Supporting Information

Light-Induced Cytosolic Activation of Reduction-Sensitive Camptothecin-Loaded Polymeric Micelles for Spatiotemporally Controlled *In Vivo* Chemotherapy

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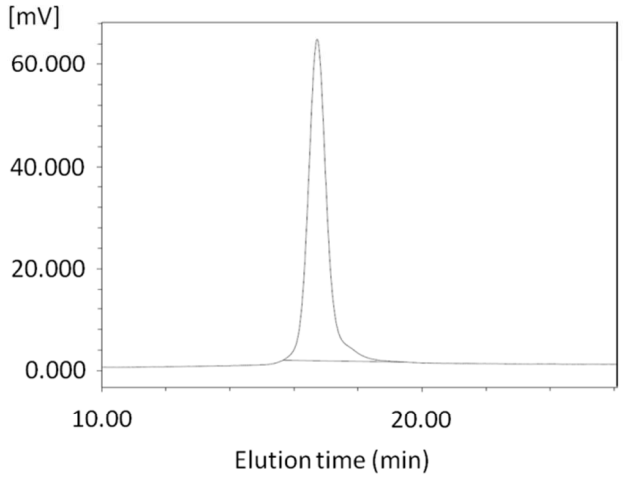
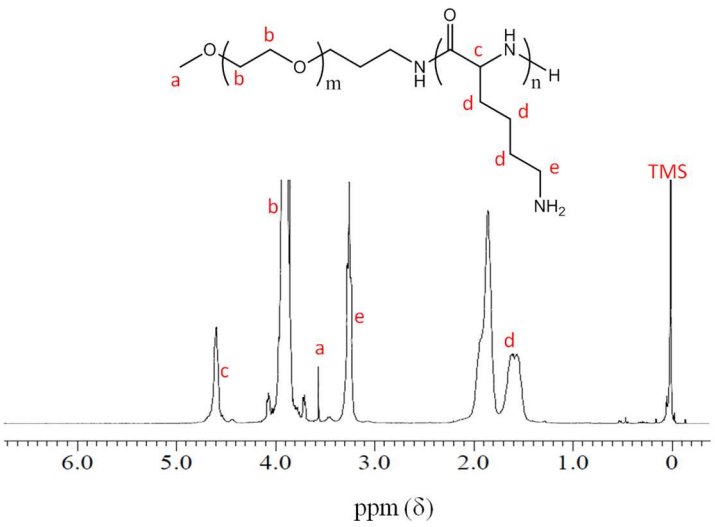
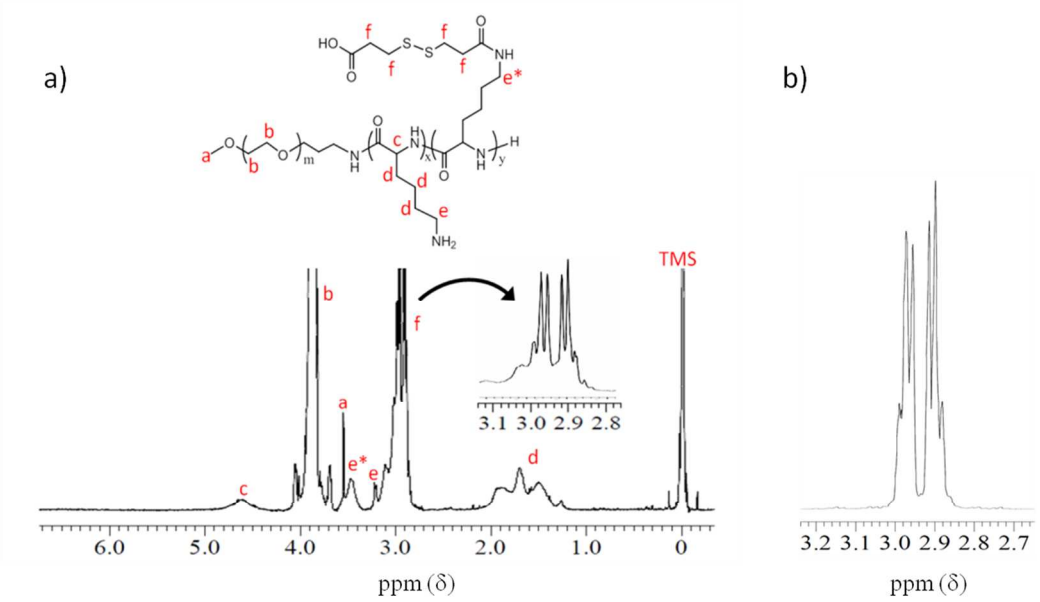


Supporting Figure S1. GPC chromatogram of PEG-P(Lys(TFA)) (12k-37DP). The result showed a narrow molecular weight distribution (M_w/M_n: 1.04). (eluent: DMF, containing 10 mM LiCl, temperature: 40 °C, flow rate: 0.8 mL/min, detector: refractive index).



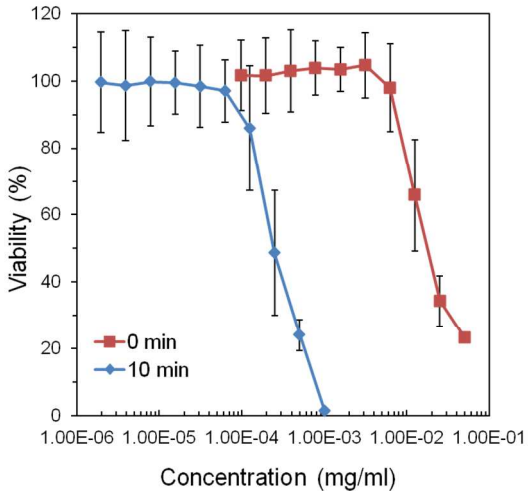
Supporting Figure S2. ^{1}H-NMR spectrum of PEG-P(Lys) (12k-37DP). (Solvent: TFA-*d*, 99.0 atom%D; temperature: 50 °C).



Supporting Figure S3. (a) ^{1}H-NMR spectrum of PEG-P(Lys(DP)); the inset plot shows an enlarge version of the peaks between δ = 2.8-3.1 ppm. (b) ^{1}H-NMR spectrum of 3,3'-dithiodipropionic acid. (Solvent: TFA-*d*, 99.0 atom %D; temperature: 50 °C)

***In Vitro* Photocytotoxicity of Photosensitizers.** To determine the non-phototoxic concentration region of Photofrin, the *in vitro* growth inhibitory activity of the photosensitizers was determined. Briefly, AY27 bladder carcinoma cells were seeded at 3,000 cells into each well of 96-well plates. After 24 h incubation, 50 μL of medium containing serial dilution of Photofrin were added into each well, and the cells were incubated for another 24 h. Then, the cells were irradiated (fluence rate: 3.0 mW/cm^2, time: 10 min) using a 300 W halogen lamp box equipped with a band pass filter (400 nm-700 nm) and the cells were post-incubated for 24 h. The cell viability was evaluated by WST-8 assay, measuring the

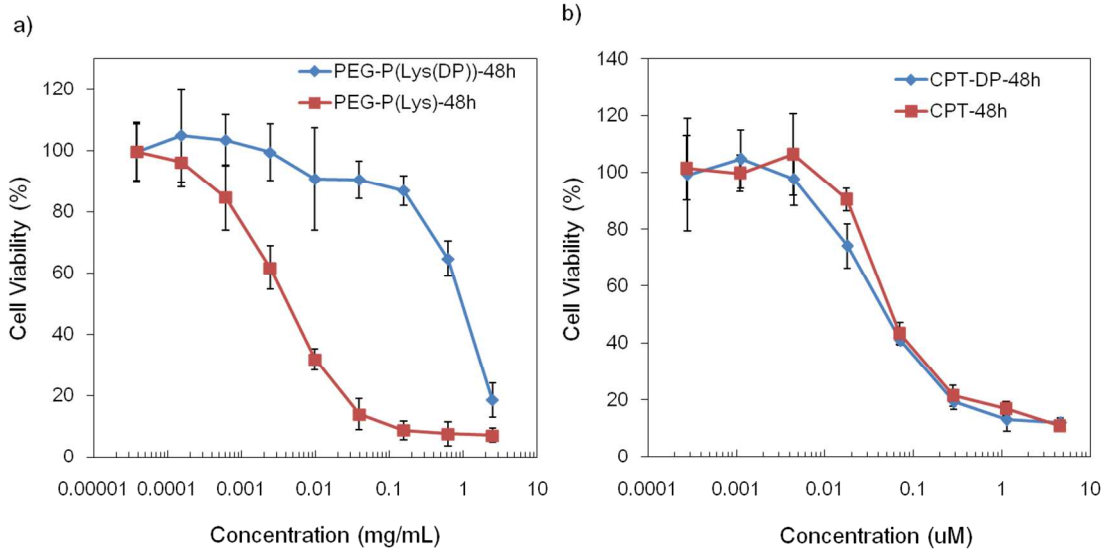
absorbance at 450 nm. The results were presented as the means and SD (n = 4). The non-toxic concentrations of 0.1 and 0.01 μg/ml of irradiated Photofrin were used for studying the PCI-enhancement of the cytotoxicity of CPT/m.



Supporting Figure S4. Cell viability of AY27 cells incubated with Photofrin with (■) or without (■) photoirradiation.

***In vitro* Cytotoxicity of Thiolated CPT and Thiolated PEG-P(Lys).** To determine the cytotoxicity of thiolated CPT (CPT-DP), which is released from the micelles after reduction of the disulfide bond, and the thiolated PEG-P(Lys) (PEG-P(Lys-DP)), the *in vitro* growth inhibitory activity of AY27 bladder carcinoma cells was examined. Briefly, AY27 cells were seeded at 3,000 cells into each well of 96-well plates. After 24 h incubation, 50 μL of medium containing serial dilution of CPT, CPT-DP, PEG-P(Lys)

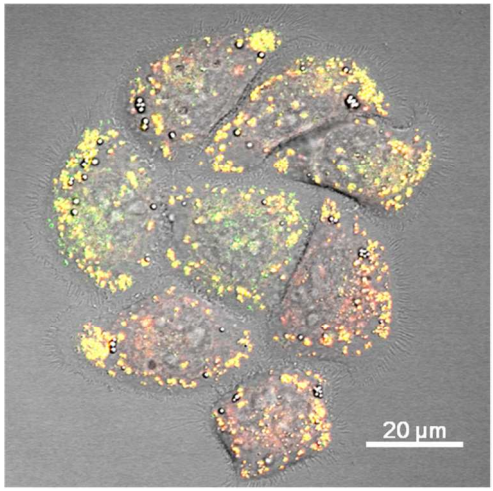
and PEG-P(Lys-DP)) were added into each well, and the cells were incubated for another 48 h. Then, the cell viabilities were evaluated by WST-8 assay, measuring the absorbance at 450 nm. The results were presented as the means and SD (n = 4). After 48 h incubation, the thiolated polymer revealed a reduced cytotoxicity than PEG-P(Lys) (Supporting Figure S5a), which may be due to the decreased positive charge of lysine after thiolation of polymer side chains. In case of CPT-DP, the cytotoxicity against AY27 cancer cells was comparable to CPT (Supporting Figure S5b).



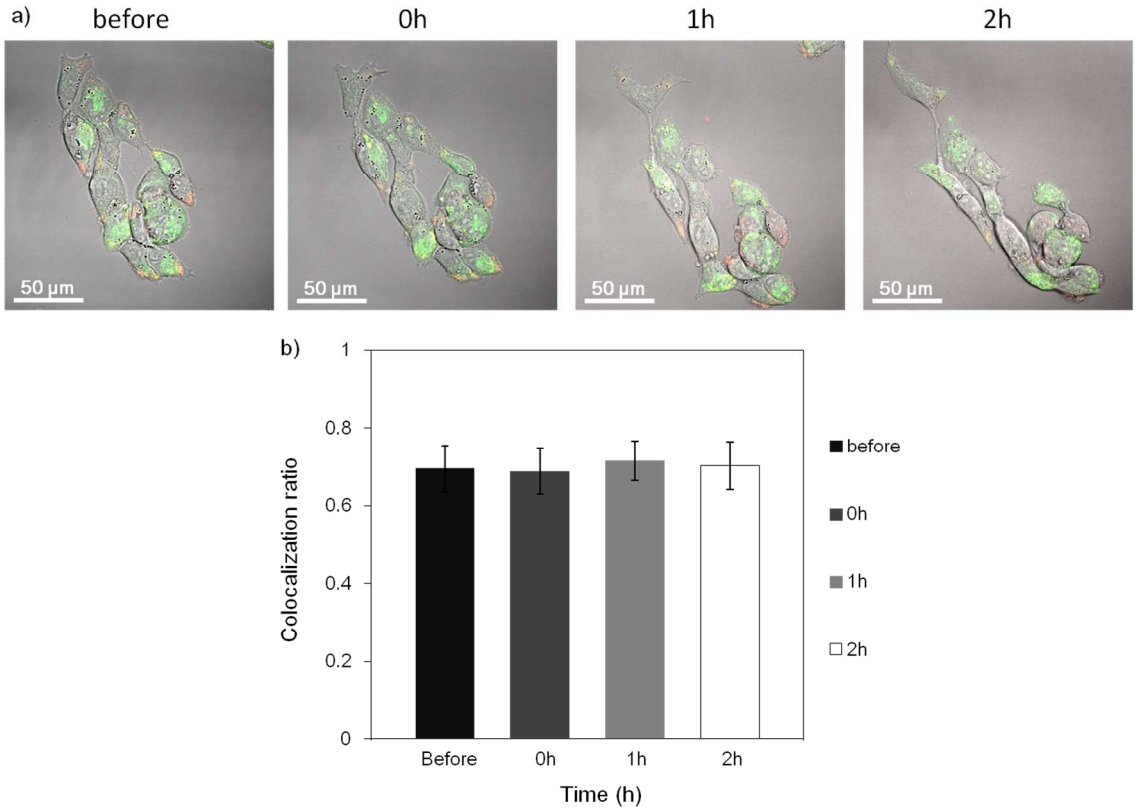
Supporting Figure S5. (a) Cell viability of AY27 cells incubated with (■) PEG-P(Lys) or (●) PEG-P(Lys(DP)) for 48 h. (b) Cell viability of AY27 cells incubated with (■) CPT or (●) CPT-DP for 48 h.

Intracellular Distribution of Photofrin. To determine the intracellular distribution of Photofrin, AY27 bladder carcinoma cells were seeded at 40,000 cells on glass-based dishes. After 24 h incubation, the

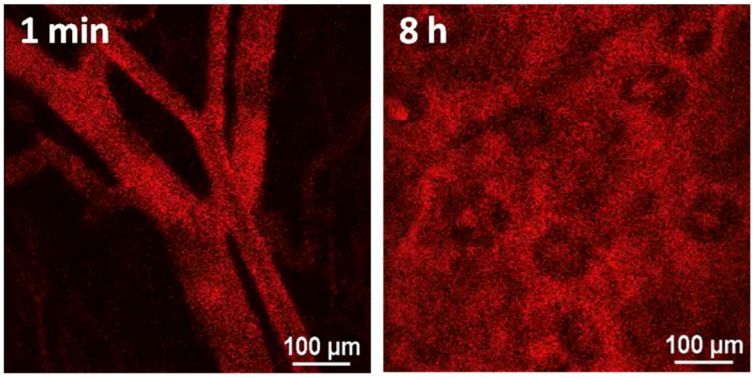
cells were treated with Photofrin (0.1 μg/ml) and incubated for another 24 h. For CLSM observation, lysosomes were pre-stained with lysotracker-green to confirm the intracellular localization of Photofrin in AY27 cells. The results revealed Photofrin can localize in lysosomes.



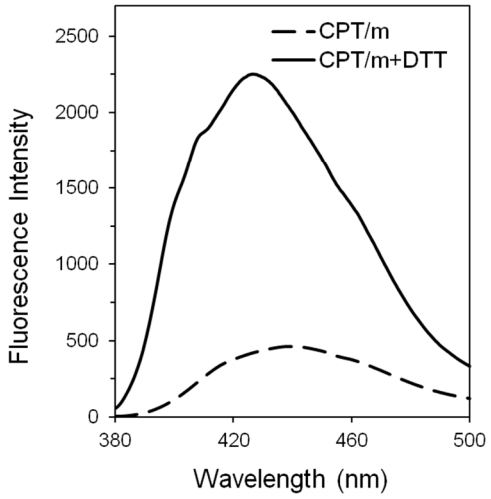
Supporting Figure S6. CLSM image of AY27 cells incubated with Photofrin for 24 h. Green: lysotracker green, Red: Photofrin, Yellow: colocalization of lysosomes and Photofrin.



Supporting Figure S7. (a) CLSM image of AY27 cells incubated with Alexa 555-labeled CPT_{10}/m were taken before and 0 h, 1 h, 2 h after light irradiation. Green: CellLight Late Endosome GFP, Red: Alexa 555-labeled CPT/m, Yellow: micelles in late endosomes. (b) Time-dependent colocalization ratio of micelles and late endosomes.



Supporting Figure S8. Snap-shots of mouse ear-lobe taken by *in vivo* CLSM recorded at 1 min and 8 h after the i.v. injection of Photofrin (red).



Supporting Figure S9. Fluorescence spectra of CPT/m before and after incubation with 10 mM DTT.