ESRF	**Experiment title:** Physical and structural study of biomimetic membranes upon the insertion of Human Neutrophil Peptide 1 (HNP1): AFM, X-Ray Reflectometry and Surface Scattering	**Experiment number**: SC-4237
Beamline: ID10	**Date of experiment**: from: 19/05/2016 to: 23/05/2016	**Date of report**:
Shifts: 12	**Local contact(s)**: Federico Zontone	*Received at ESRF:*

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Report:

The purpose of this study was to investigate the effect of the human defensin HNP1(fig. 1) on Supported Lipid Bilayers (SLBs) of different phospholipid composition, in order to mimic mammalian and bacterial membranes. The experiment aimed to integrate the structural data collected by X-Ray Reflectometry (XRR) and Surface X-ray Scattering with morphological and mechanical information measured at the nanoscale by in-situ AFM.

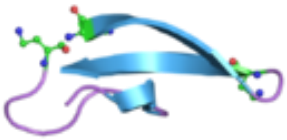

Fig. 1. Structure of HNP1 peptide

During the experiment, we succeeded to work with almost all the phospholipid compositions we wanted to characterize, with exception of few of them that were not giving enough signal most probably due to sample preparation issues. However, we could get XRR data for most of the bilayers before and after the insertion of the HNP1 peptide.

Concerning the custom X-AFM, we installed it in the diffractometer of ID10 (fig. 2) for almost an entire day. However, due to external vibrations and some technical problems we were not able to get a high quality mechanical and morphological information of our samples.

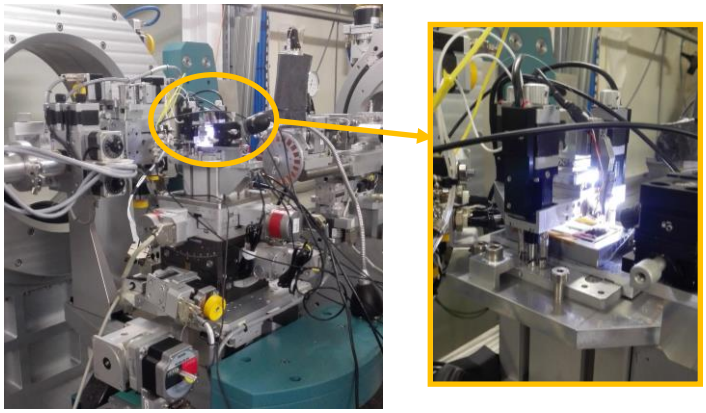

Fig. 2. X-AFM mounted on the diffractometer of ID10

No radiation damage observed:

The experiment was pretended to be performed at 24keV. We know that at this energy the phospholipid membranes suffer from radiation damage even after the first XRR scan[1], effect that we already observed in the experiment SC-4031 at ID03 (see also the correspoding report). However, thanks to the suggestion of the beamline scientist of ID10, we ended up working at 30keV with a beam size at the sample position of 10 µm x 250 µm (vertical x horizontal). We found out that at such high energy, no radiation damage on the phospholipid membranes was observed from the XRR curves. As we can see in the graph (fig. 3), we have been able to acquire three XRR dataset in the very same sample regions without observing important radiation damage effects. At our knowledge, this is the first time that SLBs are studied at such high X-ray energy and our results are very promising because of the reduced radiation damage.

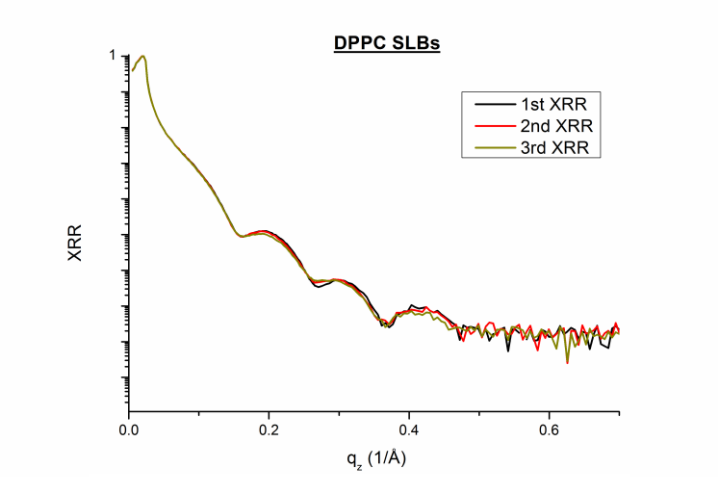

Fig. 3. XRR of DPPC SLBs showing no radiation damage

Influence of HNP1 peptide into phospholipid membranes:

We succeeded on studying the effect of the human defensin HNP1 on various phospholipid SLBs of different composition, such as DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), DPPE (1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine) and DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine).

Although an accurate data treatment and data analysis and interpretation are still ongoing, we can show preliminary results. We have been able to observe variations in the Reflectivity curves as soon as the peptide is inserted into the phospholipid membrane (fig. 4): changes in the oscillation periods suggest a modification of the bilayer thickness, while different amplitude in the oscillations suggests changes in the membrane roughness. We are currently trying to understand how the HNP1 peptide affects to the different membranes by studying the scattering electron density profiles obtained from the data.

It is worth to say that all the experiments were done in-situ, meaning that the XRR curve for the pure phospholipid membrane was acquired in the very same sample region of the XRR curve acquired after the HNP1 peptide incubation.

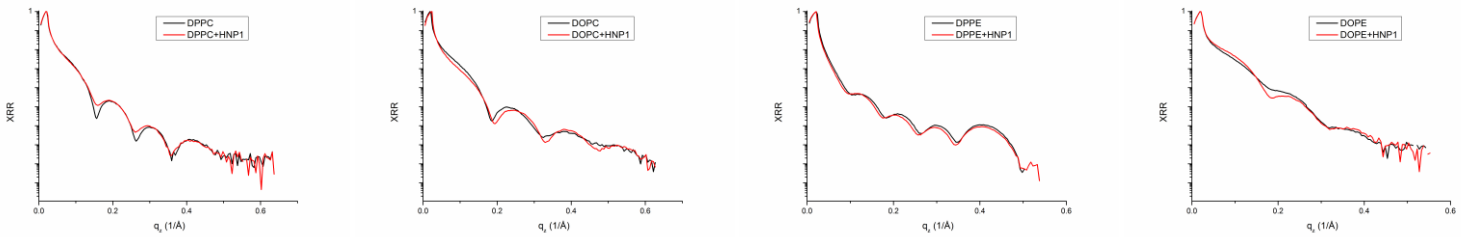

Fig. 4. XRR curves for pure phospholipid membrane (black) and phospholipid membrane with the peptide incubated (red). [From left to right: DPPC, DOPC, DPPE and DOPE]

Conclusions:

Thanks to the beamline scientist suggestions, we show that from the XRR measurements there is no radiation damage detected on the phospholipid bilayers under physiological conditions when working at 30keV.

We have studied the influence of the human defensin HNP1 into supported bilayers containing different phospholipids, observing that the peptide affects the membrane thickness or the roughness of the bilayer. However, we have still to finish the data treatment to better understand the changes produced by the human defensin into the membranes and be able to complement the resultant information with experiments performed with other techniques.

[1] B. Gumi-Audenis et al., *Journal of Synchrotron Radiation* **2015**, 22, 1364.