Sponge phase lipid nanoparticles interacting with biomimetic membranes: lipid exchange and delivery of proteins

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The development of lipid nanoparticles (LNPs) for the delivery of drugs and other functional components requires knowledge of how they interact with e.g. cell membranes as well as the mode of interaction and uptake of the compounds loaded within the LNPs. We have used neutron reflectometry and electrochemical impedance spectroscopy to reveal the interaction of lipid sponge phase nanoparticles (L_3 NPs) with model membranes composed of POPC lipid (1-palmitoyl-2-oleoyl- *sn* -glycero-3-phosphocholine) on silica substrate surfaces. The sponge phases are bicontinuous with curved flexible bilayer structures, with tuneable lipid composition, that are organised in such a way that they form relatively large (>10nm) aqueous channels [1,2]. Similar structures can also be found in cell organelles. We have previously shown that we can encapsulate myoglobin in lipid sponge phases [3]. The structural features of L_3 NPs, based on mixtures of acyl glycerides and phospholipids and their interfacial behaviour at model lipid biomembranes was explored by using scattering, microscopy, and surface-sensitive techniques [4].

Here we will discuss how neutron reflectometry and different isotopic contrast, i.e. H_2O and D_2O buffers and deuterated lipids can be used to separate between the different components, i.e. lipids and protein, to reveal whether lipid exchange and/or protein delivery occurs. Both lipids and protein cargo (myoglobin) from the L_3 NPs end up in the model POPC biomembrane. We observed that lipid exchange occurs, but the overall integrity of the POPC bilayer is preserved. This is confirmed by electrochemical impedance spectroscopy measurements, which suggest that the L_3 NPs do not induce substantial leakage of the model biomembrane. For the L_3 NPs with myoglobin, the proteins appear to end up in the upper leaflet of the bilayer. These findings provide valuable insights for optimizing protein encapsulation and controlling the release mechanisms of sponge phase delivery systems. Furthermore, we show that L_3 NPs can penetrate living cells without detrimental effect on cell viability, as shown by preliminary studies using A549 lung cancer cells.

References:

[1] M. Valldeperas, A. P. Dabkowska, G. K. Pálsson, S. Rogers, N. Mahmoudi, A. Carnerup, J. Barauskas. T. Nylander *Soft Matter* , **2019** 15, 2178-2189, DOI: 10.1039/C8SM02634C

[2] M. R. Machingauta, A. McEvoy, A. Karlsson, J. Barauskas, T. Nylander, J. Gilbert *Front. Soft Matter* **2025** , 5, 1708264, DOI: 10.3389/frsfm.2025.1708264

[3] J. Gilbert, S. Christensen, T. Nylander, L. Bulow *Front. Soft. Matter* **2023** , 3, 1201561. DOI: 10.3389/frsfm.2023.1201561

[4] M. R. Machingauta, T. Kristersdotter, A. Luchini, I. De Santis, T. Naw Sut, J. Jackman, B. Humphreys, C. Prinz, D. Berti, K. Schillen, T. Nylander *Biophys. J.* **2026** , 125, 235a-236a.