Experimental report

18/02/2025

Proposal: **8-02-998**

Council: 4/2023

Title: Characterizing the behavior of a novel archaeal membrane organizationwith connected membrane leaflets

Research area: Biology

This proposal is a new proposal

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Samples: C20-C20
C20-C25
C25-C25

Instrument	Requested days	Allocated days	From	To
D16	6	6	03/11/2023	09/11/2023

Abstract:

Extreme archaeal halophiles can grow in conditions where the activity of water is close to zero. Despite an increased intracellular osmolarity, a strong gradient exists between the environment and the cell, and the physico-chemical tolerance of the plasmic membrane to this osmotic stress remains unknown. Part of the increased membrane stability has been associated to the presence of unusual archaeal lipids which have an extended, 25 carbons long, hydrophobic chain in contrast to the 20 carbons long classic chain. These lipids harboring one extended hydrophobic chain could form a zipped bilayer membrane in which the extended chains of lipids from both faces would interact inside the midplane, which would further increase membrane stability. In this experiment, we want to determine the impact of the zip structure on membrane self-organization, structure and resistance to increased temperatures. Using known mixtures of classic and extended lipids, we will be able to quantify the relative contribution of the extended lipids to membrane physical parameters in comparison to the 'classic' archaeal bilayer.

Report on D16 experiments #8-02-998 and #8-02-1046

Bilayer-forming lipids enhance archaeal monolayer membrane stability

Membrane stability and organization are crucial for the survival of hyperthermophilic archaea, which thrive in extreme environments. To withstand the harsh conditions they inhabit, many extremophiles incorporate bipolar tetraether lipids, forming a highly rigid and resistant monolayer membrane instead of the conventional bilayer (Chong, 2010; Oger and Cario, 2013). Previous studies have highlighted the importance of the balance between diether and tetraether lipids in membrane adaptation to temperature and humidity fluctuations. However, the specific influence of lipid composition on membrane structure under varying environmental conditions remains unclear. This experiment, conducted at the Institut Laue-Langevin (ILL), aimed to investigate the structural properties of archaeal-like membranes using neutron diffraction analysis.

Project: To determine whether tetraether lipids are essential for membrane stability at high temperatures, we examined the behavior of archaeal-like membranes with varying tetraether concentrations. Using purified diether lipids from *Pyrococcus furiosus* and tetraether lipids from *Sulfolobus acidocaldarius*, we conducted neutron diffraction experiments on D16 under extreme conditions that mimic the environments inhabited by Archaea.

During the first experiment (#8-02-998), we encountered challenges in analyzing our samples. Initially, we tested fully hydrated membranes (100% relative humidity, RH) using the Cryofour setup at 25 °C, 40 °C, and 70 °C. A weak signal was detected only above 40 °C, with two observable Bragg peaks, but the lipid film was unstable at full hydration. To overcome this limitation, in our second experiment (#8-02-1046), we transferred the samples to the high-precision BerILL humidity chamber (Gonthier et al., 2019). This setup provided better control over hydration and improved data resolution. Four different membrane compositions were analyzed: pure diethers (D), pure tetraethers (T), and two diether/tetraether mixtures (D/T) at molar ratios of 1:1 and 2:1. Each sample was tested at three temperatures (60 °C, 70 °C, and 80 °C), two relative humidities (80% and 95% RH), and two contrast conditions (8% D_2O and 100% D_2O). In total, the experiment consisted of 96 hours of neutron scans.

Results: Our data from the second experiment confirmed the formation of oriented multilayer lipid membranes, as indicated by the presence of well-defined Bragg peaks (Figure 1). The number and sharpness of these peaks varied among samples. Pure diether and tetraether membranes displayed broader diffraction peaks, suggesting less-ordered structures. In contrast, mixed D/T membranes exhibited sharper and more intense peaks, indicating greater structural organization and stability (Figure 1). Preliminary neutron scattering length density (NSLD) profiles revealed a characteristic bilayer structure with a central hydrophobic core and polar headgroups at the membrane interfaces. A key finding was the presence of a density "trough" at the membrane midplane for all samples, a feature typically associated with the terminal methyl groups of diether lipids. Unexpectedly, this trough was also observed in tetraether-rich membranes (Figure 2), suggesting an unanticipated structural characteristic of tetraether monolayers. Our findings indicate that while membranes composed of a single lipid type remain structured at high temperatures, they lack the robustness of mixed-lipid systems. Moreover, NSLD profiles revealed that tetraether membranes exhibit greater rigidity and organization under high-temperature conditions compared to diether membranes (Figure 2). These results suggest that natural archaeal membranes benefit from a combination of rigid tetraethers and flexible diethers, optimizing structural integrity and stability under environmental stress. A manuscript about these results will be submitted to IJMS shortly.

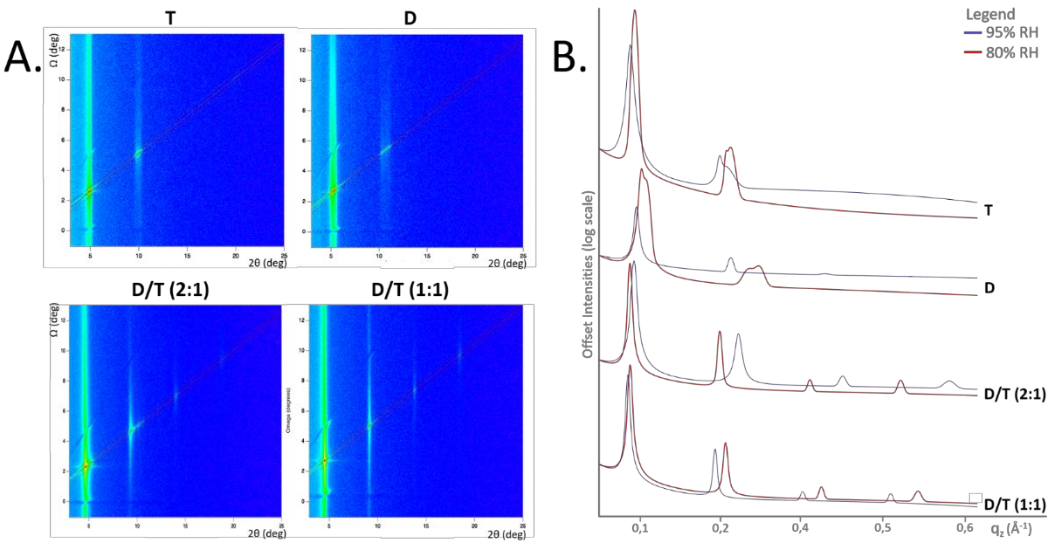

Figure 1: Neutron diffraction of multilayer samples with varying concentrations of purified tetraether (T) and diether lipids (D). *A. Reciprocal space maps resulting from typical Ω-scansl (2ϑ, Ω) showing the Bragg peak positions of the different lipid samples, run at 100 D2O 70 °C 80 RH. B. 1D integrated intensity along the Z direction of the different lipid samples highlighting Bragg peak intensities, run at 100 D2O 70 °C.*

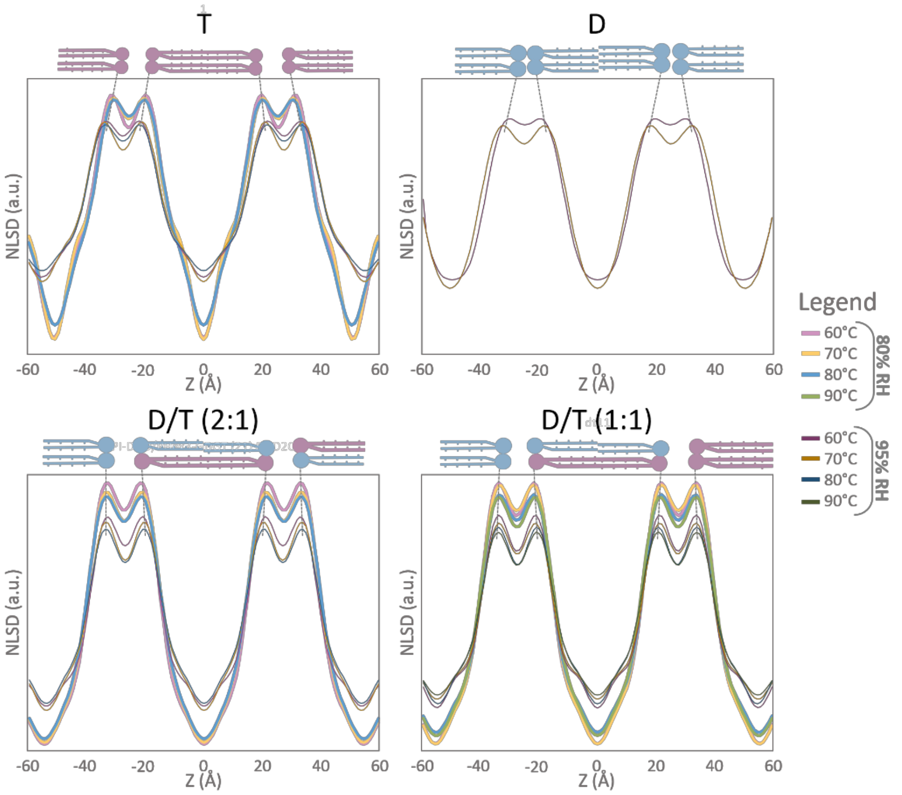

Figure 2: Overlaid NSLD profiles. Each graph corresponds to a NSLD profile at different temperatures and humidities. *The dotted black lines show the shift of the maxima in the NSLD profiles. NSLD profiles were calculated with 2 (80 % RH) or 3 (95 % RH) orders for D samples and 4 orders for T, and both D/T samples. At 80 % RH only 2 orders were detected for the diether sample (D), the NSLD profile was not calculated. A problem occurred during the diether measurement at 80 °C 95 % RH, for this reason it was not possible to calculated NLSD profile for this sample.*

Chong, P.L.-G., 2010. Archaebacterial bipolar tetraether lipids: Physico-chemical and membrane properties. Chemistry and Physics of Lipids 163, 253–265. https://doi.org/10.1016/j.chemphyslip.2009.12.006

Gonthier, J., Barrett, M.A., Aguettaz, O., Baudoin, S., Bourgeat-Lami, E., Demé, B., Grimm, N., Hauß, T., Kiefer, K., Lelièvre-Berna, E., Perkins, A., Wallacher, D., 2019. BerILL: The ultimate humidity chamber for neutron scattering. Journal of Neutron Research 21, 65–76. https://doi.org/10.3233/JNR-190109

Oger, P.M., Cario, A., 2013. Adaptation of the membrane in Archaea. Biophysical Chemistry, Biomolecular systems under extreme environmental conditions 183, 42–56. https://doi.org/10.1016/j.bpc.2013.06.020