EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Developing a model for nanoparticle-plant cell interactions: layered double hydroxide nanocarrier surface chemistry influence on mesophyll protoplast interaction	**Experiment number**: ES1316-2
Beamline: ID21	**Date of experiment**: from: Sept. 31st, 2023 to: Oct.4, 2023	**Date of report**: September 25, 2023
Shifts: 12	**Local contact(s)**: Hiram Castillo	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Vivek Kumar*, Carnegie Mellon University

Astrid Avellan*, CNRS/GET

Report:

This experiment was in continuation of our ES1316-1 experiment in July 2023. The aim here was to use a different nanoparticle formulation, with an inorganic core made of Vanadium insrzat of Terbium to ease for its detection, as Tb fluorescence emission overlaps with Fe. The aim was to study how *(i)* the protein on protoplast surface and *(ii)* the functional groups at the nanoparticles' surface are a driver for nanoparticles to cross lipidic membranes. The core in that experiment was a vanadium-phtalocyanine that was stabilized with a shell rich in either carboxyl groups, alkyl chains, or amine groups. We used a similar system as our July beamtime, where plant cells of wheat (*Triticum aestivum* cv. cumberland) were extracted and their cell wall digested to obtain for protoplasts. This time we also trypsinized the protoplasts to clive for the proteins in the protoplasts surface. This protoplast extraction was performed at ESRF, on the histology lab using an overnight enzyme digestion procedure. We followed the protol we developed during our July beamline for agar suspension, flash freezing, cryomicrotome sectionning and μ-XRF and μ-XANES using the cryostage. Thanks to our previous experiment in July we had a much better preparation protocol in place, although we still have room for cells preparation improvement. Reference XANES scanes were measured for each of the nanoparticle samples. They confirmed the V- porphyrin speciation.

The XRF maps and μ-XANES are still being analyzed, but they already indicate that *(i)* internalization of nanoparticles can occur as confirmed with the XANES spectra, *(ii)* protoplaste trypsinization influence the NP uptake indicating a role of the surface protein in NP interaction with the cell membrane and *(iii)* that NP surface functionalization does modulate their capacity to cross lipidic membranes. Further, we identified that the bulk quantification of NP association with cell membrane as currently done in the litterature (centrifugation and ICP-MS) is not a good proxi as heteroaggregation seems to occur between nanoparticles and cell debris. Thanks to these results, we are establishing a new protocol using flow cytometry to better quantify this association. These results will lead to a publication in collaboration between the ESRF, the CNRS-GET and Carnegie Mellon University (USA).