Report for Experiment SC – 4031

TITLE:

From mesoscale to nanoscale: structure and morphology of biomimetic membranes during phase transition investigated by in-situ AFM and Surface X-ray Scattering

EXPERIMENTAL SESSION: ID13 – 09 April 2015/13 April 2015

Our aim is to study the phase transitions of model lipid membranes induced by temperature changes. The experiment aims to integrate the structural data collected by Surface X-Ray Scattering and X-Ray Reflectivity (XRR) with morphological and mechanical information measured by *in-situ* Atomic Force Microscopy (AFM). The proposal was focused on DPPC:DLPC and DOPC:DPPC. However, for sample preparation issues, we decided to study solely DPPC:DLPC (1:1), pure DOPC and pure DPPC. Despite our efforts, we have not been able to characterize the membranes by Surface Scattering due to the poor S/N ration. We think this was due to the thick quantity of buffer hydrating the membranes, despite our decision to work at 22 keV. This is a high energy compared to previous studies [1], but it allows the beam to pass through the liquid without suffering of absorbtion. We have however successfully characterized the membranes both by XRR and AFM. The AFM images here reported are the first ones ever acquired during an X-Ray beamtime on hydrated samples (AFM measurement under liquid conditions). Finally, evidence of radiation damage brought us to develop strategies to use the AFM to investigate the effects on the membranes. A set of attenuators was employed to minimize the radiation damage during the acquisition of the XRR data. The beam size was 300 μm x 30 μm (horizontal x vertical). The flux at the sample position was $2x10^{13}$ photons/second. At first, we have successfully tested the capabilities of our custom-made Atomic Force Microscope at ID03 (Figure 1).

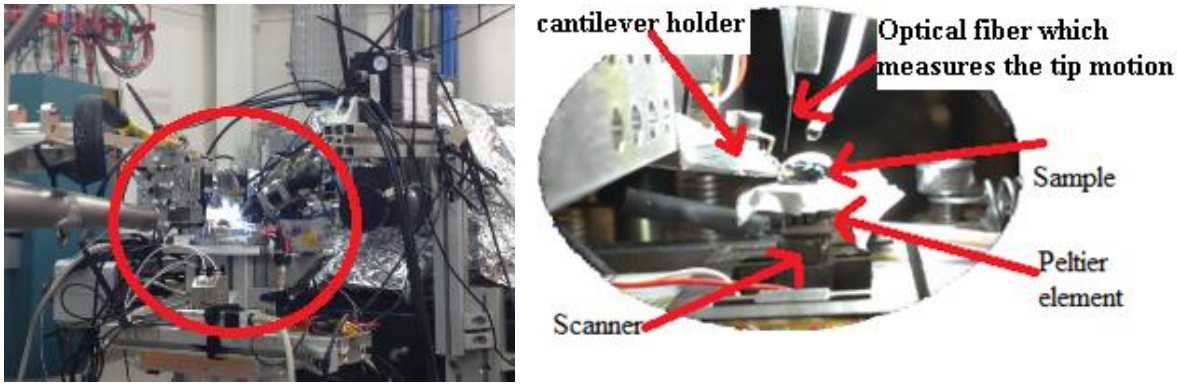

Figure 1: Left) AFM mounted at ID03. Right: zoom on the central part of the AFM: the sample holder on the top of the scanner, including the Peltier element. The cantilever holder and the fiber holder are visible on the top.

A typical experimental session included the acquisition of XRR and AFM data on the membranes as shown in Figure 2 (experiment on DPPC). After acquiring a topographical image of an interesting area (Figure 2a), the mechanical stability of the membranes (Figure 2b) was assessed by extracting the maximum force necessary to break the bilayers (20 nN *ca.*), which is a characteristic parameter for each bilayer with determined environmental conditions [2]. In the same area, the associated X-Ray Reflectivity was acquired, giving information about the thickness of the membranes (Figure 2c). The alignment between the beam and the AFM tip /cantilever was obtained by measuring the electrons flowing in the cantilever once the X-Ray beam was incident on it (Figure 2d).

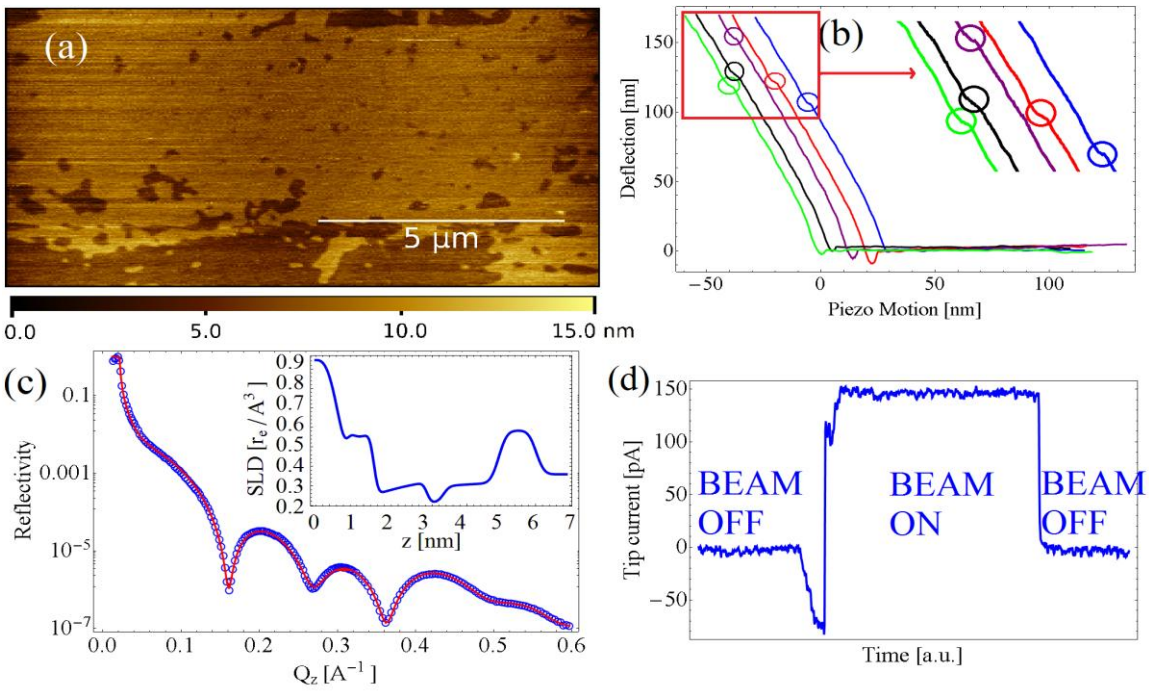

Figure 2: a) AFM image of DPPC membranes deposited onto Silicon under physiological conditions. b) Several Force-Spectroscopy curves acquired with the AFM showing the deflection of the cantilever as a function of the sample displacement (Piezo motion): we observe the rupture of the membrane. The curves have been shifted of 6 nm in the X-Axes for better clarity. c) Associated X-Ray Reflectivity from which we evaluate the bilayer thickness of 5.5 nm. Experimental data (blue circles) and best fit (continuous red line). Inset: Scattering Length Density profile evaluated from the best fit of the Reflectivity data. d) Current flowing in the cantilever once aligned with the X-Ray beam.

Phase transition study:

A typical experiment performed on DPPC membrane is reported in Figure 3.

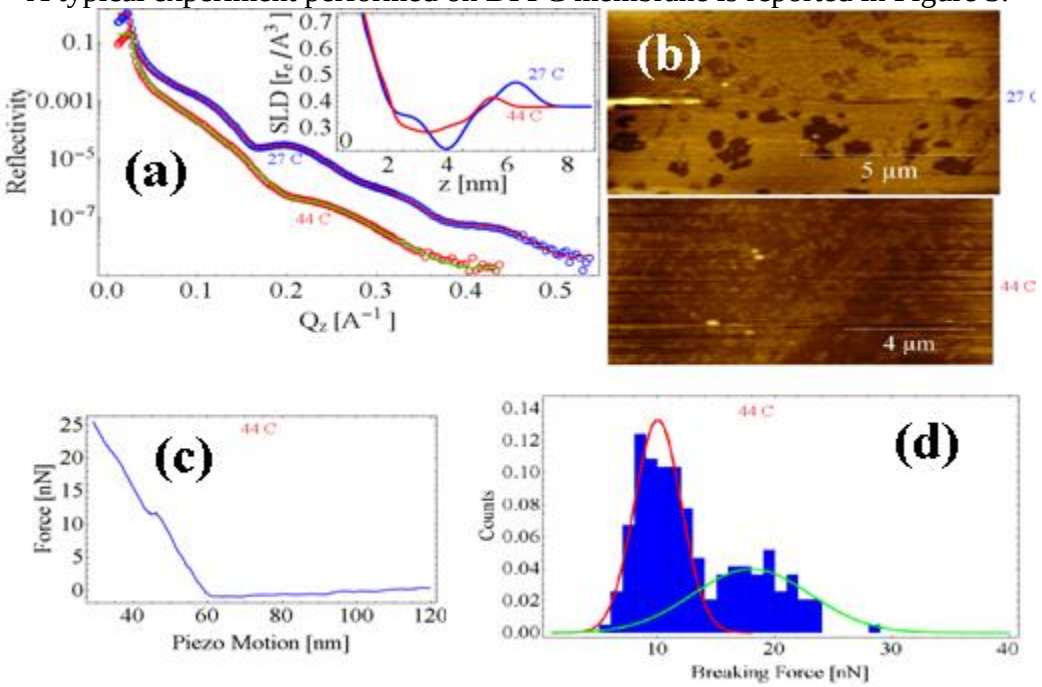

Figure 3: a) Reflectivity curves on DPPC bilayers. Blue and red: experimental data and best fit at 27 degrees. Red and green (shifted for better clarity): experimental data and best fit at 44 degrees. Inset: SLD profiles at 27 degrees (blue) and 44 degrees (red). b) AFM images at 27 degrees and 44 degrees. At room temperature the membranes are in gel phase. The observed holes are the Silicon substrate. At 44 degrees we observe the coexistence of gel and liquid phases and no substrate is visible. c) Example of indentation curve showing a membrane breaking force of 10 nN. d) Bimodal distribution of the breaking force of the membrane at 44 degrees. Liquid phase lipids show a lower breaking force of 10 nN (red fit), whereas gel lipids show a higher breaking force of 19 nN (green fit).

At 44 degrees XRR is showing a decrease of the membrane thickness, whereas the AFM image shows an increase of the substrate coverage and the coexistence of the two lipid phases. The two phases show different mechanical properties as shown in Figure 3d. Clearly, data show a loss in membrane thickness and an inclination of the lipids in the liquid phase which results in a mechanical stability with lower breaking forces compared to the gel phase. We are currently trying to understand the correlation between the SLD profiles and the mechanical properties of the membranes.

DPPC:DLPC (1:1)
The same data were acquired on DPPC:DLPC (1:1) model membranes.
A dataset is reported in Figure 4.

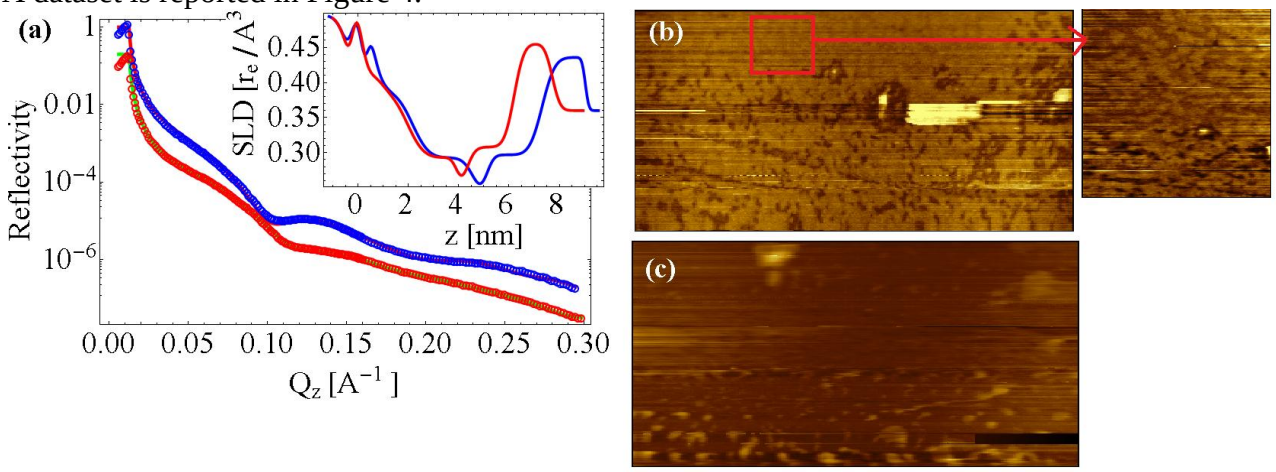

Figure 4: a) Reflectivity curves on DPPC:DLPC (1:1) membranes. Blue and red: experimental data and best fit at 27 degrees. Red and green (shifted for better clarity): experimental data and best fit at 44 degrees. Inset: SLD profiles at 27 degrees (blue) and 44 degrees (red). b) AFM image at 27 degrees. At room temperature we observe the substrate, the DPPC membrane in gel phase and the DLPC membranes in liquid phase. c) AFM image at 44 degrees: a higher coverage and a decrease of the overall membrane thickness are observed.

As observed in the case of simple DPPC, at 44 degrees XRR is showing a decrease of the membrane thickness, whereas the AFM image shows an increase of the substrate coverage. Indeed at 27 degrees there is a coexistence of DLPC in liquid phase and DPPC in gel phase (Figure 4b), whereas at 44 degrees the DPPC is mostly in liquid phase as DLPC, although some Gel parts are still present.

Radiation damage study:
After the acquisition of the first Reflectivity curves we observed the effects of the radiation damage. Figure 5 reports the morphological change observed on DPPC membranes.
In the DPPC membrane the formation of holes at the micrometric and nanometric scale is clearly visible.

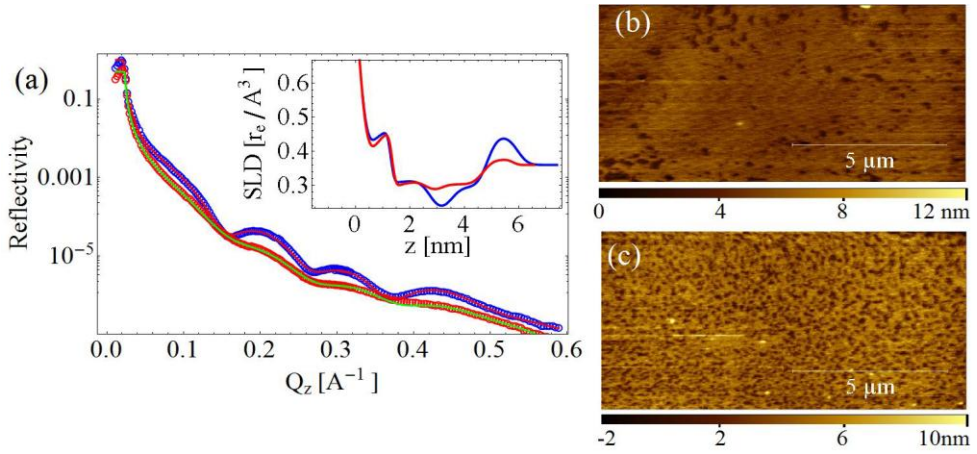

Figure 5: a) Reflectivity curves on DPPC bilayers. Blue and red: experimental data and best fit respectively for the data acquired after the AFM image shown in (b). Red and green (shifted for better clarity): experimental data and best fit respectively for the data acquired after the AFM image shown in (c). Inset: Scattering Length Density profile evaluated from the fit. Blue: data acquired after the AFM image shown in (b). Red: data acquired after the AFM image shown in (c).(b) AFM image of DPPC bilayers before being exposed to X-Rays. (c) AFM image of DPPC bilayers damaged by the X-Ray beam during the acquisition of the first Reflectivity curve. We observe the formation of holes in the membrane.

Deposition of materials was instead observed on DOPC membrane as shown in Figure 6.

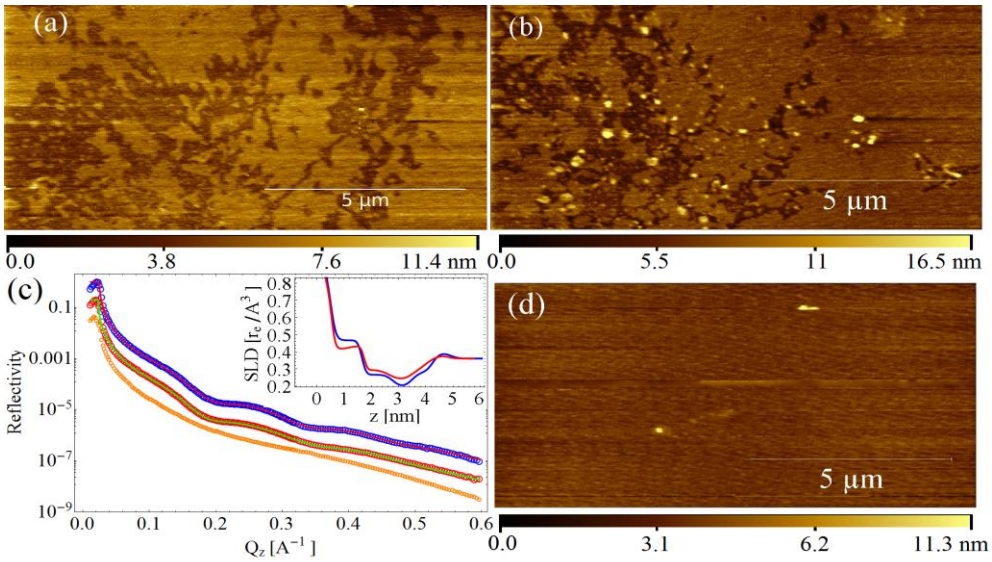

Figure 6: (a) AFM image of DOPC bilayers before being exposed to X-Rays. (b) AFM image of DOPC bilayers damaged by the X-Ray beam during the acquisition of the first Reflectivity curve which has induced the deposition of material on the top of the membranes. c) Reflectivity curves. Blue and red: experimental data and best fit respectively for the data acquired after the AFM image shown in (a). Red and green (shifted for better clarity): experimental data and best fit respectively for the data acquired after the AFM image shown in (b). Orange (shifted for better clarity): experimental data for the data acquired after exposition of 5 min full beam without attenuation at a incident angle of 0.1: the membranes have been removed by the beam and solely the substrate is observed. Inset: Scattering Length Density profile evaluated from the fit. Blue: data acquired after the AFM image shown in (a). Red: data acquired after the AFM image shown in (b). (d) AFM image acquired after exposure to full beam without attenuation. The membranes are not observed by AFM in agreement with the data shown in orange in (c).

Conclusions:

Part of the data presented in this report have been inserted in a manuscript entitled **"Custom AFM for X-Ray beamlines: in-situ biological investigations under physiological conditions"** currently accepted in *Journal of Synchrotron Radiation*.

The phase transition data have been treated and we are currently planning the setting up of a model to couple the Scattering Length Density profiles obtained with the XRR and the AFM morphological and mechanical information acquired simultaneously.

As shown, the AFM has been particularly useful to check the radiation damage on the model membrane and consequently in the setting up of the X-Ray beam attenuators during the acquisition of the XRR curves in order to minimize these effects.

Our future efforts will be devoted to study the effects of the insertion of small molecules, such as Triptophane and Melatonin, in model lipid membranes employing the strategies developed during the experiment at ID03. Since we aim to acquire X-Ray Surface Scattering data too, we will make efforts to reduce and control the amount of liquid hydrating the sample by designing a humidity chamber for our instrument.

To the best of our knowledge, the images presented in this report, together with those presented in the report of the ESRF experiment LS2339, are the first AFM images performed under liquid conditions in a synchrotron beamline.
The custom-made AFM here presented is available for any user of ID03.

References:

[1] Miller, C. E., Majewski, J., Gog, T. & Kuhl, T. L. (2005). Phys. Rev. Lett. 94, 238104.
[2] Gumì-Audenis, B., Sanz, F. & Giannotti, M. I. (2015). Soft Matter, pp.

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