Experimental report

07/02/2017

Proposal: 8-02-769 **Council:** 4/2016

Title: Probing cardiolipin layer structure on tuned surfaces with Neutron Reflectometry for the inclusion of ion transporting membrane proteins

Research area: Biology

This proposal is a new proposal

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Samples: Cardiolipin
Membrane Protein

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	3	05/12/2016	08/12/2016

Abstract:

We propose Neutron Reflectometry (NR) experiments in order to characterize at the nanoscale the structure of pure cardiolipin deposits onto flat substrates of controlled interfacial properties. Although cardiolipin is an important lipid from the mitochondrial membrane, it has seldom been characterized in its pure form. We will investigate the effect of both cardiolipin surface concentration and of the substrate interfacial properties (hydrophilicity and surface charges). These properties are easily tuned by the grafting of functional groups onto the substrates by surface chemistry or electrochemistry. We will further investigate the incorporation of the membrane transport protein NhaA, a sodium-proton antiporter into the characterized cardiolipin bilayer. The NhaA function is to specifically transport ions across a membrane to create a voltage from which energy can be recovered. This research is relevant to the design of efficient and stable biomimetic membranes for sustainable fuel cells relying on salt gradients and will be carried out within the funded collaborative project ANR bioWATTS (ANR-15-CE05-0003-01, 2016-19) between UMR CNRS 5525 (Grenoble) and 6226 (Rennes).

Exp. 8-02-769 "Probing cardiolipin layer structure on tuned surfaces with Neutron Reflectometry for the inclusion of ion transporting membrane proteins"

Experimental Team: M. Maccarini, S. Koehler, J-P. Alcaraz, E. Lebegue, T. Flinois, D. Martin
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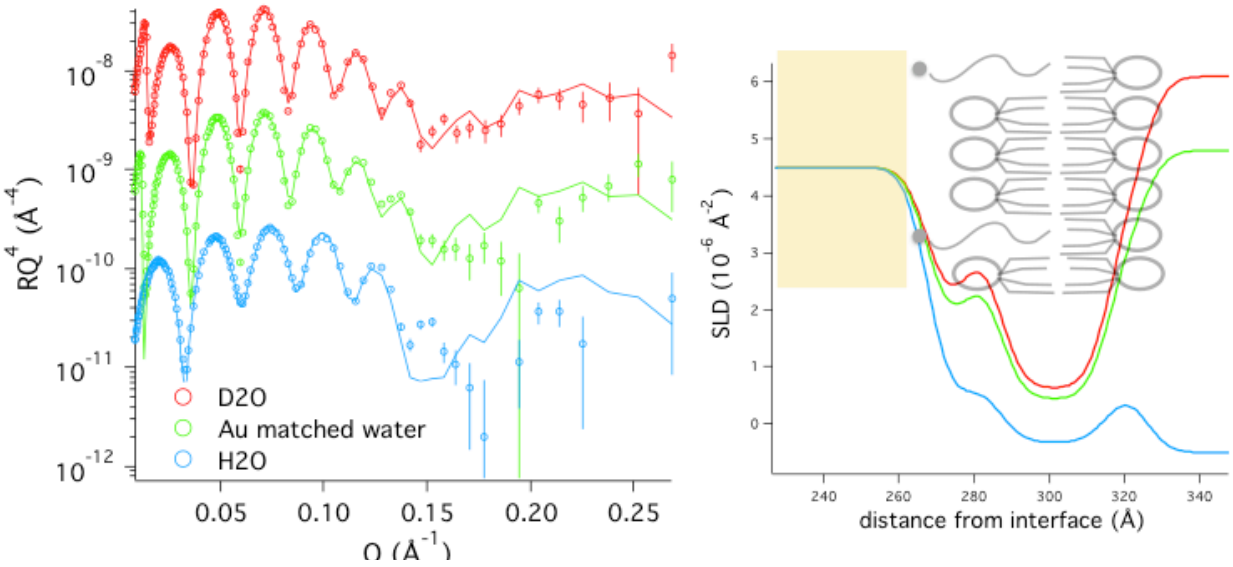
Motivation. We proposed Neutron Reflectometry (NR) experiment in order to characterise at the nanoscale the structure of cardiolipin (CP) lipid bilayers onto flat molecularly modified Au surfaces of controlled interfacial properties. CP is an important lipid from the membrane of mitochondria, organelles present in eukariotic cells involved in the production of energy and other tasks such as signalling, cell differentiation and death. The CP bilayers are used to incorporate membrane transport protein capable of translocating ions (e. g. protons, Na^+, K^+,...) across a lipid membrane with the aim of developing a biomimetic biofuel cell. Critical to the operation of the bimimetic fuel cell are the identity, properties and organisation of lipids in the bilayer and the inclusion of the transport membrane protein, in this case the NhaA antiporter that exchanges proton with sodium.

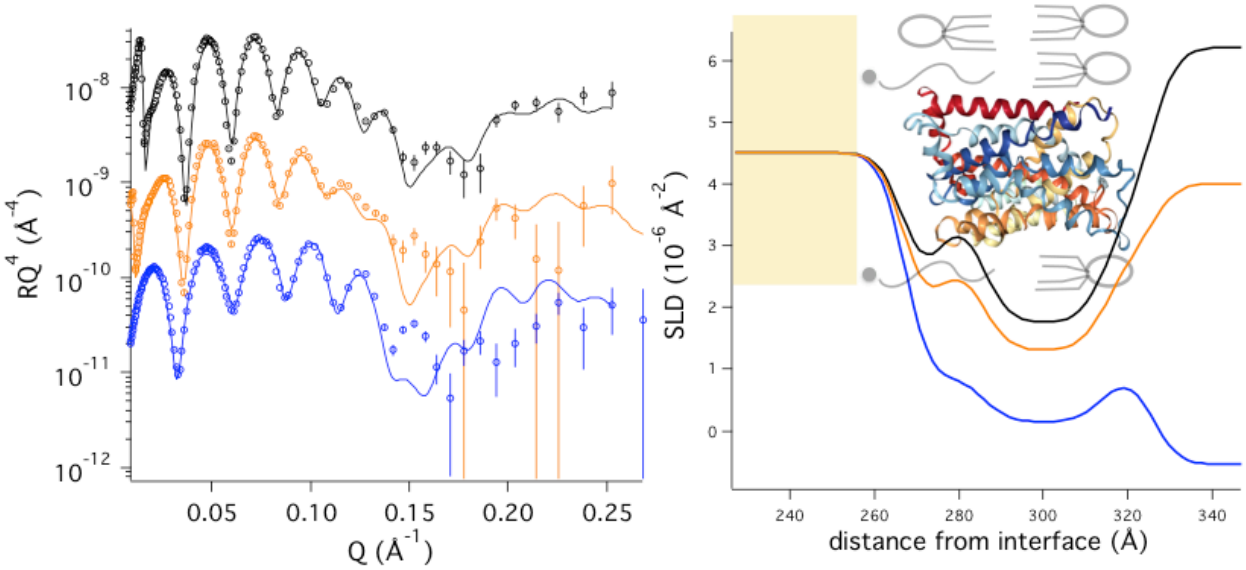
Results. Tethered bilayers were formed on gold substrates functionalised with tethering molecules following the procedure described in a previous article.[1] Shortly, following a pre-functionalisation of the gold substrates with a mixture of thiolated half-bilayer spanning phytanyls tether lipids (DLP) and thiolated polar spacer molecules, a mixed cardiolipin (CL) and DOPC lipid bilayer was deposited by fast solvent exchange. Different proportion of CL/DOPC were tested by Electrical Impedance Spectroscopy (EIS) in our laboratory performed on small samples 2x2 mm^2 and it was found that a 50:50 mixture produces a membrane that has the higher current insulating properties, i.e. maximum lipid coverage. Even content of CL up to 80:20 provided good lipid bilayers.

At ILL we scaled up the sample preparation to adapt it to large (5x8 cm^2) substrates. In Figure below we report the reflectometry profile obtained by the tethered lipid bilayer deposited on the gold substrate at three contrasts (D2O, H2O and Au matched contrast). The analysis of the NR data was obtained by simultaneous co-refinement of the NR curves obtained on the system at the three different contrasts. A model was built including a series slabs corresponding to the layers present in the system (i.e. Si substrate, SiO2, Ti, Au, Tethering molecules, lipid heads, lipid tails, lipid heads).



The results of the NR From the analysis of the NR data the CP/DOPC membrane had a total thickness of around 50 Å.

The NhaA antiporter was first incorporated into liposomes having the same CP/DOPC ratio and then transferred into the lipid bilayer by destabilisation and precipitation of the liposomes following by rinse with buffer solution. The analysis of the NR data showed an incorporation of the protein with ~ 0.2 of volume fraction (see Figure below).



The main result of this experiment is that we can form tethered lipid bilayer systems containing a high fraction of CL on gold substrates, and we can successfully incorporate the protein into the bilayer. It is now important to perform simultaneous NR and EIS experiment to relate the nanosctructural determination of the biomimetic membrane with the activity of the protein.

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[1] M. Maccarini, E. B. Watkins, B. Stidder, J.-P. Alcaraz, B. A. Cornell, D. K. Martin *Nanostructural determination of a lipid bilayer tethered to a gold substrate* The European Physical Journal E, 2016, 39, 123