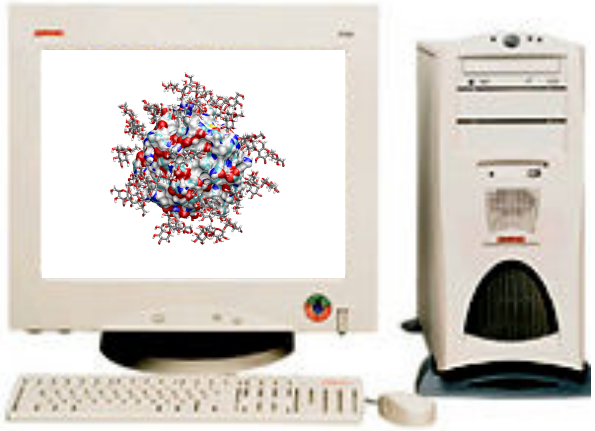
There are several topics from BFCH1 (lectures: Structure of biopolymers (2), Canonical ensemble) that are expected that students know:

- canonical ensemble
- Boltzmann probability
- free energy, enthalpy, entropy
- free energy cycle

BIOMOLECULAR SIMULATIONS



JOZEF HRITZ

CEITEC-MU

For which problems are simulations useful ?

Simulation can replace or complement the experiment:

1. Experiment is impossible — *Inside of stars*, *Weather forecast*

2. Experiment is too dangerous — *Flight simulation*, *Explosion simulation*

3. Experiment is expensive — *High pressure simulation*, *Windchannel simulation*

4. Experiment is blind — *Some properties cannot be observed on very short time-scales and very small space-scales*

5. Fantasy — *It is possible to simulate unrealistic phenomena*

For which problems are simulations useful ?

Simulations can complement the experiment:

- Simulation explains experiments

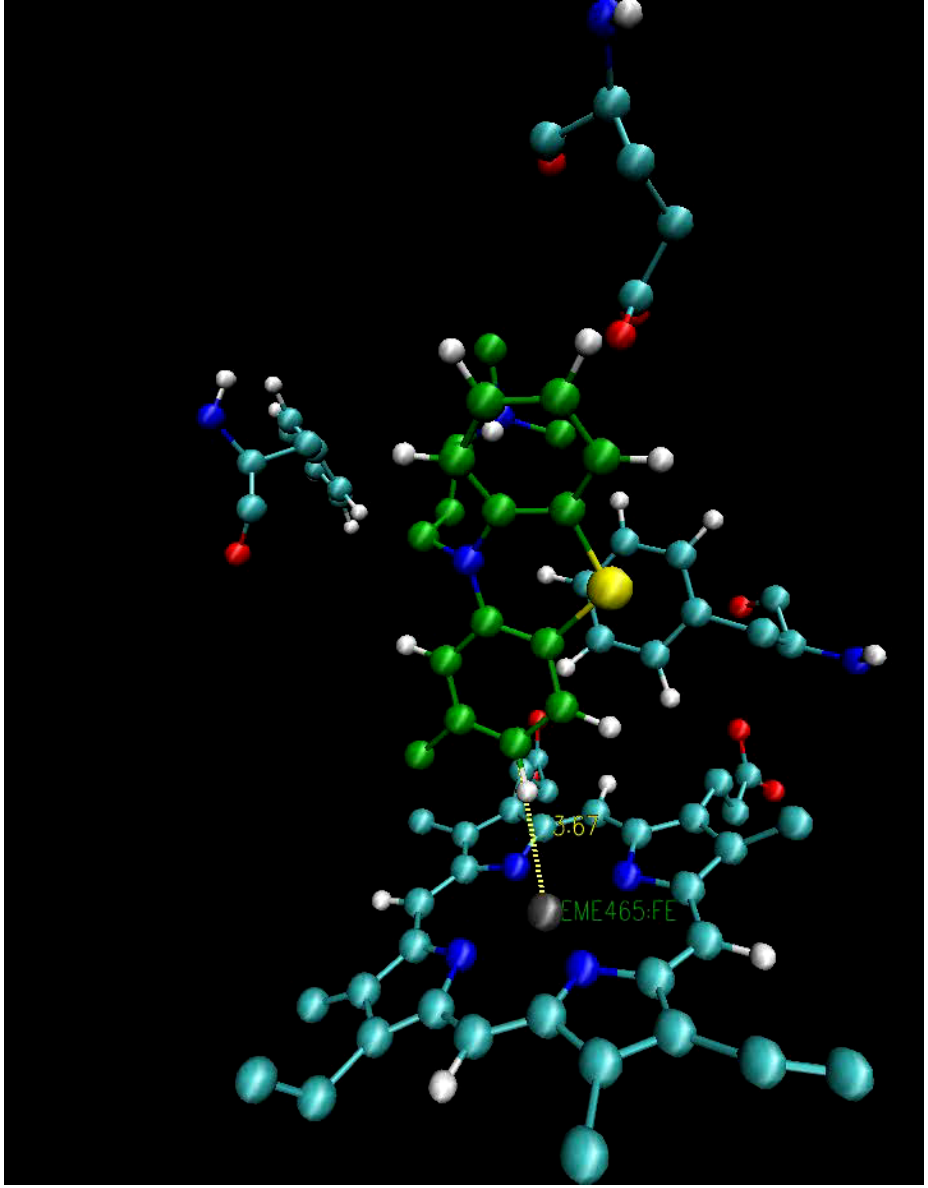
Properties of water
Folding of protein molecules

➡ knowledge
new ideas

- Simulation suggests
new experiments

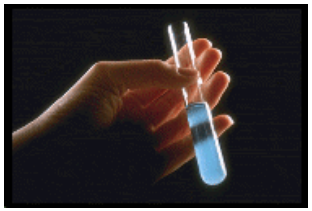
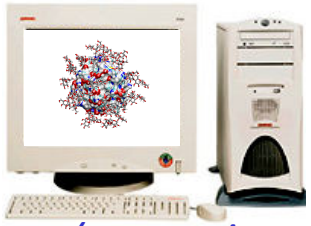
Design of drugs, enzymes
Stock market prices

➡ less experiments
better chance of success



Hritz, J.; de Ruiter, A.; Oostenbrink, C. Impact of plasticity and flexibility on docking results for Cytochrome P450 2D6: a combined approach of molecular dynamics and ligand docking. *J. Med. Chem.* **2008**, 51, 7469-7477

Molecular simulation and experiment

	experiment	***simulation***
		
Resolution*	*(restricted)*	*(unrestricted)*
size :	10^{23} molecules	1 molecule
time :	1 second	10^{-15} seconds

*: Single molecules / 10^{-15} seconds possible
(but not both in the liquid phase)

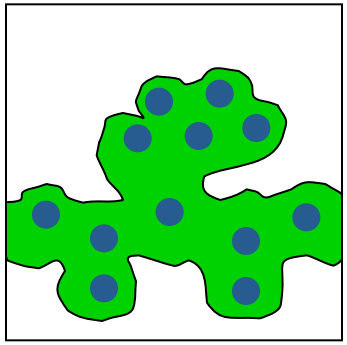
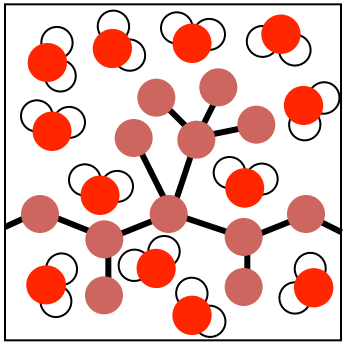
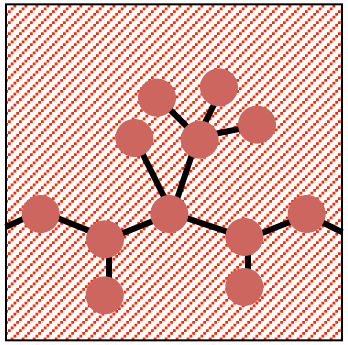
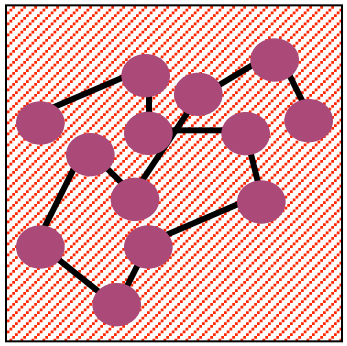
Typical space / time scales

size :	10^{-3} meter	10^{-7} meter
time :	10^{3} seconds	10^{-3} seconds

Simulation and experiment are complementing methods to study different aspects of nature

How would you calculate movement of planets in our solar system?

Choose relevant degrees of freedom: elementary particles

=

Particles:			
atomic nuclei + electrons	*all atoms*	*all atoms (excluding solvent)*	*monomers*
Description:			
quantummechanics	*classical mechanics*	*classical mechanics*	*classical mechanics*
Interactions:			
electrostatics	*Force Field (atomistic)*	*Force Field (including solvent)*	*Force Field (statistic)*

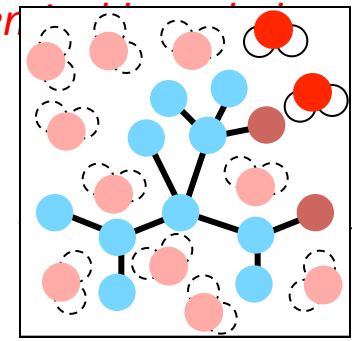
Broader applicability
Less model parameters
Physical parameters
More expensive

Restricted applicability
More model parameters
Empirical parameters
Less expensive

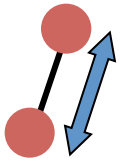
Interactions in atomic simulaties : Force Field

physico-cher

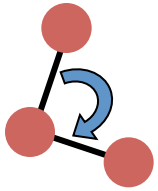
bonded interactions



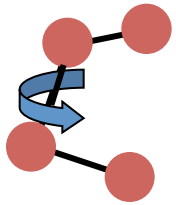
non-bonded interactions



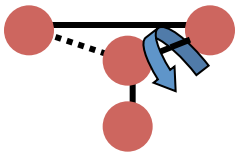
Bond stretching



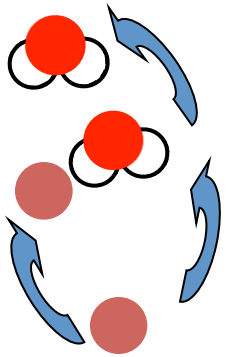
Angle bending

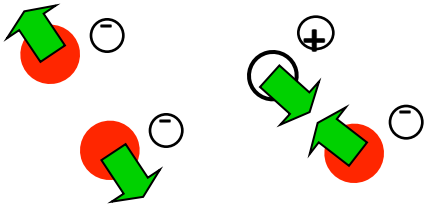


Rotation around bond

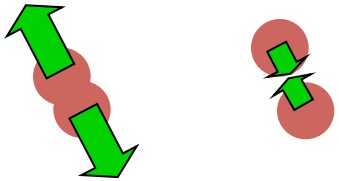


Planar atomgroups





Electrostatic interactions



van der Waals interactions

Interacting Particles

Physical Terms

$$V^{bond}\left(\vec{r}^{\,N}\right)=\sum_{bonds\ i}\frac{1}{2}K_i^b\left[b_i\left(\vec{r}^{\,N}\right)-b_i^0\right]^2$$

$$V^{v.d.Waals}\left(\vec{r}^{\,N}\right)=\sum_{pairs\ i<j}4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]$$

$$V^{angle}\left(\vec{r}^{\,N}\right)=\sum_{angles\ i}\frac{1}{2}K_i^a\left[\theta_i\left(\vec{r}^{\,N}\right)-\theta_i^0\right]^2$$

$$V^{Coulomb}\left(\vec{r}^{\,N}\right)=\sum_{pairs\ i<j}\frac{1}{4\pi\varepsilon_0\varepsilon_r}\frac{q_iq_j}{r_{ij}}$$

$$V^{torsion}\left(\vec{r}^{\,N}\right)=\sum_{torsion\ i}K_i^{\varphi}\cos\left[m_i\varphi_i\left(\vec{r}^{\,N}\right)+\delta_i\right]$$

$V^{pol}\left(\vec{r}^{\,N}\right)=$ N-body polarization energy

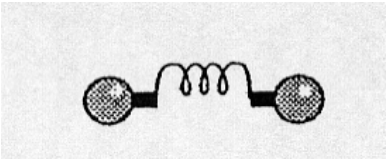
$V^{ext}\left(\vec{r}^{\,N}\right)=$ external fields energy

Special Interaction Terms examples

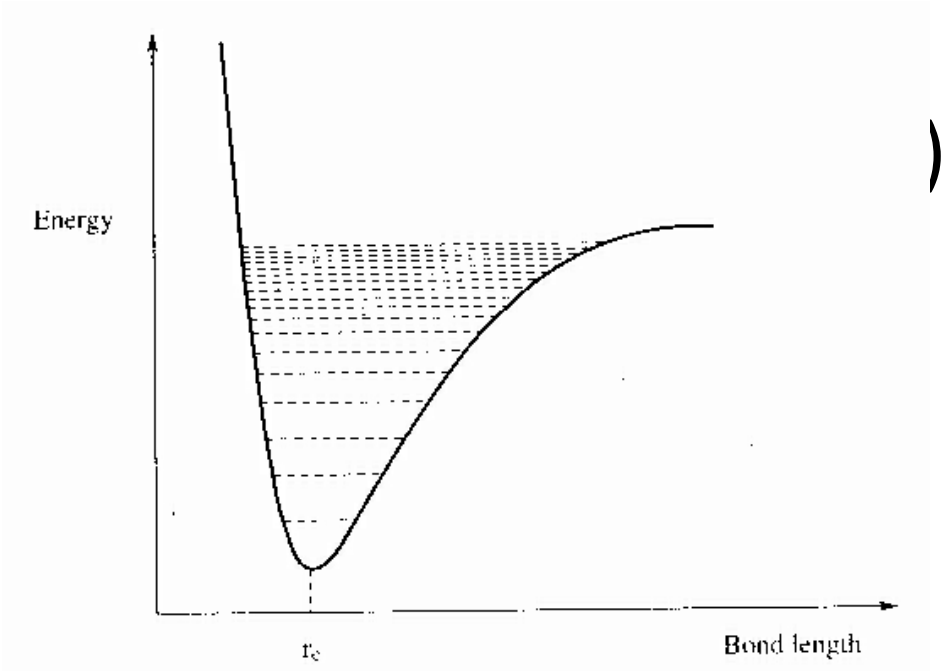
restraints on the system:

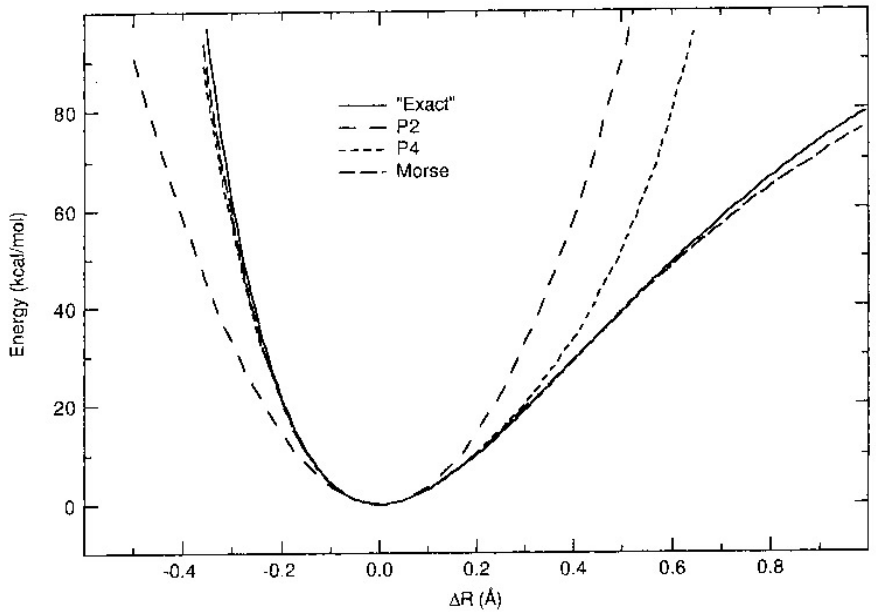
- from experimental data
- to bias the sampling

Bond stretch term

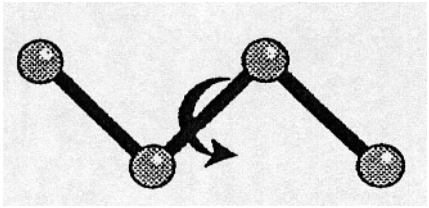


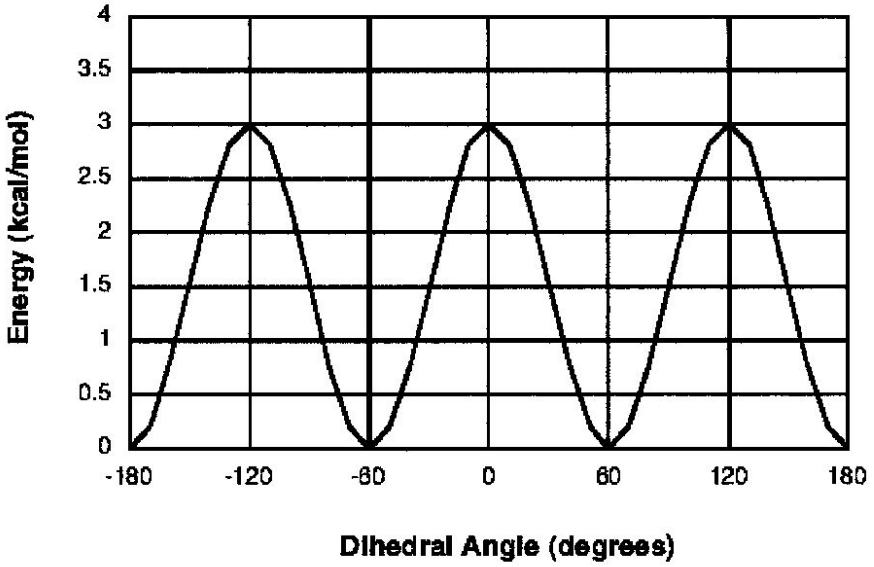
- The bond stretch term is almost always approximated with a harmonic potential:





Torsion term



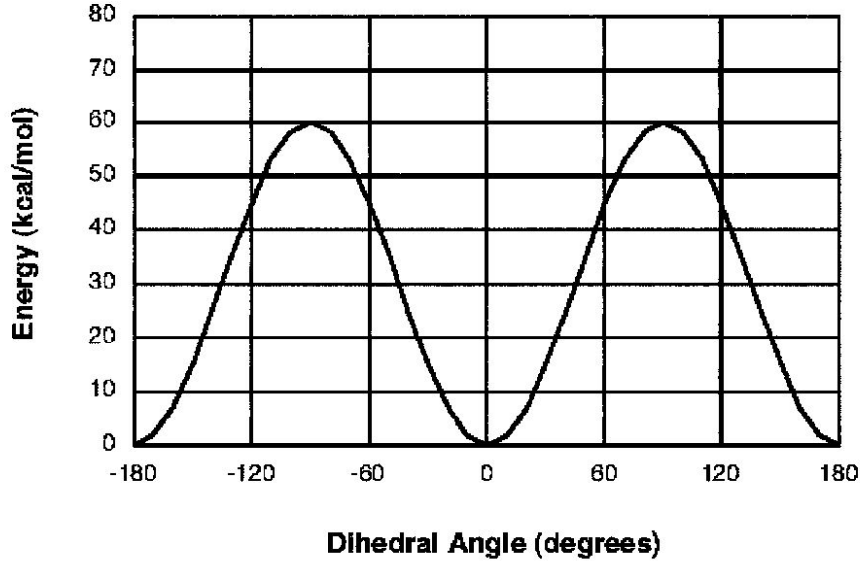


ethane: sp3-sp3 bond

CH3-CH3

periodicity = 3

phase = 0 degrees



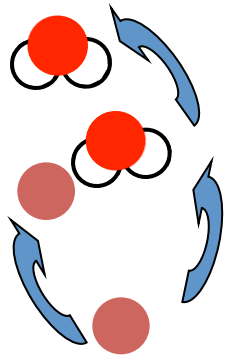
ethene: sp2-sp2 bond

CH2=CH2

periodicity = 2

phase = 0 degrees

Nonbonded interactions



- All atoms see each other through space
  ~ ½ N(N – 1) interactions

- Bound atoms (1,2 neighbours) and their neighbours (1,3 neighbours) are often excluded because the bonded interactions already take these into account
- Sometimes 1,4 neighbours are treated differently to keep the proper torsional profile

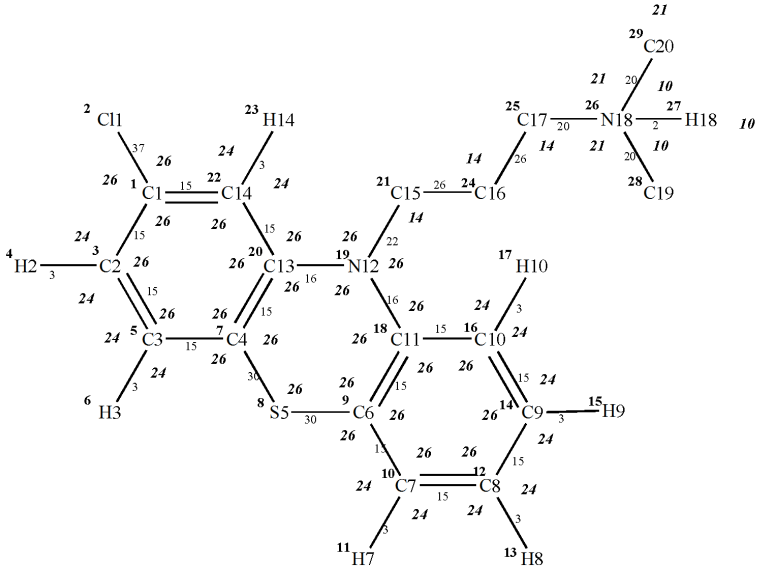
- Often we work with a cutoff for the nonbonded interaction

Other interactions

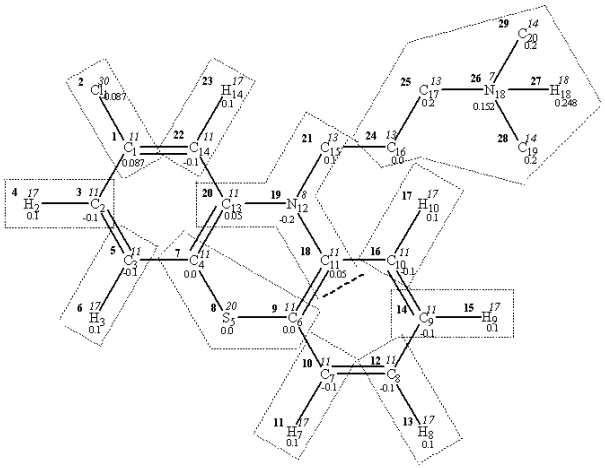
- Some force fields show alternative interactions
  - Specific hydrogen bond interactions
    usually a balance between vdw and electrostatics
  - Crossterms, e.g. Energy as function of bond length and angle: $U(r,\theta)$
    needed to reproduce vibrational spectra
  - …

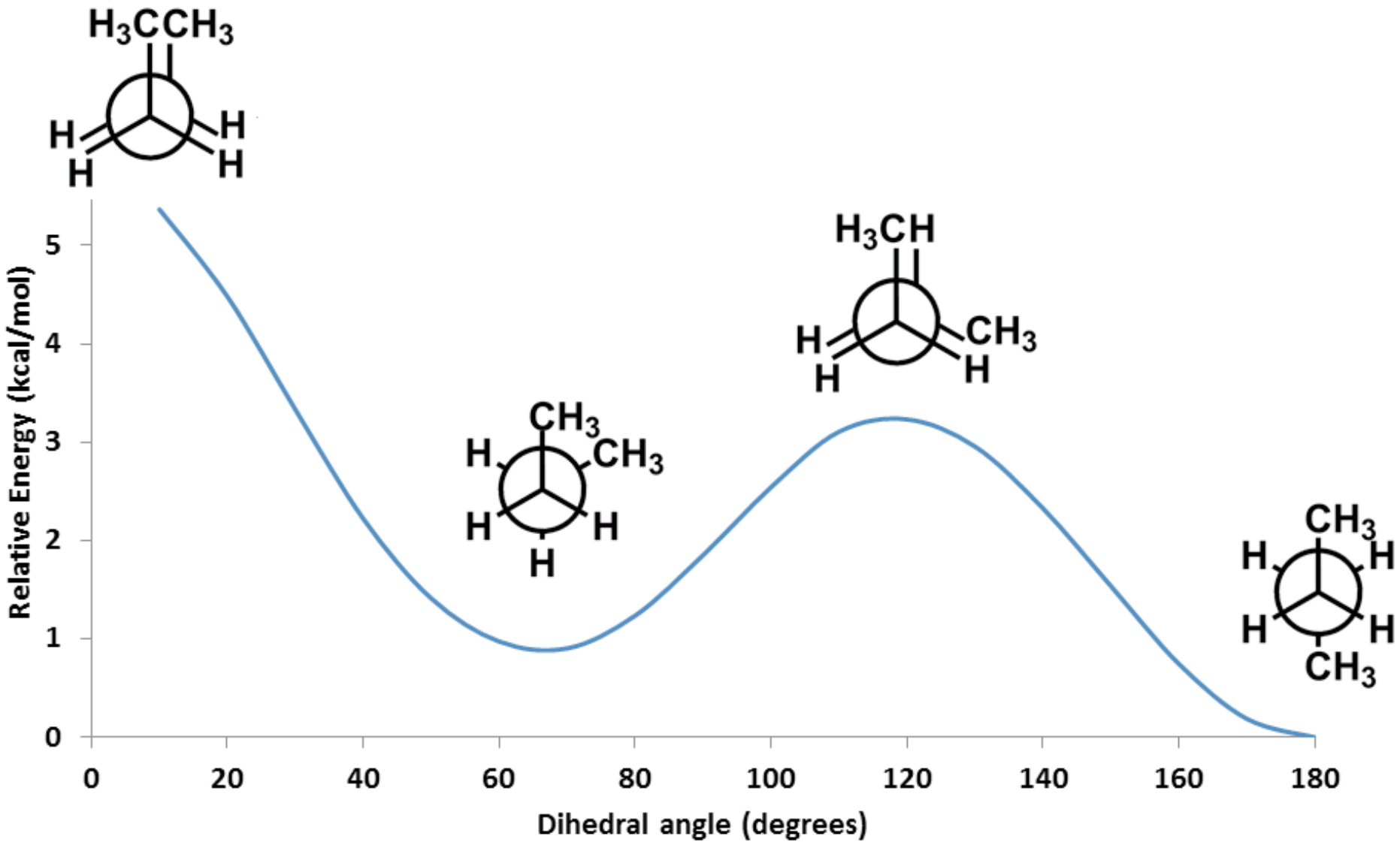
Example of force field parameters

A

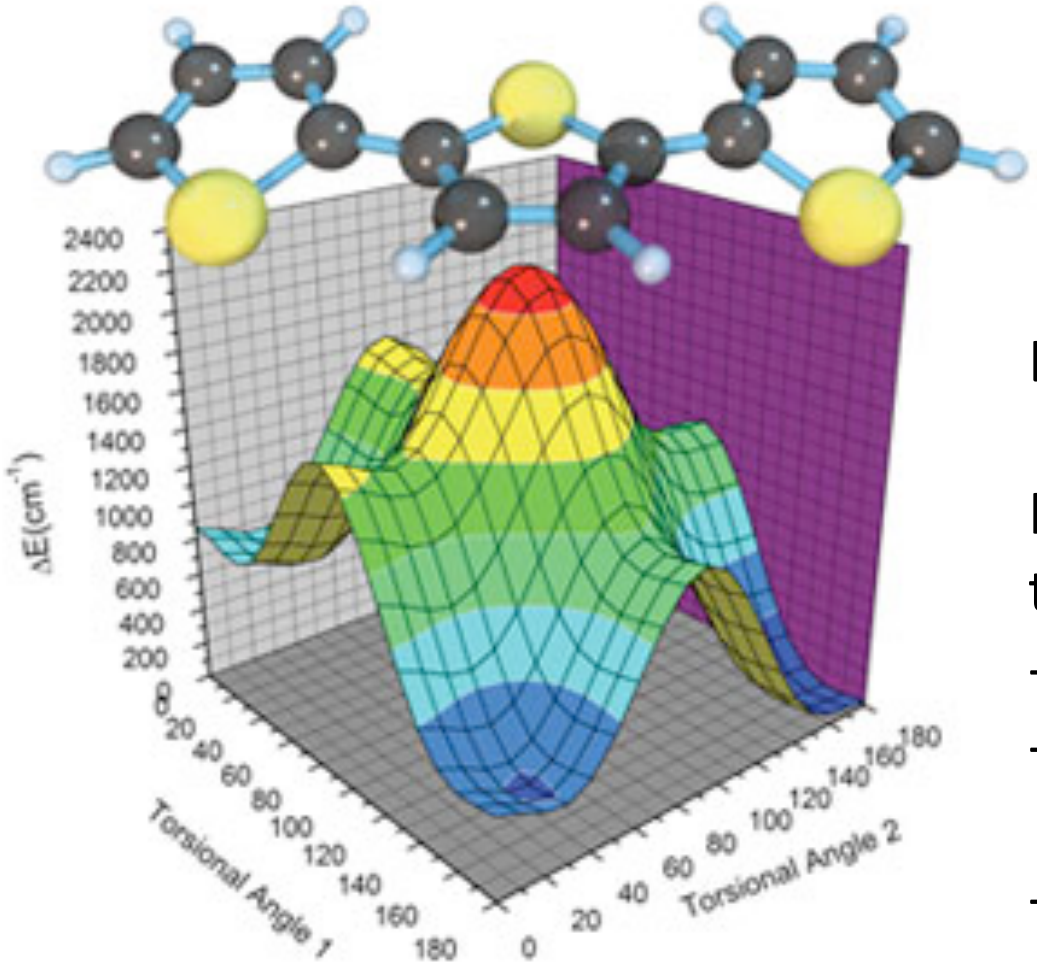


B




H3CCH3
H
H
H
H
H3CH
H
H
CH3
H
CH3
H
H
H
CH3
H
H
H
H
CH3
Relative Energy (kcal/mol)
0
1
2
3
4
5
0
20
40
60
80
100
120
140
160
180
Dihedral angle (degrees)

Potential Energy Surface (PES)



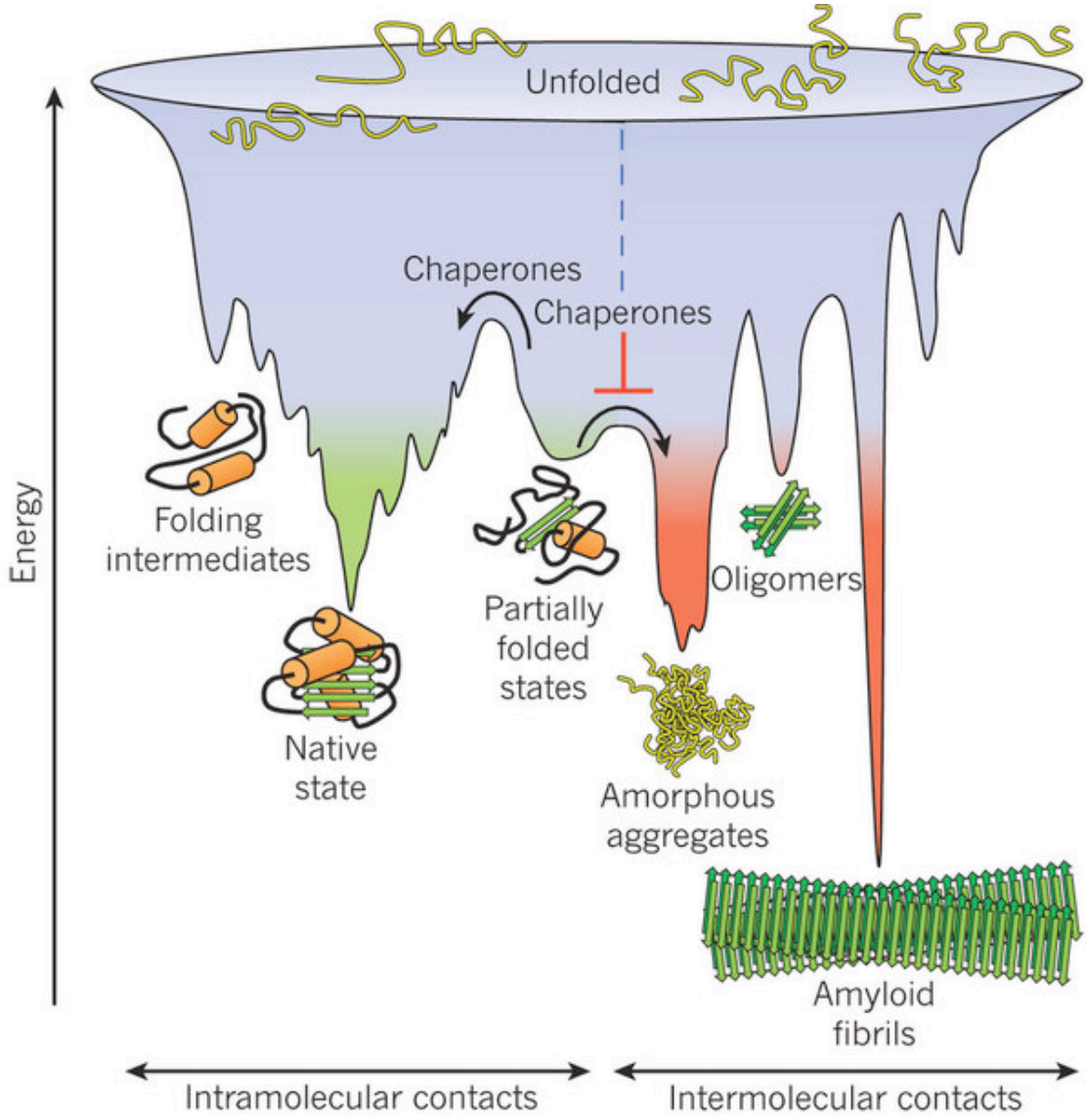
Levinthal's Paradox

Heuristic energy minimization techniques:

- Simulated annealing
- Genetic algorithm

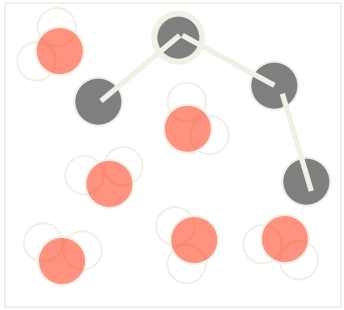
- Interpretation of EM

Potential Energy Surface (PES)



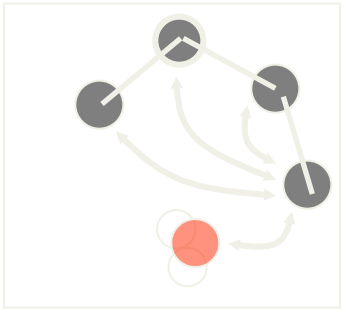
Definition of a model for molecular simulation

Every molecule consists of atoms that are very strongly attached



Degrees of freedom: atoms are the elementary particles

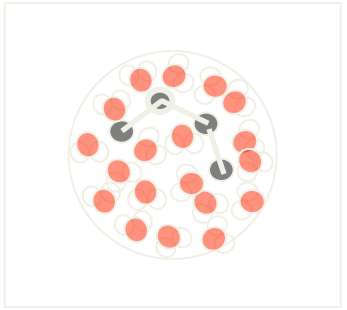
Forces or interactions between atoms



Force Field = physico-chemical knowledge

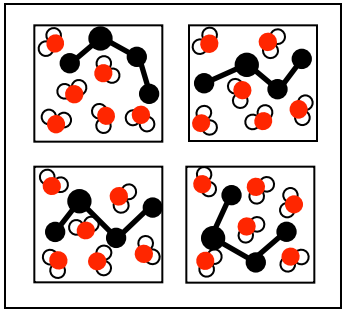
MOLECULAR MODEL

Boundary conditions

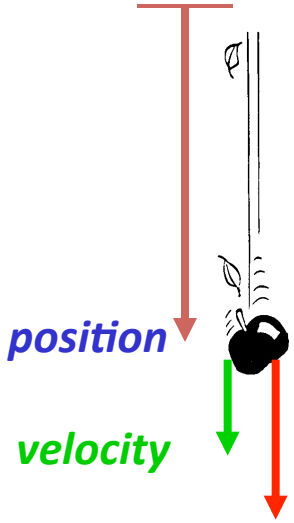


system
temperature
pressure

Methods to generate configurations of atoms: Newton



Classical dynamics



Situation at time t

Force** is determined by relative **positions

***acceleration** = **force** / mass*

Δ ***velocity** = **acceleration** × Δt*

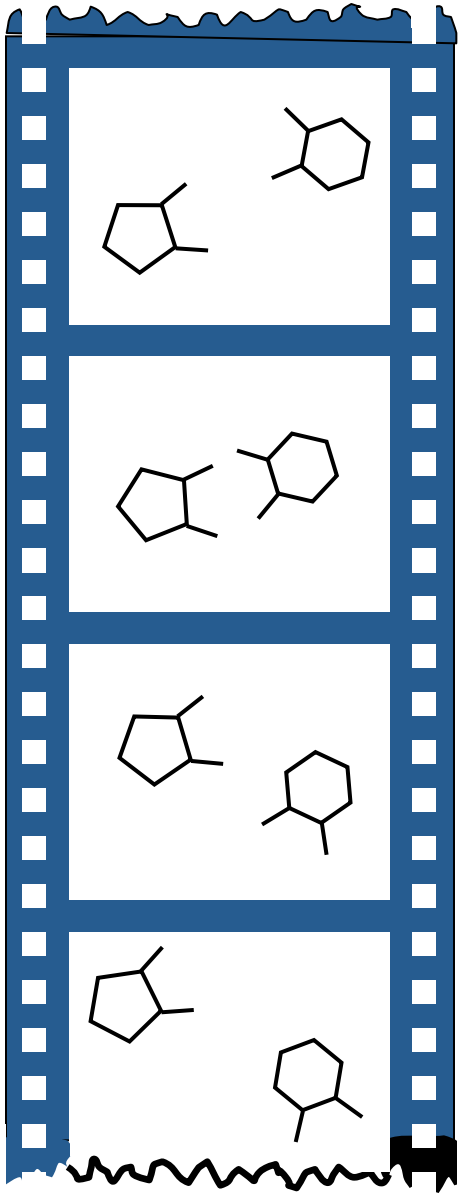
Δ ***position** = **velocity** × Δt*

Situation at time t+Δt

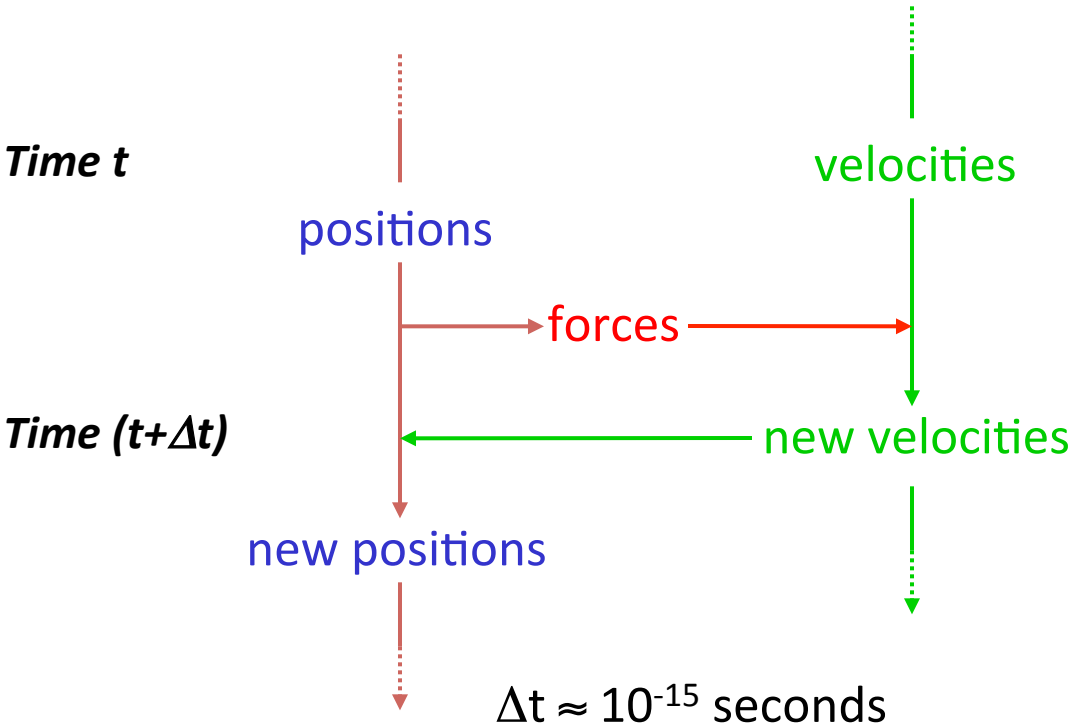
Sir Isaac Newton
1642 -1727

Determinism …

Generating configurations in atomic simulations: molecular dynamics

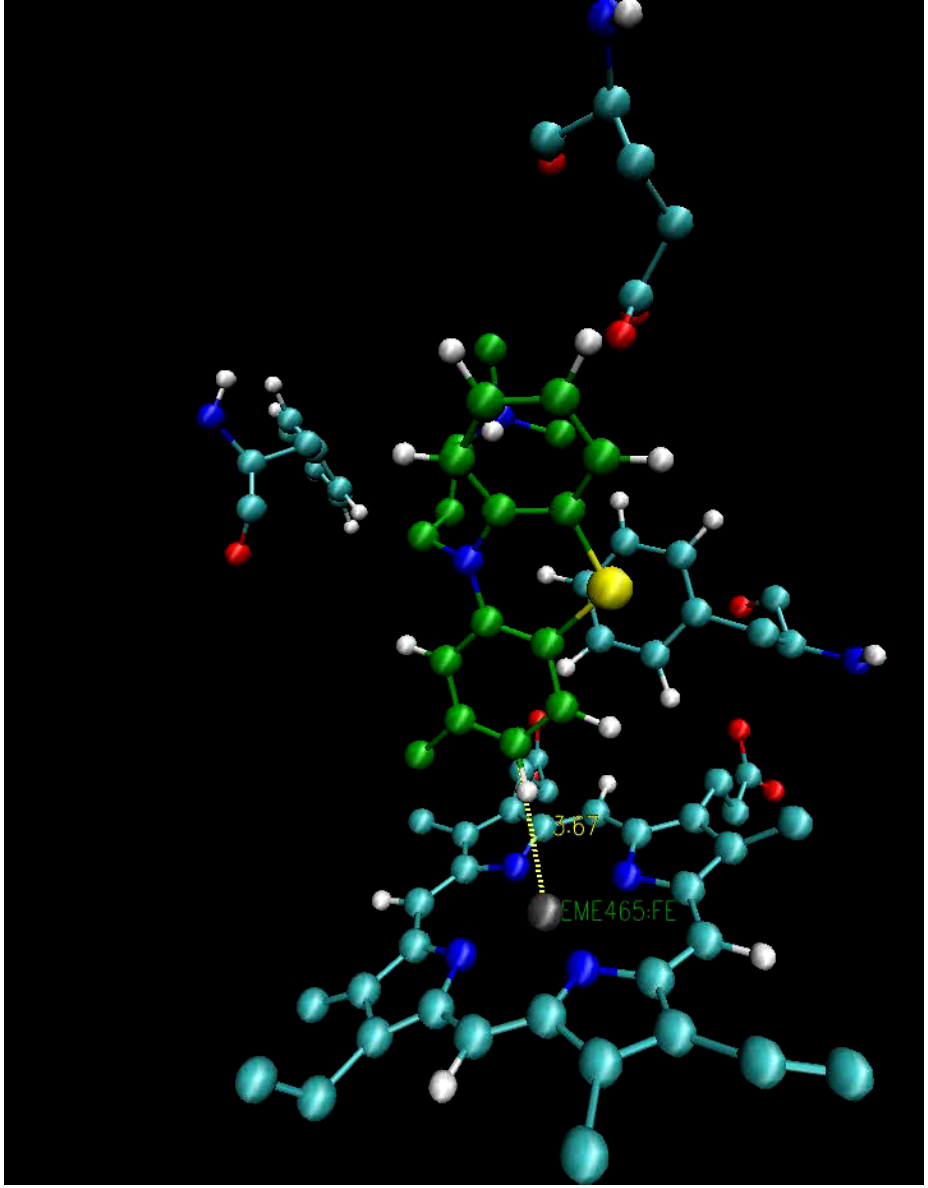


... comparable to shooting a movie of a molecular system...



$\Delta t \approx 10^{-15}$ seconds

Monte-Carlo: Alternative methodology for generating canonical



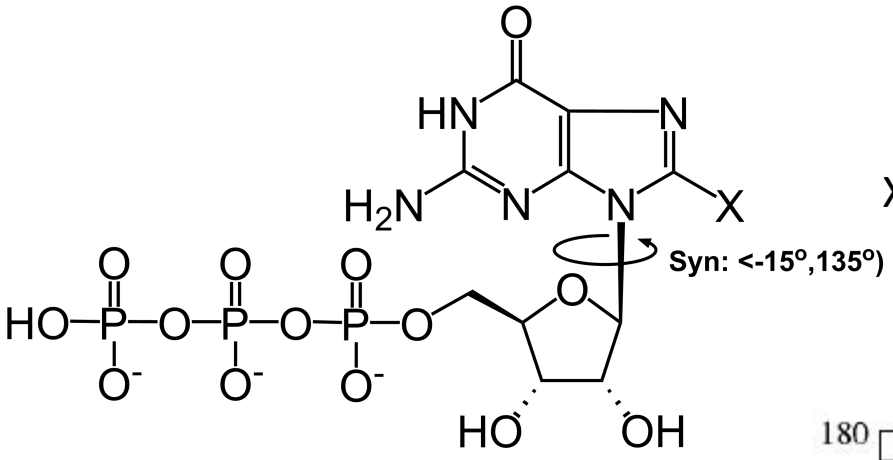
Hritz, J.; de Ruiter, A.; Oostenbrink, C. Impact of plasticity and flexibility on docking results for Cytochrome P450 2D6: a combined approach of molecular dynamics and ligand docking. *J. Med. Chem.* **2008**, 51, 7469-7477

Monte-Carlo: Alternative methodology for generating canonical structural ensemble

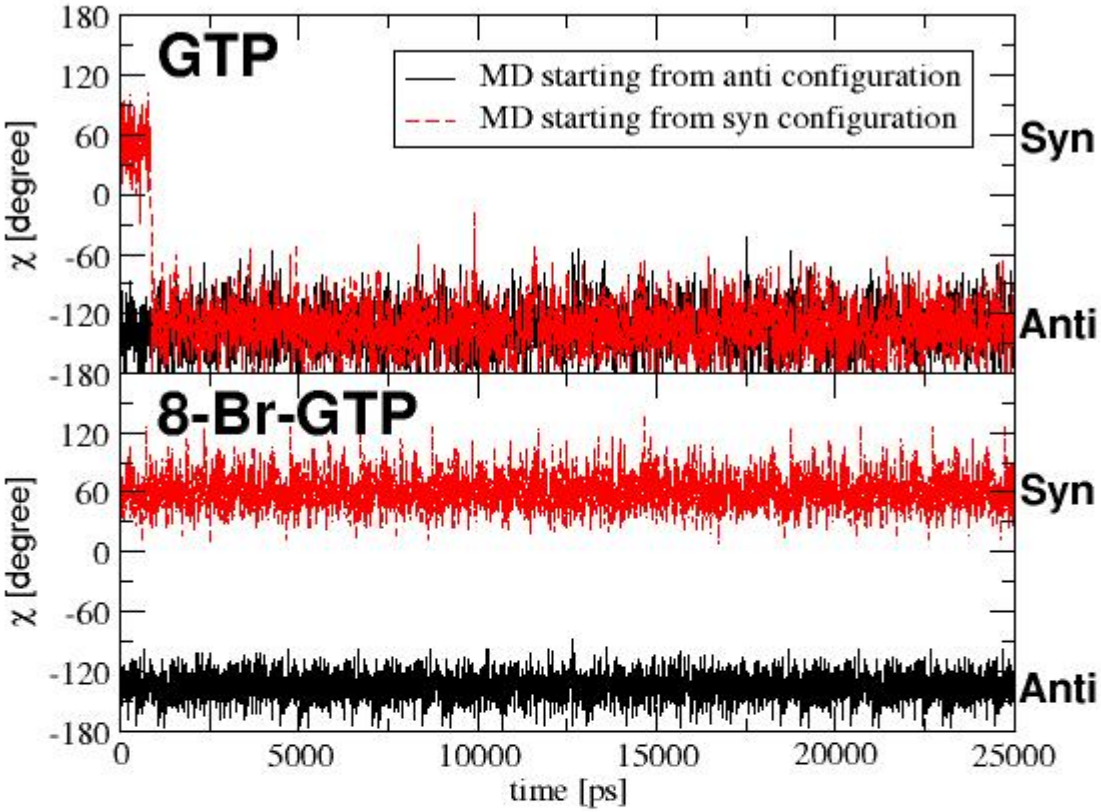
Metropolis criterion

-probability of moving from state 1 (having energy E_1) to the state 2 (having energy E_2) is:

$$p_{12}^{acc} = \min[1, e^{-\frac{E_2 - E_1}{kT}}]$$

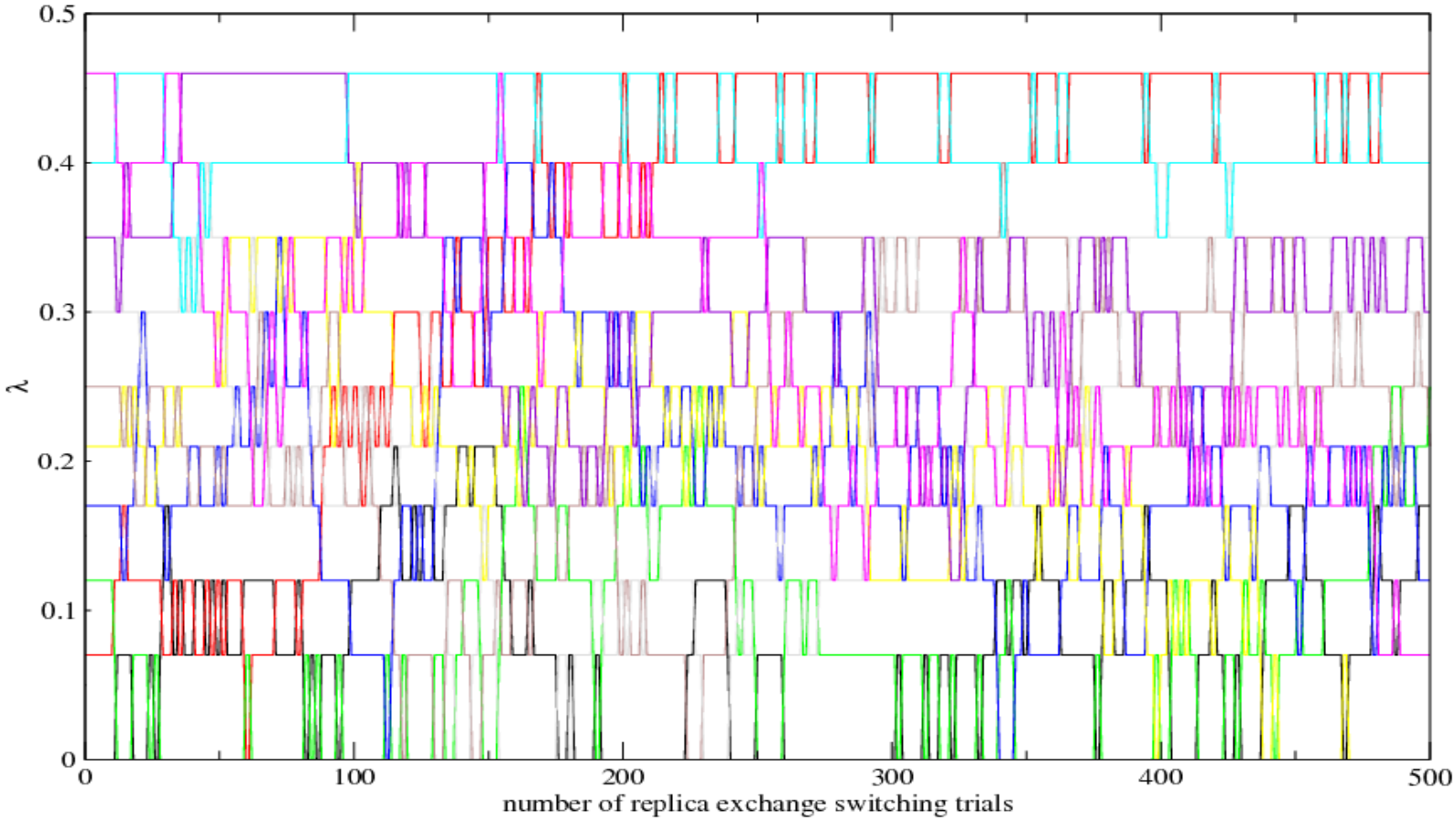

O
HN
N
H2N
N
N
X
Syn: <-15°,135°)
HO
P
O
O-
O
HO
OH

X = H, F, Cl, Br, CH_3


GTP
MD starting from anti configuration
MD starting from syn configuration
Syn
Anti
8-Br-GTP
Syn
Anti
χ [degree]
χ [degree]
180
120
60
0
-60
-120
-180
0
5000
10000
15000
20000
25000
time [ps]

Enhanced sampling by replica exchange molecular dynamics (REMD)

Hritz J., Oostenbrink C.
Hamiltonian replica exchange molecular dynamics using soft-core interactions. *J. Chem. Phys.* **2008,** 128, 144121
Hritz J, Oostenbrink C.
Optimization of Replica Exchange Molecular Dynamics by Fast Mimicking. *J. Chem. Phys.* **2007,** 127, 204104


0.5
0.4
0.3
0.2
0.1
0
λ
0
100
200
300
400
500
number of replica exchange switching trials

Replica exchange probabilities

Hansmann, U.H.E.; Chem. Phys. Lett. **1997**, 281, 140-150.

$$W(x) = \exp(-\beta H(\mathbf{r},\mathbf{p}))$$

probability of state x (Boltzmann factor)

$$W_{REM}(X) = \exp\left(-\sum_{i}^{M} \beta_i H\left(\mathbf{r}_i,\mathbf{p}_i\right)\right)$$

probability of global state X (product of single state x probabilities)

detailed balance condition on the transition probability w(X→X'):

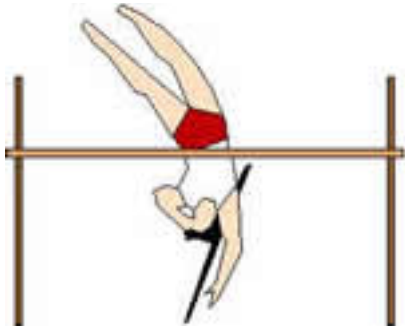
$$W_{REM}(X)w(X \to X') = W_{REM}(X')w(X' \to X)$$

Metropolis criterion for the exchange probability:

$$p(X \to X') = \frac{w(X \to X')}{w(X' \to X)} = \begin{cases} 1 & \text{for } \Delta \le 0 \\ \exp(-\Delta) & \text{for } \Delta > 0 \end{cases}$$

$$\Delta = \left(\beta_i - \beta_j\right)\left(U\left(\mathbf{r}_j\right) - U\left(\mathbf{r}_i\right)\right) \qquad \beta = 1/k_B T$$

Temperature REMD



$\Delta T \sim (\text{square root of degrees of freedom})^{-1}$

Is there alternative way how to get from one side of barrier to other one?

Hamiltonian replica exchange

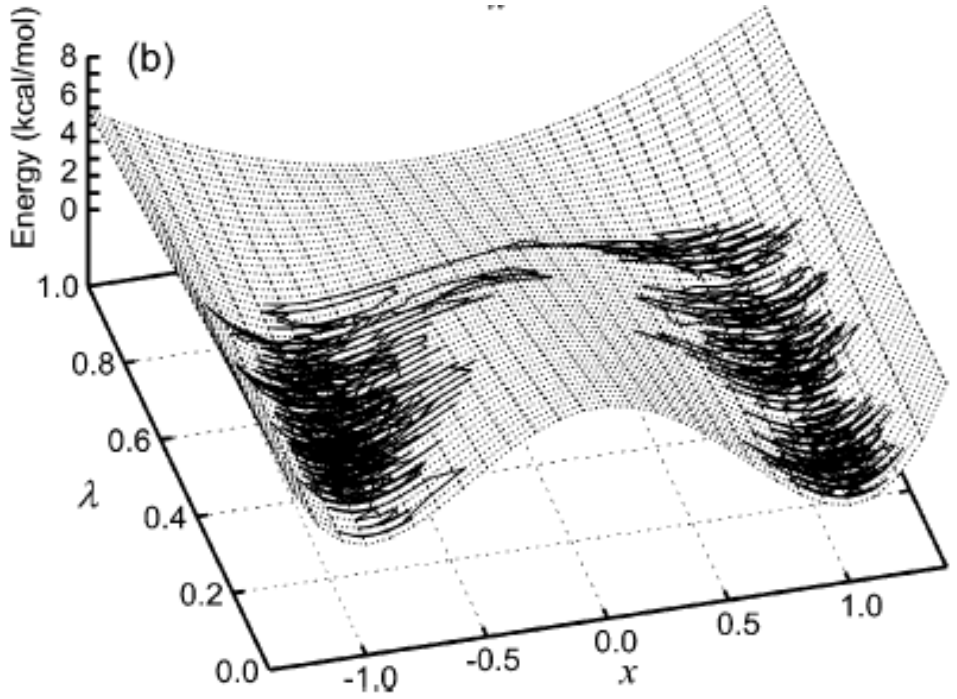
(Fukunishi, H.; Watanabe, O.; Takada, S.; J. Chem. Phys. **2002**, 113, 6042-6051.)

IDEA: instead of changing a temperature, rather alter specific interactions

$$H_i(\mathbf{r}_i, \mathbf{p}_i) = K(\mathbf{p}_i) + U_i(\mathbf{r}_i)$$

$$\Delta = \beta_i \left(U_i\left(\mathbf{r}_j\right) - U_i\left(\mathbf{r}_i\right)\right) - \beta_j \left(U_j\left(\mathbf{r}_j\right) - U_j\left(\mathbf{r}_i\right)\right)$$

Soft core interactions

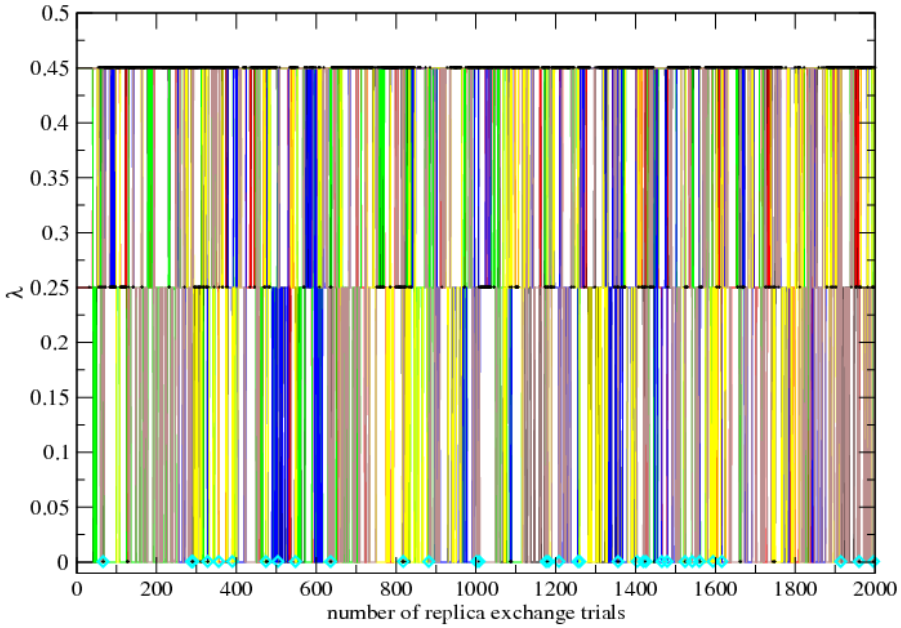


$$E_{\alpha}^{vdw}(r_{ij}) = \left(\frac{C_{12}}{A + r_{ij}^{6}} - C_{6} \right) \frac{1}{A + r_{ij}^{6}}; A = \alpha_{vdw} \frac{C_{12}}{C_{6}} \lambda^{2}$$

$$E_{\alpha}^{el}(r_{ij}) = \frac{q_i q_j}{4\pi\varepsilon} \frac{1}{\sqrt{B + r_{ij}^{2}}}; B = \alpha_{el} \lambda^{2}$$

Where else to get crazy ideas ☺?

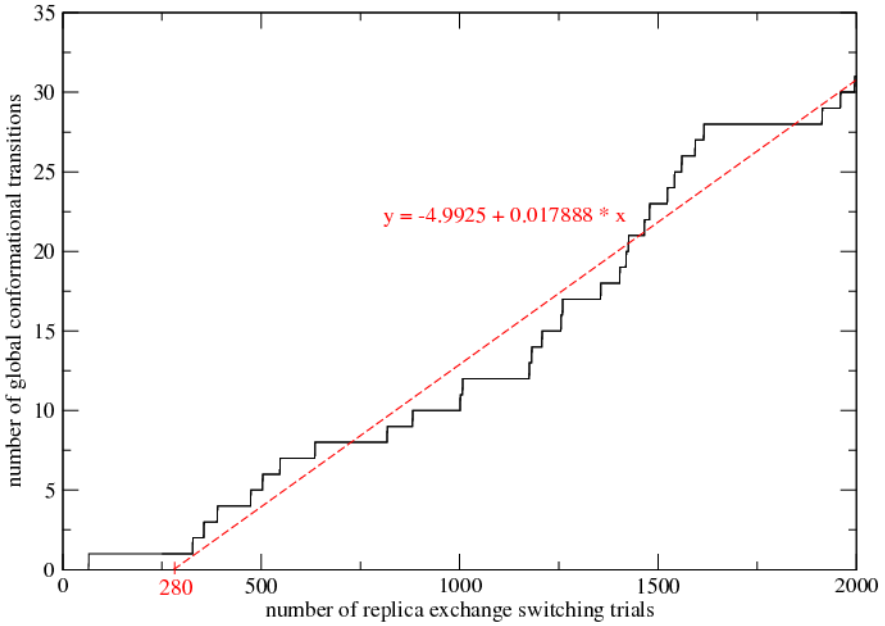
Real REMD of GTP



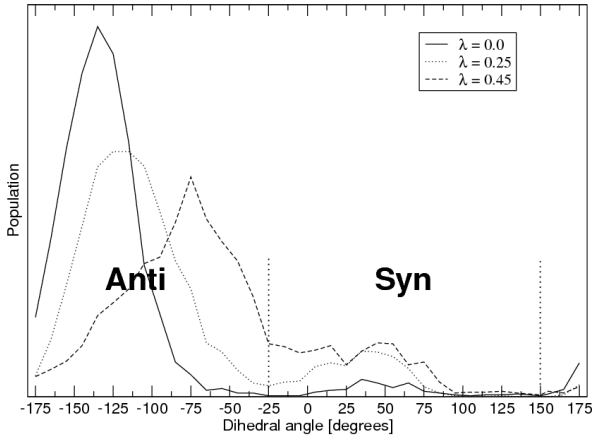
REMD costs: 6x5ns = 30ns
anti→syn transitions:16
syn→anti transitions:16

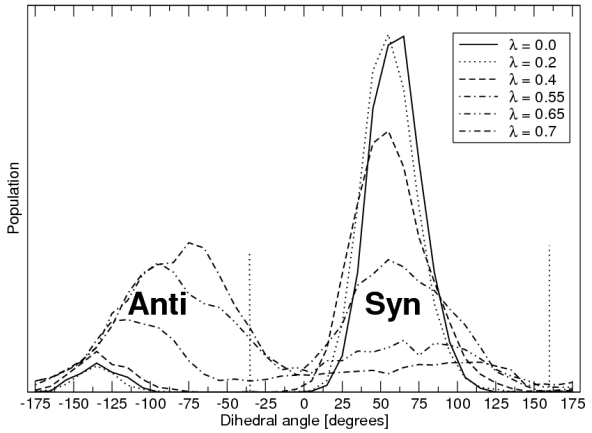
MD costs: 2x20ns = 40ns
anti→syn transitions: 0
syn→anti transitions: 1

Relaxation



Potential of mean force



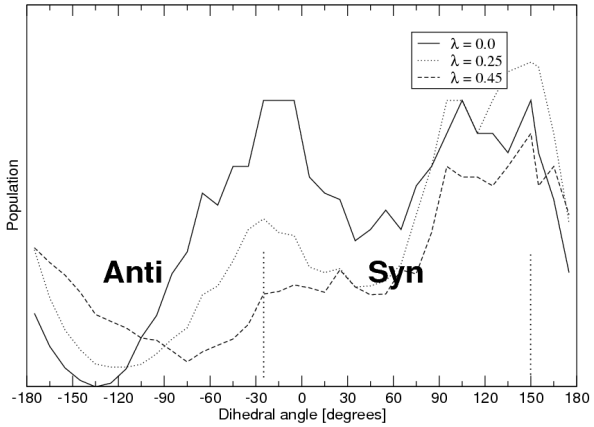


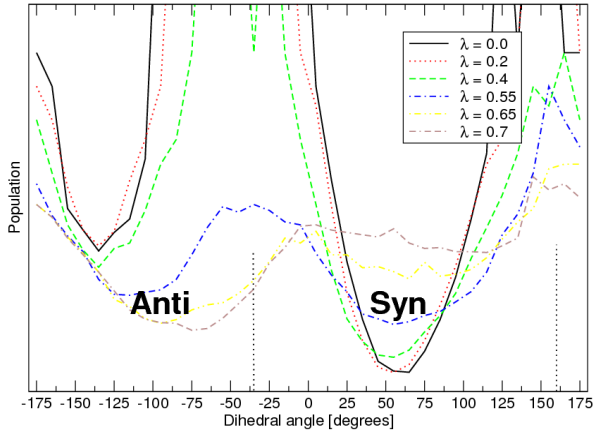
(REMD) $\Delta G^{GTP}_{syn-anti}$ 7.6 ± 0.3 kJ.mol^{-1}

(TI) $\Delta G^{GTP}_{syn-anti}$ 7.7 ± 1.2 kJ.mol^{-1}

(REMD) $\Delta G^{8-Br-GTP}_{syn-anti}$ -6.8 ± 0.9 kJ.mol^{-1}

(TI) $\Delta G^{8-Br-GTP}_{syn-anti}$ -6.8 ± 1.6 kJ.mol^{-1}





Molecular dynamics is more than super-microscope!

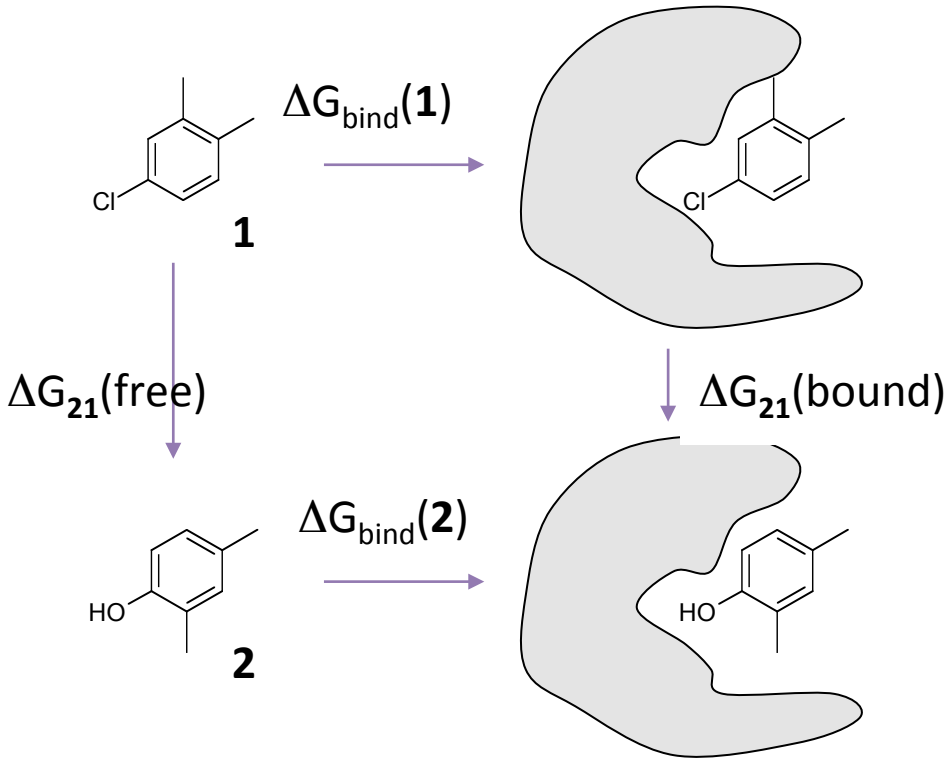
ENERGY!

Efficient free energy calculations for compounds with high intramolecular energy barriers

Hritz, J.; Lappchen T.; Oostenbrink, C.
Calculations of binding affinity between C8-substituted GTP analogs and the bacterial cell-division protein FtsZ.
Eur.Biophys. J.. **2010**, 39, 1573-1580

Hritz, J.; Oostenbrink, C.
Efficient free energy calculations for compounds with multiple stable conformations separated by high energy barriers.
J. Phys. Chem. B **2009**, 113, 12711-12720

Thermodynamic cycle



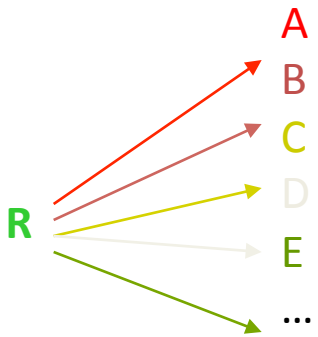
$$\Delta\Delta G_{bind} = \Delta G_{bind}(\mathbf{2}) - \Delta G_{bind}(\mathbf{1})$$
$$= \Delta G_{21}(free) - \Delta G_{21}(bound)$$

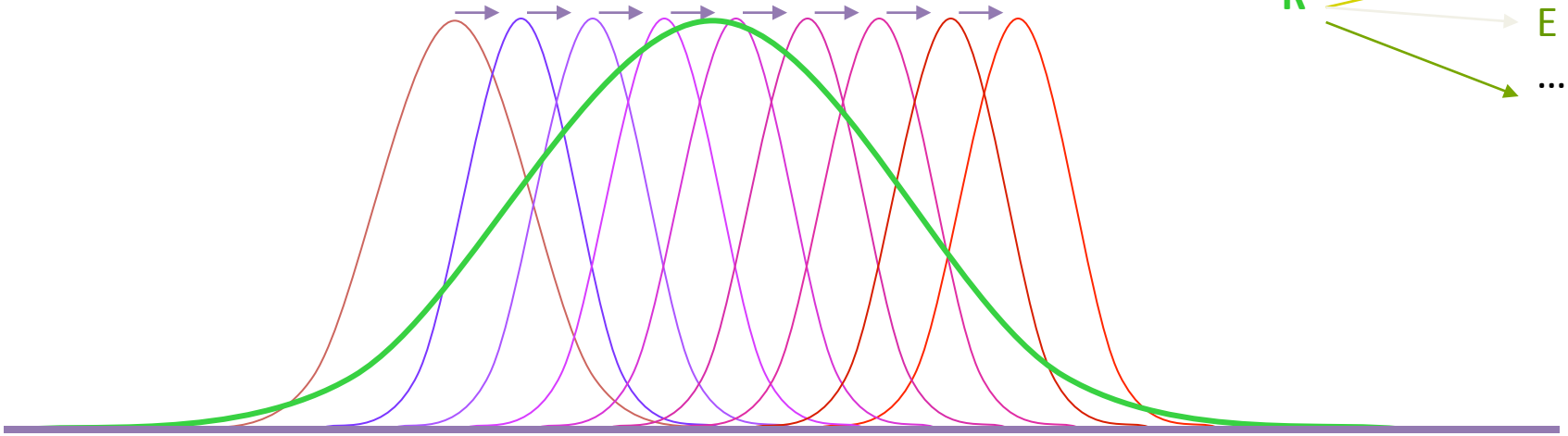
One-step perturbation

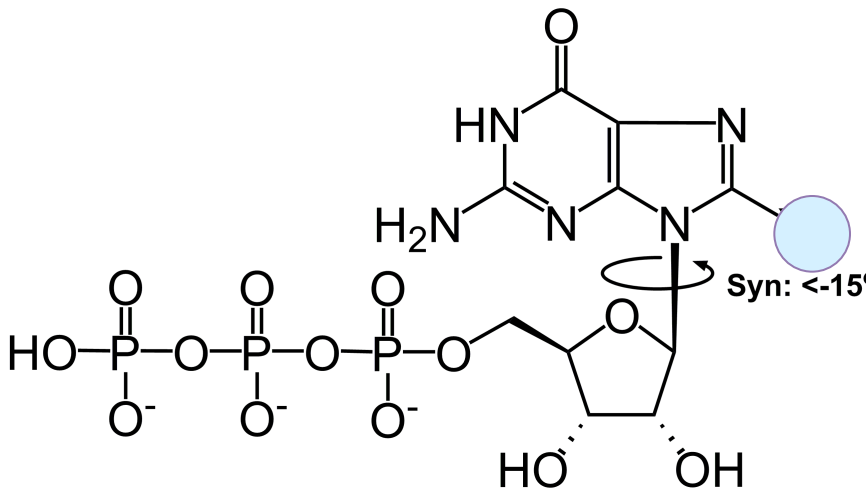
$$\Delta G_{AB} = -k_B T \ln \left\langle e^{-(E_A - E_B)/k_B T} \right\rangle_B$$

$$\Delta G_{AB} = \Delta G_{AR} - \Delta G_{BR}$$

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







X = H, F, Cl, Br, CH_3

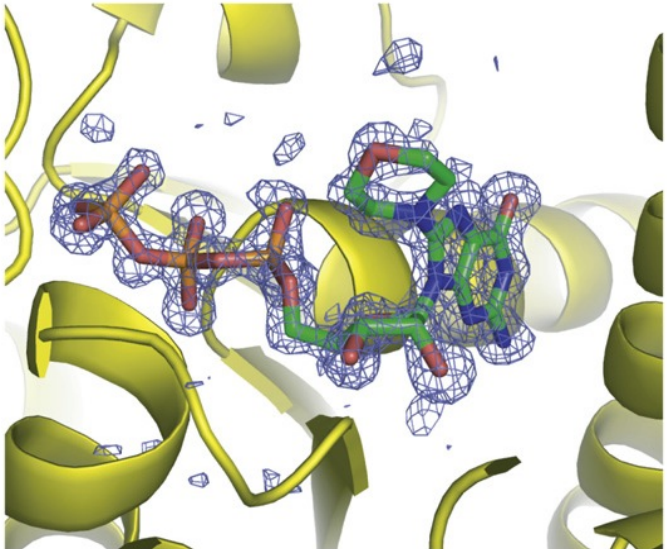
$$\Delta G_{SR} = G_R - G_S = -k_B T \ln \left\langle e^{-\frac{\left(H_R(\mathbf{q},\mathbf{p}) - H_S(\mathbf{q},\mathbf{p})\right)}{k_B T}} \right\rangle_S$$

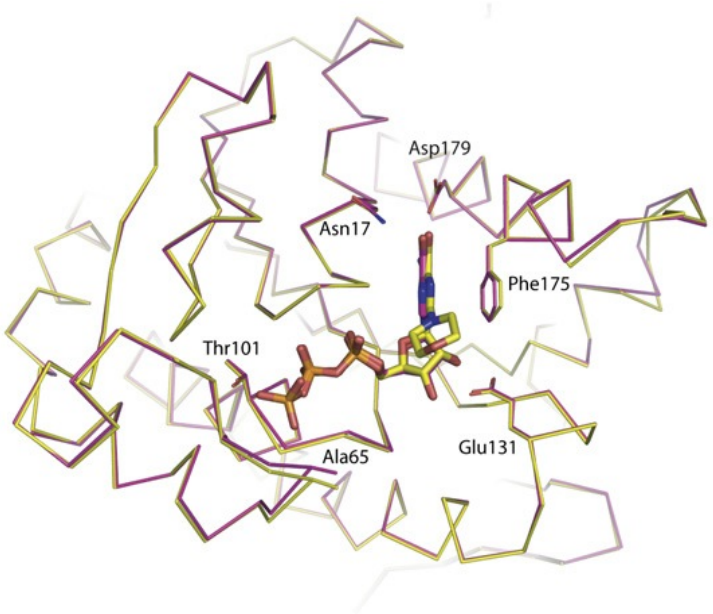
$$p_i^R = \frac{e^{-\frac{\left(H_R(\mathbf{q}_i,\mathbf{p}_i) - H_S(\mathbf{q}_i,\mathbf{p}_i)\right)}{k_B T}}}{\sum_i e^{-\frac{\left(H_R(\mathbf{q}_i,\mathbf{p}_i) - H_S(\mathbf{q}_i,\mathbf{p}_i)\right)}{k_B T}}}$$

Enhanced sampling one step perturbation method (ES-OS)

Crystal structures of FtsZ from Aquifex aeolicus

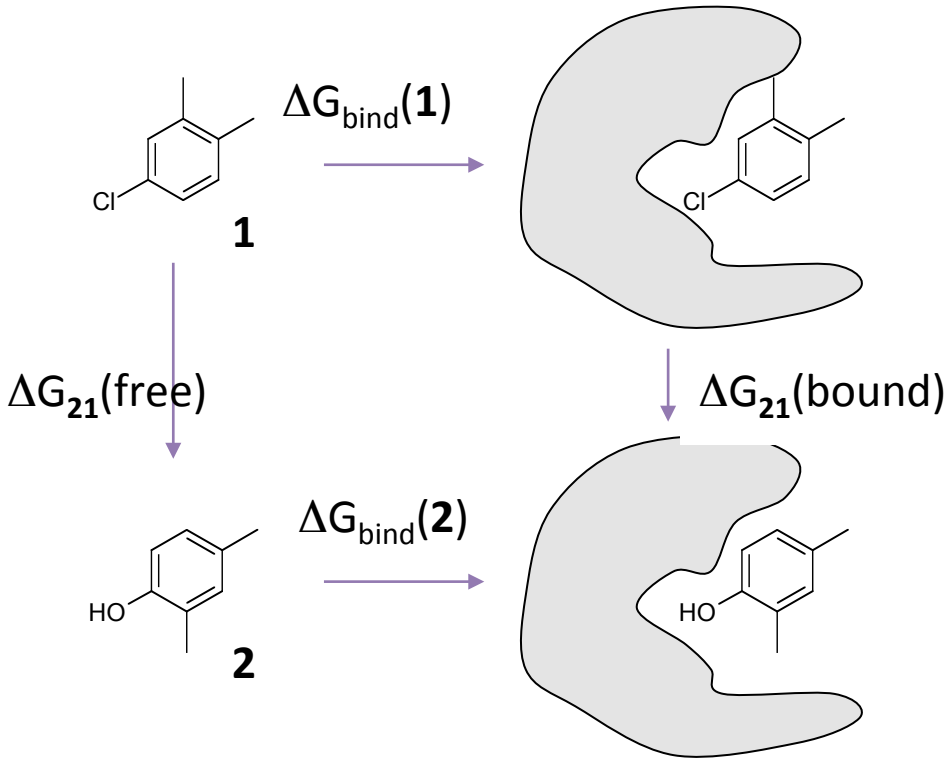
(Oliva et al. J. Mol. Biology (2007); Lappchen et al. Chemistry & Biology (2008))





Figures taken from Lappchen et al. Chemistry & Biology (2008)

Thermodynamic cycle



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

The comparison of relative binding affinities obtained from computational (Hritz, J. et al. Eur.Biophys. J. (2010)) simulations and experimental assays (Lappchen et al. Chemistry & Biology (2008)).

	$\Delta G^{OS}_{GTP,R}(FtsZ)$ [kJ.mol^{-1}]	$\Delta G^{ES-OS}_{GTP,R}(aq)$ [kJ.mol^{-1}]	$\Delta\Delta G^{calc}_{GTP,R}(bind)$ [kJ.mol^{-1}]	$\Delta\Delta G^{exp}_{GTP,R}(bind)$ [kJ.mol^{-1}]
GTP	0	0	0	0
8-F-GTP	14.56±1.08	15.03±1.38	-0.47±2.46	-----
8-Cl-GTP	16.45±1.03	12.75±1.25	3.7±2.28	8.01±4.4
8-Br-GTP	15.96±0.99	5.85±1.34	10.11±2.33	9.24±1.8
8-CH_3-GTP	23.03±1.02	12.55±1.41	10.48±2.43	~8.7±2.6[a]

[a] Value for $\Delta\Delta G^{exp}_{GTP,8-CH_3-GTP}(bind)$ was estimated from an experimental IC_{50} value of GTPase activity.

Conclusion

- Visualization of biomolecular structures, their electrostatics potential, etc. can provide very useful information
- Dynamical features can be obtained from molecular dynamics simulations. Be aware about a lot of used approximations, simplifications and restricted time length of simulation.
- Potential of molecular dynamics can be enhanced sampling methods such as replica exchange molecular dynamics: T-REMD or H-REMD.
- Free energy calculations allows for calculating the binding affinities, solvation free energies, lipophilicities, etc.