ESRF	**Experiment title:** Interaction of arachae lipid membranes derived from the thermoacidophilicarchaeon Sulfolobus Acidocaldarius with the polypeptide gramicidin D	**Experiment number**: SC-3051
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Report:

About 90% of the lipid components in the plasma membrane of the thermoacidophilic archaeon *S. Acidocaldarius* are dibiphytanyldiglycerol tetraether lipids [1], among which the polar lipid fraction E (PLFE) is one of the main constituents [2]. PLFE contains a mixture of bipolar tetraether lipids with either a glycerol dialkylcalditol tetraether (GDNT or calditoglycerocaldarchaeol; ~90% of total PLFE) or a glycerol dialkylglycerol tetraether (GDGT, or caldarchaeol; ~10% of total PLFE) skeleton [2-3]. Both GDGT and GDNT are bisubstituted in the polar headgroup regions, thus designated as bipolar tetraether lipids. The nonpolar regions of these lipids consist of a pair of 40-carbon biphytanyl chains, each of which contains up to four cyclopentane rings. Since PLFE is the major polar lipid component in the plasma membrane of *S. Acidocaldarius*, PLFE liposomes have been used as a model system for studying thermoacidophilic archaeal membranes.

Even though the properties of archaeal lipids were the subject matter of several studies now, the interaction of these lipids with peptides and proteins has not been explored so far. As suitable model system for a first investigation of the influence of a channel forming peptide on the structural properties and phase behaviour of these kind membranes, gramicidin will be chosen. Gramicidin is a polypeptide consisting of only 15 amino acid residues which have alternate L and D chirality. All side-chains of the peptide are nonpolar. Individual molecules can fold into single-stranded helices, which are stabilized by intramolecular hydrogen bonds, and can associate to helical dimers - at least in phospholipid bilayers - in which two single-stranded helices are joint end-to-end, such as the ss $\beta^{6.5}$ helical dimer with 6.5 residues per turn, which has an internal pore diameter of 3 – 4 Å and a total length of the dimer of ~25 Å [4] adapting to the thickness of the membrane.

In this study, we investigated the influence of gramicidin D on the vertical structure (electron density profile) of a single layer of bipolar tetraether lipid membranes derived from the thermoacidophilic archaeon *Sulfolobus Acidocaldarius* (grown at different temperatures) and the phase behaviour of the system at the water-air interface as a function of lateral pressure by using X-ray reflectivity measurements. In addition, we were able to test for ordered lateral structures by GIXD. The experiments were carried out on a Langmuir trough. Langmuir films of different lipids containing gramicidin D (5 %mol) at the water-air interface were prepared and the dependence of the surface pressure on the structure and phase behaviour was analyzed by X-ray reflectivity and GID as a function of the temperature. The measurements were performed using a solution at pH 6.5, mimicking the conditions inside thermoacidophilic archaeon cells.

Significant effects of the temperature of the subphase, lateral film pressure as well as growth temperature of the archae lipid could be found. Representative plots of GIXD and XRR measurements are presented in Figures 1 and 2, respectively. Detailed results will be published soon.

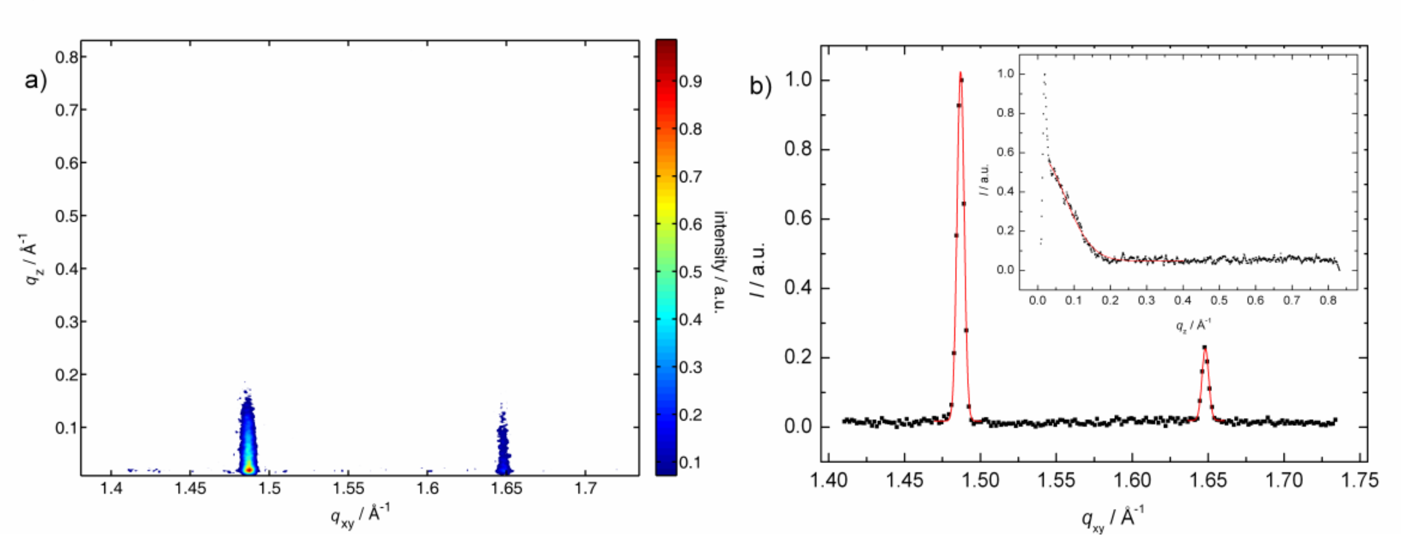

Figure 1: a) Two-dimensional contour plot of the intensity $I(Q_{xy},Q_z)$ along the horizontal (Q_{xy}) and vertical (Q_z) scattering directions as obtained from an archae lipid (grown at 76 °C) monolayer at a lateral film pressure of $\pi = 30$ mN/m. b) GIXD pattern $I(Q_{xy})$ obtained by integrating along Q_z for an archae lipid (grown at 76 °C) monolayer (squared symbols). Peaks were fitted by Gaussian functions (solid lines). (Inset) Typical Bragg rod intensity profile $I(Q_z)$ obtained by integrating along the Q_{xy} region of the first Bragg peak. The absence of a peak at $Q_z \neq 0$ indicates little or no molecular tilt.

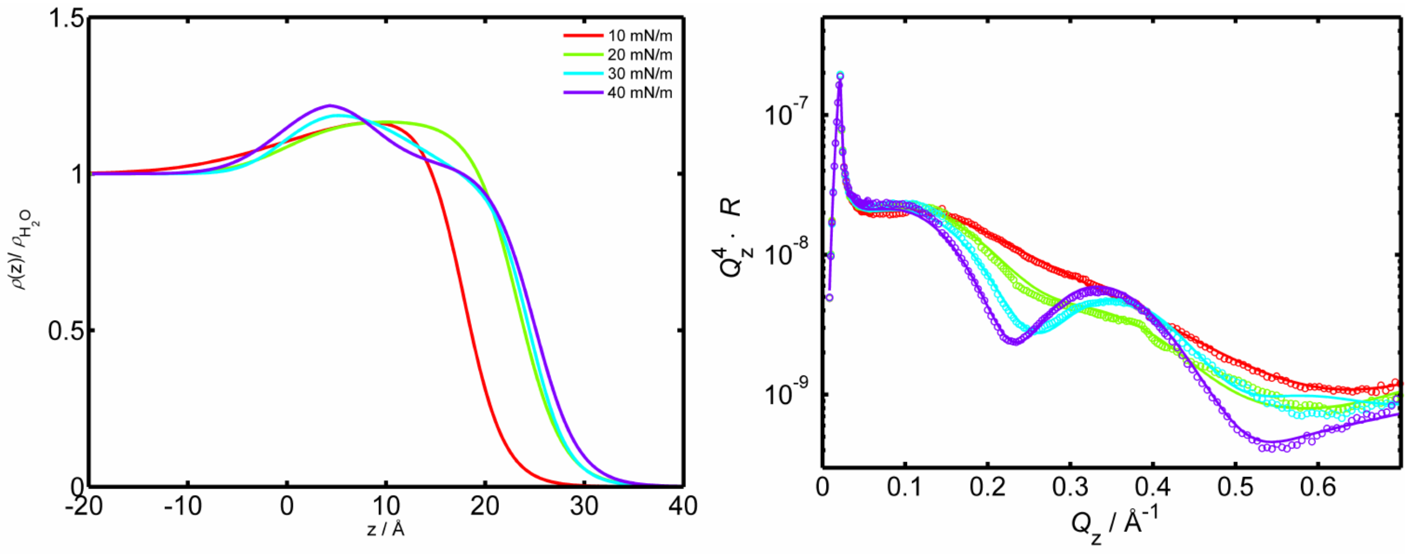

Figure 2: Normalized electron density profiles (left) and fitted reflectivity curves (right) of a PLFE monolayer at the air-water interface (pH 6.5, 10°C) as a function of lateral film pressure. With increasing film pressure the overall monolayer thickness increases and ordering of the lipid's lateral structure is observed.

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