Published in final edited form as:

FEBS Lett. 2016 October ; 590(19): 3368–3374. doi:10.1002/1873-3468.12383.

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

Xubo Lin^{#1}, Siya Zhang^{#2,3}, Hui Ding^{1,4}, Ilya Levental^{1,*}, and Alemayehu A. Gorfe^{1,*}

¹Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Texas 77030, USA

²State Key Laboratory of Medical Molecular Biology, School of Basic Medicine, Peking Union Medical College and Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China

³Department of Immunology, The University of Texas MD Anderson Cancer Center, Texas 77054, USA

⁴Department of Respiratory Medicine, Yixing Hospital affiliated Jiangsu University, Jiangsu 214200, China

These authors contributed equally to this work.

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.

Graphical abstract

*Correspondence to alemayehu.g.abebe@uth.tmc.edu (AAG) or ilya.levental@uth.tmc.edu (IL).

Supporting Information

Detailed analysis methods, additional figures and movies.

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.

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 (dipalmitoylphosphatidylcholine, 50%), 374 DLiPC (dilinoleoylphosphatidylcholine, 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 $\tau = 5$ ps and compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$. 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 μ s 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].

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]

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.

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.

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank two anonymous reviewers for insightful suggestions. We thank the National Institutes of Health (RO1 GM100078) and the Cancer Prevention and Research Institute of Texas (R1215) for financial support, Extreme

Science and Engineering Discovery Environment (XSEDE, project: TG-MCB150054) and Texas Advanced Computing Center (TACC) for computational resources.

References

1. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science*. 2010; 327:46–50. [PubMed: 20044567]
2. Ding, H. m.; Ma, Y. q. Theoretical and Computational Investigations of Nanoparticle–Biomembrane Interactions in Cellular Delivery. *Small*. 2015; 11:1055–1071. [PubMed: 25387905]
3. Brown DA. Lipid rafts, detergent-resistant membranes, and raft targeting signals. *Physiology*. 2006; 21:430–439. [PubMed: 17119156]
4. Simons K, Ehehalt R. Cholesterol, lipid rafts, and disease. *J Clin Invest*. 2002; 110:597–603. [PubMed: 12208858]
5. Róg T, Vattulainen I. Cholesterol, sphingolipids, and glycolipids: What do we know about their role in raft-like membranes? *Chem Phys Lipids*. 2014; 184:82–104. [PubMed: 25444976]
6. Krause MR, Regen SL. The Structural Role of Cholesterol in Cell Membranes: From Condensed Bilayers to Lipid Rafts. *Acc Chem Res*. 2014; 47:3512–3521. [PubMed: 25310179]
7. Li, Z.-l.; Wang, J.-j.; Ding, H.-m.; Ma, Y.-q. Influence of different membrane environments on the behavior of cholesterol. *RSC Advances*. 2014; 4:53090–53096.
8. Aittoniemi J, Róg T, Niemelä P, Pasenkiewicz-Gierula M, Karttunen M, Vattulainen I. Tilt: major factor in sterols' ordering capability in membranes. *J Phys Chem B*. 2006; 110:25562–25564. [PubMed: 17181184]
9. de Joannis J, Coppock PS, Yin F, Mori M, Zamorano A, Kindt JT. Atomistic simulation of cholesterol effects on miscibility of saturated and unsaturated phospholipids: implications for liquid-ordered/liquid-disordered phase coexistence. *J Am Chem Soc*. 2011; 133:3625–3634. [PubMed: 21341653]
10. Neuvonen M, Manna M, Morkkila S, Javanainen M, Rog T, Liu Z, Bittman R, Vattulainen I, Ikonen E. Enzymatic oxidation of cholesterol: properties and functional effects of cholestenone in cell membranes. *Plos One*. 2014; 9:e103743. [PubMed: 25157633]
11. Sodt AJ, Sandar ML, Gawrisch K, Pastor RW, Lyman E. The Molecular Structure of the Liquid Ordered Phase of Lipid Bilayers. *J Am Chem Soc*. 2014; 136:725–732. [PubMed: 24345334]
12. Toppozini L, Meinhardt S, Armstrong CL, Yamani Z, Ku erka N, Schmid F, Rheinstädter MC. Structure of Cholesterol in Lipid Rafts. *Phys Rev Lett*. 2014; 113:228101. [PubMed: 25494092]
13. Chaudhuri A, Chattopadhyay A. Transbilayer organization of membrane cholesterol at low concentrations: Implications in health and disease. *Biochim Biophys Acta-Biomembr*. 2011; 1808:19–25.
14. Nickels JD, Smith JC, Cheng X. Lateral organization, bilayer asymmetry, and inter-leaflet coupling of biological membranes. *Chem Phys Lipids*. 2015; 192:87–99. [PubMed: 26232661]
15. Devaux PF, Morris R. Transmembrane asymmetry and lateral domains in biological membranes. *Traffic*. 2004; 5:241–246. [PubMed: 15030565]
16. May S. Trans-monolayer coupling of fluid domains in lipid bilayers. *Soft Matter*. 2009; 5:3148–3156.
17. Putzel GG, Uline MJ, Szleifer I, Schick M. Interleaflet coupling and domain registry in phase-separated lipid bilayers. *Biophys J*. 2011; 100:996–1004. [PubMed: 21320444]
18. Polley A, Mayor S, Rao M. Bilayer registry in a multicomponent asymmetric membrane: Dependence on lipid composition and chain length. *J Chem Phys*. 2014; 141:064903. [PubMed: 25134595]
19. Kiessling V, Crane JM, Tamm LK. Transbilayer effects of raft-like lipid domains in asymmetric planar bilayers measured by single molecule tracking. *Biophys J*. 2006; 91:3313–3326. [PubMed: 16905614]
20. van Meer G. Dynamic transbilayer lipid asymmetry. *Cold Spring Harbor Perspect Biol*. 2011; 3:a004671.

21. Raghupathy R, Anilkumar AA, Polley A, Singh PP, Yadav M, Johnson C, Suryawanshi S, Saikam V, Sawant SD, Panda A. Transbilayer Lipid Interactions Mediate Nanoclustering of Lipid-Anchored Proteins. *Cell*. 2015; 161:581–594. [PubMed: 25910209]
22. Galimzyanov TR, Molotkovsky RJ, Bozdaganyan ME, Cohen FS, Pohl P, Akimov SA. Elastic Membrane Deformations Govern Interleaflet Coupling of Lipid-Ordered Domains. *Phys Rev Lett*. 2015; 115:088101. [PubMed: 26340212]
23. Tian J, Nickels JD, Katsaras J, Cheng X. The Behavior of Bilayer Leaflets in Asymmetric Model Membranes: Atomistic Simulation Studies. *J Phys Chem B*. 2016
24. Collins MD. Interleaflet coupling mechanisms in bilayers of lipids and cholesterol. *Biophys J*. 2008; 94:L32–L34. [PubMed: 18096628]
25. Collins MD, Keller SL. Tuning lipid mixtures to induce or suppress domain formation across leaflets of unsupported asymmetric bilayers. *Proc Natl Acad Sci U S A*. 2008; 105:124–128. [PubMed: 18172219]
26. Perlmutter JD, Sachs JN. Interleaflet interaction and asymmetry in phase separated lipid bilayers: molecular dynamics simulations. *J Am Chem Soc*. 2011; 133:6563–6577. [PubMed: 21473645]
27. Iuliano L. Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem Phys Lipids*. 2011; 164:457–468. [PubMed: 21703250]
28. Risselada HJ, Marrink SJ. The molecular face of lipid rafts in model membranes. *Proc Natl Acad Sci U S A*. 2008; 105:17367–17372. [PubMed: 18987307]
29. Marrink SJ, Risselada HJ, Yefimov S, Tieleman DP, De Vries AH. The MARTINI force field: coarse grained model for biomolecular simulations. *J Phys Chem B*. 2007; 111:7812–7824. [PubMed: 17569554]
30. Bulacu M, Goga N, Zhao W, Rossi G, Monticelli L, Periole X, Tieleman DP, Marrink SJ. Improved angle potentials for coarse-grained molecular dynamics simulations. *J Chem Theory Comput*. 2013; 9:3282–3292. [PubMed: 26584087]
31. Marrink SJ, Tieleman DP. Perspective on the Martini model. *Chem Soc Rev*. 2013; 42:6801–6822. [PubMed: 23708257]
32. Bussi G, Donadio D, Parrinello M. Canonical sampling through velocity rescaling. *J Chem Phys*. 2007; 126:014101. [PubMed: 17212484]
33. Parrinello M, Rahman A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J Appl Phys*. 1981; 52:7182–7190.
34. Hess B, Kutzner C, van der Spoel D, Lindahl E. GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J Chem Theory Comput*. 2008; 4:435–447. [PubMed: 26620784]
35. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *J Mol Graph*. 1996; 14:33–38. [PubMed: 8744570]
36. Sáenz JP, Sezgin E, Schwille P, Simons K. Functional convergence of hopanoids and sterols in membrane ordering. *Proc Natl Acad Sci U S A*. 2012; 109:14236–14240. [PubMed: 22893685]
37. Bennett WD, MacCallum JL, Hinner MJ, Marrink SJ, Tieleman DP. Molecular view of cholesterol flip-flop and chemical potential in different membrane environments. *J Am Chem Soc*. 2009; 131:12714–12720. [PubMed: 19673519]
38. Gullingsrud J, Schulten K. Lipid bilayer pressure profiles and mechanosensitive channel gating. *Biophys J*. 2004; 86:3496–3509. [PubMed: 15189849]
39. Scheve CS, Gonzales PA, Momin N, Stachowiak JC. Steric pressure between membrane-bound proteins opposes lipid phase separation. *J Am Chem Soc*. 2013; 135:1185–1188. [PubMed: 23321000]
40. McCarthy NL, Ces O, Law RV, Seddon JM, Brooks NJ. Separation of liquid domains in model membranes induced with high hydrostatic pressure. *Chem Commun*. 2015; 51:8675–8678.
41. Meinhardt S, Vink RL, Schmid F. Monolayer curvature stabilizes nanoscale raft domains in mixed lipid bilayers. *Proc Natl Acad Sci U S A*. 2013; 110:4476–4481. [PubMed: 23487780]
42. Ollila OS, Risselada HJ, Louhivuori M, Lindahl E, Vattulainen I, Marrink SJ. 3D pressure field in lipid membranes and membrane-protein complexes. *Phys Rev Lett*. 2009; 102:078101. [PubMed: 19257715]

43. Vanegas JM, Torres-Sánchez A, Arroyo M. Importance of force decomposition for local stress calculations in biomembrane molecular simulations. *J Chem Theory Comput.* 2014; 10:691–702. [PubMed: 26580046]
44. Torres-Sánchez A, Vanegas JM, Arroyo M. Examining the Mechanical Equilibrium of Microscopic Stresses in Molecular Simulations. *Phys Rev Lett.* 2015; 114:258102. [PubMed: 26197144]
45. Robalo JR, Ramalho JP, Huster D, Loura LM. Influence of the sterol aliphatic side chain on membrane properties: a molecular dynamics study. *Phys Chem Chem Phys.* 2015; 17:22736–22748. [PubMed: 26255832]
46. Scheidt HA, Meyer T, Nikolaus J, Baek DJ, Haralampiev I, Thomas L, Bittman R, Müller P, Herrmann A, Huster D. Cholesterol's Aliphatic Side Chain Modulates Membrane Properties. *Angew Chem-Int Edit.* 2013; 52:12848–12851.

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 $t = 20\mu\text{s}$ 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 $8\mu\text{s}$ of the $20\mu\text{s}$ data and error bars represent standard deviation.

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 8 μ s of a 20 μ s 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.

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 8 μs of the trajectories was used. Error bars represent standard deviation.

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 $\pm$ SD from four independent simulations and were analyzed by Student's t-test. ** $p < 0.01$, *** $p < 0.001$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript