Experimental Report

19/06/2014

Proposal:	**9-13-492**	**Council:**	10/2012
Title:	Influence of substrate roughness and preparation method on supported bilayer structure		

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **RIEU Jean-Paul**

Experimental Team: MUNTEANU Bogdan
RIEU Jean-Paul
CHARITAT Thierry
MURALI MOHAN Meera
PETIT Alexandre
CHAPUIS PIERRE

Local Contact: WATKINS Erik

Samples: Silicon
DLPC
TRIS buffer

Instrument	Req. Days	All. Days	From	To
FIGARO User-supplied	3	3	08/07/2013	11/07/2013

Abstract:

Biological contacts such as the articulating cartilage surfaces in human hips or knees often operate under severe conditions (i.e. high load and low speed), which is related to a boundary lubrication regime characterized by a very low friction coefficient. Breakdown of this lubrication can lead to wear of the cartilage and to osteoarthritis. Insights into the molecular origins of cartilage lubrication could lead to more efficient medical treatments, tissue repair and to longer-lasting prostheses.

Theoretical models predict that the membrane roughness should increase with increasing substrate roughness. Other theoretical predictions suggest that an complex series of partial unbinding transitions should be observed upon an increase the substrate roughness. As a consequence, the lipid membrane should be smoother than the substrate for large substrate roughnesss. The purpose of this proposal is to measure lipid bilayer coverage, bilayer roughness and substrate-bilayer water thickness as a function of substrate roughness, bilayer method of preparation, buffer content and eventually temperature.

Experimental Report on Experiment Number: 9-13-492

“Influence of substrate roughness and preparation method on supported bilayer structure”

Participants: Jean Paul RIEU (1), Alexandre PETIT (1), Bogdan MUNTEANU (2), Thierry CHARITAT (3), Pierre CHAPUIS (3)

(1) Institut Lumière Matière, Lyon, France; (2) LaMCoS, Lyon, France; (3) ICS, Strasbourg, France

Local contact(s): Erik WATKINS, watkins@ill.fr

Instrument FIGARO from 08/07/2013 8:30am to 11/07/2013 8:30am

Context

Nature has produced water-based lubricant systems that far outclass the best of most man-made devices [1]. Biological contacts such as the articular cartilage surfaces in human hips or knees often operate under severe conditions (*i.e.* high load and low speed), which is related to a boundary lubrication regime characterized by a very low friction coefficient (μ=0.005–0.02, for the human joint). Breakdown of this lubrication can lead to wear of the cartilage and to osteoarthritis. Insights into the molecular origins of cartilage lubrication could lead to more efficient medical treatments, tissue repair and to longer-lasting prostheses.

Studies have sought to identify the component of synovial fluid capable of playing the role of boundary lubricant. It was shown that the samples of synovial fluid contain phospholipid molecules (mostly choline) [2]. A stacking of 3 to 7 phospholipid bilayers separated by aqueous layers has been highlighted in almost all biological in-vivo rubbing surfaces and proposed to reduce the friction between biosurfaces [3]. Experiments carried out in Lyon (a collaboration between ILM/UCBL and LaMCoS/INSA de lyon) have confirmed this assumption using a home-made bio-tribometer constituted of two model hydrophilic surfaces (glass and soft hydrogel of pHEMA) covered each by a phospholipid bilayer [4-6]. The friction coefficient of model surfaces is reduced by nearly 2 orders of magnitude, to a very reproducible value μ=0.002 when surfaces are covered by a DPPC bilayer (gel phase). With only one bilayer in the contact region (gel or fluid phase), the friction coefficient was much higher and bilayers quickly degrade indicating that lubrication is ensured by the hydration layers between adjacent lipid bilayers [4]. Fluid bilayers are intrinsically less lubricant than solid ones suggesting that either lipid mobility or protrusion modes impact lubrication [6]. Finally, we had evidence that lipid bilayers might smooth substrate roughness (this is an important issue in the context of synovial joints as cartilage is very rough) [7].

Some theoretical models predict that the membrane roughness should increase with increasing substrate roughness [8]. In contrast, some other theoretical predictions [9] suggest that a complex series of partial unbinding transitions should be observed upon an increase the substrate roughness. As a consequence, the lipid membrane should be smoother than the substrate for large substrate roughnesss. In a different system, namely adhesion of graphene, both types of behaviors roughness increase [11], or smoothening via unbinding [10] have been observed, but there is up to date no clear experimental evidence in the case of lipid membranes.

The purpose of this proposal was to measure bilayer coverage, bilayer roughness and substrate-bilayer water thickness as a function of substrate roughness, bilayer method of preparation, buffer content and eventually temperature.

Methods

We recorded the specular reflectivity of a phospholipid bilayer deposited on two types of crystalline silicon (111): highly polished ones and rough ones prepared in Lyon by Reactive Ion etching (RIE).

Most of the bilayers were prepared by the vesicle fusion method. We also tested the influence of the lipid phase: fluid (POPC or DPPC at high temperature) or solid phases (DPPC RT). All incubations, rinsing periods, water contrast changes were automated thanks to a system of pumps. Up to four samples could be sequentially probed with 3 different water contrasts D20 / H20 / SMW (Silicon Match Water: 38%D20+62%H20) in order to facilitate the fitting while keeping the same bilayer. The lipid vesicles solutions used were POPC and DPPC with a 0.5 mg/ml concentration and containing vesicles measuring 100 nm in diameter. As this expected smoothing effect by bilayers may depend on the method of preparation, we also used at the end of the experimental run POPC bilayers prepared by the micelle co-adsorption method. Figure 1 shows examples of measured reflectivity curves with DPPC.

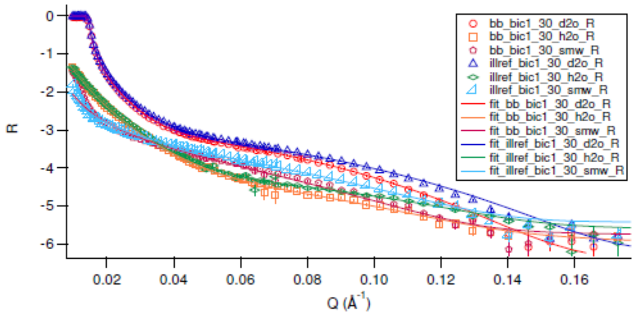

Figure.1: *Reflectivity curves of DPPC bilayers (at 30°C) deposited on various substrates with different water contrasts.*

Results

We have recorded a very complete set of data with a 0.5 nm RMS polished Si surface and a Si surface with a “mild” RIE etching (1 nm RMS) with POPC bilayers prepared by the vesicle fusion method or the micelle co-adsorption method as well as for DPPC bilayers (vesicle method) at 5 different temperatures (above and below the melting temperature at 43°C). For each we performed the three water contrasts and measured as well the reflectivity of bare substrates.

The clear trend of all these experiments is that all bilayers follow the substrate roughness and there are neither smoother neither rougher than the substrate.

Conclusions and perspectives

From theoretical estimates, we could expect a slight decoupling between substrate and bilayer roughness. However, perhaps to obtain such a decoupling, it is necessary to use rougher substrates. We are currently in Lyon measuring the diffusion constant of phospholipid fluid bilayers on different types of substrates including Si wafers, glass etched with different methods (dry or wet). These experiments should give us indications for future neutron reflectivity experiments.

References

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