Experimental report

09/02/2016

Proposal: TEST-2519 **Council:** 4/2015

Title: Lipid bilayers at different interfaces

Research area:

This proposal is a new proposal

Main proposer: Yuri GERELLI

Experimental team: Yuri GERELLI
Irena KIESEL
Ernesto SCOPPOLA
Alessandra LUCHINI

Local contacts: Yuri GERELLI

Samples:

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	14/09/2015	16/09/2015

Abstract:

Investigated Samples

A neutron reflectivity test experiment (FIGARO – ILL) was performed at the Fluorinated Oil/Deuterated water interface with or without 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) and Perfluorodecanoic acid ($C_{10}F_{19}O_2H$). The lipid bilayer was formed by vesicle fusion.

We investigated the following interfaces:

1. C_6F_{14}/D_2O+POPC (vesicles suspension at 0.5 mg/ml);
2. $C_8F_{18} + C_{10}F_{19}O_2H$ $(5 \cdot 10^{-5}\ M)/D_2O$;
3. $C_8F_{18} + C_{10}F_{19}O_2H$ $(5 \cdot 10^{-5}\ M)/D_2O$+POPC (vesicles suspension at 0.5 mg/ml);
4. $C_8F_{18} + C_{10}F_{19}O_2H$ $(5 \cdot 10^{-5}\ M)/D_2O$+POPC (vesicles suspension at 0.5 mg/ml) and NaCl (0.1 M).

To investigate the liquid/liquid interface we exploit the liquid/liquid cell developed at ILL during the three years PhD of Ernesto Scoppola, in collaboration with the CEA Marcoule, the ICSM and the University of Montpellier.

This cell is designed to exploit the top-down reflection feature on FIGARO, and thanks to the high flux and high transmission of neutron beam through fluorinated solvents we have been able to collect data in the following time:

- **1st angle:** θ=0.623°, 1 minute
- **2nd angle:** θ=1.4°, 30 minute
- **3rd angle:** θ=2.3°, 1 hours (only for sample n° 4)
-

As the fluorocarbon was denser than the aqueous phase, the FIGARO down reflection configuration was used. The combination of the three angles with the TOF technique gave us access to a Q-region up to $Q_z = 0.2$ Å^{-1}.

Results: tuning of a lipid membrane

In Figure 1 we report the comparison between reflectivity data (and best fits) for the samples n° 1, 2 and 3. No changes are observed in the reflected intensity for the two systems containing perfluorodecanoic acid. On the contrary a strong variation in the reflectivity curve is observed when an aqueous solution containing POPC vesicles is contacted with pure fluorinated oil (red curve in Figure 1 left). Moreover, the minimum observed at $Q = 0.12$ Å^{-1} it is compatible with the formation of a lipid bilayer at the liquid/liquid interface, as observed for membranes at the solid/liquid interface.

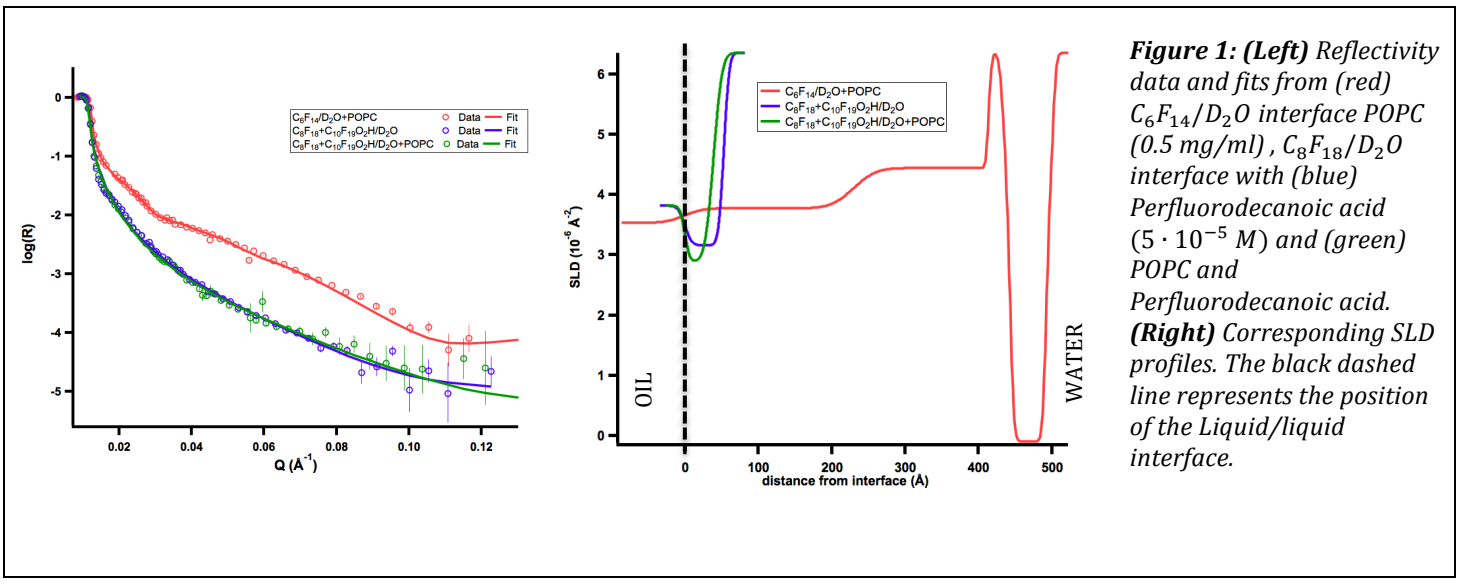

Figure 1: (Left) *Reflectivity data and fits from (red) C_6F_{14}/D_2O interface POPC (0.5 mg/ml) , C_8F_{18}/D_2O interface with (blue) Perfluorodecanoic acid $(5 \cdot 10^{-5}\ M)$ and (green) POPC and Perfluorodecanoic acid.* ***(Right)*** *Corresponding SLD profiles. The black dashed line represents the position of the Liquid/liquid interface.*

The data presented in figure 1 have been modeled to obtain the SLD profiles. For the systems containing perfluorodecanoic acid, we observe a depth in the SLD profiles of approximately 40 Å which shifts to lower SLD values when POPC is added. On the contrary, for the sample n° 1 (pure oil/water with POPC), despite the 400 Å layer (which cannot be explained with only one contrast) the SLD profile shows a depth of approximately 50 Å and with an SLD value compatible with an hydrogenated POPC membrane. The latter model is obtained with a 6 layers model but various model with 2,3 or 4 layers have been taken into account and shown in Figure 2.

Although we observe a variation of the thickness of the first layer at the liquid/liquid interface, whatever the number of layers we use to fit the data a structure compatible with the formation of a membrane appears in all the models.

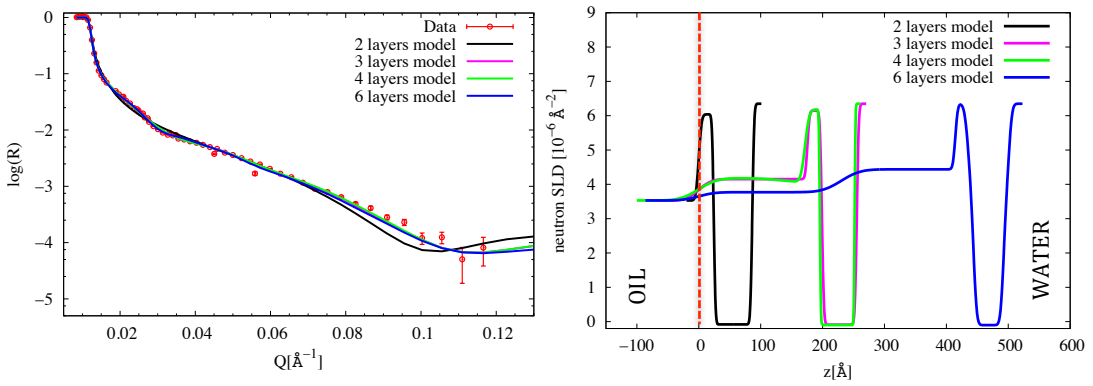

Figure 2: (Left) *Reflectivity data and fits from at (red) C_6F_{14}/D_2O interface POPC (0.5 mg/ml). With lines we report the fits obtained with (black) 2 layers, (purple) 3 layers, (green) 4 layers and (blue) 6 layers.* ***(Right)*** *Corresponding SLD profiles. The red dashed line represents the position of the Liquid/liquid interface.*

We can conclude that further investigations are required to understand how to tune the formation of a lipid bilayer at the Liquid/liquid interface. Moreover, the structure and composition of the thick layer between the interface and the region where we observe a structure compatible with a membrane has to be investigated.

Full Q-range:

The sample n°4 has been obtained injecting a Sodium Chloride aqueous solution in the sample n°3. The comparison between the two samples is reported (with best fits and SLD profiles) in Figure 3.

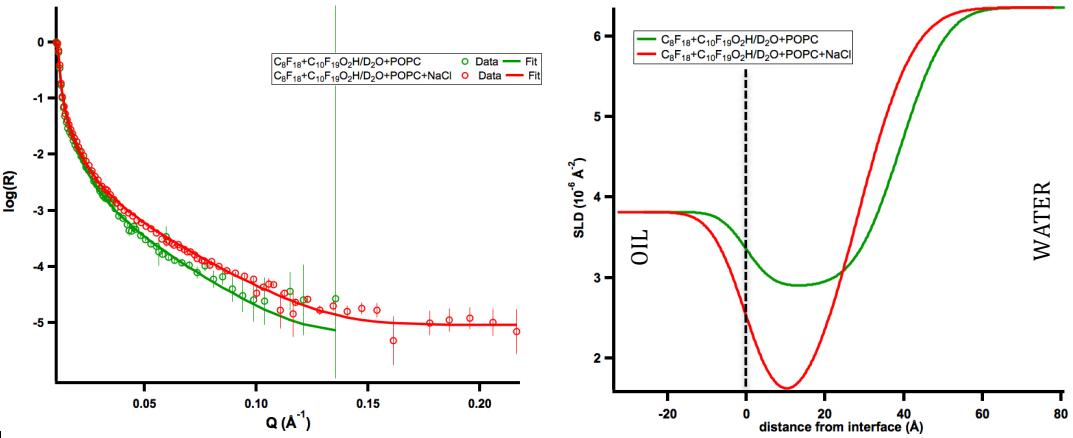

Figure 3: (Left) *Comparison between reflectivity data and fits for sample (green) n°3 and (red) n°4 .* ***(Right)*** *Corresponding SLD profiles. The black dashed line represents the position of the Liquid/liquid interface.*

With measurements at only one contrast we cannot fully explain or understand the variation in the reflected intensity when NaCl is injected, leading to a different SLD profile. Despite that in Figure 3 we show that with the exploit of fluorinated solvents, the reflected intensity is high enough to allow measurements up to $Q_z = 0.2$ Å^{-1}.

This result is new (for reference see the PhD Thesis of Ernesto Scoppola, ***"Solvent Extraction: a study of the liquid/liquid interface with ligands combining x-ray and neutron reflectivity measurements"***) and gives us the opportunity to collect data fully comparable with those obtained at solid/liquid interface.

For future measurements at 2.3° (only 1 hour for the data presented in Figure 3) longer measurements are suitable to improve the statistics and at least 3 hours are required.