ESRF	**Experiment title:** Structural and mechanical study of model phospholipid membranes upon the insertion of Melatonin, Serotonin and Cholesterol combining X-Rays Reflectometry and in situ AFM data	**Experiment number**: 25-02-871
Beamline: BM25	**Date of experiment**: from: 13/07/2016 to: 16/07/2016	**Date of report**:
Shifts: 9	**Local contact(s)**: Maria Vila	*Received at ESRF:*

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Report:

The purpose of this study was to investigate the structural change of Supported Lipid Bilayers (SLBs) upon the insertion of Melatonin (Mel), Serotonin (Ser) and Cholesterol (Chol) molecules (Fig.1). The experiment aims to integrate the structural data collected by X-Ray Reflectometry (XRR) with morphological and mechanical information measured at the nanoscale by *in-situ* Atomic Force Microscope (AFM)[1]. However, since we got less shifts than the ones asked and having a busy experimental planning, we have decided not to integrate the AFM during the beam-time and focuse more on the X-Rays part, knowing that the mounting of the X-AFM in the beamline would last at least half a day.

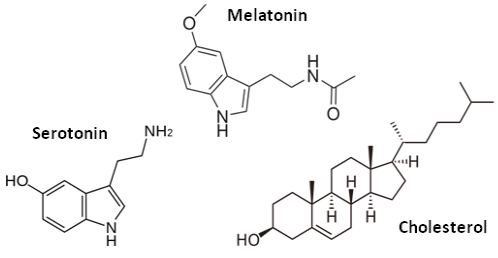

Figure 1. Chemical structures of Mel, Ser and Chol.

Following *ex-situ* experiments previously performed, we first focused on cholesterol, Tryptophan (Trp), which is the precursor molecule of Mel and Ser, and melatonin as the chosen molecules.

Concerning the phospholipid bilayers, we focused our study on DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) membranes. We also tried to

perform some measurements with other phopholipid membranes with no success, most probably due to sample preparation issues.

We expected an effect of Trp and Mel we wanted to study onto different SLBs to be quite small. We found out that the beamline was not giving enough resolution for us to observe the results we expected, so we had to adapt and change our acquisition parameters due to beam alignment and size, as well as due to some unexpected issues in the X-Rays end-station.

Cholesterol influence on DPPC SLBs:

In the experiment, we succeded in performing X-Rays Reflectometry (XRR) curves onto different bialyers by changing the DPPC:Cholesterol molar ratio (100:0, 90:10, 80:20, 60:40 and 50:50), as we can observe in Fig. 2.

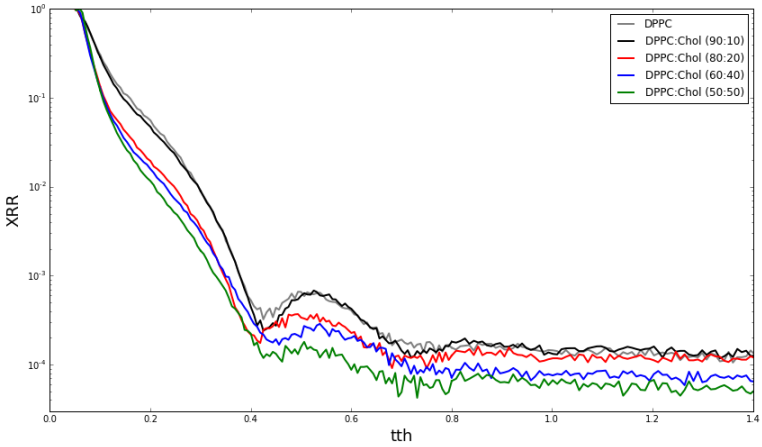

Figure 2. XRR curves of DPPC:Chol SLBs (100:0, 90:10, 80:20, 60:40 and 50:50 molar ratio)

Although an accurate data treatment and data analysis as well as interpretation of the results are still ongoing, we can show the XRR curves obtained for the different compositions. As we can observe, the first minimum is not located in the same position for all the Chol concentrations. This is an expected result, since it is known from the literature that cholesterol tends to seggregate into different domains when it is introduced into DPPC SLBs up to 20% chol content, and to be distributed into the DPPC membranes when higher concentrations of Chol are incorporated[2]. Consequently, shifts in the minimums of the XRR, as seen in the previous figure, may be related to alterations in the DPPC:Chol bilayers thickness depending on the content of Chol.

Influence of Tryptophan DOPC and DPPC membranes:

We focus on two specific concentrations of Trp: 5% and 25%. In figure 3 we can observe examples of XRR curves acquired during the experiment. However, the data treatment and analysis, as well as the interpretation of the results has still to be performed.

Looking at the different curves we observe a small change in the position of the minimum for the DOPC systems (Fig. 3 left) which suggests that Trp is modifying the thickness of DOPC bilayers, whereas differences on the oscillations amplitud are observed for the DPPC systems (Fig. 3 right), suggesting a change in the bilayer roughness or variation of surface coverage.

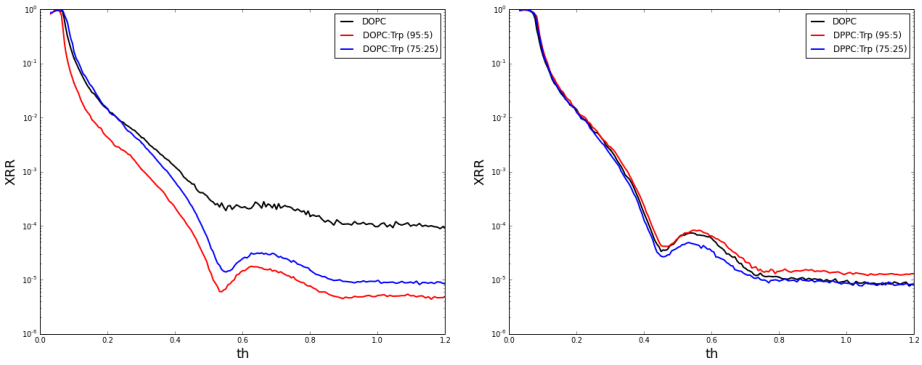

Figure 3. XRR curves of (Left) DOPC:Trp systems (100:0, 95:5, 75:25) and (Right) DPPC:Trp systems (100:0, 95:5, 75:25)

Influence of Melatonin DOPC membranes:

During the X-Rays time we could also perform few tests incorporating melatonin into DOPC bilayers, as we can observe in figure 4. As previously mentioned, data treatment and interpretation has still to be performed, so no consistent data interpretation and hypothesis can be formulated.

However, observing the graph we can clearly see a shift in the minimum position depending on the Mel concentration into the SLB. That suggests a modification of the thickness of the DOPC bilayer upon the addition of Mel.

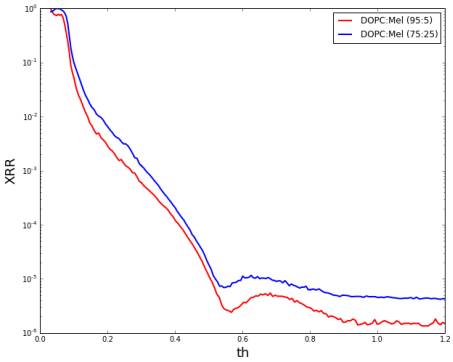

Figure 4. XRR curves of DOPC:Mel systems (95:5, 75:25)

Conclusions:

We have studied the influence of three different molecules (Chol, Trp and Mel) into phosphatidylcholine SLBs (DOPC and DPPC), observing that they affect to the membrane thickness or the roughness of the bilayer. However, we have still to finish the data treatment to better understand the changes induced by the different molecules 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.

[2] L. Redondo-Morata et al., *Langmuir* **2012**, 28, 12851.