Experimental report

14/09/2025

Proposal: 8-02-1035 **Council:** 4/2024

Title: Unravelling LNP endosome escape

Research area: Other...

This proposal is a new proposal

Main proposer: Marite CARDENAS

Experimental team: Petteri PARKKILA
Manuchar GVARAMIA

Local contacts: Nicolo PARACINI

Samples: Lipids, mRna

Instrument	Requested days	Allocated days	From	To
FIGARO	4	0		
D17	4	2	05/06/2024	07/06/2024

Abstract:

In this proposal we aim to deepen our understanding of the endosomal escape mechanism, a crucial aspect for enhancing the therapeutic efficacy of lipid nanoparticles (LNPs) in drug delivery, exemplified by the success of formulations like the Comirnaty and mRNA-1273 Covid-19 vaccines. An optical microscopy-based assay developed by the Fredrik Höök group, allow us to observe individual LNP binding events to a supported lipid bilayer (ncSLB) mimicking the endosomal membrane. Neutron reflection experiment conducted at ISIS, involving LNPs formulated with deuterated cationic lipid, provided compelling evidence of cationic lipid (CIL) fusion and molecule translocation across the ncSLB. However, uncertainties arise regarding lipid exchange and specific contributions of components. In this proposal we aim to address these uncertainties, to discern the influence of lipid exchange on LNP fusion and determine the structural changes in the ncSLB. Neutron Reflectivity study is expected to provide valuable insights into LNP behavior on a model endosome membrane, with potential implications for drug delivery applications

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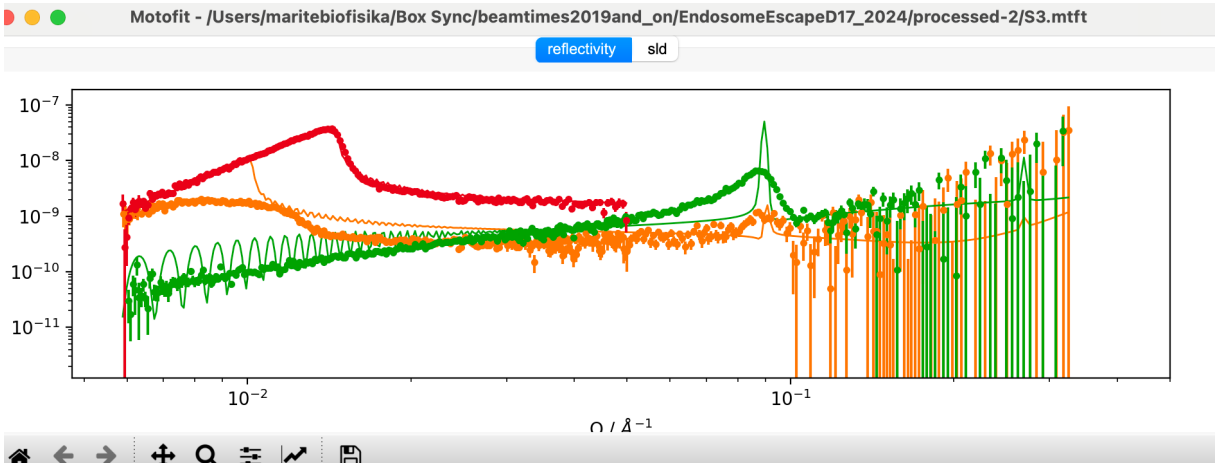
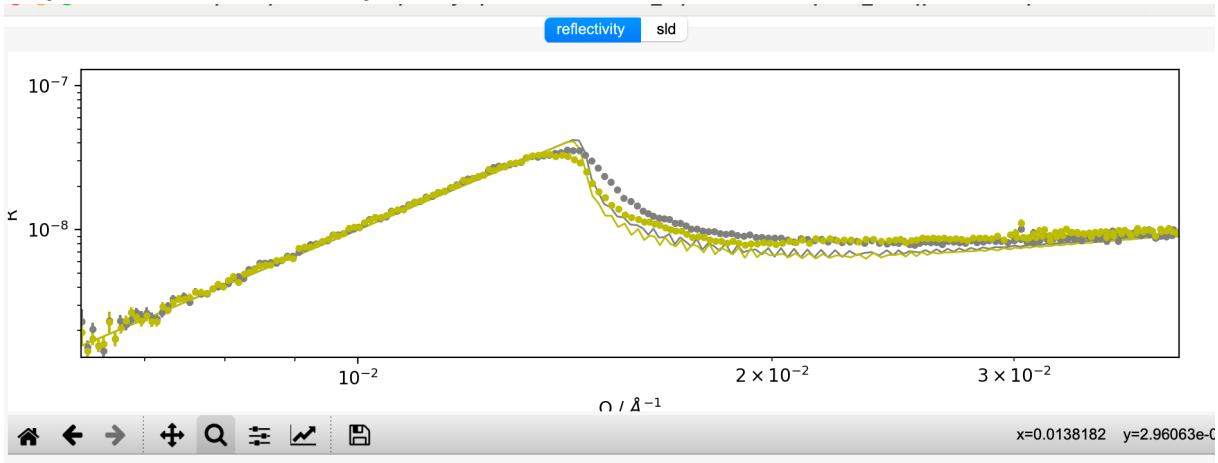
Experimental team: Petteri Parkkila and Manuchar Gvaravia
Local scientist: Ben Humphrey

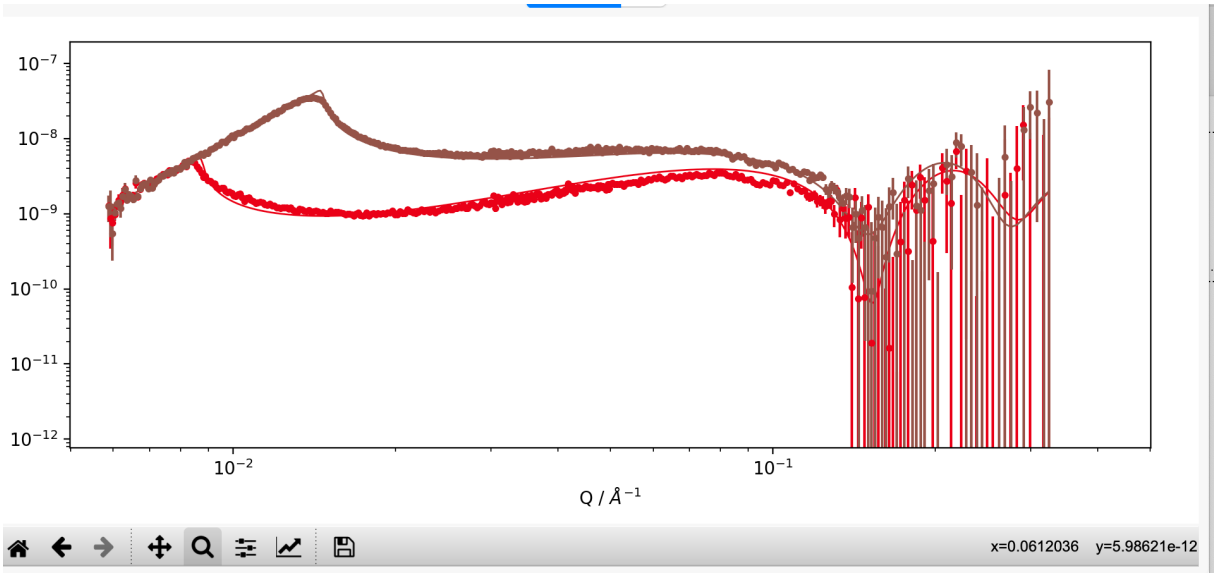
There were several problems during this experiment, data was collected in the wrong resolution to start and then the solvent exchange in the vertical configuration of the D17 reflectometer was very poor which caused a lot of problems. Here you can see an example, where the D2O measurement presents incomplete exchange, likely also a present of air inside the cell.



The poor solvent exchange implied that very little LNP adsorbed, less than 1% making very difficult to monitor any fusion and translocation event.



The data collected upon acidification did not have the correct solvent exchange either, see below. But based on the modeling of this data we could lipids been removed rather than exchanged. It could be due to the fact that we were forced to use very harsh rinsing 10 mL/min in order to get proper exchange.



In summary, the experiment was successful at showing that the SLB could be formed but we were not able to see any significant fusion or translocation due to experimental problems during the beamtime mainly due to the vertical configuration of the instrument and poor solvent exchange.