Experimental report

13/09/2016

Proposal: 9-13-580 **Council:** 10/2014

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

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: D2O
silicon
phospholipids

Instrument	Requested days	Allocated days	From	To
D17	4	3	14/09/2015	17/09/2015
FIGARO	4	0		

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. We propose here to prepare supported bilayer by GUVs spreading on
an interpenetrating hydrogel composed of polyelectrolyte multilayers and agarose and to check the quality of the formed supported bilayer, and especially the lateral homogeneity which is highly important to perform x-ray off-specular reflectivity.

Experimental Report-Experiment n° 9-13-580

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

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1 Context

Membranes are known to exhibit thermal fluctuations. Close to equilibrium properties have been widely investigated, both theoretically and experimentally [1], in living and model systems [2, 3]. In living systems, the transport of small molecules across the plasma membrane plays a crucial role. For example it is essential for the entry of nutrients, the transfer of ions through the membrane to ensure molecular motors activation such in muscle cells or signal propagation along axons, the acidification of some cell compartments.... In many cases, the 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 [4, 5].

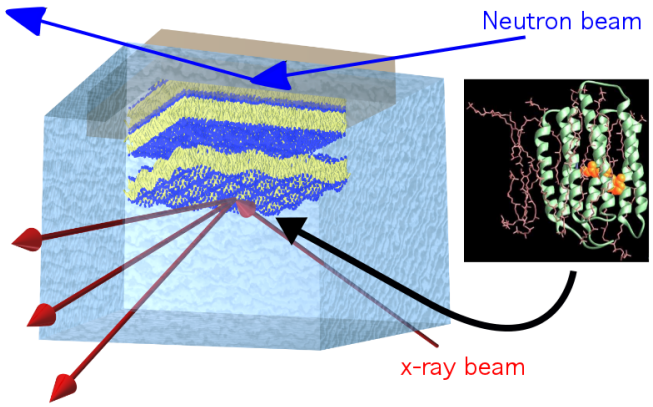

Figure 1: Schematic view of a supported double bilayer and ternary structure of bacteriorhodopsin. Fluctuations are investigated by x-ray off-specular reflectivity.

First experiments on bacteriorhodopsin (BR, see cartoon figure 1), a light-activated proton pump reconstituted in Giant Unilamellar Vesicles, were performed by using Micropipette aspiration [6] and refined video-microscopy analysis [7]. More recently, Sarcoplasmic reticulum Ca2+- ATPase (SERCA1a), an ATP activated calcium pump, was studied again by using Micropipette after reconstitution in vesicles [8]. Despite these experiments show clear evidence for the magnification of shape fluctuations when proteins are activated, the experimental technique accesses only micrometer scale information and did not provide a measurement of the fluctuation spectrum. A complete understanding of the mechanism at play in fluctuation's activation implies a fine characterization of the fluctuation spectrum at sub-micronic legnth scales.

2 Experiment

Samples were prepared in ILL using Soft Matter Lab facilities. We made supported bilayer of deuterated DPPC, POPC and DMPC on a silicon block by Langmuir-Blodgett and Langmuir-Schaefer techniques.

We have performed experiments on the D17 reflectometer, in TOF mode, with wavelength range from 2 to 20 Åand two incident angles. Using deuterated lipids on 5 bilayers (1 DPPC, 1 DOPC and 3 DMPC), we have investigated the effect of detergent and protein injection for different concentrations. We do systematic 3 contrats characterization to enhance resolution. In total we obtained 40 reflectivity curves. We present below a first preliminary analysis of these experiments. The analysis of the data has been slowed down by the student stopping his PhD at the end of 2015. A new student has been hired and will concentrate on the project as from October 2016.

3 Results

We have firstly checked the bare silicon block. We then tried to apply the detergent mediated insertion methods by investigating first the effect of detergent injection. It allow us to find conditions such as the supported bilayer remains stable. We then injected protein (bacteriorhodopsin) solutions of different concentrations (from 1 μM to 50 μM) trying to follow the kinetic of adsorption..

The insertion of the protein in the bilayer gave significant effects on the reflectivity curves, the first results are promising as shown on figure 1 where we show the reflectivity curves for DMPC bilayer at 25°C (fluid phase) before (left) and after (right) proteins injection and rinsing. First preliminary coupled fit demonstrate that the high quality of the DMPC supported bilayer. After proteins injection and rinsing, the supported bilayer is still present and we cleary observed an increase of the total thickness of the sample. First fit with a supplementary layer (thickness $\sim$ 60 Å), in qualitative agreement with the protein insertion as observed by Heinrich and Lösche [9] are presented also on figure 1. The results seems to be promising and we need now to do a systematic analysis of the datas.

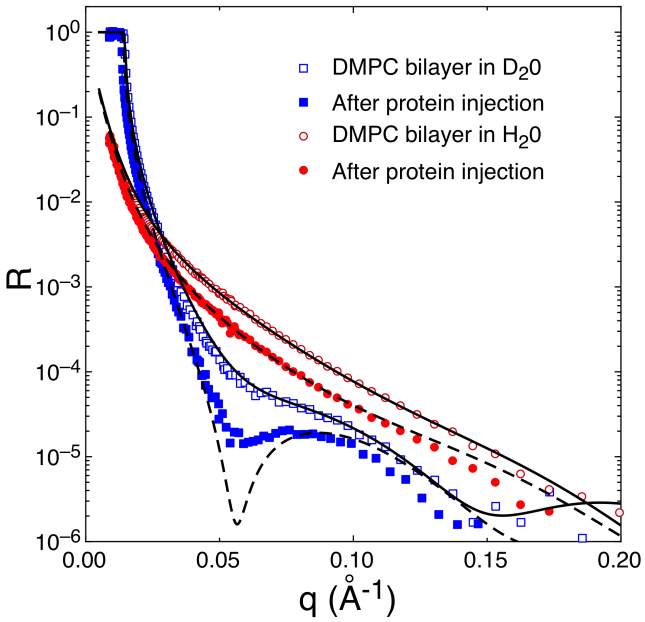

Figure 1: DMPC bilayer at 25°C (fluid phase) before (left) and after (right) proteins injection and rinsing. Lines corresponds to the best fit for supported DMPC bilayer.

4 Conclusion

We have obtained important information on the stability of supported bilayer under the action of detergent mediated method. Our preliminary results are promising and confirms the insertion of the bacteriorhodopsin in supported DMPC bilayer. These experiments need to be analyzed more finely and confirmed by fluorescence microscopy experiments. It opens important perspectives in the study of out-of-equilibrium fluctuations. The next challenging step will be now to study the insertion of the BR in double (floating) bilayers, a more free to fluctuate model system.

References

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