Experimental Report

Proposal: **8-02-632** **Council:** 10/2011

Title: Investigating tethered lipid bilayer biosensor platforms

This proposal is a new proposal

Researh Area: Biology

Main proposer: **MARTIN Donald**

Experimental Team: WATKINS Erik
STIDDER Barry
MARTIN Donald
ALCARAZ Jean-Pierre
MACCARINI MARCO

Local Contact: WATKINS Erik

Samples: DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine
VDAC ion channel

Instrument	Req. Days	All. Days	From	To
D17	4	4	05/10/2012	07/10/2012
			06/11/2012	08/11/2012

Abstract:

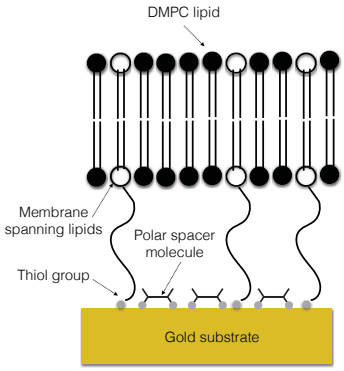
Bruce Cornell has previously developed a bilayer system that is covalently bonded to a gold substrate and which is extremely stable and optimized for incorporating transport proteins [1]. That tethered bilayer system comprises a mixture of synthetic archaebacterial double-length reservoir half-membrane-spanning phytanyl lipids (DLP) and full-membrane-spanning lipids (MSL) which are attached to a gold surface via polar linkers and sulphur-gold bonds. The surface density of those tethered species is controlled by dilution with a low-molecular weight hydrophilic spacer, mercaptoacetic acid disulphide (MAAD) or ethylene disulphide (EDS) which are directly attached to the gold surface using the same chemistry. We have used impedance spectroscopy to characterize the functional properties of membrane transport proteins that are incorporated in that tethered bilayer system. It is important to now measure the structure of the tethered lipid bilayer system, which is the reason for applying neutron reflectivity beam time. Results will enable the properties of both the membrane and the support to be tuned for the particular requirements of specific proteins.

Investigating tethered lipid bilayer biosensor platforms

M. Maccarini[1],J-P. Alcaraz[1] , B. Stidder[1], E. Watkins[2], D.K. Martin[1]

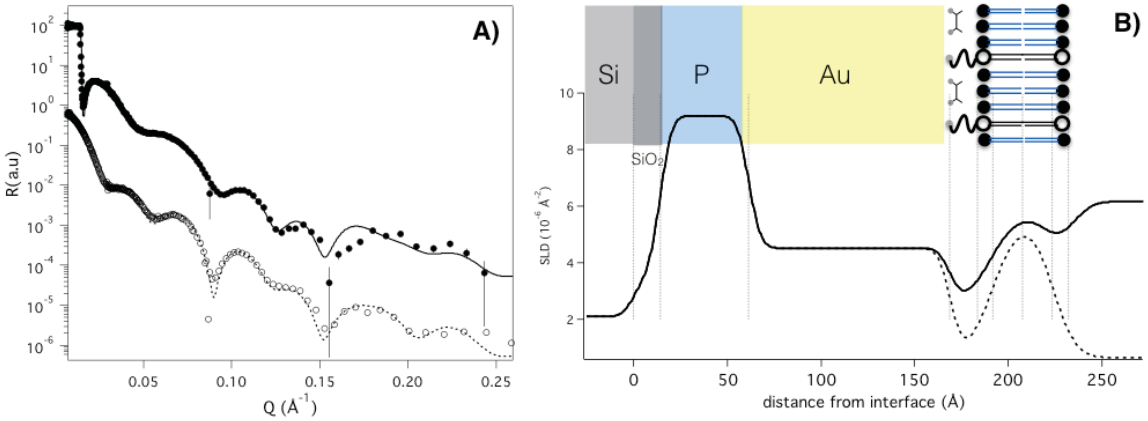
[1] UJF, TIMC-IMAG, Fr; [2] Institut Laue-Langevin, Fr; LANSCE, USA

Background. Bruce Cornell has previously developed a bilayer system that is covalently bonded to a gold substrate and which is extremely stable and optimized for incorporating transport proteins [1]. That tethered bilayer system comprises a mixture of synthetic archaebacterial double-length reservoir half-membrane-spanning phytanyl lipids (DLP) and full-membrane-spanning lipids (MSL) which are attached to a gold surface via polar linkers and sulphur-gold bonds. The surface density of those tethered species is controlled by dilution with a low-molecular weight polar spacers, mercaptoacetic acid disulphide (MAAD) which are directly attached to the gold surface using the same chemistry. This set of adsorbed molecules will act as a scaffolding structure for the DSPC bilayer, which will be formed (see figure on the right).



We have used impedance spectroscopy to characterize the functional properties of membrane transport proteins that are incorporated in that tethered bilayer system. However, recent modeling suggests that the density of MAAD spacing molecules influences whether there will be a successful incorporation of complex membrane proteins with large molecular weight. In this set of neutron reflectometry experiments we characterized the nanostructural details of the tethered bilayer systems as a function of the ratio between MSL and MAAD molecules, and characterized the inclusion of the protein OprF, isolated from *Pseudomonas Aeurginosa*, bacterium in the tethered bilayer system. The N-terminus of OprF is embedded in the membrane and the C-terminus protrudes from the membrane. OprF was synthesized using a novel cell-free approach developed at our laboratory. This protein is the target of novel drugs to treat infections from bacteria that are resistant to conventional antibiotic

Results and discussions. We characterized the structural details of two sets of tethered lipid bilayers (tLBM). One tLBM was formed with a 10% ratio between MSL and MAAD, and the other tLBM was formed with a ratio of 40% between MSL and MAAD. In order to elucidate the inner molecular details of



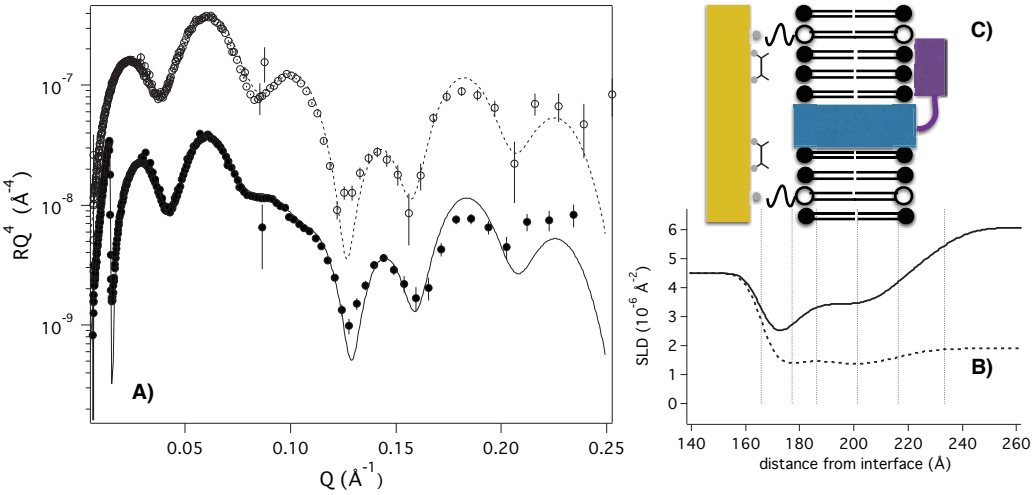
the bilayer, we introduced a contrast between the MSL and the mobile lipids by taking the former with hydrogenated tails (in black) and the latter with deuterated tails (in blue).

In the Figure above we display as an example the data obtained for d-DMPC lipid bilayer adsorbed on the MSL/MAAD 10% tethered system (A) and the scattering length density profiles corresponding to the fitting models together with a sketch of the system

Our main results are:

1) We obtained a nanoscale characterization of the lipid bilayer for the 10 and 40% MSL/MAAD systems
2) We obtained the thickness and hydration of the interstitial layer between the lipid bilayer and the substrate
3) We observed novel details in the nanostructure of the MSL, particularly that part of the MSL are bent on themselves instead of spanning fully across the bilayer. This effect was stronger on the 40% MSL/MAAD system, and this could cause problems for the inclusion of proteins in the bilayer.

Then we successfully incorporated OprF protein in the tLBM formed by the MSL/MAAD 10% tethered system, as shown in the Figure below. The protein is represented by two rectangular shapes, blue for the N-terminus andpurple for the C-terminus.



Our main results were:

1) The modeling of the reflectivity profiles were compatible with a volume fraction of the OprF in the bilayer of around 0.4.
2) Our modeling was compatible with the C-terminus pointing out only toward the bulk liquid phase.
3) OprF made using the cell-free protocol and incorporated into a tethered lipid bilayer provides a controllable (and measurable at the nanoscale) biomimetic system constructed in vitro

The next step will be to perform reflectometry measurement with in situ Impedence Spectroscopy, to monitor *in situ* the activity of the protein and establish fundamental structure/function relationships.

References:

[1] Cornell B.A., Braach-Maksvytis V.L.B., King L.G., Osman P.D.J., Raguse B., Wieczorek L. and R. J. Pace R.J. (1997). A biosensor that uses ion-channelswitches. *Nature*, **387**:580-583