Experimental report

23/05/2024

Proposal: **CRG-3046**

Council: 4/2023

Title: Coexisting planar and curved modelmembranes

Research area: Soft condensed matter

This proposal is a resubmission of 9-13-1086

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Samples: Deuterated POPC
SiO2 NP coated Si block
C18:1:cardiolipin

Instrument	Requested days	Allocated days	From	To
SUPERADAM	5	5	08/04/2024	10/04/2024

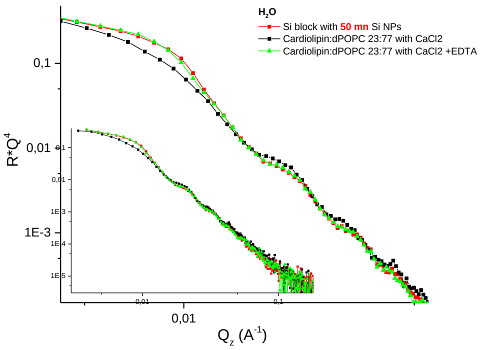
Abstract:

The vast majority of studies published in the literature regarding the curvature of biological membranes and curvature-related phenomena are based on fluorescence microscopy techniques which, in the best cases, are limited to a spatial resolution of hundreds of nanometres and require the use of lipids chemically modified with fluorophores to enable their detection. To increase the resolution down to sub nanometre distances, real-space techniques such as microscopy must be complemented with reciprocal-space methods such as neutron scattering. However, there are currently very few model membranes amenable to be used in the study of curvature-related lipid sortingusing scattering techniques. Our goal is to tackle this open challenge by creating a supported lipid bilayer on top of an ordered array of
hexagonally packed silica nanospheres and use them to study lipid demixing as a function of curvature. We have shown that we can make such samples by microscopy and neutron reflection and now we plan to study lipid demixing at different curvature using pi-gisans

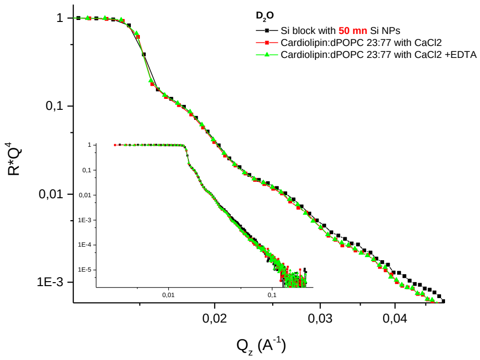
Report

Proposal CRG3046 Super Adam ILL

The supported lipid bilayer (SLB) was formed on 50nm SiO_2 NPs scaffolds with tail deuterated POPC and CL using the solvent depletion method in the presence of 1 mM $CaCl_2$. To do that CL- containing vesicles (23:77 mol% CL:tail deuterated POPC) in isopropanol produced by tip sonication and injected to solid-liquid cell. The cell was then rinsed with slow-flowing Milli-Q water and then Calcium chloride solution to form the SLB. The formed SLB was measured before and after rinsing with EDTA, which removes of calcium brings and leads to the packing parameter of CL closet to 1. Which in turn leads to decrease curvature induced demixing. EDTA leads to significant changes of NR as clear seen from Fig.1. These changes can be seen most clearly in the H_2O buffer (Fig.1a)



(a)



(b)

Fig. 1. Specular NR for NP surface scaffolds (red), SLB with CaCl2 (black) and after rinse with EDTA (green) in H2O (left) and D2O (right).

The data were analyzed using refnx python code with a mathematical model of the interface describing a silicon substrate covered with a layer of silicon oxide on top of which a monolayer of spheres was built from stacked thin slices, each one characterized by a thickness, SLD and roughness parameter. This model accounts for hexagonally packed spheres enclosed in a regular hexagon and a ‘sphere coverage’ parameter defined the total volume occupied by the prisms enclosing the spheres whilst the remaining volume fraction in between prisms was filled with solvent. The model accounts for the contribution of the lipid bilayer coating the spheres and a planar lipid bilayer adsorbed on the underlying SiO2 substrate. The analysis revealed a change in the composition of SLB both on the curve and on the flat part in the presence of Ca+ compared to SLB after washing with EDTA Fig 2. The clear changes in the SLD profile for both cases are shown at Fig.2 c,d.

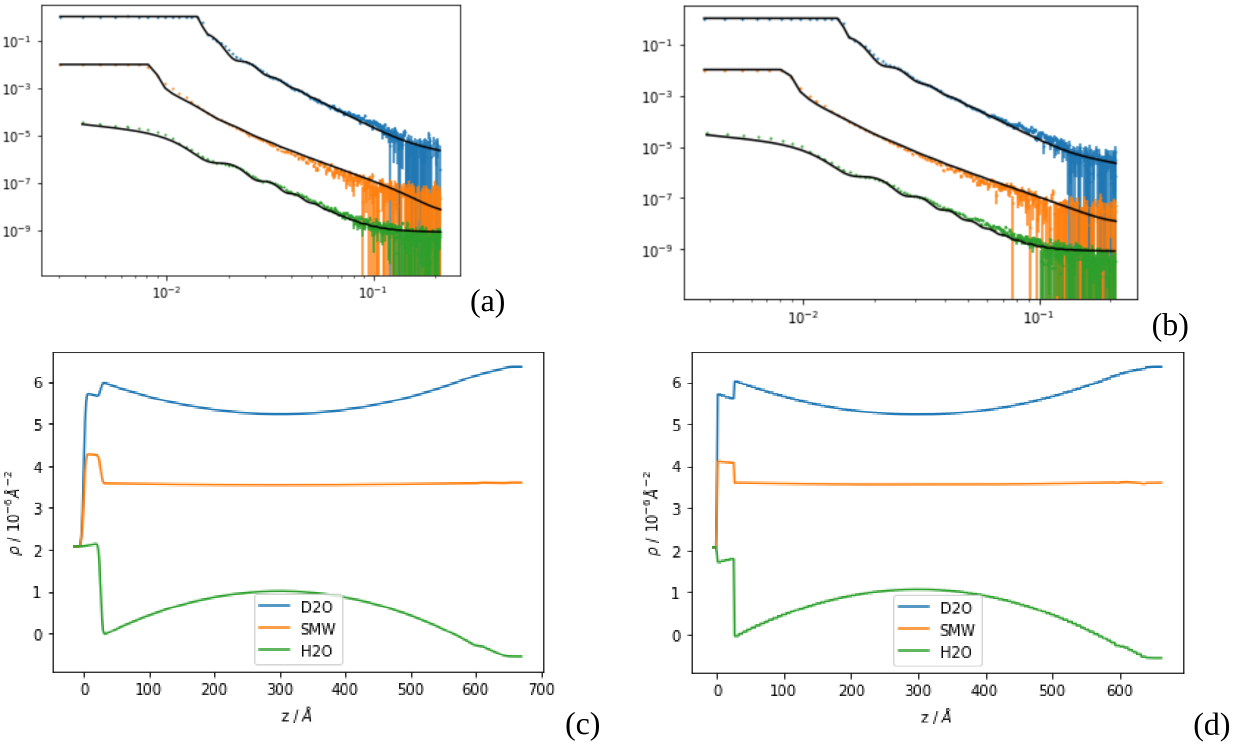


Fig.2. Specular NR for SLB (a) and SLB profile with CaCl2 (c) (left) and NR (b) and corresponding SLB profile (d) after rinse with EDTA (right) in H2O (green), D2O (blue) and SiO2MW (orange).