ESRF	**Experiment title:** Copper function in synaptogenesis: identification of Cu postsynaptic distribution and correlation with super-resolution microscopy of neuro-proteins	**Experiment number**: LS-2436
Beamline: ID16A-NI	**Date of experiment**: from: 25/11/2015 to: 01/12/2015	**Date of report**: 15/09/2016
Shifts: 18	**Local contact(s)**: Sylvain Bohic	*Received at ESRF:*

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Aims

The aim of this experiment was to identify the accurate distribution of copper in relation with specific proteins in dendritic spines of hippocampal neurons. This work is based on the correlation between high resolution maps of Cu obtained by nano-SXRF (Synchrotron X-ray Fluorescence) and distribution of proteins located in dendritic spines obtained by STED (Stimulated Emission Depletion) super resolution microscopy.

Sample Preparation and Troublesome

Primary rat hippocampal neurons were prepared in collaboration with the Interdisciplinary Institute of Neurosciences, Bordeaux university, as adapted from the Banker's method (Kaech & Banker, 2006). A specific procole enabling the sample preparation of rat primary hippocampal neurons for SXRF imaging was developed for this project and used successfully during our last experiment at ESRF (exp. LS2366), and recently published (Perrin et al., 2015). However for the present experiment we faced unexpected difficulties for sample preparation. The cell culture facility at IINS University of Bordeaux was not able to perform any neuron culture during a more than six months period. Neurons were dying, for unknown reasons, probably due to some toxic factors in the complex process of primary rat hippocampal cultures (despite the quality control procedure in function in this lab). The culture of primary glutamatergic neurons is a delicate process that can be hampered by a variety of factors. Despite all our efforts, we could only prepare few samples of incompletely mature neurons. We were therefore unable to perform STED microscopy before the SXRF experiment. In order to palliate this difficulty we prepared in parallel some glial cells (astrocytes) that were not affecetd by the toxicity troublesome.

Experimental analytical conditions

The beam size obtained at ID16A-NI beamline was 42 nm x 45 nm at 17.0 keV. Nano-SXRF was carried out at room temperature, under vacuum, using a six elements silicon drift diode detector. SXRF data treatment was performed using Pymca software to provide fitted element distribution images. Quantitative results of element concentrations were extrapolated from the analysis of thin film XRF reference sample from AXO.

Results

The results on partially mature primary neurons are presented in Figures 1 & 2. To palliate the difficulties encountered on rat primary neurons culture we also analyzed some astrocytes (Figures 3 & 4). The quality of the images in terms of spatial resoultion was outstanding thanks to the very small beam size.

Figure 1

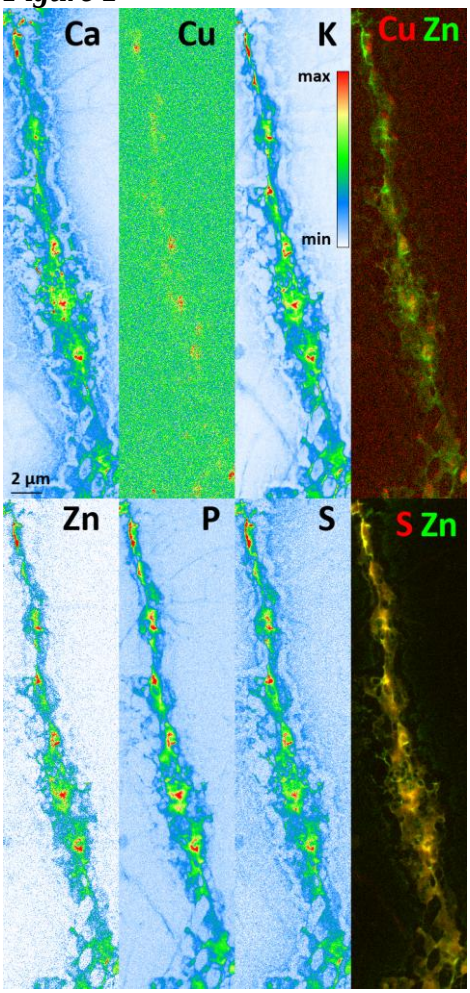

Figure 2

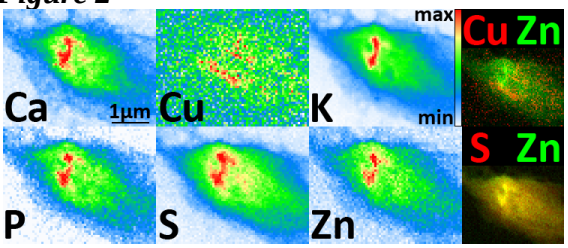

Figure 3

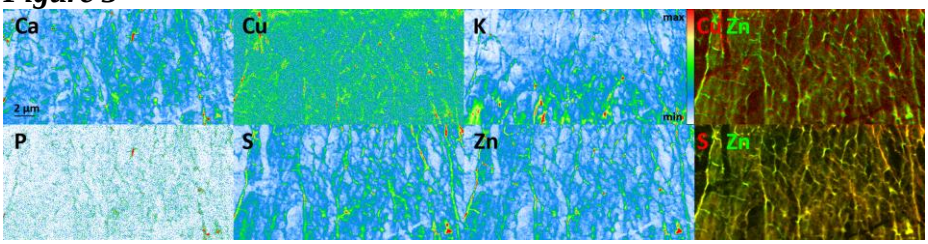

Figure 4

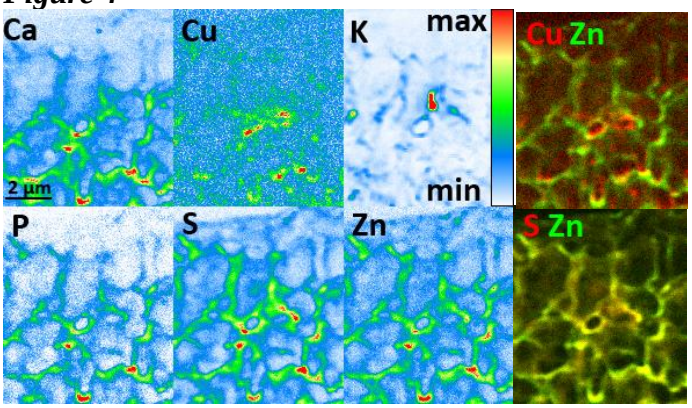

Fig. 1. *Representative examples of SXRF imaging of Ca, Cu, K, Zn, P and S, and red green correlative images in a dendrite from a rat primary hippocampal neuron. Neurons were only partially mature, showing no spines, but stubby pre-spine structures along the dendritic shaft, containg high levels of trace elements.*
Fig. 2. *Zoom on a stubby pre-spine structure of a rat primary hippocampal neuron showing that Cu and Zn distributions are different, while S and Zn are superimposed (yellow color on Red Green overlay).*
Fig. 3. *Representative examples of SXRF imaging of endogenous trace elements in the cytoplasm of an astrocyte and red-green overlay images for Zn/Cu and S/Zn.*
Fig. 4. *Zoom in the cytoplasm of an astrocyte. The red-green overlay images for Zn/Cu and S/Zn illustrate the lack of correlation between Cu and Zn, whereas Zn and s are superimposed (yellow color Red Green overlay).*

Conclusions

Every data from this experiment, either obtained from neurons or from astrocytes SXRF imaging, suggest that Cu and Zn are involved in different structural functions since their distribution is not correlated, wheras Zn and S distributions are always correlated. This result confirms the data obtained in a previous experiment on rat primary neurons (LS2366) and extend the observation to glial cells. Unfortunately, the study of rat primary hippocampal neurons using correlative STED microscopy and SXRF could not be performed. This part of the experiment should be repeated and is still of very high importance to understand in which structural proteins Cu and Zn are involved. The beamtime was however successfully employed to image, for the first time at this resolution, the distribution of trace elements in astrocytes. The image distribution of Cu and Zn suggest the role of both elements in the cytoskeleton architecture of astrocytes, a feature that was unknown up to now (article in preparation).

References

Kaech S. & Banker G. (2006) Culturing hippocampal neurons, Nature Protocols, 1, 2406-2415.

Perrin L., Carmona A., Roudeau S., Ortega R. (2015) Evaluation of sample preparation methods for single cell quantitative elemental imaging using proton or synchrotron radiation focused beams. Journal of Analytical Atomic Spectrometry, 30, 2525-2532.