ESRF	**Experiment title:** Structure and phase behaviour of bipolar tetraether lipids derived from the thermoacidophilic archaeon *Sulfolobus Acidocaldarius*	**Experiment number**: SC-2838
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Report:

We have successfully performed a grazing incidence diffraction (GID) and X-ray reflectivity (XRR) study on bipolar tetraether lipid membranes derived from the thermoacidophilic archaeon *Sulfolobus Acidocaldarius* at the air-water interface. We investigated the structural properties and phase behaviour of the lipid membranes consisting of lipids from archae bacteria grown at three different temperatures as a function of temperature, lateral pressure and pH.

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 [2–4]. 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. The number of the cyclopentane rings in each biphytanyl chain increases with increasing growth temperature [5].

In PLFE liposomes, lipids span the entire lamellar structure, forming a monomolecular spanning membrane [6], in contrast to the bilayer structure formed by monopolar diester (or diether) phospholipids in mammalian cells. 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. PLFE liposomes exhibit high thermal stability and unusually low solute permeability when compared to monopolar diester or diether liposomes [4]. The thermal stability with respect to leakage of dye originally trapped inside PLFE liposomes has been attributed to the negative charges on the membrane surface and to the tight and rigid lipid packing [7–9]. The low proton permeability in PLFE liposomes has been ascribed to the network of hydrogen bonds between the sugar residues of PLFE exposed at the outer face of the membrane [8] and to

the tight and rigid lipid packing [9,10]. Both the dye leakage and proton permeation experiments suggest that membrane packing, in either the hydrocarbon or the polar headgroup regions or both, is a central issue in understanding PLFE lipid membranes.

To study membrane packing in PLFE liposomes, lateral and rotational diffusion of membrane probes have been examined, recently. The lateral mobility of 1-palmitoyl-2-(10-pyrenyl)-decanoyl)-sn-glycero-3-phosphatidylcholine (PyrPC) in PLFE liposomes was found to be highly restricted and only became appreciable at temperatures >48°C [11]. This indicates a significant structural change near 48°C in the PLFE hydrocarbon core. These studies also suggest that the hydrocarbon region of PLFE liposomes is rigid and tightly packed below ~48°C. Above ~48°C, the hydrocarbon core of PLFE membranes begins to gain appreciable membrane fluidity, which would be required for the functionality of archaeal membranes. The polar headgroup region of PLFE membranes, on the other hand, may still be rigid and tightly packed through the hydrogen-bond network [12,13] at elevated temperatures (>48°C) so as to maintain a large proton gradient (pH 2–3 outside and pH 6.5 inside the cell) across the membrane at the growth temperature. This proposition explains why low proton permeability and appreciable membrane fluidity can occur at the same time in thermoacidophiles at high growth temperatures. This point is supported by a spin-label study, which showed that at high temperatures (~85°C) the nonitol (more precisely, calditol) headgroup of tetraether lipids from the thermoacidophilic archaeon *S. solfataricus* was relatively immobile, whereas the hydrocarbon region possessed some mobility [14].

In previous studies, we were able to reveal some structural properties of PLFE membranes in bulk solution by using SAXS [15] and to study the thermal phase transitions and volumetric properties by calorimetric methods [16]. From the lamellar *d*-spacings, no information about the packing properties and membrane thicknesses could be obtained, however, and we could not differentiate between effects of the head groups and the chain region of the lipid membrane. Determination of the vertical structure of a single lipid layer at the air-water interface is only possible by performing XRR experiments. Structural changes in the different regions of the lipid film as a function of surface pressure and temperature can be retrieved by this methodology. In addition, we determined an ordered lateral structure by GID.

The experiments were carried out using a temperature-controlled, sealed Langmuir trough available at ID10B. Langmuir films of three different PLFE lipids at the water-air interface were prepared, and the dependence of the structure and packing properties of the lipid membranes on the surface pressure were analyzed by x-ray reflectivity and GID in a temperature range between 10 and 55°C. The measurements were performed using a solution at pH 2.5, mimicking the conditions surrounding thermoacidophilic archaeon cells. The experiments were performed with PLFE lipid mixtures isolated from cells grown at 68°C, 76°C and 81°C.

Due to the little time between our measurements and the new proposal deadline (we submitted a new proposal for beamline ID10B to study the interaction of archae lipids with ion channel peptides - Ref. No 23341), very little time was left (3 days) for a first analysis of the data and writing this report. A more complete analysis of the data will be performed in the next months. In this report we give a first overview over selected results and prove that this archaebacterial membrane system is well suited for further investigations such as the proposed lipid-protein-interaction experiment.

So far it has been reported in the literature, that archae lipids may either form a monolayer consisting of “upright” standing lipids, which would have a lipid thickness of 4 – 5 nm, or adopt a U-shaped form with both hydrophilic headgroups in contact with the water surface. Such a conformation would lead to a thickness of 0.8 – 2 nm. It has also been suggested that these two conformations may coexist, but there are no data so far to verify such models [17]. Here we could clearly show that it is possible to spread the archae lipids as intact monolayers at the water air interface. Figure 1 depicts the reflectivity curves of PLFE membranes grown at all tree different growth temperatures. From the minimum of the Kiessig oscillations, the thickness of the membrane can be determined. In all measurements, we observe a value around 2.7 nm. This strongly indicates the existence of a mixture of “upright” standing and U-shaped lipids. Further analysis by fitting the electron density profiles will give more detailed information. Furthermore, Fig. 1 also reveals that the gross

structural properties of the lipid membranes grown at different temperatures are similar, but - depending of the film pressure - slight differences are clearly visible.

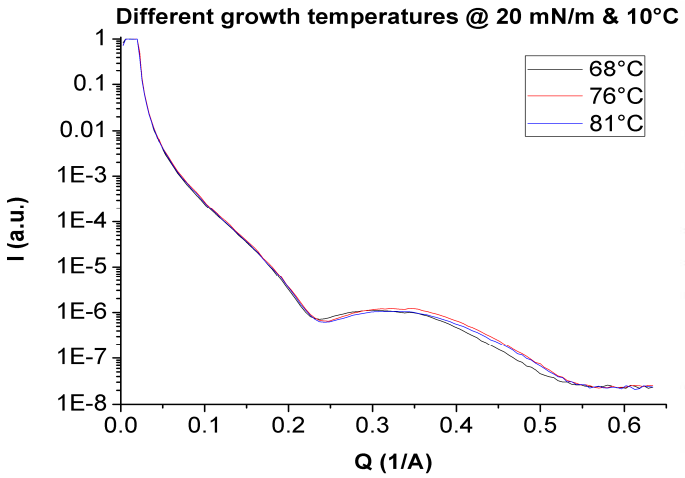

Figure 1: Reflectivity curve of archae lipids grown at different temperatures (68°C, 76°C, 81°C) at a film pressure of 20 mN/m and a temperature of 10°C.

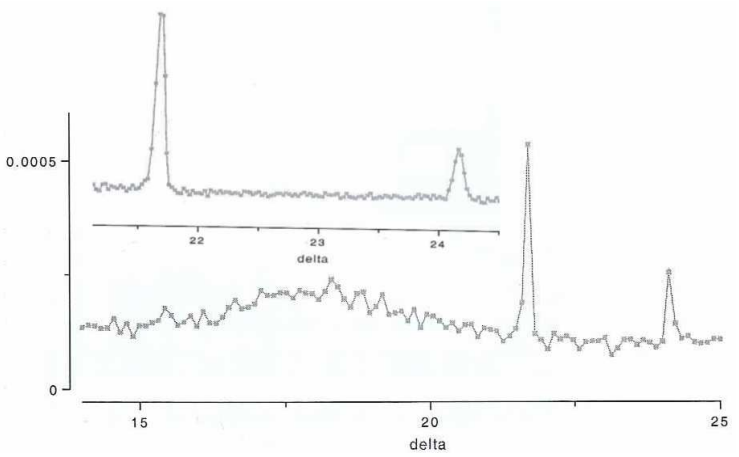

Figure 2: GID signal of PLFE grown at 68°C at a film pressure of 30 mN/m and a temperature of 10°C.

Figure 2 shows the GID scattering pattern of a PLFE sample grown at 68°C at a film pressure of 30 mN/m (mimicking a typical cellular membrane lateral pressure) and a temperature of 10°C. The inset shows the two peaks in more detail. Three different signals can be observed. The broad peak at approximately δ = 18° is probably related to fluid, relatively unordered domains within the lipid layer, most likely due to formation of U-shaped lipids. Additionally, two sharp peaks are visible that derive from ordered domains. Quantitative analysis of the GID data will give detailed information about the orientation and packing properties of the lipid headgroups as well as the sizes of these ordered domains as a function of temperature and film pressure.

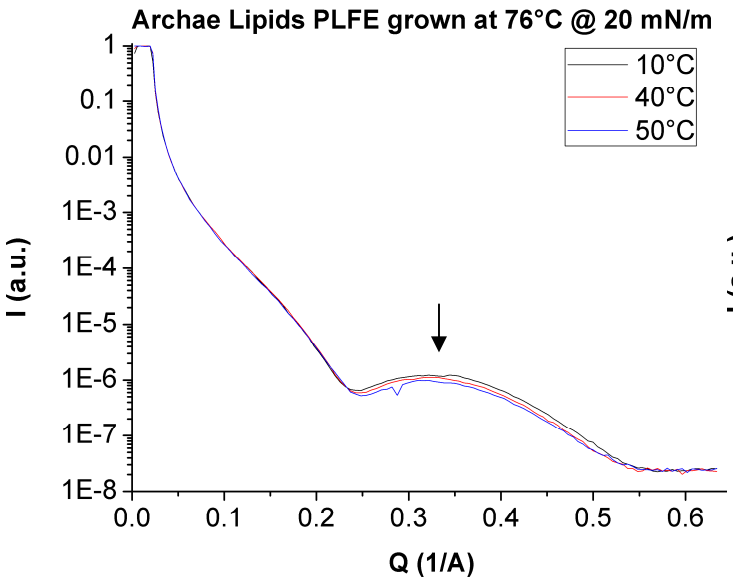

Figure3: Reflectivity curves of archae lipids grown at 76°C for different temperatures at a contant film pressure of 20 mN/m.

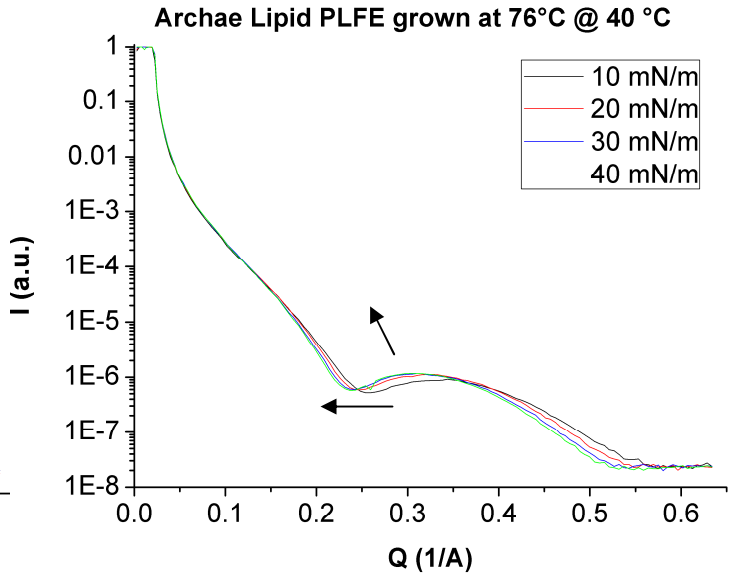

Figure 4: Reflectivity curves of archae lipids grown at 76°C for different film pressures at a constant temperature of 40°C.

In Fig. 3, the reflectivity curves of the archae lipids grown at 76°C are shown for selected temperatures at a film pressure of 20 mN/m. Small but systematic changes can be observed. The minimum of the Kiessig oscillations shifts to slightly larger *Q*-values, indicating a small decrease in the membrane thickness, possibly due to increased chain mobility and higher conformational disorder at higher temperatures. The shift of the minimum of the Kiessig oscillations is accompanied by a slight decrease in intensity with increasing temperature, indicating an increased surface roughness of the lipid monolayer at higher temperatures.

Finally, the effect of film (lateral) pressure on a monolayer of archae lipids grown at 76°C at a temperature of 40°C is depicted in Fig. 4. With increasing film pressure, a shift of the minimum to lower *Q*-values can be observed, indicating an increase in monolayer thickness. At the same time the intensity of the maximum increases due to a reduction of membrane surface roughness for higher film pressures.

The structural properties and phase behaviour of the lipids of the thermoacidophilic cell membranes are largely unexplored. Owing to their high temperature stability, they are also widely discussed for pharmacological applications. A detailed analysis of the data obtained in this study will help us to reveal detailed structural properties of archaeal lipid membranes from different growth temperatures at different surface pressures and pH values by GID and XRR experiments. These results will also lead to a better understanding of the thermal stability and the packing properties of the lipids, and will generally lead to a better understanding of the cell membranes of extremophiles *in vivo*. In addition, the knowledge gained will help in designing archaeal bipolar tetraether (e.g., from PLFE) lipid membranes for technological applications such as crystallization of membrane-bound proteins, immunoassays, vaccines or drug delivery.

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