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Electronic Supplementary Information for:

From main chain conjugated polymer photosensitizer to hyperbranched polymer photosensitizer: expansion of the polymerization-enhanced photosensitization effect for photodynamic therapy

Jingxi Cheng,‡[a] Yuping Zhou, ‡[b] Shidang Xu,[c] Yujun Xie,[a] Duo Mao*[b], Wenbo Wu*[a] and Zhen Li*[ade]

[a] Institute of Molecular Aggregation Science, Tianjin University, Tianjin, 300072, China. E-mail: wuwb@tju.edu.cn, lizhentju@tju.edu.cn or lizhen@whu.edu.cn.

[b] Precision Medicine Institute, The First Affiliated Hospital of Sun Yat-Sen University, Sun Yat-Sen University, Guangzhou, 510080, China. E-mail: maod6@mail.sysu.edu.cn

[c] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, 117585, Singapore.

[d] Department of Chemistry, Wuhan University, Wuhan, 430072, China.

[e] Joint School of National University of Singapore and Tianjin University, International Campus of Tianjin University, Binhai New City, Fuzhou, 350207, China.

‡ These authors contributed equally to this work.

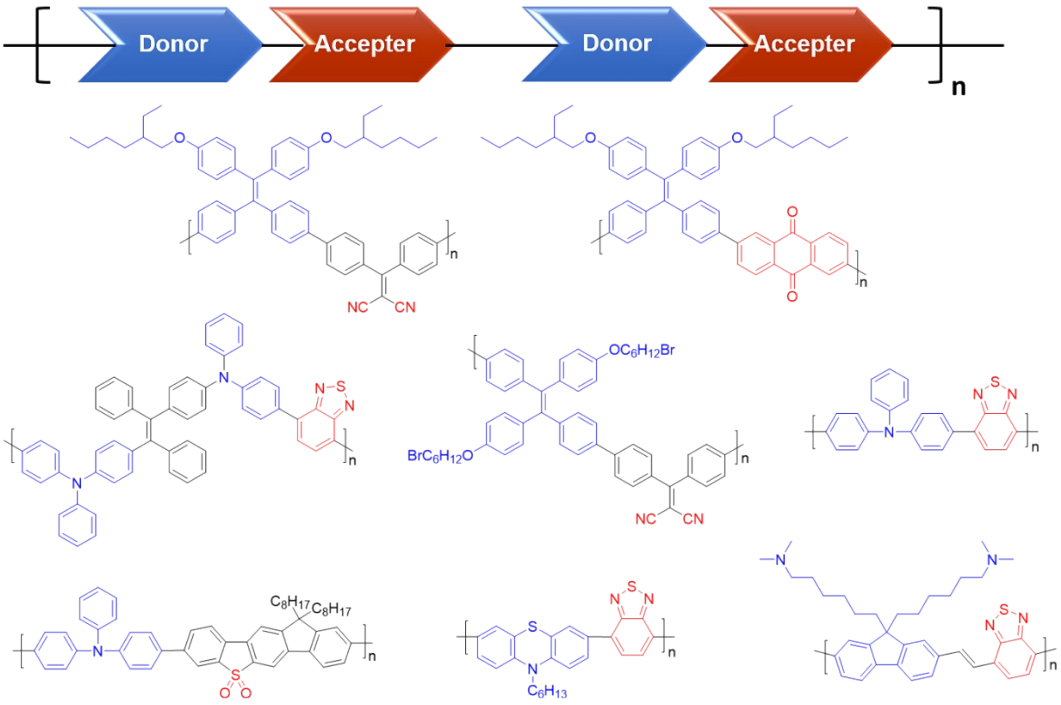

Fig. S1. The chemical structures of some reported conjugated polymer PSs[1-5].

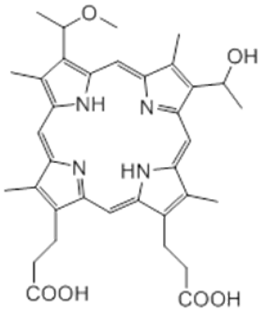

Fig. S2. The chemical structure of hemoporfin.

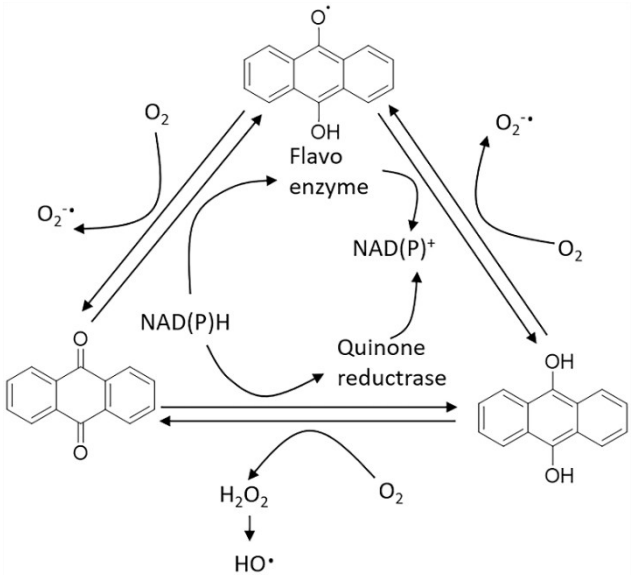

Fig. S3. The redox cycling of AQ in the cells.

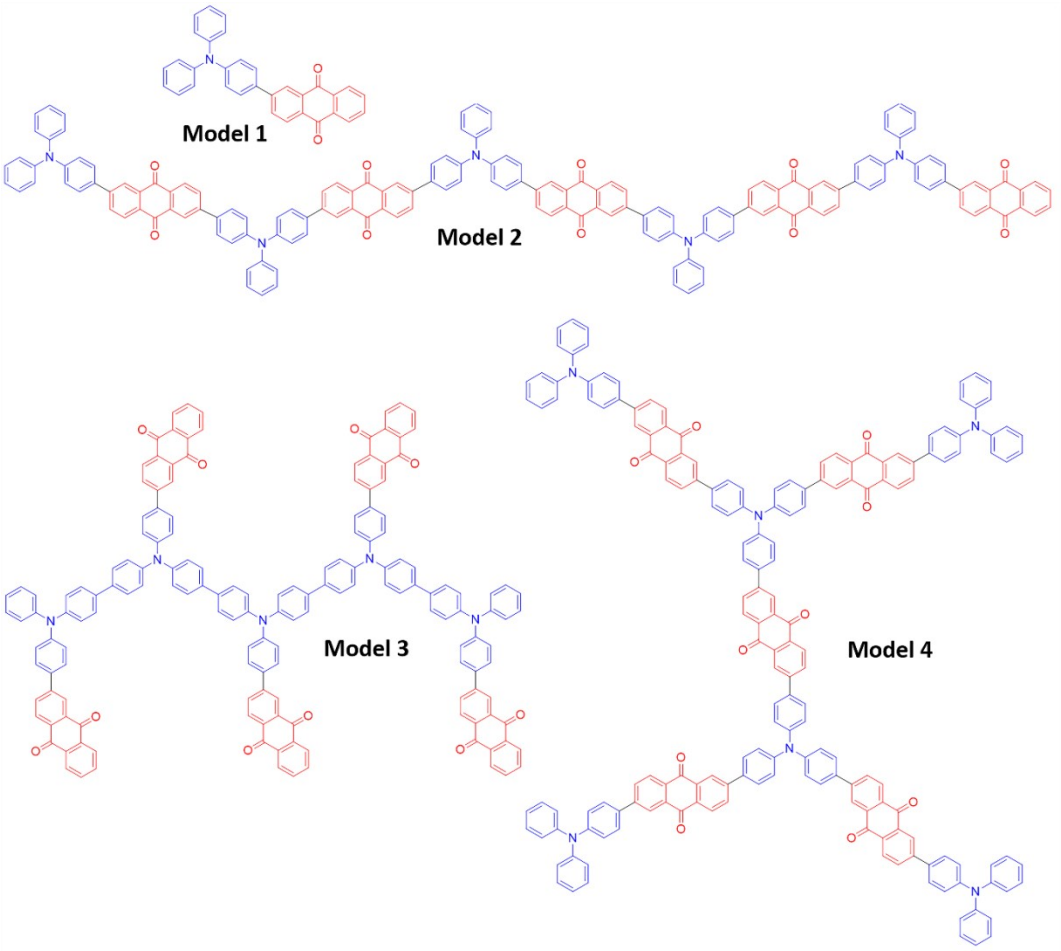

Fig. S4. The chemical structures of the model compounds. **Model 1**, **Model 2**, **Model 3** and **Model 4** refer to **TPAAQ**, and the simplified small molecule **MP**, **SP** and **HP** with 5 repeat units, respectively.

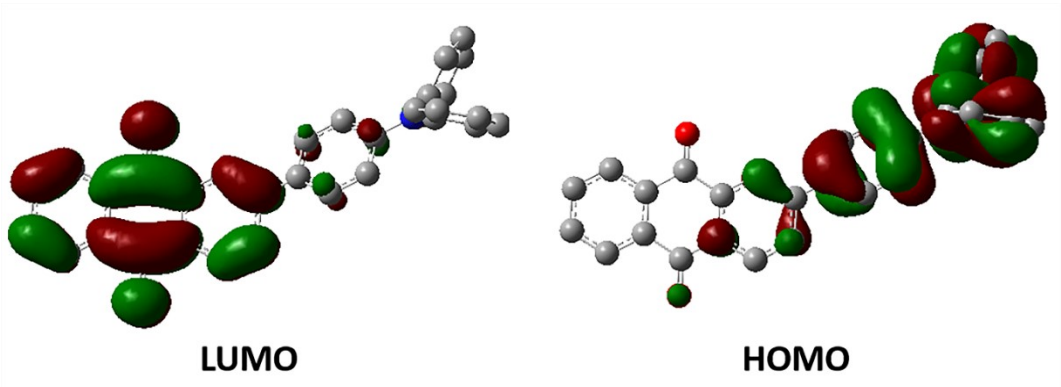

Fig. S5. HOMO-LUMO distributions of **Model 1**, which refer to small molecule PS of **TPAAQ**.

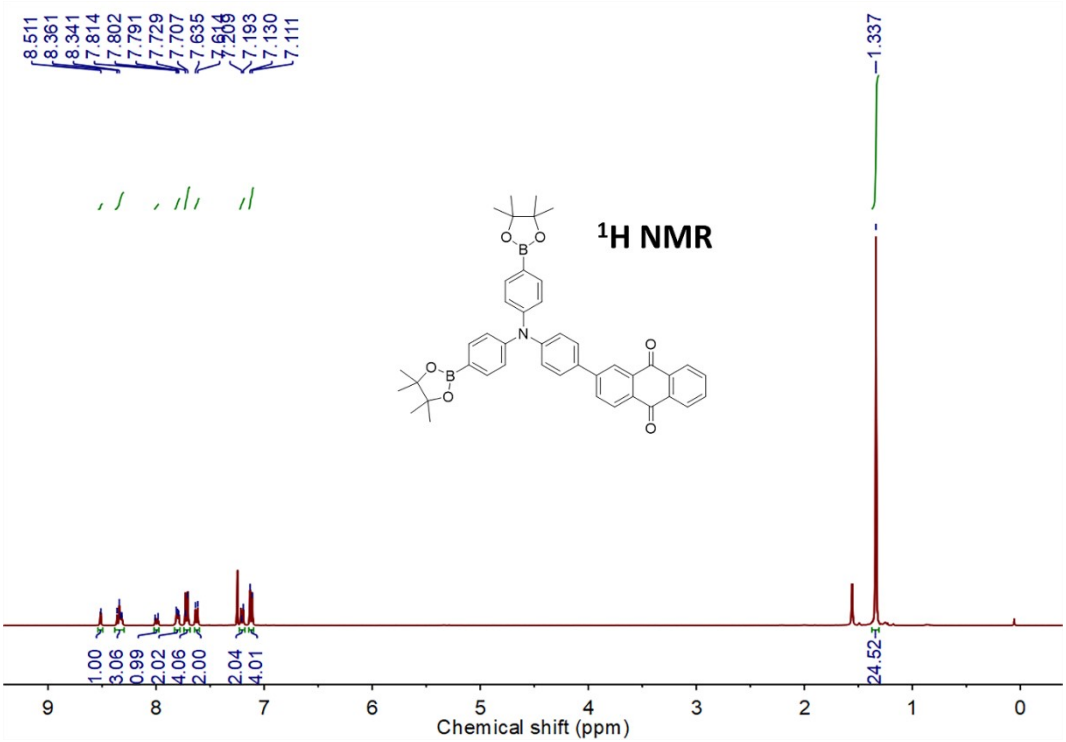

Fig. S6. ^{1}H NMR spectra of **M4** in chloroform-*d*.

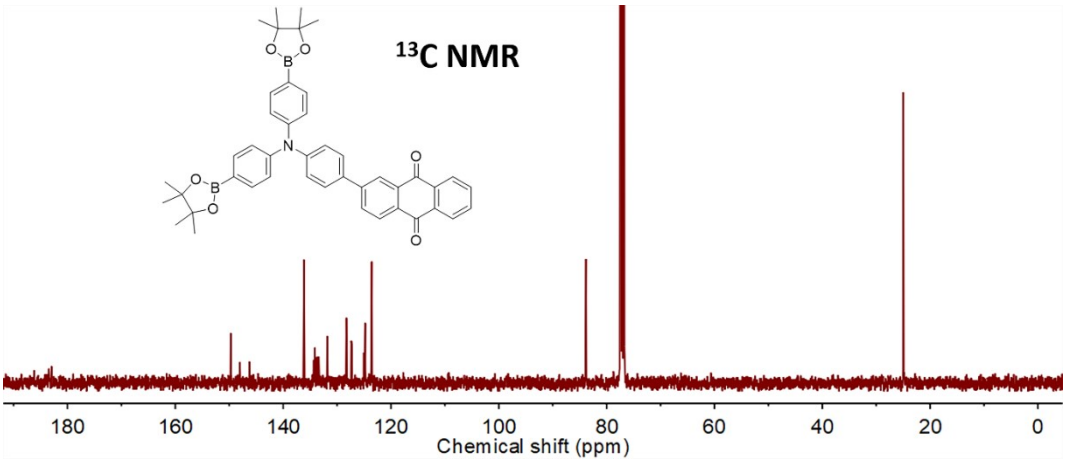

Fig. S7. ^{13}C NMR spectra of **M4** in chloroform-*d*.

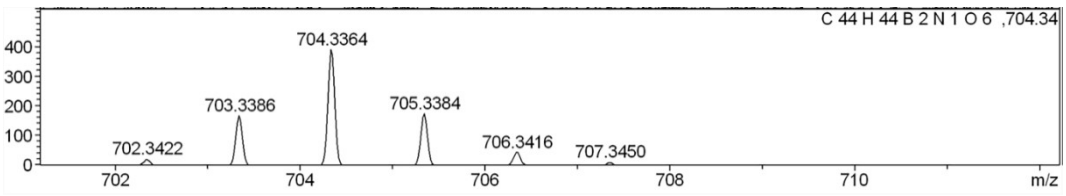

Fig. S8. High resolution mass spectrum of **M4**.

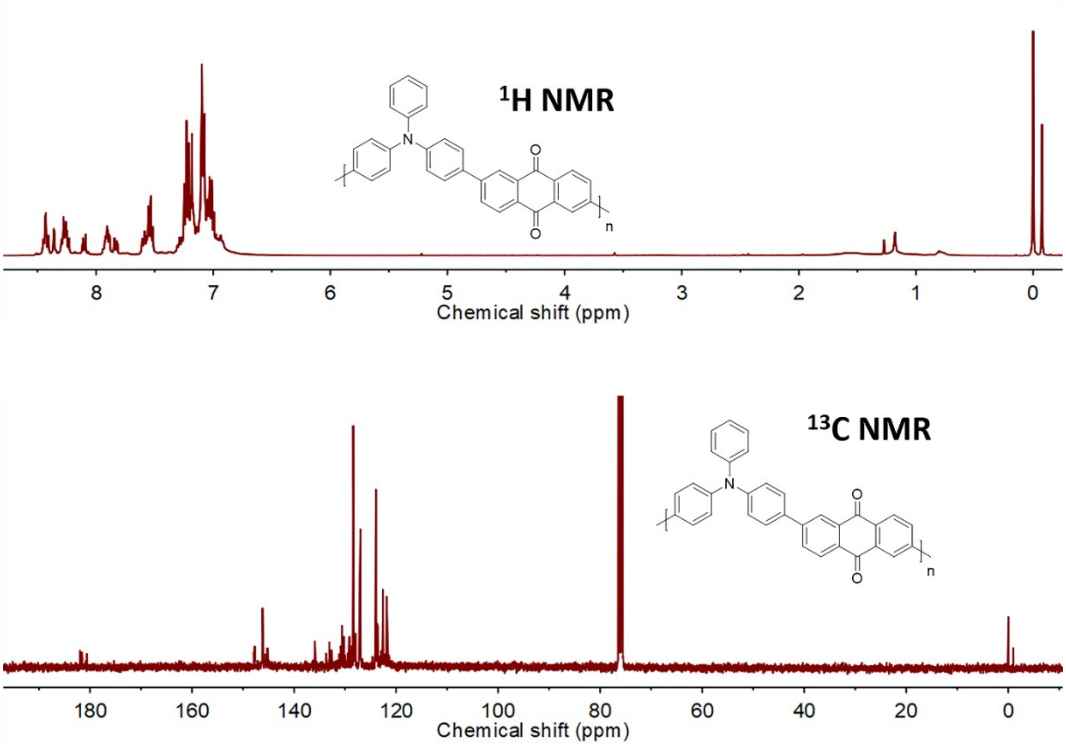

Fig. S9. NMR spectra of **MP** in chloroform-*d*.

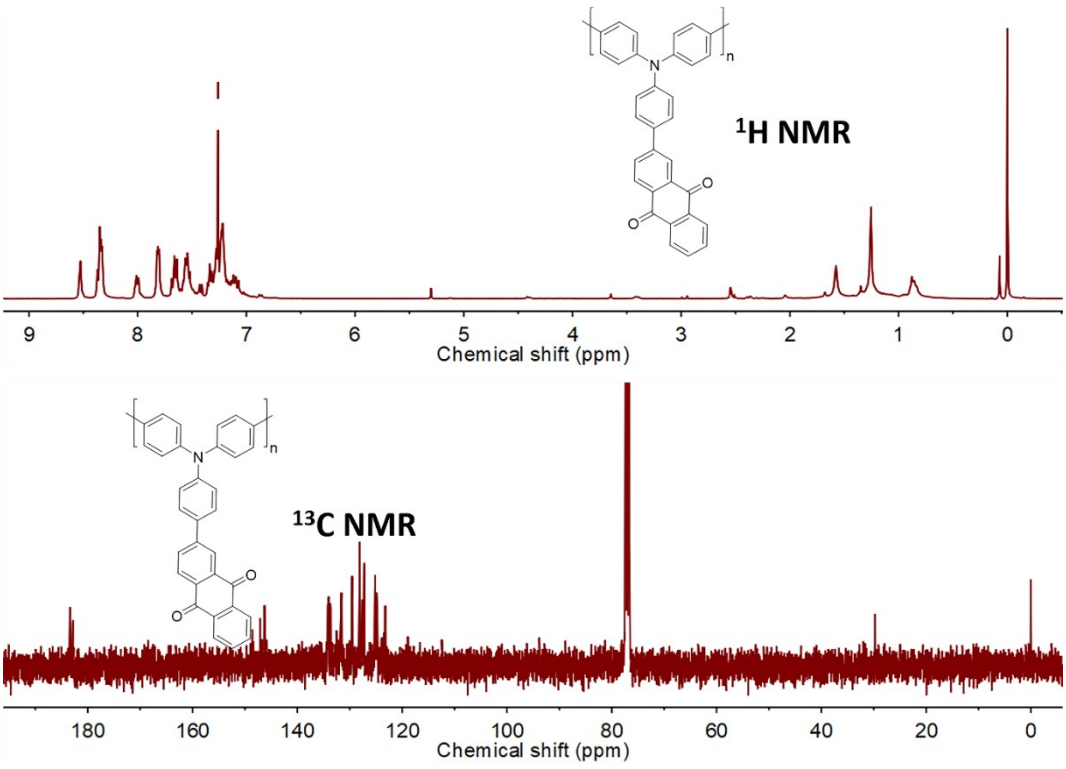

Fig. S10. NMR spectra of **SP** in chloroform-*d*.

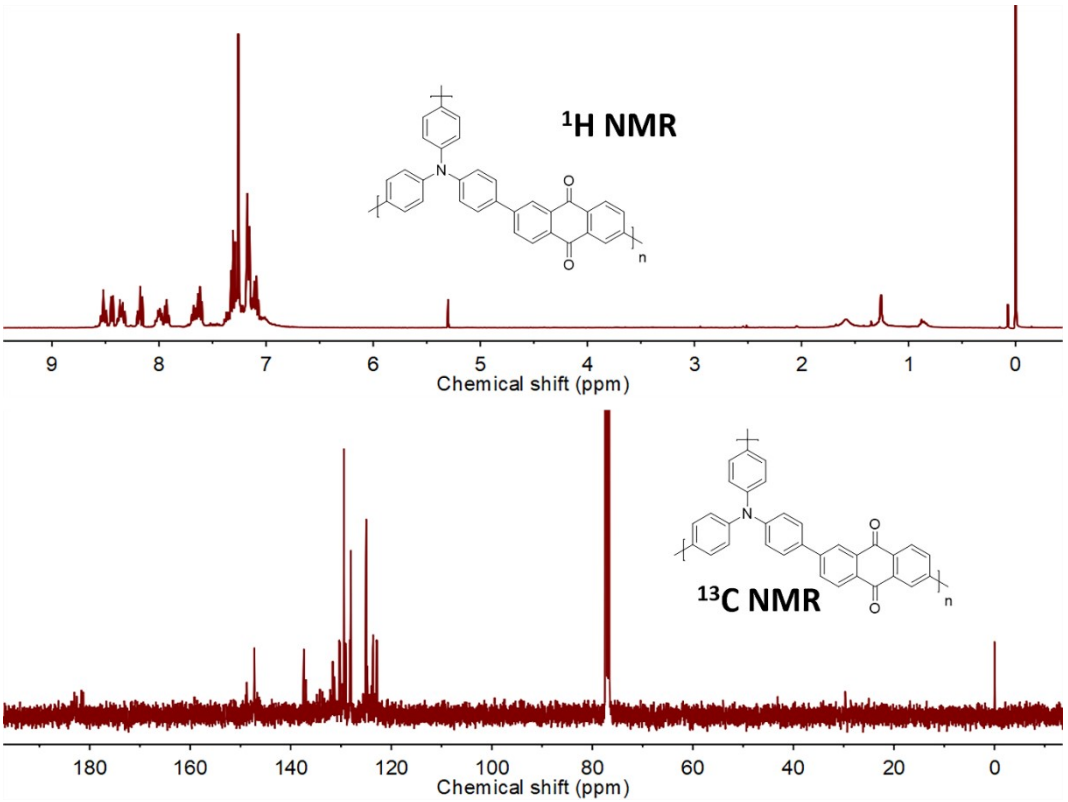

Fig. S11. NMR spectra of **HP** in chloroform-*d*.

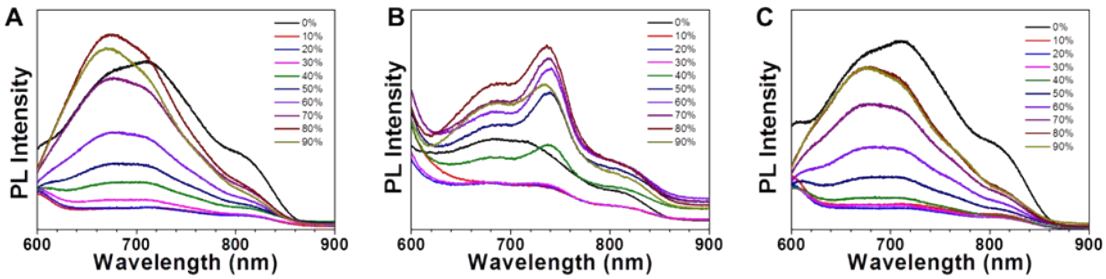

Fig. S12. Fluorescence spectra of MP (A), SP (B) and HP (C) in THF/water with different water fraction.

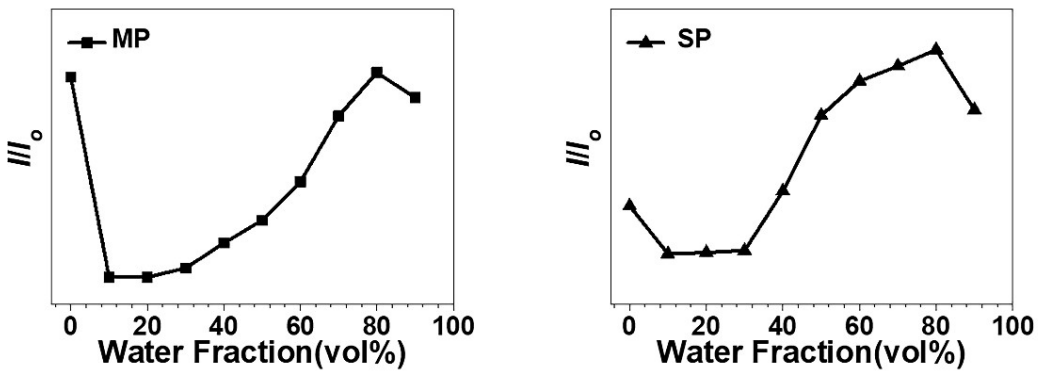

Fig. S13. The AIE properties of **MP**, **SP** and **HP**. I_0 and I are the peak PL intensities of AIEgens (10 μg mL^{-1}) in pure THF and THF/water mixtures, respectively.

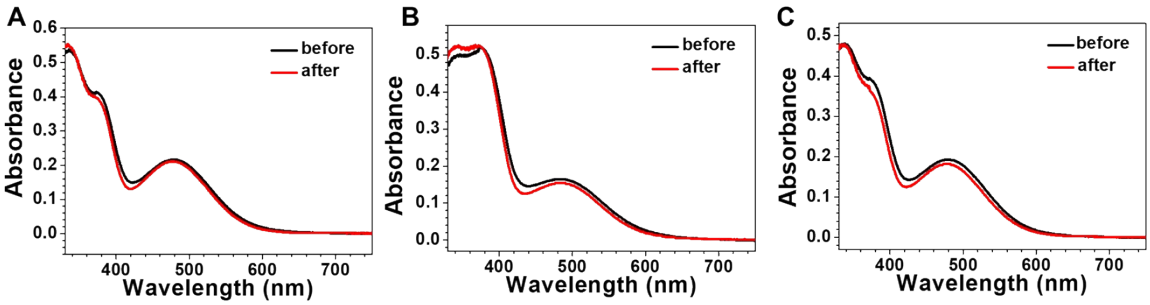

Fig. S14. UV-vis spectra of MP NPs (A), SP NPs (B) and HP NPs (C) in water before and after stored under normal conditions after 1 year. [MP, SP or HP] = 10 μg mL^{-1}.

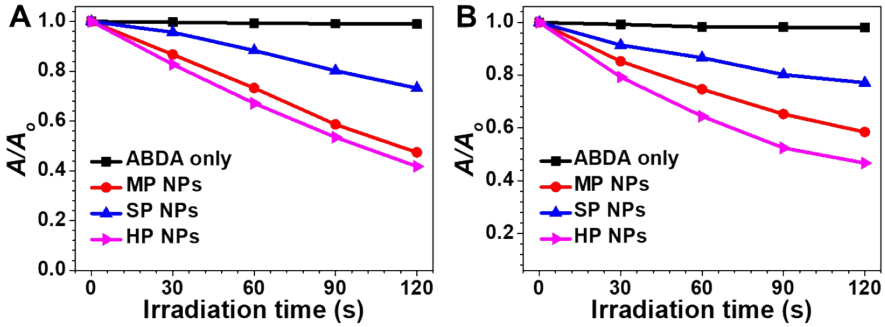

Fig. S15. Decomposition rates of ABDA in the presence of NPs under laser irradiation (100 mW cm^{-2}, 530 nm) for different times before (A) and after (B) stored under normal conditions after 1 year. Where A_0 and A are the absorbance of ABDA at 378 nm before and after irradiation. [MP, SP or HP] = 10 μg mL^{-1}, [ABDA] = 5 × 10^{-5} M.

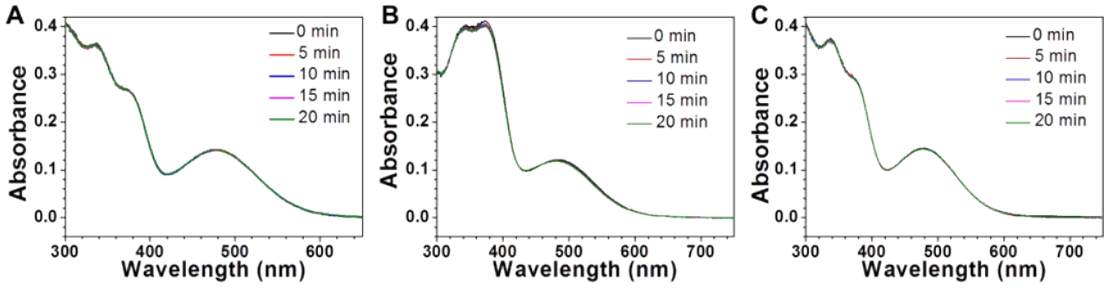

Fig. S16. UV-vis spectra of MP NPs (A), SP NPs (B) and HP NPs (C) in water under 530 nm laser irradiation (100 mW cm^{-2}) for 20 minutes. [MP, SP or HP] = 10 μg mL^{-1}.

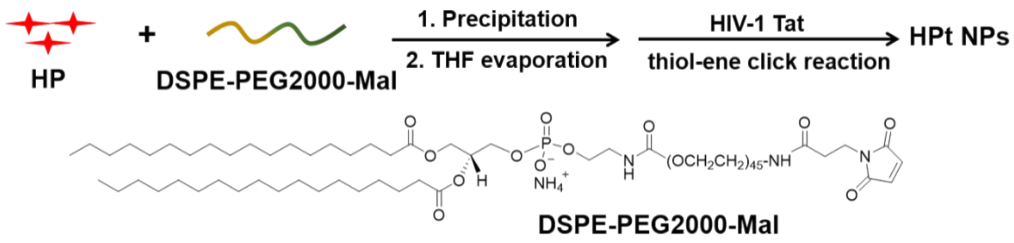

Fig. S17. The preparation of **HPt** NPs.

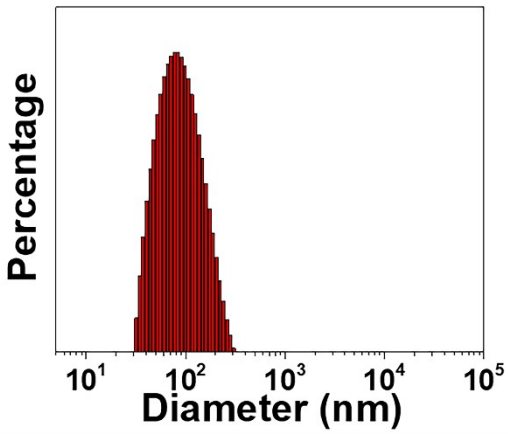

Fig. S18. The DLS results of **HPt** NPs.

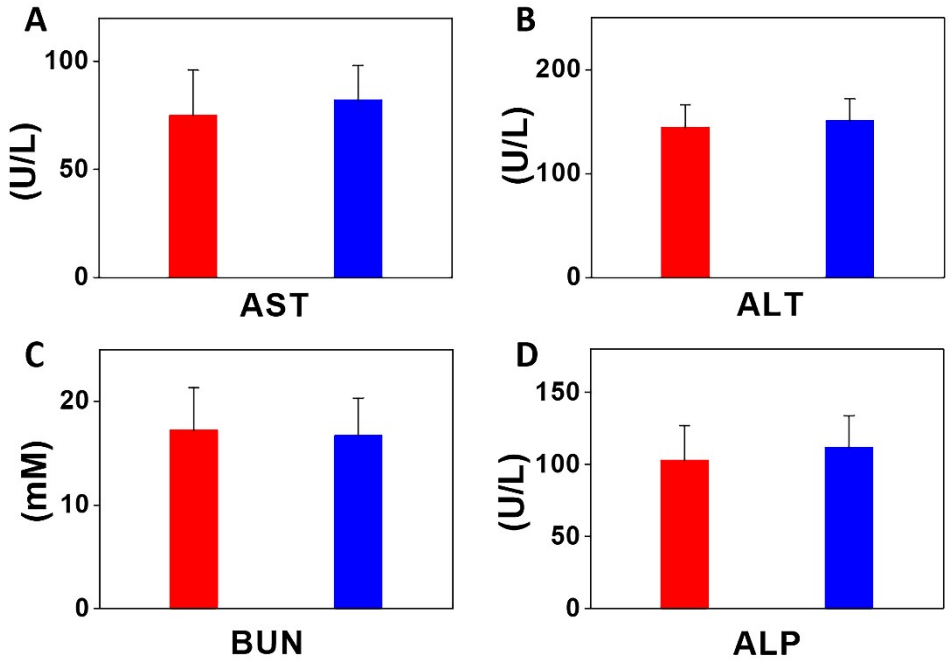

Fig. S19. Blood chemistry data of **HPt** NPs treated mice (red color) and control ones (blue color).

Supplementary Experimental Section

Materials and Instrumentation for Materials Synthesis

Anhydrous solvents (toluene and THF) were prepared by sodium treatment and distillation. Monomer **M3** were prepared by the same procedure reported in the literature[6], and its synthetic route presented in Scheme 1. All other solvents and reagents were obtained from commercial sources and used without further purification. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a 400 MHz Bruker AVANCE III spectrometer using tetramethylsilane (TMS; δ = 0 ppm) as the internal standard. High-resolution mass spectra (HRMS) were measured on a UHPLC/Q-TOF mass spectrometer. Molecular weight of the

polymers were determined by gel permeation chromatography (GPC) on a Malvern VISCOTEK TDA instrument with polystyrene as a standard and tetrahydrofuran as the eluent. UV-vis spectra were measured on a Shimadzu UV-2600 instrument. Photoluminescence spectra were measured by a Hitachi F-4700 fluorescence spectrophotometer. Hydrodynamic diameter and size distribution were measured on a Malvern Nano ZS Dynamic Laser Scattering (DLS) and Transmission Electron Microscopy (TEM) with Tecnai G2 F20 at room temperature. The instruments used for biological experiments are noted when used.

All animal procedures were performed under the Regulations for the Administration of Affairs Concerning Experimental Animals (Tianjin, revised in June 2004), which conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised in 1996).

Synthesis of Monomer M4

Monomer **M3** (609 mg, 1.0 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (635 mg, 2.5 mmol), potassium acetate (490 mg, 5.0 mmol) and $Pd(dppf)_2Cl_2$ (15 mg, 0.02 mmol) were dissolved in 1,4-dioxane (10 mL). The mixture was stirred for 12 h under an argon atmosphere at 80°C. Until the reaction completion as shown by TLC analyses, it was extracted with CH_2Cl_2, washed with water and brine, and dried over anhydrous sodium sulfate. The solvent was removed by evaporation, the crude product was purified by column chromatography on silica gel by using n-hexane/ethyl acetate (2/1, v/v) as the eluent to afford **M4** as a red solid (590 mg, 84 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (TMS, ppm): 8.51 (s, 1H, ArH), 8.36-8.32 (m, 3H, ArH), 8.01-7.98 (d, 1H, ArH), 7.81-7.79 (m, 2H, ArH), 7.73-7.71 (m, 4H, ArH), 7.64-7.61 (m, 2H, ArH), 7.21-7.19 (d, 2H, ArH), 7.13-7.11 (m, 4H, ArH), 1.34 (m, 24H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ (ppm): 149.72, 148.04, 146.22, 136.14, 134.26, 134.10, 133.72, 133.58, 133.38, 131.82, 128.20, 127.37, 125.01, 124.80, 123.57, 83.82, 24.79. HRMS (ESI), calcd for ($C_{44}H_{43}B_2NO_6$): *m*/*z* $[M+H]^+$: 703.3355; found: *m*/*z* 704.3364.

Synthesis of conjugated polymer MP

Monomer **M1** (249 mg, 0.50 mmol), monomer **M2** (183 mg, 0.50 mmol), tri(o-tolyl)phosphine (15 mg) and tris(dibenzylideneacetone)dipalladium(0) (7.5 mg) were dissolved in a mixture of toluene (8 mL) and 20% aqueous tetraethylammonium hydroxide (2 mL) under nitrogen. The mixture was refluxed with vigorous stirring for 40 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into methanol. The obtained solid was dissolved in THF, and the insoluble solid was filtered out. The filtrate was concentrated and precipitated into methanol, and the obtained solid was then washed with

acetone to yield **MP** as a red solid (77 mg, 34% yield). M_w = 9300, M_w/M_n = 1.24. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (TMS, ppm): 8.47-8.35 (ArH), 8.31-8.23 (ArH), 8.12-8.08 (ArH), 7.95-7.87 (ArH), 7.86-7.82 (ArH), 7.62-7.50 (ArH), 7.31-7.15 (ArH), 7.12-6.95 (ArH). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ (ppm): 181.99, 181.50, 180.61, 166.62, 147.70, 146.18, 145.21, 135.98, 132.99, 130.40, 129.07, 128.31, 127.89, 127.05, 124.03, 123.84, 122.57, 121.60.

Synthesis of polymer SP

The synthetic process of polymer **SP** was similar to **MP**. Monomer M3 (304 mg, 0.50 mmol), monomer M4 (352 mg, 0.50 mmol), tri(o-tolyl)phosphine (15 mg) and tris(dibenzylideneacetone) dipalladium(0) (7.5 mg) were dissolved in a mixture of toluene (8 mL) and 20% aqueous tetraethylammonium hydroxide (2 mL) under nitrogen. The mixture was refluxed with vigorous stirring for 40 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into methanol. The obtained solid was dissolved in THF, and the insoluble solid was filtered out. The filtrate was concentrated and precipitated into methanol, and the obtained solid was then washed with acetone to yield **SP** as a red solid (75 mg, 33 % yield). M_w = 4800, M_w/M_n = 1.15. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (TMS, ppm): 8.55-8.50 (ArH), 8.38-8.31 (ArH), 8.04-7.98 (ArH), 7.85-7.77 (ArH), 7.69-7.62 (ArH), 7.59-7.48 (ArH), 7.36-7.28 (ArH), 7.25-7.08 (ArH). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ (ppm): 183.50, 182.84, 148.61, 147.17, 146.18, 145.71, 134.34, 134.05, 133.56, 131.32, 129.39, 127.80, 127.62, 127.46, 125.23, 124.76, 123.00.

Synthesis of polymer HP

The synthetic process of polymer **HP** was similar to **MP**. Monomer M5 (311 mg, 0.50 mmol), monomer M2 (275 mg, 0.75 mmol), tri(o-tolyl)phosphine (23 mg) and tris(dibenzylideneacetone) dipalladium(0) (11.2 mg) were dissolved in a mixture of toluene (8 mL) and 20% aqueous tetraethylammonium hydroxide (2 mL) under nitrogen. The mixture was refluxed with vigorous stirring for 40 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into methanol. The obtained solid was dissolved in THF, and the insoluble solid was filtered out. The filtrate was concentrated and precipitated into methanol, and the obtained solid was then washed with acetone to yield **HP** as a red solid (87 mg, 39% yield). M_w = 13000, M_w/M_n = 1.36. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (TMS, ppm): 8.56-8.49 (ArH), 8.47-8.42 (ArH), 8.39-8.31 (ArH), 8.21-8.15 (ArH), 8.04-7.92 (ArH), 7.70-7.59 (ArH), 7.40-7.25 (ArH), 7.21-7.08 (ArH). ^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ (ppm): 148.72, 147.27, 137.38, 137.00, 131.56, 130.34, 130.24, 130.14, 129.45, 129.13, 129.07, 128.27, 128.02, 125.05, 123.69, 123.60, 122.91, 122.78.

Preparation of NPs

The THF (1 mL) mixture containing 1 mg of conjugated polymer (**MP**, **SP**, or **HP**) and 2 mg of DSPE-PEG2000 was poured into water with 10-fold dilution. The THF/water mixture was then sonicated for 3 min using a microtip ultrasound sonicator at 12 W output (XL2000, Misonix Incorporated, NY). After THF evaporation by stirring the obtained suspension in the fume hood overnight, NPs (10 mL, 0.1 mg mL^{-1} based on conjugated polymer) was obtained by filtration through a 0.2 μm syringe filter.

To obtain conjugated **HPt** NPs, The THF (1 mL) mixture containing 1 mg of **HP** and 2 mg of DSPE-PEG2000-Mal was poured into water with 10-fold dilution. The THF/water mixture was then sonicated for 3 min using a microtip ultrasound sonicator at 12 W output (XL2000, Misonix Incorporated, NY). After THF evaporation by stirring the obtained suspension in the fume hood overnight, NPs (10 mL, 0.1 mg mL^{-1} based on conjugated **HP**) was obtained by filtration through a 0.2 μm syringe filter. And then, 1 μmol of Tat peptide was added into the NPs solution, which was allowed to react for 12 h. The free Tat peptide was further removed by ultrafiltration.

O_2^- Generation Efficiecny Evaluation

DHR123 (5×10^{-6} M) was added to the water solution of **MP** NPs (1×10^{-4} mg mL^{-1} based on the repeat unit of **MP**), which were further irradiated with a 530 nm clinical laser. The fluorescence signal of DHR123 was monitored at different time intervals in a range of 500-620 nm with the excitation wavelength at 495 nm after the solution was irradiated 530 nm clinical laser (100 mW cm^{-2}). The fluorescence intensity at 526 nm was recorded to indicate the generation rate of O_2^-.

The other PSs were measured by the same procedure.

·OH Generation Efficiecny Evaluation

HPF (5×10^{-6} M) was added to the water solution of **MP** NPs (1×10^{-4} mg mL^{-1} based on the repeat unit of **MP**), which were further irradiated with a 530 nm clinical laser. The fluorescence signal of HPF was monitored at different time intervals in a range of 500-620 nm with the excitation wavelength at 490 nm after the solution was irradiated 530 nm clinical laser (100 mW cm^{-2}). The fluorescence intensity at 515 nm was recorded to indicate the generation rate of ·OH

The other PSs were measured by the same procedure.

$^1O_2$ Generation Efficiecny Evaluation

ABDA (5×10^{-5} M) was added to the water solution of **MP** NPs (1×10^{-4} mg mL^{-1} based on the repeat unit of **MP**), which were further irradiated with a 530 nm clinical laser (100 mW

cm^{-2}). The absorbance of ABDA at 378 nm were recorded to indicate the generation rate of $^1O_2$

The other PSs were measured by the same procedure.

Cell Culture

CT-26 cancer cells were cultured in RPMI-1640 medium (GIBCO) supplemented with 10% FBS (GIBCO) and PS (10 U/mL penicillin and 10 mg mL^{-1} streptomycin). The cells were maintained in an atmosphere of 5% CO_2 and 95% humidified air at 37 °C.

Cell Imaging

CT-26 cancer cells were seeded and cultured in glass bottom dish for 12 h. **HPt** NPs were then added into medium and incubated with cancer cells for 6 h. The fluorescent signal of **HPt** NPs within CT-26 cells were captured by confocal laser scanning microscopy (CLSM, Leica TSC SP8, Germany) with excitation at 620 nm and signal collection from 600 to 750 nm.

For intracellular ROS production imaging, **HPt** NPs labeled CT-26 cells were incubated with ROS indicator dichlorofluoresceindiacetate (DCFDA) for 15 min, followed by 530 laser exposure for 0.5 min. The post treatment cells were then imaged by CLSM.

Cell viability test

The CT-26 cancer cells were seeded in 96 well plates at a density of 3000 cells in 200 μL per well for 12 h. **HPt** NPs at different concentrations were added into the cell culture medium separately. Cells were further incubated with **HPt** NPs for 6 h, followed by 530 nm laser irradiation for 5 min. After light treatment, MTT (40 μL, 1 mg mL^{-1}) was added into medium for 3 h. The media was removed, and DMSO (100 μL) was added into each well and gently shaken for 10 min at room temperature. The absorbance of MTT at 550 nm was measured using a SpectraMax M5 Microplate Reader. Cell viability was measured by the ratio of the absorbance of the cells incubated with different NPs to that of the cells incubated with normal culture medium.

Colon Cancer Mouse Model

6-week-old BALB/c nude mice (obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China)) were used to establish breast cancer mouse model. CT-26 cancer cells (1×10^6) suspended in 30 μL of saline were injected subcutaneously into the left flank of the mouse. Tumors were grown 2-3 weeks before being used for PDT.

***In vivo* PDT**

After intratumoral injection of saline (30 μL) (n = 5) or **HPt** NPs (30 μL, 1 mg mL^{-1} based on **HP**) (n = 5) at postinjection time of 8 h, the tumor site of BALB/c mice was exposed to 530 nm laser for 10 min. The tumor size was measured every other day and calculated as follows: volume = (tumor length) × $(\text{tumor width})^2/2$.

Histological analysis

At day 14 after phototherapy, the mice in different groups were sacrificed and the tumor tissues were collected and fixed in 4% paraformaldehyde, which were then embedded into paraffin, sliced at a thickness of 5 μm. Slices were stained with hematoxylin and eosin (H&E) and imaged by optical microscopy and assessed by 3 independent pathologists. For the apoptosis staining, the tumor sections were stained following the manual instruction of In Situ Cell Death Detection Kit (Roche Applied Science) and imaged using CLSM (Leica TSC SP8, Germany).

References

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