



HAL
open science

Synchrotron Radiation X-Ray Fluorescence Nanoimaging Reveal the Intracellular Localization of Potent Anticancer Drug Osmocenyl-Tamoxifen Derivative

Florin Fus, Yang Yang, Hui Zhi Shirley Lee, Siden Top, Marie Carrière,
Alexandre Bouron, Alexandra Pacureanu, Julio Cesar da Silva, Michele
Salmain, Anne Vessieres, et al.

► **To cite this version:**

Florin Fus, Yang Yang, Hui Zhi Shirley Lee, Siden Top, Marie Carrière, et al.. Synchrotron Radiation X-Ray Fluorescence Nanoimaging Reveal the Intracellular Localization of Potent Anticancer Drug Osmocenyl-Tamoxifen Derivative. *Microscopy and Microanalysis*, 2018, 24 (S2), pp.350-351. 10.1017/S1431927618014034 . hal-02495614

HAL Id: hal-02495614

<https://hal.sorbonne-universite.fr/hal-02495614>

Submitted on 10 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Synchrotron Radiation X-Ray Fluorescence Nanoimaging Reveal the Intracellular Localization of Potent Anticancer Drug Osmocenyl-Tamoxifen Derivative.

Florin Fus¹, Yang Yang¹, Hui Zhi Shirley Lee², Siden Top², Marie Carriere³, Alexandre Bouron⁴, Alexandra Pacureanu¹, Julio Cesar da Silva¹, Michele Salmain², Anne Vessièrè², Peter Cloetens¹, Gérard Jaouen², Sylvain Bohic^{1,5,*}

¹ European Synchrotron Radiation Facility, 71 avenue des Martyrs 38000 Grenoble, France.

² Sorbonne Université, UPMC Univ Paris 06, CNRS UMR 8232, IPCM, 75005 Paris, France.

³ Laboratoire Lésions des Acides Nucléiques, Université Grenoble Alpes, CEA, INAC, LCIB, Grenoble, France.

⁴ Laboratoire de Chimie et Biologie des Métaux, UMR CNRS 5249, Université Grenoble Alpes, CEA, BIG, Grenoble, France.

⁵ EA-7442, Rayonnement Synchrotron et Recherche Médicale, Université Grenoble Alpes, Grenoble, France.

* Corresponding author, bohic@esrf.fr

Breast cancer remains nowadays, the most prevalent form of cancer in women. Administration of tamoxifen allows stabilization of patients suffering from hormone-dependent breast cancer at early stage. [1] In contrast, hormone independent, triple negative breast cancers (TNBC) suffer from genetic heterogeneity and remain difficult to cure with very few treatment options left. [2] Some of us have introduced tamoxifen-like metallocifens (TLMs) including metals of group 8 (Fe, Ru, Os). These compounds result from the replacement of the β aromatic ring in 4-hydroxytamoxifen by a metallocenic entity. The main benefit of these complexes is to display antiproliferative activity on both hormone dependent and independent cancer cells. The osmocenyl Oc-OH-Tam analogues has an IC₅₀ = 2.7 μ M. [3] Cytotoxicity is related to the unique redox properties of metallocene motifs. [4] There are strong evidences suggesting that tamoxifen-like metallocifens are prodrugs. [4-5] They are rapidly converted in cells into electrophilic quinone methides which further react selectively with thiols and selenols via a 1,8-Michael addition. [6] As such, they were shown to inhibit thioredoxin reductase, a selenoenzyme involved in the cellular redox balance, both in vitro and in Jurkat cells. [4, 7] Quantification of iron and osmium by ICP-OES in organelles resulting from subcellular fractionation of Jurkat cells incubated with the complexes revealed nearly exclusive localization in crude nuclear fraction (45–54 %) and in mitochondria (37–50%). [4] However, many artifacts are associated with organelles fractionation. Thus a cutting-edge technique providing quantitative imaging of the intracellular distribution of metal-based drugs at biologically relevant concentration in a label-free fashion is highly desirable. SR-XRF nanoimaging is well suited to meet this challenge.

In this study, we report synchrotron radiation X-ray fluorescence (SR-XRF) nanoimaging of MDA-MB-231 cells exposed to 2 μ M Oc-OH-Tam for 1 or 24 h. Oc-OH-Tam was selected since it contains the exogenous element Os and displays physico-chemical and biological properties close to those of ferrocifen. We used the high resolution synchrotron X-ray nanoprobe ID16A-NI available at ESRF, Grenoble, France, for which a nanobeam down to 13 nm at 33 keV [8] and 30 nm at 17 keV can be reached. 2D-XRF nanoanalysis were performed on chemically fixed cells and 3D X-ray fluorescence of cells were obtained on fixed cells but also on frozen hydrated cells on the recently implemented cryo-capabilities of the ID16A-NI nanoprobe. SR-XRF two-dimensional images were first recorded on chemically fixed cancer cells. Cells were fixed as quickly as possible and immediately dried upon completion of aldehyde fixation as suggested by Jin et al. [9] SR-XRF maps were also recorded at 35 nm step size as shown in

figure 1 A and C. The intrinsic presence of osmium atoms in the structure of Oc-OH-TAM allows its direct mapping through the SR-XRF emission spectrum simultaneously with physiological elements present in cells as displayed by the sum spectrum (Fig. 1C&D). The various spectral overlap interferences between the Zn K-lines and the Os L-lines were successfully deconvolved using PyMca X-ray fluorescence toolkit. Although we could not resolve the nuclear membrane while working on whole cells within the spatial resolution of the image, the Os element was found within the confined region, a pattern likely consistent with a nuclear envelope localization. Nanoanalysis of Os revealed a large perinuclear localization which likely corresponds to endoplasmic reticulum (ER) localization. Indeed ER is a well-described organelle including interconnected membrane structures (tubules, vesicles and cisternae) continuous to the outer nuclear envelope and organized as an intricate perinuclear network. XRF nanotomography let us to conclude that Os had a similar distribution as that of an ER fluorescent probe applied before chemical fixation. 3D cryo-XRF analysis of frozen hydrated cells exposed to Oc-OH-Tam demonstrated a high level of preservation of the cell volume and Os distribution corroborated a similar perinuclear distribution of this element as well as nuclear contour and a quite intense concentration in vesicular structures. Overall, this body of experiments suggests that Oc-OH-Tam undergoes fast cellular uptake in MDA-MB-231 cells with preferential accumulation in the endomembrane system.

References:

- [1] K. D. Miller *et al*, *Ca-a Cancer Journal for Clinicians* **66** (2016) 271
- [2] G. Bianchini *et al*, *Nature Reviews Clinical Oncology* **13** (2016) 674
- [3] H. Z. S. Lee *et al*, *Eur. J. Inorg. Chem.* (2015) 4217
- [4] V. Scalcon *et al*, *Metallomics* **9** (2017) 949
- [5] G. Jaouen, A. Vessieres, S. Top, *Chem. Soc. Rev.* **44** (2015) 8802
- [6] Y. Wang *et al*, *Angew. Chem. Int. Ed.* **54** (2015) 10230
- [7] A. Citta *et al*, *J. Med. Chem.* **57** (2014) 8849
- [8] J. C. Da Silva *et al*, *Optica* **4** (2017) 492
- [9] Q. L. Jin *et al*, *Journal of Microscopy* **265** (2017) 81.

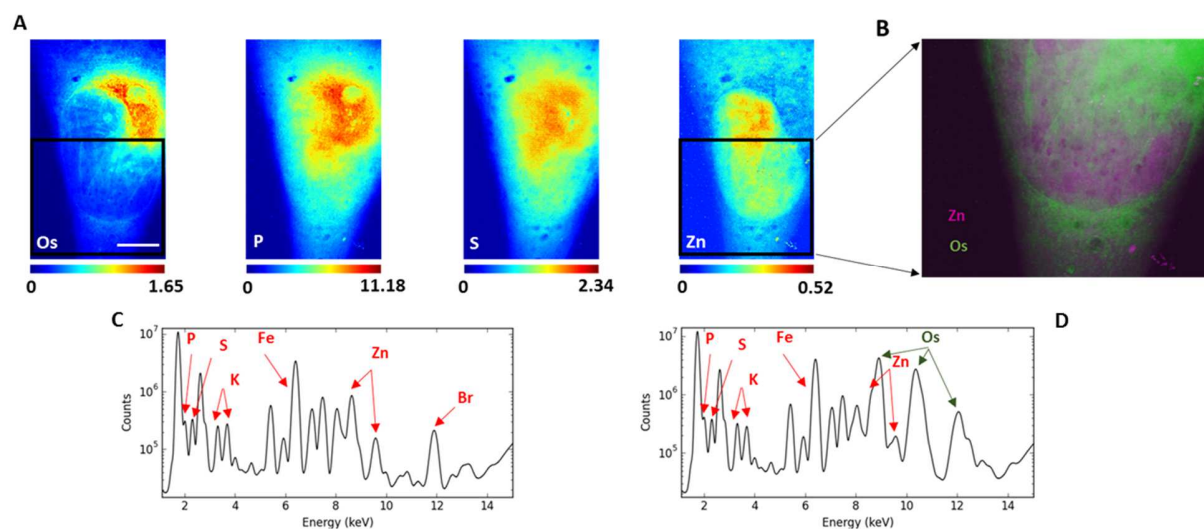


Figure 1. XRF maps of potassium (K), phosphorus (P), sulfur (S), zinc (Zn) and osmium (Os) in chemically fixed MDA-MB231 breast cancer cells. (A) Cell incubated with 2 μ M Oc-OH-Tam for 1 h and raster scan with 35x35 nm². The black square in (A) shows an enlarged view of the cytosolic – nuclear interface region with overlay of Os and Zn images in (B). (C) Sum spectra of control cell and cell exposed to Oc-OH-Tam (D). Scale bar 10 μ m.