Experimental report

04/10/2016

Proposal: **9-13-648**

Council: 4/2016

Title: Structure of interacting soft interfaces

Research area: Biology

This proposal is a new proposal

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Samples: D2O
Polyethylene glycol
synthetic phospholipids

Instrument	Requested days	Allocated days	From	To
FIGARO	2	3	09/09/2016	12/09/2016

Abstract:

The mutual interaction between the surfaces of biological membranes plays important roles in essential biological processes such as membrane adhesion, material transport in vesicles, and the formation of lamellar structures. Comprehensive understanding of the underlying physical interaction mechanisms relies on detailed and distance-dependent structural insight into molecular conformations. As demonstrated in our preliminary experiments, such distance-dependent structural information can be obtained by neutron reflectometry on amphiphilic double-monolayer architectures. Here, in order to investigate the structure of interacting membrane models at biologically most relevant weak interaction pressures, we propose a novel double monolayer architecture of interacting surfaces at the solid-liquid-liquid interface.

Experimental report for the proposal no. 9-13-648

Structure of interacting soft interfaces

1 Introduction

The aim of the performed experiments was to investigate structural properties of an amphiphilic monolayer at the oil/water interface as a function of the interaction distance with a solid substrate. In particular, we chose a monolayer made either by a neutral or a charged phospholipid mixed with PEG-decorated lipids to mimic the external leaflet of biological membranes. The lipid monolayers were DSPC+$10\%_{\mathrm{mol}}$DSPE-PEG5000 and DSPC+$10\%_{\mathrm{mol}}$DSPE-PEG5000, whose specific combination of grafting density and molecular weight (PEG degree of polymerization, N=114) leads to brush regime of the tethered polymer chains.

The experiment was performed using a quartz cell, previously adopted for the study of liquid/liquid interfaces at FIGARO [1]. The sample preparation was carried out as follows: a silicon block located at the bottom of the cell was covered by deuterated water. The monolayer was spread at the D_2O/air interface and hydrogenated dodecane was poured from the top. The oil/water interface resulted decorated by a mixed monolayer. Finally, this liquid interface is brought in proximity of the solid one (piranha etched hydrophilic SiO_2 surface) by pumping a desired amount of D_2O out of the sample container. The experimental procedure described above and the quartz cell are shown in Figure 1.

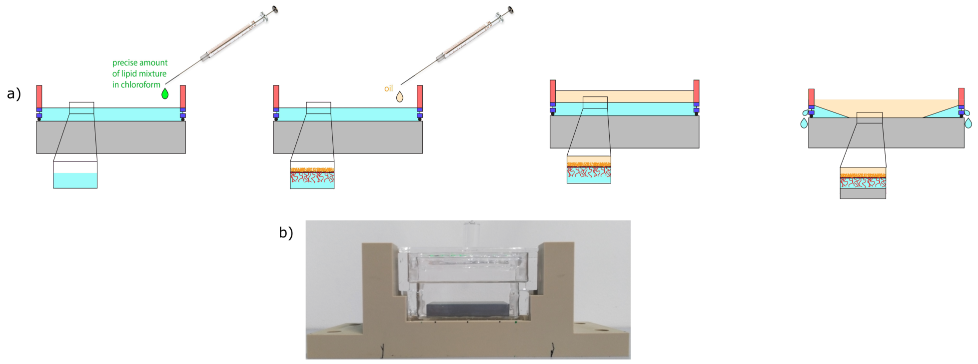

Figure 1: **a)** Schematic representation of the sample preparation for the experiment. **b)** Sample environment used for the experiment: quartz cell used for measurements at the liquid/liquid interface [1].

2 Neutron Reflectometry experiments

Reflectivity curves were collected while sampling out a small volume of D_2O until relevant changes of the profiles were noticed. When the oil phase was brought in contact with the silicon surface, a clear transition from a typical $Si/SiO_2/D_2O$ interface to a Si/SiO_2/dodecane was observed. Particular attention was paid to the intermediate states between these two boundaries. In Figure 2 the reflectivity profile for the system DSPC+10$\%_{mol}$DSPE-PEG5000 is reported. In the condition of contact between monolayer and solid substrate, the data analysis revealed the presence of a hydrogenated layer of about 10 nm thickness (SLD=$0.023 \cdot 10^{-6}$Å^{-2}) between solid surface and liquid (oil) phase, without much residual hydration water.

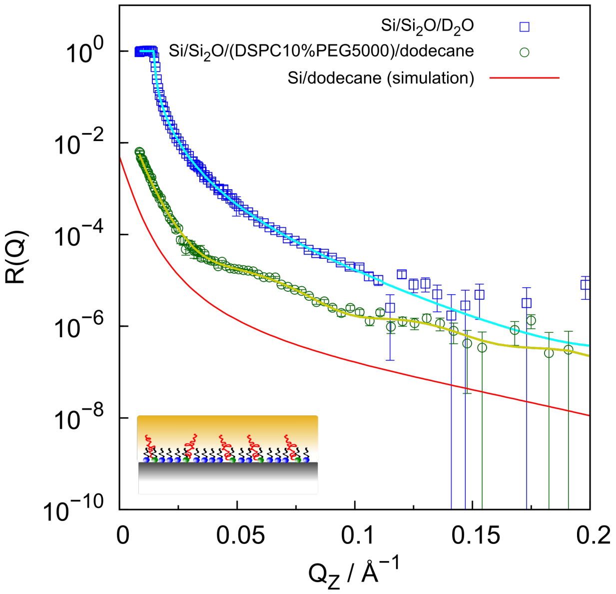

Figure 2: Reflectivity curves for the system DSPC+10$\%_{mol}$DSPE-PEG5000.

In contrast, when the DSPC in the lipid matrix was substituted by the negatively charged DPPS, keeping a constant DSPE-PEG5000 content, a nanometric D_2O layer remained sandwiched between the lipid and solid surface (Figure 3). In fact, the experimental curves indicated the presence of "bulk" water (presence of critical edge) even in condition of contact between liquid interface and solid surface. Nevertheless, such a thick water layer had a finite thickness, and the reflectivity profile was well described by a 700-nm-thick D_2O layer, as shown by the fit in Figure 3 (solid line in lilac). For the sake of comparison, on the same data set the simulated curve of an infinitely thick ($\geq$ 2000 nm) D_2O film is plotted as a dotted blue line. The deviation of this line from the data points supports the conclusion of the confinement of a finite water layer between lipid monolayer and solid substrate. Only the addition of an excess of D_2O back in the cell to macroscopically separate the two interfaces restores the $Si/SiO_2/D_2O$ profile (red curve in Figure 3). The result described above demonstrate the relevant contribution of the repulsive interactions between the equally charged lipid layer and silicon surface, which dominates the interfacial forces between the two interfaces.

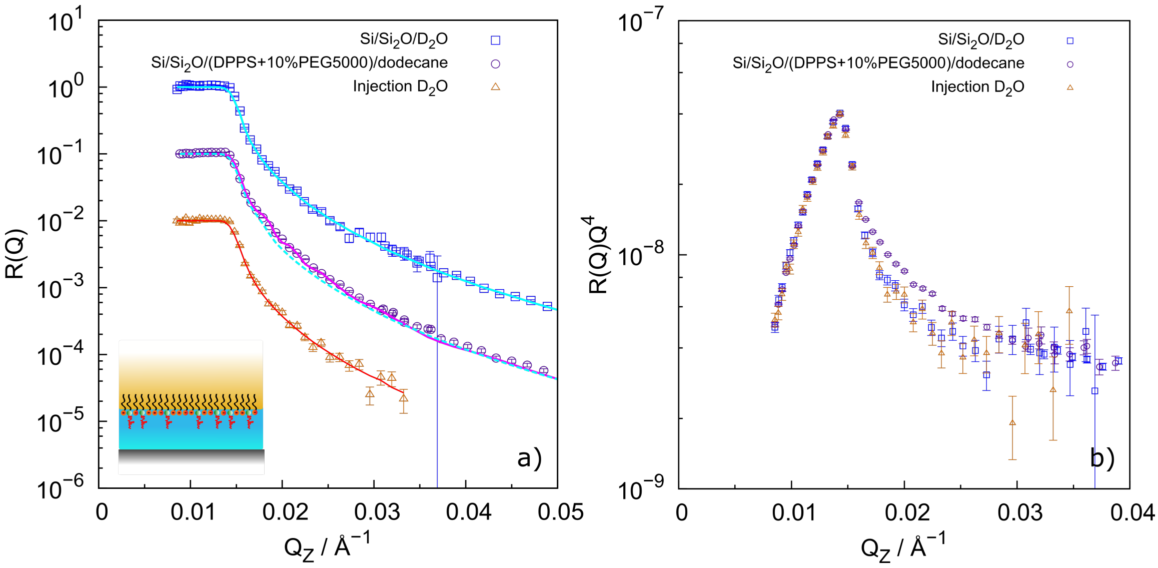

Figure 3: Reflectivity curves for the system DSPC+10%$_{\mathrm{mol}}$DSPE-PEG5000.

The minor difference between the pure silicon/water interface and the silicon/monolayer/oil interface, as highlighted in Figure 3b, can be explained by the enhancement of the internal contrast of the system, due to the proximity of the oil interface.

3 Summary

The performed experiment highlights the feasibility of this experimental method to probe the structure of fluid interfaces in proximity of a solid substrate. The existence of the two extremes, monolayer adhesion and interfacial repulsion, was determined by the nature of the phospholipid matrix, leading either to major extrusion of the hydration water between lipid and silicon surface, or to a large hydration gap between the two interfaces, to which the electrostatic repulsion might have played an important role. However, it is likely that a more complex interplay of effects governs the molecular behavior. Future experiments will be devoted to exploit the full range of interfacial interactions by the reasoned choice of surface functionality and composition of the lipid monolayer (hydrophilicity, chemical modification). Furthermore, technical aspects regarding sample environment and sample preparation will be adressed to improve the scientific outcome of the performed experiments.

References

[1] Ernesto Scoppola et al., *Structure of a liquid/liquid interface during solvent extraction combining X-ray and neutron reflectivity measurements*, Phys. Chem. Chem. Phys. (2015), 17, 15093-15097