Experimental report

23/09/2021

Proposal: 8-02-917 **Council:** 4/2020

Title: The effect of bulkiness and charges of archaeal lipid polar headgroups on bending rigidity of archaeal membranes

Research area: Biology

This proposal is a new proposal

Main proposer: Philippe OGER

Experimental team: Josephine LORICCO
Judith PETERS
Philippe OGER
Camille DALIGAULT

Local contacts: Ingo HOFFMANN

Samples: DoPhPG
DoPhPS
DoPhPI

Instrument	Requested days	Allocated days	From	To
IN15	4	4	01/09/2020	02/09/2020
			08/06/2021	11/06/2021

Abstract:

The most extremophilic organisms (able to withstand extreme conditions) all belong to the Archaea. In contrast to the familiar fatty acid based lipids of bacteria and eucarya, Archaeal membranes are composed of very particular lipids, based on ether linked polyisoprenoid chains. The ether bound and the branched chains allow for a tighter compaction of the membrane, which confers increased impermeability and resistance to the harsh conditions extremophilic Archaea live in. It is known that the polar headgroup influences the bending rigidity of the lipids, but very little work has been done thus far to characterize it with precision in the example of the archaeal bilayer. In this project we want to take opportunity of newly synthesized archaeal phospholipids with different polar headgroups (serine, glycerol, inositol, choline and ethanolamine) in order to measure the impact of these polar headgroups, with varied charge and bulkiness, on the bending rigidity of a synthetic archaeal bilayer. This will give complementary information on membrane parameters with that obtained by diffraction, SAXs and NMR, and allow to fully apprehend the behavior of the membrane under extreme conditions.

Report on Experiment # 8-02-917: The effect of bulkiness and charges of archaeal lipid polar headgroups on bending rigidity of archaeal membranes

Introduction: Archaea live at some of the most extreme conditions on Earth including temperatures of > 85°C and pressures > 400 bar. Cell membranes are especially sensitive to pressure and temperature. It has been hypothesized that Archaea use apolar lipids such as squalane to regulate their membranes in response to temperature and pressure. Previously we have characterized the structure of an archaeal-like membrane system [DoPhPC:DoPhPE + squalane] using neutron diffraction at temperatures from 25 to 85°C and pressure from 1 – 1000 bar [1]. In this work we attempt to characterize the dynamics of this system in response to increasing pressure (up to 1000 bar) using neutron spin echo (NSE). Because of the large quantity of lipid needed to perform high pressure experiments, it was performed with exclusively commercially available lipids.

Sample Preparation/Experimental Setup: Lipid films composed of a 9:1 ratio of DoPhPC:DoPhPE with or without 5 mol% squalane were prepared. These lipid films were hydrated with D_2O to a final lipid concentration of 10 mg/mL (~12 mM). After extensive vortexing the samples were extruded through a 0.1 µm polycarbonate filter 11x times to produce unilamellar vesicles (ULVs). Extrusion was performed immediately before loading the HP cell or running DLS measurement. NSE measurements were performed on IN15 using the SANE High Pressure Cell 3kbar liquid 21PL30AO2 (SANS/NSE). DLS was performed using a Malvern Zetasizer Nano Z in the partnership for soft and condensed matter (PCSM) laboratory.

Analysis: NSE data was fit using the Zilman-Granek model. In the simplest model the intermediate scattering function is fit as a function of time to a stretched exponential:

$$S(Q,t) = exp(-\Gamma_{ZG}t)^{2/3} \qquad \text{Eq. 1}$$

This method does not account for diffusion of the ULVs (which is especially important for samples that form vesicles of different sizes), and the limited amplitude of the undulation motion of the membrane [2]. Following extrusion, the sample containing squalane was much more turbid suggesting the presence of larger scattering particles. Therefore, the following corrected equation was used.

$$S(Q,t) = exp(-Dq^2t)\big(1 - a(q)\big) + a(q)\, exp(-\Gamma_{ZG}q^3t)^{2/3} \qquad \text{Eq. 2}$$

Where D is the diffusion coefficient calculated from dynamic light scattering (DLS) and a(q) is a correction for the limited amplitude of the membrane undulations. The values of Γ_{ZG} should be q-independent therefore the data at each q was fit using Γ_{ZG} as a shared parameter (Figure 1).

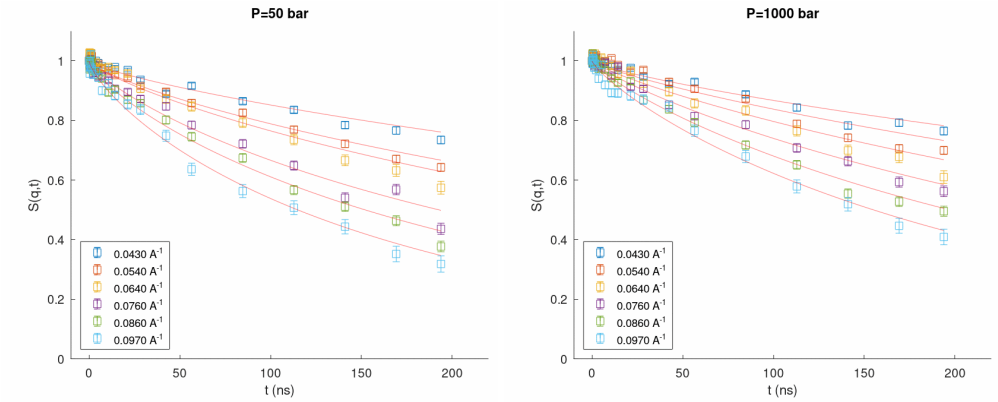

Figure 1: Global fitting of data to Eq. 2 for DoPhPC:DoPhPE (9:1) membrane at 50 bar (left) and 1000 bar (right).

DLS was performed on the samples following the NSE measurements (re-extruded) at 25°C and ambient pressure to determine the size/diffusion coefficient for these samples. In the pressure range (1-1000 bar) used in this study the assumption was made that does not change appreciably with pressure which is supported by the literature [3]. D was found to be 0.3366 $Å^2$/ns for DoPhPC:DoPhPE (9:1) at 25°C/1 bar and 0.1302 $Å^2$/ns for DoPhPC:DoPhPE (9:1) + 5 mol% squalane at 25°C/1 bar.

After fitting the intermediate scattering function to Eq. 2, the apparent bending modulus (κ) can be derived from the following equation:

$$\Gamma_{ZG} = 0.0069\gamma\sqrt{\frac{k_bT}{\kappa}}\ \frac{k_bT}{\eta(T)} \qquad \text{Eq. 3}$$

Where k_b is the Boltzmann constant, T is the temperature, η(T) is the temperature-dependent viscosity and κ is the membrane bending rigidity. γ ≈ 1 when κ >> k_BT.

Results: DoPhPC:DoPhPE (9:1) ULVs with and without 5% squalane were measured by NSE at 25°C and pressures of 50, 250, 500 and 1000 bar. The rigidity of the membrane was calculated as a function of pressure using either Eq. 1 (Simple fit) or Eq. 2 (Corrected fit).

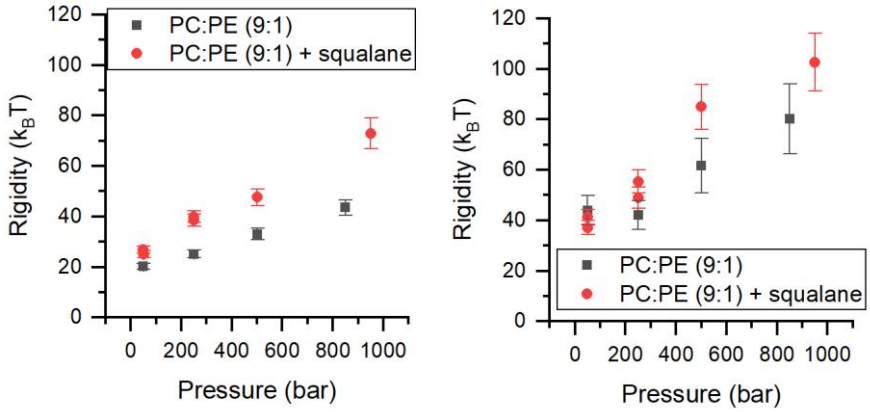

Figure 3: Bending rigidity calculated using an amplitude correction a(q). (Left) Rigidity calculated from simple fits of NSE data (equation 1). (Right) Rigidity calculated from fits using the corrected equation (equation 2).

The magnitude of the rigidity calculated without corrections at low pressure (50 bar) were within the range expected for a fluid membrane. Without using the diffusion/amplitude correction, the membrane containing squalane appears to be more rigid. However, after applying the corrections, there does not appear to be a significant difference in rigidity between the samples. The magnitude of the rigidity values calculated in this case are larger than those expected for a fluid membrane. This is a result of the chosen prefactor in Eq. 3 (0.0069) used in the analysis that was originally chosen to result in reasonable values when using the simplest method of analysis [2].

Irrespective of analysis method, the effect of pressure on the rigidity of membranes is clear. Increasing pressure leads to a corresponding increase in membrane rigidity. Increasing the pressure from 1 to 1000 bar leads to approximately a 2-fold increase in bending rigidity for both DoPhPC:DoPhPE (9:1) and DoPhPC:DoPhPE (9:1) + 5% squalane. Increasing membrane rigidity with pressure has been shown before using vesicle fluctuation analysis [4] where DOPC GUVs were shown to increase by a factor of ~2 between ambient pressure and 400 bar. Increasing rigidity with pressure has been seen for microemulsions using NSE [5], however to the best of our knowledge this is the first example of high pressure NSE done with biological membranes. Increasing pressure has been shown to lead to lateral compression of membranes and corresponding increase in membrane thickness which could explain the increased membrane rigidity [4]. An increase in membrane d-spacing with pressure has been shown previously for this system of archaeal-like lipids [1].

References: [1] LoRicco et al. (2020) *Front. Chem.*, [2] Hoffmann (2021) *Front. Phys.*, [3] Kohlbrecher et al. (2007) *Rec. Scie. Instrum.*, [4] Purushothanman et al. (2015*) J. Phys. Chem. B.*, [5] Klostermann et al. (2012) *Soft Matter*