Transition path sampling study of flip-flop transitions in model lipid bilayer membranes

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The microscopic dynamics of lipids in biomembranes is of special relevance in the study of chemical reactions produced in cells. The mechanism of the exchange of a model lipid molecule between both sides of a flexible bilayer membrane or flip-flop in an aqueous environment has been studied by computer simulation using the recently developed transition path sampling technique, since flip-flop transitions are infrequent events of the lipid dynamics. In addition, structural changes in the membrane have been investigated at ambient conditions and for increasing temperature. Our results highlight the cooperative effort of the whole system in order to allow a lipid molecule to cross the bottleneck in configuration space associated with the transition state of the flip-flop event. Within the time interval of the transition, all molecules of the system significantly change the frequency of their molecular motions.

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I. INTRODUCTION

Phospholipid membranes provide the framework for nearly all biological membranes and they are composed of bilayers of amphiphilic lipid molecules to which proteins are bound [1–3]. The formation of the bilayer is due to the location of the hydrophilic head groups of the lipids close to the water environment forming interfaces, while the hydrophobic tail groups of the lipids are expelled from the aqueous solvent and form a liquid-like inner region located between the two interfaces. Self-assembled bilayers are dynamic structures, which include protrusions or small relative displacements of individual molecules producing roughness of the interface, as it has been observed both in scattering experiments and molecular dynamics simulations [4]. Hence, local changes in the structure of the membrane can produce relevant changes in the entire system. Although structure at ambient conditions is well understood, microscopic dynamics of biomembranes is still not well known despite the amount of both experimental and theoretical work devoted to its study [5–8]. The main obstacles for the understanding of the dynamics of biomembranes are basically two: first, the enormous number of degrees of freedom to consider and second, the variety of time scales involved. Physical properties of flexible biomembranes like elasticity and mobility have also been recently studied by molecular dynamics simulations [9,10]. It has to be pointed out that molecular models employed in computer simulations, such as the model used in this work, usually consist of amphiphilic chains significantly shorter than real lipid chains, in order to have reliable system sizes from the computational point of view.

One of the phenomena relevant to the knowledge of the behavior of biomembranes is the transbilayer lipid migration or flip-flop transition [3], when a given lipid moves from one interface of the membrane to the other across the intermediate region in between. Flip-flop occurs in both natural and model phospholipid membranes. Examples of rapid flip-flop movement have been observed in the human erythrocyte membrane [11] or in photoreceptor disc membranes in retinal rods [12]. Moreover, the comprehension of such process is of central importance in the manufacture of synthetic membranes [13]. In the lipid exchange mechanism, there are different time domains that have to be considered in order to connect the microscopic phenomenon with the experimental information available. The crucial point is the fact that flip-flop motions of lipids across membranes are rare events of the lipid dynamics in most cases. A recent computational study about lateral and transverse diffusion in bilayer membranes [8] has showed that flip-flop events are extremely rare when coarse-grained models are used.

Rare events play a central role in fields like chemical kinetics, diffusion in solids, or in the electrical transport theory. The characterization of rare events is basically related to the search of the transition state of the system [14]. Molecular dynamics (MD) simulations are well suited to analyze microscopic motions in the time scale of transition state events. Nevertheless, MD can only generate trajectories lasting nanoseconds, i.e., orders of magnitude shorter than the experimentally measured time duration of flip-flop events. The aim of the present work is the study of the structure of model lipid bilayers and the dynamics of flip-flop events using a specific technique able to deal with rare events adapted to MD simulations. In a previous work [15], we analyzed the structure of the same model lipid membrane at ambient conditions and obtained the distribution of the total energy in terms of the kinetic and potential energy contributions. In addition, a preliminary analysis of the velocity distribution of each species was included. In the present work, we compare the structure and free energy profiles at ambient and high temperature conditions, together with the calculation of lateral and transverse diffusion coefficients of the

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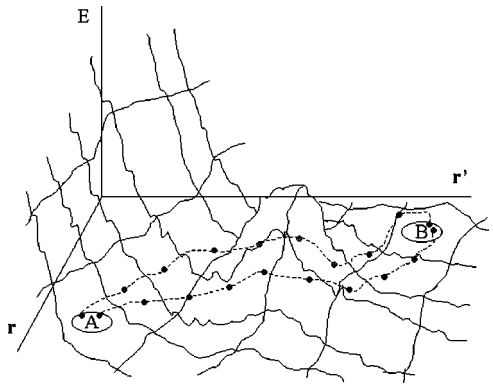

FIG. 1. Transition paths (dashed lines) between two stable states (A,B). State A corresponds to a configuration $\{\mathbf{r}(t),\mathbf{p}(t)\}$ of the system with a given lipid head located in one interface, whereas in state B such lipid head is found in the second interface after flip-flop. Here we display potential energy E as a function of configuration space coordinates $\{\mathbf{r}\}$. For the sake of simplicity, only two spatial dimensions (r,r') are plotted. The pathways cross by construction the transition state regions, defined as saddle points of the potential energy surface (network of full lines). Each black dot indicates a time slice of the transition path.

lipids and we also include a computation of the intermolecular vibrational frequencies of water molecules and lipid species.

II. THEORETICAL BASIS

A. Transition path sampling: Main aspects

A way to access the relevant dynamical mechanisms that lead to lipid transitions between interfaces is by using an enhanced computational tool able to directly focus on the physical trajectories associated with the flip-flop process, i.e., a tool capable to generate a set of flip-flop events. A useful method capable to uncover microscopic keys of rare events has recently appeared in the literature [16]. The method is called transition path sampling (TPS) and it has been successfully applied to the study of water autoionization [17], the aqueous dissociation of sodium-chloride [18–20], and to the hydrogen-bond breaking process [21], to mention a few examples. A general review of TPS can be found in Ref. [16]. Here we will only give an overview of the method, with particular attention paid to the case of the lipid flip-flop motions.

TPS works by generating a set of transition pathways connecting stable states of a system in phase space, where a path can be represented by a multidimensional vector $\{\mathbf{r}(t),\mathbf{p}(t)\}$. To have a simplified representation of such paths we will only consider configuration space, where a transition path will link two stable states of the system. Let us describe the TPS methodology in the particular case of a lipid flip-flop transition. In such case, the generation of the transition path ensemble is computationally very expensive since each transition path is associated with a flip-flop event, which is very infrequent in lipid dynamics, as it will be explained below.

We show in Fig. 1 a picture of two transition paths in a two-dimensional configuration space, where the first stable state (A) corresponds to a given lipid with its head located in one interface of the bilayer, and the second stable state (B) corresponds to the same lipid with its head placed in the opposite interface, once the flip-flop event has occurred. Both states will be associated with minima of the potential energy surface. An intermediate configuration of high energy will be associated with a transition state (TS) of the system, which in the framework of the theory is a saddle point in the potential energy surface. In our simulation, a transition path has been defined as follows: in the first time step of the path, the head group of a lipid molecule is in the first stable state, whereas in the second time step the lipid head enters the intermediate region, spending a given number of time steps in such zone, to leave it and move to the other side of the membrane in the final time step. With this definition, we assume our transition paths will have, in general, different time lengths, in opposite way as it happens in Monte Carlo TPS [18,19]. In addition, molecular dynamics TPS is well defined if some dynamic constraints are imposed [22]: total linear momentum and total angular momentum of the system have to be zero. We introduced these two constraints in our simulations, together with the control of the total energy conservation.

Defining the Z axis as the interface-interface direction, we used the transversal z coordinate of the lipid head as our mechanical order parameter able to distinguish between stable states. Nevertheless, system fluctuations that drive to flip-flop events are not uniquely concerned with the motion of a lipid chain alone but they require the participation of many molecules, as it will be shown below. This means that the true reaction coordinate of the transition dynamics is a collective variable concerning the motion of a large group of molecules, but it is in general unknown. This makes the calculation of free energy profiles very difficult, although some information about contributions to the free energy along the Z direction can be derived. The TPS method works without the requirement of the specification of the reaction coordinate: we only need to identify a pair of stable states of the system, namely a flip-flop transition and to construct an initial transition path. This path is a Newtonian trajectory and it can be sampled to produce a full ensemble of transition pathways. Once a path is altered, the subsequent dynamical evolution by MD indicates whether or not the system reaches both stable states. In the cases when a generated trajectory is able to reach both endpoints, such trajectory is considered a new transition path. Let us describe the two most usual generating procedures with more detail.

In TPS methodologies, two main tools are employed to sample the initial trajectory: the *shooting* and *shifting* procedures [16] are able to produce a number of trajectories or transition paths which start at the first stable state (lipid head in one interface) and end in the second stable state (lipid head in the second interface). A shooting move consists in the generation of a new trial trajectory from a given one. In a random time step of an equilibrated path, momenta of each particle are slightly modified, say by a small random amount δp. Integration of the equations of motion backward to time 0 and forward to time t, starting from the modified state yields a new trajectory. If the trajectory connects the two stable states, it will be accepted with nonvanishing probability. Otherwise it will be rejected.

A shifting move can be defined forward or backward in time. In a shifting move, a trial trajectory is obtained by deleting a segment of length δt from the beginning (forward move) or the end (backward move) of an existing path. New trajectory segments of length δt are grown by deterministic dynamics generating a new trial path. The condition of stable states is checked for the new path, which is accepted if the boundary conditions are fulfilled.

B. Transition state ensemble

The characterization of the TS of the system is one of the basic objectives of TPS. Transition states are located by an equal probability criterion: a configuration $\{\mathbf{r}(t_{TS}),\mathbf{p}(t_{TS})\}$ of a given path is considered to be a TS of the system if trial trajectories starting at the tagged time t_{TS} have a probability of 0.5 to reach each stable state [23]. In the present work, for instance, we used 80 trial paths per time step. The generation of trial trajectories is done again by shooting and shifting, exploring all time steps of each member of the transition path ensemble. If a particular t_{TS} is located, a member of a transition state ensemble (TSE) is found, i.e., a given configuration is considered a member of the TSE when the number of trial paths starting at such configuration and ending in each stable state is exactly the same. Hence, the TSE is a subset of the transition path ensemble because, due to the limited number of trial paths employed, the TS of a given path is not always found. This kind of calculation is computationally expensive since we need to construct auxiliary trajectories for each time slice of each path and to count the hits of such trajectories on the stable state regions. The last step in the calculation consists in the alignment of all TS, which are given the time label "0".

III. SIMULATION METHODS

The binary system that we have simulated consists of a mixture of solvent model water and amphiphilic molecule-like particles. We have not considered other species which in real systems could act as catalysts of the flip-flop transition. Amphiphilic species have been modeled with hydrophilic head groups and hydrophobic tail groups, the latter being composed of four particles with all lipid components interacting through harmonic springs,

$$V_{t-t}(r_{ij}) = K(r_{ij}-\sigma)^2,$$

where $K=500\ \varepsilon/\sigma^2$, the characteristic radius is $\sigma=1/3$ nm, the potential depth is $N_{Av}\varepsilon=2$ kJ/mol (N_{Av} being Avogadro's number), and r_{ij} is the distance between the particles i and j. The mass of all particles is the same: $m=0.036/N_{Av}$. Energy scaling of $k_BT=1.24\ \varepsilon$, $1.5\ \varepsilon$, and $2\ \varepsilon$ corresponding to 298, 360, and 480 K, respectively, have been also considered. Water molecules are represented by single particles. Water-water, head-head, and head-water interactions are of the Lennard-Jones type [24]

$$V(r_{ij}) = 4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right],$$

with the same meaning for σ, ε, and r_{ij} as above. Finally, in order to produce the different types of interactions, we truncated and shifted the Lennard-Jones forces, with cutoff distances of $R_c=2.5\sigma$ for lipid-lipid and water-water interactions and $R_c=2^{1/2}\sigma$ for lipid-water, making the hydrophobic tail-water force to be soft-core repulsive:

$$V_{t-w}(r_{ij}) = 4\varepsilon\left(\frac{\sigma}{r_{ij}}\right)^{9}.$$

In addition, a simple treatment of the long-ranged interactions (no charged particles are considered) is forced by the computationally expensive calculations we are interested to do in this work. The reliability of such a coarse-grained model is of course limited by its simplicity, although we will see below how several experimental quantities are fairly reproduced, at least at the qualitative level.

Starting from an initial mixture of amphiphiles and solvent particles, the self-assembly process of a group of amphiphilic molecules towards a cylindrical micelle and from the latter to a bilayer membrane lasts about 10^6 fs. This is in good agreement with the results of Goetz *et al.* [10]. We have used a molecular dynamics time step of $\Delta t=8.4$ fs. Our system was composed by 980 particles: 76 surfactants formed by one head and four tail molecules and 600 solvent water molecules. This system has been placed in a rectangular box of $9.9329\ \sigma\times 9.9329\ \sigma\times 14.1898\ \sigma$. All simulations were performed using a leapfrog Verlet algorithm at the three temperatures indicated above and periodic boundary conditions were adopted. We harvested more than 200 transition pathways at each temperature in order to ensure meaningful statistics.

TABLE I. Some dynamical characteristics of the membranes. Diffusion coefficients (D_{XY}, D_Z) of lipid head groups, flip-flop rates (R_{ff}), and time lengths of the transition paths (t_{TP}). T_r is a reduced temperature defined by $T_r=240.54$ K.

T/T_r	$D_{XY}(\mathrm{cm}^2/\mathrm{s})$	$D_Z(\mathrm{cm}^2/\mathrm{s})$	R_{ff}	t_{TP}(ps)
1.24	0.6×10^{-5}	2.5×10^{-7}	0.017	0.31
1.5	1.2×10^{-5}	3.4×10^{-7}	0.057	0.11
2	3.8×10^{-5}	7.7×10^{-7}	0.087	0.09

IV. RESULTS AND DISCUSSION

In real life, the observation of lipid transitions in biomembranes is very rare. So, for instance, the pass of a stearic acid across a phospholipid bilayer membrane [25] happens only once every 34 s. This time has to be associated with the mean time required to observe a flip-flop transition in the laboratory. The TPS methodology employed in this work allows the generation of flip-flop events in MD simulations with a reasonable waste of computational time. This is important due to the low rate of flip-flop realizations in ordinary MD calculations, since the lipid motion is basically produced along one of the interfaces [8], i.e., it is of the lateral type. Lateral (XY) and transversal (Z) diffusion coefficients of lipid head groups are reported in Table I together with

flip-flop rates and time lengths of the transition paths. The diffusive behavior of lipids has been studied by evaluating the averaged mean square displacements

$$D = \lim_{t\to\infty} \frac{1}{6t} \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle$$

for the traced lateral (XY) and transversal (Z) projected motions of each particle class. The flip-flop rate R_{ff} has been defined as the number of flip-flop transitions divided by the number of visits of a given lipid head to the intermediate region between the two interfaces. The length of the transition paths has been computed as the averaged number of time slices of the paths that are members of the transition path ensemble, multiplied by the value of our simulation time step.

We obtained the lateral and transversal diffusion coefficients of lipid head groups, D_{XY} and D_{Z}. Our results are consistent with computer simulation data at ambient ($T = 1.24\ T_{\mathrm{r}}$) and higher temperature conditions [8]. From the experimental side [5], a value close to 10^{-8} cm^2/s was reported for phosphatidychlorine, which cannot be directly associated with the simple lipid model we use in this work. In that case, diffusion was markedly smaller than our result due to the fact that phosphatidychlorine has a much heavier and extensive lipid structure, leading to a small diffusion coefficient. When temperature rises, we observe the increase of both diffusion coefficients at the two high temperatures. In all cases, lateral diffusion is markedly larger than the transversal one, as expected. This suggests a more important amount of flip-flop realizations as temperature increases. That point is confirmed by the flip-flop rate R_{ff}, which remarkably increases with temperature, being at 480 K about five times bigger than at room temperature conditions. Accordingly, temperature plays also a role concerning the length of the transition paths, i.e., the parameter which gives a gross measure of the time required by a lipid to shift interfaces, once this lipid starts that flip-flop process. The reader should note that this estimation of time cannot be compared neither the experimental nor the simulated ones, when standard MD simulations are considered. However, we observe that the rate of flip-flop events in lipid dynamics is indeed extremely low, compared with the number of times a lipid head group enters the intermediate region. In addition, an Arrhenius-type dependence is observed for t_{TP}, in a similar way as it was suggested by Imparato *et al.* [8]:

$$t_{\mathrm{TP}} \simeq \exp\left\{ -\frac{\Delta E}{k_{\mathrm{B}} T} \right\},$$

where ΔE is the flip-flop activation energy, k_{B} is the Boltzmann constant, and T is the temperature. This activation energy can be estimated to be of the order of 10 kJ/mol, as it will be shown below.

A. Structure

The basic structure of the lipid bilayer can be explored by means of density profiles for each of the molecular species, i.e., water molecules, lipid head groups, and lipid tail groups.

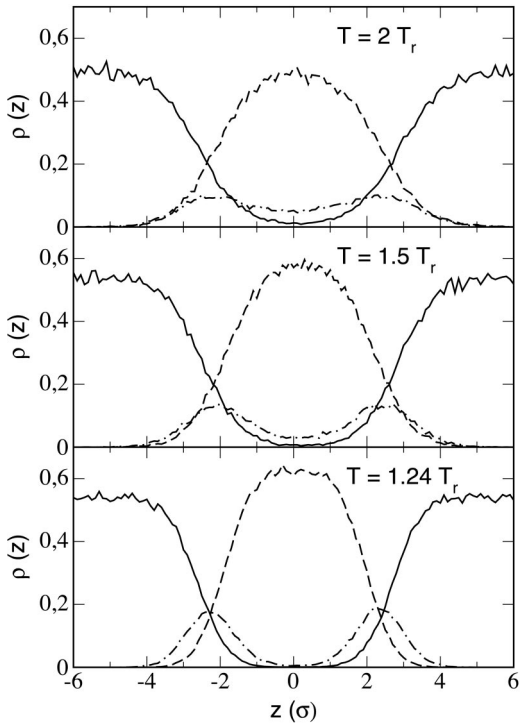

FIG. 2. Density profiles of water and lipid species in equilibrium. Water molecules (full lines), surfactant heads (dot-dashed lines), and surfactant tails (dashed lines). The center of the simulation box is at $z=0$. $T_{\mathrm{r}}=240.54$ K.

We report such profiles for each type of molecules for equilibrium configurations in Fig. 2 and for transition state configurations in Fig. 3.

We can observe that lipid head groups are symmetrically distributed around the center of the box ($z=0$). The maximum around 2.3 σ corresponds to the position of the interfaces. Lipid tail groups are located around the intermediate region, with positions spreading up to 3 σ. As expected, solvent water molecules are located at both sides of the box. As temperature grows, the most remarkable changes are found in the density profiles of head and tail groups. Both $\rho(z)$ suffer a broadening indicating some loss of the localization shown at ambient conditions. This can be regarded as a tendency of the head groups to enter the intermediate region and of the tail groups to move closer to the interfaces. This is consistent with the fact that diffusion coefficients and the flip-flop rates are higher than those at room temperature conditions.

In the TS we can see some evidence of a collective reorganization, at least at short range. Compared to the equilibrium profiles of Fig. 2 we observe that, at ambient conditions, head groups show a tendency to spread up both towards the aqueous environment and also to the central ($z=0$) region, while tail groups remain mostly around the central region although some of them could try to access the interfaces. Water positions show some fluctuations near the interfaces, with a small probability to cross them. This fact could be a signature of the migration of a water molecule across the membrane [26]. In such a case, the migrating water molecule would temporarily fill the additional space open

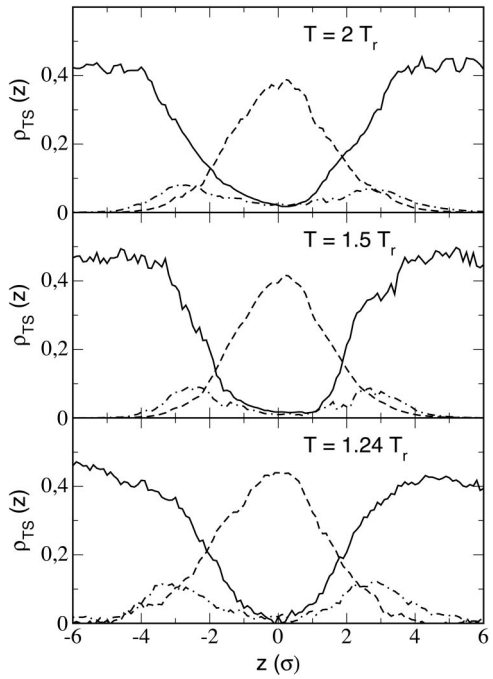

FIG. 3. Density profiles of water and lipid species for TS configurations. Water molecules (full lines), surfactant heads (dot-dashed lines), and surfactant tails (dashed lines). The center of the simulation box is at $z=0$. $T_r=240.54$ K.

by the flipping surfactant during its transition. The effect of temperature is again worth to be investigated. In a similar fashion to the case of equilibrium configurations, temperature increasing produces some broadening of the distributions of head and tail groups. Then we observe that at high temperatures flip-flop transitions are favored since the presence of lipid head groups in the intermediate region is clearly larger than at lower temperatures.

The picture of the flip-flop process can be also visualized with a series of snapshots [27] (Figs. 4 and 5). We display, only at room temperature conditions, all lipid molecules but a tagged one (which performs a flop-flop transition) in Fig. 4 and the tagged lipid plus surrounding water molecules in Fig.

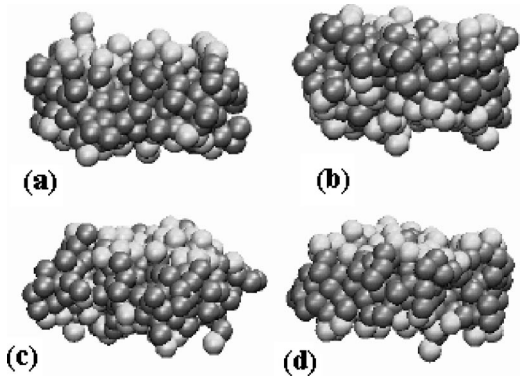

FIG. 4. Transition state event in a lipid bilayer membrane at $T=1.24\,T_r$. Lipid head groups (light gray), lipid tail groups (dark gray). (a) First stable state, (b) intermediate state, (c) transition state, and (d) second stable state. The illustrated snapshots are separated by 84 fs. Surrounding water molecules are not shown.

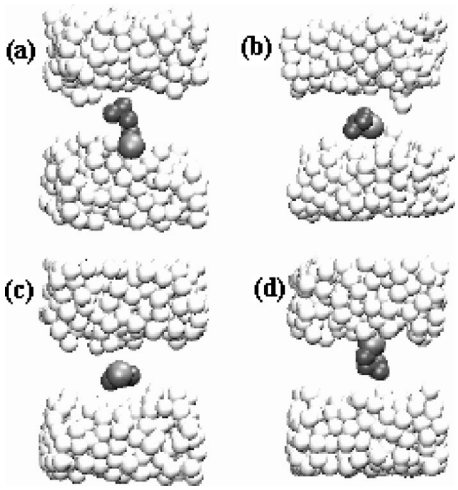

FIG. 5. Transition state event for a tagged lipid molecule at $T=1.24T_r$. Lipid head (light gray), lipid tail (dark gray), and surrounding water molecules (white). The remaining lipids are not shown. (a) First stable state, (b) intermediate state, (c) transition state, and (d) second stable state. Snapshots are separated by 84 fs.

5. The relevant information from these pictures has to be charged, in our opinion, to the gross features observed. At the stable states, lipid head groups are mostly located around $z=2.3\sigma$, although in intermediate and in the TS snapshots the degree of "disorder" is neatly larger than in the stable states: in the intermediate and TS configurations we observe a tendency of the lipid heads to penetrate the central ($z=0$) area, where a few water molecules are observed (Fig. 4).

We observe from Fig. 5 that in the first and last time steps of the transition path the tagged lipid has the head embedded in the interfaces, as expected. In the TS configuration, the whole tagged lipid is fully inside the intermediate region. The dynamic sequence reveals the motion performed by the flipping molecule, which is forced to roll up itself, in order to minimize the friction with the rest of the lipids. The calculation of the potential energy transfer [15] along the flip-flop event allows us to make an estimation of the time required to cross the transition state, which is about 0.1 ps. An averaged computation based on the transition state ensemble of trajectories produced a value of 0.137 ± 0.004 ps.

B. Free energy profile

In a previous work [15] we estimated the height of the free energy barrier associated with the flip-flop process using the density profile in equilibrium (Fig. 2) at ambient conditions. Here we include a comparison of the free energy barrier as a function of temperature. The free energy profile $w(z)$ has been obtained as the reversible work associated with the molecular displacements along the Z axis:

$$w(z) = -k_B T \ln \rho(z).$$

Assuming that this is not the full Helmholtz free energy $w(r)=-k_BT\ln\rho(r)$ of the flip-flop process but the contribution of transversal motions to $w(r)$, we can employ this result to estimate the energetic cost required by the system in order

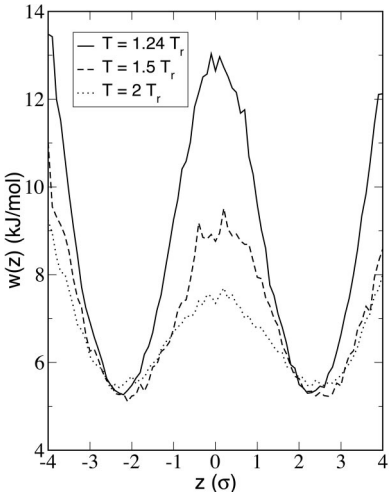

FIG. 6. Free energy profiles of the lipid-water ensemble. The center of the simulation box is at z=0. T_r=240.54 K.

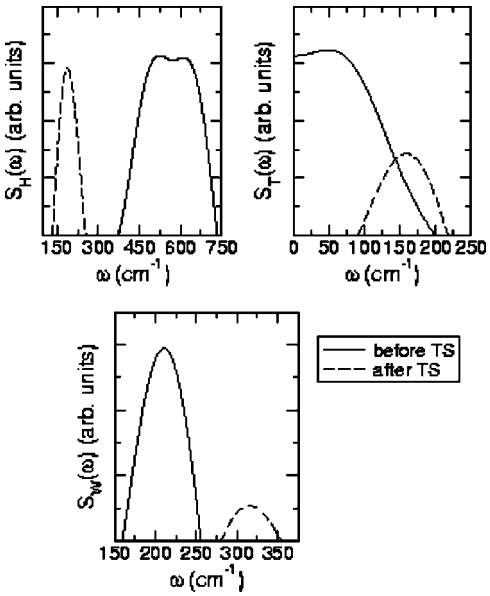

FIG. 7. Spectral densities of lipid species $S_H(\omega)$, $S_T(\omega)$, and water $S_W(\omega)$ at $T=1.24\ T_r$. Solid lines correspond to configurations started at the transition state and computed backward in time. Dashed lines are those of configurations started at the transition state and computed forward in time.

to allow a lipid flip-flop. The results at the three temperatures considered in the present paper are reported in Fig. 6.

We see that the energy barrier spreads up roughly in between the two interfaces. However, the main finding concerns the height of the energy barrier, which is lower for increasing temperature. Then the energetic cost to produce a flip-flop transition is lower at 2 T_r (about 2.5 kJ/mol) than at 1.5 T_r(4 kJ/mol) and the latter is again lower than at ambient conditions $T=1.24\ T_r$(7.5 kJ/mol). This reinforces the idea outlined above, i.e., flip-flop transitions are more likely to happen as temperature rises, at least in the temperature range explored and in the framework of the membrane model employed. Since we observe an Arrhenius-like behavior of the time required for a flip-flop as a function of temperature, we can say that flip flops are activated processes of the order of 10 kJ/mol at ambient conditions.

C. Spectroscopy

Further information on the dynamics of the system around the transition state can be obtained from spectral densities $S(\omega)$ associated with the velocity autocorrelation functions $C_v(t)$ for each particle, i.e., water, head, and tail groups, assuming that in the case of lipid tails we have considered the velocity of its center of mass. The spectral densities have been obtained [28] as the Fourier transforms of $C_v(t)$

$$S(\omega) = \int_0^\infty dt\ \cos\ \omega t\ C_\nu(t), \text{where} \quad C_\nu(t) \equiv \frac{\langle \vec{v}(t) \cdot \vec{\nu}(0) \rangle}{\langle \vec{\nu}^2(0) \rangle}$$

and they are shown in Fig. 7. To illustrate the method, we only considered ambient conditions. We computed $S(\omega)$ for lipid head groups, for the center of mass of the tail groups, and for solvating water molecules, before and after the transition state, i.e., the velocity autocorrelation functions are calculated starting at the transition state time step and evolving forward and backward in time. The meaningfulness of the computation of backward velocities is ensured by the reversibility of Newtonian classical dynamics.

First, we can observe that the typical hindered translations of water experimentally detected [29] around 180 cm^{-1} at 298 K are found around such value before the transition state is crossed, but once the bottleneck has been surmounted, a blue shift of such translational frequency is observed. This effect could be charged to the reorganization of water molecules in the interfaces once the lipid chain has been exchanged. From $S_H(\omega)$ and $S_T(\omega)$ we can see that lipids behave in a way rather different to water. Lipid heads move very quickly before the transition state is reached. However, when the barrier is surpassed they slow down their activity and vibrate at lower frequencies. Simultaneously, tail groups move slowly before the bottleneck is crossed, increasing their activity to almost equal the frequency motion of the head groups once the transition state has been surmounted. We think these frequency variations could be detected in appropriate Raman and infrared scattering experiments. A possible physical reason of such changes could be due to the different nature of all classes of particles involved. For instance, head and tail groups are particles that interact by a soft-core repulsive potential. For a successful transition, once a flipping head group particle leaves the first interface it enters the tail-group region, where repulsive interactions force it to move quickly. After the TS is crossed, the head group is significantly close to the second interface (see Fig. 5) and it moves slower than before, as it can be observed from velocity profiles [15]. Tail groups are affected by different interactions than those of head groups and this fact is reflected in the different character of the spectral shifts reported. Finally, we would like to point out that water molecules have a chance to enter the central region and, when TS occurs, the migration of a water unit has to be necessarily fast, due to the hydrophobic character of the tail groups.

V. CONCLUDING REMARKS

We have shown that molecular dynamics TPS is an adequate procedure to study the flip-flop dynamics of a lipid model molecule across a flexible bilayer membrane. Computer simulations using a simple coarse-grained model have produced structural and dynamic data in good overall agreement with other computational and experimental results at ambient conditions. A brief summary of the main findings is as follows: transversal diffusion is of the order of $10^{-7}\mathrm{cm}^2/\mathrm{s}$ at 298 K and it rises about one order of magnitude at 480 K. The structure of the membrane is dominated by temperature changes: we get distributions of head and tail groups at high temperatures significantly broader than those at room temperature. From the structural and dynamical data collected, we observed that flip-flops are more likely to occur for increasing temperatures.

The reversible work necessary to perform a flip-flop motion is estimated to be of about 7.5 kJ/mol at room temperature and it diminishes to about 2.5 kJ/mol at 480 K. The analysis of reactive trajectories has revealed a concerted motion of most of the particles of the system in order to produce the exchange of a lipid chain between the two sides of the membrane. In addition, flip-flop transitions have been observed to be activated processes with an energy barrier of the order of 10 kJ/mol, in a similar fashion as it was reported by Imparato *et al.* [8].

During a flip-flop transition, the frequency of intermolecular vibrational motions increases for the solvent water molecules and the lipid tail groups, although lipid head groups show a tendency to reduce their activity once the bottleneck has been surpassed by one of them. In essence this collective mechanism, which induces a lipid to perform a flip-flop transition is a process that can be related with the extremely fast flip-flop of fatty acids across phospholipid bilayers [6] and across human cells [7]. It has been experimentally observed that such processes do not require the participation of protein-based mechanisms, in the same fashion we assumed in the present work where we have not considered catalyst molecules.

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