EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title: Cholesterol-mediated lipid dynamics in DPPC-Cholesterol binary systems**	**Experiment number**: SC-4465
Beamline: ID28	**Date of experiment**: from: 3-May-2017 to: 9-May-2017	**Date of report**: 9-Sep-17
Shifts:18	**Local contact(s)**:Alexei Bosak	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Boris Toperverg, RUR University Bochum, Germany

Dr. Kirill Zhernenkov, JINR, Dunba, Russia

Dr Dmytro Soloviov, JINR, Dubna, Russia

Dr. Mikhail Zhernenkov, BNL, USA

Dr Yong Cai, BNL, USA

Report:

The present experiment is the continuation of a very successful project on studying lipid dynamics using inelsatic x-ray scattering. Our first experiment on pure DPPC bilayers performed at ESRF led to a high-impact publication in Nature Commun. (7, 11575) in 2016 (also featured as ESRF science highlight), where the discovery of a transverse phononic mode and the opening of a phononic gap upon the gel-fluid phase transition provide a direct evidence of the formation of short-lived local voids that enable permeation of small molecules through the membrane. We have recently extended the study to include binary systems, adding cholesterol to DPPC to study the effect of cholesterol concentration on the longitudinal and transverse membrane phonon dynamics in order to unveil the role of cholesterol in the mediation of dynamical processes in lipid bilayers. During the SC-4465 experiment at ID28 we successfully measured the series of binary mixtures of DPPC and Cholesterol at the following concentrations: DPPC:Chol(7%mol), DPPC:Chol(18%mol), DPPC:Chol(28%mol) at room temperature and at the elevated temperatures. The samples were prepared at ESRF PSCM laboratory. The measurements we performed in a custom made humidity chamber, whose mounting was made possible with help of ID28 technitians, who created custom adaptors to mount the chamber on the goniometer.

In the figure 1 we present the selected IXS spectra with the corresponding fittings using damped harmonic oscillator (DHO) model. For each sample (a single T/Chol%mol condition) we measured 2 different positions of the detector arm to achieve the ultimate Q resolution of ~1 nm^{-1} within the Q-range of up to 15 nm^{-1}, which corresponds to the first pseudo-Brillouin zone.

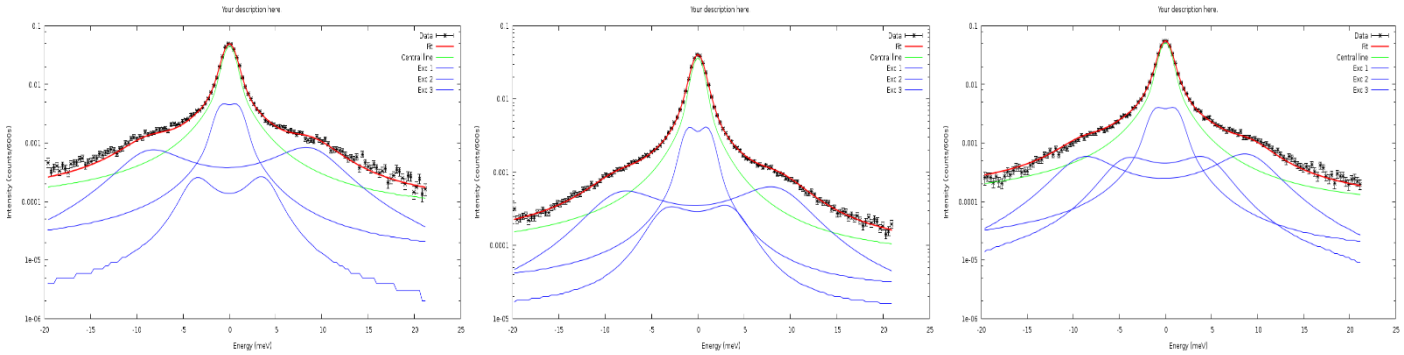

Figure 1. The examples of the IXS spectra for the ID28 analyzer #3 (which corresponds to the Q=8.6nm^{-1} at three diffferent Cholesterol concentration: 7%mol (left panel), 18% mol (central panel), 28%mol (right panel).

The raw S(q,0) scans with the analyzer #3 for three Chol concentrations at 25C are shown in figure 2 (left panel).

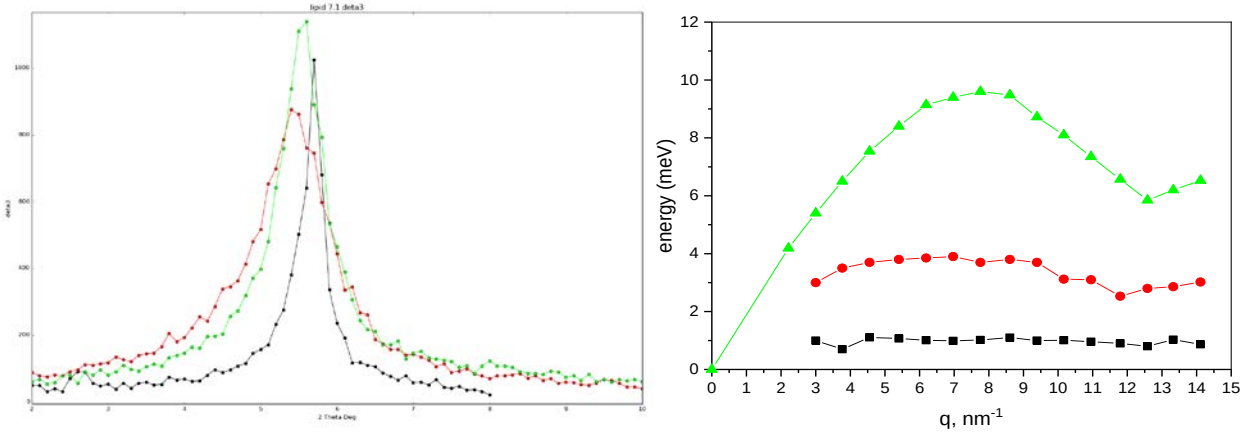

Figure 2. (left panel) The examples of the S(q,0) spectra for the ID28 analyzer #3 at three diffferent Cholesterol concentration: 7%mol (black curve), 18% mol (green curve), 28%mol (red curve). (right panel) The dispersion curves obtained with 3 DHO excitation fit for 7% Cholesterol concentration at 25 C.

As expected, with increasing the concentration of Cholesterol, the width of the S(q) peak increases, which signifies the increased degree of disorder. Such behavior proves that the sample deposition was successful. The peak shift towards smaller detector angle (or smaller Q) with Chol%mol increase implies that the size of the pseudo-Brillouin zone reduces, which is explained by the increased amount of Cholesterol between DPPC chains and ultimate increase of the pseudo-lattice parameter of the DPPC-Chol system, as expected. In figure 2 (right panel) we present an example of the complete dispersion curve for DPPC:Chol7%mol system as a result of the fitting procedure with 3 DHO excitations. Green curve denotes the regular longitudinal acoustic mode whose inclination at the small Qs corresponds to the speed of sound (point 0,0 is shown for reference). Low laying black curve denotes the transverse acoustic mode previously discovered at our previous experiment at ID28 (Nature Commun. (7, 11575)). Additional phononic branch denoted by the red curve is related to the influence of the cholesterol on the membrane dynamics and its evolution provides understanding of the dynamical signature of the lipid domains and their role in such processes like cell curvature generation, curvature sustainability and membrane bending rigidity. The results are under preparation for the publication: *M. Zhernenkov et al. "Dynamic profiling of DPPC-Cholesterol binary mixtures underpins the biological functions of a cell membrane", (2017) to be submitted to Nature Chem. Biol.*