EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Tracking the cellular intake of a novel platinum supramolecular coordination complex	**Experiment number**: CH-5994
Beamline: ID16A	**Date of experiment**: from: 08/07/2021 to: 12/07/2021	**Date of report**: 23/11/2022
Shifts: 12	**Local contact(s)**: **Peter Cloetens**	*Received at ESRF:*

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Report:

1. Abstract: We have synthesized recently the Pt(II)-based metallacycle **Pt_2L_2**, where **L** is a fluorescent pyridyl ligand.[1] Based on the differences observed between the cellular emissive behaviour of **Pt_2L_2** and **L** we initially hypothesized that **Pt_2L_2** reaches the nucleus of cells, and accumulates in the nucleoli where it binds to unconventional DNA structures called G-quadruplex. However, we cannot rule out the possibility that **Pt_2L_2** breaks partially inside cells, releasing the Pt(II) centre and ligand **L**, which could follow different cellular fates. To obtain a complete picture of the cellular trafficking of **Pt_2L_2**, we intend to use nanofocused XRF at ID16A to map Pt in cells treated with a range of concentrations of our metal complex for different times. Comparing these maps with the microscopy images previously obtained will be key to understand the mechanism of action of **Pt_2L_2**.

2. Experimental Details: Pt_2L_2 was synthesized and characterized as previously described.[1] Mouse embryonic fibroblasts (3T3-cells) were seeded on poly-L-lysine pre-treated silicon nitride windows and incubated in DMEM growth medium overnight (37°C, 5% CO_2). Then, the cells were treated with **Pt_2L_2** (25 or 50 µM). Following 30- or 60-min incubations, the medium was removed. Then, the membranes were taken out, immersed in 150 mM ammonium-acetate buffer (pH 7.1), blotted with filter paper and manually plunge-freezed in liquid ethane.[2] The freeze-dried membranes were transferred to holders that were 3D-printed inhouse for storage and transport, and kept under cryogenic conditions in liquid nitrogen till measurement.

XRF maps and tomography at ID16A were acquired under cryogenic conditions using two six elements silicon drift diode detectors. The beam energy was set to 17 keV, focused at 50 x 50 nm^2, $1.55*10^{11}$ phs/s.

Initially, two different membranes containing cells treated with 50 µM **Pt_2L_2** for 60min were loaded, but ice probed to be too thick in both of them. Following this, two different membranes containing cells treated with 25 µM **Pt_2L_2** for 30 min were loaded into the experimental hutch. Those samples showed ice layers and cells more suitable for analysis. Cells for XRF tomography were chosen by collecting initial 2D maps from the different cells on the membranes. Coarse scans were acquired using 200 x 200 nm^2 steps, and their K, Zn and Pt distribution studied. Selected cells were studied using both holotomography (using a 25 x 25 nm^2 stepsize) and XRF tomography (using stepsize between 150 x 150 nm^2 and 100 x 100 nm^2 and between 31 and 36 projection angles).

3. Results: 3D data from two different 3T3 mouse fibroblast cells treated with with 25 µM **Pt_2L_2** for 30 min was obtained. XRF imaging at ID16A allowed us to visualize and quantify different elements of interest inside cryofixed mammalian cells (Fig. 1), from biologically relevant elements (*i.e.* K, Zn or Ca) to exogenous elements of interest (*i.e.* Pt). Elemental maps from K and Zn K-emission showed the overall shape of the cells and their nuclei respectively, while Pt L-emission showed the distribution of **Pt_2L_2** complexes internalised. These maps

showed that the Pt complex studied was highly localised into small areas within the cell, suggesting some type of vesicle dependent internalisation or organelle specific concentration after cellular uptake.

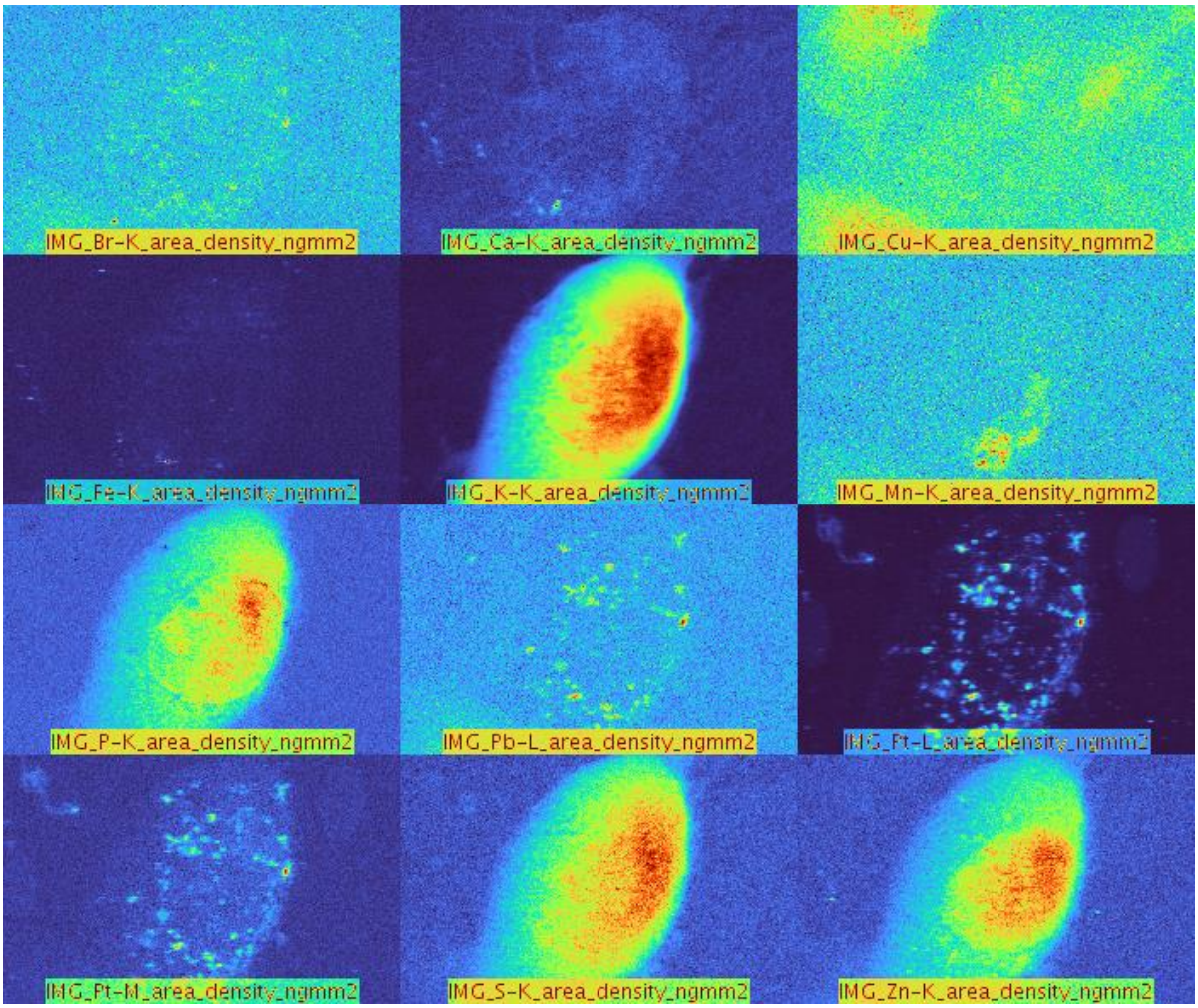

Fig. 1: XRF elemental maps of a single projection of one of the 3T3 cells treated with 25 μM **Pt_2L_2** for 30 min. Maps show distribution of different elements fitted, including Br, Ca, Cu, Fe, K, Mn, P, S and Zn K-emissions Pt and Pb L-emissions and Pt M-emissions.

Holotomography data was also collected, aiming to obtain correlative structural information that could bring light into the cellular localization of the complex. This showed the presence of limited damaged caused to cells during cryopreservation, but also confirmed nuclear localisation of the Zn and the presence of other organelles within the cytoplasm. Unfortunately, 3D reconstruction of the XRF data is still ongoing, as some problems have appeared during the alignment of the data due to a non-constant pixel size.

4. Conclusion and future work: XRF mapping at ID16A shows the intracellular localization of the Pt(II)-based metallacycle **Pt_2L_2** under cryogenic near-natural conditions. **Pt_2L_2** seems to accumulate in small vesicles or organelles within the cytoplasm of cells. The actual nuclear localization of the complex or the structural nature of the small area where it gets accumulated is yet unclear, as 3D reconstruction of the XRF tomography data is still ongoing and does not allow the correlation with holotomography data. Nevertheless, the type of cellular localisation observed is completely different to most previous imaging of G-quadruplex binding molecules which seem to get accumulated directly within the nucleolus of treated cells.[3] However, recent G-quadruplexes binders have shown to accumulate at the mitochondria of live cells, which could fit with the pattern observed for our **Pt_2L_2** complex.[4] It is unclear if such mitochondrial localisation would lead to interaction with G-quadruplexes found within the organelle, and we are performing additional optical microscopy experiments on live cells treated with **Pt_2L_2** to test this possibility.[5]

5. References

1) O. Domarco, et al, *Angew. Chemie - Int. Ed.*, **2019**, *58*, 8007.
2) C. Bissardon, *J. Vis. Exp.* **2019**, *154*, e60461.
3) P.A. Summers, et al, *Nat. Commun.* **2021**, *12*, 162.
4) P.A. Summers, et al, *Chem. Sci.*, **2021**, *12*, 14624
5) *Unpublished work.*