

Ligand-protein interactions and binding thermodynamics

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Subject

- Focus on ligand-protein binding
 - Qualitative and quantitative characterisation
 - Thermodynamics (and kinetics)
 - How to interact/influence - drug design
 - Computational support to drug discovery

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Outline

- Basic relationships
 - Measurements and computations
 - Analysis of ligand-protein binding
 - Role of water
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- Computations
 - Molecular dynamics (MD)
 - Tool for quantitative description
 - MD based applications to characterize ligand-protein binding
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- Very fast estimation of ligand-protein interactions
 - Docking-scoring
 - Drug discovery application

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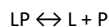
Ligand-protein binding

- Signal transduction
 - G-protein coupled receptors (GPCRs)
- Enzymatic catalysis
 - Cytochrome P450
- Transcription
 - Nuclear receptors...
- Endogenous and exogenous (e.g. drugs) ligands

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Few basic relationships



$$K_d = \frac{[L][P]}{[LP]}; pK_d = -\log(K_d)$$

$$\Delta G_{\text{bind}} = RT \ln(K_d/C_{\text{ref}})$$

$$\Delta G = \Delta H - T\Delta S \quad (\text{Gibbs}) \text{ typical experimental conditions (NPT)}$$

$$\Delta F = \Delta U - T\Delta S \quad (\text{Helmholtz}) \text{ calculations for solutions, often used (NVT, canonical)}$$

$$F = -k_B T \ln Z,$$

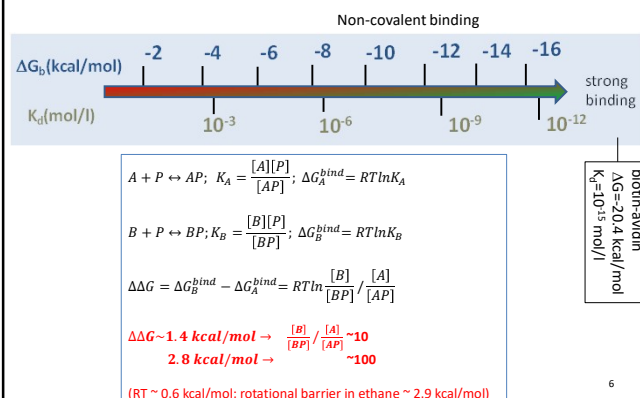
$$Z = \sum_i e^{-\frac{E_i}{k_B T}} - \text{partition function} \quad (\sim \int e^{-\frac{E(r,p)}{k_B T}} dr dp)$$

can be calculated for simple systems only

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Free energy – Equilibrium constant

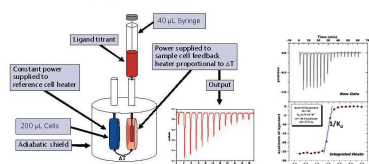


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Measuring binding thermodynamics

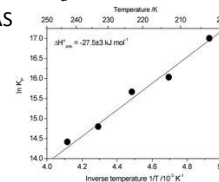
- Isothermal titration calorimetry
 - $n, K_d, \Delta H \rightarrow \Delta G, \Delta S$
 - limits:
 - solutions
 - protein quantity (10-100 μg)
 - throughput



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Measuring binding thermodynamics

- Van't Hoff analysis
 - $\ln K_d = \frac{\Delta H_b}{RT} - \frac{\Delta S_b}{R}$ ($\Delta G_b = \Delta H_b - T\Delta S_b = RT \ln K_d$)
 - Measure K_d at various $T \rightarrow \Delta H$ és ΔS
 - Experimental techniques
 - Radioligand displacement
 - Mass spectrometry
 - Chromatography
 - Surface plasmon resonance (SPR)
 - limits
 - ΔH depends on T
 - extrapolation ($\Delta S: 1/T=0$)



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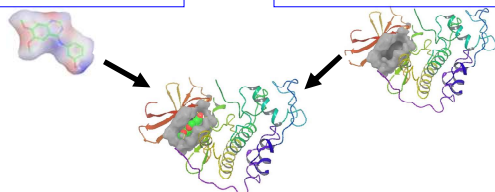
Ligand-protein binding “steps”

Ligand in water

- Conformational change
- (partial) desolvation

Protein in water

- Conformational change
- Partial desolvation



Ligand-protein complex

- Ligand-protein interactions

Water plays a substantial role in the binding process

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Qualitative binding thermodynamics

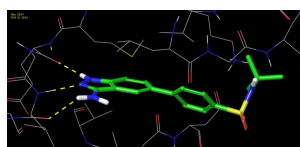
- desolvation (ligand+protein)
 - beneficial ΔS (change in water structure)
 - disadvantageous ΔH
- Conformational change (ligand+protein)
 - disadvantageous ΔH (optimal before binding)
- Ligand-protein interactions
 - beneficial ΔH (polar and van der Waals interactions)
 - disadvantageous ΔS (restricted motion)

ΔG is a sum of several terms with positive and negative signs

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Qualitative binding thermodynamics

- $\Delta G, \Delta H, \Delta S$ can be assigned to steps from one state to another – state functions
- Assigning $\Delta G, \Delta H, \Delta S$ to structural elements is problematic
 - Limited additivity
 - ΔH additivity – good approximation
 - ΔS additivity – bad approximation

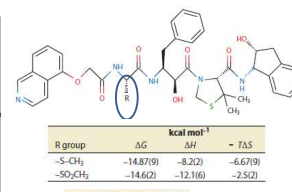
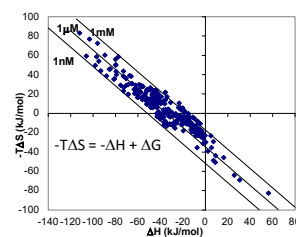


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Enthalpy-entropy compensation

Small structural changes of a ligand-protein complex results in significant $\Delta\Delta H$ és $\Delta(T\Delta S)$ changes of opposite sign and a small change in $\Delta\Delta G$

- The compensation is observed for a wide range of phenomena
- Both in water and in apolar solvents



R group	ΔG	ΔH	$-T\Delta S$
-S-CH ₃	-14.87(9)	-8.2(2)	-6.67(9)
-SO ₂ CH ₃	-14.6(2)	-12.1(6)	-2.5(2)

Annu. Rev. Biophys. 2013, 42:121-42

ΔG changes are limited (within ~35 kJ/mol)

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Hydrophobic effect

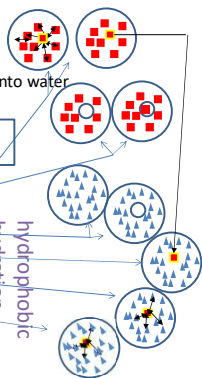
- Hydrophobic effect:

Bringing an apolar substance from its apolar solvent into water (hydrocarbon \rightarrow water)

analogy: desolvation upon ligand-protein binding (inverse)
apolar moieties - solvent \rightarrow self-interactions

- Breaking apolar contacts and removing molecule
- Filling empty space in the apolar medium
- Hole formation in water
- Inserting the apolar substance
- Formation of solute-solvent interactions
- Reorganization of water structure

ΔG positive



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Hydrophobic effect

- ΔG increase

- 20°C

- ΔH (advantageous) and $T\Delta S$ (disadvantageous) decrease; $T\Delta S$ change dominates

- higher T

- small change in ΔG

- ΔH increases and becomes dominant

- disadvantageous for enthalpy
- advantageous for free energy
- interactions are sacrificed for increased disorder

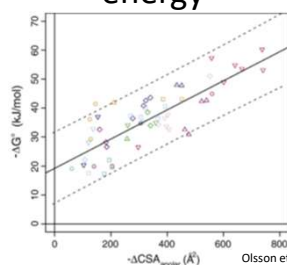
- Explanation: focuses on hydrophobic hydration

- Key factor in entropy decrease: Water structure perturbed
- Hole formation - small size of water molecules
- Water H-bonds near to the apolar solute
- No general quantitative model available!

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Apolar surface and binding free energy



Olsson et al. J. Mol. Biol. (2008) 384, 1002

- Ligand-protein binding free energy correlates with apolar surface buried in the binding ($R^2=0.65$).
- Shape fitting and polar/apolar feature mapping give significant contribution to binding
 - Directional interactions do not contribute importantly to the above correlation

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Affinity and molecular size

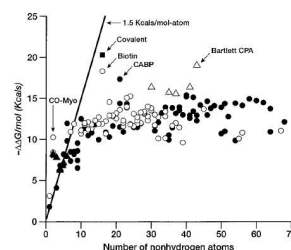


FIG. 1. Free energy of binding (in kcal/mol) for ligands and enzyme inhibitors plotted as a function of the number of nonhydrogen atoms in the ligand. See Table 1. A line with slope of 1.5 kcal/mol and an intercept of 0 is included as a visual aid to analysis. Δ , Metal ions or metalloenzymes; \blacktriangle , small anions; \circ , natural ligands; \bullet , enzyme inhibitors.

PNAS 1999, 96, 9997

Available binding affinity is

- limited
- limit does not increase with size above ~ 25 nonhydrogen atoms

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Summary

- Binding thermodynamics – characterizes ligand-protein interactions
- Key elements of binding: polar interactions and apolar desolvation
- Related phenomena: hydrophobic effect, enthalpy-entropy compensation
- Ligand size affects maximal available binding free energy
 - ΔG_{max} – available binding free energy increase fast with ligand size for small ligands and is insensitive to size for larger ligands

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