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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Interaction of Prohibitin with the inner of mitochondrial membrane	**Experiment number**: LS-3160
Beamline: ID-10	**Date of experiment**: from: 07-09-2022 to: 12-09-2022	**Date of report**:
Shifts: 15	**Local contact(s)**: Oleg Konovalov	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Abstract:

Prohibitins are composed of two subunits prohibitin-1 (PHB1) and prohibitin-2 (PHB2), each subunit possesses an N-terminal helices (NT-PHB1 and NI-PHB2). The N-terminal helices have been hypothesized to have the function of anchoring the complex to the membrane, but experimental evidence is missing. With XRR we aim to clarify how the interaction between the N-terminal helices of prohibitin and the membrane occurs, as well as the role of lipid membrane composition is still ongoing. Data analysis is not completed, however, a not strong interaction could be observed between the peptide and the membrane. Fitting of the data will allow a better understanding of this phenomenon.

Report:

Scientific background:

Prohibitins are a highly conserved protein heterodimer complex composed of two subunits prohibitin-1 (PHB1) and prohibitin-2 (PHB2), which form a large multimeric ring (12-20 dimers) in the inner mitochondrial membrane (IM). Each subunit possesses an N-terminal helices (NT-PHB1 and NI-PHB2). The formation of the Prohibitin ring is influenced by cardiolipin (CL). In the IM, Prohibitins interact with m-AAA protease [1, 2], and play a crucial role in maintaining mitochondrial homeostasis [3,4]. Prohibitins have N-terminal transmembrane helices that display a degree of amphiphilicity and are thought to anchor the proteins to the membranes [5,6]. Alteration or deletion of PHB leads to viral infection, cardiac and neurovascular diseases, and cellular ageing. Several hypotheses have been postulated with regards to the Prohibitin complex structure but their exact molecular arrangement at the inner mitochondrial membrane is still unresolved. X-ray reflectivity (XRR) will help us investigate the interaction between the N-terminal helices of prohibitin (PHB) and the membrane by locating and quantifying peptides in the bilayer. The aim of the experiment was to investigate the

concentration threshold of the peptide-membrane interactions. The role of the lipid membrane composition in the peptide-membrane interaction was also studied in this experiment.

Material and methods:

The experiment was performed at a solid-liquid interface employing a cell without a fluid flow system.
To vary the scattering vector Qz, the grazing angle was varied stepwise from 0 to 1.8 degrees to cover a Qz range from 0 to 0.8 Å employing a monochromatic beam with energy 22 keV corresponding to the wavelength 5.627 x 10^{-1} Å. The incident beam size (HxV) was 22x13 μm^2.

Bilayers with peptides were characterized in a Q-range of $1x10^{-3}$ and 1 $Å^{-1}$ with 6 scans, while the bilayer without peptides was characterized covering a Q range between $1x10^{-3}$ and 1 $Å^{-1}$, performing 3 scans
with DOPC and 6 scans with DOPC: DOPE: CL 18:1 (40:40:20% wt %) and DOPC: CL 18:1 (80: 20 wt %).

Results:

Reflectometry experiments at the solid-liquid interface were performed.
The effect of different peptide concentrations on DOPC, DOPC:DOPE: CL 18:1 bilayer was tested after having well characterized the bilayer alone. Bilayers were deposited with vesicle fusion.
Data analyses were performed with DOPC and DOPC: DOPE: CL 18:1 with motofit.
Several scans of the same sample were performed to assess the homogeneity of the samples.
The different scans performed on the two bilayers show a higher similarity for the DOPC bilayer while presenting a higher variability for DOPC: DOPE: CL 18:1. They were subsequently averaged discarding obvious outliers.
The presence of the bilayer for both systems was confirmed by the preliminary fitting of the data.
NT-PHB1, NT-PHB2, and NT-PHB1+2 were injected in the bilayers at different concentrations. The X-ray reflectivity profile with and without peptides do not present significant differences, indicating a not strong interaction interaction between them. However, data analysis is still ongoing. This experiment will be part of a manuscript that is under preparation.

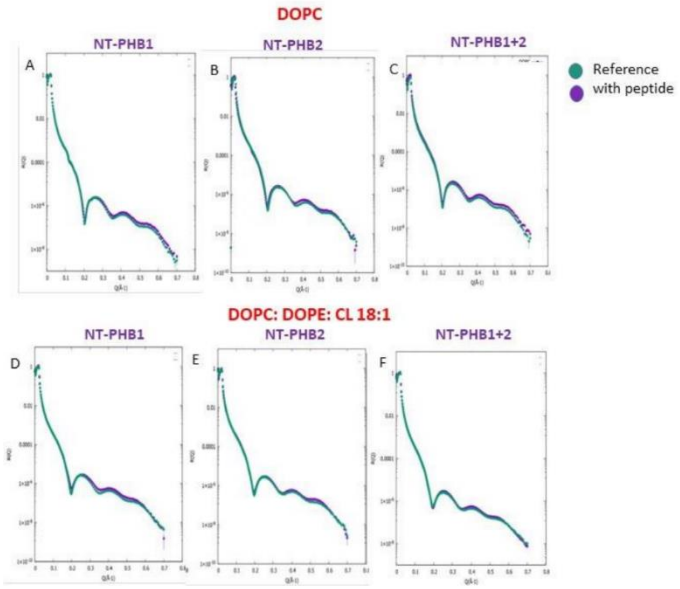

Figure 1: XRR: effect of the peptides on the membrane of DOPC and DOPC: DOPE: CL 18:1. Bilayer as reference represented in green, and bilayer with peptide represented in purple. A-B-C DOPC with and without NT-PHB1, NT-PHB2, and NT- PHB1+2. D-E-F. DOPC: DOPE: CL18:1 with and without NT-PHB1, NT-PHB2, NT- PHB1+2.