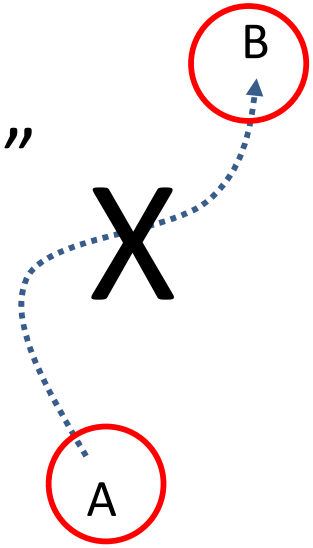
Modelling of ligand-protein binding
II. Approximate methods for estimating thermodynamic quantities

Outline

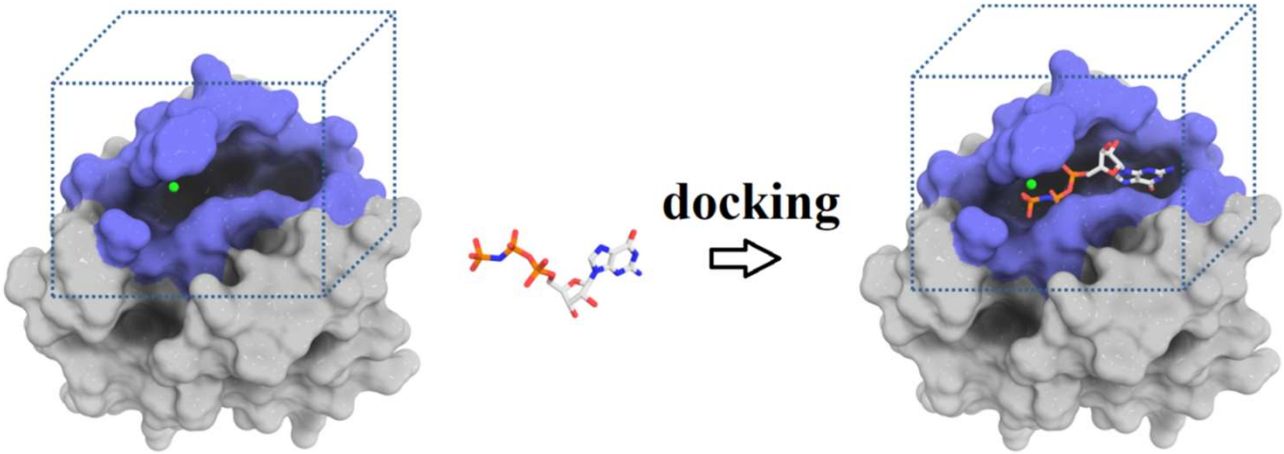
- Calculation of binding free energy by „endpoint” methods
- $\Delta G = G_b - G_A$
  - Approximate methods:
    - MM-PBSA (Molecular Mechanics Poisson – Boltzmann Surface Area) – not discussed
    - Docking and scoring

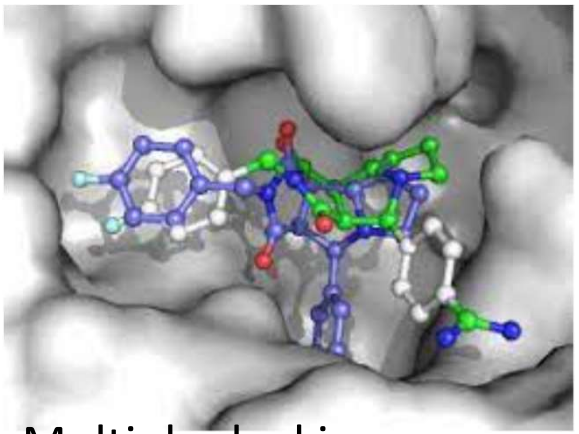


Docking and scoring

Scoring function

- Crude estimation of ligand-protein binding free energy
- Free energy vs. scoring
- Very fast – (several) ligand(s)/second
- Typically, a single protein configuration is considered
- Accompanied by docking
  - Generating the structure of complexes using minimal preliminary information





Multiple docking poses ranked by scoring

Scoring functions

- Types
  - Force field based
    - Molecular mechanics force field
  - Empirical
    - Sum of localized interactions
  - Knowledge-based
    - Based on the analysis of structural databases (Protein Data Bank, Cambridge Structural Databank)
  - Mixed
    - Combination of the types above

Force field based scoring function

- Calculation of gas-phase energy

  ($\leftrightarrow$ free energy in solvent)
- Protein field can be precomputed on a grid –> increased computational speed ($q_i/r_{ij}*q_j$)
- Structure optimization possible
- Can be complemented with
  - Solvent effect
  - entropy (?)

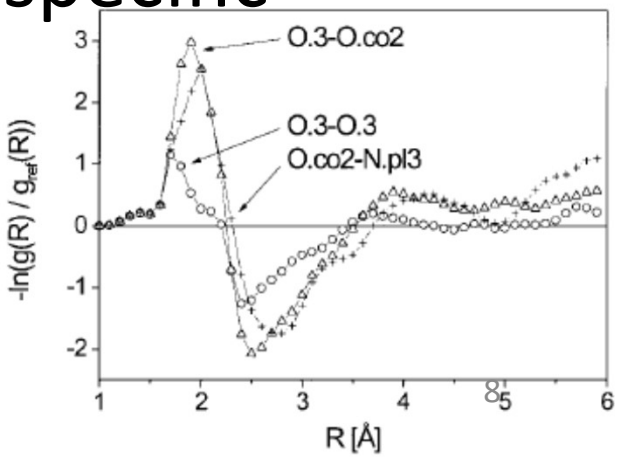
Empirical scoring functions

- Free energy estimation by an intuitive selection of interaction terms
  - Hydrogen-bond
    - Weighed sum of type dependent terms
  - Ionic interaction
  - Hydrophobic interaction
    - Proportional to the contact
- Parameters are fitted to experimental affinities

- „Sees” only terms included in the model
- Local interactions

Knowledge-based scoring function

- Derived from the statistical analysis of experimental structural data
  - $E_i = -kT\ln(p_i)$ – energy ~ observed frequency
- Protein Data Bank: over 200000 experimental structures in December 2025
- Binding affinity data not required
- Long-range sampling – solvent effect included
- Short-range sampling – emphasizes specific interactions
- Incomplete repulsion



Docking - scoring

- Generating and ranking ligand-protein complex structures
  - Single ligand-protein pair
    - finding binding mode – identify binding position with best score
  - Multiple ligands and a single protein
    - Virtual screening
      - Finding binding mode and corresponding docking score
      - Ranking ligands by docking score
- Without preliminary structural information (in principle)
- Application in pharmaceutical research – see later

Approximations of docking-scoring

Selected approximations:

- Protein is rigid or has limited flexibility
- Protonation state
  - single or few states for ligand and protein
- Interaction with water (bulk and structural)
  - Ligand dependence of structural waters is not considered
- Entropy
- Temperature
- …

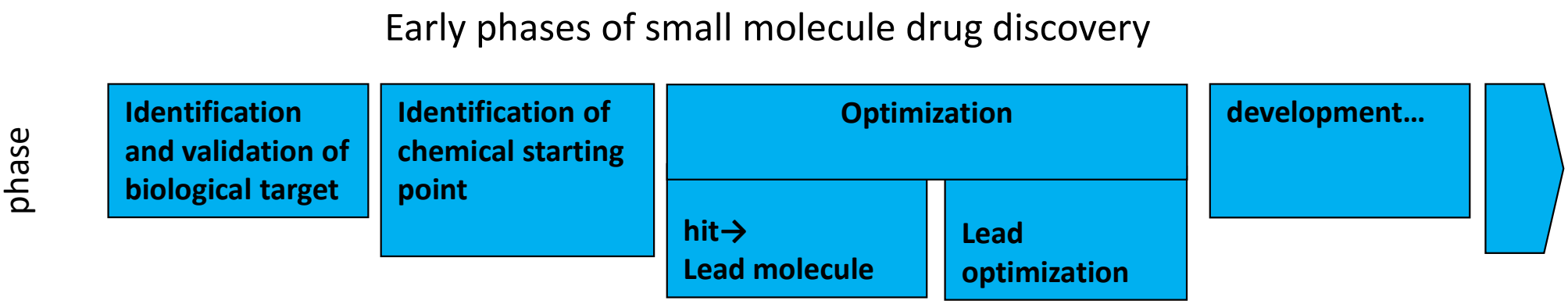
Protein flexibility – docking-scoring

- Role of protein flexibility in ligand binding
  - Selection of protein conformation advantageous for ligand binding
    - Population shift
  - Induced fit
    - Binding to a protein conformation not available for the free protein
  - No strict distinction between the above two mechanisms

Protein flexibility – docking-scoring

- Docking - taking into account protein flexibility
  - Using multiple static protein structures
    - Experimental structure – complexes with various ligands, NMR
    - Structures generated by computation (MD, MC)
    - Increased computational requirements
  - „Soft” protein structure
    - Single averaged structure derived from several structures and containing damped interactions
      - Unable to describe large movements
      - Increased binding pocket
      - Mutually exclusive binding sites appear simultaneously
  - Protein conformations generated upon binding (e.g. MD)

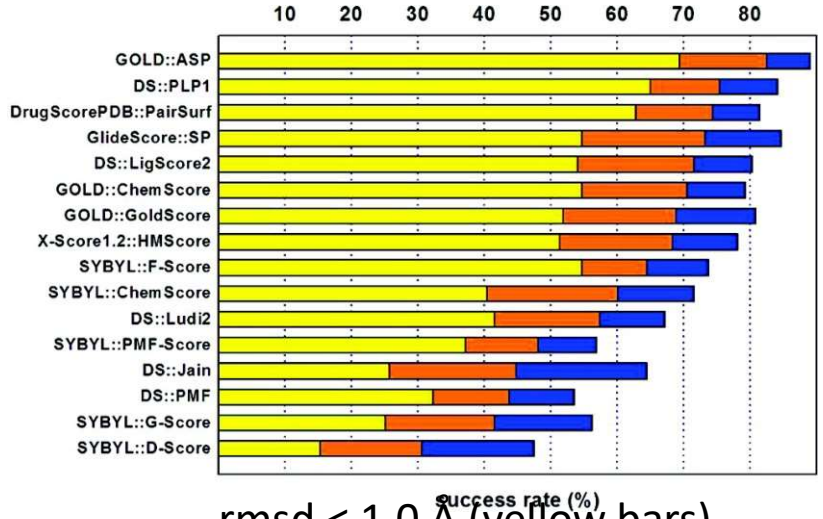
Application of docking-scoring



- Virtual screening
  - Identification of chemical starting points: good binders
- Docking – Binding mode identification
  - hit to lead

Docking

comparing scores of multiple poses of a single compound



rmsd < 1.0 Å (yellow bars),
< 2.0 Å (orange bars),
< 3.0 Å (blue bars)

J. Chem. Inf. Model. 2009, 49, 1079–1093

- Protein structure
  - X-ray crystallography
  - NMR
  - Cryo-electronmicroscopy
  - homology model
  - AlphaFold (AI)
- Ligand structure
  - Model
- Complex structure
  - Fitting the ligand into the protein binding pocket - docking
  - Ranking of binding modes using scoring functions
    - Limited protein flexibility
    - Efficient exploration of ligand conformational space
- RMSD of docked ligand < 2Å –70-80% in favourable cases

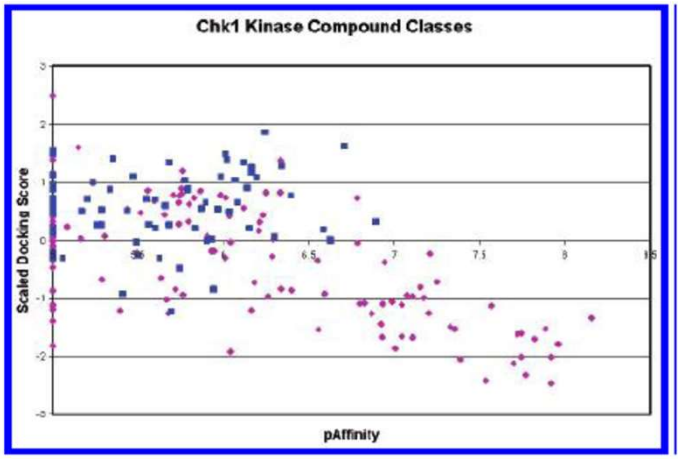
Ranking

comparing scores of several compounds

- Docking compounds into a protein and ranking the complexes (ligands) by scoring functions
  - Diverse ligands – chemical starting point identification
  - Similar ligands – hit/lead optimization
- ***weak correlation between score and experimental affinity – why is virtual screening useful?***

Best Correlation Coefficient r between the -log Affinity (pAffinity) and Docking Score

program	Chk1	FXa
Dock4	−0.33	−0.31
DockIt	−0.49	−0.19
FlexX	−0.57	−0.31
Flo+	−0.44	−0.38
Fred	−0.14	0.01
Glide	−0.47	−0.08
Gold	−0.42	−0.05
LigandFit	−0.45	−0.13
MOEDock	−0.29	0.00
MVP	−0.26	0.10



Ligands are fromtwo congeneric series

J. Med. Chem. 2006, 49, 5912

Correlation Between the Scores and Experimental Binding Affinities

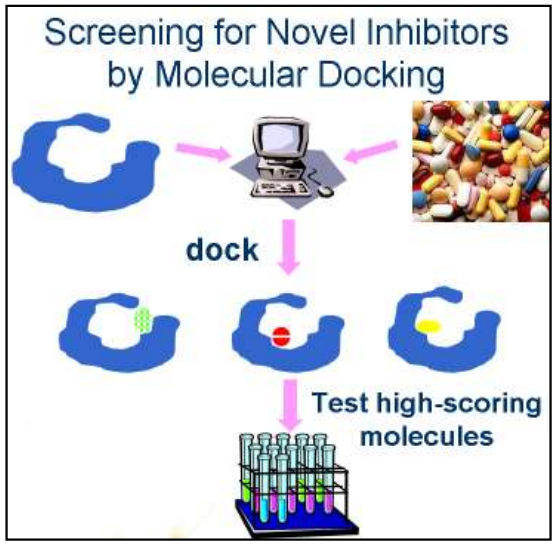
method	Pearson R	Spearman ρ
code 1	0.76 (0.80−0.71)	0.74 (0.79−0.68)
code 2	0.72 (0.77−0.66)	0.73 (0.78−0.67)
code 3	0.67 (0.72−0.60)	0.68 (0.74−0.61)
code 4	0.64 (0.70−0.58)	0.64 (0.70−0.56)
code 5	0.63 (0.69−0.56)	0.64 (0.71−0.57)
code 6	0.62 (0.68−0.55)	0.61 (0.68−0.53)
code 7	0.62 (0.68−0.55)	0.61 (0.68−0.53)
code 8	0.61 (0.67−0.54)	0.59 (0.66−0.51)
code 9	0.61 (0.67−0.53)	0.60 (0.67−0.52)
code 10	0.60 (0.66−0.52)	0.60 (0.67−0.52)
code 11	0.59 (0.66−0.52)	0.57 (0.64−0.49)
code 12	0.57 (0.63−0.49)	0.57 (0.65−0.49)
code 13	0.56 (0.63−0.48)	0.60 (0.67−0.52)
code 14	0.56 (0.63−0.48)	0.54 (0.62−0.45)
code 15	0.56 (0.63−0.48)	0.56 (0.63−0.47)
code 16	0.53 (0.60−0.45)	0.53 (0.61−0.44)
code 17	0.35 (0.44−0.25)	0.37 (0.46−0.27)

J. Chem. Inf. Model. 2011, 51, 2115

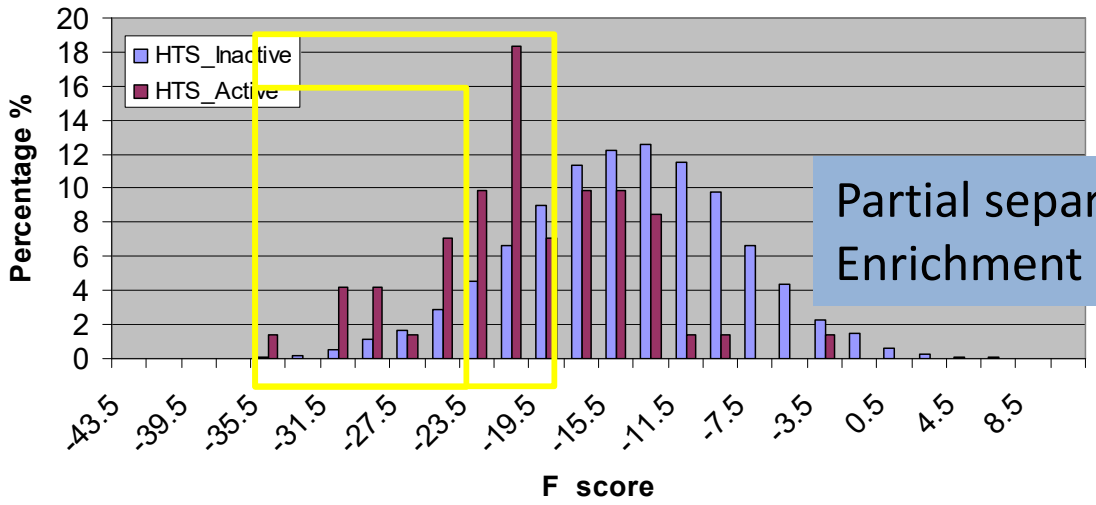
Virtual screening

comparing scores of a large number of compounds

- Identification of chemical starting points
- Computation:
  - Docking a large number of structurally divers compounds
  - Ranking the complexes (compounds) by score



- Experimental testing of top scored compounds



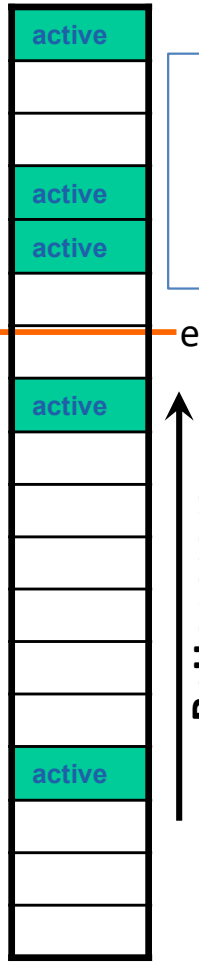
Partial separation of actives and inactives
Enrichment of actives among tip scored compounds

Identification of chemical starting point and virtual screening

- High throughput screening (HTS) - experimental
  - Finding compounds with the required effect on a target protein
  - Biochemical/biophysical methods
    - receptor binding
    - Enzyme inhibition
    - …
  - Testing 10^5-10^6 compounds
  - Number of hits: ~10^2
  - *Hit rate: 0.1%* ($10^2/10^5$)

- Virtual screening - computational
  - Objective: increase HTS hit rate by computational (cheap) prescreening
  - Docking and scoring ~10^6 compounds
  - Experimental testing of top ~10^3 compounds; typical hit rate: 1-10 %

Efficiency of virtual screening



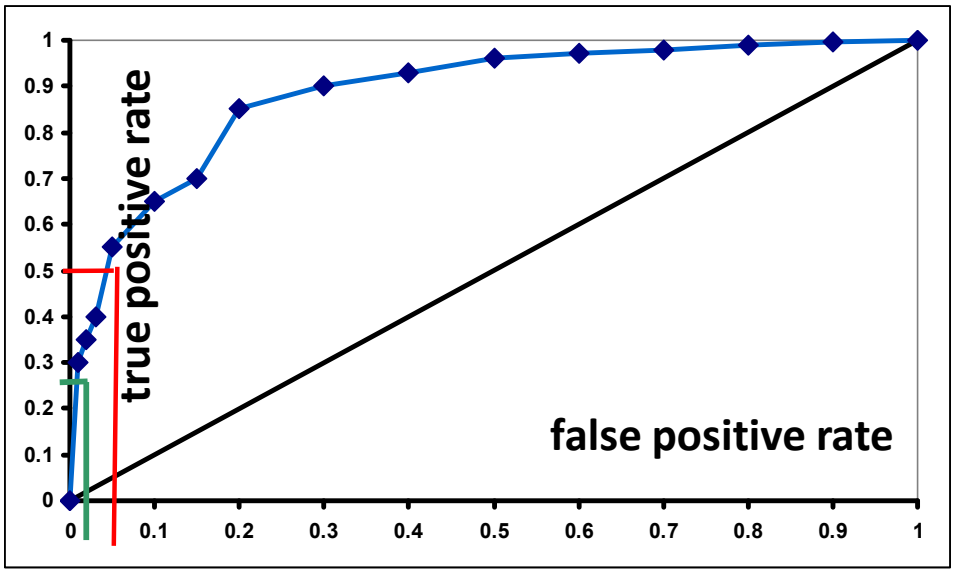
$$enrichment\ factor = \frac{\frac{active_{selected}}{inactive_{selected}}}{\frac{active_{all}}{inactive_{all}}}$$

enrichment: 3/6*18/5 ~ 5

Better score

Typical enrichment: 5-20

receiver operating characteristic (ROC)
area under curve (AUC)



10^2 actives; 10^5 inactives – 0.1%

35 actives; 2000 inactives - 1.75% EF=18

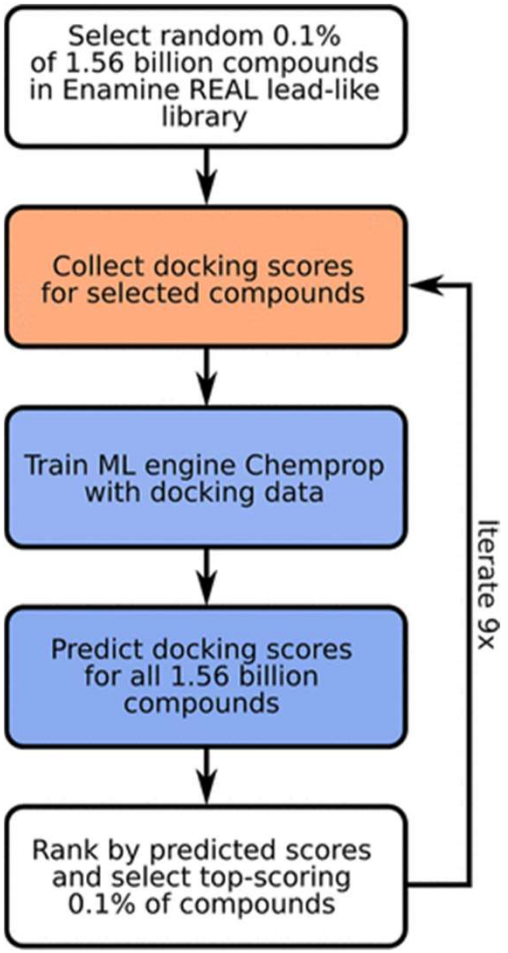
55 actives; 5000 inactives– 1% EF=10

More compounds selected for testing
- More actives found
- Lower enrichment - lower hit rate

Low hit rate (1-10%) that overcomes HTS hit rate (<1%)

Machine learning and virtual screening

- Docking and scoring for a subset of compounds
- Build a machine learning model to predict docking score by ligand structure
- Screen 10^9 molecules in 1-2 weeks



J. Chem. Inf. Model. 2023, 63, 18, 5773

Summary

Docking - scoring

- Very fast
- Good quality binding mode prediction
- Weak correlation between score and experimental affinity
- Virtual screening is an established tool in chemical starting point identification
- Intensively applied in pharmaceutical research