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

Florence Blachon[1], Frédéric Harb[2], Agnès Piednoir[1], Rémy Fulcrand[1], Olivier Pierre-Louis[1], Bernard Tinland[3] and Jean-Paul Rieu[1]

[1] Institut Lumière Matière, Unité Mixte de Recherche 5306, Université Lyon 1–Centre National de la Recherche Scientifique, Université de Lyon, 69622 Villeurbanne, France
[2] Department of Biology; Faculty of Sciences – Section II, Lebanese University, Lebanon
[3] CINaM- CNRS, Aix-Marseille Université, UMR7325, 13288, Marseille, France
florence.blachon@univ-lyon1.ftr

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 a ultrasonic bath. Supported lipid bilayers of DMPC (melting temperature at 23°C) were prepared with Langmuir-Blodgett/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

(FRAPP) 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.

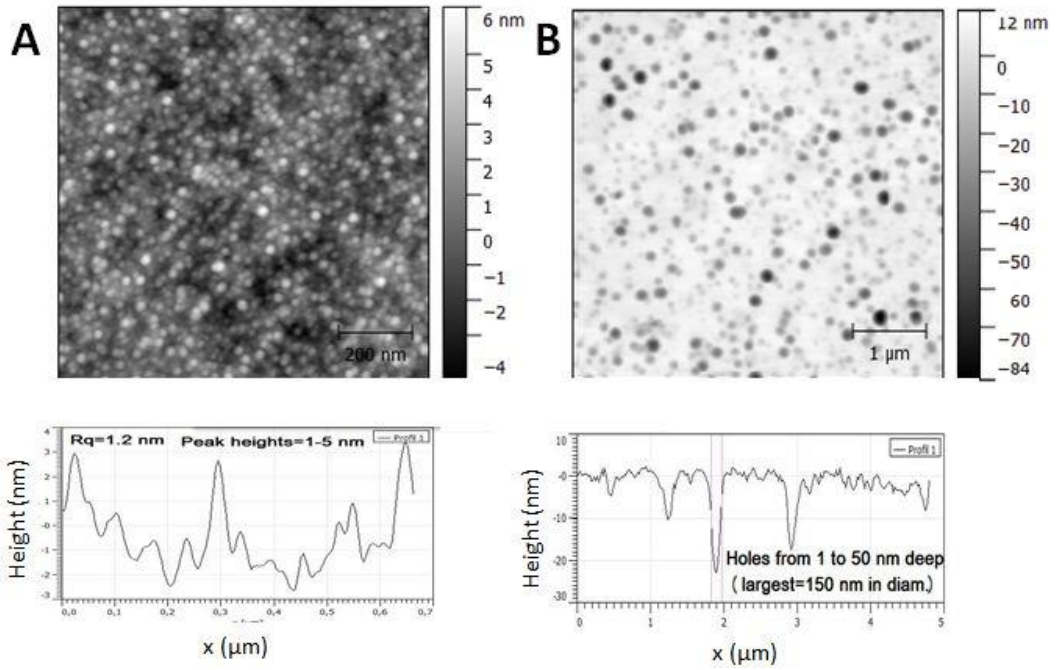

Figure 1 : (A) 1x1 µm^2 AFM image in air (tapping mode) of silicon wafer surface etched using Oxford RIE machine (RIE 80) during 30 minutes (RMS =1.76 nm) (B) 5x5 µm^2 AFM image of a borosilicate crown glass BK7 (Melles-Griot) etched in a 1:1 sodium-hydroxide:ethanol solution in a US bath.

[1] Giovanna Fragneto, Thierry Charitat, Jean Daillant. Floating lipid bilayers: models for physics and biology Eur Biophys J (2012) 41:863–874.

[2] A. V. Sarma, G. L. Powell, et M. LaBerge, « Phospholipid composition of articular cartilage boundary lubricant », *J. Orthop. Res.*, vol. 19, nº 4, p. 671-676, juill. 2001.

[3] A.-M. Trunfio-Sfarghiu, Y. Berthier, M.-H. Meurisse, et J.-P. Rieu, « Role of Nanomechanical Properties in the Tribological Performance of Phospholipid Biomimetic Surfaces », *Langmuir*, vol. 24, nº 16, p. 8765-8771, août 2008.

[4] Y Roiter, M Ornatska, AR Rammohan, J Balakrishnan, DR Heine, S Minko. Interaction of Lipid Membrane with Nanostructured Surfaces. Langmuir 2009, 25(11), 6287–6299

[5] M.-C. Corneci, F. Dekkiche, A.-M. Trunfio-Sfarghiu, M.-H. Meurisse, Y. Berthier, et J.-P. Rieu, « Tribological properties of fluid phase phospholipid bilayers », *Tribol. Int.*, vol. 44, nº 12, p. 1959-1968, nov. 2011.

[6] O. Pierre-Louis, « Adhesion of membranes and filaments on rippled surfaces », *Phys. Rev. E*, vol. 78, nº 2, p. 021603, août 2008.

[7] W. G. Cullen, M. Yamamoto, K. M. Burson, J. H. Chen, C. Jang, L. Li, M. S. Fuhrer, et E. D. Williams, « High-Fidelity Conformation of Graphene to ${\mathrm{SiO}}_{2}$ Topographic Features », *Phys. Rev. Lett.*, vol. 105, nº 21, p. 215504, nov. 2010.

[8] J. Nicolle, D. Machon, P. Poncharal, O. Pierre-Louis, et A. San-Miguel, « Pressure-Mediated Doping in Graphene », *Nano Lett.*, vol. 11, nº 9, p. 3564-3568, sept. 2011.