ESRF	**Experiment title:** Three-dimensional Analysis of Cells Containing Ir Metallodrugs by cryo-X-ray Fluorescence Microscopy	**Experiment number**: LS-2767
Beamline: ID16A-NI	**Date of experiment**: from: 25/07/2018 to: 30/07/2018	**Date of report**: 30/08/2018
Shifts: 15	**Local contact(s)**: Yang Yang	*Received at ESRF:*

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Report:

Half-sandwich IrIII anticancer complexes of general formula [(Cpx)Ir(L∧L′)Z]0/n+ (with Cpx = Cp* or Cp*R and L∧L′ = chelating C∧N or N∧N ligands) have great potency (nanomolar) toward a range of cancer cells.1 Their mechanism of action may involve both an attack on DNA and a perturbation of the redox status of cells. We have designed IrIII pro-drugs that are activated when the concentration of protons raises, at acidic pH. Those intracellular compartments with acidified media such as lysosomes and endosomes, the Golgi apparatus, and the mitochondrial intermembrane space will play a key role in the switching capability of our drugs. For this reason, localizing the IrIII pro-drugs in the intercellular space is crucial to map out the path to activation and cytotoxicity of our systems. We can shed light on such a cause-effect relationship (i.e.; intraorganelle accumulation-activation of the drug) by elucidating the drug's intracellular fate.

We used cryo Hard X-ray Fluorescence microscopy (cryo-XRF) and the high flux of focused beams at nanometric size (close to 30x30 nm2) in order to determine the intracellular localization of the Ir metallodrugs close to native conditions. To this mean, we correlated this information with the cellular structure previously revealed by cryo Soft X-ray Tomography (cryo-SXT) at the Mistral beamline (ALBA synchrotron). This unique correlative approach will allow to understand and thus control the mechanism of activation of these Ir compounds in the cells.

In this context, our goal is to understand the intracellular trafficking and the overall cellular accumulation of an Ir metallodrug with potent cytotoxic activity. For this purpose, prior to cryo-XRF measurements, we used cryo Soft X-ray tomography (cryo-SXT) to obtain the 3D ultrastructural information of whole cells treated with Ir metallodrug close to their native state at a resolution better than 50 nm. This 3D structural information was then correlated with elemental specific information from the Ir metallodrug gathered by cryo X-ray fluorescence (cryo-XRF) performed at ID16A-NI.

MCF-7 cell samples treated for 12 h with Ir metallodrug (ACC25 compound) and untreated were prepared for the correlative acquisition scheme previously detailed. This correlative approach includes cryo-epifluorescence, cryo-SXT and cryo-XRF performed on the same samples. Six cell samples treated with the compound and three without treatment were vitrified and analyzed.

Aiming at comparing the cellular morphology changes induced by the ACC25 Ir compound, prior to cryo-XRF at ID16A-NI data acquisition, we imaged treated and untreated samples by cryo-SXT (ALBA-CELLS Mistral beamline). We found that the compound alters the subcellular architecture of the cell, affecting the

mitochondria morphology and increasing the number of high density vesicles in the cytoplasm. Both events could be related with a possible induction of an apoptotic process by means of the Ir metallodrug treatment.
In order to locate unequivocally the Ir compound inside the cell and to extract quantitative information related to the elemental composition, we analyzed the same samples imaged by cryo-SXT using cryo-XRF at ID16A-NI beamline. From the correlative imaging approach followed in this work we can conclude that the Ir compound is preferably accumulated in mitochondria and not in other parts of the cell, as it was suggested by ICP-MS cell fractionation experiments. Quantitative information related to other elements like K, P, S, Ca, Mn and Zn in conjunction with the cell morphology analysis are in progress in order to relate the amount and compartmentalization of the different elements with the Ir compound treatment. This information might help elucidate the type of cell death triggered by this potent iridium metallodrug.