Experimental report

06/03/2020

Proposal: 9-13-866 **Council:** 4/2019

Title: Lipid nanoparticles-ApolipoproteinE interaction: the role of LNPs surface composition and structure

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: lipid nanoparticles
ApoE

Instrument	Requested days	Allocated days	From	To
D22	2	2	03/09/2019	05/09/2019

Abstract:

Therapeutic treatments based on the production of proteins by delivering messenger RNA (mRNA) represent a promising approach. One of the major challenges is to protect mRNA from enzymatic degradation and deliver it into the target cells. Lipid nanoparticles (LNPs) formed by a cationic ionizable lipid (CIL), DSPC, cholesterol and a pegylated lipid have been successful to deliver small interference RNA. Physical and chemical characterization of these LNPs is needed to progress from pre-clinical testing. The bio-distribution and cellular uptake of LNPs are affected by their surface composition as well as by the extracellular proteins present at the site of LNPs administration, e.g. Apolipoprotein E (ApoE). ApoE, being responsible for fat transport in the body, plays a key role in the LNP's circulation time. Our previous results show that both particle size and DSPC surface area affects the efficacy of LNPs. For an optimised LNP formulation, we aim to reveal the contribution of CIL and cholesterol to the LNP structure. Furthermore, we want to investigate the role of LNP surface structure on the ApoE binding and the structural change, in particular at the surface, due to ApoE binding.

Report on Experiment 9-13-866
3-5 September 2019

Lipid nanoparticles (LNPs) formed by a cationic ionizable lipid (CIL), DSPC, cholesterol (Chol) and a pegylated (PEG) lipid can be used to deliver mRNA.
In this experiment, we investigated: the structure and composition of LNPs in particular locating the cholesterol and the CIL.
We formulated LNPs with fully deuterated cholesterol (d-Chol, produced in collaboration with the D-lab at ILL) in order to investigate the distribution of cholesterol in the core and at the surface (LNP1). We have also prepared LNPs with d-Chol and 32% d-DSPC to make the particle invisible when the solvent contained around 39% D_2O (LNP2). We have characterized the 2 LNP samples (LNP1 and LNP2) at 5 different contrasts to determine the structure and composition in the different subparts of the nanoparticles. We have used 9.4%, 25%, 35%, 60%, and 84% or 88% D_2O buffer (respectively for LNP1 and LNP2).
Both samples and all contrasts were characterised at 3 temperatures (25, 37 and 49°C) and measured again at 25°C after cooling, we wanted to investigate the effect of temperature on the LNPs structure.
In order to find the matching conditions for LNP2 we collected data for several D_2O content between 30 and 49% and we found that 39% was the solvent composition giving the lowest intensity (Fig.1); however, we could not completely match out the LNP2, which is probably due to the structured core and/or not homogeneously mixed shell.
In Fig. 2 and 3 we show the SANS curves collected for LNP1 and LNP2 at 25°C in the different contrasts and the corresponding fitted models. For this preliminary fit a core-shell model was applied up to 25% D_2O, while to better describe the SANS profile a combined model core-shell and broad peak was used in order to describe the broad peak arising around 0.1 Å-1 clearly visible for 60 and 84% (88%) D_2O. For LNP2 in 39% D_2O the combined model needed to be used in order to get a good fit, and this is probably because at such a low scattering intensity the structure in the core plays a role even at low q. The fitted models suggest that the cholesterol fraction in the shell is higher than in the core, and the core is enriched with CIL. The deuterated CIL is now available and it will give a great opportunity to investigate LNPs formulated with d-CIL to support the current results (proposal 9-13-909, Sept 2019).
Data analysis is ongoing and these preliminary results are very promising.

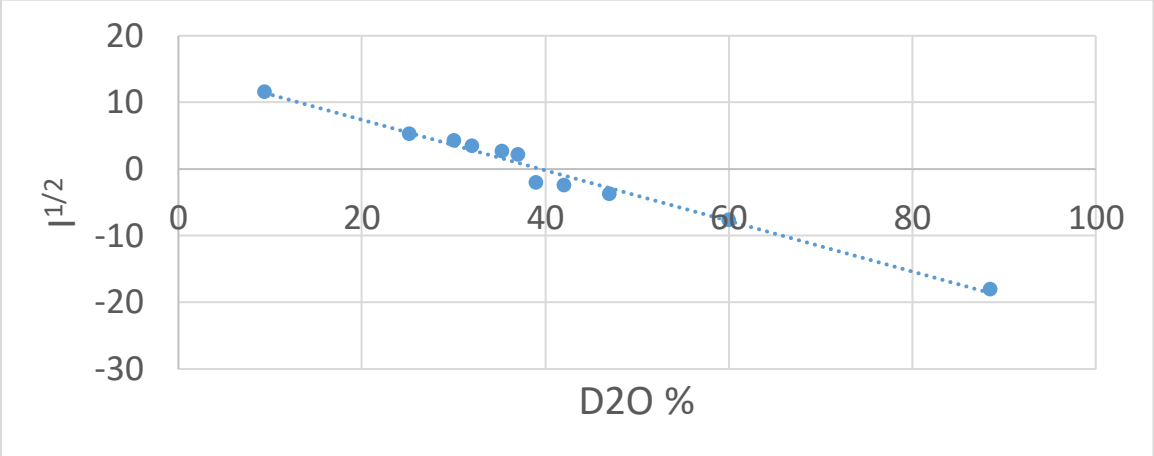

Figure 1. Neutron contrast matching plot of a squared intensity (average over low q) against D_2O content in the H-D buffer. The contrast matching point ($I_{1/2}$ close to zero) is found at 39% D_2O.

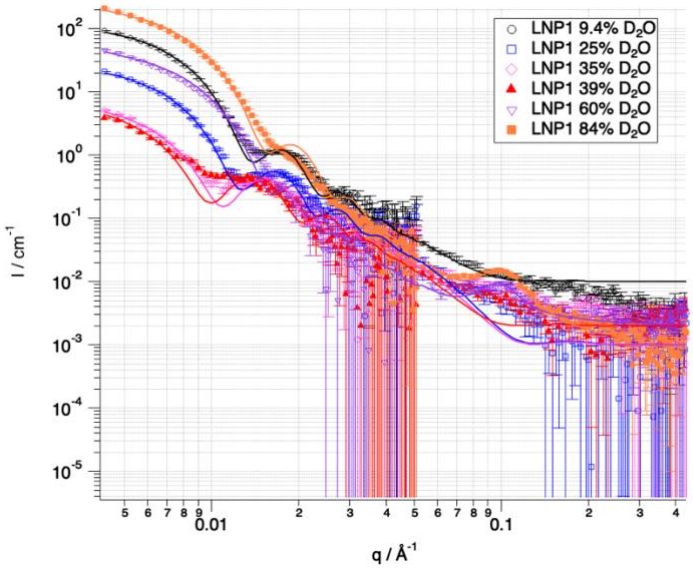

Figure 2. (a) SANS data (symbols) for mRNA containing LNPs with DSPC and deuterated Chol (LNP1) in 9.4% (black circles), 25% (blue squares), 35% (pink diamonds), 39% (red filled triangles), 60% (purple inverted triangles) and 84% (orange filled squares) D_2O buffer. The solid lines correspond to the best fit using the core shell model combined with a broad peak model for 60 and 84% D_2O buffer.

Figure 3. (a) SANS data (symbols) for mRNA containing LNPs with 32% d-DSPC and deuterated Chol (LNP2) in 9.4% (black circles), 25% (blue squares), 39% (red diamonds), 60% (purple filled triangles) and 88% (orange inverted triangles) D_2O buffer. The solid lines correspond to the best fit using the core shell model combined with a broad peak model for 39 and 88% D_2O buffer.