Introduction

Over recent decades considerable attention has been paid to the use of complexes of Ru\(^{11}\), Ir\(^{11}\) and Re\(^{11}\) d\(^{5}\) transition metal ions as luminescent probes for biological imaging.\(^1\)–\(^7\) The long-lived triplet-metal-to-ligand charge transfer (3MLCT) excited states responsible for the emission from these complexes can also sensitise singlet oxygen production which provides a basis for applications in photodynamic therapy (PDT) and the development of theranostic probes.\(^6\)–\(^11\) A noticeable drawback of these systems is their relatively high photoexcitation energy, which can result in unintended cellular damage during imaging and also limits the depth of tissue penetration. Consequently, cell-permeant luminescent imaging agents which display both low-energy electronic absorption features and deep-red phosphorescence, thus permitting excitation and luminescence imaging within the biologically-transparent region of the visible spectrum, are attractive synthetic targets.\(^12\)–\(^14\)

Osmium(II) complexes offer several advantages over their ruthenium(II) analogues. The high spin–orbit coupling constant associated with Os\(^{11}\) facilitates formally forbidden direct transitions from the ground state into 3MLCT excited states, resulting in associated absorbance bands of appreciable intensity in their optical spectra, often extending out to 700 nm.\(^15\) Excitation into these low energy absorptions results in deep-red to near-infrared (NIR) phosphorescence (\(\lambda_{\text{em}} \approx 650–900 \text{ nm}\)) and given that they are also highly kinetically inert, Os(II) complexes offer significant potential as cellular imaging agents.\(^16\) However, despite these favourable attributes, the use of Os(II) complexes as phosphorescent biological probes is still relatively scarce.

The Keyes group has reported the cellular uptake and imaging of a polyarginine-conjugated osmium(II) imidazophenanthrolinone complex\(^17\) as well as live-cell imaging of a mitochondria-targeting Os\(^{11}\) bis-(4-carboxyphenyl-terpyridine)-derived complex.\(^18\) The Zhang group has investigated a benzimidazole-containing Os\(^{11}\) complex as a NIR emissive lysosomal tracker,\(^19\) as well as a related complex that enables NIR luminescence imaging of the RNA within the nucleus of live cells.\(^20\) Very recently Zhu et al. have also exploited the deep-red luminescence of [Os(phen)\(_2\)(dppz)]\(^{2+}\) (phen = 1,10-phenanthroline, dppz = dipyridophenazine) for correlated luminescence and transmission electron microscopy (TEM) imaging of nuclear DNA.\(^21\) In this context, the Scattergood and Elliott groups have previously investigated triazole-containing Os\(^{11}\) complexes that show lysosomal/endosomal and mitochondrial localisation as optical probes,\(^22\) whilst the Thomas group has looked at DNA targeting polyppyridyl Os\(^{11}\) complexes as high-resolution contrast probes for TEM.\(^23\)

### Triazole-based osmium(II) complexes displaying red/near-IR luminescence: antimicrobial activity and super-resolution imaging†

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Cellular uptake, luminescence imaging and antimicrobial activity against clinically relevant methicillin-resistant S. aureus (MRSA) bacteria are reported. The osmium(II) complexes [Os(N\(^\text{N}^\text{N}\)N\(^\text{N}\))\(^{2+}\)] (N\(^\text{N}^\text{N}\)N = 1-benzyl-4-(pyrid-2-yl)-1,2,3-triazole (1\(^2\)+); 1-benzyl-4-(pyrimidin-2-yl)-1,2,3-triazole (2\(^2\)+); 1-benzyl-4-(pyrazin-2-yl)-1,2,3-triazole (3\(^2\)+)) were prepared and isolated as the chloride salts of their meridional and facial isomers. The complexes display prominent spin-forbidden ground state to triplet metal-to-ligand charge transfer (3MLCT) state absorption bands enabling excitation as low as 600 nm for fac/mer-\(^{2+}\) and observation of emission in aqueous solution in the deep-red/near-IR regions of the spectrum. Cellular uptake studies within MRSA cells show antimicrobial activity for 1\(^2\)+ and 2\(^2\)+ with greater toxicity for the meridional isomers in each case and mer-\(^{2+}\) showing the greatest potency (32 \(\mu\)g mL\(^{-1}\) in defined minimal media). Super-resolution imaging experiments demonstrate binding of mer- and fac-\(^{2+}\) to bacterial DNA with high Pearson’s colocalisation coefficients (up to 0.95 using DAPI). Phototoxicity studies showed the complexes exhibited a higher antimicrobial activity upon irradiation with light.

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Yet, whilst there has been interest in OsII complexes as potential anti-cancer therapeutics,\textsuperscript{19,25–32} examples of their use in bacteria are far rarer. Despite Dwyer and co-workers reporting the antimicrobial activity of RuII poly(pyridyl) complexes almost 70 years ago,\textsuperscript{33,34} interest in the antibiotic activity of d6 metal-ion complexes has only returned to some prominence over the last decade.\textsuperscript{35–41} However, the use of luminescent complexes for bacterial cell imaging remains a rarity. Following on from its earlier report that a luminescent di-nuclear RuII complex was taken up by bacteria,\textsuperscript{42} the Thomas group recently reported on the antimicrobial activity of di-nuclear analogues against a range of Gram positive and Gram negative bacteria. In these reports, several microscopy techniques were used to investigate cellular internalisation and explore the nature of the interaction of the metal complexes with the bacteria.\textsuperscript{41–44} Massi and co-workers have reported cationic IrIII-tetrazolato complexes which show good antimicrobial activity against Gram-positive \textit{D. radiodurans},\textsuperscript{45} whilst charge-neutral members of the same family have also been utilised as molecular probes for the live imaging of \textit{B. cereus}.\textsuperscript{46} Whilst there have been some scarce reports of OsII complexes showing inhibition of both Gram-positive and Gram-negative bacteria,\textsuperscript{47} to the best of our knowledge, there has yet to be any report that combines luminescence imaging with antimicrobial studies on OsIII systems despite their favourable photophysical properties.

Herein we report on the anti-microbial activity of the individual isomers of a family of water-soluble homoleptic OsII complexes featuring 1,2,3-triazole-containing ligands – Scheme 1. Strikingly, it was found that the meridional isomers of these architectures display much greater growth inhibition of Gram-positive bacteria over their facial geometrical isomers. Using the deep-red phosphorescence of these complexes we explore their cellular internalisation and identify their intracellular interaction with DNA.

### Results and discussion

#### Synthesis and characterisation

The previously reported complex [Os(pytz)\textsubscript{3}](PF\textsubscript{6})\textsubscript{2}\textsuperscript{2+} was prepared through an established procedure,\textsuperscript{23} which was also used to prepare the related homoleptic complexes [Os(pymtz)\textsubscript{3}](PF\textsubscript{6})\textsubscript{2}\textsuperscript{2+} and [Os(pytz)\textsubscript{3}](PF\textsubscript{6})\textsubscript{2}\textsuperscript{2+}. Briefly, [OsCl\textsubscript{6}][NH\textsubscript{4}]\textsubscript{2} was heated under reflux in ethylene glycol with 3 equivalents of the appropriate ligand, which after counterion metathesis with NH\textsubscript{4}PF\textsubscript{6} yielded the hexafluorophosphate salts of the complexes as deep-red coloured solids (Scheme 1).

Due to the asymmetry of the triazole-containing ligands, analysis by \textsuperscript{1}H NMR spectroscopy (Fig. S1–S8†) reveals the isolated products to be composed of a mixture of meridional and facial isomers, with formation of the former being favoured in all cases with mer:fac ratios of 1.4:1 (1\textsuperscript{2+}), 2:1 (2\textsuperscript{2+}) and 3:1 (3\textsuperscript{2+}). Whilst separation of these isomers by column chromatography is challenging, we found that efficient separation is possible on a small scale by employing preparative thin layer chromatography. Metathesis of counterions yielded chloride salts of the separated fac- and mer-isomers of 1\textsuperscript{2+}–3\textsuperscript{2+} which display excellent aqueous solubility.

The hexafluorophosphate salts of the complexes were analysed by cyclic voltammetry (Fig. 1 and Table 1). The cyclic voltammogram traces for each pair of mer- and fac-isomers are similar, showing that geometric isomerism has little impact on the electronic properties of the complexes. All complexes exhibit one electrochemically reversible oxidation process attributed to the OsII/III redox couple. Consistent with the increased electron-withdrawing character of each heterocyclic ligand, the potential of this process anodically shifts upon exchange of the pyridyl moiety for pyrimidine and pyrazine. A similar trend was reported recently for a series of bis-terdentate 1,2,3-triazole-based OsII complexes featuring pyridyl and pyrazinyl donors.\textsuperscript{48}

Scheme 1 Synthesis of 1\textsuperscript{2+}–3\textsuperscript{2+}.

Fig. 1 Cyclic voltammograms recorded at room temperature at 100 mV s\textsuperscript{-1} for 1.0 mmol dm\textsuperscript{-3} MeCN solutions of mer- and fac-1\textsuperscript{2+}–3\textsuperscript{2+} as their hexafluorophosphate salts. Solutions contained 0.2 mol dm\textsuperscript{-3} NBu\textsubscript{4}PF\textsubscript{6} as supporting electrolyte. All potentials are shown against the Fc+/Fc couple (E\textsubscript{1/2} = 0 V).
All the complexes display at least one reduction process within the available electrochemical solvent window. For mer- and fac-1\(^{2+}\), a reduction which is not completely chemically reversible is observed at −2.10 V vs. Fe\(^+/\)Fc. By comparison with previous studies on complexes of Os\(^{II}\) and Ru\(^{II}\) containing this ligand this process is attributed to a ligand-based reduction.\(^{23}\) Substitution of the pytz ligand with pymtz for both mer- and fac-2\(^{2+}\) results in the appearance of an irreversible reduction process at more positive potential. This shift is of similar magnitude to the anodic shift in the Os\(^{II/III}\) couple relative to 1\(^{2+}\), resulting in minimal change in the HOMO–LUMO energy gap. Introduction of a pyrazinyl-donor into the ligand set leads to more complex reductive electrochemistry, with the appearance of two distinct reversible processes at −1.54 and −1.77 V followed by an incompletely chemically reversible couple in the region of −2.1 V vs. Fe\(^+/\)Fc. These processes are assigned to the successive one-electron reductions of the three coordinated pytz ligands. Here the anodic shift for the ligand-based reduction is larger than for the Os\(^{II/III}\) oxidation resulting in a narrowing of the HOMO–LUMO gap.

Density functional theory (DFT) calculations on the fac- and mer-isomers of 1\(^{2+}\) to 3\(^{2+}\), carried out using the Orca 4.2 software package,\(^{49,50}\) confirm the electrochemical data (see Fig. S9, S10 and ESI for details); in all cases the HOMO has predominantly Os d-orbital character whilst the LUMO is spread over the three ligands (mostly localised over the 6-membered heterocycles) and is of π* character. In agreement with the anodic shift in the oxidation and reduction potentials, the HOMO and LUMO of fac/mer-2\(^{2+}\) are both stabilised compared to their pyridine-containing analogues resulting in near-identical HOMO–LUMO gaps (fac-1\(^{2+}/\)2\(^{2+}\); 3.51 eV; mer-1\(^{2+}/\)2\(^{2+}\); 3.48 eV). For fac/mer-3\(^{2+}\) the HOMO and LUMO are again stabilised with respect to the pyrimidine-containing complexes but with the latter stabilised to a greater extent, such that the HOMO–LUMO gap is reduced (fac-3\(^{2+}\): 3.36 eV; mer-3\(^{2+}\): 3.28 eV).

UV-Visible absorption spectra were recorded for the mer and fac chloride salts of 1\(^{2+}/3^{2+}\) in aqueous solution (Fig. 2, Table S1†). Sharp and intense bands observed for all the complexes between 220–300 nm are assigned to singlet ligand-centred (1LC) transitions, whilst bands beyond 350 nm are of MLCT character.

### Table 1

| Complex | \(E_{\text{ox}}/\text{V}\) | \(E_{\text{red}}/\text{V}\) |
|---------|-----------------|-----------------|
| mer-1\(^{2+}\) | +0.46 (73) | −2.10 (87)\(^{b}\) |
| fac-1\(^{2+}\) | +0.46 (76) | −2.10 (100)\(^{b}\) |
| mer-2\(^{2+}\) | +0.67 (80) | −1.84 |
| fac-2\(^{2+}\) | +0.66 (70) | −1.85 |
| mer-3\(^{2+}\) | +0.82 (78) | −1.54 (69), −1.77 (73), −2.17 (98)\(^{b}\) |
| fac-3\(^{2+}\) | +0.83 (68) | −1.54 (63), −1.77 (73), −2.10 (118)\(^{b}\) |

\(^{a}\) Measured at room temperature at a scan rate of 100 mV s\(^{-1}\). Potentials are shown versus the Fe\(^+/\)Fc couple. Anodic-cathodic peak separations (\(\Delta E_{\text{pc}}\)) for reversible processes are shown in millivolts within brackets. \(\Delta E_{\text{pc}}\) for Fe\(^+/\)Fc was typically 70 mV. \(^{b}\) Incompletely-reversible. \(^{c}\) Irreversible process, cathodic peak potential is quoted.

Complexes mer/fac-1\(^{2+}\) and mer/fac-2\(^{2+}\) display a series of bands between 350–420 nm which are attributed to spin-allowed 1MLCT excitations. Weaker bands, still of appreciable intensity, are observed between 430 and 580 nm and are assigned to the expected, formally spin-forbidden, direct excitations to the 3MLCT state.\(^{55,53}\) In agreement with the electrochemical data, indicating a similar HOMO–LUMO gap for mer/fac-1\(^{2+}\) and mer/fac-2\(^{2+}\) (vide supra), these bands are near coincident with each other. The analogous transitions for mer- and fac-3\(^{2+}\) are, however, red-shifted in line with the electrochemical and DFT data.

Time-dependent DFT calculations were carried out for the ground states of all complexes to determine vertical excitation energies and simulate the optical absorption spectra (Fig. S11 and Table S2†). The calculated data are in good agreement with the experimentally measured spectra. Excitations to the \(S_1\) states for 1\(^{2+}\) and 2\(^{2+}\) are near coincident appearing between 468 (fac) and 474 (mer) nm whilst those for the isomers of 3\(^{2+}\) are red-shifted to 494 (fac) and 511 nm (mer). In all cases these correspond to transitions predominantly consisting of HOMO → LUMO excitations but have relatively small oscillator strengths and will therefore have little physical significance. The calculated data show that the more intense transitions between 350 and 450 nm correspond to excitations from...
The cellular uptake properties of the compounds by a range of positive bacteria were then investigated. In these studies, we chose to investigate a methicillin resistant, clinical isolate strain of the Gram-positive bacterium Staphylococcus aureus, MRSA. We compared this to three Gram-negative bacteria strains: a uropathogenic multidrug resistant EC958 ST131 strain of Escherichia coli, a multidrug resistant clinical isolate strain of Pseudomonas aeruginosa – PA2017 and a multidrug resistant clinical isolate strain of Acinetobacter baumannii – AB184. AB184 was selected as it is the most prevalent clonal group of A. baumannii in the United Kingdom. In addition, AB184 and PA2017 belong to bacteria groups identified by the World Health Organisation as PRIORITY 1: CRITICAL for developing new antibiotics.24

The minimum inhibitory concentration, MIC, of the complexes was obtained in both nutrient rich media Mueller-Hinton-II (MH-II) and defined media – Glucose Defined Minimal Media (GDMM) – in the case of Gram-negative strains and Chemical Defined Media (CDM) for Gram-positive strains. This is the minimum concentration of compound required to halt bacterial cell proliferation. While GDMM has been used in previous studies on metal complexes,55-56 MH-II is closer to relevant biological conditions and is the bacterial growth medium recommended by the European Committee on Antimicrobial Susceptibility Testing. Data is summarised in Table 3.

As with the majority of metal-based antimicrobial compounds39,45,57 a higher activity against Gram-positive bacteria strains was observed, with all compounds showing no detectable activity on the Gram-negative strains. Strikingly, although complexes 12+ and 22+ exhibit activity against the methicillin resistant strain of S. aureus, in both cases their mer isomer is most active, with mer-12+ being comparable to standard antibiotics. More significantly still, this activity is not reduced in a pathogenic strain resistant to methicillin and ampicillin.

In recent studies, the antimicrobial activity of the direct RuII analogue of 12+ has been investigated. The Crowley group reported that both the mer and fac isomers of its hexafluorophosphate salt showed very low activity against wild-type S. aureus.39 This observation is probably due to the low water.

| Table 2 | Photophysical data for chloride salts of mer- and fac-12+ – 32+ in aqueous solution |
|--------|------------------|------------------|------------------|
| $\lambda_{em}^{2+}$/nm | $\phi_{em}^{2+}$/% | $\tau_{em}^{2+}$/ns | $\phi_{em}^{2+}$/% | $\tau_{em}^{2+}$/ns |
| mer-12+ | 621 | 3.9 (365) | 7.9 (748) |
| fac-12+ | 623 | 3.8 (350) | 8.4 (715) |
| mer-22+ | 637 | 2.3 (191) | 2.9 (242) |
| fac-22+ | 640 | 2.5 (171) | 3.2 (217) |
| mer-32+ | 696 | 0.6 (83) | 0.6 (92) |
| fac-32+ | 698 | 0.7 (98) | 0.8 (110) |

a $\lambda_{em}$ = 500 nm. b Aerated aqueous solution. c N2-equilibrated aqueous solution. d Relative to [Ru(bpy)3]2PF62, $\phi$ = 1.8% in aerated MeCN.

| Table 3 | MIC (µg mL$^{-1}$) results for E. coli pathogenic (EC958), S. aureus pathogenic (MRSA), A. baumannii pathogenic (AB184) and P. aeruginosa pathogenic (PA2017) strains in minimal media and MH-II |
|--------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| $\text{mer-1}^{2+}$ | $\text{fac-1}^{2+}$ | $\text{mer-2}^{2+}$ | $\text{fac-2}^{2+}$ | $\text{mer-3}^{2+}$ | $\text{fac-3}^{2+}$ | Control |
| Defined minimal media | EC958 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 |
| PA2017 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 |
| MRSA | 32 | 256 | 64 | 128 | 512 | 512 | 512 | >512 | >512 | >512 |
| AB184 | >512 | >512 | 256 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 |

Mueller-Hinton-II

| Defined minimal media | EC958 | PA2017 | MRSA | AB184 |
|---------------------|-------|--------|-------|-------|
| $\text{mer-1}^{2+}$ | >512 | >512 | >512 | >512 |
| $\text{fac-1}^{2+}$ | >512 | >512 | >512 | >512 |
| $\text{mer-2}^{2+}$ | >512 | >512 | >512 | >512 |
| $\text{fac-2}^{2+}$ | >512 | >512 | >512 | >512 |
| $\text{mer-3}^{2+}$ | >512 | >512 | >512 | >512 |
| $\text{fac-3}^{2+}$ | >512 | >512 | >512 | >512 |

a Ampicillin used as control.
solubility of such salts as a more recent study on the unseparated isomers of this complex, as a chloride salt, showed good activity against the non-pathogenic Gram-positive bacteria Bacillus subtilis and Staphylococcus epidermidis. The Crowley group have also varied the identity of the triazole substituent within these RuII systems, reporting that those bearing n-hexyl and n-octyl groups are the most potent against wild-type S. aureus. The chloride salts of these two complexes were then investigated against a wider panel of bacteria, which revealed that this activity was retained in pathogenic MRSA strains. However, as both the hexafluorophosphate and chloride salts display similar MIC figures against wild-type S. aureus, their activity is attributed to an optimisation of lipophilicity, rather than solubility. Promising antimicrobial activity has also been observed in related RuII analogues incorporating isoquinoline appended triazole ligands, and in quinoline-based RuII systems featuring tetrazole moieties. Interestingly, in the latter case, the fac-isomers are more active than their mer-analogues. Although structurally similar to the OsIII systems reported herein, the subtle increase in hydrophobicity achieved within these RuII systems, reporting that those bearing alkylated positive and Gram-negative bacteria, certainly a similar effect is observed in RuII complexes incorporating the alkylated ligands described above.

To determine whether the complexes cause bacterial cell death or just halt bacterial proliferation, estimates of minimum bactericidal concentration, MBC, were obtained and these data are summarised in Table 4. As with the MIC experiments, the mer isomers are the most active with mer-1\(^{2+}\) exhibiting the highest potency. The MBC values of all compounds lie within a 4-fold window of the MIC values, revealing that the compounds are classically bactericidal.

![Fig. 3 Uptake of complex mer-1\(^{2+}\) by S. aureus MRSA cells monitored through SIM microscopy. S. aureus (MRSA strain) cells were incubated with MIC concentrations of mer-1\(^{2+}\) for 60 (A) and 120 (B) minutes. Left: cross-section through the centre of the cell where peak intensity was observed. Right: 3D surface plots of selected cells in the area of the white box shown in cross-section image, revealing membrane and nuclear staining at 60 min and solely nuclear staining at 120 min. Emission was collected in the A458 channel upon excitation with the 405 nm laser. Cells were fixed using paraformaldehyde (4%) and washed in PBS. Cells were mounted using SlowFade Gold Antifade.](image_url)

Table 4 MBC (µg mL\(^{-1}\)) results for E. coli pathogenic (EC958), S. aureus pathogenic (MRSA), A. baumannii pathogenic (AB184) and P. aeruginosa pathogenic (PA2017) strains in minimal media and MH-II.

| Defined minimal media | mer-1\(^{2+}\) | fac-1\(^{2+}\) | mer-2\(^{2+}\) | fac-2\(^{2+}\) | mer-3\(^{2+}\) | fac-3\(^{2+}\) | Control* |
|-----------------------|-------------|-------------|-------------|-------------|-------------|-------------|----------|
| EC958                 | >512        | >512        | >512        | >512        | >512        | >512        | >512     |
| PA2017                | >512        | >512        | >512        | >512        | >512        | >512        | >512     |
| MRSA                  | 48          | 256         | 96          | 256         | 512         | 512         | >512     |
| AB184                 | >512        | >512        | >512        | >512        | >512        | >512        | >512     |
| Mueller–Hinton-II     | EC958       | >512        | >512        | >512        | >512        | >512        | >512     |
| PA2017                | >512        | >512        | >512        | >512        | >512        | >512        | >512     |
| MRSA                  | 128         | 256         | 256         | 256         | 512         | 512         | >512     |
| AB184                 | >512        | >512        | >512        | >512        | >512        | >512        | >512     |

* Ampicillin used as control.
bacteria, although there is significantly less membrane localisation. Again, after two hours, it largely accumulates within the bacterial DNA. Intriguingly, measured distances between the membrane, DNA and cell septum for compounds stained with the \textit{fac}-isomer were smaller on average than those for cells exposed to \textit{mer}-1\textsuperscript{2+}, suggesting that uniquely \textit{mer}-1\textsuperscript{2+} causes cell swelling, perhaps as a consequence of the cell death mechanism it provokes.

To confirm DNA localisation, co-staining experiments were carried out with the DNA stain DAPI (4',6-diamidino-2-phenylindole, Fig. 4). These images show a direct overlay between the emission of DAPI and both complexes within the bacterial DNA, which is clearly seen in the corresponding 3D surface projections and also reflected in their high Pearson’s coefficients ($r_{\text{DNA}} = 0.95; r_{\text{mem}} = 0.92$). As with the previous imaging experiments, it is clear that cells stained with the \textit{mer}-isomer are noticeably swollen.

Cell-free binding titrations on both isomers of 1\textsuperscript{2+} using their DNA-induced increase in steady-state emission (see Fig. S16\dagger), reveal they both bind to duplex DNA with relatively low affinities of $K_{d}(\text{fac}-1\textsuperscript{2+}) = 4.1 \times 10^5$ M\textsuperscript{-1} and $K_{d}(\text{mer}-1\textsuperscript{2+}) = 9.8 \times 10^4$ M\textsuperscript{-1}. However, as the DNA binding affinity of fac-1\textsuperscript{2+} is actually slightly higher than the more therapeutically active mer-1\textsuperscript{2+} it seems that this is, at best, only one factor in the cell death mechanism. It has been suggested that the mechanism of action of the previously discussed Ru\textsuperscript{II} analogues of 1\textsuperscript{2+} is due to membrane disruption and no DNA localisation was observed. However, these systems incorporate more lipophilic ligands and it is known that such physicochemical changes enhance binding to membranes over nucleic acids.$^{65,66}$

To investigate any difference in internalisation, the uptake properties of both isomers were investigated using ICP-AES (inductively coupled plasma atomic emission spectroscopy, Fig. 5). Uptake experiments were conducted in the absence of glucose over the course of an hour, as the imaging studies showed both compounds accumulate within bacteria over this period.

The ICP-AES experiments reveal that overall the \textit{mer}-isomer is preferentially taken up by \textit{S. aureus}. In addition, the uptake profiles of the isomers are different; initially, \textit{fac}-1\textsuperscript{2+} concentrations increase quite rapidly but uptake plateaus between 10 and 20 minutes. In contrast, the uptake of \textit{mer}-1\textsuperscript{2+} is rapid for 5 minutes and although the uptake rate then decreases, the concentration of \textit{mer}-1\textsuperscript{2+} continues to rise for the entire 60 minutes of the experiment. The differential uptake of \textit{mer}-1\textsuperscript{2+} and \textit{fac}-1\textsuperscript{2+} is consistent with their observed activities and the brighter emission intensity of mer-1\textsuperscript{2+} within cells.

The experiment was repeated with \textit{E. coli}, EC958, to investigate whether any change in uptake between Gram-positive and Gram-negative bacteria affected the observed differences in activity. As illustrated in Fig. 5, both complexes show levels of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{3D Z-stack projection, SIM colocalisation study using \textit{S. aureus} MRSA after 1 hour incubation of cells with MIC concentrations of \textit{fac}-1\textsuperscript{2+} (A) and \textit{mer}-1\textsuperscript{2+} (B). (i) Image obtained on excitation of 1\textsuperscript{2+} collected in the A568 channel. (ii) Images obtained by simultaneous staining with DAPI, collected in the DAPI channel. (iii) Overlay of both channels. (iv) 3D surface plots for the selected cells shown in the corresponding white boxes of the colocalisation images, showing emission intensity for 1\textsuperscript{2+} and DAPI.}
\end{figure}
uptake in *E. coli* which reflects their lowered activity in this Gram-negative species. Indeed, SIM experiments with EC958, showed little or no detectable uptake of either complex, as their emission was too weak to produce image reconstructions.

**Conclusions**

We have presented the synthesis and photophysical study of new osmium(II)-based complexes and their cellular uptake and antimicrobial activities against clinically relevant bacterial strains. The meridional isomer of 1^{2+} exhibited the highest antimicrobial activity which was competitive with conventional antibiotics and was active against meticillin- and ampicillin-resistant pathogenic *S. aureus*. Further, these antimicrobial studies were combined with super-resolution imaging studies using the red phosphorescence for 1^{2+} which demonstrated a high degree of bacterial DNA localisation. As 1^{2+} undergoes quenching of emission in the presence of air through generation of 1^O_2 the light-induced toxicity was investigated, resulting in the determination of a modest phototoxic index of 2. Fascinatingly, the fac and mer isomers of 1^{2+} display distinct differences in their biological properties, with mer-1^{2+} exhibiting both higher antibacterial activity and brighter cell staining properties. These differences are due to the higher cell uptake of this isomer compared to fac-1^{2+}.

Complexes of osmium(II) have attractive photophysical properties for biological and biomedical applications with prominent low energy absorption bands for direct ground state to \( ^3 \)MLCT state transitions and deep-red to near-IR emission. This enables increased depth of penetration in tissues for excitation with low energy light, imaging and \( ^1 \)O_2 sensitisation. The results presented here therefore demonstrate the significant potential for Os(II) complexes in (photo)therapeutic antimicrobial applications.

**Conflicts of interest**

There are no conflicts to declare.

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