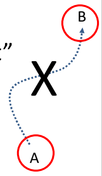
Modelling of ligand-protein binding II. Approximate methods for estimating thermodynamic quantities

1

Outline

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



2

2

Docking and scoring

3

3

Scoring function

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

4

4

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

5

5

Force field based scoring function

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

6

6

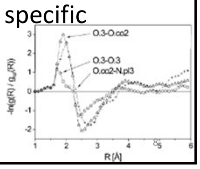
Empirical scoring functions

- 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 ~ observed frequency
- Protein Data Bank: over 180000 structures in November 2020
- 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 and structure of water
- Entropy
- Temperature
- …

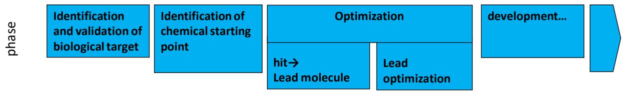
Protein flexibility – docking-scoring

- 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

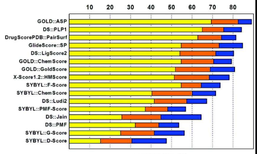


- Virtual screening
  - Identification of chemical starting point
- Docking – Binding mode identification
  - hit to lead

13

13

Docking



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
  - homology model
- 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

14

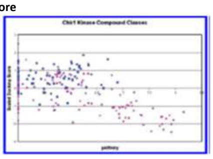
14

Ranking

- 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 Affinitiesa

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

15

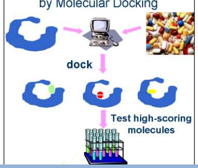
15

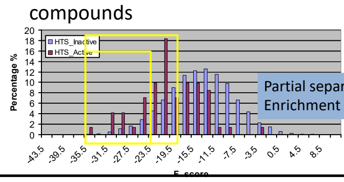
Virtual screening

- Identification of chemical starting point
- 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

16

16

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 %

17

17

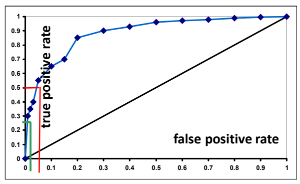
Efficiency of virtual screening

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

enrichment: 3/6*18/5 ~ 5

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 (~10^{-1}%)

18

18

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