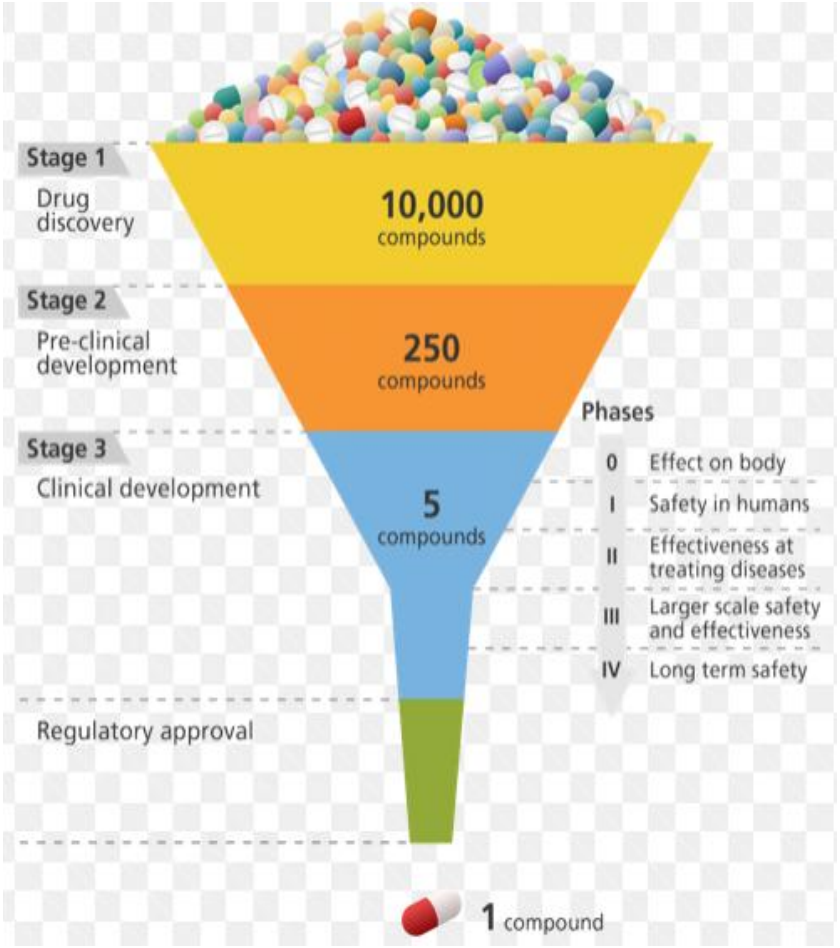
Introduction to Drug Design and Discovery



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Learning Outcomes

At the end of this lesson students will be able to

- Outline the entire process involved in the drug discovery and drug design.
- Describe molecular modelling techniques in drug design.
- Describe the ligand-based drug design and the structure-based drug design in new drug discovery.
- Explain the lead compound, its modification and combinatorial chemistry principles.
- Explain the basic concepts in receptors, drug-receptor interactions, enzymes and enzyme inhibitors.
- Explain the pre-clinical and clinical trials in drug discovery process.

Major steps involved in any drug discovery process

Target identification (to identify the potential biological target of the disease)

↓

Target validation (to make sure that the selected biological target is suitable and valid)

↓

Lead Identification (to identify suitable molecules bind to the validated target)

↓

Lead Modificiation (based on the properties, the identified lead can be modified to reduce toxicity, side effects, etc)

↓

Synthesis of compounds

↓

In-vitro evaluation by different biological assays (lab testing)

↓

In-vivo evaluation (testing new compounds using animal models for activity and toxicity)

↓

Clinical Trials (Phase-I: Drug evaluation involving human healthy volunteers
Phase-II: Drug evaluation involving small number of patients for testing activity, dosing and side effects)
Phase-III: Drug evaluation involving large number of on patients to test activity and toxicity at the long term side effects)
Phase-IV: Post marketing survey: Drug follow up after market release to report any new side effects and complications)

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Regulatory Approval

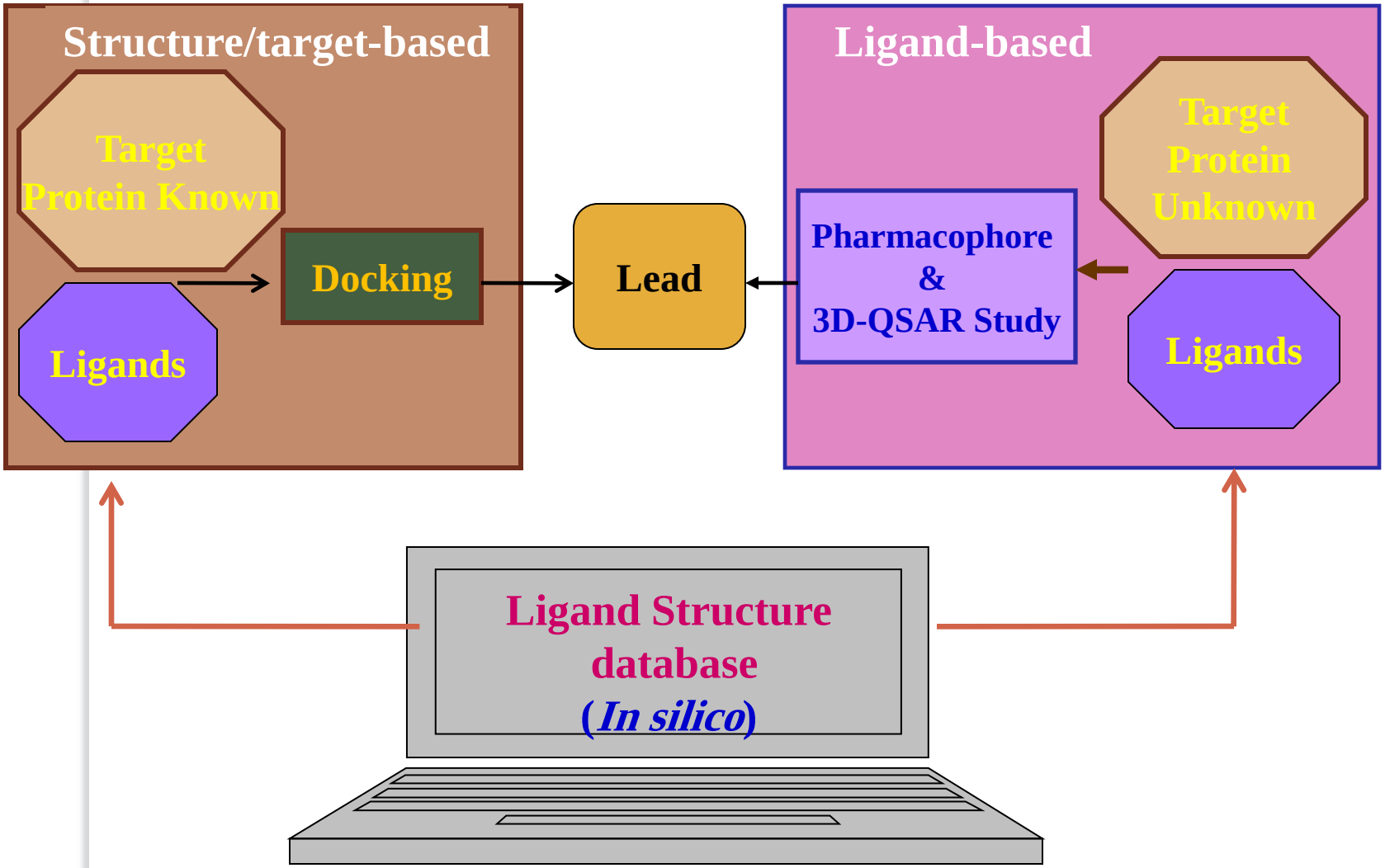
Molecular Modeling Techniques in Drug Design

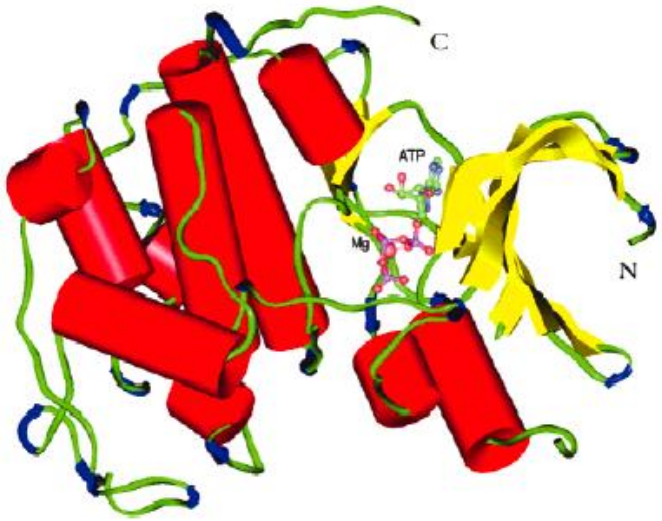
1. Quantum Mechanics

2. Molecular Mechanics

3. Molecular Dynamics

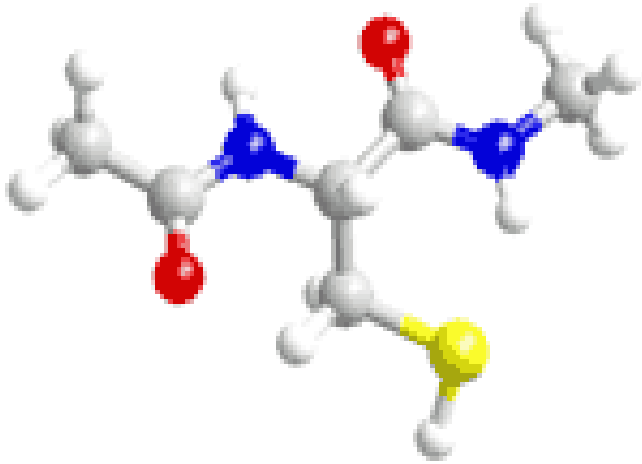
Drug Design Strategies Based on Molecular Mechanics





Target (structure)-based drug design

- Receptor structure is known
- Mechanism is known
- Ligands and their biological activities are known/ unknown
- Molecular docking

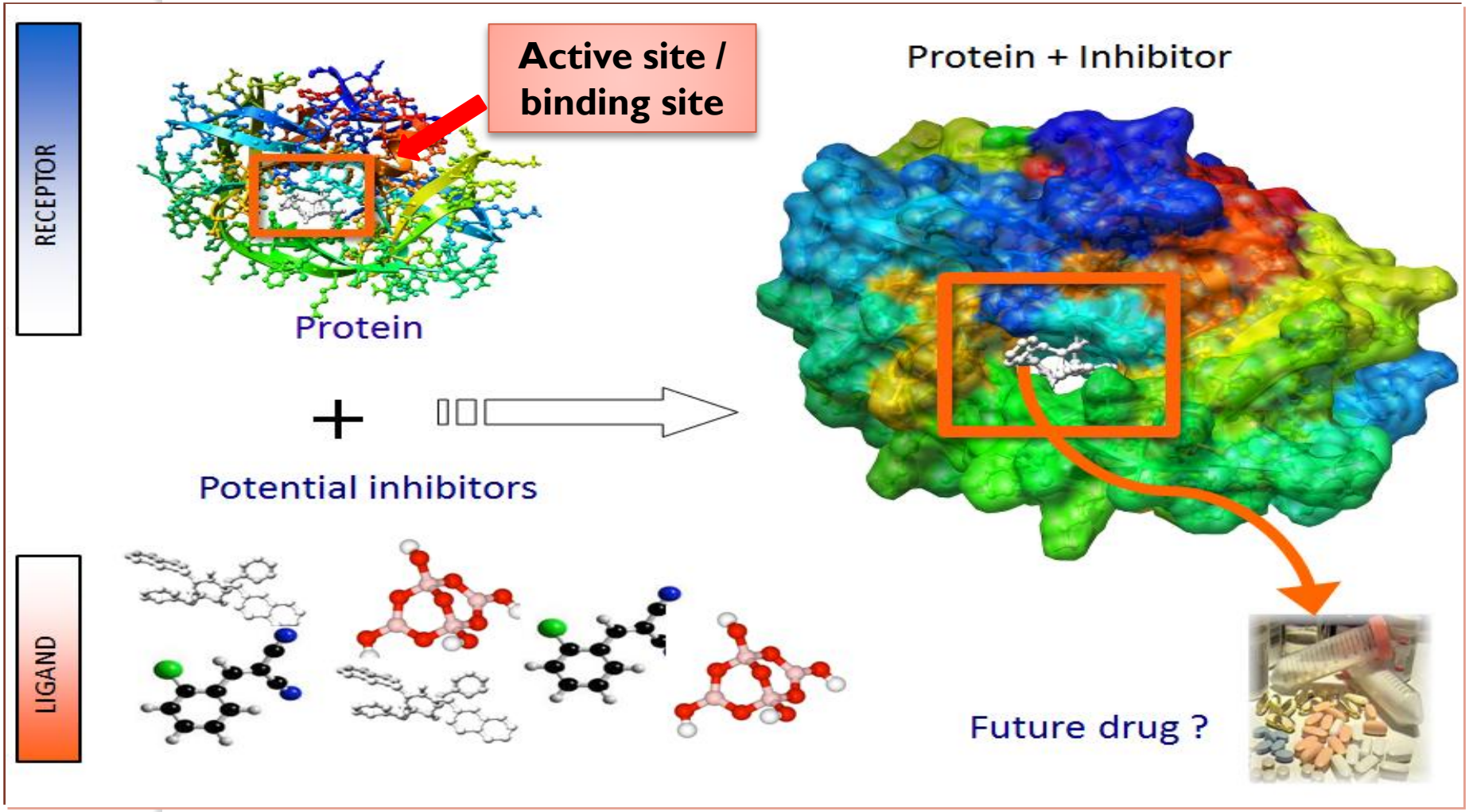


Ligand (analog)-based drug design

- Receptor structure is unknown
- Mechanism is known/ unknown
- Ligands and their biological activities are known.
- QSAR,
- Pharmacophore modelling

Structure-based (target-based) Drug Design

- It is used to **design a new drug molecule** using the **knowledge of three-dimensional (3D) structure of the biological target**.



Ligand-based Drug Design

- Here the crystal structure of the target enzyme or receptor is not available.
- But their ligands are well defined and characterized.
- This method not involves molecular docking or homology modeling methods.
- This method works based on the concept of ‘similar chemical structures have similar chemical activity’.

QSAR (Quantitative Structure-Activity Relationship)

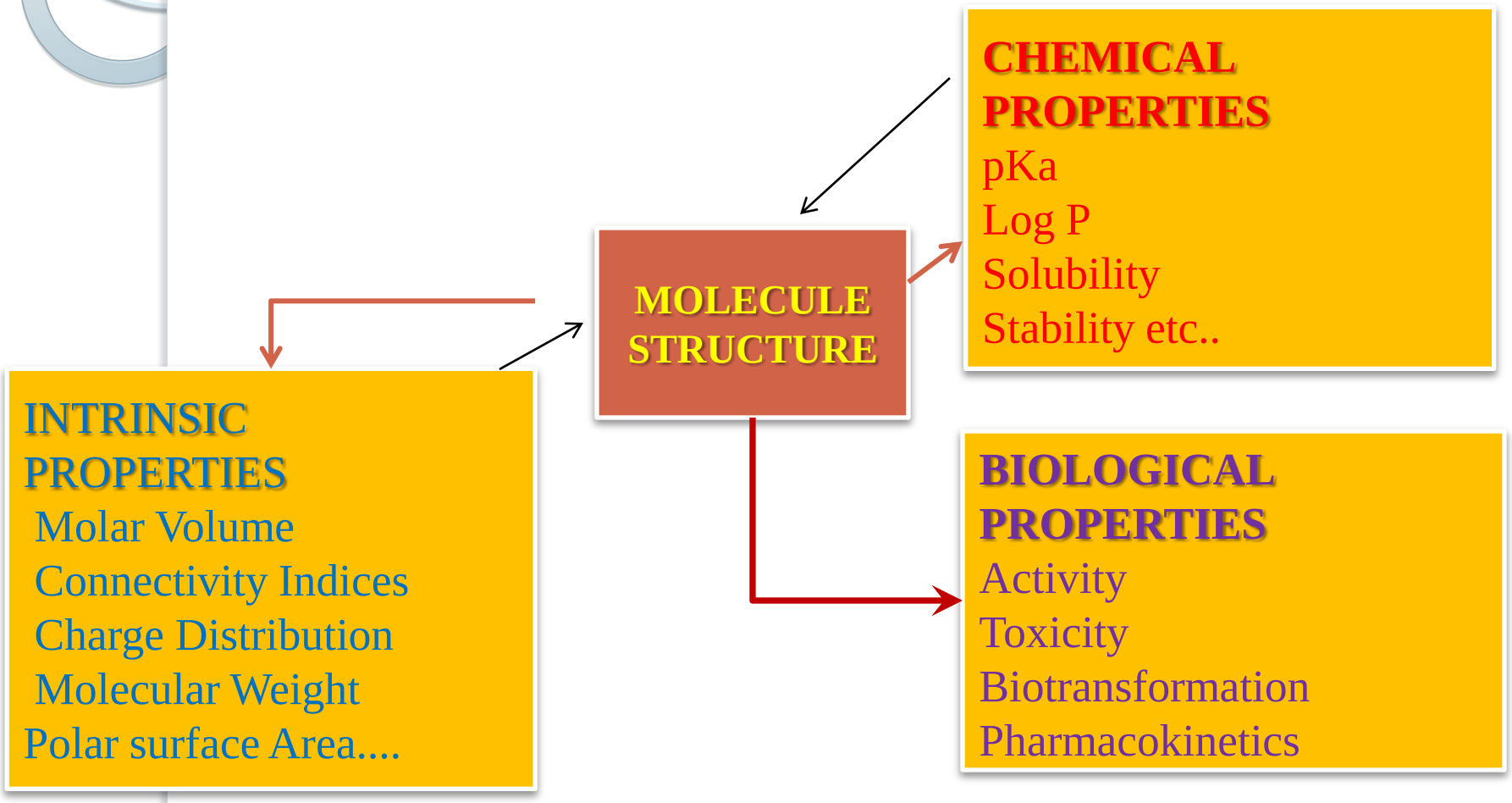
- QSAR is a mathematical relationship between a **biological activity** of a molecular system and its **geometric and chemical characteristics**.
- A general formula for a quantitative structure-activity relationship (QSAR) can be given by the following equation:

  Activity = f (molecular or fragmental properties)

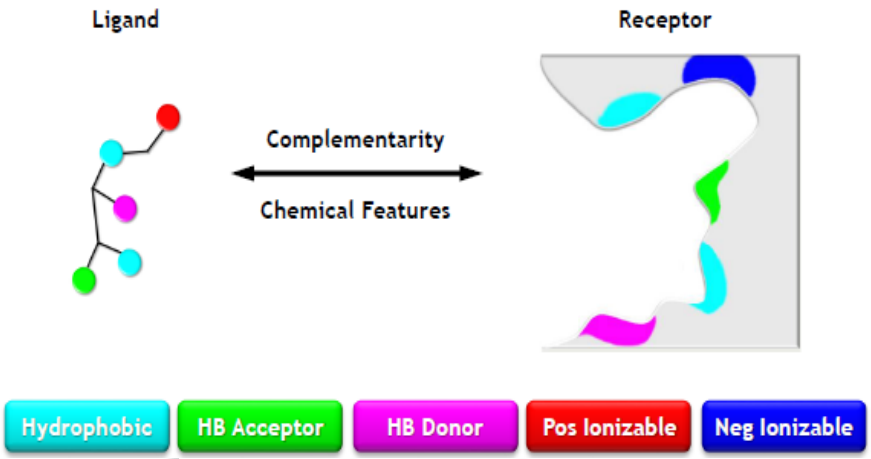
- QSAR attempts to find consistent relationship between biological activity and molecular properties, so that these "rules" can be used to evaluate the activity of new compounds.

Molecular Properties

1000's of Structure Properties for Correlation



Pharmacophore Modeling



A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal molecular interactions with a specific biological target and to trigger (or block) its biological response

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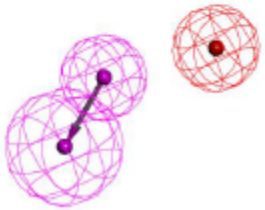
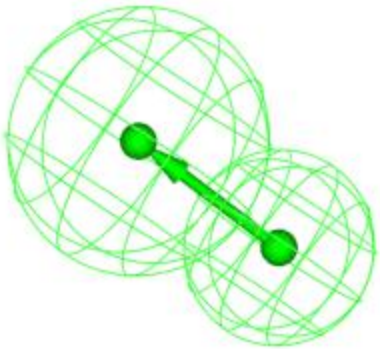
A pharmacophore feature without a location constraint only indicates the absence or presence of a chemical function

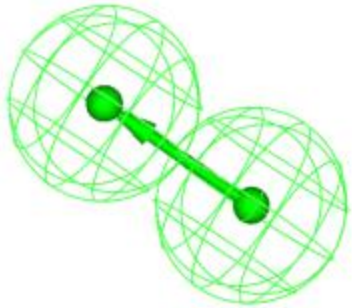
The location constraint specifies the 3D coordinates of the features and defines the spatial relationship of the features to each other



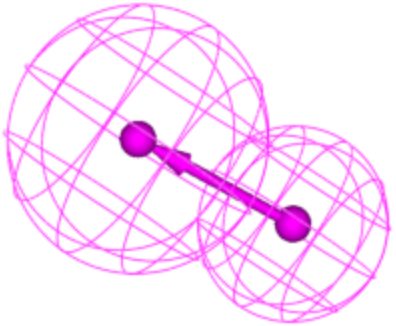
Pharmacophoric Features



H bond Acceptor



H bond Acceptor-Lipophilic

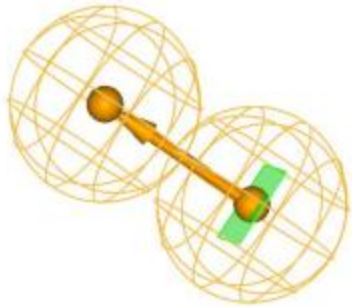


H bond Donor

Hydrophobic

Hydrophobic-Aliphatic

Hydrophobic-Aromatic



Ring aromatic

Negatively Ionizable

Positively Ionizable

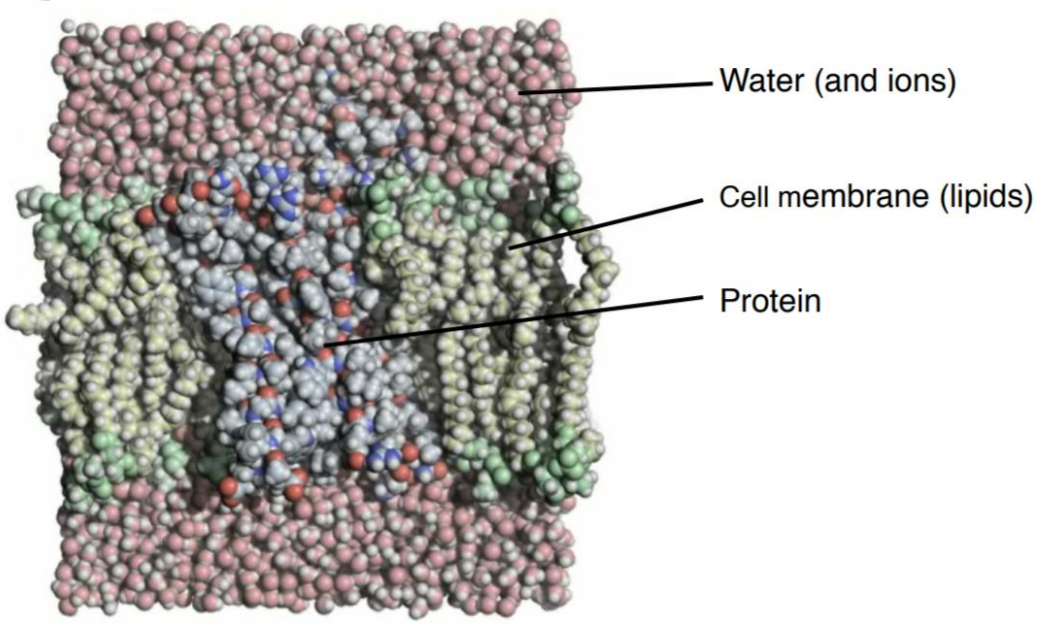
Negative Charge

Positive Charge

Molecular Dynamics

Molecular dynamics (MD) is a form of computer simulation in which atoms and molecules are allowed to interact for a period of time by approximations of known physics, giving a view of the motion of the particles.

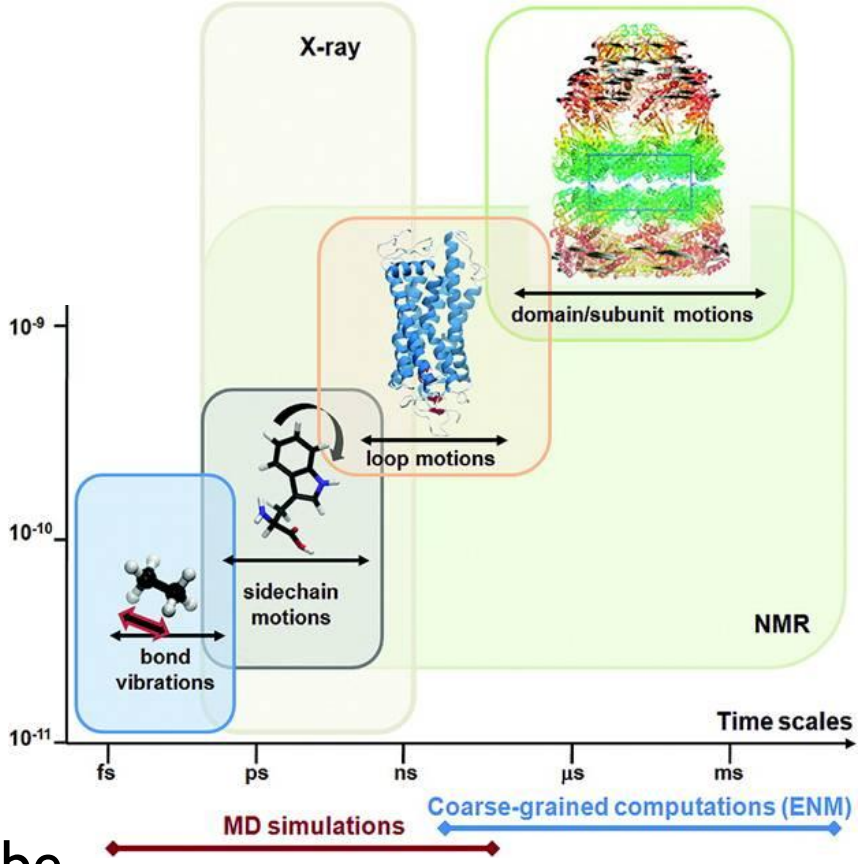
This kind of simulation is frequently used in the study of proteins and biomolecules and described as a "virtual microscope" with high temporal and spatial resolution.



Receptor/enzyme is inserted into the simulated cell membrane.

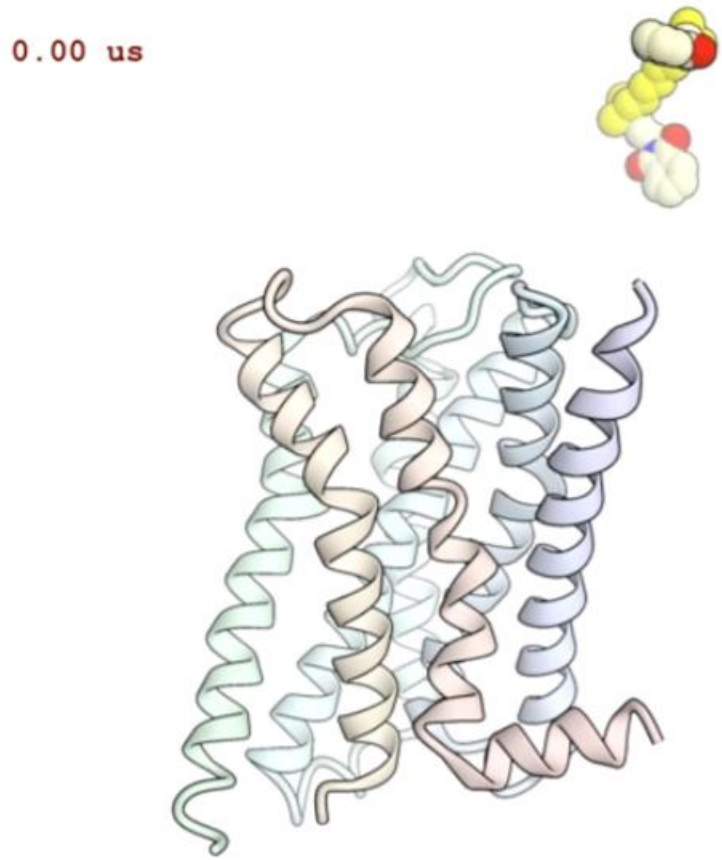
Water molecules were also added.

If any charges missing, then it can be added.



Example of MD with time scale

Molecular Dynamics (MD) Simulation helps to determine where the drug binds to receptor/enzyme



MD helps to determine

- ✓Drug binding
- ✓Change in arrangement of atoms
- ✓To alter the structure of drug
- ✓To obtain desired activity

Drug Design to Biological Evaluation

Drug design

Lead structure

Lead compound synthesis by Combinatorial Chemistry

High throughput Screening (HTS)

Lead compound or Lead

The lead is a prototype compound that has a number of attractive characteristics, including the desired biological or pharmacological activity.

Undesirable characteristics of lead are:

- High toxicity
- Other biological activities
- Absorption difficulties
- Solubility problems
- Metabolism problems

The lead optimization is to modify the chemical structure of the lead compound in order to improve the desired properties and trying to minimize the unwanted ones.

Lead Modification

Lead modification is done to improve the Pharmacological activity.

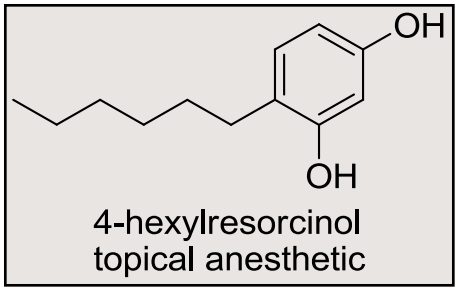
1. **Identification of the active part: PHARMACOPHORE**
2. **Functional group modification**
3. **Structure-Activity Relationship (SAR)**
4. **Structure modification to increase potency and therapeutic index**
5. **Structure modification to increase oral bioavailability**
   - **Lipophilicity.**
   - **Effect of ionization.**

Lead Modification

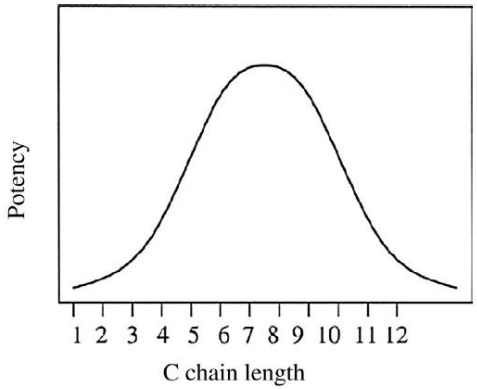
Structure modification to increase potency and therapeutic index

A. Homologation

A homologous series is a group of compounds that differ by a constant unit, generally a CH_2 (Methylene) group.



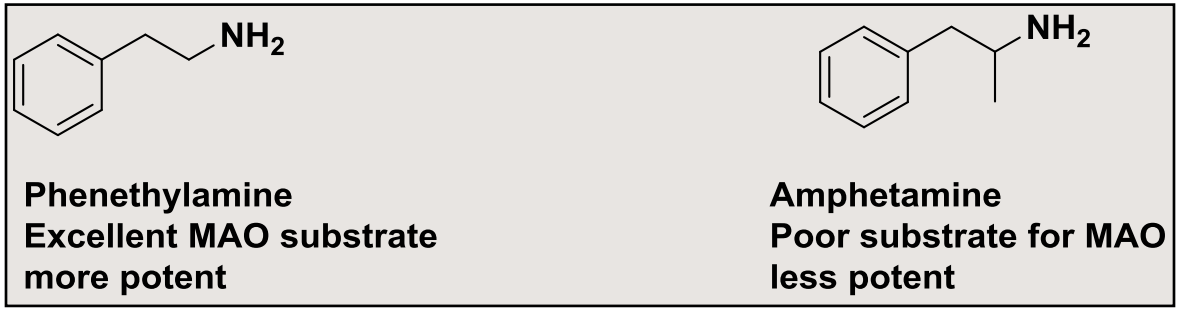
- ✓ **Chain length up to 9 carbon atom….tolerable (optimum lipophilicity and water solubility.**
- ✓ **Chain length more than 9 carbon atoms… low water solubility… low bioavailability.**



Lead Modification

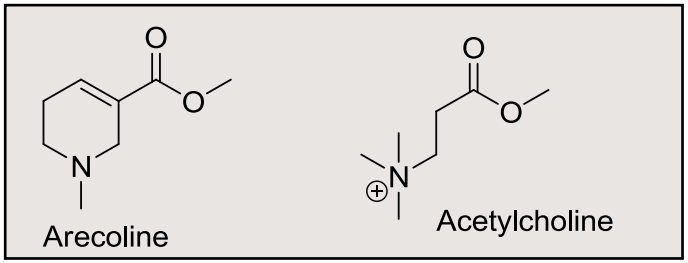
B. Chain branching

- Chain branching lowers the potency because the branched alkyl chain is less lipophilic than the straight alkyl chain.
- Chain branching may interfere with the receptor binding due to larger molar volume and shape.



C. Ring chain transformation

Affects both lipophilicity (increase) and drug metabolism (decrease).
Arecoline is more effective *in vivo*.



Lead Modification

D. Bioisosterism

- **Bioisosterism is an important lead modification approach.**

- **Bioisosteres are groups or substituents that have chemical or physical similarities, and which produce broadly similar biological properties.**

Importance or the use of Bioisosteres

- **Attenuate toxicity.**
- **Modify the activity of a lead.**
- **Alter the pharmacokinetics of lead.**
- **Potentiate activity.**

Two types of Bioisosteres

Classical Bioisosteres.

Non-classical Bioisosteres.

Lead Modification

Properties Influencing Oral Bioavailability

"Lipinski's rule of five" to improve oral bioavailability during lead modification.

The rule of five states that the orally administered drug must not have:

- **A molecular weight > 500 Dalton**
- **LogP > 5**
- **H-bond donor > 5 (expressed as the sum of OH and NH)**
- **H-bond acceptor > 10 (expressed as the sum of O and N)**

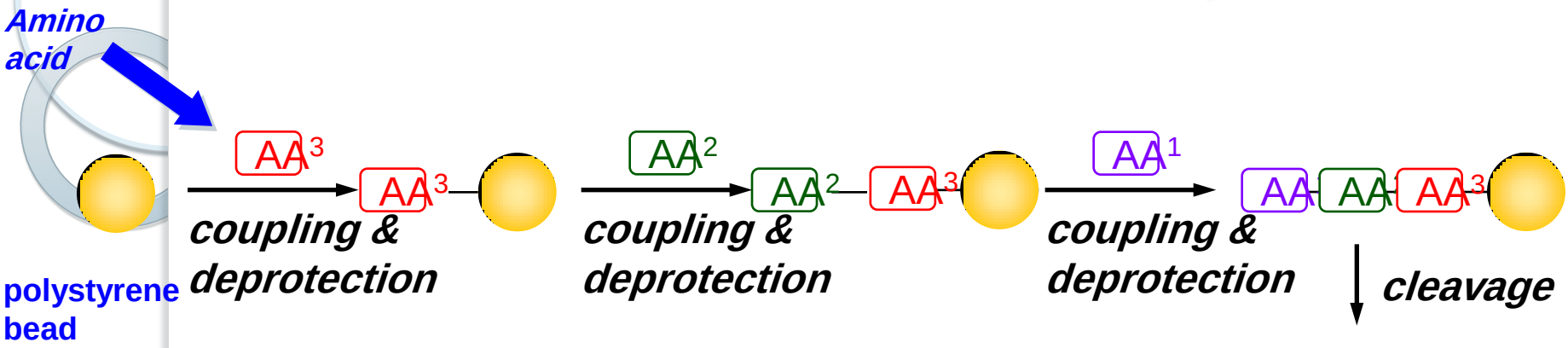
Exception of the rule of five:

- **Antibiotics**
- **Antifungals**
- **Vitamins**
- **Cardiac glycosides**

Reason for exception:

These drugs have active transporters to carry them across membrane.

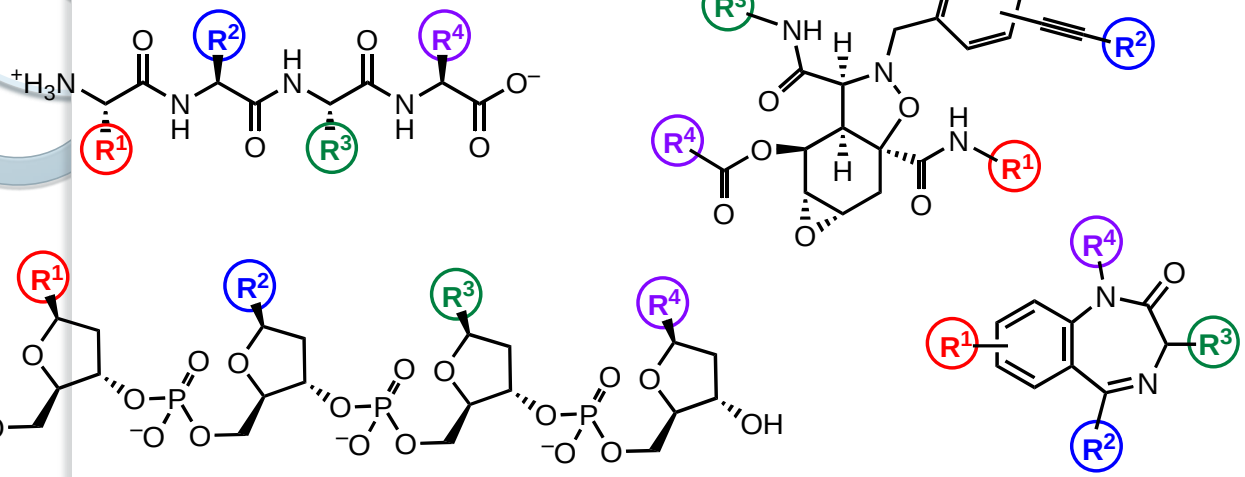
Combinatorial Chemistry



- ✓ Reactions can be driven to completion.
- ✓ Intermediates do not need to be purified.
- ✓ Final compounds are cleaved from the beads and purified by HPLC as necessary.
- ✓ Some procedures can be machine automated.

Combinatorial Chemistry

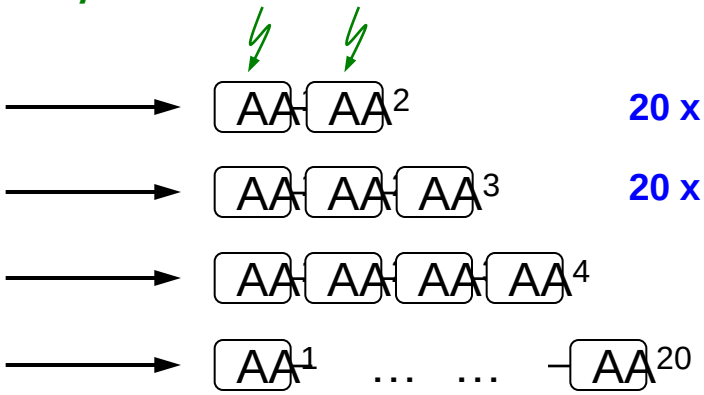
Any modular structure can be combinatorialized



- A fully **combinatorial library** includes all possible combinations of building blocks

Each position can be 1 of 20 possible amino acids

Gly	Ala	Val	Leu
Ile	Ser	Thr	Cys
Met	Lys	Phe	Tyr
His	Trp	Arg	Asp
Glu	Asn	Gln	Pro



20 x 20 = 20^2 = 400 combinations

20 x 20 x 20 = 20^3 = 8,000 combinations

= 20^4 = 160,000 combinations

= 20^{20} = 1.0 x 10^{26} combinations

Receptors

- Receptors are mostly membrane-bound proteins that selectively bind small molecules called ligands which results in physiological response.
- They are difficult to isolate because they exist in tiny amount and if isolated it will be difficult to purify.
- Receptors contain a binding site (hollow or cleft in the receptor surface) that is recognized by the chemical messenger.
- Binding of the messenger involves intermolecular bonds.

Affinity, efficacy, and potency

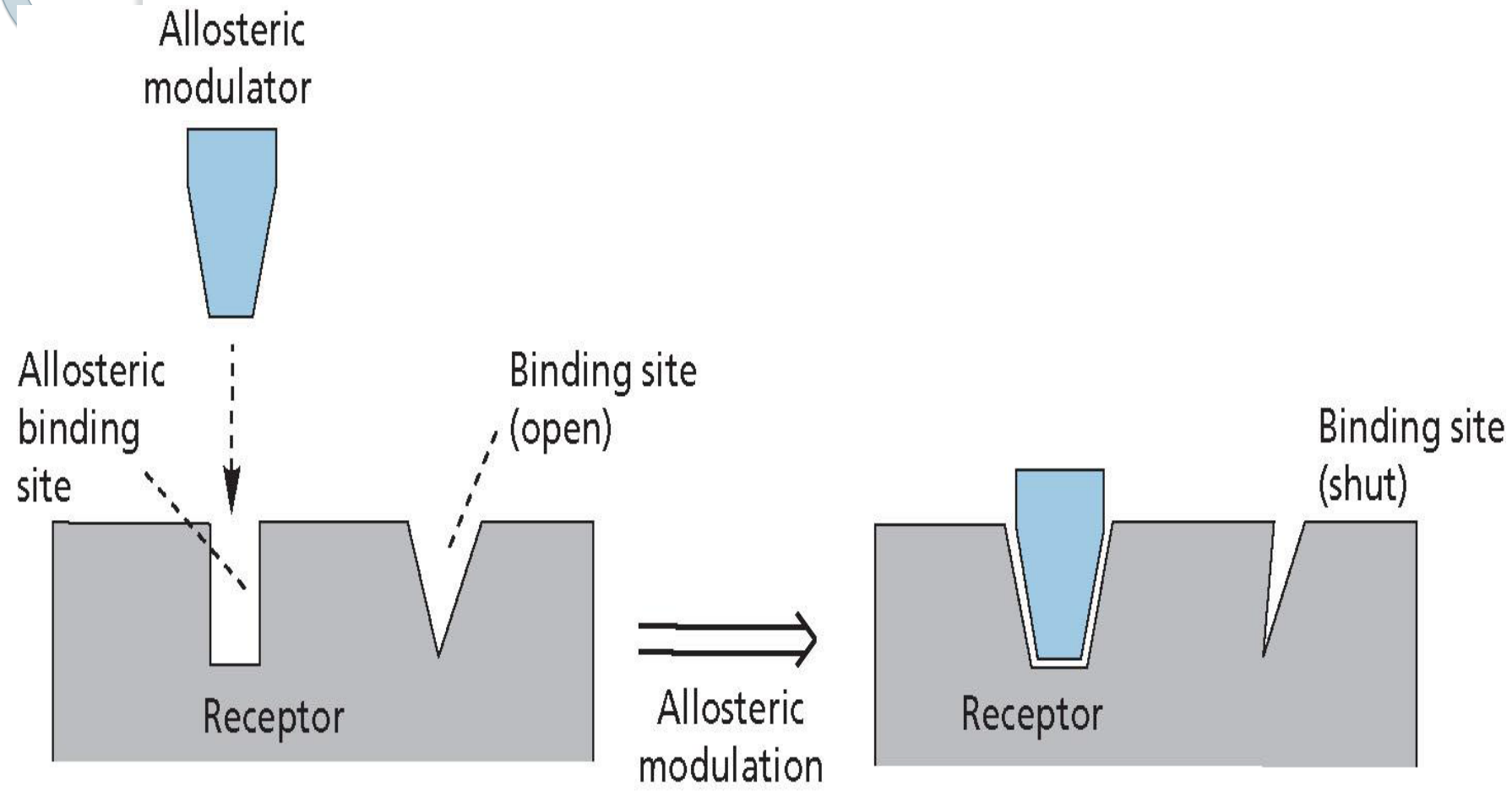
- **Affinity: how strongly the drug binds to the receptor; It depends on the molecular complementarily of drug and receptor.**
- **Efficacy: the maximum biological effect the drug can produce. A compound with high affinity does not necessarily have high efficacy (e.g. antagonists).**
- **Potency: the amount of drug needed to achieve a defined biological effect. The smaller the dose required, the more potent the drug.**

Receptor's agonist

- Agonists **mimic the natural messenger of a receptor.**
- Agonists bind **reversibly to the binding-site and produce the same induced fit as the natural messenger - receptor is activated.**
- **Similar intermolecular bonds formed as with natural messenger.**
- Agonists are often **similar in structure to the natural messenger.**

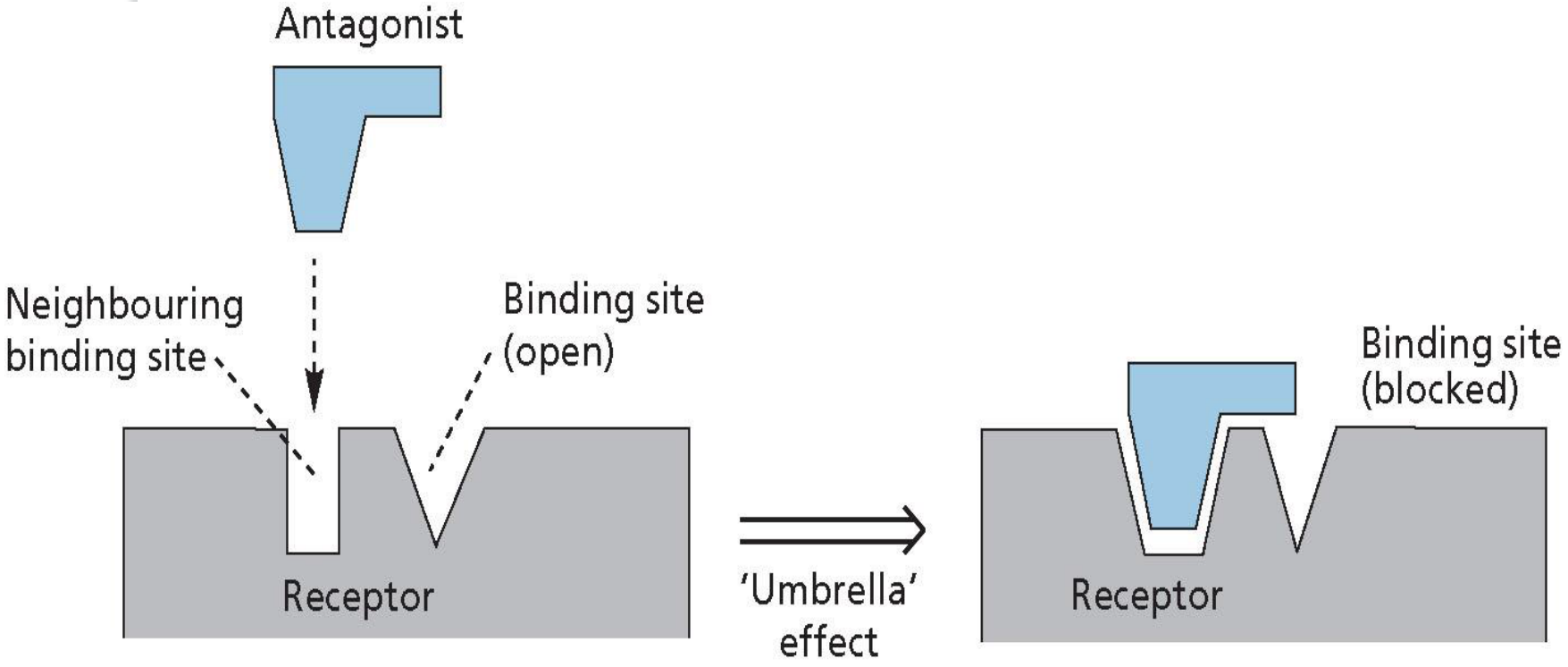
Receptor’s antagonist

Allosteric antagonists

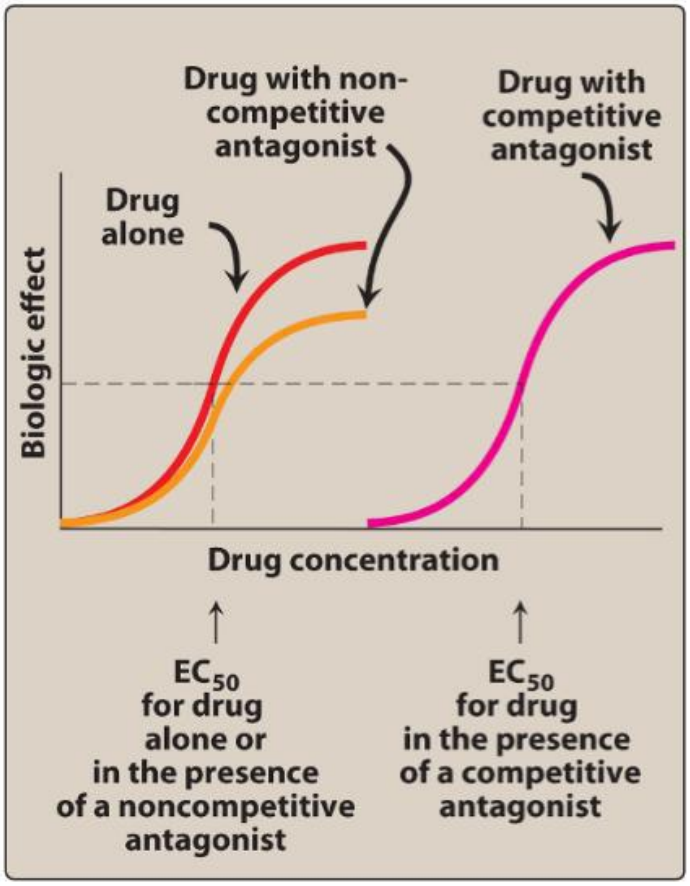


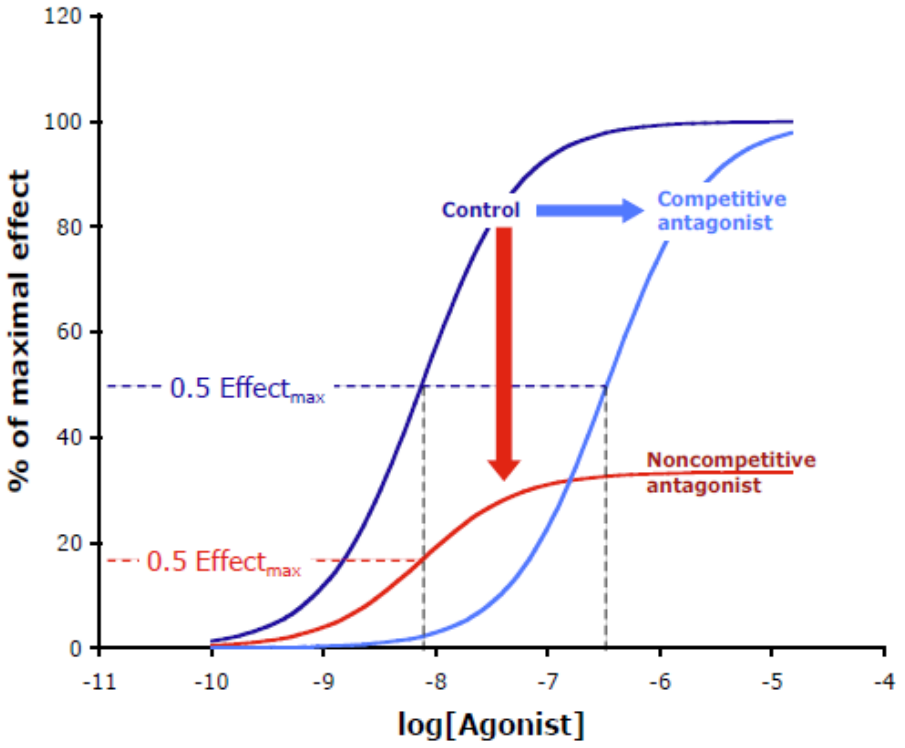
Receptor’s antagonist

Antagonism by the 'umbrella’ effect: Here the drug will bind to a region close to binding site, once bound, part of its structure (tail) will cover the opening of the binding site....preventing the normal messenger from accessing the binding site.



- **Competitive Antagonists:** Compete with agonist for receptor binding => Agonist appears less potent, but can still achieve 100% effect but at higher concentrations.
- **Non-competitive Antagonists:** Bind to receptor at different site and either prevent agonist binding or the agonist effect => maximal achievable response reduced.





Enzymes

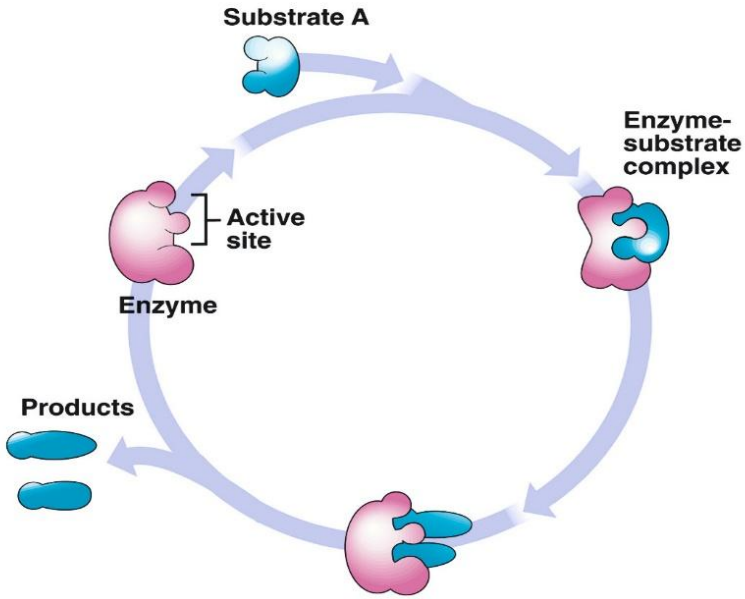
- Enzymes are soluble proteins, floating in interstitial or extrastitial fluids, cell cytosol and blood.

- 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** $\underset{K_1}{\rightleftharpoons}$ **E-S complex** $\xrightarrow{K_2}$ **Product**

E **S**

Enzyme Catalysis

It is characterized by two features:

(1) Reaction specificity.

(2) Rate acceleration.

Binding Specificity:-

(i) Very specific, Absolute: One type of substrate can fit into 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}

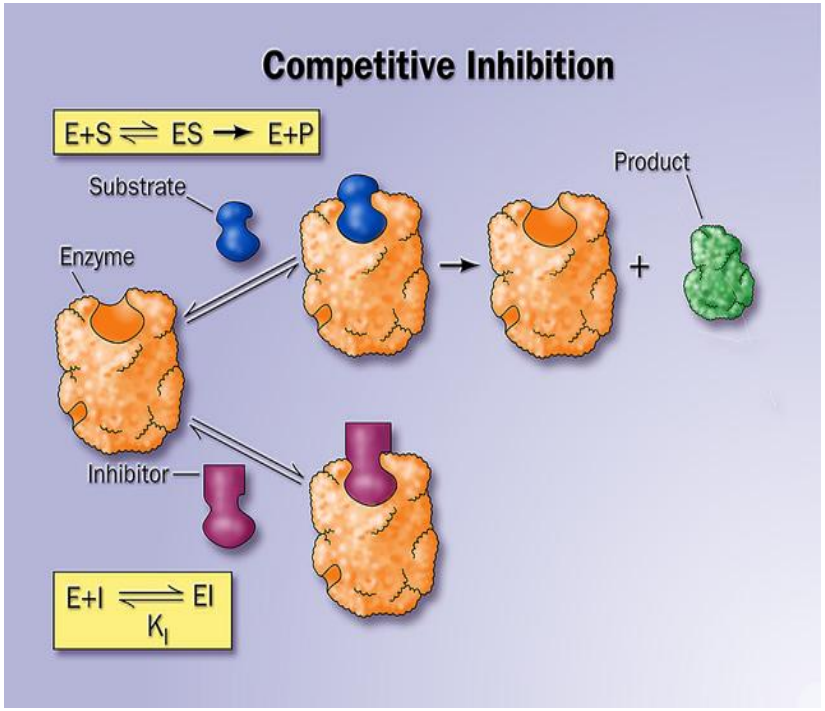
Enzyme inhibition

- 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 inhibits essential enzymes in non human cells that are unique, does not exist in human cells (penicillins).

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.

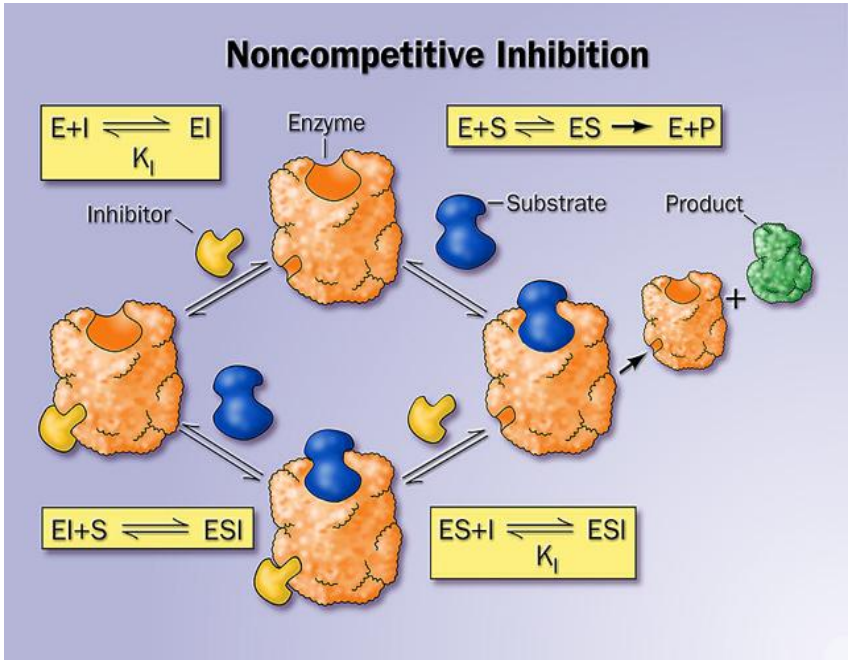


Competitive inhibition can be relieved by increasing the substrate concentration (the substrate "outcompetes" the inhibitor).

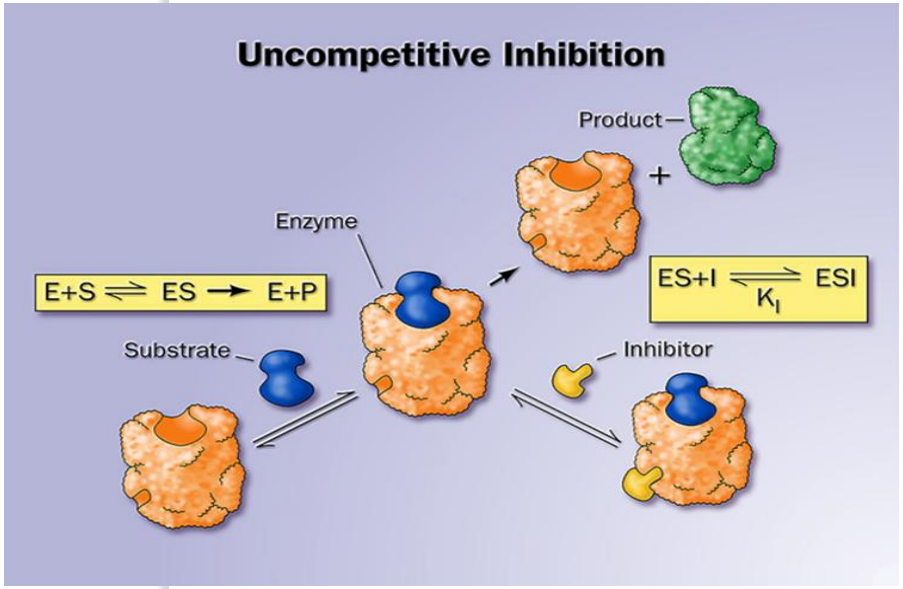
Examples: captopril, enalapril, lisinopril and oral antihypertensives (ACE Inhibitors).

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.



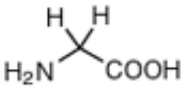
Uncompetitive inhibition



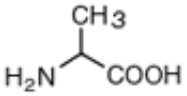
Binds at a site distinct from the substrate binding-site but only in the ES complex.

Classification of amino acids

Small

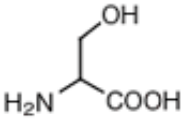


Glycine (Gly, G)
MW: 57.05

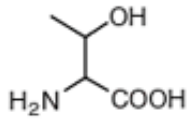


Alanine (Ala, A)
MW: 71.09

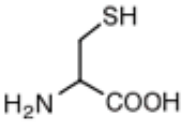
Nucleophilic



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

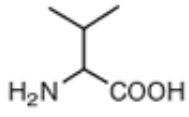


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

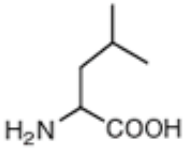


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

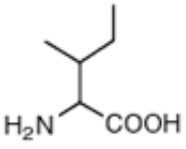
Hydrophobic



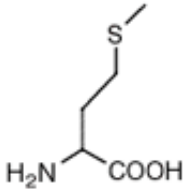
Valine (Val, V)
MW: 99.14



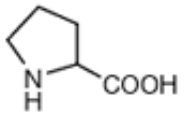
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

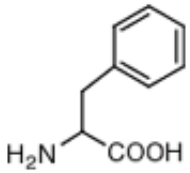


Methionine (Met, M)
MW: 131.19

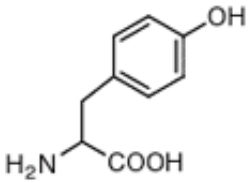


Proline (Pro, P)
MW: 97.12

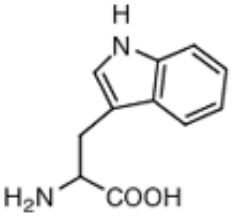
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

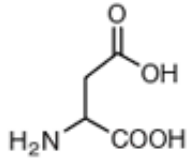


Tyrosine (Tyr, Y)
MW: 163.18

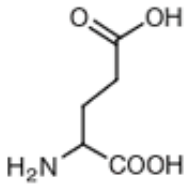


Tryptophan (Trp, W)
MW: 186.21

Acidic

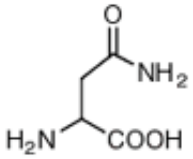


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

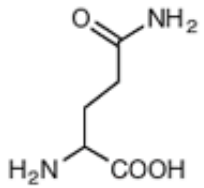


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

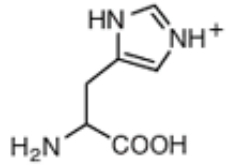


Asparagine (Asn, N)
MW: 114.11

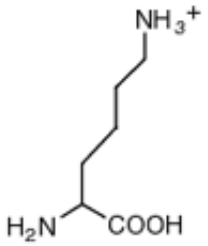


Glutamine (Gln, Q)
MW: 128.14

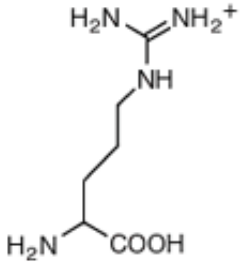
Basic



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

High throughput Screening (HTS)

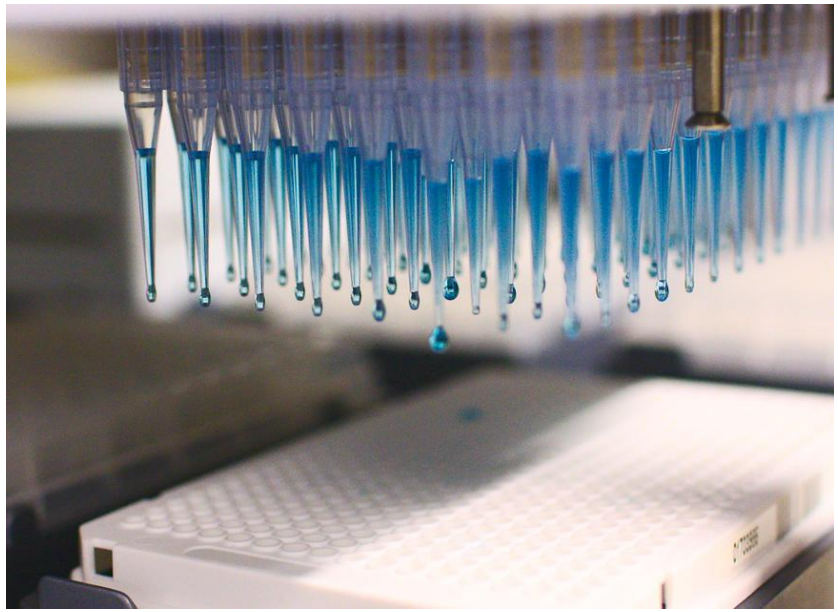
- Identification of one or more positive candidates extracted from the pool of possible candidates based on specific criteria.
- HTS provides a practical method to investigate large numbers of synthetic compounds in miniaturized *in vitro* assays to identify those capable of modulating the biological target.

Assay technology in HTS

1. Cell Growth tests
2. Tissue-response Cell-based Assay
3. Enzyme tests

Advantages of High throughput Screening (HTS)

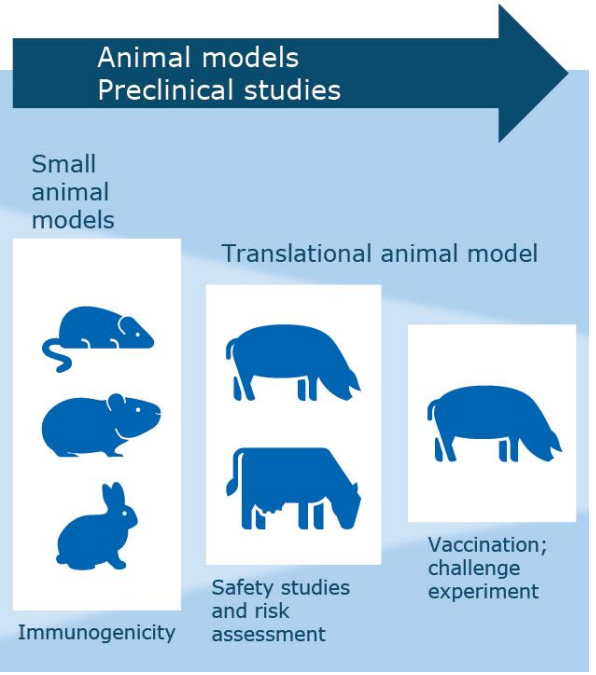
1. High sensitivity
2. High speed by Automation
3. Minimum amount of sample can be used
4. Low background signal
5. Clear results
6. Good reproducibility
7. Fast data processing
8. Acceptable cost



Preclinical Studies

Steps involved with doing a Pre-Clinical Trial:

5. File for approval as an Investigational New Drug (IND)
4. Establish Effective and Toxic Doses
3. Screen the Drug in the Assay
2. Develop a Bioassay
1. Indentify a Drug Target



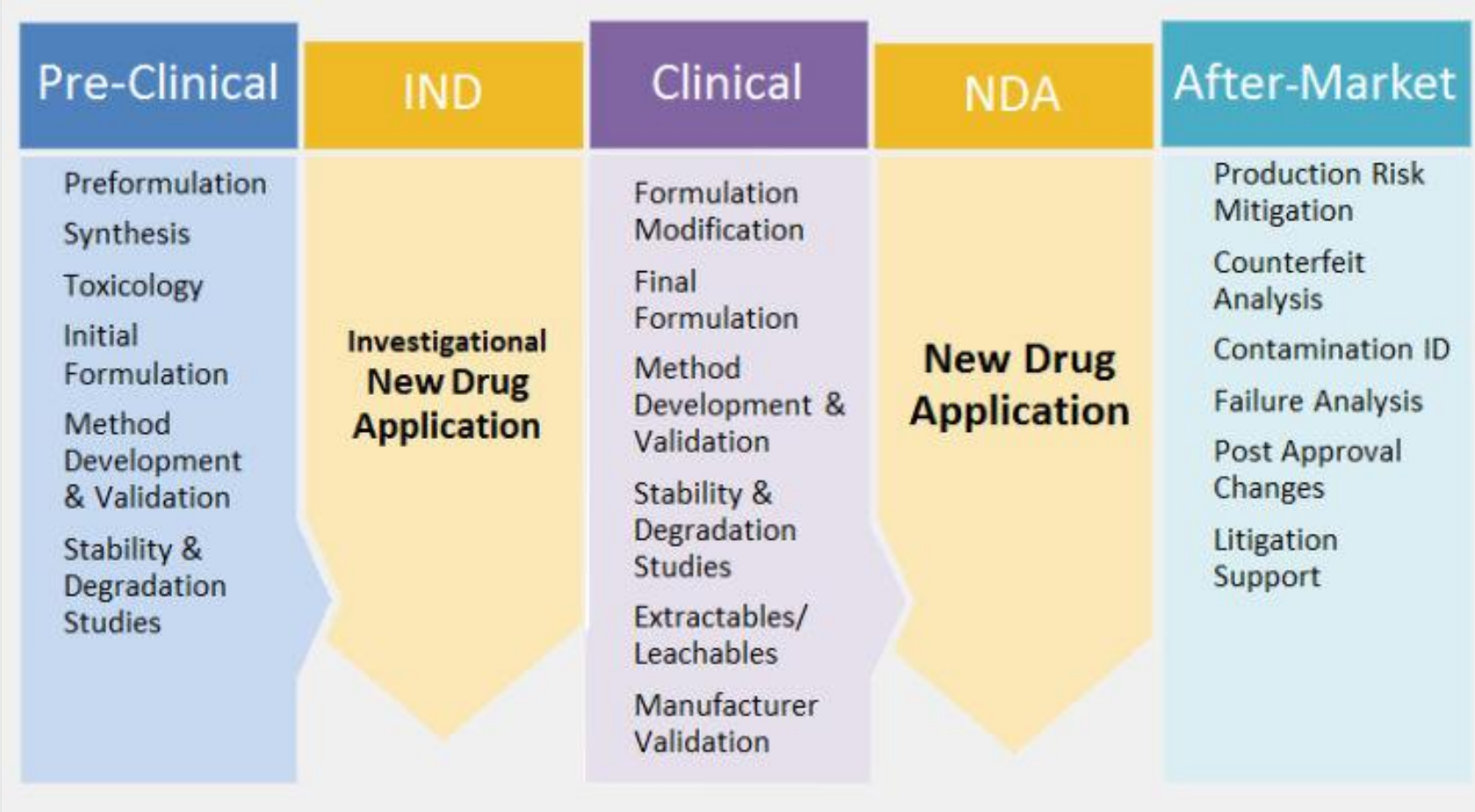
Clinical trials-Different Phases

- **Phase-I** (lasts for 1 month -1 year): Evaluation of the safety, tolerability, pharmacokinetic and pharmacological activity of drugs on 20-100 volunteers.

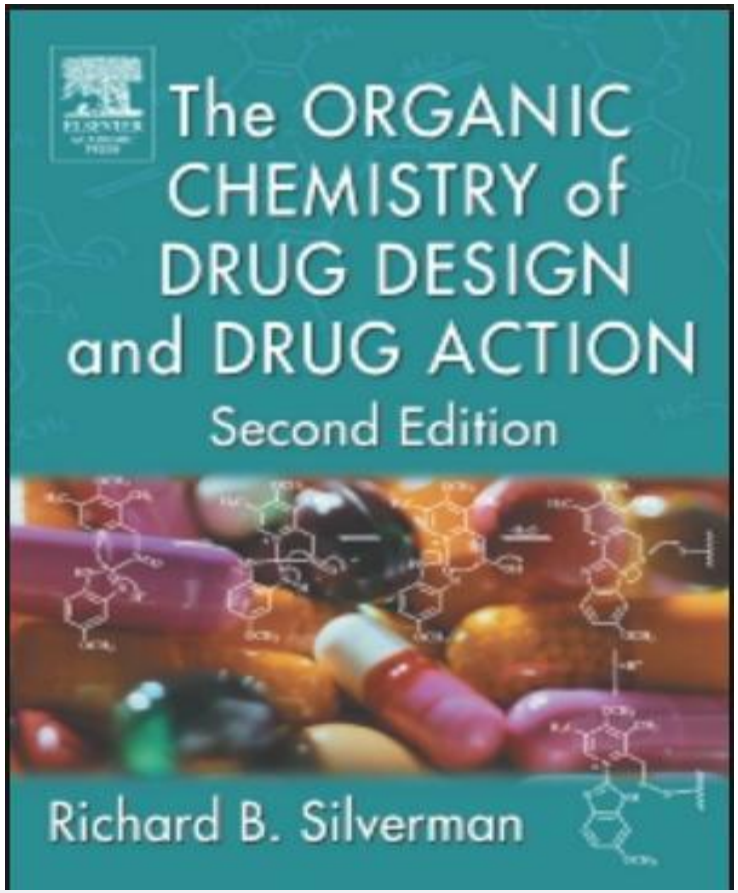
- **Phase-II** (lasts for 1-3 years): further assess the efficacy , safety of drugs in addition to dosing regimen in 300-600 patients.

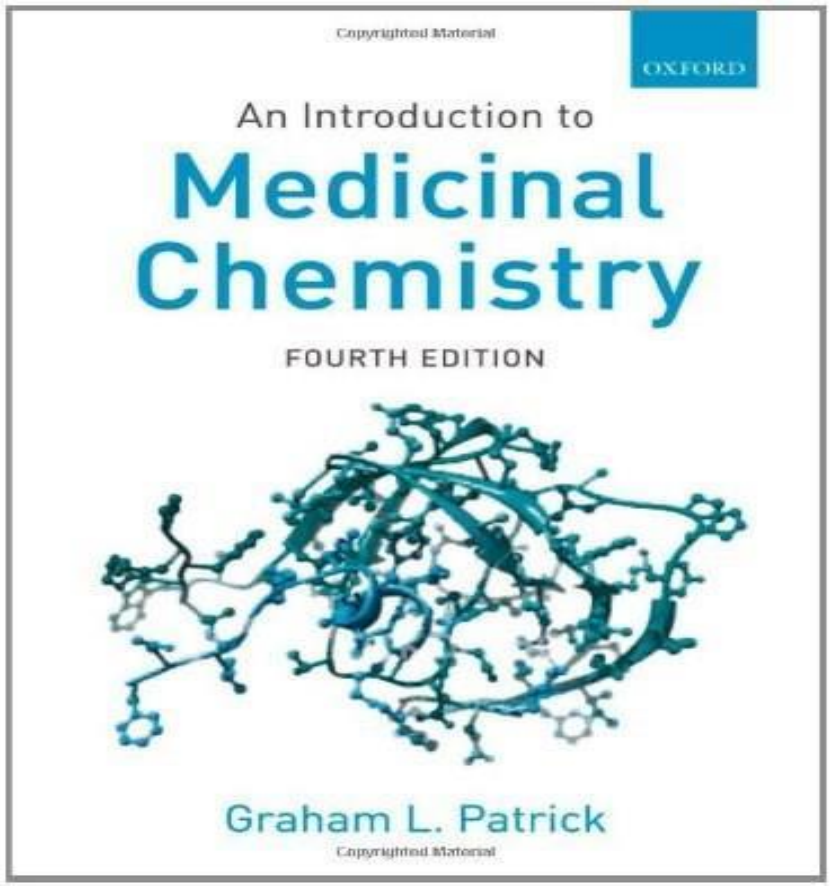
- **Phase-III** (last for 2-6 years): covers several thousands of patients in clinics or hospitals; study the activity and possible side effects on the long term.

- **Phase-IV**: Post marketing feedback, after prescribing drugs to the out patients.


Pre-Clinical
IND
Clinical
NDA
After-Market
Preformulation
Synthesis
Toxicology
Initial Formulation
Method Development & Validation
Stability & Degradation Studies
Investigational New Drug Application
Formulation Modification
Final Formulation
Method Development & Validation
Stability & Degradation Studies
Extractables/ Leachables
Manufacturer Validation
New Drug Application
Production Risk Mitigation
Counterfeit Analysis
Contamination ID
Failure Analysis
Post Approval Changes
Litigation Support

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