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

Outline

- Basic relationships
- Measurements and computations
- Analysis of ligand-protein binding
- Role of water

- 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

Ligand-protein binding

- Signal transduction
  - G-protein coupled receptors (GPCRs)
- Enzymatic catalysis
  - Cytochrome P450
- Transcription
  - Nuclear receptors…

- Endogenous and exogenous (e.g. drugs) ligands

Few basic relationships

LP ↔ L + P

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

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

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

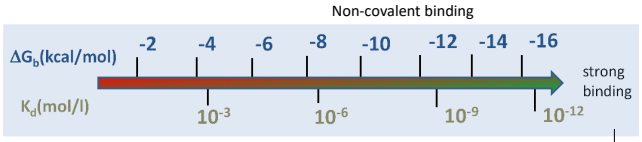
$\Delta F = \Delta U - T\Delta S$ (Helmholtz) calculations for solutions, often used (NVT, canonical)

$F = -k_B T \ln Z$,

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

can be calculated for simple systems only

Free energy – Equilibrium constant



$A + P \leftrightarrow AP;\ K_A = \frac{[A][P]}{[AP]};\ \Delta G_A^{bind} = RT \ln K_A$

$B + P \leftrightarrow BP;\ K_B = \frac{[B][P]}{[BP]};\ \Delta G_B^{bind} = RT \ln K_B$

$\Delta\Delta G = \Delta G_B^{bind} - \Delta G_A^{bind} = RT \ln \frac{[B]}{[BP]} / \frac{[A]}{[AP]}$

$\Delta\Delta G \sim 1.4\ kcal/mol \rightarrow \frac{[B]}{[BP]} / \frac{[A]}{[AP]} \sim 10$

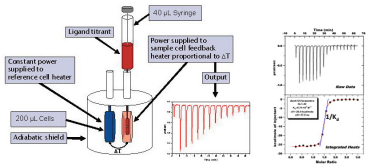
$2.8\ kcal/mol \rightarrow \sim 100$

(RT ~ 0.6 kcal/mol; rotational barrier in ethane ~ 2.9 kcal/mol)

biotin-avidin
ΔG=-20.4 kcal/mol
K_d=10^{-15} mol/l

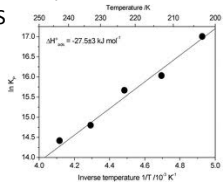
Measuring binding thermodynamics

- Isothermal titration calorimetry
  - n, K_d, ΔH →ΔG, ΔS
  - limits:
    - solutions
    - protein quantity (10-100 µg)
    - throughput



Measuring binding thermodynamics

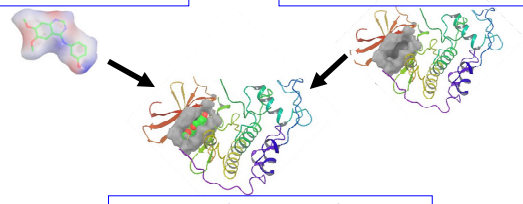
- Van't Hoff analysis
  - $lnK_d = \frac{\Delta H_b}{RT} - \frac{\Delta S_b}{R}$ ($\Delta G_b=\Delta H_b-T\Delta S_b=RTlnK_d$)
  - Measure K_d at various T → ΔH és ΔS
  - Experimental techniques
    - Radioligand displacement
    - Mass spectrometry
    - Chromatography
    - Surface plasmon resonance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



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

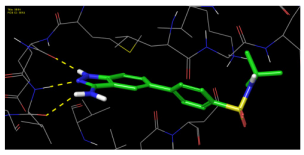
Qualitative binding thermodynamics

- 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

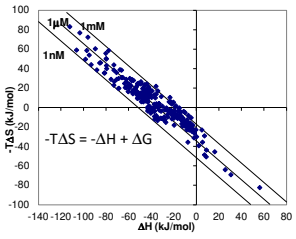
Qualitative binding thermodynamics

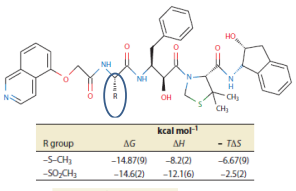
- ΔG, ΔH, ΔS can be assigned to steps from one state to another – state functions
- Assigning ΔG, ΔH, ΔS to structural elements is problematic
  - Limited additivity
    - ΔH additivity – good approximation
    - ΔS additivity – bad approximation



Enthalpy-entropy compensation

Small structural changes of a ligand-protein complex results in significant ΔΔH és Δ(TΔS) changes of opposite sign and a small change in ΔΔG
- The compensation is observed for a wide range of phenomena
- Both in water and in apolar solvents





R group	ΔG	ΔH	-TΔS
-S-CH_3	-14.87(9)	-8.2(2)	-6.67(9)
-SO_2CH_3	-14.6(2)	-12.1(6)	-2.5(2)

kcal mol⁻¹

Annu. Rev. Biophys. 2013. 42:121–42

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

Hydrophobic effect

- Hydrophobic effect:

  Bringing an apolar substance from its apolar solvent into water (hydrocarbon -> water)

  analogy: desolvation upon ligand-protein binding (inverse)

  apolar moieties - solvent -> self-interactions

  – Breaking apolar contacts and removing molecule

  – Filling empty space in the apolar medium

  – Hole formation in water

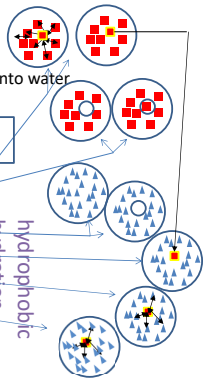
  – Inserting the apolar substance

  – Formation of solute-solvent interactions

  – Reorganization of water structure

  hydrophobic hydration

ΔG positive



Hydrophobic effect

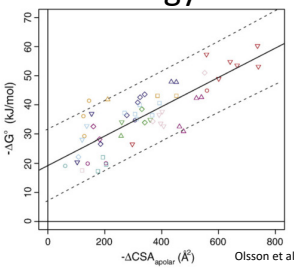
- ΔG increase
  - 20°C
    - ΔH (advantageous) and TΔS (disadvantageous) decrease; TΔS change dominates
  - higher T
    - small change in ΔG
    - ΔH increases and becomes dominant
      - disadvantageous for enthalpy
      - advantageous for free energy
      - interactions are sacrificed for increased disorder
  - Explanation: focuses on hydrophobic hydration

Hydrophobic hydration

- Key factors in entropy decrease: *Water structure perturbed*
  - Hole formation - small size of water molecules
  - Water H-bonds near to the apolar solute
    - Stronger and more H-bonds – iceberg model
    - ↕
    - Stronger, but less H-bonds – „two-state" model
- How do these factors contribute to ΔH and TΔS changes?

No general quantitative model available!

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

Affinity and molecular size

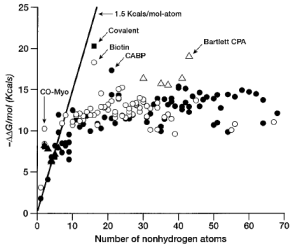


FIG. 1. Free energy of binding (in kcal/mol) for ligands and enzyme inhibitors plotted as a function of the number of nonhydrogen atoms in the ligand. See Table 1. A line with slope of 1.5 kcal/mol and an intercept of 0 is included as a visual aid to analysis. △, Metal ions or metalloenzymes; ▲, small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

PNAS 1999, 96, 9997

Available binding affinity is
- limited
- limit does not increase with size above ~25 nonhydrogen atoms

Summary

- Binding thermodynamics – characteristic to 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