Cholesterol Flip-Flop Impacts Domain Registration in Plasma Membrane Models
Thallmair, Sebastian; Ingólfssson, Helgi I.; Marrink, Siewert J.

Published in:
The Journal of Physical Chemistry Letters

DOI:
10.1021/acs.jpclett.8b01877

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Cholesterol Flip-Flop Impacts Domain Registration in Plasma Membrane Models

Sebastian Thallmair,* Helgi I. Ingólfsson,† Siewert J. Marrink,* and Siewert J. Marrink*†

1Groningen Biomolecular Sciences and Biotechnology Institute and The Zernike Institute for Advanced Material, University of Groningen, Nijenborgh 7, 9747 AG Groningen, Netherlands
2Biosciences and Biotechnology Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, 94550 California, United States

ABSTRACT: The plasma membrane is a highly complex multicomponent system that is central to the functioning of cells. Cholesterol, a key lipid component of the plasma membrane, promotes the formation of nanodomains. These nanodomains are often correlated across the two membrane leaflets, but the underlying physical mechanism remains unclear. Using coarse-grained molecular dynamics simulations, we investigate the influence of cholesterol flip-flop on membrane properties, in particular, the interleaflet coupling of cholesterol-enriched domains. We show that the cholesterol density correlation between the leaflets of an average mammalian plasma membrane is significantly reduced by suppressing interleaflet cholesterol population. Our results suggest an amplifying role of cholesterol in signal transduction across the leaflets.
Martini force field. Figure 1a shows the final snapshot of the DPPC/DLiPC/CHOL bilayer after 30 μs. The phase separation of DPPC (saturated tails) and DLiPC (double unsaturated tails) is clearly visible; CHOL preferentially resides in the DPPC-enriched Lₒ domains. The two domains are registered across the leaflets.

Table 1 lists the global membrane properties of the DPPC/DLiPC/CHOL bilayer. The average APL remains almost unchanged upon restricting CHOL flip-flop. A similar behavior is observed for the area compressibility, the tail order, as well as the bilayer thickness. Overall, the global membrane properties are virtually unaffected by the restriction of the CHOL flip-flop.

To analyze the extent by which cholesterol flip-flop affects the composition of the Lₒ and L_d domains, we evaluated the relative number of neighboring lipids for each lipid type (listed in Table S2). DPPC and CHOL prefer to be surrounded by each other or themselves, whereas they try to avoid DLiPC. On the contrary, DLiPC prefers itself in its surrounding, in line with the strong phase separation apparent from the snapshots (Figure 1a). This trend is unaffected by the CHOL flip-flop.

Table 1. Membrane Properties of the Ternary Mixtures DPPC/DLiPC/CHOL and DPPC/DOPC/CHOL

<table>
<thead>
<tr>
<th></th>
<th>DPPC/DLiPC/CHOL w/o flip-flop</th>
<th>DPPC/DOPC/CHOL w/o flip-flop</th>
<th>DPPC/DLiPC/CHOL w/flip-flop</th>
<th>DPPC/DOPC/CHOL w/flip-flop</th>
</tr>
</thead>
<tbody>
<tr>
<td>average APL (nm²)</td>
<td>0.736</td>
<td>0.737</td>
<td>0.659</td>
<td>0.660</td>
</tr>
<tr>
<td>average area compressibility (mN/m)</td>
<td>399 ± 5</td>
<td>407 ± 5</td>
<td>389 ± 4</td>
<td>392 ± 6</td>
</tr>
<tr>
<td>average tail order DPPC</td>
<td>0.634</td>
<td>0.626</td>
<td>0.533</td>
<td>0.534</td>
</tr>
<tr>
<td>average tail order DLiPC/DOPC</td>
<td>0.244</td>
<td>0.245</td>
<td>0.380</td>
<td>0.382</td>
</tr>
<tr>
<td>average bilayer thickness (nm)</td>
<td>4.071</td>
<td>4.075</td>
<td>4.191</td>
<td>4.190</td>
</tr>
<tr>
<td>CHOL flip-flop rate (10⁶ s⁻¹)</td>
<td>5.45 ± 0.08</td>
<td>0.0</td>
<td>1.77 ± 0.05</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*a* All errors are standard errors and were omitted if ≤0.002. *b*Averaged over the last 10 μs. *c*Averaged over the last 2 μs.
Finally, we analyzed the spatial correlation of the CHOL densities of the upper and lower leaflet, which provides a measure for the registration of the CHOL domains. In doing so, we extract the positions of the polar CHOL headgroups in each leaflet, convert it to a continuous density using a Gaussian kernel of $\sigma = 15 \, \text{Å}$, and calculate the Pearson correlation between the CHOL densities of the lower and upper leaflet (for details, see the SI). Figure 1d illustrates the distribution of the Pearson correlation for the DPPC/DLiPC/CHOL system in the last 20 $\mu$s of the simulations. The distributions exhibit a Gaussian shape and show no changes if CHOL flip-flop is restricted (maximum at $0.866 \pm 0.001$ with flip-flop, $0.863 \pm$...
0.001 with fb3.5, 0.866 ± 0.001 with fb1.6). The maxima were determined by fitting a Gaussian function. The temporal evolution of the correlation of all three systems is depicted in Figure S6, showing a convergence of these data on a time scale of ~4 μs. Apparently, restricting the CHOL flip-flop has virtually no effect on the interleaflet registration. To verify the CHOL density as a suitable measure of the domain registration, we also calculated the interleaflet correlation of the saturated (DPPC) and unsaturated (DLiPC) lipid densities, which are depicted in Figure S8.

An overall similar picture arises for the second ternary mixture investigated (DPPC/DOPC/CHOL). The final snapshot of the bilayer after 30 μs is depicted in Figure 1b. A non-ideal mixing of DPPC and DOPC is visible, but there is no clear domain formation. Because of the higher DPPC content, the CHOL flip-flop rate is smaller by a factor of ~3. The average APL, area compressibility, tail order, and bilayer thickness are listed in Table 1. As in the previous example, these global membrane properties are unaffected by restricting the CHOL flip-flop.

The lower tendency of the DPPC/DOPC/CHOL bilayer to form defined lateral domains is also reflected in the relative number of neighboring lipids (Table S3). The unsaturated lipid DOPC still prefers itself over DPPC or CHOL as a neighbor, but the relative preference is reduced to 1.38 compared to 2.18 for DLiPC. Again, CHOL flip-flop has no significant effect on the relative numbers of neighboring lipids.

Because the bilayer does not show a clear tendency to phase-separate in our simulations, the Pearson correlation of the CHOL densities is slightly negative (~0.011 ± 0.001 for the simulations with flip-flop; Figure 1e). It has a broader distribution compared to the DPPC/DLiPC/CHOL system and does not change upon CHOL restriction (fb3.5: −0.020 ± 0.001, fb1.6: −0.025 ± 0.001). Together, our data on ternary mixtures show that the degree of domain registration is sensitive to system composition, in line with previous results, but that cholesterol flip-flop has no significant contribution.

To approach the complexity of the PM, we investigate the quaternary lipid mixture DPPC/DOPC/DLiPC/CHOL. Because the bilayer does not show a clear tendency to phase-separate in our simulations, the Pearson correlation of the CHOL densities is slightly negative (~0.011 ± 0.001 for the simulations with flip-flop; Figure 1e). It has a broader distribution compared to the DPPC/DLiPC/CHOL system and does not change upon CHOL restriction (fb3.5: −0.020 ± 0.001, fb1.6: −0.025 ± 0.001). Together, our data on ternary mixtures show that the degree of domain registration is sensitive to system composition, in line with previous results, but that cholesterol flip-flop has no significant contribution.

Table 2 summarizes the global membrane properties of the PM with and without CHOL flip-flop. Similar to the simpler ternary mixtures, they are mostly unchanged. The APL is almost unaffected by suppressing CHOL flip-flop, with a maximum difference of 0.004 nm² lower in the case of the wide flat-bottomed potential (fb3.5). Without CHOL flip-flop, the average APL is reduced compared to the DPPC/DLiPC/CHOL mixture and is more dynamic (Figure S7 and Table S1).

The average APL, area compressibility, tail order, and bilayer thickness are listed in Table S6. These global membrane properties show an overall similar picture compared to the ternary mixtures and are unaffected by restricting the CHOL flip-flop. In the unrestrained simulation, the CHOL flip-flop rate is 4.82 × 10⁶ s⁻¹, which is slightly reduced compared to DPPC/DLiPC/CHOL (5.48 × 10⁶ s⁻¹).

The tendency to form lateral domains is also reflected in the relative number of neighboring lipids (Table S4). The unsaturated lipids DPPC and DOPC prefer themselves as neighbors over DPPC and CHOL. In addition, they prefer the same unsaturated lipid more than the other type. DPPC prefers itself and CHOL as neighbors; CHOL prefers DPPC and clearly disfavors DLiPC. Again, CHOL flip-flop has no significant effect on the relative numbers of neighboring lipids.

Because the bilayer does not show full macroscopic phase separation, the Pearson correlation of the CHOL densities, which are depicted in Figure S8, is smaller than that in the DPPC/DLiPC/CHOL system. However, the tendency to form small lateral domains can be clearly recognized in the distributions of the Pearson correlation of the CHOL densities in Figure 1f. The simulations with flip-flop show a mean value of 0.208 ± 0.002 (fb3.5: 0.246 ± 0.003, fb1.6: 0.30 ± 0.001). Whereas by restricting CHOL with the wider flat-bottomed potential, the interleaflet correlation slightly increases, the narrower one results in a drastic decrease. A control simulation using the final snapshot of the unrestrained simulation as the starting configuration shows that after 2 μs the correlation decreases to −0.013 ± 0.005 (Figure S9). This assigns a key role to the CHOL interleaflet population in driving domain registration.

Let us now take a look at the impact of CHOL flip-flop on an idealized average PM mixture, consisting of >60 different lipid types asymmetrically distributed between the two leaflets. The final snapshot of the simulated PM patch after 100 μs with CHOL flip-flop is depicted in Figure 2a. The asymmetric composition can be easily recognized. Despite this, the clustering of the glycolipids (red) is clearly noticeable.

Table 2. Membrane Properties of the Average PM

<table>
<thead>
<tr>
<th></th>
<th>w/flip-flop</th>
<th>w/o flip-flop</th>
</tr>
</thead>
<tbody>
<tr>
<td>outer average APL</td>
<td>0.503</td>
<td>0.499</td>
</tr>
<tr>
<td>inner average APL</td>
<td>0.542</td>
<td>0.538</td>
</tr>
<tr>
<td>average area comp.</td>
<td>367 ± 12</td>
<td>378 ± 12</td>
</tr>
<tr>
<td>outer average tail</td>
<td>0.429</td>
<td>0.430</td>
</tr>
<tr>
<td>inner average tail</td>
<td>0.379</td>
<td>0.376</td>
</tr>
<tr>
<td>average bilayer</td>
<td>4.166</td>
<td>4.162</td>
</tr>
<tr>
<td>CHOL flip-flop rate</td>
<td>5.48 ± 0.02</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*All errors are standard errors and were omitted if ≤0.002. *Averaged over the last 10 μs. *Averaged over the last 40 μs.
do not change when the CHOL flip-flop is restricted. The most striking effect appears for the lipids with two unsaturated tails in the outer leaflet; they have a larger tendency to be surrounded by themselves, whereas lipids with one or two saturated tails are slightly more depleted in their environment. Surprisingly, the amount of CHOL in their surroundings is not influenced. For the inner leaflet, this effect is of only minor importance.

Finally, we take a look at the Pearson correlation of the CHOL densities in the outer and inner leaflet of the PM evaluated for the last 80 μs of the simulations. The CHOL densities were calculated using a Gaussian kernel of σ = 15 Å (cf. S1). Figure 2c shows their distributions evaluated for single snapshots. Their width is somewhat smaller than the ones of the DPPC/DOPC/CHOL bilayer. In the simulated patch with snapshots. Their width is somewhat smaller than the ones of the DPPC/DOPC/CHOL bilayer. In the simulated patch with snapshots.

The Journal of Physical Chemistry Letters

Letter

In summary, we investigated the effect of CHOL flip-flop by means of CG MD simulations of four different lipid bilayers: the ternary mixtures DPPC/DLiPC/CHOL and DPPC/ DOPC/CHOL, the quaternary mixture DPPC/DOPC/ DLiPC/CHOL, as well as a more complex idealized mammalian PM model. In all four cases, no striking changes of the global membrane properties or in terms of the lipid mixing were observed. For the ternary mixtures, the correlation of the CHOL densities in the upper and lower leaflet was also unaffected by CHOL flip-flop, but distinct changes were observed for the quaternary mixture and the PM model. While a restriction with the wider flat-bottomed potential fb3.5 inflicted no changes (or even an increase for the quaternary mixture), suppressing the interleaflet CHOL population (fb1.6) resulted in a significant decrease in the CHOL density correlation. This is remarkable because it shows that it is not the flip-flop process itself that increases correlation between the leaflets but that it results from the intermediate state where CHOL is sandwiched between the leaflets. This state is significantly populated, in particular, in the presence of (poly)unsaturated lipids in line with neutron scattering data.22

Taken together, our results demonstrate a remarkable impact of CHOL flip-flop on the domain organization, most pronounced in complex lipid bilayers. A possible explanation for the dependency of this effect on the system composition is obtained by considering the different nature of the domains in the four studied systems. The DPPC/DLiPC/CHOL mixture is strongly phase-separating and the interleaflet surface tension is likely the major driving force for the strong domain coupling.27 In the case of the DPPC/DOPC/CHOL mixture, only small transient DOPC clusters are formed, which might have a too small spatial extent and a too short lifetime (Figure S7 and Table S1) to be influenced by the CHOL flip-flop. Although the domains in the quaternary mixture and the PM are also transient, their larger extent together with their dynamic flexibility allows them to react to the presence of an interleaflet CHOL population (see Figure 3). This interleaflet CHOL prefers Ld domains in its surrounding because they offer more space and enable a better embedding of the CHOL molecules. Thus interleaflet CHOL leads to a weak repulsion of Ld domains, resulting in an increased interleaflet correlation (Figure 3, bottom). A recent simulation study showing that interleaflet CHOL prefers registered Ld domains over registered Ln domains and anti-registered domains in DPPC/DLiPC/CHOL mixtures supports this idea.

On a more general note, our study shows that CHOL serves as an efficient signaling molecule transferring information between the leaflets by populating the interleaflet space. Through the alignment of (transient) domains, CHOL can quickly transfer local density gradients across the leaflets. Proteins being omnipresent in biological membranes might trigger such small local density gradients, for example, by their individual lipid fingerprint.33 Together with the help of CHOL, this could potentially steer a variety of cellular processes that depend on lateral membrane organization.

**COMPUTATIONAL METHODS**

All MD simulations were performed with the CG force field Martini (version 2.2)30,31 using the MD package GROMACS (versions 4.6.7 and 2016.1).32 We applied flat-bottomed potentials in the direction of the membrane normal to the CHOL molecules to suppress their flip-flopping between the leaflets (Figure S1). Two different widths were used: “wider” potentials with a flat region of 3.5 nm (denoted fb3.5),
allowing CHOL to populate the region between both leaflets, and “thinner” potentials with a flat region of 1.6 nm (fb1.6), restricting the polar CHOL heads to the lipid linker region. As a reference, additional simulations without any restrictions to CHOL were performed for each bilayer. For further details of the bilayer compositions and the simulation setup, see the SI.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jpcllett.8b01877.

Details of the bilayer setup, simulation details, evaluation of the Pearson correlation of the CHOL densities, additional snapshots of the membrane patches, density profiles along the membrane normal, the time-dependent behavior of the CHOL densities, Pearson correlation of the saturated and unsaturated lipid densities, control simulations of the quaternary bilayer and the PM, global membrane properties of the quaternary mixture, and the lipid mixing (PDF)

AUTHOR INFORMATION

Corresponding Authors
*E-mail: s.thallmair@rug.nl (S.T.).
*E-mail: s.j.marrink@rug.nl (S.J.M.).

ORCID
Sebastian Thallmair: 0000-0002-3396-5840
Helgi l. Ingólfsson: 0000-0002-7613-9143

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the European Commission via an ERC grant to S.J.M. (COMP-MICR-CROW-MEM, grant agreement 669723) and a Marie Skłodowska-Curie Actions fellowship to S.T. (MicroMod-PSII, grant agreement 748895). Part of this work was performed under the auspices of the U.S. Department of Energy under contract number DE-AC52-201007NA27344 (LLNL-JRNL-751356). We acknowledge a fellowship to S.T. (MicroMod-PSII, grant agreement 748895).

REFERENCES