Experimental report

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Proposal: 9-13-987 **Council:** 10/2020

Title: Inter-membrane forces between archaeal-like membranes with different polar headgroups

Research area: Biology

This proposal is a new proposal

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Samples: DoPhPG
DoPhPS
DoPhPI
DoPhPC

Instrument	Requested days	Allocated days	From	To
D16	8	6	11/03/2021	17/03/2021

Abstract:

Archaeal lipid membranes are made up of unique lipids which contain ether rather than ester linkages and branched isoprenoid hydrocarbon chains. Archaea are also known to have lipids with diverse polar headgroups but the contribution of these headgroups to bilayer structural parameters hasn't been well studied in archaea. Using neutron diffraction we can characterize how membrane structural parameters differ between archaeal lipids with different polar head groups. We propose to vary the relative humidity using the BerILL chamber to precisely increase the membrane hydration, leading to swelling of the bilayers. These swelling experiments can be then used to compare the differences in the inter-membrane forces between bilayers with different headgroup compositions.

Experiment# 9-13-987: Inter-membrane forces between archaeal-like membranes with different polar headgroups

The composition of the lipid polar head groups is an essential and yet often neglected parameter in understanding how membranes have adapted to different environmental conditions. In most Archaea, lipid chain lengths are invariable and fixed at 20 carbons for the monopolar lipids and 40 carbons for the membrane-spanning, monolayer forming lipids. Thus, we expect the nature of polar headgroups to play a critical role in the regulation of membrane parameters. The goal of this experiment was to determine structural differences between lipid bilayers containing three different charged polar headgroups phosphoinositol (PI), phosphoserine (PS) and phosphoglycerol (PG) in comparison to the neutral headgroup (PC). Diffraction was measured at 50, 80, 90, 95, 98, 99, and 100% relative humidity (RH%). At low humidity, a hexagonal phase could be detected in all samples. This agrees with findings on a DoPhPC:DoPhPE (9:1) membrane, and the related DPhPC (ester linked diphytanoyl chains) [1,2]. At >80 RH% at least one "main" lamellar phase could be seen and in some cases (PI:PC, PS-R:PC and PS-S:PC) additional lamellar phases were also present (Figure 1).

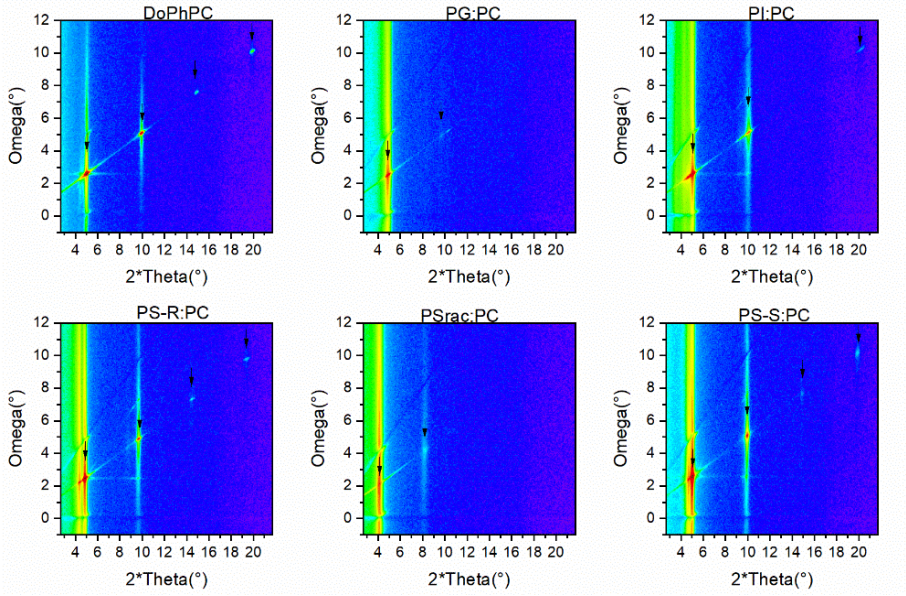

Figure 1: Diffractograms of membranes taken at 100 RH%. Contrast was 100% D_2O. Arrows are used to denote peaks from the "main" phase. Some lipid mixtures (PI:PC, PS-R:PC and PS-S:PC) contained additional phases.

Membrane d-spacing for the "main" membrane phase was plotted versus relative humidity for all the membranes measured. Pressure-distance curves were also generated using the d-spacings (Figure 2). Swelling could been seen in all the membranes as the humidity increased. The PG:PC and PSrac:PC membranes continue to swell near 100 RH%, whereas the "main" phase of DoPhPC, PI:PC, PS-R:PC and PS-S:PC membranes appear to approach a maximum d-spacing of ~52 Å.

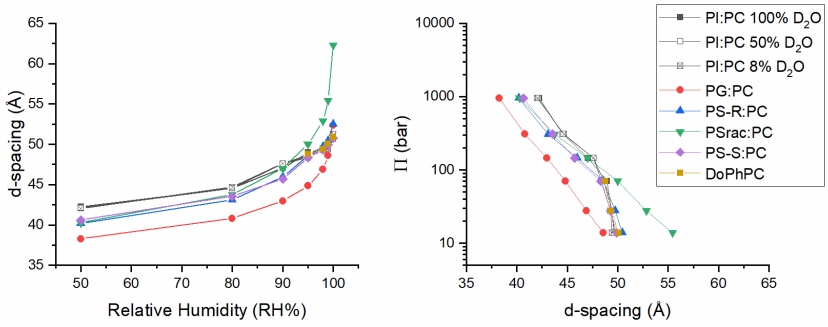

Figure 2: (Left) d-spacing a function of humidity. (Right) Pressure-distance curves. Mixtures which were well-mixed (PG:PC and PSrac:PC) continued to swell over the range of humidities measured. The "main" phase of mixtures in which additional "swollen" phases could be distinguished (PI:PC, PS-R:PC, PS-S:PC) approached a maximum d-spacing with increasing humidity.

Interestingly the lipid mixtures that approach a maximal d-spacing are seen only for mixtures in which multiple phases (phase separation) is detected (Figure 1). The mixtures which phase separated included PI:PC, PS-R:PC and PS-S:PC. The "main" phases of these mixtures behave similarly to a pure DoPhPC membrane suggesting the lipids are demixing into PC-rich and PC-poor phases. Previously we have seen that a PI:PC membrane at 25°C the membrane demixes into PC-rich and PI-rich phases (Exp. # 8-02-919). The PC-poor phases, which are rich in negatively charged lipids are more "swollen" (have a larger d-spacing) and have worse diffraction (only 1-2 diffraction peaks). Bilayers composed of PC lipids (with a net neutral charge) are known to approach a maximum spacing, while those made up of charged lipids (such as PS lipids [3,4]) have been known to swell indefinitely. It is difficult to say whether lipid demixing is present at low humidity but the phases are indistinguishable due to similar repeat spacings. The behavior of both PS-R:PC and PS-S:PC appear nearly identical at low humidity and both show phase separation at high humidity. While the "main" (and likely PC-rich) phases behave similarly, differences are seen in the "swollen" phases (likely PS-R and PS-S rich). The PS-R:PC phase has a larger swollen phase than the PS-S:PC membrane at 100 RH%. This agrees with results from Exp. # 8-02-919 where PS-R alone was found to have a larger d-spacing than PS-S alone. Surprisingly PSrac (which should be a racemic mixture of PS-S and PS-R) does not show any indication of phase separation even at 100 RH% (Figure 1). PSrac:PC exhibits a single phase at all humidity values and continues to swell with increasing humidity. This is similar to the behavior seen for PG:PC, although the magnitude of the d-spacing is smaller for the PG:PC membrane. The PG:PC membrane reached a maximal d-spacing of ~52 Å (after 16h at 100 RH%) in this experiment compared with ~60 Å found previously at full hydration (Exp. 8-02-884) suggesting this sample may not have been equilibrated for long enough.

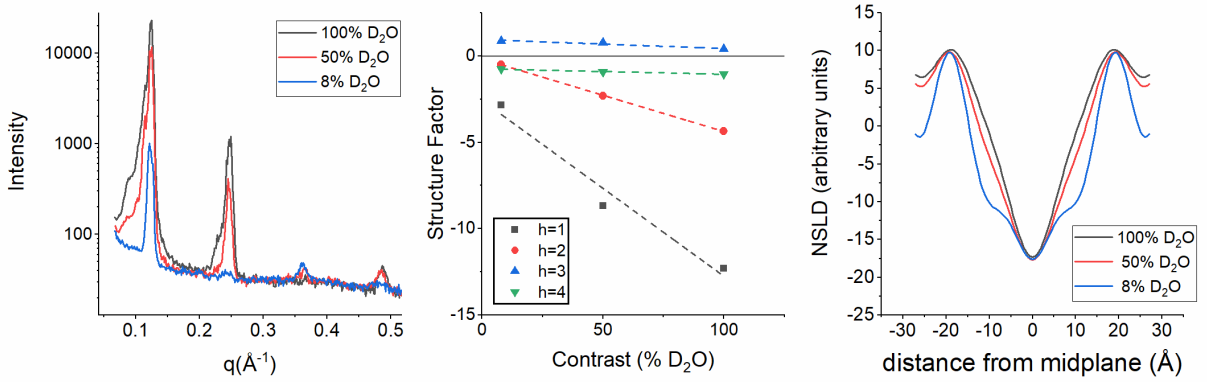

Figure 3: (Left) 1D diffractograms of PI:PC at 100 RH% at three different contrasts: 100% D_2O (black), 50% D_2O (red) and 8% D_2O (blue). (Center) Determination of structure factor phases from D_2O contrast. (Right) NSLD plots of PC-like phase of PI:PC at three different contrasts: 100% D_2O (black), 50% D_2O (red) and 8% D_2O (blue).

The PI:PC membrane was measured at three different D_2O contrasts (100%, 50%, 8% D_2O). At 100% D_2O multiple phases could be detected within the membrane. This is clearly seen at 100 RH% where at 100% D_2O, the first and second order peaks are very heterogeneous. At 8% D_2O, these additional "swollen" phases are no longer able to be detected (Figure 3). Previous experiments (Exp. # 8-02-884, #8-02-919) showed similar contrast effects for this membrane system. While multiple phases are present, only the "main" phase gave four orders of diffraction. The presence of four orders of diffraction allowed us to generate neutron scattering length density (NSLD) plots for this phase at each contrast for hydrations at which four orders of diffraction were seen (≥95 %RH). From the NSLD plots we were able to determine the bilayer thickness (d_B) and water layer thickness (d_w). Very little change was seen in the d_B as a function of humidity. Calculated values for d_B were all around 38.4 ± 0.4 Å. Because the bilayer thickness changes little with hydration, changes in the d-spacing reflect changes in the water layer thickness. As expected, the d_w increased with increasing humidity from 11.5 ± 0.4 Å at 95 RH% to 12.5 ± 0.1 Å at 98 RH%, 13.0 ± 0.1 Å at 99 RH%, and 13.7 ± 0.5 Å at 100 RH%. The values for the PI:PC "main" phase were in good agreement with those measured for the pure DoPhPC phase.

References: [1] Salvador-Castell et al. (2020) *IJMS*, [2] Hsieh et al. (1997) *Biophys. J.*, [3] Tristram-Nagle et al. (1998) *Biophys. J.*, [4] Petrache et al. (2004) *Biophys. J.*