ESRF	**Experiment title:** Testing the melanosomal casing model with nanoprobe X-ray fluorescence analysis	**Experiment number**: LS-2493
Beamline: ID16A-NI	**Date of experiment**: from: 13.07.2016 to: 16.07.2016	**Date of report**: 16.11.2016
Shifts: 9	**Local contact(s)**: Yang Yang, Peter Cloetens	*Received at ESRF:*

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Report:

In the beamtime LS-2493 'Testing the melanosomal casing model with nanoprobe X-ray fluorescence analysis' on ID16-A-NI we aimed to compare melanosomes from two different genetic types. The melanosomes were from the wildtype C57BL/J and PTP-Meg2 deficient mice. These mice are linked to the eye disease glaucoma and have an increased eye pressure at the age of 9 weeks. As the increased eye pressure is an symptom of the eye disease it is assumed that the 6 weeks old mice we chose for the experiment are not yet affected by the glaucoma.

In previous measurements at PETRAIII on the freeze-dried melanosomes of 24 weeks old mice we found out that the PTP-Meg2 mice has an increased copper content compared to the wildtype. Because of that we chose melanosomes of 6 weeks old mice for the experiment LS-2493 who do not have an increased eye pressure yet to compare the both ages of mice.

After extracting the melanosomes from the mice the organelles were vitrified and subsequently plunge-freezed on silicone nitride membranes. Because the beamline ID16A-NI offers the possibility for cryo measurements we did not need to freeze-dry the organelles and with that avoided any artifacts that might be associated with the freeze-drying process.

The energy of the beamline ID16A-NI was tuned to 17.05 keV. We used a focus of 42 nm x 39 nm.We used a pixel pitch of 0.025 µm and an exposure time of 50 ms to acquire 2D XRF data.

From all measurements of the melanosomes we got robust signals of the metals of interest calcium, copper and zinc. Due to the outstanding beam stability and the excellent beamline team support we could measure 30 melanosomes of both types during the beamtime In Fig. 1 a representative result of melanosomes of both wildtype and PTP-Meg2 are shown.

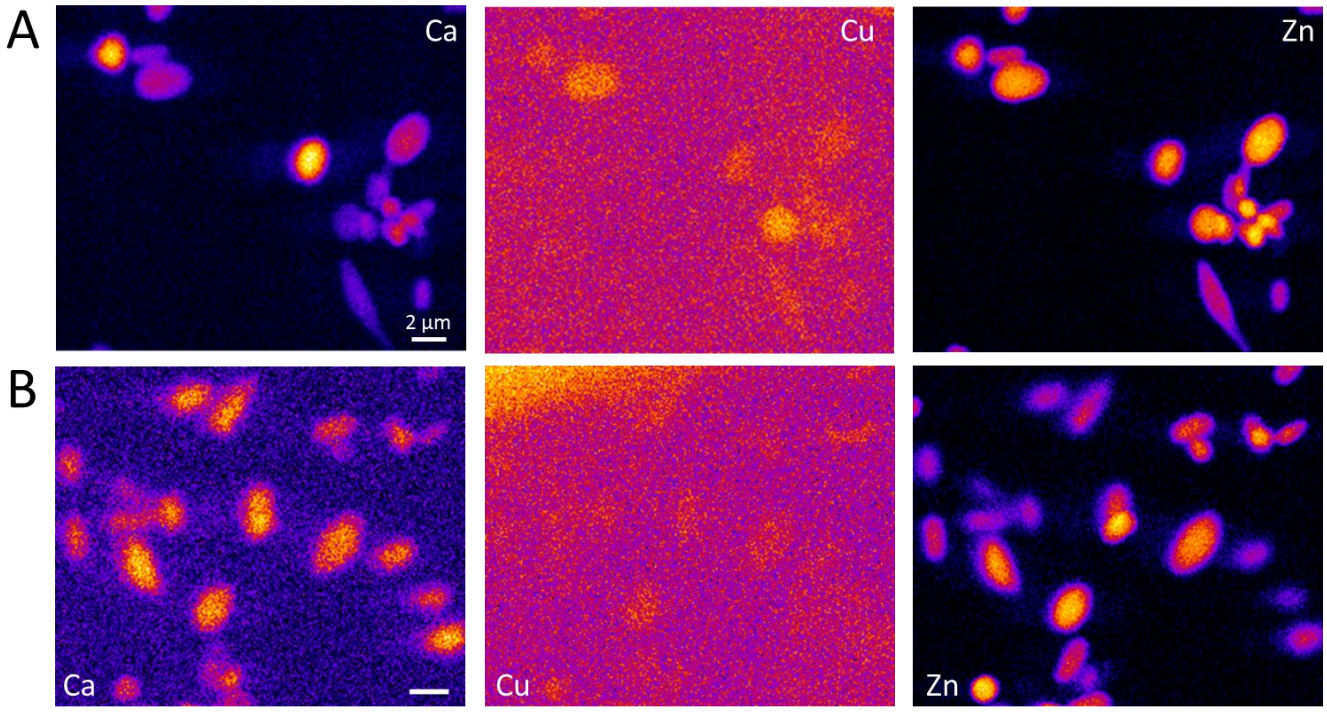

Figure 1: Fluorescence signals of melanosomes of wildtype mice (A) and from PTP-Meg2 mice (B). The signals for calcium, copper and zinc are shown. The scale bar is 2 µm in A and B.

Due to the low copper signal we focused on the correlation of zinc and calcium. We found out that in these melanosomes calcium and copper are linearly correlated in both genetic types but the correlation were not the same due to lower calcium content in some melanosomes. This is a hint that there are different types of melanosomes which we already found in former measurements [Gorniak, T., et al. (2014), Pigment Cell & Melanoma Research, 27, 831–834.]. In addition to that the calcium content was lower in the PTP-Meg2 mice.

We compared the copper signal in both types but no differences in the copper content could be found. That means that unlike in the melanosomes of 24 weeks old mice we investigated in former measurements the copper content in those melanosomes are not yet different at this young age. In addition to that the copper content varied not just within species but also in the same sample which supports the theory of different types of melanosomes.

The casing model which describes a core-shell structure with copper being a marker for eumelanin in the core and zinc being a marker for pheomelanin in the shell could not be proven or falsified due to the low copper levels.

In the next beamtime at the ESRF (LS-2557) we will compare 24 weeks old mice which were not freeze dried but also cryo prepared to this result to get closer insights to the change of the copper content during the aging of the mice and the progression of their disease.