Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Efficient Hydroxyl Radical Generation of An Activatable Phthalocyanine Photosensitizer: Oligomer Higher than Monomer and Nanoaggregate

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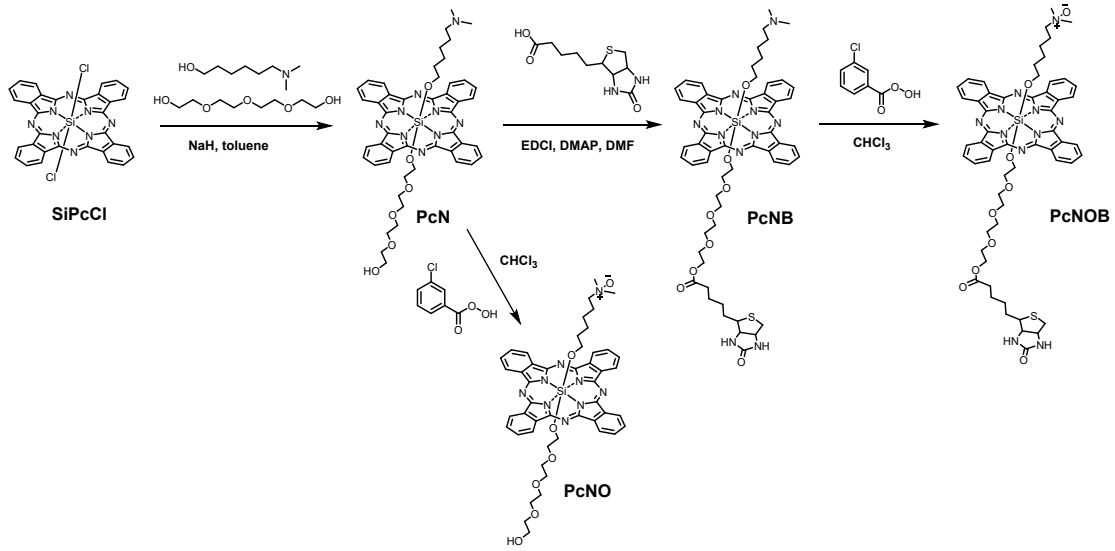
Materials and instruments

Toluene, *N, N*-dimethylformamide (DMF), chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), tetramethylene glycol, 6-dimethylamino-1-hexanol, biotin, sodium cyanide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4-dimethylaminopyridine (DMAP), 3-chloroperbenzoic acid, methylene blue (MB), Cremophor EL (CEL), aminophenyl fluorescein (APF), dihydroethidium (DHE) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich China. RPMI 1640 medium was bought from HyClone, Shanghai, China. Silicon (IV) phthalocyanine dichloride was synthesized according to our previous work. An environment of nitrogen was used for all reactions. DMF and toluene were first dried over molecular sieves and then further distilled at lower pressures before being used. Silica gel columns (100-200 mesh, Qingdao Haiyang Chemical Co., Ltd, China) were used for chromatographic purifications using the eluents mentioned. Size exclusion chromatography was carried out using Bio-Beads S-X1. Fluorescence emission and electronic absorption wavelength of these compounds were measured on an Edinburgh FL900/FS900 spectrofluorometer and Shimadzu UV-2450 UV-vis spectrometer, respectively. ^{1}H NMR spectra were determined on JEOL JNM-ECZ500R/S1 spectrometer (500 MHz) in DMSO-d6 or Chloroform-d. Relative to internal SiMe4 (δ = 0 ppm), chemical shifts were observed. An Exactive Plus Orbitrap LC/MS spectrometer from Thermo Fisher Scientific was used to record high-resolution mass spectra (HRMS). Nanoparticles were prepared using a JY92-IIN ultrasonic cell crusher. A particle analyzer (Anton Paar Litesizer 500) was used to measure dynamic light scattering (DLS). Images from a transmission electron microscope (TEM) were obtained using a TECNAI G2 F20 (FEI; Fuzhou University College of Chemistry) running at 200 kV. An IVIS Lumina III imaging system was used to capture the mice's in vivo fluorescence images.



PcN synthesis

A mixture solution of silicon (IV) phthalocyanine (SiPcCl) (100.0 mg, 0.16 mmol), NaH (24.0 mg, 1.00 mmol), 6-dimethylamino-1-hexanol (100.0 μL, 0.60 mmol) and tetraethylene glycol (100.0 μL, 0.57 mmol) in anhydrous toluene (30 mL) were stirred at 110 ℃ for 24 h. After brief cooling, the residue was purified by silica gel column chromatography using dichloromethane (DCM) and methanol (MeOH) as eluent. The first blue impurity band was removed using the solvent mixture of dichloromethane and methanol (DCM/MeOH, v/v=50:1) and then the polarity was increased to collect all the blue product of the second band, and the eluent was removed by vacuum evaporation process. Next, the compound was purified using a gel column (Bio-Beads S-X1) separation. Finally, the first blue band that remained was collected, and a small amount of ethyl acetate (EA) was used to settle it after removing the eluent. As a result, the blue solid product PcN was

obtained by filtration and vacuum drying (11.5 mg, 8.0%). ^{1}H NMR (500 MHz, Chloroform-d) δ: 9.68-9.53 (m, 8H), 8.37-8.35 (m, 8H), 3.72-3.62 (m, 8H), 3.43-3.41 (m, 2H), 3.28-3.27 (m, 2H), 3.20-3.18 (m, 2H), 2.93-2.92 (m, 2H), 2.45 (t, J = 10.0 Hz, 2H), 2.41 (s, 6H), 1.67-1.66 (m, 2H), 1.14-1.11 (m, 2H), 0.87 (t, J = 15.0 Hz, 2H), 0.66-0.61 (m, 2H), 0.42 (t, J = 10.0 Hz, 2H), -0.50- -0.57 (m, 2H), -1.39- -1.45 (m, 2H), -1.58- -1.63 (m, 2H), -1.92 (t, J = 10.0 Hz, 2H), -2.11 (t, J = 15.0 Hz, 2H). HRMS (ESI): m/z calculated for C48H51N9O6Si [M]+, 900.3631; found 900.3632. Relative error: 0.55 ppm.

PcNO synthesis

3-chloroperoxybenzoic acid (98.3 mg, 0.57 mmol) was added to a chloroform solution containing PcN (100.0 mg, 0.11 mmol) and stirred for 1 h at room temperature. The chloroform was removed by vacuum rotary evaporation at low temperature and then crude purification was carried out on a silica gel column. Finally, a gel column was used for further separation and purification to obtain a blue solid PcNO (32.0 mg, 28.3%). ^{1}H NMR (500 MHz, Chloroform-d) δ: 9.63-9.62 (m, 8H), 8.35-8.33 (m, 8H), 3.42-3.40 (m, 2H), 3.28-3.26 (m, 2H), 3.20-3.18 (m, 2H), 3.01 (s, 6H), 2.93-2.91 (m, 2H), 2.49-2.47 (m, 2H), 2.46-2.43 (m, 2H), 1.66 (m, 2H), 0.65-0.59 (m, 2H), 0.40 (t, J = 10.0 Hz, 2H), -0.50- -0.56 (m, 2H), -1.38- -1.45 (m, 2H), -1.58- -1.63 (m, 2H), -1.93 (t, J = 10.0 Hz, 2H), -2.12 (t, J = 10.0 Hz, 2H). HRMS (ESI): m/z calculated for C48H51N9O7Si [M]+,894.3761; found 894.3743. Relative error: 1.12 ppm.

PcNB synthesis

N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI, 176.8 mg, 1.14 mmol), 4-dimethylaminopyridine (DMAP, 140.0 mg, 1.14 mmol) and biotin (155.0 mg, 0.57 mmol) were mixed with DMF (10.0 mL), and stirred at room temperature for 4 h. PcN (100.0 mg, 0.12 mmol) was added to this system and continued to be stirred at room temperature for 48 h. The reaction solvent was removed by vacuum rotary evaporation. The excess of EDCI, DMAP and biotin was firstly removed by silica gel column and then further purified by gel column. It was precipitated with ethyl acetate, filtered, and dried under vacuum to obtain a blue solid PcNB (38.0 mg, 30.4%). ^{1}H NMR (500 MHz, DMSO-d6) δ: 9.68-9.66 (m, 8H), 8.52-8.50 (m, 8H), 4.27-4.24 (m, 1H), 4.08-4.05 (m, 1H), 3.95-3.93 (m, 2H), 3.58 (s, 1H), 3.12-3.10 (m, 3H), 3.02-2.98 (m, 1H), 2.84-2.80 (m, 3H), 2.77-2.73 (m, 1H), 2.32-2.29 (m, 3H), 2.16 (t, J = 15.0 Hz, 2H), 1.82 (s, 6H), 1.58-1.56 (m, 3H), 1.55-1.50 (m, 2H), 1.47-1.40 (m, 3H), 1.37- 1.33 (m, 3H), 0.32 (t, J = 10.0 Hz, 2H), 0.25-0.19 (m, 2H), -0.62- -0.68 (m, 2H), -1.62- -1.68 (m, 4H), -2.06 (t, J = 15.0 Hz, 2H), -2.17 (t, J = 10.0 Hz, 2H). HRMS (ESI): m/z calculated for C58H65N11O8SSi [M]+, 1104.4516; found 1104.4596. Relative error: 1.45 ppm.

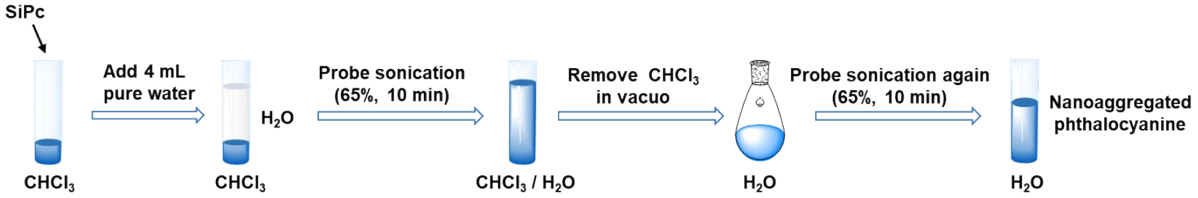
PcNOB synthesis

3-chloroperoxybenzoic acid (40.0 mg, 0.23 mmol) was added to a chloroform solution containing PcNB (50.0 mg, 0.05 mmol) and stirred for 1 h at room temperature. The chloroform was removed by vacuum rotary evaporation at low temperature and then crude purification was carried out on a silica gel column. Finally, a gel column was used for further separation and purification to obtain a blue solid PcNOB (21.0 mg, 41.4%). ^{1}H NMR (500 MHz, DMSO-d6) δ: 9.70-9.68 (m, 8H), 8.54-8.52 (m, 8H), 4.46-4.41 (m, 1H), 4.34-4.32 (m, 1H), 4.13 (s, 2H), 3.95 (t, J = 10.0 Hz, 1H), 3.63-3.59 (m, 2H), 3.54-3.51 (m, 2H), 3.12-3.10 (m, 2H), 2.85-2.82 (m, 2H), 2.72 (s, 2H), 2.42 (s, 6H), 2.35-2.31 (m, 2H), 2.23-2.19 (m, 2H), 2.15 (t, J = 10.0 Hz, 2H), 1.78-1.71 (m, 2H), 1.62-1.55 (m, 4H), 1.23 (s, 2H), 0.50-0.44 (m, 2H), 0.32 (t, J = 10.0 Hz, 2H), -0.54- -0.60 (m, 2H), -1.48- -1.54 (m, 2H), -1.64- -1.69 (m, 2H), -2.05 (t, J = 10.0 Hz, 2H), -2.16 (t, J = 15.0 Hz, 2H). HRMS (ESI): m/z calculated for C58H65N11O9SSi [M]+, 1142.4357; found 1142.4307. Relative error: 3.67 ppm.

Preparation of phthalocyanines at different degrees of aggregation

The water solutions of the three different aggregation levels were prepared as follows. Firstly, phthalocyanine and Cremophor EL (CEL) were dissolved in DMSO to reach a concentration of 2 mM and 20 mM, respectively. 5 μL of phthalocyanine solution and 5.8 μL of CEL solution were added to 2 mL of aqueous solution. This method yielded a monomeric phthalocyanine at a concentration of 5 μM in 1% CEL aqueous solution.

Secondly, 5 μL of a phthalocyanine solution with a concentration of 2 mM was taken and added directly to 2 mL of aqueous solution. This method yielded an oligomeric phthalocyanine at a concentration of 5 μM in pure aqueous solution. Finally, a certain mass of phthalocyanine solid powder was dissolved in 1 mL of chloroform and 4 mL of ultrapure water. Mixed solutions were emulsified and dispersed using an ultrasonic crusher at 65% power for 10 min. Then 1 mL of chloroform was removed using a diaphragm pump apparatus, to obtain a system with a nanoaggregated phthalocyanine at a concentration of 200 μM in pure aqueous solution. Eventually, it was diluted using pure water to 5 μM. The schematic diagram of the manufacturing process of nanoaggregated phthalocyanine is as follows.



•OH detection in aqueous solution

Aminophenyl fluorescein (APF) was used as a probe to detect •OH. 1 mg of cryopreserved APF solid powder was added to 472 μL of DMF, adjusted to a concentration of 5 mM. In the presence of •OH, the APF probe emitted bright green fluorescence (excitation wavelength around 485 nm and the fluorescence in the scanning range of 500 nm-600 nm). Phthalocyanine (5 μM) and the APF probe (20 μM) were mixed with deionized water in a quartz cuvette, and the cuvette was irradiated with red light (λ≥610 nm, 1 mW·cm^{-2}) to determine the changes in fluorescence intensity of the APF probe under different illumination times.

$O_2^{\bullet-}$ detection in aqueous solution

Dihydroethidium (DHE) was employed as an $O_2^{\bullet-}$ specific probe due to its ability to intercalate in DNA and release red fluorescence upon $O_2^{\bullet-}$ participation. Essentially, 250 g/mL of ct-DNA was present in the water solution containing phthalocyanine (5 μM) and DHE (50 μM). The control mixture consisted solely of DHE and ct-DNA. Red light (0.1 mW cm^{-2}) was applied to irradiate the mixtures. These mixtures' fluorescence spectra (excited at 490 nm) were recorded using an Edinburgh FL900/FS900 Spectrofluorometer following varying irradiation times.

Testing of cyclic voltammetric curves

A three-electrode setup was used to perform the cyclic voltammogram assay. The reference electrode and auxiliary electrode were Ag/Ag+ and Pt wire electrodes, respectively. The working electrode was a platinum-carbon compound electrode. 0.1 M tetrabutylammonium hexafluorophosphate was present in either acetonitrile or DMF during the measurement. An optimal scan rate of 100 mV/s was achieved. An external reference was Fc/Fc+.

Electron spin resonance detection

We used an electron spin resonance spectrometer, model EPR200M, to detect the generation of radical. BMPO was used to capture •OH. BMPO (1.0 mg) was dissolved in water (20 μL) to a solution concentration of 250.0 mM. Meanwhile, OligPcNB was prepared in DMF at a solution concentration of 1.0 mM. Take 20 μL from each of the above solutions and add them to water (160 μL). Finally, the mixed aqueous solution was tested immediately after exposure to light.

Dissolved oxygen removal methods

After constructing the apparatus shown in the Figure S18a, the dissolved oxygen in the aqueous solution was pumped out using a pressure-reducing device, and this process continued for 30 min.

Detection of intracellular •OH generation

Confocal dishes were used to seed hepatocellular carcinoma (HepG2) cells, which were then cultured for 24

h in either a normoxic (21% O_2) or hypoxic (1% O_2) environment. Specifically, PBS was used to wash the HepG2 cells three times after they had been incubated with 2 μM oligomer phthalocyanines for 8 h at 37°C. RPMI 1640 containing 20 μM APF (hydroxyphenyl fluorescein) was added to the culture medium, and it was incubated for 30 min. Subsequently, the RPMI 1640 was taken out and given three PBS buffer washes. Finally, the cells underwent a 5-min photosensitization experiment using a light irradiation at the power density of 15 mW cm^{-2}. Nikon single particle microscopy with an objective lens set to 40× was used to quickly capture fluorescence images of APF in staining on the cells.

Intracellular fluorescence imaging

HepG2 cells (approximately 1 × 10^5 cells per dish) were seeded in confocal dishes with RPMI 1640 medium supplemented with 10% calf serum, and they were incubated for 12 h at 37°C in a humidified 5% CO_2 environment. Following medium removal, the cells were cultured for 8 h in the dark at 0.1 μM oligomeric phthalocyanine in RPMI 1460 medium, respectively. Afterwards, the cells were twice rinsed with PBS and re-fed with PBS (0.1 mL), and finally imaged with a LEICA TCS SPE confocal microscope. Samples were observed at 640-750 nm after being excited at 635 nm. After that, the pictures were converted to digital and analyzed using the SPE ROI Fluorescence Statistics program. Additionally, the average intracellular fluorescence intensities were calculated. The above procedures are consistent with the intracellular imaging of other cells, such as 4T1 cells, and L02 cells.

Competitive inhibition assay

Except for the following information, the process for the competitive inhibition assay on HepG2 cells was the same as previously described. The cells were first incubated with extra biotin (100 nM), which was removed after 0.5 h of incubation, and then a mixed solution of oligomeric phthalocyanines (100 nM) and biotin (100 nM) was added for another 8 h co-incubation. Finally, imaged with a LEICA TCS SPE confocal microscope.

In vitro cytotoxicity

96-well plates (1×10^4 cells $well^{-1}$) were seeded with HepG2 cells, which were then cultured for 24 h in either a normoxic (21% O_2) or hypoxic (1% O_2) environment. Subsequently, 100 μL of RPMI 1640 containing various concentrations of oligomeric phthalocyanine was added to the medium. The cells were incubated for an additional 8 h, after which they were three times washed with PBS buffer. Then, the new RPMI 1640 was infused, and the cells were exposed to a red light (15 mW·cm^{-2}) for 30 min. The MTT assay was used to assess the cell viability following an additional 12 h incubation period.

Calcein AM/PI staining

Confocal plates were used to seed HepG2 cells, which were then incubated for 24 h. After that, a new culture medium containing 50 nM oligomer phthalocyanine was added, and the mixture was incubated for 8 h. Subsequently, new RPMI 1640 was added to the culture medium, which contained 5 μM propidium iodide (PI) and 5 μM Calcein AM. The Calcein AM and PI solution was removed after an additional 30 min incubation period, and it was then three times cleaned with PBS buffer. Finally, the cells were then exposed to a red light (15 mW·cm^{-2}) for 15 min or 30 min, respectively. Nikon single-particle microscopy with a 20× objective lens quickly captured fluorescent images of Calcein AM and PI staining on the cells.

Intracellular NAH(P)H/NAD(P)+ detection

Adherent HepG2 cells were cultured in six-well plates to a cell number of 1×10^6, and incubated with OligPcNOB for 2 h. Then, cells were washed three times with PBS. Subsequently, new medium RPMI 1640 was added to the six-well plate, and the cells were further cultured for 6 h. Finally, expose all cells to light for 30 min. The culture medium was aspirated, and 200 μL of NAD(P)+/NAD(P)H extraction solution was added by pipette, and the cells were gently blown to promote cell lysis. The cells were then centrifuged at 12000 rpm for 5-10 min and the supernatant was transferred to a new centrifuge tube. The supernatant was heated in a 60 °C water bath for 30 min. Heating produces insoluble material, which then needs to be centrifuged at

12000 rpm for 5 min. For testing, 20 μL of the supernatant was transferred to a 96-well plate and 90 μL of the prepared ethanol dehydrogenase working solution was added to each well. The cells were incubated for 10 min away from light to convert all the NAD+ to NADH. Then, 10 μL of the color-developing solution was added to each well and incubated for 30 min away from light until an orange-yellow color appeared. The absorption at 450 nm was then measured with an enzyme counter.

Mouse models

Mouse hepatocarcinoma (H22) cells were acquired from the China Center for Type Culture Collection (CCTCC, Shanghai, China), and the mice were procured from Wushi Animal Co. Ltd. (Fu Zhou, China). All animal studies were carried out in compliance with guidelines of the Animal Ethics Committee of Fuzhou University (2023-SG-001), and also approved by the committee. To initiate a subcutaneous tumor model, approximately 5×10^6 H22 cells in 100 μL were subcutaneously inoculated on the flank of mice weighing 18-20 g.

***In/ex vivo* fluorescence imaging**

A subcutaneous H22 tumor model in ICR mice was used to study *in vivo* imaging. Phthalocyanine (200 μM, 100 μL) was injected intravenously into the tumor-bearing mice. Next, using an IVIS Lumina III imaging system, the fluorescence was captured at various times. IVIS Lumina III imaging was also utilized to assess the *ex vivo* fluorescence imaging of phthalocyanine at 48 h post-injection. The imaging system was used to visualize the major organs of the sacrificed mice, which included the liver, spleen, lung, kidney, skin, and tumor.

***In vivo* PDT**

Mice-bearing H22 tumors were used for the *in vivo* PDT investigation. ICR mice were injected with H22 cells seven days beforehand. After that, the mice were placed in six groups to receive various treatments: Group 1: PBS injection without irradiation; Group 2: PBS injection with irradiation; Group 3: OligPcNOB injection without irradiation; Group 4: OligPcNOB injection with irradiation; Group 5: NanoPcNOB injection without irradiation; Group 6: NanoPcNOB injection with irradiation. There were five mice in each group. A laser treatment (100 $mW \cdot cm^{-2}$) was administered at 36 h after injection, irradiating the tumor area for 5 min. The effect of the different treatment groups was monitored by measuring tumor size (tumor size = $width^2 \times length/2$.) and mice body weight for 14 days after PDT treatment. Tumors were removed and weighed after 14 days.

Statistical analysis

The statistical significance was tested using a two-tailed test, and p-values were described in the captions. When $*p < 0.05$, it was regarded as significant. Herein, the smaller the p-value, the more significant is the difference between the two groups.

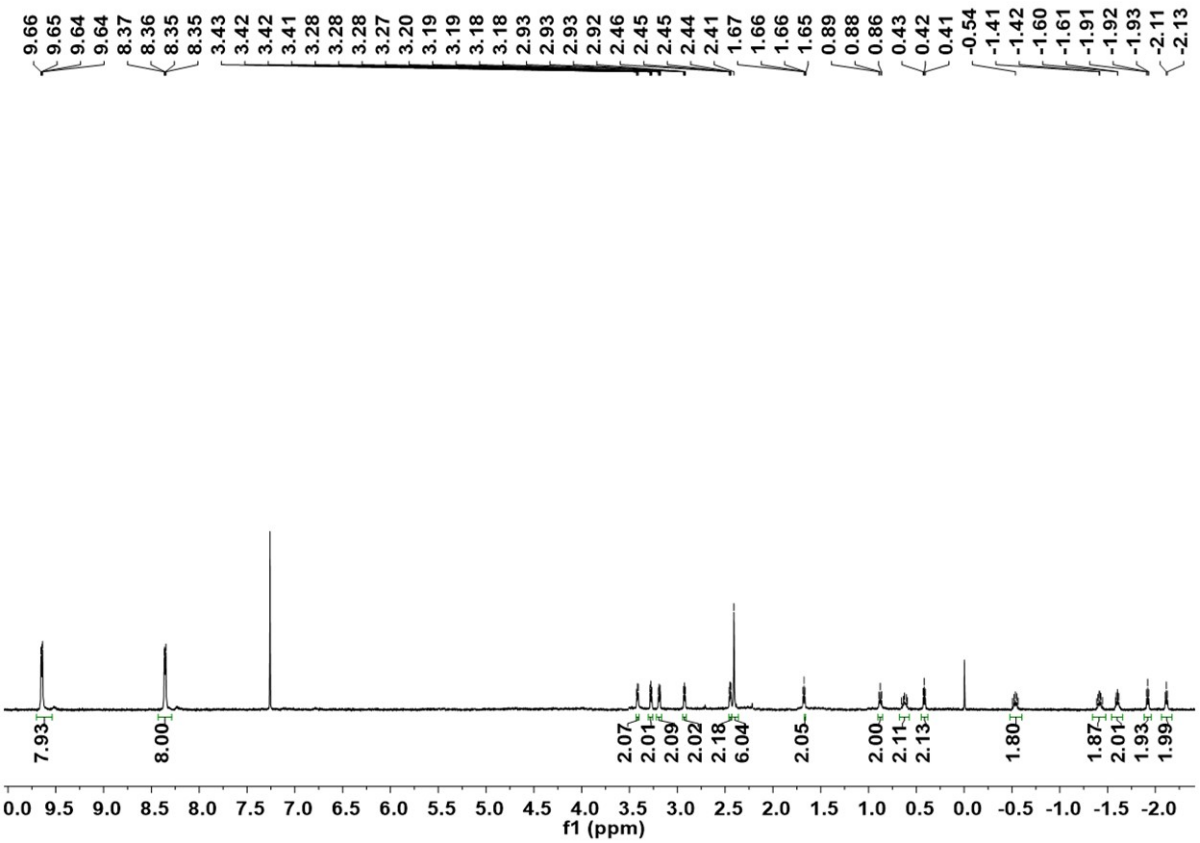


Figure S1. [1]H NMR spectrum of PcN in Chloroform-d.

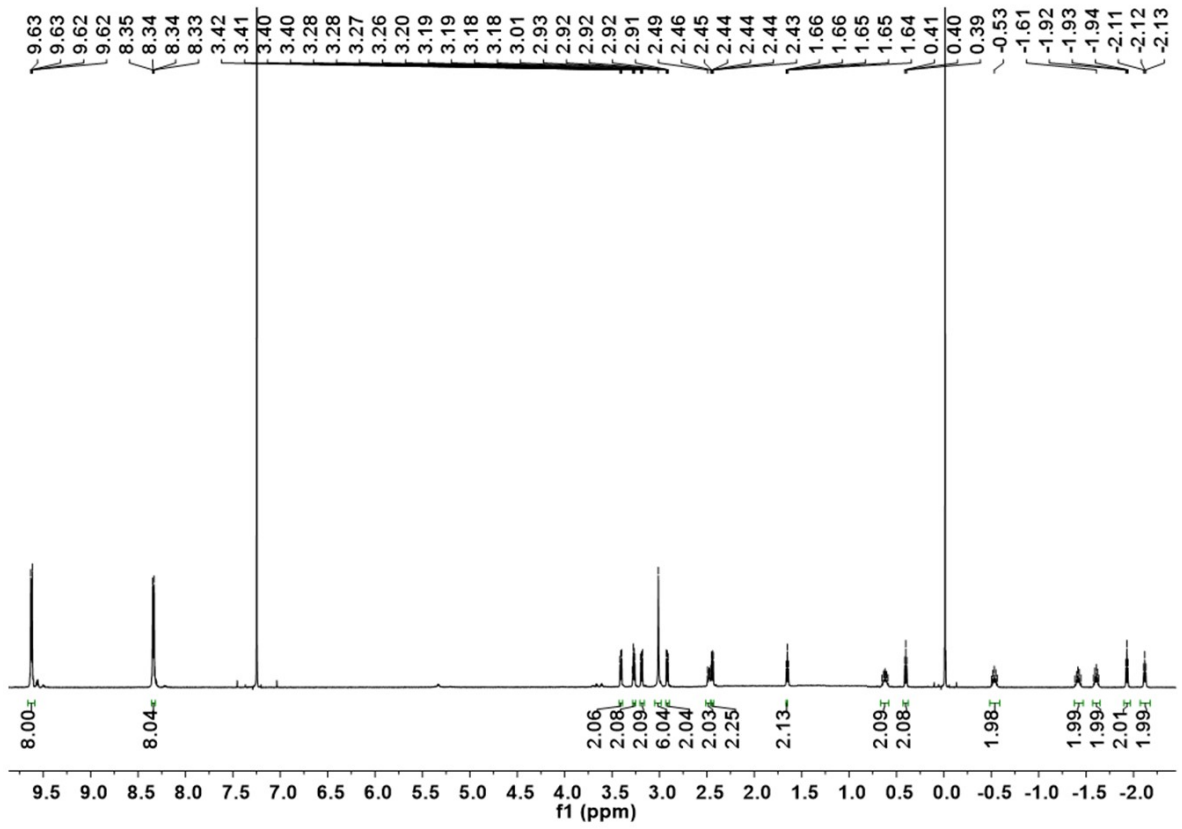


Figure S2. [1]H NMR spectrum of PcNO in Chloroform-d.

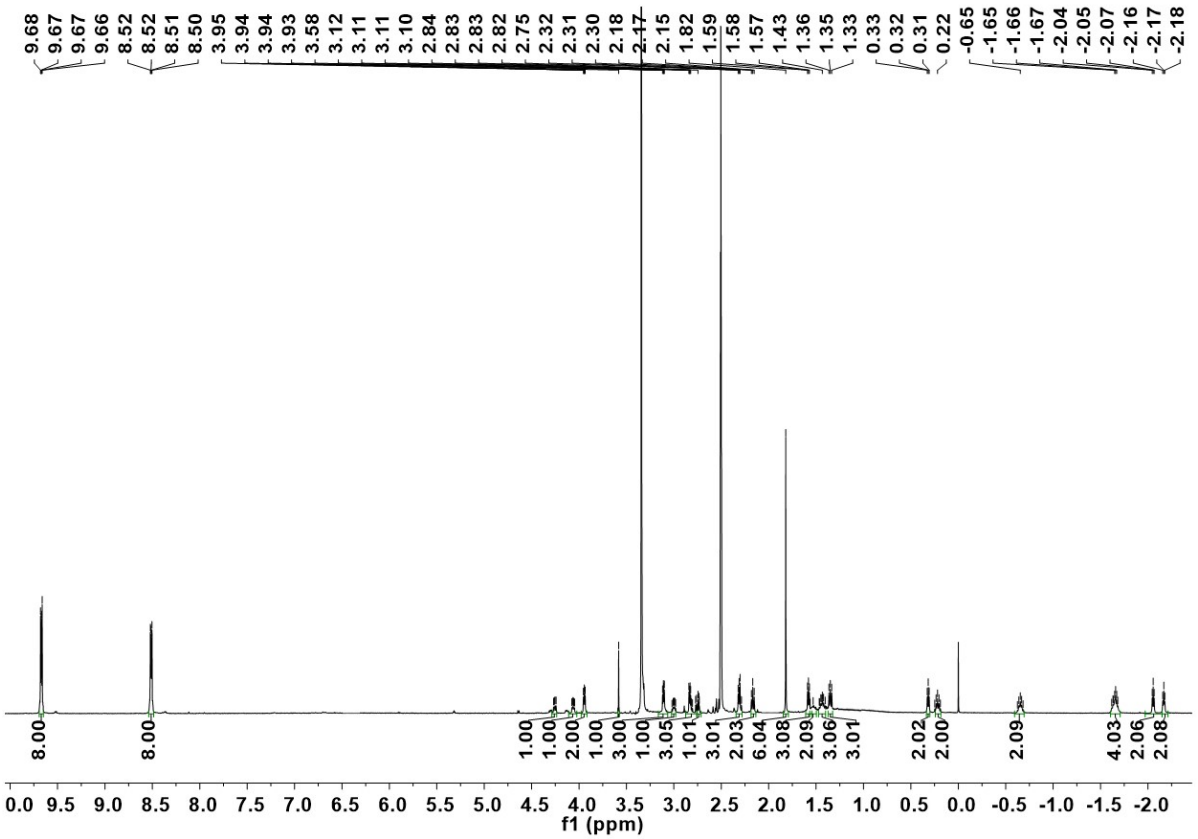


Figure S3. [1]H NMR spectrum of PcNB in DMSO-d6.

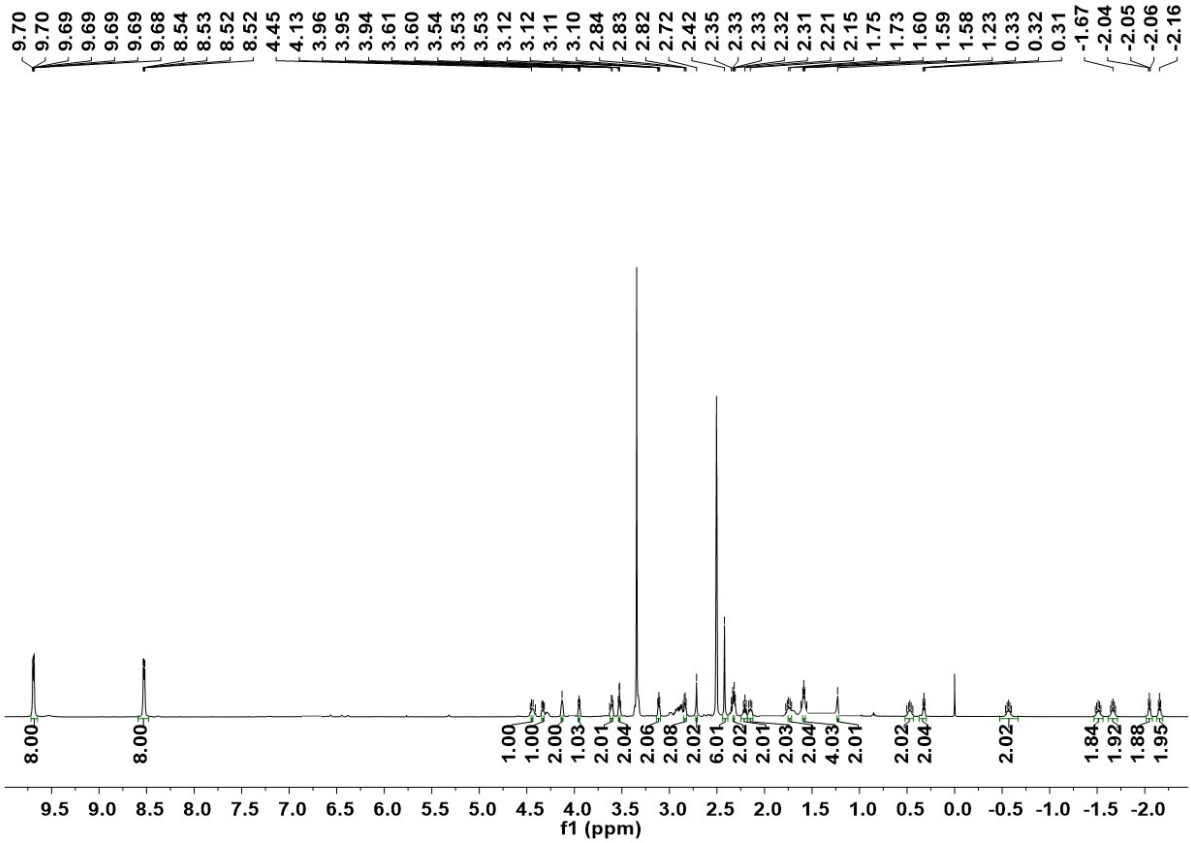


Figure S4. [1]H NMR spectrum of PcNOB in DMSO-d6.

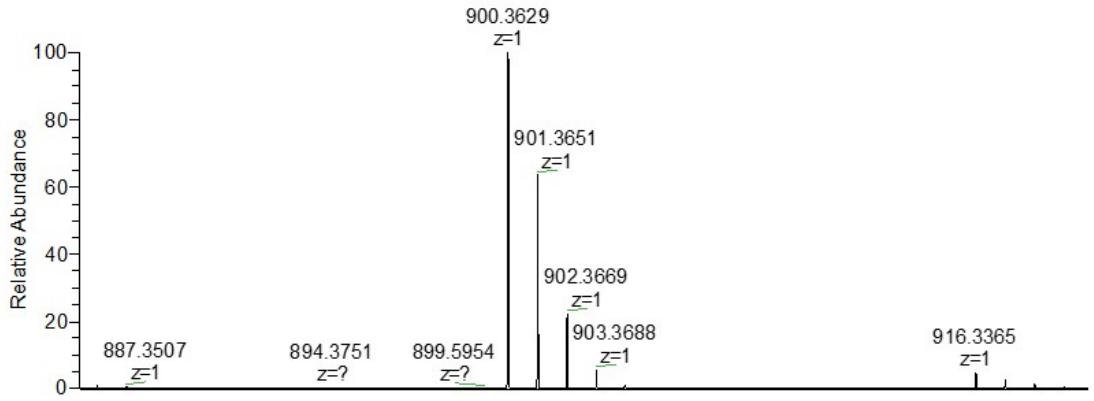


Figure S5. HRMS spectrum of PcN. The inset shows the enlarged isotopic envelope for the $[M+Na]^+$ species.

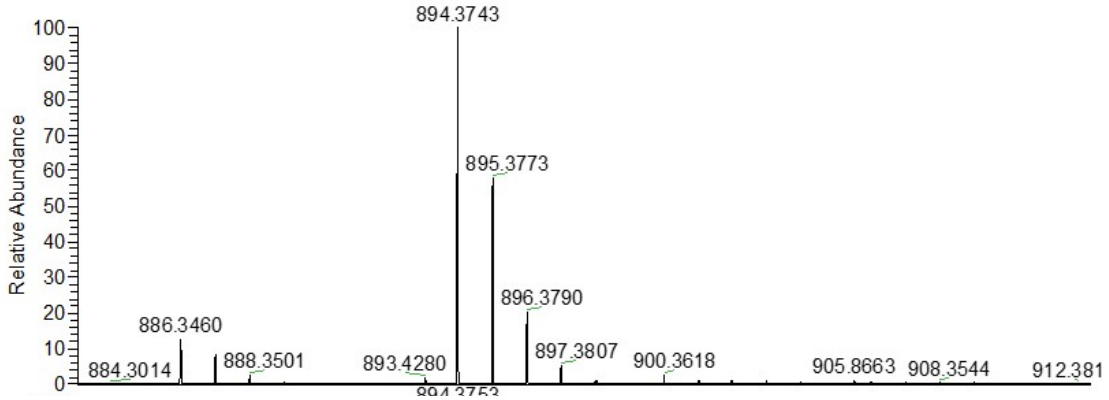


Figure S6. HRMS spectrum of PcNO. The inset shows the enlarged isotopic envelope for the $[M+H]^+$ species.

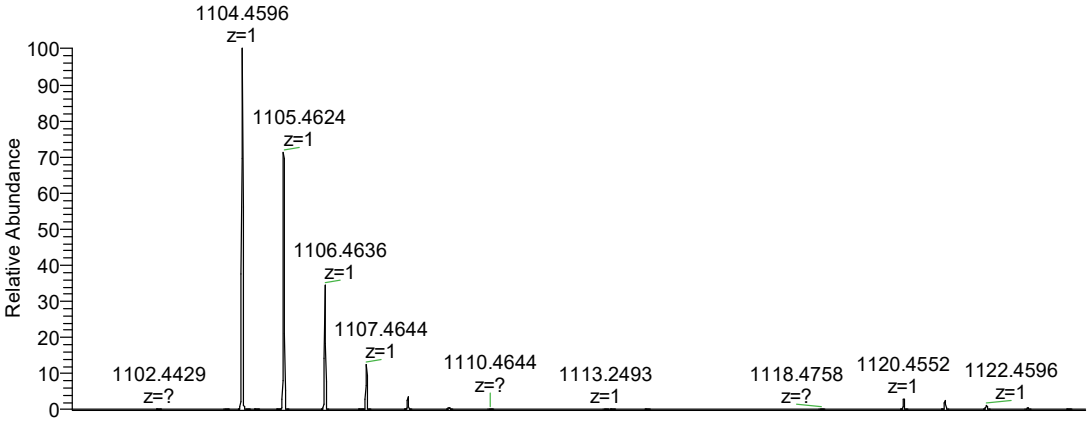


Figure S7. HRMS spectrum of PcNB. The inset shows the enlarged isotopic envelope for the $[M+H]^+$ species.

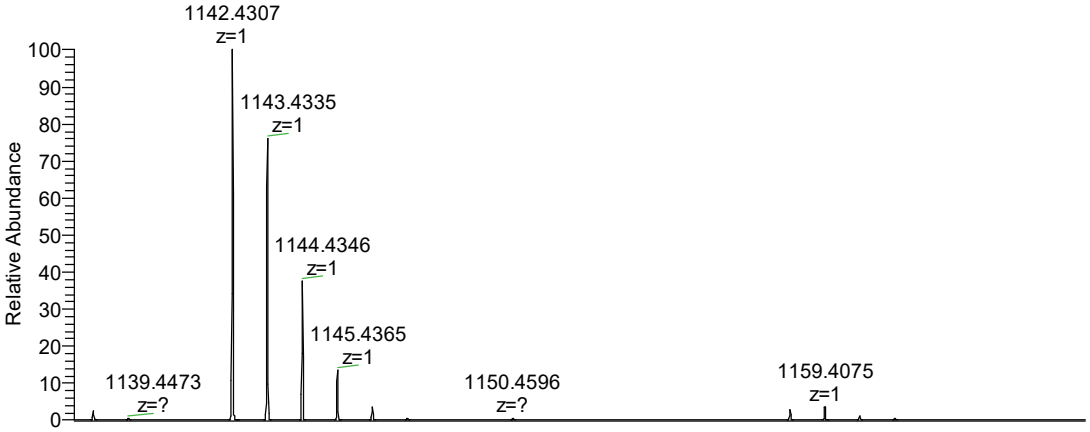
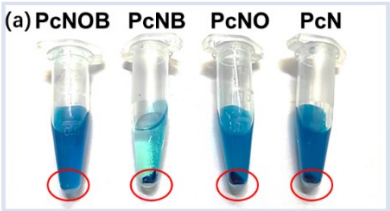


Figure S8. HRMS spectrum of PcNOB. The inset shows the enlarged isotopic envelope for the $[M+Na]^+$ species.



(b)	Solubility (mg/mL)	Log $P_{w/o}$
PcNOB	2.31±0.11	0.87±0.02
PcNB	0.21±0.02	2.81±0.13
PcNO	1.22±0.05	1.64±0.09
PcN	1.35±0.07	1.59±0.05

Figure S9. (a) Representation photos of the solubility of four phthalocyanines. (b) The specific values of solubility and lipid-water partition ratio. The detailed experimental conditions are as follows. Phthalocyanine (1.2 mg) was dissolved in 0.5 ml of purified water. It was sonicated for 5 min and centrifuged for 5 min (centrifugation force 5000 rpm). Next, the supernatant was collected and lyophilized. Then, the mass was weighed to calculate the solubility. Moreover, phthalocyanine was added to an equal volume of n-octanol and PBS solution, the mixture was shaken well and homogeneously. Finally, separating the aqueous and oil phases using a centrifuge. For testing, the same volume of solution was taken out from the n-octanol organic layer and the PBS buffer layer, and was added to DMF for the measurement of electronic absorption spectra, respectively. Calculation of the lipid-water partition ratio according to the formula Log P=lg (Co/Cw). Co and Cw represent the concentration of phthalocyanine in the organic and aqueous phases respectively.

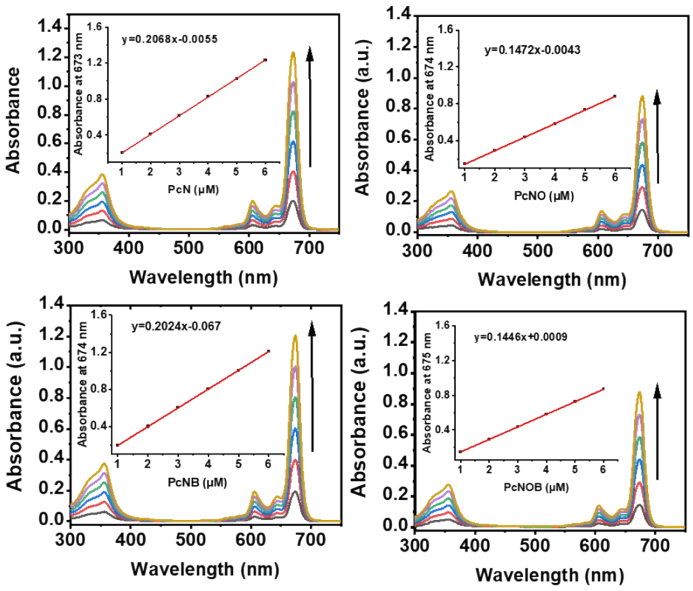


Figure S10. Electronic absorption spectra of phthalocyanines in DMF. The inset shows the relationship between the concentration of phthalocyanine and the absorbance of the corresponding Q-band, which follows Lambert-Beer's law.

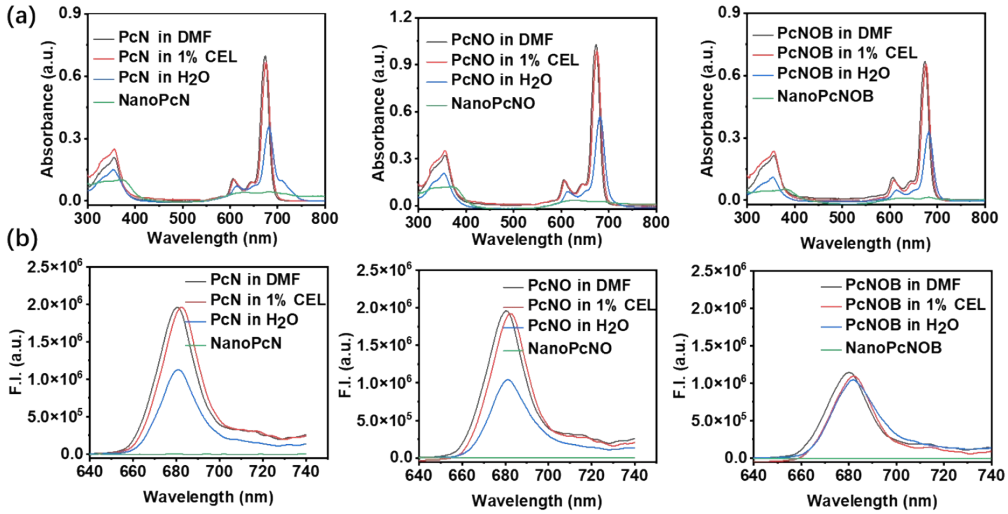


Figure S11. Electronic absorption spectra (a) and fluorescence emission spectra (b) of phthalocyanines in DMF, water containing 1% CEL and pure water, and nanostructured phthalocyanines in pure water.

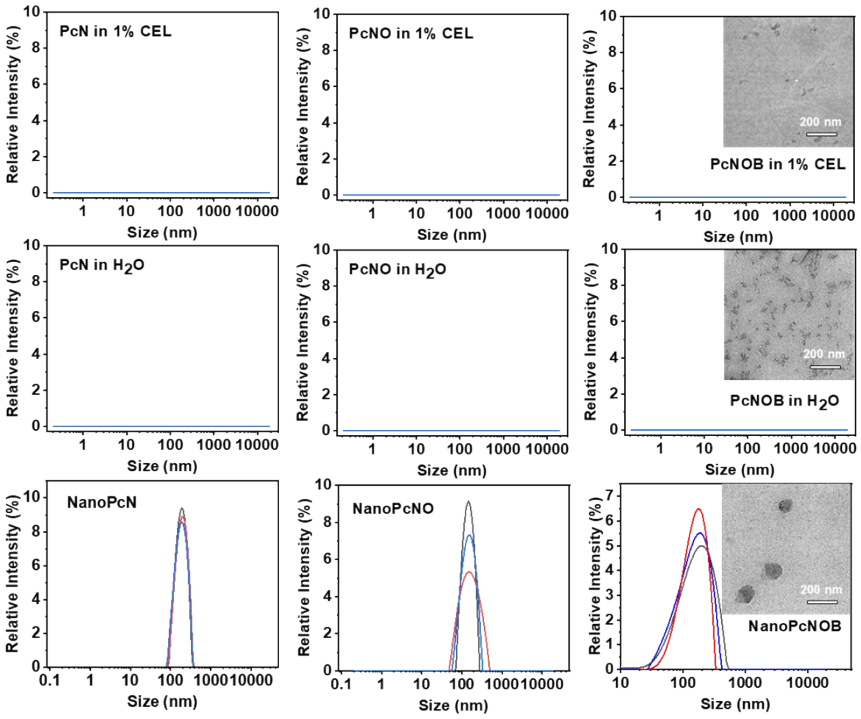


Figure S12. Hydrodynamic diameter of phthalocyanines in water containing 1% CEL (a), pure water (b), and nanostructured phthalocyanines in pure water (c). The illustration shows the transmission electron microscopy of PcNOB in water containing 1% CEL, pure water, and NanoPcNOB in pure water. scale bar: 200 nm.

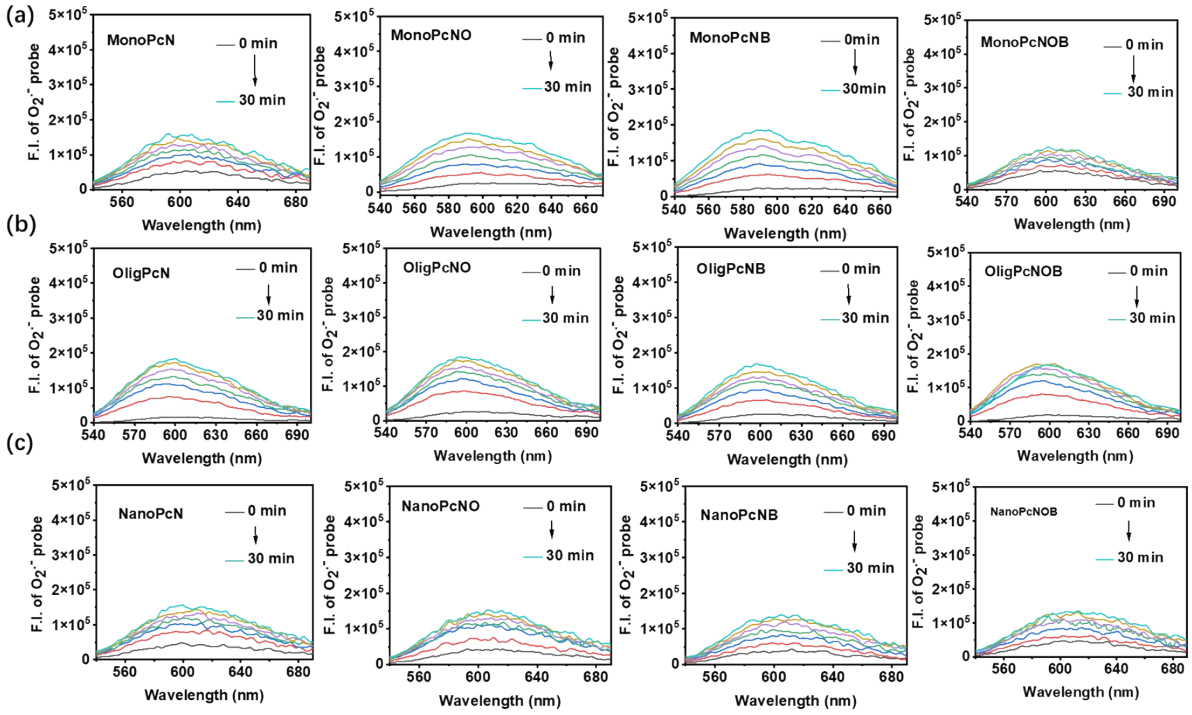


Figure S13. Fluorescence spectra of DHE probe after monomer phthalocyanines (a), oligomer phthalocyanines (b), and nanoaggregated phthalocyanines (c) (all at 5 μM) were exposed to light (λ≥610 nm) at the power density of 1 mW cm^{-2} for 30 min.

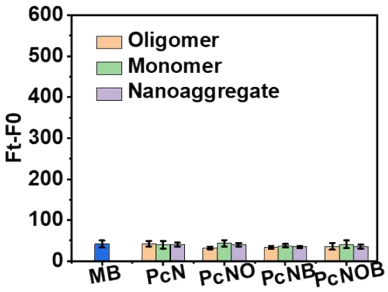


Figure S14. Rate diagrams of the DHE probe at 605 nm induced by phthalocyanines (5 μM) under different aggregation states upon a red light (λ≥610 nm) irradiation at the power density of 1 mW cm^{-2} for 30 min.

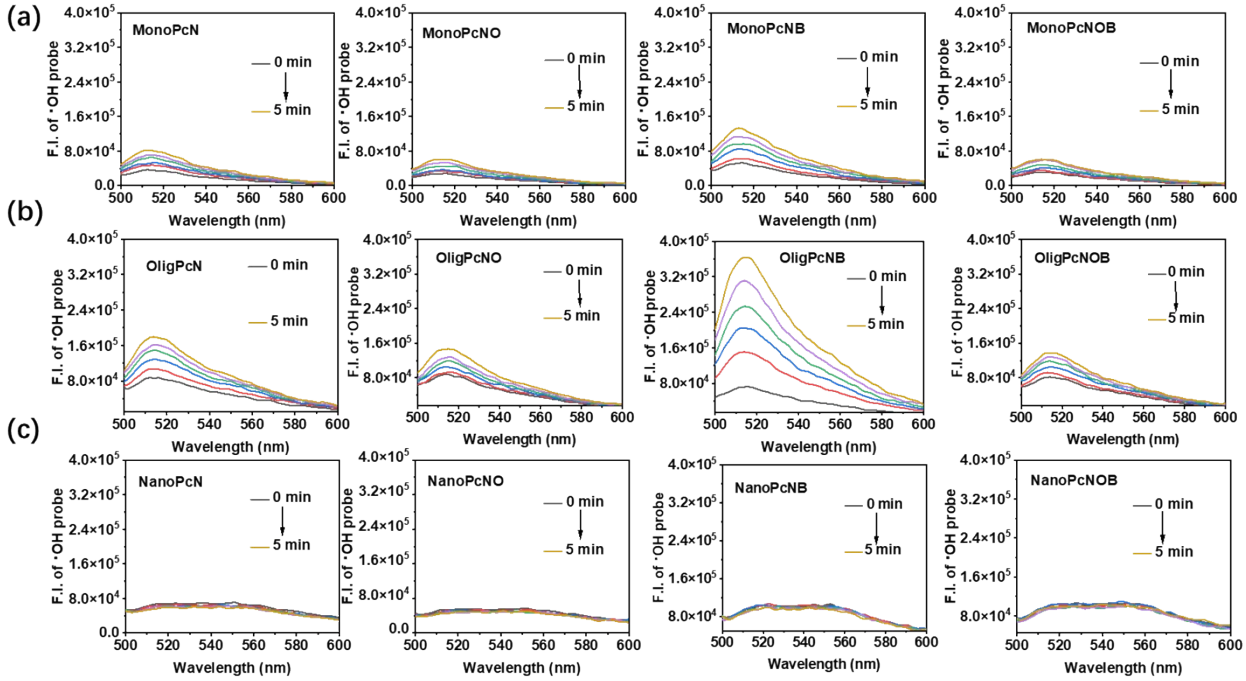


Figure S15. Fluorescence spectra of APF probe after monomer phthalocyanines (a), oligomer phthalocyanines (b) and nanoaggregated phthalocyanines (c) (all at 5 μM) were exposed to light (λ≥610 nm) at the power density of 1 mW cm^{-2} for 5 min.

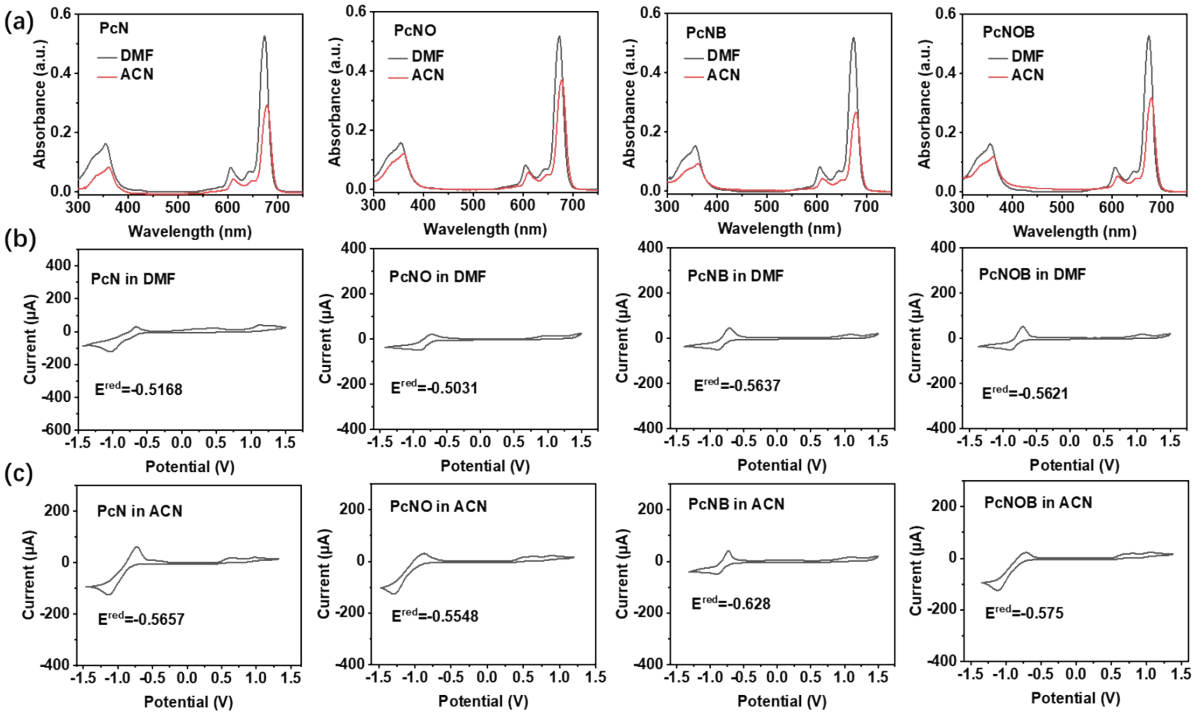


Figure S16. (a) Electron absorption spectra of phthalocyanines in DMF and acetonitrile. Cyclic voltammograms of phthalocyanines in DMF (b) and acetonitrile (c) using 0.1 M $(n\text{-}Bu)_4N^+PF^{6-}$ as a supporting electrolyte. Glassy carbon served as the working electrode, Ag/AgCl as a reference electrode, and Pt wire as the counter electrode. The scan rate was 100 mW s^{-1}.

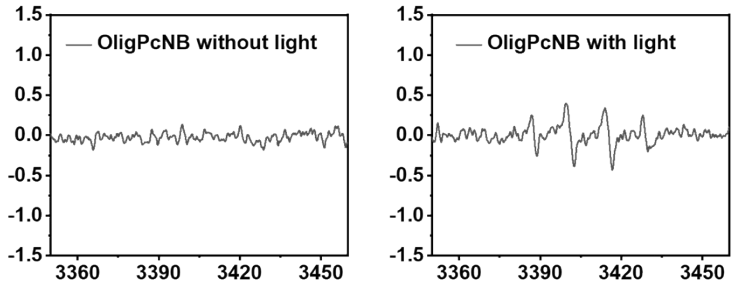


Figure S17. ESR spectra to detect •OH generated by OligPcNB (0.1 mM) in the presence or absence of light, using BMPO (25 mM) as a spin trap agent. Light conditions: λ≥610 nm, 15 mW cm^{-2}, 10 min.

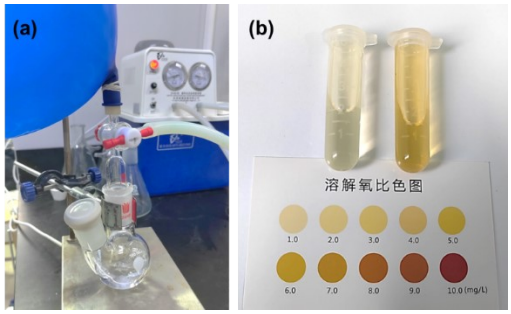


Figure S18. (a) Decompression and oxygen removal equipment. (b) Colour intensity of dissolved oxygen content indicator in water. The water in the left centrifuge tube is subjected to an oxygen removal process, and the water in the right centrifuge tube is not so treated.

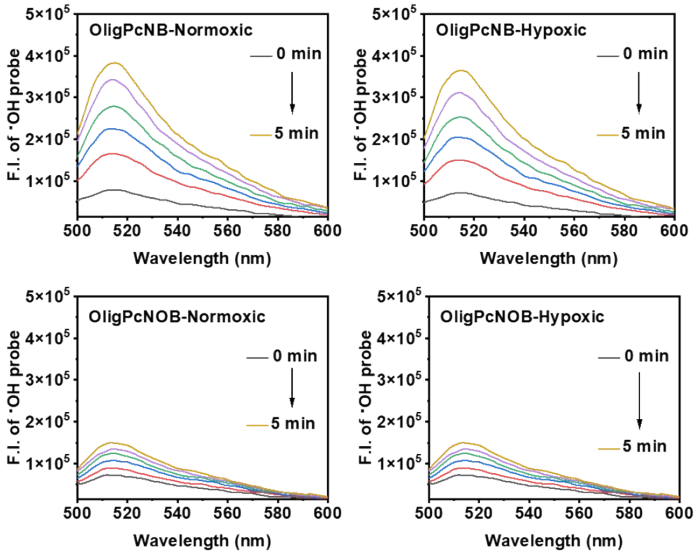
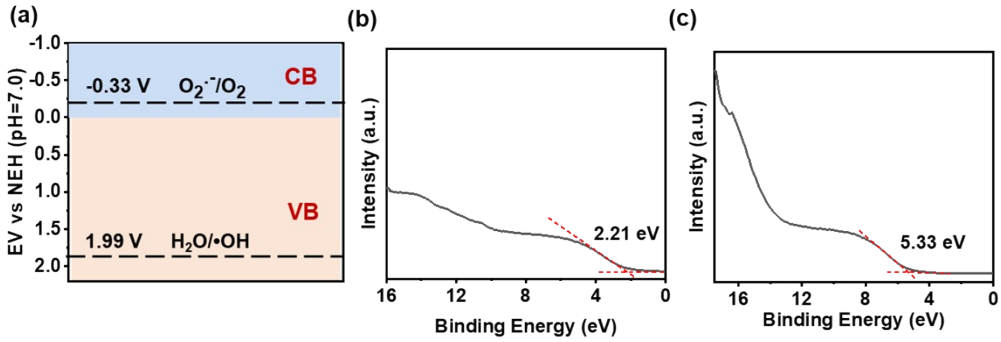


Figure S19. •OH production by oligomeric phthalocyanines (all at 5 μM) under normoxic and hypoxic conditions upon a red light irradiation (λ≥610 nm) at the power density of 1 mW cm^{-2} for 5 min.



The VB potential of phthalocyanines can be calculated using the following formula, where E_{NHE} is the VB potential of phthalocyanines vs NHE; E_F is the Fermi level; Φ is the work function. The UPS valence band spectrum of PcNB and PcNOB show that the E_F is 5.33 V and 2.21 V, respectively. The Φ of PcNB is 3.14 eV and the Φ of PcNOB is 4.10 eV. The calculated VB potential of PcNB and PcNOB is 4.03 V vs NHE and 1.87 V vs NHE, respectively.

$$E_{NHE}=E_F + \Phi - 4.44$$

Figure S20. (a) Energy positions of generation of $O_2^{\cdot-}$ and •OH. The UPS valence band spectrum of PcNOB (b) and PcNB (c).

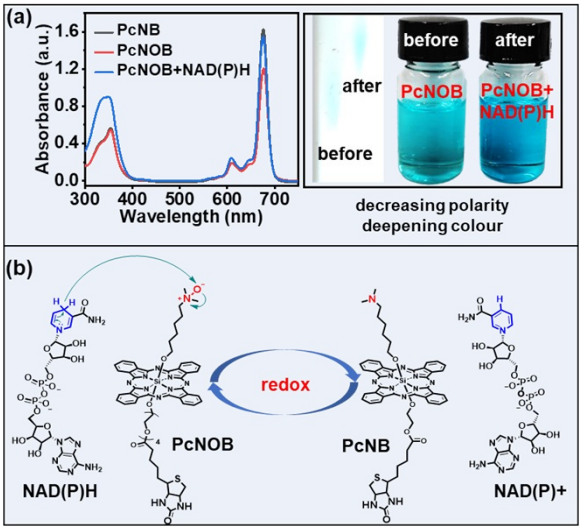


Figure S21. (a) The change of electronic absorption spectra, substance polarity, and solution color after adding NAD(P)H to a solution containing PcNOB. (b) Response mechanisms of redox occurring in NAD(P)H and PcNOB.

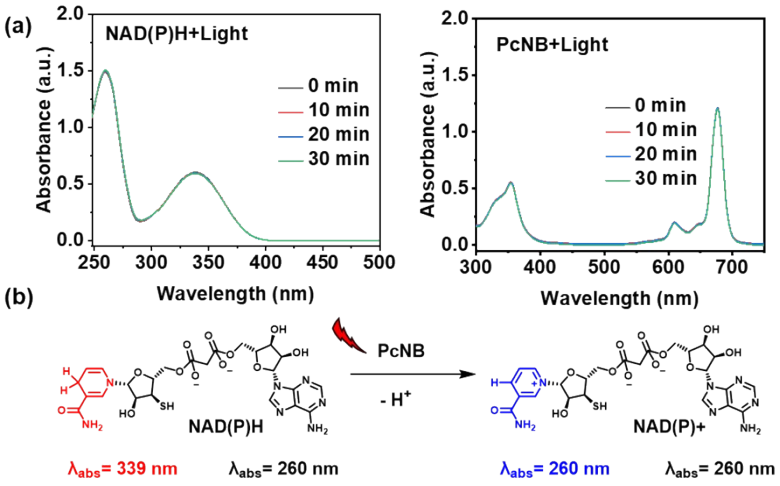


Figure S22. (a) Electronic absorption spectra of NAD(P)H or PcNB under light irradiation (λ≥610 nm) at the power density of 5.4 mW cm^{-2} for 30 min. (b) Schematic diagram of the change in chemical structure and absorption wavelength of NAD(P)H due to the photocatalytic redox reaction between PcNB and NAD(P)H.

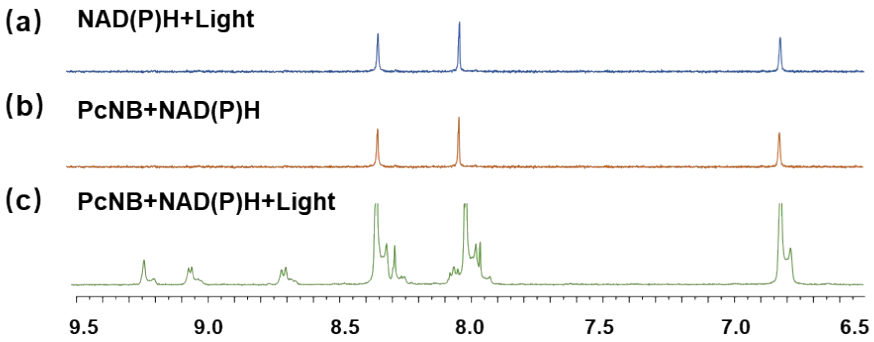


Figure S23. ^{1}H NMR spectra of NAD(P)H in the presence of light (a), NAD(P)H and PcNB in the absence of light (b), and NAD(P)H and PcNB when exposed to the light (c). Light condition: λ≥610 nm, 5.4 mW cm^{-2}, 30

min.

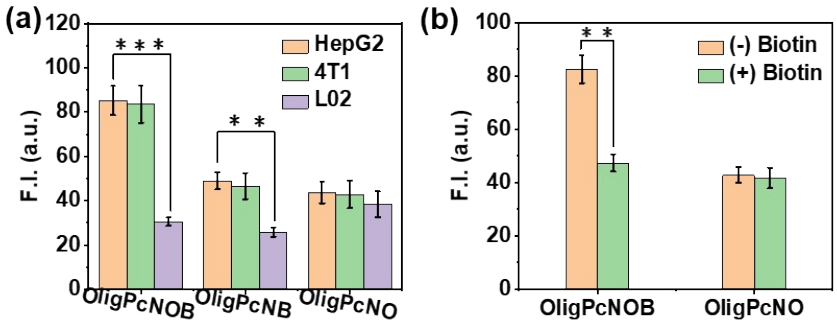


Figure S24. (a) Quantitative analysis of cellular uptake of oligomeric phthalocyanines in HepG2, 4T1 and L02 cells. (b) Quantitative analysis of intracellular fluorescence intensity of HepG2 cells after incubation with OligPcNOB or OligPcNO (100 nM) for 2 h in the presence and absence of extra biotin (10 nM). Data were presented as mean±SD derived from n=3 independent biological samples.

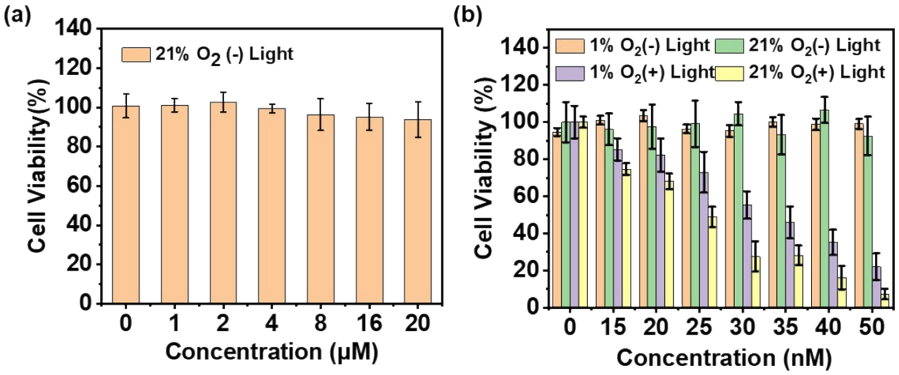


Figure S25. (a) Cytotoxicity of HepG2 cells incubated with different concentrations of OligPcNOB in the absence of light exposure. (b) Cytotoxic of OligPcNOB on HepG2 cells under normoxic condition without light, normoxic condition with light, hypoxic condition without light and hypoxic condition with light. Light condition: λ≥610 nm, 15 mW cm^{-2}, 30 min. The value represents the mean ±SD of three independent experiments (n=3).

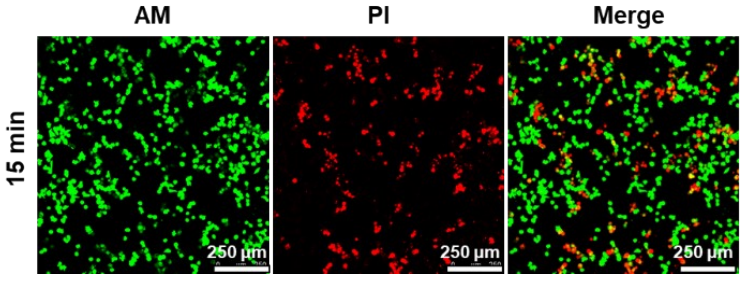


Figure S26. CLSM imaging of HepG2 cells incubated with OligPcNOB (50 nM) for 8 h under a red light (λ≥610 nm, 15 mW cm^{-2}) irradiation for 15 min using Calcein AM/PI staining. The scale bar represents 250 μm.

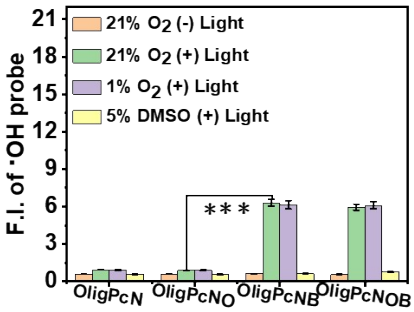


Figure S27. Quantitative analysis of •OH generation by oligomeric phthalocyanines (300 nM) using APF as the probe in HepG2 cells under normoxic condition (21% O_2) without light irradiation, normoxic condition with light irradiation, hypoxic condition (1% O_2) with light irradiation, and normoxic condition with light irradiation and in presence of 5% DMSO. Light condition: λ≥610 nm, 15 mW cm^{-2}, 5 min. Data were presented as mean±SD derived from n=3 independent biological samples.

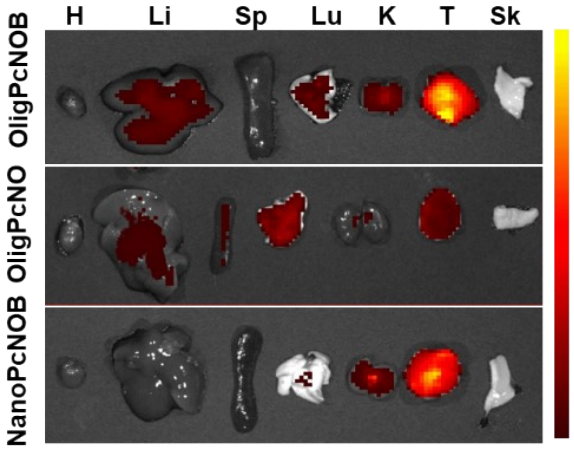


Figure S28. *Ex vivo* fluorescence images of the tumor and various organs at 48 h post-injection of OligPcNOB, OligPcNO and NanoPcNOB, respectively. The unit of fluorescence intensity: 10^8 ps^{-1} cm^{-1} sr^{-1} μW^{-1} cm^{-2}.

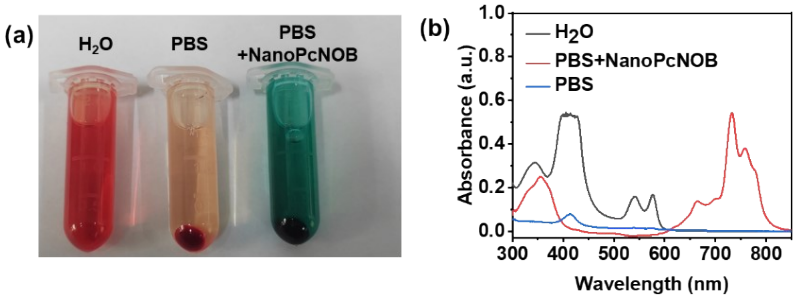


Figure S29. Representative photograph (a) and electronic absorption spectra (b) of hemolysis experiments with the NanoPcNOB in PBS buffer solution.

Table 1. Maximum absorption wavelengths and molar extinction coefficients.

Compound	λ_{max} (nm)	ε (10^5 M^{-1} cm^{-1})
PcN	**673**	**2.068**
PcNO	**674**	**1.472**
PcNB	**674**	**2.024**
PcNOB	**675**	**1.446**