Experimental Report

Proposal: **8-05-415** **Council:** 10/2012

Title: Probing membrane protein structureusing neutron reflectivity

This proposal is a new proposal

Researh Area: Biology

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Samples: cell-free expression lysate
DMPC

Instrument	Req. Days	All. Days	From	To
FIGARO	5	4	30/07/2013	02/08/2013

Abstract:

Membrane proteins represent about 30% of the proteomes of organisms and are dramatically under-represented in the structural database of the Protein Data Bank. This can be explained by their lack of stability and difficulty to crystallize. To answer this demand we propose to use a bacterial cell-free system to express hydrogenated and deuterated membrane proteins directly into a supported lipid bilayer in a neutron reflectivity (NR) solid-liquid cell. Cell-free systems are in vitro protein synthesis systems that use the extract (or lysate) from a prokaryotic or eukaryotic organism to provide the cellular machinery necessary for protein production. Here, we intend to use supported lipid bilayers to provide the lipidic environment into which the expressed membrane proteins will be directly inserted. To establish a proof of concept and demonstrate the potential of this new technique, we will investigate VDAC, a well characterized protein. Following these results, we will proceed to investigate the structure of Hepatitis C virus (HCV) proteins which have not, at this point, been crystallized.

Probing membrane protein structure using neutron reflectivity

Scientific background:

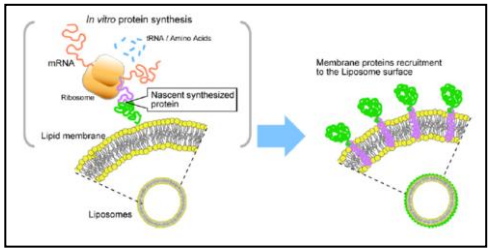

Figure 1: Schematic illustration of membrane protein direct insertion in the lipid bilayer of a liposome [1]

Membrane proteins represent about 30% of the proteomes of organisms and are dramatically under-represented in the structural database of the Protein Data Bank. This can be explained by their lack of stability and difficulty to crystallize. Here, we use a bacterial cell-free system to express hydrogenated and deuterated membrane proteins directly into a supported lipid bilayer in a neutron reflectivity (NR) solid-liquid cell to study their structure. Cell-free systems are in vitro protein synthesis systems that use the extract (or lysate) from a prokaryotic or eukaryotic organism to provide the cellular machinery necessary for protein production. Moreover, cell-free systems enable expression of proteins using isotope-labeled amino acids and have been used to produce samples for nuclear magnetic resonance [2]. Cell-free technology is ideal to overexpress membrane proteins since it offers the possibility to add lipids during protein synthesis resulting in solubilzed membrane proteins. Synthelis, a start-up company located in Grenoble, uses cell-free protein synthesis supplemented with liposomes to express membrane proteins. This approach is patented by university Joseph Fourier and Synthelis benefits from an exclusive operating license. Cell-free protein expression offers the flexibility to enable both hydrogenated and deuterated membrane protein synthesis and insertion into supported lipid bilayers within a NR cell. Typically, proteins are imbedded in the lipid membrane of liposomes. Here, we intend to use supported lipid bilayers to provide the lipidic environment required for the expression of membrane proteins. Similar methodology has been previously reported to study protein incorporation using QCM-D [3].

Preliminary characterization of p7:

The membrane protein chosen for the focus of this study was p7: a Hetatitis C viral porin involved in HCV reassembly and target for new drug candidates. p7 has been expressed with a yield of 800 µg/mL and its activity has been recorded using impedance spectroscopy and droplet lipid bilayer assays. Crystal formation has been attempted with no success, but NMR data are available [4]. We have successfully produced and functionality assessed this protein using cell-free expression. Protein yields and functionality have been determined using polyacrylamide gels and binding assays such as “pull down assays”, QCM-D and SPR analysis.

Neutron reflectivity results:

NR experiments were performed on asolectin bilayers and POPC bilayers before and after the expression of p7. Expressions were performed using either hydrogenated and deuterated amino acids with the deuterated protein providing the optimal contrast. While control experiments without DNA did not express protein, they did significantly disturb the supported bilayer structure.

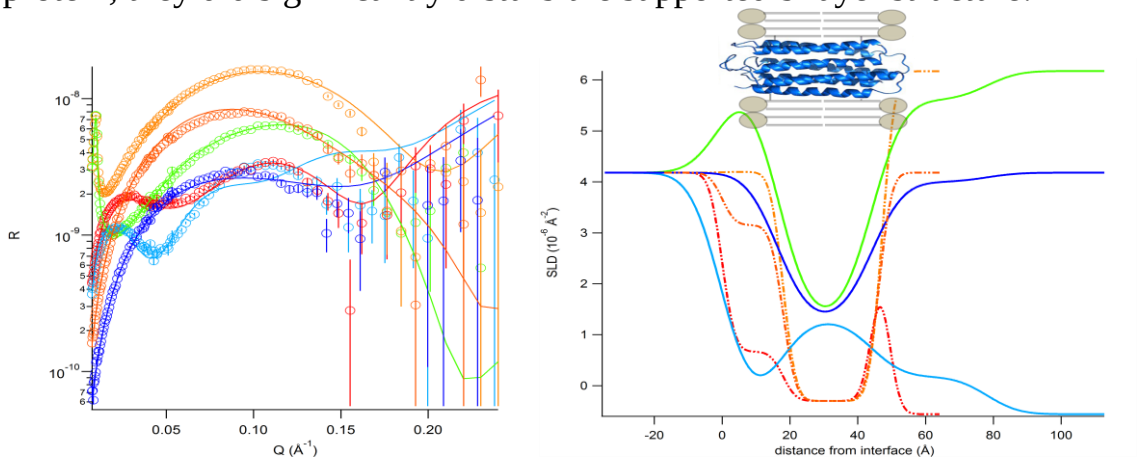

Fig 2: The left panel shows R*Qz^4 vs Qz NR data for a supported asolectin bilayer before (red data) and after deuterated p7 insertion (blue data) with three water contrasts each. The right panel shows the corresponding SLD profiles. The low SLD dip in the red curves between 20-40Å represents the lipid tail region of the bilayer. After p7 insertion, the SLD in this region of the bilayer increased dramatically for all water contrasts. This demonstrates the successful insertion of p7 at high area coverage. Since the SLD also increased for the H2O contrast, the change can't be attributed to holes filled with water.

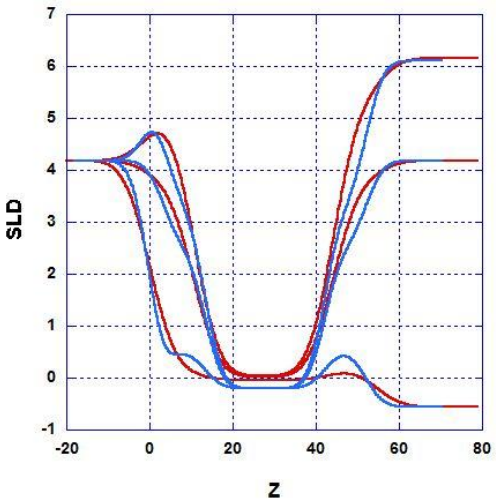

Fig 3: SLD profiles for a supported POPC bilayer before (blue profiles) and after deuterated p7 insertion (red profiles) with three water contrasts each. The low SLD dip in the red curves between 20-40Å represents the lipid tail region of the bilayer. After p7 insertion, the SLD in this region of the bilayer increased for all water contrasts indicating p7 insertion. The increase was significantly smaller than observed for insertion into an asolectin bilayer and corresponds to approximately 3% of the membrane area consisting of protein. These results demonstrate that lipid bilayer properties influence the p7 insertion mechanism.

References:

[1] Yuki Moritani et al., *The FEBS Journal* 277, no. 16 (August 2010): 3343–52,
[2] Dmitriy A Vinarov et al., *Nature Methods* 1, no. 2 (2004): 1–5.
[3] Jerome Chalmeau et al., *Biochimica Et Biophysica Acta*, January 2011,
[4] Roland Montserret et al., *The Journal of Biological Chemistry* 285, no. 41 (October 8, 2010): 31446–61,