Experimental report

24/03/2021

Proposal: 9-13-909 **Council:** 10/2019

Title: Lipid nanoparticles-ApolipoproteinE interaction: the role of pH

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: lipid nanoparticles
Apolipoprotein E

Instrument	Requested days	Allocated days	From	To
D22	2	2	01/09/2020	03/09/2020

Abstract:

Therapeutic treatments based on the protein production 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 were approved for delivery of small interference RNA. There are still concerns about how to improve LNP efficacy following endocytosis. 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. ApolipoproteinE (ApoE). ApoE has been identified as fundamental regarding the efficacy of siRNA-LNPs but its effect on the LNP structure and subsequently endosomal escape has not been studied. Our recent results suggest that ApoE binding affect the LNP structure at pH 7.4. We aim to characterise the LNP structure at pH conditions relevant to different endosomal compartments, and investigate not only how ApoE binding is regulated by the LNP surface structure but more importantly how the LNP overall structure responds to ApoE binding.

Report on Experiment 9-13-909
31st August- 3rd September 2020

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 our previous experiment (#8-13-866), we were able to describe the structure and component distribution in the LNP at pH 7.4 in presence and absence of ApoE. [1]
In this experiment, we investigated:

(1) the mRNA-LNP structure at two pH conditions for three formulations;
(2) the LNP overall structure changes due to ApoE binding for three formulations.

We formulated LNPs according to Table 1, for each composition we prepared a sample with 50%mol dDSPC and 100%mol dMC3 (dMC3 produced by collaborators in AstraZeneca) in order to investigate the distribution of MC3 (the CIL used in this work) and DSPC and highlight separately shell and core according to the solvent contrasts. An additional deuteration scheme for each sample was prepared with 50%mol dDSPC, 100%mol dMC3 and 100%mol DMPE-dPEG2000 (produced in collaboration with Lutz Willner at JCNS and Tamim Darwish at ANSTO) in order to locate the PEG and to highlight the effect of ApoE binding on the PEG layer.

Table 1 LNP compositions

	CIL	DSPC	Chol	PEG-lipid
LNP c1	50	10	38.5	1.5
LNP c2	50	10	39.75	0.25
LNP c3	53.5	4.7	41.2	0.7

All samples were measured at minimum 3 solvent contrasts, except when sample was only enough for 2. All samples were measure at pH 7.4 and 5.5. Phosphate buffer saline at 10 mM with 150 mM NaCl was used for pH 7.4, while citrate phosphate buffer 15 mM with 150 mM NaCl was used for the pH 5.5. The effect of ApoE on the LNP structure was followed for 3 hours at a single configuration and solvent contrast (46% D2O) to extract the kinetic, while the full q-range was measured after 3 hours incubation (pH=7.4).

The lower pH has a clear effect on the structure of the LNPs, Figure 1 reports the SANS curves collected for the formulation c1 at the two pH values. The characteristic bump of the core-shell structure at pH 7.4, it is not as visible when pH is 5.5. This effect is found for the other compositions as well (Figure 2 and 3), this suggests a major rearrangement of the components in the LNPs which may be crucial to understand the mRNA release mechanism once LNP enters the cell. The data analysis is ongoing.

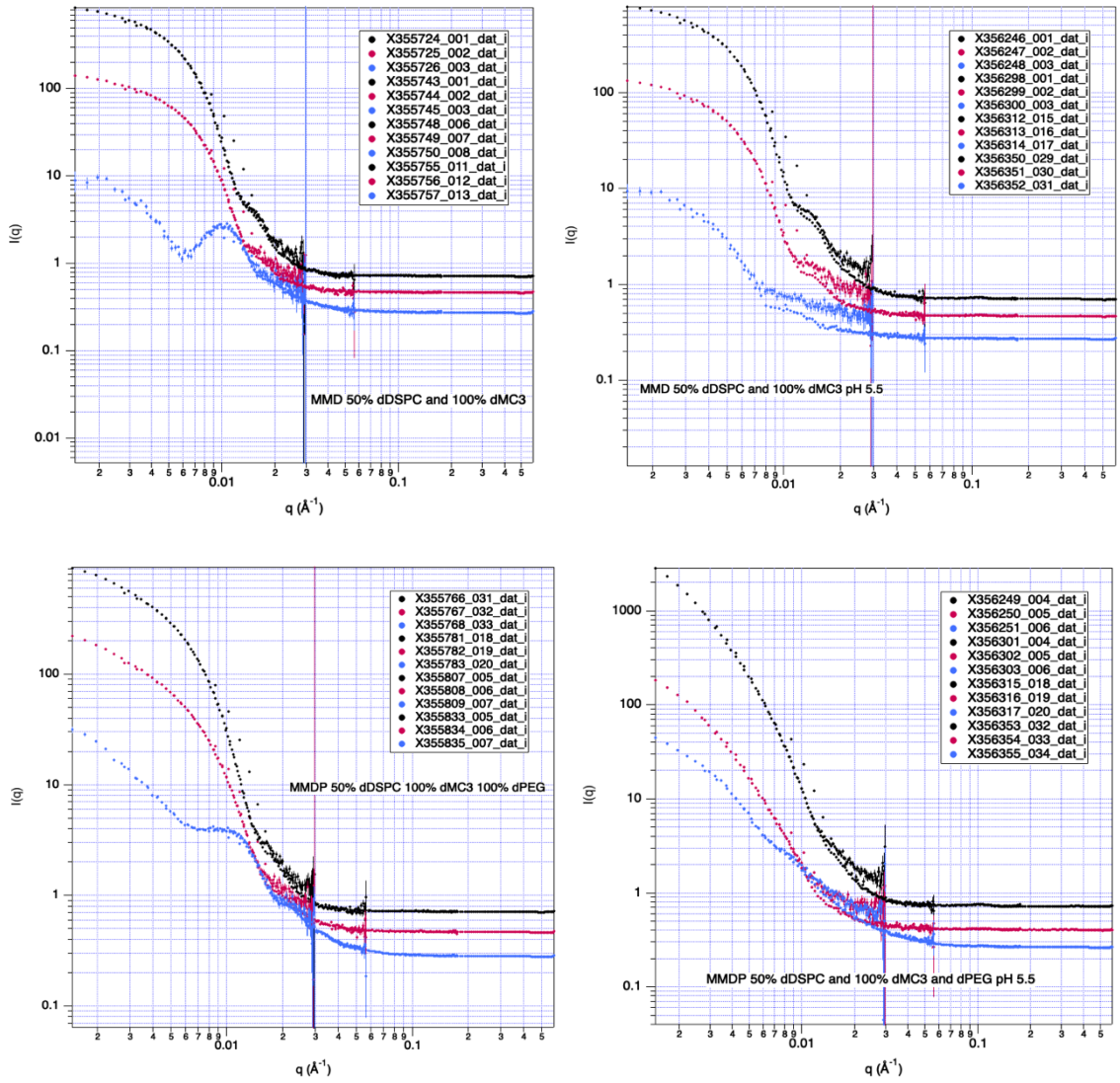

Figure 1: SANS curves measured for: LNP c1 with 50%mol dDSPC and 100%mol dMC3 (MMD) at pH 7.4 (top left) and 5.5 (top right); LNP c1 with 50%mol dDSPC, 100%mol dMC3 and 100%mol dPEG (MMDP) at pH 7.4 (bottom left) and 5.5 (bottom right). The data are not background subtracted. Samples were measured in 20 (black symbols), 46 (red symbols) and 68% d-PBS (blue symbols).

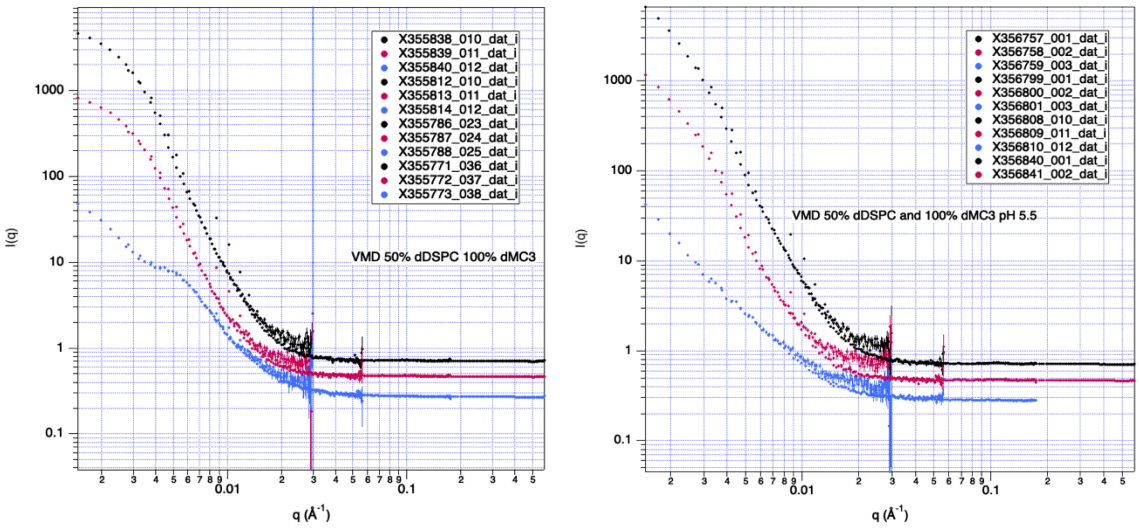

Figure 2: SANS curves measured for: LNP c2 with 50%mol dDSPC and 100%mol dMC3 (VMD) at pH 7.4 (left) and 5.5 (right). The data are not background subtracted. Samples were measured in 20 (black symbols), 46 (red symbols) and 68% d-PBS (blue symbols).

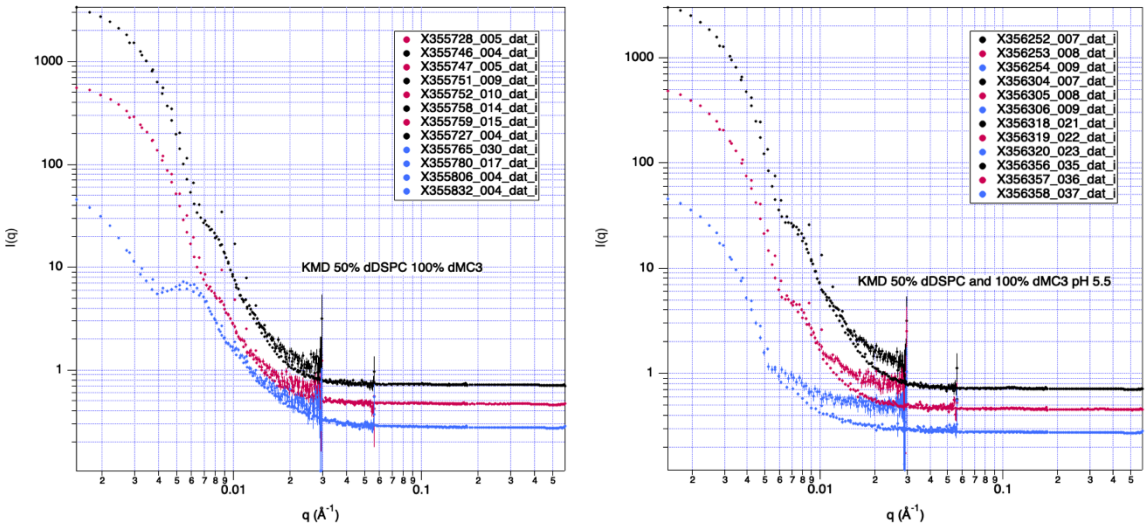

Figure 3: SANS curves measured for: LNP c3 with 50%mol dDSPC and 100%mol dMC3 (KMD) at pH 7.4 (left) and 5.5 (right). The data are not background subtracted. Samples were measured in 20 (black symbols), 46 (red symbols) and 68% d-PBS (blue symbols).

[1] F. Sebastiani *et al.*, "Apolipoprotein E Binding Drives Structural and Compositional Rearrangement of mRNA-Containing Lipid Nanoparticles," *ACS Nano*, Mar. 2021.