# Experimental report

20/04/2017

**Proposal:** 9-13-583 **Council:** 10/2014

**Title:** Cholesterol location in POPS and POPC membranes

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Local contacts:** Giovanna FRAGNETO
Yuri GERELLI


**Samples:** cholesterol
POPS
POPC

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D17 | 2 | 0 | | |
| FIGARO | 2 | 3 | 26/06/2015 | 29/06/2015 |


**Abstract:**

Using Time Resolved (TR) Small Angle Neutron Scattering (SANS), we have measured cholesterol transfer between unilamellar vesicles made of POPC and POPS. While cholesterol exchange is slower in POPS, we found that the transfer of cholesterol in POPS is also surprising and suddenly near -frozen- below 50C. Such discontinuous behavior suggests a quasi-first order change which is similar in character to the well documented case of cholesterol transport between lipid vesicles when crossing the lipid's melting transition temperature. Yet this anomalous cholesterol transport behavior is occurring with lipids in the fluid phase and, to the best of our knowledge, has never been reported. Structural analysis using SANS and X-ray scattering, combined with densitometry and DSC measurements failed to reveal any underlying transition responsible for such behavior. We plan to use neutron reflectometry to investigate the location of cholesterol in deuterated POPC and deuterated POPS supported bilayer as a function of temperature in order to characterize a possible -trapped or burried- state of cholesterol in the bilayer of POPS at low temperatures that could explain our SA

In these experiments we wanted to compare the localisation of cholesterol molecules in pure POPC and POPS lipid bilayers. The aim was to investigate any possible connection between different molecular organizations and different thermodynamic properties measured by means of other techniques (unpublished data). In order to improve the sensitivity to the position of cholesterol molecules in the bilayer the per-deuterated version of the two lipid species were used: $d_{31}$POPC and $d_{31}$POPS. Bilayers were obtained from pure phospholipid vesicles and from vesicles containing the 50% (by mol.) of cholesterol exploiting the well-known vesicle fusion method. During the first experiment (**9-13-582**) bilayers were deposited on top of hydrophilic silicon surfaces (bearing a negative surface charge) but many problems were encountered with the deposition of the negatively charged $d_{31}$POPS vesicles (with and without cholesterol) and it was not possible to obtain reproducible measurements. In this case the electrostatic repulsion, even if partially screened by the original counter-ions present in solution, was preventing the deposition of reproducible bilayers. In the experiment **TEST-2568** the same bilayers were deposited on the top of hydrophilic sapphire surfaces that because of the etching procedure were positively charged. In this case an electrostatic attraction between POPS molecules and the surface is expected promoting a better formation of bilayers but altering the molecular behaviour with respect to that of the zwitterionic POPC.

# 1 $d_{31}$POPC + Cholesterol on Silicon

Data for the d31POPC sample in presence of cholesterol (molar ratio??) were collected at 60 $^oC$. During the analysis the SLD of headgroup and tails were free to vary between the value for 100% lipids or 100% cholesterol in both regions. From a subsequent analysis the volume fraction of cholesterol in the bilayer was obtained as

$$f_{Ch} = (1 - f_w) \times \frac{\rho_{tot} - \rho Ch}{\rho_{h/t} - \rho_{Ch}} \tag{1}$$

where $f_w$ is the volume fraction occupied by water in a given $t$ (tail) or $h$ (head) region. $\rho_{Ch}$, $\rho_h$ and $\rho_t$ are respectively the SLD values for dry cholesterol, dry PC headgroup and dry d31PO tails while $\rho_{tot}$ is the total dry SLD of a given region of interest in the sample. By using nominal reference values and those obtained from the analysis for $f_w$ and $\rho_{tot}$ the volume fraction of cholesterol was estimated to be 15% in the heagroup region and 27% in the tails. The volume fraction profile of cholesterol is shown together with the resulting SLD profiles in Figure 1.

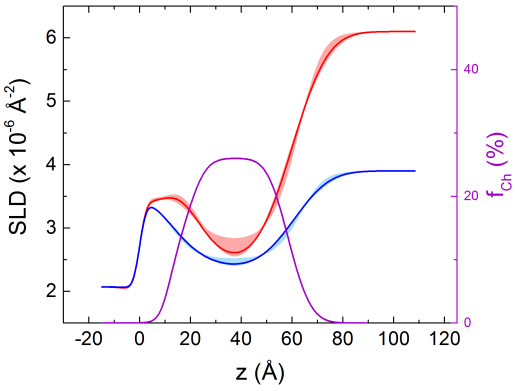



Figure 1: Scattering length density profiles resulting from the fit. Confidence intervals (1$\sigma$ from bootstrap) are indicated as shaded areas. The volume fraction of cholesterol as a function of the position along the bilayer normal is also reported.

## 2 $d_{31}$POPS + Cholesterol on Silicon

In this case we could not deposit a 100% POPS bilayer on the top of the hydrophilic silicon oxide surface probably because of the electrostatic repulsion between the negatively charged lipid headgroups and the negatively charged SiOx surface.

## 3 $d_{31}$POPC + Cholesterol on positively charged (hydrophilic) sapphire

The structure of the $d_{31}$POPC bilayer loaded with cholesterol and deposited on a sapphire surface was characterized on D17 at 50 $^oC$. The SLD profiles (see Figure 2) indicate that the cholesterol is partitioned equally between inner and outer leaflet of the membrane occupying the $\approx$ 18% of the headgroup region volume and $\approx$ 41% of the hydrophobic core region. The volume fraction profile of cholesterol in this bilayer is shown in Figure 2. It has to be noted that contrarily of what observed for the same bilayer deposited on top of a silicon oxide surface a water gap 11 Åthick was present between the surface and the inner headgroup region. This is an unexpected result, since water gaps for solid-supported lipid bilayers are, if present, typically 2-5Åthick.

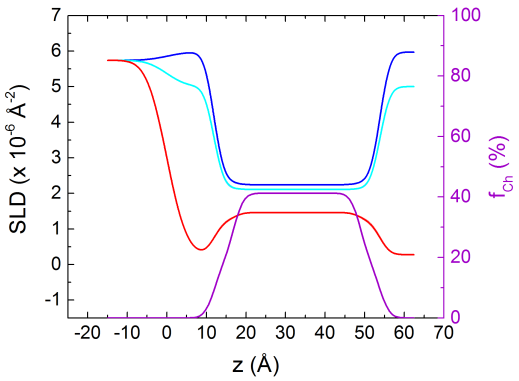



Figure 2: Scattering length density profiles resulting from the fits. Confidence intervals ($1\sigma$ from bootstrap) are indicated as shaded areas. The volume fraction of cholesterol as a function of the position along the bilayer normal is also reported.

## 4 $d_{31}$POPS + Cholesterol on positively charged (hydrophilic) sapphire

The parameters describing the structure of the pristine $d_{31}$POPS bilayer deposited on the top of a hydrophilic sapphire surface were perfectly in agreement with those reported in literature and obtained from other techniques. The coverage parameter was very close to 100% ($\pm$1%). The structure of the bilayer obtained in presence of cholesterol was clearly different: a good fit was obtained only in presence of a compositional asymmetry between inner and outer leaflet of the bilayer. This asymmetry was interpreted as induced by a different amount of cholesterol molecules in the two leaflet, larger in the external one. Moreover, no cholesterol was found in the headgroup region of both inner and outer leaflet, indicating that it was completely hosted within the hydrocarbon core of the bilayer. This asymmetry is not surprising and it can be reasonably explained as the result of the electrostatic attraction between the surface and the

phospholipid molecules, promoting a phospholipid crowding close to the surface. The volume fraction profile of cholesterol is shown together with the resulting SLD profiles in Figure 3.

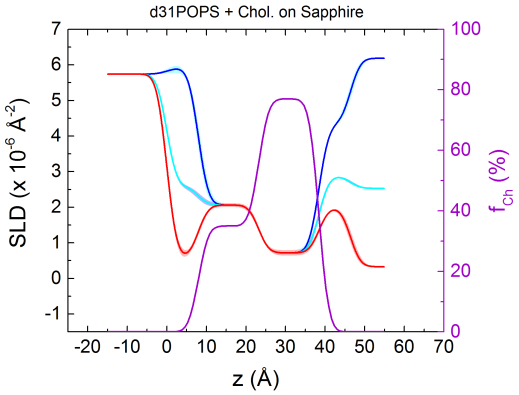



Figure 3: Scattering length density profiles resulting from the fits. Confidence intervals ($1\sigma$ from bootstrap) are indicated as shaded areas. The volume fraction of cholesterol as a function of the position along the bilayer normal is also reported.

# 5 Conclusion

The proposed experiment was performed to determine differences, if any, in the location of cholesterol molecules in $d_{31}$POPS and $d_{31}$POPC bilayers. The volume fraction profiles of cholesterol in the three investigated bilayers are shown in Figure 4. To highlight differences the profiles were shifted to be centered around $z^* = 0\mathring{A}$ . In one case (bilayer on silicon) the

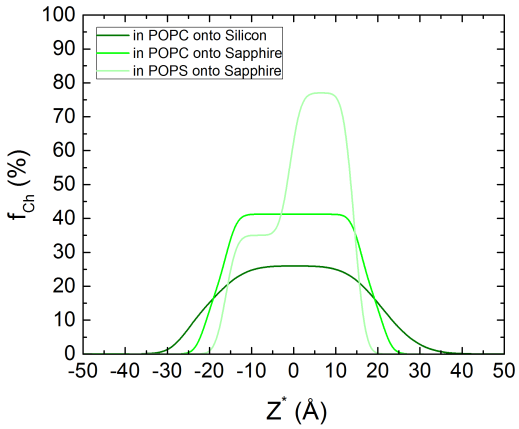



Figure 4: Volume fraction profiles for cholesterol in different bilayers. The profiles were shifted to be centered around $z^* = 0\mathring{A}$ for better comparison.

roughness was larger than in the other two cases, resulting in a broader cholesterol volume fraction profile. In the other two cases, were the roughness parameters were very similar, it is possible to remark that in the case of the POPS bilayer, despite differences in the abundance of cholesterol in inner and outer leaflet, the profile is narrower, indicating that cholesterol is present only in the hydrophobic core of the bilayer. In the case of POPC molecules, on both sapphire and silicon, a relevant fraction of the headgroup region volume resulted occupied by cholesterol suggesting a slightly different location of the molecule. In these cases the model suggest that the cholesterol molecules can protrude partially into the headgroup region.