Enzymes III

Regulation

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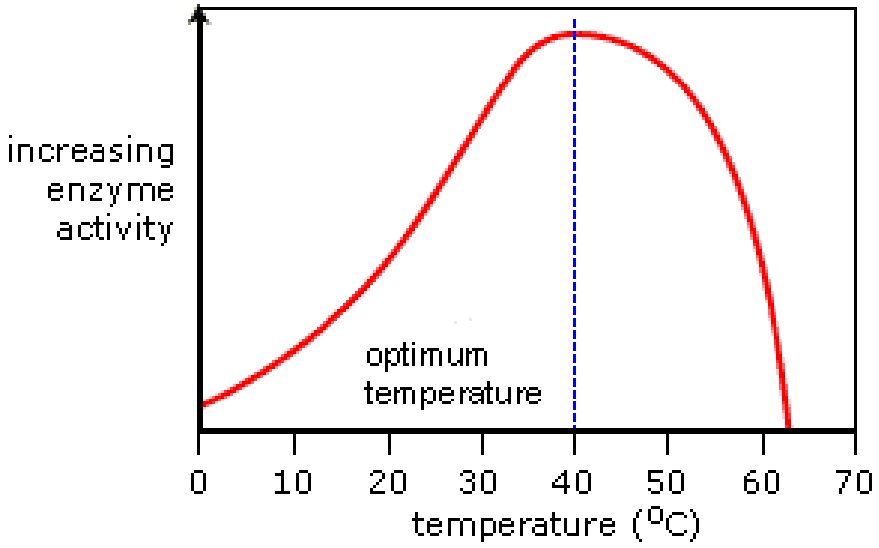
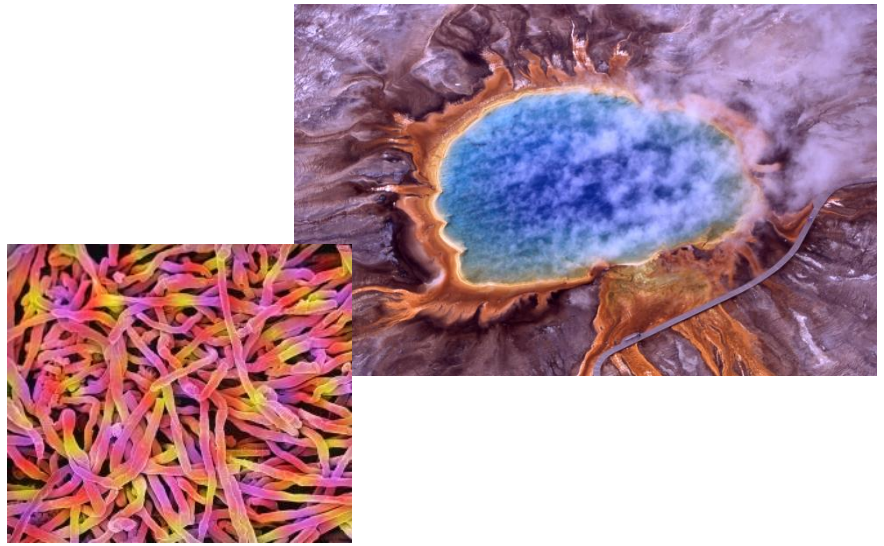
Mechanisms of regulation

- Non-specific and collective regulation (temperature, pH, diffusion, and expression)
  - Localization (compartmentalization) and enzyme complexing
  - Expression of isoenzymes
- Regulation of enzymatic activity
    - Inhibitors
    - Conformational changes
      - Modulators
      - Reversible covalent modification
      - Irreversible covalent modification
      - Allostery

Temperature

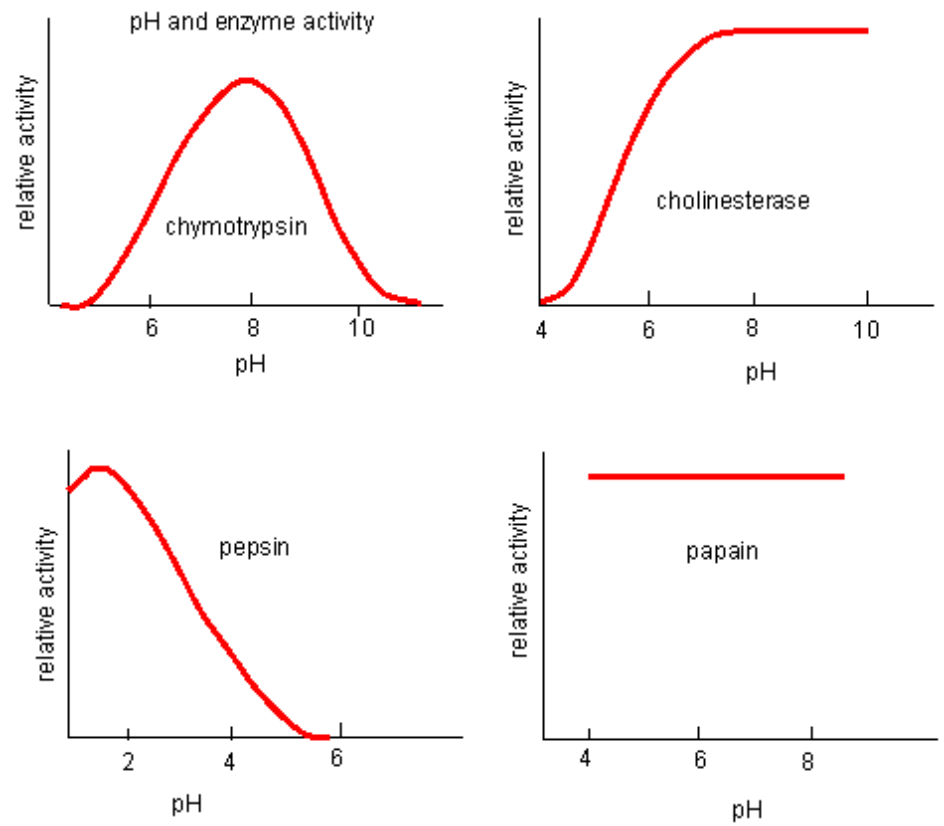
- Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates.
  - However, high temperatures lead to protein denaturation.
- Each organism has an optimal temperature for its enzymes.
  - For thermophilic bacteria, the optimal temperature is as high as 65°C.





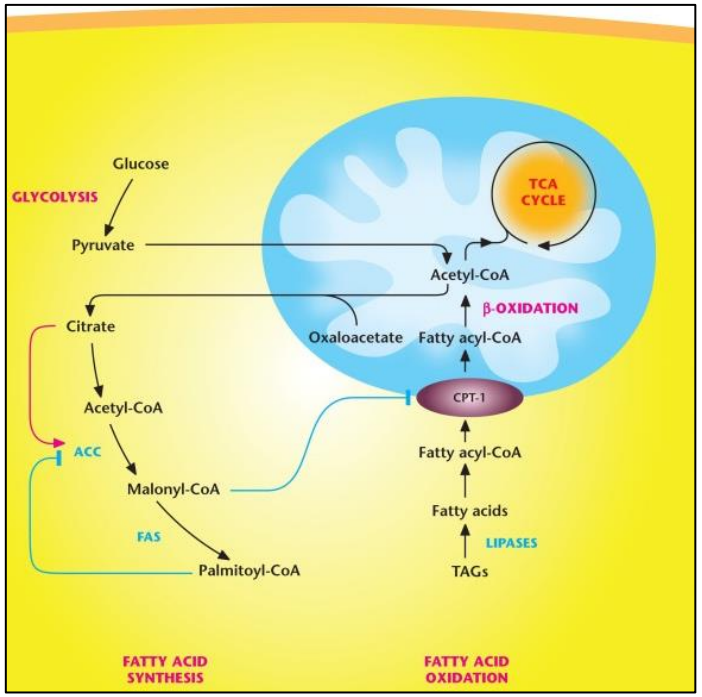
pH

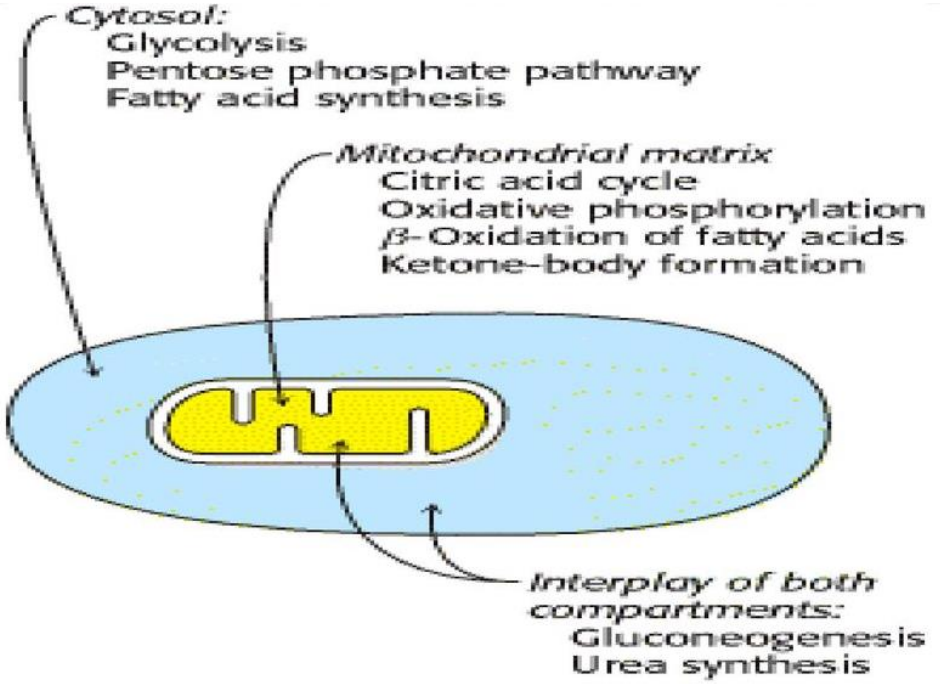
- pH alters the protonation state of the substrate and/or the enzyme and, hence, their binding.
- The effect of pH is enzyme-dependent.



Compartmentalization

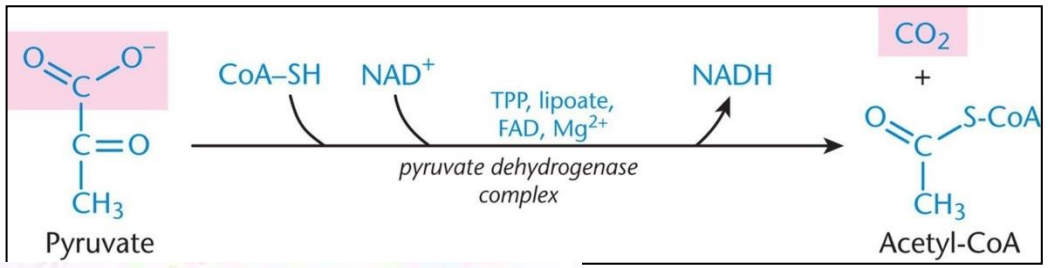
- Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide.
  - Example 1: lysosomal enzymes
  - Example 2: fatty acid metabolism
    - Synthesis occurs in cytosol, whereas break-down is mitochondrial.

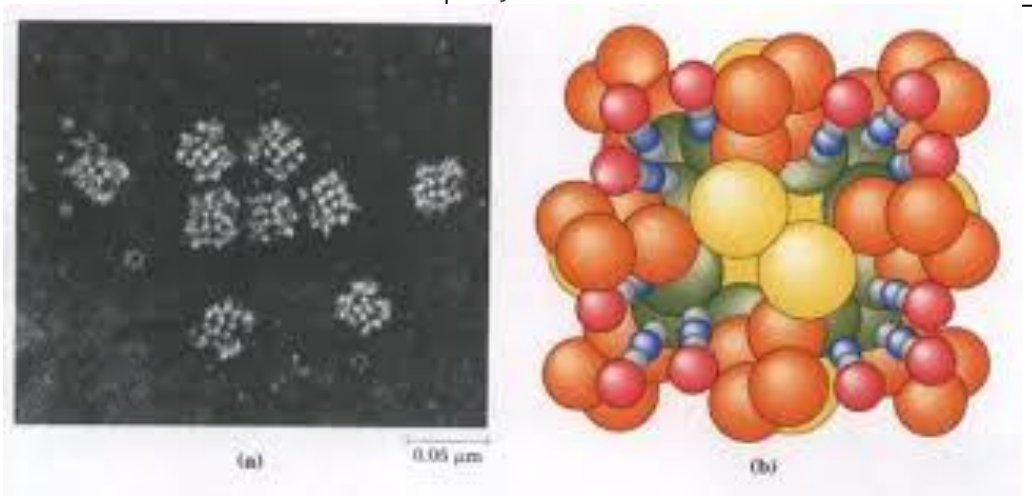


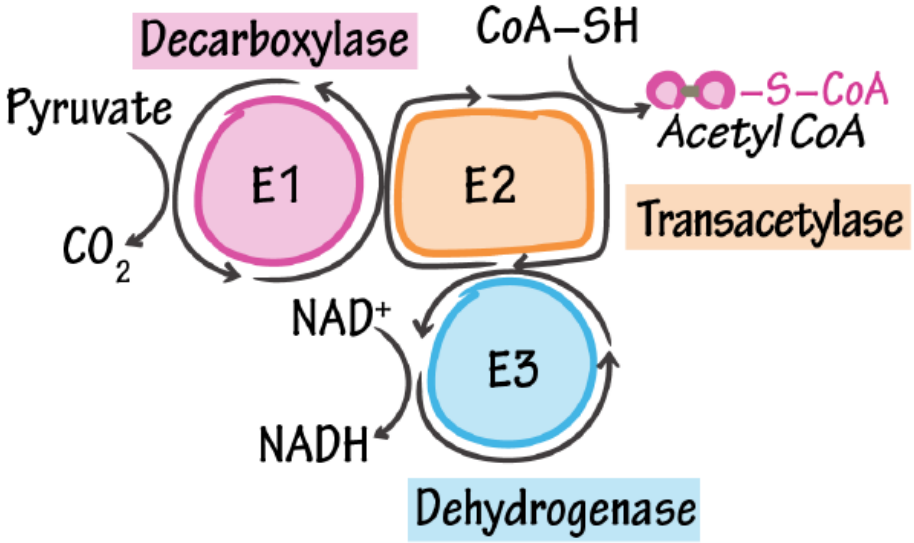


Enzyme complexing

- Formation of a complex of multiple enzymes also reduces diffusion.
- Example: Pyruvate dehydrogenase complex (mitochondria) is composed of 3 enzymes: decarboxylation, oxidation, & transfer of the acyl group to CoA.

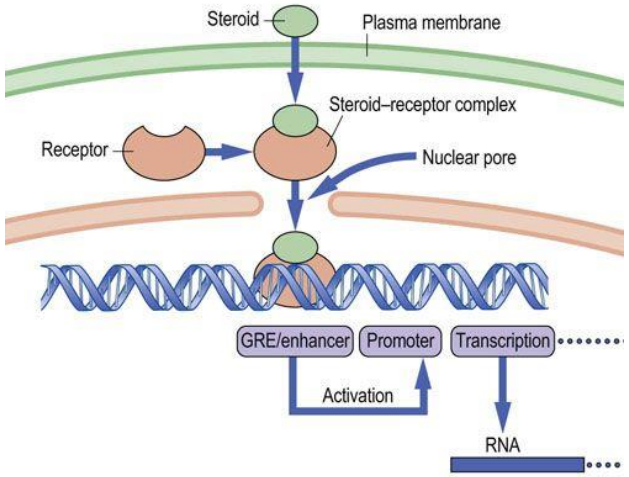






Regulation of enzyme amount

- Three mechanisms:
  - Enzyme synthesis at the gene level
  - Enzyme degradation by proteases
  - Synthesis of isozymes

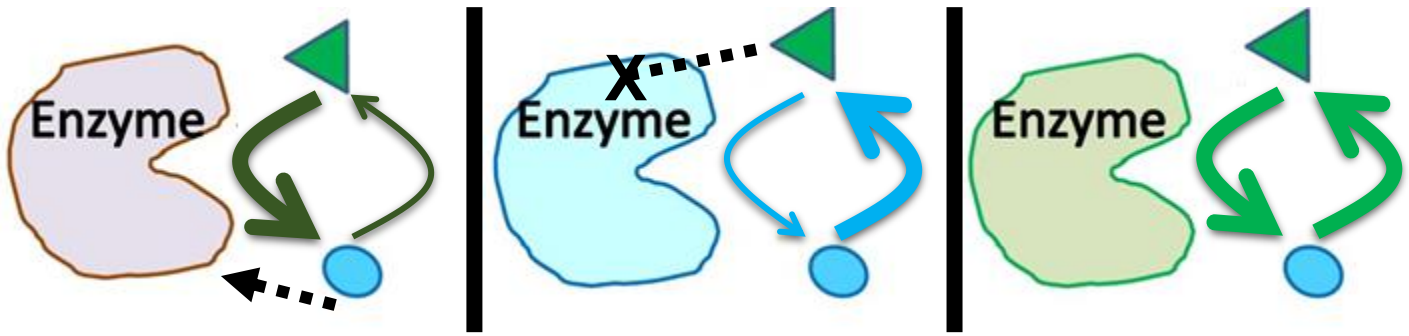


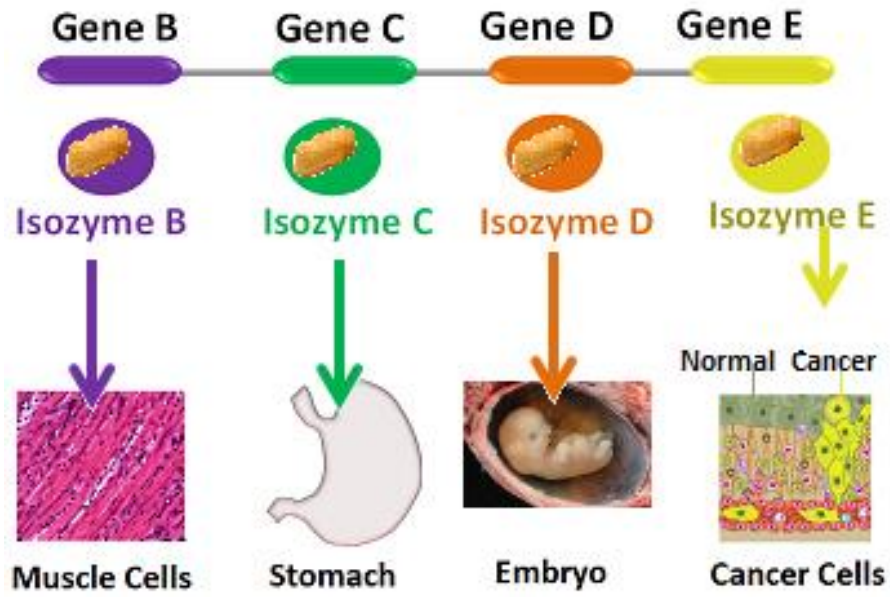
- They are comparatively slow mechanisms for regulating enzyme concentration (hours-weeks).

Enzyme	Half-life (days)
Catalase	1.4 days
Glucokinase	1.2 days
Lactate dehydrogenase	
LDH1 (heart)	1.6
LDH5 (liver)	16
LDH5 (muscle)	31

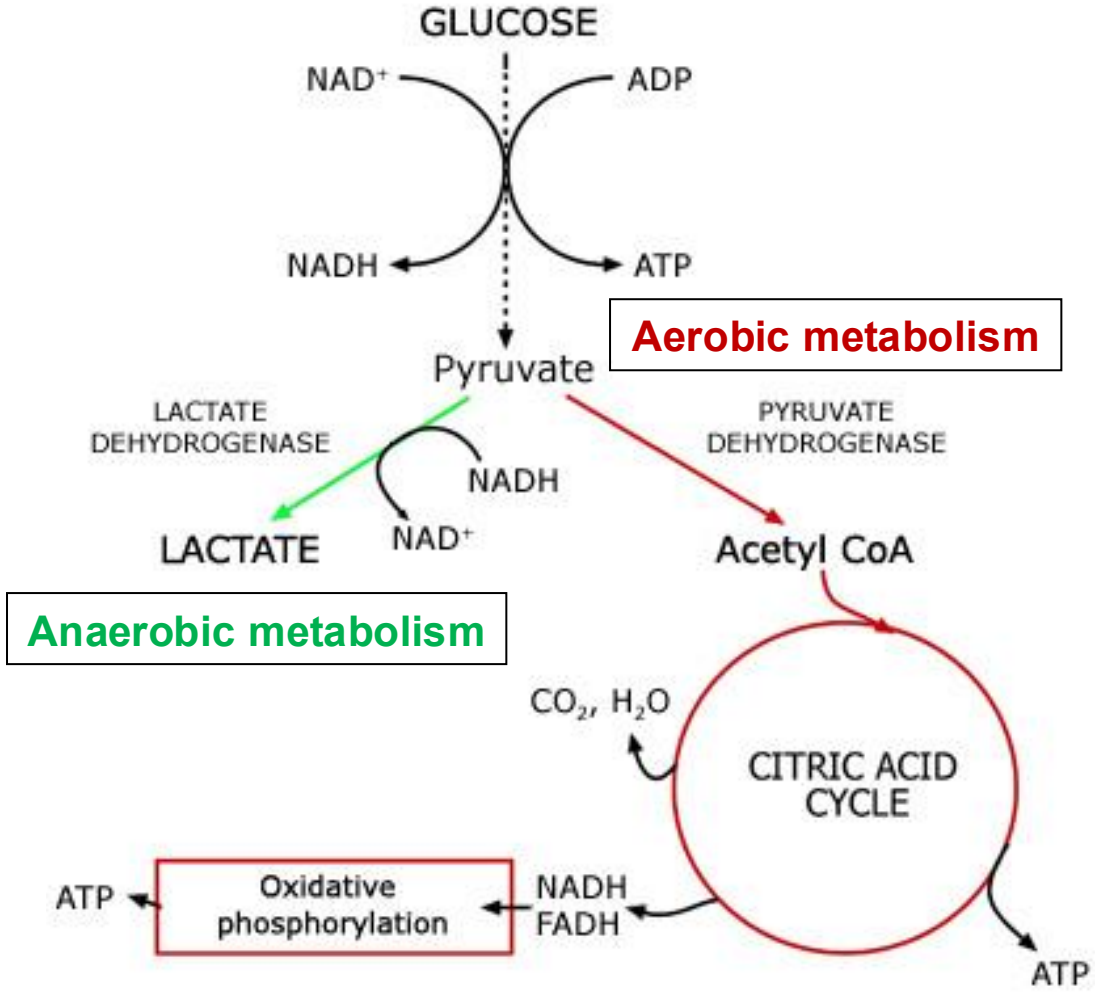
Isoenzymes (isozymes)

- Isoenzymes are enzymes that can act on the same substrate(s) and produce the same product(s).
- They are generated by different genes that are highly homologous (similar).
- Often, various isozymes are present in different tissues.
- They can be regulated differently .
- They can have different catalytic activities.



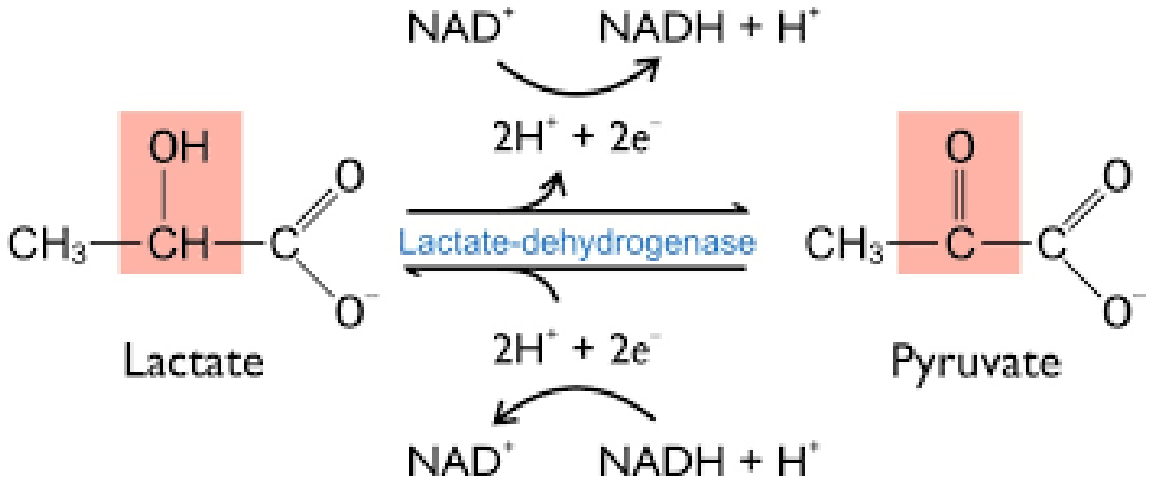


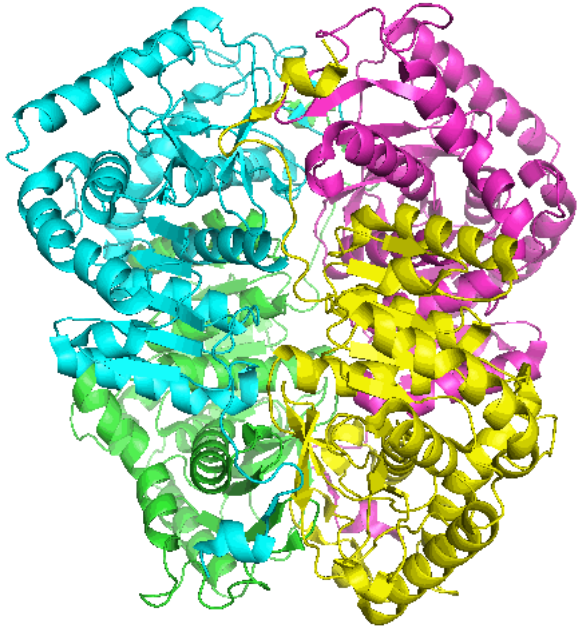
Aerobic vs. anaerobic metabolism

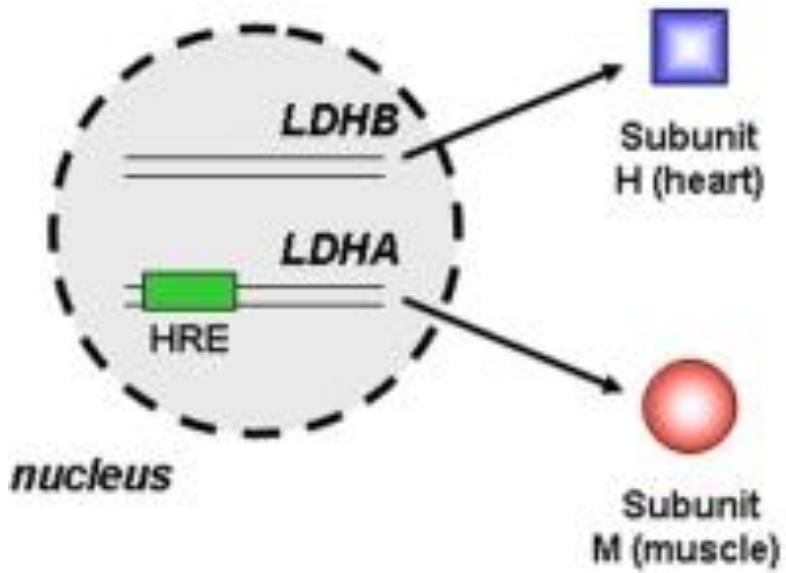


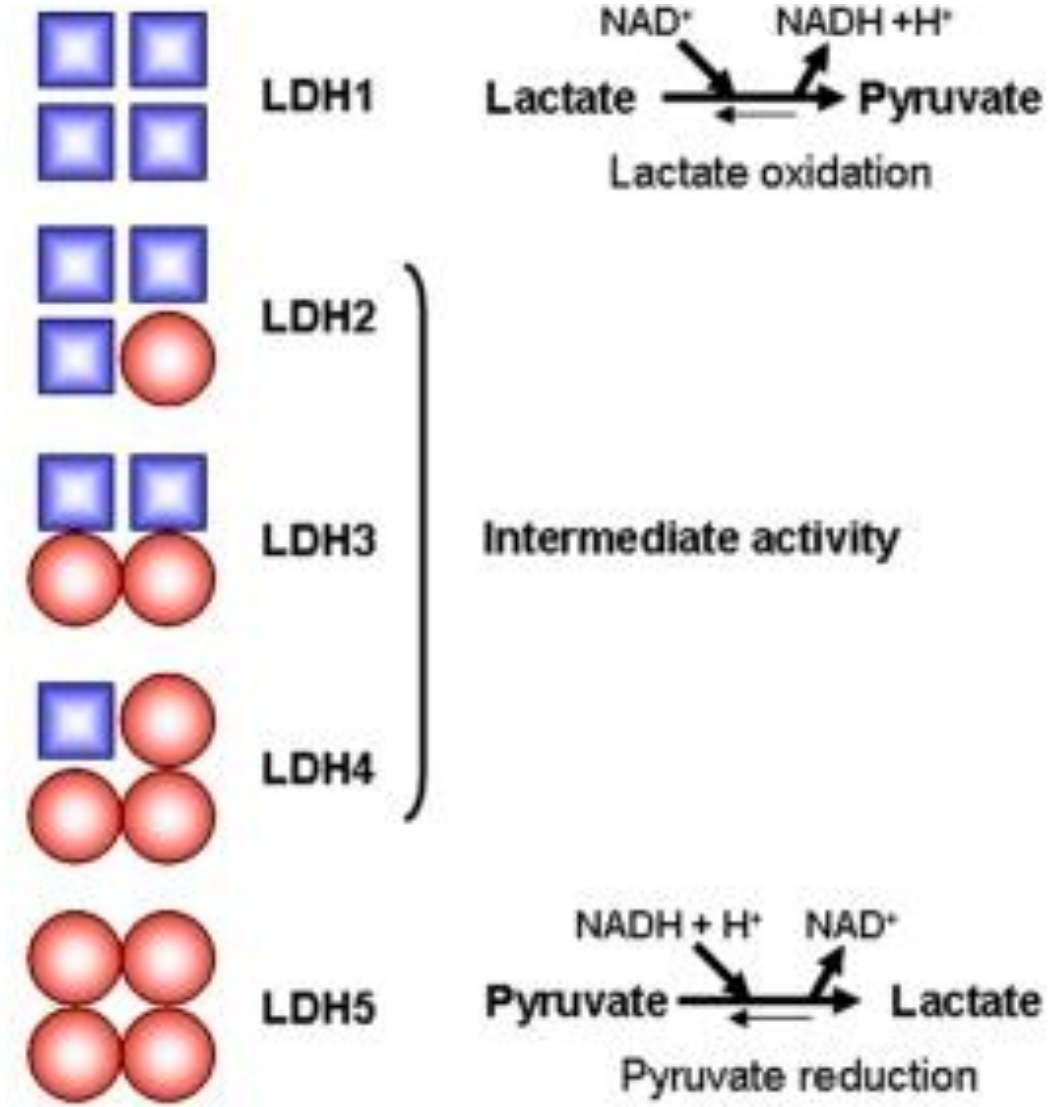
Lactate dehydrogenases (LDH)

- LDH is a tetrameric enzyme composed of a combination of one or two protein subunits: the H (heart) subunit and M (skeletal muscle) subunit.
- These subunits combine in various ways leading to 5 distinct isozymes (LDH1, LDH2, LDH3, LDH4, LDH5) with different combinations of the M and H subunits (H4, H3M. H2M2, HM3, and M4).
- The H4 isozyme or LDH1 is characteristic of that from heart tissue, and the M4 isozyme of LDH5 is typically found in skeletal muscle and liver.



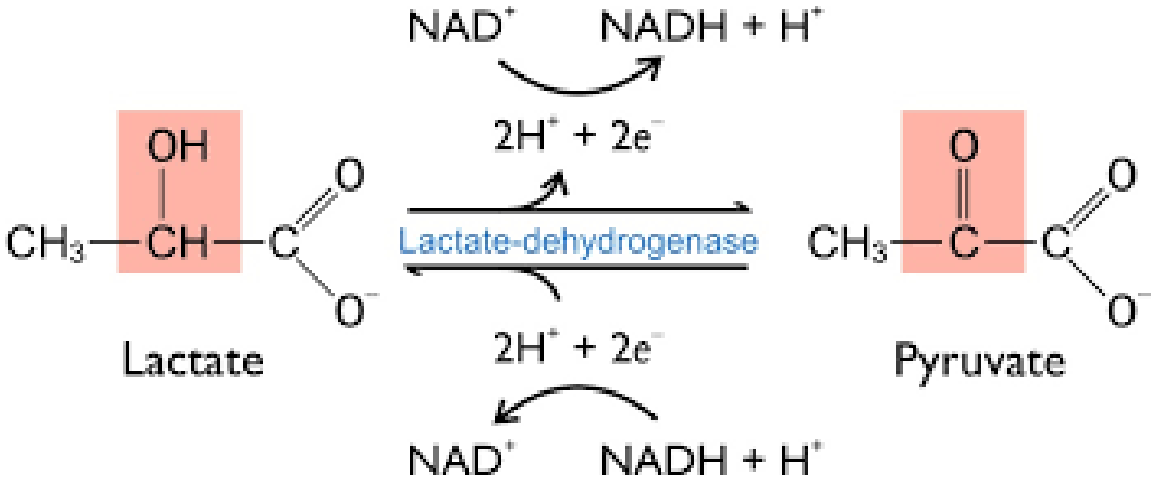






- Although the five isoforms catalyze the same reaction, they differ in their primary structure, kinetic properties, tissue distribution, affinity to the substrate (direction of the reaction), regulation, and isoelectric point.
- The M subunit has a higher affinity towards pyruvate, thus converting pyruvate to lactate (and NADH to NAD+).
- The H subunit has a higher affinity towards lactate, resulting in a preferential conversion of lactate to pyruvate (and NAD+ to NADH).

Logic behind tissue distribution



Isoenzyme	Structure	Present in
LDH1	**(H_4)**	**Myocardium**
LDH2	(H_3M_1)	RBC
LDH3	(H_2M_2)	Lungs
LDH4	(H_1M_3)	Kidney
LDH5	**(M_4)**	**Skeletal muscle, Liver**

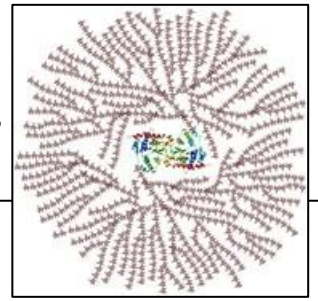
- Muscles can function anaerobically, but heart tissues cannot.
- Whereas the M4 isozyme catalyzes the reduction of pyruvate into lactate, the H4 enzyme catalyzes the reverse reaction.

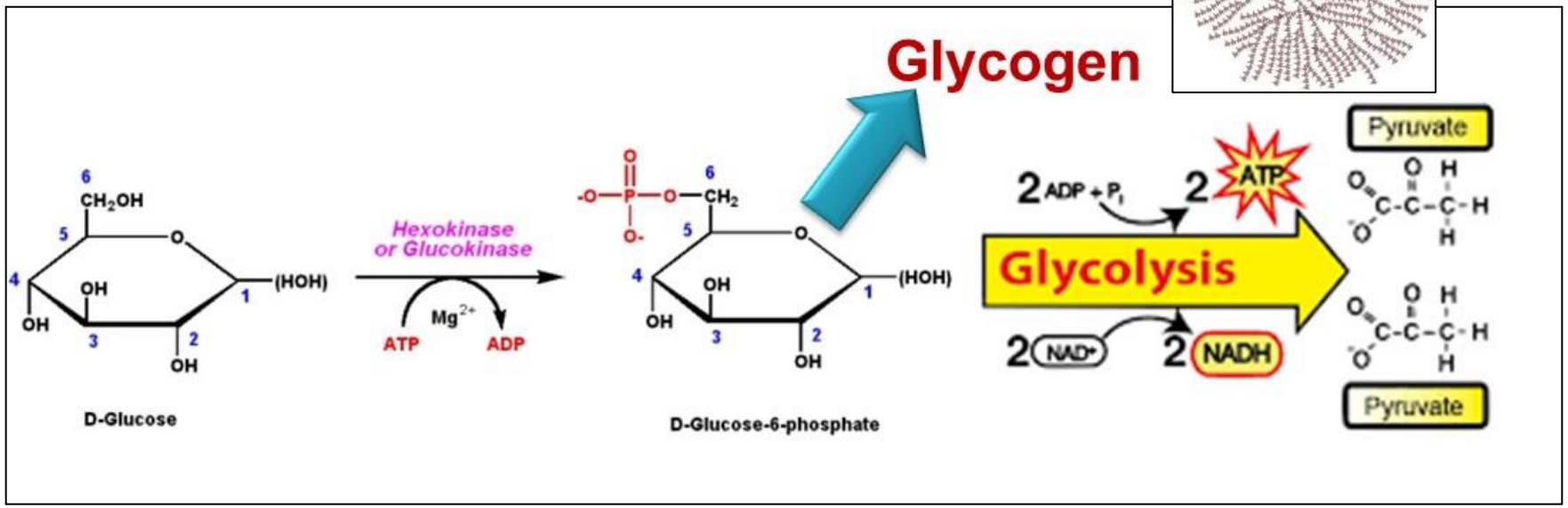
Hexokinase vs glucokinase

- Hexokinase and glucokinase (hexokinase IV) are allosteric isozymes that catalyze:

Glucose + ATP → Glucose-6-Phosphate + ADP + Pi

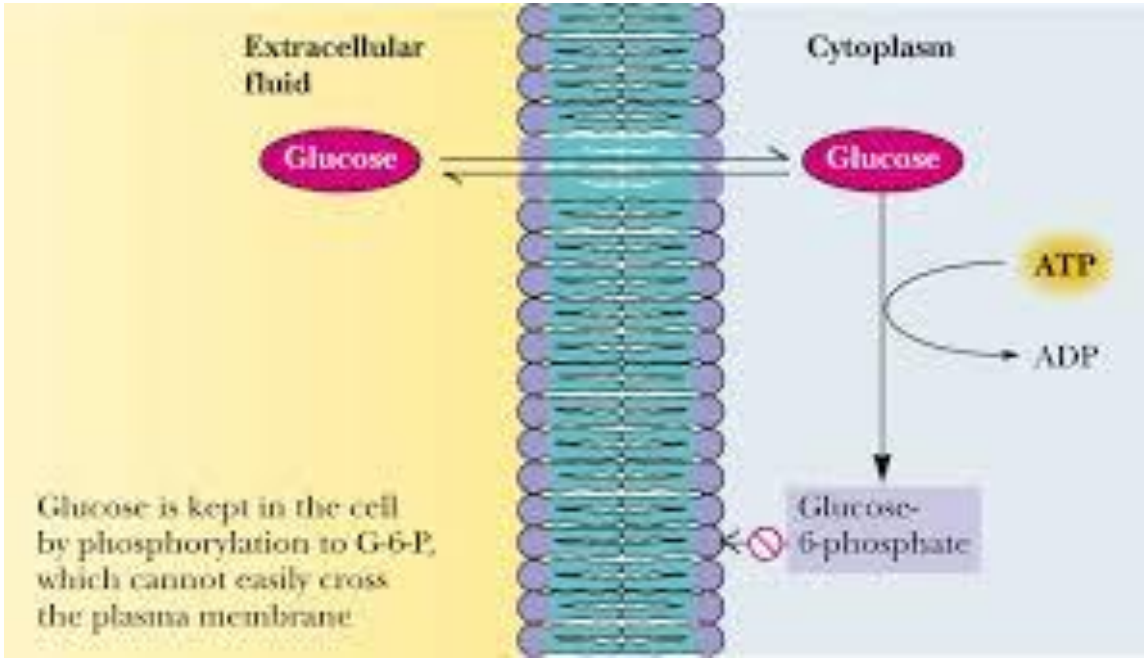
- Glucokinase is a liver (and pancreatic) enzyme, whereas hexokinase is a skeletal muscle enzyme.
  - The purpose of liver glucose is to store glucose as glycogen (or balance glucose level in the blood).
  - The purpose of skeletal muscle glucose is to produce energy.





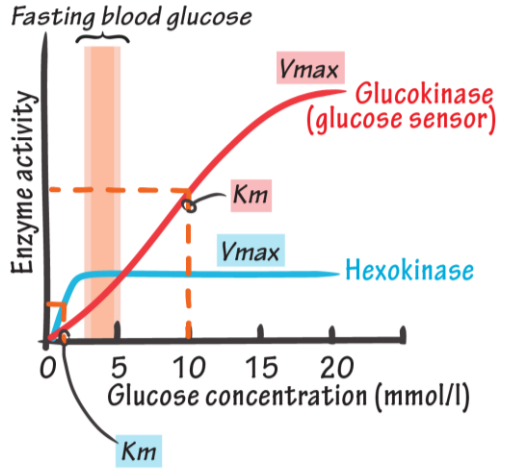
Biological significance

- Note: once glucose is phosphorylated, it cannot cross plasma membrane out of cells.
  - Liver: low efficiency enzyme to not store glucose and keep it to other organs.
  - Skeletal muscles: high efficiency enzyme to trap glucose immediately.



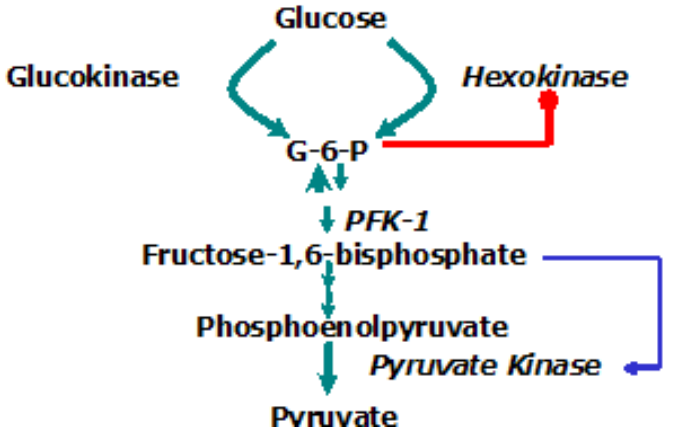
Regulation of hexokinase and glucokinase

- Note Vmax and K_M values
- Regulation
  - Hexokinase is inhibited by glucose-6-phosphate, but glucokinase is not.
- Significance:
  - At fasting state, glucose is not stored.
  - At well-fed state, skeletal muscles do not consume all glucose in blood and liver can convert excess glucose to glycogen for storage.



Km
Glucokinase >Hexokinase
Spares glucose stores for brain, muscle & other tissues.

Vmax
Glucokinase >Hexokinase
At fasting glc conc. – Hexokinase is at Vmax, Glucokinase activity varies according to glc conc.

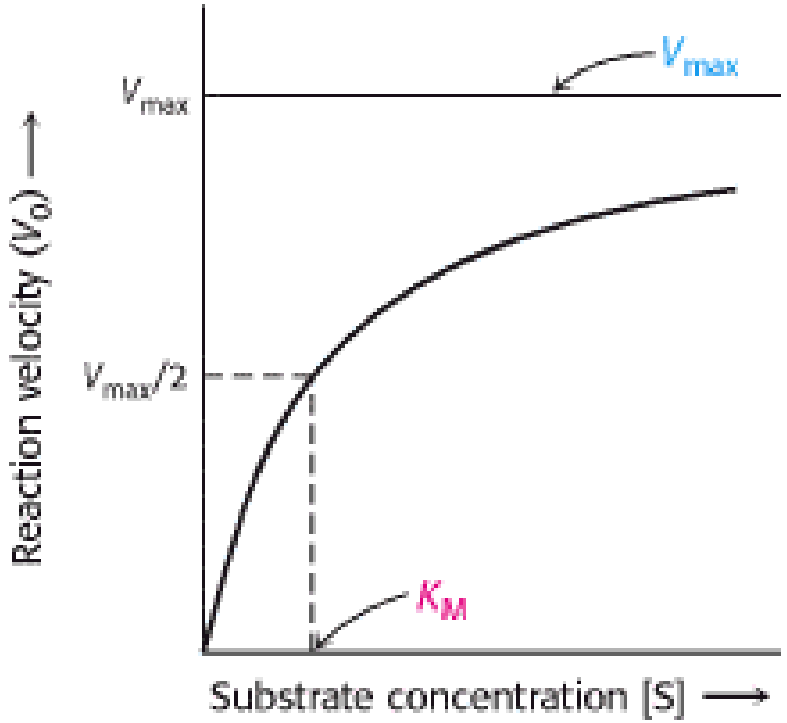


Regulation of enzymatic activity

But before, let's talk about this

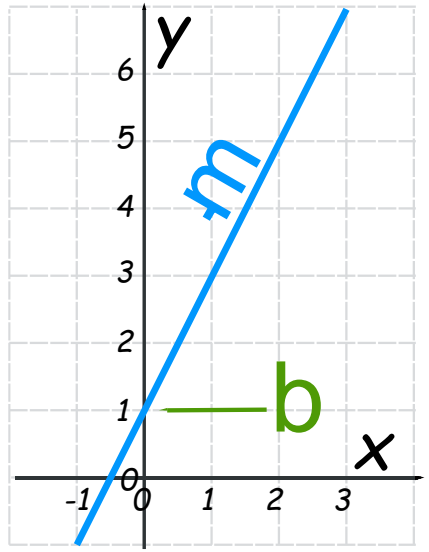
A disadvantage of the Michaelis-Menten equation

- 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

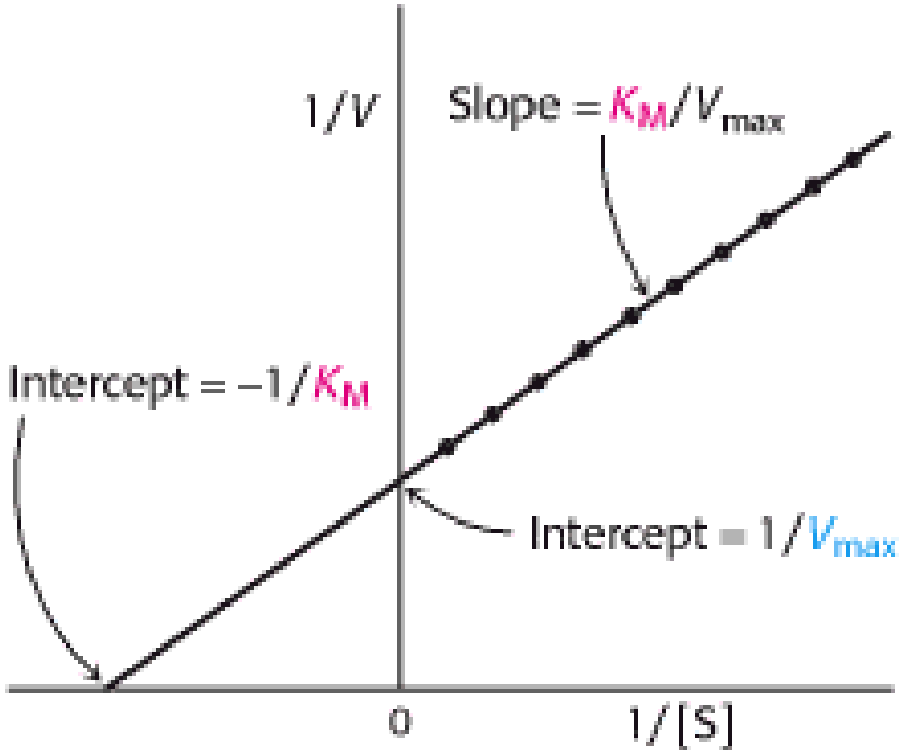


The Lineweaver-Burk or double-reciprocal plot

- 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.

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

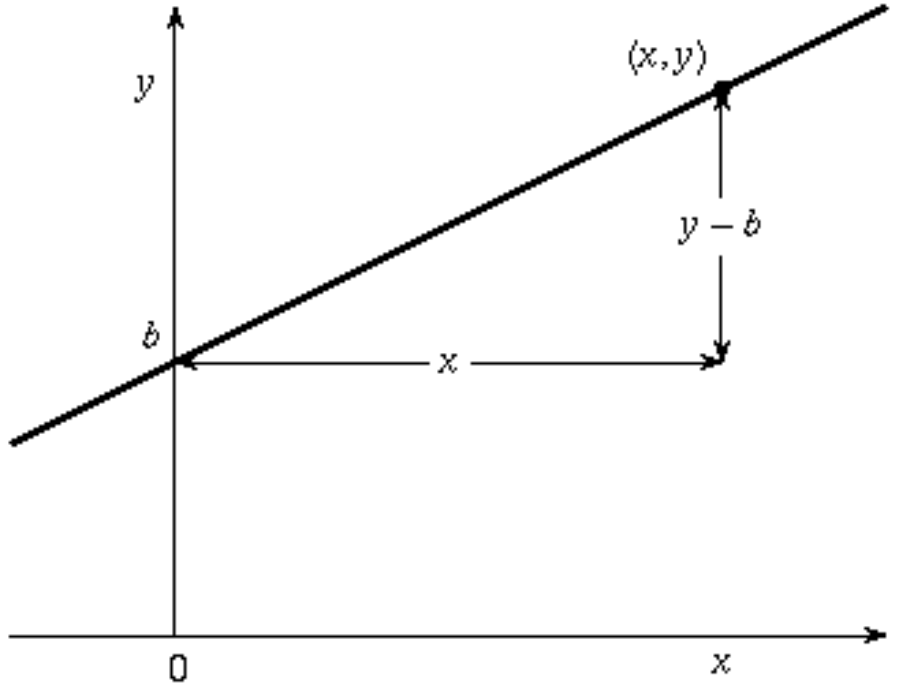
$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$



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

y= b + mx

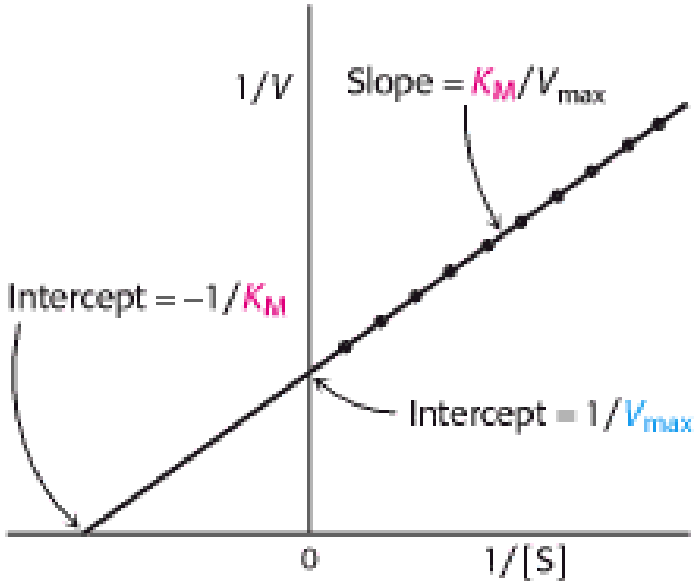
- y is y-axis = 1/ V_o
- x is x-axis = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



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

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 = -$1/K_M$)

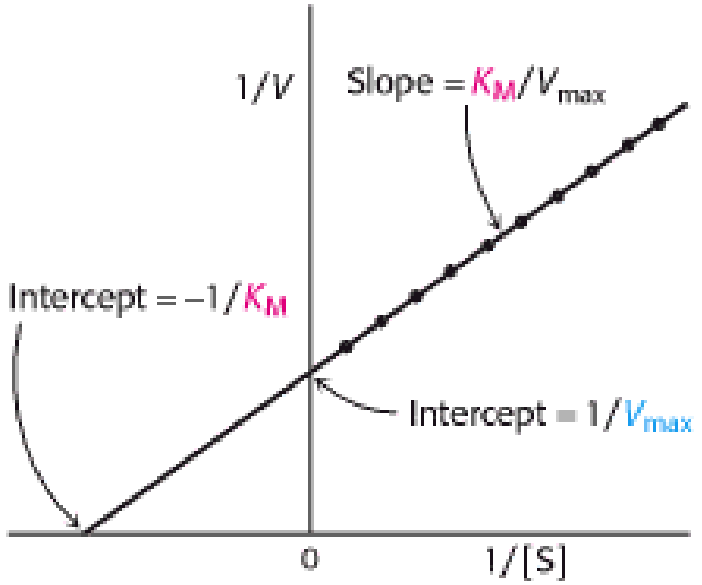
How?

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]



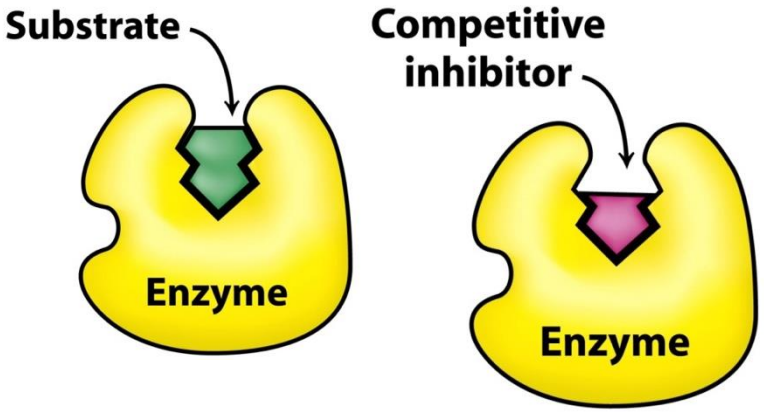
Now, we can talk about regulation of enzyme activity and we will start with inhibitors.

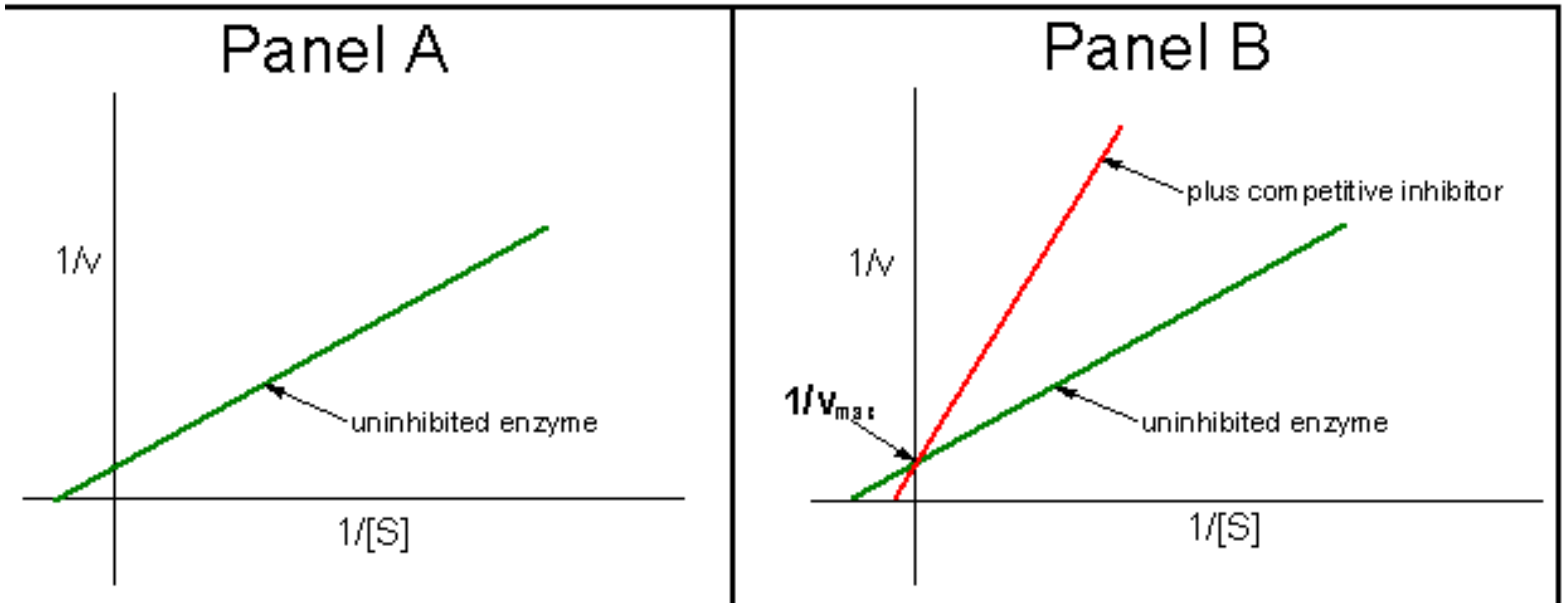
Enzyme inhibitors

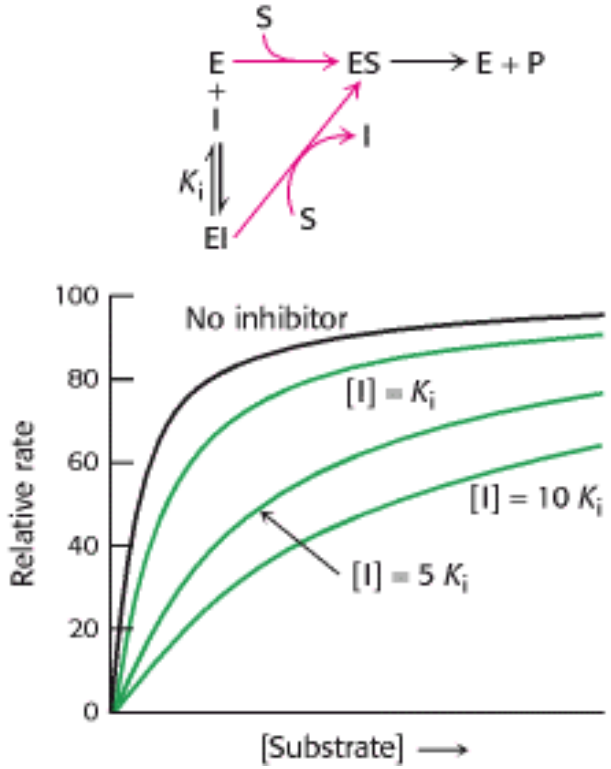
- Enzyme inhibition can be either reversible or irreversible.
  - Reversible inhibitors rapidly dissociate from enzymes (e.g. non-covalent binding).
    - Competitive, noncompetitive, or uncompetitive inhibition.
  - An irreversible inhibitor is tightly bound (e.g. covalently) to the enzyme.
    - Lower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

Competitive inhibition

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

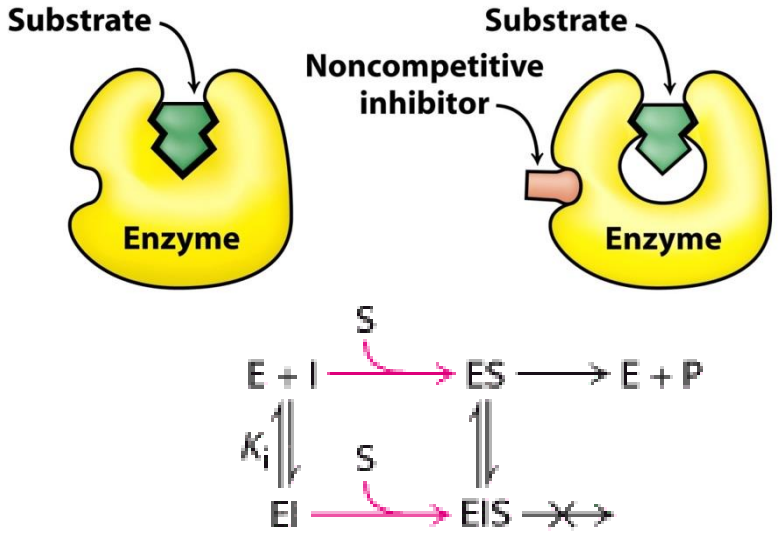


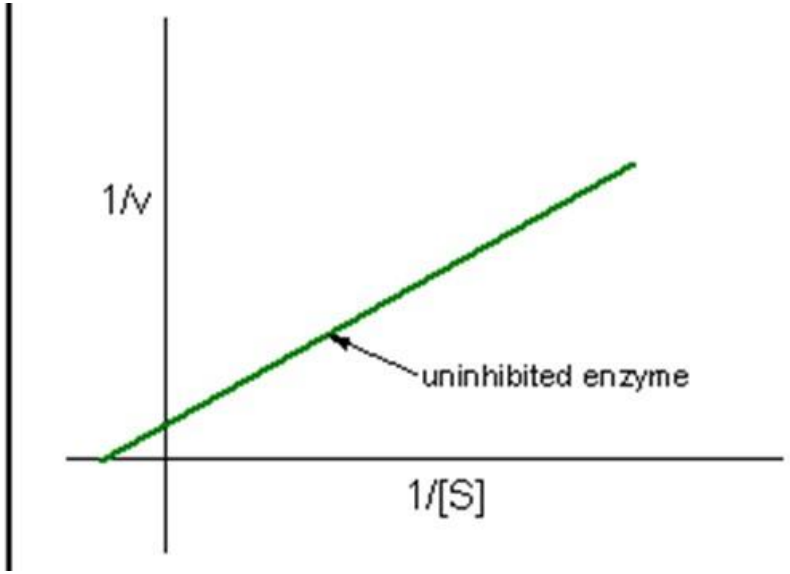


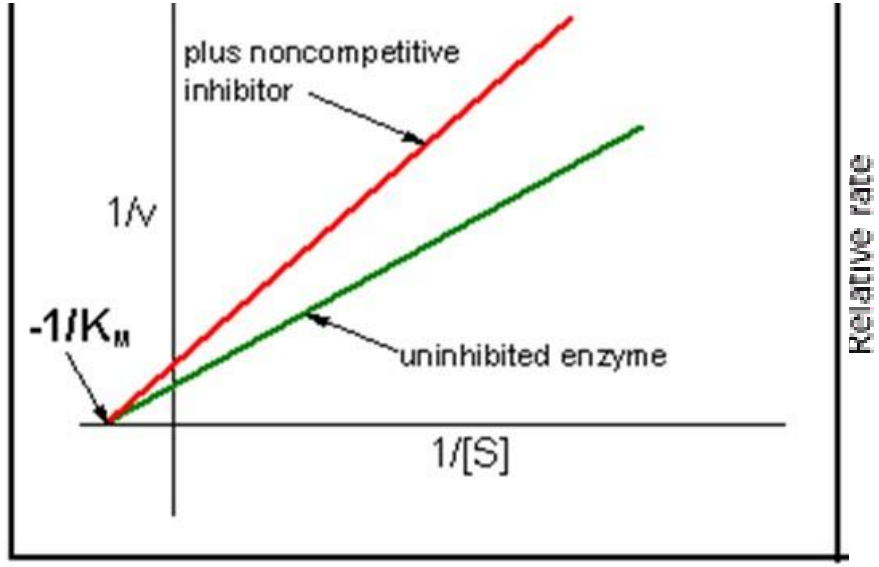


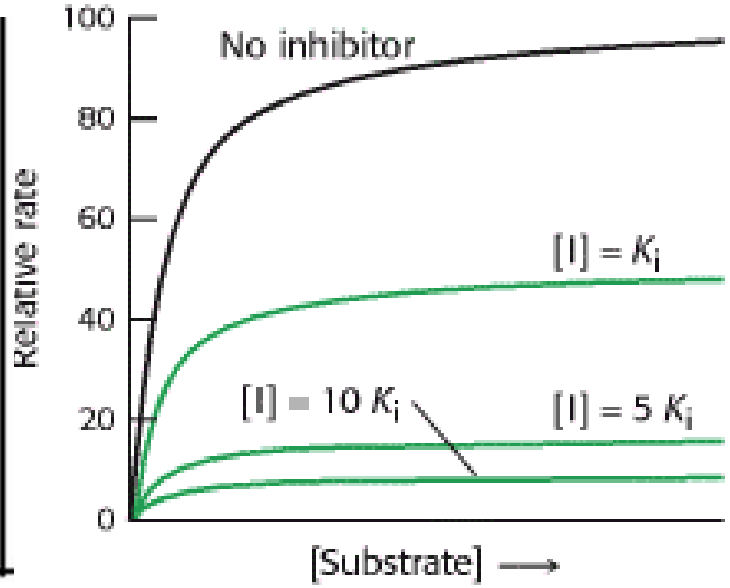
Noncompetitive inhibition

- Noncompetitive inhibitors bind E or ES complex at a site other than the catalytic site.
- Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product.
- Lower Vmax, but same K_M







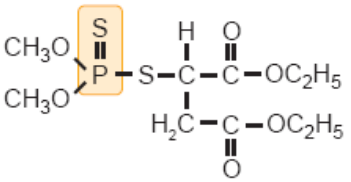


Mechanism-based inhibitors
Irreversible inhibitors

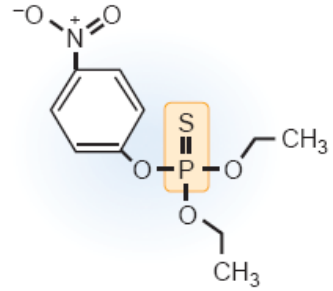
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction.
- They include:
  - Covalent inhibitors
  - Transition state analogs
  - Heavy metals
- Irreversible inhibitors decrease the concentration of active enzyme.

Covalent inhibitors

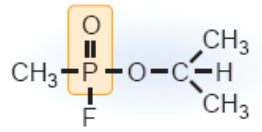
- They form covalent or extremely tight bonds with active site amino acids.
  - Example: di-isopropyl fluorophosphate (DFP) is an organophosphate
    - The nerve gas sarin
    - The insecticides malathion & parathion.
    - DFP inhibits acetylcholinesterase preventing the degradation of the neurotransmitter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



Malathion

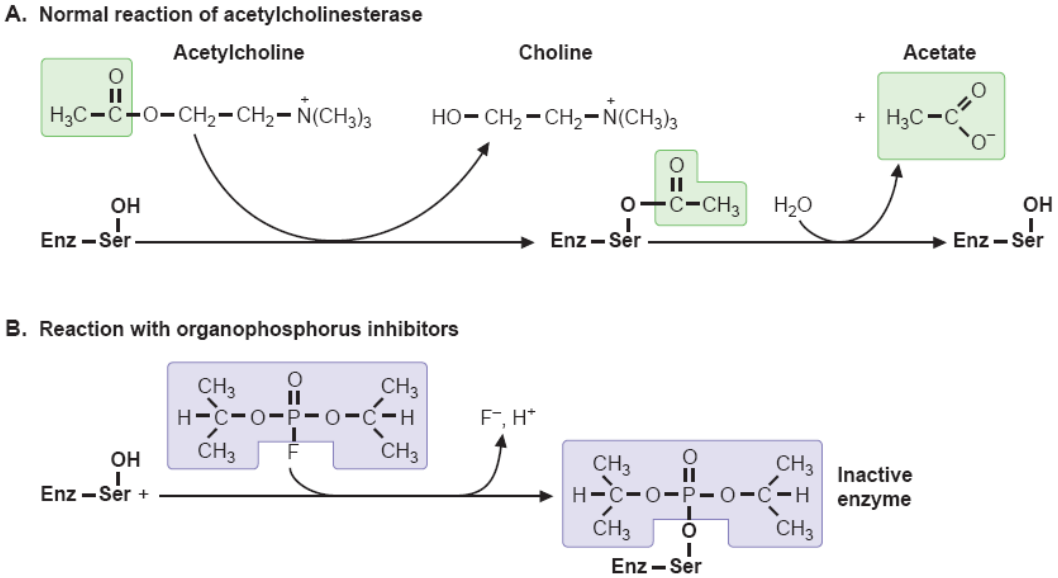


Parathion



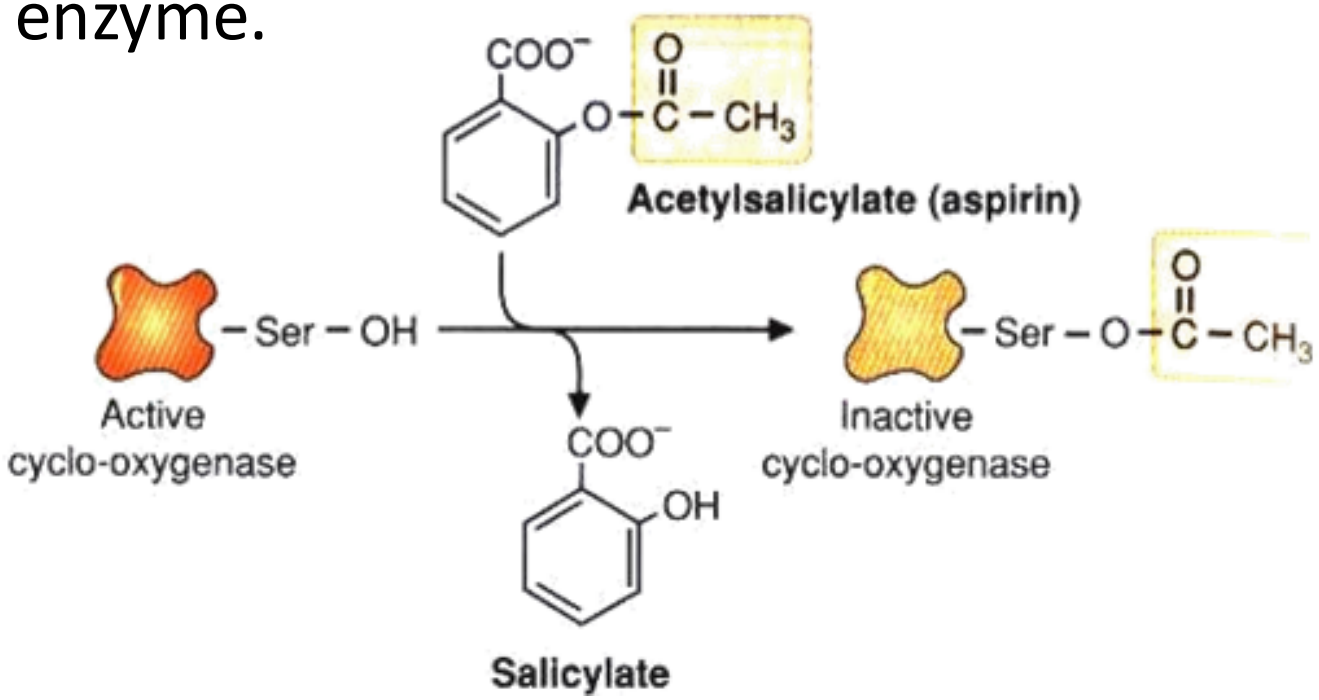
Sarin

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



Aspirin

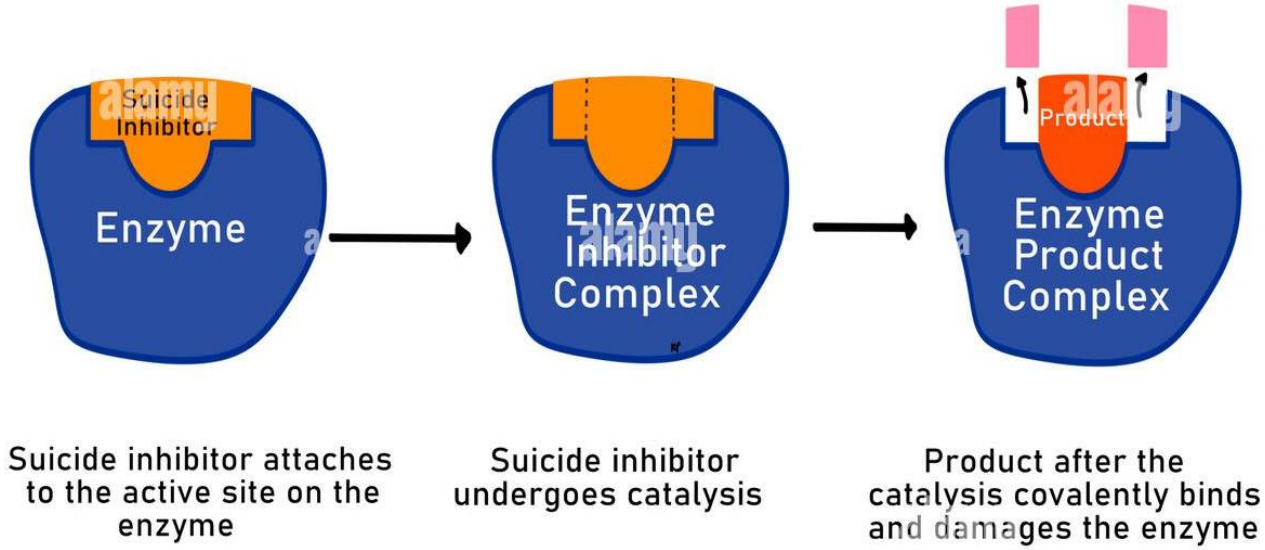
- Aspirin (acetylsalicylic acid) acetylates an active site serine of cyclooxygenase.
- Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme.



Substrate and transition-State analogs

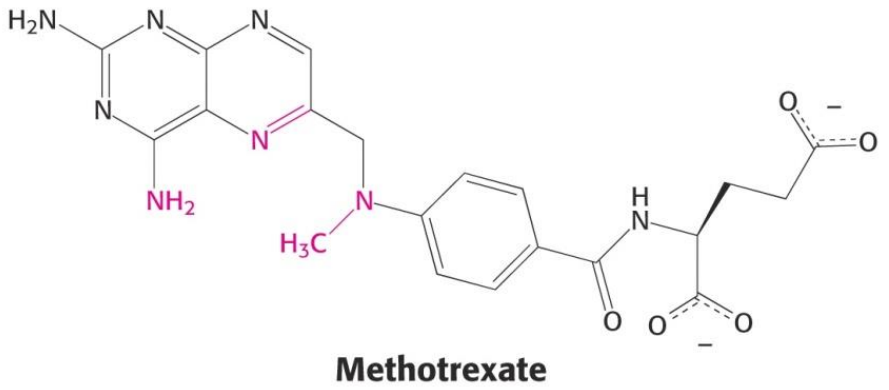
Suicide inhibitors

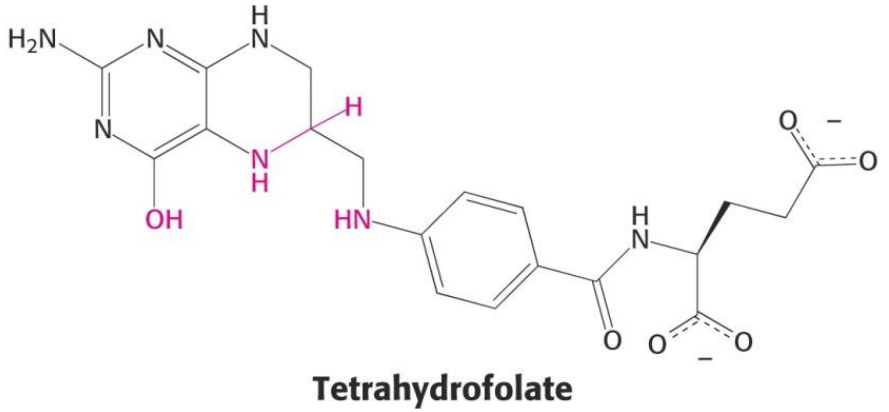
- They bind more tightly than substrates.
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure).

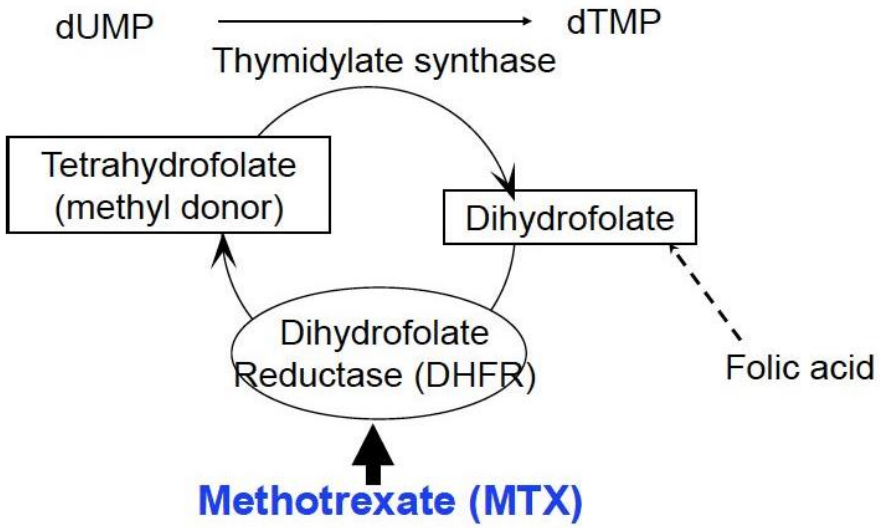


Methotrexate

- Methotrexate is a synthetic inhibitor used to treat cancer.
- 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.

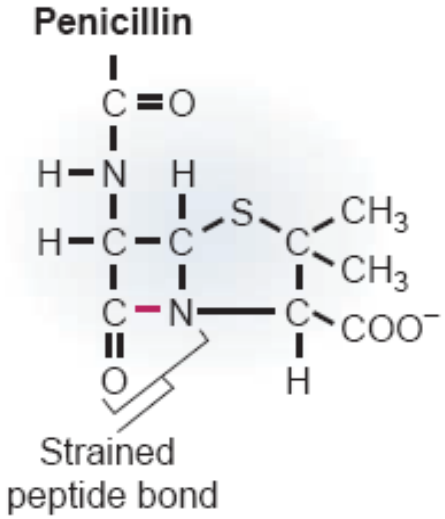


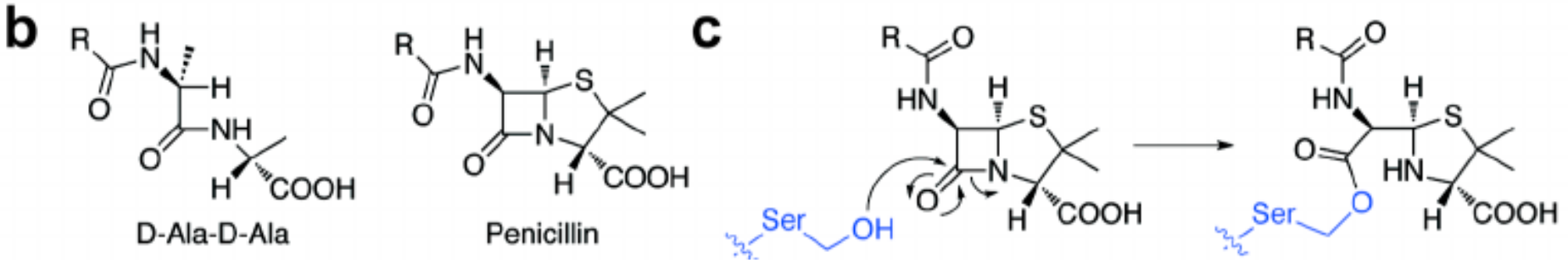




Penicillin

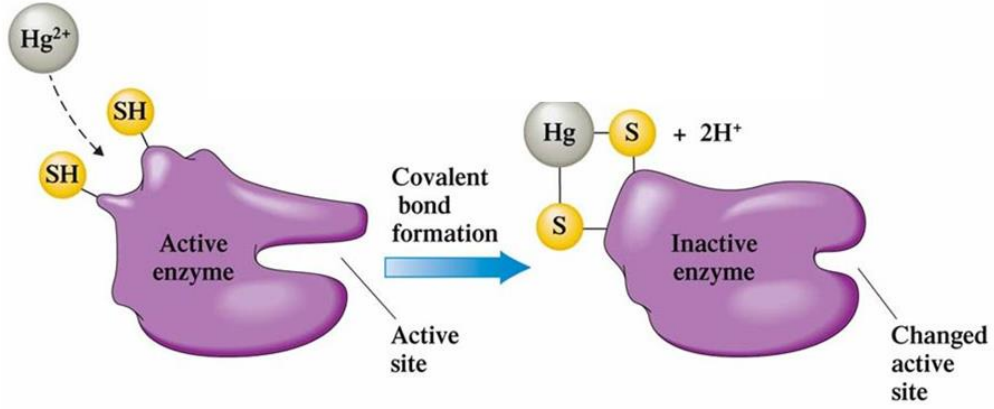
- It is a transition-state analog to glycopeptidyl transpeptidase, which is required for synthesis of the bacteria cell wall.
- The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex.
- The active site serine attacks the highly strained β -lactam ring resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme.





Heavy Metals

- 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.



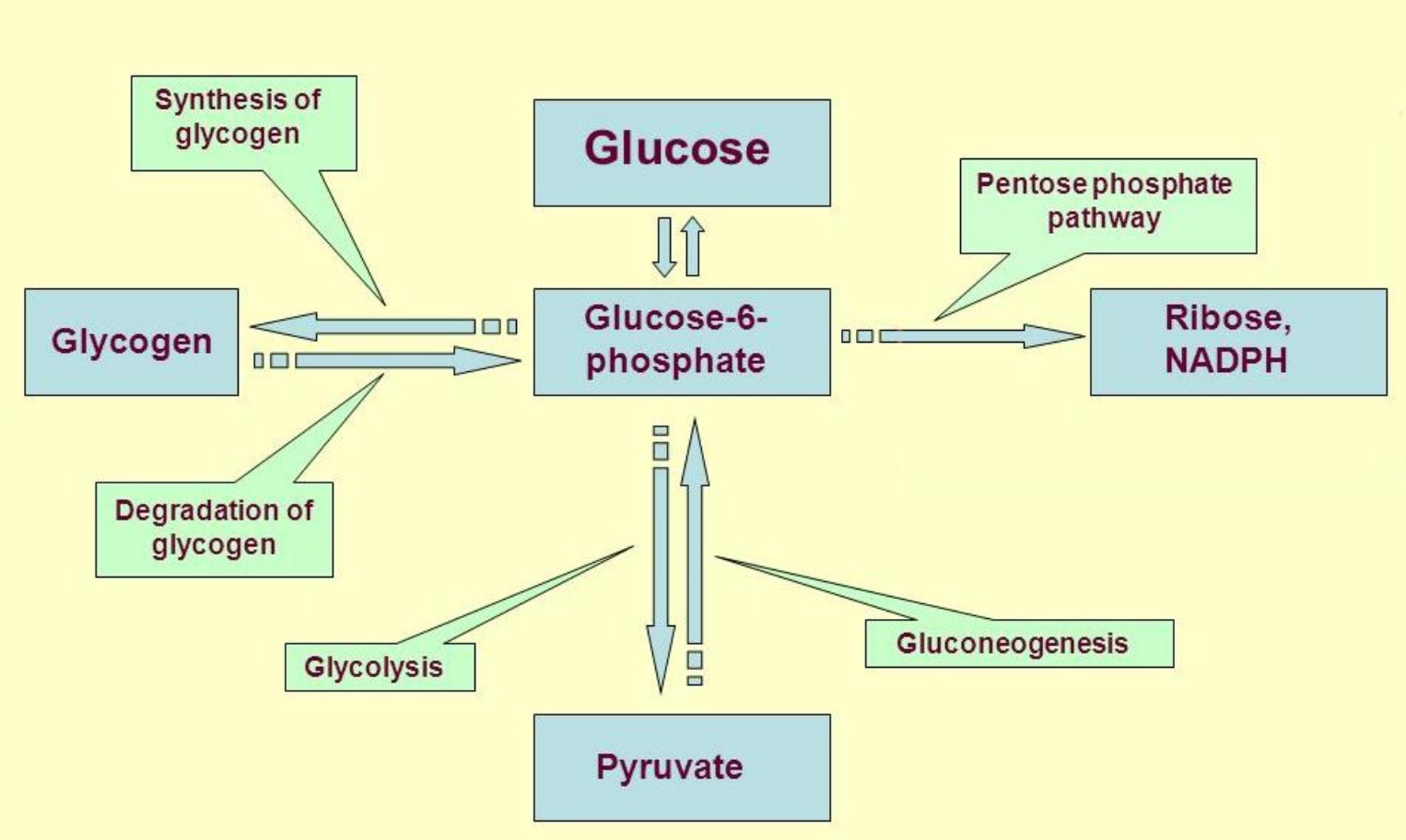
- Lead replaces the normal functional metal in an enzyme such as calcium, iron, or zinc by an irreversible mechanism.
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca+2 in several regulatory proteins that are important in the central nervous system and other tissues.

Regulation through conformational changes

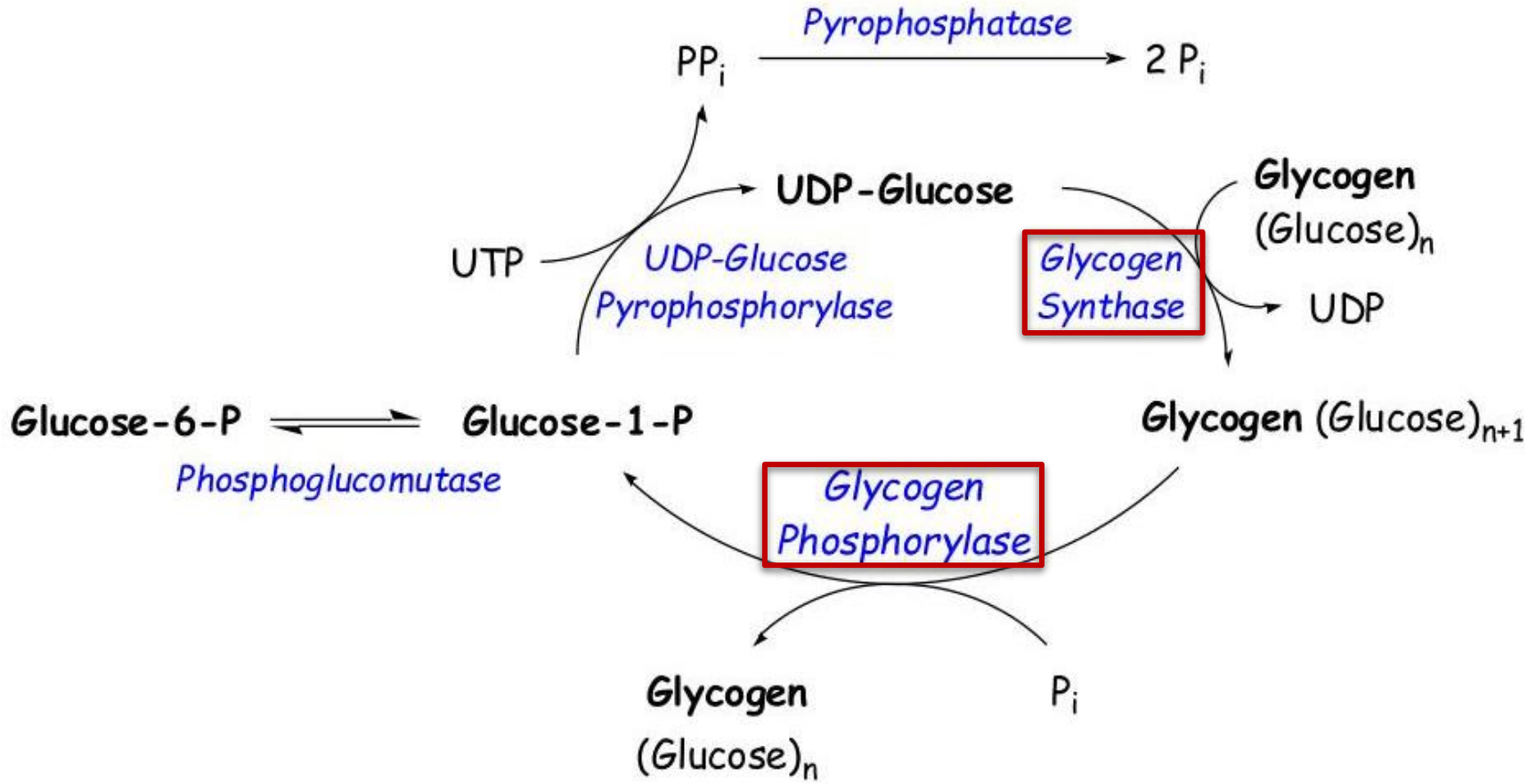
Regulation: conformational changes

- 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.

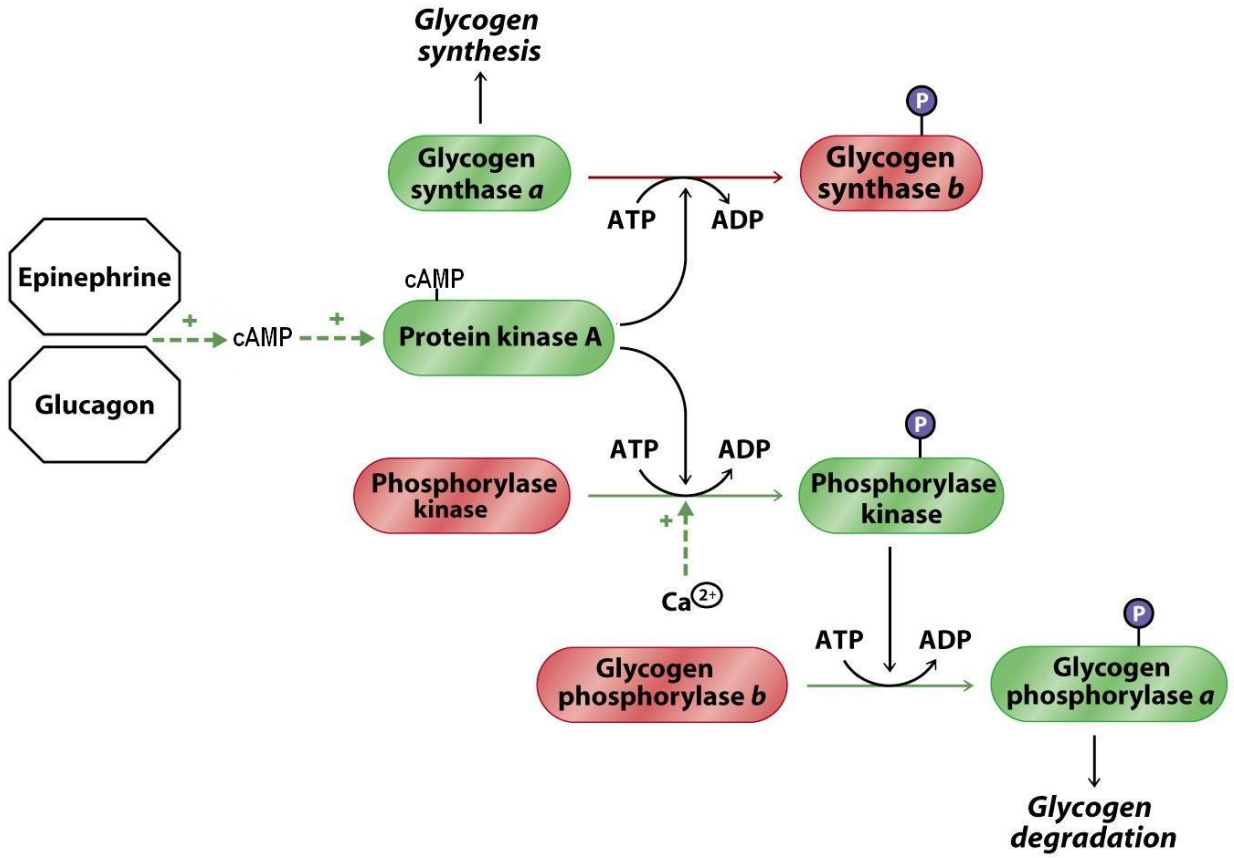
The different fates of glucose



Metabolism of glycogen



Regulation by phosphorylation



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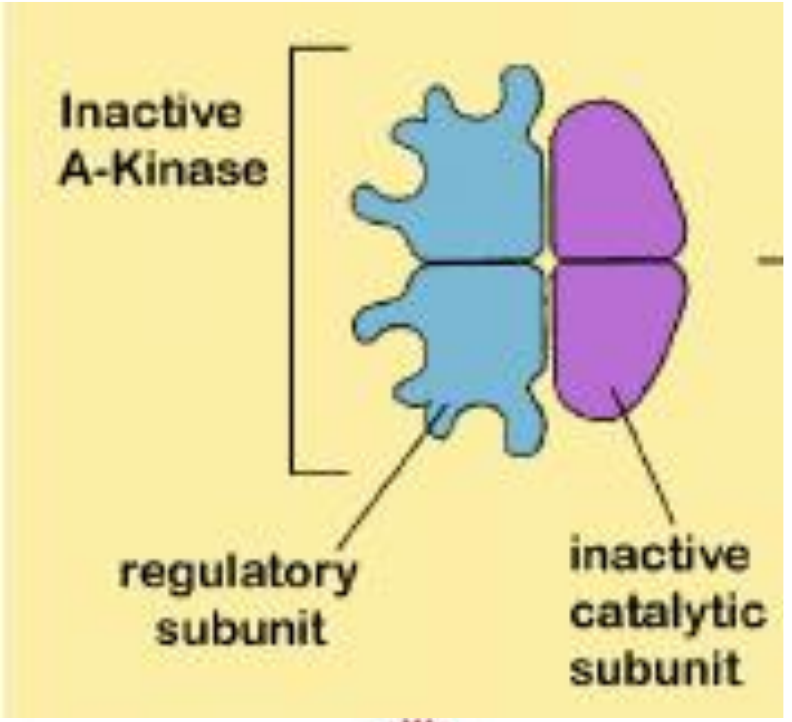
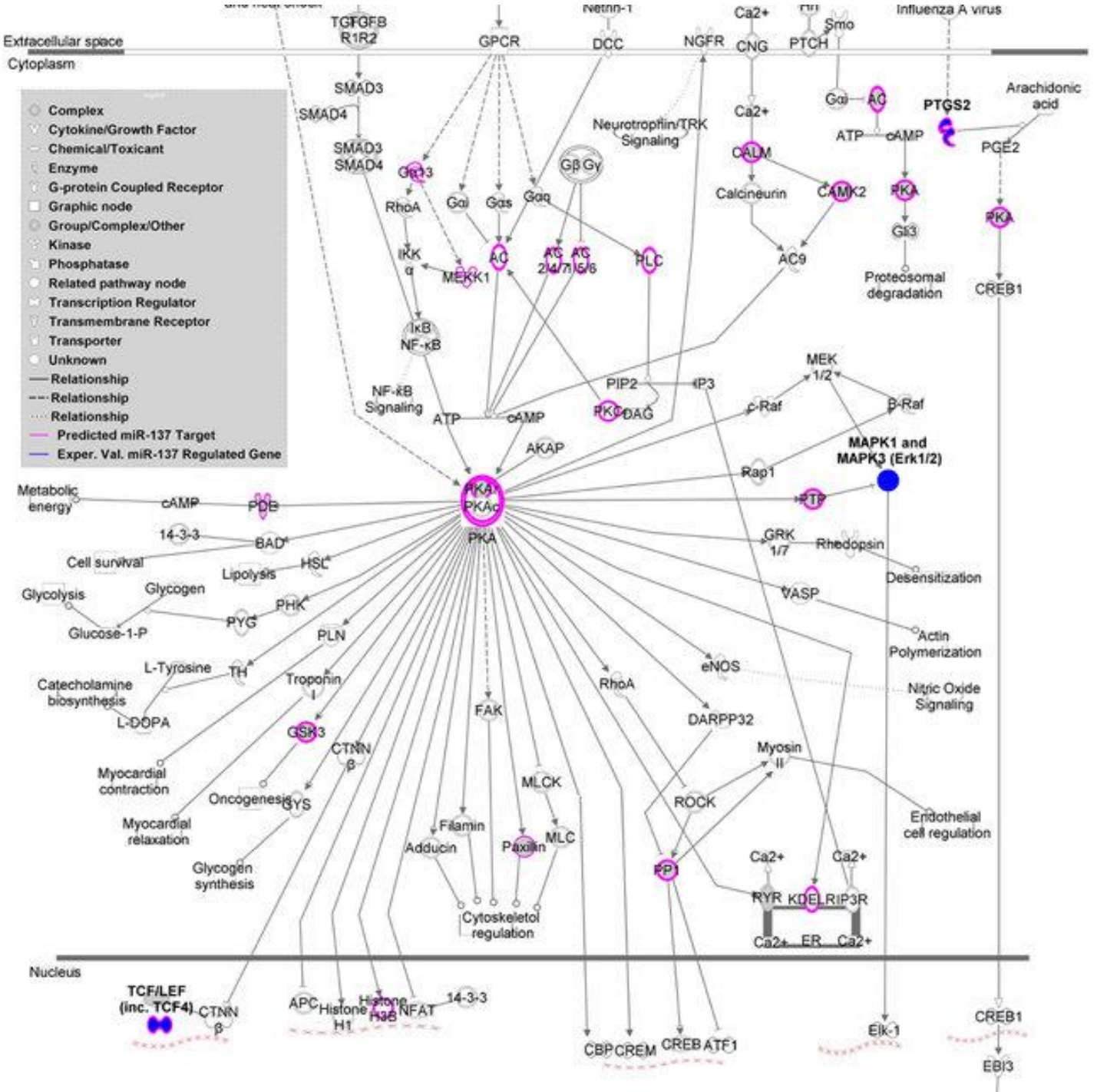
PKA-structure and regulation

- 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



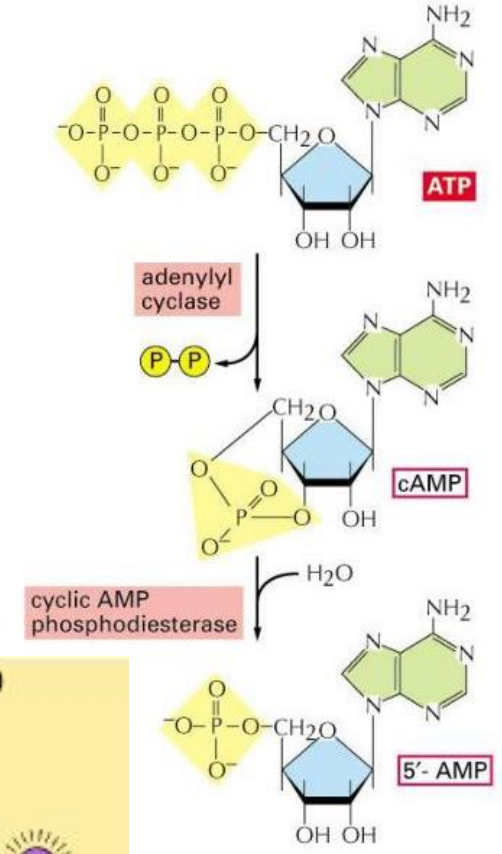
The many targets of PKA

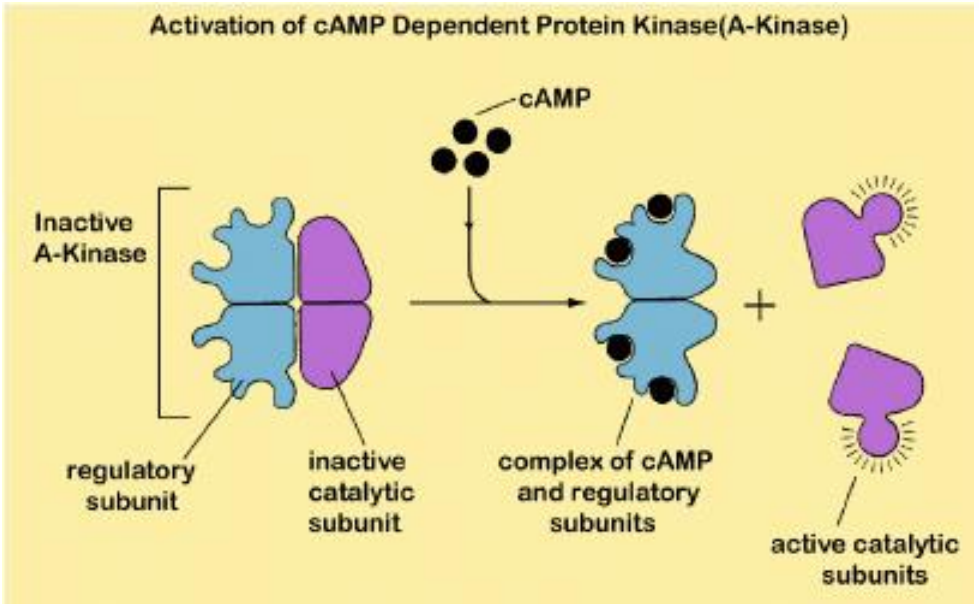


cAMP and protein kinase A (PKA)

- 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.

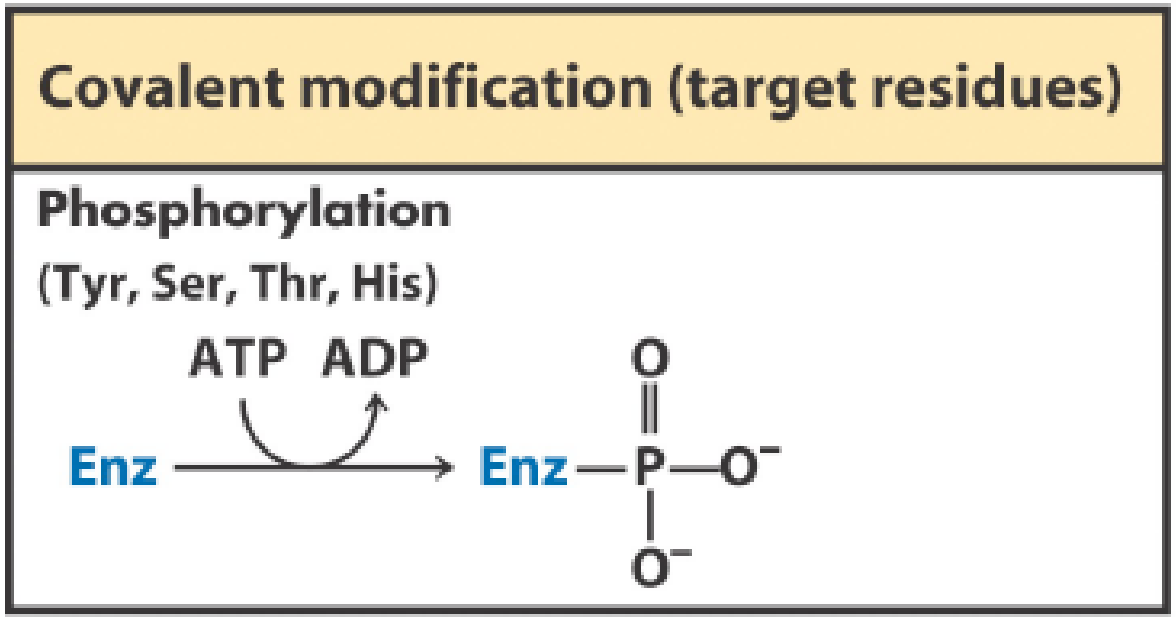




Reversible covalent modification

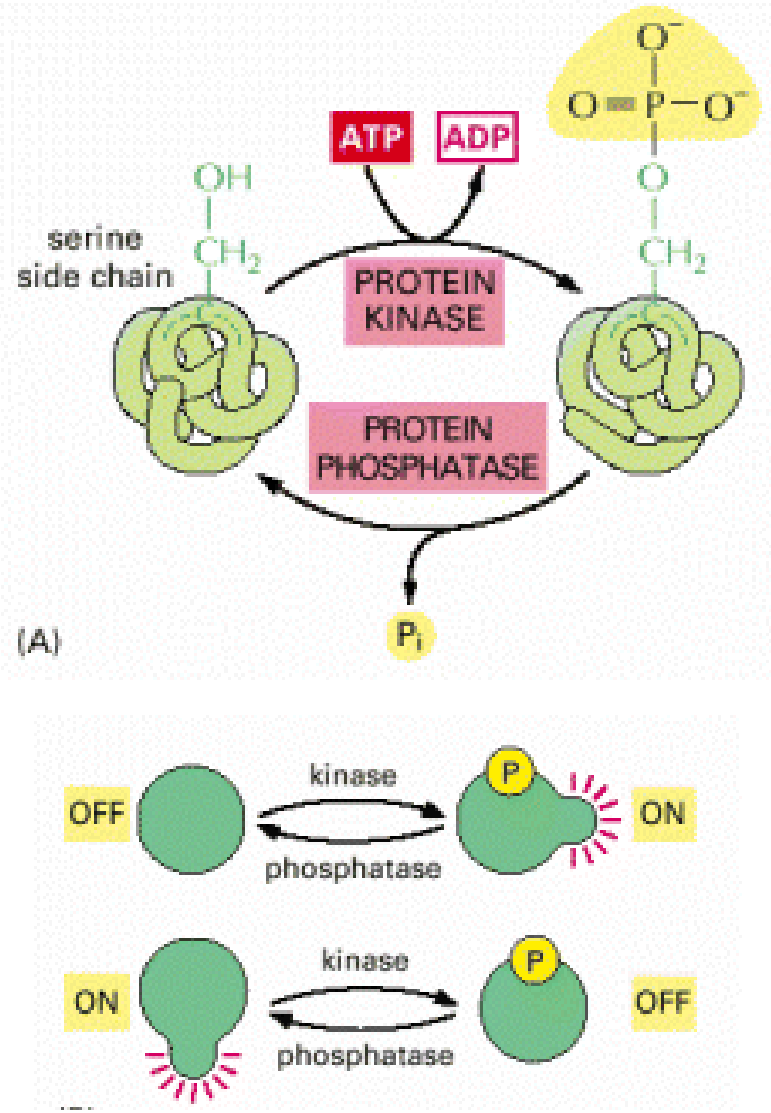
Advantage

- Rapid and transient
- 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



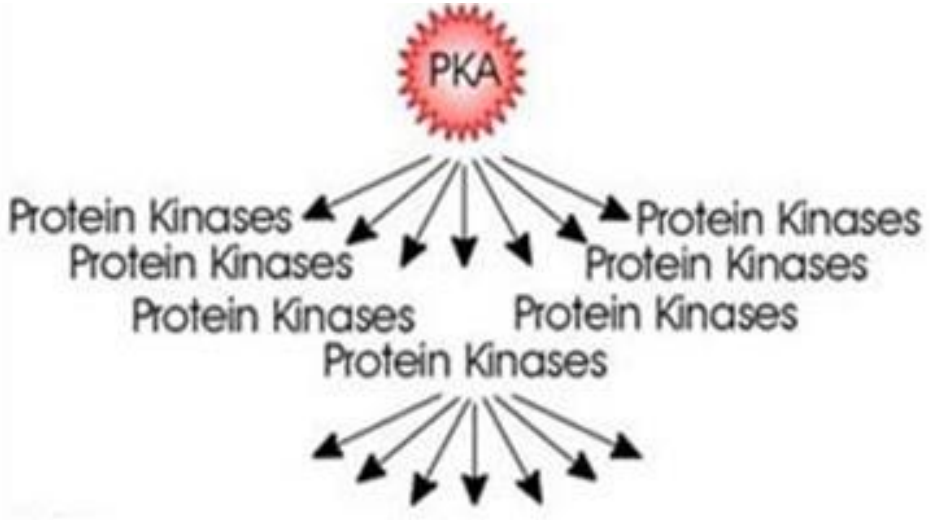
Enzymes

- 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.



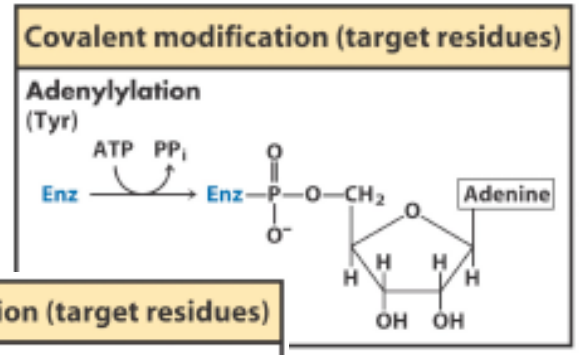
Why is phosphorylation effective?

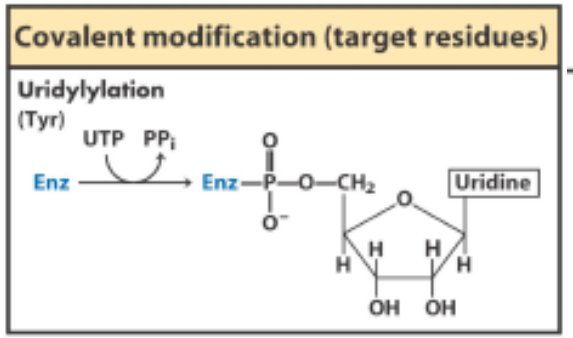
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.

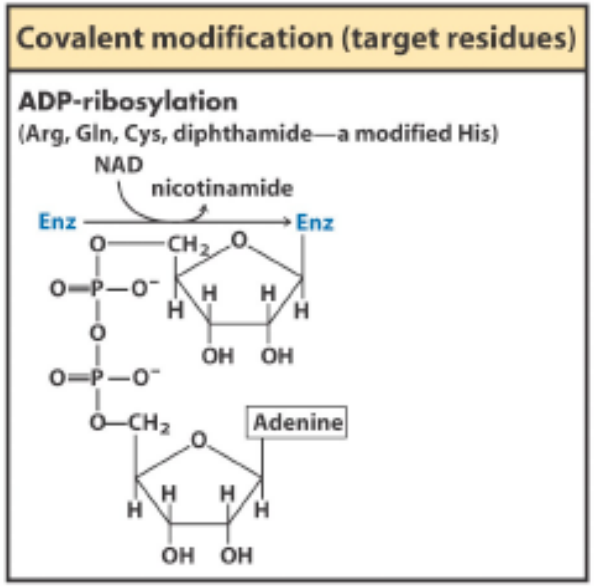


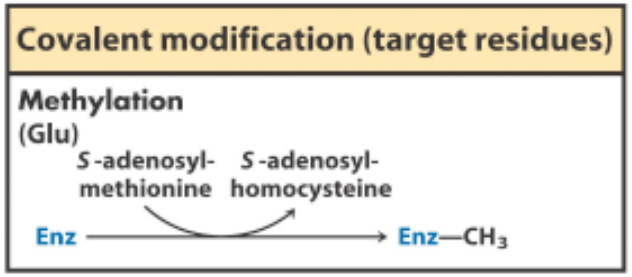
Others

- 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.







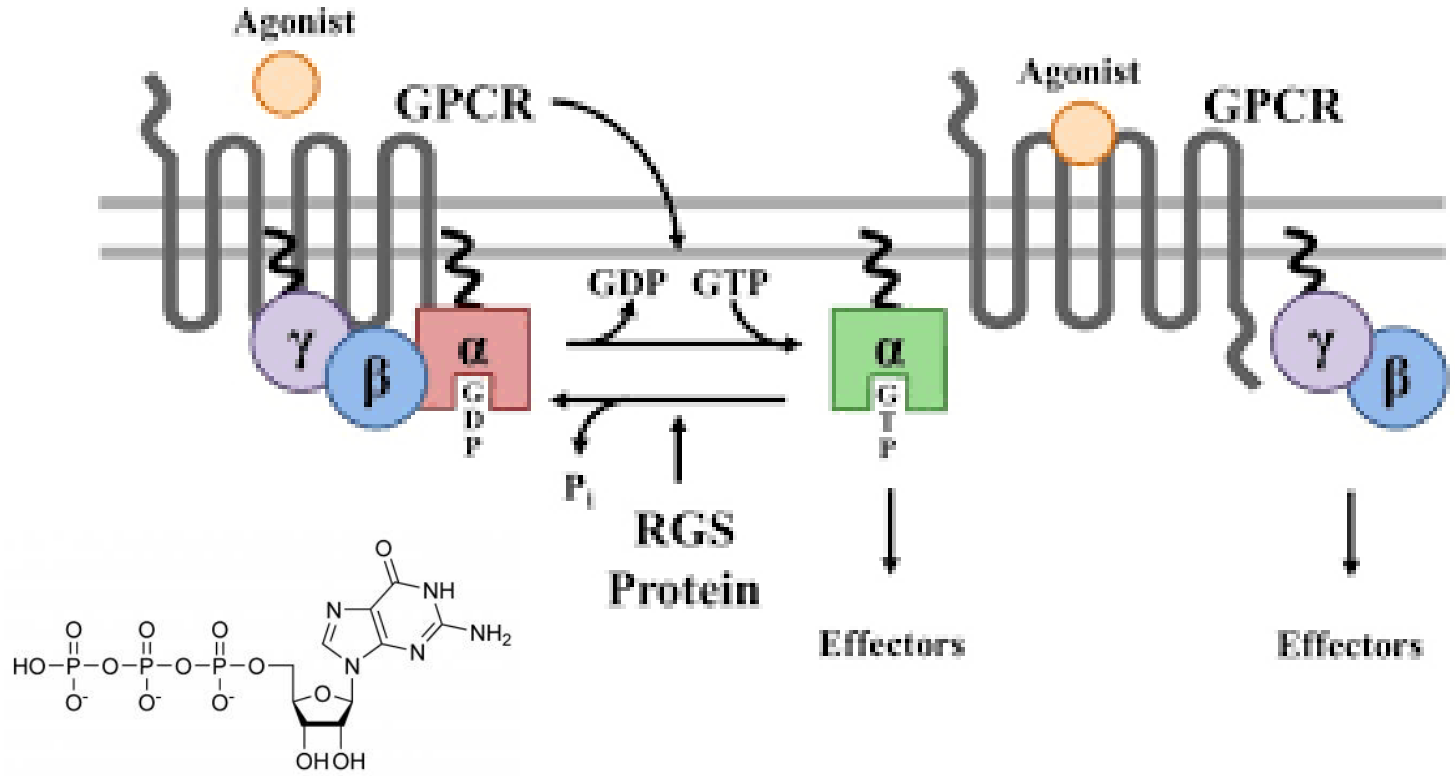


Large and small regulatory modulators

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)

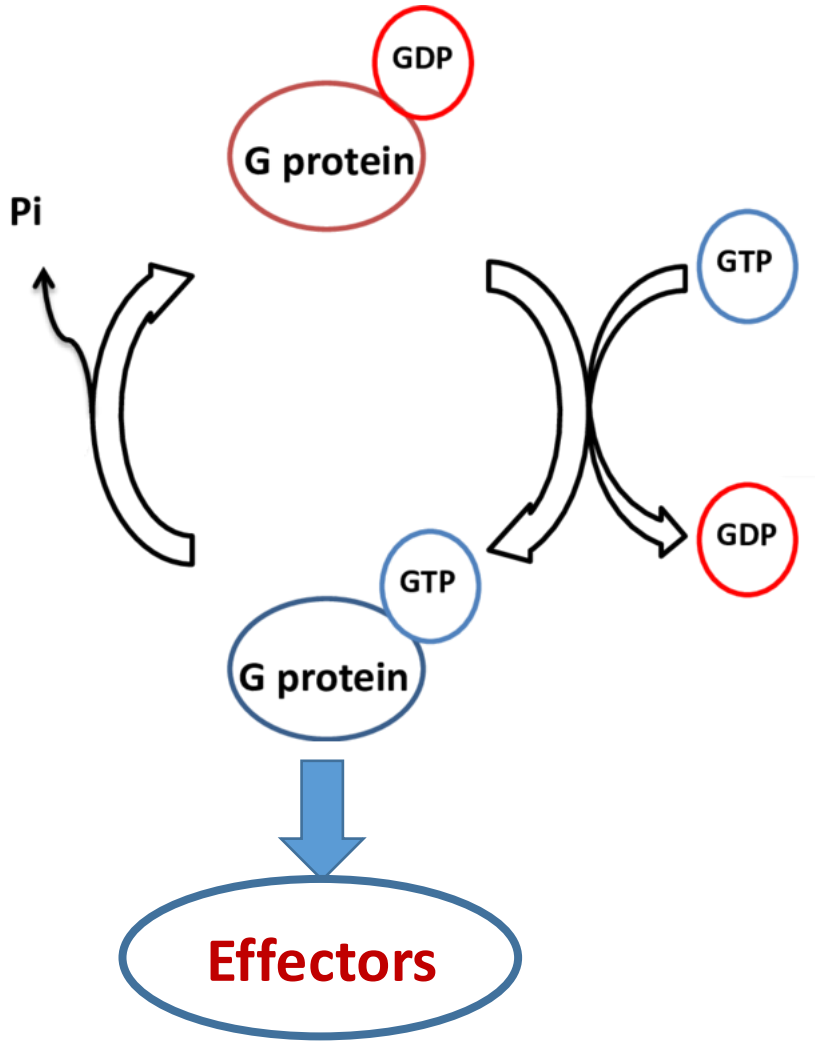
  - **When they bind GTP (small), they are 'on', and, when they bind GDP, they are 'off'.**
  - **The α subunit (large) binds to effectors stimulating or inhibiting them.**



Large and small regulatory modulators

Small monomeric G proteins

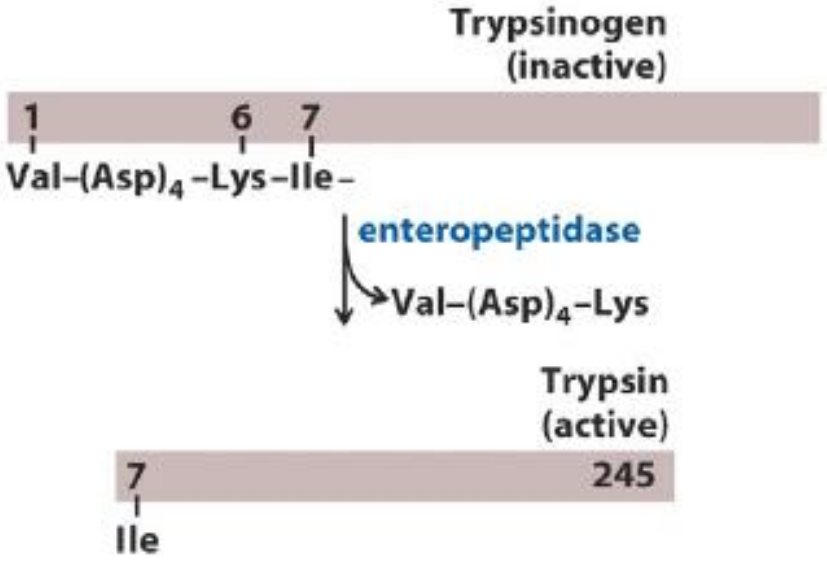
- When GTP (small) is bound, the conformation of the small monomeric G protein (large) allows it to bind target proteins, which are then activated or inhibited.
- The small monomeric G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein.
- GDP is exchanged for GTP, which reactivates the small monomeric G protein.

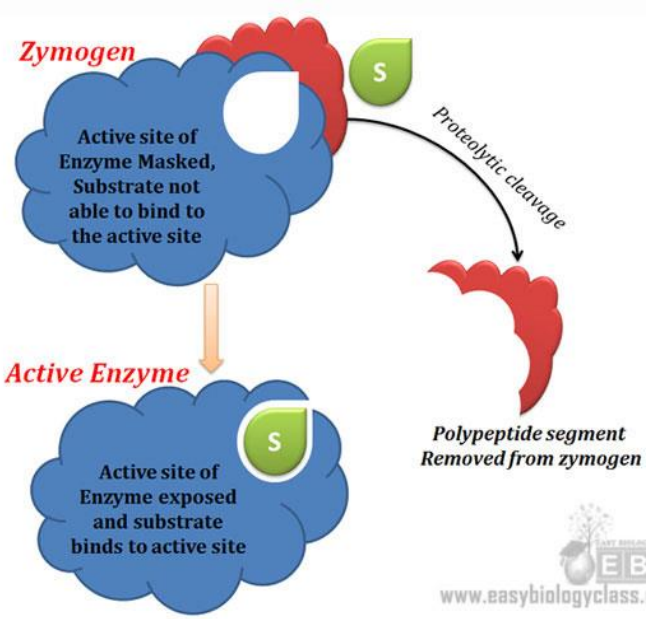


Irreversible covalent modification (Proteolytic activation)

Zymogens

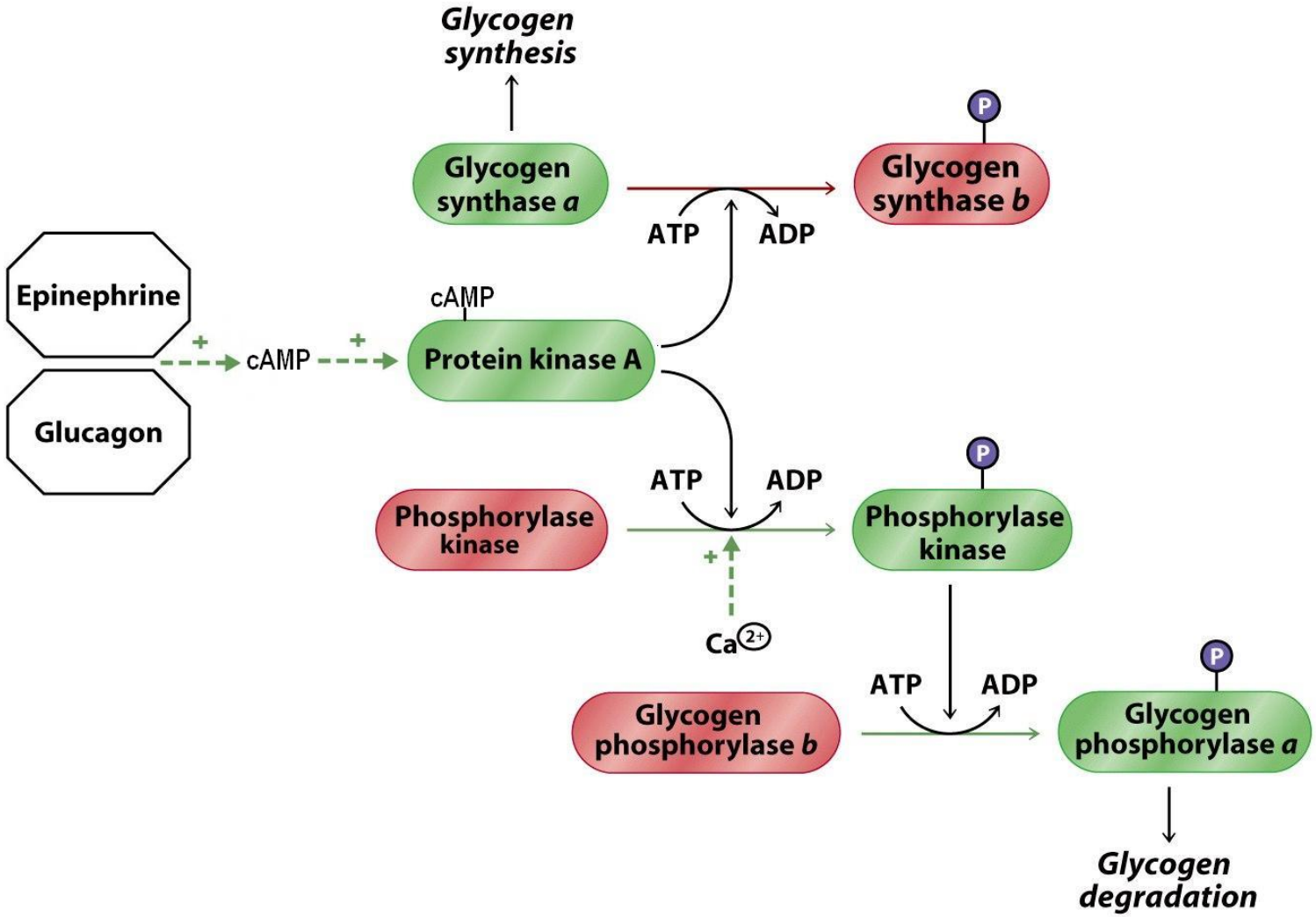
- Zymogens or proenzymes are inactive precursors of enzymes.
- Activation is done by irreversibly removing part of the enzyme (usually known as the pro region present at the N-terminus).
- Examples: digestive enzymes such as chymotrypsin, trypsin, and pepsin that get activated when food is ingested.
  - Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus.





Allosteric regulation

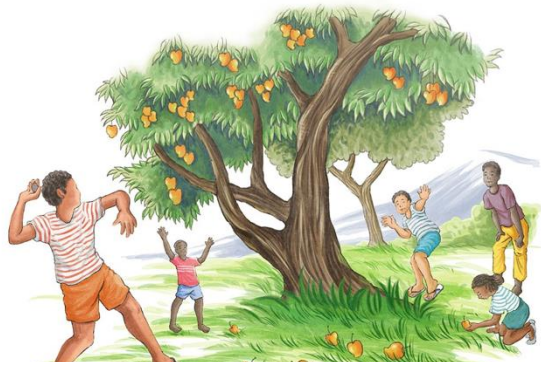
Phosphorylation cascade



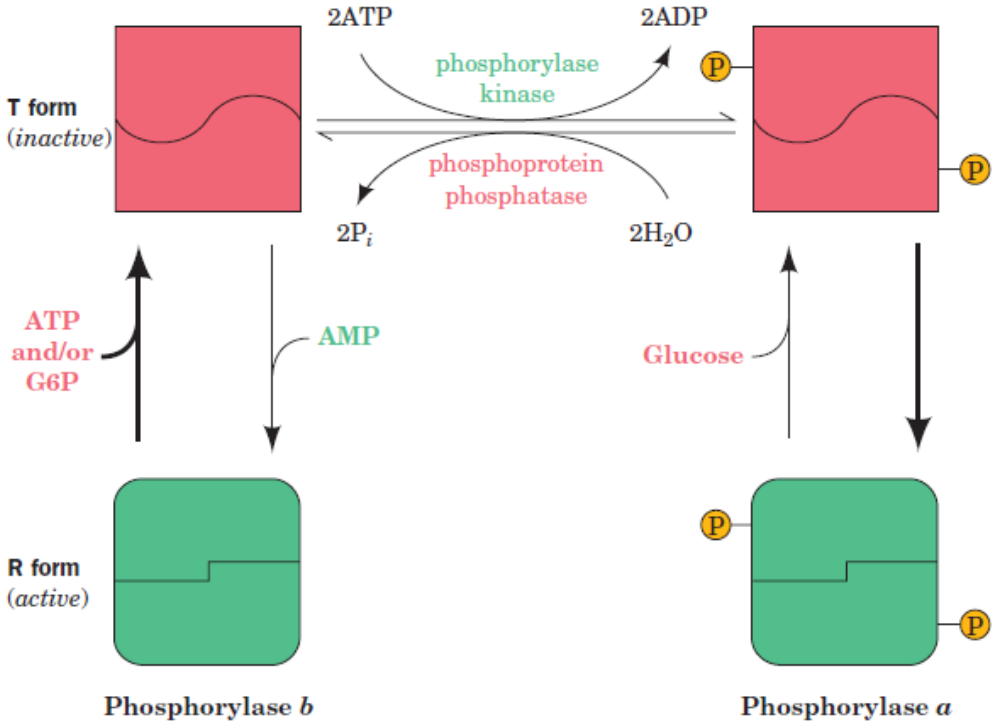
$$\text{glycogen}_{(n\ \text{residues})} + P_i \longrightarrow \text{glycogen}_{(n-1\ \text{residues})} + \text{glucose 1-P}$$

Example: Glycogen phosphorylase

- GP catalyzes removal of glucose molecules from glycogen.
- The enzyme exists in four forms:
  - T (inactive) and R (active) states
  - Phosphorylated (a) and dephosphorylated (b)

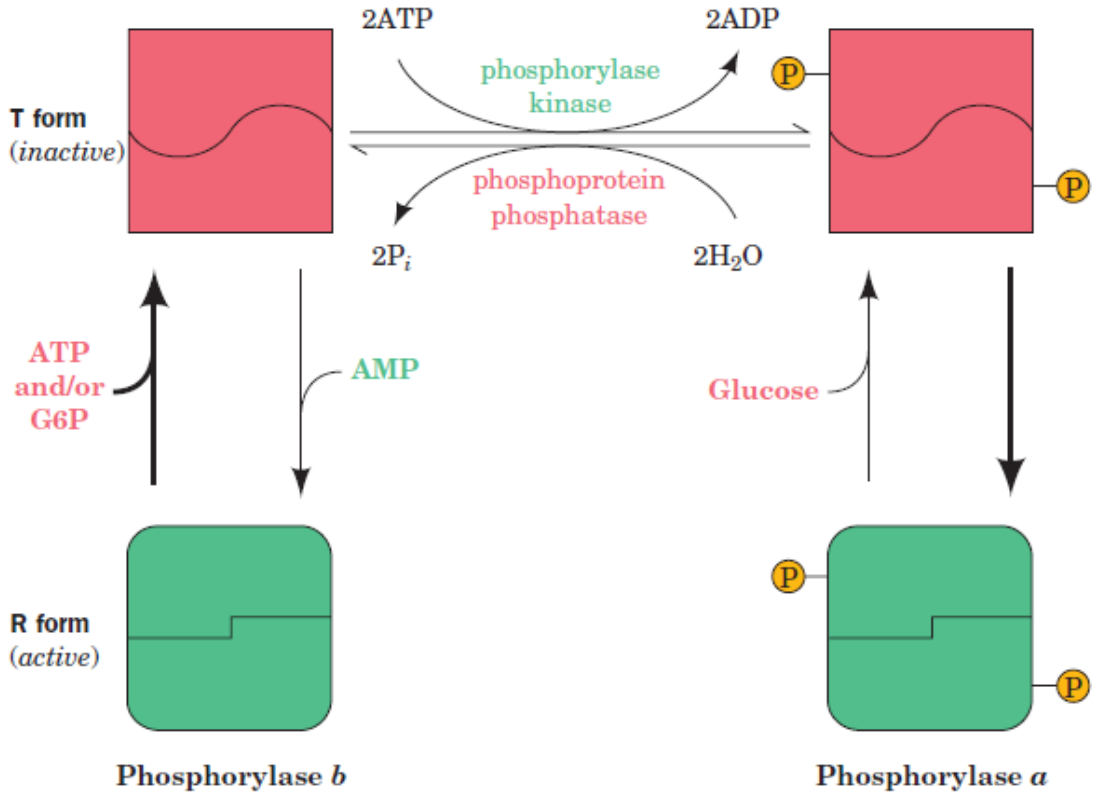


- *When phosphorylated, it is known as phosphorylase a.*
- *When dephosphorylated, it is known as phosphorylase b.*



The two forms of each form

- Both phosphorylase b and phosphorylase a exist in equilibrium between an active R state and a less-active T state.
- Phosphorylase b is usually inactive because the equilibrium favors the T state.
- Phosphorylase a is usually active because the equilibrium favors the R state.

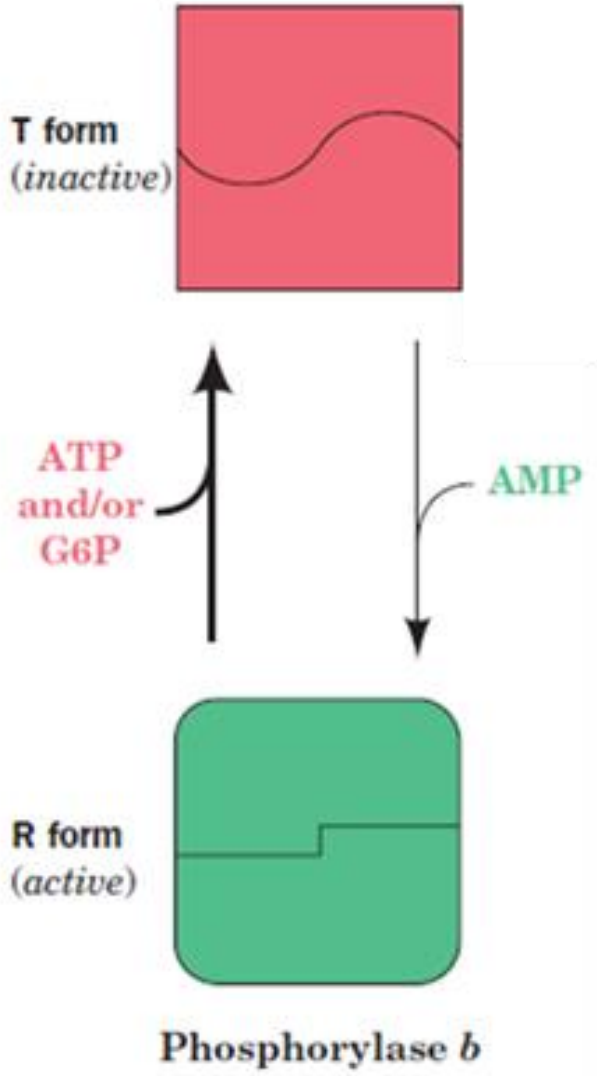


The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell and availability of glucose-6-phosphate.

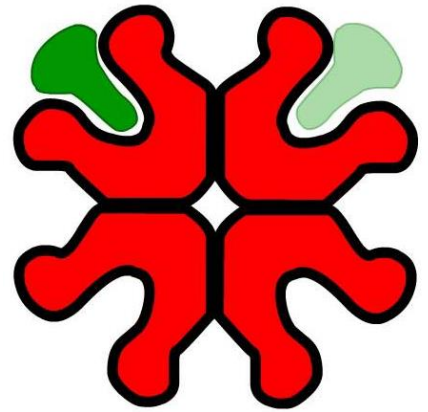
What do ATP and AMP do?

- Muscle phosphorylase b is active only in the presence of high concentrations of AMP, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase b in the R state.
- ATP acts as a negative allosteric effector by competing with AMP and so favors the T state.

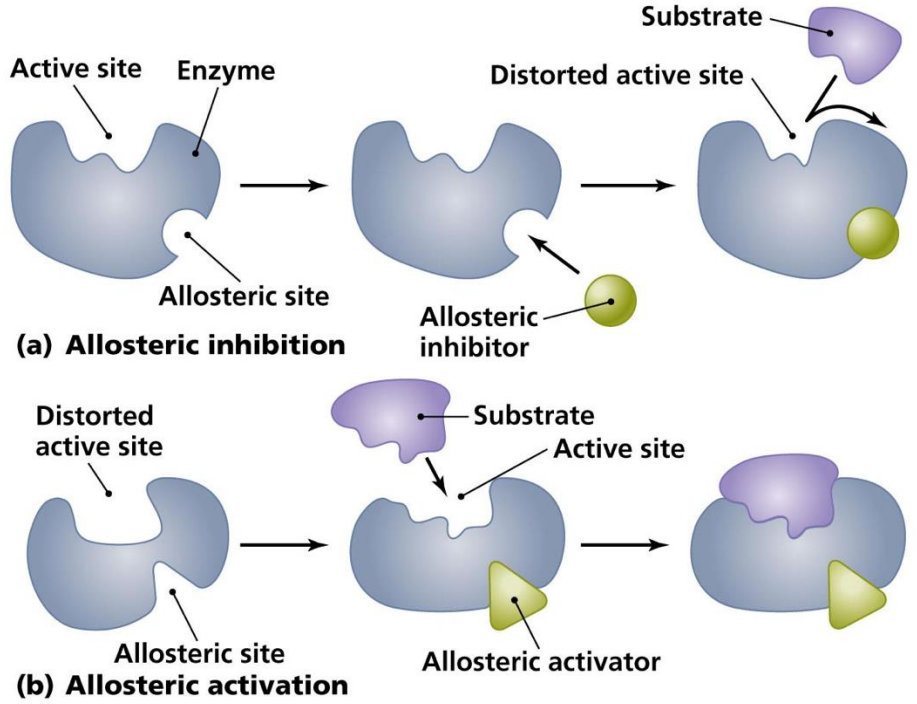
Glucose 6-phosphate also favors the T state of phosphorylase *b*, an example of feedback inhibition.



Allosteric enzymes and their modifiers

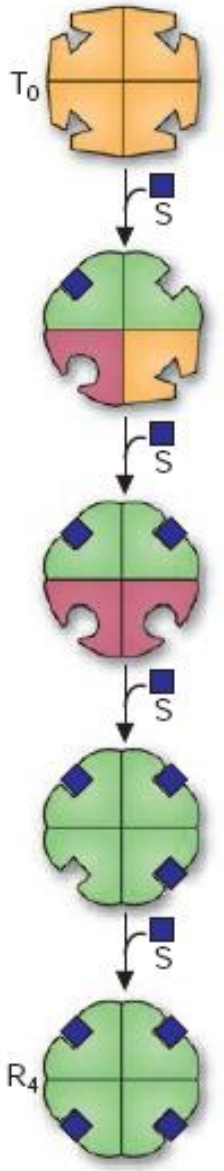


- Allosteric enzymes are multi-subunit proteins;
  - One subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).
  - Multiple active sites can exist on multiple subunits.
- The binding of regulatory molecules triggers conformational changes in the active site via modifying non-covalent interactions.
- Allosteric enzymes bind modifiers at the allosteric site, a site that is physically separate from the catalytic site.
  - A negative allosteric modifier (inhibitor) causes the enzyme to have less activity.
  - A positive allosteric modifier (activator) causes the enzyme to be more active.



More on modifiers

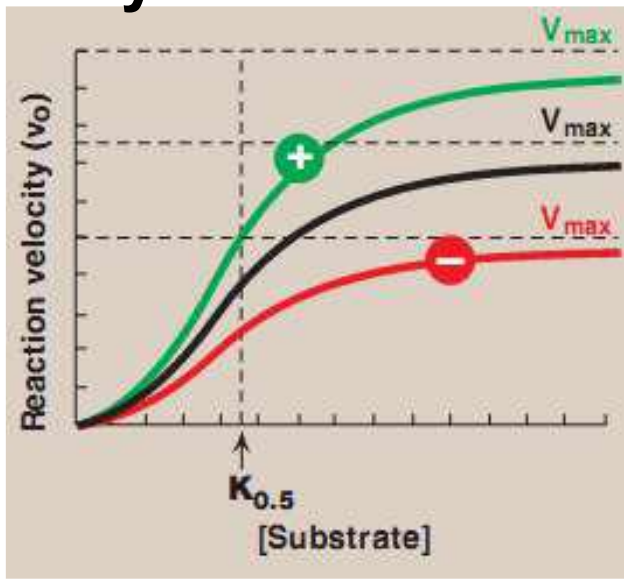
- When the modifier is a molecule other than the substrate, then it is known as heterotropic.
- If the modifier is same as the substrate, then it called homotropic.
  - The binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease.
  - This is called "positive cooperativity”.
  - T to R conformation
  - There is also negative cooperativity.



Types of allosteric enzymes

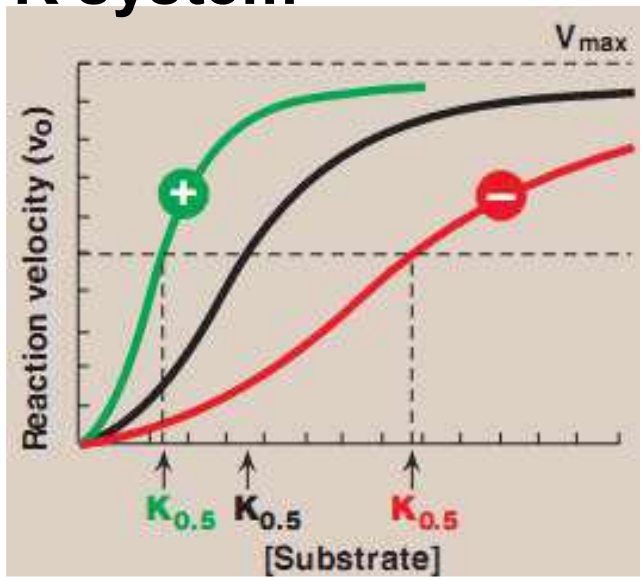
- The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes.
- $K_{0.5}$ is used instead of K_M.

V system



Same K0.5, Different Vmax.

K system



Different K0.5, same Vmax.

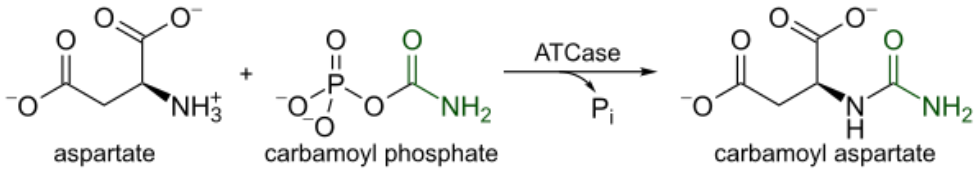
Note near-hyperbolic plot with activators

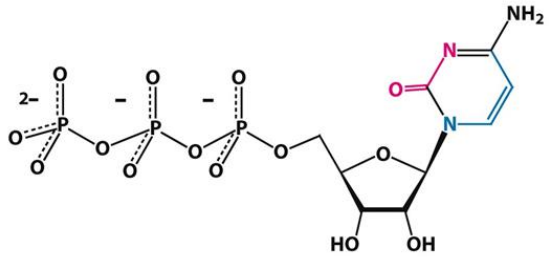
Allosteric enzymes and metabolism

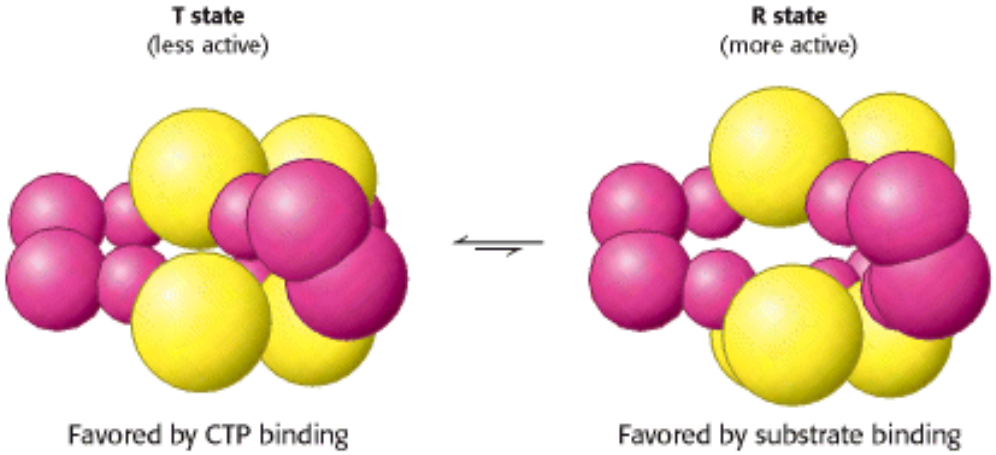
- Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors.
- Allosteric enzymes are not limited to regulation through inhibition whereby allosteric effectors may function as activators.
- The allosteric effector needs not bear any resemblance to substrate or product of the enzyme.
- The effect of an allosteric effector is rapid occurring as soon as its concentration changes in the cell.
  - Feedback regulation of metabolic pathways by end products or by signal molecules that coordinate multiple pathways.

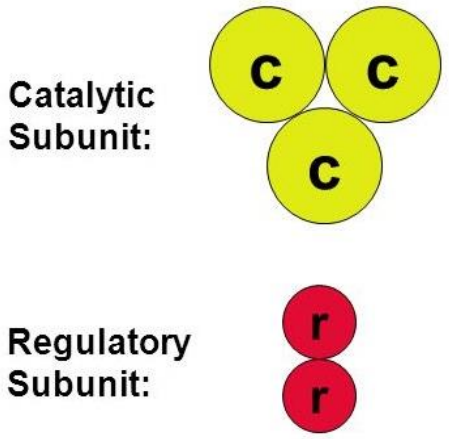
Aspartate transcarbamoylase

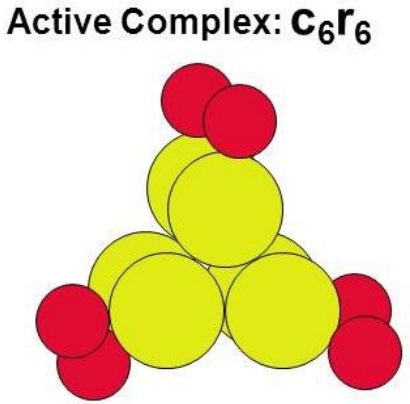
- Aspartate transcarbamoylase (ATCase) catalyzes the first step in the synthesis of pyrimidine nucleotides.
- ATCase consists of 12 polypeptide chains: six catalytic subunits (two trimers) and six regulatory subunits (three dimers).
- It exists in two forms: T state (less active) and R state (more active).





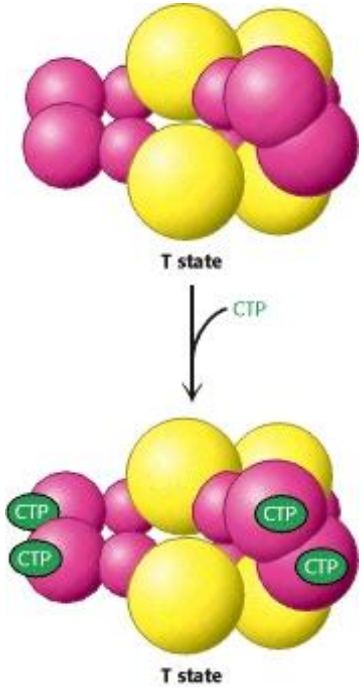


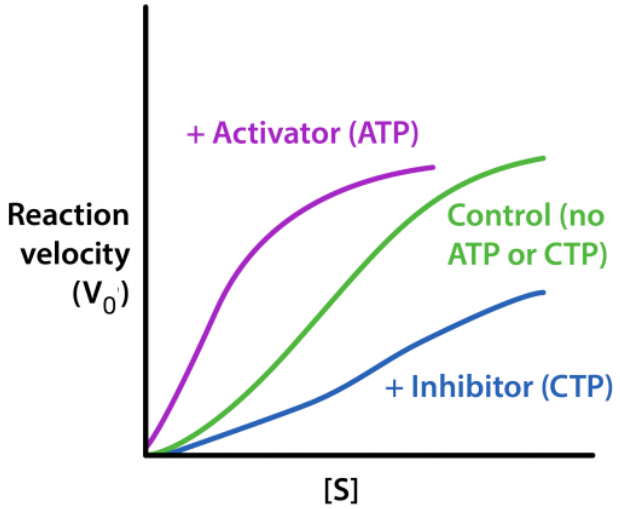




Aspartate transcarbamoylase-regulation

- ATCase is inhibited by CTP, the end-product
  - inducing a major rearrangement of subunit positions
  - stabilizing the T state of the enzyme.
  - decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits
  - increasing K0.5 (K system)
    - Note: a non-competitive inhibitor changes K0.5
    - On the other hand, ATP, a purine, heterotypically activates the enzyme in order to balance the rate of synthesis of purines and pyrimidines in cells.

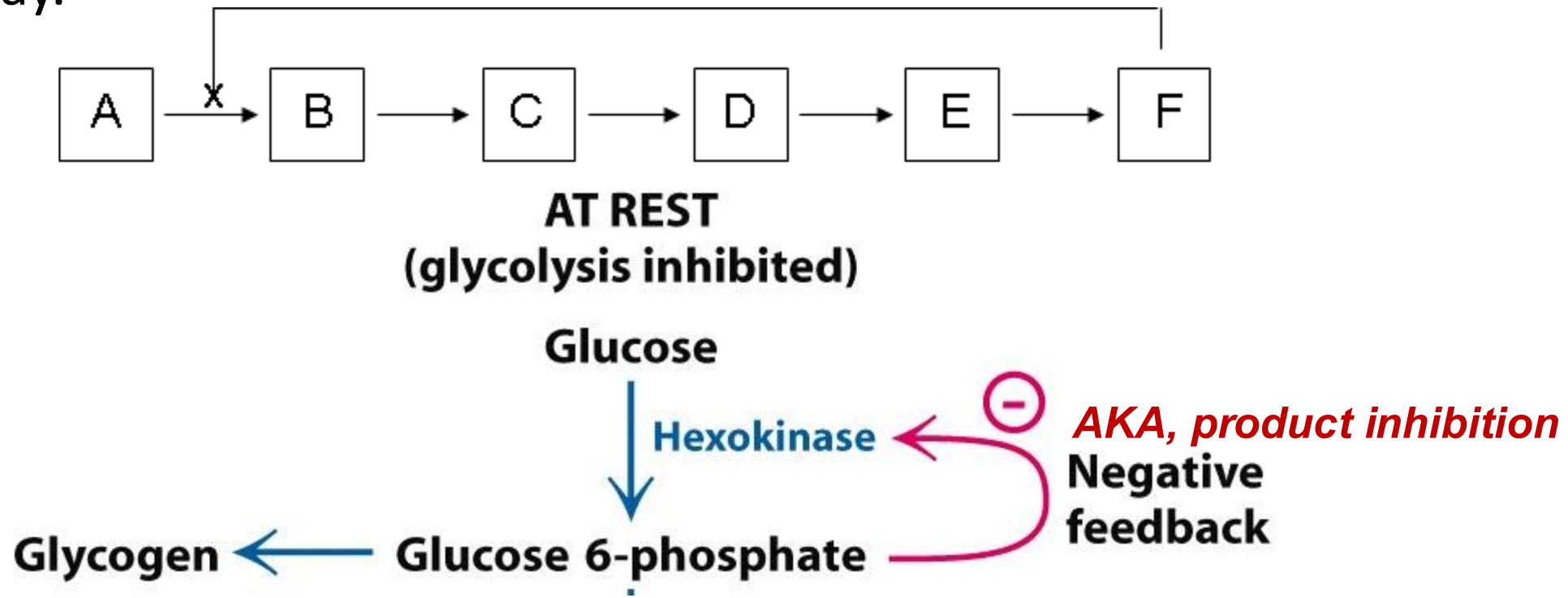




Modes of metabolic regulation

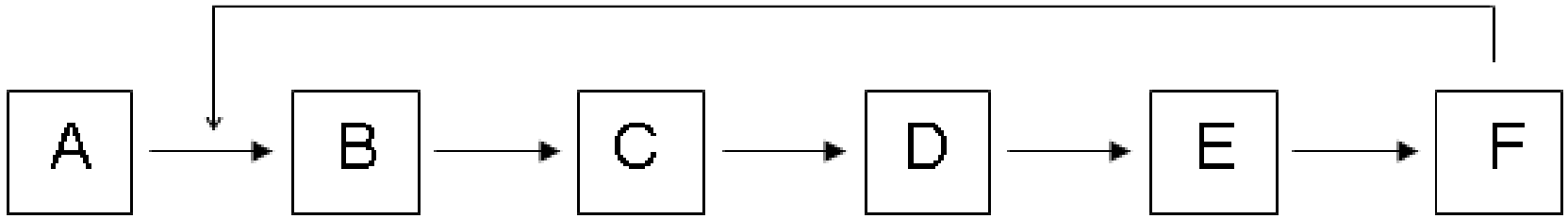
Feedback inhibition

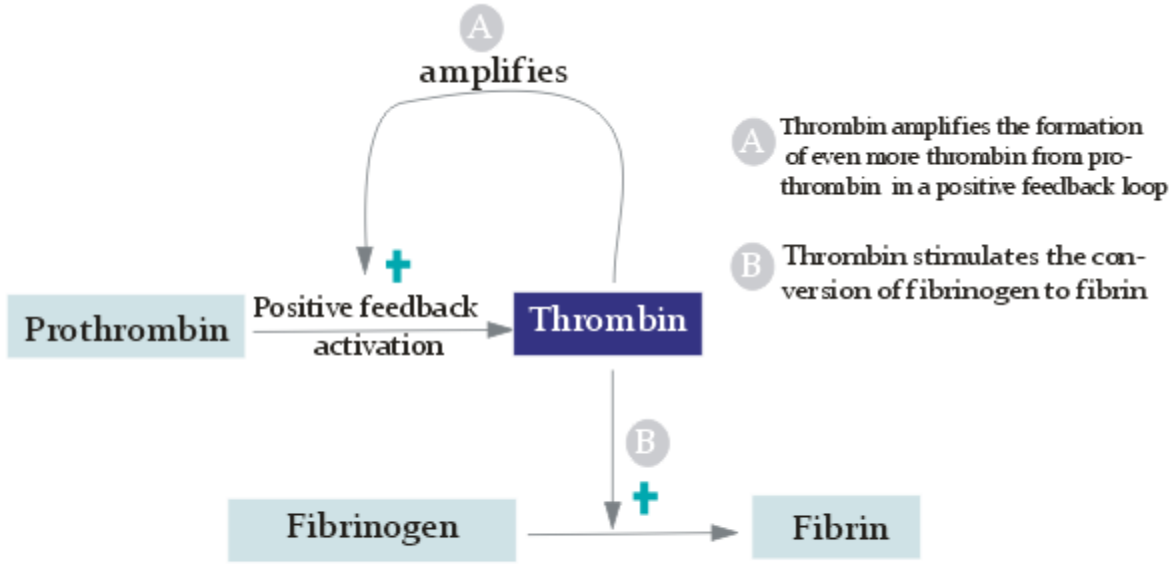
- Feedback inhibition or negative feedback regulation: an enzyme present early in a biochemical pathway is inhibited by a late product of pathway.



Feedback activation

- Positive feedback regulation: a product stimulates the activity of an enzyme.

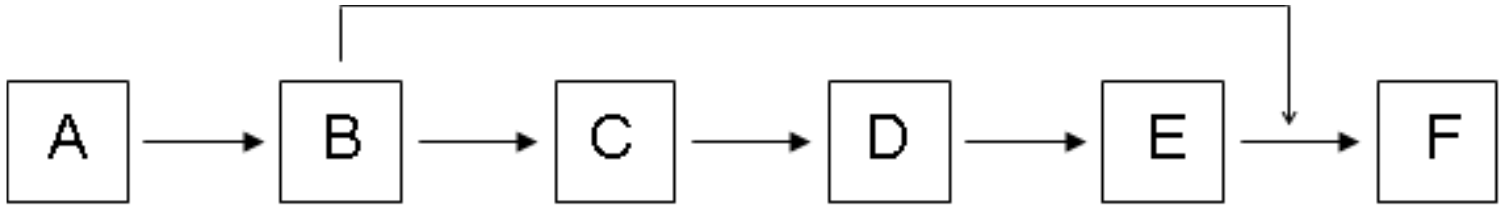




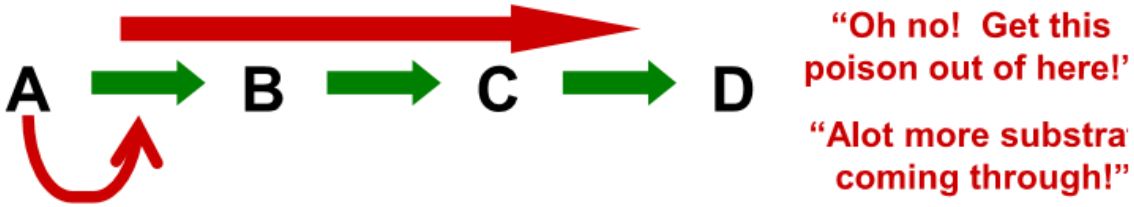
Positive feedback activation of more prothrombin into thrombin

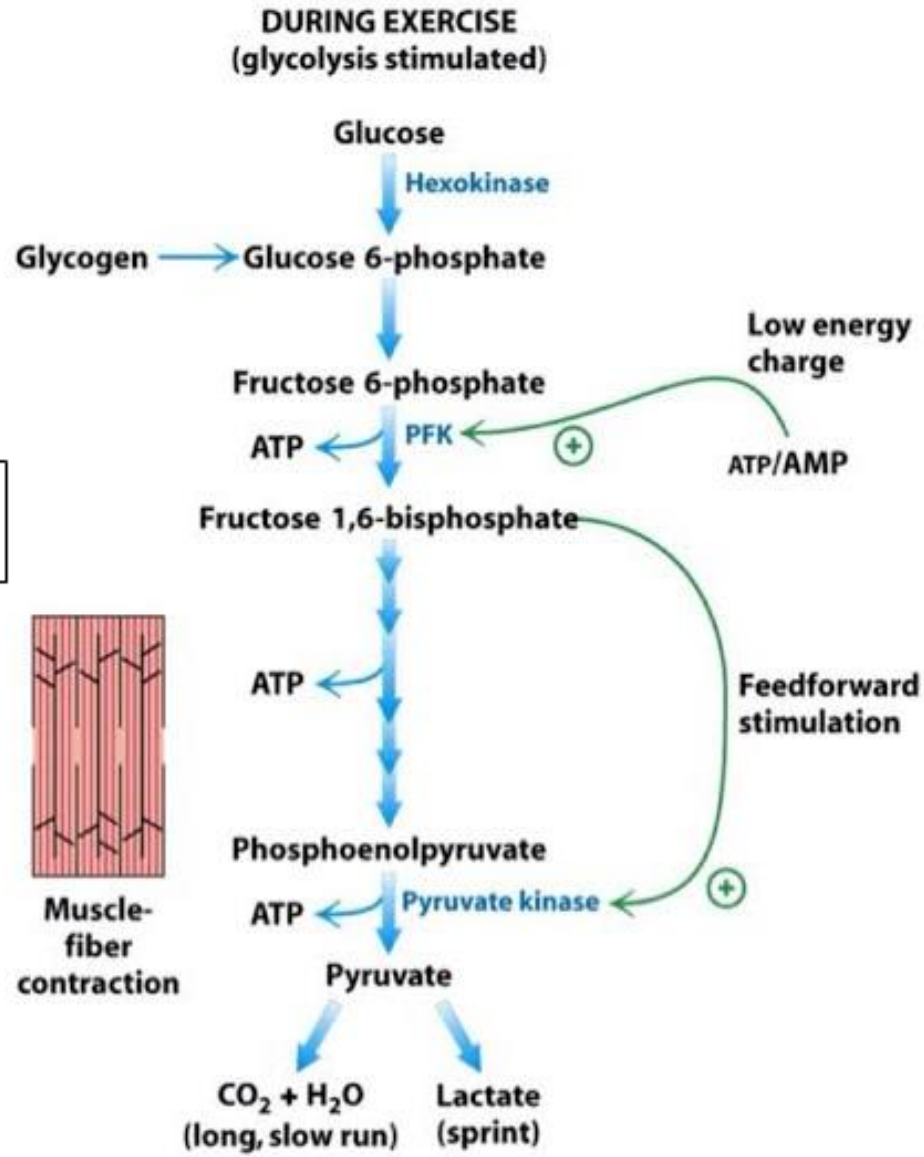
Feed-forward activation

- Feed-forward regulation: a substrate produced early in a pathway activates an enzyme downstream of the same pathway.



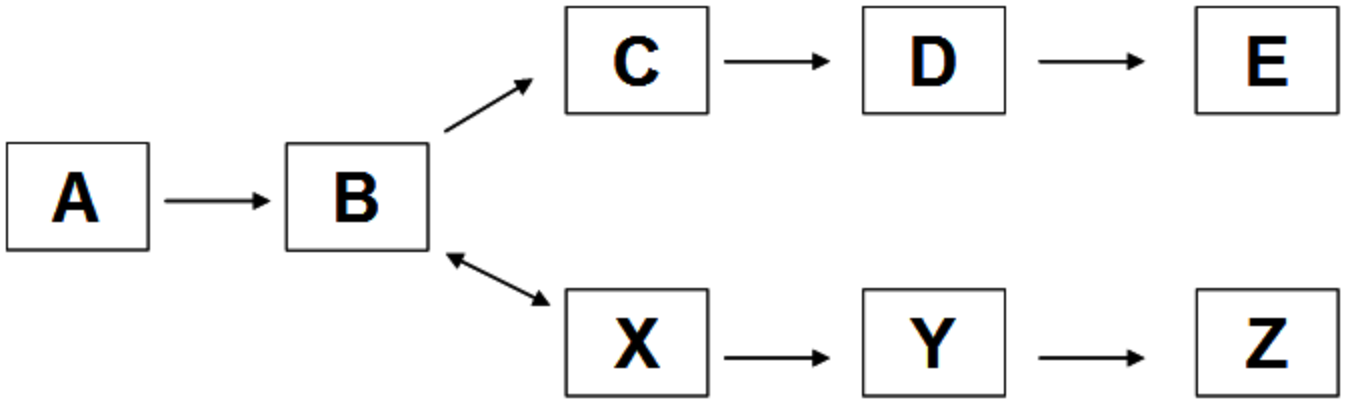
  - Glycolysis
  - Poisoning



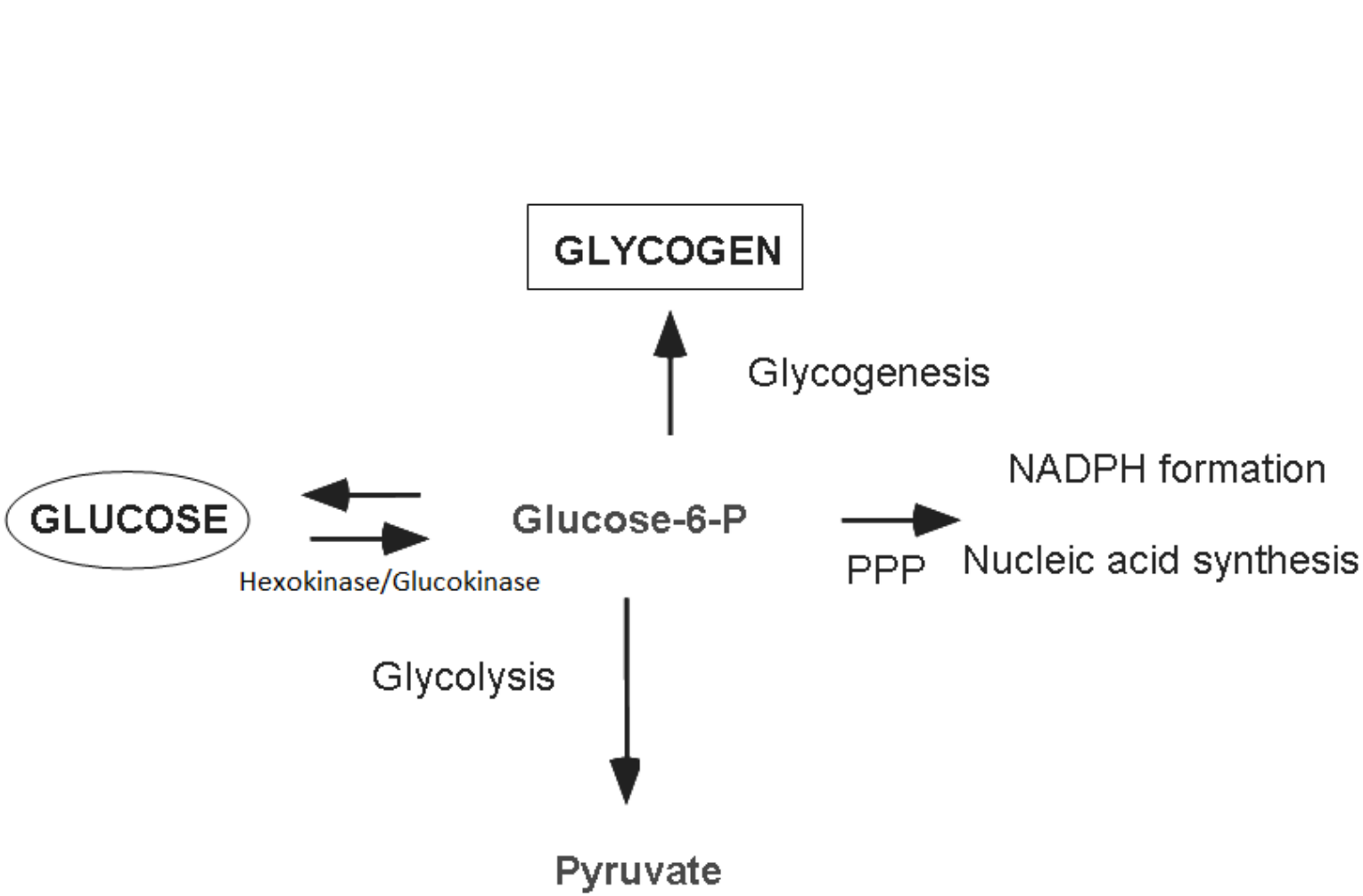


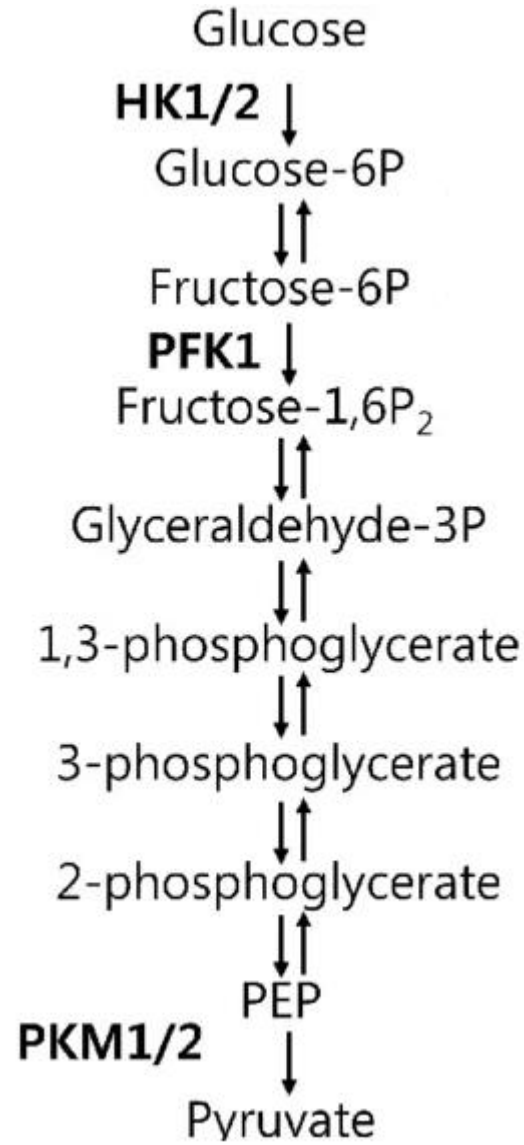
A committed step

- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.
- Committed steps are exergonic reaction.
- For example, the committed step for making product E is (B → C), not (A → B).



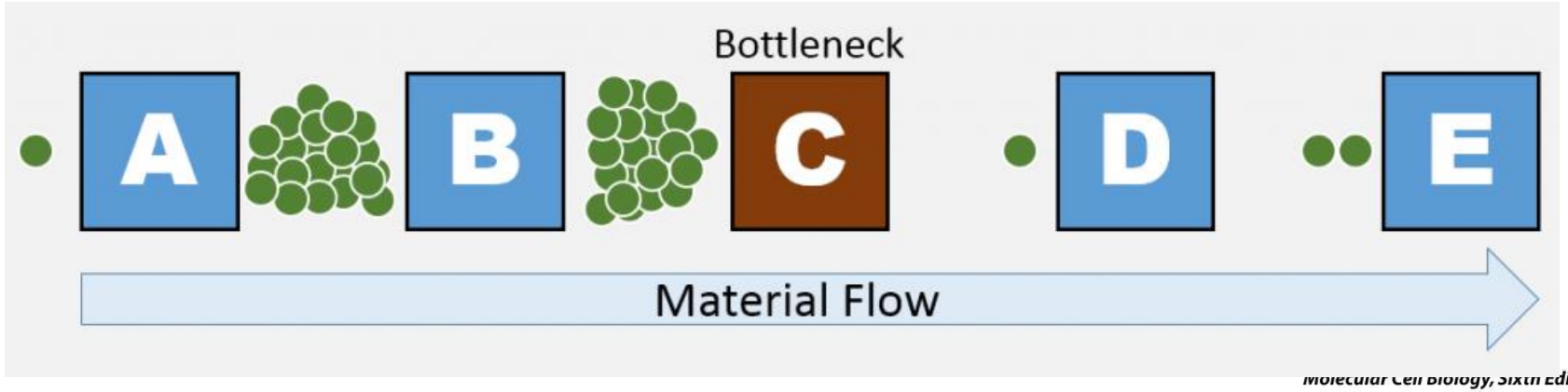
PFK, not HK/GK, is the committed step

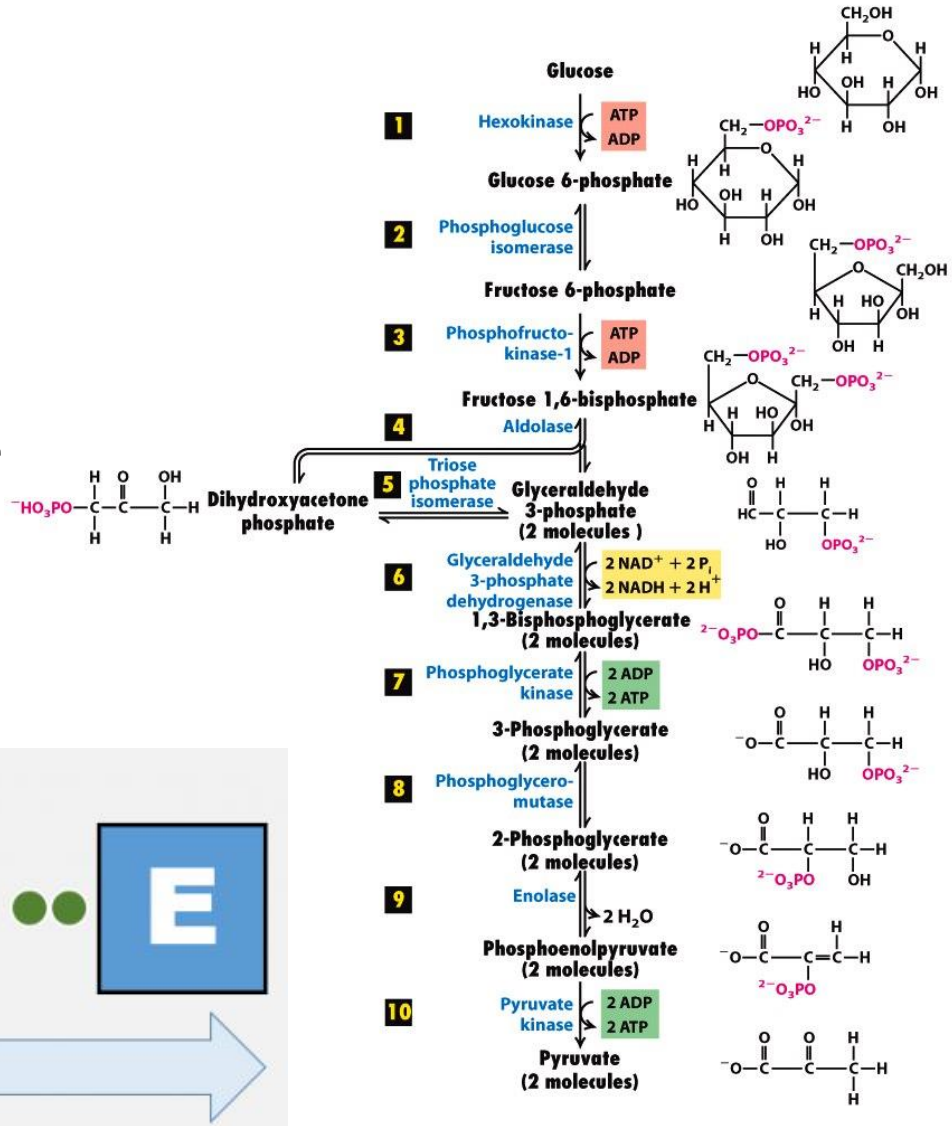




Rate-limiting reactions

- Rate-limiting reactions slow down rate of reactions because:
  - requirement for high amount of energy
  - strict regulation of enzymes
  - high Km values of enzyme towards its substrate
- These reactions are also usually, but not necessarily, committed steps.

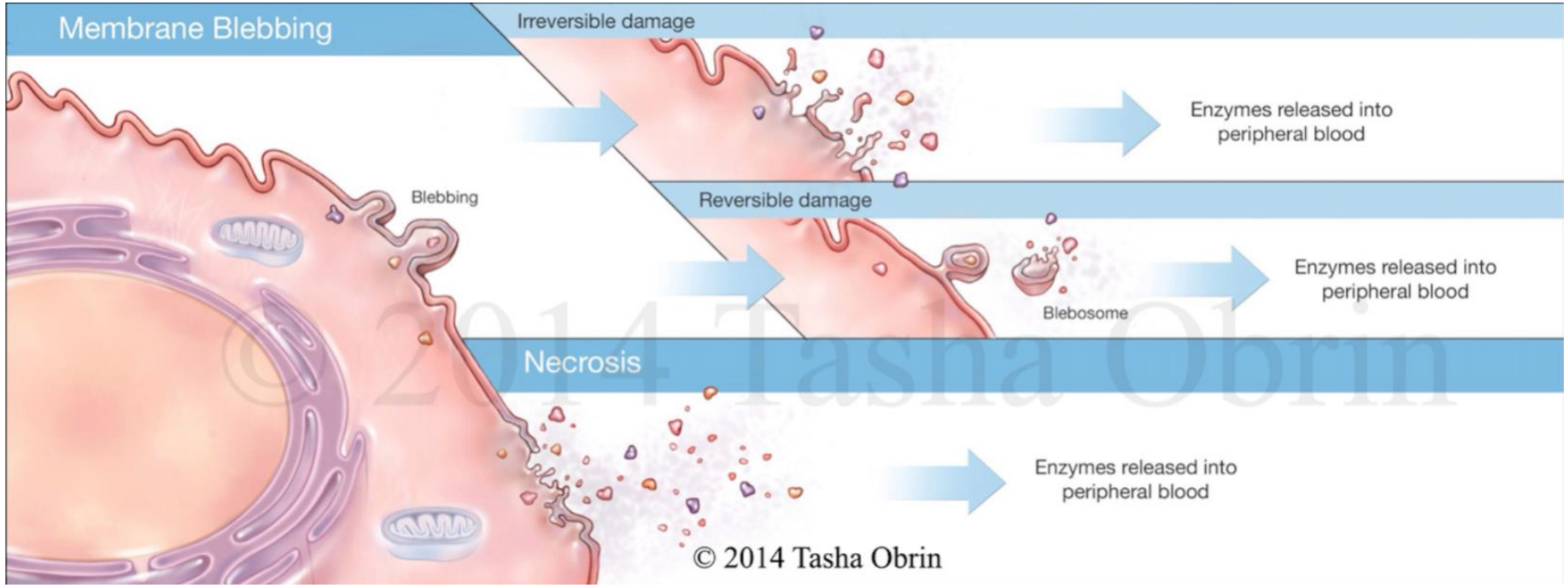




Enzymes in disease diagnosis

Concept

- The presence of enzymes in serum indicates that tissue or cellular damage.
- The measurement enzyme amount in serum is of diagnostic significance.



Example: Creatine phosphokinase (CPK)

- CPK is found primarily in heart and skeletal muscle as well as the brain.
- Three tissue-specific isozymes of CPK:
  - CPK3 (CPK-MM)
  - CPK2 (CPK-MB)
  - CPK1 (CPK-BB)

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM