# Experimental report

14/11/2017

**Proposal:** 9-13-685 **Council:** 10/2016

**Title:** There is still something to be explored on cardiolipin-containing bilayers

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** POPC, POPE, Cardiolipin

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D17 | 3 | 0 | | |
| FIGARO | 3 | 2 | 04/02/2017 | 06/02/2017 |


## Abstract:

Cardiolipin (CL) lipids are present in the inner membrane of Gram-negative and Gram-positive bacteria as well as in the one of mithocondria and chloroplasts. The unique structure of this class of lipids is responsible for peculiar physical properties. NR is a well-established technique that can be used to obtain unique and detailed information on lipid bilayers. Through the evaluation of the scattering length density profile, a clear picture of the organization of cardiolipin in lipid bilayers in the presence and in the absence of divalent ions (Ca2+) can be produced. These results will complete the characterization of the impact of CL on the organization of phospholipids. We believe that the collected results will contribute to enlarge the state-of-the-art of CL-containing lipid systems and will represent a useful reference for those who are interested in characterizing biological processes, in which CL is involved, i.e. interaction with proteins.

**EXPERIMENTAL REPORT 9-13-685-There is still something to be explored on Cardiolipin containing lipid bilayers.**

**Introduction.** Cardiolipin (CL) lipids are a group of anionic phospholipids composed of two phosphate moieties, each attached to two hydrocarbon chains *via* a glycerol backbone. CL is present in the inner membrane of Gram-negative and Gram-positive bacteria as well as in the one of mitochondria and chloroplasts [1]. The unique structure of this class of lipids is responsible for peculiar physical properties. These properties have been intensively investigated since they are believed to be pivotal for the biological role of CL [2].
In the experiment 9-13-681, bilayers composed by 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1',3'-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TMCL) or 1',3'-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TOCL), were characterized by means of NR. The main gaol of the experiment was to extract detailed structural information on the impact that TMCL and TOCL molecules have on phospholipid bilayers in the presence of different buffers (HEPES buffer pH=7.4+ NaCl,KCl (137mM, 2.7mM); HEPES buffer pH=7.4+ NaCl,KCl , $CaCl_2$ (137mM, 2.7mM, 5mM); HEPES buffer pH=7.4+ $CaCl_2$ (5mM)). Indeed, as already reported in the literature, $Ca^{2+}$ ions are able to affect the phase behaviour of CL.

**Experimental section.** Silicon crystals were cleaned through sonication in organic solvents (Chloroform, Ethanol, Acetone) and subsequently treated with Plasma Cleaner (3min). The cleaned crystals were sealed in the FIGARO NR cells and characterized in three contrasts ($D_2O$, $H_2O$, SMW). HPLC pump was used for the solvent exchange, (2 ml/min flux). Measurements were carried out with the following instrumental settings: 20 Å Frame Overlap Mirror, 7% $\Delta\lambda/\lambda$, measured angle 0.8º and 3.2º, bath temperature 25ºC. The same settings were used during the entire experiment. 3 ml of vesicle solution was injected in the NR cell after the characterization of the silicon surface. After 30 min, the cell containing the vesicle was flushed with $D_2O$ in order to induce an osmotic shock and the rupture of the vesicles. A quick measurement at 0.8º was collected in this condition to check the formation of the supported lipid bilayer. The appropriate buffer was then reintroduced in the cell and data were collected at the two angles and in the three contrasts. As representative of the collected data, Figure 1 (panel a) reports the reflectivity curves produced for the lipid bilayer with composition POPC/POPE/TMCL (40/40/20 w/w/w) in HEPES+NaCl, KCl, $CaCl_2$. Data were successfully fitted considering a 5-layers model, where 3 additional layers were added to a 12 Å silicon-oxide layer and a 6 Å water layer. These 3 layers account for the polar headgroups outer layers and the acyl chains intermediate layer of the phospholipid bilayer. A good agreement with the experimental data was achieved with similar parameters for both the inner and outer polar headgroup layers. Hence during fitting refinement these two layers were linked to have the same structural parameters (i.e. the bilayer is symmetrical).
The overall structure of the lipid bilayers resulted to be unaffected by the presence of $Ca^{2+}$ ions; very similar structural parameters were extracted for both the lipid headgroups and the acyl chains layer. However, some differences were observed in the percentage of water in the two outer-layer. This percentage of water represent the solvent volume fraction constituting the hydration water of the polar headgroups. Significative differences in this value were observed when $Ca^{2+}$ ions were added to the buffer (both in the presence and in the absence of NaCl and KCl). In particular, the water volume fraction in the absence of $Ca^{2+}$ was estimated as 0.25±0.07; it was found to be reduced to 0.09±0.05 when $Ca^{2+}$ was present in the buffer. We interpreted this result as associated to the binding of $Ca^{2+}$ ions, which hence replace some of the water molecules. We verified that this effect was strongly correlated to the presence of CL in the bilayer by repeating the same experiments on POPC/POPE bilayers. In this latter case, data collected in all the explored buffer exhibited exactly the same trend and no difference in the polar headgroup hydration were observed.
Significant structural differences occurred when TOCL was included in the POPC/POPE bilayers instead of TMCL. In particular, in the presence of $Ca^{2+}$ buffer, experimental data exhibited a different

trend with respect to the one collected for the TMCL-bilayers (Figure 1 panel c and d). Effective data analysis was no longer accessible with the previously introduced 5-layers model. Furthermore, the effect of $Ca^{2+}$ ions resulted to be particularly evident as the TOCL/POPE/POPC system in HEPES+NaCl, KCl presented the expected bilayer structure (Figure1panel c). NR data are not sufficient to fully characterize the TOCL/POPE/POPC system in HEPES+NaCl, KC+$CaCl_2$. Indeed, data reported in Figure 1 panel c and d could either correspond to the formation of patches of a floating bilayer or to the formation of ripples. In order to validate one of these hypothesis, AFM measurements are planned to complete our study.

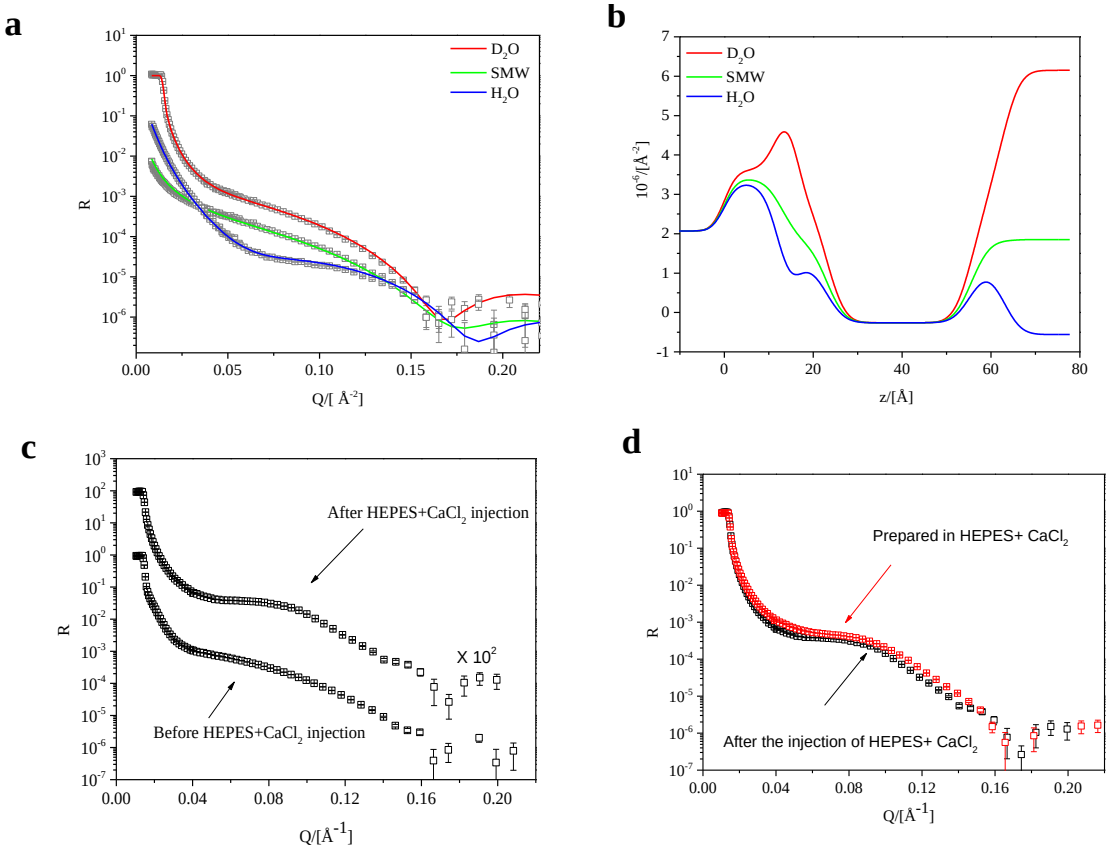


**Figure 1:** Reflectivity data together with the corresponding fitting curve collected for POPC/POPE/TMCL (40/40/20 w/w/w) in HEPES+NaCl, KCl, $CaCl_2$ (panel a). Panel b shows the scattering length density profile calculated from the analysis of the data reported in panel a and c respectively. Reflectivity data for POPC/POPE/TOCL (40/40/20 w/w/w) are reported in panels c and d; data collected for the system directly prepared in the presence of $Ca^{2+}$ and when the $Ca^{2+}$ was introduced after vesicle deposition are compared as reported in the legend.

**Conclusions.** The collected reflectivity located specifically the effect of the $Ca^{2+}$ ions as associated to the reduction of the hydration of polar headgroups and thus suggesting a selective binding when TMCL molecules are present in the bilayer. Furthermore, NR highlighted relevant differences in the system response to $Ca^{2+}$ ions when TMCL was replaced with TOCL. Even if further experiments are needed to fully shed light on the structure of TOCL/POPE/POPC, the collected data represent in general a relevant background on cardiolipin-containing lipid bilayers and more specifically the grounds for our future study which will involve potential interaction with proteins.

## References

**[1]** Ruthven N.A.H. Lewis, *et al.*, Biochimica et Biophysica Acta 1778, 2009, 2069-2079.
**[2]** J. Pan *et al.*, Soft Matter, 2015, 11, 130.