ESRF	**Experiment title:** **Cholesterol effect on the interactions between membrane inclusions**	**Experiment number:** 02-01-756
Beamline: BM02	**Date of experiment:** from: 23/01/2009 – 8:00 to: 25/01/2009 – 8:00	**Date of report:** 29/07/2009
Shifts: 6	Local contact: **Dr. Cyrille Rochas**	**Received at ESRF:**

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Report:

In a previous run (02-01-732), we had studied the membrane-mediated interaction between membrane inclusions, these latter being either antimicrobial peptides[1] or hybrid particles with an inorganic core[2]. The purpose of the present experiment was to study the effect of cholesterol (an important component of the cell membrane) and temperature on similar systems. Although very useful data was collected and a new paper is in progress[3], the goals of the proposal were not completely reached, due in part to the short time available (6 shifts granted of the 12 requested).

Setup and samples

The samples were held in 100 μm thick (and 2 mm wide) flat glass capillaries and we selected areas of homeotropic orientation (lamellae parallel to the glass walls). The scattering experiments were mostly performed in normal incidence on the capillary walls (i.e. the incident beam was parallel to the layer normal *z*), so the scattering vector *q* was contained within the (*x*,*y*) plane of the layers (some experiments were done with a varying incidence angle, see below). The X-ray energy was E = 11 keV and the sample-detector distance was 28 cm. The available *q*-range was about 0.05-0.9 $Å^{-1}$. The typical exposure times were 100−300 s.

Doped lamellar phases were prepared using the protocols already presented in previous publications: they consisted either of gramicidin-doped lipid bilayers[1] or of the zwitterionic surfactant dimethyldodecylamine-N-oxide (DDAO) doped with particles containing a tin oxide core grafted with butyl chains[2] (denoted in the following as BuSn). Each system was prepared along dilution lines with a varying density of inclusions, with and without added cholesterol.

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[1] Doru Constantin, Membrane-mediated repulsion between gramicidin pores *Biochimica et Biophysica Acta (BBA) - Biomembranes*, in press (2009).

[2] Doru Constantin, Brigitte Pansu, Marianne Impéror, Patrick Davidson, and François Ribot, Repulsion Between Inorganic Particles Inserted Within Surfactant Bilayers, *Physical Review Letters* **101**, 098101 (2008).

[3] Doru Constantin and François Ribot, The interaction of rigid nano-particles inserted within surfactant bilayers, in preparation.

Results

BuSn/DDAO: The temperature study of the BuSn/DDAO/cholesterol was mostly successful, although more time would have been needed to complete the investigation. We studied several dilution lines (with and without cholesterol) as a function of temperature, heating from room temperature to 140° C (above the melting temperature of the lamellar phase). The in-plane structure factor of the inclusions evolves substantially with the temperature T, exhibiting in particular a marked increase at small angles as T increases. This feature points towards a more attractive interaction between the articles at higher temperature. A systematic analysis is in progress, aiming to reveal changes in the interaction potential and/or the appearance of inhomogeneities in the plane of the membrane.

Reciprocal space cuts: In incidence normal to the layers, the reciprocal space is only probed in-plane (q_z~0). However, if the particles belonging to different layers interact, the structure factor is also modulated along q_z. A complete investigation requires access to the full 3D reciprocal space, which can be obtained by turning the layer normal with respect to the beam by an angle α, giving access to a reciprocal space sector with opening angle 2α. The analysis of the complete structure factor $S(q_r,q_z)$ can then yield the three-dimensional interaction potential $V(r,z)$ (work in progress). As shown in the Figure, there is some interaction across the layers (revealed by the presence of the peaks.)

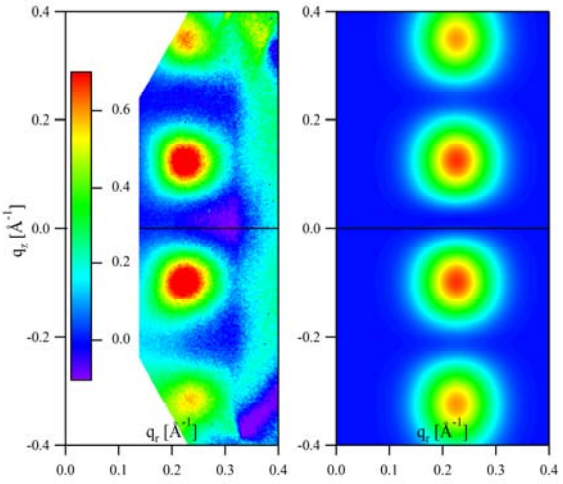

Figure: Structure factor of BuSn particles inserted within DDAO bilayers, after subtraction of the slice $S(q_z = 0)$. The peaks reveal the presence of inter-bilayer interaction. Left: experimental data. Right: theoretical model.

Gramicidin/lipid: Due to the presence of cholesterol, the samples were more difficult to orient and the homeotropic areas are smaller than for the cholesterol-free samples. Nevertheless, some data was acquired and the analysis is in progress.