Experimental report

11/02/2019

Proposal: 9-13-788 **Council:** 4/2018

Title: Temperature stability of protomembrane model vesicles by SANS

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: decanol
decanoic acid
lauric acid
dodecanol

Instrument	Requested days	Allocated days	From	To
D33	2	1	17/10/2018	18/10/2018

Abstract:

The proposal aims at characterizing the stability of single chain amphiphile vesicles as model membranes for a protocell (prebiotic living form).
This model consists in a bilayer of mid-chain fatty acid molecules along with fatty alcohols of equal chain length, with a hydrocarbon molecule inserted between the layers.
The addition of such intercalating agent in the mid-plane of the bilayer is expected to modify its physical characteristics, e.g. the membrane rigidity, permeability and overall stability. If proven, these effects will have natural impacts on the possible strategies put in place by the first living systems to maintain and protect the biological functions of its boundaries.
Previous DLS/SLS and DSC data indicated a threshold temperature (55°C), above which the single chain vesicles (lacking the hydrocarbon) become unstable and undergo a macroscopic phase separation.
Following the kinetics of both vesicle size/polydispersity (low q) and unilamellarity (high q), the functional consequences of the hydrocarbon presence will be assessed.
The study of our model can help today's science to understand how the first forms of life resisted to the harsh early Earth's conditions.

REPORT – EXPERIMENT 9-13-788 (17-18/10/2018)

The experiment was aimed at characterizing the stability of single chain amphiphile vesicles (ULVs and MLVs) as model of protocell membranes, the prebiotic form of the very first membranes.
This model consists in a bilayer of short-chain fatty acid molecules and fatty alcohols of equal chain length (C10), with various hydrocarbon molecules inserted in the bilayer: Eicosane (C20 linear alkane), Squalane (C30 branched alkane), and Triacontane (C30 linear alkane).
The addition of such intercalating agent in the bilayer is expected to modify its physical characteristics, e.g. the membrane rigidity, the friction between the two leaflets, its permeability and overall stability. If proven, these effects would reveal the possible strategies put in place by the first living systems to maintain and protect the biological functions of its boundaries at extreme temperature and pressure prebiotic conditions.
Previous DLS/SLS and DSC data indicated a threshold temperature (55°C), above which the single chain vesicles (lacking the hydrocarbon) become unstable and undergo a macroscopic phase separation.
The study of our model can help better understand how the first forms of life resisted to the harsh early Earth's conditions.

Experiment

The experiments were conducted using the standard sample changer setting available on D33, using 3 configurations to cover a wide range of Q (0.001 $Å^{-1}$ < Q < 0.6 $Å^{-1}$). The first series of measurements were performed on decanoic acid / decanol 50/50 (C10 mix) samples under the form of monodisperse unilamellar (extruded) vesicles (**ULVs**) at 20 °C < T < 60 °C, using the solvent contrast 100% D_2O/H_2O. Fig. 1 shows some of the obtained curves.

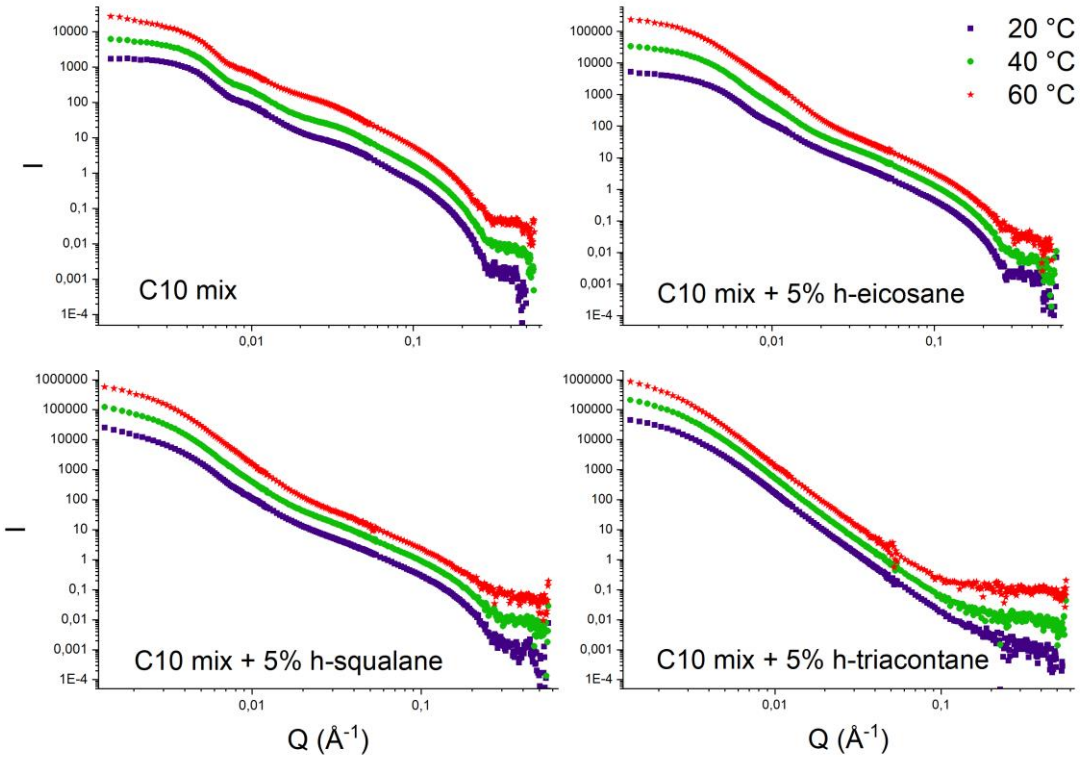

Fig. 1: SANS curves obtained at 3 different temperatures for C10 mix ULV samples with and without different apolar lipids (curves are shifted for clarity).

Already from a qualitative inspection, the ULV SANS curves show a remarkable difference in the behavior of the sample containing the triacontane with respect to the others, for which only **dense spheres** (probably oil droplets) seem to be present rather than vesicles. In addition, the samples containing eicosane and squalane, although showing a signal denoting vesicle presence in the middle Q range (with a Q^{-2} law), have clear trends as function of temperature, with the signal at low Q increasing while decreasing at middle Q.

The presence of an additional signal summed to the one corresponding to the vesicle form factor, when the apolar lipids squalane and eicosane are inserted, has been confirmed by performing additional measurements with the perdeuterated alkanes (an example in Fig 2*a*). This confirmed that a fraction of those hydrocarbons did not insert in the bilayer. Nevertheless, a difference upon addition of the apolar lipid is detected also in the fraction of signal which relates univocally to the vesicle form factor (middle-high Q range), suggesting that part of it enters the membrane and may result in a slight **increase** of the **bilayer thickness** (Fig 2*b*).

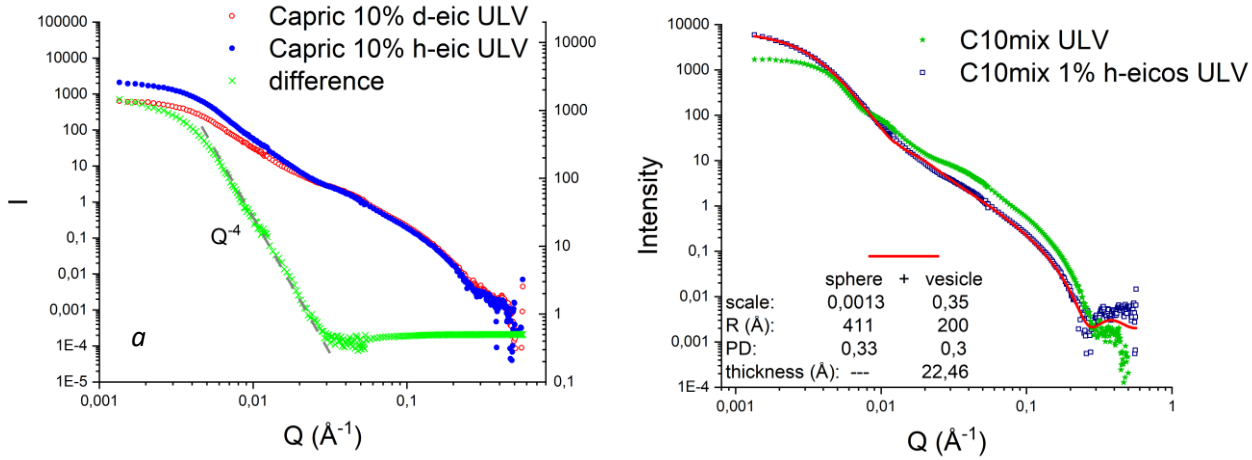

Fig. 2: *a*: SANS example curves of samples having hydrogenated and perdeuterated apolar lipids, with corresponding difference; *b*: comparison of curves measured from samples without (green) and with eicosane (blue). Red line plots a computed sum of *vesicle* and *dense sphere* form factors, assuming volume fractions proportional to the "scale" parameter (inset).

Another series of measurements was performed by using Multilamellar (non extruded) vesicles (**MLVs**) also at 20 °C < T < 60 °C, using the solvent contrast 100% D_2O/H_2O. Fig. 3 shows some of the obtained curves, which show an **effect** of the **apolar lipids** on the Bragg reflection that corresponds to the layers ordering, indicating that a fraction of them entered the membrane and helped **structuring** the multilayers.

A more detailed and quantitative data analysis using SASview is currently ongoing.

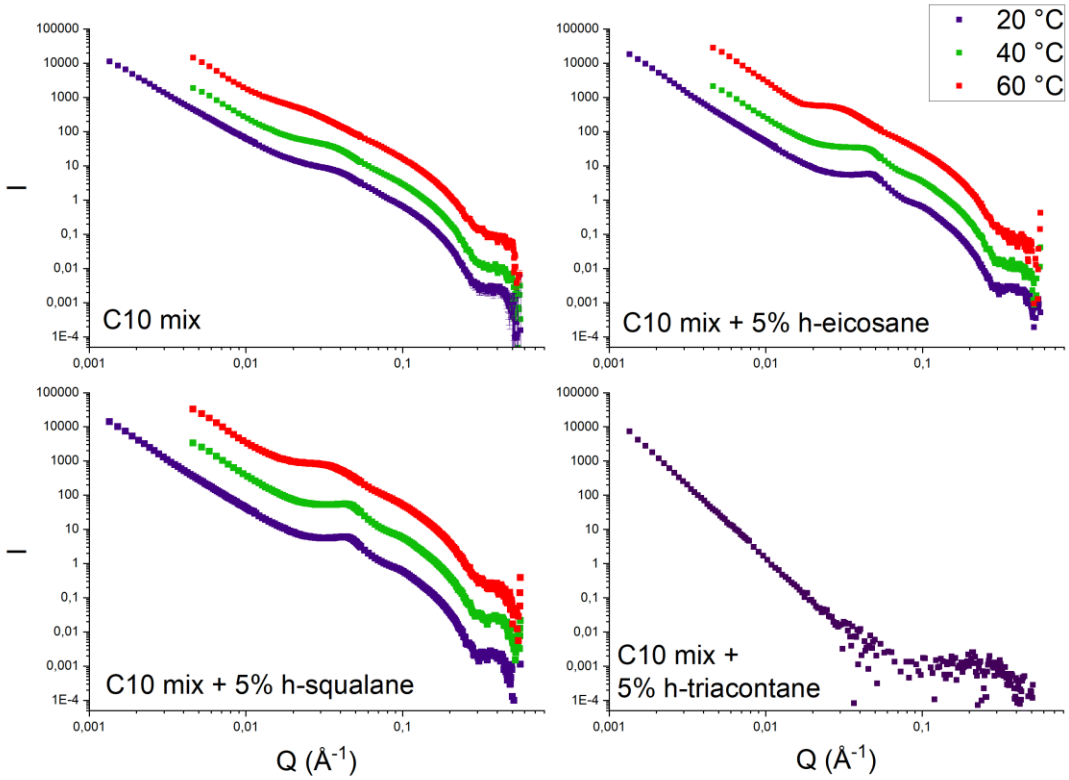

Fig. 3: SANS curves obtained at 3 different temperatures for C10 mix MLV samples without and with different apolar lipids (curves are shifted for clarity).