

Phase Behavior and Structure Properties of Supported Lipid Monolayers and Bilayers in Interaction with Silica Nanoparticles

Ndeye Rokhaya Faye, Ibtissem Gammoudi, Fabien Moroté, Christine Grauby-Heywang, and Touria Cohen-Bouhacina

Abstract—In this study we investigate silica nanoparticle (SiO_2 -NP) effects on the structure and phase properties of supported lipid monolayers and bilayers, coupling surface pressure measurements, fluorescence microscopy and atomic force microscopy. SiO_2 -NPs typically in size range of 10nm to 100 nm in diameter are tested. Our results suggest first that lipid molecules organization depends to their nature. Secondly, lipid molecules in the vicinity of big aggregates nanoparticles organize in liquid condensed phase whereas small aggregates are localized in both fluid liquid-expanded (LE) and liquid-condensed (LC). We demonstrated also by atomic force microscopy that by measuring friction forces it is possible to get information as if nanoparticle aggregates are recovered or not by lipid monolayers and bilayers.

Keywords—Atomic force microscopy, fluorescence microscopy, Langmuir films, silica nanoparticles, supported membrane models.

I. INTRODUCTION

NANOPARTICLES are widespread used in our environment, since a lot of products containing them are available in various technological fields, (paints, cosmetics, clothing,...). These last years, an increasing interest has been motivated by their physico-chemical properties differing from those of the same materials in bulk. However, an important question concerns the potential toxic effects of these NPs in cells, since it has been shown that they are able to cross the cellular membrane and to interact with intracellular biological compounds [1]. A possible mechanism of their toxicity would

lie in the interaction of NPs with membranes, since recent papers demonstrate clearly that NPs are able to induce nanoscale holes in membranes and their thinning or disruption [2], [3]. Some studies suggest that various parameters such as the size, shape, surface chemistry, composition, and aggregation state of NPs would be determining in NP-membrane interaction [2], [4], [5], [6], [7].

To date, majority of nanotoxicity studies were focused on cell cultures or *in vivo* systems [8], [9], [10]. However, because of the structural complexity and the large wide of cellular processes which take place in cell membranes, *in vivo* studies of these interactions are often difficult to assess. In particular, it is well-accepted now that membrane lipids are not randomly distributed in membranes: some of them are rather concentrated in domains called rafts, which are probably involved in different cellular processes, whereas other lipids are present in the fluid phase of membranes [11]. It explains that in recent years, new approaches have been developed to study the NP-membrane interaction by using models of biological membranes, mimicking their bilayer structure, but limited to only a few lipids in composition. Among these models, two-dimensional ones are particularly interesting, such as lipid monolayers and bilayers deposited on planar supports, usually built by Langmuir-Blodgett (LB) and Langmuir-Schaeffer (LS) transfer or vesicle spreading [12], [13], [14], [15]. Indeed they are simple to prepare and it is possible to access the lateral packing of lipid molecules (and thus the lipid phase) in the case of a LB-LS transfer by controlling the surface pressure in the monolayer during its transfer. At last, these models are particularly well-adapted to surface-sensitive physical techniques, such as atomic force microscopy (AFM) and fluorescence microscopy (FM) [16]. These two methods enable to characterize the morphology of lipid monolayers and bilayers at different lateral resolutions, in the micrometer range for FM and in the nanometer range for AFM. Moreover, AFM does not require the presence of a fluorescent probe, and offers the opportunity to study the topography of the samples by determining height profile with a sub-nanometer range. At last, this technique offers the opportunity to probe locally the mechanical properties of soft matter with less damage [17], [18], [19], [20], [21], [22].

In our case, we applied these methods to the study of lipid planar monolayers and bilayers deposited on nude mica

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surfaces and mica previously nanostructured by silica NPs (SiO_2 -NPs), in order to mimick the NPs-membrane interaction. Different lipids characterized by different transition temperatures (T_m) were tested. The T_m value, depending on different parameters such as the length of lipid chains and their degree of unsaturation, defines the lipid phase at the experimental temperature T : roughly lipids are in a disordered liquid expanded (LE) phase if $T > T_m$, whereas they are in an ordered condensed (LC) phase in the opposite case. In this work, we describe results obtained with three different lipids: POPC, an unsaturated lipid in the liquid expanded phase at ambient temperature ($T_m = -2^\circ\text{C}$); DMPC, a saturated lipid with an ambient transition temperature ($T_m = 23^\circ\text{C}$); SM, a lipid characterized by a high T_m ($T_m = 41^\circ\text{C}$) and preferentially concentrated in raft. These lipids organized in monolayer or bilayer were deposited on mica surfaces previously nanostructured with SiO_2 -NPs of various diameters from 10 to 100 nm, thus giving different local curvatures. Understanding the effects of surface defects and patterns on supported lipid monolayers or bilayers is a valuable step to assess information about NP-membrane interaction.

II. MATERIALS AND METHODS

Lipids (Fig. 1) were purchased from Avanti Polar Lipids (USA): *N*-palmitoyl-*D*-erythro-sphingosylphosphorylcholine (SM), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC). They were >99% pure and used without further purification. The fluorescent probe (2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl-amino)dodecanoyl-1-hexadecanoyl-sn-glycero-phosphocholine) (NBD-PC) was purchased from Molecular Probes (USA). Ethanol and chloroform used to solubilize lipids and the probe were both HPLC grade and purchased from Fluka and Sigma, respectively. Tris buffer and salts were purchased from Sigma. Millipore water (pH 5.5, resistivity > 18.2 M Ω .cm) was used as subphase for surface pressure measurements and buffer preparation.

Freshly cleaved ruby muscovite mica (Electron Microscopy Sciences) was used as solid support for Langmuir-Blodgett transfers. SiO_2 -NPs (10 nm, 50 nm and 100 nm in diameter) were purchased from Biovalley (Marne-la-Vallée, France). The other SiO_2 -NPs at 4 nm and 20 nm in diameter were purchased from Alfa Aesar (Schiltigheim, France). QCM-D sensor crystal (AT-cut, 5 MHz) with gold electrodes and thin upper layer of silicon oxide (30nm) were purchased from Lot-Oriel (Massy-France).

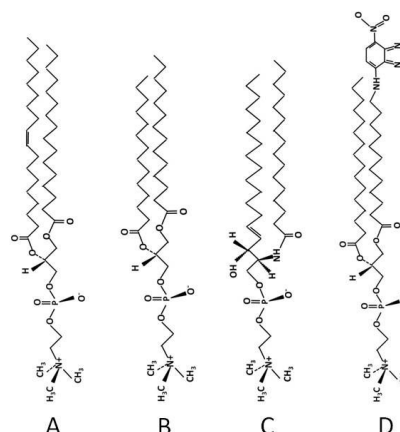


Fig. 1 Chemical structure of (A) POPC, (B) DMPC, (C) SM and (D) NBD-PC

A. Preparation of Nanostructured Surfaces

NP suspensions were first cleaned by three cycles of centrifugation, NPs being redispersed in ultra pure water after each centrifugation to ensure to remove surfactant impurity from the final colloidal suspensions. After their dilution at a final concentration of about 0.1mg/mL, 5 μL were deposited on freshly cleaved mica following two methods: in the first one, samples were simply dried, whereas in the second one, the spreading of NPs on the surface was favored by spin-coating (300 rpm, 1 minute). In all cases, these nanostructured surfaces were dried under a desiccator overnight at ambient temperature, before being used for lipid monolayers and bilayers deposition. Consequently, the main part of NPs was washed from the surface, but a sufficient amount remained on the surface, as shown by AFM experiments performed at different steps of the procedure (data not shown).

B. Surface Pressure Measurements and LB Transfer

Surface pressure experiments were carried out in air, at a temperature of $20 \pm 1^\circ\text{C}$, using a Langmuir trough (KSV NIMA, Finland) equipped with a Wilhelmy balance. Π -A isotherms were obtained by measuring the surface pressure Π ($\Pi = \gamma_w - \gamma$, where γ_w and γ are the surface tensions of water in the absence and in the presence of lipids, respectively) as a function of the mean molecular area A occupied by each lipid molecule at each step of the monolayer compression (Fig. 2). Briefly, lipids were dissolved in a 1/1 (v/v) mixture of ethanol and chloroform at a concentration of 1mM. Nude or nanostructured mica was immersed in water, perpendicularly to the interface. Lipid solution was spread at the air-water interface using a Hamilton syringe. After evaporation of solvents (15 minutes), lipids were compressed continuously at a rate of 5 $\text{cm}^2 \cdot \text{min}^{-1}$, until a surface pressure of 30 $\text{mN} \cdot \text{m}^{-1}$, this value being in the estimated range of membrane surface pressures. The surface pressure was then kept constant thanks to a control system adjusting continuously the surface occupied by the lipid monolayer. After the stabilization of the monolayer (a few minutes), the mica surface was removed

from the subphase at a rate of $2 \text{ mm} \cdot \text{min}^{-1}$ by using an automatic dipper from NIMA (Fig. 2).

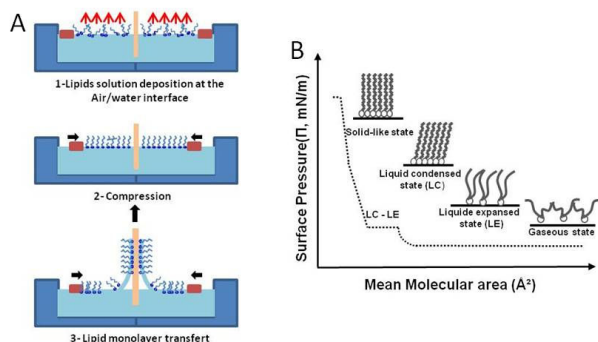


Fig. 2 Langmuir-Blodgett principle, (B) Theoretical isotherm (Π -A) obtained by compressing an insoluble lipid monolayer formed at an air / water (or buffer) interface

The LB film was then kept protected from dust under a desiccator until its imaging by AFM and FM. It is also possible to transfer a second layer, in order to have a bilayer. In this case, the mica surface with the first monolayer is placed parallel to the air-water interface and dipped in order to put it in contact with the monolayer spread at the interface. The second layer is transferred on the first one, forming a bilayer in contact with water. At least three LB films or bilayers were prepared under the same conditions to ensure reproducibility.

C. Unilamellar DMPC Vesicle Preparation

Unilamellar DMPC vesicles were prepared using the film re-hydration and extrusion methods. Briefly, an appropriate mass of lipids was dissolved in chloroform in a round bottom flask. A thin film of lipids was obtained after evaporating the solvent by rotating the flask for at least 3 hours. The lipid film was then hydrated in Tris-Buffer (10mM Tris, 150mM NaCl, 2mM CaCl_2 , pH 7.6) at a temperature of 40°C , thus above the T_m of DMPC, with a final lipid concentration of 5 mg/mL . At this step, multilamellar vesicles were obtained. To obtain unilamellar vesicles, the solution was vortexed, then extruded 15 times at 40°C through a polycarbonate membrane (pore diameter of 200 nm) (Avanti Polar Lipids Inc). Unilamellar vesicle solution was immediately stored at 4°C and used within 2 weeks. Vesicle size was characterized using dynamic light scattering (ALV-7000, Germany). The measured average hydrodynamic diameter of DMPC unilamellar vesicles was $208 \pm 6 \text{ nm}$.

D. QCM-D Monitoring

The QCM-D principle is based on real time measurement of the frequency of piezoelectric quartz crystal oscillator sensor on which a thin layer of (soft) material is deposited. The variation of the frequency is directly proportional to the change in mass (due to molecules adsorbed on the surface) and to the density and viscosity of the liquid in contact with the quartz [14]. We applied this method to the characterization of

DMPC bilayers obtained by vesicle spreading. QCM-D sensor crystal (AT-cut quartz) was rinsed in water and ethanol successively and exposed to UV-Ozone for 10 min. This process was repeated twice, and to make sure to remove all traces of water a last cleaning step was done by rinsing the crystal in iso-propanol, then exposing it to UV-ozone for 30 min. All QCM-D measurements were made at the first overtone ($n = 1$, 5MHz) using SiO_2 -coating quartz crystal (Lot Quantum Design, France), a commercial liquid cell (QCM 500W-KSV) and a homemade experimental setup. First TRIS-buffer was injected into the measurement cell thanks to the flow pump (REGLO Digital MS-2/6, ISMATEC) and changes in the frequency (Δf) and dissipation (ΔD) as function of the time were measured. After frequency and dissipation stabilization, vesicles suspension was injected and Δf and ΔD were recorded. All measurements were performed at the working temperature of 32°C , thus higher to the T_m value to promote vesicles spreading on the quartz crystal surface.

E. Fluorescence Microscopy (FM) Experiments

NBD-PC probe was dissolved in chloroform/ethanol 1/1 (v/v) and was added at 0.5 mol% in the lipid solution before spreading. This probe is currently used to characterize phase separation in lipid systems, since it concentrates in fluid phases and is expelled from condensed ones [23], [24], [25]. After its transfer, the LB film, enclosed in a black box, was placed on the stage of a fixed-stage upright microscope (BX51WI from Olympus) equipped with a 100 W mercury lamp (U-LH100HG), a BX-RFA illuminator, an U-MWBV2 filter cube (adapted to the absorption and emission spectra of NBD-PC centred at 470 nm and 540 nm, respectively) and LMPF1 x20 and x100 objectives. Images were recorded using a ORCA-Flash 2.8 CCD camera from Hamamatsu (Japan) and the HCImage Live V3.0 software. Taking into account the probe photobleaching, acquisition times were limited within 50 to 100 ms range, and several images were taken at different points of the samples to ensure homogeneity. The lateral resolution of our setup is in the $0.4\text{-}0.6 \mu\text{m}$ range.

F. Atomic Force Microscopy (AFM) Experiments

Atomic force microscopy is a high resolution scanning probe microscopy to characterize surface at the nanometer scale. Its principle consists in a sharp cantilever tip interacting with the sample surface and sensing the local forces with the sample surface (Fig. 3). Typically, these tip-sample forces are measured directly by the deflection of the cantilever on which a laser spot is reflected into an array of photodiodes. AFM can be performed through two modes: the contact and the dynamic mode. The first is used to obtain information on the topography (height signal) and rheological properties (friction signal) of different surface samples (hard, soft, conductor, non conductor,...). The second mode is in turn well suited for soft matter imaging and provides information such as the topography (height signal), mechanical and rheological properties such as heterogeneity and viscoelasticity through the phase signal. The error signal can also contain interesting

informations. Samples were investigated using two commercial AFM setups: (1) the BioscopeII AFM one (Veeco-Brucker, Santa Barbara, CA) equipped with a G scanner (maximum XYZ scan range of $150\ \mu\text{m} \times 150\ \mu\text{m} \times 12\ \mu\text{m}$), (2) the Nanoscope III one (Multimode Veeco-Brucker) equipped with a J (maximum XYZ scan range of $125\ \mu\text{m} \times 125\ \mu\text{m} \times 5\ \mu\text{m}$) and E (maximum XYZ scan range of $10\ \mu\text{m} \times 10\ \mu\text{m} \times 2.5\ \mu\text{m}$) scanners. In this work, sample surfaces were scanned in tapping mode using PPP-NCL silicon probes (NANOSensorsTM) with a spring constant and a corresponding measured resonance frequency (f_0) ranging between 21 mN/m and 98 mN/m, and 146 KHz and 236 KHz respectively. In contact mode, triangular SNL silicon nitride probes (Brucker, France) with a nominal spring constant of 0.12 N/m were used. AFM measurements were performed in air for monolayers, and in liquid (buffer) for the bilayer study, at room temperature ($20 \pm 1^\circ\text{C}$) with scan rates between 0.3 Hz and 1 Hz (according to the scan size and the scanning mode). Monolayer samples are scanned just after deposition in both tapping and contact modes whereas bilayers are scanned only in contact mode. AFM data were processed using the Nanoscope (Nanoscope 7.30, Veeco) and the Gwiddion softwares. Images at different places were recorded as in the case of FM experiments. Each time, six images were recorded at the same time: trace and retrace height images (topography), trace and retrace amplitude images (error signal) and trace and retrace phase images (mechanical properties) in tapping mode; trace and retrace height images (topography), trace and retrace deflection images (error signal) and trace and retrace friction images (mechanical properties) in contact mode. We point out that according to the expected information we want to highlight we will show one or few of these images and for clarity only flattened images (512×512 pixels) are shown in this paper.

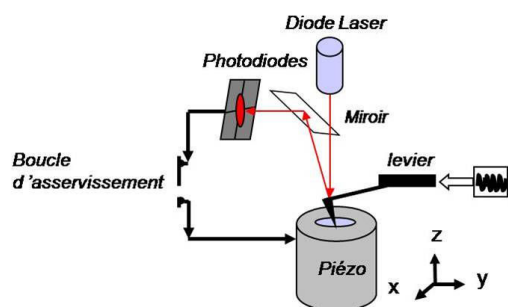


Fig. 3 Atomic force microscopy (AFM) principle

III. RESULTS AND DISCUSSION

A. Nanostructured Surfaces Characterization by AFM

Fig. 4 shows examples of AFM images obtained with 100 nm-SiO₂ NPs deposited on mica with the spin coating deposition method.

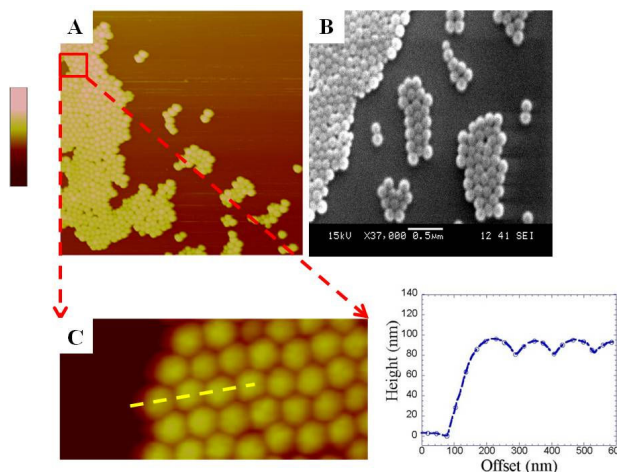


Fig. 4 AFM images ($5\ \mu\text{m} \times 5\ \mu\text{m}$) of mica nanostructured by deposition of 100-nm-SiO₂. Profile shows the height of nanoparticle corresponding to the diameter of the latter. Scale bar for height image (A) are 250 nm. Scale bar for SEM image is $0.5\ \mu\text{m}$ (only results with SiO₂-NPs with 100 nm in diameter are shown)

They show small aggregates (few hundred nanoparticles in size) where NPs are regularly organized in a one-layer structure, and isolated NPs (Fig. 4 A and Fig. 4 C). In the case of the direct deposition method, NPs are assembled in very dense and multilamellar networks (data not shown). These results are confirmed by images obtained by scanning electron microscopy (Fig. 4 B).

B. Surface Pressure Measurements

Fig. 5 shows Π -A isotherms for POPC, DMPC and SM. Both isotherms show a rather regular increase of surface pressure with compression, but the DMPC and SM isotherms are shifted to lower mean molecular areas. For instance at the surface pressure of transfer (30 mN/m), mean molecular areas are $105\ \text{\AA}^2$, $80\ \text{\AA}^2$ and $72\ \text{\AA}^2$ for POPC, SM and DMPC, respectively. Under our experimental conditions, POPC is clearly in a fluid liquid-expanded (LE) phase during all the compression.

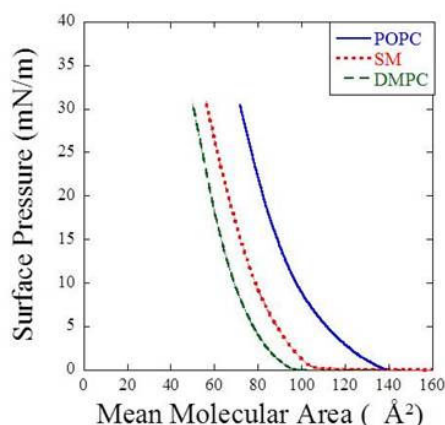


Fig. 5 Π -A isotherms for POPC, DMPC and SM Langmuir monolayers at the air-water interface

In the case of DMPC and SM, the isotherms suggest the existence of a phase transition because of a subtle change in the slopes of the isotherm. At low surface pressure, molecules would be in a LE phase. A further compression would be responsible for a transition in a more organized liquid condensed (LC) phase. Under these conditions, FM and AFM images are very useful to precise this point.

C. Supported Lipid Monolayers Study

Fig. 6 shows a very homogenous FM image of a POPC monolayer transferred at 30 mN/m, confirming that POPC is in a LE phase. On the contrary, images of DMPC and SM monolayers show clearly the co-existence of two phases, one concentrating the probe and corresponding to lipid molecules in the LE phase, and the other one expelling the probe and assigned to the LC phase. SM molecules in LC phase organize in flower-like domains with an average size of about 17.3 μm , whereas DMPC form rather circular domains of heterogeneous size and having an average diameter of about 6.4 μm . Previous studies of SM monolayers at the air-water interface or on planar support report the same heterogeneous organization and domain morphology [25]. As shown in Fig. 7, AFM images of POPC, DMPC and SM monolayers confirm fluorescence results. Indeed AFM images reveal homogeneous and flat surface for POPC monolayers with an averaged roughness of about 1 \AA , and the co-existence of LE and LC phases in the case of SM and DMPC with the specific patterns previously described.

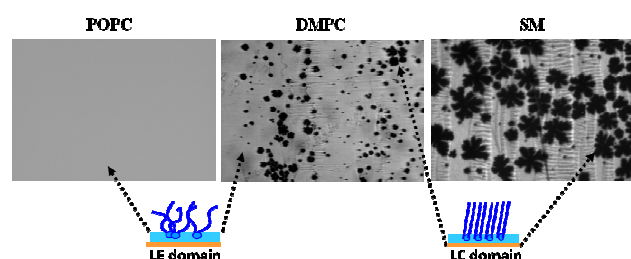


Fig. 6 FM images ($320\mu\text{m} \times 260\mu\text{m}$) of LB monolayers of POPC, DMPC and SM deposited at 30 mN/m

These results are in agreement with literature [26], [27], [28]. The different behavior of POPC and SM arises from differences in their structure. First, the *sn2* chain of POPC molecule bears a cis-double bond, preventing a regular molecular organization to install during the compression and explaining its low T_m . In the case of DMPC, the formation of domains in LC phase is favored by the saturation of its chains. SM chains are also saturated and elongated, favoring Van der Waals interactions between chains. Moreover, SM molecules are able to form intermolecular hydrogen bonds, whereas POPC and DMPC molecules are not. These bonds strengthen the molecular cohesion. For all the following results on the study of supported monolayers, we will focus POPC and SM.

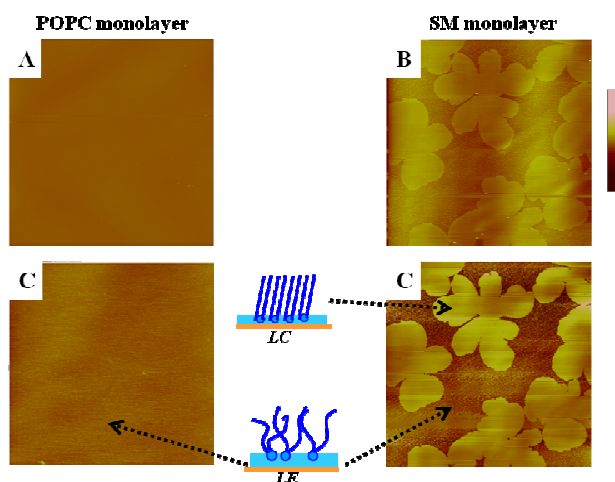


Fig. 7 AFM topography (A and B) and friction (C and D) images ($70\mu\text{m} \times 70\mu\text{m}$) of POPC and SM monolayers on nude mica. All AFM images are flattened. Scale bar for height and friction images are 10nm and 10mV respectively

After the characterization of nanostructured substrates and lipid monolayers, we studied the same monolayers transferred on nanostructured mica surfaces. In all cases, FM and AFM show no change in the organization of lipids in the presence of NPs, whatever their dimensions are, as shown for instance in Fig. 8 in the case of POPC and SM monolayers interacting with NPs of 100 nm in diameter. In particular, our imaging methods reveal the same shape of LC domains and quite the same distribution in the case of SM monolayers and DMPC (data not shown).

FM and AFM enable also to reveal that the size of the NP aggregate influence locally the lipid phase. Thus, SiO_2 -NPs small aggregates (less than 10 nanoparticles in size) creating locally a high curvature are localized in both LE and LC phases as well as in LC domain boundary regions. On the contrary, bigger aggregates are surrounded by lipid molecules in LC phase in the case of SM.

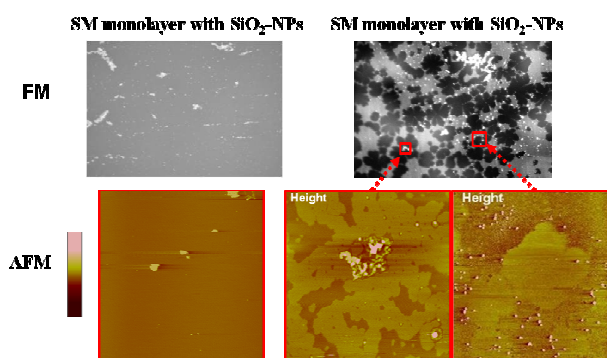


Fig. 8 FM and AFM images of POPC and SM monolayers in the presence of SiO_2 -NPs (100nm). AFM images are height images ($70\mu\text{m} \times 70\mu\text{m}$). Scale bar for height image is 10nm

D. Supported Lipid Bilayers Study

After this first step devoted to the optimization of experimental protocols (sample preparation, characterization conditions...), it is necessary to turn to supported lipid bilayers, which are more realistic membrane models, in order to extrapolate to mechanisms taking place in the interaction between NPs and membranes at the cell scale. Supported lipid bilayers consist in two lipid leaflets deposited onto a planar substrate. Two main methods are possible, a combination of LB-LS methods or the vesicles spreading. In the first method, the first monolayer is transferred by pulling vertically the solid support, whereas the second one is transferred by dipping horizontally the solid surface covered with the first leaflet. The vesicle spreading method is the most widely used method and consists basically in the adsorption and rupture of unilamellar lipid vesicles on a solid surface. We first concentrated our study on DMPC bilayers, which are widely described in literature [4], [29], [30].

In a first step, we studied by AFM the quality of DMPC planar bilayers prepared by the combination of LB-LS methods or the vesicle spreading method. AFM images (Fig 9) show that bilayers built with the first method cover bigger areas on mica surface. However, height profiles show that in some areas of the sample, the first monolayer is not covered by the second one (data not shown). Such an observation is recurrent in the case of lipid planar bilayers built by the LB-LS method. In the case of the vesicle spreading, AFM images show the presence of bilayer discontinuous patches. Their size depends on different parameters, such as the diameter of vesicles and the incubation time. These observations clearly show that the preparation method used to build the planar bilayers are particularly important, and led us to use the spreading method, which is widely applied, after optimization of different parameters such as the size of our lipid vesicles.

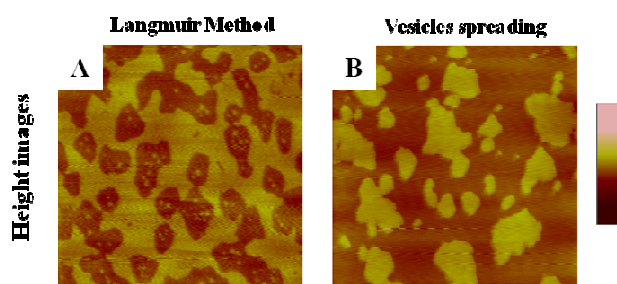


Fig. 9 AFM images of DMPC bilayers prepared by LB-LS methods (A) and by vesicle spreading (B). Scale bar for height image is 10nm

Supported lipid bilayer formation was optimized by QCM-D measurements, useful to determine the pertinent parameters for the bilayer building, by following the spontaneous adsorption and rupture of unilamellar vesicles thanks to changes of the resonance frequency and the dissipation energy, Δf and ΔD , respectively. Fig. 10 shows typical curves obtained when a SiO_2 -coated quartz crystal is put in contact with a solution of DMPC vesicles at 32°C.

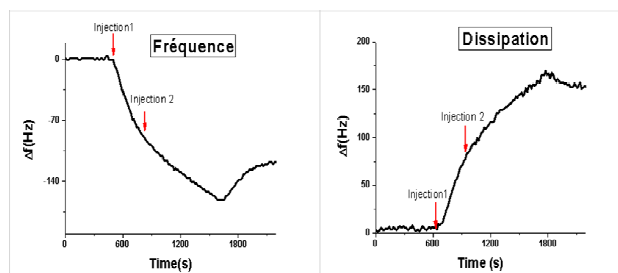


Fig. 10 QCM-D measurements (Δf and ΔD) during the formation of a DMPC bilayer on SiO_2 -coating quartz crystal at 32 °C

Just after the vesicle injection, a decrease of Δf and an increase of ΔD are systematically observed, because of the vesicle adsorption on the surface, inducing an increase of the mass in contact with the crystal surface. After a brief stabilization of both parameters, Δf increases and ΔD decreases, without reaching their initial values. These variations are due to the deformation and to the rupture of vesicles, once a critical concentration is reached on the quartz crystal surface, and more particularly to the water release during the rupture (mass of the trapped water in and between vesicles) [14], [31]. Δf and ΔD remain finally stable, once the bilayer is formed and stable. We are currently optimized supported lipid bilayer study by coupling AFM and QCM-D experiments.

The second step consists in the characterization of DMPC bilayers in interaction with nanostructured surfaces by AFM. In this case, the contrast observed in friction images can be really useful, since it can help to discriminate between NPs recovered or not by the bilayer because of different viscoelastic behaviors. Indeed, in the last case, the lipid bilayer forms a pore around the NP, this one being exposed to water. This information is thus complementary of morphological information shown previously. Some results, shown in Fig. 11, concern the interaction of DMPC bilayers with NPs of 10 nm in diameter: the contrast observed in different part of the images reveal different viscoelastic properties, suggesting two modes of interaction of these NPs with the bilayer. As shown in Fig 11A we observe both small and big aggregates interacting with DMPC bilayer.

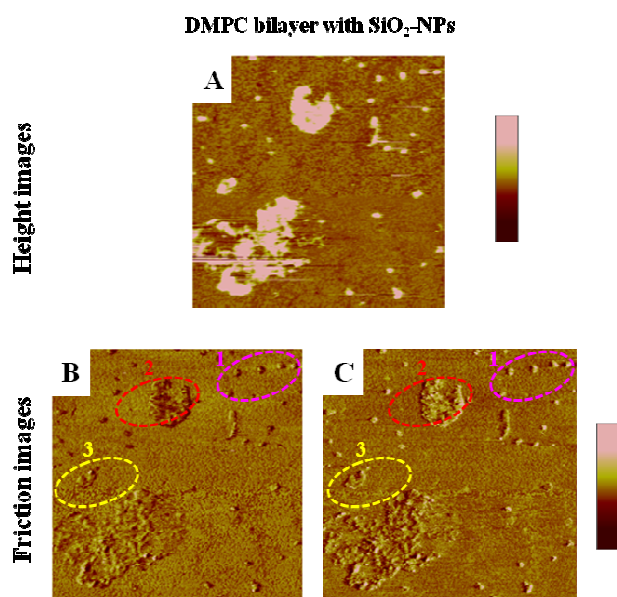


Fig. 11 DMPC supported bilayer in interaction with SiO₂-NPs (10nm).). All AFM images are flattened. Scale bar for height and friction images are 10nm and 10mV respectively

IV. CONCLUSION

In conclusion, we demonstrate in this work the potential of combining various surface-sensitive techniques to characterize qualitatively and quantitatively cell membrane models and their interaction with NPs. In particular AFM and FM enable to observe the effect of NPs on the lipid organization. AFM gives also very interesting information about the viscoelastic behavior of these systems, and thus about the possible recovering of NPs by the membrane model. Our first results encourage us to explore more deeply the effects of SiO₂-NPs on membrane models made of various lipids. At last, all the techniques described here can be applied to NPs, different in size, chemical composition.

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Ndeye Rokhaya Faye is born in Senegal in Poët. She obtained a Master 2 degree in Cutaneous Physiology, Pharmacology and Bioavailability at the University of Lyon 1 (France) in 2010. Before her master, Ndeye Rokhaya Faye got a BSc degree in biochemistry and a "Maîtrise" in structural biochemistry at the interface of biology, physics and chemistry at the University of Bordeaux 2 (France) in 2007 and 2009, respectively.

During her education, she applied her knowledge in Biochemistry and Biophysics to characterize the different conformations adopted by an antibacterial peptide during its insertion in lipid films at the air-water, during an internship in the European Institute of Chemistry and Biology of Bordeaux. During her Master, she also worked for almost 8 months at the Therapeutic Research Center of the School of Medicine-University of Queensland in Brisbane (Australia). During this internship, she studied in vivo and ex vivo redox state of keratinocytes (human skin) before and after penetration of silver nanoparticles, using multiphoton microscopy. Currently, she is in her third year of Ph.D. in the Laboratoire Ondes et Matière d'Aquitaine (LOMA, University of Bordeaux 1), funded by the Centre National de Recherche Scientifique (France) and the Aquitaine-Limousin region. Her thesis subject, at the interface between biology, physics and chemistry, focuses on the interaction of nanoparticles with simplified membrane models studied mainly by Atomic Force Microscopy (AFM) and fluorescence microscopy. To date, her results have been the subject of several oral presentations and posters in different national and international congresses. They have also been the theme of 2 publications: (1) "Oxidation of Langmuir-Blodgett films of monounsaturated lipids studied by Atomic Force Microscopy" *Int. J. Nanotechnology*. 2013; 10 (5):390 - 403, (2) "Nanoparticles and microparticles for skin drug delivery, advanced Drug Delivery" *Adv Drug Deliv Rev*. 2011 May 30; 63 (6):470-91. Two more publications are expected in the following weeks.

Ibtissem Gammoudi is born in Tunisia in February 1980. After preparing Master in Physical Chemistry of Condensed Matter (2007-2008) at the Monastir University she obtained her thesis, defended in June 2012 at the Bordeaux 1 University, entitled: on the development of bacteria based micro-biosensor for environmental monitoring, when she made her research at the IMS laboratory (laboratoire de l'Intégration du matériau au système) in collaboration with the Laboratoire Ondes et Matière d'Aquitaine (LOMA). Actually she held a temporary teaching and research position in University of Bordeaux 1-France.

Her research domain is multidisciplinary, at the interface between physics, chemistry and biology. The aim of her project is the design of a micro-biosensor based on bacteria that is sensitive and useful as a disposable sensor for environmental control and for industrial applications, using several methods such as Love wave, QCM-D, AFM and electrochemistry. Her results have been the subject of several oral presentations and posters in different national and international congresses. For example can be cited two articles where it was a first author: (1) Love wave bacterial biosensors for the detection of heavy metal toxicity in liquid medium. *Biosensors and Bioelectronics* (2010) 26 (4), pp. 1723-1726. (2). Love wave bacterial biosensors and microfluidic network for detection of heavy metal toxicity. *Sensor Letters*9 (2010) (2), pp. 816-819.

Fabien Moroté is born in France in 1987. He obtained his technical degree in physics at the University of Bordeaux 1 (France) in 2007. After this, Fabien Moroté got a professional BSc degree in physical measurements at the University of Albi in 2008.

He benefitted from two internships during his education in the Lyonnaise des eaux company, first in the metrology field, and secondly in the field of recovery and energy savings in a water treatment station. He was recruited in 2008 at the University of Bordeaux 1, in the technical staff of the Laboratoire Ondes et Matière d'Aquitaine (LOMA). He is in charge of the Nano-Spectro-

Imaging technical platform, and particularly involved in the maintenance of AFM setups and all the experimentations made on them. His collaboration with different teams led to several publications such as (1) "Oxidation of Langmuir-Blodgett films of monounsaturated lipids studied by Atomic Force Microscopy" *Int. J. Nanotechnology*. 2013; 10 (5):390 - 403, (2).

Christine Grauby-Heywang is born in France in 1971. She has a degree in biochemistry and molecular biophysics from the University of Paris 6 (France) and received her Ph.D. in molecular biophysics in this university in 1998.

After a temporary assistant-professor position at the University of Paris-Nord (France) and a post-doctoral position of one year in the group of E. Sackmann at the Technische Universität München (Germany), she became assistant-professor at the University of Bordeaux 1 (France) in 1999. Her research fields concern mainly Langmuir monolayers and supported models of cellular membranes obtained by Langmuir-Blodgett and Langmuir-Schaeffer methods or vesicle spreading, and made of lipids supposed to be present in rafts (glycolipids, sphingolipids, sterols). She studies also molecular planar systems made of organic molecules such as functionalized hemicyanines with particular optical properties, which can be applied for the specific detection of cations or light to electrical energy conversion. She turned two years ago to the interaction of nanoparticles with membrane models, studied by fluorescence microscopy and atomic force microscopy.

Grauby-Heywang (Dr) is a member of the French Biophysical Society.

Touria Cohen-Bouhacina Touria Cohen-Bouhacina is born in Algeria in September 1961. After making all her schooling (until Master 1) in Tlemcen - Algeria- she went to Toulouse-France- for continuing her graduate studies (Master 2 and PhD). Her thesis, defended in October 1989 at the Paul-Sabatier University, was focused on the relaxation in the insulating glass and semiconductors studied by electron paramagnetic resonance. She taught as master auxiliary in colleges and high schools, held a temporary assistant-professor position at the Paul-Sabatier University. In September 1991, she became assistant-professor at the University of Bordeaux 1 -France- and in September 2005 she was promoted to Professor at the same university.

Her research has always been multidisciplinary, at the interface between physics, chemistry and biology using atomic force microscopy - AFM for characterization. For example, she became interested in the physico-chemical treatment of surfaces (organosilane and / or polymers grafting, adsorption of molecules on surfaces), polymer conductors, adsorption of water on silica layers, biomaterials and cell adhesion, ATP-synthase, structure and sliding of fluid layers near a solid surface, supported models of cellular membranes ... Since 2009, Cohen-Bouhacina created her research group "Biophysics & Nanosystems" and she conducts studies related to toxicity problems: nanoparticle-membrane or bacteria interaction, bacteria-based biosensors as detectors of heavy metals, etc ... All of these studies combined different techniques of characterization such as atomic force microscopy, fluorescence microscopy, quartz microbalance dissipation, surface energy, contact angle, etc ...

Pr Touria Cohen-Bouhacina is a member of several scientific communities; she is, among other things, member of the Scientific Council of the "forum of local probes" and the "French Society of Microscopy".