

# 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

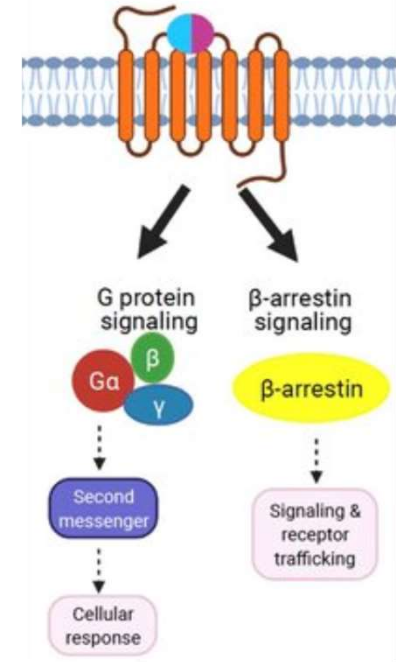
# Outline

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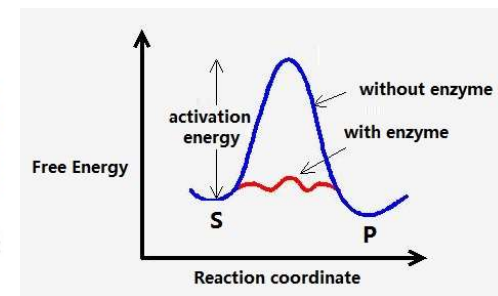
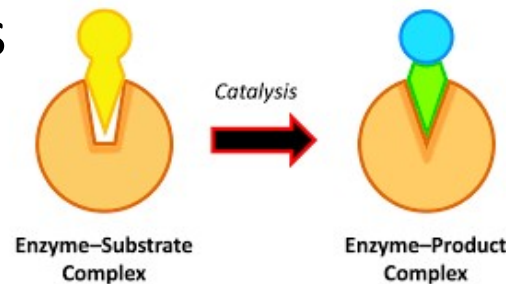
- Very fast estimation of ligand-protein interactions
  - Docking-scoring
  - Drug discovery application

# 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



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

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

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

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

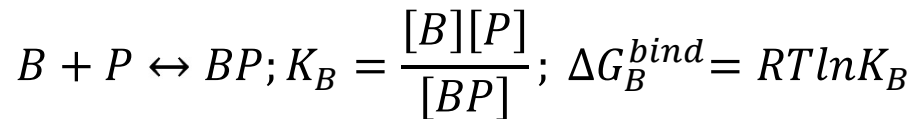
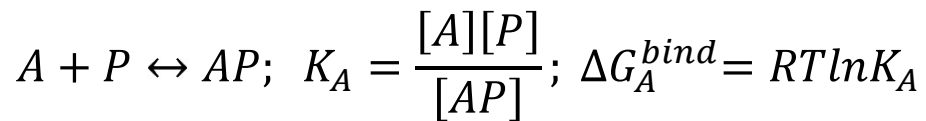
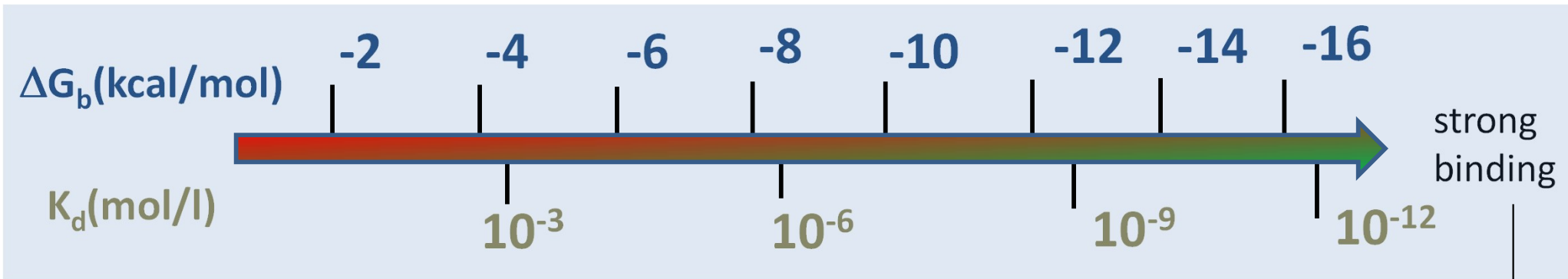
$$F = -k_B T \ln Z,$$

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

can be calculated for simple systems only

# Free energy – Equilibrium constant

Non-covalent binding



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

A binds "better"

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

$$2.8 \text{ kcal/mol} \rightarrow \sim 100$$

( $RT \sim 0.6 \text{ kcal/mol}$ ; rotational barrier in ethane  $\sim 2.9 \text{ kcal/mol}$ )

biotin-avidin  
 $\Delta G = -20.4 \text{ kcal/mol}$   
 $K_d = 10^{-15} \text{ mol/l}$

# Measuring binding thermodynamics

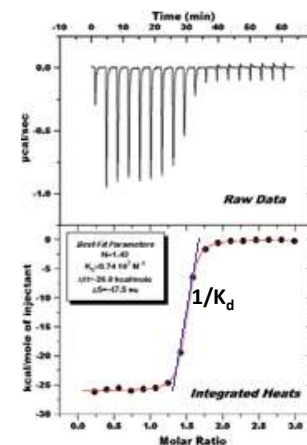
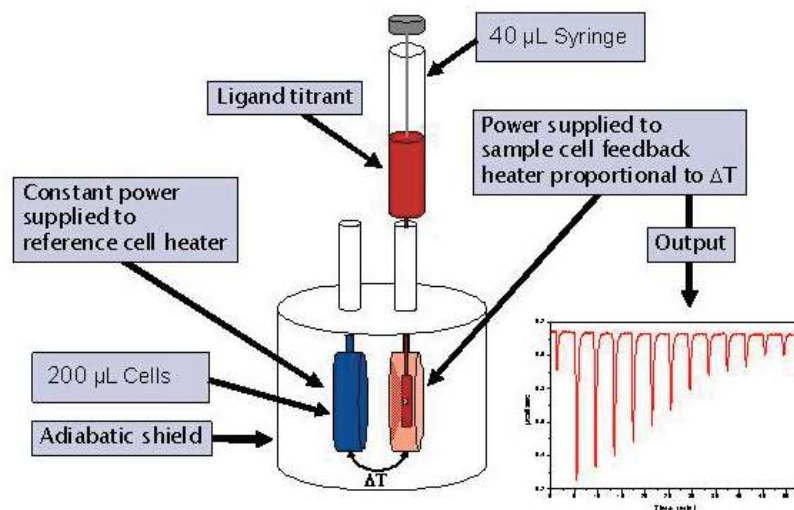
- Isothermal titration calorimetry

- $n, K_d, \Delta H \rightarrow \Delta G, \Delta S$

$$(\Delta G_b = \Delta H_b - T\Delta S_b = RT \ln K_d)$$

- limits:

- solutions
    - protein quantity (10-100  $\mu\text{g}$ )
    - throughput



# Measuring binding thermodynamics

- Van't Hoff analysis

- $\ln K_d = \frac{\Delta H_b}{RT} - \frac{\Delta S_b}{R}$  ( $\Delta G_b = \Delta H_b - T\Delta S_b = RT \ln K_d$ )

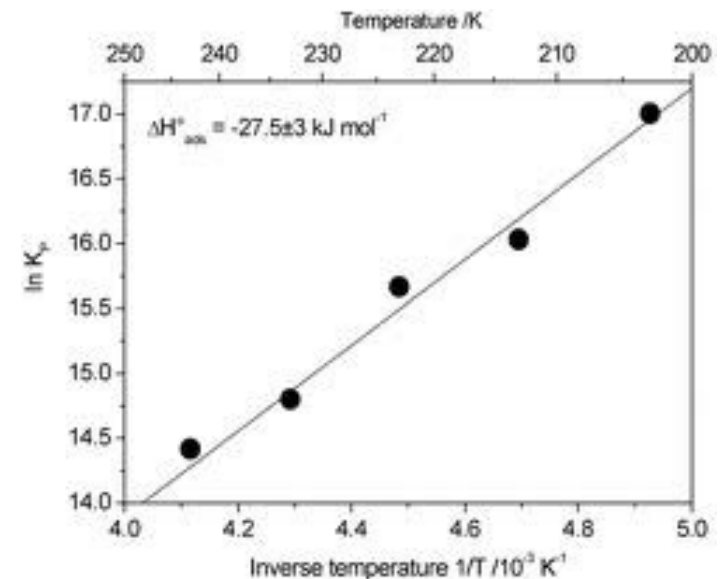
- Measure  $K_d$  at various  $T \rightarrow \Delta H$  és  $\Delta S$

- Experimental techniques

- Radioligand displacement
    - Mass spectrometry
    - Chromatography
    - Surface plasmon resonance (SPR)
    - ...

- limits

- $\Delta H$  depends on  $T$
    - extrapolation ( $\Delta S: 1/T=0$ )



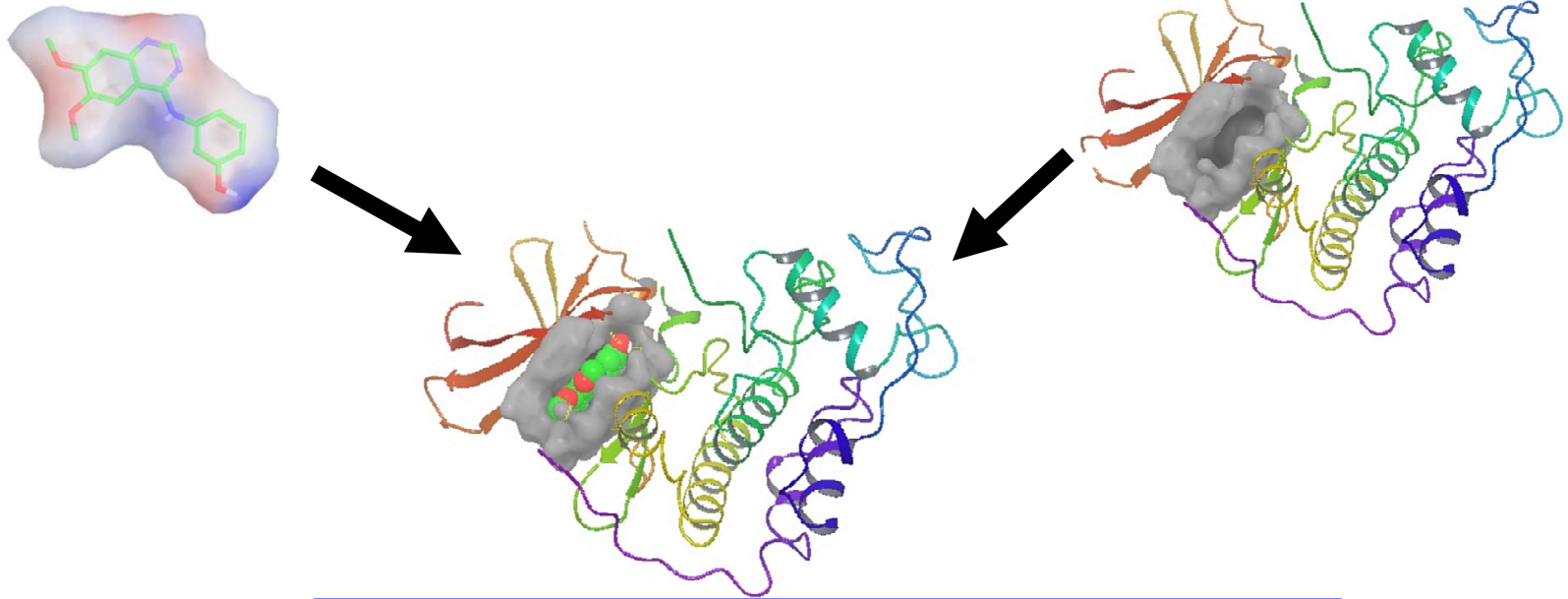
# Ligand-protein binding “steps”

## 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

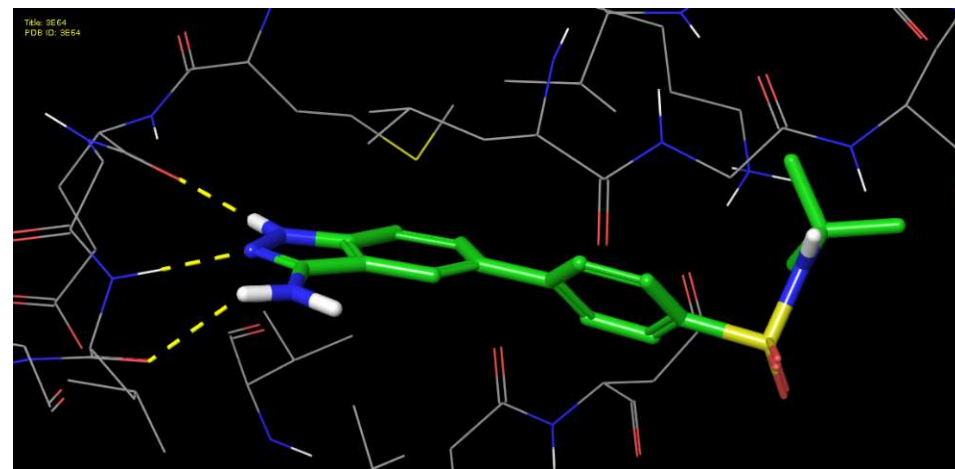
# Qualitative binding thermodynamics

- desolvation (ligand+protein)
  - beneficial  $\Delta S$  (change in water structure)
  - disadvantageous  $\Delta H$
- Conformational change (ligand+protein)
  - disadvantageous  $\Delta H$  (optimal before binding)
- Ligand-protein interactions
  - beneficial  $\Delta H$  (polar and van der Waals interactions)
  - disadvantageous  $\Delta S$  (restricted motion)

$\Delta G$  is a sum of several terms with positive and negative signs

# Qualitative binding thermodynamics

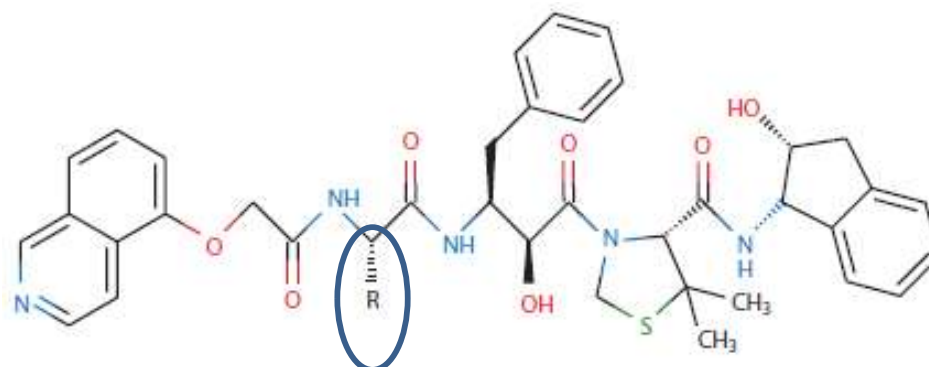
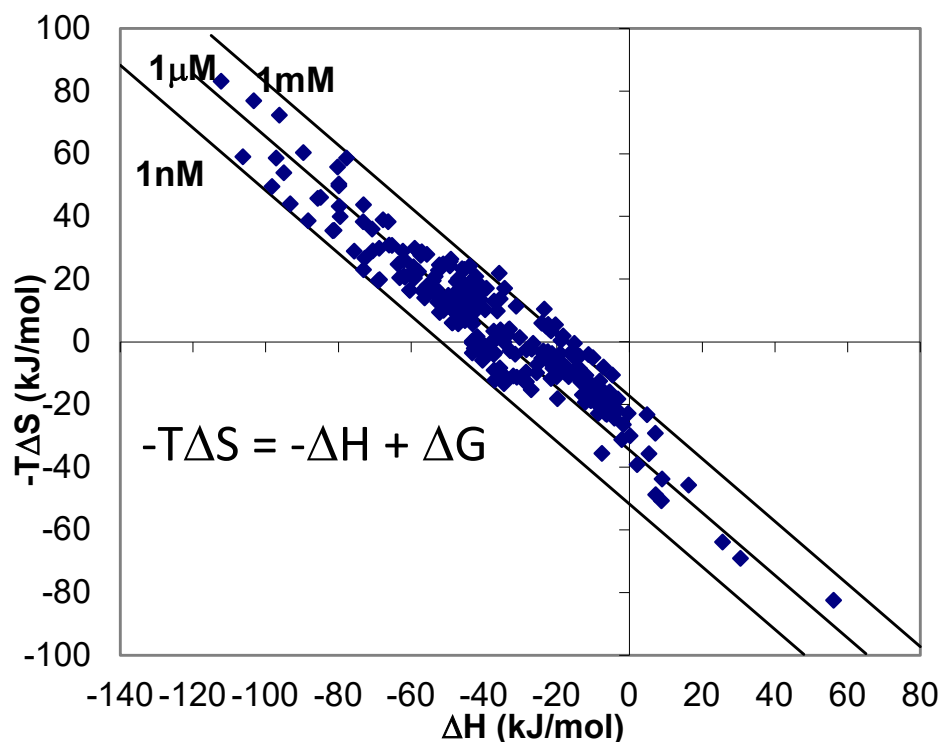
- $\Delta G$ ,  $\Delta H$ ,  $\Delta S$  can be assigned to steps from one state to another – state functions
- Assigning  $\Delta G$ ,  $\Delta H$ ,  $\Delta S$  to structural elements is problematic
  - Limited additivity
    - $\Delta H$  additivity – good approximation
    - $\Delta S$  additivity – 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	$\Delta G$	$\Delta H$	$-T\Delta S$
-S-CH <sub>3</sub>	-14.87(9)	-8.2(2)	-6.67(9)
-SO <sub>2</sub> CH <sub>3</sub>	-14.6(2)	-12.1(6)	-2.5(2)

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

**$\Delta G$  changes are limited  
(within ~35kJ/mol)**

# Hydrophobic effect

- Hydrophobic effect:

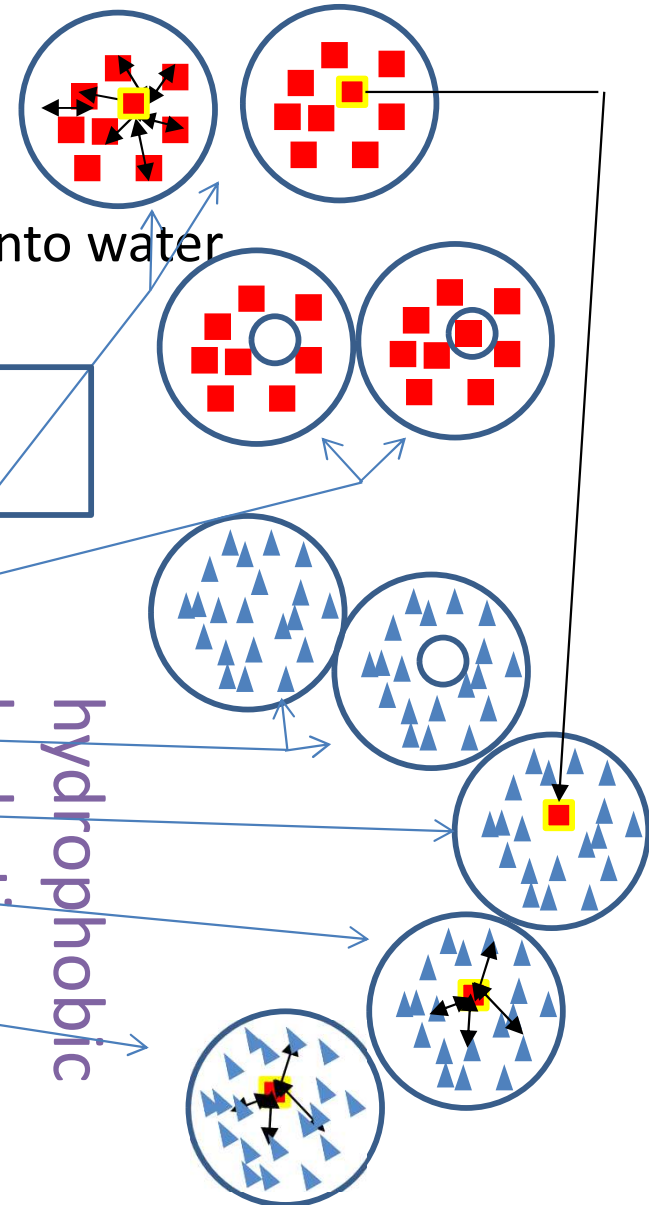
Bringing an apolar substance from its apolar solvent into water  
(hydrocarbon  $\rightarrow$  water)

*analogy: desolvation upon ligand-protein binding (inverse)  
apolar moieties - water  $\rightarrow$  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

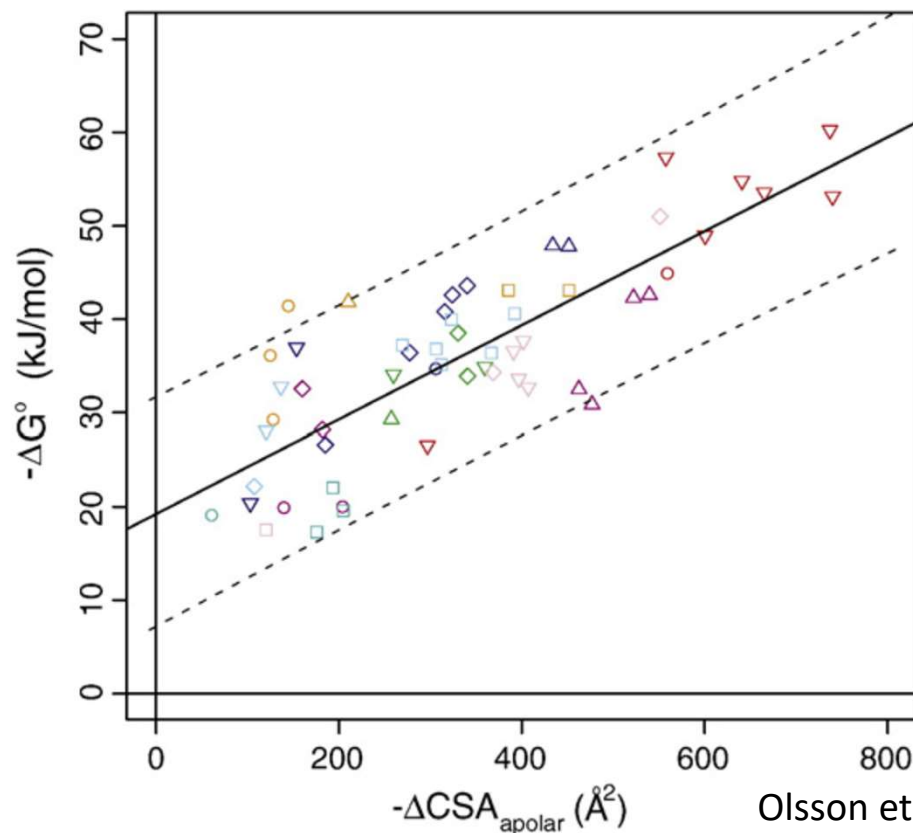
**$\Delta G$  positive**



# Hydrophobic effect

- $\Delta G$  increase
  - 20°C
    - $\Delta H$  (advantageous) and  $T\Delta S$  (disadvantageous) decrease;  $T\Delta S$  change dominates
  - higher T
    - small change in  $\Delta G$
    - $\Delta H$  increases and becomes dominant
      - disadvantageous for enthalpy
      - advantageous for entropy and 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!

# Apolar surface and binding free energy



- 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

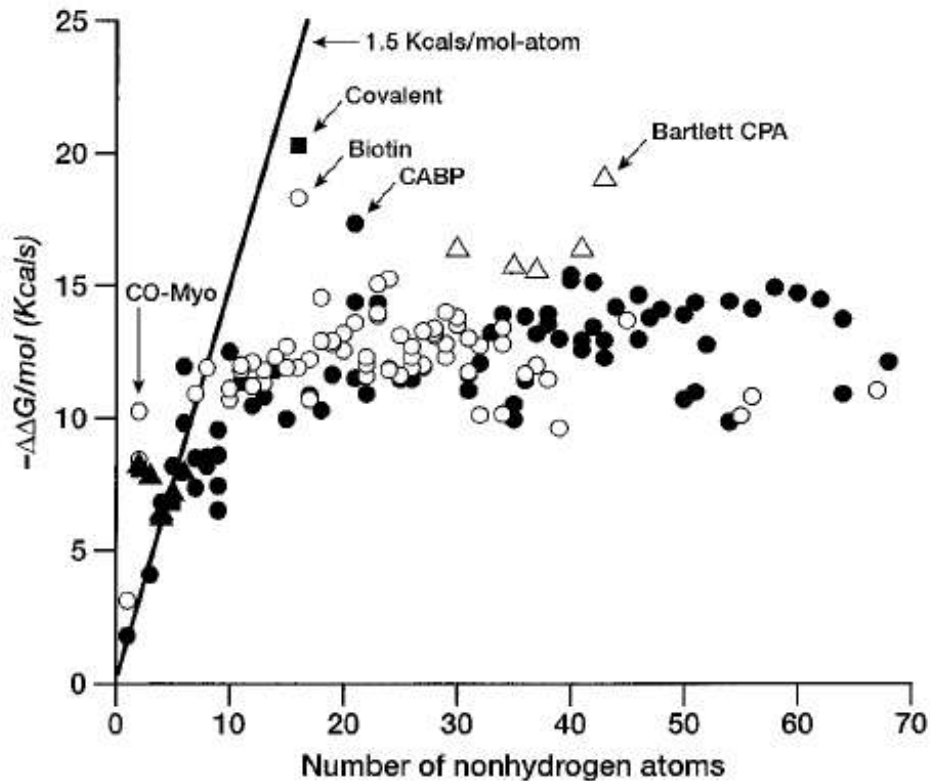


FIG. 1. Free energy of binding (in kcal/mol) for ligands and enzyme inhibitors plotted as a function of the number of nonhydrogen atoms in the ligand. See Table 1. A line with slope of 1.5 kcal/mol and an intercept of 0 is included as a visual aid to analysis.  $\Delta$ , Metal ions or metalloenzymes;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

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
  - $\Delta G_{\max}$  – available binding free energy increase fast with ligand size for small ligands and is insensitive to size for larger ligands