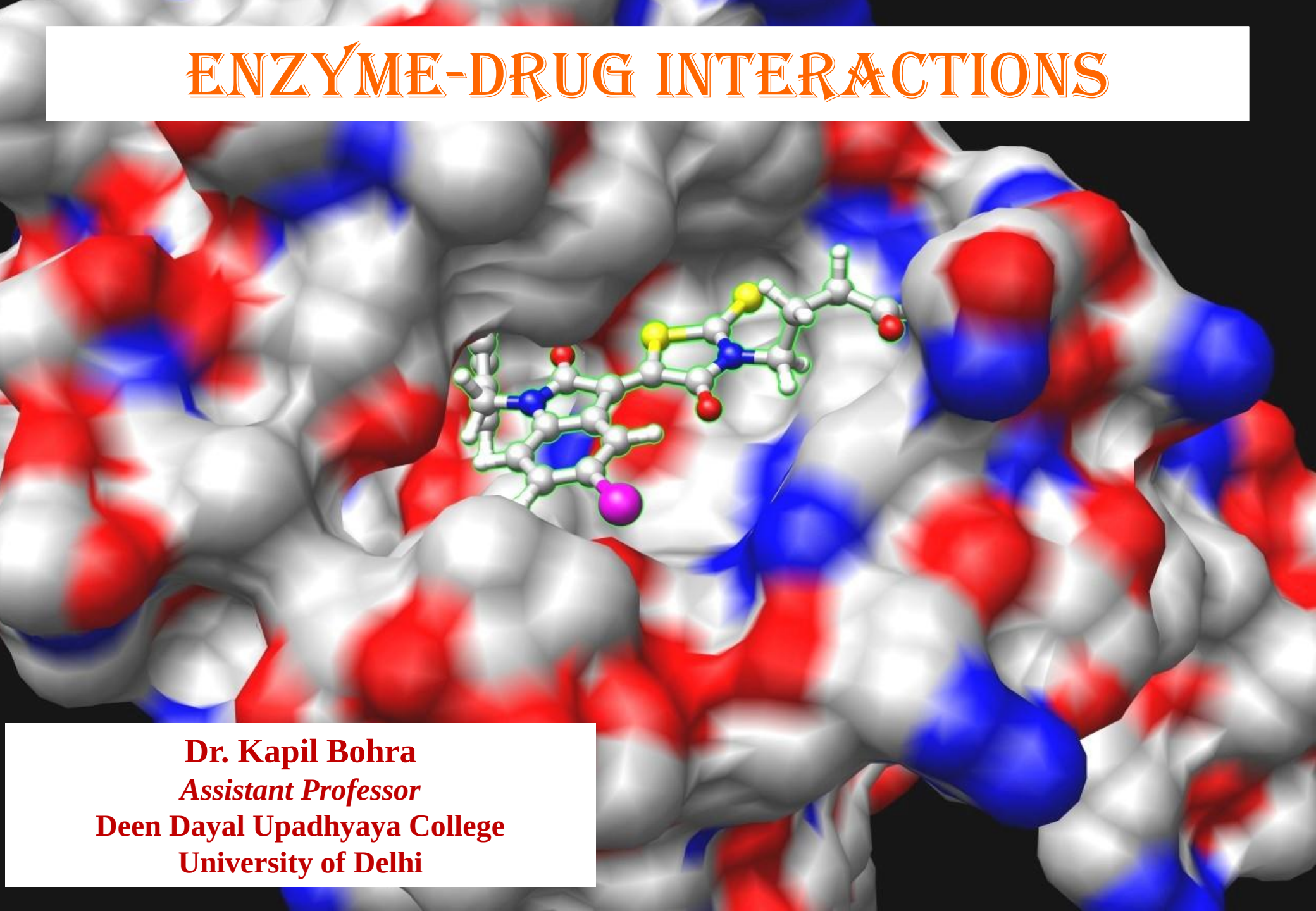
ENZYME-DRUG INTERACTIONS



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Enzymes and correlation with drug action

Contents

- Introduction of biological catalysis
- Coenzymes & cofactors and their role
- Mechanism of enzyme action
- Stereo-specificity
- Enzyme Inhibition
- Drug action-receptor theory
- SAR of drug molecules
- Binding role of -OH, $-NH_2$, double bond and aromatic.

Introduction to Enzymes

Review Bio-macromolecules

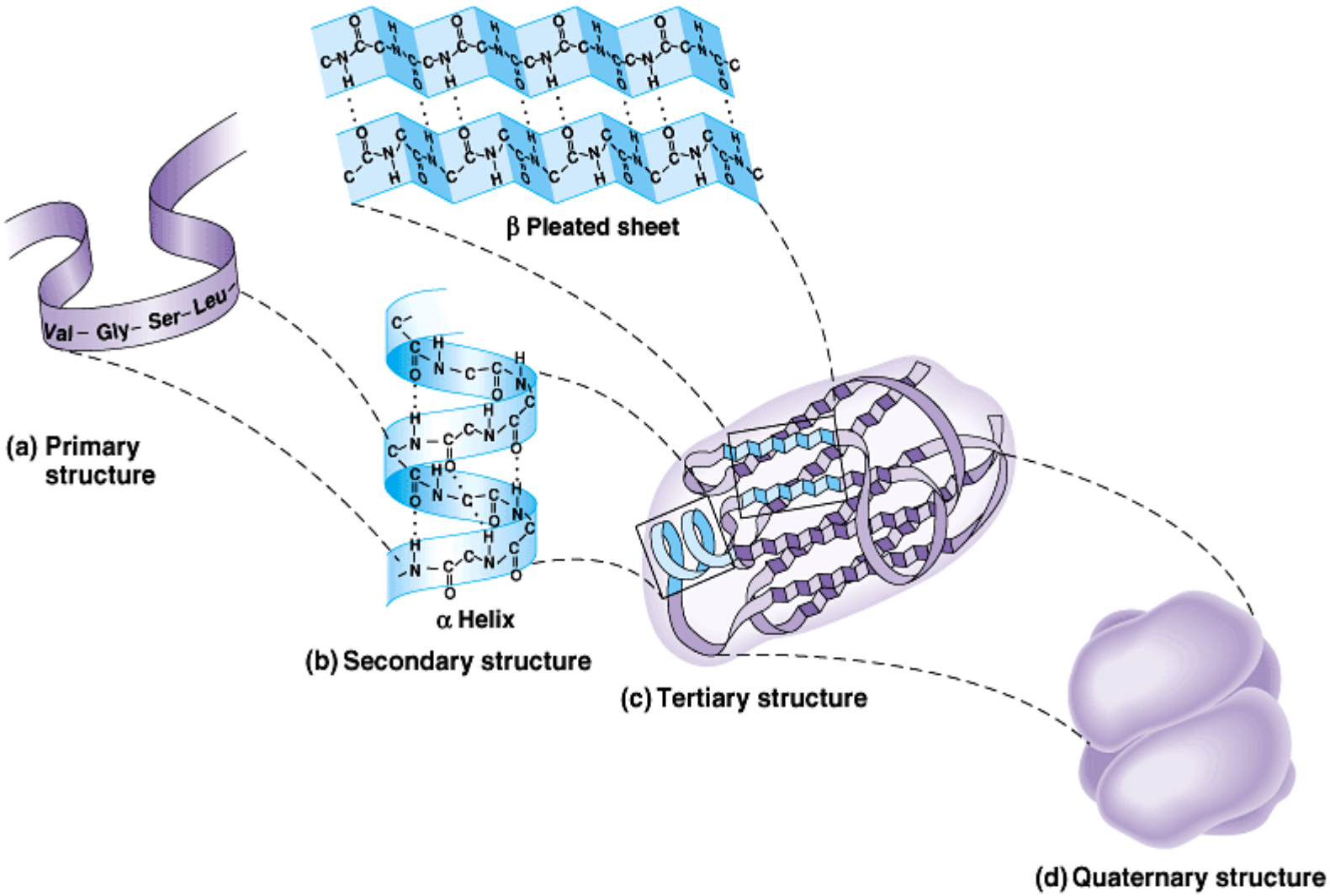
1. Proteins ⟶ Amino acids
2. Carbohydrates ⟶ Sugars
3. Lipids ⟶ Fatty acids and glycerol
4. Nucleic Acids ⟶ Nucleosides

Definition of Enzymes

• Enzymes are mostly proteins that are produced in living organisms and catalyze the chemical reactions of biological systems. For this reason, the enzymes are also termed as **Biocatalysts**.

• Enzymes are proteins that have catalytic functions indispensable to maintenance and activity of life. All chemical reactions occurring in a living organism are dependent on the catalytic actions of enzymes, and this is why enzymes are called **Biotransformation**. At present, there are about 4,000 kinds of enzymes whose actions are well known.

Structure of Proteins



Structure of Enzymes

- Enzymes are proteins which catalyze the biochemical reactions.

- Some enzymes are inactive alone (apoenzymes) and they show catalytic activity in the presence of another chemical compound (cofactors).

- Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; *i.e.*, apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

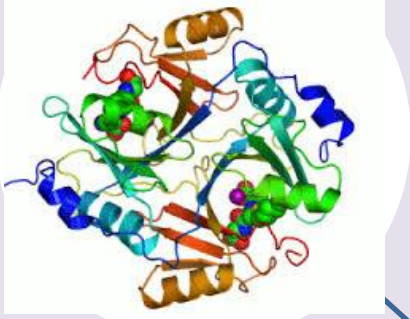
According to Holum, the cofactor may be:

1. **Coenzyme** - a non-protein organic substance which is thermostable and loosely attached to the protein part. Examples: NAD^+ or NADP, many Vitamins, Acetyl CoA.

2. **Prosthetic group** - an organic substance which is thermostable and tightly bound to the protein or apoenzyme portion. Examples: heme (Porphyrin ring) in Haemoglobin.

3. **Metal-ion-activator** - these include K^+, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}. Examples: Metalloenzymes such as carbonic anhydrase active only in the presence of a Zinc ion.

Apoenzyme (Inactive enzyme) + Cofactor (Organic or Inorganic molecule) = Holoenzyme (Active enzyme)



Enzymes

Enzymes

- Active proteins
- Proteins which have active sites

Apoenzymes

- Inactive Proteins
- Active in presence of cofactor.

Holoenzymes

- Active proteins
- Apoenzyme + Cofactors

Cofactors

Organic Compound

Coenzymes

(Vitamin B Complex, NADP, Acetyl CoA

Prosthetic Group

(Heme in Hemoglobin and Biotin)

Inorganic Metal Ions

(Zn ion in carbonic anhydrase and Mg ions in Nitrogenase)

Classification and Nomenclature of Enzymes

- Trivial names of enzymes like Trypsin, Chemotrypsin and Pepsin gives no information about the source, substrate and reaction catalyzed.
- For systematic name: According to the International union of Biochemistry an enzyme name has two parts:

  -First part is the name of the substrates for the enzyme.

  -Second part is the type of reaction catalyzed by the enzyme.This part ends with the suffix "ase".

Example: Lactate dehydrogenase

Classification: Enzymes are classified into six different groups according to the reaction being catalyzed. The nomenclature was determined by the Enzyme Commission in 1961 (with the latest update having occurred in 1992), hence all enzymes are assigned an "EC" number. The classification does not take into account amino acid sequence, protein structure, or chemical mechanism.

EC Number: EC numbers are four digits, for example a.b.c.d, where "a" is the class, "b" is the subclass, "c" is the sub-subclass, and "d" is the sub-sub-subclass. The "b" and "c" digits describe the reaction, while the "d" digit is used to distinguish between different enzymes of the same function based on the actual substrate in the reaction.

Example: for Alcohol: NAD^+ oxidoreductase EC number is 1.1.1.1

Enzymes are divided into six major classes with several sub-classes

•**EC 1. Oxidoreductases:** Enzymes which catalyze oxidation-reduction reactions. *E.g.*, Oxidases, Oxigenases, dehydrogenases and peroxidases.

•**EC 2. Transferases:** Transfer of functional groups (amino, phosphate *etc*.). *E.g.*, methyltransferase, aminotransferase, kinase and phosphorylase.

•**EC 3. Hydrolases:** Hydrolysis of substrate. *E.g.*, Protease, Phosphatase, Phosphodiesterase, Lipase, Maltase and Sucrase.

•**EC 4. Lyases:** Add or remove molecules (ammonia, water, Carbon dioxide) to or from double bond, *i.e.*, Addition-Elimination reaction. *E.g.*, Decarboxylase, aldolase and synthase.

•**EC 5. Isomerases:** Catalyze rearrangement of atoms within a molecule. Isomerization reaction. *E.g.*, Racemase and Mutase.

•**EC 6. Ligases:** Joining of two molecules. Coupling reactions. *E.g.*, Synthetase, carboxylase.

Mechanism of Action

- Enzyme speed up chemical reactions in living organisms by decreasing the energy needed to start the reaction (activation energy).
- Important Terms:

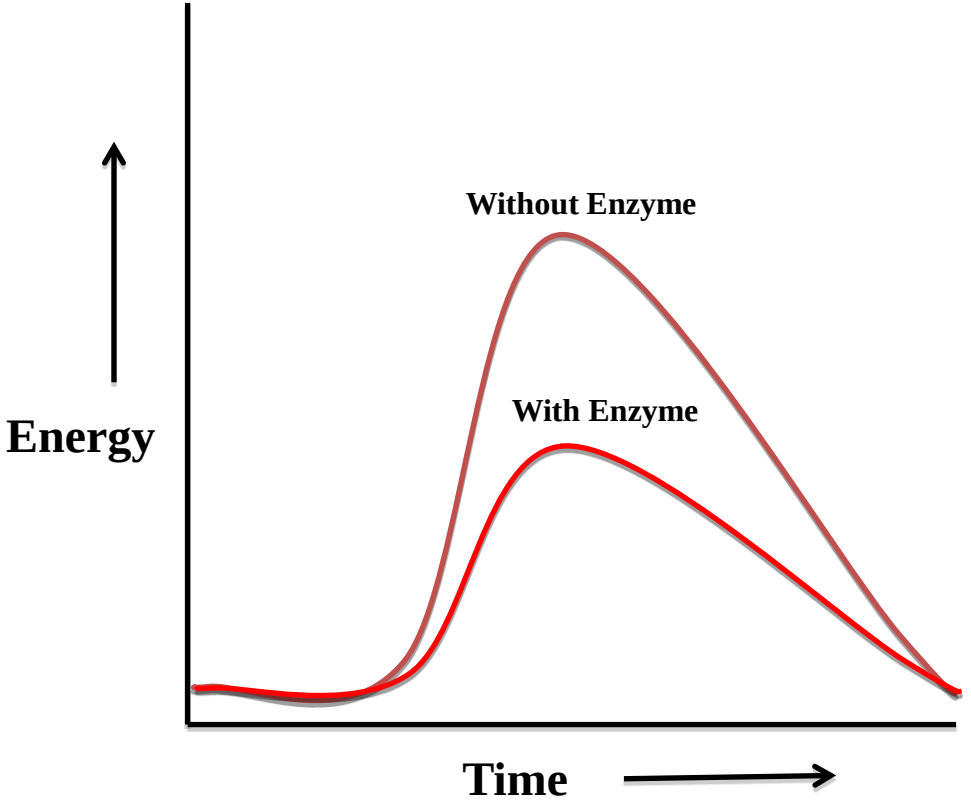
  (i) Substrate: Chemical compound that bind to the active site of an enzyme.

  (ii) Active Site: area on enzyme where substrate binds.

  (iii) Product: what the enzyme produces. Products of enzyme catalyzed reaction.

An enzyme (E) functions by combining with the reactant (termed as *substrate*) to form an activated complex known as *enzyme-substrate* complex. The complex dissociates further to form product (P) and enzyme (E). The enzyme once released from the complex is free to combine with other substrate molecules.

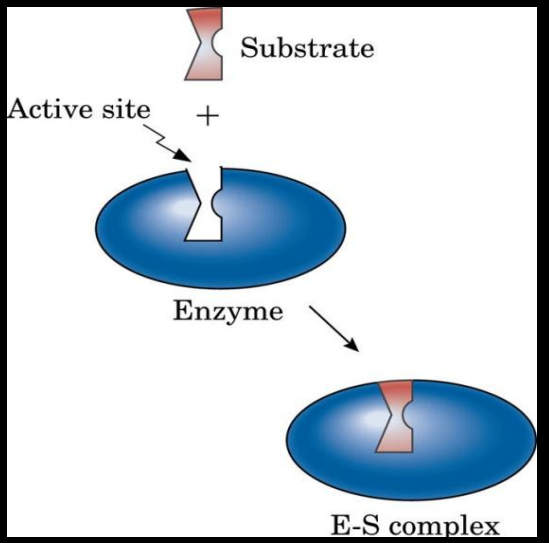
E + S ⟶ E-S ⟶ P + E



Two types of models for the formation of *Enzyme-Substrate* complex

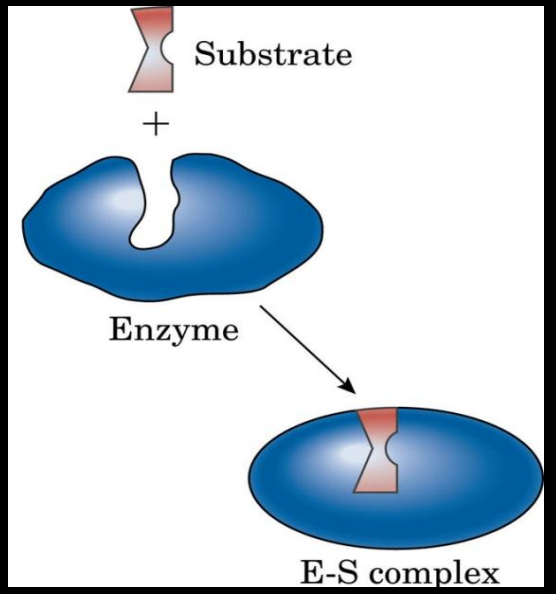
1. Lock and Key Model of enzyme mechanism

   • The enzyme is a rigid 3-D molecule.
   • The enzyme surface contains the active site.
   • Believes that each enzyme is specific to only one molecule (or somewhat identical molecule).

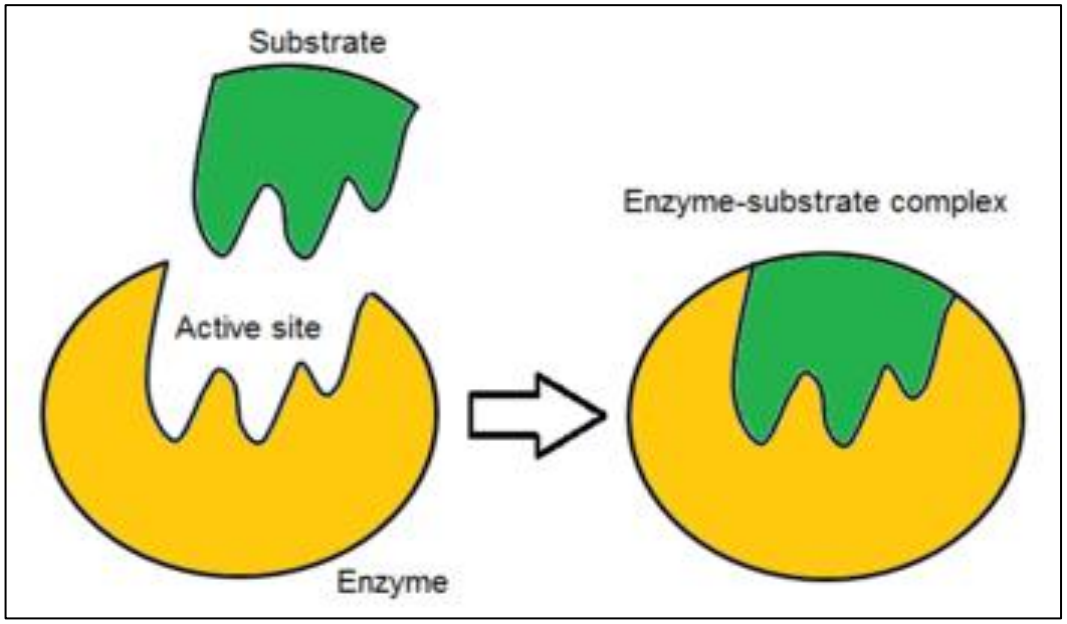


2. Induced-Fit Model of enzyme mechanism

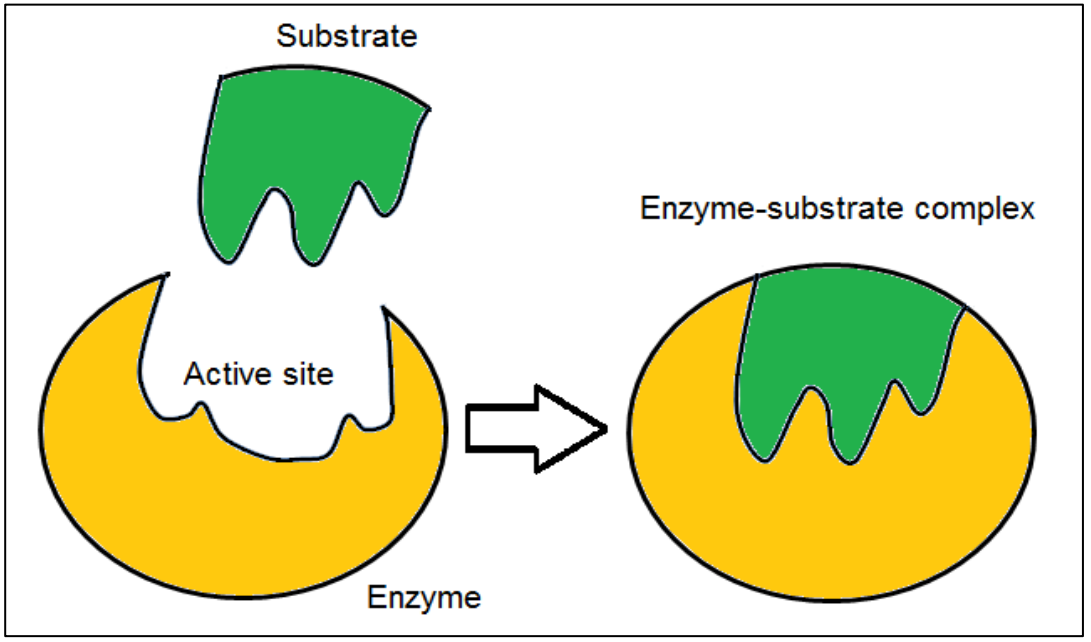
• The enzyme is flexible 3-D molecule.
• The active site becomes modified to accommodate the substrate.
• Enzyme is not rigid and conforms to the substance much like a hand grasping an object



Lock and Key Model

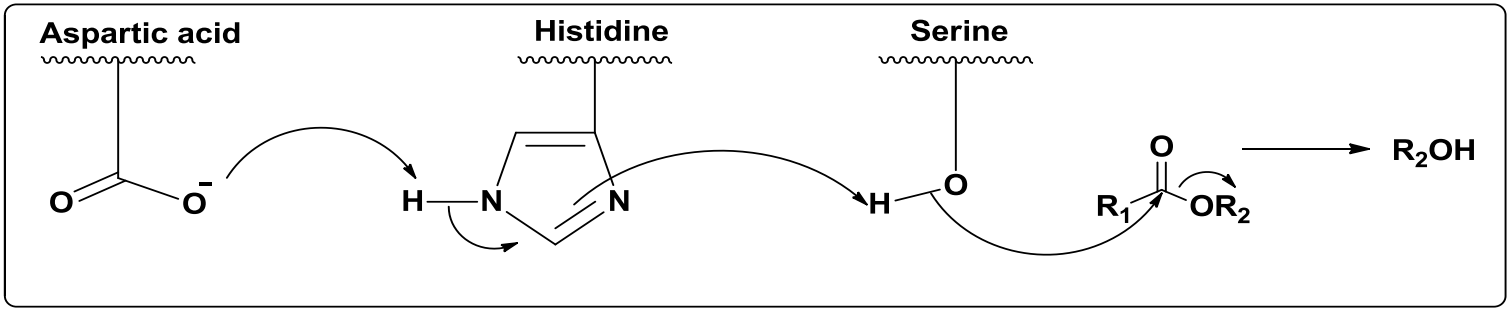


Induced-fit Model

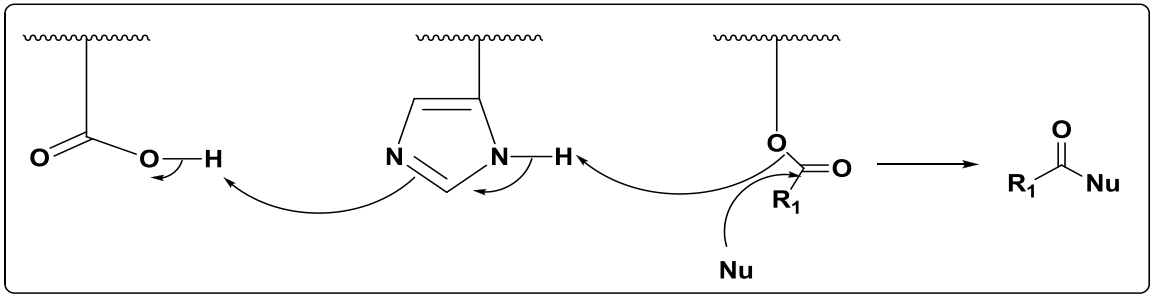


Mechanism of Lipase

Formation of enzyme substrate complex



Attack of nucleophile on activated enzyme-substrate complex



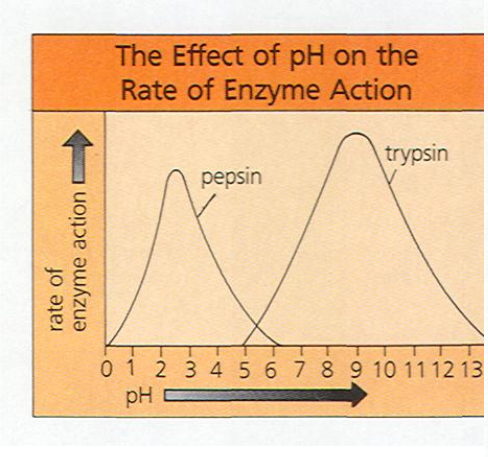
Factors affecting the enzyme activity

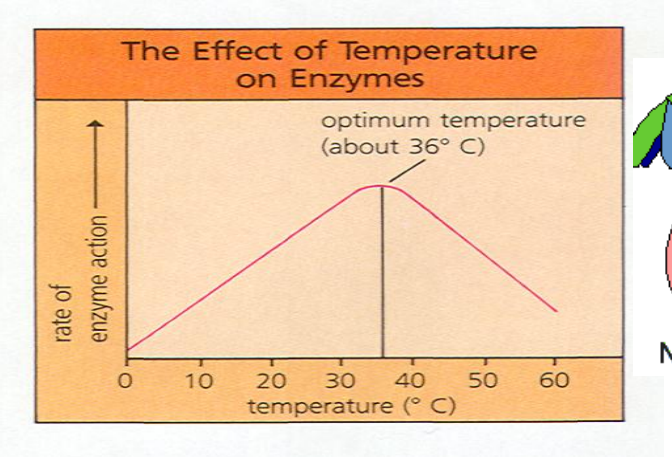
1. **Temperature**- For each enzyme, there is a specific temperature at which the activity of enzyme is a maximum. This is termed as the optimum temperature of enzyme. In general, the enzymes in human system have an optimum temperature in the range 35-40 ^{o}C.

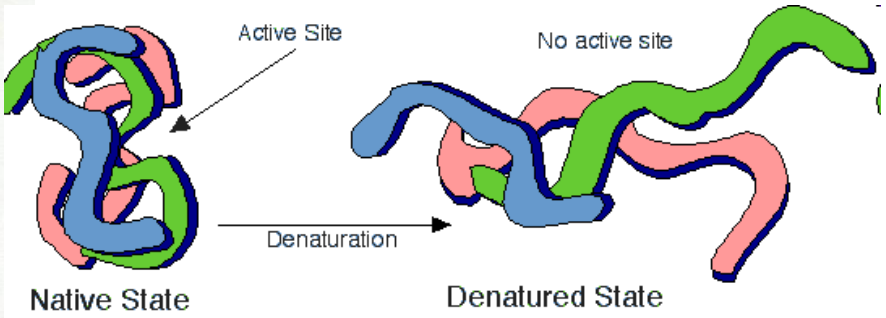
2. **pH**- The pH at which an enzyme has maximum activity is termed as its optimum pH. For most of the enzymes, the optimum pH ranges 4-9.

3. **Enzyme concentration**- The rate of enzymatic reactions increases with an increase in the concentration of enzyme.

4. **Concentration of product**- An increased product concentration lowers down the enzymatic reaction. The products formed may inhibit the active site of enzyme or can make the reaction proceed in backward direction.







Specificity of Enzyme Action

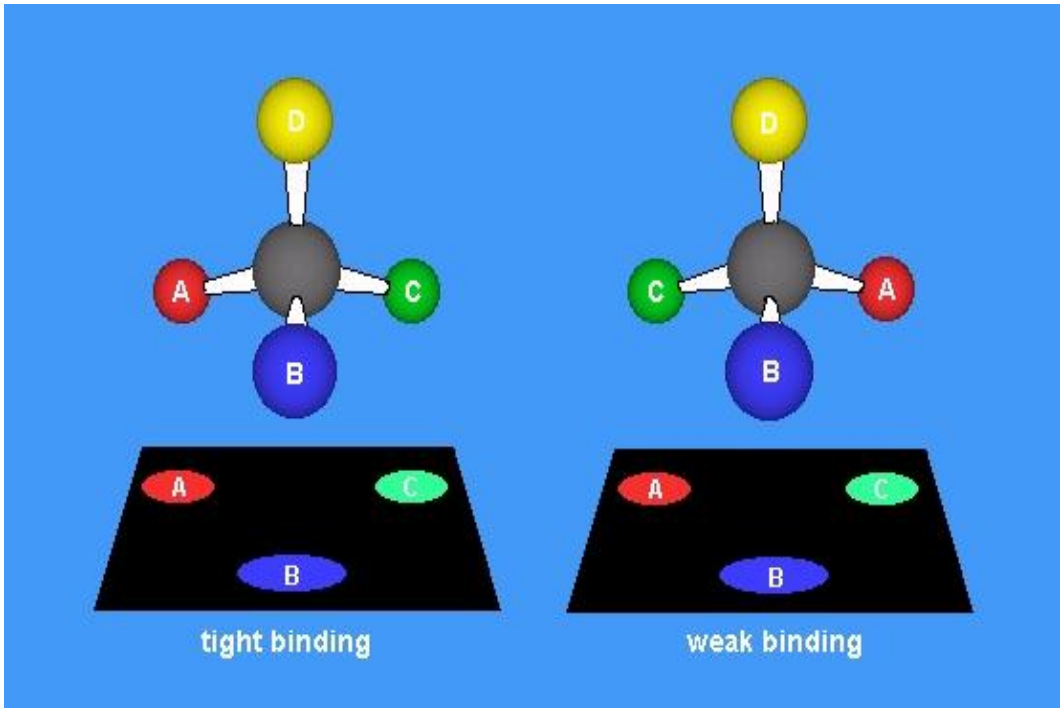
Biocatalysts are different from inorganic catalysts in that these are extremely specific in their catalytic actions. Mostly, each enzyme catalyzes a single reaction, for example, urease attack urea only and carboxypeptidase attack C-terminal peptide bond. Most of the enzymes can catalyze the same reaction with structurally related substrates.

Stereospecificity

Proteins, hormones and receptors are chiral

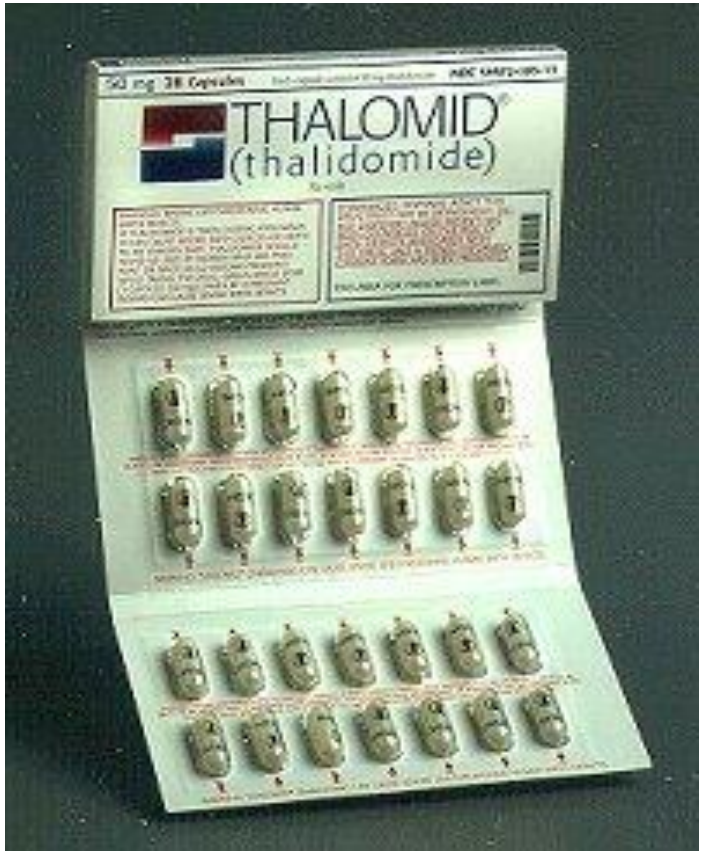
MOLECULES INTERACT WITH BIOMOLECULES STEREO SPECIFICALLY

"Nature has a way of knowing how to make things work. Reactions often run in a catalytic mode, and material use, energy, and waste are minimized. Many molecules are chiral, and their unique handedness has both intricate and dramatic influences on how they interact with biological systems."

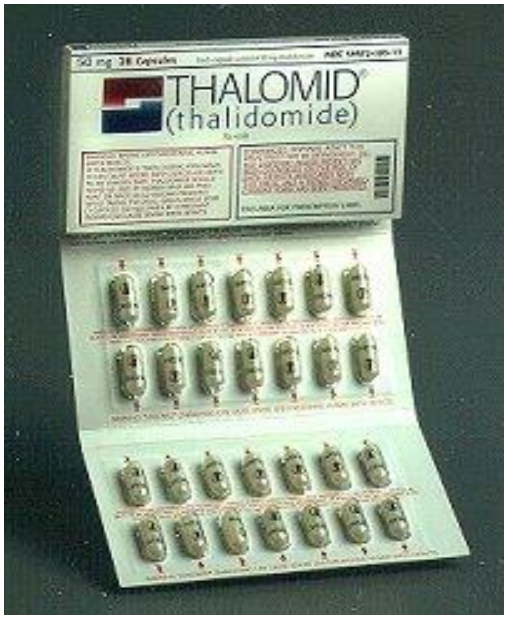


Thalidomide

Thalidomide – the sleeping pill



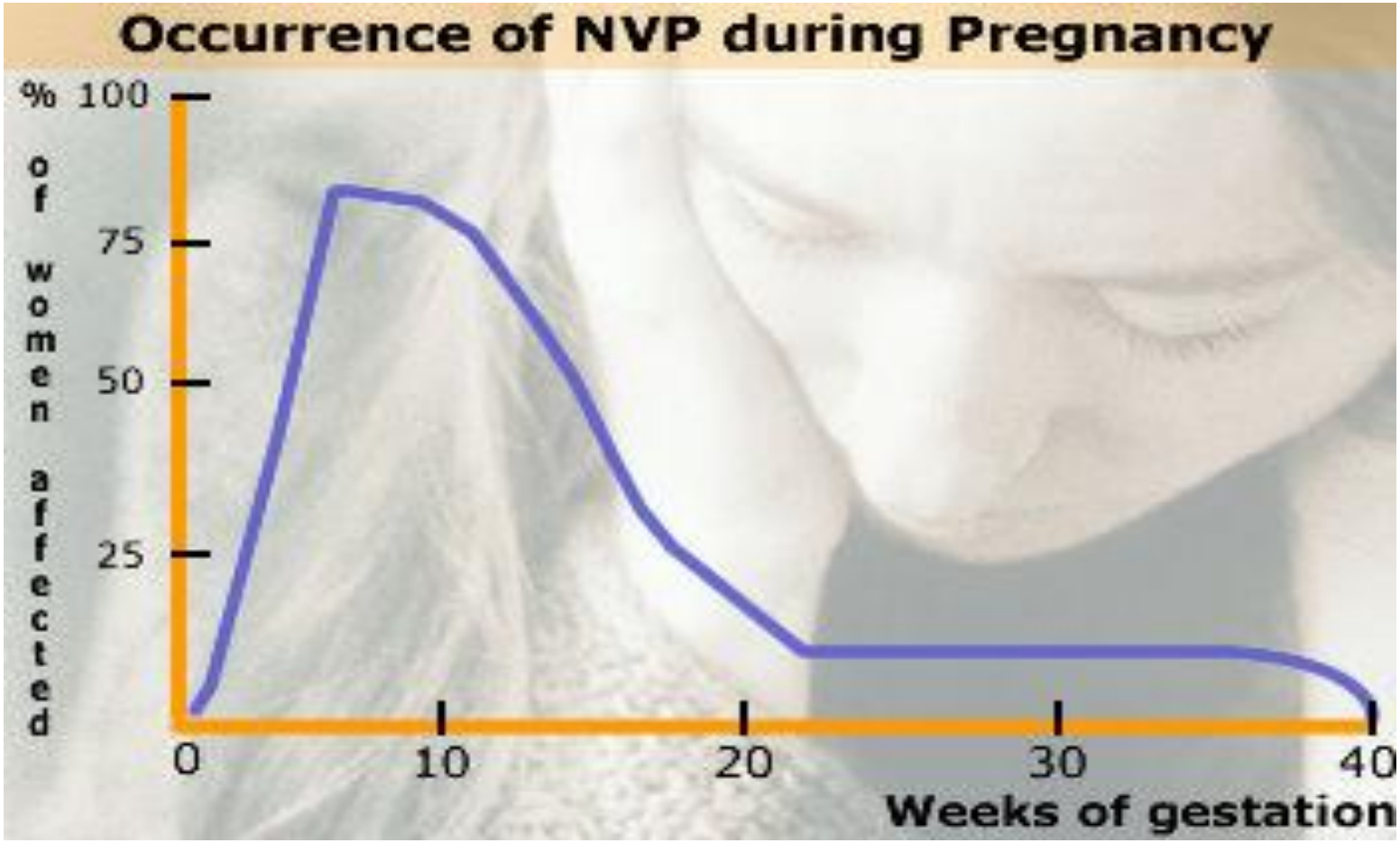
- Thalidomide was first synthesized in West Germany in 1953 by Chemie Grünenthal.



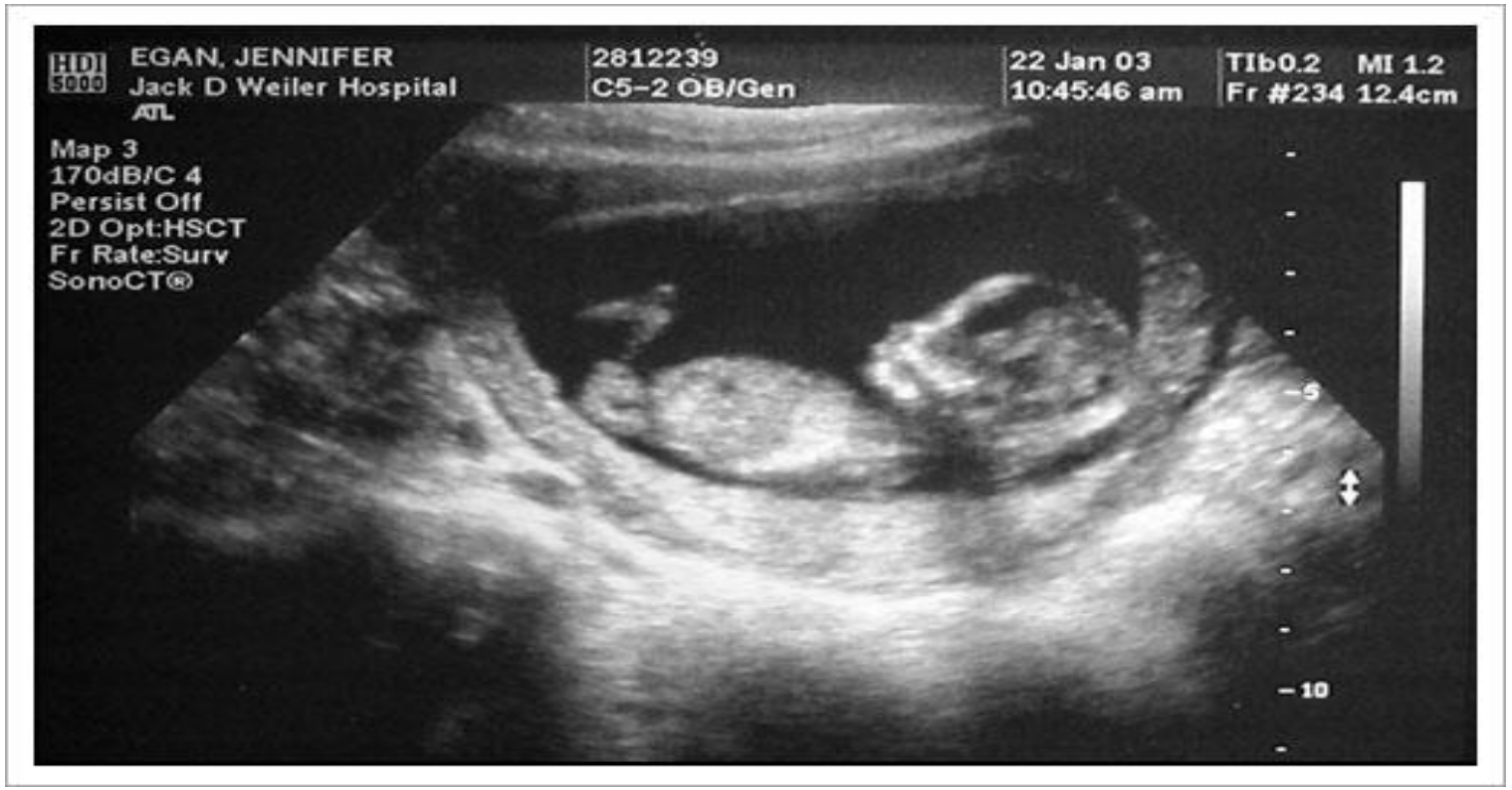
- It was hailed as a ***"wonder drug"*** that provided a ***"safe, sound sleep".***

- However the drug was also found to cure morning sickness in pregnant women

Nausea vomiting in pregnancy NVP – 1st trimester …(1/3)

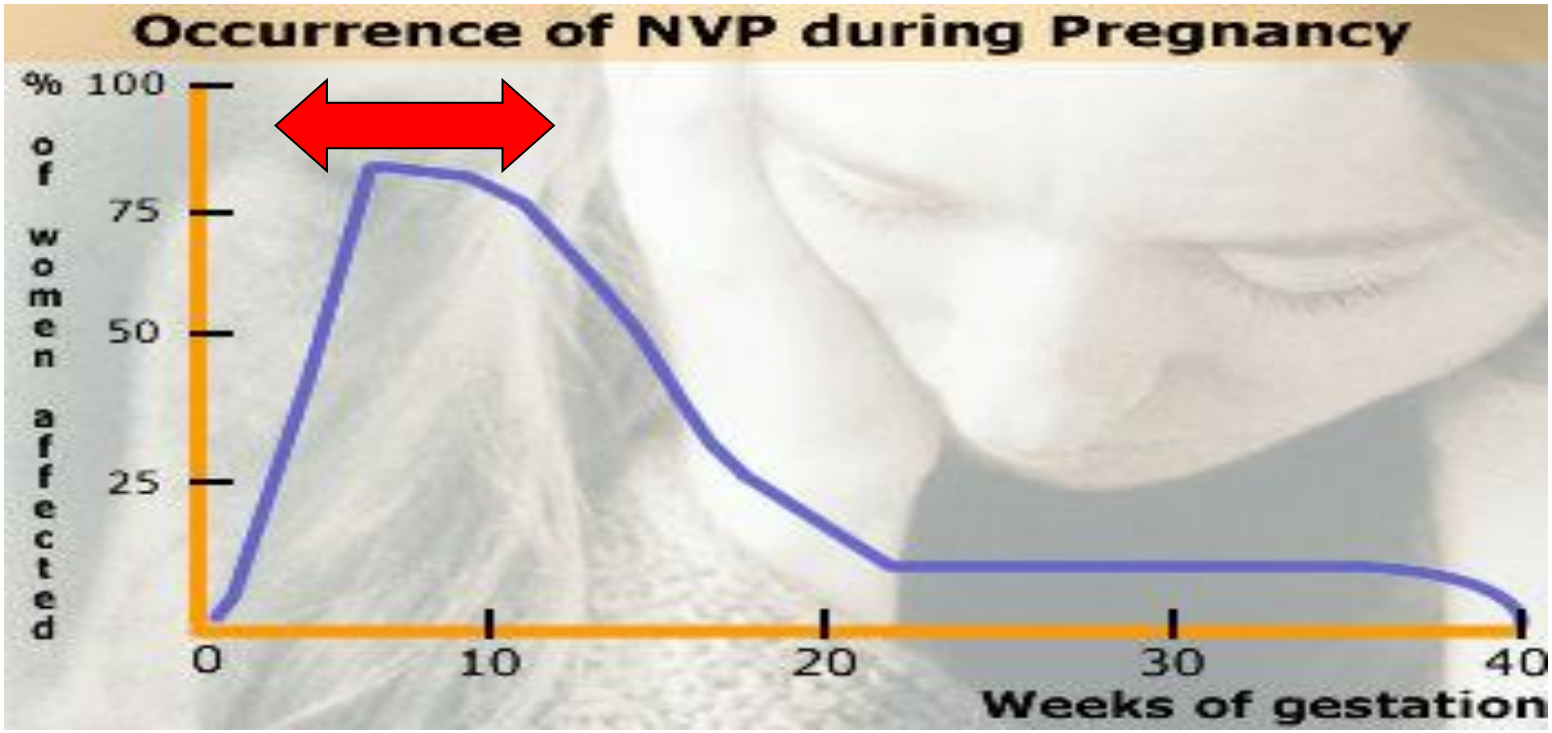


Foetal development -



- The first few weeks are a key period – any problems now will accumulate in the future

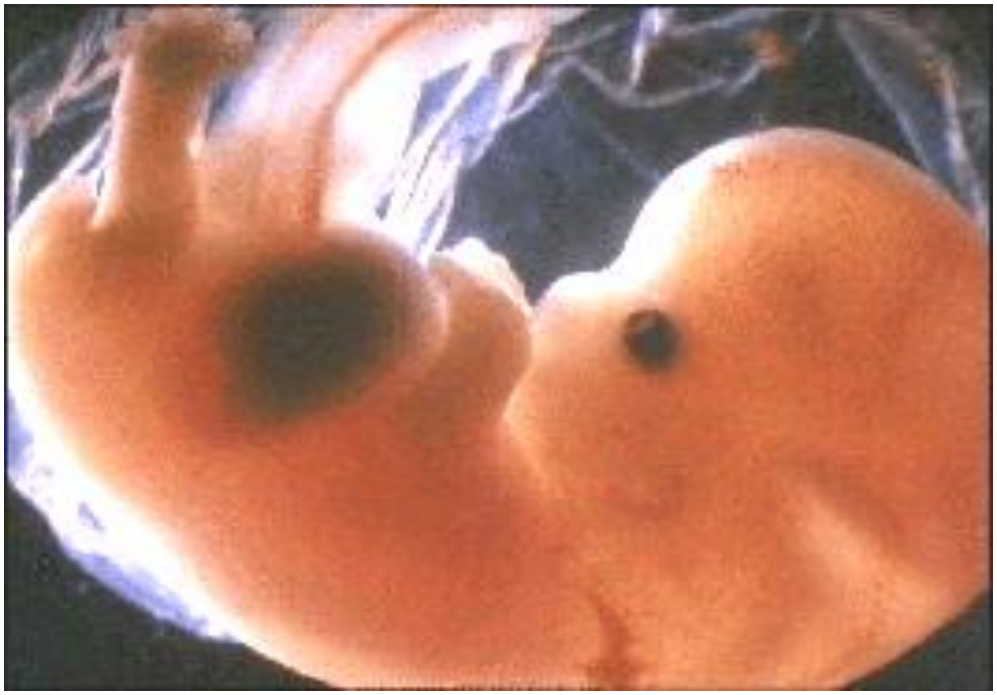
Nausea vomiting in pregnancy NVP – 1st trimester (1/3)



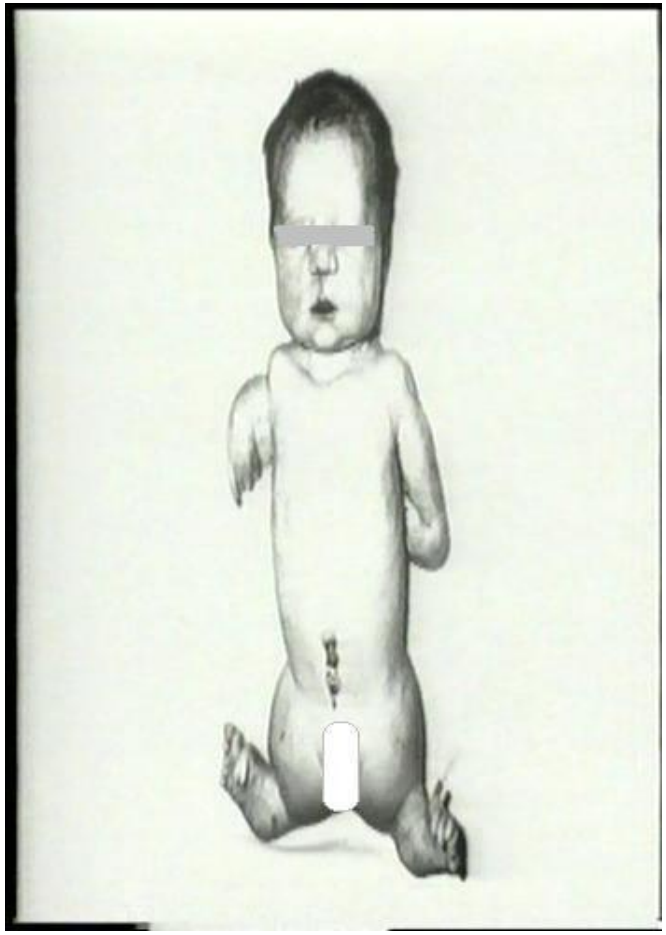
- When do you think most women would be inclined to take this drug?

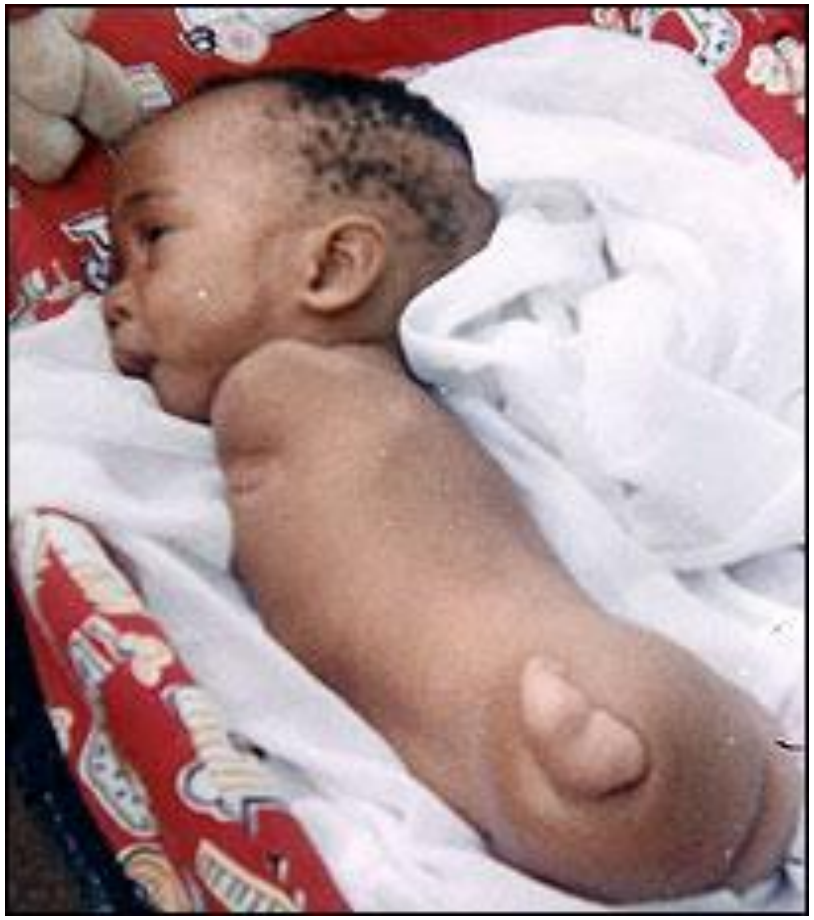
Side effects

- unfortunately molecules of the thalidomide chemical crossed the placenta and disrupt the growth patterns of the growing foetus

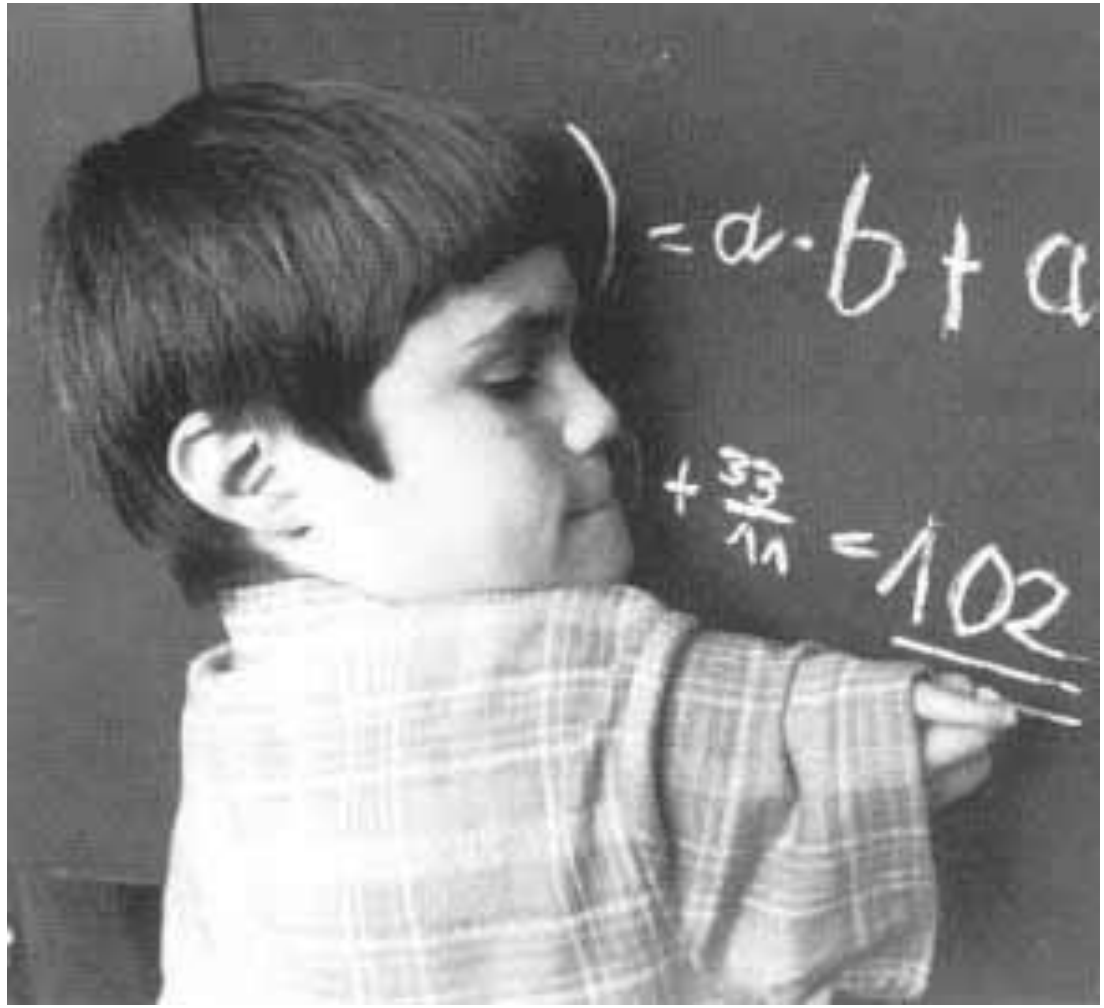


Effect = catastrophic deformations of the baby– best seen as limb abnormalities.





...if this was you – how would your life be different?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Why did this happen?

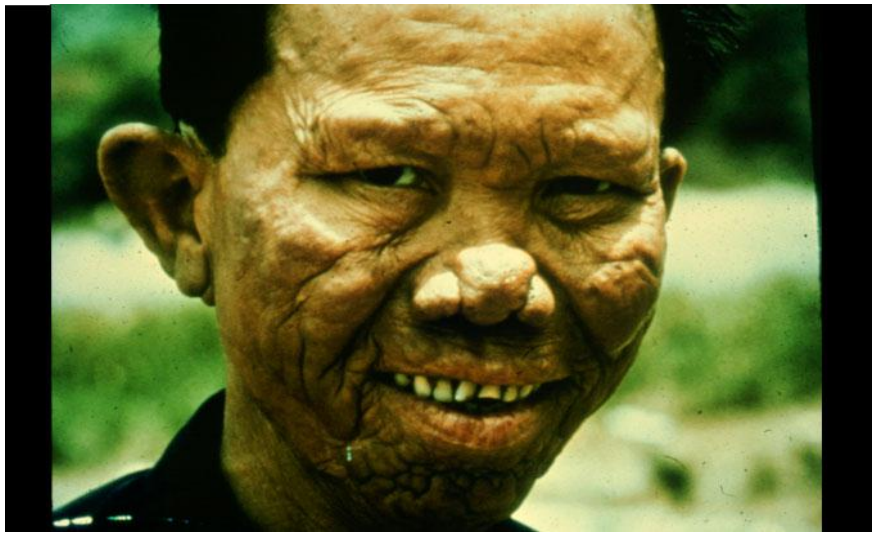
- Simple - the drug was not trialled or tested for this use on pregnant women.
- The S-enantiomer of thalidomide, caused the teratogenic effects (body mutations etc).

Thalidomide today

- Initially the drug was banned internationally for the treatment of morning sickness

- But it has now been reintroduced as a treatment for leprosy

Leprosy – bacterial infection of the skin and nerves endings

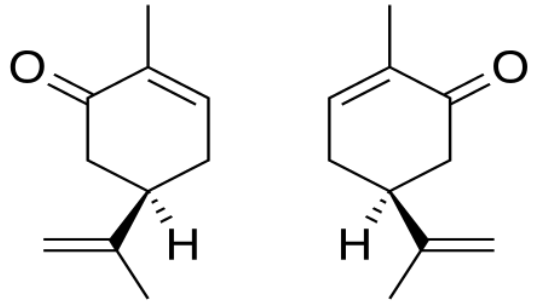


- Affects the hands, feet and features of the face.

Conclusion about the stereospecificity of enzymes

1. Active site of an enzyme is chiral, so enzyme can react with two enantiomers of a compound in different way which results into two independent physiological/biological effects.

2. Carvone forms two mirror image forms or enantiomers: *R*-(–)-carvone smells like spearmint. Its mirror image, *S*-(+)-carvone, smells like caraway. The fact that the two enantiomers are perceived as smelling differently is proof that olfactory receptors must contain chiral groups, allowing them to respond more strongly to one enantiomer than to the other.

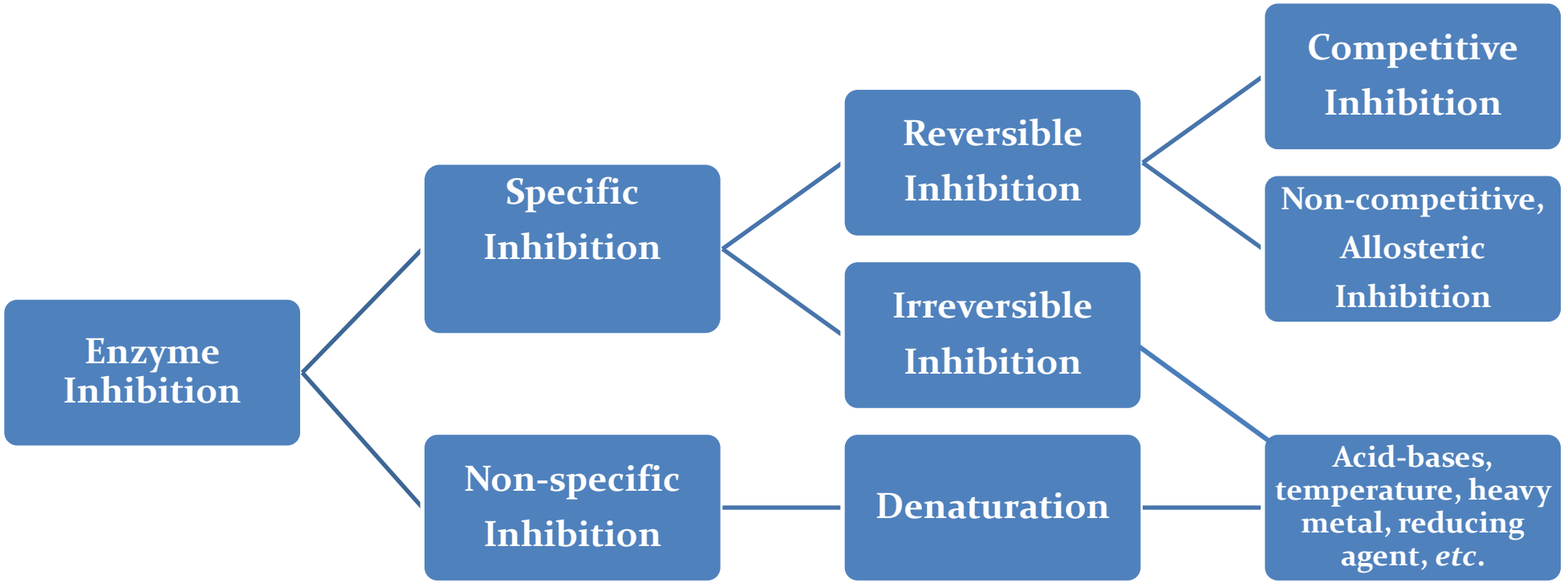


(R) (S)

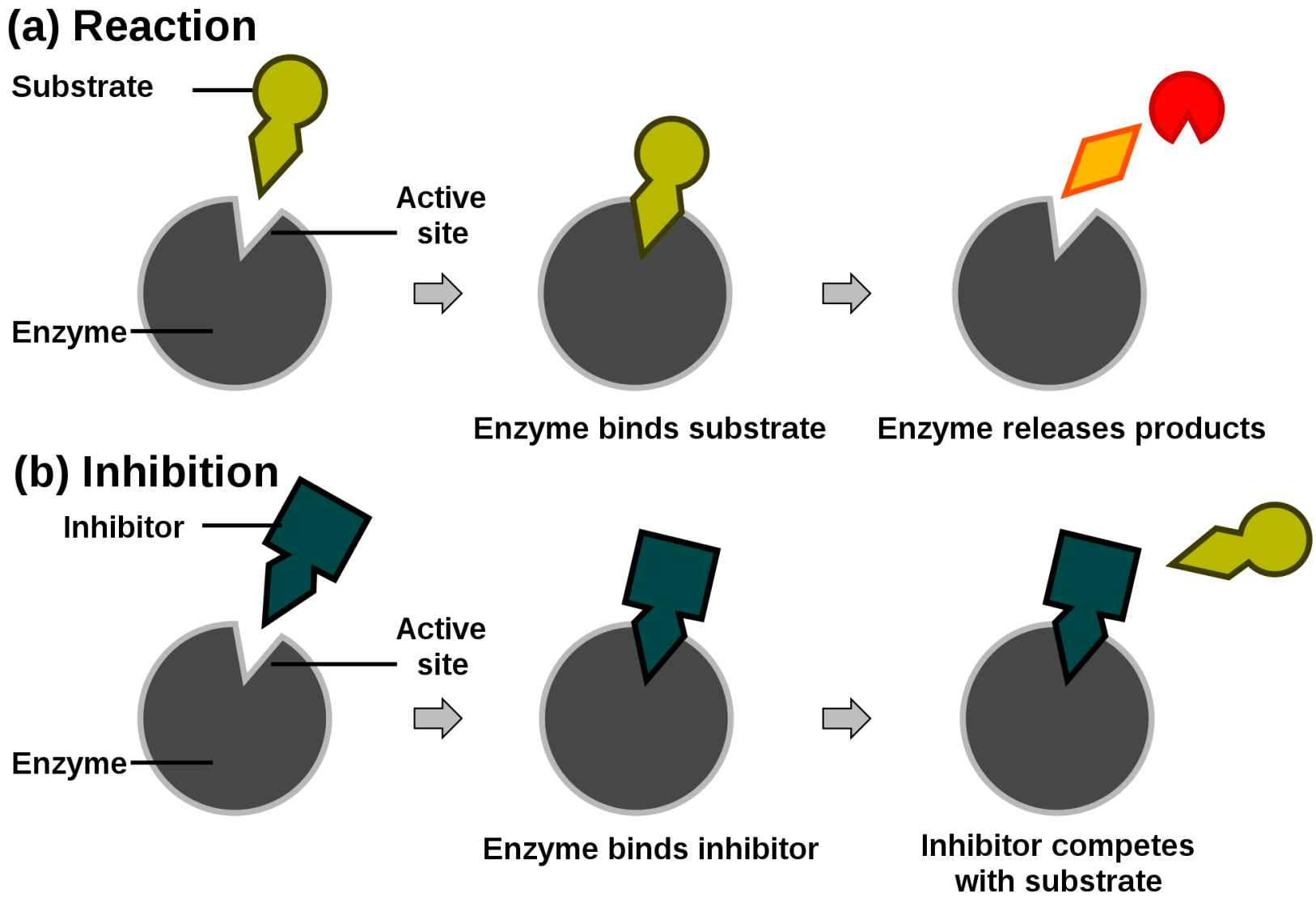
3. Many cases are known so far in which-
   -One enantiomer is active other is inactive
   -One enantiomer is active and other is more potent.
   -One enantiomer shows desirable activity and other one shows antagonistic activity.
   -Two enantiomers show entirely different beneficial activities.

Enzyme Inhibition

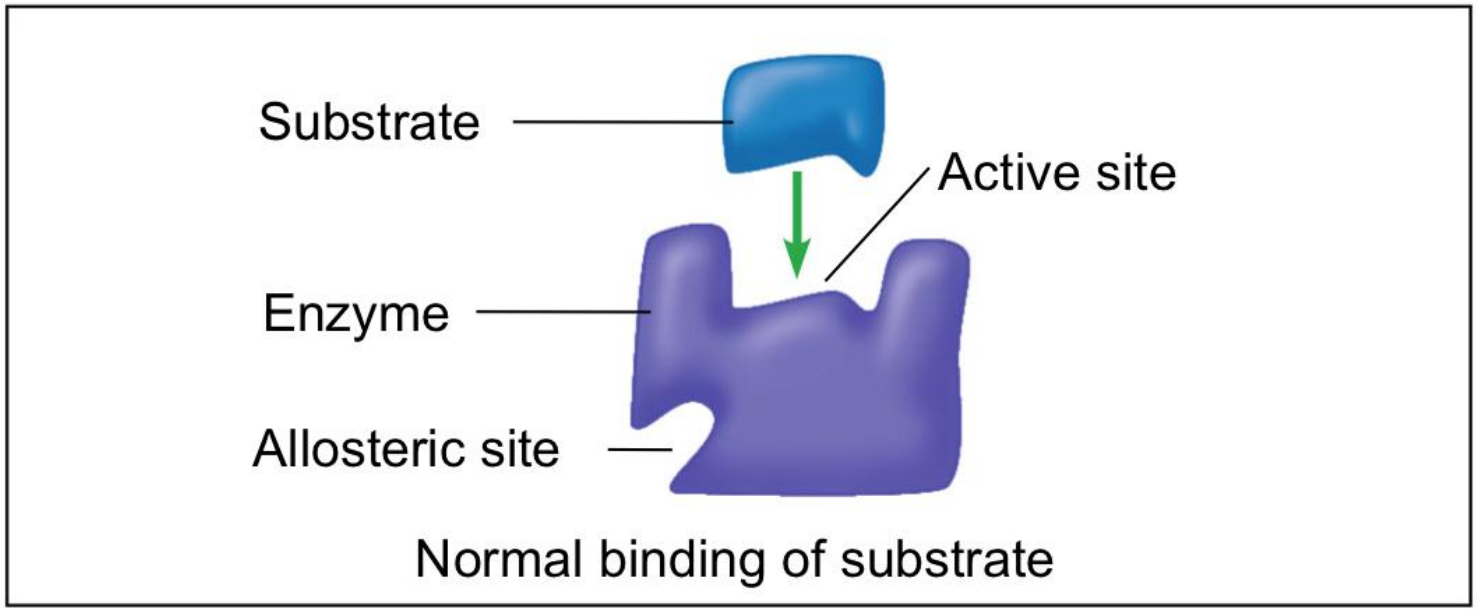
Enzyme Inhibition refers to the halting or reduction of enzyme activity. This is the opposite of enzyme induction, which triggers or increases production. Molecules or factors which reduce the enzyme activity are called **enzyme inhibitors**.

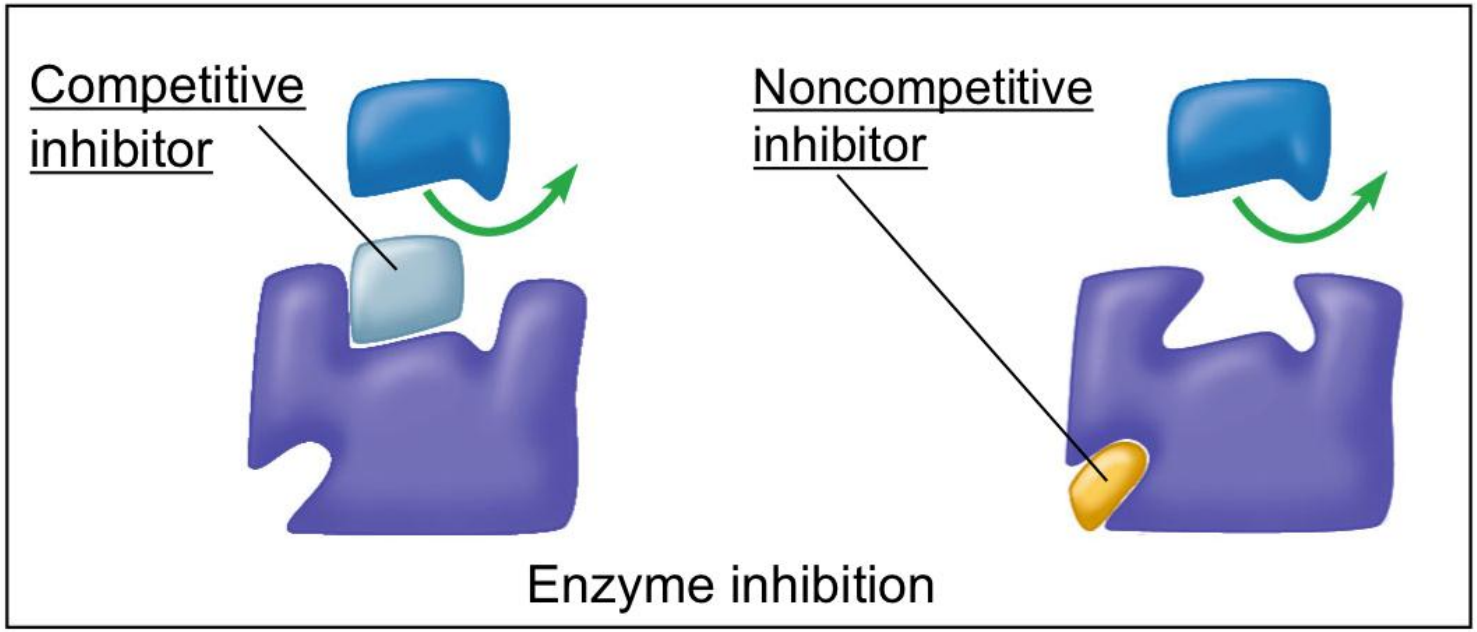


Enzyme Inhibition

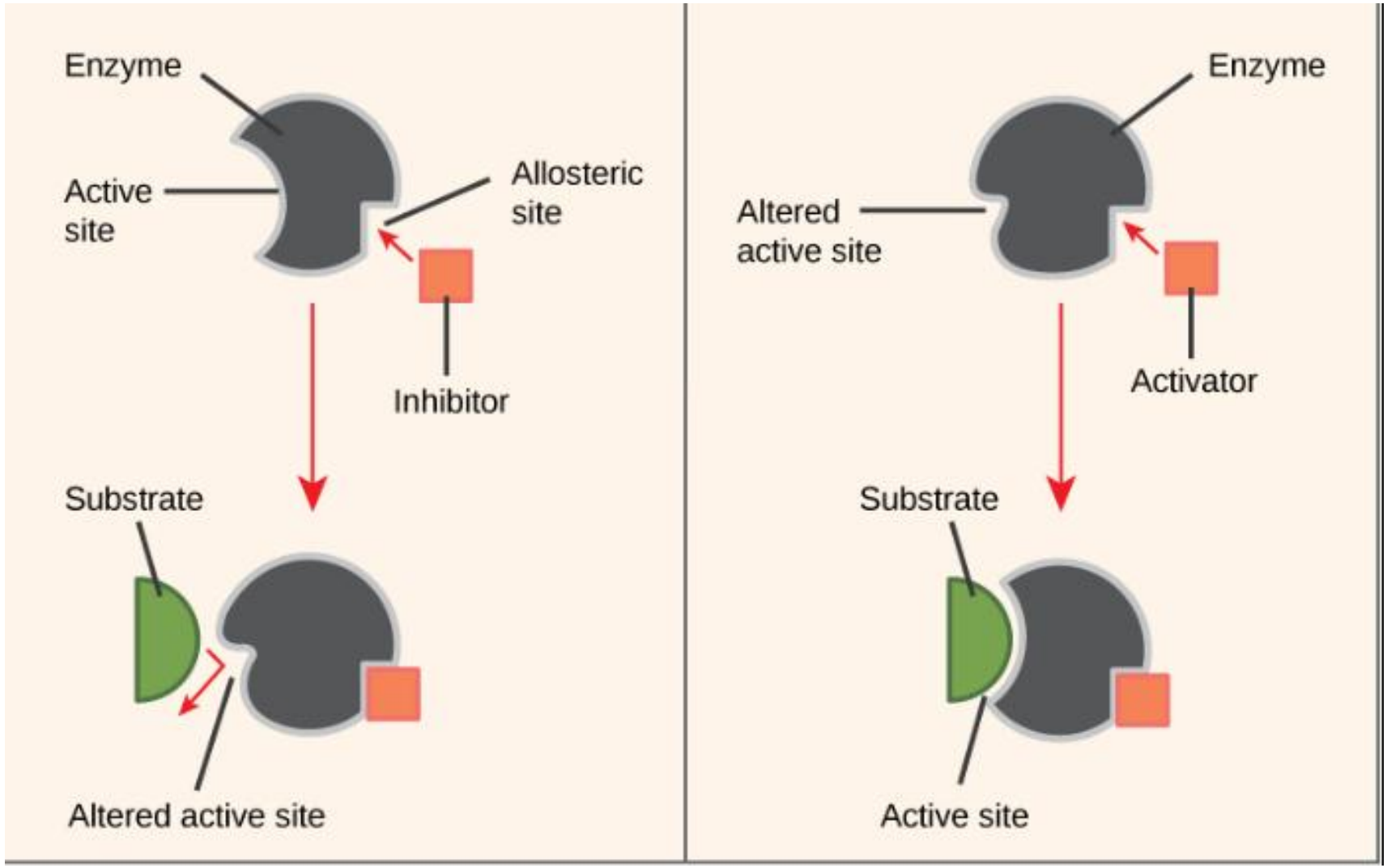


Competitive and Non-competitive Enzyme Inhibition





Allosteric Regulation



Difference Between Non-competitive inhibition and Allosteric Inhibition

•**Allosteric Site:** Any site other than the active site of an enzyme where an inhibitor or modulator can bind to the enzyme is called the allosteric site of enzyme.

•If any molecule bind to the allosteric site and inhibit the enzyme activity is called **Allosteric Inhibitor**. And if molecule bind to the allosteric site and increase (or revive) the enzyme activity is called **Allosteric Activator**.

•A **non-competitive Inhibitor** may bind to a non-substrate site on a protein and distort it to the point of non-functionality, and adding more substrate will not alleviate this inhibition. It may simple block a catalytic site. A substance that inhibits the action of an enzyme by binding to the enzyme at a location other than the active site.

•An **Allosteric Inhibitor** can be competitive or non-competitive. In allosteric inhibitor there may be competition between substrate and inhibitor (competitive allosteric inhibition). In that case concentration of substrate can affect the activity of enzyme.

•**All Non-Competitive Inhibition is Allosteric Inhibition, but not all allosteric inhibition is non-competitive.**

Drug Action- Receptor Theory

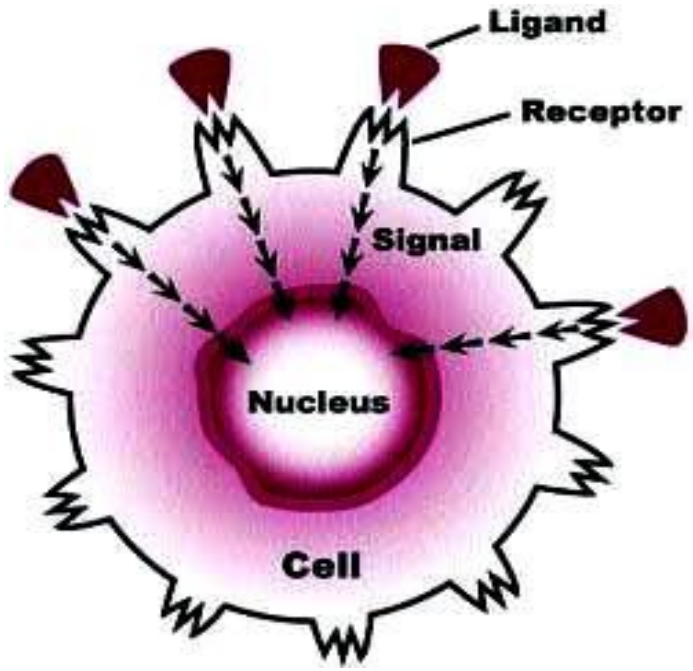
➢ A receptor is a molecule most often found on the surface of a cell, which receives chemical signals originating externally from the cell. Through binding to a receptor, these signals direct a cell to do something—for example to divide or die, or to allow certain molecules to enter or exit.

➢ Receptors are protein molecules, embedded in either the plasma membrane (cell surface receptors) or the cytoplasm or nucleus (nuclear receptors) of a cell, to which one or more specific kinds of signalling molecules may attach.

➢ A molecule which binds to a receptor is called a ligand.

Four Primary Receptor Families

1. Cell-membrane embedded proteins
2. Ligand-gated Ion Channel
3. G –protein coupled Receptor Systems
4. Transcription Factors



Drug-Receptor Theories

- **<u>Hypothesis of Clark</u>**

  " The Pharmacologic effect of the drug depends on the percentage of the receptors occupied"

  If receptors are occupied, maximum effect is obtained.

  Chemical binding follow the Law of Mass Action.

Drug-Receptor Theories

- **Hypothesis of Ariens and Stephenson**

" Effectiveness of a drug lasts as long as the receptor is occupied. Many substance possess different effect , some have high affinity for the receptor, some have low affinity and some are not effective, and those ineffective substances block or inhibit the receptor."

It is also called Occupation Theory.

Drug-Receptor Theories

- **<u>Hypothesis of Paton</u>**

  " Effectiveness of a drug does not depend on the actual occupation of the receptor but by obtaining proper stimulus"

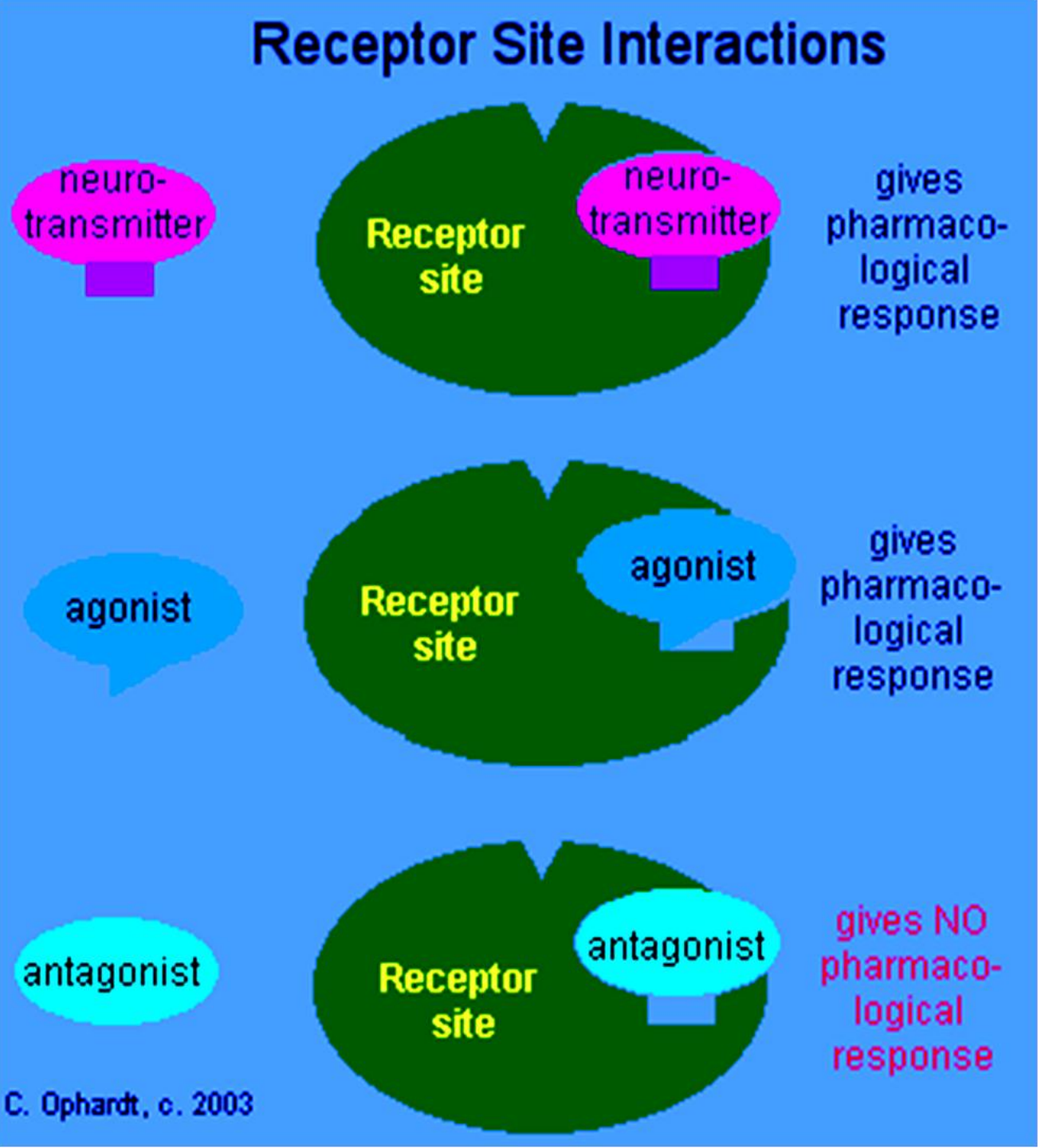
This is also known as the Rate Theory.

Drug-Receptor Theories

- **<u>Lock and Key Hypothesis</u>**

  " The drug molecule must fit into the receptor like a key fits into the lock"

Known as the Intrinsic Activity.



- If a drug causes the protein receptor to respond in the same way as the naturally occurring substance, then the drug is referred to as an agonist. Eg. morphine, nicotine.
- Antagonists are drugs that interact selectively with receptors but do not lead to an observed effect. Instead they reduce the action of an agonist at the receptor site involved.
- Receptor antagonists can be classified as reversible or irreversible. Reversible antagonists readily dissociate from their receptor. Irreversible antagonists form a stable chemical bond with their receptor (eg. in alkylation). Examples of antagonist drugs are: beta-blockers, such as propranolol.

Structure Activity Relationships (SAR) of Drug Molecules

- **Structure-activity relationship (SAR) is the relationship between the chemical or three-dimensional structure of a molecule and its biological activity.**

- **The analysis of SAR enables the determination of the chemical groups responsible for evoking a target biological effect in the organism.**

- **This allows modification of the effect or the potency of a bioactive compound (typically a drug) by changing its chemical structure.**

- **Medicinal chemists use the techniques of chemical synthesis & computational drug design to insert new chemical groups into the biomedical compound and test the modifications for their biological effects.**

- **This method was refined to build mathematical relationships between the chemical structure and the biological activity, known as quantitative structure-activity relationships (QSAR).**

Why SAR Studies are done??

It has advantages like:

1. Increase potency.
2. Greater selectivity
3. Increased or decreased the duration of action.
4. Low toxicity
5. Increased stability (bioavailability).

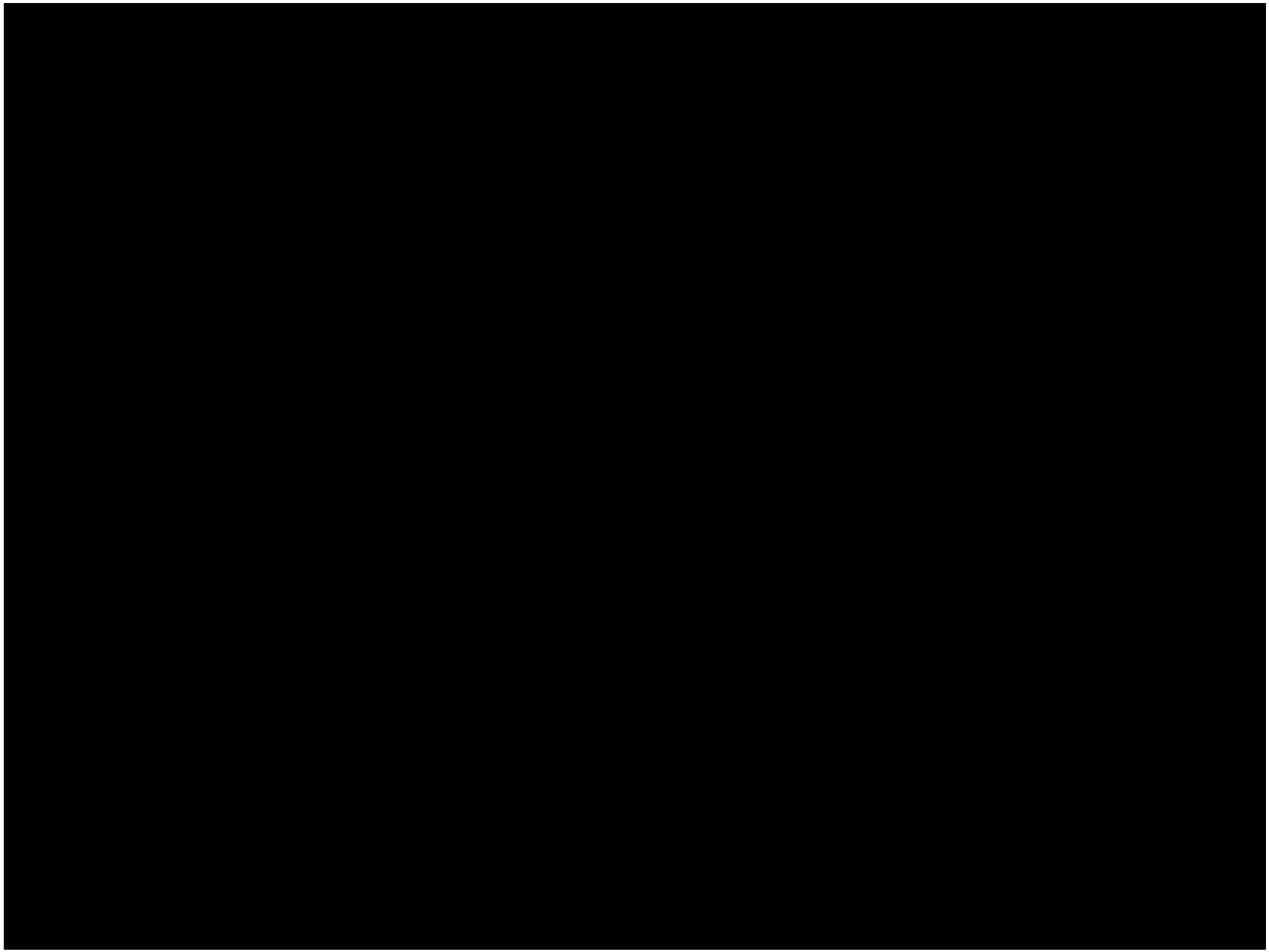
Binding Role of –OH, -NH2, Double bond and aromatic ring.

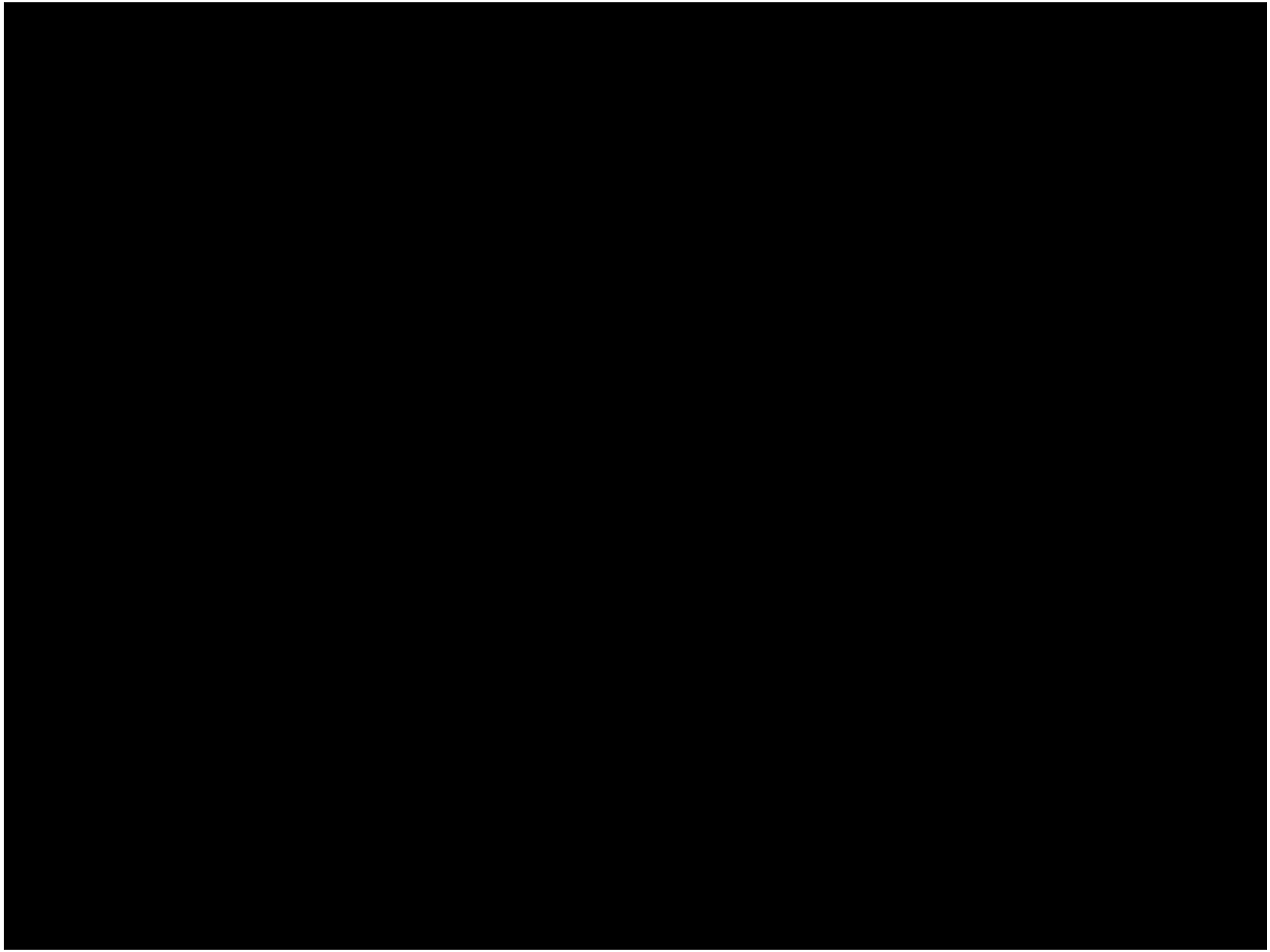
•Presence of –OH group in drug, protein's active site or at receptor site helps the substrate to bind through **Hydrogen Bonding**.

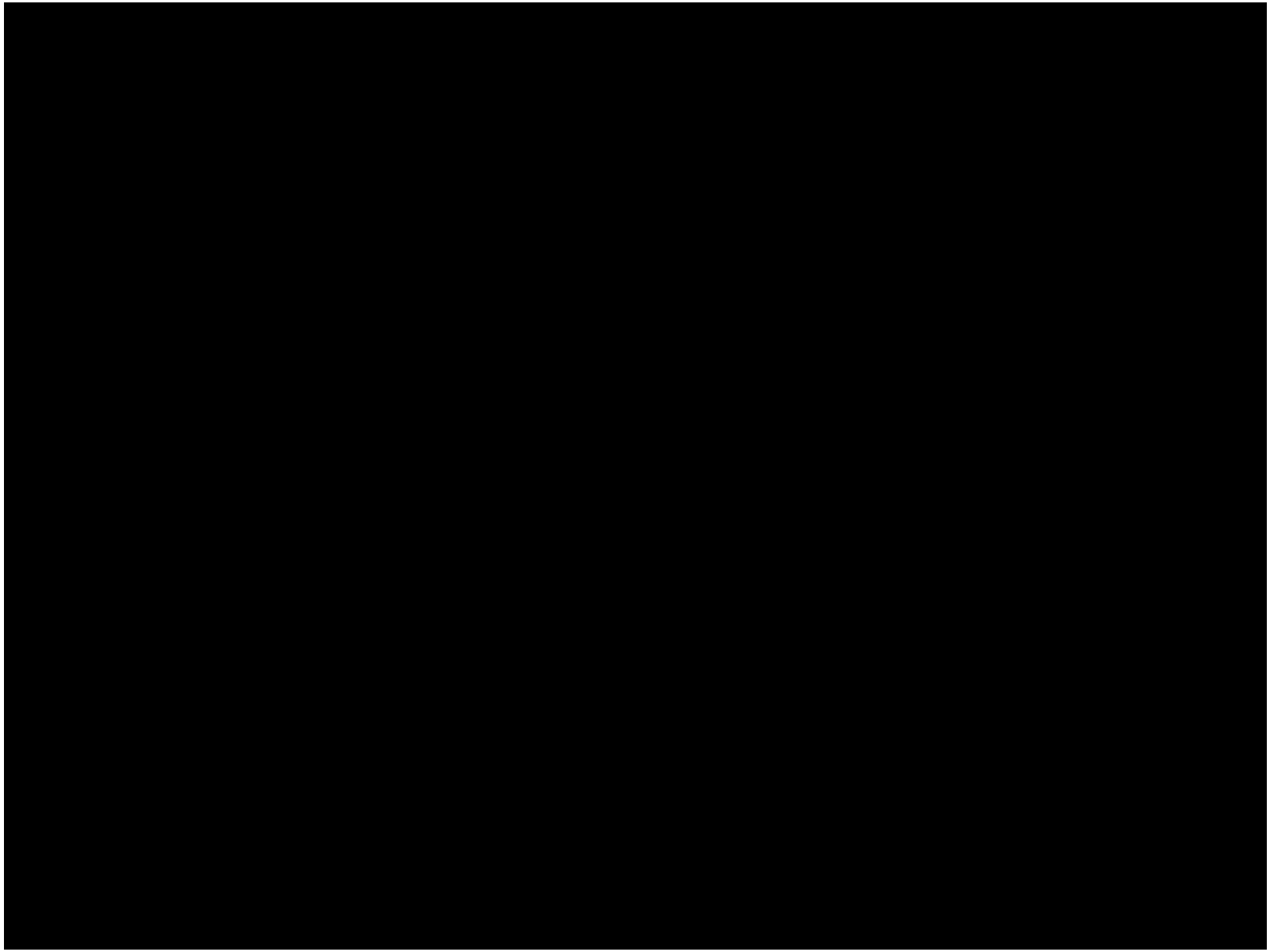
•Presence of –NH2 group in drug, protein's active site or at receptor helps the substrate to bind through **Hydrogen Bonding** and **Ionic Interactions**.

•Presence of C-C double bond in drug, protein's active site or at receptor helps the substrate to bind through **Hydrophobic Interactions**.

•Presence of aromatic ring in drug, protein's active site or at receptor helps the substrate to bind through **stacking interactions** and **hydrophobic interactions**.







Thank You