Experimental report

13/02/2019

Proposal: 8-03-942 **Council:** 4/2018

Title: What is the effect of lipid exchange on the structure of artificial HDL made from ApoE?

Research area: Biology

This proposal is a new proposal

Main proposer: Sarah WALDIE

Experimental team: Dainius JAKUBAUSKAS
Sarah WALDIE
Trevor FORSYTH

Local contacts: Yuri GERELLI
Sylvain PREVOST

Samples: deuterated phosphatidylcholine vesicles
reconstituted HDL

Instrument	Requested days	Allocated days	From	To
FIGARO	3	2		
D11	2	2	05/09/2018	07/09/2018
D17	3	0		

Abstract:

Atherosclerosis, the main killer of the West, is directly associated to the plasma levels of low and high-density lipoproteins (LDL and HDL, respectively). These particles have been traditionally considered as bad and good cholesterol, as they either deposit or remove lipids from the vessel wall, respectively. The consequences of this lipid deposition in the artery walls include fat plaque build-up leading to cardiovascular diseases such as heart attacks and strokes. Fundamental information regarding the lipid exchange kinetics and the role played by the specific apolipoprotein present in the particles is missing. Understanding and determining the importance of the lipoprotein particle composition is fundamental in the diagnosis and treatment of the disease. The goal of this project is to understand the influences on the development of atherosclerosis by following the interaction and exchange of lipids with reconstituted lipoprotein particles and model membranes.

Experimental Report for SANS contribution to experiment: **8-03-942**

Atherosclerosis is the leading cause of death in western society, its consequences of cardiovascular diseases (CVDs) such as strokes and heart attacks arise from the build-up of plaque which accumulates in the artery walls. Lipoprotein particles have been shown to play a role in this development, however mechanistic details of this process are missing. Specific apolipoprotein variants present in lipoprotein particles have been shown to either provide a protective or a detrimental effect in this development process, therefore understanding the influence of lipoprotein composition and in particular the apolipoprotein variant present is of utmost importance in understanding the initial development processes in CVDs, which in turn has knock on effects in the diagnosis and treatment possibilities of the disease, especially as clinical lipid profiling today fails to predict the risk of development to this disease. In this project, by structurally characterising reconstituted lipoprotein particles of varying composition, with initial studies focussing on apolipoprotein variants ApoE3 and ApoE4, a deeper understanding of the initial plaque build-up at the onset of atherosclerosis will be enabled

During our beam time we measured control samples of ApoE3 and ApoE4 proteins alone, which provided us with some information on their behaviour and structure in solution. We measured both samples in SANS and complemented this with SAXS on the same samples. The results gave significantly different structures even though the proteins only differ by one amino acid. Initial characterisation shows the ApoE3 to be more of a random fractal aggregate, whereas the ApoE4 seems to adopt a more rod like structure, while both showed to have the same cyclic pentomeric repeating unit. Figure 1 shows the SANS data for the ApoE3 and ApoE4 proteins without lipids in D_2O and H_2O contrasts, given in blue and green respectively. The SAXS data is given in red and for figure 1b SEC-SAXS data is given in dark orange.

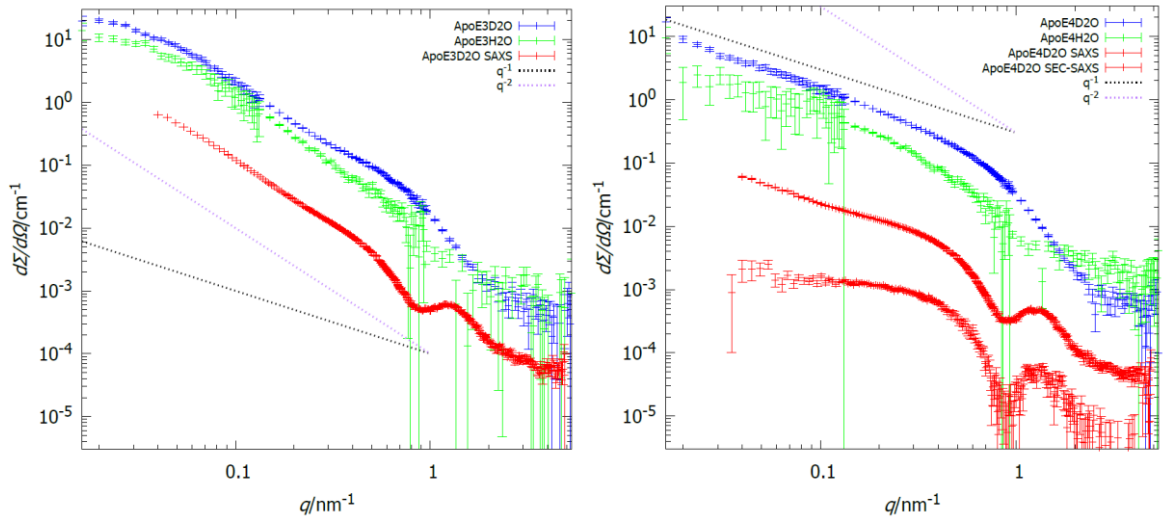

Figure 1: SANS and SAXS data collected for (a) ApoE3 and (b) ApoE4 proteins without lipids. SANS measured in D_2O and H_2O are shown in blue and green respectively, while SAXS data is shown in red and for ApoE4 SEC-SAXS data is also shown in dark orange. Samples were measured at 37 °C in H_2O or D_2O buffers of 50 mM Tris-HCl, 150 mM NaCl, pH 7.5.

Reconstituted HDL (rHDL) particles constituting of either the ApoE3 or ApoE4 proteins with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) at varying ratios and DMPC with cholesterol were measured, in addition to controls of the initial lipid vesicles. Figure 2 shows

the SANS data for the ApoE3 and ApoE4 based DMPC particles in purple and green respectively. The sizes of these particles differ by a factor of two whilst mixed at the same lipid to protein ratio, further showcasing the difference in the proteins and their consequent effects. Other particle compositions were also measured and are currently under analysis.

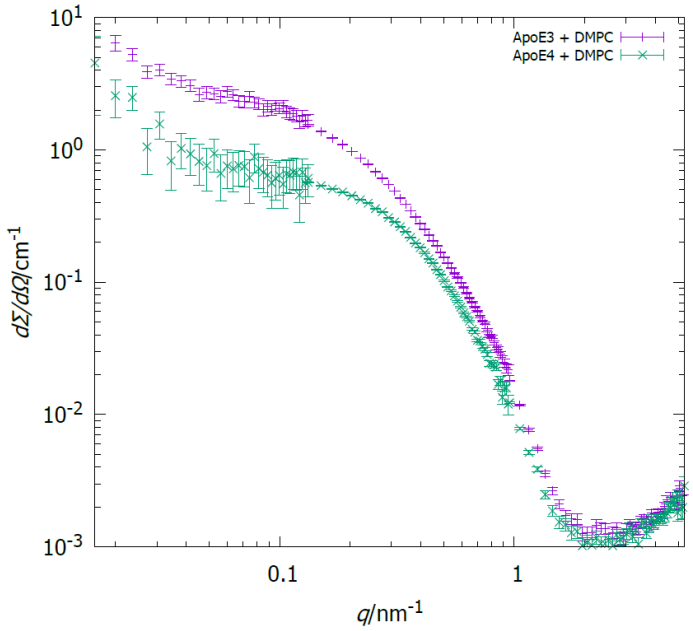

Figure 2: SANS data collected for reconstituted particles with either ApoE3 or ApoE4 and DMPC (purple and green respectively). Samples were measured at 37 °C in a D_2O based buffer of 50 mM Tris-HCl, 150 mM NaCl, pH 7.5.

Initial kinetic studies were also performed to study the rate of formation of the particles but further measurements are required to fully determine this process. Equal volume solutions of DMPC vesicles and protein were mixed together and measured over a course of 8 hours. In figure 3 the blue and red lines correspond to the initial and final measurements of the initial 2 hour period. In both cases there is a decrease in scattering from the vesicles (due to their diminishing quantity) and an increase in scattering from the reconstituted particles upon formation.

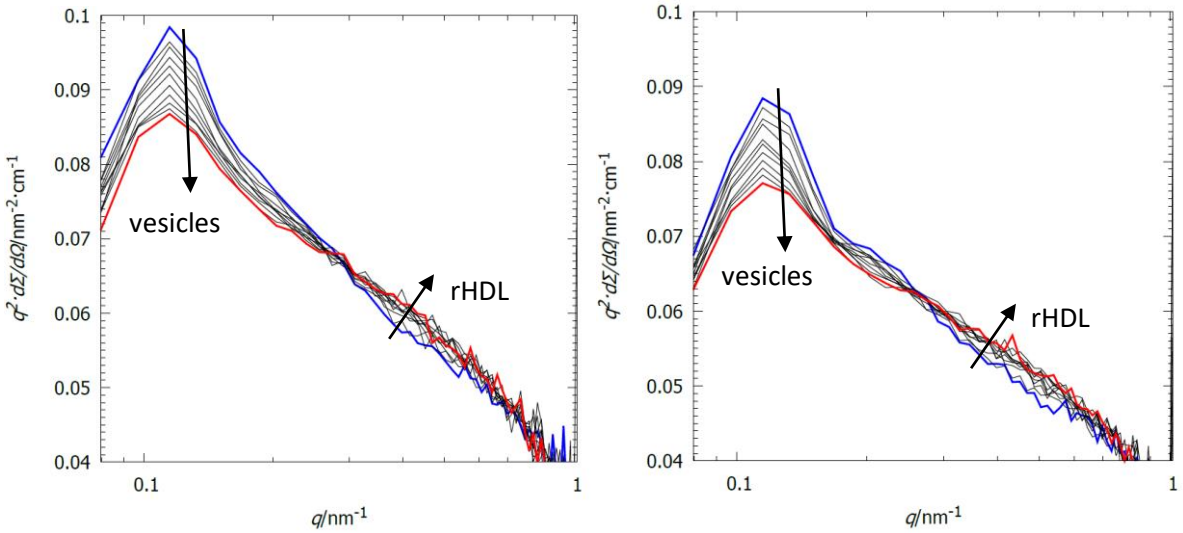

Figure 3: SANS data for the kinetics of reconstituted HDL particle formation with either (a) ApoE3 or (b) ApoE4 protein and DMPC lipids. Data was collected over a course of 8 hours and shows a decrease in scattering where the number of vesicles is diminishing and an increase in scattering where the reconstituted particles are being formed. Blue and red data lines equate to the initial and final measurements during a 2 hour window at the beginning of this time course.

On analysing the protein samples and their distinctly different scattering profiles it is clear that while their amino acid sequences only differ by one component this has a huge impact on their structure and behaviour in solution. From the reconstituted particles it has already been seen the impact this can have though further work is still required to determine the extent of this impact. Preliminary neutron reflection data has shown the interaction and lipid exchange with supported lipid bilayers is different between the 2 protein isoforms. Following on from the kinetic data, further studies are required to see the full mechanism of the particle formation and what impact the difference in the protein and the lipid cargo has.