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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:** Studies of Cu oxidation state and chemical environment in brain cancers with the use of cryo-XAS	**Experiment number**: MD-726
Beamline: BM 25A	**Date of experiment**: from: 17.04.2013 to: 23.04.2013	**Date of report**:
Shifts: 17	**Local contact(s)**: Alvaro Muñoz-Noval	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The second most common cause of death is cancer. Among the types of cancer with the smallest survival rate are brain tumours, many of which are brain gliomas. In order to successfully combat the disease, it is extremely important to learn about the mechanism of its formation. It is believed that trace elements such as Fe, Cu and Zn play a significant role in neoplastic processes. In our previous paper we showed that the oxidation state of iron changes in the process of oncogenesis: the higher the tumour grade, the more Fe^{2+} as compared to Fe^{3+} the sample contains [1]. Transition between different oxidation states of iron is controlled mainly by cuproenzymes as they have very strong redox properties. Superoxide dismutaze (Cu-Zn SOD), which accounts for 25% of the overall brain copper, is involved in the dismutation of the superoxide radical. Ceruloplasmin can oxidize Fe(II) to Fe(III). Also cytochrome c oxidase can oxidize Fe(II) to Fe(III). For this reason, information on differences in the chemical form of copper contained in tissues with various malignancy grades may significantly contribute to the knowledge of biochemical reactions involved in oncogenesis.

The samples under study were collected intraoperatively and contained brain tumours with different malignancy grades. The tissues were placed in specially designed polypropylene rings with a diameter of 10 mm and thickness of 1 mm. The front and the end of the ring were flat and covered with ultralene foil. Afterwards, the samples were immediately frozen to -80°C, to slow down biological and chemical processes, e.g., oxidation. Altogether nine samples of brain tumours with various malignancy grades were measured. Six gliomas - including glioblastoma multiforme(WHO IV), astrocytoma anaplasticum (WHO III), astrocytoma diffusum cum signis malignisationis focalis (WHO II/III) and astrocytoma diffusum partim microcysticum (WHO II) and three non-glial tumour samples including meningeoma atypicum (WHO II), meningeoma monstrocellulare et microcysticum (WHO I) and meningeoma meningotheliale (WHO I) - were analysed. Additionally, organic reference materials (Cu-Zn SOD, ceruloplasmin) and inorganic reference materials (CuO, Cu_2O and Cu foil) were examined.

The experiment was performed at beamline BM25A dedicated to X-ray absorption spectroscopy. The final beam spot size on the sample was about 4x1 mm. Special conditions were designed to avoid radiation damage. Containers with samples were placed in a liquid-helium cryostat (temperature about 20K) in a helium atmosphere. Absorption spectra of Cu were taken using incident radiation energies ranging from 8.9 to 9.6 keV. In the absorption edge area the spectrum was probed with 0.3 eV resolution and from energies 200 eV above the absrption edge, the spectrum was probed only for normalisation purpose with 3 eV resolution. The measurements were performed in fluorescent mode due to low concentration of copper in human brain tissue. The fluorescence radiation was collected by a 13-element Si(Li) detector. The noise of the tetector was 2500 cps.

The spectra were processed using the Athena program of the IFEFFIT package. For all brain samples correction for self-absorption was done. For this purpose the composition of white matter in brain tissue was estimated and assumed to be approximately $O_{16}C_{23}H_{10}N_3P_1$. Further analysis included the XANES region only because the intensity of fluorescence radiation was too low to obtain good enough statistics for EXAFS analysis.

First of all, the average oxidation state of copper in biological samples was estimated. Due to the nature of biological samples and the resulting complex shape of the spectra, the identification of the position of the appropriate maximum of the first derivative in the most widely used first-derivative method can be inaccurate. For this reason, the data were analyzed using the integration method which involved the calculation of the centre of gravity of the absorption curve within the range corresponding to the absorption edge from the following formula:

$$E_0 = E(\mu_1) + \frac{1}{\mu_2 - \mu_1} \int_{E(\mu_1)}^{E(\mu_2)} [\mu_2 - \mu(E)] dE$$

The values of the normalized absorption coefficient were set to μ_1=0.15 and μ_2=1. Figure 1 shows the energies at which the absorption edges for the tumour samples with different malignancy grades and organic standards were found. A trend can be observed that the higher the tumour malignancy grade, the higher Cu average oxidation state. If all the measured samples are taken into account, the correlation coefficient between the edge energy and the tumour malignancy grade equals 0.76. But for some histopathological reasons, glial brain tumours should be considered separately from meningeomas. If we take this restriction into account then the correlation coefficient equals 0.92.
So far we do not understand the biochemical mechanism leading to these results, but the results obtained still require further measurements to improve statistics. All the absorption edges measured for tissue samples lie between the spectra for Cu(I) and Cu(II), whereas the spectra for organic standards lie close to the spectrum for Cu(II).

As a next step, the spectra obtained for tumour tissues were compared with the spectra for organic standards measured in this experiment. Two spectras together with standards are presented in figure 2. All the spectra showed much more similarity to the spectrum of ceruloplasmin than to the spectrum of Cu-Zn SOD. These studies showed no correlation between the content of ceruloplasmin and the tumour malignancy grade. Uncertainty of the results presented above can result from the forms of copper in chemically separated ceruloplasmine and those in the ceruloplasmine naturally present in the brain. Also, considerable uncertainty of the analytical results can be causedby relativelyhigh noise of the spectra measured.

The results obtained still require further measurements to improve the statistics, but they offer hope for making one more step towards understanding the processes involved in oncogenesis.

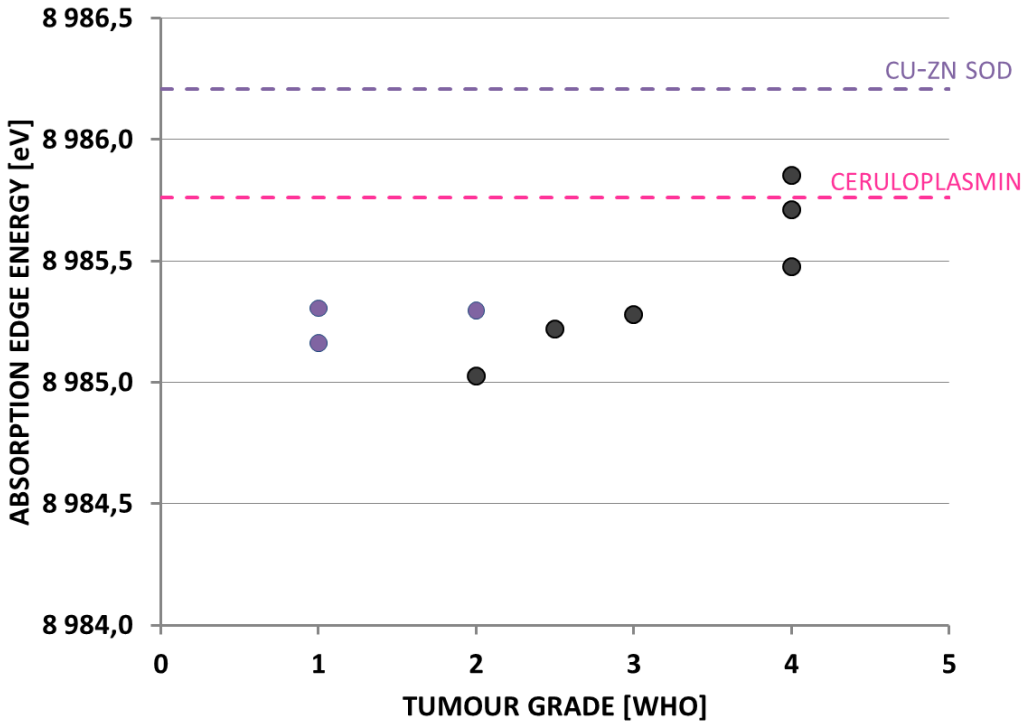

Figure 1: Absorption edge energies of Cu K for brain tissues with various malignancy grades and of organic standards. Black dots are brain gliomas and blue dots are maningeomas.

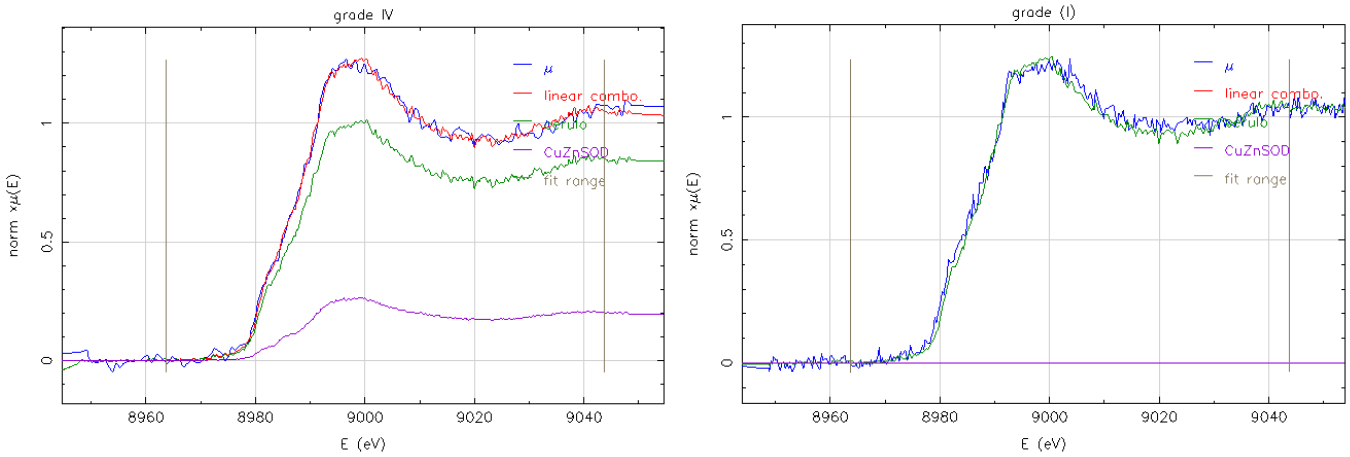

Figure 2: XANES spectrum of the brain tissue as a linear combination of organic standards: ceruloplasmin and Cu-Zn SOD.

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References:

[1] A. Wandzilak, M. Czyzycki, P. Wrobel, M. Szczerbowska-Boruchowska, E. Radwanska, D. Adamek, M. Lankosz, *Metallomics*, 2013, **5**, 1547-1553