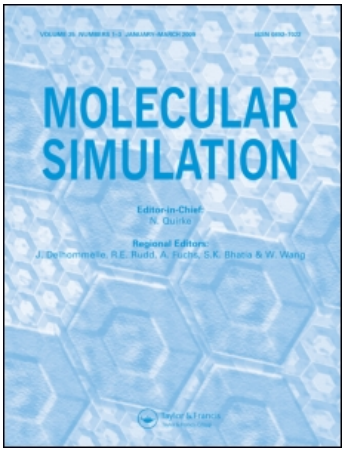

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Reversibility of Free Energy Simulations: Slow Growth May Have a Unique Advantage. (With a Note on Use of Ewald Summation)

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REVERSIBILITY OF FREE ENERGY SIMULATIONS: SLOW GROWTH MAY HAVE A UNIQUE ADVANTAGE. (WITH A NOTE ON USE OF EWALD SUMMATION)

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We review the slow-growth method for computing free energy changes for processes in conformation space or in "chemical" space, in which a system parameter, χ is changed at each integration time step, and the free energy, ΔA is approximated by accumulating the work performed at each step. The method is simple to implement and use, convergence can be monitored by performing longer simulations and by performing the simulations changing χ in both directions, and statistical error can be evaluated by performing multiple independent simulations. Because slow growth simulates a continuous process, it closely approximates the ideal isothermal quasi-static process used in defining the free energy in thermodynamics, and thus a small hysteresis in slow-growth results practically guarantees that the process is reversible, which is of course a prerequisite for the results to represent a free energy change. Whenever hysteresis is not negligible (which happens when the required long simulation times are unattainable), Boltzmann exponential averaging of slow growth results should be used to produce an upper bound on the free energy change (Jarzynski, C., *Phys. Rev. Lett.*, **78**, 2690–2693, 1997), with exponential averaging of results for change in the opposite direction giving a lower bound; it is then reasonable to choose the mean of the bounds as the best estimate. The work, W_{sg} for transfer of benzamidine from water to vacuum has been computed by insertion and extraction simulations, at different switching times. (As implemented, the molecular transformation calculation requires two evaluations of the Ewald sum; the increase in computer time required for this has been reduced by use of a multiple time step scheme in which the Ewald summations are executed at intervals of several integration time steps.) For the longest switching time, the distribution of values of W_{sg} is narrow, hysteresis is small and all methods produce a similar result for ΔA. As the switching time is reduced, (i) the distribution becomes non-Gaussian, (ii) the frictional portions and the distributions for insertion and extraction differ, (iii) the mean of the linear averages and the mean of the exponential averages for insertion and extraction both fail to give an accurate estimate of ΔA.

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INTRODUCTION

There are presently several main approaches in use for the calculation of free energy differences by simulation, as has been reviewed some time ago [1–5]. In all of these, the potential energy is perturbed in one way or another, the result of the perturbation being a change of state of the system along a specific path. If conformational variables are perturbed, the path will lie in phase space (conformational forcing), while if parameters of the energy function are perturbed, the path will be *via* the less intuitive "chemical" space (so-called molecular transformation). The methods differ in the details of how the free energy change is actually computed.

In *umbrella sampling*, the conformational variable, χ is perturbed by the application of, typically, a harmonic potential, $U_u(\chi)$ that has the effect of restraining χ near some fixed value, χ_0 where the potential is zero, $U_u(\chi_0) = 0$ [6–8]. The simulation locally samples a distribution $P_u(\chi)$, and from this local distribution the free energy's dependence on χ for the unperturbed state in the vicinity of χ_0 can be computed with

$$A(\chi) - A(\chi_0) = -kT \ln \frac{P_u(\chi)}{P_u(\chi_0)} - U_u(\chi) \tag{1}$$

Since the range of χ sampled in one of these simulations is limited, the results of simulations at many closely spaced values of χ_0 are pieced together to produce the free energy difference between end points [9].

In *stepwise perturbation*, the simulations are done with the conformational variable or energy parameter, χ fixed at some value, χ_0 and the free energy to change χ is computed from a sample of potential energy differences for changing χ to another value, χ_1 [10].

$$\Delta A = A(\chi_1) - A(\chi_0) = -kT \ln \langle \exp\{-[V(\chi_1) - V(\chi_0)]/kT\} \rangle_{\chi=\chi_0} \tag{2}$$

Again, practice has shown that accuracy requires the results of simulations at many closely spaced values of χ_0 to be pieced together [3, 11, 12].

In *thermodynamic integration*, the free energy for changing the state of the system is obtained as the work done in an isothermal quasi-static process, in which a change is forced of a conformational variable or energy parameter, χ. The free energy change is calculated as the integrated mean force applied

over the path

$$\Delta A = A(\chi_1) - A(\chi_0) = W = \int_{\chi_0}^{\chi_1} d\chi \left\langle \frac{\partial V}{\partial \chi} \right\rangle_{eq} \tag{3}$$

the average being over an equilibrium ensemble at each value of χ. This has led to two practical implementations. In *MCTI* (multi-conformational thermodynamic integration) [13], values of $\langle \partial V/\partial \chi \rangle$ at a series of values of χ are accumulated, from which the integral can be evaluated. In *slow growth* the free energy change is approximated as the work in a single simulation in which the value of χ is changed by $\delta\chi = (\chi_1 - \chi_0)/M$ at each of a total of M integration time steps [2, 11, 14]

$$W_{sg} = \sum \delta\chi \frac{\partial V}{\delta \chi} = \sum_{i=1}^{M} \frac{\chi_1 - \chi_0}{M} \left(\frac{\partial V}{\partial \chi} \right)_{\chi = \chi_0 + i\delta\chi} \tag{4}$$

Selection among these different methods can be based on a number of criteria, among which are: ease of implementation, precision, accuracy and reversibility. Ease of implementation favors slow growth, which requires executing a single simulation; on the other side, the step of piecing together the distributions obtained with different potentials in a maximally precise manner in umbrella sampling requires an especially complex procedure [15].

More important are questions of precision, *i.e.*, how, first, to limit and, second, to estimate the statistical error, and of accuracy, *i.e.*, how to avoid systematic errors.

Statistical error. Both stepwise perturbation and MCTI use averages of samples accumulated during simulations at constant potential, which makes for a straightforward analysis of statistical error, based on the autocorrelation functions of these time series [16]. This is not available with slow-growth time series, because in these the potential and therefore $\langle \partial V/\partial \chi \rangle$ changes systematically during the simulation. However, the statistical error in slow-growth calculations can be readily estimated in terms of the mean square deviation of results from several independent simulations [17–19].

Since averaging over longer time series or over a greater number of independent simulations should improve the precision by a similar factor, the effectiveness of a technique is determined by the precision achievable with a given amount of computational effort. Attempts at comparing stepwise perturbation, MCTI and slow growth have failed to determine significant differences in precision (*e.g.* [11, 12, 20]). This is perhaps not surprising because the statistical mechanical formulation of these three

methods is very similar, with stepwise perturbation converging with MCTI in the limit of very many small steps in χ, and MCTI converging with slow growth in the limit of very small steps and very short sampling time.

Systematic error. In addition, slow growth produces a systematic error: a lag between the system configuration and the (changing) potential [21] makes a positive contribution to W regardless of the direction in which χ changes. By treating this effect as due to friction, one sees that it will be eliminated in the limit of very long slow-growth simulations (or very large switching time, *i.e.*, $M \to \infty$). More useful is the fact that, at least when the friction is small, one expects it to give an equal (positive) contribution in a simulation in which the value of χ changes from χ_0 to χ_1 as in a simulation in which χ changes from χ_1 to χ_0. The systematic error due to friction produces a hysteresis, *i.e.*, in a cycle in which the potential is first changed, and then changed back to its original value, the sum of the work over the entire cycle computed according to Eq. (4) is not zero

$$H = W_{sg}(\chi_0 \to \chi_1) + W_{sg}(\chi_1 \to \chi_0) > 0 \tag{5}$$

The mean values of the work in the two directions provide upper and lower bounds of the free energy change [17–19]

$$-\langle W_{sg}(\chi_1 \to \chi_0)\rangle \le \Delta A_m(\chi_0 \to \chi_1) \le \langle W_{sg}(\chi_0 \to \chi_1)\rangle \tag{6}$$

If the friction is small, tits effect is eliminated by *subtracting* the two results and dividing by 2

$$\Delta A_m(\chi_0 \to \chi_1) = [W_{sg}(\chi_0 \to \chi_1) - W_{sg}(\chi_1 \to \chi_0)]/2 \tag{7}$$

Treatment of the statistical error and hysteresis in terms of a simple fluctuation-dissipation model has provided a basis for Eq. (7) [22, 23] and also gave as a result a relation between the hysteresis and the variance of W_{sg}

$$\mathrm{var}(W_{sg}) = kT\,H \tag{8}$$

Furthermore, this analysis indicated that both are inversely proportional to the switching time, which, according to recent work implies a linear response [33]. Jarzynski [24] has shown recently that systematic error due to friction is eliminated from slow-growth free energies differences by exponential averaging, *i.e.*,

$$\begin{aligned}\Delta A_B(\chi_0 \to \chi_1) &= -kT \ln\langle \exp[-W_{sg}(\chi_0 \to \chi_1)/kT]\rangle \\ &= kT \ln\langle \exp[-W_{sg}(\chi_1 \to \chi_0)/kT]\rangle\end{aligned} \tag{9}$$

An important consequence of this equality is that it follows that the mean value of W is an upper bound of ΔA, while the mean value of $-W$ in the opposite direction is a lower bound, *i.e.*,

$$\langle -W_{sg}(\chi_1 \to \chi_0)\rangle \leq \Delta A(\chi_0 \to \chi_1) \leq \langle W_{sg}(\chi_0 \to \chi_1)\rangle \tag{10}$$

Equation (9) has been proposed as the basis of a *fast growth protocol* to estimate ΔA_B from the results of many relatively short slow-growth simulations in a single direction [33].

It was also found that in the case where W_{sg} obeys a Gaussian distribution (as in the linear response case), Eq. (9) directly implies Eq. (8), and in that case yet another method for evaluating the free energy change from a set of independent simulations in the same direction is given by

$$\Delta A_G(\chi_0 \to \chi_1) \cong \langle W_{sg}(\chi_0 \to \chi_1)\rangle - \mathrm{msd}[W_{sg}(\chi_0 \to \chi_1)]/(2kT) \tag{11}$$

where msd stands for the mean square deviation [24].

Application of the fast growth protocol Eq. (9) would appear effective only when the probability distribution of the values of $f = W_{\mathrm{sg}}$ is narrow, as can be easily demonstrated for the case of a Gaussian probability distribution, P_G *i.e.*, when

$$\begin{aligned}\langle \exp[-f/kT]\rangle &= \int df P_G(f) \exp[-f/kT] \\ &= P_G(0) \int df \exp[-f^2/(2\sigma^2)] \exp[-f/kT]\end{aligned} \tag{12}$$

The integrand has a maximum for $f = f_{\max} = -\sigma^2/(kT)$, when the value of the probability distribution is

$$P_G(f_{\max})/P_G(0) = \exp\{-\sigma^2/[2(kT)^2]\} \tag{13}$$

Accordingly, the relative probability of sampling near the maximum of the integrand is small when $\sigma \gg kT$; for example, for $\sigma = 3kT$, the relative probability is only 0.01. In order to obtain a precise estimate of ΔA_B with use of Eq. (9) when $\sigma \gg kT$, one would need to perform a very large number of independent simulations, and thus the fast growth approach is effective only when the distribution is narrow, in which case the hysteresis, H Eq. (8), and then also the systematic error, is not large to begin with.

On the other hand, a sample of W_{sg} will have a systematic deficit of small values that contribute heavily to the average, ΔA_B computed with Eq. (9), and it has been shown that the latter also is an upper bound of the free

energy change [24],

$$\Delta A \leq \Delta A_B \tag{14}$$

(In practice, a rare instance of a low value of W_{sg} farther than, say, 3σ from the mean would have to be systematically eliminated.) Thus, ΔA_B is a better approximation of ΔA than ΔA_m.

In order to investigate how these relations hold up in a practical application, we here present results of simulations in a moderately difficult problem, the transfer from water to vacuum (extraction) of a polyatomic polar molecule having besides a positively charged end a sizable hydrophobic portion, benzamidine [C_6H_5—$C(NH_2)_2$], and the reverse (insertion). Free energies of transfer have been estimated in simulations in which the benzamidine-water interactions are slowly reduced from their full value to zero in a slow-growth protocol with non-linear scaling. We have done a sizable number of independent simulations at each of several switching times, in both directions, and analyze these here in terms of Eqs. (4) through (11).

METHODS

Simulations

Simulations were done with the program Sigma [25]. One benzamidine molecule was represented together with 2093 SPC water molecules in a cubic volume with periodic boundary conditions. Partial charges were obtained from the literature [34], nonbonded and geometric parameters were from the cedar forcefield [26, 27]. The integration time step was 2 fs, and the Shake algorithm was used to constrain all bond lengths [28]. Long-range electrostatic forces were evaluated with Ewald summation [29]. Nonbonded forces were computed at every time step for atom pairs within 8 Å, every 3 time steps for atom pairs with separation between 8 and 12 Å, and the Ewald summation was performed every 9 time steps, the long range electrostatic forces being obtained by subtracting the short- and medium range electrostatic forces from the forces computed *via* Ewald summation [30]. All other forces were computed at every time step. The integration was performed with use of a multiple time step algorithm [31]. Constant temperature (300 K) and pressure (1 bar) were maintained with Berendsen thermo- and manostats [32].

Free Energy Calculations

The free energy of transfer of benzamidine from aqueous solution to vacuum was estimated in dynamics simulations in which the force field parameters were changed by making the benzamidine-water energy dependent on a coupling parameter, λ. The beginning and end state are specified by specific potential energy functions, V_1 and V_2, the former consisting of water-water plus benzamidine-water energy and the latter containing only the water-water energy, with all intramolecular energy terms common to both. The transformation is performed using a potential energy function dependent on λ,

$$V = f_1(\lambda)V_1 + f_2(\lambda)V_2 \tag{15}$$

where

$$\begin{aligned} f_1(0) &= f_2(1) = 0 \\ f_1(1) &= f_2(0) = 1 \end{aligned} \tag{16}$$

From Eq. (3), the free energy for extracting the benzamidine molecule becomes

$$\Delta A = \int_1^0 \langle \partial V/\partial\lambda \rangle_{eq} d\lambda \tag{17}$$

Starting points for series of transformations were taken at 0.9 ps intervals from simulations of the benzamidine-water system at $\lambda = 1$ (for extractions) and at $\lambda = 0$ (for insertions).

Non-linear scaling. A non-linear scaling function (*cf*. Eq. (15)) was used, in which non-bonded interactions between benzamidine and water were scaled in proportion to λ^m. For the attractive Lennard-Jones energy and forces and the electrostatic energy and forces $m = 3$, while for the repulsive Lennard-Jones energy and forces $m = 5$ [17].

Use of Ewald Summation in Simulations that Compute Free Energies of Molecular Transformation

In the Gromos/Cedar force field and other force fields commonly used in molecular mechanics simulations, the energy is computed as a sum of many terms each involving 2 (or 3 or 4) atoms. In that case, solvent-solvent, solvent-solute and solute-solute terms occur equally in V_1 and V_2 and hence do not contribute to $\partial V/\partial\lambda$, and when the potential energy is evaluated by direct summation of terms, these need be computed only once without

scaling. In the Ewald summation, the contributions from all atomic point charges, including those from surrounding simulation cells, are combined to compute the electrostatic potential and gradient, and the *combined* gradient is used to compute the force acting on each point charge. Consequently, it is necessary to compute the Ewald sum *twice*, once as a contribution to V_1, with inclusion of the benzamidine charges, and again as a contribution to V_2, omitting the benzamidine charges. (A second evaluation is necessary also for a ligand molecule with zero net charge, and can be omitted only for a ligand that is represented without atomic partial charges.)

RESULTS

Table I summarizes the results, giving switching time, number of independent evaluations, mean value, mean square deviation and exponential mean value for insertions and extractions. Figure 1 shows progress curves from independent simulations, 20 in each direction, while Figure 2 shows distributions of simulation results at two different switching times, and Figure 3 shows convergence of two different estimates of the transfer free energy as the switching time is increased: the mean of the linear averages Eq. (7), and the mean of the exponential averages Eq. (9) for insertion and extraction.

For the 8 independent simulations with the longest switching time (180 ps), the 16 progress curves, when shifted to account for the difference in origin between insertions and extractions, are almost superimposable

TABLE I Free energy estimates for extraction of benzamidine from water from slow growth simulations of the insertion and extraction processes ("in" and "out"). Results were computed from multiple (n) independent transfers of benzamidine into and out of water in different switching times, t_s. Successively given are, linear average or mean, $\langle W_{sg} \rangle$, mean square deviation, msd (W_{sg}), mean of insertion and extraction results, ΔA_m, Boltzmann exponential average, ΔA_B and mean of insertion and extraction values, $\langle \Delta A_B \rangle$, and estimate assuming a Gaussian distribution, ΔA_G. Energies in kcal/mol

t_s (*ps*)	*in*|*out*	n	$\langle W_{sg} \rangle$	*msd* (W_{sg})	ΔA_m *Eq.* (7)	ΔA_B *Eq.* (9)	$\langle \Delta A_B \rangle$	ΔA_G *Eq.* (11)
180	in	8	56.2	0.49		56.5		56.6
180	out	8	57.1	0.10	56.7	57.1	56.8	57.0
18	in	100	52.2	4.0		55.2		55.5
18	out	100	59.7	3.6	56.0	56.8	56.0	56.7
7.2	in	200	46.5	23.9		52.6		66.4
7.2	out	200	63.1	7.0	54.8	59.1	55.9	57.3
3.6	in	400	39.2	51.2		50.3		81.9
3.6	out	400	67.5	9.8	53.4	59.1	54.7	59.3
1.8	in	800	26.7	158.		42.6		158.3
1.8	out	800	72.7	13.2	36.3	63.2	52.9	61.7

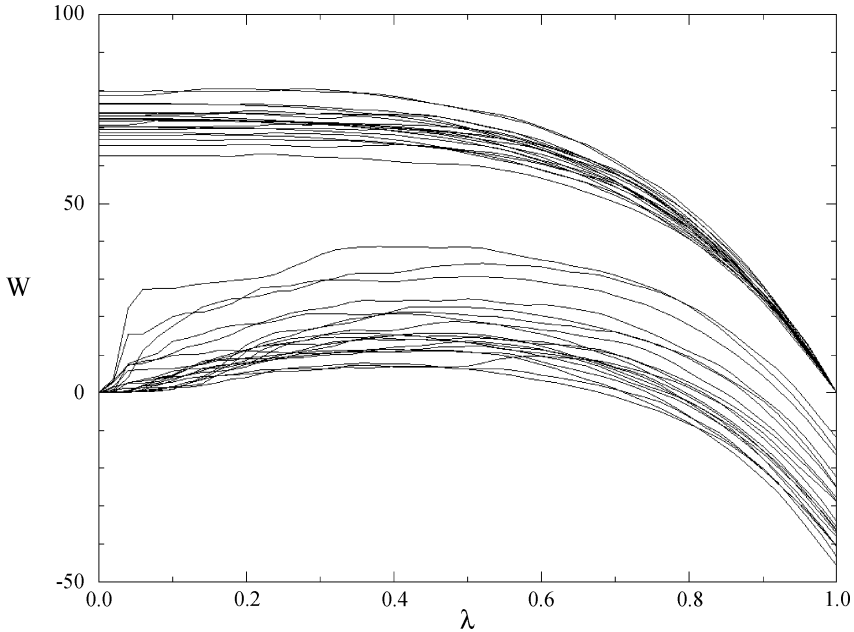

FIGURE 1 Slow growth progress curves from 20 independent extractions (top set) and insertions (bottom set) of benzamidine for a switching time of 1.8 ps. Energies in kcal/mol.

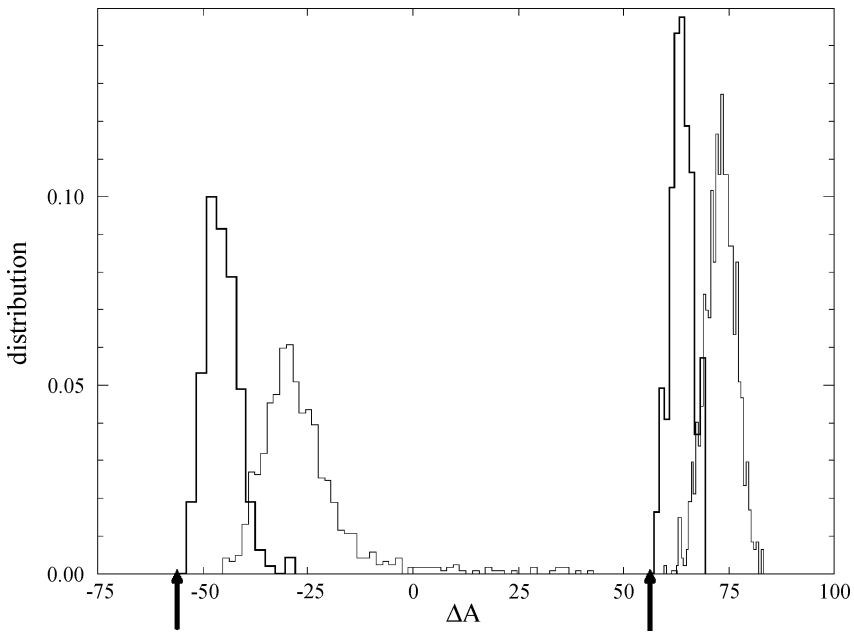

FIGURE 2 Distributions of slow growth results, W_{sg} for insertion (on left) and extraction (on right) in independent simulations, for switching times of 1.8 (thin lines) and 7.2 (heavy lines) ps. The vertical arrows indicate the estimated free energy change. Energies in kcal/mol. The distributions have been scaled to give the same area under each curve.

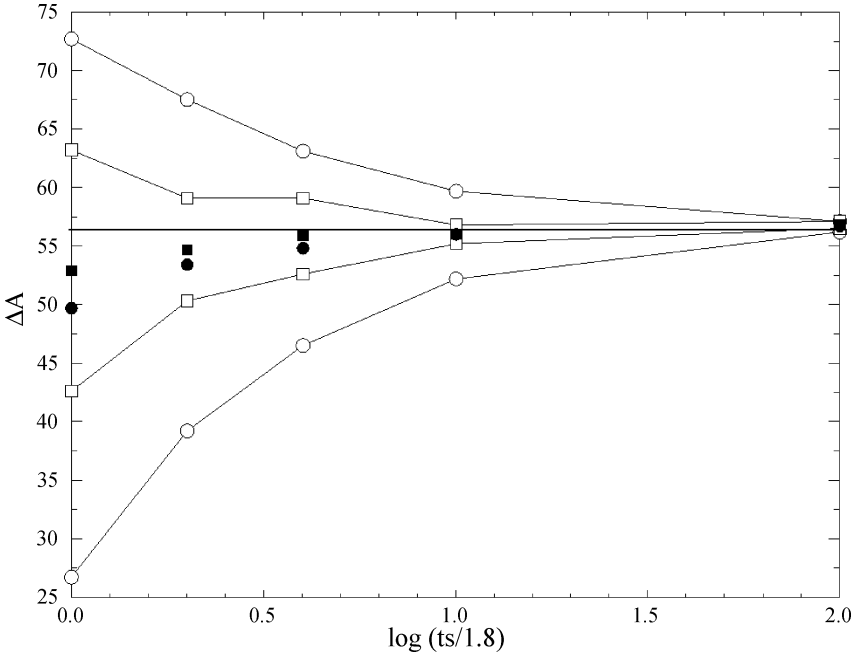

FIGURE 3 Estimates of the free energy of transfer of benzamidine from water to vacuum, ΔA (in kcal/mol) obtained with two averaging methods as a function of the switching time, t_s (in ps). Open circles represent mean values Eq. (4), and open squares represent Boltzmann exponential averages Eq. (9). Filled symbols represent the mean values for insertion and extraction of these two averages. A horizontal straight line marks the best estimate of the free energy change.

(results not shown). For a switching time of 18 ps the distributions are narrow, hysteresis is small and all methods produce a similar, reasonable accurate result for ΔA. For shorter switching times, the methods to estimate ΔA increasingly fail: (i) the distribution of W_{sg} is non-Gaussian as evidenced by the failure of Eq. (11) to give an accurate value; (ii) the distributions for insertion and extraction of W_{sg} differ markedly, and also the mean of the linear averages for insertion and extraction fails to give an accurate value; (iii) the exponential average Eq. (9) fails to give a good estimate of ΔA, presumably because needed instances of low values of W_{sg} do not occur in a sample generated by a limited number of simulations (*cf.* Eq. (13)).

Evidently, the most accurate value of ΔA is obtained from a small number of simulations, each with a correspondingly long switching time.

"*Asymmetry*" *of insertion and extraction*. It is clear from Figure 1 that insertions at short switching time give erratic results due to events that

occur, to a variable extent, at very small values of λ. In the absence of interactions between solute and solvent molecules, *i.e.*, at $\lambda = 0$, solvent molecules can very closely approach atoms of the solute, and it is well known that this creates technical difficulties if the scaling of the intermolecular forces with λ is linear [1, 2, 14]. The results reported here indicate that, when non-linear scaling is used, a randomly inserted molecule may experience a repulsive energy outside the equilibrium distribution during the initial stage of the transformation, *i.e.*, when the value of λ is small, but non-zero; this will then lead to a contribution to the "friction", for which there is no counterpart in the extraction process [21]. The consequent asymmetry between the insertion and extraction processes, has as a result that averaging of W_{sg} from insertions and extractions as in Eq. (7) does not eliminate the error due to friction. Careful inspection of the averages and mean square deviations reported in Table I shows that the asymmetry persists at even the longest switching time.

DISCUSSION

A unique drawback of slow growth is that a systematic error (which shows up as hysteresis) must be removed from the computed values of W_{sg}. At the least, the results in forward and reverse direction provide upper and lower bounds, the correct answer lying between the two extremes obtained, preferably by exponential averaging according to Eq. (9). In the limiting case in which the values of W_{sg} obey a Gaussian distribution, the corrected ΔA can be calculated according to three different methods, Eqs. (7), (9) and (11) [17, 22, 24, 33], which should produce similar answers. If they do not, and more precise results are required, longer simulations will be needed; a drawback is that if new and longer simulations are decided on, the results of the old simulations are useful only as a measure of progress towards the goal of obtaining a final set of results. Perhaps recently obtained new insight [24, 33] will encourage extension to conditions where the response is non-linear and the distributions are not Gaussian.

So far, one seems to lack a good reason to use slow growth, rather the opposite is true. There exists, however, an excellent reason for continuing to use this method, related to the reversibility of the simulated process, which is a necessary condition for the work done to bring about the process to be equated with the free energy change. In Eq. (3) the reversibility condition is satisfied by letting the force, $\partial V/\partial \chi$ be averaged over an equilibrium ensemble (Boltzmann distribution) at all values of χ. In a physical

experiment, reversibility is ensured by performing the experiment slowly, *and then performing the experiment in the opposite direction*. With slow growth one simulates the same process in both directions. A small hysteresis, furthermore one that decreases as the switching time is increased, is a direct demonstration that the simulation has followed a continuous and reversible process and that the computed result Eq. (7) indeed represents the change in the free energy of the model within a known margin of error.

Both MCTI and stepwise perturbation require the preparation of equilibrated samples at intermediate values of χ. Reversibility can be addressed by preparing two of each sample by perturbing samples that have been thoroughly equilibrated at each endpoint (χ_1 and χ_2), and computing mean values of $\langle \partial V/\partial \chi \rangle$ in two simulations, for as long as needed for convergence to the same value. In addition, when using the stepwise perturbation method, one should compute the free energy to perturb the system to *both* the next lower and the next higher value of χ, and establish reversibility by demonstrating convergence to where "up" and "down" perturbations across the same interval of χ give equal results, *i.e.*,

$$\begin{aligned} &\ln\langle \exp\{-[V(\chi_1) - V(\chi_0)]/kT\}\rangle_{\chi=\chi_0} \\ &\quad = -\ln\langle \exp\{-[V(\chi_0) - V(\chi_1)]/kT\}\rangle_{\chi=\chi_1} \end{aligned} \tag{18}$$

for all intervals.

A unique advantage of slow growth is that the change of the system is tracked through an essentially continuous process, and that every generated intermediate sample contributes to the average, $\langle \partial V/\partial \chi \rangle$. In the limit of very long switching time, the slow-growth simulated process closely approximates the quasi-static process of thermodynamics that underlies definitions of reversibility and free energy.

Even if conditions of reversibility have not been perfectly attained, it is highly desirable to have a clear indication that is has not, in the form of a non-negligible hysteresis or otherwise. Apart from establishing a level of confidence in the reported results, such knowledge may encourage new attempts to repeat the problem calculation with faster computers or with an improved algorithm, for example, by integration along a different path.

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References

[1] Beveridge, D. L. and DiCapua, F. M., "Free energy *via* molecular simulation: A primer", In: *Computer Simulations of Biomolecular Systems*, van Gunsteren, W. F. and Weiner, P. K. Eds., ESCOM: Leiden, 1989, pp. 1–26.

[2] van Gunsteren, W. F., "Methods for calculation of free energies and binding constants: Successes and problems", In: *Computer simulations of biomolecular systems*, van Gunsteren, W. F. and Weiner, P. K. Eds., ESCOM: Leiden, The Netherlands, 1989, pp. 27–59.

[3] Pearlman, D. A. and Kollman, P. A., "Free energy perturbation calculations: Problems and pitfalls along the gilded road", In: *Computer Simulations of Biomolecular Systems*, van Gunsteren, W. F. and Weiner, P. K. Eds., ESCOM: Leiden, 1989, pp. 101–119.

[4] Brooks, C. L., "Thermodynamic calculations in biological systems", In: *Computer simulations of biomolecular systems*, van Gunsteren, W. F. and Weiner, P. K. Eds., ESCOM: Leiden, 1989, pp. 73–88.

[5] Jorgensen, W. L., "Free energies in solution: The aqua vitae of computer simulations", In: *Computer simulations of biomolecular systems*, van Gunsteren, W. F. and Weiner, P. K. Eds., ESCOM: Leiden, 1989, pp. 60–71.

[6] Valleau, J. P. and Torrie, G. M., "A guide to Monte Carlo for statistical mechanics", In: *Statistical Mechanics. Part A*: *Equilibrium techniques*, Berne, B. J. Ed., Plenum press: New York, 1977, pp. 169–194.

[7] Mezei, M., Mehrotra, P. K. and Beveridge, D. L. (1985). "Monte Carlo determination of the free energy and internal energy of hydration for the Ala dipeptide at 25°C", *J. Am. Chem. Soc.*, **107**, 2239–2245.

[8] Mezei, M. and Beveridge, D. L. (1986). "Free energy simulations", *Ann. N.Y. Acad. Sci.*, **482**, 1–23.

[9] Jorgensen, W. L., Gao, J. and Ravimohan, C. (1985). "Monte Carlo simulations of alkanes in water: Hydration numbers and hydrophobic effect", *J. Phys. Chem.*, **89**, 3470–3473.

[10] Zwanzig, R. W. (1954). "High-temperature equation of state by a perturbation method", *J. Chem. Phys.*, **22**, 1420–1426.

[11] Postma, J. P. M., Berendsen, H. J. C. and Haak, J. R. (1982). "Thermodynamics of cavity formation in water", *Faraday Symp. Chem. Soc.*, **17**, 55–67.

[12] Jorgensen, W. L. and Ravimohan, C. (1985). "Monte Carlo Simulation of Differences in Free Energies of Hydration", *J. Chem. Phys.*, **83**, 3050–3054.

[13] Straatsma, T. P. and McCammon, J. A. (1991). "Multi configuration thermodynamic integration", *J. Chem. Phys.*, **95**, 1175–1188.

[14] Berendsen, H. J. C., Postma, J. P. M. and van Gunsteren, W. F., "Statistical mechanics and molecular dynamics: The calculation of free energy", In: *Molecular Dynamics and Protein Structure*, Hermans, J. Ed.; Polycrystal Book Service: Western Springs, IL, 1985, pp. 43–46.

[15] Kumar, S., Bouzida, D., Swendsen, R. H., Kollman, P. A. and Rosenberg, J. M. (1992). "The weighted histogram analysis method for free-energy calculations of biomolecules. I. The method", *J. Comput. Chem.*, **13**, 1011–1021.

[16] Straatsma, T. P., Berendsen, H. J. C. and Stam, A. (1986). "Estimation of statistical errors in molecular simulation calculations", *Mol. Phys.*, **57**, 89–95.

[17] Hermans, J., Yun, R. H. and Anderson, A. G. (1992). "Precision of free energies calculated by molecular dynamics simulations of peptides in solution", *J. Comput. Chem.*, **13**, 429–442.

[18] Reinhardt, W. P. and Hunter III, J. E. (1992). "Variational path optimization and upper and lower bounds to free energy changes *via* finite time minimization of external work", *J. Chem. Phys.*, **97**, 1599–1601.

[19] Hunter III, J. E., Reinhardt, W. P. and Davis, T. F. (1993). "A finite-time variational method for determining optimal paths and obtaining bounds on free energy changes from computer simulations", *J. Chem. Phys.*, **99**, 6856–6864.

[20] Hermans, J., Pathiaseril, A. and Anderson, A. (1988). "Excess free energy of liquids from molecular dynamics simulation. Application to water models", *J. Am. Chem. Soc.*, **110**, 5982–5986.

[21] Pearlman, D. A. and Kollman, P. A. (1989). "The lag between the Hamiltonian and the system configuration in free-energy perturbation calculations", *J. Chem. Phys.*, **91**, 7831–7839.
[22] Wood, R. H. (1991). "Estimation of errors in free-energy calculations due to the lag between the Hamiltonian and the system configuration", *J. Phys. Chem.*, **95**, 4838–4842.
[23] Hermans, J. (1991). "A simple analysis of noise and hysteresis in free energy simulations", *J. Phys. Chem.*, **95**, 9029–9032.
[24] Jarzynski, C. (1997). "Nonequilibrium equality for free energy differences", *Phys. Rev. Lett.*, **78**, 2690–2693.
[25] Hermans, J., Yun, R. H., Leech, J. and Cavanaugh, D. (1994). *Sigma documentation*. University of North Carolina: www http://femto.med.unc.edu/SIGMA/.
[26] Ferro, D. R., McQueen, J. E., McCown, J. T. and Hermans, J. (1980). "Energy minimization of rubredoxin", *J. Mol. Biol.*, **136**, 1–18.
[27] Hermans, J., Berendsen, H. J. C., van Gunsteren, W. F. and Postma, J. P. M. (1984). "A consistent empirical potential for water-protein interactions", *Biopolymers*, **23**, 1513–1518.
[28] Ryckaert, J. P., Ciccotti, G. and Berendsen, H. J. C. (1977). "Numerical integration of the Cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes", *J. Comput. Phys.*, **23**, 327–341.
[29] Darden, T. A., York, D. M. and Pedersen, L. G. (1993). "Particle mesh Ewald: An N.log(N) method for Ewald sums in large systems", *J. Chem. Phys.*, **98**, 10089–10092.
[30] Schlick, T., Skeel, R. D., Brünger, A. T., Kalé, L. V., Board, J. A., Hermans, J. and Schulten, K. (1999). "Algorithmic challenges in computational molecular biophysics", *J. Comput. Phys.*, **151**, 9–48.
[31] Tuckerman, M. E., Berne, B. J. and Martyna, G. J. (1992). "Reversible multiple time scale molecular dynamics", *J. Chem. Phys.*, **97**, 1990–2001.
[32] Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A. and Haak, J. R. (1984). "Molecular dynamics with coupling to an external bath", *J. Chem. Phys.*, **81**, 3684–3690.
[33] Hendrix, D. A. and Jarzynski, C. (2001). "A 'fast growth' method of computing free energy differences", *J. Chem. Phys.*, **114**, 5974–5981.
[34] Resat, H., Marrone, T. J. and McCammon, J. A. (1997). "Enzyme-inhibitor association thermodynamics: explicit and continuum solvent studies", *Biophys. J.*, **72**, 522–532.