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Molecular dynamic simulation study of cholesterol and conjugated double bonds in lipid bilayers

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Abstract

Conjugated linoleic acids (CLA) are found naturally in dairy products. Two isomers of CLA, that differ only in the location of *cis* and *trans* double bonds, are found to have distinct and different biological effects. The *cis* 9 *trans* 11 (C9T11) isomer is believed to have anti-carcinogenic effects, while the *trans* 10 *cis* 12 (T10C12) isomer is believed to be associated with anti-obesity effects. In this paper we extend earlier Molecular Dynamics (MD) simulations of pure CLA-phosphatidylcholine bilayers to investigate the comparative effects of cholesterol on bilayers composed of the two respective isomers. Simulations of phosphatidylcholine lipid bilayers in which the *sn*-2 chains contained one of the two isomers of CLA were performed in which, for each isomer, the simulated bilayers contained 10%, and 30% cholesterol (Chol). From MD trajectories we calculate and compare structural properties of the bilayers, including areas per molecule, thickness of bilayers, tilt angle of cholesterol, order parameter profiles, and one and two-dimensional radial distribution function (RDF), as functions of Chol concentration. While the structural effect of cholesterol is approximately the same for both isomers, we find differences at an atomistic level in order parameter profiles and in two-dimensional radial distribution functions.

Keywords

Conjugated linoleic acids; molecular dynamic simulation; cholesterol

1. Introduction

Isomers of linoleic acid that contain conjugated double bonds (conjugated linoleic acids, CLA) are found in dairy products and meats, and are associated with anti-carcinogenic effects, reduction in adiposity, and improvement of immune function [1, 2, 3] in experimental animals. 16 isomers of CLA have been identified in natural products, but two, *cis* 9 *trans* 11 (80%) and *trans* 10 *cis* 12 isomers (10%) dominate in dairy products. The two isomers differ significantly from each other in their biological effects [4, 2, 5, 6], suggesting that the location of the *cis* and *trans* double bonds in the acyl chain plays a critical role in the biological function. The mechanism of action, and the molecular basis for the divergent effects of CLA isomers remain unclear, despite these and other studies in experimental animals and cultured cells. Although it is possible that at least some of the effects of CLA are through modification of membrane structure and function [7, 8, 9, 10] our earlier MD study of pure bilayers composed of the two isomers revealed very little difference in structural and dynamical properties [11]. Since Cholesterol (Chol) comprises approximately 25–40 mol % of the lipid portion of the mammalian plasma membranes, and is thought to modulate the physico-chemical properties required for cell viability and proliferation, it is natural to next consider the interactions of the two isomers with Chol. Numerous experimental studies have demonstrated that Chol also reduces the passive permeability of membranes, increases membrane mechanical strength, and modulates membrane enzymes [12]. Because of the unique distribution of double bonds in the acyl chain, the CLA-containing phospholipids may interact with cholesterol differently from other phospholipids, resulting in the alteration of the overall function of the membrane-associated receptors, enzymes, and channels. To test this hypothesis, we conducted a molecular dynamics (MD) simulation study of bilayers of phosphatidylcholine (PC) which contained a palmitic acid at sn-1 position, and *cis* 9 *trans* 11 CLA, or *trans* 10 *cis* 12 CLA, at the sn-2 position (hereafter referred to as CLA-PC bilayers). We incorporated 10 and 30 mol% of cholesterol into the PC bilayer and studied the atomic level interactions between PC and cholesterol in the bilayer.

Molecular dynamics simulations of phospholipid bilayers are widely used to study the physical properties of specific bilayers with different lipid compositions, cholesterol, or embedded peptide/proteins and achieve good agreement with known experimental properties. Simulations can add atomic level insight into microscopic interactions between lipids, cholesterol, and water [13, 14, 15, 16, 17, 18, 19, 20, 21, 22]. Although the effect of isolated double bonds has been studied by MD simulation [23, 24, 25, 26], no studies have been conducted on the effect of conjugated double bonds on phospholipid bilayers containing cholesterol. In this paper, we focus on linoleic acids *cis* 9 *trans* 11 and *trans* 10 *cis* 12 as these two CLA isomers bear important and interesting physiological functions. We extend our earlier work [11] to study the potential interactions of cholesterol with conjugated double bonds brought to polyunsaturated lipid bilayer. We study the effect of cholesterol on the structure of CLA-PC bilayers of both isomers at different cholesterol concentrations, and we examine CLA-cholesterol interactions in atomistic detail.

2. MD Method

This paper describes MD simulations that follow on earlier simulations of pure CLA-PC bilayers of both *cis* 9 *trans* 11 and *trans* 10 *cis* 12 isomers. [11] Here we summarize simulation methodology and force fields that are described in more detail in the earlier paper. The force-field parameters used for this work were taken from the newly developed GROMOS 43A1-S3 parameter set [27]. For the CLA simulations, we added additional dihedral parameters for dihedrals that contained one or both of the CLA conjugated double bonds to the 43A1-S3 force field. The calculations of these parameters was done following

the procedure used for the 43A1-S3 force field [27]. In our earlier paper [11] we provided a detailed description of the procedure. For bonding parameters not part of the -CH=CH-CH=CH- dihedral we used existing parameters in the 43A1-S3 force field, including parameters for the dihedrals that are next to, but do not include, double bond carbons. In supplemental material we provide the energy profiles for double bonds that verify that we have included skew states as emphasized by the work of Bachar et al [23]. All force field parameters used in this paper can be uploaded from URLs given in the supplemental information.

Figure 1 shows the structures and numbering schemes for each of the CLA-PC isomers and for cholesterol. The total number of lipids in each bilayer was 200, or 100 per leaflet. The simulations were constructed as described earlier. [11] After building CLA-PC lipids for both C9T11 and T10C12 from a POPC molecular structure, bilayers were constructed by placement of 100 CLA-PC molecules per leaflet. Then, randomly selected CLA-PC molecules were replaced with Chol molecules for each simulated Chol concentration. The bilayers were hydrated with 6800 SPC-E water molecules, for a hydration level of 34 water molecules per lipid. A 200 ps MD simulation was performed on each system at 500 K. This was done to ensure proper disordering of the hydrocarbon chains. Temperatures were then brought down to the target temperature of 298 K in steps of 50 K. At each temperature step a small 200 ps MD simulation was performed on each system. Next, the systems were simulated for 180 ns of MD with regeneration of velocities from a Maxwellian distribution at 298 K after every 200 ps. Then 100 ns continuous MD trajectories were generated and used for analysis. To verify that simulation results were not dependent on initial conditions, we built two additional systems with different initial bilayer configurations, including one larger system (400 lipids), and we used a longer annealing time. All simulations were performed using the GROMACS 4.05 package [28]. The LINCS algorithm was used to constrain all the bonds in the system. [29] The integration time step was 2 fs. Periodic boundary conditions were applied in all three dimensions and long range electrostatics were calculated using the PME algorithm [30] with a real space cutoff of 10 Å. A cutoff of 16 Å was employed for van der Waals interactions, as is required for the 43A1-S3 force field. All simulations were performed at 298 K using the Nose-Hoover temperature coupling scheme. The system was simulated in an NPT ensemble using the Parrinello-Rahman pressure coupling scheme at a constant pressure of 1 bar. The systems were energy minimized to remove bad contacts. Calculated properties from those runs are quantitatively identical to results presented here. Throughout the simulations we monitored the dimensions of the simulation cells. The area per lipid in all simulations was stable, with fluctuations of ± 2.5 Å²/mol, as shown in Figure 2.

3. MD Results and discussion

Structural properties

Table 1 lists the values of the simulated areas per molecule for both isomers at 0% (from [11]), 10%, and 30% Chol. The molecular areas were calculated by dividing the simulation box area by the number of lipids per leaflet. Corrections for undulation fluctuations [31] were not made as the system sizes are sufficiently small that these terms should be small, and in any case should be the same for all systems we simulated. For binary CLA-Chol simulations, one could alternatively calculate partial molecular areas for CLA and Chol separately using the method described by Nagle and Edholm [32] but this requires at least 3–4 Chol concentrations, and the partial areas are not measured experimentally. Thus Figure 2 shows time traces, over the duration of the production runs, of the area per molecule calculated from the area of the simulation boxes for each simulation. The figure shows that, for each Chol concentration, the simulated areas per molecule of the two isomers are the same within error bars. For comparison with monolayer data we use here the total area per

molecule, that is, the area of the simulation box divided by the total number of lipids plus Chol. Figure 2 shows that the simulated areas of the two isomers are the same for pure CLA, CLA plus 10% Chol, and CLA plus 30% Chol. This means that the simulated “condensation effect” of Chol is approximately equal for both isomers.

There are no experimental data for the molecular areas of CLA-PC bilayers, so we compare the simulation results with published experimental observations of CLA-Chol monolayers. [33] Monolayer isotherms, at a temperature of 24 °C and a pressure of 15 mN/m, yield areas per molecule for both CLA isomers, with Chol concentrations of 0%, 10%, and 30%, that are comparable to the simulated areas in Figure 2. Figure 3 shows experimental isotherms (3a), molecular areas at 15 mN/m (3b), and Chol-induced condensation at 15 mN/m (3c). Condensation percentages in the monolayer experiments are calculated as described in [33], and utilize experimental areas for pure CLA and for pure Chol monolayers. Since pure Chol molecular areas are not available for the simulations, we simply compare differences in condensation percentages for each isomer at each Chol concentration with the simulation prediction that those should be very small for each isomer.

While the simulations predict that the condensation effect is the same for both isomers at 10% and 30% Chol, the experimental condensations differ somewhat for the isomers. At 15 mN/m the experimental condensation of C9T11(≈2%) is less than that of T10C12 (≈9 %) at 10% Chol. At 30% Chol the experimental condensation of C9T11(≈12%) is still less than that of T10C12(≈14 %) although the difference is smaller. While we did not run simulations at 50% Chol, we note that, at this higher Chol percentage, the experimental trend in the condensation effect is reversed, with C9T11 having a slightly greater condensation than T10C12. Possible sources of differences between experiment and simulation are likely due to (1) the inexact correspondence between monolayer and bilayer states (the monolayer chains do not interact or interdigitate with a second leaflet), and (2) a possible long time equilibration required for the mixed lipid-Chol bilayer simulations. In earlier work [19] we carried out a similar set of simulations of POPC and Chol. In that simulation, the area per molecule (again, the box area divided by half the total number of molecules) was 56.7 Å² for POPC with 10% Chol, and 44.5 Å² at 30 % Chol. Not surprisingly, the presence of a second double bond leads to an increase in the molecular areas for the CLA isomers, compared to POPC.

Lipid chain conformational flexibility can be characterized by order parameter profiles, where the order parameter for carbon m in a chain is

$$s(m) = \left\langle \left(\frac{3}{2} \cos^2 \theta_m - \frac{1}{2} \right) \right\rangle \quad (1)$$

Here, θ_m is the angle between the C-H bond vectors for carbon m . [34] Figure 4 shows the segmental deuterium order parameters for C9T11 and T10C12 chains. As is commonly observed in lipid-cholesterol simulations and experiments, the effect of Chol is to generally increase the values of the segmental order parameters, and this is found in Figure 4 as well. For the sn-1 chain, the two isomers have order parameter profiles shifted by the same amount at 30% Chol, but at 10% Chol the T10C12 sn-1 chain is slightly less affected by Chol. The same Chol-induced increase in order parameter values is found for the sn-2 chains except at the *cis* double bond positions. At these positions, the size of the dip in the order parameters due to the *cis* double bond is increased by the presence of Chol for both isomers. We note that, as in [19] C9T11 has the characteristic *cis* double bond dip in the order parameter at the C9 carbon position, while for T10C12 this dip is found at the C13 carbon position. The effect of Chol on the *cis* bond dip is to increase the magnitude of the decrease

in order, and the decrease is greater for T10C12 compared to C9T11. This observation is consistent with analysis from 2-dimensional radial distribution functions to be discussed below, that Chol is able to pack more closely to the T10C12 chain as the *trans* double bond is at a location nearer to the Chol rings. An additional result of interest in Figure 4b (and in Figure 6 of [11]) is that the location of the dip in the order parameter at the *cis* double bond appears at the carbon that is not proximal to the *trans* double bond, namely at C9 for C9T11, and at C13 for T10C12. This is due to the enhanced rigidity of the *trans* double bonds. Figure 4b also shows that the order of the *trans* double bond carbons is different from those of saturated carbons in the “plateau” region. This can be seen by comparing the plateau regions of the profiles in Figures 4a and 4b. For the saturated chains the difference profiles have the characteristic monotonically smooth and curved shape through this region while the CLA chains have flatter profile adjacent to the *cis* double bonds.

Table 1 lists bilayer thicknesses calculated from the widths of the phosphate peaks in electron density profiles (not shown) from the simulations. In cholesterol free systems, the bilayer thickness for C9T11 is 39Å, which is about 0.7 Å thicker than that of T10C12. [11] As Chol condenses the lipid areas, the thickness of bilayers increases for both isomers. Table 1 data show that the thickness of the T10C12 bilayers is now larger than that of the C9T11 bilayers at 10% and 30% Chol. The thickness difference is just outside of estimated uncertainties in thickness in the simulations. Given that the areas of the simulation boxes are the same for both isomers at both Chol levels one possibility is that there is less inter-leaflet interdigitation in T10C12 than in C9T11, as Chol is added to the bilayers. However electron and mass density plots (not shown) do not reveal a significant differences in interdigitation for the two isomers.

It is of interest to examine the average Chol tilt for the C9T11 and T10C12 isomers. We calculated tilt angles of cholesterol molecules and show the distributions for the two isomers in Figure 5. The tilt angle was defined by angle between vector of C17 and C3 carbons and the bilayer normal (*z* axis). From Figure 5, at 10% Chol, C9T11 bilayer has the largest Chol tilt angle (based on the peak of the distribution) but the difference is small. At 30%, the tilt angle distributions for the two isomers are nearly identical.

In general one expects the addition of Chol to a lipid bilayer will lead to slower lateral motions as measured by diffusion constants. We have calculated lateral diffusion constants (not shown) using the same windowing averages as described in [11]. We indeed find that lateral diffusion is slowed by the presence of Chol in the bilayers, but, within error bars, the results do not depend on the order of the CLA double bonds.

In order to examine atomistic level interactions between Chol and CLA, we calculated one dimensional and two-dimensional radial distribution functions (RDF) between Chol and carbons around double bonds for each isomer. Figure 6 shows one-dimensional radial distribution functions between the C13 Chol carbon (upper left and right), and between the C18 Chol carbon (middle left and right) and the C23 thru C27 carbons of C9T11 and T10C12. The lower two figures in Figure 6 show RDFs between the C18 Chol carbon and the C44 thru C48 carbons of C9T11 and T10C12. RDF's were calculated from both the 10% and 30% Chol-CLA trajectories, with the results from the 30% runs displayed in Figure 6. The Chol C13 carbon was chosen for these calculations as it can be presumed to interact closely with the conjugated double bonds in both isomers. The Chol C18 carbon was chosen because it is the uppermost methyl carbon on the rough side of Chol, and can also be presumed to interact closely with the conjugated double bonds in both isomers. The profiles for each Chol reference look quite similar for the two isomers. Figures 6 upper left and upper right, exhibit a first peak in all of the plots located 6 Å away from cholesterol C13. The height of the first peak for T10C12 rdf's for CLA carbons 23 – 26 (but not 27) is about

1% higher than that of C9T11. Although the difference is small, it suggests that T10C12 is able to locate more closely to Chol. Figures middle left and middle right, show RDFs between Chol 18 and CLA chain carbons, and exhibit double peaks indicating two well organized neighbor shells around the Chol C18, but again with little difference between the two isomers. We have calculated RDFs between other Chol reference atoms and the CLA carbons around the double bonds, and find in all cases that there is little or no difference in the structure of the peaks and neighbor shells between C9T11 and T01C12. For comparison, we also show RDF's between Chol C18 and carbons on the sn-1 saturated chains in Figure 6, bottom left and bottom right. The carbons chosen for these RDF's are at the same relative chain positions as those used for the sn-2 chain RDF's in the upper Figures 6. These RDF's show the same double peaks that are found in the middle figures (6), due to the C18 methyl group, and they show no clear difference between the two CLA isomers.

For anisotropic molecules, traditional RDF's do not tell the full story of lateral correlations. Cholesterol, for example, is asymmetric, with a smooth α face to the ring region and a rough, methylated β face on the opposite side of that region. One-dimensional RDF's average out any details of the asymmetry in the correlations. To examine this asymmetry we calculated two-dimensional distribution functions. These distributions are constructed as described by Pandit et al [35]. The bivariate correlation function $g(r, \varphi)$ between designated carbon atoms of C9T11 and T10C12, respectively, and Chol, defined by

$$g(r, \varphi) = \frac{N(r, \varphi)}{2\pi r \rho \delta r \delta \varphi} \quad (2)$$

ρ is the overall number density of the designated carbons, and φ is the angle made by the interatomic distance vector with respect to the positive x axis (which orients perpendicular to the ring plane and points towards the rough face) of the Chol body coordinate frame. [35] $N(r, \varphi)$ is the number of designated carbon atoms of lipids in an area element $r\delta r\delta\varphi$ at the point (r, φ) measured from the $x - y$ position of the Chol C10 atom, which serves as the origin of the coordinate system with the x axis pointing towards the Chol C19 atom. In the following figures the Chol is the light central region with the β face pointing to the right. Figure 7 shows the 2-d RDF's for which the designated lipid atoms are the C24 carbons for both isomers at 30% Chol. As can be seen in Figure 1, the C24 carbon is part of the *cis* double bond in C9T11, and just above the *trans* double bond in T10C12. Therefore these 2-d RDFs reveal details of the interactions between Chol and the double bond regions of both isomers. As revealed by the color-coded local densities in Figure 7, there is a clear differences in the packing of C9T11 compared to T10C12. With the *trans* bond beginning at the C25 position on the sn-2 chain the T10C12 isomer is able to pack more closely around the cholesterol molecules, at both the smooth and the methylated faces. The first neighbor shell is less smooth for the C9T11 isomer, indicative of greater steric hindrance to packing between the *cis* double bond carbons and Chol. For the C9T11 isomer, the second neighbor shell is more clearly outlined in Figure 7 than for T10C12. For comparison, Figure 8 shows 2-d RDF's for which the designated lipid atoms are the C45 carbons on the saturated chains for both isomers. In these 2-D RDFs the first neighbor shells are almost identical for both isomers. However, subtle differences emerge upon examination of the second neighbor shells. The second neighbor shells for T10C12 are indicative of a higher level of structure compared to those for C9T11. We note that the statistics for the higher order shells are compromised by the limited numbers of Chol molecules in the simulations.

2D RDF's have been used to characterize lipid-Chol interactions for other PC lipids. This technique was first introduced by Pandit et al [35] for the study of di-oleylol phosphatidylcholine (DOPC) -Sphingomyelin-Chol mixtures. In that study 2D-RDF's

similar to those in Figure 6 were found where the saturated SM chains packed preferentially along the smooth face of Chol leaving the unsaturated DOPC chains to pack at the edges of the Chol rings and in the vicinity of the rough Chol face. Martinez-Seara et al [26] calculated 2D RDFs between Chol and di-stearoyl phosphatidylcholine (DSPC). Martinez-Seara et al defined the 2D RDF with the x axis set as the C13–C18 vector, and they used the in-plane distances of all DSPC chain carbons in the for binning. The inclusion of all carbons improves the statistics of the binning, so that clear hydration shells are seen, and the excluded volume effect due to the Chol ring methyls is clear. For Figures 7 and 8, we use only single CLA carbons, which results in poorer statistics, but specifically examines the correlation between the double bond region of the sn-2 chains and the Chol ring.

In experimental studies, Subbaiah et al [33] find that the T10C12 isomer has decreased membrane permeability to carboxyfluorescein, and is less likely to be oxidized by free radicals, compared to C9T11. These membrane properties can be qualitatively correlated with CLA-Chol packing at an atomistic level since tight packing can act to inhibit local interactions between membrane permeants. We note that this argument likely applies only to larger permeants. Mathai et al [36] have found that the permeability of lipid membranes to water correlates most strongly with the molecular area of the bilayer.

Discussion—In this paper we extend earlier MD simulations to examine atomic level differences in structure and interactions in CLA-PC bilayers composed of the two isomers C9T11 and T10C12 at Chol concentrations between 10% and 30%. In all of the simulations, we found that the ordering of the *cis* versus *trans* double bonds does not appear to significantly affect large scale bilayer structural properties, including molecular area, chain tilt, order parameters for the saturated chain, and mass distribution. Differences were found in lipid chain order parameter profiles, and 2-d RDF plots Chol-CLA packing. As pointed out in the introduction, it is known that the biological effects of the two isomers are quite different. We conclude from the MD simulations that these effects cannot be due to large scale differences in area, mass distribution, chain tilt, or saturated chain order between the two isomers, as calculated for pure CLA or CLA-Chol bilayers. While there are currently no direct structural experimental data for the CLA - Chol bilayers we studied our results are supported indirectly by monolayer experiments that also show only small differences in lipid structure and thermodynamic properties. At an atomistic level, our results reveal that bilayers of pure CLA and CLA-Chol have different distributions of internal chain order for C9T11 and T10C12 that are induced by the different *cis* double bond location. Furthermore, the 2-d RDF plots of Figure 7 show a difference in the packing of Chol against the two CLA isomers. By elimination of all but the most subtle atomistic differences between CLA isomers interacting with Chol, our atomistic results raise the possibility that the CLA isomers may affect biological properties through differential atomistic level interactions with membrane proteins or cholesterol. The biological environment is of course highly heterogeneous compared to our simulation systems. In a biological membrane, any incorporated CLA will interact primarily with membrane proteins, cholesterol, and non-CLA membrane lipids. These interactions have the possibility of depending more strongly on the location of the *cis* double bond in CLA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- > Two isomers of a conjugated linoleic acid have different biological effects.
- > We find that the effect of cholesterol on structure of the bilayers of the two isomers is every similar.
- > One major difference lies in the order parameter profile for the unsaturated bond.
- > Another major difference lies in the radial distribution profile of lipids around cholesterols.

Figure 1.
Structures of CLA-PCs and Chol molecules, with the numbering scheme used in the simulations.

Figure 2.
Average areas per lipid versus time for all simulations, for 200 ns after equilibration.

Figure 3.

- A. Force-area compression isotherms of monolayers of 16:0-T10C12 CLA-PC in presence of (from right to left) 0, 10, 30, and 50 mol % Chol at 24 °C.
- B. Force-area compression isotherms of monolayers of 16:0-C9T11 CLA-PC in presence of (from right to left) 0, 10, 30, and 50 mol % Chol at 24 °C.
- C. Molecular areas of CLA-PC-Chol monolayers at 15 mN/m. The A-ideal line represents the theoretical molecular area if no condensation occurred.
- D. Percent condensation of T10C12 CLA-PC and C9T11 CLA-PC in presence of varying mol % Chol, as derived from Figure 3C.

Figure 4.

Deuterium order parameter (S_{CD}) profiles for C9T11 and T10C12 lipid chains at 0 %, 10% and 30% Chol concentrations. Consistent with standard formats for order parameter profiles, carbons are numbered from 2 to 17 for the LA chains (C17 and C32 in Fig 1) and from 2 to 15 for the saturated chains (C38 and C51 in Fig 1).

Figure 5.
Tilt angle distribution of Chol molecules at simulated Chol concentrations.

Figure 6. Radial distribution functions between cholesterol C13 and C18 atoms, and selected carbons of CLA-PCs, for C9T11 and T10C12, from trajectories of 30% Chol simulations. Upper left: C9T11 CLA carbons and Chol C13; Upper right: T10C12 CLA carbons and Chol C13; Middle left: C9T11 CLA carbons and Chol C18; Middle right: T10C12 CLA carbons and Chol C18. Lower left: C9T11 saturated carbons and Chol C18; Lower right: saturated chain carbons and Chol C18. Atom numbers are as defined in Figure 1.

Figure 7.

Two-dimensional probability distribution plots, $g(r,\phi)$, in the XY-plane of the Chol body coordinate system, for the C24 carbon, on the CLA chain, of both isomers 30% Chol. The bar charts show the color coded magnitude or the distributions with darker indicating higher values for the RDF's. : Left: T10C12; Right: C9T11.

Figure 8.

Two-dimensional probability distribution plots, $g(r, \phi)$, in the XY-plane of the Chol body coordinate system, for the C45 carbon, on the saturated chain, of both isomers at 30% Chol. The bar charts show the color coded magnitude or the distributions with darker indicating higher values for the RDF's. Left: T10C12; Right: C9T11

Table 1

Comparison of simulated bilayer properties for CLA isomers with Chol.

Isomer	0% Chol [11]		10% Chol		30% Chol	
	C9T11	T10C12	C9T11	T10C12	C9T11	T10C12
Area /Mol ($\text{\AA}^2$)	63.06 $\pm$ 0.69	62.43 $\pm$ 0.53	57.32 $\pm$ 0.59	57.43 $\pm$ 0.56	48.24 $\pm$ 0.74	47.66 $\pm$ 0.44
thickness (nm)	3.94 $\pm$ 0.07	3.87 $\pm$ 0.07	4.06 $\pm$ 0.05	4.17 $\pm$ 0.05	4.214 $\pm$ 0.03	4.324 $\pm$ 0.03
D ($\mu\text{m}^2/\text{s}$)	4.12 $\pm$ 1.9	5.76 $\pm$ 3.5	3.38 $\pm$ 1.76	4.42 $\pm$ 1.7	1.46 $\pm$ 0.6	1.78 $\pm$ 0.98