Experimental report

14/02/2017

Proposal: 9-13-688 **Council:** 10/2016

Title: Optimisation of lipid adsorption at the liquid/liquid interface

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: D2O
DOTAP
POPS
POPC
DSPC
Dodecane
Perfluoro-octane

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	3	3	23/01/2017	26/01/2017

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 vescicle fusion.

Experimental Report 9-13-688
Functionalization of the Liquid/Liquid interface for lipid bilayers fusion

Scientific Case

Biological membranes are vital components of all living organisms. They form the boundaries between the various compartments of cells and constitute platforms for essential biochemical processes like enzymatic reactions, molecular transport, or the formation of functional lipid domains. Importantly, structural insight is usually a prerequisite to understand the details of these processes. During the last two decades x-ray and neutron reflectometry enabled the structural characterization of model biological membranes at sub-nanometer resolution and the investigation of interactions of membranes with a variety of biomolecules [1]. This was only possible with the simultaneous development of methods for membrane immobilization in planar geometries, such as solid-supported membranes or membranes floating on polymers, lipids, or soft tethers [1,2]. However, when using these approaches, studies on molecules crossing the membrane (see for example drug delivery systems) or deeply penetrating into the bilayer chain region (see toxins, amyloid peptides, membrane proteins in general) turned out to be difficult, because membrane mobility often suffered from the presence of the solid surface. In fact, the water gap beneath the membrane is rarely thicker than around 2nm (in the case of floating bilayers) while real membranes are free to fluctuate and surrounded by soft material. Here we propose an alternative route, the immobilization of lipid membranes near functionalized liquid/liquid interfaces, which are intrinsically soft. This configuration has a number of advantages with respect to solid supported membranes. The biggest advantage is the absence of the solid surface that compromises membrane movements. Furthermore, working at the interface between two liquids offers the unique possibility for chemical manipulations from both bulk liquid phases. The interaction between interface and membrane can be adjusted, for instance, after membrane deposition without changing the aqueous medium. Finally, membranes immobilized at liquid/liquid interfaces offer unique possibilities for structural studies on molecular transport processes through lipid membranes. In this first proof of principle experiment the aim was to immobilize lipid bilayers at functionalized oil/water (perfluoro-octane/water) interfaces via vesicle fusion from the aqueous phase and to investigate their structure using specular neutron reflectometry. Fluorinated oils are interesting as they are prototypic examples of chemically inert fluids. In fact, they are even used as synthetic substitutes of blood and oxygen carriers in biomedical applications [3]. Moreover, fluorinated oils present several technical advantages for the proposed experiments (high SLD contrast with water and good performance in NR experiments) and are therefore suitable for an optimization of the methodology.

Investigated Samples

We have investigated the C_8F_{18}/Water interface functionalized with a mixture of uncharged/positively charge lipids (DSPC/18:0 TAP in a 70/30 mol/mol ratio) and the subsequently adsorption of mixture of uncharged/negatively charged lipids via vesicle fusion. For the latter we have chosen either fluid lipid vesicles (POPC/POPS in a 70/30 mol/mol ratio) or gel lipid vesicles (DPPC/DPPS in a 70/30 mol/mol ratio).
We further collected data for the adsorption of POPC/POPS vesicles onto a pre-formed DSPC/18:0 TAP monolayer at the dodecane/water interface, but these data are currently still being analyzed.

Preliminary Results

Thanks to the high transmission through perfluoro-alkanes, we have been able to record reflectivity data in Time Of Flight (TOF) at two different angles (-0.62°, -2.72°) with a resulting Q-range up until 0.2 $\AA^{-1}$. We will present here only the preliminary results obtained for the DPPC/DPPS adsorption on an interface functionalized with DSPC/18:0 TAP.
The samples have been subsequently prepared on the instrument by injecting lipids vesicles in the water phase and, after the equilibration, rinsing with pure water (both heavy water or a mixture of heavy/light water at the proper mol/mol ratio).
Samples were prepared and characterized with the following sequence:

- A1: Preparation of a bare C_8F_{18}/ D_2O interface -> Measurement
- A2: Injection of DSPC/18:0 TAP vesicles into the aqueous phase, rinsed with D_2O after few hours -> Measurement
- A3: Injection of DPPC/DPPS vesicles rinsed with D_2O after few hours -> Measurement
- B1: Rinsing with water (SLD=3.4×10^{-6} $Å^{-2}$) -> Measurement
- B2: Injection of 5 mM NaCl solution -> Measurement
- C1: Rinsing with D_2O -> Measurement

A comparison between the collected data for samples A1, A2, A3, and C1 is shown in Figure 1.

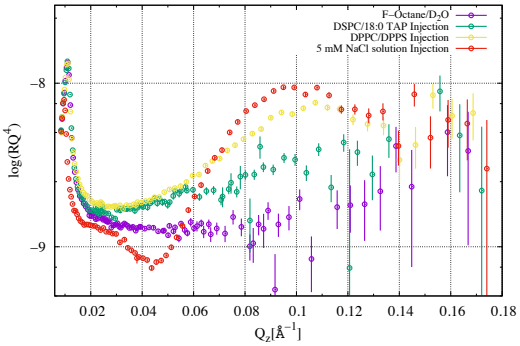

Figure 1: *Reflectivity data for C_8F_{18}/Water interface. (A1, Purple) Bare Interface. (A2, Green) After injection of DSPC/18:0 TAP vesicles. (and so on Yellow) After injection of DPPC/DPPS vesicles. (Red) After injection of 5 mM NaCl solution. The latter shows a different critical edge due to the rinsing with a mixture of D_2O/H_2O.*

The data shown in Figure 1, especially the pronounced features at low Q_z (< 0.06 Å^{-1}), evidence a strong modification of the interfacial structure after the injection of negatively charged (DPPC/DPPS) lipids vesicles, which is not anymore compatible with a simple monolayer model. A preliminary analysis of the data is presented in the following paragraphs.

Data Analysis Procedure

The aim of the data analysis is to obtain the local model distribution of chemical species across the Liquid/Liquid (L/L) interface. These distributions are affected by the intrinsic softness of the L/L interface and a simple analysis of the reflectivity data with SLD profiles would lead to an effective distribution of species convoluted by the interfacial roughness. For this reasons, during the data analysis we have fitted the data following the path described below:

1 – Model volume fraction distribution of species *i* assuming no interfacial roughness, $v_{t,i}(z)$, have been produced;

2 – All distributions $v_{t,i}(z)$ have been convoluted with a Gaussian function depending on the interfacial roughness σ_{int} to obtain the observed volumes fraction distributions $v_{o,i}(z)$. The relation between $v_{o,i}(z)$ and $v_{t,i}(z)$ follows:

$$v_{o,i}(z) = \frac{1}{\sqrt{2\pi}\sigma_{int}} \int_{-\infty}^{+\infty} v_{t,i}(\zeta) \cdot e^{-\frac{(z-\zeta)^2}{2\sigma_{int}^2}} d\zeta$$

3 – SLD profiles and reflectivity curves corresponding to the volume fraction distributions $v_{o,i}(z)$ are produced and data are fitted to obtain the best-matching distribution parameters. In the following paragraph we will show the results for the $v_{t,i}(z)$ obtained with this procedure.

Preliminary Analysis

Oil/Water interface and Lipid Monolayer

We have first analyzed the data collected at the C_8F_{18}/Water interface before (A1) and after (A2) the injection of DSPC/18:0 TAP vesicles. Data, fits and model volume fraction distribution are shown in Figure 2.

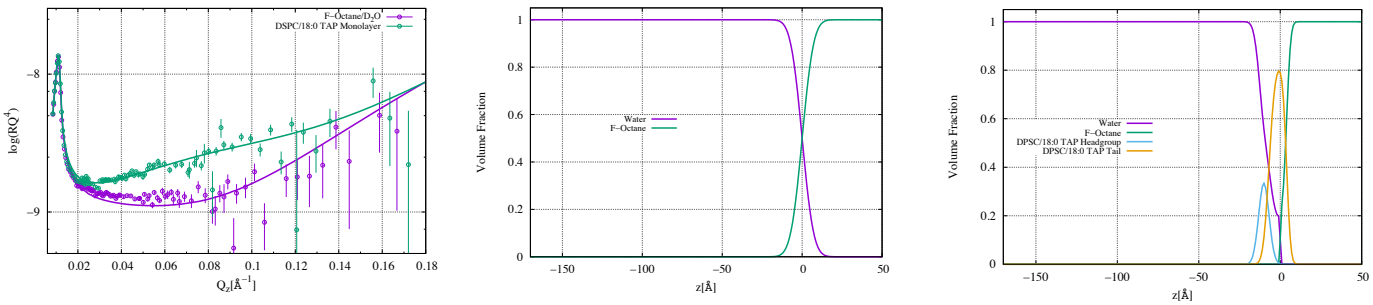

Figure 2: ***(Left)*** *Reflectivity data for C_8F_{18}/water interface (A1, purple) before and (and so on, green) after DSPC/18:0 TAP vesicles fusion. Solid lines are fits corresponding to the best-matching model parameters.* ***(Center)*** *Model distribution as a function of z for Oil and Water for sample A1.* ***(Right)*** *Model distribution as a function of z for Oil, Water, Lipid headgroup and tail for sample A2.*

Comparing the data for the samples A1 and A2 we observe a strong intensity variation for the Q_z values above Q_z=0.02 Å^{-1} due to the formation of a lipid monolayer at the L/L interface. In fact, for the best-matching lipid tail and headgroup distributions shown in Fig. 2 (right), which correspond to an area per lipid of ≈ 80 Å^2 and to a lipid orientation with the headgroup in contact with the aqueous phase, the reflectivity data are well reproduced.

Injection of DPPC/DPPS vesicles

After characterizing the positively charged lipid monolayer at the L/L interface we injected oppositely charged DPPC/DPPS lipid vesicles and measured again in two contrasts (A3 and B1). Subsequently a 5 mM NaCl aqueous

solution was injected in the sample to vary the ionic strength and the electrostatic interaction between the oppositely charged lipids and the measurements were repeated in two contrasts (B2 and C1). Reflectivity data and corresponding model volume fraction distributions are shown in Figures 3 and 4 respectively.

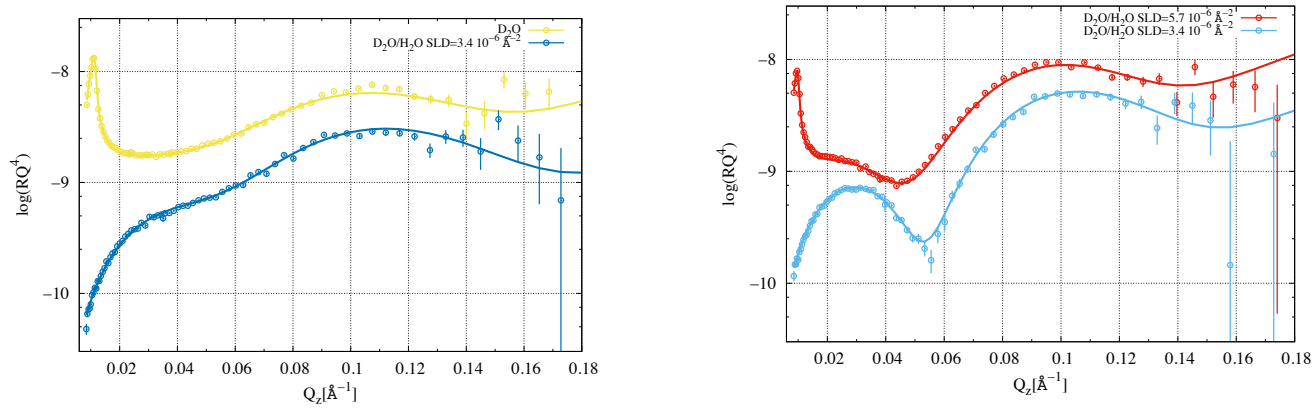

Figure 3: *Reflectivity data at two different contrast after injection of DPPC/DPPS (left) before and (right) after the injection of 5 mM NaCl aqueous solution. The data are plotted on the same scale for an easier comparison.*

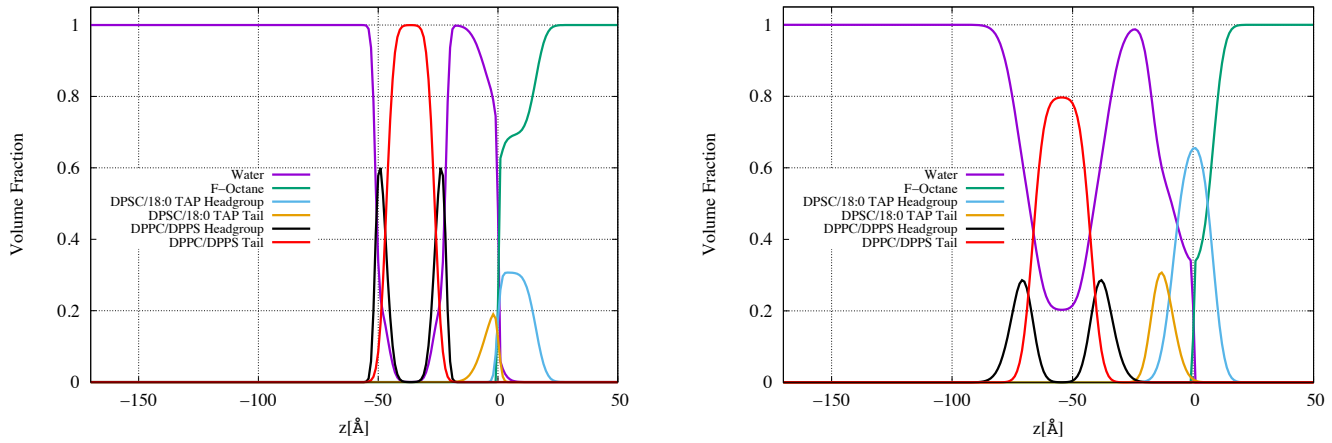

Figure 4: *Model volume fraction distribution as a function of z for samples with DPPC/DPPS (left) before and (right) after the injection of 5 mM NaCl aqueous solution. The data are plotted on the same scale for an easier comparison.*

The best-matching distributions clearly show the formation of a bilayer on top of the preformed monolayer. The injection of salts causes a displacement of the bilayer and, even in the model distributions, the specific bilayer roughness increases. Moreover, the models show that part of the lipids belonging to the bilayer may be transferred at the o/w interface after the injection of salts. This suggests the needing of vary the electrostatic interaction between the monolayer and the bilayer acting directly from the oil phase using charged/uncharged surfactant that can't exchange with the lipids in the bilayer.
It is important to underline that in both samples there no lipid vesicles left because samples have been rinsed with approximately 80 ml of water (at the proper D_2O/H_2O mixture) after each injection of vesicles or salts.

Conclusion and Perspective

This experiment has shown that is possible to form lipid trilayer structures (monolayer with floating bilayer) via pre-formation of a monolayer and subsequent vesicle fusion. Further experiments using mixtures of deuterated/hydrogenous lipids and/or fluorinated charged/uncharged surfactants are desirable in order to explore how the monolayer-bilayer interaction can be adjusted post-formation.

References

1. Wacklin H.P. - Neutron reflection from supported lipid membranes. *Curr. Opin. in Coll. & Int. Sci*. **15,** 445-454 (2010).
2. Fragneto G. , Charitat T. , Daillant J. - Floating lipid bilayers: Models for physics and biology. *E. Biophys. J*. **41,** 863-874 (2012).
3. Lowe K. C. – Fluorinated blood substitutes and oxygen carriers. *Journal of fluorine Chemistry.* **109,** 59-65 (2001).