Experimental report

23/10/2018

Proposal: 9-13-760 **Council:** 4/2018

Title: Incorporation and activation of transmembrane protein Archaerhodopsin-3 into floating phospholipid bilayers

Research area: Soft condensed matter

This proposal is a continuation of 9-13-689

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Samples: Proteins AR3
Phospholipids (DSPC and DPPC)

Instrument	Requested days	Allocated days	From	To
D17	3	3	08/09/2018	11/09/2018

Abstract:

Biological membranes consist of a complex mixture of various kinds of lipids, proteins and sugars. They undergo continuous interaction with the cytoskeleton matrix, other cells and different macromolecules and organelles present in the intracellular or extracellular cell interior.The plasma membrane is constantly subjected to the activity of its constituent components (pumping or channel activity of the proteins, isomerization processes, etc.), which changes its energy spectrum. On the contrary, pure lipid membranes exhibit only equilibrium thermal fluctuation due to the lipids' Brownian motion, so the addition of a non-equilibrium noise in physical models of lipid membranes is a necessary step towards a complete investigation of real biological membranes. In the project related to this proposal, we would like to address the question of the influence of the non-equilibrium fluctuations caused by the continuous protein activity on the structure of the phospholipid membrane. By means of NR we plan to demonstrate and fully characterize the insertion of the transmembrane protein AR3 into a floating lipid bilayer and to study the induced structural changes caused by AR3 activation.

Experimental Report-Experiment № 9-13-760

Incorporation and activation of transmembrane protein Archaerhodopsin-3 into floating phospholipid bilayers

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During the experiment on the D17 reflectometer (exp. 9-13-760, performed 08/09/18-11/09/18), we have studied the protein incorporation into the phospholipid bilayers and the activation of Bacteriorodopsin (BR) and Archaerhodopsin-3 (AR3) in floating bilayer systems. Activity of the proteins can be triggered by the absorption of the green light (wavelength 500-650 nm, with the absorption maximum at 568 nm), resulting in proton pumping action and conformational changes of BR. The setup for the illumination of samples in solid-liquid cells during NR experiment, which was recently developed at the ILL within the project of the ILL PhD student Mukhina T, was in use.

The double bilayer system was composed of the DSPC phospholipid bilayer as the first solid-supported bilayer and DPPC phospholipid bilayer as the second floating bilayer deposited on top of the first one. Such choice of the lipid composition allow us to keep the DSPC bilayer in the gel phase and DPPC bilayer in the fluid phase at the working temperature 48°C, which should promote the insertion of the proteins only into the fluid DPPC bilayer and prohibit its insertion into the DSPC bilayer. The bilayer systems were deposited on highly polished 5x8 cm^2 silicon and quartz substrates (roughness ~ 5 Å) by Langmuir-Blodgett/Langmuir-Schaefer techniques and proteins insertion into the studied membrane systems was performed by detergent-mediated incorporation method as tested previously (exp. 9-13-760, 8-02-803). This method consists in the injection of the proteins+detergent solution into the pristine bilayer system, incubation step and rinsing with buffer to remove excess of the proteins and detergent molecules, as the result membrane embedded proteins system is obtained.

The main aim of conducted experiment: The main goal of the proposed experiment is to characterize the structure of double bilayer system upon insertion and activation of the transmembrane protein AR3.

We have performed experiments on the D17 reflectometer in TOF mode with two incident angles and wavelength range from 2 to 20 Å. Samples were prepared at the ILL using PSCM lab facilities. We have prepared 12 pristine bilayers systems in total. All the systems at each step were characterized in several contrasts, i.e. using as water-phase D_2O, H_2O and Silicon or Quartz Match Water. We were tested 2 different protein concentrations of 1µg/ml and 2µg/ml in order maximize the amount of proteins inserted without compromising the integrity of the floating bilayer.

Problems encountered:

1. We had a lot of problem with the sample preparations on the freshly polished Quartz block. We had 3 freshly polished quartz blocks and for unknown reasons it was not possible to make a deposition of DSPC-DPPC phospholipid bilayer on these blocks. The problem arises during the deposition of the second layer of DSPC lipids.
2. We had one “old” quartz block, which was not polished before the experiment, so until the end of the experiment, we were preparing the samples for the experiments with illumination using only this quartz block. Even if the transfer ratio of samples made of DSPC:DPPC lipids indicated almost 100% coverage of the “old” quartz substrate, such samples appeared to be very bad quality and

unstable, so unacceptable for the continuous solvent exchange and protein injection steps. We think that the problem could come from the last step of Langmuir-Schaefer deposition techniques used for the sample preparation. As it's known, the very thick (~50 Å) porous layer could growth with time on the top of the quartz block. Such thick layer could alter the properties of the substrate and drastically change the lipid – surface interactions, leading to poor quality bilayer. To check and eliminate this possibility, we measured the reflectivity curves for the bare quartz substrate with NR. The 5 Å thick layer with the SLD of 4.2×10^{-6} Å^{-2} and water fraction of 50% was found on top of the surface. To perform the protein BR (BR is a light driven proton pump) activation it's essential to make the samples on the transparent substrate (Quartz) as the light is supplied to the sample through the block side of the solid/liquid cell.

As it's known [1,2], the presence of NaN_3 is very important for the protein activity and the enhancement of the shape fluctuation of the phospholipid bilayers. Using a solution of 1mM of NaN_3 in PBS buffer to facilitate the proton pumping activity of the proteins, we have noticed a large effect on the reflectivity curves caused by the presence of this compound on both single adsorbed and double phospholipid bilayers with and without the proteins. It is important to notice the reversible effect of sodium azide on the solid supported DPPC bilayer with incorporated BR. Changing the solution from PBS buffer to PBS buffer + 1mM NaN_3 back and forth was moving reflectivity curves reversibly in the same manner (figure 1). The effect of NaN_3 on the structure of the phospholipid bilayers is not understood yet and to our knowledge, no structural studies are available.

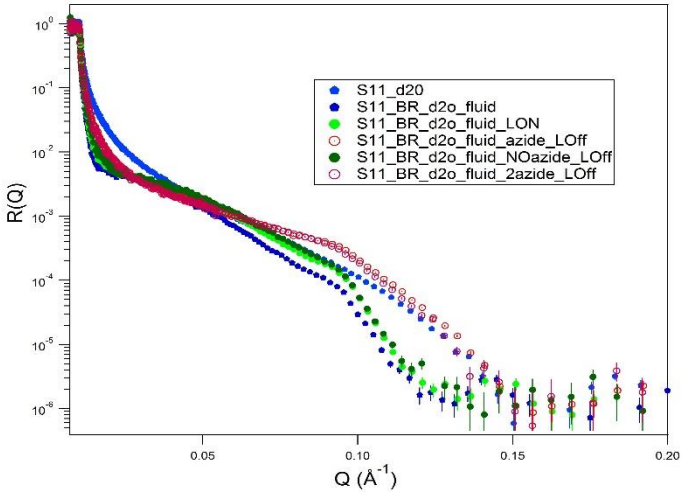

Figure 1. Reflectivity curves for the DPPC bilayer on Quartz; DPPC bilayer with incorporated BR in fluid phase in 1) D2O contrast; 2) in D2O contrast with 1mM NaN_3; 3) after the rinsing with D2O again; 4) in D2O contrast with 1mM NaN_3 again. Clear **reversible change** of the structure of the bilayer is observed due to the presents of NaN_3.

Conclusions:

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. We have uncounted many problems with sample preparation, so many prepared samples were discriminated and removed after theirs check with neutrons and did not used further. We did not notice any change in the reflectivity curves (structure of the bilayer system) with the illumination with the visible light 500-800nm to perform the protein activation. It could be due to the absence of the azide in the solution, which is consistent with the ref. [2], where no enhancement of the fluctuations of the GUV with BR were found in the absence of the azide. Furthermore, we have noticed **the strong reversible effect of NaN_3** on the bilayers structure. This effect is not understood yet and require further investigation.

References:[1] H. Steinhoff, M. Pfeiffer, T. Rink, O. Burlon, M. Kurz, J. Riesle, E. Heuberger, K. Gerwert, and D. Oesterhelt, Biophys. J. 76, 2702 (1999); **[2]** M. D. El Alaoui Faris,D. Lacoste, J. Pe´cre´aux,1,J.-F. Joanny,J. Prost, and P. Bassereau, PRL 102, 038102 (2009);