Electronic Supplementary Information

for

A Sn(IV) porphyrin with mitochondria targeting properties for enhanced photodynamic activity against MCF-7 cells

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1.1. Materials

All reagents and solvents were obtained from Sigma Aldrich. Benzaldehyde, triphenylphosphine, 3-bromopropionic acid, *N*,*N*-dicyclohexylcarbodiimide (DCC), $SnCl_2$, 9,10-dimethylanthracene (DMA), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 2′,7′-dichlorofluorescin diacetate (DCF-DA), Rhodamine 123, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), 5,10,15,20-tetraphenylporphyrinato zinc(II) (ZnTPP), Triton-100X were obtained from Sigma Aldrich. All solvents used were of analytical grade and were purified and dried by routine procedures immediately before use. Cultures of MCF-7 cells were obtained from Cellonex®. 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B and 10% (*v/v*) heat-inactivated fetal bovine serum (FBS) were obtained from Biowest®. Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's phosphate-buffered saline (DPBS) was purchased from Lonza.

1.2. Equipment

^{1}H NMR spectra were recorded with a Bruker 400 MHz instrument using trimethylsilane (TMS) as an internal standard. UV-visible absorption spectra were measured on a Shimadzu UV−2550 spectrophotometer. Fluorescence spectra were measured on a Varian Eclipse spectrofluorimeter using a 360–1100 nm filter, and fluorescence quantum yield is calculated using ZnTPP (Φ_F = 0.033 in DMF) as the standard.[1] 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. Triplet state lifetimes were determined at 500 nm in N_2 saturated DMF solutions using an Edinburgh Instruments LP980 spectrometer with a pump beam of 430 nm provided by an Ekspla NT-342B laser (2.0 mJ / 7 ns, 20 Hz) fitted with an OPO. A Thorlabs M625L3 LED mounted into the housing of a Modulight 7710-680 medical laser system was used for photoirradiation experiments. Singlet oxygen quantum yield (Φ_Δ) values were determined through a comparative method, using DMA as the singlet oxygen quencher and ZnTPP (Φ_Δ = 0.53 in DMF) as the standard.[2]

1.3. Theoretical calculations

Geometry optimizations were carried out by using the Gaussian 09 software package at the B3LYP/6-31G(d) level of theory.[3] TD-DFT calculations were carried out similarly with the CAM-B3LYP functional since it contains a long-range correction.

1.4. Lipophilicity measurements (log $P_{o/w}$)

The lipophilicity (log $P_{o/w}$) of **SnP** and **SnPH** was calculated by the Shake-flask method.[4,5] 1 mg of the compounds was added to 3 mL of 1-octanol saturated with water, and the initial absorbance was measured (A_{int}). 3 mL of water saturated with 1-octanol was added, and the mixture was stirred at room temperature for 3 h. The mixture was allowed to form separate organic and aqueous solvent phases. The absorbance of the compound in the organic phased was measured (A_{oct}). $A_{int} - A_{oct} = A_{wat}$ was calculated

to determine the amount of compound in the water phase. The log $P_{o/w}$ values were calculated from the following formula, log P = log (A_{oct}/A_{wat}).

1.5. Photostability

For photostability studies, a solution of Sn(IV) tetraphenylporphyrins in 1% DMSO/PBS was irradiated for 20 min using a 625 nm Thorlabs M625L3 LED (240 mW cm^{-2}) in the same arrangement used for the *in vitro* cell studies, and absorbance values were recorded at regular intervals.

1.6. Cell studies

The *in vitro* cytotoxicity experiments for **SnP** and **SnPH** against MCF-7 cells were carried out by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[6,7] MCF-7 cells (~1×10^5) were cultured in a 96-well culture plate in DMEM containing 10% FBS and incubated for 24 h at 37 °C in a CO_2 incubator. Stock solutions of the Sn(IV) tetraphenylporphyrins were prepared in DMSO and diluted with different volumes of DMEM media to give 0.32−20 μM. The percentage of DMSO is maintained < 0.5% in all cases. The medium was replaced with DMEM media containing compounds at different concentrations (0.32−20 μM), and the cells were incubated for 12 h in the dark. After the 12 h pre-incubation time, the old media was replaced by fresh DMEM with no phenol red, and cells were irradiated separately at 625 nm (240 mW cm^{-2}) with a Thorlabs M625L3 LED mounted into the housing of a Modulight 7710-680 medical laser system for 20 min. After photoirradiation, fresh DMEM-10% FBS was added, and cells were incubated for a further 24 h in the dark, followed by the addition of 25 μL (5 mg mL^{-1}) MTT to each well and a further 3 h of incubation in the dark. Subsequently, the culture medium was carefully discarded, and 200 μL of DMSO was added to dissolve the formazan crystals formed, and the absorbance at 545 nm was determined with a Molecular Devices Spectra Max M5 plate reader. The percentage ratio of the absorbance of the treated cells to the untreated controls provides the measurement of cytotoxicity. The IC_{50} values were determined by nonlinear regression analysis using GraphPad prism. A separate set of cells treated with the compounds was prepared, and no light treatment was performed.

1.7. Time-dependent cellular uptake

To determine the time-dependent cellular uptake of **SnP** and **SnPH**, a solution of photosensitizers (5 μM) was added to MCF-7 cells (1×10^6 cells) seeded in 24-well cell culture plates and incubated for 6, 12, 24 h. After the incubation time, the cells were carefully washed three times with PBS to remove the extracellular compound and lysed with 30 μL of Triton-100X and solubilized in 70 μL of DMSO. The relative cellular uptake was determined by measuring fluorescence intensity at 620 nm when excited at 430 nm using a Molecular Devices Spectra Max M5 plate reader. Control experiments were carried out in the absence of photosensitizers.

1.8. DCF-DA assay

Intracellular ROS generation by **SnP** and **SnPH** was measured by the 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) assay.[8] The Sn(IV) tetraphenylporphyrins (5 μM) were added to MCF-7 cells (1×10^5 cells) seeded in 24-well cell culture plates and incubated for 4 h in the dark followed by DCFDA (5 μM, final concentration) and 30 min further incubation in the dark. The cells were carefully washed with PBS (3 times) to remove extracellular compounds and irradiated at 625 nm (240 mW cm^{-2}) with a Thorlabs M625L3 625 nm LED for 30 min. Cells were analyzed using a multi-plate reader with excitation and emission wavelengths set at 485 and 535 nm, respectively. Separate dark control plates were made only with Sn(IV) tetraphenylporphyrins, and untreated cells were used as a negative control.

1.9. Mitochondria membrane potential ($\Delta\Psi_m$) – Rhodamine 123 assay

$\Delta\Psi_m$ was measured by using the Rhodamine 123 assay.[9] Briefly, the Sn(IV) tetraphenylporphyrins (5 μM) were added to MCF-7 cells (1×10^5 cells) seeded in 24-well cell culture plates and incubated for 12 h in the dark and washed with PBS (3 times). DMEM (without phenol red) was added and irradiated at 625 nm (240 mW cm^{-2}) for 15 min with a Thorlabs M625L3 LED. After irradiation, Rhodamine 123 (5 μg/ml) in PBS was added and incubated for 30 min in the dark. It was washed with PBS (3 times) and resuspended in PBS. Cells were analyzed using a multi-plate reader with excitation and emission wavelengths set at 507 and 534 nm, respectively. Cells in control wells in the dark and under illumination were prepared without the addition of the Sn(IV) tetraphenylporphyrins. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 5 μM), a well-known protonophore that can decrease the mitochondria membrane potential, was used as a positive control.

1.10. Statistical analysis

The statistical significance of differences between treatments was evaluated by using two-tailed paired student *t* tests with Microsoft Excel. Results were considered as statistically significant when $p < 0.05$. Data were reported as mean ± S.D. with $n = 3$.

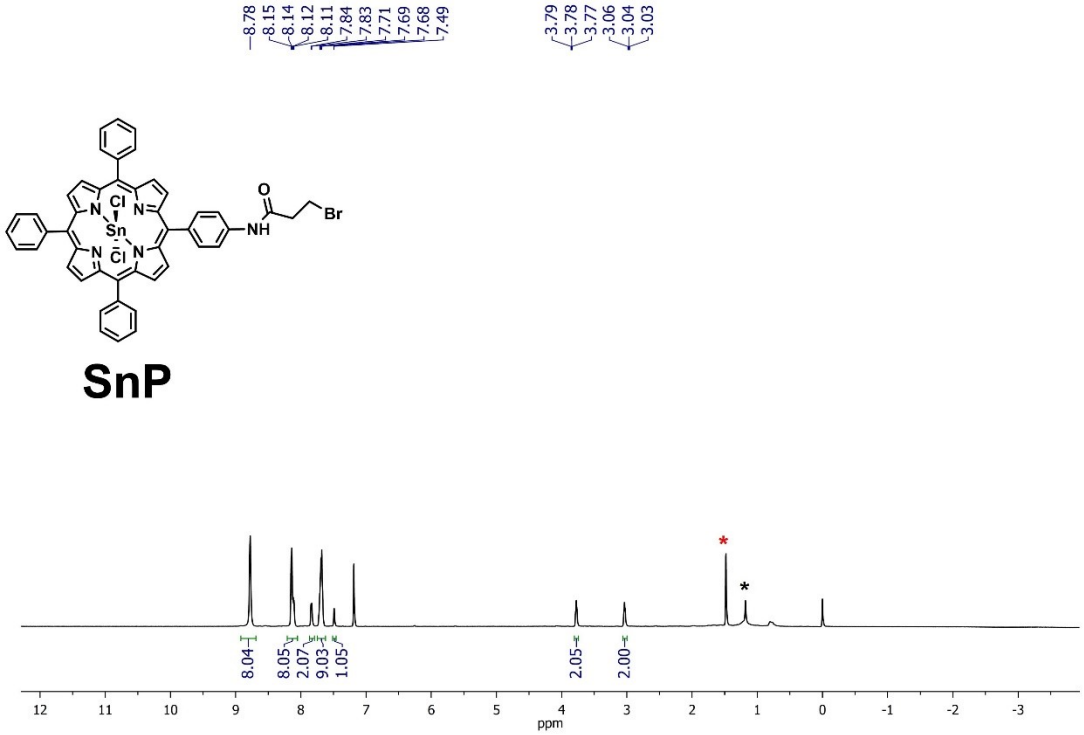

Figure S1. The ^{1}H NMR spectrum of **SnP** in $CDCl_3$ (red and black asterisks highlight the residual H_2O and grease peak, respectively).

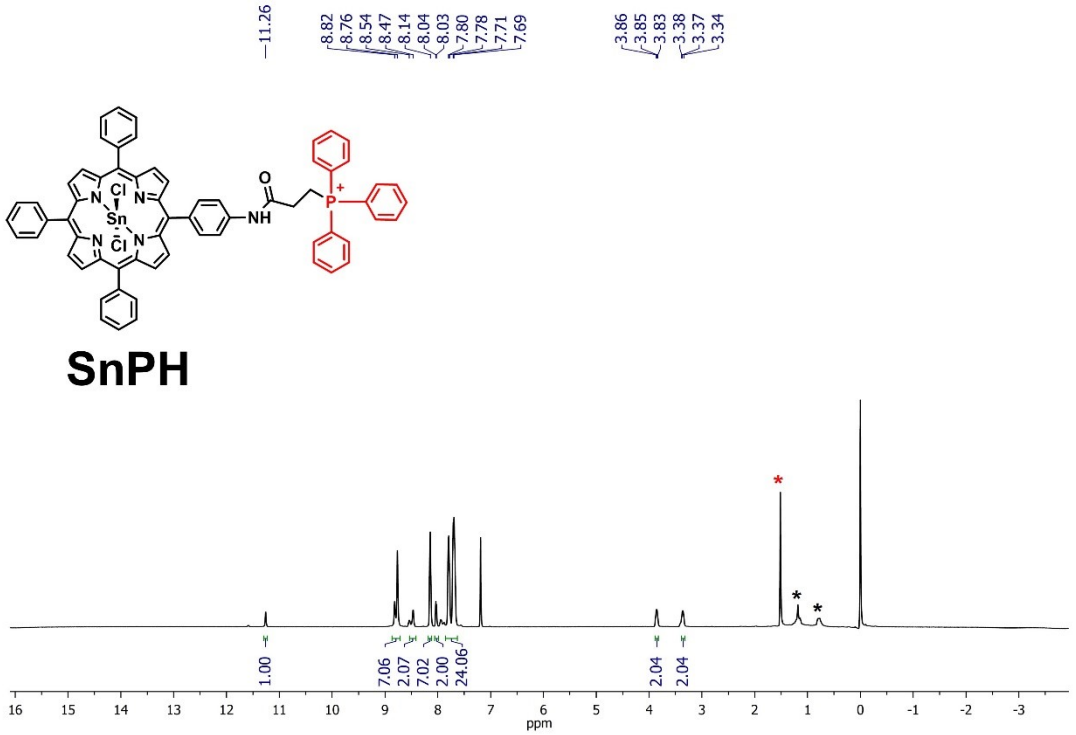

Figure S2. The ^{1}H NMR spectrum of **SnPH** in $CDCl_3$ (red and black asterisks highlight the residual H_2O and grease peak, respectively).

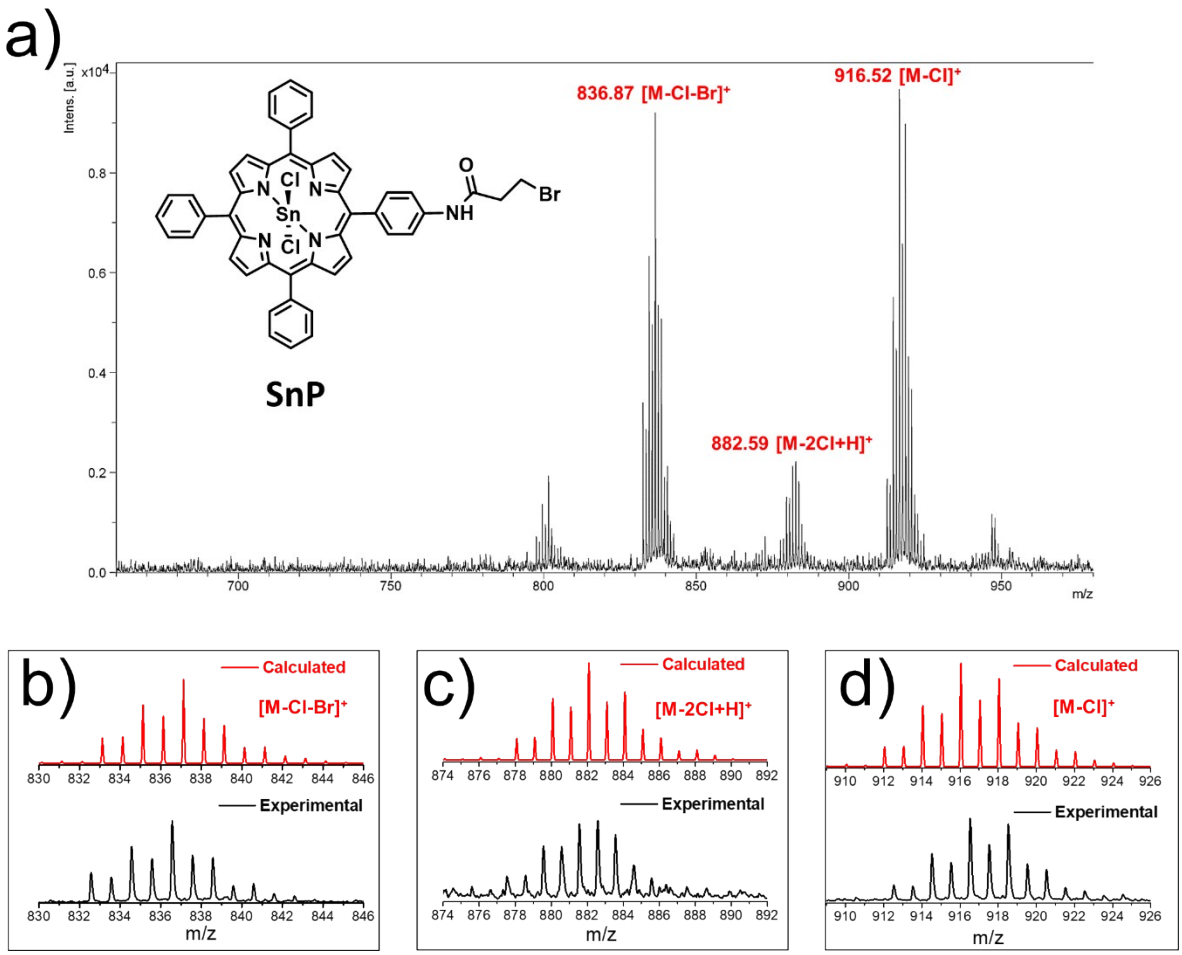

Figure S3. (a) MALDI-TOF MS data for **SnP**; (b-d) calculated and observed isotopic distribution pattern of molecular ion peaks of $[M\text{-}Cl\text{-}Br]^+$, $[M\text{-}2Cl\text{+}H]^+$, $[M\text{-}Cl]^+$ respectively.

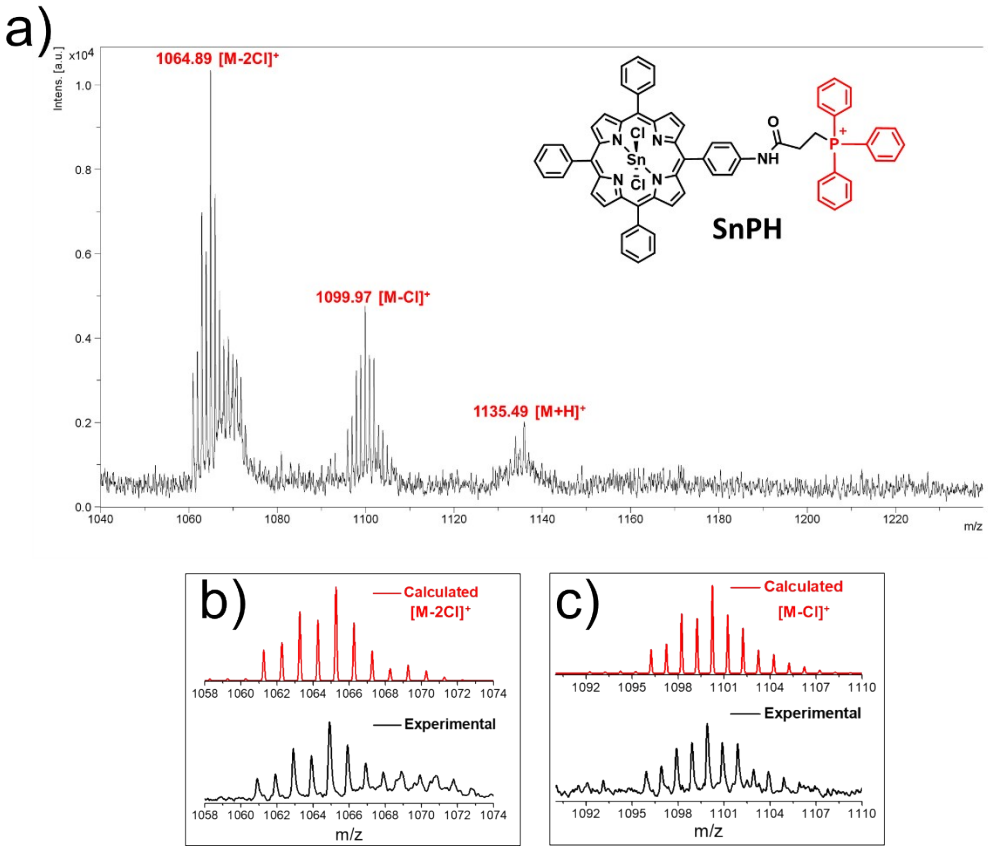

Figure S4. (a) MALDI-TOF MS data for **SnPH**; (b, c) calculated and observed isotopic distribution pattern of molecular ion peaks of $[M-2Cl]^+$, $[M-Cl]^+$ respectively.

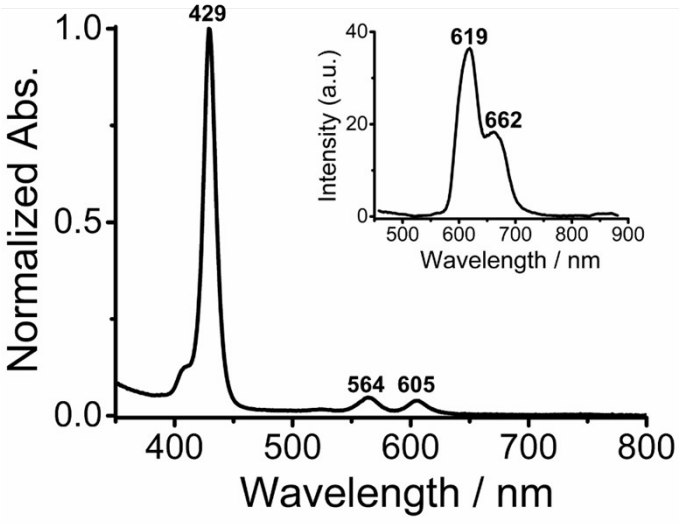

Figure S5. The UV-visible absorption spectrum of **SnP** in DMF (Inset: Emission spectrum of **SnP** excited at the B band maximum).

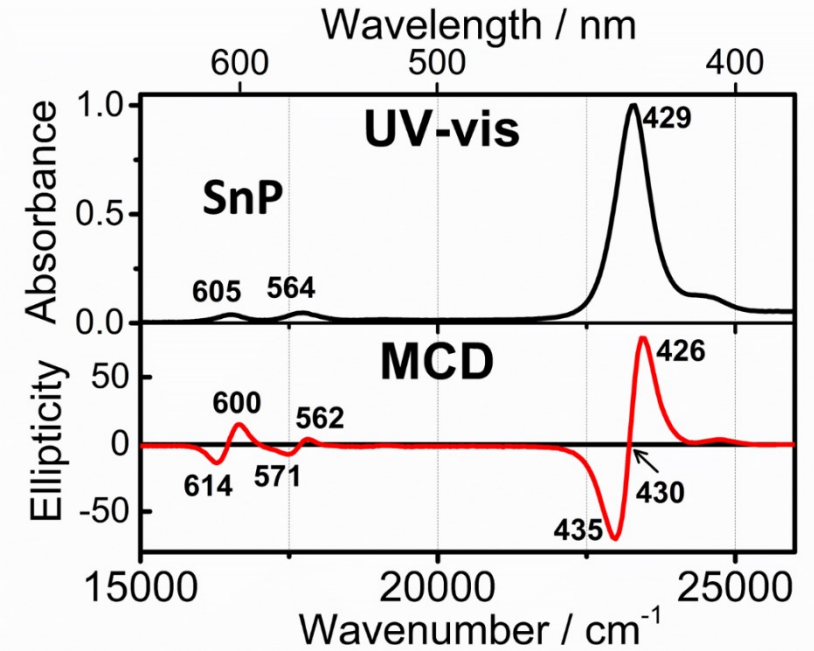

Figure S6. The UV-visible absorption (top) and MCD spectra (bottom) of **SnP** in DMF.

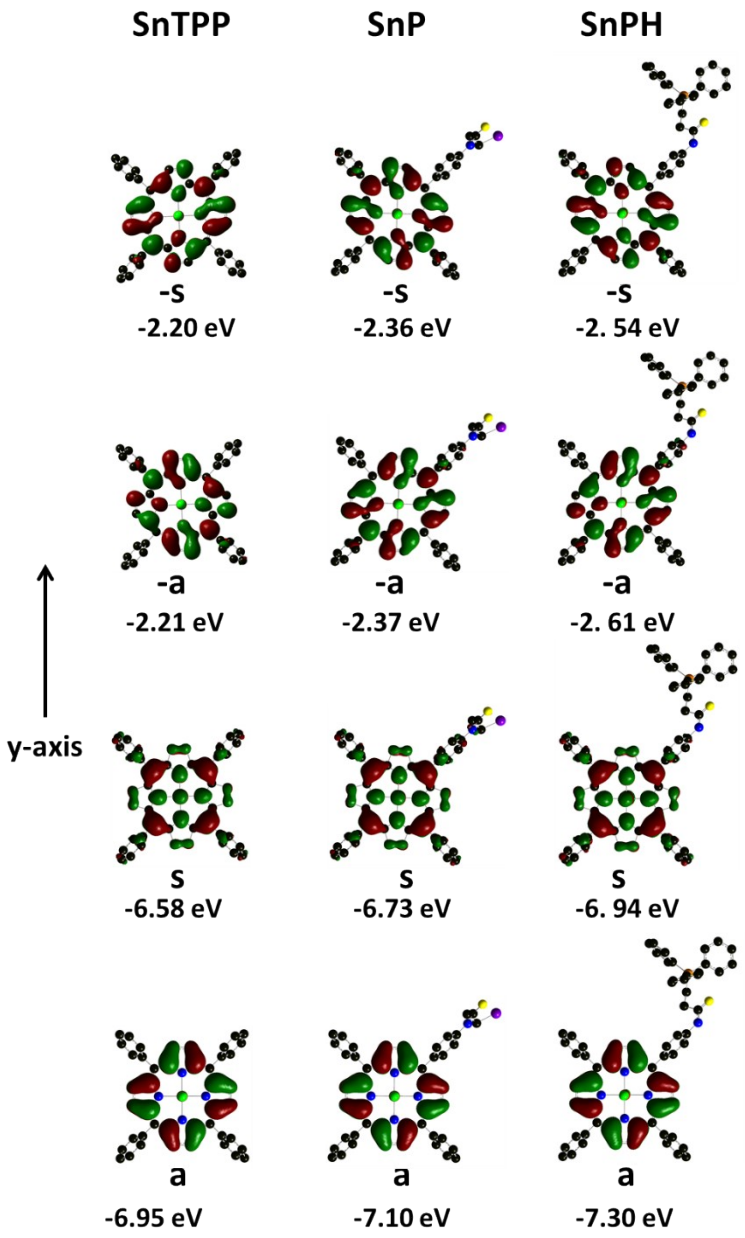

Figure S7. The angular nodal patterns and energies of the **a**, **s**, **-a** and **–s** MOs of **SnTPP**, **SnP**, and **SnPH**.

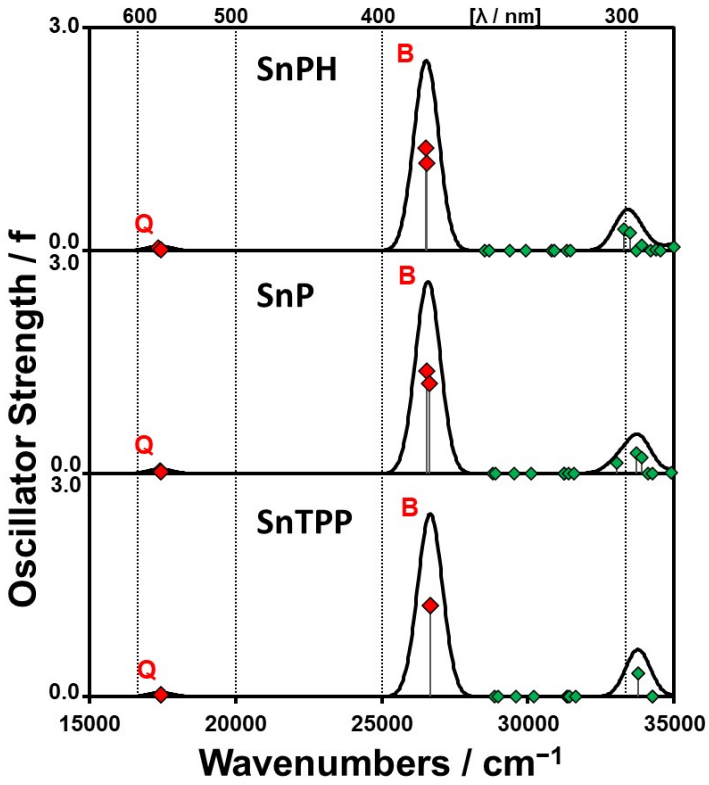

Figure S8. TD-DFT calculated spectra for B3LYP-optimized geometries of **SnTPP**, **SnP**, and **SnPH** at the CAM-B3LYP/SDD level of theory. Red diamonds are used to highlight the Q and B bands of Gouterman's 4-orbital model.[10] Simulated spectra were generated using the Chemcraft program with a fixed bandwidth of 2000 cm^{-1}.[11] Details of the calculations are provided in Table S1.

Table S1. The calculated UV-visible absorption spectra of the B3LYP optimized geometry of **SnTPP**, **SnP**, and **SnPH** were obtained by using the CAM-B3LYP functional of the Gaussian 09 software package[5] with 6-31G(d) basis sets.

SnTPP

	#[a]	λ_{exp}[b]	λ_{calc}[c]	f[d]	Wavefunction =[e]
Q	1	602	573	0.03	**61% s → -s; 36% a → -a;** …
Q	2		573	0.03	**61% s → -a; 36% a → -s;** …
B	3	428	375	1.23	**61% a → -a; 35% s → -s;** …
B	4		375	1.23	**61% a → -s; 35% s → -a;** …

SnP

	#[a]	λ_{exp}[b]	λ_{calc}[c]	f[d]	Wavefunction =[e]
Q	1	605	575	0.04	**64% s → -a; 36% a → -s**; …
Q	2		573	0.03	**63% s → -s; 37% a → -a;** …
B	3	429	377	1.38	**62% a → -s; 34% s → -a**; …
B	4		376	1.22	**61% a → -a; 36% s → -s**; …

SnPH

	#[a]	λ_{exp}[b]	λ_{calc}[c]	f[d]	Wavefunction =[e]
Q	1	604	576	0.05	**65% s → -a; 34% a → -s**; …
Q	2		573	0.02	**61% s → -s; 38% a → -a;** …
B	3	429	377	1.38	**49% a → -s; 26% s → -a; 13% a → -a; 8% s → -s**; …
B	4		377	1.18	**46% a → -a; 29% s → -s; 14% a → -s; 7% s → -a**; …

[a]Excited state number is assigned in increasing energy in the TD-DFT calculations. [b]Experimental wavelengths in nm as recorded in Table 1. [c]Calculated wavelengths in nm. [d]Calculated oscillator strengths. [e]Wavefunctions describing the MOs involved in the transition based on eigenvectors predicted by TD-DFT. Only one-electron transition contributions of more than 5% are included. **a**, **s**, **-a** and **-s** refer to the MO nomenclature of Michl's perimeter model.[12–14] One-electron transitions between these four MOs are highlighted in bold.

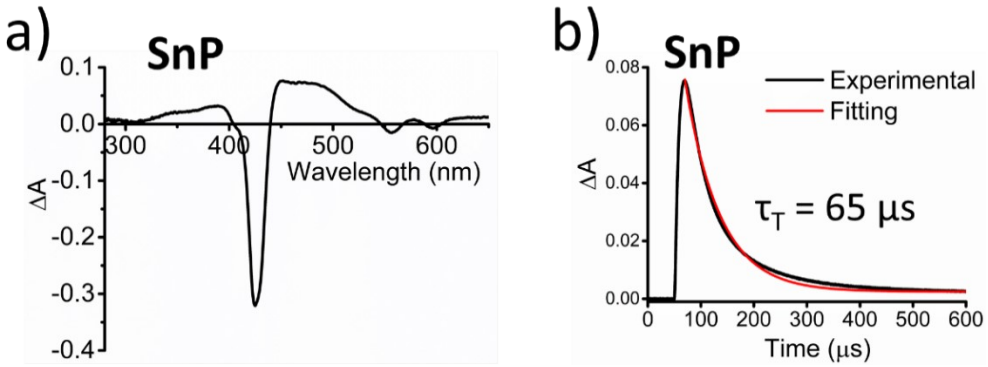

Figure S9. (a) Transient absorption spectra for **SnP**; and (b) the triplet absorption decay curve for **SnP** in N_2 purged DMF.

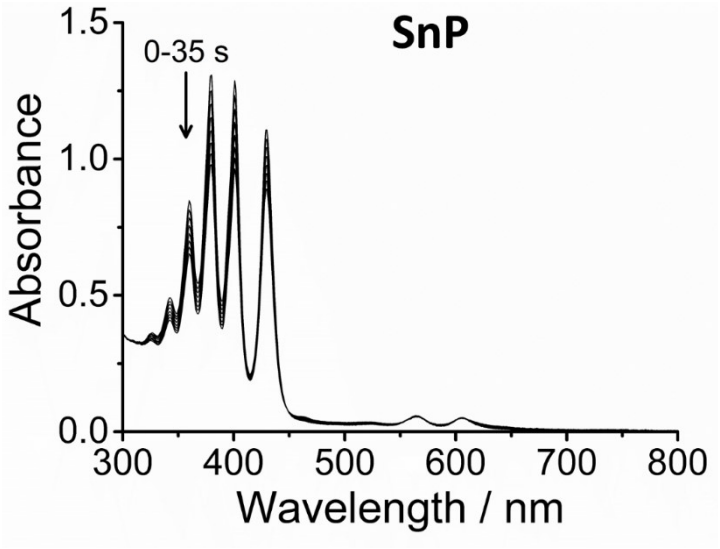

Figure S10. Change in absorbance of $^1O_2$ quencher DMA with irradiation time in the presence of **SnP** in DMF.

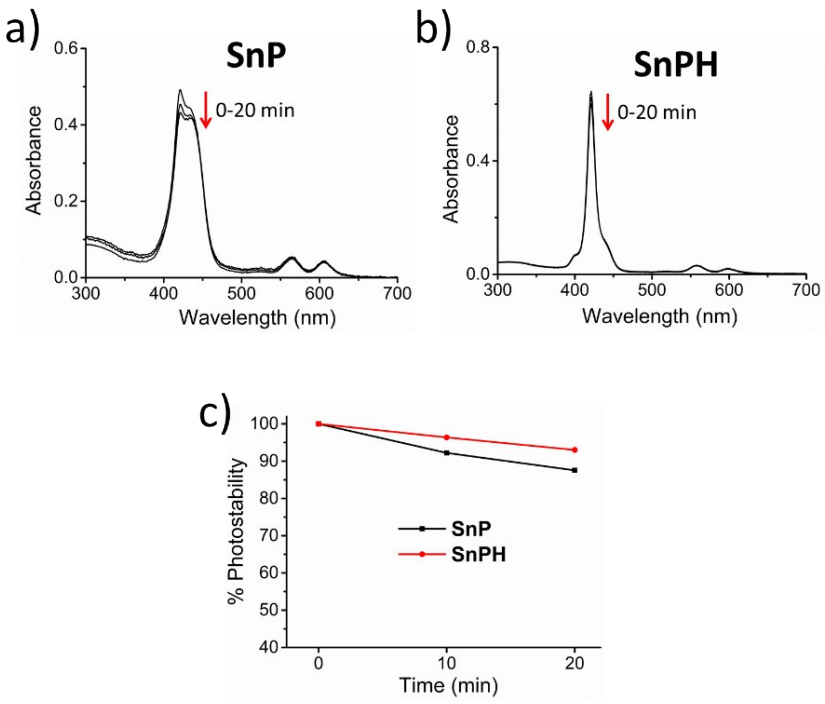

Figure S11. Absorption spectra of (a) **SnP**, (b) **SnPH** upon exposure to 625 nm LED light for 20 min, and (c) photostability plots for (a) and (b). Solvent: 1% DMSO/PBS (v/v).

References

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