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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Sulfur speciation in neuromelanin-c ontaining neurons from patients suf fering Parkinson and Lewy body dise	**Experiment number**: MD304
Beamline:	**Date of experiment**: from: 28/11/2007 to: 04/12/2007	**Date of report**: 15 february 2009
Shifts:	**Local contact(s)**: Marine Cotte	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

We are particularly interested in Parkinson's disease which is a progressive neurodegenerative disease that affects between 1-2 million people across Europe. The cause of the disease is unknown and there is no cure. The symptoms of Parkinson's disease develop as a result of the death of midbrain neurons, particularly dopaminergic neurons of the substantia nigra (SN) and the noradrenergic neurons of the locus coeruleus. These neurons are unique in that they contain a dark polymer pigment neuromelanin (NM); a body of experimental evidence suggests changes in NM underlie brain cell death in Parkinson's disease. Particularly, changes in tissue metal content are suggested to contribute to neurodegenerative cascades in Parkinson's disease (PD). In previous work we could have performed some detail intracellular analysis of this pigment thanks to the sensitive and spatially resolved microprobe and nanoprobe at ESRF. We were focusing on the variation of the chemical composition of the NM during aging (Bohic et al., 2008). We design a project to perform a detailed analysis of the NM-containing dopaminergic neurons from SN tissue sections of brain patients suffering Parkinson (PD), Incidental Lewy body diseases (ILBD) and Alzheimer (AD) compared to healthy patients. NM is known to contain relatively high S concentration ~ 2-3 %. PD is characterize by a 30–40% decrease in glutathione (GSH) concentrations, without a corresponding increase in the levels of glutathione disulfide (GSSG) (1) and a dramatic increase in the 5-S-cysteinyldopamine dopamine (DA) concentration ratio. These sulfur compounds and probably others are implicated in NM biosynthesis and any alteration could be reflected in change in sulfur species contained in NM.

XANES experiment at the sulfur K-edge was carried out on the undulator beamline ID21. Data were obtained with the scanning X-ray microscope in fluorescence mode. The beam was focused down to 0.3 x 0.3 μm2 using a Fresnel zone-plate. 8 micron thick paraffin section of post-mortem substantia nigra tissues from patients suffering PD (N=5), ILBD (N=3) and AD (N=3) and from aged-match controls (healthy patients, N=5). Sections were mounted on 4 micron thick Ultralene foil. The neuromelanin containing neurons were imaged offline using a high resolution visible microscope. 16 sulfur compounds were used as reference materials. In order to avoid photo reduction effect we experienced during the experiment, we determine the acquisition time necessary to avoid it using fast acquisition of XANES spectra (100 ms per point). The resultant sum spectrum was thus obtained from 2-3 points in NM for each dopaminergic neurons from the substantia nigra. A total of 10 cells per cases were analysed.

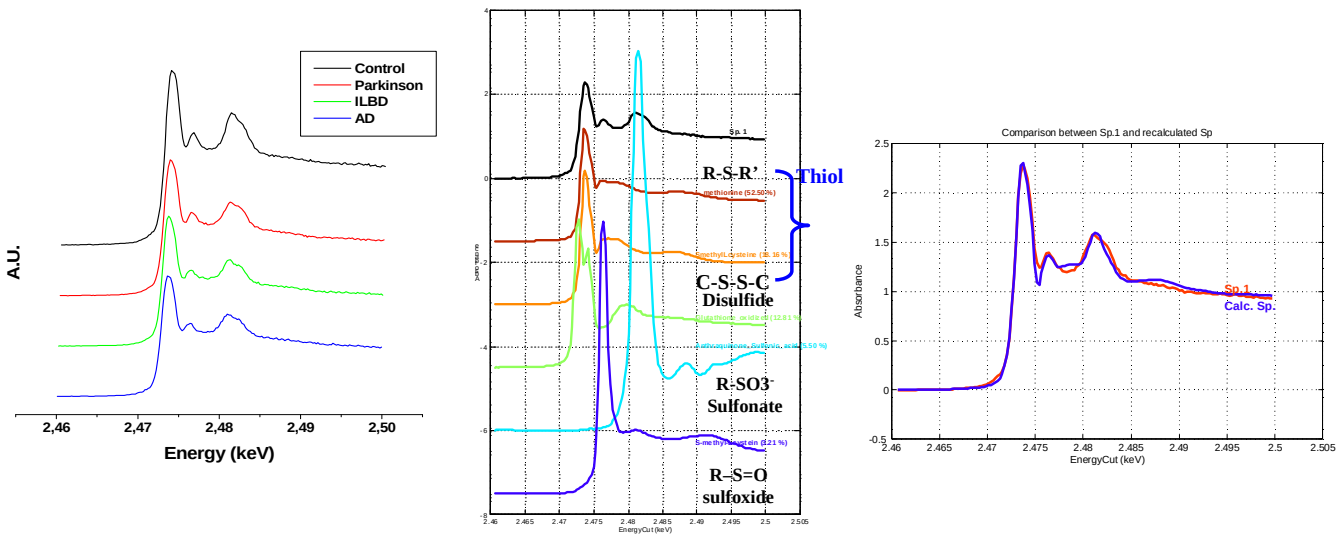

Figure 1: This panel shows the S K-edge Xanes average spectrum for the different patients' cases (left figure), model compounds used for linear combination fitting (middle) and the resultant experimental and fitted spectrum (right figure) .

Linear combination of 5 compounds best modelled the experimental spectra although specific compounds are representatives of sulfur functional groups and are not explicitly identified by this method**.** Model compounds used anthraquinone-2-sulfonic acid (formal oxidation state +5); methionine sulfoxide (formal oxidation state +2); L-methionine (formal oxidation state 0); S-methyl-L-cysteine: R-S-R' (formal oxidation state 0); Glutathione oxidised (formal oxidation state 0). We could performed a semi-quantitative analysis that shows no particular changes in the relative proportions of models compounds. Indeed, the average sum spectrum for the different groups investigated are very similar (position of the white line, edge…). Statistically, the large number of NM containing neuorns investigated let us suggest that there is no differences between NM-resting neurons of the SN of PD patients and aged-match control.

References:

1- Sofic, E., Lange, K.W., Jellinger, K., Riederer, P., 1992. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci. Lett. 142, 128–130.)

2- Jenner, P.; Dexter, D. T.; Sian, J.; Schapira, A. H. V.; Marsden,C. D. Oxidative Stress as a Cause of Nigral Cell Death in Parkinson's Disease and Incidental Lewy Body Disease. Ann. Neurol. 1992, 32, S82-S87