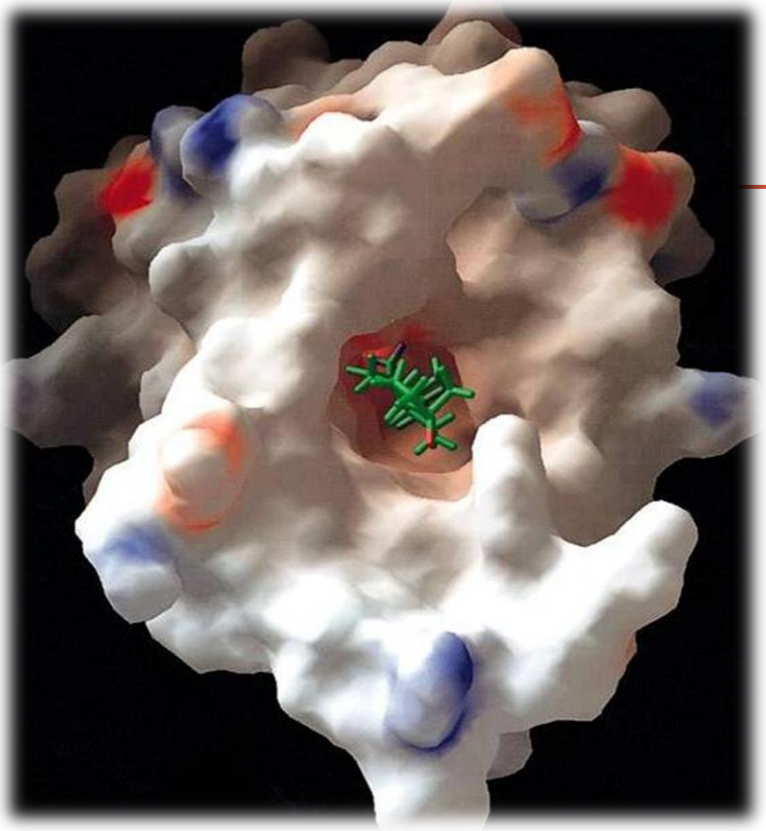
DRUG-ENZYME INTERACTION



Course: Drug Design
Course code: 0510412

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Learning Outcomes

At the end of this lesson students will be able to

- Explain the enzyme, active-site of the enzyme, enzyme catalysis, and co-enzymes.
- Define and describe the binding specificity.
- Explain various enzymatic reactions, rate acceleration, and enzyme turn over.
- Describe the enzyme inhibition mechanism and drug synergism.

Introduction

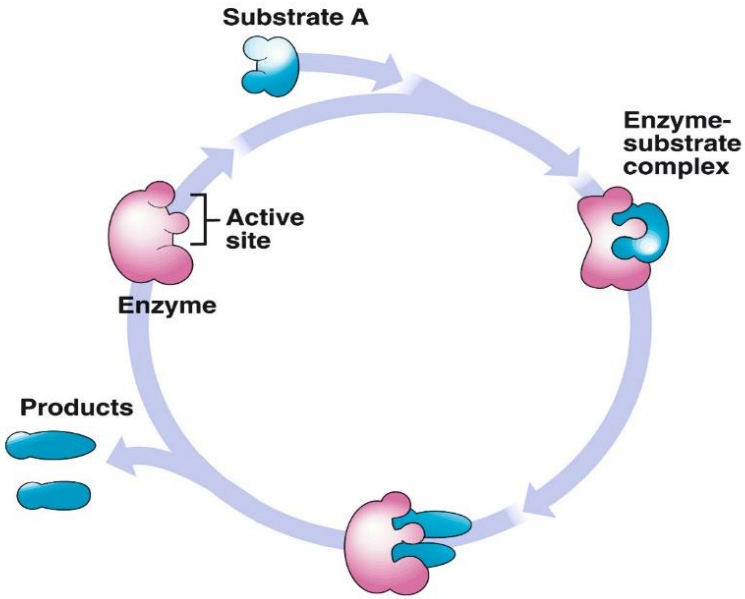
- Enzymes are soluble proteins, floating in interstitial or extrastitial fluids, cell cytosol and blood.

- Enzymes are produced by different cells of humans, animals, plants and microorganisms, which accelerate the rate of chemical reactions in living organisms.

- Enzyme can recognize the substrate and catalyzes a chemical reaction with it to release the product.

Enzyme-catalyzed reaction

- Enzymes function by lowering transition state energies and energetic intermediates and raising the ground state energy level.



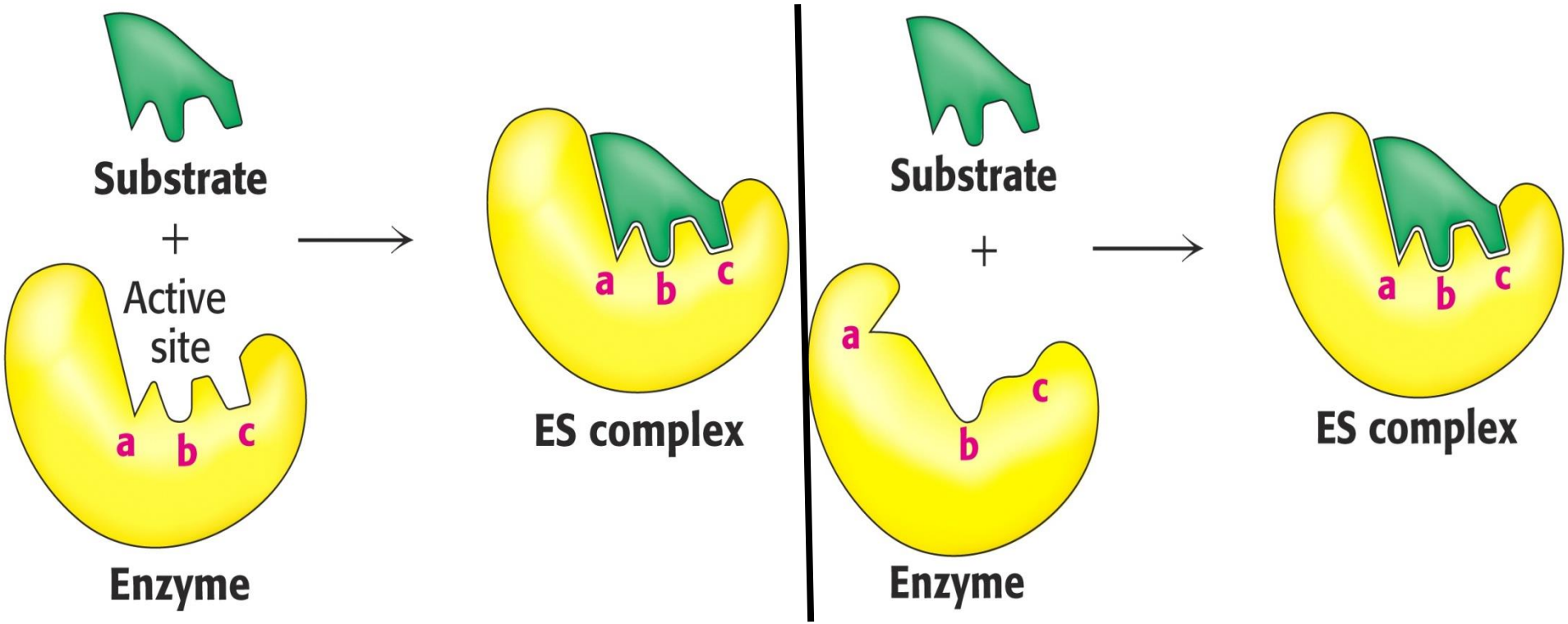
Enzyme + **Substrate** $\rightleftharpoons$ **E-S complex** $\rightarrow$ **Product**

E **S** K_1 K_2

Mechanism of enzyme action

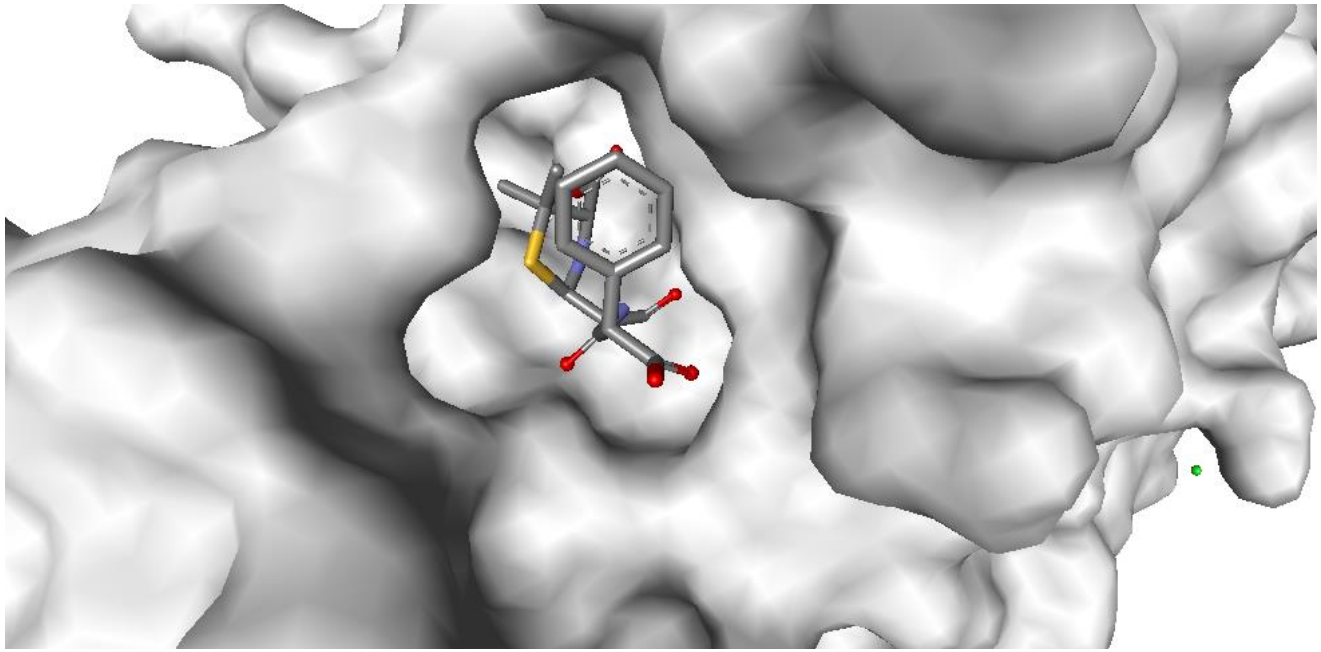
"Lock & Key" theory:

"Induced Fit" theory:



Enzyme Active-site

- Substrate binds to small area in enzyme structure known as active-site.
- Other names: Binding site, Binding domain, Active pocket



Enzyme Active site

- Two types of amino acids available inside enzyme active-site:

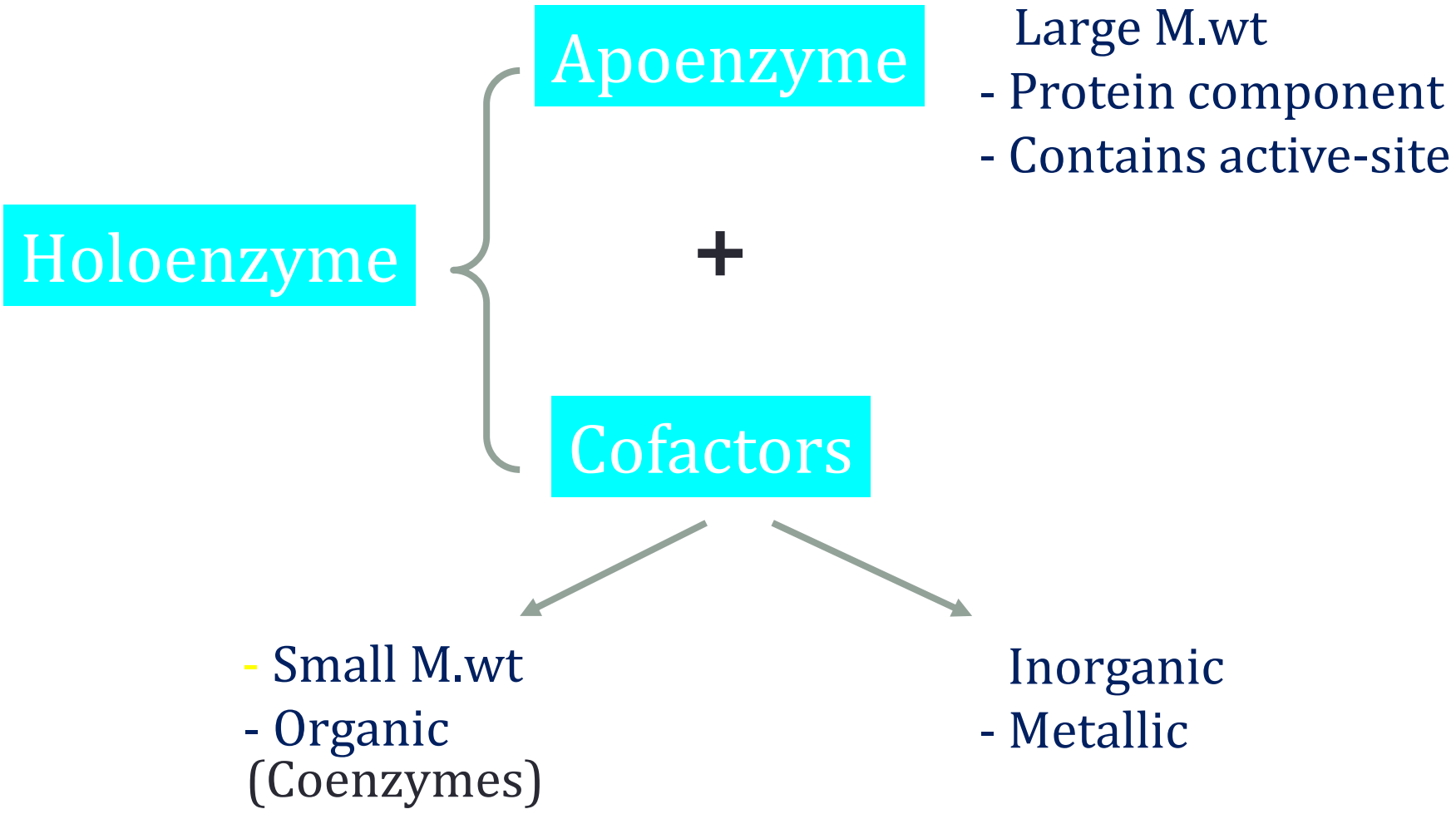
Catalytic amino acids:

They are directly or indirectly participate in the enzyme-substrate interactions.

Non-catalytic amino acids:

- Complete the construction of active-site pocket.
- Help shaping tunnels and opening to active site, especially when the pocket is deep inside.
- Might play role in binding (anchoring) of substrate to bring it close to catalytic amino acids.

Structure of Enzyme



Co-enzymes

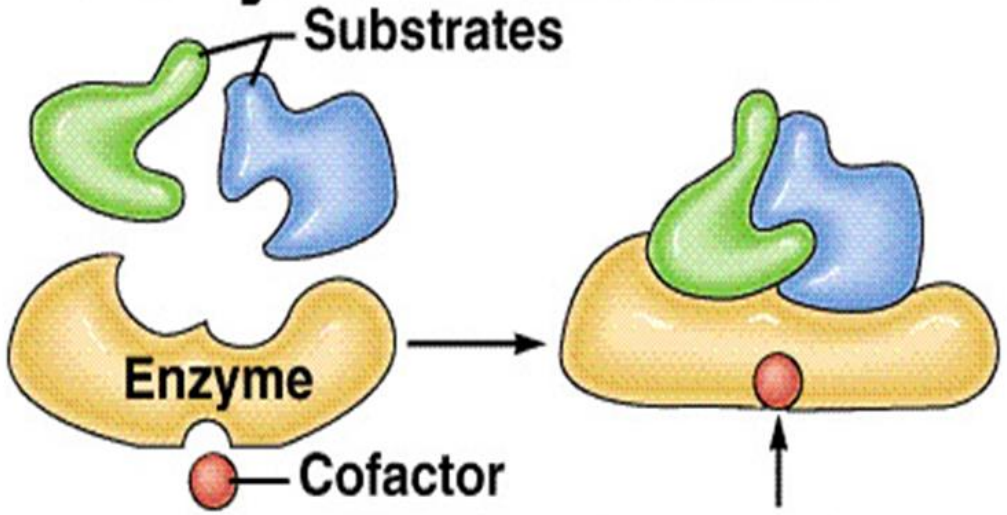
- Co-enzymes are any organic molecules (or) metal ions that are essential for catalytic action of enzyme.

Organic co-enzymes are metabolites of Vitamines.

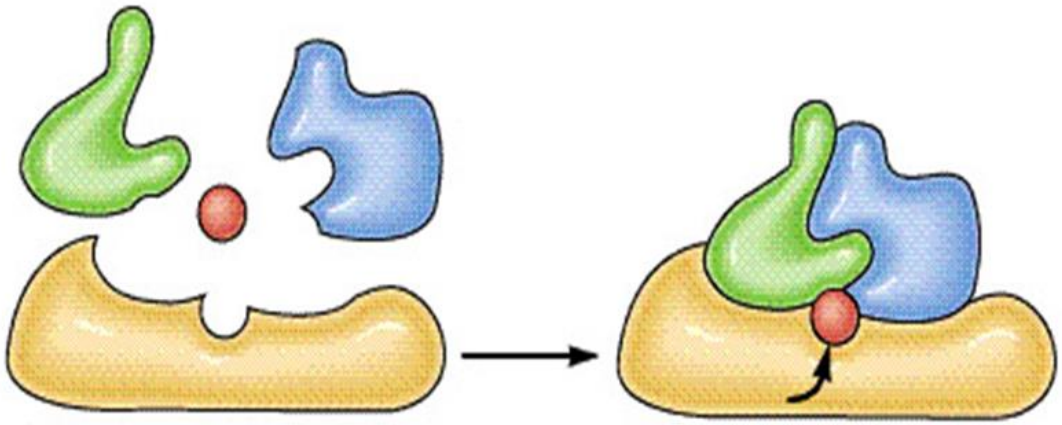
Examples:

- ATP
- Coenzyme A
- Glutathione
- Ascorbic acid
- Lipoic acid
- Zn, Co, Fe, Mg cations

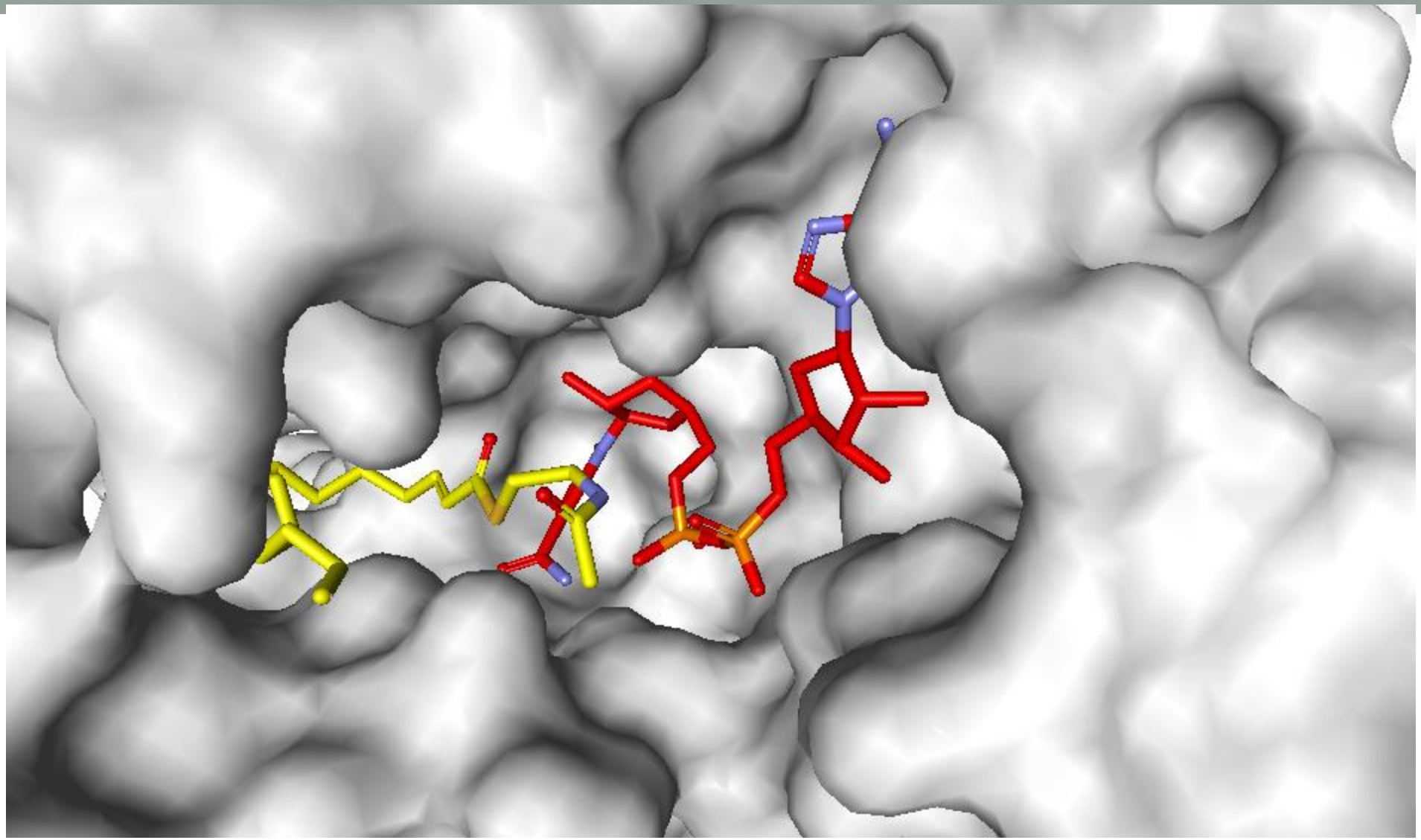
Roles of Cofactors in Enzyme Function



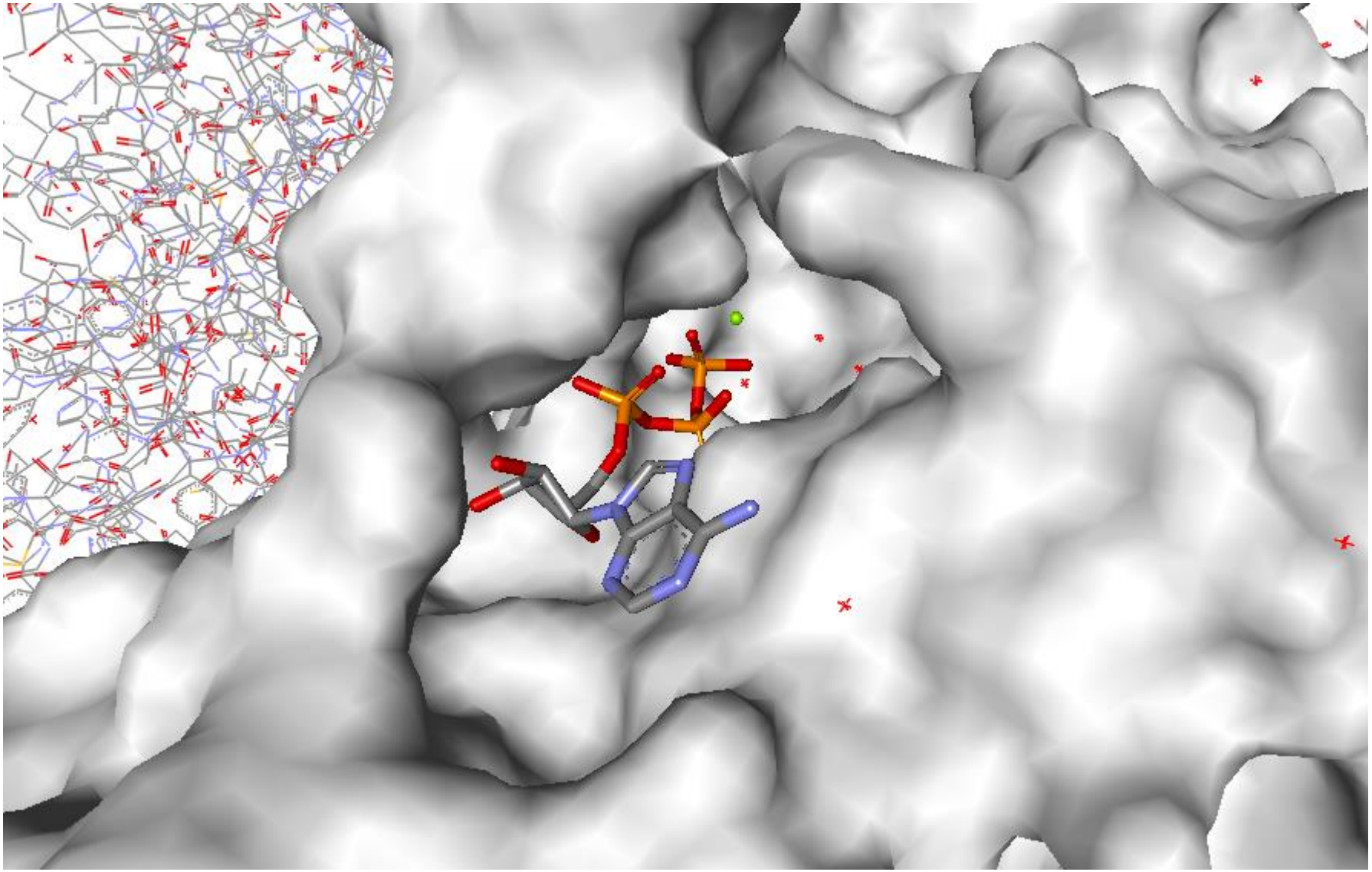
Cofactor changes conformation of active site.



Cofactor participates in temporary bonding between active site and substrates.



Substrate in yellow and NADH (co-factor) in Red



ATP (co-factor) exists in the active-site of ATP-dependent phosphofructokinase

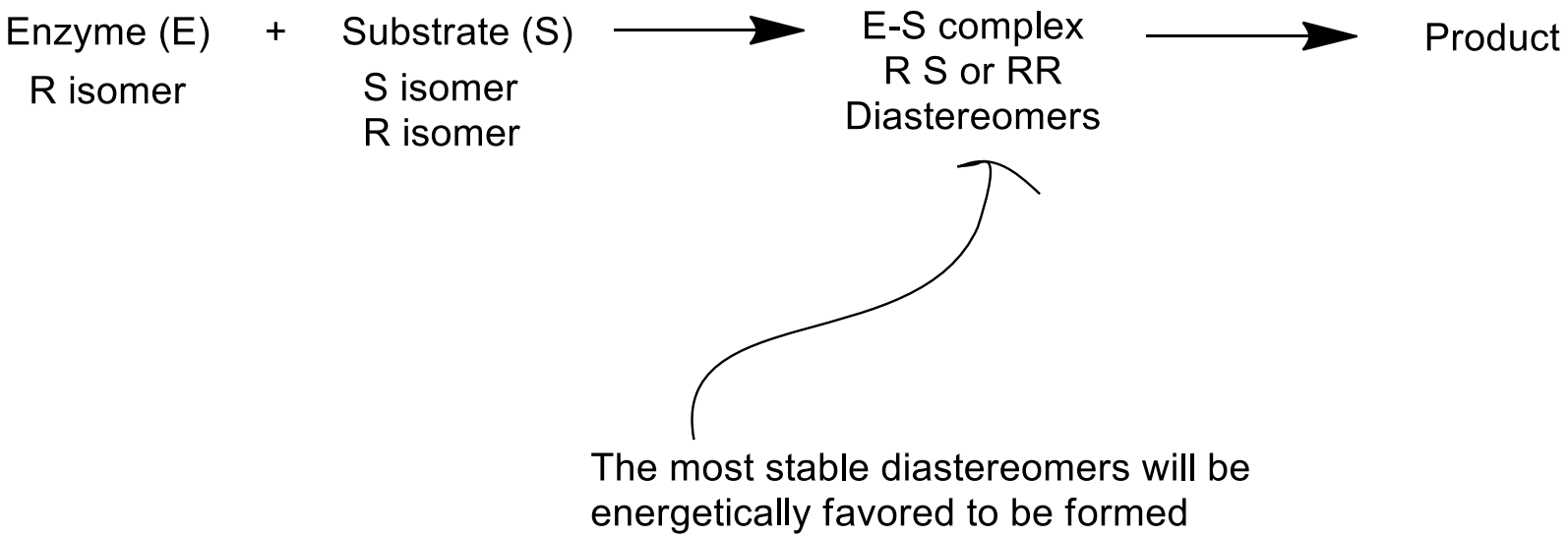
Enzyme Catalysis

- It is characterized by two features: (1) Reaction specificity.
  (2) Rate acceleration.

Reaction specificity can be classified into specificity of (A) binding and (B) specificity of reaction.

- (A) Binding Specificity:-
  (i) Very specific, Absolute: One type of substrate can fit the active-site.
  Examples: carboxyesterase, COMT, Acetylcholinesterase.
  (ii) Non-specific, Broad: More than one substrate can bind and converted into product. Example: Cytochrome P_{450}

Binding specificity



- Enzyme will bind preferentially to one isomer more than the other to form the more stable E-S complex because enzymes are chiral molecules.

- Based on steric and electronic reasons, only one isomer can give the product.

Binding specificity

- Because enzyme is a chiral molecule, only L-amino acids are available.
- All amino acids are chiral except the first amino acid Glycine.
- Chiral compounds will bind to form diastereomers (E-S complex), that have different physicochemical properties.
- The most stable diastereomer will be energetically favored to form.
- Also, amino acid residues available in the active-site play an important role in the binding specificity.

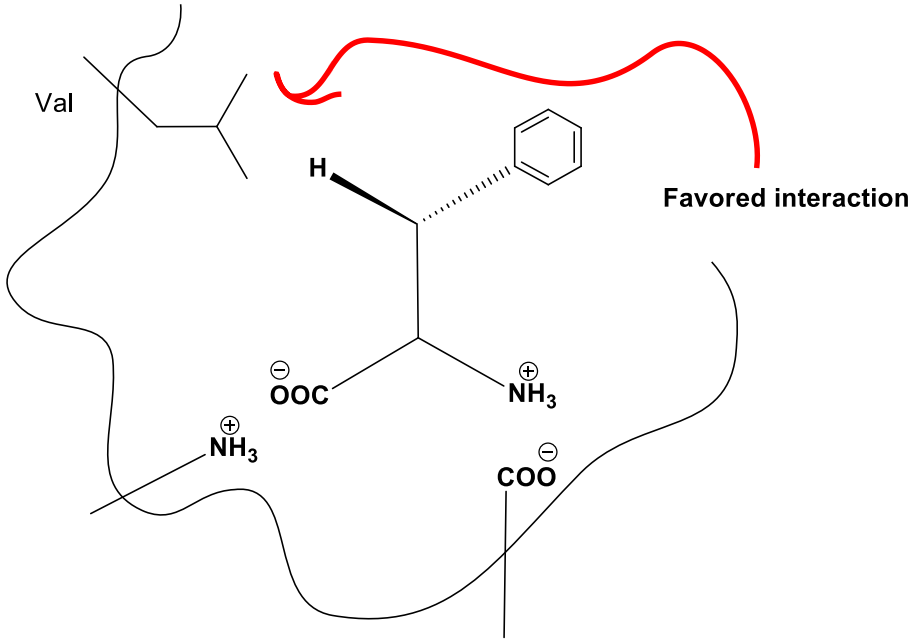
Stereo-specificity

- The enzyme will catalyze its reaction involving preferentially one particular isomer of the substrate than the other isomer.

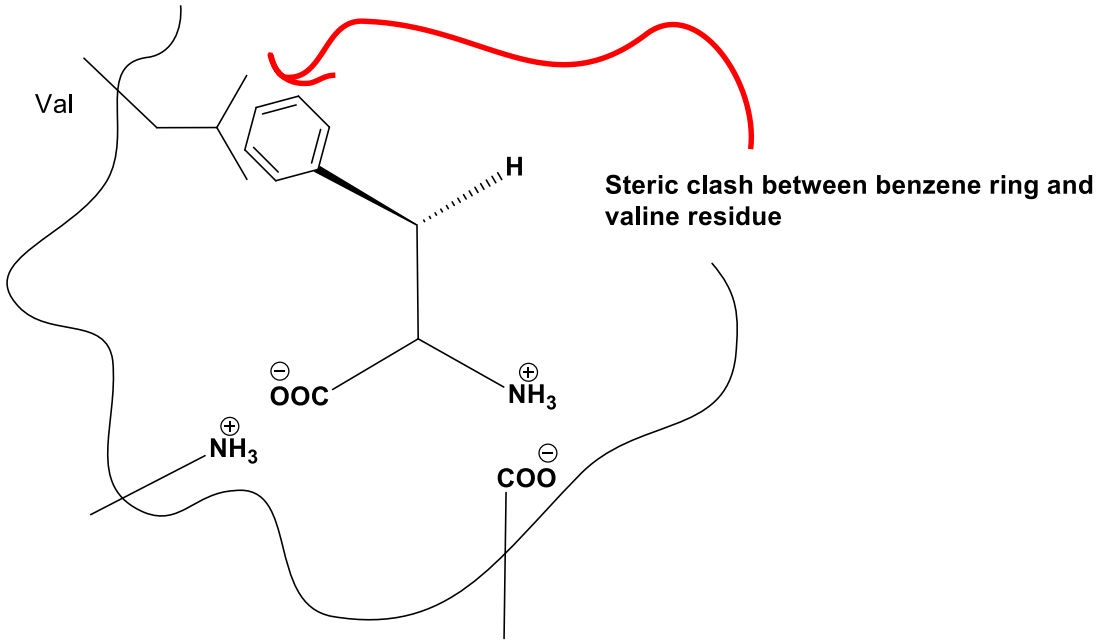
  - This is why some drugs will be active only when they are in its pure S or R isomer form.

Examples:

- R-isomer of Adrenaline is much more active than S
- R-isomer of Salbutamol is much more active than S
- S+methacoline is more active than R
- S-ibuprofen is more active than R



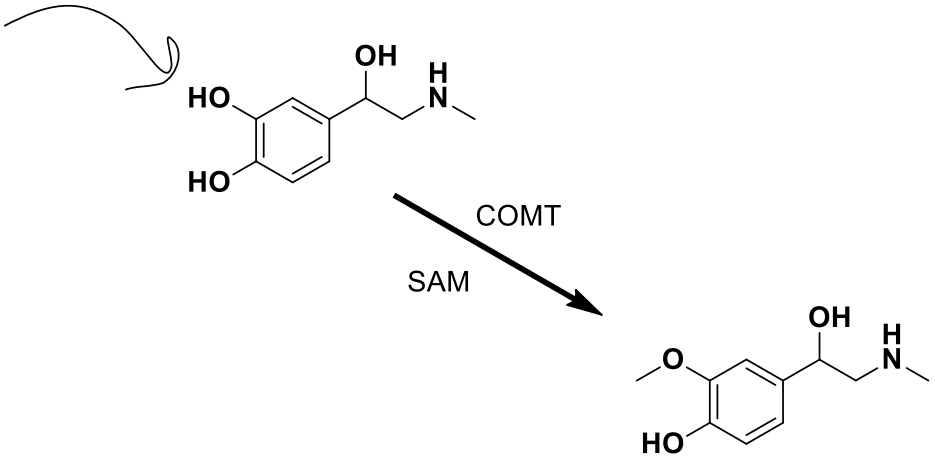
S-isomer



R-isomer

Regio-specificity

- Regio-specificity: The enzyme will catalyze its reaction on **specific group at specific position** although other identical groups are available elsewhere.
  - **Example: COMT** will methylate the hydroxyl group that is meta to amino ethylene group.



- Determinant factors that make enzymes Regio-specific are

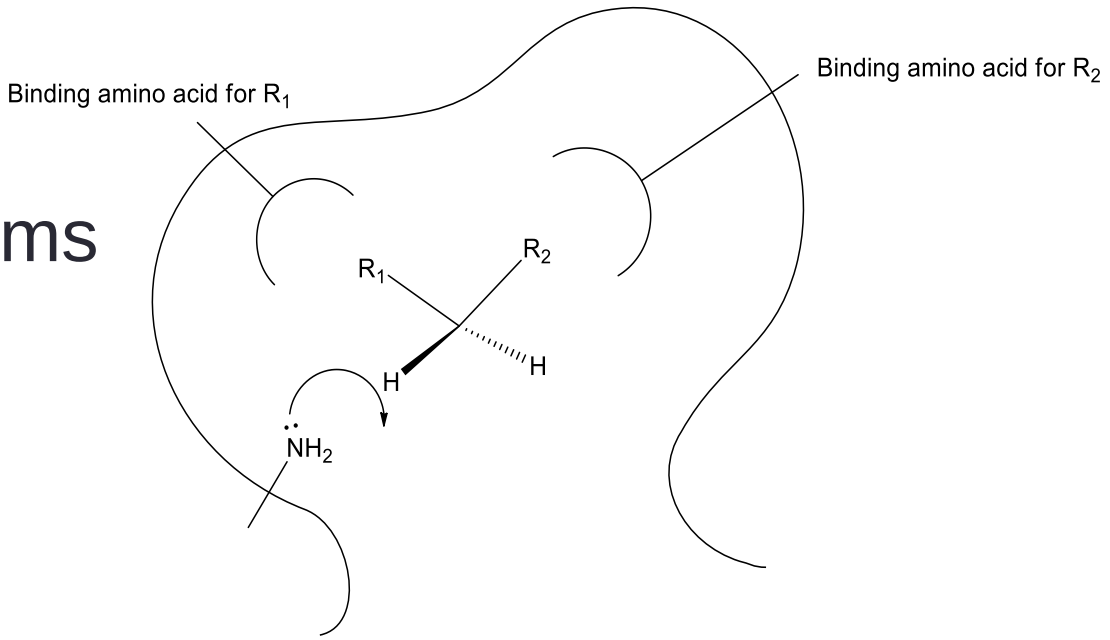
(1) Which group is the closest to the catalytic amino acids.

(2) Which group is nearest to the site where co-enzyme present.

  - ATP for phosphorylation
  - NADH/NAD^+ for redox reaction
  - SAM for methylation

Reaction specificity

- Even if substrate is achiral, enzyme can distinguish between identical hydrogen atoms or protons.



- How?

Since two pockets are specific for R_1 and R_2, the base of amino acid can reach one of the identical protons and abstract it, stereospecifically.

Enzyme can differentiate identical protons

Rate acceleration

- In general, catalysts stabilize the transition state, relative to ground state thereby decreasing the activation energy required for rate acceleration.
- Apart from catalysis, enzymes utilize binding interactions which facilitate the reactions by positioning substrates with catalytic amino acids.
- Rate acceleration is due to the presence of co-enzymes.

Rate acceleration

- Enzymes accelerate biochemical reactions by:
  - Stabilizing the transition state, thereby lowering transition state energy.
  - Destabilizing the E-S complex, thereby increasing the ground state energy.
  - Destabilizing the intermediates.

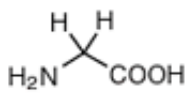
✓Finally, reaction rate could reach 10^{10} – 10^{14} than non-enzymatic reactions.

Enzyme turnover

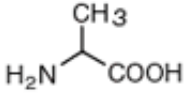
- Enzymes have turnover numbers (also known as K_{cat}). It is the number of molecules of substrate converted to product (i.e., turned over) per unit of time per molecule of enzyme active-site.
- Some enzymes have multiple subunits and each subunit can have more than one active-site.
- As the number of subunits is increased, turnover number also increased.

Classification of amino acids

Small

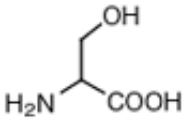


Glycine (Gly, G)
MW: 57.05

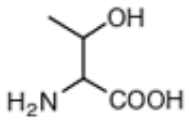


Alanine (Ala, A)
MW: 71.09

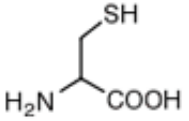
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

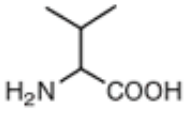


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

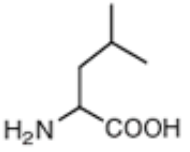


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

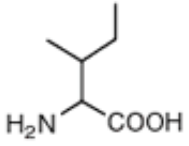
Hydrophobic



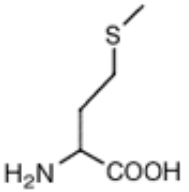
Valine (Val, V)
MW: 99.14



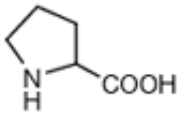
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

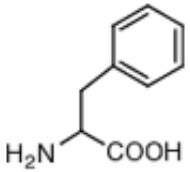


Methionine (Met, M)
MW: 131.19

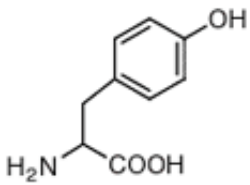


Proline (Pro, P)
MW: 97.12

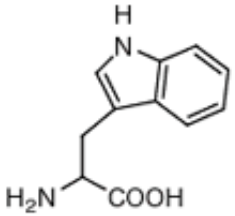
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

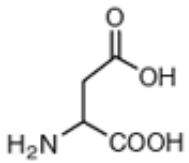


Tyrosine (Tyr, Y)
MW: 163.18

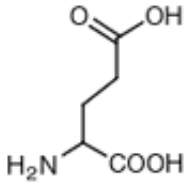


Tryptophan (Trp, W)
MW: 186.21

Acidic

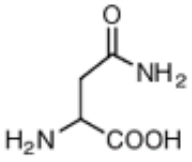


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

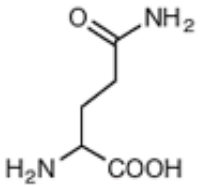


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

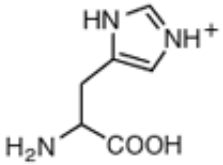


Asparagine (Asn, N)
MW: 114.11

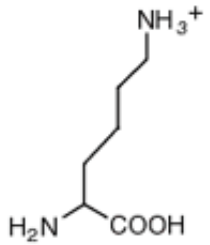


Glutamine (Gln, Q)
MW: 128.14

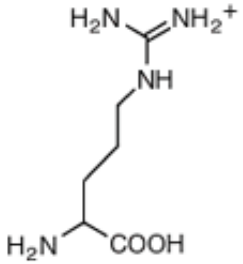
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

Enzyme inhibition

- Enzyme inhibitor will slows down or blocks enzyme catalysis.
- If the compound is an irreversible inhibitor, then it is also known as enzyme inactivator.
- The consequences of enzyme inhibition depends on the function of the enzyme.
- Examples:
  - GABA aminotransferase deactivates GABA…. Inhibition of this enzyme will accumulate GABA ….Anticonvulsant action.
  - Xanthine will be oxidized into uric acid by the enzyme Xanthine oxidase. Inhibition of this enzyme will help in decreasing uric acid level thereby treating Gout patients.

Enzyme inhibition

- Enzyme inhibitors in contrast to receptor antagonists, are closely similar in structure to enzyme natural substrate.
- They should bind strongly to active-site of enzyme and prevent the binding of normal substrate.
- Ideal enzyme inhibitor should:
  - be specific for one target enzyme.
  - target essential enzymes in essential metabolic pathway (antibacterials).
  - selectively inhibit essential enzymes in non human cells that are unique, does not exist in human cells (penicillins).

Inhibitors [I] are substances that combine with Enzymes [E] and decrease its activity.

Presence of [I] decreases the rate of Enzyme catalyzed reaction.

Enzymes are subject to reversible or irreversible inhibition.

Irreversible inhibitors usually cause stable, covalent alterations in the enzyme.

The consequence of irreversible inhibition is a decrease in the concentration of active enzyme.

Reversible inhibitors falls into three major categories

Competitive inhibitors

Binds to the enzyme active site.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

Mixed inhibitors

Binds at a separate site, but may binds either E or ES.

$$E + I \underset{}{\overset{K_I}{\rightleftharpoons}} EI \qquad ES + I \underset{}{\overset{K_I'}{\rightleftharpoons}} IES$$

Uncompetitive inhibitors

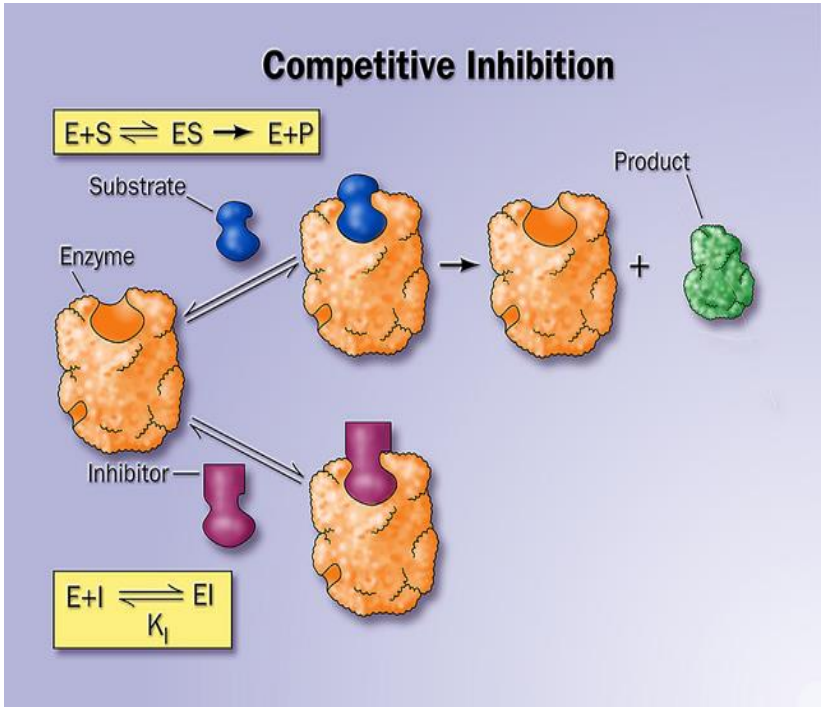
Binds at a separate site but, binds only to the ES.

$$E + I \underset{}{\overset{K_I}{\rightleftharpoons}} EI \qquad ES + I \underset{}{\overset{K_I'}{\rightleftharpoons}} IES$$

Competitive/reversible Inhibition

Compete with the substrate for the active-site of enzyme.

The overall effect of competitive inhibition is that the enzyme cannot bind with substrate when the inhibitor is present.

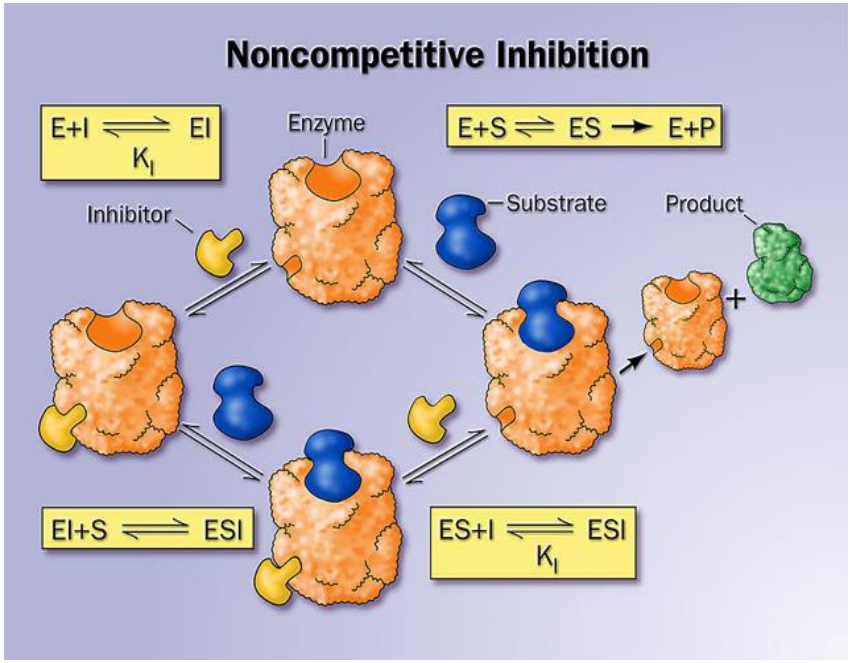


Competitive inhibition can be relieved by increasing the substrate concentration (the substrate "outcompetes" the inhibitor).

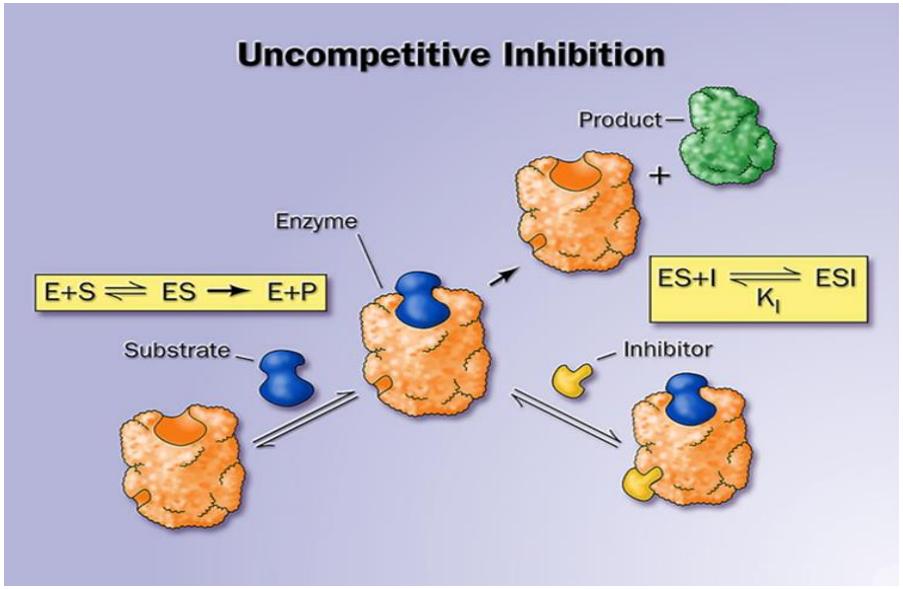
Examples: captopril, enalapril, lisinopril and oral antihypertensives (ACE Inhibitors).

Non competitive Inhibition

Non competitive or mixed inhibitors bind at a site distinct from the substrate active site (allosteric binding-site) on the enzyme, but will bind to either E or ES.

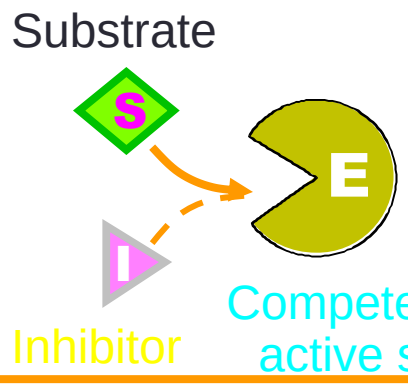
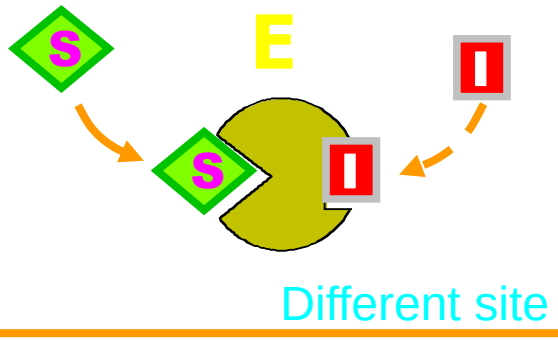
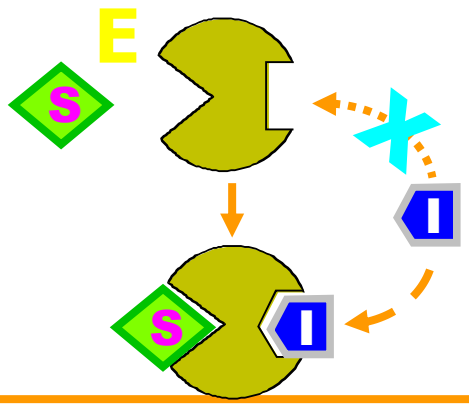


Uncompetitive inhibitors



Binds at a site distinct from the substrate binding-site but only in the ES complex.

Enzyme Inhibition (Mechanism)

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ + I ↑ EI	$E + S \rightleftarrows ES \rightarrow E + P$ I I ↑ ↑ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ I ↑ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	**[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.**	**[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].**

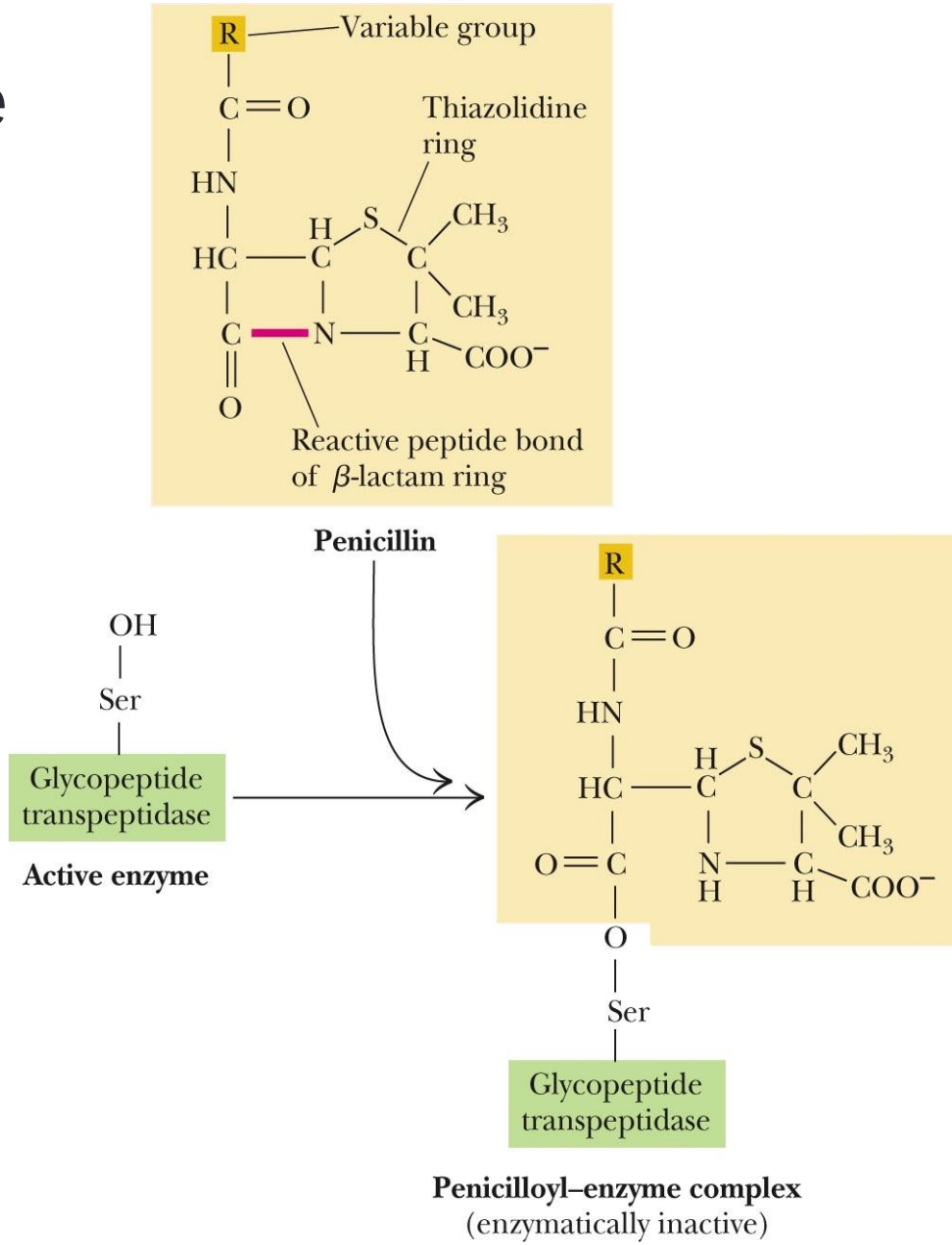
Irreversible inhibition

Irreversible inhibitors/ enzyme inactivators:

Covalently combine with and destroy a functional group on an enzyme that is essential for the enzymatic activity, or that form a stable non-covalent association that does the same thing.

Most irreversible inhibitors are toxic substances, either natural (cyanide, penicillin) or synthetic (parathion – an insecticide) compounds.

Penicillin is an irreversible inhibitor of the enzyme ***glycopeptide transpeptidase***, the enzyme which catalyzes an essential step in bacterial cell wall synthesis. Penicillin covalently blocks the active site amino acid serine present inside the glycopeptide transpeptidase.



Drug synergism

When two drugs are given in combination, the therapeutic effect of them is greater than the sum of their effects if they are given individually.

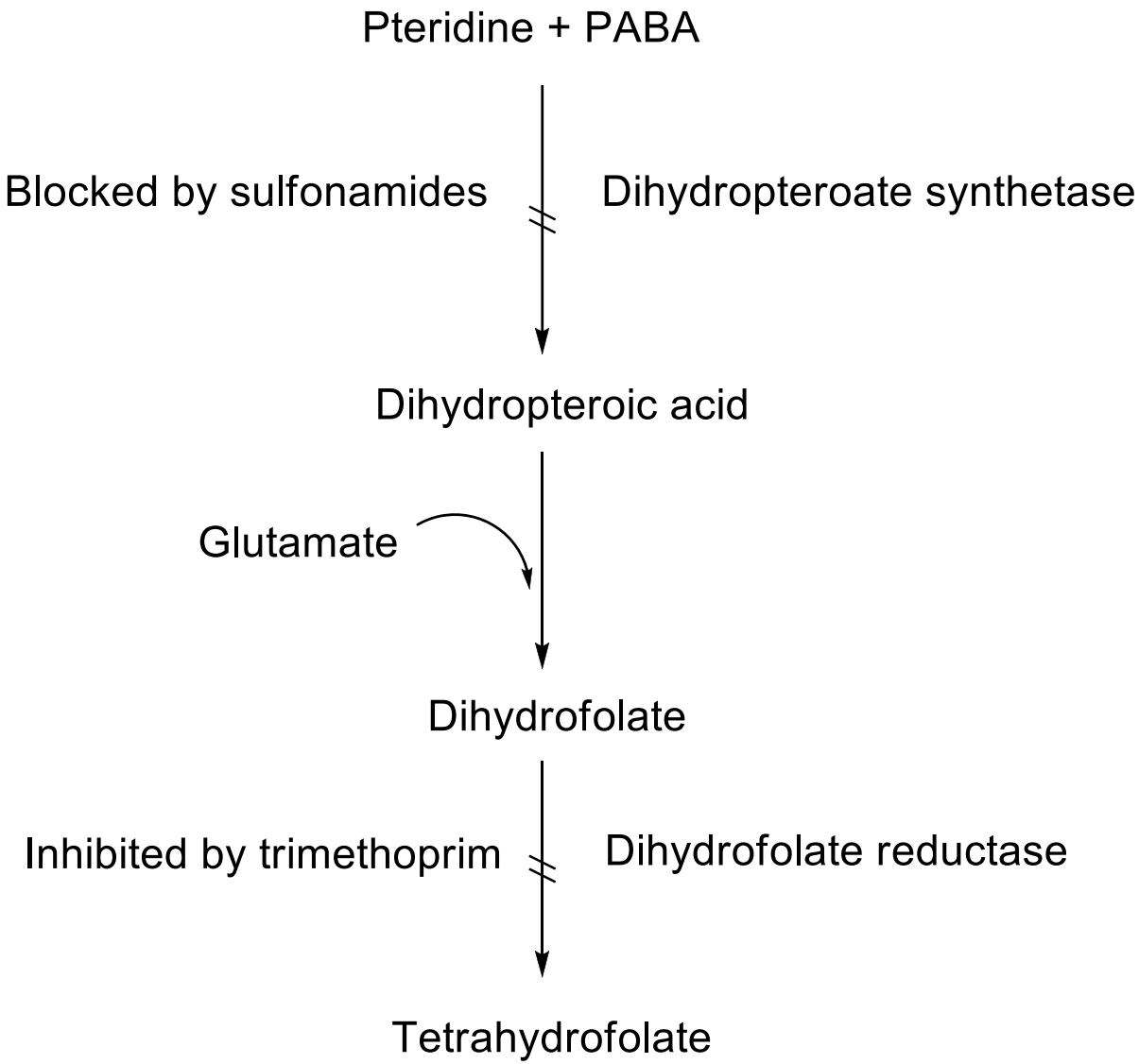
Mechanisms of enzyme synergism:-

- Inhibition of drug destroying enzymes:-

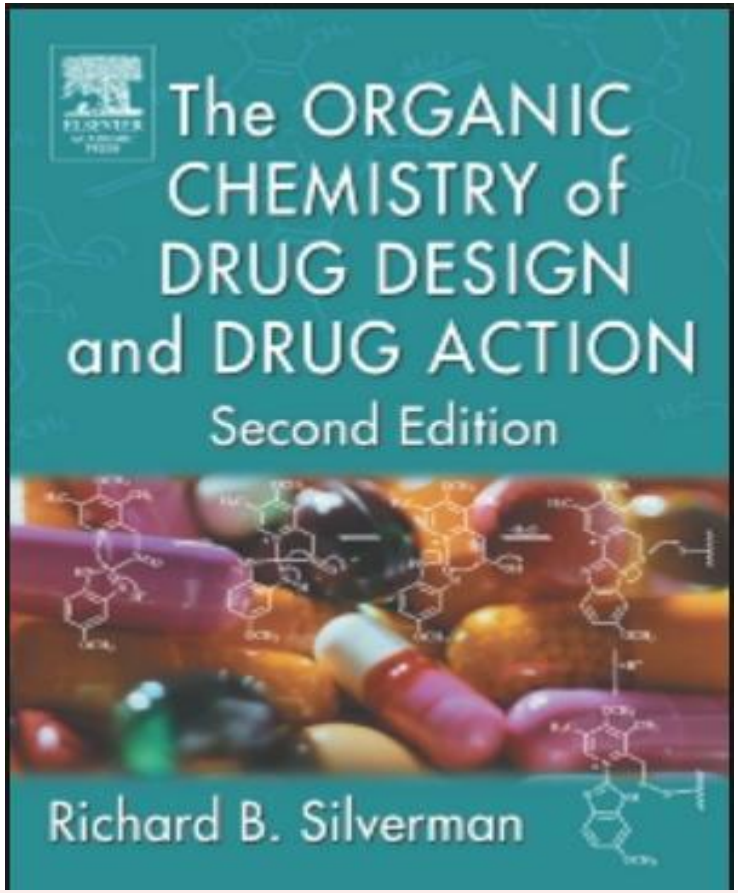
  Example: calvulanic acid with amoxicillin
- Sequential blocking:-
  - Inhibiting two or more consecutive steps or enzymes in the same metabolic pathway

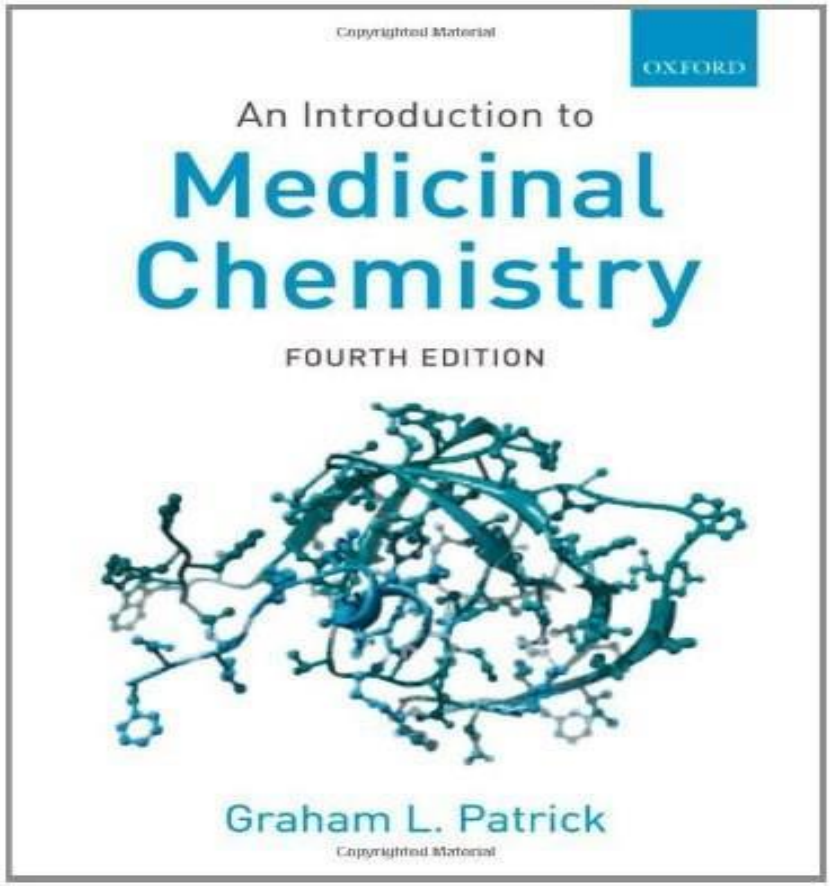
    Example: Trimethoprim with sulfamethoxazole.

Sequential blocking



Recommended Books





1. The organic chemistry of drug design by Richard B. Silverman. Second edition, Elsevier, 2004.

2. An introduction to Medicinal Chemistry by Graham L. Patrick. Fourth edition, Oxford, 2009.


Thank
You