

**Contribution aux exercices de prospective 2020-2030**  
***Contribution to the 2020-2030 prospective reflection***

**Sciences Nucléaires et Vivant**  
*Nuclear Science and Health*

**Description détaillée de la contribution**  
*Detailed description of contribution*

**Titre : Imagerie chimique de la synapse avec de nouvelles sources synchrotron**  
***Title : Chemical imaging of the synapse with upgraded synchrotron sources***

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**Objectives:** Most synchrotron radiation facilities are upgrading their sources to enhance by a factor 100 to 1,000 their brightness (ESRF delivery for 2021, APS Argonne National Lab. USA in 2024, DESY-PETRA IV in 2026, SOLEIL in 2026?). These new synchrotron sources will enable 3D hard-X-ray (>10 keV) chemical imaging with very high spatial resolution and unprecedented sensitivity. Our objective is to develop a new correlative imaging methodology for performing 3D super resolution fluorescence microscopy and **nano-SXRF (Synchrotron Radiation X-ray fluorescence)** on the same cellular area with similar spatial resolution (<50 nm). The main application of this new method will be to describe the interaction of chemical elements (i.e. biological metals such as Cu and Zn) with synaptic proteins. The expected results are a better understanding of biological metal functions in memory processes, and of disturbed mechanisms in neuropathological conditions such as Alzheimer's disease.

**Background:** The emergence of previously unknown functions for Zn and Cu in neuronal signaling has gathered considerable attention in recent years (Dodani et al., 2014; Chang, 2015). Original results could be achieved thanks to the development of new fluorescent metal sensors that can be visualized using confocal and two-photon imaging. However, the study of the labile metal pool using fluorescent metal sensors yields an important, albeit only partial, picture of metal distribution in cells. In order to gain a more comprehensive understanding of metal functions, nano-SXRF can be applied to image total (bound and labile) metal distribution in cells. Up to recently, the best resolved chemical element images in cells have been obtained with a spatial resolution around 100 nm. However, in future years, 3D element imaging will be possible at <50 nm spatial resolution thanks to the upgrade of synchrotron radiation sources. At ESRF (European Synchrotron Radiation facility) in Grenoble, the EBS (Extremely Brilliant Source) project is a 150 M€ facility upgrade centered around the construction of a low-emittance light source that will increase the brilliance and coherence of the X-ray beams produced by a factor of 100. The ESRF-EBS upgrade will be completed in 2021. Other synchrotron facilities worldwide have decided their source upgrade, such as the DESY-PETRA IV in Germany, APS at Argonne National Lab. in the US, or are under consideration for SOLEIL.

These new sources will considerably improve the capabilities of X-ray nanoprobe and will reshape the landscape of research for synchrotron-based metal imaging in cells. As a consequence, correlative approaches for biological molecules imaging at similar spatial resolution (tens of nm) must now be developed to understand element distributions in cells

at the nanoscale. STED (stimulated-emission-depletion) super resolution microscopy has already been successfully combined to transmission electron microscopy to correlate protein localization with cell ultrastructure at high spatial resolution (Watanabe, 2011). Recently, STED has been performed together with synchrotron scanning diffraction microscopy to inform about diffraction patterns in cells with a 300 nm spatial resolution (Bernhardt et al., 2018). These correlative microscopy approaches however cannot be transposed to image metals in cells since they require steps of chemical fixation known to disrupt the metal-binding as demonstrated in our group (Roudeau et al., 2014; Perrin et al., 2015). Our team has pioneered the use of synchrotron radiation for the imaging of chemical elements in cells (Ortega et al., 2009) and has also developed specific protocols for correlative X-ray fluorescence microscopy (Roudeau et al., 2014; Carmona et al., 2014 and 2019; Domart et al., 2019). The development of a correlative microscopy combining super resolution microscopy and nano-SXRF will be unique worldwide potentially leading to breakthroughs in the cell biology of metals. Once the methodology will be developed it could be applied to many research topics, our priority being the study of metal interaction with synaptic proteins.

#### **International state-of-the-art:**

At ESRF in Grenoble, a beam size of 13 nm for high energy X-ray imaging at high flux has been achieved with ID16A beamline, a world-first (Da Silva et al., 2017). The upgraded ESRF-EBS in 2021 will dramatically increase the capability of ID16A beamline allowing 3D chemical element imaging at high spatial resolution and with increased sensitivity. Our objective is to implement a long term collaboration with ESRF ID16A beamline, both on a technical and scientific perspective, to develop a 3D correlative imaging methodology. Recently, the beamline Nanoscopium at the SOLEIL French Synchrotron facility has reached a 50 nm spatial resolution with hard X-rays (<https://www.synchrotron-soleil.fr/en/beamlines/nanoscopium>). Our methodological development could also be used at Nanoscopium beamline after the upgrade of SOLEIL, potentially in 2026.

In terms of international competition, it is worth mentioning that a related project is developed by the University of Göttingen who runs a beamline at DESY-PETRA III synchrotron (Bernhardt et al., 2018). This project will also benefit from the upgrade of DESY source in 2026. However this project is based only on structural imaging, and will not include chemical element imaging due to the type of samples that can be analyzed (only chemically fixed). Our project will include both structural and chemical imaging capability. The upgrade of the APS (Advanced Photon Source, Argonne National Lab., USA) has been recently approved (\$815M). One of the six main driving topics is: *'A better understanding of the way the brain processes and stores information with neurons'*. We have no knowledge of which developments are foreseen at APS nanoprobe beamlines. The potential of combining super resolution microscopy with synchrotron chemical imaging in cell biology has also been suggested by a team from the Shanghai Synchrotron Radiation Facility in China. It is not clear if this is an on-going project or only a perspective (Zhu et al., 2017).

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