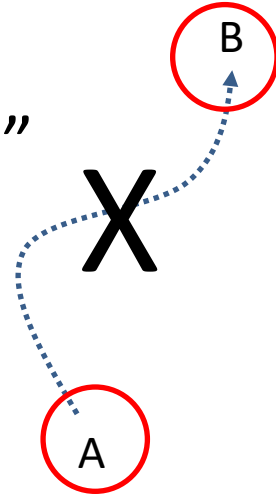


Modelling of ligand-protein binding

II. Approximate methods for estimating thermodynamic quantities

Outline

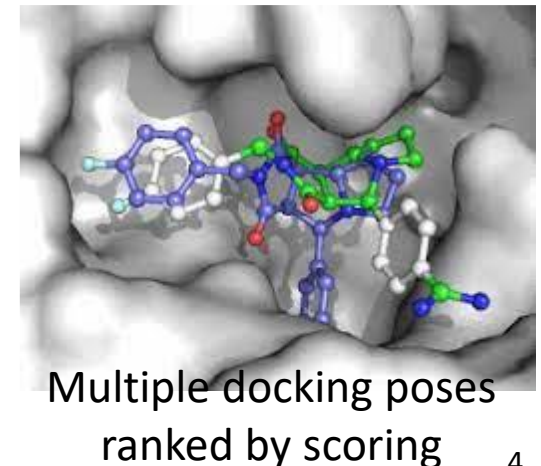
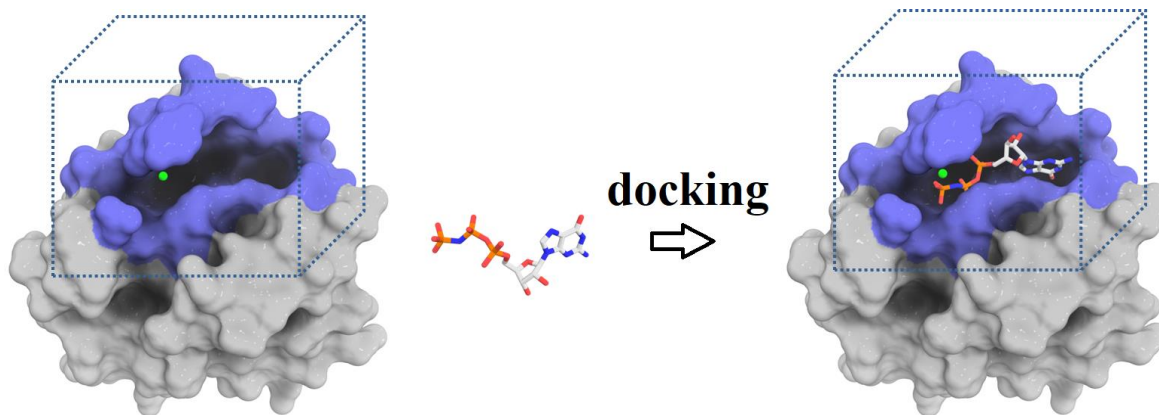
- Calculation of binding free energy by „endpoint” methods
- $\Delta G = G_b - G_A$
 - Approximate methods:
 - MM-PBSA (Molecular Mechanics Poisson – Boltzmann Surface Area) – not discussed
 - Docking and scoring



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



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

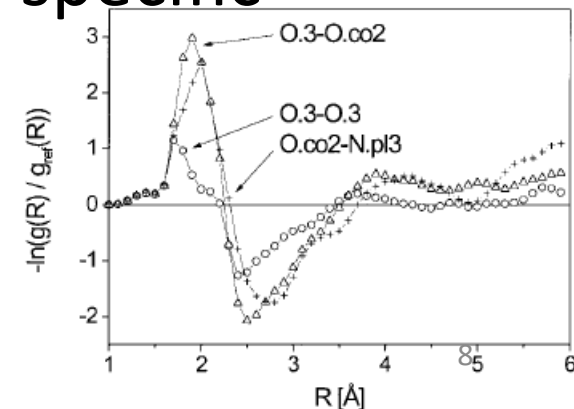
- Calculation of gas-phase energy
(\leftrightarrow free energy in solvent)
- Protein field can be precomputed on a grid \rightarrow increased computational speed
- Structure optimization possible
- Can be complemented with
 - Solvent effect
 - entropy (?)

Empirical scoring functions

- 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 \sim observed frequency
- Protein Data Bank: nearly 200000 structures in December 2022
- 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
 - Multiple ligands and a single protein
 - Virtual screening
 - Binding mode identification
 - 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
- Interaction with water (bulk and structural)
- Entropy
- Temperature
- ...

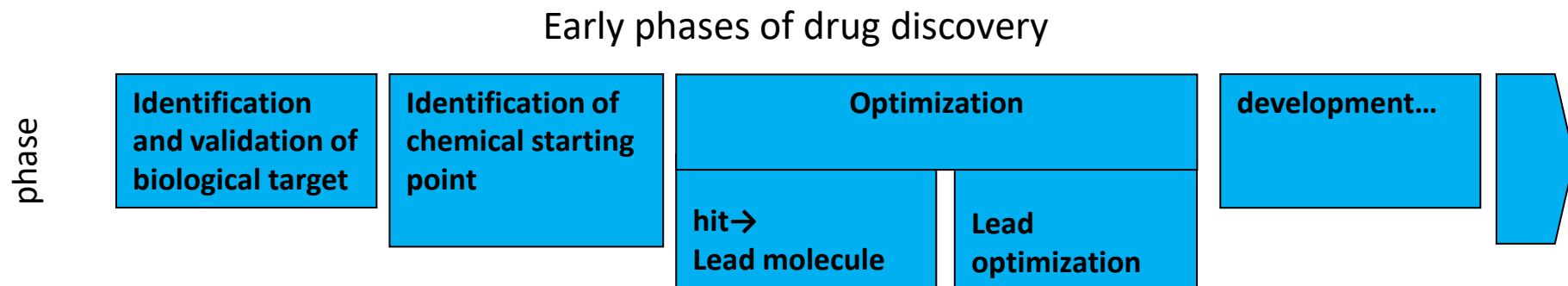
Protein flexibility – docking-scoring

- 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

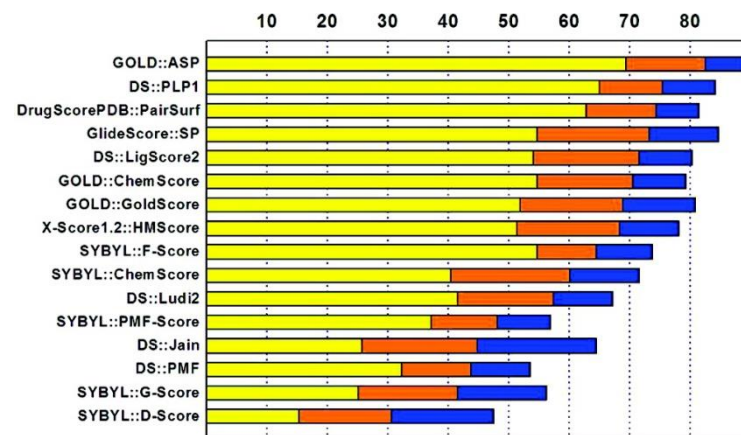


- Virtual screening
 - Identification of chemical starting point
- Docking – Binding mode identification
 - hit to lead

Docking

comparing scores of multiple poses of a single compound

- Protein structure
 - X-ray crystallography
 - NMR
 - Cryo-electronmicroscopy
 - homology model
 - AlphaFold (MI)
- 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 $< 2\text{\AA}$ – 70-80% in favourable cases



success rate (%)
rmsd $< 1.0\text{\AA}$ (yellow bars),
 $< 2.0\text{\AA}$ (orange bars),
 $< 3.0\text{\AA}$ (blue bars)
J. Chem. Inf. Model. 2009, 49, 1079–1093

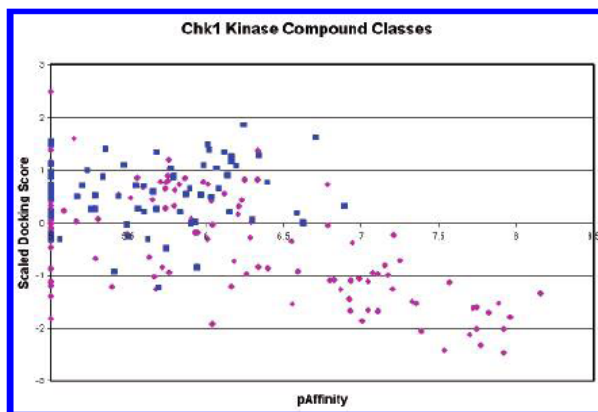
Ranking

comparing scores of several similar compounds

- Docking compounds into a protein and ranking the complexes (ligands) by scoring functions
- Studying similar compounds – lead optimization
- ***weak correlation between score and experimental affinity***

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



J. Med. Chem. 2006, 49, 5912

Correlation Between the Scores and Experimental Binding Affinities^a

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

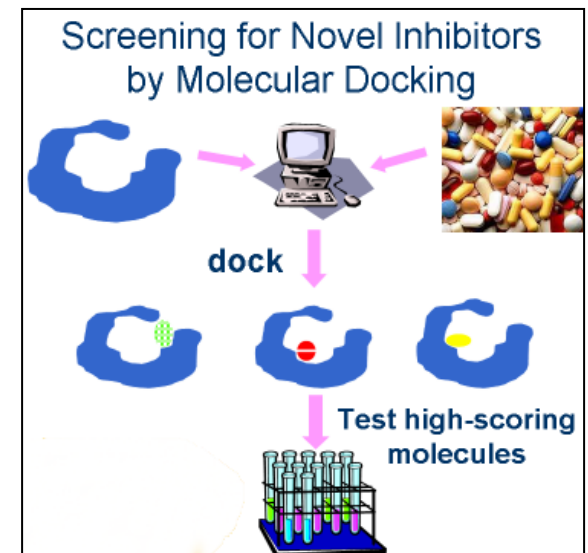
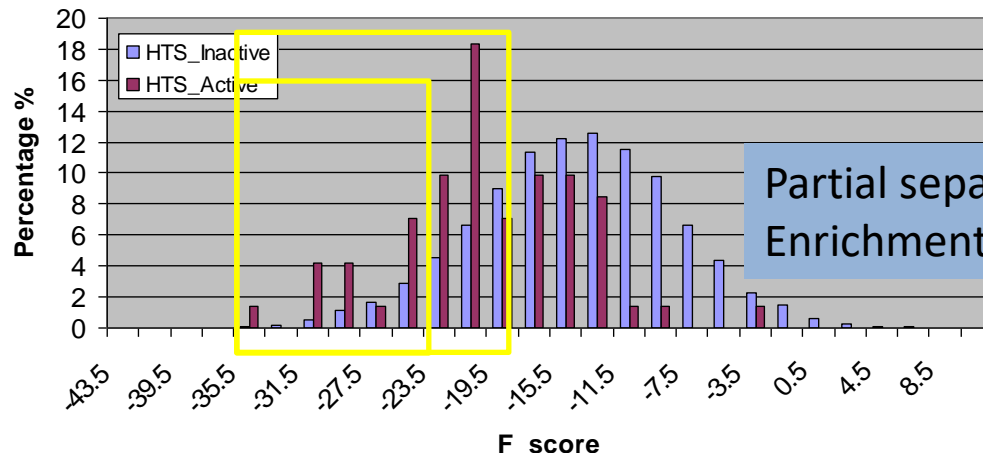
Virtual screening

comparing scores of a large number of compounds

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



- Experimental testing of top scored compounds

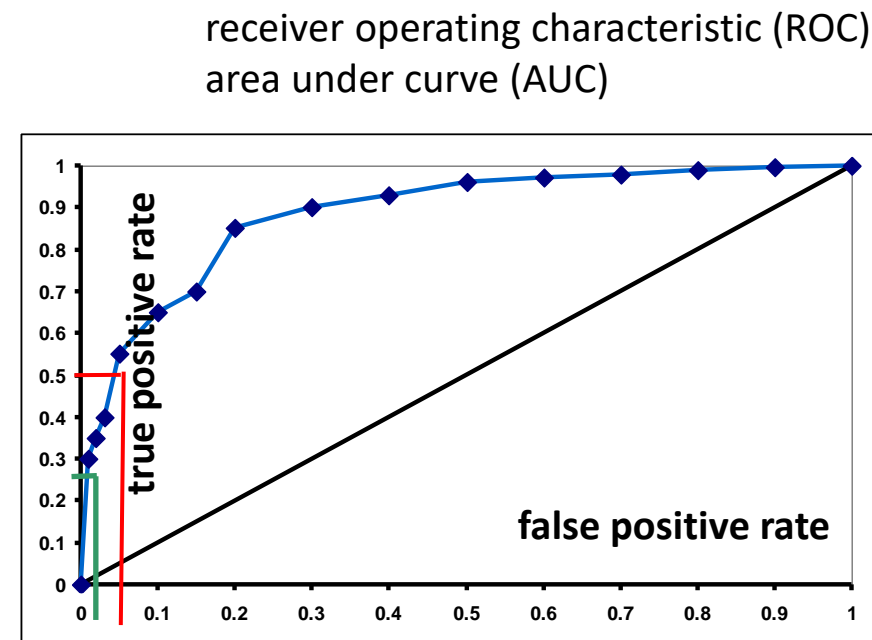
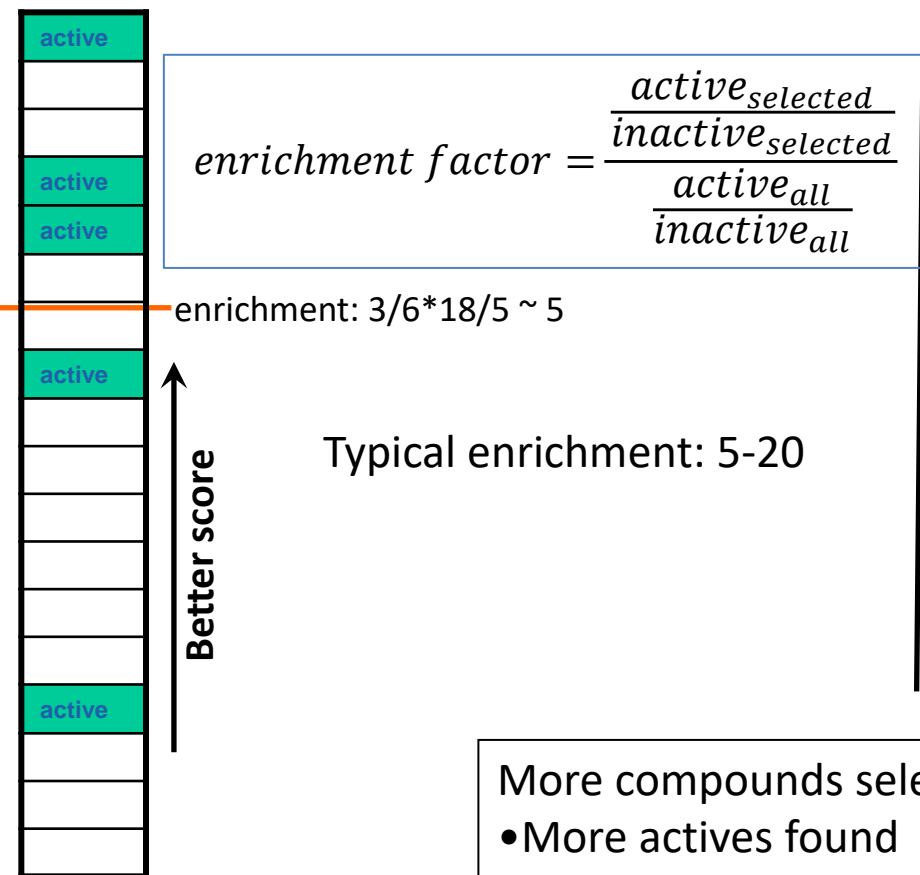


Identification of chemical starting point and virtual screening

- 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: $\sim 10^2$
 - Hit rate: 0.1% ($10^2/10^5$)
- Virtual screening - computational
 - Objective: increase HTS hit rate by computational (cheap) prescreening
 - Docking and scoring $\sim 10^6$ compounds
 - Experimental testing of top $\sim 10^3$ compounds; typical hit rate: 1-10 %



Efficiency of virtual screening



10^2 actives; 10^5 inactives – 0.1%

35 actives; 2000 inactives - 1.75%

55 actives; 5000 inactives – 1%

EF=18

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%)

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