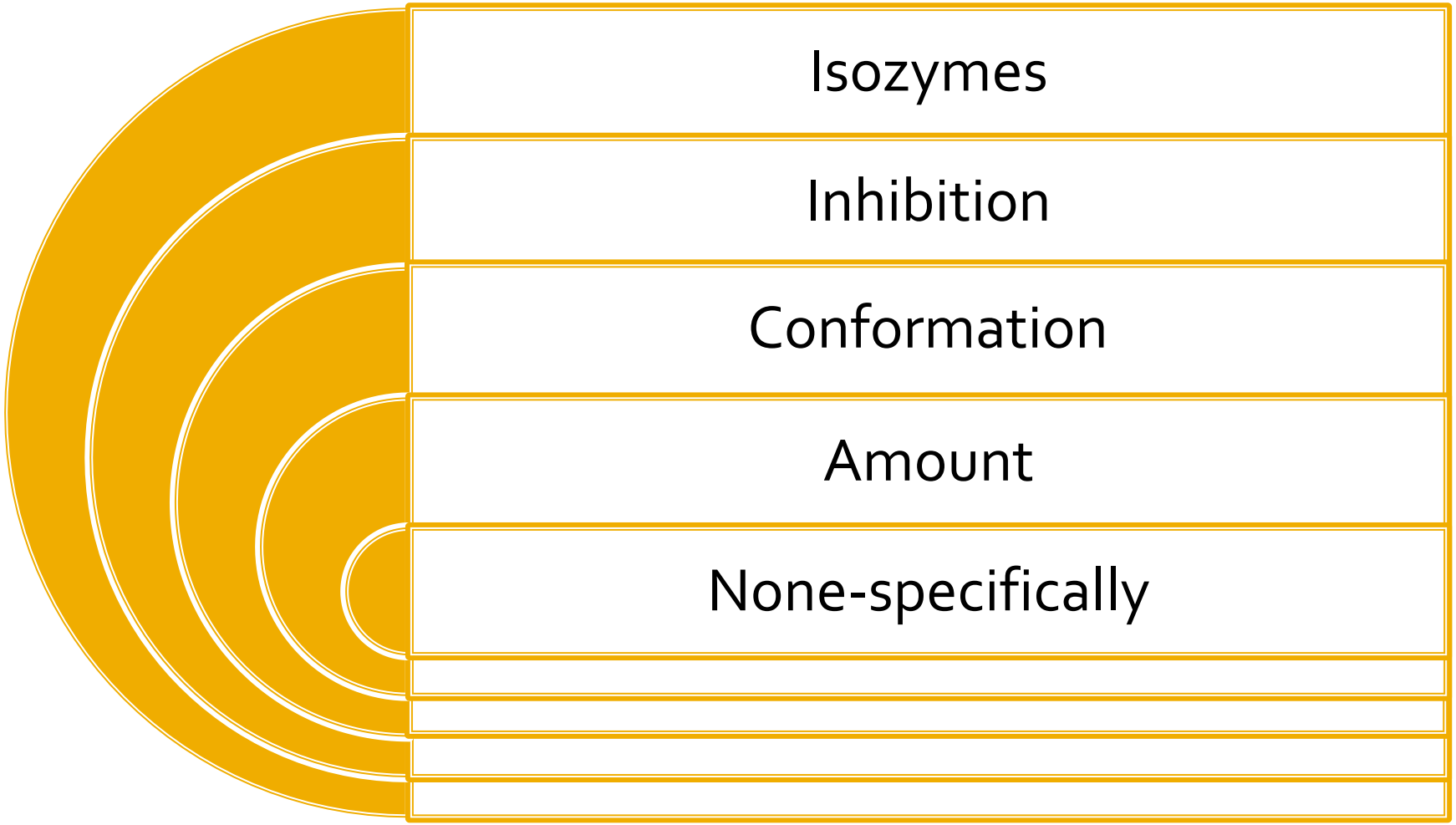
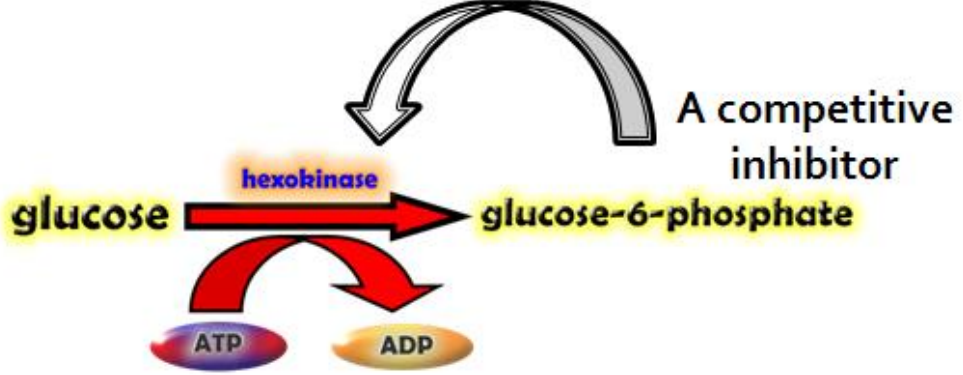
Enzymes Regulation

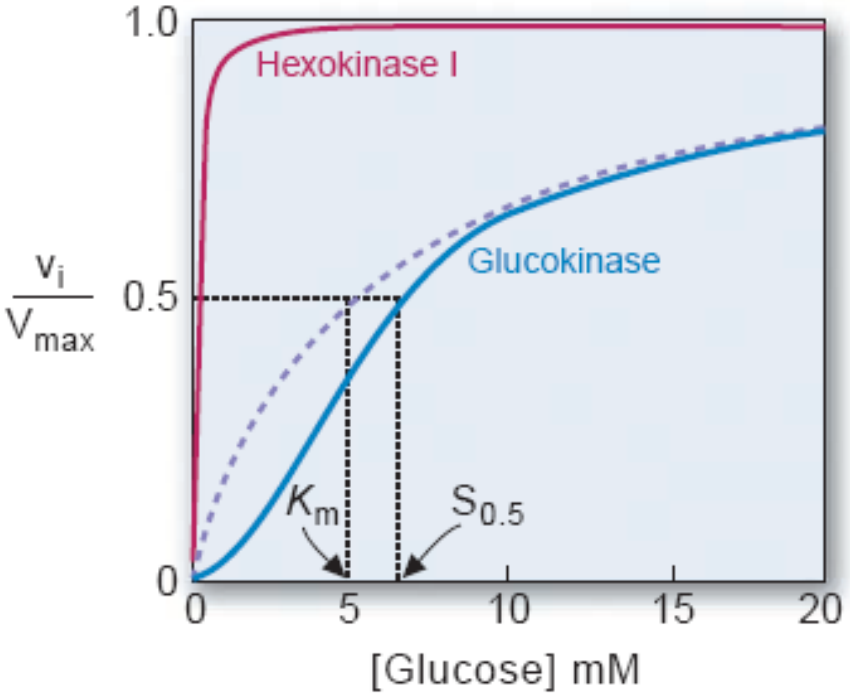
Modes of regulation



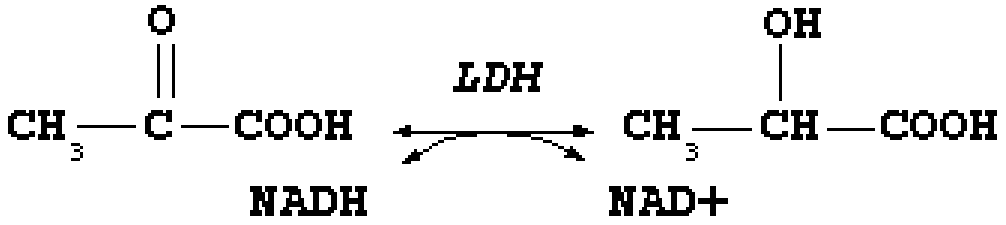
1. Isozymes (isoenzymes)
The Differential K_M Value, "Hexokinase"



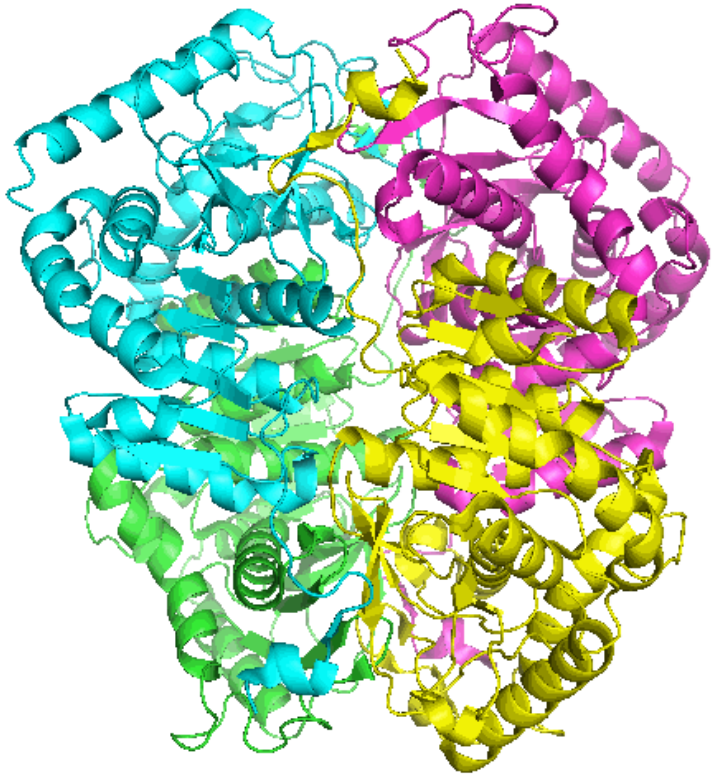
- What are isozymes? Same substrate & product, different gene, different localization, different parameters (K_m, V_{max}, k_{cat})
- Hexokinase found in RBCs & in liver
- Catalyzes the first step in glucose metabolism
- Hexokinase I (RBCs): K_M (glucose) ≈ 0.1 mM
- Hexokinase I V (glucokinase, liver, pancreas) ≈ 10 mM
- RBCs: when blood glucose falls below its normal fasting level (≈ 5 mM), RBCs could still phosphorylate glucose at rates near V_{max}
- Liver: rate of phosphorylation increases above fasting levels (after a high-carbohydrate meal)
  - High K_M of hepatic glucokinase promotes storage of glucose
- Pancreas: works as a sensor

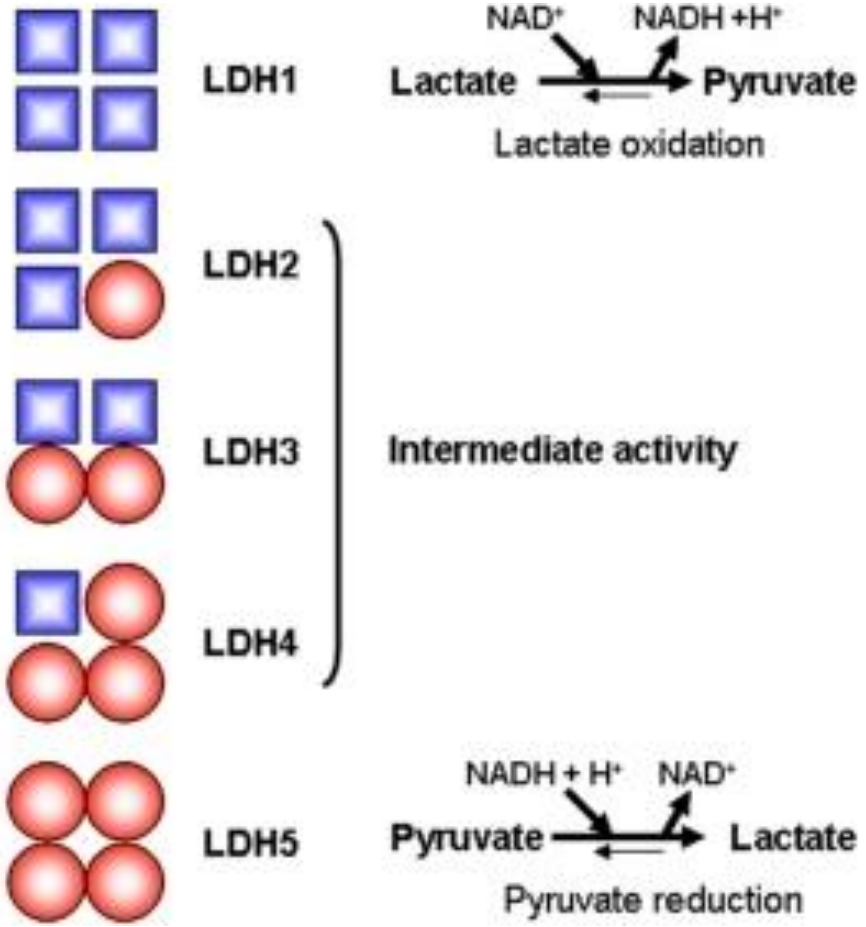


Lactate Dehydrogenase (LDH)



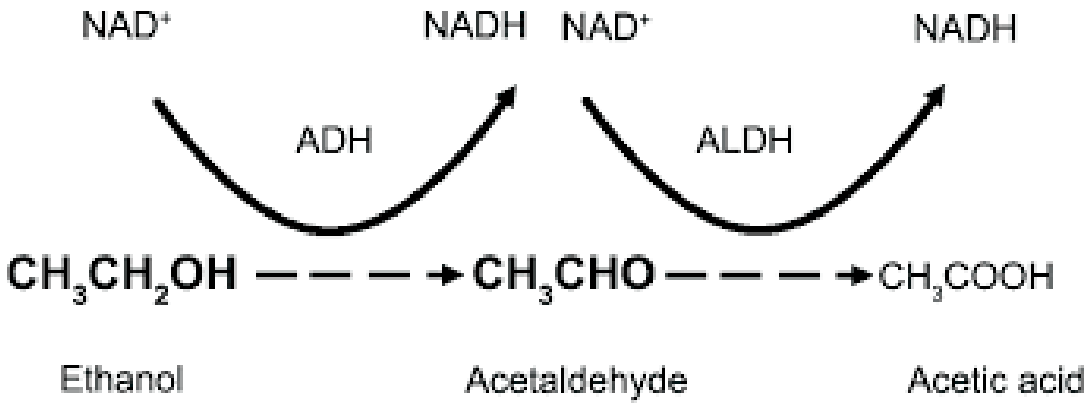
- Aerobic vs. anaerobic
- Km: H_4 >> M_4
- Inhibition: H_4 inhibited but not M_4

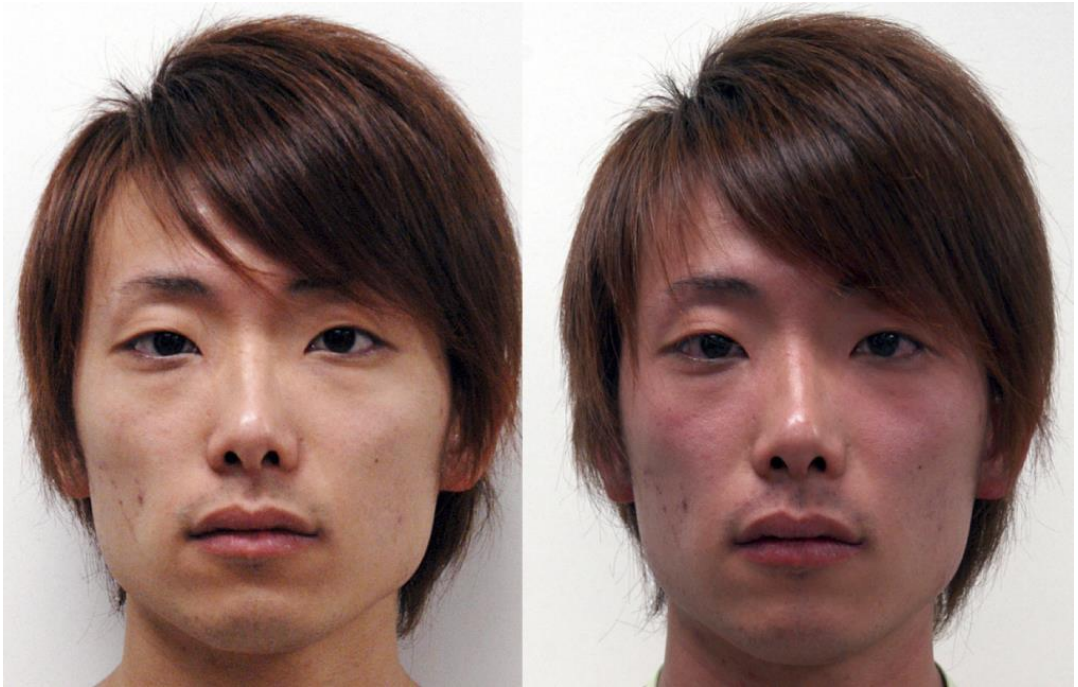




Aldehyde dehydrogenase (ALDH)

- Oxidation of acetaldehyde to acetate.
- Four tetrameric isozymes (I-IV)
- ALDH I (low Km; mitochondrial) and ALDH II (higher Km; cytosolic)
- ~50% of Japanese & Chinese are unable to produce ALDH I (not observed in Caucasian & Negroid populations)
  - Flushing response
  - Tachycardia





2. Inhbition

2.1 MECHANISM-BASED INHIBITORS

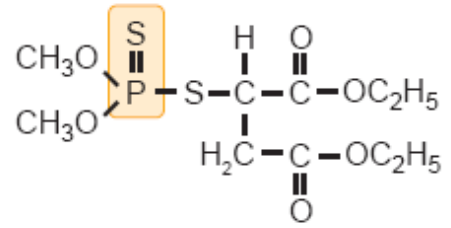
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction
- The term includes:
- A. Covalent inhibitors
- B. Transition state analogs
- C. Heavy metals

- The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme

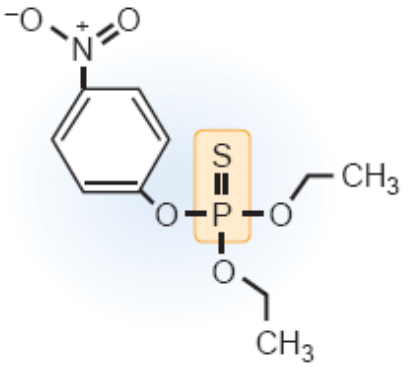
2.1.A. Covalent Inhibitors

- Covalent or extremely tight bonds with active site amino acids
- Amino acids are targeted by drugs & toxins

- The lethal compound [DFP] is an organophosphorus compound that served as a prototype for:
  - The nerve gas sarin
  - The insecticides malathion & parathion
- DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal

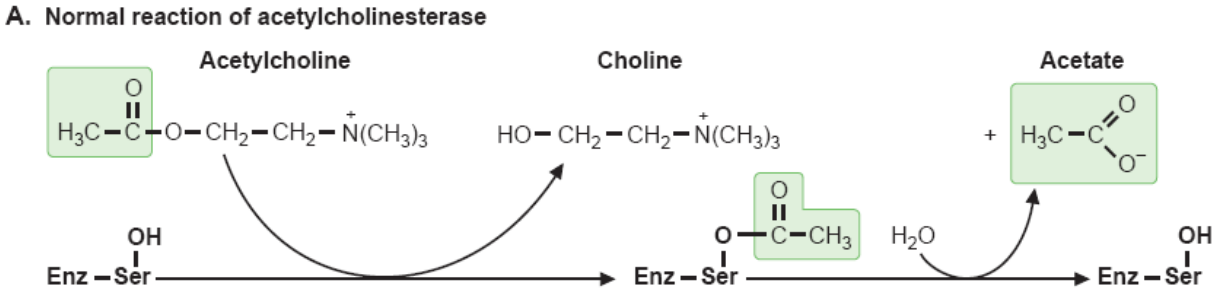


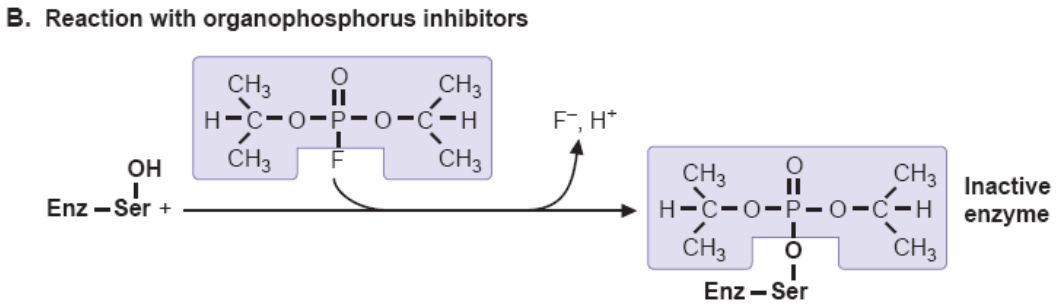
Malathion



Parathion

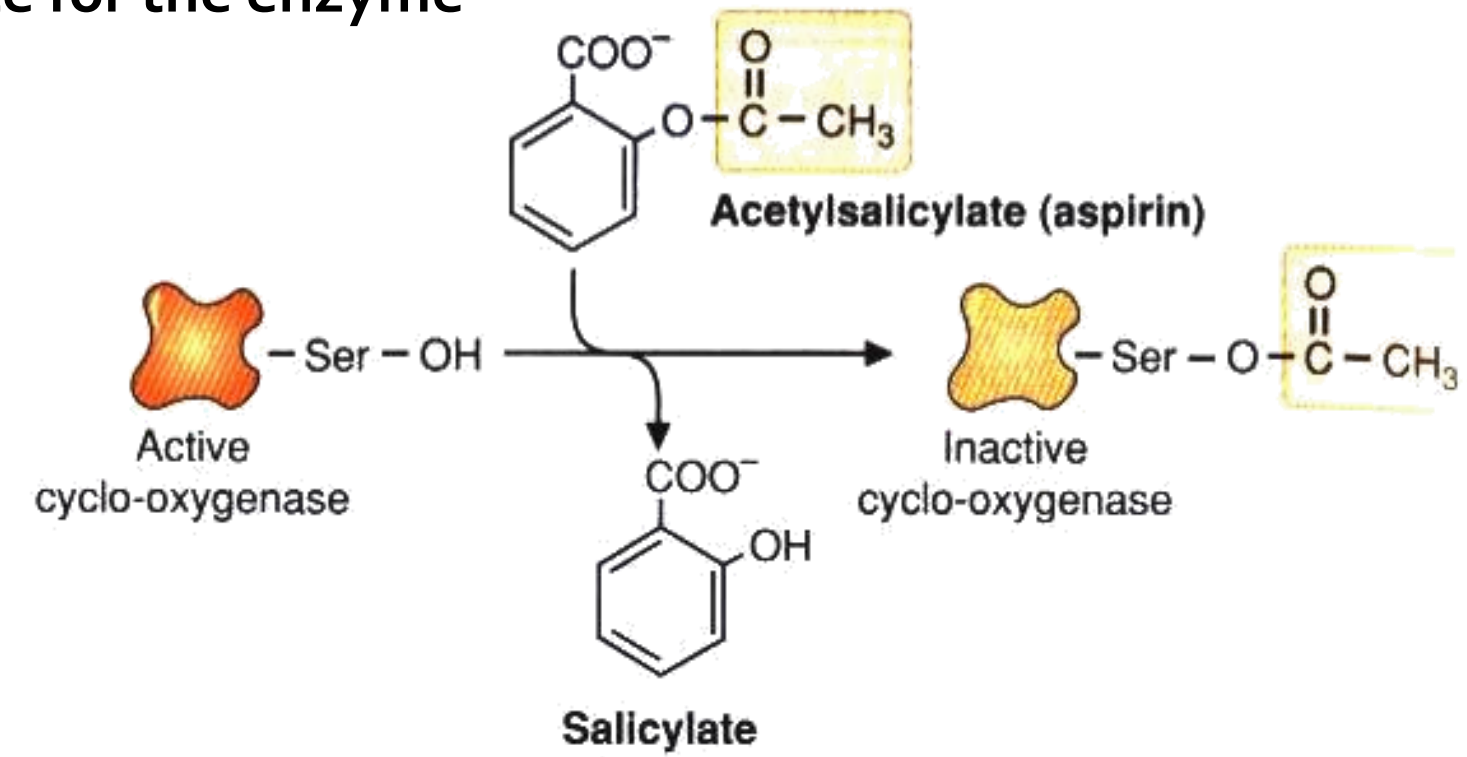
Sarin





2.1.A. Covalent Inhibitors

- Aspirin (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase)
- Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme

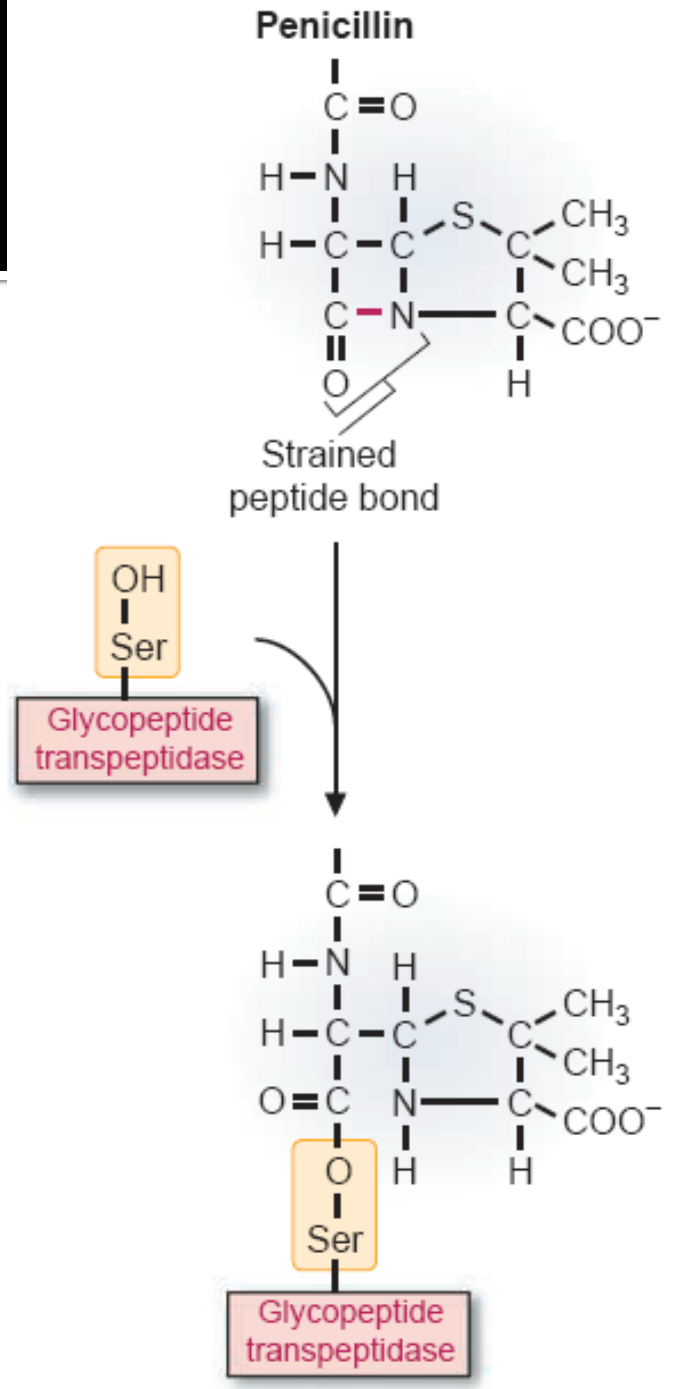


2.1.B. Transition-State Analogs & Compounds that Resemble Intermediate Stages of the Reaction

- Transition-state analogs: extremely potent inhibitors (bind more tightly)
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure)
- Substrate analogs: bind more tightly than substrates
- Known as suicide inhibitors

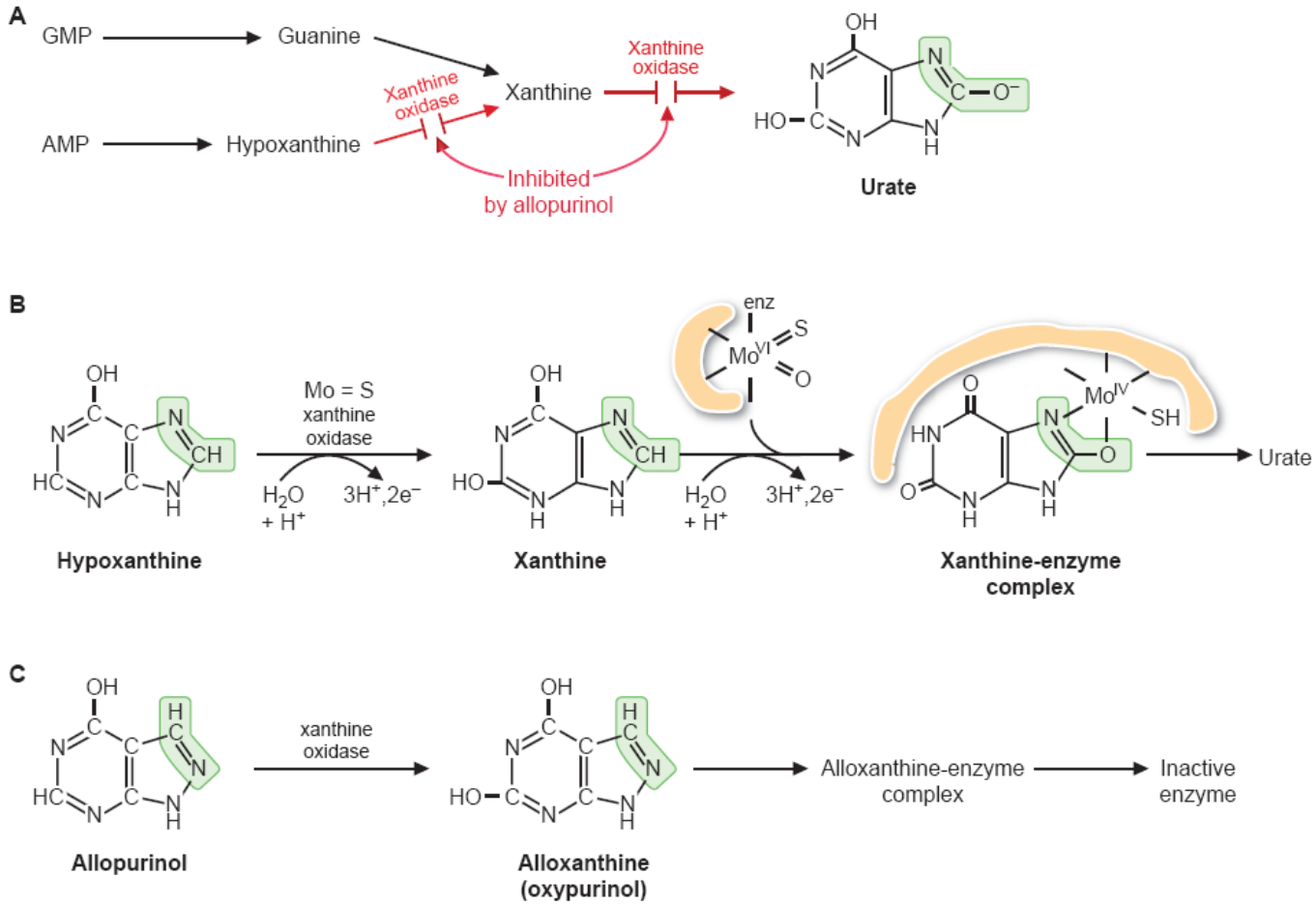
2.1.B.1 PENICILLIN

- A transition-state analog to *glycopeptidyl transferase or transpeptidase*
- Required by bacteria for synthesis of the cell wall
- The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*



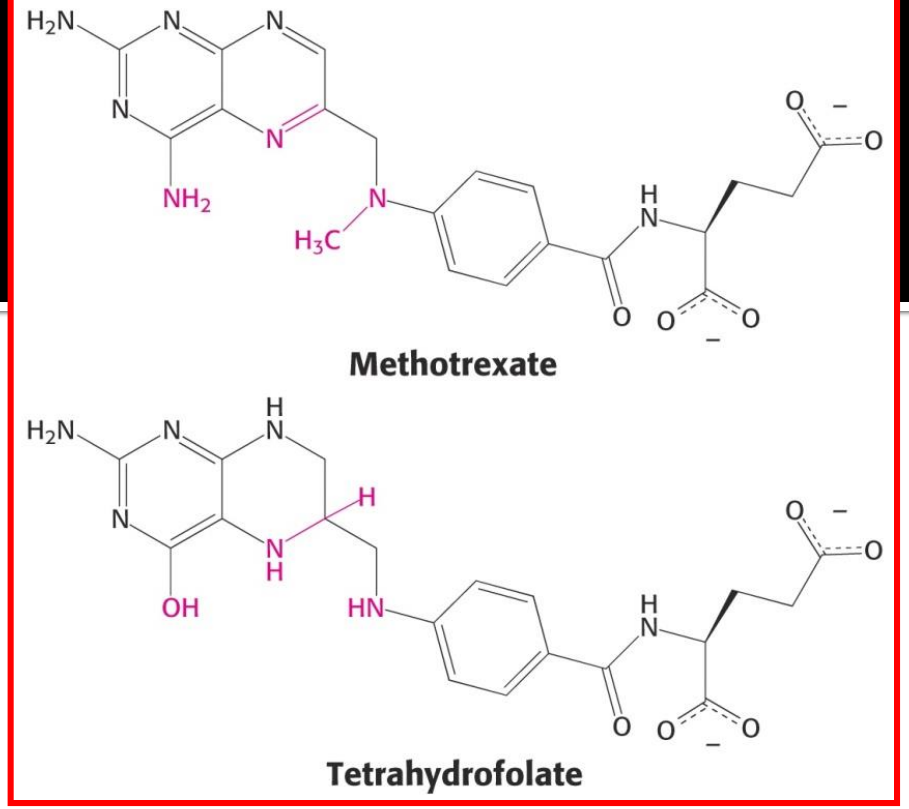
2.1.B.2 ALLOPURINOL

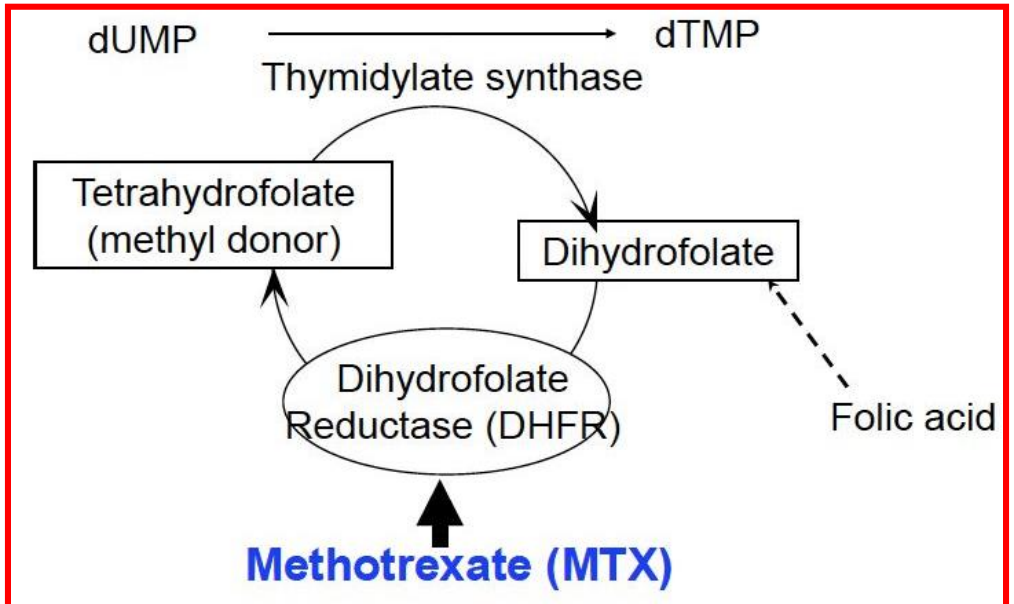
- A drug used to treat gout, Decreases urate production (xanthine oxidase)
- The enzyme commits suicide by converting the drug to a transition-state analog
- The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions
- Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site



2.1.B.3 Methotrexate

- Synthetic inhibitor
- Anticancerous
- Analog of tetrahydrofolate
- Binds to enzyme a 1000-fold more tightly
- Inhibits nucleotide base synthesis





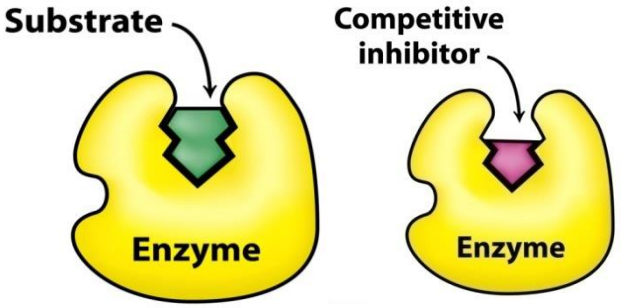
2.1.C. Heavy Metals

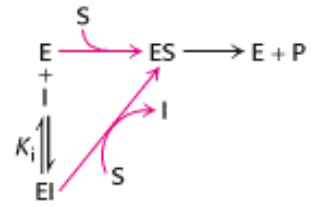
- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity
- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site
  - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc
  - 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

2.2 Reversible Inhibitors

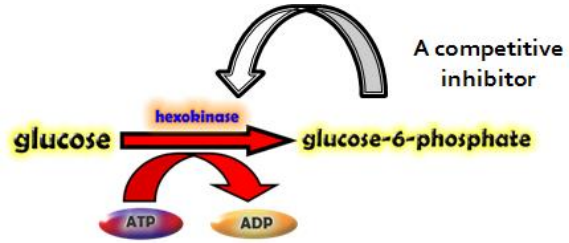
- Characterized by a rapid dissociation of the enzyme-inhibitor complex
- Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme
- Reversible inhibitors can be divided into two classes: competitive & noncompetitive
- The double-reciprocal plots are highly useful for distinguishing among these inhibitors

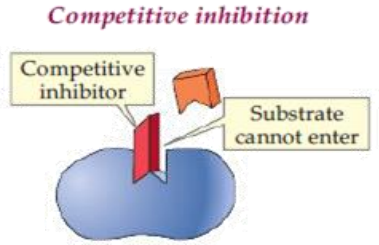
2.2.A. Competitive inhibition

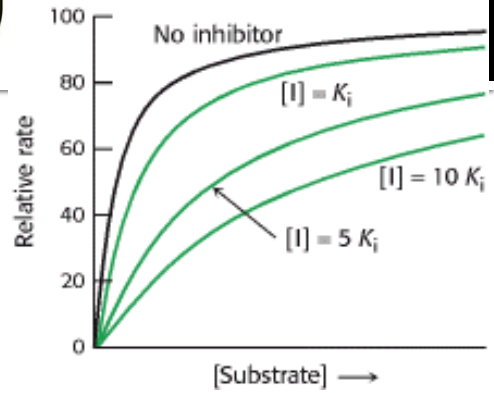


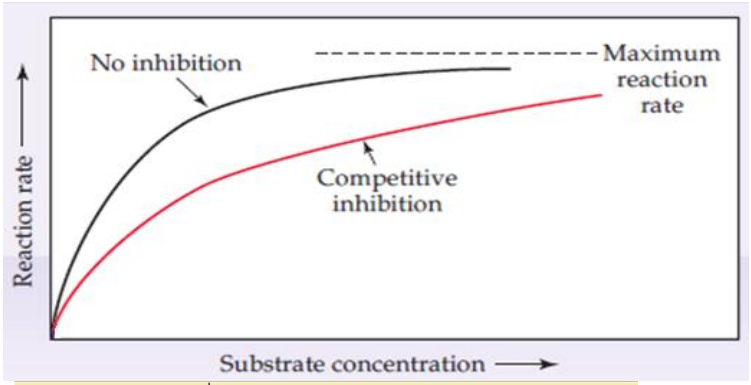


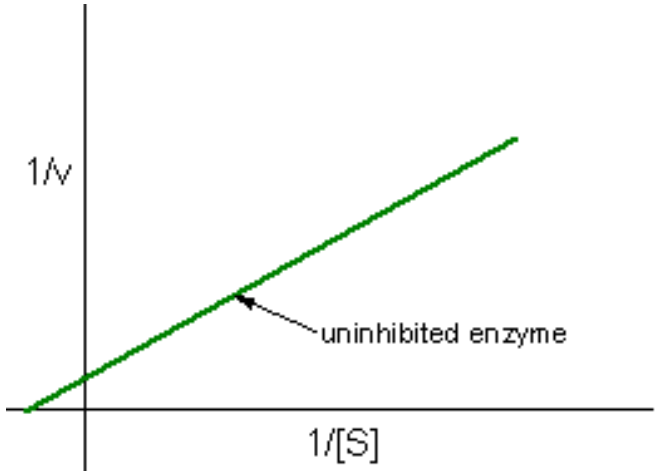
- The inhibitor competes with substrate
- Increasing [S] can overcome the inhibition (V_{max})
- Does K_M change?
- Significance (ex. Hexokinase)

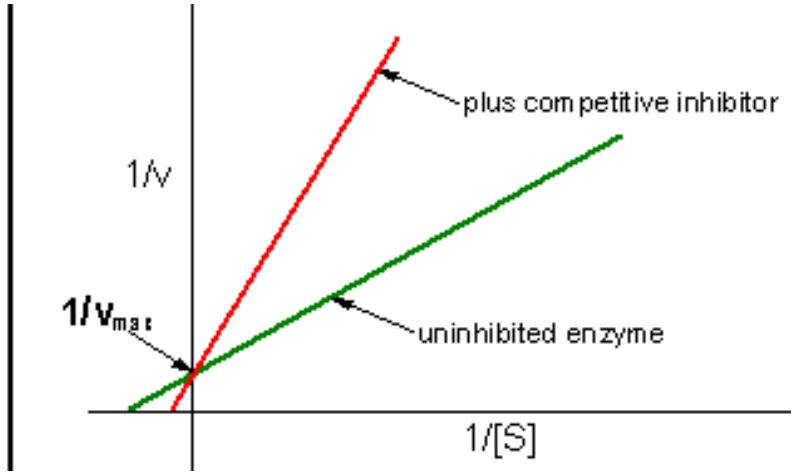


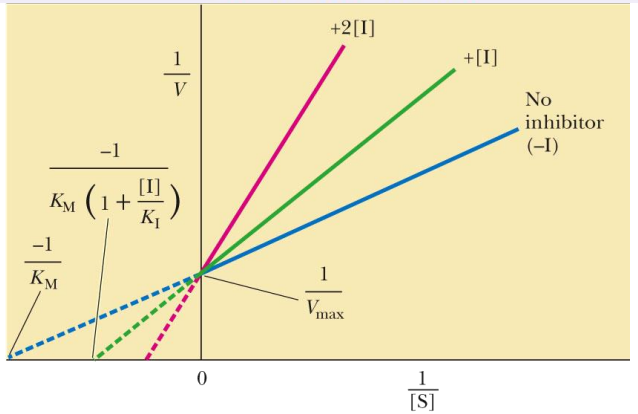






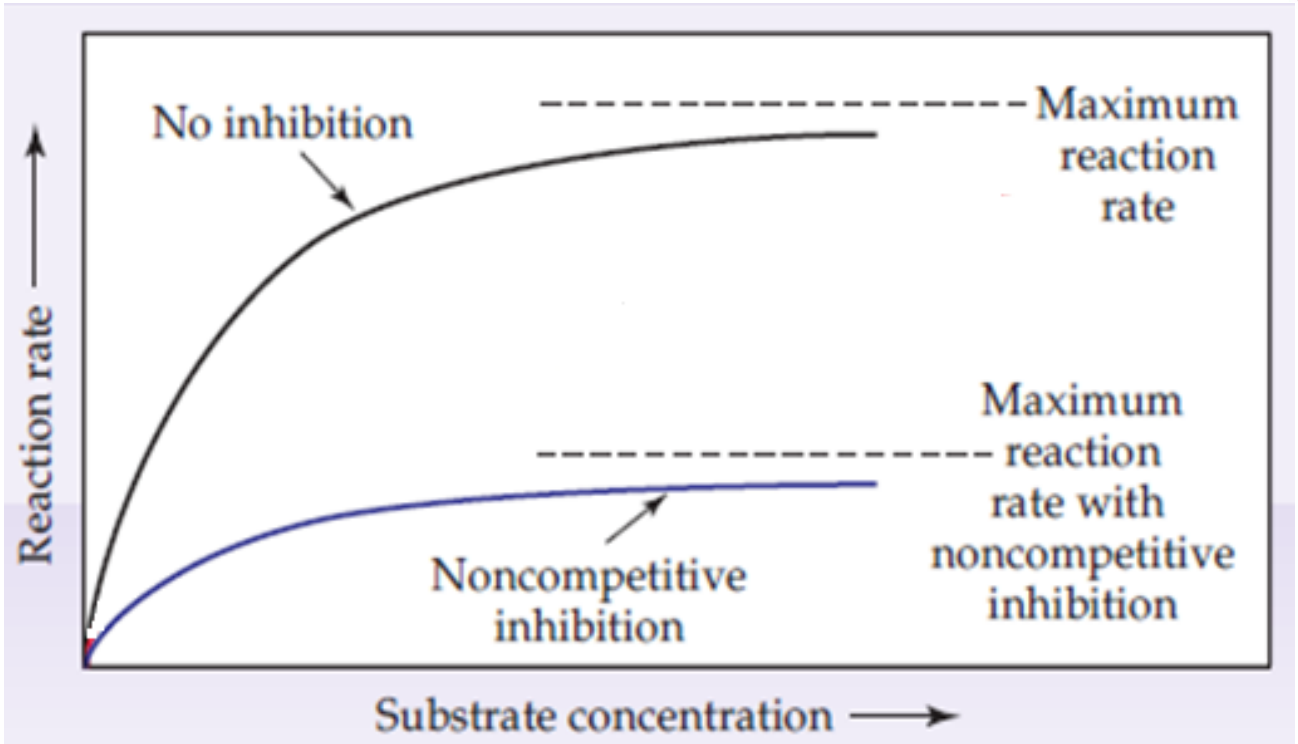


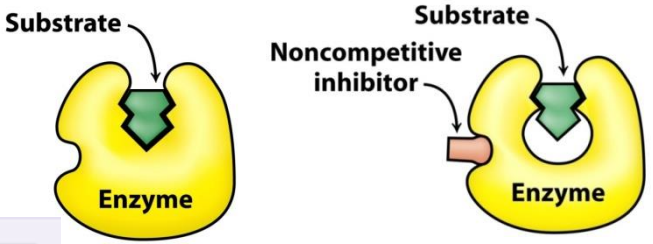


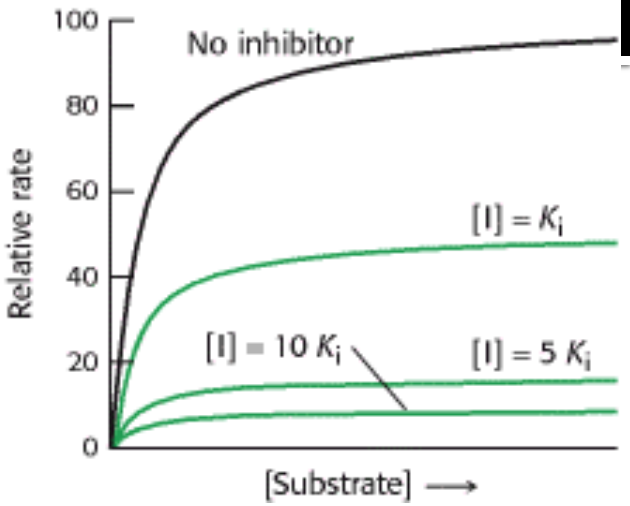


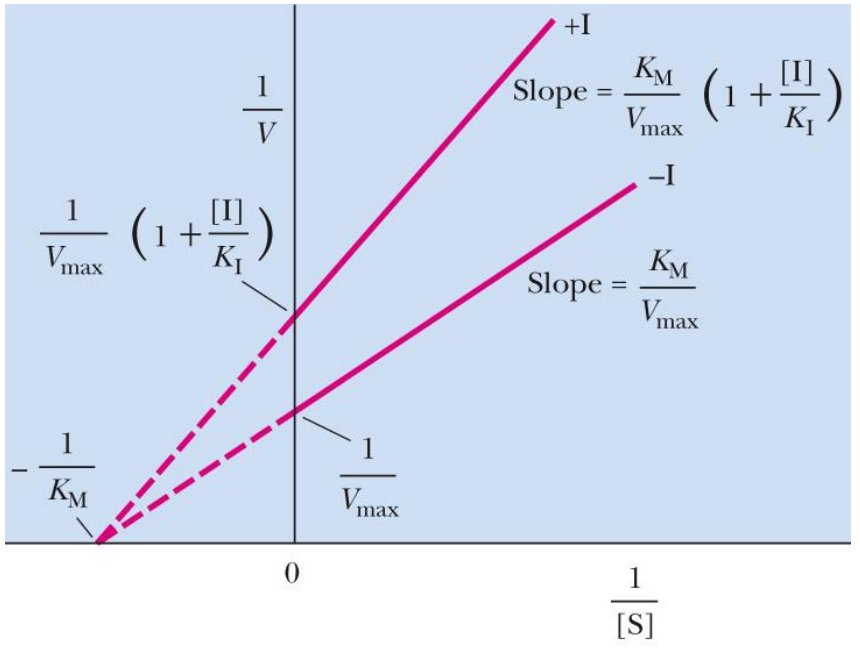
2.2.B. Noncompetitive inhibition

- The inhibitor binds at a site other than the active site
- The complex does not proceed to form product or has a lower efficiency
- V_{max} vs. K_M
- Can we reach V_{max}?









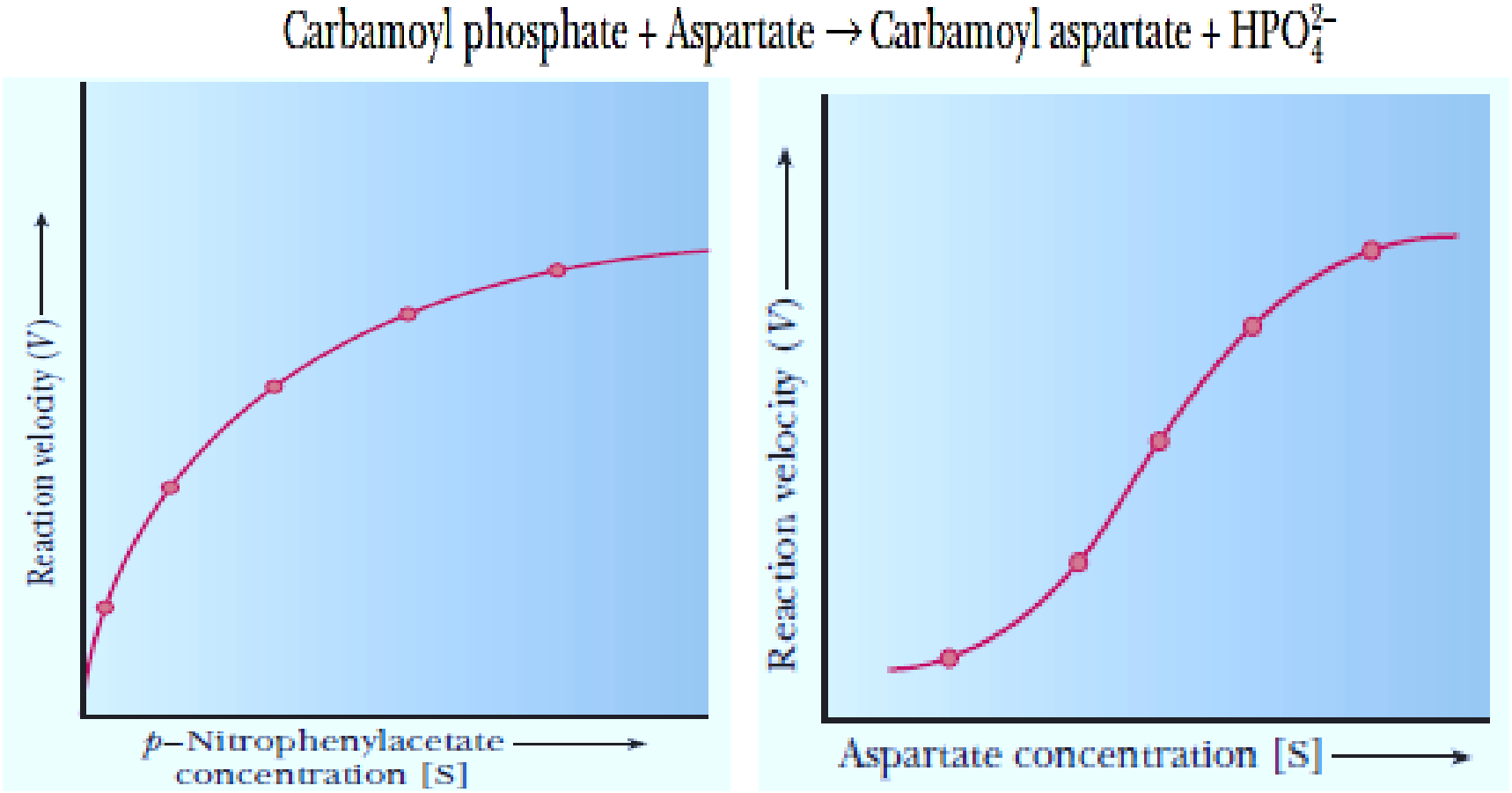
3. REGULATION THROUGH CONFORMATIONAL CHANGES

- These regulatory mechanisms include
  A. Allosteric activation and inhibition;
  B. Phosphorylation or other covalent modification;
  C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins;
  D. Proteolytic cleavage

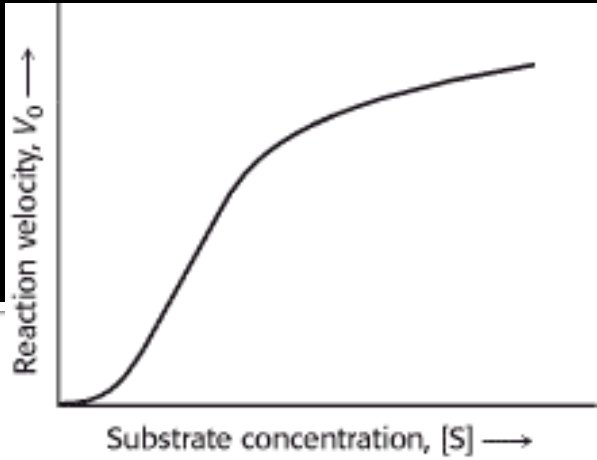
- These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation

3.A. Not all enzymes follow Michaelis-Menten equation; Chymotrypsin vs. ATCase

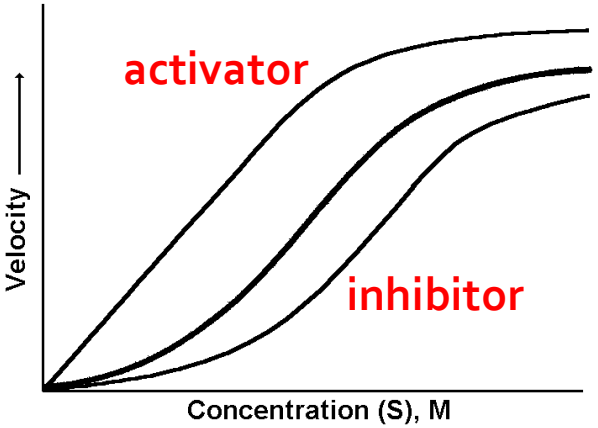
- Chymotrypsin: Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds
- Aspartate transcarbamoylase (ATCase): synthesis of CTP & UTP for RNA and DNA synthesis

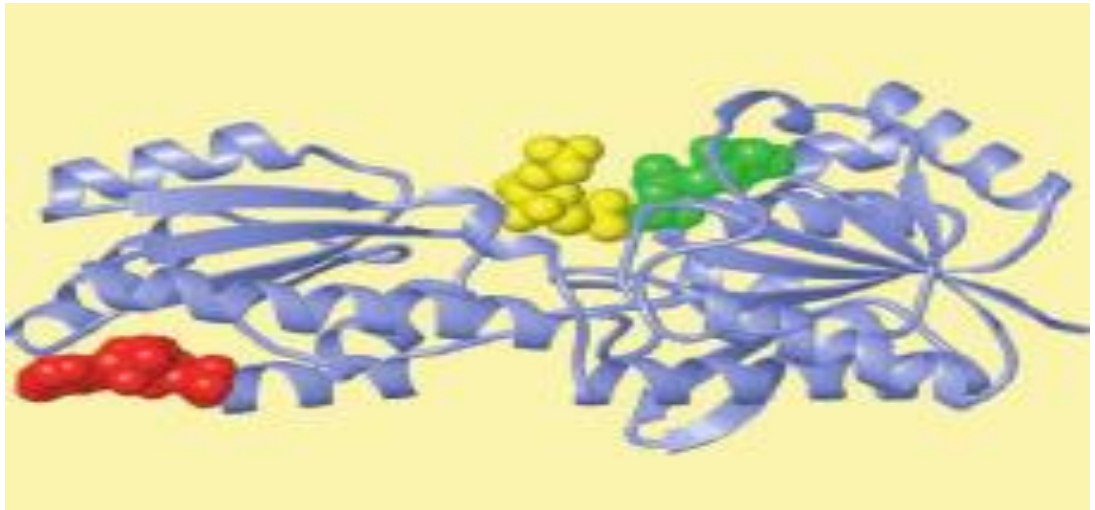


Allosteric regulation



- What are allosteric enzymes? A multi-subunit enzyme with catalytic subunit(s) and regulatory subunit(s)
- Binding triggers a conformational change in the active site
- The Michaelis-Menten model can't explain the kinetic properties
- The effect of the modulators (allosteric modifiers)
- Homotropic vs. heterotropic
- The substrate concentration at half of the V_{max} is called ($K_{0.5}$)
- Allosteric inhibitors have a much stronger effect on enzyme velocity

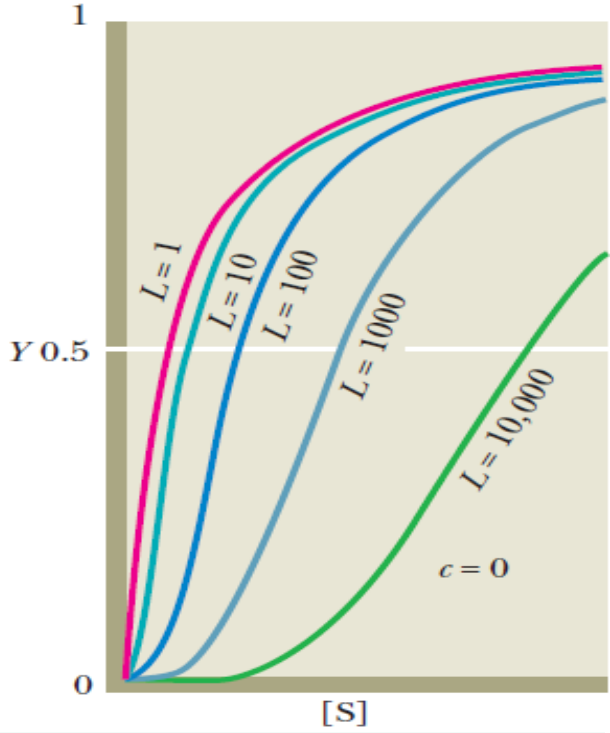


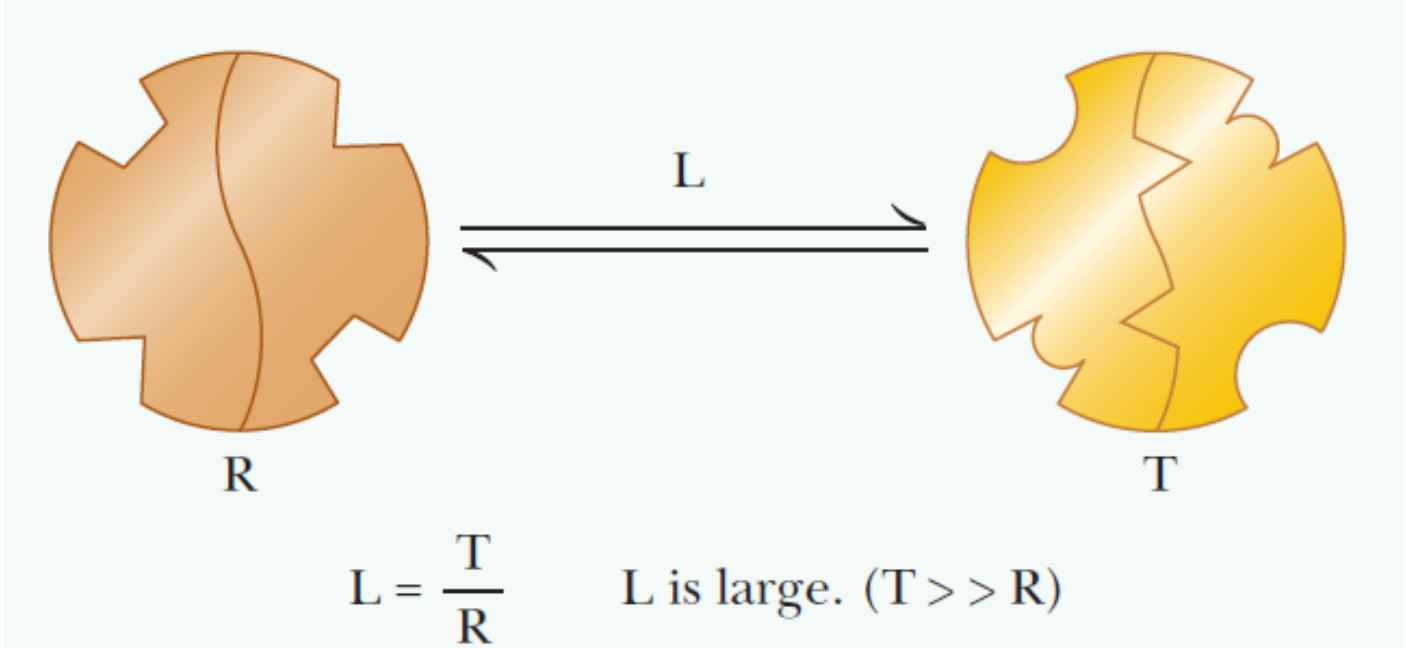


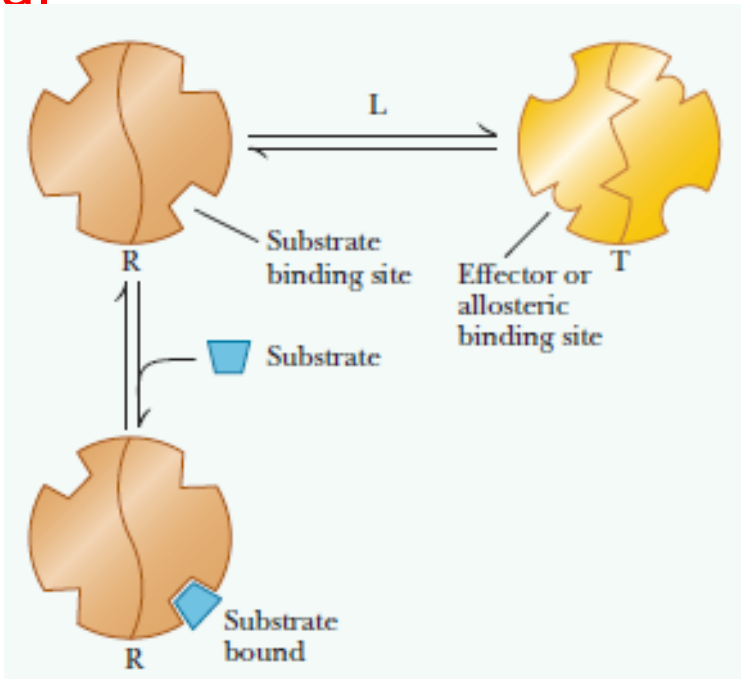
The effect of modifiers on V_{max} & $K_{0.5}$

How do allosteric enzymes work?

- Two conformations: more active (R) & less active or inactive (T),
- The equilibrium ratio (T/R) is called L and assumed to be high
- As L (T/R) increases, the shape becomes more sigmoidal

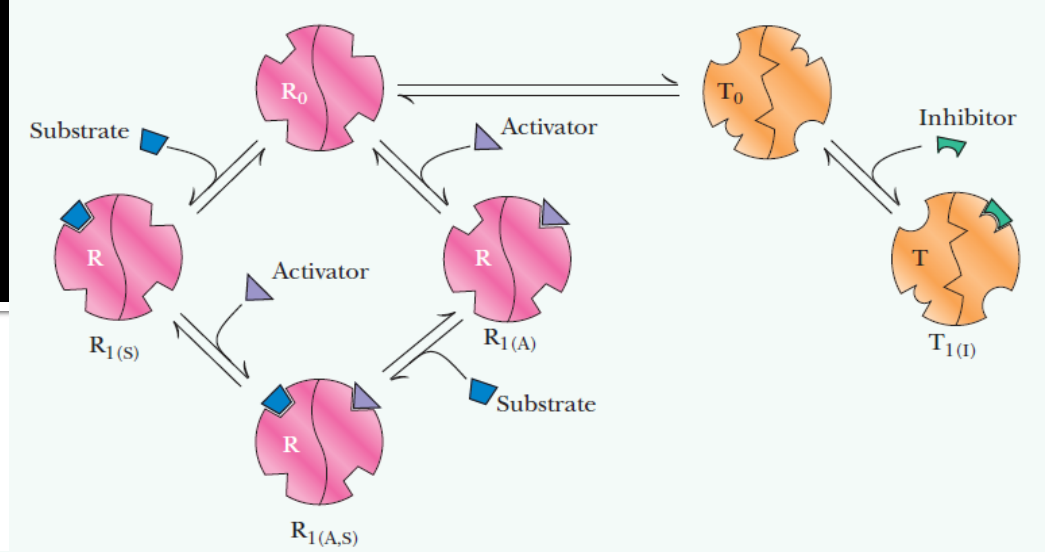




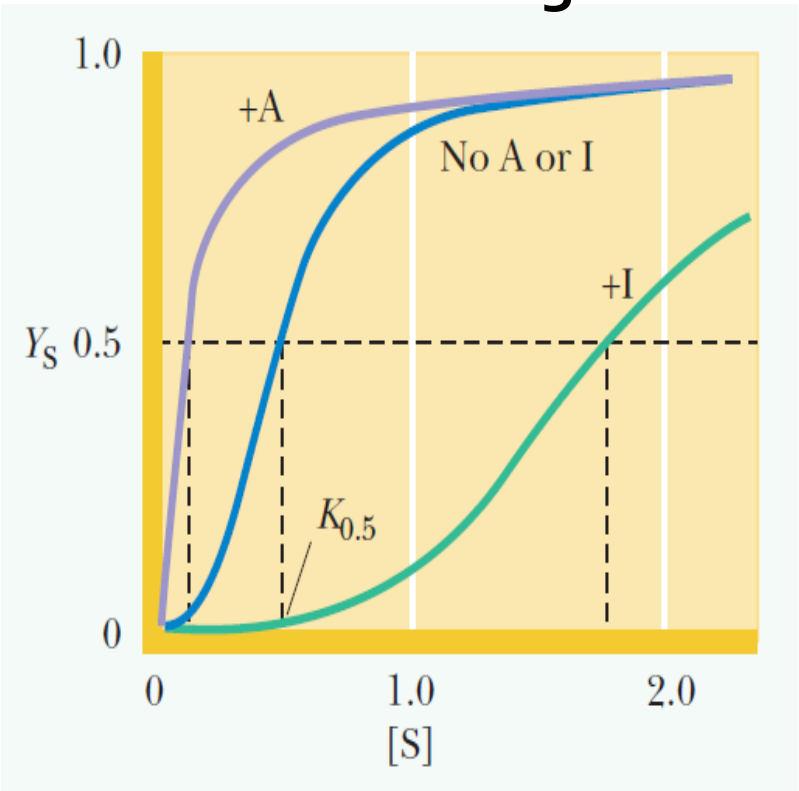


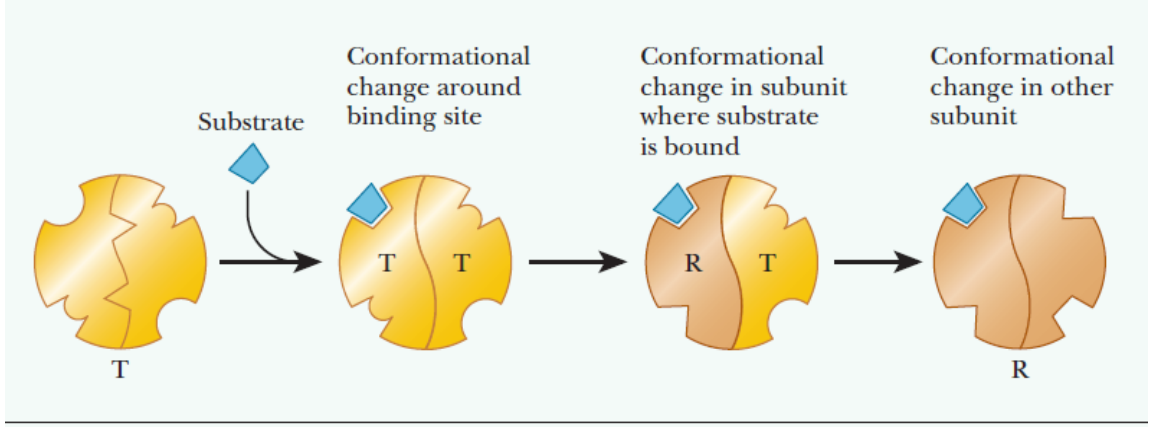
Concerted or sequential?

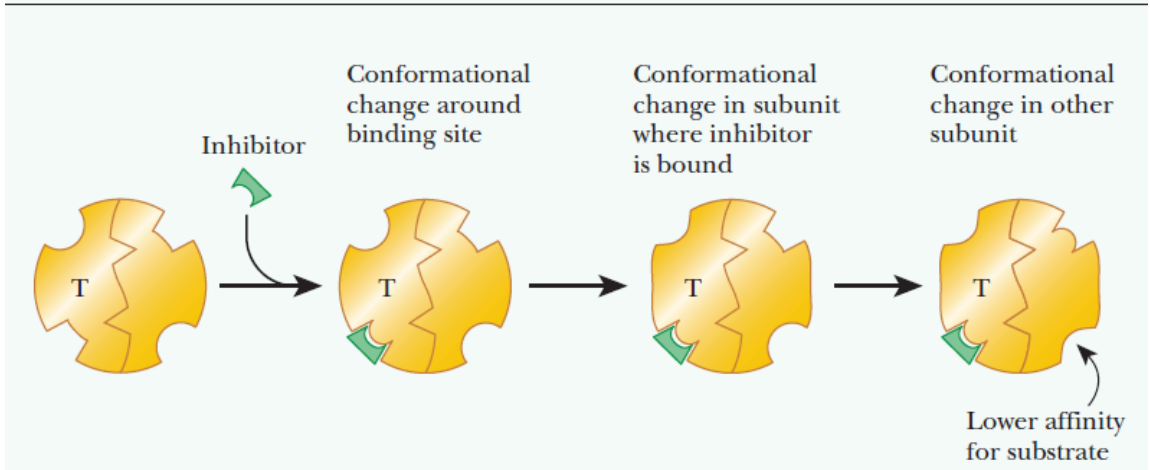
- **Either substrate or activator must be increased to overcome the effects of the allosteric inhibitor**



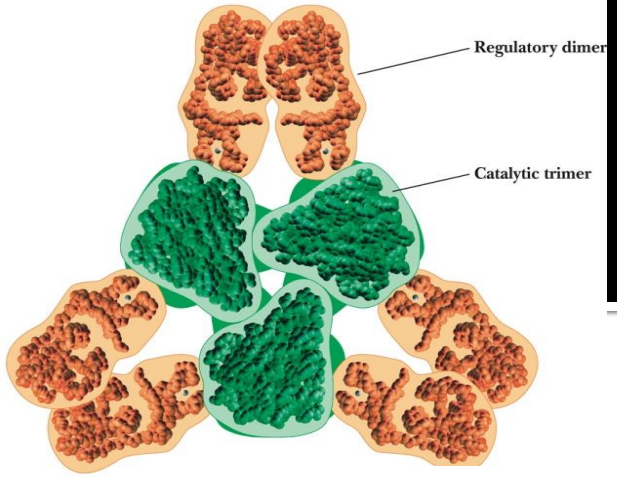
- **Conformational change**



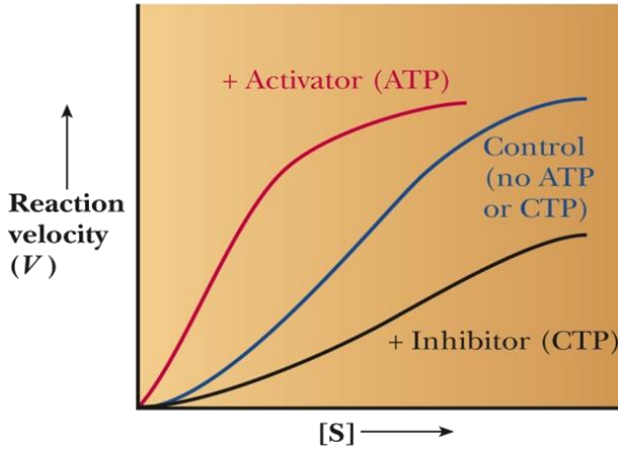


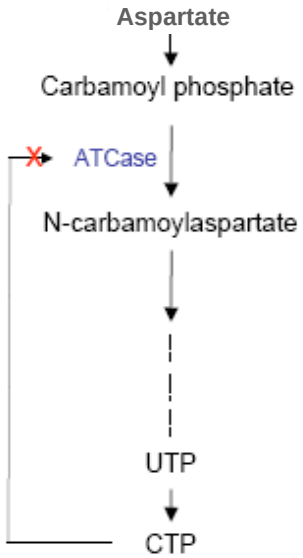


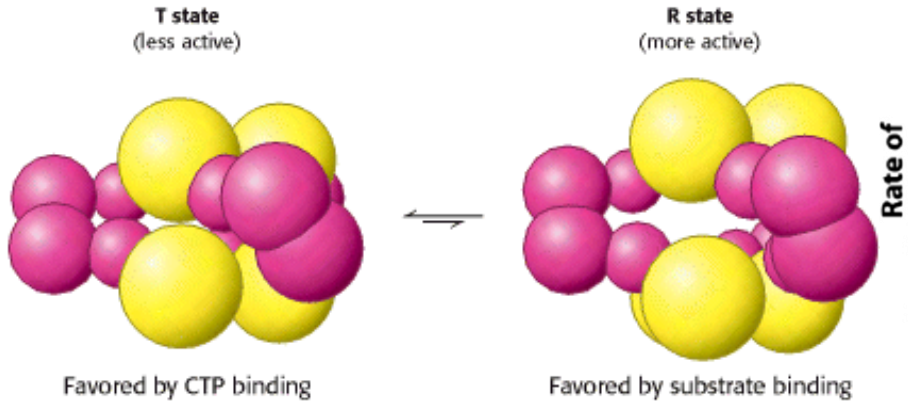
Allosteric regulation – ATCase "synthesis of pyrimidine nucleotides"

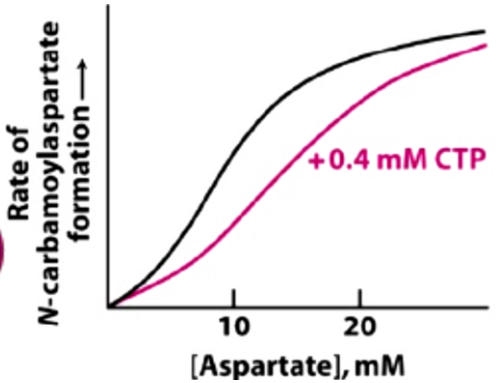


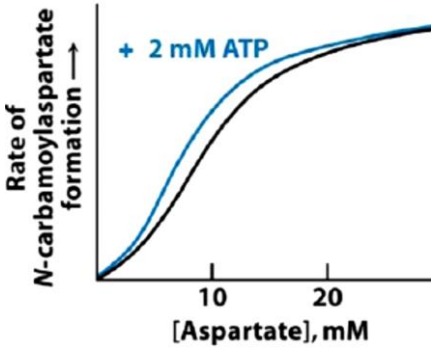
- ATCase and Hb are allosteric proteins (cooperative behavior)
- Catalytic can be separated from regulatory (hyperbolic)
- Cooperativity in relation to substrate
- CTP is an inhibitor of ATCase (feedback inhibition), ATP is an activator



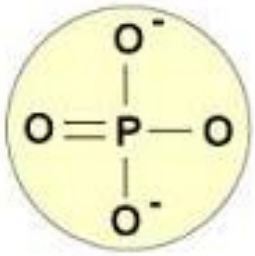








3.B. Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION



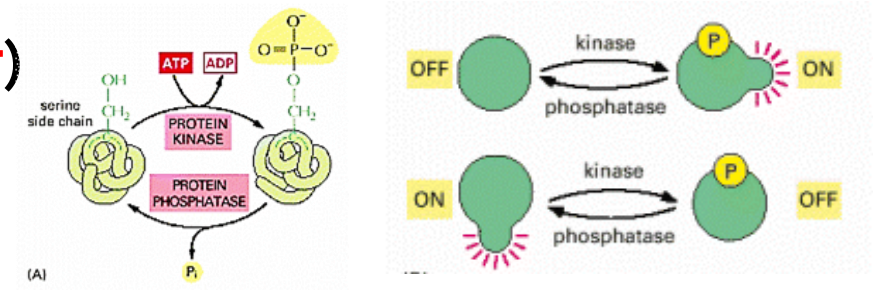
Phosphate group

- **Why is it effective?**

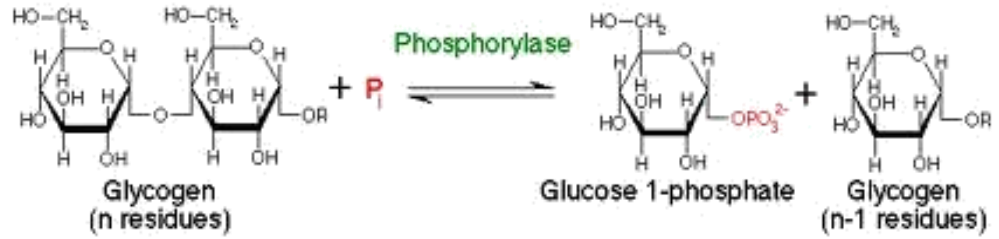
- Adds two negative charges: new electrostatic interactions and accordingly conformation
- Can form three or more hydrogen bonds: specific interactions with hydrogen-bond donors
- Can take place in less than a second or over a span of hours
- Often causes highly amplified effects

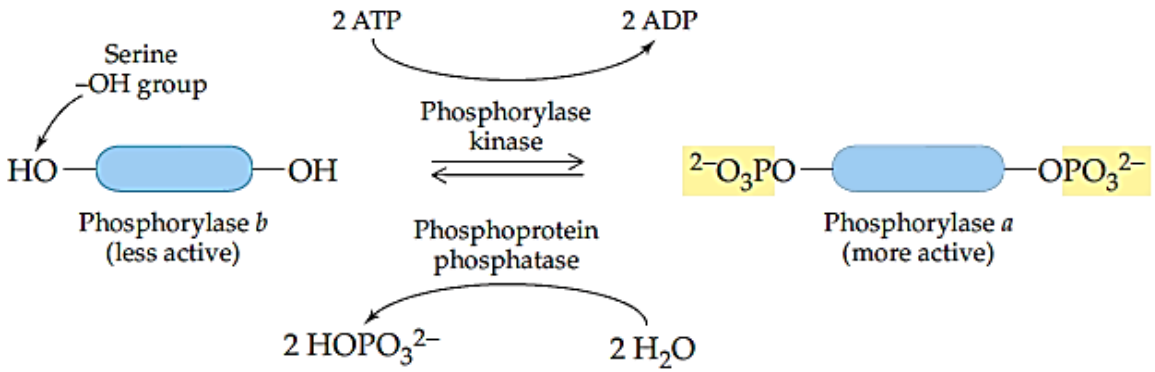
3.B. Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

- Rapid and transient regulation of enzyme activity - **REVERSIBLE**
- Phosphorylation: (**Ser, Thr, & Tyr**)
  - ✓ Mostly, ATP is the donor
  - ✓ **Kinases vs. phosphatases**



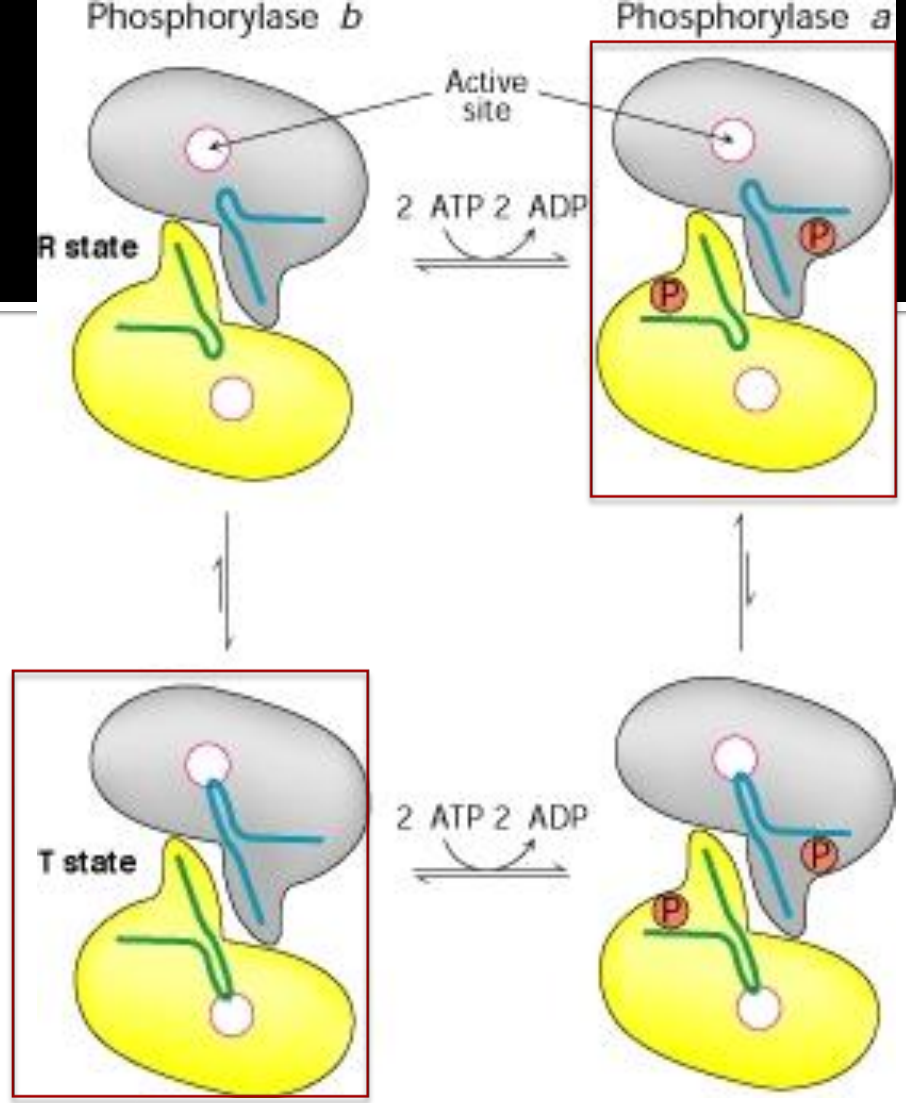
- ✓ Phosphorylation does not lead always to activation of enzymes
- ✓ **Glycogen phosphorylase-reaction** (two forms; a & b). Ser is away from the active site





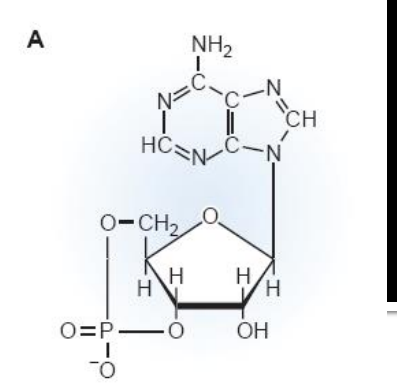
The two forms of the enzyme

- Both phosphorylase *b* and phosphorylase *a* exist as equilibria 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

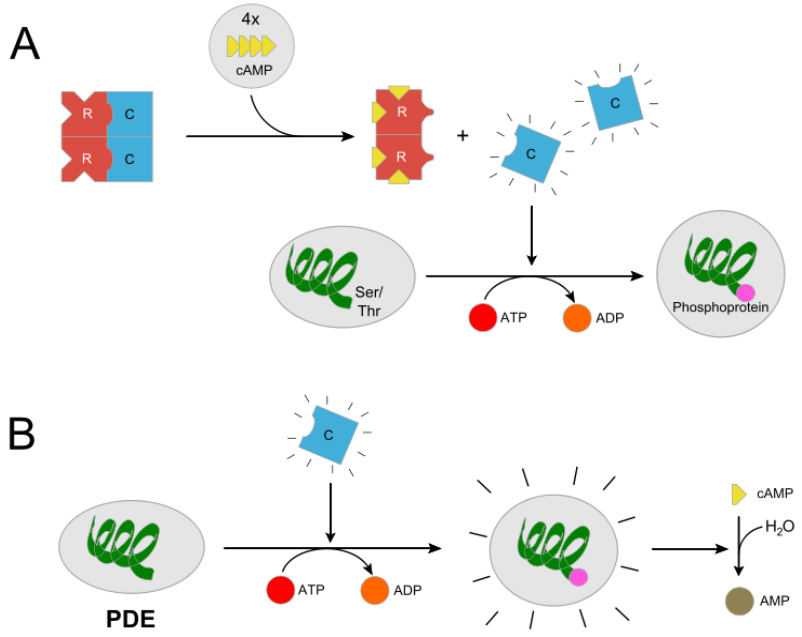


The transition of phosphorylase b between the T and the R state is controlled by the energy charge of the muscle cell.

Protein kinase A (PKA)



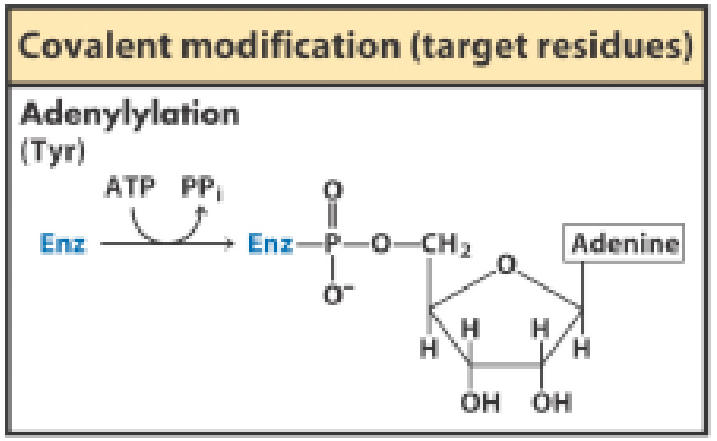
- Protein kinase A (PKA): refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP)
- cAMP: referred to as a hormonal 2nd messenger
- Either dedicated or not
- Has several functions in the cell, including regulation of glycogen, sugar, & lipid metabolism

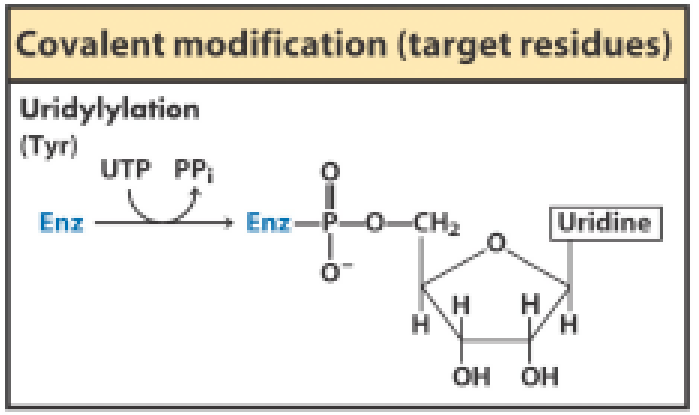


- Adrenaline (epinephrine) → ↑cAMP → activates protein kinase A → phosphorylates & activates glycogen phosphorylase kinase → phosphorylates & activates glycogen phosphorylase
- Phosphorylation cascade

Other covalent modifiers

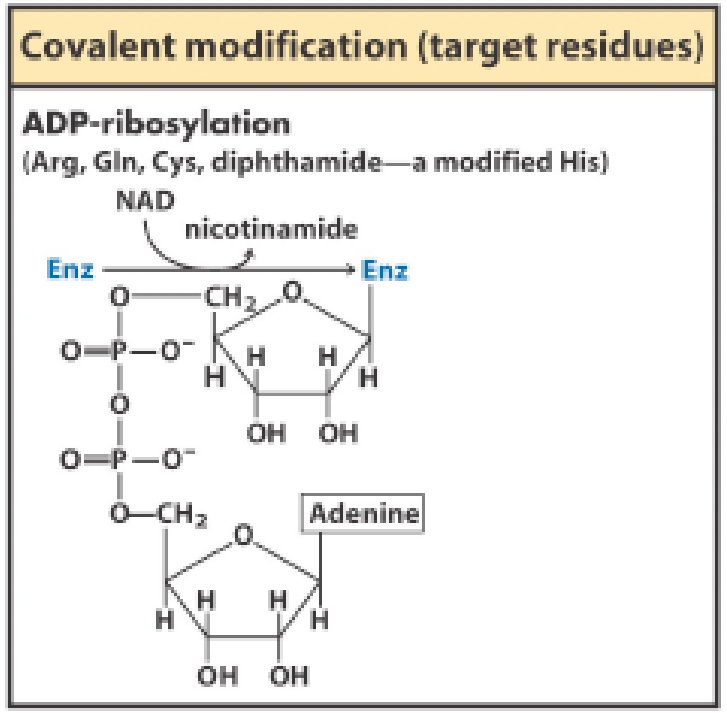
- Adenylylation (addition of adenylyl group). AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes.
- Uridylylation (addition of uridylyl group).

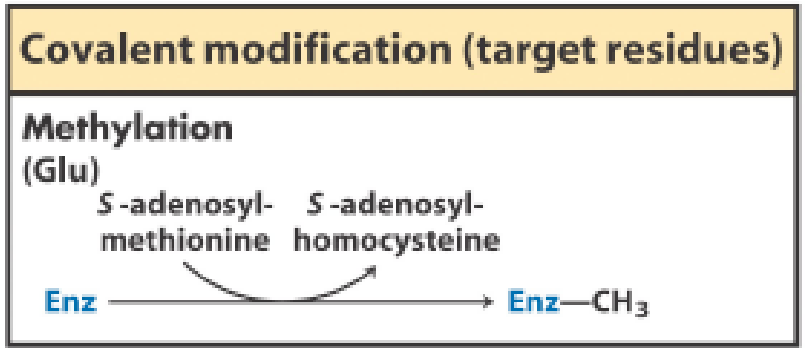




Other covalent modifiers

- ADP-ribosylation: inactivates key cellular enzymes
- Methylation: masks a negative charge & add hydrophobicity on carboxylate side chains
- Acetylation: masks positive charges when added to lysine residues



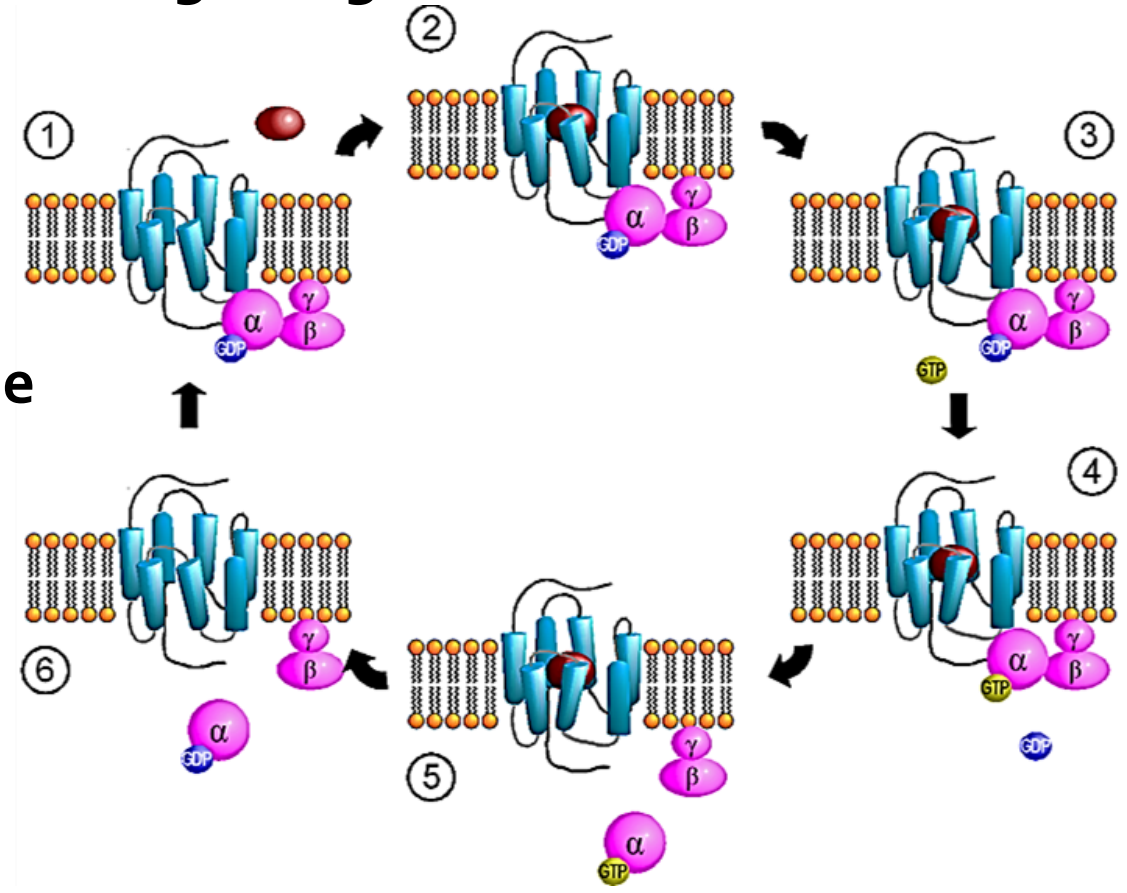


3.C. Conformational Changes from Protein–Protein Interactions

- G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors

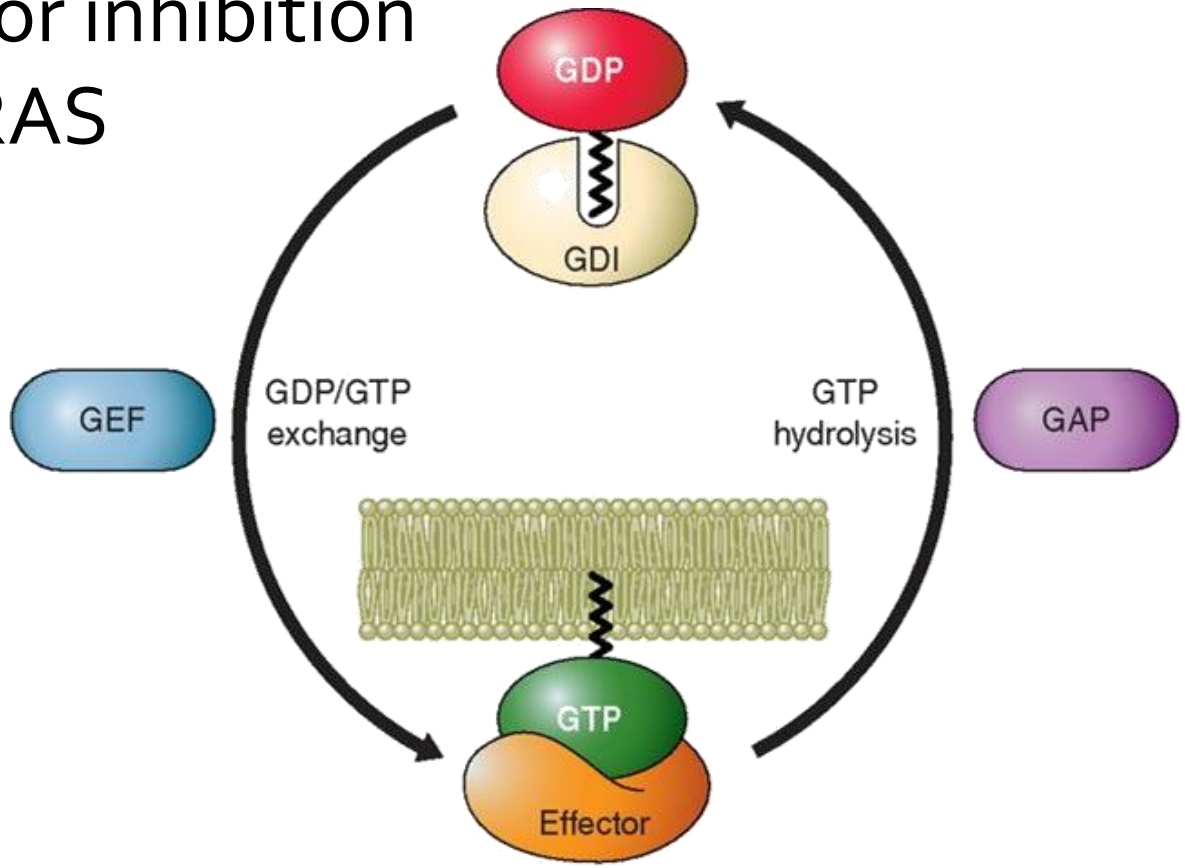
- When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'

- α-Subunit can be stimulatory or inhibitory



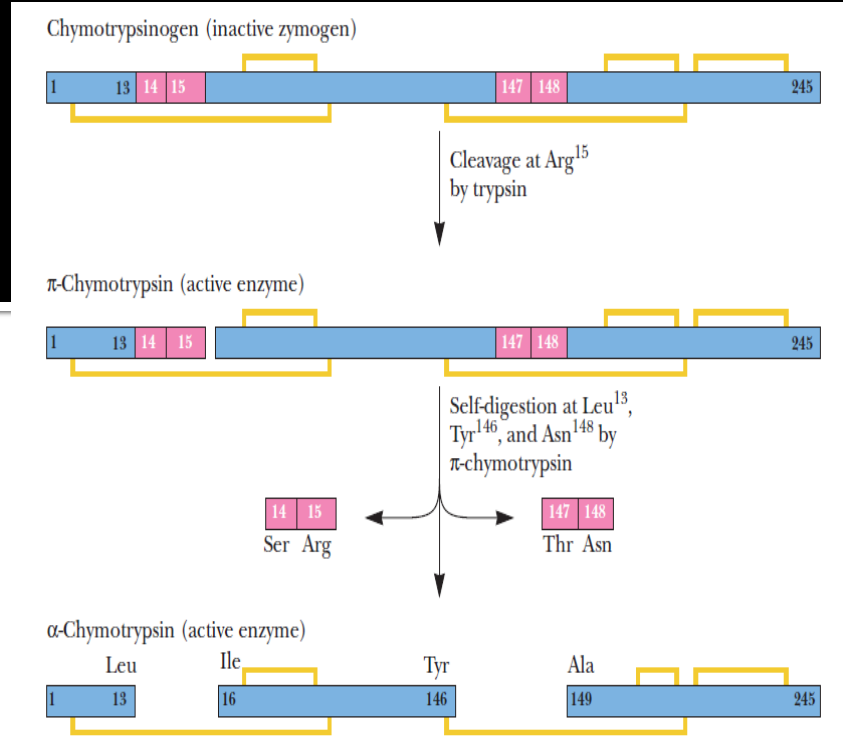
Monomeric "G" proteins

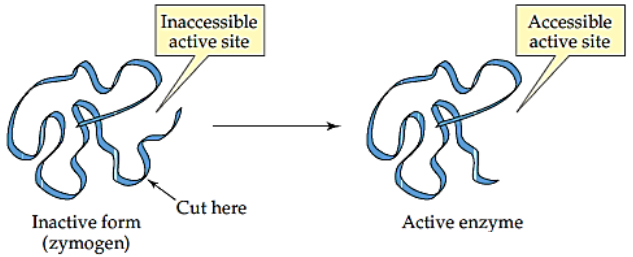
- Same as Gα
- Hydrolysis vs. exchange
- Activation or inhibition
- Example: RAS

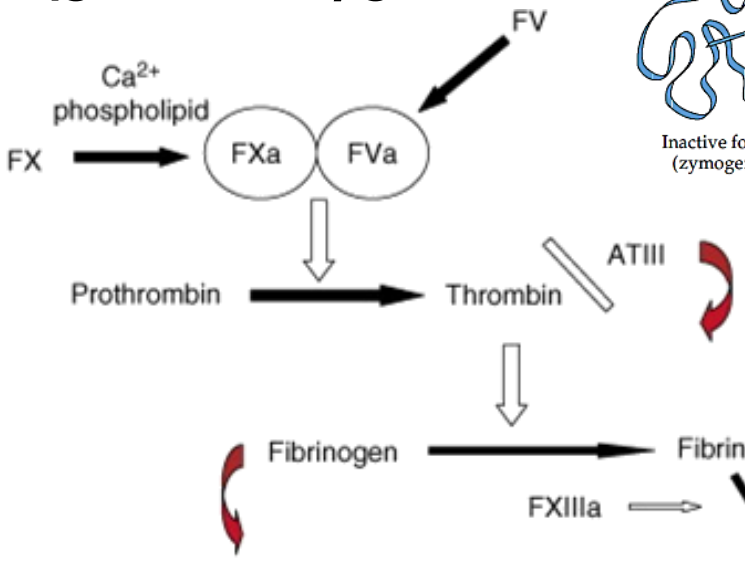


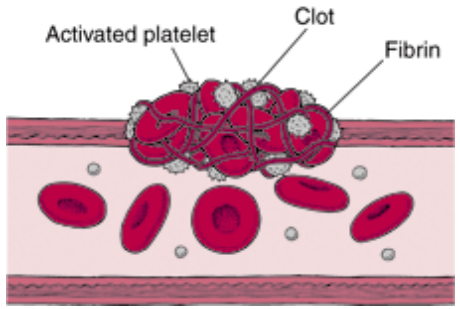
3.D. Proteolytic Cleavage Zymogens (Pro- or -gen)

- **Irreversible cut usually at the N-terminus**
- **Trypsin, chymotrypsin, pepsin (trypsinogen, pepsinogen chymotrypsinogen)**
  - ✓ Chymotrypsinogen: single polypeptide chain (245 residues), 5 (S—S) bonds
- **Blood clotting**
  - ✓ The soluble protein fibrinogen is converted to the insoluble protein fibrin









Non-specific regulators

REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

A. Regulated Enzyme Synthesis

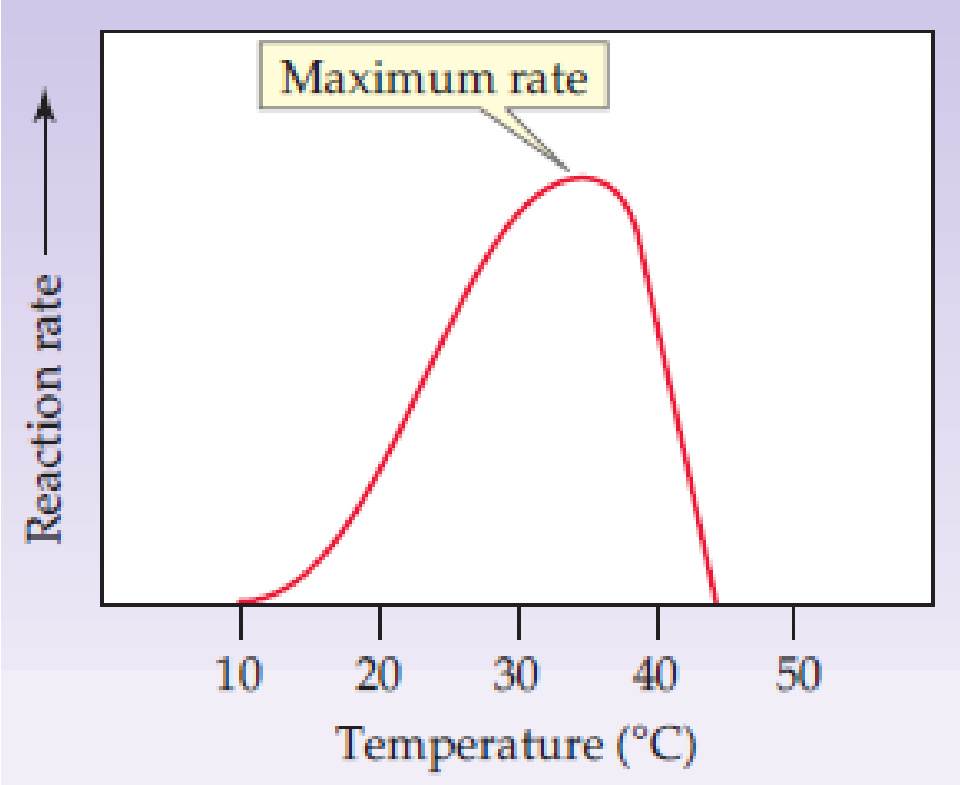
- Regulated by increasing or decreasing the rate of gene transcription (induction & repression)
  - Usually slow in humans (hours to days)
- Sometimes through stabilization of the messenger RNA

B. Regulated Protein Degradation

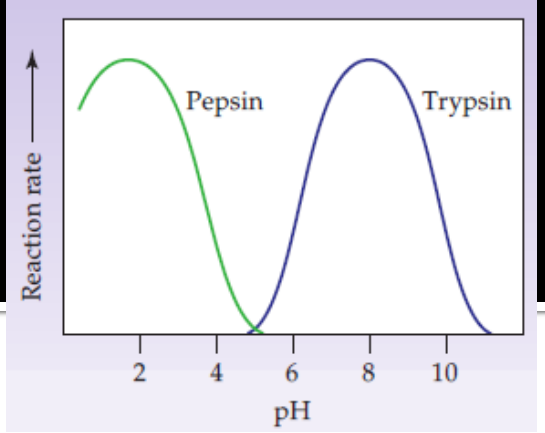
- Can be degraded with a characteristic half-life within lysosomes
  - During fasting or infective stress: gluconeogenesis increase & synthesis of antibodies (protein degradation increases)
  - Increased synthesis of ubiquitin

Effect of Temperature

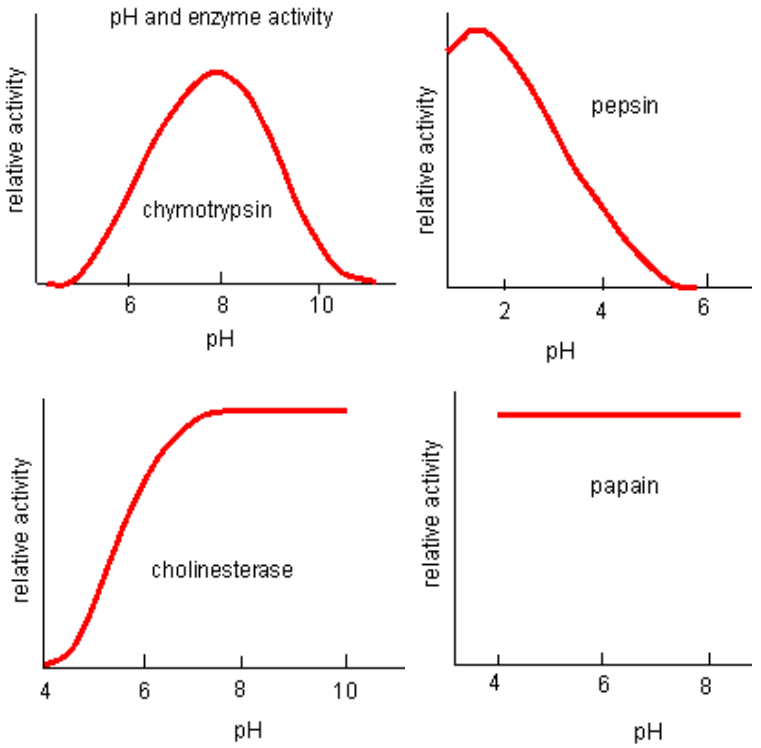
- Increase in T° increases the rate until reaches a max (≈ 50°): the optimal temperature of each enzyme is its' denaturation
- Autoclave steam heating
- Hypothermia, metabolic reactions, cardiac surgery



Effect of pH



- Usually a well defined optimum point
- Most enzymes have their max. activity between (5-9)
- Extremes of pH denatures protein
- pH can alter binding of substrate to enzyme (K_M) by altering the protonation state of the substrate, or altering the conformation of the enzyme



The effect of pH is enzyme-dependent

Extremozymes

Taq polymerase and PCR

Thermophiles (heat lovers)

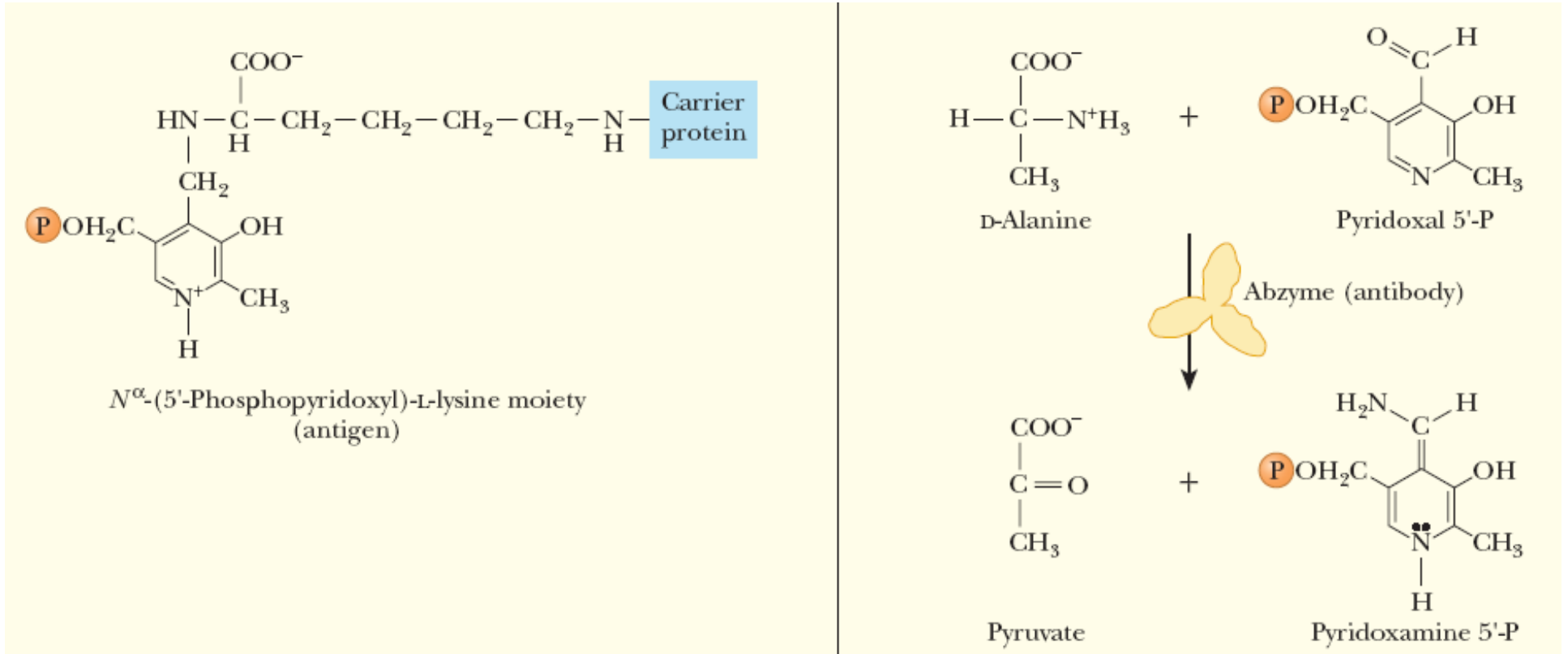
Psychrophiles (cold lovers)

Biobleaching of paper pulp using heat-stable xylanases

lipases and proteases

Abzymes – cutting edge science

- An antibody that is produced against a transition-state analog (active)
- An abzyme is created in animals



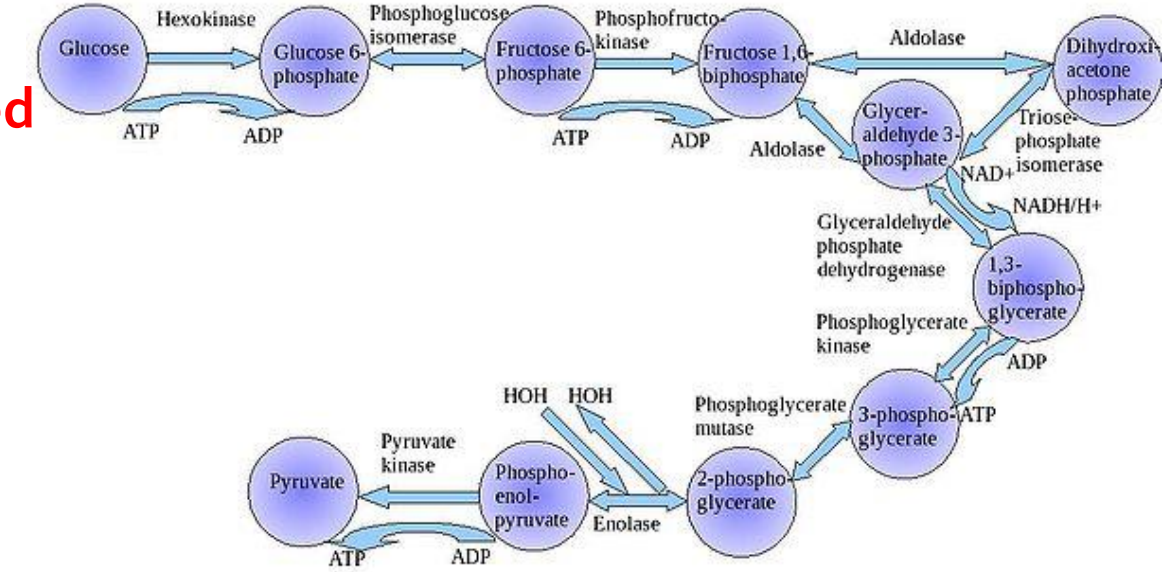
An exception to protein enzymes
Ribozymes

- RNA molecules
- Examples: telomerase & RNase P
- Catalyze splicing reactions and are involved in protein synthesis
- The catalytic efficiency of catalytic RNAs is less than that of protein enzymes, but can greatly be enhanced by the presence of protein subunits

REGULATION OF METABOLIC PATHWAYS

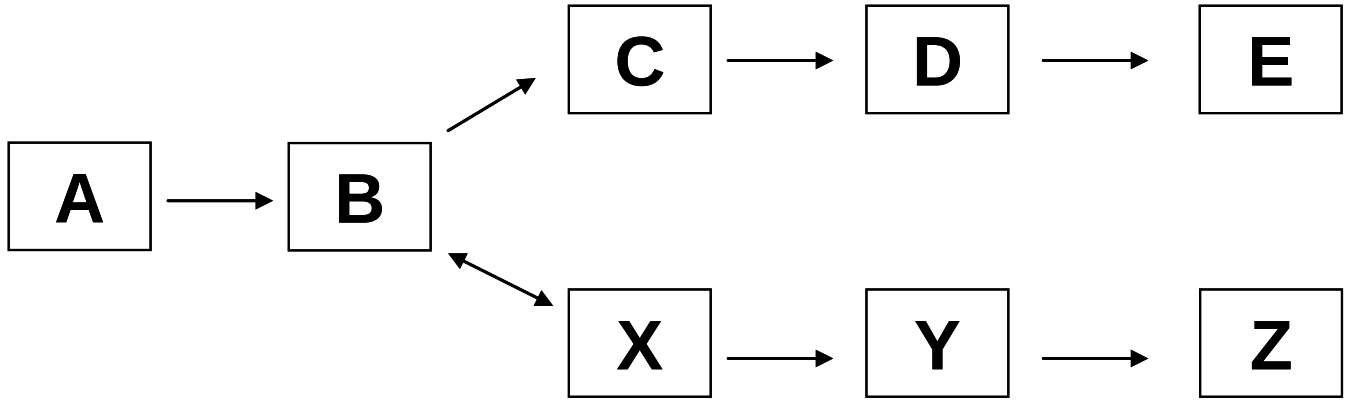
Principles of Pathway Regulation

- *1. COUNTERREGULATION OF OPPOSING PATHWAYS*
- Synthesis vs. degradation (a different regulatory enzyme)
- *2. TISSUE ISOZYMES OF REGULATORY PROTEINS*
- *3. REGULATION AT THE RATE-LIMITING STEP*
- Pathways are principally regulated at their rate-limiting step
- The slowest step & is usually not readily reversible
  - Changes in this step can influence flux through the rest of the pathway
- Usually the first committed step in a pathway
- Requirement for high amount of energy
- High K_M values of enzyme towards its substrate



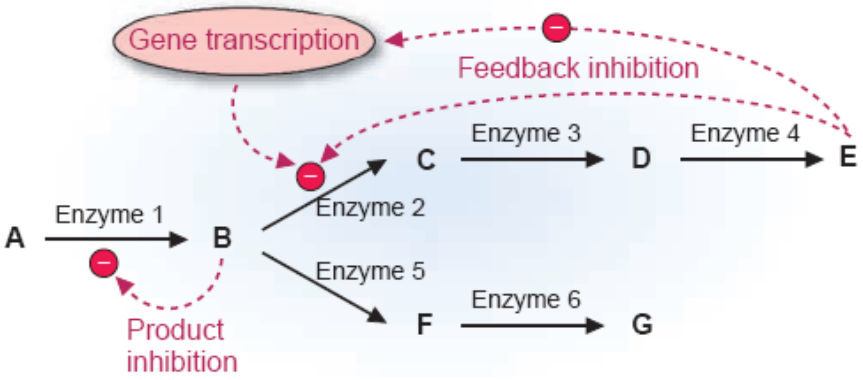
Principles of Pathway Regulation

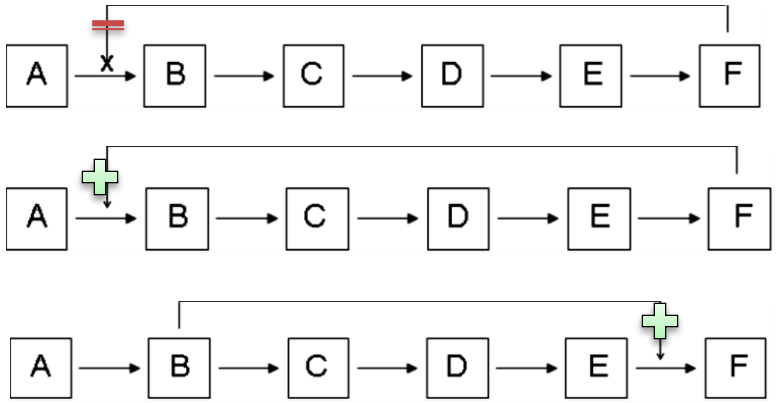
- *4. The committed step*
- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return
- Committed steps are exergonic reaction
- For example, the committed step for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

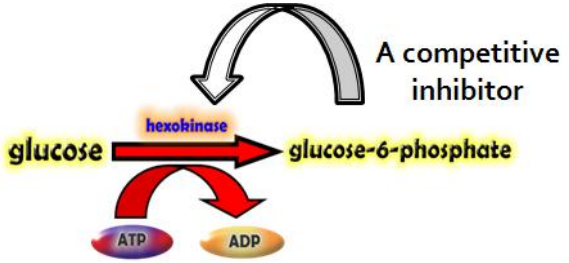


Principles of Pathway Regulation

- *5. FEEDBACK REGULATION*
- This type of regulation is much slower to respond to changing conditions than allosteric regulation
  - Negative feedback regulation (feedback inhibition)
  - Positive feedback regulation
  - Feed-forward regulation
    - Disposal of toxic compounds







Principles of Pathway Regulation

- ***6. Enzyme compartmentalization***
- **Both enzymes and their substrates are present in relatively small amount in a cell**
- **A mechanism by which rate of reactions become faster is their compartmentalization; reducing area of diffusion**
- **In this way, enzymes are sequestered inside compartments where access to their substrates is limited**
- **Lysosomes; proteins get transported to lysozymes**
- **Mitochondria; energy metabolic pathways**
- **Metabolism of fatty acids; synthesis (cytosol) vs. degradation (mitochondria)**

Principles of Pathway Regulation

- *7. Enzyme complexing (A multienzyme complex)*

- Complexing various enzymes that share one process
- Product of enzyme A pass directly to enzyme B
- Pyruvate dehydrogenase (mitochondria) 3 enzymes: decarboxylation, oxidation, & transfer of the resultant acyl group to CoA