ESRF	**Experiment title:** Structure and dynamics of lipid monolayers in interaction with trehalose	**Experiment number:** SC-2554
Beamline: ID10A	**Date of experiment:** from: 05/11/2008 to: 11/11/2008	**Date of report:** 24/02/2009
Shifts: 18	**Local contact(s):** Chiara Caronna	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Lorenzo Cordone[1*], Giorgio Schiro'[1*], Sergio Giuffrida[1], Grazia Cottone[1], Lutz Wiegart[2*]

[1]*Department of Physics and Astronomical Science of Palermo*

[2]*ESRF*

Report:

The aim of the experiment was to investigate the structural and dynamics properties of a phospholipid (dipalmitoyl-phosphatidyl-choline, DPPC) Langmuir monolayer in presence of trehalose in the acqeous subphase. Trehalose is a sugar found in large quantities in many organism that can survive adverse environments like extreme heat and desiccation. Though it is known that trehalose is responsible for the stabilization of biostructures (such as proteins and membranes) in adverse conditions, there is still an open debate on the microscopic mechanism that accounts for that [1]. Several hypotheses have been proposed: *the water entrapment hypothesis*, where it is assumed that the sugar traps residual water at the biomolecule/sugar interface, the *water replacement hypothesis*, according to which trehalose establishes hydrogen bonds directly with the biomolecules and thus stabilize the structure and the *anchorage hypotesis*. according to

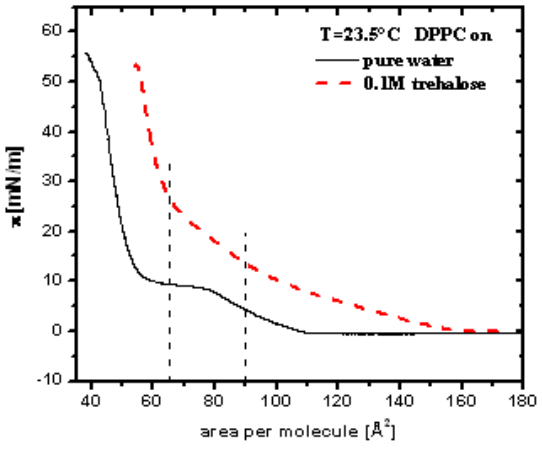

Figure 1: Isotherms of DPPC on pure water and on a 0.1M trehalose solution.

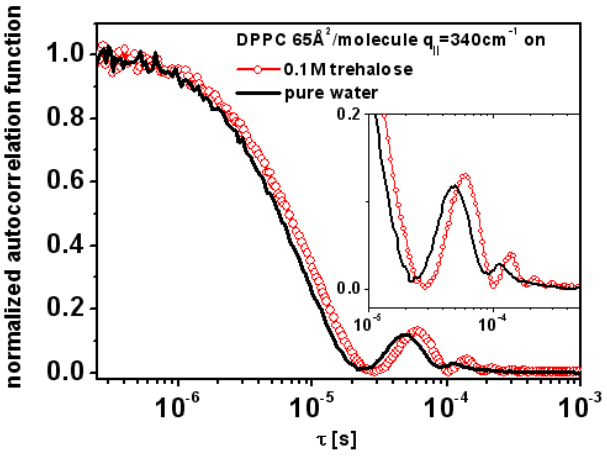

Figure 2: Correlation functions measured at T=23.5C and q=340 cm^{-1} on a high density DPPC monolayer (~65Å^2/molecule) on water and a 0.1 M trehalose solution. The inset is a zoom on the oscillations.

which a, water mediated, hydrogen bond network, whose strength increases by lowering the water content, anchors the biostructure to the surrounding water-sugar solid matrix.

Contrary to other sugars, which are expelled from the lipid layer at high compression, trehalose gets integrated into the membrane [2]. The presence of the sugar molecules certainly modifies the structural arrangements of the lipids, as can be seen from the area requirement per molecule in pressure area isotherms (Figure 1). Molecular dynamics studies [3] suggest a bridging of individual lipid molecules by the trehalose, resulting in a stabilization of the membrane. Such reinforcement of the layer will in turn alter the surface dynamics of the system, which is in the case of a lipid monolayer at the liquid-gas interface governed by propagating capillary waves, carachterized by a frequency ω and a damping constant Γ whose q-dependence is given by the viscoelastic properties of the subphase (e.g. the viscosity) and of the monolayer [4].

To address these points, we have performed an XPCS study at the beamline ID10A in grazing incidence geometry in order to follow the dynamics at the surface. We have measured the surface dynamics as a function of q of DPPC monolayers on a pure water subphase and on a threalose/water solution at different points along the corresponding isotherms, as depicted by the dashed vertical lines in figure 1.

Comparing the correlation functions obtained on monolayers with a given area per lipid molecule on pure water and a 0.1M trehalose solution an interesting effect is clearly visible. In the high density phase (65Å^2/molecule), the presence of trehalose induces a decrease of the propagation frequency (see the shift of the first maximum towards larger times in figure 2), as well as a decrease of the damping constant (as shown by the amplitude of the maximum in the inset of figure 2). A different scenario appears in the low density phase (~90Å^2/molecule), where the propagation frequency is unchanged in the presence of trehalose, while the damping constant clearly increases (figure 3). The q dependence of the frequency ω and the damping constant Γ can be obtained by fitting the correlation function with a damped cosine, $g(q,\tau) \propto \exp(-\Gamma\tau)\cos(\omega\tau)$, suitably convoluted with a resolution function [5], and are reported in figure 4. The data clearly show that over the whole q-range there is no difference in the propagation frequency between the pure water sample and the 0.1 M trehalose solution, while the damping constant of the trehalose sample is systematically higher then the one of pure water.

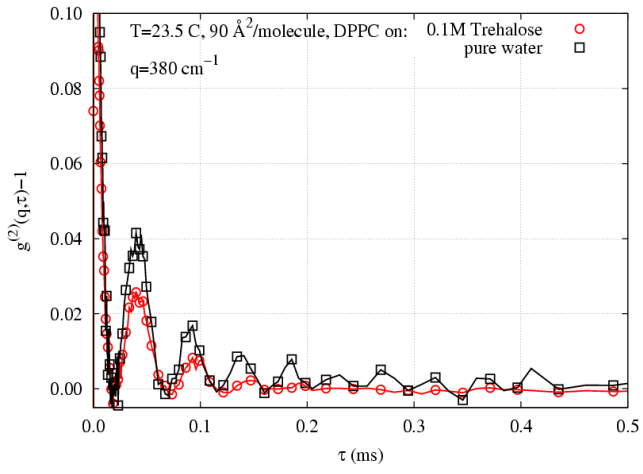

Figure 3: Correlation functions measured at T=23.5C and q=340 cm^{-1} on a low density DPPC monolayer (~90Å^2/molecule) on water and a 0.1 M trehalose solution.

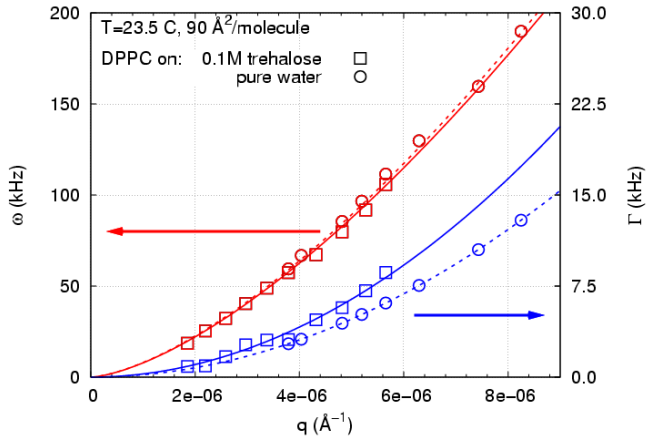

Figure 4: q-dependence of the damping constant Γ and the propagating frequency ω for a low density DPPC monolayer (~90Å^2/molecule) on water and a 0.1 M trehalose solution. Lines are fits according to the dispersion equations $\Gamma = 2\eta q^2/\rho$ and $\omega = \sqrt{\sigma q^3/\rho}$, where η is the viscosity, σ the surface tension and ρ the density.

Such preliminary results indicate a complex mechanism for the interaction of trehalose with the lipid monolayers. As a working hypothesis we assume that in the low density phase the trehalose adsorbs to the lipid molecules and causes an extra damping of the capillary waves by strengthening the interaction. At higher packing densities, the trehalose molecules act as spacers between the lipids and reduce their effective interaction, resulting in a reduced damping. A fitting of the dispersion curves with a model that takes into account the viscoelastic parameters of the monolayers is ongoing and will help in quantify the trehalose effect on the macroscopic parameters of the layer.

Clearly, the above hypothesis would also influence the structural arrangements of the lipid molecules in the monolayer. For this reason we aimed to perform simultaneous Grazing Incidence X-ray Diffraction measurements. The needed setup, where the two arms of the diffractometer are decoupled to allow to have a point detector along the diffuse scattering for recording the correlation function and at the same time a linear detector at an angle of ~20 degrees to measure the diffraction peaks has been successfully realized. Unfortunately, the diffractometer with the decoupled arms was less stable and subject to some vibrations, so that we couldn't measure the structure and the dynamics at the same time. However, we have been able to perform the GIXD measurements right after taking the XPCS data on the same sample. Figure 5 reports a GIXD scan on a DPPC monolayer (π=38mN/m) on a 0.1 M sucrose solution. The data have been collected with a linear detector during an acquisition time of 40s. It is important to note that such measurement has been realized with a coherent beam (size 10x10 μm^2), thus proving that with an enhanced stability of the diffractometer a simultaneous dynamics and static characterization of the monolayer is possible. A preliminary analysis of the diffraction pattern suggest that the 2D arrangement of the lipid molecules is not affected by the presence of the sucrose molecules, as the same structure as on pure water has been found. This goes well together with the fact that XPCS data on the same sample seem not to be different from the one in water (data not shown). A similar analysis will be done on the data collected on trehalose to retrieve the structural changes induced by this peculiar sugar.

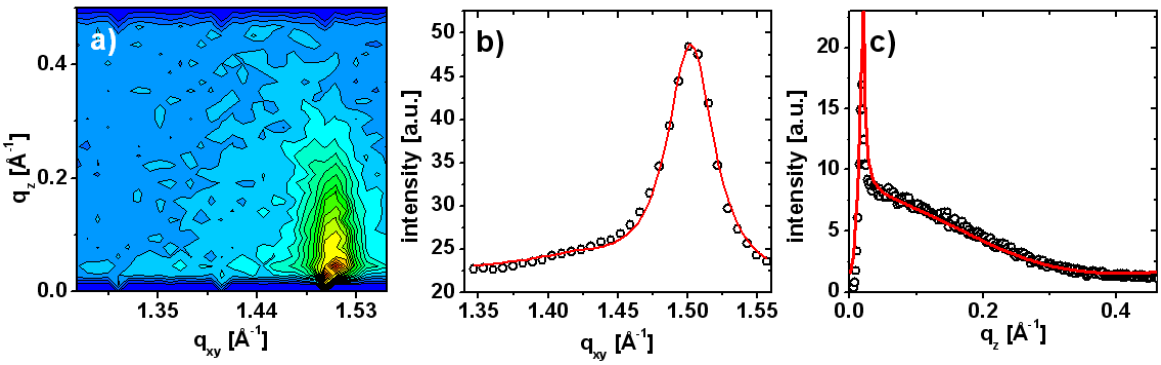

Figure 5: a) GIXD pattern of a DPPC monolayer on a 0.1 M sucrose/water solution. b) Diffraction pattern integrated along q_z and fit with Voigt model (solid line), the presence of only one diffraction peak suggest a hexagonal in-plane structure. c) Bragg rod corresponding to the diffraction peak shown in panel b), solid line is a fit with a cylinder model, the obtained parameters suggest an upright chain conformation.

References

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