A Smart Monte Carlo Technique for Free Energy Simulations of Multiconformational Molecules. Direct Calculations of the Conformational Populations of Organic Molecules

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Abstract: Metropolis Monte Carlo (MMC) can be a highly inefficient simulation technique when only a small fraction of an energy surface is populated and barriers between low-energy regions are high. In such cases, previous knowledge of the surface (*e.g.* low-energy conformations of molecules) can be used to preferentially sample the significantly populated regions. In this work we present a new MC method for accomplishing this goal. We term the method JBW for Jumping Between Wells. The JBW procedure operates by locating the various conformations of a molecule and subsequently driving an MMC-like simulation to jump repeatedly between them. Using simulations on 1- and 2-dimensional potential surfaces and on *n*-pentane, the JBW method is shown to generate ensembles of states that are indistinguishable from the canonical ensembles generated by classical MMC in the limit. Integration of JBW into the recently described MC/SD hybrid simulation algorithm enables rapidly converged simulations of conformationally flexible molecules including cyclic molecules in all degrees of freedom. The new method (MC-(JBW)/SD) gives converged comformational populations at a rate that is essentially independent of the energy barriers between conformations. We use the method to evaluate free energy differences between the conformers of various substituted cyclohexanes and of the larger ring hydrocarbons cycloheptane, cyclooctane, cyclononane and cyclodecane on several widely used potential energy surfaces. Such conformational free energies are compared with simple molecular mechanics steric energies both with and without rigid rotor–harmonic oscillator free energy corrections. In general, we find that assumptions of harmonicity do not lead to good approximations of the actual anharmonic free energies. In the case of cyclohexane derivatives at room temperature, the MC(JBW)/SD method is estimated to generate converged ensembles of all conformations at a rate $\sim 10^6$ times faster than methods based on simple molecular or stochastic dynamics.

Introduction

Impressive successes in reproducing experimental free energies have both demonstrated the power of free energy simulations and validated the reliability of the force field methods and solvation models used in these calculations.[1] However, when the system studied has multiple conformers separated by significant energy barriers, adequate sampling of the full potential energy surface commonly becomes the limiting step in the application of free energy simulation methods.[2] The problem is that converged free energies (and other averaged molecular properties) can be obtained only when all significantly populated conformations are sampled with their correct statistical weights. Unfortunately, the two most commonly used methods in the field, molecular or stochastic dynamics (MD or SD) and Metropolis Monte Carlo (MMC),[3] have difficulty in sampling all the low-energy regions of conformational space when there are large barriers between minima or when conformational space is large and sparcely populated. Consequently, such methodologies often require impractically long simulations to achieve usefully precise, converged results.

A short while ago, we described a new hybrid simulation technique termed MC/SD that effectively addresses the sampling problem for certain multiconformational molecules.[4] The technique generates a canonical ensemble of molecular states via alternating MC and SD steps—the MC steps being used to make large random moves through conformational space and the SD steps being used to explore each potential energy well. With simple acyclics such as *n*-pentane at 300 K, MC/SD was found to be $\sim 10^3$ times faster than pure SD at achieving a given level of simulation convergence. Although superior to both simple MMC and SD, the MC/SD technique still suffers from a major deficiency of MMC, namely slow convergence on sparcely populated potential surfaces. Such surfaces are common and are characteristic of complex molecules having many internal degrees of freedom (*e.g.* rotatable torsion angles) but relatively few low-energy conformational states. MMC handles such systems poorly because it explores conformational space by random moves that all too frequently end up in high-energy regions of space and are rejected. This high move rejection rate leads to slow interconversion of conformers and thus slow simulation convergence. Clearly, much could be gained by replacing MMC with a more efficient MC procedure—*e.g.* one that utilizes prior knowledge of the potential surface to direct the simulation to interconvert low-energy conformations and thus to preferentially sample its low-energy regions.

Several such modifications of MMC have been described in the literature[5] and are usually referred to as Smart MC methods. While these methods speed convergence in simple systems, none seemed generally applicable to complex molecular systems having multiple low-energy conformers. For such systems, we

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envisioned a new Smart MC procedure that would first locate all low-energy regions of conformational space and then use that information to generate the correct, Boltzmann-weighted populations of molecular conformations.

In this paper we describe such a simulation method. We term it the JBW method (for Jumping Between Wells) because it begins with a conformational search and then uses the resulting minimum energy conformers to drive an MMC-based procedure to jump between them. The method is similar in spirit to several recently described simulation methods that also use prior knowledge of the potential surface to speed barrier crossings in double-well potentials[6a] or linear chain molecules.[6b] However, the JBW methodology is simpler and appears applicable to virtually any molecular structure. As we will demonstrate, the JBW method can be used in place of MMC in our hybrid MC/SD simulation method. The resulting MC(JBW)/SD method can be used to generate canonical ensembles efficiently with variation of all degrees of freedom. As such, it may be used with conformationally heterogeneous molecules as an ensemble-generating method in free energy calculating procedures such as free energy perturbation. We expect that the new method will solve some of the convergence problems that often plague such calculations.[2] In the following paragraphs, however, we use the method to generate room temperature ensembles with multiconformational molecules that we analyze directly for conformational populations. In particular, we have used our MC(JBW)/SD method to compute conformational populations and free energies for derivatives of cyclohexane and several larger ring hydrocarbons on MM2 and MM3 potential surfaces. We believe this work is the first to provide high-precision calculations of conformational free energies of such systems without the need for approximations (*e.g.* harmonicity) to the nature of the conformational energy wells.

JBW Method

The JBW method was developed to solve convergence problems that we encountered in simulations of organic host molecules and their complexes. Such systems typically have multiple low-energy conformations that are separated by large energy barriers and their conformers often differ substantially in multiple internal coordinates. With these systems, common simulation techniques (MC, MD, or SD) rarely interconvert conformations and therefore lead to incorrect final results. The key problem is that such simulations spend too much time in a subset of the conformational states. If the simulation gets stuck and never samples some significant, low-energy conformation(s), then the simulation appears converged but does not generate the correct ensemble. If the simulation moves between conformations but does so only infrequently, then ensemble averages drift slowly and continuously throughout the simulation. In our experience, both of these situations are common with pure MC and dynamics simulations of complex molecular systems. Only when a simulation samples all significantly populated minima many times will the ensemble generated approximate the correct, Boltzmann-weighted populations of states. Unfortunately, such sampling with multiconformational molecules is not always easy.

To be sure that our simulations adequately sample all known conformational states, we envisioned a new algorithm that operates in two stages: (1) carry out an extensive conformational search to find all low-energy minima, and (2) use the structures of the minima to drive a simulation to repeatedly sample each and every minimum. While methods for carrying out conformational searches are well-known, it was less clear how to use known conformers to direct a simulation method to sample them all with generation of a standard canonical ensemble. The idea we developed is based on the standard MMC procedure[3] with added internal coordinate movements that promote interconversion of conformers. These added movements are always the same for a given pair of conformers and bias each MC trial step to move from the system's current conformation toward a randomly chosen conformer. This procedure results in generation of a Markov chain with the Metropolis transition probabilities and consequently a canonical ensemble. The specific steps in the JBW algorithm are the following:

Step 1: Carry out a conformational search to find the set of low-energy conformers—call these $\mathbf{X}_i$. Evaluate the internal coordinate transformations that interconvert all pairs (i,j) of the conformers in the $\mathbf{X}_i$ list—call these transformations $\mathbf{T}_{ij}$.

Step 2: Pick an initial conformation—call this structure $\mathbf{Y}_0$.

Step 3: Find the conformer on the $\mathbf{X}_i$ list that is closest to $\mathbf{Y}_0$—call this conformer $\mathbf{X}_0$.

Step 4: Randomly choose a conformer from the $\mathbf{X}_i$ list—call this conformer $\mathbf{X}_T$.

Step 5: Apply transformation $\mathbf{T}_{\mathbf{X}_0\mathbf{X}_T}$ to structure $\mathbf{Y}_0$ to generate structure $\mathbf{Y}_1$.

Step 6: Apply small random variations to internal coordinates of $\mathbf{Y}_1$ to generate the new trial structure $\mathbf{Y}_2$.

Step 7: Compare energies of $\mathbf{Y}_0$ and $\mathbf{Y}_2$, accepting $\mathbf{Y}_2$ with a probability defined by Metropolis;[3] $p = \min\{1, \exp[-(E(\mathbf{Y}_2) - E(\mathbf{Y}_0))/kT]\}$.

Step 8: Define the resulting structure as $\mathbf{Y}_0$ and go back to Step 3.

To find all conformers in Step 1, we use the internal coordinate SUMM conformational search method.[7] We typically use all minimum energy conformers within 5 kcal/mol of the global minimum in the $\mathbf{X}_i$ set. For molecules having symmetry, these conformers include both members of enantiomeric conformational pairs and all possible molecule numbering systems. Only distinct conformers having at least one significantly differing internal coordinate should be included in the $\mathbf{X}_i$ set. It is unnecessary to include conformers that are separated from any member of $\mathbf{X}_i$ by energy barriers as small as $\sim 3kT$ because these will be sampled via conventional barrier crossing events. The transformation matrix $\mathbf{T}_{ij}$ has elements that are a list of internal coordinate changes that convert minimum energy conformer i into (approximately) minimum energy conformer j. We have found it adequate to include only bond angles and torsional angles in $\mathbf{T}_{ij}$. We have also found it adequate to store in $\mathbf{T}_{ij}$ only those bond angles and torsion angles that differ significantly between conformers i and j. The best test of angle significance is application of a $\mathbf{T}_{ij}$ to minimum energy conformer i to generate a conformation ($\sim j$) that is structurally very similar to the actual conformer j. If the energy of $\sim j$ is within $4kT$ of

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the energy of j, then that element of the $\mathbf{T}_{ij}$ matrix is adequate for use in the JBW algorithm.

In Step 3 of the algorithm, we determine the conformational family a given structure is in by a Cartesian coordinate least-squares superimposition[13] of the structure's atoms with those of members of the $\mathbf{X}_i$ list. Occasionally, high-energy structures are encountered that do not match any conformers in the $\mathbf{X}_i$ list very well (*e.g.* rms > 1 Å). In such cases, Steps 4 and 5 may be omitted and the algorithm takes a standard MMC step.

Tests with 1-Dimensional and 2-Dimensional Surfaces

We started by testing the JBW method using simulations of a particle on simple one- and two-dimensional potential surfaces (potentials 1a–1d, Figure 1). Several of these surfaces were designed to reflect situations in which we believe our method will have a distinct advantage over MMC. Thus potentials 1b and 1c are characterized by narrow, deep energy wells that are separated by large energy barriers and should not be efficiently sampled by a purely random sampling procedure such as MMC. As measures of the properties of the ensemble averages, we took the first two moments of the potential energy distribution (average and standard deviation) and the populations of the various wells in each potential. Due to the simplicity of the test potentials 1a–1d, we were able to obtain the correct results both by numerical integration (NI) using the Trapezoid method and by lengthy MMC simulations. Furthermore, the two wells of potentials 1a and 1b are equivalent and thus should be characterized by equal populations. Our MMC and JBW simulations were initiated from arbitrary points on the potential surfaces and consisted of 10^7 and 10^8 steps for the one-dimensional (potential 1a) and two-dimensional (potentials 1b and 1c) surfaces, respectively. For MMC, we used a maximum step size of 10 Å along all axes to force complete coverage of each surface. For JBW, the conformers ($\mathbf{X}_i$) of Step 1 corresponded to the wells in the simple test potentials and the transformation matrices ($\mathbf{T}_{ij}$) consisted of simple translation vectors given by differences in the coordinates of the wells. The JBW Step 6 randomization consisted of 0.0–(±)0.5 Å translations along each axis. All of our test simulations were carried out at a temperature of 300 K.

Data showing the results of simulations with potentials 1a–1c are listed in Table 1. These results indicate that MMC and JBW methods converge to the same ensemble averages in the limit of long simulation times and that they coincide with high precision to results obtained by NI. Thus the well populations and the ensemble potential energy averages and standard deviations are indistinguishable whether computed by NI, MMC, or JBW. The JBW method is therefore shown to generate the correct canonical ensembles for test potentials 1a–1c.

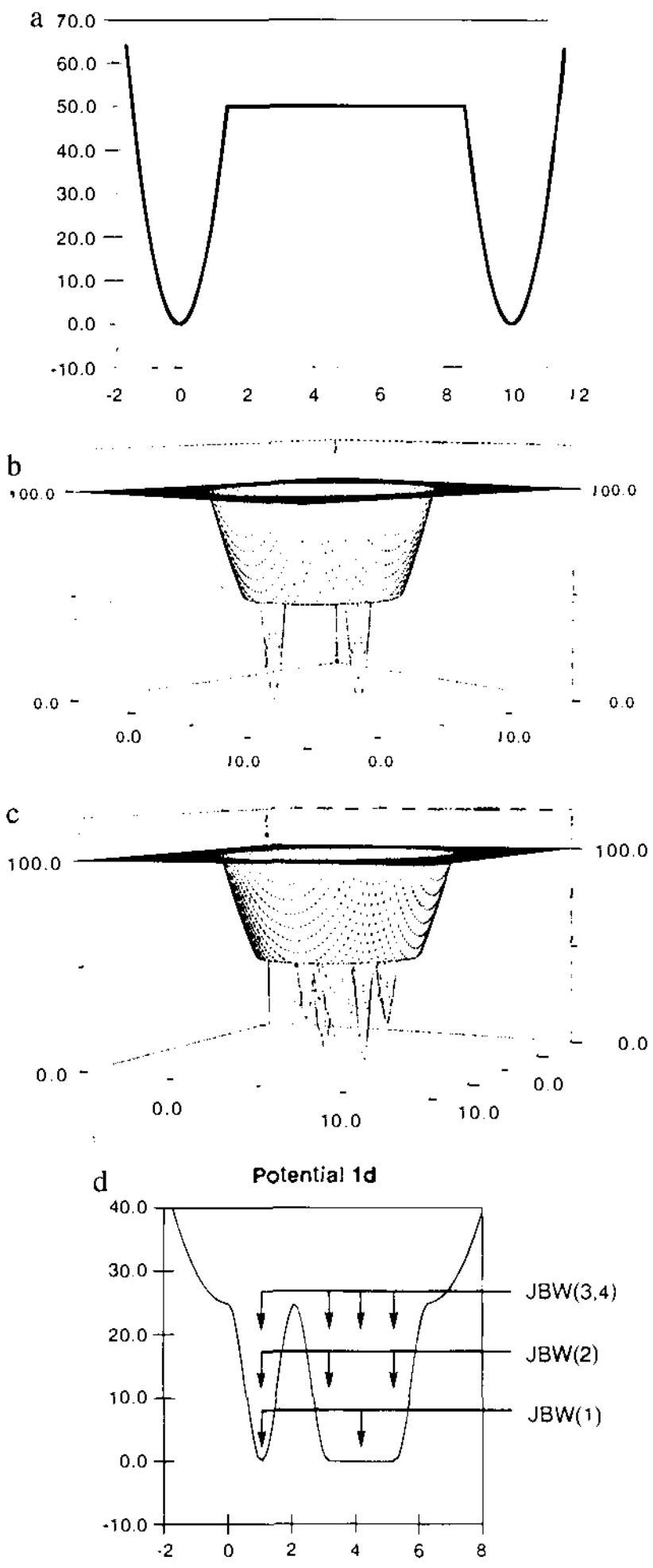

Figure 1. Graphic representations of potentials 1a–d with energy (y axis) in kJ/mol vs particle position (x axis) in Å.

Next, we consider the relative efficiency of MMC and JBW. One-dimensional potential 1a is the simplest case—it consists of two identical wells separated by a constant energy plateau set at 12 kcal/mol. Because of the symmetry of the potential, the populations of the two wells should be equal at convergence. By monitoring populations during the course of our simulations, we find that MMC gives equal well populations within 1% after 1×10^5 steps while JBW achieves the same result after $3 \times$

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Table 1. A Comparison of Ensemble Average Potential Energy (PE; kJ/mol), Standard Deviation (SD), Wells Population and Acceptance Rates (% Acceptance) between Numerical Integration (NI), Metropolis Monte Carlo (MMC), and Jumping between Wells (JBW) for Potentials **1a–c**

	1a			1b			1c		
	NI	MMC	JBW	NI	MMC	JBW	NI	MMC	JBW
⟨PE⟩	1.247	1.251	1.247	2.494	2.490	2.494	3.700	3.705	3.698
⟨SD⟩	1.764	1.765	1.765	2.494	2.490	2.494	3.778	3.777	3.780
well 1	0.500	0.500	0.500	0.500	0.500	0.500	0.021	0.021	0.020
well 2	0.500	0.500	0.500	0.500	0.500	0.500	0.841	0.842	0.841
well 3							0.004	0.004	0.004
well 4							0.135	0.134	0.135
well 5							0.0001	0.0001	0.0001
acceptance		5.3	59.6		0.08	15.3		0.07	4.7

10^4 steps. Furthermore, the trial step acceptance rate with JBW (60%) is an order of magnitude larger than with MMC (5%).

Two-dimensional potential 1b is a more demanding case, since the two identical but narrow parabolic wells occupy only a small fraction (2.4%) of the potential surface area below the 25-kJ/mol level. Consequently, we expect JBW to have a significant advantage over MMC with this potential. Indeed, while it takes MMC 5×10^6 steps to equalize the well populations to within 1%, JBW reaches the same degree of convergence in only 5×10^4 steps. Again, the JBW step acceptance rate (15%) is significantly greater than that of MMC (0.08%).

Potential 1c is still more complicated because it has five energy wells of differing depths and widths. Here, we monitored the potential energy average (PE) and standard deviation (SD) to assess the convergence rate. Again, our JBW method proved much more efficient than MMC. As shown in Table 2, convergence to within 0.5% of the correct PE and SD was reached by MMC in 6×10^6 steps and by JBW in 3×10^5 steps. Here too, the JBW step acceptance rate (5%) substantially exceeds that of MMC (0.07%).

Finally, we consider the situation of potentials having several minima separated by small energy barriers. This situation is modeled by potential 1d (Figure 1) in which the broad, right-hand well is actually composed of two wells (at ~3 and ~5 Å) separated by a tiny energy barrier ($0.01kT$). The issue is whether this system should be considered as a two-well or a three-well system by the JBW procedure—or does it make any difference? To answer this question, we carried out 10^9-step JBW simulations of potential 1d using different numbers of wells in the JBW conformer list $\mathbf{X}_i$. In these simulations, the randomization step (algorithm Step 6) consisted of 0.0–(±)0.5 Å translations except as noted below. The various $\mathbf{X}_i$ situations are shown in the potential 1d diagram. JBW(1) is the case where the $\mathbf{X}_i$ list includes two minima (at ~1 and 4 Å). In JBW(2), the $\mathbf{X}_i$ list has three minima (at ~1, 3, and 5 Å), and in JBW(3,4) the $\mathbf{X}_i$ list has four "minima" as shown. The results of these simulations along with comparison data from numerical integration (NI) and MMC are given in Table 3.

The data indicate that the ensemble averages were virtually identical for NI, MMC, JBW(1), and JBW(2). Thus the correct results are obtained whether one considers potential 1d to be a 2-well or a 3-well system. If, however, the broad, right-hand well is further subdivided yielding three submina and these are included in the $\mathbf{X}_i$ list (JBW(3)), then the ensemble averages diverge from the correct values due to oversampling of the right-hand well. This oversampling results generally from biasing of the JBW algorithm by the $\mathbf{X}_i$ list to sample some energy wells more frequently than others. The problem occurs when an algorithm jump (Step 5) to one entry of the $\mathbf{X}_i$ list plus (Step 6) randomization enters the space of a different $\mathbf{X}_i$ entry. This oversampling problem may be avoided by including only structures in the $\mathbf{X}_i$ list that are significantly different from one another and by making the randomization step small relative to the distance in appropriate coordinates between structures in the $\mathbf{X}_i$ list. Thus by reducing the maximum randomization step to (±)0.01 Å, results very close to the correct ones can be obtained even when potential 1d is considered by the JBW procedure to be a four-well system (Table 3, JBW(4)).

These results indicate that the JBW method generates ensembles that are indistinguishable from the correct Boltzmann-weighted ensembles when the potential surface has well-defined minima and each such minimum is included only once in the $\mathbf{X}_i$ list. In systems where minima are ill-defined, a variety of low-energy sampling points from the same large well may be included in the $\mathbf{X}_i$ list provided that the differences in the coordinates of the points are significantly larger (≥ 10-fold) than the randomization step (JBW Step 6). In systems suspected of having poorly-defined minima, lengthy simulations should be carried out with several different extents of randomization and with different points in the $\mathbf{X}_i$ list to test for potential oversampling as noted in JBW(3) above. Such tests described

Table 2. A Comparison of Potential Energy (kJ/mol) Average (PE) and Standard Deviation (SD) during MMC and JBW Simulations with Potential 1c[a]

	energy (MMC)		energy (JBW)	
steps ($\times 10^4$)	⟨PE⟩	⟨SD⟩	⟨PE⟩	⟨SD⟩
1	4.36	3.96	3.76	3.81
5	3.81	3.70	3.79	3.84
10	3.57	3.67	3.75	3.80
20	3.72	3.72	3.73	3.80
30	3.61	3.79	**3.72**	**3.78**
50	3.48	3.69	3.72	3.78
100	3.64	3.73	3.71	3.79
200	3.69	3.73	3.70	3.78
300	3.67	3.74	3.70	3.78
500	3.67	3.64	3.70	3.78
600	**3.68**	**3.74**	3.70	3.78
1000	3.71	3.78	3.70	3.78

[a] Bold values indicate the points where both PE and SD are converged to within 0.5% of the correct values (see text).

Table 3. A Comparison of Potential Energy (kJ/mol) Average (PE), Standard Deviation (SD), and Well Populations with Potential 1d from Various Simulation Methods (see text)

	energy		populations	
	⟨PE⟩	⟨SD⟩	well 1	well 2
NI	0.347	1.154	0.134	0.866
MMC	0.347	1.15(4)	0.134	0.866
JBW(1)	0.347	1.14(8)	0.134	0.866
JBW(2)	0.346	1.14(7)	0.134	0.866
JBW(3)	0.282	0.99(6)	0.123	0.877
JBW(4)	0.337	1.09(5)	0.133	0.867

Table 4. A Comparison of Ensemble Average Energy (PE; kcal/mol), Standard Deviation (SD), Skew (SK), Kurtosis (KU), Acceptance rate (% Acceptance) and Minima Populations between MMC and JBW (5×10^8 steps) and between MC/SD and JBW/SD (100 ns) for United Atom *n*-Pentane

	MC (torsions only)		MC/SD (all degrees of freedom)	
	MMC	JBW	MMC	JBW
⟨PE⟩	0.913	0.911	2.97	2.97
⟨SD⟩	0.813	0.811	1.37	1.37
⟨SK⟩	0.311	0.306	0.20	0.20
⟨KU⟩	0.655	0.645	0.27	0.26
acceptance	14	41	14	43
populations				
aa	0.434	0.435	0.409	0.410
ag^+	0.117	0.117	0.118	0.118
ag^-	0.117	0.117	0.118	0.118
g^+a	0.117	0.117	0.118	0.118
g^-a	0.117	0.117	0.118	0.118
g^+g^+	0.029	0.029	0.038	0.038
g^-g^-	0.029	0.029	0.038	0.038
g^+g^-	0.00061	0.00060	0.0030	0.0030
g^-g^+	0.00061	0.00061	0.0029	0.0029

in the sections below imply that oversampling in simulations of molecules having many degrees of freedom is not a significant problem.

Tests with a Molecular System: *n*-Pentane

To test the JBW algorithm on molecular systems, we incorporated it into the MacroModel V5.0 distribution of our simulation program BatchMin.[8] In the first implementation, the JBW method was programmed to handle simulations in torsion angle space only. Thus all molecular conformations ($\mathbf{X}_i$) and all transformations ($\mathbf{T}_{ij}$) between them were defined in terms of torsional degrees of freedom. The randomization step of the JBW algorithm consisted of $0-(\pm)1°$ torsional rotations. We chose *n*-pentane as a simple polyatomic molecule having multiple, significantly populated conformers for our torsional simulation tests. Following a conformational search of *n*-pentane using the united atom AMBER[9] force field, we ran lengthy (5×10^8 steps) MMC and JBW simulations at 300 K with variation of *n*-pentane's two torsion angles. To judge the ensembles generated, we accumulated the first four moments of the potential energy distribution (average, standard deviation, skew, and kurtosis)[10] and the populations of *n*-pentane's nine distinct conformers. Results for our torsional *n*-pentane simulations are summarized in the left two columns of Table 4 and confirm that both MMC and JBW generate ensembles in the limit of long simulations that are virtually indistinguishable.

We then integrated the JBW algorithm into our hybrid MC/SD simulation method to allow simulations in all degrees of freedom. The torsional JBW algorithm served as a smart Monte Carlo replacement for the torsional MMC part of the original MC/SD method.[4] Again using *n*-pentane as a test system, we carried out 100-ns simulations with the original MC(MMC)/SD and the new MC(JBW)/SD at 300 K and compared the results. The results reflecting exploration of all degrees of freedom are given in the right two columns of Table 4 and confirm that the new MC(JBW)/SD algorithm indeed generates the correct canonical ensemble. Convergence of these simulations can be estimated by comparing populations of molecular conformations which are equivalent by symmetry. Thus for a converged simulation, populations of the symmetry equivalent ag^+, ag^-, g^+a, g^-a (a = *anti*; g = *gauche*) conformers must be the same in the limit, as must be the populations of g^+g^+, g^-g^- and g^+g^-, g^-g^+. The data at the bottom of Table 4 indicate that both MC(MMC)/SD and MC(JBW)/SD methods give conformational populations that are converged to three significant figures within the 100-ns simulations. Other experiments show that the MC(JBW)/SD results are also insensitive to changes in the extent of randomization (JBW Step 6)—the same results being obtained whether the torsional randomization is $0-(\pm)1°$ or $0-(\pm)5°$.

The results with *n*-pentane and with the one- and two-dimensional oscillator models establish that our JBW method generates virtually the same ensembles produced by more classical methods such as MMC. We have also shown that the JBW method can be integrated into our MC/SD procedure to give an algorithm that explores all degrees of freedom and generates molecular ensembles that are indistinguishable from those produced by classical methods in the simulation time limit. In the following paragraphs, we show that MC(JBW)/SD can be used to carry out converged free energy simulations of organic molecules that would be virtually impossible by previously described dynamics or Monte Carlo methods.

Direct Calculations of Conformational Populations and Free Energies

Molecular mechanics energy minimization is the most common method for computing energy differences between the various conformers of a flexible molecule. From such conformational energy differences, conformational populations are frequently computed based on a Boltzmann distribution. When simply applied, this approach involves the assumption that the entropies and heat capacities of conformational isomers are equal. Though such an assumption does not appear to introduce serious errors into calculations on simple molecules composed of five- and six-membered ring systems, conformational entropy differences might be expected to be more significant with larger and more flexible molecules (*e.g.* macrocycles). Some of the effects of free energy can be included in a calculation by assuming that minimum energy conformers behave like harmonic oscillators—*i.e.* that energy wells defining conformations are parabolic in shape. However, the realism of such an approximation has yet to be established with complex or highly flexible molecules.[11] There is a worry that the harmonic approximation may be rather crude, because calculations on molecules as simple as *n*-butane show that anharmonic effects can make significant contributions to entropy.[15] Similar concerns arise with quantum mechanical calculations of geometry-optimized conformers.

Because conformational energy calculations are commonly carried out on flexible molecules where the approximations regarding conformational entropies are of unknown accuracy, we have used our new simulation methodology to evaluate fully anharmonic, conformational free energies for comparison with conformational energies available from molecular mechanics. The approach we used involved directly monitoring the populations of the various conformers of a molecule during a single MC(JBW)/SD simulation in which the conformers are frequently interconverted (*i.e.* are in rapid equilibrium). Conformational free energies follow simply from populations using $\Delta G = -RT \ln K_{eq}$. Given that such simulations produce converged, Boltzmann-weighted ensembles of conformational states, the energies evaluated by such a procedure will represent the actual free energies of the system that are defined by the molecule, the temperature, and the force field.

Our conformational free energy calculating procedure assumes that the conformers are sampled with their correct

(15) Bell, D. C.; Harvey, S. C. *J. Phys. Chem.* **1986**, *90*, 6595.

Table 5. Conformational Energies of *n*-Pentane Calculated Using the United Atom AMBER Force Field

conformation	energy (kcal/mol) SE[a]	$G_{300K-Harmonic}$[b]	G_{300K}[c]
anti-anti (aa)	0.0	0.0	0.0
anti-gauche (ag)	0.62	0.63	0.74
(+)gauche-(+)gauche (+g+g)	1.18	1.20	1.42
(+)gauche-(−)gauche (+g−g)	2.64	2.64	2.94

[a] Relative steric energies from molecular mechanics (MM). [b] Relative steric energies plus free energy effects calculated at 300 K by normal mode analysis using the rigid rotor, harmonic oscillator approximation excluding corrections for zero point energy differences. [c] Relative free energies computed using $\Delta G = -RT \ln K_{eq}$ and populations from MC(JBW)/SD simulation at 300 K.

statistical weights (*i.e.* that the simulation is converged) and that it is always possible to determine which conformation the system is in. With respect to the convergence issue, previous analogous approaches to conformational energies have been limited by slow conformational interconversion to flexible molecules having particularly low barriers between conformations.[12] Here, the conformation hopping MC(JBW)/SD methodology should provide a major advantage. In the following paragraphs, we establish convergence by investigating symmetrical systems and tabulating populations of symmetry-equivalent conformations. With respect to the conformational identity question, we compare structures from the MC(JBW)/SD simulation with each of the known minimum energy conformers ($\mathbf{X}_i$ list) using a least-squares superimposition in Cartesian coordinates.[13] The conformer having the smallest root mean square (rms) deviation from the simulation structure is taken to define the conformation of that structure.

Using this approach with *n*-pentane and the populations given in Table 4, the relative AMBER free energies of the four different types of conformations at 300 K can be computed. The free energies (G_{300K}) of specific examples of each of these conformers are tabulated in Table 5 along with molecular mechanics (MM) steric energy differences (SE) and molecular mechanics conformational free energy differences ($G_{300K-Harmonic}$) in the rigid rotor, harmonic oscillator approximation. In these and the other harmonic oscillator results below, conformational zero point energies and rotational symmetry corrections are not included to allow comparisons with our simulation results that do not incorporate such effects. As indicated in the table, MM steric energies (SE) and the actual free energies from MC/SD simulation (G_{300K}) give similar trends for *n*-pentane's conformational energies though results with the two methods differ quantitatively. The increase in *gauche-anti* energy difference that occurs when entropy is included likely follows from sterically-induced restrictions to independent torsional and vibrational motion in the *gauche* conformer. Similar conclusions have been drawn from conformational entropy calculations on *n*-butane.[14] Though these differences amount to only 0.1−0.3 kcal/mol, the harmonic oscillator approximation does little to modify the MM steric energies toward the actual, fully anharmonic free energies as defined by the force field.

To apply our MC(JBW)/SD methods to cyclic molecules, we define a ring closure bond for each cyclic array of atoms to generate a pseudoacyclic equivalent of the molecule to be studied.[16] So that jumps between ring conformations do not significantly alter the length of a ring closure bond (and therefore lead to MC move rejection), all torsion angles and bond angles in any ring being conformationally modified by the JBW procedure are included in the $\mathbf{T}_{ij}$ transformation matrix (see JBW Method Step 1).

Table 6. *A* Value of Methylcyclohexane[a]

force field	energy (kcal/mol) ΔSE[b]	$\Delta G_{300K-Harmonic}$[c]	ΔG_{300K}[d]
MM2 (all atom)	1.78	1.89	1.9(9)
MM3 (all atom)	1.77	1.85	1.9(5)
AMBER (united atom)	1.45	1.51	1.6(2)
AMBER (united atom + Du)[e]	1.34	1.43	1.5(1)

[a] *A* value = free energy difference between axial and equatorial substituted cyclohexane. [b] Relative steric energies from molecular mechanics (MM). [c] Relative steric energies plus free energy effects calculated at 300 K by normal mode analysis using the rigid rotor, harmonic oscillator approximation excluding corrections for zero point energy differences. [d] Free energies from MC(JBW)/SD simulation at 300 K. [e] Dummy atom (Du) having nonbonded parameters set to 0.0 and attached to the methine carbon.

Using this approach we studied a basic conformational analysis problem, the free energy difference between axial and equatorial methylcyclohexane (the so-called *A value* for methyl). Experimentally, this value is estimated from ^{13}C NMR measurements in $CFCl_3-CDCl_3$ solution at low temperature to be 1.6−1.8 kcal/mol at 300 K.[17] Our MC(JBW)/SD methylcyclohexane simulations were run *in vacuo* for 10 ns using the MM2,[18] MM3,[19] and AMBER[9] force fields and resulted in ~6000 (MM2, MM3) and ~17000 (united atom AMBER) interconversions between the two alternative chair conformations. This extent of conformational interconversion allowed populations to be established to within 2%. Such rapid interconversions between alternate chair forms (once every picosecond on average) are remarkable in comparison with the behavior of a real molecule—its 10 kcal/mol conformational barrier implies conformer interconversion occurs only every 3 μs.[20] This comparision suggests that with cyclohexanes at room temperature, MC(JBW)/SD simulations converge at a rate ~10^6 times faster than methods based on simple molecular or stochastic dynamics.

Results of this free energy simulation and corresponding MM calculations are given in Table 6. Consistent with calculations on *gauche* hydrocarbons described above, axial methylcyclohexane incorporates two *gauche*-butane interactions and is disfavored entropically with all force fields studied by 0.6−0.7 cal/(deg·mol) *in vacuo*. The experimental conformational entropy difference is found to be somewhat smaller in solution at -0.03 ± 0.25 cal/(deg·mol).[17] With methylcyclohexane, harmonic free energy corrections of steric energy make a greater contribution to the free energy difference than was found with *n*-pentane. However, these corrections still significantly underestimate the actual free energy differences found by our MC(JBW)/SD simulations.

The *A* value of methylcyclohexane is also a particularly good problem for calculation by an alternative technique, free energy

(16) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

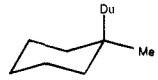
(17) Booth, H.; Everett, J. R. *J. Chem. Soc., Perkin Trans. 2* **1980**, 255.

(18) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. Our MM2 calculations were carried out using our BatchMin V5.0 computer program and our MM2* force field. For the saturated hydrocarbons described here, MM2* is identical in all respects to MM2 as described by Allinger. Our 1-methyl-1-phenylcyclohexane MM2* force field calculations differ from Allinger's MM2 in our use of atomic partial charges instead of bond dipoles for electrostatic terms.

(19) Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551, 8566, 8576. The comments in ref 18 regarding our MM2 implementation apply to MM3 as well.

(20) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons: New York, 1994; p 635.

perturbation.[21] Thus the chair conformers of methylcyclohexane (below) can be interconverted by mutating an appropriate dummy atom (Du) and united atom methyl (Me) into one another (Du → Me, Me → Du). The dummy atom was attached to the methine carbon by a bond of the same length as the C−Me bond (1.526 Å) and had all nonbonded parameters set to zero. We carried out our mutation over 21 stages using stochastic dynamics at 300 K with the united atom AMBER force field using the BatchMin computer program. During the course of this simulation, the two chair conformations of the cyclohexane ring did not interconvert. The full mutation spanned a total of 4.2 ns and gave an *A* value of 1.54 (±0.05) kcal/mol. This value is statistically indistinguishable from the *A* value of 1.51 kcal/mol found for the same system by direct MC(JBW)/SD simulation at 300 K.



Isopropylcyclohexane is generally similar in its conformational behavior to methylcyclohexane except that entropy exerts a larger effect. This difference follows from the mobility of the isopropyl substituent which is greater in the equatorial form. As a result, the *A* value of isopropyl exceeds that of methyl. Experimentally, the isopropyl *A* value is estimated from low-temperature ^{13}C NMR measurements in $CFCl_3-CDCl_3$ solution to be 2.0−2.4 kcal/mol at 300 K.[17] Using the same methods employed for methylcyclohexane above, 10 ns MC(JBW)/SD simulations of isopropylcyclohexane gave free energy *A* values of 2.6 kcal/mol at 300 K using both the MM2 and MM3 force fields. In comparison, simple molecular mechanics SE calculations give an isopropyl *A* value of 2.3 kcal/mol (with both MM2 and MM3). Applying the rigid rotor, harmonic oscillator approximation, molecular mechanics gives slightly larger *A* values (2.4 and 2.5 kcal/mol for MM2 and MM3, respectively).

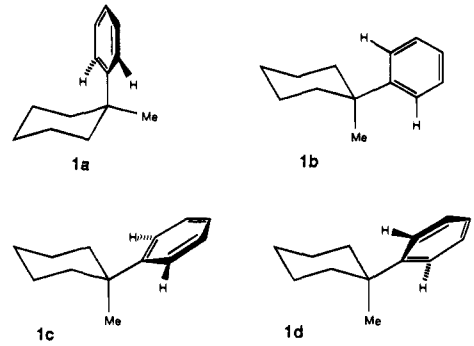
1-Methyl-1-phenylcyclohexane (**1**) is an interesting molecule that shows how *A* values are nonadditive with geminally disubstituted cyclohexanes: though the *A* value of phenyl (2.8 kcal/mol) is greater than that of methyl (1.7 kcal/mol), the conformer having the phenyl axial is found to be more stable by 0.3 kcal/mol at 200 K in CD_2Cl_2.[22] At room temperature, the ^{13}C NMR chemical shift data are consistent with a conformational energy difference closer to zero. While the axial phenyl conformation populates primarily one structure (**1a**), the equatorial phenyl conformation incorporates various phenyl rotomers including minima **1b** and the enantiomeric **1c** and **1d**.

Our MC(JBW)/SD simulations of **1** consisted of 10 ns, 200 and 300 K runs using the 15 minimum energy conformers found by conformational searching[7] as the JBW method's $\mathbf{X}_i$ set. All of these calculations employed the MM2 force field. The 300 K simulation spent ~99% of its time exploring the conformational space of the four lowest energy conformations (**1a−d**) and interconverted these conformers ~57 000 times. The population data for symmetry-equivalent conformation pairs (*e.g.* **1c**, **1d**) suggested that populations were converged to within 5%. For example, the populations of **1c** and **1d** at 300 K were computed to be 22% and 24%, respectively. The conformational energy results of our calculations are given in Table 7. Given that the room temperature free energy difference between axial and equatorial phenyl conformers can be estimated from experiment to be near zero, the calculations of simple SE (0.1 kcal/mol favoring axial phenyl) and 300 K free energy by MC(JBW)/SD (0.1 kcal/mol favoring equatorial phenyl) are in excellent agreement with experiment. In contrast, applying the harmonic appoximation makes axial phenyl more stable by 0.8 kcal/mol at 300 K—a significant deviation from experiment. At low temperature (200 K) where the conformational ratio was evaluated by NMR, the experimental conformational energy of 0.3 kcal/mol favoring axial phenyl matches very well with the calculated 0.23 kcal/mol favoring the same conformer from our MC(JBW)/SD simulation at 200 K.

Table 7. Conformational Energies of 1-Methyl-1-phenylcyclohexane (**1**) Using the MM2 Force Field *in Vacuo*

	energy (kcal/mol)			
conformation	SE[a]	$G_{300K-Harmonic}$[b]	G_{300K}[c]	G_{200K}[c]
1a (Ax phenyl)	0.0	0.0	0.0	0.0
1b (Eq phenyl)	1.30 (1.31)[d]	2.10	1.0(4)	1.5(6)
1c (Eq phenyl)	0.59 (0.61)[d]	1.29	0.4(4)[e]	0.7(4)[f]
1d (Eq phenyl)	0.59 (0.61)[d]	1.29	0.3(9)[e]	0.6(8)[f]

[a] Relative steric energies from molecular mechanics (MM2). [b] Relative steric energies plus free energy effects calculated at 300 K by normal mode analysis using the rigid rotor, harmonic oscillator approximation excluding corrections for zero point energy differences. [c] From 10 ns MC(JBW)/SD simulation. [d] MM2 energies from ref 22b. [e] These pairs of energies would be equal at full convergence. [f] These pairs of energies would be equal at full convergence.



Cycloheptane was one of the first molecules to be studied[23] by molecular mechanics and it has two low-energy minima on the MM2 potential surface, a twist chair (TC, SE = 14.31 kcal/mol) and a boat (B, SE = 17.46 kcal/mol). The high symmetry of such cyclic hydrocarbons simplifies their conformational behavior by reducing the number of distinct conformers relative to unsymmetrical derivatives. To provide for the symmetry of these molecules in our calculations, conformers in the $\mathbf{X}_i$ list (JBW Step 1) include all possible numbering systems. In the case of cycloheptane, there are 14 such numbering systems for each energetically distinct conformer. In our simulations, we separately tabulated the populations of each such conformer and numbering system to judge the extent of simulation convergence. The results of our 10 ns MC(JBW)/SD simulation of cycloheptane at 300 K are given in Table 8. During this simulation, each of the symmetry equivalent conformers were found to have comparable populations—a result indicative of good simulation convergence. Thus, the 14 equivalent twist chairs were each populated to the extent of 6.8−7.3% (total 99.2%) while the 14 boats had populations of 0.05−0.07% each (total 0.8%). As indicated in the table, the harmonic approximation greatly overestimates the higher entropy of the relatively flexible boat conformer.

Analogous simulations were carried out on the medium-ring hydrocarbons cyclooctane, cyclononane, and cyclodecane and

(21) Zwanzig, R. W. *J. Chem. Phys.* **1954**, *22*, 1420. See also ref 1; Anderson, A.; Carson, M.; Hermans, J. *Ann. N.Y. Acad. Sci.* **1986**, *42*, 51; Tobias, D. J.; Brooks, C. L.; Fleischman, S. H. *Chem. Phys. Lett.* **1989**, *156*, 256.

(22) (a) Eliel, E. L.; Manoharan, M. *J. Org. Chem.* **1981**, *46*, 1959. (b) Hodgson, D. J.; Rychlewska, U.; Eliel, E. L.; Manoharan, M.; Knox, D. E.; Olefirowicz, E. M. *J. Org. Chem.* **1985**, *50*, 4838.

(23) Hendrickson, J. B. *J. Am. Chem. Soc.* **1961**, *83*, 4537; **1967**, *89*, 7036, 7047.

Table 8. Conformational Energies of Cyclic Hydrocarbons Using the MM2 Force Field *in Vacuo*

molecule	conformation	weight[a]	energy (kcal/mol) SE[b]	$G_{300K-MM}$[c]	$G_{300K-Harmonic}$[d]	G_{300K}[e]
cycloheptane	TC[f]	1	0.0	0.0	0.0	0.0
	B[g]	1	3.15	3.15	1.66	2.8(1)
cyclooctane	BC[h]	2	0.0	0.0	0.0	0.0
	TCC[i]	1	0.97	1.38	0.86	0.6(5)
	TBC[j]	2	1.66	1.66	1.47	1.2(6)
	S4[k]	1	3.12	3.53	3.37	3.4(4)
cyclononane	TBC[l]	1	0.0	0.0	0.28	0.3(4)
	m	3	0.75	0.10	0.0	0.1(0)
	TCC[n]	3	0.77	0.12	0.11	0.0
	TCTC[o]	6	2.22	1.15	0.93	0.8(7)
cyclodecane	BCB[p]	1	0.0	0.0	0.04	0.4(2)
	q	2	0.42	0.01	0.0	0.0
	r	2	1.12	0.71	0.73	0.9(0)
	TCCC[s]	1	1.13	1.13	1.05	1.2(3)
	TBCC[t]	4	1.53	0.70	0.66	0.8(0)

[a] Statistical correction for conformational symmetry. [b] Steric energy relative to global minimum from molecular mechanics (MM2). [c] Molecular mechanics SE plus entropy of mixing effects from statistical weights at 300 K. [d] G_{300-MM} plus free energy effects calculated at 300 K by normal mode analysis using the rigid rotor, harmonic oscillator approximation (symmetry number and zero point energy contributions not included). [e] Actual conformational free energy from 10 ns MC(JBW)/SD simulation. [f] Torsion angles −39, 88,-73, 55, −73, 88, −39°. [g] Torsion angles 0, −70, 31,57, −57, −31, 70°. [h] Torsion angles −68, 68, −102, 44, 65, −65, −44, 102°. [i] Torsion angles 85, −63, 85, −111, 85, −63, 85, −111°. [j] Torsion angles 92, −49, −47, 117, −47, −49, 92, −89°. [k] Torsion angles −36, −65, 36, 65, −36, −65, 36, 65°. [l] Torsion angles 56, −125, 56, 56, −126, 56, 56, −125, 56°. [m] Torsion angles 70, −67, −67, 70, 51, −103, 86, −103, 51°. [n] Torsion angles −118, 73, −86, 122, −86, 73, −118, 65, 65°. [o] Torsion angles −148, 90, −56, 89, −118, 103, −97, 47, 60°. [p] Torsion angles 55, 66, −66, −55, 151, −55, −66, 66, 55, −151°. [q] Torsion angles 151, −64, −58, 130, −58, −64, 151, −96, 54, −96°. [r] Torsion angles −53, 138, −61, −77, 68, 68, −77, −61, 138, −53°. [s] Torsion angles 145, −145, 84, −68, 84, −145, 145, −84, 68, −84°. [t] Torsion angles 90, −156, 61, 72, −58, −52, 143, −135, 85, −62°.

their conformational free energies are also summarized in Table 8. Also included in the table are molecular mechanics steric energy differences (SE), SE corrected for differential entropies of mixing based on conformer degeneracy ($G_{300K-MM}$), and SE corrected both for differential entropies of mixing and for the effect of harmonic motion ($G_{300K-Harmonic}$). None of these calculations were corrected for rotational free energy effects of conformer symmetry number (σ) because our free energy simulations would not include such effects. Zero point vibrational energies were omitted for the same reason. Thus all the energies given in the table refer to the same models and should be comparable. While the anharmonic conformational free energies (G_{300K}) from MC(JBW)/SD simulation are qualitatively similar to the appropriately weighted molecular mechanics steric energies (G_{300-MM}), there are differences between the two energies that often amount to 0.2−0.5 kcal/mol. As with the molecules described above, harmonic approximations do not always improve the correlation between molecular mechanics energies and the fully anharmonic free energies from simulation.

Though free energy simulations are useful in evaluating conformational free energy differences, the exact results obtained will depend upon the procedure used to assign the simulation structures to particular conformational families. In our work, we used Cartesian coordinates and least-squares superimpositions to evaluate rms deviations between simulation structures and minimum energy conformers from the $\mathbf{X}_i$ list—taking the conformer with the smallest rms to define the conformational family. Occasionally, however, structures far from any minimum are generated by simulations and using such structures in conformational population evaluations could lead to errors. Consequently, we required in our studies that superimposition rms's be no greater than 0.6 Å for assigning structures to conformational families. This value for maximum superimposition RMS was found by examinations of the conformational assignments with cyclononane at 300 K. The maximum rms used to define conformational families can also be too small. For example, if a maximum rms of 0.3 Å is used with cyclononane, the free energies at 300 K of the four conformers in Table 8 were found to be 0.16, 0.23, 0.0, and 1.01 kcal/mol, respectively. These energies and those in the table differ because the two calculations accumulate conformer population statistics from different regions of the potential energy surface. The rms = 0.3 Å calculation includes only structures that are relatively close to minimum energy forms (*i.e.* are close to the bottoms of conformational energy wells) whereas the rms = 0.6 Å calculations include virtually the entire surface, at least with cyclononane. Thus with a maximum rms = 0.3 Å, only 30% of the cyclononane structures at 300 K are assigned to conformational families while simulations with rms = 0.6 Å result in 99.6% of the structures being assigned.

Cyclononane is also interesting because two of its minima, the TCC and TCTC conformers, have very similar arrays of torsion angles—the largest difference being 33°. Furthermore, these conformers are reported to be separated by a barrier of only 0.1 kcal/mol.[24] Because these conformers are unusually similar in structure, we tested our simulation for possible oversampling (see discussion of potential 1d above) of these minima. To test for such oversampling, we repeated the MC-(JBW)/SD cyclononane simulation with increased torsional randomization (JBW Step 6, 0−(±)5°). After the 10 ns, 300 K simulation, we obtained populations for all conformations that were statistically indistinguishable from those described above using the smaller randomization. As with *n*-pentane, the cyclononane ensemble averages are insensitive to the size of the randomization step and thus appear converged to the correct values.

In another test for oversampling, we eliminated the relatively high energy TCTC conformation from the $\mathbf{X}_i$ list of cyclononane conformations and carried out another 10 ns, 300 K MC(JBW)/SD simulation. Because the TCTC conformer can be considered to lie within the TCC conformational well, we would expect the population of the TCC conformation in this new, three-state simulation to equal the total population of TCC and TCTC conformations in the previous four-state simulation. Indeed, whereas the previous simulation gave TCC and TCTC populations of 0.37 and 0.08, the new simulation gave the population of the (composite) TCC conformation as 0.44—a result very close to 0.37 + 0.08. The populations of the other two cyclononane conformations were identical to within 1% in both the four- and three-state simulations. Thus we did not detect evidence of oversampling in a potentially problematic molecular system where two conformers on the $\mathbf{X}_i$ list are geometrically similar and separated by only a small energy barrier.

The results described above indicate that the MC/SD simulation method using the JBW procedure gives full coverage of conformational space and results in converged simulations of all the molecules studied on the nanosecond simulation time scale. It is also clear from these simulations that harmonic approximations to the real potential energy surface are not particularly accurate with simple molecules and common molecular mechanics force fields at 300 K. In fact, the fully anharmonic conformational free energy differences from MM2 force field simulations were somewhat better correlated to simple MM2 steric energies with entropy of mixing corrections (correlation coefficient $R^2 = 0.92$, slope = 0.90) than to free

(24) Anet. F. A. L. *J. Am. Chem. Soc.* **1990**, *112*, 7172.

energies based on the rigid rotor, harmonic oscillator approximation ($R^2 = 0.77$, slope = 0.94).

In the simple examples we have studied, the 300 K conformational free energy differences from simulation deviated from those based on molecular mechanics SE with entropy of mixing effects by as much as 0.6 kcal/mol. Thus the notion that conformational isomers of simple molecules have equal entropies is not generally supported by experiments using the MM2 force field. The largest conformational entropy differences were noted with the flexible medium ring compounds and it is here that free energy simulations like ours should be of particular value. It is noteworthy, however, that SE calculations of conformational energies on smaller, relatively rigid cyclohexane derivatives are somewhat closer to experiment than are the computed free energies from simulation. This situation may result from the use of experimental conformer populations (*i.e.* free energy data) for parametrization of the conformational properties of common organic substructures, *e.g.* *n*-butane-like hydrocarbon networks.

Conclusion

In this paper we have described a Smart Monte Carlo technique that we term JBW and that explores conformational space by Jumping Between (energy) Wells. Though the JBW procedure can be used alone as in our torsional *n*-pentane simulations, we have implemented it as part of a hybrid algorithm termed MC(JBW)/SD that alternates between JBW and dynamics (SD) steps. The JBW part of the algorithm uses knowledge of the global potential surface in the form of the $\mathbf{X}_i$ list of minima while the SD part uses knowledge of the local potential surface in the form of potential energy derivatives. Because the method uses available knowledge of the low-energy regions of a potential surface to create trial conformations, it should be applicable to any molecule in which all low-energy conformations are known or can be found. With most multiconformational molecules, MC(JBW)/SD simulations are enormously more efficient than standard dynamics methods (MD or SD) which are typically slow to interconvert conformers.

The simple organic molecules we have studied here show not only that entropy can have a significant effect on conformational populations at typical laboratory temperatures but also that harmonic entropy can be a poor approximation to the actual anharmonic entropy. Thus the common practice of computing approximate conformational energies by simple energy minimization without applying harmonic corrections for entropy is justified by results with the systems studied here. It is also clear that assuming that conformers always have equal entropies can be dangerous. With simple molecules, the errors associated with ignoring entropy may not be large—in part because the steric energies (SE) of many molecular mechanics force fields were parametrized to reproduce experimentally determined populations and thus *free* energies. However, with larger, more flexible molecules at ambient temperatures, conformational energy errors greater than 0.5 kcal/mol may be encountered. Systems having conformers separated by low barriers are particular suspects for having significant conformational entropy differences.

Our results also suggest that the MC(JBW)/SD simulation methodology can be applied to a wide range of flexible organic molecules and can efficiently generate converged ensembles for use in other calculations. These might include calculations of such ensemble-averaged observables as NMR coupling constants or nuclear Overhauser effects. The methodology should also be useful as an ensemble-generating technique for free energy calculations (*e.g.* free energy perturbation) on multiconformational molecules.

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