Supplementary Information (SI) for Journal of Materials Chemistry B.

Smart Polydopamine Nanodots-Knotted Hydrogels for Photodynamic Tumor Therapy

Zhan Li[a], Bo Liang[b], Jianhua Zhang[a], Tianyou Wang[a], Pengyu Liu[a], Zhipeng Gu[a], Lei Yang[a], Wei Zhang[c], Yiwen Li[a, *] and Zhen Yang[d, *]

a. College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, China.

b. School of Materials Science and Engineering, Hubei University of Automotive Technology, Shiyan 442002, China

c. Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA 1526

d. Department of Radiology, Huaxi MR Research Center, Functional and Molecular Imaging Key Laboratory of Sichuan Province, National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu 610041, China.

*Corresponding author. E-mail: ywli@scu.edu.cn (Y.L.); zhenyang@scu.edu.cn (Z.Y.);

Materials and Experiments.

Dopamine hydrochloride (DA·HCl, 98%), 5,10,15,20-tetrakis(4-aminophenyl) porphyrin (TAPP, 97%), Allyl bromide (98%), 4-Dimethylaminopyridine (DMAP, 98%), N,N'-Diisopropylcarbodiimide (DIPC, 98%), Sodium thiosulfate ($Na_2S_2O_3$, 99%), tert-Butyl chloroacetate (97%), Polyethyleneimine (PEI, Mw: 10000) and Sodium iodide (NaI, 99%) were all purchased from Energy Chemical. Potassium ethyl xanthogenate (95%), Trifluoromethanesulfonic anhydride (98%), 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%), 1,4-Dioxane (99.5%, SuperDry), Potassium acetate (99%), Bis(pinacolato)diboron (98%), Trifluoroacetic acid (TFA, 99%), 4-Acetylbenzoic acid (98%), 2′,4′-Dihydroxyacetophenone (98%), 1,1'-Bis(diphenylphosphino)ferrocene palladium dichloride [$Pd(dppf)Cl_2$, 98%], 1,1'-Bis(diphenylphosphino)ferrocene (dppf, 98%) were purchased from J&K Scientific. 8-ARM-PEG-OH (Mw: 10000) was obtained from Tansh-tech (China). NaOH (99%), Na_2CO_3 (98%), $NaHCO_3$ (98%), Na_2SO_4 (98%), Triethylamine (TEA, 99%), Molecular sieves 3A (particle size: 3 - 5 mm), Dichloromethane (DCM), N,N-Dimethylformamide (DMF), Ethyl acetate (EA), Ethyl Alcohol and Acetone were purchased from Kelong chemical (China).

The molecules to be tested were dissolved in the appropriate deuterated reagents, and then characterized by ^{1}H NMR (400 MHz) and ^{13}H NMR (100 MHz) using a Bruker AV III HD instrument, and TMS was selected as the internal standard. Deuterated reagents: $CDCl_3$ (Aldrich, 99.8 % D), the characteristic signal peaks of ^{1}H NMR and

^{13}C NMR are δ 7.66 ppm and δ77.00 ppm, respectively; D_2O (Aldrich, 99.8% D), the characteristic signal peak of ^{1}H NMR is δ 4 .79 ppm. The fluorescence spectra of samples were detected using an F98 fluorescence spectrophotometer (Lengguang technology, china), the slits width of excitation and emission was both set to 10 nm. In detail, 2 mL PDs solution was placed in a four-way quartz cuvette, and then the fluorescence emission spectra of samples were measured by setting a specific excitation wavelength. Transmission electron microscopy (TEM) images were performed on FEI transmission electron microscope (F20). The hydrodynamic diameter (Dh) and zeta potential of PDs was determined by dynamical light scattering (DLS, Malvern Nano ZS ZEN3690). The UV-Vis spectra of sample solution were measured by using a UV-Vis spectrophotometer (PerkinElmer Lambda 650 UV/Vis) with a wavelength range of 250-800 nm and a slit of 1 nm. ESR spectra were measured at room temperature on a Bruker EMXplus EPR spectrometer. The FT-IR spectra of KBr tablet samples were acquired using a NICOLET-560 spectrometer. The wavenumber was set over the range of 4000-500 cm^{-1}, the resolution was 1 cm^{-1}, and the number of scans was 32 times. Rheological data were measured with MCR302.

Preparation polyphenol-based nanodots (PDs). PD&TCPP: 50 mg DA·HCl and 4 mg TCPP were added to a 250 mL round bottom glass flask containing 45 mL deionized water, and stirred at room temperature until completely dissolved. Next, a 5 mL aqueous solution of PEI (10 mg/mL) was added to the above mixed solution, and resulting solution was stirred under dark and atmospheric conditions for 48 hours. Afterwards, the solution was centrifuged at 15,000 rpm for 20 minutes. The supernatant

was then transferred to a dialysis bag (molecular weight cutoff: 14,000 Da) and dialyzed repeatedly against deionized water. Finally, the dialysate was concentrated to the desired concentration and stored for later use. The yield of nanodots was 53%.

PD: 50 mg DA·HCl was added to a 250 mL round bottom glass flask containing 45 mL deionized water, and stirred at room temperature until completely dissolved. Next, a 5 mL aqueous solution of PEI (10 mg/mL) was added to the above mixed solution, and resulting solution was stirred under dark and atmospheric conditions for 48 hours. Afterwards, the solution was centrifugated at 15000 r/min for 20 min, then the supernatant was transferred to a dialysis bag (molecular weight cutoff 14000 Da) and dialyzed repeatedly with deionized water. Finally, the dialysate was concentrated to the desired concentration and stored for late use.

***In Vitro* Singlet Oxygen Evaluation.** Singlet oxygen ($^1O_2$) generation was quantified by monitoring the absorbance decrease of 1,2-diphenylisobenzofuran (DPBF) at 416 nm. A 4 mM DPBF solution in DMF was prepared and mixed with PD&TCPP to achieve a final PD&TCPP concentration of 0.2 mg/mL. The mixture was subjected to intermittent irradiation with a 450 nm laser (1.5 W cm^{-2}) at 2-minute intervals. UV-Vis spectroscopy was employed to measure the absorbance at 416 nm at each time point.

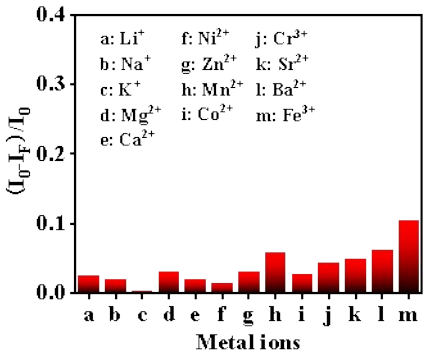
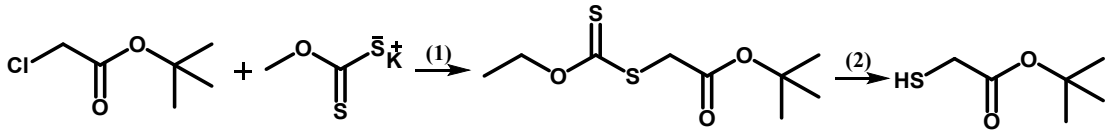


Fig. S1 The effect of different metal ions on the fluorescence intensity of PD&TCPP.

Synthetic Procedures



Scheme S1 Synthesis scheme of tert-butyl 2-mercaptoacetate.

Synthesis of tert-butyl 2-((ethoxy carbonothioyl)thio)acetate: Potassium ethyl xanthogenate (30 g, 205.5 mmol) was added to a 250 mL round bottom glass flask containing 120 mL acetone and stirred until complete dispersion. tert-Butyl chloroacetate (28 g, 186.8 mmol) was slowly added into the above mixed solution with a pressure-equalizing addition funnel, and reacted at room temperature for 24 hours. Afterward, the mixed reaction solution was filtered and evaporated under vacuum to remove in-solution solids and solution, and the obtained solid was redissolved with 300 mL ethyl ether, and then the solution was sequentially washed with 5% $NaHCO_3$ solution (3×100 mL) and H_2O (3 ×100 mL). Finally, the organic phase was collected and removed water with anhydrous Na_2SO_4, then filtered and evaporated under vacuum to obtain the products (37.5 g, 85%).

Synthesis of tert-butyl 2-mercaptoacetate: tert-Butyl 2-((ethoxy carbonothioyl) thio)

acetate (35 g, 148.3 mmol) was added to a 250 mL round bottom flask and stirred continuously. Under ice-bath conditions, ethanolamine (9.95 g, 163.1 mmol) was added into the above solution, and then reacted at room temperature for 24 hours. After the reaction, 300 mL EA was added to the reaction mixture and washed sequentially with 0.5 mM HCl solution (3×100 mL) and saturated NaCl solution (1×100 mL). The organic phase was collected and removed water with anhydrous Na_2SO_4, then filtered and evaporated under vacuum to obtain the crude product. Finally, the crude product was further purified by column chromatography (eluent: PE/EA=8:1) to obtain the liquid target samples (15.8 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 1.49 (m, 12H, -C(CH_3)$_3$), 1.95 (t, 1H, -SH), 3.19 (d, 2H, -CH_2SH); 13C NMR (100 MHz, $CDCl_3$, ppm, δ) : 169.93, 81.79, 34.66, 27.61.

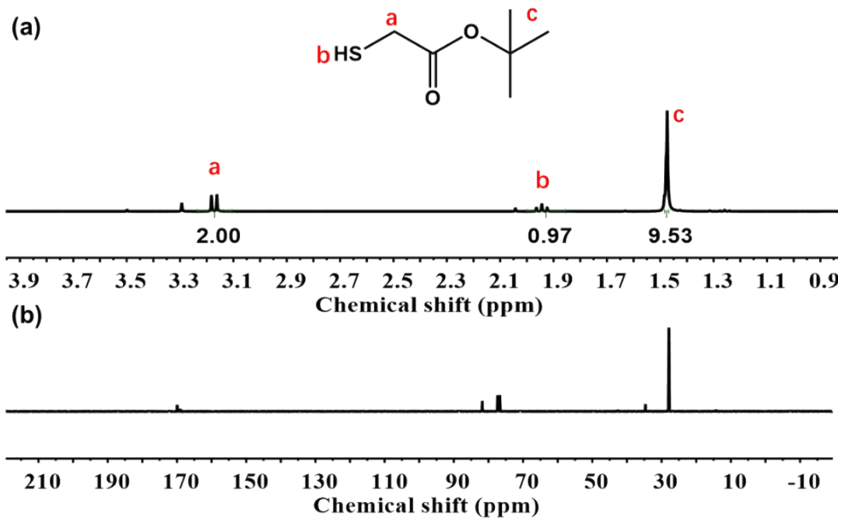
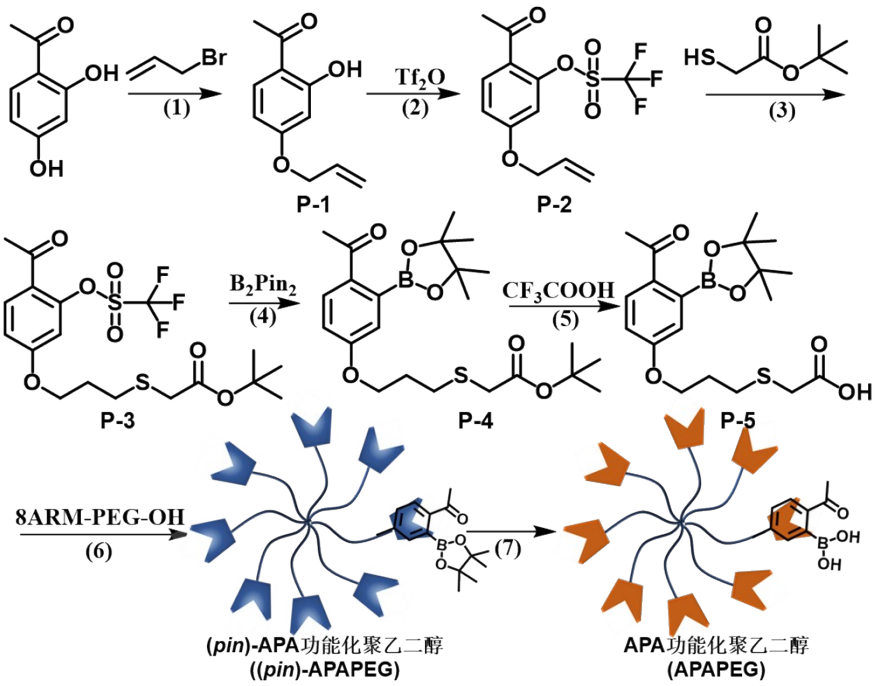


Fig. S2 (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of tert-butyl 2-mercaptoacetate.



Scheme S2 Synthesis scheme of (*pin*)-APAPEG and APAPEG.

P-1: Allyl bromide (25 mL, 289.3 mmol) and NaI (43.4 g, 289.3 mmol) were added to a round bottom flask containing 250 mL of acetone, and stirred and refluxed for 1 hour at 60 °C. Then the 2′,4′-Dihydroxyacetophenone (29.3 g, 192.9 mmol), K_2CO_3 (26.1 g, 189.1 mmol) and 150 mL acetone were sequentially added into the above reaction system, and the reflux reaction was continued for 20 hours. After the reaction, the acetone was removed by rotary evaporation under reduced pressure, the residue was dissolved with 250 mL deionized water. Then it was extracted with ethyl ether (3×200 mL), and the organic phase was collected and sequentially washed with 5% Na_2SO_4 (2×150 mL) and saturated NaCl (150 mL). The organic phase was evaporated under vacuum to obtain the crude product. And the crude product was further purified by column chromatography (eluent: PE/EA=6:1) to obtain the liquid target samples (27.5 g, 75%).^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 2.54 (s, 3H, - C$\underline{H}_3$), 4.56 (dt, 2H, -

OC<u>H</u>$_2$CH=CH$_2$), 5.37 (m, 2H, 2H, -OCH$_2$CH=C<u>H</u>$_2$), 6.02 (m, 1H, -OCH$_2$C<u>H</u>=CH$_2$), 6.43 (m, 2H, -C<u>H</u>=C-), 7.62 (d, 1H, -C<u>H</u>=C-); ^{13}C NMR (100 MHz, $CDCl_3$, ppm, δ) :202.57, 165.16, 132.32, 118.35, 113.98, 107,98, 101.67, 68.95, 26.21.

P-2: P-1 (25 g, 131.6 mmol) and TEA (54.8 mL, 394.8 mmol) added to a round bottom flask containing 250 mL anhydrous DCM. The mixed system was stirred and reacted at -78 ℃ for 15 min, and then the trifluoromethanesulfonic anhydride (47.5 mL, 289.5 mmol) was slowly added using pressure-equalizing addition funnel. After transferring to room temperature, the reaction was continued in inert atmosphere for 0.5 hours, and then the saturated 150 mL Na_2CO_3 was added to terminate the reaction. The organic phase was collected and evaporated to obtain crude product. Then the crude product was further purified by column chromatography (eluent: PE/EA=5:1) to obtain the liquid target samples (34.7 g, 82%).^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 2.59 (s, 3H, -C<u>H</u>$_3$), 4.62 (dt, 2H, -OC<u>H</u>$_2$CH=CH$_2$), 5.41 (m, 2H, 2H, -OCH$_2$CH=C<u>H</u>$_2$), 6.02 (m, 1H, -OCH$_2$C<u>H</u>=CH$_2$), 6.84/6.98 (m, 2H, -C<u>H</u>=C-), 7.82 (d, 1H, -C<u>H</u>=C-); ^{13}C NMR (100 MHz, $CDCl_3$, ppm, δ) :195.01, 162.58, 148.47, 132.69, 131.56, 124.11, 118.98, 114.06, 109.58, 69.60, 29.12.

P-3: P-2 (20 g, 62.1 mmol), tert-butyl 2-mercaptoacetate (13.7 g, 93.2 mmol) and DMPA (1.6 g, 6.2 mmol) were added to a beaker containing 300 mL THF. After stirring and dissolving, the mixed solution was irradiated with a 365 nm ultraviolet lamp (LUYOR-3109) for 40 min. Following the solvent was evaporated under reduce pressure and further purified by column chromatography (eluent: PE/EA=5:1) to obtain the solid target samples (25.7 g, 88%).^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 1.46 (s,

9H, -C(C$\underline{H}_3$)$_3$), 2.13 (m, 2H, -OCH$_2$C$\underline{H}_2$CH$_2$S-), 2.60 (s, 3H, - C$\underline{H}_3$), 2.84 (t, 2H, -OCH$_2$CH$_2$C$\underline{H}_2$S-), 3.15 (s, 2H, -SC$\underline{H}_2$O-), 4.14 (t, 2H, -OC$\underline{H}_2$CH$_2$CH$_2$S-), 6.84/6.98 (m, 2H, -C$\underline{H}$=C-), 7.82 (d, 1H, -C$\underline{H}$=C-); ^{13}C NMR (100 MHz, $CDCl_3$, ppm, δ) :194.99, 169.50, 162.85, 148.49, 132.75, 124.10, 113.63, 109.41, 81.74, 67.04, 39.94, 29.15, 28.83.

P-4: P-3 (5 g, 10.6 mmol), bis(pinacolato)diboron (6.5 g, 25.4 mmol), Pd(dppf)Cl_2 (660 mg, 0.883 mmol), dppf (490 mg, 0.883 mmol), potassium acetate (3.2 g, 31.8) and 3A molecular sieve (1 g) were added to a 100 mL Schlenk flask containing 30 mL dioxane and stirred to disperse completely. The reaction mixture was degassed with three freeze-pump-thaw cycles and then stirred at 102 ℃ under N_2 atmosphere for 3 hours. After the reaction, the organic phase was filtered and rotary evaporated under reduce pressure to obtain the crude product. And then the crude product was further purified by column chromatography (eluent: PE/EA=5:1) to obtain the solid target samples (3.4 g, 72%).

P-5: P-4 (3.4 g) was added to a 50 mL round bottom flask containing 10 mL DCM and 5 mL TFA, and then stirred at room temperature for 6 hours. After the reaction, the reaction was diluted with 200 mL DCM, and then washed with deionized water (3×100 mL) and saturated NaCl (1×100 mL). The organic phase was collected, dried with anhydrous Na_2SO_4, filtered, and rotary evaporated under reduced pressure to obtain the target solid product (2.6 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 1.42 (s, 12H, -C(C$\underline{H}_3$)$_2$), 2.09 (m, 2H, -OCH$_2$C$\underline{H}_2$CH$_2$S-), 2.57 (s, 3H, - C$\underline{H}_3$), 2.84 (t, 2H, -OCH$_2$CH$_2$C$\underline{H}_2$S-), 3.24 (s, 2H, -SC$\underline{H}_2$O-), 4.14 (t, 2H, -OC$\underline{H}_2$CH$_2$CH$_2$S-), 6.84/6.98 (m, 2H, -C$\underline{H}$=C-), 7.78 (d, 1H, -C$\underline{H}$=C-); ^{13}C NMR (100 MHz, $CDCl_3$, ppm, δ) :199.38,

174.68, 162.82, 133.45, 130.69, 117.88, 114.42, 83.72, 66.17, 33.51, 29.15, 28.47, 24.89, 24.43.

(*pin*)-APAPEG: 8Arm-PEG-OH (2 g, 0.2 mmol), P-5 (940 mg, 2.4 mmol) and DMAP (439 mg, 3.6 mmol) were added to a 25 mL round bottom flask containing 6 mL anhydrous DCM and covered with rubber stopper. After the reaction mixture was stirred for 10 min under ice-bath conditions, DIPC (0.56 mL, 3.6 mmol) was added with a syringe, and then reacted at room temperature for 24 hours. After the reaction, the reaction system was concentrated, and the precipitation was repeated three times in a mixed solution of ice isopropanol/diethyl ether (v/v=1:2), and then dried under reduced pressure to obtain the solid target sample (2.2 g). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 1.42 (s, 12H, -C(C$\underline{H}_3$)$_2$), 2. 09 (m, 2H, -OCH$_2$C$\underline{H}_2$CH$_2$S-), 2.54 (s, 3H, - C$\underline{H}_3$), 2.84 (t, 2H, -OCH$_2$CH$_2$C$\underline{H}_2$S-), 3.27 (s, 2H, -SC$\underline{H}_2$O-), 3.65 (m, -OC$\underline{H}_2$C$\underline{H}_2$-), 4.12 (t, 2H, -OC$\underline{H}_2$CH$_2$CH$_2$S-), 6.84/6.98 (m, 2H, -C$\underline{H}$=C-), 7.78 (d, 1H, -C$\underline{H}$=C-).

APAPEG: (*pin*)-APAPEG (400 mg, 0.02 mmol) and phenylboronic acid (390 mg, 3.2 mmol) were added to a 20 mL glass vial containing 5 mL acetonitrile/HCl (1 M) (v/v=9:1) mixed solution, and stirred at room temperature for 24 hours. Subsequently, the reaction mixture was transferred to a dialysis bag (1000 MWCO), and after repeated dialysis with CH_3OH for three times, the samples in the dialysis bag were collected and dried in a vacuum state to obtain a solid target sample (178 mg). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 1.9 (s, 3H, - C$\underline{H}_3$), 2. 11 (m, 2H, -OCH$_2$C$\underline{H}_2$CH$_2$S-), 2.82 (t, 2H, -OCH$_2$CH$_2$C$\underline{H}_2$S-), 3.28 (s, 2H, -SC$\underline{H}_2$O-), 3.62 (m, -OC$\underline{H}_2$C$\underline{H}_2$-), 4.18 (t, 2H, -OC$\underline{H}_2$CH$_2$CH$_2$S-), 6.94/7.1 (m, 2H, -C$\underline{H}$=C-), 8.00 (d, 1H, -C$\underline{H}$=C-).

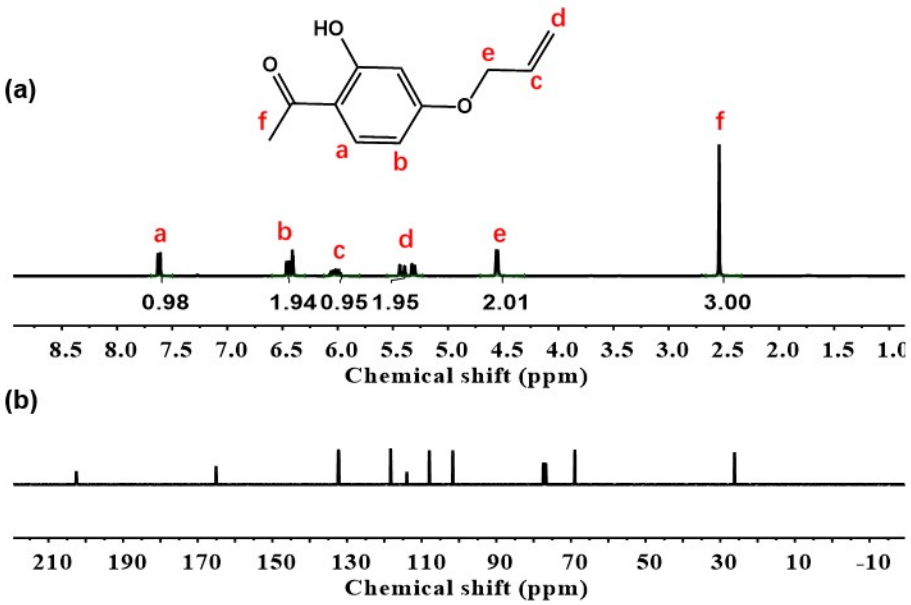


Fig. S3 (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of P-1.

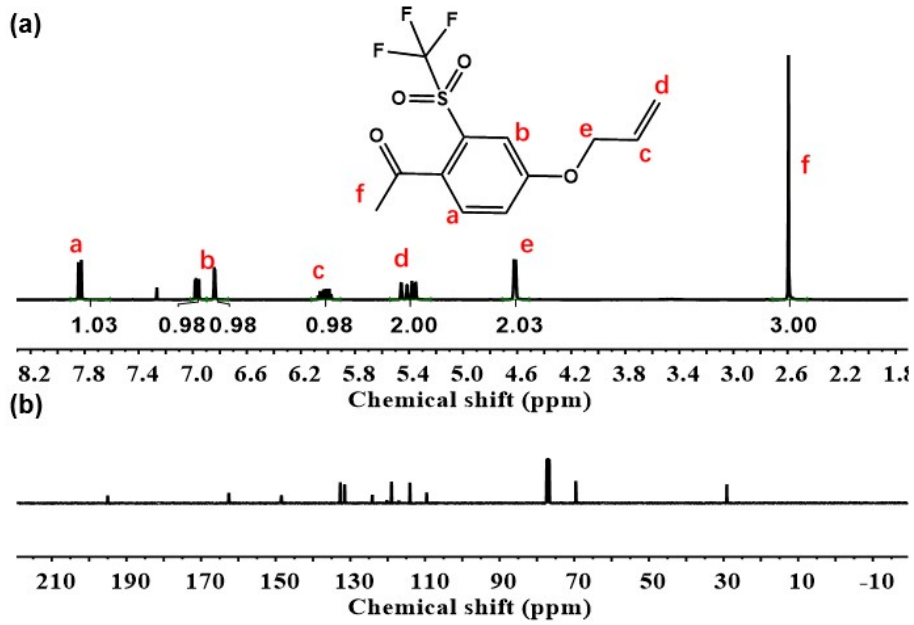


Fig. S4 (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of P-2.

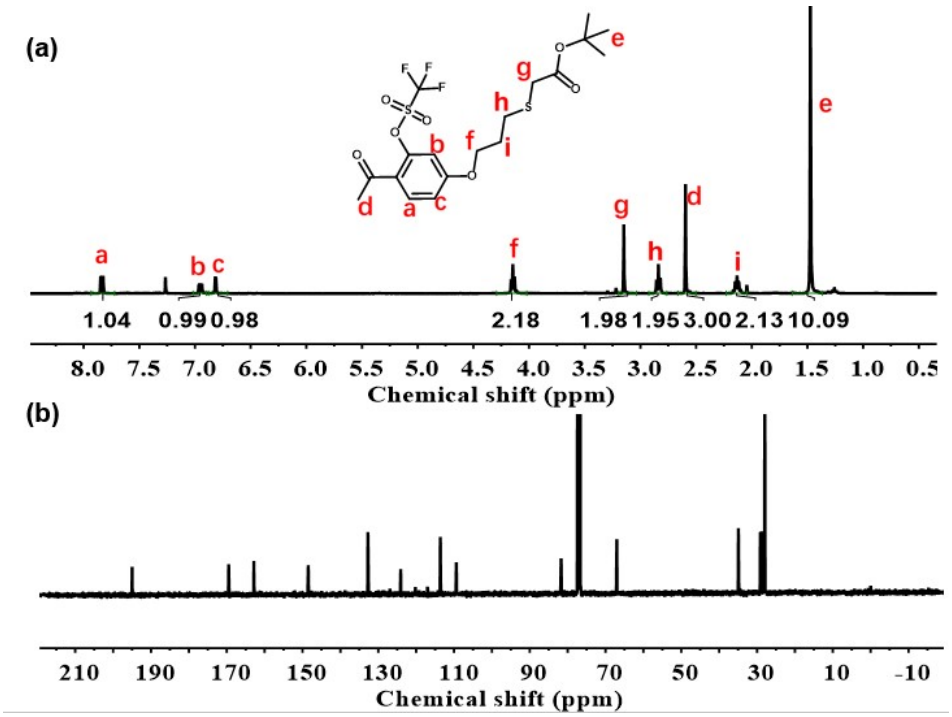


Fig. S5 (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of P-3.

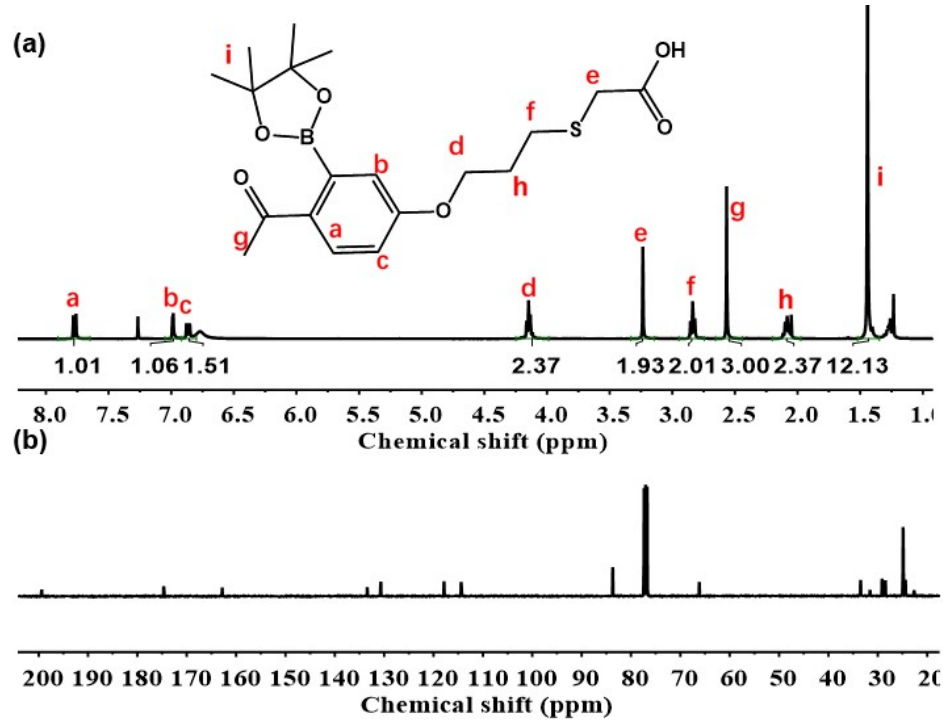


Fig. S6 (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of P-5.

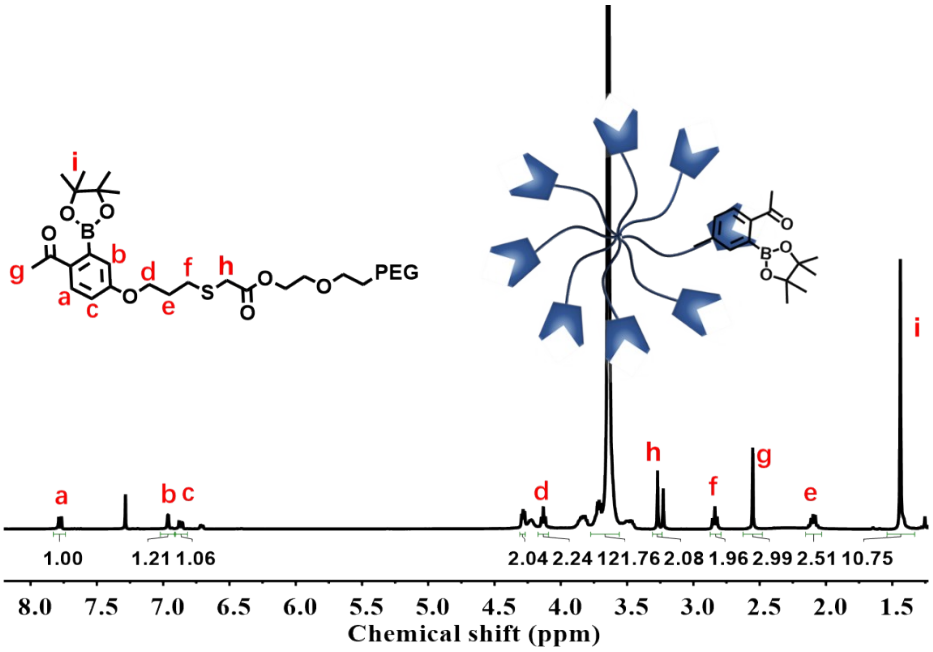


Fig. S7 ^{1}H NMR spectra of (*pin*)-APAPEG.

The Preparation of (pin)-APT hydrogel: A series of PD&TCPP (8, 12, 16, 20, 24, 28, 32, 36 and 40 mg/mL) and (pin)-APAPEG (50, 75, 100, 125, 150, 175, 200, 225 mg/mL) solutions at different concentrations were configured in PBS buffer (pH=7.4). And then, the (pin)-APT hydrogel was prepared by rapid mixing of PD&TCPP and (pin)-APAPEG solutions with different concentrations at room temperature. Note that the typical gelation condition (PD&TCPP: 20 mg/mL; (pin)-APAPEG: 175 mg/mL) was used to prepare model hydrogel sample for further investigations. The control hydrogel samples were prepared by rapid mixing of PD&TCPP (20 mg/mL) and APAPEG or APEG (175 mg/mL) solutions at room temperature, separately.

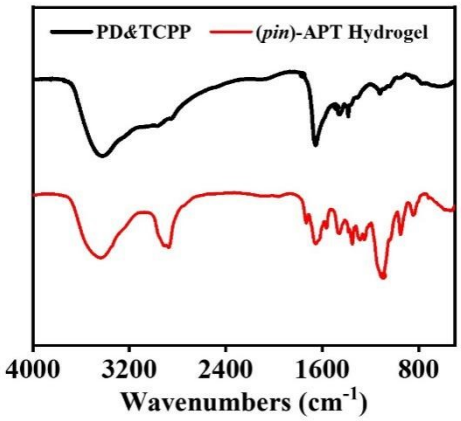


Fig. S8 The FT-IR spectra of the PD&TCPP and (*pin*)-APT hydrogel.

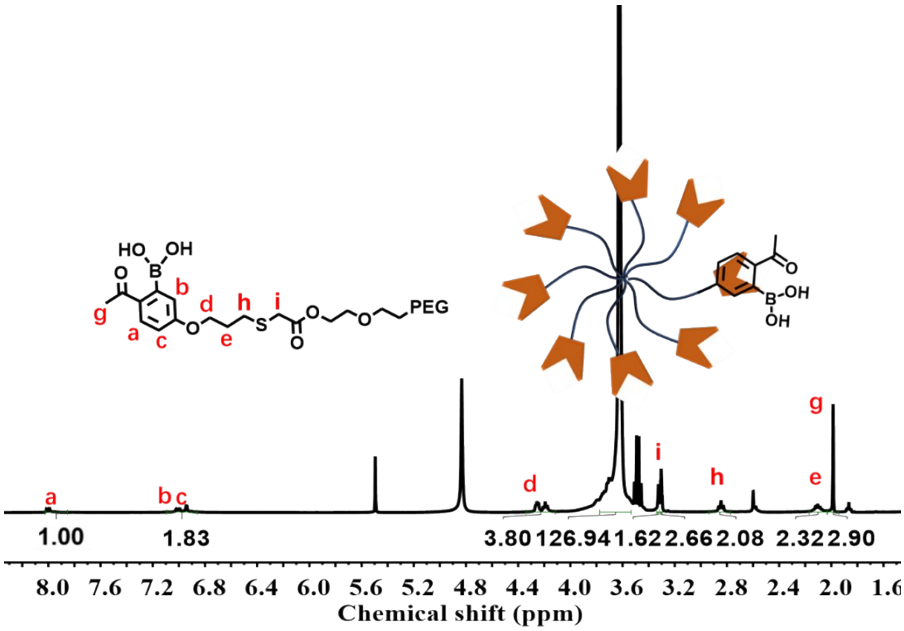
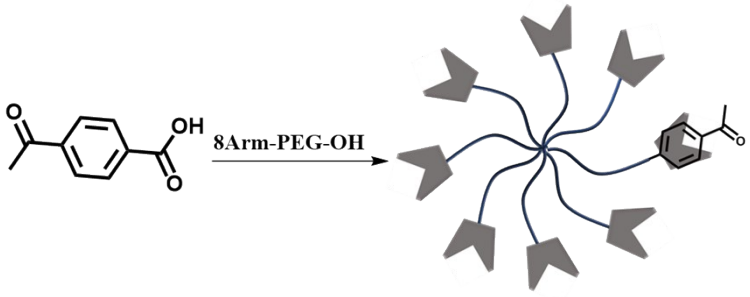


Fig. S9 ^{1}H NMR spectra of APAPEG.



Scheme S3 Synthesis scheme of APEG.

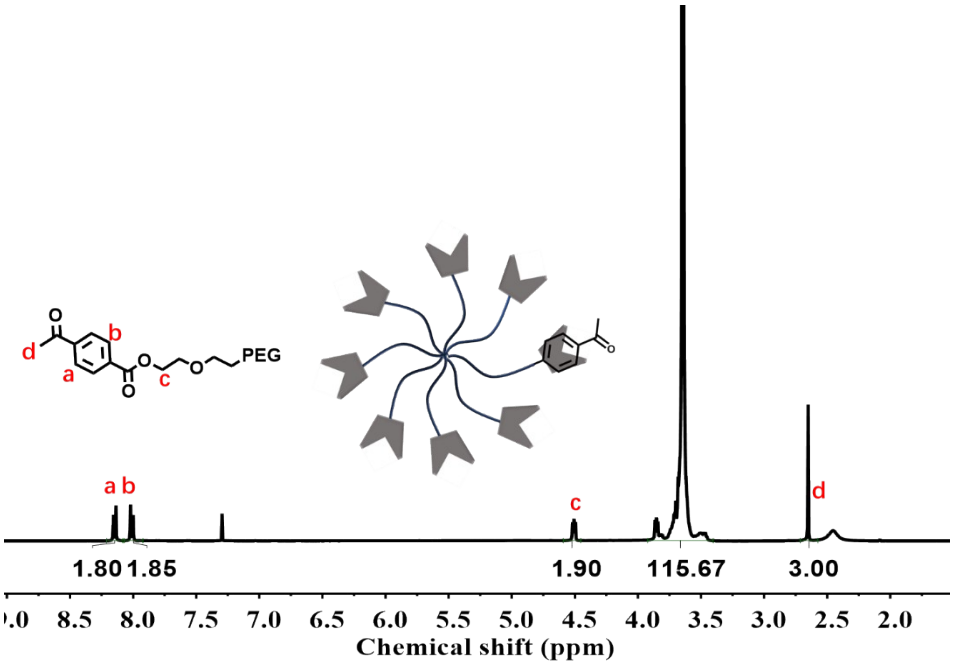


Fig. S10 ^{1}H NMR spectra of APEG.

APEG: 8Arm-PEG-OH (1 g, 0.1 mmol, Mw=10000), 4-Acetylbenzoic acid (196.8 mg, 1.2 mmol) and DMAP (220 mg, 1.8 mmol) were added to a 25 mL round bottom flask containing 6 mL anhydrous DCM and covered with rubber stopper. After the reaction mixture was stirred for 10 min under ice-bath conditions, DIPC (0.28 mL, 1.8 mmol) was added with a syringe, and then reacted at room temperature for 24 hours. After the reaction, the reaction system was concentrated, and the precipitation was repeated three times in a mixed solution of ice isopropanol/diethyl ether (v/v=1:2), and then dried under reduced pressure to obtain the solid target sample (985 mg). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 2.64 (s, 3H, $C\underline{H}_3CO$-), 3.65 (m, -$OC\underline{H}_2C\underline{H}_2$-), 4.50 (t, 2H, -$OCOC\underline{H}_2$-), 8.01/8.15 (dd, 4H, -$C\underline{H}$=CH-).

The Preparation of (pin)-AP hydrogel

(*pin*)-AP hydrogel: the (*pin*)-AP hydrogel was prepared by rapid mixing PDs (20 mg/mL) and (*pin*)-APAPEG (175 mg/mL) solutions at room temperature.

Cell Experiment

Biocompatibility experiment: NIH 3T3 cells and CT-26 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ℃ and 5% CO_2. Cell viability was assessed by MTT assay. In brief, NIH 3T3 cells or CT-26 cells was seeded into 96-well plates at a density of 2000 cells per well and incubated overnight. The cells were the incubated with different concentrations of hydrogel extract for another 24 hours, and the cells viability was assessed using the MTT assay. Additionally, NIH 3T3 cells or CT-26 cells was seeded into 24-well plates at a density of 50000 cells per well and subjected to the same procedure. The cells were stained with Calcein-AM/PI to visualize living and dead cells. The different concentrations of polyphenol-based nanodots (PDs and PD&TCPP) used in all cell experiments were obtained by diluting the model hydrogel (PD&TCPP: 20 mg/mL and (*pin*)-APAPEG: 175 mg/mL) degradation solution.

Cellular photodynamic therapy experiment. CT-26 cells were seeded into multiple 96-well plates at a density of 2000 cells per well and incubated overnight. Then medium containing different concentrations of PD&TCPP was added and incubated for another 6 hours. After incubation, the medium in the well plates was replaced with PBS and then irradiated with blue light (450 nm, 1.5 W/cm²) for a specific time. Subsequently, the medium was replaced with DMEM and incubated another 12 hours. Cell viability was assessed using MTT assay. Additionally, CT-26 cells were seeded into 24-well plates at a density of 50000 cells per well and subjected to the same procedure. The cells were stained with Calcein-AM/PI to visualize living and dead cells. The various

concentrations of PD&TCPP test solutions were prepared based on the concentration of nanodots in the degradation solution after the completely degraded model hydrogel (PD&TCPP: 20 mg/mL and (*pin*)-APAPEG: 175 mg/mL).

***In Vivo* Anticancer Therapy.** The mouse experiment was approved by the Medical Ethics Committee of West China Hospital of Sichuan University (Approval No.: SCU42-2407-02). Thirty BALB/c mice (6-8 weeks; body weight: 20g) were purchased from Shuoda Biotech Co., Ltd. (Sichuan, China) and adapted for 7 days. The hair in the axillary region of the mice was shaved, followed by subcutaneous incubation of CT-26 cells (10^6 cells in 100 μl of PBS per mouse) to establish a solid tumor model. One week later, the mice were randomly divided into 5 groups (Blank, (*pin*)-APT, Light, Light+(*pin*)-AP and Light+(*pin*)-APT groups), and 20 μL of PBS, (*pin*)-APT, PBS, (*pin*)-AP and (*pin*)-APT were separately injected into the tumor on the 8th and 11th. Meanwhile, the mice in the latter three groups (Light, +Light+(*pin*)-AP, Light+(*pin*)-APT) received 6 min of 450 nm light irradiation for 6 consecutive days. The tumor volume and body weight of the all mice were monitored daily for 13 days. The formula for calculating tumor volume is: $V=W2\times L/2$, where W and L represent the width and length of the tumor, respectively. Finally, the mice were euthanized, and the histological analysis was performed using H&E and TUNEL staining, as well as immunohistochemical analysis was conducted using KI-67 and HIF-1α staining.

Statistical analysis: The data are statistically analyzed and presented using Prism software or origin. Results are expressed as mean ± standard deviation (SD). For comparisons involving more than two groups, one-way ANOVA with Tukey's multiple

comparison tests is applied, while t-tests were used for two-group comparisons. A P value of < 0.05 was considered statistically significant, with *** indicating a P value less than 0.0005.

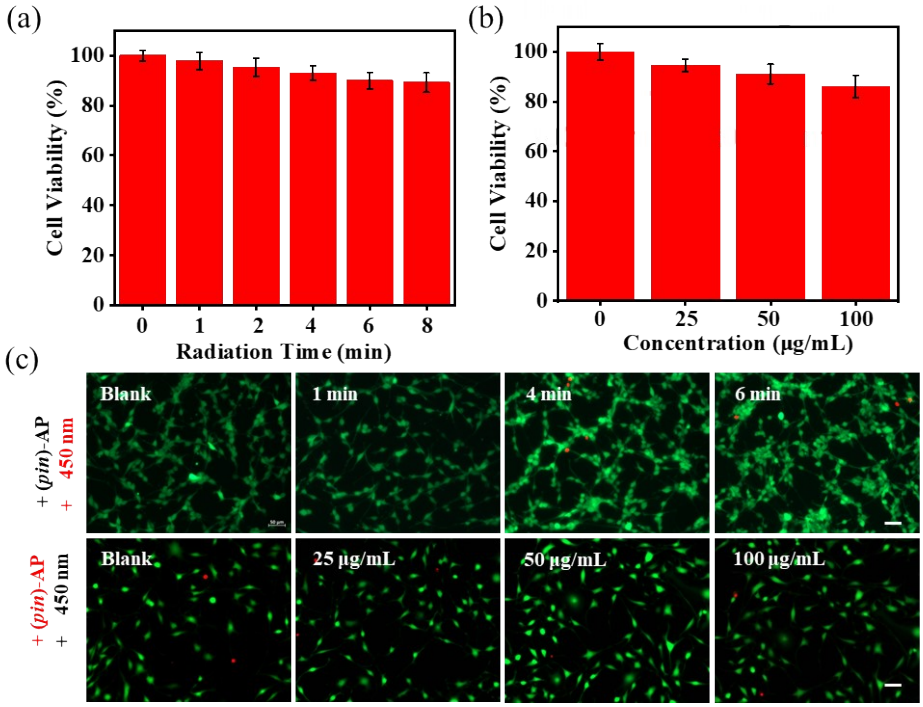


Fig. S11 (a) Cell viability of CT-26 cells co-incubated with PDs (50 μg/mL) exposed to 450 nm light for different times and then incubated for 12 hours; (b) Cell viability of CT-26 cells co-incubated different concentrations of PDs exposed to 450 nm light for 6 min and then incubated for 12 hours; (c) Fluorescence pictures of live-dead staining of NIH 3T3 and CT-26 cells of the corresponding experimental groups (Red markers indicate variables). The various concentrations of PDs test solutions were prepared based on the concentration of nanodots in the degradation solution after the completely degraded model hydrogel (PDs: 20 mg/mL and (*pin*)-APAPEG: 175 mg/mL). Scale bar: 200 μm.

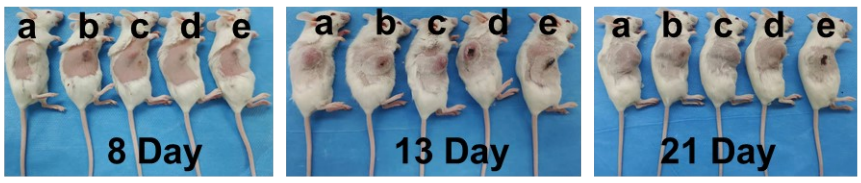


Fig. S12 Representative macroscopic images of tumor sizes in mice across treatment groups at days 8, 13, and 21 post-inoculations. a-e represent Blank, (*pin*)-APT, Light, Light+(*pin*)-AP, and Light+(*pin*)-APT groups, respectively.