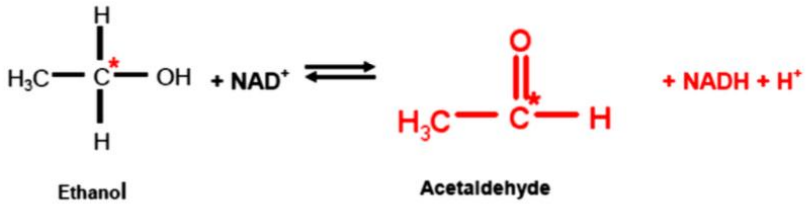
1)Dehydrogenases:

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

-Lactate dehydrogenase

Lactate + NAD+ Pyruvate + NADH + H+

-Alcohol dehydrogenase



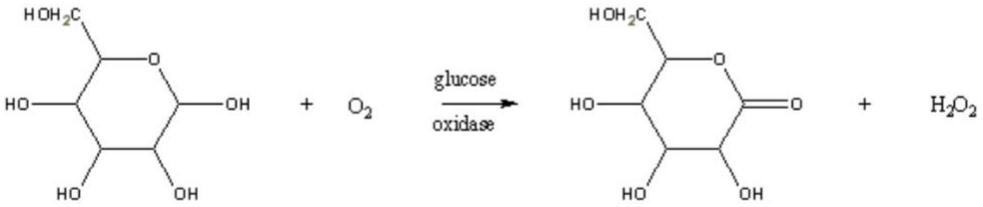
2)Oxidases:

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

-they oxidize molecules using **oxygen**

-Glucose oxidase

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

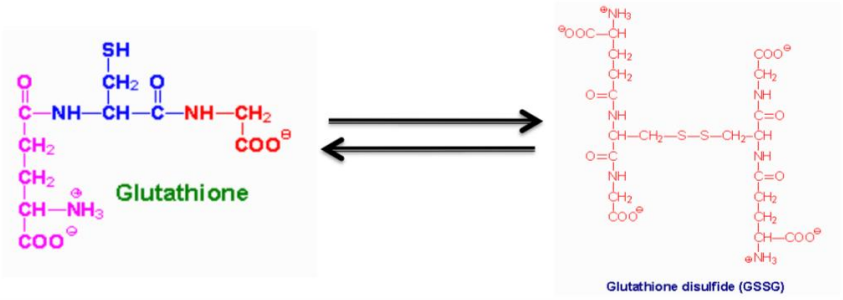


3)Peroxidases:

-catalyze oxidation of a substrate by hydrogen peroxide

Example: Oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide

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



4)Oxygenases:

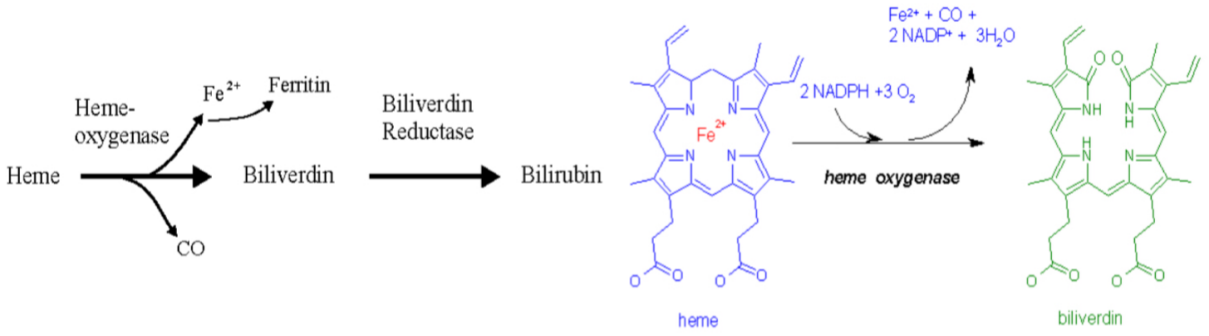
-Catalyze substrate oxidation by molecular O2.

-The reduced product of the reaction in this case is water and not H2O2

-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



-The rest will be continued in the next sheet.

BEST WISHES