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Application for beam time at ESRF – Experimental Method

This document should consist of a maximum of two A4 pages with a minimal font size of 12 pt.

Proposal Summary (should state the aims and scientific basis of the proposal) :

Cholesterol metabolism and transport is of increasing interest, due to the fact that it is implicated in cardiovascular diseases. The reason for the increasing interest is very clear in the latest data from the U.S. National Vital Report. Here it is clearly shown that cardiovascular disease is the main cause of death in the U.S (1). A main player in the human cholesterol metabolism and transport is the Apolipoprotein A1 (ApoA1). This protein is found in high amounts in human plasma (1mg/ml) and is responsible for transport of lipids and cholesterol. ApoA1 has a remarkable ability to make structural changes to accommodate varying amounts of lipid and cholesterol cargo. Insight into how lipids and cholesterol is transported around the human body by ApoA1 is paramount in further understanding of how this is accomplished.
This experiment falls well in line with our research into lipid:protein particles, previously performed at ERSF and ILL(2,3). Our previous research has enabled us to model the obtained data from these discoidal lipid:protein particles very precisely and extract structural information of previously unprecedented detail.

Scientific background :

As stated in the proposal summary, cholesterol is a main component in cardiovascular diseases. Seen from a pharmaceutical point of view, this makes cholesterol transport and metabolism highly interesting. Any new insight into either how cholesterol is transported or metabolized in the human body has the potential of leading to better treatments. The protein ApoA1 is known to be the main protein involved in lipid and cholesterol in humans. The ability for ApoA1 to make rearrangements to accommodate more and more cholesterol is well known. How this is accomplished structurally by the ApoA1 protein is still unknown even though the properties of discoidal ApoA1 has been studied since the early 70s (4). A large body of *in vivo* data has been accumulated over the past decades regarding the function of the discoidal lipoprotein particle. In light of this, there is very little known about the structure of the ApoA1 and even less about how the rearrangement is accomplished. Different structural hypotheses exist in the medical science literature for this cholesterol effect. However none of these are strongly experimentally supported by direct structural data.
Besides from investigating the native ApoA1, a truncated version of the protein will be investigated. This will be done to get information on the role of the N-terminal part of the protein. This N-terminal part is encoded by its own exon in the human genome, and the function of this part of the protein is at present unknown.

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc) :

These lipoprotein particles at present not possible to crystallize, but their size are about 10-12nm, which makes them perfectly suited for investigation by SAXS. The experiment will be comprised of lipoprotein particles of protein, phospholipids and varying amounts of cholesterol. The proteins used will be either native ApoA1 protein or an N-terminal truncated version. The accommodation of cholesterol will be tested by gradually incorporating more and more cholesterol into the particles, up to a previously documented upper limit of 20% mol/mol cholesterol. A total 20 samples plus backgrounds will be investigated. We would like to use the standard BioSAXS setup including sample changer robot and Pilatus detector optimized for a q-range from 0.008 to 0.5 Å-1. We ask for a total of 8 hours of beam time for the experiment. We expect to obtain high-quality SAXS data from multiple preparations of protein:lipid discs.

Beamline(s) and beam time requested with justification :

For the purpose of getting the best SAXS data possible, we apply for SAXS beamtime at the BioSAXS beamline. If granted, we hope that this beamtime can be co-scheduled with another (16 hours) SAXS proposal by the same proposers.

Results expected and their significance in the respective field of research :

The central objective of the proposed experiment is to see the structural changes of the ApoA1 protein upon the addition of cholesterol. The specific question is how the structure adapts to accommodate the incorporated cholesterol.

ApoA1 with and without cholesterol was previously measured in a pilot experiment performed at ESRF, ID14-3. The obtained data are plotted in figure 1. While a difference may be observed between the ApoA1:phospholipid particle with and without 5% cholesterol, we found, to our surprise no indications of a major cholesterol induced structural reorganization of the particles. However, while the particles without cholesterol could be fitted well with an adapted version of our previously published model for discoidal particles (see fig 1A), the cholesterol containing particles could not (see fig 1B).

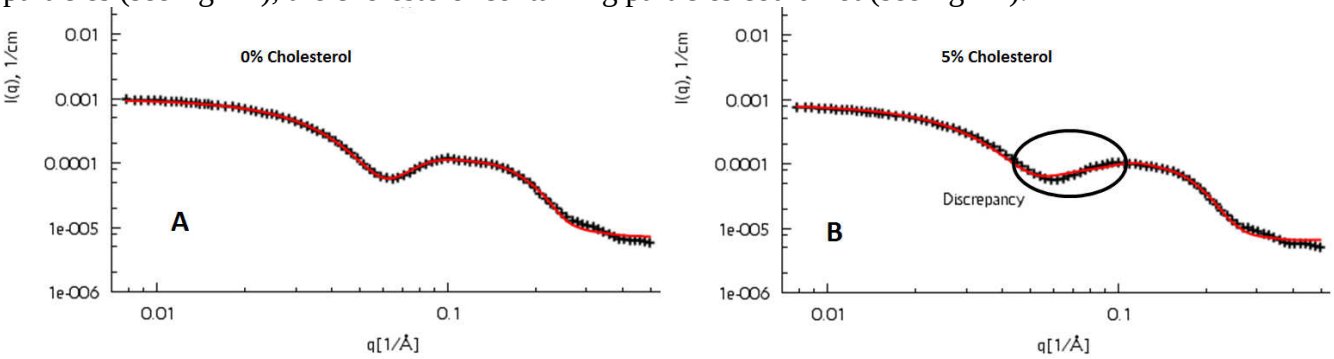

Figure 1. SAXS data from the pilot experiment showing data (black points) and model fits (red line) from particles without cholesterol (A) and particles containing 5% cholesterol (B) While our model could fit the particles without cholesterol to the experimental data, the cholesterol data could not be fitted satisfactorily.

The series of measurements outlined in this proposal will allow us to see the progress of the structural changes upon addition of cholesterol. This will provide valuable direct structural insight into how ApoA1 accommodate the addition of more and more cholesterol. Furthermore, information about the role of the N-terminal domain in cholesterol uptake will be available in the data. Our hypothesis for the general cholesterol uptake is that the cholesterol incorporates into the central lipid bilayer and makes the discoidal particle swell to a more ellipsoidal structure.

Even though these particles has been studied for a couple of decades, there methodology for interpreting scattering data and extracting detailed structural information has not been available before now(2,3). We believe that this new knowledge will enable us to make a significant new contribution to this important field of research.

References

(1) Sherry L. Murphy, B.S.; Jiaquan Xu, M.D.; and Kenneth D. Kochanek, M. A. . Nad. Vital Stat. Rep. 2012, 60, 1-68.

(2) Nicholas Skar-Gislinge et al. (2010) Elliptical Structure of Phospholipid Bilayer Nanodiscs Encapsulated by Scaffold Proteins: Casting the Roles of the Lipids and the Protein, JACS, 132, 13713–13722.

(3) Nicholas Skar-Gislinge et al. (2011) Small-angle scattering from phospholipid nanodiscs: derivation and refinement of a molecular constrained analytical model form factor, Phys. Chem. Chem. Phys., 13, 3161-3170

(4) Forte, T.; Norum, K. R.; Glomset, J. a; Nichols, a V. Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy. The Journal of clinical investigation 1971, 50, 1141-8.