

## NANO-BUBBLES AT POLYMER SUPPORTED HYBRID BIOMEMBRANES. NEUTRON REFLECTOMETRY AND AFM INVESTIGATIONS.

T.Gutberlet<sup>1,2</sup>, R.Steitz<sup>2,3</sup>, R.Krastev<sup>2</sup>, A.C.Simonsen<sup>4</sup> & B.Kloesgen<sup>4</sup>

<sup>1</sup>Paul Scherrer Institut, LNS, Villigen, Switzerland. <sup>2</sup>Hahn-Meitner-Institut, Berlin, Germany.

<sup>3</sup>Technische Universität Berlin, Berlin, Germany. <sup>4</sup>MEMPHIS, Physics Dept., University of Southern Denmark, Odense, Denmark.

**INTRODUCTION:** The controlled design of supported lipid bilayer probes as model biomembranes is a central challenge in the development of biosensors or as tools for basic biophysical investigations. To guide and control the fabrication of such probes at solid interfaces in-situ surface sensitive spectroscopic and microscopic techniques can be used.

In recent work we have investigated the adsorption of dimyristoyl phosphatidylcholine (DMPC) in water (D<sub>2</sub>O) onto a planar hydrophobic polystyrene interface, deposited by spin-coating on a Si-crystal (PS/Si), using neutron reflectivity [1]. Here, formation of a lipid monolayer should be observed at the surface of the soft cushion support and precipitation of further lipid molecules in time. Thus, a free floating fluid membrane should be formed in the vicinity of the solid/liquid interface. The quality of the adsorption of the lipid molecules to the interface is affected by the wetting behavior of the polymer support. Depletion of the boundary layer of water against hydrophobic substrates may occur interfering with the evolution of lipid layers at the solid/liquid interface. By neutron reflectivity and AFM we have studied this behavior at the above PS/Si system [2].

**METHODS:** PS/Si samples were composed of deuterated or protonated polystyrene layer (PS, M~65kD) deposited onto silicon single-crystal blocks with native oxide layer by spin coating (3500 rpm, toluene solution (5mg/ml)). Thereupon, lipid layers were adsorbed by exposure of the polymer surface to a vesicle suspension prepared by sonication of DMPC in water (0.5 mg/ml). The deposition was monitored by taking consecutive neutron reflectivity patterns at the neutron reflectometer V6 with  $\lambda=4.66\text{\AA}$  at BENSC, HMI Berlin [1]. The wet samples were investigated by tapping mode AFM, performed with the PicoSPM (Molecular Imaging, Phoenix, AZ) inMACmode (magnetic excitation of the cantilever) and using the medium range scanner (~30  $\mu\text{m}$  maximal scan width) [2].

**RESULTS & DISCUSSION:** Immediately after injection of the phospholipid vesicle suspension into the sample cell, formation of a lipid monolayer was observed in the reflectivity curve obtained. Further adsorption of an additional lipid bilayer occurred slowly in time. A thickness for each monolayer of about 16  $\text{\AA}$  was observed in the samples [1] in good agreement with small angle neutron scattering experiments of suspended large unilamellar vesicles. In contrast to measurements of LB deposited phospholipid double bilayers at the bare hydrophilic Si-crystal interface no distinct water gap between the adsorbed lipid monolayer and bilayer was revealed. This may be due to hydrophobic interactions between the polymer interface and the deposited lipid layers. Neutron reflectivity and AFM measurements of the PS/Si interface clearly demonstrated the existence of a nano-meter scale depletion layer before deposition of the phospholipid (Fig. 1) [2]. The origin of this depletion layer has been related to the existence of nanoscopic gas bubbles at the polymer/water interface. They may interfere with the adsorption of lipids to the interface leading to incomplete surface coverage or defects and produce artifacts in the analysis of the reflectivity measurements.

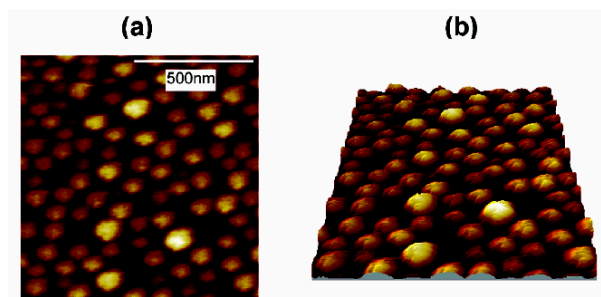


Fig. 1: Tapping mode topology image of nanobubbles on a  $1\ \mu\text{m}^2$  of the surface of a d-PS coated silicon substrate (film) in distilled water (a) and 3D projection (b).

**REFERENCES:** <sup>1</sup>T. Gutberlet, R. Steitz, J. Howse et al. (2002) *Appl. Phys. A* **74**[Suppl.]: S1262-63. <sup>2</sup>R. Steitz, T. Gutberlet, T. Hauss et al. (2003) *Langmuir* **19**:2409-18.