# Experimental report

17/02/2017

**Proposal:** **8-02-748**

**Council:** 4/2015

**Title:** Investigating tethered lipid bilayer biosensor platforms: structure/function relationships

**Research area:** Biology

**This proposal is a resubmission of 8-02-714**


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**Samples:** DMPC

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| FIGARO | 4 | 0 | | |
| D17 | 4 | 4 | 26/01/2017 | 30/01/2017 |


## Abstract:

We propose to perform combined measurement of neutron reflectometry (NR) and impedance spectroscopy (IS) to determine the structure function/relationship of tethered lipid bilayer biosensor platforms built up by tethered bilayer systems with embedded membrane transport proteins.
The interpretation and modeling of IS data are related to structural detailed of the membranes. These can be obtained by modeling NR data to improve/constraint IS data, and vice versa. Hence, IS in situ monitoring of the biomimetic membrane activity during NR experiments would boost the ability of a full characterization of the biomimetic membrane obtained singularly with each of the two techniques.
We will apply this combined NR/IS measurement to obtain a characterize lipid bilayer systems tethered to gold substrates, with proteins membrane proteins like OprF or VDAC.

**Exp. 8-02-748 "Investigating tethered lipid bilayer biosensor platforms: structure/function relationship"**

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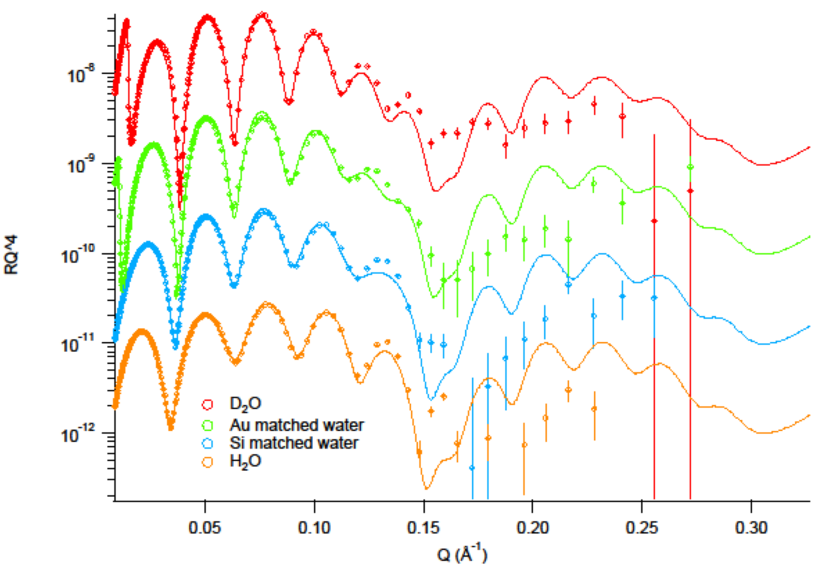
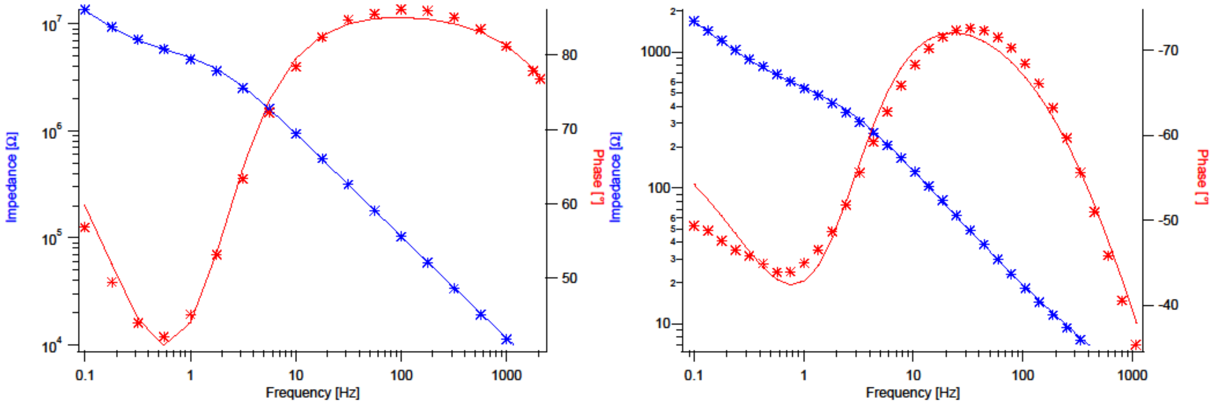
In this experiment we tested the possibility to perform simultaneous electrical impedance spectroscopy (EIS) and neutron reflectometry (NR) to characterise the structure and the electrical behaviour of tethered lipid bilayer system. Normally we perform EIS on very small cells of about 2.1 $mm^2$. The real challenge here is to scale up the size of the membrane to fit the requirements imposed by a NR experiment which require membranes of larger surfaces 30-40 $cm^2$.
We approached this using a system that consists of a 5x8cm^2 titanium and gold coated silicon wafer functionalized with a thiolated half-bilayer spanning phytanyl tether lipid (DLP) and thiolated polar spacer molecules. On top of that lipid bilayers of different composition were created using the fast solvent exchange method.
For combined EIS and NR experiments the standard solid-liquid interface cell for D17 and Figaro has been modified with a gold electrode in the bottom plate. The gold layer on which the tethered membrane was formed functioned as the second electrode enabling the application of voltage in between. For electric control a Bio-Logic SP200 Potentiostat was used.
Reflectometry measurements have been performed on bilayers containing Cardiolipin (CL) and DOPC. The figure below shows reflectivity curves for a 50:50 mol% CL:DOPC bilayer measured at 4 different contrasts.




| | Layer Thickness [Å] |
|---|---|
| SiO2 | 11 |
| Ti | 40.5 |
| Au | 203.66 |
| Tethers | 14.35 |
| Lipid heads | 9.49 |
| Lipid chains | 20.07 |
| Lipid heads | 8.96 |

A seven layer slab model was fitted to the curves by simultaneous co-refinement to characterize the bilayer system. The respective thickness for each layer is shown in the table above.

With the modified sample chamber we were able to record EIS measurements and reflectometry curves simultaneously. For that we scanned AC voltage cycles of frequencies between 200 kHz and 100 MHz and measured the systems response. In the figure below we show the obtained results (left) in comparison to an in-house EIS measurement in or lab (right) of the same sample composition.




The phase curve recorded with the ILL sample chamber shows the shape typical for a lipid membrane with a minimum in phase shift around 1 Hz. By fitting an equivalent circuit to the EIS data we deduced bilayer parameters like membrane capacity and conductance.

For the sample corresponding to the neutron reflectivity curves above we found values of 0.95 μS/cm^2 conductance and 6 μF/cm^2 membrane capacitance. Compared to typical results of in-house measurements for the same sample composition of 0.11 μS/cm^2 conductance and 1 μF/cm^2 membrane capacitance these are on the same order of magnitude. **This shows that it is possible to create bilayers of roughly the same electrochemical properties using this sample system and the electrochemical properties are comparable on these two different length scales**.

The **next step** would be to use simultaneous EIS and neutron reflectivity measurements to determine the activity of ion channels proteins, as they alter the electrochemical properties of the membrane when active by transporting charge across the membrane.