Experimental report

08/04/2019

Proposal: 9-13-727 **Council:** 4/2017

Title: Lipid Bilayers at Soft Liquid/Liquid interfaces

Research area: Soft condensed matter

This proposal is a continuation of 9-13-688

Main proposer: Giovanna FRAGNETO

Experimental team: Candice HALBERT
Nicolas FAERBER
Leah MURPHY
Giovanna FRAGNETO
Emanuel SCHNECK
Ignacio RODRIGUEZ LOUREIRO
Ernesto SCOPPOLA

Local contacts: Armando MAESTRO

Samples: D2O
POPS
POPC
C8F18
DSPC
18:0 TAP

Instrument	Requested days	Allocated days	From	To
FIGARO	4	8	11/06/2018	15/06/2018
			05/10/2018	09/10/2018

Abstract:

In the last two decades reflectometry techniques have allowed the study of model biological membranes at sub-nanometer resolution. Studies on model systems have proliferated with the final aim to structurally characterize interactions of model membranes with a variety of biomolecules. When this interaction is limited to the headgroup region of the lipid bilayer present in the membrane, both studies of monolayers in water or studies of bilayers adsorbed on solid substrates are useful.
A great deal of work in the last 15 years has thus concentrated on the development of soft cushions on the solid substrate supporting floating bilayer systems. This has turned out to be challenging and often the mobility of the bilayer suffered either from the presence of tethered cushions or pinning of the bilayer on the underlying layer, or other factors.
Exploiting the Liquid/liquid cell developed at ILL and FIGARO's features (top-down geometry, high flux) we propose to study the functionalization of
soft interfaces (perfluorocarbon/water, alkane/water interfaces) with charged lipids and a deposition of a second layer on top of it by vesicle fusion.

EXPERIMENTAL REPORT 9-13-727
Floating Lipid Bilayer at the Liquid/Liquid Interface

The aim of the proposal was to test the possibility of forming floating lipid bilayers onto lipid monolayers at the Liquid/Liquid (LL) interface and the possibility of studying them at different isotopic contrasts. For this purpose, perfluorooctane/water interface has been functionalized with DSPC/18:0 TAP monolayers formed by vesicle fusion. Subsequently, h-POPC/POPS vesicles are injected to form a floating bilayer by vesicle fusion.

The experiment has shown the feasibility of working at various heavy/light water mixtures by using peristaltic pumps. For controlling better the water exchange a new electronic apparatus controlled by NOMAD has to be developed in the future.

Results

At first we have collected reflectivity data of an oil/water interface decorated with h-DSPC/18:0 TAP in heavy water and OCMW (Oil Contrast Matched water), as shown in Figure 1.

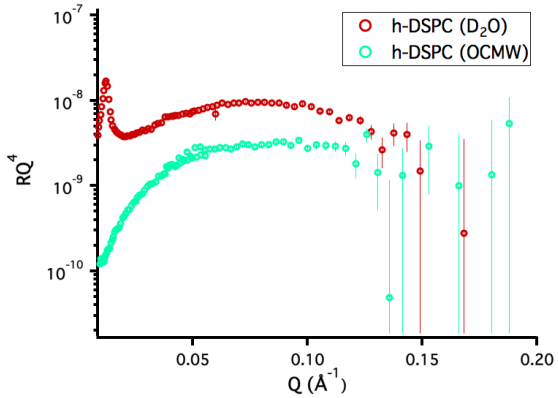

Figure 1: Reflectivity data obtained h.DSPC/18:0 TAP monolayer at LL interface at two different contrast: D_2O (red), OCMW (cyan).

The monolayer signature can be observed especially in the OCMW data in which we would not get any signal if lipids were not absorbed.
Subsequently, an hydrogenous POPC/POPS bilayer was formed by vesicle fusion and again measured at two contrasts, as show in Figure 2.

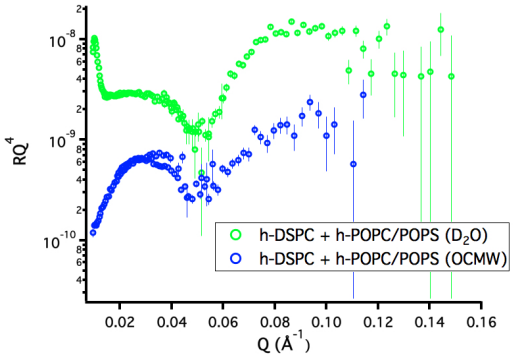

Figure 2: Reflectivity data obtained for h-POPC/POPS bilayers deposited h-DSPC/18:0 TAPmonolayer at different contrast (D_2O (green), $OCMW$ (blue)).

A comparison with Figure 1 evidences the presence of minima, clear signature of structure on the length scale of few nanometers (see minima in Figure 2).
In order to resolve all the bilayer/monolayer details, the same measurements have been performed on a sample with a monolayer of deuterated DSPC/18:0 TAP and h-POPC/POPS bilayer, as shown in Figure 3.

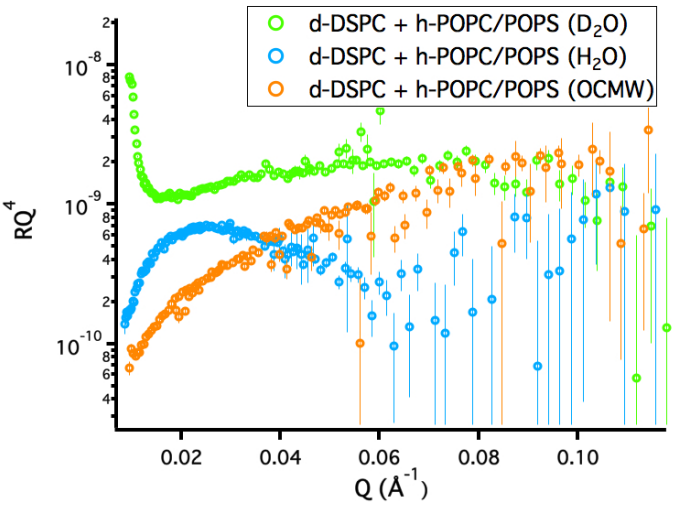

Figure 2: Reflectivity data obtained for h-POPC/POPS bilayers deposited onto functionalized LL interface by d-DSPC/18:0 TAP 2 monolayer at various water contrast.

Minima in the data (Figure 3) are less evident due to the different interference pattern created by the deuterated DSPC/18:0 TAP monolayer which is now less "visible" at the LL interface.

More samples have been measured as floating bilayers onto PEG brushes at the LL interface and bilayers onto LL interface functionalized with fluorinated surfactant. Data are not reported here for lack of space but they are in the analysis process. Future experiment will help for testing new developments of the ILL Liquid/Liquid cell (temperature control, pumping setup for solvent exchange and automatic alignement).