Electronic Supporting Information

for

Sn(IV) N-confused porphyrins as photosensitizer dyes for photodynamic therapy in the near IR region

Balaji Babu, John Mack* and Tebello Nyokong*

Institute for Nanotechnology Innovation. Department of Chemistry, Rhodes University, Makhanda, South Africa.

Materials and methods

All reagents and solvents were procured from Sigma Aldrich. Benzaldehyde, $SnCl_2$, Methane sulfonic acid, 2′,7′-dichlorofluorescin diacetate (DCF-DA), N-acetyl-L-cysteine (NAC), 5,10,15,20-tetraphenylporphyrin (H_2TPP), 5,10,15,20-tetraphenylporphyrinato zinc(II) (ZnTPP), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 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. ^{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. 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)[S1] as the standard. Singlet oxygen quantum yield (Φ_Δ) values were determined by photooxidation of 1,3-diphenylisobenzofuran (DPBF) in dimethylsulfoxide (DMSO) by using a comparative method with 5,10,15,20-tetraphenylporphyrin (H_2TPP) was used as the reference.[S2] Steady-state phosphorescence measurements of singlet oxygen phosphorescence (*ca.* 1270 nm) were carried out on a Picoquant FluoTime 300 spectrometer equipped with a Near-IR PMT. Triplet state lifetimes were determined in nitrogen saturated DMSO solutions at 500 nm by using an Edinburgh Instruments LP980 spectrometer and pump beams of 444 and 428 nm for **SnNCP** and **SnTPP**, respectively, provided by an Ekspla NT-342B laser (2.0 mJ / 7 ns, 20 Hz).

Cultures of the MCF-7 cell were obtained from Cellonex®. 10% (v/v) heat-inactivated 10% fetal bovine serum (FBS) and 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B were obtained from Biowest®. Dulbecco's phosphate-buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Lonza®.

Cell studies

The *in vitro* anticancer activities of the **SnNCP** and **SnTPP** were evaluated against MCF-7 cell line by using the MTT assay.[S3] The MCF-7 cells cultured in Dulbecco's modified Eagle's medium (DMEM) containing l-glutamine and phenol red, and supplemented with 10% heat-inactivated fetal calf serum (FCS) and 100 unit/ml-penicillin–100 µg/ml-streptomycin-

amphotericin B. When the cells were 80% confluent, they were collected and seeded in 96-well plates at a density of 1×10^4 cells per well in DMEM medium. The cells were then incubated for 24 h at 37°C in the incubator under 5% CO_2. The medium was replaced with DMEM media containing compounds at different concentrations (0.4−25 μM) and the cells were incubated for 24 h in the dark. Control cells were given fresh DMEM medium. After 24 h, the original medium was removed and fresh DMEM with no phenol red was added and cells were irradiated at 660 nm (280 mW.cm^{-2}) and 780 nm (440 mW.cm^{-2}) with Thorlabs M660L3 and Thorlabs M780L3 LEDs, respectively, which is mounted into the housing of a Modulight 7710-680 medical laser system for 30 min. Fresh DMEM medium was added and cells were incubated for a further 24 h in the dark. A separate set of cells treated with the compounds were prepared and no light treatment was performed. Cell viability was determined by MTT assay.[S3] 20 μL of MTT (5mg/mL) solution was added to each well and incubated for 3 h to form purple formazan crystals. The medium was discarded carefully and 200 μL of DMSO was added to dissolve formazan crystals. Data were quantified by measuring the absorbance at 540 nm with a Molecular Devices Spectra Max M5 plate reader. The cytotoxicities of the complexes were measured as a percentage ratio of the absorbance of the treated cells relative to the untreated controls. The IC_{50} values were determined by nonlinear regression analysis (GraphPad Prism 5).

Cellular uptake

MCF-7 cells (1×10^5 cells) were seeded in 24-well cell culture plates and incubated for 24 h. The cells were exposed to 10 μM of **SnNCP** and **SnTPP** at regular time intervals (12, 24, 48 h). After the incubation time, the cells were washed three times with PBS, lysed with 30 μL of Triton-100X and solubilized in 70 μL of DMSO. The relative cellular uptake was measured by determining the absorption at 445 nm for **SnNCP** and 430 nm for **SnTPP** with an ELISA reader. Control experiments were carried out in the absence of compound treatment.

DCF-DA assay

The intracellular production of reactive oxygen species (ROS) was detected using the 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assay.[S4] 1×10^5 MCF-7 cells were seeded in a 96 well plate and incubated for 24 h. Cells were incubated with 10 μM of Sn(IV) porphyrins for 24 h in dark. DCFDA (10 μM, final concentration) were added and incubated further for 30 min in the dark. Cells were washed with PBS three times to remove any extracellular compounds and DCF-DA and were irradiated with 660 nm (280 mW.cm^{-2}) and 780 nm (440

mW.cm^{-2}) mounted into the housing of a Modulight 7710-680 medical laser system for 30 min. Control experiments were carried out in parallel in the dark. Cells were analyzed using a multi-plate reader with excitation and emission wavelengths of 485 and 535 nm, respectively.[S4] The same experiment was also performed in the presence of 1 mM N-acetyl cysteine (NAC), as a ROS quencher.

Theoretical calculations

Geometry optimizations were carried out for **SnNCP** and **SnTPP** by using the Gaussian 09 software package[S5] at the B3LYP/6-31G(d) level of theory. TD-DFT calculations were carried out in a similar manner with the CAM-B3LYP functional since it contains a long-range correction.

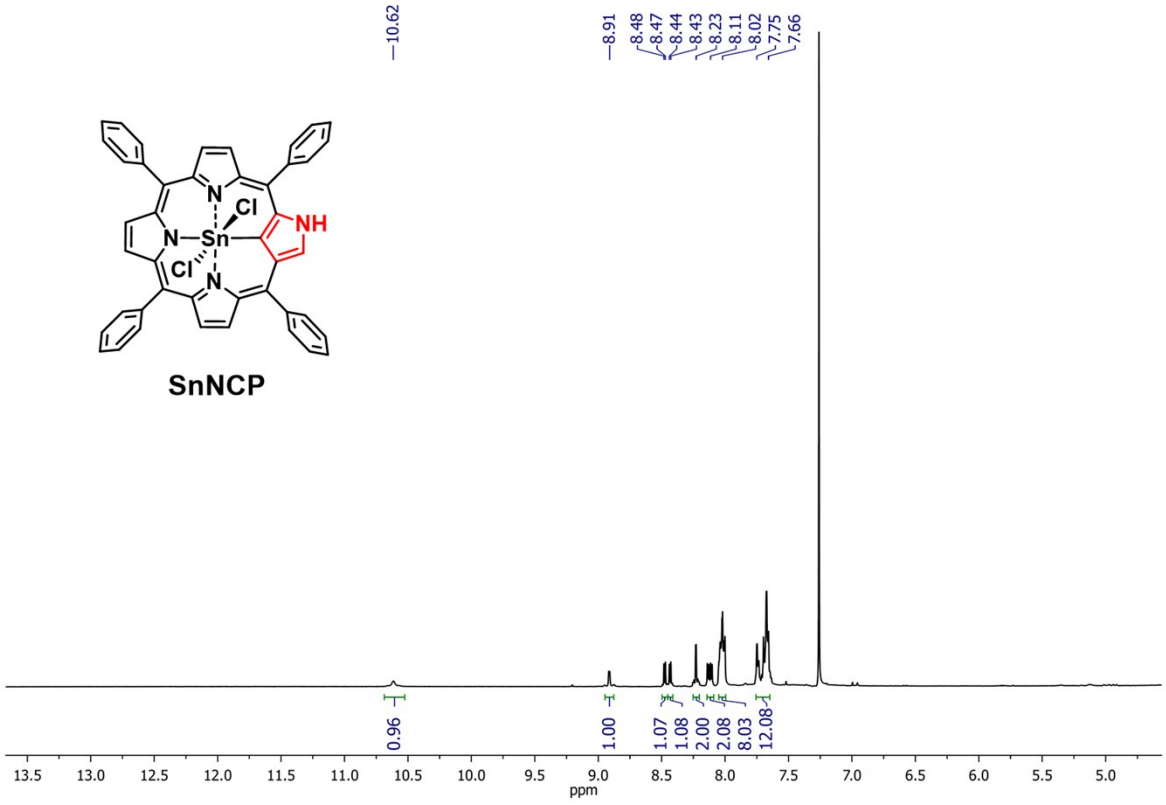

Figure S1. ^{1}H NMR (400 MHz) spectrum of **SnNCP** in $CDCl_3$.

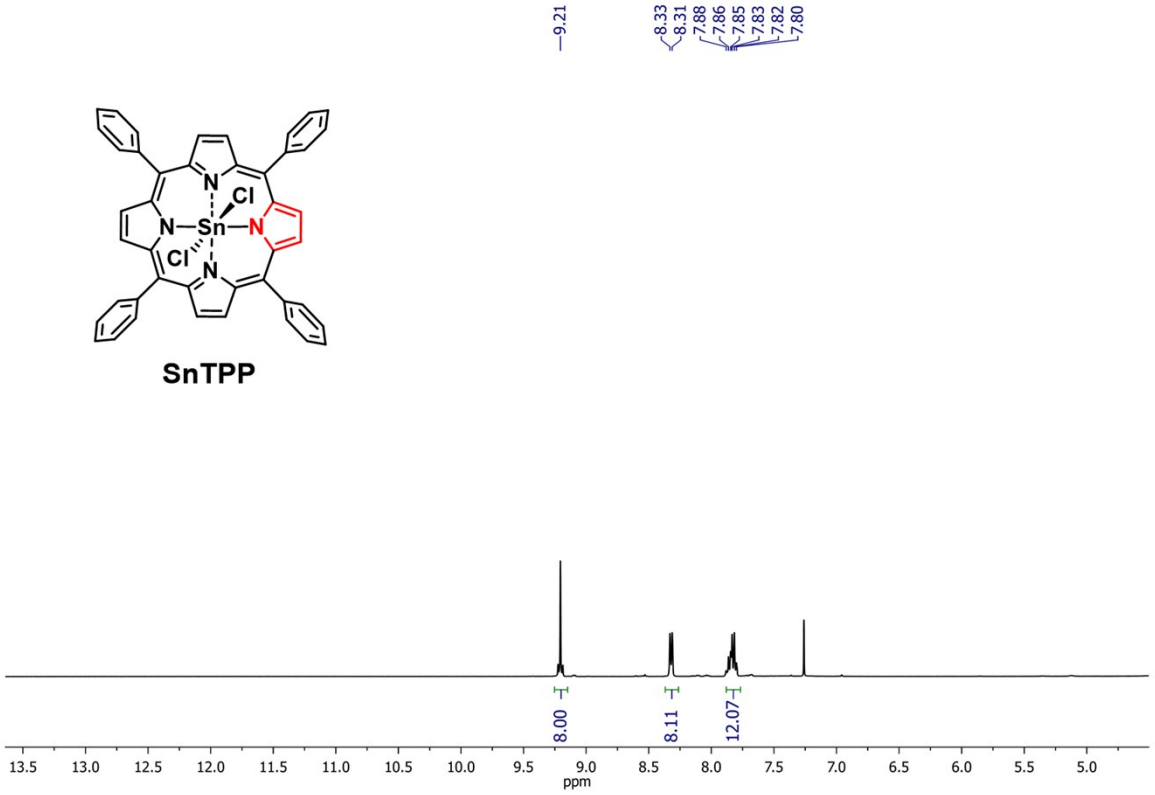

Figure S2. ^{1}H NMR (400 MHz) spectrum of **SnTPP** in $CDCl_3$.

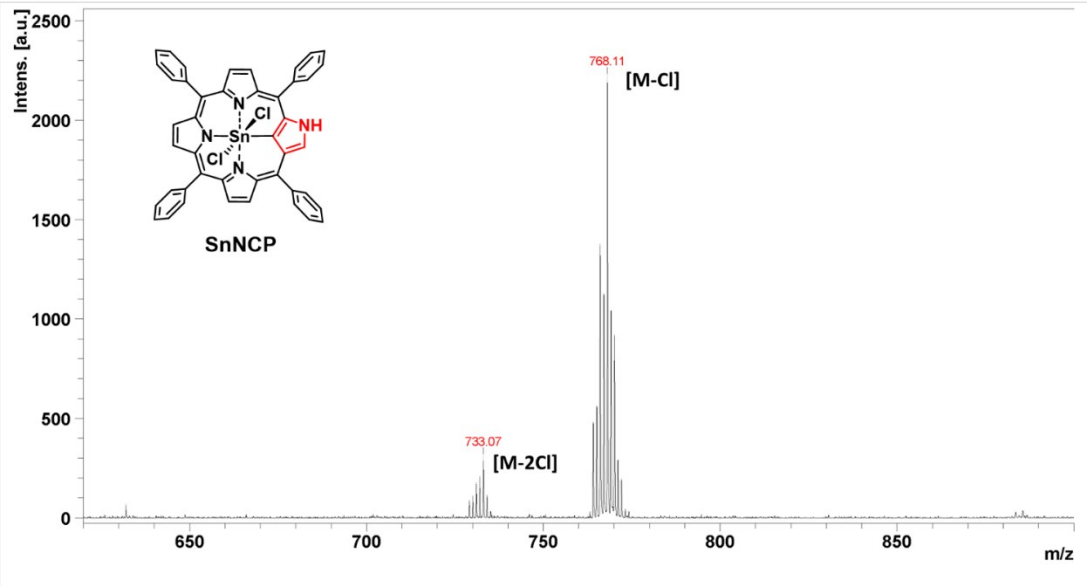

Figure S3. MALDI-TOF MS data for **SnNCP**.

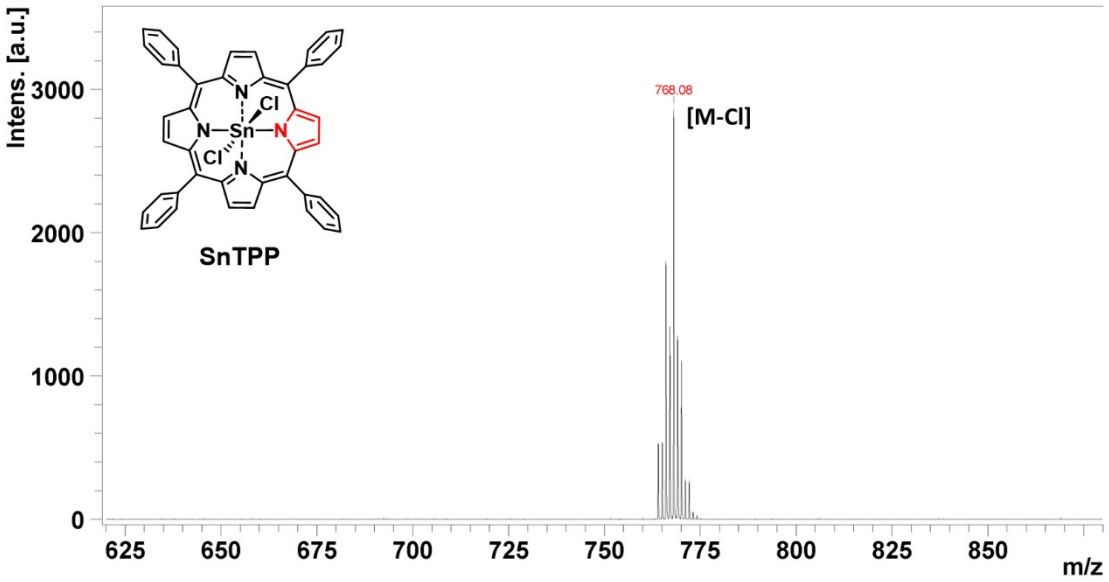

Figure S4. MALDI-TOF MS data for **SnTPP**.

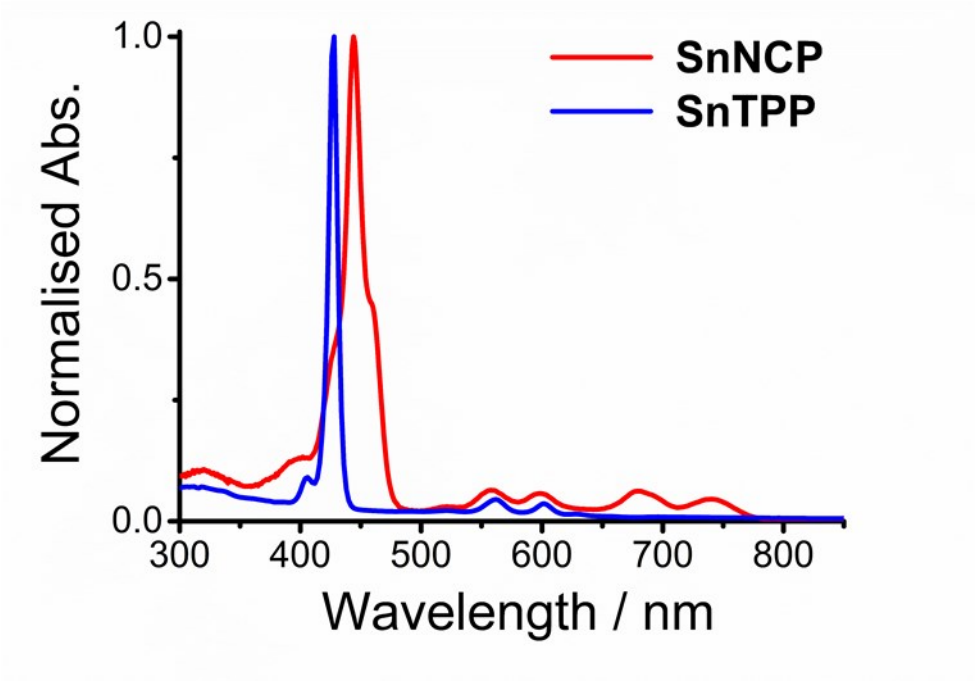

Figure S5. UV-visible absorption spectra of the **SnNCP** (red line), **SnTPP** (blue line) in DMSO.

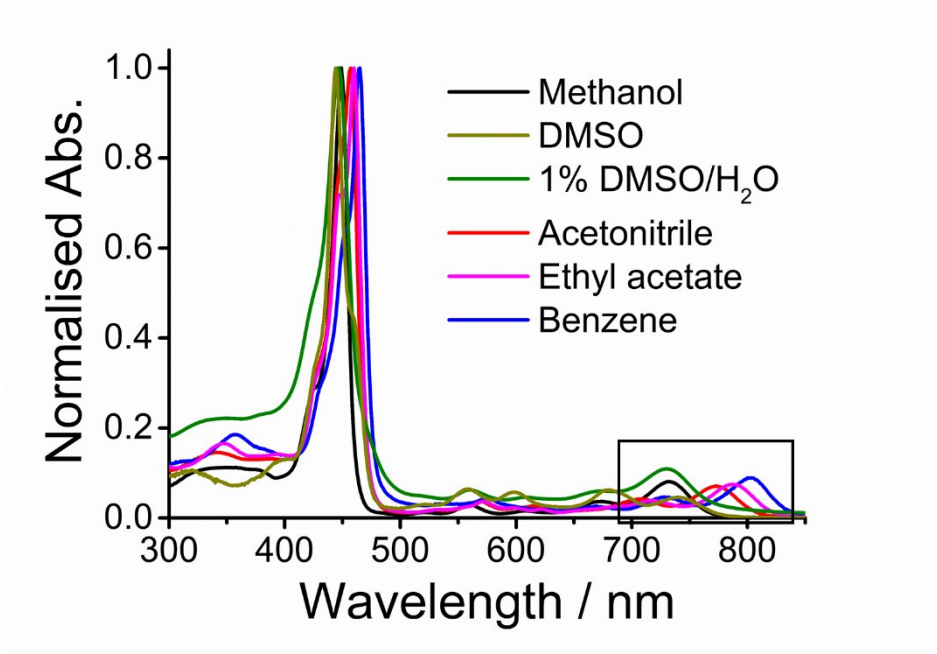

Figure S6. Normalized electronic absorption spectra of **SnNCP** in different solvents.

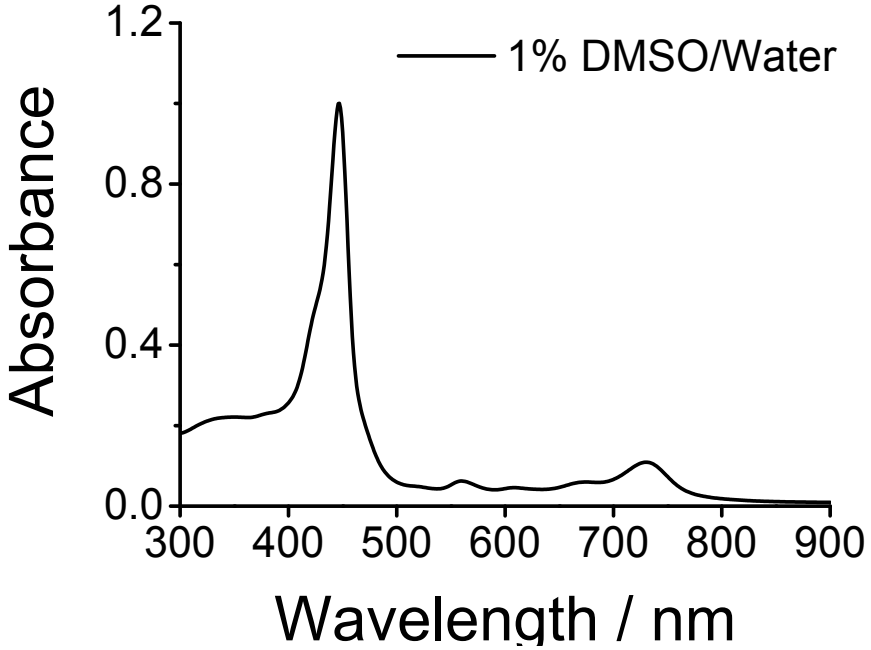

Figure S7. Absorption spectrum of **SnNCP** in 1% DMSO/Water (v/v).

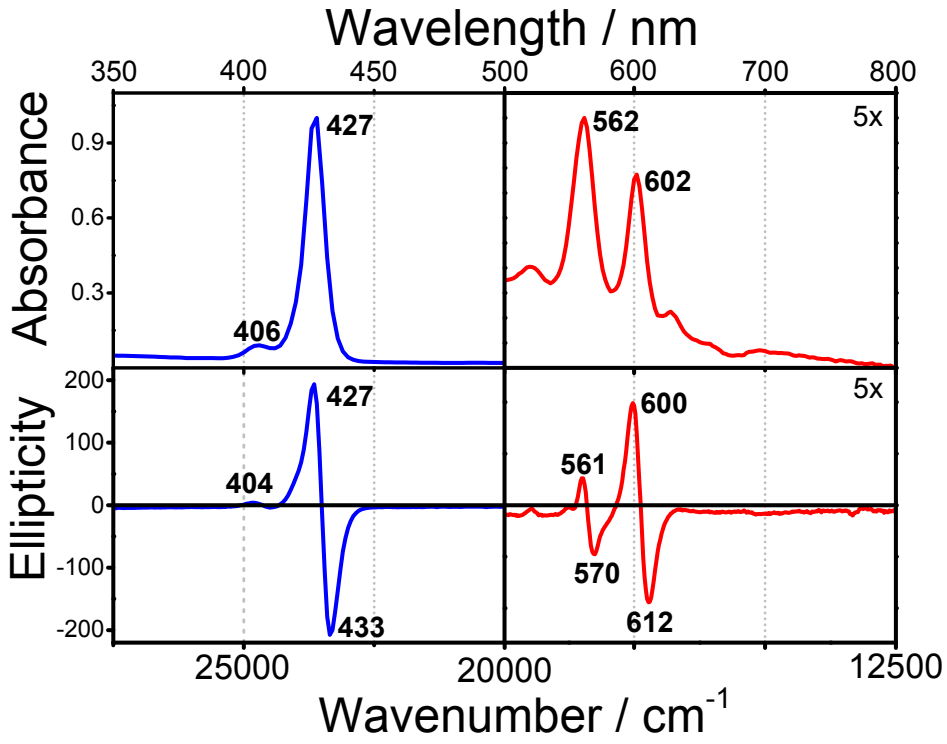

Figure S8. UV-visible absorption and MCD spectra of **SnTPP** in DMSO.

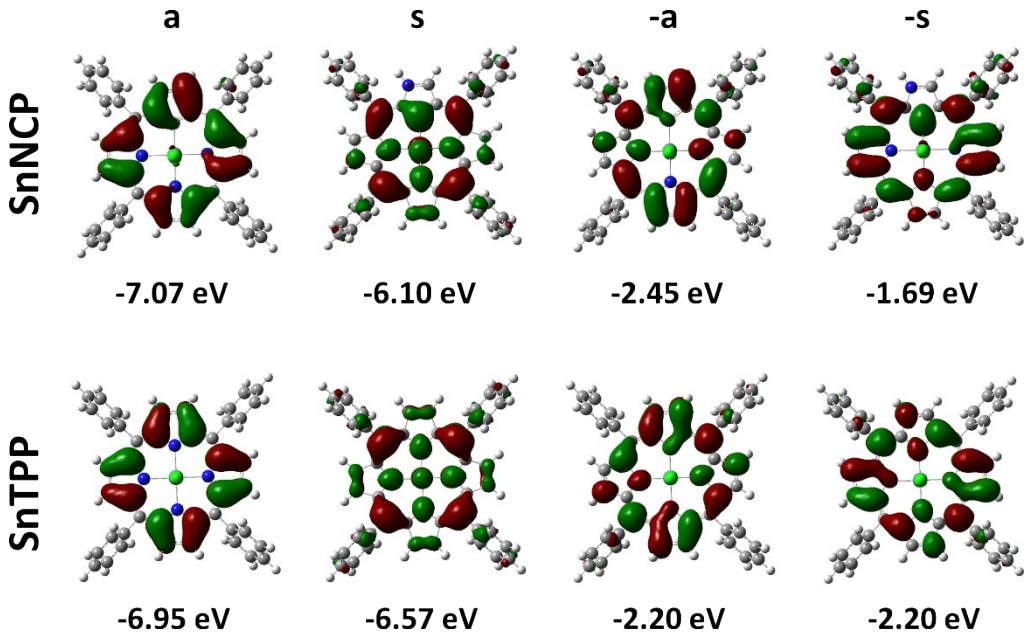

Figure S9. The angular nodal patterns and energies of the **a**, **s**, **-a** and **-s** MOs of **SnNCP** and **SnTPP**.

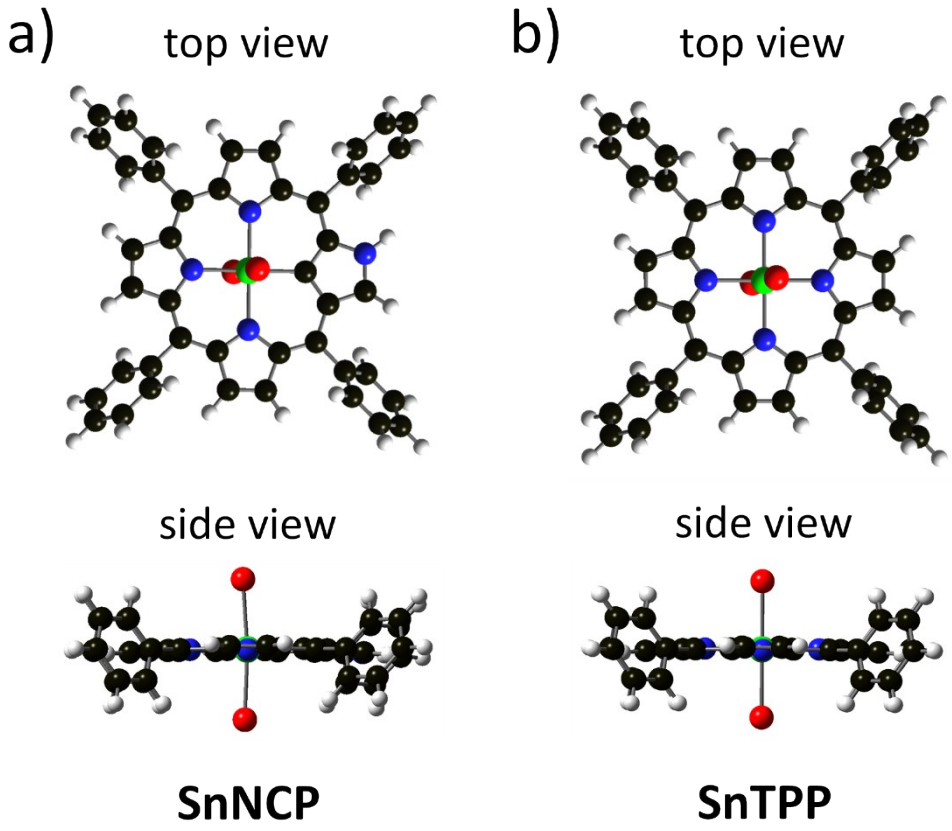

Figure S10. B3LYP optimized geometries showing top and side views of (a) **SnNCP** and (b) **SnTPP.**

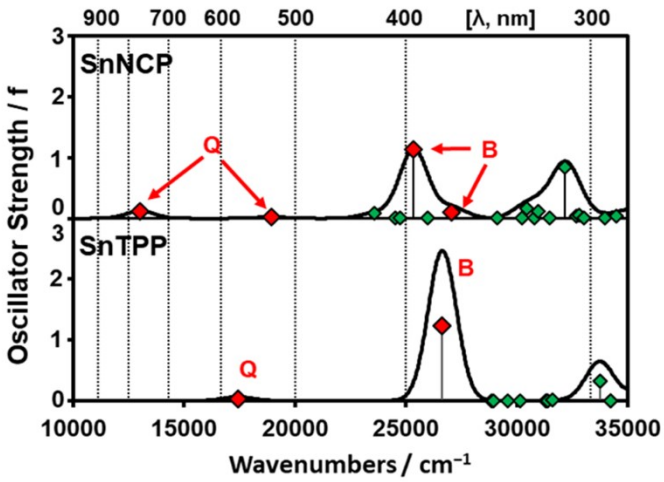

Figure 11. Calculated TD-DFT spectra for B3LYP-optimized geometries of **SnNCP** and **SnTPP** at the CAM-B3LYP/SDD level of theory. Red diamonds are used to highlight the Q and B bands of Gouterman's 4-orbital model.[S6] Simulated spectra were generated using the Chemcraft program with a fixed bandwidth of 2000 cm^{-1}.[S7] Details of the calculations are provided in Table S1.

Table S2. The calculated UV-visible absorption spectra of the B3LYP optimized geometry of **SnNCP** and **SnTPP** obtained by using the CAM-B3LYP functional of the Gaussian 09 software package[S5] with 6-31G(d) basis sets.

			SnNCP		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	740	767	0.12	**92% s → -a; 5% a → -s;** …
Q	2		528	0.03	**66% s → -s; 32% a → -a;** …
B	3	444	394	1.13	**58% a → -a; 28% s → -s;** …
B	4		369	0.09	**48% a → -a; 38% a → -s;** …
			SnTPP		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	602	574	0.03	**32% s → -a; 31% s → -s; 18% a → -s; 18% a → -a**; …
Q	2		574	0.03	**32% s → -s; 31% s → -a; 18% a → -s; 18% a → -a**; …
B	3	427	374	1.23	**32% a → -a; 29% a → -s**; **18% s → -s**; 17% **s → -a**; …
B	4		375	1.23	**32% a → -s; 29% a → -a**; **18% s → -a**; 17% **s → -s**; …

[a]Excited state number assigned in increasing energy in the TD-DFT calculations. [b]Experimental wavelengths in nm, 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** refers to the MO nomenclature of Michl's perimeter model.[S8] One-electron transitions between these four MOs are highlighted in bold.

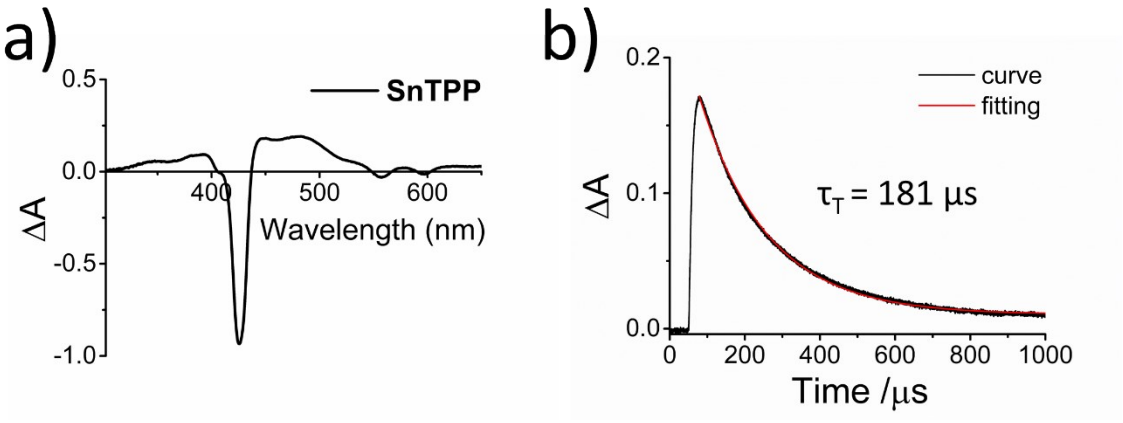

Figure S12. (a) Transient absorption spectra for N_2 purged **SnTPP**; and (b) the triplet absorption decay curve for **SnTPP** in DMSO.

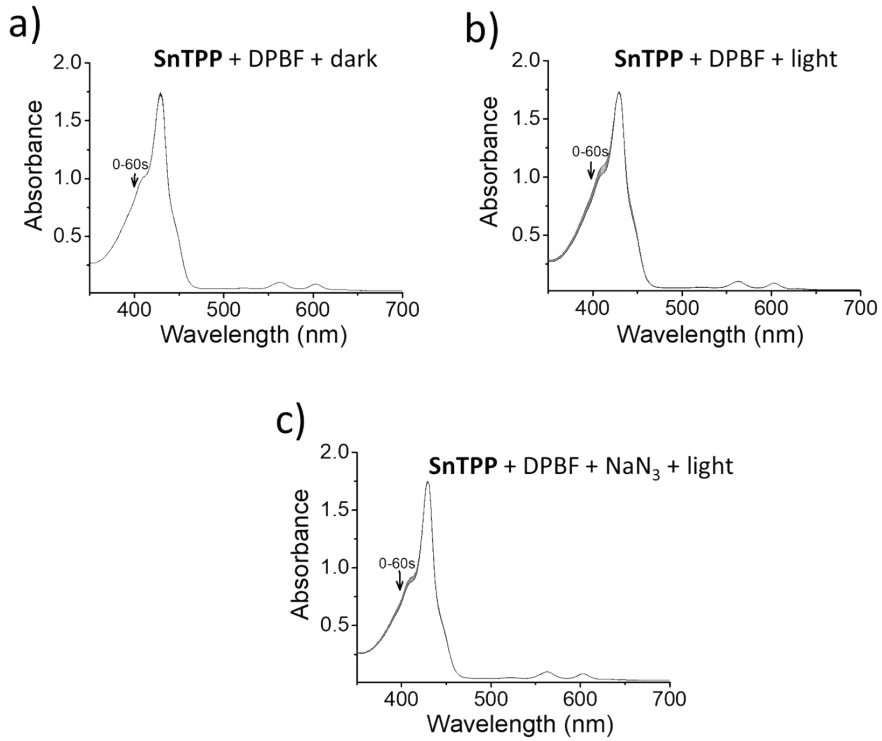

Figure S13. Change in the absorption spectra of DPBF in the presence of **SnTPP** (a) without light irradiation (dark); (b) under light irradiation; and (c) under light irradiation in the presence of NaN_3.

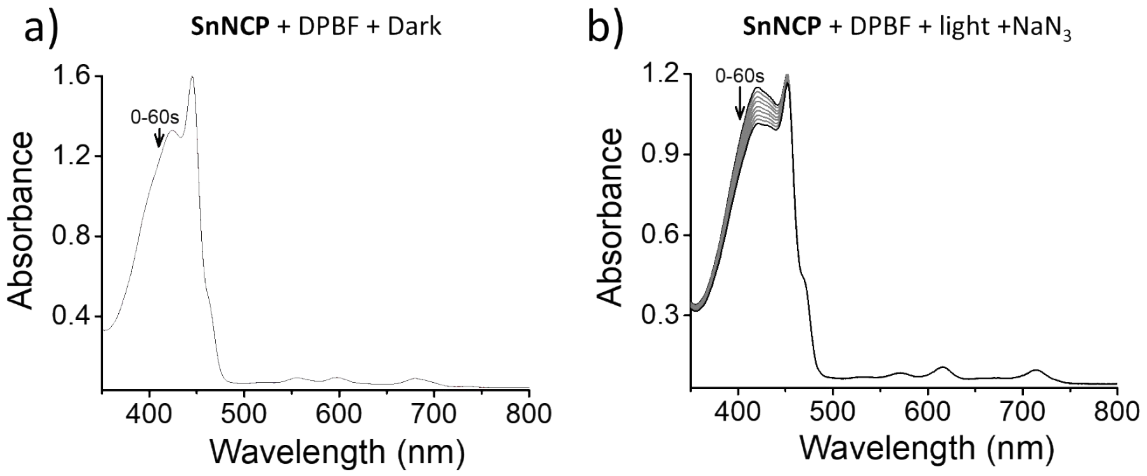

Figure S14. Change in the absorption spectra of DPBF in the presence of **SnNCP** (a) in the absence of irradiation (dark); and (b) upon irradiation with light in the presence of NaN_3.

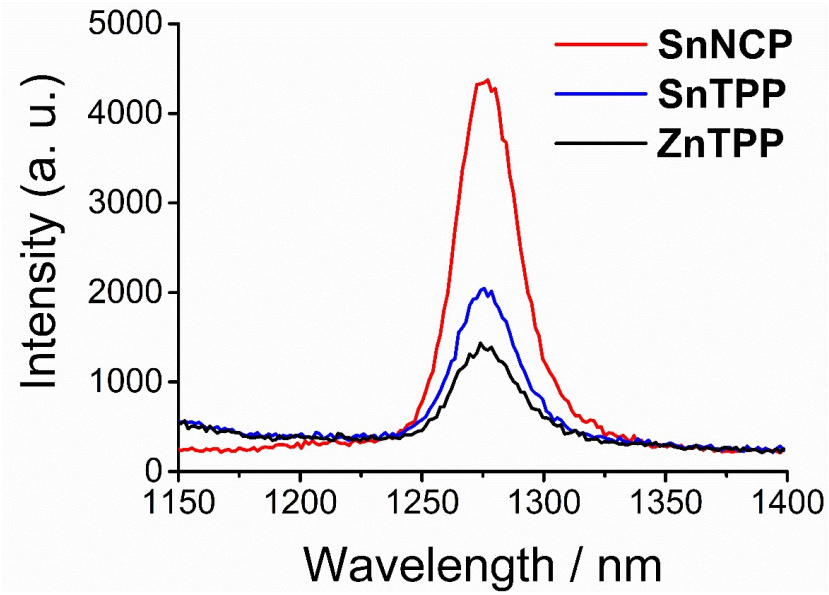

Figure S15. Singlet oxygen phosphorescence spectra produced by **SnNCP** (red line), **SnTPP** (blue line), and standard ZnTPP (black line) upon excitation at the B band maxima in air saturated DMSO.

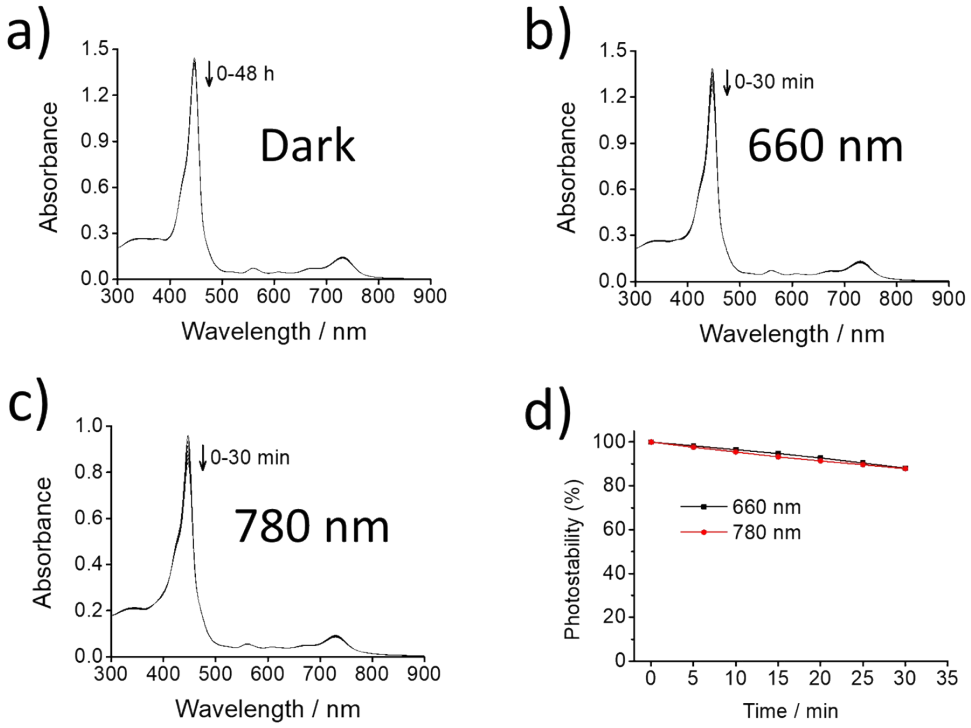

Figure S16. Absorption spectra of **SnNCP** measured (a) in the dark for 48 h; (b) under 660 nm LED irradiation; (c) under 780 nm LED irradiation; and (d) as a photostability plot for (b) and (c). Solvent: 1% DMSO/H_2O (v/v).

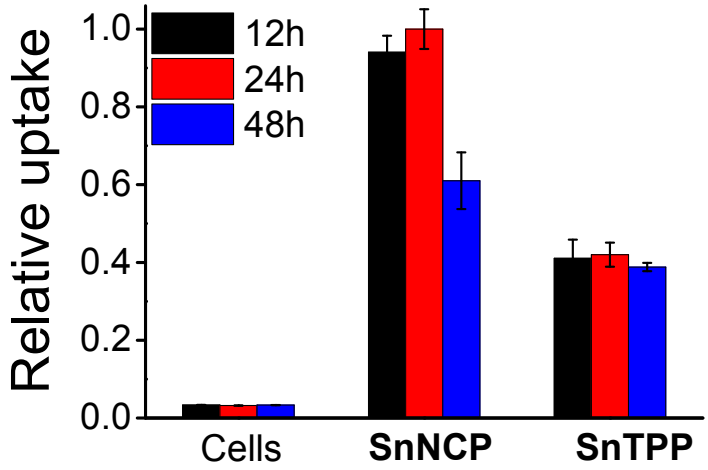

Figure S17. Time-dependent cellular uptake of **SnNCP** and **SnTPP** (10 μM) by MCF-7 cells as measured by absorption spectroscopy.

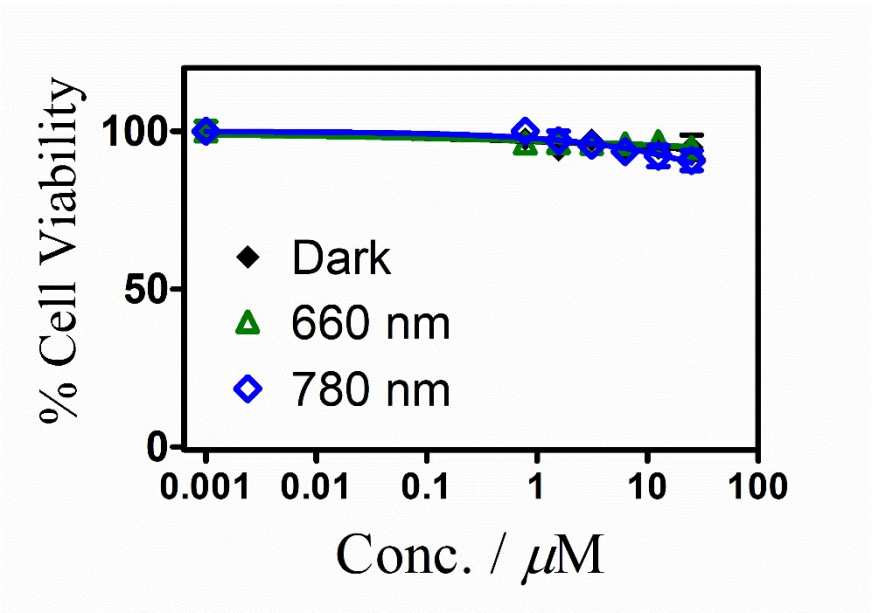

Figure S18. MTT assay cytotoxicity profiles for **SnTPP** against MCF-7 cells upon 24 h incubation in the dark followed by photoirradiation for 30 min with 660 (280 mW.cm^{-2}) and 780 nm (440 mW.cm^{-2}) Thorlabs LED light.

References

[S1] R. L. Brookfield, H. Ellul, A. Harriman and G. J. Porter. *J. Chem. Soc., Faraday Trans. 2*, 1986, **82**, 219.

[S2] R. W. Redmond, J. N. Gamlin. *Photochem. Photobiol.* 1999, **70**, 391.

[S3] M. V. Berridge, P. M. Herst and A. S. Tan, *Biotechnol. Ann. Rev.*, 2005, **11**, 127.

[S4] (a) M. H. Shen, C. Y. Shi, Y. Shen, C. N. Ong, *Free Radical Biol. Med.*, 1996, **21**, 139; (b) C. A. Hageh , M. A. Assaad , Z. E. Masri, N. Samaan, M. El-Sibai, C. Khalil and R. S. Khnayzer, *Dalton Trans.*, 2018, **47**, 4959.

[S5] Gaussian 09, Revision E.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2013.

[S6] M. Gouterman, Optical Spectra and Electronic Structure of Porphyrins and Related Rings. In *The Porphyrins*, vol. III, D. Dolphin (Ed.) Academic Press: New York, 1978, 1-165.

[S7] Chemcraft - graphical software for visualization of quantum chemistry computations. https://www.chemcraftprog.com

[S8] (a) J. Michl, *J. Am. Chem. Soc.* 1978, **100**, 6801. (b) J. Michl, *J. Am. Chem. Soc.* 1978, **100**, 6812. (c) J. Michl, *Pure Appl. Chem.* 1980, **52**, 1549. (d) J. Michl, *Tetrahedron* 1984, **40**, 3845.