بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

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

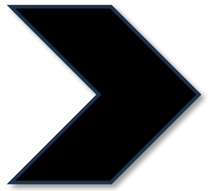
Final | Lecture 15

Enzymes pt.5 (Regulation pt.2)

Written by : Ali Alshareah
Yaman Feras

Reviewed by : Yamen Aljarrah

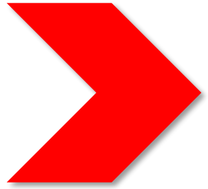
Color coding used in the modified:



Black: the original slides

Blue: the doctor’s explanation/words

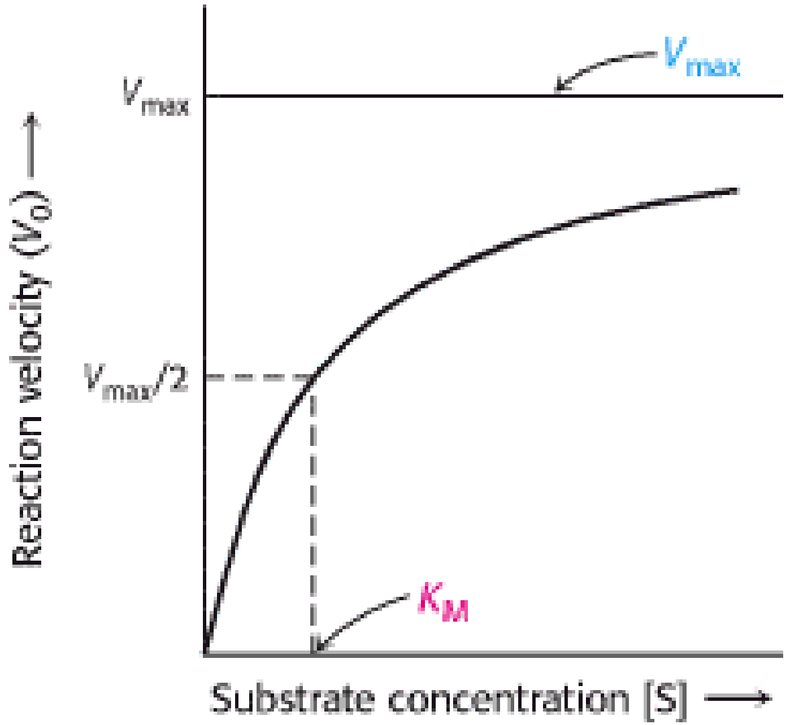
Gray: additional information and explanation



Red: important information

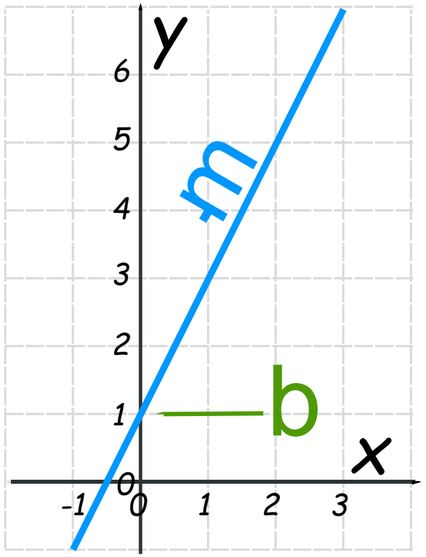
A disadvantage of the Michaelis-Menten equation

- Determination of K_M from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach Vmax.
- This prevents the calculation of both Vmax and K_M.



$y = mx + b$	m is the slope b is the y-intercept

Solution



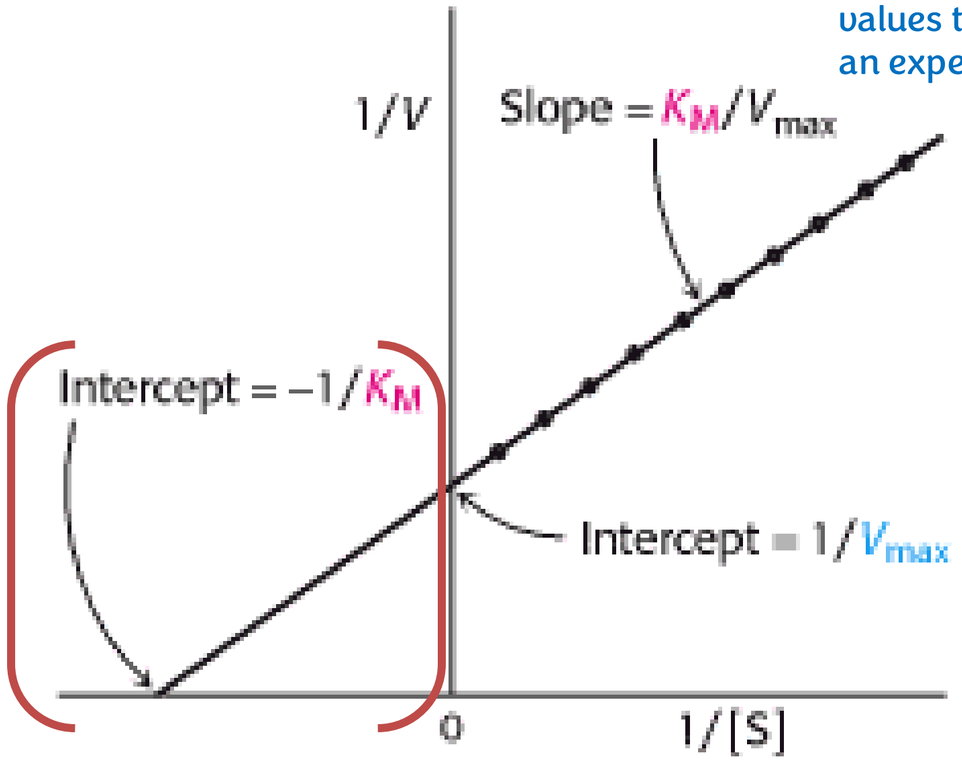
The Lineweaver-Burk or double-reciprocal plot

- A plot of 1/ V_o versus 1/[S], called a Lineweaver-Burk or double-reciprocal plot, **yields a straight line with an intercept** of 1/Vmax and a slope of K_M /Vmax.
- The intercept on the x-axis is -1/ K_M.

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

Constructed from values taken from an experiment

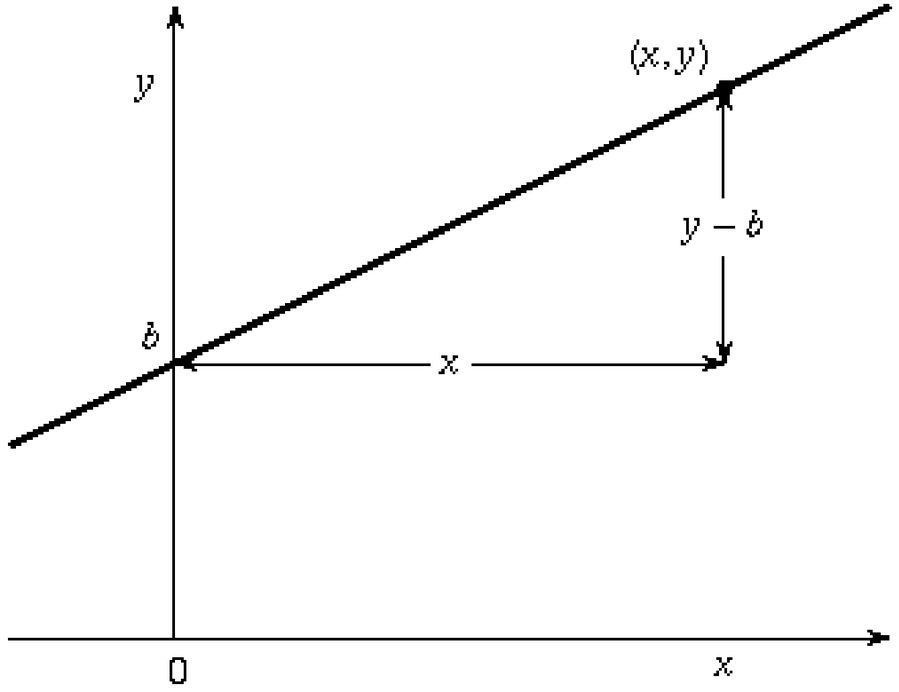


This part is hypothetical

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

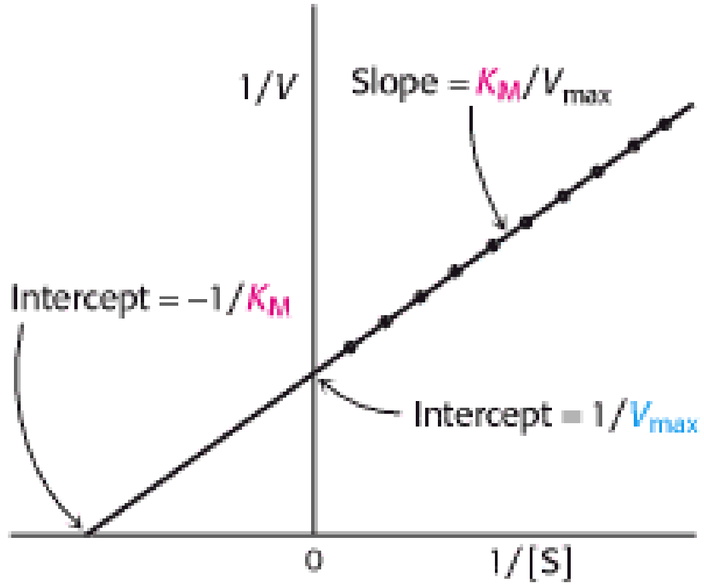
- y is y-axis (0) = 1/ V_o
- x is x-axis (0) = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

- If x = 0, then y = b (x-axis is 0, then y-intercept = 1/Vmax)



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

If y = 0, then mx = -b (y-axis is 0, then x-intercept = $-1/K_M$)

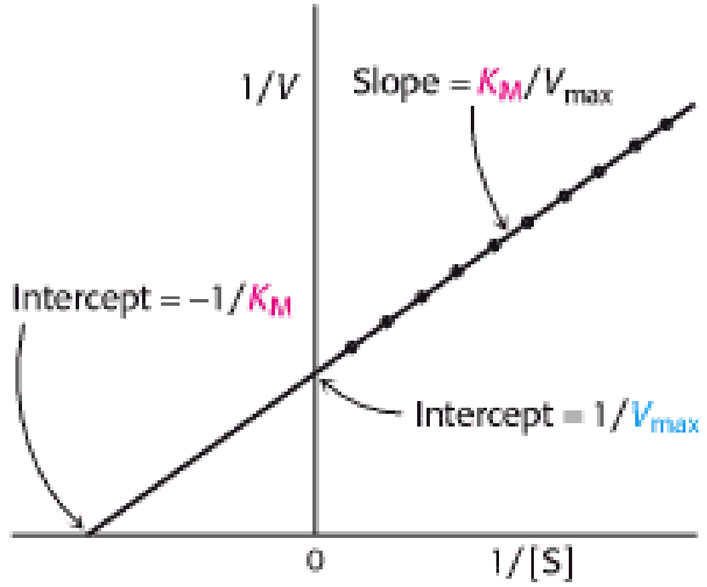
How?

0 = 1/Vmax + (K_M/Vax) . (1/[S])

-1/Vmax = (K_M/Vax) . (1/[S])

-1 = K_M . (1/[S])

-1/ K_M= 1/[S]



Now, we can talk about regulation of enzyme activity and we will start with inhibitors.

Enzyme inhibitors: small molecule or compounds that binds to enzymes and inhibit them .

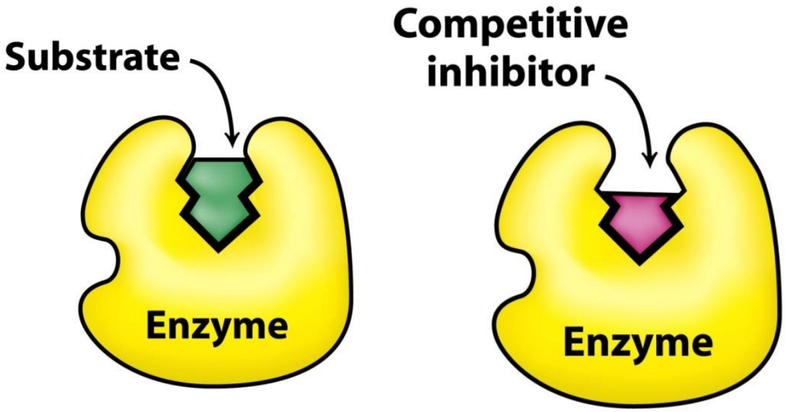
- Enzyme inhibition can be either reversible or irreversible.
  - **Reversible** inhibitors rapidly dissociate from enzymes (e.g. **non-covalent binding**).
    - **Competitive, noncompetitive, or** uncompetitive inhibition.
  - An **irreversible** inhibitor is tightly bound (e.g. covalently) to the enzyme.
    - Lower concentration of active enzyme.

Irreversible inhibitiors usually form covalent bonds with the enzyme , but they can bond with very strong non-covalent interactions aswell.

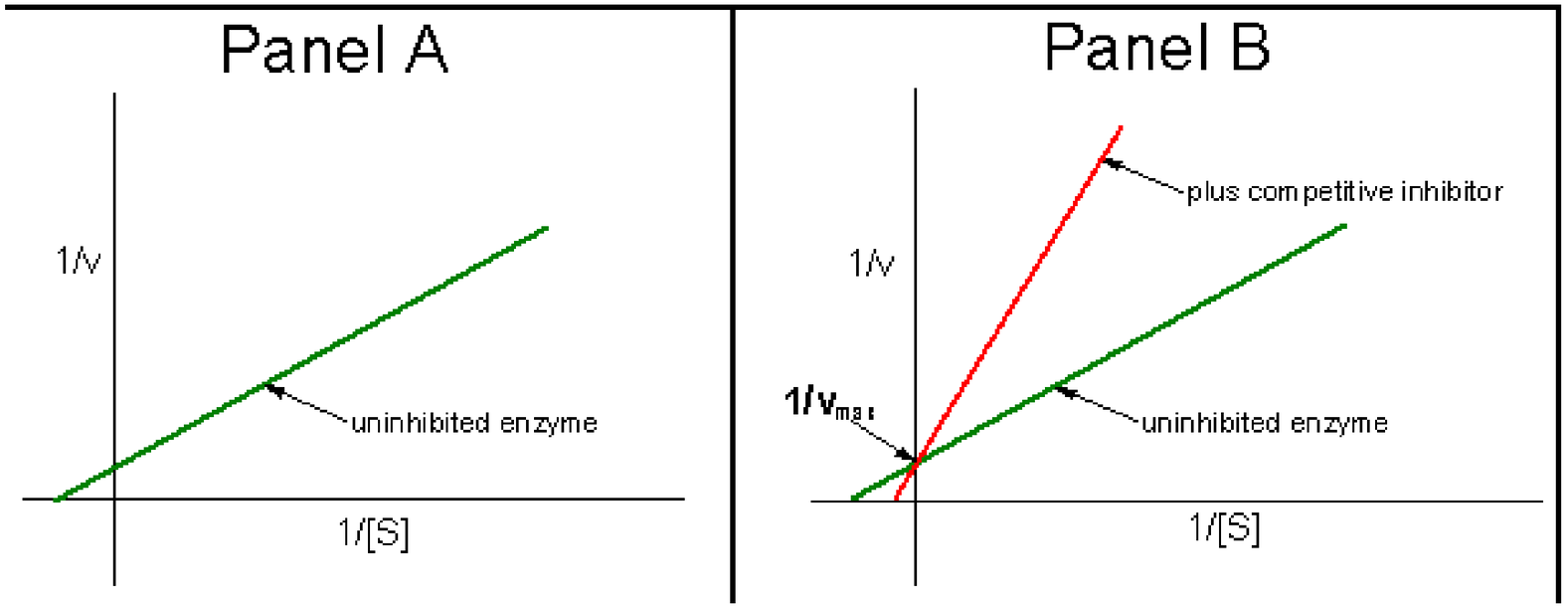
All inhibitors that are found in our bodies are reversible inhibitors, while irreversible inhibitors are usually not physiological (found in drugs and in nature but not originally in our bodies).

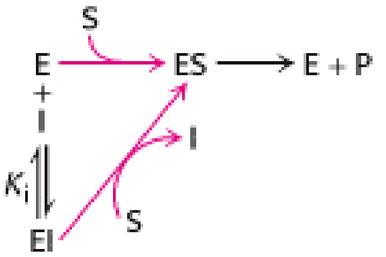
Competitive inhibition

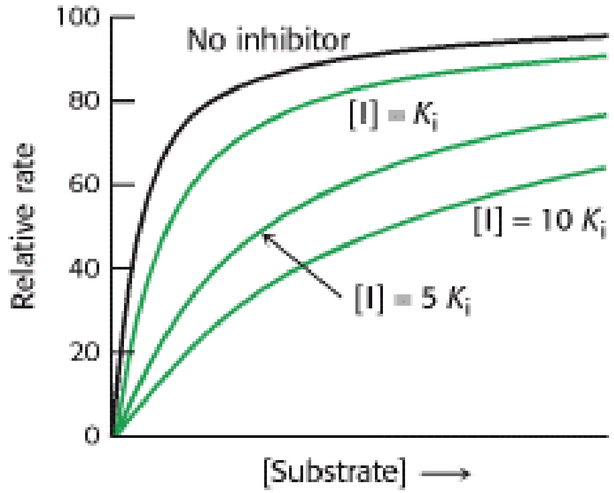
- Competitive inhibitors compete with the substrate for the active site.
  - Increasing substrate can overcome inhibition.
- Same Vmax, but higher K_M



We get the same Vmax in competitive inhibition because of the competition between the inhibiter and substrate, increasing the substrate amount will eventually lead to reaching Vmax, but Km will be affected (increases), affinity decreases.

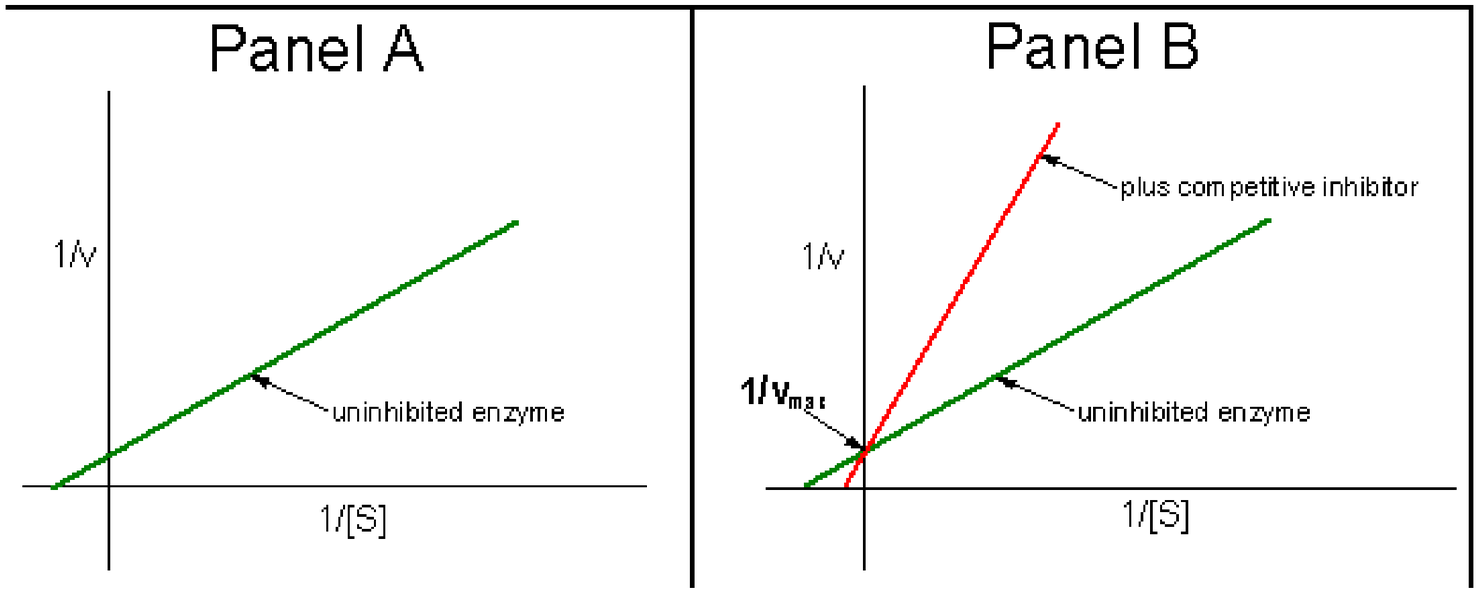


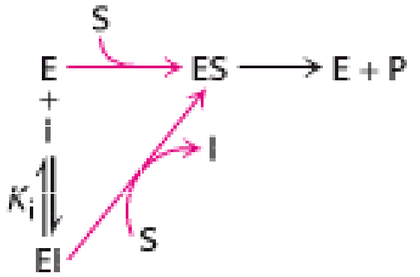


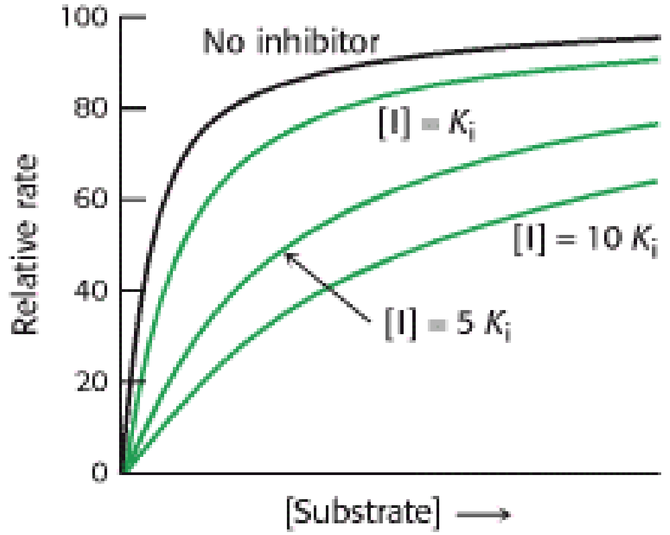


Competitive inhibition

The graphs are very important to study for the exam.



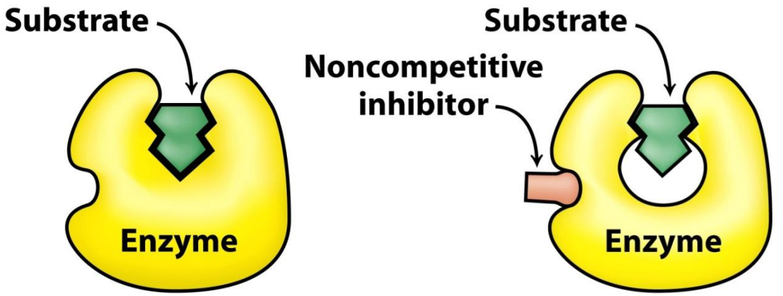




As shown in the Lineweaver-Burk plot , the y-intercept (1/Vmax) is the same if we have competitive inhibitors or not, while the x-intercept (-1/Km) is bigger when we add competitive inhibitors.

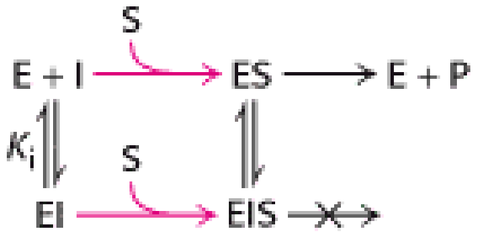
As you can see in this mechaelis-menten graph, Vmax stays the same whether you add competitive inhibitors or not, but Km for the substrate increases the more you add competitive inhibitors (you will need a higher concentration of substrates in order to reach Vmax).

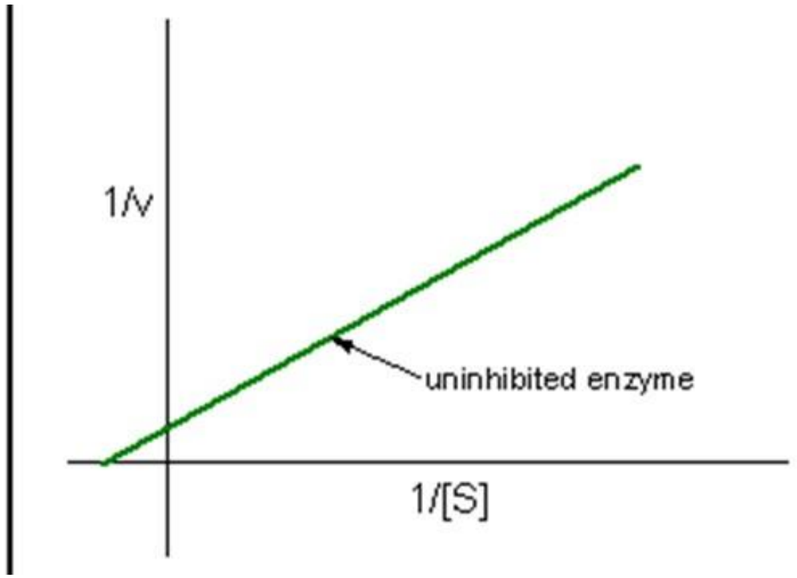
Noncompetitive inhibition

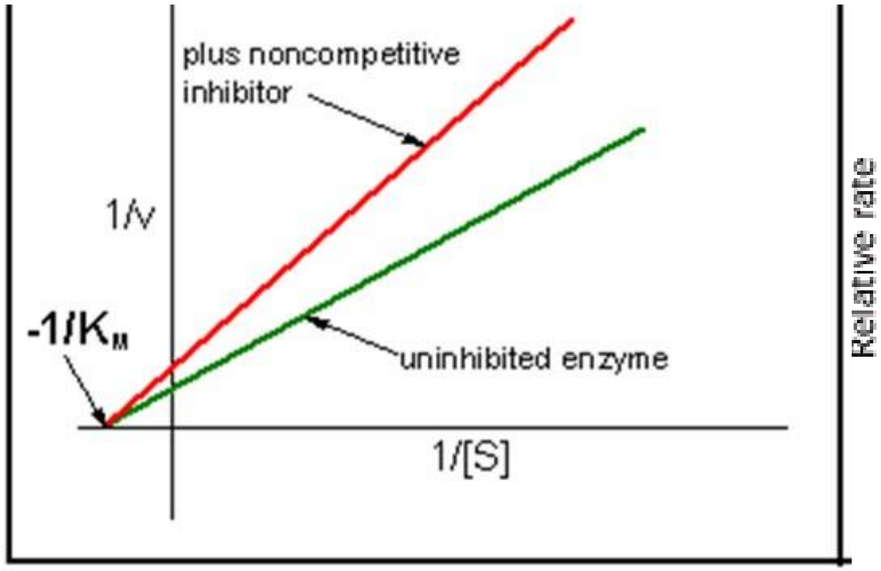


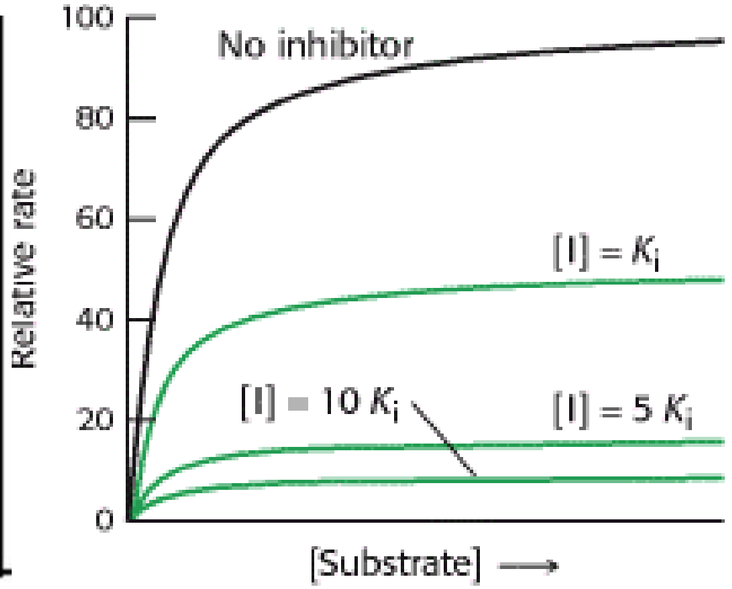
- Noncompetitive inhibitors bind E or ES complex at a site **other than the catalytic site (active site)**. (no competition with the substrate)
- Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product.
- Lower Vmax, but same K_M

Km won't change because the binding between the substrate and the enzyme won't be affected, but producing products and enzyme activity will be less (Vmax will be less).

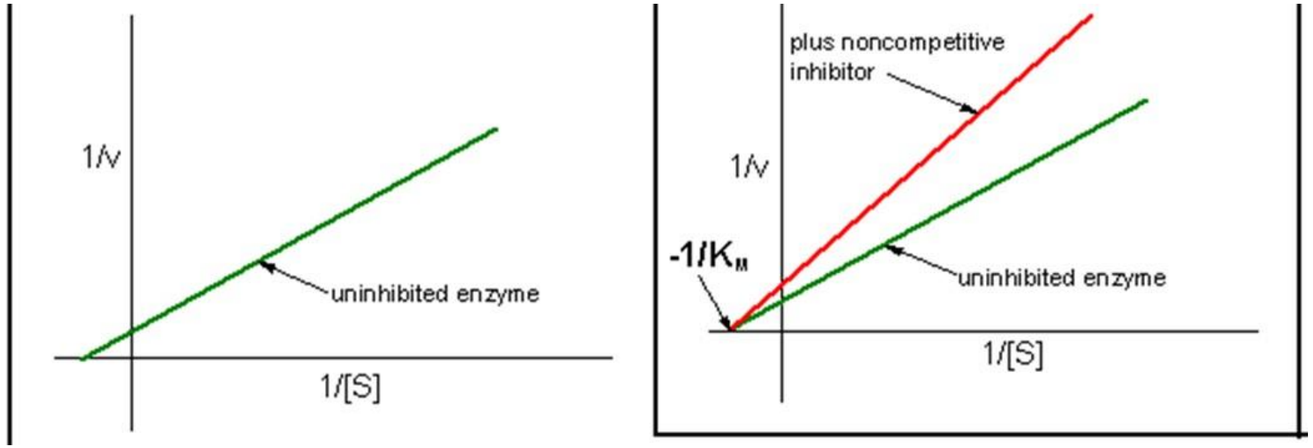




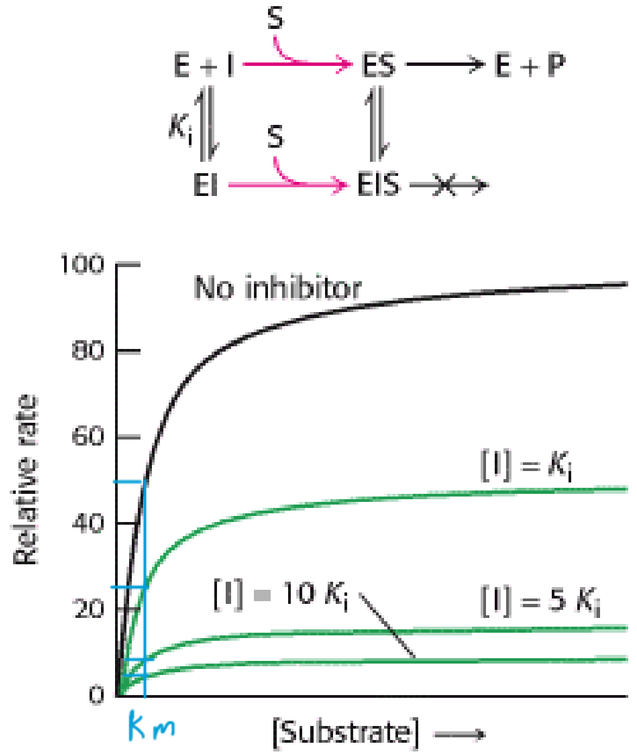




Noncompetitive inhibition



In this plot, we can see that adding a noncompeptitive inhibitor doesn't change the x-intercept (-1/Km), while adding this inhibitor makes the Y-intercept bigger (1/Vmax) "which means smaller Vmax for non competitive inhibitor"



As shown in this graph, Vmax decreases the more we add non competitive inhibitors, but Km stays the same (the amount of substatre needed to reach 0.5Vmax doesn't change!)

The prof asked in the lecture:
"What would happen if a non competitive inhibitor binds to an enzyme and changes the structure of the active site so the substrate binds less to the enzyme?"
Answer : Vmax will become lower, and Km will become higher.

The prof also mentioned that he might bring 2 random graphs in the test and ask us what changed when adding the inhibitor.

Mechanism-based inhibitors

Irreversible inhibitors

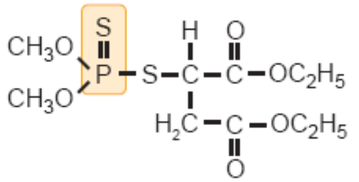
Non-physiological inhibitors

They affect the mechanism which the enzyme works by

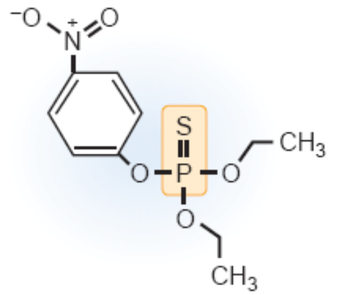
These inhibitors do not exist in our body "remember all the inhibitors that our body makes are reversible inhibitors"

- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction.
- They include:
  - Covalent inhibitors
  - Transition state analogs (they look like the transition state)
  - Heavy metals
- Irreversible inhibitors decrease the concentration of active enzyme.

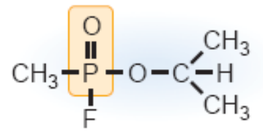
Covalent inhibitors



Malathion



Parathion

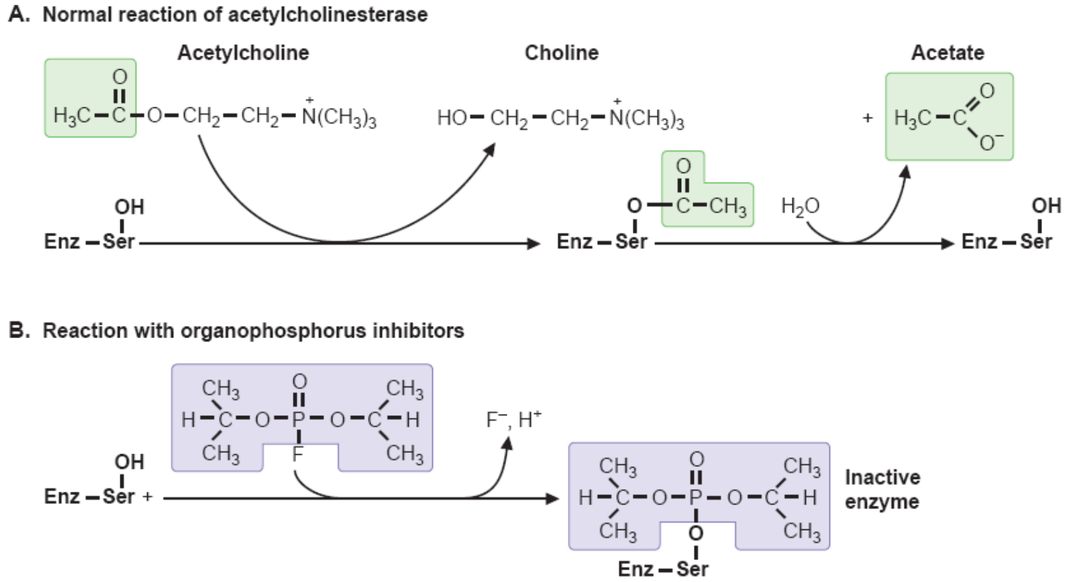


Sarin

- They form covalent or extremely tight bonds with active site amino acids (active site of the enzyme).
  - Example: di-isopropyl fluorophosphate (DFP) is an organophosphate
    - The nerve gas sarin
    - The insecticides مبيدات حشرية malathion & parathion.
    - DFP inhibits acetylcholinesterase preventing the degradation of the neurotransmitter acetylcholine.

DFP also inhibits other enzymes that use serine (ex. serine proteases), but not lethal.

When the substrate wants to bind to the enzyme, it will find that the active site is occupied so it will not be able to bind there, even if we change the substrate concentration nothing will be affected because the enzyme is already dead (irreversible inhibitors they will never be released. They can't be separated from the enzyme).



DFP يستخدم مبيد من أجل طرد الحشرات لكنه مثبط للانزيمات في اجسامنا أيضا فهو سام

The nerve gas sarin can be used to kill people (like what happens in Syria before)

DFP has the same effect as the nerve gas, they both cause inhibition for the enzyme acetylcholinesterase.
(acetylcholine is one of the neurotransmitters in our central nervous system, it stimulates different synapses, so our body regulates and synthesizes the enzyme acetylcholinesterase which breaks acetylcholine so the signal will be terminated (it stops) because of the degradation of acetylcholine).

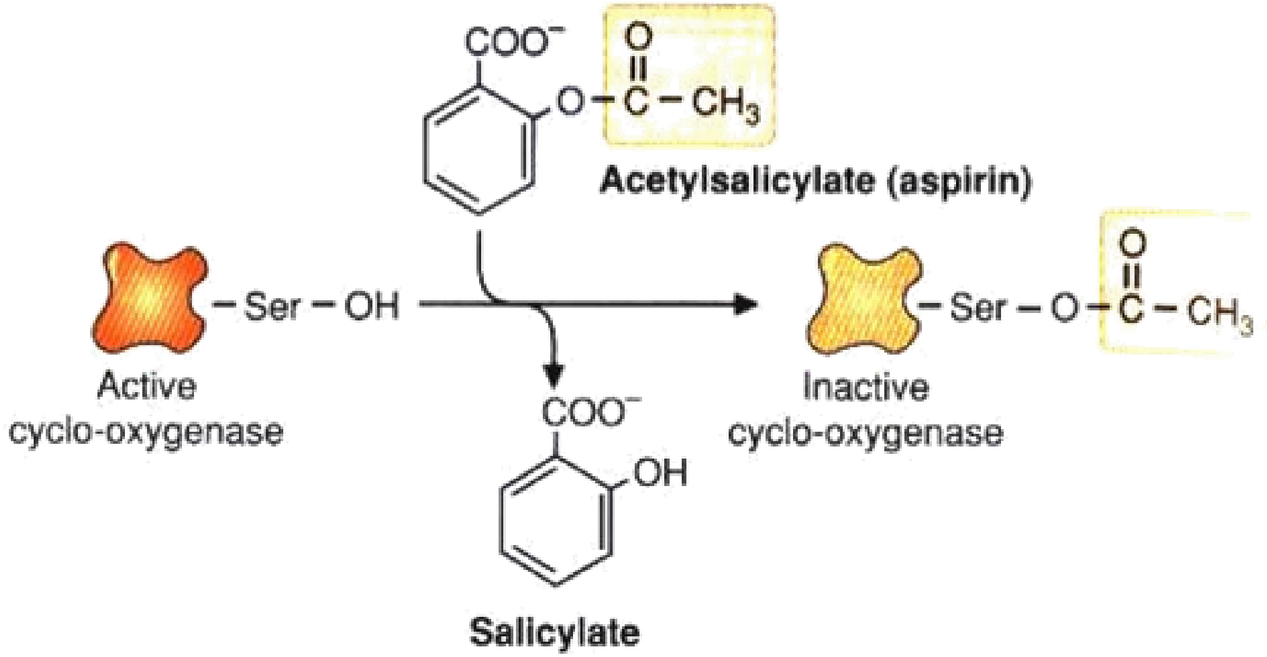
DFP causes an inhibition for the acetylcholinesterase so acetylcholine will remain in the synapse and the stimulation won't stop. A person who gets exposed for the sarin gas or DFP spasm يتشنج (his muscles spasm) because there is no relaxation. There is no termination for the signal because neurotransmitters are acting all the time because of the inhibition of the enzyme acetylcholinesterase.

DFP causes the inhibition for other enzymes in our bodies as well.

Aspirin

- Aspirin (acetylsalicylic acid) acetylates an active site serine of cyclooxygenase (enzyme)
- Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme.
- its mechanism also depends on the covalent inhibition.
- It does the inhibition by adding an acetyl group to the serine in the active site so the active site is modified and cannot function normally and does not produce (synthesize) eicosanoids.

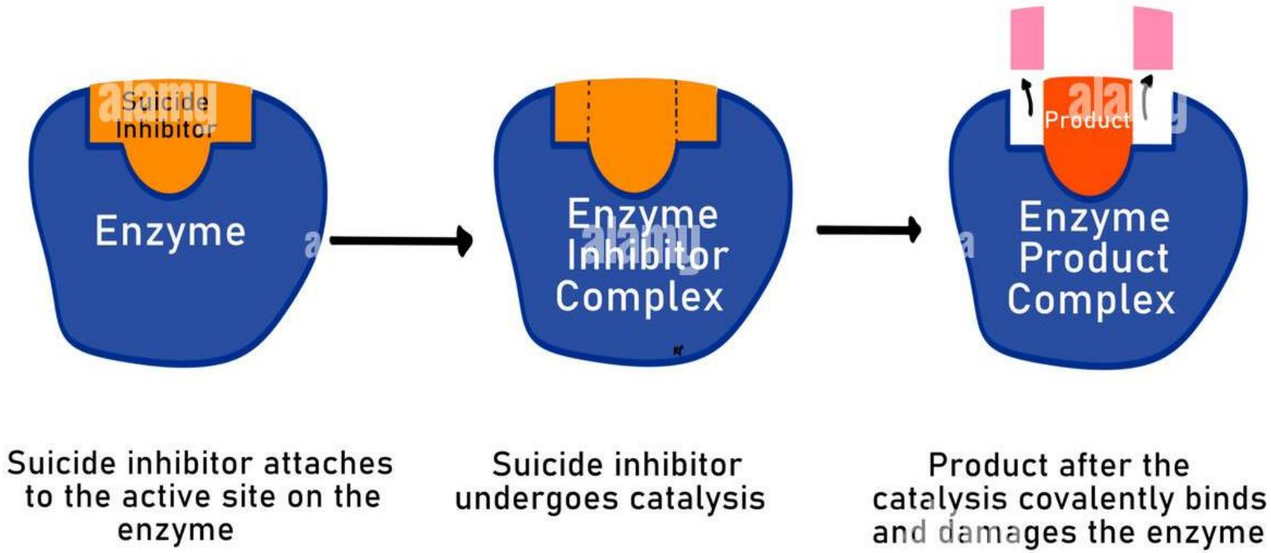
We talked about aspirin in lipids lecture when we said that the aspirin causes the inhibition of COX-1 & COX-2 so they invented another drug called celebrex (celecoxib) that inhibits COX-2 so it is an selective inhibitor.



Substrate and transition-State analog Suicide inhibitors

- They bind more tightly than substrates.
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure).

Recall that the transition state is an intermediate between the substrate and the product And it has very high energy because it's unstable, so their formation is really hard because they are unstable. They bind with very high affinity with enzymes. Some suicide inhibitors can be designed so they bind to the active site and the enzyme starts its mechanism of action , then it stops which leads to the suicide inhibitor and the enzyme being stuck and binded together.

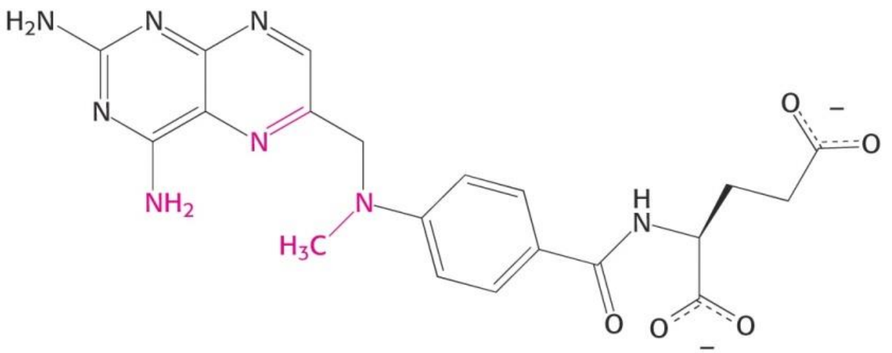


For example: The enzyme removes an OH group from the inhibitor and puts it in another place and then tries to remove a proton but it can't be found. so the reaction remains stuck " it cannot get back and cant be completed and that's why they are named suicide inhibitors because they start the reaction and cannot complete it."

Methotrexate

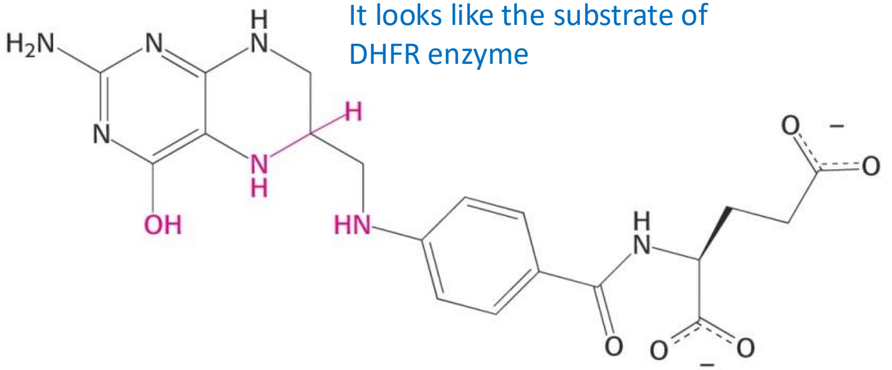
- Methotrexate is a synthetic inhibitor used to treat cancer & it is also used for the Rheumatoid arthritis (which is an inflammatory disease where the immune system attacks the joints).
- It is a structural analog of folate, a substrate for the enzyme dihydrofolate reductase, and a coenzyme for thymidylate synthase, both of which are responsible for the synthesis of nucleotides.
- It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis.

It targets that cells that proliferate, because these cells need to synthesize DNA to split into daughter cells. So it inhibits the cell division by inhibiting DNA synthesis. people who take this drug will have all the cells in their bodies that proliferate affected and that's why the side effects for the chemotherapy that is given for the cancer patients are devastating. (Chemotherapy contains Methotrexate).

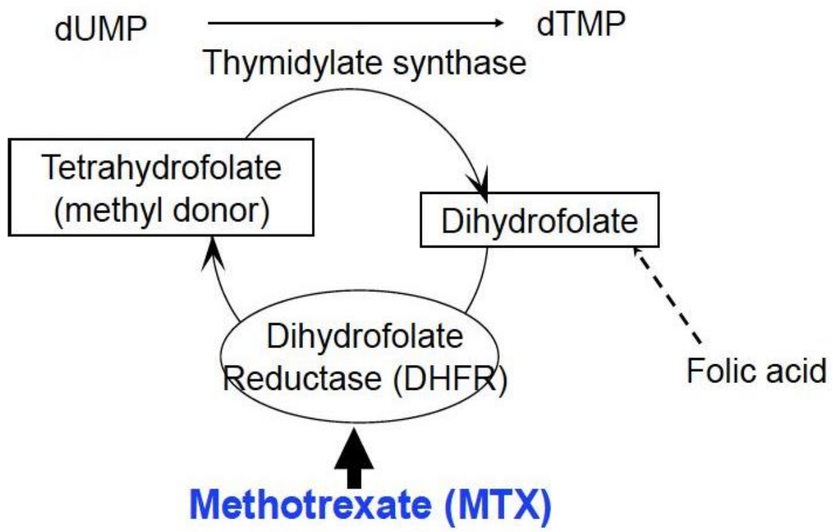


Methotrexate

It looks like the substrate of DHFR enzyme



Tetrahydrofolate



- Patients who take methotrexate cannot even eat because the lining of the intestines and the esophagus are renewed all the time but now they won't be able to because of this drug.

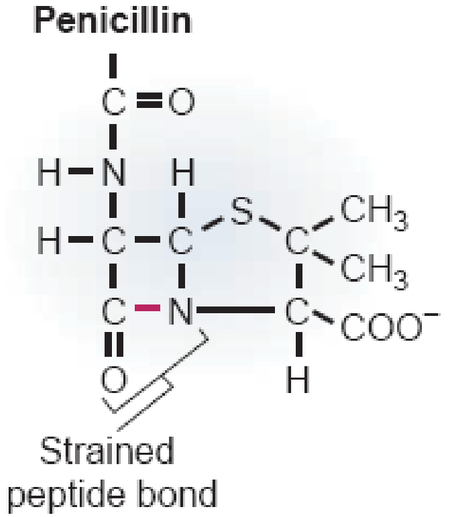
- The immune system will be weaker because immune cells will die.

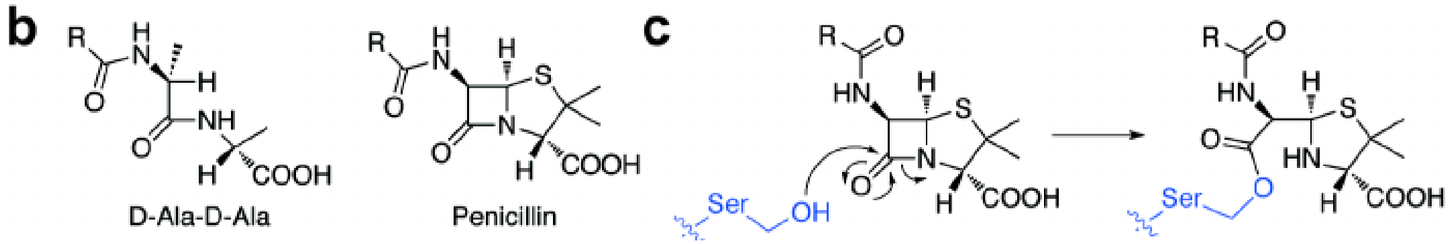
- The blood also will be weaker because the platelets will be reduced.

- Mechanism of action: folic acid (a type of vitamin) is renewed normally in cells, so methotrexate tricks the enzyme dihyrofolate reductase (DHFR) and binds with it instead of the substrate (dihydrofolate), so the intermediate (tetrahydrofolate) will not exist for the synthesis of nucleotides (the monomers of the DNA), so building blocks for DNA will be missing and DNA cannot copy itself. As a result ,DNA synthesis will stop.

Penicillin it is an antibiotic drug

- It is a **transition-state analog** to glycopeptidyl transpeptidase, (enzyme) which is required for synthesis of the bacteria cell wall. Which is made of (GAGs) and there is oligopeptides between different GAGs chains, so the cell wall will be very rigid. (Check midterm lecture 8 slide 16 for further explanation)
- The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex.
- The active site serine attacks the highly strained β -lactam ring resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme.
- The penicillin bonds to the active site so the bacteria won't be able to synthesize a cell wall. (Then our body can kill the bacteria so the bacteria will be vulnerable ضعيفة يمكن مقاومتها).

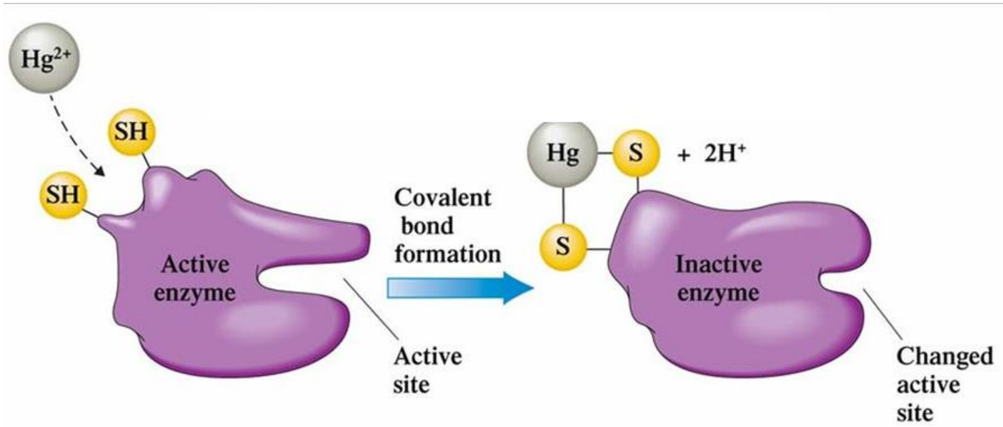




Recently , some bacteria in our bodies has developed resistance to different antibiotics, this is because the wrong and excess usage of antibiotics so the bacteria was able to adapt to it.

Heavy Metals Irreversible inhibitors

- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) result in tight binding to a functional group in an enzyme .
  - **Nonspecific inhibition** at high doses.
- Mercury binds to reactive sulfhydryl groups **away** from the active site and affect the binding of substrates.
  - Unknown enzymes in mercury toxicity.



- Lead replaces the normal functional metal in an enzyme such as calcium, iron, or zinc by an irreversible mechanism.
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca+2 in several regulatory proteins that are important in the central nervous system and other tissues.

Regulation through conformational changes

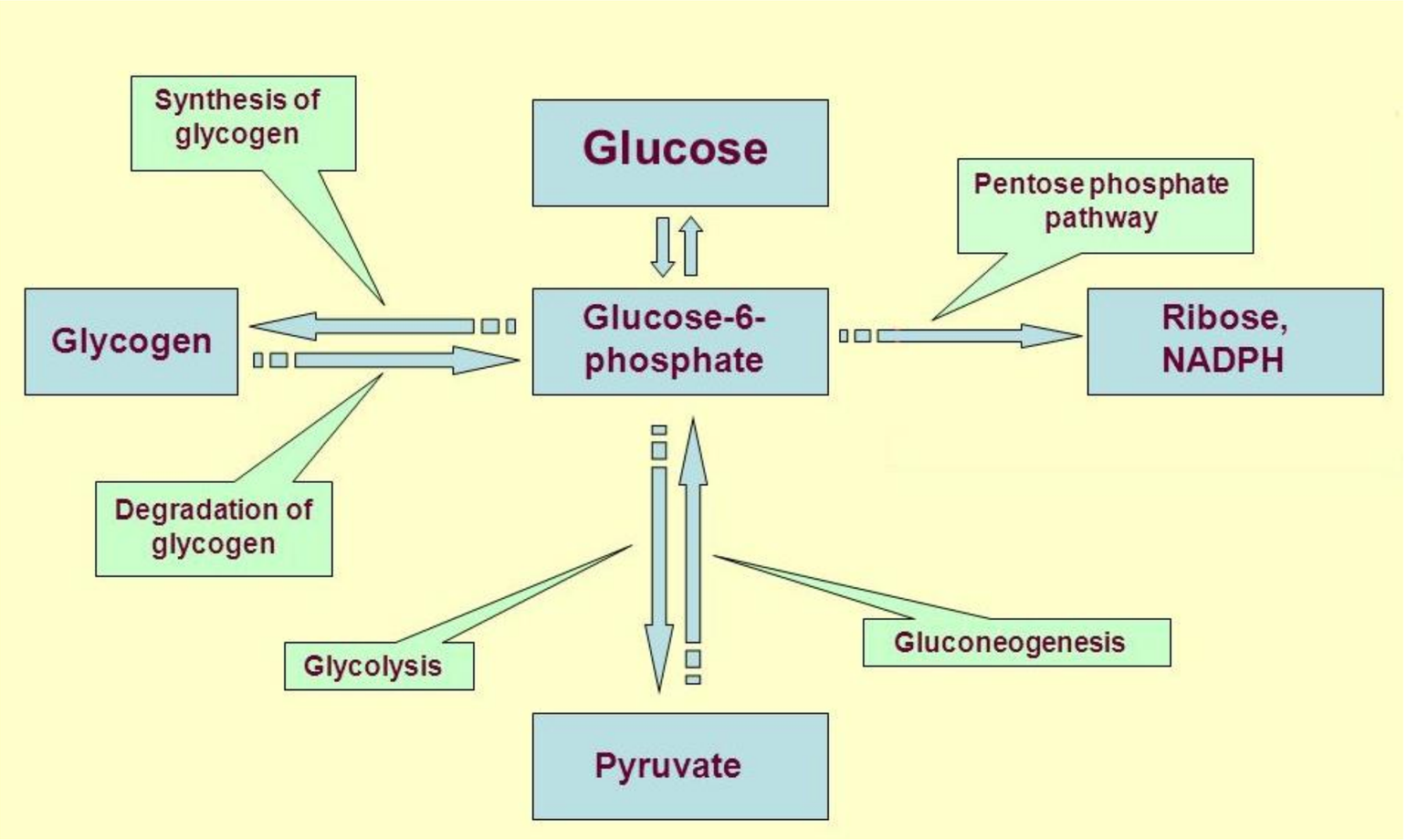
Changing “the shape” of the enzyme , thus, its activity changes.

Regulation: conformational changes

- These regulatory mechanisms include
  - Covalent modification
  - Major structural changes (regulatory & catalytic subunits)
  - Protein-protein interactions
  - Proteolytic cleavage
  - Allostery
- Rapidly change from inactive to fully active enzyme.

The different fates of glucose

You won't be asked about these different metabolic pathways in the exam, but it's very very important to understand them now.



Glucose enters the cells , and the first thing that happens to it is phosphorylation by kinases (transferases) (kinases are the enzymes that do phosphorylation , giving phosphate group to the substrate) . So glucose becomes glucose-6-phosphate ,This occurs in all cells.

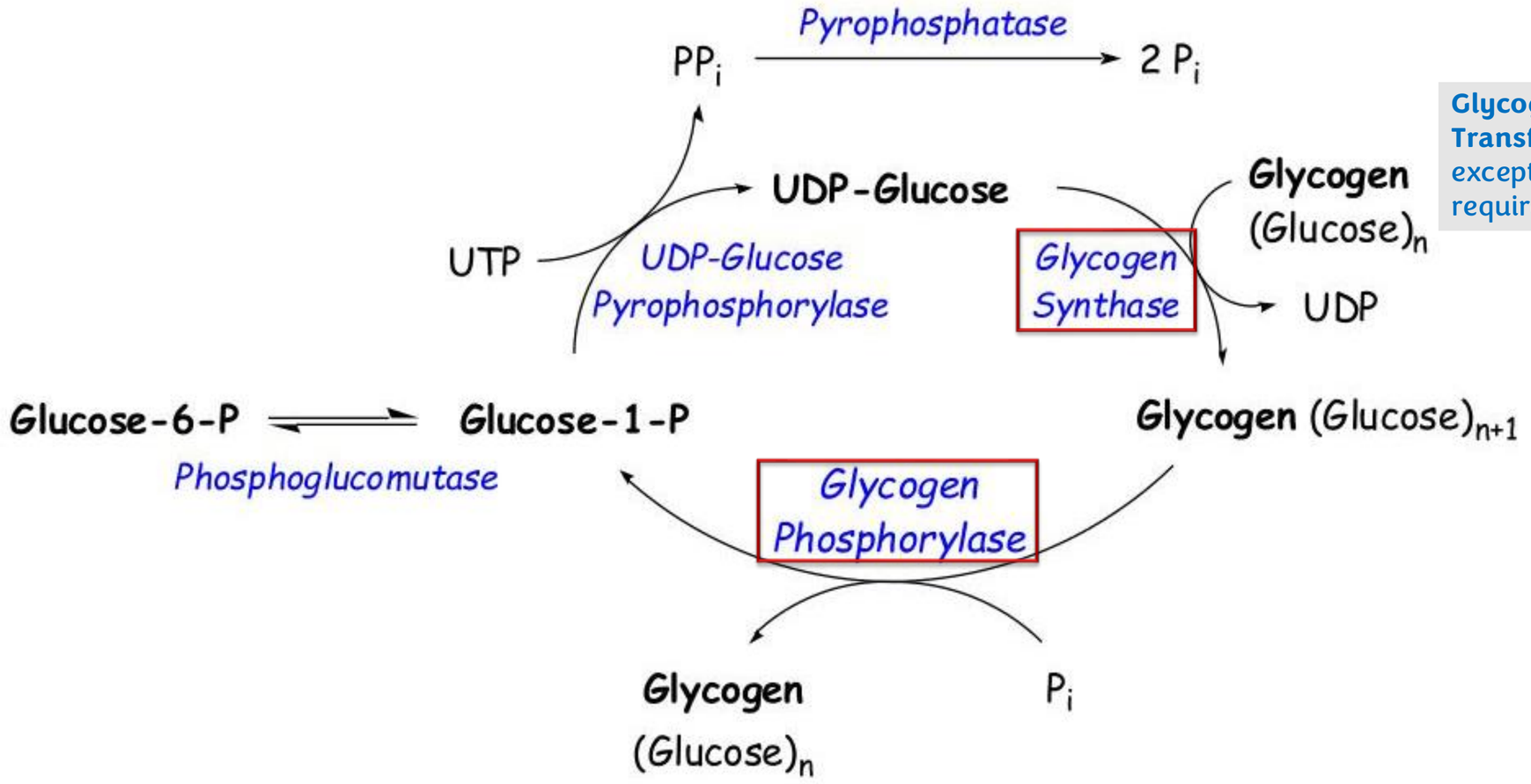
Recall , Liver has glucokinase and muscles have hexokinase that do the same thing (isoenzymes),but the efficiency of phosphorylation is different (the liver slows down prior phosphorylating glucose , because once glucose is phosphorylated it can't leave the cell)

The cells chooses the pathway for glucose-6-phosphate, **first** , it can produce pyruvate to make energy (ATP) , pyruvate can go either aerobic (acetyl Co-A , then enters Krebs cycle,etc...) or unaerobic (becomes lactate) metabolism. A **second pathway** is adding the glucose-6-phosphate to glycogen ,happens in the liver and some muscles (some skeletal muscles storing glycogen)

The **Third pathway** is converting glucose-6-phosphate into ribose (5-carbon sugar) by removing one carbon from glucose , 5-carbon sugars (ribose) are used in DNA & RNA , thus , cells undergoing **proliferation** (growing and dividing) needs ribose to synthesize DNA for division , it makes it from glucose-6-phosphate.

The first 2 pathways can be reversed by the cell depending on it's need .
The third pathway (Glucose into ribose) isn't reversible .

Metabolism of glycogen



Glycogen synthase is Transferase not lyase it's an exception (details aren't required)

Memorise the enzymes in red boxes.

In order to add glucose-6-phosphate to glycogen, it must be converted to glucose-1-phosphate (changing the position of the phosphate group from C6 to C1).After that , the enzyme **Glycogen synthase** adds glucose-1-phosphate to glycogen.

When the cell needs glucose , the liver has the glycogen , the enzyme **Glycogen phosphorylase** removes glucose-1-phosphate from glycogen, then it is converted to glucose-6-phosphate, then converted into glucose to leave the liver.

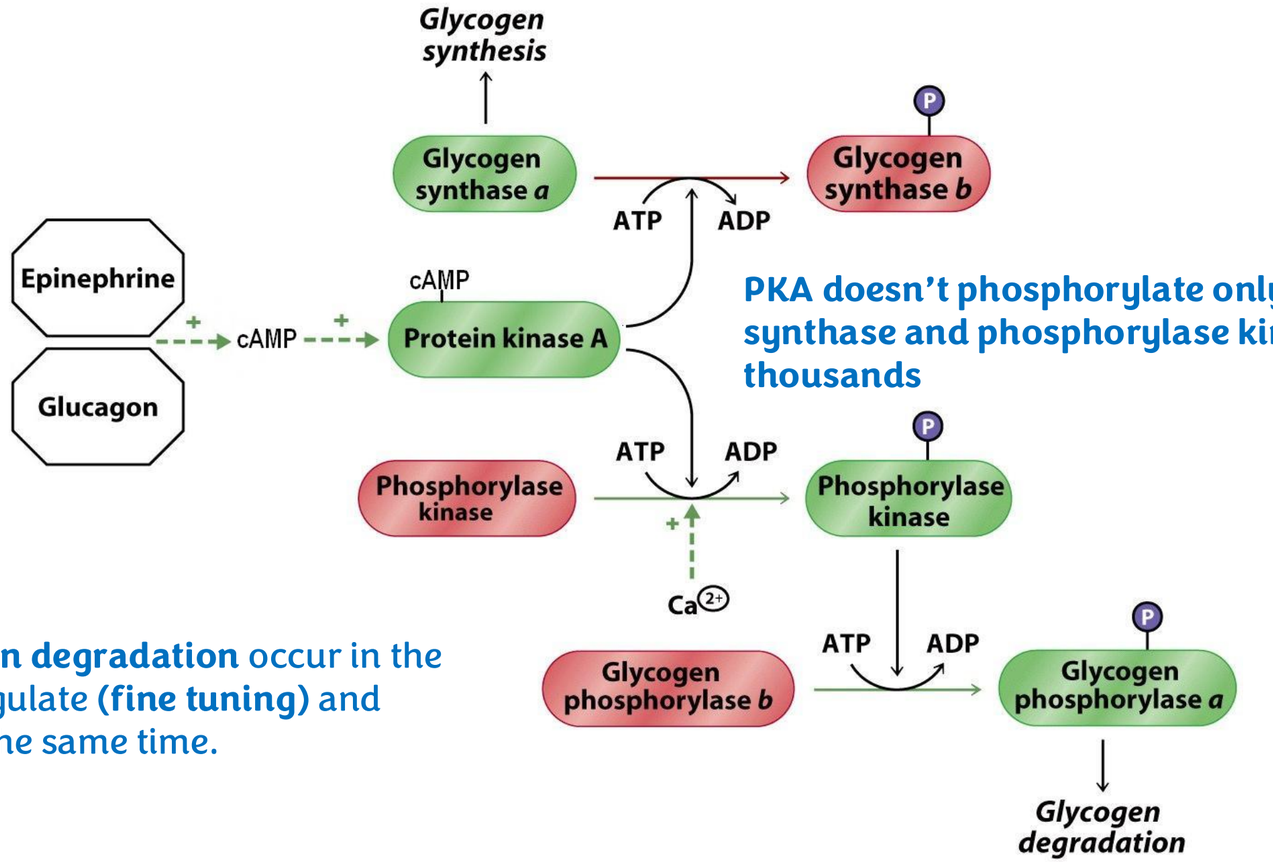
So basically , we have two **antagonistic enzymes** (having the opposite mechanisms of each other), they are **Glycogen synthase & Glycogen phosphorylase** , both reactions occur in the **Cytosol**

Recall , part of the enzyme regulation is conducting opposite reactions in different places . For example , fatty acids metabolism , fatty acid synthesis occurs in the **cytosol** , but fatty acid breakdown occurs in the **mitochondria.**

But sometimes antagonistic reactions can occur at the same place , like **Glycogen synthase & Glycogen phosphorylase .** So how can the cell regulate both while occurring on the same place ?

Regulation by phosphorylation

Phosphorylation is a reversible covalent modification



PKA doesn't phosphorylate only one molecule of glycogen synthase and phosphorylase kinase , rather , it phosphorylates thousands

Both **glycogen synthesis and glycogen degradation** occur in the **cytosol**, allowing the liver to finely regulate **(fine tuning)** and balance these opposing pathways at the same time.

Principles of Biochemistry, 4/e
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When the body is hungry, glucagon acts on the liver to Inhibit glycogen synthesis, Stimulates glycogen degradation and release glucose from the liver to supply the other tissues.

After eating, the body secretes **insulin** from **pancreas** , which is considered a **satiety hormone**. When the body is hungry and needs energy, it secretes **glucagon** from the **pancreas**, but from different cells.

In a fight-or-flight situation, such as seeing a lion or during an exam, the body secretes **epinephrine (adrenaline).** Glucagon acts mainly on the liver, whereas epinephrine acts mainly on skeletal muscle. However, they use essentially the **same signaling mechanism in different cell types.**

Signaling Pathway
Glucagon or epinephrine binds to a receptor on the cell surface ,This activates the enzyme **adenylyl cyclase.** Then , Adenylyl cyclase converts **ATP → cyclic AMP (cAMP)**. After that **cAMP binds to and activates Protein Kinase A (PKA).**PKA is a **kinase**, so it carries out **phosphorylation**.

PKA does this :

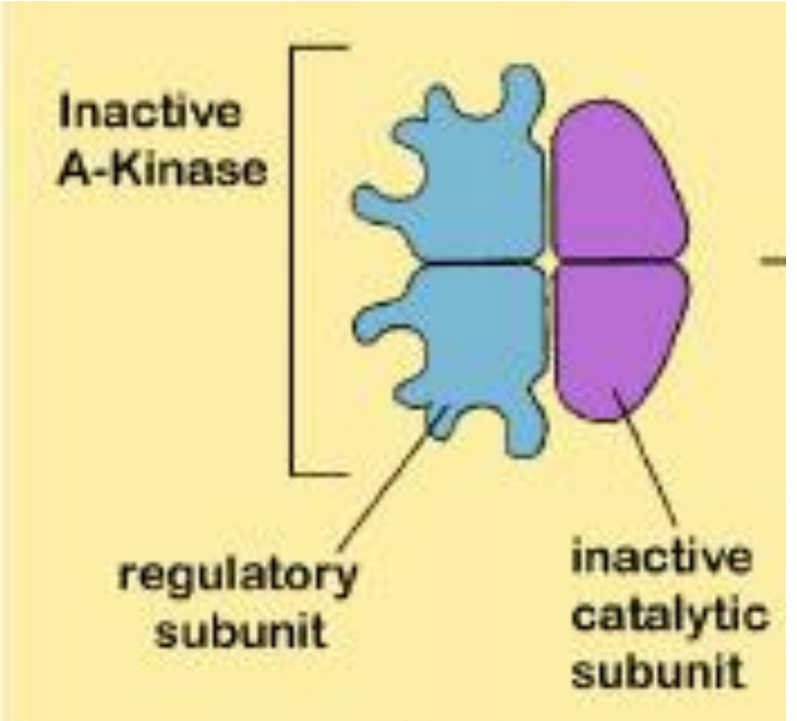
- PKA phosphorylates glycogen synthase → inactive , Therefore, glycogen synthesis is inhibited.
- PKA phosphorylates phosphorylase kinase → active ,THEN Phosphorylase kinase phosphorylates glycogen phosphorylase → active.
- Active glycogen phosphorylase promotes glycogen degradation to produce Glucose-1-P.

Overall:
Glucagon/epinephrine → adenylyl cyclase → cAMP → PKA → ↓ glycogen synthesis + ↑ glycogen degradation.

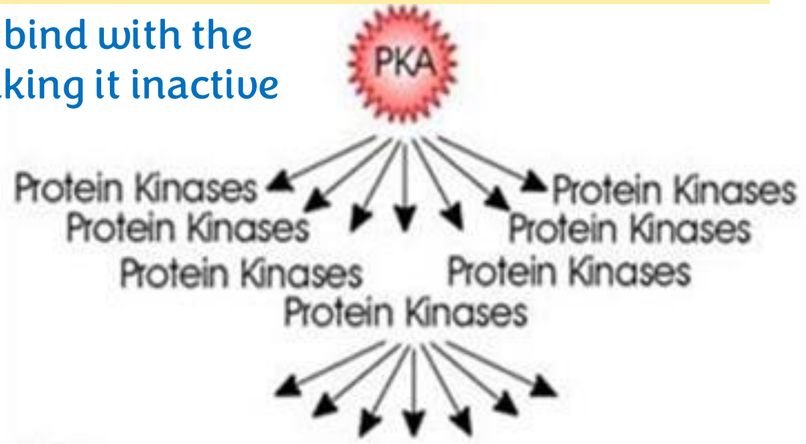
PKA-structure and regulation

Tetramer (four subunits) , 2 regulatory & 2 catalytic , regulatory subunits do not have an active site , only the catalytic parts have .

- Protein kinase A (PKA), a serine/threonine protein kinase, phosphorylates several enzymes that regulate different metabolic pathways.
  - Example: glycogen phosphorylase kinase
- When inactive, PKA consists of four subunits
  - Two regulatory (R) subunits with high affinity for cAMP,
  - Two catalytic (C) subunits



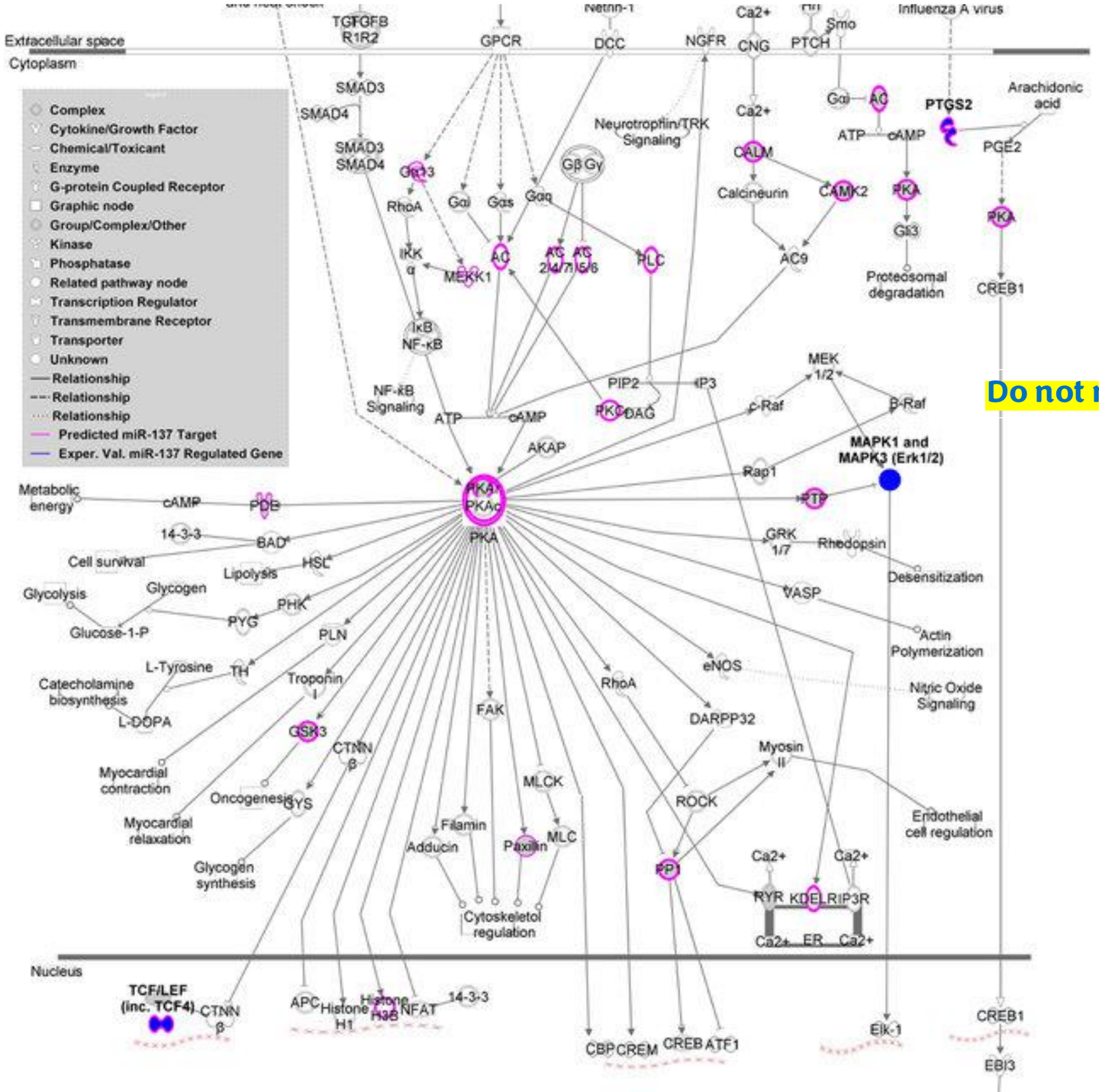
Regulatory subunits bind with the catalytic subunits making it inactive



activation of PKA leads to:
↑ Glycogen degradation
↓ Glycogen synthesis
↓ Cholesterol synthesis
↓ Fatty acid synthesis
And other metabolic effects.

The many targets of PKA

Protein Kinase A (PKA) can phosphorylate a large number of different enzymes and protein targets. When PKA is activated, it can simultaneously cause activation or inhibition of multiple targets, allowing different metabolic pathways to work together in a coordinated manner.



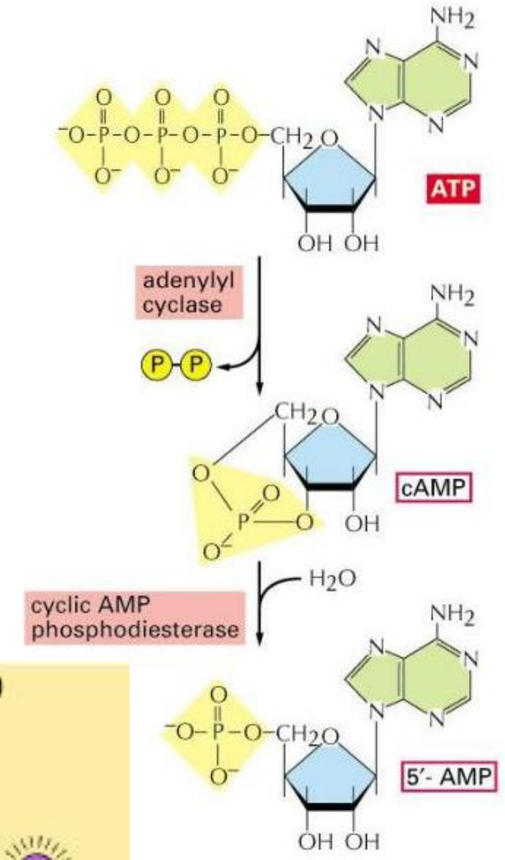
Do not memorize

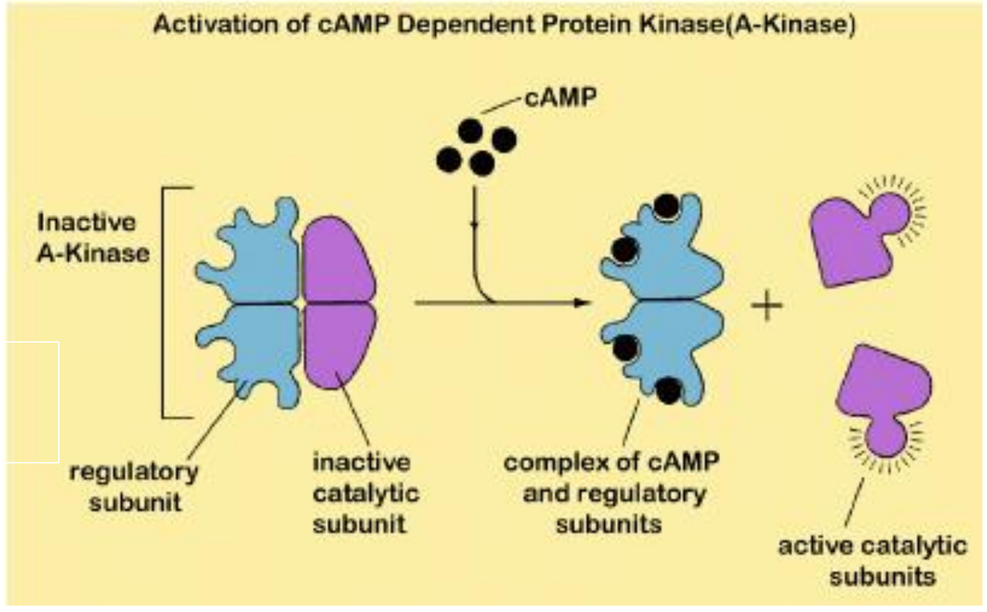
cAMP and protein kinase A (PKA)

- Small-molecule modulators can have dramatic effects on enzymes.
- For example, cAMP, which is structurally modified AMP, can activate protein kinase A (PKA).

- The binding of two molecules of cAMP to each regulatory subunit leads to the dissociation of R2C2 into an R2 subunit and two active C subunits.

This is **major structural changes** which is a way of **conformational regulation**





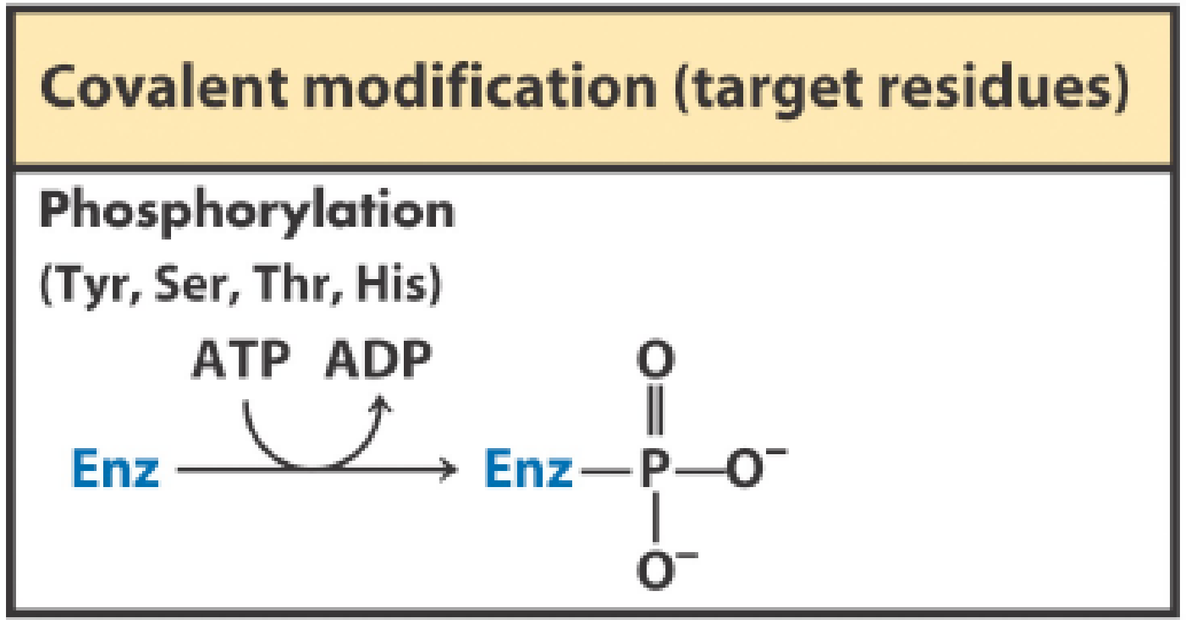
Reversible covalent modification

Most important is **Phosphorylation**

Advantage

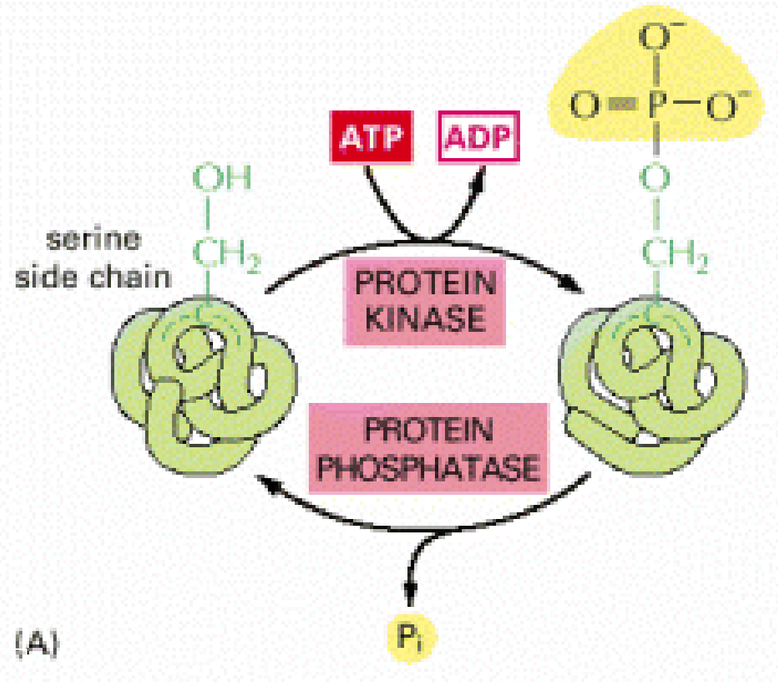
- Rapid and transient phosphorylation and dephosphorylation reactions can occur within **milliseconds** or even **microseconds**.
- A most common mechanism is enzyme phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains).
  - Usually serine, threonine, and tyrosine

Tyrosine kinases → phosphorylate tyrosine residues.
Serine/threonine kinases → phosphorylate serine or threonine residues.



Enzymes

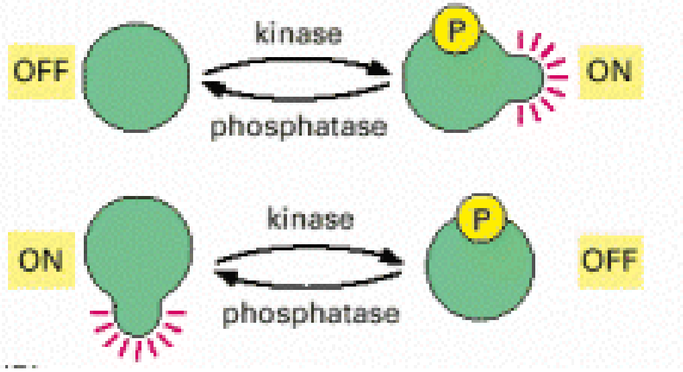
- ATP mostly is the phosphoryl donor in these reactions, which are catalyzed by protein kinases.
- The removal of phosphoryl groups (dephosphorylation) by hydrolysis is catalyzed by protein phosphatases.
- Note: dephosphorylation is not the reversal of phosphorylation.
- The addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes.



effect of phosphorylation **depends on the specific enzyme being phosphorylated.**
A phosphate group is relatively small, but it carries **three negative charges.**
These negative charges can significantly **change the three-dimensional structure of the protein** by causing:

- Attraction between charged groups
- Repulsion between charged groups
- Changes in the position of different groups within the protein

As a result, phosphorylation can **change the shape of the active site.**

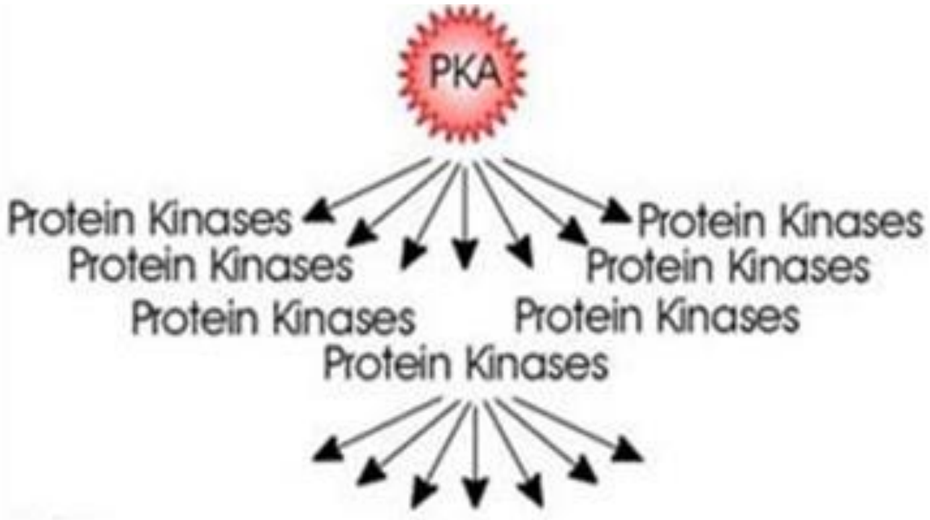


Remember that the amino acids surrounding the active site determine its shape and structure.
Therefore, adding a phosphate group to the enzyme can alter the arrangement of these amino acids and consequently change the shape of the active site.

Why is phosphorylation effective?

Because it is Rapid and transient

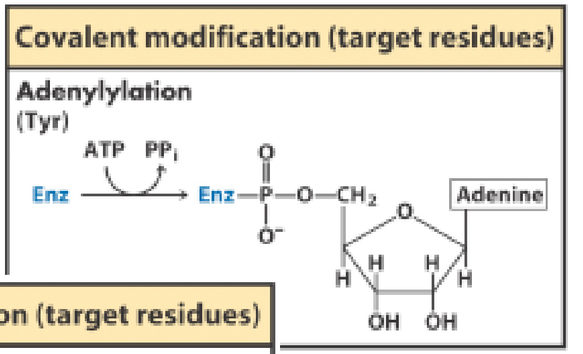
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects. **Can phosphorylate many targets , controlling many pathways , an active kinase can cause phosphorylation to vast number of targets**

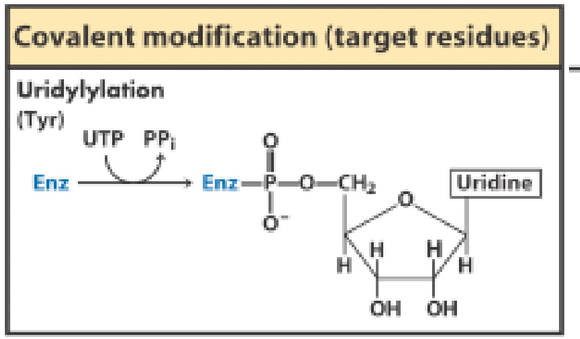


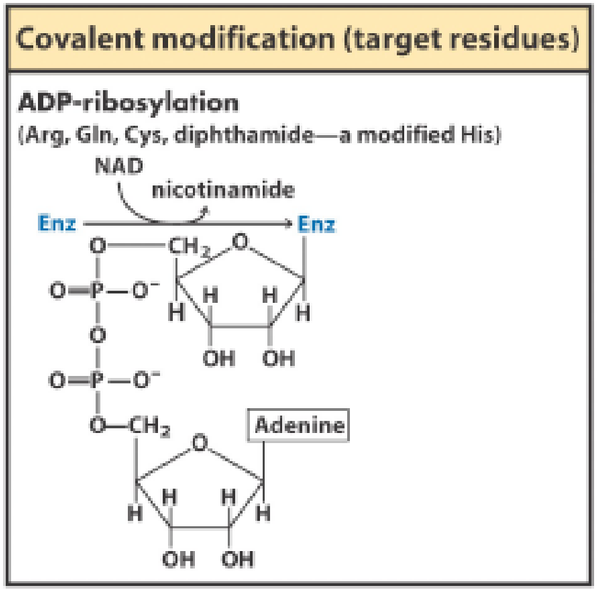
Others

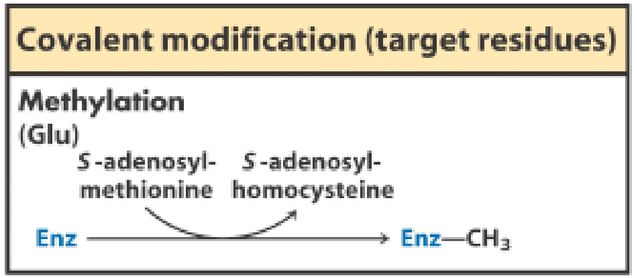
Large molecules can be added to the enzyme , changing it's activity

- Adenylylation (addition of adenylyl group (AMP)).
- Uridylylation (addition of uridylyl group).
- ADP-ribosylation (addition of adenosine diphosphate ribosyl group).
- Methylation of carboxylate side chains masking negative charges.
- Acetylation (from acetyl CoA) masking positive charges.







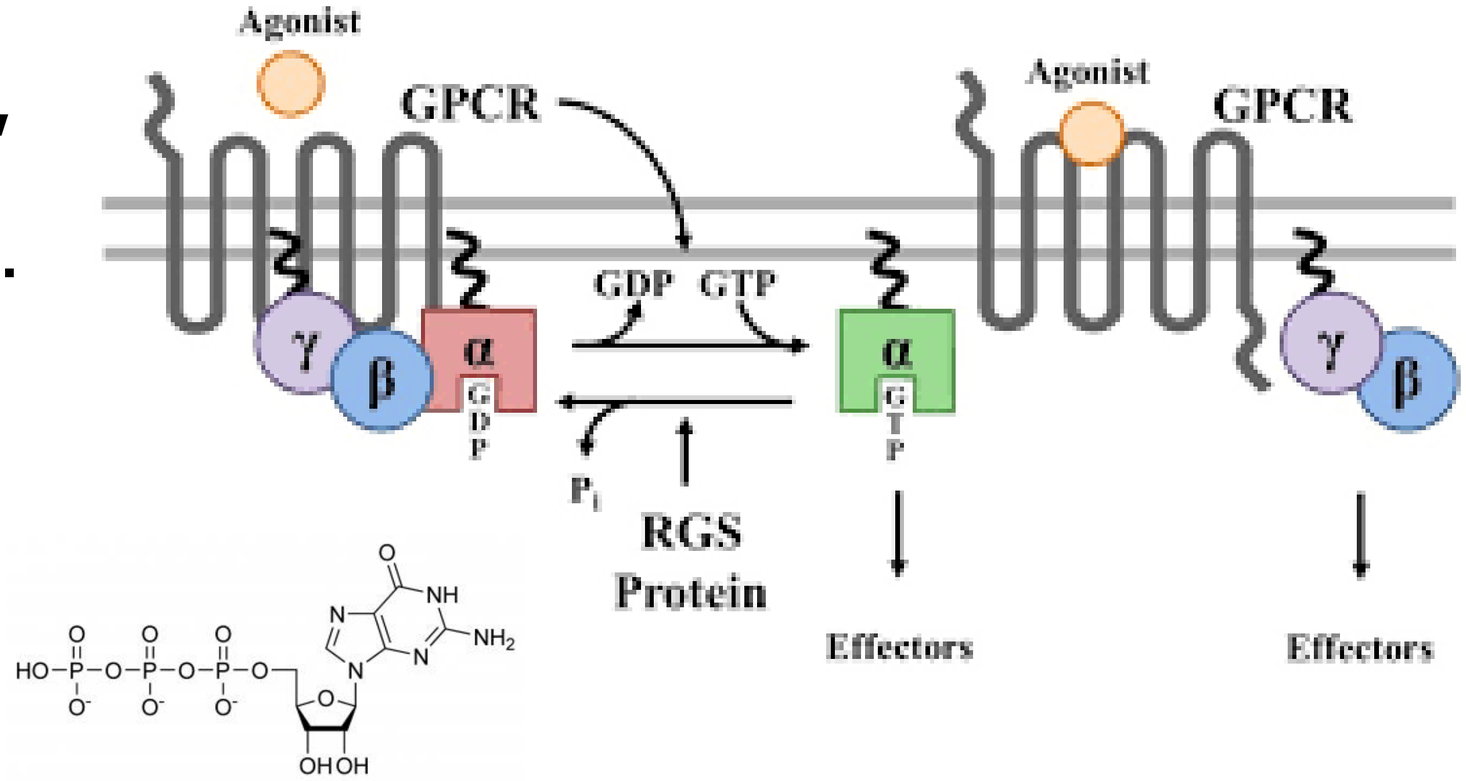


Large and small regulatory modulators

Trimeric large G proteins

- Trimeric G proteins: a family of membrane-bound proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors through G protein-coupled receptors (GPCRs)

- **When they bind GTP (small), they are 'on', and, when they bind GDP, they are 'off'.**
- **The α subunit (large) binds to effectors stimulating or inhibiting them.**



G proteins, also known as large trimeric G proteins, are extremely important physiologically because they regulate many cellular functions.
Examples of physiological processes that depend on trimeric G proteins include:

- Hearing
- Taste
- Sensory perception in the skin

- Many drugs target trimeric G proteins and their receptors, known as G protein-coupled receptors (GPCRs). Therefore, these proteins and receptors are highly important in medicine

How Trimeric G Proteins Work?
A G protein-coupled receptor (GPCR) is located on the cell surface , a ligand/agonist binds to the receptor, causing a conformational change in the receptor.
The receptor is associated with a trimeric G protein, which consists of three subunits: **α (alpha). β (beta) , γ (gamma)**

In the **inactive state**, the **α-subunit is bound to GDP** and is associated with the β and γ subunits.

When the ligand binds to the receptor, the receptor changes its conformation and causes the **α-subunit** to **dissociate** from the β and γ subunits , The **GDP bound to the α-subunit is released and replaced by GTP**. This is a **GDP-GTP exchange.**
The **α-subunit becomes active and can interact with downstream effectors, including other enzymes.**

Although the β and γ subunits were traditionally considered **inhibitory toward the α-subunit,** it is now known that **β and γ can also have their own targets and effects.**

Note: trimeric G proteins (especially α-subunit) and the enzymes they stimulate are a good example of regulation of enzymes by compartmentalization as they all exist in the cell membrane (which is important to have less diffusion and faster reactions).

Quiz!

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			

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي:

اللَّهُمَّ إِنِّي أَعُوذُ بِكَ مِنْ الْهَمِّ وَالْحَزَنِ،
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