We report the synthesis of novel tetraplatinated metalloporphyrin-based photosensitizers (PSs) for photodynamic therapy (PDT), their characterization, cellular uptake and localization, as well as the determination of their in vitro light-induced anticancer properties. The PSs show excellent phototoxic indexes up to 5800 against HeLa cells, which is, to the best of our knowledge, the highest value reported for any porphyrin so far. Furthermore, isotopic labelling of the porphyrin with a highly enriched $^{67}$Zn isotope was performed in order to determine the distribution ratio of zinc to platinum by ICP-MS, allowing to differentiate between naturally occurring zinc and $^{67}$Zn that was introduced into the cells by the PS. We conclude that the platinum units within the platinum-PS conjugates help to solubilize the PS and, at the same time, act as cell-penetrating vectors, enhancing the efficiency of the PS without causing a significant dark toxicity.
Title: Studying the cellular distribution of highly phototoxic platinated metalloporphyrins using isotope labelling

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Abstract:

We report the synthesis of novel tetraplatinated metalloporphyrin-based photosensitizers (PSs) for photodynamic therapy (PDT), their characterization, cellular uptake and localization, as well as the determination of their in vitro light-induced anticancer properties. The PSs show excellent phototoxic indexes up to 5800 against HeLa cells, which is, to the best of our knowledge, the highest value reported for any porphyrin so far. Furthermore, isotopic labelling of the porphyrin with a highly enriched $^{67}$Zn isotope was performed in order to determine the distribution ratio of zinc to platinum by ICP-MS, allowing to differentiate between naturally occurring zinc and $^{67}$Zn that was introduced into the cells by the PS. We conclude that the platinum units within the platinum-PS conjugates help to solubilize the PS and, at the same time, act as cell-penetrating vectors, enhancing the efficiency of the PS without causing a significant dark toxicity. This study opens up new avenues in effective transportation and cellular targeting of hydrophobic drug candidates by reaction with transplatin.

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**Introduction:**

In cancer therapy, “classical” chemotherapeutic drugs such as cisplatin ($\text{cis}\cdot[\text{PtCl}_2(\text{NH}_3)_2]$) are still widely used, despite the impressive recent developments and applications of antibody-based\(^1\) and immunotherapy-based\(^2\) treatments. Cisplatin displays high activity against a variety of cancer types including testicular, ovarian, cervical and bladder tumors.\(^3\) Although cisplatin is extremely successful as an anti-cancer drug, undesirable side effects such as nephrotoxicity, neurotoxicity, hepatotoxicity, gastrointestinal dysfunction and cardiotoxicity are prevalent.\(^4\) Hence, there is scope for much improvement, with respect to increasing the clinical effectiveness of the drug, elimination of side effects, and improving tumor localization. The fact that more than 900 planned or on-going clinical trials include cisplatin mirrors the intensive interest in further expanding the possible applications of this drug.\(^5\)

Another approach to overcome the aforementioned limitations of cisplatin is to combine the action of two curative modalities, i.e. photodynamic therapy (PDT) and cisplatin-based chemotherapy. A combination of chemotherapy and PDT is one of the several preclinical attempts, which has yielded promising results.\(^3a, 6\)

PDT is a clinically approved and minimally invasive therapeutic treatment for dermatological, ophthalmic, infectious diseases, as well as cancer, which involves the application of a photosensitizer (PS) that ideally localizes disease-specifically. The application is followed by low energy irradiation to activate the PS leading to the generation of reactive oxygen species (ROS) which in turn induce cell death.\(^7\)

The starting point of the current study was our previous work in which we reported a series of easily accessible tetrapyridylporphyrins ($\text{tPt}-\text{H}_2\text{PyP}$, $\text{cPt}-\text{H}_2\text{PyP}$, $\text{dPt}-\text{H}_2\text{PyP}$) that were coordinated by four platinum(II) complexes to yield highly phototoxic agents (Figure 1). These compounds displayed a light toxicity down to $19\ \text{nM}$ and at the same time low dark toxicities of around $45\ \mu\text{M}$ in human cancer cell lines (Figure 1).\(^8\) The ratio of dark to light toxicity is referred to as the phototoxic index (PI), and it should be as high as possible. Our systems displayed PI values higher than 1000 in HeLa cells after being excited with light of a wavelength at 420 nm. These PI values are better than the current clinically used PSs that display a phototoxicity of $>10$ or $>260$ for the first\(^9\) and second\(^10\) generation PSs, respectively.
Figure 1: Already reported tetraplatinated porphyrins tPt-H24PyP, cPt-H24PyP and dPt-H24PyP and novel ones tPt-Zn4PyP, tPt-67Zn4PyP, tPt-Cu4PyP, cPt-Zn4PyP, cPt-Cu4PyP, dPt-Zn4PyP and dPt-Cu4PyP.

Based on our previous study,8 we investigated a set of important follow-up questions:

- How does the insertion of either zinc or copper modulate the phototoxicity of tPt-H24PyP, cPt-H24PyP and dPt-H24PyP? The singlet oxygen quantum yield of porphyrins was reported to increase11 or not to change at all12 after insertion of zinc into the macrocycle. In another case, the metal free porphyrin generated mainly free hydroxy radicals, which were in vitro more cytotoxic than the singlet oxygen produced by the zinc porphyrin.13
- Is the complex tPt-H24PyP stable when it reaches the cell?
- What is the reason for the low dark toxicities of our platinum-porphyrin conjugates?
- What is the origin of the slow reaction of tPt-H24PyP with guanosine?

Experimental Section:

The chemicals were purchased from Acros Organics (Belgium), Chempur (Germany), Fluorochem (United Kingdom) and Sigma-Aldrich (Switzerland). 67Zinc as a metal was obtained from ISOFL Ex USA (enrichment level of 89.6%). The solvents used in analysis were of reaction grade and purchased from EMSURE, the remaining solvents were of technical grade and purchased from Honeywell. Reactions were monitored for completion by analysing a small sample by TLC. Thin layer chromatography (TLC): Merck TLC plates silica gel 60 on aluminium with the indicated solvent system; the spots were visualized by UV light (254 nm and 366 nm). UV-Vis spectra: Specord® 250 PLUS spectrophotometer (Analytic Jena, Germany); λ in nm. IR spectra: SpectrumTwo FT-IR Spectrometer (Perkin-Elmer, USA) equipped with a Specac Golden Gate™ ATR (attenuated total reflection) accessory; applied as neat samples; 1/λ in cm⁻¹. 1H-NMR spectra in the indicated solvent; Bruker AV-400 (400 MHz); δ in ppm rel. to TMS (δ 0.00), J in Hz. 13C-NMR spectra in the indicated solvent; Bruker AV-500 (125.7 MHz); δ in ppm rel. to TMS (δ 0.0). 195Pt-NMR spectra in the indicated solvent; Bruker AV-500 (107.5 MHz); δ in ppm rel. to K₂PtCl₆ in D₂O (δ 0). EI mass spectrometry was performed on a DFS double-focusing (BE geometry) magneticsector mass spectrometer DFS (ThermoFisher Scientific, USA). Mass spectra were measured with electron ionization (EI) at 70 eV, solid probe inlet, source temperature of 200°C, acceleration voltage of 5 kV, and resolution of 2500. The instrument was scanned between m/z 30 und 900 at scan rate of 2 s/decade in the magnetic scan mode. Perfluorokerocene (PFK, Fluorochem) served for calibration. ESI mass spectra were recorded on a Bruker maXis mass spectrometer. Samples were dissolved in an appropriate solvent at a concentration of around 1 µmol/mL and measured at continuous flow at 3 µL/min. The mass spectrometer was operated in the positive electrospray ionization mode at 4’000 V capillary voltage, -500 V endplate offset, with a N₂ nebulizer.
pressure of 0.8 bar and dry gas flow of 4 L/min at 180°C. MS acquisitions were performed in the mass range from \( m/z \) 50 to 20'000 resolution (full width at half maximum) and 1.0 Hz spectra rate. Masses were calibrated between \( m/z \) 158 and 1450 or 2721 prior analysis below 2 ppm accuracy, with a 2 mM solution of sodium formate or with a Fluka electrospray calibration solution (Sigma-Aldrich) that has been 100 times diluted with acetonitrile, respectively. Elemental analysis was performed on a LECO Truespec CHNS(O)-microanalyser. The elemental analysis of the platinated species was done at the Mikrolabor of the Laboratorium für Organische Chemie at the ETHZ. The synthesis of the platinated porphyrins \( tPt-Zn4PyP, tPt-Cu4PyP, cPt-Zn4PyP, cPt-Cu4PyP, dPt-Zn4PyP \) and \( dPt-Cu4PyP \) is described in the electronic supplementary information.

Crystal structure determination of the cationic \([trans-PtCl(NH_3)_2]_4-5,10,15,20\text{-tetra(4’-pyridyl)}\text{-zinc(II)}\text{porphyrin unit. A saturated solution of } tPt-Zn4PyP \text{ in DMF (about 1 mg in 0.5 mL of DMF)} \text{ was subjected to the under oil crystallization following the procedure recently described by us}^{14}. \text{In short, 3.35 } \mu\text{L of the } tPt-Zn4PyP \text{ DMF solution and 3.5 } \mu\text{L of each of the 96 aqueous solutions of the Small Molecule Anion screen}^{15} \text{ were pipetted in 100 } \mu\text{L of silicone oil by a Crystal Gryphon LCP pipetting robot with a custom made Hamilton syringe holder. After one day, needle shaped crystals appeared in the drop originating from a 0.2 M aqueous solution of sodium tetraphenyl borate. Repeating the same procedure with } tPt-Zn4PyP \text{ dissolved in water did not yield any crystals, most likely due to the too solubility of } tPt-Zn4PyP \text{ in water. Crystallographic data were collected at 160.0(1) K on a Rigaku OD XtaLAB Synergy Dualflex diffractometer equipped with a Pilatus 200 K detector and a PhotonJet Cu K\( \alpha \) source (\( \lambda = 1.54184 \) Å). Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), placed on a nylon loop that is mounted in a CrystalCap Magnetic\textsuperscript{TM} (Hampton Research) and immediately transferred to the diffractometer. The program suite } CrysAlisPro \text{ was used for data collection, numerical and empirical absorption correction as well as data reduction.}^{16}\text{ The structure was solved with direct methods using } Shelxtl^{17} \text{ and was refined by full-matrix least-squares methods on } F^2 \text{ with SHELXL-2014}^{18} \text{ using the GUI OLEX2 (Table S1)}.^{19} \text{ The asymmetric unit contains one half of the molecule. Therefore, the central zinc metal center and the coordinated dimethylformamide solvent molecules are disordered in a 1:1 ratio. Furthermore, two more DMF molecules were disordered in a ratio of 58:42 and 56:44 respectively and had to be treated with suitable restraints. Graphical output was produced with the help of the program Mercury.}^{20} \text{ CCDC 1977523 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.}

\textbf{67Zinc(II) chloride dihydrate (67ZnCl}_2\cdot 2 \text{H}_2\text{O)}

67\text{Zn (100.0 mg, 1.494 mmol) was dissolved in 5.0 mL of an aqueous solution of 16 % HCl and the mixture was stirred at room temperature until the solid had totally dissolved (30 minutes). The mixture was dried \textit{in vacuo} by warming up the system to 55°C. The product was obtained as a white solid in a}
yield of 81%. Analytical Data: $^{67}$ZnCl₂·2 H₂O, Mol. Wt.: 173.86 g/mol. MS (EI): $m/z = 136.87$ (100%), 138.86 (66.9%), 140.86 (8.7%).

5,10,15,20-tetra(4'-pyridyl)$^{67}$zinc(II)porphyrin ($^{67}$Zn₄PyP)
The reaction of 5,10,15,20-tetra-(4'-pyridyl)-porphyrin with either zinc chloride or zinc acetate was optimized with respect to yield and minimal required excess of zinc. For these reactions, unlabelled zinc was used (Table S2). The best conditions applied with $^{67}$Zn were as follows: 5,10,15,20-tetra-(4'-pyridyl)-porphyrin (40.0 mg, 0.051 mmol) was suspended in a mixture of 7.0 mL of DMF/water (1:1), then added to $^{67}$ZnCl₂·2 H₂O (40.0 mg, 0.232 mmol). The mixture was stirred at 120°C for 26 h. After cooling down to room temperature, the product was precipitated with water, filtered and washed with water. The solid was taken up in a mixture of chloroform/methanol (3:2) and dried in vacuo to yield 34.0 mg of the porphyrin as a violet solid in a yield of 97%. Analytical Data: C₄₀H₂₄N₈$^{67}$Zn, Mol. Wt.: 683.62 g/mol. UV-Vis (DMF, $\lambda$; nm): 424.5 (Soret band), 404.9, 557.4, 596.3 (Q bands). MS (ESI): $m/z = 684.14567 \ [M + H]^+ \ (44\%), \ 342.57721 \ [M + 2H]^{2+} \ (100\%).$ 

[trans-PtCl₂(NH₃)₂]₄-5,10,15,20-tetra(4'-pyridyl)$^{67}$zinc(II)porphyrin nitrate (tPt-$^{67}$Zn₄PyP)
Transplatin (38.6 mg, 0.129 mmol) and silver nitrate (21.9 mg, 0.129 mmol) were dissolved in 2.0 mL DMF and stirred in the dark at room temperature for 24 h. The resulted suspension was centrifuged to remove the white silver chloride. The light yellow colored solution was added to a suspension of $^{67}$Zn₄PyP (20.0 mg, 0.029 mmol) in 2.0 mL DMF and stirred in the dark at 50°C for 48 h. The mixture was cooled down to room temperature, then 50% of the solvent was evaporated in vacuo and the porphyrin was subsequently precipitated with methyl tert-butyl ether. The precipitate was filtered and further washed with methanol, dichloromethane and methyl tert-butyl ether. Then, the solid was further dissolved in 2.0 mL DMF and centrifuged to remove insoluble impurities, before it was precipitated again with methyl tert-butyl ether. After having pipetted out the supernatant, the product was dried under vacuum and 28.5 mg of the product were obtained in 49% of yield as a violet solid. Analytical Data: C₄₀H₄₈Cl₄N₂O₁₂Pt₄$^{67}$Zn, Mol. Wt.: 1990.02 g/mol. ¹H-NMR (400 MHz, DMF-d₇): $\delta$ (ppm) 9.63 (d, $\omega$-pyridyl, 8 H, $J = 4 \text{ Hz}$), 9.28 ($\beta$-pyrrole, s, 8H), 8.72-8.71 (m-pyridyl, d, 8 H, $J = 4 \text{ Hz}$), 5.01 (NH₃, s, 24 H). ¹³C-NMR (125 MHz, DMF-d₇): $\delta$ (ppm) 153.37, 151.92, 149.09, 132.35, 132.06, 117.24. ¹⁹⁵Pt-NMR (107 MHz; DMF-d₇): $\delta$ (ppm) -2309. MS (ESI): $m/z = 435.52079 \ [M]^{4+}$. Elemental analysis calcd. (%) for C₄₀H₄₈Cl₄N₂O₁₂Pt₄$^{67}$Zn·2(H₂O): C 23.71, H 2.59, N 13.83; found C 24.16, H 2.81, N 13.40.

Singlet oxygen quantum yields:
The singlet oxygen quantum yields ($\Phi_{\Delta}$) of tPt-H₂₄PyP, cPt-H₂₄PyP, tPt-Zn₄PyP and cPt-Zn₄PyP were determined analogously to a method reported by our group. The detailed procedure is described in the Supporting Information.
Cell Culture:

Human cervical carcinoma (HeLa) cells were cultured in DMEM (Gibco) supplemented with 5% fetal calf serum (FCS, Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin. The normal human fetal lung fibroblast cell line (MRC-5) was grown in MEM medium (Gibco) supplemented with 10% FCS (Gibco), penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were cultured at 37 °C and in 6% CO₂ humidified atmosphere.

Cytotoxicity studies:

Cytotoxicity studies in the dark and after light irradiation of complexes \( \text{tPt-Zn4PyP}, \text{tPt-Cu4PyP}, \text{cPt-Zn4PyP}, \text{cPt-Cu4PyP}, \text{dPt-Zn4PyP}, \text{dPt-Cu4PyP} \) and cisPt(DMSO)₂Cl₂ (dPt) were performed on the HeLa cervical cancer cell line and the MRC5 fibroblast non-tumorigenic cell line by a fluorometric cell viability assay using resazurin (PromoCell GmbH, Germany). Cells were grown in triplicates in 96-well plates at a density of \( 4 \times 10^3 \) cells/well in 100 μL. One day later, cells were treated with increasing concentrations of the metal porphyrin complexes for 4 h, following which the growth medium was replaced with fresh medium. The plates were irradiated for 15 min at 420 nm in a Rayonet Chamber Reactor Complex (6.95 J / cm²). Cells were further incubated for 44 h, the medium was removed, and 100 μL of growth medium containing resazurin (0.2 mg / mL final concentration) was added. After 4 h, the fluorescence of the resorufin product was quantified at 590 nm emission with 540 nm excitation wavelength in a SpectraMax M5 microplate Reader. The results are expressed as mean ± error bar of independent experiments (Table 1). A series of negative controls (with cells treated with the porphyrin metal complexes in the dark) and positive controls (the porphyrin ligand together with cisplatin (cPt) to evaluate their possible combined action) were also performed.

Localization studies using laser scanning confocal microscopy:

HeLa cells were seeded in Ibidi μ-Slide 8-well glass bottom dishes. Upon ≈70 % confluence, the growth medium was replaced with medium containing freshly prepared solutions of drugs at a final concentration of 1 μM. After 2 h incubation with compounds, cells were washed three times with PBS (0.5 mL) and stained with the GOLGI ID® Green assay kit (ENZ-51028, Enzo Life Sciences, Inc., Switzerland) according to the manufacturer’s protocol. Prior to imaging, cells were incubated for 5 min with Hoechst 33258 (1.5 μM final concentration). Finally, cells were kept in fresh media containing 10 % FBS for live-cell imaging. The fluorescence of the GOLGI-ID® Green dye was visualized using excitation at 488 nm and emission at 550-600 nm, the one of the photosensitizers using excitation at 514 nm and emission at 650-750 nm (using a longpass filter) and the one of Hoechst 33342 using excitation at 405 nm and emission at 450-500 nm.
Immunofluorescence:
Coverslips, sterilized by washing them with 70% EtOH and brief flaming, were placed in 60 mm cell culture dishes. Cells were grown on coverslips to attain 80% confluence and were incubated with the corresponding photosensitizer for 14 hours at 37°C. Next, the cells on the coverslips were washed three times with PBS in order to remove the non-uptaken PS. The control samples and samples were kept in the dark and were then directly fixed, while samples undergoing light irradiation were first exposed to light for 15 min at 420 nm (light dose 6.95 J / cm²). Cells were fixed for 5 minutes at room temperature with a freshly-prepared 4% (v/v) formaldehyde solution from a 37% stock solution diluted in PBS buffer. Coverslips were washed with PBS and permeabilized with 0.2% (v/v) Triton X-100 from a 25% stock solution diluted with PBS for 5 min at room temperature. Coverslips were washed twice with PBS and blocked for 20 minutes at room temperature in a 2% (w/v) BSA solution in PBS. The primary antibody (mouse anti-γH2AX(S139), 1:800) was diluted in 2% (w/v) BSA in PBS solution. Antibody solution (35 μL per coverslip) were pipetted per coverslip onto parafilm in a humidifying chamber. Coverslips were placed and incubated with the antibody mix over night at 4 °C, were washed the next day twice with PBS and incubated with the secondary antibody (goat anti-mouse Alexa fluor 594, 1:800) in the dark at 37°C for 1 h. After that, coverslips were washed twice with PBS and incubated with 1 μg/mL DAPI in PBS for 10 min at room temperature in the dark. Finally, coverslips were washed twice with PBS and once with H₂O and mounted with 5 μL Vectashield® mounting medium onto glass slides. The slides were left at 4°C for 1 h and then the edges were sealed with nail polish. Image acquisition was performed on CLSM - Leica SP8 inverse STED 3X maintained by the Center for Microscopy and Image Analysis, University of Zurich, and the images were analyzed using Fiji (version 2.0.0- r-49/1.52i).

Sample Preparation for ICP-MS:
HeLa cells were seeded a week before treatment at a concentration of 1 × 10⁶ cells / mL in a 15 cm² cell culture Petri dish, allowed to grow until 80% confluence and incubated in cell culture medium with the target compounds (previously dissolved in DMF as vehicle, v/v < 0.1%) at a concentration of 10.0 μM for 4 h. The medium was removed, the cells washed with PBS and trypsinized. After re-suspension in PBS, the pellet was washed with ice cold PBS and collected per centrifugation at 600 g for 5 min at 4°C. The organelles were isolated via differential centrifugation. Briefly, the collected pellets were re-dissolved in 2.0 mL of extraction buffer containing protease inhibitor cocktail (Cat. Nr: LYSISO1, Sigma-Aldrich, Switzerland) and incubated for 15 min on ice. The samples were then homogenized with a pre-chilled Dounce homogenizer (7 mL, tight pestle A, ~25 strokes) and centrifuged at 1000 g for 10 min at 4°C. After centrifugation, the pellet obtained was re-dissolved in 2 mL of a sucrose solution (0.25 M sucrose, 10 mM MgCl₂) and layered with 2 mL of a second hypertonic sucrose solution (0.55 M sucrose, 0.5 mM MgCl₂). The suspension was centrifuged twice at 1450 g and 4°C for 5 min. The pellet was re-suspended in 3 mL of the second sucrose solution and centrifuged at 1450 g and 4°C for 5 min.
to obtain the nuclear extract. These steps of the isolation procedure were monitored under phase contrast microscope on Menzel-Gläser coverslips (Olympus IX81 microscope). The supernatant phases removed during the isolation of nuclei were collected and formed the “residual” fraction. An aliquot of crude lysate after homogenization, nuclear and residual fraction was each used for protein quantification using the Bradford method. The isolated samples were then lyophilized on an Alpha 2-4 LD plus (CHRIST, Germany). The resulting samples underwent chemical digestion with 10 mL of a 2% nitrohydrochloric acid solution for 24 h. The resulting suspensions were filtered on 0.20 μm non-pyrogenic sterile Filtropur filters (Sarstedt, Switzerland) and the obtained samples were injected in ICP-MS.

ICP-MS Studies:
ICP-MS measurements were performed on an Agilent QQQ 8800 Triple quad ICP-MS spectrometer (Agilent Technologies, Switzerland) with an ASX200 autosampler (Agilent Technologies, Switzerland), equipped with standard nickel cones and a “micro-mist” quartz nebulizer fed with 0.3 mL/min analytic flow (as a 2 % HNO₃ aqueous solution). Platinum and zinc were measured against a platinum and zinc single element standard (Merck 1703410100 and Merck 1703890100, respectively) and verified by a control (Agilent 5188-6524 PA Tuning 2). Metals content of the samples was determined by means of a 9-step serial dilution in the range between 0 and 500 ppb in metals (R>0.99) with a background equivalent concentration of BEC: 8.4 ppt and a detection limit of DL: 20 ppt. The isotopes ¹⁹⁴Pt (32.97 % abundance), ¹⁹⁵Pt (33.83% abundance) and ⁶⁷Zn (4.1% abundance) were evaluated in “no-gas” mode and He-gas mode. Spiking the samples with untreated negative controls (to account for eventual carbon content from the biological samples) resulted in equivalent values within error ranges. A solution of indium (500 ppb) and tungsten (500 ppb) was used as internal standard. The results are expressed as ng metals / mg protein (correction due to the different mass of the observed cellular compartments), as mean ± standard deviation error of different independent experiments.

Results and Discussion:
To study the effect of a metal inside the center of the porphyrins tPt-H₂₄PyP, cPt-H₂₄PyP and dPt-H₂₄PyP, the complexes tPt-Zn₄PyP, tPt-Cu₄PyP, cPt-Zn₄PyP, cPt-Cu₄PyP, dPt-Zn₄PyP and dPt-Cu₄PyP were synthesized following the previously published procedures. Subsequently, these complexes were characterized by ¹H- and ¹⁹⁵Pt-NMR, IR, UV-Vis, MS and elemental analysis (Supporting Information). Additionally, the cationic unit of tPt-Zn₄PyP could be crystallized as the tetraphenylborate salt using the under-oil technique (see experimental part) and was studied by X-ray analysis (Figure 2). We are particularly pleased to obtain this structure, as this structure is the first example of expanding our robotic, aqueous crystallization trials towards organic solvents. The zinc metal center is in a square pyramidal geometry with one DMF molecule as the fifth ligand. This is
only the third crystallographic report about a porphyrin with an exocyclic, non-organometallically bound platinum within the porphyrin plane.\textsuperscript{24}

\textbf{Figure 2}: Ellipsoidal plot of the crystal structure of $[\textit{trans}\text{-PtCl(NH}_3\text{)}_2]_4\text{-5,10,15,20-tetra(4'-pyridyl)-zinc(II)porphyrin}$ tetraphenylborate $\cdot$ 7 DMF. Hydrogen atoms attached to a carbon atom and DMF molecules not coordinated to the zinc metal center were omitted for clarity.

The compounds were then tested in the HeLa cell line and the non-cancerous MRC-5 cell line composed of fibroblasts derived from lung tissue to assess their anti-proliferative properties. The results of the cytotoxicity studies of the compounds are presented in Table 1. The insertion of zinc into the complex $\text{tPt-H}_2\text{4PyP}$ to yield the zinc complex $\text{tPt-Zn4PyP}$ improves the phototoxicity and lowers the dark toxicity at the same time. Together these two effects improve the PI by almost a factor of five. These results can be attributed to the higher singlet oxygen quantum yield of $\text{tPt-Zn4PyP}$ compared to $\text{tPt-H}_2\text{4PyP}$ (Table 2) and are in line with previous reports about other (metallo)porphyrin containing photosensitizers.\textsuperscript{11-12}
| Complex                  | IC_{50} MRC5 dark (µM) | IC_{50} HeLa dark (µM) | IC_{50} HeLa 420 nm (µM) | PI (HeLa) | Reference   |
|-------------------------|------------------------|------------------------|--------------------------|-----------|-------------|
| photofrin               | n.d.                   | >41                    | 4.3 ± 0.2                | >9.5      | this work   |
| transplatin (tPt)       | 86 ± 8                 | ~130                   | n.d.                     | n.d.      | 25,26       |
| tPt-H_{2}PyP             | 93.4 ± 6.5             | 44.9 ± 8.0             | 0.037 ± 0.02             | 1210      | 8           |
| tPt-Zn_{4}PyP            | >100                   | >100                   | 0.017 ± 0.004            | >5882     | this work   |
| tPt-Cu_{4}PyP            | 82.5 ± 17.5            | 56.4 ± 6.5             | 14.1 ± 6.3               | 4.0       | this work   |
| cisplatin (cPt)          | 7.9 ± 1.2              | 11.5 ± 2.9             | 22.3 ± 5.7               | 0.52      | 8           |
| cPt + H_{2}PyP (1:1 ratio) | >100                   | >100                   | 5.78 ± 1.9               | >17       | 8           |
| cPt-H_{2}PyP             | 50.2 ± 0.6             | 35.4 ± 4.4             | 0.054 ± 0.01             | 655       | 8           |
| cPt-Zn_{4}PyP            | 65.0 ± 0.6             | 17.1 ± 5.1             | 0.25 ± 0.02              | 68.4      | this work   |
| cPt-Cu_{4}PyP            | >100                   | 13.9 ± 1.6             | >100                     | <0.14     | this work   |
| cisPtDMSO (dPt)          | >100                   | >100                   | >100                     | n.a.      | this work   |
| dPt-H_{2}PyP             | >100                   | >100                   | 0.15 ± 0.02              | >680      | 8           |
| dPt-Zn_{4}PyP            | >100                   | >100                   | 1.28 ± 0.4               | >78       | this work   |
| dPt-Cu_{4}PyP            | >100                   | 61.4 ± 1.5             | >100                     | <0.61     | this work   |

Table 1: Anti-proliferative effects of the various compounds on non-cancerous MRC5 and cancerous HeLa cells in the dark and upon irradiation; PI = phototoxic index, n.d. = not determined, n.a. = not applicable.

However, the zinc complexes cPt-Zn_{4}PyP and dPt-Zn_{4}PyP are less phototoxic than their corresponding metal-free porphyrins cPt-H_{2}PyP and dPt-H_{2}PyP. This difference in behaviour can be explained for cPt-Zn_{4}PyP. After the insertion of zinc, the complex becomes rather photosensitive, which leads to photo-bleaching under irradiation and therefore to a lower singlet oxygen quantum yield (Table 2). The higher phototoxic indexes of complexes tPt-H_{2}PyP, tPt-Zn_{4}PyP and tPt-Cu_{4}PyP compared with cPt-H_{2}PyP, cPt-Zn_{4}PyP and cPt-Cu_{4}PyP, respectively are explained further below. Furthermore, tPt-Cu_{4}PyP is, to the best of our knowledge, only the second ever reported copper containing PS\textsuperscript{7c}, which is phototoxic, as normally copper porphyrinoids are non-phototoxic. The DMSO containing complexes dPt-H_{2}PyP and dPt-Zn_{4}PyP have comparable PIs to the cisplatin derived...
complexes cPt-H$_2$4PyP and cPt-Zn4PyP. For a careful discussion on the influence of DMSO upon any platinum containing drug, the reader is directed to the excellent study by Gottesman.$^{27}$ The singlet oxygen quantum yields ($\Phi_\Delta$) of tPt-H$_2$4PyP, cPt-H$_2$4PyP, tPt-Zn4PyP and cPt-Zn4PyP are shown in Table 2.

|                  | $\Phi_\Delta$ (D$_2$O) |
|------------------|------------------------|
| tPt-H$_2$4PyP    | 0.65 ± 0.04            |
| tPt-Zn4PyP       | 0.74 ± 0.03            |
| cPt-H$_2$4PyP    | 0.65 ± 0.06            |
| cPt-Zn4PyP       | 0.47 ± 0.02            |
| dPt-H$_2$4PyP    | n.d. (insoluble)       |
| dPt-Zn4PyP       | n.d. (insoluble)       |

Table 2. Singlet oxygen quantum yields ($\Phi_\Delta$) of tPt-H$_2$4PyP, cPt-H$_2$4PyP, tPt-Zn4PyP and cPt-Zn4PyP determined by the direct method in D$_2$O with irradiation at 420 nm (TPPS as standard$^{28}$); n.d. = not determined. The $\Phi_\Delta$ of dPt-H$_2$4PyP and dPt-Zn4PyP could not be determined due to their insolubility in D$_2$O).

As previously reported by us, tPt-H$_2$4PyP reacts extremely slowly with guanosine, as the platination of N7 progressed to less than 50% completion within 10 days.$^8$ We speculate that the high charge (4+) of the cation of tPt-H$_2$4PyP might be responsible for this slow reaction, since the platinum chloride bond must be hydrolysed before the platinum can react with N7 of guanosine. The hydrolysis yields an intermediate that possesses an even higher charge of +5 (Figure S1). In order to test this hypothesis, we synthesized trans-[Pt(NH$_3$)$_2$(pyridine)Cl]NO$_3$ (tPt-Py)$^{29}$ and repeated the earlier mentioned reaction with guanosine (Figure 3). The reaction of tPt-Py and one equivalent of guanosine was monitored by $^1$H-NMR spectroscopy in a 1:1 DMF-d$_7$:D$_2$O mixture with a substance concentration of 618 µM. The spectra were recorded at intervals of 2.5 h, and 65 spectra were recorded in total. In the $^1$H-NMR spectrum of the free guanosine, the H8 and H1’ resonances are located at 8.11 and 5.95 ppm, respectively.$^8$ Binding of tPt-Py that has lost its chloride ion results in a downfield shift for the two signals: for H8 the new signal is located at 8.95 ppm and for H1’ the new signal (highlighted in bold in Figure 3) is located at 6.13 ppm. The recorded spectra are shown in Figure S2. The integrals of the signal at 5.95 ppm and the signals at 6.13 ppm are equal in the 27th spectrum recorded, showing that 50% of the binding occurred after nearly three days. Compared to previous results of [trans-PtCl(NH$_3$)$_2$]-5,10,15,20-tetra-(4'-pyridyl)-porphyrin nitrate, the reaction of tPt-Py is considerably faster. This supports the assumption that the rather slow reaction rate of tPt-H$_2$4PyP with guanosine is caused by unfavourable electrostatic interactions.
Figure 3: Reaction of trans-[Pt(NH$_3$)$_2$(pyridine)Cl]NO$_3$ (tPt-Py) with guanosine.

Since guanosine and tPt-Py react with a 1:1 stoichiometry, the assumption was made that the reaction can be treated as a second order reaction. For second order reactions, the inverse of the concentration vs. time plots results in a straight line, where the slope $S$ is equal to $k$, the reaction rate constant. This was applied to the signal at 5.95 ppm, where the decay was plotted as the inverse of the concentration vs. time (Figure S3). The values of the first six spectra were not considered due to a high background. The plotted values result in a straight line, showing that the reaction was a second order reaction. The determined slope $S$ is equal to a second order reaction constant $k$ of 0.0034 M$^{-1}$s$^{-1}$ ± 0.0001 M$^{-1}$s$^{-1}$. This value compares well with the pseudo first order reaction rate ($k_1$ of 0.00036 s$^{-1}$ ± 0.00002 s$^{-1}$) for the reaction of the mono aquated phenanthriplatin with 9-methylguanine.$^{30}$ If one takes the concentration of the excess 9-methylguanine into account, one can convert the pseudo first order reaction rate into a second order rate ($k_2$ of 0.012 M$^{-1}$s$^{-1}$ ± 0.007 M$^{-1}$s$^{-1}$). This rate is about 3.5 times faster than the determined rate of tPt-Py with guanosine, which is reasonable since tPt-Py must first be hydrolysed before it can react with guanosine.

Next, we investigated the uptake and distribution of the zinc complex in HeLa cells using ICP-MS. The expected ratio of zinc to platinum is 1:4, provided the compound tPt-Zn$_4$PyP does not disintegrate. To study the uptake, we first synthesized a highly enriched isotope $^{67}$Zn complex tPt-$^{67}$Zn$_4$PyP. Using the highly enriched zinc isotope allows to differentiate between naturally occurring zinc and $^{67}$Zn$^{31}$ that was introduced into the cells with compound tPt-$^{67}$Zn$_4$PyP. As $^{67}$Zn is expensive, initially, a careful and lengthy series of optimizations of the synthesis of zinc-5,10,15,20-tetra-(4'-pyridyl)-porphyrin starting from elemental zinc with a natural distribution of isotopes was performed (Table S2). Using these optimized conditions, the synthesis of tPt-Zn$_4$PyP was then repeated using elemental $^{67}$Zn, which delivered complex tPt-$^{67}$Zn$_4$PyP (Figures S4 - S9). The uptake study shows that only one quarter of all applied platinum entered the HeLa cells, whereas three quarters of $^{67}$Zn entered the cells (Figures S12 and S13). Subsequently, we studied the distribution of platinum and $^{67}$Zn in the different cell compartments (Tables 3 and S3).
Table 3. Pt and $^{67}\text{Zn}$ content in the different cellular compartments of HeLa cells treated for 4 h at 10 μM with complex $\text{tPt-}^{67}\text{Zn4PyP}$; results are expressed in nmol metal / mg protein.

|       | Nucleus | Non-nucleus |
|-------|---------|-------------|
| $^{67}\text{Zn}$ | 11.9 ± 4.4 | 11 ± 2.8 |
| Pt    | 3.8 ± 1.0 | 4.7 ± 1.7 |

In the nucleus, the ratio of $^{67}\text{Zn}$ to Pt was found to be 3:1 instead of 1:4. These results clearly demonstrate that the complex $\text{tPt-}^{67}\text{Zn4PyP}$ is disintegrated into the $^{67}\text{Zn}$ containing porphyrin and the relatively non-toxic mono-activated transplatin.$^{32}$ The analogous complex $\text{tPt-Zn4PyP}$ with the natural Zn isotope distribution is expected to behave the same as $\text{tPt-}^{67}\text{Zn4PyP}$. The transplatin moiety helps to improve solubility and transport the PS into the cell while not exhibiting a toxicity after the cleavage of the conjugate. Transplatin is known to be even faster effluxed from the cell than cisplatin.$^{33}$ This explains why there is less platinum found inside of the cells than $^{67}\text{Zn}$.

We also investigated the differences between the dark cytotoxicities of the trans-platinum series of compounds, $\text{tPt-H24PyP}$, $\text{tPt-Zn4PyP}$ and $\text{tPt-Cu4PyP}$ and the cis-platinum series, $\text{cPt-H24PyP}$, $\text{cPt-Zn4PyP}$ and $\text{cPt-Cu4PyP}$. The trans-platinum series generates, as described before, the trans-diamminemonochloroplatinum(II) moiety$^{34}$ after breakage of the N(pyridine)–platinum bond. This fragment has a much lower cytotoxicity than cis-diamminemonochloroplatinum(II), which is the first intermediate of cisplatin that must be generated in order to achieve any biological impact of cisplatin. Both, fragments of cis- and trans-diamminechloroplatinum(II) in the conjugates $\text{c/tPt-H24PyP}$, $\text{c/tPt-Zn4PyP}$ and $\text{c/tPt-Cu4PyP}$ serve as a cancer-cell penetrating vector. However, the trans moiety has a much lower dark toxicity after hydrolysis. The cis-diamminemonochloroplatinum(II) fragment has been previously used as a chemotoxic part of a dual acting reagent.$^{36}$ Our results imply that the trans-diamminemonochloroplatinum(II) conjugate would be even more interesting to study as well, maybe yielding in a photosensitizer with an improved PI. Odani and co-worker reported a higher phototoxic index for their cis complex with chlorido gallium inside of the porphyrin ring $\text{cPt-GaCl4PyP}$ than for the corresponding trans complex $\text{tPt-GaCl4PyP}$. Unfortunately, the authors of that study dissolved their platinum complexes in DMSO, which should never be done with chlorido-platinum complexes, as it leads after a chloride to DMSO exchange to the corresponding DMSO platinum complexes. These are known to behave very differently than the original chloride complexes; in all cases so far, they were reported to have a much lower cytotoxicity.$^{27}$

Next, we examined whether the compounds induce damages to the DNA. It is established, that platinum drugs form drug-DNA adducts, leading to DNA damage, which triggers cell cycle arrest and DNA repair.$^{35}$ The drug-induced DNA damage is followed by the phosphorylation of the histone H2AX,
which is involved in the recruiting and localizing of the DNA damage repair proteins. Therefore, \( \gamma \text{H2AX} \) is considered an important marker to observe DNA damage, in particular double strand breaks.\(^{36}\) To determine the effectiveness of light-triggered DNA damage by the metal porphyrin conjugates, we treated HeLa cells with 500 nM \( \text{tPt-H}_2\text{PyP} \) and \( \text{tPt-Zn}_4\text{PyP} \) for 14 h and subsequent light irradiation. Cells were then stained with \( \gamma \text{H2AX}-\text{specific Alexafluor 594 antibody} \) and visualized by confocal microscopy.

As shown in the immunofluorescent images (Figures 4 and S14), we could observe that \( \text{tPt-H}_2\text{PyP} \) and \( \text{tPt-Zn}_4\text{PyP} \) both induced severe DNA damage upon light irradiation, as observed by the increased accumulation of \( \gamma \text{H2AX} \). This means that \( \text{tPt-H}_2\text{PyP} \) and \( \text{tPt-Zn}_4\text{PyP} \) generate significantly more DNA damage after light irradiation compared with the experiments in the dark.

![Immunofluorescent images of HeLa cells treated with 500 nM \( \text{tPt-Zn}_4\text{PyP} \) for 14 h, then treated with light compared to the non-treated control. Cells were stained with \( \gamma \text{H2AX}-\text{Alexafluor 594 antibody} \) (green) and DAPI. \( \text{tPt-Zn}_4\text{PyP} \): 458 nm ex., 630-750 nm em.; \( \gamma \text{H2AX}-\text{Alexafluor 594} \): 594 nm ex., 610-630 em.](image)

**Figure 4:** DNA damage induced by light activated photosensitizer \( \text{tPt-Zn}_4\text{PyP} \). Immunofluorescent images of HeLa cells treated with 500 nM \( \text{tPt-Zn}_4\text{PyP} \) for 14 h, then treated with light compared to the non-treated control. Cells were stained with \( \gamma \text{H2AX}-\text{Alexafluor 594 antibody} \) (green) and DAPI. \( \text{tPt-Zn}_4\text{PyP} \): 458 nm ex., 630-750 nm em.; \( \gamma \text{H2AX}-\text{Alexafluor 594} \): 594 nm ex., 610-630 em.

To assess the intracellular localization of the photosensitizers, the compounds were co-stained with a marker for the Golgi organelle. \( \text{tPt-Zn}_4\text{PyP} \) co-localized in the Golgi apparatus (Figures 5 and S14). This experimental finding is pretty inspiring, as some reported drugs directed against the Golgi complex have been shown to be effective in both androgen-dependent and androgen-independent prostate cancer, through targeting abnormal glycosylation.\(^{37}\)
Figure 5: Co-localization of \( \texttt{tPt-Zn4PyP} \) with the Golgi apparatus. Representative images of live-cell imaging of HeLa cells treated with 50 \( \mu \text{M} \) \( \texttt{tPt-Zn4PyP} \) and stained with GOLGI-ID\textsuperscript{®} Green dye (ENZO ENZ-51028).

**Conclusions:**

In summary, the insertion of a zinc cation into the phototoxic photosensitizer \( \texttt{tPt-H24PyP} \) yielded an even superior PS \( \texttt{tPt-Zn4PyP} \) with a phototoxic index of higher than 5880. This is likely due to the higher singlet oxygen quantum yield (\( \Phi_\Delta \)) of the zinc porphyrin platinum complex \( \texttt{tPt-Zn4PyP} \) compared to the porphyrin platinum complex \( \texttt{tPt-H24PyP} \). The \(^1\text{H}-\text{NMR} \) experiments to follow the kinetics of the reaction of \( \textit{trans}-[\text{Pt}(\text{NH}_3)_2(\text{pyridine})\text{Cl}]\text{NO}_3 \) (\( \texttt{tPt-Py} \)) with guanosine revealed that the monocation \( \textit{trans}-[\text{Pt}(\text{NH}_3)_2(\text{pyridine})\text{Cl}]^+ \) reacts faster than the tetracation \( \texttt{tPt-H24PyP} \). The tetracation has to overcome the barrier of generating an even five-fold charged state during the reaction with N7 of the guanine base. ICP-MS studies of the \(^{67}\text{Zn} \) labelled complex \( \texttt{tPt-67Zn4PyP} \) showed that the \( \textit{trans}\)-diamminemonochloroplatinum(II) units of the complex \( \texttt{tPt-Zn4PyP} \) dissociate from the conjugate when localized inside the HeLa cells. For the first time, such a \(^{67}\text{Zn} \) labelled conjugate was employed to monitor the fate of the zinc conjugate within cells. The \( \textit{trans}\)-diamminemonochloroplatinum(II) unit is thought to act as a cell-penetrating vector for the photosensitizer, which permits its activation inside the cancer cell by light. We could demonstrate that within the cell, the PS co-localizes with the Golgi apparatus and additionally, induces DNA damage in the nucleus. Therefore, we propose the further investigation of transplatin-drug conjugates for PDT and other oncological treatments.

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**Electronic Supplementary Information:**

Synthetic procedures to yield platinated porphyrins $\text{tPt-Zn}_4\text{PyP}$ and $\text{tPt-Cu}_4\text{PyP}$, $\text{cPt-Zn}_4\text{PyP}$ and $\text{cPt-Cu}_4\text{PyP}$, $\text{dPt-Zn}_4\text{PyP}$ and $\text{dPt-Cu}_4\text{PyP}$ and trans-$[\text{Pt(NH}_3)_2(\text{pyridine})\text{Cl}]\text{NO}_3$ ($\text{tPt-Py}$), Tables S1 to S2 and Figures S1 to S14.

**Notes:**

The authors declare no competing financial interest.

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Supporting information for

Title: Studying the cellular distribution of highly phototoxic platinated metalloporphyrins using isotope labelling

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Content:

Synthetic procedures to yield platinated porphyrins \( \text{tPt-Zn4PyP, tPt-Cu4PyP, cPt-Zn4PyP, cPt-Cu4PyP, dPt-Zn4PyP, dPt-Cu4PyP and tPt-Py} \). Singlet oxygen quantum yield determination.

Table S1: Crystal data of \([\text{trans-PtCl(NH}_3\text{)}_2\text{]_4-5,10,15,20-tetra(4'-pyridyl)-zinc(II)porphyrin tetraphenylborate - 7 DMF}\)

Table S2: Optimisation of the zinc(II) insertion into 5,10,15,20-tetra-(4'-pyridyl)-porphyrin

Table S3: Pt and \( ^{67}\text{Zn} \) taken up in the different compartments of HeLa cells treated for 4 h at 10 \( \mu\text{M} \) with porphyrin \( \text{tPt-}^{67}\text{Zn4PyP} \); results expressed in ng metal / mg protein

Fig. S1: Reaction mechanism of the platination of a guanine nucleobase by platinated porphyrins

Fig. S2: Overview of the 65 recorded \(^1\text{H-NMR} \) spectra of \( \text{tPt-Py} \) reacting with one equivalent of guanosine in a 1:1 DMF-d\(_2\):D\(_2\)O mixture

Fig. S3: Inverse of the concentration vs. time plot for the signal at 5.95 ppm of the reaction of \( \text{tPt-Py} \) with guanosine in a 1:1 DMF-d\(_2\):D\(_2\)O mixture

Fig. S4: HR-MS-ESI of \( ^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)-porphyrin (}^{67}\text{Zn4PyP)} \)

Fig. S5: UV-Vis of \( ^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)-porphyrin (}^{67}\text{Zn4PyP)} \) in DMF

Fig. S6: HR-ESI-MS of \([\text{trans-PtCl(NH}_3\text{)}_2\text{]_4-}^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)porphyrin nitrate (}^{67}\text{Zn4PyP)} \)

Fig. S7: \(^1\text{H-NMR} \) of \([\text{trans-PtCl(NH}_3\text{)}_2\text{]_4-}^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)porphyrin nitrate (}^{67}\text{Zn4PyP)} \) in DMF

Fig. S8: \(^{13}\text{C-NMR} \) of \([\text{trans-PtCl(NH}_3\text{)}_2\text{]_4-}^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)porphyrin nitrate (}^{67}\text{Zn4PyP)} \) in DMF

Fig. S9: \(^{195}\text{Pt-NMR} \) of \([\text{trans-PtCl(NH}_3\text{)}_2\text{]_4-}^{67}\text{Zn(II)-5,10,15,20-tetra-(4'-pyridyl)porphyrin nitrate (}^{67}\text{Zn4PyP)} \) in DMF

Fig. S10: UV-Vis of \( \text{tPt-H}_2\text{4PyP, tPt-Zn4PyP, cPt-H}_2\text{4PyP, cPt-Zn4PyP, dPt-H}_2\text{4PyP, dPt-Zn4PyP} \) in DMF

Fig. S11: Example of \( ^{67}\text{zinc calibration curve in ICP-MS and quantification lines in no gas mode} \)

Fig. S12: Ratio of intra- and extracellular content of \( ^{67}\text{Zn and Pt after treatment of HeLa cells with} \( ^{67}\text{Zn4PyP} \)

Fig. S13: \( ^{67}\text{Zn and Pt biodistribution between nucleus and cytoplasmic fractions after treatment of HeLa cells with} \( ^{67}\text{Zn4PyP} \)

Fig. S14: Characterization of DNA double-strand breaks by \( \gamma\text{H2AX induced by light activated photosensitizer} \( ^{67}\text{H24PyP} \)

S2
Synthetic procedures to yield platinated porphyrins \( tPt-Zn4PyP \) and \( tPt-Cu4PyP \), \( cPt-Zn4PyP \) and \( cPt-Cu4PyP \), \( dPt-Zn4PyP \) and \( dPt-Cu4PyP \) and \( trans-[Pt(NH3)2(pyridine)Cl]NO3 \) (\( tPt-Py \))

5,10,15,20-tetra(4'-pyridyl) porphyrin zinc (\( Zn4PyP \))\(^1\)

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{Zn} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\]

To a solution of \( H_{24}PyP \) (0.032 mmol, 20.0 mg) in DMF:water (1:1, 3.0 mL) Zn(OAc)\( \_2 \) \( \cdot \) 2 H\text{H}2O (0.081 mmol, 18.0 mg) was slowly added. The solution was refluxed for 60 min (completion of reaction was monitored by HPLC) and then cooled to room temperature. Water was added to the solution to precipitate the product, which was further filtered and washed thoroughly with water, methanol and ether. The resulting violet solid product was obtained in 18.0 mg, 82 % yield. IR (KBr, cm\(^{-1}\)): 1604, 1594, 1409, 1338, 1205, 1072, 992, 790, 669; MS (ESI): \( m/z = 681 \) \([M + H]^+\)

5,10,15,20-tetra(4'-pyridyl) porphyrin copper (\( Cu4PyP \))\(^{1b}\)

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{Cu} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\]

To a solution of \( H_{24}PyP \) (0.032 mmol, 20.0 mg) in acetic acid (3.0 mL) Cu(OAc)\( \_2 \) \( \cdot \) H\text{H}2O (0.202 mmol, 40.4 mg) dissolved in water (0.4 mL) was added. The solution was refluxed for 60 min (completion of reaction by monitored by HPLC) and then cooled to room temperature. Ammonia (25 %, 3.0 mL) was added to neutralize the solution and precipitate the product, which was further filtered and washed thoroughly with water, methanol and ether. The resulting violet solid product was obtained in 17.0 mg, 77 % yield. IR (KBr, cm\(^{-1}\)): 1590, 1404, 1347, 1002, 990, 798, 671; MS (ESI): \( m/z = 680 \) \([M + H]^+\)
[trans-PtCl(NH3)2]4-5,10,15,20-tetra(4'-pyridyl)-zinc(II)porphyrin nitrate (tPt-Zn4PyP)

Transplatin (0.117 mmol, 35.3 mg) and silver nitrate (0.117 mmol, 19.9 mg) were dissolved in 2.0 mL of DMF and stirred in the dark at room temperature for 24 h. The resulted turbid solution was centrifuged to remove the white silver chloride. The light yellow colored solution was added to suspension of Zn4PyP (0.029 mmol, 20.1 mg) in 2.0 mL DMF and stirred in the dark at 50°C for 24 h. After that, the mixture was precipitated with diethyl ether. The solid was filtered and further washed with methanol, dichloromethane and diethyl ether. The solid was further dried in vacuum and the product was obtained in 51.0 mg, 87 % yield. IR (KBr, cm⁻¹): 3186, 3107, 1614, 1313, 994, 800, 720, 662; ¹H NMR (400 MHz; DMF-d7): δ 9.42 (d, 8H, J = 6.4 Hz), 9.06 (s, 8H), 8.50 (d, 8H, J = 6.5 Hz), 4.79 (s, 24H); ¹⁹⁵Pt NMR (107 MHz; DMF-d7): δ -2309; MS (ESI): m/z = 1272 [M - 3(NO3) - 2{PtCl(NH3)2}]+, 945 [M - 4(NO3) - 3{PtCl(NH3)2}]+, 892 [M - 4(NO3) - 3{PtCl(NH3)2} - Cl - NH3]+, 873 [M - 4(NO3) - 3{PtCl(NH3)2} - Cl - 2(NH3)]+, 605 [M - 4(NO3) - 2{PtCl(NH3)2}]²⁺; Elemental analysis calcd (%) for C₄₀H₄₈Cl₄N₂₀O₁₂Pt₄Zn: C 23.73, H 2.59, N 13.84; found: C 23.34, H 2.40, N 13.52.

[trans-PtCl(NH3)2]4-5,10,15,20-tetra(4'-pyridyl)-copper(II)porphyrin nitrate (tPt-Cu4PyP)

Transplatin (0.058 mmol, 17.7 mg) and silver nitrate (0.058 mmol, 9.9 mg) were dissolved in 1.0 mL of DMF and stirred at room temperature in the dark for 24 h. The resulted turbid solution was centrifuged to remove the white silver chloride. The light yellow colored solution was added to suspension of
**Cu4PyP** (0.015 mmol, 9.9 mg) in 1.0 mL DMF and stirred at 50°C in the dark for 24 h. After that, the mixture was cooled down to room temperature and precipitated with diethyl ether. The solid was filtered and further washed with methanol, dichloromethane and diethyl ether. The solid was further dried in vacuum and the product was obtained in 23.0 mg, 79 % yield. IR (KBr, cm⁻¹): 3183, 3105, 1617, 1320, 999, 803, 721, 659; MS (ESI): \( m/z = 1271 \) [M – 3(NO₃) – 2{PtCl(NH₃)₂}⁺], 944 [M – 4(NO₃) – 3{PtCl(NH₃)₂}]⁺, 891 [M – 4(NO₃) – 3{PtCl(NH₃)₂} – Cl – NH₃]⁺, 604 [M – 4(NO₃) – 2{PtCl(NH₃)₂}]²⁺; Elemental analysis calcd (%) for C₄₀H₄₈Cl₄N₂₀O₁₂Pt₄Cu: C 24.18, H 2.44, N 14.10; found: C 24.39, H 2.48, N 14.12.

\[ \text{[cis-PtCl(NH₃)₂]}₄\cdot{}5,10,15,20\text{-tetra(4'-pyridyl)-zinc(II)porphyrin nitrate (cPt-Zn4PyP)} \]

Cisplatin (0.029 mmol, 8.8 mg) and silver nitrate (0.029 mmol, 5.0 mg) were dissolved in 0.5 mL of DMF and stirred at room temperature in the dark for 24 h. The resulted turbid solution was centrifuged to remove the white silver chloride. The light yellow colored solution was added to suspension of Zn4PyP (0.007 mmol, 5.0 mg) in 0.5 mL DMF and stirred at 50°C in the dark for 24 h. After that, the mixture was cooled down to room temperature and precipitated with diethyl ether. The solid was filtered and further washed with methanol, dichloromethane and diethylether. The solid was further dried in vacuum and the product was obtained in 12.0 mg, 82 % yield. IR (KBr, cm⁻¹): 3202, 3111, 1614, 1312, 994, 795, 718, 662; \(^1\)H NMR (500 MHz; DMF-d₇): \( δ = 9.32 \) (d, 8H, \( J = 4.7 \) Hz), 9.01 (s, 8H), 8.48 (d, 8H, \( J = 4.2 \) Hz), 5.29 (s, 12H), 4.73 (s, 12H); \(^{195}\)Pt NMR (107 MHz; DMF-d₇): \( δ = -2292.6 \) ppm; MS (ESI): \( m/z = 1510.3 \) [M – 4(NO₃) – {PtCl(NH₃)₂} + Cl]⁺, 1228.6 [M – 4(NO₃) – 2{PtCl(NH₃)₂} – NH₃ + Cl]⁺, 910 [M – 4(NO₃) – 3{PtCl(NH₃)₂} – Cl]⁺; Elemental analysis calcd (%) for C₄₀H₄₈Cl₄N₂₀O₁₂Pt₄Zn·(MeOH)·(DMF): C 25.24, H 2.84, N 14.05; found: C 25.26, H 2.60, N 13.77.
Cisplatin (0.029 mmol, 8.8 mg) and silver nitrate (0.029 mmol, 5.0 mg) were dissolved in 0.5 mL of DMF and stirred in the dark at room temperature for 24 h. The resulted turbid solution was centrifuged to remove the white silver chloride. The light yellow colored solution was added to suspension of Cu4PyP (0.0074 mmol, 5.0 mg) in 0.5 mL DMF and stirred in the dark at 50°C for 24 h. After that, the mixture was cooled down to room temperature and precipitated with diethyl ether. The solid was filtered and further washed with methanol, dichloromethane and diethyl ether. The solid was further dried in vacuum and the product was obtained in 11.0 mg, 74 % yield. IR (KBr, cm⁻¹): 3190, 3107, 1614, 1305, 999, 797, 716, 660; MS (ESI): m/z = 1509.3 [M – 4(NO₃) – {PtCl(NH₃)₂} + Cl]⁺, 1226.6 [M – 4(NO₃) – 2{PtCl(NH₃)₂} – NH₃ + Cl]⁺, 909 [M – 4(NO₃) – 3{PtCl(NH₃)₂} – Cl]⁺; Elemental analysis calcd (%) for C₄₀H₄₈Cl₄N₂₀O₁₂Pt₄Cu·(MeOH)₀.₄(H₂O)₁₅: C 23.94, H 2.62, N 13.82; found: C 23.99, H 2.29, N 13.51.

Cis-PtCl(NH₃)₂|₄·5,10,15,20-tetra(4'-pyridyl)-copper(II)porphyrin nitrate (cPt-Cu₄PyP)

[trans-PtCl₅(DMSO)]₄·5,10,15,20-tetra(4'-pyridyl)-zinc(II)porphyrin (dPt-Zn₄PyP)

cis-Pt(DMSO)₂Cl₂ (0.058 mmol, 24.7 mg) and Zn₄PyP (0.015 mmol, 10.0 mg) were dissolved in 5.0 mL of dichloromethane and stirred in the dark for 4 h at 50°C. The reaction mixture was centrifuged...
and solvent was removed. Subsequently 2.0 mL of dichloromethane were added before the mixture was centrifuged and the solvent was removed again (to remove unreacted $cis$-$\text{Pt(DMSO)}_2\text{Cl}_2$). The remaining solvent was removed in vacuo. The solid was further dried and the product was obtained in 27.0 mg, 89 \% yield. IR (KBr, cm$^{-1}$): 1611, 1418, 1145, 1017, 995, 791, 695; $^1$H NMR (400 MHz; DMF-$d_7$): $\delta$ 9.25 (dd, 8H, $J = 5.2, 1.2$ Hz), 9.04 (s, 8H), 8.62 (d, 8H, $J = 5.2, 1.4$ Hz), 3.65 (s, 24H); $^{195}\text{Pt}$ NMR (107 MHz; DMF-$d_7$): $\delta$ -3050; MS (ESI): $m/z$ = 2094.5 [M + K]$^+$, 2080.6 [M + Na]$^+$, 1752 [M – Pt – DMSO – 2Cl + K]$^+$, 1736 [M – Pt – DMSO – 2Cl + Na]$^+$, 1408 [M – 2Pt – 2DMSO – 4Cl + K]$^+$, 1392 [M – 2Pt – 2(DMSO) – 4Cl + Na]$^+$, 1064 [M – 3 Pt – 4(DMSO) – 6Cl + K]$^+$; Elemental analysis calcd (%) for C$_{48}$H$_{48}$Cl$_8$N$_8$O$_4$Pt$_4$S$_4$Zn: C 28.01, H 2.35, N 5.44; found: C 27.77, H 2.33, N 5.29.

[trans-$\text{PtCl(DMSO)}_2$]$_4$-5,10,15,20-tetra(4'-pyridyl)-copper(II)porphyrin (dPt-Cu4PyP)

$cis$-$\text{Pt(DMSO)}_2\text{Cl}_2$ (0.064 mmol, 27.3 mg) and Cu4PyP (0.016 mmol, 11.0 mg) were dissolved in 5.0 mL of dichloromethane and stirred in the dark for 4 h at 50°C. The reaction mixture was centrifuged and solvent was removed. Subsequently 2.0 mL of dichloromethane were added before the mixture was centrifuged and the solvent was removed again (to remove unreacted $cis$-$\text{Pt(DMSO)}_2\text{Cl}_2$). The remaining solvent was removed in vacuo. The solid was further dried and the product was obtained in 32.0 mg, 92 \% yield. IR (KBr, cm$^{-1}$): 1610, 1417, 1147, 1019, 999, 696; MS (ESI): $m/z$ = 2015.7 [M-Cl]$^+$, 1752 [M – Pt – DMSO – 2Cl + K]$^+$, 1674.0 [M – Pt – 2(DMSO) – 2Cl + K]$^+$, 1408 [M – 2Pt – 2(DMSO) – 4Cl + K]$^+$, 1330 [M – 2 Pt – 3(DMSO) – 4Cl + K]$^+$, 1064 [M – 3Pt – 4(DMSO) – 6Cl + K]$^+$; Elemental analysis calcd (%) for C$_{48}$H$_{48}$Cl$_8$N$_8$O$_4$Pt$_4$S$_4$Cu: C 28.03, H 2.35, N 5.45; found: C 27.95, H 2.31, N 5.37.
$\text{trans-}[\text{Pt(NH}_3\text{)}_2(\text{pyridine})\text{Cl}]\text{NO}_3$ ($\text{tPt-Py}$)$_2$

Transplatin (0.331 mmol, 100.0 mg) and silver nitrate (0.331 mmol, 56.0 mg) were dissolved in 5.0 mL of DMF and stirred in the dark at 50°C for 24 h. The resulted turbid solution was centrifuged to remove the white silver chloride. The light yellow colored solution was added to pyridine (24.0 µL, 0.302 mmol) and stirred at 50°C in the dark for 24 h. After that, the mixture was cooled down to room temperature and the solvent was evaporated with the aid of a rotary evaporator. The residue was dissolved in 10 mL methanol and subsequently filtered. Diethyl ether was added to the filtrate, the formed precipitate was collected and washed with diethyl ether. The precipitate was dissolved again in methanol and added dropwise to vigorously stirred diethyl ether to obtain $\text{tPt-Py}$ after collecting and drying in vacuum as a white solid in 65.0 mg, 49 % yield. IR (Golden-Gate, cm$^{-1}$): 3260, 3119, 1652, 1611, 1586, 1456, 1346, 1304, 1242, 1162, 1078, 1021, 944, 871, 824, 760, 687, 661. $^1$H-NMR (400 MHz; DMF-d$_7$): 9.00 ($dd$, $J = 6.6, 1.5$ Hz, 2H, 2 x arom. N-CH); 8.15 ($t$, $J = 7.7$ Hz, 1H, 1 x arom. N-CH-CH-$CH$); 7.69 ($t$, $J = 7.0$ Hz, 2H, 2 x arom. N-CH-$CH$); 4.52 (broad. s, 6H, 2 x NH$_3$). $^{13}$C-NMR (125 MHz; DMF-d$_7$): 154.1 ($d$, 2 x arom. N-CH); 149.5 ($d$, 1 x arom. N-CH-CH-CH); 127.1 ($d$, 2 x arom. N-CH-CH). $^{195}$Pt-NMR (107 MHz; DMF-d$_7$): -2306 (s, Pt). MS (ESI): $m/z = 343$ [M – NO$_3$]$^+$. Elemental analysis calcd (%) for C$_5$H$_{11}$ClN$_4$O$_3$Pt: C 14.80, H 2.73, N 13.81; found: C 14.89, H 2.70, N 13.68.
Singlet oxygen quantum yields:

The singlet oxygen quantum yields (ΦΔ) of \( \text{tPt-H}_2\text{4PyP}, \ c\text{Pt-H}_2\text{4PyP}, \ \text{tPt-Zn}_4\text{PyP} \) and \( \text{cPt-Zn}_4\text{PyP} \) were determined analogously to a method reported by our group. The PSs were dissolved in D\text{2}O and the solutions were placed in a glass cuvette (114F-10-40, 10 mm × 4 mm dimensions, Hellma Analytics, Germany). Two different concentrations were prepared for each PS, corresponding to maximum absorption intensities of approximately 1.0 and 0.5, with the cuvette oriented in a way that the light path equals to 10 mm. The cuvettes containing solutions of the same concentration as used for the corresponding UV-Vis spectroscopy were placed in a CUV-UV/VIS-TC-ABS temperature-controlled Qpod sample compartment (Avantes, The Netherlands) and cooled to 20°C; temperature control was performed with the use of a TC-125 controller (Quantum Northwest, USA) and Q-Blue software. Emission spectroscopy was conducted in a custom-built setup, that is based on the setup described by Bonnet and co-workers. Excitation was performed using a high-power-LED 420 nm light source (FC5-LED-WL, Prizmatix Ltd., Israel). The light source was connected with an optical fibre (1000 \( \mu \text{m} \) core diameter, Avantes) to the cuvette holder over a SMA 905 fibre optic connector. The intensity of the light source was measured to be 22.8 mW / cm\(^2\) at the position of the cuvette. The excitation power was measured using a S310C thermal sensor connected to a PM100USB power meter (Thorlabs, Germany). The connection piece used to insert the SMA connector into the cuvette holder was replaced by an in-house custom-built connection piece that allows the fibre to be inserted at a distance of 2.0 cm from the cuvette. The detector (AvaSpec-NIR256-1.7TEC, Avantes) was set to 0°C and connected to the cuvette holder with an optical fibre (600 mm diameter, Avantes). Emission spectra were collected at a 90° angle with respect to the excitation beam from 1050 nm to 1500 nm after two measurement runs; every measurement run consisted of five averaged measurements each lasting 9 s. All spectra were recorded using AvaSoft 8.9 software from Avantes and further processed using Microsoft Office Excel and Origin 2018 software.

The singlet oxygen quantum yields were calculated by comparison with meso-tetrakis(4'-sulphonatophenyl)porphine tetraammonium (TPPS), according to equation E1. It was assumed that the singlet oxygen quantum yield of this compound is identical to the one of the analogous tetrasodium salt (\( \Phi_{\Delta} = 0.62 \) in H\text{2}O\text{5}).

\[
\Phi_{\Delta(x)} = \Phi_{\Delta(\text{std})} \left( \frac{I_{420}}{I_{420x}} \right) \left( \frac{E_x}{E_{\text{std}}} \right)
\]

In E1, the subscript “x” designates the corresponding photosensitizer and “std” the standard (TPPS), respectively. “\( \Phi_{\Delta} \)” is the singlet oxygen quantum yield. “\( I_{420x} \)” is the rate of light absorption calculated as overlap of the absorption spectrum of either PS or standard and the emission spectrum of the LED light source at 420 nm (\( I_0 \)). The absorption intensity depends exponentially on absorbance A (E2). For A, the measured absorbance values were scaled by a factor of 0.4. E is the integrated emission peak of
singlet oxygen at around 1270 nm. For these emission spectra, the integrated values were obtained by applying a manual background correction in Origin.

\[ I_{420} = I_0 (1 - 10^{-A}) \]  
(E2)

E1 can be rewritten as:

\[ \Phi_{\Delta(x)} = \Phi_{\Delta(\text{std})} \left( \frac{S_x}{S_{\text{std}}} \right) \]  
(E3)

In E3, “S” designates the slope when “E” is plotted against “I_{420}” for the two measured concentrations, with a fixed intercept at 0. Errors of the singlet oxygen quantum yields were calculated by error propagation from the standard errors of “S_x” and “S_{std}”.
Table S1: Crystal data of [trans-PtCl(NH₃)₂]⁺₅,10,15,20-tetra(4'-pyridyl)-zinc(II)porphyrin tetraphenylborate • 7 DMF

| Property                                      | Value                   |
|-----------------------------------------------|-------------------------|
| Empirical formula                             | C₁₆₀H₁₈₄B₄Cl₄N₂₄O₈Pt₄Zn |
| Formula weight                                | 3602.07                 |
| Crystal system                                | Triclinic               |
| Space group                                   | P-1                     |
| a [Å]                                         | 12.1513(2)              |
| b [Å]                                         | 17.9898(2)              |
| c [Å]                                         | 19.21888(13)            |
| α [°]                                         | 77.5748(8)              |
| β [°]                                         | 80.9900(10)             |
| γ [°]                                         | 89.3233(12)             |
| Volume [Å³]                                   | 4051.16(9)              |
| Z                                             | 1                       |
| Density (calculated) [Mg/m³]                  | 1.476                   |
| Temperature [K]                               | 160.00(10)              |
| Wavelength [Å]                                | 1.54184                 |
| Absorption coefficient [mm⁻¹]                 | 7.556                   |
| F(000)                                        | 1806                    |
| Crystal size [mm³]                            | 0.321 x 0.034 x 0.03    |
| Crystal description                           | red needle              |
| Theta range for data collection [°]           | 3.684 to 80.096         |
| Index ranges                                  | -15≤h≤15, -22≤k≤22, -23≤l≤24 |
| Reflections collected                         | 144826                  |
| Independent reflections                       | 17213 [R(int) = 0.0697] |
| Reflections observed                          | 15220                   |
| Criterion for observation                     | I > 2 σ (I)             |
| Completeness to theta                         | 99.9 % to 67.684°       |
| Absorption correction                         | Gaussian                |
| Max. and min. transmission                    | 1.000 and 0.204         |
| Data / restraints / parameters                | 17213 / 104 / 950       |
| Goodness-of-fit on F²                         | 1.037                   |
| Final R indices [I > 2 σ (I)]                 | R1 = 0.0684, wR2 = 0.1917 |
| R indices (all data)                          | R1 = 0.0736, wR2 = 0.1964 |
| Largest diff. peak and hole [e.Å⁻³]           | 6.611 and -2.481        |
Table S2: Optimisation of the zinc(II) insertion into H$_4$PyP

| Zinc(II) salt       | Equivalents of Zn(II) | Time | Yield  |
|---------------------|-----------------------|------|--------|
| Zn(OAc)$_2$·2 H$_2$O | 6                     | 48 h | 88 %   |
| ZnCl$_2$·2 H$_2$O   | 6                     | 26 h | 96 %   |
| ZnCl$_2$·2 H$_2$O   | 5                     | 26 h | 87 %   |
| ZnCl$_2$·2 H$_2$O   | 4                     | 26 h | 90 %   |

The reactions were monitored by UV-Vis spectroscopy and $^1$H-NMR.

Table S3: Pt and $^{67}$Zn taken up in the different compartments of HeLa cells treated for 4 h at 10 μM with porphyrin t$^{15}$Pt-$^{67}$Zn$_4$PyP; results expressed in ng metal / mg protein

|        | Nucleus | Residual | Total  |
|--------|---------|----------|--------|
| $^{67}$Zn | 795 ± 298 | 763 ± 190 | 1558 ± 23 |
| Pt      | 745 ± 200 | 926 ± 326 | 1671 ± 345 |
Fig. S1: Reaction mechanism of the platination of a guanine nucleobase by platinated porphyrins

\[
\text{M = H}_2, \text{M}'
\]
Fig. S2: Overview of the 65 recorded $^1$H-NMR spectra of tPt-Py reacting with one equivalent of guanosine in a 1:1 DMF-d$_7$:D$_2$O mixture
Fig. S3: Inverse of the concentration vs. time plot for the signal at 5.95 ppm of the reaction of tPt-Py with guanosine in a 1:1 DMF-d7:D2O mixture

**Equation:** \( y = a + b \times x \)

| Plot        | 1/1          |
|-------------|--------------|
| Weight      | No Weighting |
| Intercept   | 2230.41066 ± 35.19462 |
| Slope       | 0.00343 ± 1.12831E-4 |
| Residual Sum of Squares | 1.09538E6 |
| Pearson’s r  | 0.97911   |
| R-Square(COD) | 0.94092  |
| Adj. R-Square | 0.9399  |
Fig. S4: HR-ESI-MS of $^{67}$Zn$^4$PyP

Fig. S5: UV-Vis of $^{67}$Zn$^4$PyP in DMF
Fig. S6: HR-ESI-MS of \( {\text{tPt}}^{67}\text{Zn4PyP} \)

Fig. S7: \( \text{\textsuperscript{1}H-NMR of tPt}^{67}\text{Zn4PyP} \) in DMF-d\textsubscript{7}
Fig. S8: $^{13}$C-NMR of tPt-$^{67}$Zn$_4$PyP in DMF-d$_7$.

Fig. S9: $^{195}$Pt-NMR of tPt-$^{67}$Zn$_4$PyP in DMF-d$_7$. 
Fig. S10: UV-Vis of tPt-H24PyP, tPt-Zn4PyP, cPt-H24PyP, cPt-Zn4PyP, dPt-H24PyP, dPt-Zn4PyP in DMF
Fig. S11: Example of $^{67}\text{Zn}$ calibration curve in ICP-MS and quantification lines (67, 194 and 195) in no gas mode.

Indium and tungsten were used as internal standard.

Fig. S12: Ratio of intra- and extracellular content of $^{67}\text{Zn}$ and Pt after treatment of HeLa cells with $t\text{Pt-}^{67}\text{Zn4PyP}$

HeLa cells were treated with 10 µM of target porphyrin $t\text{Pt-}^{67}\text{Zn4PyP}$ for 4 h. Left panel: Total $^{67}\text{Zn}$ content in the treatment solution (whole pie) and $^{67}\text{zinc}$ content detected in the cell (dark gray part of the pie); Right panel: Total platinum content in the treatment solution (whole pie) and platinum content detected in the cell (dark gray part of the pie).
Fig. S13: $^{67}$Zn and Pt biodistribution between nucleus and cytoplasmic fractions after treatment of HeLa cells with tPt-$^{67}$Zn4PyP

HeLa cells were treated with 10 µM tPt-$^{67}$Zn4PyP for 4 h. Left panel: $^{67}$Zinc biodistribution between nuclear and cytoplasmic fractions; Right panel: Platinum biodistribution between nuclear and cytoplasmic fractions.
Fig. S14: DNA damage induced by light activated photosensitizer $tPt-H_24PyP$.

Immunofluorescence images of HeLa cells treated with 500 nM of $tPt-H_24PyP$ for 14 h, followed by light irradiation, compared to unirradiated control. Cells were stained with $\gamma H2AX$-Alexafluor 594 antibody (green) and DAPI. ($tPt-H_24PyP$: 458 nm ex., 650-750 nm em.; $\gamma H2AX$-Alexafluor 594: 594 nm ex., 610-650 nm em.).

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