Electronic Supplementary Information

for

Non-aggregated lipophilic water-soluble tin porphyrins as photosensitizers for PDT and PACT

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Materials and methods

All reagents and solvents were procured from Sigma Aldrich. All solvents used were of analytical grade and were purified and dried by routine procedures immediately before use. ^{1}H NMR spectra were recorded with a Bruker 600 MHz instrument using trimethylsilane (TMS) as an internal standard. UV-visible absorption spectra were measured on a Shimadzu UV−2550 spectrophotometer. MALDI-TOF mass spectra were recorded on Bruker® AutoFLEX III Smart-beam TOF/TOF mass spectrometer by using α-cyano-4-hydroxycinnamic acid as the matrix.

Steady state fluorescence spectra were obtained with a Varian Cary-Eclipse spectrofluorimeter. The fluorescence quantum yield (Φ_F) values were estimated from the emission and absorption spectra by using the comparative method with ZnTPP (Φ_F = 0.039 in DMSO[1]) as the standard.[2] Fluorescence lifetimes were measured by time-correlated single photon counting (TCSPC) (FluoTime 300, Picoquant GmbH) with a diode laser (LDH-P-420, Picoquant_GmbH, 20 MHz repetition rate, 44 ps pulse width). Singlet oxygen quantum yield (Φ_Δ) values were determined by photooxidation of 9,10-anthracenediyl-bis(methylene)dimalonic acid (ADMA) in dimethylsulfoxide (DMSO) and water by using a comparative method.[3] Rose Bengal (RB) was used as the reference.[4] Triplet state lifetimes were determined in nitrogen saturated DMSO and aqueous solutions at 500 nm by using an Edinburgh Instruments LP980 spectrometer and a pump beam of 425 nm provided by an Ekspla NT-342B laser (2.0 mJ/7 ns, 20 Hz).

Synthesis and Experimental Section

Trans-dihydroxo[*meso-tetrakis*(4-pyridyl)porphyrinato] tin(IV) [Sn(IV)(TPyP)$(OH)_2$] (**3**), trans-*bis*(2-napthlato) [*meso*-tetrakis (4-pyridiyl)porphyrinato] tin(IV) (**4**) were prepared by following a previously reported literature method.[5]

***Trans-bis*(2-napthlato)[*meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrinato] tin(IV)-SnM (1)**

4 (102 mg, 0.1 mM) and iodomethane (0.25 mL, 20 mM) were dissolved in dry DMF and refluxed for 24 h under a N_2 atmosphere. The reaction mixture was cooled, and excess diethyl ether was added to precipitate out **SnM** (**1**) as a purple solid. The product was filtered, washed with diethyl ether and dried.

Yield: 131 mg (83%). MS (MALDI-TOF): m/z calcd for [Sn-(2-napthalato)$_2$-CH_3] 780.50; found 779.95, [Sn-(2-napthalato)$_2$-2CH_3] 765.47; found 765.92, [Sn-(2-napthalato)$_2$-3CH_3] 751.14 ; found 752.18, [Sn-(2-napthalato)$_2$-4CH_3] 736.11; found 736.45. ^{1}H NMR (600 MHz; D_2O): 9.59 (s, 8H, β), 9.44(d, *J* = 6.1 Hz, 8H, pyridyl), 9.15 (d, *J* = 6.1 Hz, 8H, pyridyl), 4.87 (s, 12H,N-CH_3), 3.22 (s, 6H, naptholate), 3.04 (s, 2H, naptholate), 2.89 (s, 4H, naptholate), 2.75 (s, 4H, naptholate) ppm.

***Trans-bis*(2-napthlato)[*meso*-tetrakis(*N*-n-hexylpyridinium-4-yl)porphyrinato] tin(IV)-SnH (2)**

4 (102 mg, 0.1 mM) and 1-iodohexane (2.9 mL, 20 mM) were dissolved in dry DMF and refluxed for 24 h under a N_2 atmosphere. The reaction mixture was cooled, and excess diethyl ether was added to precipitate out **SnH** (**2**) as a purple solid. The product was filtered, washed with diethyl ether and dried.

Yield: 142 mg (76%). MS (MALDI-TOF): m/z calcd for [Sn-(2-napthalato)$_2$-2C_6H_{13}] 905.74; found 905.48, [Sn-(2-napthalato)$_2$-3C_6H_{13}] 820.57; found 820.26, [Sn-(2-napthalato)$_2$-4C_6H_{13}] 736.11; found 736.62. ^{1}H NMR (600 MHz; D_2O): 9.55 (s, 8H, β-pyrrolic H), 9.50 (d, *J* = 6.4 Hz, 8H, pyridyl), 9.15 (d, *J* = 6.5 Hz, 8H, pyridyl), 5.09 (t, *J* = 7.3 Hz, 8H, N-CH_2-), 3.05 (s, 2H, naptholate), 2.89 (s, 2H, naptholate), 2.75 (s, 10H, naptholate), 2.43 (m, 8H, -CH_2-), 1.70 (m, 8H, -CH_2-), 1.57 (m, 8H, -CH_2-), 1.50 (m, 8H, -CH_2-), 1.02 (t, 12H, -CH_3) ppm.

n-Octanol/H_2O partition coefficients

The n-octanol/H_2O partition coefficients were measured by following the shake-flask method.[6] The Sn(IV)porphyrins were dissolved in 10 mL of n-octanol. The absorbance of the Soret band of this solution was measured (A_o). 3 mL of this solution was mixed with 3 mL water and stirred for 4 h at room temperature. The mixture was allowed to form two separate solvent layers. The octanol layer was collected, centrifuged and the absorbance of the Soret band was measured (A_{final}). From this, $A_w = A_o - A_{final}$ is calculated. Partition coefficients (log $P_{o/w}$) were calculated on the basis of $\log(A_o / A_w)$.

Biological studies

Cell culture

MCF-7 (human breast adenocarcinoma) cells were cultured in Dulbecco's modified Eagle's medium-10% fetal bovine serum (DMEM-FBS), 100 unit.mL^{-1} of penicillin, 100 μg/mL of streptomycin and 2.0 mM of Glutamax at 37 °C in an incubator (5% CO_2). The cultures were grown as a monolayer. They were passaged once over 4−5 days by trypsinizing with 0.25% trypsin-EDTA.

Cell cytotoxicity studies

Cytotoxicity of the Sn(IV)porphyrins (**SnM** and **SnH**) was assessed by using the standard protocols of the MTT assay.[7] The tetrazolium ring of MTT is cleaved by mitochondrial reductases in viable cells to form formazan crystals which are solubilised in DMSO so that cell death can be estimated by measuring absorbance at 540 nm. The concentration of formazan formed hence provides a measure of the number of viable cells.

Approximately, 1×10^4 cells were seeded in a 96-well culture plate in DMEM containing 10% FBS and allowed to adhere overnight. Sn(IV)porphyrins of different concentrations (0.78−50 μM) in DMEM were added to the cells and the solutions were incubated for 24 h in the dark. The medium was replaced with PBS and photoirradiated with a Thorlabs M595L3 595 nm light emitting diode (LED) for 30 min (450 J.cm^{-2}). The LED was mounted into the housing of a Modulight 7710-680 medical laser system. An irradiance value of 250 mW.cm^{-2} was measured in this context with a Coherent FieldmaxII TOP energy/power meter fitted with a Coherent Powermax PM10 sensor.

After irradiation, phosphate-buffered saline (PBS) was replaced with DMEM-FBS and incubation was continued for a further period of 24 h in the dark. A similar 96-well plate was prepared with the compounds and kept in dark. After incubation, 25 μL of a 4 $mg.mL^{-1}$ solution of MTT in PBS was added to each well and incubated for a further 3 h. The culture medium was subsequently discarded and 200 μL of DMSO was added to dissolve the formazan crystals. Absorbance at 540 nm was determined by using a Biorad Elisa plate reader. The cytotoxicity of the complexes was measured as a percentage ratio of the absorbance of the treated cells to the untreated controls. The IC_{50} values of the complexes were determined by a nonlinear regression analysis (GraphPad Prism 5.1). The signal was normalized to 100% viable cells (when untreated with a photosensitizer dye) and 0% viable cells (when treated with 50 μM of the Sn(IV) porphyrin compounds).

Time-dependent cellular uptake:

Approximately 10^4 cells were seeded on 96-well plates and allowed to grow for 24 h. The cells were treated with **SnM** (10 μM) and **SnH** (10 μM) in PBS over different time intervals (12, 24 and 48 h). After the incubation, cells were washed twice with PBS to remove any extra cellular compounds. Cells were solubilized in 100 μL of 30% Triton X-100 in PBS. The cell accumulated Sn(IV)porphyrin was detected by fluorescence using an ELISA reader with excitation and emission wavelengths of 420 and 650 nm, respectively.

Inverted fluorescence microscopy

Approximately 1×10^5 MCF-7 cells were seeded on 24-well plates and were allowed to adhere for 24 h. Cells were treated with 10 μM of the Sn(IV) porphyrins and incubated for 24 h. Cells were then washed twice with PBS, and photoirradiated with a Thorlabs M595L3 mounted into the housing of a Modulight 7710-680 medical laser system for 30 min to observe the PDT effect. Dark treated control cells were kept in an incubator. The cells were incubated for a further 20 h and imaged with a Zeiss-AxioVert inverted microscope.

ROS measurement (DCFDA assay)

For ROS detection, 1×10^5 MCF-7 cells were seeded in a 96 well plate and were incubated for 24 h. Cells were incubated with 10 μM of **SnM** and **SnH** for 4 h in the dark. 2',7'-Dichlorodihydrofluorescein diacetate (DCFDA, 10 μM, final concentration) was added and the cells were incubated for a further 30 min. Cells were washed thrice with PBS and were irradiated with a Thorlabs M595L3 595 nm LED for 30 min. A similar 96 well plate was kept

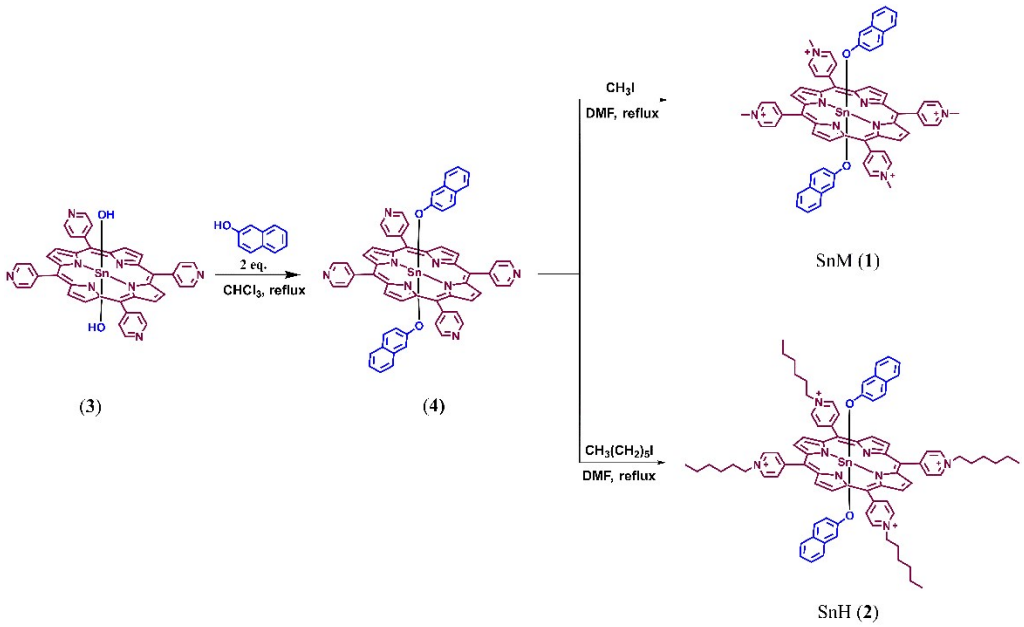
in the dark. Cells were analyzed using a multi-plate reader with excitation and emission wavelengths of 485 and 535 nm, respectively. Hydrogen peroxide-treated cells and untreated cells were used as positive and negative controls, respectively.

Antimicrobial Studies

The microorganism (*S. aureus*) was grown on an agar plate according to the manufacturer's specifications to obtain an individual colony. The bacteria culture was prepared according to a procedure described in the literature.[8] The colony was inoculated into the nutrient broth and agitated on a rotary shaker (ca. 200 rpm) overnight at 37°C. Aliquots of the culture were transferred to 5 mL of fresh broth and incubated at 37°C to obtain a mid-logarithmic phase (OD 620 nm ≈ 0.6). The optical densities of the bacteria culture were determined using a Ledetect 96 from Labxim Products so that log reduction values could be calculated in units of colony forming units per millilitre ($CFU.mL^{-1}$). Through centrifuging for 15 min at 3000 rpm to remove the broth, the bacterial culture in the logarithmic phase of growth was harvested and washed three times in PBS. The bacteria culture was diluted by a factor of 10^3 in PBS to provide a working stock solution.

Stock solutions of the Sn(IV)porphyrins were prepared in PBS. 5 mL aliquots of mixtures of the Sn(IV)porphyrins and *S. aureus* were prepared to provide the final study concentration of 0.5 μM for the Sn(IV) porphyrins. The mixtures were incubated in an oven equipped with a shaker for 30 min in the dark at 37°C. 2.5 mL of the incubated bacterial solutions were irradiated with a Thorlabs M595L3 595 nm LED in a 24 well plate for 90 min, while the other 2.5 mL was kept on a plate in the dark to provide a control. The LED was mounted into the housing of a Modulight 7710-680 medical laser system. An irradiance value of 250 $mW.cm^{-2}$ was measured in this context with a Coherent FieldmaxII TOP energy/power meter fitted with a Coherent Powermax PM10 sensor. After 15, 30, 60 and 90 min irradiation time, a 100 μL sample was pipetted out and spotted on agar plates with a micropipette. The plates were kept in the dark and were incubated at 37ºC for 24 h. A Scan 500 Automatic Colony Counter from Healthcare Technologies was used for the colony forming unit (CFU) determinations. The survival fractions were calculated through comparisons with a control solution containing no photosensitizer dye. The experiments were carried out in triplicate, so that standard deviations could be calculated.

Schemes



Scheme S1. Synthetic procedures for **SnM** (**1**) and **SnH** (**2**), and precursors **3** and **4**.

Figures

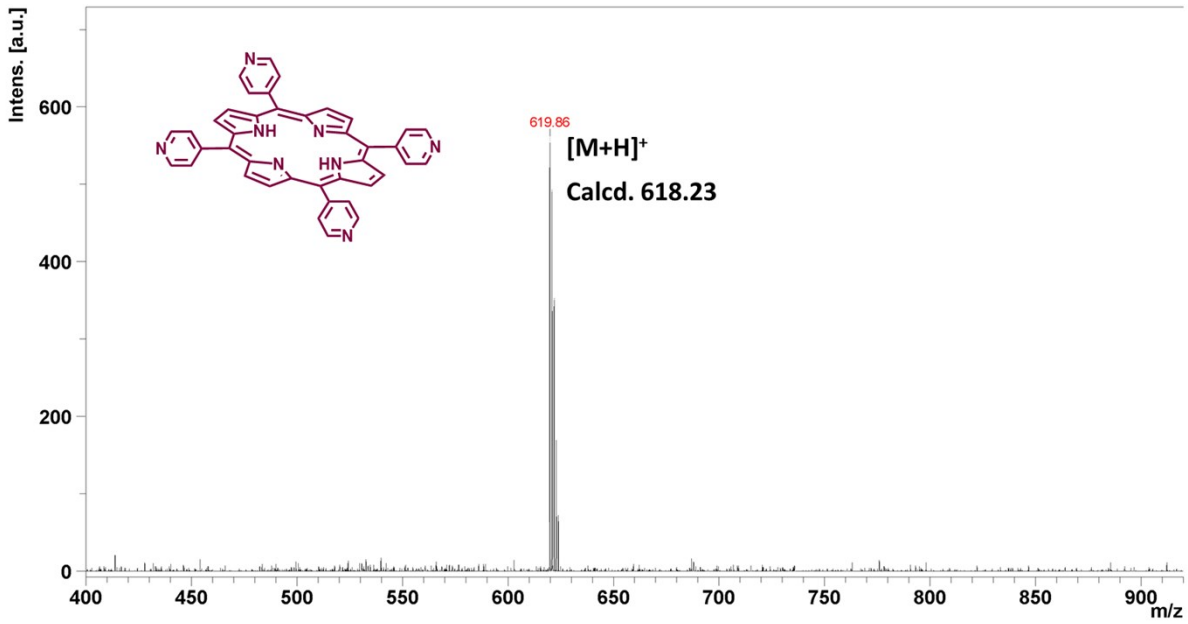


Figure S1. MALDI-TOF MS data for *meso-tetrakis*(4-pyridyl)porphyrin.

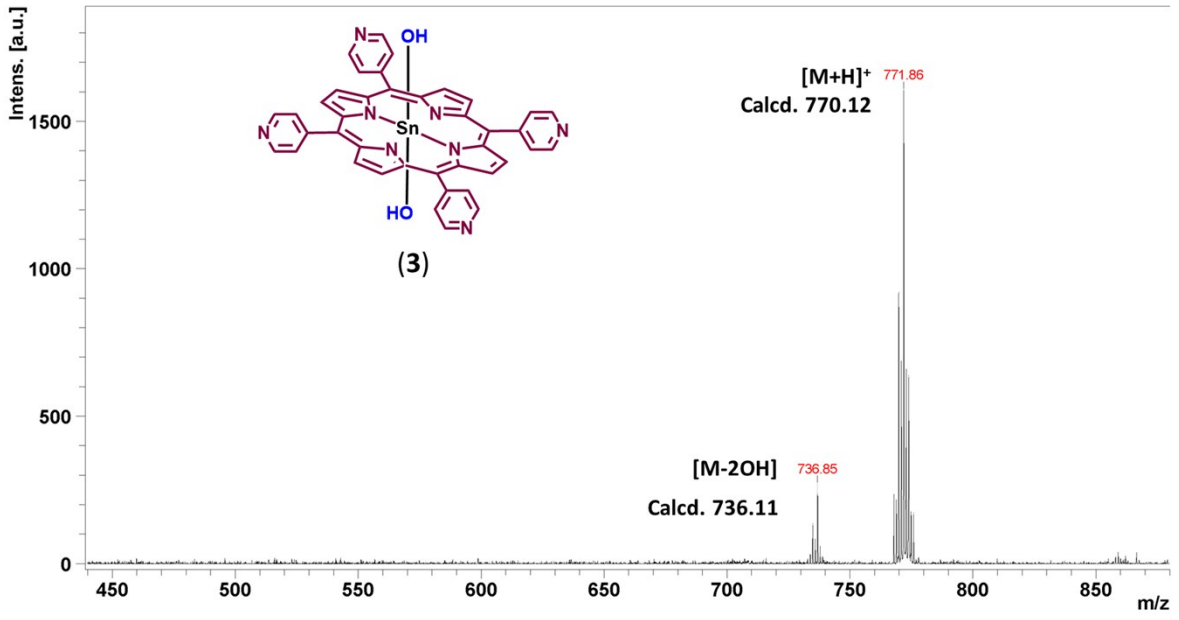


Figure S2. MALDI-TOF MS data for **3**.

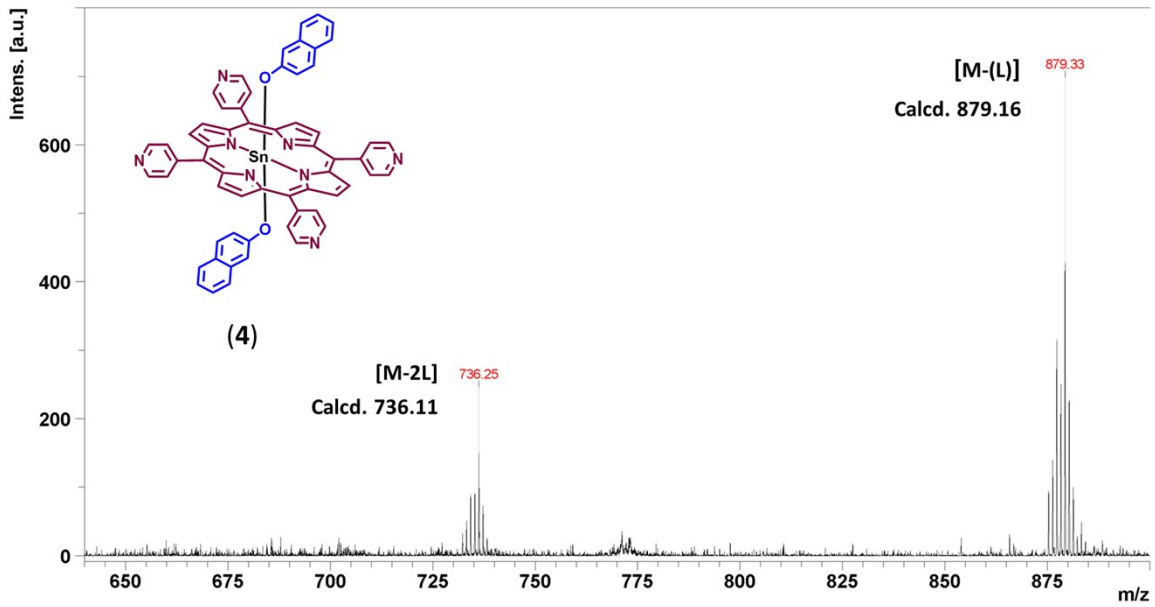


Figure S3. MALDI-TOF MS data for **4**.

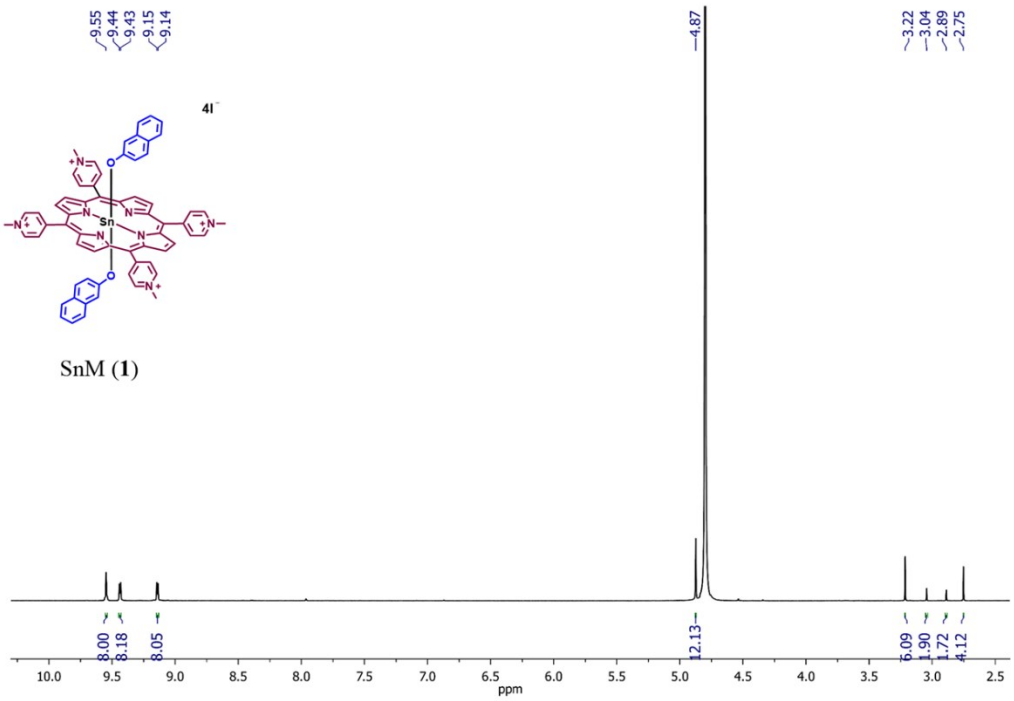


Figure S4. ^{1}H NMR (600 MHz) of **SnM** (**1**) in D_2O.

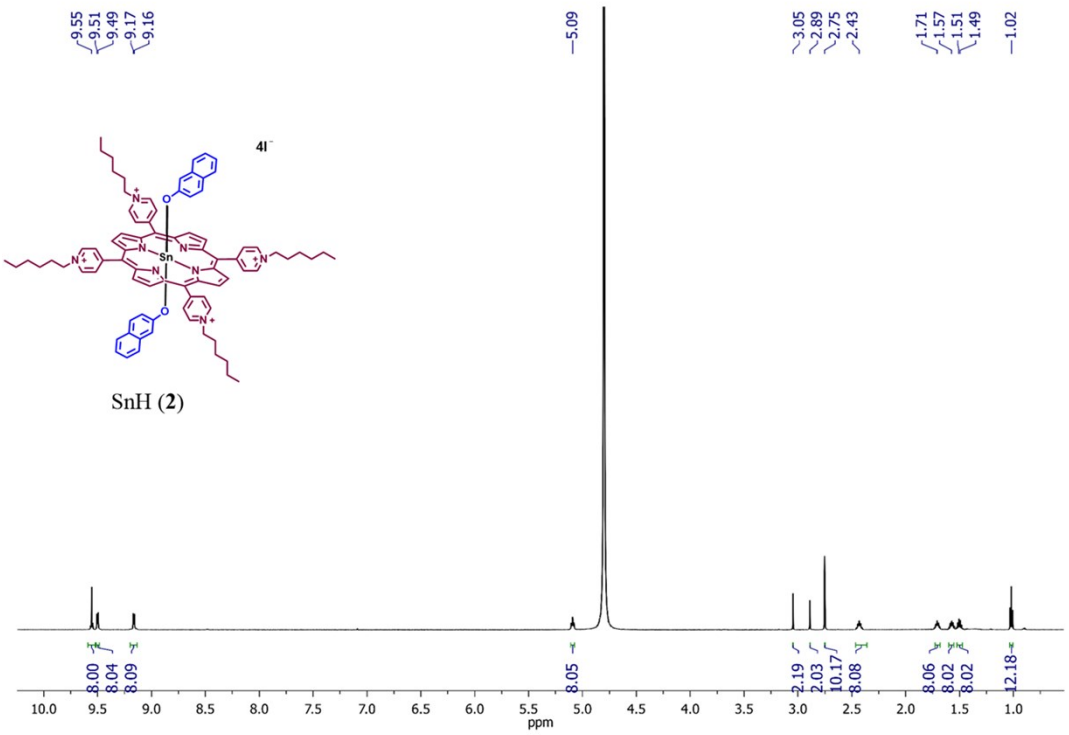


Figure S5. ^{1}H NMR (600 MHz) of **SnH** (**2**) in D_2O.

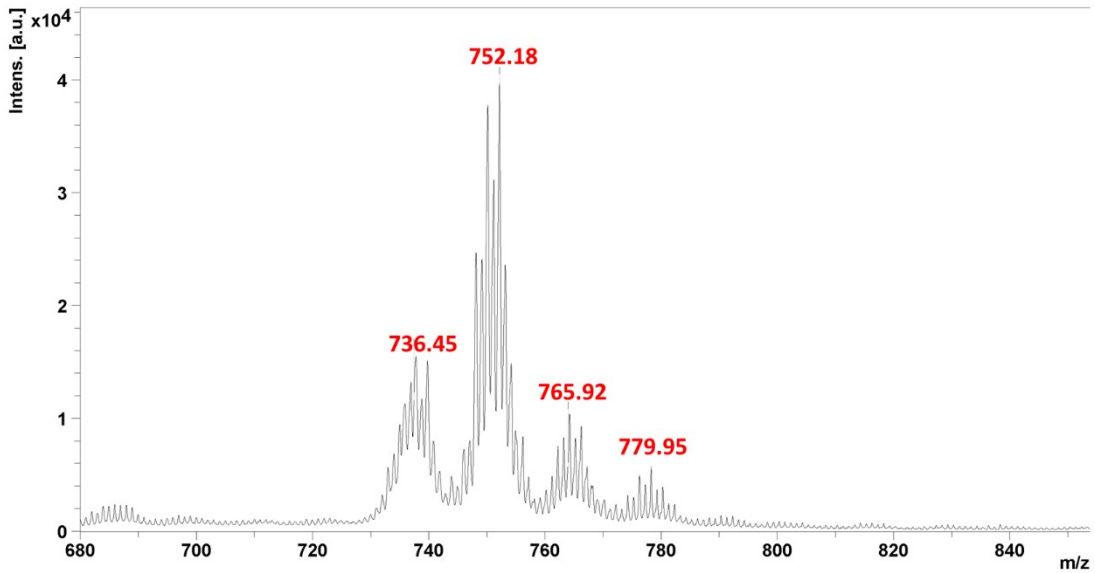


Figure S6. MALDI-TOF MS data for **SnM**.

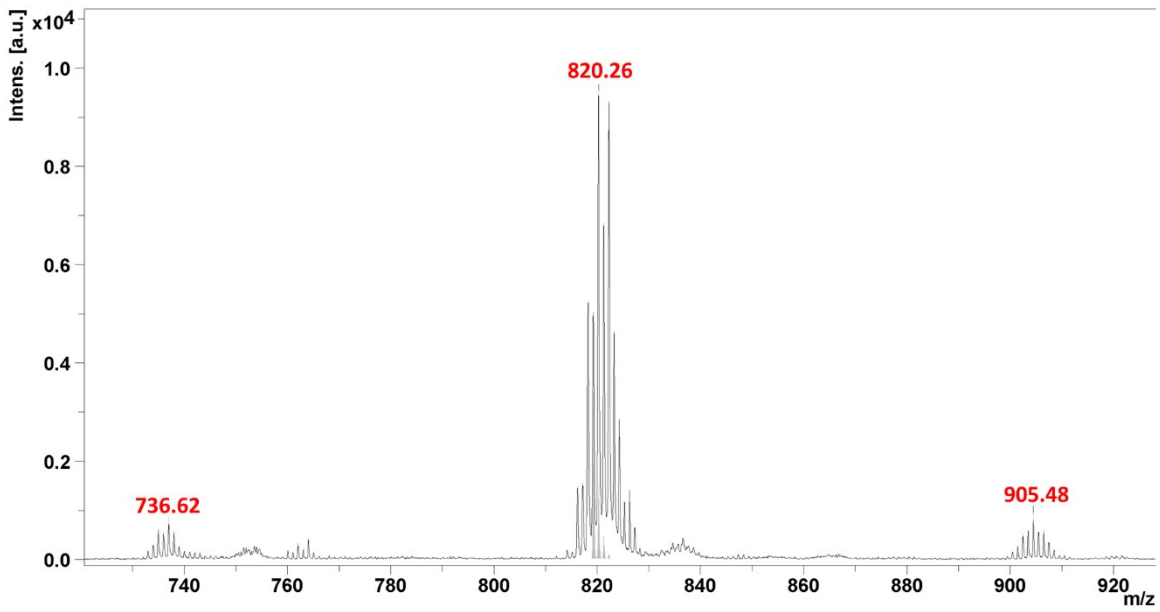


Figure S7. MALDI-TOF MS data for **SnH**.

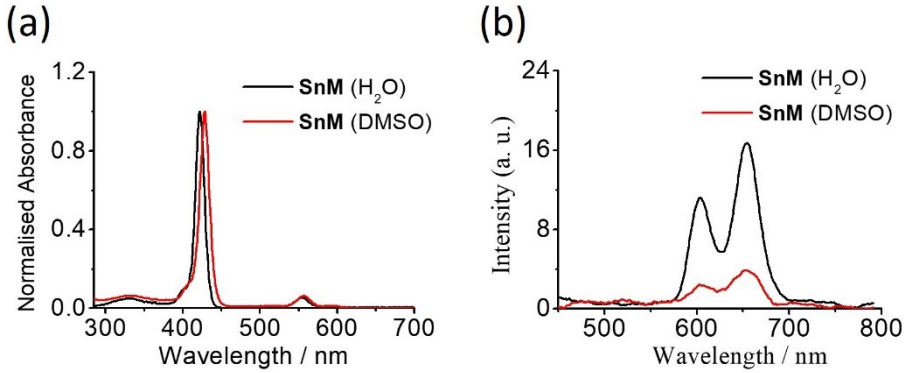


Figure S8. (a) UV-Visible and (b) emission spectra of **SnM** in water and DMSO.

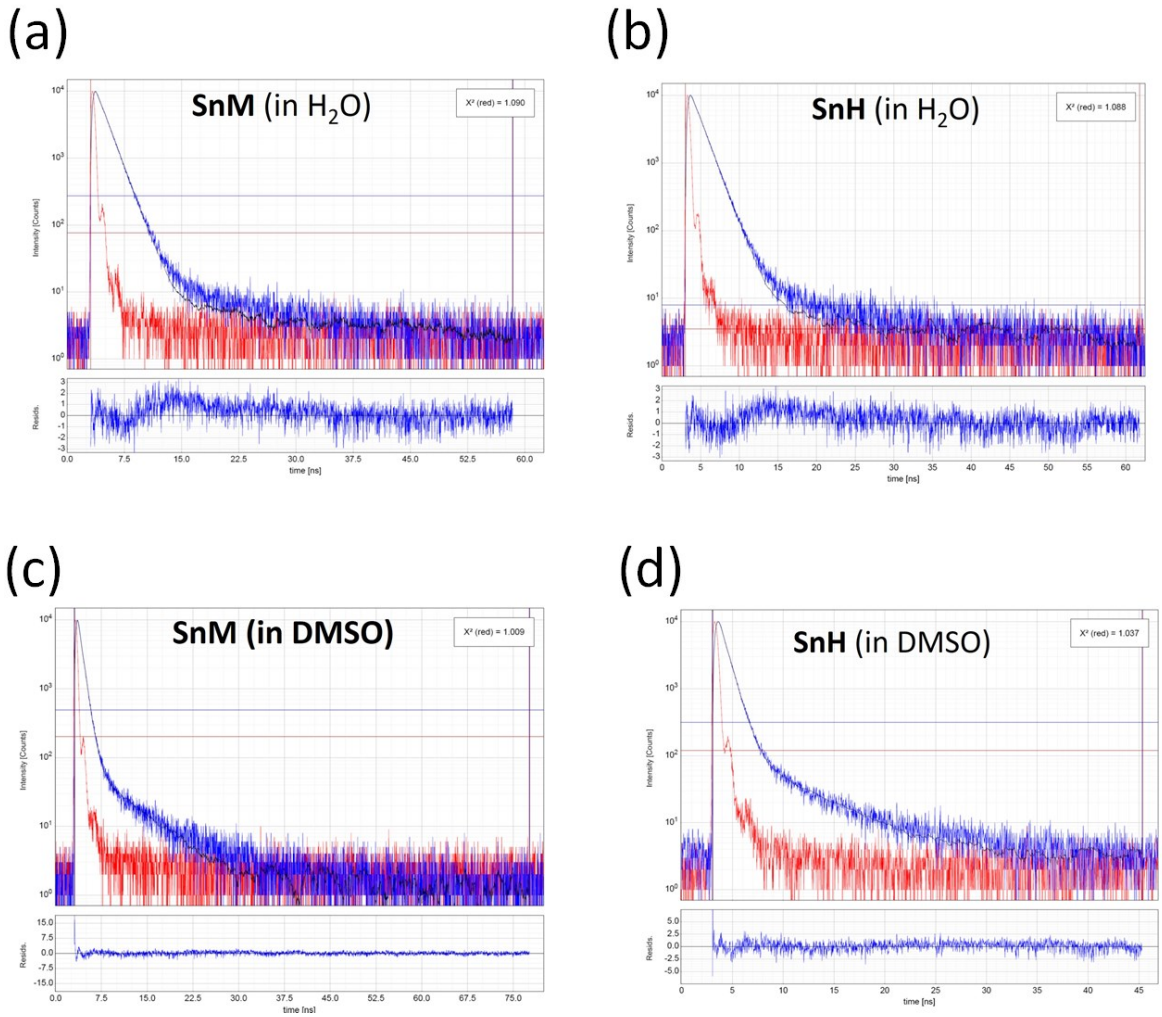


Figure S9. Fluorescence decay (blue) and instrument response function (red) curves for (a) **SnM** and (b) **SnH** in H_2O, and (c) **SnM** and (d) **SnH** in DMSO.

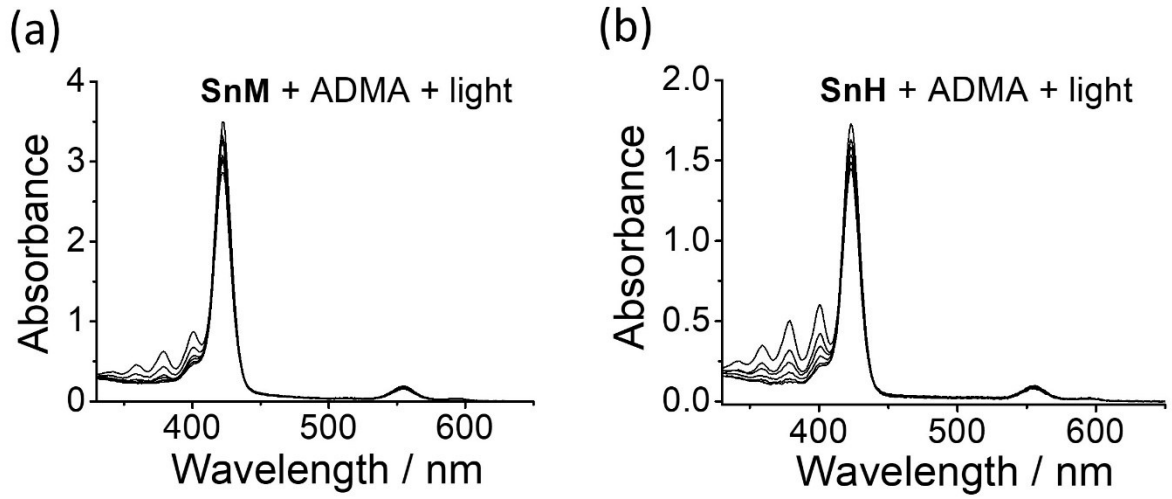


Figure S10. Changes observed in the absorption spectrum of ADMA upon light irradiation in the presence of (a) **SnM** and (b) **SnH** in H_2O.

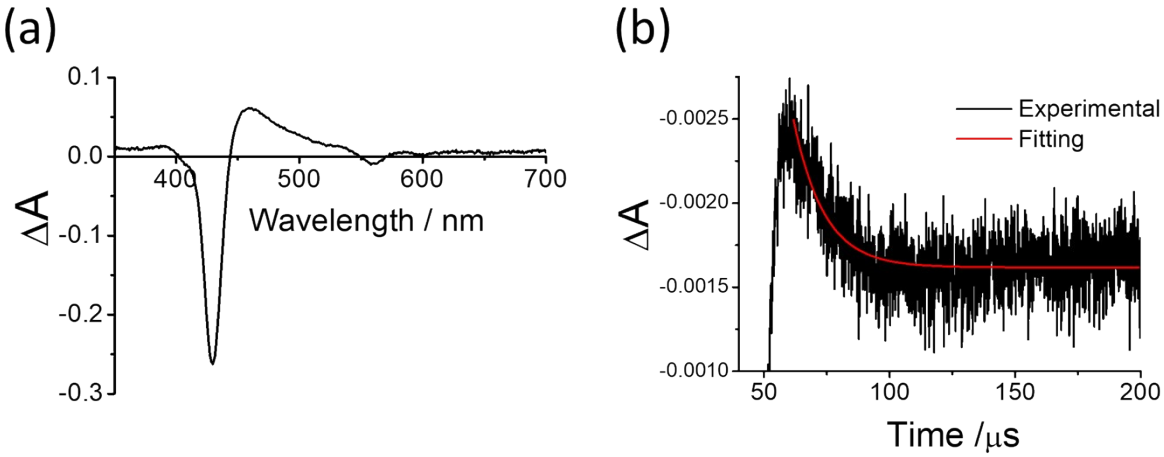


Figure S11. (a) Transient absorption curve observed for **SnM** in DMSO upon irradiation at 425 nm, and (b) the triplet absorption decay curve at 460 nm.

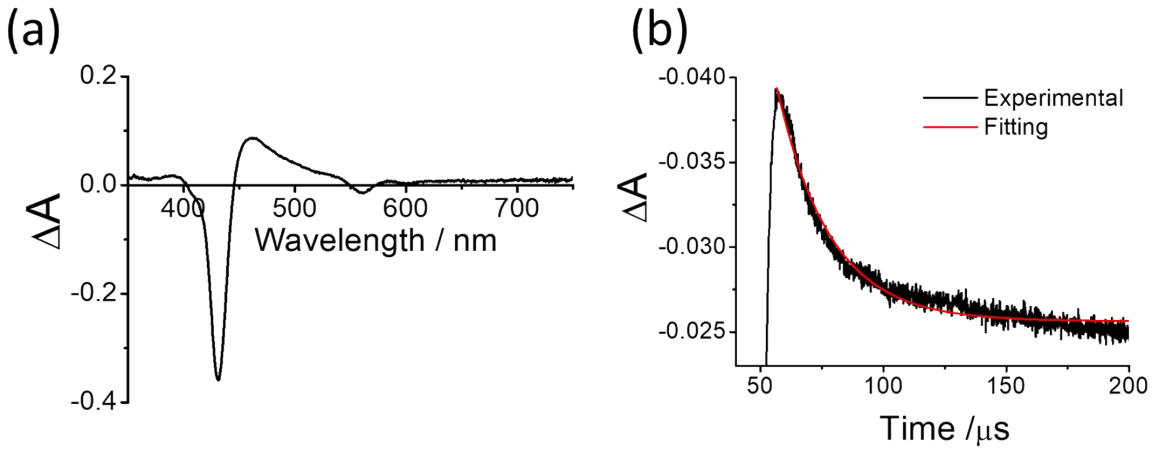


Figure S12. (a) Transient absorption curve for **SnH** in DMSO observed upon irradiation at 425 nm, and (b) the triplet absorption decay curve at 460 nm.

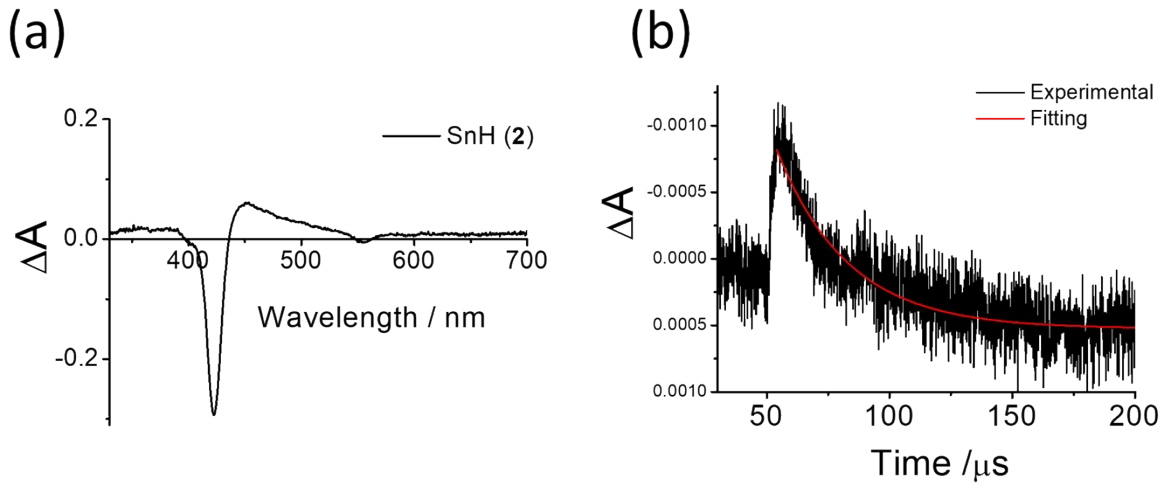


Figure S13. (a) Transient absorption curves for **SnH** in H_2O observed upon irradiation at 425 nm, and (b) the triplet absorption decay curve at 460 nm.

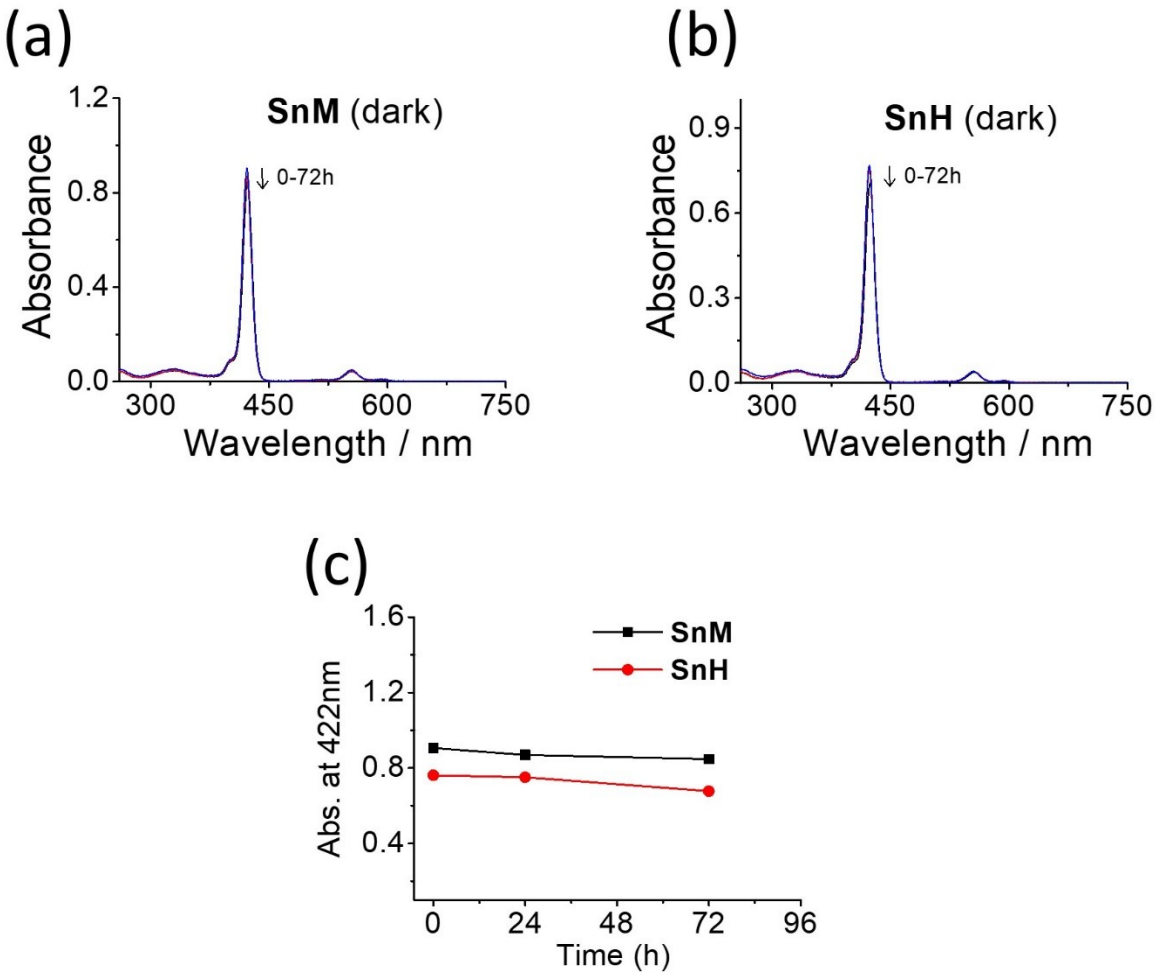


Figure S14. Changes in absorbance at 422 nm in the UV-visible absorption spectra of (a) **SnM** and (b) **SnH** with time in the dark in H_2O, and (c) a plot of absorbance at 422 nm vs time. Solvent: H_2O.

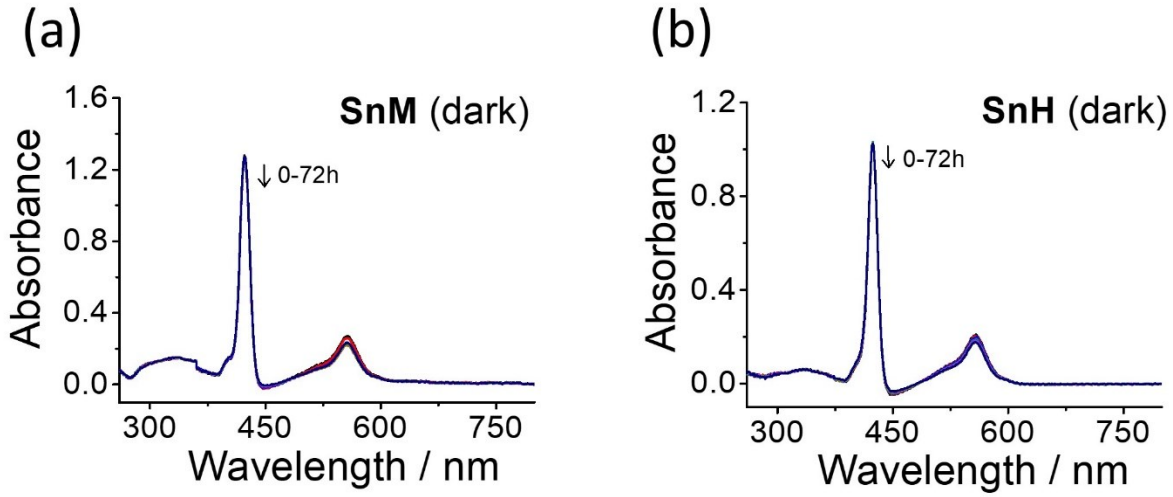


Figure S15. Changes in the UV-visible absorption spectra of (a) **SnM** and (b) **SnH** in DMEM cell growth media with time in the dark.

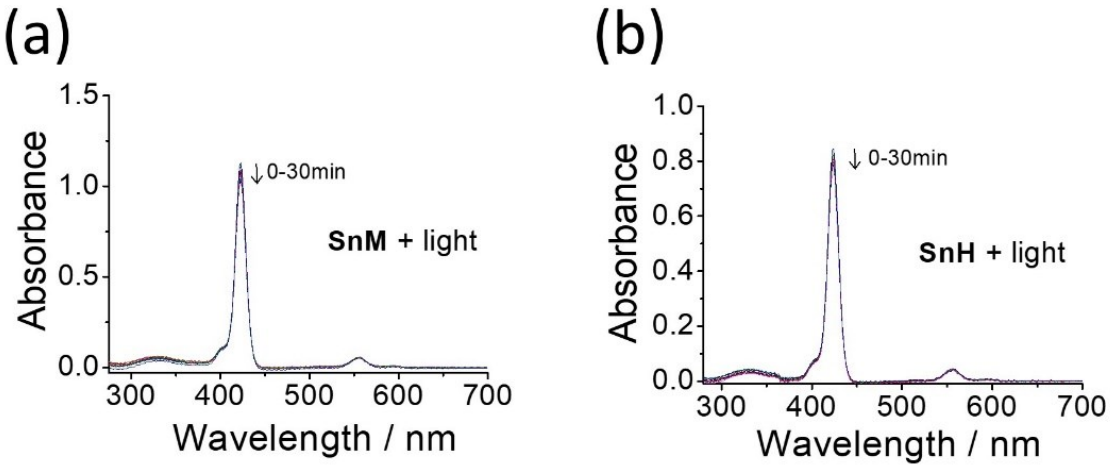


Figure S16. Time-dependent UV-visible absorption spectra of (a) **SnM** and (b) **SnH** upon irradiation with a Thorlabs 595 nm LED at 250 mW.cm^{-2} in H_2O.

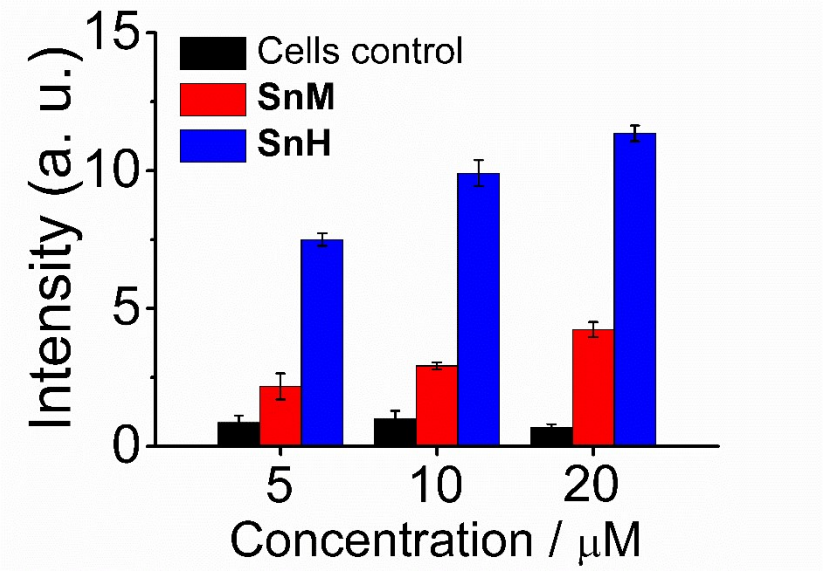


Figure S17. Concentration-dependent cellular uptake of **SnM** and **SnH** into MCF-7 cells as measured by fluorescence intensity using excitation and emission wavelengths of 422 and 650 nm, respectively.

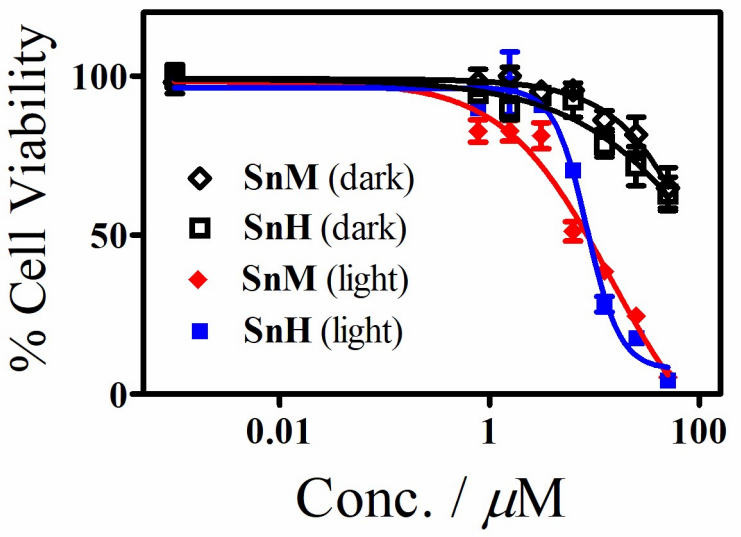


Figure S18. Cytotoxicity of **SnM** and **SnH** against MCF-7 cells as determined with the MTT assay after 24 h incubation in the dark followed by photoirradiation for 30 min with a Thorlabs 595 nm LED at 250 mW.cm^{-2}.

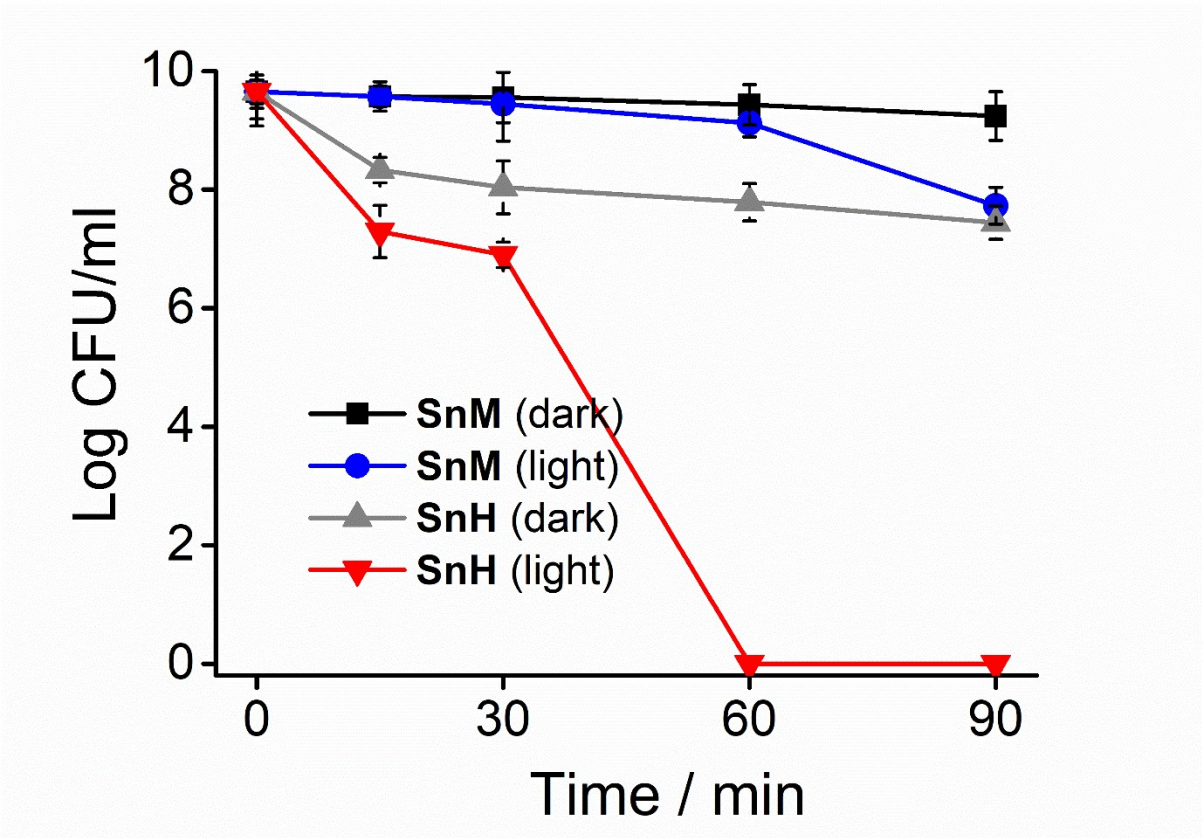


Figure S19. Logarithmic reduction of *S. aureus* treated with Sn(IV) porphyrins **SnM** and **SnH** (0.5 μM) in the dark and after irradiation with a Thorlabs 595 nm LED at 250 mW.cm^{-2}.

References

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