DE-NOVO DRUG DESIGN

DE NOVO APPROACHES

- De novo design is the approach to build a customized Ligand for a given receptor.
- This approach involves the ligand optimization.
- Ligand optimization can be done by analyzing protein active site properties that could be probable area of contact by the ligand.
- The analyzed active site properties are described to negative image of protein such as hydrogen bond, hydrogen bond acceptor and hydrophobic contact region.

DE NOVO DRUG DESIGN

- De novo means **start afresh, from the beginning, from the scratch.**
- It is a process in which the 3D structure of receptor is used to design newer molecules.
- It involves structural determination of the lead target complexes and lead modifications using molecular modeling tools.
- Information available about target receptor but no existing leads that can interact.

Procedure

- Crystallise target protein with bound ligand
- (e.g. enzyme + inhibitor or ligand)
- Acquire structure by X-ray crystallography
- Identify binding site (region where ligand is bound)
- Remove ligand
- Identify potential binding regions in the binding site
- Design a lead compound to interact with the binding site
- Synthesise the lead compound and test it for activity
- Crystallise the lead compound with target protein and identify the actual binding interactions
- Structure based drug design

Disadvantages

- The position of atoms in the crystal structure is accurate only to 0.2–0.4 A and allowance should be made for that.
- It is possible that the designed molecule may not bind to the binding site exactly as predicted.
- It is worth leaving scope for variation and elaboration of the molecule. This allows fine tuning of the molecule's binding affinity and pharmacokinetics.

Types of De Novo Drug Design And Differences

Manual Design

slow

A single novel structure

Automated Design

much faster

large numbers of diverse structures

Impotant Points In De Novo Drug Design

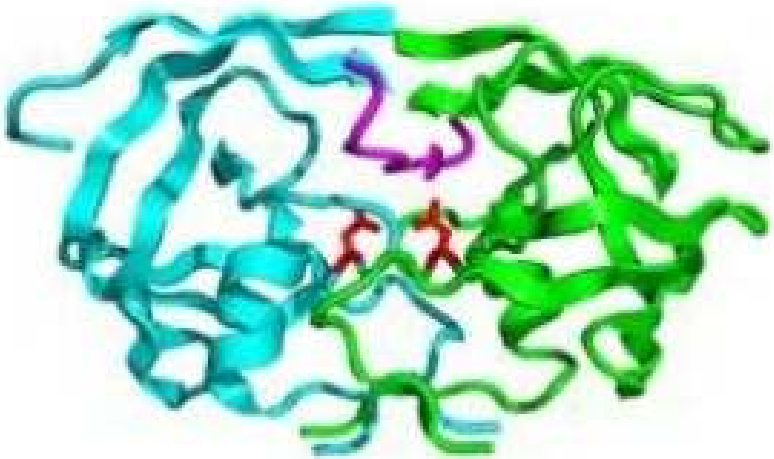
- Flexible molecules are better than rigid molecules.
- It is pointless designing molecules which are difficult or impossible to synthesize.
- Similarly, it is pointless designing molecules which need to adopt an unstable conformation in order to bind.
- Consideration of the energy losses involved in water desolvation should be taken into account.
- There may be subtle differences in structure between receptors and enzymes from different species. This is significant if the structure of the binding site used for *de novo* design is based on a protein that is not human in origin.

Problems of Automated De Novo

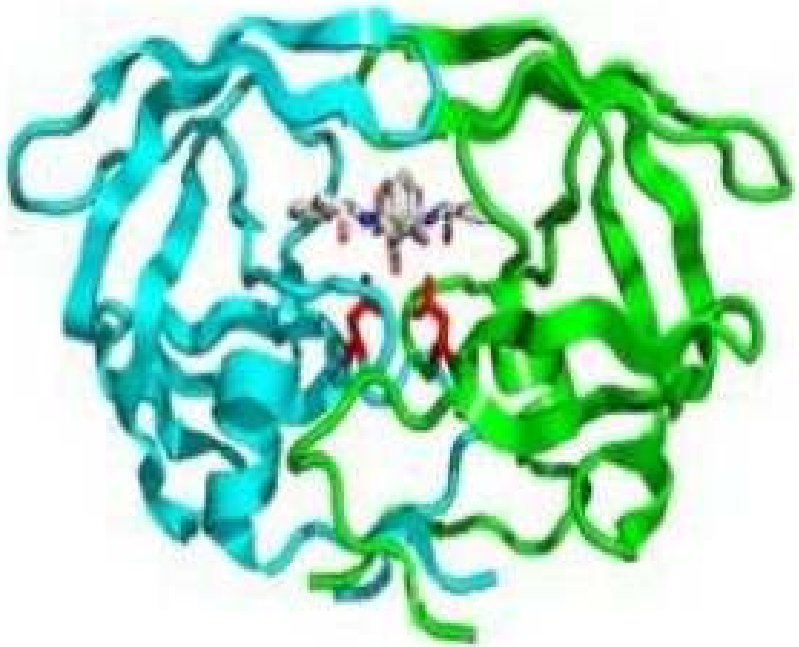
- automated *de novo* drug design is prone to generating structures which are either difficult or impossible to synthesize.
- automated *de novo* programs revolves around the scoring functions used to estimate binding affinities.

Applications

- Design of HIV 1 protease inhibitors
- Design of bradykinin receptor antagonist
- Catechol ortho methyl transferase inhibitors
- Estrogen receptor antagonist



Structure of enzyme



Enzyme with inhibitor

OTHER METHODS FOR DE NOVO DRUG DESIGN

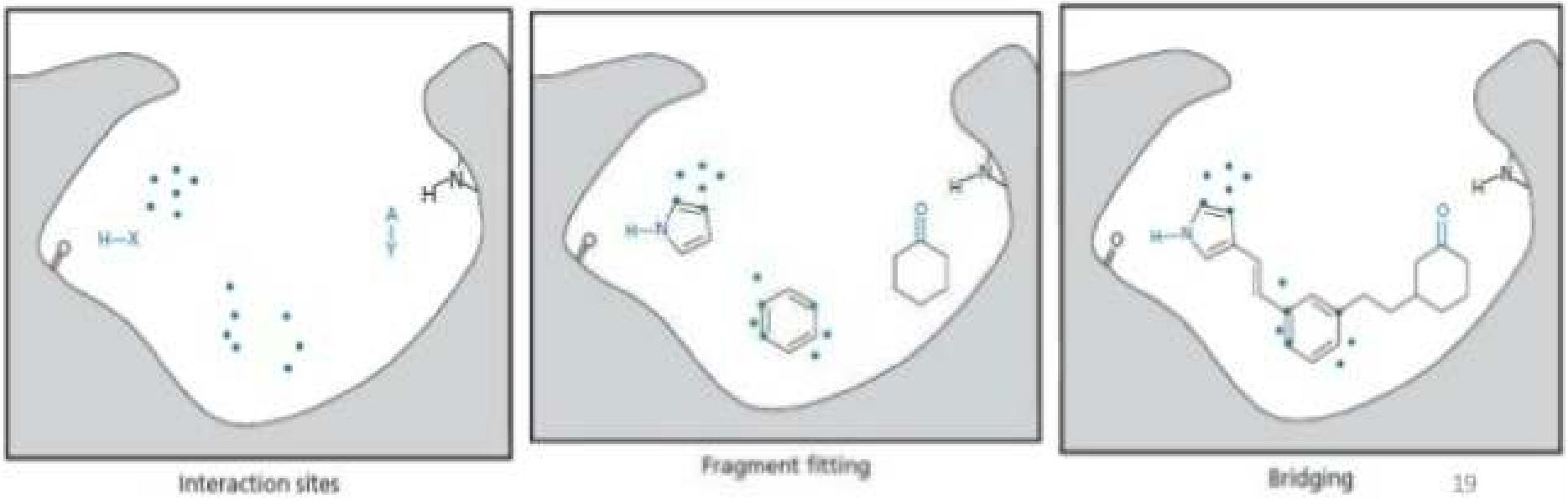
METHOD	PROGRAMS AVAILABLE
Site point connection method	LUDI
Fragment connection method	SPLICE, NEW LEAD, PRO-LIGAND
Sequential build up methods	LEGEND, GROW, SPORUT
Random connection and disconnection methods	CONCEPTS, CONCERTS, MCDNLG

LUDI

Stage 1: identification of interaction sites

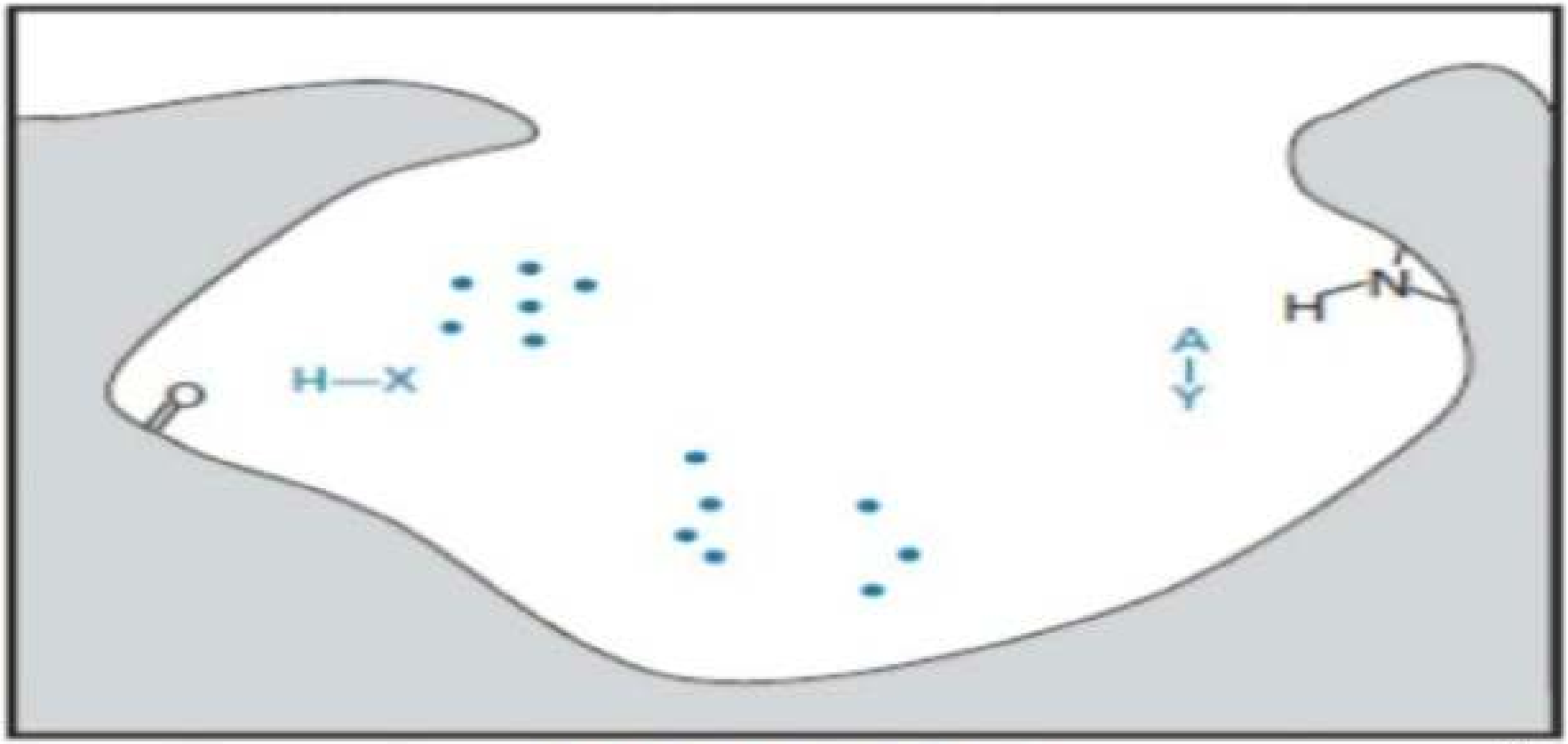
Stage 2: fitting molecular fragments

Stage 3: fragment bridging



Stage 1: Identification of interaction sites

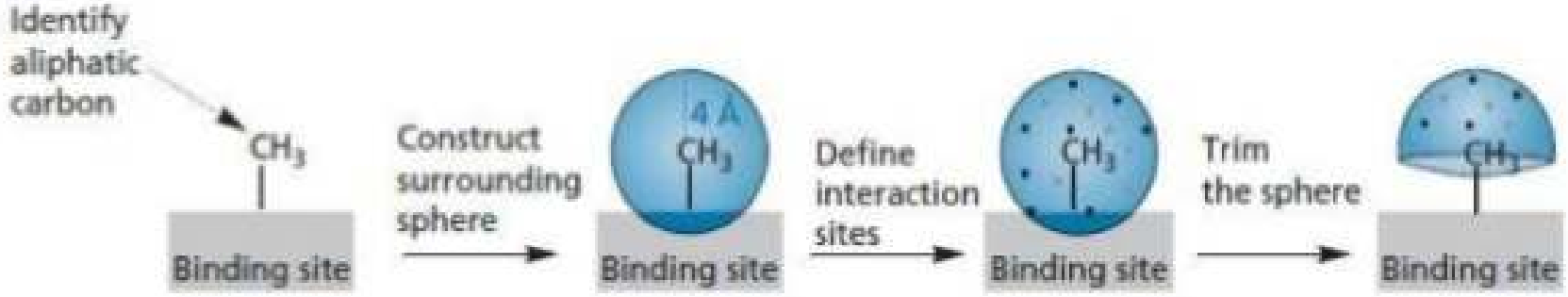
The atoms present in the binding site are analysed to identify those that can take part in hydrogen bonding interactions, and those that can take part in van der Waals interactions.



Interaction sites

Examples

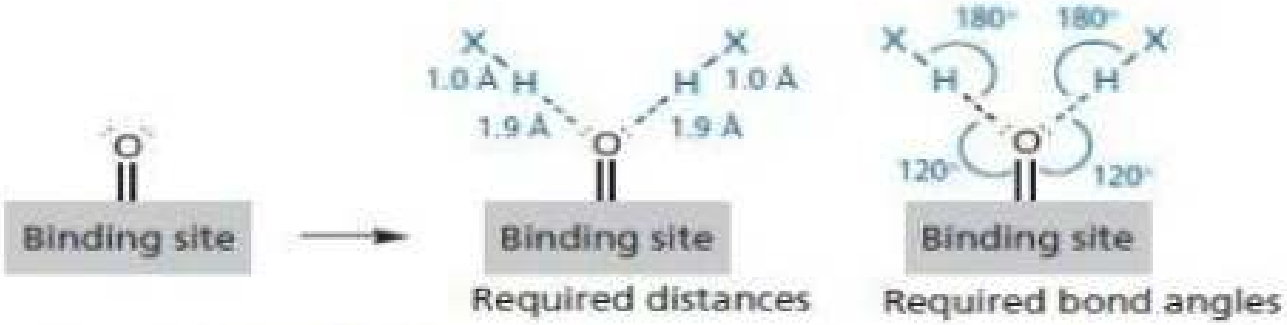
- The program would identify the carbon of that group as an aliphatic carbon capable of taking part in van der Waals interactions.
- This is a non-directional interaction, so a sphere is constructed around the carbon atom with a radius corresponding to the ideal distance for such an interaction(4 A).
- A number of points are placed over the surface of the sphere to define aliphatic interaction sites.
- Regions of the sphere which overlap or come too close to atoms making up the binding site are rejected.
- The remaining points are used as the aliphatic interaction sites.



Identifi cation of aliphatic interaction sites around a methyl group (LUDI).

Identifying interaction sites for hydrogen bonds is carried out in a different fashion.

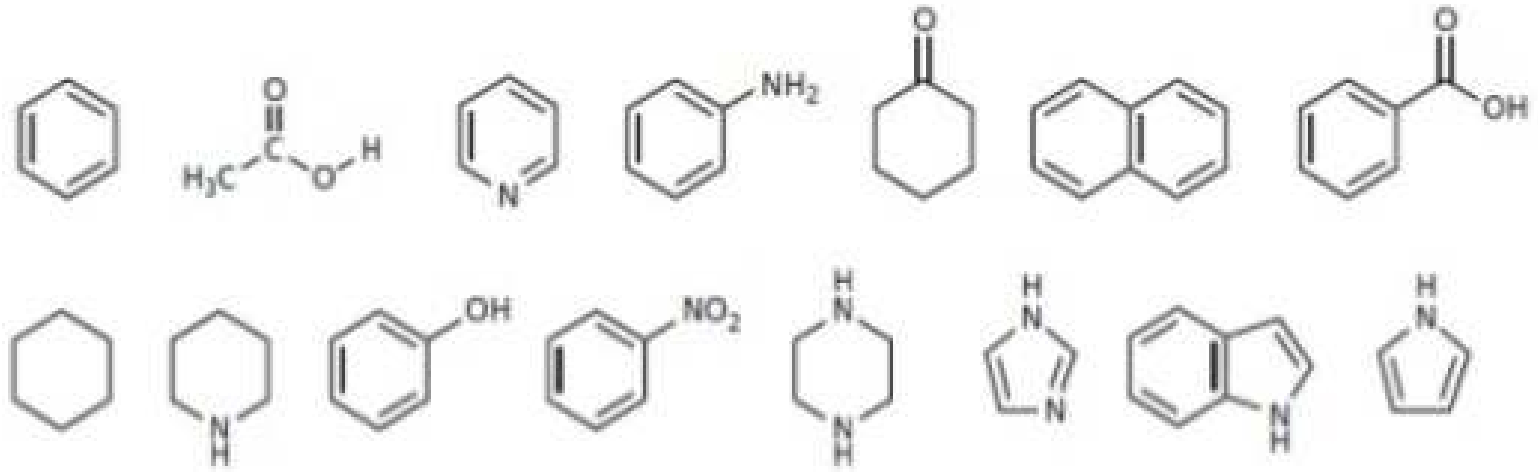
- As hydrogen bonds are directional, it is important to define not only the distance between the ligand and the binding region, but also the relevant orientation of the atoms.
- This can be done by defining the hydrogen bond interaction site as a vector involving two atoms.
- The position of these atoms is determined by the ideal bond lengths and bond angles for a hydrogen bond.



The interaction sites for a hydrogen bond donor, represented by H–X (LUDI).

Stage 2: fitting molecular fragments

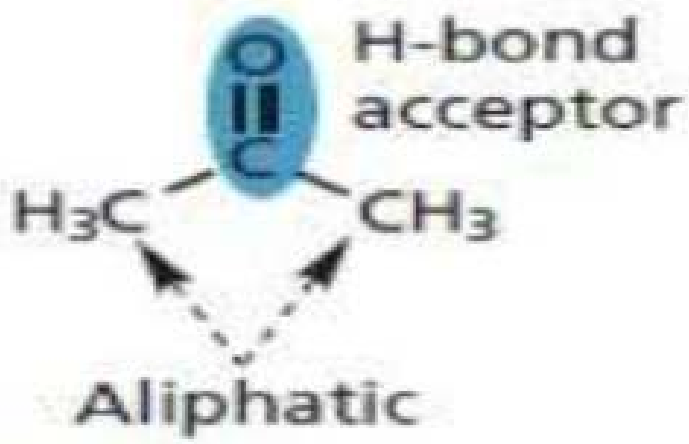
- The LUDI program accesses a library of several hundred molecular fragments.
- The molecules chosen are typically 5–30 atoms in size and are usually rigid in structure because the fitting procedure assumes rigid fragments.
- Some fragments are included which *can* adopt different conformations.



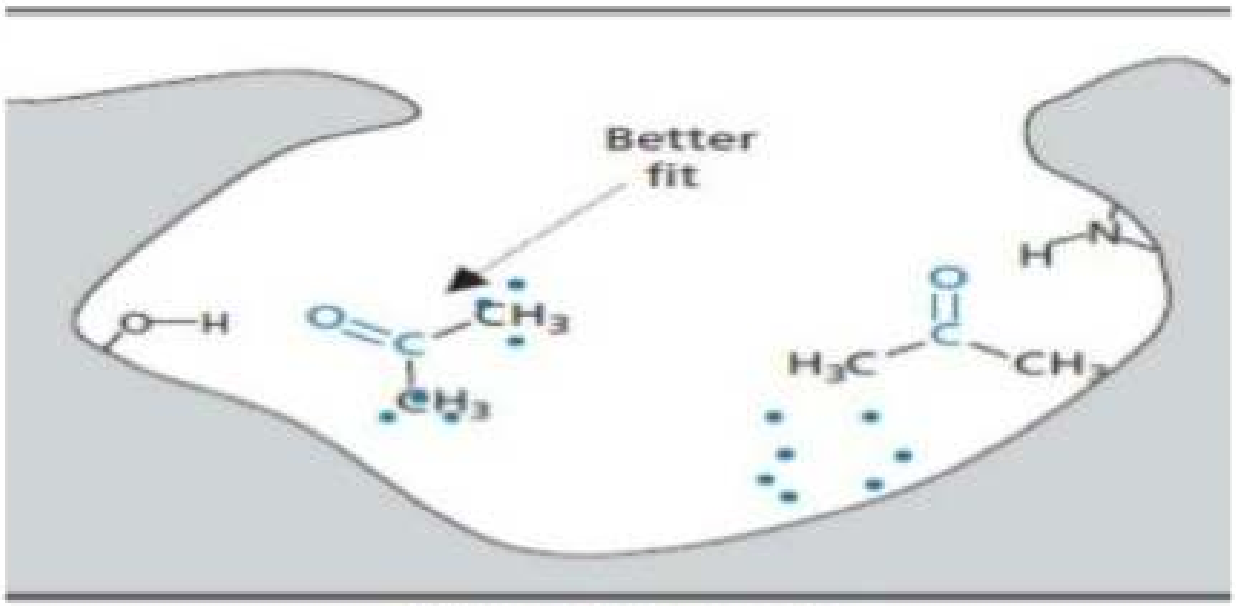
Examples of molecular fragments used by LUDI.

Examples

- The methyl carbons of an acetone fragment are defined as aliphatic and can only be fitted onto aliphatic interaction sites.
- The carbonyl group is defined as a hydrogen bond acceptor and can only be fitted onto the corresponding interaction site.



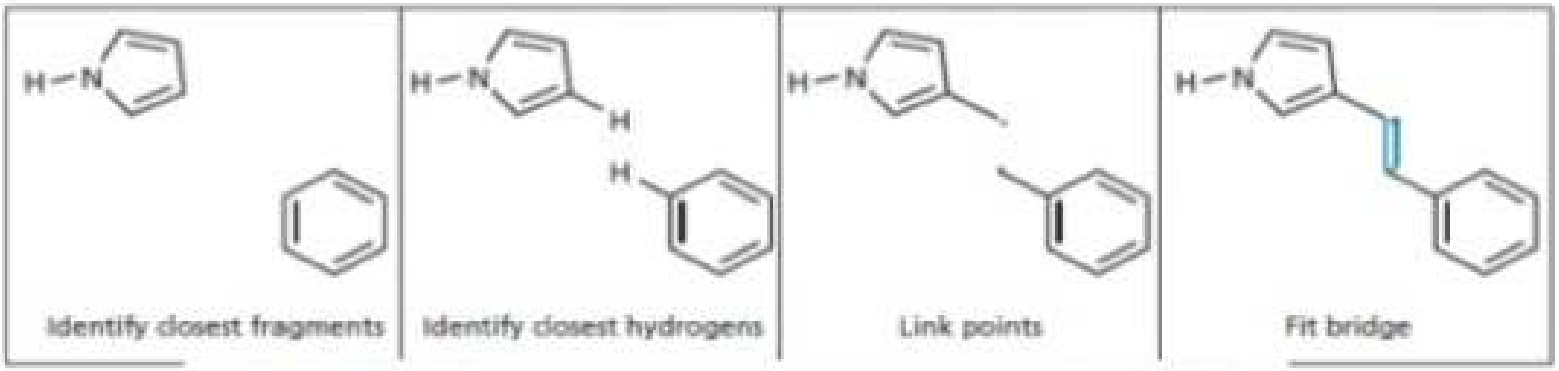
- Th e best fit will be the one that matches up the fragment with the maximum number of interaction sites.
- The program can 'try out' the various fragments in its library and identify those that can be matched up or fitted to the available interactionsites in the binding site.



Fitting fragments

Stage 3: fragment bridging

- Fragments have been identified and fitted to the binding site, the final stage is to link them up.
- The program first identifies the molecular fragments that closest to each other in the binding site, then identifies the closest hydrogen atoms.
- These now define the link sites for the bridge. The program now tries out various molecular bridges from a stored library to find out which one fits best.
- A suitable bridge has been found, a final molecule is created.



The bridging process (LUDI).

SPROUT

- Like LUDI , the program fits fragments to interaction sites, but there are differences in the way that the process is carried out.
- Uses **templates** to represent molecular fragments.
- Each template is defined by vertices and edges, rather than by atoms and bonds.
- A vertex represents a generalized sp-, sp2, sp3 hybridized atom.
- An edge represents a single, double, or triple bond, depending on the hybridization of the vertices at either end.

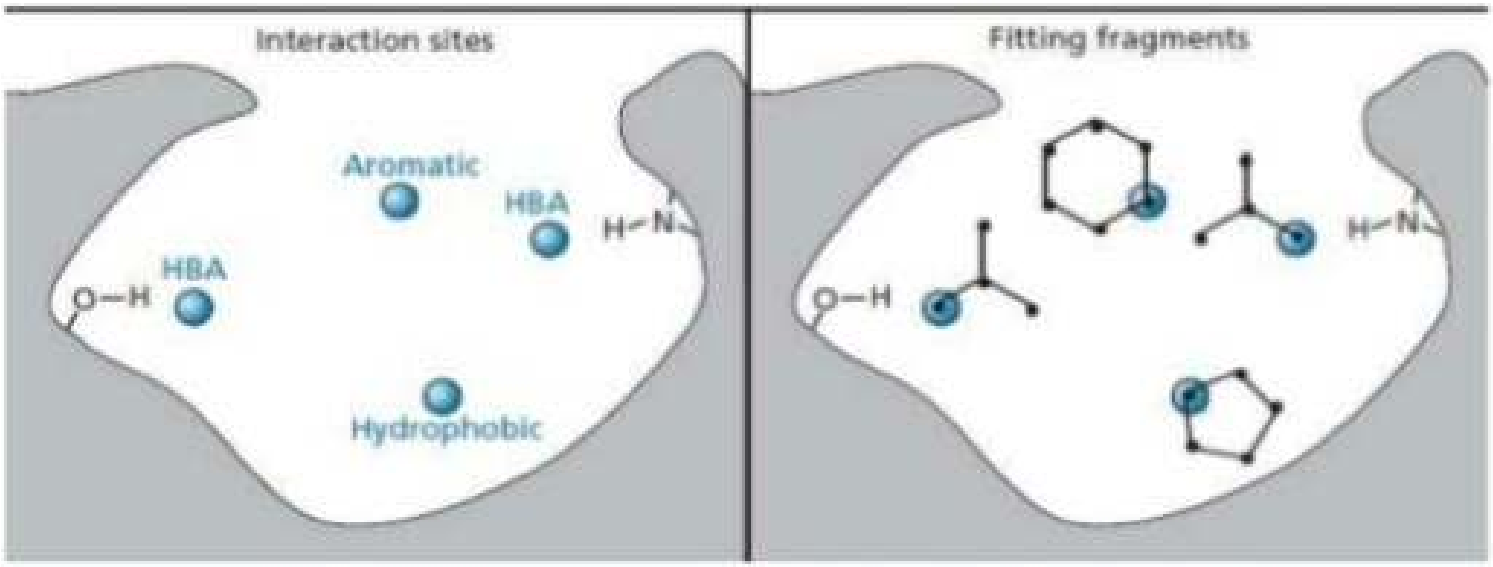
Fragment template

Examples of structures represented by a template used in SPROUT.

Stages of the generation of the structures

1- Generate fragment templates that will fit the binding site.

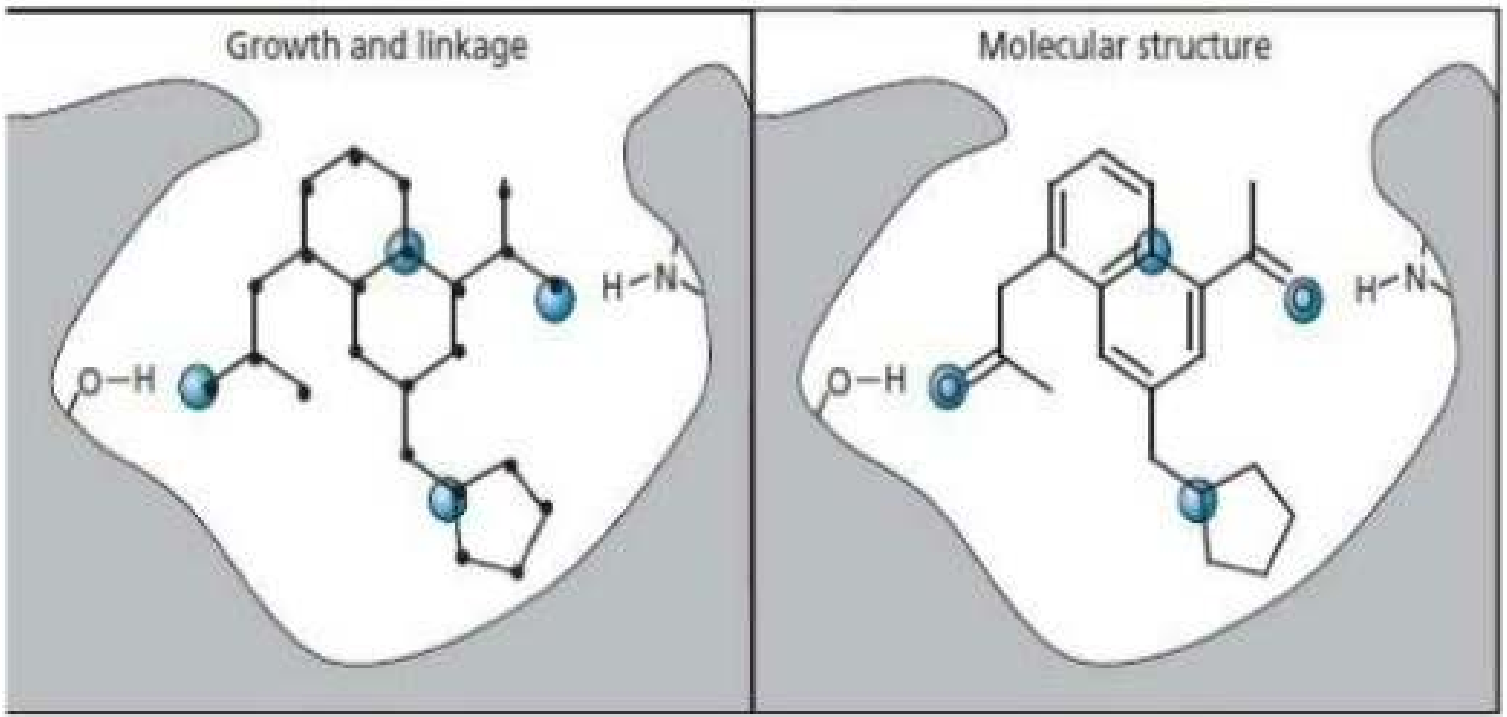
The program selects a fragment template randomly and positions it into the binding site by placing one of the vertices at the center of a sphere.
Fragment templates are placed at all the spheres and grown towards each other until they are finally linked.



Generating structures using SPROUT.

2- Create specific molecules from the molecular templates that have been produced.

This involves replacing the vertices with suitable atoms to allow favourable hydrogen bonding and vander waals interactions whith the binding site.



Generating structures using SPROUT.

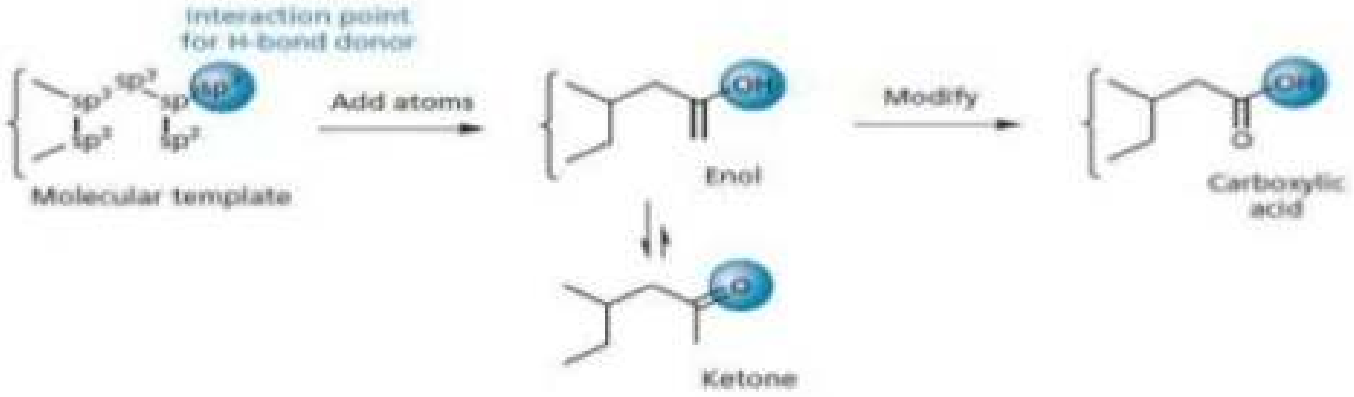
- It radically cuts down the number of different fragments that have to be stored in the program, making the search for novel structures more efficient.

- The growth of fragment templates allows a molecular template to be constructed which bridges interaction sites that are some distance apart.

In the **LUDI** methode, single fragments are placed at each interaction point and are then linked. If there is a large seperation between the interaction sites, there might not be a sufficiently long linker to connect the fragments.

- Sprout has the capacity to identify certain structural features that might be unrealistic and then modify them.

For example, an OH might be generated during the second stage in order to introduce a hydrogen bond donor, but if the OH is linked to a double bond this results in an enol which would tautomerize to a ketone. The latter would not be able to act as a hydrogen bond donor. The programme can identify an enol and modify it to a carboxylic acid which can still act as a hydrogen bond donor.



Modification of an enol to a carboxylic acid by SPROUT.

Advantage

- The programe also has the ability to modify structures such that they are more readily synthesized.

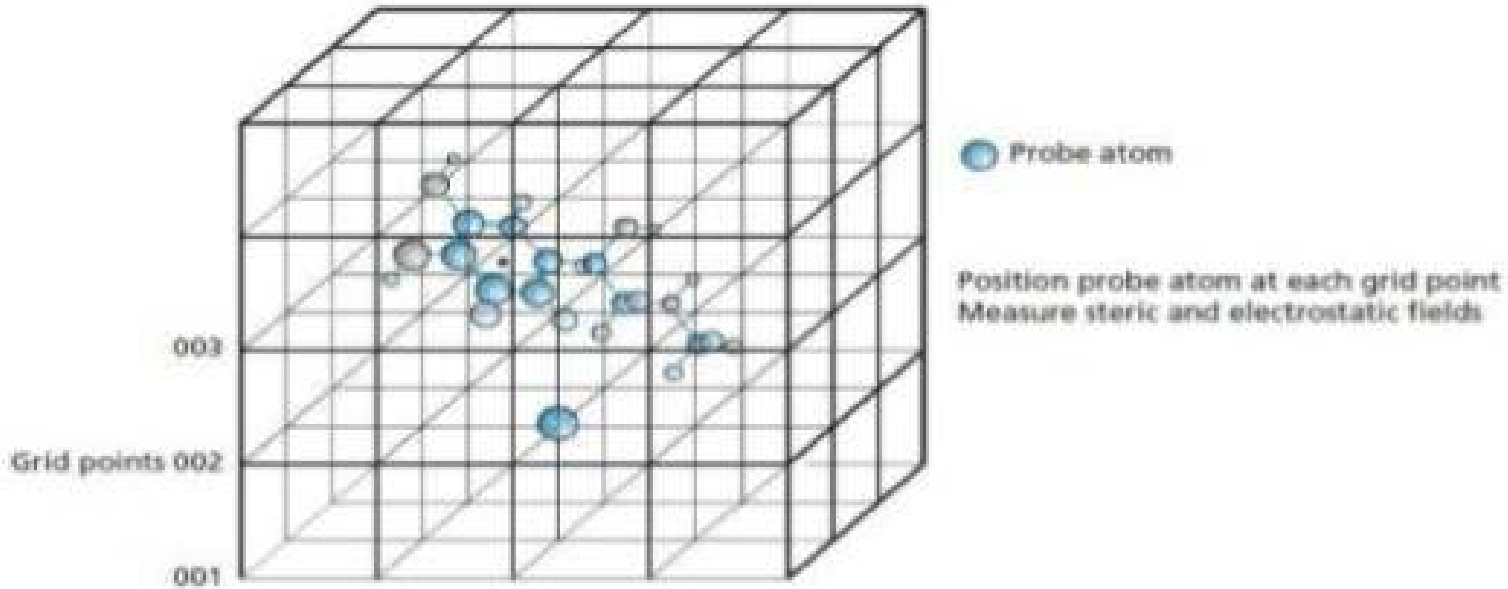
For example, introducing a heteroatom into a two-carbon link between two rings generates a structure which can be more readily synthesized.

Modification by SPROUT to generate a more synthetically feasible structure.

- The strucures that are finally generated by **sprout** are then evaluated in silico for a variety of properties, including possible toxicity and pharmacokinetic properties.

LEGEND

A grid is set up within the binding site to identify steric and electrostatic interaction energies between each grid point and the binding site.



Measuring fields around a molecule by placing a probe atom at grid points.

- These are tabulated for different types of atom and are used to estimate vander waals interactions for the growing skeletons that are generated by the program, as well as for structure optimization of final structures.

- The operator has the choice of starting from a single heteroatom, placed in such a position that it can form a hydrogen bond with the binding site.

DIFFERENCE

Unlike LUDI and SPROUT, LEGEND does not use fragments or templates to generate skeletons.

GROW

It is a program that uses molecular fragments to generate novel ligands for binding sites. The fragments used represent aminoacides and so the structures that are generated are limited to peptides.

SYNOPSIS

It is designed to generate synthetically feasible structures. Fragments can only be linked if there is a known reaction which will allow it.

CONCLUSION

- Although a relatively new design method, de novo design will play an ever-increasing role in modern drug design. Though yet not able to automatically generate viable drugs by itself, it is able to give rise to novel and often unexpected drugs.
- Rather slow and inefficient.

- Ignores synthetic feasibility while constructing structures.

- Cannot be a sole basis for drug design.

Thank you