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Electronic supporting information

for

A heavy-atom-free π-extended N-confused porphyrin as a photosensitizer for photodynamic therapy

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Experimental

Materials

Pyrrole, benzaldehyde, methanesulfonic acid, DDQ, 1,3-diethyl-2-thiobarbituric acid, 1,3-diphenylisobenzofuran (DPBF), 2′,7′-dichlorofluorescein diacetate (DCFDA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and Triton-X-100 were obtained from Sigma Aldrich. All other reagents and solvents were purchased from commercial suppliers and were of analytical grade and used without further purification. 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.

Equipment

UV-visible absorption spectra were recorded on a Shimadzu UV−2550 spectrophotometer. MS data were obtained on a Bruker® AutoFLEX III Smart-beam TOF/TOF mass spectrometer in positive ion mode by using α-cyano-4-hydroxycinnamic acid as the matrix. ^{1}H NMR spectra were recorded at room temperature with a Bruker 400 MHz spectrometer. Photoirradiation experiments were carried out with Thorlabs M530L3 and M660L3 light emitting diodes (LEDs) mounted into the housing of a Modulight 7710-680 medical laser system.

Synthesis

Synthesis of **NCP** (**1**)

N-confused tetraphenylporphyrin (**NCP**, **1**) was synthesized as described previously in the literature.[S1] MS (MALDI-TOF): *m/z* for $[M+H]^+$ = 615.99 (calc. 615.25). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ_H 8.99 (d, *J* = 4.8 Hz, 1H), 8.92 (d, *J* = 4.7 Hz, 1H), 8.76 (s, 1H), 8.61 (d, *J* = 4.8 Hz, 1H), 8.56 (m, 3H), 8.37 (d, *J* = 7.2 Hz, 2H), 8.34 (d, *J* = 7.2 Hz, 2H), 8.16 (m, 4H), 7.89–7.69 (m, 12H), -2.44 (br s, 2H), -5.00 (s, 1H) ppm.

Synthesis of **NCP-TB** (**2**)

NCP-TB (**1**) was synthesized by following the previously reported procedures.[S2] 0.06 g (0.3 mmol) of 1,3-Diethyl-2-thiobarbituric acid was added to a solution of **NCP** (**1**) (0.15 g, 0.25 mmol) in THF (10 mL) and stirred at room temperature for 15 min. The color of the reaction mixture changes from green to purple. The solvent was evaporated to dryness and subjected to column chromatography (silica) with chloroform/methanol (100:1 *v*/*v*) as eluent to give **NCP-TB** as purple crystalline solid (Yield, 0.16 g, 74%). MS (MALDI-TOF): *m/z* for $[M+H]^+$ = 813.38 (calc. 813.29). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ_H 12.63 (s, 1H), 8.72 (d, *J* = 5.08 Hz, 1H), 8.59 (d, *J* = 4.88 Hz, 1H), 8.45 (d, *J* = 6.84 Hz, 2H), 8.38 (d, *J* = 7.16 Hz, 2H), 8.28 (m, 3H), 8.18 (d, *J* = 5.04 Hz, 2H), 8.07 (m, 4H), 7.97 (t, *J* = 7.48 Hz, 2H),

7.88 (t, J = 7.42 Hz, 1H), 7.74 (m, 6H), 8.59 (m, 3H), 1.26 (m, 4H), 1.13 (t, J = 6.92 Hz, 6H), -3.84 (br s, 1H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ_C 14.30, 42.99, 88.85, 91.74, 111.93, 119.86, 126.42, 127.45, 127.48, 127.73, 127.82, 128.14, 128.74, 128.84, 129.27, 130.12, 130.61, 133.12, 134.64, 135.01, 135.21, 135.33, 136.40, 136.64, 137.93, 139.84, 140.17, 140.84, 141.17, 141.80, 144.15, 145.85, 149.84, 161.34, 172.11, 177.54 ppm.

Theoretical calculations

Geometry optimizations were carried out by using the Gaussian 09 software package[S3] at the B3LYP/6-31G(d) level of theory. TD-DFT calculations were carried out similarly with the CAM-B3LYP functional since it contains a long-range correction.

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

The lipophilicity of **NCP** and **NCP-TB** was calculated by the Shake-flask method following the previously reported procedure for calculating log $P_{o/w}$ values.[S4] Approximately 1 mg of the compounds was dissolved in 3 mL of 1-octanol saturated with water, and an absorbance value was measured (A_{int}). 3 mL of water saturated with 1-octanol was added, and the mixture was shaken for 3 h at room temperature. The mixture was allowed to form separate organic and aqueous solvent phases and the absorbance of the compound in the organic phase (A_{oct}) was measured. $A_{int}-A_{oct} = A_{wat}$ is 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}).

Cell studies

The *in-vitro* cytotoxicity experiments for **NCP** and **NCP-TB** were carried out towards MCF-7 cells by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[S5] MCF-7 cells ($\sim 1 \times 10^5$) 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. The medium was replaced with DMEM media containing compounds at different concentrations (0.8−50 μM), and the cells were incubated for 12 h in the dark. After the incubation time, the old media was replaced by fresh DMEM with no phenol red, and cells were irradiated separately at 530 nm (110 mW.cm^{-2}) and 660 nm (280 mW.cm^{-2}) with Thorlabs M530L3 and M660L3 LEDs, respectively, mounted into the housing of a Modulight 7710-680 medical laser system for 30 min. After photoirradiation, fresh DMEM-10% FBS was added, and cells were incubated for a further 24 h in the dark. A 25 μL portion of 5 mg mL^{-1} MTT was then added to each well and incubated for an additional 3 h. The culture medium was carefully discarded, 200 μL of DMSO was added to dissolve the formazan crystals formed, and the absorbance was measured at 545 nm with a Molecular Devices Spectra Max M5 plate reader. The percentage ratio of the absorbance of the treated cells to that of the untreated control cells provides the measurement of cytotoxicity. The IC_{50} values were determined by

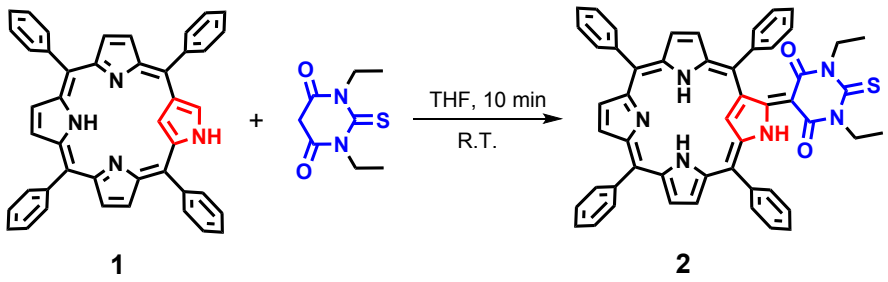
nonlinear regression analysis. A separate set of cells treated with the compounds was prepared, and no light treatment was performed to determine the dark toxicity.

Cellular uptake

The solution of photosensitizers (10 μM) was added to MCF-7 cells (1×10^6 cells) seeded in a 24-well cell culture plate and was incubated for 12 h. After the incubation time, the cells were carefully washed three times with PBS to remove extracellular compound and lysed with 30 μL of Triton-100X and solubilized in 70 μL of DMSO. The relative cellular uptake was measured by determining the absorbance value at 444 nm with an ELISA reader. Control experiments were carried out in the absence of the photosensitizer dyes.

DCF-DA assay

2′,7′-Dichlorodihydrofluorescein diacetate (DCFDA) was used to measure intracellular ROS generation.[S6] The solution of photosensitizer (10 μM) was added to MCF-7 cells (1×10^5 cells) seeded in a 24-well cell culture plate and was incubated for 4 h in the dark. DCFDA (5 μM, final concentration) was then added and incubated for a further 30 min in the dark. The cells were carefully washed with PBS (3 times) and irradiated at 530 (110 mW.cm^{-2}) and 660 nm (280 mW.cm^{-2}) with Thorlabs M530L3 and M660L3 LEDs, respectively, mounted into the housing of a Modulight 7710-680 medical laser system 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 prepared with only the photosensitizer, and untreated cells were used as a negative control.



Scheme S1. Synthesis procedure for conversion of **NCP** (**1**) to **NCP-TB** (**2**).

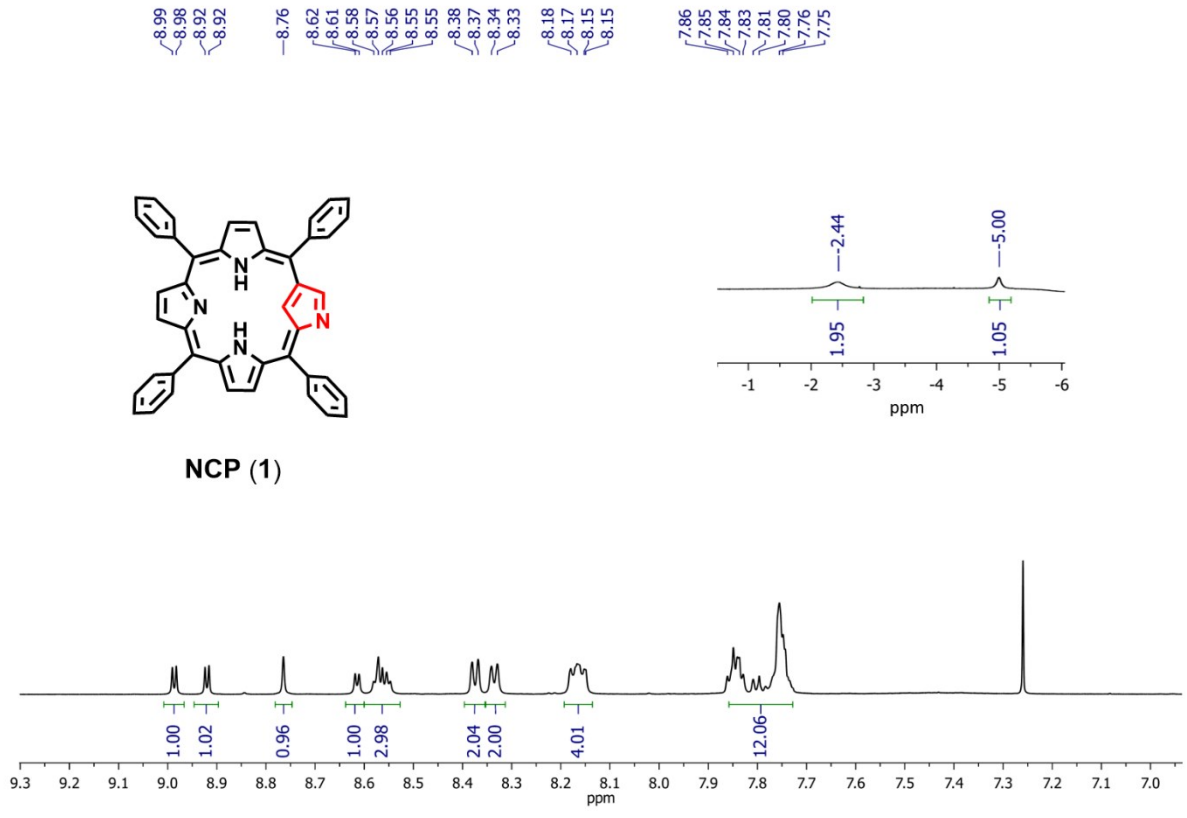


Figure S1. ^{1}H NMR (400 MHz) spectrum of **NCP** (**1**) in $CDCl_3$. Inset shows the low field region of the spectrum.

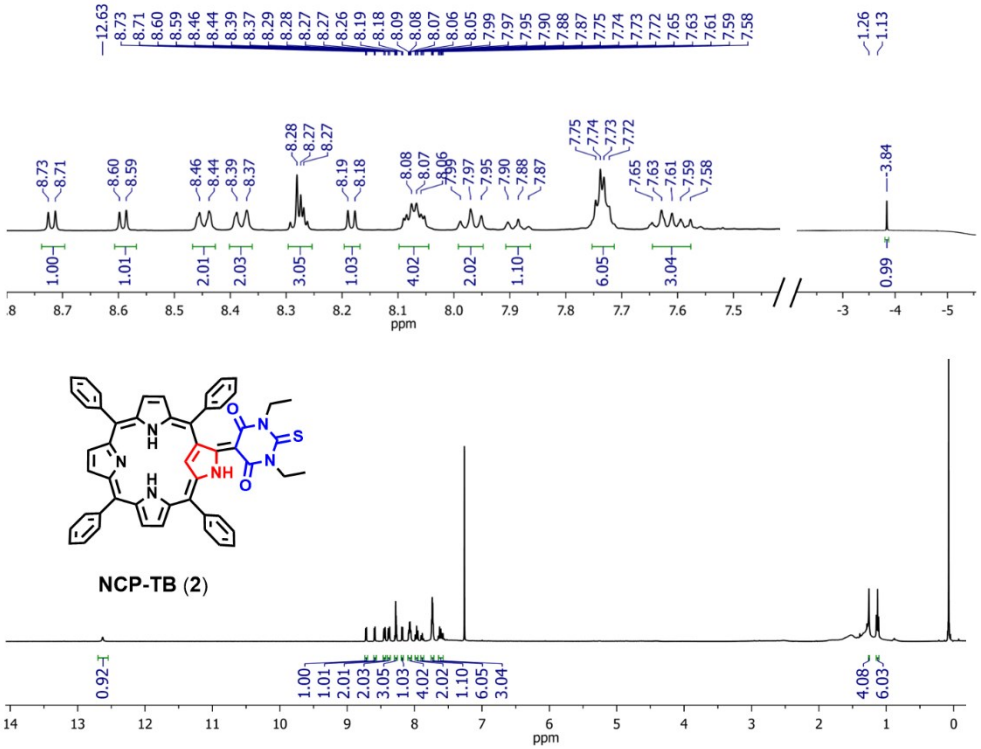


Figure S2. ^{1}H NMR (400 MHz) spectrum of **NCP-TB** (**2**) in $CDCl_3$ at 298 K. The left and right insets show an expansion of the low field and high field regions, respectively.

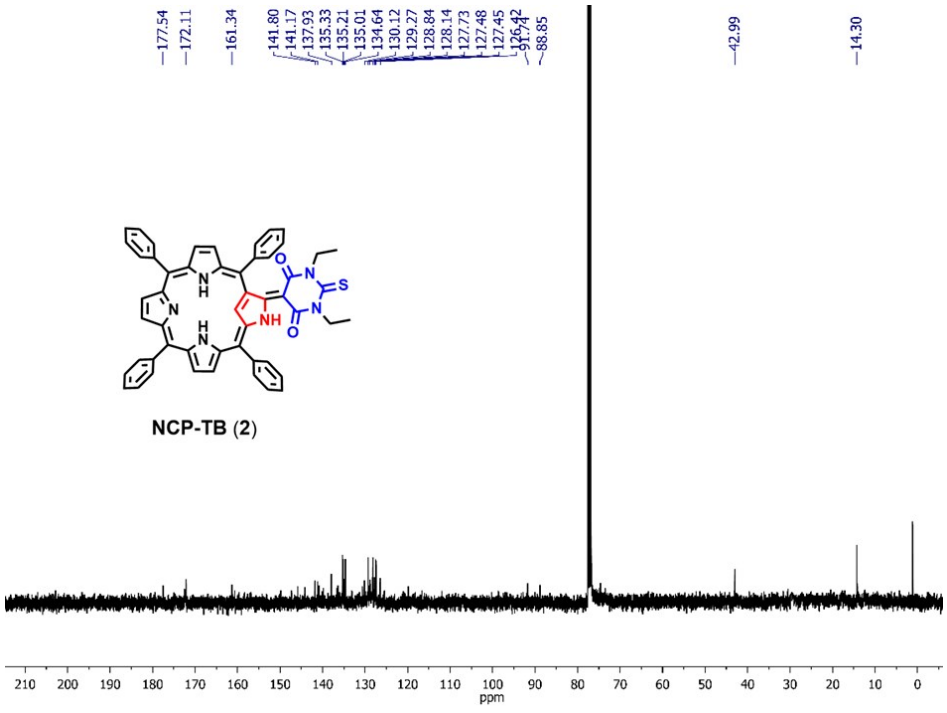


Figure S3. ^{13}C NMR (400 MHz) spectrum of **NCP-TB** (**2**) in $CDCl_3$ at 298 K.

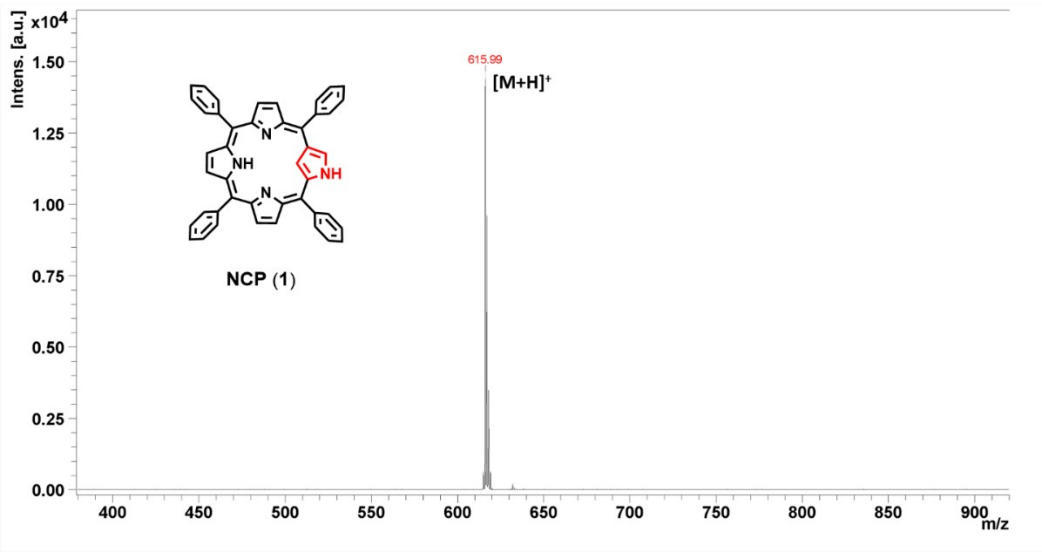


Figure S4. MALDI-TOF MS data for **NCP** (**1**).

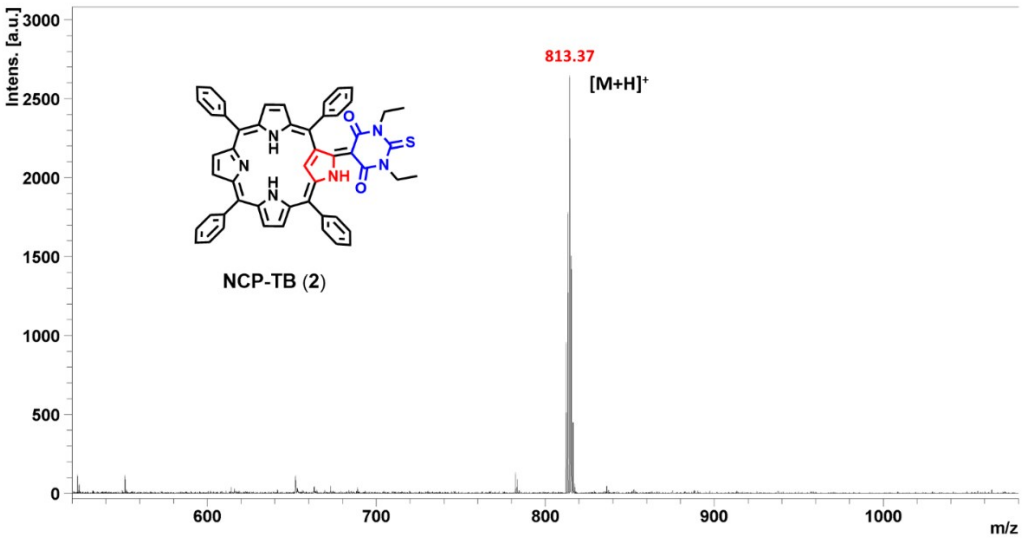


Figure S5. MALDI-TOF MS data for **NCP-TB** (**2**).

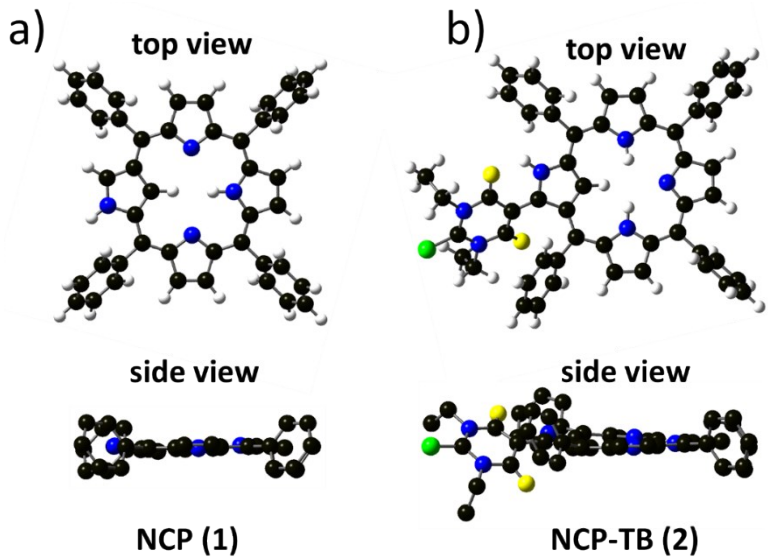


Figure S6. B3LYP optimized geometries showing top and side views of (a) **NCP** (**1**) and (b) **NCP-TB** (**2**). Hydrogens omitted in the side view for clarity.

Table S1. The calculated UV-visible absorption spectra of the B3LYP optimized geometry of **NCP** and **NCP-TB** obtained by using the CAM-B3LYP functional of the Gaussian 09 software package[S3] with 6-31G(d) basis sets.

			NCP		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	700	647	0.16	**90% s → -a;** …
Q	2	644	503	0.01	**60% s → -s; 38% a → -a;** …
B	3	444	376	1.38	**60% a → -a; 36% s → -s;** …
B	4		357	0.26	**77% a → -s;** …
			NCP-TB		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	868	672	0.03	**69% s → -a; 27% a → -s**; …
Q	2	644	594	0.08	**58% a → -a; 38% s → -s;** …
B	3	540	450	1.14	**40% a → -s; 17% s → -a**; **14% s → -s**; …
B	4	444	420	0.99	**40% s → -s; 29% a → -a**; **22% a → -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.[S7] One-electron transitions between these four MOs are highlighted in bold.

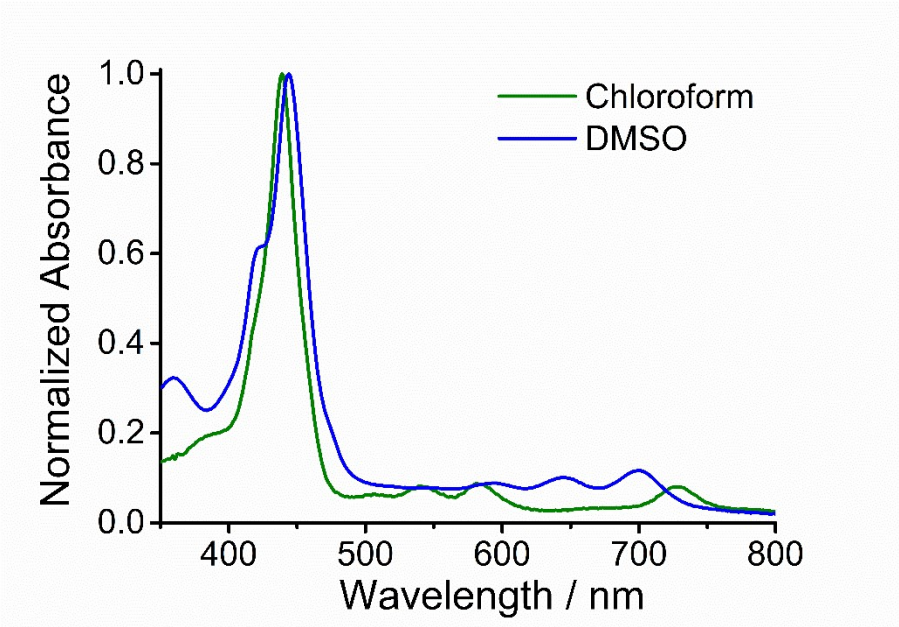


Figure S7. Absorption spectrum of **NCP** (**1**) in DMSO and $CHCl_3$.

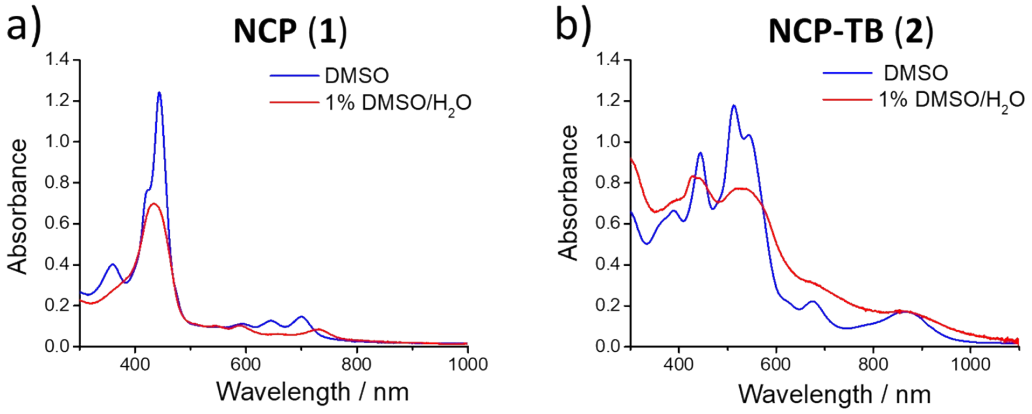


Figure S8. Absorption spectra of (a) **NCP** (**1**), (b) **NCP-TB** (**2**) in DMSO and 1% DMSO/H_2O.

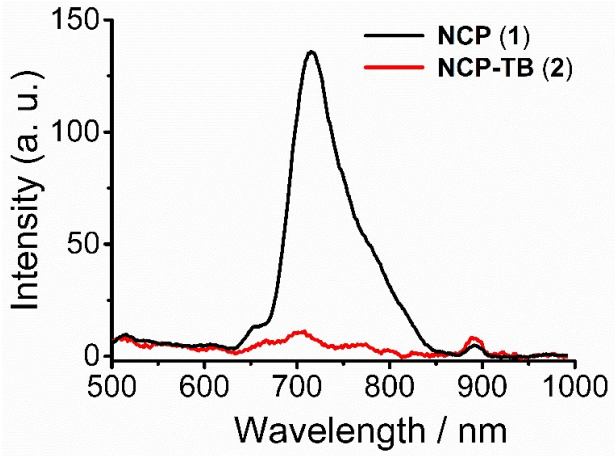


Figure S9. Emission spectra of the **NCP** (black line), **NCP-TB** (red line) in DMSO with λ_{ex} set at the Soret band maximum.

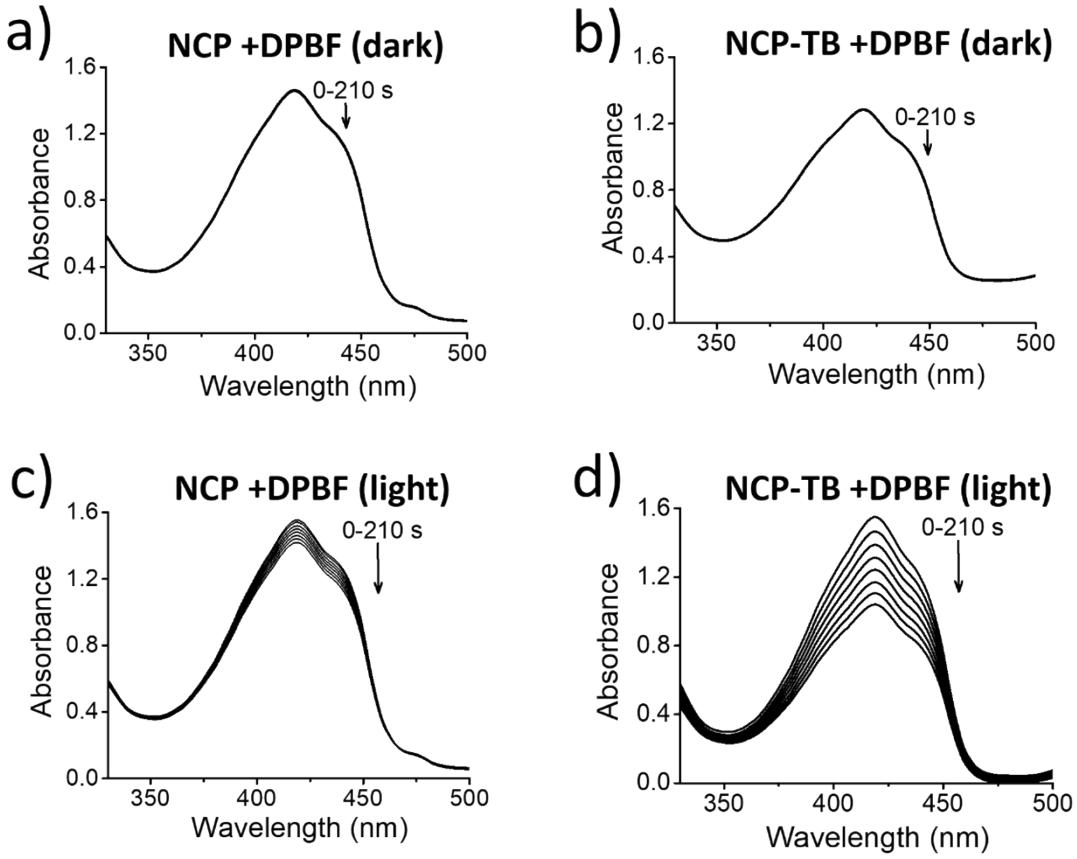


Figure S10. Changes in the absorbance of DPBF in DMSO in the presence of (a) **NCP** in the dark, **(b) NCP-TB** in the dark, (c) **NCP** after photoexcitation, (d) **NCP-TB** after photoexcitation.

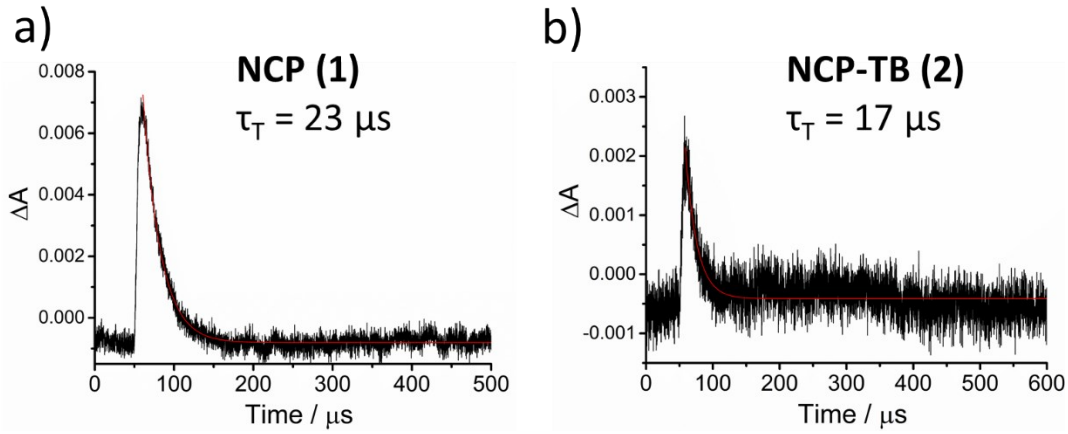


Figure S11. Triplet absorption decay curve for (a) **NCP** (**1**) and (b) **NCP-TB** (**2**) in N_2 purged DMSO.

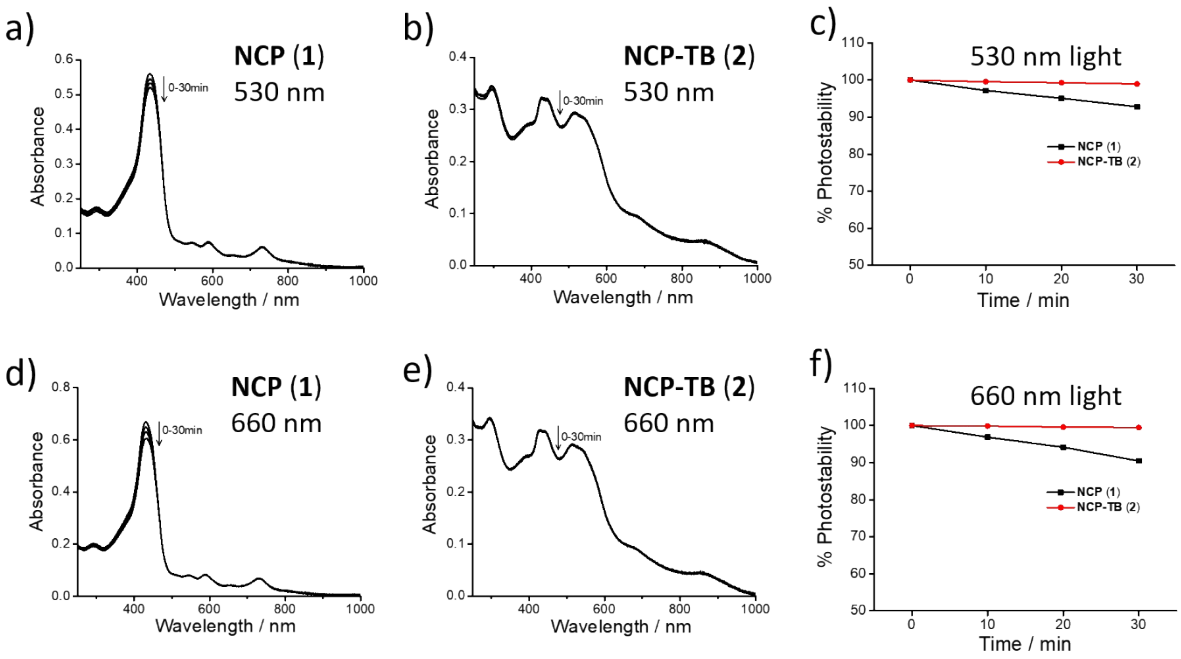


Figure S12. Absorption spectra of (a) **NCP** (**1**), (b) **NCP-TB** (**2**) measured under 530 nm LED irradiation, and (c) photostability plots for (a) and (b); (d) **NCP** (**1**), (e) **NCP-TB** (**2**) measured under 660 nm LED irradiation, and (f) photostability plots for (d) and (e). Solvent: 1% DMSO/H_2O (v/v).

References

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