Experimental report

09/02/2016

Proposal: 8-04-740 **Council:** 10/2014

Title: Investigation of water diffusion in brain tissue under stress conditions

Research area: Biology

This proposal is a new proposal

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Samples: rait brain tissue and cell culture

Instrument	Requested days	Allocated days	From	To
IN13	8	8	20/04/2015	28/04/2015
IN5	3	3	20/04/2015	23/04/2015

Abstract:

The proposal aims to investigate the distribution of water pools (which strongly differ in dynamical properties) across the brain tissue heterogeneity. For that, we propose a neutron scattering investigation on rat brain tissue samples, submitted to different challenges to modify cell structures and/or volumes, two potential contributors to water diffusion dynamics. In parallel to that, in order to investigate whether the results obtained on brain tissue, which represents a mixture of cell types, are the same as in more simple systems, the same study will be performed on Neuro2a (N2a) cells, a fast-growing mouse neuroblastoma cell line, often used as model for neurons.

Experimental report 8-04-740

Title: Exploring cell biodiversity

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The experiment 8-04-740 aimed at studing the dependence of macromolecules dynamical behaviour upon cell biodiversity using elastic neutron scattering on IN13 between 280 and 320 K and to discriminate between fast water and slow water molecules with different diffusivities propriety at 300 K by quasi-elastic neutron scattering on in5 at two energy resolutions, 10 and 60 μeV.

The temperature dependence of the elastic neutron scattering (ENS) scans of different cell types were investigated: E. coli is a rod-shaped bacteria about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3. Yeasts are eukaryotic microorganisms classified in the kingdom Fungi, typically measuring 3–4 μm in diameter. Instead glioma 9-L are tumor cells obtained in vitro and very useful for in vivo studies to investigate the effects of various therapeutic modalities on brain tumors or to implant tumor cell in healthy rat. PC12 is a cell line derived from a pheochromocytoma (is a neuroendocrine tumor) of the rat adrenal medulla, that have an embryonic origin from the neural crest that has a mixture of neuroblastic cells and eosinophilic cells.

In fig. 1 we report images acquired with an optical microscope available at EMBL, Grenoble. The chosen sampling of cells represents a variety in size and shape.

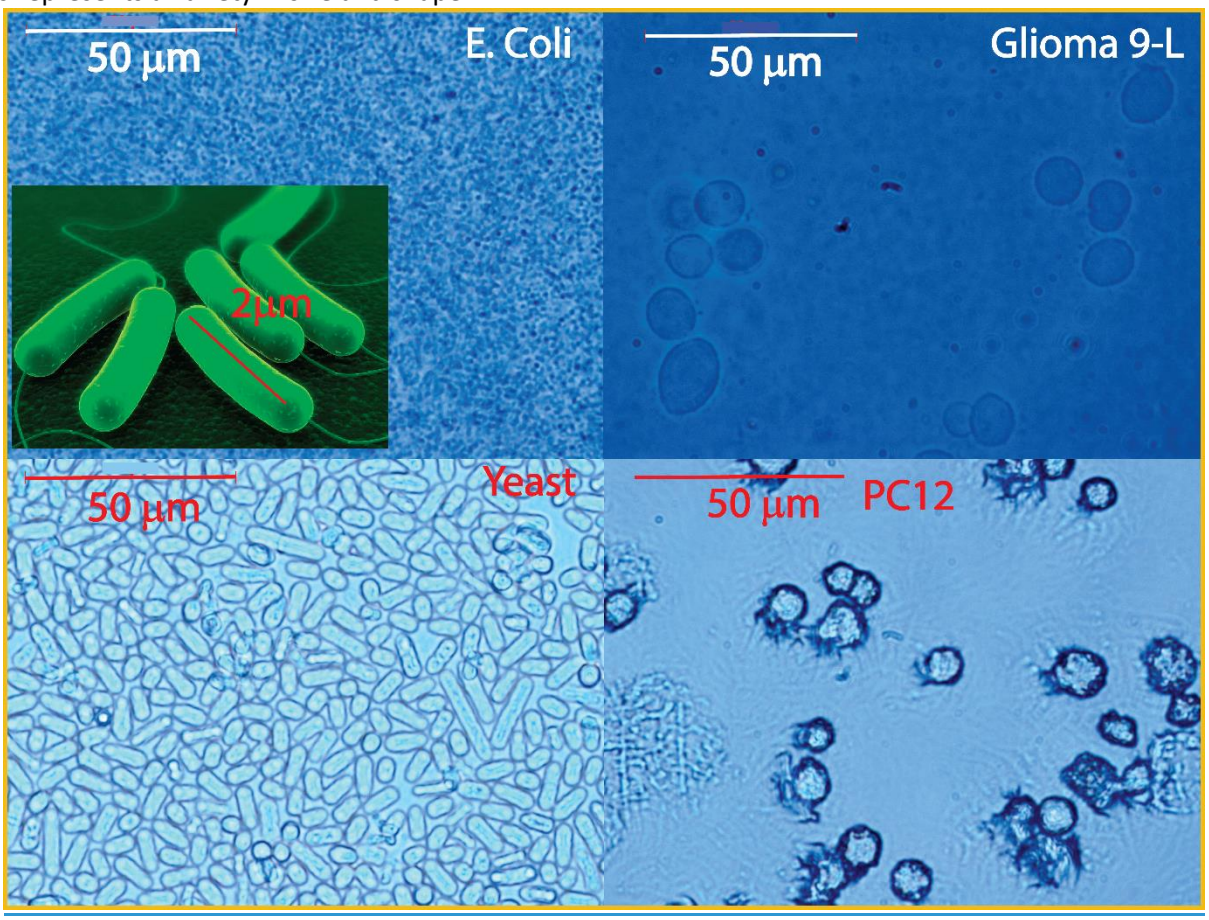


Figure 1 Image of cells at 100X

The sample were furnished cell cultures at the neuroscience Institute of the CHU (GIN, Grenoble). During the transport to ILL, the samples were kept in PSB buffer to guarantee physiological condition thus to prevent cell death. Prior to the experiment, cells were centrifuged in order to obtain a cell paste.

Weight measurements of samples reveal that Escherichia coli bacteria, yeast, PC12 and Glioma (9-L) contain a range between 35% and 85% in weight of removed water calculated on the total mass of the sample after the experiment.

ENS experiment on IN13

Elastic scan were performed in the 280-310 K temperature region. The elastic response was also monitored during cycles of heating-cooling to check the existence of eventual hysteresis. Several controls measurement at 300 k were also performed in order to check the process reversibility. Strong hysteresis were observed in yeast and PC12, less prominent one in Glioma and almost absent in E.coli.

A first insight to the dynamical behaviour is given in fig. 2 where the summed intensities, integrated over the accessible Q range, are shown in function of the sample temperature. For comparison purpose, data have been normalised to one.

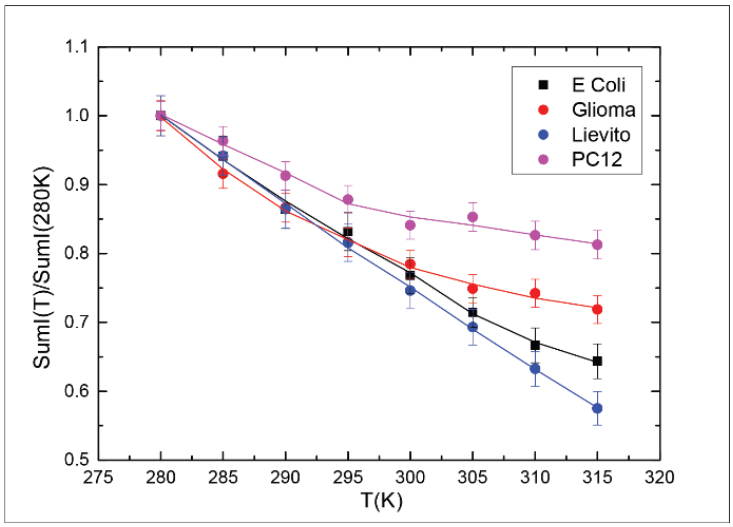


Figure 2 Summed intensities as function of temperature

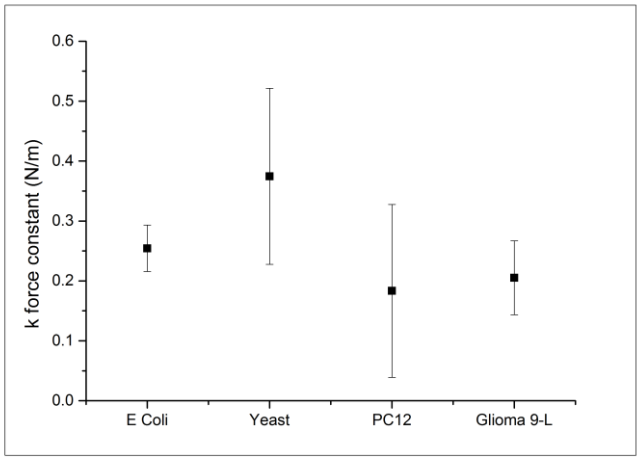


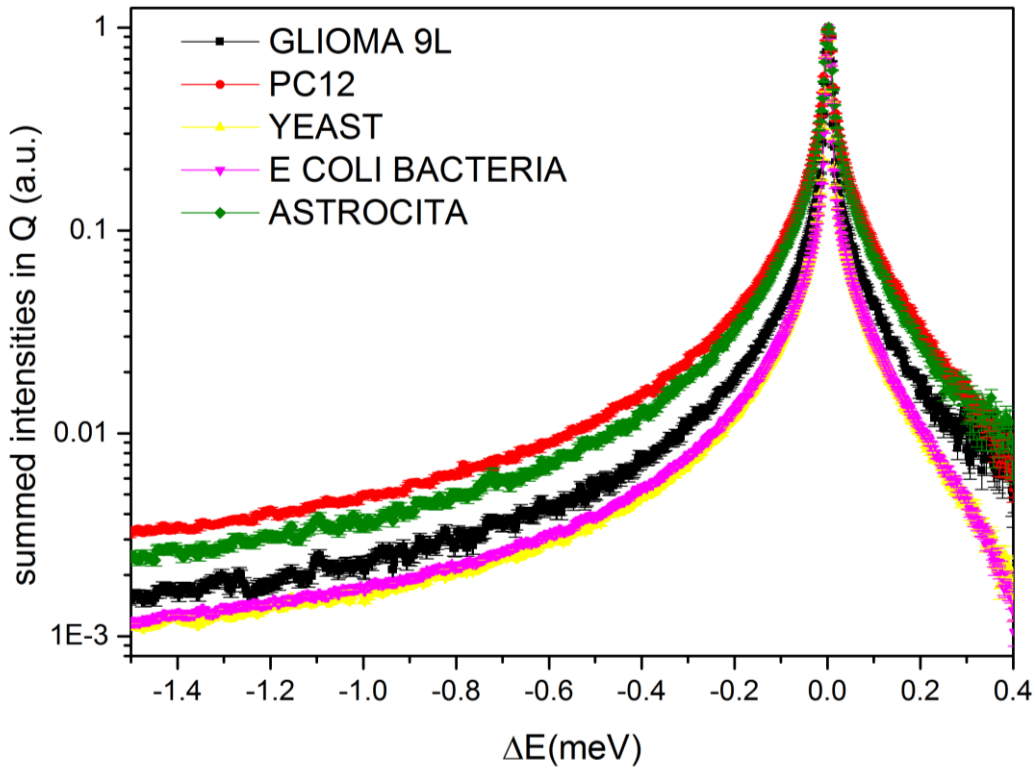
Figure 3 k-force constant for cell investigated

The analysis in terms of force constants derived as a linear fit of the mean square displacements [1] (fig. 3) support the strong dependence of cell softness/stiffness upon cellular composition.

Data should be extended to large sampling to better explore the cellular biodiversity.

QENS experiment on IN5

An additional sample were investigated in QENS experiment: astrocytes, a kind of neural cell. Quasi-elastic neutron scattering spectra on IN5 at high resolution (ΔE~ 10μeV) explore the interaction between 0.1 and 1.01 $Å^{-1}$. We show the total sum on Q-values for E. Coli bacteria, yeast, glioma-9L, PC12 and astrocyte as function the energy transfer during the scattering event. The spectra are normalized to the maximum values and in logarithmic scale.



E. Coli bacteria and yeast seem have a similar diffusion. The highest diffusion seems present in PC12 cancer cell, followed from astrocytes and Glioma-9L A preliminary analysis has done trying to associated such property and to discriminate between fast and slow water populations.

REFERENCE

1. Zaccai et al., Science, 228(5471): 1604-1607, (2000).