

# Experimental report

25/01/2024

**Proposal:** 9-13-1093

**Council:** 4/2023

**Title:** Probing the structure and pH responsiveness of next-generation mRNA vaccines based on lipid-polymer hybrid nanoparticles

**Research area:** Chemistry

**This proposal is a new proposal**

**Main proposer:** Camilla FOGED

**Experimental team:** Zhenning SHI  
Federica SEBASTIANI

**Local contacts:** Lionel PORCAR

**Samples:** mRNA  
lipid-polymer nanoparticles

| Instrument | Requested days | Allocated days | From       | To         |
|------------|----------------|----------------|------------|------------|
| D33        | 2              | 0              |            |            |
| D22        | 2              | 1              | 30/11/2023 | 01/12/2023 |

## Abstract:

RNA therapeutics are expected to revolutionize medicine. However, one of the biggest barriers to RNA therapy is to safely deliver the drugs into the correct cells. Lipid-polymer hybrid nanoparticles (LPNs), composed of ionizable cationic lipid and a polymer, have an ability to deliver RNA to target tissues, but little is known about the structure-activity relationship of these LPNs and how they interact with the biological environment after administration. We have designed monodisperse mRNA-loaded LPNs using the microfluidics manufacturing method that display very high in vitro and in vivo transfection efficiency and immunogenicity in initial vaccination studies in mice. CryoTEM imaging suggests that these LPNs display structural characteristics that depend on: (i) the lipid:polymer ratio, (ii) the lipid:mRNA ratio, and (iii) the solvents used for preparation. For an optimized mRNA-loaded LPN formulation, we aim to reveal the structure and component distribution of LPNs for a range of formulations (lipid weight % and type of RNA cargo) in conditions relevant to the extracellular and endosomal compartments (physiological and acidic pH).

# Report on Experiment 9-13-1093

30 Nov- 01 Dec 2023

We have designed lipid-polymer hybrid nanoparticles (LPNs), composed of ionizable cationic lipid (C12-200) and a polymer (PLGA), helper lipid DOPE and a pegylated (PEG) lipid can be used to deliver mRNA. The data analysis is ongoing. We are using SasView to fit models to the experimental data and exploring core-shell sphere model for detailed core and shell radius and thickness (example in Figure 1).

In this experiment, we measured the structure and component distribution of LPNs for a range of formulations (different flow rate ratios, FRR) under conditions relevant to extracellular (pH 7.4) and endosomal (pH 5.5) compartments.

We used microfluidics to prepare monodisperse LPNs loaded with eGFP mRNA as model RNA based on ionizable cationic lipid (C12-200). We systematically varied the flow rate ratio of LPNs (5:1 and 7:1) for mRNA. Then, we measured this LPNs set in buffer solution with pH 5.5, reflecting the endosomal environment.

All LPNs samples were measured at 3 solvent contrasts (20%, 45%, 80%). Citrate phosphate buffer 10 with 150 mM NaCl was used for both pH 7.4 and 5.5. As can be seen from Figure 1, different flow rate ratios have an impact on the core-shell structure of LPNs, but not significantly at 20%. However, at 45% and 80% D<sub>2</sub>O, it can be clearly seen that the FRR 5:1 curve is flat while 7:1 shows a more obvious core-shell structure (Figure 3 and 4). The pH value did not affect the core-shell structure much at 20% and 80% contrast, and at 45% contrast, the structure of the nanoparticles has been affected to a certain extent, which may also be related to the proximity to the match point (Figure 5). Temperature seems to have an impact on the core-shell structure of 45% and 80% of nanoparticles, which may come from the expansion of the shell caused by heating. (Figure 6)

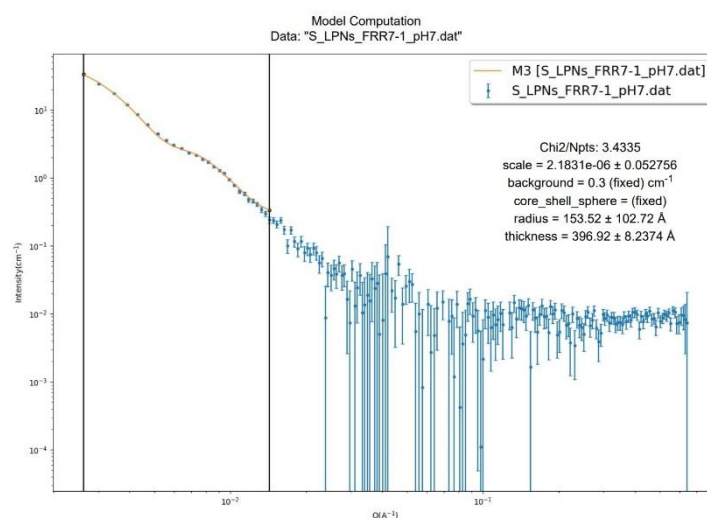


Figure 1: SANS model fitting for LPNs with flow rate ratio 7:1 at 80% contrast.

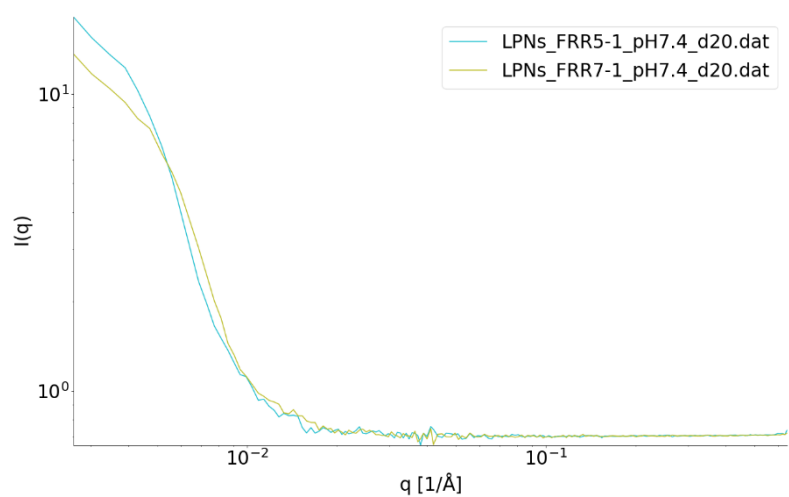


Figure 2: SANS curves measured for LPNs with flow rate ratios (7:1 and 5:1) at 20% contrast.

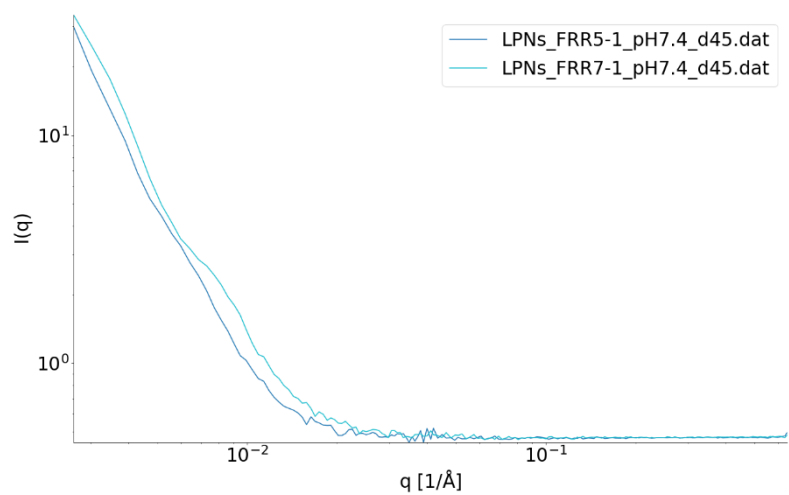


Figure 3: SANS curves measured for LPNs with flow rate ratios (7:1 and 5:1) at 45% contrast.

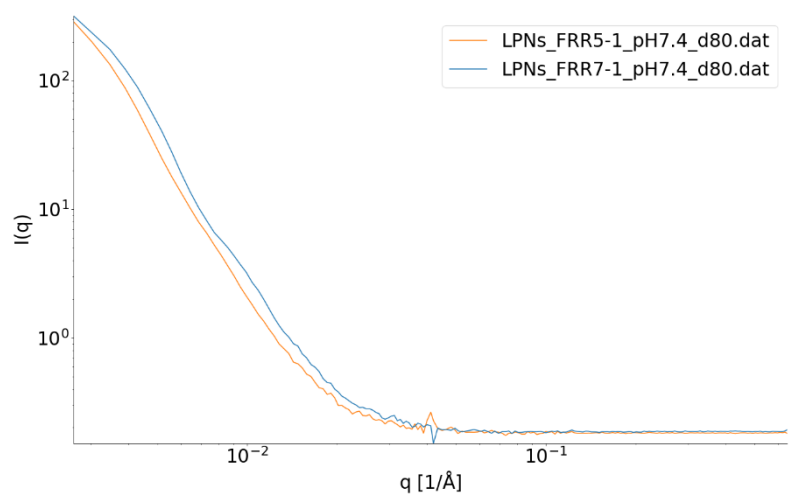


Figure 4: SANS curves measured for LPNs with flow rate ratios (7:1 and 5:1) at 80% contrast.

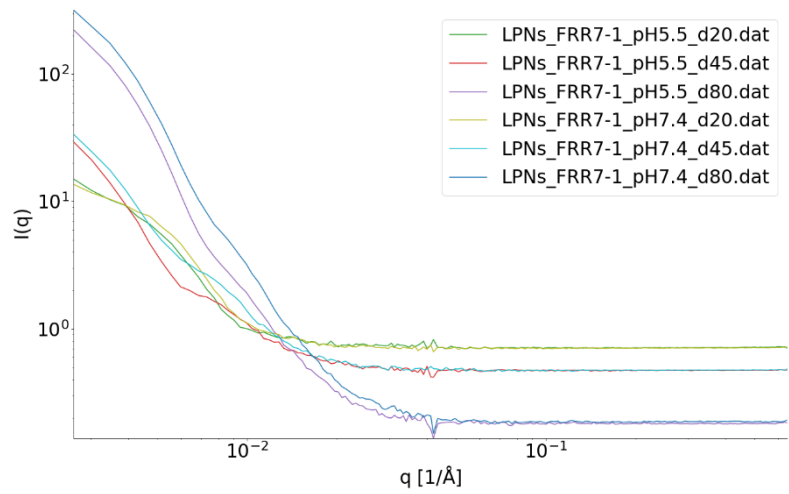


Figure 5: Example of SANS curves measured for nanoparticles at different pH values.

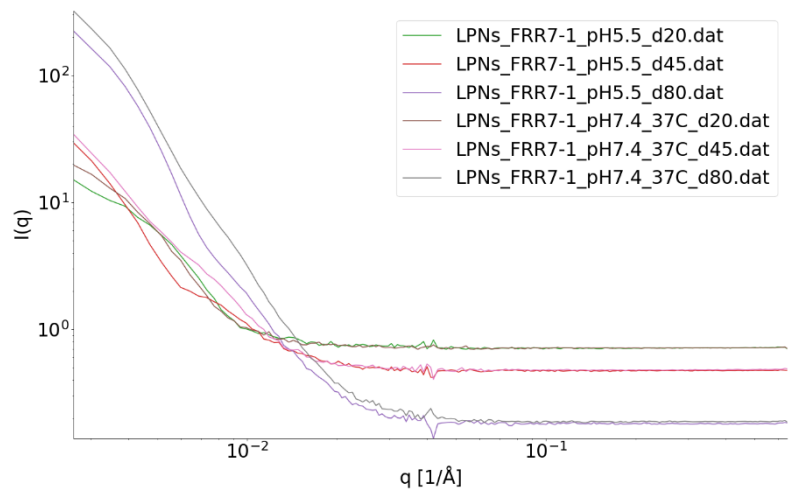


Figure 6: Example of SANS curves measured for nanoparticles at different temperature.