SUPPORTING INFORMATION

Traceable Cancer Cell Photoablation with A New Mitochondria-Responsive and -Activatable Red-Emissive Photosensitizer

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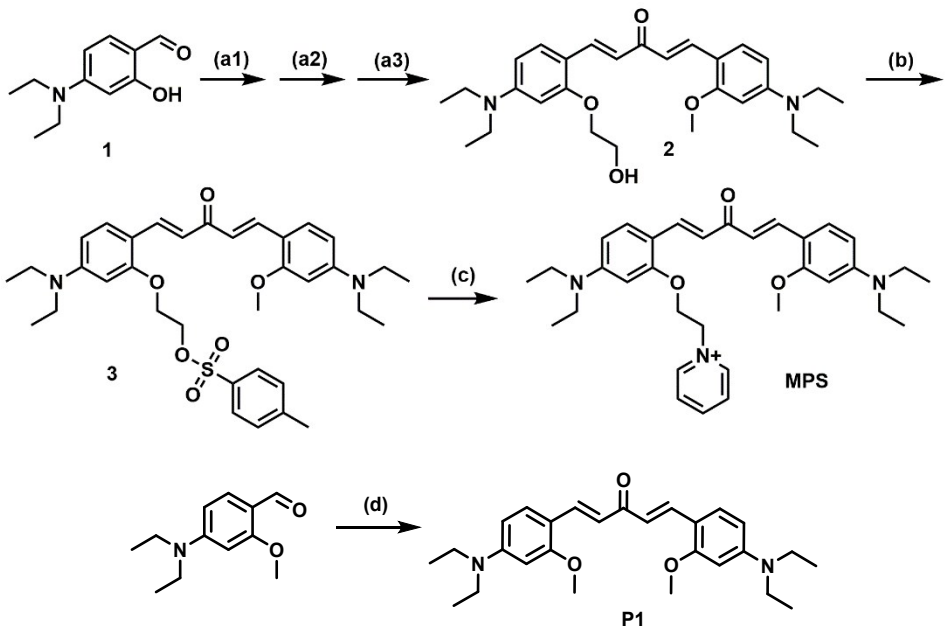
1. General information

All the chemical reagents were obtained from inno-chem and used without further purification. Bioreagents, MitoTracker Green FM, 2',7'-dichlorodihydrofluorescin diacetate, and Annexin V-FITC conjugate were purchased from Beijing BioDee Biotechnology Company. The cell lines including A431cells and Hela cells were obtained from the National Infrastructure of Cell Line Resource in China. ^{1}H-NMR spectra were recorded on Bruker Avance 400 spectrometer. Ultrapure water (18 MΩ/cm) was used for the preparation of all aqueous solutions. MALDI-TOF-MS spectrum were measured by a Bruker BIFLEX III spectrometer. UV-vis absorption spectra were recorded with a Hitachi U-3010 spectrophotometer. Photoluminescence spectra were obtained on a HitachiF-7000 spectrophotometer. Fluorescence quantum yields were

determined by using Nile Red (0.38 in methanol) as the standard. King Box-50W LED flood light supplied by Samvol Lighting Co.Ltd was used for the intracellular ROS generation.

2. Compound Synthesis

Synthetic scheme



Scheme S1. Synthesis of MPS and P1. Reagent and conditions: (a1) 1.5 eq NaOtBu, 1.5 eq 2-bromoethanol and DMF, 100 °C, 12 h, (a2) acetone, aqueous NaOH, RT, 1h. (a3) 1 eq 4-(diethylamino)-2-methoxybenzaldehyde, aqueous NaOH, ethanol, rt, 1h, (b) 1.5 eq TsCl, 1.5 eq TEA, DCM, RT, 3 h, (c) pyridine, 100 °C, 12 h. (d) 0.5 eq acetone, ethanol, aqueous NaOH, RT, 1h.

Synthesis of compound 2

A three-neck flask was charged with 4-(diethylamino)salicylaldehyde (1.93g, 10mmol) and sodium tert-butoxide (1.44g, 15mmol) and purged with argon. To the flask was added DMF (30mL) and 2-bromoethanol (1.05mL, 15mmol), and the mixture was heated to 100 °C with stirring for 12 hours. The mixture was cooled to room temperature. To the mixture was added water (100mL) and then ethyl acetate (100mL). The organic layer was separated and was

evaporated under reduced pressure, giving a brown semi-solid. The product was diluted with acetone (20mL) and transferred to a round-bottom flask. To the flask was added several drops of aqueous 1M NaOH. The mixture was stirred at room temperature for 1 hour. Then to the mixture was added water (100mL) and extracted with dichloromethane (50mL) three times. The solvent was evaporated under reduced pressure and the residue was diluted with ethanol (20mL) and transferred to a new round-bottom flask. To the solution was added 4-(diethylamino)-2-methoxybenzaldehyde (2.07g, 10mmol) and several drops of aqueous 1M NaOH. The the mixture was stirred at room temperature for 1 hour. Then to the mixture was added water (100mL) and extracted with dichloromethane (50mL) three times. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using hexane-ethyl acetate (4: 1, v/v) as an eluent to give a red solid compound 2 (2.9g, 65%). ^{1}HNMR (300 MHz, DMSO) δ 7.85 (s, 1H), 7.80 (s, 1H), 7.55 (d, J = 3.0 Hz, 1H), 7.52 (d, J = 3.0 Hz, 1H), 7.02 (d, J = 15.8 Hz, 1H), 6.94 (d, J = 15.8 Hz, 1H), 6.32 (d, J = 8.8 Hz, 2H), 6.21 (d, J = 5.7 Hz, 2H), 4.11 (t, J = 4.8 Hz, 2H), 3.88 (s, 3H), 3.85 (d, J = 4.6 Hz, 2H), 3.77 (d, J = 4.5 Hz, 1H), 3.41 (m, 8H), 1.13 (m, 12H). HRMS (MALDI): Calcd for $[M+H]^{+}$, 467.29077, Found, 467.29043.

Synthesis of compound 3

A round-bottom flask was charged with compound 2 (2.33g, 5mmol) and tosyl chloride (1.9g, 10mmol). To the flask was added dichloromethane (50mL) and triethylamine (2mL, 15mmol). The mixture was stirred at room temperature for 3 hours. Then to the mixture was added water (100mL) and extracted with dichloromethane (80mL) three times. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using

hexane-ethyl acetate (8: 1, v/v) as an eluent to give a dark red solid compound 3 (2.8g, 90%). ^{1}HNMR (400 MHz, DMSO) δ 7.85 (d, J = 4.3 Hz, 1H), 7.82 (d, J = 2.8 Hz, 1H), 7.77 (d, J = 15.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 1H), 7.52 (d, J = 8.9 Hz, 1H), 7.43 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 15.8 Hz, 1H), 6.94 (d, J = 15.9 Hz, 1H), 6.32 (d, J = 8.7 Hz, 1H), 6.24 (d, J = 8.9 Hz, 1H), 6.19 (s, 1H), 6.12 (s, 1H), 4.44 (d, J = 3.2 Hz, 2H), 4.29 (d, J = 1.8 Hz, 2H), 3.86 (s, 3H), 3.44 – 3.35 (m, 8H), 2.37 (s, 3H), 1.10 (q, J = 6.7 Hz, 12H). HRMS (MALDI): Calcd for $[M+H]^+$ 621.29963, Found, 621.29928.

Synthesis of compound MPS

A three-neck flask was charged with compound 3 (0.62g, 1mmol) and purged with argon. To the flask was added pyridine (10mL), and the mixture was heated to 100 °C with stirring for 12 hours. The mixture was cooled to room temperature. The solvent was evaporated under reduced pressure and the residue was washed with hexane three times to give a dark red solid MPS (0.6g, 85%). ^{1}HNMR (300 MHz, DMSO) δ 9.28 (d, *J* = 5.3 Hz, 2H), 8.62 (d, *J* = 6.9 Hz, 1H), 8.17 (s, 2H), 7.89 (d, *J* = 15.7 Hz, 1H), 7.63 (d, *J* = 8.9 Hz, 1H), 7.61 - 7.46 (m, 4H), 7.10 (d, *J* = 7.1 Hz, 2H), 6.96 (d, *J* = 15.7 Hz, 1H), 6.80 (d, *J* = 15.7 Hz, 1H), 6.33 (d, *J* = 6.0 Hz, 2H), 6.19 (d, *J* = 11.0 Hz, 2H), 5.19 (s, 2H), 4.64 (s, 2H), 3.89 (s, 3H), 3.39 (s, 8H), 2.27 (s, 3H), 1.12 (d, *J* = 6.1 Hz, 12H). HRMS (ESI+): Calcd for $[M]^+$ 528.32219, Found, 528.32207.

Synthesis of compound P1

A three-neck flask was charged with 4-(diethylamino)-2-methoxybenzaldehyde (2.07g, 10mmol). To the flask was added ethanol (30mL), acetone (5mmol) and several drops of aqueous 1M NaOH. The mixture was stirred at room temperature for 3 hours. Then to the mixture was added water (100mL) and extracted with dichloromethane (80mL) three times.

The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using hexane-ethyl acetate (8: 1, v/v) as an eluent to give a dark red solid compound P1 (1.9g, 88%). ^{1}HNMR (400 MHz, DMSO) δ 7.83 (d, *J* = 15.7 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 2H), 6.93 (d, *J* = 15.8 Hz, 2H), 6.30 (d, *J* = 8.7 Hz, 2H), 6.19 (s, 2H), 3.87 (s, 6H), 3.39 (d, *J* = 6.8 Hz, 8H), 1.12 (t, *J* = 6.8 Hz, 12H). HRMS (MALDI): Calcd for $[M+H]^+$ 437.27259, Found, 437.27231.

3. Singlet Oxygen Detection in Solution

The singlet oxygen generation was studied by using 1, 3-diphenylisobenzofuran (DPBF) as a singlet oxygen indicator through measuring the absorbance decreases of DPBF upon reaction with singlet oxygen. For singlet oxygen detection, the DPBF (20 μM) in ethanol or in 1:1 (v/v) ethanol-water mixture and exposed to 532 nm laser diode irradiation with power density of 80 mW cm^{-2}. The absorbance decrease of DPBF at 410 nm was recorded for different durations of light irradiation to obtain the rate of DPBF consumption. The rate of DPBF consumption was calibrated in terms of the relative number of absorbed photons at the maximum absorbance of each dye, and this calculated value corresponds to the relative efficiency of $^{1}O_2$ generation by the irradiated photosensitizer. Rose Bengal (RB) was used as the standard photosensitizer (ΦRB = 0.86 in ethanol).

4. Cell culture and imaging

A431 and HeLa cells were grown at 37 °C under 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serium. Fluorescence microscopy images were obtained on a confocal microscope FV1000-IX81 and were analyzed with FV10-ASW software with an equal parameter.

Intracellular ROS Detection

The intracellular ROS generation was detected by using 2', 7' -dichlorofluorescin diacetate (DCF-DA) as an indicator and studied by confocal images. HeLa and A431 cells in confocal dishes were firstly incubated with MPS (5 μM) for 30 min in the dark, then the cells were rinsed with PBS for 3 times and stained with 10 μM of DCF-DA. After 15 min incubation, the cells were exposed to LED white light irradiation (15 s, 40mW cm^{-2}). After irradiation, the cells were incubated for 30 min and then studied by confocal microscope. For DCF detection, the excitation wavelength was 488 nm, and the emission filter was 500 − 520 nm.

Cell Apoptosis Detection by Annexin V-FITC Staining.

HeLa and A431 cells in confocal dishes were firstly incubated with MPS (5 μM) for 30 min in the dark, then the cells were rinsed with PBS for 3 times and were exposed to LED white light irradiation (15 s, 40mW cm^{-2}). After irradiation, the cells were incubated for 90 min and then stained with Annexin V-FITC according to the manufacturer's instruction. Afterwards, the cells were studied by confocal microscope. For FITC detection, the excitation wavelength was 488 nm, and the emission filter was 500−520 nm.

Cell Apoptosis Induced by Addition of Hydrogen Peroxide

A431 cells in confocal dishes were firstly incubated with MPS (5 μM) for 30 min, and then divided into two parts: one was treated with 400 μM hydrogen peroxide for 30 min to induce cell apoptosis and the other was untreated as control. All cells then stained with Annexin V-FITC according to the manufacturer's instruction. Afterwards, the cells were studied by confocal microscope. For FITC detection, the excitation wavelength was 488 nm, and the emission filter was 500−520 nm.

Cytotoxicity Studies.

MTT assays were used to assess the cell viability of HeLa and A431 cells after incubation with MPS or P1 and exposed with LED white light irradiation. The cells in 96-well plates (Corning, USA) were first incubated with the probe for designated time in the dark. The fluorescence intensity at 600 nm was firstly recorded by the microplate reader (Genios). Then the cells were washed with PBS twice and exposed to white light irradiation for 15 s or 45 s at a power density of 40 mW cm^{-2}. The cells were further incubated in fresh medium for 24 h and washed with PBS and the fluorescence intensity at 600 nm was recorded again. Then MTT in PBS solution (10 μL, 5 mg mL^{-1}) was added into each well. After incubation for 3 h, the supernatant was discarded and the precipitate was dissolved in DMSO (100 μL) with gentle shaking. The absorbance of MTT at 490 nm was monitored by the microplate reader (Genios). The cells without any treatment were used as control.

5. Supplementary Figures and Tables

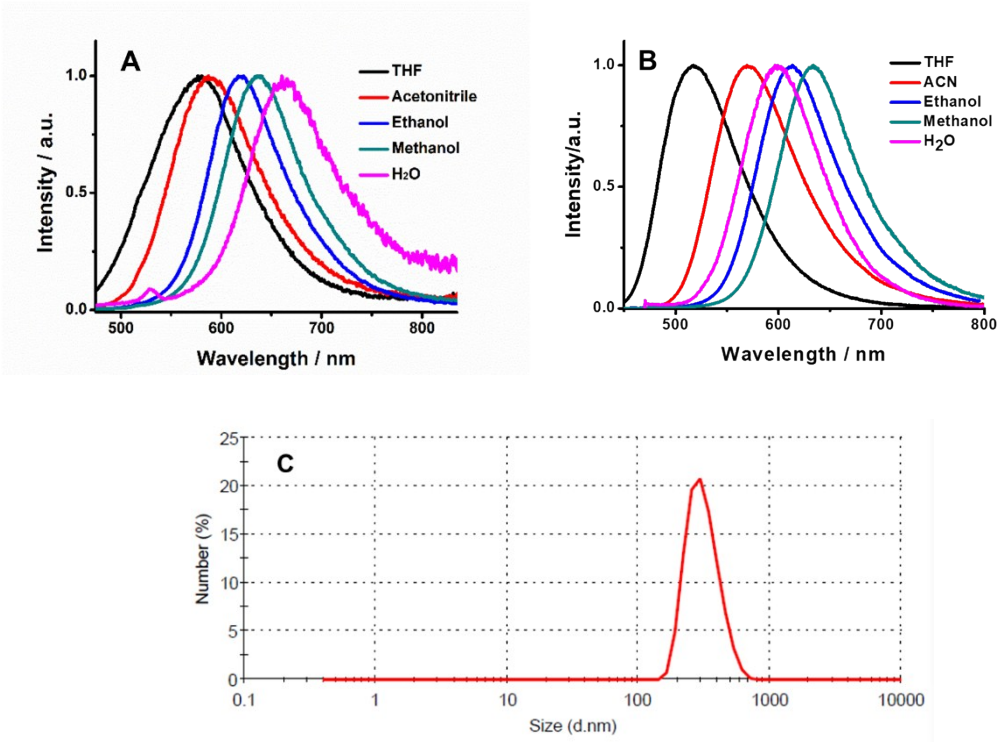


Figure S1. Normalized fluorescence spectra of MPS (A) and P1 (B) in various solvents with the concentration of 5 μM, respectively excited at the absorption peaks and Dynamic light scattering (DLS) analysis of P1 (5 μM) in water (C).

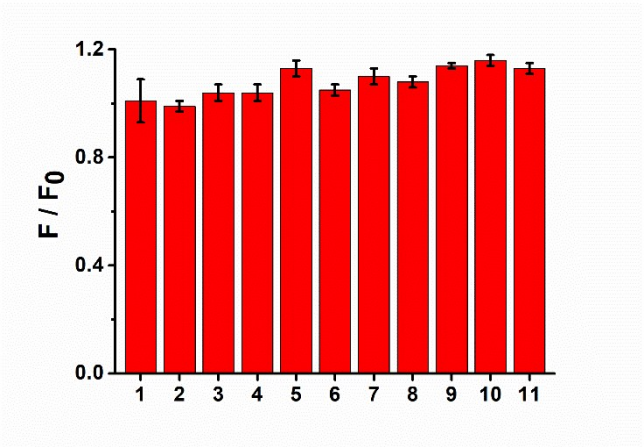


Figure S2. Fluorescence intensity of MPS (5 μM) at 650 nm before and after the addition of various biologically relevant species in PBS buffer (pH = 7.4): (1) $CaCl_2$ (10 mM), (2) $FeCl_3$ (1 mM), (3) $CuBr_2$ (1 mM), (4) KCl (10 mM), (5) H_2O_2 (0.1 mM), (6) Gly (0.1 mM), (7) Cysteine (0.1 mM), (8) Vitamin C (1 mM), (9) GSH (1 mM), (10) NaCl (10 mM), (11) H_2S (0.1 mM).

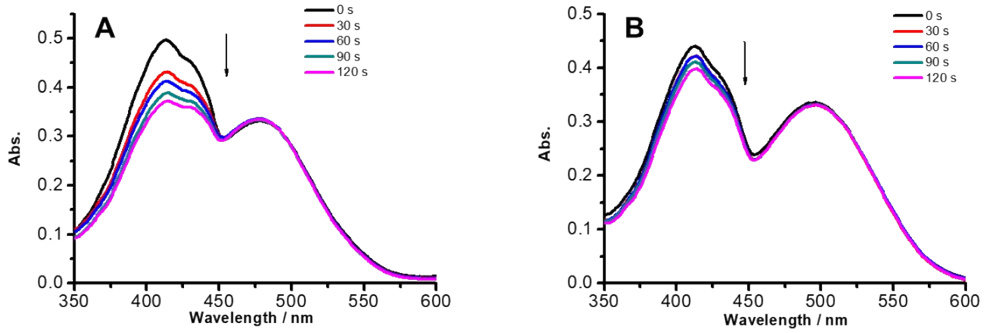


Figure S3. Time dependent absorption of DPBF (20 μM) mixed with P1 (10 μM) in ethanol (A) and 1:1 (v/v) ethanol-water mixture (B) upon 532 nm laser diode irradiation with power density of 80 mW cm^{-2}.

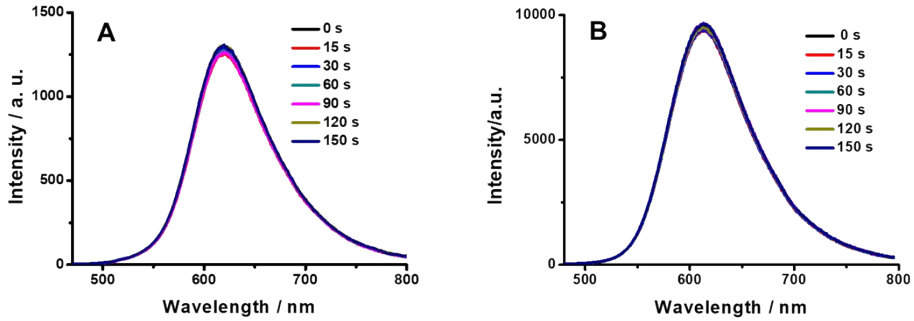


Figure S4. Time-dependent fluorescence spectra of **MPS** (A) and P1 (B) in ethanol with the concentration of 10 μM irradiated under the white light LED with power density of 40 mW cm^{-2}

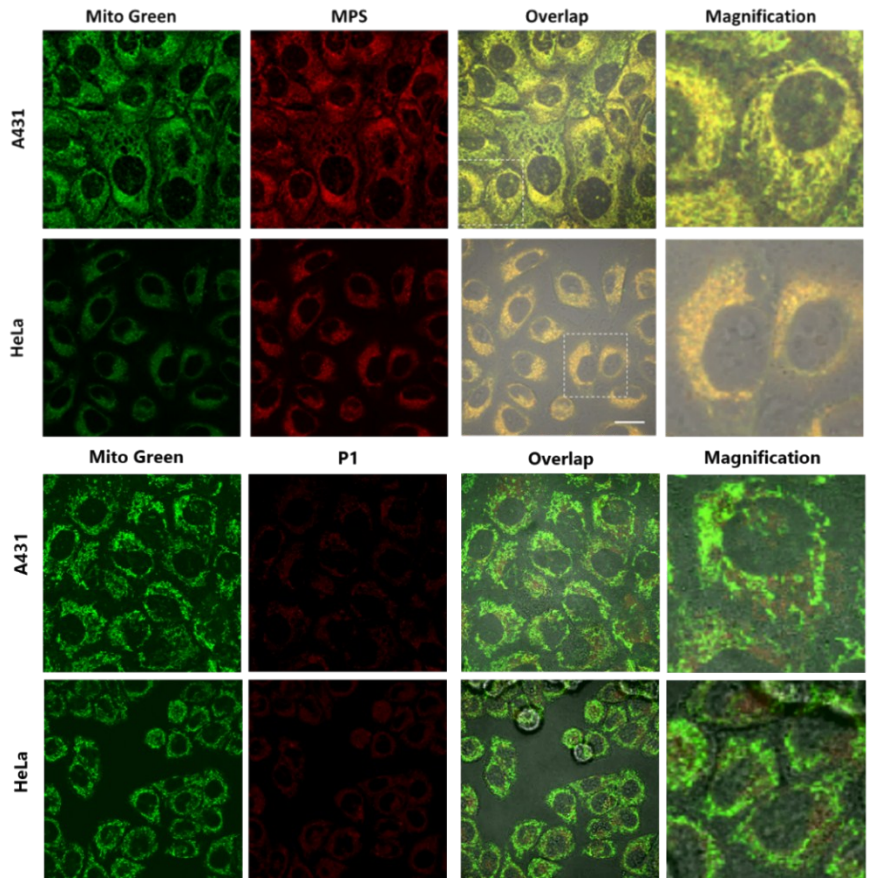


Figure S5. Confocal fluorescence images of A431 cells and HeLa cells coincubated with MPS (5 μM) and MitoTracker Green FM (0.1 μM) as well as P1 (5 μM) and MitoTracker Green FM (0.1 μM): Green channel (λex: 488 nm, λem: 500-520 nm) for MitoTracker Green FM. Red channel (λex: 488 nm, λem: 580-640 nm) for MPS and P1. The scale bar represents 20 μm for all the images. The Pearson correlation coefficients of MPS with MitoTracker and P1 with MitoTracker calculated based on the imaging overlap of red channel and green channel are 0.96 and 0.76 for A431 cells, 0.95 and 0.77 for HeLa cells.

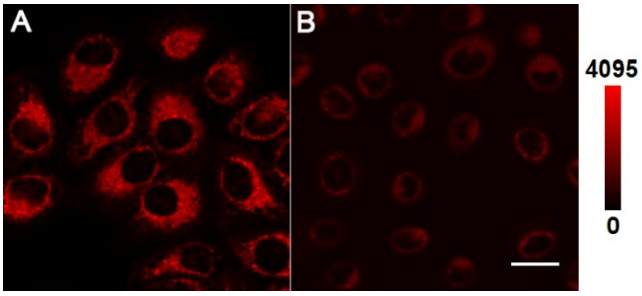


Figure S6. Confocal fluorescence images of HeLa cells incubated with MPS (5 μM) without (A) and with (B) treatment of carbonyl cyanide m-chlorophenyl hydrazine (CCCP, 100 μM) for 30min. Red channel (EX: 488 nm, Em: 580-640 nm). The scale bar represents 20 μm for all the images.

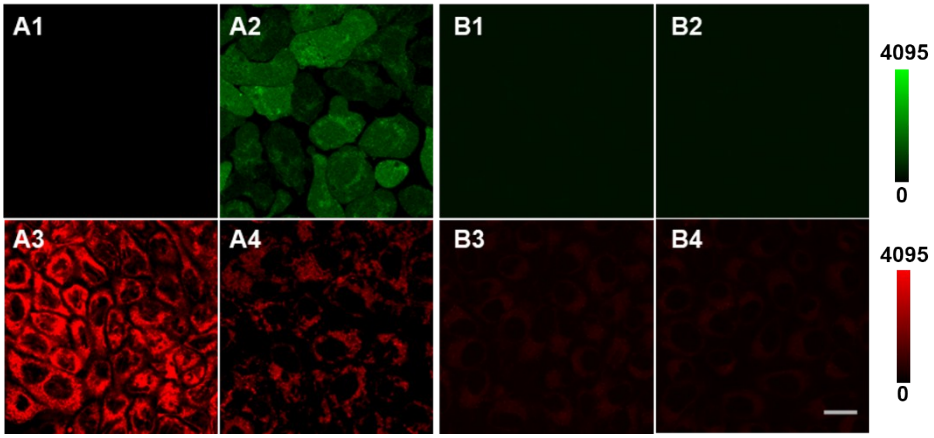


Figure S7. Images of MPS (A) and P1 (B) incubated A431 cell lines co-stained with DCHF-DA in the absence (1 and 3) and presence (2 and 4) of white light LED irradiation (15 s, 40 mW cm^{-2}). Cell lines were first incubated with MPS (5 μM) or P1 (5 μM) for 30 min then subjected to irradiation for 15 s. After another 15 min incubation, all cells were stained with DCHF-DA (10 μM). (1-2) Green channel for DCF (EX: 488 nm, Em: 500-520 nm). (3-4) Red channel for MPS or P1 (EX: 488 nm, Em: 580-640 nm). The scale bar represents 20 μm for all the images.

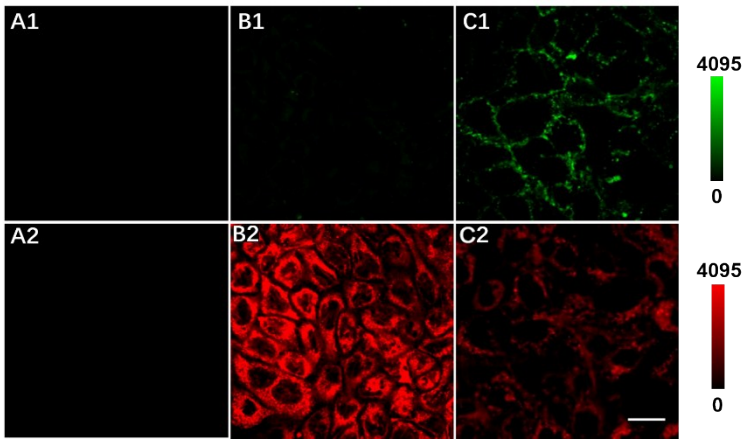


Figure S8. (A) Confocal fluorescence images of A431 cells without MPS treatment in the presence of white light irradiation (15 s, 40 mW cm^{-2}), A431 cells with MPS treatment (5 μM) in the absence (B) and presence (C) of white light irradiation (15 s, 40 mW cm^{-2}). A431cell lines were first incubated with MPS (5 μM) for 30 min then subjected to irradiation for 15 s. After another 90 min incubation, all cells were stained with Annexin V-FITC. (A1-C1) Green channel for Annexin V-FITC (EX: 488 nm, EM: 500-520 nm); (A2-C2) Red channel for MPS (EX: 488 nm, EM: 580-640 nm); The scale bar represents 20 μm for all the images.

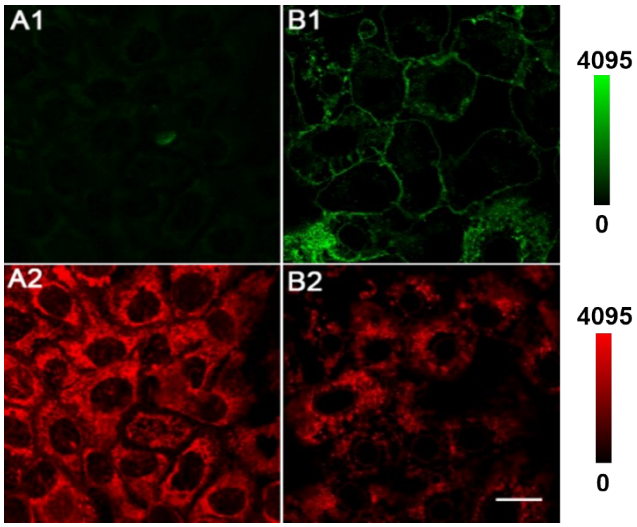


Figure S9. Confocal fluorescence images of MPS (5 μM) -incubated A431 cells untreated (A) and treated with (B) hydrogen peroxide (400 μM). After a 90 min incubation following the irradiation, all cells were stained with Annexin V-FITC. (A1-B1) Green channel for Annexin V-FITC (EX: 488 nm, Em: 500-520 nm). (A2-B2) and red channel for MPS (EX: 488 nm, Em: 580-640 nm). The scale bar represents 20 μm for all the images.

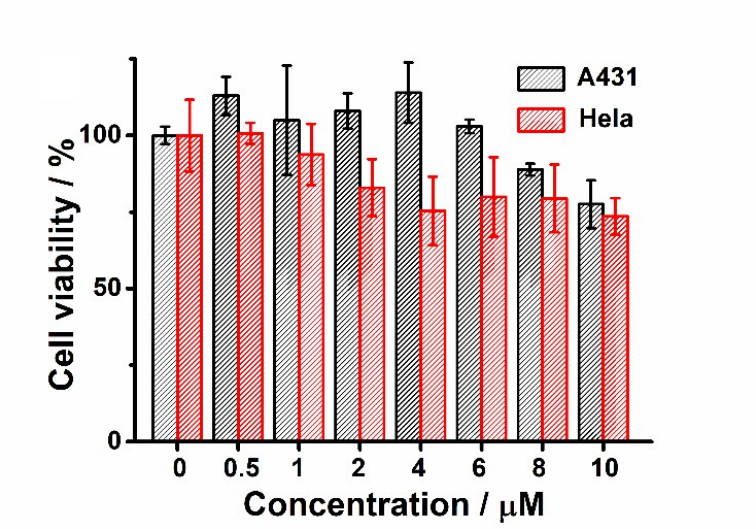


Figure S10. The dark cytotoxicity of MPS towards HeLa and A431 cells.

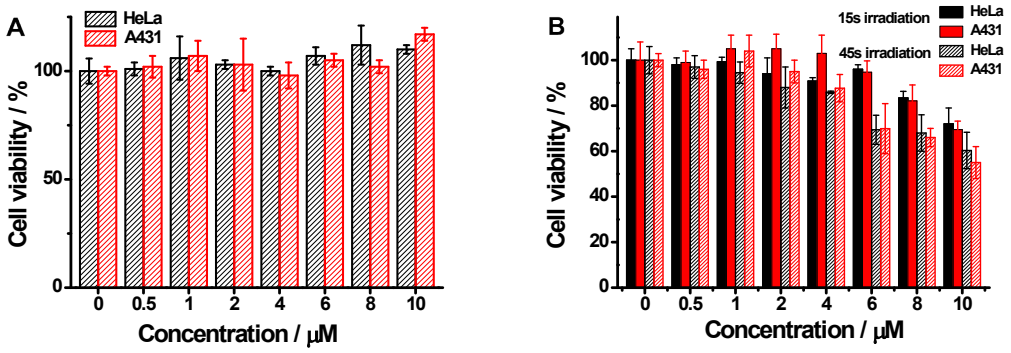


Figure S11. The cytotoxicity of P1 nanoaggregates with various concentrations towards HeLa and A431 cells(A) in dark and (B) upon white light irradiation (40 mW cm^{-2}) for 15 and 45 s, respectively.

Table S1. Photophysical properties of MPS and P1.

	Φ_{on} [a]	Φ_{off} [b]	F_{on} / F_{off} [c]	$\Phi_{on}(^1O_2)$ [d]	$\Phi_{off}(^1O_2)$ [e]
MPS	0.09	0.02	44	0.15	0.02
P1	0.34	0.11	11	0.16	0.03

[a] Fluorescence quantum yield recorded in ethanol. [b]Fluorescence quantum yield recorded in water. [c]The ratio of fluorescence intensity at 600 nm of the dye in ethanol and in water. [d]Singlet oxygen quantum yield measured in ethanol. [f]Single oxygen quantum yield measured in 1:1 (v/v) ethanol-water mixture.