Experimental report

15/02/2021

Proposal: 8-02-889 **Council:** 10/2019

Title: Curvature-induced lipid segregation studied by grazing incidence neutron scattering on nanoparticle-supported lipid bilayers (NP-SLB)

Research area: Biology

This proposal is a new proposal

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Samples: d54DMPC
Cardiolipin
Silica nanoparticles

Instrument	Requested days	Allocated days	From	To
FIGARO	0	3	18/09/2020	21/09/2020
SUPERADAM	5	0		

Abstract:

Biological membrane in plane organisation is a challenging topic, specially since it has been suggested to depend on curvature. So far the main approach to study curvature-induced in plane phase separation has been through the approach of fluorescent techniques and liposomes. Here we propose to use grazing incidence neutron scattering to explore the signal from a well ordered nanoparticle coated surface and use it as a solid substrate to form curved supported lipid bilayers by vesicle fusion. The curvature of the membranes will be tuned by the size of the nanoparticles in the surface coating and specular and off-specular reflectivity will be used to probe the in-in plane lipid organisation and the SLD distribution normal to the surface.

Report for experiment 8-02-889 performed on 18-21 Sept 2020 on FIGARO

AIM: The aim of the first part of this experiment was to measure the specular and off-specular reflectivity from samples made of a silicon wafer functionalised with a monolayer of hexagonally packed, spherical silica nanoparticles (SiNP) at the solid liquid interface. Three different samples were prepared functionalized with SiNP of 50, 100 and 200 nm in diameter (Ø).

After the characterization of the substrates, lipids (hPOPC) were deposited via vesicle fusion onto the SiNP monolayers and again characterized by specular and off-specular NR. In situ removal of the lipids was performed with ethanol and a second type of lipids (dPOPC) deposited and characterized.

Lastly the substrates were cleaned once again with ethanol and a mixture of hydrogenous and deuterated lipids was deposited on the surface.

RESULTS:

- Specular reflectivity: From the preliminary analysis of the specular reflectivity profiles the 100 and 200 nm Ø SiNP monolayers were found to have a SiNP coverage of around ~70% of the available area. SiNP of 50 nm Ø yielded a lower coverage of around ~50% (**Figure 1**). Addition of hPOPC to the 200 nm Ø SiNP sample resulted in the formation of a full coverage lipid bilayer around the particles as well as a lower-coverage (~50%) bilayer on the flat surface of the silicon wafer embedding the lower part of the spheres (**Figure 2**). In the case of the 100 nm Ø SINP, addition of lipids also resulted in the full coverage of the particles with a bilayer but a much lower coverage of the flat bilayer on the silicon wafer (~10%) (**Figure 3**). Analysis of the remaining samples compositions is in progress
- Off-Specular reflectivity: All samples gave a strong off-specular signal. The preliminary simulations of the off-specular signal from the 200 nm Ø SiNP, both before (**Figure 4A**) and after the addition of the lipids (**Figure 4B**) show promising correspondence between the measured signal and the calculated one.

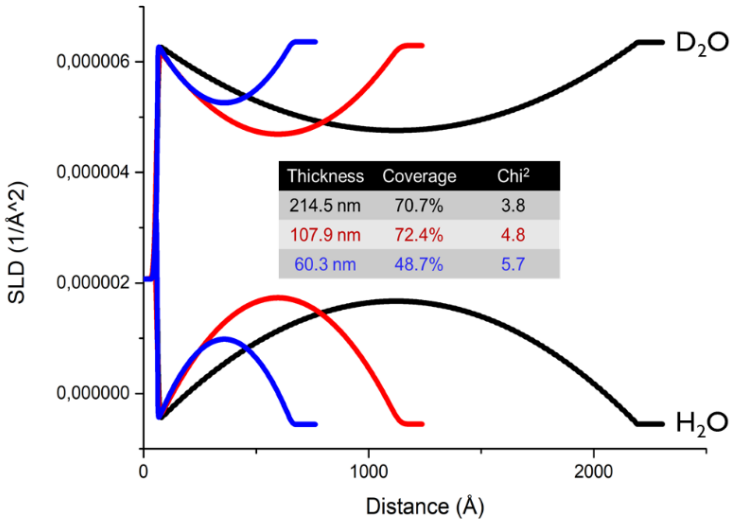

Figure 1 SLD profiles of the SiNP monolayers obtained from the fit to the specular reflectivity data

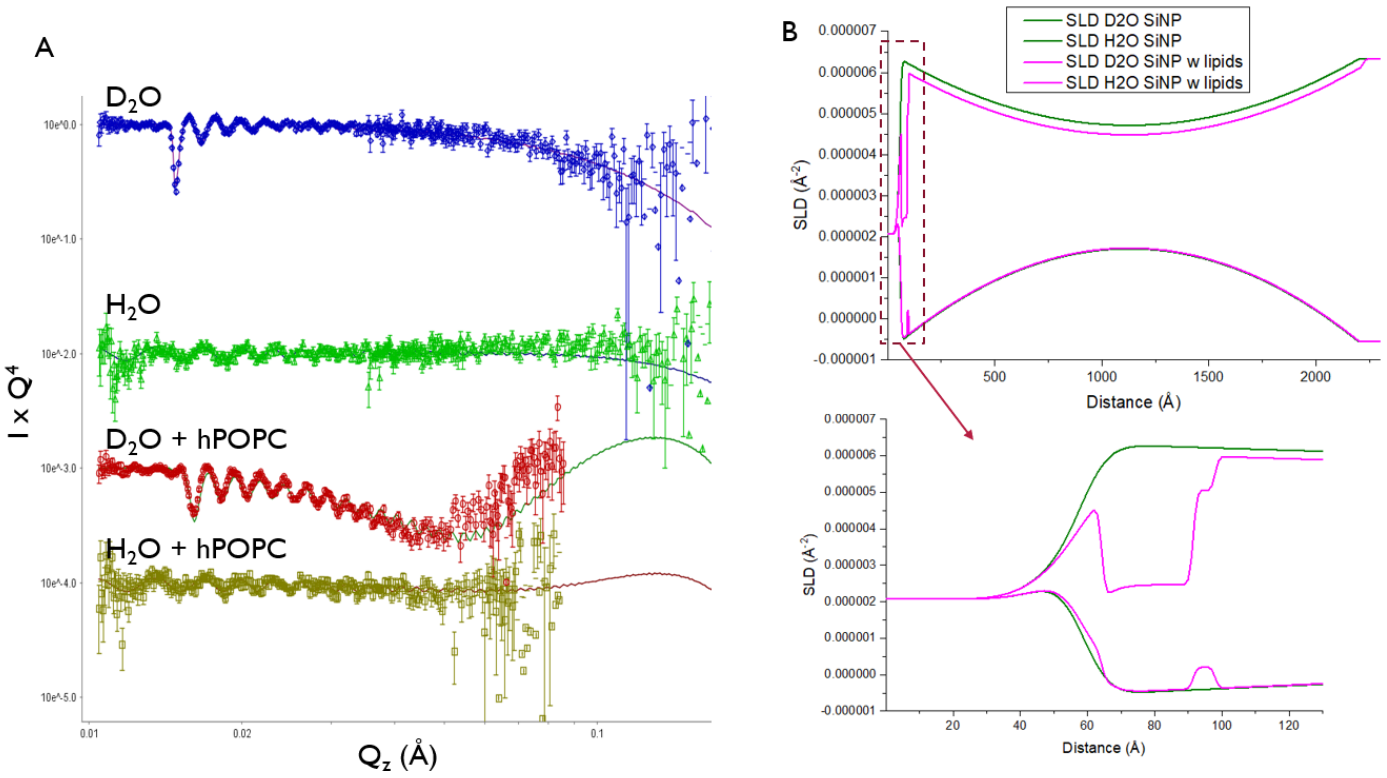

Figure 2 Specular reflectivity analysis of 200 nm Ø SiNP before and after coating with hPOPC. (**A**) Reflectivity data points and corresponding fit lines measured in H_2O and D_2O before and after the addition of the lipids to the 200 nm Ø SiNP. (**B**) SLD profiles relative to the constrained fits shown in **A**.

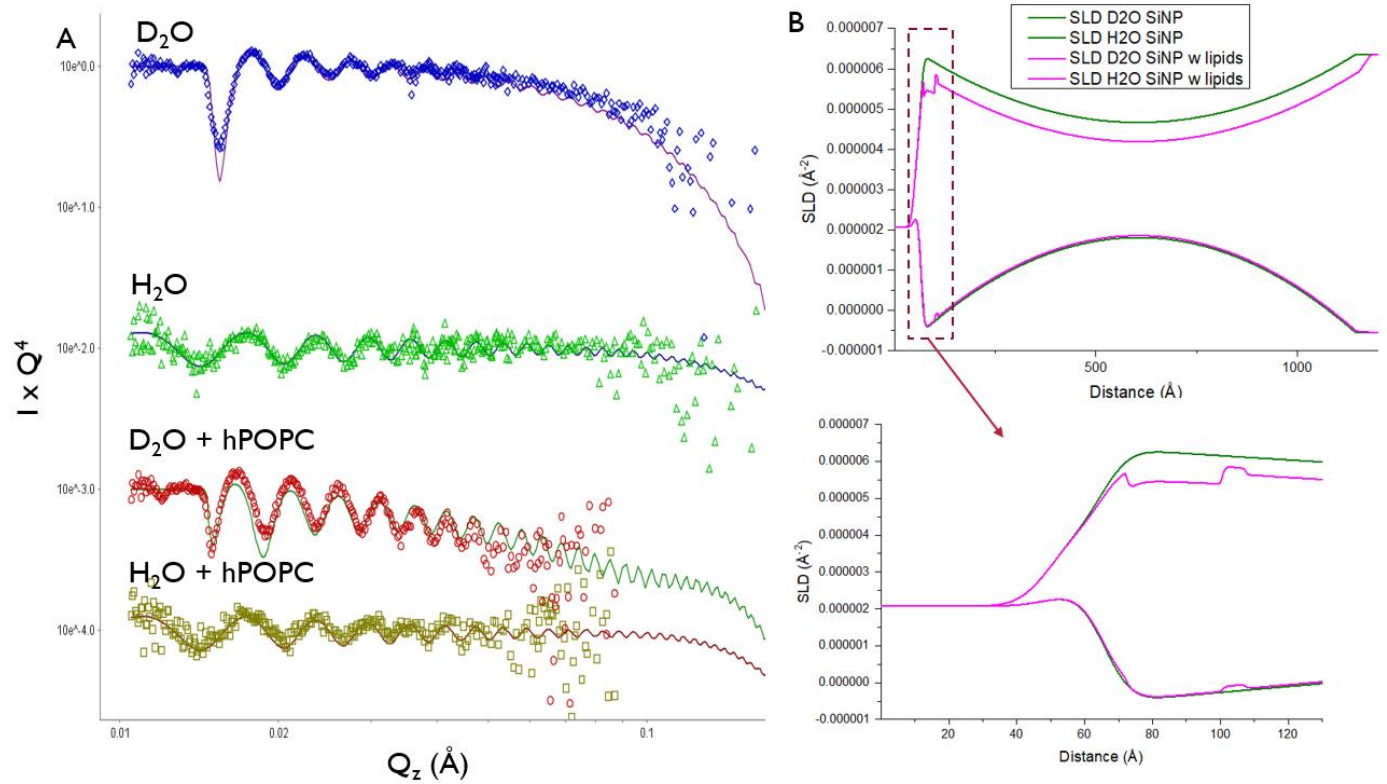

Figure 3 Specular reflectivity analysis of 100 nm Ø SiNP before and after coating with hPOPC. (**A**) Reflectivity data points and corresponding fit lines measured in H_2O and D_2O before and after the addition of the lipids to the 100 nm Ø SiNP. (**B**) SLD profiles relative to the constrained fits shown in **A**.

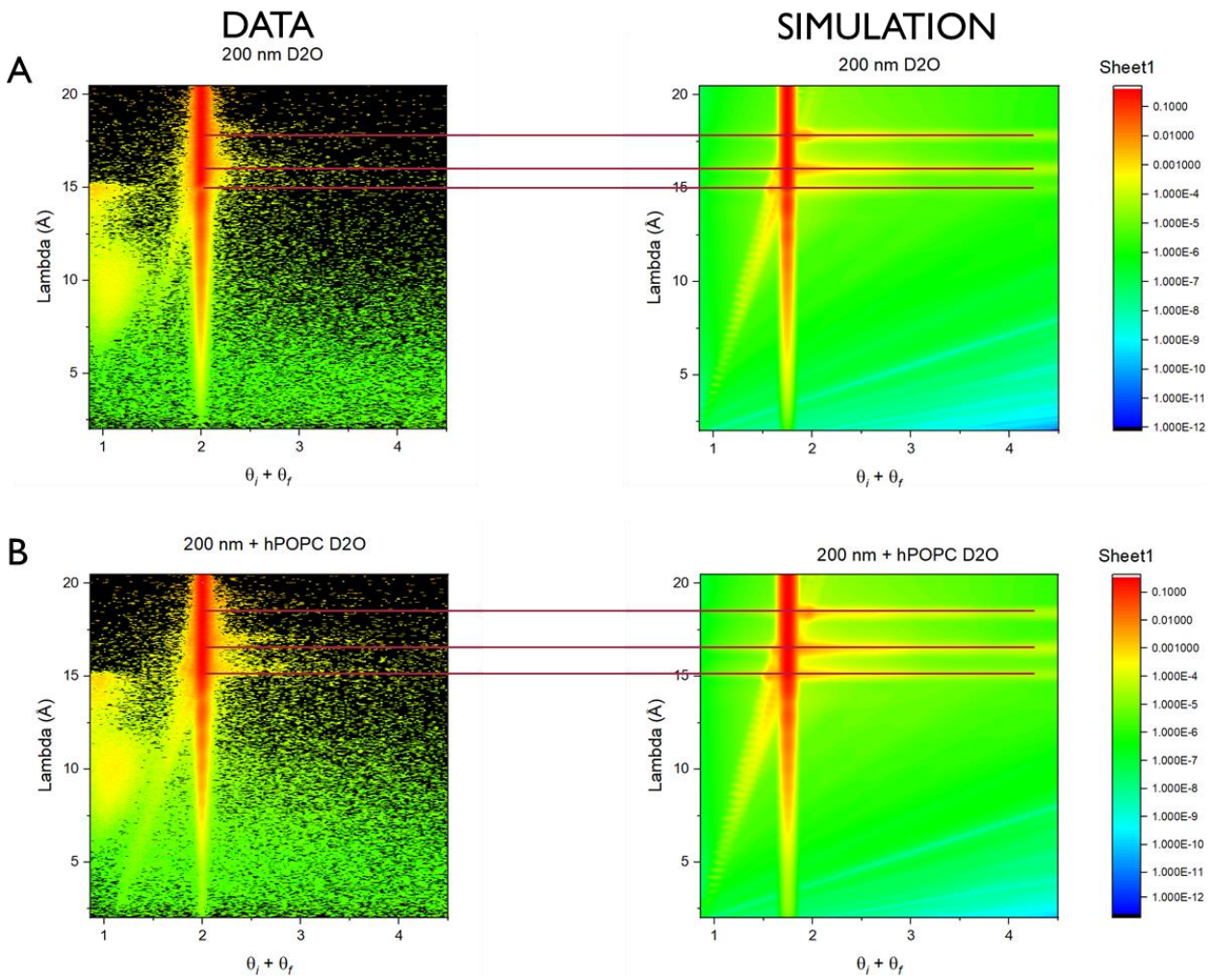

Figure 4 Off-specular reflectivity analysis of 200 nm Ø SiNP (**A**) before and (**B**) after coating with hPOPC