X-ray fluorescence nano-imaging traces anticancer compound in human cells

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X-ray fluorescence nano-imaging was used to map the distribution of an iridium-based anticancer compound in human cells. The compound was found in intracellular structures, consistent with identified protein targets. This study enhances understanding of the compound's mechanism of action and supports metallodrug discovery.

Much cancer research today is focused on finding metal-based alternatives to platinum anticancer drugs, aiming for molecules with novel mechanisms of action and fewer side effects. Iridium(III) complexes are promising candidates **[1]**, particularly 'half-sandwich' complexes that include a penta-substituted cyclopentadienyl ligand, a bidentate chelating ligand and a halogeno ligand. These can interact with reactive biomolecules.

A recent study introduced 10 iridium complexes and identified the most effective one for its cytotoxicity, anti-proliferative properties, and apoptosis induction **[2]**. This compound appears to work by generating hydrogen peroxide in cells and binding to yet-unknown cellular protein targets. To track this compound in cells, researchers attached a fluorescent BODIPY entity to the complex, revealing its presence in lipid droplets, mitochondrial membranes and endoplasmic reticulum, where a specific response to stress was elicited **[3]**. However, in this setting, the distribution was likely restricted by the BODIPY's lipophilic nature, necessitating an unbiased technique for high-resolution imaging of the compound's true intracellular distribution.

To achieve an unbiased analysis of iridium compound accumulation in human cells, researchers utilised cryo-fixation followed by high-resolution X-ray fluorescence (XRF) element mapping at beamline **ID16A**. This method preserves all intracellular elements, including highly diffusible ions like potassium, and highlights compartments such as the nucleus and Golgi apparatus.

Cultured cells were stained with fluorescent dyes to mark various structures including the actin network, mitochondria, and nucleus. The cells were then exposed to the iridium compound (5 µM) or a control for 15 minutes, sufficient time for the compound to accumulate in cells and interact with target biomolecules. The samples were vitrified to maintain element distribution and cell integrity,

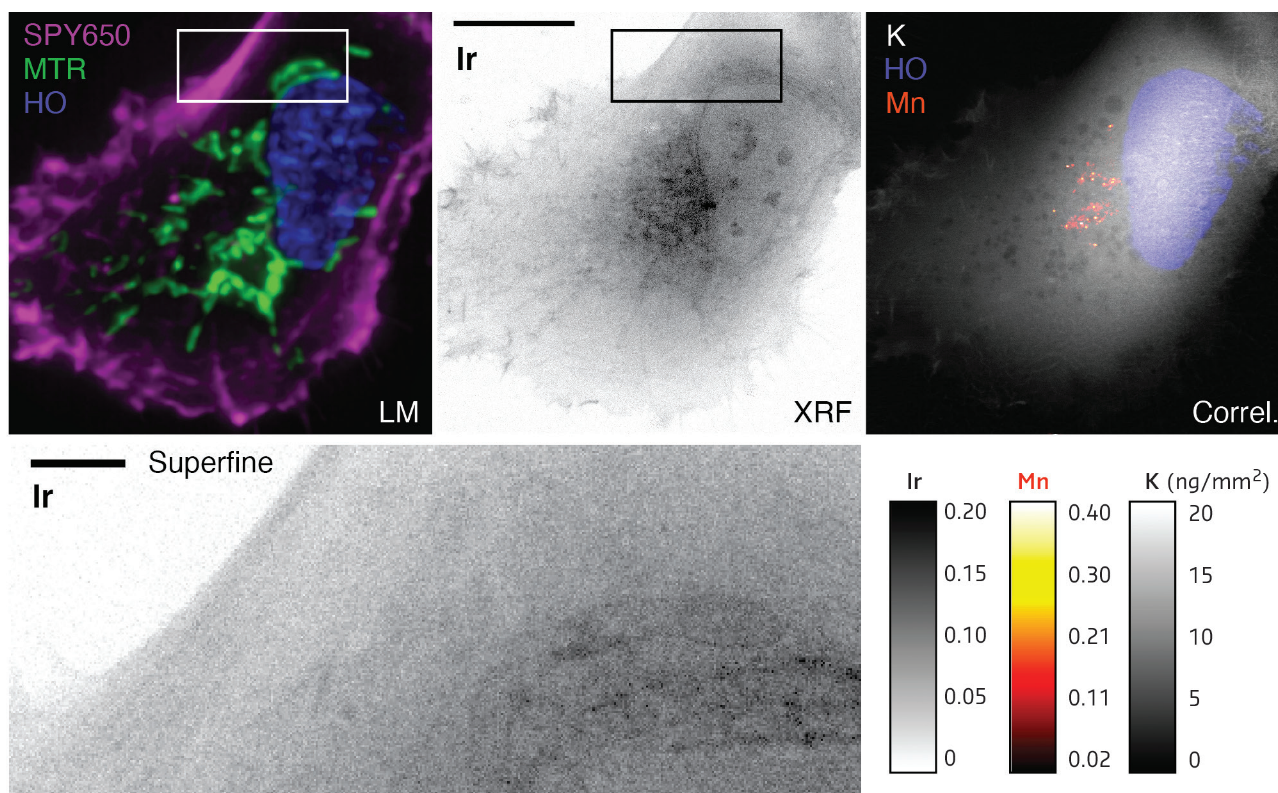

Fig. 1: Correlative analysis of a frozen-hydrated RPE1-hTert human cell treated with 5 µM Iridium-based compound for 15 min and imaged at 88 K. Light cryo-microscopy (LM) of the actin cytoskeleton (SPY650-FastAct, magenta), mitochondria (Mitotracker Green, MTR), and the nucleus (Hoechst 33342, blue, HO). Cryo-X-ray fluorescence imaging (XRF) of elemental Ir, Mn, and K expressed in ng/mm^2 as colour scales of grey, fire, and white respectively, acquired at 'fine' 50 nm/px/50 ms or 'ultra-fine' 30 nm/px/100 ms resolution. Scale bars: 10 and 1 µm.

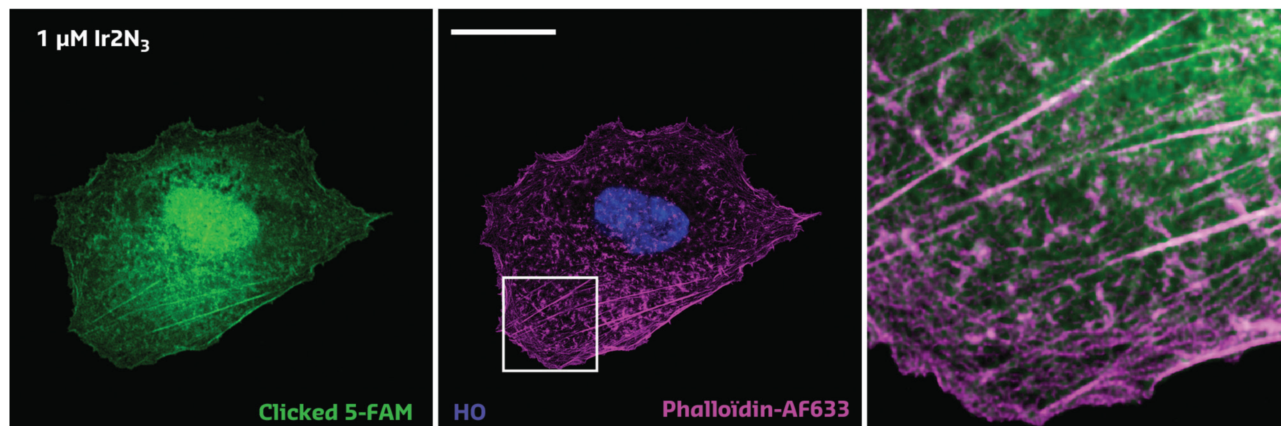

Fig. 2: Super-resolution confocal images of cells exposed to 1 µM bioorthogonal probe $Ir2N_3$ for 1 h, fixed and labelled *in situ* thanks to a click reaction with fluorescein-alkyne (5-FAM, green). Staining of the actin cytoskeleton (phalloidin-AF633, magenta) and DNA (Hoechst 33342, HO, blue). Right: zoom on a selected region of 200 µm^2 showing colocalization of probe and microfilaments (white) on the merged images. Scale bar: 20 µm.

before being analysed at **ID16A**. After nanopositioning, regions of interest were first rapidly scanned to check for cell integrity, indicated by uniform potassium distribution. 'Fine' (50 nm) and 'ultra-fine' (30 nm) XRF scans were then conducted for detailed imaging of selected cell areas. The XRF spectra were processed, resulting in elemental areal mass density maps.

The XRF mapping showed that iridium was present in both the cytoplasm and the nucleus, with notable enrichment in mitochondria, actin bundles, and the nuclear envelope. This distribution was compared to cryo-optical fluorescence microscopy, which used vital markers for visualization (**Figure 1**). The iridium content was estimated at approximately 70 fg per cell, consistent with another analysis performed in parallel that found 130 ± 2 fg per cell. This pattern matches the protein targets identified through chemical proteomics and proximity ligation assays, which pinpointed mitochondrial proteins and actin-binding proteins like filamin B as high-affinity targets. The binding of the compound to the actin meshwork was also confirmed by in-cell fluorescence labelling and super-resolution confocal imaging (**Figure 2**).

In conclusion, X-ray fluorescence nano-imaging of briefly exposed human cells enabled high-resolution mapping of iridium and identification of its target structures, aided by correlative fluorescence microscopy using organelle trackers. Combined with cellular protein target identification through chemical proteomics, this study uncovered the primary cellular processes affected by the iridium complex and provided a molecular basis for its cytotoxicity. Additionally, a general methodological workflow was established for metallodrug discovery.

PRINCIPAL PUBLICATION AND AUTHORS

Identification of Cellular Protein Targets of a Half-Sandwich Iridium(III) Complex Reveals Its Dual Mechanism of Action via Both Electrophilic and Oxidative Stresses, R. Ramos (a,b), A. Karaiskou (b), C. Botuha (a), S. Amhaz (b), M. Trichet (c), F. Dingli (d), J. Forté (a), F. Lam (c), A. Canette (c), C. Chaumeton (c), M. Salome (e), T. Chenuel (f), C. Bergonzi (f), P. Meyer (f), S. Bohic (g), D. Loew (d), M. Salmain (a), J. Sobczak-Thépot (b), *J. Med. Chem.* **67**, 6189-6206 (2024); https://doi.org/10.1021/acs.jmedchem.3c02000
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