

# What determines the thickness of biological membrane?

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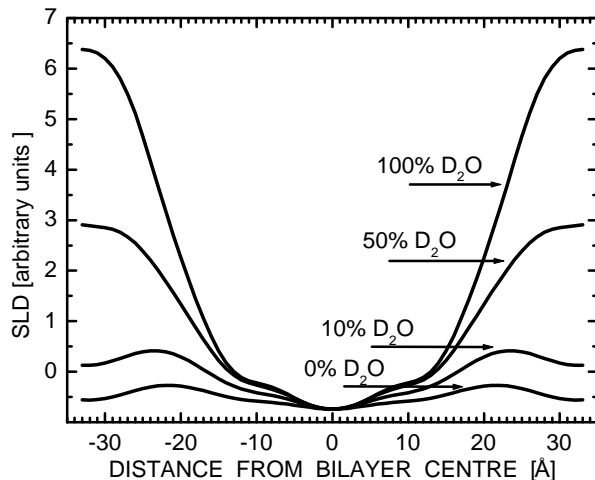
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For various reasons, the thickness of biological membranes is known to vary, though one exciting speculation is that it acts as a signal for membrane proteins. The insertion and orientation of polypeptides, as well as the activity of integral membrane proteins critically depends on bilayer thickness [1]. On the other hand, the bilayer can adjust its thickness to match the protein's hydrophobic surface [2]. In general, it is assumed that bilayer thickness is a key factor for proper protein function. Having a basic understanding of how a membrane modulates its thickness is essential in understanding the relation between structure and function of biological membranes.

Based on its ubiquitous presence in animal cell membranes, it has long been assumed that the dominant player in determining membrane thickness, is cholesterol [3]. However, the saturation level of the lipid's acyl chain has also a marked effect on bilayer properties and protein-lipid interactions. In mixtures of saturated and monounsaturated lipids, cholesterol partitions preferably into the saturated part, increasing lipid acyl chain order and resulting in an increased bilayer thickness. Such results were reported for a wide range of saturated lipids, while remaining relatively obscure in the case of unsaturated lipids. The integration of cholesterol into comparable length dioleoyl-phosphatidylcholine (diC18:1PC) bilayers, induces minimal change in bilayer thickness. On the other hand, recent data for a polyunsaturated lipid (diC20:4PC) bilayer shows cholesterol being sequestered in the bilayer center, resulting in a thinning of the lipid membrane [4]. Using even longer, but monounsaturated lipids (e.g. diC22:1PC) resulted in practically no increase in bilayer thickness [5] - although the result was somewhat inconclusive due to large experimental errors.

Although these results seem, at first, conflicting, this is not necessarily the case. The effect of cholesterol on extremely short (e.g. diC14:1PC) and extremely long (e.g. diC22:1PC) lipid bilayers can lend some insight into the presently poorly understood mechanism of lipid-cholesterol interactions. To address this question, the primary aim of our study was to determine the effect of cholesterol on the thickness of "thin" and "thick" monounsaturated bilayers. The addition of cholesterol to these bilayers provides direct evaluation of membrane response to the hydrophobic mismatch between the lipid and cholesterol.

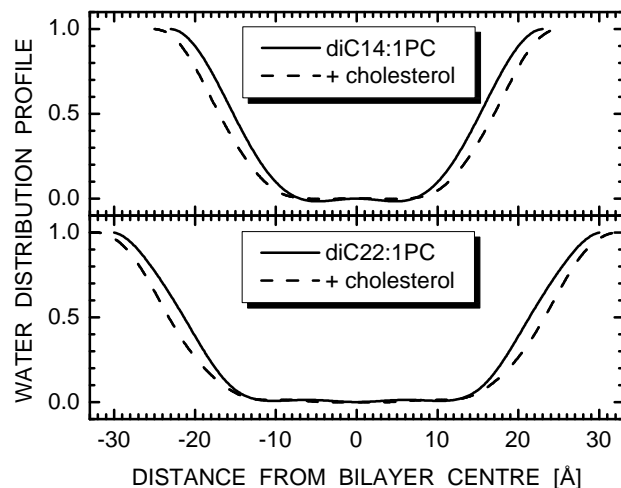
We have used neutron scattering from oriented stacks of bilayers to determine the relative changes in thickness upon the addition of cholesterol. Neutron diffraction measurements were carried out using the N5 spectrometer, located at the National Research Universal (NRU) reactor (Chalk River, ON). Bilayers of di-C14:1PC (diMyristoleoylPC: 9-cis-tetradecenoic) and di-C22:1PC (diErucoylPC: 13-cis-docosenoic), with and without 40% cholesterol, were oriented on silicon crystal substrates and hydrated using four different D<sub>2</sub>O/H<sub>2</sub>O mixtures (Figure 1). Contrast variation is widely used for determining the phases of the various structure factors [6-8], although the straightforward application of this rule breaks down under certain conditions (e.g., the thickness of the bilayer and water layer) [9]. The choice of experimentally obtained phases was supported with data obtained from molecular dynamics simulations.



**Figure 1:** 1D scattering length density (SLD) profiles obtained from the Fourier reconstruction of diffraction data from oriented multilayers of diC22:1PC lipid with 40% cholesterol. The bilayers were hydrated with different D<sub>2</sub>O/H<sub>2</sub>O mixtures (D<sub>2</sub>O volume fractions of 0, 10, 50 and 100 %) in order to obtain the phase component of the structure factor.

The bilayers of interest were studied at 30°C, at which point the bilayers are well in the fluid thermodynamic phase. Comparison of lipid bilayers was done at the same absolute temperature, based on the fact that in a biological membrane all of the different lipids coexist at a given temperature. Thus, the intra- and inter- lipid interactions that define the bilayer structure are taking place at the same absolute temperature.

Up to seven orders of Bragg diffraction were collected for the various samples studied. The data were subsequently Fourier transformed into neutron scattering length density (SLD) profiles. These profiles reflect the high contrast between the fully protonated lipid and the 100% D<sub>2</sub>O solvent, while the headgroup component becomes more pronounced in solutions with lesser D<sub>2</sub>O content (Figure 1). Even though the four SLD profiles differ considerably, they correspond to a bilayer system having the same distribution of lipid components and water molecules across the bilayer. These distributions are then individually evaluated from the subtraction of SLD profiles obtained at different contrast conditions. Figure 2 shows the distribution of water molecules for the systems studied.



**Figure 2:** Water distribution probabilities obtained from difference profiles. The top panel compares the bilayer made up of the short-chain lipid di-C14:1PC, while the bottom panel shows the effect of cholesterol on long-chain lipid di-C22:1PC. The figure suggests cholesterol having the same influence on the two different bilayers.

The distribution of water across the bilayer directly reflects the lipid distribution, as the two profiles (i.e. water and lipid) are complementary probabilities. The central region consists, almost exclusively, of lipid molecules, while the probability of water distribution achieves maximal values outside this region. The gradual increase from zero to one corresponds to the penetration of water, and its central value defines the total thickness of the bilayer. Consistent with our expectation, lipid bilayers made up of di-C14:1PC are thinner than di-C22:1PC bilayers (Figure 2), while the addition of cholesterol resulted in increasing both bilayers by about 3 Å.

These results suggest that cholesterol has the same effect on bilayers comprised of di-C14:1PC and di-C22:1PC lipids. This is surprising, especially in the case of the diC22:1PC lipid whose hydrocarbon region seems to be longer than the overall length of a cholesterol molecule [10]. The widely accepted model of lipid-cholesterol interactions, is that cholesterol affects membrane structure in two ways: a) due to its rigid structure cholesterol increases lipid acyl chain order. For a highly flexible fluid phase lipid molecule, such an interaction results in an increased bilayer thickness; b) it is believed that the rigid hydrophobic molecule of cholesterol determines the thickness of the hydrocarbon chain region. Therefore, in the case of long-chain lipids, cholesterol is expected to decrease their overall thickness. Our results, in an agreement with [11], suggest otherwise, implying that cholesterol prefers to further order the lipid's hydrocarbon chain over the possibility of rectifying the hydrocarbon chain mismatch.

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