Can mRNA - Lipid Nanoparticle Surface Composition Regulate Apolipoprotein Binding From Serum?

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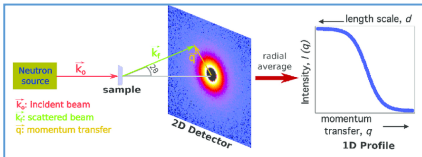
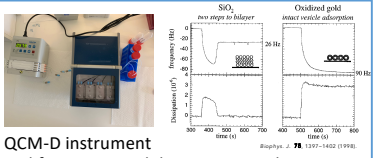
Background: Lipid nanoparticles (LNPs) are promising vehicles for mRNA delivery and are formed by a cationic ionizable lipid (CIL), DSPC, cholesterol (Chol) and a pegylated (PEG) lipid. It is important to understand the relation between LNP physico-chemical properties and their ability to collect proteins. A common component found in the "protein corona" of LNPs is Apolipoprotein E (ApoE), which is responsible for the transport of fats in the systemic circulation and it triggers the fat uptake by cell-rich in low-density lipoprotein (LDL) receptors. This recognition step is critical to control the LNP's circulation time and thus its pharmacological efficiency.



Principle of a SANS experiment

Methods: We employed small angle neutron scattering (SANS) and deuteration of lipids and cholesterol to investigate the distribution of components in the LNP and the effect that ApoE has on the LNP structure. In addition, we have developed a sensor platform based on Quartz Crystal Microbalance with Dissipation (QCM-D) to assess the binding affinity of serum protein to LNPs.



QCM-D instrument and frequency and dissipation results upon adsorption of a lipid bilayer and intact vesicles.

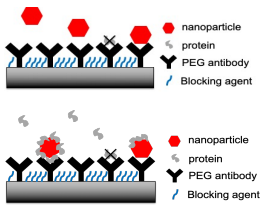
Results and Discussion: Previous studies reported the core shell structure of LNPs (Yanez Arteta et al. PNAS 2018 115(15)) , and highlighted the enrichment of the shell with the saturated lipid DSPC.

In this work, we reveal the precise location of cholesterol and CIL across the LNPs by means of SANS. The SANS curves (Fig. 1-2) were fitted to a core shell model which allowed to determine size and composition of core and shell; the broad peak, visible when d-chol and d-DSPC are present (Fig. 1), suggest an internal structure with a characteristic distance of (6.35 ± 0.02) nm determined by the CIL-mRNA arrangement.

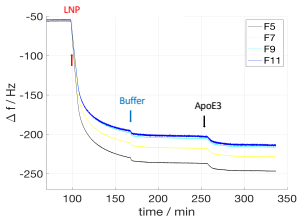
Additionally, we measured the LNP upon incubation with ApoE in a solvent contrast where ApoE is invisible (39% d-buffer, Fig. 3-4). The modelling of the data highlighted the effect that protein binding exerts on the lipid distribution within the LNP particle (LNP schematics).

In parallel, we developed a sensor platform based on QCM-D that allows to quantify the binding affinity of proteins to the LNPs; a decrease in frequency corresponds to an increase in wet mass adsorbed on the sensor. We show that, in line with what has been reported in literature, ApoE has a higher binding affinity to LNP compared to HSA and ApoA1.

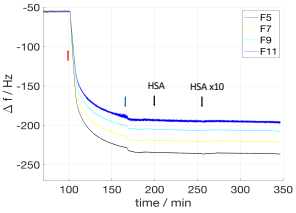
QCM-D sensor platform



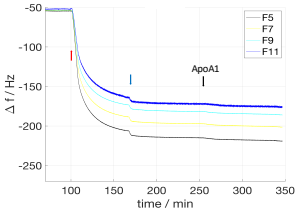
Schematics of the working principle of the sensor



mRNA-LNP binding to ApoE (5 μg/ml)



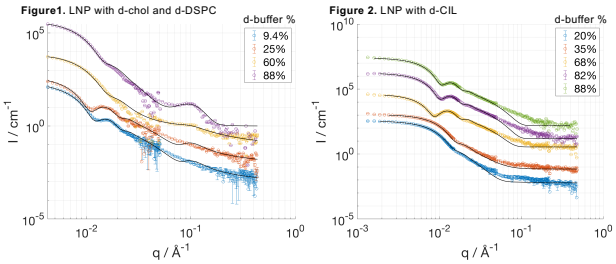
mRNA-LNP binding to Human Serum Albumin (5 and 50 μg/ml)

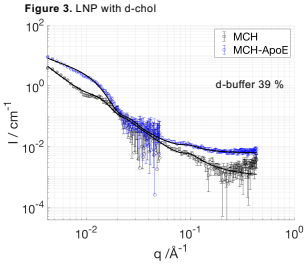


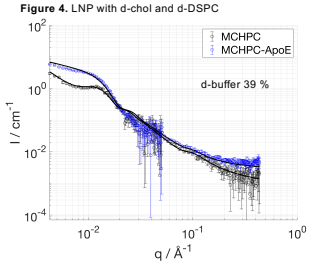
mRNA-LNP binding to ApoA1 (5 μg/ml)

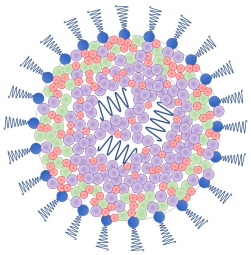
SANS experiments

The curves were shifted for clarity in Fig.1 and 2.
The black solid lines are the results of model fitting, while symbols represent the experimental data.

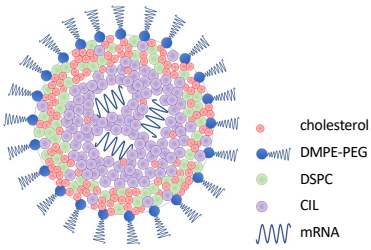








Schematics of mRNA-LNP



Schematics of mRNA-LNP upon 3 hours ApoE incubation

Conclusions

- We elucidated the structure of mRNA-LNPs and the localization of the different components across the particle inner core and shell.
- We demonstrated that ApoE binding affects the structure of LNPs.
- We developed a tool to assess the ability of the LNPs to bind proteins.

Combining these approaches, we can determine how changes in the LNP formulation, and hence structure, affect the protein binding to LNPs.

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