Experimental report

15/02/2021

Proposal: 9-13-681 **Council:** 10/2016

Title: Characterising supported lipid bilayers from selectively deuterated PC and cholesterol, and their interactions with human HDL

Research area: Soft condensed matter

This proposal is a continuation of 9-13-609

Main proposer: Marite CARDENAS

Experimental team: Florian WEBER
Josefine Eilsoe NIELSEN
Marite CARDENAS
Selma MARIC
Sarah WALDIE
Trevor FORSYTH
Kathryn BROWNING
Abdullah LONE

Local contacts: Armando MAESTRO

Samples: Cholesterol
Phospholipids
HDL

Instrument	Requested days	Allocated days	From	To
FIGARO	3	5	22/02/2017	25/02/2017
			13/04/2018	15/04/2018
D17	3	0		

Abstract:

Atherosclerosis is the leading cause of death in western society, its consequences of cardiovascular diseases such as strokes and heart attacks arise from lipoprotein deposition onto cell membranes in the artery walls. Essential information regarding molecular mechanisms resulting in plaque build-up, is missing. Neutron reflection with selective deuteration studies provide crucial and unique information about lipid exchange and lipoprotein binding to model cell membrane. We have so far established a successful protocol to follow such exchange processes based on perdeuterated lipids. However, the availability of deuterated lipid types and molecular species is quite limited. Thanks to a collaboration with the Life Sciences group at the ILL using the D-Lab, a recently started ILL PhD studentship and chromatographic protocols already established at our home institution, full extracts of D2O-contrast matched phosphatidylcholine (PC) and POPC as well as their mixtures with cholesterol will be used to form supported lipid bilayers and follow the interactions with high density lipoprotein (HDL). This will unravel the effect of lipid composition of model cell membranes and HDL interactions.

Experimental Report for experiment: **9-13-681**

Characterising supported lipid bilayers from selectively deuterated PC and cholesterol, and their interactions with human HDL

The aim of the experiment was to structurally characterise PC-cholesterol bilayers to determine the localisation of the cholesterol within the bilayers. The second part of the experiment was following the incubation of HDL with the previously characterised bilayers to determine quantities of lipids exchanged.

During our beam time we determine the localisation of cholesterol in the bilayers. We did this with various model membranes to ensure the effect of deuteration of the cholesterol and phospholipids was accounted for. We used naturally derived PC lipids which have a contrast match point equal to that of D_2O to highlight the positioning of the cholesterol. Different quantities of cholesterol were incorporated into the bilayers to follow the effect of increasing cholesterol mole percentage. Two main phenomena were found: the first was that increasing levels of cholesterol gave an increasingly thickened bilayer and the second was that while at only 10 mol% cholesterol the bilayers were symmetrical and equal levels of cholesterol were found in both leaflets, at 20 mol% higher quantities of cholesterol were found in the outer leaflet of the membrane than the inner leaflet.

A model for the positioning of the cholesterol in the membranes was also determined. Initially a simple three-layer model for the bilayer was tested, however this did fit with the data and did not allow for the asymmetry of the cholesterol to be apparent. Asymmetrical four-layer models were also tested, however these were also not suitable. Eventually we used a five-layer model, consisting of an outer headgroup, three core layers and an inner head group. The three core layers were split this way to allow for the cholesterol to locate towards the tail-head interface rather than towards the centre, as seen with the differences in SLD in these regions associated with the differences in SLD between the cholesterol and the PC tails. No differences were seen in the models needed for the membranes with deuterated or non-deuterated cholesterol, verifying the suitability of the deuterated cholesterol in these experiments.

This data has been published: *Langmuir* 2018, 34, 1, 472–479

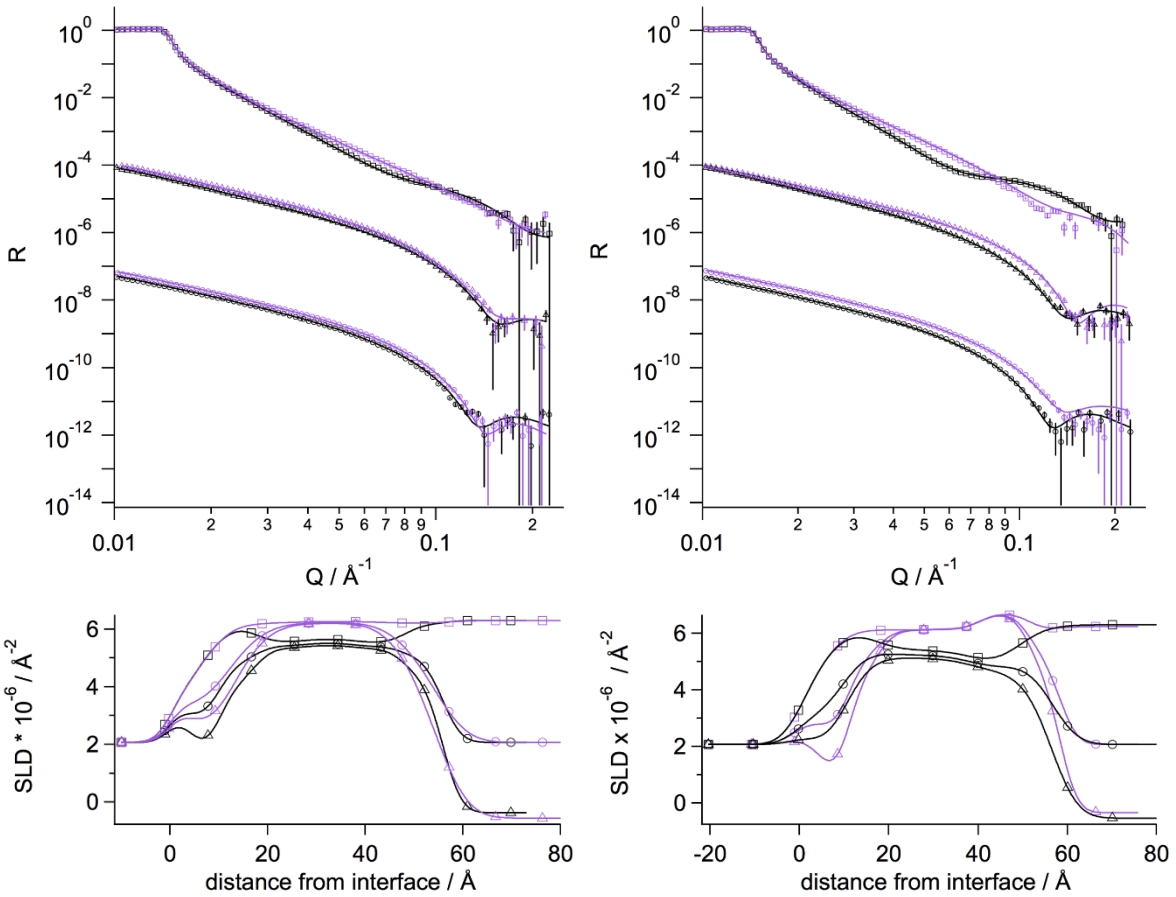

Figure 1. NR (upper)and SLD (lower) profiles for the PC with 10 mol% (left) and 20mol% (right) cholesterol.

The second part of the beamtime focussed on the interaction of HDL with the model membranes and the effect cholesterol in the bilayer had on the ability of HDL to exchange and remove lipids. It was found that the presence of cholesterol had little effect on the ability of HDL to remove and exchange lipids. A solution of HDL was incubated with the model membranes for 8 hours during which measurements were taken to follow the decrease in reflectivity signal corresponding to the removal and/or exchange of lipids in the membrane.

The second allocated beamtime was used to determine the effect of cholesterol in the membranes when incubated with LDL when in saturated membranes (DMPC) as opposed to unsaturated which were used before (PC). Very different results were seen here, while the presence of cholesterol in the membranes did not make a huge difference to the amount of lipids exchanged when incubated with LDL, the cholesterol did reduce the overall amount of lipids removed from the bilayers.

Further experiments were required to complete the datasets needed to publish this data, namely interaction of HDL with saturated lipids, and interaction of LDL with unsaturated lipids. The LDL incubation with unsaturated lipids gave similar results to those seen with the HDL, whereby the cholesterol did not affect the amount of lipids exchanged or removed. Whereas the HDL with saturated lipids gave the most dramatic difference, whereby the presence of

cholesterol drastically reduced the number of lipids both removed and exchanged and to a greater extent when increased levels of cholesterol were in the bilayer, see figure 2.

This data has been published: *Scientific Reports* volume 9, Article number: 5118 (2019)

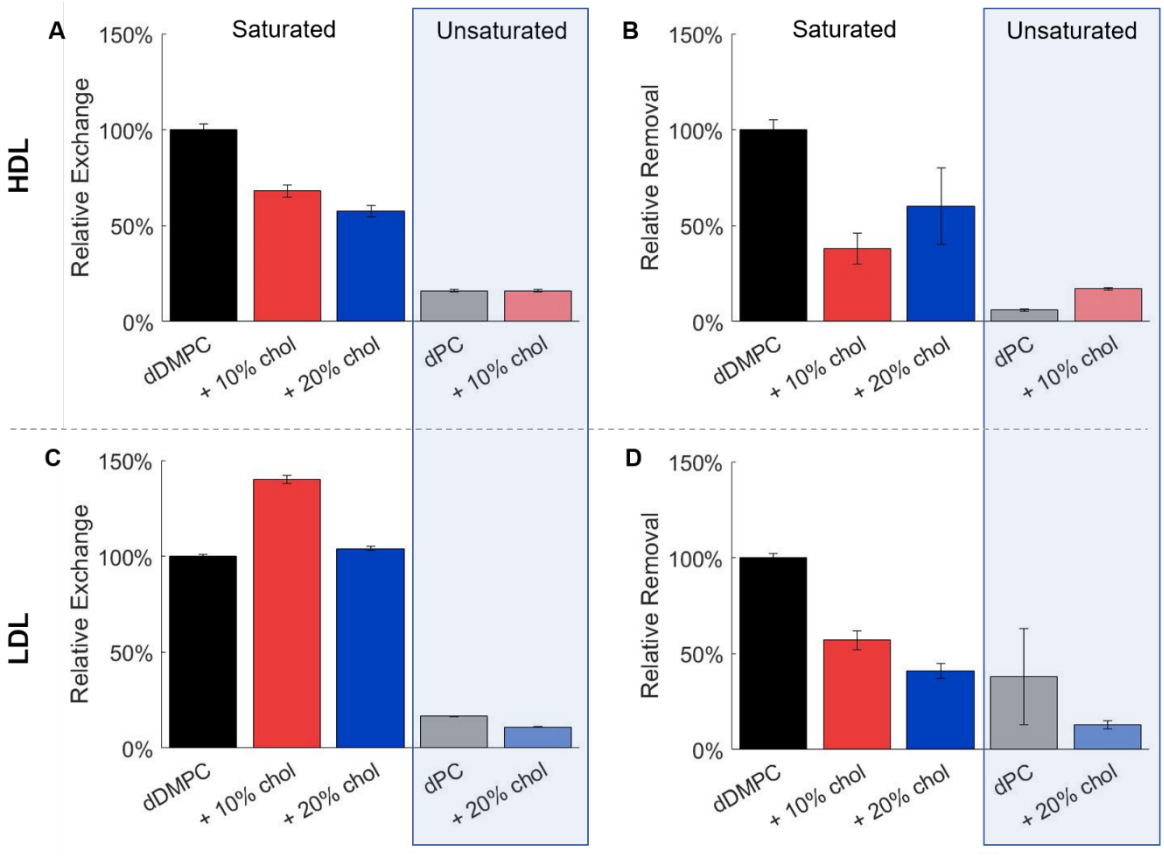

Figure 2. RN data of HDL and LDL incubated with saturated (DMPC) and unsaturated (PC) model membranes in the presence and absence of cholesterol. Lipids exchanged (A,C) and removed (B,D) with HDL and LDL respectively.