Experimental Report

Proposal: **8-04-666** **Council:** 4/2012

Title: Proton dynamics in malignant glioma tumor

This proposal is a new proposal

Researh Area: Biology

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Samples: healthy and tumoral mice brains

Instrument	Req. Days	All. Days	From	To
IN5	6	3	09/11/2012	12/11/2012
IN13	20	10	04/11/2012	14/11/2012

Abstract:

The proposed experiment represents a benchmark in the study of biological tissue with neutrons. From the elementary building blocks of the cell (proteins, membranes etc) to bacteria and in vivo cells, and now to complex brain tissue, neutrons have proven to be unique in pinpointing proton dynamics at atomic scale in biological compounds regardless of the macromolecular complexity involved. The results will be relevant for a deeper understanding of the physical basis of diffusion magnetic resonance imaging technique, nowadays widely used to diagnose brain diseases such as ischemia and tumors.

Exp. Report n. 8-05-466 and CRG-1943
Dates of exp.: 09/11/2012 – 12/11/2012 (IN5), 04/11/2012 - 14/11/2012 (IN13), 14/11/2012 – 22/11/2012 (IN13).
Title: Proton dynamics in malignant glioma tumor
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When seen at the micron-scale, brain tissues (belonging to the Central Nervous System, CNS) are heterogeneous systems containing numerous compartments (i.e. glia cells, neurons, myelin sheaths and extracellular space) of different size and shape separated by impermeable and semipermeable membranes. The major tissue constituent is the water (> 70%). Interacting with cell membranes during their random motion, water molecules can be used as a tool to probe tissue structure at microscopic scale, and thus, provide unique information on the functional architecture of tissues. Nowadays, diffusion magnetic resonance imaging technique (dMRI), based on water diffusion (~$10^{-3}$$mm^2$/s = ~$10^{-5}$$cm^2$/s), is widely used to detect variation at the micron scale in the tissue contrast induced by brain diseases such as ischemia, tumors etc. [1,2]. However, at micron scale, the cellular contributions are averaged hiding a correct interpretation of the diagnostic images obtained through DMRI.
Using neutron scattering techniques, the measuring distance is reduced to the scale of the macromolecular separation. This allows having access to atomic and picosecond distance-time scales.
Through quasi-elastic neutron scattering experiments (QENS) performed recently IN5 at ILL we were able to prove that neutrons are unique in pinpointing proton dynamics at atomic scale in biological compounds regardless of the macromolecular complexity involved. Results clearly suggest that water dynamics is different on micrometric and atomic scales [3,4].
Here we performed elastic neutron scattering (ENS) and QENS experiments (on IN13 and IN5 respectively) using the same energy resolution (10 microeV), which allowed us to discriminate and quantify at atomic scale free and restricted water diffusion processes, on healthy and pathogenic rat brain hemispheres.
The aggressive primary malignant tumor 9L *gliosarcoma* has been taken as tumor model. *Glioma,* arising from the *glial* tissue, is the most common form of primary brain tumor (~60%). It occurs predominantly in the cerebral hemispheres of middle-life adults (mainly men) and is highly malignant and rapidly progressing.
The 9L gliosarcoma cells were grown with complete medium at 37°C and inoculated into the right hemisphere of the rat brain. The brain were removed in few minutes, immediately weighed and frozen in liquid isopentane at -50°C and stored at -80°C. Coronal brain sections (50µm) were cut at -20°C on a cryostat (Microm HM 80, France) and stained with hematoxylin/erythrosine (HE) (Fig. 1).

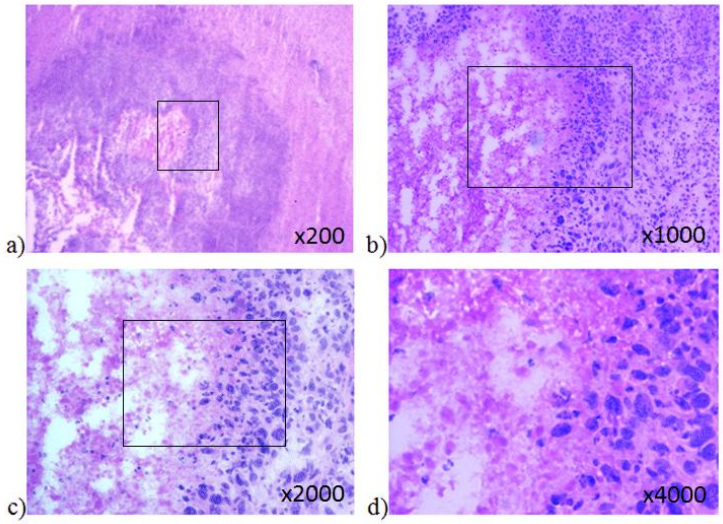

Fig. 1. Photomicrographs, at different magnifications, of glioma tumor (hematoxylin-eosin, HE, stain coloration), showing regions of necrosis. Taken at the Biomedical facility, ESRF, Grenoble.

ENS and QENS scan were acquired at 10 microeV resolution on 9L tumors sampled at Day 24 and 47 after tumor implantation. Healthy brain tissues have been also measured and taken as references (control brains). Data analysis, currently under progress, aims at determining changes in the roto-translational diffusion coefficients and resident times of the free-like and restricted water pools using the Sears model [5]. In this framework, in fig. 2 we report the comparison of the widths of the QENS contributions associated to the translational diffusions of free-like (left panel) and restricted (right panel) water pools for healthy (red symbols) and tumor suffering (after 47 days of implantation, blue symbols) rat brain tissues.

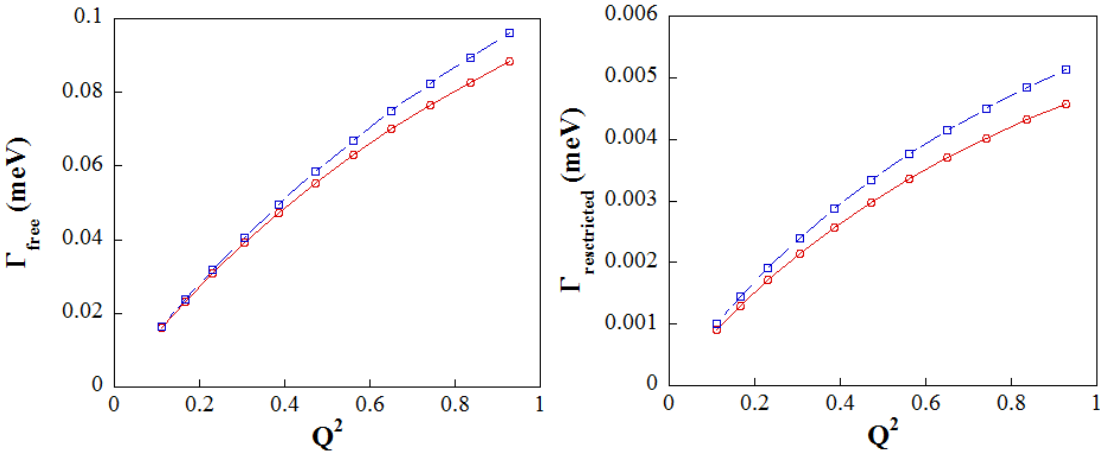

Fig. 2. Comparison of widths of the QENS contributions associated to the translational diffusions of free-like (left panel) and restricted (right panel) water pools. Control brain tissue: red symbols; tumor suffering brain tissue (at 47 days after implantation): blue symbols.

Preliminary results clearly demonstrate that cellular water diffusion is affected by the presence of *glioma*. In particular, occurrence of necrosis, typically associated to high tumor grade, speeds up water diffusion as indicated by the enhancement of the QENS broadening. The results are in agreement with dMRI findings. Indeed, average water diffusion constants (ADC) obtained through dMRI measurements have shown enhanced ADC for *glioma* when compared to healthy tissue [6].

References

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