

Computer Simulation of Chemical and Biomolecular Systems

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Free Energy Simulations ^a

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INTRODUCTION.

Monte Carlo or molecular dynamics simulations involve the numerical determinations of the statistical thermodynamics and related structural, energetics and (in the case of MD) dynamical properties of an atomic or molecular assembly on a high speed digital computer. Applications to molecular systems range from the study of the motions of atoms or groups of atoms of a molecule or macromolecule under the influence of intramolecular energy functions to the exploration of the structure and energetics of condensed fluid phases such as liquid water and aqueous solutions based on intermolecular potentials. The quantities determined in a typical Monte Carlo or molecular dynamics simulation include the average or mean configurational energy (thermodynamic excess internal energy), various spatial distribution functions for equilibrium systems and time correlation functions for dynamical systems, along with detailed structural and energetic analyses thereof. Diverse problems in structural and reaction chemistry of molecules in solution, such as solvation potentials, solvent effects on conformational stability and the effect of solvent on chemical reaction kinetics and mechanism via activated complex theory also require a particular knowledge of the configurational free energy, which in principle follows directly from the statistical thermodynamic partition function for the system.

Considerations on free energy in molecular simulations take a distinctly different form for intramolecular and intermolecular degrees of freedom. For the intramolecular case, the problem involves vibrational and librational modes of motion on the intramolecular energy surface. We will discuss briefly at the end of this paper the harmonic and quasiharmonic approximation used to compute vibrational contributions to the free energy but restrict the focus herein to the intermolecular case, where the particles of the system undergo diffusional motion and a harmonic or quasiharmonic treatment breaks down. These considerations

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apply also in the case of a flexible molecule where conformational transitions are effectively an intramolecular “diffusional mode”.

Conventional Monte Carlo and molecular dynamics procedures for diffusional modes, although firmly grounded in Boltzmann statistical mechanics and dynamics, do not proceed via the direct determination of a partition function due to well-known difficulties in convergence. The Metropolis method used in Monte Carlo procedures is a Markov process designed specifically to avoid the partition function in the calculation of mean energy and related properties in a simulation. In molecular dynamics, the physical nature of the calculated particle trajectories serves this purpose equally as well. However, in the absence of a partition function, one is unable to compute the free energy of the system directly and thus under ordinary circumstances molecular simulations lack access to the fundamental index of thermodynamic stability of the system.

This article reviews the area of free energy simulation in the forms which are currently being pursued for systems of many molecules. Several new aspects of the methods discussed will be also presented. We begin with an elementary definition of the free energy problem as encountered for this case and discuss two procedures, umbrella sampling and the coupling parameter approach, which are quite useful in this area. Then the diverse individual approaches to free energy simulations are described, considering for each in turn the genesis, methodological details, advantages and disadvantages, and applications currently reported. We will emphasize applications to molecular liquids and solutions at the expense of the large body of work on simple fluids. These, however have been reviewed by Barker and Henderson¹ and Levesque and Weiss². The interested reader is also referred to reviews related to this subject by Valleau and Torrie³, Quirke⁴, Gubbins⁵ and Pohorille and Pratt⁶ which have appeared in recent years. A complete simulation of molecular liquids and solutions requires of course a consideration of both vibrational and diffusive modes of motion. Some free energy studies where both intramolecular and intermolecular aspects are involved are reviewed at the conclusion of this article.

BACKGROUND.

In computer simulations on molecular liquids and solutions, N molecules, typically of $O(10)$ - (10^3) , are configured in an elementary cell subject to periodic boundary conditions, for which various options (simple cubic, face-centered cubic, etc.) are available. The configurational energy EN^7 of individual complexions of the N -particle system are evaluated from analytical energy functions parametrized from experimental data, quantum-mechanical calculations or sometimes a combination of both, truncated at certain predefined limits for computational efficiency. All simulations involve generating large numbers of possible configurations of the N -particle system and forming estimates of ensemble averages such as mean energy. On digital computers circa 1985, it is practical to think in terms of sampling $O(10^6)$ configurations, or $O(10)$ - $O(10^2)$ picoseconds of atomic and molecular motions. The current generation of supercomputers and/or attached processors make an order of magnitude or so increase in sampling immediately feasible.

Important statistical thermodynamic quantities for a discussion of computer simulations are

a) the configurational partition function Z of the system,

$$Z_N = \int \dots \int \exp(-E_N/kT) dR^N \quad (1)$$

where k is Boltzmann's constant and T is the absolute temperature, V is the volume of the system, and the integration extends over all space dR^N of the N particle system;

b) the Boltzmann probability function $P(E_N)$ for a configuration corresponding to a particular E_N ,

$$P(E_N) = \exp(-E_N/kT)/Z_N \quad (2)$$

c) the average or mean energy expression

$$U = \int \dots \int E_N P(E_N) dR^N = \langle E_N \rangle \quad (3)$$

which corresponds to the excess or configurational thermodynamic internal energy of the system; and

d) the excess or configurational free energy

$$A = -kT \ln(Z_N/V^N), \quad (4)$$

the focus of interest in this article. Our discussion here is presented in terms of the constant volume (T, V, N) canonical ensemble, but the arguments can be extended to the microcanonical or isothermal/isobaric ensemble with no real difficulty. The grand canonical ensemble affords a particular alternative approach to free energy simulation considered briefly at the end of this article.

The essential problem involved in the determination of mean energy via simulation can be appreciated simply from Equations(3 and 4). The determination of mean energy via simulation involves essentially calculating the area under the curve $E_N P(E_N)$ or $E_N \exp(-E_N/kT)$ by generating various configurations of the system and calculating their respective energies and probabilities. Only a narrow range of E_N values result in significant values of the integrand since the region in which E_N and $\exp(-E_N/kT)$ are simultaneously large is relatively limited. A random selection of configurations in a simulation (a crude Monte Carlo method) is thus an inefficient approach to the numerical determination of mean energy since inordinate amount of time would be spent sampling configurations which make relatively insignificant contributions to the integrand of Equation(3).

This problem in Monte Carlo theory was taken up some years ago by Metropolis *et al.*⁸, who devised an importance sampling scheme which is now followed in essentially all Monte Carlo studies of fluids. The Metropolis method is a Markov walk through configuration space sampling complexions of the system with a frequency proportional to the

corresponding Boltzmann factor. In this realization, the evaluation of mean energy reduces to the simple summation

$$\sum_{j=1}^n E_N(R_j)/n \quad R_j \in \mathcal{M} \quad (5)$$

where the n configurations R_j are chosen by the Metropolis method (\mathcal{M}). Convergence studies indicate that the Metropolis method makes mean energy determinations feasible at presently accessible sampling rates. Other properties of the system computed in parallel with mean energy each found to have their own profile of convergence, and in general structural properties converge more rapidly than energetic properties and simple average quantities such as mean energy converge more rapidly than fluctuation properties such as heat capacity. Higher moments of the energy can be expected to converge even slower. Detailed examples of convergence profiles for liquid water simulations are available in recent papers from this Laboratory⁹⁻¹¹ and by Rao *et al.*¹² where these effects are evident. Generally, the mean energy of a pure liquid can be determined to a precision of ± 0.05 kcal/mol or better from a simulation of $O(10^6)$ configurations. However, other energetic quantities such as transfer energies of a solute molecule into solvent water are susceptible of $O(10)$ kcal/mol statistical noise engendered in finding small differences between large, relatively noisy numbers. Free energy simulations, as described later in this account, do not encounter this problem as severely.

A corresponding consideration of free energy follows from the ensemble average expression for the excess free energy,

$$A = kT \ln \langle \exp[+E_N/kT] \rangle \quad (6)$$

a completely equivalent statement of Equation(4). Expanding the exponential in powers of E_N and rearranging terms, it can be shown that

$$A = kT \ln \{ [1 + U/kT + (U/2kT)^2 + C'_v/(2k) + O(\langle E_N^3 \rangle / (kT)^3) + \dots] / V^N \}. \quad (7)$$

Thus the convergence problem in free energy is equivalent to problem of determining ensemble averages of the mean energy (first moment) and higher moments of the energy distribution mentioned above. The convergence difficulties in fluctuation properties and by implication higher moments of the energy distribution described above indicate that a significant increase in sampling would be required to achieve convergence in free energy relative to mean energy in a simulation.

A broader range of sampling could of course be accomplished by a crude Monte Carlo approach, but in practice configuration space for a system of even $O(10^2)$ molecules is so immense that convergence for the partition function integral cannot reasonably be expected. Metropolis sampling, concentrated in regions where $E_N \exp(-E_N/kT)$ is large, is optimal for internal energy but would not sample broadly enough to provide accurate estimates of the partition function and the free energy. Similar considerations apply in molecular dynamics simulations. Thus we are left with no viable means of the determination of free energy by the

usual numerical methods for molecular systems of interest from a mean energy simulation, and with the clear indication that an extent of sampling significantly beyond that suitable for mean energy determinations must be involved in free energy simulations.

In concluding this section, we note that an effective means of extending the range of sampling in a simulation, Monte Carlo or molecular dynamics, has been devised by Valleau, Patey and Torrie^{13,14}. The range of sampling in a simulation can be altered in a special form of importance sampling by running the Metropolis procedure or molecular dynamics with modified energy function

$$E'_N = E_N + E_W. \quad (8)$$

Here E_W is the modification of the energy that serves to extend the sampling. E_W takes various forms depending upon the particular application. However, introducing E_W carries the simulation to a non-Boltzmann regime i.e., sampling based on E'_N rather than E_N , and thus the ensemble averages produced would be inappropriate for the determination of statistical thermodynamic quantities. Valleau and coworkers demonstrated that appropriate Boltzmann weighted ensemble averages $\langle Q \rangle$ for any property Q can be extracted from a simulation carried out on the modified energy function E'_N by collecting the additional ensemble average $\langle \exp(E_W/kT) \rangle_W$ and forming the quotient

$$\langle Q \rangle = \langle Q \exp(E_W/kT) \rangle_W / \langle \exp(E_W/kT) \rangle_W \quad (9)$$

where the subscript W denotes an ensemble average based on the energy function E'_N . Eq.(9) can be verified by writing out explicitly the expectation values and recognizing that the $\exp(E_W/kT)$ from Eq.(9) cancels the $\exp(-E_W/kT)$ from the modified Boltzmann factor. This approach is widely known as “umbrella sampling” (US), and as discussed below finds wide applicability in free energy simulations.

One obvious limitation of the umbrella sampling procedure is as follows: the more the sampling is to be extended, the larger will be the range of E_W making the calculation of $\langle \exp(E_W/kT) \rangle_W$ prone to roundoff errors. In fact, if the variations are large, the computed average will be dominated by contributions from only a few configurations, a clearly undesirable effect. Therefore, a limit has to be set for the variations allowed in E_W which effectively puts a limit to the extension of sampling with this technique. That is, convergence of the E'_N -based ensemble averages still requires careful numerical attention.

The selection of appropriate E_W is generally a nontrivial matter since it essentially presupposes knowledge about the respective probabilities of configurations with different values of the parameters of interest. The more complex these parameters, the more difficult is the determination of an efficient E_W . This, however, suggest an iterative approach to the determination of an effective E_W . Paine and Scheraga¹⁵ have described an adaptive importance sampling technique applied to the conformational study of a free dipeptide molecule and a procedure of this type, called “Adaptive US” has recently been developed and tested on a conformational study of the alanine dipeptide in aqueous solution in this Laboratory¹⁶. Besides possible savings in computational effort, the procedure produces, at

least in principle, a uniform distribution of the parameters of interest and is self-checking by construction.

THE COUPLING PARAMETER APPROACH

In molecular systems we are frequently confronted not only with the determination of the absolute excess free energy A , but the free energy difference ΔA between two well-defined states. Here for states denoted 0 and 1, ΔA is given in terms of the ratio of the partition functions for the two states, Z_1 and Z_0 , as

$$\Delta A = A_1 - A_0 = -kT \ln(Z_1/Z_0) \quad (10)$$

A straightforward approach to the free energy difference ΔA would require independent determinations of Z_0 and Z_1 based on energy functions E_0 and E_1 , which individually are subject to all the numerical difficulties detailed in the preceding section. Several proposed solutions are based on a very useful construct, the ‘‘coupling parameter’’. Notice that here and in the following the subscript N is dropped for brevity. Let us assume that the potential E depends on a continuous parameter λ such that as λ is varied from 0 to 1, $E(\lambda)$ passes smoothly from E_0 to E_1 . A free energy function $A(\lambda)$ can be defined as

$$A(\lambda) = -kT \ln Z(\lambda), \quad (11)$$

and calculation of ΔA can be performed by integrating the derivative of $A(\lambda)$ along λ (thermodynamic integration), by designating intermediate states $A(\lambda_i)$ spaced closely enough and use the defining equation of the free energy to compute ΔA in a stepwise manner (direct method, sometimes called perturbation method) or actually developing $A(\lambda)$ in the $[0,1]$ interval from simulations where λ is variable (probability ratio method). The function $A(\lambda)$ is equivalent to the potential of mean force in the statistical thermodynamics of fluids.

In a modern sense the coupling parameter approach originates in the derivation of an important integral equation in liquid state theory by Kirkwood¹⁷, but the seeds of this idea can be traced to the work of de Donder on chemical affinity and the degree of advancement parameter for a chemical process¹⁸. The coupling parameter λ is a generalized extent parameter or analytical continuation and defines a physical or sometimes non-physical path between states 0 and 1. On a physical path, a knowledge of $A(\lambda)$ can be used to determine the free energy of activation ΔA^+ as well as ΔA . A non-physical path is admissible in the case where the quantity of interest is a state function like ΔA , which of course is independent of the path. There is a considerable freedom in the choice of the path and decisions on the selection of $E(\lambda)$ is usually made by combining physical and numerical requirements. Some applications of the coupling parameter approach of interest in current computer simulation studies involve topographical changes in the system, and of course we refer this to the molecular topography of the system. We consider here three broad classes of topographical transition coordinates: structural transition coordinates, simple and complex reaction coordinates, and creation/annihilation coordinates; c.f. FIGURE 1.

Type	$\mathcal{M} = 0$	$\mathcal{M} = 1$
A.1 Conformational transition coordinate		
A.2 Correlated conformational transition coordinate		
A.3 Structural transition coordinate		
B.1 Simple reaction coordinate		
B.2 Complex reaction coordinate		
C.1 Functional group (or residue) creation/annihilation coordinate		
C.2 Molecule creation annihilation coordinate		

FIGURE 1. Topographical transition coordinates.

Structural Transition Coordinates

On a structural transition coordinate, one three-dimensional form of a molecule or macromolecule is carried over into another three dimensional form.

a.) λ defines a “conformational transition coordinate”. The study of molecular conformation in solution requires the free energy of the system as a function of one or more internal coordinates of the system. One of the simplest cases of interest is the torsion angle $\phi/(C1-C2-C3-C4)$ in butane which can be written in terms of coupling parameter as

$$\phi = (1 - \lambda)\phi_G + \lambda\phi_T \quad (12)$$

where $\phi_T = 180^\circ$ and $\phi_G = 60^\circ$. Here λ can be considered a “conformational transition coordinate”, a special case of a structural transition coordinate. This can obviously be generalized to many dimensions.

b.) λ as a “correlated conformational transition coordinate”. Our recent studies of solvent effects on the conformation stability of the alanine dipeptide¹⁹ provide a two dimensional example of this utilization of the coupling parameter approach. In the system, the various conformations of interest differ in values of the Ramachandran torsion angles ψ and ϕ . We mapped the structural change involving the conformational coordinates ψ and ϕ onto a single λ by means of the equation

$$(\psi, \phi) = (1 - \lambda)(\psi_0, \phi_0) + \lambda(\psi_1, \phi_1) \quad (13)$$

where $\lambda=0$ selects the reference state (ψ_0, ϕ_0) and $\lambda=1$ selects the (ψ_1, ϕ_1) ; here λ is a “correlated conformational transition coordinate”. As discussed below, to access computationally tractable sampling procedures in computer simulations it will be frequently desirable to map changes in the structure involving many internal coordinates onto a single λ if possible. The coupling parameter here can be considered essentially a virtual bond coordinate of the sort used extensively in diverse biomolecular conformation problems.

c.) λ as a structural transition coordinate. This use of coupling parameter can be extended to structural changes in bond lengths and bond angles. Combinations thereof with conformational changes, mapped onto a single λ would produce a “correlated structural transition coordinate”. This can be useful for studies of helix-coil transition in polypeptides, protein denaturation, and structural interconversions in nucleic acids involving the A, B, C ... Z forms.

Reaction Coordinates

A reaction coordinate is a coordinate upon which the evolution of a chemical reaction can be described.

a.) λ as a simple reaction coordinate. Let the simulation be carried out on a segment of coordinate R beginning at R_0 and R_1 . Then for any point R between R_0 and R_1

$$R = (1 - \lambda)R_0 + \lambda R_1, \quad (14)$$

defining the coupling parameter such that for $\lambda=0$, $R = R_0$ and for $\lambda=1$, $R = R_1$. This is convenient for describing a simple association process in biological systems such as hydrophobic bonding, ion pairing and hydrogen bonding and can be easily generalized to many dimensions if necessary.

b.) λ as a complex reaction coordinate. In a complex reaction system such as a proton transfer studied by Warshel^{20–22} or the reaction of CH_3Cl and Cl^- treated recently by Jorgensen and coworkers^{23,24}, distinct reactant and product species are involved. The use of the coupling parameter in this case is a straightforward generalization of that described above and coincides fully with the definition of reaction coordinate in the transition state theory of chemical reactions²⁵. Here as in the case of a structural transition it will be useful to map concerted changes onto a single λ and work with a “correlated complex reaction coordinate”.

Creation/Annihilation Coordinates

A creation/annihilation coordinate carries the molecular topography of the system smoothly from one structural entity to another. Others in the field refer to this as “Hamiltonian warping”²⁶ or “mutation”²⁷.

a.) λ as a functional group, residue or subunit creation annihilation coordinate. Here the coupling parameter can be used to incorporate one well defined moiety in the system while simultaneously removing another. This type of creation/annihilation coordinate could be used to pass from one derivative to another in studies of the relative thermodynamics of a homologous series of molecules and is of potential use in the study of biological activity of related sets of molecules via simulation. Possible applications are found in enzyme-inhibitor, drug-receptor and in fact many other types of problems in biological systems. Recent works by McCammon, Jorgensen and coworkers are in this category^{27–29}.

b.) λ as a molecule creation/annihilation coordinate. Here we distinguish the case where λ removes an entire molecule from the system and creates another in its place. Kirkwood’s initial use of the coupling parameter can be considered a special case of this category in which only the creation branch is active. A creation/annihilation coordinate can be useful for studying the thermodynamics of hydration of a series of amino acids or nucleotide bases for example, but actually diverse applications are possible. There are in fact interesting topographical transition coordinates to define in this class, individually as well as in combination with the other forms defined above. Considering the coupling parameter approach in this generalized form admits considerable imagination into the design of computer simulation studies on chemical and biological systems.

THERMODYNAMIC INTEGRATION

Applying a basic mathematical identity to the free energy function $A(\lambda)$ gives the following (exact) equation:

$$\Delta A = \int_0^1 (\partial A(\lambda)/\partial \lambda) d\lambda. \quad (15)$$

Substituting Equation(1) into Equation (10) gives

$$\partial A(\lambda)/\partial\lambda = \langle\partial E(\lambda)/\partial\lambda\rangle_\lambda \quad (16)$$

where the subscript λ implies a Boltzmann average based on the function $E(\lambda)$. The direct numerical evaluation of Equation (15), integrating between initial and final states using some thermodynamic relationship, is called “thermodynamic integration”. The simplest application of the coupling parameter approach with λ identified with the volume or with the inverse temperature of the system, lead to the virial expression for pressure and the van’t Hoff equation, respectively, textbook cases in physical chemistry. The integration variable however need not be restricted to thermodynamic quantities, and thus Equation (15) can be used to determine the free energy difference between two states which differ in many conceivable ways, as long as the energy is a smooth function of λ ; however, pathways crossing phase transitions have discontinuities that will be problematic.

Linear TI

An important simple particular case is encountered when $E(\lambda)$ is linear in λ :

$$E(\lambda) = (1 - \lambda)E_0 + \lambda E_1 \quad (17)$$

If the reference state is an ideal gas or otherwise defines the zero of a configurational energy scale,

$$E(\lambda) = \lambda E_1 \quad (18)$$

and

$$\Delta A = \int_0^1 U(\lambda)d\lambda \quad (19)$$

where $U(\lambda)$ is a mean energy-like quantity developed as a function the coupling parameter, e.g., $\langle E_1 \rangle_\lambda$. Implementation of Equation (19) in simulations is quite simple. A series of simulations corresponding to successive discrete values of λ are carried out, giving $U(\lambda_i)$ for $i = 1, 2, \dots$. The final integration over λ is carried out by an additional numerical procedure. When E_0 is non-zero, Equation (18) becomes

$$E(\lambda) = E_0 + \lambda(E_1 - E_0) = E_0 + \lambda\Delta E \quad (20)$$

and the free energy difference is then

$$\Delta A = \int_0^1 \langle\Delta E\rangle_\lambda d\lambda \quad (21)$$

The computational procedure, a succession of simulations for discrete values of λ , is essentially similar to that described above.

An important advantage of linear TI is that it only involves computing ensemble averages of energies, not their derivatives, and these are the fastest converging quantities. Also, since the energy calculations are needed for the regular computer simulation runs, the

implementation of linear TI into existing programs is a rather simple task. As an additional advantage, it is very easy to demonstrate, that for the case of linear λ -dependence, the integrand in Equation(15) is a monotonic function of λ ³⁰. Differentiation gives

$$\frac{\partial}{\partial \lambda} \left\langle \frac{\partial}{\partial \lambda} E(\lambda) \right\rangle_{\lambda} = - \left[\left\langle \left(\frac{\partial}{\partial \lambda} E(\lambda) \right)^2 \right\rangle_{\lambda} - \left\langle \frac{\partial}{\partial \lambda} E(\lambda) \right\rangle_{\lambda}^2 \right] + \left\langle \frac{\partial^2}{\partial \lambda^2} E(\lambda) \right\rangle_{\lambda}. \quad (22)$$

Obviously, the term containing the second derivative is zero for linear $E(\lambda)$ and the remaining terms in the bracket form a fluctuation expression that is always nonnegative. This result ensures that the interpolation implicit in any numerical quadrature should be reliable for linear TI.

Particular care must be exercised, however, when a creation/annihilation coordinate is involved where the integrand $U(\lambda)$ or $\Delta E(\lambda)$ may diverge at $\lambda=0$ and/or $\lambda=1$. This divergence occurs for example when a particle is coupled to the system at a location where no particle previously existed. In this event, at $\lambda=0$ the just decoupled particle becomes invisible to the generation of new configurations and therefore the rest of the system is free to overlap with it, producing arbitrarily large E_1 or ΔE values. Mruzik *et al.* remarked³¹ that a particle interacting with a system through an r^{-12} potential will give $\langle E_1 \rangle_{\lambda} \propto \lambda^{-3/4}$ for small values of λ . In general, it can be shown by standard technique that for a particle interacting with a potential of r^{-n} in d dimensions, the limiting behaviour of $\langle E_1 \rangle_{\lambda}$ for $\lambda \rightarrow 0$ is proportional to $\lambda^{(d/n)-1}$. This, unfortunately, makes the integral in Equation(19) or (21) an improper one for $d < n$. As a result, direct application of numerical integration may lead to significant error, since no matter how small the smallest λ_0 value used in the numerical quadrature, the contribution from the $[0, \lambda_0]$ interval will depend on the limiting behaviour of $\langle E_1 \rangle_{\lambda}$ about which the numerical quadrature has no information at all. A possible procedure is then to find a λ_0 such that for $\lambda < \lambda_0$ the limiting behaviour is observed and use numerical integration only for the range $[\lambda_0, 1]$. The contribution from the interval $[0, \lambda_0]$ should be obtained by exploiting the limiting behaviour of $\langle E_1 \rangle_{\lambda}$. Writing $\langle E_1 \rangle_{\lambda} = \lambda_0^{(d/n)-1}$, we obtain

$$\int_0^{\lambda_0} \langle E_1 \rangle_{\lambda} d\lambda = (n/d) \lambda_0 \langle E_1 \rangle_{\lambda}. \quad (23)$$

A helpful fact in this respect, verifiable by direct substitution, is that $E(\lambda)$ defined in terms of E_0 and E_1 is equivalent to $E(\lambda')$ defined in terms of $E'_0 \equiv E(\lambda_0)$ and $E'_1 \equiv E(\lambda_1)$ where

$$\lambda' = (\lambda - \lambda_0) / (\lambda_1 - \lambda_0). \quad (24)$$

Equation(24) allows calculation of ΔA with different choices of λ_0 without having to obtain new ensemble averages.

A different approach, also based on the recognition of the limiting behaviour of $\langle E_1 \rangle_{\lambda}$, was proposed by Mruzik *et al.*³¹. The integration was carried out by introducing a new variable, λ^m :

$$\Delta A = (1/m) \int_0^{0.5^m} \lambda^{1-m} \langle E_1 \rangle_{\lambda} d(\lambda^m), \quad (25)$$

where the exponent m is chosen so that the λ^{1-m} factor in the integrand makes it finite for all λ . They used $m=0.25$, appropriate for potentials with an r^{-12} repulsive core. While this transformation makes the integrand finite everywhere, it also puts heavy emphasis on the small λ range. 0.231, This suggest that the transformation (25) be carried out only in a small interval near the singularity. The λ^m transformation has the further advantage that by choosing different m values a consistency check is obtained on ΔA . For simultaneous creation and annihilation it is also possible that a similar discontinuity exists at $\lambda=1$. This case should be handled analogously, either by obtaining the basic integral in the range $[\lambda_0,1]$ from the limiting behaviour of the integrand, as in Equation(23) or by transforming the integration variable as Mruzik *et al.* proposed. In this case, the integration has to be broken into two parts and different variables have to be introduced:

$$\int_0^1 \langle E_1 - E_0 \rangle_\lambda = (1/m) \int_0^{0.5^m} d(\lambda^m) \lambda^{m-1} \langle E_1 - E_0 \rangle_\lambda + (1/m) \int_{0.5^m}^1 d((1-\lambda)^m) (1-\lambda)^{1-m} \langle E_1 - E_0 \rangle_\lambda \quad (26)$$

Andersen *et al.*³² and Wilson *et al.*³³ in recent research eliminated the singularity problem by truncating the repulsive part of the potential above a few kT , arguing that the strongly repulsive part of the potential is never sampled by the system anyway. However, as demonstrated earlier, the calculation of the free energy requires sampling of a larger than usual region of the configuration space and thus it is not immediately clear that the error caused by the truncation is negligible.

Calculations based essentially on Equation(15) for simple liquids have been reviewed by Barker and Henderson¹. Mruzik, Abraham, Schreier and Pound reported a quite early application of this approach to ion hydration energies³¹. The free energy of liquid water was computed using this method by Mezei, Swaminathan and Beveridge³⁴ by integrating between the liquid and an ideal fluid at liquid density on a transcritical tieline. Frischleder computed the free energy of solvation for the dimethyl phosphate anion³⁵. Mezei computed the free energy differences between soft spheres and the MCY water and between MCY and ST2 as well as between MCY and SPC waters²⁶. Subsequently the free energy difference between the ST2 and SPC waters was determined directly and was found to agree with the indirectly calculated value within the stated error limits³⁶. Swope, Andersen, Berens and Wilson described related procedures for the calculation of formation constants for water clusters³⁷. Berens, Makay, White and Wilson described the incorporation of this approach in a molecular dynamics study of liquid water³⁸. Postma³⁹ calculated the free energy of cavity formation in liquid water and obtained the integrand in a molecular dynamics calculation where λ was “forced” to grow from 0 to 1 during the simulation.

Non-linear TI

Several possible pathways between two states involve an $E(\lambda)$ such that the λ dependence of when $E(\lambda)$ is non-linear. In these cases TI becomes more complex, since the

required derivative will be a function of λ . A simple example of non-linear TI is the thermodynamic pressure integration mentioned before where the volume of the system is scaled up to infinity. Here we present the formalism for some other cases, and discuss their possible utility. A general description of altering a molecule in the course of the λ -integration can be obtained with the following energy function:

$$\begin{aligned} E(\lambda) &= E(\mathbf{R}(\lambda)), \\ \mathbf{R}(\lambda) &= \lambda\mathbf{R}_1 + (1 - \lambda)\mathbf{R}_0. \end{aligned} \tag{27}$$

Here \mathbf{R} represents all the parameters of the energy function that change. For this choice, we have for the basic integrand:

$$\frac{\partial}{\partial\lambda} E(\lambda) = \sum_{\mathbf{k}} \frac{\partial E}{\partial R_{\mathbf{k}}} (R_{1,\mathbf{k}} - R_{0,\mathbf{k}}) \tag{28}$$

Implementation of Equation(28) into existing programs is more complicated than the implementation of linear TI, but should not lead to significant increase in computational expense for programs that calculate atomic forces anyway (like molecular dynamics or force bias Monte Carlo or variations thereof) since in general most terms going into the R -derivatives are already computed as a partial result.

Equation(28) can describe a conformational transition of a molecule or the change of a molecule into a new one. In the first case, \mathbf{R} describes the atomic coordinates of the molecule to be changed as well as the potential parameters that undergo change during the switch from system 0 to system 1. When applied to the special case of system 1 being an ideal gas particle, a new molecule can be created without the singularity problem⁴⁰. A possible advantage of nonlinear TI instead of linear TI lies in the fact that the singularity problem is avoided. Also, there is no need to calculate two energy functions as in ETI. However, the configurational average to be calculated is not the energy itself, but its derivative, therefore convergence is likely to be more expensive to achieve and the monotonicity of the integrand is lost.

The non-linear TI method can be used to change the simulation unit cell size. Conformational transition of large solutes may require a change in the shape of the simulation box, particularly when the periodicity of the solute is built into the periodicity of the solvent system. Changing the unit cell affects directly the energy function since the position of the periodic images will change. Let \mathbf{L} describe the three lengths of the rectangular unit cell. The transition from \mathbf{L}_0 to \mathbf{L}_1 can be described as

$$\begin{aligned} E(\lambda) &= E(\mathbf{L}(\lambda)), \\ \mathbf{L}(\lambda) &= \lambda\mathbf{L}_1 + (1 - \lambda)\mathbf{L}_0. \end{aligned} \tag{29}$$

The derivative with respect to λ is given (for pairwise additive potentials) as

$$\frac{\partial}{\partial\lambda} E(\lambda) = \sum_{i < j}^N \frac{\partial}{\partial\lambda} E_{ij}(\lambda) \tag{30}$$

$$\frac{\partial}{\partial \lambda} E_{ij}(\lambda) = \sum_{k=1}^3 \frac{\partial}{\partial L_k} (L_{1,k} - L_{0,k}) \quad (31)$$

$$\frac{\partial}{\partial L_k} E_{ij}(\lambda) = \frac{\partial}{\partial r_{ij,k}} E_{ij}(\lambda) \frac{\partial}{\partial L_k} r_{ij,k} \quad (32)$$

$$\frac{\partial}{\partial L_k} r_{ij,k} = r_{ij,k} \delta(|L_k| - |r_{ij,k}|) + \left\{ \begin{array}{l} 1 \text{ if } r_{ij} = r_{ij}^0 + L_k \\ 0 \text{ if } r_{ij} = r_{ij}^0 \\ -1 \text{ if } r_{ij} = r_{ij}^0 - L_k \end{array} \right\} \quad (33)$$

where r_{ij}^0 is the difference between the position vector of i and j and r_{ij} is the difference translated to the nearest image. The first term is a consequence of the possible discontinuity in $E_{ij}(\lambda)$ at $|r_{ij,k}| = L_k$, that arise when the potential cutoff is not less than the simulation cell's inscribed sphere radius. However, its contribution to the TI integrand is zero since the sign of $r_{ij,k}$ is independent of $\partial/\partial r_{ij,k} E(\lambda)$. Therefore, it can be dropped from further considerations.

The implementation of Equations(30-33) requires similar amount of programming effort as that of Equation(28) but again will not increase the actual computational expenses significantly for gradient bias calculations since all terms in Equations(30- 33) are either simple to compute or are already computed during the force calculation.

An other possible use of non-linear TI comes in the change in the “rate” of coupling during the λ -integration. A simple modification of the linear coupling involves introducing the k -th power of the coupling parameter which can be done in two ways:

$$E(\lambda) = [(1 - \lambda^k)E_0 + \lambda^k E_1] \quad (34)$$

or

$$E(\lambda) = [(1 - \lambda)^k E_0 + \lambda^k E_1]. \quad (35)$$

The derivative required is obtained simply as

$$\frac{\partial}{\partial \lambda} E(\lambda) = k\lambda^{k-1}(E_1 - E_0) \quad (36)$$

or

$$\frac{\partial}{\partial \lambda} E(\lambda) = k(1 - \lambda)^{k-1} E_0 + k\lambda^{k-1} E_1, \quad (37)$$

respectively. For creation/annihilation coupling, both methods will produce an integrand with limiting behaviour $\lambda^{(kd/n)-1}$ at $\lambda=0$. For the limiting behaviour at $\lambda=1$, important when creation and annihilation is done simultaneously, the first gives the same as the linear TI, $(1 - \lambda)^{(d/n)-1}$, and the second version gives the same as the first version at $\lambda=0$: $(1 - \lambda)^{(d/n)-1}$. Therefore, for $k \geq n/d$ the singularity is avoided while the simplicity of the linear TI is maintained. Actually, the first version is a reformulation the integral transform suggested by Mruzik *et al.*, while the second version can be considered its generalization. The

monotonicity of the integrand is, of course maintained for the first version since it is related to the linear TI by a change of variables via a monotonous function. It is not clear, however, if the same is true for the second version and further studies are required at this point. There is an additional point of interest for the second version when applied to changing one large solute into an other large but rather different one: It can be expected that in the region $\lambda=0.5$ the systems pressure will be rather high, since the presence of both solutes is felt by the solvents. This large pressure may slow down convergence. Since $\lambda^k + (1 - \lambda)^k < 1$ for $k > 1$ in the (0,1) interval, the coupling above would alleviate this problem as well.

Independent expression of the entropy in TI

Generally, the entropy change is calculated from ΔA and the internal energy difference $\langle E_1 \rangle_1 - \langle E_0 \rangle_0$. However, it can also be obtained from a TI calculation directly as follows. By taking the derivative of $\langle E(\lambda) \rangle_\lambda$, expressed by Equation(20), we obtain

$$\left\langle \frac{\partial E(\lambda)}{\partial \lambda} \right\rangle_\lambda - \frac{\partial \langle E(\lambda) \rangle_\lambda}{\partial \lambda} = \left[\left\langle E(\lambda) \frac{\partial E(\lambda)}{\partial \lambda} \right\rangle_\lambda - \langle E(\lambda) \rangle_\lambda \left\langle \frac{\partial E(\lambda)}{\partial \lambda} \right\rangle_\lambda \right] / kT \quad (38)$$

Integrating Equation(38) from 0 to 1 and using Equation(15) gives

$$\Delta A - (\langle E_1 \rangle - \langle E_0 \rangle) = -T \Delta S = \int_0^1 \left[\left\langle E(\lambda) \frac{\partial E(\lambda)}{\partial \lambda} \right\rangle_\lambda - \langle E(\lambda) \rangle_\lambda \left\langle \frac{\partial E(\lambda)}{\partial \lambda} \right\rangle_\lambda \right] d\lambda / kT \quad (39)$$

The ensemble averages in Equation(39) are either already computed during the calculation of ΔA or are trivial to obtain. Notice also that if the internal energy difference is calculated from independent (TVN) ensemble calculations, Equation(39) offers a consistency check.

PERTURBATION METHOD

An alternative expression for ΔA can be obtained by inserting unity into Z_1 of Equation(10) in the form $\exp(E_0/kT) \exp(-E_0/kT)$ leading directly to the equation

$$\Delta A = -kT \ln \langle \exp[-(E_1 - E_0)/kT] \rangle_0. \quad (40)$$

Reversing the role of systems 0 and 1 we obtain the mirror expression of Equation(40):

$$\Delta A = kT \ln \langle \exp[-(E_1 - E_0)/kT] \rangle_1. \quad (41)$$

Use of these equations have been also referred to as perturbation method (PM) since E_1 and E_0 differ by a small ‘‘perturbation’’ in successful application. Bennett⁴¹ recognized that Eqs.(40,41) are equivalent to an infinite order perturbation expansion, that is, they are exact. Therefore, they do not correspond to a ‘‘perturbation theory’’ in the usual sense of the word.

The methodology of the PM involves essentially a simulation carried out by Metropolis - Monte Carlo or molecular dynamics procedures based on the energy function E_0 (or E_1), in

which E_1 (or E_0) is also computed at each step and the average of the exponential quantity of Equations (40) and (41) is formed. Successful numerical calculation of these ensemble averages via simulations requires that states 0 and 1 be not too dissimilar. In the event of difficulties with the direct application of Equations(40) and (41), it is possible to use the coupling parameter approach to define a numerically viable path involving intermediate states between states 0 and 1 and compute the free energy difference as

$$\Delta A = \sum_i \Delta A_i \quad (42)$$

where

$$\Delta A_i = \ln \langle \exp[-(E_{i+1} - E_i)/kT] \rangle_i. \quad (43)$$

Here the interval between successive states can in principle be maintained small enough that the similarity condition is always sufficiently satisfied and the ensemble average can be successfully determined; of course if the states were too different the number of intermediate steps would become prohibitively large.

The two alternative expressions for ΔA , Equations(40) and (41), allow the calculations of ΔA_i and ΔA_{i+1} in the same simulation step. Interestingly, performing a simulation with $E(\lambda_i)$ and computing ΔA_i and ΔA_{i-1} from Equations(40) and (41) is exactly equivalent to an umbrella sampling calculation between $A(\lambda_{i-1})$ and $A(\lambda_{i+1})$ using $E_W = E(\lambda_i) - E(\lambda_{i-1})$ as the non-Boltzmann bias. The combined use of Equations(40) and (41) was actually called half umbrella sampling by Scott and Lee⁴². The recognition of this fact, however, also implies that the general umbrella sampling may be superior to half umbrella sampling, in particular if an efficient way of determining the non-Boltzmann bias can be found.

Equations(40) and (41) can not only used to effect computational savings but also for a consistency check as an alternative. This check is not too strong, however, since if the two states involved in ΔA_i are too distant the inadequacy of both Equation(40) and (41) is of the same degree and the true error may not show up. As has been remarked earlier³⁰, a stonger test for the adequacy of sampling can be obtained by computing in the $(i - 1)$ th run

$$\langle E_i \rangle_\lambda = \langle E_i \exp[(E_i - E_{i-1})/kT] \rangle_{\lambda_{i-1}} / \langle \exp[(E_i - E_{i-1})/kT] \rangle_{\lambda_{i-1}} \quad (44)$$

and comparing it with the value computed in the i th run directly. An additional point is that the PM is particularly advantageous when the dependence of $E(\lambda)$ on λ is complex since it does not require derivatives of $E(\lambda)$. Care must be taken, however, to make sure that the range of ΔE is limited to a few kT since otherwise the sum of their exponential will be dominated by a few terms, a clearly undesirable effect.

The PM was used very early by Dashevsky *et al.*⁴³ to compute ΔA between liquid water and an ideal gas in a single step. Owicki and Scheraga pointed out in an argument similar to that given in the BACKGROUND section based on Equations(6,7) that $\langle \exp(+E_N/kT) \rangle$ can not be expected to converge in a mean energy calculation⁴⁴. Miyazaki, Barker and

Pound used this technique to calculate the surface tension of the Lennard-Jones liquid by separating two slabs of liquid in a stepwise manner⁴⁵. Torrie and Valleau introduced US to enhance the efficiency of Equations(40) and (41) in calculating ΔA between soft spheres and the Lennard Jones fluid and between Lennard-Jones fluid at various temperature¹⁴. Owicki and Scheraga performed calculations on the probability of finding a cavity in liquid water⁴⁶. Scott and Lee calculated the surface tension of the MCY water⁴² by combining US with the technique of Miyazaki *et al.*⁴⁵. The difficulty of obtaining efficiently the non-Boltzmann bias was recognized and the half umbrella sampling was proposed. Nakanishi and coworkers⁴⁷ computed the free energy of hydration of a methane molecule into liquid water in one step. US was used and the exponential of the E_W function they used varied between 1 and 1056. Numerical problems may arise when the range of the weighting function is so large. Mezei attempted to calculate the ΔA between the ST2 and MCY liquids using US but repeated attempts at the determination of an effective EW function failed to give consistent results even when two intermediate states were used, pointing to a serious limitation of the perturbation method³⁰. Recently, Postma, Berendsen and Haak used this approach to determine the free energy of cavity formation in water⁴⁸. Sussman, Goodfellow, Barnes and Finney calculated ΔA between liquid water at various temperatures⁴⁹, using US. Jorgensen and Ravimohan computed the free energy difference between ethane and ethylalcohol²⁷. McCammon, Tembe, Lybrandt and Wipf calculated the free-energy difference of changing a coenzyme in aqueous solution^{28,29} and were able to calculate the free energy of solvation between $[\text{Cl}^-]_{\text{aq}}$ and $[\text{Br}^-]_{\text{aq}}$ in one step⁵⁰, but this involves only a relatively small alteration in an ionic radius parameter.

THE POTENTIAL OF MEAN FORCE

The expression for Helmholtz free energy, Equation(4), for the special case of two of the N particles of the system fixed in space at a distance R takes the form

$$A(R) = -kT \ln \int \dots \int \exp[-E_N(R)/kT] dR^{N-2}. \quad (45)$$

The radial distribution function for the system is defined as

$$g(R) = [N(N-1)/\rho] \int \dots \int \exp[-E_N(R)/kT] dR^{N-2} \quad (46)$$

and thus

$$A(R) = -kT \ln g(R) + \text{constant}. \quad (47)$$

The quantity $A(R)$ is the force acting between the fixed particles due to the remaining $N-2$ other particles of the system⁵¹ and thus $A(R)$, frequently denoted $w(R)$ in the statistical mechanics literature, is known as the potential of mean force. Equation(47) is generally true for any parameter R fixed. The knowledge of $A(R)$ is particularly useful for conformational changes, molecular associations and chemical reactions.

In general, $g(R)$ is obtained as the ratio of the probability of sampling the coordinate R , obtained from a simulation where R is also allowed to vary, and the volume element of the configuration space corresponding to the coordinate R :

$$g(R) = P(R)/V(R). \quad (48)$$

When the R coordinate is considered as simply another degree of freedom in an otherwise conventional mean energy calculation serious sampling problems arise. The simulation, seeking to describe the equilibrium state dictated by the Boltzmann factor, would end up sampling only a small region of the R -space rather than the full space. Thus sampling of R requires umbrella sampling techniques to cover the less probable regions of R . The volume element can be interpreted as a quantity proportional to the probability of sampling the parameter R with the potential function set to zero. For example, if R is an intermolecular distance, $V(R) = 4\pi R^2$ and if R is a torsion angle, $V(R) = \text{const}$. Its determination becomes progressively more complex as the dimensionality of R is increased.

Several recent simulation studies to compute $A(R)$ have been reported. Apart from the original study of Patey and Valleau¹³ where a tabulated weighting function was used, all recent works carried out a series of simulations, each constrained to sample the local region about points R_1, R_2, \dots respectively. In an individual simulation, a distribution $g(R)_i$ is obtained. A particular simulation is constrained to sample a region about R_i by adding a harmonic constraint to the configurational energy, via

$$W(R) = k_{\text{H}}(R - R_i)^2, \quad (49)$$

as first employed by Pangali Rao and Berne⁵². Successive points R_i are chosen so that $g(R)_i$ are overlapping. Overlapping points in the distribution correspond in principle to the same absolute value but in practice differ by a normalization constant. Thus the various computed $g(R)_i$ can be arbitrarily shifted up or down, and ultimately matched up to produce a $g(R)$ for the entire range of R . The matching can in principle be carried out for any overlapping points, but in practice one chooses those points with relatively low statistical noise levels. Ideally, the matching should be based on all overlapping points, with higher weight given to points that were sampled more extensively. A formalism, applicable to multidimensional R , has been presented in References^{16,19}.

Other potential of mean force determinations on R as a reaction coordinate include an early calculation of $A(R)$ for the association of ion pairs by Patey and Valleau¹³ and by Berkowitz et al.⁵³ where successive minima in $A(R)$ correspond to contact and solvent-separated ion pairs. The calculation of $A(R)$ for the association of apolar atoms and molecules has been carried out in studies of the hydrophobic effect by Pangali *et al.*⁵² and by Ravishanker, Mezei and Beveridge^{54,55}. Here as well contact and solvent-separated forms were identified with the latter having an unexpectedly high statistical weight, indicating that the hydrophobic effect may act over a longer range of distance than previously suspected by means of solvent-mediated structures. A series of potential of mean torsion

studies have been carried out on the n-butane system by Rebertus, Berne and Chandler⁵⁶, Rosenberg, Mikkilineni and Berne⁵⁷ and Jorgensen⁵⁸ as reviewed recently by Jorgensen⁵⁹. Most recently, Chandrashekar, Smith and Jorgensen have determined the potential of mean force on the complex reaction coordinate of the organic SN2 reaction of CH₃Cl and Cl⁻ in water²³ DMF²⁴. The reaction is predicted to be concerted in water but to proceed via a reaction intermediate in DMF, a previously unanticipated result. Warshel used umbrella sampling to compute the potential of mean force along a proton dissociation coordinate described by a coupling parameter^{20–22}. Case determined the potential of mean force for a carbonmonoxide molecule approaching the hemoglobin active site⁶⁰.

PROBABILITY RATIO METHOD

If the free energy function $A(R)$ is evaluated as a function of a coupling parameter λ on the 0,1 interval the free energy difference ΔA can be simply obtained as

$$\Delta A = A(1) - A(0) = kT \ln[g(R_0)/g(R_1)]. \quad (50)$$

In view of the interpretation of $g(R)$ as a probability per unit volume, the procedure based on Equation(50) will be called probability ratio method (PRM). Also, it can be shown that for the one-dimensional coupling parameter λ ,

$$V(\lambda) = \text{constant}, \quad (51)$$

and therefore

$$\Delta A = kT \ln P(\lambda_0)/P(\lambda_1), \quad (52)$$

where the probability ratio appears explicitly. The advantages of the PRM are twofold. First, there is no volume-element ratio to deal with. This appears to contradict to the above mentioned necessity of using $V(R) = 4\pi R^2$ for the intersolute distance R . However, the R^2 factor comes in only if the two solutes are allowed to move freely in the 3-dimensional space, implying that $R = |R|$, a parameter depending on three degrees of freedom. If instead the simulation is performed by directly varying the intersolute distance (that is, restrict the movement of the solute to the intersolute line), no volume element correction is needed. This statement can be justified also by recognizing that while the *a priori* probability of moving from R to $R + \Delta R$ and from R to $R - \Delta R$ is the same in the second case, for the first case they are unequal and their ratio is just $(R + \Delta R)^2/(R - \Delta R)^2$. Second, we can apply PRM for computing ΔA between systems described with different potentials (as already pointed out by Bennett earlier⁴¹). Since it can be generally expected that

$$|\langle E(\lambda + \Delta) \rangle_\lambda - \langle E(\lambda) \rangle_\lambda| > |\langle E(\lambda + \Delta) \rangle_{\lambda+\Delta} - \langle E(\lambda) \rangle_\lambda| \quad (53)$$

it is very likely that the creation of a new particle can be performed efficiently without any sudden increase in the integrand. The above derivation also provides a justification for allowing changes in the energy function when a conformational coordinate is varied. This was the case on Ref.19 where the atomic charges were also varied with the solute conformation.

A special application of PRM is to systems where the free energy difference between two solute conformations is required. The PRM method only provides the solvent contribution to this free energy difference. The solute contribution has to be computed separately. In that respect it should be pointed out that including the intramolecular energy into the simulation is equivalent to calculating ΔA with the intramolecular energy contribution set zero during the simulation and correcting the free energy difference with the intramolecular energy difference assuming that there is no coupling between the intermolecular and intramolecular terms²⁰. This result can also be obtained if one assumes that the calculation is using the total energy $E + E_{\text{intra}}$ but also employs umbrella sampling with $W(R) = -E_{\text{intra}}(R)$ where E_{intra} is the intramolecular energy contribution¹⁹.

In a recent work from this Laboratory¹⁹ using the PRM, λ took the form of a correlated conformational transition coordinate defined by Equation(19) as a linear tie-line from the C7 conformation to a “final” structure α_{R} or P_{II} of the Ala dipeptide in water. In subsequent studies we calculated the solvent contribution to the free energy difference between various conformations of the dimethyl phosphate anion⁶¹ and between the *cis* and *trans* conformation of *N*-methyl acetamide⁶² in water.

FULL FREE ENERGY SIMULATIONS

We use the terminology “full free energy simulations” or “complete free energy simulations” to refer to theoretical studies where both intramolecular and intermolecular contributions are included. For the case of determining ΔA between two states which correspond to well-defined minima on the intramolecular potential surface, the approximation

$$A = A_{\text{inter}} + A_{\text{intra}} \tag{54}$$

can be pursued. The vibrational entropy can be obtained in the harmonic approximation from the calculated vibrational frequencies and vibrational partition function by simple extension of the Einstein oscillator problem⁶³, or in the quasiharmonic approximation via an entropy obtained from the covariance matrix of atomic displacements^{64,65,66}. The free energy is then computed from the entropy and the calculated mean of the intramolecular internal energy. As the conformational flexibility of the molecule increases, the likelihood of the system going from the region of one minimum to another is larger and in these instances, harmonic and quasiharmonic methods fail. However, a limited perspective can be still developed from this approach by a detailed study restricted to a few conformations. In these cases, it is reasonable to consider the neighbourhood of these conformations independently and define conformational free energy assuming that only the neighbourhood of this selected conformation is accessible to the system, and proceed via Equation(56). Limitations arise due to the well-known multiple minimum problem. At the extreme of complete conformational flexibility, one has to proceed by means of a molecular simulation involving simultaneously both intramolecular and intermolecular degrees of freedom and obtain the free energy of the system via the procedures described in the preceding sections. Ravishanker et al.⁶⁷ have recently carried out here a series of calculations on the intramolecular thermodynamics of

the Ala dipeptide in the C_7 , C_5 , α_R and P_{II} conformations using the quasiharmonic Monte Carlo method^{64,68} with energy functions carried over from the CHARMM program⁶⁹, and combined these with our estimates of the free energy of hydration as determined with the PRM described in the preceding section. The C_7 form is indicated to be preferentially stabilized in the isolated molecule due to the 7-atom intramolecular ring structure closed with the $NH\dots OC$ hydrogen bond. The open forms are indicated to be entropically favored, but this contributes little to the intramolecular free energy. In water, the hydration stabilizes the open forms with favorable carbonyl-water hydrogen bonds, mitigated partially by a solvent entropy compensation effect. The net (intramolecular + hydration) free energy of three of the conformational forms turns out to be similar, indicating the molecule to be conformationally flexible in water. This is generally consistent with the experimental results⁷⁰, and suggests that the well-known conformational flexibility of numerous small peptides in water arises as a consequence of hydration competing successfully with intramolecular hydrogen bonds to stabilize open conformational forms.

DISCUSSION

No single method for free energy simulations can be considered as clearly superior to the others and the proper choice depends very much on the system under consideration. At one extreme of the spectrum, where the systems 0 and 1 are very similar, the PM is clearly optimal. However, the number of stages required is roughly proportional to the variation in ΔE . Therefore, when the systems 0 and 1 differ considerably, thermodynamic integration methods are likely to be the method of choice as demonstrated by the MCY-ST2 water calculation where three quadrature points were found to be adequate but the PM with US failed when tried in three stages. However, further studies are required for the optimal handling of the singularity problem. At this point little experience is accumulated with the use of the PRM. It has been proposed in this paper that PRM may be rather efficient in “creating” new particles. It is quite possible that for larger systems a combination of the methods would turn out to be optimal - we have in mind a technique where the “seeds” of a new system are introduced with, say, the PRM method (to avoid the singularity problem) and the rest of the system is “grown” with linear TI (to be able to rely on the monotonicity of the integrand). Answers to these questions will come from extensive comparative calculations in future work.

A TI calculation that determines the value of the integrand at one λ value can be considered also as a PM calculation between intermediate states $E_0 = E(\lambda - \Delta)$ and $E_1 = E(\lambda + \Delta)$ for small enough λ . If the points λ_i in the numerical integration are chosen close enough that a small enough λ can be found such that $\lambda_{i-1} + \Delta = \lambda_i - \Delta$ then ΔA can be calculated from the same set of calculations in two different ways (using either Equation(40) or (41)), providing a very useful consistency check. However, in most of the cases the integrand is “smooth” enough that the integral can be approximated adequately by evaluating the integrand at only a relatively few points which demonstrates one of the basic strengths of the TI method.

The error characteristics of the different approaches are also different. First, it can be generally said that free energy differences are likely to be more reliable than internal energy differences since the free energy difference calculations involve the energy differences before the averaging while the internal energy differences are usually obtained as a small difference between large quantities with their respective statistical uncertainties. Comparing the three different approaches discussed above, in calculations that require the definition of intermediate states, the errors of individual calculations contribute additively to the error in the final result. By use of thermodynamic integration, however, a weighted sum of the error of the individual quadrature points gives the error of the final result. Therefore, if a single calculation has an unusually large error, its effect will be present fully in the final result for the PM or PRM but in a TI calculation its effect will be scaled down.

The estimation of the error in a free energy simulation has two aspects. First, the error of the individual calculations is to be assessed and second, the propagation of the error to the final results is to be determined. The error of the individual calculations can generally be obtained from the method of batch means^{71,72}. Special care must be taken for both the PM and the PRM since these approaches are rather sensitive to the long range correlations. The propagation of the error is rather straightforward, as discussed above.

An alternative approach to the overall error problem is the use of consistency checks. We showed several examples where the same quantity can be computed in different ways. In fact, the alternative approaches mentioned can be considered as such as well. There is also the possibility to compute free energy differences along different paths. A simple example for this is the check employed by Scott and Lee on their PM/US calculations where a calculation between λ_i and λ_{i+1} was checked by computing the free energy difference between systems λ_i and $\lambda_i + (\lambda_{i+1} - \lambda_i)/2$ plus the difference between systems $\lambda_i + (\lambda_{i+1} - \lambda_i)/2$ and λ_{i+1} or the calculations on the ST2, MCY and SPC waters.

There are a number of other free energy techniques that have been developed and tested on relatively simple liquids. The first class of methods are based on a paper by Widom⁷³. These methods require the addition or deletion of a particle from the system^{74–77}. Simulations in the grand-canonical ensemble also fall into this class^{78–80}. These methods, however, can only be applied for small particles or low densities. A different class of methods, originated by Bennett⁴¹, are based on Equation(52). They do not actually change the coupling parameter, but rely instead of the comparison of energy distributions. The Virtual Overlap and the Overlap Ratio methods of Quirke and Jacucci also fall into this category^{81,82}. Their main drawback is that they require accurate estimate of the tail of the energy distributions which are known to be particularly sensitive to the small but well documented long range correlations that exist in simulations^{9,10}. Voter⁸³ published an interesting variation of the original Bennet method but it is only applicable to systems with a small number of extrema. It is unlikely that any of these methods can be applied efficiently to systems consisting of large molecules.

CONCLUSIONS

The results of reserach investigations described in this article clearly indicate that the time is now at hand for the calculation of free energies of molecular systems via computer simulation. We expect with the advent of supercomputers that the sampling problems inherent in the numerical determination of free energy can be overcome and the goal of producing a full thermodynamic description of molecular assemblies in condensed phase system can be more fully realized.

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