Supporting Information

ROS-Sensitive Biomimetic Nanocarriers Modulate Tumor Hypoxia for Synergistic Photodynamic-Chemotherapy

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Materials and characterization.

Polymers were characterized by NMR Bruker AVANCE III 400 MHz spectrometer (Bruker Scientific Corporation Ltd., Switzerland) in deuterated reagent ($CDCl_3$ or DMSO-d_6). Molecular weights of the samples were determined by gel permeation chromatography (GPC, Waters, Milford, MA). The size and zeta potential measurements were carried out in aqueous solutions using a Malvern ZS90 dynamic light scattering (DLS) instrument (Malvern Instruments Ltd., England) with a He-Ne laser (633 nm) and 90 collecting optics. The data was analyzed using a Malvern Dispersion Technology Software 5.10. Morphology of formulations was examined by Tecnai TEM (FEI, Hillsboro, OR) at an accelerating voltage of 120 kV.

Synthesis of Monomer 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl acrylate (BBA).

Monomer BBA was synthesized according to previous literature (Scheme S1).[1] Briefly, 4-(hydroxymethyl) phenylboronic acid (30.4 g, 0.20 mol) and pinacol (23.5 g, 0.20 mol) were mixed into a round-bottle flask with freshly activated molecular sieves (4 Å, 10 g), and then toluene (240 mL) was added. The reaction was carried out at 120 ºC for 24 h until the reaction system became clear. The reaction solution was filtered and evaporated to remove toluene under reduced pressure, and white powder (P1) was produced with a yield of 90%.

P1 (11.7 g, 50 mmol) was dissolved in anhydrous dichloromethane (60 mL), followed by adding TEA (6.1 g, 60 mmol). After cooling to ~0 ºC, 5.4 g (60 mmol) of acryloyl chloride in ~5 mL dried dichloromethane was added dropwise within 1 h.

Then, the reaction mixture was warmed to room temperature, stirred for 12 h, and filtered. The filtrate was concentrated on a rotary evaporator and diluted by ethyl acetate, and washed with saturated sodium chloride thrice. After drying by $MgSO_4$, the organic solution was concentrated and purified by silica column chromatography using hexane and ethyl acetate (v/v = 20/1) as the eluent. The monomer BBA was obtained as colorless crystal with a yield of 74%, and was characterized by ^{1}H and ^{13}C NMR.

Synthesis of Block Copolymers mPEG-*b*-PBBA.

mPEG-*b*-PBBA was prepared *via* reversible addition-fragmentation chain transfer (RAFT) polymerization of BBA monomers using mPEG-based macro-RAFT agent as the chain transfer agent. Briefly, the macro-RAFT agent (mPEG-CPAD) was synthesized by the esterification reaction of methoxy poly(ethylene glycol) (mPEG-OH, Mn = 5000 g mol^{-1}) and 4-(4-cyanopentanoic acid) dithiobenzoate (CPAD). The CPAD conjugation efficiency with mPEG-OH were calculated to be approximately 83.5%, according to ^{1}H NMR spectrum. Then, mPEG-CPAD (200 mg, 0.036 mmol), AIBN (3 mg, 0.018 mmol), BBA monomer (524 mg, 1.8 mmol), and 1,4-dioxane (2 mL) were charged into a 10 mL flask. The reaction system was degassed by three freeze-pump-thaw cycles and sealed under vacuum. Then, the flask was placed in a preheated oil bath at 75 °C. After 12 h, the reaction mixture was added dropwise into hexane. The precipitate was then collected and dried in a vacuum oven, yielding a pink solid (yield of 50.1%). The degree of polymerization (DP) of PBBA segments was 33 determined by ^{1}H NMR analysis.

The Synthesis of Octanoic Tirapazamine Prodrug (TPZp). Octanoic acid (0.193 g,

1.12 mmol) was dissolved in dichloromethane (20 mL), followed by addition of triethylamine (0.6 mL), and then N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 0.215 g, 1.12 mmol), N-hydroxysuccinimide (NHS, 0.128 g, 1.12 mmol), and TPZ (0.200g, 1.12 mmol) were added. The resulting mixture was stirred overnight. After treatment, the residue was purified by silicon column chromatography to give octanoic tirapazamine prodrug (TPZp, 30% yield).

Extraction of RBCs.

RBCs were first extracted following previously published protocols with modifications.[2] Whole blood was first withdrawn from female Balb/c mice (6-8 w) obtained from Vital River Laboratories (Beijing, China) by removing eyeball using a tube containing a 100 μL of heparin solution (100 U/mL). The whole blood was then centrifuged (5000 rpm, 5 min, 4 °C), following which the serum and the buffy coat were carefully removed. The resulting RBCs were washed with cold 1× PBS for three times and were collected by centrifugation.

Cell Culture.

Murine breast cancer 4T1 cells were obtained from American Type Culture Collection (ATCC) and cultured at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were cultured in normal RPMI 1640 culture medium (Gibco®, Thermo Fisher Scientific, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT) and 1% penicillin/streptomycin (Gibco®, Life Technologies, Grand Island, NY).

Cellular Uptake of NPs_{Ce6}, $NPs@RBM_{Ce6}$, and $NPs@i\text{-}RBM_{Ce6}$.

4T1 cells were seeded in 24-well plates at a density of 4×10^4 cells per well. After culturing overnight, the cells were treated with PBS, Ce6, NPs_{Ce6}, $NPs@RBM_{Ce6}$, or $NPs@i\text{-}RBM_{Ce6}$. After incubation for 4 h, the cells washed thrice with cold PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. Subsequently, the cells were stained with DAPI and Alexa Fluor 488 phalloidin (1:50) to indicate cell nucleus and cytoskeleton, respectively, according to the manufacturer's instructions. Coverslips were mounted on glass microscope slides with a drop of anti-fade mounting media, and then visualized by CLSM.

***In Vitro* Light-Triggered Release of TPZp from NPs@i-RBM$_{Ce6+TPZp}$.**

1 mL 1×PBS containing $NPs_{Ce6+TPZp}$, $NPs@RBM_{Ce6+TPZp}$, or $NPs@i\text{-}RBM_{Ce6+TPZp}$ (TPZp, 20 μg/mL) was added to a 1.5 mL centrifuge tube and shaken gently at 37 °C. At different time intervals after irradiation with 660 nm (0.5 W/cm^2) laser for 10 min, the supernatant was collected after centrifugation, and was detected using UV-Vis spectroscopy. Similar procedure also applied to the negative control groups without laser irradiation.

***In Vitro* Cytotoxicity Examined with MTT Assay.**

4T1 cells were seeded in 96-well plates at 5×10^3 cells per well in 100 μL of RPMI 1640 medium, and incubated at 37 °C overnight. The culture medium was then replaced by 100 μL of medium containing Ce6, NPs_{Ce6}, $NPs@RBM_{Ce6}$, $NPs@i\text{-}RBM_{Ce6}$, or $NPs@i\text{-}RBM_{Ce6+TPZp}$ at varying concentrations. After incubation for 4 h, the culture medium was replaced with fresh medium followed with 660 nm laser irradiation (0.5 W/cm^2, 10 min). After incubation for another 48 h, MTT stock solution was added to

the wells to a final concentration of 1 mg/mL. After incubation for additional 4 h, 100 μL of extraction buffer (20% SDS in 50% DMF, pH 4.7, prepared at 37 °C) was added. The absorbance was measured at 570 nm using ELx800™ Absorbance microplate readers (BioTek Instruments, Winooski, VT). Cell viabilities were normalized to that of cells cultured in medium with PBS.

Animals and Tumor Model.

Female Balb/c mice at 6-8 weeks of age were obtained from Vital River Laboratories (Beijing, China). All animals received are in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the University of Science and Technology of China Animal Care and Use Committee. The xenograft tumor model was generated by injection of 2×10^5 4T1 cells (100 μL) with 20% Matrigel (BD Bioscience, Franklin Lakes, NJ) into the right flank of Balb/c mice.

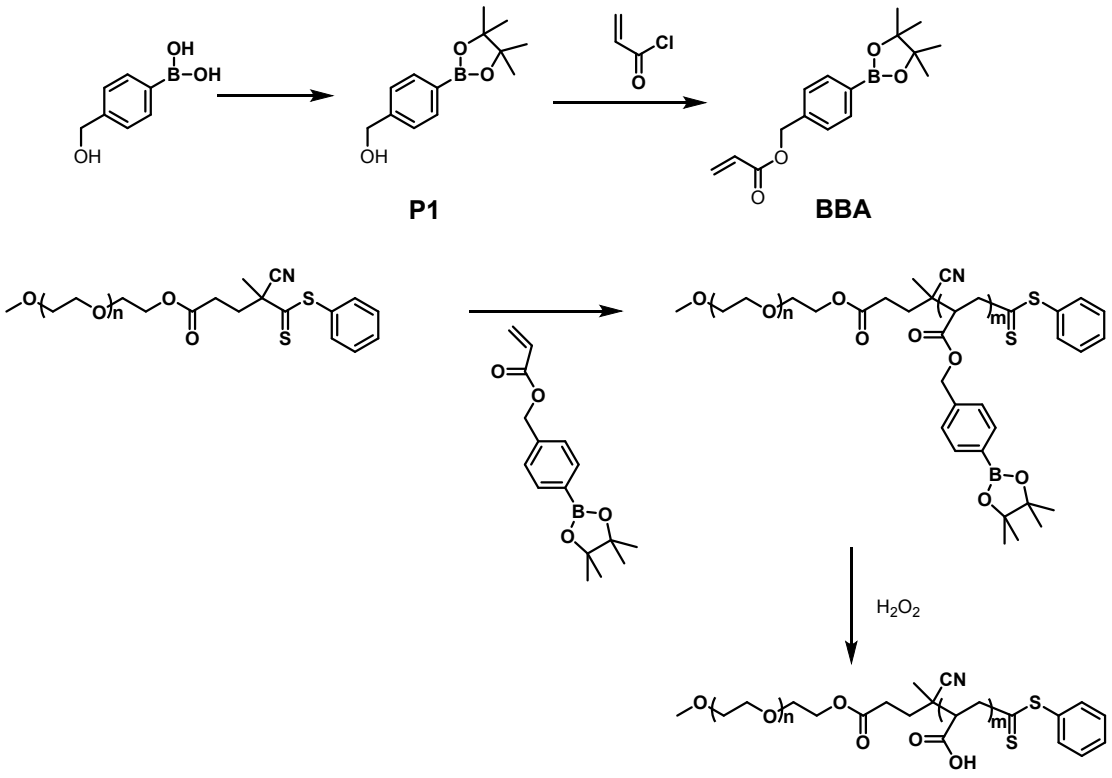
Biodistribution of NPs@i-RBM.

Female Balb/c mice bearing 4T1 xenografts were intravenously injected with DiD-labeled NPs, NPs@RBM, or NPs@i-RBM. The dose of DiD was 0.5 mg per kg mouse body weight. At the preset times, *in vivo* fluorescence images were acquired on the Xenogen IVIS Lumina system (Caliper Life Sciences, Alameda, CA). Moreover, at 72 h post-injection, the organs of mice including heart, lung, liver, spleen, kidney, and tumor were collected. Then fluorescence images were also acquired on the Xenogen IVIS Lumina system as well. The parameters of mice fluorescence images *via* Xenogen IVIS Spectrum was listed below, including exposure time 0.5 s, emission filter 680 nm,

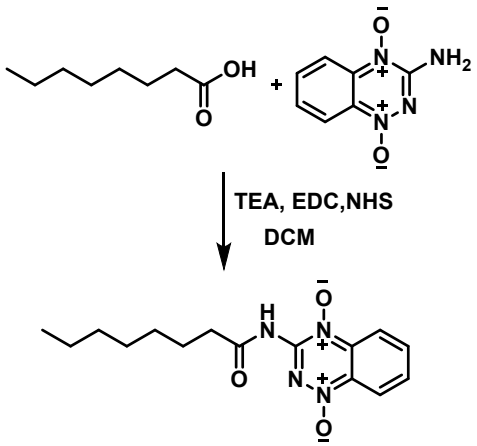
excitation filter: 640 nm, filter position: 13, binning factor: 8, binning factor: 8, field of view: 22.5, and measured temperature -90 ℃.

Antitumor Study.

When the tumor volumes were around 100 mm^3, the 4T1 tumor-bearing mice were *i.v.* injected with various formulations (n = 5 for each group). At 24 h post-injection, the mice were received irradiation with 660 nm laser at power density of 0.5 W/cm^2 for 10 min. Seven formulations were used as below: (I) PBS (−), (II) NPs@i-$RBM_{Ce6+TPZp}$ (−), (III)Ce6 (+) , (IV) NPs_{Ce6} (+), (V) NPs@RBM_{Ce6} (+), (VI) NPs@i-RBM_{Ce6} (+), and (VII) NPs@i-$RBM_{Ce6+TPZp}$ (+) at an equivalent dose of 2.0 mg Ce6 per kg mouse weight and 2.0 mg TPZp per kg mouse weight. The tumor growth was monitored by measuring the perpendicular diameter of the tumors (*i.e.*, length and width, respectively) using calipers every two days. The estimated volume was calculated according to the formula: Tumor volume (mm^3) = 0.5 × length × $width^2$. Weight of each mouse was also measured every two days.



Scheme S1. The synthetic route of mPEG-*b*-PBBA and degradation under H_2O_2.



Scheme S2. The synthesis of octanoic TPZ prodrug.

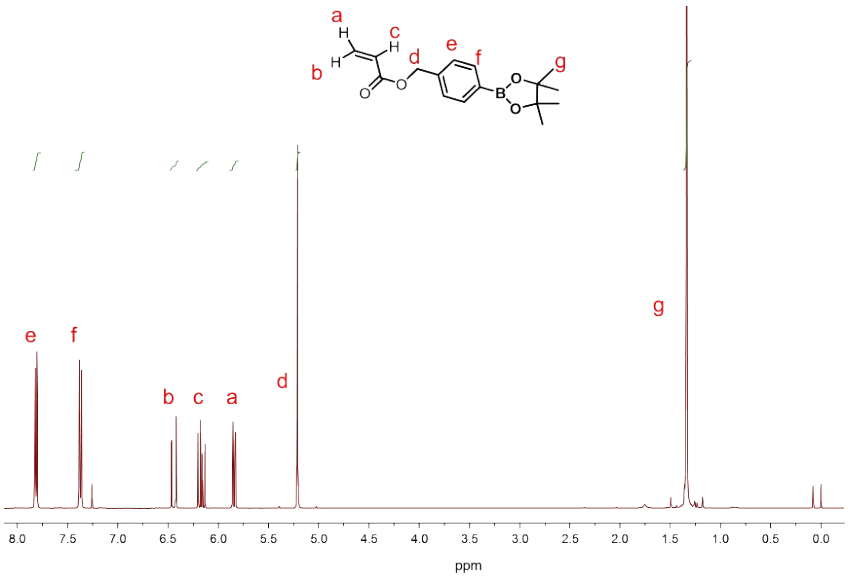


Figure S1. ^{1}H NMR spectrum of BBA monomer in $CDCl_3$.

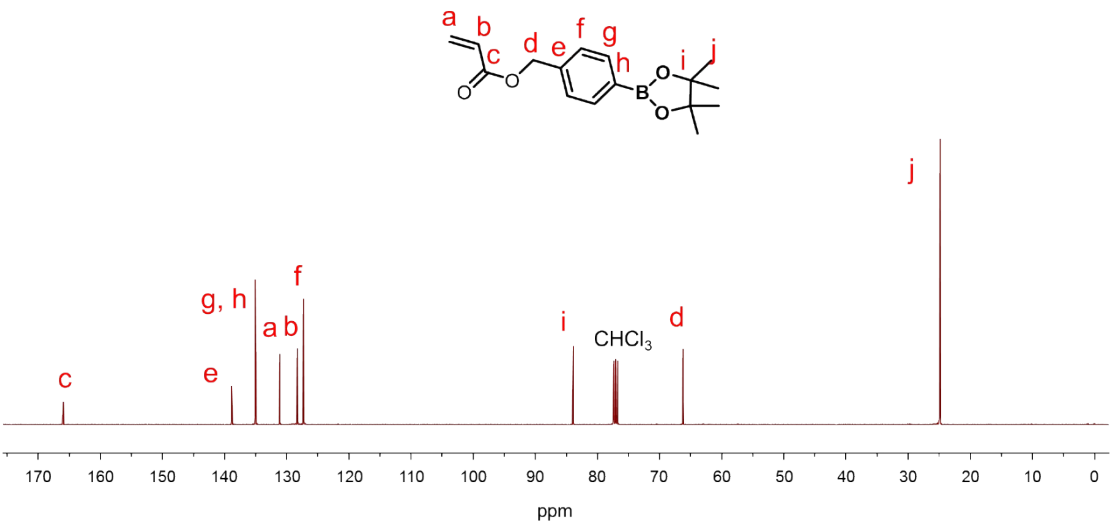


Figure S2. ^{13}C NMR spectrum of BBA monomer in $CDCl_3$.

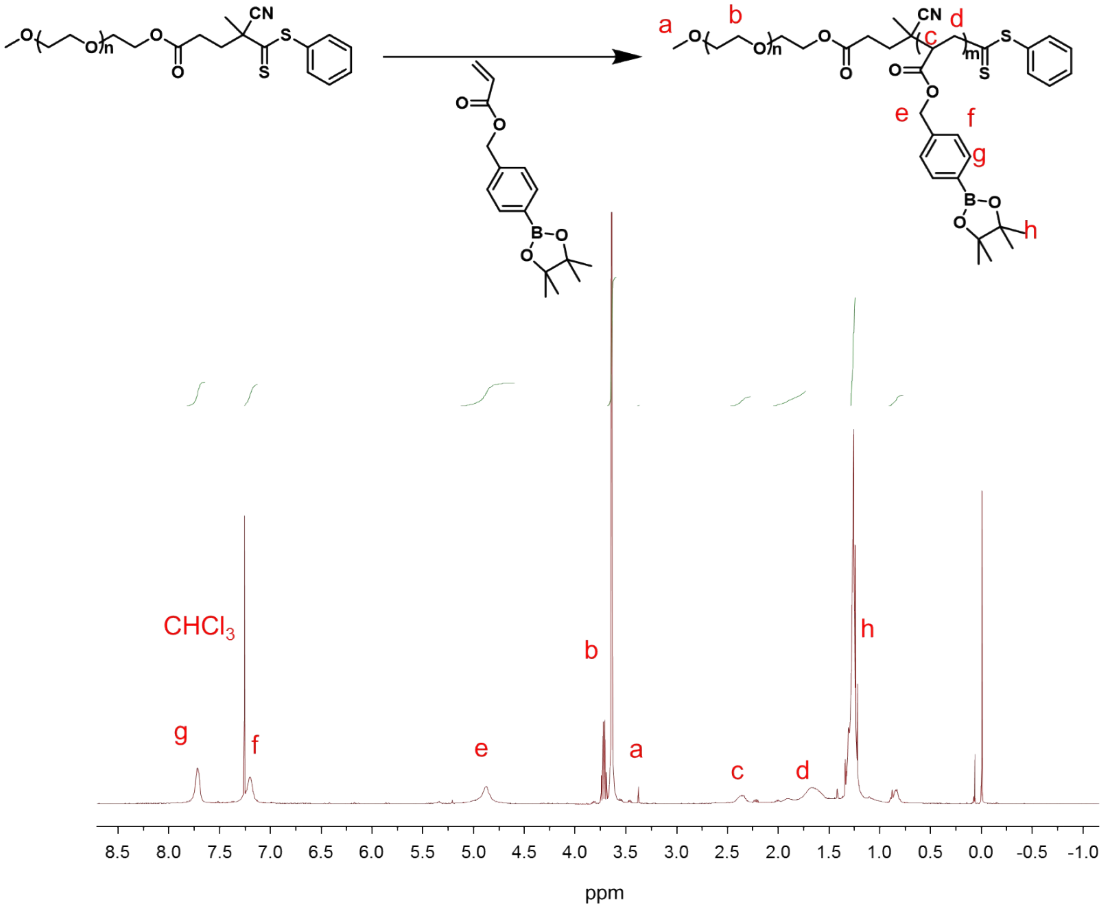


Figure S3. ^{1}H NMR spectrum of mPEG-*b*-PBBA in $CDCl_3$.

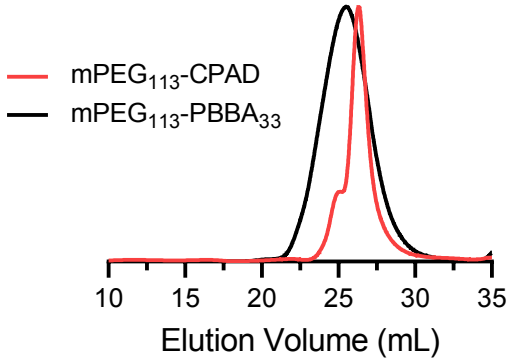


Figure S4. GPC chromatograms of $mPEG_{113}$-CPAD and $mPEG_{113}$-$PBBA_{33}$ in DMF.

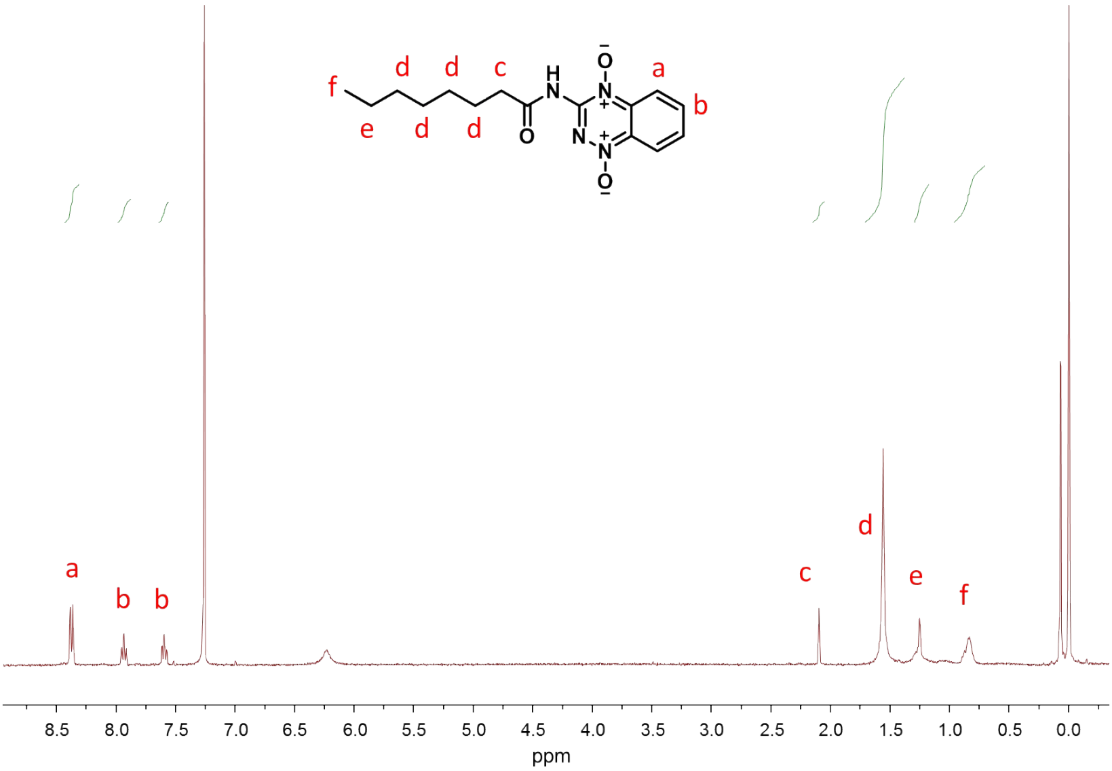


Figure S5. ^{1}H NMR spectrum of TPZ prodrug (TPZp) in $CDCl_3$.

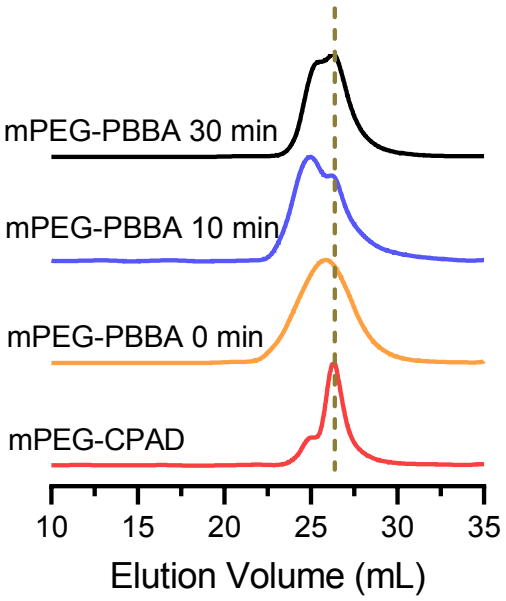


Figure S6. GPC analysis of mPEG-PBBA incubtated in50 mM H_2O_2 for different times.

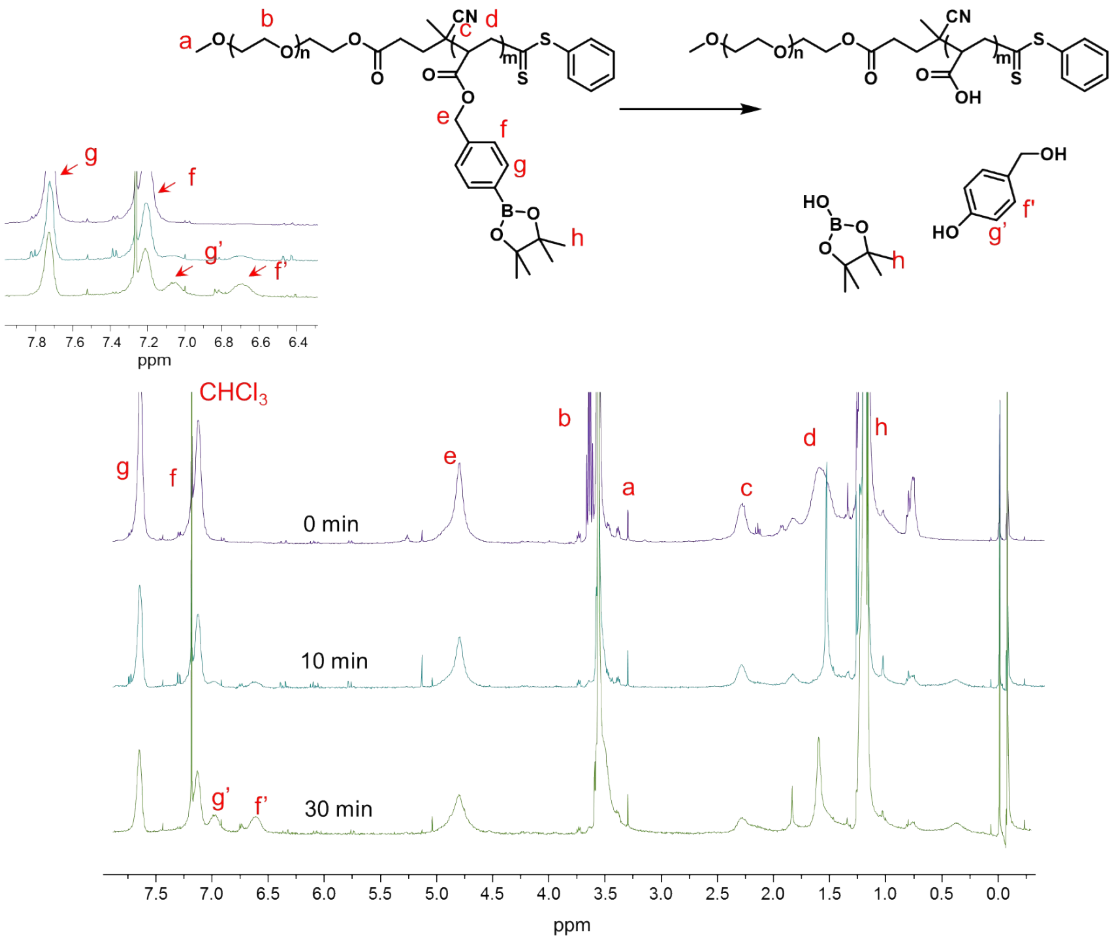


Figure S7. Time-dependent ^{1}H NMR spectra of mPEG-PBBA incubtated in 50 mM H_2O_2 for different times.

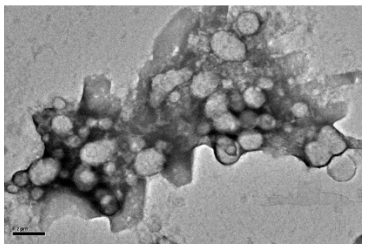


Figure S8. TEM image of NPs@i-RBM after laser irradiation (660 nm, 0.5 W/cm^2, 10 min) (scale bar=200 nm).

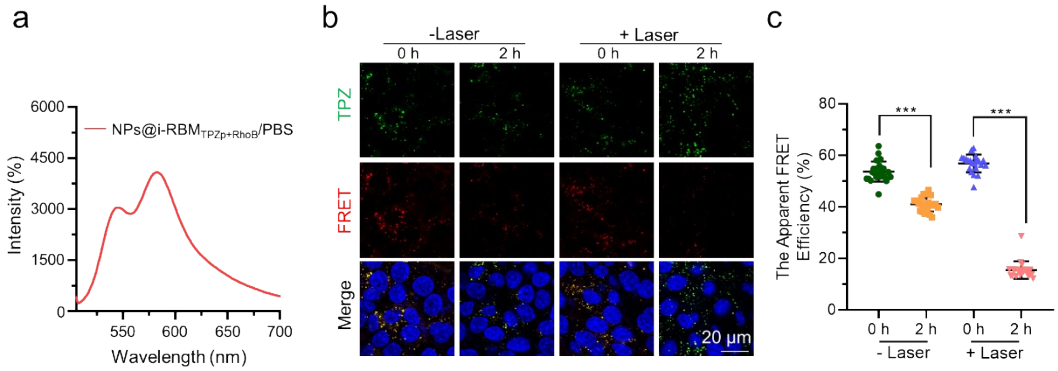


Figure S9. (a) Emission fluorescence spectra of FRET dyes (TPZp and RhoB) encapsulated in NPs@i-RBM in PBS. (b) Representative images of FRET efficiency analysis of NPs@i-RBM in 4T1 cells with or without laser irradiation at 0 h and 2 h, respectively. (c) The apparent FRET efficiency analysis of NPs@i-RBM from plane b.

References

1. C.-C. Song, R. Ji, F.-S. Du, D.-H Liang, Z.-C. Li , *ACS Macro. Letters.*, 2013, **2**, 273-277.

2. C. M. Hu, L. Zhang, S. Aryal, C. Cheung, R. H. Fang, L. Zhang, *P. Natl. Acad. Sci. USA*, 2011, **108**, 10980-10985.