Experimental report

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Proposal: 8-03-853 **Council:** 4/2015

Title: Lipoprotein particles from human serum- Implications of structure and dynamics on atherosclerosis development.

Research area: Biology

This proposal is a new proposal

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Samples: lipoprotein particles
d-POPC

Instrument	Requested days	Allocated days	From	To
D11	0	2	30/11/2015	02/12/2015
D22	2	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 others). 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

Lipoprotein particles from human serum- Implications of structure and dynamics on atherosclerosis development.

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Background and Aim

In atherosclerosis, plaques of fat and fibrous elements accumulate in the arteries leading to heart disease and stroke. Atherosclerosis and related cardiovascular diseases constitute the leading cause of death in westernized societies. The role of the main clinical indicator of today, the low density lipoprotein (LDL), in depositing fat to the vessel wall is believed to be the onset of the process. However, many subfractions of the LDL, which differ both in structure and composition, are present in the blood and amongst different individuals. Understanding the relationship between LDL structure and composition is key to unravel the specific role of various LDL in the development and/or prevention of atherosclerosis. The use of neutron small angle scattering techniques, in combination with selective deuteration, can provide unique information on both structure and molecular exchange between lipids and proteins in the lipoprotein particle. The goal of this project is to provide unique information into the molecular mechanisms behind the biological markers such as LDL, as well as (high density lipoprotein (HDL) with the hopes of developing novel strategies in the fight against cardio-vascular diseases. More precisely we want to relate the structure of the lipoprotein particles with dynamics of lipid exchange and capacity to release their load at blood vessel surfaces.

Preliminary Results

In this experiment we characterized the lipid exchange kinetics between a pooled sample of native LDL particles extracted from healthy humans and model cellular membranes (liposomes made of “invisible” phosphatidylcholine (PC) lipids), see Fig. 1.

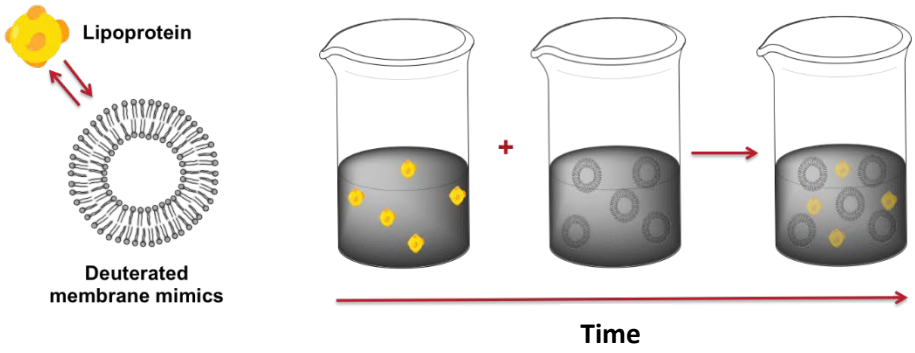

Fig. 1. Set-up of the SANS experiment for monitoring the lipid exchange between native LDL and invisible PC-membranes

The goal of the experiment was to determine the appropriate solvent conditions and q-range where the signal from the deuterated vesicles was negligible and if possible to only monitor the decrease in lipoprotein signal as it took up deuterated lipids. We characterized the particles at intermediate and high q in 100% D_2O and subsequently performed a time-dependent characterization at high q up to 24-48 hours. In this way, we were able to observe a decrease in scattering intensity over time, at a q-range where the scattering was dominated by that of lipoprotein particles.

As a first attempt to analyse the data, we plotted the intensity as a function of time at a specific q (Fig. 2.). This showed two clear kinetic regimes, one of which occurred on a 10 time faster scale than between lipid vesicles alone.

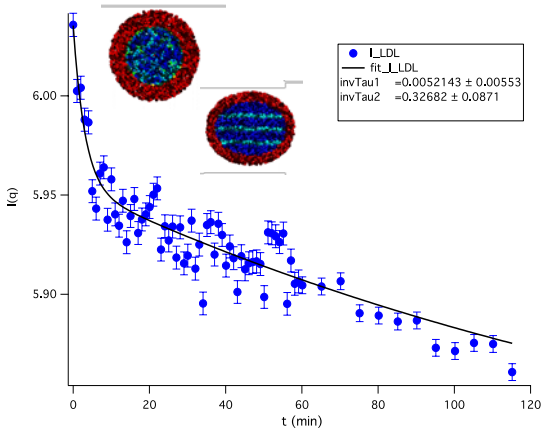

Fig. 2. Intensity as a function of time showing the kinetics of lipid exchange between LDL and D_2O matched POPC vesicles at different particle ratios at a high q setting. The experiments were performed at 37°C.

The measurements were done at 37°C, which is physiologically relevant, in a temperature-controlled sample rack. A time delay between the loading of the sample after the mixing and the start of the measurements led to the loss of the initial part of the kinetics curve which we think could possibly be circumvented using a stopped-flow set up.

Briefly, the current study suggests that the apolipoprotein has a key role in lipid dynamics and is possibly enhancing lipid exchange. The incubation of the LDL particles with deuterated phospholipid vesicles lead to a change in the scattering pattern (Fig. 3.) which could either be assigned to a structural change in the LDL particle or a contrast change in the LDL upon lipid exchange.

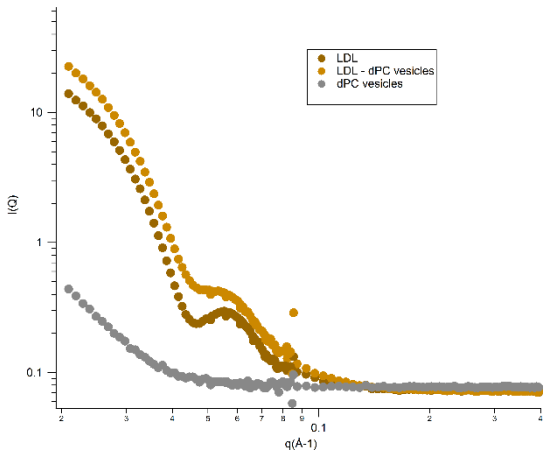

Fig. 3. SANS data of LDL (brown), D_2O matched POPC vesicles (grey), and LDL during the exchange with D_2O matched POPC vesicles (orange). All experiments were performed at 37°C.

We are currently working on relating the changes in structure and/or contrast of the particles with the observed lipid exchange events.