Chemically Induced Phospholipid Translocation Across Biological Membranes

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Chemical means of manipulating the distribution of lipids across biological membranes is of considerable interest for many biomedical applications as a characteristic lipid distribution is vital for numerous cellular functions. Here we employ atomic-scale molecular simulations to shed light on the ability of certain amphiphilic compounds to promote lipid translocation (flip-flops) across membranes. We show that chemically induced lipid flip-flops are most likely pore-mediated: the actual flip-flop event is a very fast process (time scales of tens of nanoseconds) once a transient water defect has been induced by the amphiphilic chemical (dimethylsulfoxide in this instance). Our findings are consistent with available experimental observations and further emphasize the importance of transient membrane defects for chemical control of lipid distribution across cell membranes.

Introduction

Lipid molecules are known to be distributed asymmetrically across most biological membranes.[1,2] This asymmetry is vital for cellular function and a failure to maintain the asymmetric distribution of lipids can have dramatic consequences. For instance, the externalization of anionic phosphatidylserine lipids, which are normally localized in the inner leaflet of plasma membranes, is able to trigger programmed cell death.[3] Consequently, there is considerable interest in developing physical and chemical means for manipulating the lipid distribution across cell and organelle membranes. Such an ability could give rise to a array of biomedical applications and possibly new therapeutic agents.

To alter the asymmetric transmembrane lipid distribution, one needs to translocate (flip-flop) lipid molecules from one membrane leaflet to another. Physiologically, the asymmetry is maintained largely by active lipid translocation driven by specific membrane proteins, flippases;[4] this is complemented by passive transport mechanisms.[5] Flip-flops can also be induced artificially, e.g., by chemical means or through electroporation.[6] Of these methods the more versatile approach, particularly with a view to developing therapeutic agents, is probably the chemical means of flip-flop induction. Chemicals that have been shown to enhance flip-flop activity include local anesthetics[7−9] and amphiphilic compounds.[10−12] How these chemicals exert their effect at the molecular level is still a mystery, though it has been proposed that they induce transient defects in plasma membranes and in so doing promote flip-flop activity.

Table 1. Summary of MD Simulations of DMSO-Induced Lipid Flip-Flops

system	lipids[a]	T [K][b]	$N_{\text{flip-flop}}$[c]	$T_{\text{flip-flop}}$ [ns][d]
1	DPPC	350	60	13 ± 7
2	DPPC	323	40	25 ± 13
3	DMPC	323	75	17 ± 8
4	POPC	310	29	30 ± 16

[a] Type of phospholipids in a bilayer. [b] Temperature. [c] Number of successful flip-flop events. [d] Average time of a lipid flip-flop. Errors are estimated as standard deviations.

To address this problem, we have employed molecular dynamics (MD) simulations, which provide unprecedented atomic resolution, and investigated how the aprotic solvent, dimethylsulfoxide (DMSO), promotes transmembrane lipid translocations in a series of single-component phospholipid membranes comprising different phosphatidylcholine lipids. DMSO was chosen as it is known to interact strongly with membranes being widely employed in cell biology as a penetration enhancer,[13] cryoprotectant,[14] and cell fusogen.[15] It has also been observed in simulations that DMSO is able to induce water pores in membranes.[16−18] On the basis of the simulations we demonstrate that small amphiphilic solutes do indeed promote flip-flop activity and present a detailed molecular mechanism of how this happens. The mechanism turns out to be two-step: first, a hydrophilic pore spanning the entire membrane is induced by the chemical and then individual lipids translocate from one lipid monolayer to the other via the pore.

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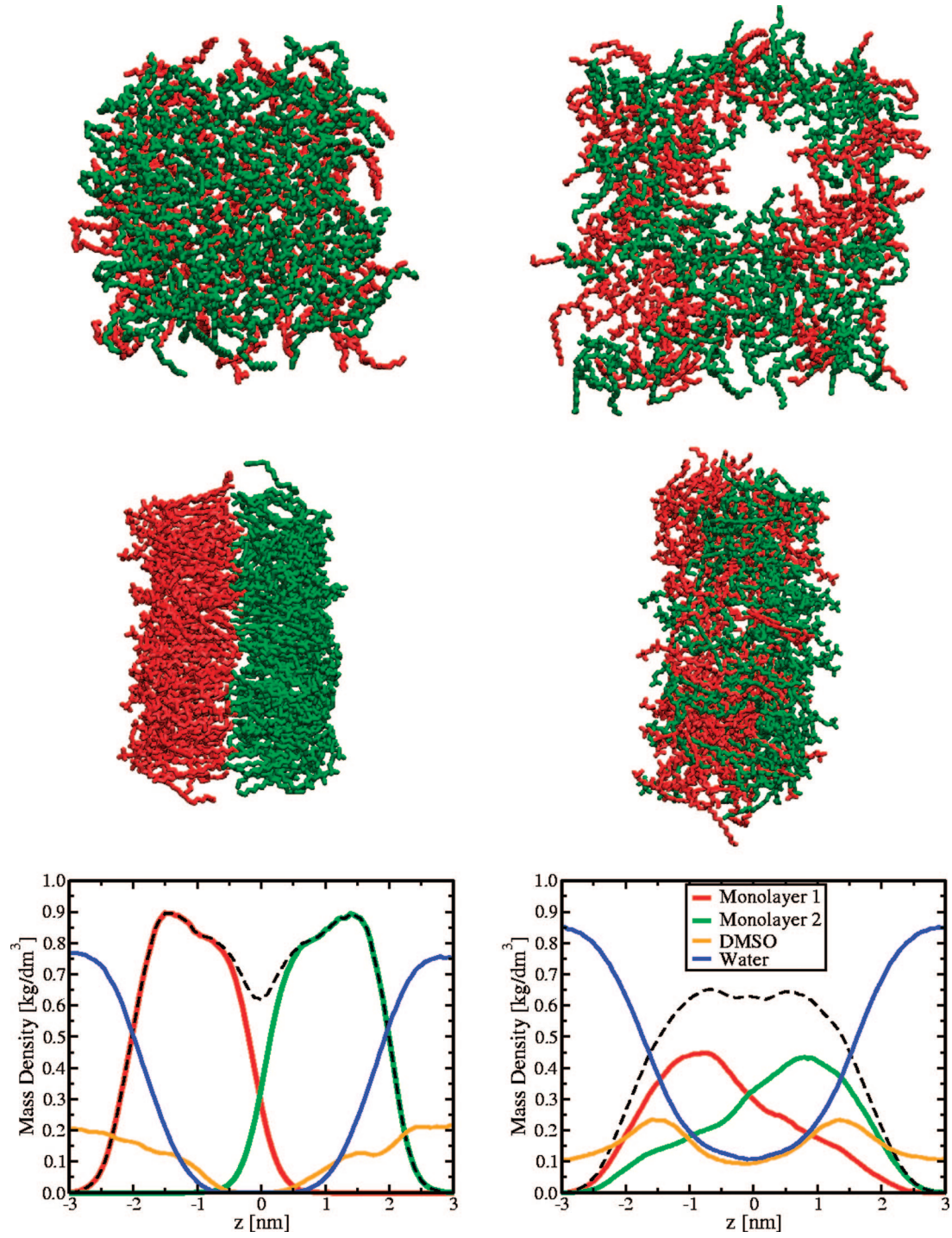

Figure 1. DMSO-induced lipid flip-flops: A DPPC membrane in water with 7 mol % DMSO at $T = 323$ K (system 2, see Table 1) at $t = 0$ (left) and 100 ns (right). Shown are membrane snapshots, top view (top) and side view (middle), and component-wise density profiles (bottom) calculated over first and last 5-ns parts of the 100-ns trajectory. Lipids in opposite leaflets are shown in red and green.

Methods

We have performed atomic-scale molecular dynamics (MD) simulations of a series of single-component phosphatidylcholine lipid membranes in aqueous solution with 7 mol % DMSO (lipid-free basis). In all, we studied four different bilayer systems comprised of dipalmitoyl-phosphatidylcholine (DPPC, $T = 350$ K and $T = 323$ K), dimyristoyl-phosphatidylcholine, (DMPC, $T = 323$ K), and palmitoyl-oleoyl-phosphatidylcholine (POPC, $T = 310$ K) lipids, see Table 1. Typically, the simulation system consisted of 128 lipids and about 7000 solvent molecules (water and DMSO). Force-field parameters for PC lipids were taken from the united atom force-field of Berger et al.,[19] water was modeled using the simple point charge model,[20] and for DMSO we used the force-field of Bordat et al.[21] The Lennard-Jones interactions were cut off at 1 nm. For the electrostatic interactions the particle-mesh Ewald method[22,23] was used. The simulations were performed in the NpT ensemble; pressure

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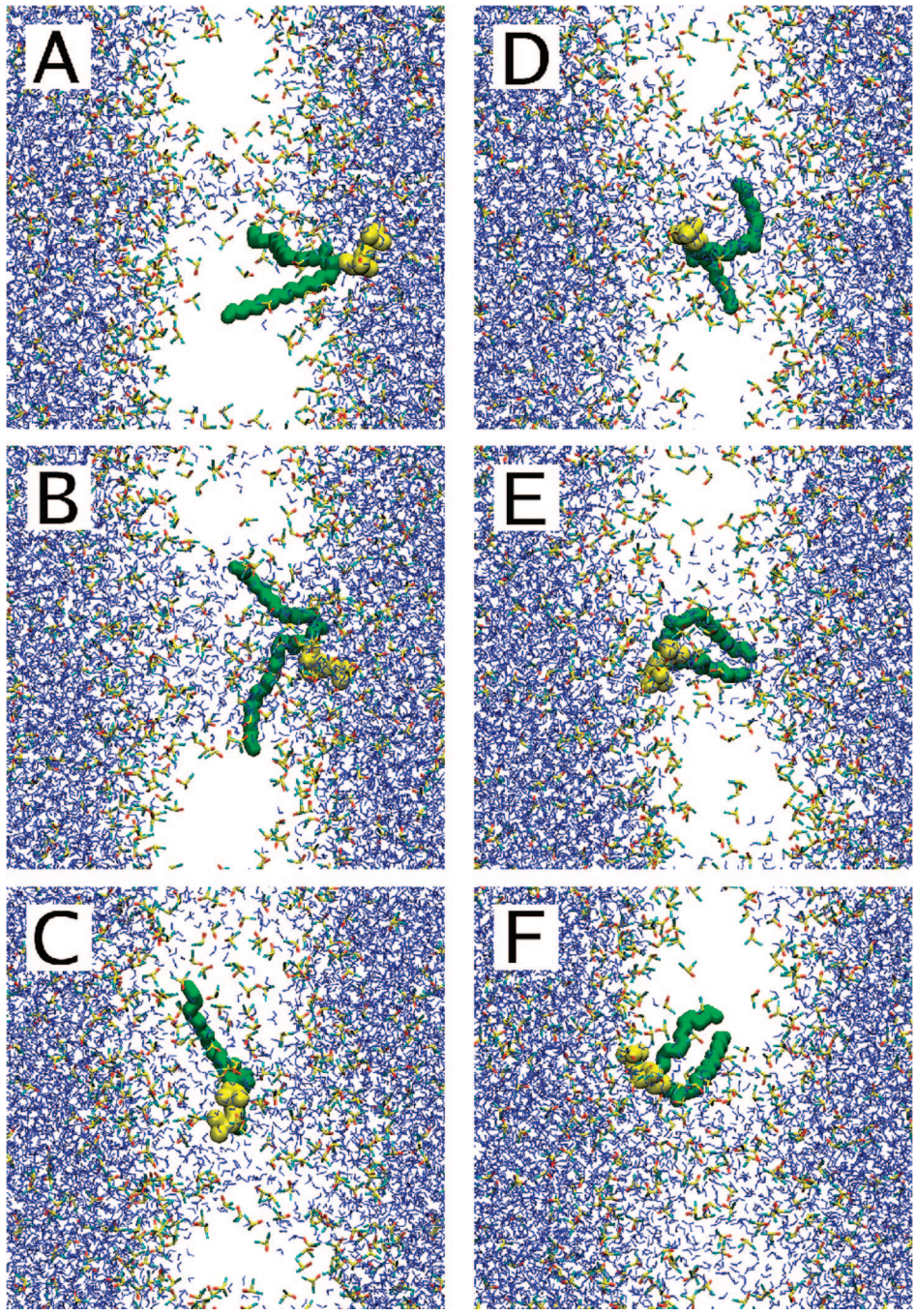

Figure 2. DMSO-induced translocation of a lipid across a DPPC membrane ($T = 350$ K): (A) 42 500 ps, the highlighted lipid is far away from the water pore; (B) 51 400 ps, the lipid diffuses to the pore site and enters the pore; (C) 56 950 ps, diffusional translocation of the lipid starts, this is accompanied by the overall lipid reorientation; (D) 62 150 ps, the lipid is still within the pore but its hydrocarbon chains are now oriented the same way as lipid chains in the opposite leaflet; (E) 65 650 ps, the lipid enters the opposite leaflet; the translocation is accomplished; (F) 76 750 ps, the lipid diffuses away from the pore site. Lipids (except for the flip-flopped one) are not shown; water is shown in blue, DMSO molecules are shown in bond representation and colored in red-yellow-cyan, acyl chains of the flip-flopped lipid are shown in green, and its headgroup in yellow.

was set to 1 bar. Temperature and pressure were kept constant by the Berendsen scheme.[24] The time step used in all simulations was 2 fs. Every bilayer system was simulated for 100 ns except the POPC bilayer whose simulation was extended to 120 ns due to slower pore formation and flip-flop process. All simulations were performed using the GROMACS suite.[25]

Results and Discussion

The simulations reveal an overall picture which is very similar for all PC lipid membranes studied. Adding 7 mol % of DMSO to a membrane/water system leads to expansion and thinning[17,26] of the membrane followed by the formation of hydrophilic pores that are lined by lipid head groups and span the bilayer.[16–18] This process occurs on a time scale of tens of nanoseconds. The formation of a water pore can be easily observed visually and

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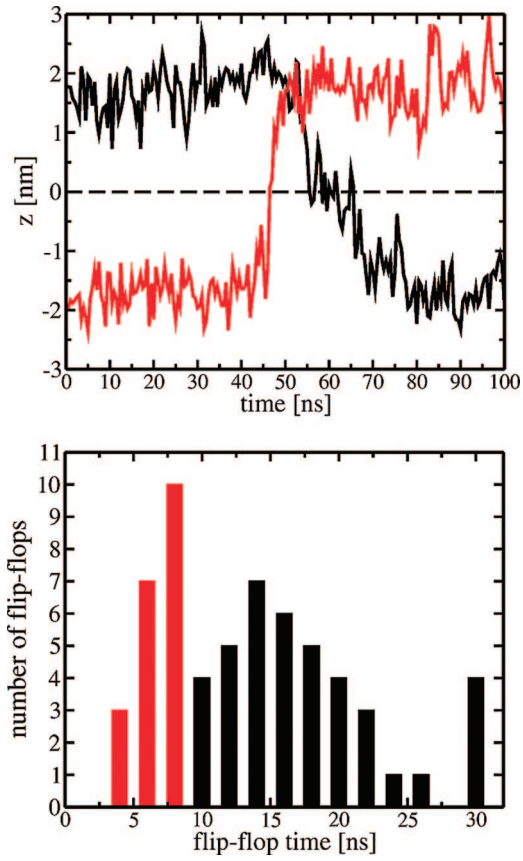

Figure 3. (Top) Time evolution of the center of mass (CM) of head groups for 2 representative flip-flopped lipids of system 1 (the DPPC membrane at $T = 350$ K). The two lipid trajectories are colored in black and red; $z = 0$ corresponds to the center of the membrane. (Bottom) The distribution of flip-flop times for system 1; the contribution of fast flip-flops with translocation times notably shorter than the average value (13 ns, see Table 1) are shown in red.

through the component-wise density profiles of the membrane system (Figure 1).

Once a water pore (defect) has formed, one observes spontaneous translocation (flip-flop) of lipids across the membrane, leading to considerable mixing up of the lipids that belong to the opposite membrane leaflets (Figure 1 (right)). On the basis of more than 200 successful flip-flop events resolved in atomic detail, we were able to develop a molecular picture of the trajectory followed by a typical lipid molecule as it translocates across membrane monolayers, identify the driving force involved, and characterize the kinetics.

It is clear that the key event leading to DMSO-promoted flip-flops is the spontaneous formation of a water pore, which serves to provide a suitable pathway for the lipid molecule translocation events. A typical translocation event involves lateral diffusion of the lipid molecule to the pore, desorption of its hydrocarbon chains from its native membrane leaflet and entry into the pore structure (where the headgroup lines the pore wall), followed by reorientation of the chains which are then accommodated in the opposite leaflet; the overall process is visualized in Figure 2. The evolution of the position of the center of mass of the lipid headgroups during two such representative events is shown in Figure 3 (top).

As there are no external forces applied to the bilayer system, the transmembrane translocation of lipid molecules should be purely diffusive, i.e., driven exclusively by thermal fluctuations. Indeed, analysis of the flip-flop statistics for two DPPC membranes (see Table 1) shows that reducing temperature from 350 to 323 K leads to a considerable drop in the overall number of flip-flops (by a factor of 1.5) and makes the flip-flops themselves two times slower (13 vs 25 ns). The POPC membrane at physiological temperature ($T = 310$ K) exhibits an even longer flip-flop time (~30 ns). We note that the flip-flop time was defined as the time required for the center of mass of a lipid headgroup to translocate from one leaflet ($z > 1.8$ nm) to another leaflet ($z < 1.8$ nm) or vice versa, see, e.g., Figure 3 (top).

While not readily apparent from the average time periods presented in Table 1, the flip-flop times for individual lipids show considerable scatter. The distributions of flip-flop times were typically bimodal as seen in Figure 3 (bottom) for the DPPC membrane at 350 K. The translocation times of the majority of lipids are rather broadly distributed and follow a near-Gaussian distribution with the peak close to the average time of flip-flops (the black part of the histogram in Figure 3 (bottom)). This reflects the stochastic nature of the flip-flop process. The typical translocation trajectory of a lipid across a membrane is shown in Figure 3 (top) in black and clearly indicates that a lipid involved in a flip-flop can spend significant time within the membrane interior (in the pore) before it eventually diffuses to the opposite leaflet. The distributions also show a notable population of rather fast flip-flops with the distribution peak located in the domain of times shorter than the average value (these are shown in red in Figure 3). This type of flip-flop can be co-operative in nature being associated with extensive fluctuations of the size of DMSO-induced pores; such fluctuations can be seen in Figure 2. In particular, an increase in the pore size can lead to a significant intake of lipid head groups by the membrane interior. In turn, a drop in the size of a pore can push some lipids from the pore towards the membrane leaflets. Inspection of several flip-flop events indicates that a lipid which has just entered the membrane interior can be forced by pore size fluctuations toward the opposite leaflet, resulting in translocation across the entire membrane in an almost continuous manner. Clearly, the translocation processes involve co-operativity (akin to that noted for pore formation, lateral diffusion of lipids,[29] and membrane undulations[30]) whereby collective motions of the surrounding lipids enable particular lipids to undergo desorption/adsorption events from/into a leaflet as well as reorientation.

The effect of the length of hydrocarbon lipid chains is also noteworthy. Comparison between DPPC and DMPC bilayer systems studied at the same temperature ($T = 323$ K) reveals that the probability (based on number of events, see Table 1) of the DMPC flip-flop is two times higher than that for a DPPC one; the flip-flops themselves are also faster in the case of DMPC (17 vs 25 ns). This difference correlates well with the differences in the acyl chain region of the lipids: DMPC hydrocarbon chains are two hydrocarbons shorter than those of DPPC. This means that the energy required for lipid desorption out of the monolayer is smaller in the case of DMPC. Furthermore, the DMPC membrane has a smaller hydrophobic core and therefore presents a shorter pathway for lipid translocation.

While this is the first time flip-flop activity has been characterized for a chemically induced pore, the picture that emerges is consistent with an earlier computational study of lipid flip-flops associated with water pores preformed by transmembrane ionic charge imbalance.[27] The observations also agree with a single instance of a defect-mediated lipid flip-flop observed in MD simulations of lipid membranes under the influence of butanol.[28]

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It is instructive to compare the characteristic times of *pore-mediated* diffusive flip-flops in lipid membrane systems with and without DMSO. The value of 60 ns was reported in ref 27 where the pore was formed using ionic charge imbalance for DMPC bilayers at $T = 323$ K. Comparison of this characteristic time with current simulations of DMPC membranes containing DMSO at the same temperature (see Table 1) reveals flip-flop times for the latter to be 4 times shorter. There are probably several reasons behind such a pronounced effect of DMSO. First, DMSO inclusion into a membrane enhances its fluidity and, therefore, increases the lateral mobility of lipids. Indeed, the lateral diffusion coefficient D_L of DMPC lipids under the influence of 7 mol % of DMSO (estimated for an intact membrane prior to pore formation) was found to be 4.87×10^{-7} cm^2/s, which is about three times larger than the value $D_L = 1.53 \times 10^{-7}$ cm^2/s observed for a DMPC bilayer without DMSO at the same temperature.[31] Secondly, DMSO molecules are able to efficiently decrease interactions between the lipid molecules (by virtue of them being able to penetrate into the lipid/water interface), as a result of which less energy is required for a lipid to desorb out of a leaflet, and for subsequent reorientation and accommodation in the opposite leaflet. Finally, DMSO makes the membrane considerably thinner, hence reducing the translocation path.[16,17,26] Our estimates show that adding 7 mol % of DMSO to a DMPC membrane leads to a drop in the membrane thickness from 3.35 nm (see ref 31) to 2.74 nm, i.e., by around 18%. Clearly, in the search for solutes that could significantly enhance the rate of flip-flops a water-pore forming capability seems to be a key requisite, which in turn is linked to an ability to reduce both the area compressibility (this implies a reduction in lipid−lipid interaction) and the bending rigidity moduli,[16] and possibly to an ability to induce a positive spontaneous curvature. Solutes that do not induce water pores but which increase the general fluidity of the membrane (normally accompanied by thinning and expansion of a membrane) are also expected to enhance flip-flops, albeit in a limited manner.

Summarizing, we have shown by means of atomic scale molecular simulations that chemically induced water pores can significantly promote flip-flop activity. These findings lend support to the idea put forward by experimental observations that chemically induced flip-flops are essentially pore-mediated. Assuming that pore formation is a general characteristic of chemicals that induce flip-flops, the rate limiting step then appears to be the formation of a pore. Once a pore has been formed, the actual flip-flop event appears to be a rapid process occurring on a time scale of tens of nanoseconds. The study has also identified the key molecular steps that are involved in translocating a lipid molecule via a water pore. These insights coupled with our increasing understanding of the molecular features of pore inducing molecules[16–18] should aid the identification of chemicals, possibly therapeutic,[32] that modulate the lipid distribution across cell membranes.

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