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Hybrid neMD/MC Lipid Swapping Algorithm to Equilibrate Membrane Simulation with Thermodynamic Reservoir

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Molecular dynamics (MD) simulations based on detailed all-atom models offer a powerful approach to study the structure and dynamics of biological membranes. However, the complexity of biological membranes in terms of chemical diversity presents an outstanding challenge. Particularly difficulties are encountered when a given lipid type is present at very low abundance. While considering a very large simulation system with a small number of the low abundance lipid may offer a practical solution in some cases, resorting to increasingly large system rapidly becomes computationally costly and impractical. More fundamentally, additional issue may be encountered if the low abundance lipid displays a high affinity for some protein in the simulation system. What is needed is to treat the simulation box as an open system in which the number of lipid can naturally fluctuate, as in the Grand Canonical Monte Carlo (GCMC) algorithm. However, this approach, in which a whole lipid molecule needs to be inserted or annihilated is essentially impractical in the context of an all-atom simulation. To enforce equilibrium between a simulated system and an infinite surrounding bath, we propose a hybrid nonequilibrium molecular dynamics - Monte Carlo (neMD/MC) algorithm, in which a randomly chosen lipid molecule in the simulated system is swapped with a lipid picked in a separate system standing as a thermodynamic “reservoir” with the desired mole fraction for all lipid components. The neMD/MC algorithm consists in driving the system via short nonequilibrium trajectories to generate a new state of the system that are subsequently accepted or rejected via a Metropolis MC step. The probability of exchanges in the context of an infinite reservoir with the desired mole fraction for all lipid components is derived and tested with a few illustrative systems for a PC and PG lipid mixtures.

I. INTRODUCTION

Membranes in living cells are complex inhomogeneous systems, literally comprising hundreds of chemically distinct lipid species.¹ This presents an outstanding challenge to efforts aimed at simulating these systems using detailed atomistic models. The lipidome of living organism comprises a considerable number of chemically different lipids.² Lipid composition affects not only the mechanical properties of membranes but also modulates the function of membrane proteins through distinct mechanisms.^{3–6} The wide ranging composition of biological membranes is such that some components dominate the overall structure of the membrane, while others are present at extremely low abundance.⁷ Because of the rich compositional diversity, some molecules with low abundance nonetheless find themselves involved in local organizational structures such as lipid rafts,⁸ or affect the properties of cancer cells.⁹ The partitioning of hundreds of lipid species, some at a very low abundance, may be associ-

ated with bilayer asymmetries and various functional complexities.^{10–12}

From a computational point of view, the sampling challenges of biological membrane using all-atom molecular dynamics (MD) simulations has long been recognized.¹³ However, the accessible simulation timescales are not sufficient to allow a satisfactory sampling of inhomogeneous multi-component membranes where lateral diffusion of the lipid molecules is very slow.^{14,15} Efforts were dedicated to develop and adapt various enhanced sampling algorithms to more efficiently explore the accessible configurations of membranes.¹⁶ Very few methods aimed at sampling the configuration space of inhomogeneous bilayer using all-atom simulations have been proposed in recent years.^{17,18} Alternatively, the use of simplified models is one approach that can be used to simulation large and complex cellular membranes.¹⁹ To decrease the computational time, many simulation studies of lipid-protein association in complex mixtures are based on coarse-grained (CG) models, in which groups of atoms are reduced to a single effective particle.^{15,20–22}

A particularly difficult situation is encountered when simulating a system in which some lipid components are meant to be at very low abundance. To

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illustrate the situation, let us consider a concrete system in which one lipid type is supposed to be at 0.1% mole fraction. In setting up a simulation, one might want to include one copy of this molecule in a system with 1000 lipids. While this may seem reasonable, the fixed number does not account for the considerable fluctuations that could occur at low mole fraction. Furthermore, this naive treatment may become invalid if some protein or other component is present that recruits and increase the present of the lipid at low abundance. For example, the mean number of negatively charged lipids may increase in the neighborhood of a positively charged protein, but this cannot occur in a finite system if the number of those lipids is too small. Fundamentally, the mean response to a local perturbation is associated with number fluctuations, and simulations with a fixed composition are inherently unable to adapt in response to a local perturbation. Typically, the practical solution is to simulate a very large system, for example, with 10,000 lipids including 10 copies of the lipid component at low abundance. However, resorting to increasingly large system can become computationally prohibitive.

Fundamentally needed is a representation of the simulation box as an open system, in which the number of lipid can naturally fluctuate in equilibrium with an infinite bath or “reservoir” with the desired mole fraction for all lipid components. In principle, Grand Canonical Monte Carlo (GCMC) simulation algorithms can address this type of issue.^{23–27} However, attempting the full insertion or annihilation of an entire lipid molecule with a non-zero acceptance Metropolis probability in the context of an all-atom membrane simulation is nearly impossible. These practical difficulties can be partly alleviated by substituting the insertion/annihilation with a swapping process, in which the two different types of lipids are exchanged. This is the essence of the hybrid nonequilibrium MD Monte Carlo algorithm (neMD/MC) for lipid exchange that has been proposed recently to better sample the configurations of all-atom membrane models.²⁸ The general idea of neMD/MC consists in driving a system via a short nonequilibrium trajectory to generate a new state that is then subsequently accepted or rejected via a Metropolis MC step.^{29–32} Stern first introduced the neMD/MC method to simulate systems at a constant pH,³³ though the original idea dates back to an extension of Widom’s test particle method by Athenes.³⁴

These hybrid simulation methods combining the advantages of MC with the strengths of MD offer promising strategies to efficiently sample the configurations of complex molecular systems. The ability of neMD/MC simulations to sample equilibrium configurations provide an important tool for studying complex biomolecule systems.^{35–40} More specifically, the concept of enforcing equilibrium with an exter-

nal thermodynamic reservoir with exchanges driven by a hybrid neMD/MC algorithm been also proposed in the case of aqueous electrolytes.^{41,42} Kindt and coworkers reported the first example of a hybrid MD/MC simulation of a bilayer involving lipid mutations,^{43–45} followed by Fathizadeh and Elber with the MDAS algorithm (Molecular Dynamics with Alchemical Steps).^{46–48}

To enforce equilibrium between a simulated system and an infinite surrounding bath, we propose a hybrid neMD/MC algorithm, in which a randomly chosen lipid molecule in the simulated system is swapped with a lipid picked in a separate system serving as a thermodynamic “reservoir” with the desired mole fraction for all lipid components. Theoretical developments regarding the probability of exchanges in the context of an infinite reservoir with the desired mole fraction for all lipid components are presented in the next section. The neMD/MC reservoir algorithm is then examined and tested for few illustrative systems with exchanges of the zwitterionic dilauroylphosphatidylcholine (DLPC) and anionic dilauroylphosphatidylglycerol (DLPG) lipids.

II. THEORETICAL DEVELOPMENTS

We consider an extended combined system corresponding to a membrane bilayer comprising a simulation box (s) together with a very large external reservoir (r). There are two type of lipids in the extended system, a and b . We want to swap the lipids of type a and b between the simulation box and the large external reservoir, as depicted in Figure 1.

In the extended combined system, the total number of lipids of type a is N_a , and the total number of lipids of type b is N_b . The number of lipids of type a and b in the simulation system is n_a and n_b , and the total number of lipids in the simulation system, $N_s = n_a + n_b$, is fixed. The number of lipids of type a and b in the external reservoir is $N_a - n_a$ and $N_b - n_b$. For clarity, the formal development is pursued with a finite number of lipids in the extended system. At the final stage, we will take the limit that the external reservoir is much larger than the simulated system, with $N_a \gg n_a$ and $N_b \gg n_b$.

We write the constrained partition function of the extended system as,

$$\Xi = \sum_{n_a \geq 0} \sum_{n_b \geq 0} \delta_{n_a + n_b, N_s} \frac{N_a!}{n_a!(N_a - n_a)!} \frac{N_b!}{n_b!(N_b - n_b)!} \int_s d\mathbf{R}_s e^{-\beta U_s(n_a, n_b)} \int_r d\mathbf{R}_r e^{-\beta U_r(N_a - n_a, N_b - n_b)} \quad (1)$$

where N_a and N_b are the total number of molecules. Introducing the scaled coordinates $\mathbf{X} \equiv \{\mathbf{x}_a, \dots, \mathbf{x}_n\}$

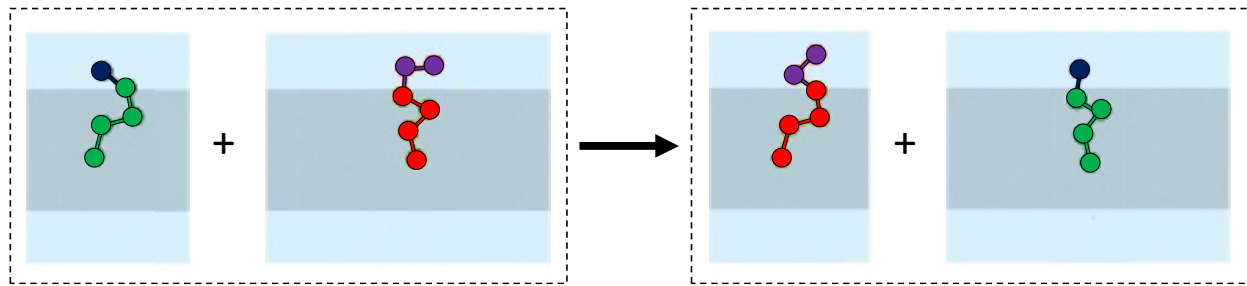


Figure 1. Schematic representation of lipid swapping within a combined system (dashed line box) comprising a finite simulation system (left) and a thermodynamic reservoir (right). Depicted is a lipid exchange between a simulation box (left) and a large external thermodynamic reservoir (right) of the polar head group of a lipid of type a (blue) with a lipid of type b (purple) while retaining the original conformation of the hydrocarbon chains (green for type a and red for type b).

such that the coordinates become dimensionless and varies between 0 and 1, we re-write this as,

$$\Xi = \sum_{n_a \geq 0} \sum_{n_b \geq 0} \delta_{n_a+n_b, N_s} \frac{N_a!}{n_a!(N_a - n_a)!} \frac{N_b!}{n_b!(N_b - n_b)!} (V_s)^{n_a+n_b} \int_s d\mathbf{X}_s e^{-\beta U_s(n_a, n_b)} (V_r)^{N_b-n_b+N_a-n_a} \int_r d\mathbf{X}_r e^{-\beta U_r(N_a-n_a, N_b-n_b)} \quad (2)$$

The probability of a given configuration with n_a and n_b particles in the inner region is

$$\mathcal{P}(n_a, n_b) = \frac{1}{\Xi} \frac{N_a!}{n_a!(N_a - n_a)!} \frac{N_b!}{n_b!(N_b - n_b)!} (V_s)^{n_a+n_b} (V_r)^{N_b-n_b+N_a-n_a} e^{-\beta[U_s(n_a, n_b) + U_r(N_a-n_a, N_b-n_b)]} \quad (3)$$

An important prerequisite in constructing a valid non-equilibrium simulation algorithm is that the system relaxes to the correct statistical properties when the channel is submitted to equilibrium boundary conditions. Let us construct a Markov chain for the system in which the fraction of particles 1 and 2 can vary by +1 (creation) or -1 (destruction) via random transitions. This random walk in the number of particles can be indicated schematically as,

$$\dots \leftrightarrow (n_a - 1, n_b + 1) \leftrightarrow (n_a, n_b) \leftrightarrow (n_a + 1, n_b - 1) \leftrightarrow \dots$$

Stepping toward the right replaces a lipid b by a lipid a , Stepping toward the left replaces a lipid a by a lipid b . There is an infinite number of Markov chains with transition probabilities $k_{n_a, n_b \rightarrow n_a+1, n_b-1}$ and $k_{n_a+1, n_b-1 \rightarrow n_a, n_b}$ converging towards the equilibrium probabilities given by Eq. (3). A sufficient condition to insure that the transition probabilities will yield the correct equilibrium probabilities is to impose the condition of detailed balance, i.e.,

$$\mathcal{P}(n_a, n_b) k_{n_a, n_b \rightarrow n_a+1, n_b-1}$$

$$= \mathcal{P}(n_a + 1, n_b - 1) k_{n_a+1, n_b-1 \rightarrow n_a, n_b} \quad (4)$$

To proceed further, we need to determine the ratio of the equilibrium probabilities, $\mathcal{P}(n_a + 1, n_b - 1)/\mathcal{P}(n_a, n_b)$. If we add one molecule of type a and remove a molecule of type b ,

$$\frac{\mathcal{P}(n_a + 1, n_b - 1)}{\mathcal{P}(n_a, n_b)} = \frac{n_a!(N_a - n_a)!}{(n_a + 1)!(N_a - n_a - 1)!} \frac{n_b!(N_b - n_b)!}{(n_b - 1)!(N_b - n_b + 1)!} e^{-\beta \Delta W} = \frac{(N_a - n_a)}{(n_a + 1)} \frac{n_b}{(N_b - n_b + 1)} e^{-\beta \Delta W} \quad (5)$$

where ΔW is the neMD work to go from the potential energy $[U_s(n_a, n_b) + U_r(N_a - n_a, N_b - n_b)]$ to the potential energy $[U_s(n_a + 1, n_b - 1) + U_r(N_a - n_a - 1, N_b - n_b + 1)]$. Therefore, we have that

$$\frac{k_{n_a, n_b \rightarrow n_a+1, n_b-1}}{k_{n_a+1, n_b-1 \rightarrow n_a, n_b}} = \frac{\mathcal{P}(n_a + 1, n_b - 1)}{\mathcal{P}(n_a, n_b)} = \frac{(N_a - n_a)}{(n_a + 1)} \frac{n_b}{(N_b - n_b + 1)} e^{-\beta \Delta W} \quad (6)$$

Detailed balance provides only a constraint on the relative magnitude of the transition probabilities. We write

$$k_{n_a, n_b \rightarrow n_a+1, n_b-1} = \left(\frac{(N_a - n_a) n_b}{C} \right) \min\{1, e^{-\beta \Delta W}\} \quad (7)$$

and

$$k_{n_a+1, n_b-1 \rightarrow n_a, n_b} = \left(\frac{(n_a + 1)(N_b - n_b + 1)}{C} \right)$$

$$\min\left\{1, e^{-\beta\Delta W}\right\} \quad (8)$$

Setting the constant, $C = (N_a - n_a) n_b + (n_a + 1) (N_b - n_b + 1)$, we get,

$$k_{n_a, n_b \rightarrow n_a+1, n_b-1} = \left(\frac{(N_a - n_a) n_b}{(N_a - n_a) n_b + (n_a + 1) (N_b - n_b + 1)} \right) \min\left\{1, e^{-\beta\Delta W}\right\} \quad (9)$$

and

$$k_{n_a+1, n_b-1 \rightarrow n_a, n_b} = \left(\frac{(n_a + 1) (N_b - n_b + 1)}{(N_a - n_a) n_b + (n_a + 1) (N_b - n_b + 1)} \right) \min\left\{1, e^{-\beta\Delta W}\right\} \quad (10)$$

which can be shifted to the initial state n_a, n_b by decreasing n_a by 1 and increasing n_b by 1,

$$k_{n_a, n_b \rightarrow n_a-1, n_b+1} = \left(\frac{n_a (N_b - n_b)}{(N_a - n_a + 1) (n_b + 1) + n_a (N_b - n_b)} \right) \min\left\{1, e^{-\beta\Delta W}\right\} \quad (11)$$

to obtain an expression for the removal of a lipid of type a . Now, we take the limit that the external reservoir is much larger than the simulated system, with $N_a \gg n_a$ and $N_b \gg n_b$, and that it can essentially be treated as an infinite reservoir with fixed mole fractions $f_a = N_a/(N_a + N_b)$ and $f_b = N_b/(N_a + N_b)$,

$$\lim_{N_a, N_b \rightarrow \infty} k_{n_a, n_b \rightarrow n_a+1, n_b-1} = P_a^{\text{incr}} \times \min\left\{1, e^{-\beta\Delta W}\right\} \quad (12)$$

and

$$\lim_{N_a, N_b \rightarrow \infty} k_{n_a, n_b \rightarrow n_a-1, n_b+1} = P_a^{\text{decr}} \times \min\left\{1, e^{-\beta\Delta W}\right\} \quad (13)$$

where

$$P_a^{\text{incr}} = \left(\frac{(N_a/N_b) n_b}{(N_a/N_b) n_b + (n_a + 1)} \right) = \left(\frac{(f_a/f_b) n_b}{(f_a/f_b) n_b + (n_a + 1)} \right) \quad (14)$$

and

$$P_a^{\text{decr}} = \left(\frac{n_a}{(N_a/N_b) (n_b + 1) + n_a} \right)$$

$$= \left(\frac{n_a}{(f_a/f_b) (n_b + 1) + n_a} \right) \quad (15)$$

are the probability for attempting to increase or decrease the number of lipid of type a , respectively.

Similar statistical prefactors accounting for the number density of the molecule of interest appear also in the Grand Canonical Monte Carlo algorithm.⁴⁹ It is noted that the probabilities for increasing or decreasing are symmetric with respect to a and b . These expressions were derived assuming that only lipid a and b are exchanged. However, if the lipid of type a is anionic, it may be necessary to simultaneously swap a cation (c) with a water molecules (w) to maintain charge neutrality in the simulated system. Further analysis shows that accounting for this additional exchange introduces a factor of $((n_c + 1)/n_w) (f_w/f_c)$ multiplying $(n_a + 1)$ in the expression for P_a^{incr} , or the n_a in the expression for P_a^{decr} , where $f_c = N_c/(N_c + N_w)$ and $f_w = N_w/(N_c + N_w)$ are the mole fraction of cations and water molecules in the reservoir, respectively. This analysis shows that, as long as the salt solution in the simulated system remains stably at the same concentration as the reservoir, the multiplicative factor should be very close to unity with $((n_c + 1)/n_w) \approx (f_c/f_w)$. For the sake of simplicity, this additional factor was not included in the present simulations. The basic steps of the algorithm are given in Scheme II. [!h]

Python pseudocode to run the MC-swap

```

for i in range(1, nstep):
    PaInc = (Fa/Fb)*nb/((Fa/Fb)*nb+(na+1))
    PaDec = na/((Fa/Fb)*(nb+1)+na)
    rand_1 = numpy.random.rand()
    if rand_1 < PaInc:
        # Attempt to increase "na" and decrease "nb"
        run alchemical md switch to calculate the work W
        rand_2 = numpy.random.rand()
        if rand_2 < min(1, exp(-W/kT)) : THEN
            na = na + 1
            nb = nb - 1
            #Identify the specific lipids for this exchange
    elif (rand_1 > PaInc) and (rand_1 < (PaInc+PaDec)) :
        #Attempt to decrease "na" and increase "nb"
        rand_2 = numpy.random.rand()
        run alchemical md switch to calculate the work W
        if( rand_1 < min(1, exp(-W/kT)) ):
            na = na - 1
            nb = nb + 1
            #Identify the specific lipids for this exchange
    ELSE
        #No attempt to change "na" and "nb"
        continue to run equilibrium MD

```

Fundamentally, Scheme II separates the probability of the complete exchange event into two distinct steps. In the first step, the probabilities P_a^{incr} and P_a^{decr} from Eqs. (14) and (15) are used to determine whether one should attempt increasing or decreasing the number of lipid of type a . This is then followed in a second step by calculating the computa-

tionally expensive nonequilibrium work to accept or reject this attempted exchange using the Metropolis probability criterion.³¹ The resulting exchange probability is the product of the probability of each step. A similar two-step scheme involving a probability associated with the inherent pK_a of ionizable residues was also included in a neMD/MC constant pH simulation algorithm.^{37,38}

III. COMPUTATIONAL METHODOLOGY

A. Swapping protocol

Following Scheme II, the character of the attempted swapped is randomly chosen based on the probabilities P_a^{incr} and P_a^{decr} based on Eqs. (14) and (15). Figure 2 shows a detailed representation of the swapping procedure. If the attempt is to increase (decrease) the number of lipid of type a , then a lipid of type a (type b) is randomly chosen in the simulation system (called s in Figure 2). Simultaneously, a lipid of type b (type a) is randomly chosen in the reservoir (called r in Figure 2). To maintain charge neutrality, a negative lipid is associated with a random cation, and the neutral lipid is associated with a random water molecule. The lipids are swapped in the membrane, and the water molecule and ion are exchanged in the bulk. The exchanges follow the two steps “constrained dual-topology” procedure previously developed by the authors.²⁸ For the molecular system s , the lipid a (b) is chosen, then copied in vacuum. The position of all the atoms of a (b) is restraint. The lipid b (a) chosen in r is then copied in this box of vacuum, such that the carbon tails, common between the two lipids, are aligned, the polar heads are approximately superposed, and the oxygen of the water is aligned on the ion. A weak restraint is added between the polar heads and another one between the ion and water. A simulation in vacuum is run to equilibrate the conformation of the molecule b (a). Then, an alchemical simulation is run in the molecular system s , with the common atoms of a and b restraints, and the conservation of the weak restraint between the polar heads, and the water and ion. During this alchemical exchange, the molecule a (b) is decoupled, and the molecule b (a) are coupled. From this simulation, the nonequilibrium work associated with the exchange carried out over the finite switching time t_{sw} is computed using a Thermodynamic Integration (TI) procedure,

$$W_{\text{int}}^s = \int_0^{t_{\text{sw}}} \left(\frac{\partial U_{\text{int}}}{\partial \lambda} \right) \dot{\lambda}(t) dt \quad (16)$$

The same method is used for the molecular system r . During the alchemical simulation r , the molecule b (a) is decoupled and the molecule a (b) are coupled, and the work associated with the exchange W_{int}^r is

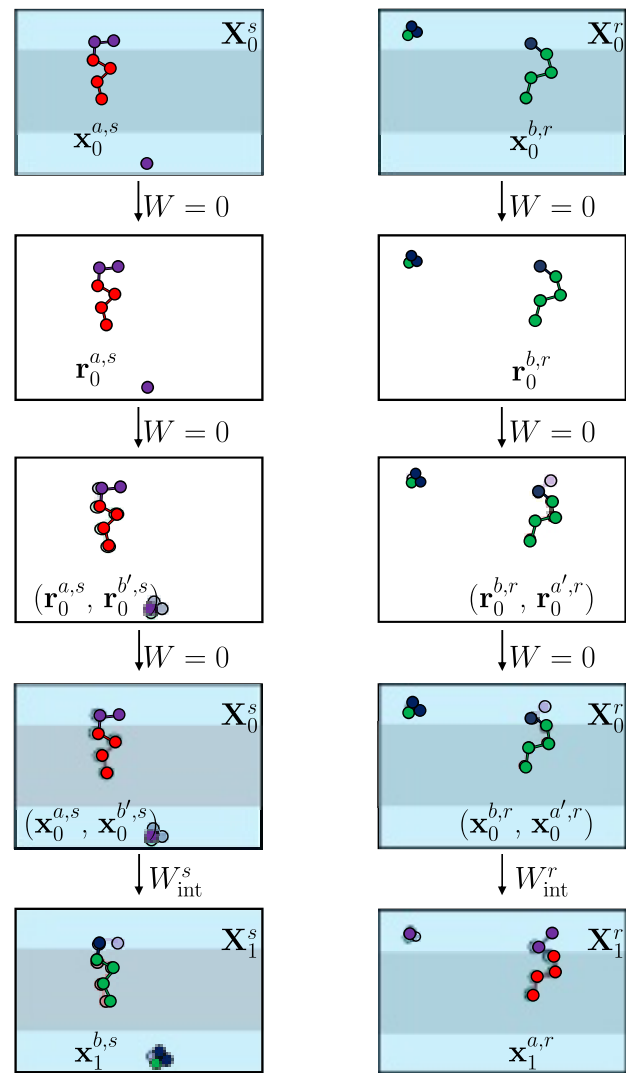


Figure 2. Schematic representation of the “constrained dual-topology” exchange methodology. The variables s and r represent the simulation system (s) and the reservoir (r), $\mathbf{x}_0^{a,s}$ and $\mathbf{x}_0^{b,r}$ are the coordinates and velocities of the molecules a and b in their initial systems, $\mathbf{r}_0^{a,s}$ and $\mathbf{r}_0^{b,r}$ are the coordinates of the molecules in the vacuum, $\mathbf{r}_0^{a',r}$, $\mathbf{r}_0^{b',s}$, $\mathbf{x}_0^{a',r}$ and $\mathbf{x}_0^{b',s}$ are the coordinates (and velocities if written as \mathbf{x}) of the molecules in their new conformation in the other molecular system before the exchange, $\mathbf{x}_1^{a,r}$ and $\mathbf{x}_1^{b,s}$ are the positions and velocities of a and b in their final state after the exchange, \mathbf{X}^s and \mathbf{X}^r are the coordinates and velocities of the rest of the systems, indexed as 0 for the initial values, and 1 for the final values.

computed using the same equation 16. The free energy associated with swapping configurations in the constrained dual-topology paradigm depicted in Figure 2 is believed to be small in the present case and is neglected.²⁸ Then, a Metropolis test is run on the nonequilibrium work to accept or reject the exchange:

$$T^{(a)}(\mathbf{x}_0^{a,s}, \mathbf{X}_0^s, \mathbf{x}_0^{b,r}, \mathbf{X}_0^r \rightarrow \mathbf{x}_1^{a,r}, \mathbf{X}_1^s, \mathbf{x}_1^{b,s}, \mathbf{X}_1^r)$$

$$= \min \left[1, e^{-\Delta W/k_B T} \right] \quad (17)$$

where $\Delta W = W_{\text{int}}^s + W_{\text{int}}^r$. This Metropolis test ensures the microscopic detailed balance, and thus the convergence toward the equilibrium Boltzmann distribution.³¹ Symmetric momentum reversal conditions are applied before and after the attempted exchange.³⁰

Following Scheme II, there is a first test using the probabilities based on Eqs. (14) and (15) to know if a given exchange should be attempted (and which lipids would be exchanged). The Metropolis test involving the nonequilibrium work ΔW is executed as a second step, only if an exchange is attempted. It is worth noting that there is no systematic bias in the two-step algorithm, with the probabilities P_a^{incr} and P_a^{decr} from Eqs. (14) and (15). For instance, it can be verified that the desired mole fraction f_a is obtained exactly in a simulation with an “ideal gas” representation of the system setting ΔW artificially to zero (Supporting Figure S1).

B. Simulation details

Four examples are being studied. The lipids considered are DLPG and DLPC. Two patches are being equilibrated, each of them with 50 DLPC per layer. One is a pure membrane, the other one has a peptide in the middle of the membrane. The peptide is a helix of 22 leucines in the membrane, with 9 arginines at both ends. The arginines of this peptide should have a high affinity with negatively charged lipids. These two patches are equilibrated with two reservoirs: one is a homogeneous mixture of DLPG and DLPC, with 50 lipids of each kind per layer and the other one is a membrane with 99 DLPC and 1 DLPG per layer. The two patches and the two reservoirs are shown Figure 3. The four examples studied are the equilibration of each patch with the two reservoirs.

All the systems are created using the CHARMM-GUI membrane builder.⁵⁰ The two reservoirs are approximately $80 \text{ \AA} \times 80 \text{ \AA} \times 80 \text{ \AA}$, with about 50,000 atoms. The small patch without peptide is $55 \text{ \AA} \times 55 \text{ \AA} \times 80 \text{ \AA}$, with about 25,000 atoms, and the one with a peptide is $50 \text{ \AA} \times 60 \text{ \AA} \times 94 \text{ \AA}$ with about 35,000. In each system, the concentration of NaCl is set up to be at 0.1 M. All simulations are performed using NAMD⁵¹, in the NPT ensemble. The water is described using the TIP3P model⁵², the lipids, ions and peptide are described using the nonpolarizable force field CHARMM36⁵³. To keep the temperature fixed at 315 K and the pressure fixed at 1.0315 bar, Langevin thermostat and piston are used⁵⁴. To handle long-range interactions, the Particle-Mesh Ewald algorithm is used⁵⁵, and the van der Waals and electrostatic interactions are truncated above 12 Å at

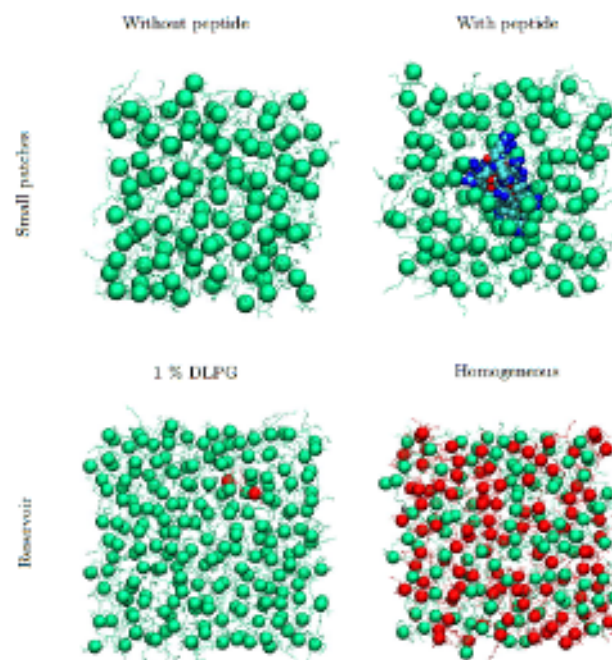


Figure 3. Simulated systems used for the study. On the first row, the two patches that need to be equilibrated. On the second row, the two reservoirs. In green: DLPC, in red: DLPG, in blue and cyan: the peptide. The beads correspond to the position of the phosphate group of each lipid.

a switching distance of 14 Å. To constrain covalent bonds, the SHAKE/RATTLE^{56,57} algorithm is used, and the SETTLE algorithm⁵⁸ is used for the water. The Hydrogen Mass Repartitioning (HMR) scheme⁵⁹ is used, and all equilibrium MD and neMD/MC simulations were carried out with a time-step of 2 fs.

The alchemical exchanges are performed over 50 ps, and the equilibrium MD simulations are 100 ps long. The length of the MD is independent of the switches, so can be made longer if desired. The main focus of this project being to exchange lipids between two systems, it was not necessary to make it long in this context. The alchemical exchange is done without soft-core potential^{60,61}, with a linear modification of the van der Waals and electrostatic interactions for λ varying from 0 to 1. The alchemical TI code developed by Radak³⁹ was used.

It was shown previously that the efficiency of the neMD/MC algorithm decreases if the switch is too long, even though it would increase the probability to accept and exchange.³² Therefore, the length of the alchemical switch has been chosen to be as short as possible. The two alchemical simulations (the one in the small patch and the one in the reservoir) are done using the same parameters. The equilibrium MD is performed after 65 attempts. One attempt starts with

the probability test described by the equations (14) and (15). If this test does not allow to increase or decrease the number of lipids, the attempt ends here. If it allows to change the lipid distribution, the attempt then continues to the corresponding alchemical exchange and the Metropolis test described by the equation (17).

The concentration of the lipids in the reservoir is supposed to be constant. Therefore, it is not necessary to keep the lipid distribution after the exchange. To limit the computational cost associated with the reservoir, a unique trajectory of the membrane is generated using brute force MD. At each exchange, a frame is randomly selected in the trajectory, and from this frame, the coordinates of the molecules as well as their velocities are extracted. These are used during the alchemical exchange, but, after the exchange, once the simulation is done and the work is computed, the newly generated lipid distribution is not conserved. The next attempt is done using another frame of the initial trajectory. While Eqs. (14) and (15) are written for an infinite reservoir, in practice, a finite simulation system is used to calculate the nonequilibrium work ΔW associated with the lipid exchange. This approximation is expected to be valid as long as the simulation system is sufficiently large.

IV. RESULTS

Figure 4 shows the work associated with the exchanges of lipids between the patch with peptide and the reservoir at 1 % of DLPG. The histograms for the other simulations are all similar (not shown). Figure

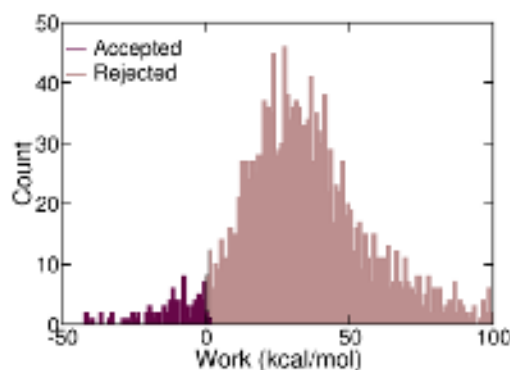


Figure 4. Histogram of the work associated with the exchanges of lipids between the membrane system with a peptide and the reservoir at 1 % of DLPG. Brown and indigo stand for rejected and accepted exchanges, respectively.

5 shows the evolution of the mole fraction of DLPG in each membrane equilibrated with the reservoir at 1 % of DLPG. The observed mole ratio in the simulation

is about 0.56 % in the membrane without the peptide, and 0.75 % in the membrane with the peptide, which is somewhat lower than the expected value of 1 % set by the reservoir.

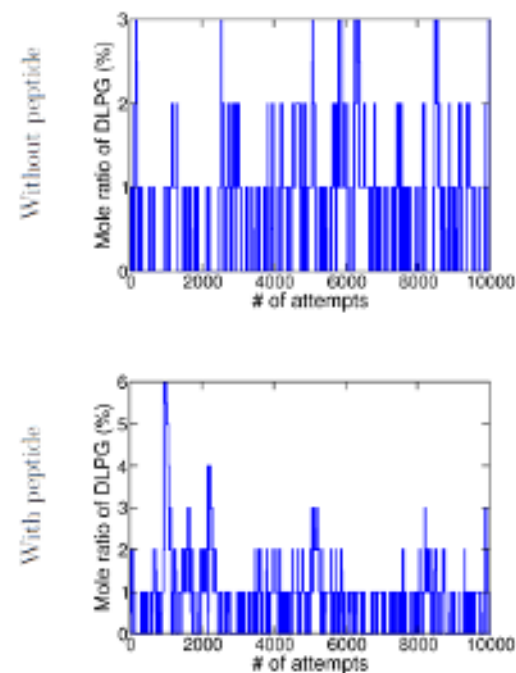


Figure 5. Evolution of the number of DLPG in the simulated systems equilibrated with a reservoir at 1 % of DLPG. Shown are the membrane system without a peptide (top) and with a peptide (bottom). The average without peptide is 0.56 %. The average with peptide is 0.75 %.

Further analysis indicates that this discrepancy is reduced when increasing the duration of the equilibrium MD trajectory between attempted exchanges or the frequency. A close examination of the work associated to the exchange attempts reveals that decreasing the number of DLPG is associated with a lower value of the work compared to increasing the number of DLPG (Supporting Figure S2). The observed mole ratio of DLPG is larger when an equilibrium MD simulation carried out is every 10 moves (Figure 5) compared to when it is carried out only every 65 moves (Supporting Figure S3). While this does not indicate the presence of a systematic bias in the algorithm, it suggests that convergence is slower if the successive attempted exchanges are too strongly correlated. Presumably, the local environment in the membrane must be allowed to relax to a more stable configuration after adding a new DLPG. In this case, the algorithm would benefit from a longer equilibrium MD between the attempted exchanges to converge toward the correct mole fraction.

Figure 6 shows the evolution of the mole fraction of DLPG in each membrane equilibrated with the reservoir at 50 % of DLPG. Both membranes show fluctu-

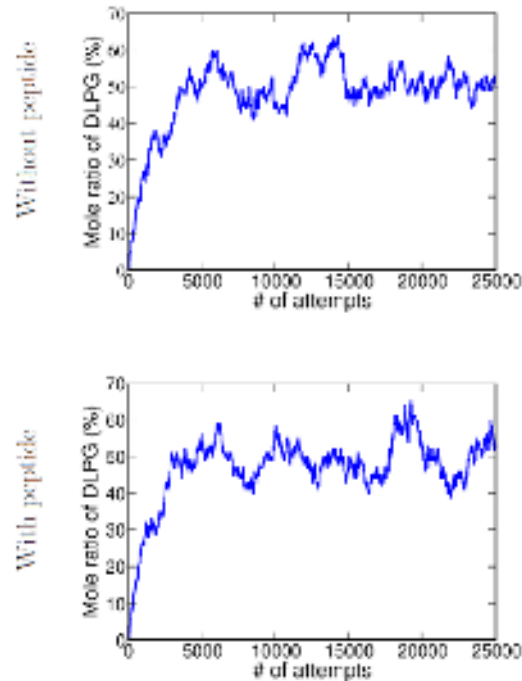


Figure 6. Evolution of the number of DLPG in the simulated systems equilibrated with a reservoir at 50:50 of DLPG and DLPC. Shown are the membrane system without a peptide (top) and with a peptide (bottom). The average without peptide is 50.6 %. The average with peptide is 50.6 %.

tuations around 50 % of DLPG. The membrane with the peptide converges faster to this value than the system without the peptide. It indicates that the peptide drives and stabilizes the composition of membrane. Figure 7 shows the lipid distribution within 2 Å of the peptide. When the membrane is equilibrated with the homogeneous reservoir, the attempts to increase the number of DLPG are the majority, at least until a homogeneous composition of the membrane is reached. The DLPG inserted in the membrane have more time to relax before there is an attempt to get removed, so they diffuse to bind to the peptide. The specificity of protein-lipid binding is reproduced by the neMD/MC method.

V. CONCLUDING DISCUSSION

Adopting a finite model that is representative of a complex biological lipid membrane to carry out MD simulations is often challenging. The difficulties are further heightened when considering an inhomogeneous system in which some components are present at low abundance. In practice, simulation of membranes with a fixed composition are inherently unable to adapt in response to a local perturbation. For example, the mean number of negatively charged

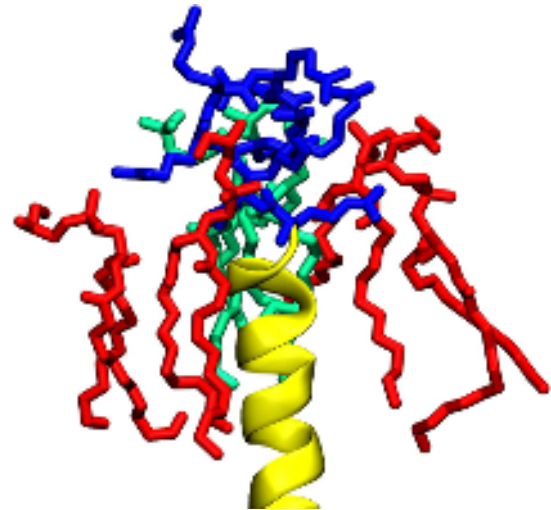


Figure 7. Local enrichment of DLPG in the neighborhood of the peptide resulting from the neMD/MC simulation. The lipid molecules within 2 Å of the peptide (in yellow) in the membrane equilibrated with the homogeneous reservoir at 50% DLPG mole ratio. The blue sticks represent the arginine, the DLPG are in red and the DLPC are in green.

lipids may increase in the neighborhood of a positively charged protein, but this cannot occur in a finite system if the number of those lipids is too small. The mean response to a local perturbation is associated with number fluctuations, which are ignored in a closed system with fixed composition.

To enforce equilibrium between a simulated system and an infinite surrounding bath, we designed a novel hybrid nonequilibrium molecular dynamics - Monte Carlo (neMD/MC) algorithm, in which a randomly chosen lipid molecule in the simulated system is swapped with a lipid picked in a separate system standing as a thermodynamic “reservoir” with the desired mole fraction for all lipid components. In essence, the algorithm is akin to standard experimental procedures, where the lipid composition of a sample in terms of the mole fraction is chosen deliberately.

Admittedly, there is a computational cost associated with explicitly simulating the lipid exchange in the reservoir at every attempted move. However, while this decreases somewhat the efficiency of the neMD/MC method relative to using a tabulated exchange free energy for the reservoir, the latter approach could give rise to difficult calibration issues. In practice, explicitly simulating the lipid exchange in the reservoir is simpler and more advantageous.

The neMD/MC exchange algorithm was tested with a few illustrative systems with a DLPC:DLPG lipid mixture. In practice, the exchange algorithm attempts to swap the PC and PG polar head groups while retaining the conformation of the identical hydrocarbon chains. A cation picked randomly was associated with the anionic DLPG molecule to preserve

charge neutrality.

The tests show that the algorithm is able to populate the simulation system in a manner consistent with the mole fraction present in the thermodynamic reservoir, and enable number fluctuations consistent with the finite size. A particular advantage of a neMD/MC formulation based on exchange between a simulated system and a reservoir is that it bypasses the need to determine the excess chemical potential of the lipid of type *a* and *b* that would be required in a Grand Canonical Monte Carlo algorithm. It is our hope that the algorithm will provide a useful methodology to generate realistic simulations of complex multi-component membranes.

In the immediate future, the neMD/MC exchange algorithm will be expanded to treat multi-component systems with variations in polar head groups and hydrocarbon chains.^{7,10–12} One specific focus will be the phosphatidylinositol biphosphates (PIP2) molecule, which makes up around 1% of the plasma membrane composition but is found in higher concentrations near intrinsic proteins and separate membrane domains.^{62,63}

SUPPLEMENTARY MATERIAL

Three Supplementary Figures are included: (S1) the mole ratio of DLPG in equilibrium with a reservoir at 1% of DLPG for an gas neMD/MC simulation, (S2) a histogram of the work associated with the exchanges of lipids between the patch with peptide and the reservoir at 1 % of DLPG, (S3) the evolution of the number of DLPG in the simulated systems. The systems are equilibrated with a reservoir at 1 % of DLPG with a shorter MD simulations between attempted exchanges.

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