

Staging Is More Important than Perturbation Method for Computation of Enthalpy and Entropy Changes in Complex Systems

Nandou Lu,^{*,†,‡} David A. Kofke,[†] and Thomas B. Woolf^{‡,§}

Department of Chemical Engineering, University at Buffalo, The State University of New York, Buffalo, New York 14260-4200, and Departments of Physiology and of Biophysics and Biophysical Chemistry, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205-2185

Received: December 5, 2002; In Final Form: March 24, 2003

Entropy and enthalpy compensation are frequently observed in thermodynamic measurements. To understand the molecular details of these compensating changes, molecular dynamics or Monte Carlo methods may, in principle, be used. Yet, in practice, computational methods to evaluate changes in entropy and enthalpy usually have much greater (e.g. order of magnitude worse) error than relative free energy calculations. In this paper, we examine ways to improve the computational ability to determine changes in both enthalpy and entropy. Toward that end we consider five different perturbation schemes for enthalpy determination and also consider the relative importance of staging the computation (i.e., dividing the entire perturbation calculation into a series of consecutive smaller ones) along the reaction coordinate. Two model systems are used for evaluation: a system of Lennard-Jones spheres, where the computation is directed to the addition/deletion of a sphere, and the alchemical transformation of an anion in explicit TIP3P water from one type to another. The results show only subtle differences among the better perturbation methods and much larger improvement in relative accuracy with the use and careful selection of staged intermediates along the reaction coordinate. As a result of these calculations, we suggest that approximate calculations of relative entropy be used to guide the staging of intermediates for computation of free energy, entropy, and enthalpy differences. We suggest that for these purposes the single state perturbation (SSP) method is the best choice. For the pure purpose of enthalpy and/or entropy evaluation, we suggest multistage SSP and direct approach, respectively, for systems with small and large differences.

1. Introduction

Observations from experiment and molecular simulation indicate that there often exists a compensation between large counter changing entropy and enthalpy values, which balance to create a much smaller change in relative free energy.^{1–11} Free energy is the principal driving force for thermodynamic phenomena, and an understanding of its behavior can be improved by examination of the enthalpy and entropy contributions. However, the reliable computation, from detailed molecular conformations, of enthalpy and entropy changes in complex systems is a real challenge. Even the best approaches suggest an error of nearly 10 times greater uncertainty than that associated with relative free energy changes. Thus, we hope, with this study, to provide analysis of ways to improve this situation.

An important category of methods for the computation of thermodynamic properties from molecular dynamics and Monte Carlo simulations is based on the use of perturbations. In its simplest form, the free-energy perturbation (FEP) method computes the free-energy difference ΔA between two systems (designated 0 and 1) of interest according to¹²

$$\beta\Delta A = \beta(A_1 - A_0) = -\ln\langle\exp(-\beta u)\rangle_0 \quad (1)$$

* Corresponding author. E-mail: nlu@groucho.med.jhmi.edu (N.L.); kofke@eng.buffalo.edu (D.A.K.); woolf@groucho.med.jhmi.edu (T.B.W.).

[†] Department of Chemical Engineering, University at Buffalo.

[‡] Department of Physiology, School of Medicine, Johns Hopkins University.

[§] Department of Biophysics and Biophysical Chemistry, School of Medicine, Johns Hopkins University.

where $u = U_1(\Gamma) - U_0(\Gamma)$ is the potential energy difference encountered at a FEP trial with Γ representing the phase-space point, $\beta = 1/kT$ is the reciprocal temperature with k Boltzmann's constant, and T is the absolute temperature. The angle brackets $\langle \dots \rangle$ indicate a canonical-ensemble average specified by the subscript.

The direct (or single-stage) perturbation method is limited to applications with a small difference between the two systems. A multistage^{13–15} ("multiple-window" is an equivalent term used in some literatures) FEP calculation can be conducted to handle a perturbation that is too large for any single-stage calculation. In a multistage calculation, one or more intermediate systems are introduced along the reaction coordinate connecting the systems 0 and 1, and the overall difference is computed as the sum of the smaller successive differences. The reliability and efficiency of the overall calculation is closely related to the staging design (e.g., number of stages, definition of each intermediate). For the most part, the history of relative free energy calculations shows concentration on issues related to sampling and convergence within windows and less on addressing whether an optimal staging along the reaction coordinate is necessary.^{15,16} In fact, the usual rule of thumb has been to simply adopt uniform steps along the reaction coordinate. Recent results suggest that changes in entropy (S) could play an important role in determining the reliability (i.e., the precision and accuracy) of FEP results.^{17–20} In particular, the results suggested that entropy information provides a guideline for optimal staging design increasing both reliability^{18–20} (precision as well as accuracy) and efficiency²⁰ (the number of stages and the simulation length for each stage) for a calculation. These

previous results further suggested a heuristic that the optimal staging of a multistage FEP calculation should correspond to an equal-entropy difference between all stages, i.e., $\Delta(\Delta S/k)_{ij} = \Delta S_j - \Delta S_i = 0$, where subscripts i and j represent two different perturbation pairs. Thus, for free energy changes, the earlier results showed¹⁸ that application of the entropy heuristic can improve the precision of free-energy calculation over consideration of equal free energy differences, i.e., $\Delta(\Delta A)_{ij} = \Delta A_j - \Delta A_i = 0$.²¹

While we initiated this study with the concept of the importance of entropy in staging perturbation calculations, we also entered the calculations with a realization that computation of entropy and enthalpy is known to occur with large errors and thus may be less important in practice than in principle. That is, we expect that, with the guidance of relative entropy information, a multi-stage FEP calculation for relative free energy could be designed to produce efficiently precise results with a particular level of accuracy. However, this requirement of entropy information may hinder the application of this principle, because of the great difficulty in computing entropy effectively in molecular simulation.^{16,22–24} Thus, one goal of the present paper was to determine the best way to calculate entropy (or analogously enthalpy) changes for a multistage FEP calculation.

The entropy difference ΔS , free energy difference ΔA , and potential energy (or enthalpy; we treat the terms equivalently in this paper) difference ΔU are coupled according to the thermodynamic relationship

$$T\Delta S = \Delta U - \Delta A \quad (2)$$

where T is the absolute temperature. Information on any two of these three quantities gives knowledge of the third. Compared to free energy calculation, the methodology and performance of relative enthalpy and entropy calculations via molecular simulation has received much less attention. This may be reasonable, since evaluating the energy U is a straightforward task in molecular simulation. The average configurational energy of a system can be simply computed as an ensemble average

$$\langle U \rangle = \frac{1}{Q} \int U(\Gamma) \exp[-\beta U(\Gamma)] d\Gamma \quad (3)$$

where Q is the partition function of the canonical ensemble. In general, the configurational energy difference can be determined by evaluating the average energy of the two systems under interest (0 and 1) separately then taking the difference between them,

$$\Delta U = \langle U_1 \rangle_1 - \langle U_0 \rangle_0 \quad (4)$$

where the subscript of the angled brackets indicates the system where the ensemble average is computed. This direct approach is straightforward and has been used in the past.^{25,26} However, it can be far less useful than might first appear, due to imprecision (uncertainty) of the averages, especially when the difference between systems 0 and 1 is small. The uncertainty is magnified because average energies are typically large numbers (of order N) with proportionally large fluctuations, while the difference between them is typically much smaller (of order 1)—the problem is exacerbated with increasing system size (the fluctuation grows as $N^{1/2}$). As a consequence, the uncertainty in ΔU (hence ΔS) is typically large; when the 0 and 1 systems are very similar to each other, ΔU determined in this way may be completely unreliable. Some efforts have been

made to improve the precision of the calculation by carefully considering a subset of molecules involved in the calculation, therefore reducing the fluctuations in the energy. For example, in a study of solvation, Gallicchio et al.²⁷ decomposed the solute–solvent and solvent–solvent interaction energies and adapted a solvation shell approximation^{28,29} in computing the solvent reorganization energy. (They employed this idea for a perturbation method—the single state perturbation described below—where the fluctuation grows with the system size at a rate of $N^{1/2}$.)

In contrast, the free energy difference ΔA is computed as an ensemble average involving the potential difference u (refer to eq 1) rather than as the difference of two ensemble averages. Furthermore, in computing u , any common features in the two perturbation systems cancel out and therefore make no contribution to the uncertainty in ΔA . Consequently, computation of ΔA is more precise than that of ΔU (and hence ΔS). It has been shown, for certain systems, that the uncertainty of ΔU is about 1–2 orders of magnitude larger than that of ΔA .¹⁶ It should be remembered that calculation of ΔA has problems as well. The free energy difference calculation is prone to inaccuracy (systematic error), sometimes giving a result that is reproducible but incorrect. This is much less of an issue in computing ΔU , where concerns of precision (stochastic error) dominate.

Entropy and energy information can be of interest by themselves in many problems, such as in solvation, binding affinity, and phase coexistence (e.g. refs 1–11, 30). There already exist different methods for computing the entropy difference and absolute entropy, such as adiabatic switching,³¹ integration of correlation functions,^{32–34} vibrational normal modes analysis,^{35–38} and local states and hypothetical scanning.³⁹ Each of these approaches has advantages and application ranges. Another category of the methods derives from the perturbation idea; thus, we refer to them as perturbation approaches. The advantage of a perturbation method is, when incorporated with FEP calculations, it requires almost no additional computational effort in computing ΔU or ΔS . Moreover, the formulation of these methods promotes a partial cancellation of the fluctuations that give rise to the imprecision in the energy calculation. And in principle, it works for all kinds of systems provided the perturbation size is within an appropriate range. In this study, we focus solely on these perturbation approaches. We also consider a multistage scheme for computing ΔU and ΔS in a staged FEP calculation. Although some of the perturbation methods presented here have been used widely in the past,^{16,23,40–43} systematic study of the methodologies and comparisons of the performance of different methods is lacking. It is a goal of this study to examine the performance of five different perturbation methods, and their staging, for computing the enthalpy (or potential energy, and hence entropy) in the context of FEP calculations and to obtain improved understanding of the common behavior and limitations for these methods. Note that the application of the methods described in this paper is not limited to FEP calculations. Some of the formulas, such as that for the single state perturbation and β -perturbation (see below), can be easily modified to fit the thermodynamic integration calculation and for pure energy difference evaluation purposes.

This paper is organized as follows: five perturbation methods (including two new methods), for the enthalpy (or energy) difference and entropy difference evaluation are presented in section 2; descriptions of a Monte Carlo simulation of Lennard-Jones (LJ) fluid and molecular dynamics simulation of an alchemical change between two anions is given in section 3;

Section 4 presents the results and discussion, and this is followed by concluding remarks in section 5.

2. Perturbation Methods

All the perturbation methods start with the assumption that an ensemble of molecular conformations of the system is to be collected with a fixed value of the reaction coordinate. The relative changes in entropy, enthalpy and free energy are then computed through a series of (ideally one step) perturbations from the initial ensemble to the final ensemble. In each simulation we also conduct a series of perturbations from one side of the reaction coordinate to the other or vice-versa.

2.1. Finite Difference (FD) Method. One of the more frequently used^{23,43} perturbation approaches is the finite difference (FD) method, based on the thermodynamic relationship

$$\Delta S = -\left(\frac{\partial \Delta A}{\partial T}\right)_{N,V} \quad (5)$$

Assuming that the free energy difference is a linear function of T (or, equivalently, the heat capacity $\Delta C_v = T(\partial S/\partial T)_V$ is independent of temperature) over a small temperature interval ΔT , the entropy difference can be computed using a finite difference approximation

$$\Delta S(T) = -\frac{\Delta A(T + \Delta T) - \Delta A(T - \Delta T)}{2\Delta T} \quad (6)$$

where thermodynamic quantities are written explicitly as a function of the temperature T . Strictly speaking, this method does not belong to the perturbation category, but it can be called a perturbation approach when ΔA is computed using a perturbation, as is done for this paper. It has been reported that the FD method provides better estimates of ΔS than the direct approach when ΔT does not to exceed 30–50 K.¹⁶ This approach does have disadvantages: three independent simulations (i.e., at the temperature T , $T + \Delta T$, and $T - \Delta T$) are needed in order to compute ΔA and ΔS (thereafter ΔU). With this heavier computational expense, however, does not come reliable ΔS (and ΔU), since the errors in separate measurements of ΔA accumulate. Details regarding this point are discussed in the Results and Discussion section. It is possible to compute the differential of eq 5 using only data collected at T and $T + \Delta T$ (or $T - \Delta T$) (i.e., Euler difference) to reduce the computational cost; however, in general doing this will degrade the quality of the result.⁴⁴ A hybrid version incorporating the ideas of finite difference and perturbation on T will be presented in section 2.3, where only one simulation under a single temperature is needed.

2.2. Single State Perturbation (SSP) Method. The enthalpy difference may be computed from a simulation on a single ensemble with a standard statistical mechanical perturbation formula. The ensemble average $\langle U_1 \rangle_1$ is

$$\begin{aligned} \langle U_1 \rangle_1 &= \frac{1}{Q_1} \int U_1 \exp(-\beta U_1) d\Gamma \\ &= \frac{Q_0}{Q_1} \frac{1}{Q_0} \int U_1 \exp(-\beta u) \exp(-\beta U_0) d\Gamma \\ &= \frac{1}{\langle \exp(-\beta u) \rangle_0} \langle U_1 \exp(-\beta u) \rangle_0 \end{aligned} \quad (7)$$

where $u(\Gamma) = U_1(\Gamma) - U_0(\Gamma)$ is the energy change of perturbing system 0 to system 1 under a common configuration. Thus, we have

$$\Delta U \equiv \langle U_1 \rangle_1 - \langle U_0 \rangle_0 = \frac{\langle U_1 \exp(-\beta u) \rangle_0}{\langle \exp(-\beta u) \rangle_0} - \langle U_0 \rangle_0 \quad (8)$$

Equation 8 is the simplest perturbation formula for computing the enthalpy difference. It has been used in the past by many researchers^{23,27,42,45–47} often with different names. For example, Smith and Haymet²³ call this procedure the “unified” method. Here we name it “single state perturbation” (SSP) to better reflect the nature of this approach. The method can also be used in thermodynamic integration with straightforward modifications.

Equation 8 shares the flaw present in eq 4, namely that it involves an additive term $\langle U_0 \rangle_0$ which is of order N and therefore may exhibit fluctuations of a magnitude that overwhelms the difference ΔU being calculated. The uncertainty in ΔU will suffer less if all averages in eq 8 are collected in a single simulation (i.e., using the same set of configurations), leading to partial cancellation of random errors in the two terms on the right-hand side of eq 8. Unfortunately, the cancellation of the irrelevant fluctuations is not total, because contributions to the first term arise only when the 0 system is sampling configurations important to the 1 system. When such is not the case, the term in $\exp(-\beta u)$ is small, and any energy fluctuation in the second average is unchecked by a corresponding fluctuation in the first average.

2.3. β -Perturbation (BP) Method. As mentioned in section 2.1, the finite difference method can be used with a perturbation on temperature T (or more effectively, on reciprocal temperature $\beta = 1/kT$), and the perturbation on temperature is then computed in the same FEP calculation; thus, only a single simulation under one system condition is needed (as in the SSP method).

Instead of using eq 5, we start from the thermodynamic relationship between ΔU and ΔA . The energy difference ΔU is expressed as a β -derivative of the free-energy difference

$$\Delta U = \left(\frac{\partial \beta \Delta A}{\partial \beta}\right)_{N,V} \quad (9)$$

Applying a finite difference estimate

$$\Delta U = \frac{\beta^+ \Delta A(\beta^+) - \beta^- \Delta A(\beta^-)}{2\Delta \beta} \quad (10)$$

where $\beta^+ = \beta + \Delta \beta$ and $\beta^- = \beta - \Delta \beta$. We realize

$$\begin{aligned} \exp[-\beta^+ \Delta A(\beta^+)] &= \langle \exp(-\beta^+ u) \rangle_{0,\beta^+} \\ &= \frac{\int \exp(-\beta U_0 - \Delta \beta U_0) \exp(-\beta^+ u) d\Gamma}{\int \exp(-\beta U_0 - \Delta \beta U_0) d\Gamma} \\ &= \frac{\langle \exp(-\Delta \beta U_0 - \beta^+ u) \rangle_{0,\beta}}{\langle \exp(-\Delta \beta U_0) \rangle_{0,\beta}} \end{aligned} \quad (11)$$

where $\langle \dots \rangle_{0,\beta}$ indicates the ensemble average is taken on the 0 (reference) system at the reciprocal temperature β . Similarly,

$$\exp[-\beta^- \Delta A(\beta^-)] = \frac{\langle \exp(\Delta \beta U_0 - \beta^- u) \rangle_{0,\beta}}{\langle \exp(+\Delta \beta U_0) \rangle_{0,\beta}} \quad (12)$$

Substituting eqs 11 and 12 into eq 10, we have

$$\Delta U = \frac{1}{2\Delta\beta} \ln \left[\frac{\langle \exp(-\Delta\beta U_0) \rangle_{0,\beta} \langle \exp(+\Delta\beta U_0 - \beta^- u) \rangle_{0,\beta}}{\langle \exp(+\Delta\beta U_0) \rangle_{0,\beta} \langle \exp(-\Delta\beta U_0 - \beta^+ u) \rangle_{0,\beta}} \right] \quad (13)$$

This forms the working equation of the β -perturbation (BP) method. Note that both perturbations in β and U are performed in a single simulation on reference system 0 at the reciprocal temperature β .

Fleischman and Brooks⁴⁰ proposed an equivalent formula in terms of temperature derivatives (with a different derivation)

$$\Delta U_{\lambda \rightarrow \lambda'} = \frac{kT^2}{2\Delta T} \times \ln \left[\frac{\left\langle \exp \left[\frac{-U(\lambda')}{k(T+\Delta T)} + \frac{U(\lambda)}{kT} \right] \right\rangle_{\lambda,T}}{\left\langle \exp \left[\frac{-U(\lambda')}{k(T-\Delta T)} + \frac{U(\lambda)}{kT} \right] \right\rangle_{\lambda,T}} \times \frac{\left\langle \exp \left[\frac{-U(\lambda)L_-(T)}{k} \right] \right\rangle_{\lambda,T}}{\left\langle \exp \left[\frac{-U(\lambda)L_+(T)}{k} \right] \right\rangle_{\lambda,T}} \right] \quad (14)$$

where the perturbation is conducted from a state specified by parameter λ to another state specified by λ' (e.g. 0 and 1 in our context), and

$$L_{\pm}(T) = \frac{1}{T \pm \Delta T} - \frac{1}{T} \quad (15)$$

This formula has been used in several studies.^{41,48,49}

The performance of the BP method will depend on the perturbation magnitude in the Hamiltonian as well as the magnitude of $\Delta\beta$ (or ΔT); this issue will be discussed in detail in the Results and Discussion section. The simplicity of eq 13 enables a direct extension to the modified β -perturbation method to apply multiple $\Delta\beta$ steps during the calculation to produce more precise (but not necessarily more accurate) and robust (less sensitive to the choice of $\Delta\beta$) results; this extension is described below.

2.4 Modified β -Perturbation (MBP) Method. A single estimate of ΔU can be obtained from eq 13 for each given $\Delta\beta$. An obvious extension is to use more than one value of $\Delta\beta$ during the same perturbation trial in the FEP simulation, because the choice of the perturbation in β is independent of the free energy perturbation. All the ΔU obtained in such a calculation could be used to enhance the estimate. For example, if we take K values of $\Delta\beta$ during the simulation, the overall average of ΔU from the modified β -perturbation (MBP) version is then expressed as

$$\Delta U = \frac{1}{K} \sum_{k=1}^K \frac{1}{2\Delta\beta_k} \times \ln \left[\frac{\langle \exp(-\Delta\beta_k U_0) \rangle_{0,\beta} \langle \exp(+\Delta\beta_k U_0 - \beta_k^- u) \rangle_{0,\beta}}{\langle \exp(+\Delta\beta_k U_0) \rangle_{0,\beta} \langle \exp(-\Delta\beta_k U_0 - \beta_k^+ u) \rangle_{0,\beta}} \right] \quad (16)$$

We should note that while some improvement in the precision may occur over the original BP method, eq 16 does require additional calculations in order to evaluate the ensemble averages involving all the $\Delta\beta_k$. But this computational requirement could be greatly reduced. For example, one could use an

elementary $\Delta\beta$, designated as $\delta\beta$, to form a set of $\Delta\beta$ according to

$$\Delta\beta_k = k\delta\beta \quad (17)$$

for $k = 1, \dots, K$, eq 16 then becomes

$$\Delta U = \frac{1}{2K(\delta\beta)} \sum_{k=1}^K \frac{1}{k} \times \ln \left[\frac{\langle \exp[-k(\delta\beta)U_0] \rangle_{0,\beta} \langle \exp[+k(\delta\beta)(U_0 + u)] \exp(-\beta u) \rangle_{0,\beta}}{\langle \exp[+k(\delta\beta)U_0] \rangle_{0,\beta} \langle \exp[-k(\delta\beta)(U_0 + u)] \exp(-\beta u) \rangle_{0,\beta}} \right] \quad (18)$$

Clearly an expensive evaluation of exponential operations is required for $(\delta\beta)U_0$ and $(\delta\beta)u$, as in the BP method, but all other terms involving different k can be computed by simple multiplication. Thus the computational cost is reduced relative to the original BP method.

2.5 Perturbation and Correction (PC) Method. The fifth and final perturbation approach analyzed in this paper is the “perturbation and correction” (PC) method. The key idea is to split the difference between two quantities with large fluctuations into ones with much smaller fluctuations and to maximize the utilization of error cancellation. Typically, a perturbation calculation has some built-in error cancellation (see section 2.2), but here we take an explicit approach to cancel common large fluctuations between U_0 and U_1 . To do this we rearrange eq 4 as

$$\begin{aligned} \Delta U &= \langle U_1 \rangle_1 - \langle U_0 \rangle_0 = \langle U_1 - U_0 + U_0 \rangle_1 - \langle U_0 \rangle_0 \\ &= \langle u \rangle_1 + [\langle U_0 \rangle_1 - \langle U_0 \rangle_0] \end{aligned} \quad (19)$$

Notice that the term $\langle U_1 \rangle_1$ has been split into two components. The first component, $\langle u \rangle_1$, is referred to as the “perturbation” term here—it is the ensemble average of the energy difference u taken over the target system in a perturbation calculation. Since the 0 and 1 systems share a common configuration during the perturbation, irrelevant (and large) fluctuations of the configurational energies are canceled out *exactly*. Therefore $\langle u \rangle_1$ does not suffer the imprecision problem of the direct approach (providing the configuration is within the common important phase space region of both the 0 and 1 systems; choosing the 1 system to be the lower entropy one between the two—as it is in this paper—is required to satisfy this condition). When the perturbation from the 0 to the 1 system is small, a favorable outcome for a FEP calculation, $\langle u \rangle_1$ would typically be the major contribution to ΔU . In this case, the terms in the square brackets can be considered as a (small) correction to $\langle u \rangle_1$ in order to obtain the correct ΔU . For this reason, we call it the “correction” term and the method “perturbation and correction” (PC).

Physically, the correction term is the average energy of the 0 system when sampled over the 1 system, minus the same when averaged over the 0 system. This difference will be small if the configurations important to the 1 system are representative of those important to the 0 system. One requirement for this situation to hold is that the configurations important to the 1 system are a subset of those important to 0. If configurations of 1 are not important to 0, they cannot be representative of the 0 system, and we cannot expect this difference to be small. Consequently, we must expect the performance of the PC method to be highly asymmetric—it may work in one direction, but it will fail if the roles of the 0 and 1 systems are switched.

We may further consider a perturbation approach for the second term, leading to

$$\Delta U = \langle u \rangle_1 + \frac{\langle U_0 \exp(-\beta u) \rangle_0}{\langle \exp(-\beta u) \rangle_0} - \langle U_0 \rangle_0 \quad (20)$$

We expect the major imprecision of eq 20 will be from the correction term. Note that the correction term expressed in eq 20 is quite close to the formula used in the SSP method. Thus, it is expected that the current version of the PC method will have similar performance to the SSP method. Likewise, while we expect some cancellation of fluctuations in taking the difference using data from a single simulation, the cancellation (unlike that for $\langle u \rangle_1$) is not total. The real advantage of the PC method is realized if it can be used without the correction at all, thereby eliminating a great deal of the imprecision in the result. The consequence, of course, is that the result may be systematically incorrect, but the hope would be that this loss in accuracy would be less than the gain in precision, in other words, that the correction term is in fact zero within its confidence limits.

Another development for considering the energy change distributions in the FEP calculation leads to essentially the same formalism as eq 20. The energy-change distributions were first studied by Shing and Gubbins⁵⁰ in the context of chemical potential calculation. Here, we denote the energy-change distribution function as p_i , where $i = 0$ or 1 is the reference system for the FEP calculation. It has been shown¹⁹

$$\Delta U = \int du \left[u - \frac{\partial \ln p_0(u)}{\partial \beta} \right] p_1(u) \quad (21)$$

and

$$\frac{\partial \ln p_0(u)}{\partial \beta} = - \left[\frac{\langle U_0 \delta \rangle_0}{\langle \delta \rangle_0} - \langle U_0 \rangle_0 \right] \quad (22)$$

where δ is the Dirac delta function applied to the energy change u ; the ensemble average then gives the distribution functions $p_0(u) = \langle \delta \rangle_0$ and $p_1(u) = \langle \delta \rangle_1$.

We have

$$\Delta U = \int u p_1(u) du + \frac{1}{\exp(-\beta \Delta A)} \int [\langle U_0 \delta \rangle_0 - \langle U_0 \rangle_0 \langle \delta \rangle_0] \exp(-\beta u) du \quad (23)$$

Equation 23 is equivalent to eq 20, but it reveals the nature of the correction term as a fluctuation average, in the sense of the heat capacity or compressibility.

Again, to obtain accurate results, the perturbation term should be computed on the lower entropy system (system 1 in this study), unless the important phase space regions of 0 and 1 are very close to each other. It is also necessary that the important configurations of the 1 system form a subset of the important configurations of the 0 system.

The names and working equations of all methods discussed above are summarized in Table 1 for further reference throughout this paper.

3. Model and Simulation Methods

We performed simulation tests for the five perturbation methods discussed in the previous section using both a Lennard-Jones (LJ) fluid system and an alchemical change from one anion in solution to another. Due to the large fluctuation in the

TABLE 1: Summary of the Name and the Working Equation for All Methods Discussed in This Paper

method name	working equation
direct method	4
finite difference (FD)	6
single state perturbation (SSP)	8
β -perturbation (BP)	13 or 14
modified β -perturbation (MBP)	18
perturbation and correction (PC)	19 or 23

relative enthalpy evaluation, typically very long simulations are required to produce statistically meaningful results (i.e., the convergence rate is very slow). The simplicity of both of these systems is thus a particularly welcome feature, because it enables us to get good results using reasonable amounts of computation. With these model systems we can perform systematic investigation of the different methods, helping us to understand and reveal possible common behavior. These features can be lost when applied to more complex systems, such as full-scale protein and protein:ligand systems. Without a good understanding of the results from different methods obtained from simple systems, fundamental phenomena would be hard to analyze in more complex systems.

For LJ fluids, the energy difference between different states can be computed with reasonable accuracy using the direct method for a wide range of perturbations, even when the perturbation is small. (In general, given enough computer time the direct method is considered to be more reliable for large perturbations where a perturbation method over the same scale would fail.) Thus the computation of ΔU by the direct method provides a standard for measuring the accuracy of the other methods. Our previous work^{19,51} on free energy perturbation methods suggested that the change in entropy may be the appropriate quantity to characterize the magnitude of the perturbation, and here we consider whether it may also be the key quantity for determining the reliability of enthalpy-difference calculations. Thus, we started the analysis aiming to determine how the calculation of enthalpy (or energy) differences are affected by changes in entropy along the reaction coordinate within each system.

Molecular dynamics (MD) computations on relative solvation between two anion types were examined as the second test system for the methods of this paper. For these calculations the CHARMM program⁵² was used with the TIP3P water model.⁵³ Perturbations were performed with the BLOCK array feature of CHARMM, and initially 13 equally spaced λ points (12 intervals) were used along the reaction coordinate that takes one anion type into the other.

Further details of both simulation studies are provided in the Appendix.

4. Results and Discussion

We performed Monte Carlo simulations of a LJ fluid and molecular dynamics simulations of anion solvation with five different perturbation schemes and different choices for staging designs. In both sets of simulations the relative differences between the five different perturbation formulas for the enthalpy are less extreme than the differences found from improper staging of the intermediates. That is, the best choice for improving the relative overall error of enthalpy and entropy calculations comes from the best placement of the intermediates of each perturbation.

To set the scale for our MC simulations, the effect of the alchemical LJ particles' radius was first considered. Thus, the entropy change ΔS for a perturbation is investigated as a

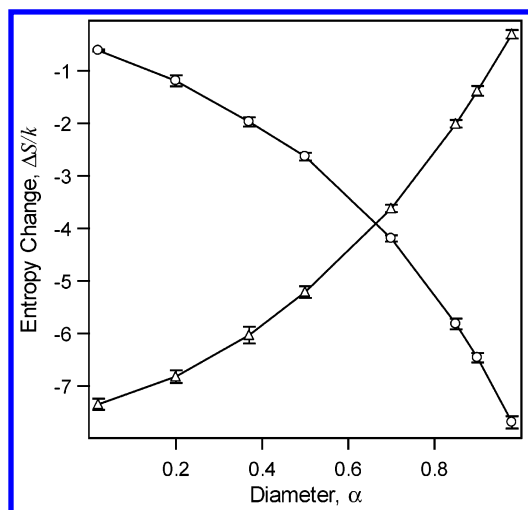


Figure 1. Entropy change ($\Delta S/k$) in the first-stage (open circle; 0 to W) and second-stage (open triangle; W to 1) FEP calculation of a Lennard-Jones (LJ) system as a function of the diameter (α) of the special alchemical LJ particle in the W system. Reduced units are applied for entropy and diameter. This calculation enables us to choose a particular entropy change by setting the α diameter.

function of the diameter α of the alchemical LJ particle in the W state. $\Delta S/k$ is calculated using eq 2, where ΔA is computed using FEP in the generalized insertion direction^{18,19} (i.e., the direction with negative entropy change; in this particular case, it is from 0 to W for the first stage and from W to 1 for the second) and ΔU is computed using the direct method (believed to be reasonably accurate under current system definition and simulation conditions). Figure 1 shows the $\Delta S/k$ results from both stages. The error bar of $\Delta S/k$ is very small, indicating a precise calculation. From this figure we can examine the range of perturbation methods as a function of $\Delta S/k$. We are especially interested in times when $\Delta S/k$ has an absolute value around 1–2—previous work has suggested that changes in this range of $\Delta S/k$ will give the most reliable estimates of free energy differences with a single stage perturbation.²⁰

Our results for computing enthalpy changes (ΔU) with the five different methods, as well as their error bars, are presented in Figure 2 as a function of $\Delta S/k$ for MC perturbation changes from both 0 to W (part a of the figure) and 1 to W (part b) and for two directions within MD for the anion system (parts c and d). The results with the BP method in the plots used $\Delta\beta = 0.1$; the MBP method used $K = 10$ and $\delta\beta = 0.01$. From all four parts of Figure 2, note that the absolute value of enthalpy change (ΔU) becomes larger as the magnitude of ΔS increases (note that Figure 2c,d has the smallest changes on the left, while Figure 2a,b has the smallest changes on the right). This can be rationalized, since the energy difference between the perturbation systems depends on the perturbation size—characterized by the entropy difference. If we use the results of the direct method as a standard for measuring the accuracy of the perturbation results for the LJ system, we see that the inaccuracy is an increasing function of $\Delta S/k$. For small changes in $\Delta S/k$, these perturbation methods do well, but the quality of the result degrades as the magnitude of $\Delta S/k$ increases; finally, at large $\Delta S/k$, the results become unreliable. A major finding of this figure is that the precision of ΔU calculated by all five perturbation methods is well-determined by the magnitude of $\Delta S/k$ (see parts a–d). These observations suggest that the entropy change for the perturbation step is an important quantity for determining the reliability of the computational change in both enthalpy and entropy.

An important point to note about the perturbation methods is that their precision depends on the perturbation size, since they are collected at one state and extended to the other state. This does not apply for the direct method (with sufficiently long CPU time), since the uncertainty is constant, regardless of the value of $\Delta S/k$. This is expected, since the method uses a difference from two independent simulation measurements—its uncertainty depends only on the statistics of each of the measurements, but not the magnitude of the difference. Thus, while perturbation methods can provide more precise estimates of ΔU when the perturbation is small, the direct method outperforms when the perturbation becomes large (see Figure 2). This point has been commented on before, for example, in a recent study by Gallicchio et al.,²⁷ where it is noted that when the difference between two systems are large, it is better to use the direct approach to evaluate ΔU . What is illuminating about the current results is that it provides insight into when the relative performance of the direct method is improved or worse relative to the perturbation approach. We expect that the switching point (or switching range) can be specified in terms of entropy. Still, we do emphasize that the current results do not comment on whether any common switching point/range exists for different kinds of systems. For the present LJ system, we find that a value of 3 for $|\Delta S/k|$ (and around 2 for anion system, see Figure 2c,d) can roughly indicate the switching point. Considering that the best application range of the free-energy perturbation is for $|\Delta S/k| = 2$, it is likely that the perturbation method can give better results for ΔU than the direct approach in situations where a FEP calculation is effective.

When examining the performance of the five different perturbation methods by looking at Figure 2, another point one may notice is the discrepancy between the forward and reverse results and their error bars of the SSP, BP, and MBP methods. Using the changes in ΔU by the direct method as a standard, the reverse calculation results are generally less accurate than the forward counterpart. Meanwhile, the uncertainty of the reverse results can be much larger than that of the forward results. From the plots, neither the accuracy nor the precision of the forward/reverse results is symmetric. The asymmetry of the free energy perturbation calculation has been noted in previous studies,^{18,19,51} and it is not surprising to note that the relative changes in enthalpy have similar behavior. In the case of free energy calculations, it was found that the calculation performed in the generalized insertion direction is more reliable than that in the generalized deletion direction. In the current case, the definition of the forward direction is coincident with the generalized insertion direction (entropy decreases). One observation is thus that the forward (generalized insertion) direction is superior for both free energy and enthalpy difference calculations. For small perturbations, calculations in both directions may produce practically equivalent results, because the asymmetry issue is less strong. In general, calculation in the insertion direction is more reliable.

Within the same context of forward or reverse calculations, the performance of different methods, i.e., the single stage perturbation (SSP), β -perturbation (BP), and its modified version (MBP) are quite close. The perturbation and correction (PC) method almost always produces identical results as the SSP method, both in terms of accuracy and precision, except in the very large perturbation region. (But note that the PC method requires independent simulations for both 0 and 1 states; thus, it needs double the computational time relative to the SSP method.) As mentioned in section 2.5, this is the outcome of our current implementation for the correction term, i.e., the same

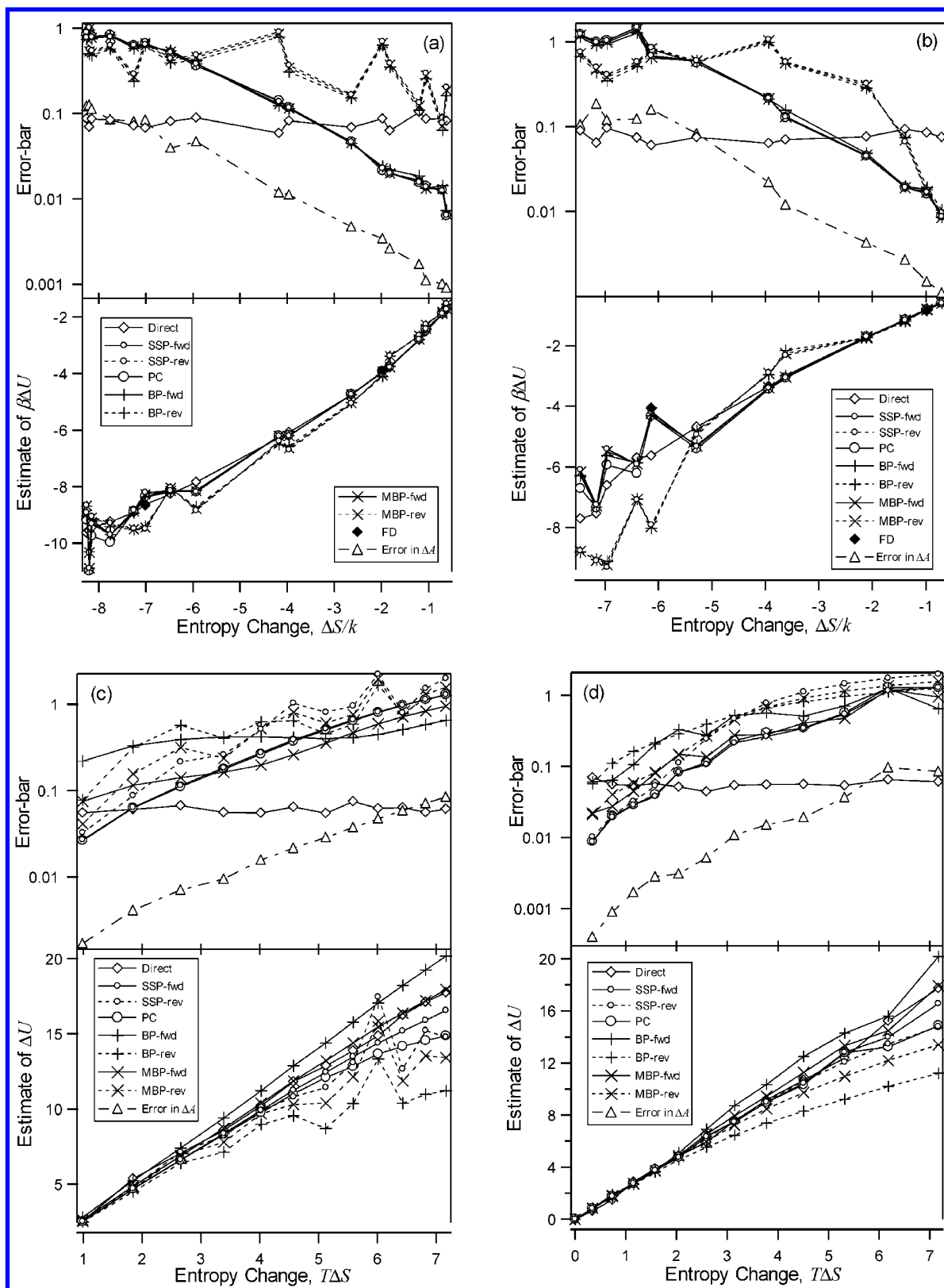


Figure 2. Enthalpy difference ΔU computed by five different methods as a function of entropy change (representing the perturbation magnitude) with the perturbation. Estimates of ΔU are shown in the lower half, and corresponding error bars, as well as the error bar of ΔA , are shown in the upper half. Note that for small entropy changes (about 1–2) all methods give similar estimates for the enthalpy change, while the error estimates change for the different methods. All quantities in parts a and b are in reduced LJ parameter units, while kcal/mol is used in parts c and d. Note for the results in parts c and d, only solvent–solute interaction energy is counted in computing ΔU . For the FD results presented in parts a and b, the parameters are $\beta^+ = 1.1$, $\beta^- = 0.9$ for the data points located near $\Delta S/k = -2$ in part a and $\Delta S/k = -6$ in part b, and $\beta^+ = 1.05$, $\beta^- = 0.95$ for the data points located near $\Delta S/k = -7$ in part a and $\Delta S/k = -1$ in part b.

way that the SSP method is formulated. In Table 2, some example results from the first-stage calculation for the LJ system

are presented to demonstrate the contribution of the perturbation term to the overall ΔU computed by the PC method, and the

TABLE 2: Contribution of the Perturbation Term to the Final Results of ΔU by the Perturbation and Correction (PC) Method^a

$ \Delta S/k ^b$	$\langle u \rangle_1/\Delta U_{PC},^c \%$	$\delta_{\langle u \rangle_1}/\delta \Delta U_{PCd}$	$\delta_{\langle u \rangle_1}/\delta \Delta A^e$
0.6	99.0	0.095	0.67
1.0	96.5	0.065	0.60
1.8	98.6	0.061	0.75
2.0	98.8	0.056	0.43
4.0	95.3	0.041	0.43
6.5	88.8	0.005	0.06

^a The data are taken from the first stage FEP calculation for the LJ system. ^b The absolute value of $\Delta S/k$ for the perturbation. ^c The percentage of the ΔU contributed by the perturbation term. ^d The ratio of error bars of the perturbation term and that of ΔU . ^e The ratio of the error bars of the perturbation term and that of the free energy difference computed in the forward direction.

TABLE 3: Error Bar of ΔU ($\delta_{\Delta U}$) Computed using SSP and Those of Two Terms (δ_{T1} and δ_{T2}) in the Right-Hand Side of Eq 8 for the LJ System^a

series	$ \Delta S/k $	forward			reverse		
		δ_{T1}	δ_{T2}	$\delta_{\Delta U}$	δ_{T1}	δ_{T2}	$\delta_{\Delta U}$
1	0.7	0.060	0.058	0.012	0.091	0.070	0.067
2	1.0	0.072	0.073	0.016	0.055	0.049	0.016
3	2.0	0.064	0.059	0.024	0.034	0.057	0.035

^a Results of both calculations in the forward and reverse directions are shown. The smaller error bar in ΔU than that in the first term in the right-hand side of eq 8 indicates some error is canceled out in the minus operation. All quantities are in reduced units according to LJ energy and diameter parameters. Note the results of series 1 and 3 are for the first stage of calculation and that of series 2 is for the second stage calculation.

ratio of the error bars of $\langle u \rangle_1$ and ΔU , as well as the ratio of the error bar of $\langle u \rangle_1$ and ΔA , is also shown. As one can see, the error bar in the perturbation term $\langle u \rangle_1$ is very small—even smaller than that in ΔA . As a result, the precision of the PC results is mainly decided by the uncertainty of the correction term. In addition, $\langle u \rangle_1$ seems able to contribute most of the ΔU ; therefore, in some cases it is possible to only compute $\langle u \rangle_1$ and use it as a quick and dirty estimate of ΔU . However, we cannot say for sure when such an estimate would be effective in most cases.

Careful examination shows that the SSP method (and PC) performs the best among all the perturbation methods (we are not including the FD method here). The MBP method shows improvement over the BP version; its results are typically very close to those of SSP. In some cases, it could be even slightly better than SSP in terms of precision, but such improvement is very minor and inconsistent and more likely to be random events. In general, the SSP method sets up a limit for the performance of the BP and MBP methods.

We believe that the cancellation of random errors between the first and second term in the right-hand side of eq 8 contributes to the performance of the SSP method. The data in Table 3 demonstrate such a cancellation effect: for example, the overall error bar in ΔU is less than that in the first term of the right-hand side of eq 8.

To enable further comparison of the five methods, we plot in Figure 3 (a and b) estimates of enthalpy change and their error for both MC and MD simulations. In these plots the height of the box is the estimate and the error bars are shown as stippled features on the box ends. Plotted in this way, the relative differences between the five methods are made clearer. Equally interesting is the differences between the MC and MD datasets. For example, the MC results show the marked asymmetry between those determined in the forward versus the reverse

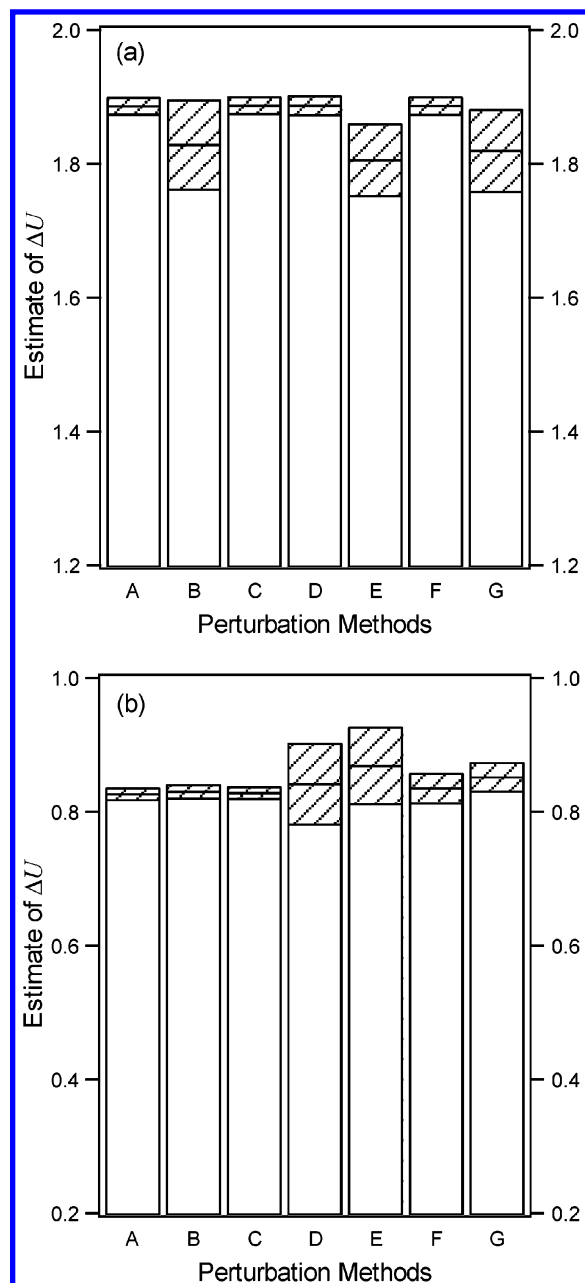


Figure 3. A direct comparison of ΔU estimates by the different perturbation methods at small perturbation sizes. The perturbation for part a is the diameter going from zero to 0.05 for the LJ system; the perturbation for plot b is λ changing from 0.917 to 1.0 for the anion system. The units are in reduced unit for LJ system, and kcal/mol for anion system. Key to methods: (A) SSP in the forward direction, (B) SSP in the reverse direction, (C) PC method, (D) BP in the forward direction, (E) BP in the reverse direction, (F) MBP in the forward direction, and (G) MBP in the reverse direction.

directions. The SSP, BP, and MBP methods are all reflecting much larger errors and a clear difference in estimated change in ΔU between the forward and reverse directions, with the forward direction being much the better of the two. Note also for the MC datasets that the error size and prediction quality is about the same for a forward perturbation for the SSP, PC, BP, and MBP methods. In contrast, for the MD results, the largest error is for the BP method in both forward and reverse directions. The next largest is for MBP in both forward and reverse directions. The smallest error, and that is seemingly independent of direction for this perturbation, is from the SSP and PC calculations.

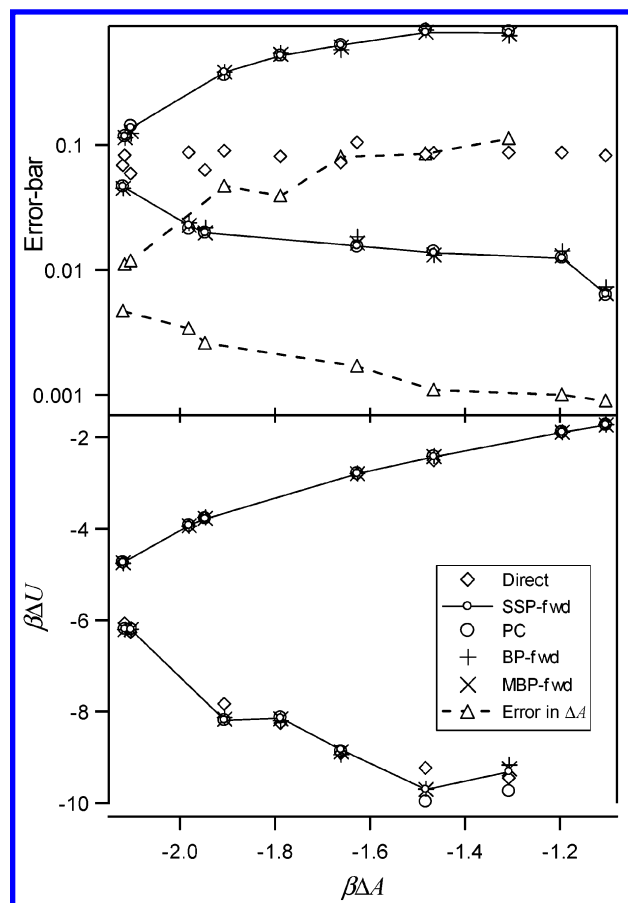


Figure 4. Enthalpy differences ΔU (lower half) and their error bars (upper half) as a function of the free energy difference ΔA for the first-stage perturbation (0 to W) of the LJ system. The differences between the methods are smaller in their estimates of enthalpy change, while the error estimates are more variable. For ΔU , only the results of the direct method, the PC method, and the SSP, BP, and MBP methods in the forward direction are shown. The curve is bifurcated due to multiple values of ΔU for a given change in ΔA .

We note that the relative performance of different perturbation methods applies over the entire simulation length being investigated. The same conclusion would be drawn had we reported the results using another set of simulations with different length, say, half of the current value. The error decay rate as a function of simulation length is roughly the same for all the methods conducted in the same direction (if applicable).

In contrast to our finding of the importance of the size of the change in entropy during the perturbation, a similar approach using ΔA is not as strong a predictor for characterizing the quality of each perturbation method. For example, in Figure 4, the same set of perturbations in ΔU and the error bars of Figure 2a are replotted as a function of ΔA . No general relationship is observed. Instead, both the values of ΔU and its error bar form two branches as ΔA changes. The common behavior of the methods would have been hidden if ΔA was examined as the only staging metric.

The performance of the BP method (similar for MBP) depends on the magnitude of $\Delta\beta$ ($\{\delta\beta, K\}$ for MBP), as well as the magnitude of perturbation in the Hamiltonian. It is possible to improve the reliability of the result by tuning the value of $\Delta\beta$. Numerical results show that the smaller $\Delta\beta$ is, the better the accuracy and precision for the results. When $\Delta\beta$ approaches zero, the results of BP approach those of SSP (both in precision and accuracy). Figure 5 demonstrates two examples of such numerical results—the MBP results are also shown. For

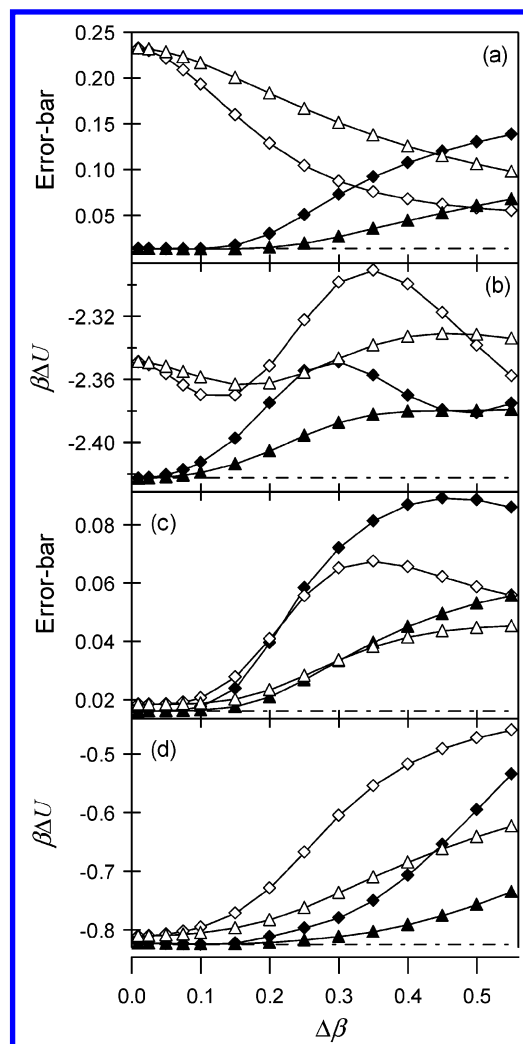


Figure 5. The performance of the BP and MBP methods as a function of parameter $\Delta\beta$ applied to the LJ system. The forward SSP results of ΔU with error bar are used as for a standard of comparison. (a) Enthalpy difference ΔU , (b) error bar of ΔU for a first-stage calculation having $\Delta S/k \sim -1.0$, (c) enthalpy difference ΔU , and (d) error bar of ΔU for a second-stage calculation having $\Delta S/k \sim -1.0$. Labels: filled diamond, forward BP; open diamond, reverse BP; filled triangle, forward MBP; open triangle, reverse MBP; dot-dashed line, forward SSP. Note the differences between forward and reverse and between the computed enthalpy change for the first relative to the second stage.

the MBP results in the plots, parameter $\delta\beta = \Delta\beta/K$ with $K = 10$ used. As one can see, MBP does not break the SSP limit. But it always reaches the SSP limit at a larger $\Delta\beta$ than the BP method, for both the accuracy and precision, and it is less sensitive to the choice of $\Delta\beta$ —on the plots, the MBP curves are flatter than those of BP. Thus the MBP method is more robust than the BP version.

Another finding, already seen at this point of the study (Figure 2), is that the best precision of ΔU obtained by a perturbation approach is about 10+ times worse than that of ΔA (whose error bar is represented there by the dotted line with open squares). Interestingly, the curve of the uncertainty of ΔU is found almost parallel to that of ΔA (in log scale).

Two set of results computed using the FD methods are also presented in Figure 2 (as filled circles). The FD method may be able to provide more precise results than the other perturbation methods, because the large fluctuation problem in the direct method is avoided by using the information of ΔA directly, but this improvement is not always going to happen. Even when it does a better job, the improvement may not be significant and

may not be quite worth the additional simulations under two different temperatures. The performance of the FD method depends on two components: first, the perturbation size of the FEP calculation and, second, the validity of the linear assumption of $\Delta A \sim T$. The perturbation size affects the precision and accuracy of the free energy difference, and any such effects on ΔA will be propagated to ΔU . The choice of ΔT or $\Delta\beta$ will mainly affect the accuracy of the calculation. When ΔT or $\Delta\beta$ is too large, the linear assumption of $\Delta A \sim T$ may break down and the FD method would produce an inaccurate estimate of ΔU . To better satisfy the linear relationship, a small ΔT or $\Delta\beta$ is preferred. However, a smaller ΔT (or $\Delta\beta$) may lead to a larger propagation of error from ΔA , as indicated by the following analysis. Assuming that calculations of ΔA under different temperatures are conducted independently, the propagation error in ΔS is

$$\delta_{\Delta S/k} = \frac{\delta_{\Delta A}}{\sqrt{2}\Delta T} \quad (24)$$

and in ΔU is

$$\delta_{\Delta U} = \frac{\delta_{\Delta A}}{\sqrt{2}} \sqrt{1 + \left(\frac{T}{\Delta T} - 1\right)^2} \rightarrow \frac{\delta_{\Delta A} T}{\sqrt{2}\Delta T} \text{ for small } \Delta T \quad (25)$$

where the uncertainty of ΔA under different temperatures has been treated to be the same ($\delta_{\Delta A}$). Clearly, when ΔT becomes small, the uncertainty in ΔU and $\Delta S/k$ could be huge. Similarly, in terms of β , the uncertainty of ΔU can be written as

$$\delta_{\Delta U} = \frac{\delta_{\Delta A}}{\sqrt{2}} \sqrt{1 + \left(\frac{\beta}{\Delta\beta}\right)^2} \rightarrow \frac{\delta_{\Delta A}\beta}{\sqrt{2}\Delta\beta} \text{ for small } \Delta\beta \quad (26)$$

Like eq 25, eq 26 indicates that the uncertainty in ΔU is a decreasing function of $\Delta\beta$, and the uncertainty in ΔU can be significant when $\Delta\beta$ is small. This analysis suggests that in order to reduce the propagation error in ΔU , one should choose a larger possible value of ΔT or $\Delta\beta$, while such a choice should still maintain the linear approximation of $\Delta A \sim T$ to avoid severe inaccuracy problems. The optimal choice of ΔT or $\Delta\beta$ will be related to the specific system under investigation; therefore, we do not carry further analysis to pursue a common solution.

Further insight into the nature of our analysis and results at this stage can be seen by reflection in Figure 6. In this figure, we plot the changes in entropy, enthalpy, and free energy as a function of the reaction coordinate. Notice that both systems clearly have entropy–enthalpy compensation. In Figure 6a, the changes in free energy are consistently small, while the diverging entropy and enthalpy values grow much larger as the diameter decreases. For the anion system, a similar conclusion results, yet the change in relative free energy grows quickly and then starts to plateau as the relative alchemical point between the two anion types is increased. For this system as well, the enthalpy dominates over the entropy, with the larger contribution becoming more marked as the scale of the alchemical change is increased toward the end point.

Many readers might be tempted to argue, even at this point, that staging of a complex reaction could as readily be performed by looking for equal changes in free energy ($\Delta\Delta A$) as in entropy ($\Delta\Delta S$) or enthalpy ($\Delta\Delta U$). That might be true for some particular systems, but will not generally be true. We note that, by examining these two relatively simple systems (Figure 7 a and b), not all three quantities change in the same way or meet at a common zero point. This is especially true for the LJ system,

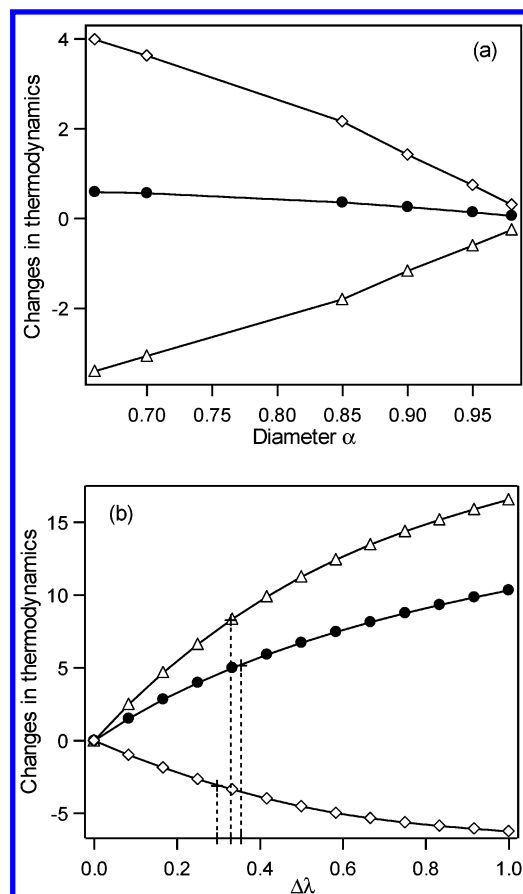


Figure 6. Changes in entropy, enthalpy, and free energy, as well as the entropy/enthalpy compensation in both model systems. (a) The Lennard-Jones system. The perturbation is from state W to state 1. The abscissa indicates the diameter α of the alchemical LJ sphere. Quantities are in the LJ reduced units. (b) The anion system. The perturbation is conducted from a $\lambda = 0$ state to a state with $0 \leq \lambda \leq 1.0$. Quantities have units of kcal/mol. The dotted vertical lines with plus signs at ends in plot b indicate the locations of $\Delta\lambda$ for the middle values of the change in thermodynamics quantities. Labels: diamond, ΔS ; triangle, ΔU ; circles, ΔA .

where the zero of $\Delta\Delta S$ change is at 2.76 on $\Delta\Delta A$ and the slopes of both $\Delta\Delta S$ and $\Delta\Delta U$ versus $\Delta\Delta A$ are not identical. By contrast, for the anion system the relative changes in $\Delta\Delta A$, $\Delta\Delta U$, and $\Delta\Delta S$ meet near a common origin and are more closely similar. Yet, it is clear that the three do not meet at a common origin (see the blowup in Figure 7b; also see Figure 6b for different positions of middle values marked by dotted vertical lines), and that only one choice should be used to give the optimal staging size for steps along the reaction coordinate.

The most important observation from the perturbation results presented in this paper is the relationship between the overall error of ΔU for the calculation as a function of the step in perturbation. This issue relates to the optimal design of multistage calculations for obtaining the most precise estimate of ΔU . We computed the overall error in ΔU for a two-stage calculation using the SSP (forward) method according to the standard error propagation formula. The resulting overall error is plotted as a function of $\Delta\Delta S/k$ for the LJ and $\Delta(T\Delta S)$ for the anion systems (defined as the difference of $\Delta S/k$ between the first and second stage) in Figure 8 (a and b). A minimal overall error is found near the point where $\Delta\Delta S = 0$. At this point it is worth remarking that in previous work we proposed a heuristic for optimal staging of FEP calculation,¹⁸ viz., the minimal overall error in ΔA roughly corresponds to the entropy difference between stages to be zero, i.e., $\Delta\Delta S/k = 0$. For reference, the

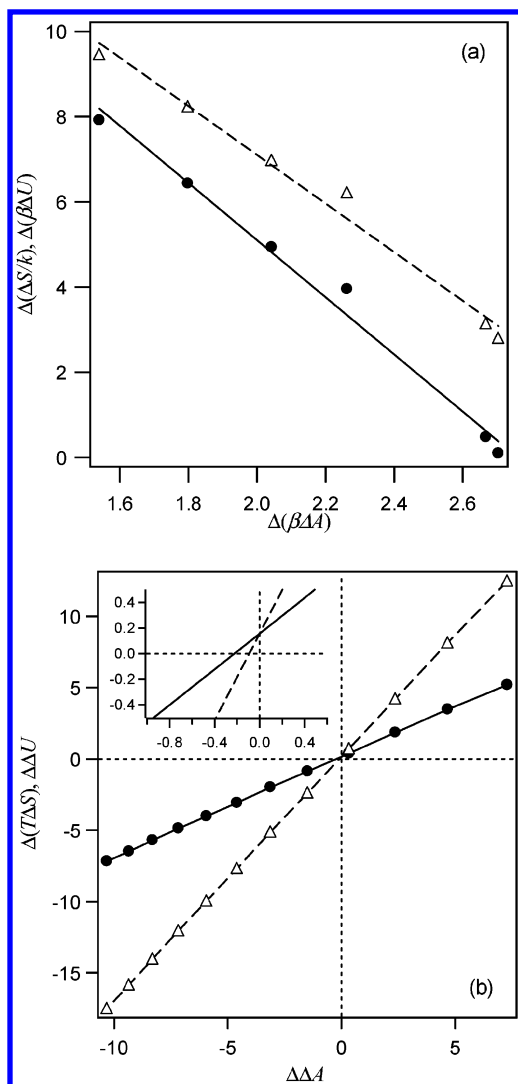


Figure 7. Demonstration of the lack of correlation between $\Delta\Delta$ changes in entropy, enthalpy and free energy. The results of $\Delta\Delta A$, $\Delta\Delta S$, and $\Delta\Delta U$ correspond to the difference between the two-stage calculations. (a) The Lennard-Jones system. Quantities are in the LJ reduced units. The straight lines are obtained by linear fitting. (Only those data on the lower branch of $\Delta U \sim \Delta A$ Figure 4 are shown.) (b): The anion system. The two-stage calculations are conducted as $\lambda = 0 \rightarrow \lambda_w$ and $\lambda = \lambda_w \rightarrow 1.0$, where λ_w has a values between 0 and 1. Quantities have unit of kcal/mol. The inset shows the enlargement of the region of $\Delta\Delta A$ and $\Delta\Delta S$ close to zero. Zero lines are explicitly shown in plot b. Labels: filled circles, $\Delta\Delta S$; triangles, $\Delta\Delta U$.

overall error bar of ΔA is also plotted in Figure 8. Similarly to the results of Figure 7, we do not see a similar trend for $\Delta\Delta A = 0$. Such behavior in ΔU related to $\Delta\Delta S/k$ is not surprising, given what we know about ΔA : in Figure 2, we observed that the $\delta_{\Delta U}$ vs $\Delta S/k$ curves are almost parallel to that of $\delta_{\Delta A}$ vs $\Delta S/k$.

Given similar computer time, a natural question is whether the direct method is to be preferred to a multistage perturbation method. As we argue below, we cannot answer this question for the general case, but it is illustrative to consider it for the present system. To this end, we evaluated energy changes in the anion system using different methods with equal amounts of production time. We selected stages of equal $\Delta S/k$ and considered a range of reference–target system pairs separated by multiples of the chosen $\Delta S/k$. Thus, we use a reference of $\lambda = 0$ and consider target systems differing from it by values of λ such that the total entropy difference is $\Delta S/k$ or $2\Delta S/k$ or $3\Delta S/k$, etc. We use a simulation of $M/2$ MD steps (where M corresponds to a 1 ns simulation) to compute the energy for

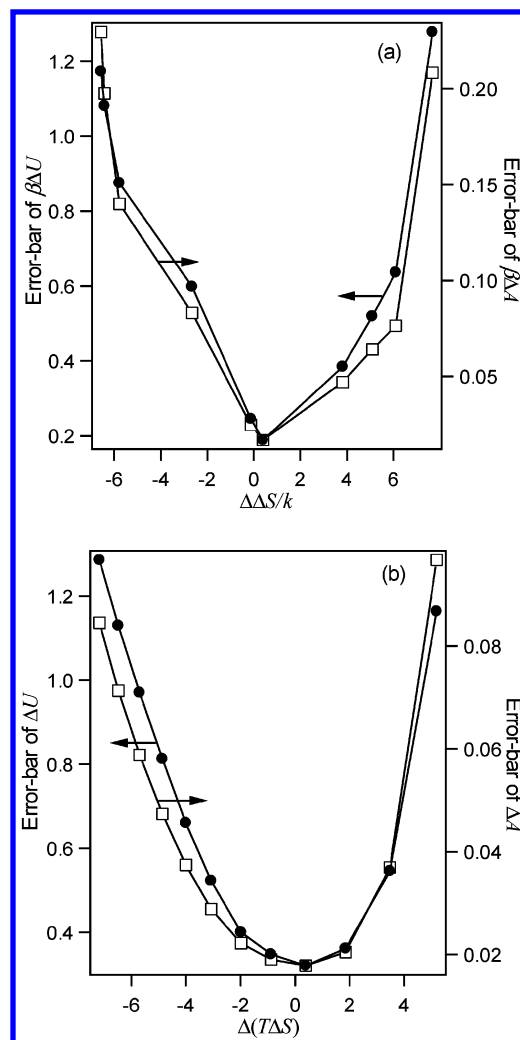


Figure 8. The overall error of enthalpy (ΔU ; filled circle) and free energy (ΔA ; open square) for the two-stage calculation as a function of the difference of entropy changes $\Delta\Delta S$ in the two perturbation stages. $\Delta\Delta S$ is defined as the ΔS of stage 2 minus that of stage 1. Thus a low value of $\Delta\Delta S$ reflects a balance between two stages. (a) The Lennard-Jones system. Quantities have LJ reduced unit. (b) The anion system. Quantities have units of kcal/mol.

each system and thus apply a total of M MD steps to compute ΔU using the direct method, taking the difference in the energy measured for a reference–target pair. For the staged perturbation method, we chose the SSP scheme in the forward direction, since it produces more precise results than other perturbation methods. Then, for the reference–target pair having total entropy difference $n\Delta S/k$, the total energy difference was computed using n simulations each of duration M/n , each giving the incremental energy difference for one $\Delta S/k$ stage. The error bar for the total energy is given by the root-sum-square of the error of the individual steps; 15 simulations are repeated independently for each perturbation to generate the precision information. As an additional comparison, we applied a single-stage SSP calculation of duration M to compute the total energy difference for each $n\Delta S/k$ reference–target pair. We examined two values of the elementary entropy step, $\Delta S/k = 1.5$ and $\Delta S/k = 2.0$; because the maximum total $\Delta S/k$ for this anion system is about 6.0, we can examine the use of up to $n = 4$ and $n = 3$ SSP stages, respectively. For perspective, it can be noted that our previous study of free energy calculations has shown that the staging size for optimal precision of a total free energy difference has $\Delta S/k = 2.0$.

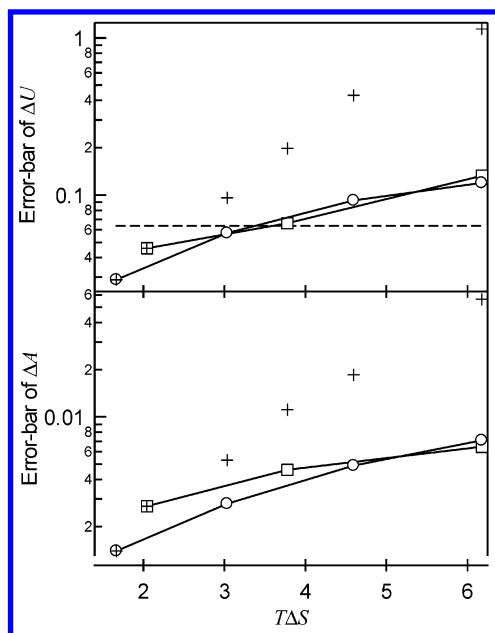


Figure 9. Comparison of precision for the anion system results computed by the staged perturbation (SSP in the forward direction) method and the direct approach given the same computational time for both (upper half). For staged SSP, the staging is roughly formed according to two choices of ΔS : $\Delta S = 1.5$ (open circle) and $\Delta S = 2.0$ (open square). The dashed line indicates the (constant) error bar of the single-stage SSP results (plus sign). The error bars of free energy difference ΔA computed in the same perturbation direction are given in the lower half of the plot; labels have the same meanings as those for ΔU .

Results of this calculation are presented in Figure 9; for reference, the error bars of single-stage and multistage ΔA results are also presented. Recall that the direct approach produces a constant error bar, regardless of the perturbation magnitude, while the imprecision of perturbation methods (both single and multiple staged) is an increasing function of $|\Delta S|$. However, the rate of increase in imprecision for the staged method is much lower than that of the single-stage perturbation—this results in a broader working range ($\Delta S/k \sim 4$) for the perturbation method relative to the direct method, whereas the single-stage method is viable only to about $\Delta S/k \sim 2.5$. The indication here that the direct method is to be preferred to staging methods for total entropy changes $\Delta S/k$ larger than about 4.0 cannot be considered conclusive. First, we have not considered thoroughly the effect of the size of the elementary step, although it is unlikely that this consideration will affect the conclusion much. More important is the likely dependence of this break-even point with the nature of the system, even something as simple as the system size. If we had (for example) doubled the size of the system being studied here, the magnitude of the energy and entropy changes would be largely unaffected, so we could use the same number and type of stages. However, the error bar of the energy difference computed by the direct method would increase proportionally with the square-root of system size, and thus by a factor of $\sqrt{2}$; the imprecision of the staged calculations would increase as well, but probably not to the same extent. So as regards whether and when staging is preferred to direct ΔU calculation, the general question remains open.

5. Concluding Remarks

One might expect that the approach to the computation, i.e. what perturbation formula to use, is far more important than the staging of the computation. By staging, we mean the size

of the changes along a predefined reaction coordinate. In this study, consistent with other results, we argue that the main determinant of error in the calculation is the size of the perturbation step measured in entropy change units. The formula for the perturbation matters less, by this standard. We further argue, in agreement with previous results, that the direction of the change is important and needs to be considered.

In terms of the perturbation formula used, the perturbation and correction method (PC) brings some ideas for tackling the precision of the calculations, but no large improvement is seen in this study. Due to the nature of perturbations, a method will work best when the difference between two systems is small (staging again). When the difference is large, it is better to use the direct approach to compute the energy or entropy difference. It is likely that these perturbation methods have the same application range as free-energy perturbation calculations, implying that perturbation approaches will produce better estimates of ΔU and ΔS than the direct method in conjunction with FEP calculation. The finite difference method, when the value of ΔT or $\Delta \beta$ is optimally tuned, may be able to produce superior results with additional calculations under two different temperatures; however, such an optimal value may not be easily determined. In general, considering its simplicity of implementation and reliability in results, the single state perturbation (SSP) method is our recommendation among all perturbation methods for computation of relative enthalpy studied in this paper.

This study contributes to the fundamental understanding of perturbation methods. The change in entropy presents an appropriate quantity for characterizing the expected reliability of the perturbation. In a multistage FEP calculation, staging designed according to $\Delta \Delta S/k = 0$ will give the most precise overall estimate of ΔU as well as ΔA (thus ΔS itself). Also, the perturbation calculation conducted in the opposite direction shows an asymmetric nature, as that has been found in ΔA calculations.

Since approximate methods for computing entropy changes exist and since the entropy change itself can be monitored from the perturbation, we thus suggest that perturbation information should be used to estimate the error at each step along the reaction path. This information is then used to collect new information to adjust the perturbation step. That is, we envision an interactive procedure wherein the error estimates at each step of a perturbation series are used to inform the next calculation and the entire calculation is performed with some knowledge of the overall error estimate. This continues other studies along these lines and improves the computational abilities of the algorithm to comment on changes within complex systems.

We conclude that staging of enthalpy and entropy changes has a strong impact on the quality of the result, as demonstrated by comparison of the five different perturbation schemes. Thus, the most important single quantity to characterize the predicted reliability of a relative enthalpy or relative entropy calculation is the size of the perturbation step, measured in entropy change units.

Acknowledgment. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research of the U.S. Department of Energy (contract DE-FG02-96ER14677 to D.A.K.) and by a grant from the American Cancer Society (ACS-RSG-01-048-01-GMC to T.B.W.). Computing facilities were provided by the University at Buffalo Center for Computational Research and by a Beowulf cluster in the Woolf lab.

Appendix. Simulation Details

Lennard-Jones MC. We performed the Monte Carlo simulations of a LJ fluid using a *NVT* ensemble. To conveniently study the relationship between perturbation magnitude and performance, a two-stage FEP calculation was adopted. In this FEP calculation, three states, 0, *W* and 1, are involved. The 0 state is defined to contain ($N - 1$) identical LJ particles plus one ideal gas (IG) sphere (or, equivalently, a zero interaction-diameter LJ particle); the 1 system has N identical LJ particles; and the intermediate, *W*, includes a special "alchemical" LJ particle with smaller diameter besides ($N - 1$) full-sized LJ particles (as those in the 0 and 1 systems). For the calculation, the interaction diameter α of the alchemical particle is adjusted to have a value between 0 (noninteracting IG) and 1 (the diameter of a full-sized LJ particle), thus controlling the perturbation size of the FEP calculation. The first-stage FEP calculation is conducted between the 0 and *W* states, and the second stage is between the *W* and 1 states. For each stage of the calculation, the free energy difference ΔA is computed using the standard perturbation formula, and the enthalpy difference ΔU is evaluated using all five of the methods discussed above. Results of both stages are collected and analyzed. For the SSP, BP, and MBP methods, the calculations in the two opposite directions are conducted independently. Here, we arbitrarily define the forward direction as the one from 0 to *W* in the first stage, and from *W* to 1 in the second stage. The reverse direction therefore is the opposite.

For the MC studies, we used the following state parameters: $N = 108$, density $\rho = 0.8$, and reciprocal temperature $\beta = 1.0$. Note that reduced units (defined according to the LJ energy and diameter parameters) were applied on all quantities. For each simulation the production run contains 1×10^6 MC cycles (a cycle is defined as 108 trial translational moves). At the end of each MC cycle, one FEP trial is performed and the associated energy change is computed and recorded. The statistics of the results are obtained by dividing the run into 20 blocks each with 5×10^4 perturbation trials. The error bar of the result is reported as the standard deviation of the mean.

Anion MD. The simulations were performed with 216 explicit waters in a box that was started with a periodic edge of 18.856 Å. An anion was then placed at the very center of the box and fixed to remain there throughout the calculation. Periodic boundary conditions were enforced with all three edge lengths changing together. Dynamics were performed under constant temperature (300 K) and constant pressure (1 atm). The temperature is controlled using the Nose–Hoover^{54,55} thermostat. Nonbonded interactions were represented using shifted-force Lennard-Jones potentials⁵⁶ with a cutoff distance of 9 Å (similar to other reduced models). The anion parameters were based on the Lybrand et al. parametrization⁵⁷ and are taken to represent two particular anion states, rather than being a model of any particular anion type. They are thus close, in spirit, to other calculations of anion change (e.g. Br to Cl and vice versa). For the purposes of the current paper, we selected this system due to computational tractability and not for direct comparison with anion solvation data sets.

Molecular dynamics calculations proceeded from the pre-equilibrated water box and then performed an additional 800 ps of equilibration of the system with one or the other anion type present in the system. The time step of integration was 2 fs and SHAKE⁵⁸ (with tolerance of 1.0×10^{-10}) was used on all bonds involving hydrogen. After the equilibration of the system with the anion present, production runs of 1 ns were conducted and conformations saved for the relative enthalpy,

entropy, and free energy calculations every 0.1 ps. This created a data set of 80 000 conformational points for our analysis. We also looked carefully at the use of the solvation shell approximation introduced by Gallicchio et al.²⁷ In this approach, the solvent:solvent and solute:solvent interactions are separated out and the solute:solvent interactions are computed within a solvation shell around the solute. We note that the MD simulation itself is performed under periodic boundary conditions and that this is a postprocessing step. While the size of the solvation shell may have some effect on the absolute value of our thermodynamic perturbations, it will not have an effect on the trend. In fact, we observe similar trends for computations using all solute:solvent and solvent:solvent interactions for thermodynamic quantities. Since the solvation shell approximation, for this system, also showed better efficiency with smaller error bars, we use this method for the presentation of the anion results.

Further processing of the MD results is started by computing the relative entropy changes from $T\Delta S = \Delta U - \Delta A$. For example, in the MD situation, ΔU is estimated by combining all the data available for 12 substages. The free energy change (ΔA) is estimated according to the overlap sampling method^{59,60} (related to Bennett's method). To estimate the errors in ΔU , ΔS , and ΔA , the whole set of data is divided into 16 blocks, each containing 5000 randomly resorted data points. A two-stage scheme that is analogous to that of the LJ system is used to study the staging problem. One of the 11 intermediate states with λ between 0 and 1 is used as the *W* system. The overall errors reported are the sum of those for both substages (e.g. from 0 to *W* and from *W* to 1).

References and Notes

- Boots, H. M. J.; Bokx, P. K. d. *J. Phys. Chem.* **1989**, *93*, 8240.
- Lui, Y.; Tong, L.-H.; Huang, S.; Tia, B.-Z.; Inoue, Y.; Hakushi, T. *J. Phys. Chem.* **1990**, *94*, 2666.
- Kuroki, R.; Nitta, K.; Yutani, K. *J. Biol. Chem.* **1992**, *267*, 24297.
- Sturtevant, J. M. *Curr. Opin. Struct. Biol.* **1994**, *4*, 69.
- Lee, B. *Biophys. Chem.* **1994**, *512*, 271.
- Grunwald, E.; Steel, C. *J. Am. Chem. Soc.* **1995**, *117*, 5687.
- Gill, P.; Ferretti, V.; Giolli, G.; Borea, P. A. *J. Phys. Chem.* **1994**, *98*, 1515.
- Petruska, J.; Goodman, M. F. *J. Biol. Chem.* **1995**, *270*, 746.
- Qian, H.; Hopfield, J. J. *J. Chem. Phys.* **1996**, *105*, 9292.
- Vailaya, A.; Horvath, C. J. *Phys. Chem.* **1996**, *100*, 2447.
- Gallicchio, E.; Kubo, M. M.; Levy, R. M. *J. Am. Chem. Soc.* **1998**, *120*, 4526.
- Zwanzig, R. W. *J. Chem. Phys.* **1954**, *22*, 1420.
- Bennett, C. H. *J. Comput. Phys.* **1976**, *22*, 245.
- Torrie, G. M.; Valleau, J. P. *J. Comput. Phys.* **1977**, *23*, 187.
- Radmer, R. J.; Kollman, P. A. *J. Comput. Chem.* **1997**, *18*, 902.
- Pearlman, D. A.; B. G., R. Free Energy Calculations: Methods and Applications. In *Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Ed.; John Wiley & Sons: New York, 1998.
- Kofke, D. A.; Cummings, P. T. *Fluid Phase Equil.* **1998**, *150*, 41.
- Lu, N.; Kofke, D. A. *J. Chem. Phys.* **1999**, *111*, 4414.
- Lu, N.; Kofke, D. A. *J. Chem. Phys.* **2001**, *114*, 7303.
- Lu, N.; Kofke, D. A. *J. Chem. Phys.* **2001**, *115*, 6866.
- Pearlman, D. A.; Kollman, P. A. *J. Chem. Phys.* **1989**, *90*, 2460.
- Reynolds, C. A.; King, P. M.; Richards, W. G. *Mol. Phys.* **1992**, *76*, 251.
- Smith, D. E.; Haymet, A. D. J. *J. Chem. Phys.* **1993**, *98*, 6445.
- Mark, A. E. Free-energy perturbation calculations. In *Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Ed.; John Wiley & Sons: New York, 1998; Vol. 2, p 1070.
- Jorgensen, W. L.; Gao, J.; Ravimohan, C. *J. Phys. Chem.* **1985**, *89*, 3470.
- Kusaka, I.; Oxtoby, D. W. *J. Chem. Phys.* **2000**, *113*, 10100.
- Gallicchio, E.; Kubo, M. M.; Levy, R. M. *J. Phys. Chem. B* **2000**, *104*, 6271.
- Matubayasi, N.; Gallicchio, E.; Levy, R. M. *J. Chem. Phys.* **1998**, *109*, 4864.
- Nemethy, G.; Sheraga, H. *J. Chem. Phys.* **1962**, *36*, 3401.
- Ratnaparkhi, G. S.; Varadarajan, R. *Biochemistry* **2000**, *39*, 12365.

- (31) Watanabe, M.; Reinhardt, W. P. *Phys. Rev. Lett.* **1990**, 65, 3301.
(32) Lazaridis, T.; Paulaitis, M. E. *J. Phys. Chem.* **1992**, 96, 3847.
(33) Lazaridis, T.; Karplus, M. *J. Chem. Phys.* **1996**, 105, 4294.
(34) Ashbaugh, H. S.; Paulaitis, M. E. *J. Phys. Chem.* **1996**, 100, 1900.
(35) Chandler, D.; Pratt, L. R. *J. Chem. Phys.* **1976**, 65, 2925.
(36) Karplus, M.; Ichiye, T.; Pettitt, B. M. *Biophys. J.* **1987**, 52, 1083.
(37) Schlitter, J. *Chem. Phys. Lett.* **1993**, 215, 617.
(38) Schafer, H.; Mark, A. E.; van Gunsteren, W. F. *J. Chem. Phys.* **2000**, 113, 7809.
(39) Meirovitch, H. *J. Chem. Phys.* **2001**, 114, 3859.
(40) Fleischman, S. H.; Brooks, C. L., III *J. Chem. Phys.* **1987**, 87, 3029.
(41) Brooks, C. L., III; Fleischman, S. H. *Int. J. Quantum Chem.: Quantum Biol. Symp.* **1988**, 15, 221.
(42) Liu, H.; Mark, A. E.; van Gunsteren, W. F. *J. Phys. Chem.* **1996**, 100, 9485.
(43) Tsunekawa, N.; Miyagawa, H.; Kitamura, K.; Hiwatari, Y. *J. Chem. Phys.* **2002**, 116, 6725.
(44) Press, W. H.; Teukolsky, S. A.; Vetterling, W. T.; Flannery, B. P. *Numerical Recipes: The Art of Scientific Computing*, 2nd ed.; Cambridge University: Cambridge, 1992.
(45) Wallqvist, A.; Berne, B. J. *J. Phys. Chem.* **1995**, 99, 2893.
(46) Guillot, B.; Guissani, Y.; Bratos, S. *J. Chem. Phys.* **1991**, 95, 3643.
(47) Guillot, B.; Guissani, Y. *J. Chem. Phys.* **1993**, 99, 8075.
(48) Brooks, C. L., III; Fleischman, S. H. *J. Am. Chem. Soc.* **1990**, 112, 3307.
(49) Mazon, M. H.; McCammon, J. A.; Lybrand, T. P. *J. Am. Chem. Soc.* **1990**, 112, 4411.
(50) Shing, K. S.; Gubbins, K. E. *Mol. Phys.* **1982**, 46, 1109.
(51) Lu, N.; Kofke, D. A. Simple model for insertion/deletion asymmetry of free-energy calculations. In *Foundations of Molecular Modeling and Simulation, AIChE Symp. Ser.*; Cummings, P., Westmoreland, P., Eds.; 2001; Vol. 97, p 146.
(52) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, 4, 187.
(53) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, 79, 926.
(54) Nosé, S. *Mol. Phys.* **1984**, 52, 255.
(55) Hoover, W. G. *Phys. Rev. A* **1985**, 31, 1695.
(56) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids*; Clarendon Press: Oxford, 1987.
(57) Lybrand, T. P.; Kollman, P. A. *J. Chem. Phys.* **1985**, 83, 2923.
(58) Ryckaert, J. P.; Cicotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, 23, 327.
(59) Lu, N.; Singh, J. K.; Kofke, D. A. *J. Chem. Phys.* **2003**, 118, 2977.
(60) Lu, N.; Kofke, D. A.; Woolf, T. B. Manuscript in preparation.