CAN mRNA - LIPID NANOPARTICLE SURFACE COMPOSITION REGULATE APOLIPOPROTEIN BINDING FROM SERUM?

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Background: Therapeutic treatments based on the production of proteins by delivering messenger RNA (*mRNA*) represent a promising approach to treat many diseases that currently lack other alternatives. One of the major challenges for designing such treatments is the adequate protection of these macromolecules from enzymatic degradation and their safe deliver into the target cells. 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. Even though some LNPs were recently FDA approved for the treatment of peripheral nerve disease by delivery of small interference RNA (*siRNA*), there are still concerns about the safety profile of these nanoparticles. A good understanding of the physical and chemical characteristics of the LNPs under study is necessary to progress from pre-clinical testing. In addition, 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, such as proteins in the plasma. Therefore, it is also important to understand the relation between LNP physical 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.

Methods: We employed small angle neutron scattering (SANS) to investigate the distribution of components in the LNP and the effect that ApoE could have 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 with different size and surface composition.

Results: Previous studies reported the core shell structure of LNPs, and highlighted the enrichment of the shell with the saturated lipid DSPC. By means of SANS, we reveal the precise location of cholesterol and CIL across the LNPs. Additionally, we determine the extent of ApoE binding to LNP and subsequently the effect that protein binding exerts on the lipid distribution within the LNP particle. The structural studies provided fundamental information to understand how ApoE mediates cell uptake via LDL receptor.

Additionally, with the sensor platform based on QCM-D, we show that, in line with what has been reported in literature, ApoE has a higher binding affinity compared to HSA and ApoA1.

Conclusions: The structure of mRNA-LNPs was studied by SANS yielding the localization of the different components across the particle inner core and shell. 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.