ESRF	**Subcellular distribution of iron in alpha-synuclein cellular models of Parkinson's disease (PD).**	**Experiment number**: MD383
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The aim of this experiment was to determine whether iron distribution is altered in dopaminergic cells overexpressing α-synuclein, a protein involved in the aetiology of Parkinson's disease (PD). Alpha-synuclein can aggregate to form insoluble amyloid fibrils in pathological conditions characterized by Lewy bodies, such as PD.

Anomalous iron handling has been proposed to be involved in the selective loss of dopaminergic neurons from the substantia nigra pars compacta (SNpc) in PD (for review see: Crichton & Ward, 2006; Berg & Hochstrasser, 2006). In vitro studies suggest that iron at low micromolar concentration can dramatically increase α-synuclein aggregation and induce formation of large oligomers which are suspected to represent the principal toxic species in dopaminergic neurons (Uverski et al., 2001; Kostka et al., 2008; Pandey et al., 2008). However the interaction of iron with α-synuclein has not been evidenced yet in cells. Our aim is to determine if iron is present in α-synuclein aggregates formed in dopaminergic cells overexpressing this protein.

In a previous experiment at ESRF (experiment report MD178) we performed X-ray fluorescence imaging of chemical elements on PC12 dopaminergic cells with a 90 nm spatial resolution (Ortega et al., 2007; Carmona et al., 2008). Nano-chemical imaging indicates that iron accumulates into dopamine vesicles, within the cytoplasm, neurites, and distal ends of cultured dopaminergic cells. These results suggest that Fe is bound to dopamine in neurovesicles of normal dopaminergic neurons. Now we aimed to investigate if Fe distribution could be modified in a cellular model of PD, the PC12 cell line overexpressing alpha-synuclein. Our hypothesis, as well as others (Smythies, 1999), is that dopamine-iron complexes may wrongly relocate to the cytosplasm in PD dopaminergic neurons leading to cell death through redox cycling due to the oxidative stress induced by the highly reactive iron-catechol compounds. This redistribution of Fe-dopamine could be associated to the overexpression of alpha-synuclein, as already shown for dopamine alone (Lashuel & Hirling, 2006).

In this experiment, a setup for high spatial resolution X-ray fluorescence microanalysis based on a Kirkpatrick-Baez lens and a piezo sample stage was used on ID22NI beamline, thus leading to the achievement of a pink photon beam at the same time highly spatially resolved (100 nm x 100 nm - v x h beam size) and with a high flux of photons ($5.5 \cdot 10^{10}$ ph/s). The characteristics of the beam fulfilled the

requirements for mapping of biological trace elements (in the µg/g range) at a sub-micrometer scale (size of most intracellular organelles). The energy of the incoming X-ray beam was set at 17.5 keV allowing to image Fe and most trace elements in cells (Figure 1).

Figure 1 shows an example of analysis. Cells were exposed to an excess of iron and to overexpression of α-synuclein. We found that iron is redistributed into the cytoplasm in the form of large grains of 1-2 µm size mostly in the perinuclear region. Whereas, in previous studies, (Ortega et al., 2007; Carmona et al., 2008) iron in control PC12 cells is distributed within the cytoplasm and neurite outgrowth but into small grains of <200 nm corresponding to neurosecretory vesicles. The distribution of Fe in alpha-synuclein overexpressing cells follows the distribution of alpha-synuclein which in known to accumulate in the perinuclear region when overexpressed (Pandey et al., 2008). All together, these results strongly support the interaction of Fe with alpha-synuclein which is evidenced for the first time in cells.

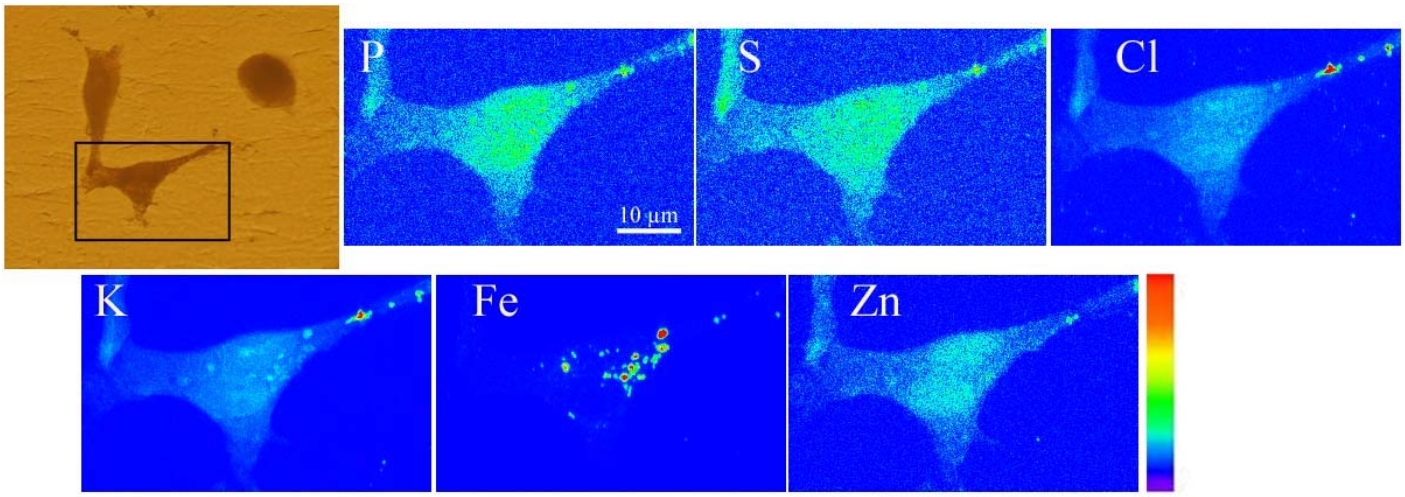

Figure 1: elemental distributions in a single PC12 cell (left, black square) exposed to 300 µM of $FeSO_4$ during 24 hours and overexpressing α-synuclein. Scan size is 37 x 60 µm (scale bar = 10 µm). Color bar ranging from blue to red (min to max) is proportional to the number of X-rays detected.

References

Berg D., Hochstrasser H. (2006) Iron metabolism in parkinsonian syndromes. *Movement Disorders*, 21, 1299-1310.

Carmona A., Cloetens P., Devès G., Bohic S., Ortega R. (2008) Nano-imaging of trace metals by synchrotron X-ray fluorescence into dopaminergic single cells and neurite-like processes. *Journal of Analytical Atomic Spectrometry,* 23, 1083-1088.

Crichton R.R., Ward R.J. (2006) In: *Metal-based Neurodegeneration: From Molecular Mechanisms to Therapeutic Strategies*. Wiley, Chichester, 227 p.

Kostka M et al. (2008) Single-particle characterization of iron-induced pore-forming B-synuclein oligomers. *Journal of Biological Chemistry*, 283, 10992-11003

Lashuel H.A., Hirling H. (2006) Rescuing defective vesicular trafficking protects against alpha-synuclein toxicity in cellular and animal models of Parkinson's disease. *ACS Chemical Biology*, 1, 420-424.

Ortega R., Cloetens P., Devès G., Carmona A., Bohic S. (2007) Iron storage in neurovesicles revealed by chemical nano-imaging. *PLoS ONE*, 2(9), e925.

Pandey N., Strider J., Nolan W.C., Yan S.X., Galvin J.E. (2008) Curcumin inhibits aggregation of alphasynuclein. *Acta Neuropathologica*, 115(4), 479-489.

Smythies J. (1999) The neurotoxicity of glutamate, dopamine, iron and reactive oxygen species: functional interrelationships in health and disease: a review-discussion. *Neurotox Res*. 1(1), 27-39.

Uversky V.N., Li J., Fink A.L. (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human B-synuclein. *Journal of Biological Chemistry*, 276, 44284-44296.