

Improving Sampling by Exchanging Hamiltonians with Efficiently Configured Nonequilibrium Simulations

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S Supporting Information

ABSTRACT: Molecular simulations aim to sample all of the thermodynamically important states; when the sampling is inadequate, inaccuracy follows. A widely used technique to enhance sampling in simulations is Hamiltonian exchange. This technique introduces auxiliary Hamiltonians under which sampling is computationally efficient and attempts to exchange the molecular states among the auxiliary and the original Hamiltonians. The effectiveness of Hamiltonian exchange depends in part on the probability that the trial exchanges can be accepted, which involves good choices of auxiliary Hamiltonians and a good method of generating the trial exchanges. In this paper, we investigate nonequilibrium simulations as trial exchange generators and develop a theoretical model for the efficiency of Hamiltonian exchange and an algorithm to better configure such simulations. We show that properly configured nonequilibrium simulations can modestly increase the overall efficiency of Hamiltonian exchange.

1. INTRODUCTION

A major problem in many molecular simulations is inadequate sampling.^{1,2} Sampling methods such as molecular dynamics (MD) and Monte Carlo (MC) typically generate a series of molecular states by small moves, such that in the long time limit, the states are sampled with probabilities corresponding to the equilibrium distribution of the underlying Hamiltonian. In many molecular systems, however, the thermodynamically important states are separated by high energy barriers, and crossing these barriers in a simulation is rare, which, for computations of feasible length, leads to incorrect probability densities of the sampled molecular states. Inadequate sampling can be manifested in poorly converged estimates of thermodynamic averages of physical quantities; it can prevent simulations from reproducing molecular events—such as protein folding and conformational changes—observed in experiments. Poor sampling can cause inaccurate results to appear deceptively precise,³ leading to a false assumption of convergence. Many techniques have been developed to tackle the problem of inadequate sampling,¹ including the popular and powerful Hamiltonian exchange method. In this work, we propose a protocol to significantly improve the efficiency of Hamiltonian exchange and provide a systematic framework for measuring the relative benefits of the variations of Hamiltonian exchange methods.

A common approach used to enhance sampling is to introduce auxiliary Hamiltonians under which the energy barriers are significantly reduced and the sampling is efficient. The equilibrium distribution of the original Hamiltonian can be obtained either by reweighting the molecular states sampled under the auxiliary Hamiltonians⁴ or by coupling a simulation of the original Hamiltonian with simulations of the auxiliary Hamiltonians, so that the molecular states sampled under the auxiliary Hamiltonians can be “exchanged” into the simulation of the original Hamiltonian. This latter approach—with many variations—is referred to as the generalized ensemble method or

Hamiltonian exchange. In such simulations, normal MD or MC moves are interrupted by attempts to exchange molecular states generated under one Hamiltonian into the simulation under a different Hamiltonian, and such trial exchanges are accepted or rejected such that the equilibrium distribution at each Hamiltonian is preserved. Early attempts of Hamiltonian exchange, such as simulated tempering^{5,6} and parallel tempering,^{7,8} reduce enthalpic barriers by raising the simulation temperatures, in effect linearly scaling the energy function. Other Hamiltonian modifications,^{6,9,10} such as softening nonbonded interactions,^{11,12} altering dihedral terms,¹³ or reducing the effective degrees of freedom,^{14,15} have since been proposed.

In order for Hamiltonian exchange to work well, trial exchanges must be accepted with a high enough probability to allow simulations at the original Hamiltonian to benefit from the enhanced sampling at the auxiliary Hamiltonians. The efficiency of Hamiltonian exchange can be improved through the judicious selection of auxiliary Hamiltonians and through informed construction of trial exchanges. The simple trial exchange—given a molecular state under Hamiltonian H_a , switch on a different Hamiltonian H_b —has been a *de facto* choice in most reported Hamiltonian exchange simulations, in which case the only issue is to optimize the selection of auxiliary Hamiltonians. More sophisticated trial exchanges,^{16–18} in which the molecular state and the Hamiltonian are updated together in such a way that the new molecular state has increased probability in the equilibrium distribution of the new Hamiltonian, can enhance the acceptance probabilities of the trial exchanges, as illustrated in Figure 1a. Recently, nonequilibrium simulations have been proposed as a method for generating trial exchanges.^{19,20} In Hamiltonian exchange with nonequilibrium trials (HENT), both the nonequilibrium simulations in the trial exchanges and the selection of

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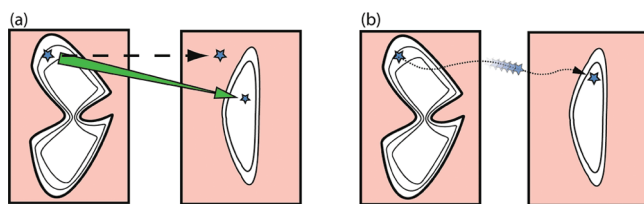


Figure 1. Benefits of sophisticated trial moves. The large rectangles represent two energy surfaces on the same two-dimensional domain, with the white and pink regions showing low and high energy regions, respectively. (a) A simple trial exchange (dotted line) keeps the coordinates (blue star) the same, producing a move unlikely to be accepted. A more efficient move (green arrow) connects the low energy regions, and has a much higher acceptance rate without the need for intermediate Hamiltonians. The changing width of the green arrow indicates a scaling of coordinates, and is accounted for by the Jacobian term in the acceptance criterion. (b) In many-atom systems, it is often unclear how to construct the most efficient trial exchanges. Instead, shortened molecular dynamics with a time-dependent Hamiltonian can be used to make a sophisticated trial exchange. This comes with an associated cost, so the challenge is to determine when the benefits outweigh the added expense. Both a and b apply to serial exchange; in replica exchange, there is a simultaneous move from the right system to the left, i.e., an arrow in the reverse direction connecting a different pair of points.

auxiliary Hamiltonians need to be planned carefully to achieve efficient exchanges. How to plan them effectively is an open question that we address in this work; our protocol optimizes the two aspects together.

To generate a trial exchange using nonequilibrium simulations, the molecular system undergoes a simulation governed by a time-dependent Hamiltonian that changes in a nonequilibrium fashion from the current Hamiltonian into the target Hamiltonian over a prescribed period of time (Figure 1b). These trial exchanges are more likely to be accepted than simple trial exchanges because the molecular state has changed gradually following the evolution of the Hamiltonian, so that at the end of the simulation, the molecular state is one that is more likely to be found in the equilibrium distribution corresponding to the new Hamiltonian. On the other hand, increased acceptance does not necessarily translate to increased computational efficiency, as the nonequilibrium simulations entail additional computational cost. The question remains whether the benefit of increased acceptance outweighs the cost of the additional simulation associated with each trial exchange. This paper lays out an objective framework to measure the overall efficiency of HENT and compares HENT to Hamiltonian exchange using simple trial exchanges.

In this work, we illustrate our method and demonstrate its effectiveness in a common application: computing the free energy associated with the transfer of a flexible molecule from the gas phase into an aqueous solution. In order to obtain the correct free energy, all of the conformations of the solute molecule must be sampled according to the Boltzmann distribution, both in the gas phase and in the solution phase, but inadequate sampling can occur when there are high energy barriers between different solute conformations. Here, we compare the effectiveness of several variations of Hamiltonian exchange in dealing with this problem. We show that at the same total computational cost, nonequilibrium trial exchanges configured using our protocol modestly enhance the overall efficiency of Hamiltonian exchange compared with methods

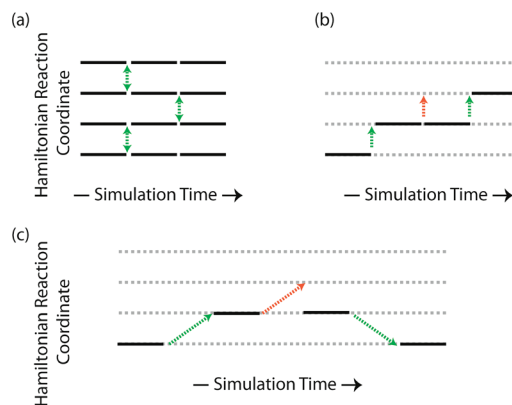


Figure 2. Three types of Hamiltonian exchange. (a) Replica exchange requires one simulation per Hamiltonian level of interest, with all simulations running simultaneously. In one common version of replica exchange, swap moves (green arrows) are attempted at regular intervals, with decisions made independently for disjoint pairs of neighbors. Swaps require no simulation time, and regardless of their outcome, there is always exactly one simulation per Hamiltonian level. (b) Serial exchange uses a single simulation that can hop between different Hamiltonian levels. If a Hamiltonian swap is rejected (red arrow), the simulation stays at the same level. One parameter per level controls the average amount of time spent simulating at each Hamiltonian. (c) Serial exchange with nonequilibrium trials replaces the direct Hamiltonian swap attempts with a simulation under a time-dependent Hamiltonian, which improves acceptance rates at the expense of additional simulation time. A similar strategy can also be applied to replica exchange (not shown).

based on simple trial exchanges, as evidenced by more accurate free energy estimates. We expect that HENT under our protocol can be a superior sampling method in general applications.

2. METHODS

2.1. Review of Hamiltonian Exchange and Nonequilibrium Simulations. Hamiltonian exchange is a technique for simulating the equilibrium distributions of a system for two or more related energy functions (Hamiltonians). By coupling multiple Hamiltonians into a single simulation, coordinates explored with one energy function can be shared with all of the other energy functions. There are two basic categories of Hamiltonian exchange simulations: replica exchange and serial exchange. Given a system with N different Hamiltonians, replica exchange methods require N simulations to be run in parallel, one per Hamiltonian. At regular or randomized intervals, the N simulations attempt to exchange energy functions (or equivalently, atomic coordinates) in some predetermined manner. A common exchange scheme⁸ for an ordered set of Hamiltonians is to have pairs of adjacent neighbors attempt Hamiltonian swaps (Figure 2a). These exchange attempts are accepted or rejected probabilistically such that the equilibrium distribution at each of the N Hamiltonians is preserved. While replica exchange requires N simultaneous simulations, serial exchange requires only one. This single simulation can traverse all N Hamiltonians by regularly attempting to change Hamiltonians (Figure 2b). Compared to replica exchange, serial exchange requires $N - 1$ additional parameters for the simulation to run efficiently.⁵ Further comparisons between serial exchange and replica exchange are included in the Supporting Information.

A sufficient condition for ensuring that exchange moves preserve the equilibrium distribution is detailed balance. This property can be defined in terms of a transformation of coordinates:

$$\pi(\hat{x}) p(T) a(\hat{x} \rightarrow T[\hat{x}]) = \pi(T[\hat{x}]) J_T(\hat{x}) |p(T^{-1}) a(T[\hat{x}] \rightarrow \hat{x})| \quad (1)$$

Here, \hat{x} is a generalized variable that includes the atomic coordinates, velocities, and other state information for all of the systems being simulated, T is a one-to-one transformation, $p(T)$ is the probability of attempting T , $J_T(\hat{x})$ is the corresponding Jacobian determinant, and $a(\hat{x} \rightarrow T[\hat{x}])$ is the probability of accepting the transformation. A simple serial exchange transformation is to keep atomic positions and velocities constant and to increment or decrement the Hamiltonian level. The Jacobian determinant of this transformation is simply 1 and is often omitted from the detailed balance equation. Individual moves that satisfy detailed balance can be strung together in a sequence to form a single combined move. While this combined move is unlikely to satisfy detailed balance, Manousiouthakis and Deem²¹ showed that such a sequence will still drive the system toward the equilibrium distribution. This justifies alternating sampling steps with a series of exchange attempts, as depicted in Figure 2a.

In molecular simulations, efficient trial moves such as those in Figure 1a are often difficult to generate. Nonequilibrium molecular dynamics can be used—in this case with a reversible, time-dependent energy function that smoothly links two target Hamiltonians^{22,23}—to generate trial moves (Figure 2c), similar to how equilibrium molecular dynamics is used to generate trial moves in hybrid Monte Carlo.²⁴ The change in the total energy of the system before and after the nonequilibrium simulation determines the acceptance probability of the trial move. In this work, such trial moves are referred to as *nonequilibrium trial exchanges*, and methods that use these simulation-based moves fall into a category referred to as *Hamiltonian exchange with nonequilibrium trials*. In contrast, direct coordinate swaps without simulation-based moves are referred to as *simple trial exchanges*, and methods that use only these moves are collectively referred to as *simple Hamiltonian exchange*.

2.2. Efficient Configuration of Hamiltonian Exchange by Minimizing Mean Round Trip Time. In a typical Hamiltonian exchange simulation, there are two energy functions of interest: a standard molecular dynamics force field meant to mimic physical properties and a modified force field meant to enhance sampling. To efficiently perform Hamiltonian exchange, one needs to have a reasonable chance of accepting exchanges between the two. In simple Hamiltonian exchange, this is traditionally accomplished by choosing a reaction coordinate connecting these two Hamiltonians and placing enough *intermediate Hamiltonians* along this path to ensure that nearest-neighbor exchanges are accepted at a reasonable rate. Much has already been written on the optimal placement of intermediates for various simple Hamiltonian exchange methods.^{7,25–28} When performing HENT, efficiently setting up the simulation becomes more complicated. Is it better to have only two Hamiltonians connected by a long nonequilibrium trial exchange, or a few intermediate Hamiltonians connected by shorter trial exchange simulations, or many intermediates without any nonequilibrium trial exchanges? A framework for answering this question is the main methodological contribution of this work and provides a way to configure efficient HENT simulations.

Scheme 1. Pseudocode for Selecting Hamiltonian Exchange Parameters

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inputs :
 $s_i$  for all candidate levels, 1 to  $L$ 
Candidate exchange probabilities,  $a_{i,j,t}$ 

outputs :
Cheap paths from 1 to  $i$ ,  $\forall i \in [2, L]$ 

subroutine bestT ( $i, j, \text{sumST}, \text{sumA}$ ):
# Find locally optimal  $t$  connecting  $i, j$ 
# Start with  $t = 0$ 
 $\text{bestScore} = 2(\text{sumST} + s_j)(\text{sumA} + 1/a_{i,j,t})$ 
 $\text{bestTij} = 0$ 
 $\text{bestAijt} = a_{i,j,0}$ 
forall ( $i, j, t$ ) # Loop over available  $t$  for fixed ( $i, j$ )
 $\text{score} = 2(\text{sumST} + s_j + t)(\text{sumA} + 1/a_{i,j,t})$ 
if  $\text{score} < \text{bestScore}$ 
 $\text{bestScore} = \text{score}$ 
 $\text{bestTij} = t$ 
 $\text{bestAijt} = a_{i,j,t}$ 
return ( $\text{bestScore}, \text{sumST} + s_j + \text{bestTij}, \text{sumA} + 1/\text{bestAijt}$ )

#Main Algorithm
for  $i = 2$  to  $L$ 
# Solve for a cheap path from 1 to  $i$ 
# First try simple trial exchange
( $\text{bestScore}, \text{sumST}, \text{sumA}$ ) = bestT (1,  $i, s_i, 0$ )
# Store  $\sum s + \sum t$  and  $\sum 1/a$ 
 $\text{bestSumST}[i] = \text{sumST}$ 
 $\text{bestSumA}[i] = \text{sumA}$ 
for  $j = 2$  to  $i - 1$ 
# Try adding node  $i$  to existing best path to  $j$ 
( $\text{score}, \text{sumST}, \text{sumA}$ ) = bestT ( $j, i, \text{bestSumST}[j], \text{bestSumA}[j]$ )
if  $\text{score} < \text{bestScore}$ 
# New best path from 1 to  $i$ 
 $\text{bestScore} = \text{score}$ 
 $\text{bestSumST}[i] = \text{sumST}$ 
 $\text{bestSumA}[i] = \text{sumA}$ 

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Given a predetermined reaction coordinate, one needs to decide the number and placement of intermediate levels, as well as the lengths of any nonequilibrium trial exchanges. This is a nontrivial task, for which it is necessary to have a simple quality metric to assist parameter selection. One such metric for parameter quality is the *mean round trip time* (mrtt), a measure of the average simulation time required for the system (or a particular replica of the system in the case of replica exchange) to diffuse from one end of the reaction coordinate to the other end and back again.²⁶ Conceptually, minimizing the mrtt allows the simulation to quickly diffuse to a Hamiltonian where sampling is fast, and then back to the physical Hamiltonian of interest. The main theoretical result of this paper is an inexpensive estimate of the mrtt for an N -level serial exchange process:

$$\widehat{\text{mrtt}} = 2 \left(\sum_{i=1}^N s_i + \sum_{i=1}^{N-1} t_{i,i+1} \right) \left(\sum_{i=1}^{N-1} 1/a_{i,i+1} \right) \quad (2)$$

where s_i is the amount of time spent simulating at Hamiltonian i before attempting an exchange, $a_{i,i+1}$ is the mean acceptance rate between two adjacent levels, $t_{i,i+1}$ is the length of a nonequilibrium trial exchange, and $\widehat{\text{mrtt}}$ is an estimator of the true mean round trip time. Throughout this paper, Hamiltonian exchange simulations are configured by choosing parameters, i.e., the number and placement of intermediate Hamiltonians, and the length of the nonequilibrium simulations, that minimize the value of this estimator. Equation 2 is exact for the case of a simplified Markov process with an equilibrium distribution that visits all N Hamiltonian levels equally. While it may not be

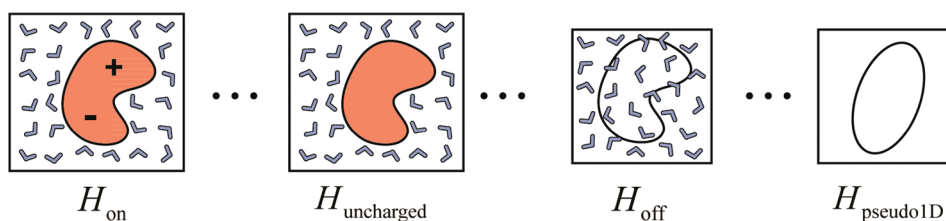


Figure 3. Hamiltonian exchange reaction coordinate. Starting with the full Hamiltonian, H_{on} , the first stage linearly scales down all ligand charges. $H_{\text{uncharged}}$ has all ligand charges set to zero but still interacts with the water through Leonard-Jones forces. The ligand–solvent interactions are then scaled down using a softcore potential, with the forces reduced to zero (white ligand) in H_{off} . The goal of the free energy calculation described here is to compute the free energy difference between H_{on} and H_{off} . To overcome energetic barriers between slowly converting conformers, the reaction coordinate is extended to H_{pseudo1D} , where the intramolecular Leonard-Jones terms in the ligand have also been removed. This greatly simplifies the ligand potential energy (depicted by the simpler shape) such that the ligand's conformations can interconvert directly, without the need for molecular dynamics. Water is not shown in H_{pseudo1D} because it has already been decoupled from the ligand. Triple dots indicate an unspecified number of intermediate Hamiltonians.

optimal to equally visit all levels,^{26,29} this is a reasonable way to simplify parameter selection. The derivation of eq 2 is provided in the Supporting Information.

In this work, the s_i values in eq 2 are fixed at 1 ps for almost all i (see section 2.3 for the exception). There is no clear consensus at this point on the optimal frequency of exchange attempts,^{30,31} the s_i values used in this study were not chosen according to any particular optimality criterion but fall well within the (rather wide) range of values that have been employed in various studies reported in the literature.^{32–34}

Equation 2 applies specifically to serial exchange but can also be adapted to replica exchange with a few small modifications. Viewing replica exchange as N loosely coupled serial exchange runs,³⁵ eq 2 still holds with the exception that all $s_i \leftarrow \max_i(s_i)$ and all $t_{i,i+1} \leftarrow \max_i(t_{i,i+1})$, due to the synchronous nature of replica exchange. The mrrt is based solely on simulation time used, and it is assumed that all other computations, such as energy evaluations, have negligible costs. This assumption breaks down for extremely small values of s_i but is reasonable for the examples presented here.

Practically, eq 2 provides a simple metric for parameter optimization, under the assumption that mrrt is a reasonable predictor of Hamiltonian exchange efficiency. Given L possible Hamiltonian levels, along with estimated $a_{i,j}$ for given $t_{i,j}$, a simple dynamic program³⁶ can quickly choose an mrrt-optimal subset of these intermediates, along with optimal lengths for nonequilibrium trial exchanges. (A method for estimating $a_{i,j}$ as a function of $t_{i,j}$ is described in section 2.5.) The total number of levels selected is an output of the algorithm and need not be specified beforehand. This optimization algorithm is similar to finding the minimum cost path connecting points 1 and L , given $L - 2$ possible intermediates and various pairwise edge costs. The problem here is slightly more difficult, as the total cost of a path is not simply the sum of independent edge costs, but similar ideas can still be used. A simple, near-optimal algorithm is given by the pseudocode in Scheme 1. While this algorithm is likely sufficient for practical purposes, refinements to guarantee optimality are discussed in the Supporting Information.

2.3. Alchemical Free Energy Calculations with Hamiltonian Exchange. Hamiltonian exchange can be used to compute the free energy required to transfer a flexible small molecule from the gas phase to an aqueous environment.³⁷ One part of this calculation³⁸ is the conversion of a fully solvated molecule to an uncharged small molecule with no interaction with the solvent. These two states are depicted in Figure 3 as Hamiltonians H_{on}

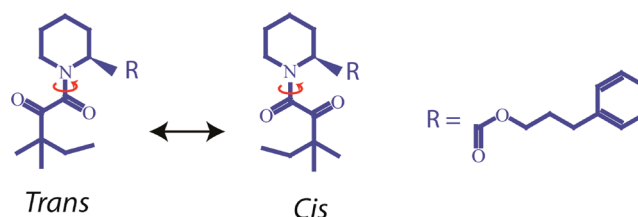


Figure 4. The FKBP ligand used in this study. Under standard conditions, this ligand exists as a mixture of *trans* and *cis* isomers. A large potential energy barrier prevents rapid rotation about the amide bond.

and H_{off} respectively. Although Hamiltonian H_{off} should provide some enhanced sampling by removing the effects of solvent viscosity on the small molecule, this effect is small. To speed up sampling further, an auxiliary Hamiltonian, H_{pseudo1D} , is introduced and allows for faster conformational changes (see below).

The small molecule studied here is a ligand for FK506-binding protein (Figure 4). This ligand is one of several that have previously been examined³⁹ and is known to have at least two slowly interconverting conformers. The experimental conversion time between these forms is on the order of milliseconds to seconds, and sampling of both states by plain molecular dynamics is unlikely on the time scales typically used for free energy calculations.

There are several methods for overcoming energy barriers^{40–42} that could be used to facilitate transitions among the slowly converting conformers. The method that we use in this work extends the reaction coordinate to a new Hamiltonian, H_{pseudo1D} , where the ligand is not only decoupled from the solvent but all ligand–ligand nonbonded interactions have also been turned off. As a result, the potential energy of a ligand under H_{pseudo1D} is simply the sum of bond, angle, and dihedral terms. If bond lengths and bond angles are temporarily fixed, each rotatable bond in the molecule, with the exception of those in a ring, has an energy function that is independent of all the others, and the one-dimensional probability distributions around each bond can be easily computed. This decomposition into independent, one-dimensional problems allows for direct resampling of all non-cyclic rotatable bonds using standard numerical techniques such as acceptance–rejection sampling.⁴³ While bond lengths and angles are not varied with this type of move, molecular dynamics at other Hamiltonian levels should provide sufficient sampling for these other degrees of freedom. Thus, when a serial exchange

simulation reaches H_{pseudo1D} , plain molecular dynamics is replaced with a direct resampling of rotatable bonds, and $s_{\text{pseudo1D}} = 0$. In replica exchange, the same direct resampling can be performed, but since all other levels are still performing molecular dynamics, there are no wall-clock savings by skipping the simulation step only at this level. Thus, when performing replica exchange at H_{pseudo1D} , plain molecular dynamics is used along with direct resampling of all noncyclic rotatable bonds.

2.4. Reaction Coordinate Details. To connect H_{on} and H_{off} , a reaction coordinate⁴⁴ is chosen (Figure 3) that first linearly scales the charges to zero, yielding an intermediate Hamiltonian, $H_{\text{uncharged}}$. Next, the ligand–solvent Leonard–Jones interactions are turned off with a softcore potential⁴² (see Supporting Information), described by a parameter λ_{vdw} . When $\lambda_{\text{vdw}} = 0$, this is the standard Leonard–Jones potential, and when $\lambda_{\text{vdw}} = 1$, this potential is zero everywhere. The segment of the reaction coordinate joining $H_{\text{uncharged}}$ to H_{off} linearly scales λ_{vdw} from zero to one. In order to overcome energy barriers, the reaction coordinate is further extended to H_{pseudo1D} by turning off all ligand–ligand Leonard–Jones interactions, using a similar softcore potential.

2.5. Initialization Data and Input Estimation. The results of the parameter selection algorithm will only be as good as the $a_{i,j,t}$ supplied to it. To estimate these $a_{i,j,t}$, the reaction coordinate is first subdivided into a large number of possible intermediates. In the reaction coordinate segment connecting H_{on} and $H_{\text{uncharged}}$, there are 21 candidate intermediates for the charge scaling parameter, ranging from one to zero. In the region $H_{\text{uncharged}}$ to H_{off} , there are 51 candidate intermediates for the ligand–solvent softcore parameter, ranging from zero (full strength) to one (completely off). In the final segment from H_{off} to H_{pseudo1D} , ligand–ligand interactions are disabled with 21 candidate intermediates. Such a fine subdivision would not be necessary for actual applications, but for method comparison purposes, a conservative approach is taken. Further detail is provided in the Supporting Information.

Using the candidate intermediates described above, a series of 300 ps molecular dynamics runs are performed, starting with H_{pseudo1D} and ending with H_{on} . In the first leg, H_{pseudo1D} to H_{off} , water is unnecessary, as it is decoupled from the ligand, and the ligand is simulated in an otherwise empty box and run with a constant volume, constant energy integrator. Every 1 ps, the velocities are resampled to generate a constant volume, constant temperature ensemble. An equilibrated water box is then overlaid onto the ligand, and the remaining two legs are run analogously to the first, but with an NPH integrator, and velocity resampling (including an Andersen piston⁴⁵) to maintain an NPT ensemble. After the first 60 ps of each run, the potential energy of the system is periodically evaluated at all other Hamiltonians within the leg. From these data, relative free energy estimates are made for all levels, using Bennett analysis⁴⁶ or multi-Bennett analysis.⁴⁷ (In this study, only minor differences exist between standard and multi-Bennett, so for the remainder of the work, standard Bennett analysis is used, as it is computationally cheaper.) These relative free energy estimates directly give the necessary parameters needed for serial exchange.^{5,48} Furthermore, these same data are used with the free energy estimates to approximate the mean acceptance rates, $a_{i,j}$, of direct exchanges ($t_{i,j} = 0$) between all pairs of levels within a leg. These same data are also analyzed to estimate replica exchange acceptance rates. In simple Hamiltonian exchange, these acceptance rates are sufficient for mrvt-optimal level placement, using the minimum-cost path algorithm outlined above.

To estimate the impact of nonequilibrium trial exchanges, additional simulations are needed for pairs of possible intermediates. Exhaustive testing of all pairs is not practical, so only a limited set of pairs is explored. These tests involve running two-state serial exchange runs for select pairs, with separate simulations needed for each $t_{i,j} \neq 0$ of interest. Analyzing the work values⁴⁹ from these simulations gives estimated mean acceptance rates as a function of simulation length. The choice of which (i, j) pairs and $t_{i,j}$ to test is done by hand, and the specific cases examined are shown in the Supporting Information. In the end, the parameter selection algorithm can only choose nonequilibrium trial exchanges that are explicitly tested. Including additional nonequilibrium exchanges might further improve the performance of these methods relative to simple serial exchange.

One final aspect of initialization leverages the use of H_{pseudo1D} . While the initialization procedure outlined above may work well for a single ligand conformation, it may miss other important conformers. To account for this, the above procedure is repeated multiple times. Starting with a snapshot of the system under Hamiltonian H_{pseudo1D} , the system is quickly minimized by running 60 ps of constant volume, constant temperature molecular dynamics at 5 K. From this minimized structure, the equilibrium probability densities for rotation around each noncyclic rotatable bond are independently analyzed and divided into rotamers, using a $5 k_B T$ minimum peak to trough distance as a rotamer definition. The free energies of these rotamers are then estimated (see Supporting Information), and the free energy of a conformer is approximated as the sum of its constituent rotamer energies. Here, the six lowest free energy conformers (with Hamiltonian H_{pseudo1D}) include three *trans* and three *cis* rotamers (Figure 4) and are chosen for further analysis. The initialization procedure from the preceding paragraphs is performed on all six of these conformers, and the data are combined in a straightforward way to give revised input parameters, appropriately averaged (see Supporting Information) across all tested conformers. The nonequilibrium trial exchange tests are only performed at the end, once the composite free energy estimates are made using all six conformers. This rather elaborate procedure is unnecessary for the correctness of the method, but helps in selecting parameters for fair comparisons among different variants of Hamiltonian exchange.

2.6. Production Data and Analysis. Once all of the necessary parameters are chosen by the optimization algorithm, production data are generated for three methods: simple replica exchange, simple serial exchange, and serial exchange with nonequilibrium trials. The optimized replica exchange simulation uses 19 Hamiltonian levels and is run for 200 ns. The starting conformations for each level are picked from the initialization simulations and include a roughly equal number of structures from each of the six selected conformers. In both versions of serial exchange, 19 independent runs of 100 ns are performed using the same starting coordinates as in replica exchange. The simulation costs of any nonequilibrium trial exchanges are included in the total run time. When comparing the three methods, only the first 100 ns of replica exchange are used. The additional 100 ns of replica exchange are used to help create a gold standard computational result (see Results). Free energies for these long simulations are computed using versions of Bennett's method,^{44,47,50} with details given in the Supporting Information.

Mean absolute errors in free energy estimates are computed for varying amounts of total simulation time, which for HENT includes the simulation time used to generate nonequilibrium

Table 1. Estimated Free Energy Differences and 90% Confidence Intervals

method	total simulation time	relative free energy (kcal/mol @ 300 K)
simple replica exchange (data from 19 replicas lumped together)	3.8 μ s	−55.41 (gold standard)
simple replica exchange (first half only, data analyzed as 19 independent trajectories)	1.9 μ s	−55.41 \pm 0.05
simple serial exchange (19 independent runs)	1.9 μ s	−55.47 \pm 0.05
serial exchange with nonequilibrium trials (19 independent runs)	1.9 μ s	−55.40 \pm 0.03

trial exchanges. Each simulation is divided into nonoverlapping time intervals, and free energies are estimated for each time interval. The differences between these estimates and the gold standard are averaged across all of the time intervals to obtain the mean absolute error. The 90% confidence intervals are calculated subject to the assumption that the data in different time intervals are independent. This assumption is justified when the correlation between neighboring intervals is small; here, the total simulation time is comparable to the mrtt, and the data are gathered across 19 replicas.

2.7. Molecular Dynamics Details. Simulations are run using the molecular dynamics package, Desmond,⁵¹ using periodic boundary conditions, with long-range electrostatics handled by particle mesh Ewald.⁵² Calculations are performed with a reference temperature of 300 K and a reference pressure of 1 bar. A custom plugin is used to handle Hamiltonian exchange. Simulations use a 1:1:3 RESPA schedule with an inner time step of 2 fs, and bonds involving hydrogen atoms are constrained using a numerically superior implementation⁵³ of M-SHAKE.⁵⁴ Additional molecular dynamics and Hamiltonian exchange details are provided in the Supporting Information.

3. RESULTS AND DISCUSSION

3.1. Method Validation. To validate the correctness of nonequilibrium simulations as trial exchange generators, the relative free energy defined in section 2.3 is computed using three different types of Hamiltonian exchange. The two control methods, simple replica exchange and simple serial exchange, hold system coordinates constant while attempting Hamiltonian exchanges. The third method, serial exchange with nonequilibrium trials, adjusts system coordinates via molecular dynamics in order to improve acceptance rates. Table 1 shows that all three methods produce statistically equivalent free energy differences. The first row of the table gives the free energy estimate from replica exchange, using all 3.8 μ s of aggregate simulation time. Since simple replica exchange is the most commonly used Hamiltonian exchange method, this free energy estimate is used as the gold standard for all future analysis. Other choices of the gold standard, such as a maximum-likelihood weighted average of all three methods' results, yield similar results (a difference of only 0.01 kcal/mol in the gold standard) to those presented in this paper.

3.2. Efficiency of Nonequilibrium Trial Exchanges. As a measure of the efficiency of nonequilibrium trial exchanges, the mean absolute errors in free energy estimates are compared for varying amounts of total simulation time. Figure 5 graphs these errors for the three Hamiltonian exchange methods described above. Serial exchange with nonequilibrium trials is the clear winner and provides a roughly 20% reduction in mean absolute error for all time lengths considered.

Less direct measures of efficiencies are the estimated and observed mean round trip times for each Hamiltonian exchange

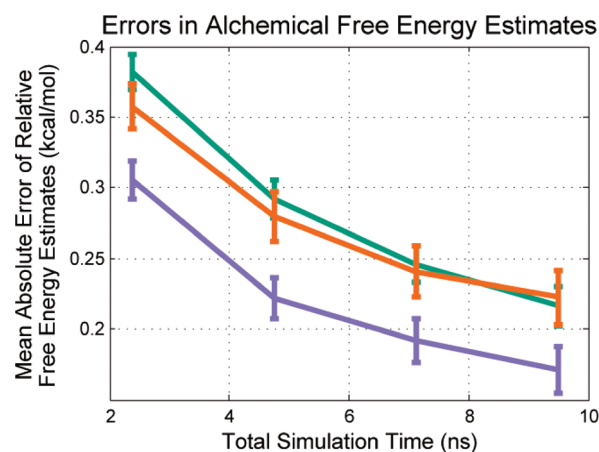


Figure 5. Error reductions from nonequilibrium trial exchanges. The mean absolute error (relative to a long replica exchange control) is shown as a function of total simulation time used. In simple replica exchange (green), this involved a single run with 19 replicas, while the two serial exchange methods (orange, purple) combined data from 19 independent runs each. Simple serial exchange (orange) performed comparably to simple replica exchange (green), while serial exchange with nonequilibrium trials (purple) reduced the average error by roughly 20% across the board. Error bars represent 90% confidence intervals.

simulation. As described previously, the estimated mean round trip time is the quality metric used to choose the number and placement of intermediate Hamiltonians along the reaction coordinate, as well as the lengths of any nonequilibrium exchange simulations. Table 2 shows the results of minimizing the estimated round trip times, using short initialization data and the algorithm outlined in section 2.2. Comparing simple replica exchange and simple serial exchange shows that the latter uses fewer intermediates and is predicted by eq 2 to reduce the mean round trip time by a factor of 2. Allowing for nonequilibrium trial exchanges further reduces both the number of intermediates and the estimated mean round trip time. Actual mean round trip times for the production simulations are shown alongside the predicted values, and the relative speeds of these three methods appear to be accurately captured by the model. This correlation between estimated and observed mean round trip times suggests that the parameter selection algorithm described here is a reasonable way to set up and compare different Hamiltonian exchange methods. Replica exchange with nonequilibrium trials was not directly tested, but the relative benefits of nonequilibrium simulations will likely be similar to those seen for serial exchange. Finally, although simple serial exchange has shorter mrtt than replica exchange, the former does not appear to significantly outperform the latter with respect to the mean absolute error of free energy estimates (Figure 5). Thus, mrtt alone is not always a perfect measure of sampling efficiency.

Table 2. Theoretical and Observed Mean Round Trip Times and Speedup Factors Relative to Replica Exchange

method	optimal number of levels	theoretical time (ps)	theoretical speedup factor	observed time with 90% confidence intervals (ps)	observed speedup factor
simple replica exchange	19	3189		4423 ± 156	
simple serial exchange	14	1529	2.1 x	1967 ± 65	2.2×
serial exchange with nonequilibrium trials	8	993	3.2 x	1286 ± 34	3.4×

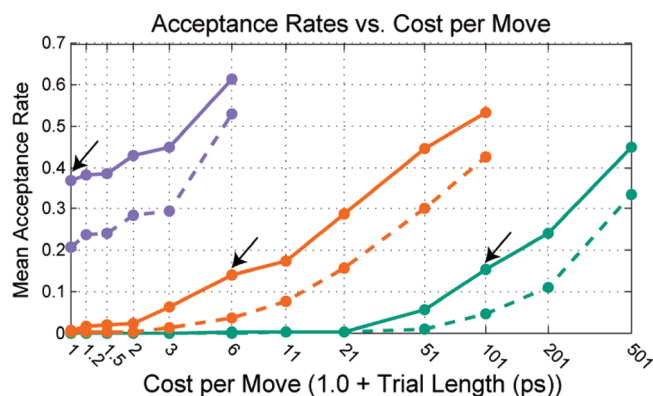


Figure 6. Approximating the efficiency of nonequilibrium trial exchanges. All curves show estimates of the mean acceptance rate for changing the Leonard-Jones interactions between an uncharged ligand and the solvent molecules, with solid lines indicating serial exchange and dashed lines indicating replica exchange. The purple curves represent an easy case: switching between a softcore coefficient of 0.88 and 1.0. The orange curves switch between 0.82 and 1.0, and the green curves switch between 0.0 and 1.0. The arrows indicate the point where the mean acceptance rate divided by the cost per move is maximized for serial exchange. In the hardest case (green), the maximum ratio occurs for 100 ps trial moves, whereas for the easiest case (purple), the maximum occurs with direct swaps between levels. These data were generated by performing two-level serial exchange simulations on a single conformer of the FKBP ligand. Replica exchange estimates were made based on these same data.

3.3. Effects of Nonequilibrium Simulation Lengths and Intermediate Hamiltonian Placement. Estimates of the mean acceptance rates for select nonequilibrium exchange attempts are shown in Figure 6. In all cases, as the nonequilibrium simulation time increases, the mean acceptance rates go up. Black arrows in Figure 6 indicate the point at which the *efficiency ratio*, defined here as the mean acceptance rate divided by the move generation cost, is at a maximum. Clearly, there is no single optimal length for nonequilibrium trial exchanges, as the answer depends on the location of the two end points along the reaction coordinate. In cases where acceptance rates are already high with a simple trial exchange, performing a nonequilibrium simulation decreases the efficiency ratio. When acceptance rates are extremely low for simple swaps, adding nonequilibrium trial exchanges can significantly boost the efficiency. Looking at the green curve, the efficiency ratio increases by a factor greater than 10^{10} when comparing 100 ps nonequilibrium trial exchanges to simple trial exchanges. This dramatic improvement is highly misleading, as someone setting up a simple Hamiltonian exchange simulation would never choose adjacent levels so far apart that the mean acceptance rates were near zero. Instead, more intermediate levels would be introduced, drastically reducing the benefit of nonequilibrium trial exchanges for any pair of adjacent levels.

Table 3. Hamiltonian Levels and Parameters for Serial Exchange with Nonequilibrium Trials

level	ligand charge coefficient	softcore λ_{vdw} (ligand–solvent)	softcore λ_{vdw} (ligand–ligand)	$t_{i,i+1}$ (ps)
0 (H_{on})	1	0.000	0.0	2
1 ($H_{\text{uncharged}}$)	0	0.000	0.0	5
2	0	0.775	0.0	0
3	0	0.825	0.0	0
4	0	0.875	0.0	0
5 (H_{off})	0	1.000	0.0	0.5
6	0	1.000	0.7	0
7 (H_{pseudo1D})	0	1.000	1.0	

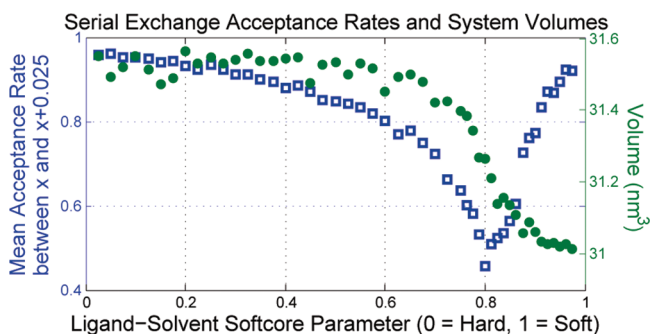


Figure 7. Trial move acceptance is not uniform. Using the initialization data from a single conformer of the FKBP ligand, the estimated mean acceptance rate (blue squares) of equidistant swaps, and the estimated mean box volume (green circles) are plotted as a function of the reaction coordinate. When x equals zero, the ligand–solvent Leonard-Jones interactions are at full strength, whereas at $x = 1$, these terms are zero. Mean acceptance rates are not uniform along the reaction coordinate, highlighting the need to carefully distribute intermediate levels. The lowest mean acceptance rates occur near the inflection point of the volume curve.

This example highlights why it would be unfair to simply choose a single set of Hamiltonian levels as the basis for comparison between simple Hamiltonian exchange and Hamiltonian exchange with nonequilibrium trials. Instead, eq 2 is used as a basis to choose reasonable parameters for each method. Along these lines, Table 3 shows the location and nonequilibrium simulation times for the eight Hamiltonian levels used with serial exchange. Of the seven possible nonequilibrium trial exchanges, four are chosen to be instantaneous (i.e., the same as simple serial exchange), and the remaining three have lengths of 0.5, 2, and 5 ps. The details of the Hamiltonian levels selected for the other two methods are given in the Supporting Information.

Figure 7 shows both the average simulation box volume and the estimated acceptance rates of serial exchanges without

Table 4. Estimated Mean Acceptance Rates for Simple Trial Exchanges between a Fully Charged and a Partially Charged Ligand

ligand charge coefficient	serial exchange acceptance rate	replica exchange acceptance rate
0.95	0.82	0.74
0.85	0.48	0.32
0.75	0.26	0.11
0.55	0.12	0.03
0.45	0.05	<0.01

nonequilibrium trials, plotted as functions of the reaction coordinate. The shape of the acceptance rate curve shows that equal size moves along the reaction coordinate do not have an equal chance of success, reinforcing the need for care when choosing intermediate level placement, even when not using nonequilibrium trial exchanges. Strikingly, the acceptance rates dip to their lowest point just when the volume of the box changes the most rapidly. This volume reduction corresponds to a phase change, wherein the ligand–solvent interaction has become soft enough that solvent molecules can stably occupy the same physical space as the ligand. The corresponding drop in acceptance rates supports the assertion that additional intermediate levels are needed close to phase transitions.^{7,26}

Table 4 compares estimated mean acceptance rates of simple replica exchange and simple serial exchange for different amounts of charge scaling. In all cases, not just those shown in Table 4, the estimated mean acceptance rates were higher for serial exchange, a result consistent with work on temperature-based exchange methods.⁴⁶

4. CONCLUSIONS

In this work, a method is developed for efficiently configuring HENT. In the computation of solvation free energy studied here, the introduction of nonequilibrium trial exchanges reduces the mean absolute error of free energy estimates, without increasing the overall simulation cost. This method can be readily adapted to problems besides free energy calculations and is applicable wherever Hamiltonian exchange is employed.

The observed reductions in statistical error reported here are encouraging, yet several additional factors should be considered. First, the comparisons described in Figure 5 exclude the cost of parameter selection, as the initialization data generated are likely overkill for typical applications. While the use of nonequilibrium simulations does require extra initialization runs to estimate the benefits of nonequilibrium trial exchanges, it is unclear how much this would actually cost. If general rules could be developed for the most likely placement and length of nonequilibrium simulations, the cost of testing them may be minimal. Furthermore, if production runs are significantly longer than the fixed cost of initialization—and they usually are—this cost will be negligible. A caveat is that our method chooses parameters to minimize estimated mean round trip times, which does not guarantee minimized sampling error. One important parameter is the simulation interval between Hamiltonian exchange attempts, fixed here as $s = 1$ ps. Equation 2 suggests that larger values of s will favor (and smaller values will disfavor) the use of nonequilibrium trial exchanges.

In summary, we have presented here a method for efficiently incorporating nonequilibrium trials into Hamiltonian exchange. Although it has thus far not been clear whether nonequilibrium methods are capable of achieving greater computational efficiency than traditional equilibrium approaches, the results presented here suggest that under certain circumstances, properly configured HENT does have the potential to achieve at least a modest gain in efficiency. Equation 2 and the accompanying algorithm provide a reasonable way to test whether nonequilibrium trial exchanges are beneficial. To fully validate and gauge the significance of the improvements we have observed, however, further testing involving a variety of systems will be needed.

■ ASSOCIATED CONTENT

S Supporting Information. Included are (1) additional details on hybrid Monte Carlo, (2) a derivation of \overline{mrtt} , (3) further discussion of the parameter selection algorithm, (4) detailed descriptions of the reaction coordinate and softcore potential, (5) the setup of free energy calculations, (6) technical details related to the simulations performed, (7) technical details related to the Hamiltonian exchange performed, (8) a method for combining conformer data, (9) final setup parameters for simple replica and serial exchange, and (10) a list of nonequilibrium trial exchanges tested during initialization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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