Experimental report

25/01/2024

Proposal: 8-02-991 **Council:** 10/2022

Title: Impact of tetraethers on the stability of archaeal membranes at high temperature

Research area: Biology

This proposal is a new proposal

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Samples: archaeal lipid

Instrument	Requested days	Allocated days	From	To
D16	5	5	09/11/2023	14/11/2023

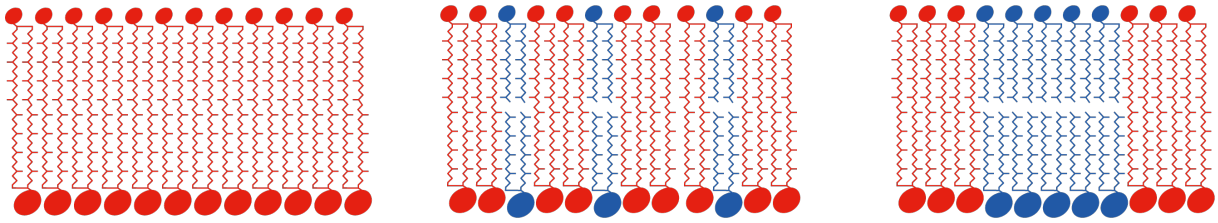
Abstract:

Archaeal monolayer forming lipids have long been thought to be the major adaptation of the archaeal membrane to extreme conditions. We have proposed and demonstrated a novel membrane architecture model to explain the stability of lipid bilayers in Archaea at high temperatures (> 70°C) and high pressures (400 bar). In this architecture, the increase in membrane stability/rigidity is due to the presence, in the midplane of the bilayer, of apolar hydrocarbons. In our previous experiments (Salvador-Castell 2021), we demonstrated that the presence of the apolar molecule induces phase separation and the appearance of novel phases in the membrane with a P- and T-dependence (ILL report 8-02-852, and LoRicco 2021). We have recently obtained an archaeal strain mutant which is impaired in the synthesis of monolayer forming lipids, and thus can no longer form monolayers. Here, we would like to take this opportunity to explore the relative contribution of the two different types of lipids, diethers and tetraethers, in order to draw a better understanding of bilayer stability in Archaea, and the exact role played by the tetraethers.

Impact of tetraethers on the stability of archaeal membranes at high temperature

Abstract

Archaeal monolayer forming lipids have long been thought to be the major adaptation of the archaeal membrane to extreme conditions. We have proposed to test this hypothesis experimentally. We have recently obtained an archaeal strain mutant which is impaired in the synthesis of monolayer forming lipids, and thus can no longer form monolayers. Here, we took this opportunity to explore the relative contribution of the two different types of lipids, diethers and tetraethers, in order to draw a better understanding of bilayer stability in Archaea, and the exact role played by the tetraethers.



100% diether lipids | 75% diether lipids 25% tetraether lipids | 50% diether lipids 50% tetraether lipids

Figure 1: Archaeal membrane structures with increasing proportions of tetraether, monolayer forming lipids (from left to right). We expect increased thermal stability with increasing T, and a lowering of the mean d spacing. To date the spatial disposition of the lipid, as a homogenous mix as shown here in the figure, or phase separation be tween the two types of lipids, is unknown. The demonstration of a putative tetraether induced lateral organization is a possible outcome of the measures.

In this experiment, we have used the same approach as we have used in experiment 8-02-8052, e.g., multi-layered bilayers composed of archaeal lipids. Multi-layered membranes were constructed from mixtures of pure lipids to generate 4 different membrane compositions (100T, 50/50 D/T, 75/25 D/T, 100D), as solvent deposited films using 3 mg of lipid mix on ultraclean silicium wafers, cut to fit inside the aluminum sample holder. The solvent was evaporated under vacuum overnight and the layers were rehydrated at the correspondent D_2O contrast for at least 48h at 50°C inside the aluminum sample holder.

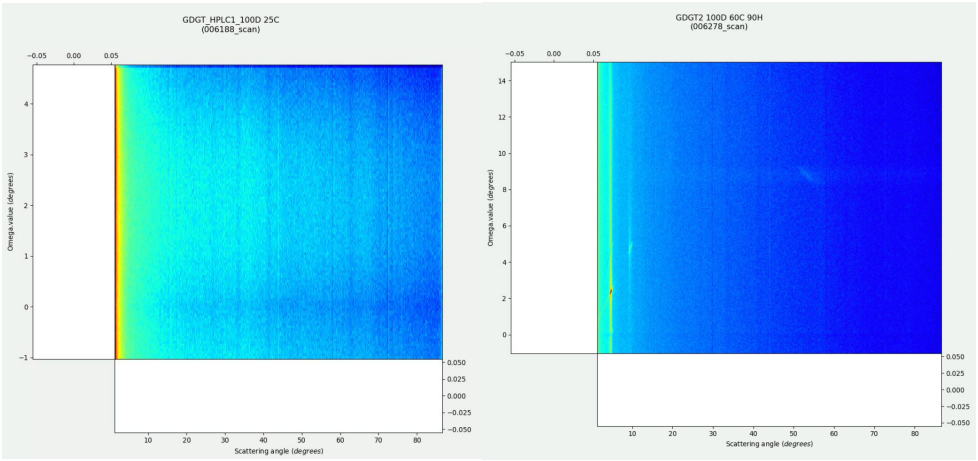


Figure 2: Raw, uncorrected spectra for sample GDGT1 (100% monolayer forming lipids) in the aluminum sample holder (left) or in the BerILL humidity chamber at 60°C.

Samples were scanned at 5 temperatures (25, 40, 55, 70 and 85°C) in the orange cryofurnace. In contrast to our previous results with similar lipids [1,2,3], none of the samples yielded a very organized membrane stack, as shown by the presence of a single Bragg peak on the spectra (Figure 2, left panel). With experience, we supposed that there might have been a problem with the hydration of the layers. Indeed, when hydration is over 100%, membrane stacks tend to lose their organization. Furthermore, upon opening of the aluminum cells we noticed water droplets oat the surface of the wafers. In order to circumvent the problem with hydration, we used the last 1 and a half day of our beamtime to change the instrument setup to accept the BerILL humidity chambers and explore the influence of hydration and temperature on the organization of the multistacked lipid layers. As can be seen in the right panel of figure 2, we have been able to see at least 4 Brags peaks with the samples if temperature was over 60°C and hydration over 80%. We did not have time to explore the complete relative humidity/temperature range space for all lipid mixtures, but 1) we could get a good diffraction signal for all lipid mixtures, 2) we could test the BerILL at 70°C and get good signal without damage to the BerILL chamber. In addition, we tested whether samples prepared for experiment 8-02-991 could be used in a further experiment after long term storage as a multistack of layers. To do so, we took samples prepared for experiment 8-02-973, and which have been stored at -80°C for 3 years. The spectra that we obtained showed 4 Bragg peaks, which is similar to the original data, demonstrating that the samples already prepared can be used in the eventuality that beamtime is available to further this work in the BerILL environment.

We have tried to understand why the lipids behaved differently than during previous experiments. One of the differences with these previous experiments is that the lipids here are isolated from natural sources, and not synthetic. The lipids were supposedly purified to contain a single lipid molecule of identical molecular structure, as shown by the chemical data. However, there is the possibility that impurities caried along the extraction/purification procedure impact on the structure of the membrane reconstructed from natural lipids. There also is the possibility that the natural lipids have a different structure than the synthetic ones, and that it went unnoticed in the RMN and HPLC-MS spectra. We have started to explore this last hypothesis, and we have some evidence that indeed, the phospho-lipid produced by the cells does not have the exact structure as the one proposed in the literature. The one we synthesized, which corresponds to the described structure, harbors a myo-inositol polar headgroup, while the natural one harbors another C6 ose of yet undetermined structure.

References

[1] Salvador-Castell, M *et al.* «Lipid phase separation induced by the apolar polyisoprenoid squalane demonstrates its role in membrane domain formation in archaeal membranes ». Langmuir 36, n° 26 (2020): 7375-82.

[2] Salvador-Castell, M *et al*. «Characterization of a synthetic archaeal membrane reveals a possible new adaptation route to extreme conditions ». Commun. Biol. 4 (2021): 653.

[3] LoRicco JG *et al.* Apolar Polyisoprenoids Located in the Midplane of the Bilayer Regulate the Response of an Archaeal-Like Membrane to High Temperature and Pressure. Front Chem. (2020) 8:594039.