Effect of cholesterol vs. ergosterol on DPPC bilayer properties: insights from atomistic simulations

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Abstract

Cholesterol and ergosterol are two dominant sterols in the membranes of eukaryotic and yeast cells, respectively. Although their chemical structure is very similar, their impact on the structure and dynamics of membranes differs. In this work, we have explored the effect of these two sterols on binary mixtures with 1,2-dipalnitoyl-sn-glycerol-3-phosphocholine (DPPC) lipid bilayer at various sterol concentration and temperatures, employing molecular dynamics simulations. The simulations revealed that cholesterol has a stronger impact on the ordering of the lipid chains and leads to more condensed membranes with respect to ergosterol. This difference likely arises from a more planar structure of the ring part as well as the better alignment of cholesterol among the DPPC chains with respect to ergosterol. The degree of the planarity of the ring system affects the orientation of the methyl groups on the rough side and distribute the lipid chains on the two sides of the sterols differently. Similar to the structural observations, cholesterol also has a stronger influence on the dynamics, and consistently, establishes stronger DPPC-sterol interactions when compared to ergosterol. Although our findings are consistent with some previous simulations as well as recent experiments, they are at odds with some other studies. Therefore, the presented results may shed new lights on the impact of sterols on the saturated lipids bilayers with implications for binary mixtures of lipids as well as lipid rafts.

Significance

Cholesterol and ergosterol are crucial lipid molecules of eukaryotic and prokaryotic cells, respectively, with an important role for the characteristics of the membranes. Surprisingly, many experimental studies have reported opposing results concerning their relative impact. Our work aims to understand the molecular mechanism behind the influence of these sterols on the properties of saturated DPPC chains via a systematic computational approach at atomic resolution. The results show that cholesterol has a higher impact on the ordering, condensing and dynamics of the lipid chains and closely interact with them due to its more planar structure as compared to ergosterol. These effects can have implications in lipid rafts and the interaction of therapeutic drugs with membranes.

1 Introduction

Biological membranes are self-assembled structures in liquid environment and form the boundaries of the cells and organelles, separating the internal compartment of the cells from external environment. They also function as a barrier which controls the transport of ions and molecules across the cell membrane. Membranes of the cells are comprised of complex mixtures of hundreds of various types of lipids, proteins and other molecules, depending on the cell type and its functionality. It is evidenced that specific domains or lipid rafts are created within the membrane which are enriched in saturated lipids and cholesterol [1–5].

Cholesterol is the most prominent sterol in eukaryotic cells, and due to its functionality in lipid rafts, has attracted much attention during the last decades.

The occurrence of these domains is due to the different interaction of cholesterol with saturated and unsaturated lipids. The mechanism of these interactions is still unclear. It is assumed, however, that it is due to the condensing and ordering effect of cholesterol on saturated lipids, which can have an important role in the stability and permeability as well as the interaction of specific peptides and therapeutic molecules with membranes [6–10]. Therefore, understanding the lipid-lipid interaction and, in general, the effect of sterols on phospholipids is crucial for clarifying the mechanism behind the segregation of lipids occurring in lipid rafts.

As a minimum bilayer system to unravel the properties of the impact of cholesterol on lipids, mixtures of a saturated lipid with a variable concentration of cholesterol are frequently studied. Without cholesterol, at low temperatures the lipid molecules arrange themselves in an ordered manner in a two-dimensional (2D) hexagonal lattice where the lipid chains are ordered [11, 12]. This phase is called 'gel' or S_o (solid-ordered). As the temperature increases, the lipid chains melt and the bilayer enters the liquid crystalline phase (L_d). When cholesterol is added to the bilayer, interaction of cholesterol with lipids drives the system into a new complex phase, L_o (liquid-ordered), where the sterol molecules reduce the flexibility and increase the order of the saturated lipid chains. Due to the ordering effect of cholesterol on the saturated lipid chains, it has been shown to have a condensing effect with the immediate consequence of decreasing the area per lipid and increasing the membrane thickness [13–22]. Below the phase transition, however, cholesterol has been shown to have an opposite effect and decreases the high order of lipid chains [23–25]. Therefore, cholesterol is of dual nature: it promotes the fluidity of membrane in the gel phase and decreases it in the liquid phase. Accordingly, cholesterol modifies the lateral diffusion of lipids as well as their permeability [15, 26–29].

The structure of cholesterol is composed of three main parts: the hydrophilic OH group, the rigid ring part composed of 4 rings, and a hydrocarbon tail attached to the planar part (Figure 1a,c). In the planar part, two out-off plane methyl groups make the cholesterol molecule asymmetric. The side with the two methyl groups is called "rough side", while the other side is referred to as "smooth side" (Figure 1d). The concentration of this sterol is usually around 20-30% and can reach as high as 50% and 70% in blood cells and eye lens membranes, respectively [30, 31]. It is established that cholesterol can modulate structural and dynamical properties of membranes depending on the composition of this molecule in the bilayer.

Other sterols found in the membranes of eukaryotic and prokaryotic cells have also been studied in lipid mixtures, although not as extensively as cholesterol. Even though they have similar chemical structure with respect to cholesterol, they have distinct impacts on the structural and dynamical properties of the membranes due to their different 3-dimensional (3D) structure and flexibility [21, 24, 32–35]. Ergosterol is the predominant sterol in the membrane of yeast and other fungi [36], and is different from cholesterol with the addition of two double bonds, one in the ring part and one in the tail part of the sterol as well as one extra methyl group in the tail region [37] (Figure 1b). It is hypothesized that the extra double bonds increase the flatness and promotes the interaction of this sterol with the lipid chains [24]. In this paper we inspect this hypothesis and will see how these extra double bonds can affect the planarity of the ring part. Furthermore, it has been shown that ergosterol also promotes the

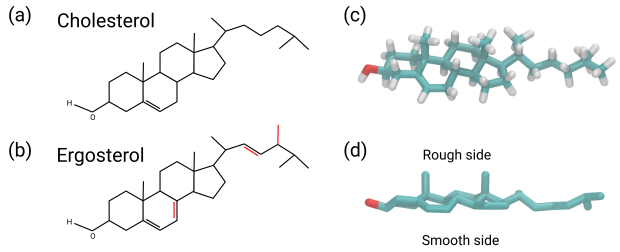

Figure 1: The chemical structure of cholesterol and ergosterol are shown respectively in (a) and (b) panels. The three-dimensional structure of cholesterol along with hydrogen bonds (c) and without hydrogens, showing the rough and the smooth side of the sterol (d) is depicted in Licorice representation.

formation of the L_o phase [5, 23, 33, 38], and is nearly as effective as cholesterol in this respect [5, 33, 39–42].

A number of computational and experimental studies have focused on the comparison of binary mixtures of cholesterol and ergosterol with saturated lipids including DPPC (16:0) and DMPC (14:0). The outcome of these studies, however, are not in agreement with one another [6, 15, 16, 18, 22, 24, 39, 40, 42–48]. A similar disagreement is also observed at the ternary level [33, 35, 38].

In one set of studies, the ordering and condensing effects have been compared for cholesterol and ergosterol. At the binary level, two MD simulations have indicated that ergosterol has a stronger impact on the compactness and order of the lipids hydrocarbon chains of the DMPC and DPPC membrane with respect to cholesterol [15, 16]. These studies are in line with several experimental works. In an NMR experiment, largest quadrapolar splitting and lower relaxation times have been obtained for DPPC/ergosterol systems, suggesting higher order for the systems including ergosterol [39]. Another NMR experiment estimated the deuterium order parameter for the DPPC/sterol systems and, interestingly, found that at low temperature the order parameter of the systems with ergosterol is indeed higher, whereas the opposite trend was obtained at higher temperatures [45]. Using a wide range of temperatures and sterol concentrations, the steady-state fluorescence anisotropy has been calculated for the DPPC/sterol system and based on the data, ergosterol was inducing more order [24]. Other experiments including NMR, fourier-transform infrared and fluorescence anisotropy experiments on DPPC/sterol or DMPC/sterol mixtures have also reported higher ordering effect for ergosterol as compared to cholesterol [6, 23, 40, 41]. At the ternary level, using fluorescence quenching and detergent insolubility techniques, it was also shown that ergosterol is stronger in domain-promoting in DPPC/12SLPC/sterol bilayers when compared to cholesterol [33]. Furthermore, in a very recent study, using atomic force microscopy, it was found that ergosterol has a profound impact on the thickness of the ordered domains in SM/POPC/sterol ternary bilayer, whereas cholesterol does not effectively differentiate between saturated and unsaturated chains [35]. Via the use of MD simulations and quantum mechanical calculations, the authors concluded that the molecular flexibility is the reason for this difference, and showed that at the ab-initio level the minimum energy conformers of ergosterol have a more linear

structure as compared to the boat-shaped structure of cholesterol [35].

In contrary to the above-mentioned studies, a number of groups have reported the opposite effects of the sterols, i.e. stronger effects for cholesterol. At the binary level, in an NMR study on DPPC/sterol systems at relatively high temperatures, cholesterol had higher impact on the order of the lipid chains [45]. In a recent work, using steady-state fluorescence polarization for DPPC/sterol mixtures in monolayers and bilayer vesicles above the phase transition temperature of the pure DPPC bilayer, lower fluidity values were obtained for cholesterol, meaning its higher ordering effect as compared to ergosterol [42]. Also recently, using the X-ray diffraction method on the DMPC/sterol binary mixtures, ergosterol was found to be three times less potent than cholesterol to condense the DMPC bilayers [22]. In agreement with this study, in an MD simulation work on DMPC/sterol mixtures, a higher impact of cholesterol was reported in terms of thickness, order parameter and sterol tilt angle with respect to ergosterol [44]. Another MD simulation study simulated multi-component bilayers and it was revealed that membranes containing ergosterol have a lower compressibility modulus than the models with cholesterol, although other properties such as are per lipid and the tilt angle were not statistically different [49]. Also very recently, using fluorescence probes it was shown that cholesterol decreases the membrane fluidity in DOPC/DPPC/sterol systems more effectively than ergosterol [38]. There are further experimental studies, which are in accordance with the higher effect of cholesterol in terms of condensing effects [21, 46, 47, 47, 48].

The above discussion indicates that the comparative studies of cholesterol and ergosterol even considering only DPPC/sterol systems have reported different results [6, 24, 39, 40, 42, 45, 47]. Among the different methods of study, computer simulations can have an essential role to provide insights into the underlying mechanisms in lipid bilayer systems at the atomic resolution even though they are limited in terms of simulation time and system size as well as the accuracy of the present force fields. However, to the best of our knowledge, extensive simulations are missing in literature for the effect of sterols on saturated lipids, and the previous MD simulations on this specific subject go back to more than a decade ago [15, 16, 44]. These simulation studies have used older versions of the current available force fields, which have now been improved noticeably. Therefore, in this work we aim to fill this gap and to better understand the respective effects of the sterols on the saturated lipids as well as the different reported results in literature. For this purpose, we adopt an all-atom molecular dynamics simulation approach with the use of a highly reliable force field and studied the respective impact of cholesterol and ergosterol on the binary mixtures of DPPC with the two sterols in a relatively wide range of concentrations and temperatures. Accordingly, we perform a systematic analysis to connect structural, dynamical and energetical insights to formulate a consistent picture of the different effect of these sterols on the DPPC bilayer and to relate these differences to the different microscopic structure of these molecules. The consistency of the proposed results may provide insights into the understanding of the effect of sterols on the binary bilayers as well as the lipid-lipid interactions.

2 Methods

2.1 The model

The binary mixtures of a DPPC bilayer with different mole fractions of sterols, 10, 20 and 30% (cholesterol and ergosterol) molecules were constructed using the web-based CHARMM-GUI membrane builder and were solvated by water molecules [50]. One additional pure DPPC bilayer system was also created as a control simulation. The total number of lipid molecules for the constructed systems varies between 350 and 400 and the number of water molecules is close to 13000.

2.2 Simulation protocol

MD simulations were performed using the version 2018.6 of GROMACS [51, 52], the CHARMM36 force field [53], and the TIP3 model for water molecules [54]. Periodic boundary conditions were applied in all directions. The long-range electrostatic interactions were treated using particle mesh Ewald method [55], with 1.2 nm cutoff distance and the compressibility of 4.5×10^{-5}. For van der Waals (vdW) interactions, the cut-off schemes with the cutoff distance of 1.2 nm were used, which is smoothly truncated between 1.0 and 1.2 nm. The electrostatic interactions were treated by the particle mesh Ewald method [55]. Constant pressure was controlled using the Parinello-Rahman barostat [56] with the semi-isotropic pressure of 1 bar. The temperature was controlled by coupling the system to the Nosé-Hoover thermostat [57, 58]. The LINCS algorithm [59] was utilized to constrain the bonds. The systems were first minimized in 10000 steps and were subsequently equilibrated using first the NVT (500 ps) and then the NPT (16 ns) protocol. During the course of equilibration, restraints were applied according to the CHARMM-GUI procedure on the head and the tail groups which were gradually decreased to zero in the course of the NPT equilibration. For the equilibration procedure of the gel phase of the systems containing 10% of cholesterol, we had to use larger restraints of 1000 $kJ.mol^{-1}.nm^{-2}$ during the equilibration in order to prevent the formation of the ripple phase. For each system, 600 ns production simulations were produced in the NPT ensemble with the time step of 2 fs. All the systems were simulated in the temperature range of 290-350 K with temperature steps of 10 K. Two sets of independent simulations were performed for the DPPC/ergosterol system. The last 400 ns of each production run was used for the analysis and the error bars were estimated by the block analysis method with the block size of 100 ns, unless otherwise stated.

For the simulations of the single sterols, the systems were first minimized in 1000 steps and then the simulations were performed in NVT ensemble at 300 K in vacuum without applying periodic boundary conditions. The same thermostat, cutoff distances and constraints were used as the ones for the bilayer systems. The equations of motions were integrated using 1 fs time step. The total length of the trajectory for each molecule is 50 ns.

All the simulations data have been analyzed using the python routines incorporating the MDAnalysis package [60, 61] and GROMACS tools. The VMD software was used for the visualization of the structures and the trajectories [62].

3 Results

3.1 Structural properties

In order to understand how the two sterol molecules modify the DPPC bilayer, we have calculated several parameters such as area per lipid, order parameter of the DPPC and sterols, electron density profiles, tilt angle of the DPPC molecules, the planarity of the sterols and the orientation of the methyl groups as well as the density of the carbon atoms of DPPC around sterols, which are presented as follows.

3.1.1 Area per lipid and condensing effect

One of the most commonly calculated parameters in lipid bilayer systems is the area per lipid. Many different ways have been proposed to quantify this parameter. The definition we have used here is the total area per DPPC molecules, which is calculated by dividing the cross-sectional area of the box along the xy plane ($L_x L_y$) divided by the total number of DPPC molecules, therefore excluding the sterols. This quantity estimates how much space is occupied by sterols among the lipid molecules, and therefore, it evaluates the sterols condensing effect. In pure DPPC bilayer, this quantity readily gives the area of DPPC molecules. As the temperature increases there is a considerable change in the area per DPPC in the pure system between T=310 and 320 K (Figure 2), meaning that the transition temperature lies in that range. The addition of sterols shifts the transition temperature to higher values, i.e. between 320 and 330 K. For the systems containing 30% sterols, the transition is continuous. When the sterols are added to the bilayer, they are mainly placed at the level of lipid chains due to the hydrophobic mismatch and being smaller than lipid molecules. Accordingly, the sterols first occupy the voids between the lipid chains and do not change the area per DPPC molecule, reflecting the condensing effect. At some point, the addition of more sterols increases this quantity and the spacing effect induced by more sterol molecules dominates the condensing effect. The results show that, interestingly, above the phase transition the addition of cholesterol does not change the area per DPPC up to 20% mole fraction, while this is not the case for ergosterol (Figure 2). These results indicate that cholesterol has a higher ability to condense the bilayer than ergosterol. The condensing effect of the sterols and their respective behavior can also be observed in the radial distribution function (RDF) of the lipid chains. The RDF profiles of the center of mass of the single lipid chains show that the positions of the RDF peaks along the radial distance are slightly closer to each other in the DPPC/sterol system with respect to the pure DPPC system, meaning that the presence of sterols condenses the lipid bilayer (Figure 3). Furthermore, the heights of the RDF peaks is relatively higher for the cholesterol system denoting higher packing of the lipid chains in the DPPC/cholesterol systems. This conclusion is in accordance with an x-ray diffraction experiment showing that cholesterol is stronger in condensing DMPC bilayer [22]. Consistently, ATRIR spectroscopy, detergent solubility and zeta potential measurements have also shown that the condensing capacity of cholesterol is stronger than that of ergosterol due to the less negative zeta potential [21]. Furthermore, at the monolayer level, cholesterol was found to induce smaller surface areas than ergosterol [63]. The higher con-

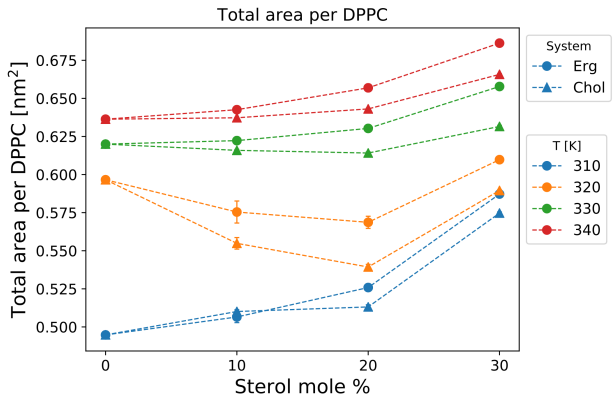

Figure 2: Area per DPPC molecules as a function of sterols mole fraction in the temperature range of T=310-340 K.

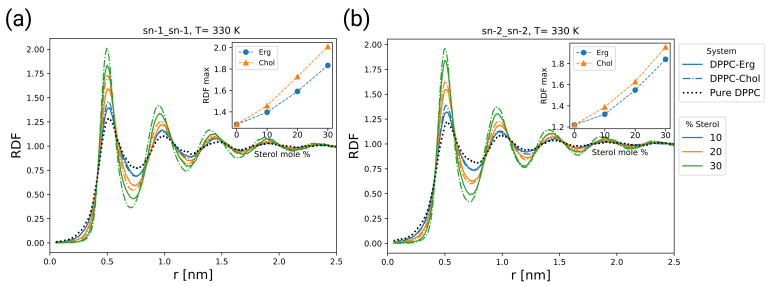

Figure 3: DPPC-DPPC RDF profiles for the pure DPPC bilayer as well as the binary mixtures with different mole fraction of sterols. The RDF profiles are based on the center of mass of the sn-1 (a) and sn-2 (b) chains of the DPPC molecules. The inset shows the height of the first peak in the RDF profiles at different mole fraction of sterols.

densing effect of cholesterol compared to ergosterol and lanesterol has also been reported in the experiments on monoloyer mixtures of DPPC and DMPC with the sterols, and cholesterol has been able to create more stable monolayers [48]. There are, however, a number of other simulations and experimental studies, which have obtained contrary results, stating that ergosterol is more effective in this respect than cholesterol [6, 15, 16, 23, 33, 39–41, 64].

3.1.2 DPPC order parameter

Another extensively used parameter to inspect the structural properties of the lipid bilayers is the order parameter. It is closely related to the condensing effect since more ordered chains leave less space in the bilayer. This quantity measures how the lipid chains are oriented with respect to the membrane normal and quantifies the degree of their orientational order. According to ref. [65] the

order parameter is defined based on the formula

$$S = \left\langle \frac{1}{2}(3\cos^2\Theta - 1) \right\rangle \tag{1}$$

where Θ is calculated for each segment of the lipid chains and is the angle between the vector constructed by C_{i-1} and C_{i+1}, connecting the carbon atoms $i-1$ and $i+1$, and the membrane normal (z-axis). The angular brackets represent the ensemble and time average. A random orientation corresponds to vanishing order. However, this value does not only describe random fluctuations but it is also reduced due to the tilting of the alkyl chains. Our idea is to disentangle the effects of tilting and random fluctuations. Therefore, we proceeded in two steps. First, we determined the average tilt vector of all the DPPC molecules. Whereas in the disordered phase this average tilt angle is close to the membrane normal, this is no longer the case in the gel phase where it expresses the collective tilting effect. Second, in the calculation of the order parameter we defined the angle Θ with respect to that average tilt vector. As a consequence, S is a measure for the fluctuations around that vector. The results for the newly defined order parameter is shown in Figure 4a, the tilt angle in Figure 4b and the order parameter, using the standard definition of Θ with respect to the membrane normal in Figure S1.

In the gel phase, i.e. below the phase transition, the pure DPPC bilayer represents the highest order and the addition of sterols slightly decreases the order of the lipid chains (Figure 4a). As a much stronger effect, the addition of sterols dramatically decreases the collective tilt angle (Figure 4b). Again here the impact of cholesterol is much stronger. Above the transition temperature, however, the addition of sterols considerably increases the order of the lipid chains. This increasing order also gives rise to a slight increase in membrane thickness according to the electron density profiles (Figure S2). These results reflect the dual nature of sterols that was discussed in the Introduction and are in qualitative agreement with a number of experimental data [6, 23–25]. Considering the relative behavior of cholesterol and ergosterol, the former displays stronger effects, which is in accordance with the condensing strength of cholesterol discussed in the previous section. This comparative result is also in agreement with the simulations of Smondyrev et al. [44] and some other NMR, X-ray diffraction and fluorescence experiments [22, 42, 45]. Contrary results, however, have been reported in some other simulations and experiments [6, 15, 16, 39, 66]

3.1.3 Sterols order parameter

Of key relevance is the orientational behavior of sterols since their tilt angle is commonly attributed to the ability of ordering the surrounding lipid chains. Naturally, in the low-temperature phase, the average tilt vector of the DPPC molecules again serves as an appropriate reference frame for the sterols. Therefore, we proceeded in full analogy to the case of pure DPPC and defined the angle Θ for the orientation of the individual sterol molecules with respect to that average tilt vector. For a better comparison with the corresponding data for DPPC we expressed the orientational behavior of the cholesterol molecules in terms of an order parameter rather than a tilt angle.

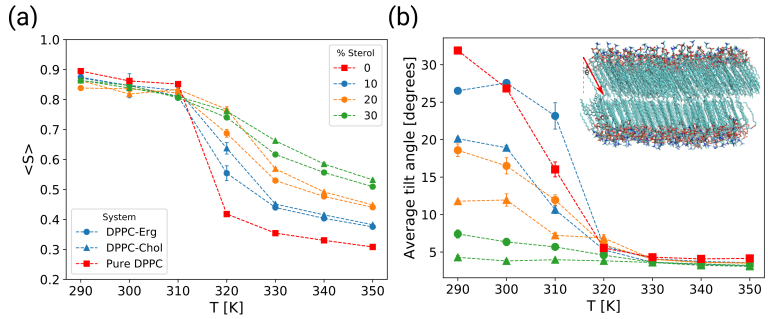

Figure 4: Temperature dependence of the average order parameter at different mole fraction of sterols. Due to the asymmetric distributions of the order parameter, median has been used instead of the average values. (b) The temperature dependence of the average tilt angle of the DPPC molecules with respect to the membrane normal for the pure DPPC bilayers as well as the binary systems is shown. To calculate this angle, the vector connecting the first and the last carbon atom of each chain is averaged over all the DPPC molecules. In the inset of panel (b) the snapshot of the lipid bilayer with 10% sterol at T = 290 K is represented. The average tilt vector of the DPPC molecules for the upper layer is schematically represented via a red vector, which makes the angle of Θ with the z-axis.

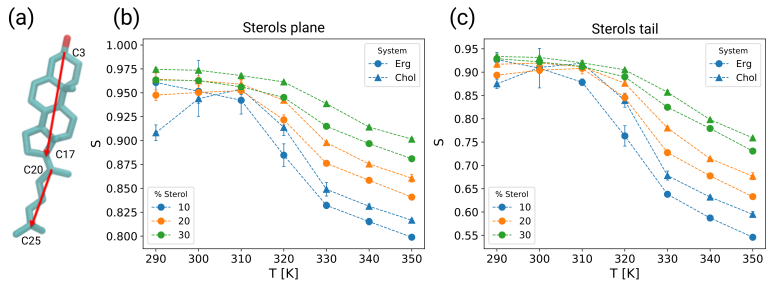

Figure 5: Average order parameter of the sterol molecules. (a) The cholesterol molecule and the defined vectors for the calculation of the tilt angle of the planar and the tail part of cholesterol (similar for ergosterol) are represented. The order parameter is calculated separately for the planar (b) and the tail part (c).

More specifically, we individually analyzed the planar and the tail part. For the planar part, the vector from atom C3 (first carbon in the planar part) to C17 (last carbon in the planar part with a methyl group) was considered, whereas for the tail part, the vector from atom C20 (first carbon in the tail) to C25 (last carbon in the tail) was used (Figure 5a).

Our analysis allows for a very specific comparison of DPPC (Figure 4a) and sterol behavior (Figure 5a and Figure 5b) with respect to the temperature and concentration dependent order parameter. Most notably, except for the absolute values, all the three graphs display a similar temperature and concentration

dependence.

In the low-temperature phase, the order parameter only weakly depends on temperature or concentration. This underlines that it was useful to determine the order parameter of these quantities in the tilted coordinate frame. Thus, the change of both temperature and concentration only modifies the tilt angle but not the size of fluctuations of both DPPC and sterol relative to this average tilt angle. This holds for both cholesterol and ergosterol so that their differences only matter for the overall tilt angle as already discussed above.

Above approximately 320 K there is no collective tilt angle any more and all order parameters decrease with increasing temperature. The order parameter of the planar part is considerably higher as compared to the DPPC chains and the tail part. This reflects the ability of the sterols to generate order even at high temperatures (Figure 5b). Naturally, the order parameter of the tail is lower than the planar part due to its flexibility. The decrease in sterol order parameter with increasing temperature and decreasing concentration is fully equivalent with the behavior of the DPPC chains, except for the absolute values. This similarity can be even extended to exchanging cholesterol with ergosterol. For the order parameters of the sterols as well as DPPC, this results in a lower value. This strong correlation as well as the high order parameter of the planar part corroborates the impact of the sterols to induce order for the DPPC chains. Consistently, MD simulations on the DMPC/sterol systems as well as more complex bilayers have also reported less ordering for ergosterol as compared to cholesterol in the modeled bilayer systems [44, 49].

The common property of both the low- and high-temperature phase is that DPPC is more strongly aligned along the membrane normal upon increasing sterol content. For all temeperatures cholesterol is more efficient in doing so. Surprisingly, in one other MD simulation study [15], cholesterol is only more efficient in the low-temperature phase.

Going beyond the description via the order parameter it is also instructive to analyse the whole distribution of tilt angles as can be frequently found in literature; see, e.g., ref. [67]. In Figure 6 we indicate the distribution of tilt angles of the planar parts of the sterols for different concentrations and temperatures but display it as a function of $\cos(\Theta)$ rather than Θ as typically done. Whereas in the low-temperature phase there is a maximum for intermediate values, in the high-temperature regime one observes just a fast decaying distribution with a maximum close to zero tilt, i.e. $\cos(\Theta) = 1$. In physical terms this means that the entropic factor proportional to $\sin(\Theta)$ is taken out. Thus, the sterol molecules have a strong tendency to align parallel to the membrane normal apart from the fluctuations and it is just the trivial entropic factor which in reality gives rise to the most probable non-zero tilt angle. Consistent with the results based on the order parameter, the distribution is more centered for cholesterol than for ergosterol so that the resulting order parameter is larger for cholesterol. These arguments are inspired by ref. [68] where the width of that distribution is expressed in terms of the tilt sterol modulus.

3.1.4 Planarity of the sterols

Having identified the higher efficiency of cholesterol as compared to ergosterol to alter the properties of DPPC, one may wonder which structural feature is responsible for this behavior. Here we study the planarity as a possible structural

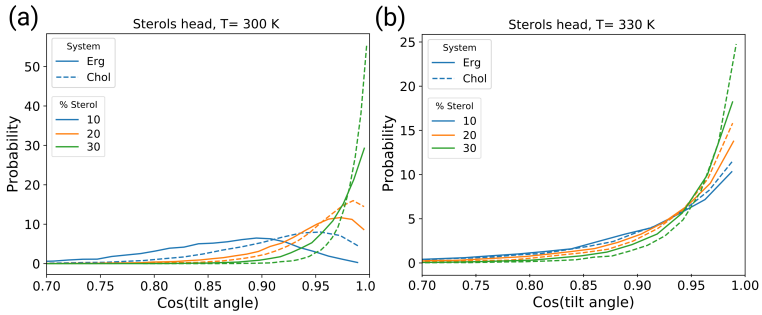

Figure 6: Distribution of the cosine of the tilt angle of the sterols for the simulations performed at T=300 K (a) and T=330 K (b). The tilt angle is calculated as the angle between the z-axis and the vector defined by C3 and C17 atoms of the planar part.

candidate. Indeed, although the chemical structures of the sterols are similar, their 3D structure and dynamics varies and may lead to different impacts on the lipid membranes [21, 24, 32–35]. It is hypothesized that the presence of double bonds modifies the planarity. Coprosterol, which is a fully saturated analog of cholesterol has been shown to either abolish domain formation completely or reduce the temperature of mixing, depending on the lipid type [33]. In agreement, the ab-initio calculations predicted a boat-shape structure for this sterol, far from a flat molecule [35]. In the mixtures of DPPC/sterols, the introduction of an extra double bond in the planar part of cholesterol, the so-called 7-dehydrocholesterol (7DHC), has lead to a drastic increase in the ordering capacity. In ref. [24] it turned out that ergosterol with its extra double bond and the methyl group in its tail had the highest ordering capacity among the three sterols. Similarly, ergosterol and 7DHC were more domain-promoting than cholesterol in raft model membranes. Conversely, however, in the liposomes containing DPPC/sterols, the condensing effect of ergosterol and 7DHC was found to be lower than cholesterol [21]. There are also other studies, in which the relative effects of cholesterol and 7DHC is different, both in the monolayers and bilayers [69–73]

The above discussion shows that the presence of at least one double bond is required for the efficiency of the sterols, however, there is a debate on whether the existence of the extra double bond in the planar part results in a more efficient sterol. Furthermore, it has been shown previously that the role of the planar part is stronger than the tail part [24], which is naturally due to its bulkier and rigid structure. Therefore, we examined the planarity of the ring system of the two sterols in all the simulated systems. For this purpose, we quantified the planarity by aligning a hypothetical plane with optimum overlap to the ring system of the sterols (Figure 7a) and calculated the sum of the distances of all the atoms in this part from the imaginary plane (Figure 7b). In general, this quantity has higher values for ergosterol relative to cholesterol, meaning that cholesterol is more planar. Below the phase transition, this quantity is relatively dependent on the concentration, specifically for ergosterol. Above the phase transition, however, there is a systematic difference between the planarity

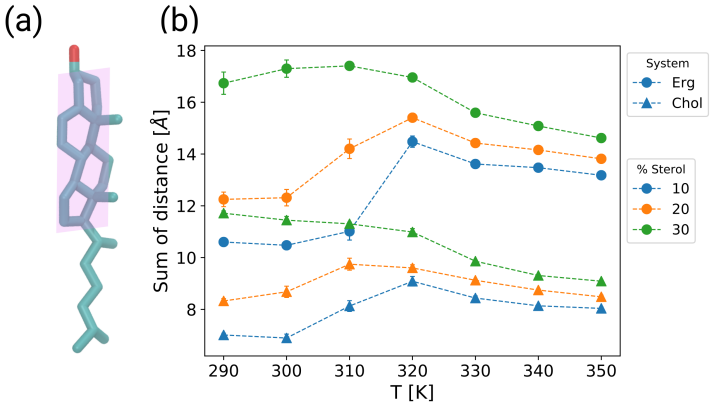

Figure 7: Planarity of the sterols. (a) Cholesterol molecule is depicted with the schematic optimum aligned plane to its ring part. (b) The sum of the distances of all the carbon atoms of the sterol planar part from the aligned plane.

of the two sterols, and the dependence on the concentration is relatively low. Interestingly, the planarity of the sterols is lowest for the higher concentration, especially for ergosterol. Therefore, we checked if this quantity is affected by the number of neighboring sterol molecules around each sterol. The result shows that the decrease of planarity with the sterol content is not a local effect rather a global one, both in the gel phase and above the phase transition (Figure S3). In summary, we may conclude that the higher planarity of cholesterol serves as a reasonable structural motif, inducing the stronger impact of cholesterol than ergosterol, as discussed so far (and also later).

Now in order to check whether the planarity is modified by the lipid environment, we performed the MD simulations of the individual sterols in vacuum and calculated their planarity (Figure S4). We obtained for ergosterol and cholesterol 8.70 ± 0.67 and 5.76 ± 0.72 Å, respectively. Consequently, the planar part is flatter in vacuum for both sterols. Although the order is conserved, the strong deviations from the sterols in the lipid bilayer indicate that the binding ability of the sterols is somewhat reduced due to the surrounding lipids. It also indicates that the information, obtained from vacuum simulations, has to be taken with care.

3.1.5 Orientation of Methyl groups

The role of the smooth and the rough face of cholesterol has been studied in several computational and experimental works. These studies showed that the smooth side of cholesterol promotes the order more than its rough side [14, 39, 74–76], although the presence of the methyl groups on the rough side is also essential for its functionality [77, 78]. Since the planarity of the two sterols was found to be different, as discussed in the previous section, it might also translate to a different state of the methyl groups. By superimposing the cholesterol and ergosterol molecules over each other, we observed that the orientation of the methyl groups are significantly different for the two sterol molecules (Figure 8a).

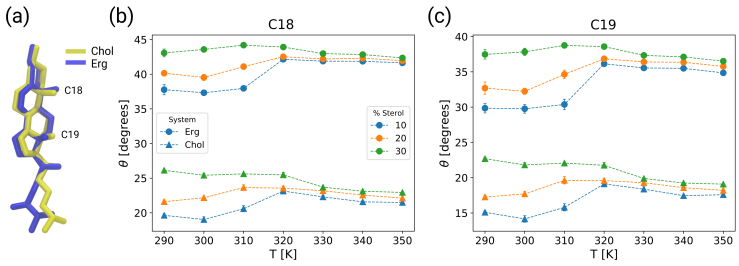

Figure 8: (a) The superposition of cholesterol and ergosterol is represented in yellow and blue, respectively. The temperature dependence of the average angle between C18 and C19 methyl groups with the normal vector of the optimum fitted plane to the sterols ring part are shown in panel (b) and (c), respectively, at different concentrations of sterols.

To quantify this observation, we calculated the average angle between each methyl group and the normal vector of the fitted plane to the ring part of the sterols, which was used for the estimation of planarity. In accordance with the inspection by superposition, these angles are remarkably higher for ergosterol than cholesterol (Figure 8b,c). This difference, as expected, likely arises from the different 3D structure and the planarity of the ring system of the two sterols, and may also contribute to the higher efficiency of cholesterol as compared to ergosterol with respect to the DPPC properties.

3.1.6 Anisotropic DPPC-sterol structure

The differences in the structural properties of the sterols molecules discussed in the previous sections can have a significant impact on the distribution of the lipid chains on the two sides of the sterols. Therefore, we study the details of lateral ordering and the arrangement of the lipid chains around the sterol molecules, and probe how they are affected by the planarity and the orientation of the methyl groups. For this purpose, we have calculated the average 2D density maps of the carbon atoms of the DPPC chains around all the sterol molecules, superimposed with respect to a frame of reference. This particular density profile provides additional information as compared to the RDF since it is not averaged over the angular direction. Furthermore, it represents the details of the density around the sterol with respect to the smooth and the rough side of the sterol (Figure 1d). The significance of each side in inducing order has been discussed elsewhere [14, 39, 74–78]. The density maps shown here are related to the DPPC membranes with 20% sterol at T=330 K (Figure 9). In order to clarify the density at the two faces of the sterol molecules, we have tagged three atoms of the sterol, two of which are located in the planar part (C7 and C11), and one is the carbon atom in the first methyl group of the sterols (C18) (Figure 9a) and represented them in the density map.

For the case of cholesterol and considering the lateral distribution, the RDF profiles based on the density map show that the packing of the carbon atoms are not the same for the smooth ($0<\theta<180°$) and the rough side ($180°<\theta<360°$) (Figure 9a). The position of the peaks for the first and the second shell on the

rough side are located at ∼6 and ∼10.5 Å, respectively, and the third shell is nearly smeared out (Figure 9b,c). The position of the peaks on the smooth side are, however, located at closer distances, ∼5.4 and ∼10 Å, respectively for the first and the second shell. This means that the smooth side is more densely packed with carbon atoms than the rough side. This result is consistent with a computational work, in which a detailed RDF analysis showed a more populated packing of carbon atoms on the smooth side with respect to the rough side [79].

A closer look at the first shell revealed that the distribution of the carbon atoms is not monotonic and there are preferred locations for the lipid chains around the sterol. In order to clarify these locations, we have monitored how the density changes as a function of θ, by considering an annulus around the sterol with the radius $3.5<r<7.0$ Å. Since the densities are not symmetric, the annulus does not ideally capture the strength of all the peaks. The variation of density along the annulus represents approximately seven peaks, three peaks on the smooth side ($\theta \approx 90°$, 70° and 130°) and four peaks on the rough side ($\theta \approx 200°$, 260°, 300° and 330°) (Figure 9d). This type of 2D analysis has already been performed for the DSPC/cholesterol system by tracking one sterol molecule, and the position of the peaks is nearly similar [76].

For the case of the ergosterol system, there are distinctive differences with respect to cholesterol (Figure 9e). According to the RDF profiles, the average lateral distribution of the carbon atoms represents almost no difference between the smooth and the rough side of ergosterol (Figure 9f), and are almost the same as the rough side of cholesterol (Figure 9c) with only slightly lower values. This means that the smooth side of ergosterol is less densely populated by the carbon atoms of the acyl chains. The first coordination shell also indicates distinctive differences. Consistent with the RDF profiles of the smooth and the rough side, the shells around ergosterol have a more circular shape, compared to a more triangular shape of the shells around cholesterol. The variation of density along the first shell at different angular directions shows the same number of peaks as for the case of cholesterol although the position of some of the peaks is slightly different (Figure 9e,g). There are also two distinctive differences on the two sides of the sterols. On the smooth side of ergosterol, a minimum is observed (corresponding to $\theta \approx 45°$) and the second and the third shells are more disrupted in this region, while this is not the case for cholesterol. On the rough side, the distribution is more homogeneous for ergosterol, while the peaks on the rough side of cholesterol are more pronounced, particularly the one located at $\theta \approx 290°$ (Figure 9g). Furthermore, opposite to the case for ergosterol, the second and the third shell of cholesterols are slightly disturbed on the rough side rather than the smooth side at $\theta \approx 260°$. The differences observed on the rough side are likely due to the different deviation of the methyl groups in the two sterol molecule, and for the case of ergosterol, they tend to distribute the carbon atoms more uniformly in this region as they deviate more from the normal of the plane fitted to the planar part.

3.2 Dynamical properties

3.2.1 Flexibility of the sterols

In this section, we intend to probe the respective rigidity of the two sterols and their flexibility. For this purpose, we calculated the root-mean-squared deviation

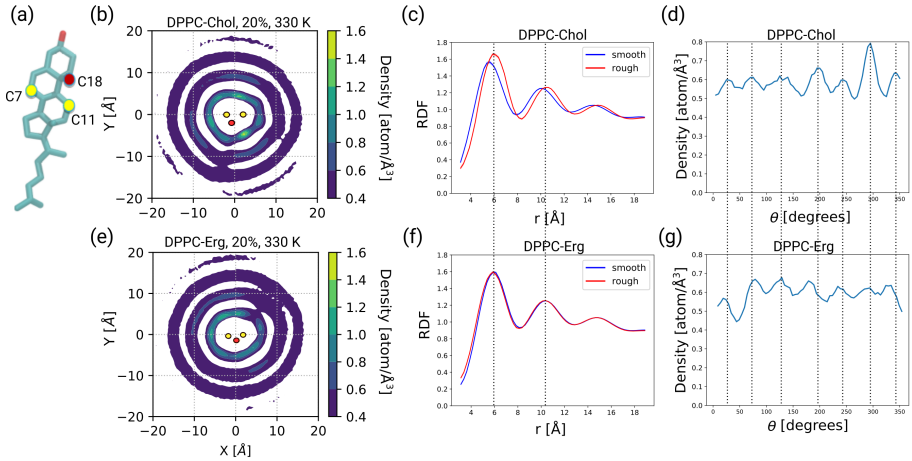

Figure 9: The 2D density maps of the superposition of the carbon atoms of the DPPC chains around all the sterol molecule in the simulated systems with 20% sterol at T=330 K. (a) Cholesterol is represented with three labeled atoms (C7, C11 and C18) which have been used to show the orientation of the sterol molecules with respect to its smooth and rough side. The 2D density maps of the carbon atoms are shown, respectively for DPPC/Erg (b) and DPPC/Chol (e) systems. The RDF profiles based on the 2D densities for the smooth (blue) and rough (red) side for cholesterol (c) and ergosterol (f) are plotted. The variation of density for the first coordination shell ($3.5<r<7.0$ Å) along different angles is represented for cholesterol (d) and ergosterol (g). The density plots were produced by superposition of the carbon atoms of the DPPC chains around each sterol molecule over the trajectory, rotated and translated with respect to the frame of reference defined by the coordinates of the planar part of a specific sterol in the first configuration.

(RMSD) for the planar part. The RMSD of the planar part of ergosterol is lower with respect to cholesterol which, as expected, is likely due to the extra double bond in this region of ergosterol, which renders this molecule more rigidity (Figure 10). This property is nearly independent of concentration with a slight increase as the temperature rises (Figure 10).

In order to inspect again whether these properties are dependent on the lipid environment, we calculated the RMSD of the sterols in the simulations of the individual sterols in vacuum. For the RMSD of the planar part of the sterols we obtained 0.23 ± 0.02 nm and 0.38 ± 0.01 nm, respectively, for ergosterol and cholesterol. This shows that the planar part of ergosterol is more rigid than cholesterol also in vacuum, where the rigidity of the two molecules is lower in comparison to the lipid environment.

Concerning the tail part, the RMSD values were not significantly different for the two sterols, with surprisingly higher values for ergosterol, even though the extra double bond in this region is also supposed to restrict its motion. For the systems with 20% sterol at T=300 K, the RMSD is 0.16 and 0.18 nm for cholesterol and ergosterol, respectively. The RMSD of the tail in vacuum simulations, however, was lower for ergosterol (0.19 nm), compared to cholesterol

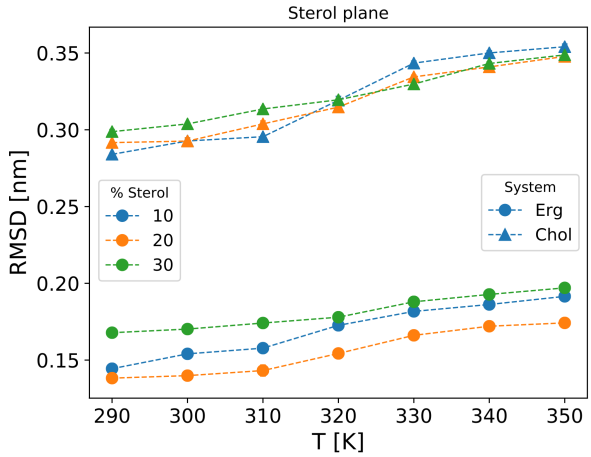

Figure 10: The temperature dependence of the average RMSD of the planar part at different mole fraction of sterols.

(0.21 nm), opposite to the behavior in the lipid environment. We further looked at the configurations of the tail part in the vacuum simulations by superimposing the structures of each molecule along the trajectory by fitting them with respect to the planar part. Accordingly, we observed that the tail of cholesterol is more flexible than that of ergosterol and can completely bend towards the smooth side. For ergosterol, however, this movement is more restricted (Figure S4). Nevertheless, we also detected a number of linear configurations of cholesterol, in which the tail part is stretched along the planar part (Figure S4). These observations in vacuum simulations are in agreement with the previous MD simulations on individual sterols [34]. In one MD study, however, the tail of cholesterol had noticeably higher fluctuations although the authors have adopted the same force field as the one we have used [35]. Nonetheless, the results of the vacuum simulations should be interpreted with caution as the interaction of the sterols with the lipid chains can modify their behavior, as it was also the case with planarity, not forgetting also the higher flexibility of the tail part with respect to the planar part and a higher probability for a different behavior among the lipid chains.

3.2.2 Fluidity of the lipids

As noted in the Introduction, the addition of sterols within the lipid chains modifies the fluidity of the lipid bilayers and the lipids dynamics. To quantify this effect, we calculated the mean-squared displacement (MSD) of the lipids at varying temperatures and concentrations. In Figure S5a, the MSD for T=330 K is represented at different concentration of sterols. The results show that at this temperature, which is above the phase transition, the lipid molecules can reach the diffusive regime during the simulation time scale, while below the phase transition (T≤310 K) they behave in a sub-diffusive manner (Figure S5a). The sub-diffusive behavior at low temperatures is the consequence of the gel phase,

in which the movement of lipids is restricted and the fluidity of the membrane is low. Furthermore, the addition of sterols to the pure bilayer reduces the fluidity of the membrane above the phase transition (Figure S5b). In order to represent the mobility of the lipids both below and above the phase transition and to probe the effect of the sterol content, we looked at the average MSD values at a specific part of the trajectory, between 100 and 150 ns. As Figure 11 shows, incorporating more sterols decreases the fluidity above the phase transition, whereas in the gel phase, they induce more fluidity in the membrane. Similar MSD values were also observed for the sterol molecules (Figure S6). This dual nature has been previously reported for cholesterol [23–25], and here the MD simulations further confirm this characteristic for both sterols. Moreover, the comparison of the two sterols indicates that the effect of cholesterol on the mobility of the DPPC molecules is stronger for the systems with 30% sterol above the phase transitions, where the lipid molecules are in the diffusive regime (Figure 11inset). This is also reflected in the mobility of the sterol molecules (Figure S6inset). Consistent with these results, in one MD simulation study, a higher diffusion coefficient was reported for ergosterol than cholesterol [44]. This result is also in accordance with the fluorescence experiments, showing that the effect of ergosterol is less pronounced both in rigidifying fluid membranes and fluidizing gel phase membranes [46]. The higher impact of cholesterol on dynamics has also been reported in the fluorescence experiment on the binary and ternary systems including DPPC and sterols via fluidity measurements [38, 42]. The results proposed here are, however, contrary to the quasi-elastic neutron scattering experiment, showing that ergosterol has a lower lateral diffusion coefficient compared to cholesterol [64]. Furthermore, in the MD simulation study by Czub et al., a stronger influence of ergosterol compared to cholesterol has been reported on the dynamics of the DMPC molecules [15].

3.3 Intermolecular interactions

In order to better understand the lipid-lipid interactions at the molecular level, here we estimated the average pair interaction energy, i.e. enthalpy, as a function of the average order parameter of the respective pairs. This quantity measures the vdW and electrostatic energy contributions of the lipid pairs in the DPPC/sterol systems. This function was obtained by averaging over all the potential energies of all the nearest neighbors of each lipid as a function of the average order parameter of the DPPC molecules in the corresponding pair. For this purpose, we need to define the cut-off radius within which the nearest neighbors are defined. For the DPPC-DPPC and DPPC-sterol interaction, this radius can be obtained using, respectively, the radial distribution function (RDF) of the P-P and P-O3 pairs (P is the phosphorous atom of DPPC and O3 is the oxygen atom of the sterols). For the P-P and P-O3 RDF profiles, the position of the first minimum can be found at ∼10 and ∼8 Å, respectively (Figure S7). After setting the neighborhood distance, we looked at the average interaction energy as a function of the average order parameter of the two DPPC molecules in the pair for the DPPC-DPPC interaction, and the order parameter of the DPPC molecule in the pair corresponding to the DPPC-sterol interaction. These energies were calculated for the systems containing 20 and 30 % sterol, which were simulated at the temperatures of T=330 and 340 K, and the results presented here for each concentration are the averaged values over

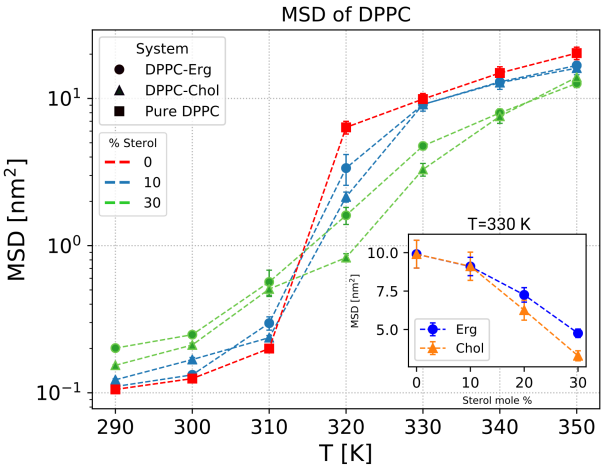

Figure 11: The temperature dependence of the average lateral MSD of the DPPC molecules for the pure DPPC bilayer as well as the bilayers with 10% and 30% sterols is represented. (inset) The MSD values for the DPPC molecules in the DPPC/sterol systems at different mole fractions of sterols are shown for the simulations performed at T=330 K. For all the data points, the MSD values corresponding to the 100 to 150 ns trajectory part were averaged. The error bars are standard deviations, which were computed by dividing the considered trajectory part into two parts.

the two temperatures. The DPPC-DPPC interaction energies for both systems decrease with the increase in order parameter, meaning that the interaction energy is more favorable at higher order parameters (Figure 12a,b). Indeed, for higher order parameter the chains adjust themselves in a way that optimal vdW interaction is fulfilled, while for the disordered chains, only a few carbon atoms manage to displace in optimum positions. A similar behavior has been observed in a previous work [80]. It is also worth noting that, as it has been previously shown, almost all the order parameter dependence comes from the enthalpic term [80]. For DPPC-DPPC interaction, the behavior is similar for the two systems, except that the average value of the energy is lower and more favorable for the systems containing cholesterol (Figure 12a,b), which is related to the higher order parameter of this sterol (Figure 4a). This effect is noticeably more pronounced for the DPPC-sterol interaction both for 20 and 30% of sterols (Figure 12c,d): in the systems containing ergosterol, the DPPC-sterol interaction is less sensitive to the DPPC configuration and the order of the DPPC chains, particularly in the systems containing 30% sterol; for cholesterol, however, this interaction is more sensitive to the order of the lipid chains and is more favorable with respect to the corresponding energies in the ergosterol systems. Therefore, the stronger impact of cholesterol is due to its stronger order parameter dependence of the energies particularly for the DPPC-sterol interactions. This result is expected due to the condensing effect of cholesterol as well as the more linear and flat structure of this molecule when compared to ergosterol. Indeed, the more planar and linear structure of cholesterol allows closer interaction with the

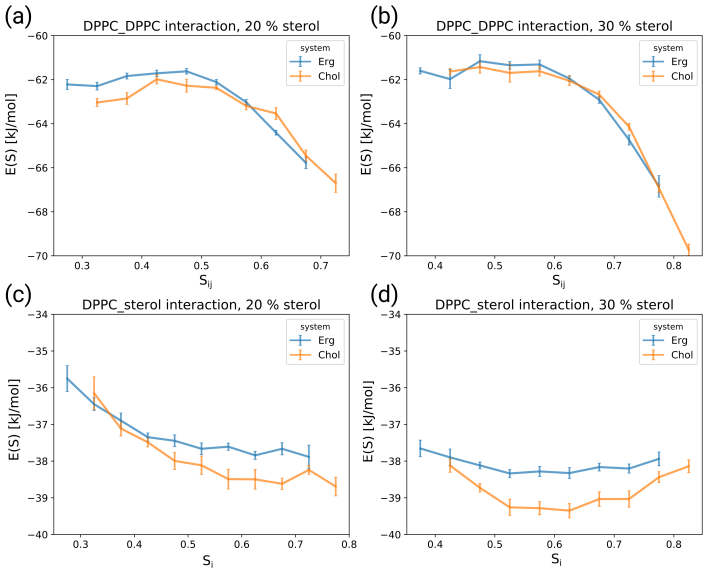

Figure 12: The average DPPC-DPPC interaction energy as a function of the average order parameter of the DPPC molecules in each pair for the bilayers with 20% (a) and 30% (b) of sterol is represented. The average DPPC-sterol interaction energy as a function of the order parameter of the DPPC molecule in each pair for the bilayers with 20% (c) and 30% (d) of sterol is shown. For all the interactions, the averages are taken over the two simulations performed at T=330 and 340 K.

lipid chains. Consistently, in a Langmuir monolayer study the excess free energy of mixing measurements revealed attractive interaction of cholesterol with DPPC, while the interaction with ergosterol was found to be less favorable [63]. In another study on monolayers containing DPPC or DMPC with sterols, again the excess free energy analysis showed that cholesterol enhances the stability of the monolayers as compared to other sterols including ergosterol [48].

4 Discussion and conclusions

Sterol molecules have a critical role in biological membrane properties and functionality, resulting from their specific structural features. The main functional elements of these molecules are the rigid and planar ring system, the small hydrophilic hydroxyl group, the short flexible tail and the asymmetric nature due to the presence of the smooth side and the rough side, which includes the methyl groups, as well as the respective existence and the position of double bonds in the ring system and the tail. It has already been established that any modification to these structural features diminishes the effect of cholesterol on the lipid bilayers [4, 14, 24, 33, 39, 41, 67, 81–83].

In literature it has been argued that the resulting tilt angle of the planar part of sterol molecules is strongly connected to its ability to modify the membrane properties [67]: the smaller the tilt angle, the stronger the impact. Note that

for the DPPC/sterol system in the low-temperature regime, the tilt angle is basically identical to the DPPC tilt angle which approaches zero for high sterol concentrations. Indeed, for all temperatures the sterol aims to be parallel to the membrane normal. This observation might be captured by the sterol tilt modulus as used in ref. [68]. Actually, the finite average tilt angle, often reported in publications, is mainly an effect of the entropic factor, suppressing the phase space of small tilt angles. Thus, a stronger localization around $\cos(\Theta) = 1$ in the distribution as a function of $\cos(\Theta)$ is equivalent to a lower average tilt angle if analysed in dependence of Θ.

As shown in this work, the tilt angle is smaller for cholesterol both in the low- and high-temperature phase. In agreement with the proposal from literature, this higher efficiency translates to all membrane properties, studied in this work. Specifically, we have analysed the condensing effect, the impact on the order parameters, the degree of structuring around the sterol molecules, and the impact on the mobility. Note that the observed effects can be very different in both phases, e.g. the mobility being increased upon addition of sterols in the low-temperature phase and decreased in the high-temperature phase. However, in all cases except the MSD of lipids in the low-temperature regime, we obtained a stronger impact of cholesterol as compared to ergosterol. This observation is also in line with the phase diagram of ergosterol, which implies that a higher mole fraction of ergosterol is required in order to generate the L_o phase and to be as effective as cholesterol [23]. Furthermore, it also agrees with more recent experiments [22, 42].

One may argue that the different tilt angles of cholesterol and ergosterol is not the *cause* of the observed differences in the membrane properties but is already a *consequence* of the underlying structural distinctions in the two sterols. Naturally, the information from simulations may be explored to get additional insight about these structural effects. Concerning the planar part, it has been shown that coprosterol, which does not have any double bond in its structure, is completely inefficient with respect to cholesterol [24, 33]. Therefore, the presence of at least one double bond in the planar part is necessary as a more planar structure resulting from this double bond is crucial for its condensing and ordering capability. The simulations have revealed that the planar part for both sterols only shows very small fluctuations around the collective tilt in the low-temperature phase (for all concentrations) and significantly smaller fluctuations than the alkyl chains of DPPC in the high-temperature phase. Interestingly, the additional double bond of ergosterol in the planar part reduces the planarity significantly. Naturally, a lower planarity may give rise to weaker van der Waals interactions and thus explain the weaker impact of ergosterol. This effect was quantified in this work by the systematic analysis of the interaction energies of nearest neighbors, showing that the DPPC-cholesterol interaction is particularly strong for DPPC with high order parameters. As a comparison, 7DHC, which also has an extra double bond in its planar part with respect to cholesterol, has been shown to have lower effects when compared to cholesterol [21, 24, 33, 69–73]. Furthermore, it is known that ergosterol is more rigid and stiffer due to the two extra double bonds in the ring and the tail part and the rigidity is linked to the ordering capability. Our RMSD analysis of the planar part proved that ergosterol indeed has a more rigid structure as compared to cholesterol. Thus, the higher rigidity cannot balance the lower degree of planarity to have a strong impact on the surrounding lipid molecules.

Related to the properties of the planar part, the specific orientations of the methyl groups seem to be essential for the ordering effect [78], yielding, e.g., different packing behavior on the smooth and the rough side [77] and the relevance of both sides for its functionality [76]. Thus, it is remarkable that for ergosterol the two methyl groups show very different orientations which, correspondingly, results in a more uniformly distribution of the surrounding lipid chains.

Concerning the tail part of the sterols, it has been shown that the introduction of a double bond in this region counterbalances the drastic effect of the sterols with bulky tail on their condensing capability [24]. It has also been shown that desmosterol, which is a direct precursor of cholesterol in its biosynthetic pathway, is not able to replace cholesterol in rafts [82]. It is different from cholesterol only in one double bond in the tail region, close to the end of the tail, and it has been shown that its tilt angle is 7° higher than that for cholesterol [82]. The same behavior has been observed in an MD simulation work, where higher tilt angles of desmosterol and lower order of DPPC lipids has been reported [67]. Thus, there is an additional contribution of the tail part to modify the membrane properties. The tail regions of ergosterol display lower order parameters in the high-temperature phase as compared to cholesterol. Thus, the different chemical structure of the tail of ergosterol brings in disorder maybe in analogy to the comparison of unsaturated and saturated lipids. However, based on the higher correlation of the orientation in the low-temperature phase, as reflected by $S > 0.95$, one may tentatively conclude that the planar part is of key importance to understand the impact of sterols and, in particular, to compare different sterols.

As already mentioned above, there are several studies on the binary systems of saturated lipids with sterols that have obtained contradictory results about the relative behavior of cholesterol and ergosterol. In terms of MD simulations, the discrepancies might be due to the use of various force fields with different levels of accuracy as well as the limitations on the system size and the simulation time scales. The analysis of our work was performed with the latest version of CHARMM, a well-accepted force field for bio-simulations [84]. However, in a recent study discrepancies were reported when comparing MD simulation of individual sterol molecules in vacuum with ab-initio calculations [35]. It was suggested that the force fields should be improved in particular for ergosterol. Naturally, there should exist strong correlations between MD simulations of single molecules and ab-initio calculations. For example, the linear and stretched configurations of cholesterol, seen in our vacuum simulations, have been already detected as low energy configurations in previous single-molecule studies at the ab-initio level [34, 35]. Nevertheless, we would like to stress that this type of comparison requires careful interpretation since finally the quality of the force fields should be judged based on the features of the entire system rather than the single molecules. For instance, we observed in our simulations that the degree of planarity was nearly half as small for both sterols in vacuum simulations; and with respect to the fluctuations of the tail, the ranking of both sterols even changed. Therefore, the behavior of the sterols can be different in the lipid environment.

Naturally, one always has to keep in mind that there is no perfect force field and improvement is always possible. Independent of the specific force field, used in our work, the results of our systematic analysis suggest that the planar

part of a sterol molecule has a key impact on the membrane properties. Often the rigidity is regarded as a central property of a sterol molecule [6, 16, 35, 39, 43, 49]. Indeed, in agreement with literature we also find that ergosterol is more rigid than cholesterol due to the additional double bond. However, for the modelled systems in this work it is the lower planarity rather than the increased rigidity that finally determines the degree of the impact of the sterol on the membrane properties. Thus, if in future work a differently parametrized consistent set of force field parameters would reverse the degree of planarity of cholesterol and ergosterol, we would expect a systematic modification of all other properties, discussed in this work, as well.

In summary, the results presented in this simulation work highlight the stronger impact of cholesterol over ergosterol in the DPPC binary bilayers in terms of ordering capacity, condensing effect, lateral diffusion and DPPC-sterol interaction, just to mention the most relevant ones. They also clarify how the structural and dynamical properties of the lipid molecules are governed by different structural features of the sterols. It was revealed how the rigidity, planarity of the ring system and the orientation of the methyl groups as well as the orientation and movements of the tail region can be responsible for the stronger impact of cholesterol to order the lipid chain and modify their dynamics as compared to ergosterol. The outcomes of the present work is relevant to better understand the regulation of saturated chains of DPPC lipids with sterols in the binary mixture of bilayers and lipid rafts.

Author Contributions

AA, FK, and AH conceived and designed the analysis. AA performed the computer simulations and the subsequent analysis. AA and FK wrote the analysis tools. AA wrote the initial version of the manuscript. To the final version there were additional contributions also from AH and FK.

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