Grand canonical ensemble Monte Carlo simulation of a lipid bilayer using extension biased rotations

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The cavity-biased grand-canonical ensemble method was applied to the simulation of a lipid bilayer using an enhanced Monte Carlo sampling technique. The enhancements include controlling the torsion and molecular rotation step size based on the lipid's conformation and controlling the order of torsion change attempts. It was found that the proposed sampling technique significantly enhances the rate of sampling of the lipid conformations while the grand-canonical ensemble implementation ensures that the water can both penetrate and escape pockets in the bilayer. The latter will be particularly important for simulating bilayers with embedded molecules. © *1999 American Institute of Physics.* [S0021-9606(99)52448-3]

I. INTRODUCTION

Computer simulation methods can provide a unique opportunity to get an insight into the structure of molecular assemblies of vital biological importance, such as lipid membranes, in atomic detail and complement the data obtained by various experimental methods.[1] However, besides its certain success there are also serious limitations of using computer simulation methods in the study of lipid membranes.[2] The predominant methodology, molecular dynamics (MD) is limited by the time scale of the biological processes: while the usual MD time step is in the femtosecond range, the characteristic time of the rotation of the lipid molecules in a membrane is in the nanosecond range.[3] Similarly, the flux of the water molecules crossing the membrane in a nanosecond[4] is of the order of 10^{-5} molecules/Å^2, and hence the transmembrane diffusion of water can hardly be seen in the computationally accessible scale of a customary MD simulation (i.e., 10^{-9} s and 10^3 Å^2). Additional difficulties arise when membrane-bound proteins are simulated. In such systems, waters can be trapped in unfavorable positions and internal cavities would not be able to exchange and thus equilibrate their waters with the bulk region.[5] These limitations imply that any advancement in the efficiency of the exploration of the configurational space can largely improve the reliability of the lipid membrane simulations and are particularly welcome.

An alternative simulation technique is the Monte Carlo (MC) method.[6] While its stochastic nature excludes the calculation of time-dependent properties, this limitation can also give an edge to the MC methodology over MD: being uncoupled from the time evolution of the system the size of the configurational space exploring steps is not limited by any fundamental reasons, only by the practical requirement of obtaining a sufficient number of accepted moves and it can be increased by introducing appropriate biasing.

Simulations in the grand-canonical (μ,V,T) ensemble (GCE)[7] have the promise of reducing convergence difficulties. It has been shown earlier that GCE MC simulations can provide well converged results for waters in crystal hydrates[8–10] and produce good convergence in calculations of the potential of mean force in aqueous systems.[11,12] GCE simulations, in general, encounter the difficulty posed by changing the number of molecules. Operationally, GCE simulations require periodic attempts to delete and insert molecules into the system—a difficult proposition since insertions into a dense system are rarely successful. This difficulty has been overcome by the cavity-biased insertion method (CB/GCE)[13] for MC simulations (that attempts insertions only into suitable cavities and modifies the acceptance expression accordingly) and by the introduction of an additional degree of freedom for MD simulations (that allows for the gradual creation of a new molecule).[14] A recent comparison[15] has found that for aqueous systems the CB/GCE method is an order of magnitude more efficient in sampling the density fluctuations than the MD technique. The promise of the GCE methodology in lipid membrane simulations is based on the fact that pairs of deletion and insertion steps represent large displacements of molecules and such large displacements are *not* hindered by intervening energy barriers. Such barrier crossing is very unlikely in (N,V,T) or (N,p,T) ensemble MC as well as in standard MD simulations, where the molecules have to go physically through the barrier. Furthermore, the beneficial effect of the GCE for potential of mean force calculations[11,12] indicate that the periodic removal and insertion of waters would lead also to better sampling of the lipid headgroup conformations by eliminating the need of ''squeezing out'' waters from tight spaces before a headgroup conformation change can take place.

In this Communication we demonstrate the feasibility of simulating a lipid bilayer in the grand canonical ensemble using cavity-biased insertions and Monte Carlo sampling of

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lipid conformations. We also present a biasing scheme to improve the efficiency of torsion angle changing and molecular rotation attempts, called *extension-biased rotation steps*. It is to be noted that the cavity-biased GCE technique can be used in conjunction with any other Monte Carlo scheme (e.g., the configuration biased method,[16] recently applied to lipid bilayers[17]).

II. COMPUTATIONAL DETAILS

A. The system simulated

We report here results of simulations of a bilayer containing 50 dimyristoylphosphatidylcholine (DMPC) molecules (i.e., 25 in each layer) in an aqueous environment. The simulations have been performed using the code MMC[18] in the canonical (N,V,T) as well as in the grand canonical (μ,V,T) ensemble in a hexagonal prism-shape basic simulation cell under periodic boundary conditions. The hexagonal shape of the cross section of the cell maximizes the distance between two periodic images of a given molecule in two dimensions, and therefore this cell shape is optimal for simulating systems in which the molecules are arranged in a planar way. The edge length of the basic hexagon and the length of the prism have been 24.8 and 72.0 Å, respectively. The temperature of the system was 37 °C, well above the gel/liquid crystalline transition temperature of DMPC, in both cases. In the (N,V,T) ensemble system the lipid bilayer has been surrounded by 2033 water molecules. The chemical potential value μ used in the (μ,V,T) ensemble simulations has been obtained from a set of preliminary test runs, in which the water density in the 10-Å-thick layer farthest from the lipids was monitored while the chemical potential was varied. The μ value producing the experimental density in this layer has been used in the simulations.

The interaction parameters of the lipid and water molecules have been represented by the all-atom CHARMM22 force field optimized for phospholipides[19] and the TIP3P model,[20] respectively. The long-range interactions have been treated by a group-based spherical cutoff. Lipid–water and water–water interactions beyond 20 Å have been truncated to zero. In order to prevent the direct interaction of the two lipid layers through the aqueous phase and thus avoid simulating an infinite stack of lipid layers this cutoff distance of the group centers has been set to 12 Å for lipid–lipid interactions. The conformation of the lipid molecules has been sampled from their torsion angle space, while their bond lengths and bond angles have been kept fixed at their equilibrium values. Every water displacement step, in which the chosen water molecule has been translated by a maximum distance of 0.3 Å and rotated around a randomly selected space-fixed axis by a maximum angle of 20°, has been followed by a lipid move. In the grand canonical ensemble simulation every water move included a water displacement and an insertion or deletion step. Insertion attempts were restricted to cavities having a radius of at least 2.5 Å. The lipid molecules have been selected in a shuffled cyclic order[21] both for displacement and for torsion angle change. The torsion angles have been selected sequentially but subject to a probability filter resulting in less frequent moves of torsions near the end of the chains. We found that the sampling can be improved when going from the end of a chain toward the center of the lipid, because in this case the part of the chain moved by a torsion angle is more likely to have been changed from its conformation at the time of the previous move of the same torsion, which increases the chance of the acceptance. The step size parameters of the torsional rotation steps have been optimized individually for each of the 41 torsions. This procedure, applied also for the overall lipid displacement steps, is discussed in detail in Sec. III.

B. Extension-biased rotations

To improve the sampling of both orientational and torsional degrees of freedom we have developed a new biasing technique, called *extension biased rotation*. It can be applied both for the overall rotation of the lipid molecules and for torsional rotations around a bond. This method belongs to the group of the variable step size methods, as the maximum angle of the rotation is varied according to the shape of the rotated unit or molecule. The principle underlying this method is rather simple: The farther an atom is from the axis of rotation, the larger displacement is caused by its rotation with a given angle. Thus, in an extension biased rotation step the closer the rotated atoms are to the axis of rotation (i.e., the smaller the extension of the molecule perpendicular to this axis is) the larger we set the angle range of random rotation.

We have tested several functional forms of the dependence of the maximum angle of rotation $\Delta\alpha_{\max}$ using both the average and maximum extension of the rotated unit perpendicular to the rotation axis. The best results so far are obtained using the relation $\Delta\alpha_{\max}=c^{*}R_{\max}^{-1/2}$, where $R_{\max}$ is the distance of the farthest rotated atom from the axis of rotation and c is the stepsize parameter.

In general, simulations with variable step sizes require the modification of the acceptance probability by the factor $P_{j\to i}^{\mathrm{sel}}/P_{i\to j}^{\mathrm{sel}}$, the ratio of the probabilities of selecting a move and of its reverse. However, as long as only one torsion angle is changed on the same molecule, or only one molecule is rotated as a rigid body in one MC step, this factor is one since these changes do not affect the distance of the rotated atoms from the axis of rotation, leaving the value of $R_{\max}$ unchanged. Hence, the standard Metropolis acceptance criterion[6] can be applied unmodified. As a result, the extra computational effort required by the new sampling method is negligible.

III. RESULTS AND DISCUSSION

A. Extension biased rotations

The computational gain obtained by using extension biased (EXB) instead of unbiased (UNB) rotations is demonstrated by Table I, in which the two methods are compared through the results of 10^5 MC steps long (N,V,T) ensemble runs. The step size parameters c of the overall rotation of the molecules and the torsional rotations as well as the relative frequency of these two kinds of moves have been independently optimized in both cases. The final values of the 41 torsional step size parameters and the values for the probabil-

TABLE I. Comparison of the sampling efficiency of the extension biased (EXB) and unbiased (UNB) rotations. $P^o_{\rm acc}$ and $P^t_{\rm acc}$ are the acceptance rates obtained for the overall lipid and torsional moves, respectively. For the definition of the other quantities see the text. Distances are in Angstroms, angles in degrees.

	$P^o_{\rm acc}$	$P^t_{\rm acc}$	D_{α_x}	D_{α_y}	D_{α_z}	D_L	D_T	$D_{T_{\rm HG}}$	$D_{T_{\rm HC1}}$	$D_{T_{\rm HC2}}$
EXB	0.211	0.181	2.23	3.06	3.19	0.22	8.01	3.29	4.94	4.82
UNB	0.410	0.314	1.82	2.43	2.60	0.25	6.68	2.64	4.44	3.92

ity filter are summarized elsewhere[22] both in the UNB and the EXB case. It was found during the step size optimization that the sampling efficiency is remarkably sensitive to the step sizes.

The sampling efficiency was characterized by the overall displacement of the center of the lipid molecules during the run (averaged over all lipids), D_L; the mean overall rotation of the molecule around the space-fixed *x*, *y*, and *z* axes (the bilayer is in the $y-z$ plane), D_{α_x}, D_{α_y}, D_{α_z}, respectively; and the mean overall displacement of the entire molecule, the headgroup chain and the two hydrocarbon chains due to the torsion angle changes, D_T, $D_{T_{\rm HG}}$, $D_{T_{\rm HC1}}$, $D_{T_{\rm HC2}}$, respectively. These torsion-related displacements were obtained for each lipid molecule by first overlaying the fixed core of the lipid (the glycerol carbon the headgroup is attached to and its neighbors) in its initial and final conformation and calculating the root mean square differences of the atomic positions in each chain separately. The results listed in Table I clearly show that extension biasing indeed improves the efficiency of rotational sampling significantly. Although it has a slight negative effect on the translation of the molecules it improves the efficiency of both the overall rotation steps and the torsional rotations by 20%–25%. Moreover, in the sampling of the torsional space of the headgroup region the gain of the extension biasing is even larger than in the other cases; the $D_{T_{\rm HG}}$ value of the EXB run being about 35% larger than that of UNB. The adequate sampling of headgroup conformation is of key importance, since the headgroup region, unlike that of the hydrocarbon tails, is largely inhomogeneous. It is also notable that the acceptance rates found to be optimal follow no particular pattern and are far from the old 50% prescription.

In order to demonstrate the performance of such enhanced sampling methods in a MC simulation, a 30-ps-long trajectory of a MD simulation[23] has been compared with a 10^7 MC steps long (N,V,T) ensemble MC run with extension biasing—each about a week long run on a single SGI R10000 CPU for each system. For each calculation, the first nine most changed lipid molecules are displayed in their initial and final position, orientation and conformation in Fig. 1. It can be clearly seen that the lipids underwent significantly larger conformational changes in the MC than in the MD simulation.

B. Grand-canonical ensemble simulations

With the torsional sampling technique calibrated, we performed simulations in the (μ,V,T) ensemble. A 10^7 MC step long simulation in the (μ,V,T) ensemble took only 15% longer than the corresponding (N,V,T) ensemble run. It included 5×10^6 attempts to insert or delete (in alternating order) a water molecule, out of which 4048 were accepted. The number of waters in the system fluctuated between 2014 and 2068. Figure 2 shows the density profiles of water as obtained after these short runs of 10^7 MC steps in both ensembles, as well as results of another 10^7 MC step long (N,V,T) ensemble run, performed after a 1.5×10^8 MC steps long equilibration. The GCE profile follows well the canonical ensemble results but shows deeper solvent penetration. It can also be seen that the two ensembles converge to the same results, but this convergence is at least an order of magnitude

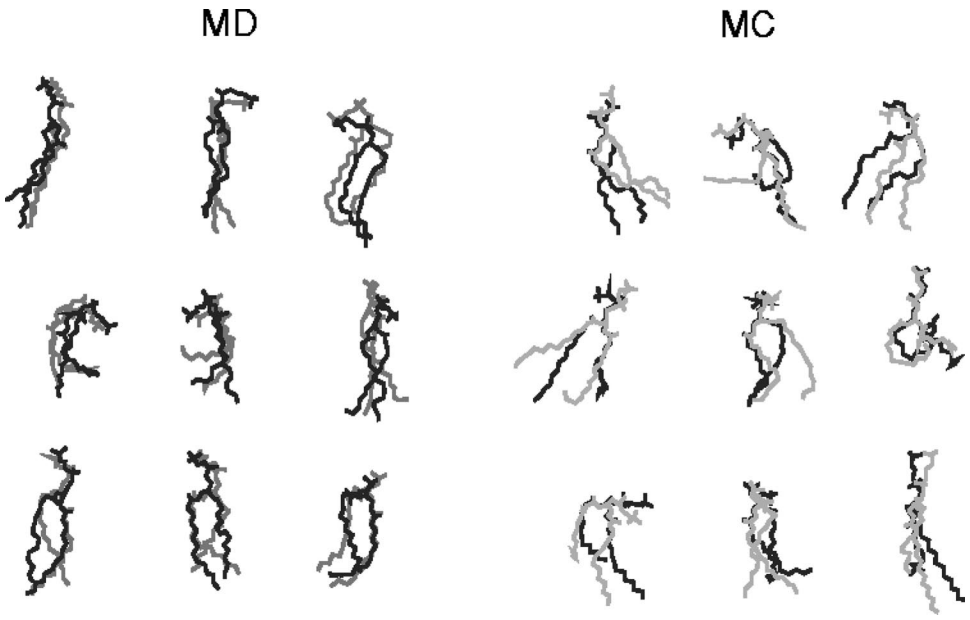

FIG. 1. Changes in conformation during a 30 ps molecular dynamics run and a 10^7 MC step long (N,V,T) ensemble Monte Carlo run for the nine most changed lipids in each calculation.

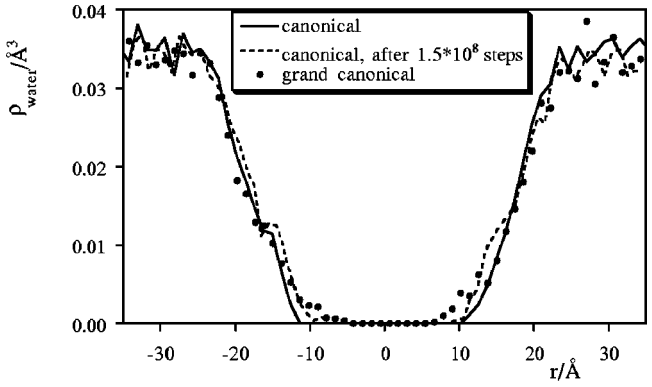

FIG. 2. The density profile of water in the direction perpendicular to the lipid bilayer, calculated in short canonical and grand-canonical ensemble simulations.

slower in the canonical ensemble.[24] This comparison thus serves both as validation of the GCE methodology and a demonstration of its superior ability in equilibrating the solvent.

The GCE methodology can also rapidly *detect* (although not necessarily *eliminate*) hidden artifacts in the system. This was demonstrated by a GCE simulation started from a configuration in which a lipid molecule was translated toward the middle of the bilayer by 2 Å. Within 5×10^7 MC steps the headgroup of this molecule flipped backwards, i.e., deep into the hydrophobic region, providing a ''scaffold'' for waters. As a consequence, 20–30 water molecules penetrated the hydrocarbon region providing a diagnostic of the artifact. On the other hand, even a five times longer (N,V,T) run failed to detect this anomaly.

The results presented in this Communication demonstrate the advantages of the use of the Monte Carlo methodology using the grand-canonical ensemble and including an appropriately biased sampling technique in lipid membrane simulations. Obviously, sampling enhancements are not limited to the ones shown here and further studies in this direction are currently in progress.

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