ESRF	**Experiment title:** Subcellular distribution of Mn in HeLa cells based on expression of SLC30A10 and mutation related to Parkinsonism	**Experiment number**: LS-2640
Beamline: ID16A-NI	**Date of experiment**: from:28/06/2017, to: 03/07/2017	**Date of report**: 22/01/2018
Shifts: 15	**Local contact(s)**: Murielle Salomé	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Asuncion CARMONA

Stephane ROUDEAU

Richard ORTEGA

Florelle DOMART

Report:

The objective of this project was to elucidate manganese distribution in cells expressing or not the SLC30A10 protein and in one of its pathological mutant SLC30A10-delta 105-107. For that purpose we used Synchrotron X-ray Fluorescence imaging (SXRF) at ID16A.

Recently a new form of familial parkinsonism has been attributable to mutations in protein SLC30A10 and clinical analyses reveals an increase of manganese (Mn) in patients liver, blood and brain. At cellular level the effect of SLC30A10 mutation on Mn distribution is unknown. According to our previous results (Carmona 2010, 2014) we hypothesized that Mn can be detoxified by the Golgi apparatus when SLC30A10 protein is expressed properly while SLC30A10 mutations could alter Mn distribution, leading to Mn mislocation into the cytosol especially when available at high concentrations.

HeLa cells have been selected since they do not express constitutively the SLC30A10 protein. HeLa cells were transfected under 3 different conditions using the fluorescent molecular constructs developed at University of Texas in Austin (Leyva-Illades et al., 2014): 1) GFP-Rab5 (a control plasmid), 2) with GFP-SLC30A10-wildtype protein, and 3) with GFP-SLC30A10-delta-105-107 mutant protein. For each condition cells were exposed to 500 µM of $MnCl_2$, corresponding to a toxic exposure concentration. Cells were cultured on 500 nm thick silicon nitride membranes, a substrate compatible with SXRF imaging. Samples were prepared according to recently updated cryogenic protocols (Perrin et al., 2015).

Prior to cryofixation we imaged the entire sample under fluorescence and also classical optical microscopy and we reconstructed both maps. By superposition of both images we could discriminate fluorescence cells from the others. We scanned by SXRF only fluorescent cells to be sure that cells expressed the protein of interest.

Samples were cryofixed, shipped and stored under nitrogen vapours until to be mounted in the ID16A cryogenic chamber. At this step and during the analyses, samples were maintained at -160°C by thermal contact. Under cryogenic condition mounting the samples is the most delicate and longer process, it took at least 2 hours to change the sample holder and reach the adequate vacuum each time. X-rays emitted by light elements such as P, S and Cl were attenuated into the ice in front of the sample and could not be imaged. The formation of ice onto the sample is a normal fact that cannot be avoided when working with cryogenic samples.

The X-ray beam of 17 keV was focused down to 50 nm x 58 nm while maintaining a very high flux of photons (1.6 10^{11} ph/s). Cell imaging at high resolution was performed with a dwell time analysis of 50 ms per point, and with a step size of 50 nm (close to the beam spatial resolution). To scan one cell with an area of about 30 µm x 30 µm took around 5h of effective beam time analysis. We could scan 3 cells for each of the 3 conditions.

For cells not expressing the SLC30A10 protein, and for cells expressing the SLC30A10-delta-105-107 mutant, manganese accumulated into the Golgi apparatus. This result confirmed our hypothesis. Moreover, the very high spatial resolution of the beamline enabled to distinguish manganese location in single vesicles of the Golgi complex, of about 50 nm size. This late result suggest that manganese could be stored more specifically into the trans Golgi network which consists in secretory vesicles. On the other hand, when cells express the SLC30A10-WT protein, as expected, manganese efficiently excreted by the cells, and is not present in the Golgi complex. The content of manganese was lower than in the other cases, indicating that cells expressing SLC30A10-WT protein accumulates less manganese.

Thanks to the unique high spatial resolution and high flux of ID16A beamline, SXRF imaging enabled to elucidate how this protein mutation affects manganese storage into the cell.

Carmona A, et al. (2010) Manganese Accumulates within Golgi Apparatus in Dopaminergic Cells as Revealed by Synchrotron X-ray Fluorescence Nanoimaging. ACS Chem Neurosci. 1(3):194-203.

Carmona A, et al. (2014) Environmental manganese compounds accumulate as Mn(II) within the Golgi apparatus of dopamine cells: relationship between speciation, subcellular distribution, and cytotoxicity. Metallomics.6(4):822-32.

Leyva-Illades D, et al. (2014) SLC30A10 is a cell surface-localized manganese efflux transporter, and parkinsonism-causing mutations block its intracellular trafficking and efflux activity. J. Neurosci. 34(42):14079-14095.

Perrin L, et al. (2015) Evaluation of sample preparation methods for single cell quantitative element imaging using proton or synchrotron radiation focused beams. J. Anal. At. Spectrom. 30:2525-2532.