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Curvature-Induced Sorting of Lipids in Plasma Membrane Tethers

Svetlana Baoukina, Helgi I. Ingólfsson, Siewert J. Marrink, and D. Peter Tieleman*

Membrane curvature controls the spatial organization and activity of cells. Lipid sorting in cell membranes can be explained by matching lipid molecular shape to regions of different curvatures. A molecular view of curvature-induced lipid sorting is obtained using coarse-grained molecular dynamics. A model membrane consisting of an asymmetric bilayer of multiple lipid species is simulated. Curvature is induced by pulling a tether, that is, a bilayer nanotube, from a flat membrane. Pulling is performed both from the inner and outer leaflets, corresponding to directions in and out of the cell. Redistribution of different lipid types between the tether and the bilayer is observed, leading to spatial variations in the composition of both leaflets, and, in turn, softening of the tether. Depending on the direction of pulling, the lipid distributions and the tether properties differ. Formation of a tether from the planar membrane thus induces lipid sorting without phase separation.

Membrane curvature controls the spatial organization and activity of cells. Membrane-bound organelles in eukaryotic cells, including endoplasmic reticulum, Golgi, and endosomes, contain highly curved vesicular and tubular structures.^[1,2] Trafficking between cells and their organelles involves these highly curved transport intermediates.^[3] In plasma membranes, high curvatures are generated dynamically during membrane remodeling.^[4]

The spatial distribution of lipids and proteins is coupled to membrane curvature. A variety of membrane-binding proteins are involved in curvature sensing and generation.^[5–7] The ability of proteins to generate curvature is based on a number of mechanisms, including scaffolding, wedging, protein oligomerization into coats, protein crowding, and applying a localized pulling force to the membrane.

The coupling of lipid distribution to curvature is less understood. Each lipid has a spontaneous (or intrinsic) curvature resulting from its effective molecular shape. Theoretically, lipids can generate membrane curvature by their shape and by asymmetry in composition or area between the two leaflets. In practice,

spontaneous curvatures of lipids alone are insufficient; high curvatures of intracellular organelles and transport intermediates ($\approx 1/50 \text{ nm}^{-1}$) require significant leaflet asymmetry, achievable only by special mechanisms and conditions.^[2] However, lipids can sense the curvature generated by specialized proteins and change their distribution in the membrane. The sorting of lipids by curvature can be explained by different mechanisms depending on either individual or collective behavior.

Segregation of individual lipids into regions of different membrane curvature is based on their molecular shape and flexibility.^[8–14] Molecular shape is expected to match the curved membrane, minimizing the (bending) deformation energy. By

convention, lipids with headgroups larger than tails have a positive spontaneous curvature. A lipid with only one hydrocarbon chain—lysolipid—matches positive curvature regions, while a lipid with a small headgroup, for example, dioleoyl phosphatidylethanolamine or cholesterol, fits negative curvature regions. Molecular flexibility of a lipid is associated with the saturation of its hydrocarbon chains. Unsaturated and, in particular, polyunsaturated lipids are more flexible and are expected to partition to curved regions to lower the energetic cost of membrane deformation, while more rigid saturated lipids are expected to be depleted from curved regions.

Segregation of individual lipids is counteracted by mixing entropy. To overcome the entropic cost of de-mixing, lipid–protein and lipid–lipid interactions are required,^[15–18] resulting in collective behavior. Examples include proximity to a phase transition, clustering of lipids by proteins, or formation of ordered domains ("rafts") by specific lipids and proteins. Curvature can induce sorting of ordered and disordered domains based on differences in their bending rigidity, where the softer disordered phase segregates into curved or stressed regions.^[10,19–21] In cells, sorting of raft domains into transport carriers is facilitated and regulated by protein machinery.^[22,23]

Here we demonstrate lipid sorting by membrane curvature in the absence of proteins and phase separation. We perform molecular dynamics (MD) simulations with the coarse-grained force field Martini^[24] to model a generic plasma membrane. Curvature is induced by pulling a tether, that is, a bilayer nanotube, from a flat membrane, using a previously established protocol.^[25] The tethers are pulled in two directions: from the inner and from the outer leaflet of the plasma membrane, and at different temperatures. The formation of tethers leads to redistribution of

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Figure 1. Tethers formed from a plasma membrane in water by pulling “out” and “in.” Water and ions are left out of clarity. The tether is sliced through on the right-hand side of the image to show its bilayer structure. The headgroup and chain composition of the outer and inner leaflets are shown at the top and bottom, respectively. The tether snapshots are simulations at 290 K after 40 μ s. The lipid headgroups are colored based on the lipid “type” and the tails by the level of unsaturation (see pie charts).

individual lipid components between the tether and the remaining membrane. At the same pulling conditions, the tether properties and the distribution of lipids differ, depending on the direction of pulling.

The idealized plasma membrane model is represented by an asymmetric bilayer of multiple lipid species.^[26] Previous simulations of this membrane have shown that at 310 K it forms a liquid phase with transient Lo-like domains coupled across the two leaflets.^[26] In addition, clustering of gangliosides and phosphoinositides was observed. In our control simulations, the unperturbed plasma membrane shows lateral heterogeneity due to composition fluctuations, but remains in one phase between 270 and 323 K during 50 μ s simulation.

The simulation setup for pulling tethers consists of a bilayer of ≈ 6600 lipids surrounded by solvent (water with ions) of $\approx 500\,000$ particles, in a simulation cell of $\approx 40 \times 40 \times 40$ nm³. A tether is pulled from this bilayer by applying an external force along the bilayer normal to the center of mass of a circular lipid patch. As the composition of the two bilayer leaflets differs, we distinguish two pulling directions: “out,” that is, from the outer leaflet (toward the extracellular space), and “in,” that is, from the inner leaflet (toward the cytoplasm; **Figure 1**). Pulling in both directions is performed at 280, 290, and 310 K. The simulation times are 40 μ s for each condition. Simulation details are given in Supporting Information.

Tether formation initiates from a protrusion, followed by elongation, as in previous MD simulations.^[25,27,28] Tethers develop within the first microsecond of the simulation under all conditions. The tether length is defined by the balance of the external force and forces arising from local and nonlocal bilayer bending. The contribution of nonlocal bending forces becomes significant in simulations due to the small bilayer area compared to experiments.^[25]

Depending on temperature and the pull direction, tether properties differ (**Table 1**). With increasing temperature, the tether radius, R , decreases and tether length, L , increases. This trend is expected and can be understood as softening of the tether (decrease of the local and nonlocal bending moduli) with temperature. As the tether has a cylindrical shape, its curvature equals $1/R$ and for the considered conditions constitutes $0.185\text{--}0.2$ nm^{−1}.

Interestingly, at the same temperature and pulling force, the properties differ depending on the pulling direction. Pulling membranes “in” produces wider and shorter tethers compared to pulling membranes “out.” The asymmetry of bilayer composition yields distinct elastic moduli of the leaflets. As the degree of (bending) deformation is higher in the inner leaflet than in the outer leaflet,^[11,29] the leaflets give unequal contribution to the energetic cost of deformation. In addition, steric/geometric constraints could play a role, for example, accommodating the bulky ganglioside headgroups inside the tether in the “in” direction.

The tether geometric properties also change noticeably over the course of simulation: the tethers become longer and thinner. These changes indicate softening of the tethers related to significant changes in the tether composition due to lipid sorting.

Lipid sorting is induced by the curvature gradient between the highly curved tether and the flat remainder of the membrane. Lipid sorting is quantified by the concentration of lipids in the tether, normalized by those in the unperturbed bilayer (**Figure S1**, Supporting Information). Resulting lipid fractions are shown in **Figure 2** (and **Figure S2**, Supporting Information): values above and below unity indicate preferred partitioning to the tether and flat membrane, respectively. Lipid species are grouped by type and chain unsaturation.

Figure 2 shows lipid sorting on average and by leaflet. For both pulling directions, polyunsaturated lipids are enriched and fully saturated lipids are depleted in the tether. The unsaturated and, in particular, polyunsaturated chains are softer and reduce the energetic cost of deformation. A minor decrease in cholesterol concentration in the tether could be related to its stiffening effect on the unsaturated lipids.

The tethers formed by pulling the plasma membrane “out” are enriched in lysolipids (LPC). The LPCs are present only in the outer leaflet, located outside of the tether in this pulling configuration. This enrichment is thus evident as LPCs have only one hydrocarbon chain and prefer a positive curvature. The depletion of the sphingomyelin (SM) in the tether is likely related to their long and mostly saturated chains, which stiffen the membrane. The enrichment of phosphatidylcholine (PC) lipids is possibly correlated with the depletion of SM and cholesterol, as these types constitute the major groups in the membrane. Other notable features include depletion of the anionic phosphatidylserine (PS) and phosphatidylinositol (PI) lipids, depletion of gangliosides (GM) and enrichment of phosphatidylinositol phosphates (PIP) in the tether.

The tethers formed by pulling the membrane “in” are depleted in LPC, PIP, and SM lipids, and enriched in phosphatidylethanolamine (PE), PS, and diacylglycerol (DG) lipids. Some of these trends are expected: a positive curvature of the outer membrane leaflet inside the tether is unfavorable for the one-chain LPC and favorable for DG, having no headgroup. The depletion of SM is more pronounced here because of its stiffening effect and its larger fraction in the outer membrane leaflet,

Table 1. Tether properties: radius, R , and length, L .

Time	1–2 μ s				39–40 μ s			
	Pull out		Pull in		Pull out		Pull in	
	R [nm]	L [nm]	R [nm]	L [nm]	R [nm]	L [nm]	R [nm]	L [nm]
310	5.0 $\pm$ 0.1	33.4 $\pm$ 0.8	5.3 $\pm$ 0.1	30.1 $\pm$ 0.8	4.6 $\pm$ 0.1	38.1 $\pm$ 1.9	5.2 $\pm$ 0.2	33.2 $\pm$ 0.6
290	5.2 $\pm$ 0.1	29.4 $\pm$ 0.7	5.5 $\pm$ 0.2	26.4 $\pm$ 0.6	4.8 $\pm$ 0.1	33.7 $\pm$ 1.3	5.2 $\pm$ 0.1	28.4 $\pm$ 0.7
280	5.4 $\pm$ 0.1	27.6 $\pm$ 0.5	6.0 $\pm$ 0.2	24.7 $\pm$ 0.4	5.0 $\pm$ 0.1	30.0 $\pm$ 0.6	5.4 $\pm$ 0.1	27.6 $\pm$ 0.5

Figure 2. Lipid sorting by curvature: the fractions of the lipid types for pulling membrane “out” (left column) and “in” (right column) calculated on a) average and b) by leaflet at 290 K. The inner and outer leaflets refer to the plasma membrane: as the membrane is pulled “out” the outer leaflet is located on the outside of the tether, as the membrane is pulled “in” the inner leaflet is located on the outside of the tether. CH, cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; CE, ceramide; PA, phosphatidic acid; DG, diacylglycerol; PI, phosphatidylinositol; PIP, phosphatidylinositol mono-, bi-, and tri-phosphate; GM, gangliosides (GM1 and GM3); LPC, lysolipids; Saturated, containing two fully saturated hydrocarbon chains; Polyunsat, two or more double bonds in both chains. Results for 280 and 310 K are shown in Figure S2, Supporting Information.

which is pulled inside the tether. The distribution of other components may appear the opposite from expected. For example, PE lipids have a smaller headgroup compared to other lipid types, combined with (poly)unsaturated chains they have a negative spontaneous curvature. However, this group is found mainly in the inner leaflet of the membrane, located outside of the tether in this pulling configuration and having a positive curvature.

The analysis by leaflet (Figure 2b) shows significant enrichment of PE lipids in the outer leaflet located inside the tether

on pulling the membrane “in.” This enrichment is expected considering the negative spontaneous curvature of PE and the negative curvature of the outer leaflet in this pulling configuration. A moderate increase in PE concentration in the positively curved inner leaflet located outside of the tether can be related to the unsaturation of the chains. The PE lipids have a high fraction of soft polyunsaturated chains that decrease the deformation energy upon partitioning to the tether. A tendency of polyunsaturated chains to partition to positively curved regions has been

previously observed in simulations and was related to their conformational flexibility.^[11] Interestingly, the concentration of cholesterol in the tether is always increased in the leaflet located inside the tether consistent with its negative spontaneous curvature.^[30] The distributions of lipids along the tether (Figure S3, Supporting Information) show gradual changes in the local concentrations.

These results demonstrate that pulling a tether from a plasma membrane induces sorting of lipids between the tether and the membrane. The observed redistribution is caused by a spatial variation in curvature. An unperturbed plasma membrane of the same composition does not undergo a clear phase separation in a temperature range comprising the considered interval (270–323 K) on the time scales of tens of μ s. This supports curvature-induced segregation of individual lipids versus collective behavior in our simulations. The observed segregation can be explained entirely by the lipid effective shape (spontaneous curvature) and chain flexibility.

Note that the tethers in our simulations have a very high curvature due to their small radius (≈ 5 nm). This radius is smaller than that of cellular structures and the radius achieved by pulling tethers experimentally ($R \approx 20$ – 100 nm).^[31] The resulting higher curvature in our simulations leads to stronger driving forces for lipid sorting. Lipid segregation is thus expected to be less strong for smaller curvatures. In addition, stronger pulling forces and smaller radius in simulations result in a higher surface tension in the tether, contributing to its deformation. The resulting stretching of the tether surface likely increases lipid sorting, in particular, related to molecular flexibility and chain saturation. Nevertheless, our results demonstrate the tendency for curvature-induced redistribution for all lipid types in a complex mixture relevant for cellular membranes.

In summary, we observe a curvature-induced lipid sorting on pulling a tether from an idealized plasma membrane. The sorting occurs in the absence of proteins or a phase separation and leads to softening of the curved membranes. The sorting can be explained by the effective shape and chain saturation of the lipid molecules.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

bilayer nanotubes, cellular trafficking, curvature sensing, molecular shape, transport intermediates

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