DRUG DESIGN

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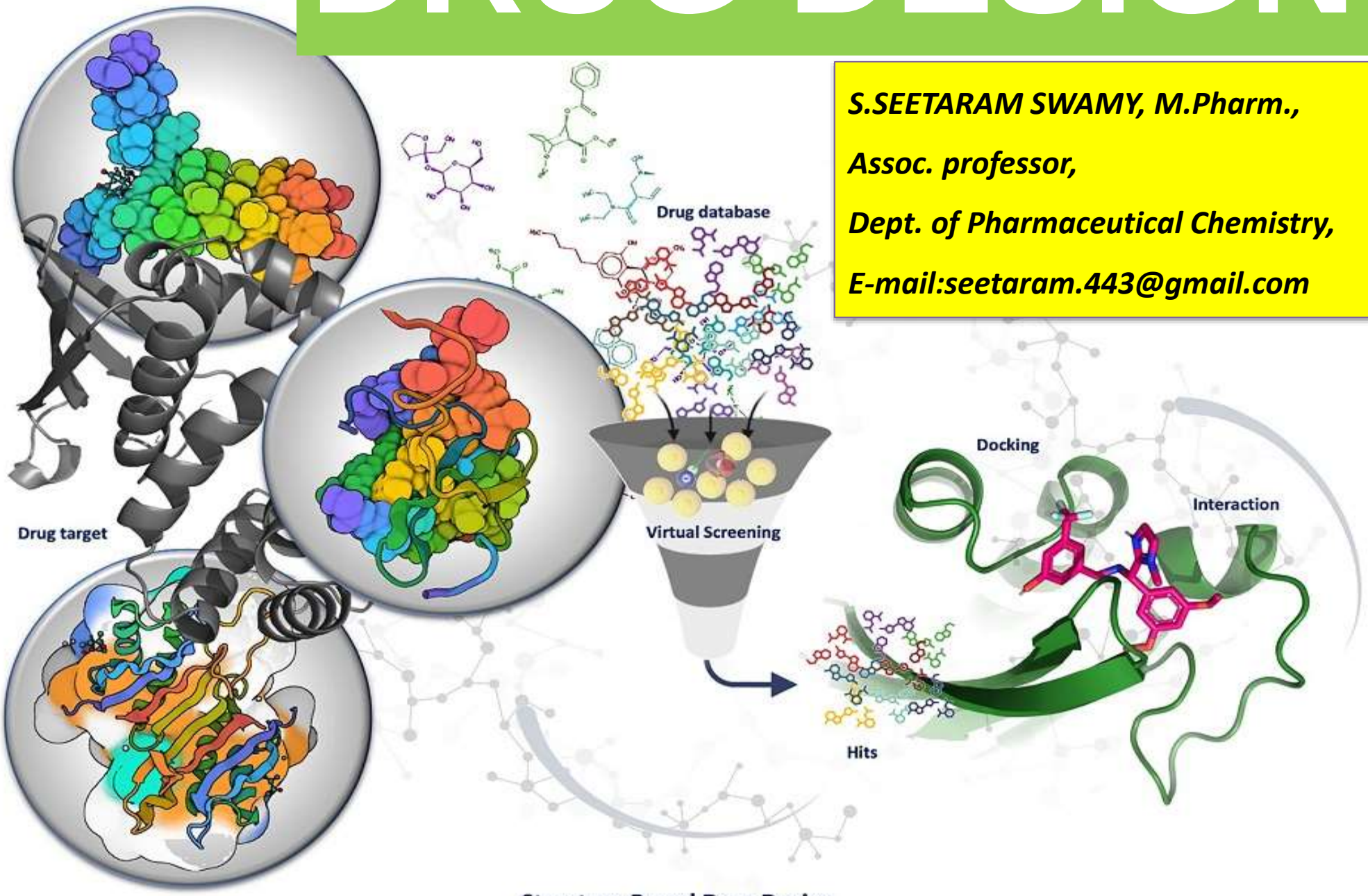
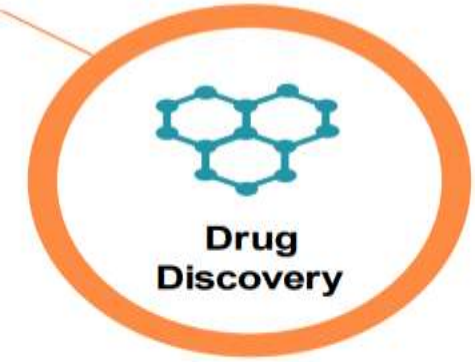


Drug Discovery
Stage 1 (Drug Design)

Target Selection

Select a Defined Target Proteins: Receptors, Enzymes, Ion Channels

Systemic planning a project to discover a new drug from a unknow or known sources.
- Novel drugs
- Me too version



Lead Findings

Selection and Finding of Lead compounds: Synthetic/Natural products

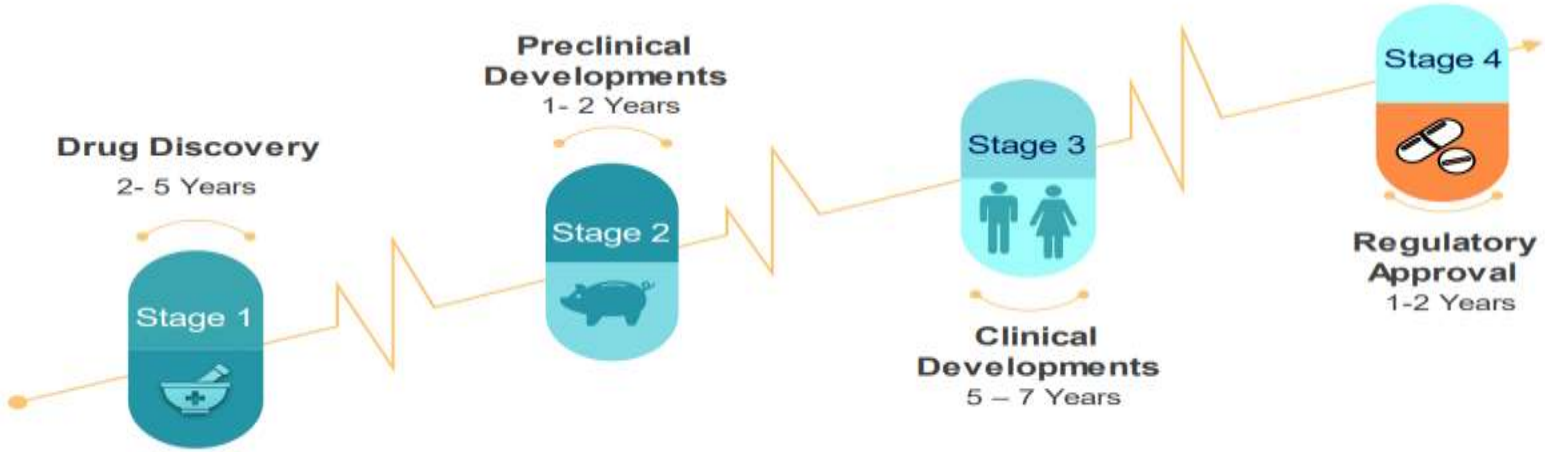
Lead Optimization

Optimize the lead activity on selected target protein: selectivity, metabolic stability, potency

"To discover a effective medicine, select a proper target & lead"

Drug Discovery
Stages

With the development of the pharmaceutical world towards the end of 19 th Century, Drug Discovery become a highly focused and manages process.



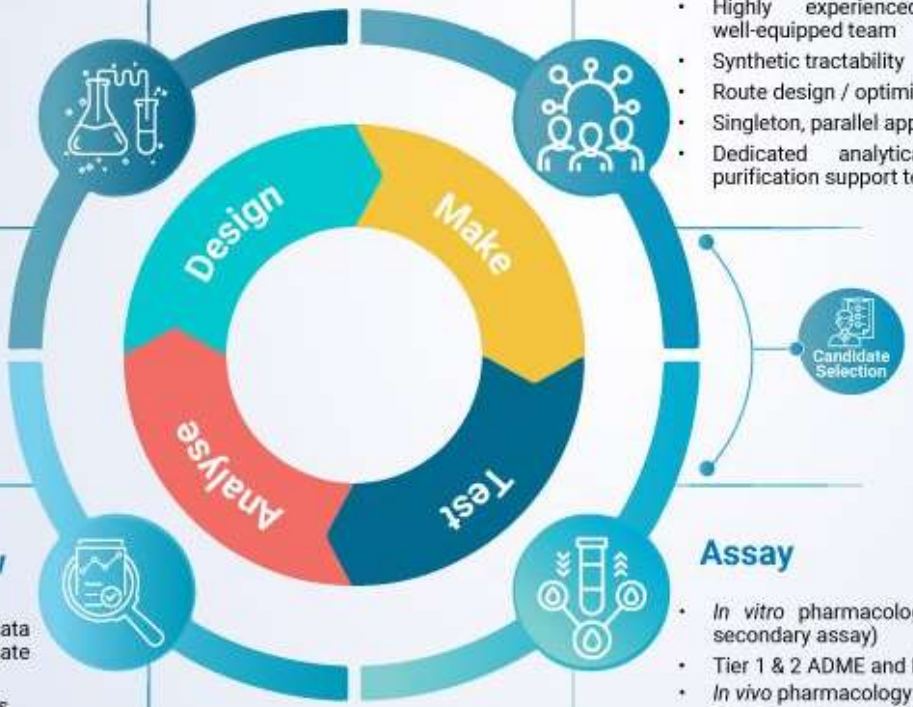
Target ID → Target knowledge → Hit identification → Hit to lead → Lead optimization → Candidate selection

Medicinal Chemistry

- Literature review
- Pharmacophore-based /scaffold-hopping, SBDD, virtual screening
- *In Silico* property prediction, Target ID & Target knowledge
- IP review & FTO searches

Synthetic Chemistry

- Highly experienced and well-equipped team
- Synthetic tractability
- Route design / optimization
- Singleton, parallel approach
- Dedicated analytical & purification support team



Strategic Review

- SAR, SPR analysis & data interpretation vs candidate drug target profile
- Joint project team meetings
- Decision on next step

Assay

- *In vitro* pharmacology (primary, secondary assay)
- Tier 1 & 2 ADME and PK
- *In vivo* pharmacology
- Preliminary tox profiling
- Clear screening cascade with well defined acceptance criteria to support progression

- Projects covering from discovery to pre-clinical candidate (PCC)
- Catering US / European / Japanese pharma / biotech companies
- Designed potential hit scaffolds
- Synthetic team made analogues of initial hits to generate leads
- Lead optimization led to identification of PCC
- Preclinical R&D group scouted routes and delivered multi-gram quantities of the PCC

VARIOUS APPROACHES USED IN DRUG DESIGN

The various approaches used in drug design (**Ligand based or Structural based**) include the following.

1) Drug discovery via Random screening of synthetic compounds or chemicals and natural products by bioassay procedures.

2) Drug discovery via metabolic studies

3) Drug discovery via Novel compounds preparation based on the known structures of biologically active, natural substances of plant and animal origin, i.e., lead skeleton.

4) Drug discovery via Preparation of structural analogs of lead with increasing biological activity and Application of bio isosteric principle.

There are two major types or approaches to drug design.

- Ligand based drug design (Indirect drug design)
- Structure based drug design (Direct drug design)

Ligand based drug design (Indirect drug design): Ligand based drug design is based on the knowledge of other molecules that bind to the biological target of interest so as to derive a **pharmacophore** which will bind to the target.

(A) Q SAR

(B) Analog drug design

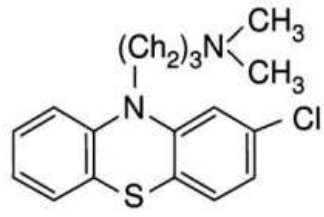
(C) Combinatorial chemistry

(D) Natural Products as a lead, etc

Structure based drug design (Direct drug design): Structure based drug design is based on the knowledge of the three dimensional structure of the biological target. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed.

Imipramine

Chlorpromazine



16.2. RATIONAL APPROACHES & CONCEPTS

Drug design seeks to explore:

- ✓ Effects of biological compounds on the basis of molecular interaction
- ✓ Explore the various process involve in drug discovery
- ✓ Explore drug-protein interaction to elicit biological response
- ✓ Probable relationship between biological activity and chemical structure.

Concept of LEAD

Lead is the prototype bioactive molecule subjected to drug design and drug discovery and needs to exploration and exploitation.

- ✓ **Exploration of Lead:** The search for new lead.
- ✓ **Exploitation of Lead:** Requires assessment, improvement and extension of lead.

Concept of Analogue

- ✓ Chemical Derivatives or structural analogue having similar structure with little medication

Concepts of Prodrug

- ✓ Prodrug is the appropriative derivatives which converts active metabolite invo

Pharmacophore

- The original concept of the pharmacophore was developed by Paul Ehrlich during the late 1800s.
- The word pharmacophore was coined much later, by Schueler in his 1960 book Chemobiodynamics and Drug Design, and was defined as "a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity."
- The definition of a pharmacophore was therefore no longer concerned with "chemical groups" but "patterns of abstract features.

Pharmacophore: is a group of atoms in the molecule of a drug responsible for the drug's action

Pharmacophore

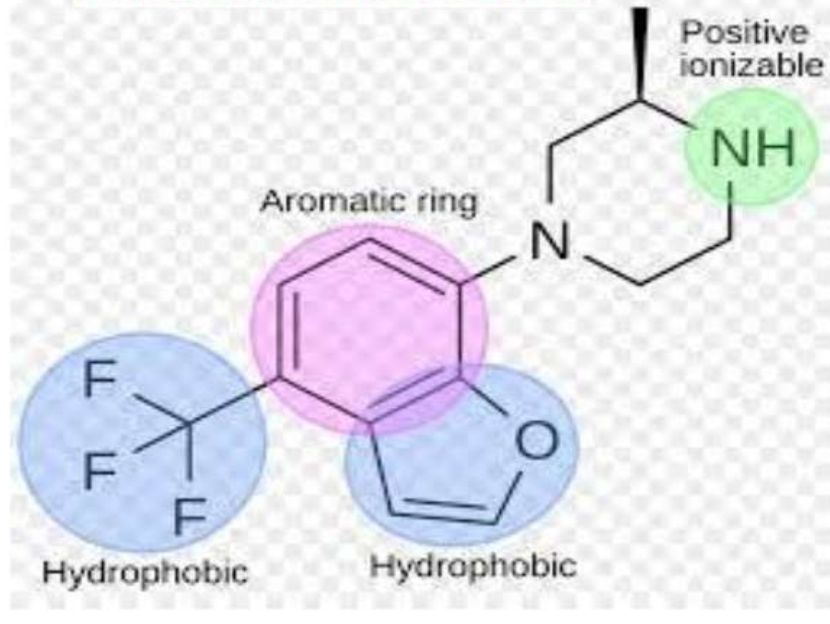
- International Union of Pure and Applied Chemistry "A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response"
- Significance
  - Pharmacophores can be used to represent and identify molecules on a 2D or 3D level by schematically.
  - The most common application of pharmacophores is virtual screening, and different strategies are possible depending on the prior knowledge
  - The pharmacophore concept is also useful for ADME-tox modeling, side effect, and off-target prediction as well as target identification.

Pharmacophore Features

- Hydrogen Bond Donor
- Hydrogen Bond Acceptor
- Aromatic
- Hydrophobicity
- Hydrophilic

- Pharmer
- Pharmapper
- Pharmagist
- Boomer
- ZincPharmer

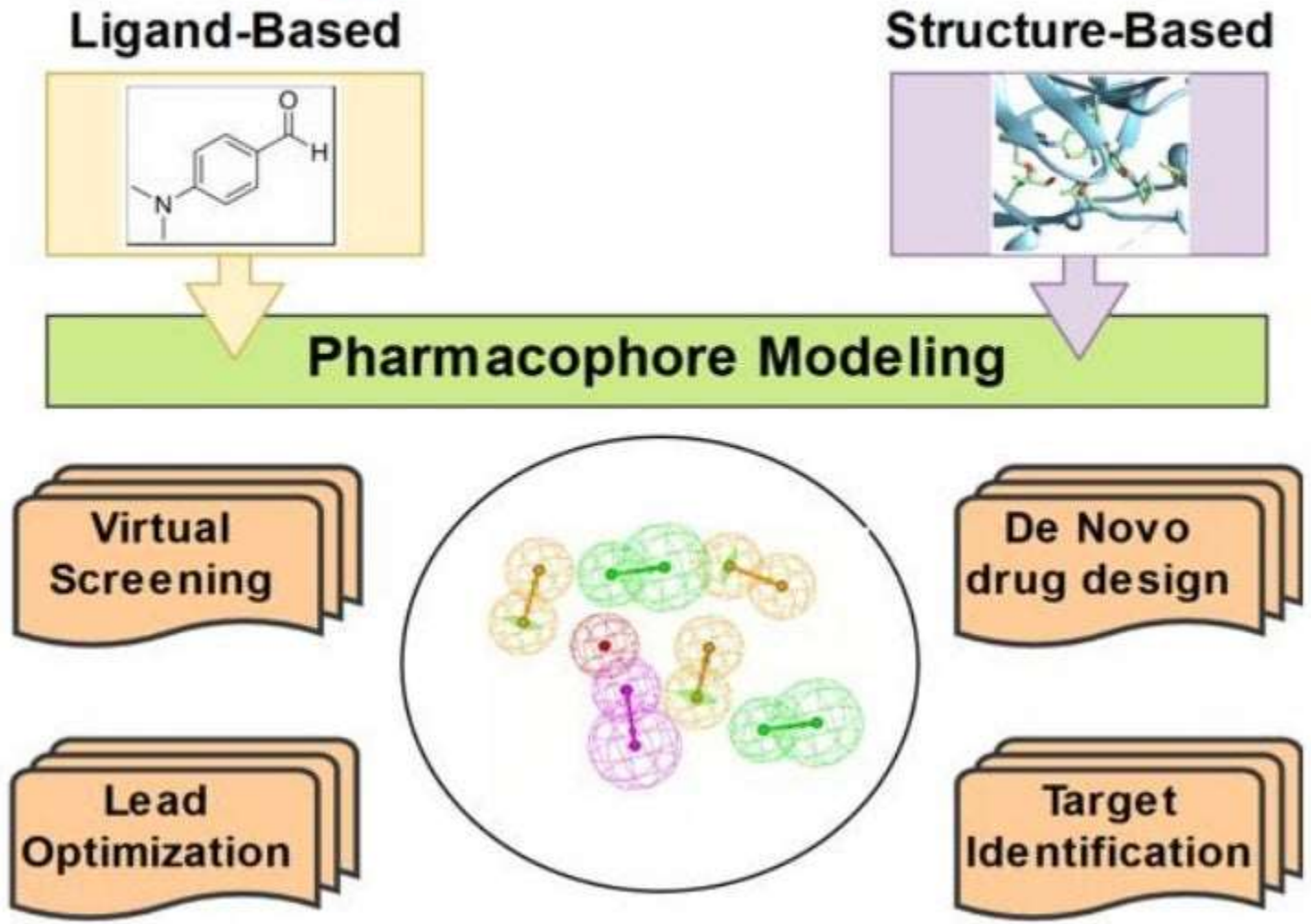
Pharmacophore identification



Pharmacophore modeling

- Pharmacophore modeling is most often applied to virtual screening in order to identify molecules triggering the desired biological effect.
- For this purpose, researchers create a pharmacophore model (query) that most likely encodes the correct 3D organization of the required interaction pattern. Depending on how much is known about the particular protein target, different options are available to construct such a query

Types of Pharmacophore modeling



Pharmacophore modeling

- A pharmacophore model is the ensemble of common steric and electronic features that are necessary to ensure the optimal molecular interactions with a specific biological target and to trigger (or block) its biological response. It can be used to represent and characterize molecules on schematic 2D or 3D level by identifying the essential properties of molecular recognition.

Pharmacophore modeling

Ligand-based pharmacophore modeling:

- In the absence of the macromolecular target structure, ligand-based pharmacophore modeling is an essential strategy for drug discovery.
- In this method, the common chemical characteristics from 3D structures of multiple known ligands are extracted through ligand alignment, which would represent the essential interactions between ligand and potential macromolecular target.

Pharmacophore modeling

Structure-based pharmacophore modeling:

- The structure-based pharmacophore modeling generates chemical features of the active site and the sterical relationships from 3D structure of macromolecular target or macromolecule-ligand complex.
- It probes the possible interaction sites between the macromolecular target and the ligands.

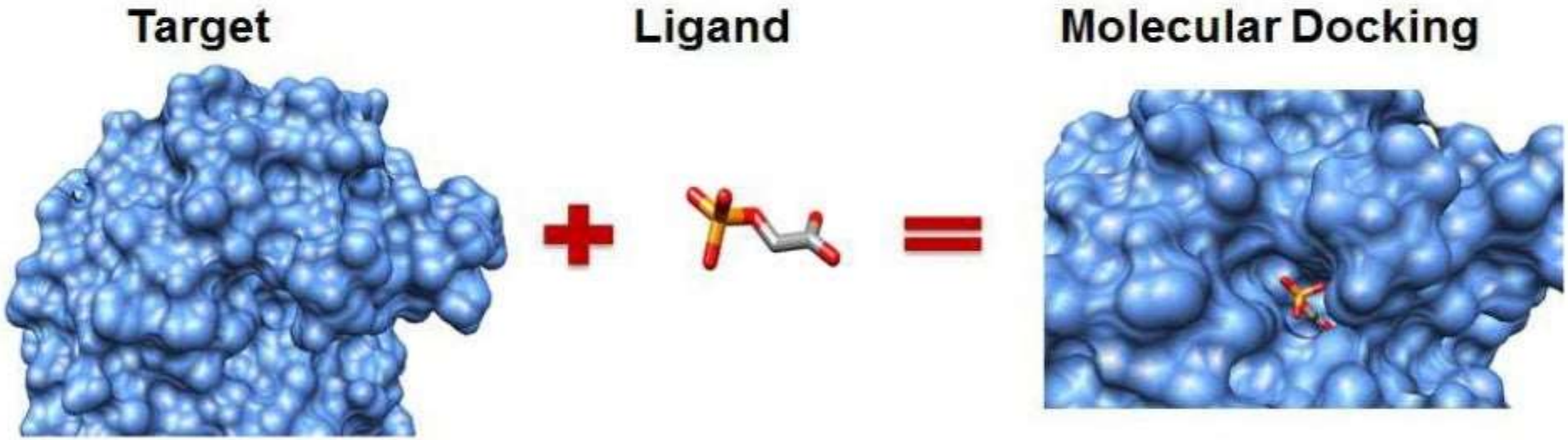
Docking Techniques

Introduction

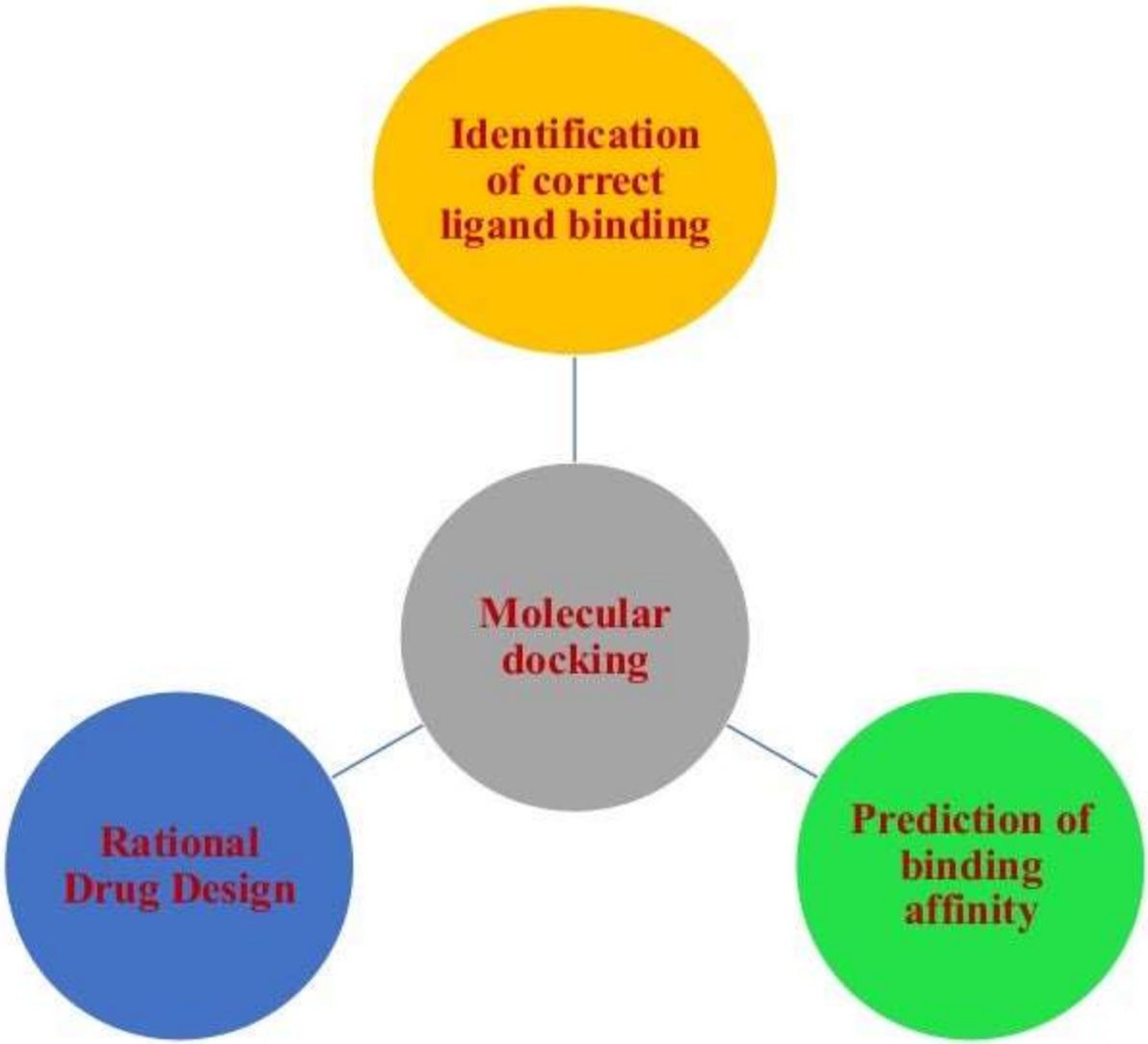
- Docking is an attempt to find the best matching between two molecules.
- A more serious definition….
  - Docking is a method which predicts the preferred orientation of one ligand when bound in an active site to form a stable complex.
- Finding the correct relative orientation of the "key" which will open up the "lock".
- The protein can be thought of as the "lock" and the ligand can be thought of as a "key".

Introduction

Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings

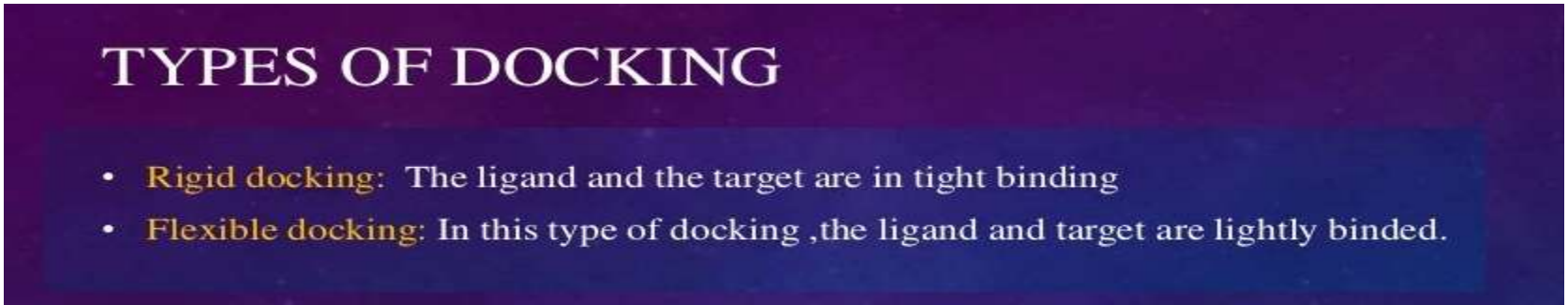


Molecular Docking



Types of Docking

- Rigid Docking (Lock and Key)
- In rigid docking, the internal geometry of both the receptor and ligand are treated as rigid.
- Flexible Docking (Induced fit)
- An enumeration on the rotations of one of the molecules (usually smaller one) is performed. Every rotation the energy is calculated; later the most optimum pose is selected.



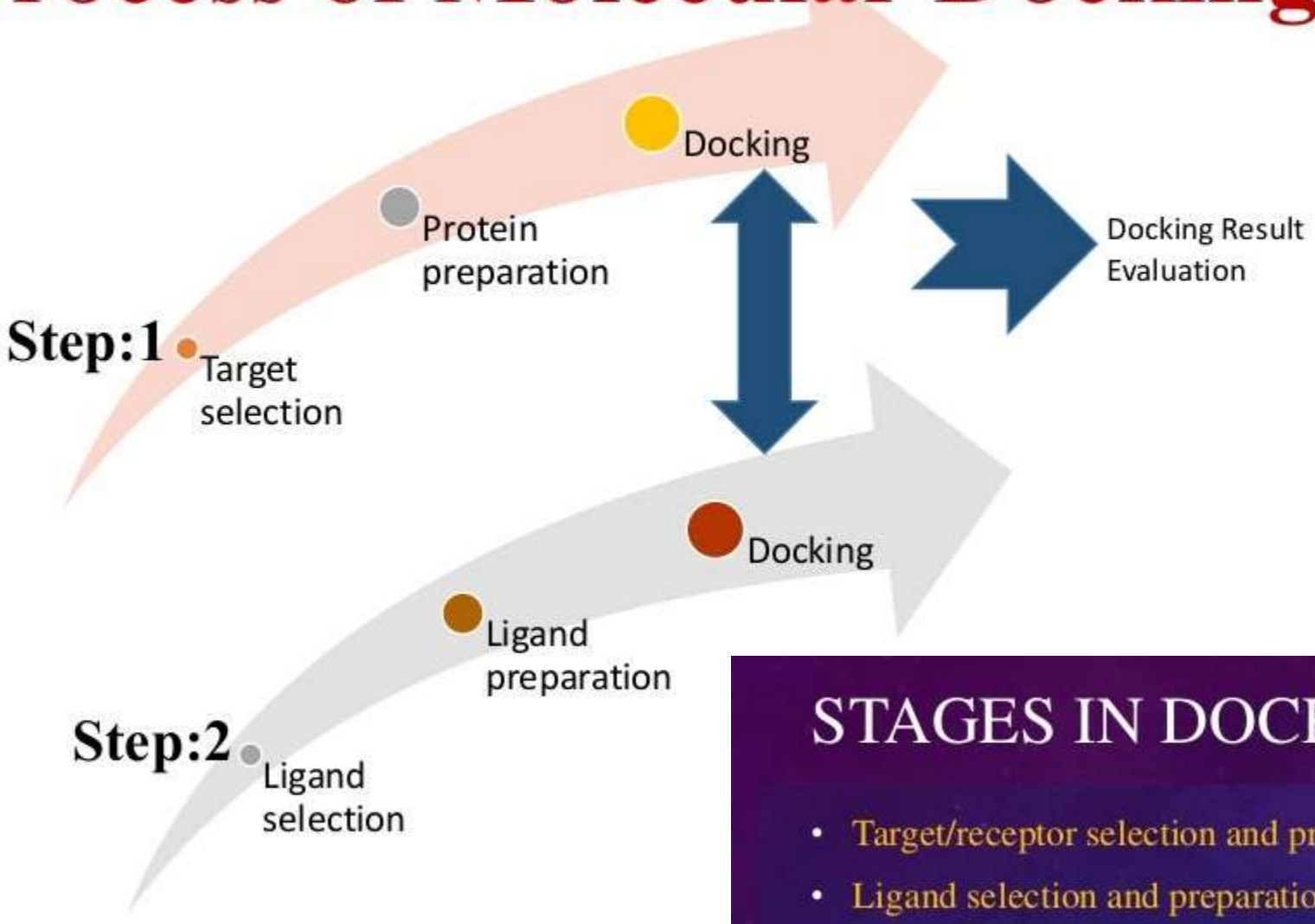
Docking can be between....

- Protein – Ligand
- Protein – Protein
- Protein – Nucleotide

Requirements

- Protein (Enzyme, peptide)
- Ligand (Drug, novel compound, testing compound, organic compound)
- Docking Software (Autodock)
- Result analysis

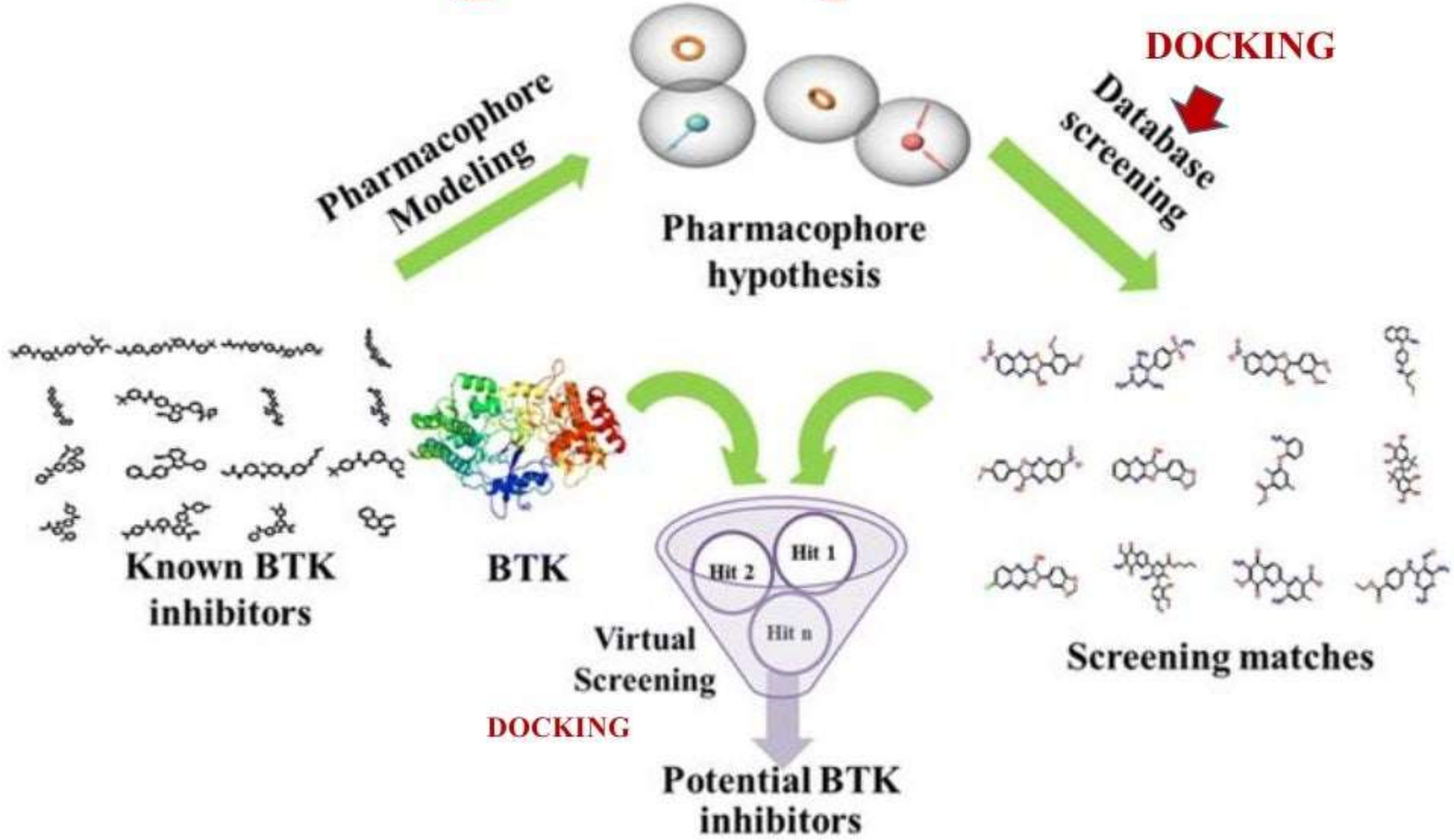
Process of Molecular Docking



STAGES IN DOCKING

- Target/receptor selection and preparation
- Ligand selection and preparation
- Docking
- Evaluation of docking results.

Screening of Compounds



Bruton's tyrosine kinase inhibitors

Docking Software

- **SANJEEVINI** – IIT Delhi
- **GOLD** – University of Cambridge ,UK
- **AUTODOCK** - Scripps Research Institute,USA
- **GemDock(Generic Evolutionary Method for Molecular Docking)** A tool, developed by Jinn-Moon Yang, a professor of the Institute of Bioinformatics, National Chiao Tung University, Taiwan
- **Hex Protein Docking** - University of Aberdeen, UK
- **GRAMM (Global Range Molecular Matching) Protein docking** - A Center for Bioinformatics, University of Kansas, USA

Limitations(Pharmacophore & Docking)

- The major limitation in virtual screening by pharmacophore is the absence of good scoring metrics.
- Whereas docking simulations are based on scoring functions trying to predict the affinity, and similarity searches utilize similarity metrics.
- Pharmacophore queries do not have a reliable, general scoring metric.
  - Most commonly, the quality of fitting the ligand into a pharmacophore query is expressed by the root mean square deviation between the features of the query and atoms of the molecule.

Application

- Pharmacophore approaches are successful subfields of computer-aided drug design (CADD) which have become one of the major tools
  - hit identification
  - lead optimization
  - rational design of novel drugs.
- Virtual screening (hit identification)
- Drug Discovery (lead optimization)
- Bioremediation

QSAR PARAMETERS

QSAR (QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS)

- QSAR is a computational modeling method for revealing relationships between structural properties of chemical compounds and biological activities
- Hansch, (1964)- Structural properties of a chemical influence its biological activity and similar compounds behave similarly.
- So QSAR is mathematical or statistical approaches to define the relationship between biological activity (experimental data) of a molecular system and its geometrical, physical, electronic, and chemical properties

Activity = function (property 1 , property 2.....)

Activity = function (xi)

xi- descriptor

Property (xi)- size, shape, no. of H-bond, electrostatic etc

INTRODUCTION

Principles of drug designing

- Improving the selectivity
- Increasing the selectivity
- Reduce side effects
- Arrangement functional groups and identification of a pharmacophore

WHAT IS QSAR ?

- A QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics.

- 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.

QSAR involves the derivation of mathematical formula which relates the biological activities of a group of compounds to their measurable physicochemical parameters. These parameters have major influence on the drug's activity.QSAR derived equation take the general form:

- Biological activity = function (parameters)
  - Activity is expressed as log(1/c). C is the minimum concentration required to cause a defined biological response

Physicochemical Parameters

Various parameters used in QSAR studies are:

- **Hydrophobicity:** partition coefficient, π-substitution constant
- **Steric Parameters:** Taft's constant, verloop steric parameter
- **Electronic Parameter:** Hammet constant, dipole moment

HYDROPHOBICITY

- Hydrophobic character of a drug is crucial to how easily it crosses the cell membrane and may also important in receptor interactions.

- Hydrophobicity of a drug is measured experimentally by testing the drugs relative distribution is known as partition coefficient

Partition coefficient:

Partition coefficient P usually expressed as logP

It is defined as $$p = \frac{(X)ocatanol}{(X)aqueous}$$

- P is a measure of the relative affinity of a molecule for the lipid and aqueous phase in the absence of ionization.
- 1-Octanol is a most frequently used lipid phase in pharmaceutical research

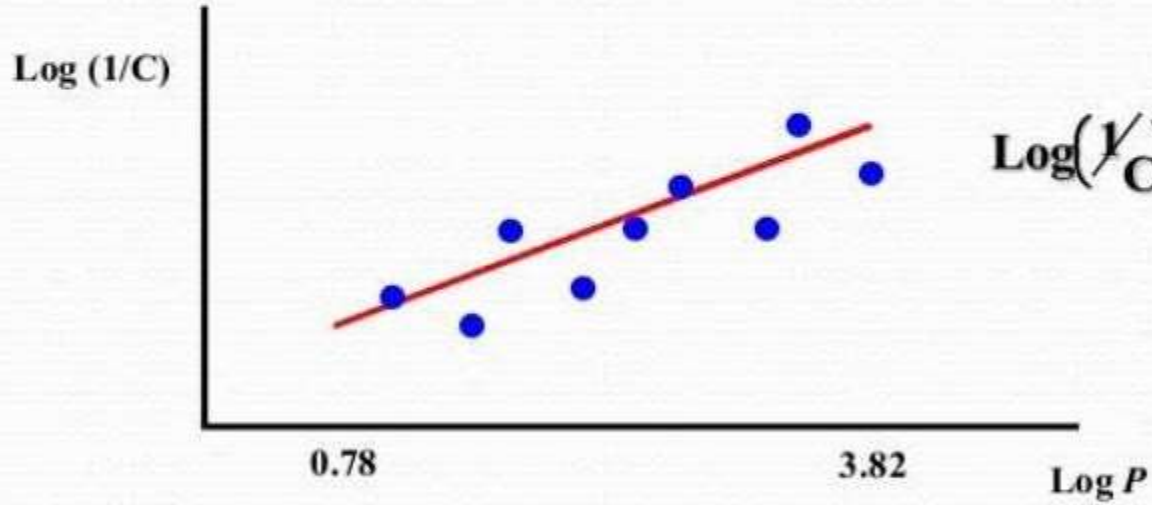
LogP for a molecule can be calculated from a sum of fragmental or atom based terms plus various corrections.

$$\text{LogP} = \Sigma \text{ fragments} + \Sigma \text{ corrections}$$

Relationship between LogP and Log1/C

- **Activity of drugs is often related to *P***

  e.g. binding of drugs to serum albumin
  (straight line - limited range of log *P*)



$$\mathrm{Log}\left(\frac{1}{C}\right) = k1 \log P + k2$$

- **Binding increases as log *P* increases**
- **Binding is greater for hydrophobic drugs**

π-substituent constant

The π-substituent constant defined by hansch and co-workers by the following equation.

Partition coefficient can be calculated by knowing the contribution that various substituent , is known as substituent hydrophobicity constant.

$$\pi x = \log Px - \log PH$$

A positive π value indicates that the π substituent has a higher hydrophobicity than hydrogen

A negative π value indicates that the π substituent has a lower hydrophobicity than hydrogen and the drug favors the aqueous phase.

π identify specific regions of the molecule which might interact with hydrophobic regions in the binding sites.

ELECTRONIC EFFECT

- The electronic effect of various substituent will clearly have an effect on drug ionization and polarity.

- Have an effect on how easily drug can pass through the cell membrane or how strongly it can interact with a binding site.

The Hammett constant (σ)

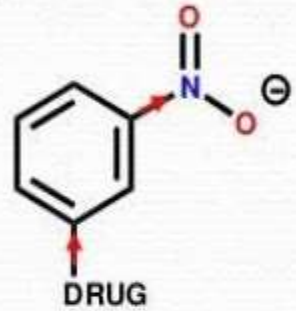
$$sx = \log (Kx/K \text{ benzoic})$$

Hammett constant takes into account both resonance and inductive effects; thus, the value depends on whether the substituent is *para or meta* substituted

- -*ortho* not measured due to steric effects

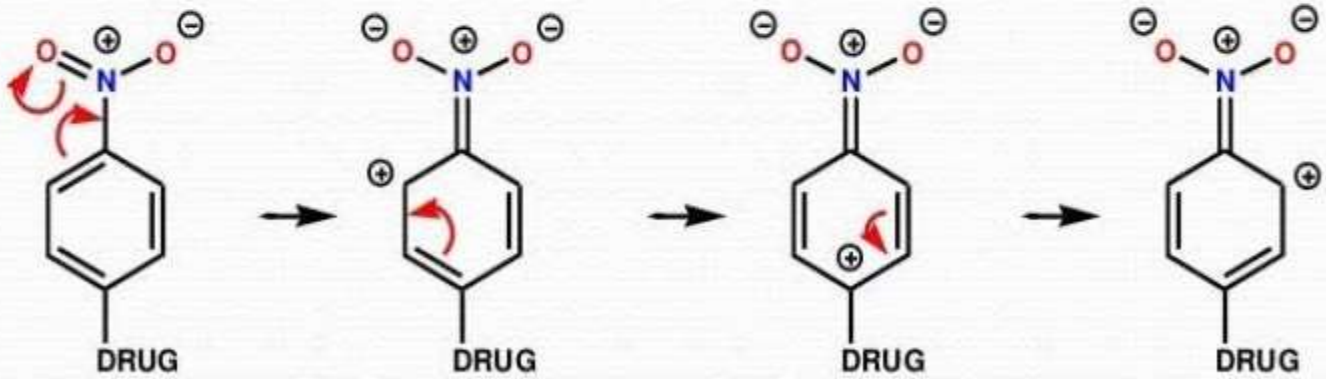
EXAMPLES: $\sigma_p\ (NO_2) = 0.78$ $\sigma_m\ (NO_2) = 0.71$

meta-Substitution



e-withdrawing (inductive effect only)

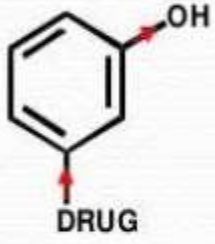
para-Substitution



e-withdrawing (inductive + resonance effects)

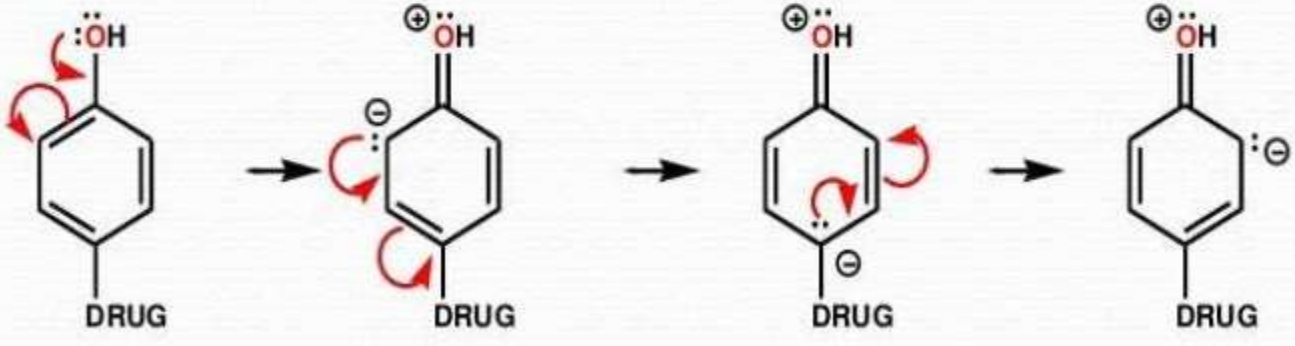
EXAMPLES: σ_m (OH) = 0.12 σ_p (OH) = –0.37

meta-Substitution



e-withdrawing (inductive effect only)

para-Substitution



e-donating by resonance more important than inductive effect

STERIC SUBSTITUTION CONSTANT

It is a measure of the bulkiness of the group it represents and it effects on the closeness of contact between the drug and receptor site

Bulky substituent may help to orient a drug property for maximum binding and increase activity.

STERIC SUBSTITUTION CONSTANT

It is a measure of the bulkiness of the group it represents and it effects on the closeness of contact between the drug and receptor site. It is much harder to quantitate.

❖ **Taft's steric factor (Es')**

•Measured by comparing the rates of hydrolysis of substituted aliphatic esters against a standard ester under acidic conditions

$$E_s = \log k_x - \log k_o$$

k_x represents the rate of hydrolysis of a substituted ester

k_o represents the rate of hydrolysis of the parent ester

- **Molar refractivity** (MR)--measure of the volume occupied by an atom or group--equation includes the MW, density(d), and the index of refraction(n)–

$$MR=(n^2-1)MW/(n^2+2)d$$

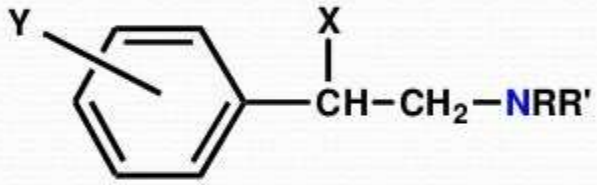
- **Verloop steric parameter**--computer program uses bond angles, van der Waals radii, bond lengths

Hansch Equation

- A QSAR equation relating various physicochemical properties to the biological activity of a series of compounds
- Usually includes log *P*, electronic and steric factors
- Start with simple equations and elaborate as more structures are synthesised
- Typical equation for a wide range of log *P* is parabolic

$$\mathrm{Log}\left(1/C\right) = -k_1(\log P)^2 + k_2 \log P + k_3 \sigma + k_4 E_s + k_5$$

Example: **Adrenergic blocking activity of β-halo-β-arylamines**



$$\text{Log}(1/C) = 1.22\,\pi - 1.59\,\sigma + 7.89$$

Conclusions:

- **Activity increases if π is +ve (i.e. hydrophobic substituents)**
- **Activity increases if σ is negative (i.e. e-donating substituents)**

Free-Wilson Approach

Method

- The biological activity of the parent structure is measured and compared with the activity of analogues bearing different substituents
- An equation is derived relating biological activity to the presence or absence of particular substituents

$$\text{Activity} = k_1X_1 + k_2X_2 + \ldots k_nX_n + Z$$

- X_n is an indicator variable which is given the value 0 or 1 depending on whether the substituent (n) is present or not
- The contribution of each substituent (n) to activity is determined by the value of k_n
- Z is a constant representing the overall activity of the structures studied

Advantages

- No need for physicochemical constants or tables
- Useful for structures with unusual substituents
- Useful for quantifying the biological effects of molecular features that cannot be quantified or tabulated by the Hansch method

Disadvantages

- A large number of analogues need to be synthesised to represent each different substituent and each different position of a substituent
- It is difficult to rationalise why specific substituents are good or bad for activity

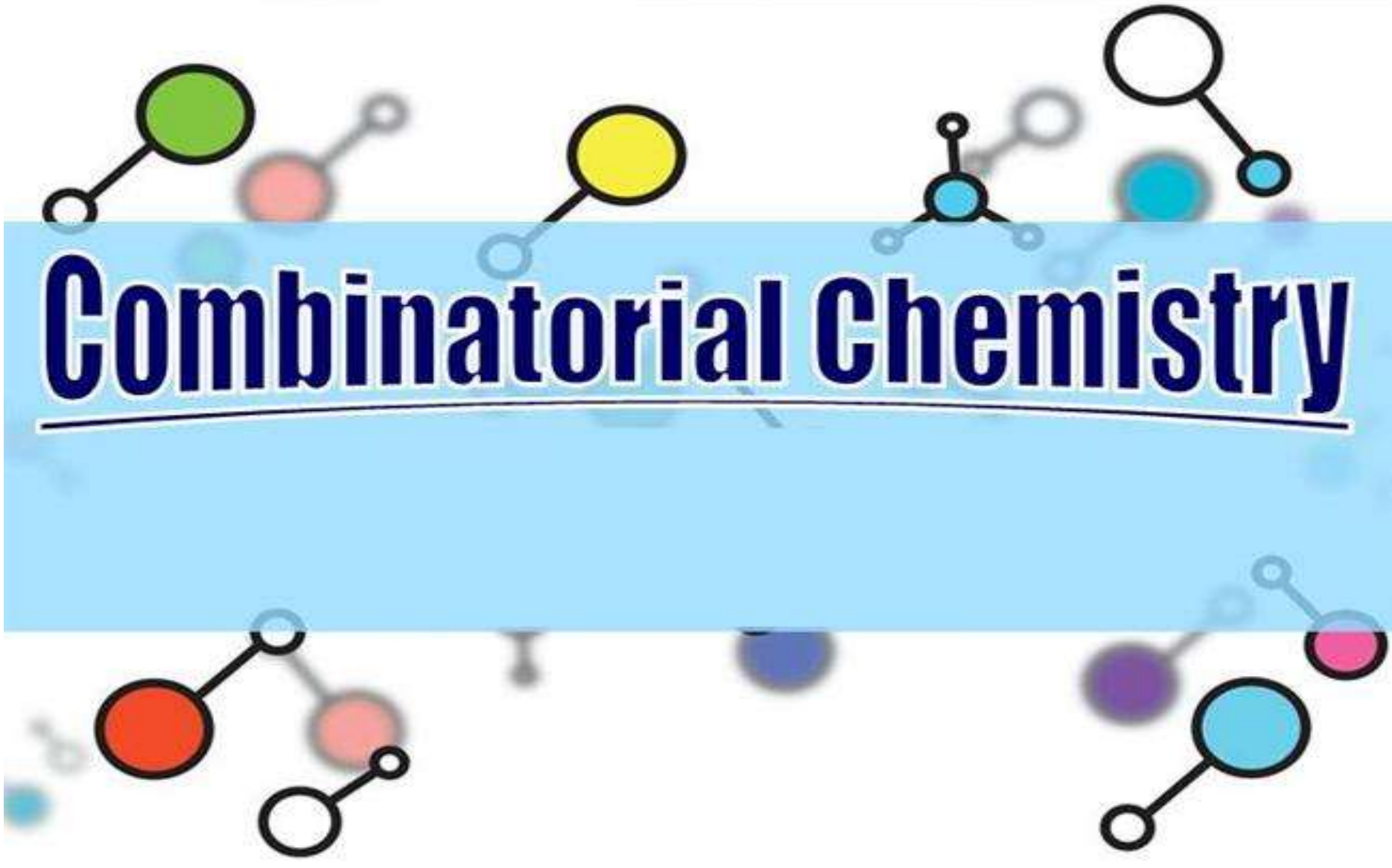
Taft's steric factor (Es)

It is measure by the comparing the rate of hydrolysis of substituted aliphatic esters against a standard ester under acidic condition

$$E_s = \log k_x - \log k_o$$

k_x represents the rate of hydrolysis of a substituted ester

k_o represents the rate of hydrolysis of the parent ester


Combinatorial Chemistry

Introduction:

•Combinatorial Chemistry is a new method developed by academics and researchers to reduce the time and cost of producing effective, marketable and competitive new drugs.

• Scientists use Combinatorial Chemistry to create large numbers of molecules that can be detected efficiently.

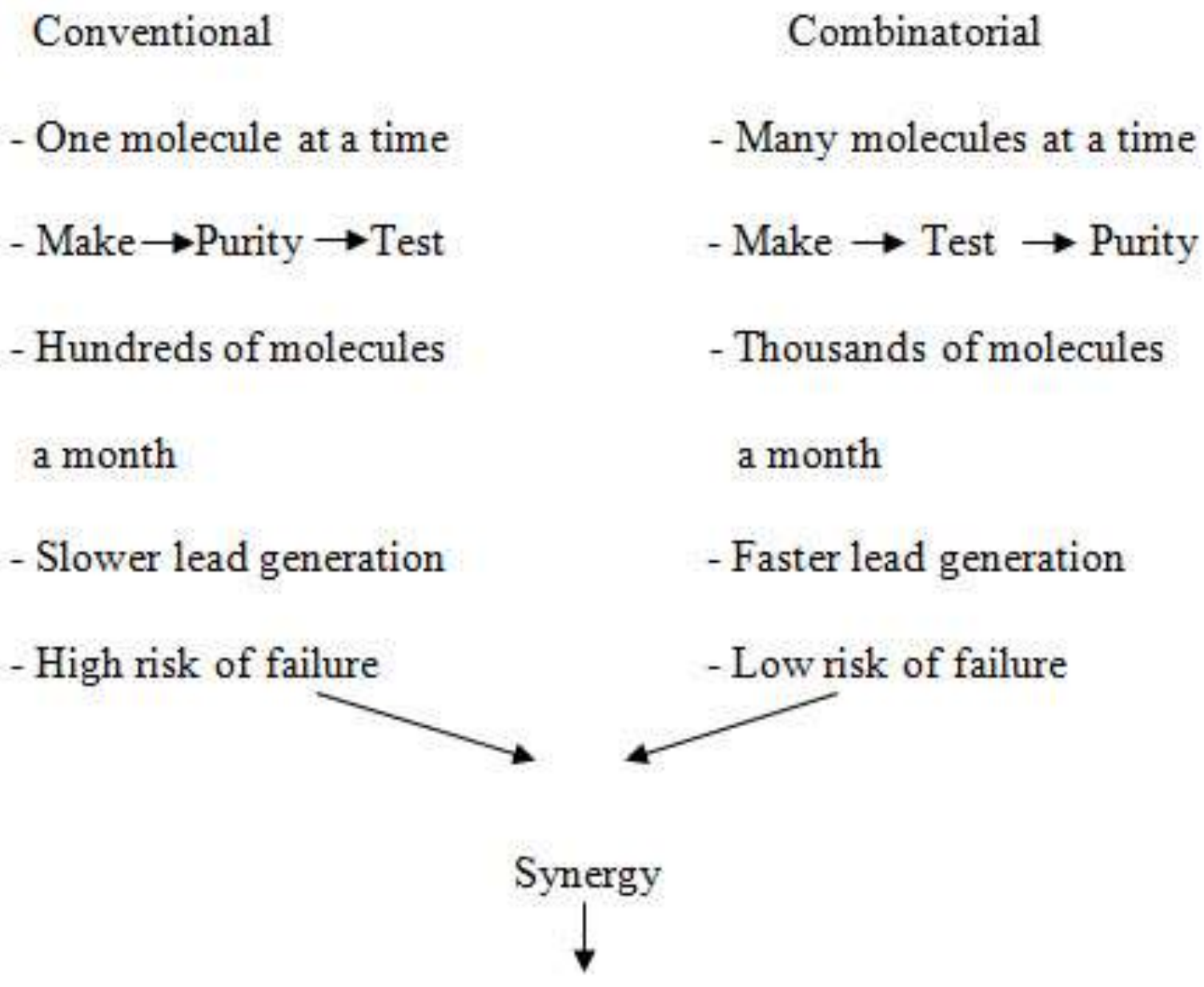
•This technique has captured the attention of many areas such as Pharmaceutical chemistry, Biotechnology and Agro chemistry.

Definition:

•Combinatorial chemistry is a technique by which large numbers of different but structurally similar molecules are produced rapidly and submitted for pharmacological assay.

•This technique uses the same reaction conditions with the same reaction vessels to produce a large range of analogues.

• Technique invented in the late 1980s and early 1990s to enable tasks to be applied to many molecules simultaneously


Conventional
- One molecule at a time
- Make → Purity → Test
- Hundreds of molecules a month
- Slower lead generation
- High risk of failure
Combinatorial
- Many molecules at a time
- Make → Test → Purity
- Thousands of molecules a month
- Faster lead generation
- Low risk of failure
Synergy
LEAD IDENTIFICATION

Application:

- Applications of combinatorial chemistry are very wide Scientists use combinatorial chemistry to create large populations of molecules that can be screened efficiently.
- By producing larger, more diverse compound libraries, companies increase the probability that they will find novel compounds of significant therapeutic and commercial value.
- Provides a stimulus for robot-controlled and immobilization strategies that allow high-thrughput and multiple parallel approaches to drug discovery

Advantages:

Fast

Combinatorial approach can give rise to million of compound in same time as it will take to produce one compound by traditional method of synthesis .

Economical

A negative result of mixture saves the effort of synthesis, purification & identification of each compound

Easy

Isolation purification & identification of active molecule from combinatorial library is relatively easy.

Drug Discovery

Mixed Combinatorial synthesis produces chemical pool. Probability of finding a molecule in a random screening process is *proportional* to the number of molecules subjected to the screening process

Drug Optimization

Parallel synthesis produces analogues with slight differences which is required for lead optimization

Disadvantages:

Efficiency is highly affected by compound's size, solubility and function group.

Compounds produced tend to be Achiral of Racemic

Combinatorial Chemistry within drug design (1)

1. SOLID PHASE TECHNIQUES

- Reactants are bound to a polymeric surface and modified whilst still attached. Final product is released at the end of the synthesis

Advantages

- Specific reactants can be bound to specific beads
- Beads can be mixed and reacted in the same reaction vessel
- Products formed are distinctive for each bead and physically distinct
- Excess reagents can be used to drive reactions to completion
- Excess reagents and by products are easily removed
- Reaction intermediates are attached to bead and do not need to be isolated and purified
- Individual beads can be separated to isolate individual products
- Polymeric support can be regenerated and re-used after cleaving the product
- Automation is possible

1. SOLID PHASE TECHNIQUES

Requirements

- A resin bead or a functionalised surface to act as a solid support
- An anchor or linker
- A bond linking the substrate to the linker. The bond must be stable to the reaction conditions used in the synthesis
- A means of cleaving the product from the linker at the end
- Protecting groups for functional groups not involved in the synthesis

1. SOLID PHASE TECHNIQUES

Examples of Solid Supports

- Partially cross-linked polystyrene beads hydrophobic in nature

  causes problems in peptide synthesis due to peptide folding

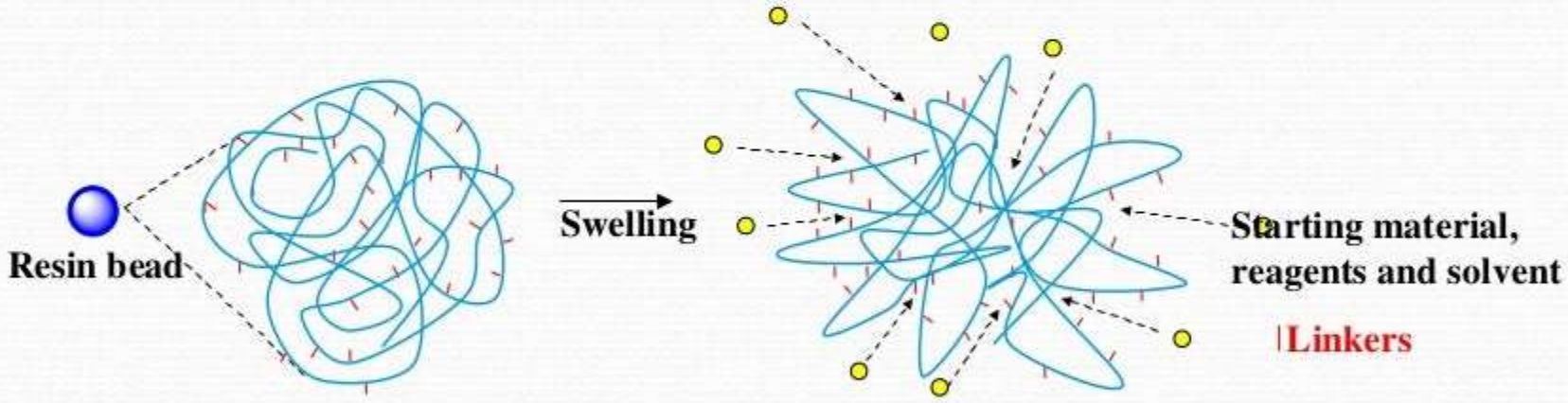
- Sheppard's polyamide resin - more polar

- Tentagel resin - similar environment to ether or THF

- Beads, pins and functionalised glass surfaces

1. SOLID PHASE TECHNIQUES

- Beads must be able to swell in the solvent used, and remain stable
- Most reactions occur in the bead interior



1. SOLID PHASE TECHNIQUES

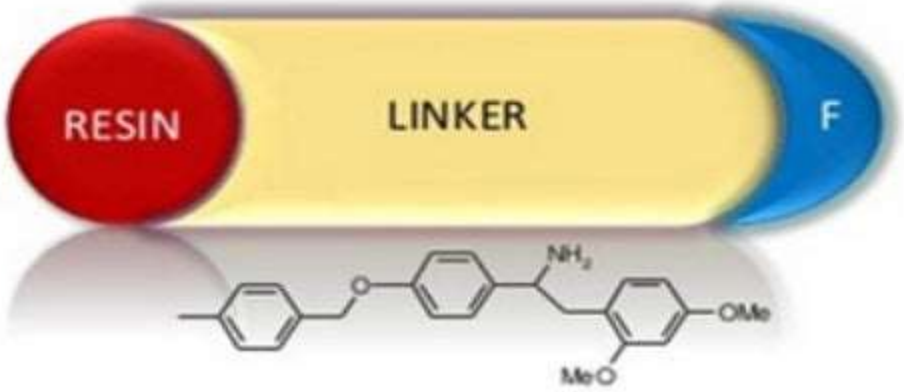
Anchor or linker

- A molecular moiety which is covalently attached to the solid support, and which contains a reactive functional group
- Allows attachment of the first reactant
- The link must be stable to the reaction conditions in the synthesis but easily cleaved to release the final compound
- Different linkers are available depending on the functional group to be attached and the desired functional group on the product
- Resins are named to define the linker e.g.

Merrifield, Wang, Rink

WANG RESIN: linker suitable for attachment & release of carboxylic acids.

MERRIFIELD RESIN : linker suitable for peptide products.



RINK RESIN : Linker suitable for attachment & release of carboxamide.

Solid phase synthesis: protecting groups

- A few protecting groups used in solid phase synthesis.

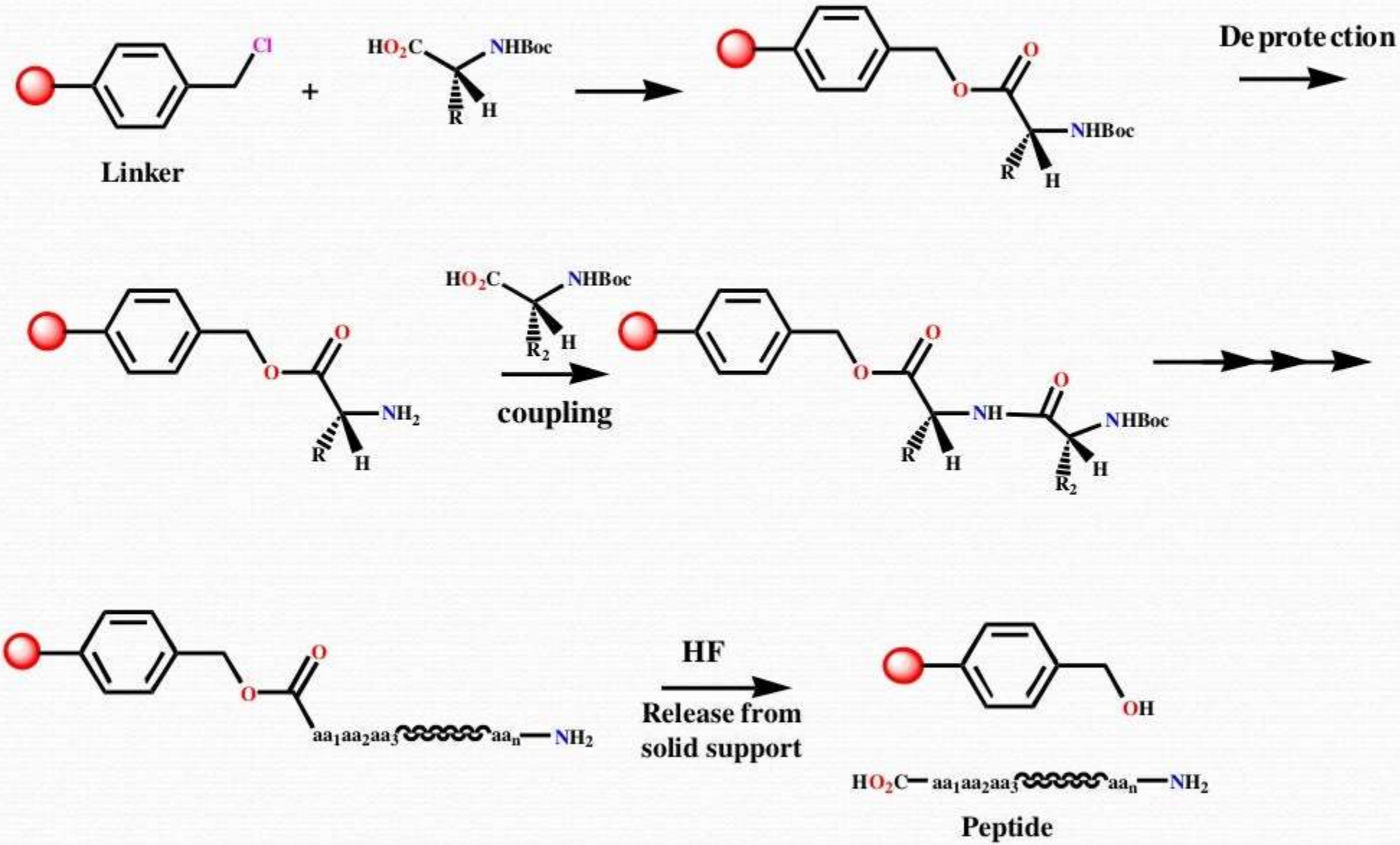
- **For amines.**
- Boc (t-butoxycarbonyl)
- Fmoc (9-fluorenylmetoxy carbonyl)
- Tmsec (2 [trimethylsilyl] ethoxycarbonyl)

- **For carboxylic acids.**
- Tert Bu ester(t-butyl ester)
- Fm ester(9-fluronyl methyl ester)
- Tmse ester(2 [trimethylsilyl] ethyl)

17

ield resin for peptide synthesis (chloromethyl group)

= resin bead



equipment for Solid Phase Peptide Synthesis

4.Solution phase synthesis

- Unlike one bead - one compound synthesis ,solution phase synthesis often lead to mixture of products in one pool.

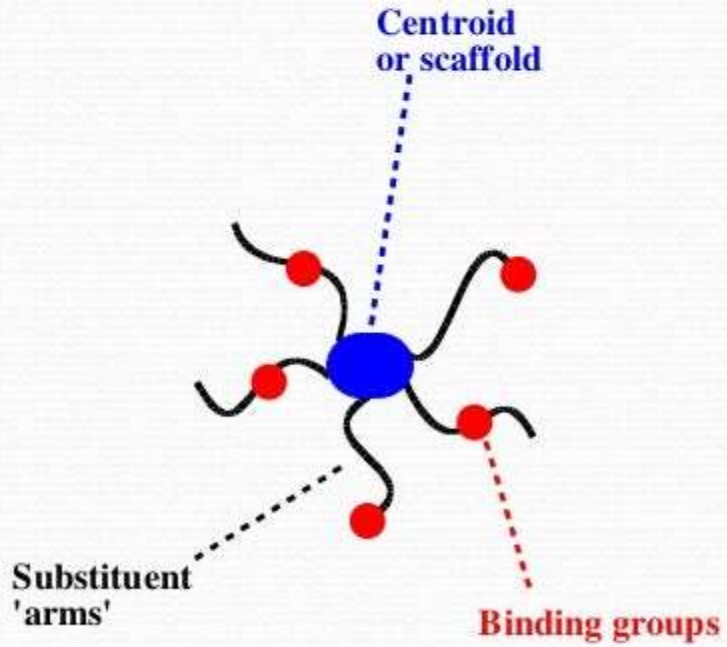
- Most of the org reaction occurs in solution phase . For this reason there has been much interest in solution phase synthesis.

- The main problem here is the difficulty of removing unwanted impurities at each step in synthesis.

7. Planning a Combinatorial Synthesis

7.1 Aims

- To generate a large number of compounds
- To generate a diverse range of compounds
- Increase chances of finding a lead compound to fit a binding site
- Synthesis based on producing a molecular core or scaffold with functionality attached



7. Planning a Combinatorial Syntheses

7.1 Aims

Target molecules should obey Lipinski's 'Rule of Five' for oral activity

- a molecular weight less than 500
- a calculated log *P* value less than +5
- no more than 5 H-bond donating groups
- no more than 10 H-bond accepting groups