Experimental report

19/10/2016

Proposal: **TEST-2599** **Council:** 4/2016

Title: Kinetic measurements of depositionof fully and partially deuterated lipid mixtures

Research area:

This proposal is a new proposal

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Samples: Natural Pichia pastoris deuterated lipids

Instrument	Requested days	Allocated days	From	To
FIGARO	1	3	23/05/2016	24/05/2016
			23/06/2016	25/06/2016

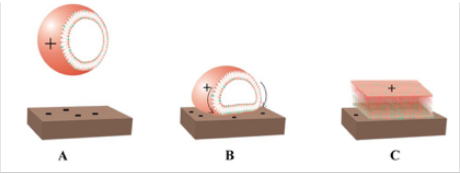
Abstract:

Report FIGARO
Test Experiment n°2599

Objectives:

The structure of yeast membranes differs considerably from typical model lipid bilayers composed of synthetic lipids and depends on the degree of lipid polyunsaturation. In order to investigate the interactions of such complex mixtures with neutron reflectometry techniques, it is usually needed to demonstrate the ability to deposit single bilayers on a substrate. Mostly Silicon substrates. This is performed using Quartz Crystal Microbalance with Dissipation measurements. Nevertheless, we have seen differences on the behaviour of the deposition of the samples between the laboratory (QCM-D) and NR experiments. The occasion was given to us to monitor deposition processes using complex mixtures directly on Figaro for 2 days.

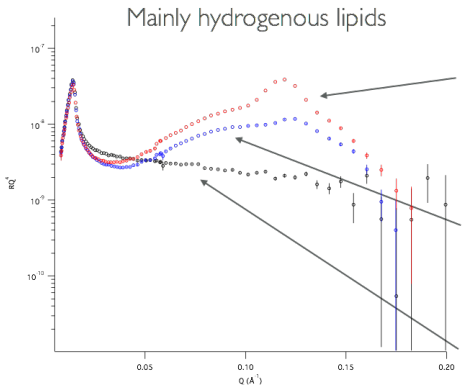
Results:



Schematic of vesicle fusion on a solid substrate
(A) Vesicles with a charge opposite to the surface charge are attracted to the substrate; (B) Vesicles adhere to the substrate and its shape is distorted to a disk-like one; (C) After reaching a certain threshold concentration on the surface the vesicles rupture to form a bilayer.

When dealing with complex natural lipid mixtures, the bilayer deposition is dependent on the salts used in the sample solution. The divalent Ca2+ salt have been shown to play a huge role in the deposition process since in its absence, or low concentration, no interactions, and thus disruption, of vesicles with the substrate was observed by QCM-D. The time dependence is also of importance as well as the temperature, maintained at 52°C during the deposition and lowered once a single bilayer formed.

Those were the only three parameters monitored and controlled for the deposition of our system. Nevertheless, during previous experiment, we could observe different behaviour depending on the geometry of the instrument used, and this raised an interrogation, investigated during this test.

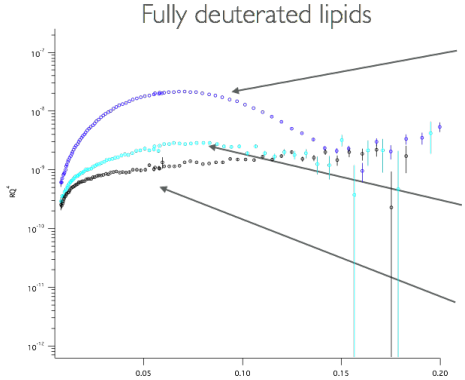


DEPOSITION: h-Polar dErg in H_2O, **reflection up** geometry,
30 min at 65°C, then D_2O rinsing and cell at 30°C.
= 150% deposition (BRAGG peak)

DEPOSITION: h-Polar dErg in H_2O, **reflection down** geometry,
30 min at 65°C, then D_2O rinsing and cell at 30°C.
= 102% deposition

Bare substrate (Si), D_2O contrast

With the example presented above, we see the impact of the geometry of the instrument used, or if you prefer, the location of the substrate, either above the sample (reflection up geometry) or below it (reflection down geometry)

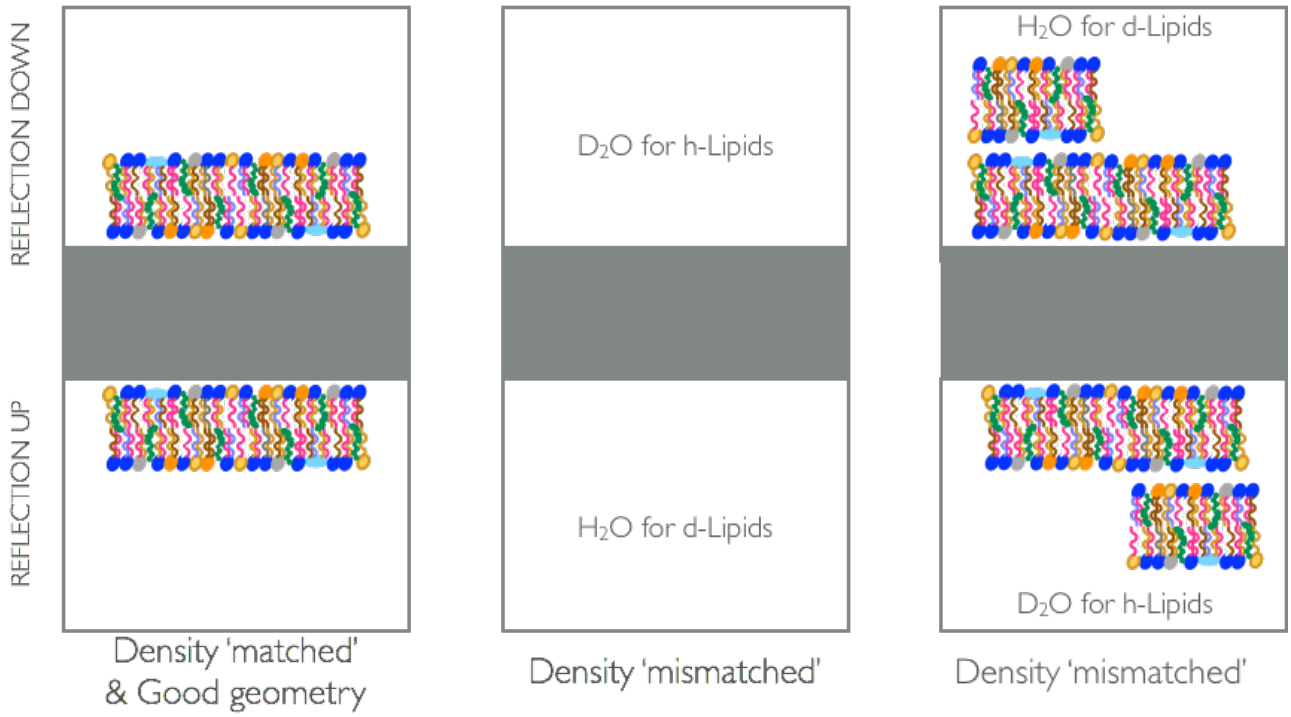


DEPOSITION: d-Lipids in D_2O, **reflection down** geometry,
30 min at 65°C, then H_2O rinsing and cell at 30°C.
= 100% deposition (perfect!)

DEPOSITION: d-Lipids in D_2O, **reflection up** geometry,
30 min at 65°C, then H_2O rinsing and cell at 30°C.
= Less than 50% deposition

Bare substrate (Si), H_2O contrast

With this new example, we observe the opposite behaviour. Deposition is enhanced in the opposite conformation. But, the reflection down geometry gives the best results in both cases. And this is due to the fact that we matched the overall density of the samples with the water density. The deposition of a fully deuterated sample in H_2O gives no results in reflection up geometry but gives stacks of bilayers in reflection down. The opposite is true for an hydrogenated sample in D_2O. The results obtained are represented as following:



These observations are probably due to the fact that, because of the quite important concentration of CaCl2 (20mM) necessary to deposit a bilayer, the sample never goes limpid once sonicated and the vesicles formed by sonication are big enough to scatter light. Increasing the time of sonication or its strength does not resolve this issue. Thus, on such large vesicles, forming back aggregates in time after a stop in sonicating, the gravity should have a significant impact. The observations shown above were called ‘gravity effect’ as it is though that lipids will ‘fall’ or ‘float’ depending on the density of the solution they are sonicating in. In conclusion, in order to deposit a single bilayer, monitoring salt concentrations temperature, density matches, it is also important to place the substrate below the solution remaining for deposition for a given time of 30 min.