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The Aliphatic Chain of Cholesterol Modulates Bilayer Interleaflet Coupling and Domain Registration

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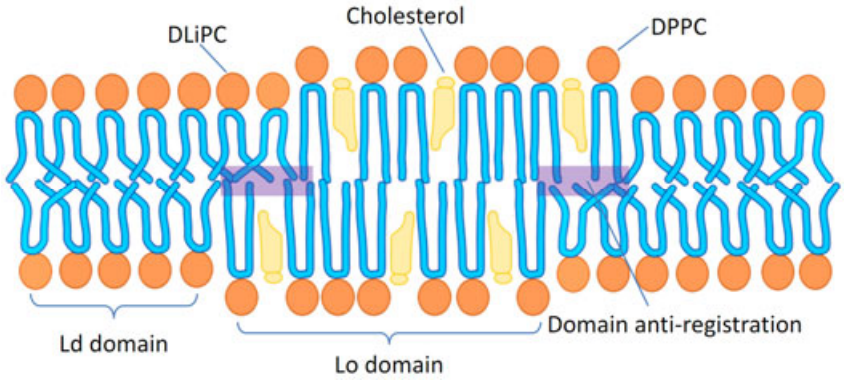
TOC GRAPHICS



Cholesterol aliphatic chain length modulates domain anti-registration.

Abstract

Cholesterol is a necessary component and critical regulator of liquid-ordered membrane domains. However, the structural features that determine its unique physicochemical behaviors are not fully understood. In particular, very little is known about the specific functions of the terminal aliphatic chain of cholesterol, since previous studies have focused mainly on the rigid sterol ring structure and its hydroxyl head. In the current work, we used coarse-grained molecular dynamics simulations to investigate the effect of cholesterol aliphatic chain length on the dynamics and structure of co-existing lipid domains. We found that the aliphatic chain has no appreciable effect on phase separation per se, but it significantly affects the rate of cholesterol flip-flop and intermonolayer interaction. These effects are accompanied by changes in domain dynamics, lateral pressure, and interleaflet coupling. Our study provides useful insight into how biological sterols modulate communication between the outer and inner surfaces of the plasma membrane and, therefore, cellular signaling.

Keywords: Lipid domains; Cholesterol; Interleaflet Coupling; Domain Anti-registration; Molecular Dynamics Simulation; Coarse-grained Model.

Introduction

Cell membranes are composed of a broad variety of lipids and proteins that laterally segregate into so-called lipid rafts, nanoscale domains of lipids and proteins with distinct structure and composition.[1, 2] Lipid rafts play an important role in signal transduction by facilitating protein-protein interactions[3], and their deregulation is potentially associated with diseases.[4] Cholesterol is a major component of mammalian plasma membranes and is essential for maintaining membrane integrity and organization. Specifically, cholesterol is a necessary component of the liquid-ordered (L_o) phase, which is believed to be the physical analog of biological lipid rafts. To gain insights into the structure and organization of biological membranes, cholesterol-containing model membranes have been widely studied experimentally and in simulations. Cholesterol consists of a 3β-hydroxyl group, a rigid sterol ring, and a flexible aliphatic chain. Cholesterol's ability to enhance ordering of lipid hydrocarbon chains, or its condensing effect,[5-7] was suggested to arise from the tilting of the sterol ring.[8-10] Other studies showed that the flat α-face of cholesterol preferentially interacts with saturated tails of high-melting lipids while the rough β-face has no preference for tail unsaturation.[5, 6, 11, 12]

Inter-leaflet coupling is important for membrane structural integrity and information transmission,[13-16] domain registration[17-20] and other functions. For example, trans-bilayer lipid interactions that couple outer-leaflet long-chain lipids with inner-leaflet phosphatidylserine were found to modulate protein clustering.[21] An excellent review by Nickels and

coworkers[14] enumerates some of the key factors that regulate inter-leaflet coupling and domain registration, including bilayer asymmetry, transmembrane protein content, acyl chain interdigitation, composition-dependent curvature, line tension, electrostatics and cholesterol flip-flop. For example, Galimzyanov *et al*. proposed that inter-leaflet coupling is regulated by membrane deformation and line tension,[22] in agreement with a subsequent simulation study.[23] Similarly, cholesterol flip-flop between leaflets was suggested to modulate domain registration.[24, 25] Moreover, several studies examined domain registration at the atomic/near-atomic level using all-atom (AA) or coarse-grained (CG) molecular dynamics (MD) simulations. These suggested that differences in acyl chain inter-digitation[18] and length[26] drive inter-leaflet anti-registration. However, to the best of our knowledge, there has been no detailed study on how or if the rate of cholesterol flip-flop might affect domain registration.

Systematic experimental examination of cholesterol flip-flop rates can be complicated by the difficulties in accurate quantification of the degree of domain registration, and by the spontaneous oxidation of cholesterol and its analogues[27] that may have different flip-flop rates[10]. Molecular simulations enable direct modulation of cholesterol flip-flop rates via the use of artificial cholesterol models with varying aliphatic chain lengths, which can have different rates of flip-flop due to steric effects. Therefore, these can serve as a suitable model system to quantitatively characterize the effect of cholesterol aliphatic chain on membrane domain dynamics and inter-leaflet coupling by CGMD, a molecular simulation approach that allows access to long timescale processes such as raft formation and inter-leaflet coupling.[26, 28]

Model and Methods

In the current work we have simulated four different three-component membrane systems (DPPC/DLiPC/Sterols) using the standard Martini CG force field (version 2.1) [29, 30], which maps, on average, four heavy atoms into one single interaction bead. Details of the force field can be found in ref [29], and the feasibility of the force field especially for lipid membrane systems has been widely validated[31]. In order to study the effects of cholesterol aliphatic chain length, we designed model sterols of different aliphatic chain lengths by adding 1, 2, 3 or 4 CG beads (i-C4, i-C8, i-C12, i-C16) to the sterol ring of natural cholesterol (CHOL), which we named as CHOL1, CHOL2 (natural cholesterol), CHOL3 and CHOL4 (**Fig 1a**). Parameters for these cholesterol analogs with different aliphatic chain lengths were derived from the parameters of cholesterol (CHOL2) and lipid acyl chains in Martini CG model[29]. Each of the four three-component membrane systems consisted of 624 DPPC (dipalmitoylphosphatydilcholine, 50%), 374 DLiPC (dilinoleaylphosphatydilcholine, 30%), 250 sterols (20%), 21494 CG water, 0.15M NaCl. Initial models were constructed by randomly placing lipids in a planar bilayer and running 100ns CGMD simulations at T=400K, which helped to achieve complete lipid mixing. For brevity, we will refer to these systems also as *system* CHOL1, CHOL2, CHOL3 and CHOL4.

The MD simulations were conducted as follows. For van der Waals (vdW) interactions, the Lenard-Jones potential was smoothly shifted to zero between 0.9 nm and 1.2 nm (cutoff) to reduce cutoff noise. For electrostatic interactions, the columbic potential, with a cutoff of 1.2 nm, was smoothly shifted to zero from 0 to 1.2 nm. The default dielectric constant of 15 was used.[29] Simulations were conducted in the isothermal-isobaric ensemble (NPT ensemble). Temperature was controlled by V-rescale heat baths[32] at $T = 298K$ with the constant $\tau = 1\ ps$ for lipids and water/ions separately. Pressure was kept at $1\ bar$ by semi-isotropic Parrinello-

Rahman pressure coupling scheme[33] with $\tau = 5ps$ and compressibility of $3 \times 10^{-4}\ bar^{-1}$. The neighbor list for non-bonded interactions was updated every 10 steps with a cut-off of 1.4 nm. Simulations with periodic boundary conditions were performed by GROMACS 4.5.4[34] for 5 μs with a time step of 20 fs. The effective time (4 times simulation time) was used for analysis in current work. And details of the analysis techniques are provided as supporting information. All snapshots in this work were rendered by VMD[35].

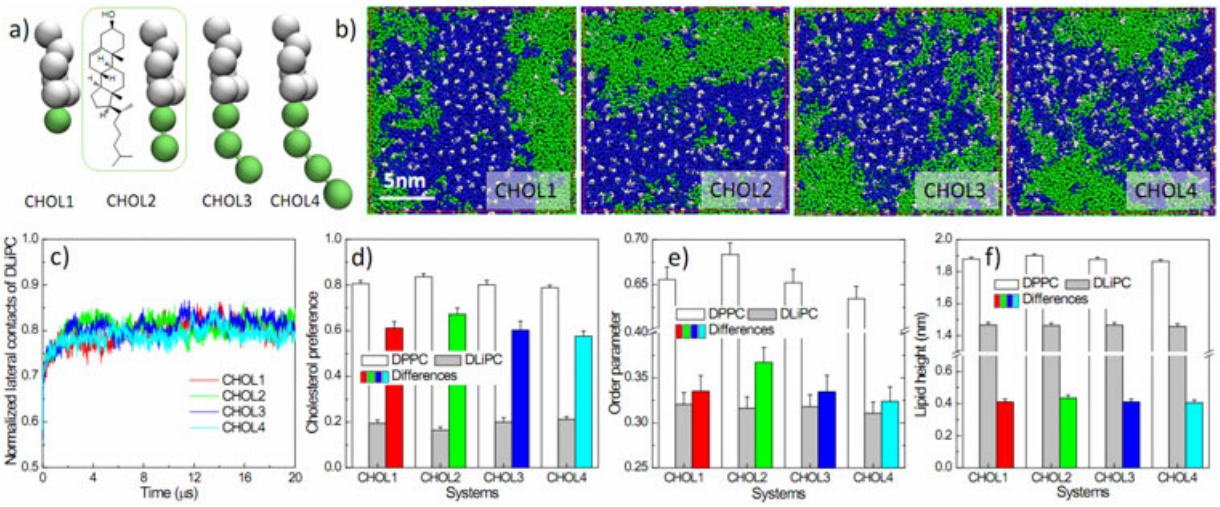


Figure 1. **(a)** Martini configuration of cholesterol with different aliphatic chain lengths. The chemical structure of natural cholesterol is shown for reference. **(b)** Snapshots at $t = 20\mu s$ of the four systems with increasing cholesterol aliphatic chain length (CHOL1, CHOL2, CHOL3 and CHOL4) with DPPC colored in blue, DLiPC in green and cholesterol in white). **(c)** Time-evolution of normalized lateral contacts between unsaturated lipids. **(d-f)** Normalized cholesterol preference **(d)**, order parameter **(e)** and lipid height **(f)** of saturated DPPC lipids, unsaturated DLiPC lipids and differences between the two. Results in **(d-f)** are based on the last 8μs of the 20μs data and error bars represent standard deviation.

Results and Discussion

The final snapshot of each system shown in **Fig. 1b** indicates that all four cholesterol models enabled lipid phase separation. To quantify potential differences in the extent of lipid phase separation, we estimated relative domain sizes by the normalized number of lateral contact among the unsaturated DLiPC lipids. **Fig. 1c** shows that the aliphatic chain length of cholesterol has no appreciable effect on domain size. This is further supported by the lack of effects on the rigid sterol ring orientation (**Fig. S2b**), an important indicator of condensation/ordering of lipids.[8-10] Furthermore, cholesterol preferences, order parameters, and heights of the saturated DPPC and the unsaturated DLiPC lipids are all comparable among the different sterol systems (**Fig. 1d-f**). Taken together, these data clearly show that cholesterol aliphatic chain length has little effect on membrane domain formation. This is consistent with previous observations. For example, diplopterol -- a cholesterol analog with a shorter aliphatic chain and more complex head group – has the same capacity as cholesterol to facilitate L_o phase formation in model membranes.[36]

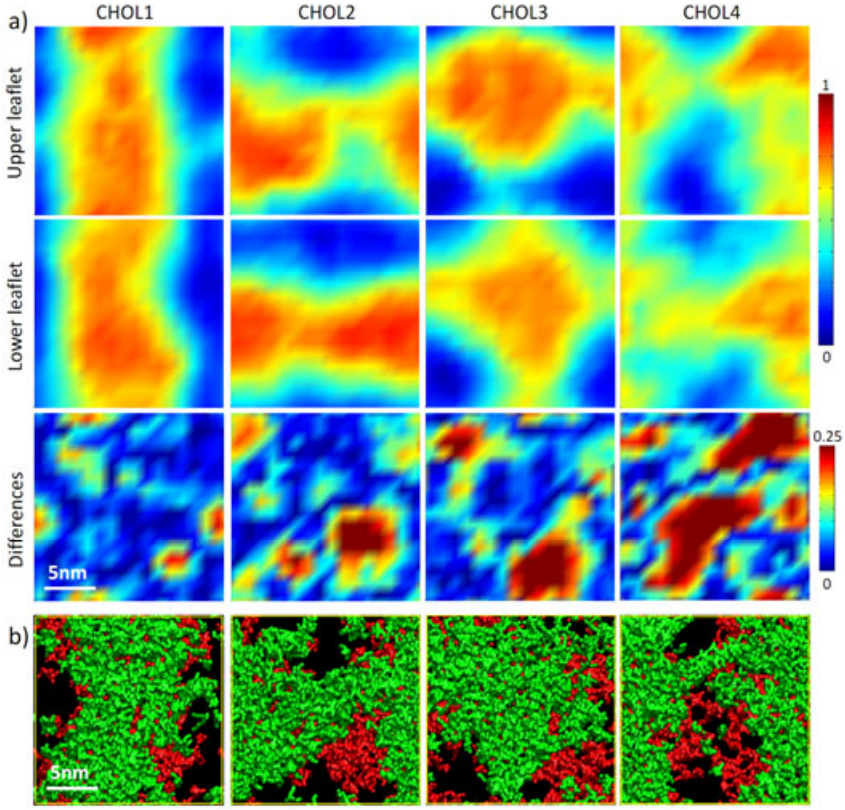


Figure 2. **(a)** Two-dimensional (2D) DPPC localization probability maps of the upper and lower leaflets (P_{upper}, P_{lower}) and their absolute differences ($|P_{upper} - P_{lower}|$) based on data from the last 8μs of a 20μs simulation. **(b)** Top-view of snapshots illustrating domain anti-registration using (for clarity) only DPPC molecules with upper leaflet in green and lower leaflet in red.

To examine the effect of cholesterol aliphatic chain length on domain registration, we analyzed the two-dimensional (2D) DPPC localization probability maps for the upper and lower leaflets, and the differences between the two (**Fig. 2a**). In these maps, red regions represent highly DPPC-enriched areas, i.e. L_o domains, while blue regions represent disordered membrane domains that are largely devoid of DPPC molecules. The intermediate color regions (cyan-green-yellow) can be regarded as domain boundaries whose width is a correlate for the dynamics of the lipid domains. **Fig. 2a** shows that the boundary width subtly increases from CHOL1 to CHOL4, suggesting that longer cholesterol aliphatic chains enhance domain dynamics. More strikingly,

aliphatic chain length seems to promote membrane domain anti-registration, as can be seen from the 2D maps of DPPC number density differential between the upper and lower leaflets (also **Fig. S5**). **Fig. 2b** shows snapshots illustrating increased domain anti-registration (warmer colors) in membranes containing cholesterol analogs with longer aliphatic chains (see also *Supplementary movie S1-S4*). This observation is also supported by the average number of contact between DPPC molecules across the membrane mid-plane (**Fig. 3a**). Thus, our CGMD results show a clear relationship between cholesterol aliphatic chain length and membrane domain registration.

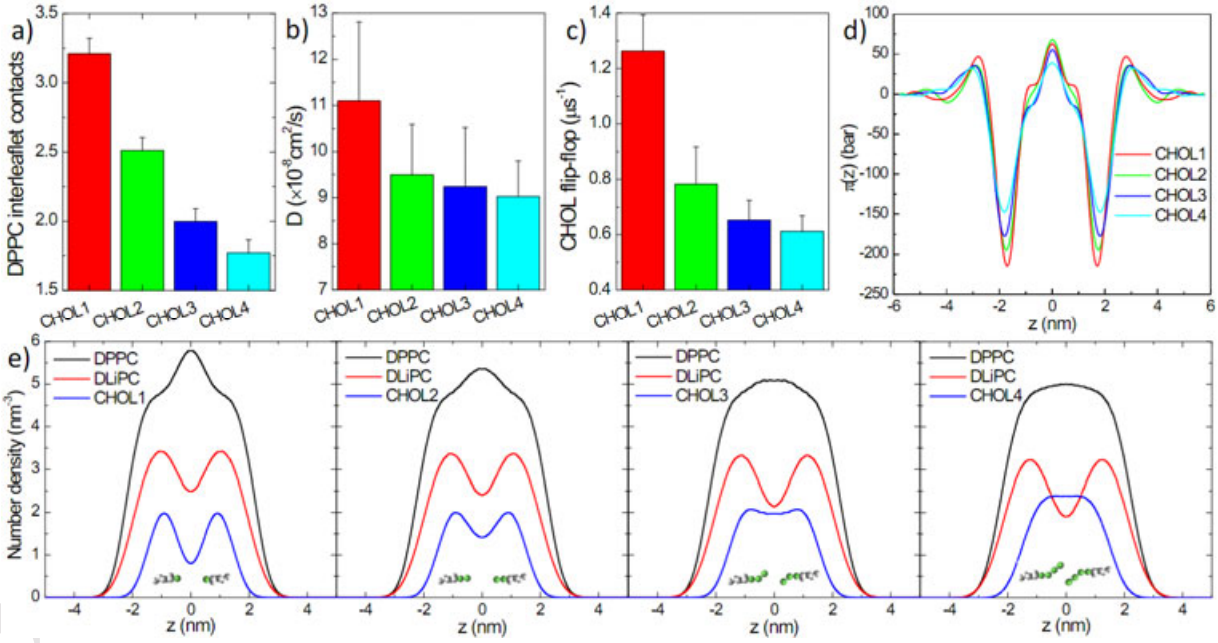


Figure 3. **(a)** Number of DPPC beads in one leaflet that are in contact with DPPC beads in the other leaflet across the bilayer mid-plane, normalized by the number of DPPC molecules per leaflet. **(b)** 2D diffusion coefficient. **(c)** Rate of cholesterol flip-flop. **(d)** Lateral pressure profile across the bilayer. **(e)** Number density of each lipid species across the bilayer, which monitors the effect of cholesterol aliphatic chain length on lipid packing. The last 8μs of the trajectories was used. Error bars represent standard deviation.

In order to examine the mechanism by which cholesterol aliphatic chain length modulates interleaflet coupling and domain registration (**Fig. 2** and **Fig. 3a**), we focused on the dynamics of the four cholesterol analogs and lipid packing, which are thought to be responsible for domain dynamics[18, 20, 24, 37]. As shown in **Fig. 3b, c**, there was a statistically significant decrease in cholesterol diffusion and flip-flop rate upon increasing aliphatic chain length, as might be expected from the increased molecular mass and complexity engendered by a longer aliphatic chain. Sterols have preferred interactions with the saturated DPPC lipids (**Fig. 1f**) and therefore mainly localize in the L_o domain (**Fig. 1b**). Their enhanced diffusion and flip-flop might be intuitively assumed to correlate with a general increase in the dynamics of the L_o domain, and therefore to a potentially weaker interaction at the membrane mid-plane. However, inter-leaflet DPPC contact is highest for CHOL1 (Fig 3a) despite its fastest rates of diffusion and flip-flop (Figs 3a,b). In fact, the rates of lateral diffusion and flip-flop of sterols appear to be anti-correlated with the interaction of saturated lipids across the bilayer mid-plane (Figs 3a-b), suggesting that sterol dynamics is not the driving force of domain registration.

To check if cholesterol aliphatic chain length affects lipid packing and lateral pressure, two physical parameters that are closely associated with membrane structure,[38-41] we analyzed the average number density of lipids and pressure profiles (see refs [42-44]) along the membrane normal. We found that the magnitude of the lateral pressure progressively decreases with increasing cholesterol aliphatic chain length **(Fig. 3d**). This is accompanied by a small but significant progressive increase in membrane thickness as measured by the shift in the location of the peaks in **Fig. 3d**. Combined with the absence of any effect on the average height of each bilayer component (**Fig. 1f**), this result suggests reduction in inter-leaflet interaction at the bilayer mid-plane (i.e. reduced acyl chain interdigitation). The impact of cholesterol aliphatic

chain length on lipid packing is captured most vividly by the number density plots shown in **Fig. 3e**, where the density of DPPC and DLiPC beads at the bilayer center (z=0) dramatically decreases while that of the sterol increases with increasing aliphatic chain length. We confirmed the impact of cholesterol aliphatic chain length on lipid acyl chain inter-digitation by quantifying the overlap areas in the number densities of the upper and lower leaflet DPPC and DLiPC lipids. As shown in **Fig. 4**, there is a significant decrease in the overlap areas upon increasing the aliphatic chain length of the sterols. We conclude that inter-leaflet acyl chain inter-digitation, which decreases upon increasing cholesterol aliphatic chain length, is the primary driver of the enhanced domain anti-registration upon increasing the aliphatic chain length of model sterols.

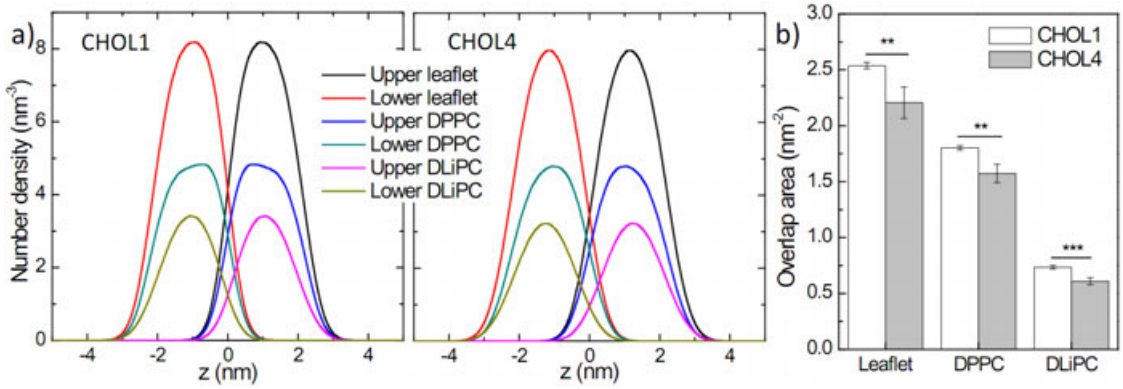


Figure 4. **(a)** Number density of upper and lower leaflet DPPC and DLiPC lipids for systems CHOL1 and CHOL4. **(b)** Overlap area between the upper and lower leaflet total, DPPC and DLiPC number densities for systems CHOL1 and CHOL4. The longer sterol aliphatic chain length (CHOL4) significantly reduced the overlap areas at the center of the membrane and thus reduced lipid acyl chain interdigitation. Data are mean ± SD from four independent simulations and were analyzed by Student's t-test. **p < 0.01, ***p < 0.001.

Conclusion

In summary, we used coarse-grained MD simulations to quantitatively characterize the effect of cholesterol aliphatic chain length on lipid domain stability and dynamics using a three-component model membrane made up of DPPC, DLiPC and analogs of cholesterol with various aliphatic chain lengths. Consistent with a previous AAMD simulation[45] and experiment [46], our simulations indicated that cholesterol's aliphatic chain length has little effect on phase separation but significantly alters inter-leaflet coupling and domain registration. A major source of this effect is the impact of the aliphatic chain on interaction of lipids across the bilayer mid-plane. Inter-leaflet coupling and domain registration play important roles in transmission of signal across the membrane[21]. Our results provide insights into how this process can be modulated by the aliphatic tail of cholesterol.

Supporting Information

Detailed analysis methods, additional figures and movies.

Acknowledgment

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Author contributions

XL, AAG, SZ, and IL designed the study; XL, SZ, and HD analyzed the data; XL, AAG, SZ and IL wrote the manuscript.

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