

Using overlap and funnel sampling to obtain accurate free energies from nonequilibrium work measurements

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Two concepts are presented for accurate nonequilibrium work free-energy measurements, realized both in molecular simulation and experiment. First, the need for an intermediate important to both the reference and the target systems (overlap) is indicated. Second, the use of a soft path from each end point to the intermediate (funnel) is demonstrated. Schemes implementing these concepts dramatically improve efficiency and accuracy of free energy calculations, as shown by calculation of the free energy of ion charging in water, and the free energy change in mutation of an adenosine molecule.

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Free energy is a key quantity for characterizing and modeling chemical, physical, and biological behavior, and consequently the measurement of free energies—by molecular simulation or experiment—is of great importance to science and technology. Yet, the design of robust and efficient methods for free energy measurement remains a “grand challenge” problem in molecular simulation [1], this despite decades of development and application. Experimental measurements of free energies for molecular-scale processes have, on the other hand, emerged only recently [2], spurred largely by advances in the formulation of nonequilibrium methods [3]. Their practice is also problematic, and attempts are now being made to understand and thereby improve them [4].

The goal of free-energy measurement is to determine the free energy difference ΔF between two systems A and B ($\Delta F \equiv F_B - F_A$). The main difficulty arises from the tendency of a measurement to exhibit large (many times kT) systematic error or bias. Usually the error is reproducible, so the standard error measures—which gauge precision, not accuracy—do not hint at the problem. Even bootstrap methods, which do target inaccuracies, are not effective [5]. The only sure way to uncover the error is to measure the same ΔF using different algorithms or protocols, and examine the consistency of the results. Discrepancies are remedied by applying sufficient effort (e.g., computation) to remove the inconsistencies, or by applying unjustified heuristics, such as averaging the differing results. Such practices can be inefficient or unreliable.

Recently, in the context of free-energy perturbation (FEP) methods [6], we have shown how a proper conception of the nature of the measurement and the mechanism causing inaccuracy can guide one to apply methods that are much less prone to systematic error [7–9]. A key point is the consideration of the phase-space relations of the A and B systems, which leads to the notion of overlap sampling as an effective strategy for conducting staged FEP calculations [5,10]. The same conceptual basis can be applied to improve the more

general class of work-based free-energy methods, in both their experimental and computational realizations. There is one rule to follow: the nonequilibrium path must proceed “down the funnel,” that is, the sequence of systems traversed in the measurement must be successive phase-space subsets. Two general problems arise in connection with this requirement. First, it may be that the systems of interest have only partial overlap, and one is not a subset of the other in phase space; second, the systems may relate more as a pinhole than a funnel, meaning that one may be an extremely small phase-space subset of the other. This report lays out these concepts and presents general protocols to address them. We show that the former problem is addressed by overlap sampling, and the latter by funnel sampling. First, some more background.

The general formulation of work-based non-equilibrium free-energy calculation methods was presented by Jarzynski [3] and further developed by Crooks [11]. Jarzynski established an equality between the equilibrium free energy difference and the Boltzmann-weighted ensemble average of nonequilibrium (finite-time) work (W) for the switching from A to B along a path at a finite rate:

$$\exp(-\beta \Delta F) = \langle \exp(-\beta W_{A \rightarrow B}) \rangle_A, \quad (1)$$

where $\beta = 1/kT$ is the reciprocal temperature, and the angle brackets indicate an ensemble average over initial configurations of the equilibrated A system. The method based on Eq. (1)—referred to here as the nonequilibrium work (NEW)—is remarkably versatile [3]: it can be adapted to a broad range of simulations, including adiabatic and isothermal molecular dynamics simulations, Monte Carlo simulations, and experimental studies of molecular-scale systems [2]. In addition, the NEW generalizes several well-established free-energy methods, thereby unifying them, and presents avenues to follow for the development of new calculation techniques. FEP is found as a limiting case of NEW, in which the switching from A to B is instantaneous, while the other limiting case arises when the process occurs reversibly: $\Delta F = W_{A \rightarrow B}^{\text{rev}}$. The

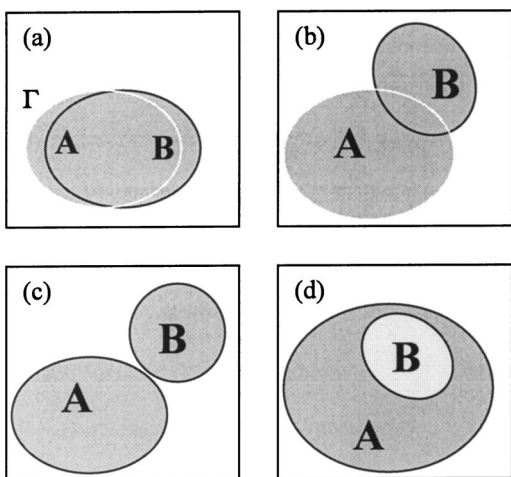


FIG. 1. Schematic depiction of four ways that important phase space regions (indicated by ovals) of two systems can relate: (a) coincidence, both spaces are roughly the same; (b) partial overlap; (c) no overlap; (d) subset.

NEW method suffers from certain limitations common to FEP, and if used improperly it too will provide results that are systematically incorrect [3]. In particular, since the ensemble averaging of Eq. (1) involves a nonlinear quantity in the exponential, these systematic errors can be viewed as a form of sampling that is very sensitive to the tails of the distribution [4,8,12].

Consider the classical phase space Γ [13] occupied by the A and B systems. The contribution of each phase-space point Γ_i to the partition function is Boltzmann-weighted according to its energy $E(\Gamma_i)$. We refer to those Γ_i having significant contributions to the partition function as the “important” phase space regions (denoted Γ^*) of a system. We can define $\Gamma_i \in \Gamma^*$ if $E(\Gamma_i)$ is less than a characteristic energy, e.g., the most-likely energy, or the average energy for the system [8]. Each system has its own set of important molecular configurations (Γ_A^* and Γ_B^*), which are sampled in a simulation governed by the system, and the differences between the systems typically cause their preferred configurations to differ.

The four ways that Γ_A^* and Γ_B^* can relate [9] are illustrated in Fig. 1: (a) coincident, (b) partial overlap, (c) no overlap, or (d) subset. We note that in general (a) and (d) are the only cases in which a simple (single-stage) FEP calculation will produce an accurate result [7–9]. An analogous argument can be made in connection with the NEW calculation. Let us define a parameter γ ($\gamma \in [0, 1]$, $\gamma=0$ and 1 for the A and B systems, respectively) that describes progress along the path $A \rightarrow B$, such that for a particular value of γ the energy at phase-space point Γ_i is $E_\gamma(\Gamma_i)$, and the set of important configurations for the system defined by γ is denoted Γ_γ^* . As the system steps from γ_n to γ_{n+1} , the same issue arises as with FEP [7–9]. Any parts of $\Gamma_{\gamma_{n+1}}^*$ outside of $\Gamma_{\gamma_n}^*$ are rarely (if ever) sampled, yet if they were encountered they would make a large, negative contribution to $W_{A \rightarrow B}$, and thus a significant contribution to the NEW average. If such points exist, the failure to sample them introduces inaccuracy in the calculated free energy. The conclusion is that for an

accurate result, the sequence of systems traversed in a NEW calculation must proceed such that *each* successive system obeys a phase-space subset relation with the ones that precede it. Adopting a term from the protein-folding literature [14] describing a related concept, we say that a path following such a trajectory moves “down the funnel.” The funnel requirement is attenuated to the degree that the path approaches reversibility. For a reversible path γ is incremented differentially, and the phase-space relation for adjacent values of γ will look more similar to that depicted in Fig. 1(a). In this manner it is possible to go from A to B even if these end states relate as in Figs. 1(b) or 1(c). If the path is not traversed reversibly, then it is necessary that A and B relate as in Fig. 1(d) in order for the subset relation to be satisfied all along the path $A \rightarrow B$.

The definitions for systems A and B are typically set by the context of the problem motivating the free energy measurement, thus they cannot readily be selected to ensure that the phase-space subset relation can be satisfied. This problem may be overcome by setting up the calculation in stages [7,15], defining one or more intermediate system(s) (designated M) and computing ΔF_{AB} as the sum of, e.g., ΔF_{AM} (defined as $F_M - F_A$) and ΔF_{MB} . The foregoing considerations prescribe the selection of M : it must obey a subset relation with both A and B (for simplicity here we assume the case requiring only a single intermediate M). Such an M can be formulated in two ways. One is to define the M such that Γ_M^* “contains” both Γ_A^* and Γ_B^* ; this is the umbrella sampling technique [16] in the context of FEP. The other, overlap sampling (OS) [5,10,17], defines M so that Γ_M^* is a subset of both Γ_A^* and Γ_B^* simultaneously (i.e., a subset of the overlap region). The OS method requires A and B to satisfy the partial-overlap relation [see Fig. 1(b)]. This relationship is common in many free energy problems, and if not, higher-order staging methods can be formulated using similar considerations. These staging methods have been formulated and studied in the context of FEP, and we note that they apply equally well in the context of the more general NEW. Here, we consider the OS staging method because certain features of its implementation make it better suited for the calculation. In the OS method, the free energy formula is

$$e^{-\beta\Delta F} = \frac{\langle e^{-\beta W_{A \rightarrow M}} \rangle_A}{\langle e^{-\beta W_{B \rightarrow M}} \rangle_B}. \quad (2)$$

Equation (2) indicates two separate simulations starting with equilibrated A and B systems, respectively, and proceeding to a common destination, the system M .

Now we proceed with the construction of the intermediate M and the switching paths taken to M from A and B . Bennett [17] considered the formulation of an optimal M for an OS calculation (for FEP), but he did not present it this way. His “acceptance ratio” perspective instead considered the best way to combine separate FEP calculations $A \rightarrow B$ and $B \rightarrow A$, and not on finding a free-energy staging intermediate. The different perspectives on Bennett’s method lead to different implementations when it is generalized to NEW calculations. Following Bennett, Crooks [18] proposed a corresponding formula to blend NEW calculations from A to B

and back. Crooks' "generalized acceptance ratio" makes no statement regarding the path taken between A and B . In contrast, in the OS perspective, the path must pass through an intermediate M , which we wish to define consistently with Bennett's (implicitly defined) M . To this end, we propose the following path between the A and B systems:

$$e^{-\beta E_\gamma} = [(1 - \gamma)e^{+\beta E_A} + \gamma e^{+\beta E_B}]^{-1}. \quad (3)$$

For $\gamma=0,1$ we recover systems A and B , respectively. At intermediate γ , configurations important to *both* A and B are increasingly important. Thus for a suitably chosen γ , defining the intermediate M , we should expect the path from A and B to M will proceed down a funnel. If we adopt Bennett's prescription for an intermediate, then the optimal γ will satisfy

$$\gamma^*/(1 - \gamma^*) = \exp(-\beta \Delta F). \quad (4)$$

Equations (2)–(4) complete the NEW-OS method, whereby the free energy difference ΔF can be obtained by solving these equations self-consistently using the following procedure. Perform NEW calculations from A to B ($\gamma=0 \rightarrow 1$ with a predefined set of $\{\gamma_i\}$) and separately from B to A (using the same $\{\gamma_i\}$ but in reverse) following paths defined by Eq. (3); for each switch, record the partial work values at each point of γ along the path; compute ensemble averages and thus ΔF_γ using Eq. (2); finally, select the γ (therefore ΔF) satisfying Eq. (4).

Thus the OS formulation assures that each element of the overall free-energy difference calculation is formed as a funnel sampling (FS) calculation. The second consideration is the shape of the funnel, which focuses on the paths taken from A and B to M . A "pinhole" funnel is unlikely to provide good results, because most contributions to the averages in Eq. (2) are near zero, except for those rare instances in which the A (or B) $\rightarrow M$ transition happens to start in the Γ_M^* region. The path should provide a smooth, broad funnel-like transition from A and B to M . Within this picture the performance of the calculation is affected by many variables, so it is difficult to develop a general, "optimal" result of the type presented by Bennett. Instead we propose one possible approach, with the idea that future effort can focus on improving even more this aspect of the calculation. In particular, we modify the Bennett-inspired finite-time switching path as follows:

$$e^{-\beta E_\gamma} = e^{-\beta E_A} [(1 - \gamma) + \gamma e^{+\alpha(E_B - E_A) - D}]^{-\beta/\alpha}. \quad (5)$$

The parameter α affects the softness of the transition from A and B to M . We find that a reasonable choice for its value causes $\alpha(E_B - E_A)$ to be of order unity for typical values of $(E_B - E_A)$ when sampling the A or B systems. This causes the weight [Eq. (5)] to decay over a broader range of γ for energies of this magnitude. Note that α is a fixed parameter, and does not change with the configuration or with γ . One disadvantage in this modification is the loss of the rigorously optimized Bennett's intermediate, which is no longer encountered in this path. Nevertheless, we retain Eq. (4) as the criterion for selecting the value of γ that defines M . Also in this version we have introduced a parameter D , which is a

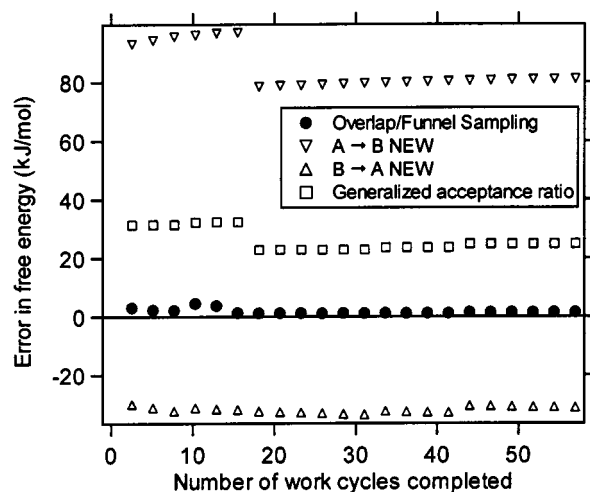


FIG. 2. Error in free energy measured by several NEW implementations [20].

constant and is added for convenience of the calculation. It can be used to ensure that the optimal γ is found at a reasonable distance from the values 0 or 1. Otherwise it is likely (for large ΔF) that the work path will jump over γ^* in the first step, making its identification difficult. If D is selected to be equal to $\beta \Delta F$, then the optimal γ is [using a modification of Eq. (4)] 0.5.

We demonstrate with two calculations: the first measures the free-energy change associated with charging an ion within SPC water, and the second measures the free-energy change between two alchemical states of an adenosine molecule in aqueous solution. In the charging simulation, the A system consists of a single Lennard-Jones atom with charge of $+1 e$ in a system of 216 SPC water molecules at 298 K and 1 g/cm^3 , and the B system is the same but with no charge on the solute atom. All interactions are truncated at a distance of 9 \AA and no additional treatment is applied for long-range electrostatic interactions. The free energy differences are calculated using four methods: NEW $A \rightarrow B$ and $B \rightarrow A$ each by themselves, Crooks' generalized acceptance ratio, and the proposed algorithm with $\alpha=10^{-3}\beta$. All calculations follow the same path [Eq. (5)] and are based on a common set of data, so the same amount of simulation is applied for all methods. The correct value for this free-energy difference (as given by equilibrium-work calculations using molecular dynamics simulation [19]) is $420.81 \pm 0.3 \text{ J/mol}$. For such a large ΔF , the parameter D must be selected to be within a few percent of the actual free energy, or else the optimal γ is too close to 0 or 1 to be identifiable. Free energy results are shown in Fig. 2, where the difference from the literature value is plotted as a function of the amount of sampling performed in the simulation [20]. The proposed method yields impressive results, with convergence observed very early in the simulation. In contrast, the other methods display a systematic error that shows little sign of improvement over the course of the calculations. The failure of the generalized acceptance ratio method indicates that minimization of the statistical error is not sufficient for a reliable free energy calculation, and that consideration of phase space sampling—which is a built-in feature of OS—is a key component for an optimal calculation.

The ΔF calculated for mutation of adenosine was measured with the paths of Eq. (3) (details for the simulation setup are in Ref. [5]). The result from the NEW-OS method matches the correct ΔF of 35.3 kJ/mol, while the separate forward and reverse NEW give values of 38.1 and 29.9 kJ/mol, respectively. With work values sampled along the paths defined by Eq. (3), the acceptance-ratio implementation gives result comparable to that by NEW-OS, but it yields 34.7 kJ/mol when employing a conventional sampling path.

To summarize, a reliable work-based free-energy measurement requires barrier-free sampling of important phase space regions, and systematic errors due to inappropriate sampling, if not prevented in the first place, cannot be easily overcome afterwards. For each step of the calculation, the reference and target systems should obey a subset relation for their important phase space, and the work should accompany a path that satisfies the subset criterion along its entire length. The OS and FS techniques presented in this report provide a general approach to meet these criteria.

Concepts presented here have concentrated on molecular

simulation, but they may also be applied to advantage in experimental realizations of NEW methods [2]. The OS idea in particular can be implemented experimentally by appropriately combining work calculations from the *A* and *B* systems to a suitably chosen intermediate. This practice cannot be followed to the detail permitted in simulation [i.e., precisely according to Eq. (3)], but it may be sufficiently helpful to adhere only to a reformulation of Eq. (4) appropriate to the system. Otherwise this step can be taken immediately, and requires no redesign of the experiments except to ensure that the NEW averages are recorded throughout the path. At a minimum, it should be standard practice to combine the forward and reverse results according to the acceptance-ratio formulation presented by Crooks.

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- [20] A "work cycle" consists of 100 nonuniform steps in γ from 0 to 1 and back (100 each way). One simulation sweep (N MC trials) is performed after each step in γ and 1000 equilibration sweeps are conducted after reaching each endpoint, before beginning the next work half cycle.