Supporting Information

Dual-Targeted Cascade-Responsive Prodrug Micelle System for Tumor Therapy *in Vivo*

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List of Contents

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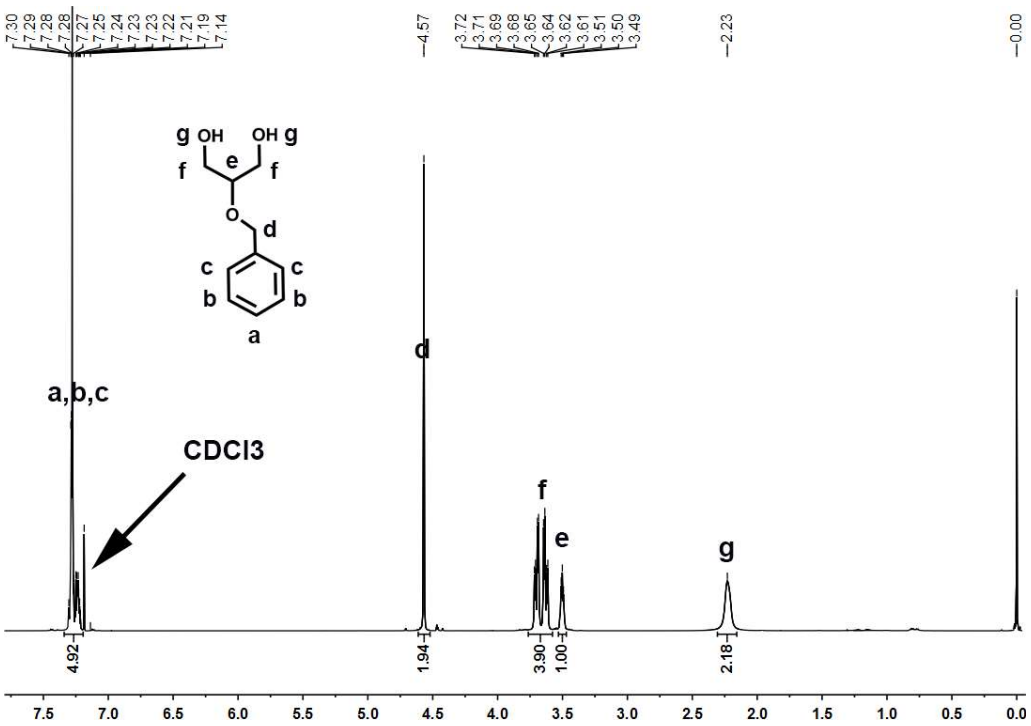
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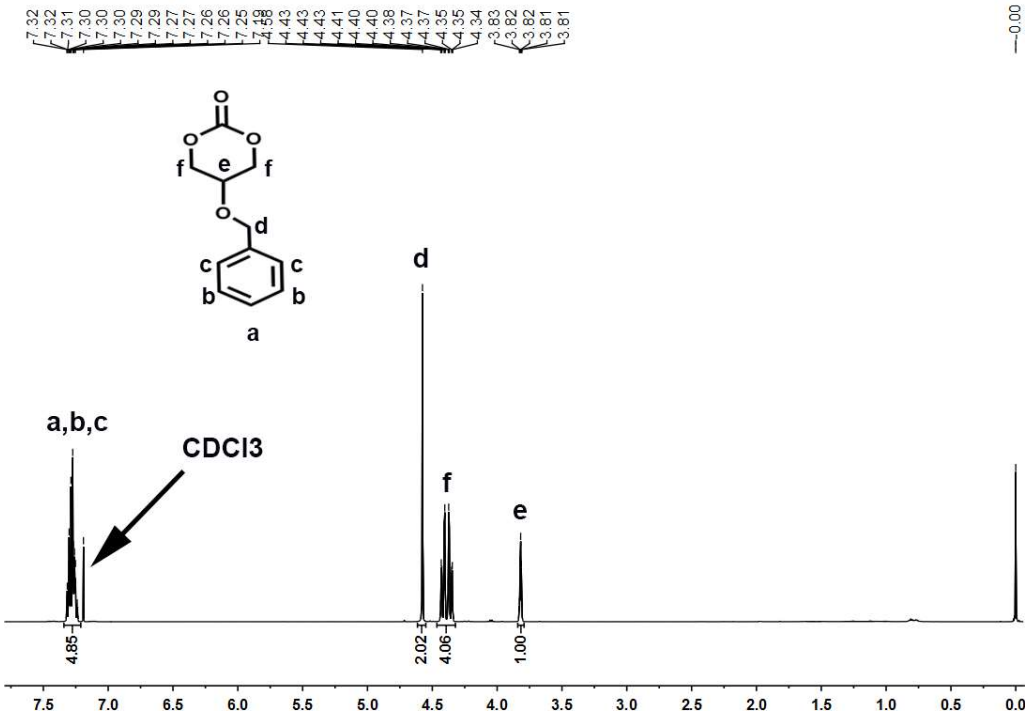
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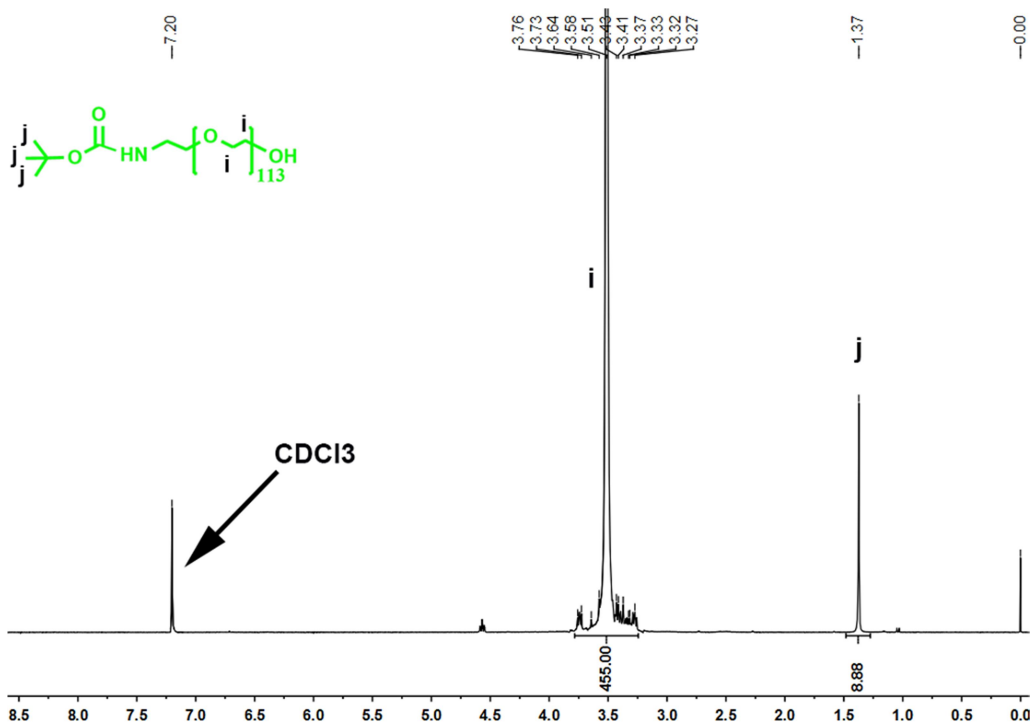
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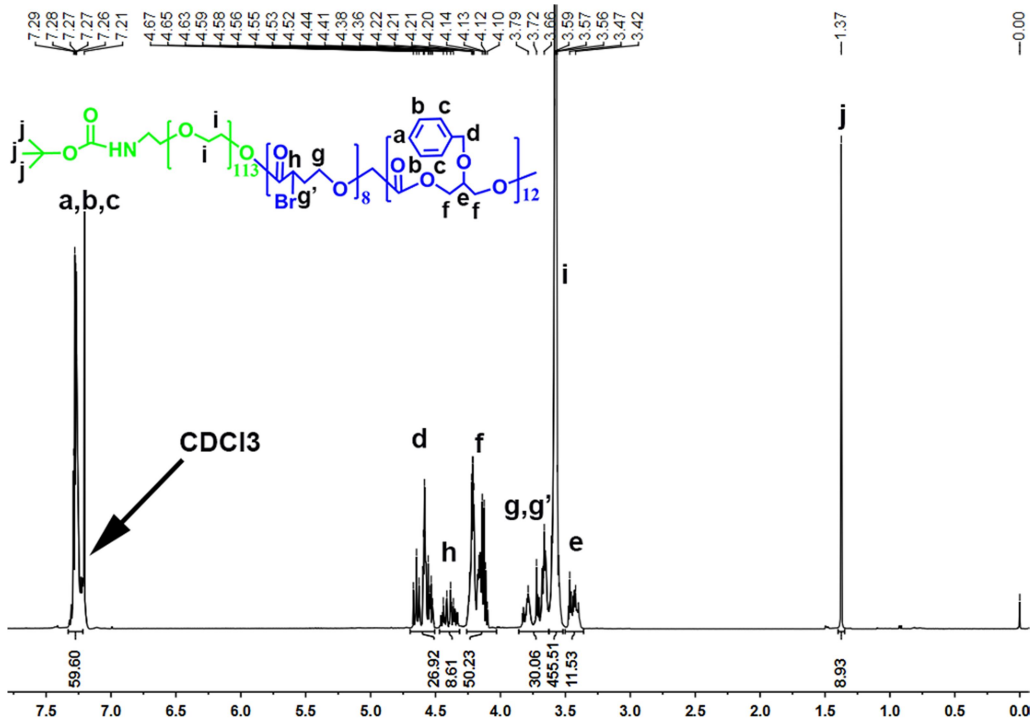
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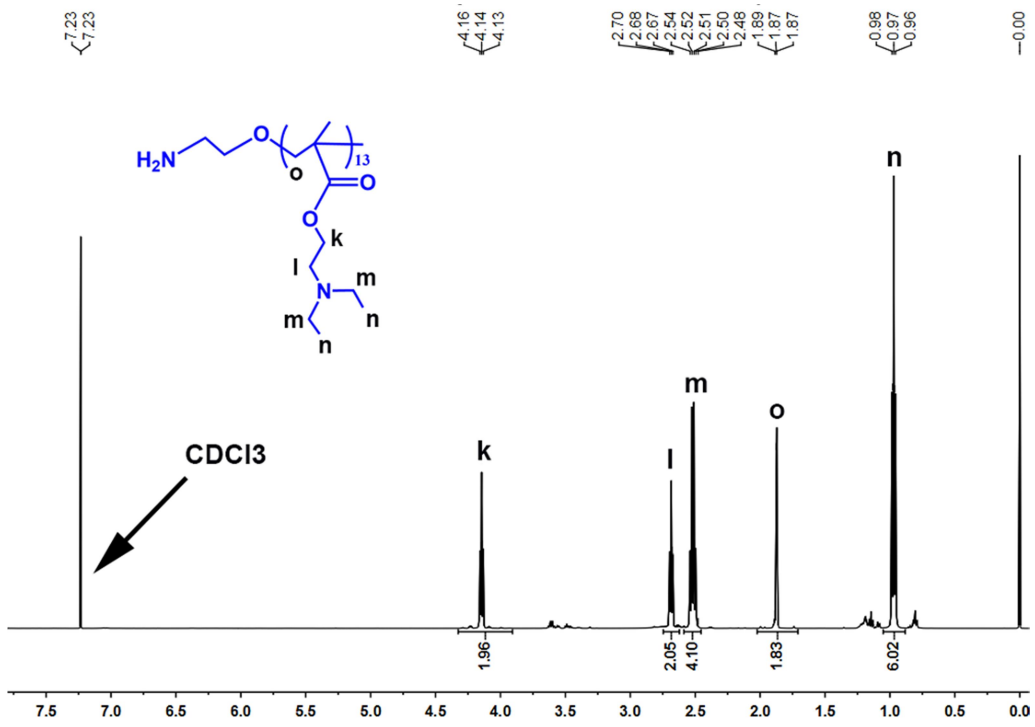
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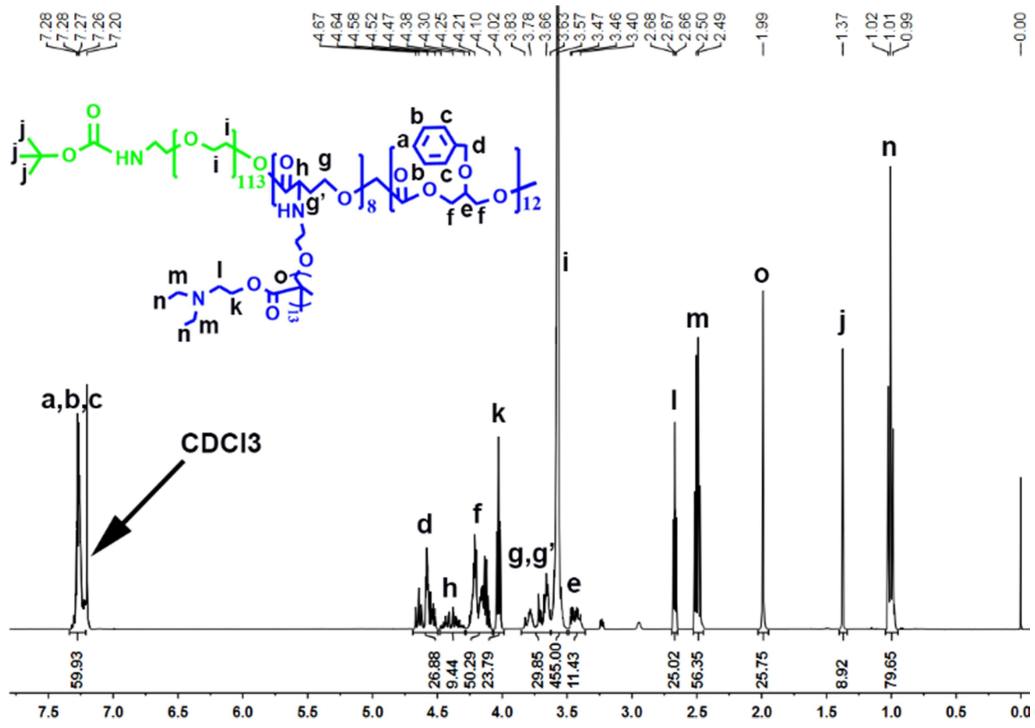
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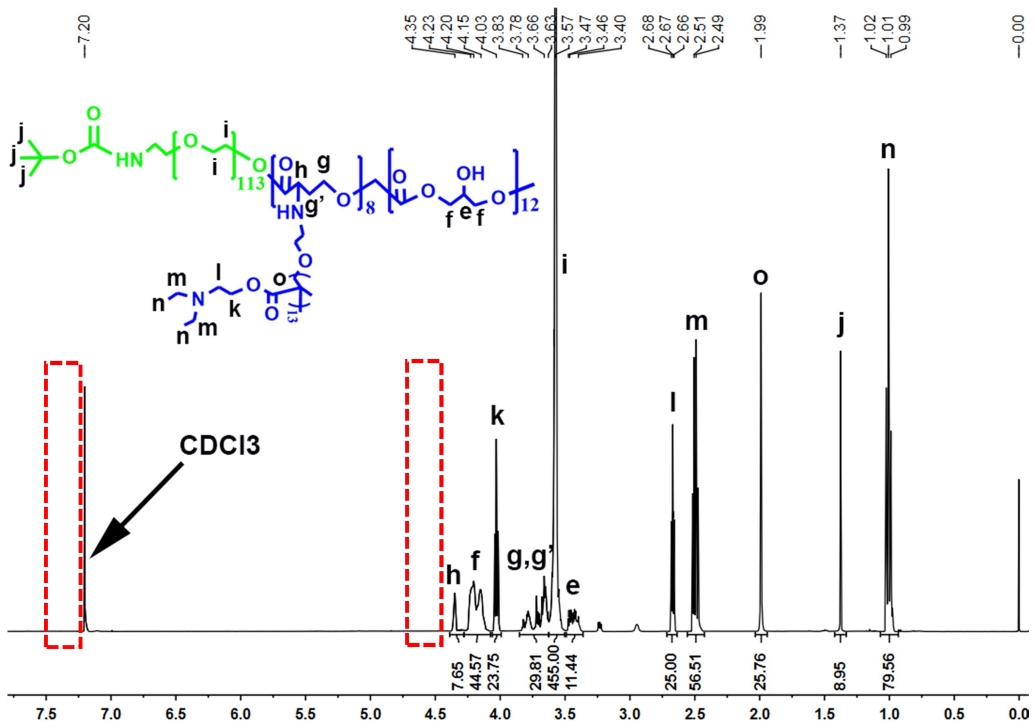
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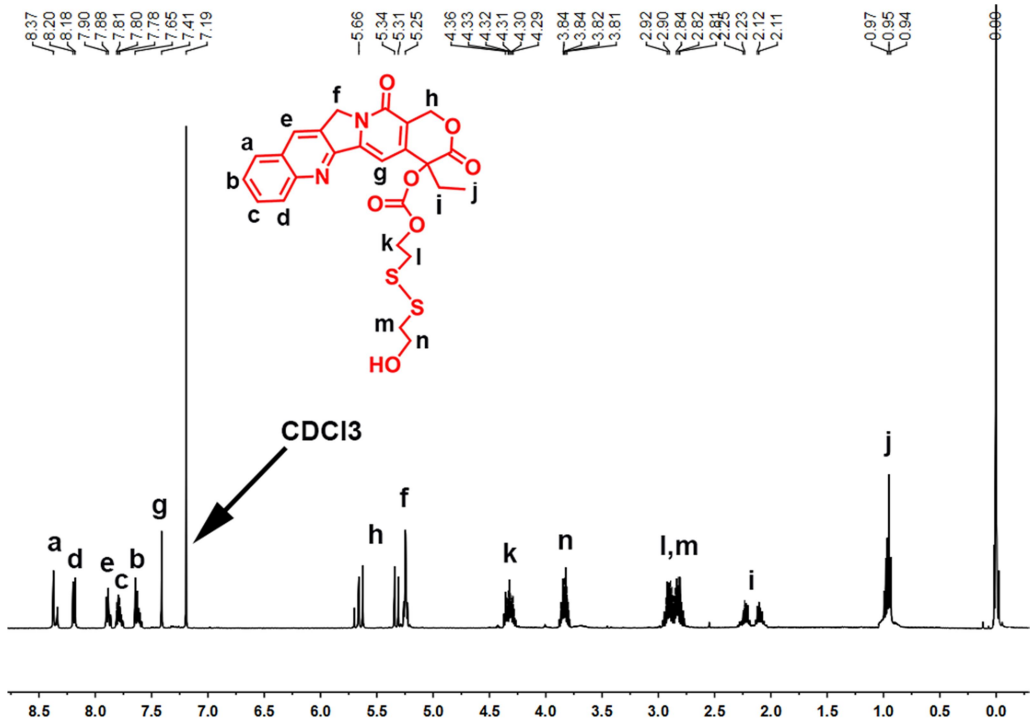
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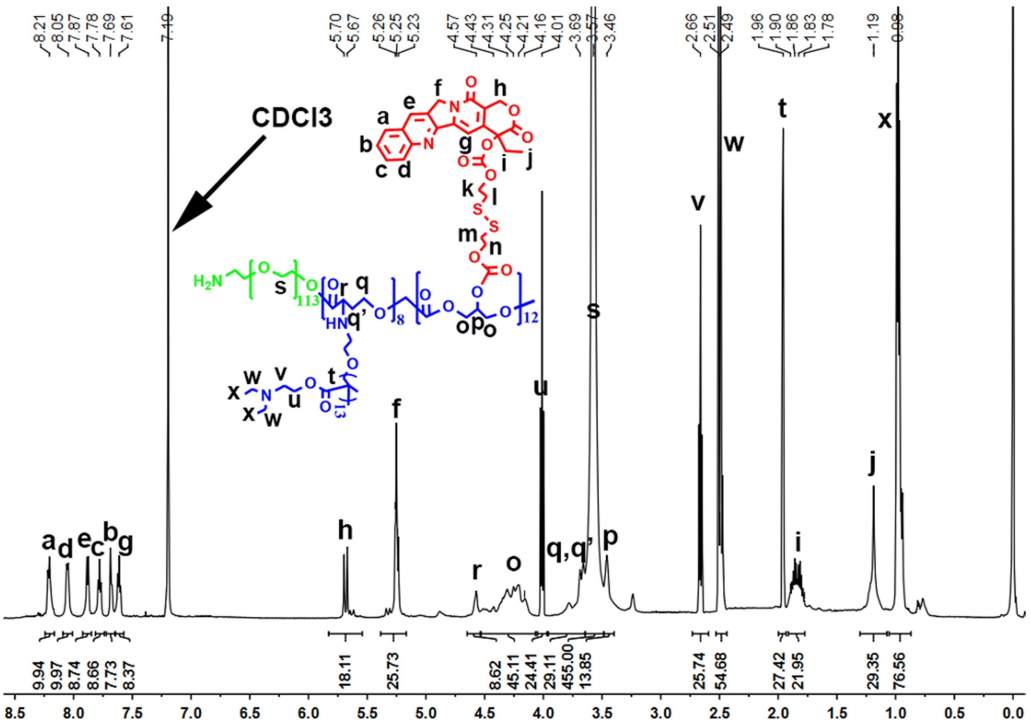
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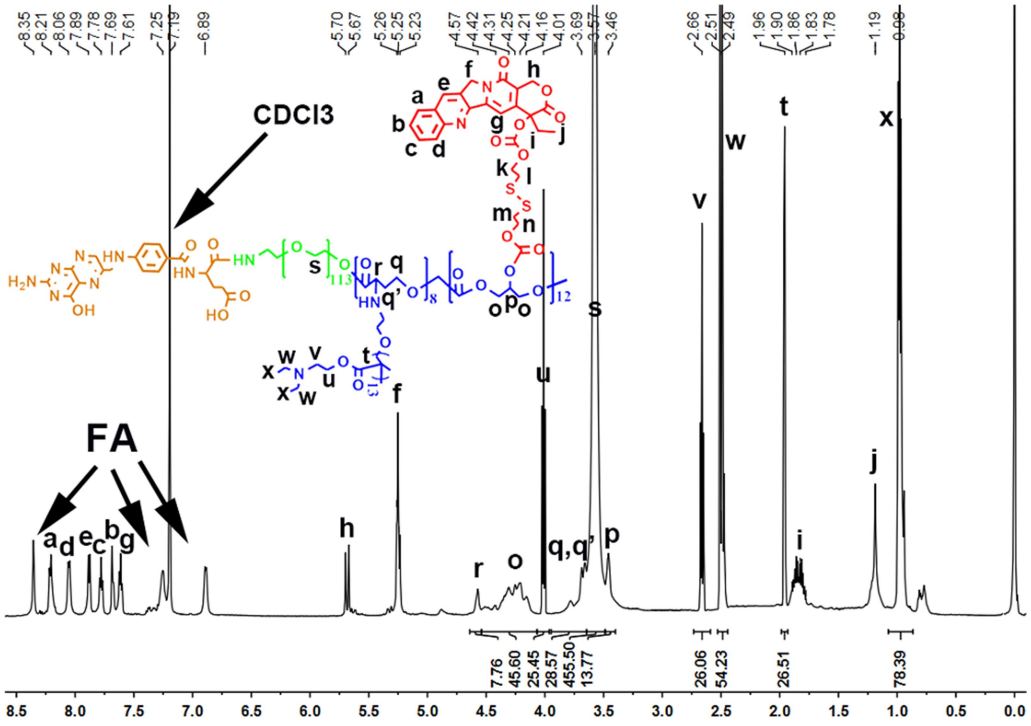
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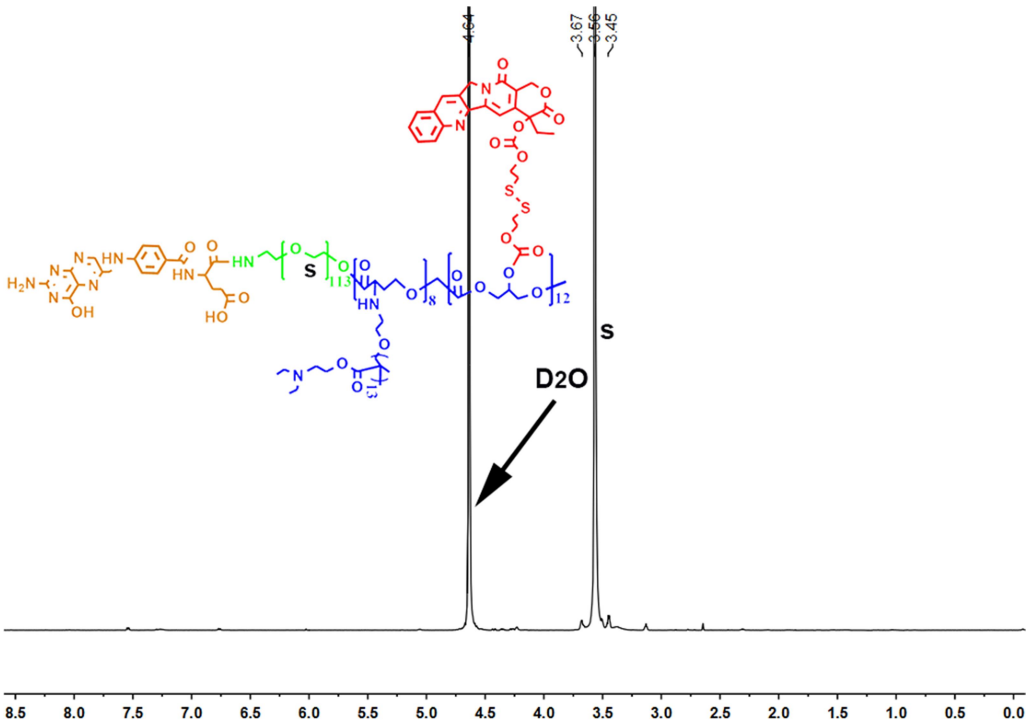
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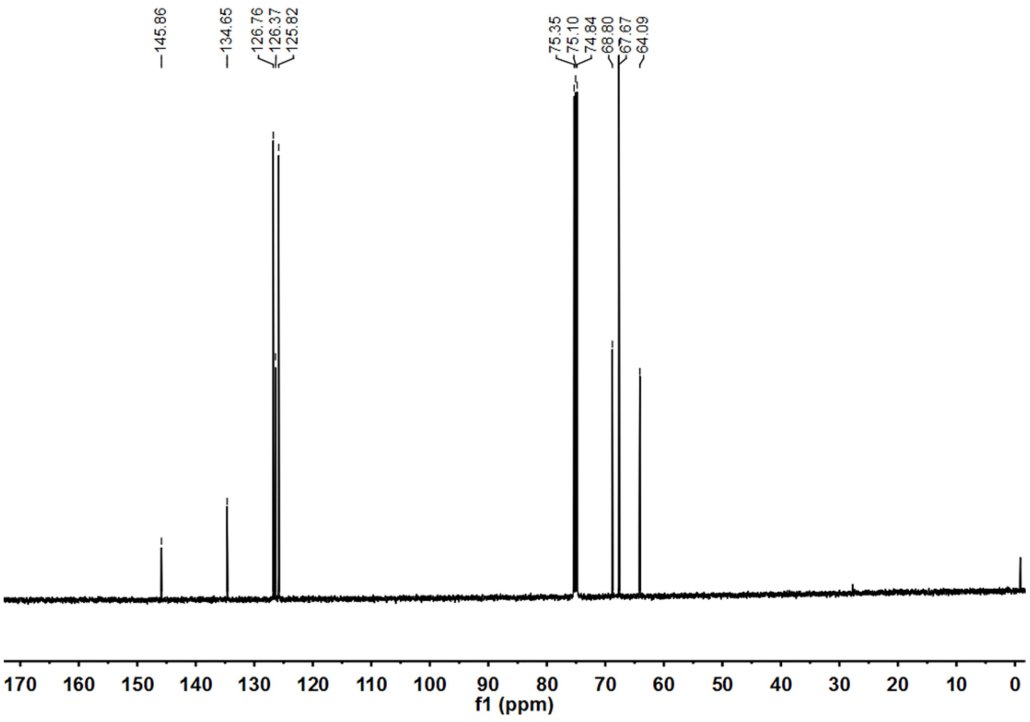
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m

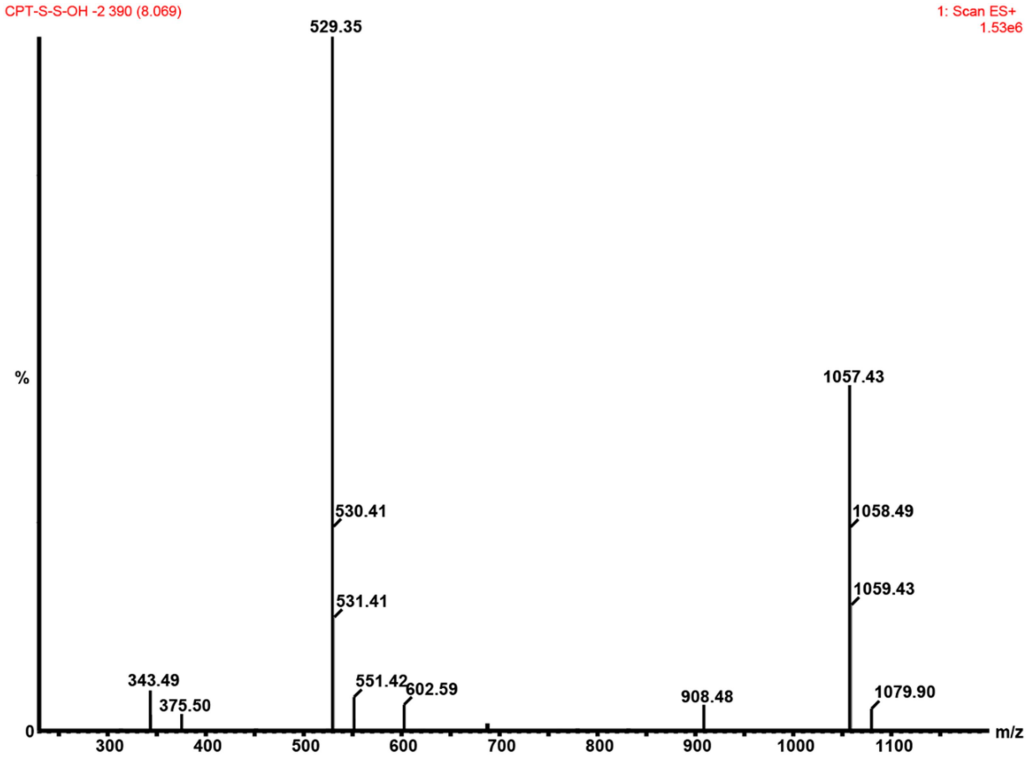


Figure S1. Characterization of FA-PEG-PDBO-BPT copolymer: 1H NMR spectra (400 MHz, 298 K) of relevant products from the synthesis process of FA-PEG-PDBO-BPT copolymer, including BPD (a), BDO (b), boc-PEG (c), bPEG-BBO-BDO (d), PDEA (e), bPEG-PDBO-BDO (f), bPEG-PDBO-BOH (g), CPT-S-S-OH (h), PEG-PDBO-BPT (i), and FA-PEG-PDBO-BPT (j & k, copolymer dissolved in CDCl3 and D2O, respectively); ^{13}C NMR spectra of BDO (l); and ESI-MS spectrum of CPT-S-S-OH (m).

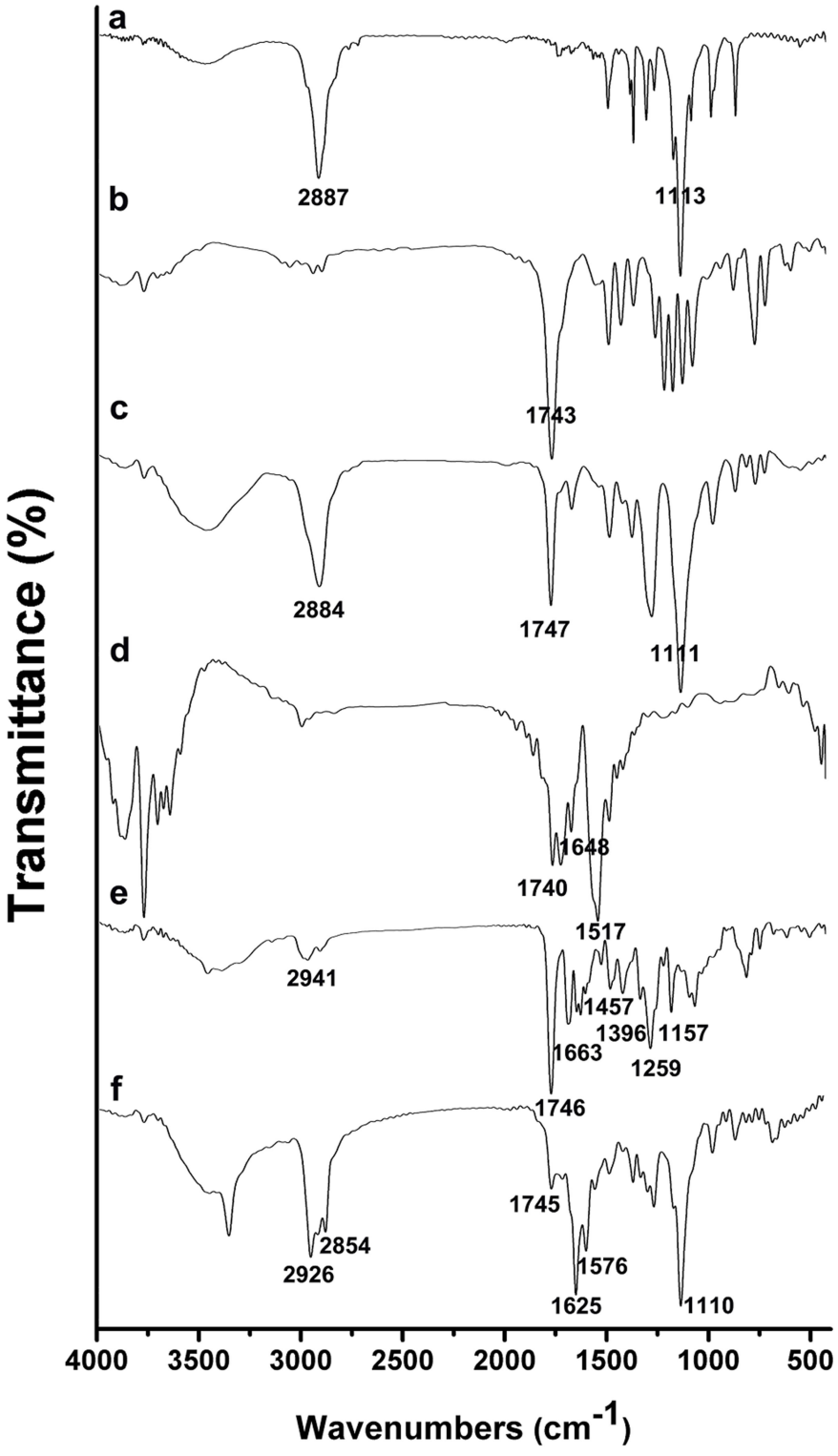
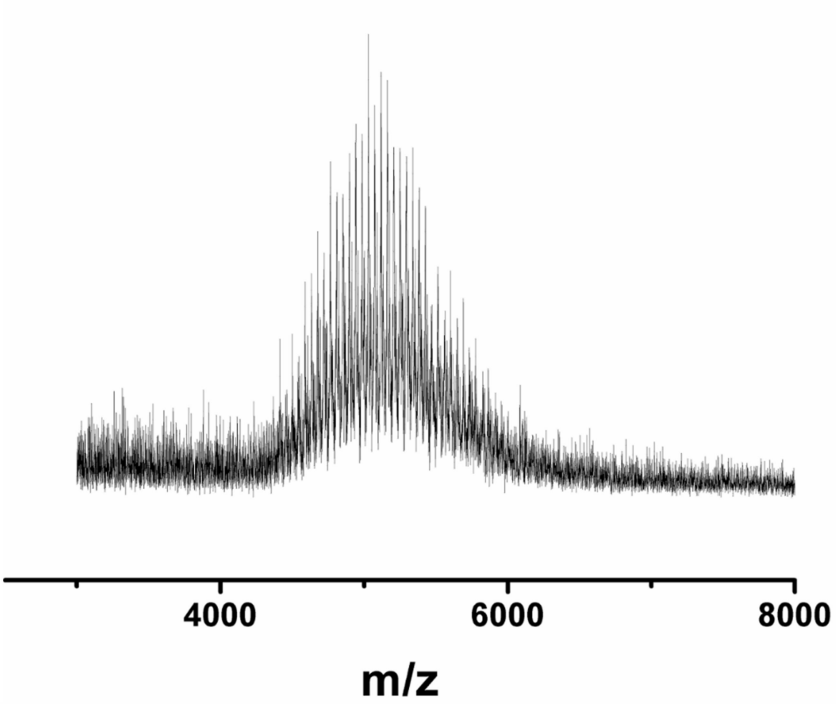


Figure S2. FTIR spectra of (a) boc-PEG, (b) BDO, (c) bPEG-BBO-BDO, (d) PDEA, (e) CPT-S-S-OH, and (f) FA-PEG-PDBO-BPT, respectively.

A



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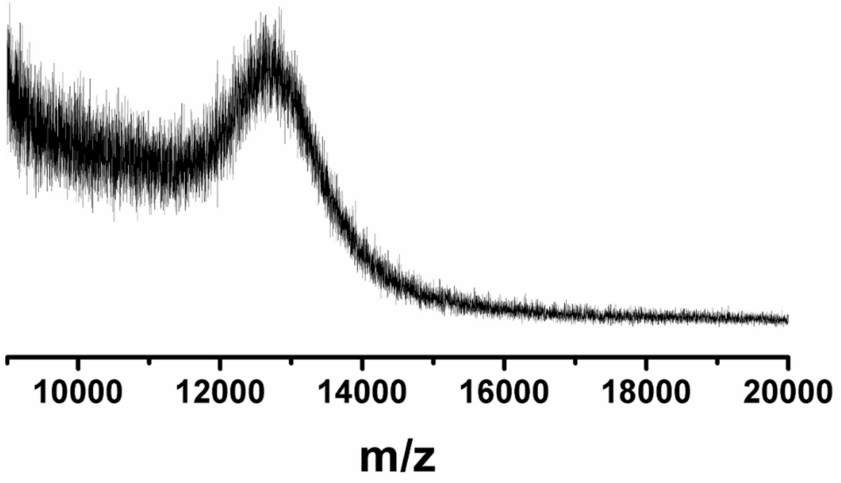


Figure S3. MALDI-TOF mass spectra of boc-PEG and FA-PEG-PDBO-BPT copolymer, respectively. Saturated MALDI matrix solution was prepared in MeOH /water solutions (80/20, v/v) containing 0.1% TFA. Sinapinic acid was used as matrix.

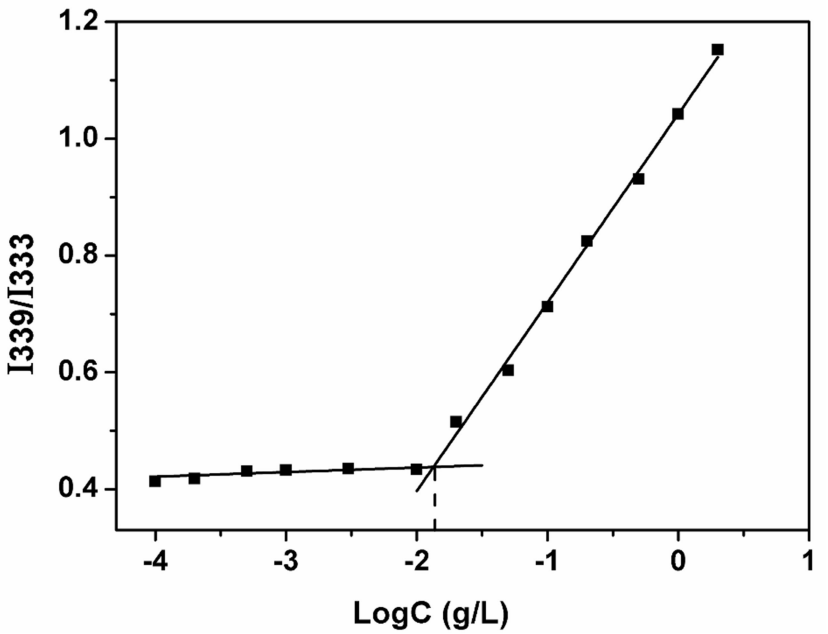


Figure S4. Critical micelle concentration (CMC) of FA-PEG-PDBO-BPT micelle.

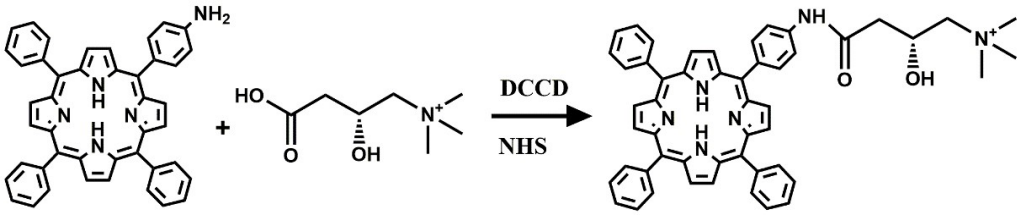


Figure S5. Chemical structure and synthesis route of the mitochondria-targeting photosensitizer MTPP.

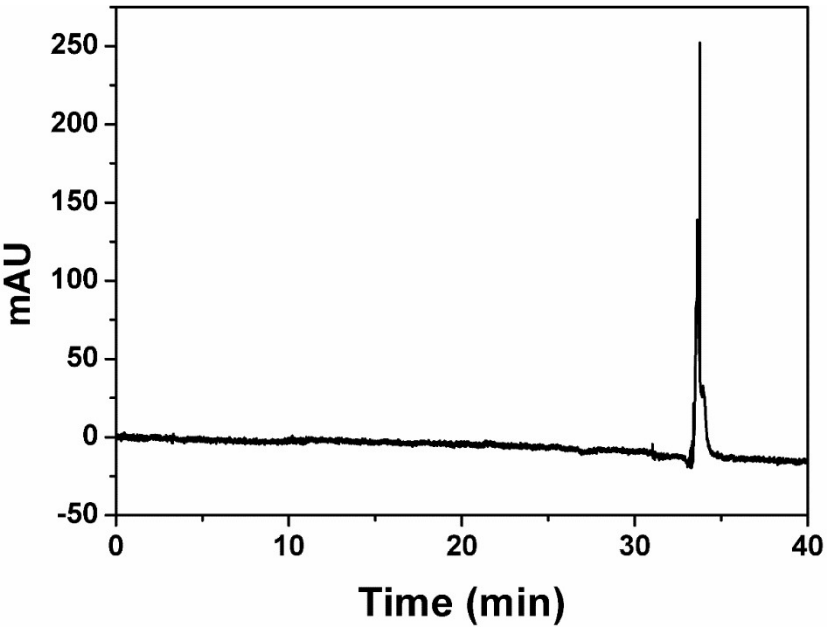


Figure S6. HPLC curve of mitochondria-targeting photosensitizer MTPP.

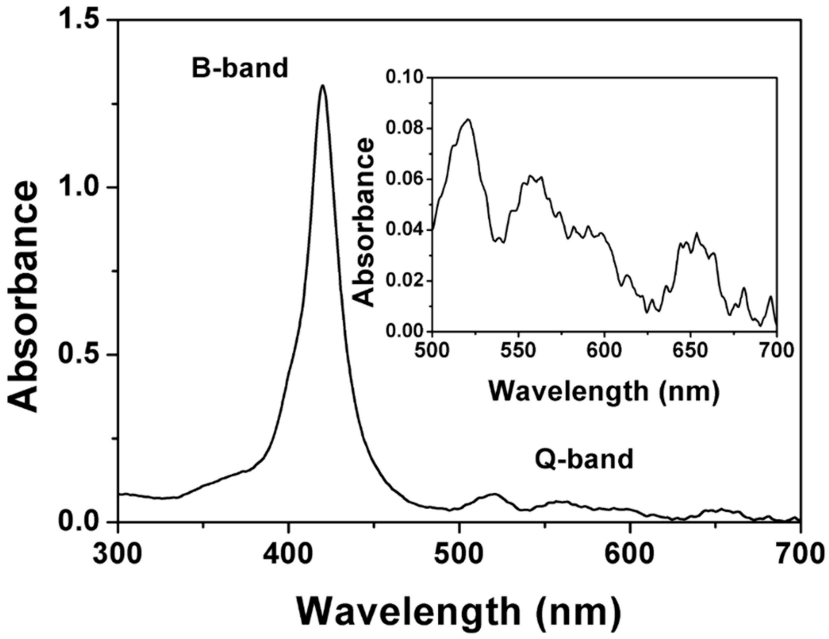


Figure S7. Absorption spectrum for MTPP in DMSO. The inset is the amplified Q band.

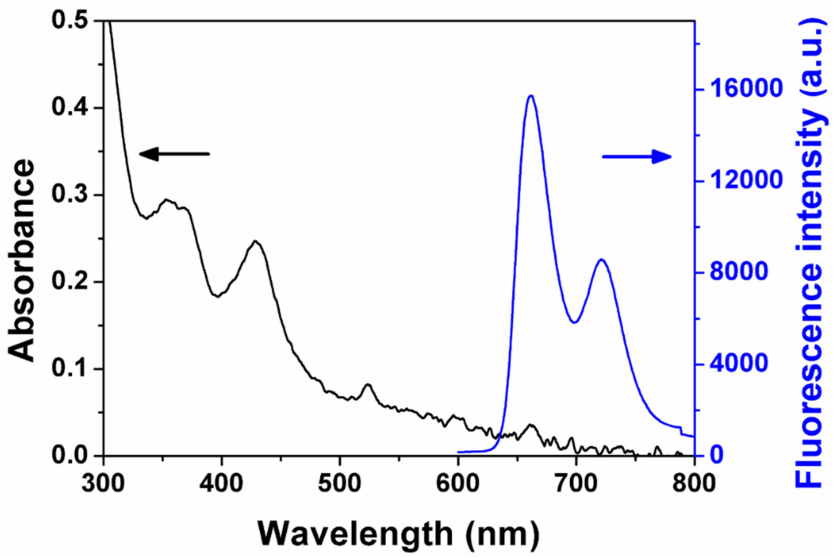


Figure S8. Absorption (black) and emission (blue) spectra of the FA-PEG-PDBO-BPT@MTPP system (excited at 421 nm) dissolved DMSO/H_2O (1/4, v/v) solution.

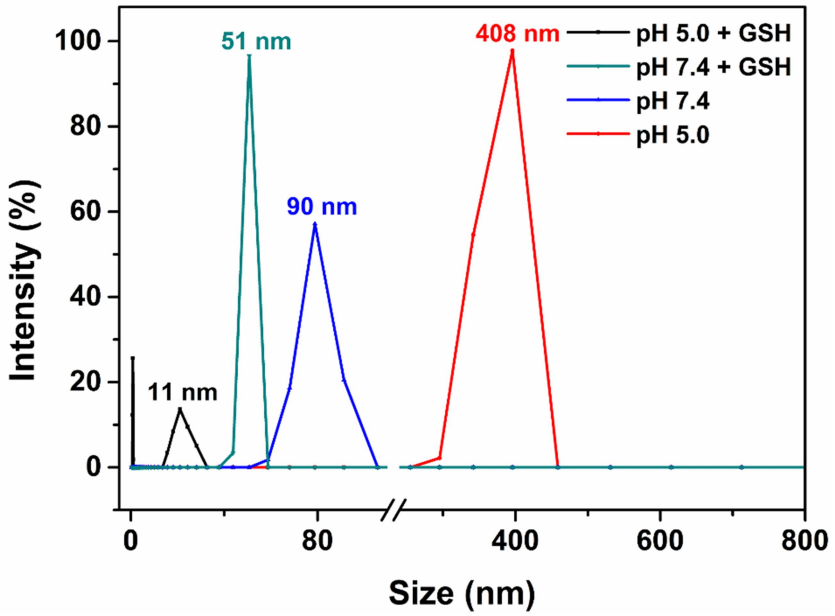


Figure S9. DLS size distribution of the FA-PEG-PDBO-BPT micelles dissolved in pH 7.4 (control) and pH 5.0 PBS solution in the absence and presence of GSH (10 mM) for 2 h, respectively.

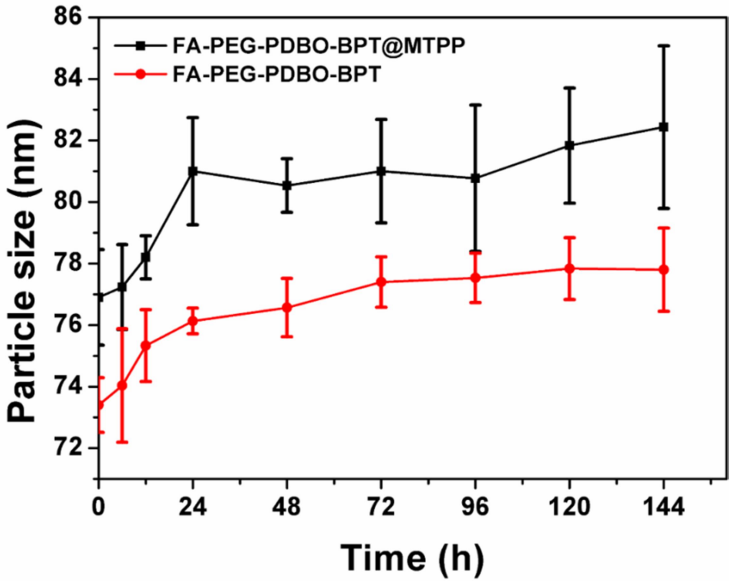


Figure S10. Stability of FA-PEG-PDBO-BPT micelles and MTPP loaded micelles incubated in serum for 6 days.

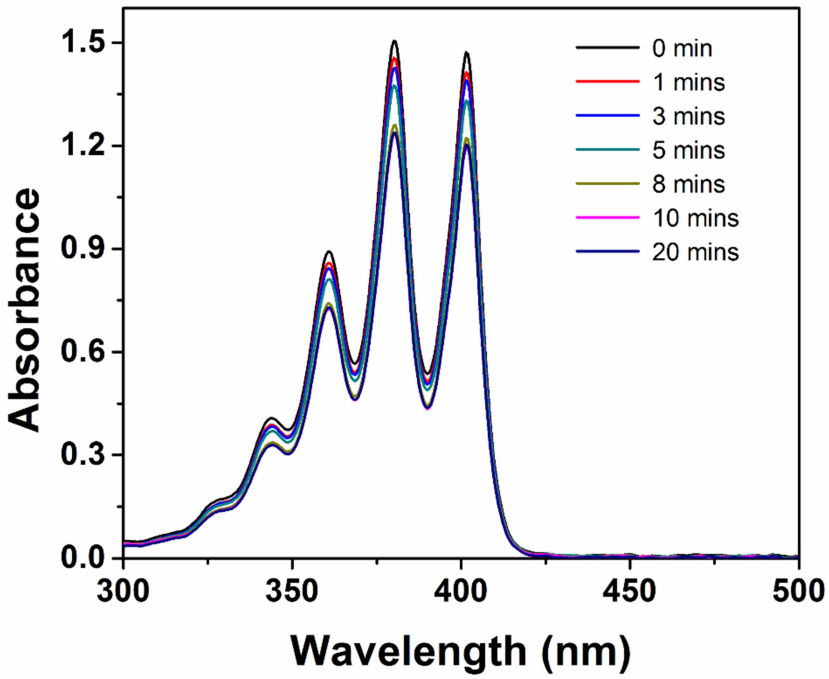


Figure S11. Absorption spectra of ABDA in presence of micelles@MTPP dissolved in pH 7.4 buffer solution under laser irradiation over different periods of time.

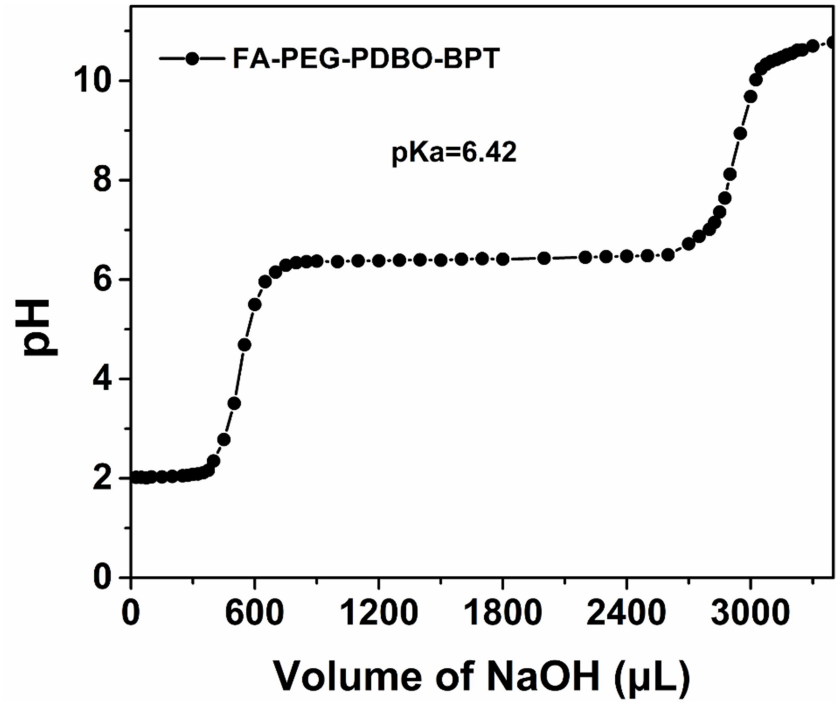


Figure S12. Potentiometric titration curve of FA-PEG-PDBO-BPT copolymer.

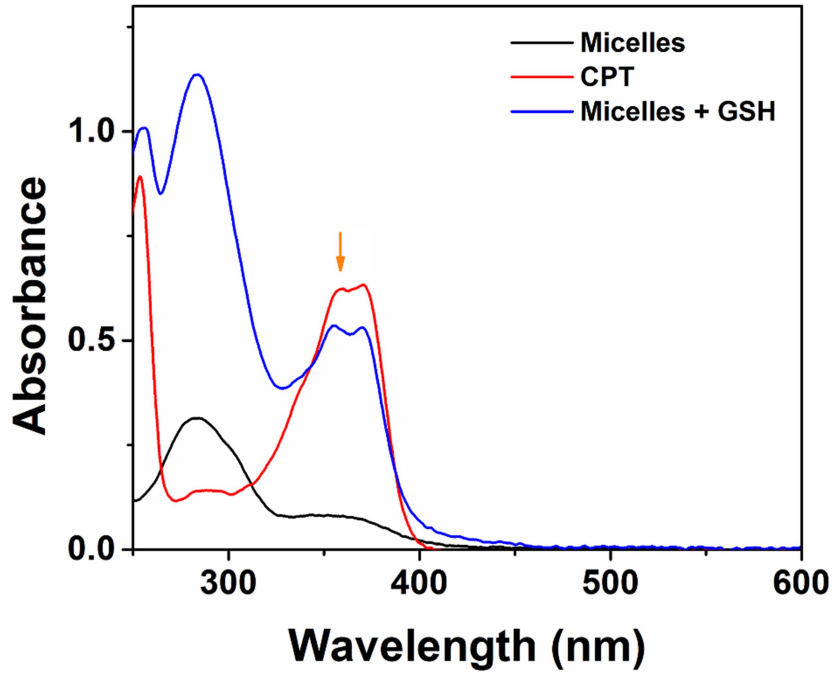
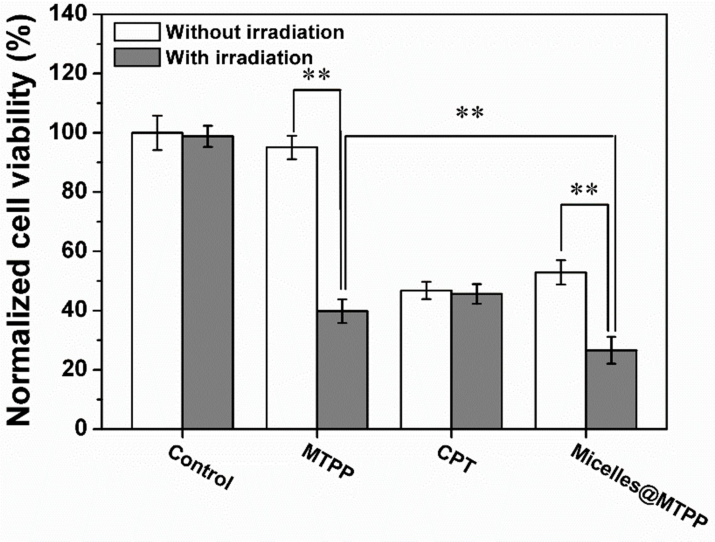


Figure S13. The absorbance spectra of free CPT, FA-PEG-PDBO-BPT micelles, and micelles treated with 10 mM GSH for 2 h in MeOH/H_2O (1/4, v/v) solution.

A



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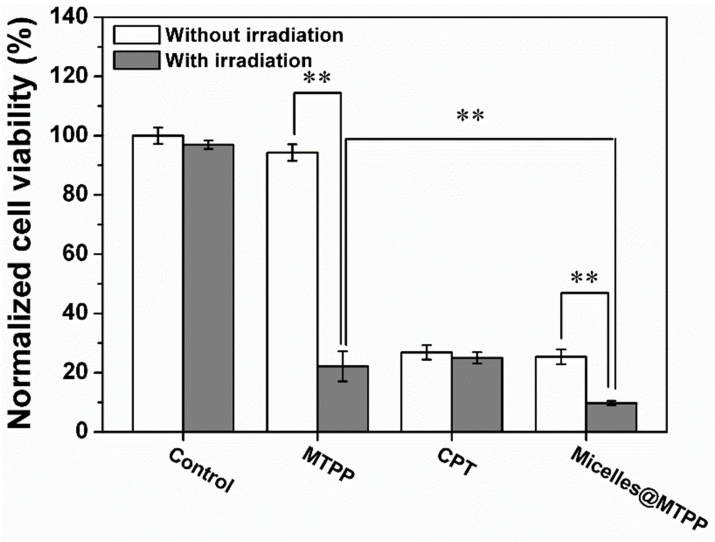


Figure S14. Cytotoxicity assay of HeLa cells treated with MTPP (5 μM), CPT (15 μg/mL) and FA-PEG-PDBO-BPT@MTPP micelles (abbreviation as micelles@MTPP, 76.6 μg/mL) with and without irradiation (20 min) for 12 h (A) and 24 h (B), respectively. Error bars present as mean ± SD (n=4), **p < 0.01.

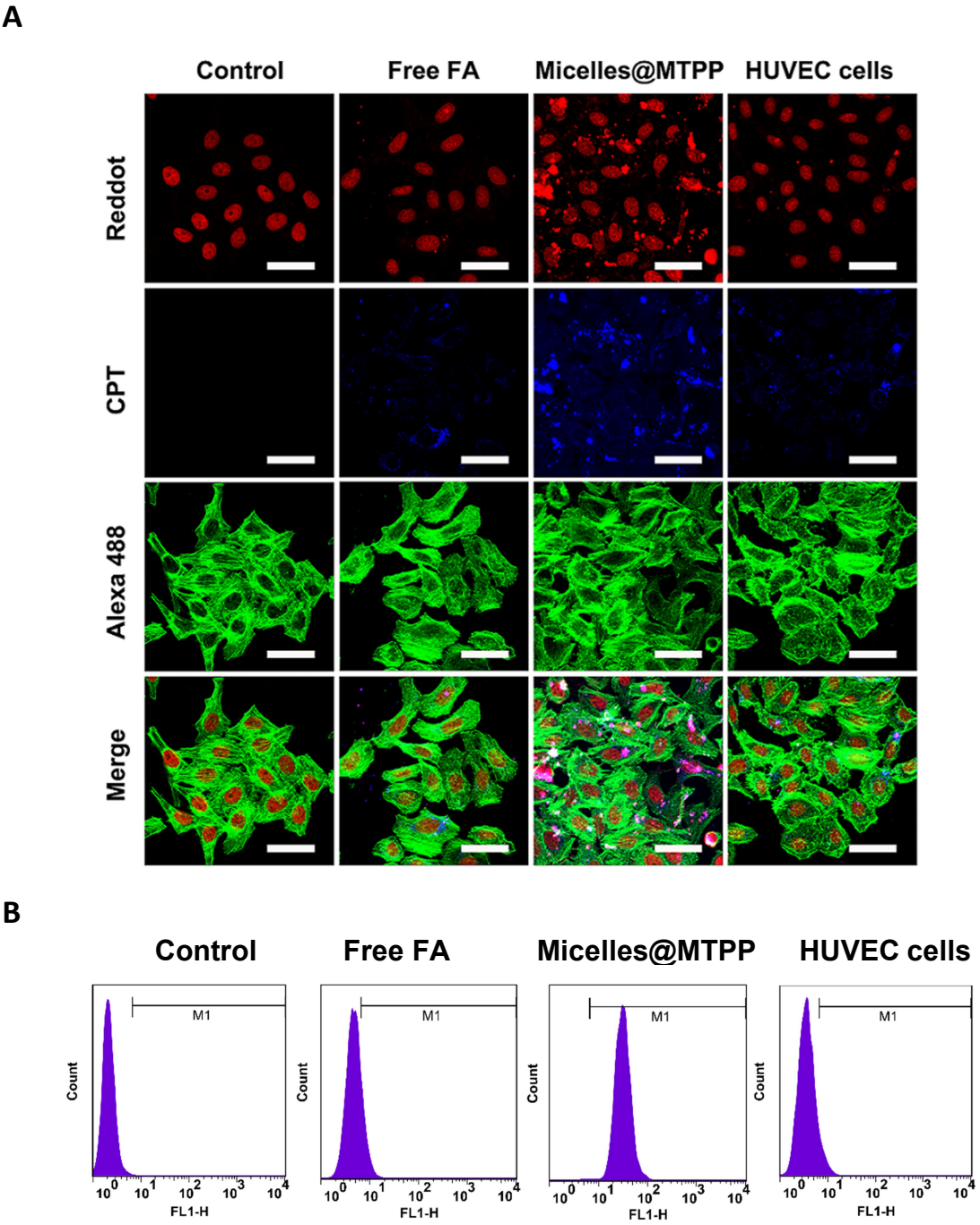
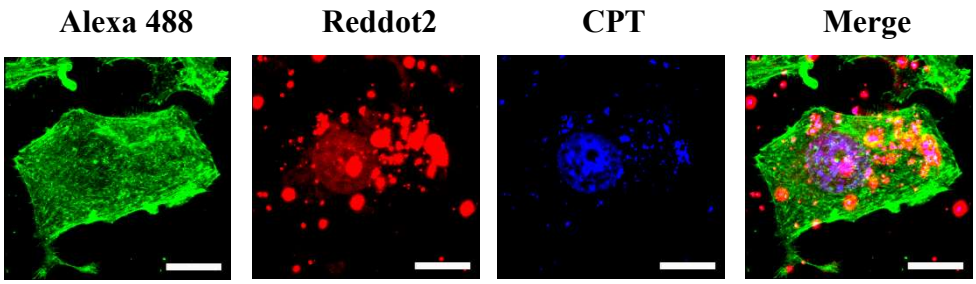


Figure S15. Endocytosis efficiency of FA-PEG-PDBO-BPT@MTPP micelle by HeLa and HUVEC cells after incubation for 4 h, and detected by CLSM and FCM. Prior to drug treatment, HeLa cells were pre-incubated with excessive free FA molecules to block FA receptors of HeLa cells. Scale bar: 50 μm.

A



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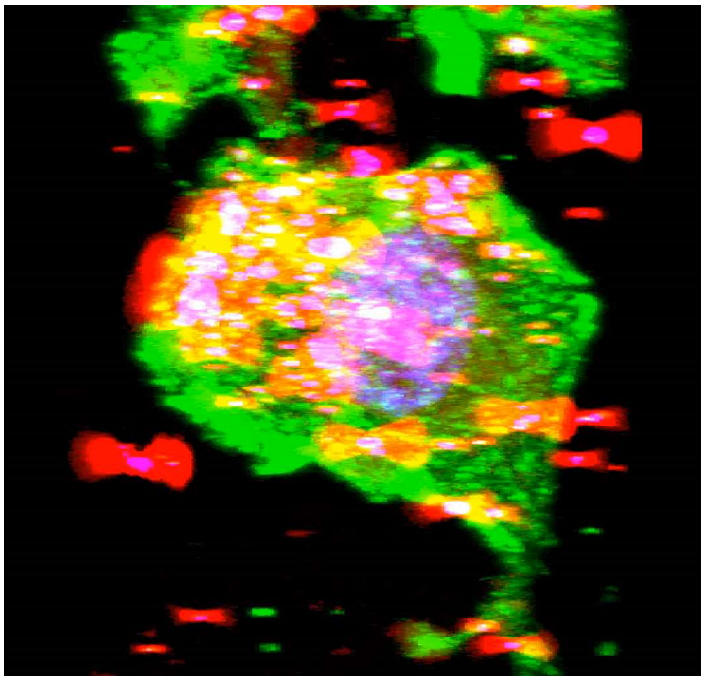

Figure S16. 3D reconstructed images and video of intracellular release and distribution of CPT after treatment with FA-PEG-PDBO-BPT@MTPP for 12 h. Scale bar: 50 μm.

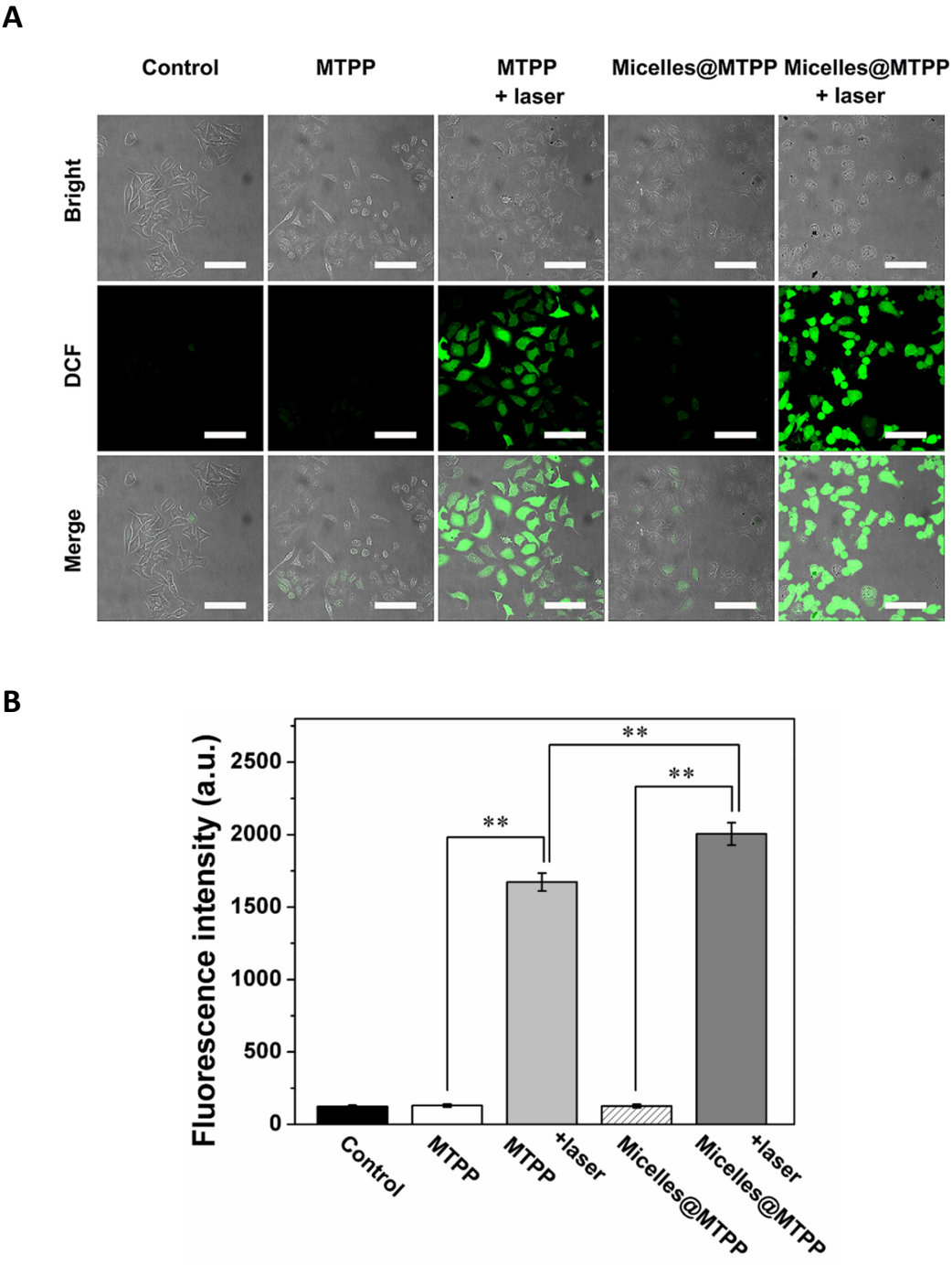

Figure S17. (A) CLSM images and (B) quantitative measurement of intracellular ROS. HeLa cells were incubated with free MTPP, FA-PEG-PDBO-BPT@MTPP micelles (micelles@MTPP) for 12 h, and then stained with ROS detection probe dichlorofluorescin-diacetate (DCFH-DA, green) plus/without laser irradiation (10 min). Error bars present as mean ± SD (n=4), **p < 0.01. Scale bar: 100 μm.

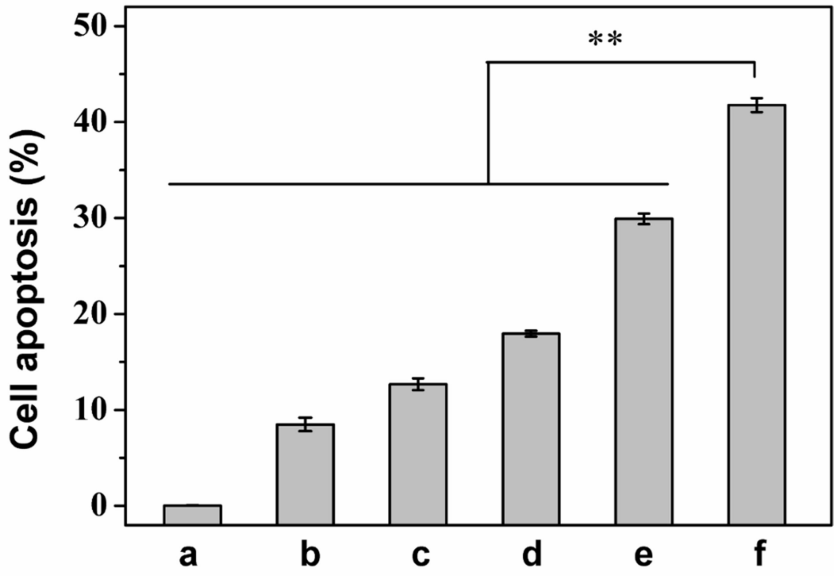

Figure S18. Quantitative analysis of HeLa cells apoptosis after treatments with PBS (control, a), MTPP (b), CPT (c), micelles (d), and micelles@MTPP without (e)/plus (f) laser irradiation (10 mins) for 12 h. Error bars present as mean ± SD (n=4), **p < 0.01.

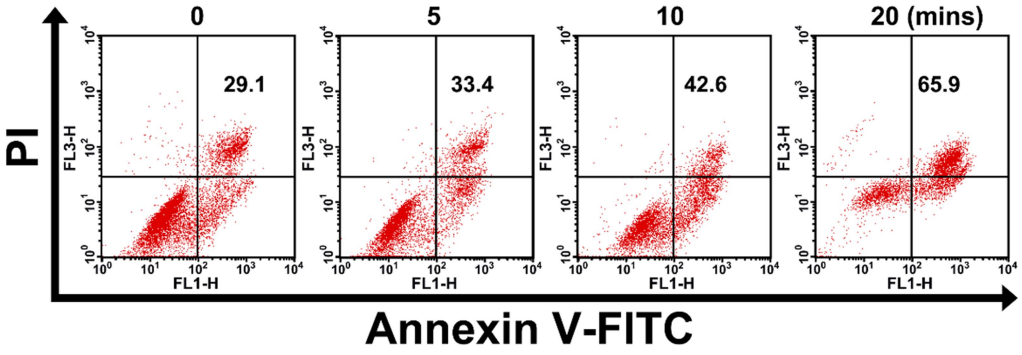

Figure S19. The irradiation time-dependent apoptosis of HeLa cells after treatments with micelles@MTPP for 12 h with various irradiation times (0, 5, 10 and 20 min) and detected by FCM.

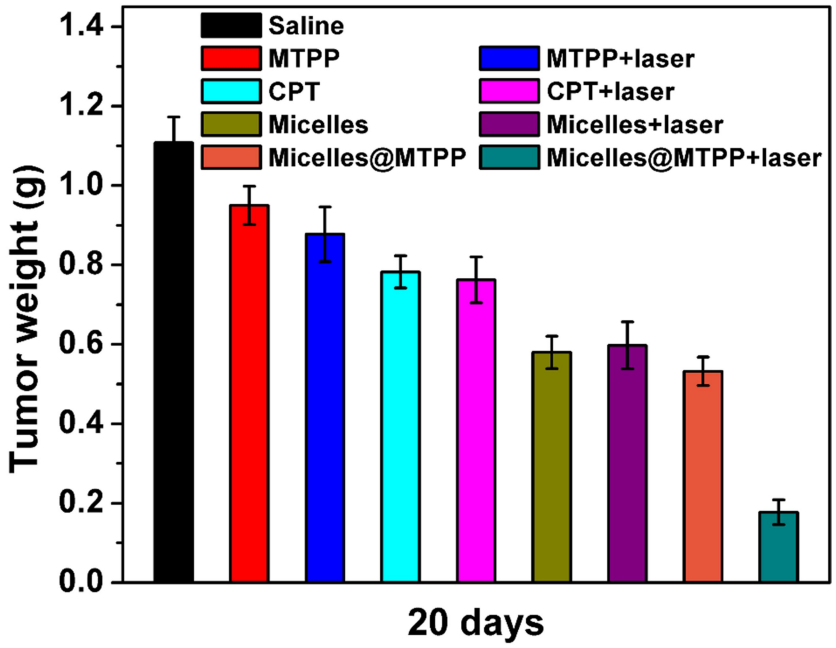

Figure S20. Final weights of tumors after different treatments.

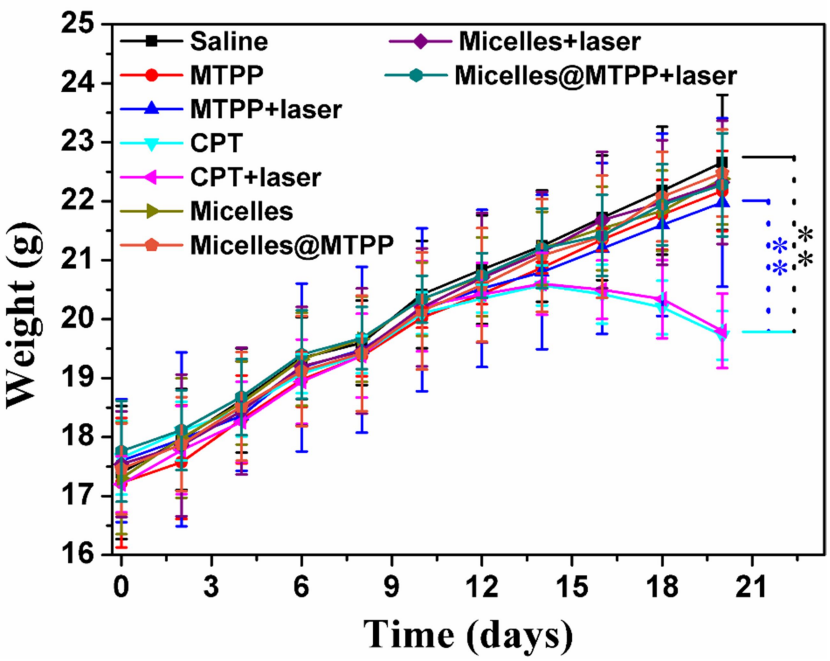

Figure S21. The curve of weight change of mice bearing HeLa tumors after different treatments. Error bars present as mean ± SD (n=6), **p < 0.01.

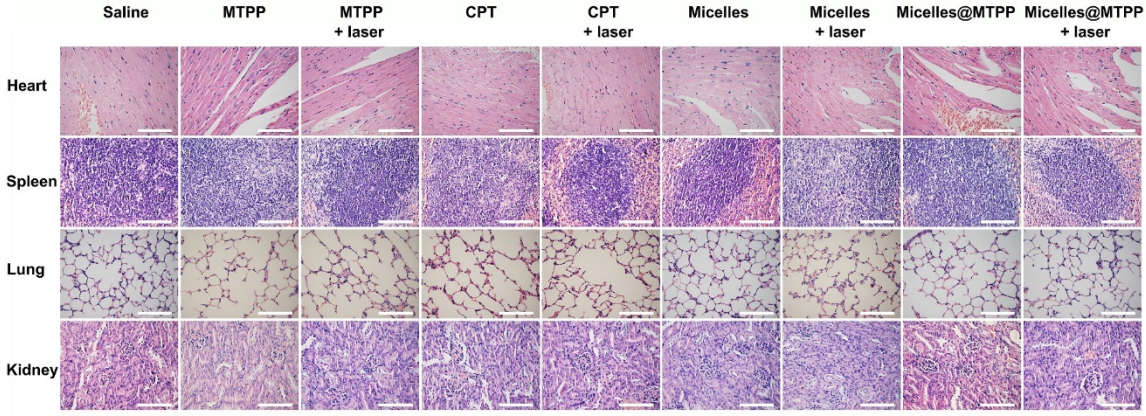

Figure S22. H&E analyses of major tissues (heart, spleen, lung, and kidney) of the mice after treatments with various formulations for 21 days. Scale bar: 100 μm.

Table S1. Molecular weight data of various intermediate products in the synthesis process of FA-PEG-PDBO-BPT copolymer.

Polymer	$Mn-_{theory}$	$Mn-_{NMR}$	$Mn-_{GPC}$	PDI
bPEG-BBO-BDO	8840	8620	7900	1.21
PDEA	2500	2480	2200	1.32
bPEG-PDBO-BOH	10254	10019	9400	1.28
FA-PEG-PDBO-BPT	15378	14882	13600	1.26

Mn-theory: Theoretical molecular weight calculation based on monomer conversion.

$Mn-_{NMR}$: Molecular weight based on ^{1}HNMRanalysis.

$Mn-_{GPC}$: Number-average absolute molecular weight determined by GPC equipped with refractive index and multi-angle light scattering detectors.

PDI: Polydispersity index determined by GPC analysis.