Experimental Report

Proposal: **8-02-696** **Council:** 4/2014

Title: Self-organisation of RNA in lipid multilayers

This proposal is a new proposal

Researh Area: Biology

Main proposer: **MAUREL Marie Christine**

Experimental Team: ZACCAI Giuseppe
DA SILVA Laura
MAUREL Marie Christine

Local Contact: DEME Bruno
SEYDEL Tilo

Samples: AMP, phospholipid

Instrument	Req. Days	All. Days	From	To
IN16B	4	2	02/10/2014	04/10/2014
D16	10	7	25/11/2014	02/12/2014

Abstract:

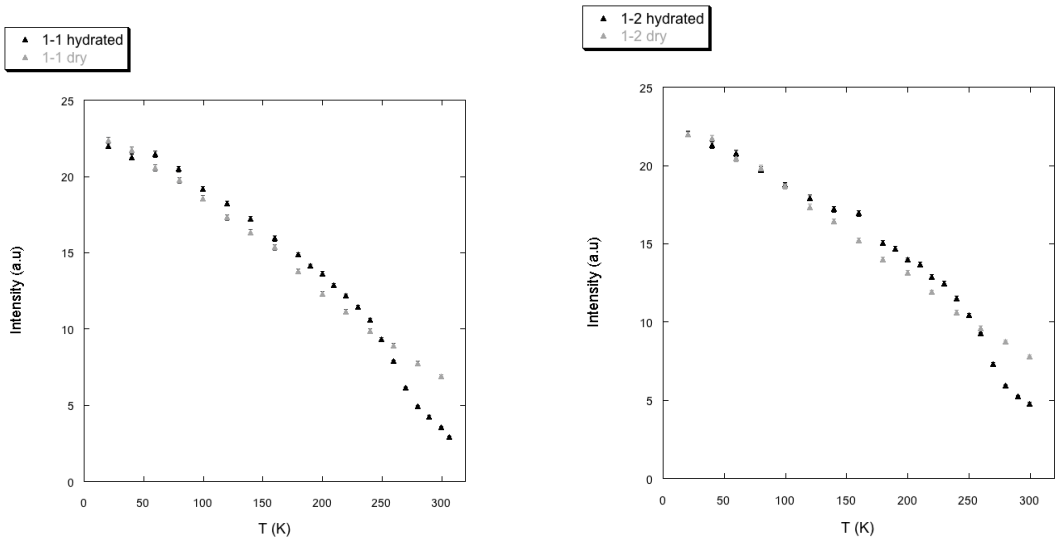
RNA displays a diversity of properties, which are now being developed for medical applications. A significant limitation to progress is that RNA can only be synthesized in small quantities using specific enzymes and costly substrates. A recent discovery is that guided polymerization in an anhydrous lipid environment can promote a non-enzymatic reaction in which oligomers of single stranded ribonucleic acids are synthesized from ordinary mononucleotides such as AMP. The observation is also highly relevant for origin of Life studies of how nucleic acids first assembled and then were incorporated into the earliest forms of cellular life. The presence of multilamellar phospholipid as an organizing matrix markedly enhanced the yield of polymeric products, presumably because the matrix serves to concentrate and organize the mononucleotides as well as allowing a degree of diffusional mobility required for extensive polymerization. The proposal is to characterize AMP/phospholipid multilamellar structures and dynamics on D16 and IN16B, respectively, with the aim of furthering our understanding of the RNA polymerization process.

Self-organisation of RNA in lipid multilayers

Background. RNA displays a rich diversity of properties, and the nucleic acid field is flourishing with the discovery and characterisation of multiple biological functions involving RNA molecules. However, a significant limitation to progress is that RNA can only be synthesized in small quantities using enzyme catalyzed reactions and costly nucleoside triphosphates (ATP, TTP, GTP and CTP). A recent discovery is that guided polymerization in an anhydrous lipid environment can promote a non-enzymatic reaction in which oligomers of single stranded ribonucleic acids are synthesized from ordinary mononucleotides [1-3]. The observation is also highly relevant in addressing a fundamental question of biology concerning how nucleic acids first assembled and then were incorporated into the earliest forms of cellular life 4 billion years ago. The presence of multilamellar phospholipid as an organizing matrix markedly enhanced the yield of polymeric products, presumably because the liquid crystalline matrix serves to concentrate and organize the mononucleotides as well as allowing a degree of diffusional mobility required for extensive polymerization.

The primary **aim** was to determine how mononucleotides are organized within a multilamellar lipid structure that is produced when liposomes and solutes undergo controlled dehydration.

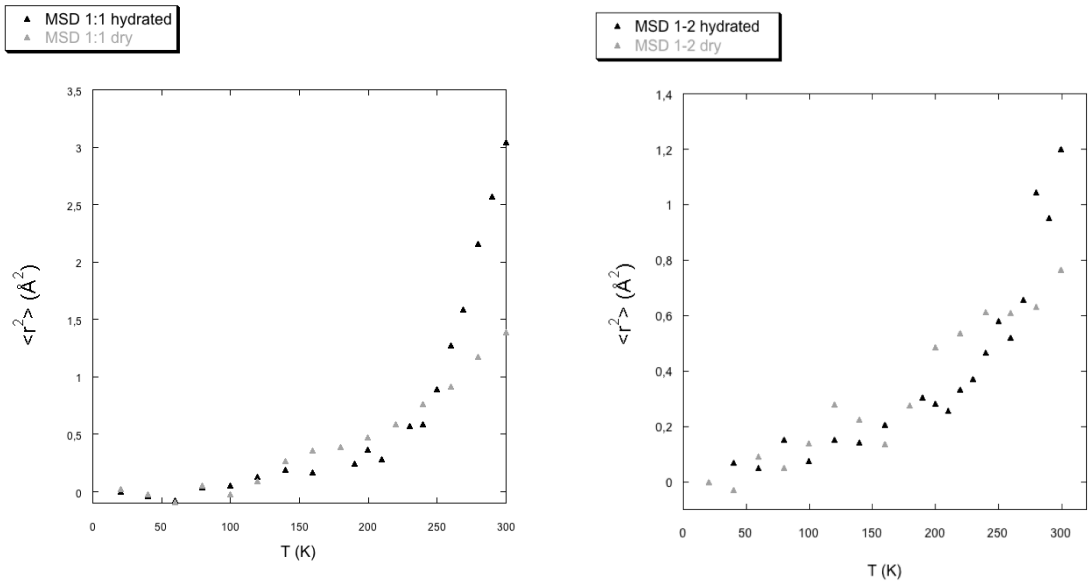
Experiments 8-02-696 on IN5, IN16B, IN13 and D16 spectrometers were performed to determine the dynamic mobility and structures of AMP molecules in the inter-lamellar space as a function of hydration. Here, we report experiments done on IN13 and D16. Samples were examined on the IN13 spectrometer (time window 100ps) for 1:1 and 1:2 DMPC:AMP mole ratio and different sample states: dried and hydrated at 0.4 g D_2O/g of product. The summed elastic intensities values are plotted in Figure 1.



A B

Figure 1. Summed elastic intensities measured as a function of temperature in a heating cycle for A) DMPC:AMP 1:1 and B) DMPC:AMP 1:2 dry and hydrated at 0.4 g D_2O/ g of product. The ratios are given as molar ratios.

A transition phase appeared at 250 K for both hydrated AMP:DMPC systems tested confirming an effect of AMP on lipids dynamics because no transition at 250 K was observed in DMPC lipids alone. Moreover, one observes more motion in hydrated lipid samples and less in dry ones. The motion was more important in samples where AMP was less concentrated suggesting we were observing AMP rather than lipid dynamics (Figure 2).

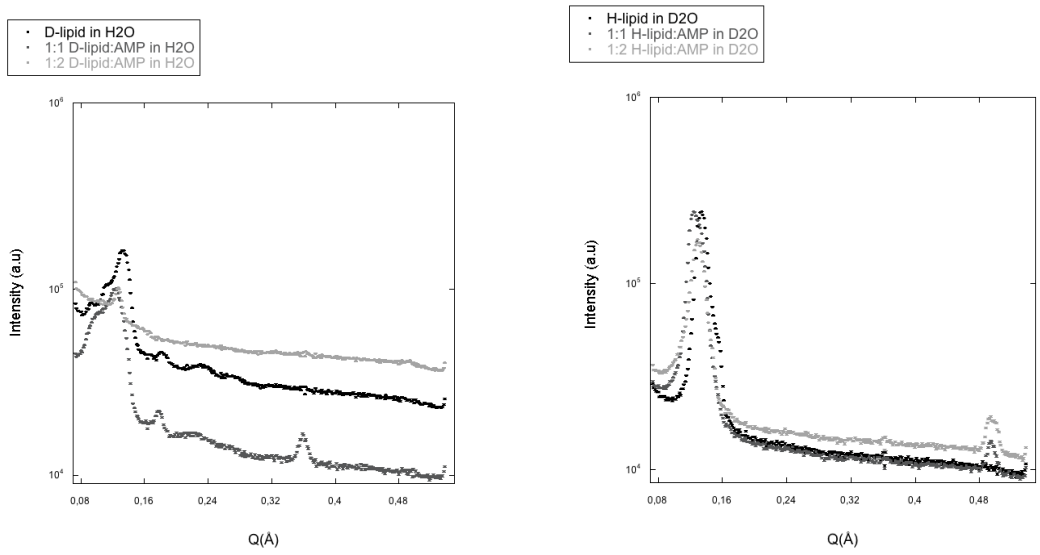


A B

Figure 2: Atomic mean-squared displacements as a function of temperature for A) DMPC:AMP ratio 1:1 and, B) DMPC:AMP ratio 1:2 dry and hydrated at 0.4 g D_2O/ g of product.

Quasi-elastic intensities were measured with IN5 instrument at two different energy resolutions: 100 μeV corresponding to the incident wavelength of 5 Å with an observable time-scale in the order of 7 ps and 12 μeV corresponding to the incident wavelength of 10 Å with an observable time-scale in the order of 55 ps, both at 310 K. Elastic scans from 20 to 315 K with IN16B instrument were also measured. The spectrometer IN16B has an energy resolution of 0.9 μeV which corresponds to a time-scale in the order of 1 ns. It allows studying the global dynamic of the structure on slower motion compared to IN13 and IN5. The data are still to be analyzed.

Preliminary results of diffraction experiments performed on the small momentum transfer diffractometer D16 show that the presence of AMP molecules has an effect in increasing the order of the lipids multilayers systems (Figure 3).



A B

Figure 3: Diffraction data for A) 1:1 D-lipids:AMP hydrated sample with H_2O and B) 1:1 H-lipids:AMP hydrated samples with D_2O at low relative humidity .

References

[1] Rajamani S, Vlassov A, Benner S, Coombs A, Olasagasti F, Deamer D. 2008 Lipid-assisted synthesis of RNA-like polymers from mononucleotides. Orig Life Evol Biosph. 38:57-74.
[2] Laura Toppozini, Hannah Dies, David W. Deamer, Maikel C. Rheinstädter (2013) Adenosine Monophosphate Forms Ordered Arrays in Multilamellar Lipid Matrices: Insights into Assembly of Nucleic Acid for Primitive Life PLOS ONE | www.plosone.org 1 May 2013 | Volume 8 | Issue 5 | e62810.
[3] Deamer D. 2012. Liquid crystalline nanostructures: organizing matrices for non-enzymatic nucleic acid polymerization. Chem Soc Rev. 41:5375-9.