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A molecular dynamics transition path sampling study of model lipid bilayer membranes in aqueous environments

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Abstract

The transversal motion of lipids across biological membranes or flip–flop in aqueous media is of fundamental importance in understanding chemical reactions produced in cells. It is also an example of a rare event in a biological system. Such a process has been investigated by molecular dynamics transition path sampling, which is a powerful tool designed to deal with rare events in a wide range of systems. The study covers a wide range of temperature conditions and includes the analysis of flip–flop free energies together with a detailed study of vibrational densities of states associated with flip–flop motions.

1. Introduction

Most biological membranes are formed by bilayers of amphiphilic lipid molecules bound to proteins in aqueous-like environments [1–4]. The structure of the bilayer is basically organized with the lipid hydrophilic head-groups in the close vicinity of the surrounding water molecules, forming interfaces, whereas the hydrophobic tail-groups form an internal region in between the interfaces. Lipid bilayer membranes usually perform protrusions (small relative displacements of individual molecules) in such a way that some roughness of the interface is produced, as has been observed both in scattering experiments and molecular dynamics simulations [5]. Despite the increasing amount of both theoretical and experimental work, the structure and, more specifically, dynamics of biomembranes are not yet fully understood. The biggest obstacles for the comprehension of microscopical dynamics are first, the wide variety of timescales relevant to both longitudinal and transversal motions of lipid molecules, and second, the huge number of degrees of freedom involved. Some properties such as elasticity and mobility [6, 7] as well as diffusion of water and amphiphiles [8] have recently been studied by molecular dynamics simulations.

The molecular models employed in most computer simulations of lipid membranes consist of short rigid or flexible amphiphilic chains surrounded by water-like molecules [8]. In other

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words, it is usual to deal with simplified models which are feasible to be treated from the computational point of view, instead of the complex long-chains appearing in real biological systems. A process of fundamental importance concerning the dynamics of biomembranes is the so-called transbilayer lipid migration or flip–flop transition [3]. This consists of the exchange of a given lipid chain between the two interfaces of the system. Flip–flops occur in both natural and model phospholipid membranes. Rapid flip–flop motions have been observed in the human erythrocyte membrane [9] or in photoreceptor disc membranes in retinal rods [10]. Further, the understanding of such processes from the microscopical point of view is of basic importance in the manufacture of synthetic membranes [11].

Flip–flop transitions are challenging phenomena due to the multiplicity of timescales involved in the process and because such motions of phospholipids across membranes are rare events, i.e. transversal transitions are only a tiny fraction of the motions of lipid chains in biomembranes, where the usual motions are related to longitudinal diffusion. The recent computational study of Imperato *et al* [8] has demonstrated that flip–flop motions are extremely rare when coarse-grained models are used. For that reason, it is crucial to employ a specific tool able to select and even generate those flip–flop configurations from an equilibrated sample. The technique employed in the present work, transition path sampling (TPS), has revealed itself to be very successful in dealing with rare events in physical and chemical systems. As a few examples, we can mention water autoionization [12, 13], the aqueous dissociation of sodium chloride [14–17], or the hydrogen-bond breaking process [18]. Detailed reviews and developments of the method can be found in the literature [19–23], and for that reason we will only include an overview adapted to the particular case of flip–flop transitions. Preliminary studies of transbilayer migration at ambient [24] and high temperature conditions [25] have been reported. Here we will focus our attention on the analysis of a wide range of thermodynamic states, including low- and high-temperature conditions, and we will devote special attention to the vibrational motions associated with the system in each case.

2. Computational details

Lipid membrane systems are usually modelled by means of simplified potentials which can be studied from the computational point of view in a reasonable timescale. Our binary system consists of a mixture of solvent model water and amphiphilic lipid-like particles. The amphiphiles have been modelled with single hydrophilic head-groups and hydrophobic tail-groups composed of four centres. The pseudo-particles composing the tails are assumed to internally interact through harmonic springs:

$$V_{\mathrm{tt}}(r_{ij}) = k(r_{ij} - \sigma)^2, \tag{1}$$

where $k = 500\varepsilon/\sigma^2$, the characteristic radius is $\sigma = 1/3$ nm, the potential depth is $N_{\mathrm{Av}}\varepsilon = 2$ kJ mol^{-1}, N_{Av} being Avogadro's number, and r_{ij} the distance between the particles i and j. All particles have a mass $m = 0.036N_{\mathrm{Av}}$. An energy scaling of $k_{\mathrm{B}}T = \alpha\varepsilon$, with k_{B} the Boltzmann constant and $\alpha = 0.5, 1, 1.24, 1.5, 1.75, 2, 2.5$, respectively corresponding to the macroscopic values of 120.27, 240.54, 298.27, 360.81, 420.95, 481.08 and 601.35 K, has been assumed. A reduced temperature $T_{\mathrm{r}} = 240.54$ K has been defined for the case $\alpha = 1$. Water molecules are represented by single particles. Water–water, head–head and head–water interactions are modelled by means of Lennard-Jones potentials [26]:

$$V(r_{ij}) = 4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right]. \tag{2}$$

In order to generate different types of interactions, we truncated and shifted the Lennard-Jones forces, using cut-off distances of $R_c = 2.5\sigma$ for lipid–lipid and water–water interactions and $R_c = 2^{1/2}\sigma$ for lipid–water forces, which produces the tail–water forces to be soft-core repulsive:

$$V_{tw}(r_{ij}) = 4\varepsilon \left(\frac{\sigma}{r_{ij}}\right)^9. \tag{3}$$

Despite its simplicity, the reliability of such a coarse-grained model is remarkable since several experimental quantities can be qualitatively well reproduced. In all simulations we employed a time step of $\Delta t = 8.4$ fs. Our systems were composed of 980 particles: 76 surfactants formed by one head and four tail particles and 600 solvent water-like molecules. The systems were placed in a rectangular box of $9.9329\sigma \times 9.9329\sigma \times 14.1898\sigma$. This means that pressure variations have been large across the full thermodynamical range studied. We used a leap-frog Verlet algorithm and periodic boundary conditions in all cases. In addition, more than 200 transition pathways have been collected at each temperature, in order to have statistically meaningful results.

3. Transition path sampling: basic aspects

In the present study of lipid transitions between interfaces in model biological membranes, we have used a powerful computational tool able to generate a set of flip–flop events, namely TPS. This is particularly important since flip–flops are rare events of lipid dynamics. Let us describe specific details of TPS adapted to the lipid flip–flop process.

The TPS method is based on the generation of a set of transition pathways linking stable states of a physical system in phase space. Each path is represented by a multidimensional vector $\{\vec{r}(t), \vec{p}(t)\}$. If we just consider the configuration space $\{\vec{r}(t)\}$, a simple representation of the method can be described: the two transition paths depicted in figure 1 connect two stable states of the system (A, B). In the particular case of a lipid flip–flop transition, the first stable state (A) corresponds to a given lipid with its head-group placed in one interface of the bilayer, whereas in the second stable state (B) the same lipid has its head-group located in the second interface, after a flip–flop transition has occurred. These stable states are defined as minima of the potential energy surface, indicated in the figure as a grid. Each path connects the two stable states in configurational space. In addition, a time step of a path which crosses a saddle point in the potential energy surface is the time step associated with a transition state (TS) of the flip–flop process.

In order to distinguish between states A and B we employed the transversal z-coordinate of the lipid head as our mechanical order parameter, where the Z-axis is the direction along the interfaces. In our case, transition paths are defined in the following way: in the first time step of the path the head-group of a lipid must be located in one interface (state A); in the second time step the lipid head enters the middle region; later on, the lipid head-group lives in such a zone for several time steps and in the final time step the tagged head-group has left the intermediate region and it is located in the second interface (state B), i.e. the flip–flop transition has been completed. With such a definition the number of time steps of each transition path will, in general, be different.

Once two stable states A and B are located, TPS works by generating a set of transition paths, starting from a given initial Newtonian trajectory. This can be done by means of several procedures, but it has been observed that the most successful ones, from the computational point of view, are the so-called shooting and shifting [23]. These are the methods employed in the present work. 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, the momenta of each particle

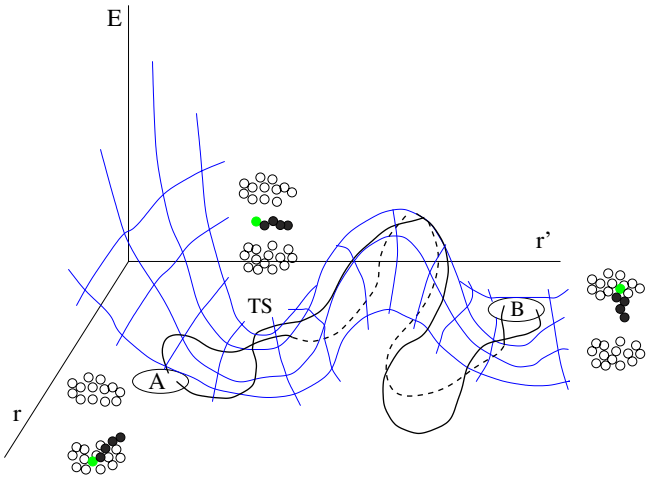

Figure 1. Transition paths (bold curves) between two stable states (A, B). The pathways cross the transition states regions defined as saddle points on the potential energy surface (thin curves). Sketches of configurations corresponding to stable and transition states are included. The tagged lipid performing the flip–flop motion has a black tail and a grey head. The rest of the lipids are not shown. Surrounding water molecules are depicted as white circles.

(This figure is in colour only in the electronic version)

are slightly modified, say by a small random amount $\mathrm{d}p$. Integration of the equations of motion backwards to time 0 and forwards 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 non-vanishing probability. Otherwise it will be rejected. A shifting move can be defined forwards or backwards in time. In a shifting move, a trial trajectory is obtained by deleting a segment of length $\mathrm{d}t$ from the beginning (forwards move) or the end (backwards move) of an existing path. New trajectory segments of length $\mathrm{d}t$ 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. The crucial fact is that, in summary, we can harvest a full bundle of paths which represent an event (flip–flop motion) which is extremely rare in ordinary molecular dynamics (see [8], for instance).

After the set of transition paths is obtained, the search for the time step associated with the transition state of the system is carried out through an equal probability criterion [27]: a configuration $(\vec{r}(t_{\mathrm{ts}}), \vec{p}(t_{\mathrm{ts}}))$ of a given path is considered to represent the TS of the system at time t_{ts} if trial trajectories starting at t_{ts} have a probability of 0.5 to reach each stable state. Here we used 80 trial paths per time step and, with the equal probability criterion, we aligned our paths in order to match the TS at the same time label, namely time label '0'. A detailed analysis of the TS at ambient conditions has been previously reported [24].

4. Results and discussion

In the present work we are basically interested in the study of the energetic and dynamic aspects of transversal motions of lipids in bilayer membranes under temperature changes. In figure 2, the rates of flip–flop transitions and the averaged flip–flop times τ_{ff} are presented. The flip–flop rates have been computed as

$$r_{\mathrm{ff}} = \frac{N_{\mathrm{ff}}}{N_{\mathrm{inter}}}, \tag{4}$$

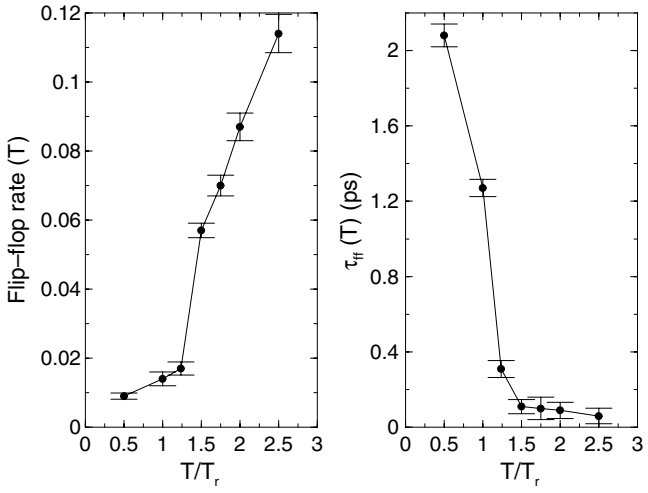

Figure 2. Flip–flop rates and the time elapsed for a flip–flop motion ($\tau_{\rm ff}$) as a function of temperature.

where $N_{\rm ff}$ is the number of flip–flop realizations and $N_{\rm inter}$ is the number of visits to the intermediate region, for a given lipid head-group. Flip–flop times are simply computed as the time length of the transition paths. These are not equivalent to the experimental times observed for flip–flop motions in systems such as stearic acids in phospholipid membranes [28]. However, some flip–flop transitions in natural systems are extremely fast, such as in fatty acids [29] or across human cells [30], like those observed in the present work.

We can observe that both properties show a basically inverse trend: as the temperature increases, the flip–flop rates also increase, whereas the time elapsed for a flip–flop realization rapidly decreases. This can be considered basically as the result of thermal effects leading to the breaking of the bilayer ordering at ambient conditions when high temperatures are considered [25]. In the present case, only a few flip–flop realizations are observed at $0.5T_{\rm r}$. The flip–flop rate slightly increases in a monotonic-like way while the temperature is below room value, whereas an important change in the shape of the flip–flop rate is observed when the temperature is over 298 K. In the change from $1.24T_{\rm r}$ to $1.5T_{\rm r}$ we find that the rate of flip–flop realizations increases by about a factor three. Remarkably, in the same temperature interval, the time elapsed for a flip–flop transition decreases rapidly from more than 2 ps at $0.5T_{\rm r}$ to about 0.3 ps at $1.24T_{\rm r}$. In the change from $1.24T_{\rm r}$ to $1.5T_{\rm r}$ the flip–flop time is reduced by a factor three. In the high-temperature regime ($T \geqslant 1.5T_{\rm r}$), the dependence of both properties on the temperature can be approximated by an Arrhenius behaviour, at least on the basis of the simple lipid model employed here. A similar observation was reported by Imperato *et al* [8]. Interestingly, this trend is not observed for $T < 1.24T_{\rm r}$.

Another dynamic property of especial relevance in the study of biomembranes is the self-diffusion coefficient of lipids. We have obtained lateral $D_{XY}(T)$ and transversal $D_Z(T)$ diffusion coefficients as the long-time slopes of the mean square displacements for the lipid head-groups:

$$D = \lim_{t\to\infty} \frac{1}{6t}\langle|\vec{r}(t) - \vec{r}(0)|^2\rangle. \tag{5}$$

The results are presented in figure 3. As a gross feature, we can observe that $D_{XY}(T)$ is about two orders of magnitude larger than $D_Z(T)$. This is in agreement with results

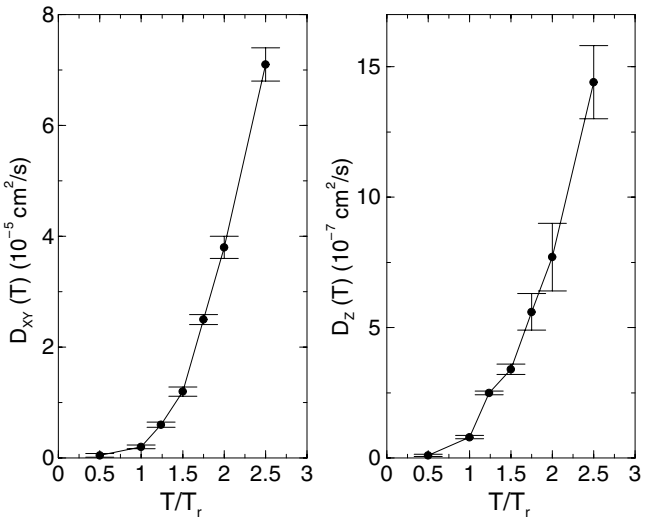

Figure 3. Diffusion coefficients for longitudinal (D_{XY}) and transverse motions (D_Z) as a function of temperature.

from other simulations [8]. Despite the impossibility of a direct comparison of our results with experimental values, due to the simplicity of the model employed here, it should be mentioned that values of about 10^{-8} cm^2 s^{-1} have been found for the transversal diffusion of phosphatidychlorine [31]. Both diffusion coefficients reveal a general trend: their temperature dependence can be modelled as an Arrhenius behaviour. At $0.5T_r$, the diffusion of lipid head-groups is extremely low, in good accordance with the results of the flip–flop rate and time length reported above. Lipid mobility is only important when the room temperature is surpassed, when a remarkable increase is produced for both lateral and transverse diffusion coefficients.

The Helmholtz free energy $w(r)$ can be obtained from equilibrium density profiles in the following way:

$$w(r) = -k_B T \ln \rho(r), \tag{6}$$

where $\rho(r)$ is the equilibrium density profile as a function of a molecular displacement r. In our case, we have obtained the contribution to the free energy due only to transverse motions, i.e. $w(z) = -k_B T \ln \rho(z)$. A detailed discussion of the density profiles $\rho(z)$ in equilibrium and in the TS has been previously reported [24, 25]. Here we display $w(z)$ in a semi-logarithmic plot in figure 4 for several temperatures. The cases of $T = 1.5T_r$ and $2T_r$ were reported in a preliminary communication [25]. A broad free energy barrier appears in all cases but the highest temperature, where only a little residual energy waste is required to move along the centre of the box, due to the big fluctuations of the z-coordinate of the lipids. In that particular case, the energetic cost for a lipid head-group to cross the coordinate $z = 0$ would be of the same order of magnitude as the energy required to move in the vicinity of the two interfaces. Conversely, that barrier appears already at $T = 1.75T_r$ and its height increases gradually until it reaches a very high value for $T = 0.5T_r$, when the cost to cross the intermediate region is about 55 kJ mol^{-1}. This is in good agreement with the fact that, in the case of 'frozen' membranes ($T = 0.5T_r$), the time required for a flip–flop transition is about 20 times longer than the time needed in the case of very 'hot' membranes ($T = 2.5T_r$).

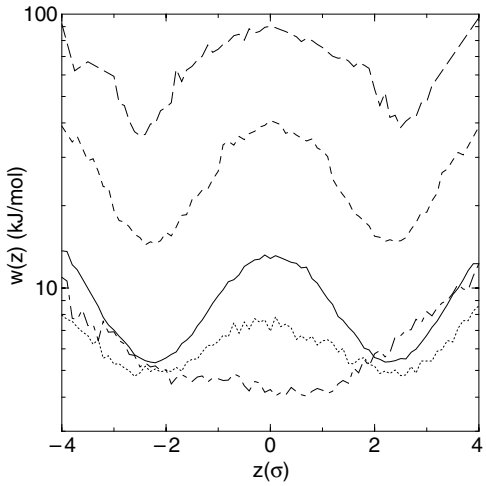

Figure 4. Logarithm of the free energy $w(z)$ as a function of temperature. $T = 0.5T_r$ (long-dashed curve), $T = T_r$ (dashed curve), $T = 1.24T_r$ (full curve), $T = 1.75T_r$ (dotted curve), $T = 2.5T_r$ (dot–dashed curve). The centre of the simulation box is at $z = 0$.

The study of the spectroscopy of biological membranes can be carried out in an approximated way, within the methodology and modelling employed in this work. Spectral densities $S(\omega)$ of lipid species (head-, tail-groups) and water molecules can be computed by Fourier-transforming the molecular velocity correlation functions [25]. It has to be pointed out that the spectral densities obtained are purely classical objects, while the experimental spectra are quantum properties. Nevertheless, the fundamental frequency of a quantum harmonic oscillator and the natural frequency of the corresponding classical counterpart are exactly the same and, for that reason, we will be able to properly compare the positions of the spectral bands with experimental data. Conversely, the widths and heights of such calculated bands will be totally arbitrary.

From the determination of the TS for each transition path, we have computed two different spectral densities, $S(\omega)_{\rm back}$ and $S(\omega)_{\rm forw}$ given by

$$S(\omega) = \int_0^\infty {\rm d}t\ \cos\omega t C(t), \tag{7}$$

where the velocity correlation functions $C(t)$ are defined as:

$$C_{\rm back}(t) = \frac{\langle \vec{v}_{\rm back}(t) \cdot \vec{v}_{\rm back}(0)\rangle}{\langle v^2_{\rm back}(0)\rangle}, \qquad C_{\rm forw}(t) = \frac{\langle \vec{v}_{\rm forw}(t) \cdot \vec{v}_{\rm forw}(0)\rangle}{\langle v^2_{\rm forw}(0)\rangle}. \tag{8}$$

Here $\vec{v}_{\rm back}(t)$ and $\vec{v}_{\rm forw}(t)$ are the molecular velocities backwards (forwards) in time (starting from the TS), respectively. All velocities are those of the centre of mass of all types of particle. This kind of calculation allowed us to distinguish the effect of the flip–flop transition on the microscopic dynamics. We report the spectral densities for water and lipid molecules in figures 5–7. In the case of water (figure 5), the maximum of $S(\omega)$ before the TS is located around 200 wavenumbers, in good agreement with experimental data at 298 K [32]. Once the bottleneck in phase space is crossed, the band suffers a blue-shift of about 100 cm^{-1}. The temperature dependence reveals lower values for the band maxima at low temperatures and a remarkable increase of such a peak in the high-temperature regime for the 'backwards' case, in a similar fashion as occurs in liquid bulk water [33]. After the TS is surpassed, we observe an anomalous red-shift in the $T = 2.5T_r$ case.

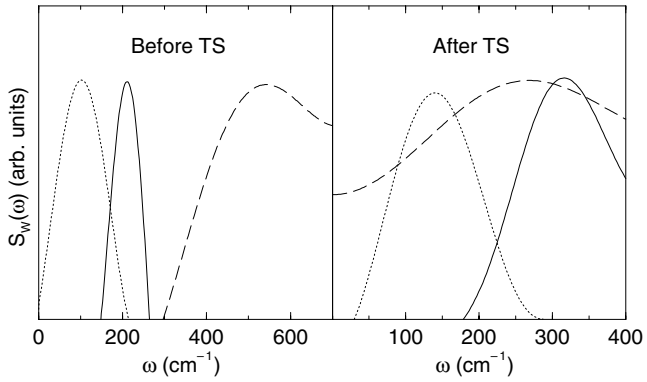

Figure 5. Spectral densities of water molecules as a function of temperature for configurations starting at the transition state and computed backwards in time (left) and configurations starting at the transition state and computed forwards in time (right). $T = 0.5T_{\mathrm{r}}$ (dotted curve), $T = 1.24T_{\mathrm{r}}$ (full curve), $T = 2.5T_{\mathrm{r}}$ (dashed curve).

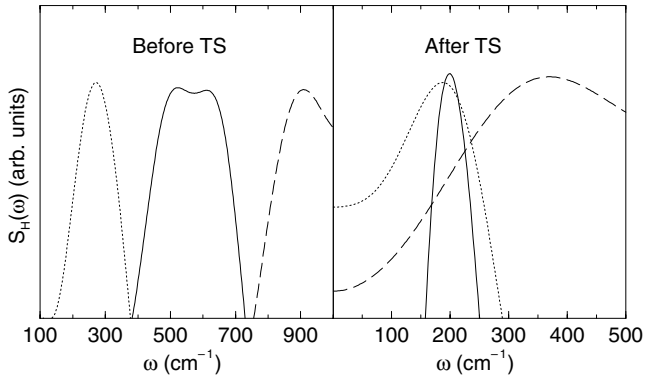

Figure 6. Spectral densities of lipid head-groups as a function of temperature. Configurations starting at the transition state and computed backwards in time (left), configurations starting at the transition state and computed forwards in time (right). $T = 0.5T_{\mathrm{r}}$ (dotted curve), $T = 1.24T_{\mathrm{r}}$ (full curve), $T = 2.5T_{\mathrm{r}}$ (dashed curve).

Spectral densities of the lipid head-groups (figure 6) reveal important differences with temperature changes and remarkable spectral shifts when the TS of the system is surpassed. Before the TS, lipid head-groups vibrate with higher frequency as the temperature increases, from 270 cm^{-1} at $T = 0.5T_{\mathrm{r}}$ to nearly 900 cm^{-1} at $T = 2.5T_{\mathrm{r}}$. Once the TS is surpassed and the flip–flop transition is nearly completed, i.e. in the 'forwards' spectral densities, the maxima of the spectral bands are clearly red-shifted. However, it is interesting to note that both $T = 0.5T_{\mathrm{r}}$ and $1.24T_{\mathrm{r}}$ spectra show a maximum roughly at the same frequency, around 200 wavenumbers. The situation for the spectra of tail-groups (figure 7) shows a relevant difference for both 'backwards' and 'forwards' cases: the maxima of the spectral bands at $T = 0.5T_{\mathrm{r}}$ are located at frequencies larger than those at $T = 1.24T_{\mathrm{r}}$. This reversed behaviour should be due to the fact that, at low-temperature, the lipids move as a whole at any instant of the transition process, whereas at room temperature and high temperature the head-groups show a tendency to vibrate quickly before the TS is crossed and to relax their motion to low frequencies. Meanwhile, tail-groups show a tendency to speed up their vibrational motions

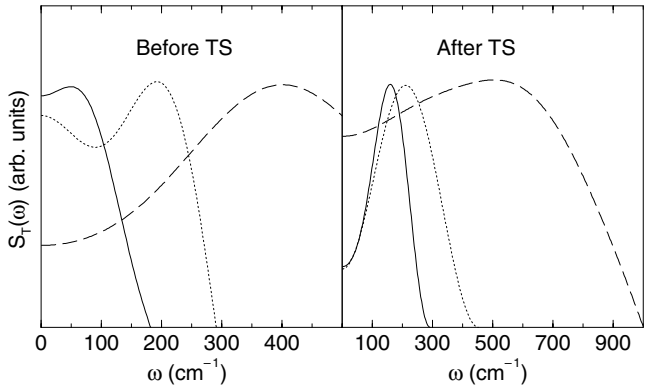

Figure 7. Spectral densities of lipid tail-groups as a function of temperature. Configurations starting at the transition state and computed backwards in time (left), configurations starting at the transition state and computed forwards in time (right). $T = 0.5T_r$ (dotted curve), $T = 1.24T_r$ (full curve), $T = 2.5T_r$ (dashed curve).

after the TS is surmounted. This indicates a non-concerted vibrational behaviour of the lipid species when thermal energies are high, and it is probably related to the structure loss of the whole bilayer membrane at those conditions.

5. Concluding remarks

In the present work, we have reported a molecular dynamics transition path sampling study of the dynamics of biomembranes with the aid of a simplified lipid bilayer model. Despite its simplicity, such a model is able to reproduce the correct order of magnitude of a few experimental values. In particular, the transversal diffusion coefficient of a small lipid chain and the low-frequency maximum of liquid water are qualitatively well described. However, the study of rare events by computer simulations requires the use of specific tools able to explore the small regions of phase space associated with those infrequent phenomena. Here we employed TPS as the enhanced tool capable of sampling a bundle of reactive paths which connect a first stable state of the lipid dynamics, i.e. a lipid head-group embedded in one of the interfaces of the membrane, with a second stable state where the lipid head has been transferred to the second interface, after a flip–flop transition has occurred. The generation of a sufficiently large (from the statistical point of view) set of transition paths has given us the opportunity to analyse detailed aspects of the lipid exchange process. We have considered seven thermodynamic states, ranging from 120.27 to 601.35 K, and studied dynamic and energetic properties.

The change of temperature has revealed itself to be a crucial factor with influence both on the rate and on the time required for the flip–flop transitions. So, a dramatic reduction of the elapsed time for flip–flop in parallel with a big increase of the flip–flop rate are observed for temperatures higher than 298 K. The behaviour concerning diffusion coefficients is nearly an Arrhenius-type dependence, being transverse diffusion (associated with flip–flops) at least one order of magnitude smaller than longitudinal diffusion. Finally, the effect of temperature is also remarkable when spectral densities are considered: the maxima of the low-frequency bands of lipid-like molecules tend to blue-shift about 200–300 cm^{-1} when the temperature is raised from 298 to 601.35 K. At the lowest temperature of 120.27 K, all spectral bands are located at frequencies lower than their high-temperature counterparts, with the exception of the

bands corresponding to the tail-groups of lipids. Interestingly, the main spectral features are sensitive to the dynamic state of the lipid chains: when power spectra are obtained from velocity correlation functions begun at the TS and computed backwards in time, the bands appearing in the spectral densities are clearly located at frequencies different from those observed in spectral densities from correlation functions of velocities begun at the TS and directed forwards in time. This can be summarized as follows: the vibrational motions of lipid and water molecules are both affected by temperature changes and by the occurrence of flip–flop motions and, as such, the spectral shifts observed could be detected in experimental measures of lipid membranes in aqueous environments.

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