Evidence for an unbinding transition for supported phospholipid bilayers on rough surfaces: combined FRAPP and AFM studies

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Supported lipid bilayers have been extensively used as model systems for cell membranes offering the possibility of applying surface sensitive techniques such as atomic force microscopy (AFM), fluorescence microscopy or X-rays and neutron scattering techniques [1]. Most of these techniques require atomically flat surfaces. But in vivo, the surfaces to which lipid layers interact are generally not flat. For instance, it was proposed that phospholipid multi-bilayers which are localized on the cartilage surface [2] may explain the exceptional lubricant properties of joints [3]. But the cartilage surface is very rough. Bilayers might span over nanoholes or cover nanoparticles with a diameter larger than about 20 nm [4]. Such an issue is very important for instance to understand how inhaled nanoparticles could penetrate the first pulmonary surfactant layers or to build lipid based sensors on nanoporous surfaces.

We obtained indirect evidences from tribological experiments that bilayer might smooth substrate roughness [5]. Furthermore, some theoretical predictions suggest that a complex series of partial unbinding transitions should be observed upon an increase in substrate roughness (i.e., increase in corrugation curvature). The bending modulus and bilayer-substrate adhesion are the important parameters triggering the transitions [6-7]. In a different field, namely adhesion of graphene on surfaces decorated with nanoparticles, both types of behaviors, roughness smoothening via unbinding [7] or, increase [8], have been observed, but there was up to date no clear experimental evidence in the case of lipid membrane.

The purpose of this work is to investigate whether such an unbinding transition occurs for supported lipid bilayers on rough surfaces. We worked with two different kinds of surfaces with a root mean squared (rms) roughness between 0.15 and 10 nm. Silicon wafers were etched using reactive-ion technique, and BK7, a borosilicate crown glass, was prepared by a chemical etching process with a 1:1 sodium hydroxide:ethanol solution in an ultrasonic bath. Supported lipid bilayers of DMPC (melting temperature at 23°C) were prepared with Langmuir-Blogett/Langmuir-Schaeffer deposition techniques on such substrates. Two AFM images of a roughened silicon wafer and BK7 glass prior to lipid deposition are shown in Figures 1A-B. Notice that the silicon sample shows a dense forest of peaks (whose shape is currently limited by AFM tip convolution effects) while the glass sample show rather a flat surface with large and deep holes (up to 50nm in depth). After the deposition, we did not find any significant topographical difference both in the gel and fluid phases but it could be due to the too large force exerted by the tip during scanning. We have hence performed a non-contacting technique, namely Fluorescence Recovery after Patterned Photobleaching.
In order to measure the apparent diffusion coefficient $D$ of phospholipids in bilayers deposited on rough surfaces. We studied the influence of temperature on diffusion of DMPC lipids on rough surface and the effect of calcium.

We will show that our results evidence for a possible unbinding transition especially in the gel phase while in the fluid phase our observations will be discussed in the framework of current theoretical models.

Figure 1: (A) 1x1 $\mu$m$^2$ AFM image in air (tapping mode) of silicon wafer surface etched using Oxford RIE machine (RIE 80) during 30 minutes (RMS =1.76 nm) (B) 5x5 $\mu$m$^2$ AFM image of a borosilicate crown glass BK7 (Melles-Griot) etched in a 1:1 sodium-hydroxide:ethanol solution in a US bath.