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Free Energy Perturbation (FEP)

A method for doing free energy (or free enthalpy) calculations in which an ensemble average of an exponential of the free energy divided by the product of the gas constant and absolute temperature is evaluated. See ***Free Energy Calculations: Methods and Applications*** and ***Free Energy Perturbation Calculations***.

Free Energy Perturbation Calculations

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Abbreviations

PMF = potential of mean force; SPC = simple point charge; US = umbrella sampling; WHAM = weighted histogram analysis method.

1 INTRODUCTION

The calculation of free energy has been described as the 'Holy Grail' of computational chemistry. Many, if not most,

of the physical properties a chemist (or biochemist) could wish to determine by calculation or experiment depend on the free energy of the system. Essentially the free energy is a measure of the probability of finding a system in a given state. A difference in free energy gives the relative probability of finding the system in one state as opposed to another. For example, the binding affinity of a ligand to its acceptor is given by the difference in free energy between the complex and the ligand plus acceptor free in solution. In a similar manner the direction and extent to which a chemical reaction will proceed, the phase behavior of a compound, the mixing behavior of different compounds, or even the response of a system to the application of external pressure all depend on the relative free energy of different states of a system.

From basic physical chemistry we know that the Gibbs (free) energy of association ΔG_A, of a ligand X interacting with a receptor Y with an association constant, K_A is given by

$$\Delta G_A = -k_B T \ln K_A$$
$$K_A = \frac{[XY]}{[X][Y]} \tag{1}$$

where k_B is Boltzmann's constant, T is the absolute temperature, and the brackets [ ] indicate concentration.[1] That is, the difference in free energy is proportional to the logarithm of the relative probability of finding the system in one of the two possible states at equilibrium. To determine the absolute free energy of a system all possible states must be considered. Quantum mechanically we can express the Helmholtz free energy, F, of a system of N particles in a volume V at a temperature T, in terms of the canonical partition function, Q, as,

$$F(N,V,T) = -k_B T \ln Q(N,V,T)$$
$$= -k_B T \ln \left[\sum_j e^{-E_j(N,V)/k_B T} \right] \tag{2}$$

where the energy of a quantum mechanical state, j, of the system is given by $E_j(N,V)$.[2] The absolute free energy of a system is a sum over all possible electronic and nuclear degrees of freedom and for most systems of interest cannot be calculated. What can be determined is the difference in free energy between two (closely) related states of a system. Experimentally, a difference in free energy is determined either from the relative probability of finding the system in a given state as in equation (1), or from the reversible work required to go from an initial state to a final state. Computationally, the same basic approaches are used. To estimate the free energy of association in equation (1) by simulating a mixture of the two components and counting how often the complex is formed is, however, extremely inefficient. In practice, a perturbation is applied to the system to force the transition from one state to another. Statistical mechanical procedures are then used to correct for the effect of the perturbation or to calculate the work done on the system by the perturbation.

Free energy perturbation calculations have been extensively reviewed in many places.[3-17] The aim of this article is, therefore, primarily to introduce the basic principles underlying free energy perturbation calculations and to help the reader place the various implementations of the methodology into a consistent framework. In addition, the main factors which determine the reliability of a given calculation are discussed and a brief review of the current state of the field provided. For a more complete discussion of the underling theory it is strongly recommended that the reader refer to Refs. 3, 5, 9, 12, 13, and 17.

2 BASIC PRINCIPLES

2.1 Statistical Mechanics of a Classical System

In terms of the energy, E, and the entropy, S, the Helmholtz free energy, F, of a system of N particles in a volume V at a temperature T is given by[2]

$$F(N,V,T) = E(N,V,T) - TS(N,V,T) \tag{3}$$

In terms of the canonical partition function, $Z(N,V,T)$, it is given by

$$F(N,V,T) = -k_B T \ln Z(N,V,T)$$
$$= -k_B T \ln \left[(h^{3N} N!)^{-1} \int\int e^{-H(p,r)/k_B T} \, dp \, dr \right] \tag{4}$$

where h is Planck's constant and the classical Hamiltonian,

$$H(p,r) = \sum_{i=1}^{N} p_i^2/(2m_i) + U(r) \tag{5}$$

expresses the total energy of the system in terms of the coordinates $r = (r_1, r_2, \ldots, r_N)$, and the conjugate momenta, $p = (p_1, p_2, \ldots, p_N)$ of the N (indistinguishable) particles of the system. m_i indicates the mass of particle i, and $U(r)$ is the interaction function.[2,13] Classically, the free energy is given by a double integral of the (positive) Boltzmann factor $\exp[-H(p,r)/k_B T]$ over all possible values of p and r which define the volume of phase space accessible to the system. To estimate the absolute free energy of a system from a numerical simulation using equation (4) is not possible. Molecular dynamics (MD) or Monte Carlo (MC) simulations of large molecular systems necessarily sample only a limited set of configurations. Any calculation of the free energy via equation (4) using a set of MD or MC configurations suffers in a systematic way from the incomplete sampling of phase space. Specifically, F is overestimated. The Boltzmann factor in equation (4) is necessarily positive so each additional part of phase space that is included in the integral gives a negative contribution to the free energy and a positive contribution to the entropy. Even if a set of configurations representative of the complete ensemble could be obtained from a simulation, the integral in equation (4) would still not be accurate.[13]

The essential difficulty in estimating the total free energy of a system using simulation techniques may be more readily seen if the total free energy is re-expressed as an ensemble average. Integrating over the momenta p in the partition function and using $\int dr = V^N$, we find

$$F(N,V,T) = -k_B T \ln \left[\frac{V^N \int e^{-U(r)/k_B T} \, dr}{\int e^{+U(r)/k_B T} e^{-U(r)/k_B T} \, dr} \right]$$
$$- k_B T \ln\{[2\pi m k_B T/h^2]^{3N/2}/N!\}$$
$$= +k_B T \ln\langle e^{+U/k_B T}\rangle_{N,V,T}$$
$$- k_B T \ln\{V^N [2\pi m k_B T/h^2]^{3N/2}/N!\} \tag{6}$$

where the ensemble average $\langle\chi\rangle$ of a microscopically defined quantity $\chi(\boldsymbol{p},\boldsymbol{r})$ is defined by

$$\langle\chi\rangle_{N,V,T} \equiv \frac{\int\int \chi(\boldsymbol{p},\boldsymbol{r})\mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r})/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}}{\int\int \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r})/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}} = \int\int \chi(\boldsymbol{p},\boldsymbol{r})\rho(\boldsymbol{p},\boldsymbol{r})\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r} \tag{7}$$

$\rho(\boldsymbol{p},\boldsymbol{r})$ is the probability of finding the system in the state characterized by $\boldsymbol{p}$ and $\boldsymbol{r}$. In an analogous manner the entropy, S, and the energy, E, can also be expressed in terms of ensemble averages.[13] The accurate calculation of the free energy (or entropy) is not possible due to the occurrence of the ensemble average $\langle\exp[+U/k_\mathrm{B}T]\rangle$ in equation (6). The probability $\rho(\boldsymbol{r})$ of a molecular configuration being sampled is proportional to the Boltzmann factor $\exp[-U(\boldsymbol{r})/k_\mathrm{B}T]$. This will be small when the function to be averaged, $\exp[+U(\boldsymbol{r})/k_\mathrm{B}T]$, is large and vice versa.

2.2 The Coupling Parameter Approach

To calculate the absolute free energy (or entropy) of a complex molecular system is virtually impossible. It is possible, however, to calculate the difference in free energy between two (closely) related states of a system using the so-called coupling parameter approach.[3] To determine the difference in free energy between two states A and B of a system using this approach the Hamiltonian is made a function of a coupling parameter, λ, such that when $\lambda = \lambda_A$ the Hamiltonian of the system corresponds to that of state A, $H(\boldsymbol{p},\boldsymbol{r};\lambda_A) = H_A(\boldsymbol{p},\boldsymbol{r})$, and when $\lambda = \lambda_B$, the Hamiltonian of the system corresponds to that of state B, $H(\boldsymbol{p},\boldsymbol{r};\lambda_B) = H_B(\boldsymbol{p},\boldsymbol{r})$. The partition function

$$Z(N,V,T;\lambda) = (h^{3N}N!)^{-1}\int\int \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r};\lambda)/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r} \tag{8}$$

thus becomes a function of λ, as does the free energy.

$$F(N,V,T;\lambda) = -k_\mathrm{B}T\ln Z(N,V,T;\lambda) \tag{9}$$

2.2.1 *Thermodynamic Integration*

If the free energy is a function of λ we may express the difference in free energy between the two states A and B (at constant N, V, and T) as an integral

$$\Delta F_{BA} \equiv F(\lambda_B) - F(\lambda_A) = \int_{\lambda_A}^{\lambda_B} F'(\lambda)\,\mathrm{d}\lambda \tag{10}$$

where $F'(\lambda) \equiv \mathrm{d}F/\mathrm{d}\lambda$. For simplicity, the indication of constant N, V, and T in the notation has been dropped. Differentiating equation (9) with respect to λ we find

$$F'(\lambda) = \int\int \frac{\partial H(\boldsymbol{p},\boldsymbol{r};\lambda)}{\partial\lambda}\rho(\boldsymbol{p},\boldsymbol{r};\lambda)\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r} = \left\langle\frac{\partial H(\lambda)}{\partial\lambda}\right\rangle_\lambda \tag{11}$$

where the probability of occurrence of a molecular configuration (and momenta) $\rho(\boldsymbol{p},\boldsymbol{r};\lambda)$ and the ensemble average $\langle\ldots\rangle_\lambda$ defined in equation (7) are dependent on λ. Formulae (10) and (11) form the so-called thermodynamic integration formula.[13] The name originates in analogy with thermodynamic methods to obtain differences in free energy between states of different temperature or volume.[12] The advantage of formulae (10) and (11) over formulae (4) or (6) is that the relative free energy is computed as an integral over the ensemble average of the derivative of the Hamiltonian with respect to the coupling parameter λ. This ensemble average does not suffer from the sampling problems which prohibit the computation of the absolute free energy via formulae (4) or (6) because the most probable configurations also dominate the average. Nevertheless, a representative ensemble must be sampled for each λ.

2.2.2 *Thermodynamic Perturbation*

Thermodynamic integration treats the change from the initial to the final state as continuous. Alternatively, the change can be considered as one (or more) discrete steps or perturbations. The initial derivation of the so-called thermodynamic perturbation formula, due to Zwanzig,[18] dealt with distinct states but a stepwise perturbation may also be thought of as numerical solution to the derivative in equation (10).[13] Using equation (4), the free energy difference ΔF_{BA}, between two states A and B of a system, can be expressed as the ratio of the respective partition functions and rearranged to give,

$$\begin{aligned}\Delta F_{BA} \equiv F(\lambda_B) - F(\lambda_A) &= -k_\mathrm{B}T\ln\frac{Z(\lambda_B)}{Z(\lambda_A)} \\ &= -k_\mathrm{B}T\ln\left\{\frac{\int\int \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r};\lambda_B)/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}}{\int\int \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r};\lambda_A)/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}}\right\} \\ &= -k_\mathrm{B}T\ln\left\{\frac{\int\int \mathrm{e}^{-[H(\boldsymbol{p},\boldsymbol{r};\lambda_B)-H(\boldsymbol{p},\boldsymbol{r};\lambda_A)]/k_\mathrm{B}T}\times \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r};\lambda_A)}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}}{\int\int \mathrm{e}^{-H(\boldsymbol{p},\boldsymbol{r};\lambda_A)/k_\mathrm{B}T}\,\mathrm{d}\boldsymbol{p}\,\mathrm{d}\boldsymbol{r}}\right\}\end{aligned} \tag{12}$$

which has the form of an ensemble average over state A

$$\Delta F_{BA} = -k_\mathrm{B}T\ln\langle\mathrm{e}^{-[H(\lambda_B)-H(\lambda_A)]/k_\mathrm{B}T}\rangle_{\lambda_A} \tag{13}$$

Equally we could have written ΔF_{BA} as an ensemble average over state B,

$$\Delta F_{BA} = +k_\mathrm{B}T\ln\langle\mathrm{e}^{-[H(\lambda_A)-H(\lambda_B)]/k_\mathrm{B}T}\rangle_{\lambda_B} \tag{14}$$

Equations (13) and (14) are formally exact for any perturbation. For large perturbations, however, this formulation suffers similar convergence problems to equation (6). In fact equation (13) reduces to equation (6) if the interactions between the atoms in state B are zero. Using equation (13) the free energy is calculated from the relative probability, a given configuration sampled in an ensemble at λ_A being sampled in the ensemble at λ_B. To obtain convergence, significant overlap of the low energy regions of the two ensembles is required. The effect of the perturbation on the configurational space sampled must be small. For this reason the thermodynamic perturbation formula is often expressed more generally as a sum over

a series of small steps in λ (windows),

$$\Delta F_{BA} = \sum_{\lambda=\lambda_A}^{\lambda_B} -k_B T \ln\langle e^{-[H(\lambda+\Delta\lambda)-H(\lambda)]/k_B T}\rangle_\lambda \qquad (15)$$

Sometimes equation (15) is also referred to as the exponential formula. This is in order to distinguish equation (15) which is formally exact from perturbation approaches in statistical mechanics which formally depend on the change being small (see *Perturbation Theory*). For simplicity equations (11) and (15) have been derived in the canonical ensemble (N, V, T). The corresponding equations for the Gibbs free energy in the isothermal isobaric ensemble (N, P, T) have, however, the same form.[13] Equations (11) and (15) have also been expressed in terms of the total Hamiltonian. Commonly, the ensemble averages are expressed only in terms of the potential energy of the system. In Cartesian space and without constraints the terms in the Hamiltonian arising from the potential energy and the momenta are formally separable and can be integrated independently. At constant temperature the kinetic contribution to the free energy is a constant and will cancel within a thermodynamic cycle (see Section 3.3).[13]

The coupling parameter approach transforms the basic problem from the determination of the absolute free energy of two different systems, which requires knowledge of all possible states of each system, to the determination of the difference in free energy between two specific states of one overall system. At the same time it provides a defined path along which the difference in free energy may be calculated. This is a great simplification. It does not, however, mean that free energy or the difference in free energy no longer depends on all phase space. The difference in free energy does depend on all phase space but is dominated by the low (free) energy regions along the pathway linking the states of interest. The implicit assumption in all free energy calculations is that the contributions from the regions of phase space not sampled in the simulations to the absolute free energy of the two end states effectively cancel.

2.3 Special Cases

2.3.1 *Widom Particle Insertion*

The method of Widom[19] to calculate the excess chemical potential of a system by random insertion of a test particle, particle insertion, is in essence a special case of the thermodynamic perturbation formula. The excess chemical potential (or free energy per particle), μ^{excess}, is the chemical potential of a system in excess of that of an ideal (noninteracting) system at the same density i.e., $\mu^{\text{excess}} = \mu^{\text{total}} - \mu^{\text{ideal}}$.[1,2] The excess chemical potential can be determined using the thermodynamic integration or perturbation formula from the change in free energy as all intermolecular interactions are gradually reduced to zero, normalized by the number of particles, N. Alternatively, if N is large, this can be approximated by the free energy of inserting (or removing) one additional particle. Treating the addition of a particle as a one-step perturbation we obtain from equation (13)

$$\mu^{\text{excess}} = -k_B T \ln\langle e^{-\Delta U/k_B T}\rangle_{N,V,T} \qquad (16)$$

where ΔU is the interaction energy of the test (ghost) particle with the rest of the system.[12] As the position of the test particle is independent of the configuration of the rest of the system it may be randomly inserted multiple times in each configuration to improve statistics. Equation (16) will converge so long as low energy configurations for the test particle, that is a configuration with an appropriately sized cavity, are sampled. In practice particle insertion can be highly efficient but fails if the density is such that the ghost particle (almost) never samples an appropriate cavity. This should be contrasted to the inverse of particle insertion, particle deletion. In this case

$$\mu^{\text{excess}} = +k_B T \ln\langle e^{+\Delta U/k_B T}\rangle_{N,V,T} \qquad (17)$$

where ΔU is now the interaction energy of a given (real) particle with the rest of the system. Using particle deletion μ^{excess} never converges to the correct value. This is because configurations in which a cavity does not exist at the position of the real atom can never be sampled. This highlights a fundamental property of the perturbation approach. If configurations which correspond to low energy configurations of the perturbed state are sampled, the method is highly efficient. If not, the method does not converge. The method is not symmetric. Convergence of the forward mutation is no guarantee that the reverse mutation also converges (see Section 3.11).

2.3.2 *Potential of Mean Force*

If the parameter linking the initial and final states corresponds to a spatial coordinate, i.e., an internal coordinate of the system or a coordinate in Cartesian space, it follows from equation (11) that the change in free energy as a function of this coordinate is the ensemble average of the derivative of the potential energy function with respect to the given coordinate or simply the average force acting along the coordinate. The change in free energy as a function of a spatial coordinate, ξ, is thus commonly referred to as a potential of mean force (PMF). A PMF can be straightforwardly evaluated using equation (11). Often, however, a potential of mean force, $w(\xi)$, is expressed in terms of a relative probability function, $P(\xi)$

$$w(\xi) = -k_B T \ln P(\xi) + C \qquad (18)$$

The constant, C, corresponds to the work required to constrain the system to the coordinate of interest. Because adequate sampling is only required in respect of the coordinate in question, direct determination of the probability function is frequently possible. For example, in the case of two ions in solution the free energy as a function of the ion–ion interatomic distance, r, is simply given by

$$w(r) = -k_B T \ln g(r) \qquad (19)$$

where the ion–ion radial distribution function, $g(r)$, can be readily obtained from an MD or MC simulation. In the case of a highly complex spatial coordinate, however, for example one associated with a major change in the conformation of a molecule, or where there are barriers in the free energy profile in excess of a few $k_B T$, adequate sampling will not be achieved in an unbiased simulation.

2.3.3 *Umbrella Sampling*

Using the coupling parameter approach the system is forced to move along a particular reaction coordinate. This may be a spatial coordinate or a coordinate in parameter space. The system is forced because it will not spontaneously sample the

relevant regions of phase space efficiently. Essentially, the coupling parameter is treated as a constraint. An alternative, but closely related, approach is to modify the Hamiltonian of the system so that the sampling is biased in favor of particular (important) regions of phase space. This is achieved by including in the Hamiltonian an additional biasing or umbrella potential. In effect a restraint as opposed to a constraint is applied. The inclusion of a biasing potential in the Hamiltonian means that the configurations sampled in the simulation no longer have appropriate Boltzmann weights. Torrie and Valleau showed, however, that the (unbiased) ensemble average of any quantity χ, $\langle\chi\rangle_U$, could be obtained from a biased ensemble using the relation

$$\langle\chi\rangle_U = \frac{\langle\chi e^{+U_W/k_BT}\rangle_W}{\langle e^{+U_W/k_BT}\rangle_W} \tag{20}$$

where U_W is the biasing potential energy term and $\langle\ldots\rangle_W$ represents an ensemble average over the biased ensemble.[12,13,20] Umbrella sampling (US) techniques can be readily combined with other free energy perturbation methods. For example, in potential of mean force calculations, maxima in the free energy profile will be poorly sampled in the probability distribution. To overcome this problem an umbrella potential may be used to increase sampling in the less favored regions. Combining equations (18) and (20) we obtain

$$\begin{aligned} w(\xi) &= -k_BT \ln\left[\frac{P_W(\xi)e^{+U_w(\xi)/k_BT}}{\langle e^{+U_W(\xi)/k_BT}\rangle_W}\right] + C \\ &= -k_BT \ln P_W(\xi) - U_W(\xi) + k_BT \ln\langle e^{+U_W(\xi)/k_BT}\rangle_W + C \\ &= -k_BT \ln P_W(\xi) - U_W(\xi) + F_W + C \end{aligned} \tag{21}$$

where $P_W(\xi)$ is the relative probability of sampling ξ in the biased ensemble and $U_W(\xi)$ is the contribution of the biasing term to the total potential energy. F_W corresponds to the work done on the system (the change in free energy) by the imposition of the biasing potential. F_W depends on the ensemble average of a positive exponent and for the same arguments as apply to equation (6) cannot readily be determined.

Biasing potentials may take different forms. If chosen to be the negative of the free energy profile the sampling of any point along the coordinate will be equally probable. This is, however, not always desirable. Alternatively, an umbrella potential can be used to restrict the sampling to a small region of phase space to improve statistics. Different umbrellas may be used to cover a range of interest and the free energy profiles combined. Because F_W is dependent on the biasing potential and cannot readily be determined, the free energy profiles must be matched empirically. This may be achieved by exploiting regions of overlap between adjacent umbrellas. Alternatively, the weighted histogram analysis method (WHAM) can be used to obtain a self-consistent solution combining all data.[21–23] If we have N_W biased simulations it follows from equation (21) that the unbiased probability function $P_{(i)}(\xi)$ is given by

$$P_{(i)}(\xi) = \frac{P_{W(i)}(\xi)}{e^{-[U_{W(i)}(\xi) - F_{W(i)}]/k_BT}} \tag{22}$$

where i indicates a specific biasing potential. If each of the $F_{W(i)}$ are known, the combined unbiased probability density function is simply

$$P(\xi) = \frac{\sum_{i=1}^{N_W} n_i P_{W(i)}(\xi)}{\sum_{j=1}^{N_W} n_j e^{-[U_{W(j)}(\xi) - F_{W(j)}]/k_BT}} \tag{23}$$

where n_i is the number of independent points used to construct the given biased distribution function. If $P(\xi)$ is known for all ξ the constants $F_{W(i)}$ can be estimated from

$$e^{-F_{W(i)}/k_BT} = \int P(\xi) e^{-U_{W(i)}(\xi)/k_BT}\, d\xi \tag{24}$$

Equations (23) and (24) form the basis of the WHAM procedure. An initial set of estimates of $F_{W(i)}$ (typically $F_{W(i)} = 0$) is used to obtain an estimate of $P(\xi)$ which is used to obtain improved estimates of $F_{W(i)}$. The iteration is continued to self-consistency. The WHAM procedure has proved very powerful. It simultaneously combines all data, it is robust, and is easily extended to multiple coordinates.[20,21] Nevertheless, to obtain reliable estimates of $F_{W(i)}$ the sampling along ξ must be continuous and sufficient for all possible ξ.

3 PRACTICAL CONSIDERATIONS

3.1 Enthalpy and Entropy

Why calculate the change in free energy rather than the change in internal energy and/or the change in entropy? The answer to this question comes by noting that the derivative in equation (11) and the difference in energy in equation (15) are dependent only on those interactions which change as a function of the coupling parameter. The difference in free energy is expressed only in terms of perturbed interactions, not in terms of the system as a whole. The same is not true for either the change in internal energy or the change in entropy. The change in internal energy between two states, A and B, of a system is given by

$$\Delta E_{BA} = E(\lambda_B) - E(\lambda_A) = \langle H(\lambda_B)\rangle_{\lambda_B} - \langle H(\lambda_A)\rangle_{\lambda_A} \tag{25}$$

That is, the difference in the average total energy of the two end states. The change in internal energy can be readily estimated in free energy calculations. However, because it is directly dependent on all interactions in the system and given by the difference of what are usually two very large numbers (with large fluctuations), the calculated change in internal energy is considered to be more than an order of magnitude less statistically reliable than the change in free energy. The same is true for the change in entropy. Using the thermodynamic integration formalism the change in entropy is given by[7]

$$\begin{aligned} \Delta S_{BA} &= S(\lambda_B) - S(\lambda_A) \\ &= [k_BT^2]^{-1} \int_{\lambda_A}^{\lambda_B} \left[\langle H(\lambda)\rangle_\lambda \left\langle \frac{\partial H(\lambda)}{\partial\lambda}\right\rangle_\lambda - \left\langle H(\lambda)\frac{\partial H(\lambda)}{\partial\lambda}\right\rangle_\lambda\right] d\lambda \end{aligned} \tag{26}$$

which is again dependent on the total system. An alternative expression based on the perturbation approach is available.[24] In summary, though it is possible to independently estimate the change in internal energy and/or the change in entropy

from a simulation, in practice these quantities are much less statistically reliable than the change in free energy.

3.2 Thermodynamic Perturbation versus Thermodynamic Integration

The thermodynamic integration formula (10–11) and the thermodynamic perturbation formula (15) are in principle equivalent. Both depend on the evaluation of an ensemble average. The perturbation formula is, however, often described as exact, whereas the integration formula is called approximate because of the numerical integration in equation (10). This is misleading. The perturbation formula is exact only in the limit of infinite sampling or perfectly overlapping ensembles for $H(\lambda)$ and $H(\lambda + \Delta\lambda)$. Either method can be used to determine a given change in free energy to any desired precision. What does differ is the convergence properties of the two approaches. Which method is most appropriate strongly depends on the problem.[16,25] Perturbation performs well if the ensembles of the initial and final states closely overlap. Otherwise, small increments in λ and long simulation times are required to avoid large systematic errors. Consider the case of a particle of mass, m, in a one-dimensional harmonic well. The potential energy, U, is given by

$$U = \tfrac{1}{2}K(x - x_0)^2 + C \tag{27}$$

where K is the harmonic force constant, x_0 the equilibrium position and C simply an offset. In this case the free energy, which may be expressed analytically as

$$F = -k_B T \ln[2\pi h^{-1} kT(m/K)^{1/2}] + C \tag{28}$$

is independent of x_0. No work is required to move the equilibrium position of the oscillator. For mutation **A** in Figure 1, $\Delta F = 0$. This mutation is analogous to changing a bond length in a vacuum. The average $\langle \partial U/\partial x_0 \rangle = 0$. Thus, using the thermodynamic integration formula, $\Delta F = 0$ for any change in x_0. Using the perturbation formula ΔF is always positive and dependent on Δx_0. ΔF will only approach zero, for finite sampling, as Δx_0 approaches zero. In contrast, estimating the ΔF associated with a change in the offset C (mutation **B** in Figure 1) using the perturbation formula is highly efficient. The ensembles for the initial and final states indicated by the dotted lines overlap exactly and ΔF will converge rapidly for any change in C. Using the thermodynamic integration formula, the derivative at intermediate C values may be required to evaluate the integral. This example also illustrates a potential danger in optimizing $\Delta\lambda$ or the window size in a perturbation calculation based on the calculated change in free energy.[26] The convergence of equation (15) does not depend on the difference in free energy, but on the degree of overlap between the ensembles.

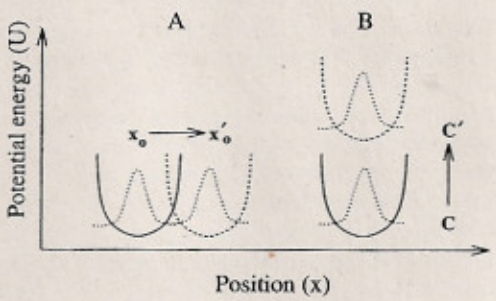

Figure 1 Two potential mutations of an isolated harmonic oscillator which illustrate the difference in the convergence properties of the perturbation and integration formula. **A** represents a shift in the position, x_0, of the oscillator and **B** represents a shift in the offset, **C**, of the potential energy. The parabolic curves correspond to the potential energy, $U(x)$ (equation 25) and the Gaussian curves correspond to the probability distribution of the particle

In summary, in the limit of infinite sampling the perturbation and integration formulae are equivalent. The convergence properties, however, differ markedly. If the ensembles closely overlap perturbation is most efficient. However, because the convergence of the ensemble average does not depend on the magnitude of the change in λ, the integration formula often offers the better opportunity to reduce and monitor errors in practice.[13]

3.3 Slow Growth versus Numerical Quadrature

The integral in (10) may be evaluated in one of two ways. (i) The coupling parameter, λ, may be made a function of time and slowly changed throughout a simulation. The integral is then approximated by a sum over each configuration. This procedure is commonly referred to as slow growth or single configuration thermodynamic integration. (ii) Separate ensemble averages may be determined at specific values of λ and the integral evaluated numerically (multi-configurational thermodynamic integration).[13,17,27] Using slow growth one configuration is sampled for each value of λ. The ensemble average in equation (11) is approximated by a set of configurations over a small range of λ. As λ is continuously changed throughout the simulation the system is never truly in equilibrium, but lags behind the changing Hamiltonian.[28,29] Excess work is done on the system and the free energy is systematically overestimated. Averaging the results for the forward and reverse mutation to correct for this overestimation is unreliable.[30] A minimum prerequisite in slow growth calculations is that the difference between the forward and reverse processes or hysteresis is small. The hysteresis indicates only the degree of reversibility, not that the system is in equilibrium nor that a representative ensemble has been sampled for each λ. If mutated much faster than it can respond, the system will remain trapped in a local state. The mutation will appear reversible and show a small hysteresis. However, if the length of the simulation is increased the apparent hysteresis will also increase.[29] Slow growth performs well in rapidly equilibrating systems where a single configuration approximates an equilibrium ensemble, e.g., a single ion in water or the simultaneous mutation of a large number of molecules. If the mutated molecule can adopt multiple configurations, the method converges slowly.

The alternative approach, to perform different simulations at discrete λ values and integrate numerically, allows effects due to equilibration and sampling to be largely separated.[17,27,30] A simulation is followed until the ensemble average $\langle \partial H/\partial\lambda \rangle_\lambda$ converges within the desired precision. To perform the integration it is assumed that the derivative is a slowly changing continuous function of λ. However, depending on how the Hamiltonians of the initial and final states are coupled, the change in free energy as a function of λ may be highly nonlinear or contain singularities when atoms are created or destroyed. Even when the same coupling scheme is used for

all interactions, the derivatives of different terms within the Hamiltonian may exhibit a different dependence on λ. For this reason, the convergence of the integral must be tested independently of the convergence of the derivatives to avoid systematic errors.[30] Higher-order derivatives of the free energy with respect to λ may improve the estimation of the integral but require longer simulation times to converge.[31]

3.4 Thermodynamic Cycles

Since the free energy is a state function, the difference in free energy between two states of a system is independent of the path used to go between them. The change in free energy for any cyclic mutation is zero. This has led to the concept of a thermodynamic cycle in which the change in free energy along one leg of a circular pathway is expressed in terms of the change in free energy along each of the other legs.[3] This is illustrated in Figure 2. The difference in the free energy of binding of the two ligands, X and Y, to a receptor P ($\Delta\Delta G_{YX}$) can be determined in one of two ways. Either the physical process of complexation $X + P \rightarrow X{:}P$ (ΔG_1) and $Y + P \rightarrow Y{:}P$ (ΔG_2) can be simulated (horizontal arrows) and the difference in binding energy determined as $\Delta\Delta G_{YX} = \Delta G_2 - \Delta G_1$, or the nonphysical mutations, $X \rightarrow Y$ free in solution (ΔG_3) and $X{:}P \rightarrow Y{:}P$ in the complex (ΔG_4), can be performed (vertical arrows) in which case $\Delta\Delta G_{YX} = \Delta G_4 - \Delta G_3$. The results are equivalent as $\Delta G_1 + \Delta G_4 - \Delta G_2 - \Delta G_3 = 0$.

Thermodynamic cycles are used for reasons of computational efficiency.[3,13,17] Simulating the process of complexation between a ligand and a protein with the associated rearrangement of the protein and a large number of solvent molecules is generally not practical. In contrast, the nonphysical mutation of X into Y (computer alchemy) involving the conversion of a small number of atoms from one type to another is straightforward. A thermodynamic cycle also has the advantage that systematic errors in the calculation may cancel. Systematic

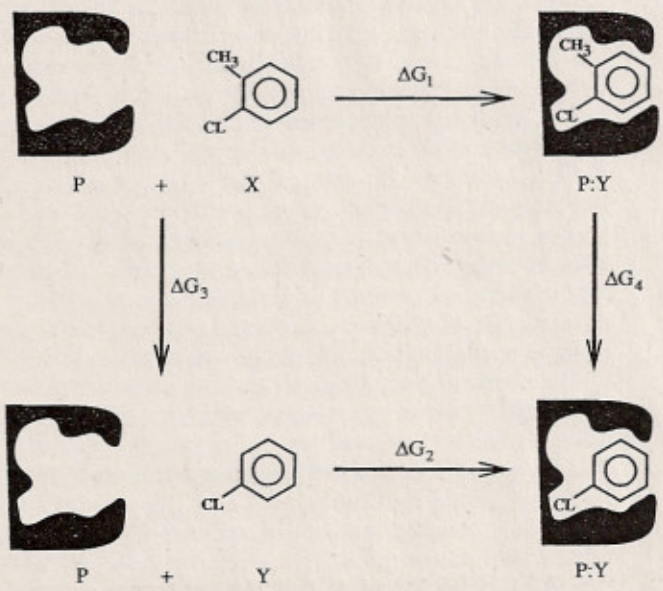

Figure 2 A thermodynamic cycle that would be used to determine the difference in binding free energy of the ligands X and Y to the receptor P

errors may arise from the failure to include quantum mechanical effects, limitations in the force field, the imposition of boundary conditions, cutoff radii, etc. For cancellation to occur the simulation conditions must be as similar as possible throughout the cycle.[13,32,33]

Within a thermodynamic cycle, terms which make no net contribution to the free energy will cancel and can effectively be ignored. For example, at constant temperature and in the absence of mass-metric tensor effects,[13] the kinetic energy contribution to the free energy associated with a change in the mass of a particle is a constant, the inclusion of which only introduces noise. Extreme caution is, however, required when neglecting components. The work required to create a charge during a simulation will depend on the dielectric constant of the medium. Long-range electrostatic contributions can only be ignored if the dielectric properties of the environments on both sides of the thermodynamic cycle are equivalent. This is not the case when one side corresponds to the low dielectric interior of a protein and the other to a high dielectric medium such as water. The influence of internal terms must also be considered. Frequently, internal contributions to the free energy are neglected. However, if the conformational freedom in the initial and final states is different, internal terms will make a net contribution to the overall free energy and cannot be ignored.[13,32,33]

3.5 Equilibration and Sampling

A primary source of error in free energy calculations is a failure to sample a representative (equilibrium) ensemble.[34–37] To sample the complete ensemble composed of all possible configurations is not possible. Free energy calculations aim not to determine the complete ensemble but to sample a representative ensemble of a specific (metastable) state, e.g., a ligand bound to a folded protein. As only a single or at most a small number of molecules may be included in a simulation, the ensemble average in equations (11) or (15) is replaced by a time average (MD) or an average over a set of sequentially generated configurations (MC). This means that unless the sampling time, τ_{sampling}, is much longer than the relaxation time of the system, the calculated free energy will be correlated in time and dependent on the starting configuration. For a single molecule equilibrium can also only be defined in terms of a time average. The starting conformation must not only be part of an equilibrium ensemble but also represent a common configuration. The equilibration and sampling time required at each λ value will depend on the properties of the specific system. It cannot be expressed in terms of MD simulation time or MC steps. Sampling can be considered sufficient only if the calculated free energy or the partial derivatives of the potential energy function with respect to λ no longer change with time. However, such convergence in itself is no guarantee that the system is in equilibrium (i.e., the free energy is at a minimum) or that a representative ensemble has been sampled. In the case of the perturbation formula, convergence of the free energy is also no guarantee that the configurations sampled in the reference state correspond to low energy configurations in the perturbed state.

3.6 Choice of Pathway

The λ dependence of the Hamiltonian defines the pathway from the initial to the final state. The choice of path does not

affect the change in free energy but strongly affects computational efficiency. A direct path is not necessarily the most efficient.[38] The most efficient path is one along which the relaxation time of the system with respect to the change in the Hamiltonian and the time required to sample a representative ensemble are both minimized. As only terms in the Hamiltonian that change as a function of λ contribute to the change in free energy, the number of such changes should be minimized. The unnecessary introduction of additional degrees of freedom should be avoided. In many cases involving the creation or deletion of a substituent atom only the van der Waals and Coulomb interactions need be mutated. If possible bond, angle and dihedral angle terms should be held constant. This avoids the additional work required to modify the associated force constants and the additional sampling required along these degrees of freedom. If the covalent terms are held constant, the end state will differ from the case when the force constants are reduced to zero. The calculated free energy will also differ. This difference will, however, cancel within the context of a thermodynamic cycle.[13,17]

3.7 Creation and Deletion of Atoms

The Lennard-Jones and Coulomb potential energy terms standardly used in atomic force fields contain a singularity in the energy and the force $(\partial U/\partial r)$ when the interaction distance between two atoms i and j, r_{ij}, is zero. For fully interacting atoms this singularity is never sampled due to the repulsive term in the Lennard-Jones interaction and it does not contribute to an ensemble average of the potential energy. The singularity in the potential energy function when $r_{ij} = 0$ means, however, that there is a singularity in the derivative of the potential energy function with respect to λ, when $r_{ij} = 0$ for atoms that are created or destroyed. If a linear coupling between the Hamiltonian of the initial and the final states is used, the singularity in the associated derivative may be sampled in the initial or the final state when the effective van der Waals radius of the mutated atom is zero. In this case the ensemble averages in equations (11) and (15) cannot be determined. In practice the sampling of the singularity is often avoided by creating or deleting an atom within the effective van der Waals radius of an atom to which it is bound.[26]

Alternatively, the dependence of the Hamiltonian on λ may be chosen so that the derivative of the potential energy function with respect to λ at the end states is zero or finite. For example, when using a 6–12 Lennard-Jones potential the effective diameter of the particle, σ, may be scaled at the same time as the well depth, ε.[39] This is equivalent to scaling the total interaction by a higher-order power of λ.[40,41] Using such an approach to remove the singularity in the derivative with respect to λ is applicable where single atoms are created or removed and the ensemble is generated using MC. If MD is used to generate the ensemble, linear or nonlinear coupling of the Hamiltonian will lead to numerical instabilities in the simulation as exposed atoms are created or destroyed. This is because the singularity in the force is unaffected. If a finite step size is used to integrate the equations of motion, then when the effective radius of the mutated atom is small compared to the length of flight of an atom per timestep, high energy regions of the potential, including $r = 0$, may be sampled.[41] To avoid such numerical problems a form of potential energy function that does not contain a singularity at $r = 0$ may be used.[41,42] Such numerical instabilities do not arise in MC simulations because the force is not used to propagate the system. Nevertheless, sampling problems may still arise if clusters of bonded atoms are created or destroyed simultaneously. Finally, sometimes the calculation is simply truncated at a given value of λ and the free energy extrapolated to the end states.[26,43] This is the least satisfactory of the possible approaches as the magnitude of the error that is made in a particular case is unknown.

3.8 Metric Tensor Corrections

If constraints are used, for example to maintain fixed bond lengths during a simulation, the Hamiltonian of the system is different from the unconstrained case. This in turn leads to a (slightly) different ensemble being generated. To correct for the effects of such constraints a so called mass-metric tensor correction can be applied.[13] In practice as metric tensor corrections are often small and/or cancel within a thermodynamic cycle, they are frequently ignored. However, if the constraint significantly alters the volume of phase space accessible to the system, metric tensor effects can make a major contribution to the free energy.

3.9 Constraint Contributions

Metric tensor corrections arise directly from the application of constraints and are a correction to the total free energy of the system. Sometimes, however, reference is made to an additional contribution or correction to the free energy associated with a change in a constrained bond length. This additional 'correction' is not due to the imposition of the constraints as such but due to the manner in which the thermodynamic integration or perturbation formula has been implemented. Using the coupling parameter approach all parts of the Hamiltonian that change as a function of λ contribute to the change in free energy. A constraint on the system is formally part of the Hamiltonian. Thus, if a bond length parameter is changed there will be a contribution from the bond to the change in free energy. This is true whether or not the bond is treated as a constraint or as a harmonic oscillator and irrespective of the method used to generate the ensemble.[44] In the case of a constraint this contribution to the free energy will include the difference in the metric tensor correction. If an analytical derivative of the Hamiltonian $(\partial H/\partial\lambda)$ is used in conjunction with the thermodynamic integration formula, the derivative of the potential along the direction of the bond must be determined. This is simply the force from the environment acting along the direction of the bond which is opposed by the constraint (see Section 2.3.2). This force can be readily calculated using the constraint resetting procedure SHAKE and has been referred to by some as a SHAKE contribution.[13,45] It has, however, nothing intrinsically to do with SHAKE. The same contribution is present irrespective of the method used to reset the constraints. If a numerical derivative is calculated or if the perturbation formula is used, $H(\lambda)$ and $H(\lambda + \Delta\lambda)$ are evaluated for a given ensemble. As the geometric parameters (e.g., bond lengths) form part of the Hamiltonian when constraints are used, the bond lengths must correspond to $H(\lambda)$ when evaluating $H(\lambda)$ and $H(\lambda + \Delta\lambda)$ when evaluating $H(\lambda + \Delta\lambda)$.[13] If only the interaction function parameters and not the geometric constraints are changed when

evaluating $H(\lambda + \Delta\lambda)$ the contribution to the free energy associated with the change in bond length is neglected and an additional correction is required.[46]

3.10 Free Energy Components

A physically meaningful separation of the free energy into specific components is, in general, not possible. The total free energy of the system can only be expressed in terms of a sum of unique components in so far as the total system can be separated into a series of independent subsystems (e.g., two groups widely separated in space). This is a direct consequence of the statistical mechanical definitions of free energy and entropy.[2,13] In a number of studies, however, analyses of a breakdown of free energy components based on the thermodynamic integration formula have been presented. It may be easily shown that if the Hamiltonian, H, can be expressed as a linear combination of terms, we may rewrite the integration formula (10–11) in the form

$$\begin{aligned}\Delta F_{BA} &= \int_{\lambda=\lambda_A}^{\lambda_B} \left\langle \frac{\partial H_1}{\partial \lambda} \right\rangle_\lambda d\lambda + \int_{\lambda=\lambda_A}^{\lambda_B} \left\langle \frac{\partial H_2}{\partial \lambda} \right\rangle_\lambda d\lambda \\ &\quad + \cdots + \int_{\lambda=\lambda_A}^{\lambda_B} \left\langle \frac{\partial H_n}{\partial \lambda} \right\rangle \\ &= \Delta F_{BA}(1) + \Delta F_{BA}(2) + \cdots + \Delta F_{BA}(n) \end{aligned} \quad (29)$$

where H_1 to H_n can refer to any separation of the Hamiltonian either in terms of force field parameters, residue–residue interactions or solvent–protein interactions.[43] The problem with such a breakdown is that the type of separation possible and the magnitudes of the calculated free energy components $\Delta F_{BA}(1)$ to $\Delta F_{BA}(n)$ depend on the λ-dependence of the Hamiltonian which defines the pathway taken to go from the initial to the final state. The choice of the λ-dependence of the Hamiltonian or pathway is not unique, hence the calculated free energy components are not unique.[17,47,48]

Nevertheless, some authors argue that there are 'natural' (nonphysical) pathways along which components can be defined.[49–52] Sharp and co-workers have shown that if all interactions are uniformly scaled to zero, the (entropic) contributions to the free energy arising from correlations between various force field terms partition evenly.[49,50] This does not mean that there are no correlations nor that they can be determined, only that the contributions partition in a defined manner as is true for any pathway. Another pathway frequently used for component analysis is to remove the electrostatic interactions of an atom with the rest of the system before removing the van der Waals interactions.[51] The electrostatic contribution may then be compared to continuum electrostatic models. The physical relevance of this is, however, uncertain as the van der Waals and electrostatic interactions are highly correlated.[48] It is not possible, for example, to consider the pure electrostatic contribution to the free energy of an ion in water. If the van der Waals interactions are removed before the electrostatic interactions, a singularity in the potential energy function is exposed and the contributions of the van der Waals and the electrostatic interactions to the free energy are undefined.

Perturbation always involves a linear combination of the initial and final states, therefore can the perturbation formula be used to define unique free energy components? Expanding equation (13) we obtain

$$\begin{aligned}\Delta F_{BA} = &+\langle H(\lambda_B) - H(\lambda_A)\rangle_{\lambda_A} - (2k_BT)^{-1}[\langle [H(\lambda_B) - H(\lambda_A)]^2\rangle_{\lambda_A} \\ &- \langle H(\lambda_B) - H(\lambda_A)\rangle + O[(k_BT)^{-2}] \end{aligned} \quad (30)$$

which can be further expanded in terms of specific force field terms and correlations between these terms.[49,50] Applying the equivalent expansion to equation (14) for the identical change in free energy we obtain

$$\begin{aligned}\Delta F_{BA} = &-\langle H(\lambda_A) - H(\lambda_B)\rangle_{\lambda_B} + (2k_BT)^{-1}[\langle [H(\lambda_A) - H(\lambda_B)]^2\rangle_{\lambda_B} \\ &- \langle H(\lambda_A) - H(\lambda_B)\rangle_{\lambda_B}^2] - O[(k_BT)^{-2}] \end{aligned} \quad (31)$$

Although equations (28) and (29) refer to the same change in free energy, depend on a linear combination of the initial and final states, and are based on the same series expansion, the individual terms are not equivalent,

$$\langle H(\lambda_A) - H(\lambda_B)\rangle_{\lambda_B} \not\equiv -\langle H(\lambda_B) - H(\lambda_A)\rangle_{\lambda_A} \quad (32)$$

The averages are over different ensembles. Component analysis based on the perturbation formula is not unique but dependent on the reference ensemble. It is possible to manipulate the reference ensemble and hence manipulate the components.[52]

To summarize, although the total free energy of a system is a state function, free energy components are not. Free energy components are essentially a reflection of the path (or the reference ensemble) that has been imposed and any analysis or physical interpretation of free energy components based on such calculations is subjective.

3.11 Error Estimation

To estimate the statistical error in free energy calculations is nontrivial. The reason is twofold. First, the sampling of phase space in MD or MC simulations is not random and ergodic but uneven and highly correlated in time, especially in macromolecular systems. Second, the magnitude of the fluctuations and correlation times for different terms which contribute to the change in free energy can vary dramatically. Using a given sampling period, the free energy may well appear converged simply because the simulation contains no information in regard to processes that occur on a longer time scale. To estimate statistical error in free energy calculations it is generally assumed that the sampling time is longer than the relevent correlation time and/or that the fluctuations in the free energy are normally distributed about the mean. Frequently neither is the case. Estimates of the statistical error in a free energy calculation thus tend to be dominated by terms with large fluctuations and short correlation times leading to the systematic underestimation of the true uncertainty. When a calculation is repeated using different starting structures, the difference in the change in free energy may be much greater than the statistical error in any given simulation. The degree of closure in a thermodynamic cycle is a simple measure of the *minimum* statistical (and/or systematic) error. If several molecules in different environments are to be compared the closure of thermodynamic cyles in each environment and between as many molecules as possible should be checked.

In perturbation calculations the change in free energy for the forward $(\lambda + \Delta\lambda)$ and reverse $(\lambda - \Delta\lambda)$ mutations can be determined and compared for each point along the pathway.

This is one measure of the uncertainty. However, as discussed in regard to particle insertion and deletion (see Section 2.3.1) the convergence properties of the forward and reverse mutations are not necessarily equivalent. In slow growth calculations the free energy as a function of λ is clearly correlated in time. In perturbation and multiconfigurational thermodynamic integration calculations the simulations at specific values of λ are formally independent. However, if intermediate states are generated sequentially or from a single starting configuration they will be correlated along the pathway and not represent independent samples. Different methods to estimate the statistical error in free energy calculations are discussed in Refs. 29, 34, 53, and 54.

3.12 The Force Field

Whether the calculated and experimental free energy differences agree depends critically on the model used to describe the interatomic interactions in the system, that is, the force field. The empirical force fields commonly used in macromolecular simulations, e.g., CHARMM,[55] AMBER,[56] and GROMOS,[57] have been primarily derived and tested using structural criteria (see ***AMBER: A Program for Simulation of Biological and Organic Molecules; CHARMM: The Energy Function and Its Parameterization;*** and ***GROMOS Force Field***). Few force fields are directly parameterized to reproduce relative free energies. This situation is changing. The OPLS force field (see ***OPLS Force Fields***), fitted to reproduce the heat of vaporization and density at a given temperature and pressure for simple liquids, is perhaps the best example of a force field parameterized against thermodynamic data.[58] The most recent version of the GROMOS force field (GROMOS96) is in part also parameterized to reproduce thermodynamic properties including free energies of hydration.[57] No force field is, however, correct in an absolute sense. All force fields, even *ab initio* quantum mechanical force fields, are models and a model can only predict those properties which it encompasses. If electronic polarization effects dominate the change in free energy, electronic polarization effects must explicitly (or implicitly) be part of the model. In free energy calculations the critical factor is the difference between the force fields of the initial and final states. Different parts of the force field must also be compatible. A model of liquid water parameterized to reproduce the heat of vaporization at a given density and pressure will yield the correct excess free energy of water as may a similarly parameterized model of methanol. This does not mean that, when combined, the models will necessarily reproduce the hydration free energy of methanol. The treatment of long-range interactions by the application of a cutoff, Ewald summation or a reaction field is also part of the overall force field. The sensitivity of different properties to specific force field parameters will vary. The excess free energy of the simple point charge (SPC) water model is comparatively insensitive to the treatment of long range interactions.[59] In contrast the free energy of hydration of an ion in SPC water is very sensitive to the treatment of long-range interactions.[60] To summarize, force field parameterization is a contentious issue for which there is no unique solution; however, if a force field is to be used to predict free energies, thermodynamic properties should be considered in the parameterization and testing.

4 APPLICATIONS

The concept of free energy is fundamental to our understanding of chemistry. Thus, when considering possible applications of free energy calculations the primary question is not which properties of a system can be analyzed but for which systems can the requirements of equilibrium and adequate sampling be realized. The most comprehensive listing of studies involving free energy calculations to date is the review of Kollman.[11] The following simply illustrates some classes of potential applications and provides a starting point for further reference.

4.1 Solvation

The most straightforward application of free energy calculations is the determination of solvation free energies. This includes the estimation of the excess free energy of simple liquids,[59] the estimation of hydration free energies,[58,60] and the estimation of partition coefficients.[61] The systems involved in such calculations are characterized by two features. First, the relaxation time of the environment to the perturbation, for a simple solvent such as water, methanol or chloroform, is short compared to the accessible simulation time. Second, mutations primarily involve changes to the solute–solvent nonbonded interactions. Adequate sampling can readily be achieved and the difference in free energy estimated to high precision (<1 kJ mol^{-1}). The results depend primarily on how the interactions in the system are modeled. Such calculations are increasingly used to verify the utility (or otherwise) of specific force fields,[58–62] to test methodology,[61,62] and to address questions as diverse as the distribution of water across a biological membrane[63] and the nature of the hydrophobic effect.[64]

4.2 Molecular Association

The greatest potential application of free energy calculations in chemistry is the estimation of the free energy of association between two compounds. For example, free energy calculations can be used to help understand the difference in binding affinity of different compounds in structure based drug design[11,15,65] (see ***Drug Design***). To calculate the difference in binding affinity between two compounds a thermodynamic cycle of the type shown in Figure 2 is used. The calculation of the difference in solvation free energy for the two compounds is straightforward. The sampling of the compound within the binding site is more problematic. If the receptor is a protein the environment of the ligand may relax slowly compared to the available simulation time. Normally, sampling all possible orientations of the ligand within the binding site is not possible. Demonstrating that the results are converged is thus a major challenge. Even in simple systems the accuracy of the results is limited as much by insufficient sampling as by the force field. Examples of the use of free energy calculations to study molecular association include ion-chelation,[66] inclusion complexes,[30] protein–ligand interactions,[51] and protein–DNA interactions[67] to list but a few.

4.3 Conformational Equilibria

The estimation of the difference in free energy associated with a change in conformation, or where the mutation results in a change in conformational equilibria, represents a class of

problems dominated by sampling considerations.[68–72] Even for a simple model system, such as liquid butane, the frequency of barrier crossings is such that long simulation times are required to adequately sample the *trans*, *gauche+*, and *gauche−* states.[38] If a given mutation alters the probability of the *trans* and *gauche* states being sampled, the complete equilibrium distribution must be sampled at every value of λ. This places severe constraints on the type of system that can be treated rigorously without resorting to special techniques to enhance sampling.[70–72]

4.4 Protein Stability

The estimation of the change in protein stability associated with the substitution of a given amino acid represents a class of problems where the use of free energy calculations is questionable.[33] To estimate a change in protein stability the difference in free energy of a given mutation performed in the folded state and the unfolded state must be determined. Many studies in the literature have addressed this question, and results in good agreement with experiment have been published.[43,73] Nevertheless, serious questions remain in regard to the validity of the model used for the unfolded state and whether adequate sampling can be achieved. To fulfil the requirements of the theory a representative ensemble of unfolded configurations must be sampled. However, no experimental structure for the unfolded state of a protein exists. This does not mean that such applications should never be attempted. In some cases the simplifying assumptions used may well be appropriate. However, when judging the validity of the results (or interpretation) from any free energy calculation it must be considered to what extent the assumptions on which the methodology is based are met. If the basic assumptions of the methodology are not met any apparent agreement with experiment is simply fortuitous.

5 RECENT TRENDS

5.1 Free Energy Extrapolation

In principle, the difference in free energy between a reference state and any other state of a system can be determined if the equilibrium fluctuations of the reference state are completely known. Essentially, the free energy of an alternative state can be extrapolated from the behavior of the system in the reference state. For many applications, most notably in structure based drug design, such an approach has many advantages. One is that a single simulation (ensemble) can be used to estimate free energy differences between multiple alternate states.

Methods to estimate the change in free energy associated with multiple perturbed states from a single, or a small number of simulations are based on either: (i) a series expansion of the free energy around a given reference state, (ii) an assumption with respect to the functional form of the free energy or, (iii) the application of the thermodynamic perturbation formula. The first approach is conceptually the most simple. The free energy is expanded as a function λ into a Taylor series around a given reference state, $\lambda = 0$, as follows,

$$\Delta F(\lambda) = F(\lambda) - F(0)$$
$$= F'|_{\lambda=0}\lambda + \frac{1}{2!}F''|_{\lambda=0}\lambda^2 + \frac{1}{3!}F'''|_{\lambda=0}\lambda^3 + \cdots \quad (33)$$

where the values of the higher-order derivatives F'', F''' ... at $\lambda = 0$ are computed as averages over the ensemble of the reference state. Smith and van Gunsteren, using a 1 ns simulation, showed that the change in free energy associated with substantial charge rearrangements ($\pm 0.25\ e$) of a model diatomic dipolar molecule in water could be predicted truncating the series beyond the second- or third-order terms.[31] Higher-order derivatives, however, converged slowly. A potential advantage of this approach is that the convergence of the series will depend on the λ dependence of the potential energy function.

An alternative to a series expansion is to assume a specific functional dependence for the free energy. Levy et al., using linear response theory, which essentially assumes that the fluctuations of the system have a Gaussian distribution, derived an expression for the change in electrostatic free energy which was equivalent to including only the first- and the second-order terms in the Taylor series.[31,74] The method of Jayaram and Beveridge (renormalization on the unit interval) also assumes a particular (normal) distribution for the fluctuation in the total energy of the system.[75] Estimating free energy differences based on linear response theory using simulations at both the initial and final states have also been proposed by a number of workers.[76]

If the series expansion in equation (33) converges for a linear combination of the initial and final states then the approach is formally equivalent to the application of the perturbation formula (13). To obtain a meaningful estimate of a free energy difference using the perturbation formula, low energy regions of the ensemble generated for the reference state must overlap with low energy regions of the alternative state. Such overlap does not occur when atoms are created or deleted and the mutation must normally be broken into a number of intermediate steps. The essential difficulty is sampling. Liu et al. have demonstrated, however, that a well chosen localized biasing potential (i.e., placing soft-core interaction sites at positions where atoms were to be created or deleted), permitted the accurate prediction of changes in the free energy of hydration for a series of substituted phenols associated with the creation and removal of multiple atoms and substantial charge rearrangement from a single 300 ps simulation.[77]

5.2 Coordinate Transformations to Enhance Sampling

High energy barriers in the potential energy surface can prevent rapid equilibration and the sampling of a representative ensemble. For example, a compound can become sterically trapped because two atoms are unable to pass through each other. In some cases coordinate transformations can be used to circumvent such barriers. One general approach is to extend the dimensionality of the system during the free energy calculation.[78] All atoms in the end states are constrained to be in three-dimensional (3D) Cartesian space but some or all atoms in intermediate states may move in four dimensions. This is nothing other than choosing a pathway that facilitates the relaxation of the system. The only requirement is that the work done on the system when changing the dimensionality of the system is included in the calculation. The use of a soft-core potential or the separated shifted scaling method when atoms are created or deleted is analogous to the inclusion of a fourth dimension. Increasing the dimensionality of the system may facilitate the sampling of alternate configurations but the volume of phase space accessible to the system will also increase.

Both factors must be considered when applying any method to enhance sampling in free energy calculations. In practice it is necessary to constrain the system very close to 3D Cartesian space.

Other forms of coordinate transformations can also be used to enhance sampling. Severance et al. demonstrated that an appropriate coordinate transformation applied to every configuration of a given ensemble can be used to dramatically extend the range for which the perturbation formula converges.[79] Note, however, that the same coordinate transformation must be applied to every configuration in the ensemble. Although this was not done in the original work, Severance et al. demonstrated an important general principle.[79]

5.3 Dynamics in λ Space

The introduction of the coupling parameter, λ, into the Hamiltonian effectively increases the degrees of freedom available to the system. Normally, λ is treated as a constraint. The system is forced to move along a defined pathway in λ space from one physically relevant state to another. An alternative is to treat the coupling parameter as a dynamic variable and perform MC or MD in λ, or parameter, space. This space will be multidimensional if separate coupling parameters, λ_i, are assigned to individual parts of the Hamiltonian. The set of coupling parameters, λ_i, may be treated as any other set of degrees of freedom in the system.

To perform MC in λ_i space a number of MC moves may be attempted in Cartesian space during which the λ_i are held constant. Then a number of moves in λ_i space may be attempted during which the Cartesian coordinates are held constant. The same acceptance criteria can be used for both types of move.[80] To perform MD in λ space the system must be described in terms of an extended Lagrangian. The λ_i parameters are assigned fictitious masses and the force on each λ_i is given by the partial derivative of the potential energy function with respect to a given λ_i. The normal equations of motion may then be used to generate an appropriate trajectory. Performing dynamics in λ space the system will spontaneously move toward regions of lower free energy. The method can, therefore, be used to search a range of parameter space in the same manner as a normal MD or MC simulation is used to search configurational space. Optimization methods such as simulated annealing may also be applied.[80] The free energy difference between two states, however, can no longer be determined by directly integrating along the path. Instead it must be determined from the probability distribution in λ space analogous to a PMF (see Section 2.3.2). One attraction of performing dynamics in λ space is that neither the exact pathway nor the end states need be defined. For example, all force field parameters describing the binding of a ligand to a receptor could in principle be treated as variables and optimized during a simulation to determine the tightest binding ligand. However, the introduction of a nonphysical degree of freedom into a system means the minimum free energy need not necessarily correspond to a physical state unless additional constraints are applied. Adding nonphysical degrees of freedom also increases the volume of phase space accessible to the system, compounding sampling problems. Another application of dynamics in λ space is to obtain a pathway between two states along which the free energy is always a minimum.[81] In some cases this may be an advantage but efficient sampling of configurational space and the lowest free energy state are not necessarily synonymous.[38]

6 CONCLUSIONS

Free energy perturbation calculations are very powerful. They provide one of the few practical means by which the difference in free energy between two states of a system can be calculated directly using MC and MD simulation techniques. The basic methodology is well established and potentially highly accurate. Free energy is, however, a statistical mechanical quantity. This means that the free energy is critically dependent on the sampling of phase space. A reliable estimate of the free energy difference between two states of a system will only be obtained if the underlying assumptions on which the methods described in this entry are met. There are two basic requirements. First, a representative equilibrium must be sampled. Second, the force field or model used to describe the system must be appropriate. It is against these two criteria that all free energy calculations should be judged.

7 RELATED ARTICLES

AMBER: A Program for Simulation of Biological and Organic Molecules; Carbohydrate Force Fields; CHARMM: The Energy Function and Its Parameterization; Drug Design; Force Fields: CFF; Free Energy Calculations: Methods and Applications; Free Energy Changes in Solution; Free Energy Simulations; GROMOS Force Field; Molecular Dynamics and Hybrid Monte Carlo in Systems with Multiple Time Scales and Long-range Forces: Reference System Propagator Algorithms; Molecular Dynamics: Techniques and Applications to Proteins; Monte Carlo Simulations for Liquids; OPLS Force Fields.

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Free Energy Simulations

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Abbreviations

MCTI = multi-configuration thermodynamic integration; PMF = potential of mean force; SCTI = single-configuration thermodynamic integration.

1 INTRODUCTION

Computer simulation has become a key scientific tool in the study of chemical and biochemical systems. Molecular modeling techniques are routinely being used in the study of a wide variety of chemical systems, such as proteins and DNA, and their interaction with potential pharmaceutical agents. The use of molecular simulations has become possible as a result of advances in theoretical and computational chemistry and the rapid development of cost-effective computing resources. Among the most popular tools in computer simulation studies of complex chemical systems are the thermodynamic cycle free energy methodologies.

The foundation of the methodological development of these free energy techniques lies in statistical mechanics, providing the connection between the detailed, microscopic description used in the computer simulation of the molecular system, and measurable macroscopic properties. The fundamental statistical mechanical quantity is the partition function, from which the thermodynamic quantities of interest are derived. These thermodynamic properties can be expressed in terms of statistical mechanical ensemble averages which are approximated by calculated ensemble or time averages from molecular simulations. Much of the recent development is in the design of computational techniques to overcome the difficulty of obtaining converged values for these averages, rather than in the derivation of the methodology itself. This difficulty lies in the inability of practical simulations to traverse the important regions of phase space within a reasonable amount of simulation time, and is commonly referred to as the sampling problem.

This article describes thermodynamic perturbation and thermodynamic integration. These methods form the basis of the most popular computational techniques for free energy difference evaluation with molecular simulations.

2 STATISTICAL MECHANICAL BACKGROUND

Consider a system of N particles with generalized coordinates $\mathbf{q}^N$ and conjugate momenta $\mathbf{p}^N$. The classical canonical partition function for the system at temperature T is given by[1,2]

$$Q = \frac{1}{h^{3N}N!}\int\int \exp\left(-\frac{\mathcal{H}(\mathbf{q}^N, \mathbf{p}^N)}{k_B T}\right) d\mathbf{p}^N d\mathbf{q}^N \qquad (1)$$

where h is Planck's constant, k_B is Boltzmann's constant, and $\mathcal{H}(\mathbf{q}^N, \mathbf{p}^N)$ is the classical Hamiltonian describing the interactions in the system in terms of coordinates and momenta of all particles. The factor $N!$ is only applicable if the N particles are indistinguishable.

Each point in phase space is characterized by a unique set of coordinates and momenta. The normalized phase space probability $\pi(\mathbf{q}^N, \mathbf{p}^N)$ is proportional to the Boltzmann factor and given by

$$\pi(\mathbf{q}^N, \mathbf{p}^N) = \frac{\exp[-\mathcal{H}(\mathbf{q}^N, \mathbf{p}^N)/k_B T]}{\int\int \exp[-\mathcal{H}(\mathbf{q}^N, \mathbf{p}^N)/k_B T]\, d\mathbf{q}^N d\mathbf{p}^N} \qquad (2)$$

For any property Ψ the expectation value can be written as

$$\mathcal{E}(\Psi(\mathbf{q}^N, \mathbf{p}^N)) = \int\int \Psi(\mathbf{q}^N, \mathbf{p}^N)\pi(\mathbf{q}^N, \mathbf{p}^N)\, d\mathbf{p}^N\, d\mathbf{q}^N \qquad (3)$$

The Hamiltonian of a conservative system can be separated into a kinetic energy contribution depending solely on the momenta and a potential energy contribution that depends only on the coordinates:

$$\mathcal{H}(\mathbf{q}^N, \mathbf{p}^N) = \mathcal{T}(\mathbf{p}^N) + \mathcal{V}(\mathbf{q}^N) \qquad (4)$$

Then the phase space probability can be integrated over the momenta to obtain the normalized coordinate space probability,

$$\pi(\mathbf{q}^N) = \int \pi(\mathbf{q}^N, \mathbf{p}^N)\, d\mathbf{p}^N = \frac{\exp[-\mathcal{V}(\mathbf{q}^N)/k_B T]}{\int \exp[-\mathcal{V}(\mathbf{q}^N) k_B T]\, d\mathbf{p}^N} \qquad (5)$$

which allows the expectation value of any property that depends on the coordinates only to be written as

$$\mathcal{E}(\Psi(\mathbf{q}^N)) = \int \Psi(\mathbf{q}^N)\pi(\mathbf{q}^N)\, d\mathbf{q}^N \qquad (6)$$