Conformational sampling via a self-regulating effective energy surface

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The difficulty of efficiently sampling the phase space of complex systems with rough energy surfaces is well known. Typical solutions to the problem involve accelerating the crossing of barriers, but such methods often have the secondary problem that the low-energy states of interest are inadequately sampled, unless the parameters of the search algorithm are modified as the system evolves. A method is presented to improve the sampling with particular emphasis on the low-energy conformations, which make the most important contributions to the thermodynamics of the system. The algorithm proposed here samples the details of the minima, while easily surmounting barriers. This is achieved by introducing a self-regulating sampling variable which depends on the current state of the system. Two replicas of the system are introduced and the sampling variable is treated as a particle coupled to the physical system. The method is illustrated with a simple model system and is applied to the realistic example of barrier crossing in a protein-ligand complex.

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I. INTRODUCTION

Molecular simulations are now a standard method for the study of mesoscopic systems. Of particular interest are biological macromolecules such as proteins, which are highly inhomogeneous systems with complicated potential energy surfaces (PESs). One area of practical importance, as well as of theoretical concern, is the determination of positions where candidate ligands are likely to bind. The nature of the PES of biomolecules or of biomolecule-ligand complexes leads to well-known sampling problems, such as the problem of broken ergodicity in molecular dynamics simulations (i.e., effective confinement of the system to a certain basin of attraction) and the correlate problem of terminating the search in a local minimum in global optimization algorithms. These problems have been studied by many groups. Nearly all approaches to the sampling problem in complex systems involve accelerating the crossing of barriers. However, the usual treatment of this issue generates a secondary problem, namely, that low-energy regions are explored inadequately. The latter are important if the simulation is to be used either to find low-energy positions of candidate ligands (the primary concern of this paper) or to calculate thermodynamic properties of the system.

An illustration of this dilemma is given by multicanonical or adaptive umbrella sampling. In the two approaches, which are formally equivalent, the aim is to sample uniformly in energy space, i.e., for the system to perform a random walk in the space of the total energy or potential energy. This distribution assures that low-energy regions are sampled (as in low temperature canonical simulations) and that barriers are crossed (as in high temperature canonical simulations). In the standard implementation of multicanonical sampling, the sampling is initiated in the high-energy regime and the probability surface $P(E)$ is progressively leveled “top down.” The major problem encountered in this approach is that the low-energy states of the system are typically undersampled (i.e., the surface persistently remains “top heavy”); hence, convergence to the uniform surface is very slow. Xu and Berne discussed this problem and proposed a solution, that of combining J-walking with multicanonical sampling to improve the sampling of the low-energy states. In the method of adaptive umbrella sampling, the sampling is initiated in the low-energy regime (as in standard umbrella sampling), the difference from the latter being that the umbrella “coordinate” is the potential energy rather than (say) a dihedral angle. In this case, the probability surface is leveled “bottom up” and the corresponding problem encountered is that the barriers are undersampled (i.e., the surface persistently remains “bottom heavy”). Hence in both cases (which illustrate the two horns of the dilemma) the convergence to the desired $P(E)$ distribution is very difficult for complex systems, such as large peptides or noble gas clusters. This problem has
been addressed in Ref. 17, where a geometric coordinate, in addition to the potential energy, was introduced. However, as pointed out in that paper, such an approach requires some knowledge of the essential coordinates of the system.

As these authors discussed, what is required for the efficient sampling of a complex system is a multifaceted sampling method that leads to the rapid crossing of large barriers (i.e., \( W \gg kT \)), but that, having done so, samples finely the low-energy region on the far side of the barrier. In short, a different behavior of the sampling algorithm is required when the system is in the neighborhood of barriers from that around minima. The challenge is to accomplish this type of sampling without a priori knowledge of the system. The difficulty in doing this is illustrated by methods that depart from standards. These methods involve diverse energy regimes and as such involves a rather strong assumption concerning the ideal surface for sampling efficiency. This is practical for very simple systems, but can be quite unrealistic for complex systems, since the latter commonly involve diverse energy regimes (as indicated, e.g., by “disconnectivity graphs”\(^{24}\)), the variety of which is not amenable to any simple scheme. It follows that efficient sampling in complex systems requires a method that is self-guided rather than knowledge based (as SGAs are), but which also is capable of adjusting the type of sampling to the current state of the system.

As we show in this report, this can be accomplished by the use of a “sampling variable” that evolves with the configuration of the system. Such sampling variables, referred to as “\( \lambda \) dynamics,” have been introduced for a variety of purposes. Brooks and co-workers have demonstrated their ability for determining the relative binding free energies of a set of ligands.\(^{4-8}\) Our purpose in the present paper, as already mentioned, is to find low free energy conformations for potential ligands by the use of a sampling variable. The sampling variable is incorporated into the system by extending the Hamiltonian; specifically, it is treated as a one-dimensional particle which couples to the physical coordinates so as to scale the potential energy function of the system. The result is that an effective energy surface for the physical coordinates is generated dynamically as the system and sampling variable evolve. The optimal design of this surface for efficient sampling is realized by coupling the configurational degrees of freedom of the system to the sampling variable such that when the system encounters a barrier, the value of the sampling variable adjusts so as to reduce the effective barrier height, thereby increasing the transition probability, and, conversely, when the system nears a local minimum, the sampling variable adjusts so as to localize the system, thereby focusing sampling in this region. The effective energy surface generated by the sampling variable differs most from the real PES near barrier regions and is most like the real PES near minima. As a result, the crossing of barrier regions (which are relatively unimportant thermodynamically, but essential to the efficient exploration of phase space) is enhanced relative to the real system, while the sampling of low-energy regions (which contribute significantly to the thermodynamic properties of the system) occurs on a PES that is close to that for the actual system. In so doing, the method in principle preserves the correct relative sampling of the low-energy regions given a suitable separation between the motion of the physical variables and that of the (slower) sampling variable. In practice, it is possible to accelerate the sampling of the low-energy regions further by varying the sampling variable more rapidly than that required to maintain the canonical distribution. The latter approach has been used here, consistent with the focus of the present work on the mapping of low free energy positions. The conditions under which the canonical distribution is sampled by this method, and the significance of deviations from this distribution, will be discussed separately.\(^{25}\)

The balance between sampling minima versus barrier regions is essential for the success of the method. This balance can be controlled through the use of auxiliary sampling parameters (as it is in the related methods of Refs. 21 and 26); the limitation of such an approach lies in the need to modify (update) these parameters to optimize sampling as the simulation proceeds. A different approach, on which we focus here, is to incorporate the regulation of the sampling into the algorithm itself; i.e., to develop a sampling algorithm that is fully self-regulating. As we argue, in principle, and demonstrate by example, one way to accomplish this is by replicating the system (including its sampling variable) and having it compete against itself. This approach draws its motivation from the work of Tidor\(^{27}\) and Brooks and co-workers,\(^{4-8}\) who showed that having multiple, distinct ligands compete against each other for the same protein target allowed optimal interactions to be identified. We have chosen a replicated system in which the sampling variable competes against itself and, thereby, regulates itself. The regulation in this case is a mutual effect of the interacting replicas. For this reason, it requires no a priori knowledge of the system (as in the case of umbrella coordinates) nor does it make any assumptions about the type of sampling required (as in SGAs and standard deformation methods).

The organization of this paper is as follows. In the Theory section, the formalism of the method is presented in its general form and then specialized to the case in which only a part of the system is replicated. The Results section first illustrates the basic features of the method by the use of a one-dimensional model system with a single deep minimum and surrounding shallow minima. In the second part, the method is applied to a realistic example of conformational sampling in the neighborhood of a barrier in a protein-ligand system. A brief summary is given in the Conclusions.
II. THEORY

A. Use of a sampling variable: Unreplicated system

Consider a molecular system in thermal equilibrium in which the potential energy function $U(q)$ is known. The Hamiltonian for this system is $H(p,q) = K(p) + U(q)$. (For simplicity, we do not show the additional terms describing the thermostat; see Ref. 28 for details.) In the case of an unreplicated system coupled to a sampling variable, the extended Hamiltonian $H(p,p\theta,q,\theta)$ is

$$H(p,p\theta,q,\theta) = K(p) + K(p\theta) + U_{\text{eff}}(q,\theta),$$  \hspace{1cm} (1)

where $q$ describes the configuration of the physical system, $\theta$ is the sampling variable, and $U_{\text{eff}}(q,\theta)$ is the extended effective potential energy function. The functional dependence of $U$ on $q$ and $\theta$ is general; in the present work, we use the simple multiplicative form

$$U_{\text{eff}}(q,\theta) = f(\theta)[U(q) - U_{\text{ref}}],$$  \hspace{1cm} (2)

where $U_{\text{ref}}$ is a fixed auxiliary sampling parameter. As can be seen from Eq. (1), $\theta$ is treated as a virtual particle in an extended Hamiltonian formulation; i.e., it has mass $m_\theta$ and a “position” that is propagated with the physical coordinates $q$. Thus, each dynamics step yields a new value of the sampling variable, as well as a new configuration for the physical system; the former is determined using $F_\theta = -\nabla_\theta U_{\text{eff}}$. We further specify that $f(\theta)$ is a monotonically increasing function of $\theta$ [in the examples presented here, we use the simple form $f(\theta) = \theta$] and we impose the constraint $0 \leq f(\theta) \leq 1$ on the system. The use of a parameter $U_{\text{ref}}$ in conjunction with the prescribed range for $f(\theta)$ allows the simulator to control which regions are sampled (i.e., around minima versus barriers and other high-energy states).

The role of $f(\theta)$ is best understood as an operator that transforms the physical PES $U(q)$ into an effective energy surface $f(\theta)U(q)$. Since $\theta$ is time dependent, the effective energy surface evolves during the simulation. Further, since $f(\theta)$ multiplies the physical potential energy function and is between zero and one, a given value of $f(\theta)$ linearly reduces the magnitude of all points on the physical PES. Specifically, when $f(\theta) = 1$, the effective PES is identical to the physical PES (possibly minus an arbitrary constant $U_{\text{ref}}$) and, as $f(\theta)$ decreases, the effective surface is flattened with respect to the physical surface. The result of this leveling is that the relative heights of all barriers are reduced and the relative depths of all minima are elevated. At the lower bound $f(\theta) = 0$, the effective surface is perfectly flat; i.e., it has a constant value of zero.

To understand how the effective energy surface alters the probability distribution (i.e., how the sampling variable affects the sampling), we consider the effect of $f(\theta)$ on the configurational probability distribution at thermal equilibrium. For the physical system, the equilibrium probability $P_i$ of a configurational $i$ at temperature $T$ is given by its normalized Boltzmann factor $(Z_q)^{-1}\exp[-U(q_i)/kT]$, where $Z_q$ is the configuration partition function. Correspondingly, the effective probability of configuration $i$ is $(P_i)^{f(\theta)}$. Thus, the effective probability distribution is flattened relative to the physical distribution in a nonuniform manner, i.e., if $P_j < P_k$, then $(P_j)^{f(\theta)}/P_j > (P_k)^{f(\theta)}/P_k$; in other words, the higher-energy configurations (such as $j$) become relatively more likely at the expense of the lower-energy configurations (such as $k$). This effect obtains for all values of $f(\theta)$ less than 1 and becomes exponentially more significant as $f(\theta)$ decreases to zero. Consequently, transitions to and between higher-energy configurations are relatively more common on the effective energy surfaces generated by the sampling variable, especially at small values of $f(\theta)$. The value of $f(\theta)$ at a given time determines what energy regime the physical system samples at that moment, i.e., if $f(\theta)$ is near one, a low-energy regime is sampled; if it is near zero, a high-energy regime is sampled. Since the extended Hamiltonian $H(p,p\theta,q,\theta)$ is propagated at constant temperature, it follows that in the absence of $U_{\text{ref}}$ (i.e., when $U_{\text{eff}} = f(\theta)U(q)$), the Boltzmann distribution of states $(q,\theta)$ strongly favors states in which $f(\theta)$ is small (i.e., near zero). For this reason, it is necessary to include $U_{\text{ref}}$ to focus (as appropriate) the sampling on the low-energy regions. Specifically, the balance between the system staying in a current basin (and on average, near the minimum) and exploring more broadly a higher-energy regime is modulated by the magnitude of the energy of the local minimum $U(q_{\text{min}})$, which we want to explore, relative to the reference value $U_{\text{ref}}$. When $U(q_{\text{min}}) < U_{\text{ref}}$, the extended system spends much of its time near $q_{\text{min}}$ since the effective potential energy function $f(\theta)[U(q) - U_{\text{ref}}]$ is, for a given value of $f(\theta)$, at its minimum value when $U(q) = U(q_{\text{min}})$. Thus, in the extended system, it is possible (through the judicious choice of $U_{\text{ref}}$) to focus sampling around individual minima, which is important because such regions contribute significantly to the thermodynamic properties of the system. However, barrier crossings are required to access the multiple minima present on a complex energy surface and such crossings are made unlikely when $U_{\text{min}} < U_{\text{ref}}$. To efficiently accomplish barrier crossing, it is necessary to modify the parameter $U_{\text{ref}}$ such that $U_{\text{min}} > U_{\text{ref}}$. In this case, the extended system will spend more time (relative to the physical system) exploring the higher-energy regime, since the term $f(\theta)[U(q) - U_{\text{ref}}]$ is minimized when $f(\theta) = 0$, i.e., so that all values of $U(q)$ are essentially equally probable. In a typical energy landscape the density of states $\Omega(E)$ increases very rapidly with $E$; i.e., the high-energy configurations vastly outnumber the low-energy ones. Consequently, it follows that the system will primarily explore high-energy configurations when $f(\theta) \rightarrow 0$. Thus, after a barrier is surmounted, it is necessary to again modify $U_{\text{ref}}$ so that the minimum region on the far side of the barrier is well sampled. This discussion makes clear that the adjustment of $U_{\text{ref}}$ can be a significant problem in the full exploration of a PES (see, for example, Ref. 21).

B. Replicated system

It is clear from the preceding discussion that one way to achieve optimal sampling (rapid crossing of barriers and fine sampling around minima) is to modify the value of parameter $U_{\text{ref}}$ as the simulation proceeds, i.e., to keep $U_{\text{ref}}$ large relative to $U_{\text{min}}$ in a given basin for a while (for fine sampling around the minima), and then to decrease it in order to
exit that basin. An alternative approach is to replicate the system, which as we shall show obviates the need for the use of the auxiliary sampling parameter \( U_{\text{ref}} \). In the latter case, the system (or possibly a part of the system) is replicated and the replicas are propagated independently to first order (i.e., in the presence of the physical Hamiltonian), but are coupled at second order through their respective sampling variables \( \{ \theta \} \). The coupling is introduced by imposing a holonomic constraint on the sum of the instantaneous values of \( f(\theta) \), specifically \( \Sigma f(\theta) = 1 \). For the purpose of illustration, we consider a system consisting of two replicas. In this case, the two sampling variables \( \theta_1 \) and \( \theta_2 \) associated with the two replicas share a single degree of freedom \( \theta \), which is treated as a one-dimensional virtual particle, in addition to the replica coordinates \( q_1 \) and \( q_2 \) and is propagated simultaneously with them, consistent with the constraint. As before, the time-dependent value, \( \theta(t) \), of this degree of freedom is given by the position at time \( t \) of the virtual particle, which interacts with the configurational degrees of freedom according to an extended potential energy function \( U(q_1, q_2, \theta) \). The function \( U(q_1, q_2, \theta) \) is separable in the present formulation, i.e., \( U(q_1, q_2, \theta) = f(\theta)U(q_1) + [1 - f(\theta)]U(q_2) \). Hence the Hamiltonian for the two-replica system is

\[
H(p_1, p_2, p_\theta, q_1, q_2, \theta) = K(p_1) + K(p_2) + K(p_\theta) + f(\theta)U(q_1) + (1 - f(\theta))U(q_2),
\]

if the auxiliary sampling parameters are set equal to zero. The values of \( f(\theta) \) and \( 1 - f(\theta) \) at a given time determine what energy regime the replicas 1 and 2 sample at that moment (i.e., if \( f(\theta) \) or \( 1 - f(\theta) \) is near one, a low-energy regime is sampled by replica 1 or 2, respectively; if near zero, a high-energy regime is sampled). Again, \( f(\theta) \) is best understood as an operator that transforms the original PES for replica 1 into the effective energy surface \( f(\theta)U(q_1) \) and for replica 2 into \( (1 - f(\theta))U(q_2) \).

We now consider the dynamics of the sampling degree of freedom \( \theta \) in the two-replica system. To show that having the system compete against itself results in a self-regulation of the sampling, we assume, for simplicity, that \( f(\theta) = \theta \). In this case, \( \Phi_{\theta} = -\nabla_{\theta}U = U(q_2) - U(q_1) \). This equation implies that when (say) replica 2 approaches a barrier and its energy increases, the contribution to the change in the force, \( \partial \Phi_{\theta} / \partial \theta \), is positive, i.e., it tends to accelerate \( \theta \). To illustrate this effect, assume that at \( t = 0 \), \( U(q_1) = U(q_2) \), and therefore, that the acceleration of \( \theta \), \( a_{\theta} = 0 \); the sign of the velocity of \( \theta \), \( v_{\theta} \), will depend on the prior conditions of the trajectory. At \( t = 1 \), assume that replica 2 approaches a barrier, i.e., \( U(q_2) > U(q_1) \). This means that \( \Phi_{\theta} > 0 \), i.e., \( \theta \) accelerates. In the case when \( v_{\theta} > 0 \), as \( \theta \) accelerates, the value of \( (1 - \theta) \) decreases faster, i.e., the effective energy surface for replica 2 is rapidly flattened and consequently the barrier height is reduced. Concomitantly, for larger \( \theta \), the effective energy surface for replica 1 is “sharpened,” i.e., the minima are deepened, so that the replica is more localized. Hence, while one replica samples the phase space more broadly, the other samples more finely. [The situation is similar when \( v_{\theta} < 0 \), except that in this case it is the progress of the system in the “wrong” direction that is halted, i.e., as \( \theta \) accelerates, the rate of increase in the value of \( (1 - \theta) \) is lessened; in other words, the sharpening of the effective energy surface for replica 2 is halted. If replica 2 remains near the barrier, \( v_{\theta} \) will continue to increase, and eventually become positive, at which point the barrier will be leveled.] The key to the self-regulation is that every \( \Delta f(\theta) \) for replica 1 is coupled with a corresponding \( \Delta f(\theta) \) for replica 2. Since \( \Phi_{\theta} \) is given by \( U(q_2) - U(q_1) \), the (instantaneous) rate at which the effective energy surface changes depends on the relative values of \( U(q_1) \) and \( U(q_2) \); i.e., when the two values are similar, the acceleration of \( \theta \) (whether positive or negative) is smaller, i.e., the effective energy surface is relatively stationary. This “competition” between the two replicas continues until one replica attains a significantly better energy, at which point the speed of \( \theta \) increases again. That is, the different low-energy regions of phase space, which are separated by barriers, are explored successively in a relatively short simulation time; barrier crossing is accelerated but not at the expense of fine sampling around minima.

We describe the multiple-replica ansatz as self-regulating in that the type of sampling, which is possible in the unreplicated system (i.e., if the parameter \( U_{\text{ref}} \) is perfectly chosen for a given minimum or barrier), is achieved naturally in the replicated system. By contrast, in the majority of existing methods \(^{21,23} \) for enhanced sampling, the parameters are updated by the simulator as the algorithm proceeds. In the latter case, of which the unreplicated ansatz [Eq. (2)] is an example, the success of the method depends in part on the intuition or knowledge of the simulator; i.e., in the optimal adjustment of the values for the sampling parameters. The use of a self-regulating method obviates this problem. In the first example presented in the Results section, these points are illustrated by a simple model system that is simulated with both the unreplicated ansatz [Eq. (2)] and the multiple-replica ansatz [Eq. (3)].

### C. Replicated subsystem

We now consider a specialization of the method, in which only a part of the system is replicated. In what follows, the formalism for the case of a replicated subsystem is developed in terms of a protein-ligand system. Such a system forms the second example given in the Results section.

In this case, multiple replicas of a subsystem are propagated simultaneously in the field of the rest of the system (i.e., the “environment”). The partitioning of the system into a replicated subsystem moving in a common environment relies on the use of the time-dependent Hartree approximation adopted for classical dynamics. Specifically, each replica feels the full force of the environment but is invisible to the other replicas, while the environment feels an average force due to the replicas. This approach was introduced by Elber and Karplus, \(^{29} \) who showed that the sampling of pathways of carbon monoxide through myoglobin could be significantly enhanced if multiple copies of CO moved simultaneously in the field of a single dynamic myoglobin molecule. Special cases of this method are locally enhanced sampling (LES), in which the replicas remain in the same region throughout the
simulation\textsuperscript{30} and the multiple-copy simultaneous search approach (MCSS) approach for ligand design.\textsuperscript{2,3}

In standard multiple-copy sampling, the replicas do not interact directly with each other, so that their effect on each other is second order; i.e., it is exerted by way of their respective influence on the environment, which interacts with (and thereby couples) all copies. If the rest of the system is rigid, then the second-order coupling effect is zero; if it is mobile, then the effect is nonzero, but typically small.\textsuperscript{29} Thus, in general the replicas effectively do not “communicate” with each other. This was regarded as an advantage in the original development of the method, since it reduces the chances so introduced between copies will distort the protein structure. However, for most ligands (or fragments thereof), the range over which such correlations distort the structure is expected to be small (especially when only a few replicas are used, as here), while the benefit of such communication vis-à-vis the enhancement of sampling is potentially significant. The essential point for optimizing sampling is to structure the communication such that the sampling of copy positions is self-regulating, in the manner described for the two-replica example. This is achieved by coupling the ligand or ligand fragment replicas by way of their adjustable scaling factors (lambda weights) through the normalization condition enforced on their sum.

When only a part of the system is replicated, the method is formally equivalent to the lambda dynamics method developed first in a molecular dynamics context by Brooks and co-workers\textsuperscript{4–8} and applied using a Monte Carlo (MC) algorithm by Kollman and co-workers.\textsuperscript{31,32} The published applications of the method have primarily concerned the competition among chemically distinct subsystems (e.g., candidate ligands); identical subsystems were briefly considered in Ref. 7 to validate the precision of the probability ratio calculation. The present paper concerns the competition among chemically identical subsystems. The method is presented and applied in terms of a Monte Carlo algorithm, though it could be used equally well with molecular dynamics simulations. We adopt the terminology established for lambda dynamics and refer to the sampling variable as “\(\lambda\).”

The potential energy function \(U\) of the extended system depends on the conformation \(q\) of the protein and the conformations of the copies \(\{q_j\}\), as well as on the lambda values of the copies \(\{\lambda_j\}\); i.e.,

\[
U(q, \{q_j\}, \{\lambda_j\}) = U_{\text{prot}}(q) + \sum_{j=1}^{N} \lambda_j (U_{\text{sys}}^{j}(q) + U_{\text{int}}^{j}(q, q_j)),
\]

where \(\Sigma \lambda_j = 1\). The self-energy of the protein is given by \(U_{\text{prot}}(q)\). The energy of copy \(j\), \(U_j\), is defined as its self-energy \(U_{\text{sys}}^{j}(q)\) plus its interaction with the protein \(U_{\text{int}}^{j}(q, q_j)\). Scaling \(U_j\) by the lambda weight \(\lambda_j\) of the copy gives the scaled copy energy, \(\lambda_j U_j\). In the present application, protein (side chain) positions \(q\), the copy positions \(\{q_j\}\), and the lambda weights \(\{\lambda_j\}\) are subject to MC moves. When a lambda move is accepted for copy \(i\), i.e., \(\lambda_i \rightarrow \lambda'_i\), the remaining lambda values are adjusted, consistent with the criterion \(\Sigma \lambda_j = 1\). In the present implementation, the adjustment is distributed uniformly over the remaining \(N-1\) copies; i.e., for all \(j \neq i\),

\[
\lambda_j \rightarrow \lambda_j' \left(1 - \lambda_j'/1 - \lambda_i'\right) + \lambda_i' = 1.
\]

The Metropolis criterion\textsuperscript{33} specifies that a move be accepted with probability \(\min[1, \exp(-\beta \Delta U)]\), where \(U = U(q, \{q_j\}, \{\lambda_j\})\) is the potential of the extended system [Eq. (4)]. When copy \(i\) attempts a position move \((\lambda_i \rightarrow \lambda'_i)\), \(\beta \Delta U\) is given by

\[
\beta \Delta U = (kT)^{-1} \left( \lambda'_i - \lambda_i \right) U_i + \sum_{j \neq i}^{N} \lambda_j \left( \frac{(1 - \lambda'_j)}{1 - \lambda_j} - 1 \right) U_j.
\]

The trial moves are isotropic and have a fixed maximum size and, therefore, the implementation of lambda moves satisfies detailed balance. Trial moves which increase a single lambda value to greater than 1 are always rejected.

From Eq. (4) the other \(N-1\) copies are invisible to copy \(i\) (i.e., they do not interact with \(i\) in Cartesian space), but each copy moves in the full force field of the protein (as in the standard multiple-copy method). Thus, the copies are coupled exclusively through their respective lambda values. On the basis of Eq. (5), if copy \(i\) is the one with the most favorable energy, increasing \(\lambda_i\) is accepted with unit probability; if it is not, then the lambda move is accepted with probability \(\exp(-\beta \Delta U)\). When copy \(i\) attempts a position move, \(\beta \Delta U\) is given by

\[
\beta \Delta U = \left( kT \right)^{-1} \left( \frac{U_i'}{\lambda_i} - \frac{U_i}{\lambda_i} \right),
\]

where \(U_i\) and \(U_i'\) are the copy energies before and after the trial move. In this case, \(\lambda_i\) does not change, but it does scale the energy change associated with the position move. As a result, an unfavorable position move is relatively more likely to be accepted if the copy has a smaller lambda value.

Earlier we described the probability distribution for the system in which the potential energy function is scaled by \(f(\theta)\) (cf. Sec. II A). The same argument holds when only a part of the potential function (i.e., that of a given copy \(i\)) is scaled (i.e., by \(\lambda_i\), as in the case of a replicated subsystem. We now consider, for a given copy at a given lambda value, the typical ways in which changes in lambda and the copy energy are coupled. For sampling in the low-energy regime (i.e., when lambda is large), a rapidly decreasing lambda value more readily allows a copy to change its position, often by way of close contacts or barriers which increase its interaction energy, while a rapidly increasing lambda value encourages the local optimization of the copy, thus a decrease in its energy. For sampling in the high-energy regime (i.e., when lambda is small), an increasing lambda value usually follows an improvement (decrease) in the copy energy.

As in the general case for the replica approach, the various replicas compete during the simulation. When a single replica attains a comparatively good interaction (i.e., relative to the strength of the interactions of the other replicas in a given multiple copy configuration), the lambda values of the other replicas are lower, hence the latter sample more broadly (in the high-energy regime). If a replica happens to find a position that is better than that of the currently “dominant” copy, it is likely to replace the latter as dominant (i.e.,
its lambda value is likely to increase toward 1). In short, the sampling of replica positions and conformations is inherently self-regulating. One “price” to be paid for this is that the algorithm on occasion may “lose” certain replicas into irrelevant high-energy regions; the risk of this increases with the number of replicas, and one correction for it (employed by Brooks and co-workers in the context of multiligram studies \(^7,^8\)) involves the use of restraining potentials. With this in mind, we note that the present method and the standard MCSS approach (i.e., with constant copy weights) are two complementary approaches for conformational searching.

III. RESULTS
A. Model system

By the use of a simple (effectively one-dimensional) model system we compare the results obtained for different values of \(U_{\text{ref}}\) in Eq. (2) with those obtained by the self-regulating two-replica method [Eq. (3)]. We consider a “butane” molecule which consists of four extended carbon atoms with the central torsion angle \(\phi\) having the potential in Fig. 1; the potential is constructed as a sum of cosine terms (see Fig. 1 caption). The simple linear form \(f(\theta) = \theta\) is used for the sampling variable. The remaining internal terms (i.e., bond lengths and bond angles) are not scaled by \(f(\theta)\) and are described by harmonic terms, with force constants sufficiently large that these degrees of freedom are, in effect, adiabatically separated from the torsion degree of freedom. The global potential energy minimum for this system was arbitrarily chosen to equal 6 kcal/mol at \(\phi = \pi\).

Using the c28 version of the CHARMM program,\(^{34}\) molecular dynamics simulations with the unreplicated ansatz [Eq. (2)] were done at 300 K with a Nosé-Hoover thermostat.\(^{35}\) The time step used was 1 fs and the coordinates \((\mathbf{q}, \theta)\) were saved every ten steps. Three simulations were done, each using a different value of \(U_{\text{ref}},\) viz., 3, 6, or 12 (values in kcal/mol). The first value of \(U_{\text{ref}}\) was chosen to be less than the energy of the global minimum, the second equal to this value, and the third greater than this value. The effect of \(U_{\text{ref}}\) is mediated by the value of \(f(\theta)\); i.e., the effective biasing constant is the product \(f(\theta)U_{\text{ref}}.\) In the case where \(U(\mathbf{q}) < U_{\text{ref}},\) the global minimum of the effective potential \(f(\theta)(U(\mathbf{q}) - U_{\text{ref}})\) occurs when \(U(\mathbf{q}) = U(\mathbf{q}_{\text{min}})\) and \(f(\theta) = 1;\) the system then is expected to remain mainly in the neighborhood of this point. When \(U(\mathbf{q}_{\text{min}}) > U_{\text{ref}},\) the global minimum includes all points in the subspace in which \(f(\theta) = 0,\) so that the system will typically remain in that region.

Sampling with fixed reference potential. The simulation in which \(U_{\text{ref}} = 3\) kcal/mol [an example of \(U(\mathbf{q}) > U_{\text{ref}}\)] is illustrated in Fig. 2(a); a representative interval of 100 steps is shown. Figure 2(a) (top) shows the time series of \(f(\theta),\) Fig. 2(a) (middle) is the time series of the bare potential energy \(U(\phi),\) and Fig. 2(a) (bottom) is the time series of the effective potential \(f(\theta)U(\phi).\) A periodicity in the variation of \(f(\theta)\) is evident, viz., \(f(\theta)\) oscillates between 0 and about 0.01. The observed range of \(f(\theta)\) is consistent with the fact that the lowest energy of the system occurs for \(f(\theta) = 0.\) It can be inferred from Fig. 2(a) (top) that \(\phi\) also oscillates, in this case over its complete range \((0, 2\pi),\) and with a somewhat lower frequency than \(\theta;\) i.e., the torsion angle rotates essentially continuously during this interval. This reflects the fact that, due to the consistently small values of \(f(\theta),\) the effective potential in this interval [Fig. 2(a) (bottom)] has no barriers greater than \(kT.\) The behavior in this interval is characteristic of the entire simulation; occasionally, \(f(\theta)\) transiently increases (including variation up to one) but then rapidly decreases to its small value. The behavior of \(\theta\) and \(\phi\) can readily be understood by considering the extended PES on which the system moves. For the effective potential energy function \(U_{\text{eff}}(\phi, \theta)\) [cf. Eq. (2)] in which \(U(\phi) > 6\) and \(U_{\text{ref}} = 3,\) the global potential energy minimum is \(U_{\text{eff}} = 0,\) which occurs at any point in the subspace in which \(\theta = 0,\) i.e., the region of values \([\theta = 0, \phi = 0 - 2\pi].\) Hence it is reasonable that the system spends most of its time in that neighborhood. This means that the effective energy surface \(U_{\text{eff}}(\phi, \theta)\) does not discriminate energetically between different conformations of the “molecule;” hence the system undersamples the true low-energy regions on the real surface \(U(\phi).\) Thus, the effective surface with \(U_{\text{ref}} = 3\) does not improve sampling of important conformations of the real system. We note that this two-dimensional system (i.e., \(\phi\) and \(\theta\) appears to be sufficiently close to integrable that the trajectory is quasiperiodic, i.e., oscillatory behavior is seen for both \(\phi\) and \(\phi.\) The periods for \(\theta\) and \(\phi\) are likely to be established by the requirement that the closed orbit be probable at thermal equilibrium, i.e., that the barriers of \(U(\phi)\) be less than \(kT,\) which, in particular, sets the upper bound for the value of \(\theta.\) For \(\theta \sim 0.017,\) the highest barrier of \(U(\phi),\) which is 36 kcal/mol, is equal to the average thermal energy \((\sim 0.6\) kcal/mol). It is possible that the system is effectively microcanonical, as is sometimes seen in low-dimensional systems coupled to a Nosé bath.\(^{36}\)

A very different behavior is observed in the simulation with \(U_{\text{ref}} = 12\) kcal/mol [i.e., an example of \(U(\mathbf{q}_{\text{min}}) < U_{\text{ref}}\)]. This simulation is illustrated in Fig. 2(b); 500 000 steps are shown. Figure 2(b) (top) shows the time series of \(f(\theta),\) Fig. 2(b) (middle) is the time series of the bare potential energy \(U(\phi),\) and Fig. 2(b) (bottom) is the time series of the effec-
tive potential \( f(\theta)U(\phi) \). In this case, the global potential energy minimum of the extended system is \( U_{\text{eff}}=-6 \), which occurs at the single point \([f(\theta)=1, \phi=\pi]\). Consequently, the system spends most of the time around this point; after an initial period of sampling, it is “trapped” around the minimum. The problem found here is the reverse of that in the previous simulation; i.e., the trajectory on the effective surface does not explore broadly the physical energy surface. For realistic energy surfaces with multiple minima similar in energy, but separated by large barriers, this constitutes a serious problem (“broken ergodicity”).

In the third simulation, \( U_{\text{ref}}=6 \) kcal/mol is used [i.e., \( U(q_{\text{min}})=U_{\text{ref}} \)]. The results are illustrated in Fig. 2(c); 1000 steps are shown. Figure 2(c) (top) shows the time series of \( f(\theta) \), Fig. 2(c) (middle) is the time series of the bare potential energy \( U(\phi) \), and Fig. 2(c) (bottom) is the time series of the effective potential \( f(\theta)U(\phi) \). In this case, the value of the biasing potential \( U_{\text{ref}} \) is equal to the energy of the global minimum on the original PES. Thus, the minimum energy on the extended surface is zero, which occurs in two regions on this surface; i.e., when \([\phi=\pi, f(\theta)=0–1] \) and when \([\phi=0–2\pi, f(\theta)=0] \). These two regions intersect at the point \([\phi=\pi, f(\theta)=0] \). The system sequentially visits both regions, passing from one to the other via the mentioned vertex. In this case, the sampling is ideal; i.e., broad sampling of the phase space [which occurs when \( f(\theta) \) is around zero] is balanced with fine sampling around minima [which occurs when \( f(\theta) \) is near one].

The three simulations presented show that, in using the unreplicated ansatz, the choice of the biasing constant is crucial to the type of sampling achieved, with the optimum choice being the minimum potential energy of the original system. Of course, this quantity in general is not known and often is difficult to estimate.

**Self-regulating potential.** We now use a two-replica system to show that no reference potential is needed to obtain the same optimal sampling as with the ideally biased unreplicated ansatz. A molecular dynamics simulation was done using the two-replica extended Hamiltonian [Eq. (3)]. Figure 3 illustrates the results; 1000 steps are shown. Figure 3(a) shows the time series of \( f(\theta) \), the sampling variable for replica 1; Fig. 3(b) is the time series of the potential energy.
U₁(φ) of replica 1; Fig. 3(c) is the time series of [1−f(θ)], the sampling variable for replica 2; and Fig. 3(d) is the time series of the potential energy U₂(φ) of replica 2. The results in this simulation are comparable to those shown in Fig. 2(c), i.e., broad sampling is balanced with fine exploration around minima. The difference between the two is that in the unreplicated simulation, the system sequentially searches broadly, then concentrates around minima; in the two-replica simulation, by contrast, the broad exploration by one replica occurs simultaneously with the fine exploration of the second replica. This is consistent with what is expected based on the development presented in the Theory section.

B. Application to a protein-ligand system

We now consider the crossing of a free energy barrier in a protein binding site using the self-regulating approach. The simulations are done using the replicated subsystem ansatz (presented in Sec. II C). For comparison, the MCSS approach is applied to the same system.

Unliganded human nonpancreatic secretory phospholipase A₂ (PLA₂) was used for this study. The protein structure was modeled using the polar hydrogen representation in the CHARMM program. The Metropolis Monte Carlo method was used to propagate the atomic positions and the positions of the virtual particles representing the lambda values. All calculations were done using the MC module in the c27 version of CHARMM, which was modified to include the lambda variables. In the calculations, the protein main chain was fixed, but 52 side chains in the binding pocket were allowed to move. Cartesian and torsional moves were allowed in the MC calculation. The Cartesian moves were limited to a maximum displacement of 0.1 Å/move and the torsion moves a maximum rotation of 1°/move. The functional group used in this study is indole; it was selected because it is similar to a major fragment of a series of ligands for which crystal structures in complex with PLA₂ have been determined. The indole groups were allowed to move only via rigid-body translations and rotations; i.e., their internal geometry was fixed. The rigid translation moves had a maximum displacement of 0.5 Å and the rigid rotation moves a maximum rotation of 10°. Lambda moves

FIG. 3. Two-replica simulation as a function of time (in 10⁻¹⁴ s). (a) The time series of f(θ), the sampling variable for replica 1, (b) the time series of the potential energy U₁(φ) (in kcal/mol) for replica 1, (c) the time series of (1−f(θ)), the sampling variable for replica 2, and (d) the time series of the potential energy U₂(φ) (in kcal/mol) for replica 2.
also were allowed for the indole groups, with a maximum change per lambda move of 0.2. The state of the system (i.e., the instantaneous position and lambda values) is recorded every 1000 MC moves, an interval which for convenience we define as a single “step.” In the set of calculations, which concern crossing a specific free energy barrier and use two indole copies, an average of approximately 900 protein position moves and 100 copy position moves (i.e., 50 per copy) plus 3 lambda moves are done per step. A total of 100 such steps are done. The MCSS simulations are identical except for the absence of the lambda variables. That is, the MCSS potential energy function is given by Eq. (4) where by definition the lambda parameters are fixed at \(\lambda_1=\lambda_2=1/2\) throughout the simulation. (We note that this use of multiple-copy searching differs somewhat from the standard application of MCSS to determine optimal positions via minimization or quenched dynamics. The present work represents the first use of MC with MCSS.)

We study the entry of the indole fragment into the binding pocket of PLA$_2$. Figure 4 shows a view of the binding site in PLA$_2$ pertinent to the present problem. In Fig. 4(a), the native structure of PLA$_2$ is shown and four residues in the binding site are emphasized, namely, Leu 2 and Val 30 (in yellow), His 6 (light blue), and Asp 48 (red). The backbone of the residues that are allowed to move during the simulation is shown in green and that of the fixed residues is shown in dark blue. In Fig. 4(b), a bound structure of PLA$_2$ consisting of a transition state analog (in green) plus the protein is shown; the latter covers Asp 48 in this view.
His 6, Leu 2, and Val 30, the energy of the indole fragment is much worse (i.e., the cluster in this region is high energy). The migration of indole from a position on the surface near His 6 to a better one near Asp 48 requires that the fragment move along a spatially constricted path; i.e., essentially a narrow interior “tube” connects these two positions. For this reason, there is a free energy barrier (i.e., primarily entropic in origin) which prevents the facile movement of indole along this path. We use the crossing of this specific free energy barrier to find the stable (low energy) minimum as the test case for the algorithm.

Two indole groups, each with an initial lambda weight of 0.5, were placed in an identical position on the surface near His 6. The initial position is shown (in yellow) in Fig. 5; note that the orientation of the protein in this figure is related to that in Fig. 4 by an ~180° rotation. In Fig. 5, a surface model is drawn (in green) to represent the protein, with the key residues colored as before. The “tube” connecting the surface near His 6 with the interior near Asp 48 runs vertically in this view. Note that the side chain of Asp 48 is indicated explicitly at the bottom of the tube. The goal of these simulations is to examine the conditions under which the indole fragment is able to overcome the free energy barrier impeding its passage along the tube to a better position and orientation near Asp 48. The latter position is shown (in light blue) in Fig. 5; the NH moiety in this case is drawn in dark blue (for N) and white (for H) to emphasize the correct orientation, i.e., with the indole NH interacting with the carboxylate group of Asp 48. It is expected that below a critical temperature, the self-regulating two-copy system (SRS) will manifest an advantage over the standard fixed potential two-copy system (MCSS), since the barrier height in the former method can be reduced by adjusting the lambda value; i.e., a higher effective temperature can be transiently maintained.

In general, the effective temperature for a part of the system (say replica $j$) that is simulated with a potential scaled by $\lambda_j$ is given by $T/\lambda_j$, where $T$ is the physical temperature of the system; i.e., this is the factor which multiplies $U_j$ in the argument of the exponent in the Metropolis criterion. (We note that it is also possible to have different temperatures for the protein, $T_{pr}$, and for the groups, $T_{gr}$, as we do here.) $T$ is a MC parameter which is constant during the simulation. In accord with Sec. II C, $\lambda_j$ is a variable which evolves with accepted lambda “moves.” Thus, a lambda move is equivalent to a temperature-changing move, such that the effective temperature of one copy increases, and those of the remaining copies decrease, or vice versa [see Eq. (5)]. At the start of the simulation, the two copies have the same lambda value and, therefore, the same effective temperature. During the simulation, a copy $j$ can (i.e., if $\lambda_j \rightarrow 0$) reach a nearly infinite effective temperature, i.e., it can pass through phase space completely unimpeded. It will be shown in the examples given below that the ability in SRS to access transiently higher effective temperatures is the key to its success in improving sampling.

Twenty simulations were done using SRS and 20 parallel simulations using standard MCSS. In the simulations, Leu 2 and Val 30 were held rigidly in place to make the problem more challenging; other residues nearby were free. Each simulation consists of 100 “steps” (as defined above). The acceptance ratios were as follows: for protein Cartesian moves, ~50% for both SRS and MCSS; for protein torsion moves, ~90% for both SRS and MCSS; for ligand translations, ~50% for SRS but ~35% for MCSS; and for ligand rotations, ~55% for SRS but ~40% for MCSS. The acceptance ratio for lambda moves in SRS was ~40%. In both sets, the temperature for the protein moves, $T_{pr}$, is 300 K; while $T_{gr}$ for the indole fragments is 75 K. This means that, in the MCSS simulations, in which $\lambda_1 = \lambda_2 = 0.5$ throughout the simulation, the effective temperatures of the replicas are constant at 150 K; in the SRS simulations, the initial effective replica temperatures also are 150 K, but vary inversely with the lambda values during the simulation. The final effective temperature for the dominant replica is ~75 K (i.e., as $\lambda_i \rightarrow 1$, $T_{eff} \rightarrow 75$ K). At 150 K, an indole group has sufficient thermal energy to cross the barrier on occasion. Thus, of the 40 copies used in each method, 6 copies in the MCSS simulations and an equal number in the SRS simulations cross the barrier region to attain low-energy positions and orientations near Asp 48. While the SRS copies on average move farther from their initial positions (i.e., sampling in general is broader, consistent with the higher acceptance ratios in SRS for ligand moves), a difference in the ability to cross the barrier is not observed in this set of simulations. To discern an effect, the temperature was reduced.

When the two-copy simulation was repeated with $T_{gr}$ set at 30 K, a clear difference was observed between the two approaches. As already described, the effective temperature is a constant for the MCSS copies, and in this case is 60 K. This also is the initial effective temperature for the SRS copies, whereas the final effective temperature of a dominant SRS copy is ~30 K. Again 20 SRS and 20 MCSS simulations were done. The acceptance ratios were as follows: for
protein Cartesian moves, ~50% for both SRS and MCSS; for protein torsion moves, ~90% for both SRS and MCSS; for ligand translations, ~40% for SRS but ~20% for MCSS; and for ligand rotations, ~40% for SRS but ~25% for MCSS (note that the acceptance ratios for ligand moves again are higher for SRS compared with MCSS, but on the whole the ratios are smaller than in the previous set due to the decreased temperature at which the ligands are simulated). The acceptance ratio for lambda moves in SRS was ~55% (which is higher than the ratio in the previous set, consistent with the increased role played by lambda moves when the physical coordinates are simulated at lower temperature, i.e., are less mobile). In the SRS simulations, six copies crossed the barrier and attained low-energy positions and orientations near Asp 48; in MCSS, none of the copies crossed the barrier region. Figure 6 is a plot of the final copy energies [the energy of copy $j$, $U_j$, is defined as $U^\text{hel}(q_j) + U^\text{int}(q, q_j)$ versus the root-mean-square distance of the final positions of the copies relative to their initial position. From this plot it can be seen that the MCSS copies (shown as squares) cluster into two groups, viz., one within 1 Å of the original position and the other about 4 Å from the original position. The copy energies in the first group are similar to the copy energy at the start of the simulation, i.e., ~4 kcal/mol. The second group of copies is located midway between the initial copy position and Asp 48; this latter “halfway” position is shown in red in Fig. 5. The halfway position is energetically better than the initial position; i.e., the energies of copies in this group are ~1 kcal/mol (cf. Fig. 6), whereas kcal/mol less than those of the first group, but still about 10 kcal/mol higher than the SRS copies that are ideally located with the NH moiety interacting with Asp 48. We note that in none of the 26 trajectories in which a MCSS copy moves to this halfway position is a potential energy barrier encountered (data not shown); this conclusion follows from a plot of the physical (unscaled) energy versus step number for these paths. Rather, what is typically seen is that the entry into the binding pocket occurs very rapidly; i.e., over a few steps, the unscaled copy energy falls from ~4 to ~0 kcal/mol.

Figure 6 also plots the copy energy versus rms distance for the SRS copies (shown as diamonds in this figure). The latter show much greater variety in the final copy positions; that is, a larger region in phase space is explored in SRS compared with MCSS (as was also seen in the previous set of simulations). Figure 6 indicates that a few SRS copies have energies greater than ~10 kcal/mol; these copies have escaped from the protein surface and, since they are essentially isolated in vacuum, their energies are close to the vacuum self-energy of indole. The remaining SRS copies are variously distributed on the surface and the interior of the protein. In particular, several of the copies located at ~7 Å are near Asp 48 (cf. Fig. 5). Three of these replicas have attained a good orientation, i.e., in which the indole NH interacts with the carboxylate moiety of Asp 48 (NH...O distance ~2.7 Å); the energies of these copies are around ~12 kcal/mol. Three other replicas in this group are nearby but have different orientations; the common feature in this latter set is that the indole nitrogen is relatively distant from Asp 48 and, as a result, these copies have significantly higher energies, i.e., around ~4 kcal/mol.

The details of the SRS simulations in which a copy crosses the free energy barrier are instructive and consistently show that energetically different paths are taken by replicas in SRS versus MCSS, even if both end up in the same positions. In the first set of simulations described, i.e., that with $T_g=75$ K, it was observed that no potential energy barriers were encountered in any of the trajectories of the MCSS copies that crossed the barrier. The situation is quite different for the SRS simulations in that same set of simulations, as well as in the present set of simulations (i.e., with $T_g=30$ K). Nearly all trajectories of SRS replicas that cross the free energy barrier involve large fluctuations in the potential energy, most notably when passing through the narrower parts of the pocket. It is clear that in SRS, the replicas in general explore a higher-energy regime. This of course would not be beneficial, as such, unless the system also were capable of being “quenched” at the right time, i.e., when a low-energy position is sampled. This is precisely what happens in each of the three SRS trajectories that reach Asp 48; namely, one of the copies transiently explores a high-energy regime (which allows it to cross the barrier) and then is quenched (i.e., its effective temperature is lowered) upon reaching the low-energy position near Asp 48. This process exemplifies the self-regulating nature of the sampling algorithm, as described in the Theory section.

We now consider briefly a few of the individual SRS simulations. As noted, three simulations culminate with an indole copy located at a very good position and orientation. We consider two of the three simulations.

(a) First illustrative trajectory. The results of the first simulation are shown in Figs. 7 and 8. Figures 7(a) and 7(c) are plots of the potential energy and lambda value, respectively, for the successful copy, which is called “copy 1.” Figs. 7(b) and 7(d) are plots of the same for the other copy.
Figure 8 shows the trajectories for copies 1 and 2. In what follows, we give a description of the trajectories for both copies which connects the observed copy motions with their energies and lambda values.

In the simulation (Fig. 8), copy 2 initially moves away from the binding pocket and thereafter erratically explores the protein surface; as a result, its unscaled energy (i.e., $U_2$) increases up to $\sim 10$ kcal/mol. This behavior continues until around step 25, at which point its position becomes more stable; i.e., it interacts with Val 30 on the protein surface and $U_2$ drops to $\sim 1$ kcal/mol. This latter event is coupled with a rapid increase in $\lambda_2$, up to $\sim 0.7$. Meanwhile, for the first 20 steps of the simulation, copy 1 maintains its initial position and energy ($\sim 5$ kcal/mol). During this period, $\lambda_1$ is dominant because the copy 1 energy ($\sim 5$ kcal/mol) is significantly better than that of copy 2 ($\sim 10$ kcal/mol). However, after copy 2 finds the aforementioned good position around step 25, i.e., a position that is lower in energy than that of its competition, copy 1, $\lambda_1$ falls and $\lambda_2$ rises. This type of competition is typical in SRS simulations and is in accord with the presentation in Secs. II B and II C. From its good position achieved at step 25, copy 2 then moves over steps 30–37 to a better one; correspondingly, $\lambda_2$ increases from $\sim 0.7$ to $\sim 1$ and $\lambda_1$ falls to nearly zero. In detail, over steps 30–37, copy 2 adjusts its orientation so that its nitrogen is near the carbonyl oxygen of Val 30 and it remains in this favorable orientation for the rest of the simulation; hence $U_2$ also is constant ($\sim 5$ kcal/mol) for the remainder of the simulation. From this stable position, copy 2 provides a fairly constant competition for copy 1, rather like a biasing potential.
albeit more nuanced in that it indeed is capable of subtle adjustments in its position (and in principle of gross adjustments as well, though such behavior is not seen here). We note that the occurrence of such adjustments will depend primarily on the stiffness of the competition posed by the other copy.

The very small value of $\lambda_1$ following step 35 permits copy 1 to develop an unfavorable close contact (i.e., van der Waals repulsion) with a surface residue. At this point, the copy is highly “indeterminate;” i.e., its lambda weight is very low and it lacks a favorable position to “anchor” it (two features which typically go together), and as a result its mobility is greatest. Copy 1 at this point is capable of moving essentially anywhere in the phase space. As it happens, it enters into the binding pocket around step 40, reaching the “halfway” position; at this point, $U_1$ is $\sim -2.5 \text{ kcal/mol}$ (typical for this position) and thus copy 1 becomes somewhat more competitive with copy 1 ($U_2 \sim -5 \text{ kcal/mol}$). Consequently, $\lambda_1$ rises sharply, though it continues to fluctuate considerably. Copy 1 remains at the halfway position from steps 40 to 80, and its lambda value fluctuates throughout this period. The stochastic fluctuation in lambda values during this stage indicates that the two copies are competing closely, due to the fact that their physical energies are similar. After step 75, $\lambda_1$ happens (i.e., stochastically) to become very small and, as usual, unfavorable Van der Waals (vdW) contacts develop soon thereafter.

Based on the MCSS results, it is clear that passing from the halfway position to the one near Asp 48 involves a free energy barrier. Specifically, at an effective temperature of 60 K, none of the MCSS copies is able to cross this barrier. In the present SRS simulation, copy 1 crosses the barrier during steps 80–95. The barrier crossing is facilitated by the very small value of $\lambda_1$ at this point, which means that the effective temperature of copy 1 is greater than 60 K while it crosses. We note that if copy 2 had not been dominant at this point, copy 1 would be less likely to cross the barrier; i.e., in the latter situation, $\lambda_1$ would already have become $\sim 1$ concomitant with the entry of copy 1 into the binding pocket (i.e., to the halfway position), and as a result lack of sufficient mobility to cross the barrier. (Indeed, such behavior was observed in other simulations in this set [not shown]). Following the barrier crossing, the terminal event in this trajectory is the reorientation of copy 1 vis-à-vis Asp 48, which occurs during the last ten steps of the simulation.\textsuperscript{41}

(b) Second illustrative trajectory. The second SRS simulation with a successful replica is similar to the first in its general features. Figure 9(a) shows the time series of the energy of the successful copy (called “copy 1”) and Fig. 9(c) shows its lambda value. Figures 9(b) and 9(d) show the energy and lambda value of the second replica (“copy 2”). The trajectory is described in detail in Ref. 41. In this simulation, copy 2 also crosses over the free energy barrier and (at first) correctly orients its nitrogen near Asp 48. Hence, from step 50 to the end of the simulation, both copies are located at the correct position but continue to compete for an optimal orientation. Copy 1 eventually dominates and, as a result, copy 2 after that point ($\sim$ step 75) transiently loses and regains its favorable contact with Asp 48.

The two simulations described illustrate the nature of the competition between replicas in the SRS approach. In particular, the behavior of the successful copy can be understood only in connection with that of the other copy, which closely competes with it during key intervals in a given simulation. Both simulations illustrate the self-regulating nature of the sampling algorithm; i.e., competition between the replicas results in a continuing improvement in the replica energy until the ideal position and orientation is achieved by copy 1. It is significant to note that what is seen here is similar in spirit to the “competition” that obtains in genetic algorithms (GAs). In the latter, however, the evolution of the system is unphysical. In the SRS approach, the probability distribution law that governs the average properties of the system is known and, as a result, the sampling obtained corresponds to a specific thermodynamic ensemble.

In the two representative simulations, the free energy barrier is crossed by a copy when its lambda value is quite small. Typically, $\lambda$ is $<0.2$, which corresponds to an effective temperature at least 2.5 times greater than it is in the corresponding MCSS simulations. The effective reduction in the barrier heights in SRS simulations results in a significant difference in the sampling ability of the two approaches. We mention in closing that it is possible to use more than two replicas in the present approach. In our studies, we have found that the use of additional replicas renders the competition between all replicas less acute, since it tends to make it more difficult for any one nondominant replica to become sufficiently strong to challenge the dominant one. This situation differs somewhat from that which obtains when chemically distinct copies are used (as in the work of Brooks and co-workers), in which case more replicas means more chemical diversity, and so is advantageous for this reason alone.
IV. CONCLUSIONS

Efficient sampling of conformations in mesoscopic systems is a difficult problem which has been addressed extensively in recent years. Sampling in the high-energy regime (e.g., by using a high temperature), which permits the facile crossing of barriers, usually leads to undersampling of the low-energy conformations that contribute predominantly to the thermodynamic properties of the system. A method that satisfies these conditions has been developed. It relies on the use of multiple replicas of the system (or of a part of the system), which sample the different energy regimes by the introduction of dynamic sampling variables associated with each of the copies. The dynamics of the sampling variables are coupled (using a holonomic constraint), such that when one replica is searching finely around a minimum, the other is searching broadly in the configuration space; and if the latter happens in so doing to find a better minimum, then the relationship is reversed. Since the reversal occurs naturally in this method, the sampling achieved is self-regulating. The basic features of the method were illustrated using a simple one-dimensional model system. An application to a protein-ligand problem showed how the cross-

FIG. 9. Second illustrative simulation (as a function of MC step number) in which the indole group (“replica 1”) crosses the free energy barrier. All energies are reported in kcal/mol. (a) The time series of the bare potential energy $U_1(q_1)$ for replica 1 (heavy line) and the scaled (effective) potential energy $\lambda_1 U_1(q_1)$ (broken line). (b) The time series of the bare potential energy $U_2(q_2)$ for replica 2 (heavy line) and the scaled (effective) potential energy $\lambda_2 U_2(q_2)$ (broken line). (c) The time series of the lambda value $\lambda_1$ for replica 1. (d) The time series of the lambda value $\lambda_2$ for replica 2.
ning of a free energy barrier is improved with the result that the lowest-energy position is found by one of the replicas. We envision that the method will be useful for sampling rough energy surfaces, such as are found in peptides and in connection with the protein folding problem. It also will be of interest to compare the present method with certain existing methods, such as replica exchange or adaptive umbrella sampling.

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42. See EPAPS Document No. E-ICPSA6-124-510607 for a MCSS map of the binding site, a description of the last ten steps of the first illustrative trajectory, and a complete description of the second illustrative trajectory. This document can be reached via a direct link in the online article’s HTML reference section or via the EPAPS homepage (http://www.aip.org/pubservs/epaps.html).