Experimental report

03/02/2016

Proposal: 9-13-609 **Council:** 4/2015

Title: Lipoprotein particles from human serum, dynamics of lipid exchange with lipid bilayers: Implications on atherosclerosis development

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: **Marite CARDENAS**

Experimental team: Selma MARIC
Marite CARDENAS
Kathryn BROWNING
Tania LIND

Local contacts: Robert BARKER

Samples: Lipids
lipoproteins

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	3	3	27/11/2015	30/11/2015
D17	3	0		

Abstract:

In westernized societies, atherosclerosis and its clinical consequences such as heart disease and stroke constitute the leading cause of death, accounting for around 16.7 million deaths/year. Current knowledge recognizes a whole range of indicators associated with atherosclerosis, including concentrations of various lipoprotein particles (low density lipoprotein (LDL), oxidized LDL (oxLDL), high density lipoprotein (HDL), and the lipoprotein-like particle lp(a) or apolipoproteins among others1,2). To date, we still do not know the impact that the apolipoprotein isoform, the apolipoprotein oxidation state, the lipid cargo and the presence of divalent ions have on the structure and stability of the lipoprotein particles, and therefore on the subsequent effects on interactions with blood vessel components. Neutron reflection can provide major insights into the exchange process occurring at mimics of biological interfaces. This project will thus provide unique information into the molecular mechanisms behind the importance of biological markers such as LDL, HDL total concentrations as well as the LDL/HDL ratio, advancing novel strategies in the fight against atherosclerosis.

Experimental Report for Experiment 9-13-609: Lipoprotein particles from human serum- Implications on atherosclerosis development.

The aim of this experiment was to study the interactions of two biological particles known to play a role in the development and progress of atherosclerosis with supported lipid bilayers using neutron reflection.

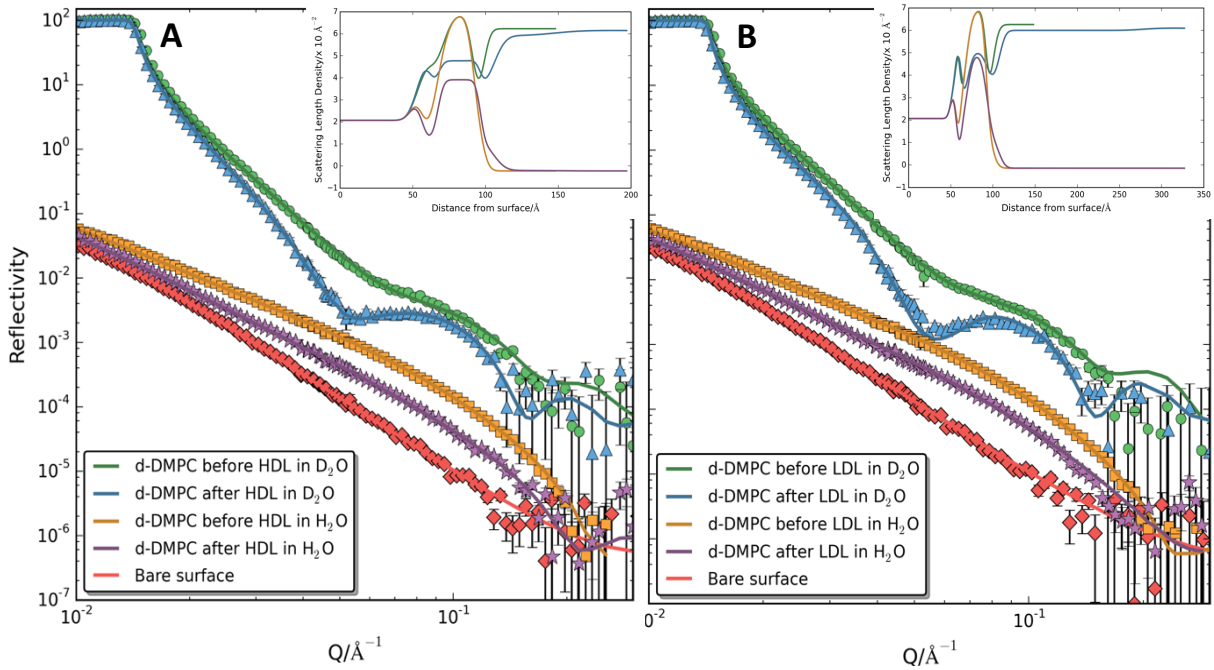

Figure 1: Experimental data (points) and calculated fits (lines) for the change in reflectivity upon introduction of lipoproteins to a dDMPC bilayer. A shows the reflectivity change upon introduction of 0.132 mg L^{-1} HDL, inset shows the scattering length density (SLD) profile for the fitted HDL data. B shows the reflectivity change upon introduction of 0.1 mg L^{-1} LDL and inset the SLD profile for the fitted LDL data. The total number of LP is constant in these experiments.

Briefly, the experiment consisted of contacting either high density (HDL) or low density (LDL) lipoprotein (LP) with a lipid bilayer consisting of tail deuterated dDMPC or a 75:25 mixture of dDMPC and tail deuterated dDMPS. A final experiment looked at how the bilayer changed when contacted with a 2:1 mixture of LDL:HDL. Fast kinetics (min) were measured at the point of injection for one hour with further monitoring over the next 7 hours as changes in reflectivity continued to occur within this time frame. This was followed by washing with H_2O, D_2O and CMSi buffers for full characterisation.

Case 1: HDL or LDL adsorbed onto a dDMPC bilayer. Figure 1 shows the data obtained from contacting the dDMPC with lipoproteins, either HDL or LDL. It can be seen that the difference in reflectivity is rather similar between the two LP particles with the fringe in the D_2O data becoming more pronounced in the presence of LP. From our recent SANS experiments, we know that LP and lipids from vesicles exchange. Therefore, our first model fit assumed the LP was only exchanging deuterated material for hydrogenated material in the bilayer. This

was modelled by allowing the lipid tail region to decrease in SLD. This exchange-only model fit the LDL data (B) very well however this was not the case for HDL. A better fit to the HDL data was achieved by also allowing the SLB to decrease its coverage by 13% suggesting HDL removes lipids from the SLB.

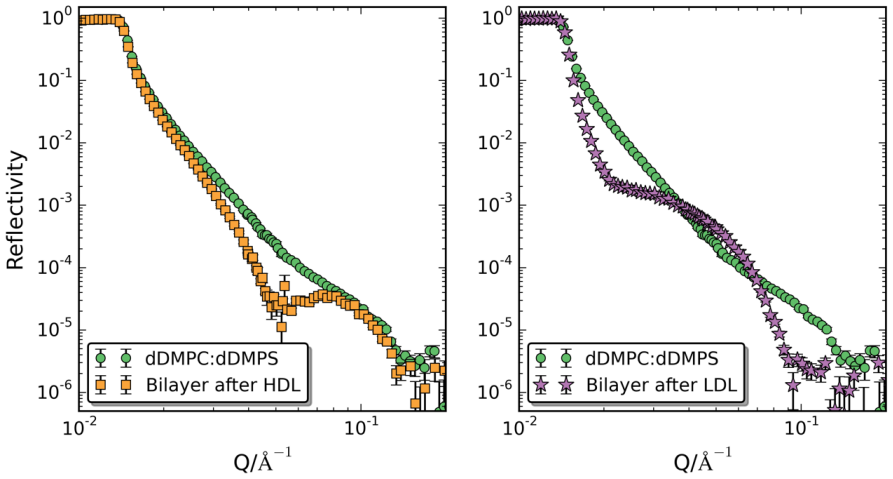

Figure 2: Experimental neutron reflectivity data for the change in reflectivity upon introduction of lipoproteins to a 75:25 dDMPC:dDMPS bilayer after 8h of incubation time. (yellow squares) shows the reflectivity change upon introduction of 0.132 mg L^{-1} HDL and (purple stars) shows the reflectivity change upon introduction of 0.1 mg L^{-1} LDL. The total number of LP is constant in these experiments.

Case 2: HDL and LDL adsorbed onto a 75:25 dDMPC:dDMPS bilayer. Figure 2 shows the experimental reflectivity curves of the change in reflectivity upon contact with HDL and LDL. The introduction of LP to a highly negatively charged bilayer (25% DMPS) produced much more distinct differences in the reflectivity profiles between HDL and LDL, possibly suggesting that differentiation is driven by interactions with negative charges in the lipid bilayer. The same models as case 1 were used to fit the data although it was not possible at this stage to obtain a sufficient fit to ensure the model is adequate. More experiments using a hydrogenated bilayer to contrast match out lipid exchange will be necessary to help produce a better fit.

Case 3: HDL and LDL in a 2:1 ratio adsorbed onto a 75:25 dDMPC:dDMPS bilayer. The ratio of 2:1 was chosen as that represents the lowest atherosclerotic risk category for both men and women. The difference between the mixture and single components was immediately clear as the reflectivity profile began to change over the course of the first 5 minutes upon injection. In comparison, there was little change in the first hour when studying the LDL or HDL separately. After 8h incubation, the reflectivity profile is markedly different to that obtained with either LDL or HDL. Therefore, our data suggest that the presence of both HDL and LDL indeed affect the way these LP interact with cellular membranes.

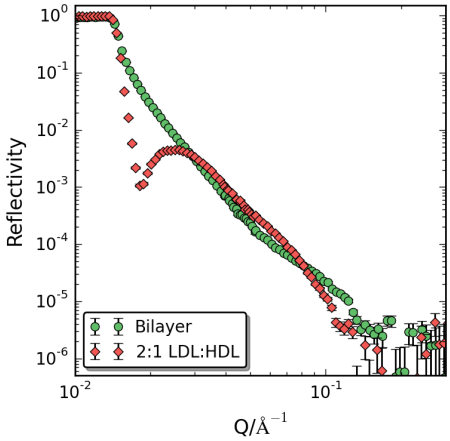

Figure 3: Experimental neutron reflectivity data for the change in reflectivity upon introduction of a 2:1 ratio of LDL:HDL to a 75:25 dDMPC:dDMPS bilayer.