Applied Statistical Thermodynamics

# Calculation of free energy from molecular simulations

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# Schedule

| Time | Mon. 20/11/17 | Tue. 21/11/17 | Wed. 22/11/17 | Thur. 23/11/17 | Fri. 24/11/17 | Mon. 27/11/17 | Tue. 28/11/17 | Wed. 29/11/17 | Thur. 30/11/17 | Fri. 01/12/17 |
|---|---|---|---|---|---|---|---|---|---|---|
| 9:00 | Free Time | Welcome JAG | Free Time | Free Time | Free Time | Free Time | Free Time | Free Time | Free Time | Free Time |
| 9:30 | | Lecture 1: Overview and Introduction JAG | Lecture 4: Thermodynamics JAG | Lecture 7: Classical Mechanics I JAG | Lecture 10: Ensembles I CO | Lecture 13: Free energies: reaction coordinates JAG | Lecture 16: Calculating properties from simulations JAG | Lecture 19: Electrostatics WvG | Lecture 22: On the ethics of the academic endeavour: where do we go? | Lecture 24: Left-overs/ questions and future perspectives WvG |
| 10:15 | | Break | Break | Break | Break | Break | Break | Break | | Break |
| 10:30 | | Lecture 2: Molecular Simulations MD/SD/MC CO | Lecture 5: Force-Field Development CO | Lecture 8: Classical Mechanics II JAG | Lecture 11: Ensembles II JAG | Lecture 14: Boundary Conditions I WvG | Lecture 17: Comparison with Experiments WvG | Lecture 20: Polarization WvG | WvG | Lecture 25: Students plans I |
| 11:15 | | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break |
| 11:45 | | Lecture 3: How to simulate using GROMOS CO | Lecture 6: Structure Refinement CO | Lecture 9: Analizing with GROMOS CO | Lecture 12: Free energies: alchemistry CO | Lecture 15: Boundary Conditions II WvG | Lecture 18: Searching & Enhanced Sampling WvG | Lecture 21: Multi -resolution simulations WvG | Lecture 23: QM/MM WvG | Lecture 26: Students plans II |
| 12:30 | | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Break for lunch, self-study, discussion.* | Tutorial 10: ** |
| 14:00 | Registration<br>Tutorial 1 Linux, NLHPC and OS-dongle installation | Tutorial 2: Running MD GROMOS Tutorial | Tutorial 3: Running MD Students Plans | Tutorial 4: Running MD Students Plans | Tutorial 5: Running MD Students Plans | Tutorial 6: Statistical Mechanics exercises | Tutorial 7 Analizing MD: GROMOS Tutorial | Tutorial 8: Analizing MD: Students Plans | Tutorial 9: Analizing MD: Students Plans | How to prepare a barbecue |
| 16:00 | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | Coffee Break | |
| 17:30 | End of session | End of session | End of session | End of session | End of session | End of session | End of session | End of session | End of session | |
| 20:30 | Free time | Free time | Free time | Free time | Beer, Science & Friendship Good Stock Bar | Free time | Free time | Free time | Free time | Farewell |

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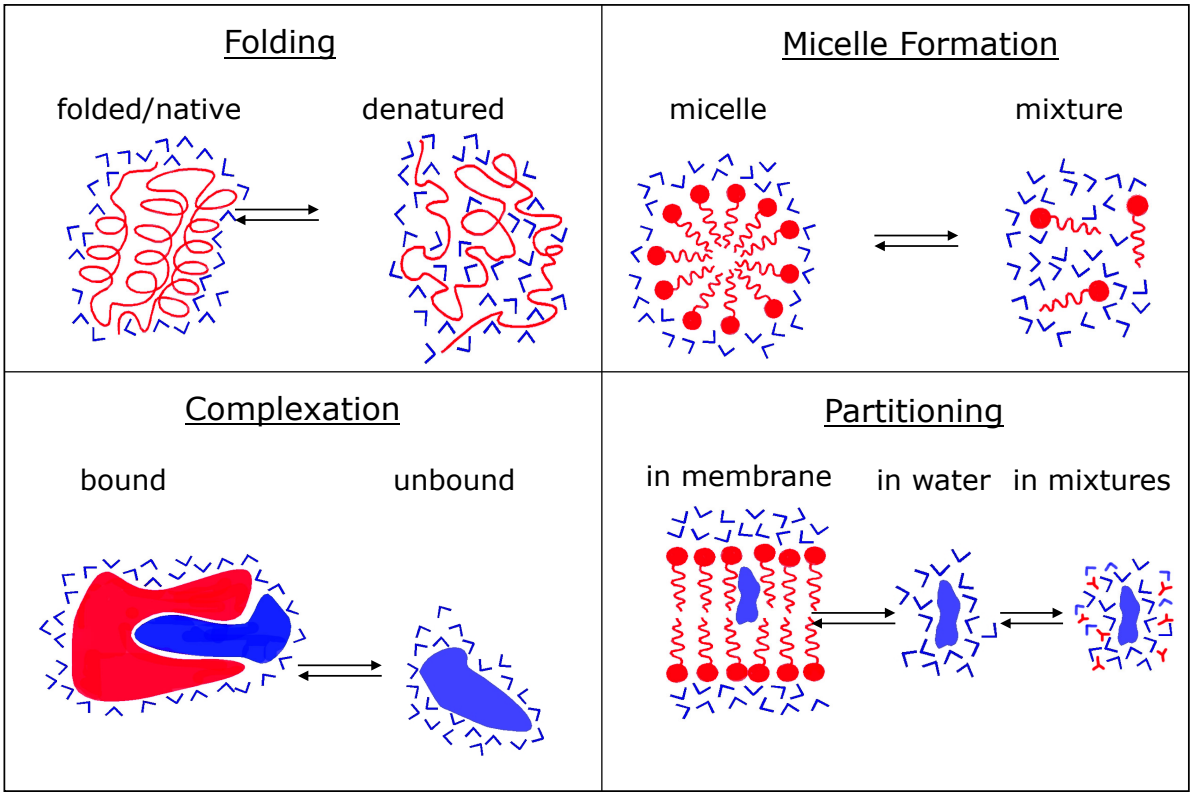
# Outline

- **Free energies and drug design**
  - Enthalpy, Entropy and Free Energy
  - Actors and effects
- **Calculation of free energies from statistical mechanics**
  - Free energy perturbation
  - Thermodynamic integration
  - Slow / fast growth
  - One step perturbations
- **Conclusions**

# Processes: Thermodynamic Equilibria



# Definitions

**free energy**

The driving force for all physical processes

Free energy $\Delta A$; Free enthalpy $\Delta G$ ($=\Delta A + p\Delta V$)

**energy**

The internal energy of the systems

Energy $\Delta E/\Delta U$; Enthalpy $\Delta H$ ($=\Delta E + p\Delta V$)

**entropy**

"The number of realization possibilities"

Entropy $\Delta S$

**Helmholtz / Gibbs equations**

$\Delta A = \Delta E - T\Delta S$; $\Delta G = \Delta H - T\Delta S$

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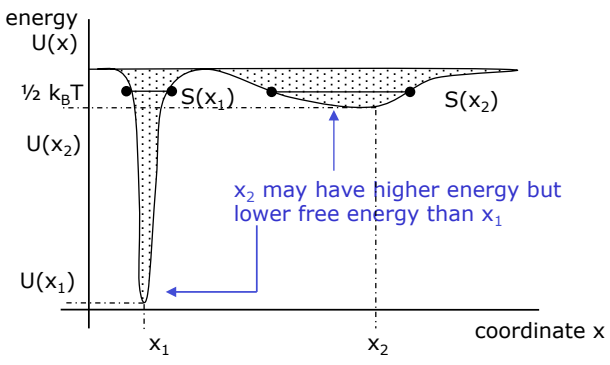
# Statistical mechanics



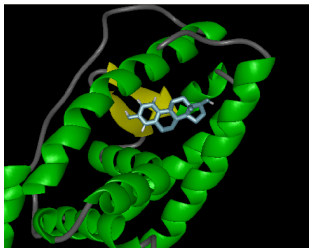

mechanics: a state is characterised by ***one* minimum energy structure** (global minimum)

statistical mechanics: a state is characterised by ***an ensemble* of structures** or configurations or conformations

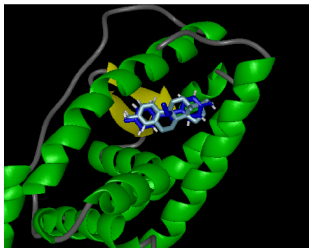
# Lock and key: binding affinity

- Predict binding affinity to prioritize compounds,
  safe money on the experiments

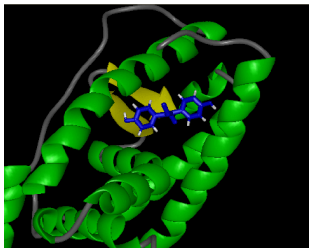
The keyhole: the *active site* in a protein



A fitting key: the *active site* with a known binder



Old and new molecules superposed



A new key? The *active site* containing a new molecule

- Interaction between the protein and a ligand
- Affinity is determined by the Free Energy, (≠ potential energy)
- Free energy includes entropy (number of possible conformations, sampling!)

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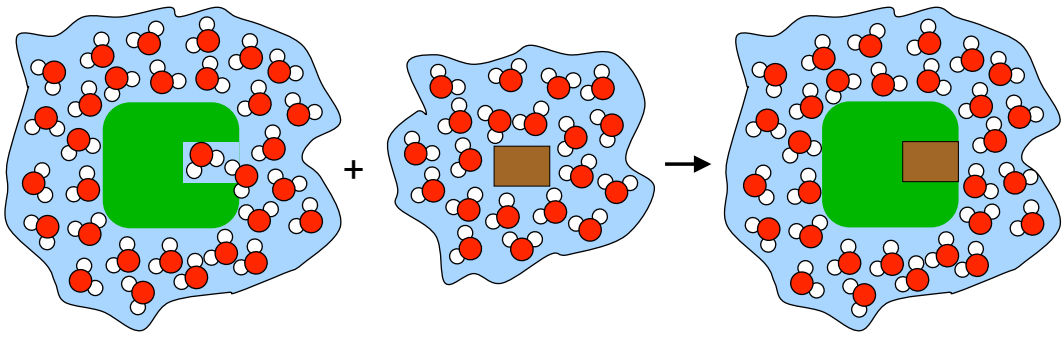
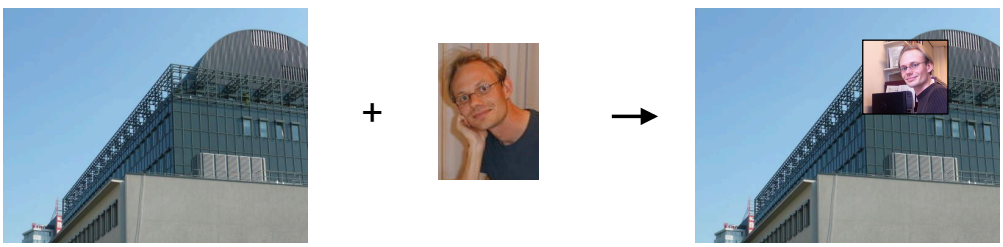
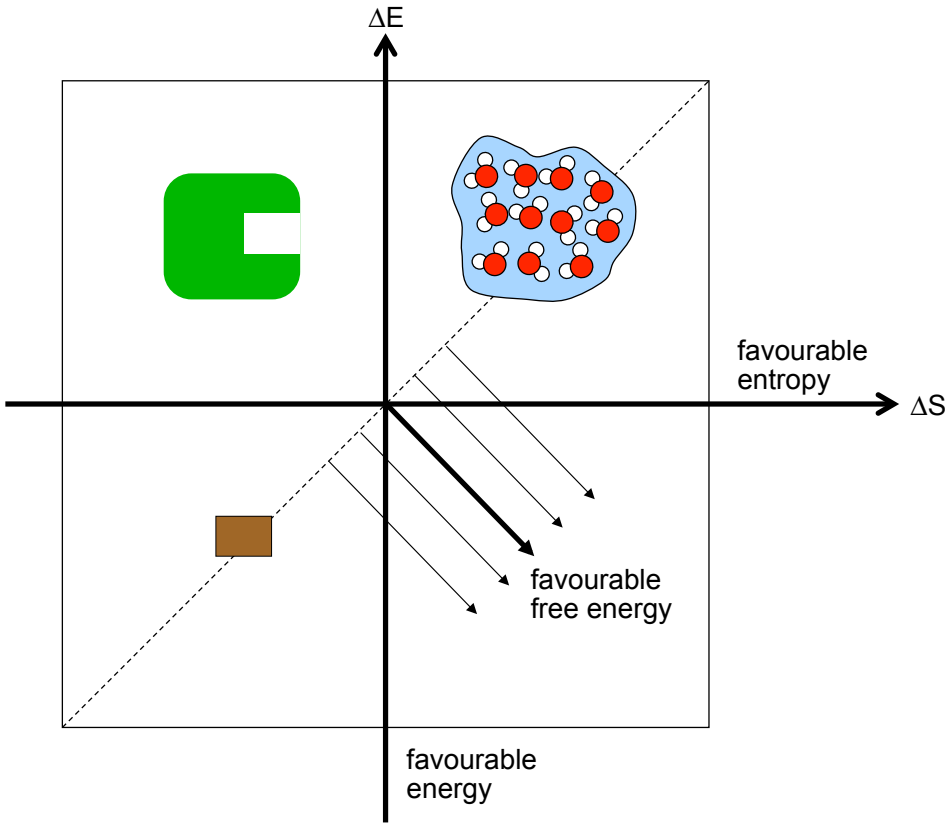
# The system





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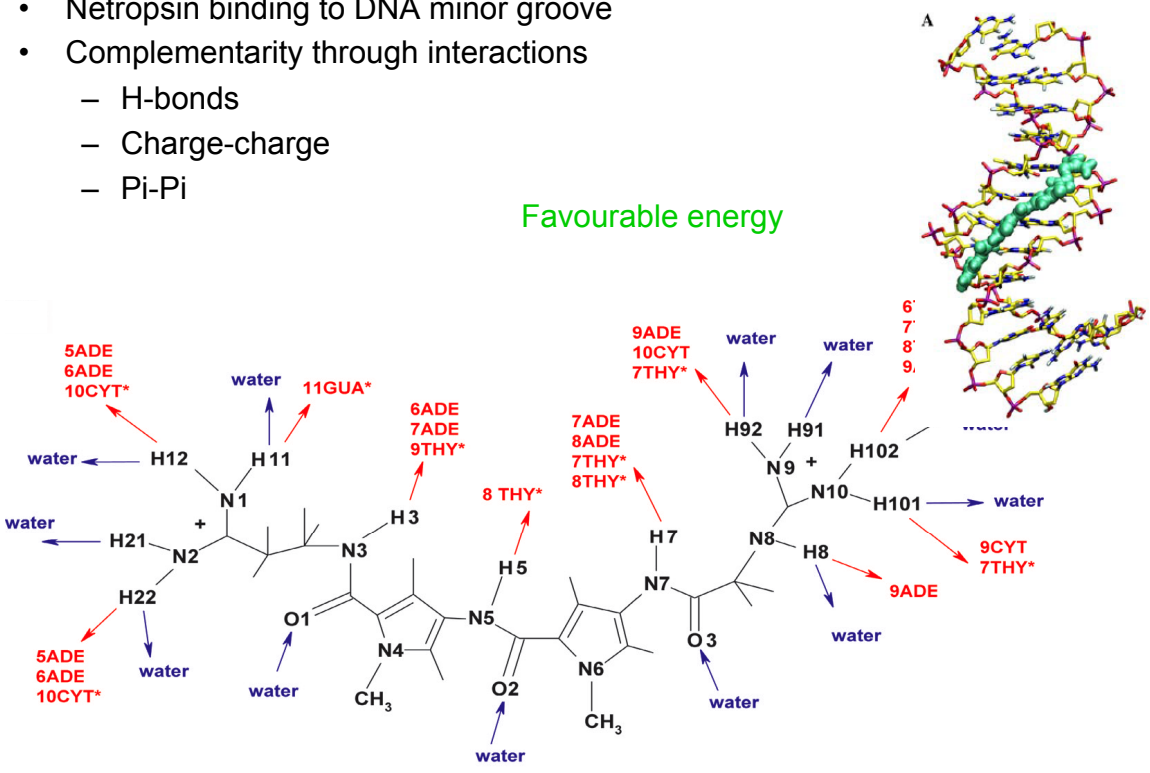
# Guest-host complementarity

- Netropsin binding to DNA minor groove
- Complementarity through interactions
  - H-bonds
  - Charge-charge
  - Pi-Pi

Favourable energy



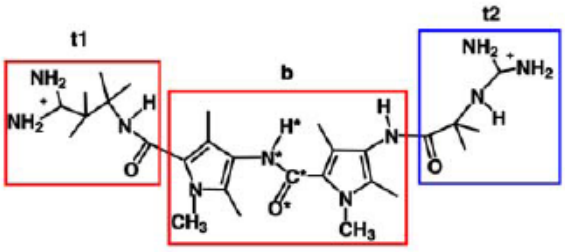

J. Dolenc, C. Oostenbrink, J. Koller, W.F. van Gunsteren, *Nucl. Acid Res.* (2005) 33:725

# Entropy loss upon binding

- Conformational entropy calculated using Schlitter's formula
  - Netropsin and Distamycin A
  - In solution and when bound to DNA

| | $S_{free}$ | $S_{bound}$ | $\Delta S_{bind}$ |
|---|---|---|---|
| Netropsin | 862 | 735 | -127 |
| Distamycin | 902 | 798 | -104 |

in J/K/mol




Unfavourable entropy

Entropy loss mostly in the tails of the molecules

J. Dolenc, R. Baron, C. Oostenbrink, J. Koller and W.F. van Gunsteren, *Biophys J.* (2006) 91:1460

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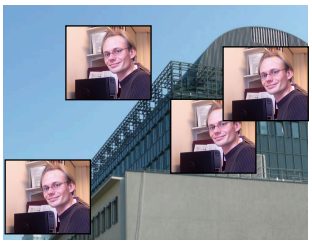
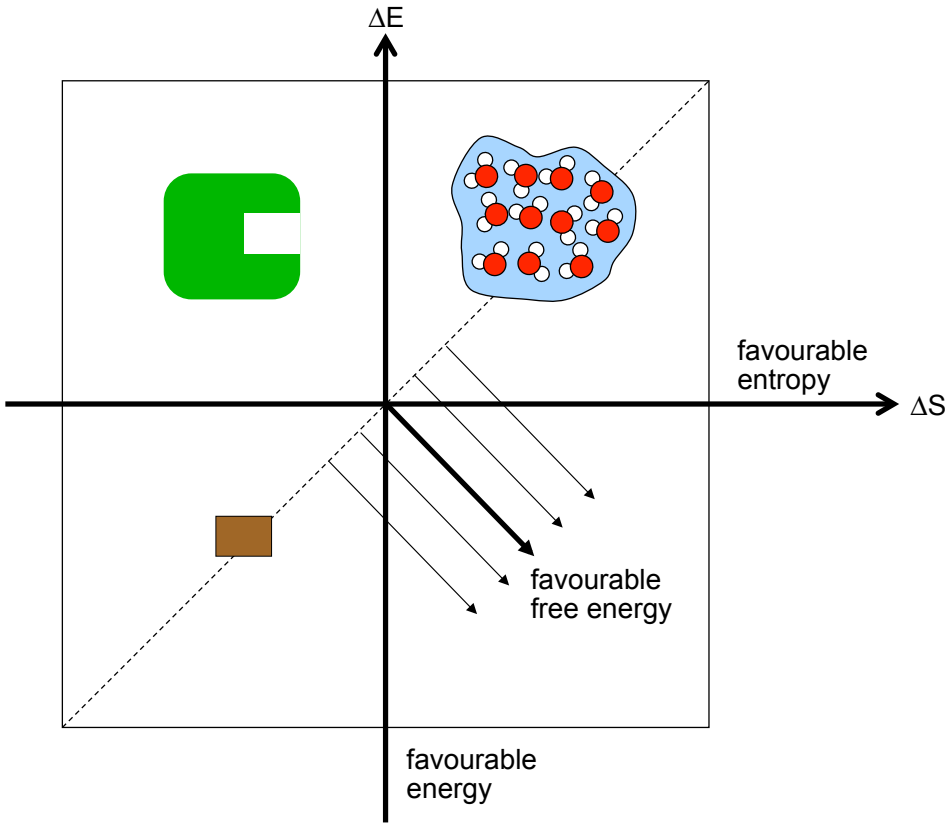
# My affinity for BOKU

- Affinity is a combination of energy and entropy
  - Energy:
    Interactions with people at BOKU are mostly favourable
    (and I am even getting paid for it!)
  - Entropy:
    How much freedom do I have? At BOKU and elsewhere?

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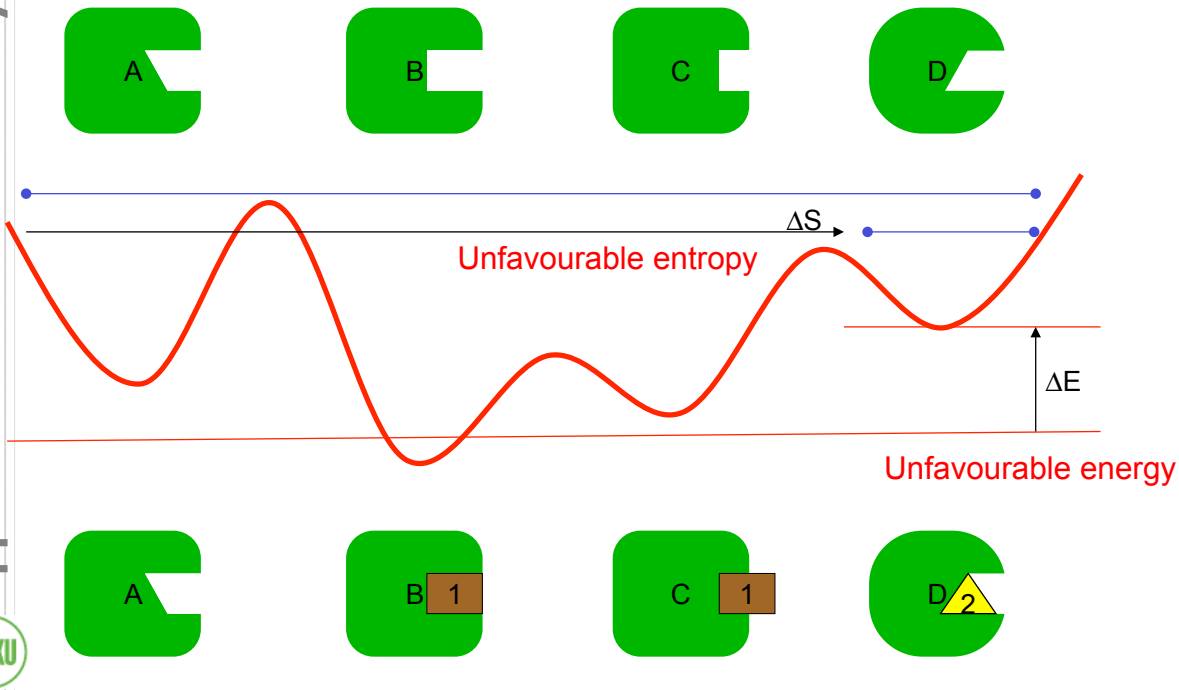
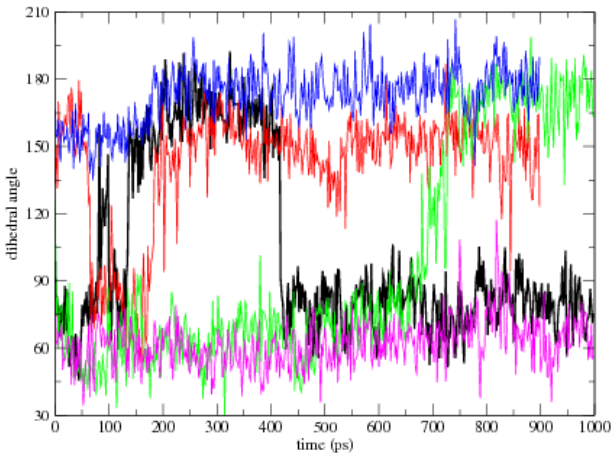
## Conformational selection

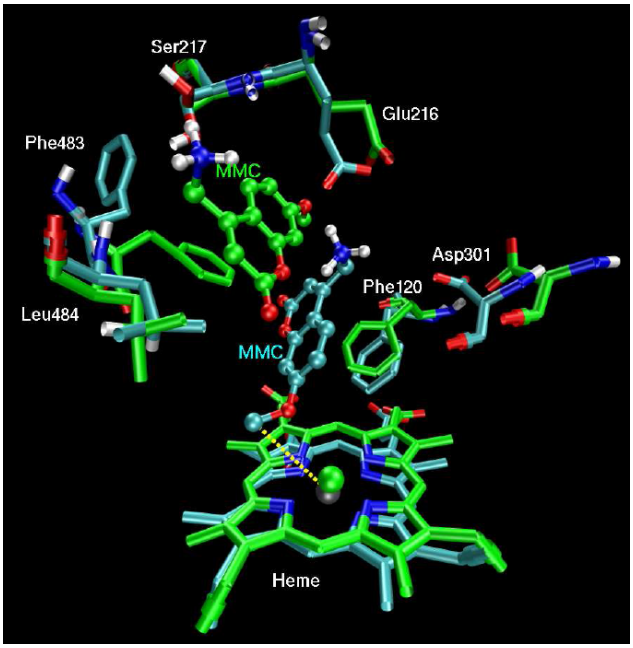


# Binding to multiple structures

- Docking of 65 substrates in 2500 protein CYP2D6 structures
- Side-chain of Phe483 occupies multiple conformational states

- Different substrates have different preference




J. Med. Chem. 51 (2008) 7469 - 7477

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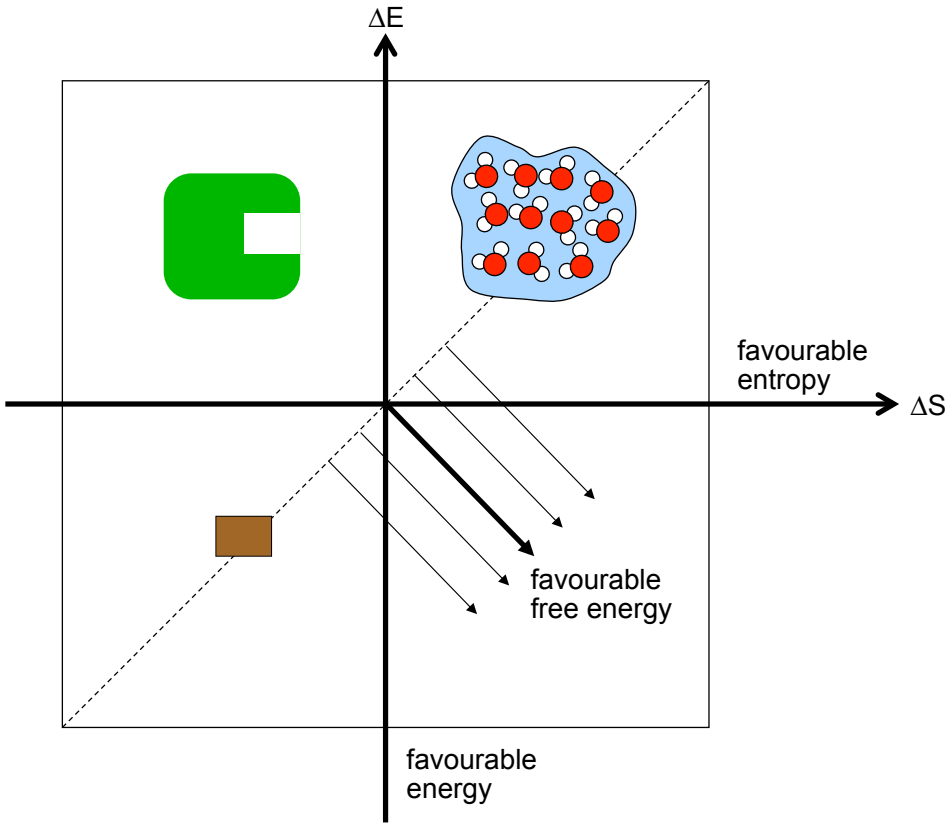
BOKU

# The shape of the host

- Before we could come, people had to move and squeeze together
- Furniture was moved around

Unfavourable energy
Unfavourable entropy

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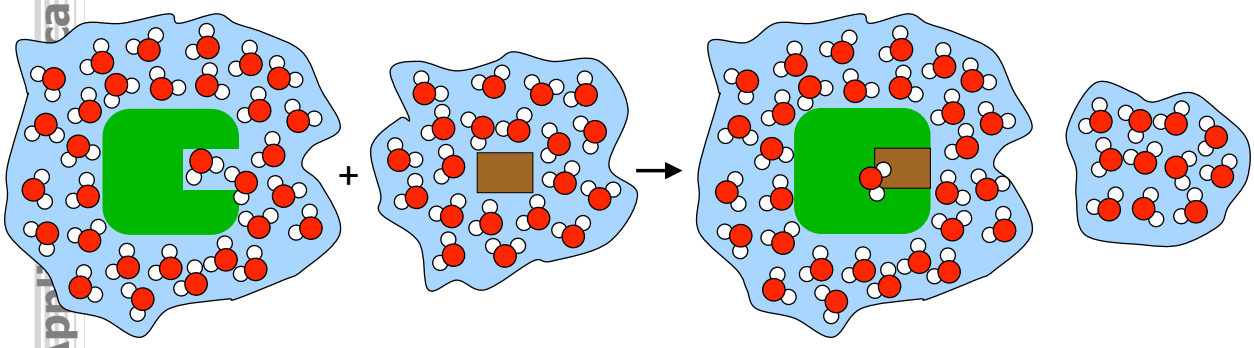
# Desolvation of protein and ligand

- Loss of solute-solvent hydrogen bonds
- Release of ordered water to bulk
  - Hydrophobic effect
- Structural water molecules in the active site

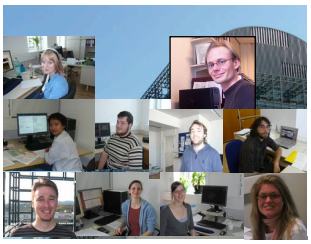
Unfavourable energy
Favourable entropy



# People and knowledge

+

students and co-workers…



strengthen the interaction

+

even if it hurts to say goodbye…
they have lot's of possibilities

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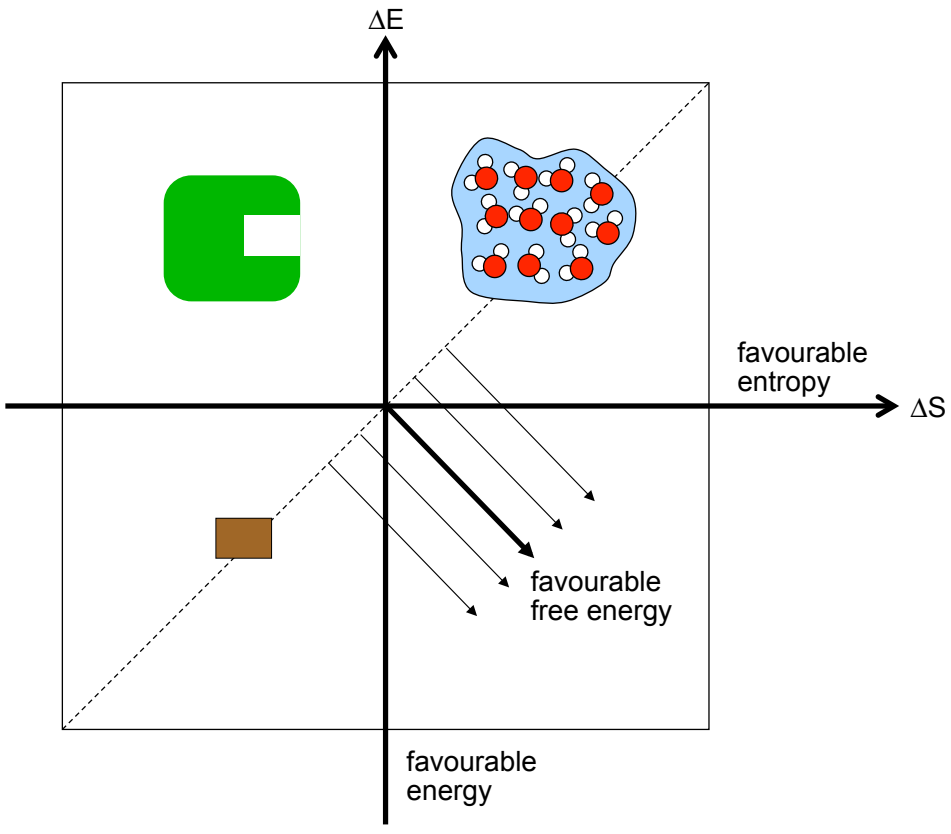
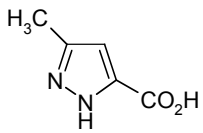
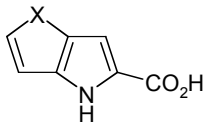


# Example: DAAO inhibitors

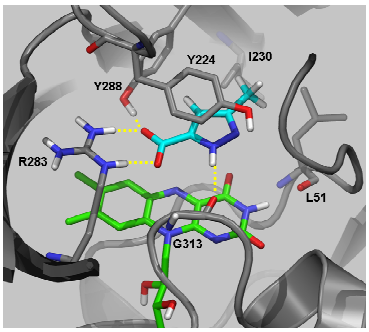
- Three inhibitors of the enzyme D-amino acid oxidase were studied

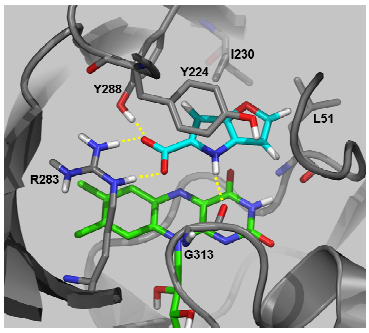



**1**




**3** X = O
**4** X = S




- Molecular dynamics simulations of the ligands in solution and bound to the protein, using GROMOS (parameter set 45A4)

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# Analysis of the simulations

Number of hydrogen bonds

| | **1** | **3** | **4** |
|---|---|---|---|
| *Free in solution* | 9.98 | 8.47 | 7.60 |
| *In complex:* | | | |
| Tyr228 OH | 1.00 | 1.00 | 1.00 |
| Arg283 HE | 1.03 | 0.99 | 1.00 |
| Arg283 HH | 1.28 | 1.35 | 1.34 |
| Gly313 | 0.91 | 0.50 | 0.50 |
| $H_2O$ | 2.15 | 1.87 | 0.61 |
| Loss of H-bond | 3.61 | 2.76 | 3.15 |

water is being released

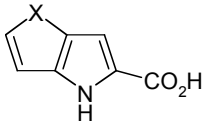
water still plays a role

Entropy (kJ/mol)

| | calculated conformational entropy ligand | calculated conformational entropy protein | full entropy experiment |
|---|---|---|---|
| $-T\Delta S$ (3) | 15.3 | 17.0 | -5.0 |
| $-T\Delta S$ (4) | 15.1 | 3.3 | -15.9 |
| $-T\Delta\Delta S$ | 0.2 | 13.7 | 10.9 |

the ligands are equally rigid

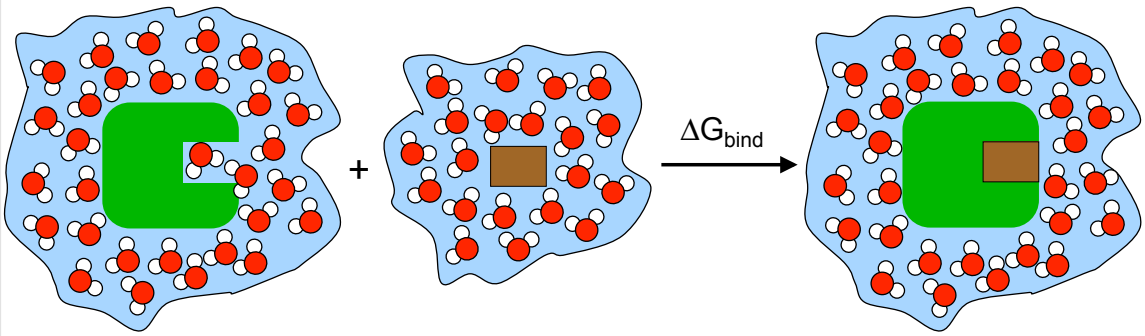
protein loss of entropy explains experiment?




**3** X = O
**4** X = S

# Free energies of binding

- We hope to calculate the free energy of ligand binding
  - And need to consider all energetic and entropic contributions




$$\Delta G_{bind} = G_{complex} - G_{ligand} - G_{protein}$$

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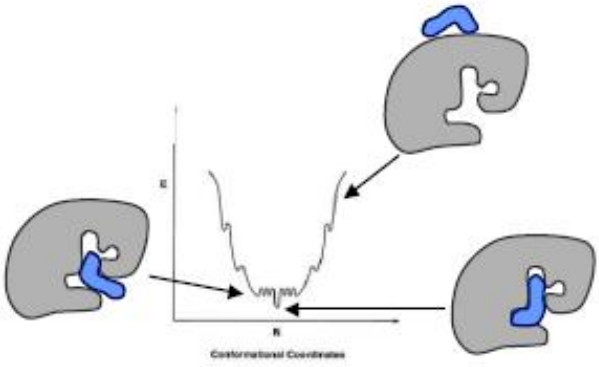
# Scoring problem

- Affinity is determined by free energy
- Needs to be calculated fast and accurately
- The details are on a small energy scale!

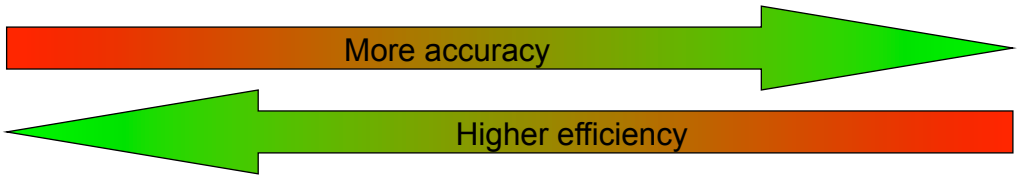
Goal: estimate binding affinity for given protein, ligand, pose, and conformations



# Protein ligand interactions

- Methods to calculate the interactions for different problems

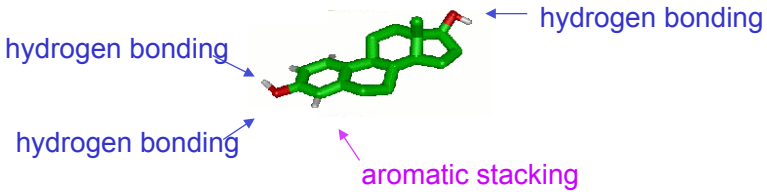
| | DOCKING: | LIE method: | ONE STEP: | FEP/TI: |
|---|---|---|---|---|
| Sampling | e.g. Genetic algorithm | Molecular dynamics | Molecular dynamics enhanced by soft atoms | Molecular dynamics (extensive sampling) |
| Scoring | Empirical scoring function | Linear response | Statistical mechanics | Statistical mechanics |
| Characteristics | Many empirical parameters | Few empirical parameters | No empirical parameters | No empirical parameters |
| | Empirical function | Theoretical basis | Theoretically correct | Theoretically correct |
| | | | Restricted to one framework | |
| | Rigid protein | Protein flexibility | Protein flexibility | Protein flexibility |
| | No solvation | Solvation | Solvation | Solvation |






# Scoring functions

Estimate the binding free energy of a compound with a protein

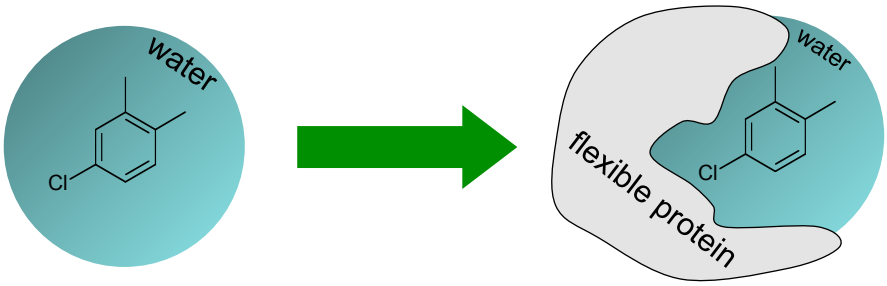



$$\Delta G^{bind} = \Delta G^{HB} + \Delta G^{arom} + \Delta G^{hydr} - T\Delta S^{flex} + \ldots - \ldots$$

Count the number of hydrogen bonds, aromatic interactions etc.

Entropy taken into account in a very rough (and wrong) way: number of rotatable bonds, fuzzy factors to allow for protein flexibility

# Linear interaction energies



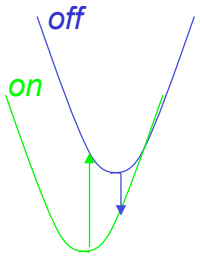

$$\Delta G_{bind} = \beta\left(\left\langle V_{l-s}^{el}\right\rangle_{bound} - \left\langle V_{l-s}^{el}\right\rangle_{free}\right) + \alpha\left(\left\langle V_{l-s}^{vdw}\right\rangle_{bound} - \left\langle V_{l-s}^{vdw}\right\rangle_{free}\right)$$

Derived from theory, $\beta$=1/2, but $\langle V_{l\text{-}s}\rangle_{off}$ = 0 assumed

No theoretical background, empirical equation

- From linear response

$$\Delta G_{sol}^{el} = \tfrac{1}{2}\left(\left\langle \Delta V_{on->off}^{el}\right\rangle_{on} - \left\langle \Delta V_{off-on}^{el}\right\rangle_{off}\right)$$





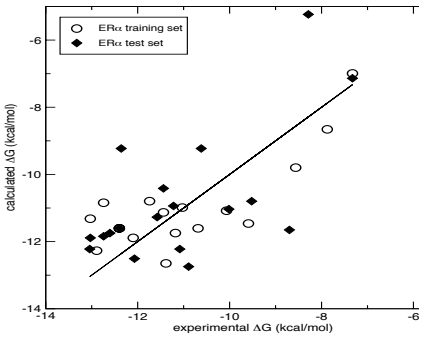

# Direct estimates of $\Delta G_{bind}$

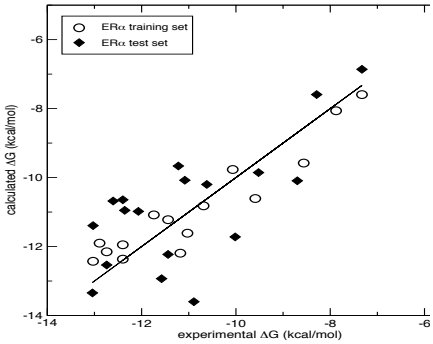
- Can MD simulations be used in a lead optimisation project?
- Estrogen receptor a
  - 36 compounds, 18 training set, 18 test set
  - comparison of
    - Linear Interaction Method from MD simulation

$$\Delta G_{bind} = \beta\Delta\left\langle V_{l-s}^{el}\right\rangle + \alpha\Delta\left\langle V_{l-s}^{vdw}\right\rangle + \delta\Delta\left(\left\langle V_{l-l}^{el}\right\rangle + \left\langle V_{l-l}^{vdw}\right\rangle\right)$$

    - the best scoring function (selected out of 10) : HS score
      linear regression from score to exp. binding free energies

# LIE vs scoring?




| | | HS score | LIE |
|---|---|---|---|
| $r^2$ | train | 0.62 | 0.87 |
| rmsd | train | 1.05 kcal/mol | 0.63 kcal/mol |
| rmsd | test | 1.54 kcal/mol | 1.30 kcal/mol |
| Spearman-rank | all | 0.55 | 0.65 |

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# Definitions

**free energy**

$$A(N,V,T) = -k_B T \ln\left[N! h^{3N}\right]^{-1} \iint \exp\left(-H(\vec{p},\vec{r})/k_B T\right) d\vec{p} d\vec{r}$$
$$= U - TS$$

**energy**

$$U(N,V,T) = \left(\frac{\partial A/T}{\partial 1/T}\right)_{N,V} = \left\langle H(\ ,\ )\right\rangle_{\vec{p},\vec{r}}$$

**entropy**

$$S(N,V,T) = -\left(\frac{\partial A}{\partial T}\right)_{N,V} = \frac{U-A}{T}$$

**Partition function**

$$Z(N,V,T) = \left[N! h^{3N}\right]^{-1} \iint \exp\left(-H(\vec{p},\vec{r})/k_B T\right) d\vec{p} d\vec{r}$$

Free energy, energy and entropy are defined from statistical thermodynamics

# Statistical mechanics

- Equation to calculate free energy is simple:

$$G = -k_B T \ln Z_{NpT}$$

- Where $Z_{NpT}$ is the partition function of the system

$$Z_{NpT} = \frac{1}{h^{3N} N!} \iiint e^{-(H(\mathbf{r},\mathbf{p})+pV)/k_B T} \, d\mathbf{p}\, d\mathbf{r}\, dV$$

Simulation samples over positions, momenta and volumes in physically relevant way

Integral over all possible positions, and all possible momenta of all particles in all different volumes

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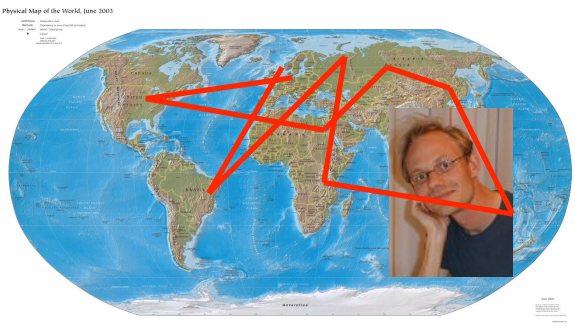
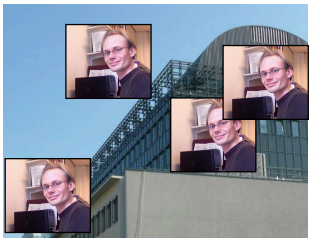
# Sampling of all positions

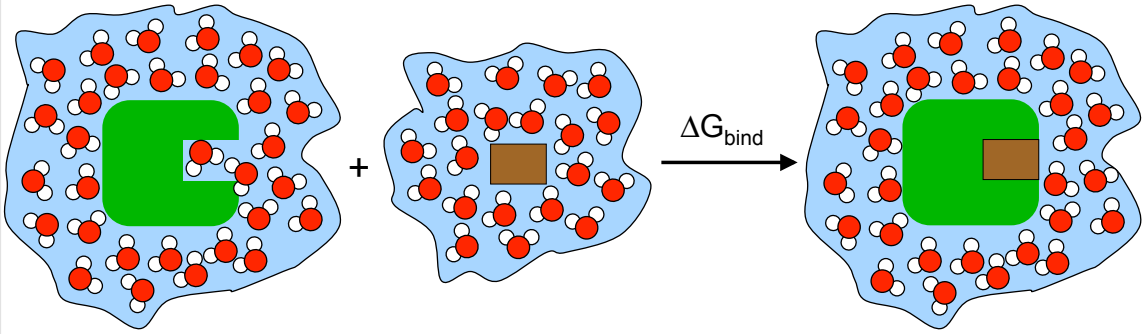
- Sampling of all positions to calculate absolute free energy is practically impossible
- For molecules and for me



# Do we really want that? (I)

- We only really care about the difference, $\Delta G$




- The perturbation formula calculates free energy differences directly

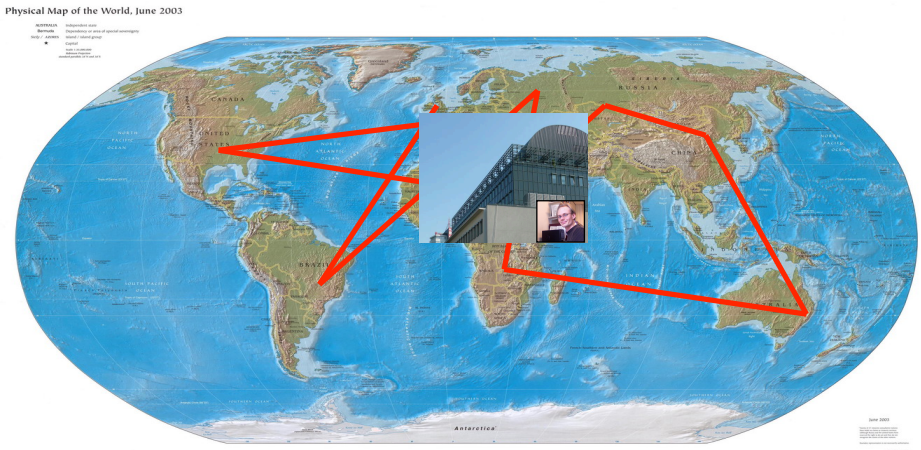
$$\Delta G = -k_B T \ln \left\langle e^{-(H_{complex} - H_{ligand} - H_{protein})/k_B T} \right\rangle$$

Works if the positions, momenta and volumes of both sides are not too different

Applied Statistical Thermodynamics

# Directly consider the difference

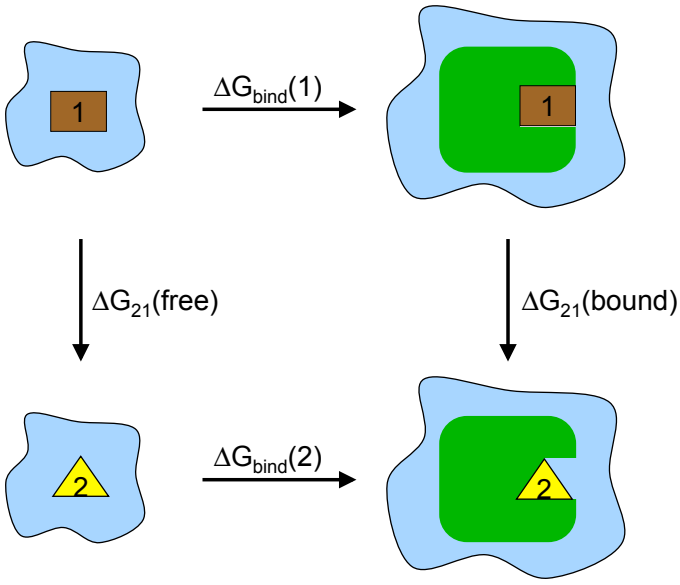
- Consider my presence at BOKU as one of many possibilities




- Is still very difficult and unlikely to work

# Do we really want that? (II)

- Free energy is independent of the path (state function)
- Thermodynamic cycle
- Relative free energies
- Computational alchemy

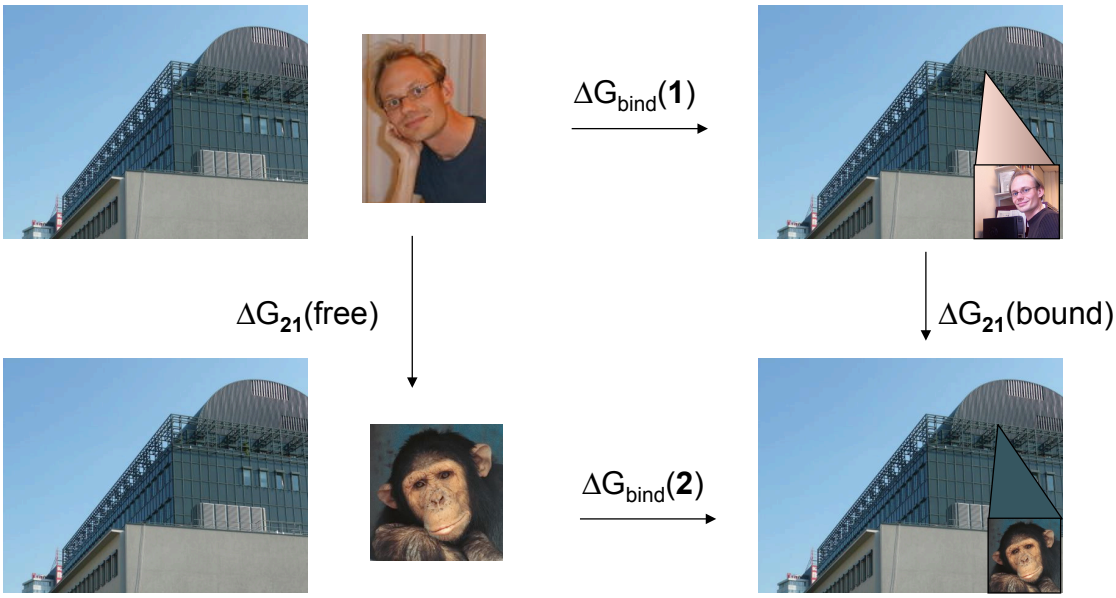



$$\Delta\Delta G_{bind} = \Delta G_{bind}(2) - \Delta G_{bind}(1)$$
$$= \Delta G_{21}(bound) - \Delta G_{21}(free)$$

Applied Statistical Thermodynamics

# Who fits better at BOKU?

- Are there others that are more suitable?




- Compare two employees when they are free and at BOKU

# Free energy difference

## Free energy perturbation

$$\Delta A_{BA} = A_B - A_A = -k_B T \ln \frac{Z_B(N,V,T)}{Z_A(N,V,T)}$$

$$= -k_B T \ln \frac{\iint e^{-H_B(\mathbf{r},\mathbf{p})/k_B T} d\mathbf{p}d\mathbf{r}}{\iint e^{-H_A(\mathbf{r},\mathbf{p})/k_B T} d\mathbf{p}d\mathbf{r}}$$

$$= -k_B T \ln \frac{\iint e^{-(H_B(\mathbf{r},\mathbf{p})-H_A(\mathbf{r},\mathbf{p}))/k_B T} e^{-H_A(\mathbf{r},\mathbf{p})/k_B T} d\mathbf{p}d\mathbf{r}}{\iint e^{-H_A(\mathbf{r},\mathbf{p})/k_B T} d\mathbf{p}d\mathbf{r}}$$

$$= -k_B T \ln \left\langle e^{-(H_B - H_A)/k_B T} \right\rangle_A$$

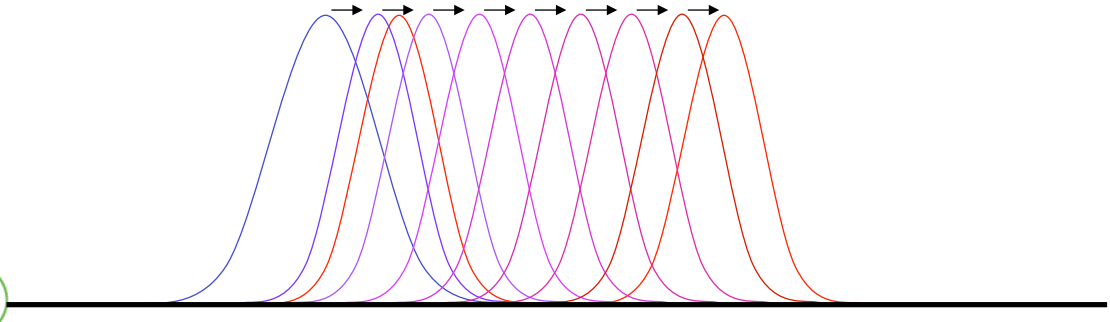
Zwanzig, J. Chem. Phys (1954) 22:1420

Applied Statistical Thermodynamics

# Free energy calculation

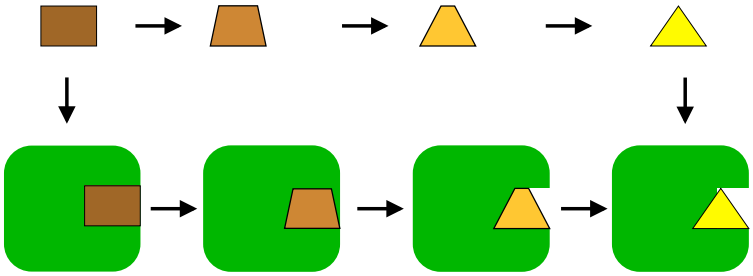
$$\Delta G_{AB} = -k_B T \ln \left\langle e^{-(E_A - E_B)/k_B T} \right\rangle_B$$

$$\Delta G_{AB} = \sum_{\lambda} \Delta G_{\lambda,\lambda+\delta\lambda}$$

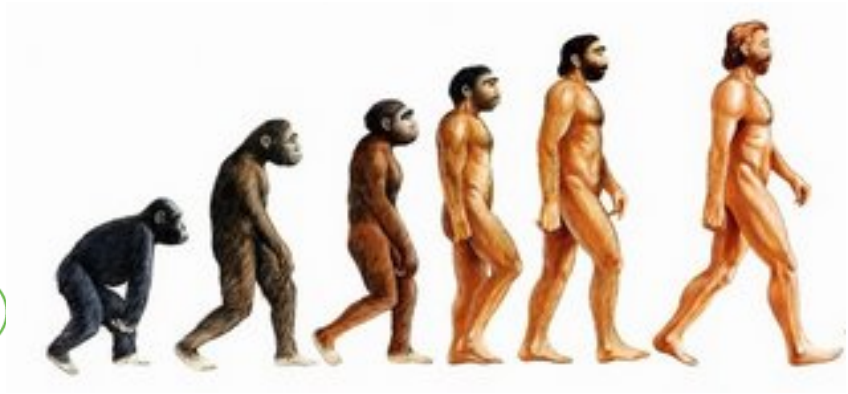


conformational space

# Gradually change one in the other



Change ligand 1 into ligand 2, in solution and when bound to the protein

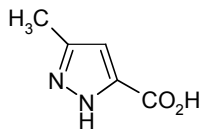


As long as the end-states are defined, the intermediates do not have to be physically possible

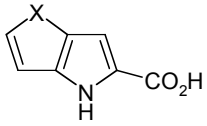
Applied Statistical Thermodynamics

# Example: DAAO inhibitors

- Three inhibitors of the enzyme D-amino acid oxidase were studied



**1**



**3** X = O
**4** X = S

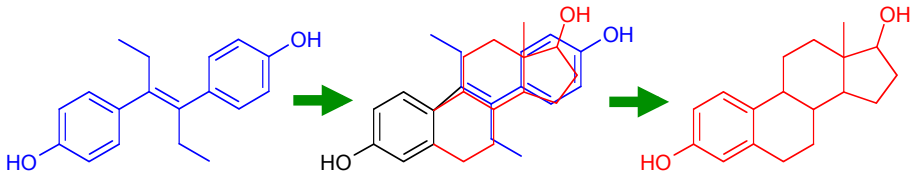
| | **3->1** | **3->4** | **4->1** |
|---|---|---|---|
| *Calculated values*: | | | |
| $\Delta G_{free}$ | 106.3 ±1.5 | 86.1 ±0.8 | 20.4 ±1.1 |
| $\Delta G_{complex}$ | 113.8 ± 2.2 | 87.3 ±3.5 | 36.7 ±2.0 |
| $\Delta\Delta G_{bind}$ | 7.5 ± 3.7 | 1.2 ± 4.3 | 16.3 ± 3.1 |
| *Experimental* $\Delta\Delta G_{bind}$ *based on:* | | | |
| $IC_{50}$[a] | 8.2 | -0.9 | 9.1 |
| $IC_{50}$[b] | 4.6 | 0.1 | 4.6 |
| ITC | 9.4 | 0.8 | 8.6 |
| SPR[c] | 14.1 | 1.6 | 12.4 |

Overall, the relative binding free energies are very well reproduced

J.H.M. Lange, J. Venhorst, M.J.P. van Dongen, J. Frankena, F. Bassissi, N.M.W.J. de Bruin, C. den Besten, S.B.A. de Beer, C. Oostenbrink, N. Markova and C.G. Kruse, *Eur. J. Med. Chem.* (2011) **46**, 4808 - 4819

# Computational alchemy

- Modify one compound into another one in small steps




- In a formula: $E(\mathbf{q},\mathbf{p},\lambda) = (1-\lambda)E_A(\mathbf{q},\mathbf{p}) + \lambda E_B(\mathbf{q},\mathbf{p})$

$\lambda = 0 \rightarrow E = E_A \qquad \lambda = 1 \rightarrow E = E_B$

Along the way? The protein 'sees' a mixture of A and B

$$\Delta G_{AB} = \sum_{\lambda=0}^{1} -k_B T \ln \left\langle e^{-\Delta E(\lambda \rightarrow \lambda + d\lambda)/k_B T} \right\rangle$$

Applied Statistical Thermodynamics

# Thermodynamic integration

- Slightly more convenient

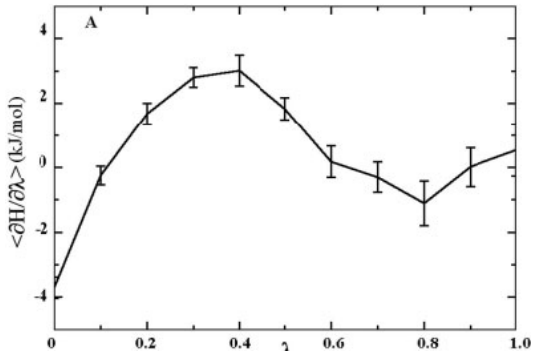
$$\Delta A_{BA} = \int_0^1 \frac{dA(\lambda)}{d\lambda} d\lambda$$

$$\frac{dA(\lambda)}{d\lambda} = \frac{d}{d\lambda}\left( -k_B T \ln \frac{1}{h^{3N} N!} \iint e^{-H(\mathbf{r},\mathbf{p},\lambda)/k_B T} d\mathbf{p} d\mathbf{r} \right)$$

$$= -k_B T \frac{\frac{d}{d\lambda} \iint e^{-H(\mathbf{r},\mathbf{p},\lambda)/k_B T} d\mathbf{p} d\mathbf{r}}{\iint e^{-H(\mathbf{r},\mathbf{p},\lambda)/k_B T} d\mathbf{p} d\mathbf{r}}$$

$$= \frac{\iint \frac{\partial H(\mathbf{r},\mathbf{p},\lambda)}{\partial \lambda} e^{-H(\mathbf{r},\mathbf{p},\lambda)/k_B T} d\mathbf{p} d\mathbf{r}}{\iint e^{-H(\mathbf{r},\mathbf{p},\lambda)/k_B T} d\mathbf{p} d\mathbf{r}}$$

$$= \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_\lambda$$




Kirkwood, J. Chem. Phys (1935) 3:300

TI uses the derivative of the Hamiltonian to calculate free energies

# Example: ER

- Relative free energy of three compounds
- In three different media (vacuum, solution, protein)
- In 11 discrete steps, forward and backward TI

**Table 4.** TI Results (kJ mol$^{-1}$)[a]

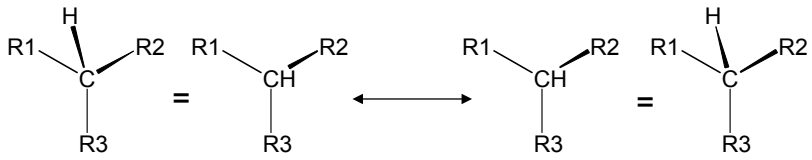
| | DES ↔ E2 | | | DES ↔ GEN | | |
|---|---|---|---|---|---|---|
| TI | for-ward | back-ward | hysteresis | for-ward | back-ward | hysteresis |
| vacuum | 76.3 | 76.1 | 0.2 | 187.1 | 186.9 | 0.2 |
| solvent | 79.0 | 81.6 | −2.6 | 151.5 | 157.3 | −5.8 |
| protein | 80.4 | 78.2 | 2.2 | 173.1 | 165.3 | 7.8 |
| $\Delta\Delta G_{solv}$ | 2.8 | 5.5 | −2.7 | −35.6 | −29.5 | −6.0 |
| $\Delta\Delta G_{bind}$ | 1.4 | −3.4 | 4.8 | 21.6 | 8.0 | 13.6 |
| $\Delta\Delta G_{bind}$ | 3.8[b] | | | 11.3[b] | | |
| (expt) | 0.79[c] | | | 21.69[c] | | |

Applied Statistical Thermodynamics

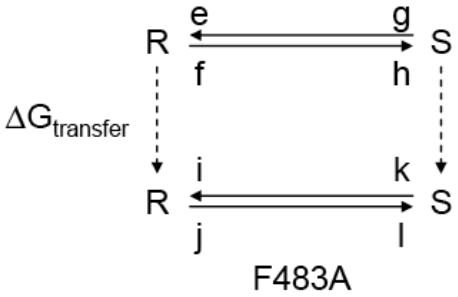
# Stereospecific propranolol binding

- R- and S-Propranolol have similar affinity for CYP450 2D6
- 20 fold decrease of affinity of R-Propranolol to F483A mutant

- Free energy calculation to convert R-propranolol into S-propranolol




WT

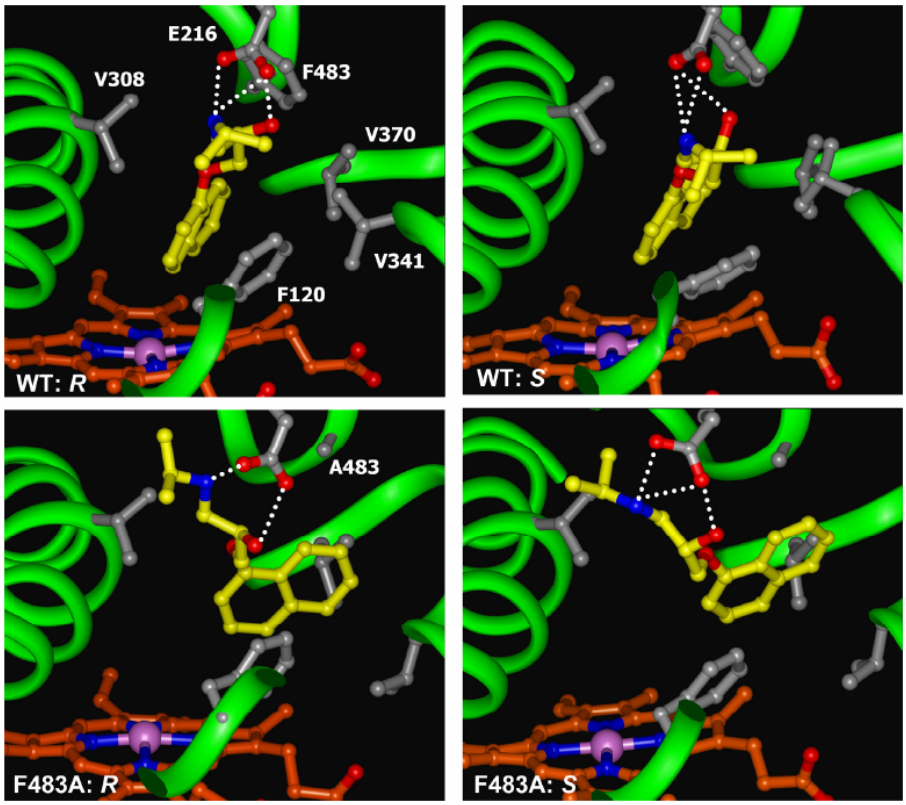



F483A

$\Delta\Delta G_{bind}$ = $\Delta G_{bind}$(R) - $\Delta G_{bind}$(S)
= -7.7 kJ/mol (experimental)

= $\Delta G_{inv}$(F483A) - $\Delta G_{inv}$(WT)
= -7.5 kJ/mol (simulation)

# Molecular picture

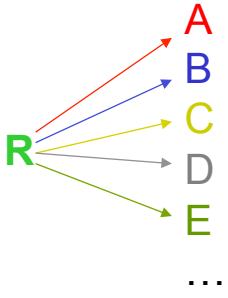





# One step perturbation
# Enveloping Distribution Sampling

$$\Delta G_{AB} = -k_B T \ln \left\langle e^{-(E_A - E_B)/k_B T} \right\rangle_B$$

$$\Delta G_{AB} = \sum_{\lambda} \Delta G_{\lambda,\lambda+\delta\lambda}$$

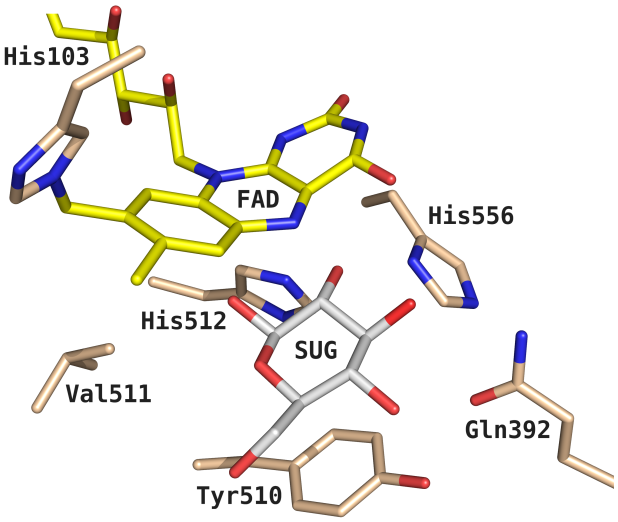




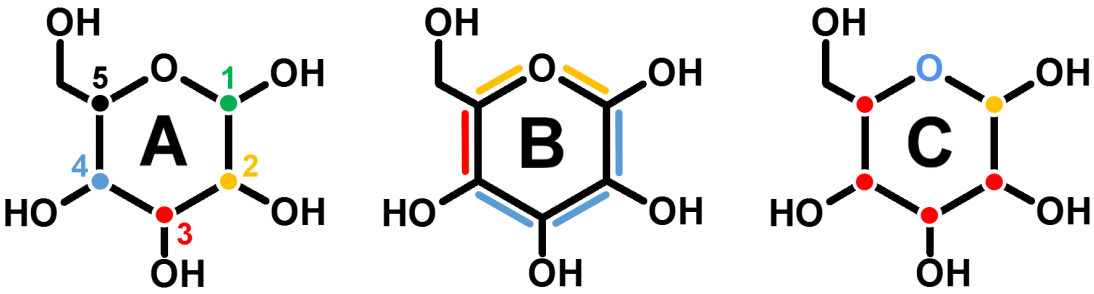

conformational space

# Pyranose dehydrogenase

- fungal dehydrogenase
- litter degrading enzyme
- promiscuous substrate binding and product formation

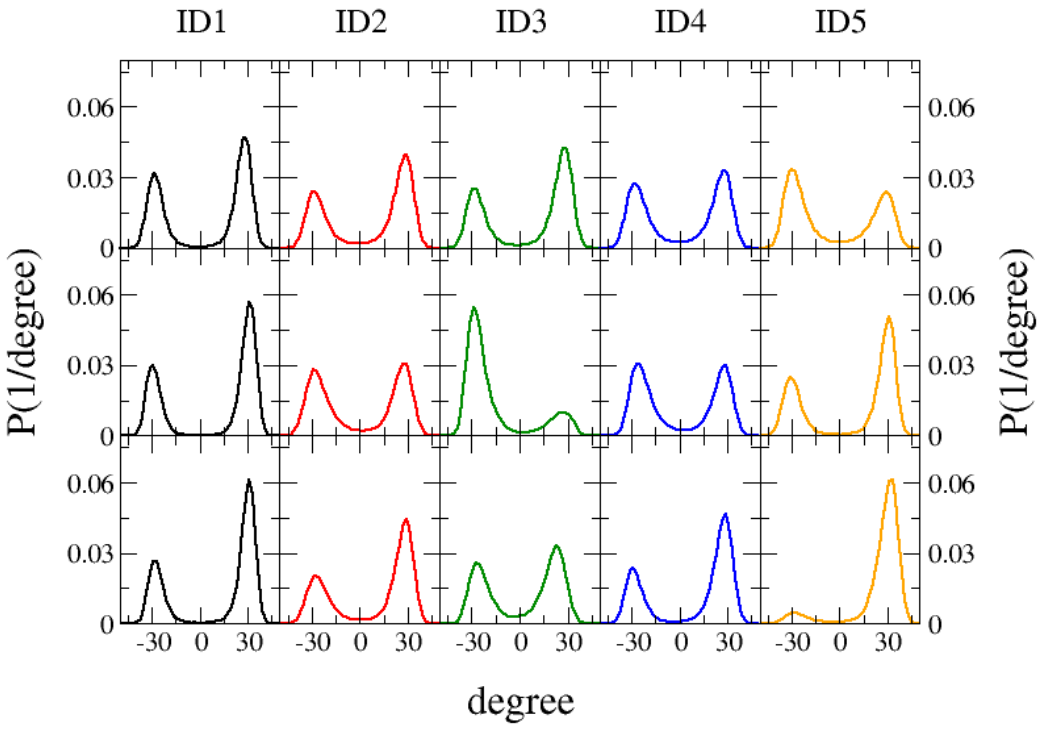



- **Floppy reference states:**
  - A. Improper dihedrals removed
  - B. + proper dihedrals weakened
  - C. + angle bending weakened




Applied Statistical Thermodynamics

# Occurrences




- water one simulation; in protein multiple reference states used

# Free energies

| Sugar | $\Delta G_{water}$ | $\Delta G_{prot}$ | $\Delta\Delta G_{bind}$ [kJ/mol] |
|---|---|---|---|
| β-D-glucose | 31.7 | 22.5 | -9.1 |
| β-D-mannose | 37.9 | 46.7 | 8.7 |
| β-D-galactose | 34.5 | 29.2 | -5.3 |
| β-D-talose | 40.8 | 41.7 | 0.8 |
| α-D-glucose | 34.4 | 31.5 | -2.9 |
| α-D-mannose | 33.9 | 35.9 | 2.1 |
| … | | | |
| β-D-gulose | 27.6 | 26.6 | -1.0 |
| β-D-idose | 32.7 | 40.0 | 7.3 |
| α-D-allose | 31.6 | 35.8 | 4.2 |
| α-D-altrose | 29.7 | 38.2 | 8.6 |
| α-D-gulose | 31.3 | 36.0 | 4.7 |
| α-D-idose | 32.6 | 36.3 | 3.7 |
| β-L-glucose | 30.8 | 40.1 | 9.3 |
| β-L-mannose | 30.1 | 35.3 | 5.2 |
| β-L-galactose | 28.0 | 44.3 | 16.3 |
| β-L-talose | 30.4 | 29.9 | -0.5 |
| α-L-glucose | 31.2 | 62.0 | 30.8 |
| α-L-mannose | 27.2 | 39.1 | 12.0 |

| Sugar | $\Delta G_{\beta-\alpha}$ exp. | $\Delta G_{\beta-\alpha}$ calc. |
|---|---|---|
| D-glucose | -1.2 | -2.8 |
| D-mannose | 1.8 | 4.1 |
| D-galactose | -1.7 | -1.7 |
| D-talose | 1.0 | 3.0 |
| D-allose | -4.1 | -4.7 |
| D-altrose -1.1 | -1.0 | |
| D-gulose -4.8 | -3.7 | |
| D-idose | -0.3 | 0.1 |

| Sugar | $\Delta\Delta G_{bind}$ exp. | $\Delta\Delta G_{bind}$ calc. |
|---|---|---|
| D-glucose | 0.0 | 0.0 |
| D-mannose | 12.3 | 10.9 |
| D-galactose | 0.6 | 3.7 |
| D-talose | 11.5 | 5.6 |

Applied Statistical Thermodynamics

# Conclusions

- Free energy calculations for e.g. drug design / lead optimisation
  - Enthalpic and entropic effects should be included
  - Statistical mechanics and thermodynamic cycles
    - Efficient calculations
    - Unphysical intermediates
- Different methods available
  - Docking and scoring
  - Empirical free-energy methods
  - Free energy perturbation
  - Thermodynamic integration
  - One-step perturbation from a specialized reference state
- Protein flexibility and multiple binding conformations contribute
- Accuracy ~ 2.5 kJ/mol