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Rhenium carbonyl complexes bearing methylated triphenylphosphonium cations as antibody-free mitochondria trackers for X-ray fluorescence imaging†

Gabrielle Schanne,^a Lucas Henry,^a How Chee Ong,^b Andrea Somogyi,^c Kadda Medjoubi,^c Nicolas Delsuc,^a Clotilde Policar,^a Felipe García,^{a,b} and Helene C. Bertrand^{a,b}

Synchrotron Radiation X-ray Fluorescence (SXRF) imaging is a powerful technique for the visualization of metal complexes in biological systems. However, due to the lack of an endogenous elemental signature for mitochondria, probes for the localization of this organelle are required for colocalization studies. In this work, we designed and synthesized rhenium pyta tricarbonyl complexes conjugated to methylated triphenylphosphonium TP*P⁺ cations as multimodal probes for the visualization of mitochondria, suitable for fluorescence and SXRF imaging and quantification. Accumulation of the methylated triphenylphosphonium TP*P⁺-based conjugates in cells was observed in fixed A549 cells, and the amount of mitochondrial uptake was linked to the lipophilicity of the TPP⁺ vector. Our work highlights a convenient rhenium-based multimodal mitochondrial-targeted probe compatible with SXRF nano-imaging.

Introduction

The subcellular detection, localization, and quantification of small molecules in cellular medium and in particular of transition metal complexes are crucial in their development in biology and medicine and in the understanding of their biological mode of action. Several methods can be used to study the subcellular localization of a compound of interest: study of pharmacological effects, chemical analysis and the most widely employed microscopic imaging studies using spectroscopic probes specific for individual organelles.¹ Among the common approaches, fluorescence microscopy using fluorescent probes is pivotal as a non-invasive imaging technique for its convenience, good sensitivity and high spatial resolution, resulting in the routine use of organelle-specific fluorophores as conventional markers in fluorescence imaging.^{2–4} Despite their popularity and utility, fluorescent probes still

suffer from some drawbacks such as photobleaching and limited laser penetration depths. Moreover, fluorescence imaging is dependent on environmental factors such as medium polarity, pH, binding status, *etc.*, that can modify the quantum yield and/or emission spectrum, possibly rendering apparent subcellular distribution inaccurate.^{5,6} More importantly, the commonly used strategy of appending a large organic fluorophore on a non-fluorescent compound of interest to enable its detection may modify its molecular parameters and hence alter subcellular distribution.^{7,8}

The developments of imaging techniques and organelle-specific probes based on complementary modalities are hence of primary importance. In the context of metal imaging, X-ray fluorescence (XRF) nano-imaging is highly promising as it allows for the specific and direct imaging of the metal centre. Hard X-ray scanning XRF spectroscopy enables the simultaneous mapping of all elements having $Z > 14$ provided their edge energy is lower than the incident X-ray energy. Thus, information on both the chemical environment and the studied metal centre's distribution in biological environments and cells⁹ is readily available. Using highly focalised synchrotron beams, resolutions as good as a few 10 nm's can be obtained. Moreover, scanning Synchrotron Radiation X-ray Fluorescence (SXRF) imaging has a high analytical sensitivity (\leq ppm). SXRF spectroscopy thus exhibits a high detection sensitivity, a high specificity and a high spatial resolution. SXRF nano-imaging in studies of metallodrugs have received increas-

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ing attention in the last decade^{10–15} and provided valuable information on the fate of the metal complexes in the cellular context. XRF imaging is, hence, highly promising as a tool in the growing field of inorganic medicinal chemistry.

For co-localization studies by XRF, some endogenous elements can be indicative of a subcellular compartment – e.g. phosphorus and zinc reveal nucleus localization,¹⁶ while manganese has been shown to accumulate in the Golgi apparatus in neural cells¹⁷ – although this is not the case for all the organelles. 3D XRF nano-imaging was recently used to determine the subcellular localization of anticancer metal complexes.^{18,19} Sealed carbon nanotubes filled with heavy metals and decorated with organelle-specific peptides were described for the XRF mapping of cell membrane, nucleus and endoplasmic reticulum.²⁰ Mitochondria localization, up to now, has only been identified by XRF using correlative imaging with fluorescence²¹ or electron microscopy²² or using an immunolabeling technique involving a secondary antibody functionalized with gold nanoparticles.^{23,24} Hence, systems of easier access such as low molecular weight XRF imaging molecular probes with specific organelle targeting in the manner of conventional fluorophores, with an additional modality such as classical fluorescence for validation, would be highly valuable tools for colocalization studies and convenient access to the accurate localization of metal complexes by XRF.

One promising system for that purpose is multimodal rhenium(I) carbonyl-based probes, that can be visualized through infrared (IR), fluorescence and XRF imaging. These biologically-stable octahedral d^6 low-spin Re(I) tricarbonyl complexes, of general formula $[\text{Re}(\text{CO})_3(\text{N}^{\wedge}\text{N})\text{X}]^{n+}$ (with $\text{N}^{\wedge}\text{N}$ a diimine ligand with low-energy π^* orbitals), show exciting and valuable photophysical properties such as low toxicity, large Stokes shifts and long luminescent lifetimes that render them suitable for bio-imaging.^{25–27} These complexes have thus been developed to target different cell compartments as luminescent organelle trackers as described in recent reviews.^{28,29} $\text{Re}(\text{CO})_3$ complexes also display IR absorptions in the range 1800–2200 cm^{-1} , a spectral region in which biological media are transparent. This combination of properties enables the use of these complexes as Single Core Multimodal Probe for Imaging (SCoMPI) in cells and tissues,^{30–33} while the IR signature of the complexes enabled their quantification inside cells,⁵ as demonstrated in correlative fluorescence and infrared imaging studies. Most notably, these $\text{Re}(\text{CO})_3$ complexes can also be mapped by XRF nano-imaging, as recently demonstrated.^{12,34,35} Rhenium is highly suitable for this function as it is highly emissive in X-fluorescence (after irradiation at >12.53 keV), and its natural abundance is very low (ultra-trace element in urine and plasma ($<1 \mu\text{g g}^{-1}$)),³⁶ leading to a good signal-to-noise ratio.

Ideally, to exert their biological effect, compounds must reach their subcellular targets while reducing accumulation in non-targeted sites to reduce associated side effects. There is therefore a high interest in drug targeting strategies to direct a compound to its specific site of action.^{37,38,39} One of such targets that have recently drawn attention has been mitochondria organelle due to its increasing association with metabolic

diseases and neurodegenerative diseases *inter alia*.^{40–42} To confer mitochondrial selectivity,^{43,44} lipophilic cations such as mitochondrial-targeting peptides (MTPs)^{45–47} or triphenylphosphonium (TPP⁺)^{48,49} derivatives can be conjugated to the cargo of interest, thus exploiting the mitochondrion's strong negative potential (up to 180–200 mV), allowing accumulation within the mitochondrial matrix according to the Nernst equation.⁵⁰ In this context, the triphenylphosphonium (TPP⁺) moiety – which is the “gold standard” among mitochondrial delivery vectors – has been broadly used as a molecular vector for selective mitochondrial delivery owing to its high lipophilicity, cationic nature, high stability in physiological conditions and its ease of conjugation to molecular cargo.⁴⁹ The versatility of this platform is exemplified by the wide range of molecules that has been delivered into the mitochondria through TPP⁺ conjugation, such as spin-traps, antioxidants, prodrugs, protonophores, fluorophores, photodynamic therapy sensitizers as well as positron emitters.⁴⁹

Although metal-based probes – mainly Ir(III), Ir(I) and Ru(II)⁵¹ and a handful of $\text{Re}(\text{CO})_3$ complexes^{52–55} – have been described to target mitochondria, to the best of our knowledge, none were used as molecular probes in XRF imaging. As mentioned above, the techniques used so far require the most advanced 3D XRF processes or immunolabeling techniques that could be cumbersome. Finally, there is no XRF-detectable endogenous and ubiquitous element characteristic for the mitochondria, making a mitochondria targeting XRF probe of particular interest.

In light of the need for an organelle-specific multimodal probe compatible with XRF techniques, we have designed and synthesized three triarylphosphonium- $[\text{Re}(\text{CO})_3(\text{pyta})\text{X}]$ mitochondria-targeted probes suitable for X-fluorescence mapping and quantification. We explored methylated TPP derivatives (*i.e.*, TP*P⁺) that were shown in recent studies to display enhanced performance as compared to the conventionally used TPP⁺ moiety. For instance, TP*P⁺ species have shown a two-fold performance enhancement in *in vitro* photodynamic therapy (PDT) studies, and have also enabled the accumulation of dicationic species previously described as unable to permeate the mitochondrial membrane.^{56,57} The increase in lipophilicity by enhancement of the molecular volume and solvent accessible surface area (SASA) of the cations was proposed as key determinant to improve mitochondria accumulation.^{56,57}

Here, we report the synthesis of a series of rhenium pyta tricarbonyl complexes conjugated to triarylphosphonium cations. Toxicity and imaging studies were performed in A549 cells, revealing lipophilicity-dependent toxicity associated with increasing internalization of the compounds. Mitochondria localization was assessed by colocalization studies in fluorescence imaging using a conventional mitochondria stain Mitotracker Deep Red. Subcellular XRF mapping of the compounds was successfully achieved by SXRF imaging using the Re-L β edge of Re. We describe here the conjugate with the bis-methyl TP*P⁺ cation C3 as a low toxicity mitochondria-targeted metal-based multimodal probe that can be mapped and quantified inside cells using SXRF. Our study confirms the potential

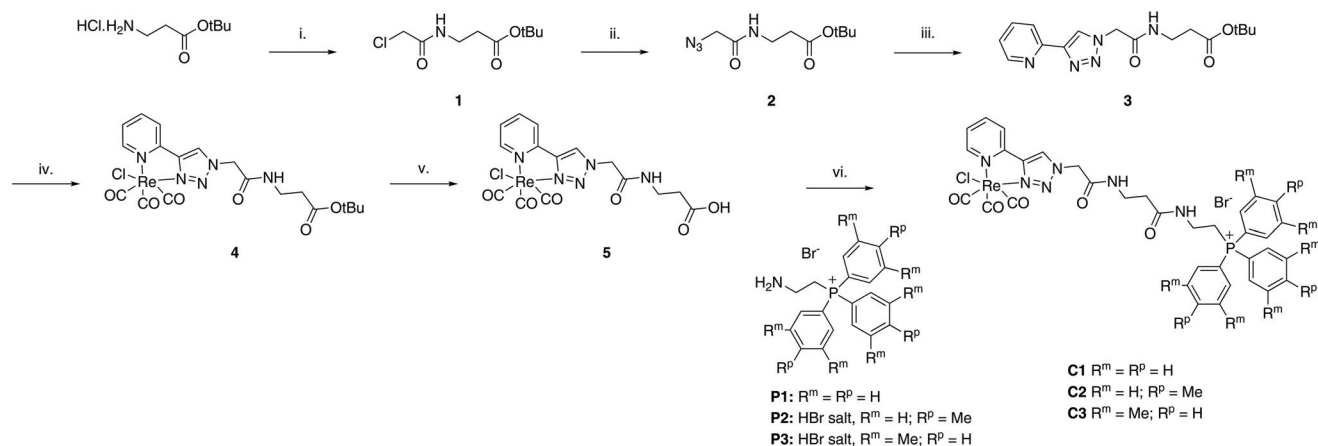
of methyl functionalized TPP⁺ as mitochondrial delivery vectors with enhanced properties.

Synthesis

We synthesized conjugates **C1–3** comprising three triphenylphosphonium cation derivatives with a rhenium pyta tricarbonyl complex^{58–60} based respectively on the classical TPP⁺ cation (R^m = R^p = H), the modified TP*P⁺ cation bearing a methyl group on phenyl *para* positions (R^m = H, R^p = Me) and

the modified TP*P⁺ cation bearing methyl groups on all phenyl *meta* positions (R^m = Me, R^p = H) (Scheme 1). A beta-alanine spacer was chosen to bridge the pyta ligand with the aminoethyl phosphonium cations. Increasing the alkyl chain length between the cargo and the TPP⁺ cation vector is known to increase the cell penetration of the construct.^{61–63} For practical reasons in the synthesis and consistent data interpretation, we chose to keep the spacer constant and only the nature of the phosphonium vector was changed.

Therefore, β-alanine *tert*-butyl ester hydrochloride was first reacted with chloroacetyl chloride in the presence of diiso-



Scheme 1 Synthesis of conjugates **C1–3**. Conditions: i. chloroacetyl chloride (1.0 eq.), DIEA (2.5 eq.), DCM, 1 h, 25 °C (quantitative); ii. NaN₃ (1.97 eq.), NaI (0.1 eq.), acetone/H₂O 3 : 1 v : v, 17 h, 50 °C (89%); iii. 2-ethynylpyridine (1.2 eq.), CuSO₄·5H₂O (0.26 eq.), sodium ascorbate (1.0 eq.), acetone/H₂O 2 : 1 v : v, 2 h, 25 °C (85%); iv. Re(CO)₅Cl (1.1 eq.), toluene, 5 h, 80 °C (99%); v. TFA/DCM 1 : 1 v : v, 1 h, 25 °C then conc. HCl, 10 min, 25 °C (86%); vi. (2-aminoethyl)triphenylphosphonium bromide derivative **P1–3** (1.1 eq.), HOBt (1.5 eq.), EDC·HCl (1.5 eq.), DMF, 24–48 h (20–70%).

Table 1 Photophysical properties of conjugates **C1–3** in acetonitrile and IC₅₀ from MTT assay

Complex	Excitation (nm)	Emission (nm)	Range (nm)	Quantum yield ^a (%)	IC ₅₀ (μM)	Hydrophobicity as per rt in RP-HPLC (min)
C1	330	530	450–620	0.27	413 ± 65	6.40
C2	330	530	450–620	0.27	100 ± 26	7.93
C3	330	530	450–620	0.25	46 ± 10	8.52

^a Quinine sulfate in 0.1 N sulfuric acid was used as a standard with a known quantum yield of 54.6% (λ_{exc} 320 nm).

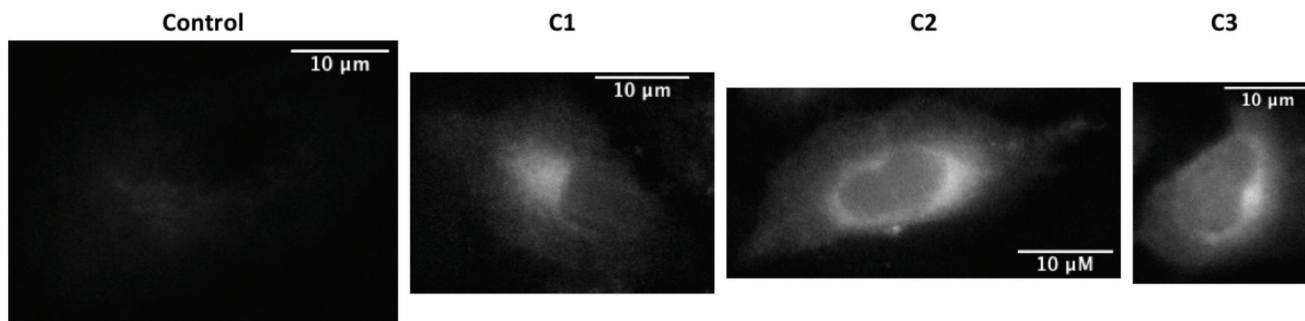


Fig. 1 Fluorescence imaging of A549 control cells (control) and cells incubated with conjugates **C1–3** (at 20 μM for 4 h) (λ_{exc} 350 nm). The signal-to-noise ratio were equal to 23.5 dB, 32.0 dB, 34.5 dB and 36.0 dB for control cells, cells incubated with **C1**, **C2** and **C3**, respectively. Fixed fluorescence intensity scale (0–7500 a.u.). Scale bar: 10 μm (fixed at 3.1 cm).

propylethylamine (DIEA) in dichloromethane (Scheme 1, step i). Chloride nucleophilic substitution with sodium azide followed by a copper catalyzed azide alkyne cycloaddition (CuAAC) using copper sulfate pentahydrate and sodium ascorbate with ethynyl pyridine generated the pyridine-triazole

(pyta) ligand functionalized with a *tert*-butyl ester terminated linker 3 (steps ii-iii).³⁴ The corresponding rhenium carbonyl complex 4 was obtained by reaction with rhenium pentacarbonyl chloride in toluene under heating (step iv). Hydrolysis of the ester function was performed at this stage by treatment in

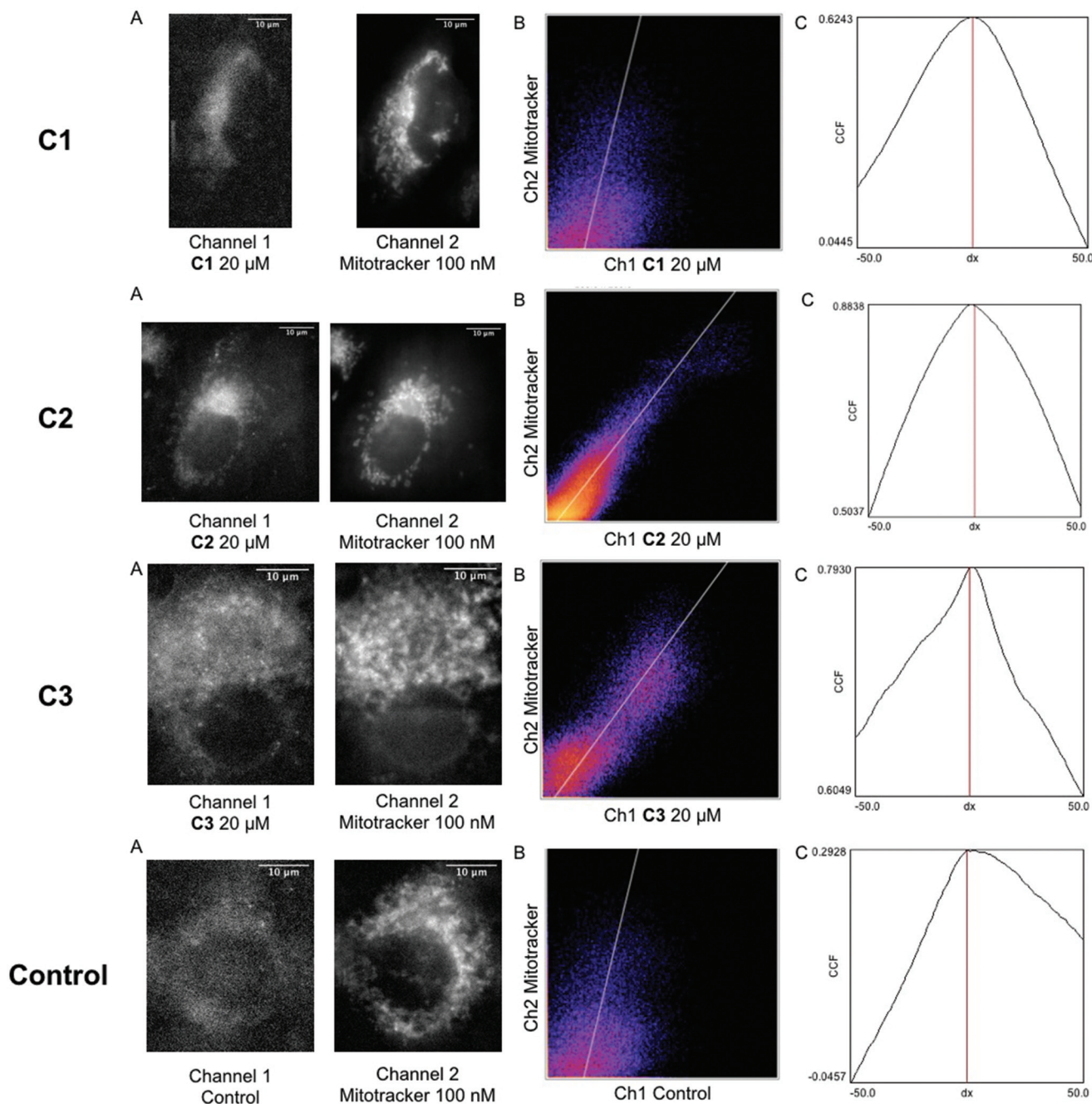


Fig. 2 Colocalization analyses with the fluorescence signal of Mitotracker Deep Red. A549 cells were incubated with C1–C3 probes at 20 μ M for 4 hours or without incubation (control). (A) Left: channel 1 – fluorescence image with excitation at λ_{exc} 350 nm, obtained with a gain of 0 over an exposure time of 3 s. The signal-to-noise ratio is equal to 17 dB for C1, 22 dB for C2, 20 dB for C3 and 12 dB for control cells; right: channel 2 – fluorescence image of the mitotracker Deep Red (λ_{exc} 644 nm) obtained with a gain of 0 over an exposure time of 5 s. (B) Scatter plot or 2D-histogram with a linear regression representing the signal intensity relationship of the two fluorescence images. (C) Van Steensel curve (see ESI† for details). The cross correlation function is maximal for a shift dx equal to 0, 2, 2 and 1 pixels for C1, C2, C3 and control cells, respectively. The Pearson coefficient is equal to 0.62 for C1, 0.88 for C2, 0.79 for C3 and 0.30 for control cells.

a mixture of TFA in DCM, followed by treatment with concentrated HCl to afford a complex **5** with a chloride as unique X ligand (step v). The conjugates **C1–3** were finally obtained *via* an amide coupling with aminoethylphosphonium bromide derivatives **P1–3** prepared as previously described⁵⁶ using HOBt and EDC·HCl as coupling agents in the presence of DIEA in DMF (step vi).

Photophysical properties

The photophysical properties of the conjugates **C1–3** were characterized in acetonitrile and are summarized in Table 1 and Fig. S1.† The three conjugates show typical absorptions of rhenium carbonyl complexes with a MLCT band centered at 330 nm in acetonitrile (Fig. S1†).^{5,58,60} Excitation at 330 nm led to a broad emission band centered at 530 nm. **C1–3** showed

similar low quantum yields around 0.25–0.27%, consistent with rhenium carbonyl complexes with substitutions of comparable features.^{58,64}

Toxicity

The toxicity of the conjugates was evaluated in A549 non-small cell lung cancer cell line using a classical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were incubated with a range of concentrations (1–1000 μM) for 4 h. The conjugates showed differential toxicity with IC_{50} comprised between $46 \pm 10 \mu\text{M}$ for **C3** and $413 \pm 65 \mu\text{M}$ for **C1** (Table 1 and Fig. S2, S3†). The higher toxicity with increasing methyl functionalization of the phenyl rings of the phosphonium targeting moieties (**C1** to **C3**, with **C3** 10 times more cytotoxic than **C1**) is consistent with an increased hydrophobicity, as sup-

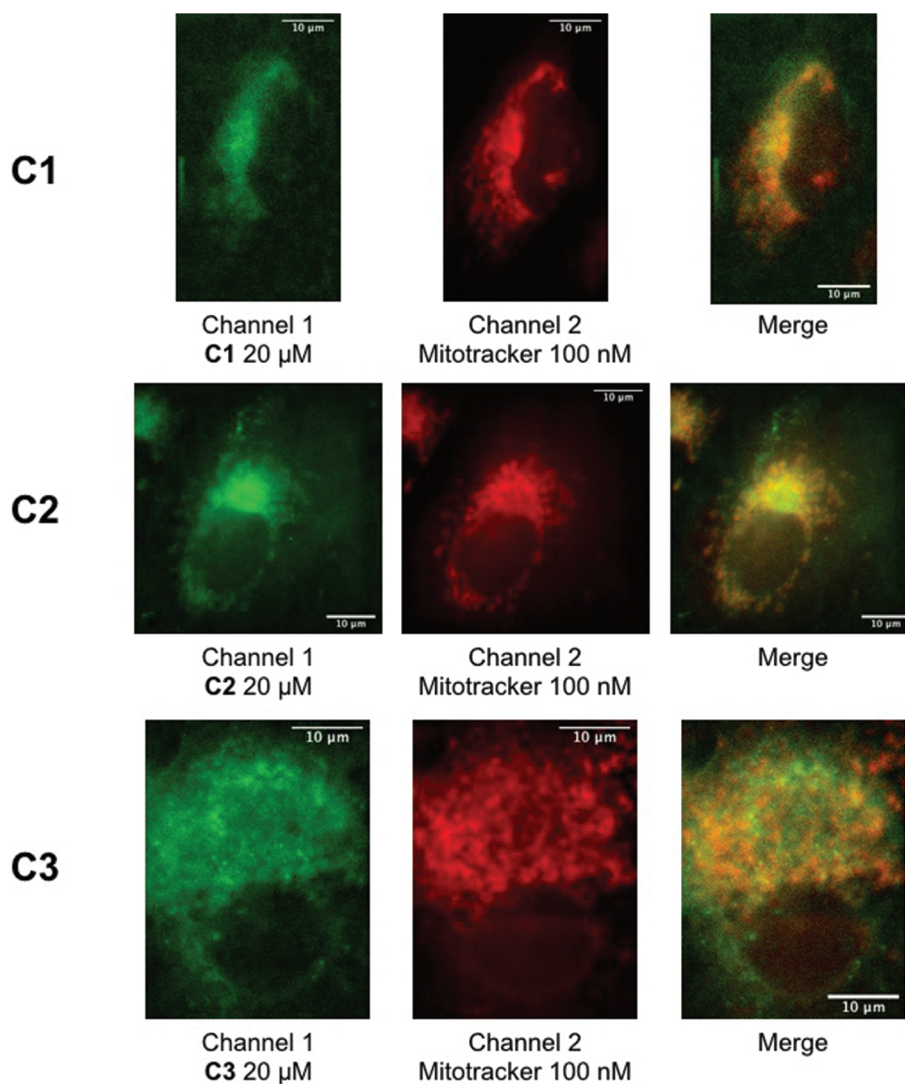


Fig. 3 A549 cells were incubated with **C1–C3** probes (20 μM , 4 h). Left: fluorescence signal of the conjugate (λ_{exc} 350 nm); middle: fluorescence signal of Mitotracker Deep Red (λ_{exc} 644 nm); right: merge of conjugate (green) and Mitotracker Deep Red (red) with their overlay in yellow.

ported by the increased retention time in reverse phase analytical HPLC (6.40, 7.93, 8.52 min for C1, C2, and C3, respectively). This may be, in turn, consistent with a greater cell penetration. The viability at 10 μM and 20 μM , concentrations used for the subsequent imaging experiments, were >80% for the three conjugates, and the slight toxicity observed at these concentrations did not preclude the imaging studies. Consequently, imaging studies using fluorescence and X-ray fluorescence spectroscopy were then performed to investigate the conjugates' sub-cellular localisation and quantify their accumulation in A549 cells.

Fluorescence imaging

A549 cells were incubated with 10 μM or 20 μM of the conjugates C1–C3 for 4 h, fixed with 4% PFA and imaged by single photon excitation at 350 nm (Fig. 1 and Fig. S4–S7†). For all the conjugates, a significant fluorescence signal was detected and localized around the nucleus. Mitochondrial labeling was then investigated using co-incubation with a conventional mitochondrial marker, MitoTracker Deep Red, characterized by an excitation maximum at 644 nm and an emission in the 650–750 nm range. The use of MitoTracker green (excitation 490 nm, emission 516 nm) as alternative mitochondria stain was not compatible with the $\text{Re}(\text{CO})_3$ conjugates. During the imaging studies, a

loss in intensity of the luminescence signal detected for the three conjugates C1–C3 was observed when coincubated with MitoTracker Deep Red. Further studies in solution (see ESI, Fig. S8†) showed that, although there is no overlap in the excitation spectra of the conjugates and the Mitotracker, there is a partial overlay between the emission of the conjugates (450–650 nm, centered at 530 nm in water) and the excitation of the organic fluorophore. An enhancement of Mitotracker fluorescence signal, along with a loss in luminescence signal of C3 (λ_{exc} 320 nm) was consistently observed with an increasing concentration of Mitotracker (ESI Fig. S9–S11†). This suggests the existence of a fluorescence resonance energy transfer (FRET) between the two compounds, implying in turn, that the conjugates and the Mitotracker are in close proximity in cells, since FRET is distance-dependent. Despite this intensity loss, the luminescence of the conjugates could still be detected without parasite signal (the signal emitted by the Mitotracker upon excitation at the λ_{exc} of the conjugates (Fig. S10†) is filtered out with a filter cut-off above 600 nm). Moreover, the FRET phenomenon is expected to occur to the same extent for complexes of similar photophysical properties, which allows us to qualitatively compare the cellular luminescence images in all three cases. Co-localization results were analyzed using the Van Steensel curve and Pearson value methods between the labeling of C1–C3 and Mitotracker Deep Red in cells.

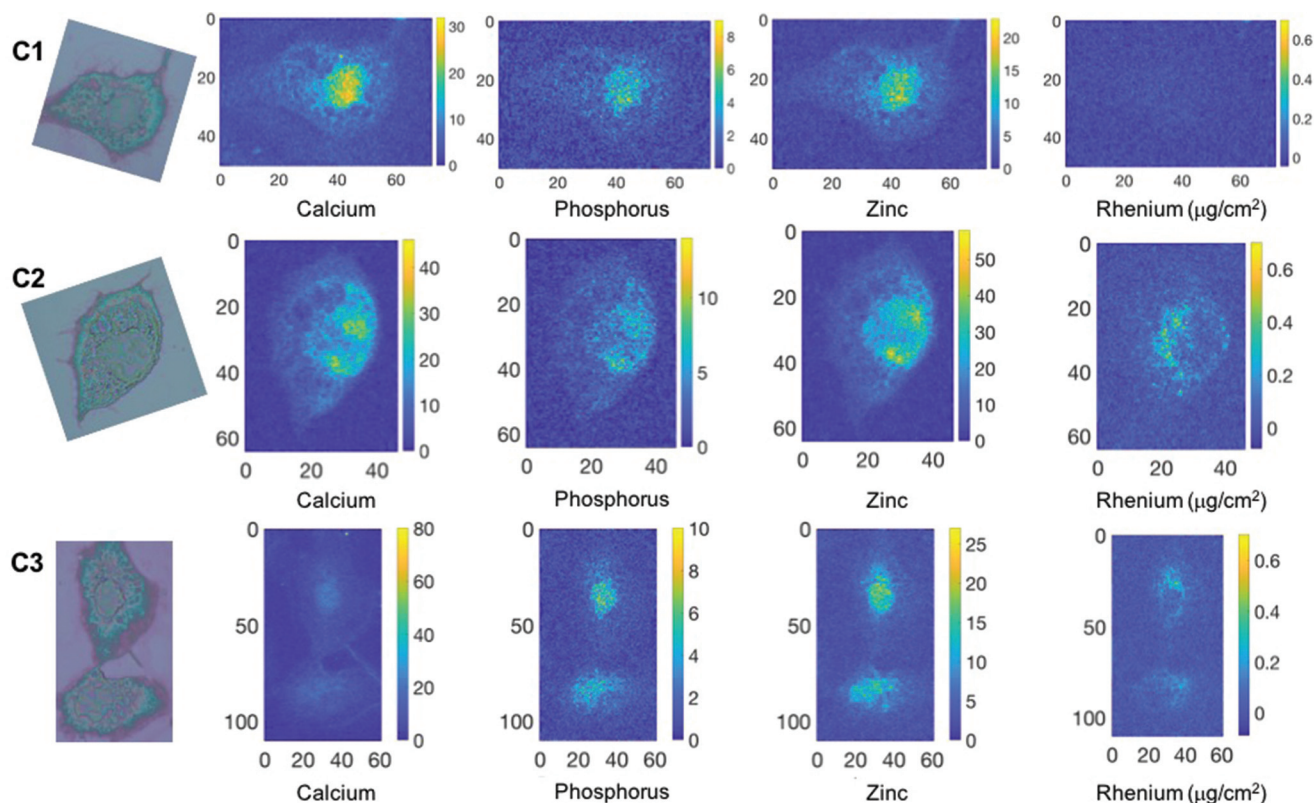


Fig. 4 Transmission optical microscope images (left) and elemental distributions of Ca, P, Re, and Zn in A549 cells incubated with C1–C3 (with color coded map (intensity) (right)). The phosphorus (P), and zinc (Zn) maps, are used to identify the nucleus area. Re was mapped using the L β lines. A549 cells were incubated for 4 hours with C1–C3 (20 μM) before fixation and air-drying (excitation at 14 keV; integration time, 300 ms per pixel; pixel size, 500 nm). Scale axis in μm .

In cells incubated with the conjugate C1 with a classical TPP vector, the luminescence signal observed appeared less clearly than that of C2 and C3. This may be explained by a poor internalization in these conditions. In contrast, a clear luminescence signal could be detected in the case of the conjugate C2 compared to control cells. Gaussian maxima shifted from the zero position of dx (red line) were obtained in the Van Steensel method and a mean Pearson coefficient of 0.83 ± 0.04 was calculated, both suggesting a partial overlay of C2 labeling with the mitochondrial marker. The conjugate C3 gave a luminescence signal qualitatively stronger than C2, with a comparable sub-cellular distribution as shown by the similar results in colocalization methods (mean Pearson coefficient 0.68 ± 0.06 , Fig. 2 and Fig. S12–S16†).

The fluorescence studies are consistent with toxicity studies suggesting a cell penetration increasing in the order $C1 < C2 < C3$. They show a partial overlay of the labeling of the conjugates with that of the MitoTracker Deep Red, pointing to a partial preferential localization at the mitochondria, particularly for C2 and C3 (Fig. 3).

Synchrotron radiation scanning X-ray fluorescence nano-imaging (SXRF)

SXRF was used to study the intracellular distribution of rhenium in A549 cells incubated with the probes (20 μM for 4 hours). The cells were seeded on Si_3N_4 silicon nitride membranes, fixed with 4% paraformaldehyde and air-dried (see ESI†). Chemical fixation and air drying could be used here, the focus of the study not being endogenous metals that can diffuse upon this treatment.^{34,65} Fig. 4 shows the elemental distributions of calcium, phosphorus, zinc and rhenium in a single incubated A549 cell (see ESI Fig. S16–S26† for the mapping of other incubated cells and control cells). In order to avoid spectral overlapping between the Zn-K α and Re-L α (~ 8.6 keV) XRF lines, we use the Zn-K β (~ 9.6 keV) and Re L β (~ 10.15 and 10.28 keV) spectral lines to produce the Re and the Zn distribution maps³⁴ (see ESI†). The localization of the nucleus is indicated by the phosphorus and zinc mapping. As rhenium is an ultratrace element in biological samples,³⁶ the rhenium signal can therefore be attributed to the conjugates C1–C3.

Rhenium could not be significantly detected in cells incubated with C1 (Fig. 4 and S16–S18† in comparison to control cells (Fig. S24–S26†)). By contrast, the probes C2 and C3 can be unambiguously detected in incubated cells. They show a perinuclear and punctuate distribution that qualitatively matches that observed by fluorescence imaging.

Finally, the amount of rhenium in cells incubated with the three conjugates C1–C3 was quantified by XRF using a rhenium standard (see ESI†). The average concentrations expressed in μg per cell are shown in Fig. 5. A differential accumulation of the probes in cells in the order $C1 < C2 < C3$ was confirmed, supporting a reduced internalization of C1. The amount of C1 was not significant compared to control

Accumulation of Re probes in cells

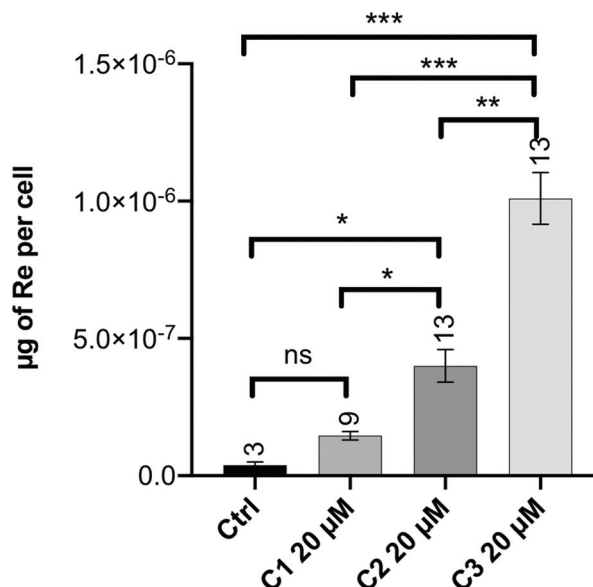


Fig. 5 Quantification of rhenium accumulation in cells by X-ray fluorescence. A549 cells were incubated in presence of the rhenium probes C1–C3 at 20 μM for 4 h. Data represent mean \pm SEM. The number of measurements is indicated above each column. The p -values were calculated from the Kruskal–Wallis test (non-parametric ANOVA test) using Prism software. Each comparison stands alone. (***) $p < 0.001$, (**) $p < 0.002$ and (*) $p < 0.033$ and ns means non-significant.

cells. The average amount of rhenium in cells incubated with C2 and C3 was 4.00×10^{-7} μg per cell and 1.01×10^{-6} μg per cell, respectively, indicating a more than 2-fold increase in cellular accumulation for the C3 conjugate.

Conclusions

We designed and synthesized rhenium pyta tricarbonyl complexes with triphenylphosphonium cation derivatives as mitochondria targeting probes. The classical TPP⁺ was evaluated along with two poly-methylated derivatives TP*P⁺ that were proposed as valuable alternative for mitochondria accumulation.^{56,57} The appendage of the non-polar methyl groups enhances the cation's lipophilicity while increasing its solvent accessible surface area and molecular volume, suggested as a key parameter to predict targeting ability. The conjugates displayed typical photophysical properties of rhenium carbonyl complexes with a MLCT absorption band centered at 330 nm and an emission at 530 nm with low quantum yields below 1% in acetonitrile. An increasing toxicity on A549 cells was observed with the lipophilicity of the conjugates, the C3 complex with a bis-methyl TP*P⁺ being the more toxic with an IC_{50} of 46 μM , still suitable for bio-imaging. Fluorescence imaging studies in fixed A549 cells and colocalization studies showed a partial localization at the mitochondria for TP*P⁺ conjugates C2 and C3 while no significant signal was observed for TPP⁺ complex C1 when co-incubated

with a Mitotracker. The conjugates were finally mapped in fixed dried cells and quantified using XRF spectroscopy. Compared to C1, TP*P⁺ conjugates C2 and C3 were unambiguously detected in incubated single cells with a perinuclear accumulation of punctuate appearance consistent with a partial mitochondrial localization. A higher penetration and accumulation in cells was confirmed with the use of TP*P⁺ cations, with twice as much bis-methyl cation C3 compared to mono methyl cation C2. Our study expands the use of this alternative family of mitochondria targeting agents and further supports its potential for enhanced mitochondrial therapies. Besides, we identify two Re tricarbonyl complexes as multimodal imaging probes targeting mitochondria as observed by fluorescence and X-ray fluorescence spectroscopies. These probes are a very convenient alternative to immunolabeling involving gold-modified secondary antibodies and compatible with chemical fixation and air drying easily applied for SXRF. Further studies are underway to further demonstrate mitochondrial localization in live cells and in cryofixed samples. Finally, this paves the way towards the development and applications of organelle specific XRF molecular probes that would undoubtedly bring invaluable insights in medicinal inorganic chemistry approaches.

Conflicts of interest

There are no conflicts to declare.

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