Biomolecular modeling III

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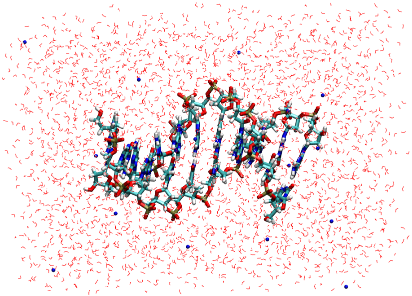
Déjà vu

Biomolecular simulation

Each atom – x, y, z coordinates



Déjà vu

Expression for energy – the force field

$$E(R^N) =$$

$$= \frac{1}{2}\sum_i k_i(r_i - r_i^0)^2 + \frac{1}{2}\sum_j k_j^\vartheta(\vartheta_j - \vartheta_j^0)^2 + \frac{1}{2}\sum_n V_n \cdot \cos\left[n\omega - \gamma_n\right]$$

$$+ \sum_i^N \sum_{j=i+1}^N \left\{ 4\varepsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right) + \frac{1}{4\pi\varepsilon_0}\frac{q_i q_j}{r_{ij}} \right\}$$

Déjà vu

Equations of motion

$$m \cdot \ddot{r} = F$$

Verlet integration

$$\begin{aligned} \ddot{r}(t) &= a(t) = \frac{F(t)}{m} = -\frac{1}{m}\frac{\partial V}{\partial r}(t) \\ r(t+\Delta t) &= 2 \cdot r(t) - r(t-\Delta t) + \ddot{r}(t)\Delta t^2 \end{aligned}$$

Enhanced sampling

How to save time, and time is money

Problem

with normal nanosecond length MD simulations:

It is difficult to overcome barriers to conformational transitions,
and only conformations in the neighborhood of the initial structure may be sampled,
even if some other (different) conformations are more relevant, i.e. have lower free energy

Special techniques are required to solve this problem.

Energy barriers in simulations

Energy landscapes in large (bio)molecular systems
– multitude of almost iso-energetic minima,
separated from each other by energy barriers of various heights

Each of these minima $\equiv$ one particular structure (conformation);
neighboring minima correspond to similar structures

Structural transitions are barrier crossings, and
the transition rate is determined by the height of the barrier.

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the transition rate is determined by the height of the barrier.

Normal MD – only nanosecond time scales are accessible,
so only the smallest barriers are overcome in simulations,
and only small structural changes occur. $k \propto \exp\left[-E_{\mathrm{A}}/kT\right]$

The larger barriers are traversed more rarely
(although the transition process itself may well be fast),
and thus are not observed in MD simulations.

Using quotations by Helmut Grubmüller

Note – do not be afraid of Arrhenius

How often does something happen (in a simulation)?

$k = A \times \exp\left[-E_\mathrm{A}/kT\right]$, let us have $A = 1 \times 10^9\ \mathrm{s}^{-1}$

E_A kcal/mol	k 1/s	$1/k$ μs
1	0.19×10^9	0.005
3	6.7×10^6	0.15
5	0.24×10^6	4.2
7	8.6×10^3	120

So, if the process has to overcome a barrier of 5 kcal/mol,
we will have to simulate for 4 μs to see it happen once on average.

Replica-exchange molecular dynamics

REMD (or parallel tempering) – method to accelerate the sampling of configuration space, which can be applied
even if the configurations of interest are separated by high barriers.

Several (identical) replicas of the molecular system are simulated at the same time, with different temperatures.

The coordinates+velocities of the replicas may be switched (exchanged) between two temperatures.

Probability of replica exchange

The probability of the replica exchange between T_1 and T_2 is determined in (regular) time intervals from the instantaneous potential energies U_1 and U_2 in the corresponding simulations as

$$\mathcal{P}(1 \leftrightarrow 2) = \begin{cases} 1 & \text{if } U_2 < U_1, \\ \exp\left[\left(\frac{1}{k_B T_1} - \frac{1}{k_B T_2}\right) \cdot (U_1 - U_2)\right] & \text{otherwise.} \end{cases}$$

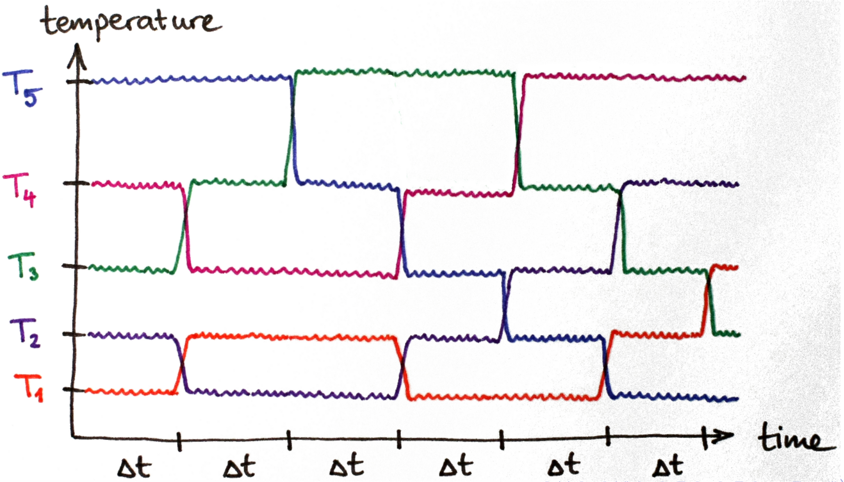
Then, if $\mathcal{P}(1 \leftrightarrow 2)$ is larger than a random number from $(0, 1)$, the replicas in simulations at T_1 and T_2 are exchanged.

Setup of the simulation of replicas

Simulated one replica at the temperature of interest ($T_1 = 300$ K) and several other replicas at higher temp. ($T_1 < T_2 < T_3 < \ldots$).

After (say) 1000 MD steps, attempt exchanges $1 \leftrightarrow 2$, $3 \leftrightarrow 4$ etc., and after next 1000 steps do the same for $2 \leftrightarrow 3$, $4 \leftrightarrow 5$ etc.
so only try to exchange replicas at “neighboring” temperatures

Setup of the simulation of replicas



Advantages of REMD

- due to the simulations at high temperatures
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- first application – protein folding (Sugita & Okamoto, Chem. Phys. Lett. 1999)

Choice of temperatures to simulate

Important – suitable choice of temperatures T_i – criteria:

- how frequent exchanges we wish (average prob. $\mathcal{P}(1 \leftrightarrow 2)$)
- the size of the system (degrees of freedom N_{dof})
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For protein/water systems with all bond lengths constrained:

- $N_{\text{dof}} \approx 2N$ (N – number of atoms)
- average probability is related to $T_2 - T_1 = \varepsilon T_1$ as

$$\overline{\mathcal{P}(1 \leftrightarrow 2)} \approx \exp\left[-2\varepsilon^2 N\right]$$

- set of temperatures may be designed to suit the problem

Disadvantages of parallel tempering REMD

- large number of atoms: low exchange probability
  $\rightarrow$ low efficiency
- high temperature – sensitive biostructures may not survive
  (membranes etc.)

how to apply the replica-exchange idea and avoid these issues?

Hamiltonian replica exchange – HREX

also called ‘Replica exchange with solute tempering’ (REST)

$$P = \exp\left[-\frac{U}{kT}\right] = \exp\left[-\beta U\right]$$

- note: $\frac{1}{2}U$ would be the same as $2T$

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- note: $\frac{1}{2}U$ would be the same as $2T$
- force field energy U is combined from many individual terms
  - let us scale selected terms (not all of them!)
  - is not possible for temperature scaling (a single T)
  - 'heating' of a (small) part of the system
  - typically, a group of atoms – a ligand, or several AAs. . .

Hamiltonian replica exchange – HREX

Simulations 1 and 2 are performed
with different force fields U_1 and U_2
How to calculate the probability of exchange?
(q_1 and q_2 – coordinates of atoms in simulations 1 and 2)

$$\begin{aligned} \Delta &= \frac{U_1(q_2) - U_1(q_1) - U_2(q_1) + U_2(q_2)}{kT} \\ \mathcal{P}(1 \leftrightarrow 2) &= \begin{cases} 1 & \text{if } \Delta \leq 0, \\ \exp\left[-\Delta\right] & \text{otherwise.} \end{cases} \end{aligned}$$

Then, if $\mathcal{P}(1 \leftrightarrow 2)$ is larger than a random number from $(0, 1)$, the replicas in simulations with U_1 and U_2 are exchanged.

HREX – a good variant

- divide the system into two parts:
- hot – small, will be subject to extended sampling
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- additional scaling of dihedral angles

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Then, the 'effective' temperatures are

- inside hot: $T/\lambda_m > T$
- interactions between hot and cold: $T/\sqrt{\lambda_m}$
- inside cold: T is retained

HREX

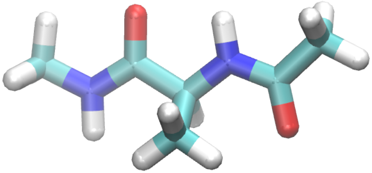
Meaning of temperature

- kinetic energy $\leftarrow$ velocities
  - does not change, is the same in hot and cold (300 K)
  - simulation settings need not be adjusted (time step!)
  - unlike in parallel tempering
- factor affecting the population of states
  - we play with this

HREX – example

Solute tempering – dialanine

- alanine dipeptide – 22 atoms, 1 pair of $\varphi - \psi$
- 5 replicas, $\lambda = 1 \ldots 0.18$ i.e. $T_m = 300 \ldots 1700$ K
- exchange every 0.1 ps, observed $\overline{\mathcal{P}}$ =0.25–0.50



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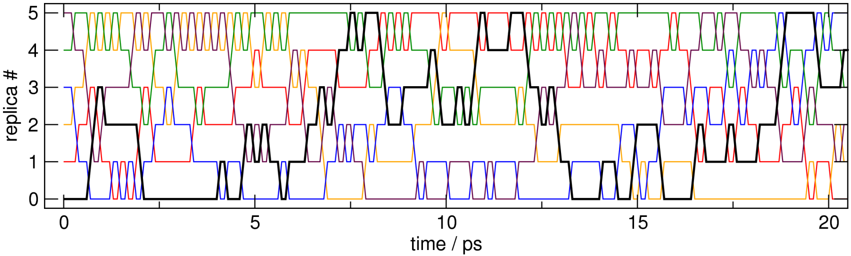
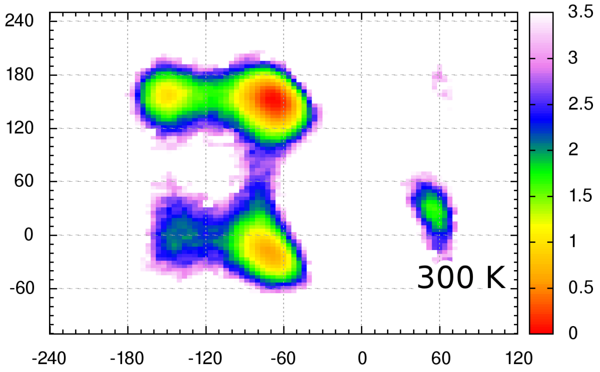


HREX – example

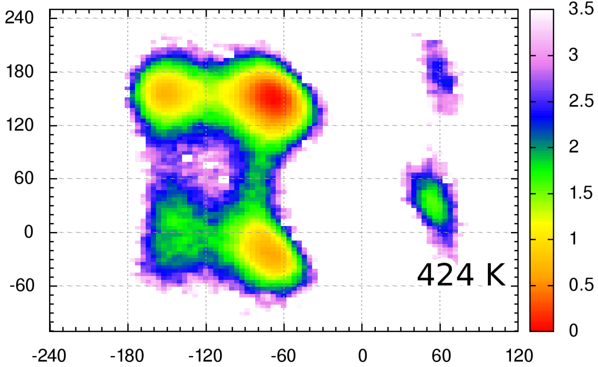
Solute tempering – dialanine – replica #0



$\varphi - \psi$ in degrees, ΔF in kcal/mol

HREX – example

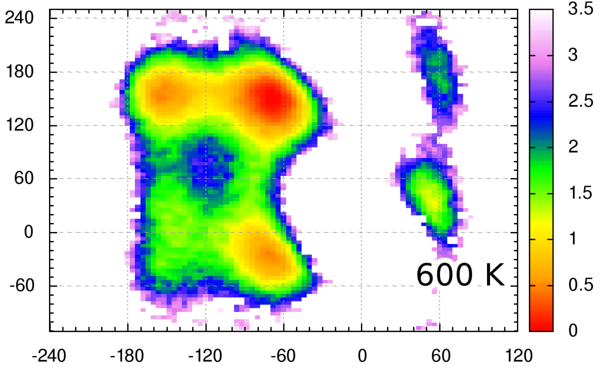
Solute tempering – dialanine – replica #1



$\varphi - \psi$ in degrees, ΔF in kcal/mol

HREX – example

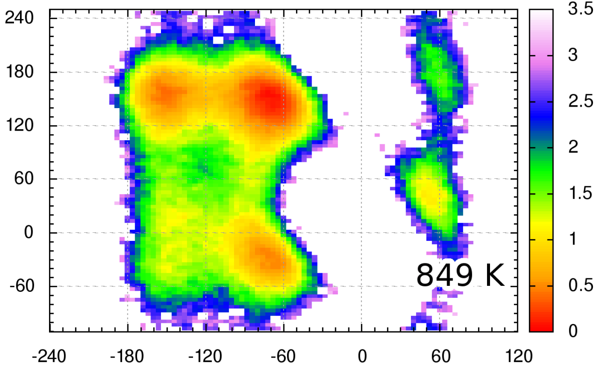
Solute tempering – dialanine – replica #2



$\varphi - \psi$ in degrees, ΔF in kcal/mol

HREX – example

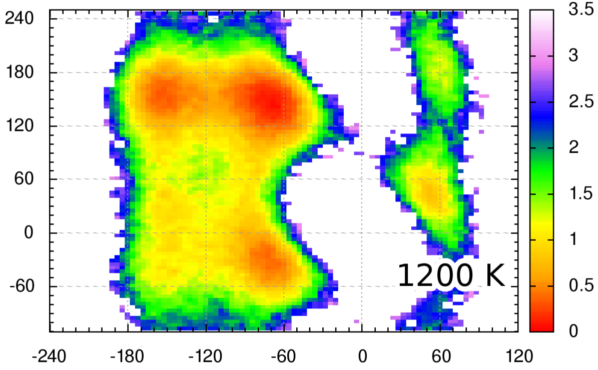
Solute tempering – dialanine – replica #3



$\varphi - \psi$ in degrees, ΔF in kcal/mol

HREX – example

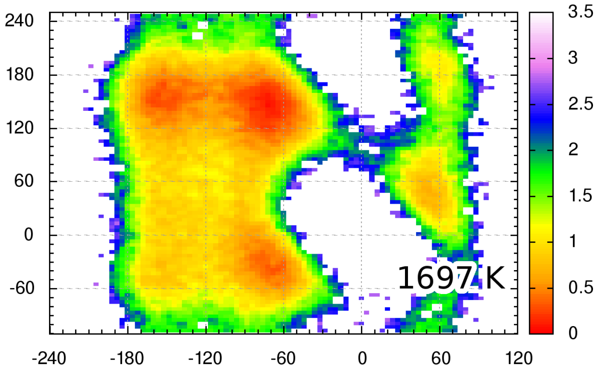
Solute tempering – dialanine – replica #4



$\varphi - \psi$ in degrees, ΔF in kcal/mol

HREX – example

Solute tempering – dialanine – replica #5



$\varphi - \psi$ in degrees, ΔF in kcal/mol

Methods using biasing potentials

Other approaches use a different idea:

It is easy to introduce an additional contribution
to the potential energy of the molecule

Example – the extra potential may force the molecule
over an energy barrier, to explore other conformations

It is 'unrealistic' – we do not simulate a real molecule
but this bias may be removed by a right post-processing

Note: use of NMR-based distance restrains in MD simulations
$\rightarrow$ 'NMR-refined' structure of the molecule (e.g. PDB ID 1AC9)

Conformational flooding

– way to accelerate conformational transitions in MD simulations by several orders of magnitude
– brings slow conformational transitions into the scope

First – we generate a trajectory with a normal MD simulation
Then – using this ensemble of structures, we construct
a localized artificial flooding potential V_{fl}:

- V_{fl} shall affect only the initial conformation and vanish everywhere outside of this region of conformational space
- V_{fl} shall be well-behaved (smooth) and 'flood' the entire initial potential-energy well

Flooding potential

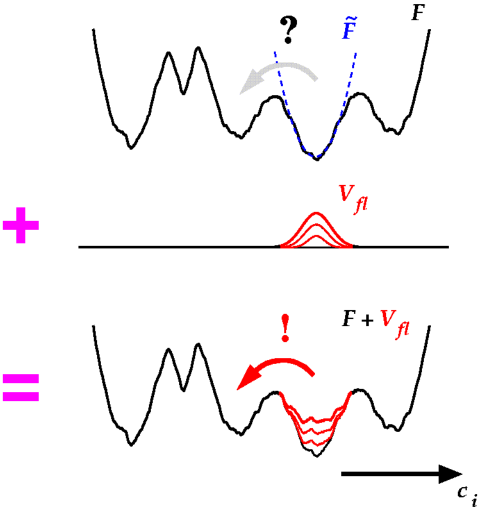
a multivariate (n-dimensional) Gaussian function is good:

$$V_{\text{fl}} = E_{\text{fl}} \cdot \exp\left[-\frac{E_{\text{fl}}}{2k_{\text{B}}T} \cdot \sum_{i=1}^{n} q_i^2 \lambda_i\right]$$

E_{fl} – strength of the flooding potential (constant)
q_i – coordinates along the first n essential dynamics modes (PCA)

Here, the first n essential dynamic modes with eigenvalues λ_i will be flooded

The course of flooding simulation



The course of flooding simulation

The flooding potential is added to the force field, and 'flooding' (biased) simulations are performed.

The energy minimum of the initial conformation is elevated
→ the height of barriers is reduced
→ the transitions are accelerated (TS theory)

Note: we have modified only the energy landscape within the minimum where the dynamics is already known, i.e. uninteresting
The barriers and all the other minima – which we are interested in – are not modified at all.

CF is expected to induce unbiased transitions – those which would be observed without flooding, on a much longer time scale.

Metadynamics

– aimed at reconstructing the multidimensional free energy of complex systems (Laio & Parrinello 2002)

– based on an artificial dynamics (metadynamics) performed in the space of a few collective variables S (e.g. normal modes)

– at regular time intervals during the simulation,
an additional biasing energy function is added to the force field
  – a Gaussian that is centered on the current structure

using quotations by Alessandro Laio

Metadynamics – how it works

a new Gaussian is added at every time interval t_G,
and the biasing potential at time t is given by

$$V_G(S(x),t) = \sum_{t'=t_G,2t_G,3t_G,\ldots} w \cdot \exp\left[-\frac{(S(x)-s_{t'})^2}{2\cdot\delta s^2}\right]$$

w and δs – height and width of the Gaussians
$s_t = S(x(t))$ – value of the collective variable at time t

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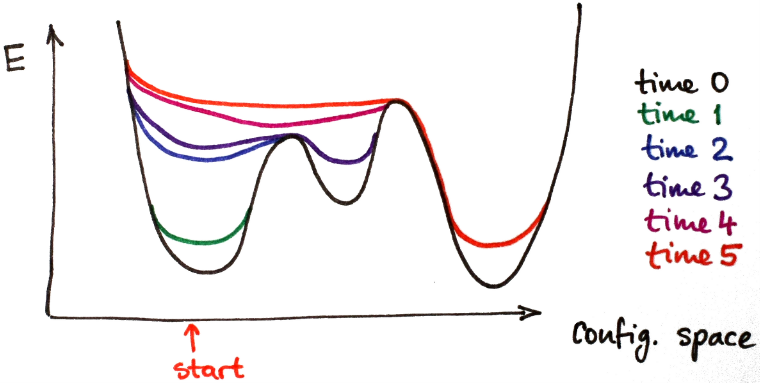
In the course of the simulation, this potential is filling the minima on the free energy surface that the system is traveling through.

So, the MD has a memory via the biasing potential

Metadynamics – what it looks like



https://www.youtube.com/watch?v=IzEBpQ0c8TA
https://www.youtube.com/watch?v=iu2GtQAyoj0

Properties of metadynamics

Metadynamics – to explore new reaction pathways,
accelerate rare events,
and also to estimate the free energies efficiently.

- The system escapes a local free energy minimum through the lowest free-energy saddle point.
- The dynamics continues, and all of the free-energy profile is filled with the biasing Gaussians.
- At the end, the sum of the Gaussians provides the negative of the free energy.

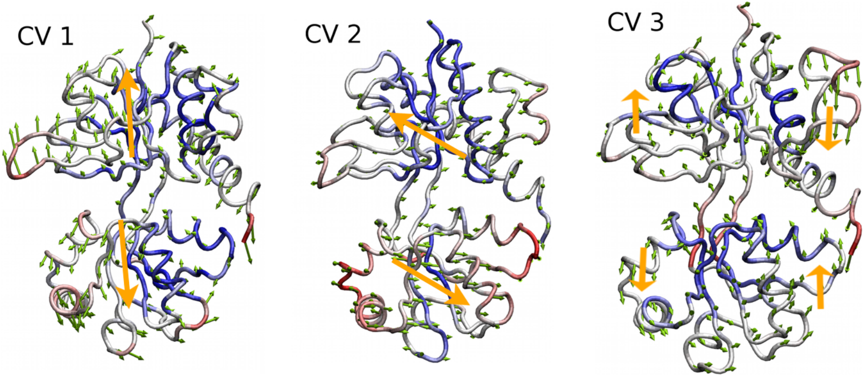
Properties of metadynamics

Crucial point – identify the collective variables of interest
that are difficult to sample because of high barriers

These variables $S(x)$ are functions of the coordinates of the system;
practical applications – up to 3 such variables,
and the choice depend on the process being studied.

Typical choices – principal modes of motion obtained with PCA
Still, the choice of S may be difficult

Example – opening of a protein binding pocket



clamshell twisting rocking

courtesy Tino Wolter

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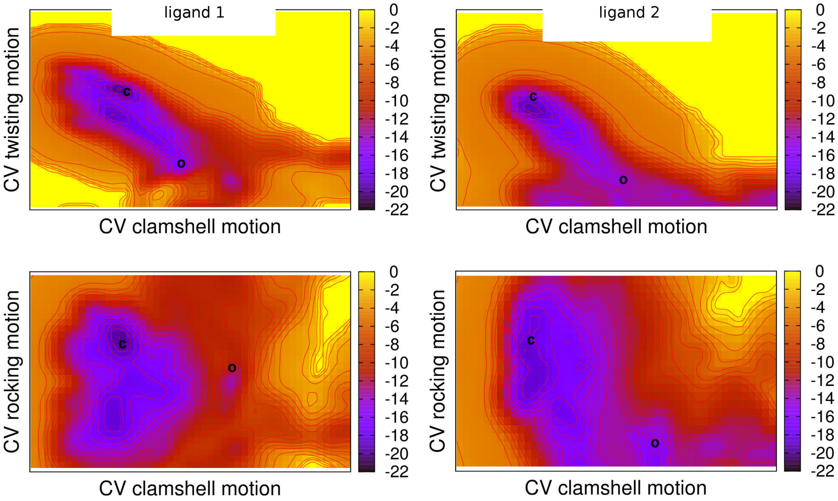

courtesy Tina Walter

Enhanced sampling methods – comparison

Biasing potential methods – metadynamics, umbrella sampling

- required: a priori choice of reaction coordinate(s) to be biased
- problem – success depends on that choice, possibly non-trivial

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REMD with parallel tempering

- $+$ no such required, can be used rather blindly
- $-$ all of the system heated $\rightarrow$ may destroy something
- $-$ no knowledge of the system may be embedded
- $-$ poor efficiency for big systems: $\overline{\mathcal{P}(1 \leftrightarrow 2)} \approx \exp\left[-2\varepsilon^2 N\right]$
  $\rightarrow$ critical problem

Enhanced sampling methods – comparison

Hamiltonian replica exchange (HREX)

- in intermediate position
  between metadynamics/US and REMD-PT
- simpler to use than metadynamics/US
  – results depend not so strongly on the choices to be made
- efficiency does not depend on the overall system size

Coarse-grained models

United-atom force fields

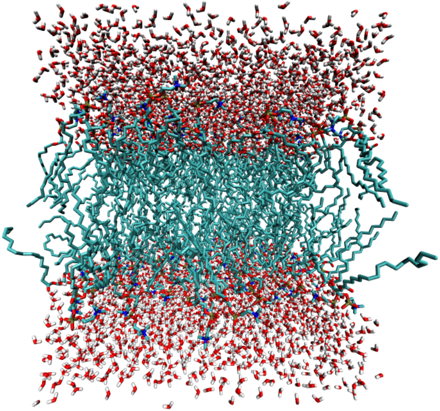
Early biomolecular force fields (e.g. Weiner 1984)
– united-atom approach
– hydrogen atoms considered as condensed to the heavy atom
– mass and charge represent such a group of atoms as a whole
– number of atoms reduced considerably relative to all-atom FF
– popular in the 1990's

This approach works very well for non-polar C–H bonds,
so a methyl group constituting of one united atom works good.

A substitution of a polar O–H group by a single particle
would be very crude (without any correction terms in FF)
$\rightarrow$ only non-polar hydrogens are usually condensed with heavy

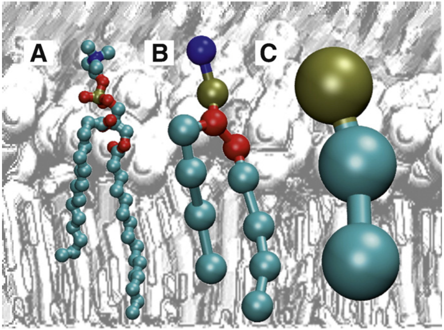
United-atom force fields

– still used e.g. to describe lipids, where each CH_2 is a united atom



– simulation of a DOPC bilayer in water – Berger FF for the lipid

United-atom and coarse-grained force fields



(A) united-atom, (B) specific and (C) generic coarse-grained

from Marrink et al., Biochim. Biophys. Acta 2009

Coarse-grained models

Coarse graining – an advanced and sophisticated approach to reduce the computational expense of simulations

The same idea – reduction of the number of particles
Considered are particles composed of several atoms – beads
The number of inter-particle interactions decreases, reducing the computational expense largely.

The necessary parameters of the force field are often obtained by fitting to all-atom force fields.

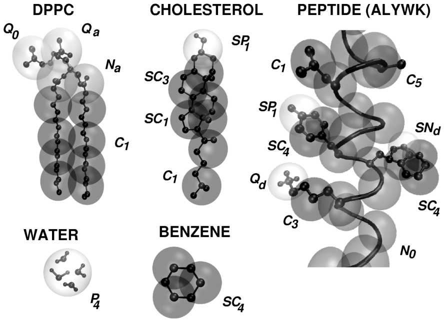
Coarse-grained models

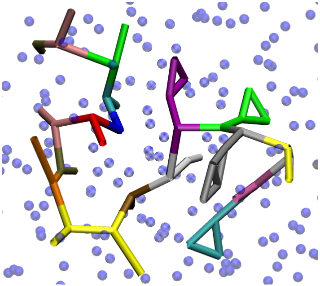
Every bead usually represents several atoms,
and a molecule is composed of several beads.
For the solvent, there is e.g. a 'water bead'
composed of four H_2O molecules.

Note that some of the transferability of all-atom FF is lost
– e.g. secondary structure of proteins is fixed with Martini FF
Also, hydrogen bonding cannot be described with beads!
solution – compensation with Lennard-Jones contributions

Such CG force fields are particularly useful for simulations
of large-scale conformational transitions, which involve
exceedingly large molecular systems, excessive time scales,
or both.

Martini force field





left – mapping of beads onto molecular fragments with Martini FF

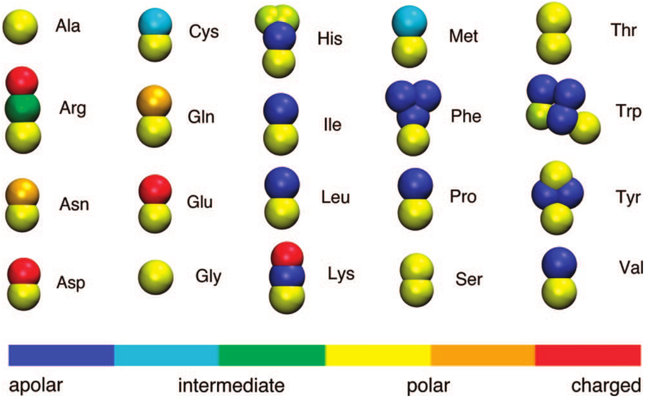
– 3 to 4 heavy atoms compose one bead ('4-to-1 mapping')

– mass of beads – 72 u ($= 4\ H_2O$), or 45 u in ring structures

right – a solvated peptide with Martini

from the Martini website

Martini force field



The CG force field Martini – amino acids

from Monticelli et al., J. Chem. Theory Comput. 2008

Acceleration of the simulation

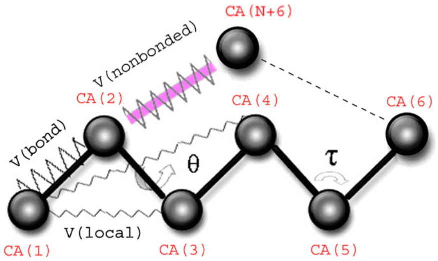
Why does a coarse-grained simulation run faster?

- smaller number of particles → fewer interactions
- long integration time step due to large masses of beads
  – 25 fs with Martini (i.e. 100 fs effectively, see below)
- FF often constructed for use with faster simulation algorithms
  – e.g. cut-off for electrostatics with Martini
- smaller number of DoF → smoother free energy surfaces
  → fewer barriers → acceleration of all processes
  (by a factor of 3 to 8 for Martini, but not uniformly!
  – factor of 4 for acceleration of diffusion in water)

"... length and time scales that are 2 to 3 orders of magnitude larger compared to atomistic simulations, providing a bridge between the atomistic and the mesoscopic scale."

Coarse-grained models

Another example – Vamm force field for proteins,
where every amino acid is represented by a single bead at C-α.



from Korkut & Hendrickson 2009

Free energy simulations

Motivation

a physical quantity that is of most interest in chemistry?

free energies – Helmholtz F or Gibbs G

– determine whether processes (reactions) run spontaneously or not

– holy grail of computational chemistry,
both for their importance
and because they are difficult to calculate

Convergence issue

(all of the formulas come from statistical thermodynamics)

– especially desperate for free energies:

$$F = k_\mathrm{B} T \cdot \ln \left\langle \exp \left[\frac{E}{k_\mathrm{B} T} \right] \right\rangle$$

serious issue – the large energy values enter an exponential,
and so the high-energy regions may contribute significantly!
$\rightarrow$ if these are undersampled, then free energies are wrong

– calculation of free energies impossible, special methods needed!

Tackling the issue

two fundamental approaches:
free energy perturbation and thermodynamic integration

several computational tricks for particular types of reactions:
alchemical simulations or umbrella sampling

important: not necessary to find the absolute value of free energy;
for a chemical reaction, we only need
the free energy difference (ΔF, ΔG) of reactant and product

"reaction" – not necessarily chemical bonds created or broken
– ligand binding a protein, passage of a molecule through membrane, protein folding

Tackling the issue

Note on ΔF vs. ΔG:

ΔF is obtained in NVT simulations
ΔG is obtained in NPT simulations
– automatically, with otherwise identical simulation protocols

In this presentation, we write F.
Everything applies to G as well.

Free energy perturbation

Zwanzig formula (1954):

$$\Delta F(A \to B) = -k_\mathrm{B} T \ln \left\langle \exp[-\beta(E_B - E_A)] \right\rangle_A$$
$$\Delta F(B \to A) = -k_\mathrm{B} T \ln \left\langle \exp[-\beta(E_A - E_B)] \right\rangle_B$$

Simulate the state A (reactant) and obtain the free energy
by averaging the exponential of the difference
of energies of states B and A
or vice versa
(simulate the product, and evaluate the exp of energy difference)

Examples of use

Free energy of deprotonation (pK)
of an amino acid side chain in a protein

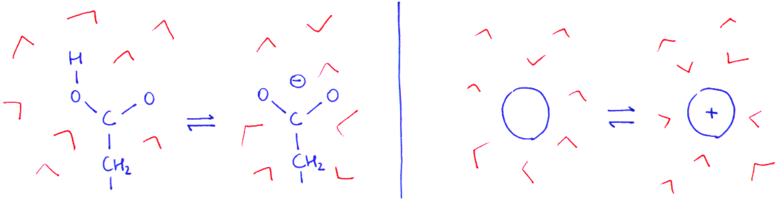
– we would simulate the protonated species,
and then evaluate the energy difference between protonated and unprotonated species to get the average of $\exp[-\beta(E_B - E_A)]$.

The ionization of a molecule

– we would simulate the neutral species and evaluate the energy differences.

Examples of use

Deprotonation of amino acid (left), ionization of molecule (right), both hydrated



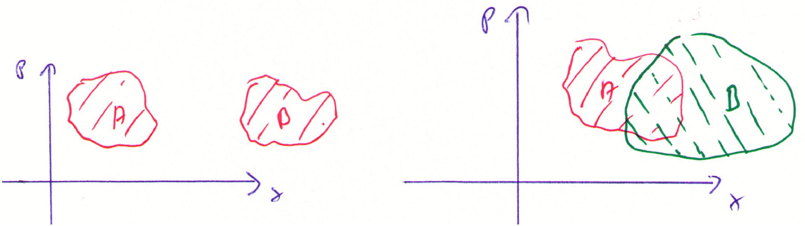
Advantage of FEP

- evaluate directly the difference of energies,
  no need to sample for the (large) total energies first
- evaluate the free energy difference directly in one simulation
  – it is not important what happens outside of the region where the reaction takes place (no contrib. to $E_B - E_A$)
- the cluster of structures that have to be sampled thoroughly is much smaller, and shorter simulation length is required

FEP in use – requirements

overlap of structural clusters of the reactant and the product
(similar structures of the reactant and product)
– this includes the close neighborhood of the 'reaction center'

scheme of structural clusters ('phase space densities')



FEP in use – requirements

What happens if this is not the case?

The simulation of reactant hardly gives molecular structures for which the product has low energy

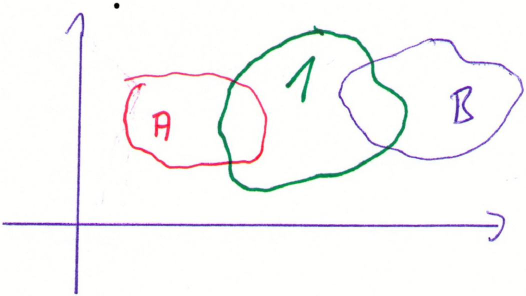
$\rightarrow$ this structural cluster is undersampled, the averaging of the energy E_B is wrong $\rightarrow$ no convergence

We can expect this problem whenever

$$|E_B - E_A| > k_\mathrm{B} T$$

FEP in use – connecting the end states

How to overcome this problem?
insert an intermediate overlapping with both reactant and product:



free energy is a state function, and so

$$\Delta F(A \rightarrow B) = \Delta F(A \rightarrow 1) + \Delta F(1 \rightarrow B)$$

FEP in use – connecting the end states

We can perform two MD simulations, one for each of the states A and 1, and evaluate free energies for the two reactions.

These may be expected to converge better,
and their sum gives the free energy of $A \to B$:

$$\Delta F(A \to B) = \Delta F(A \to 1) + \Delta F(1 \to B)$$

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If the difference is large, we can insert more than one intermediate, and for N intermediates $1, 2, \ldots, N$, we obtain

$$\Delta F(A \rightarrow B) = \Delta F(A \rightarrow 1) + \Delta F(1 \rightarrow 2) + \ldots + \Delta F(N \rightarrow B)$$

and we have to perform $N + 1$ simulations of states $A, 1, 2, \ldots, N$.

FEP in use

FEP looks complicated, but it is rather straightforward, and the common simulation programs run FEP calculations conveniently.

We can change the chemical identities of atoms or functional groups – computational alchemy.

Using a parameter λ, the force-field parameters of state A are changed to those of state B gradually:

$$E_\lambda = (1 - \lambda) \cdot E_A + \lambda \cdot E_B$$

Examples

The hydration free energy difference of argon and xenon

The two atoms differ only in the vdW parameters
– the well depth ε and the radius σ.

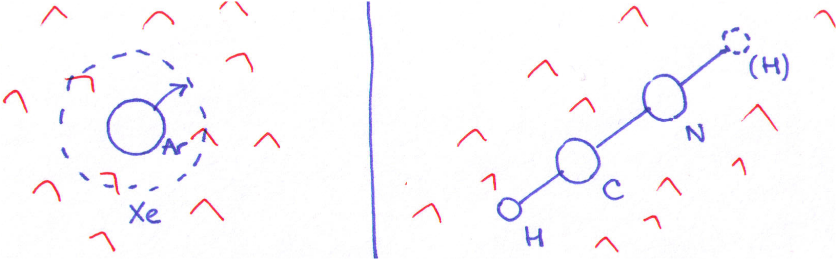
We interpolate between the parameters for the two elements:

$$\begin{aligned} \varepsilon_\lambda &= (1-\lambda)\cdot\varepsilon_A + \lambda\cdot\varepsilon_B \\ \sigma_\lambda &= (1-\lambda)\cdot\sigma_A + \lambda\cdot\sigma_B \end{aligned}$$

In the simulation, we start from $\lambda = 0$, i.e. an argon atom, and change it in subsequent steps to 1.

For each step (window), we perform an MD simulation with the corresponding values of the vdW parameters, and calculate the free energy difference.

Examples



Examples

A true chemical reaction: HCN $\rightarrow$ CNH

More complicated
– we have both molecules simultaneously in the simulation.
We gradually switch off the interaction of one species
with the solvent during the simulation
while we switch on the other at the same time.

Thermodynamic integration

TI – an alternative way to free energies.

Think of free energy as function of λ: $F = F(\lambda)$,
with $\lambda = 0$ for reactant, $\lambda = 1$ for product

$$\Delta F = F(B) - F(A) = \int_0^1 \frac{\partial F(\lambda)}{\partial \lambda} \mathrm{d}\lambda$$

Essence of TI – the derivative of free energy F with respect to λ is calculated as the average of derivative of total MM energy E, which can be directly evaluated in the simulation:

$$\Delta F = \int_0^1 \left\langle \frac{\partial E_\lambda}{\partial \lambda} \right\rangle_\lambda \mathrm{d}\lambda$$

How to do it practically

We perform a MD simulation for each chosen value of λ:
usually, equidistant values in the interval (0,1) are taken:

0, 0.05, . . . , 0.95 and 1.

Each of these simulations produces a value of $\left\langle \frac{\partial E}{\partial \lambda} \right\rangle_\lambda$,
so we obtain the derivative of F in discrete points for $\lambda \in (0, 1)$.
This function is then integrated numerically,
and the result is the desired free energy difference ΔF.

Example

Free energy of hydration of rare gas (neon)

van der Waals parameters of the neon are gradually switched off by means of λ, so that the atom is effectively disappearing

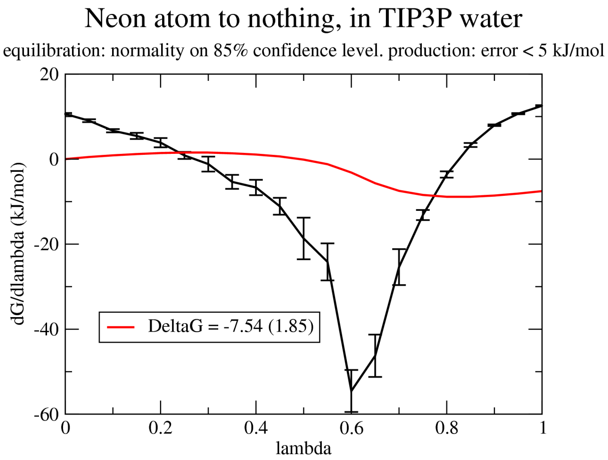
The derivative of total energy with respect to λ is evaluated for 21 values of λ ranging from 0 to 1.

Then, TI gives the Gibbs energy difference of two states:

- a neon atom in water
- no neon atom in water $\equiv$

  $\equiv$ a neon atom outside of the solution, in vacuo

Example


Neon atom to nothing, in TIP3P water
equilibration: normality on 85% confidence level. production: error < 5 kJ/mol
dG/dlambda (kJ/mol)
lambda
DeltaG = -7.54 (1.85)
20
0
-20
-40
-60
0
0.2
0.4
0.6
0.8
1

Choice of reaction coordinate

Both FEP and TI require a coupling parameter λ, representing the reaction coordinate ($\lambda = 0$ is reactant; $\lambda = 1$ is product).

Free energy is a state function
$\rightarrow$ the result is independent of the chosen path
between the reactant and the product.

We are free to use even an unphysical process as the reaction coordinate – a change of chemical identity of one or more atoms (in the alchemical simulations).

Choice of the number of windows

– we would like to have as few as possible,
without compromising numerical precision of the calculation.
– the factors affecting the choice are different in FEP and in TI:

FEP: the assumption is that while simulating the state A, the low-energy regions of state B are sampled well. The closer the windows are, the better this condition is met.

TI: the free energy derivative is always evaluated for one λ-value, and the problem present in FEP does not occur here. However, numerical inaccuracy may be due to the numerical integration of the free energy derivative

Differences of differences

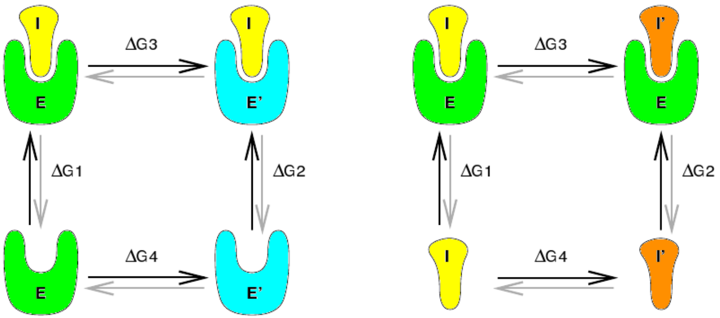
Often – we are interested not in the absolute free energies
and not even in the reaction free energies,
but rather in the difference (Δ) of reaction free energies (ΔF)
of two similar reactions:

$$\Delta\Delta F \text{ or } \Delta\Delta G$$

Reaction free energy difference

Example left: binding of an inhibitor molecule I to an enzyme E, difference of binding free energies to similar enzymes E and E′:

$$\begin{array}{rclc} \mathrm{E} + \mathrm{I} & \rightleftharpoons & \mathrm{EI} & \Delta G_1 \\ \mathrm{E}' + \mathrm{I} & \rightleftharpoons & \mathrm{E}'\mathrm{I} & \Delta G_2 \end{array}$$



Reaction free energy difference

The simulation of the ligand binding process itself – very difficult (possibly large structural changes in the enzyme upon binding)

Solution of the problem – do not simulate the reaction of binding, but rather the alchemical transmutation of enzyme E to E′.

E to E′ are very similar so this may be easy to do.
(example: mutation of a single AA, e.g. leucine to valine)
Then, the structure of complexes EI and E′I may be similar as well, and the simulation may provide converged free energy.

Reaction free energy difference

Free energy is a state function → the sum of free energies
around a thermodynamic cycle vanishes:
(e.g. clockwise in figure left):

$$\Delta G_1 + \Delta G_3 - \Delta G_2 - \Delta G_4 = 0$$

The difference of binding free energies equals
the difference of free energies calculated in alchemical simulations:

$$\Delta\Delta G = \Delta G_1 - \Delta G_2 = \Delta G_3 - \Delta G_4$$

Geometric reaction coordinate

Sometimes, we need to know how the free energy changes along a geometric reaction coordinate q within a certain interval.

The free energy is then a function of q
while it is integrated over all other degrees of freedom.

Such a function $F(q)$ is called the potential of mean force.

Geometric reaction coordinate

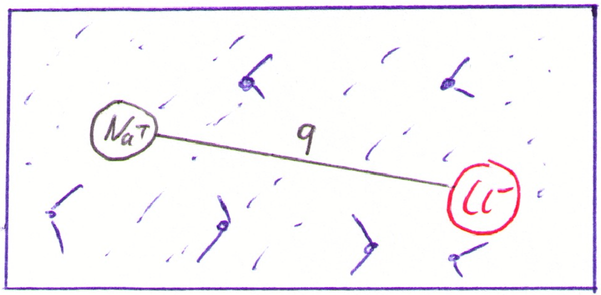
Examples:

- distance between two particles in a dissociating complex
- the position of a proton for a reaction of proton transfer
- the dihedral angle when dealing with conformational changes

Looking for the free energy at a certain value of q,
remaining degrees of freedom are averaged over (integrated out).
One could think of performing an MD simulation
and sampling all degrees of freedom except for q.

Example

free energy of formation of an ion pair in solution:



we need to know the value of free energy
for every value of the reaction coordinate q.

Straightforward approach

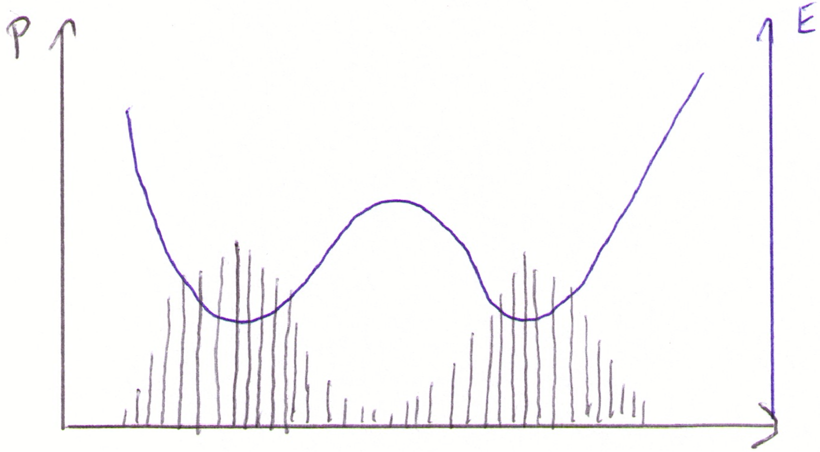
We perform an MD simulation for the system,
and then count how many times q takes the value q_0:
we calculate the probability $P(q_0)$ of finding the system at q_0.

Then, the free energy difference of two states A and B
(with different values of coordinate q) is

$$F_B - F_A = -k_\mathrm{B} T \ln \frac{P(q_B)}{P(q_A)}$$

which contains the equilibrium constant $P(B)/P(A)$.

Energy profile and probability distribution along the reaction coordinate. Note the undersampled region of the barrier.



Problem to be solved

What to do:
perform an MD simulation, specify the reaction coordinate, and then just count how many times the reaction coordinate takes the values in the specified bins (intervals)
→ the ratio of the counts gives the free energy difference

The problem:
If a high barrier has to be crossed to come from A to B, a pure (unbiased) MD simulation will hardly make it
→ the high-energy region (barrier) is described poorly (for sure)
→ we may not obtain the product at all (possibly)

Working principle

A straightforward solution:
apply an additional potential, also called biasing potential
to restrain the system to values of reaction coordinate
that would otherwise remain undersampled.

This is the principle of the umbrella sampling.

The additional potential will become a part of the force field,
and it shall depend only on the reaction coordinate: $V = V(q)$

Working principle

... free energy follows as function of reaction coordinate, or PMF:

$$F(q) = -k_B T \ln P^*(q) - V(q) + K$$

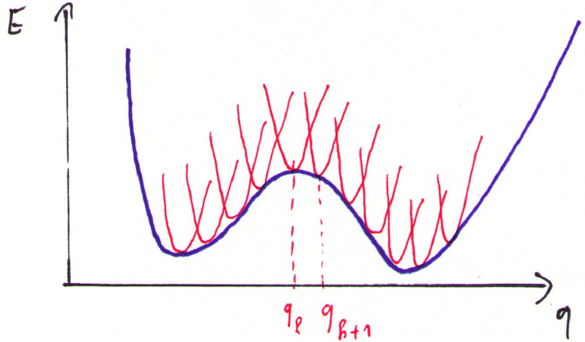
An arbitrary potential $V(q)$ is added to the system.

We obtain the biased probability $P^*(q)$ of finding the system at the value of the reaction coordinate for the ensemble, which differs from the real, unbiased probability $P(q)$, obviously.

Still, we obtain the right, unbiased free energy $F(q)$, once we take the biased probability $P^*(q)$, subtract the biasing potential $V(q)$ and add the term K (which has to be determined yet).

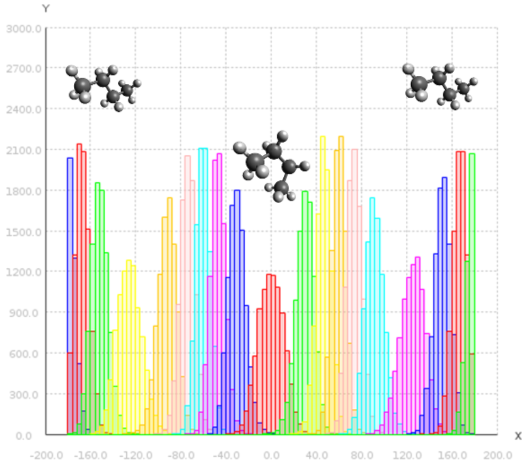
Practical PMF

We can use this scheme efficiently, by way of moving a biasing harmonic potential along the reaction coordinate:



Practical PMF

Example – probabilities from biased simulations – histograms



http://people.cs.uct.ac.za/~mkuttel/images/projectImages/WHAM.png

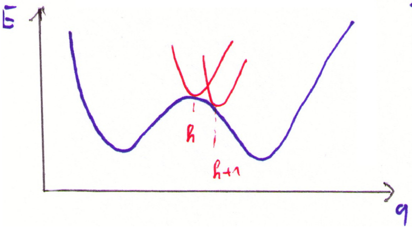
Practical PMF

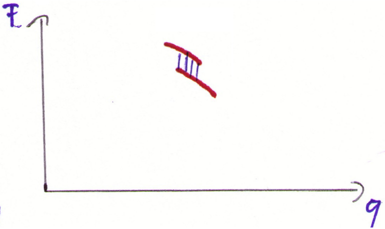
We perform k simulations with biasing potentials V_k and obtain

$$F(q) = -k_\mathrm{B} T \ln P^*(q) - V_k(q) + K_k$$

For each of the k simulations, we extract the probability $P^*(q)$ for every value of q and easily calculate $V^k(q)$.

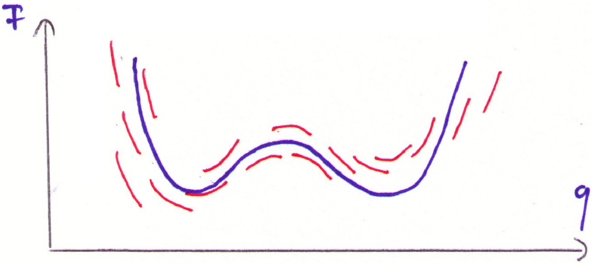
The curves of $-k_\mathrm{B} T \ln P^*(q) - V^k(q)$ for simulations k and $k+1$ differ by a constant shift, which corresponds to the difference of K:





Practical PMF

The main task – to match the pieces of the curve together.
One way – to fit the values K_k to obtain a total $F(q)$ curve that is as smooth as possible.
Requirement – the pieces k and $k+1$ must ‘overlap’ sufficiently.



the WHAM method – included in modern simulation programs

Molecular modeling in the drug design

Drug design

– to construct new chemical compounds interacting in a defined way with natural materials – proteins, NA, carbohydrates. . .
– typical example – find a potent inhibitor of an enzyme, which does not interact harmfully with other substances in the organism

– typical difficulties:

- the drug has to be a potent inhibitor
- it must not interact with other enzymes (might be lethal)
- it must not decompose too early (to reach destination)
- its metabolites must not be (too) toxic

hard and $$$ business

Molecular docking

"Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex." Wikipedia

Typical pharmacological problem – find a ligand molecule to bind to a protein as strongly and specifically as possible

Molecular docking

"Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex." Wikipedia

Typical pharmacological problem – find a ligand molecule to bind to a protein as strongly and specifically as possible

Good news: the binding site (pocket) is usually known
– often, the active or allosteric place of the protein

Bad news:

- many DoF – transl., rot. and internal flex. of the ligand
- only a small number of molecules can be docked manually, once the binding mode of a similar molecule is known (and, even similar molecules sometimes bind differently)

Molecular docking

a sequence of tasks:

1. Generate the pool of compounds to test – database of compounds, construction from a database of moieties,. . .
2. For each compound, find the binding mode. For this, try out several/many orientations and conformations (poses), and determine the most favorable
3. Evaluate the strength of the interaction. Accurate determination of ΔG_{bind} impossible; instead, a scoring function is employed

Molecular docking

Various levels of approximation may be employed

The simplest approach – exploit a database of molecules, and try to fit each molecule as a rigid body into the binding pocket
A natural expansion – consider the flexibility of the ligand

How to generate different configurations of the molecule?

- simple minimization or molecular dynamics
- Monte Carlo, perhaps combined with simulated annealing
- genetic algorithms

Efficient alternative – incremental construction of the ligand, which is partitioned into chemically reasonable fragments
– natural account for the conformational flexibility of the molecule

Molecular docking

problem of docking – it is all about sampling

No way to do molecular dynamics for every candidate molecule:

- MD takes much longer than what is affordable (would be OK for one ligand, but there are too many)
- MD would probably work only for quite rigid molecules moving relatively freely in the binding pocket (usually not the case)

Difference:

- If the goal is to dock a single molecule – a thorough search is affordable, involving MD, enhanced sampling. . .
- If we have to dock and assess many candidate ligands
  – simpler approaches have to be chosen
  – current state of the art – consider the flexibility of ligands
  – flexibility of protein (side chains) under development

Scoring function

needed: extremely efficient way to quantify the strength of binding

1. to find the right binding mode of each ligand
2. to compare the strength of binding of various ligands.

the quantity of interest – binding free energy
problem with free energy methods – too inefficient for docking
what we need here – a simple additive function to approximate ΔG_{bind}, which would give a result rapidly, in a single step

Scoring function

$$\Delta G_\text{bind} = \Delta G_\text{solv} + \Delta G_\text{conf} + \Delta G_\text{int} + \Delta G_\text{rot} + \Delta G_\text{t/r} + \Delta G_\text{vib}$$

ΔG_solv – change of hydration (ligand, protein) upon binding
ΔG_conf – deformation energy of the ligand (forced by the pocket)
ΔG_int – 'interaction energy' – a favorable contribution
due to the specific ligand–protein interactions
ΔG_rot – loss of entropy due to the frozen rotations
approx. $+RT \log 3 = 0.7$ kcal/mol per 3-state rotatable bond
$\Delta G_\text{t/r}$ – loss of trans. and rot. entropy upon association
– approx. the same for all ligands of similar size
ΔG_vib – change of vibrational modes – difficult, often ignored

Scoring function

- a 'force field' for the free energy of binding
- problem – although approximative, it is still too costly
- usually, very simple constructions, looking over-simplified in comparison with MM force fields; example (Böhm, 1994):

$$\begin{aligned}\Delta G = \Delta G_0 \quad &+ \quad \Delta G_{\mathrm{Hbond}} \cdot \sum_{\mathrm{Hbonds}} f(R,\alpha) + \Delta G_{\mathrm{ionpair}} \cdot \sum_{\mathrm{ionpairs}} f'(R,\alpha) \\ &+ \quad \Delta G_{\mathrm{lipo}} \cdot A_{\mathrm{lipo}} + \Delta G_{\mathrm{rot}} \cdot N_{\mathrm{rot}}\end{aligned}$$

$\Delta G_{\mathrm{Hbond}}$ – ideal hydrogen bond
$f(R,\alpha)$ – penalty function for a realistic hydrogen bond
$\Delta G_{\mathrm{ionpair}}$ and $f'(R,\alpha)$ – dtto for ionic contacts
ΔG_{lipo} – due to hydrophobic interaction; non-polar SA A_{lipo}
ΔG_{rot} – due to a rotatable bond that freezes upon binding

Scoring function

Further concepts present in other scoring functions:

- partitioning of the surface areas of both the proteins and the ligand into polar and non-polar regions, and assigning different parameters to the interactions of different kinds of regions (polar-polar, polar-nonpolar, nonpolar-nonpolar)
- statistical techniques to parametrize the scoring function

Problem – such s.f. only describe well ligands that bind tightly
Modestly binding ligands
– of increasing interest in docking studies
– more poorly described by such functions

Possible solution – 'consensus scoring' – combining results from several scoring functions; performs better than any single s.f.

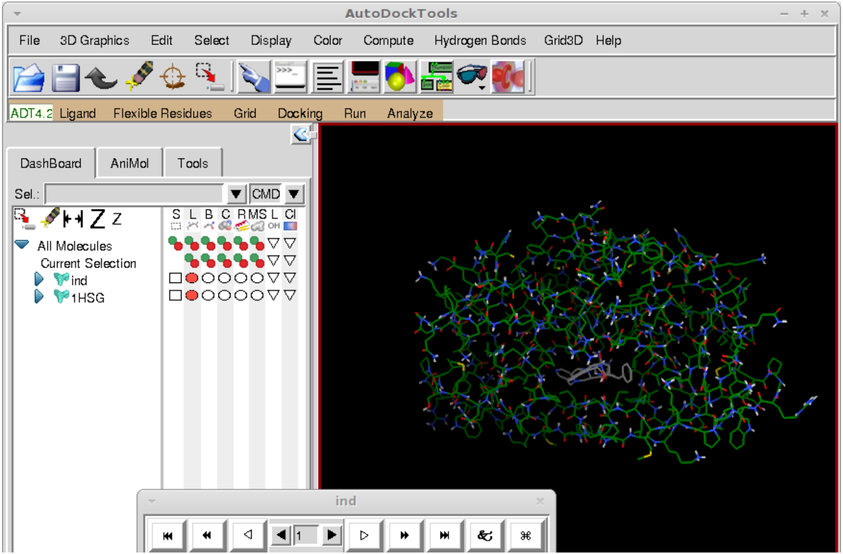
Scoring function

Comment on accuracy

an error of ΔG_{bind} of 1.4 kcal/mol
$\rightarrow$ ten-fold increase/decrease of the inhibition constant

or: as little as 4.2 kcal/mol of ΔG_{bind} lies
between a micro- and a nanomolar inhibitor

Therefore, the requirements on the accuracy of s.f.
are actually rather big


AutoDockTools
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Hydrogen Bonds
Grid3D
Help
ADT4.2
Ligand
Flexible Residues
Grid
Docking
Run
Analyze
DashBoard
AniMol
Tools
Sel.:
CMD
S L B C R MS L Cl
All Molecules
Current Selection
ind
1HSG
ind

De novo design of ligands

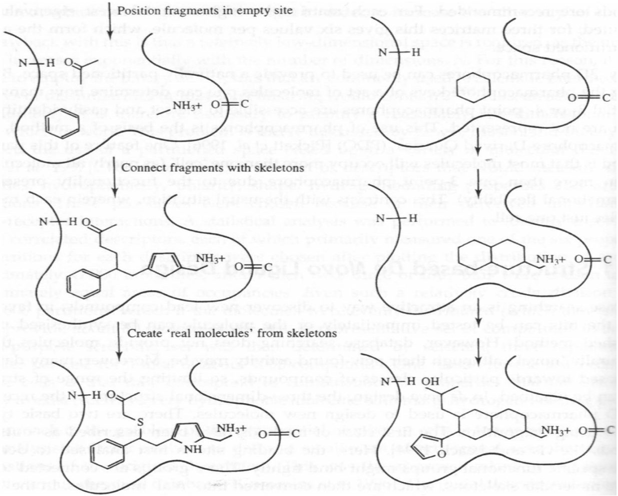
It may be a good idea to construct the ligand 'from scratch'
– without relying on the content of a database.

2 basic types of *de novo* design:

- outside–in: Binding site is analyzed and tightly-binding ligand fragments are proposed. They are connected (db of linkers) $\rightarrow$ molecular skeleton of the ligand $\rightarrow$ actual molecule.
- inside–out: 'growing' the ligand in the binding pocket, driven by a search algorithm with a scoring function.

De novo design of ligands

OUTSIDE IN



INSIDE OUT

Molecular docking

Glossary of terms

- receptor / host / lock
- ligand / guest / key
- docking
- binding mode – position and orientation of ligand
- pose – a candidate for the binding mode
- scoring – determine how favorable a pose is
- ranking – of the poses to determine the binding mode