Structural organisation of artificial water channels

The experiment aimed at characterizing the structural organization of artificial water channels in a planar lipid bilayer formed by a set of molecules (I-quartet imidazole) that have demonstrated to have high transport potentiality and ionic selectivity. These molecules mimic natural aquaporine-like behavior, and have strong technological potentiality in the field of water purification [1-6]. The techniques of XRR and GISAX are suited to this aim since they can investigate the out of plane and in-plane structure of lipid membranes with a sub-nanometrer resolution.

Since this was the first experiment on these systems, we devoted a significant part of the beam time to find the best conditions to optimize the X-ray measurements and assess the conditions to minimize the radiation damage. After these conditions were founds, we performed XRR scans of the pristine DOPC lipid bilayer adsorbed on Si substrates before and after the addition of the imidazole molecules on different I-quarted imidazole molecules (HC8, HC4, S-HC8 and HC6). A GISAX measurement was performed for the HC6.

LUV preparation: The liposomes were prepared using the film rehydration method. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in $CHCl_3$ (25mg/mL, 4mL) was used as lipid support for the vesicles. The solvent was evaporated on a rotary evaporator and dried in a vacuum desiccator for 3h. The lipid film resulted was rehydrated under vortex with 5 mL 10 mM PBS (pH = 7.4) at 25 °C for 1h to give a milky suspension. The resulting suspension was subjected to a 10 freeze-thaw cycles using liquid N_2 to freeze and warm water bath to thaw. The suspension was extruded (using an Avanti Polar Lipids, Inc. extruder) through 100 nm polyethersulfone membrane (Whatman, UK, 0.1 µm) for 21 times to obtain monodisperse unillamelar vesicles and then diluted with the same buffer into 25 mL.

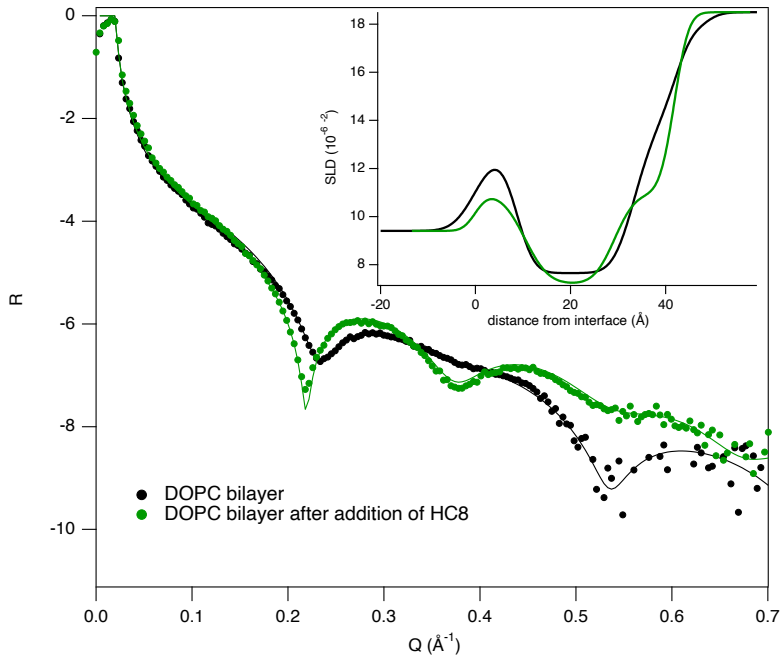

Figure 1*. XRR curves of a DOPC bilayer before and after the incorporation of HC8 and corresponding fits with a slab model.*

Lipid bilayer deposition: Vesicles solution (LUV with diameter 100 nm, concentration of DOPC was 4 mg/ml in PBS buffer solution 7.4) was poured out on cut silicon wafer with dimension 20mm*30mm and left to form bilayer for 15 min, after that 4 mL of the same PBS buffer solution was added to the cell in order to stop the forming of lipid bilayer by dilution of vesicle solution.

Incorporation of imidazole compounds: Injection of compound was done by adding of DMSO solution to the sell containing the lipid bilayer formed on silicon substrate and PBS buffer

solution. Compound concentration was fixed as 0.5 mM, the volume of aliquot was 200 µL.

Measurements on ID10: we performed a set of XRR measurements on supported lipid bilayer before and after the addition of all the different imidazole molecules: HC8, HC4, S-HC8 and HC6. In Figure 1 we report the XRR measurement of a representative scan of DOPC bilayer before and after the addition of HC8. The black line is the best fit to a slab model with features similar to a DOPC bilayer (lipid tail thickness ~ 25 A, heads head thickness ~ 7A). The aliquot of imidazole compound used doesn't form aggregation and doesn't destroy the lipid bilayer deposed on the Si wafer as it can be seen from the green curve that still can be fitted with a slab model similar to that of a DOPC bilayer with a remodulation of its structural properties that can be precisely quantified. In the inset we report the different scattering length density profiles before and after the incorporation of the HC8. In this particular case, the incorporation remodulates the structural parameter of the lipid bilayers. We evidenced in particular a shrink of the tail region (from 25 to 19 Å) and a widening of the head regions (from 7 to 10-12 Å), while keeping the total thickness of the lipid bilayer constant. The extent of the remodulation depends on the particular I-quarted imidazole molecules. The analysis of XRR data provided a detailed quantification of how the incorporation of the particular artificial pores remodulated the nanostructure of the lipid bilayer in the direction orthogonal to the bilayer.

We had the time to perform only one GISAXS measurement on the sample DOPC sample treated with the HC6 (Figure 2). Compared to the reference DOPC sample, the sample after the incorporation of HC6 shows a supplemental shoulder around 0.1-0.2 $Å^{-1}$, probably due to in-plane lateral correlation of a single patch of channels formed by HC6 molecules. This shows that it is important to extend GISAXS to all the different I-quartet imidazole molecules incorporated in the lipid bilayer to see how the molecular structure impact the formation of the channels. It is furthermore important to extend the GISAXS study toward lower Q to detect possible structural arrangement of different patches of channels at larger lengthscales.

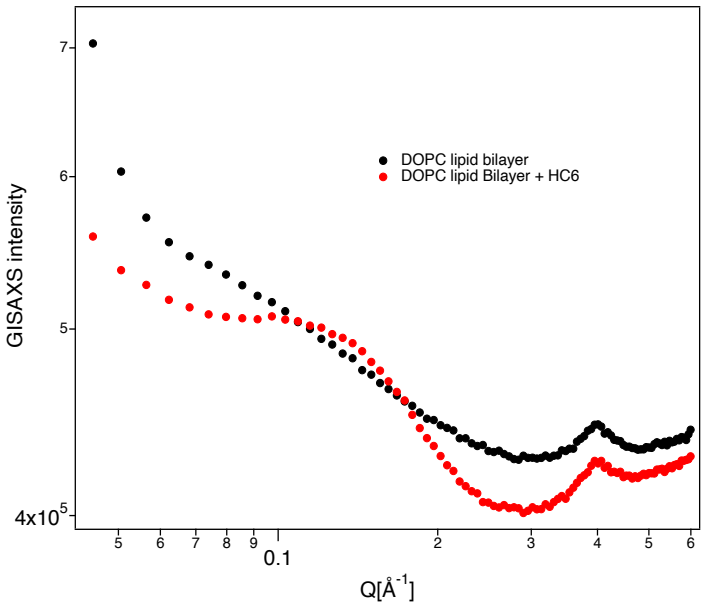

***Figure 2**. GISAXS curves obtained on a DOPC lipid bilayer before and after the incorporation of HC6.*

Conclusion and outlook: This first set of experiments shows that the combination of XRR and GISAXS is an optimum tool to elucidate how the incorporation of different pore forming molecules remodulate the structural properties of lipid bilayer formed by single lipid species. It is important now to extend this study to mixtures of lipids that are needed to optimize the transport properties and ion selectivity of the artificial channels.

References

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