ESRF	**Experiment title:** Ubiquitous role of zinc and copper in cytoskeleton architecture	**Experiment number**: LS-3030
Beamline: ID16A	**Date of experiment**: from: 10/12/2021 to: 14/12/2021	**Date of report**: 04/03/2022
Shifts: 12	**Local contact(s)**: Peter Cloetens	*Received at ESRF:*

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Report:

Zinc (Zn) and copper (Cu) are essential metals that participate in cell differentiation and neurogenesis processes. We have developed a new protocol for correlative imaging of metals by nano-SXRF (Synchrotron X-ray Fluorescence), and of proteins by Stimulated Emission Depletion (STED) super resolution microscopy, both with a spatial resolution of 40 nm. Our previous studies using ID16A at ESRF highlighted the colocalization of Zn and microtubules at the dendritic level. We also shown the co-localization of Cu and actin-F in synaptic compartments. These imaging results and additional quantitative immunofluorescence analyses revealed the essential role of Zn and Cu in the cytoskeleton architecture of primary rat hippocampal neurons (Domart et al., 2020). Some studies suggest the possible involvement of Zn and Cu directly in the organization of the cytoskeleton for various cellular systems other than neurons.

The aim of the present experiment was to investigate if this interaction between metals and proteins of the cytoskeleton could also exist for other cell types and could play a major role in the processes of cell differentiation and division. Our project aimed to investigate the interaction of metals with cytoskeleton proteins, for two cellular models. First, we selected COS-1 african green monkey kidney cells as a model of study because of the known organization of the cytoskeleton structure into easily identifiable microtubules and F-actin fibers. Second, in rat primary astrocytes because they express a different isoform of actin (α) vs neurons (β and γ isoforms).

Cells were labelled with Silicon-Rhodamine STED fluorofores and both confocal and STED microscopy were peformed on living cells to image tubulin and actin-F. Immediately after confocal and STED microscopy, samples were cryofixed by fast inmersion in cooled ethane (using a FEI vitrobot) and maintained in a cryodewar until analysis. The analyses were carried out on cells maintained in their frozen-hydrated state using the dedicated ID16A cryostation. We used a 17.1 keV X-ray beam focused down to 40 nm with a resulting photon flux of 2.2 10^{11} ph/s.

By confocal microscopy we imaged areas were both proteins showed a clear segregation and we imaged by STED smaller areas at higher spatial resolution (Fig. 1A and B). Tubulin (red) forms filaments inside the cell,

while actin (green) is located at the cellular edges. We imaged by cryo-nano-XRF at ID16A the same cell areas than those imaged by STED. In COS-1 cells, Cu was below the detection limit. Zinc was quite homogeusly distributed in the cell area, not showing evident correlation with cytoskeleton proteins (Fig. 1). In the case of astrocytes, protein compartmentalization is even more pronounced. We can see thick actin fibers spreading throughout the cell, while tubulin is confined to the interior of the cell. Tubulin network consists of numerous fibers, thinner that actin (Fig. 2). In astrocytes, again, we could not evidence a correlation between Zn distribution and F-actin or tubulin. Cu could not be detected either suggesting a higher requirement for Cu in neuronal synaptic compartments compared to astrocytes or COS-1 fibroblasts.

In conclusion, this experiment benefited from the outstanding capabilities of ID16A beamline in terms of spatial resolution, sensitivity and imaging of frozen-hydrated cells, close to their native state. This experiment enabled to clearly conclude that the expected interaction of Cu and Zn with cytoskeleton proteins, previously described in neuronal synapses, are not observed in COS-1 fibroblats and primary rat astrocytes. This result suggests the involvement of metals in the dynamic phases of cytoskeletal modelling in synaptic compartments that is not found in static phases as observed in cells such as fibroblasts and astrocytes. This a very important information that suggests a specific function for Cu and Zn in the synaptic cytosqueletal plasticity.

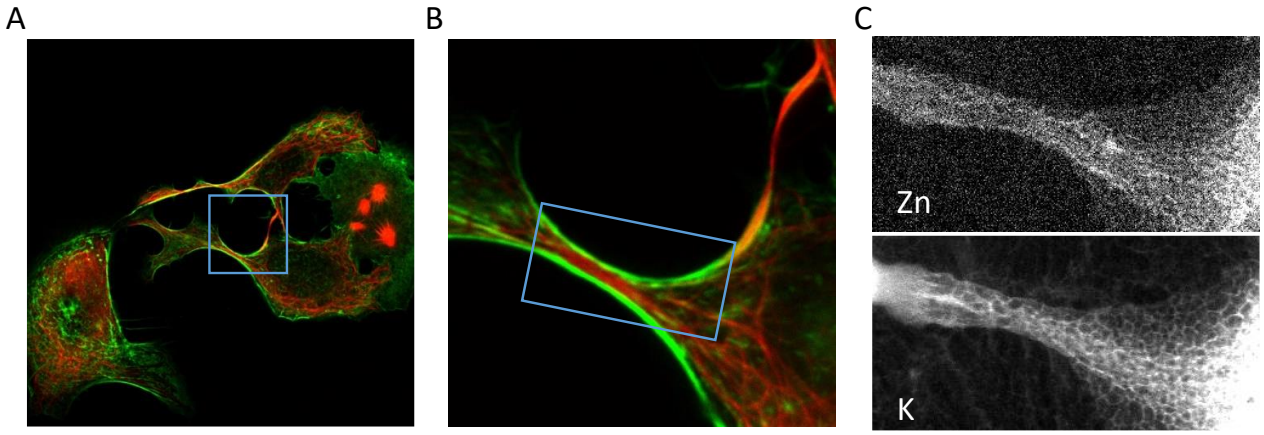

Fig. 1. A) Live-cell confocal microscopy of tubulin (red) and F-actin (green) in a COS-1 fibroblast; B) Live-cell STED image of the blue framed region in (A); C) SXRF cryo-nano-imaging of Zn and K of the blue framed region in (B).

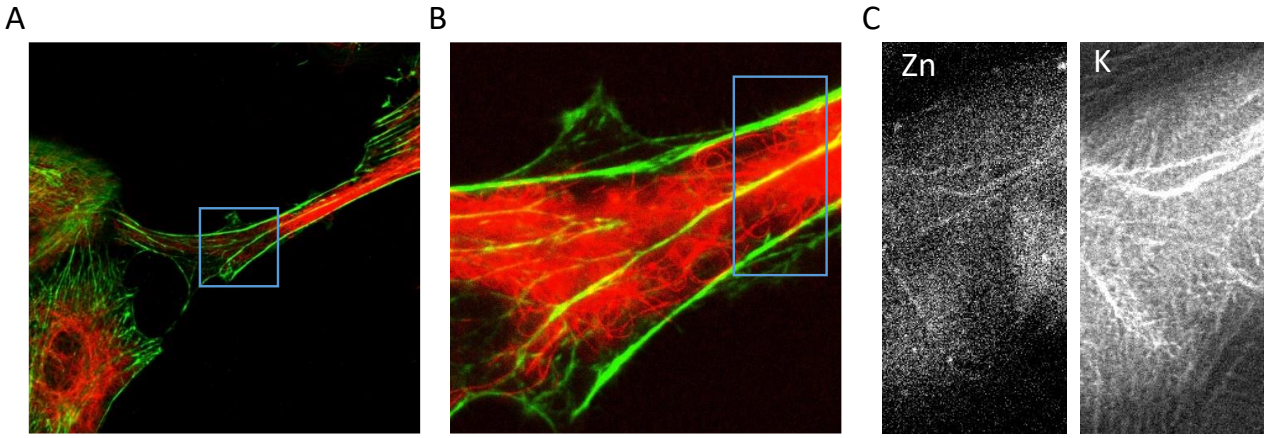

Fig. 2. A) Live-cell confocal image of tubulin (red) and F-actin (green) in a primary rat actrocyte; B) Live-cell STED image of the blue framed region in (A); C) SXRF cryo-nano-images of Zn and K of the blue framed region in (B).

Reference

Domart F., Cloetens P., Roudeau S., Carmona A., Verdier E., Choquet D., Ortega R. (2020) Correlating STED and synchrotron XRF nano-imaging unveils cosegregation of metals and cytoskeleton proteins in dendrites. *eLife*, 9:e62334. DOI: 10.7554/eLife.62334