Structural Properties of Phospholipid-based Bilayers with Long-Chain Alcohol Molecules in the Gel Phase

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ABSTRACT: The structural properties of two-component gel-phase bilayers of distearylphosphatidylcholine (DSPC) and alcohol molecules with different compositions and chain lengths (12−24 carbons long) are studied via molecular dynamics simulations. Several bilayer properties, including area per lipid, tilt angle, chain interdigitation, and headgroup offset, are studied for each system and compared, revealing important structural implications depending upon headgroup size and chain length. While tail tilt is the primary mechanism for single-component bilayers to balance tail attraction and headgroup repulsion, our results demonstrate that the lipid mixtures studied adjust this balance via an offset between the depths of the different molecular species in the bilayer; this behavior is found to depend both on composition and on the length of alcohol molecules relative to the length of DSPC tails. It is shown that the structural properties of bilayers with asymmetric tail lengths depend strongly on the bilayer composition, while the composition has less influence on mixed-component bilayers with nearly symmetric tail lengths. These findings are explained on the basis of the interdigitation between bilayer leaflets and how interdigitation is related to other structural properties.
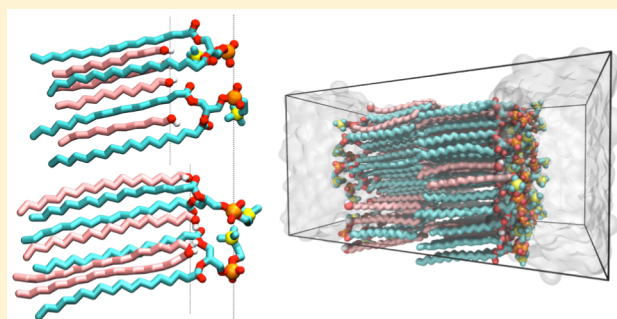
1. INTRODUCTION

Phospholipid-based lipid membranes have been extensively studied in large part because of the prominence of phospholipids within cell membranes and their use in the pharmaceutical, cosmetic, and food industries. In particular, previous work has focused on bilayers consisting of a single phospholipid or phospholipids combined with other molecules, such as cholesterol[1] or sphingolipids,[2] and to a lesser extent free fatty acids or alcohols. These phospholipid-based systems typically occur in a liquid-crystalline phase, in which the lipid tails are disordered. Tail saturation and low temperature can however result in a gel phase in which the lipids are densely packed and the tails highly ordered. Mixing different lipid species together within the bilayer can lead to significant changes in bilayer behavior and provide means for tuning membrane properties, such as permeability and phase transitions. For example, the chain packing in pure phosphatidylcholine (PC) gel-phase bilayers is known to result in a tilted phase, while an untilted gel phase forms when PC lipids are mixed with a large concentration of free fatty acids with tail lengths equal to the phospholipids.[3] Similarly, experiments have shown that the addition of a small amount of DOPC or cholesterol eliminates the tilt of gel-phase DPPC

bilayers.[4] Such a structural transformation from a tilted to an untilted phase can have severe consequences for other bilayer properties, since the tilt of the lipid tails is closely related to the deformation of the hexatic packing of the acyl chains, which has been suggested to be a key factor in determining membrane permeability.[5] However, the influence that mixing multiple lipid species has on the bilayer structure can strongly depend on the phase of the bilayer, e.g., fluid-like (liquid-crystalline) versus the gel-phase. Gel-phase bilayers are characterized by a dense and highly ordered hexatic chain packing in the bilayer interior, in contrast to fluid-like bilayers, which are more disordered. When considering mixtures of cholesterol and phospholipids, experiments and molecular simulations have shown that cholesterol increases the rigidity and packing density of fluid-like lipid bilayers,[1] while the rings and methyl groups in cholesterol disrupt the dense chain packing of bilayers in the gel phase.[6,7] Even in the absence of rings, molecules such as alkanes[8] can have a significantly different effect on a liquid-crystalline bilayer than on a gel-phase bilayer.[9] For example, Hishida et al.[9,10]

found via X-ray diffraction and differential scanning calorimetry studies that the addition of *n*-alkanes increased the chain packing density of a dimyristoylphosphatidylcholine (DMPC) bilayer in the gel phase, with the density-enhancing effect of the alkanes increasing with chain length. Conversely, alkanes were found to have little effect on chain packing density in the liquid-crystalline phase.

Mixtures of phospholipids with alcohol molecules are of particular interest as they are important additives in synthetic phospholipid-based systems and have been shown to demonstrate permeability-modifying[11] and anesthetic[12] effects for fluid-phase bilayers.[13] For example, Patra et al.[14] showed via molecular dynamics simulations that the presence of short-chain alcohol molecules increases the fluidity and permeability of a dipalmitoylphosphatidylcholine (DPPC) bilayer in the liquid-crystalline phase. Similarly, simulations[13,15] and experimental studies[15,16] have demonstrated that short-chain alcohols increase the area per lipid and decrease the chain order in a fluid-like DMPC bilayer; a reduction in area per lipid is observed if the chain length of the alcohol molecule is at least half of the phospholipid tail length,[16] in which case the attraction between the hydrocarbon chains is found to rigidify the bilayer.[10,17] However, a robust understanding of the composition-dependent structural behavior of gel-phase phospholipid-alcohol mixtures is currently lacking. While experimental techniques, such as X-ray diffraction,[18] X-ray scattering,[19] and Fourier transform infrared spectroscopy,[20] can provide some insight into the structure of lipid bilayers, the results from these methods often require significant interpretation or do not provide molecular-level resolution. In contrast, such information can be gleaned directly from molecular dynamics (MD) simulations as highlighted in the discussion above.[21,22] However, MD studies typically focus on liquid-crystalline systems over gel-phase mixtures because of the challenges related to slow dynamics in gel-phase bilayers,[23−25] and as of yet, no studies have focused on gel-phase mixtures of phospholipids and alcohol molecules.

In this work, MD simulations are used to investigate the structural properties of gel-phase distearylphosphatidylcholine (DSPC) bilayers combined with a homologous series of saturated alcohol molecules. The influence of composition and alcohol chain length are systematically investigated to provide insight into the impact of the alcohol molecules, as well as the general structural behavior of gel-phase bilayers, neither of which have been extensively studied with MD. The remainder of the paper is organized as follows. In the Simulation and Analysis Details Section details of the molecular dynamics simulations and analysis are provided. Simulation results are shown and discussed in the Results and Discussion Section and the findings and conclusions are summarized in the Conclusions Section.

2. SIMULATION AND ANALYSIS DETAILS

The structural properties of 15 two-component gel-phase bilayers have been investigated (Table 1). The bilayers each consist of DSPC and long-chain alcohol molecules (Figure 1a), with the composition and alcohol molecule length being varied to elucidate the effect of these parameters on the mixed-lipid bilayer structure. The alcohol molecules considered are 12−24 carbons long, while the DSPC tails are 18 carbons long. The simulations were performed at 305 K and 1 atm. We note that this is below the experimentally determined melting point of DSPC (327.9 K)[26] and the melting temperatures of the alcohol

Table 1. Overview of the Simulated Bilayers in This Study[a]

	1:1	1:2	1:4	1:9
C12	√		√	
C14	√			
C16	√	√	√	√
C18	√			
C20	√		√	
C22	√			
C24	√	√	√	√

[a]Each system contains DSPC mixed with an alcohol of chain length between 12 and 24 carbon atoms long. Four stoichiometries are considered in this study, with the columns indicating the different DSPC-alcohol molar ratios.
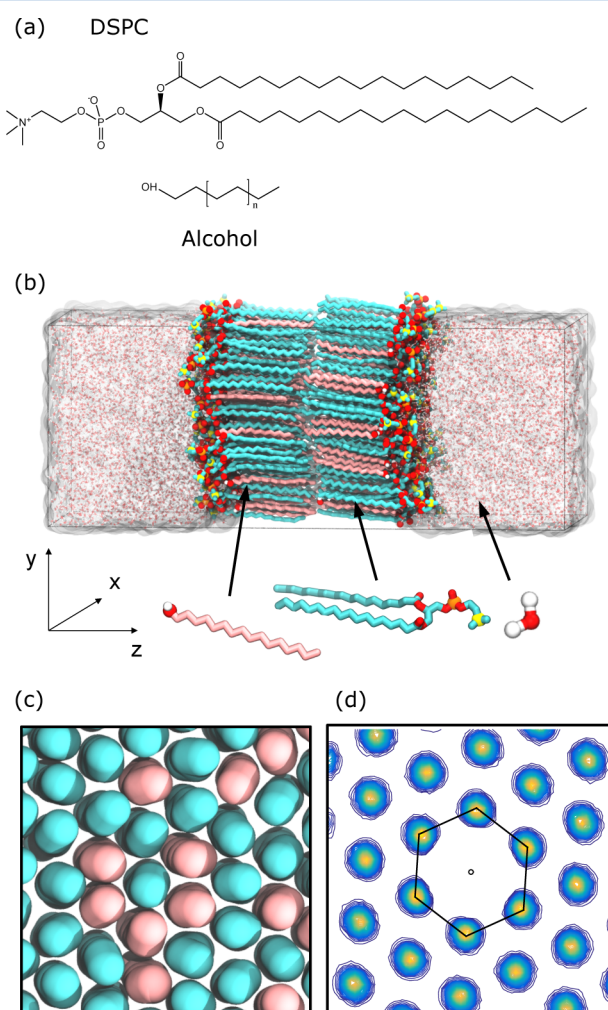

Figure 1. (a) Chemical structure of the DSPC and alcohol molecules examined in this study. (b) Snapshot of a typical bilayer configuration of an equimolar mixture of DSPC and C18 immersed in water. Hydrocarbons are shown in cyan (DSPC) and pink (alcohol), oxygen in red, phosphorus in orange, nitrogen in yellow, and hydrogen in white. (c) Render of the in-plane packing of DSPC (cyan) and alcohol (pink) tails. (d) The in-plane pair distribution function of tails.

molecules 14−24 carbons long (311, 322, 332,[27] 345.5, 350 K,[28] respectively); C12 alcohols despite having a melting temperature of 297 K,[27] are not in sufficient quantities to prevent the formation of gel phase bilayers when mixed with DSPC at 305 K. Varying the alcohol molecule length in this range allows for a comprehensive study of tail-length

asymmetry between the two lipid components. The effect of tail length is studied for equimolar mixtures and for 1:4 mixtures of DSPC and alcohol molecules, since the effect of the tail length could also depend on composition. Finally, the effect of composition is studied in more detail for bilayers containing C16 and C24 alcohol molecules, with DSPC molar fractions of 0.1, 0.2, 0.35, and 0.5 simulated to gain insight into how the composition modulates the bilayer structure for bilayers with symmetric and asymmetric tail lengths.

The bilayers were assembled by placing two opposing leaflets, each containing 100 lipids organized in a square lattice, in a bath of 4000 water molecules. The lattice had an initial area per lipid of 36 Å^2. The lipids in either leaflet were randomly rotated about their long axis and lipids of each type randomly distributed over the lattice sites to mimic a mixed system with two dissimilar leaflets. The headgroup of the alcohol molecules was placed at the same depth in the leaflet as the phosphate group of the phospholipid molecules. DSPC and alcohol molecules were both represented by the united atom GROMOS 53a6 force field[29] with partial charges from Chiu et al.[30] for the DSPC molecules. The C16 alcohol molecule was created using the automated topology builder version 2.1,[31] which was also used as the basis for the other alcohol molecules in this study by manually adding or removing CH_2 beads. Water was modeled with the simple point charge model, with the rigid structure preserved using the SHAKE algorithm. MD simulations were performed using the LAMMPS simulation engine,[32] with the simulation box being orthogonal and fully periodic. Dispersion interactions were described by a Lennard-Jones potential, with a cutoff distance of 14 Å. The particle–particle particle–mesh method was used to calculate electrostatic interactions, with the real part truncated at 14 Å.[33] The Nosé–Hoover thermostat and anisotropic barostat were used to maintain a constant temperature of 305 K and a pressure of 1 atm, respectively.

The atom positions and the size of the simulation box throughout the simulation were used to calculate the area per lipid (APL), the offset distance, the average tail tilt angle θ, and the interdigitation between the bilayer leaflets. The APL was calculated from the cross-sectional area of the simulation box, divided by the number of lipids in the leaflet. The offset distance was determined as the difference between the average depth (z-position) of the phosphate group of the DSPC molecules and the hydroxyl headgroup of the alcohol molecules (Figure 3). The tilt of the lipid chains was taken as the average angle between the long axis of each molecule tail and the transmembrane axis, with the long axis of a chain defined as the eigenvector corresponding to the minimum eigenvalue of the inertia tensor.[34] The tilt was found to be uniform across the bilayer leaflets. The interdigitation between the bilayer leaflets was calculated from the overlap in density profiles of the bilayer leaflets:[35]

$$I_{LR} = \int_{-\infty}^{\infty} \frac{4\rho_L(z)\rho_R(z)}{(\rho_L(z) + \rho_L(z))^2} dz \tag{1}$$

where the subscripts "L" and "R" indicate the left and right bilayer leaflets, respectively.

The bilayers were simulated for 160 ns, with two different initial random configurations used for the equimolar mixtures to ensure that the results converged independently of the initial configuration. This validation was performed for equimolar mixtures because they were found to be more likely to get stuck

in a local energy minimum than bilayers with higher alcohol concentrations, since the single-tail alcohol molecules move around more easily than the two-tailed phospholipids. The last 60 ns of simulation time was used for analysis. These simulation times are comparable to those reported in other recent molecular dynamics studies of gel-phase bilayers[36,37] and were based on convergence of the APL, lipid tail tilt angle, and the difference ("offset") between the alcohol and DSPC headgroup depths in the bilayer. Agreement between the tail tilt distributions of both bilayer leaflets was used as further indication that the lipids are not stuck in an undesirable configuration, given that the two leaflets were individually randomized. Figure 1 shows a simulation snapshot from an equilibrated gel-phase bilayer containing an equimolar mixture of DSPC and C16 alcohol molecules and the hexatic in-plane tail packing characteristic of gel-phase bilayers.

3. RESULTS AND DISCUSSION

Influence of Alcohol Tail Length. For DSPC-alcohol ratios of 1:1 and 1:4 the APL, offset distance, average tail tilt angle, and interdigitation between the bilayer leaflets are shown as a function of the alcohol tail length in Figure 2a−d, respectively. Each of the two-component bilayers considered here show much smaller tilt than the 32.5° and 36.3° reported for a pure DSPC bilayer by X-ray scattering[38] and MD simulation,[24] respectively; the observed structure depends strongly on the alcohol tail length and on the bilayer composition. Focusing first on the equimolar DSPC-alcohol mixtures, we note that the APL is largest when the DSPC and alcohol tails are similar in length, although differences between the APLs are small since the APL is largely determined by the headgroup size. Furthermore, if both molecular species have similar tail lengths, a small offset is found between the depths of the alcohol and DSPC headgroups in the bilayer, along with a large tilt angle and a small interdigitation, compared to mixtures with more asymmetric tail lengths. The fact that the depths of the DSPC and alcohol headgroups in the equimolar mixtures are offset by at least 5 Å (offset shown schematically in Figure 3), distinguishes the structure of these bilayers from those of multicomponent phospholipid gel-phase bilayers (i.e., mixtures of DSPC and DMPC), which exhibit smaller offset distances.[23] This offset was also found in gel-phase bilayers of phospholipids and emollients[24] and for a liquid-crystalline bilayer of short-chain phospholipids mixed with free fatty acids.[39] These studies indicate that tail-length asymmetry and headgroup offset affect bilayer properties. However, the influence of this offset mechanism on the bilayer structure and its dependence on bilayer composition has not been systematically studied for mixed-lipid gel-phase bilayers.

The data in Figure 2 suggest a stronger effect of tail-length asymmetry on the bilayer properties of the equimolar mixtures for short-chain alcohol molecules (i.e., short relative to the 18 carbons in the DSPC tails) than for long alcohol molecules, with the exception of the interdigitation. Large interdigitation is found when the alcohol tails are longer than the DSPC tails. Though, somewhat surprisingly, the interdigitation increases superlinearly with the alcohol chain length, as a consequence of the increasing offset distance. When the alcohol tails are shorter than the DSPC tails, the interdigitation increases with decreasing alcohol tail length. Since the alcohol headgroup is always located deeper in the bilayer than the DSPC headgroup, as shown for two tail lengths in Figure 3, an increase in the offset can increase the protrusion of long alcohol molecules into
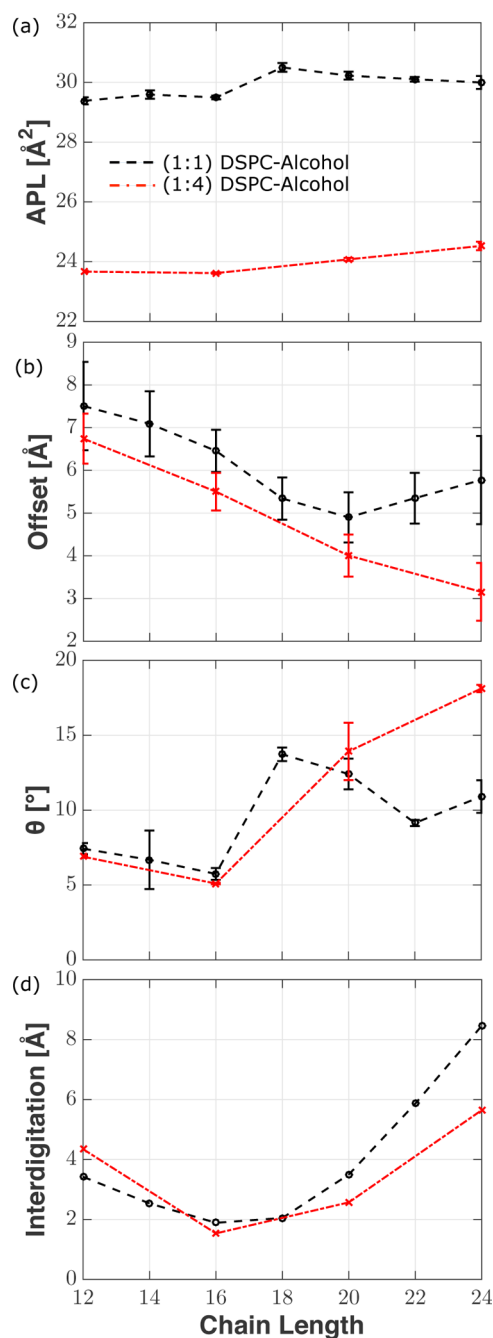
Figure 2. Bilayer structural properties for 1:1 (black) and 1:4 (red) mixtures of DSPC and alcohols of varying chain length. (a) The area per lipid, (b) the offset distance, (c) the average tail tilt angle, and (d) the interdigitation between the bilayer leaflets. The error bars denote the standard error calculated from block averages, with 10 blocks of 6 ns each.

Figure 3. (a) A schematic illustrating the offset distance between the headgroup depths of the phosphates of the DSPC molecules and the hydroxyl headgroups of alcohol molecules. Hydrocarbons are shown in the DSPC and alcohol molecules in cyan and pink respectively, the oxygens in red, phosphorus in orange, nitrogens in yellow, and hydrogens in white. (b) Structures of DSPC and C12 and C24 alcohol bilayers with asymmetric tail lengths at two different DSPC-alcohol ratios. Only a small section of each bilayer is shown for clarity. The numbers indicate the interdigitation of the bilayers, with the dashed lines showing the approximate interdigitation length in the middle of the bilayer.

the middle of the bilayer, or decrease the protrusion of DSPC tails if the alcohol molecules are short.

The offset between headgroup depths enables the lipids to be densely packed in the bilayer plane (i.e., small APL). In recent MD simulations[24] we have shown that the headgroup offset mechanism contributed to a denser lipid packing in systems with phospholipids and emollients compared to pure phospholipid or emollient bilayers. This packing mechanism essentially increases the tail attraction without the large penalty of increasing the steric repulsion between headgroups and as
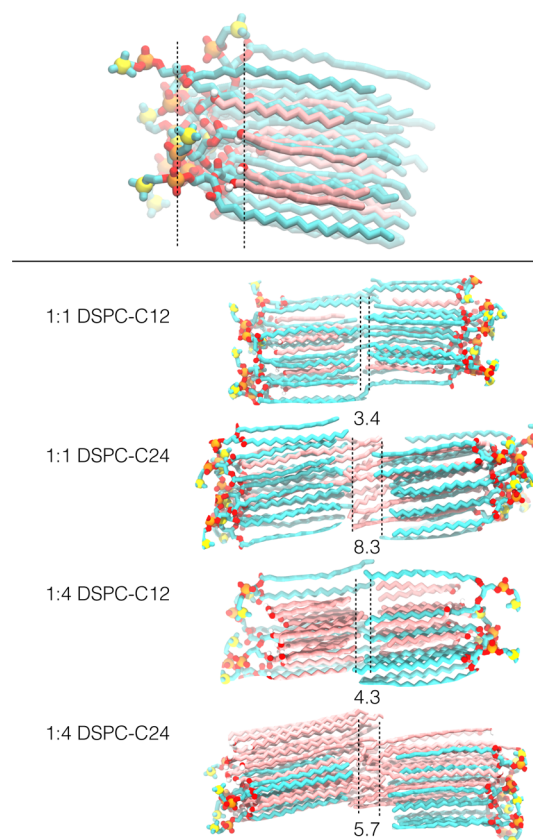
such is analogous to chain tilt in single-component gel-phase bilayers. Multicomponent bilayers have the advantage of being able to combine headgroup offset and chain tilt to reach a thermodynamically stable configuration. If the offset decreases, the APL typically increases in order to avoid overlap between headgroups. The larger APL in turn requires a larger tilt of the tails to maintain the desired distance between the hexagonally packed chains and maximize van der Waals interactions.[24] Thus, if the offset for a bilayer with a certain alcohol chain length and composition decreases, the tilt tends to increase; however, Figure 2 shows that this relation does not fully extend to a comparison between bilayers of different compositions and chain lengths. For example, a shorter alcohol chain length can increase the offset, without further decreasing the tilt angle or the APL.

For the 1:4 mixtures of DSPC and alcohol molecules, the structural properties in Figure 2 display a different dependence on the alcohol tail length than seen in the equimolar systems. These differences between the compositions manifest particularly for long-chain alcohols, as most clearly seen from the offset distance, which drops to 3.1 Å for the 1:4 mixture with C24 alcohol molecules, while the corresponding equimolar mixture has an offset of 5.8 Å. The APL increases with the
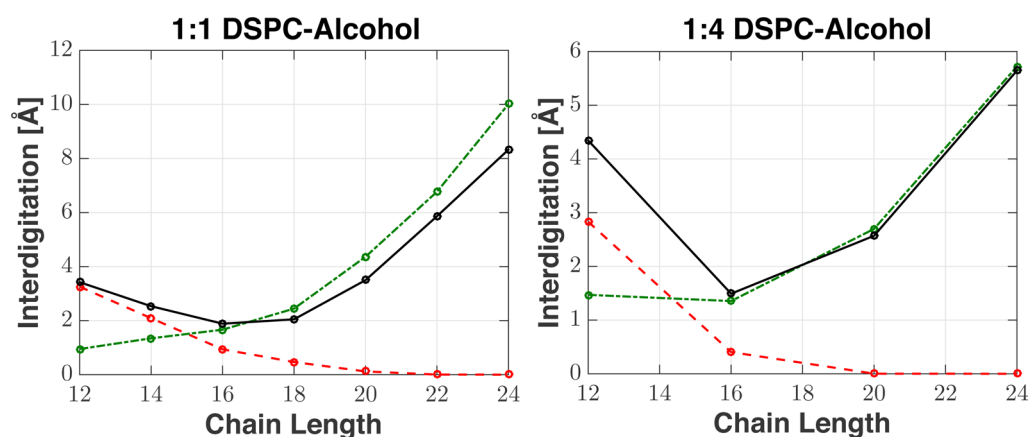
Figure 4. Interdigitation of individual components in the 1:1 (left) and 1:4 (right) DSPC-alcohol bilayers. The bilayer (i.e., considering all lipid species) interdigation is shown in black, the DSPC interdigitation in red, and the alcohol interdigitation in green.

alcohol tail length when long alcohol molecules are mixed with DSPC molecules in a stoichiometry of 1:4, resulting in a larger spacing between DSPC headgroups. This larger spacing allows for a smaller offset distance and leads to larger chain tilt angles, driven by the desire to reduce the distance between tails and increase van der Waals interactions. The interdigitation of the 1:4 mixtures demonstrate qualitatively the same behavior as the 1:1 mixtures, although the quantitative differences are an important indication of composition-dependent structural differences. For short alcohol molecules, the protruding DSPC tails of the two leaflets interdigitate, suggesting that a smaller DSPC concentration would reduce the interdigitation when the alcohol tails are short; however, the opposite behavior is seen in Figure 2d, where the 1:4 mixtures with short-chain alcohol molecules show larger interdigitation than the 1:1 mixtures. Similarly, alcohol tails protrude and interdigitate when these are longer than the DSPC tails. A larger alcohol concentration would thus be expected to increase the interdigitation, but the opposite behavior is again observed, with an interdigitation of 5.7 Å for the 1:4 mixture containing C24 alcohol molecules, against 8.3 Å for the equimolar mixture with the same alcohol tail length. The foundation for these counterintuitive results lies in how the headgroup offset is related to other structural properties and how it depends on tail-length asymmetry. The data for both compositions in Figure 2 demonstrate that the relation between structural properties is different for bilayers with short alcohol molecules than for bilayers with long alcohol molecules. The behaviors of mixtures with long and short alcohol molecules are therefore explained separately.

Starting with the case of the alcohol molecules being longer than the DSPC tails, an increase in APL is compensated by a combination of the tilt and offset in Figure 2. A decrease in the offset increases the steric repulsions, which are mitigated by a larger APL, which can lead to a larger tilt. This decrease in offset can lead to an increase of the preferable tail–tail contact area, depending on the interdigitation between the leaflets, which in turn depends on the composition. If half of the tails in the leaflets belong to alcohol molecules (which would be the case for a 1:2 mixture), the number of alcohol tails from the two leaflets combined equals the total number of tails in either leaflet, such that the protruding alcohol tails form an ordered interdigitated region in the middle of the bilayer. The 1:1 mixtures investigated in Figure 2 have fewer alcohol tails than DSPC tails, while the alcohol tails outnumber the DSPC tails in

1:4 mixtures. In fact, the number of protruding alcohol molecules in the 1:4 mixtures is so large that there is not enough space for the protruding tails from both leaflets to fully interdigitate. Since the tail–tail contact is not maximized via interdigitation in these bilayers, the headgroup offset decreases to increase tail–tail contact (Figure 2b). Furthermore, the increased disorder in the protruding tails (Figure 3) contributes to a higher average tilt of the tails, shown in Figure 2c. The equimolar mixtures show a different behavior, with the long alcohol molecules interdigitating, such that the offset can increase, thus reducing steric effect between headgroups, without decreasing the tail–tail contact.

For bilayers in which the alcohol molecules are shorter than the DSPC tails, the offset distance increased with a decreasing alcohol molecule length (Figure 2b), and thus with an increasing tail-length asymmetry. This trend in the offset is explained by the fact that the short alcohol molecules do not protrude from the leaflets. Therefore, an increase in the offset distance does not decrease the tail–tail contact, while it reduces the steric headgroup repulsions. A smaller offset distance, on the other hand, would cause DSPC tails to protrude further from the leaflets, which again reduces the tail–tail contact and is thus unfavorable. The fact that the interdigitation in Figure 2d is smaller for bilayers with short alcohol molecules than for those with long alcohol molecules demonstrates that the offset reduces the effect of tail-length asymmetry on the interdigitation in the case of short alcohol molecules, while it effectively increases the tail-length asymmetry in bilayers with long alcohol molecules. However, the fact that the interdigitation of bilayers with short alcohol molecules increased with a decreasing alcohol molecule length indicates that the offset does not fully compensate for the tail-length asymmetry. While both compositions studied here show the same trend in interdigitation for mixtures with short alcohol molecules, the amount of interdigitation depends on the number of protruding tails from both leaflets combined, as was also the case for mixtures with long alcohol molecules. In the equimolar lipid mixtures, two-thirds of the tails belongs to DSPC molecules, such that there is not enough space for the protruding DSPC tails of both leaflets to interdigitate in the middle of the bilayer. This is confirmed by the smaller interdigitation values compared to the lipid mixtures with a 1:4 composition (3.4 Å against 4.3 Å, for the C12 alcohol mixtures). In the latter mixtures, only one-third of the tails belongs to DSPC
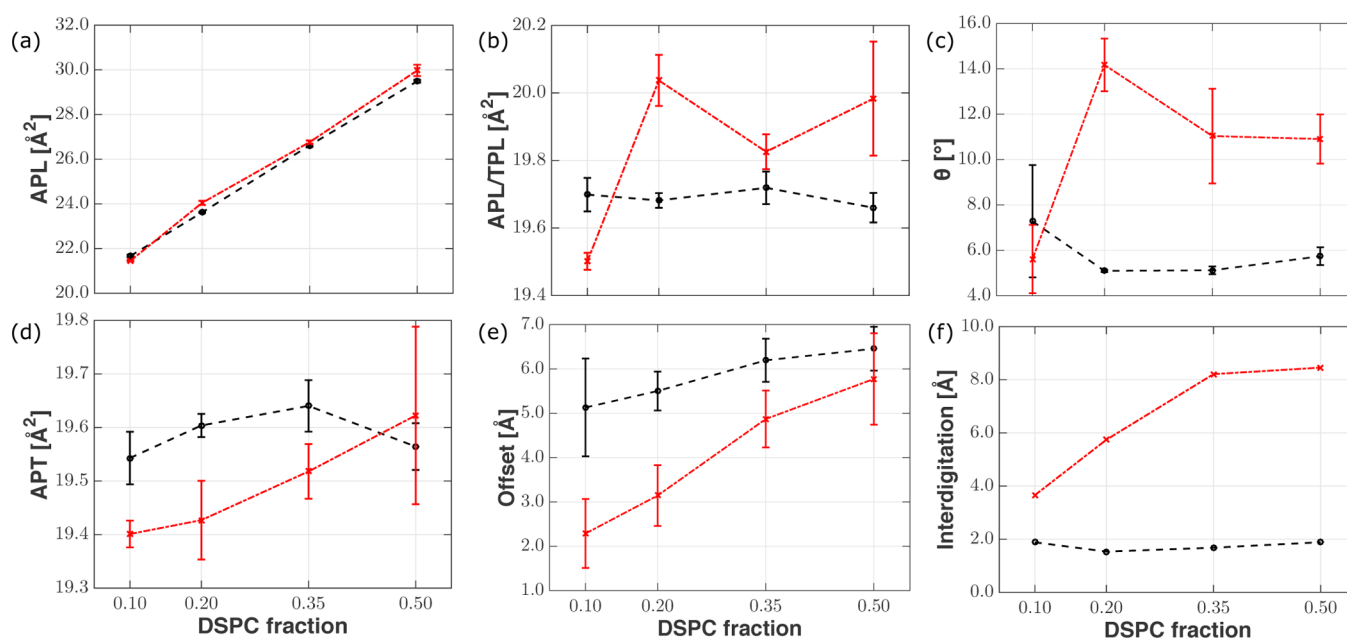
Figure 5. Bilayer structural properties for mixtures of DSPC and C16 (black) or C24 (red) alcohols as a function of the DSPC fraction. (a) The area per lipid, (b) the area per lipid normalized by the average number of tails per lipid, (c) the average tail tilt angle, (d) the area per tail in the plane perpendicular to the chain orientation, (e) offset distance, and (f) the interdigitation between the bilayer leaflets. The error bars denote the standard error calculated from block averages, with 10 blocks of 6 ns each.

molecules, such that there is enough space for these protruding tails to interdigitate more.

The interdigitation of the 1:1 and 1:4 mixtures is further investigated in Figure 4, by comparing the interdigitation of only the DSPC tails and only the alcohol molecules. The interdigitation of a subset of the bilayer is calculated in the same way as the total interdigitation of the bilayer, with the mass density profiles of the lipid component of interest inserted in eq 1. Figure 4 shows that the interdigitation of the DSPC tails decreases with the alcohol chain length, while the interdigitation of alcohol increases with its chain length. The combination of the trends in the DSPC and alcohol interdigitation causes the convex shape of the bilayer interdigitation profile as a function of the alcohol chain length. Note that the interdigitation of alcohol molecules can be larger than the interdigitation of the whole bilayer, due to the different mass density profiles used in the normalization of eq 1. The interdigitation of alcohol or DSPC is thus not a fraction of the total, but rather the bilayer interdigitation is a weighted average of the interdigitation of its components. Figure 4 shows that the interdigitation of alcohol molecules is larger than that of the DSPC tails when the alcohol molecules are at least 16 carbon atoms long, which is shorter than the DSPC tail length of 18 carbon atoms long. This again highlights the influence of the offset mechanism on the bilayer structure. Furthermore, the interdigitation of the C12 and the C16 alcohol molecules in the 1:4 mixtures are similar because the C12 alcohol molecules sink deeper into the bilayer, as evidenced by the large offset distance in Figure 2b.

Influence of Composition. The influence of composition on the bilayer structure is further investigated for mixtures of DSPC with C16 or C24 alcohol molecules. Shorter alcohol molecules were excluded from this comparison because Figure 2 showed that, although the amount of interdigitation depends on the bilayer composition, the other structural properties are not affected for mixtures with short alcohol molecules, since there is no driving force decreasing the offset distance. The

structural bilayer properties are presented in Figure 5 for DSPC fractions of: 0.1 (1:9), 0.2 (1:4), 0.35 (~1:2), and 0.5 (1:1). Figure 5a shows that the APL of the bilayers increases approximately linearly with the DSPC fraction, since the APL is determined predominantly by the headgroup volume. Deviations from the linear trend and differences between the C16 and C24 alcohol molecules become evident in Figure 5b, which shows the ratio between the APL and the average number of tails per lipid. This measure indicates which bilayers have a dense lipid packing in the bilayer plane and which bilayers require a large tail tilt to adopt a dense chain packing. A large cross-sectional area per chain is unfavorable from the viewpoint of tail interactions, causing tails to tilt relative to the bilayer normal direction in order to increase the chain packing density. Consequently, the tilt angles in Figure 5c follow similar trends as the data in Figure 5b, with both figures showing a nonmonotonic trend with the DSPC fraction for the bilayers with C24 alcohol, while no strong dependence on composition is found for the bilayers with C16 alcohol. The small tilt angles for the bilayers with C16 alcohol are consistent with the findings of Seddon et al.,[3] who deduced from X-ray diffraction measurements that 1:2 mixtures of PC lipids and free fatty acids with equal tail lengths formed an untilted gel phase. The bilayers with C24 alcohol tilt most at a DSPC fraction of 0.2, for which alcohol molecules comprise two-thirds of the tails in the leaflet, which is too many to form an ordered interdigitated layer; instead, a partially interdigitated region forms, causing large chain tilt. The lipid packing and tilt are smaller for DSPC fractions of 0.1 and 0.35, where there are no strong mismatches between the number of tails in the leaflets and in the middle of the bilayer. A DSPC fraction of 0.1 corresponds to 9 out of every 11 tails belonging to an alcohol molecule, such that the tail endings need to tilt only slightly further than the rest of the leaflet to compensate for the lower density of tails near the middle of the bilayer, but not as much as some other bilayers. A DSPC fraction of 0.35 is near the ideal value at which the

number of tails in the middle of the bilayer is the same as the number of tails per leaflet.

Multiplying the data in Figure 5b with the cosine of the tilt angle denotes the cross-sectional area per tail (APT) in the plane perpendicular to the chain orientation, shown in Figure 5d. These values between 19.4 and 19.7 Å^2 are smaller than the 19.8 (0.1) Å^2 measured via X- scattering on pure DSPC bilayers,[38] for which the large headgroups prohibit a denser packing. On the other hand, Seddon et al.,[3] found via X-ray diffraction an APT of 20.6 Å^2 for an untilted 1:2 mixture of DSPC and C18 free fatty acids at approximately 335 K. The data in Figure 5d suggest that the APT of the DSPC-alcohol mixtures decreases with increasing alcohol chain length for the bilayers with DSPC fractions below 0.5. This dependence on chain length is consistent with X-ray scattering studies[38] that have found that the APT for pure phospholipid bilayers at 298 K decreased from 20.2 (0.2) Å^2 for $DC_{16}PC$ to 19.3 (0.2) Å^2 for $DC_{24}PC$. This effect of tail–tail interaction also causes the APT of bilayers with C24 alcohol to increase with the DSPC fraction, since the amount of tail interaction decreases with a decreasing fraction of long chains. Although the interaction between tails largely determines the APT, offset and tilt are both needed to facilitate a configuration in which the tails are able to pack optimally.

At each DSPC fraction, the offset distance (Figure 5e) is smaller for bilayers with C24 alcohol than for those containing C16 alcohol, due to the increased tail–tail interaction. The offset increases with the DSPC fraction for both alcohol molecule lengths, due to the steric demands of increasing the presence of the large DSPC headgroup. This increase in offset causes long alcohol molecules to protrude further, while it has little effect on protrusion in bilayers with alcohol molecules that are shorter than the DSPC tails. This is concluded from the fact that the interdigitation of the bilayers containing C16 alcohol (in Figure 5f) does not depend on the ratio between alcohol and DSPC tails, since neither molecule protrudes far from the leaflet. However, calculation of the interdigitation of the individual molecular species (Figure 4) indicated that, due to the offset mechanisms, the C16 alcohol molecules contribute more to the interdigitation than the DSPC tails for each of the compositions studied here. In contrast to the C16 alcohol mixtures, the interdigitation of bilayers with C24 alcohol increases strongly with the DSPC fraction. While this trend is assisted by the offset increasing, the number of protruding tails is again the dominant factor determining the amount of interdigitation. It was suggested above that a 1:2 ratio of DSPC and alcohol is optimal for a dense interdigitated region to form, since there are as many protruding tails from both leaflets combined, as there are tails in either leaflet. Full interdigitation is unfeasible if there are more alcohol tails present (i.e., a DSPC fraction below 0.33), while a smaller number of alcohol tails (a DSPC fraction above 0.33) would result in a more dilute and disordered interdigitated region. This is confirmed by a transition in the interdigitation behavior for a DSPC fraction of 0.35 (near the optimal 1:2 ratio) in Figure 5f. The interdigitation of alcohol tails varies linearly from 8.2 Å at a DSPC fraction of 0.35, down to 3.7 Å at a DSPC fraction of 0.1. The interdigitation increases only from 8.2 to 8.3 Å when the DSPC fraction increases from 0.35 to 0.5. The protruding alcohol tails in both systems are interdigitated, with the slightly larger offset of the latter bilayer causing the larger interdigitation.

4. CONCLUSIONS

The effects of lipid composition and chain length on the structural properties of gel-phase bilayers have been investigated in this study for mixtures of distearylphosphatidylcholine (DSPC) and alcohol molecules. Such mixed-component bilayers form a more complicated three-dimensional structure than that seen in single-component systems. The area per lipid, chain tilt, headgroup offset distance, and interdigitation were analyzed for bilayers with four different compositions and seven chain lengths, and the complex relations between these measures explained.

Tail-length asymmetry was shown to be important for the balance between interdigitation, tilt, offset, and area per lipid of mixtures of DSPC and alcohol. If the alcohol molecules were approximately of the same length as the DSPC tails, the area per lipid was large, while the tilt angle, the offset distance, and the interdigitation were small compared to bilayers with alcohol molecules that are either shorter or longer than the DSPC tails. The effect of tail-length asymmetry on structural properties was found to be different for short alcohol molecules than for long ones, because the alcohol headgroups are always located deeper in the bilayer than the DSPC headgroups. Consequently, an increase in offset would cause long alcohol molecules to protrude further into the center of the bilayer, while an increase in offset decreases the protrusion length of the DSPC tails in bilayers with short alcohol molecules. More importantly, there is no driving force to reduce the offset distance in bilayers with short alcohol molecules, since the alcohol molecules are fully buried in the DSPC tail region. Conversely, in the case of long alcohol molecules, a smaller offset distance helps to reduce the unfavorable protrusion of alcohol tails, while resulting in a larger area per lipid.

To better understand the role of composition, multiple DSPC fractions were simulated for lipid mixtures in which the tails were almost equal in length (DSPC with C16 alcohol) and for bilayers in which the (C24) alcohol molecule was much longer than the DSPC tails (which are 18 carbon atoms long). Composition was found to be especially important in the latter case, since the fraction of protruding tails largely determined the interdigitation behavior in the middle of the bilayer. An ordered interdigitated region cannot form in bilayers that contain more long alcohol tails than DSPC tails (for a DSPC fraction <0.33), resulting in a partially interdigitated region and larger tail tilt. However, if the DSPC fraction is very small (≈0.1), the leaflets contain sufficient alcohol molecules to form a dense ordered region without the need for interdigitation. If the DSPC fraction is above 0.33, less than half of all tails corresponds to alcohol molecules, such that the protruding tails from both leaflets interdigitate. The interdigitation for the bilayers with long alcohol molecules was assisted by the offset, which increased the protrusion length of the alcohol tails.

The findings in this study suggest that the composition and tail lengths of different molecules can modulate the structure of binary phospholipid-alcohol mixtures in the gel phase, by shifting the balance between headgroup repulsion and tail attraction. A dense chain packing can be facilitated by combining a phospholipid with alcohol molecules that are shorter than the phospholipid tails, or by combining phospholipid molecules with a large amount of longer alcohol molecules. In both cases, the tail length asymmetry is found to play an important role. These findings contribute to a better understanding of the structure of multicomponent bilayers in

the gel phase and have potential application to biological and commercial systems that employ mixtures of phosphatidylcholines with long chained alcohols and potential utility in formulation design, where lipid-packing characteristics need to be optimized.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b10192.

> Tabulated numerical values of the reported bilayer properties (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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