Supporting information

Near Infra-Red Luminescent Osmium Labelled Gold Nanoparticles for Cellular Imaging and Singlet Oxygen Generation

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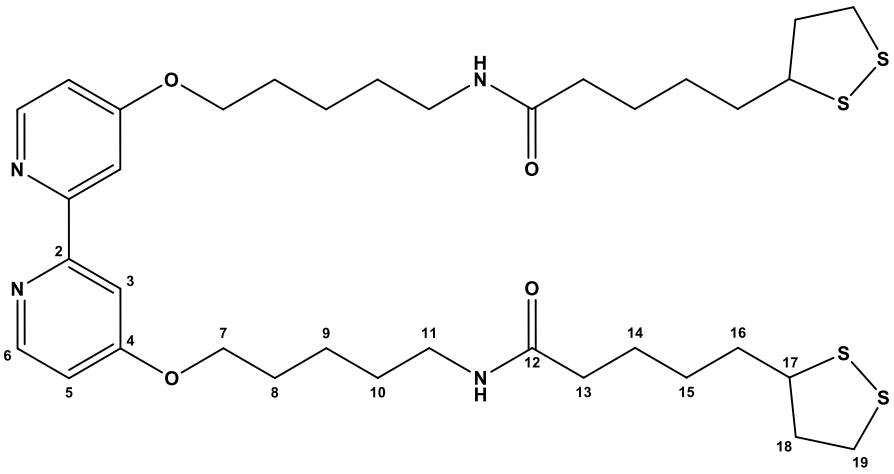
This file includes:

BpySS Characterisation



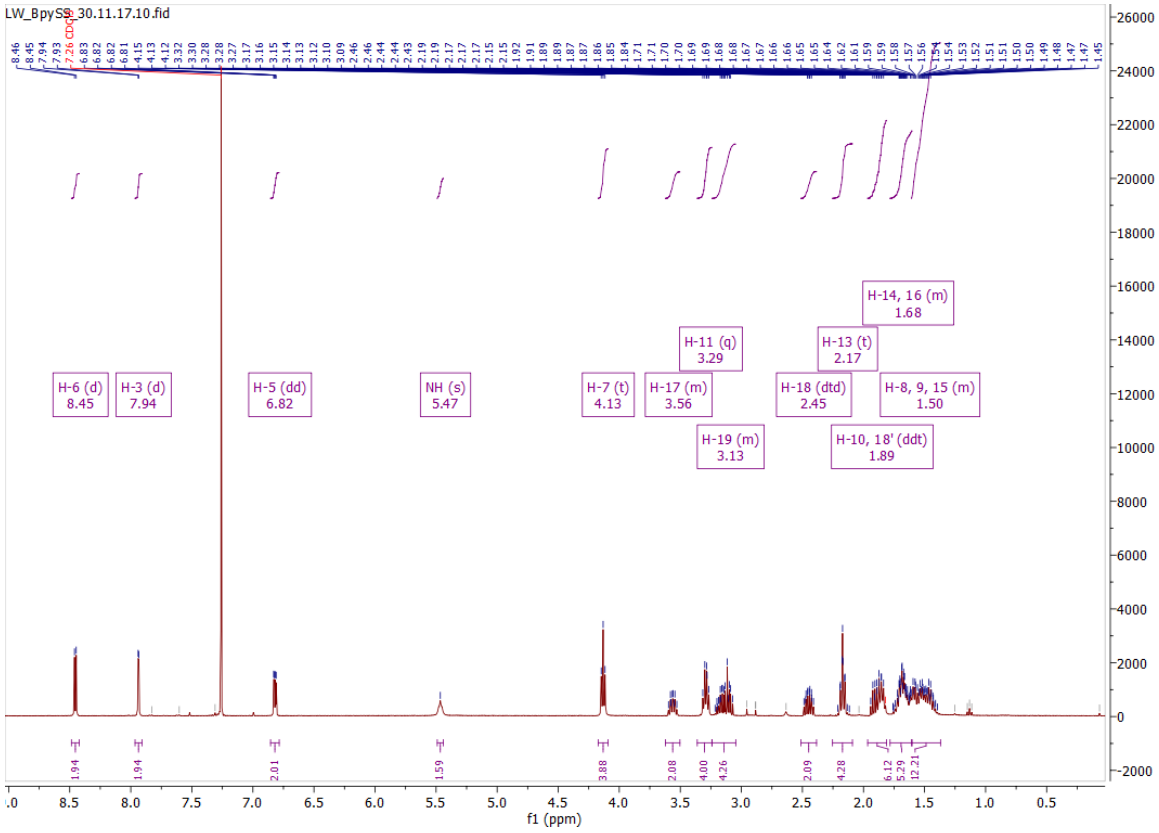


Figure S1. ^{1}H NMR spectrum of BpySS in $CDCl_3$.

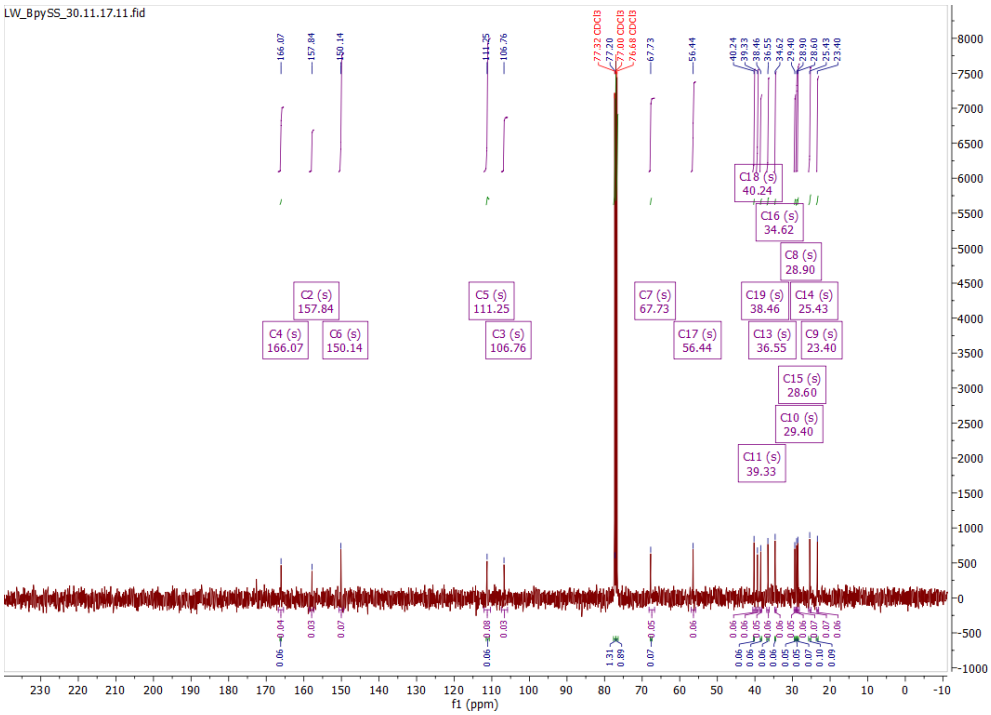


Figure S2. ^{13}C NMR spectrum of BpySS in $CDCl_3$.

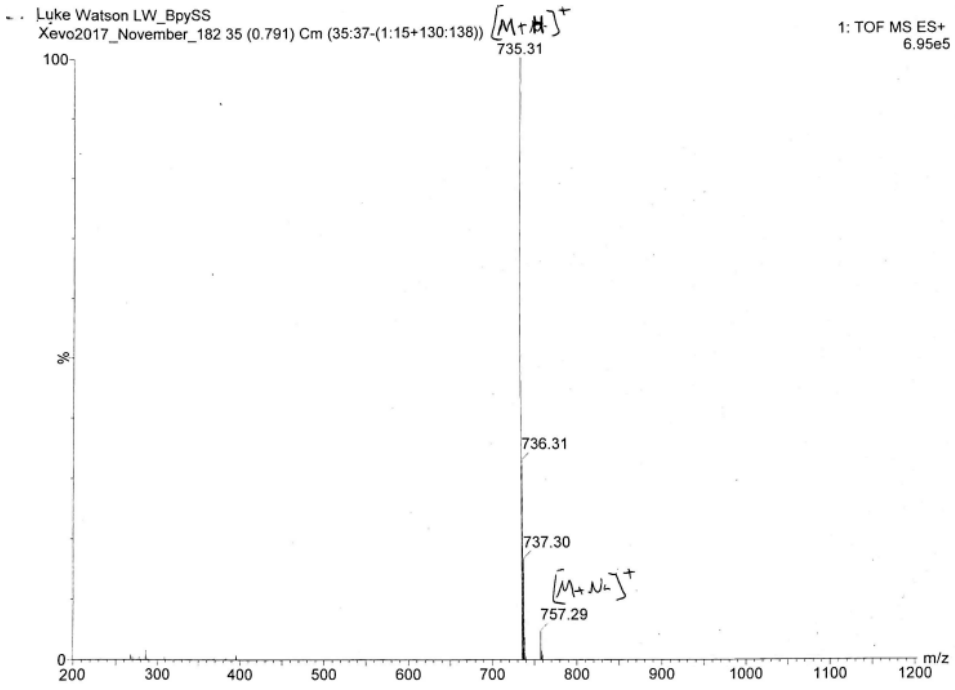


Figure S3. ESI (+) mass spectrum of BpySS.

$Os(phen)_2Cl_2$ Characterisation

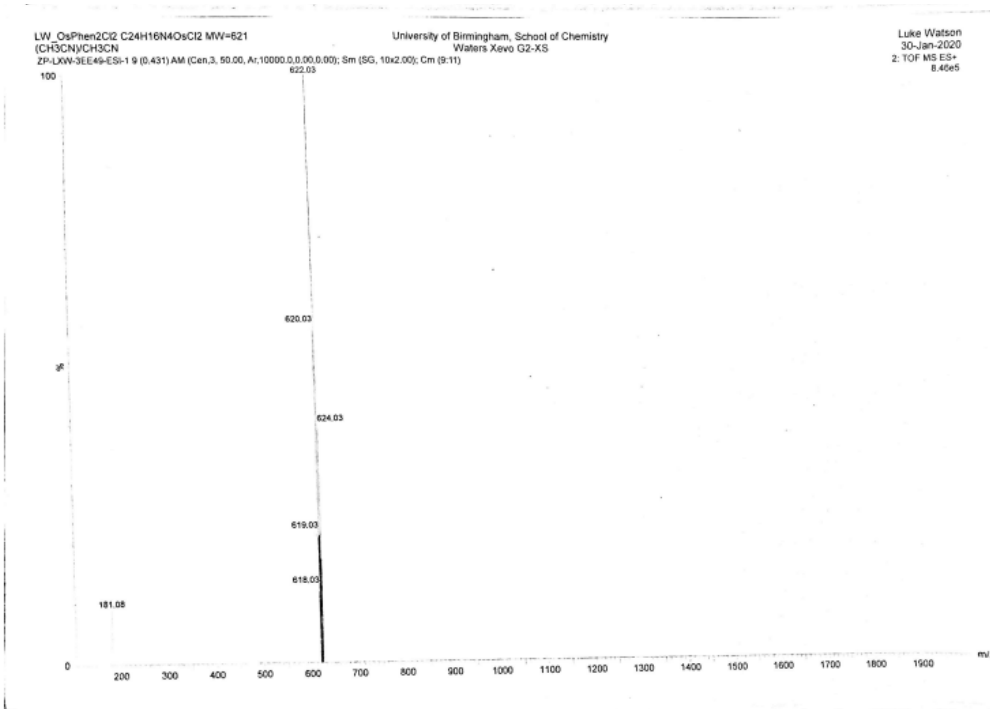


Figure S4. ESI(+) mass spectrum of $Os(Phen)_2Cl_2$.

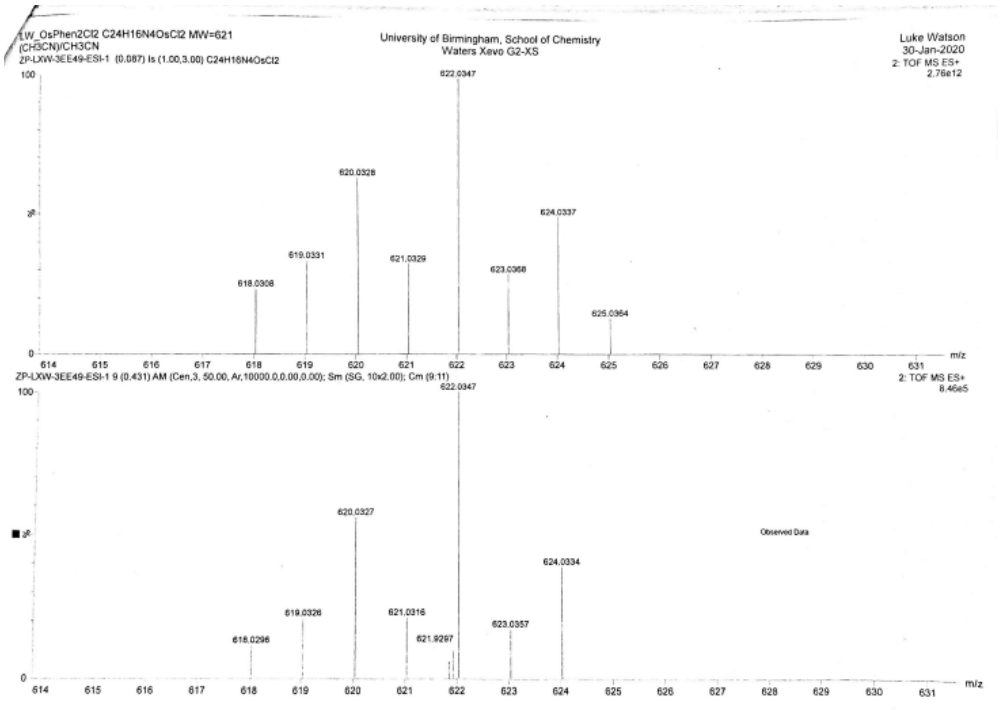
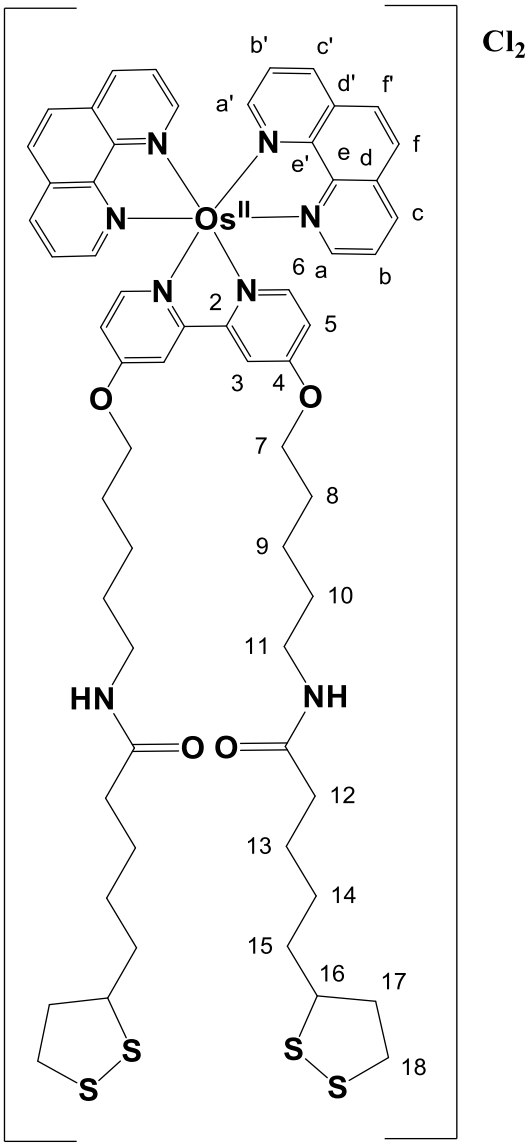


Figure S5. ESI (+) mass spectrum of $Os(Phen)_2Cl_2$ of experimental mass vs theoretical mass.

OsPhenSS Characterisation



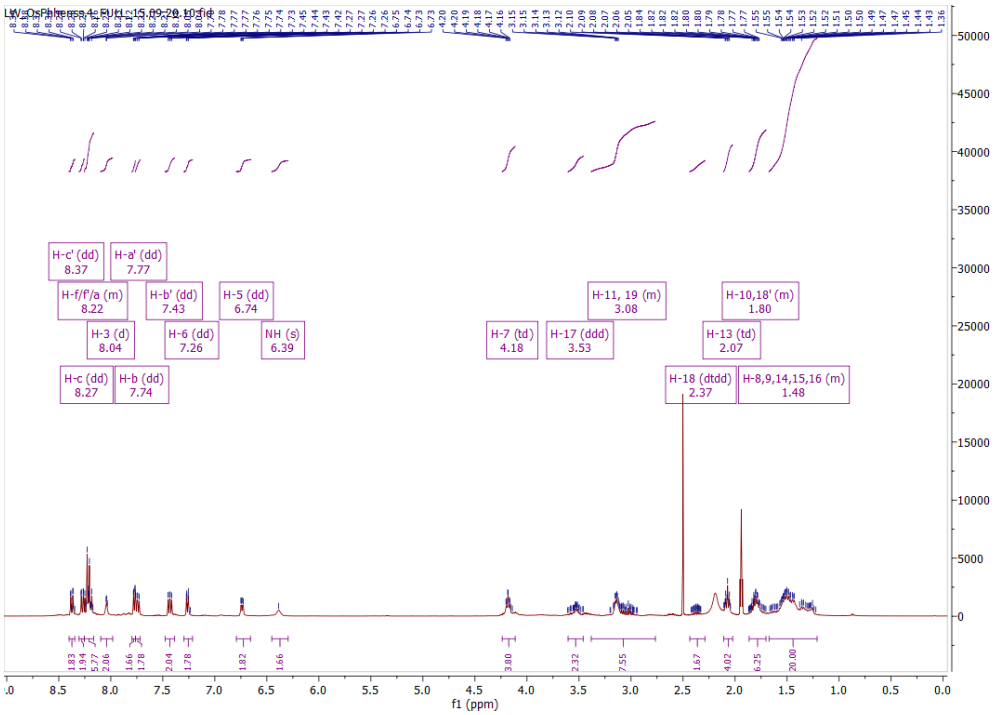


Figure S6. ^{1}H NMR spectrum of **OsPhenSS** in CD_3CN.

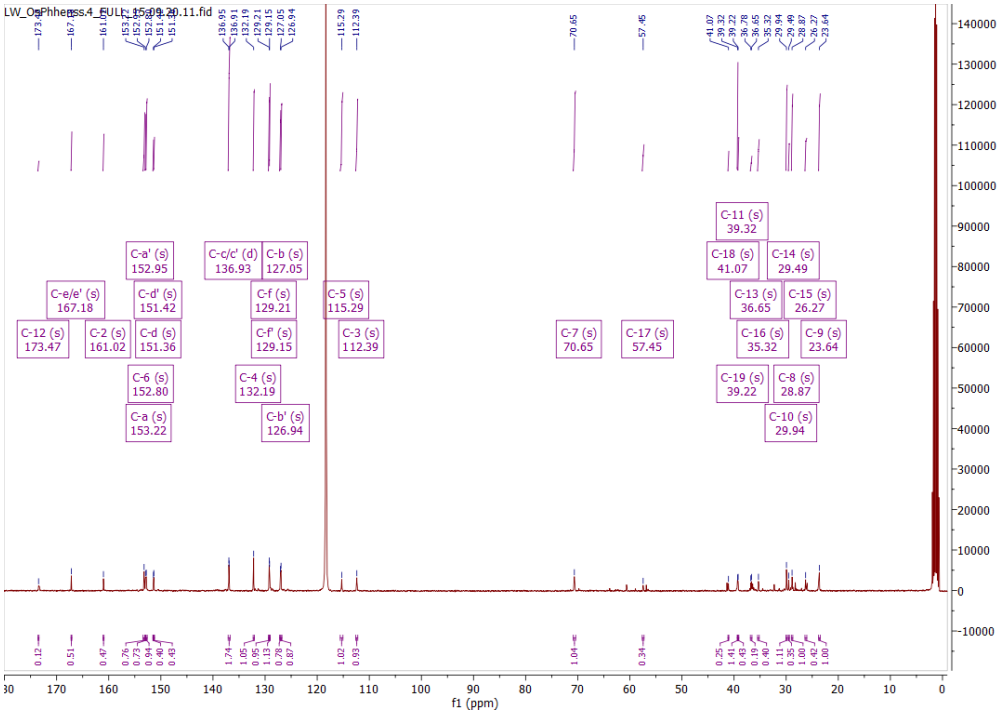


Figure S7. ^{13}C NMR spectrum of **OsPhenSS** in CD_3CN.

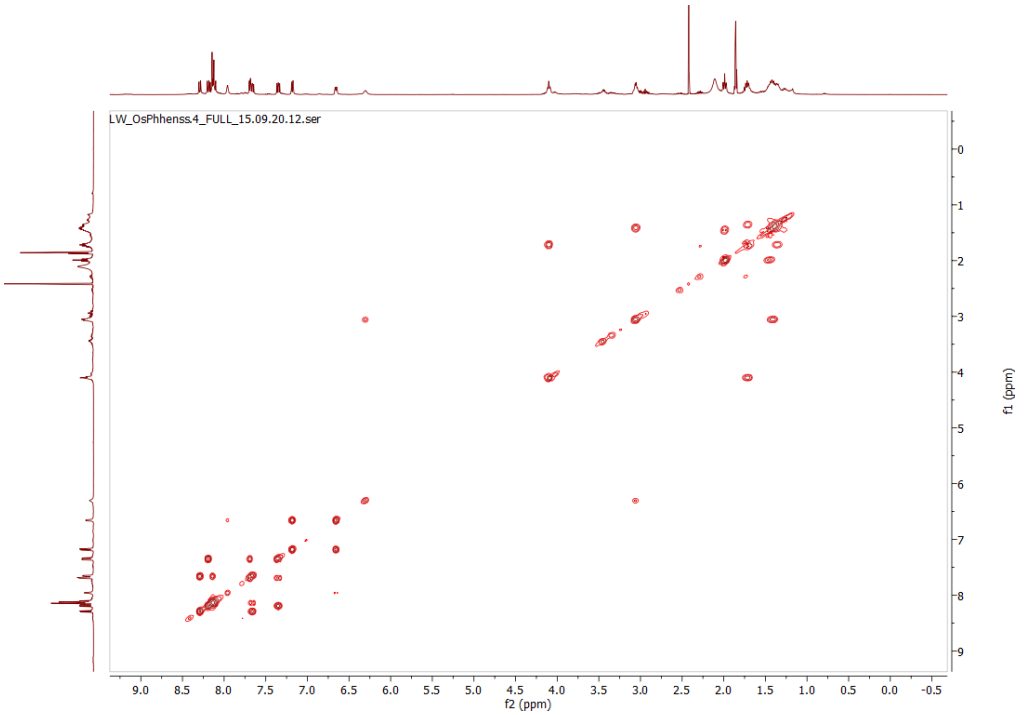


Figure S8. COSY spectrum of **OsPhenSS** in CD_3CN.

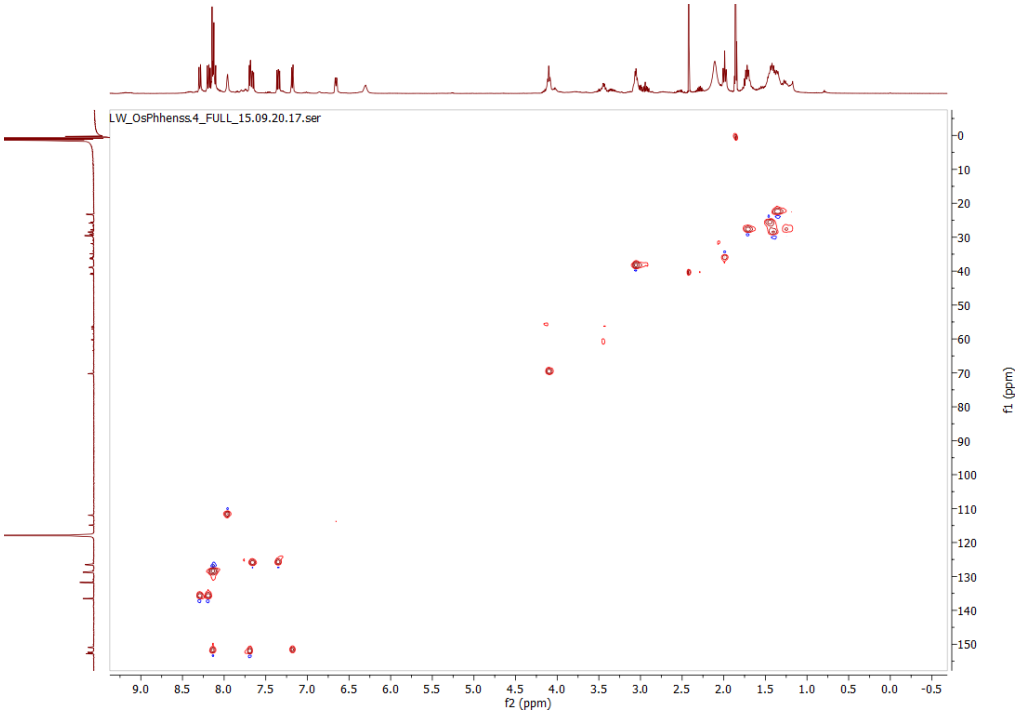


Figure S9. HSQC spectrum of **OsPhenSS** in CD_3CN.

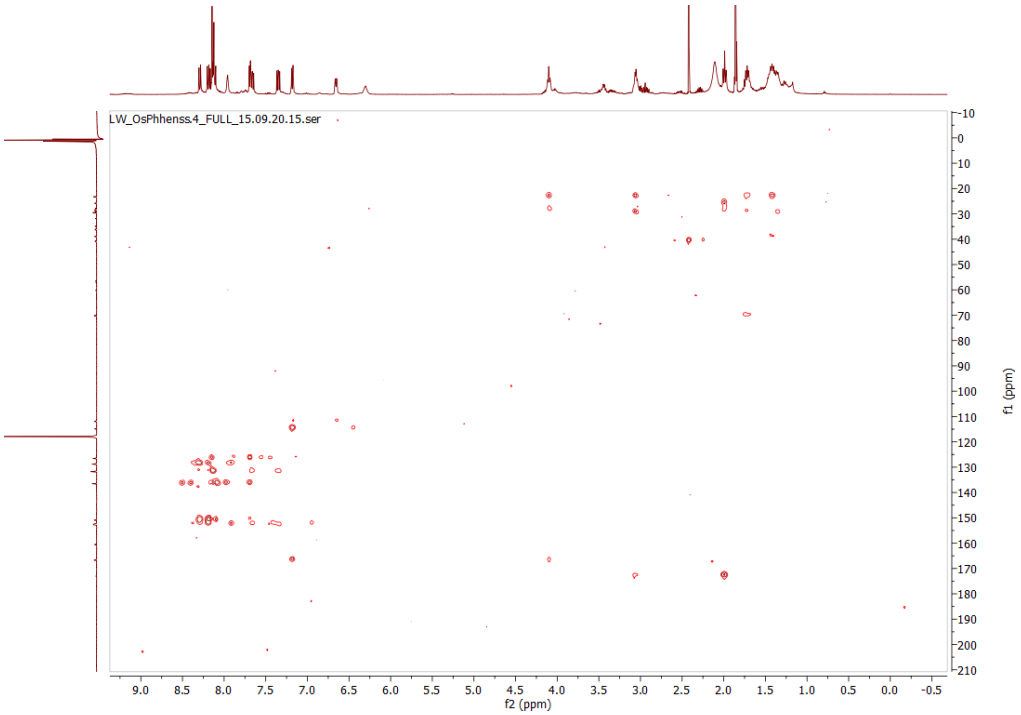


Figure S10. HMBC spectrum of **OsPhenSS** in CD_3CN.

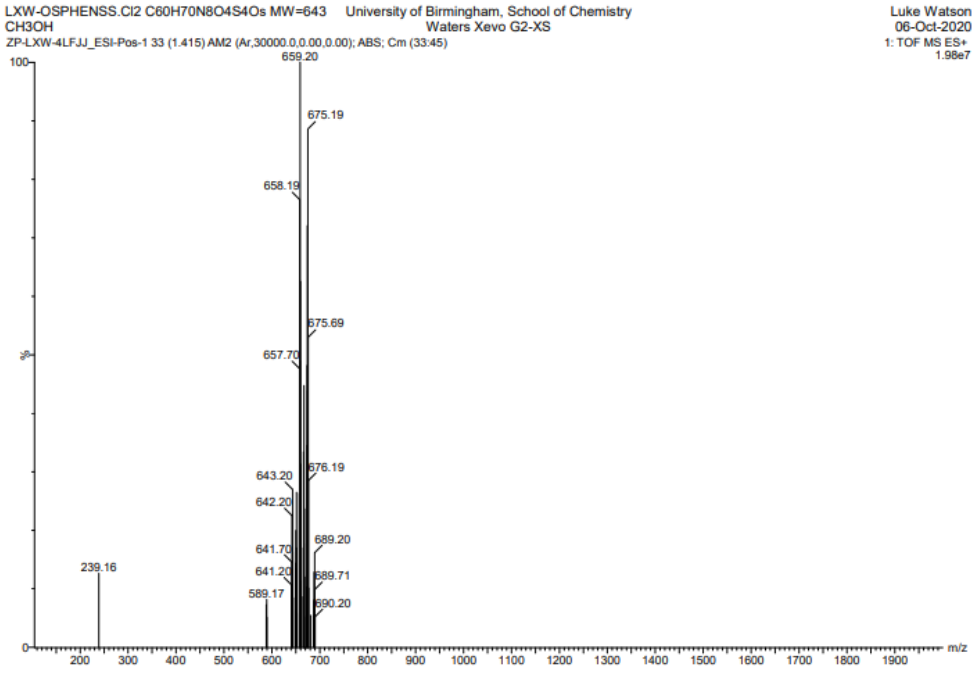


Figure S11. ESI (+) mass spectrum of **OsPhenSS**.

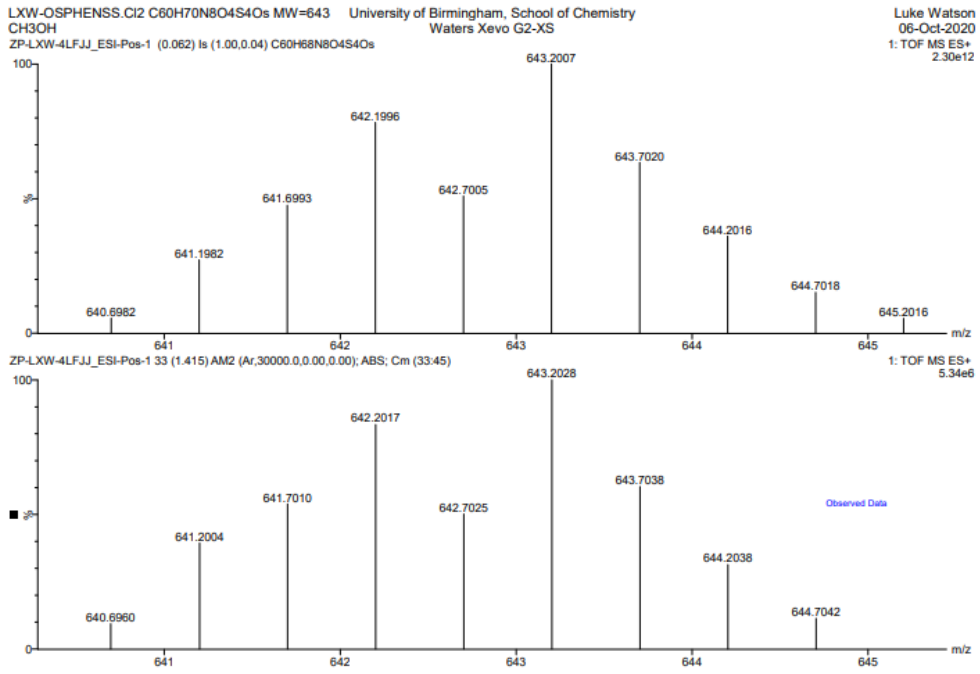


Figure S12. ESI (+) mass spectrum of **OsPhenSS.** Theoretical mass vs experimental exact mass.

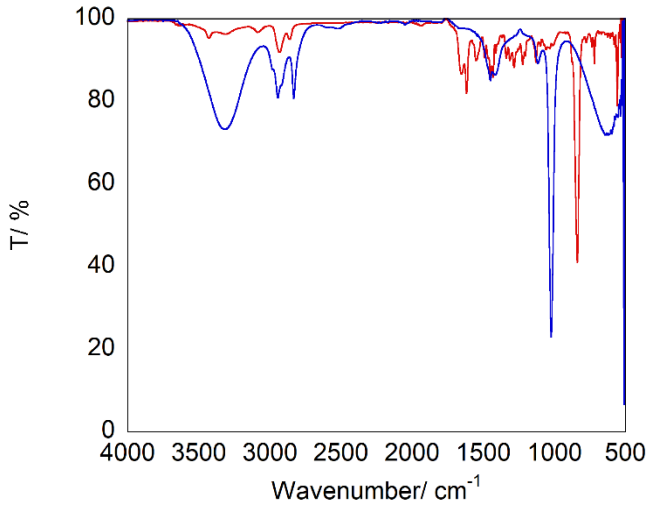


Figure S13. FTIR spectrum of **OsPhenSS** as the PF_6 counter ion (red) and the Cl counter ion (blue) showing successful ion exchange.

Photophysical Characterisation of OsPhenSS in presence of fluorosurfactant

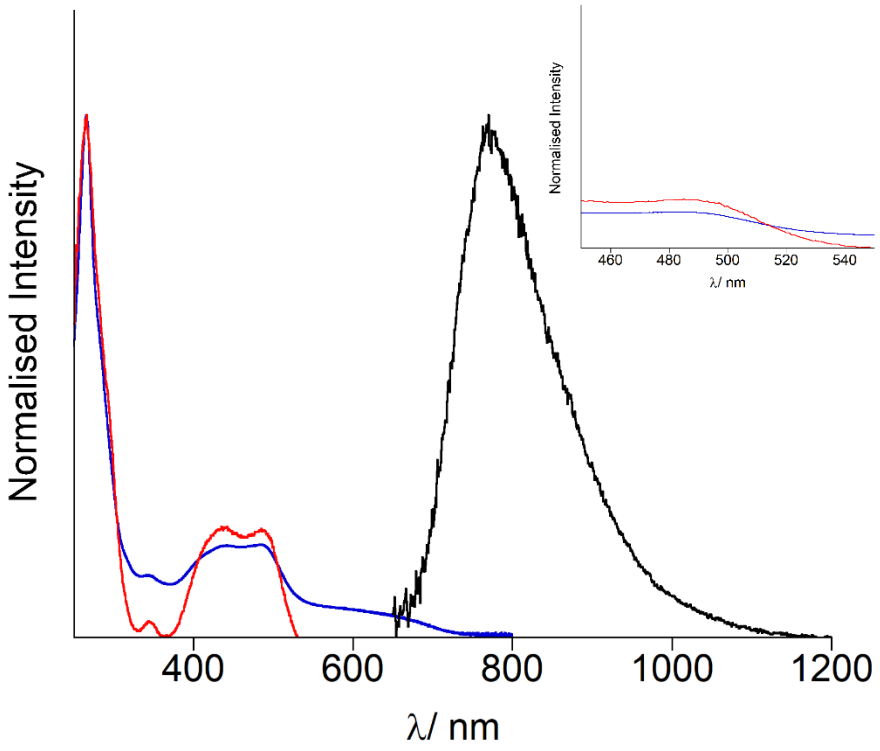


Figure S14. Normalised emission (black), excitation (red) and UV Vis (blue) of 10 µM **OsPhenSS** in the presence of 485 µM Zonyl FSA in 1mL aerated water (λ_{ex} = 485nm, λ_{em} = 770nm). Insert is magnification of excitation spectra 450 – 550 nm showing tail off.

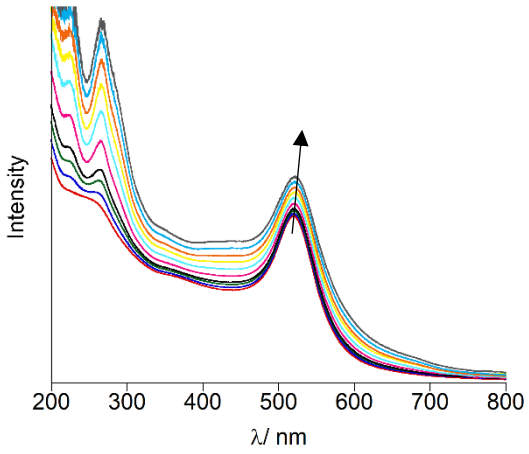


Figure S15. UV-Vis titration of 5 µL quantities of 1 mM **OsPhenSS** into 2 nM **Z•AuNP25** in water (35 µL added).

Data summary of gold nanoparticles' characterisation data

(A)

	λ_{max} / nm	Shift / nm
AuNP13	517	0
Z•AuNP13	518	1
OsPhenSS•AuNP13	521	4
AuNP25	518	0
Z•AuNP25	519	1
OsPhenSS•AuNP25	524	6

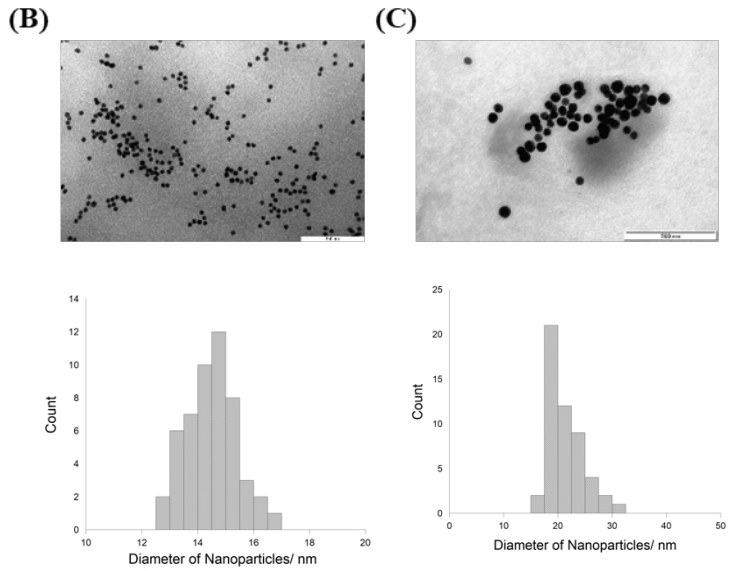


Figure S16. (A) Summary of UV-Vis shifts of gold nanoparticle samples; TEM images of (B) **OsPhenSS•AuNP13** and (C) **OsPhenSS•AuNP25** and associated histogram plots (n = 50). Scale bar = 200 nm.

(A)

	Number/ nm	Volume/ nm	PDI	Zeta potential / mV
AuNP13	14 ± 3	17 ± 4	0.06	-35 ± 4
Z•AuNP13	20 ± 5	23 ± 5	0.12	-49 ± 5
OsPhen•AuNP13	18 ± 5	31 ± 14	0.27	-39 ± 8
AuNP25	21 ± 5	25 ± 7	0.07	-34 ± 4
Z•AuNP25	29 ± 8	34 ± 13	0.26	-42 ± 4
OsPhen•AuNP25	27 ± 8	40 ± 25	0.32	-36 ± 3
AuNP13 in D_2O	13 ± 3	16 ± 6	0.095	-32 ± 8
Z•AuNP13 in D_2O	21 ± 6	27 ± 10	0.271	-76 ± 12
OsPhenSS•AuNP13 in D_2O	22 ± 6	28 ± 11	0.265	-36 ± 8

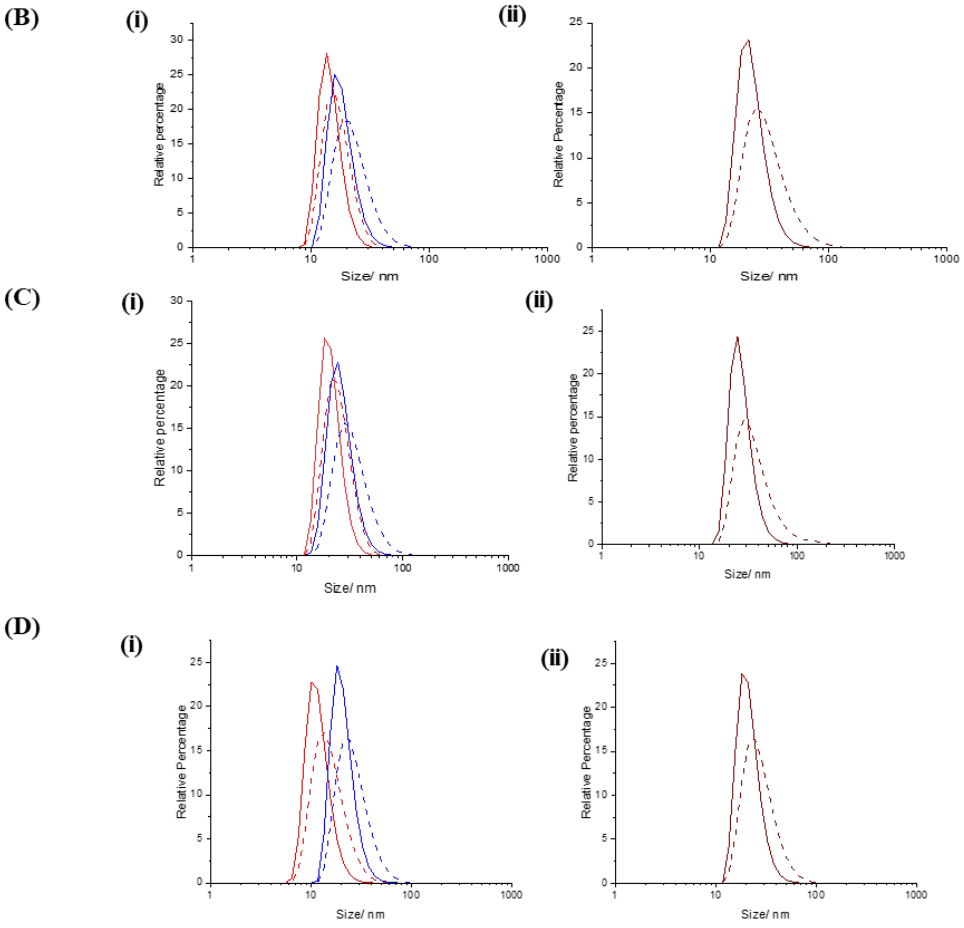


Figure S17. (A) Summary of Dynamic Light Scattering (DLS)data of gold nanoparticle samples (n = 5 for size and n = 3 for ζ-potential); **(B)** Number (solid line) an Volume (dashed line) size distributions for (i) citrate-capped **AuNP13** (red) and **Z•AuNP13** (blue) and (ii) **OsPhen•AuNP13** (brown); (C) Number (solid line) an Volume (dashed line) size distributions for (i) citrate-capped **AuNP25** (red) and **Z-AuNP25** (blue) and (ii) **OsPhen•AuNP25** (brown); (D) Number (solid line) an Volume (dashed line) size distributions for (i) citrate-capped **AuNP13** in D_2O **(**red) and **Z•AuNP13** in D_2O (blue) and (ii) **OsPhen•AuNP13** in D_2O (brown).

Photophysical characterisation of OsPhenSS•AuNP25

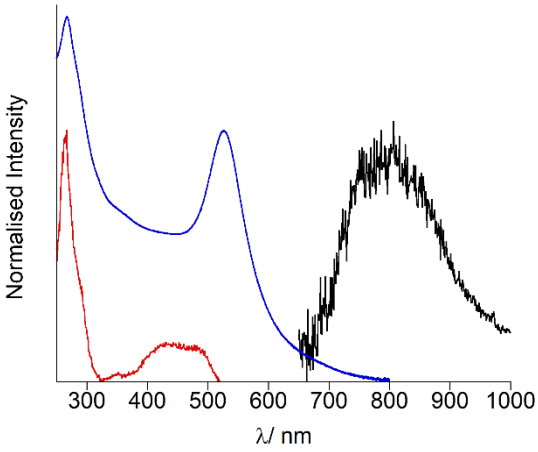


Figure S18. Normalised emission (black, λ_{exc} = 485 nm), excitation (red, λ_{em} = 785 nm) and UV-Vis (blue) spectra of **OsPhenSS•AuNP25** (2 nM) in aerated water.

Cell viability assays of OsPhenSS•AuNP in live A549 cells

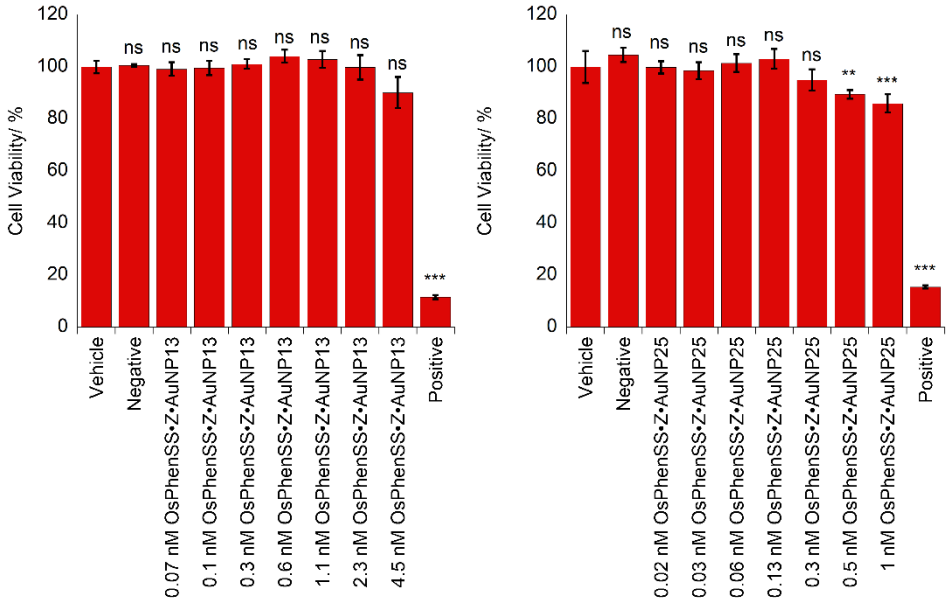


Figure S19. MTT assays of A549 cells treated with serial particle dilutions of (a) **OsPhenSS•AuNP13** and (b) **OsPhenSS•AuNP25** for 24 hours at 37°C 1-way ANOVA followed by a Dunnett's t-test corrected for multiple comparisons. *=P<0.05, **=P<0.01, ***=P<0.001, n = 3.

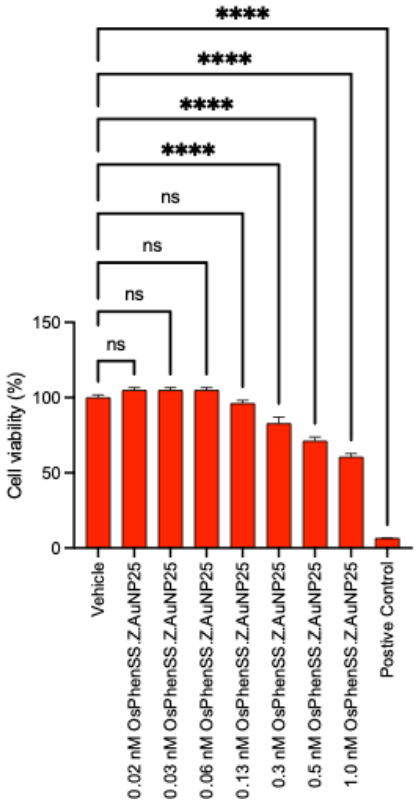


Figure S20. MTT cytotoxicity assay of MRC5 cells (embryonic human lung fibroblasts) treated with **OsPhenSS•AuNP25** for 24 h. Data is plotted as percentage control relative to vehicle alone. As a positive control cells were treated with 0.1% w/v Triton-X-100 for 24 h. The results represent the mean +/-SEM of six technical replicates. ****, statistically significant from vehicle control as assessed by a one-way ANOVA followed by a Dunnett's post-hoc t-test corrected for multiple comparisons, $P<0.0001$.

Additional Confocal microscopy images of OsPhenSS•AuNP25

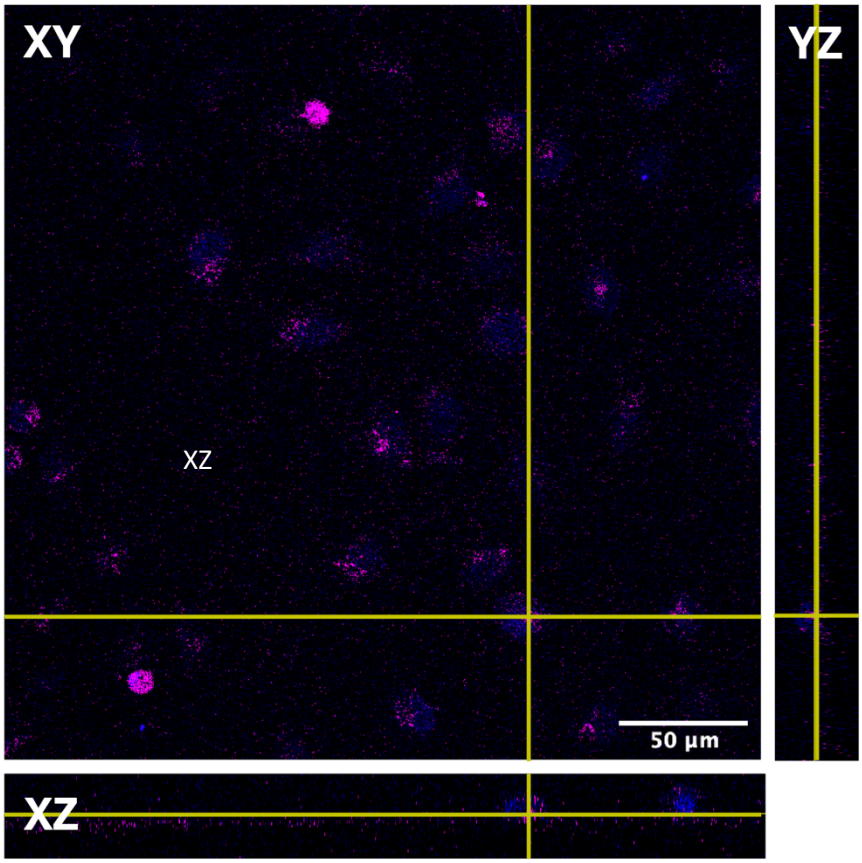


Figure S21. Orthogonal reconstruction of selected central plane from z-stack images of A549 cells showing osmium signal inside cells after incubation with 0.2 nM **OsPhenSS•AuNP25** for 18 h at 37°C. Purple channel, osmium emission (λ_{exc} = 488 nm, λ_{em} = 650 – 800 nm); blue channel, Hoescht 3368 nuclear staining (λ_{exc} = 402 nm, λ_{em} 420−470 nm).

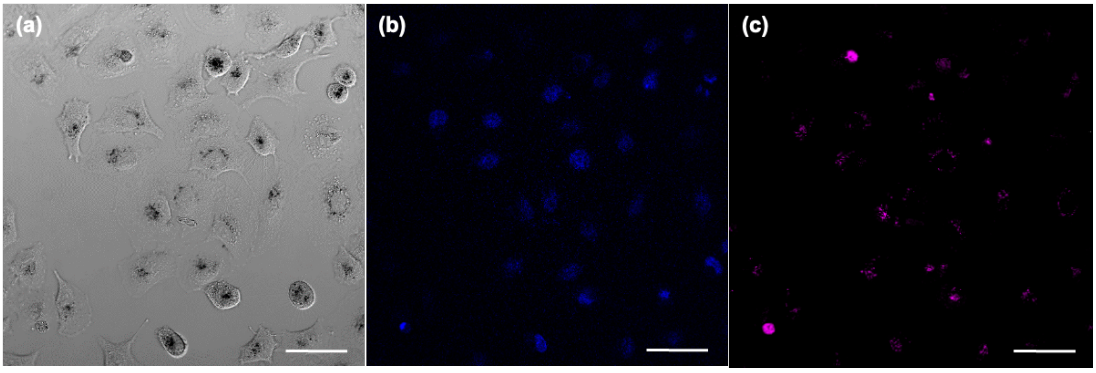


Figure S22. Separate channels (Figure 5) in the live cell images of A549 cells incubated with **OsPhenSS•AuNP25** (0.2 nM, incubation for 18 h). (a) brightfield, (b) blue channel, Hoescht 3368 nuclear staining (λ_{exc} = 405 nm, λ_{em}= 420 − 470 nm), (c) purple channel, osmium-based emission (λ_{exc} = 488 nm, λ_{em} = 650−800 nm). Scale bar is 50 µm.

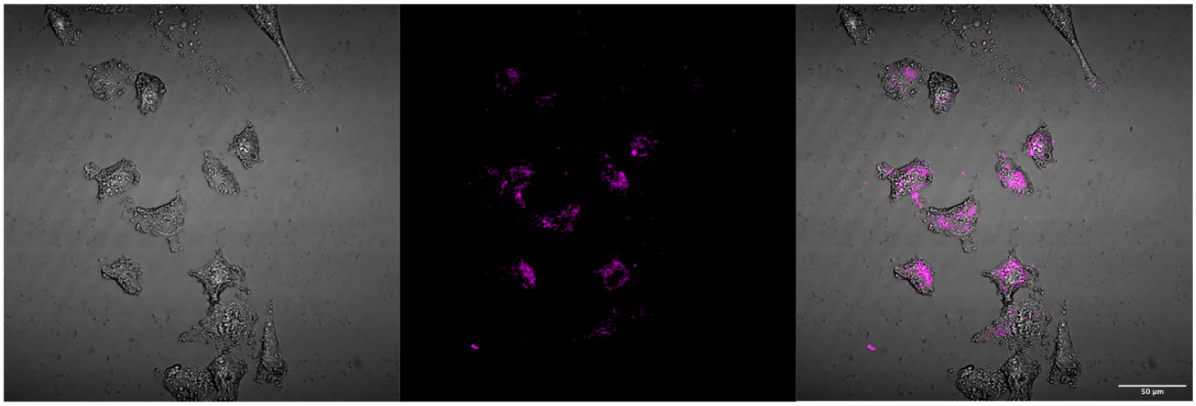


Figure S23. Confocal microscopy of **OsPhenSS•AuNP25** in fixed A549 cells (0.2 nM **OsPhenSS•AuNP25** at 4 h). Brightfield (left); Purple channel, **OsPhenSS•AuNP25** emission (λ_{exc} = 488 nm, λ_{em} = 650−800 nm) (middle) and overlap of brightfield and osmium emission (right). Scale bar is 50 µm.

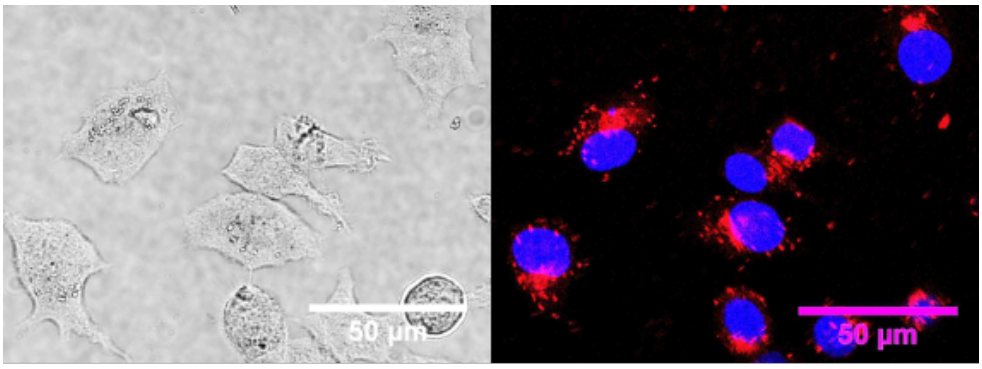


Figure S24. Dual channel fluorescence image of MCF-7 cells with corresponding brightfield image incubated with **OsPhenSS•AuNP25** at 0.1 nM incubated for 24 h (red channel, λ_{exc} = 552 nm, λ_{em} = 680-710 nm) and Nuclear Violet (Cambridge Bioscience, UK) incubated for 1 h at 10µM prior to imaging (blue channel, λ_{exc} = 405 nm, λ_{em} = 430-470 nm) to stain the nucleus. The luminescence shows punctate intracellular and perinuclear distribution. Scale bar is 50 µm.

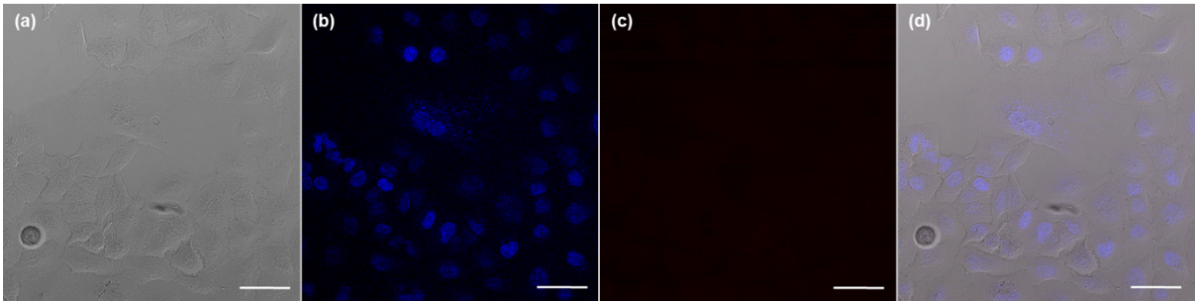


Figure S25. Confocal image of untreated control A549 cells. (a) Brightfield, (b) blue channel, Hoechst 3368 nuclear staining (λ_{exc} = 405 nm, λ_{em}= 420 − 470 nm), (c) purple channel, osmium (λ_{exc} = 488 nm, λ_{em} = 650 − 800 nm) and (d) overlap of brightfield, blue channel (Hoechst 3368) and purple channel (osmium). Scale bar is 50 µm.

Calculation of number (*N*) of gold atoms per nanoparticle

Assuming the gold nanoparticles are perfect spheres and atoms are packed in a uniform face-centred cubic crystalline structure, and taking the density of gold as ρ_{gold} = 19.32 g cm^{-3}

$$\boldsymbol{N = \frac{\rho \text{ x } \pi D^3}{6 \text{ x } M_r} \text{ x } N_A}$$

Where Mr of gold =196.96657 g mol^{-1}, Avogadro's constant N_A = 6.022140857 × 10^{23} mol^{-1}

The equation simplifies:

$$N = 30.8969 \times D^3$$