Free-energy calculations

Measuring free-energy differences using computer simulations

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Synopsis

1. Free-energy perturbation calculations

2. Reaction coordinate–based free-energy calculations

3. Good practices

Free-energy differences can be estimated computationally following four different routes:

- **Methods based on histograms,**[1]
- **Non-equilibrium work simulations,**[2]
- **Perturbation theory,**[3]
- **Measuring the derivative and integrating it.**[4]

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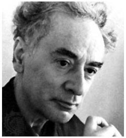
- Perturbation theory is one of the oldest and most useful, general techniques in applied mathematics.

- Its initial applications to physics were in celestial mechanics, and its goal was to explain how the presence of bodies other than the sun perturbed the elliptical orbits of planets.

- Although applications of perturbation theory vary widely, the main idea remains the same: One starts with an initial problem, called the *unperturbed* or *reference* problem.

- The problem of interest, called the *target* problem, is represented in terms of a *perturbation* to the reference problem.

- The effect of the perturbation is expressed as an expansion in a series with respect to a small quantity, called the *perturbation parameter*.



- Approach followed by the pioneers of free energy perturbation (FEP) theory.[5]

- The Hamiltonian of the target system was represented as the sum of the reference Hamiltonian and the perturbation term.

- The free energy difference between the two systems was expressed exactly as the ensemble average of the appropriate function of the perturbation term over the reference system.

- This statistical average was represented as a series.

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[5] BORN (1920); KIRKWOOD (1935); LANDAU (1938); ZWANZIG (1954)

- FEP is not only the oldest but also one of the most useful, general purpose strategies for calculating free energy differences.
- In the early years of molecular–level computer simulations in chemistry and biology it was applied to small solutes dissolved in water.[6]
- Today, it is used for some of the most challenging applications, such as protein–ligand interactions and *in silico* protein engineering.[7]
- It can also be applied to examine the effect of force fields on the computed free energies.
- It forms the conceptual framework for a number of approximate theories.

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[6] JORGENSEN, RAVIMOHAN (1985)

[7] SIMONSON ET AL. (2002)

- N–particle reference system described by Hamiltonian $\mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)$, function of $3N$ Cartesian coordinates, $\mathbf{x}$, and conjugated momenta $\mathbf{p}_x$.

- Goal: Calculate the free energy difference between the reference system and the target system characterized by Hamiltonian $\mathscr{H}_1(\mathbf{x}, \mathbf{p}_x)$:

$$\mathscr{H}_1(\mathbf{x}, \mathbf{p}_x) = \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x) + \Delta\mathscr{H}(\mathbf{x}, \mathbf{p}_x)$$

- *Example*: Free energy of solvation of a chemical species at infinite dilution. $\Delta\mathscr{H}(\mathbf{x}, \mathbf{p}_x)$ then consists of all terms in $\mathscr{H}_1(\mathbf{x}, \mathbf{p}_x)$ that describe solute-solvent interactions.

- *Example*: Calculate the difference between the hydration free energies of sodium and argon described as Lennard–Jones particles with and without a charge.

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- Difference in the Helmholtz free energy between the target and the reference systems:

$$\Delta A = -\frac{1}{\beta}\ \ln \frac{Q_1}{Q_0}$$

- Partition function:

$$Q = \frac{1}{h^{3N} N!} \int\!\!\int \exp\left[-\beta \mathscr{H}(\mathbf{x}, \mathbf{p}_x)\right]\ \mathrm{d}\mathbf{x}\ \mathrm{d}\mathbf{p}_x$$

- Combining the expressions of ΔA and Q,

$$\begin{aligned} \Delta A &= -\frac{1}{\beta}\ \ln \frac{\displaystyle\int\!\!\int \exp\left[-\beta \mathscr{H}_1(\mathbf{x}, \mathbf{p}_x)\right]\ \mathrm{d}\mathbf{x}\ \mathrm{d}\mathbf{p}_x}{\displaystyle\int\!\!\int \exp\left[-\beta \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)\right]\ \mathrm{d}\mathbf{x}\ \mathrm{d}\mathbf{p}_x} \\ &= -\frac{1}{\beta}\ \ln \frac{\displaystyle\int\!\!\int \exp\left[-\beta \Delta\mathscr{H}(\mathbf{x}, \mathbf{p}_x)\right] \exp\left[-\beta \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)\right]\ \mathrm{d}\mathbf{x}\ \mathrm{d}\mathbf{p}_x}{\displaystyle\int\!\!\int \exp\left[-\beta \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)\right]\ \mathrm{d}\mathbf{x}\ \mathrm{d}\mathbf{p}_x} \end{aligned}$$

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- Probability density function of finding the reference system in a state defined by $\mathbf{x}$ and $\mathbf{p}_x$:

$$P_0(\mathbf{x}, \mathbf{p}_x) = \frac{\exp\left[-\beta \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)\right]}{\displaystyle\int\!\!\int \exp\left[-\beta \mathscr{H}_0(\mathbf{x}, \mathbf{p}_x)\right] \, \mathrm{d}\mathbf{x} \, \mathrm{d}\mathbf{p}_x}$$

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- Fundamental FEP formula for the *forward*, 0 → 1 transformation:

$$\boxed{\Delta A = -\frac{1}{\beta} \, \ln \langle \exp\left[-\beta \Delta \mathscr{H}(\mathbf{x}, \mathbf{p}_x)\right] \rangle_0}$$

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- If the kinetic term cancels out,

$$\boxed{\Delta A = -\frac{1}{\beta}\ \ln\langle\exp\left(-\beta\Delta U\right)\rangle_0}$$

excess Helmholtz free energy of the solute over that in the ideal gas.

- Simplification true for any two systems of particles with the same masses.

- If masses differ, additional term due to the change in the kinetic energy.

- Reversing the reference and the target systems,

$$\boxed{\Delta A = \frac{1}{\beta}\ \ln\langle\exp\left(\beta\Delta U\right)\rangle_1}$$

- Although *forward* and *backward* expressions are formally equivalent, their convergence properties may be different.[8]

- Preferred direction to carry out the required transformation between states.

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[8] Widom (1963)

- Since ΔA is calculated as the average over a quantity that depends only on ΔU, this average can be taken over the probability distribution $P_0(\Delta U)$ instead of $P_0(\mathbf{x}, \mathbf{p}_x)$:[9]

$$\boxed{\Delta A = -\frac{1}{\beta} \ln \int \exp\left(-\beta \Delta U\right) \; P_0(\Delta U) \, \mathrm{d}\Delta U}$$

- If U_0 and U_1 were the functions of a sufficient number of identically distributed random variables, then ΔU would be Gaussian–distributed (CLT).

- In practice, the probability distribution $P_0(\Delta U)$ deviates somewhat from the ideal Gaussian case, but still has a “Gaussian–like” shape.

- The integrand, the probability distribution multiplied by the Boltzmann factor $\exp\left(-\beta \Delta U\right)$, is shifted to the left and, hence, depends on the low-energy tail.

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[9] SHING, GUBBINS (1982)

- Substitute

$$P_0(\Delta U) = \frac{1}{\sqrt{2\pi}\sigma}\ \exp\left[-\frac{\left(\Delta U - \langle \Delta U \rangle_0\right)^2}{2\sigma^2}\right]$$

where

$$\sigma^2 = \left\langle \Delta U^2 \right\rangle_0 - \langle \Delta U \rangle_0{}^2$$

to the expression of ΔA.

- A Gaussian $P_0(\Delta U)$ obviates the need to carry out a numerical integration:

$$\boxed{\Delta A = \langle \Delta U \rangle_0 - \frac{1}{2}\beta\sigma^2}$$

- This analytical expression of ΔA has a broader significance:
  - The first term, which is simply equal to the average energy difference measured in the reference state, can be either positive or negative.
  - The second term, which depends on fluctuations of ΔU, is always negative.

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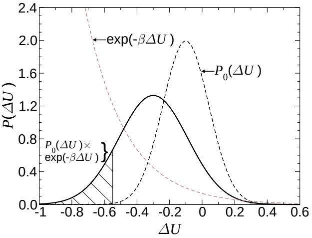
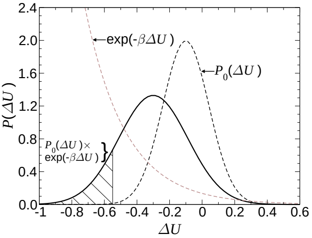


- $\exp(-\beta\Delta U)\,P_0(\Delta U)$ is a Gaussian, as is $P_0(\Delta U)$, but is not normalized and shifted towards low ΔU by $\beta\sigma^2/2$.
- Reasonably accurate evaluation of ΔA *via* direct numerical integration is possible only if the probability distribution function in the low ΔU region is sufficiently well known up to two standard deviations from the peak of the integrand or $\beta\sigma/2 + 2$ standard deviations from the peak of $P_0(\Delta U)$, located at $\langle\Delta U\rangle_0$.
- If σ is small, *e.g.* equal to k_BT, 97% of sampled values of ΔU are within $\pm 2\sigma$ from the peak of $\exp(-\beta\Delta U)\,P_0(\Delta U)$ at room temperature.
- If σ is large, *e.g.* equal to $6k_{\mathrm{B}}T$, this percentage drops to 16%. Most of these samples correspond to ΔU larger than the peak of the integrand, $\langle\Delta U\rangle_0 - \beta\sigma^2/2$. For this value of σ, ΔU smaller than the peak of the integrand will be sampled, on average, only 27 out of 10^4 times.



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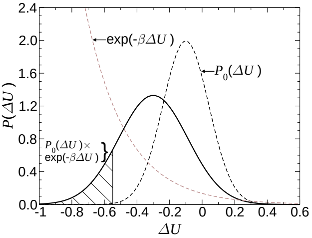

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- **A small value of ΔA does not imply that this quantity is easy to estimate in computer simulations**. In fact, if $\langle \Delta U \rangle_0$ and $-\beta\sigma^2$ / 2 were equal but large, an accurate estimate of ΔA would be evidently hard to achieve.

- One consequence of the positivity of σ is that $\Delta A \leq \langle \Delta U \rangle_0$. repeating the same reasoning for the backward transformation, then $\Delta A \geq \langle \Delta U \rangle_1$.

- These inequalities, the Gibbs–Bogoliubov bounds on free energy, hold *not only for Gaussian distributions*, but for any arbitrary probability distribution function.

- An uncharged Lennard–Jones particle immersed in a large container of water acquires a charge q.

- The free energy change associated with charging is given by the *forward* FEP equation, in which state 0 refers to the reference state of the solvated Lennard–Jones sphere.

- Electrostatic energy of interaction between charge q and all water molecules:

$$\Delta U = qV$$

  V is the electrostatic potential created by the solvent and acting on the charge in the center of the cavity.

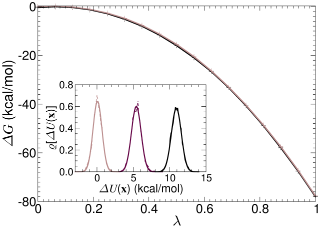
- Second-order perturbation theory:

$$\Delta A = q\langle V\rangle_0 - \frac{\beta}{2} q^2 \left[\left\langle (V - \langle V\rangle_0)^2 \right\rangle_0 \right]$$

- If water is considered as a homogeneous dipolar liquid, $\langle V\rangle_0 = 0$, the expression for the free energy change further simplifies to:

$$\Delta A = -\frac{\beta}{2}\, q^2 \, \left\langle V^2 \right\rangle_0$$

- This result implies that ΔA should be a quadratic function of the ionic charge.

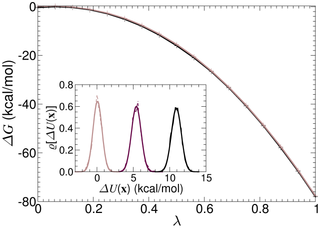


- Born model: The ion is a spherical particle of radius a and the solvent is represented as a dielectric continuum characterized by a dielectric constant ε:[10]

$$\Delta A = -\frac{\varepsilon - 1}{\varepsilon} \frac{q^2}{2a}$$

- Comparison between prediction and computer simulations requires care. In practice, the computed values of ΔA exhibit considerable system–size dependence, *i.e.* they vary with the size of the simulation box, because charge–dipole interactions between the solute and solvent molecules decay slowly as $1/r^2$.

[10] BORN (1920)



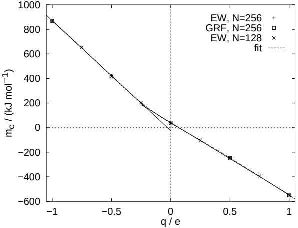
- System–size effects can be greatly reduced by properly correcting for the self–interaction term, which is due to interactions between the charge and its images and the neutralizing background.[11]

- This is true for both Ewald lattice summation and generalized reaction field treatments of finite–size effects.[12]

- In general, free energy calculations, in which the system is transformed such that its electrical charge changes, should include system–size corrections.

[11] HUMMER ET AL. (1996)

[12] HÜNENBERGER, MCCAMMON (1999)



- Monte Carlo simulations, in which a non-polar particle is progressively charged to $q = 1$ or $q = -1$ in intervals of 0.25 e, indicated that the predicted quadratic dependence of ΔA on q is essentially correct.[13]

- In agreement with experimental data[14] negative ions are, however, better hydrated than positive ions, reflected by the different slopes of the straight line. This can be ascribed to different arrangements of water molecules in the vicinity of the ion.

- The positively charged hydrogen atoms of water, which possess small van der Waals radii, can approach negative ions closer than large, negative oxygen atoms approach positive ions.

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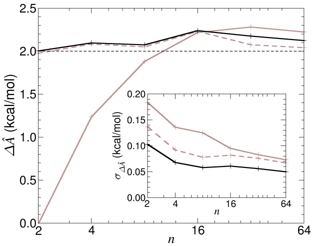
[13] HUMMER ET AL. (1996)

[14] MARKUS (1991)

- Direct use of the forward FEP equation can be successful only if $P_0(\Delta U)$ is a narrow function of ΔU.

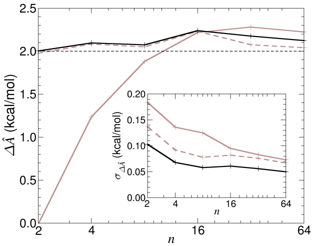
- This does not imply that the free energy difference between the reference and the target states must be small.

- *Example*: Although the hydration free energy of benzene is only -0.767 kcal/mol at 298 K, this quantity cannot be successfully calculated by direct application of the FEP equation to a simulation of a reasonable length, because low–energy configurations in the target ensemble, which do not suffer from the overlap between the solute and solvent molecules, are not sampled in simulations of the reference state.



- The difficulty in applying FEP theory can be circumvented through a *stratification* strategy, or *staging*.
- It relies on constructing several intermediate states between the reference and the target state such that $P(\Delta U_{i,i+1})$ for two consecutive states i and $i+1$ sampled at state i is sufficiently narrow for the direct evaluation of the corresponding free energy difference, $\Delta A_{i,i+1}$.
- With $N-2$ intermediate states,

$$\Delta A = \sum_{i=1}^{N-1} \Delta A_{i,i+1} = -\frac{1}{\beta} \sum_{i=1}^{N-1} \ln \left\langle \exp\left(-\beta \Delta U_{i,i+1}\right)\right\rangle_i$$



- Intermediate states do not need to be physically meaningful, *i.e.* they do not have to correspond to systems that actually exist.
- More generally, the Hamiltonian can be considered to be a function of some parameter, λ.
- Without loss of generality, λ can be defined between 0 and 1, such that $\lambda = 0$ and $\lambda = 1$ for the reference and target states, respectively.
- A simple choice for the dependence of the Hamiltonian on λ, the *coupling parameter*:[15]

$$\mathscr{H}(\lambda_i) = \lambda_i \mathscr{H}_1 + (1 - \lambda_i)\mathscr{H}_0 = \mathscr{H}_0 + \lambda_i \Delta\mathscr{H}$$

$\Delta\mathscr{H}$ is the perturbation term in the target Hamiltonian, equal to $\mathscr{H}_1 - \mathscr{H}_0$.

[15] Kirkwood (1935)

- If $N-2$ intermediate states are created to link the reference and the target states such that $\lambda_1 = 0$ and $\lambda_N = 1$,

$$\begin{aligned}\Delta\mathscr{H}_i &= \mathscr{H}(\lambda_{i+1}) - \mathscr{H}(\lambda_i) \\ &= (\lambda_{i+1} - \lambda_i)\,\Delta\mathscr{H} \\ &= \Delta\lambda_i\Delta\mathscr{H}\end{aligned}$$

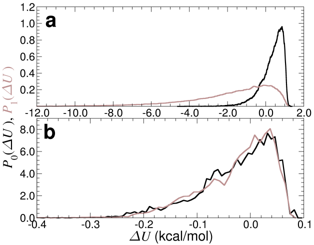
  with $\Delta\lambda_i = \lambda_{i+1} - \lambda_i$.

- Total free energy difference:

$$\Delta A = -\frac{1}{\beta}\sum_{i=1}^{N-1} \ln\langle\exp(-\beta\Delta\lambda_i\Delta\mathscr{H})\rangle_{\lambda_i}$$

- Integrating out the kinetic term in the Hamiltonian,

$$\boxed{\Delta A = -\frac{1}{\beta}\sum_{i=1}^{N-1} \ln\langle\exp(-\beta\Delta\lambda_i\Delta U)\rangle_{\lambda_i}}$$



- As a function of the problem of interest, choosing intermediate states separated by a constant $\Delta\lambda$ may not be the best possible choice — *e.g.* charging of a Lennard–Jones particle, for which ΔA is a quadratic function of the charge.

- **How should N and $\Delta\lambda_i$ be chosen?**

- One method for optimizing both N and $\Delta\lambda_i$ consists in starting with short runs with a large N and select the number of intermediate states and the corresponding values of the coupling parameter on the basis of these runs, such that the estimated variances in $P(\Delta U_{i,i+1})$ are sufficiently small and approximately equal.[16]

- N and ΔA are related through the work dissipated in the process:[17] $N_0 \propto \exp(\beta W_1)$ and $N_1 \propto \exp(\beta W_0)$, where $W_0 = \langle\Delta U\rangle_0 - \Delta A$ and $W_1 = -\langle\Delta U\rangle_1 + \Delta A$.

[16] PEARLMAN, KOLLMAN (1989)

[17] KULLBACK, LEIBER (1951); POHORILLE ET AL. (2010)

- In FEP calculations, configurations are sampled according to the probability, $P_0(U)$, of finding the reference system in a state corresponding to the potential energy U.
- *Does it guarantee that the key quantity for calculating ΔA, i.e. $P_0(\Delta U)$, is estimated accurately?*
- Unfortunately, this does not have to be the case.
- FEP will only provide accurate estimates of free energy differences under the *sine qua non* condition that the target system be sufficiently similar to the reference system.

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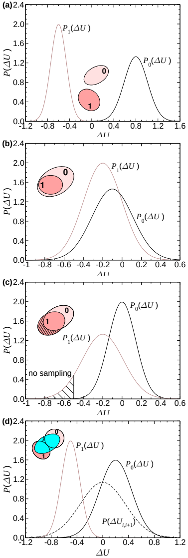
- *Important regions* are volumes that encompass configurations of the system with highly probable energy values.

- A configuration in the important region should have the potential energy, the probability of which is higher than a given, predefined value.

- Configurations that belong to the important regions are expected to make significant contributions to the free energy and ought to be adequately sampled.



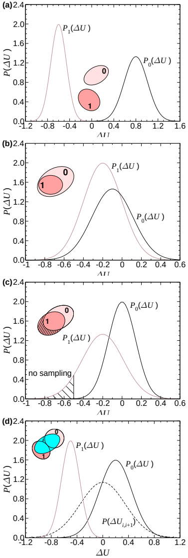
- A pictorial representation of the relationship between the reference and the target systems may constitute a useful tool to detect inaccuracies caused by incomplete sampling.[18]

- If the important region of the target system fully overlaps, or is a subset of the important region of the reference system, $P_0(\Delta U)$ estimated from FEP calculations should be reliable.

- Good sampling of the important region in the reference system will also yield good sampling of the important region in the target system.

- If the important regions of the two systems do not overlap, the important region of the target state is not expected to be sufficiently sampled during a simulation of the reference system. Satisfactory estimates of ΔA are unlikely to be obtained.

[18] LU, KOFKE (2001)



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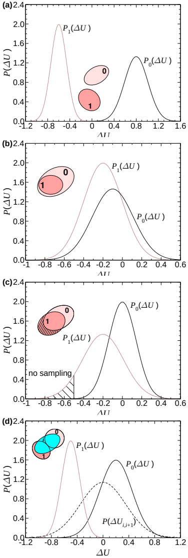
[18] Lu, Kofke (2001)



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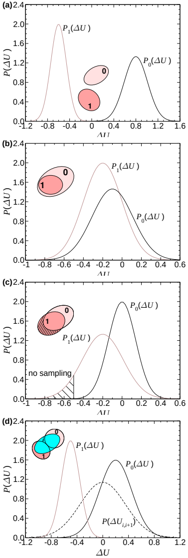

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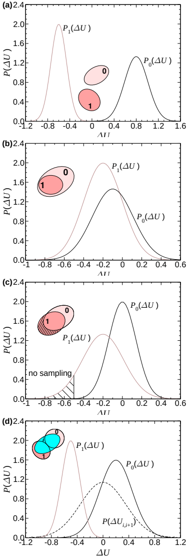
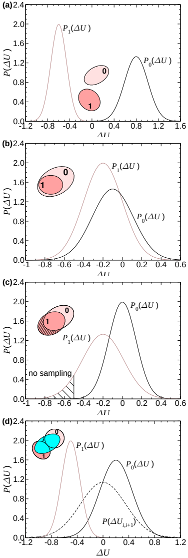


- In many instances, the important region of the reference system overlaps with only a part of the important region of the target system.
- The poorly sampled reminder of these important region contributes to inaccuracies in the estimated free energy differences, which, in some circumstances, could be substantial.
- If the two important regions do not overlap, or overlap only partially, it is usually necessary to stratify.
- The optimal enhanced sampling strategy is largely determined by the relationship between the two important regions in phase space.[19]

[19] Lu, Kofke (2001)



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[19] Lu, Kofke (2001)

- How can the free energy differences associated with the transformation of a chemical species into a different one be calculated?

- Computational process often called “alchemical transformation” refers to the the inaccessible dream of the proverbial alchemist to transmute matter.

- The potential energy function is sufficiently malleable from a computational standpoint, that it can be gently altered to transform one chemical system into another, slightly modified one.[20]

- FEP applications of this approach include protein–ligand binding, host–guest chemistry, and solvation properties.[21]

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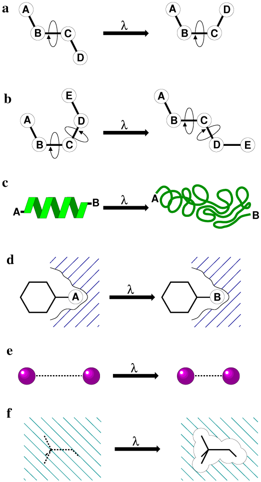
[20] Chipot, Pohorille (2007)

[21] Kollman (1993); King (1993); Kollman (1996); Simonson et al. (2002)

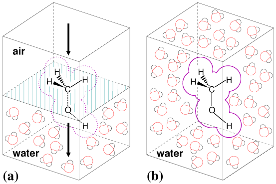


- The concept of *order parameter* is central to free energy calculations: They are collective variables used to describe transformations from the initial, reference system to the final, target one.

- An order parameter may, albeit does not necessarily have to correspond to the path along which the transformation takes place in nature, and would be called the *reaction coordinate* or the *reaction path* if such were the case.

- There is more than one way to define an order parameter. *How to make the best, or at least an appropriate, choice of an order parameter?*

- The choice of order parameters may have a significant effect on the efficiency and accuracy of free energy calculations. Some order parameters may map a *smooth* path between the reference and target states whereas others may lead through a *rough* energy landscape.

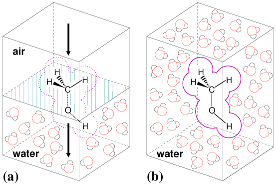


- Central to the application of FEP to alchemical transformations is the concept of a *thermodynamic cycle*.
- The cycle may be *hypothetical*, as individual transformations can be carried out only on the computer.

$$\begin{tikzcd}
{[\text{solute}]_{\text{vac.}}} \arrow[r, "\Delta A_{\text{hydration}}"] \arrow[d, "\Delta A^0_{\text{annihilation}}"'] & {[\text{solute}]_{\text{aq.}}} \arrow[d, "\Delta A^1_{\text{annihilation}}"] \\
{[\mathit{nothing}]_{\text{vac.}}} \arrow[r, "\Delta A=0"'] & {[\mathit{nothing}]_{\text{aq.}}}
\end{tikzcd}$$

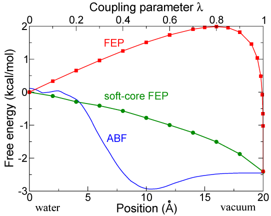
- Considering that all transformations are reversible, the cycle can be run in both directions and the free energy of interest can be calculated from either *forward* or *backward* transformations.
- The computational efficiencies of carrying out these transformations may, however, differ.[22]

[22] Pohorille et al. (2010)



$$\begin{tikzcd}
{[\text{solute}]_{\text{vac.}}} \arrow[r, "\Delta A_{\text{hydration}}"] \arrow[d, "\Delta A^{0}_{\text{annihilation}}"'] & {[\text{solute}]_{\text{aq.}}} \arrow[d, "\Delta A^{1}_{\text{annihilation}}"] \\
{[\textit{nothing}]_{\text{vac.}}} \arrow[r, "\Delta A{=}0"'] & {[\textit{nothing}]_{\text{aq.}}}
\end{tikzcd}$$

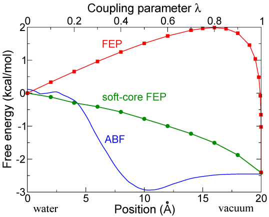
- In the FEP framework, hydration can be described by turning on the perturbation term in the Hamiltonian responsible for solute-solvent interactions.
- $\Delta A_{\text{hydration}}$ is the *excess* free energy over that in the gas phase.
- When solute–solvent interactions are turned on, the solvent undergoes substantial reorganization, and conformational equilibria in flexible solutes may also be affected.



- $\Delta A_{\text{hydration}}$ can be also obtained from the *reverse* process, in which solute–solvent interactions are turned off.

- This corresponds to moving the solute from the aqueous solution to the gas phase. and the calculated quantity is the negative of $\Delta A_{\text{hydration}}$.

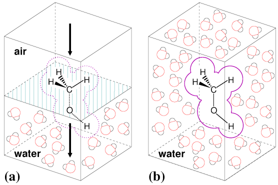
- If the same order parameter, λ, is used for the *forward* and the *reverse* transformations, the changes in the free energy with λ should be reversible, and, consequently, the sum of the calculated free energies differences should be *zero*.



- Discrepancies between the forward and the reverse transformations yield the hysteresis of the reaction, which constitutes a measure of the error in the free energy calculation.

- If the hysteresis is markedly larger than the estimated statistical errors, it is usually indicative of *ergodicity issues during the transformations.*

- Even though the hysteresis is negligible and statistical errors are small, it does not imply that the calculated free energy difference is accurate, because it can be burdened with systematic errors, due, for instance, to unsuitable potential energy functions.



$$\begin{tikzcd}
{[\text{solute}]_{\text{vac.}}} \arrow[r, "\Delta A_{\text{hydration}}"] \arrow[d, "\Delta A^0_{\text{annihilation}}"'] & {[\text{solute}]_{\text{aq.}}} \arrow[d, "\Delta A^1_{\text{annihilation}}"] \\
{[\textit{nothing}]_{\text{vac.}}} \arrow[r, "\Delta A=0"'] & {[\textit{nothing}]_{\text{aq.}}}
\end{tikzcd}$$

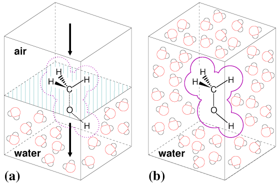
- Alternative approach: Double annihilation of the solute,

$$\Delta A_{\text{hydration}} = \Delta A^0_{\text{annihilation}} - \Delta A^1_{\text{annihilation}}$$

- The transformation to *nothing* should not be taken literally. The perturbed Hamiltonian contains not only terms responsible for solute–solvent interactions, but also all the terms that involve intramolecular interactions in the solute.

- Symmetrically,

$$\Delta A_{\text{hydration}} = \Delta A^1_{\text{creation}} - \Delta A^0_{\text{creation}}$$



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{[\text{solute}]_{\text{vac.}}} \arrow[r, "\Delta A_{\text{hydration}}"] \arrow[d, "\Delta A^{0}_{\text{annihilation}}"'] & {[\text{solute}]_{\text{aq.}}} \arrow[d, "\Delta A^{1}_{\text{annihilation}}"] \\
{[\textit{nothing}]_{\text{vac.}}} \arrow[r, "\Delta A=0"'] & {[\textit{nothing}]_{\text{aq.}}}
\end{tikzcd}$$

Comparison of the “alchemical” scheme with the direct transformation [23] reveals two important differences:

- The “alchemical” transformations require two set of simulations instead of one, one of them involving only the solute in the gas phase and is much less computationally intensive. Ignoring intra-perturbed interactions in the implementation obviates the need to run separate gas-phase calculations (**caveat emptor**).

- The two methods differ in their description of the solute in the reference state. In both cases, the solute does not interact with the solvent. In the “alchemical” transformations, all interactions of atoms forming the solute vanish, whereas in the direct transformation, the molecule remains intact.

[23] Pohorille, Wilson (1993); Chipot et al. (1997)

$$\begin{tikzcd}
\text{protein + ligand} \arrow[r, "\Delta A_{\text{binding}}"] \arrow[d, "\Delta A^{0}_{\text{annihilation}}"'] & \text{protein} \cdots \text{ligand} \arrow[d, "\Delta A^{1}_{\text{annihilation}}"] \\
\text{protein} + \textit{nothing} \arrow[r, shift left, "\Delta A{=}0"] & \text{protein} \cdots \textit{nothing} \arrow[l, shift left, "\Delta A_{\text{restrain}}"]
\end{tikzcd}$$

- The binding free energy of two molecules, $\Delta A_{\text{binding}}$, defined as the free energy difference between these molecules in the bound and the free, unbound states, can be determined experimentally through the measurement of binding constants using isothermal microcalorimetry,[24] electrospray ionization mass spectrometry, [25] or BIAcore.[26]

- The computationally equivalent procedure corresponds to calculating $\Delta A_{\text{binding}}$ directly, by means of an order parameter that measures the separation between the ligand and the binding center of the protein.

- Definition of a relevant order parameter to describe protein-ligand association may be difficult, in particular when the ligand is buried deep in the binding pocket, and access to the latter involves *large* conformational changes in the protein.

[24] WADSÖ (1995)

[25] FENN ET AL. (1989)

[26] KARLSSON, LARSSON (2004)

$$\begin{tikzcd}
\text{protein + ligand} \arrow[r, "\Delta A_{\text{binding}}"] \arrow[d, "\Delta A^{0}_{\text{annihilation}}"'] & \text{protein} \cdots \text{ligand} \arrow[d, "\Delta A^{1}_{\text{annihilation}}"] \\
\text{protein + } \textit{nothing} \arrow[r, shift left, "\Delta A=0"] & \text{protein} \cdots \textit{nothing} \arrow[l, shift left, "\Delta A_{\text{restrain}}"]
\end{tikzcd}$$

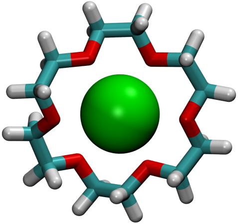
- Alternative route: Double annihilation of the ligand,

$$\Delta A_{\text{binding}} = \Delta A^{0}_{\text{annihilation}} - \Delta A^{1}_{\text{annihilation}}$$

- This implies obtaining $\Delta A^{0}_{\text{annihilation}}$ and $\Delta A^{1}_{\text{annihilation}}$ in the thermodynamic cycle.

- FEP is the appropriate technique, which, however, some care.

$$\begin{tikzcd}
\text{protein + ligand} \arrow[r, "\Delta A_{\text{binding}}"] \arrow[d, "\Delta A^{0}_{\text{annihilation}}"'] & \text{protein}\cdots\text{ligand} \arrow[d, "\Delta A^{1}_{\text{annihilation}}"] \\
\text{protein + }\textit{nothing} \arrow[r, shift left, "\Delta A=0"] & \text{protein}\cdots\textit{nothing} \arrow[l, shift left, "\Delta A_{\text{restrain}}"]
\end{tikzcd}$$



- The ligand in the binding pocket is annihilated from a strongly constrained position, whereas the free, unbound ligand can *translate*, *rotate* and *isomerize* freely during annihilation.
- This means that the thermodynamic cycle reflecting micro-reversibility of the transformations may not zero out unless proper corrections for the loss of *translational*, *rotational* and *conformational* entropies are taken into account.[27]
- Under favorable circumstances, these corrections can be evaluated analytically.[28]

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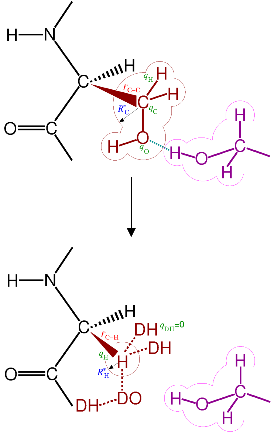
[27] Gilson et al. (1997); Hermans, Wang (1997); Dixit, Chipot (2001)

[28] Deng, Roux (2009)

$$\begin{tikzcd}
\text{protein + ligand A} \arrow[r, "\Delta A^{\mathrm{A}}_{\mathrm{binding}}"] \arrow[d, "\Delta A^{0}_{\mathrm{mutation}}"'] & \text{protein} \cdots \text{ligand A} \arrow[d, "\Delta A^{1}_{\mathrm{mutation}}"] \\
\text{protein + ligand B} \arrow[r, "\Delta A^{\mathrm{B}}_{\mathrm{binding}}"'] & \text{protein} \cdots \text{ligand B}
\end{tikzcd}$$

- In many instances, determining relative binding free energies for a series of ligands is preferred over a single binding free energy.
- This is the case, for example, potential inhibitors of a target protein are sought in the context of computer–aided drug design.[29]
- This can be handled by repeating the “absolute” transformations for each ligand of interest.
- There is an alternate pathway, likely to be more efficient: Mutation of ligand A into ligand B in both the bound and the free states, following a different thermodynamic cycle.

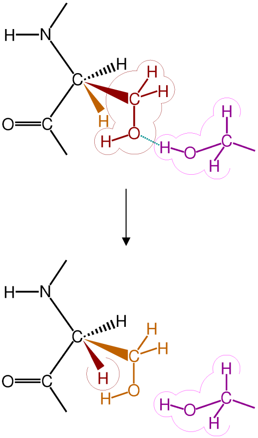
[29] Pearlman, Charifson (2001); Chipot et al. (2005)



- In the *single-topology* paradigm, a common topology is sought for the initial state and the final states.[30]
- The most complex topology serves as the common denominator for both states, and the missing atoms are treated as vanishing particles, the non–bonded parameters of which are progressively set to zero as λ varies from 0 to 1.
- *Example*: In the mutation of ethane into methanol, the former serves as the common topology.[31] As the carbon atom is transformed into oxygen, two hydrogen atoms of the methyl moiety are turned into non–interacting, “ghost” particles by annihilating their point charges and van der Waals parameters.

[30] Jorgensen, Ravimohan (1985); Pearlman (1994)

[31] Jorgensen, Ravimohan (1985)



- In contrast with the single-topology paradigm, the topologies of the reference, 0, and the target, 1, states coexist in the *dual–topology* scheme, throughout the “alchemical transformation”.[32]
- Using an exclusion list, atoms that are not common to 0 and 1 never interact during the simulation.
- Their intra– and intermolecular interaction with other atoms in the system are scaled by λ, which varies from 0 to 1.
- In the initial state, only topology 0 interacts with the rest of the system, whereas in the final state, only topology 1 does:

$$U(\mathbf{x};\lambda) = \lambda U_1(\mathbf{x}) + (1-\lambda)U_0(\mathbf{x})$$

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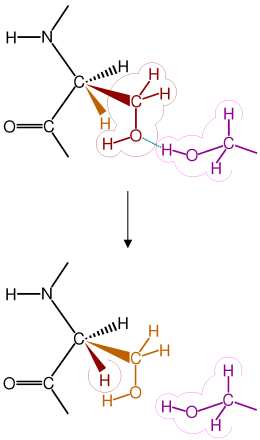
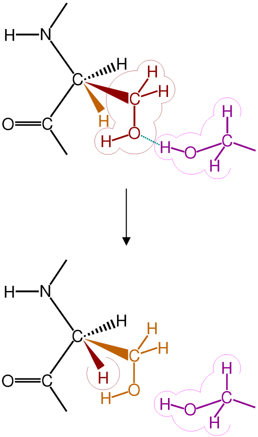
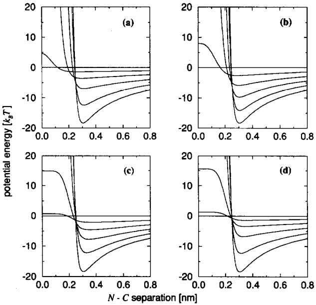
[32] Gao et al. (1989); Boresch, Karplus (1999a,b)



- The dual–topology paradigm avoids several complications inherent to the single-topology approach.
  - The problem of growing or shrinking chemical bonds is not present here.
  - Decoupling electrostatic and non-electrostatic contributions during simulations is no longer needed.
- Dual–topology also suffers from problems when λ approaches 0 or 1, which are often referred to as “end-point catastrophes”.
- At these end points, interaction of the reference, or the target topology with its environment is extremely weak, yet still non–zero, which in turn allows the surrounding atoms to clash against the appearing or vanishing chemical moieties.



- The resulting numerical instabilities cause large fluctuations in the estimated $\langle \Delta U \rangle$, attenuated only after extensive sampling.
- The most trivial one consists in splitting the reaction pathway into windows of uneven widths, $\delta\lambda$, and using a large number of narrow windows when λ approaches 0 or 1.
- Equivalent to adopting a non-linear dependence of the interaction potential energy on λ.
- Clashes between the appearing atoms and the rest of the system occur even for windows as narrow as $\delta\lambda \simeq 10^{-5}$.



- Solution: Modification of the parametrization of the van der Waals term in the potential energy function that governs the interaction of an appearing, or disappearing, atom, i, with an unaltered one, j:[33]

$$U_{ij}^{\text{vdW}}(r_{ij};\lambda) = 4\,\epsilon_{ij}\,\lambda^n \times \left\{ \frac{1}{\left[\alpha_{\text{vdW}}\,(1-\lambda)^2 + \left(\frac{r_{ij}}{\sigma_{ij}}\right)^6\right]^2} - \frac{1}{\alpha_{\text{vdW}}\,(1-\lambda)^2 + \left(\frac{r_{ij}}{\sigma_{ij}}\right)^6} \right\}$$

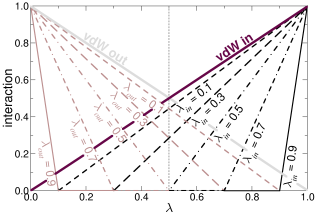
- Or equivalently,[34]

$$U^{\text{vdW}}(r_{ij};\lambda) = 4\epsilon_{ij}(1-\lambda)\left[\left(\frac{\sigma_{ij}^2}{r_{ij}^2+\alpha\lambda}\right)^6 - \left(\frac{\sigma_{ij}^2}{r_{ij}^2+\alpha\lambda}\right)^3\right]$$

- Possibility to scale interactions with different schedules.

[33] BEUTLER ET AL. (1994)

[34] ZACHARIAS ET AL. (1994)



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- Or equivalently,[34]

$$U^{\mathrm{vdW}}(r_{ij};\lambda) = 4\epsilon_{ij}(1-\lambda)\left[\left(\frac{\sigma_{ij}^2}{r_{ij}^2+\alpha\lambda}\right)^6 - \left(\frac{\sigma_{ij}^2}{r_{ij}^2+\alpha\lambda}\right)^3\right]$$

- Possibility to scale interactions with different schedules.

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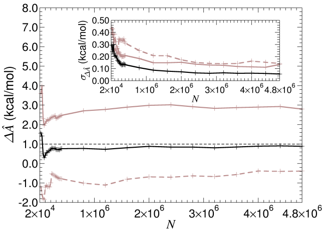
[33] BEUTLER ET AL. (1994)

[34] ZACHARIAS ET AL. (1994)

1. Build the topologies representative of state 0 and state 1, and establish an exclusion list to prevent atoms that are not common to 0 and 1 from interacting.
2. Generate an ensemble of configurations that are representative of the reference state, λ.
3. For each configuration, evaluate the potential energy using the reference state Hamiltonian, $U(\mathbf{x};\lambda)$.
4. Repeat the same calculations using the Hamiltonian of the target state.
5. For each configuration, evaluate the potential energy difference.
6. Compute the ensemble average $\langle \exp\left\{-\beta\left[U(\mathbf{x};\lambda+\Delta\lambda)-U(\mathbf{x};\lambda)\right]\right\}\rangle_{\lambda}$, from which the free energy difference $\Delta A = A(\lambda+\delta\lambda) - A(\lambda)$ can be derived.
7. Increment λ and go to stage 2.



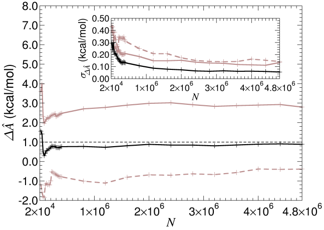
- Computing ΔU for several different values of λ might be helpful in optimizing choices of intermediate states for stratification.

- The simplest implementation is to calculate $\Delta A_{i,i+1}$ in the forward and backward directions, starting from i or $i+1$ and using the relevant FEP formulas. The results of these two calculations may then be combined, for example by simple averaging.

- This procedure has a serious drawback: In general, the accuracies of estimating $\Delta A_{i,i+1}$ from the forward and the backward simulations are not identical.[35]

- In fact, it is common that they differ substantially, because the corresponding probability distributions, $P_i\left(\Delta U_{i,i+1}\right)$ and $P_{i+1}\left(\Delta U_{i+1,i}\right)$ have different widths.

[35] WIDOM (1963); POHORILLE ET AL. (2010)



- Performing a forward calculation from state i, corresponding to λ_i, to an additional intermediate at $\lambda_i + \Delta\lambda/2$, and a backward calculation from state $i+1$ — which corresponds to $\lambda_i + \Delta\lambda$ — to the same additional intermediate.

- The difference in the free energies obtained from these calculations is equal to $\Delta A_{i,i+1}$. Combining forward and backward FEP equations,[36]

$$\Delta A_{i,i+1} = -\frac{1}{\beta} \ln \frac{\langle \exp\left(-\beta \Delta U_{i,i+1}/2\right)\rangle_i}{\langle \exp\left(\ \beta \Delta U_{i,i+1}/2\right)\rangle_{i+1}}$$

- Simple overlap sampling (SOS) method[37] — one of the oldest techniques for improving FEP calculations.[38] In general, markedly more accurate than simple averaging.

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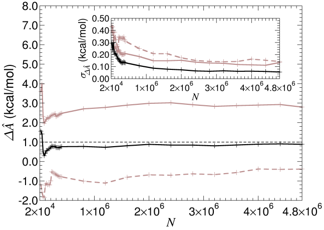
[36] Pohorille et al. (2010)

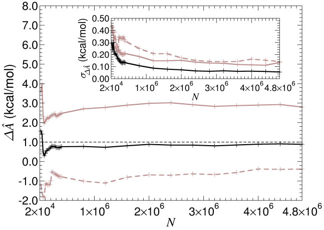
[37] Lu et al. (2003, 2004)

[38] Lee, Scott (1980)



- It requires that one forward and one backward calculation be performed at every intermediate state.
- Additional intermediate does not have to be located at $\lambda_i + \Delta\lambda/2$, but, instead, may be chosen at any value comprised between λ_i and $\lambda_i + \Delta\lambda$.
- Ideally, the location of the intermediate should be found to minimize the statistical error of $\Delta A_{i,i+1}$.[39]

[39] BENNETT (1976)



- This is tantamount to calculating

$$\boxed{\begin{aligned} \exp(-\beta \Delta A_{i,i+1}) \quad &= \quad \frac{\left\langle \{1+\exp[\beta(\Delta U_{i,i+1}-C)]\}^{-1}\right\rangle_i}{\left\langle \{1+\exp[-\beta(\Delta U_{i,i+1}-C)]\}^{-1}\right\rangle_{i+1}} \\ &\times \quad \exp(-\beta C) \end{aligned}}$$

- C is a constant that determines the position of the additional intermediate,

$$C = \Delta A_{i,i+1} + \frac{1}{\beta}\ln\frac{n_i}{n_{i+1}}$$

- C cannot be accessed directly because it involves the unknown value of $\Delta A_{i,i+1}$. Instead, the equations for $\exp(-\beta\Delta A_{i,i+1})$ and C are solved iteratively at a post–processing stage.

- Estimating the error associated to a computed free-energy difference has been recurrently overlooked, or simply ignored.
- Convoluted nature of the sources of error responsible for the discrepancy between theoretical and experimental measures.
- Common belief that a single simulation cannot provide both a free-energy difference and the error associated to it with an equal accuracy.
- Error associated to a free-energy change has a systematic origin, which primarily stems from finite sampling, and a statistical origin related to the width of the underlying probability distributions.[40]
- In a more general sense, systematic errors also embrace force-field inaccuracies, as well as algorithmic artifacts arising primarily from flawed numerical integration of the equations of motion and inappropriate treatment of intermolecular interactions.

---

[40] KOFKE, CUMMINGS (1998); CHIPOT, POHORILLE (2007)

- The statistical error can be expressed in terms of the variance of the ensemble average, *viz.* $\exp(-\beta\Delta\widehat{A}) = \langle \exp(-\beta\Delta U)\rangle_0$, measured over the n samples accrued in a single simulation.

- Using the propagation of uncertainty,[41]

$$\boxed{\sigma^2_{\Delta\widehat{A}} = \frac{(1+2\kappa)}{n\beta^2}\,\frac{1}{\langle \exp(-\beta\Delta U)\rangle_0^2}\,\sigma^2_{\langle \exp(-\beta\Delta U)\rangle_0}}$$

  where κ is the correlation length of the series and $(1+2\kappa)$, the sampling ratio, corresponds to the minimum number of samples found between two independent observables.[42]

- A similar formula can be devised for the BAR[43] estimator,

$$\sigma^2_{\Delta\widehat{A}} = \frac{(1+2\kappa_0)}{n_0\beta^2}\left(\frac{\langle f^2(x)\rangle_0}{\langle f(x)\rangle_0^2} - 1\right) + \frac{(1+2\kappa_1)}{n_1\beta^2}\left(\frac{\langle f^2(-x)\rangle_1}{\langle f(-x)\rangle_1^2} - 1\right)$$

  where $f(x) = 1/(1+e^x)$ and $x = \beta(\Delta U - C)$.

---

[41] MEYER (1992)

[42] STRAATSMA ET AL. (1986); FLYVBJERG, PETERSEN (1989)

[43] BENNETT (1976); HAHN, THEN (2009); POHORILLE ET AL. (2010)

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[41] Meyer (1992)

[42] Straatsma et al. (1986); Flyvbjerg, Petersen (1989)

[43] Bennett (1976); Hahn, Then (2009); Pohorille et al. (2010)

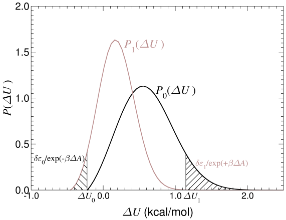
- Measuring the systematic, finite-sampling error is less straightforward.

- To a large extent, the accuracy of a free-energy estimate is intimately related to the sampling of the associated probability distribution.

- Simulations of finite length are necessarily prone to sample preferentially states of energy close to the peak of the probability distributions, *i.e.* either $\langle \Delta U \rangle_0$ or $\langle \Delta U \rangle_1$, at the expense of their tails.

- Yet, insufficient sampling of the latter is tantamount to ignoring the most significant contributions to the free-energy difference.

- In a forward transformation, these contributions are found in the lower tail of $P_0(\Delta U)$. Symmetrically, they correspond to the higher tail of $P_1(\Delta U)$ in a backward transformation.

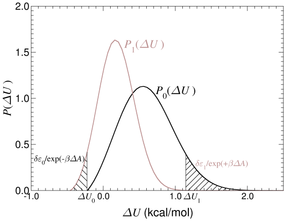


- A naive view of this problem can be put forth in terms of a frontier value of the perturbation, ΔU_0, below which, in a forward transformation, states characterized by an energy difference $\Delta U < \Delta U_0$ are never sampled.

- The systematic error then arises from failure to explore important regions of configurational space that correspond to $\Delta U < \Delta U_0$.

- In the ergodic limit, the *exact* free-energy difference, ΔA, of a forward transformation can be expressed as a sum of two contributions reflecting sampling of regions above and below the frontier value, ΔU_0,

$$\begin{aligned}\exp(-\beta\Delta A) &= \int_{-\infty}^{\Delta U_0} P_0(\Delta U)\ \exp(-\beta\Delta U)\ \mathrm{d}\Delta U \\ &+ \int_{\Delta U_0}^{+\infty} P_0(\Delta U)\ \exp(-\beta\Delta U)\ \mathrm{d}\Delta U\end{aligned}$$



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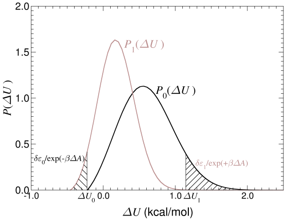

- In simulations of finite length, where regions contributing significantly to the free-energy difference are ignored, only the second term obviously prevails.

$$\exp(-\beta\Delta\widehat{A}_0) = \int_{\Delta U_0}^{+\infty} P_0(\Delta U)\ \exp(-\beta\Delta U)\ \mathrm{d}\Delta U$$

- The systematic error is the difference between the *exact* free-energy difference and its finite-sampling biased estimator,

$$\delta\epsilon^0_{\mathrm{bias},\Delta\widehat{A}} = -\int_{-\infty}^{\Delta U_0} P_0(\Delta U)\ \exp(-\beta\Delta U)\ \mathrm{d}\Delta U$$

- Of practical interest is the relative systematic error associated to the measure of the forward free-energy difference,

$$\frac{\delta\epsilon^0_{\mathrm{bias},\Delta\widehat{A}}}{\exp(-\beta\Delta A)} = -\int_{-\infty}^{\Delta U_0} P_1(\Delta U)\ \mathrm{d}\Delta U$$

Free-energy differences can be estimated computationally following four different routes:

- **Methods based on histograms,**[44]
- **Non-equilibrium work simulations,**[45]
- **Perturbation theory,**[46]
- **Measuring the derivative and integrating it.**[47]

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[44] WIDOM (1963); TORRIE, VALLEAU (1977)

[45] JARZYNSKI (1997); IZRAILEV ET AL. (1998); CROOKS (1999)

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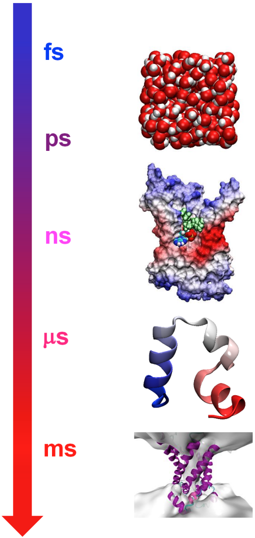
Synopsis

1. Free-energy perturbation calculations
2. Reaction coordinate–based free-energy calculations
3. Good practices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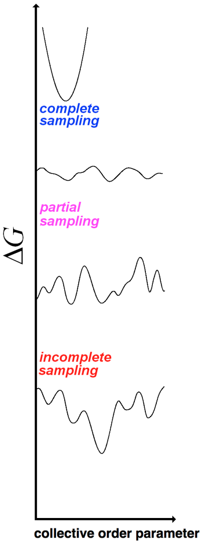
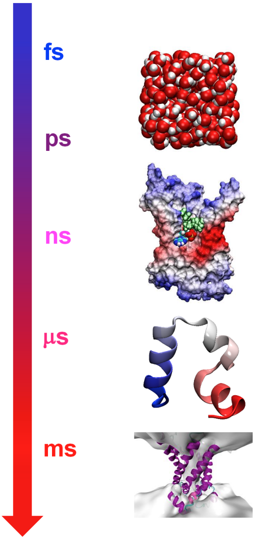

ΔG
complete sampling
partial sampling
incomplete sampling
collective order parameter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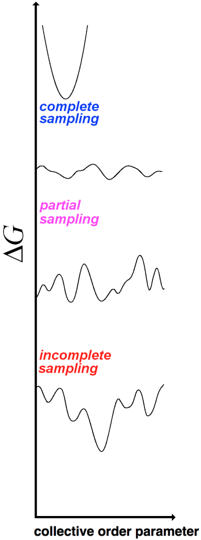
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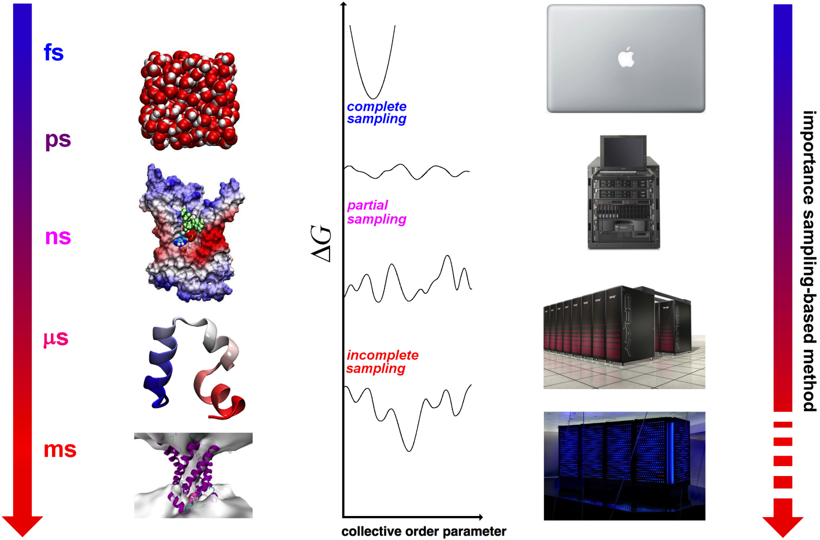
Bridging biological and computational time scales



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  Carter et al. (1989)
  den Otter, Briels (2000)
- Non-equilibrium MD
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  Park (2003)
- Adaptive umbrella sampling
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- Local elevation/flooding
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- Generalized extent parameter
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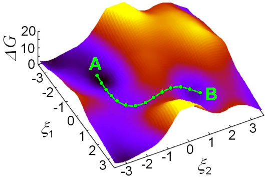

- The reaction coordinate is the committor function in $\mathbb{R}^{3N}$, defined between 0 and 1.[48]

- The definition of *order parameter* $\xi : \mathbb{R}^{3N} \rightarrow \mathbb{R}^{n}$, with n small, is reasonable if ξ is essentially decoupled from the slow manifolds.

- The choice of an order parameter, or combination thereof, unlikely to embrace concerted, collective movements is prone to yield quasi non-ergodicity scenarios.

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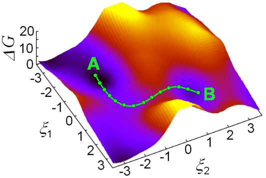
[48] Bolhuis et al. (2002); E et al. (2005); Zhao et al. (2010)



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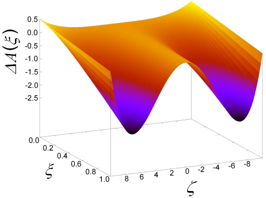

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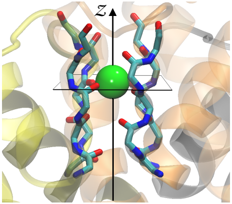

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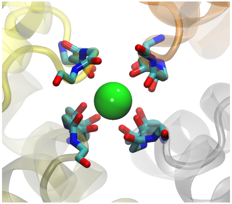

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Generalization of the classical definition of $w(r)$, based on $g(r)$, $w(r) = -1/\beta \ln g(r)$, is not straightforward.[49]

Turn to an alternate definition:

$$A(\xi) = -\frac{1}{\beta} \ln P_\xi + A_0$$

Probability to find the system at ξ along the order parameter:

$$P_\xi = \int \delta[\xi - \xi(\mathbf{x})] \; \exp[-\beta \mathscr{H}(\mathbf{x}, \mathbf{p}_x)] \; \mathrm{d}\mathbf{x} \; \mathrm{d}\mathbf{p}_x$$

First derivative of the free energy with respect to the order parameter:

$$\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} = \left\langle \frac{\partial \mathscr{H}(\mathbf{x}, \mathbf{p}_x)}{\partial \xi} \right\rangle_\xi$$

---

[49] CHANDLER (1987)

ξ and $\{\mathbf{x}\}$ are not independent variables:

$$P_\xi = \int |J| \ \exp[-\beta U(\mathbf{q};\xi)] \ \mathrm{d}\mathbf{q} \ \int \exp[-\beta T(\mathbf{p}_x)] \mathrm{d}\mathbf{p}_x$$

Derivative of the probability distribution with respect to ξ:

$$\begin{aligned}\frac{\mathrm{d}P_\xi}{\mathrm{d}\xi} &= \int \exp[-\beta U(\mathbf{q};\xi)] \left\{ -\beta |J| \frac{\partial U(\mathbf{q};\xi)}{\partial \xi} + \frac{\partial |J|}{\partial \xi} \right\} \mathrm{d}\mathbf{q} \\ &\times \int \exp[-\beta T(\mathbf{p}_x)] \ \mathrm{d}\mathbf{p}_x\end{aligned}$$

The kinetic contribution vanishes in $\mathrm{d}A(\xi)/\mathrm{d}\xi$:

$$\begin{aligned}\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} &= -\frac{1}{\beta} \ \frac{1}{P_\xi} \int \exp[-\beta U(\mathbf{q};\xi^*)] \ \delta(\xi^* - \xi) \\ &\times \left\{ -\beta |J| \frac{\partial U(\mathbf{q};\xi^*)}{\partial \xi} + \frac{\partial |J|}{\partial \xi} \right\} \mathrm{d}\mathbf{q} \ \mathrm{d}\xi^*\end{aligned}$$

Back transformation into Cartesian coordinates:

$$\begin{aligned}\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} &= -\frac{1}{\beta}\,\frac{1}{P_\xi}\int \exp[-\beta U(\mathbf{x})]\;\delta[\xi(x)-\xi] \\ &\times \left\{-\beta\frac{\partial U(\mathbf{x})}{\partial \xi} + \frac{1}{|J|}\;\frac{\partial |J|}{\partial \xi}\right\}\,\mathrm{d}\mathbf{x}\end{aligned}$$

The derivative of the free energy with respect to the order parameter may be expressed as a sum of configurational averages at constant ξ: [50]

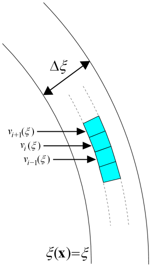
$$\boxed{\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} = \left\langle \frac{\partial U(\mathbf{x})}{\partial \xi}\right\rangle_\xi - \frac{1}{\beta}\left\langle \frac{\partial \ln|J|}{\partial \xi}\right\rangle_\xi := -\langle F_\xi\rangle_\xi}$$

---

[50] Carter et al. (1989); Chipot, Pohorille (2007)

Derivative of the free energy with respect to the order parameter:

$$\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} = -\frac{1}{\beta}\,\frac{1}{Q(\xi)}\,\frac{\mathrm{d}Q(\xi)}{\mathrm{d}\xi}$$



Partition function:

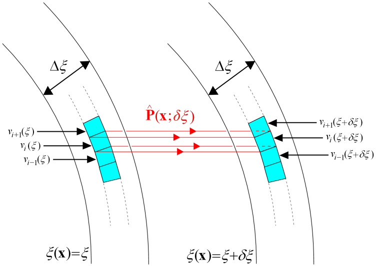
$$Q(\xi) = \int \delta[\xi - \xi(\mathbf{x})]\ \exp[-\beta U(\mathbf{x})]\ \mathrm{d}\mathbf{x}$$

The δ–function can be viewed as a square pulse of width $\Delta\xi$. $Q(\xi)$ is an integral over a thin slab of configurational space, i.e. a sum of the Boltzmann weight over the volume elements $v_i(\xi)$, so that:

$$Q(\xi) = \frac{1}{\Delta\xi}\sum_i \exp[-\beta U_i(\xi)]\ v_i(\xi)$$

Increase the order parameter by $\delta\xi$. The partition function, $Q(\xi+\delta\xi)$, is obtained from summing all volume elements in the new slab bounded by surfaces $\xi(\mathbf{x}) = \xi + \delta\xi \pm 1/2\Delta\xi$:

$$Q(\xi+\delta\xi) = \frac{1}{\Delta\xi} \sum_i \exp[-\beta U_i(\xi+\delta\xi)]\ v_i(\xi+\delta\xi)$$

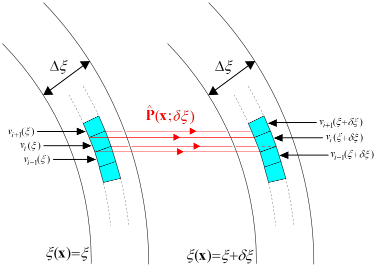


Assuming that the changes in the volume elements is sufficiently small, $\delta Q(\xi)$ can be approximated from a Taylor expansion:

$$\begin{aligned}\frac{\delta Q(\xi)}{\delta\xi} &= \frac{1}{\Delta\xi} \sum_i \left[-\beta \frac{\delta U_i(\xi)}{\delta\xi} + \frac{1}{v_i(\xi)} \frac{\delta v_i(\xi)}{\delta\xi}\right] \\ &\times \exp[-\beta U_i(\xi)]\ v_i(\xi)\end{aligned}$$

Procedure to move and transform $v_i(\xi)$ into $v_i(\xi+\delta\xi)$. It converts a grid of points in the old slab into another grid of points in the new slab. In continuous space, this is tantamount to:

$$\xi[\mathbf{P}(\mathbf{x};\delta\xi)] = \xi(\mathbf{x}) + \delta\xi$$



For a small projection:

$$\mathbf{P}(\mathbf{x};\delta\xi) = \mathbf{x} + \delta\xi\ \mathbf{V}(\mathbf{x})$$

$\mathbf{V} = (\mathbf{v}_1^{\mathrm{T}}, \dots)^{\mathrm{T}}$ is an arbitrarily chosen vector field of $\mathbb{R}^{3N} \to \mathbb{R}^{3N}$, which verifies:

$$\mathbf{v}_i \cdot \boldsymbol{\nabla}_{\mathbf{x}}\xi_j = \delta_{ij} \qquad\qquad \forall i,j$$

From $\mathbf{P}(\mathbf{x};\delta\xi) = \mathbf{x} + \delta\xi\ \mathbf{V}(\mathbf{x})$, it follows to first order in $\delta\xi$, that the potential energy changes by:

$$\frac{\delta U_i(\xi)}{\delta\xi} = \mathbf{V}\cdot\boldsymbol{\nabla}_{\mathbf{x}} U(\mathbf{x})$$

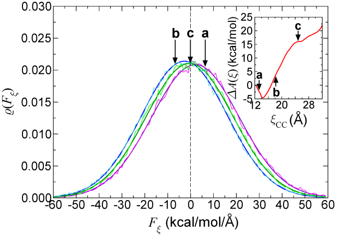
From the fact that vector $\mathbf{x}_2 - \mathbf{x}_1$ is projected onto $\mathbf{x}_2 - \mathbf{x}_1 + \delta\xi(\mathbf{x}_2 - \mathbf{x}_1)\cdot\boldsymbol{\nabla}_{\mathbf{x}}\mathbf{V}(\mathbf{x}_1)$, it follows to first order in $\delta\xi$, that the kinetic term changes by:

$$\frac{1}{v_i(\xi)}\frac{\delta v_i(\xi)}{\delta\xi} = \boldsymbol{\nabla}_{\mathbf{x}}\cdot\mathbf{V}$$

Derivative of the free energy with respect to the order parameter:[51]

$$\boxed{\frac{\mathrm{d}A(\xi)}{\mathrm{d}\xi} = \left\langle \mathbf{V}\cdot\boldsymbol{\nabla}_{\mathbf{x}} U(\mathbf{x}) - \frac{1}{\beta}\boldsymbol{\nabla}_{\mathbf{x}}\cdot\mathbf{V}\right\rangle_{\xi}}$$

[51] den Otter (2000)



- Accrue the instantaneous force exerted along ξ in bins of finite size, $\delta\xi$, and provide an estimate of $\mathrm{d}A(\xi)/\mathrm{d}\xi$.
- The biasing force, $-\langle F_\xi \rangle_\xi \, \boldsymbol{\nabla}\xi$, is applied after a user-defined number of steps.
- ABF erases the roughness of the free energy surface along ξ.
- The system is in principle driven only by its self–diffusion properties, granted that ξ is essentially decoupled from the slow manifolds.[52]
- ABF formally converges.[53]

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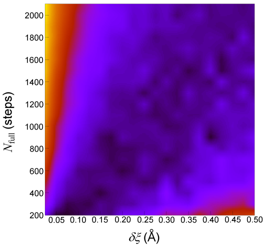
[52] Darve, Pohorille (2001); Hénin, Chipot (2004); Chipot, Hénin (2005)

[53] Lelièvre et al. (2007)



- Accrue the instantaneous force exerted along ξ in bins of finite size, $\delta\xi$, and provide an estimate of $\mathrm{d}A(\xi)/\mathrm{d}\xi$.
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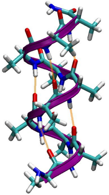

- Pulling of deca–alanine to investigate the equilibrium between the native, α–helical conformation and an ensemble of extended structures.

- Steered molecular dynamics and the application of the Jarzynski equality with a cumulant expansion:[54]

$$\exp[-\beta\ \Delta A(\xi)] = \langle \exp[-\beta\ w(\xi)] \rangle$$

Reference reversible pulling:	200.0 ns
Jarzynski:	100 × 2.0 ns (v = 10.0 Å/ns)
	100 × 0.2 ns (v = 100.0 Å/ns)

- Unconstrained dynamics with the formalism of the average force. The chosen order parameter, ξ, is the distance separating the first and the last α–carbon atoms.[55]

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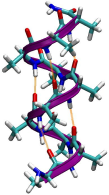
[54] PARK ET AL. (2003)

[55] HÉNIN, CHIPOT (2004); CHIPOT, HÉNIN (2005)



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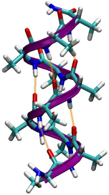

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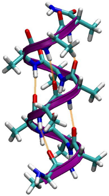

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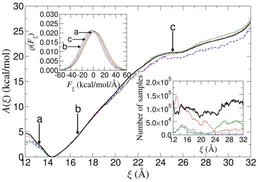

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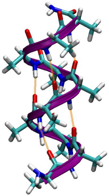

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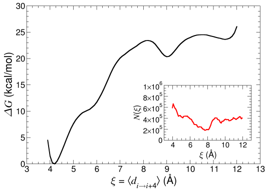

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[54] Park et al. (2003)

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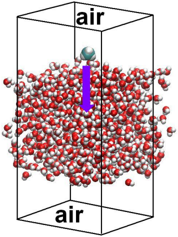


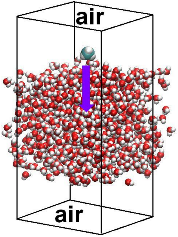
- Translocation of a single methane molecule across a water–air interface, using unconstrained MD and the average force exerted along ξ, the direction normal to the aqueous interface.

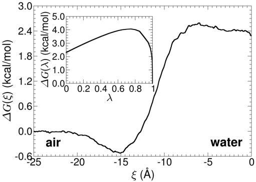
- The free energy difference between the tail–ends of the PMF yields the hydration free energy: $+2.4\pm0.4$ kcal/mol (exp. $+2.0$ kcal/mol).

- FEP creation and annihilation of methane in vacuum and in water. 1.02 ns forward and reverse simulations. $\Delta G_{\text{hydr}} = +2.5\pm0.4$ kcal/mol.

- This agrees nicely with the recent estimate of $+2.44$ kcal/mol, based on a 73.2 ns simulation.[56]

[56] Shirts et al. (2003)





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[56] Shirts et al. (2003)

- Midpoint rule: $\Delta A \simeq -\Delta\xi \sum_{i=1}^{p} \langle F_\xi \rangle_{\xi_i}$
- Average and variance of the force over the reaction pathway:

$$\begin{cases} \mu & = & \langle F_\xi \rangle_\xi = \dfrac{1}{p} \displaystyle\sum_{i=1}^{p} \langle F_\xi \rangle_{\xi_i} = \dfrac{1}{p} \displaystyle\sum_{i=1}^{p} \mu_i \\ \sigma^2 & = & \dfrac{1}{p} \displaystyle\sum_{i=1}^{p} \sigma_i^2 + (\mu - \mu_i)^2 \end{cases}$$

- Fluctuation on the free-energy difference:

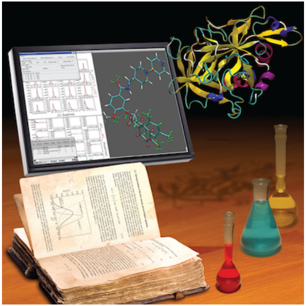
$$\begin{aligned} \langle |\Delta A - \overline{\Delta A}|^2 \rangle &= (\Delta\xi)^2 \sum_{i,j=1}^{p} \sum_{k=1}^{n_i} \sum_{l=1}^{n_j} \frac{1}{n_i n_j} \langle (F_{\xi,l}^i - \mu)(F_{\xi,k}^i - \mu) \rangle \\ &\simeq (\Delta\xi)^2 \left\{ \frac{p}{N} \sum_{i=1}^{p} \left[\sigma_i^2 + (\mu - \mu_i)^2 \right] + 2\sigma^2 \frac{p^2}{N} \kappa \right\} \end{aligned}$$

- Standard deviation:[57] $\boxed{\sigma_{\Delta A} \simeq \Delta\xi \, \dfrac{\sigma}{N^{1/2}} (1 + 2\kappa)^{1/2}}$

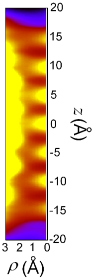
[57] RODRIGUEZ-GOMEZ ET AL. (2004)

Synopsis

1. Free-energy perturbation calculations
2. Reaction coordinate–based free-energy calculations
3. Good practices



1. All free-energy calculations should be accompanied by an error estimate. A distinction between *statistical* and *systematic* error ought to be made.
2. Since the reliability of free-energy estimates depends on the overlap between $P_0(\Delta U)$ and $P_1(\Delta U)$, these distributions should be monitored to assess the degree of overlap.
3. Stratification provides an effective, general method for reducing the variance and improving overlap at each stage.
4. Combining forward and backward simulations using, for instance, the BAR estimator is strongly recommended.



1. The continuity of the derivative $\mathrm{d}A(\xi)/\mathrm{d}\xi$ across the reaction pathway imposes that $\langle F_\xi \rangle_\xi$ be continuous throughout.
2. Since the dynamics of ξ corresponds to a random walk at zero mean force, sampling across the reaction pathway ought to be completely uniform. A markedly uneven sampling may be symptomatic of a poorly chosen order parameter.
3. Like any free-energy measure, potentials of mean force should be provided with error bars.
4. Since the kinetic properties of the system depend on the choice of the order parameter, it is recommended to ascertain that the latter constitutes a reasonable model of the reaction coordinate.

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