Experimental report

23/01/2024

Proposal: **9-13-1058** **Council:** 10/2022

Title: Tribology of charged and zwitterionic lipids layer: effect of hydration on structural properties

Research area: Soft condensed matter

This proposal is a new proposal

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Samples:
DSPC lipids
DPPS lipids

Instrument	Requested days	Allocated days	From	To
D17	5	4	30/06/2023	04/07/2023

Abstract:

A stack of hydrated phopsholipid bilayers lubricates biological rubbing surfaces. Its exceptional frictional behavior appears to originate from the confined nanoscopic water layer via the hydration lubrication mechanism. However, other energy dissipation path- ways are plausible. To elucidate the shear of confined lipids, we have developed a system combining Tribology and FRAPP (TriboFRAPP), giving at the same time an insight into the friction and hydrodynamics of the sheared film. We study charged (DPPS) and zwitterionic (DSPC) trilayers deposited on a glass substrate (solid/air interface) which allows us to play on the hydration level. With the TriboFRAPP device, we hope to locate the shear slip plane. The precise measurement of the thickness of the confined water by neutron reflectivity would complete our results by demonstrating the structure/friction relation and would reinforce our data concerning the velocity field obtained with the TriboFRAPP setup. Finally, the structural knowledge of the trilayer would allow us to compare our experimental work with molecular dynamics simulations performed by collaborators working on the same ANR project (ANR Banana_Slip, PRC, AAPG2021).

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1 Context

Many biological processes are governed by the complex balance between surface forces. At the nanometer scale, solvated surfaces experience hydration repulsion, preventing the collapse of biological matter triggered by Van der Waals attraction. Hydration forces provide lubrication between surfaces, while Van der Waals forces contribute to stability. Together, these forces create a framework that allows life to function even in the most confined part of cells. The stacking of phospholipid membranes serves as a perfect example of this molecular forces equilibrium [1]. Recently, we have demonstrated that within like-charged phospholipid membranes, a strong electrostatic attraction is introduced into the system [2]. This counterintuitive attractive force typically arises in the presence of highly correlated multivalent ions [3]. Surprisingly, in our system, only monovalent ions were sufficient to reveal it. We attributed this phenomenon to the low value of the dielectric constant of the interfacial water, which reduces the screening of electrostatic interactions. While a significant amount of theoretical and numerical work has been devoted to explaining the electrostatic strong coupling attraction, experimental validation remains limited. We argue that solid-supported phospholipid membranes offer a valuable platform for the study of membrane-membrane interactions and, in particular, are well suited to probe the emergence of electrostatic attraction in nanoconfined water layers. In the context of this study, we propose an innovative approach that involves an odd number of layers deposited on a silica substrate. Specifically, at the air/solid interface, we can control an osmotic pressure by adjusting air relative humidity. Using the D17 neutron reflectometer, we measured the interlayer spacing for membranes with varying surface charge densities. From this data, we deduced the disjoining pressure between the layers and identified an experimental crossover between electrostatic attraction and repulsion.

2 Experiment

Samples were prepared at the ILL using the Soft Matter Lab facilities (PSCM). We used two types of phospholipids: DPPC, a zwitterionic lipid with a surface charge density of $\sigma_{\mathrm{PC}} = 10^{-3}\ \mathrm{e}^-/\mathrm{nm}^2$, and DPPS, an anionic lipid with a surface charge density of $\sigma_{\mathrm{PS}} = 1\ \mathrm{e}^-/\mathrm{nm}^2$. The lipids were deposited on a silicon block using the Langmuir-Blodgett technique, at a surface pressure of 40 to 45 mN/m, corresponding to the gel phase of both DPPC and DPPS. Depending on the fraction of DPPS, we were able to produce two types of supported phospholipid layers. At low charge density, the deposition method limited the number of layers to 3 (tri-layer). However, above a fraction of 60% DPPS, it was possible to stack more layers, although we restricted ourselves to 5 layers (penta-layer). The first layer in contact with the substrate was always pure DPPC, as it allows to obtain a better transfer ratio for the subsequent layers. Using an in-house humidity chamber at ILL, we were able to control the temperature and vary the humidity of the sample for different vapor contrasts, and characterize the evolution of the structure with high resolution. Experiments were carried out at a fixed temperature of 25°C on 9 samples (silicon substrate, DPPC tri-layer, DPPS tri-layer, DPPC 80%/DPPS 20% tri-layer, DPPC 40%/DPPS 60% tri-layer, DPPC 20%/DPPS 80% tri-layer, DPPC 30%/DPPS 70% penta-layer, DPPC 20%/DPPS 80% penta-layer, DPPS penta-layer) for 2 different contrasts (H_2O and D_20). In total we generated 76 reflectivity curves in four and a half days. A preliminary analysis of these experiments is presented below.

3 Results

We initially investigated a bare silica substrate under low humidity conditions (RH=3-4%). From the reflectivity curve, we extracted the thickness and roughness of the silica oxide layer on our substrate (not shown in this report). We measured the reflectivity of a DPPC tri-layer at various humidity levels (RH=3, 30, 38, 45, 50, 60, 70, 90%). Two different contrasts, D_2O and H_2O, were employed to enhance fitting accuracy. The reflectivity curves at RH=30% and RH=90% are de-

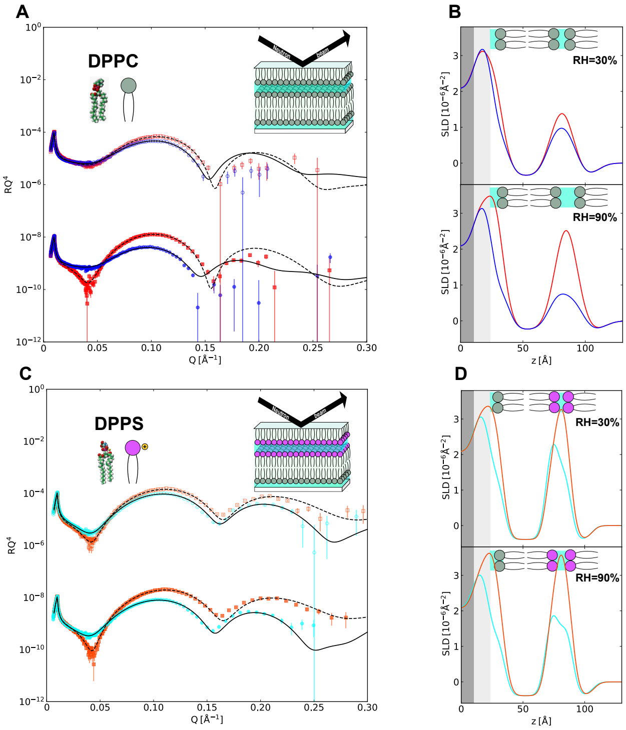

Figure 1: A and C represent reflectivity curves of DPPC and DPPS tri-layers under two relative humidity conditions at 25°C. Empty markers correspond to RH=30%, while filled represent RH=90%. To enhance clarity, reflectivity curves at RH=30% have been shifted by 4 decades. D_2O contrasts are red and orange squares and H_20 contrasts are represented by blue and cyan circles. B and D depict the SLD profiles corresponding to reflectivity curves fitted with black lines. The gray and light gray bands correspond to silica and silica oxide, respectively.

picted in Figure 1.A. From the extracted SLD profiles shown in Figure 1.B, we quantified the thickness variations of water layers in response to changes in humidity. To investigate the impact of negatively charged phospholipids on these variations, we conducted the same analysis for a DPPS tri-layer (Figure 1.C and Figure 1.D), for which only the second and third layers are changed with DPPS. The observed variations in the water layers indicate the signature of a strong coupling regime, leading to attraction between the layers. Subsequently, we aimed to investigate the emergence of this attraction by repeating the experiment with a tri-layer containing a DPPC:DPPS mixture, aiming to lower the surface charge density. We successfully characterized three DPPC / DPPS tri-layers with different compositions(20%, 40%, 80% charge fraction), but these results will not be shown in this report. Beyond 40% of charged fraction, it was possible to deposit more than three layers on the substrate, prompting us to perform reflectivity experiments on three types of penta-layers (70%, 80%, and 100% DPPS fraction). Part of the analysis of the 80% penta-layer is shown in Figure 2.

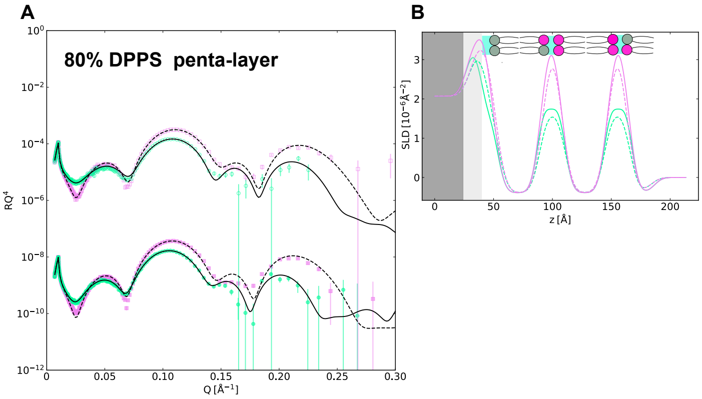

Figure 2: A) represents reflectivity curves of 80% DPPS penta-layers under two relative humidity conditions at 25°C. Empty markers correspond to RH=38%, while filled correspond to RH=90%. To enhance clarity, reflectivity curves at RH=38% have been shifted by 4 decades. D_2O contrasts are violet squares and H_2O contrasts are represented by green circles. B) depicts the SLD profiles corresponding to reflectivity curves fitted with black lines. Dashed lines are for RH=38%, plain for RH=90%. The gray and light gray bands correspond to silica and silica oxide, respectively.

We can then construct a graphical representation that correlates the DPPS fraction (so surface charge density), to dw_2, the second water layer (see Figure 3). The model we proposed (depicted by the dashed line in Figure 3) captures the balance of surface forces in nanoconfined water layers at charged interfaces. The essential ingredients to elucidate the behavior rely on the interplay between hydration repulsion and electrostatic attraction.

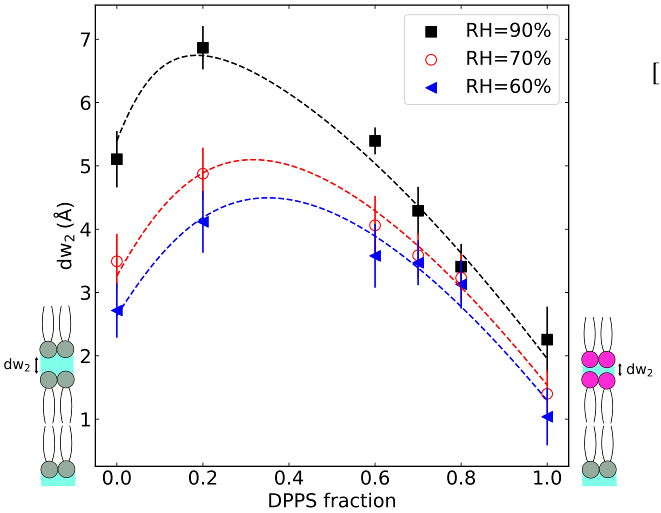

Figure 3: Variation of the second water layer dw$_2$ with changes in DPPS fraction at three fixed relative humidity: RH=90%,70%,60%.

4 Conclusion

Thanks to neutron reflectivity, we were able to confirm the effects of charges on the supported phospholipid odd-layers. These experiments will serve as a foundation for comparison to theoretical models. They also provide us with insights for future neutron reflectivity experiments. Furthermore, these structural analyses will be beneficial when coupled with our friction experiments conducted with the TriboFRAPP setup—a homemade system coupling a tribometer with velocimetry. All in all, we believe this study will shed light on the emergence of electrostatic like-charges attraction in water-confined layers.

References

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