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Neutron Scattering Strategies for Investigating Internal Architecture of mRNA Lipid Nanoparticle

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Extended Abstract

Rationale. Since the onset of the COVID-19 pandemic in 2019, mRNA vaccines have been in the global research spotlight [1], expanding into diverse therapeutic applications for diseases, including cancers and monogenetic disorders [2]. However, lipid nanoparticle (LNP) formulations (a complex assemblage of neutral helper lipid, cholesterol, polyethylene glycol (PEG)-lipid, and ionizable lipid) are designed primarily for mRNA vaccines. LNP formulations for specific therapeutic mRNA applications might require systematic reconfiguration to impart tailored properties suitable for their intended use. This entails an in-depth understanding of their complex self-assembly mechanisms and nanoarchitectures, challenging with conventional imaging methods alone. To address this, our study employs small-angle neutron scattering approach with selective deuteration and contrast variation strategies[3], offering detailed insights into LNP structures and compositional distributions.

Methodology. In the current work, LNPs based on the ionisable lipid DLin-KC2-DMA, cholesterol, DSPC and DMG-PEG2000 were fabricated with mRNA payload coding for the enhanced green-fluorescent protein. Selectively deuterated LNPs were also fabricated using deuterated versions of cholesterol and DSPC. Using different deuterium oxide (D_2O)–water mixtures (0, 25, 50, 75, and 100% D_2O), series of systematically neutron contrast-varied dispersions were prepared and measured using the Bilby small-angle neutron scattering (SANS) beamline at ACNS, ANSTO. The combination of selective deuteration and contrast variation allowed us to match out individual components at different contrast match points, providing neutron scattering data with different "filters", thereby enabling us to deduce the internal particle architecture and distribution of LNP components.

Results. Through systematic model fitting, employing both sphere and core–shell models, we successfully estimated average apparent scattering length densities (SLDs) of the particles. LNPs exhibit a core–shell structure, characterised by complex mixed phases of water, mRNA, and lipid components distributed within both the shell and the core. Presence of a significant volume of water (45%–60%) was detected within the LNP matrix. Notably, our investigation revealed the dynamic water exchange between the surrounding environment and the encapsulated water within the LNPs. Combining model fitting with Invariant analysis and cryo-TEM, indicate that mRNA molecules predominantly occupy the shell,

coexisting with water and the ionizable lipid, encapsulating a cholesterol-rich core. Acidification of dispersing medium to pH 4 was shown to significantly increase the size of LNPs, signifying a destabilising impact on these particles. This observation aligns with the previously documented pH-dependent mRNA release mechanism of LNPs, emphasising the role of endosomal escape within cells.

Conclusions and Future Work. Taken together, this research unveils various structural features of a model mRNA-loaded LNPs. An in-depth understanding of LNP internal structures serves as a roadmap for revolutionizing mRNA nanomaterials, expanding therapeutic approaches, and broadening their utility to address various prevalent diseases and conditions. Further studies are warranted to validate the transferability of SANS and selective deuteration strategies to other LNP systems, incorporating the influence of additional variables (lipid components, mRNA concentration, and fabrication process parameters) on the internal structure of LNPs.

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