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 change - 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
    - Drug discovery application

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

$LP \leftrightarrow L + P$

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

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

$\Delta G = \Delta H - T\Delta S$ (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 $\left(\sim \int e^{-\frac{E(r,p)}{k_B T}} dr dp\right)$

can be calculated for simple systems only

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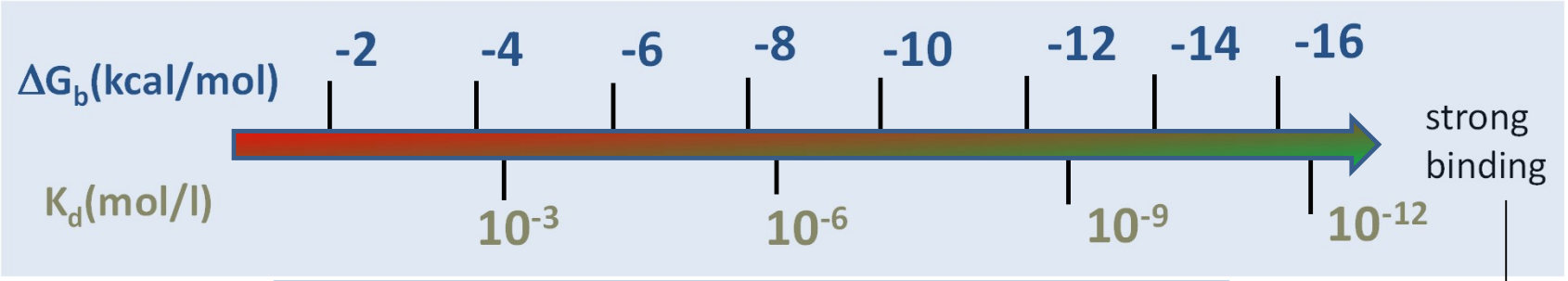
Free energy – Equilibrium constant

Non-covalent binding



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

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

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

A binds "better"

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

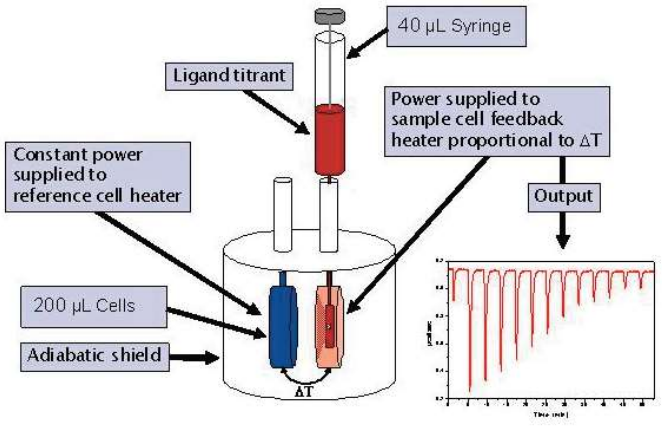
$2.8\ kcal/mol \rightarrow$ ~100

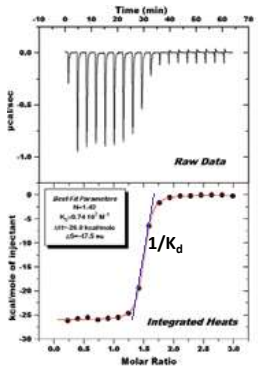
(RT ~ 0.6 kcal/mol; rotational barrier in ethane ~ 2.9 kcal/mol)

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

Measuring binding thermodynamics

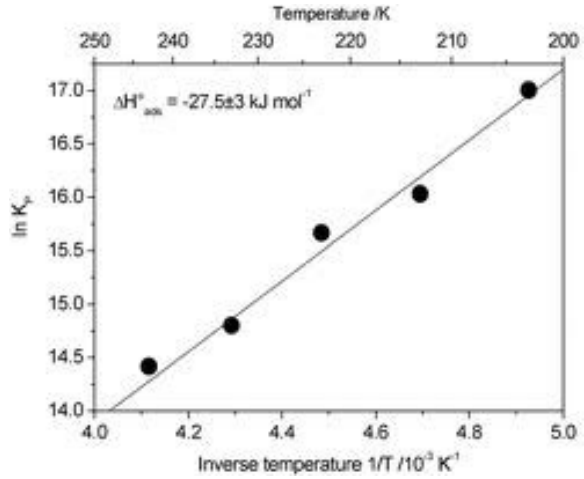
- Isothermal titration calorimetry
  - n, K_d, ΔH $\rightarrow \Delta G$, ΔS ($\Delta G_b = \Delta H_b - T\Delta S_b = RT\ln K_d$)
  - limits:
    - solutions
    - protein quantity (10-100 μg)
    - throughput





Measuring binding thermodynamics

- Van't Hoff analysis
  - $lnK_d = \frac{\Delta H_b}{RT} - \frac{\Delta S_b}{R}$ ($\Delta G_b = \Delta H_b - T\Delta S_b = RT\ln K_d$)
  - 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)



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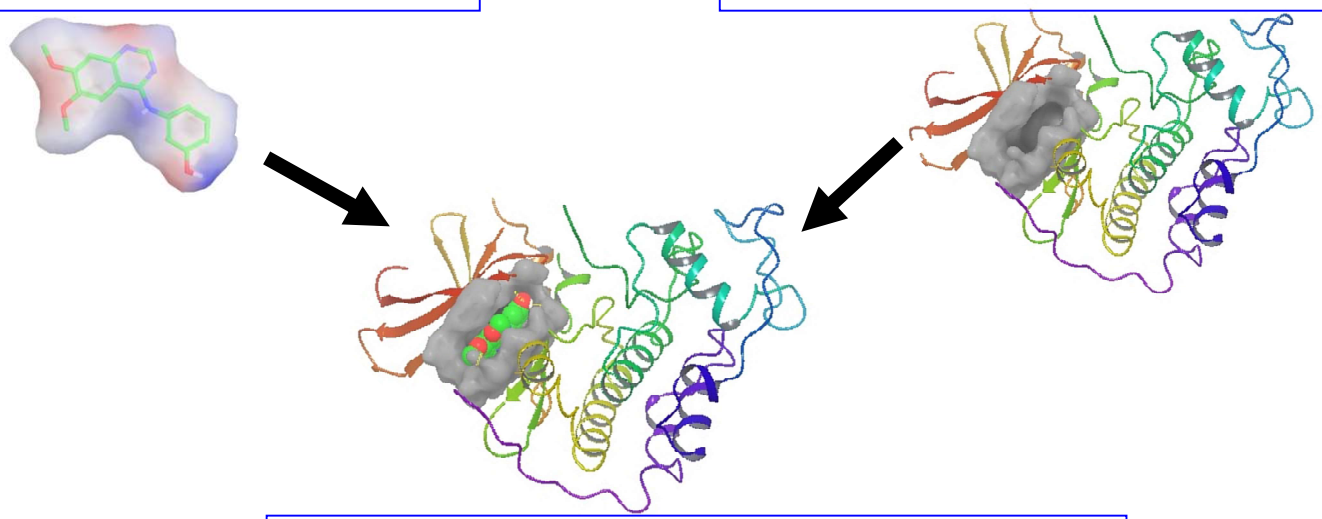
Ligand-protein binding “steps”

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

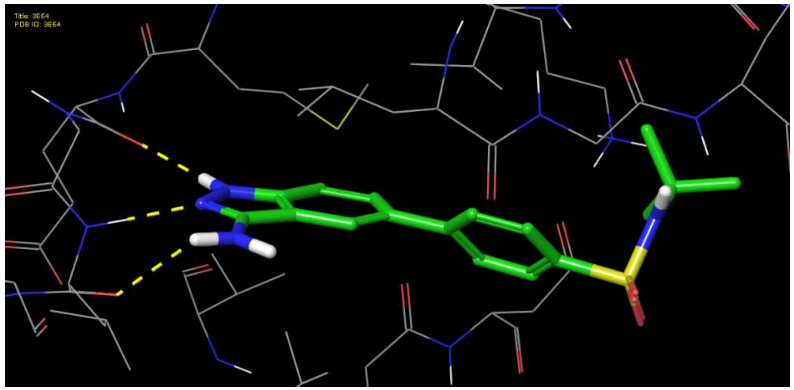
- desolvation (ligand+protein)
  - beneficial ΔS (change in water structure)
  - disadvantageous ΔH
- Conformational change (ligand+protein)
  - disadvantageous ΔH (optimal before binding)
- Ligand-protein interactions
  - beneficial ΔH (polar and van der Waals interactions)
  - disadvantageous ΔS (restricted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

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

Qualitative binding thermodynamics

- ΔG, ΔH, ΔS can be assigned to steps from one state to another – state functions

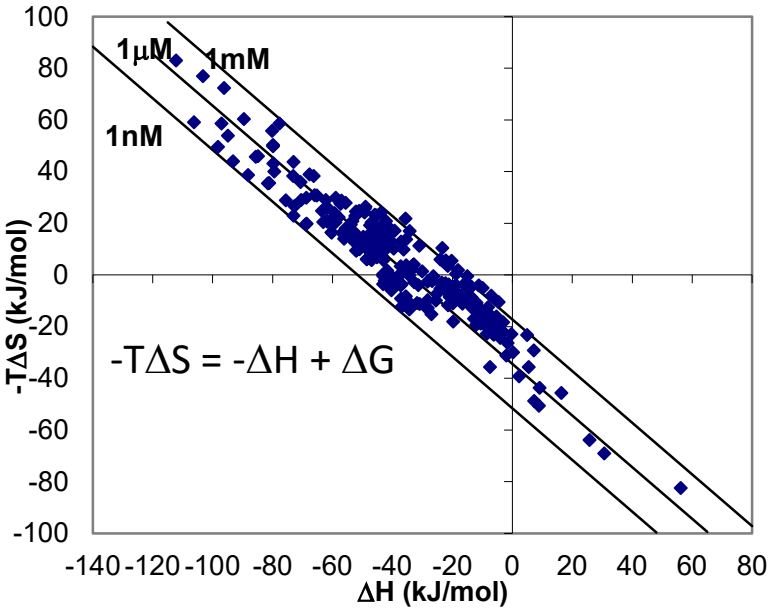
- Assigning ΔG, ΔH, ΔS to structural elements is problematic
  - Limited additivity
    - ΔH additivity – good approximation
    - ΔS additivity – bad approximation

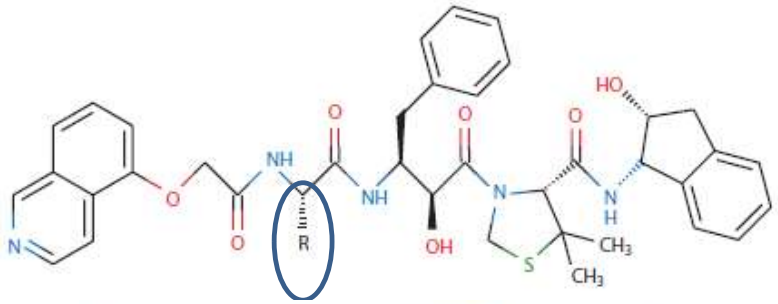


Enthalpy-entropy compensation

Small structural changes of a ligand-protein complex results in significant $\Delta\Delta H$ 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	kcal mol^{-1} ΔG	ΔH	$-T\Delta S$
$-S-CH_3$	−14.87(9)	−8.2(2)	−6.67(9)
$-SO_2CH_3$	−14.6(2)	−12.1(6)	−2.5(2)

Annu. Rev. Biophys. 2013. 42:121–42

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

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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 - water -> apolar-apolar, water-water

  – Breaking apolar contacts and removing molecule

  – Filling empty space in the apolar medium

  – Hole formation in water

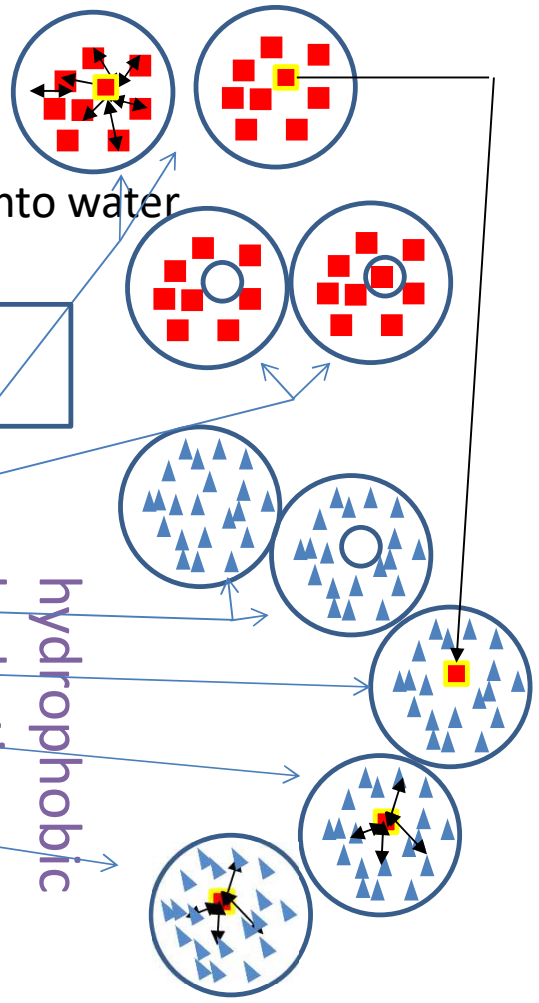
  – Inserting the apolar substance

  – Formation of solute-solvent interactions

  – Reorganization of water structure

  hydrophobic hydration

  ΔG positive



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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
    - 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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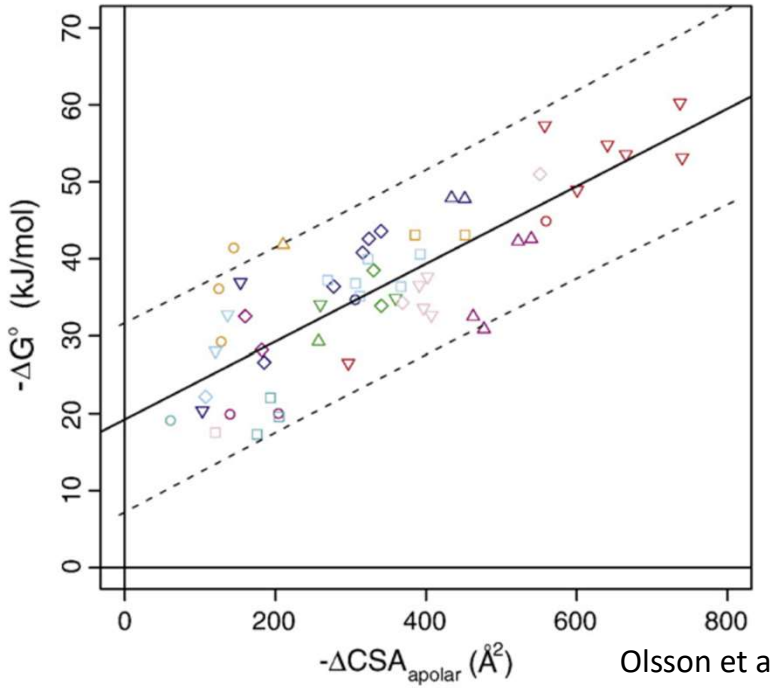
Apolar surface and binding free energy



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

Affinity and molecular size

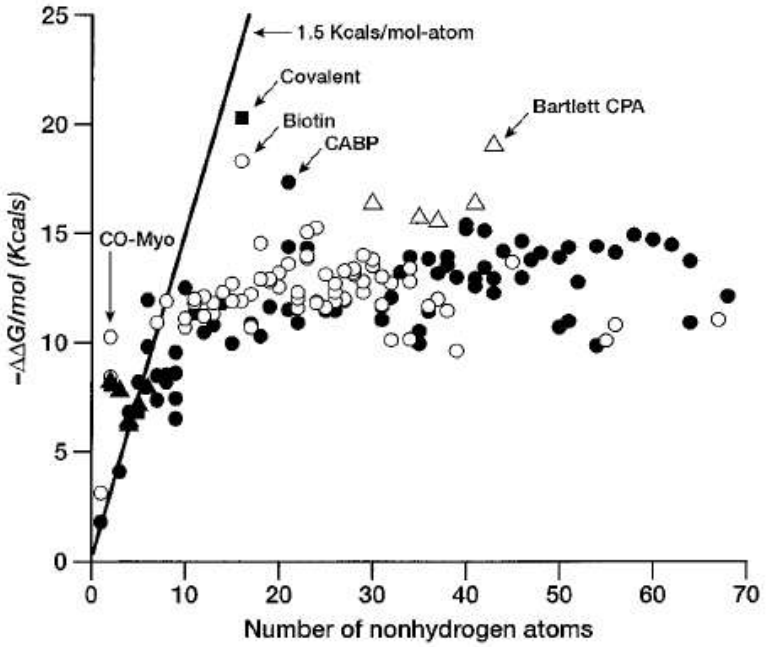


FIG. 1. Free energy of binding (in kcal/mol) for ligands and enzyme inhibitors plotted as a function of the number of nonhydrogen atoms in the ligand. See Table 1. A line with slope of 1.5 kcal/mol and an intercept of 0 is included as a visual aid to analysis. △, Metal ions or metalloenzymes; ▲, small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