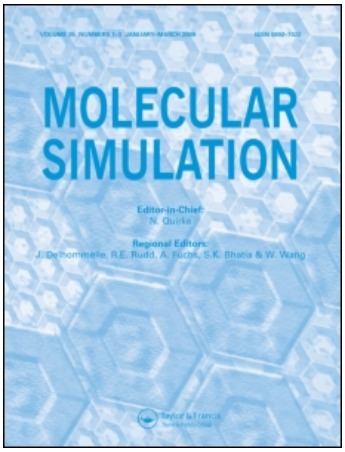

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Membrane Crossing by a Polar Molecule: A Molecular Dynamics Simulation

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MEMBRANE CROSSING BY A POLAR MOLECULE: A MOLECULAR DYNAMICS SIMULATION

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This paper is concerned with the permeability of a glycerolipid bilayer to a polar molecule. We have simulated by molecular dynamics the interaction between a molecule of dimethyl sulfoxide (DMSO) and a membrane of glycerol monoleate in solution. We find that the structure of the membrane is affected only locally by the crossing of the polar molecule. The DMSO molecule opens a hole at the surface of the bilayer which allows a water molecule to penetrate inside. This water is then expelled as the DMSO penetrates more deeply inside. Finally we find that the energetics of crossing is affected by structural transformations occuring on a timescale of 20 ps and over.

KEY WORDS: Numerical simulation, molecular dynamics, lipid bilayer, membrane, permeability.

1 INTRODUCTION

The transport of drugs in the human body is directly related to drug-membrane interactions and the crossing of cellular membranes [1]. To study in a controlled environment this phenomenon many investigations in the past have focused on the transport of small molecules through artificial lipid membranes. Although insights on mass and charge exchange through bilayers have been gained by a variety of experimental techniques, the molecular mechanism for such events is still not completely understood.

In this paper we present a study of a bilayer formed by glycerol monoleate (9-Octadecenoic monoester with 1,2,3-propanetriol) (GMO) in aqueous solution and its interactions with a polar molecule. The choice of an uncharged glycerolipids such as GMO avoids technical complication associated with the presence of counterions, while the presence of a single-chain allows a faster relaxation than in double-chain lipids. The static and the dynamic properties of a GMO membrane were recently investigated by M.A. Wilson and A. Pohorille [2]. The main objective of this paper is to address the problem of transfer of a small polar molecule of dimethyl sulfoxide (DMSO) through

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the model membrane and investigate the molecular mechanism involved with this transfer by molecular dynamics (MD) simulation. We have studied the structural changes occurring in a GMO bilayer when a DMSO molecule is transferred across by carrying out a series of simulations in which the distance between molecule and membrane was constrained. Anticipating our results, we find that the GMO bilayer maintains its structural integrity during the transfer of the DMSO molecule. On average one water molecule penetrates the membrane bound to DMSO and escapes only when DMSO reaches 10 Å inside the membrane. Additionally, we reveal the existence of structural transformations occuring on a timescale in the order of 10–20 ps.

The paper is organized as follows: In Section 2 the potential model and the simulation technique are discussed. In Section 3 we give details on the reaction coordinate and the technique used to constrain it. The simulation results are presented in Section 4. The paper ends with a discussion.

2 MODEL AND SIMULATION TECHNIQUE

2.1 System and Potential

We have studied a model membrane composed of two monolayers of 36 GMO molecules solvated on each side by 1152 water molecules which correspond roughly to eight layers of water. The dimensions of the simulation box are 36.94 Å × 36.94 Å × 150 Å. The direction of the z-axis corresponds to the normal to the plane of the bilayer. The thickness of each layer of GMO and water is respectively 12 Å and 16.5 Å on average. The corresponding vacuum layer of approximately 46.5 Å among the water lamellae prevents the water molecules of different lamellae from interacting with each other. This ensures that at least in the z-direction the pressure was low and that fluctuations along the same direction are not hindered by periodic boundary effects.

Wilson and Pohorille have developed potential parameters for GMO [2] from the OLPS force field of Jorgensen and co-workers [3] and an *ab initio* calculation on glycerol and ethylene glycol. In their model potential the molecule is flexible only in the torsion angles, while angle bendings and bond stretching are kept rigid by holonomic constraints. In order to improve the computational efficiency of the simulation, we replaced angle bending constraints with a harmonic bending potential and the constraint on the planarity of the carbonyl group with a harmonic improper torsion term. Thus, we added the following two terms to the potential energy function of Ref. [2]:

$$\frac{1}{2}\sum_{\text{bendings}} K_\theta(\theta-\theta_0)^2+\frac{1}{2}\sum_{\text{imp. tors.}} K_\phi(\phi-\phi_0)^2.$$

In Table 1 and 2 we use the atom numbering of Figure 1 to give the corresponding extra potential parameters for GMO. For water and DMSO we have used respectively the TIP4P model [4] and a simple point charge model developed recently by Luzar *et al.*

Table 1 Angle bending potential parameters for GMO.

Atom 1	*Atom 2*	*Atom 3*	θ_0	K_θ/kJ mol^{-1} deg^{-1}
C(sp^3)	C(sp^3)	C(sp^3)	112.50	209.20
C(sp^3)	C(sp^3)	C(sp^2)	112.90	188.28
C(sp^3)	C(sp^2)	C(sp^2)	122.00	251.04
C(sp^3)	C(sp^3)	C(carbonyl)	109.47	334.72
C(sp^3)	C(carbonyl)	O(carbonyl)	122.60	276.14
C(sp^3)	C(carbonyl)	O(ester)	115.00	251.04
C(carbonyl)	O(ester)	C(sp^3)	120.50	194.56
O(carbonyl)	C(carbonyl)	O(ester)	124.00	338.90
O(ester)	C(sp^3)	CH(sp^2)	109.47	334.72
C(sp^3)	C(sp^2)	O(alcohol)	109.47	334.72
C(sp^3)	C(sp^2)	C(sp^3)	112.50	209.20
C(sp^2)	O(alcohol)	H	110.00	221.75
C(sp^2)	C(sp^3)	O(alcohol)	109.47	334.72
C(sp^3)	O(alcohol)	H	110.00	221.75

Table 2 Improper torsion potential parameters for GMO.

Atom 1	*Atom 2*	*Atom 3*	*Atom 4*	ϕ_0	K_ϕ/kJ mol^{-1} deg^{-1}
C(sp^3)	C(carbonyl)	O(carbonyl)	O(ester)	0.00	615.05

[5]. Of the proposed sets in Ref. [5] we have used set P2. Both molecules are kept rigid by holonomic constraints.

2.2 *Molecular Dynamics*

Molecular dynamics simulations were performed in the microcanonical ensemble at an average temperature of about 300 K utilizing the ORAC program [6]. Periodic boundary conditions were used throughout the runs. Forces and energies were computed using spherical group cut-off (molecules are subdivided into neutral groups of atoms and the truncation is applied to the distance between the centers of mass of each group) at 8.0 Å and a cubic spline between 7.5 Å and 8.0 Å. The importance of long range electrostatic forces in simulations of lipid membranes is still to be investigated. However, since the GMO molecule is not charged, Coulomb effects are likely to be less important than in phospholipids. The *Verlet* algorithm [7] was used to integrate the equations of motion. A timestep of 3 fs guaranteed a good conservation of

$$\overset{1}{C}H_3-(CH_2)_7-\overset{9}{C}H=\overset{10}{C}H-(CH_2)_7-(\overset{18}{C}=\overset{19}{O})-\overset{20}{O}-(\overset{21}{C}H_2)-(\overset{22}{C}H-\overset{23}{O}-\overset{24}{H})-(\overset{25}{C}H_2-\overset{26}{O}-\overset{27}{H})$$

Figure 1 The GMO molecule. Hydrocarbon groups of the tail region are numbered 1–16. The ester group (17–21) and alcohol groups (22–24 and 25–27) compose the head region. The carbonyl oxygen which is used to define the water-bilayer interface is numbered 19.

the total energy with a fluctuations of the total energy relative to the fluctuation of potential energy in the order of 10^{-5}. The SHAKE algorithm [8] with a tolerance of 10^{-7} Å was used to take care of the bond constraints. By using angle bendings and improper torsions instead of constraints we were able to reduce the computer time used by the SHAKE routine by a factor five [9].

3 SIMULATION

The projection on the z axis of the distance between the DMSO sulphur and the center of mass of the carbonyl oxygens near the closest water-bilayer interface was chosen as the reaction coordinate, ξ. This reaction coordinate takes positive values when the DMSO molecule is in the water region and negative values when the molecule is inside the bilayer. A precise location of the water-bilayer interfaces is not unique. Wilson *et al.* [2], for instance, defined it as the equimolar surface of water, namely the surfaces where the density on the bilayer side is balanced by the depletion of density on the water side, but other definitions can be used. Fortunately, the exact definition of *reaction coordinate* is not very important as long as it identifies a reaction path for the crossing of the DMSO molecule through the bilayer.

Since the spontaneous bilayer crossing of a DMSO molecule is a rare event, in our simulations we constrained the system to a series of different values of ξ. Thus, we observed the structural changes in the lipid membrane when the molecule was dragged across the membrane. With this technique the free energy associated with the crossing can in principle be calculated [10].

The starting point for our runs was an equilibrated configuration of the water-bilayer system taken from the simulation of Ref. [2] which was provided to us by Michael A. Wilson and Andrew Pohorille. At first, we carried out 300 ps of additional simulation without DMSO. We verified by calculating torsional angles distribution, next neighbor order parameter and bilayer thickness that the system was only minimally affected by the modification we introduced in the potential field. At a subsequent time, we injected a DMSO molecule from one of the vacuum-water interface into the corresponding water lamella constraining ξ to allow contact between DMSO and the surface water. Then the reaction coordinate was decreased sequentially in steps of 0.5 Å at a time. In order to let the system equilibrate we waited for 30 ps before the next push. The constraint on ξ was satisfied using the SHAKE algorithm. For a few values of the constraint, after having prepared the system as previously described, we ran additional equilibrations of 90 ps and then extracted averages over trajectories from 150 to 300 ps long.

4 RESULTS

4.1 Interior of the Bilayer

A description of the structure of the membrane interior in presence of a DMSO molecule is given by the pair distribution function $g_{dc}(r)$. This distribution function is proportional to the probability of finding a carbon atom of the GMO tail region, (those labeled 1–16 in Fig. 1) at distance r given that the DMSO's oxygen is in the origin. We find that $g_{dc}(r)$ has little dependence on the reaction coordinate. In Figure 2 we show

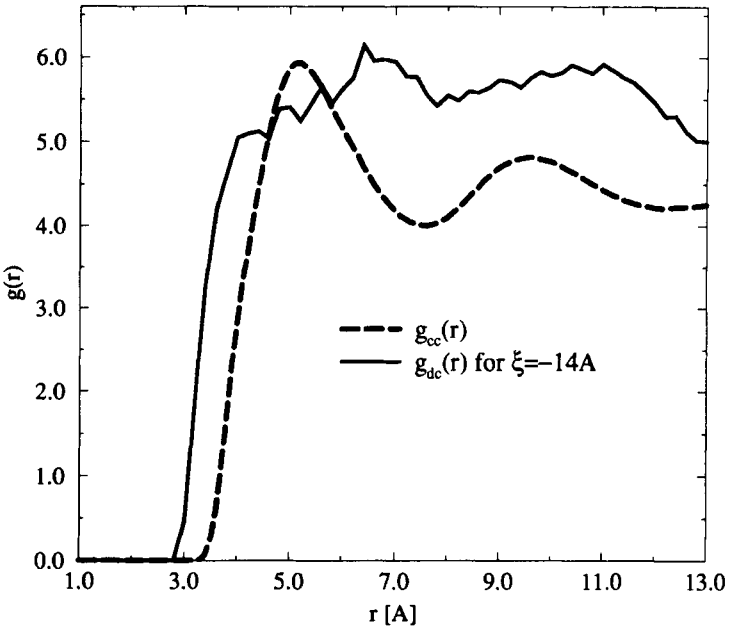

Figure 2 Oxygen (DMSO)-Carbon (GMO tail) and Carbon (GMO tail) pair distribution function (called respectively $g_{dc}(r)$ and $g_{cc}(r)$) when DMSO is inside the membrane and far from the interface ($\xi = -14$ Å). For $\xi = -8$ and -10 Å, we obtain comparable results. The pair distribution function is defined for an homogeneous mixture as $g_{\alpha\beta}(r) = V\langle\sum_i^{N\alpha}\sum_j^{N\beta}\delta(|\mathbf{r} - \mathbf{r}_{ij}|)\rangle/N_\alpha N_\beta$ where N_α, N_β are the number of particles of species α and β respectively and V is the volume of the simulation box.

$g_{dc}(r)$ at $\xi = -14$ Å. In the same figure we present the tail carbon pair distribution function $g_{cc}(r)$. The direct comparison of $g_{cc}(r)$ and $g_{dc}(r)$ shows that while $g_{cc}(r)$ becomes non-zero past 3.5 Å, $g_{dc}(r)$ does the same at 3.0 Å. This suggests that the excluded volume around the DMSO molecule is decreased with respect to the average excluded volume around the tail carbons of GMO. For all the values of ξ we find that g_{dc} does not show a well defined first peak and has a structureless shape beyond it. This implies the absence of any real ordering induced by the presence of DMSO on the tail hydrocarbons.

Furthermore, we find that the orientational disorder in the interior of the bilayer is unaffected by the presence of the small polar molecule and the change in the intramolecular potential we introduced. In Figure 3 we plot the next-neighbor order parameters for $\xi = -10$ Å and compare it with the result of Wilson *et al.* The next-neighbor order parameter is related to the orientational preference of the different amphiphilic chain segments and shows that the orientational order of the hydrocarbon groups increases going from the center of the bilayer through the interface.

4.2 Interaction of DMSO with Water and the Bilayer

The presence of the DMSO molecule in the immediate surroundings or interior of the bilayer represents a small perturbation in the structure of the water-GMO interface.

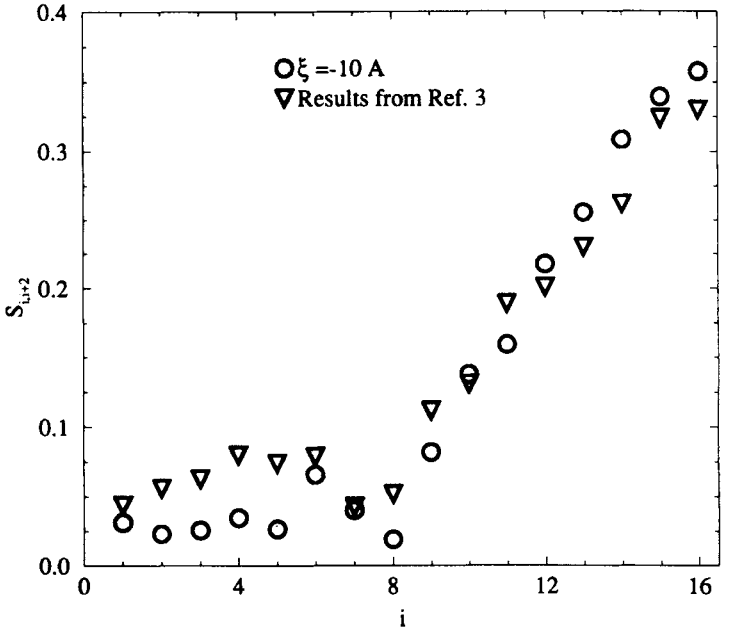

Figure 3 Next-Neighbor Order Parameter for $\xi = -10$ (circles) compared to the same function computed in Ref. [2] (triangles). The next-neighbor order parameter is defined as $S_{i,i+2} = (3\langle\cos^2\theta\rangle - 1)/2$ where θ is the angle formed by the three adjacent hydrocarbon groups $i, i+1, i+2$. The estimated error is around 0.25 units. Our result does not depend on the exact position of the DMSO inside the membrane.

Although there are no visible changes in the density profiles of water and GMO in any of our simulations as compared to Ref. [2], the passage of the polar molecule produces a local effect on the nearby molecules of the bilayer. In particular, we find that a hole is formed in the membrane at the interface with water. In Figure 4 we compare the DMSO oxygen-GMO carbonyl oxygen pair correlation function at $\xi = 0$, $g_{do}(r)$, with the GMO carbonyl oxygen pair distribution function $g_{oo}(r)$. From the position of the first peak of the $g_{do}(r)$, we find an average hole size in the membrane of ~ 4.5 Å which compares with average radius of GMO heads of ~ 4.0 Å.

The hole in the bilayer surface formed at crossing persists until the DMSO is deep inside the hydrophobic region of the bilayer disappearing at ξ smaller than -8 Å. According to Table 3, where we report the average water coordination number of DMSO, water penetrates the membrane during crossing: DMSO remains coordinated by at least a water molecule until deep inside the hydrophobic region ($\xi < -8$ Å). It must be pointed out that during our simulations without DMSO or when DMSO is in the water region, no water molecules are found in the hydrocarbon region of the bilayer. The water molecule coordinated to DMSO is found inside the channel produced by DMSO in the membrane. When DMSO is pushed beyond 8 Å inside the membrane this water molecule is expelled from the bilayer. This finding might be due to an artifact of our nonbonded cutoff at 8 Å. The inclusion of long range interactions using techniques such as Ewald summation should clarify this last point.

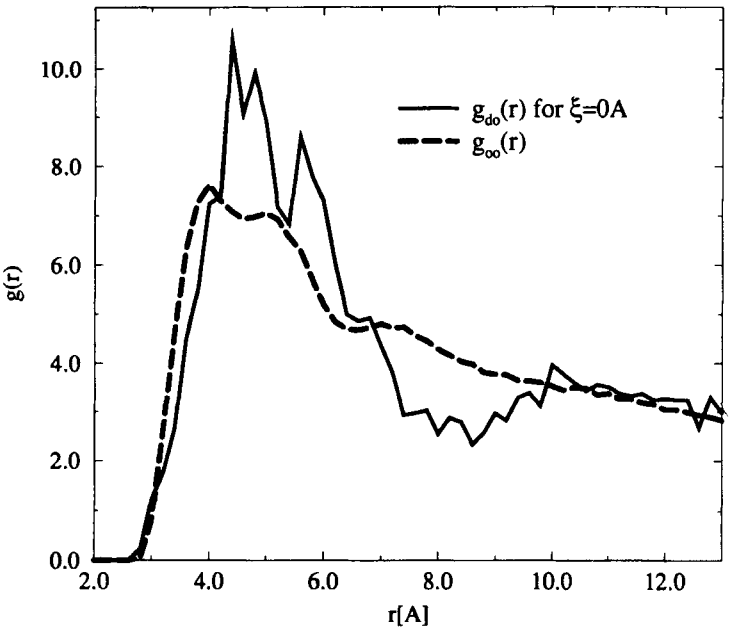

Figure 4 Oxygen (DMSO)-Oxygen (GMO carbonyl) pair distribution function $g_{do}(r)$ when the DMSO is at the interface ($\xi = 0$) and Oxygen (GMO carbonyl) pair distribution function $g_{oo}(r)$.

Table 3 Radius r_c of the first coordination shell and number $n(r_c)$ of water oxygens contained in a sphere of radius r_c around the DMSO oxygen for different values of the constraint ξ. For $\xi = -10$ and $\xi = -14$, during the whole trajectory, no water molecules were found inside the bilayer.

ξ/Å	r_c/Å	$n(r_c)$
6	3.1	2.3
0	3.2	1.8
−4	3.3	1.6
−8	3.6	1.1

4.3 Energetics

For all the values of the constrained reaction coordinate we have calculated the total average potential energy for the DMSO molecule. In Figure 5 we present the mean energy profile as a function of ξ. The calculated total height of the barrier is $\simeq 80$ kJ $\simeq 32 k_B T$ at 300 K. If the entropic contribution is not relevant, spontaneous crossings of the membrane at room temperature are unlikely. This does not agree with experimental evidence that DMSO penetrates very efficiently in membranes. By

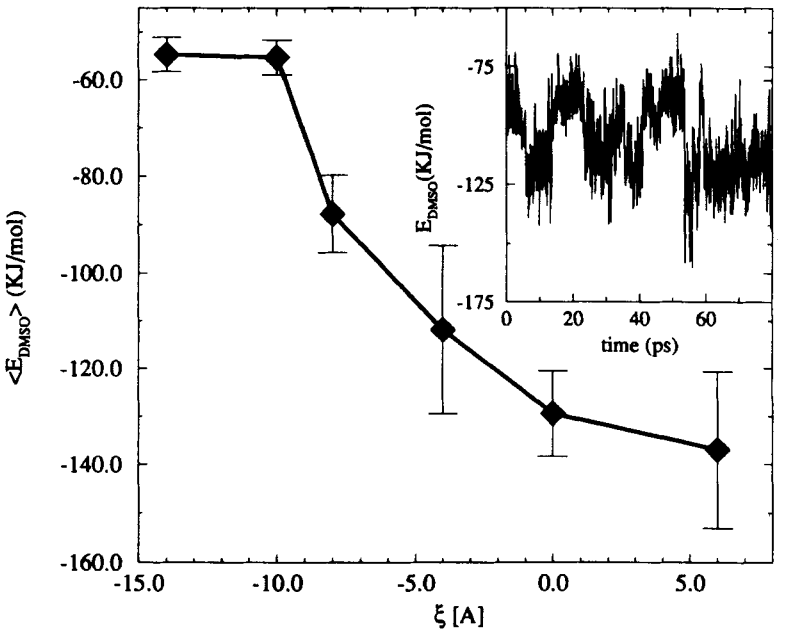

Figure 5 ξ-dependent potential energy as a function of ξ. In the inset: potential energy as a function of time when ξ is constrained at 0 Å.

calculating the mean force acting on the molecule constrained at different values of the reaction coordinate, one could in principle calculate the free energy profile of crossing [10, 11]. Unfortunately, we find this mean force being very sensitive to microscopic rearrangements of the membrane, especially of the polar heads. We find that even after trajectories longer than 300 ps this mean force is far from being converged. This convergence problem is a direct consequence of oscillations in the potential energy which occur in time scales of the order of 20 ps and more. In the inset in Figure 5 we show a typical behavior of the DMO potential energy.

To see how the DMSO is expelled from the bilayer interior once the constraint on the reaction coordinate is released, we performed two more simulations. In Figure 6 we report the trajectory of the reaction coordinate as a function of time. Starting from $\xi = -8$ the DMSO molecule is ejected after about 200 ps. It takes instead 600 ps to expel a DMSO molecule from $\xi = -14$.

5 CONCLUDING REMARKS

In this paper we have studied the diffusion of polar solute such as DMSO in a glycerolipid bilayer. We find that the structural properties of the lipid membrane are not affected by the presence in its interior of a polar solute. In particular, the typical ordering of the interior hydrocarbon chains and the structure of the water membrane interface does not change with respect to simulations without the polar molecule. We

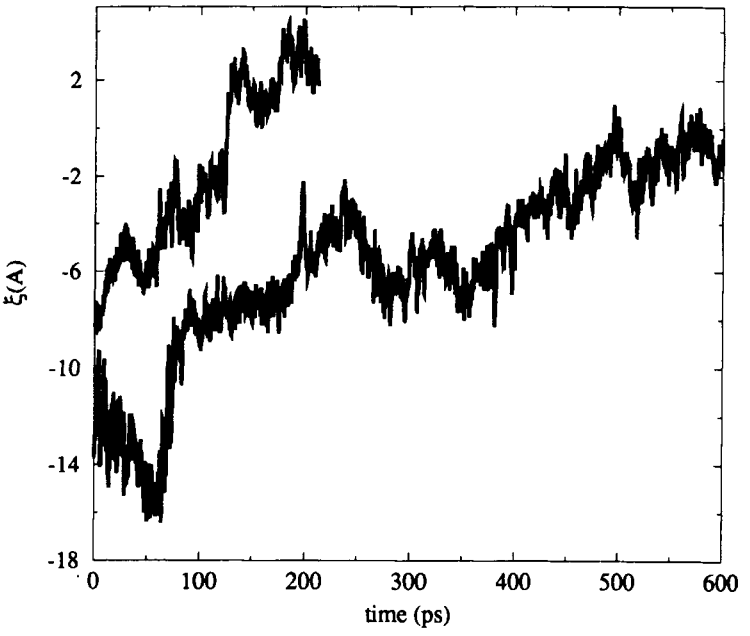

Figure 6 Expulsion of DMSO molecule from inside the membrane.

find that the structural modifications induced by the solute molecule are local. In particular DMSO opens a hole at the surface of the bilayer which allows a water molecule to go inside. As the DMSO arrives approximately half way between the two interfaces, the hole closes and the water molecule is expelled.

Finally, the energy profile for the membrane crossing shows a strong dependence on structural transformations occuring in the membrane which have timescales in the order of 20 ps. This behavior makes it difficult to estimate in a reliable fashion the entropic contributions to the free energy profile of crossing.

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