Bilayer Thickness and Thermal Response of Dimyristoylphosphatidylcholine Unilamellar Vesicles Containing Cholesterol, Ergosterol and Lanosterol: A Small-Angle Neutron Scattering Study

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ABSTRACT

Small-angle neutron scattering (SANS) measurements are performed on pure dimyristoyl phosphatidylcholine (DMPC) unilamellar vesicles (ULV) and those containing either 20 or 47 mol % cholesterol, ergosterol or lanosterol. From the SANS data we were able to determine the influence of these sterols on ULV bilayer thickness and vesicle area expansion coefficients. While these parameters have been determined previously for membranes containing cholesterol, to the best of our knowledge, this is the first time such results have been presented for membranes containing the structurally related sterols, ergosterol and lanosterol. At both molar concentrations and at temperatures ranging from 10 to 45 ^{o}C, the addition of the different sterols leads to increases in bilayer thickness, relative to pure DMPC. We observe large differences in the influence of these sterols on the membrane thermal area expansion coefficient. All three sterols, however, produce very similar changes to membrane thickness.

INTRODUCTION

Membrane hydrophobic thickness has been identified as an important modulator for the insertion [1], folding [2], multimeric assembly [3], and function [4-7] of trans-membrane proteins. Since membrane protein reconstitution and activity studies often utilize unilamellar vesicles (e.g. [8]), ULV have become the focus of a number of studies pertaining to the bilayer's hydrophobic thickness [9-11], and its modulation by cholesterol [12], proteins [13], solutes [14] and other additives [15]. Both SANS and small-angle x-ray scattering (SAXS) have proven particularly useful for such studies, since the scattering data yielded by these techniques are directly related to vesicle structural parameters (e.g. [10,13]). It has been hypothesized that the correlation between protein function and cholesterol levels in membranes may be related to the modulation of the membrane's hydrophobic thickness (e.g. [7]). However, while cholesterol has been identified as a modulator of membrane protein function ([16,17]), such modulation could occur via any combination of factors including: hydrophobic mismatch between the membrane and transmembrane protein segments ([4-7]), membrane dynamics [18], membrane elasticity [7], membrane lateral pressure profile [19], and membrane lateral organization [20], since cholesterol affects all of these membrane properties. Thus, the identification of the particular mechanisms for cholesterol modulation of protein function clearly requires knowledge of how cholesterol modulates membrane properties, such as the hydrophobic thickness, elasticity, and lateral organization.

Synthetic and naturally derived sterols that are structurally related to cholesterol have been utilized as biomolecular probes allowing researchers to identify the specific structural features of cholesterol that enable it to aid in the function of a variety of membrane proteins (e.g., [21,22]). Such probes also present the opportunity to identify the structural features responsible for cholesterol's abilities to modulate membrane permeability [23], elasticity [24,25], lateral organization [26,27], and acyl chain order [24,28-30]. As such, sterols that are structurally related to cholesterol can help us to elucidate cholesterol's role in modulating the function of transmembrane proteins.

Ergosterol and lanosterol are two specific sterols that have attracted some interest in comparative studies with cholesterol [24, 29-31]. Ergosterol differs from cholesterol in having a double bond at the base of its tail, joining C22 and C23, and an additional methyl group attached to C24 (Fig. 1). On the other hand, lanosterol has two additional methyl groups on the otherwise flat alpha face, attached to C4 and C14 and one additional methyl group on its beta face, also attached at the C4 position (Fig. 1). Similar to ergosterol, this sterol also has a double bond in its tail, joining C24 and C25. The dimensions of ergosterol and lanosterol (total length of the molecule and length of the rigid steroid portion) are comparable to cholesterol.

Interest in comparisons of ergosterol and lanosterol to cholesterol does not only arise from their structural similarities, but also their biosynthetic relationships. Ergosterol is the final step in the biosynthetic pathway for sterols of a variety of yeasts, thus presenting a functional evolutionary alternative to cholesterol [32,33]. Lanosterol, on the other hand, is a biosynthetic precursor to both cholesterol and ergosterol and rarely appears as a final product of sterol biosynthesis in organisms (see, e.g., [34, 35]). Despite the small differences in their molecular architecture, these sterols differ significantly in their modulation of membrane properties, which could be

associated with their differing roles in biological membranes. Studies of the three sterols have shown that they vary in their influence on membrane acyl chain order [28-30, 36], phospholipid lateral organization [26], and membrane material properties, such as elasticity and bending rigidity [24, 25, 31].

Studies of sterol-containing membranes have also shown that these lipid mixtures exhibit complex phase behaviour, which is sterol dependent [37-39]. Such mixtures can exhibit a liquid-ordered (lo) phase, characterized by high sterol content, molecular order similar to a gel phase and lipid dynamics and mobility characteristic of fluid phase bilayers. Differences in the phase diagrams of membranes containing cholesterol, ergosterol and lanosterol are manifest as variations in the position of phase boundaries, as well as differences in the qualitative shapes of the phase diagrams.

Because of the potential importance of hydrophobic mismatch in modulating protein function, an understanding of the mechanisms by which sterols modulate protein function requires knowledge of how they induce changes in membrane thickness. Extensive studies by various scattering methods have shown that the addition of cholesterol to fluid phase membranes increases the membrane bilayer thickness (e.g. [12, 40-42]). Despite the large number of studies on cholesterol containing membranes, to the best of our knowledge there have only been a handful of diffraction studies on membranes containing other sterols [43-47], only one of which characterized sterol-induced changes to membrane thickness [47]. Knowledge of how sterols other than cholesterol influence membrane structural parameters, such as membrane thickness, is important in identifying the relative contribution of hydrophobic mismatch to sterol-modulated protein activity. As such, there is a clear need for structural studies on membranes containing sterols other than cholesterol.

In this study, we use SANS to investigate differences in the influence of various sterols on DMPC ULV thickness. We examine mixtures at two levels of sterol content, 20 and 47 mol %. Based on the phase diagrams for these mixtures, we expect 20 mol % sterol mixtures to exhibit either coexisting solid ordered (so) – liquid ordered (lo) or liquid disordered (ld) - lo phases (depending on temperature) while 47 mol % sterol mixtures should be in the pure lo phase between 10 and 45 ºC. [37-39]. Fits to SANS data yield the scattering length density-weighted average membrane thickness, vesicle size and polydispersity, as well as changes in these parameters induced by the addition of sterols and alteration of temperature. From this data, we also obtain details about changes in vesicle area resulting from changes in temperature. For ULV with the various sterols at 47 mol % concentration, we find significant variations in the thermal area expansion coefficients. However, while all three sterols produce increases in DMPC ULV bilayer thickness, the extent of thickness increase varies only slightly among sterols.

MATERIALS AND METHODS

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), solubilized in chloroform was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and used without further purification. Upon arrival, ampoules containing the lipid were stored at - 40 ºC. Cholesterol, ergosterol and lanosterol were purchased as lyophilized powders from Sigma-Aldrich (St. Louis, MO), with purities of > 99%, ~ 99%, ~ 97%, respectively, and also used without further purification. D_2O

(99% purity) was purchased from Cambridge Isotope Labs, Inc. (Andover, MA). All other chemicals were reagent grade. (Reference to commercial sources and products used in this study does not constitute endorsement by the National Institute of Standards and Technology (NIST), nor should it be inferred that the products mentioned are necessarily the best available for the purpose used.)

300 Å radius ULV were prepared by extrusion using the method of Nayar, et al. [48]. For DMPC – sterol mixtures, sterols were dissolved in chloroform and mixed with DMPC to the appropriate molar ratios. Lipid or lipid-sterol mixtures solubilized in chloroform were then transferred to round bottom flasks. Solvent was subsequently removed from the samples by careful flow of N_2 across the dispersions to yield lipid films. The thin films of lipid adhering to the flasks were then placed under vacuum to remove any residual chloroform. The dry lipid films were then dispersed into D_2O, and the lipid dispersions were subsequently extruded under N_2 at approximately 30 ^oC using a pressure of ~ 700 kPa. Total lipid concentrations were 10 mg/ml prior to extrusion, except for samples with 20 mol % lanosterol, which had a concentration of 5 mg/ml. Vesicles were extruded using three different pore radius polycarbonate filters and a total of 27 passes [e.g., 1000-Å (9 times), 500-Å (9 times) and 250-Å (19 times)].

SANS measurements were performed using the NG1 8 m SANS [49] located at the National Institute of Standards and Technology (Gaithersburg, MD). A sample-to-detector distance (SDD) of 3.84 m and neutron wavelength, λ, of 6 Å ($\Delta\lambda/\lambda$ = 12%) were used, resulting in a total range in scattering vector, $0.015 < q < 0.16$ Å^{-1}, where $q = 4\pi \sin(\theta/2)/\lambda$, and θ is the scattering angle.

Vesicle size, polydispersity and bilayer thickness were determined from fits to the data using the form factor for spherical vesicles and lipid bilayers of uniform scattering length density. This form factor, as determined for neutron scattering via the Born approximation (or via the Rayleigh-Gans-Debye approximation for light scattering), is given by [50]:

$$P(q,R) = (\rho - \rho_0)^2 \left[R_o^3 \frac{j_1(qR_o)}{qR_o} - R_i^3 \frac{j_1(qR_i)}{qR_i} \right]^2 , \quad (2)$$

where ρ is the average scattering length density (SLD) of the membrane, ρ_0 is the SLD of the medium, R is the distance between the center of the vesicle and that of the bilayer, t is the bilayer thickness, $R_o = R+t/2$, $R_i = R-t/2$, and $j_1(x)$ is the first-order spherical Bessel function:

$$j_1(x) = \frac{\sin(x)}{x^2} - \frac{\cos(x)}{x} \qquad (3)$$

In order to take into account the influence of vesicle size polydispersity, Eq. (2) is integrated over the Schulz, or Gamma distribution, given by

$$G(R) = \left(\frac{z+1}{R_a} \right)^{z+1} \frac{R^z}{\Gamma(z+1)} \exp\left(\frac{-R(z+1)}{R_a} \right), \quad (4)$$

where the number average vesicle radius is equal to R_a, the variance is $\sigma^2 = R_a^2/(z+1)$ and the polydispersity (relative variance) is $\Delta^2 = 1/(z+1)$. The scattered intensity from a suspension of ULV is then given by:

$$I(q) \propto \int G(R) P(q,R) dR \, . \quad (5)$$

Fits to the data also include the effects of instrumental smearing, as described by Glinka, et al. [51] and a constant incoherent background. Data reduction was performed and data were fit using Igor Pro and macros provided by NIST [52]. The ULV surface areas, A, are calculated from the mean radii, R_a, as $A=4\pi R^2$.

Note that the representation of the membrane SLD as a uniform region with sharp interfaces is an approximation to a more complex SLD; as observed in [53], a more realistic representation of the membrane SLD includes a continuous change in SLD from a uniform hydrophobic core to the membrane water interface. However, Kučerka, et al. [53] also show that a meaningful determination of the hydrophobic thickness and thickness of the interfacial region either depend on simplifications of the model of the membrane profile, or rely on accurate knowledge of the scattering lengths and component volumes of the lipids making up the membrane. Elsewhere, it has been shown that the apparent thickness of ULV in D_2O and changes to that thickness follow the same trends as the hydrophobic thickness (e.g. [9, 15]), although the apparent thickness has a numerical value somewhat lower than the hydrophobic thickness [10]. Meanwhile, other studies have shown that the apparent membrane thickness will depend not only on the hydrophobic thickness, but also the extent of water penetration into the interfacial region [53-55]. Thus, our observation of changes to the apparent thickness will likely reflect either changes to the hydrophobic thickness or the extent of water penetration into the membrane, or some combination of the two.

RESULTS

Vesicle Size and Polydispersity

Fits to SANS data, as a function of sterol content and temperature, yield ULV size, polydispersity and membrane thickness. We were able to determine the mean vesicle sizes and polydispersities with a reasonable degree of accuracy (errors < 5 %). However, the changes in these parameters over 5 ˚C increments were smaller than the fit uncertainties, making it difficult to quantitatively assess vesicle size changes over small temperature intervals. Nevertheless, as shown below, we were able to assess temperature induced vesicle size changes by fitting data over large temperature intervals (e.g. spanning an interval ~ 30 ˚C).

In order to verify that the extrusion process resulted in well-defined populations of ULV for all lipid – sterol mixtures, we first examined ULV at 30 ˚C. Representative fits are shown in Fig. 2 and fit parameters are given in Table 1. Error bars quoted in Table 1 correspond to fit errors. We find that all samples examined consist of low polydispersity, monomodal distributions of ULV, and that the presence of the various sterols in DMPC vesicles does not significantly influence either their size or polydispersity. For all samples we find mean vesicle radii of approximately 300 Å with polydispersities of ~ 0.2. For pure DMPC ULV at 30 ˚C, we find somewhat different values for the mean size and polydispersity, than those obtained in another recent SANS study (http://arxiv.org/abs/physics/0507140). However, as noted by Patty and Frisken [56], the mean size and polydispersity of extruded vesicles can vary, depending on both extrusion pressure and membrane composition.

Membrane Thickness

In contrast to changes in ULV size and area, changes to membrane thickness as a function of temperature are much more readily observable (Figs. 3A and B). The values for the apparent membrane thickness are shown in Fig. 3, for cholesterol, ergosterol and lanosterol, respectively. On heating from 10 to 30 ˚C, we observe a substantial reduction, 4 Å, in the apparent thickness of pure DMPC ULV, consistent with observations elsewhere of the membrane structural changes that accompany the gel-fluid phase transition [e.g. 57, 58]. For the DMPC sterol mixtures, we also observe thickness reductions on heating, ~ 4 Å and ~ 2 Å for 20 and 47 mol % sterol concentrations, respectively. The addition of sterols produces an increase in the membrane thickness at both 20 and 47 mol % at all temperatures examined. Surprisingly, for the different sterols at temperatures less than 25 ˚C, the addition of 20 mol % sterol produces a greater change in bilayer thickness than at 47 mol % sterol content. However, above 35 ˚C, this trend is reversed.

While the influence of all three sterols on membrane thickness is similar, there are small differences. At all temperatures, lanosterol appears to produce slightly greater thickness increases than the other two sterols at 20 mol %, and ergosterol appears to produce a somewhat larger thickness increase than the other sterols at 47 mol %. Studies elsewhere, using more sophisticated models of membrane SLD profiles have shown that the apparent membrane thickness will depend on both the membrane hydrophobic thickness and level of hydration [53-55]. Thus the small differences in apparent thickness that we observe among the various lipid-sterol mixtures could be due to either differences in hydrophobic thickness or the extent of membrane hydration. Unfortunately, we cannot distinguish between these two possibilities here, since the q range of the present data is not sufficient to obtain unambiguous fits using the more sophisticated models described in [53-55]. In any case, since the differences among the lipid-sterol mixtures are small, it is clear that the corresponding differences in either hydrophobic thickness or hydration level must also be small.

Thermal Area Expansion Coefficients

The area changes of the ULV membranes containing 47 mol % sterol have, to a first approximation, a linear dependence with temperature. This linear dependence allows us to determine thermal area expansion coefficients, as in Needham et al. [59] via linear regression fits. In Fig. 4, the area vs. temperature dependence is shown for ULV containing 47 mol % cholesterol, ergosterol and lanosterol, respectively, plotted against that of pure DMPC. The surface areas are normalized to those values found at 20 ˚C, as in Needham, et al. [59]. The linear dependence allows us to determine the thermal area expansion coefficient, $\Delta A/(A_0 \Delta T) = \alpha$, for the various membranes. We assess the value α at 25 ˚C, in order to make comparisons with the results of [59], and find that the sterols reduce the thermal area expansion coefficient, relative to pure DMPC [59], in the order ergosterol > cholesterol > lanosterol (Table 2). Note that our measured value of α for DMPC at 25 ˚C is significantly larger than that of Needham, et

al. [58] at 35 ˚C. The reason for this difference is simply that the value of α rapidly increases near the phase transition.

DISCUSSION:

The sterol concentrations used in this study, 20 and 47 mol %, likely produce mixtures corresponding to distinct regions within the lipid – sterol phase diagrams. As mentioned previously, the former should exhibit phase coexistence, while the latter exists within a pure lo phase over the temperature range examined. Since there is considerable experimental evidence that the phase behaviour for PC-cholesterol mixtures is generic [60, 61] we use the phase diagrams given by [37-39] to estimate temperatures corresponding to phase boundaries for the mixtures used in our study. For 20 mol % cholesterol and ergosterol, we expect so-lo phase coexistence below ~ 20 ˚C, and ld-lo coexistence above this temperature. ULV containing 20 mol % lanosterol should show so-lo coexistence below ~ 15 ˚C, and ld-lo coexistence above this temperature. At 47 mol %, all three mixtures should form a pure lo phase over the entire temperature range examined. Thus, while parameters obtained for membranes containing 47 mol % sterol correspond to those in the lo phase, the thickness obtained for membranes with 20 mol % sterol will constitute an average of the lo and either the so or ld phase, depending on temperature.

As discussed above, at 47 mol % concentration, the variation of vesicle area with temperature is nearly linear, which allows us to estimate the thermal area expansion coefficients via linear regression fits to the data. The sterols reduce the area expansion coefficients in the order ergosterol > cholesterol > lanosterol, suggesting that ergosterol has the greatest condensing effect on DMPC membranes while lanosterol has the least. Since all three sterols are fairly rigid, compared to DMPC, they all have an ordering influence on the lipid. Acyl chain ordering of DMPC, as well as attractive van der Waals interactions, likely lead to closer phospholipid packing, or condensation. Since ergosterol is more conformationally restricted than cholesterol, it is likely to be more effective in condensing DMPC. Lanosterol, on the other hand, is likely to be less effective in condensing the membrane, since the two methyls attached to C4 and C14 will disrupt close packing between the sterol and lipid. These results are not surprising, since similar observations have been made by Urbina, et al., with respect to the ordering of DMPC by the three sterols [30].

Consistent with other studies of phospholipids, we observe a substantial reduction in the apparent lipid thickness on heating ULV from the gel phase to the fluid phase. As discussed above, it is not a priori clear whether our observations reflect a change only in hydrophobic thickness or may also reflect changes in the thickness of the membrane water interface. Detailed structural studies of pure DMPC membranes in [57, 58] report a change in d_{HH} (the distance between headgroups on either side of a bilayer) on heating from 10 to 30 ºC of 40.1 to 35.3 Å and concomitant change in the steric thickness, d_b', of 48.3 to 43.4 Å. In our case, the values of the apparent thickness at 10 and 30 ºC are 44.2 and 40.2 Å, respectively. Comparison of our results with those in [57, 58], clearly shows that the thickness we obtain from SANS is intermediate between the hydrophobic and steric thickness of the bilayer, which must therefore contain contributions from both the hydrophobic thickness and thickness of the hydration layer. Our observation of a smaller

thickness change, 4 Å, than that observed in [57, 58], 5 Å, likely reflects the uncertainty that arises due to the combined effects of changes to the hydrophobic and hydration layer thicknesses.

When cholesterol, ergosterol and lanosterol are added to DMPC, we find the surprising result that the sterols produce a thickness increase of the ULV membrane below T_m of the pure lipid. Since the addition of these sterols to the pure lipid gel phase reduces the mean acyl chain order [37-39], and acyl chain order is correlated to membrane thickness [61, 62], one might expect that the addition of these sterols to the gel phase should reduce the membrane thickness. However, diffraction studies show that the addition of cholesterol to the gel phase of pure DMPC increases the thickness of the membrane [63]. In this study, it is suggested that the cholesterol-induced thickness increase in the gel phase is likely a consequence of the disruption of lipid tilt [64, 65]. A reduction of acyl chain tilt in the membrane could lead to an increase in the bilayer thickness, even with a reduction in the acyl chain order. Léonard, et al. [63], show that if there is no chain conformational disorder, complete disruption of the lipid tilt in the gel phase of DMPC should lead to a membrane thickness increase of about 5 Å. On the other hand, the thickness reduction on going from the gel to fluid phase of DMPC, due to disordering of the acyl chains is also about 5 Å [57, 58]. Thus, the addition of sterol to DMPC in the gel phase could result in membrane thickening or thinning depending on the degree of chain disorder compared to the effect of disruption of the acyl chain tilt. Since our results show that membranes containing sterols are thicker than pure gel phase lipid, we see that for all three sterols, the reduction of lipid tilt dominates over the reduction of acyl chain order in influencing membrane thickness. This observation is consistent with the previous results of Léonard, et al. [59], who observe an increase in hydrophobic thickness of about 3.5 Å with the addition of 30 mol % cholesterol to the gel phase of DMPC.

Below the T_m of pure DMPC, we observe that the 20 mol % mixtures show greater thicknesses than either the 47 mol % mixtures or pure gel phase DMPC bilayers. From this result we can infer that, on average, at lower concentrations the sterols have a greater effect on lipid tilt than acyl chain order. It is likely that the sterols disrupt lipid tilt in the gel phase of the mixtures as well as the lo phase. Since the acyl chains in the gel phase will still show a high degree of order, disruption of lipid tilt will result in a significant increase in membrane thickness, much more so than in the lo phase. Thus, the average thickness of the untilted gel phase plus that of the lo phase will be larger than either the sterol free tilted gel phase or pure lo phase.

The addition of and differences in the overall concentration of sterols also contribute to changes in the thermal variation of membrane thickness. At 20 mol % concentration, we see that the variation of thickness with temperature shows a steep decrease near T_m of the pure lipid, while at 47 mol % the thickness of the sterol-containing membranes varies almost linearly with temperature (Fig. 3-5). The steep decrease in the case of 20 mol % sterol reflects the melting of the gel phase while, as has been discussed, there is no chain melting transition observed for 47 mol % sterol mixtures, which are in the pure lo phase. Likewise, the linear variation in membrane area with temperature at 47 mol % sterol also shows that the membrane is in the pure lo phase at this molar fraction of cholesterol.

CONCLUSIONS:

In examining the influence of the sterols, cholesterol, ergosterol and lanosterol, on DMPC ULV, we find that the sterols differ in their influence on the membrane thermal expansion coefficient, following the same trend as observed by Urbina, et al, [30] with respect to the influence of the sterols on acyl chain order, ergosterol > cholesterol > lanosterol. However, we find only small differences among the sterols in their influence on membrane thickness. We thus conclude that, while differences in sterol architecture may play a significant role in their modulation of membrane material properties, it is likely that the sterol-induced changes to membrane thickness are dominated by the overall length of the sterol relative to the hydrophobic thickness of the membrane.

Differences observed here and elsewhere [24-31] in the influence of cholesterol, ergosterol and lanosterol on material properties of membranes show the potential use of this series of sterols to determine the importance of hydrophobic thickness relative to other membrane properties in modulating membrane protein function. Clearly, in the cases where membrane thickness is the determining factor in protein function, we can expect that cholesterol, ergosterol and lanosterol will perform in a similar manner in their modulation of protein function. The observation of Urbina, et al. [30] that lipid unsaturation also modulates the effects of the sterols on membranes, suggests that lipid unsaturation could increase the differences among sterols in their influence on membrane thickness. Nevertheless, our thickness measurements, taken together with previous results on DMPC – sterol mixtures, provide useful information over a range of conditions for such studies in the case of DMPC model membranes.

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Table 1: Fitting results for DMPC ULV at 30 ºC as a function of sterol content. Error bars cited correspond to uncertainties in the fitting parameters.

Composition	$\langle R \rangle$ (Å)	σ	t (Å)
Pure DMPC	313.4 ± 8.4	0.23 ± 0.01	40.1 ± 0.1
20 mol % chol	315.6 ± 6.1	0.20 ± 0.01	43.8 ± 0.1
47 mol % chol	312.3 ± 6.4	0.19 ± 0.01	44.1 ± 0.1
20 mol % erg	316.6 ± 6.7	0.21 ± 0.01	43.6 ± 0.1
47 mol % erg	322.2 ± 5.7	0.20 ± 0.01	44.3 ± 0.1
20 mol % lan	314.2 ± 6.7	0.20 ± 0.01	44.2 ± 0.1
47 mol % lan	322.4 ± 6.7	0.20 ± 0.01	43.7 ± 0.1

Table 2: Thermal area expansion coefficients for DMPC ULV containing 47 mol % cholesterol, ergosterol and lanosterol, at 25 ºC. [a]Values taken from Needham, et al. (1988).

Composition	α (x 10^{-3} ºC^{-1})
Pure DMPC	10.1 ± 0.5
47 mol % chol	1.9 ± 0.1
47 mol % erg	0.5 ± 0.7
47 mol % lan	2.9 ± 0.5
Pure DMPC (35 ºC)[a]	4.2 ± 0.2
40 mol % chol (35 ºC)[a]	2.3
50 mol % chol (22 ºC)[a]	1.3

Figure Captions:

Figure 1: Space filling models (left) and chemical structures of (top to bottom) cholesterol, ergosterol and lanosterol. The C28 methyl group of ergosterol is coloured blue, as are the C28, C29 and C30 methyl groups of lanosterol.

Figure 2: SANS curves for DMPC vesicles at 30 ^{o}C with and without 20 mol % cholesterol, ergosterol and lanosterol. Also shown are fits to the data (solid lines) using the RGD approximation. Fit results are summarized in Table 1.

Figure 3: A. Membrane thickness vs. temperature for pure DMPC ULV (squares), and DMPC ULV containing 20 mol % cholesterol (circles), ergosterol (triangles), or lanosterol (inverted triangles). B. Membrane thickness vs. temperature for pure DMPC ULV (squares), and DMPC ULV containing 47 mol % cholesterol (circles), ergosterol (triangles), or lanosterol (inverted triangles). Lines joining the various data points are not fits to the data, but are provided to better show the various trends. Error bars shown correspond to uncertainties in the fits based on the assumption of uniform membrane scattering length density (SLD). As discussed in the text, given that the membrane SLD is not uniform, the uncertainties may be much larger, ~ 0.5 Å.

Figure 4: Vesicle surface area (normalized to that at 20 ˚C) as a function of temperature for DMPC ULV (squares) and vesicles containing 47 mol % cholesterol (circles), ergosterol (triangles), or lanosterol (inverted triangles). Also shown is a linear regression fit to the data for vesicles containing cholesterol. DMPC vesicle data is fit over the temperature interval from 20 to 30 ˚C.

Figure01

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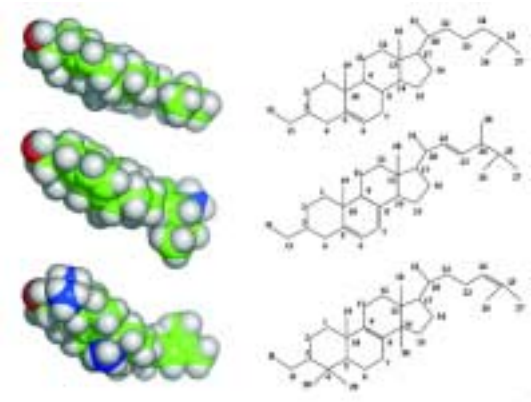

Figure02

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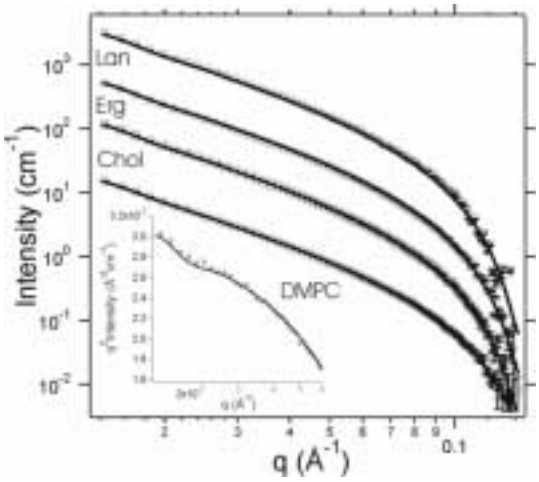

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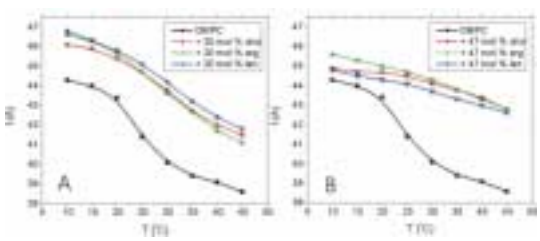

Figure04

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