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:**<br>Testing the melanosomal casing model with nanoprobe X-ray fluorescence analysis | **Experiment number**:<br>LS-2557 |
|---|---|---|
| **Beamline**:<br>ID16A-NI | **Date of experiment**:<br>from: 29.01.2017 to: 02.02.2017 | **Date of report**: |
| **Shifts:**<br>9 | **Local contact(s)**:<br>Yang Yang, Sylvain Bohic | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

**Susan Stuhr***

*Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum Germany*

**Christoph Rumancev***

*Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum Germany*

**Cindy Beyer***

*Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum Germany*

**Prof. Axel Rosenhahn**

*Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum Germany*


**Report:**

In the beamtime LS-2557 'Testing the melanosomal casing model with nanoprobe X-ray fluorescence analysis' we aimed to compare melanosomes of 24 weeks old wildtype mice to mice which are linked to the eye disease glaucoma by using X-ray fluorescence nanoanalysis. The PTP-Meg2 deficient mice start to get an increased eye pressure at the age of 9 weeks. As we measured melanosomes of mice at an age of 6 weeks before in LS-2493 we aimed to compare the melanosomes of mice of different ages. The melanosomes were extracted from the mice of two different genetic backgrounds, vitrified and subsequently plunge-freezed on silicon nitride membranes. We were able to use the opportunity to measure the organelles at the beamline under cryo conditions minimizing potential artifacts caused by sample preparation.

In the beamtime LS-2557, the beamline was tuned to an excitation energy of 17.05 keV. We used the focus of 43 nm x 50 nm with a pixel pitch of 0.025 µm and an exposure time of 50 ms to acquire 2D XRF data. Fortunately we could use all 9 shifts due to the outstanding beam stability and the excellent beamline team support.

We got clear and robust signals of all three metals desired: calcium, copper and zinc. In most of the melanosomes of the wildtype mice a core-shell structure as predicted in the casing-model could be found (around 60%). The shape of most melanosomes was ellipsoidal but also some melanosomes with a roundish shape were detected (see Fig. 1). The correlation between Ca and Zn was linear, while there was different correlation for Ca and Cu.

Most of the melanosomes of the PTP-Meg2 mice were also ellipsoidal and an even clearer casing model due to higher copper content could be shown. In addition to that, two further

types were found. The first type which were around 19% percent of all melanosomes investigated had a very low copper content while the second type had a deformed appearance. As the latter type was not found in the wildtype mice we suspect that this is characteristic for the PTP-Meg2 mice.

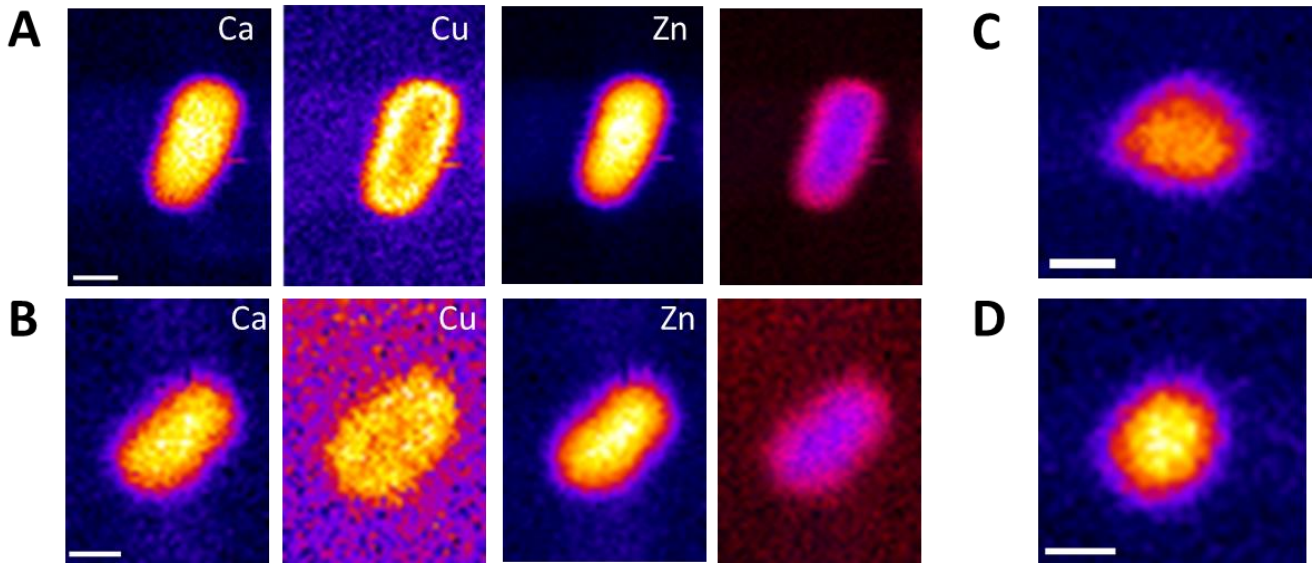


***Figure 1:Fluorescence signals of melanosomes of PTP-Meg2 mice (A, C) and from wildtype mice (B,D). The signals for calcium, copper and zinc are shown. The scale bar is 250 nm***

In comparison to the measurement of 6 weeks old mice (LS-2493) we got a very strong copper signal for all melanosomes. While we could not find any differences between the two genetic backgrounds, obvious differences were be found in this beamtime. In the majority of the investigated melanosomes, the casing-model was confirmed in both wildtype and heterozygote mice. While three different types of melanosomes were found for the heterozygotes, two were investigated for the wildtype. These differences show that during the aging process and with increasing eye pressure due to progression of the disease the metal content within the melasonomes is changed and the course of the disease affects their shape.
In our next beamtime at the ESRF (LS-2708) we want to make a direct comparison to another murine glaucoma model (DBA/2J) and also compare two different ages at two different progression stages of the disease to prove that our observations have a general validity for glaucoma disease progression.