Supporting Information

Copper Coordination-Based Conjugated Polymer Nanoparticles for Synergistic Photodynamic and Chemodynamic Therapy

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1. Experimental Section

1.1 Materials and Instruments

All chemicals were purchased from suppliers and used as received. HeLa cells were obtained from Xiangya Hospital at Central South University. ^{1}H NMR spectra were acquired using a Bruker 400 spectrometer, with chemical shifts reported in ppm (TMS as an internal standard). On a Waters 1515 liquid chromatography system equipped with a Waters 2414 refractive index detector and Phenogel Gel Permeation Chromatography (GPC) columns, GPC was performed. UV-vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. The size of material was measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS instrument (Malvern, UK). IR spectra were recorded on Fourier exchange infrared spectroscopy (FTIR, Nicolet IS 10, USA). Images of fluorescence imaging were captured using an inverted fluorescent microscope (IX83, Olympus, Japan). An LED lamp (DJ, Xuzhou Ai Jia electronic technology Co. Ltd., China) was used as the light source. To detect singlet oxygen, the cuvette was illuminated with a 400-800 nm LED lamp (20 mW/cm^2). To assess the efficacy of PDT in cells, the cells were illuminated with a 430-440 nm LED lamp (20 mW/cm^2).

1.2 Detection of Singlet Oxygen

The $^1O_2$ quantum yields (Φ_Δ) of different PPEs were determined using 9, 10-dibenzanthracene or 9, 10-anthracenediyl-bis(methylene)-dimalonic acid (ABDA) as the indicator and Rose Bengal (RB) as the standard. The solution containing 9, 10-dibenzanthracene or ABDA and the PPEs was placed in a cuvette and irradiated with LED light for different times. The absorbance at 378 nm was recorded by a UV-Vis absorption spectrophotometer. Φ_Δ was determined by the following formula:

$$\Phi_{\Delta PPE} = \Phi_{\Delta RB} * (k_{PPE}/k_{RB}) * (A_{RB}/A_{PPE})$$

where k_{PPE} and k_{RB} are the decomposition rate constants of 9, 10-dibenzanthracene or ABDA in the presence of PPEs or RB respectively; A_{PPE} and A_{RB} represent the integration area of absorption bands ranging from 400 to 800 nm of different PPEs or RB, respectively.

1.3 Extracellular Hydroxyl Radicals (•OH) Generation

The production of extracellular •OH was measured with Methylene Blue (MB). **PPE-Cu NPs** (30.0 μM) and GSH (5.0 mM) were dissolved in 2.0 mL PBS (pH = 6.5) for 15 min. MB (27.0 μM) and H_2O_2 (0.5 M) were then added to the mixture described above. The absorbance variation of MB at 664 nm was then measured.

1.4 Cell Culture and Measurement of Intracellular ROS

Hela cells with an exponential growth rate were cultured at 37 °C and 5% CO_2 atmosphere for 24 h in a high-glucose Dulbecco's Modified Eagle Medium (DMEM). In a 96-well plate, cells were seeded for 12 h before being incubated with **PPE-COOEt-NMe$_3$** (20.0 μM) and **PPE-Cu NPs** (20.0 μM) for 12 h. The cells were then washed with PBS and incubated for an additional 30 min with DCFH-DA (10.0 μM). The cells were then divided into four groups: 1) **PPE-COOEt-NMe$_3$**; 2) **PPE-COOEt-NMe$_3$** + L; 3) **PPE-Cu NPs**; 4) **PPE-Cu NPs** + L. After irradiating the second and fourth groups with LED light for 10 min, all groups were imaged with an inverted fluorescent microscope. λ_{ex} = 500 nm, λ_{em} = 525 nm.

1.5 Cytotoxicity Test

The toxicity of photosensitizers on HeLa cells with or without light exposure was evaluated by determining the cell viability. In 96-well plates, cells were seeded at a density of 1.0×10^4 cells per well and incubated for 24 h. Then different concentrations (0, 5.0, 10.0, 20.0 and 50.0 μM) of PPEs were added to each well, and the cells were incubated for 12 h. The cells were washed three times with PBS, irradiated for 10 min with LED light, and incubated for an additional 24 h. The viability of the cells was then measured using the MTT assay according to protocol.

1.6 Live/Dead Cell Staining

Calcein-AM and propidium iodide (PI) were utilized as co-staining agents for living and nonliving cells, respectively. HeLa cells were incubated in a culture dish for 24 h and then divided into six groups with different treatments: 1) cells were incubated with PBS for 12 h in the dark; 2) cells were incubated with PBS for 12 h, followed by irradiation with LED light for 10 min; 3) cells were incubated with **PPE-COOEt-NMe$_3$** (20.0 μM) for 12 h in the dark; 4) cells were incubated with **PPE-COOEt-NMe$_3$** (20.0

μM) for 12 h, followed by irradiation with LED light for 10 min; 5) cells were incubated with **PPE-Cu NPs** (20.0 μM) for 12 h in the dark; 6) cells were incubated with **PPE-Cu NPs** (20.0 μM) for 12 h, followed by irradiation with LED light for 10 min. After 24 h of incubation, each group of cells was stained for 30 min with AM/PI. The fluorescence images were captured using an inverted fluorescent microscope (calcein-AM: λ_{ex} = 490 nm, λ_{em} = 515 nm; PI: λ_{ex} = 535 nm, λ_{em} = 617 nm).

1.7 Flow Cytometry Analysis

Flow cytometry sets were divided into six groups including 1) PBS; 2) PBS + L; 3) **PPE-COOEt-NMe$_3$**; 4) **PPE-COOEt-NMe$_3$** + L; 5) **PPE-Cu NPs**; 6) **PPE-Cu NPs** + L, similar to the experimental procedure for live/dead cell staining. HeLa cells were subjected to apoptosis assays using the AV-FITC/7-AAD Apoptosis Detection Kit according to the manufacturer's instructions, and the results were captured using an inverted fluorescent microscope.

1.8 Subcellular Localization

Hela cells were cultured in 35 mm glass-bottom culture dishes for 12 h. Cells were incubated with **PPE-Cu NPs** (20.0 μM) for 12 h, and then the cells were washed three times with PBS. Lyso-tracker Red (100.0 nM) and Mito-tracker Red (100.0 nM) were added and co-incubated for 15 min. Next, the cells were washed three times with PBS before confocal luminescence imaging was carried out. Green channel: λ_{ex} = 408 nm, λ_{em} = 450-550 nm; red channel: λ_{ex} = 561 nm, λ_{em} = 580-650 nm.

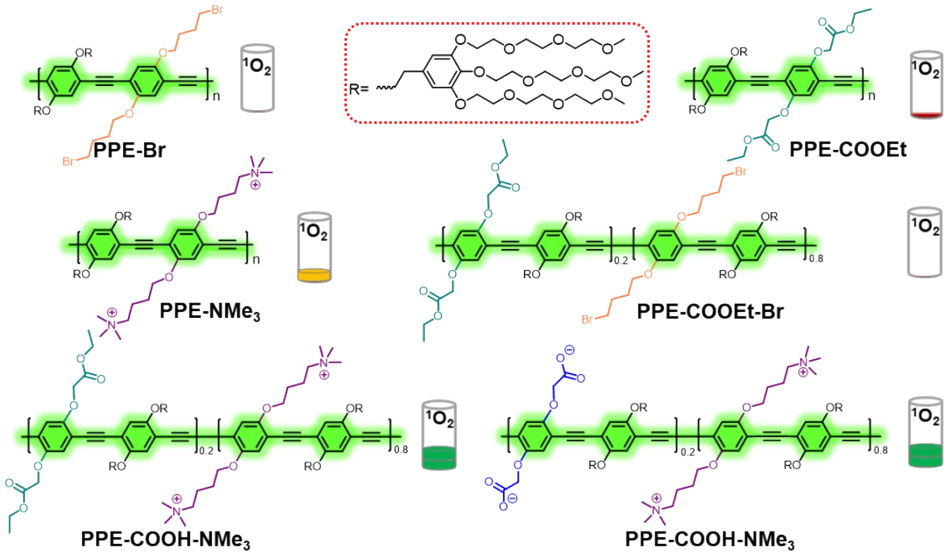
1.9 Mitochondria Disruption Assay

JC-1 is a dual-emission fluorescent probe widely used to detect mitochondrial membrane potential (MMP). In normal mitochondria, JC-1 accumulates in the mitochondrial matrix to form aggregates that emit intense red fluorescence. In unhealthy mitochondria, JC-1 is present as a monomer due to a decrease or loss of membrane potential, producing green fluorescence.[1] Therefore, we used JC-1 Assay Kit to investigate whether MMP changes during photodynamic and chemodynamic therapy.

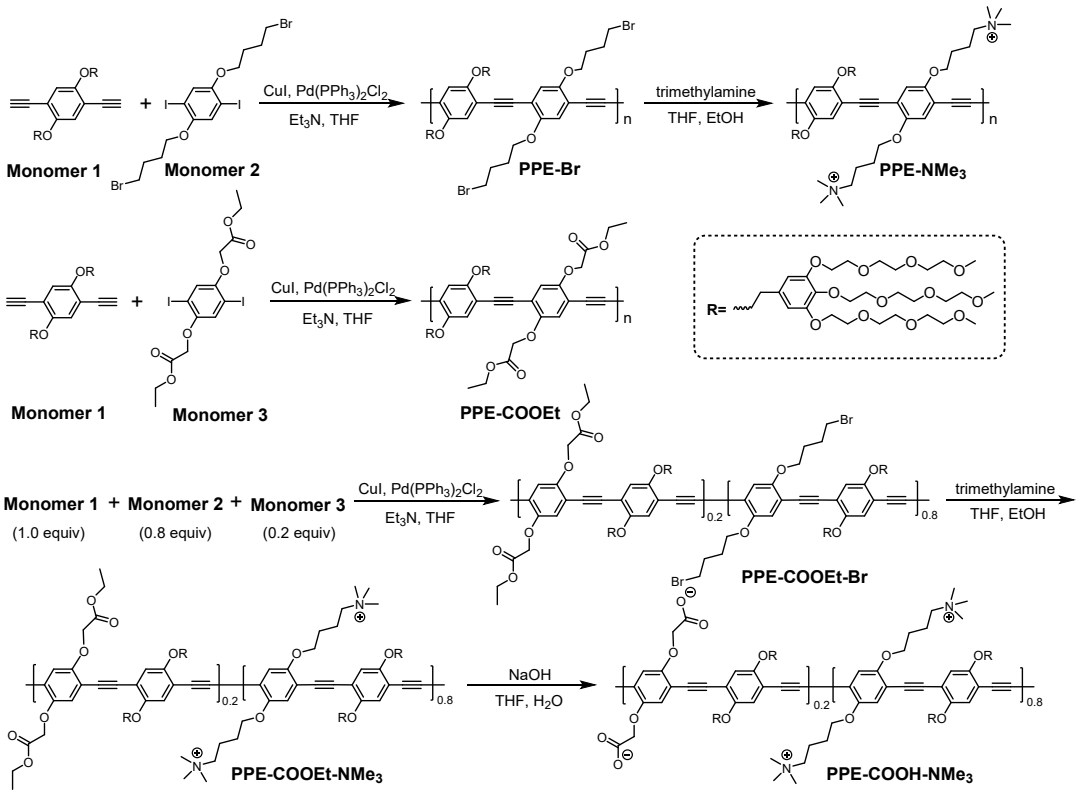
HeLa cells were incubated in a culture dish for 14 h and then divided into three groups with different treatments: 1) cells were incubated with PBS for 12 h in the dark; 2) cells

were incubated with **PPE-Cu NPs** (20.0 μM) for 12 h in the dark; 3) cells were incubated with **PPE-Cu NPs** (20.0 μM) for 12 h, followed by irradiation with LED light for 10 min. After 12 h of incubation, cells were washed with PBS for three times and stained for 15 min with JC-1 Assay Kit according to the manual. Next, the confocal luminescence imaging was carried out and images were collected. Green channel: λ_{ex} = 488 nm, λ_{em} = 500-550 nm; red channel: λ_{ex} = 561 nm, λ_{em} = 580-630 nm.

2. Synthesis



Scheme S1. Chemical structures of conjugated polymers.



Scheme S2. The synthetic routes of conjugated polymers.

Sonogashira coupling reactions of the corresponding monomers in a mixture of dry THF and triethylamine catalyzed by $Pd(PPh_3)_2Cl_2$ and CuI were used to produce the neutral polymers **PPE-Br**, **PPE-COOEt**, and **PPE-COOEt-Br**. The cationic polymers **PPE-NMe$_3$** and **PPE-COOEt-NMe$_3$** were produced by the quaternary amination of **PPE-Br** and **PPE-COOEt-Br**, respectively. After saponification, dialysis, and freeze-drying **PPE-COOEt-NMe$_3$**, the zwitterionic polymer **PPE-COOH-NMe$_3$** was obtained. Due to the superposition of hydrogen chemical shifts on aromatic rings, it is challenging to determine the precise ratio of each monomer. As the ratio of monomers in the polymers **PPE-COOEt-Br**, **PPE-COOEt-NMe$_3$** and **PPE-COOH-NMe$_3$**, the theoretical proportion was utilized.

Synthesis of Monomers 1, 2 and 3. Monomers **1, 2** and **3** were prepared according to the reported methods.[2-4]

Synthesis of PPE-Br. In a mixture of degassed THF/triethylamine (10.0 mL/5.0 mL), monomers **1** (262.2 mg, 0.2 mmol) and **2** (125.4 mg, 0.2 mmol) were dissolved. Under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (70.0 mg, 0.10 mmol) and CuI (70.0 mg, 0.37 mmol) were added. After 17 h of stirring at 37 ℃, the mixture was extracted with DCM

(50 mL × 6) and successively washed with saturated NaCl and NH_4Cl solutions. The organic phases were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in a small amount of DCM and added slowly to an excess of *n*-hexane to produce polymer **PPE-Br** as a brown solid (251.0 mg, 75.0 %). GPC (in DMF): M_w = 2.85 × 10^5, M_n = 2.10 × 10^5, PDI = 1.36. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63-7.24 (m, 4 H), 6.77-6.56 (s, 4 H), 5.14-4.89 (s, 4 H), 4.18-3.80 (m, 16 H), 3.79-3.30 (m, 64 H), 3.30-3.17 (m, 18 H), 2.15-1.69 (m, 8 H).

Synthesis of PPE-NMe$_3$. Polymer **PPE-Br** (158.0 mg, 0.1 mmol) was dissolved in 10.0 mL/5.0 mL of degassed THF/EtOH. Slowly adding 2.0 mL of trimethylamine, the mixture was allowed to reflux for 24 h at room temperature. After the solvents were evaporated, the mixture was extracted with DCM (50 mL × 6) and successively washed with saturated NaCl and NH_4Cl solutions. The organic phases were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in water and dialyzed for seven days against DI water. A brownish-yellow solid (140.0 mg, 86.3%) was obtained after freeze-drying. The GPC data are generated by **PPE-Br**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.63-7.29 (m, 4H), 6.78-6.63 (m, 4 H), 5.09-4.85 (s, 4 H), 4.26-3.87 (m, 16H), 3.65-3.52 (m, 64 H), 3.33-3.20 (m, 18 H), 2.25-1.85 (m, 8 H),1.31-1.25 (m, 18 H).

Synthesis of PPE-COOEt. In a mixture of degassed THF/triethylamine (4.0 mL/2.0 mL), monomers **1** (131.1 mg, 0.1 mmol) and **3** (53.5 mg, 0.1 mmol) were dissolved. Under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (30.0 mg, 0.04 mmol) and CuI (30.0 mg, 0.16 mmol) were added. After 17 h of stirring at 37 °C, the mixture was extracted with DCM (50 mL × 6) and successively washed with saturated NaCl and NH_4Cl solutions. The organic phases were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in a small amount of DCM and added slowly to an excess of *n*-hexane to produce polymer **PPE-COOEt** as a brownish yellow solid (111.1 mg, 70 %). GPC (in DMF): M_w = 4.18 × 10^5, M_n = 3.30 × 10^5, PDI = 1.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61-7.40 (m, 4 H), 6.70-6.60 (s, 4

H), 5.09-4.85 (s, 4 H), 3.99-3.79 (m, 20 H), 3.65-3.36 (m, 60 H), 3.33-3.18 (m, 18 H), 1.31-1.27 (m, 6 H).

Synthesis of PPE-COOEt-Br. In a mixture of degassed THF/triethylamine (8.0 mL/4.0 mL), monomers **1** (131.1 mg, 0.1 mmol), **2** (50.2 mg, 0.08 mmol) and **3** (10.7 mg, 0.02 mmol) were dissolved. Under an argon atmosphere, Pd $(PPh_3)_2Cl_2$ (40.0 mg, 0.057 mmol) and CuI (40.0 mg, 0.21 mmol) were added. After 17 h of stirring at 37 °C, the mixture was extracted with DCM (50 mL × 6) and successively washed with saturated NaCl and NH_4Cl solutions. The organic phases were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in a small amount of DCM and added slowly to an excess of *n*-hexane to produce polymer **PPE-COOEt-Br** as a brown solid (113.0 mg, 68.0 %). GPC (in DMF): M_w =2.43 × 10^5, M_n = 1.93 × 10^5, PDI = 1.26. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71-7.09 (m, 4.0 H), 6.80-6.65 (s, 4.0 H), 5.19-4.95 (s, 4.0 H), 4.46-3.89 (m, 16.8 H), 3.85-3.46 (m, 63.3 H), 3.40-3.30 (m, 18.1 H), 1.88-1.76 (m, 6.4 H), 1.27-1.25 (m, 1.3 H).

Synthesis of PPE-COOEt-NMe$_3$. Polymer **PPE-COOEt-Br** (100.0 mg, 0.05 mmol) was dissolved in 10.0 mL/5.0 mL of degassed THF/EtOH. Slowly adding 2.0 mL of trimethylamine, the mixture was allowed to reflux for 24 h at room temperature. After the solvents were evaporated, the mixture was extracted with DCM (50 mL × 6) and successively washed with saturated NaCl and NH_4Cl solutions. The organic phases were combined, dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in water and dialyzed for seven days against DI water. A brownish-yellow solid (87.2 mg, 86.2%) was obtained after freeze-drying. The GPC data are generated by **PPE-COOEt-Br**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.71-7.46 (m, 4.0 H), 6.80-6.66 (s, 4.0 H), 5.19-4.91 (s, 4.0 H), 4.28-3.87 (m, 16.9 H), 3.71-3.42 (m, 63.1 H), 3.37-3.28 (m, 18.0 H), 2.07-1.88 (m, 6.4 H), 1.41-1.28 (m, 14.5 H), 1.31-1.27 (m, 1.2 H).

Synthesis of PPE-COOH-NMe$_3$. Polymer **PPE-COOEt-NMe$_3$** (50.0 mg, 32.0 μmol) was suspended in 2.5 N NaOH (0.64 mL, 1.6 mmol) and refluxed for 24 h at 50 °C. After cooling down to room temperature, the pH of the solution was adjusted to 7.0 by

HCl. The solution was then dialyzed for 3 days against DI water. A yellow solid (43.0 mg, 89%) was obtained after freeze-drying. The GPC data are generated by **PPE-COOEt-Br**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.68-7.36 (m, 4.0 H), 6.80-6.66 (s, 4.0 H), 5.16-5.02 (s, 4.0 H), 4.32-3.97 (m, 16.8 H), 3.81-3.49 (m, 63.2 H), 3.39-3.31 (m, 18.1 H), 2.11-1.88 (m, 6.4 H), 1.51-1.38 (m, 14.4 H).

Synthesis of PPE-Cu NPs. After dissolving 20.0 mg of **PPE-COOH-NMe$_3$** in 40.0 mL of MeOH/H_2O (2/1, *v/v*), 1.0 mL of glacial acetic acid was added to the solution. This mixture was stirred for 2 h at room temperature. The $CuCl_2$ solution (10.0 mg $CuCl_2$ dissolved in 5.0 mL H_2O) was then added, and the reaction mixture was maintained at 90 °C for 24 h. Organic solvents were removed from the colloidal dispersion by dialyzing it with ultrapure water for 48 h using a dialysis tube (molecular weight cut-off = 3.0 kDa). The water was changed every 8 h during dialysis, and the PPE-Cu nanoparticle solution was collected from the dialysis tube.

3. Supplemental Figures

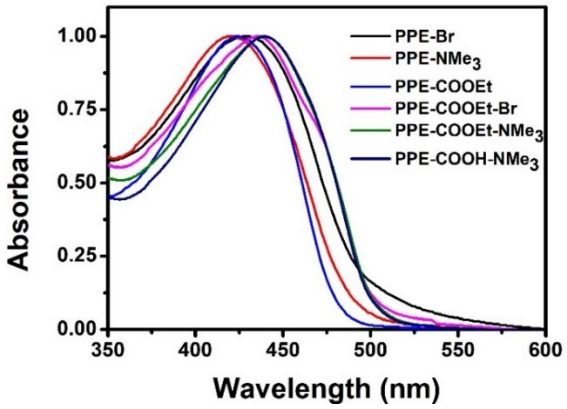


Fig. S1 Normalize UV-vis absorption spectra of PPEs.

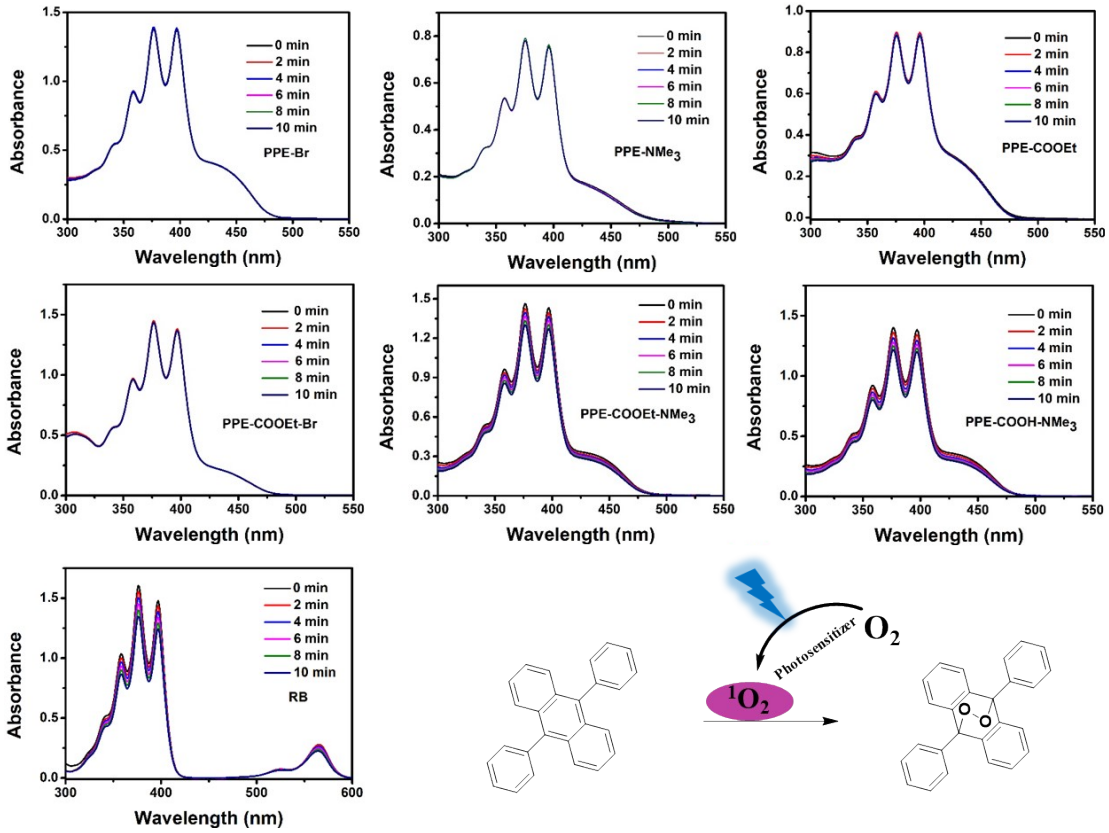


Fig. S2 UV-vis absorption spectra of 9, 10-diphenylanthracene in the presence of various PPEs in ethanol before and after LED light irradiation (400-800 nm, 20 mW/cm^2) for different time. Bottom panel: the mechanism of 9, 10-diphenylanthracene as an indicator for monitoring $^1O_2$ production.

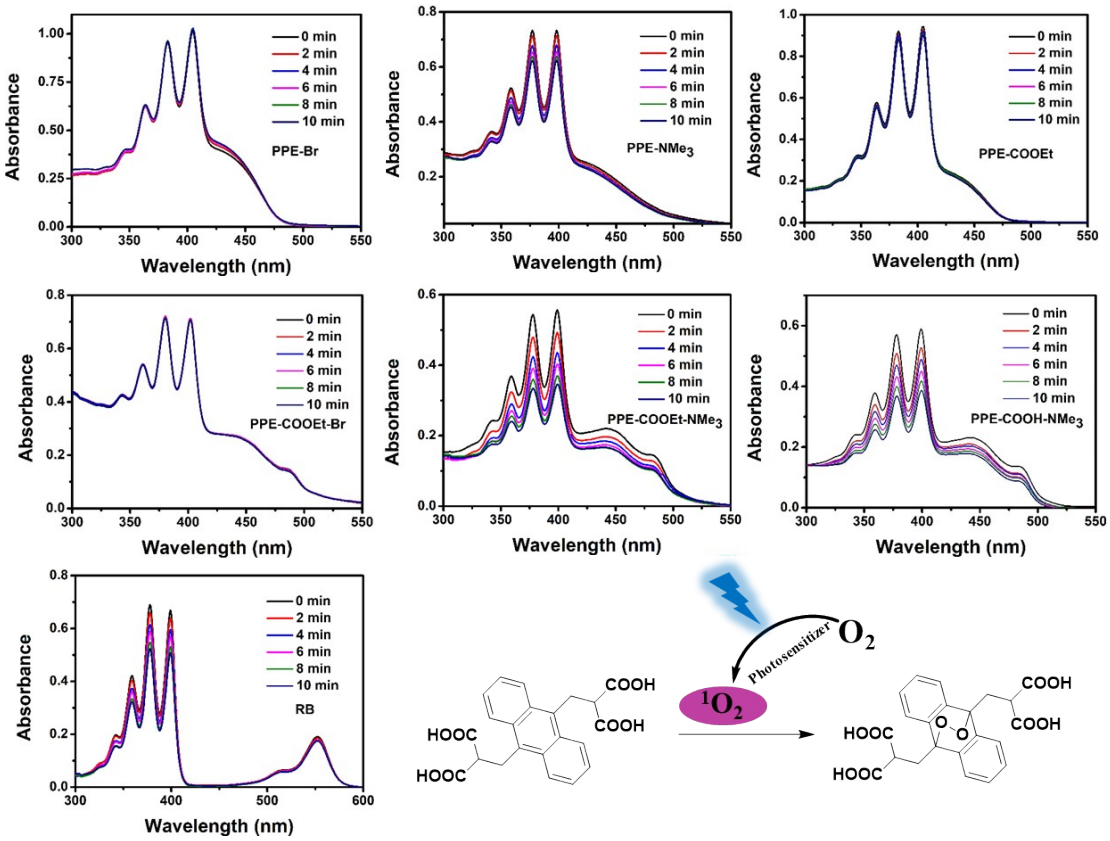


Fig. S3 UV-vis absorption spectra of ABDA in the presence of various PPEs in ethanol/water (1:9, *v/v*) before and after LED light irradiation (400-800 nm, 20 mW/cm^2) for different time. Bottom panel: the mechanism of ABDA as an indicator for monitoring $^1O_2$ production.

Table S1. The quantum yields of singlet oxygen for various PPEs.

PPEs	Φ_Δ[a]	Φ_Δ[b]
PPE-Br	–	–
PPE-COOEt	0.05	0.09
PPE-NMe$_3$	–	0.24
PPE-COOEt-Br	0.03	–
PPE-COOEt-NMe$_3$	0.41	0.41
PPE-COOH-NMe$_3$	0.48	0.39

[a] determined by 9,10-diphenylanthracene as the $^1O_2$ trapping agent in ethanol; [b] determined by ABDA as the $^1O_2$ trapping reagent in ethanol/water (1:9, *v/v*).

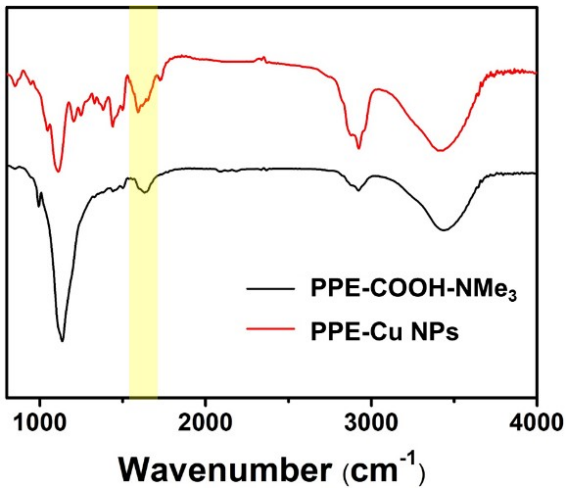


Fig. S4 IR spectra of **PPE-COOH-NMe$_3$** and **PPE-Cu NPs**.

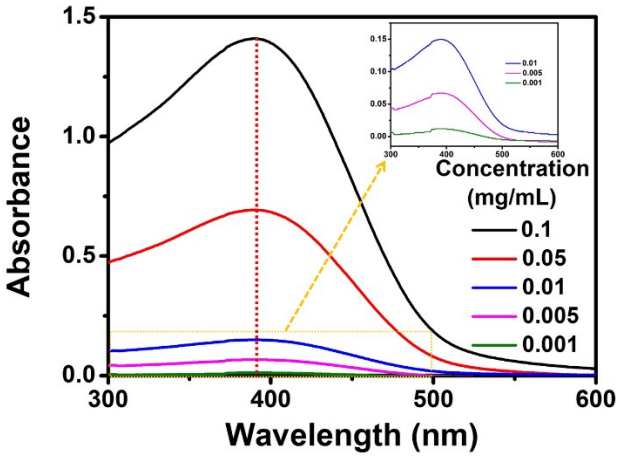


Fig.S5 Absorption spectra of **PPE-Cu NPs** at different concentrations.

Table S2. Particle sizes of **PPE-Cu NPs** at different concentrations measured by DLS.

Concentration (mg/mL)	Particle Size (nm)
0.1	92.8
0.05	91.7
0.01	86.9
0.005	89.6
0.001	87.1

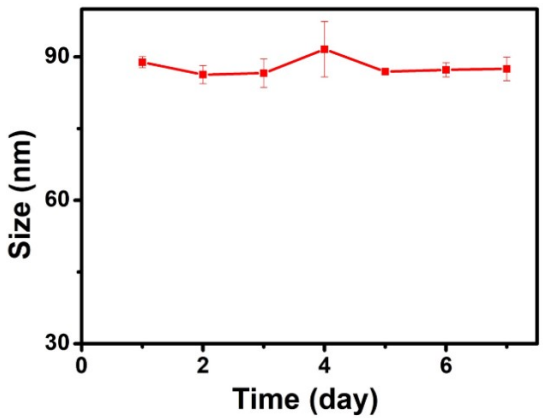


Fig.S6 Variation of the particle size of **PPE-Cu NPs** (0.1 mg/mL) versus time.

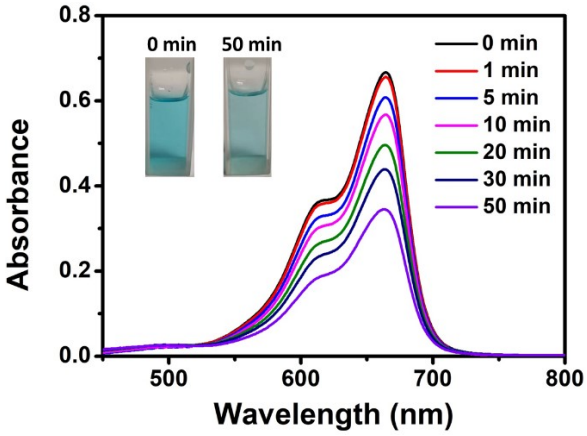


Fig.S7 Absorption spectral changes of MB at different time points in the presence of Cu^{2+} (30.0 μM), GSH (5.0 mM), and H_2O_2 (0.5 M). Inset: the color changes of MB at 0 min and 50 min.

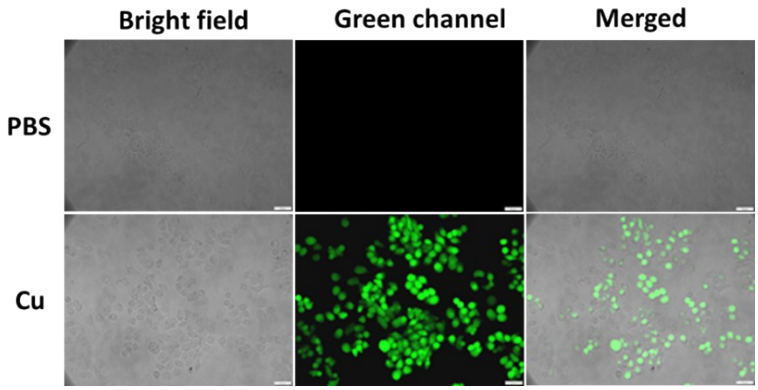


Fig.S8 Images of Hela cells treated with DCFH-DA (10.0 μM) under PBS or Cu^{2+} (20.0 μM). Scale bar = 50 μm.

We investigated the ability of Cu^{2+} to generate hydroxyl radicals (•OH) in solution and cells. As shown in Fig. S7, the absorbance of MB located at 664 nm gradually decreases with time and the color of MB fades, which indicates the generation of •OH.

In addition, the ability of Cu^{2+} to generate •OH in Hela cells was evaluated by using DCFH-DA as a fluorescent ROS indicator. Compared with PBS group, the cells incubated with Cu^{2+} exhibited strong green fluorescence, which was caused by the generation of •OH (Fig. S8).

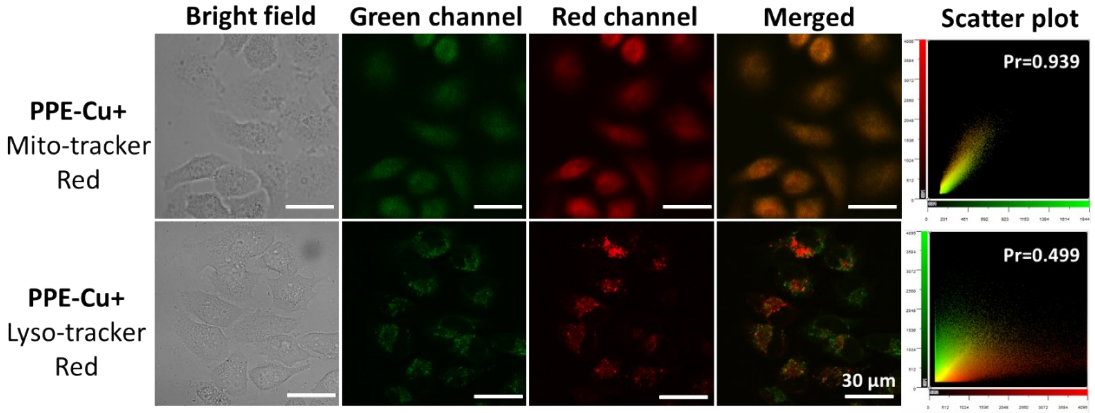


Fig.S9 Subcellular colocalization images of Hela cells incubated with **PPE-Cu NPs** (20.0 μM) for 12 h, followed by organelle staining dyes (100 nM) for 15 min. Green channel: λ_{ex} = 408 nm, λ_{em} = 450-550 nm; Red channel: λ_{ex} = 561 nm, λ_{em} = 580-650 nm. Scale bar: 30 μm.

4. ^{1}H NMR Spectra

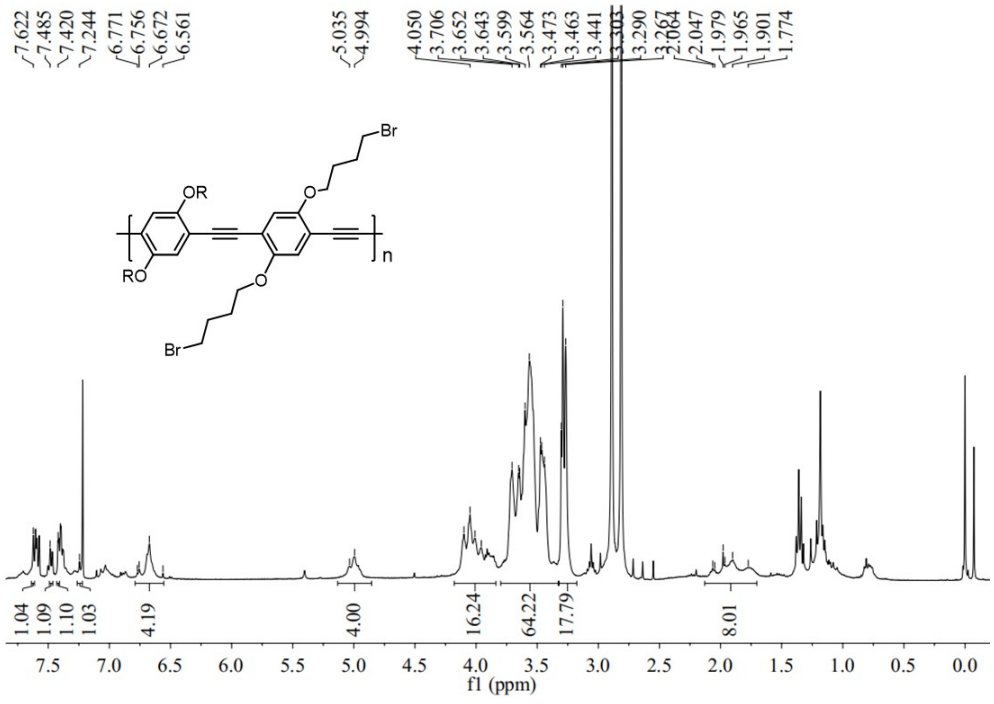


Fig. S10 ^{1}H NMR spectrum of **PPE-Br** in $CDCl_3$.

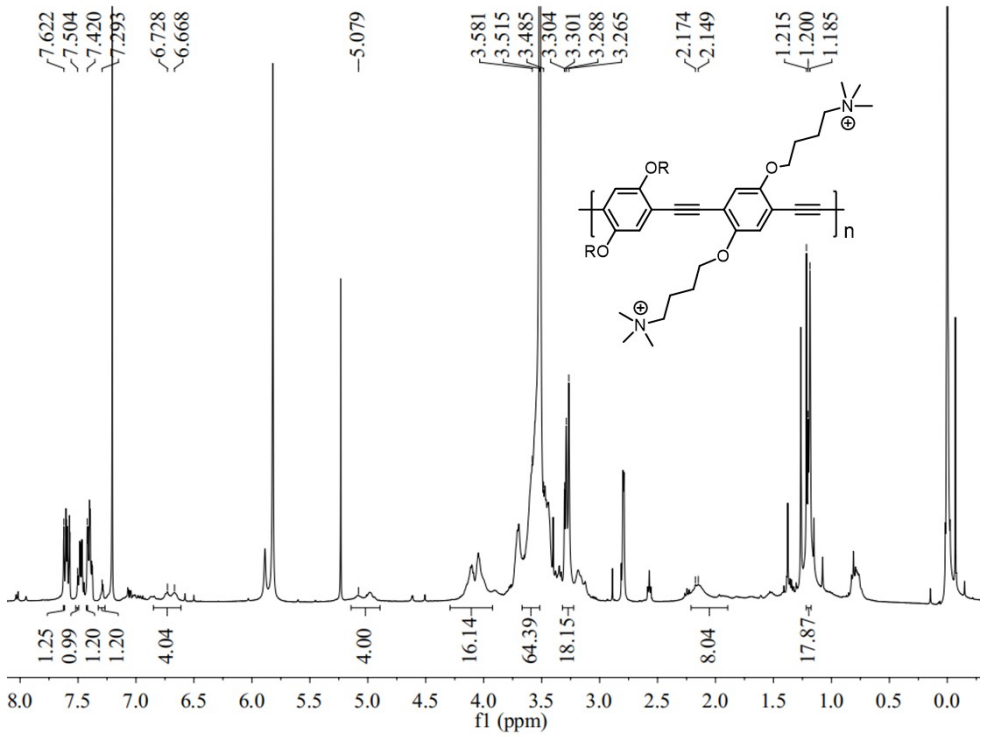


Fig. S11 ^{1}H NMR spectrum of **PPE-NMe$_3$** in $CDCl_3$.

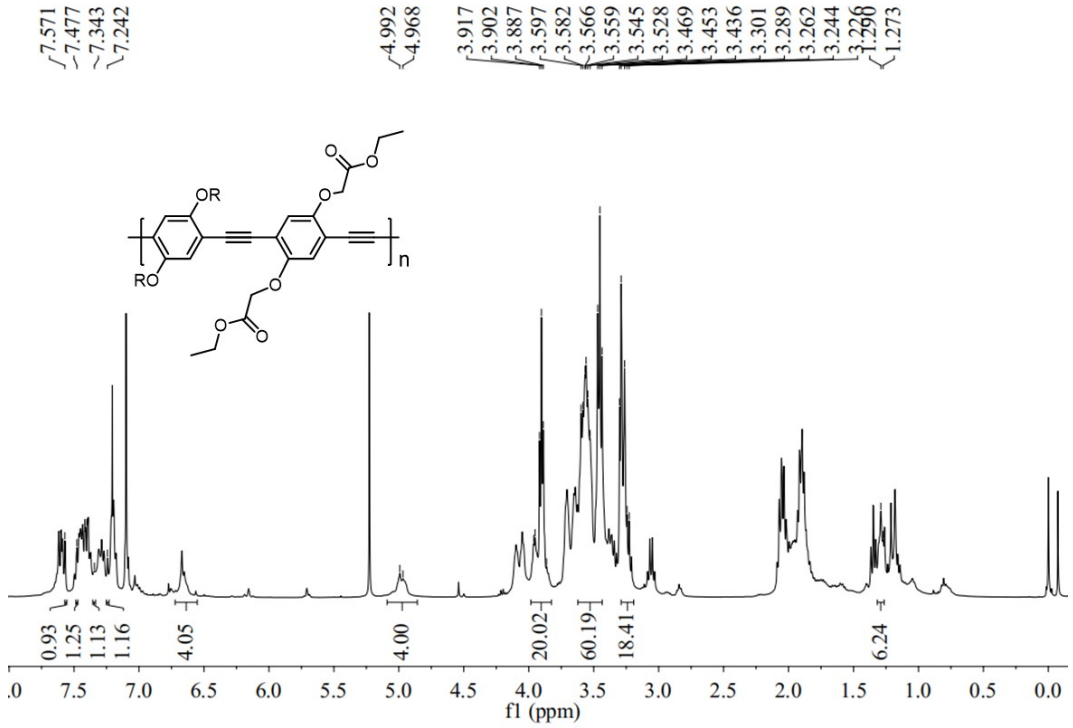


Fig. S12 ^{1}H NMR spectrum of **PPE-COOEt** in $CDCl_3$.

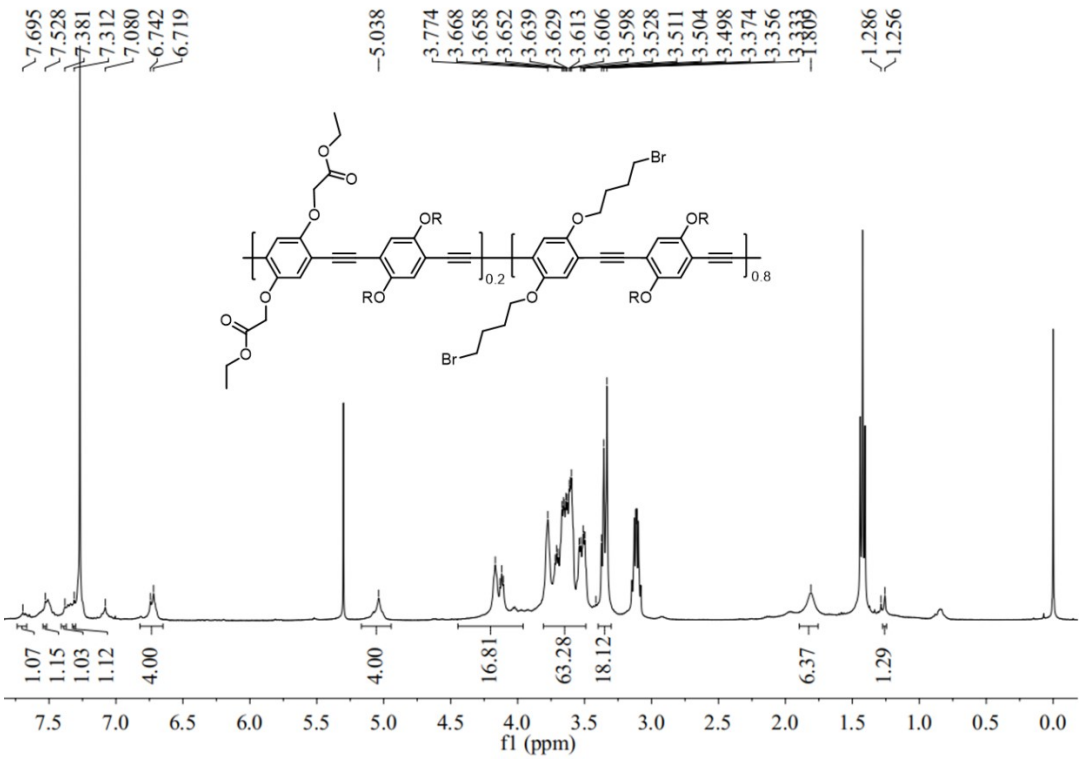


Fig. S13 ^{1}H NMR spectrum of **PPE-COOEt-Br** in $CDCl_3$.

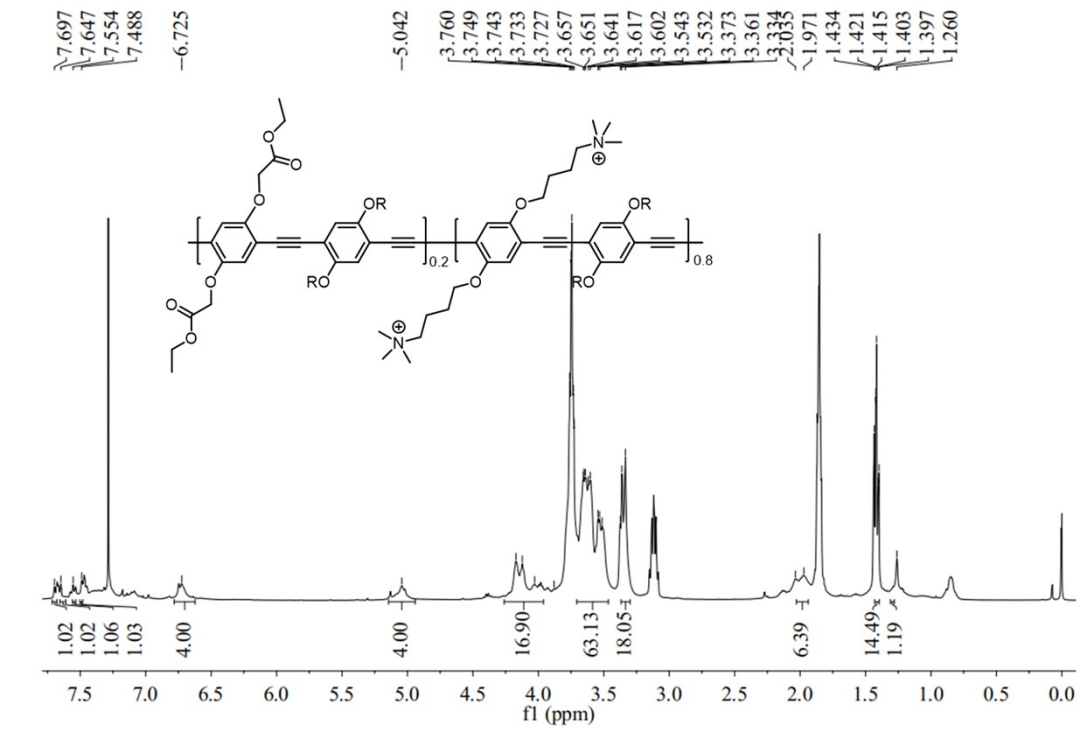


Fig. S14 ^{1}H NMR spectrum of **PPE-COOEt-NMe$_3$** in $CDCl_3$.

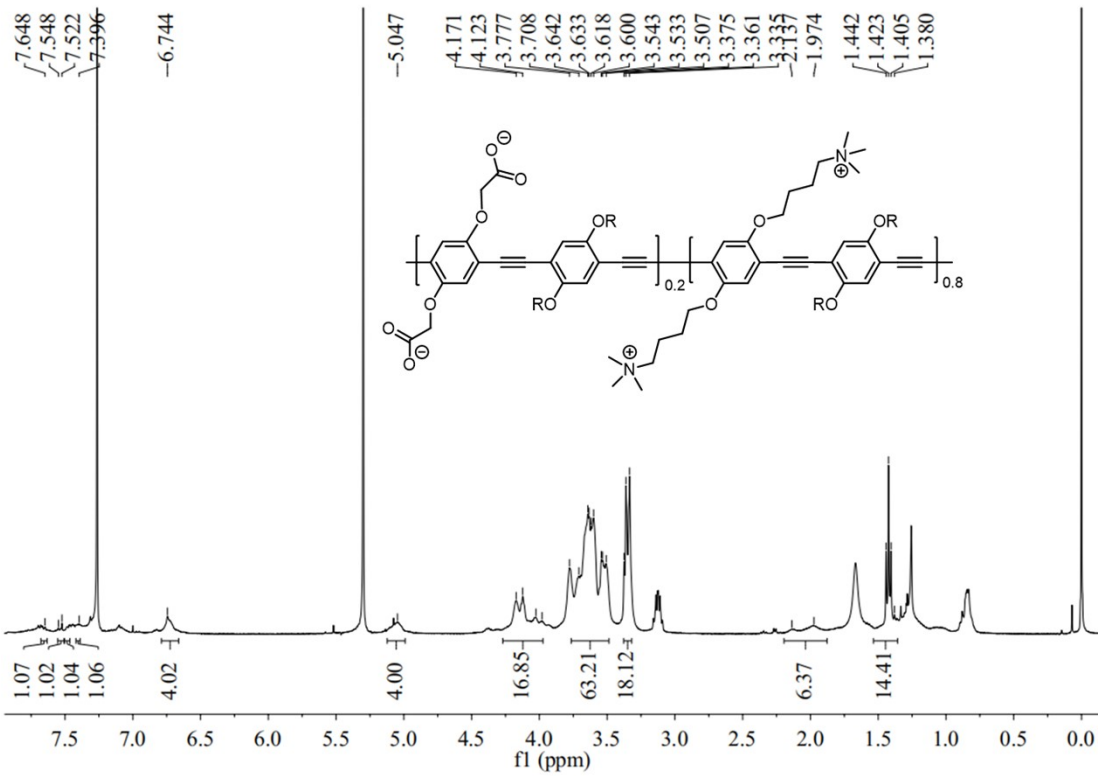


Fig. S15 ^{1}H NMR spectrum of **PPE-COOH-NMe$_3$** in $CDCl_3$.

5. References

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