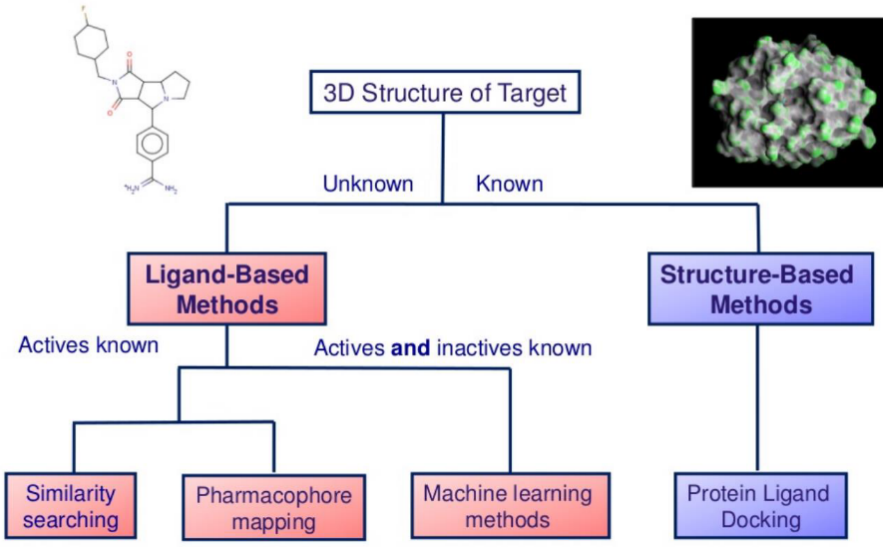
7.2 Small molecule-protein interactions

Virtuell screening

Range of *in-silico* techniques for searching large compound databases to select a smaller number for biological testing, mainly selecting those with a large binding capacity against a target of interest.



Ligand based methods

Similarity searching

Structurally similar proteins tend to have similar properties

Three components are used for measuring the similarity between 2 molecules:

- Molecular descriptors:
  - Physicochemical properties
  - 2D and 3D properties:
    - 2D **fingerprints** encode in a binary (boolean) array the presence of subfragments in the molecule (i.e CHOH terminal group)
    - 3D fingerprints: Encode information about the 3D structure of the molecule, such as concrete relationships of atoms and distances, torsion angles...
- Similarity coefficient: Quantitative measure of the simiilarity between molecular descriptors.
  - **Tanimoto coefficient**: Used to transform vectorial similarity into a numeric similarity measure: $SIM_{RD} = \frac{Sharedbits}{Bits_{molA} + Bits_{molB} - Sharedbits}$

  - Aligment-based 3D similarity: Molecules are aligned in 3D and their shared volumes are compared: $SIM_{AB} = \frac{V_C}{V_A+V_B-V_C}$
- Weighting function to integrate different data.

Resources: https://chem.libretexts.org/Courses/Intercollegiate_Courses/Cheminformatics_OLCC_(2019)/6%3.

https://sci-hub.se/https://doi.org/10.1016/B978-0-12-801505-6.00008-9

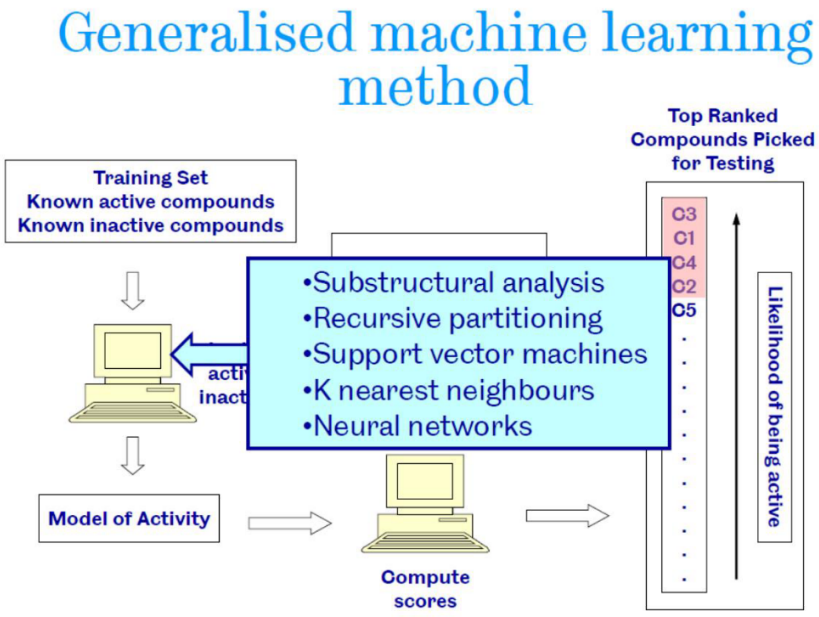
Pharmacophore

Pharmacophore: fingerprint representation of the necessary properties for a molecule (ligand) in order to be able to interact with a given target (protein).

Resources: https://sci-hub.se/https://doi.org/10.1016/B978-0-12-801505-6.00010-7

Machine learning methods

Make use of QSARs: Quantitative-Structure Activity Relationships: Databases of compounds with presence or abscence of activity that can be used for training the algorithms and SARs, where activity is treated quantitatively.



Resources:

https://www.sciencedirect.com/science/article/pii/S1359644617304695

https://sci-hub.se/10.1016/b978-0-12-801505-6.00006-5

Protein ligand docking

Similar ligands bind to the same binding site or to dissimilar proteins if they have similar binding sites. So, binding site prediction is vital. Some facts about binding site that can be used for predicting them:

- There's no standard definition of what's a **pocket**, so geometric descriptors are used.
- Biggest cleft corresponds to binding sites in most cases (but not all)
- Specific aminoacids tend to be more present (Arg, His, Trp, Tyr).
- The size of the binding site is not related to the size of the protein, but the number of binding sites is.

Energetics driving the protein-ligand binding

- Ligand-receptor binding is driven by
  - electrostatics (including hydrogen bonding interactions)
  - dispersion or van der Waals forces
  - hydrophobic interactions
  - desolvation: surfaces buried between the protein and the ligand have to be desolvated
  - Conformational changes to protein and ligand
  - ligand must be properly orientated and translated to interact and form a complex
  - loss of entropy of the ligand due to being fixed in one conformation
- Free energy of binding

$$\Delta G_{bind} = \Delta G_{solvent} + \Delta G_{conf} + \Delta G_{\text{int}} + \Delta G_{rot} + \Delta G_{t/r} + \Delta G_{vib}$$

Measurement of the binding strength between protein and ligand

K_d: Disociation constant, measures the rate of disociation of the complex protein ligand, and measures the strength of the binding. The lower the constant the stronger the binding. Related to the Boltzmann constant.

$\frac{1}{k_d} = e^{-\frac{\Delta G}{RT}} \rightarrow \Delta G = RTln(K_d)$

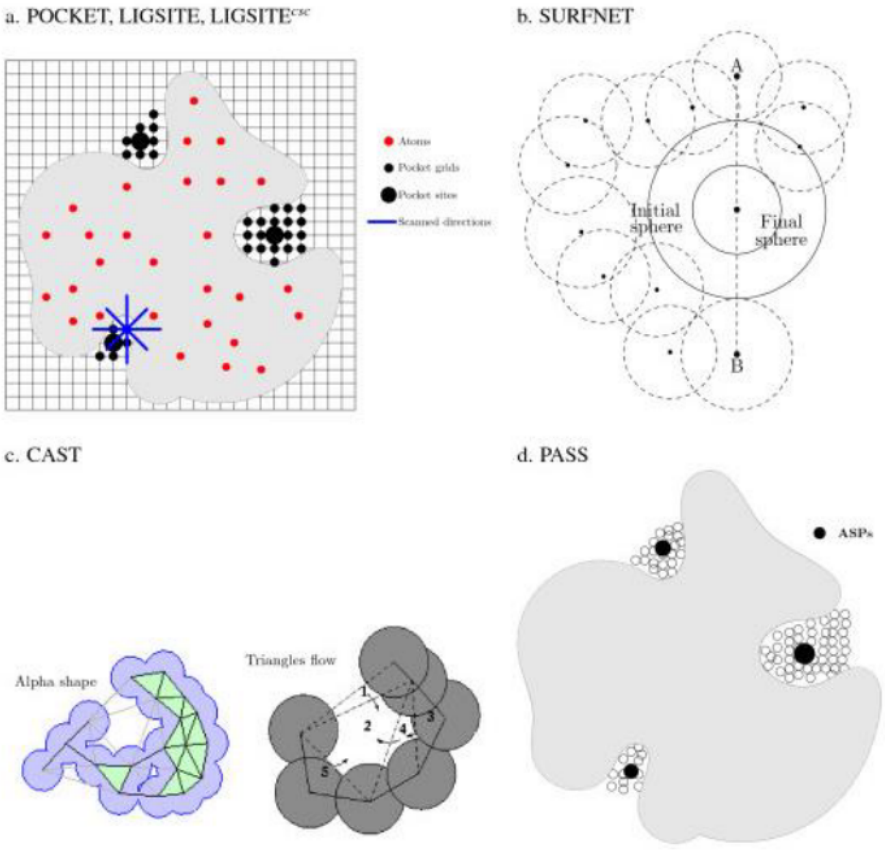
Low k_d corresponds to highly negative ΔG

Specifity: Specifity of ligand binding is also of high interest for drug design: Ligands with low specificity are more likely to have big side effects or toxicity.

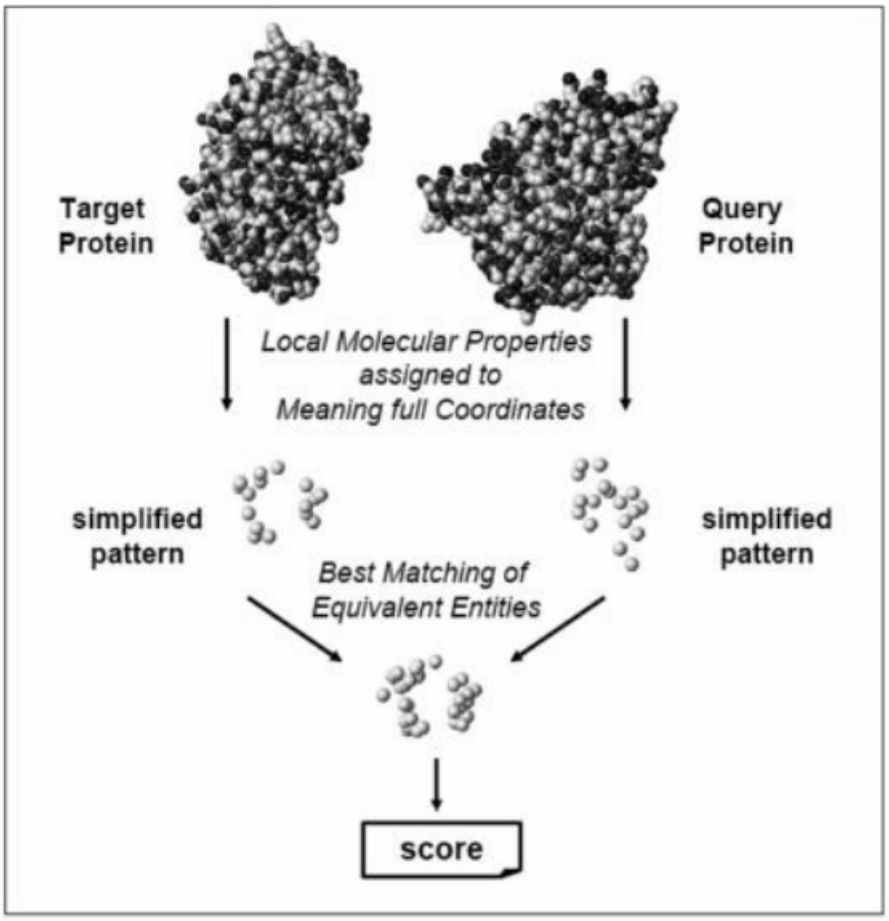
Pocket identification methods

Several algorithmic approaches are used for the identification of pockets.

- a. Grid scan methods: They search for protein-solvent-protein and surface-solvent-surface events.
- b. Sphere placement: Pocket is filled with spheres occupying the highest volume
- c. Triangulation of the surface: Merging small to large neighbors.
- d. Iterative coating of the molecular surface searching for atom contacts. Then we select the cavities with more beads as potential ligand-binding sites.



Methods for geometric pocket comparisons



Simplified representation

First, pockets are indentified, from the surface shape or from the distance between the residues involved and a crystallized ligand. Residues involved in pockets are then transformed into a simplified representation of the 3D coordinates of the atoms involved or of **pseudoatoms** (properties of groups of atoms related to the pharcopore, such as aromatic groups, H-bond donors. . .)

Resources:

https://pubs.acs.org/doi/10.1021/acs.accounts.5b00516

Whole section: https://www.sciencedirect.com/science/article/pii/S2001037014600179

Search for the best structural alignment of the simplified pocket

Different possible approaches:

Exhaustive search

Iterative search for the best translation/rotation, done in two steps:

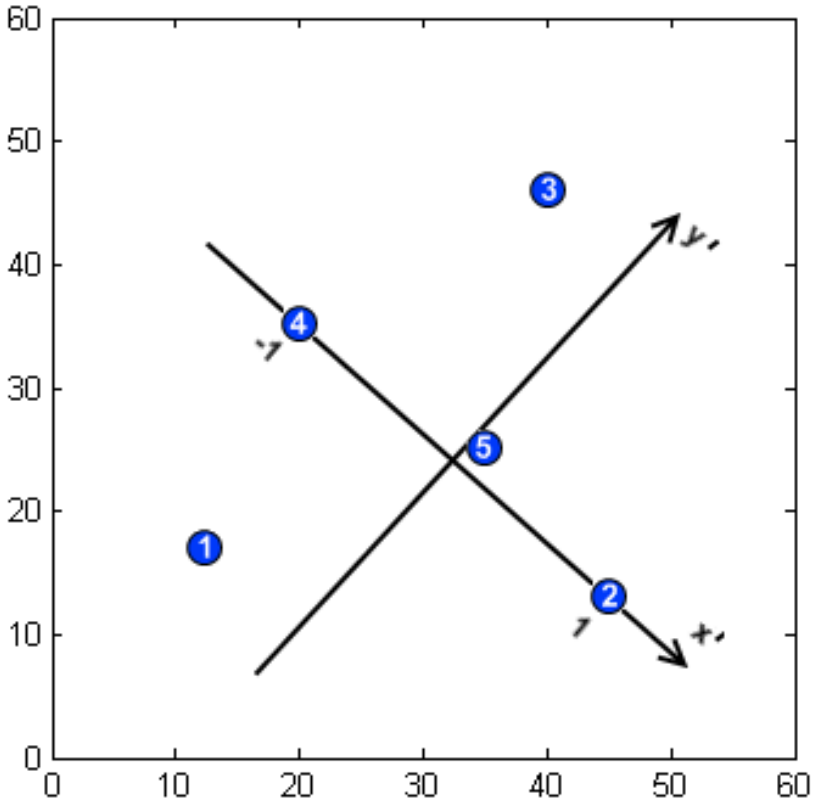
- Search for similar regions
- Exploration of those regions

Is simple conceptually but is slow.

Geometric searches

- Geometric hashing:
  - Original object is decomposed into descriptors
  - Each atom's coordinates are expressed in different coordinate systems spanned by other atom combinations, and stored into a hash table.
  - This hash table can be used for querying other pockets for similarities, using efficiently all different possible coordinate systems.
  - It's rotation, scale and translation invariant.

https://user.ceng.metu.edu.tr/~tcan/ceng465/Spring2006/Schedule/geohash.pdf



- Graph based: Graph representation is invariant to rotations and translations. Patterns are represented as nodes, and they are connected by edges with weights corresponding to their physical distances. Then, the maximal subgraph isomorphism is found, using graph product and finding the maximum clique in the graph product, using for example Bron-Kerbosch algorithm.

Scoring similarities

Similarity between predicted cavities have to be scored in order to find candidate proteins with similar functions, etc. There's not a single best measure of structural similarity, so several can be used, for example:

- Tanimoto coefficient, RMSD, sequence similarity measured by Smith-Waterman, comparison of cavity fingerprints (projection of cavity onto a sphere that is then used for querying)... Each has advantages and datasets where are good predictors of homology, and others where they behave similarly to random predictions.

The effect of protein flexibility

Constant conformational changes in proteins add a lot of complexity to the computational problem, when it's possible to predict correctly, so they are modeled as rigid objects. Proteins suffer conformational changes due to allosterism and ligand binding. Usually the proteins used for training algorithms are in holo state (bound to a ligand). That adds additional imprecision.

Main algorithms

Searching binding sites:

- *Geometric and genomic:* LigSite^{CSC}, SURFNET,ConSurf
- *Geometric:* CAST, CASTp, CAVER, Fpocket, LigSite, PASS, POCKET, PocketPicker, SURFNET, etc.
- *Geometric and energy-based:* SiteMap, etc.
- *Energy-based:* SITEHOUND, AutoLigand, GRID, etc.

Evaluating binding site similarities:

- CavBase, CPASS, CSC, eF-seek, FINDSITE, IsoCleft, MultiBind, PROSURFER, SiteAlign, SiteBase, SiteEngine, SuMo, etc.

References

Binkowski et al,. 2003, *Journal of Molecular Biology*
Schmitt et al., 2002, *Journal of Molecular Biology*
Huang et al., 2006, *BMC Structural Biology*
Kellenberger et al., 2008, *Current Computer-Aided Drug Design*
Perot et al., 2010, *Drug Discovery Today*

Protein-ligand docking

Two steps:

- Search algorithm: Generate different feasible dispositions of the ligand relative to the active site, altering its conformation, position and orientation.
- Scoring function: Quantify an estimation of the binding affinity.

Search algorithm

It has to deal with many degrees of freedom:

- 6 due to translation and rotation
- Conformational degrees of freedom of the protein and ligand.
- The solvent is often ignored, but if considered adds complexity to the model.

Different combinations are called **poses**.

It tries to cover the search space as exhaustively as possible, but there's a tradeoff between time and space coverage.

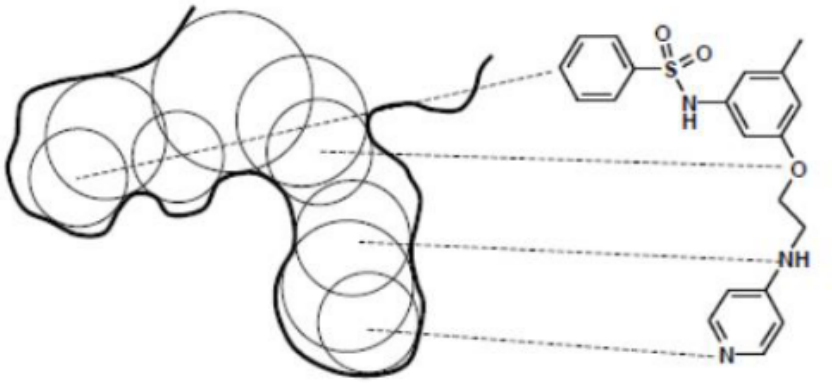
Conformational space can be explored before docking or at runtime:

- Before docking: An ensemble of ligand conformations is created before docking and they are treated as rigid bodies.
- At runtime, additional degrees of freedom related to the conformation of the ligand are considered and explored using methods such as MCMC.

Examples of Docking Search Algorithms

- First attempts: Platzer *et al.* (1972) who performed conformational energy calculations for a set of substrates binding to chymotrypsin.
- DOCK: first docking program by Kuntz et al. 1982
  - Based on shape complementarity and rigid ligands
- Current algorithms
  - Fragment-based methods: FlexX, DOCK (since version 4.0)
  - Monte Carlo/Simulated annealing: QXP(Flo), Autodock, Affinity & LigandFit (Accelrys)
  - Genetic algorithms: GOLD, AutoDock (since version 3.0)
  - Systematic search: FRED (OpenEye), Glide (Schrödinger)

- DOCK(1982): Rigid docking. Fills the cavity with spheres touching two atoms. The spheres become potential sites for ligand atoms. Ligand are matched to these spheres. If it's feasible it's scored. The algorithm returns the pose with the best score.



- FLEXX(1996):
  - A base is selected (a rigid core of the molecule, such as aromatic chains to start the algorithm from)
  - The base is placed in the binding site independent of the rest of the ligand
  - The ligand is constructed in an incremental way adding iteratively more molecules and accomodating its position to the binding site.
  - Then the pose is scored
  - Pose clustering using complete linkage hierarchical clustering based on RMSD distance.

Scoring function

- RMSD can be used to measure spatial proximity between the protein and the ligand. The idea is that lower RMSD correspond to lower K_d
- Lipinski's rule of five: max. 5 hydrogen bond donors, 10 bond acceptors, molecular mass less than 500 daltons, octanol-water partition coefficient $\log P \leq 5$

Overview of Docking Techniques

Connolly Surface	
Connolly	1986
Bacon, Moult	1992
Fischer et al.	1995
Lin et al.	1994
Norel et al.	1995
Sandak et al.	1995
Campbell et al.	1996
Ackermann et al.	1995

Fuzzy Logic	
Extner, Brickmann	1997

Cube Representation	
Jiang, Kim	1991

Graph Representation	
Shoichet et al.	1992
Shoichet, Kuntz	1996
Kasinos et al.	1992

Correlation	
Katchalski-Katzir et al.	1992
Vakser, Aflalo	1994
Vakser	1996
Gabb et al.	1997
Meyer et al.	1996

Monte Carlo Approach	
Cherfils et al.	1991
Totrov, Abagyan	1994

Slices Representation	
Walls, Sternberg	1992
Helmer-Citterich et al.	1994
Ausiello et al.	1997

Genetic Algorithms	
Levine et al.	1997

Database	
Ester et al.	1995