Experimental report

14/02/2018

Proposal: 9-13-689 **Council:** 10/2016

Title: Out-of-equilibrium active membranes: incorporation of bacteriorhodopsin in floating lipid bilayer

Research area: Soft condensed matter

This proposal is a continuation of 9-13-580

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Samples: Silicon crystals
phospholipids (DSPC, DMPC, POPC)

Instrument	Requested days	Allocated days	From	To
FIGARO	4	0		
D17	4	3	20/02/2017	23/02/2017

Abstract:

Transport process through the membrane involves specific membrane proteins, which use metabolic energy as ATP hydrolysis or photochemical reaction to process conformational changes. This protein activity breaks the fluctuation-dissipation theorem leading to out-of-equilibrium fluctuations. These active fluctuations have been widely described theoretically but less is known on the experimental point of view.
In the last 15 years, we have developed a new model system (fluid floating bilayer) and original off-specular reflectivity experiments allowing us to study the fluctuations of a single floating bilayer near a substrate as well as membrane-membrane interactions. It opens a wide range of perspectives to achieve a better understanding of active membranes properties which is the purpose of a PhD financed by ILL, in collaboration between ILL.

In a recent experiment (9-13-580), we have obtained strong evidences of proteins (bacterio-rhodopsin) in supported DMPC bilayer. The next challenging step is to insert the proteins in floating bilayer and to investigate fluctuation enhancement by protein activation. This is the purpose of this proposal.

Experimental Report-Experiment № 9-13-689

Out-of-equilibrium active membranes: incorporation of bacteriorhodopsin in supported lipid bilayer

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Introduction/State of art

Transmembrane proteins play a pivotal role in the large number of cellular processes and contribute to essential functions of the cell membranes. Membranes exhibit thermal fluctuations, but transmembrane proteins activity breaks the fluctuation-dissipation theorem leading to out-of-equilibrium fluctuations. Active fluctuations have been widely described theoretically [1], but there are only few techniques for their experimental study. The main aim of this project is to investigate out-of-equilibrium fluctuations of phospholipid membranes induced by the active transmembrane protein bacteriorhodopsin (BR). As a starting point, the structural characterization of the protein embedding membrane system at sub-micron length scales is necessary. In fact, the direct study of membrane proteins by neutron reflectometry requires creation of rational models, which mimic the cell membrane structurally, and in which the protein retains its structural integrity and functional activity.

Model systems such as solid-supported single and floating phospholipid bilayers, prepared by Langmuir-Blodgett/Langmuir-Schaefer techniques, are in use to mimic and study phospholipid membranes, their interactions and the protein incorporation process. The detergent-mediated incorporation method [2] was adapted to perform the insertion of BR in the phospholipid bilayer at the interfaces, using sugar- based detergents as DDM and DOTM.

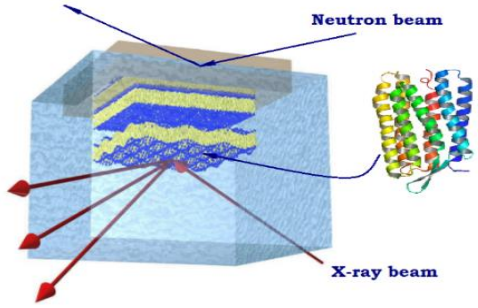

Figure 1: *Schematic view of a supported double bilayer and ternary structure of bacteriorhodopsin (BR).*

The main aim of conducted experiment was to demonstrate clearly the possibility of inserting BR in floating bilayer systems by mean of the detergent-mediated incorporation method and to study the induced structural changes caused by BR insertion.

Experiment and Results:

We have performed experiments on the D17 reflectometer in TOF mode with two incident angles and wavelength range from 2 to 20 Å. The phospholipid bilayers systems were deposited on highly polished 5x8 cm^2 silicon blocks by Langmuir-Blodgett/Langmuir-Schaefer technique. Samples were prepared at the ILL using PSCM lab facilities.

We have prepared 12 pristine bilayers in total:

i. 4 double bilayer systems consisting of a supporting DSPC bilayer and of a floating POPC:DPPC (1:1 or 0.7:0.3 by mol) bilayer.
ii. 1 single DPPC bilayer
iii. 1 single DPPC:POPC(1:1 by mol) bilayer
iv. 4 DSPC/DPPC double bilayers
v. 2 DSPC/(d62)DPPC double bilayers.

Even if the transfer ratio of samples made of DPPC: POPC mixture ((i) as a floating lipid bilayer and (iii)), indicated an almost 100% coverage of the substrate, such samples appeared to be very fragile and unstable during continuous solvent exchange and protein injection steps. This unexpected behavior demonstrates the interaction between the proteins and the bilayer, but also the difficulty of the insertion process. This is the first important result of our experiment, and only NR can give us

such an information. We thus focused on more robust samples containing saturated phospholipid moieties (ii, iv and v) obtaining consistent results.

These experiments were performed at 48 °C to keep the floating bilayer in the fluid biologically relevant phase and to avoid the insertion of the BR in the first supported bilayer (in gel phase at 48 °C). In order to maximize the amount of the inserted BR into the floating bilayer without compromising its integrity, we performed measurements for different **detergent concentrations** and **different incubation times**. We did systematically at least 3 contrasts measurements before and after injection of the protein/detergent solution to get a refined characterization of the structure of the samples with and without proteins.

The insertion of the protein in the double bilayer systems resulted in significant changes in the reflectivity curves. As example, reflectivity, fits and resulting SLD profiles for DSPC/DPPC double bilayer, before (closed symbols) and after (opened symbols) proteins injection, are shown in Figure 1. The obtained fit demonstrates the high quality of the DSPC / DPPC double bilayer samples. The protocol for the sample preparation leads to very reproducible samples, being the reflectivity of all DSPC/DPPC double bilayer samples almost identical.

After protein injection and rinsing, a small increase of the total thickness of the floating bilayers was observed. The SLD value of the tail region of the floating DPPC bilayer increases from 0.353×10^{-6} Å^{-2} to 0.7×10^{-6} Å^{-2}, which can be interpreted as the result of the insertion of BR in the bilayer. The amount of BR can be estimated as the 45% (by volume) of the floating bilayer.

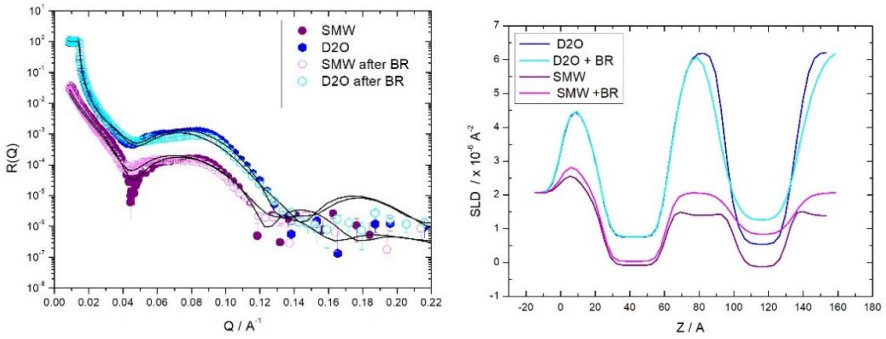

Figure 1: *(Left) Reflectivity curves for the DSPC:DPPC double bilayer at 48 °C (DSPC supported bilayer is in gel phase, DPPC floating bilayer is in fluid phase) before (open symbols) and after (closed symbols) protein BR incorporation. (right) SLD profiles corresponding to the fits. The floating bilayer of the final system consists of 45 % of proteins BR and 55% of DPPC lipids, the first DSPC bilayer was not changed.*

Problems encountered:

- After BR injection, mixing between hydrogenated and deuterated lipids was detected for the DSPC/(d62)DPPC double bilayer samples. Because of this, the samples were ruined.
- One of the DSPC/DPPC bilayers was lost after the injection of BR in combination with too long incubation time.
- High Q data showed a poor signal/noise ratio. The problem was investigated with the help of the instrument responsible during the beamtime, but no solution was found. Once the experiment was finished, it was discovered that this problem was caused by a problem with the detector electronics.

Conclusion

We have obtained important information on the stability of double bilayer systems after its destabilization with the detergent and protein/detergent mixture to perform the protein incorporation step. Our results are promising and demonstrate the possibility of inserting of the bacteriorhodopsin in the DSPC/DPPC phospholipid double bilayer systems. The next challenging step is to study the induced structural changes caused by BR activation in floating phospholipid bilayers. The new cell for reflectometry experiments with the ability to expose the sample to light to activate BR is being under development.

References: [1] J. Prost, J.-B. Manneville, and R. Bruinsma. EPJB, 1:465–480 (1998).

[2] M. Dezi, A. Di Cicco, P. Bassereau, and D. L´evy. PNAS, 110(18):7276-7281 (2013).