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## Report:

**Introduction.** We report a complementary grazing incidence diffraction (GIXD) and X-ray reflectivity (XRR) study on lipid monolayers at the liquid-gas interphase in interaction with different sugars.
The aim of the experimental study was to investigate the structural changes in lipid Langmuir monolayers due to the interaction with different sugars from the aqueous subphase and to draw conclusions about the underlying interaction schemes. More specific, we were looking for the microscopic mechanism accounting for the peculiar properties of trehalose, a disaccharide of glucose present in large quantities in various extremophiles which can resists extreme temperatures and water scarcity. A possible theory explaining this peculiarity involves the anchoring of the bio-membrane via, water mediated, hydrogen bonds networks to the surrounding water-sugar solid matrix. Indeed, molecular dynamics simulations have pointed towards a bridging between individual lipid molecules by trehalose. To shed light on the role of the hydrogen bond (HB) networks the present study employs sugars with different hydrogen bonding properties with the surroundings: trehalose and sucrose. Both sugars act as HB acceptors and donors, but while trehalose cannot perform internal HB, sucrose can, besides hydrogen bonding with its surrounding, also establish two internal HB. Accordingly, under conditions of water scarcity, like e.g. in the liquid compressed phase of a Langmuir monolayer, trehalose keeps an extended HB network of increasing hardness, while sucrose increases the number of internal HB and thus reduces the connectivity of the film. To vary in addition the number of hydrogen bonding partner in the lipid, two different lipids, namely a phospholipid and a sphingolipid were chosen. While the first exhibits various binding locations in the glycerol backbone and the phosphocholine headgroup, these groups are absent in sphingolipids (see figure 1a) for lipids and sugars used in this study).

**Materials and Methods.** The lipids 1,2-dipalmitoyl-phosphatidyl-choline (DPPC) and N-palmitoyl-D-*erythro*-sphinganine (sphinganine) where purchased from Sigma Aldrich and Avanti Polar Lipids, respectively. Solutions for spreading with a microliter syringe (Hamilton) where prepared with highly pure chloroform (Fluka) at lipid concentrations of 0.2mg/ml. Ultra pure water (Millipore) was used for the subphase

preparation. Sucrose was purchased from Fluka and used without further purification. Trehalose was purchased from Hayashibara Shoji, and used after recrystallization. Due to the presence of some surfactant impurities, trehalose solution were left for a minimum of two days in a separatory funnel, to allow emergence of residual impurities to the surface, which was then removed. Additional cleaning was provided prior to monolayer preparation by leaving the aqueous sugar subphases in the Langmuir trough for an hour before closing the barrier and removing the top layer by means of a membrane pump.
X-ray measurements were carried out at ESRF beamline ID10B at an X-ray energy of 8.07keV (λ=1.536Å). A combined setup for GIXD and XRR was used, where the tilt angle of the incident beam with respect to the liquid surface was provided by a Ge-deflector. The incidence angle for GIXD was chosen as 0.12deg., thus below the critical angle of the aqueous subphase. Grazing incidence diffraction used a 150mm linear position sensitive detector (PSD) and a Soller collimator providing an in-plane resolution of 1.4mrad. The reflectivity signal was detected by a NaI point detector with two-pairs of horizontal and vertical slits as pre-detector collimation. The X-ray measurements used a custom build Langmuir trough equipped with a commercial film-balance (Nima). The subphase temperature adjusted by a circulating water bath monitored by a Pt100 temperature sensor. Off-line measurements of Langmuir pressure-area isotherms used a commercial Langmuir trough (Nima) and similar temperature control.

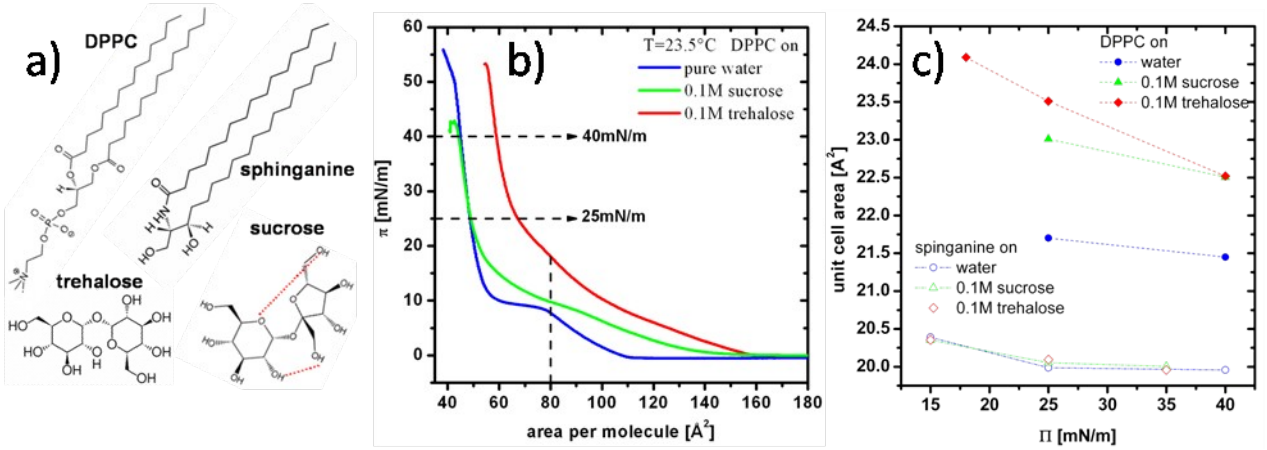


Figure 1. a) sketch of sugars and lipids used in this study. The red dashed lines in the case of sucrose indicate the internal hydrogen bonds. b) Langmuir pressure-area isotherms for DPPC monolayers on the different subphases. The dashed lines indicate the isobares and the isochore along which the GIXD measurements were performed. c) unit cell area for DPPC and sphinganine on the different subphases

**Results.** Langmuir pressure-area isotherms for DPPC on pure water and 0.1M sucrose and trehalose subphases are shown in figure 1 b). Besides the altered phase behavior at low surface pressure, the shift of the isotherm along the x-axis is evident in the presence of trehalose, pointing towards the sugar getting embedded in to the lipid film. Contrary, sucrose causes a similar alteration of the low surface pressure phases, but the corresponding isotherm resembles the one on pure water for the high pressure phase. While from a bio-physical point of view a comparison of the different systems at a given molecular density would be preferable, the shift in the isotherm for DPPC on 0.1M trehalose makes such comparison impossible for the high surface pressures. Consequently, the structures were compared at a molecular coverage of 80Å$^2$/molecule and along two isobares at 25 and 40mN/m. For sphinganine no distinct differences were found in the isotherms (not shown) and structures were compared along two isobares at 25 and 35mN/m. GIXD was used to get information about the lipid's in-plane ordering in the ordered phase at high surface pressures. Complementary XRR measurements reveal the layering of the film, including a potential absorption layer of sugar, perpendicular to the surface. However, analysis of the XRR data is still pending and the current report thus focuses on the GIXD. For a monolayer, GIXD does not only reveal the in-plane ordering, but also allows to probe directly the molecular form factor via the so-called Bragg rods. Together with apriori knowledge about the lipid film, like e.g. the fact that the observed reflections steam from an ordered alkane chain lattice, GIXD gives information about the 2d unit cell and the alignment of the alkane chains within. In order to obtain the in-plane reflections, the GIXD spectra were integrated along the PSD (corresponding to the $q_z$ direction) and fitted to a Voigt profile. The Bragg rods were then extracted as PSD spectra at fixed in-plane angle, corresponding to the position of the Bragg reflection. With the position of the maxima of the molecular form factor, all relevant parameters like the 2d unit cell, tilt angle and tilt direction of the alkane chains can be analytically calculated. As a last step, the Bragg rods were modeled with a cylinder model, which allows to

determine the length of the ordered part of the alkane chain. In general, two in-plane reflections were found for the DPPC monolayers, indicating a centered rectangular lattice, while for sphinganine only one reflection was found, fingerprint of a hexagonal lattice. These differences in the 2d lattice are not unexpected, as the known deviation of DPPC from a hexagonal lattice arises from its bulky headgroup and glycerol backbone. In the case of sphinganine the alkane chains are upright while for DPPC they exhibit a distinct tilt angle with a tilt direction towards the nearest neighbour. A comparison of the 2d unit cells for the investigated systems as a function of surface pressure is shown in figure 1 c). For sphinganine the influence of sucrose and trehalose on the 2d packing in the condensed phase is negligible, while for DPPC in the presence of both trehalose and sucrose the unit cell of the 2d lattice is significantly increased. The data point for DPPC on 0.1M trehalose indicated with an arrow corresponds to a lipid surface coverage of 80Å$^2$/molecule (from Langmuir isotherm) which only in the presence of trehalose corresponds to an ordered phase. Figure 2 a) shows the tilt angles for DPPC on the various subphases, which in general decreases upon compression. In the presence of sucrose and trehalose the tilt angle is greatly increased by up to 50%. For sphinganine on water, 0.1M sucrose and 0.1M trehalose as well as for DPPC on water is the alkane chain cross-section decreasing upon compression (see figure 2 b)). Contrary, for DPPC on 0.1M trehalose and 0.1M sucrose, the alkane chain cross section increases upon compression. This unusual behavior steams from the tilt angle reducing much more than the area of the 2d unit cell.

A possible model to understand the specific interactions between the two sugars and DPPC is sketched in figure 2 c). In the presence of sucrose, the sugar molecules bind to the headgroup of the phospholipid, leading to an effectively increased headgroup size. Consequences are a larger 2d unit cell and increased tilt angle in the ordered phases, as compared to DPPC on pure water. The effectively increased headgroup size of the lipid alters the Langmuir isotherm in the non-ordered phases, however, there is no increase in the molecule-molecule interaction and consequently no ordering is present at a molecular coverage of 80Å$^2$/molecule.

In the case of trehalose, the sugar also binds to the lipid headgroup, but contrary to sucrose, trehalose is able to form hydrogen bonds with itself, enabling the formation of a linked sugar matrix. The lipid bound to this matrix experiences not only an effectively increased headgroup size as in the case of sucrose, but moreover increased molecule-molecule interactions steaming from the sugar cross-links. Consequently, a shift in the phase diagram appears, allowing for an ordered phase already at the low average coverage of 80Å$^2$/molecule. For the ordered phases the effect of trehalose is similar to the one of sucrose, namely an increased area of the 2d unit cell and tilt angle. The shift in the isotherm towards a larger area per molecule which is observed for all phases points towards the sugar cross-links being embedded into the plane of the lipid layer.

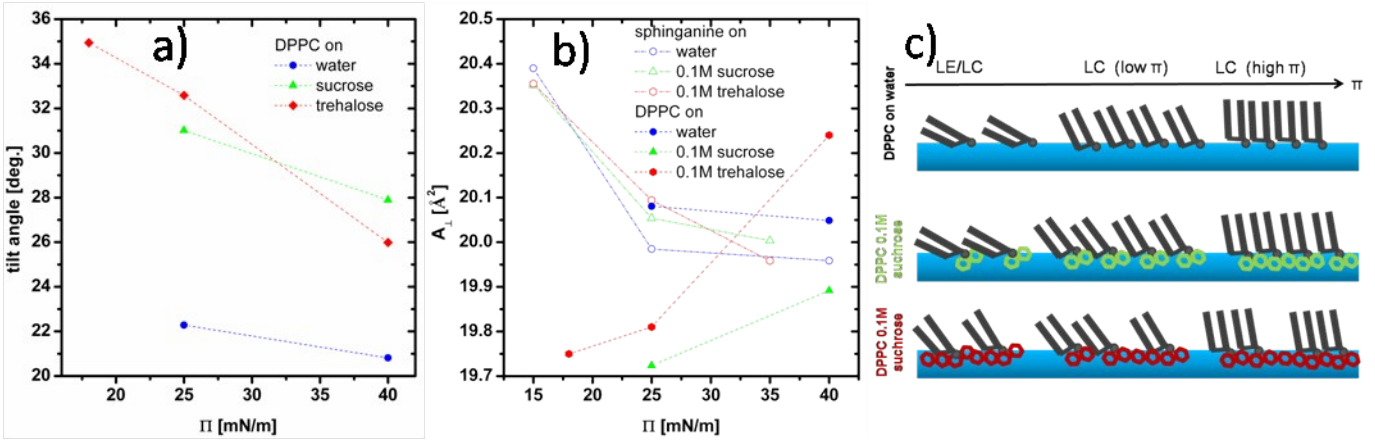


Figure 2. a) tilt angle between the lipid's alkane chains and the surface normal for DPPC. b) packing density (alkane chain cross section) for DPPC and sphinganine on different subphases. c) sketch of a model explaining the structural differences for DPPC on water and in the presence of sucrose and trehalose.