Experimental report

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Proposal: 8-02-803 **Council:** 4/2017

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

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Bacteriorhodopsin
synthetic phospholipids

Instrument	Requested days	Allocated days	From	To
D17	4	4	09/04/2018	13/04/2018
FIGARO	4	0		

Abstract:

Membrane proteins are associated with the lipid membranes in different ways, thus performing numerous specific functions. Using metabolic energy as photochemical reaction, transmembrane proteins frequently undergo conformational changes to perform their functions. Such protein activity leads to out-of-equilibrium fluctuation of the complex systems consisting in integrated proteins and lipid bilayers. Measurement of the fluctuation spectrum at sub-µm length scales is needed to complete understanding of the undergoing active processes.

To investigate out-of-equilibrium fluctuations of phospholipid membranes induced by active transmembrane proteins, we propose to study the insertion of bacteriorhodopsin (BR) in floating phospholipids bilayers. The detergent-mediated incorporation method is in use to accomplish the most crucial step for this project – incorporate BR, a light driven proton pump, in the floating bilayer. NR will allow us to finely characterize the induced changes in the static structure of the double bilayer system caused by the protein incorporation and activity. This experiment will be a major step to achieve a better understanding of out-of-equilibrium fluctuations.

Experimental Report-Experiment № 8-02-803

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

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The main aim of conducted experiment was to demonstrate the possibility of inserting a protein, bacteriorhodopsin (BR) in floating bilayer systems by mean of detergent-mediated incorporation method and to study the structural changes caused by BR activation. The double bilayer systems studied were composed of a DSPC phospholipid bilayer as the solid-supported bilayer and a DPPC phospholipid bilayer as the second *floating* bilayer. This system was deposited either on polished 5x8 cm^2 silicon and quartz blocks by Langmuir-Blodgett/Langmuir-Schaefer techniques. Samples were prepared at the ILL using PSCM lab facilities. In total, during this beamtime we have prepared and investigated 12 different bilayers systems. **Neutron reflectometry measurements** were performed at 48 °C to keep the floating bilayer in the fluid, biologically relevant, phase. In order to maximize the amount of BR inserted into the floating bilayer without compromising its integrity, we performed measurements at different **protein concentrations.** We collected data systematically in at least 3 contrasts conditions before and after protein injection.

To confirm that all the structural changes of the double bilayer system upon the injection of the protein+detergent solution come from the interaction of BR molecules with the membrane we have performed the experiment to investigate the effect of 0.05mM DDM solution on the structure of the bilayers (fig. 1). No big changes in the reflectivity curves of the pristine bilayer were noticed upon addition of detergent. However, the insertion of the proteins in the double bilayer system resulted in significant changes in the reflectivity curves. As example, reflectivity curves, fits and resulting SLD profiles for DSPC/DPPC double bilayer, before (closed symbols) and after (opened symbols) proteins injection, are shown in Figure 2. The obtained fit demonstrates the high quality of the pristine DSPC : DPPC double bilayer and partial protein insertion only into the floating bilayer.

Once inserted, the activity of BR was triggered by illumination of the sample with visible light (wavelength 500-650 nm, having BR absorption maximum at 568 nm) using new sample environment developed for the D17 reflectometer: new solid/liquid cells, in combination with transparent substrates (quartz in this case), allows the exposure of the sample to the light with different wavelength range (adjustable by using different filters) and different intensity (regulated by the lamp settings). BR activation results in proton pumping action and conformational changes of protein, which has an influence on the structure of the membrane. NR experiments allowed us to reveal the induced changes in the static structure of the system caused by the protein activity. For one of the samples we have noticed the small change in the reflectivity curves of the bilayer system upon illumination with 500-800 nm wavelength light. This is consistent with the increased protein activity. However, for the majority of samples changes upon illumination were not present.

Problems encountered:

1. During the NR experiments a very thick (~50 Å) porous layer on the quartz block surface was found for the some of the blocks. We suppose that such layer could growth with the time on the quartz surface. It was not possible to remove this layer with the cleaning procedure applied, so re-polishing of the blocks is required to remove this layer. The presence of such layer harmfully alter the quality

of the deposited double bilayer systems and many samples were not acceptable for the further use during the experiment.

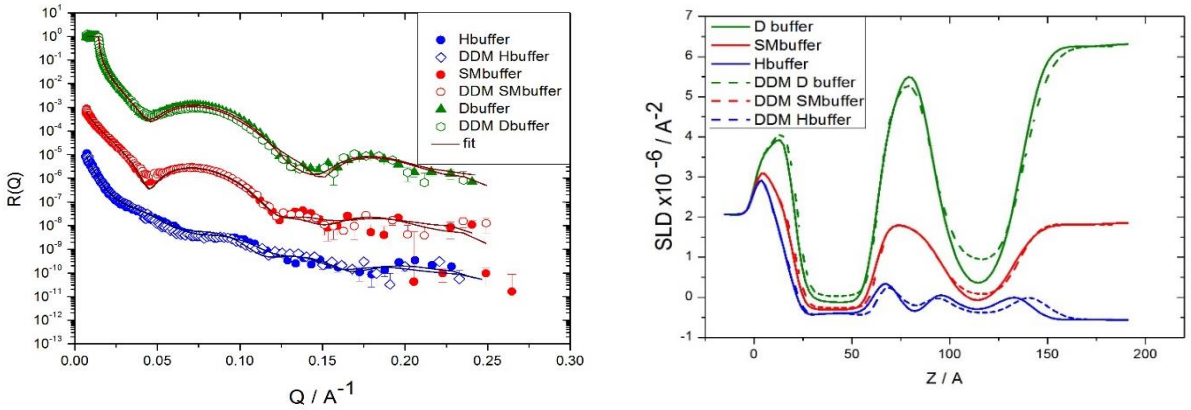

Figure 1: *(Left) Reflectivity curves for the DSPC:DPPC double bilayer at 48 °C (DSPC supported bilayer is in gel phase, DPPC floating bilayer is in fluid phase) before (open symbols) and after (closed symbols) 0.05mM DDM solution injection. (right) SLD profiles corresponding to the fits. The floating bilayer of the final system is disturbed (small increase in roughness and water content), the first DSPC bilayer was not almost changed.*

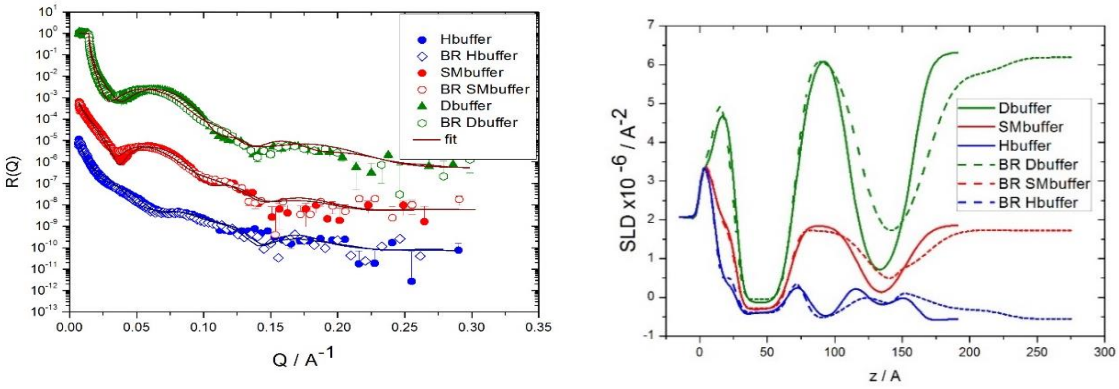

Figure 2: *(Left) Reflectivity curves for the DSPC:DPPC double bilayer at 48 °C (DSPC supported bilayer is in gel phase, DPPC floating bilayer is in fluid phase) before (open symbols) and after (closed symbols) protein BR incorporation. (right) SLD profiles corresponding to the fits. The floating bilayer of the final system consistent with the partial insertion of BR into the DPPC bilayer, the first DSPC bilayer was not changed.*

2. For some period of the experiment, we had a problem with the thermal bath (temperature reading), so it was not possible to control precisely the temperature of the samples, which is crucial for such fragile systems as lipid bilayers.
3. Some of the samples were removed after the protein +detergent injection step. For these samples the floating DPPC bilayer was lost upon the BR injection due to the strong interaction of the proteins with the bilayer and very fluctuating nature of the DPPC bilayer itself at 48 °C, in fluid phase. For the three samples, the changes of the reflectivity curve were very drastic that the qualitative analysis with fitting becomes almost impossible.

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 and its activation using the newly built set up for the NR experiments.