ESRF	**Experiment title:** Out-of-equilibrium active membrane: detergent- mediated incorporation of bacteriorhodopsin in supported lipid bilayer	**Experiment number:** SC-4329
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Shifts:	**Local contact(s):** Oleg Konovalov	*Received at ESRF:*

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Report:

This experiments is part of a large project, in a close collaboration between ILL (Giovanna Fragneto), Synchrotron SOLEIL (Jean Daillant) and Strasbourg University (Thierry Charitat). It is now the central work of an ILL Phd student. Initially, the purpose of this experiment was to investigate the properties of floating bilayers (quite free to fluctuate bilayer on top of a supported one) with incorporated membrane proteins: α-hemolysin (α-H, quite easy to insert) and bacteriorhodopsin (BR, more difficult to insert). α-H is a passive protein that is supposed to change bilayer fluctuations mainly by modifying their mechanical properties (bending rigidity...). BR is a more complex proteins that is able to pump some protons under illumination at the good wavelength and to bring the bilayer out-of-equilibrium (see proposal for more details).

The main objective of this first experiments was to check the insertion of the two proteins (α-H an BR) in floating bilayers (a quite free to fluctuate bilayer on top of a supported one) by specular x-ray reflectitity and to analyze their fluctuations by off-specular reflectivity in passive conditions (no illumination of BR).

Unfortunately we encoutered several problem in sample preparations. Floating bilayers are very fragile samples that need to be prepared during the x-ray experiments, by a combination of Langmuir-Blodgett and Langmuir-Schaeffer depositions using PSCM facilities. In the case of zwitterionic lipids, they are highly sensitive to any pollution of the bulk water. We have made many experiments at ILL and ESRF (see [1,2,3]) with such samples. This time, despite our numerous efforts (we try to do 12 samples changing cleaning procedure, silicon blocks, lipids, milliQ set-up...), but we did not

succeed in forming floating DSPC bilayer. To avoid loss of beamtime when we were trying to prepare samples and solve this problem, we have made experiments on fully charged floating bilayer of the negatively charged phospholipid DPPS (di-palmitoyl-phosphatidyl-serine), that are, what may seem surprising, more robust. This is the purpose of another project we are working on, that deals with interactions between highly charged membrane. In that case, samples where very nice and we obtained very interesting specular and off-specular, confirming our previous results (see experimental report SC-3590) and allowing us to check the effect of salt. These data are highly promising and complement nicely our previous experiments (see Figure 1). We are still working on the analysis because the strong electrostatic interaction between the bilayers, in the regime of strong correlation and beyond the Poisson-Boltzmann theory, imply extensive modeling effort to analyze off-specular experiments.

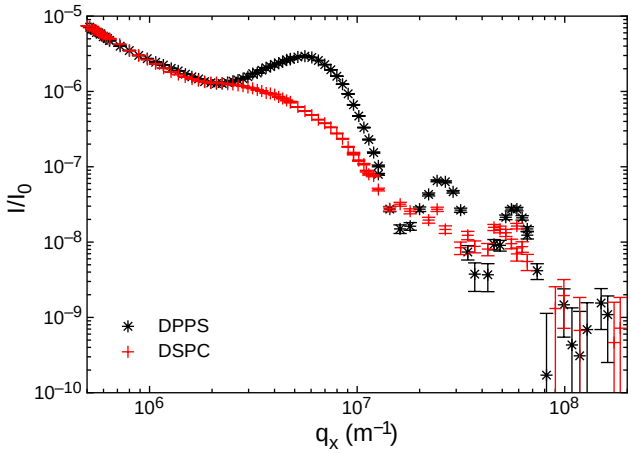

Figure1: Off-specular reflectivity data for a DPPS double bilayer (red cross, this experiment) and a DSCPC double bilayer (black star, previous experiments at ESRF). The data clearly shows that DPPS bilayers are highly correlated.

We also succeeded in preparing samples of single supported DSPC bilayers, in which we inserted α-hemolysin. We were able to do 4 bilayers samples with inserted α-hemolysin at different concentrations. We are still analyzing these experiments that are very difficult to treat because the fluctuation of the supported bilayers are very small compared to the floating one.

To conclude, the main goals of the initial proposal were not achieved because of the difficulties with sample preparations. Although the reasons for the sample preparation failure during this experiment are still unclear, the problem has been sorted out by now as both ourselves and colleagues have succeeded recently to prepare floating bilayers therefore we believe that the initial planned experiment should be repeated. Finally, we haven't lost any beamtime since we performed successful measurements on a different system but with the same aim of studying at the fluctuations of floating bilayers.