Experimental report

26/07/2022

Proposal: 9-13-906 **Council:** 10/2019

Title: Alkane localisation inside a compliant protomembrane model at an atomic scale

Research area: Soft condensed matter

This proposal is a continuation of 9-13-758

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Samples: squalane
eicosane
1,2-Didecanoyl-sn-glycero-3-phosphocholine
triacontane

Instrument	Requested days	Allocated days	From	To
D16	8	6	12/08/2020	18/08/2020

Abstract:

A novel membrane architecture is proposed as a model for the first forms of life.
This model consists on a bilayer of short chain amphiphiles (readily available at the onset of life), with small percentages of alkane molecules inserted inside the membrane. The latter would help sustain the extreme environmental conditions (high T, high p) experienced by the first protocells on the early planet.
We propose to perform neutron membrane diffraction to study, at atomic scale, the positioning of different lengths (20 - 30 carbons) and types (linear - isoprenoid) of alkanes. This will be done by employing both hydrogenated and deuterated alkanes, as well as different D2O/H2O contrasts, and will allow to perform the 1D Fourier analysis to construct the membrane scattering length density profiles.
Besides, humidity scans will be performed using the humidity chamber available as sample environment on D16, to study the membrane response to different water pressure conditions in the cases where the alkanes are presents (and which) or not.
A precise alkane localization inside the membrane will help us understanding the mechanisms for which these molecules affect the membrane properties.

REPORT – EXPERIMENT 9-13-906 (12-18/08/2020)

Loreto Misuraca, Judith Peters, Philippe Oger, Bruno Demé

Background The emergence of life on our planet contains many intriguing, open questions. Some of them, in the particular case of the protocell membranes, are given by two strict limiting factors of the prebiotic environment: 1) the lack of molecular complexity on the early planet, so that the only compounds able to form proto-cellular compartments would have been short, single chain fatty acids [1] and alcohols. 2) The most probable scenarios for life appearance, likely to be the oceanic hydrothermal vents [2] with extreme environmental conditions (T up to 350 °C, p up to 800 bar). Fatty acid vesicles are known to be more sensitive to their surrounding environment [3], making their effectiveness as compartmentalization molecules questionable.

In our project, we study a possible architecture for protocell membrane, consisting on a mixture of decanoic "Capric" acid with a fatty alcohol of equal chain length, the decanol (named hereafter C10 mix) (Figure 1 left). These short, single chain amphiphilic molecules have been found able to form stable vesicles [4]. To account for the supposed extreme environmental condition, the membrane model contains and additional idrophobic molecule, such as the eicosane (linear alkane C20) (Figure 2 right). It has been previously proven, by means of Neutron Membrane Diffraction [5], that such kind of hydrophobic molecules (in the literature example, the isoprenoid C30 squalane) are able to enter inside a DOPC/DOPG lipid membrane, in a position perpendicular to the lipid acyl chains.

However, it is still unknown whether even linear and shorter alkanes, more abundant in the early planet, would enter the membrane placing in the same perpendicular position and hence modifying the bilayer properties.

Although we found evidences on the actual insertion of the alkane molecules inside the C10 mix sample in form of vesicles (by means of SANS on D33 and NSE on IN15), the study of the alkane positioning inside the membrane has revealed many difficulties. In fact, the many preparation attempts to obtain ordered multilayers on a flat silicon wafer did not lead to Bragg peak observations on D16 neutron beamtime when the C10 mix sample was used, while the Capric acid alone (lacking the decanol) allowed us to observe only the first 2 orders (ILL Exp. Report: 9-13-758, Figure 2 left). The reasons for that lies probably in the need of a high concentration buffer (1:1 C10 mix:buffer molar ratio) to stabilize the membranes. For an effective alkane localization through the 1-dimensional Fourier analysis, a minimum of 4 peaks is needed, thus even the use of the pure Capric acid (without the decanol) was enough for our purposes.

For the above reasons, a similar compound has to be employed to be able to localize the alkanes inside the membrane having the same lipid chain length as the C10 mix, but without the issues related to the lipid film preparation: the 1,2-Didecanoyl-*sn*-glycero-3-phosphocholine (DCPC). The study will also complement and generalize what it has been found by [5] to different chain lengths and different length/type of inserted alkanes.

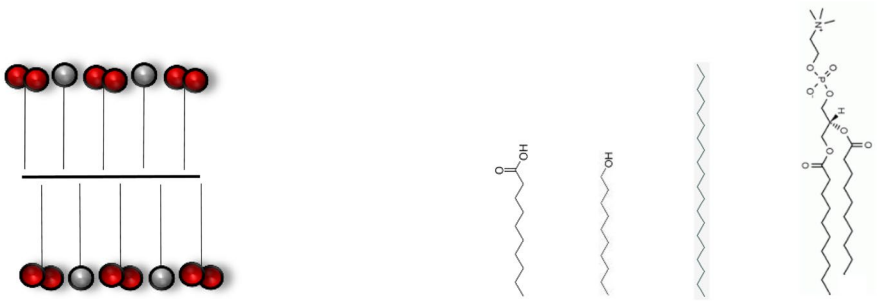

Figure 1: Left: sketch of the C10 mix model protomembrane. Right: the molecular formulas of the compounds of interest, Capric acid, decanol, eicosane and DCPC, respectively.

Experiment and results The experiment was performed in August 2020 on the diffractometer D16. The DCPC samples, pure or containing 4% of either hydrogenated or deuterated alkanes (eicosane, squalane or triacontane), were measured. Samples were incubated at a constant relative humidity (RH) of 97% for 2 hours before starting each scan. Every sample was incubated three times inside the humidity chamber BERIII at different D_2O contrasts, with the reservoir filled with 20 ml of different mixtures of D_2O/H_2O (8%, 70% or 100%). The different contrasts were used to extract the phase of each Bragg reflection required to determine the membrane neutron scattering length density (NSLD) profile [6].

An example of reciprocal space map obtained by merging two ω-scans is shown in Figure 2. The first visible feature is the occurrence of a series of reflections located at the same periodicity (q) on top of the line q// = (q)/2 that corresponds to the specular condition. The periodicity identified by the reflections results from the lamellar ordering of the multilayer, with a repeat distance given by one bilayer plus one water layer.

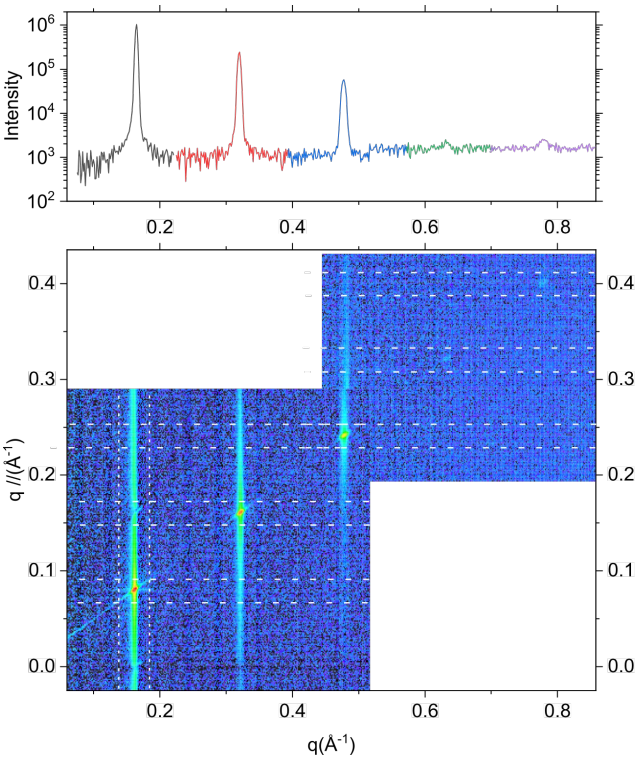

Figure 2. Example of reciprocal space map (q// vs q) of DCPC at 8% D_2O contrast and 30% relative humidity showing 5 lamellar diffraction orders.

Figure 3 shows the NSLD profiles of the samples at 8% and 100% D_2O/H_2O contrasts respectively. The 8% contrast allows easy definition of the position of the headgroup centres, as local maxima located at z ≈ ± 11.5 Å from the bilayer core. On the other hand, in the 100% contrast the higher NSLD of D_2O gives local maxima in the middle of the water region at z ≈ ± 21.3 Å from the bilayer mid-point.

By superposing the NSLD profiles in the water regions for the samples containing the hydrogenated and deuterated form of the same alkane (h- and d-eicosane; h- and d-squalane), one can calculate the difference profiles and get information about the average alkane location inside the membrane. As shown in Figure 3, both alkanes are found to be incorporated and sit inside the bilayer in the hydrocarbon region, although a more precise localization (e.g. whether the molecules are placed perpendicularly or parallel to the acyl chains of the bilayer) is not possible. This is potentially due to two reasons:

1. The limited resolution of the NSLD profiles (here drawn using up to 5 Bragg reflections, the only ones experimentally visible);

2. Intrinsic disorder in the samples at the used alkane/lipid ratio of 4% (e.g. if portions of the alkanes position differently).

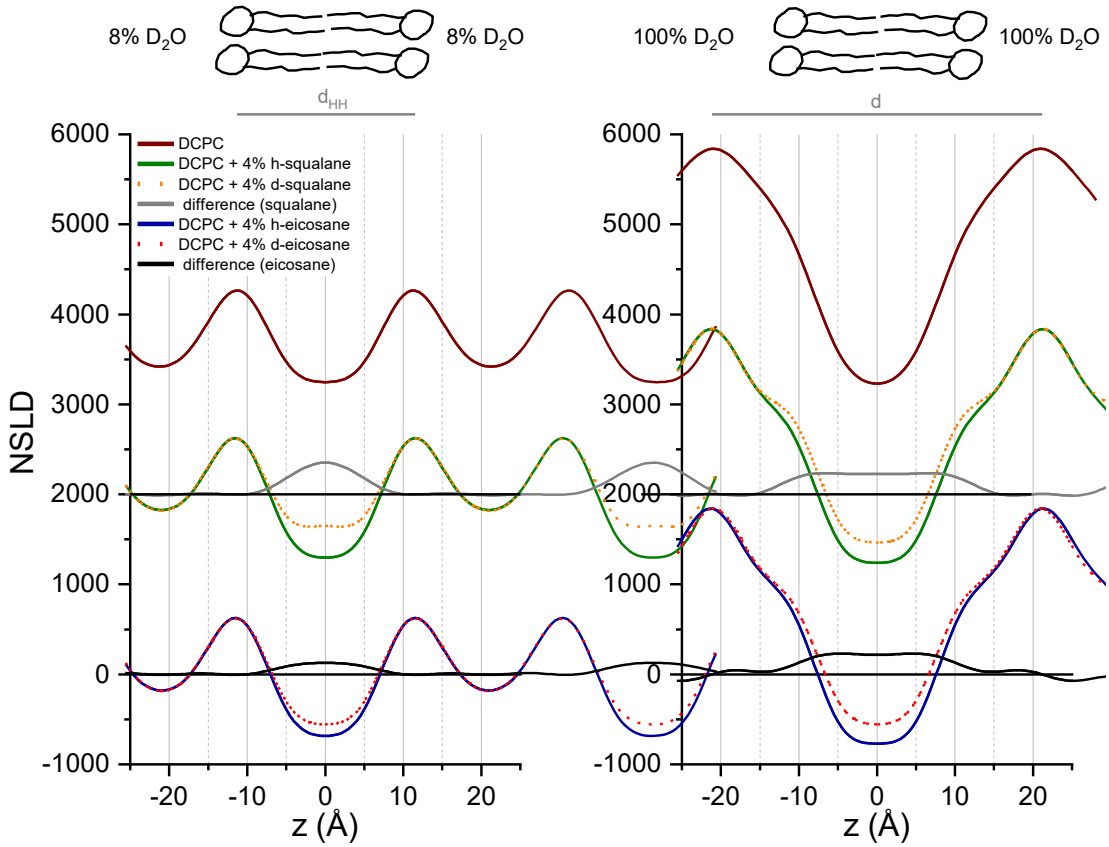

Figure 3. NSLD profiles of samples measured at 97% RH. Left: 8% D_2O/H_2O contrast; right: 100% contrast. The y-axis refers to the alkane containing samples, while the other NSLD profiles were shifted for clarity. Top image shows a sketch of the two bilayers mapped by the NSLD profiles in the z range shown and the quantities d_{HH} and d identified by the position of the maxima in the two cases.

Further data analysis is actually under progress.

References

[1] P.A. Monnard, D.W. Deamer, Met. Enzym. (2003) 372:133-51.
[2] M.S. Dodd; D. Papineau ; T. Grenne ; J.F. Slack; M. Rittner; F. Pirajno; J. O'Neil; C.T.S. Little, Nature (2017) 543, 60–64.
[3] K. Morigaki, P. Walde, Curr. Opin. Coll. Inter. Sci (2007) 75:80.
[4] S. Kapoor, M. Berghaus, S. Suladze, D. Prumbaum, S. Grobelny, P. Degen, S. Raunser, R. Winter, Angewandte Chemie, (2014), 53, 8397:8401.
[5] T. Hauss, S. Dante, N.A. Dencher, T.H. Haines, BBA (2002), 1556, 149-154.
[6] Worcester, D. L. & Franks, N. P. J. Mol. Biol. 100, 359–378 (1976).