EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY
INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report CH 4717

ESRF	**Experiment title:** Quantitating neuronal and astrocytic iodine speciation linked to an iodoacetate-dependent blockade of glycolysis in the living rat brain	**Experiment number**: CH-4717
Beamline:	**Date of experiment**: from: 23 March 2016 to: 29 March 2016	**Date of report**: 14 June 2016
Shifts: 18	**Local contact(s)**: Murielle Salomé	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Carole Poitry-Yamate

Centre d'Imagerie Biomédicale

Ecole Polytechnique Fédérale de Lausanne

Report:

In 2013, the systemic delivery of iodoacetate (IAA), a compound of choice to inhibit the metabolism of glucose through glycolysis, was successfully performed for the first time in mammals, in vivo, using non-invasive positron emission tomography (PET) and the radiolabeled glucose analogue (18FDG) at the CIBM. Such experiments were repeated under identical physiological conditions without the radiolabeled 18FDG, enabling brain slices to be removed and processed for HEXRF of I (in IAA) measurements, with the goal of identifying the cellular locus of cerebral IAA uptake. The tissue fixation procedure was previously tested at other beamlines to ensure tissue structure and the stabilization of diffusible substances. At ID21, a fluorescence signal corresponding to the L line of the element I, albeit a weak one, was detected from the epon, far from brain tissue, and we attributed this to either the epon itself, or alternatively, to the counter stain, methyl blue. In contrast, the fluorescence signal attributed to I from IAA from regions of cortical brain was spatially heterogeneous and disproportionately high in neurons, as shown in the corresponding experimental report. A manuscript was subsequently submitted with the accord of all scientists involved. Although the manuscript was considered very promising and timely, but not accepted, one of the peer reviewers raised the question as to whether IAA had effectively entered cellular glycolysis.This led the principal investigator to submit a subsequent proposal in 2015 that addressed that question, and the measurements were performed in March of this year, using the un-used sections of counter-stained brain tissue initially transported to the beamline in 2013.

The global results obtained in March showed a weak fluorescence emission signal corresponding to the L lines of the Z element I from the counter-stain, with and without an epon background. Surprisingly, and unexpectedly, the level of this signal was virtually unchanged when cortical brain areas were HEXRF scanned. We concluded that the counter-stain, methyl blue, was likely contaminated with added iodine from the chemical supplier, which it also sells, but under a different name. Subsequently, we performed HEXRF measurements on previously unused and un-stained brain sections; and here, the I fluorescence signal was

weak to absent. This result was unexpected. We raised the possibility that (1) the 2013 results from brain tissue were a combination of biological/metabolic iodine (from IAA) and counter-stain; and (2) that the biological iodine signal had degraded within the epon matrix of the brain tissue since 2013. Even if one takes into account what iodine is known to counter-stain, i.e., starch, the spatial distribution of I fluorescence from the 2013 results does not correspond readily nor reproducibly to intracellular glucose (or glycogen) in the neurons. We further attempted fluorescence measurements using unused brain sections from successful HEXRF Gd measurements performed at IDE at Argonne National Laboratory in 2013, and again, no fluorescence signal was obtained at ID21. Finally, with the main safety officer and principal beamline scientist, we constructively discussed how to remove any unwanted (non-biological) background signals from the preparations, with minimal handling of the tissue following the in vivo experiments, tissue fixation and transport to the ESRF. This is the subject to be proposed for the next proposal period in September in support for additional beam time, as resubmission of the combined PET and synchrotron x-ray manuscript hinges on the aforementioned goal and experimental results.