Electronic Supplementary Information

Hydrogen peroxide degradable conjugated polymer nanoparticles for fluorescence and photoacoustic bimodal imaging

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General Information: 2,5-Diiodo-1-methyl-1*H*-imidazole was purchased from Combi-blocks Inc. All other chemicals and reagents were purchased from Aldrich and used as received.

Characterization: NMR spectra were collected on a Bruker Avance 500 NMR spectrometer (500 MHz for ^{1}H, referenced to TMS at $\delta = 0.00$ ppm). The transmission electron microscopy images were captured on a JEM-2010F (JEOL, Japan) microscope. UV-vis-NIR spectra were recorded by a UV-1700 spectrometer (Shimadzu, Japan). Photoluminescence (PL) spectra were collected on a Perkin Elmer LS-55 equiped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 ^{o}C angle detection for solution samples. All UV-vis and PL spectra were collected at 24 ± 1 ^{o}C. Fisher brand regenerated cellulose dialysis tubing with 12~14 kDa molecular weight cutoff was used for polymer dialysis. Milli-Q water (18.2 MQ) was used for all the experiments.

Preparation of PDPPID NPs

The preparation of PDPPID NPs involves six steps: i) 2,5-Diiodo-1-methyl-1*H*-imidazole

(3.3 mg, 1.0 μmol), 2,5-bis(2-ethylhexyl)-3,6-bis(5-ethynylthiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (5.7 mg, 1.0 μmol), $Pd(PPh_3)_2Cl_2$ (0.5 mg) and CuI (2 mg) were dissolved well in THF (3 mL); ii) the mixture was added to water (10 mL) containing Tween 80 (500 mg) under argon atmosphere and followed by sonication for 5 min; iii) the resultant mixture was stirred at room temperature for 0.5 h before THF was removed under reduced pressure; iv) triethylamine (1 mL) was added to initiate the Sonogashira reaction and the reaction was kept at 50 ^oC for 24 h; v) the mixture was used for dialysis against water using dialysis membrane (molecular weight cut off = 12-14k) for two days; and vi) the mixture was further purified by centrifugation and concentrated by ultrafilter to be 1 mg/mL further use. In order to characterize the chemical structure, we used chloroform to extract the polymer PDPPID from PDPPID NPs followed by precipitation from methanol after 5 times repeated centrifugation and redispersion in water. The obtained organic solvent soluble fraction was used for NMR. ^1H NMR ($CDCl_3$, 400 MHz, δ) 8.89 (br, 2 H), 7.67-7.47 (br, 3 H), 4.01 (br, 4 H), 3.70 (br, 3 H), 2.03 (br, 2 H), 1.26 (br, 16 H), 0.84 (br, 12 H).

Cell Culture: HeLa cells were cultured in folate-free Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. Before experiments, the cells were precultured until confluence was reached.

PA imaging of PDPPID NPs in solution

The PA imaging of the PDPPID NPs in aqueous solution after incubation with H_2O_2 was measured by a multispectral optoacoustic tomographic (MSOT) imaging system (MSOT EIP 10, iThera Medical, Germany). The aqueous solution of PDPPID NPs (100 μg/mL) was mixed with H_2O_2 (50 μM) and loaded into a polyethylene tube for PA imaging. The PA signals of the NPs were excited by a pulsed laser with a tunable wavelength from 670 to 900 nm with an interval of 5 nm. The PA images of the solution of PDPPID NPs were recorded

after 0, 3, 6, 12 and 24 h incubation of H_2O_2.

Cellular PA imaging

HeLa cells were used to investigate the in vitro degradation of PDPPID NPs by PA imaging. Cells were seeded in cell culture flask and incubated overnight, followed by replacing the old culture medium with fresh medium containing 100 μg/mL PDPPID NPs. After additional incubation for 12 h, the cells were washed with 1× PBS for three times. Then fresh culture medium containing 20 μM H_2O_2 was added into the flask and cells were further incubated for different time period (0, 3, 6, 12 and 24 h). After the treatment with H_2O_2, the cells were washed three times with 1× PBS and digested by trypsin and fixed with 4% paraformaldehyde for 15 min. Finally, the cells were collected by centrifugation and the tube was filled with ultrasound gel to fix the cell pellet. HeLa cells without any treatment were used as control. All the cell samples were taken for PA imaging on the MSOT imaging system.

Cellular fluorescence imaging

The cellular fluorescence imaging was utilized to investigate the degradation of PDPPID NPs by H_2O_2. HeLa cells were seeded in an 8-well chamber and incubated overnight. The old culture medium was removed and fresh culture medium containing 100 μg/mL PDPPID NPs was added. After additional incubation for 12 h, the medium was discarded and the cells were washed with 1× PBS. Then fresh culture medium containing 20 μM H_2O_2 was added into the well and cells were taken for real-time confocal fluorescence imaging (Leica TCS SP8). The fluorescence of PDPPID NPs in HeLa cells was excited at a wavelength of 600 nm and collected within 620-720 nm range. Confocal images were recorded every 30 min.

Fluorescence imaging of PDPPID NPs in solution

The aqueous solution of PDPPID NPs (100 μg/mL) was mixed with H_2O_2 (50 μM) and was sucked into a glass capillary tube for confocal imaging. The fluorescence of PDPPID NPs

was excited at a wavelength of 600 nm and collected within 620-720 nm range. Confocal images were recorded every 5 min.

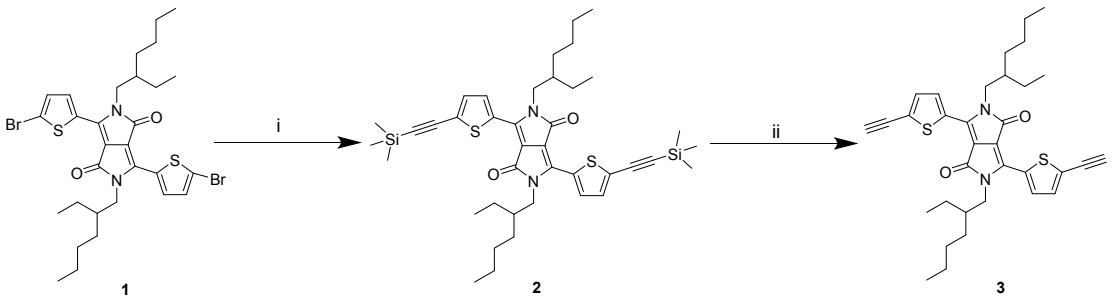
Cytotoxicity and PTT of PDPPID NPs

The PTT performance of PDPPID NPs towards HeLa cells was evaluated by using MTT method. HeLa cells were seeded in a 96-well plate with a density of 5×10^3 cells per well and incubated overnight. The old culture medium was replaced by fresh culture medium containing various concentrations of PDPPID NPs. After incubation with NPs for 12 h, the HeLa cells were washed three times with 1× PBS to remove the un-internalized NPs. The PTT treatment was performed by irradiate the selected HeLa cells with a laser at 808 nm (1.6 W/cm^2, 10 min). After the PTT experiment, cells were further incubated for 24 h and the viabilities were evaluated by a MTT assay. For the cytotoxicity evaluation of PDPPID NPs without light exposure, HeLa cells were incubated with various concentrations of NPs for 24 and 48 h and then subjected to MTT assay.

2,5-Bis(2-ethylhexyl)-3,6-bis(5-((trimethylsilyl)ethynyl)thiophene-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (2). This compound was synthesized according to literature with modified procedures.[1] To a solution of 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-ethylhexyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione **1** (272 mg, 0.4 mmol), copper iodide (15 mg, 80 μmol) and $Pd(PPh_3)_2Cl_2$ (15 mg, 21 μmol) in triethylamine (30 mL) under argon atmosphere was added trimethylsilylaceylene (117 mg, 1.2 mmol). The reaction was performed at 80 ^oC overnight. The mixture was diluted with dichloromethane, filtered through a celite pad, concentrated under reduced pressure and purified by silica gel column chromatography using hexane/dichloromethane (6/4) as eluent to afford **2** as a dark purple solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 8.79 (d, J = 4.0 Hz, 2 H), 7.30 (d, J = 4.0 Hz, 2 H), 4.02–3.89 (m, 4 H), 1.88–1.78 (m, 2 H), 1.39–1.16 (m, 16 H), 0.90–0.82 (m, 12 H), 0.26 (s, 18 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.5, 139.6, 135.2, 133.5, 130.5, 128.4, 108.9, 104.2,

96.7, 46.1, 39.1, 30.2, 28.4, 23.6, 23.0, 14.0, 10.5, –0.30

2,5-Bis(2-ethylhexyl)-3,6-bis(5-ethynylthiophene-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (3). This compound was synthesized according to literature with modified procedures.[1] A round bottle flask was charged with **2** (215 mg, 0.3 mmol), potassium carbonate (414 mg, 3 mmol), THF (50 mL) and methanol (25 mL). The mixture was stirred at room temperature under argon atmosphere for 10 h. After solvent removal, the residue was subsequently redissolved in dichloromethane, washed with water and dried over $MgSO_4$. The crude product was purified by silica gel column chromatography using hexane/dichloromethane (6/4) as eluent to yield **3** as a dark purple solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.80 (d, J = 4.0 Hz, 2 H), 7.35 (d, J = 4.0 Hz, 2 H), 4.02–3.88 (m, 4 H), 3.57 (s, 2 H), 1.89–1.77 (m, 2 H), 1.39–1.15 (m, 16 H), 0.90–0.81 (m, 12 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 161.4, 139.6, 135.2, 134.0, 130.7, 127.0, 108.9, 85.5, 76.3, 46.0, 39.1, 30.1, 28.3, 23.5, 23.0, 14.0, 10.4.



Scheme S1 Synthetic route to 2,5-bis(2-ethylhexyl)-3,6-bis(5-ethynylthiophene-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione **3**. Conditions and reagents: i) $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, trimethylsilylaceylene, 80 ^{o}C, overnight; and ii) K_2CO_3, THF, MeOH, r.t., 10 h.

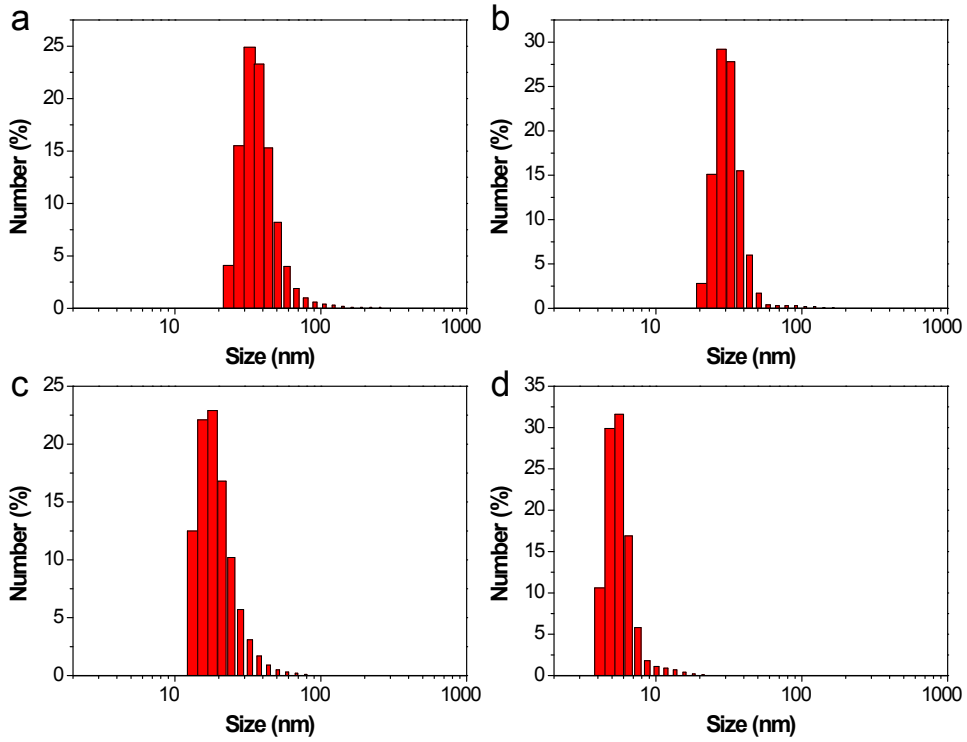


Fig. S1 The size distributions of PDPPID NPs after treatment with H_2O_2 for 0, 1, 3 and 6 h, measured by dynamic light scattering technique.

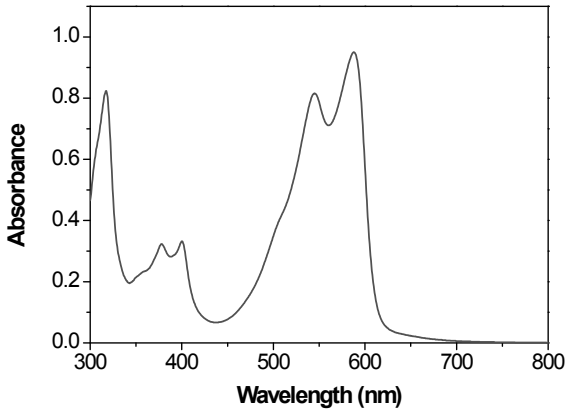


Fig. S2 UV-vis-NIR spectrum of 2,5-bis(2-ethylhexyl)-3,6-bis(5-ethynylthiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione in THF.

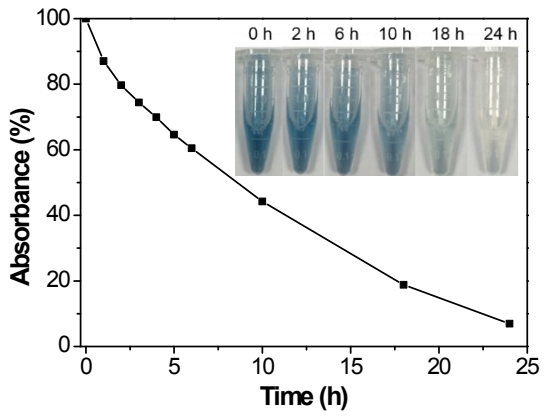


Fig. S3 The absorbance of PDPPID NPs at 620 nm versus treatment time with H_2O_2. Inset: photographs of PDPPID NPs treated with H_2O_2 for 0, 2, 6, 10, 18, and 24 h, respectively.

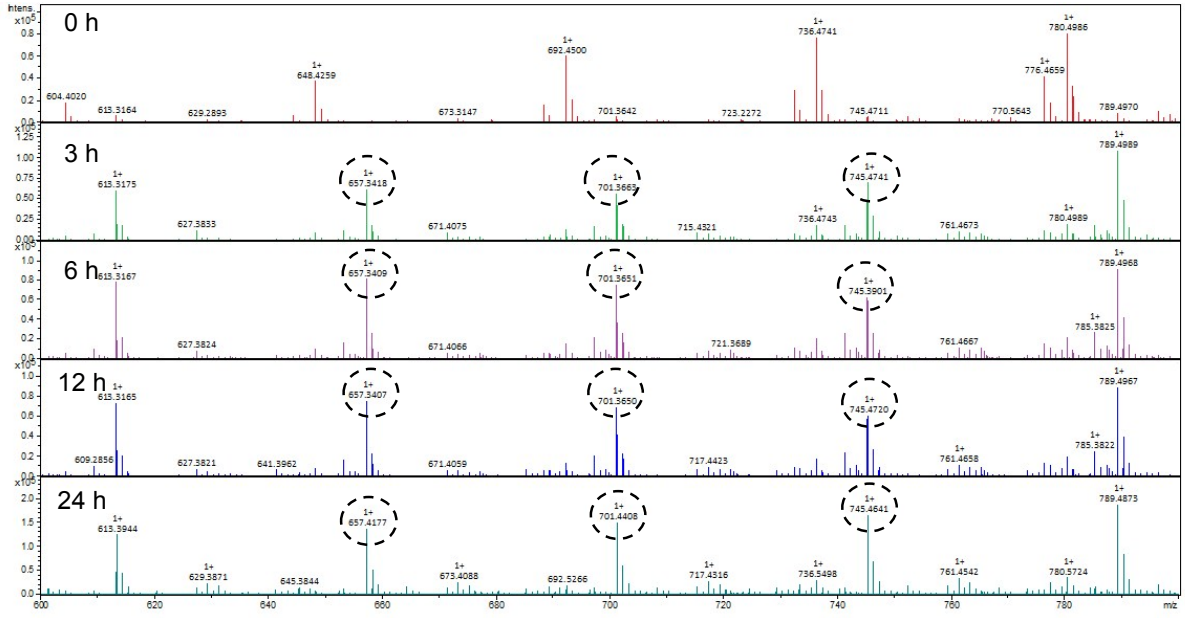


Fig. S4 ESI-MS results of the degradation products at time 0, 3, 6, 12 and 24 h, respectively. The proposed products are indicated by circles.

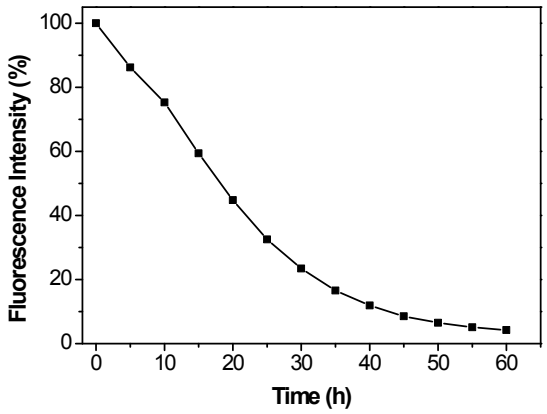


Fig. S5 Fluorescence intensity at 632 nm versus treatment time with H_2O_2.

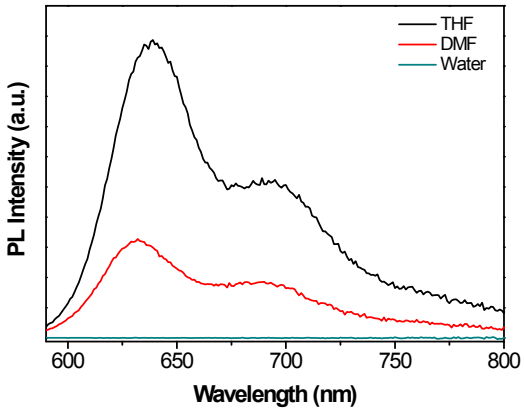


Fig. S6 PL spectra of PDPPID extracted from the PDPPID NPs in THF, DMF and water.

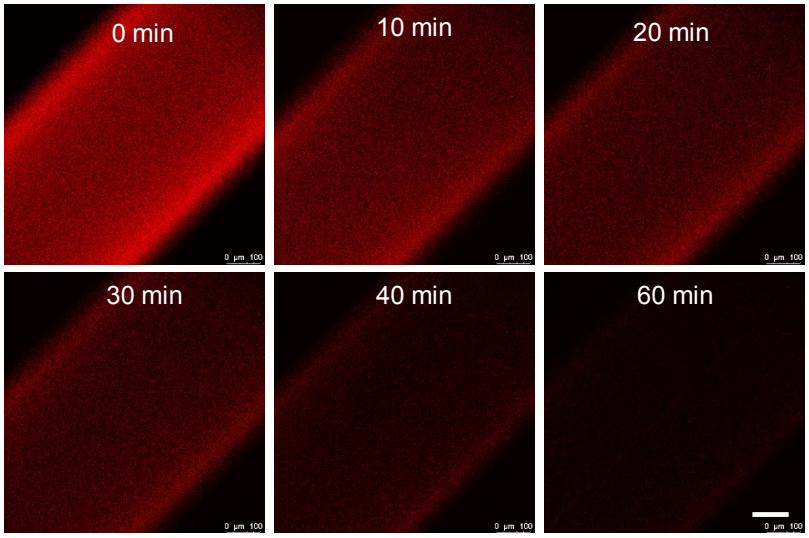


Fig. S7 CLSM images of H_2O_2-treated PDPPID NPs sealed in capillary.

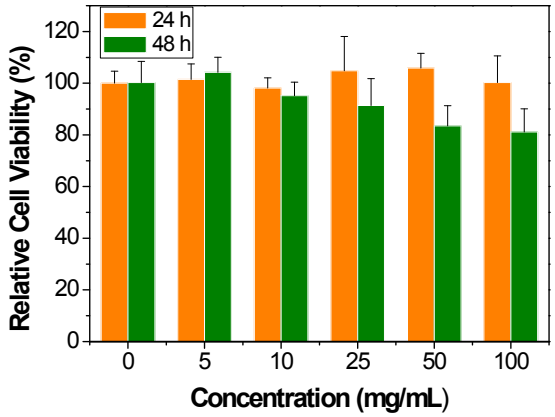


Fig. S8 Viabilities of HeLa cells incubated with various concentration of PDPPID NPs for 24 and 48 h, respectively.

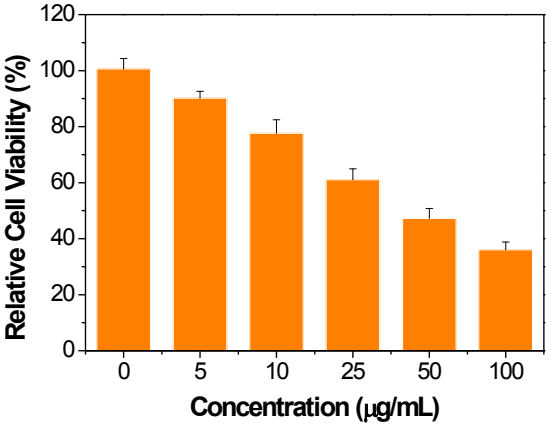


Fig. S9 Cell viability of HeLa cells incubated with PDPPID NPs for 12 h at various concentration followed by 808 nm laser irradiation for 10 min, and then the cells were further incubated for 24 h.

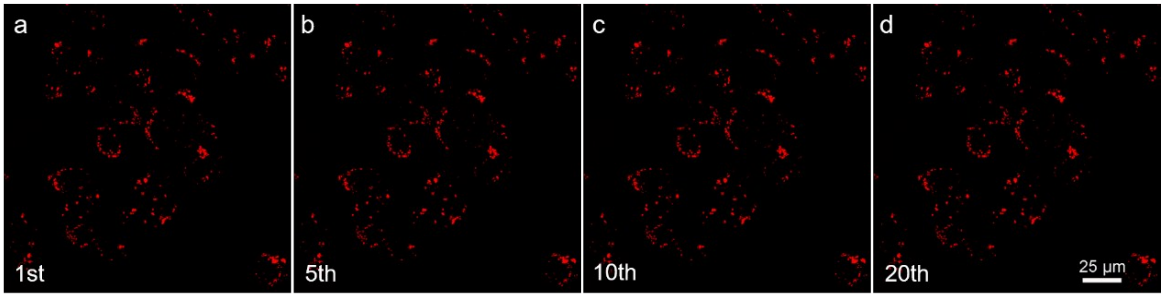


Fig. S10 Confocal fluorescence images of HeLa cells incubated with PDPPID NPs after 1st (A), 5th (B), 10th (C), and 20th (D) scans. This result indicated that there is no obvious photobleaching when performing multiple scans.

Reference

1. A. Punzi, E. Maiorano, F. Nicoletta, D. Blasi, A. Ardizzone, N. Ventosa, I. Ratera, J. Veciana and G. M. Farinola, *Eur. J. Org. Chem.*, **2016**, 2617.