ESRF	**Experiment title:** Characterisation of the sub-cellular elemental distribution of osmium and iridium-based antitumor drugs using X-ray fluorescence nanoprobe.	**Experiment number**: CH-4283
Beamline: ID16A	**Date of experiment**: from: 28th January 2015 to: 31st January 2015	**Date of report**: 17/02/2015
Shifts: 9	**Local contact(s)**: Dr. Sylvain Bohic and Dr. Yang Yang	*Received at ESRF:*

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We aimed to investigate the cellular distribution of a series of Os(II) and Ir(III) drug candidates, namely [Os(η^6-*p*-cym)(Azpy-NMe$_2$)I]PF$_6$ **[1]**, [Os(η^6-bip)(Azpy-F)I]PF$_6$ **[2]** [Ir(η^5-cp^{xph})(Azpy-NMe$_2$)Cl]PF$_6$ **[3]** and [Ir(η^5-C^{pxbiph})(phpy)(py)]PF$_6$ **[4]** (*p*-cym = *p*-cymene, Azpy-NMe$_2$ = 2-(*p*-[dimethylamino]phenylazo) pyridine, bip = biphenyl, Azpy-F = 2-(*p*-[dimethylamino]phenylazo)-5-Fluoropyridine, cp^{xph} = tetramethyl(phenyl)cyclopentadienyl, cp^{xbiph} = tetramethyl(biphenyl)cyclopentadienyl, phpy = 2-phenylpyridine, and py = pyridine), at ID16A using X-ray fluorescence nanoprobe (XRFN). These potent anticancer complexes show interesting *in vitro* and *in vivo* antiproliferative properties due to alteration of the cellular redox balance (possibly involving mitochondrial pathways),[1,2,3] and identifying their intracellular target sites, would be key to understanding their mechanism of action, and contribute to their advance towards phase I of clinical trials against platinum resistant ovarian cancer.

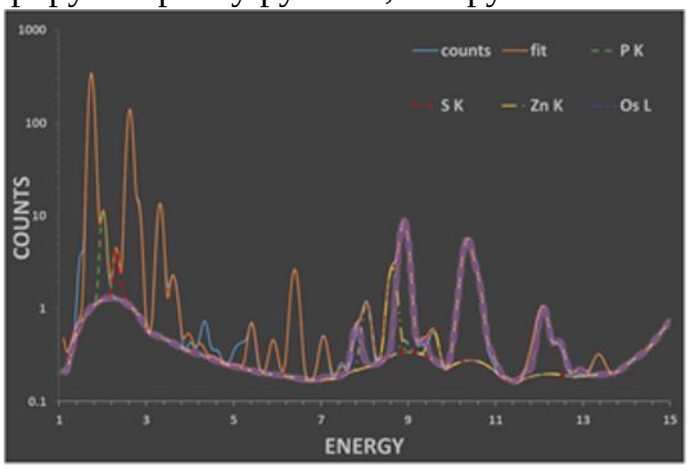

Fig.1 XRF spectrum of A2780 cells treated with **1**

A2870 ovarian carcinoma cells treated with different concentrations of **1-4** (IC_{50} to 10 µM) were prepared on 5x5 mm carbon frames with 500 nm silicon nitride windows (Silson Ltd, UK), both as epon embedded sections (500 nm thickness) and whole cells (fixed with Methanol or PFA). Untreated cells were prepared similarly as negative controls. The frames were placed under vacuum (in a sample holder with capacity for four frames) and irradiated individually using ID16A standard energy setting (17 KeV). XRF emission was collected using two six elements silicon drift diode detectors. Fast coarse scans were taken to identify interesting areas (beam size 400x400 nm, dwell time 100 ms), that were then studied using higher resolution (beam size 50x50 or 20x20 nm, dwell time 40ms or 50 ms). No phase

constrast images could be obtained due to lack of time, so a density of 2 µm or 500 nm was estimated (for whole cells and sections respectively) for the processing of the images (that were normalised according to the beam flux).

A clear and distintive set of peaks corresponding to Os-L emission was observed in samples treated with **1** (Fig. 1 and Fig. 2). Unfortunately, no Ir emission could be detected on cells treated with **3** or **4** and we did not have time to study samples treated with **2**. Initial scans also showed that PFA is better preserving the structure of A2780 cells than methanol (Fig. 3), and that our fixation proceedure did not managed to dehydrate completely cells, as some of them exploded when irradiated.

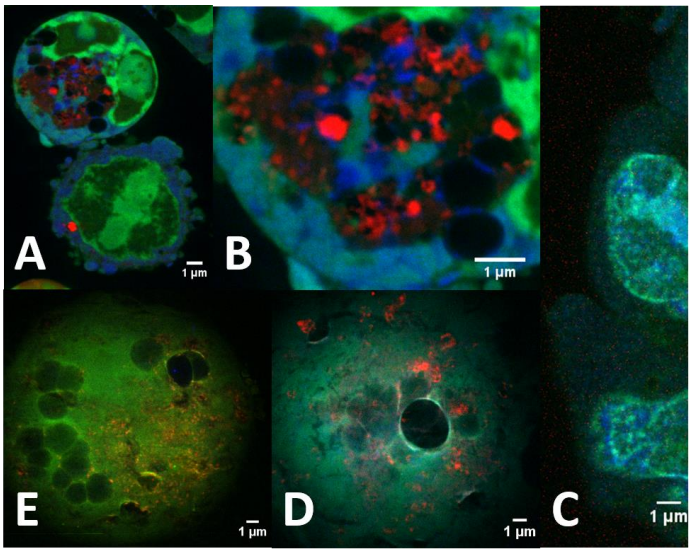

Fig.2 XRF maps of A2780 cells showing Os (red), Zn (green) and Ca (blue), treated with: A) 1 µM **1** (section, 50 nm res.); B) 1 µM **1** (section, 20 nm res.); C) untreated cells (section; 50 nm res); D) IC_{50}:0.14 µM **1** (PFA fixed, 50 nm res.); E) 1 µM **1** (PFA fixed, 50 nm res.).

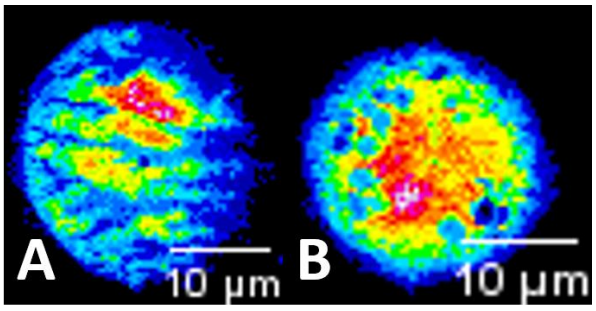

Fig.3 XRF maps of K in A2780 cells treated with 1 µM **1** and fixed with: A) Methanol; B) PFA.

Images of cells incubated with **1 (**1 µM or IC_{50}: 0.14 µM; Fig. 2 and Fig. 4) showed that Os did not overlap with Zn or P maps. This suggests that the drug does not localise in the nucleus. Cells treated with **1** also showed abnormal shaped nuclei (Fig. 2 and Fig. 4; compared with untreated cells, Fig. 2C), and possible membrane blebbing; which might indicate that they are going through an apoptotic process.

Remarkably, **1** was concentrated in small elliptical areas around 0.3-0.4 µm in length, similar in size and shape to the mitochondria of fast growing tumour cells (as A2780), but also to vesicles and other organelles. Unfortunately, mitochondrial localisation could not be assesed as planned in the proposal (using antibody-conjugated nanogold) due to lack of experimental time. Nevertheless, these results are the first to bring light on the intracellular distribution of this kind of compounds and a fast publication is envisioned.

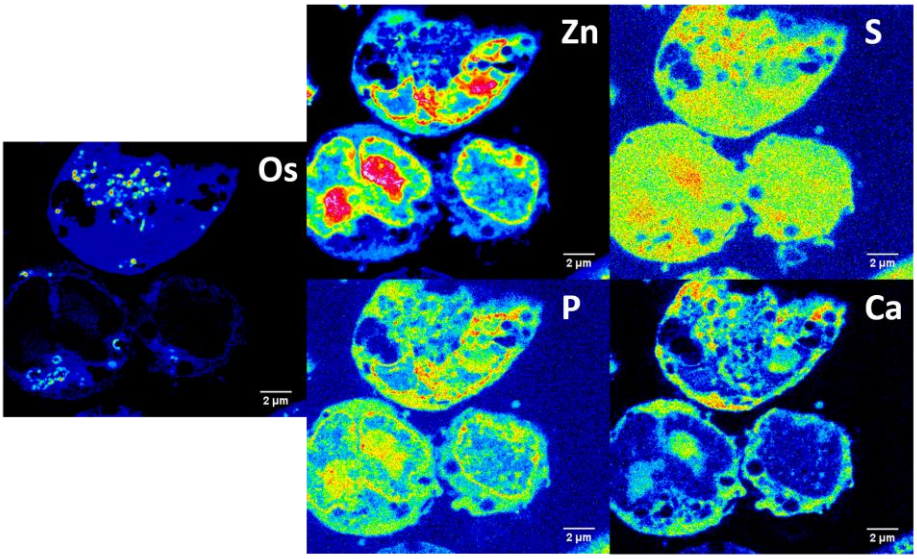

Fig.4 XRF maps of sections of A2780 cells treated with 1 µM **1** showing cellular distribution of Os, Zn, P, S and Ca.

1) Fu Y, et al. *J Med Chem*. **2010**, *53*, 8192; 2) Hearn JM, et al. *ACS Chem Biol*. **2013**, *8*, 1335; 3) Shnyder SD, et al. *Med Chem Comm*. **2011**, *2*, 66.