

Supporting Information

Design of Tumor Acidity-Responsive Sheddable Nanoparticles for Fluorescence/Magnetic Resonance Imaging-Guided Photodynamic Therapy

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Synthesis of copolymer PCL₄₅-*b*-PAEP₃₅.

The macroinitiator PCL₄₅-OH (the subscript number represents the degrees of polymerization of the PCL) was synthesized by ring-opening polymerization of ϵ -caprolactone in THF using aluminum isopropoxide as the initiator [1]. The average degree of polymerization is 45, which was calculated based on the integration ratio of methylene protons from the PCL block (2H, 2.95 ppm) and methyl protons of isopropanol (6H, 1.31 ppm) from ¹H NMR (Figure S1A). Then, the block copolymer PCL₄₅-*b*-PAEP₃₅ was synthesized by ring-opening polymerization of AEP using PCL₄₅-OH as the initiator and TBD as the catalyst [2]. As a typical example, PCL₄₅-OH (2.57 g, 0.5 mmol), hydrophobic cyclic phosphate monomers AEP (3.28 g, 20.0 mmol), and 20 mL THF were added into a fresh flamed and nitrogen purged round-bottomed flask in a glove box. After stirring for 10 min, TBD (69.4 mg, 0.5 mmol) was added and stirred for 5 min. Then benzoic acid (0.122 g, 1.0 mmol) was added to terminate the reaction. The sample was concentrated and precipitated into a cold diethyl ether/methanol mixture (10/1, v/v) twice. The product was dried under vacuum at room temperature to a constant weight to obtain the product with a yield of 89.7%. The average degree of polymerization of AEP is 35, which was calculated based on the integration ratio of methylene protons from the PAEP block (4H, 4.21 ppm) and methylene protons from the PCL block (2H, 2.95 ppm) from ¹H NMR (Figure S1A).

Synthesis of amino-functionalized polymer PCL₄₅-*b*-PAEP₃₅-Cya.

PCL₄₅-*b*-PAEP₃₅-Cya was synthesized by thiol-ene 'click' reaction of PCL₄₅-*b*-PAEP₃₅ with Cysteamine (Cya) according to our previously reported procedure [2]. The reaction was performed as follows: 1.08 g of PCL₄₅-*b*-PAEP₃₅ (0.1 mmol), 3 equivalents (to alkene) of Cya (1.19 g, 10.5 mmol) and 0.05 equivalent (to alkene) of DMPA (44.85 mg, 0.175 mmol) were dissolved in dry Dimethylformamide (DMF, 5.0 mL). Then, the reaction solution was purged by N₂ for 20 min, and irradiated under UV ($\lambda_{\text{max}} = 365$ nm) for 30 min. Subsequently, the sample was transferred into the dialysis membrane tubing (MWCO 14000 Da) and dialyzed against distilled water at 4 °C for 24 h to remove impurities, and PCL₄₅-*b*-PAEP₃₅-Cya was obtained by lyophilization (yield: 83.2%).

Synthesis of PCL₄₅-*b*-PAEP₃₅-Cya/DTPA.

The PCL-*b*-PAEP/Cya grafted with Diethylenetriaminepentaacetic dianhydride (DTPA) was synthesized as follows: PCL-*b*-PAEP/Cya (100 mg, 0.24 mmol NH₂) and DTPA (125.8 mg, 0.35 mmol) was dissolved in DMSO (10 mL). Under N₂ atmosphere, trimethylamine (TEA, 0.1 mL) and pyridine (0.1 mL) was added. The mixture was stirred at room temperature for 24 h. Resulting solution was precipitated into diethyl ether twice Then dialyzed against ultrapurified water using Spectra dialysis tubing (molecular weight cutoff of 3500 Da, Spectrum Laboratories). The product was dried under vacuum at room temperature (yield: 85.3%), and the obtained polymer was denoted as PCL₄₅-*b*-PAEP₃₅-Cya/ DTPA.

Synthesis of tumor acidity-responsive polymer PPC-DA.

The polymer PPC-DA was synthesized by a two-step method. The parental diblock copolymer monomethoxyl poly(ethylene glycol)-*b*-poly(allyl ethylene phosphate) (mPEG-*b*-PAEP) was first synthesized by ring-opening polymerization of AEP using mPEG₄₅-OH as the initiator and TBD as the catalyst [3]. The average degree of polymerization of AEP is 75, which was calculated based on the integration ratio of methylene protons from the PAEP block (4H, 4.25 ppm) and methylene protons from the PEG block (4H, 3.64 ppm) from ¹H NMR (Figure S2A). And then, amino-functionalized polymer mPEG₄₅-*b*-PAEP₇₅-Cya (PPC) was prepared by thiol-ene 'click' reaction of mPEG-*b*-PAEP with Cya under UV irradiation [3]. Subsequently, PPC-DA was then synthesized by reaction of PPC with 2,3-dimethylmaleic anhydride (DMMA). The reaction was performed as follows: 0.200 g of PPC was dissolved in phosphate buffer (0.2 M, pH 8.0), and 3 equivalents (to amino groups) of DMMA (0.249 g) was added in several portions. The pH of solution was kept in the range of 8-9 by addition of NaOH solution (1 M). The reaction was continued at room temperature for 4 h. After ultrafiltration using Amicon YM-30 centrifugal filter devices (Millipore, MWCO 3000 Da), the solution was freeze-dried to yield PPC-DA [2].

Preparation of Ce6 and Gd³⁺ ions-loaded cationic nanoparticles

[⊕]NP_{Ce6&(DTPA-Gd)}

The photosensitizer Ce6 (1.0 mg) and PCL₄₅-*b*-PAEP₃₅-Cya/DTPA (10.0 mg) were dissolved in 1.0 mL of DMF, and then, the mixed solution was added into 10 mL of ultrapure water under stirring. After additional stirring for 2 h, the solution was dialyzed against (MWCO: 14000 Da) ultrapure water for 24 h to remove DM, the final solution was filtered through a 0.45- μ m filter (Millipore) to remove unloaded Ce6 and obtain Ce6-loaded cationic nanoparticles, \oplus NP_{Ce6}. The concentration of Ce6 was determined using a UV-Vis spectrometer (UV-2802 PC, UNICO Instruments) at a wavelength of 405 nm and referring to a standard curve of free Ce6 concentrations in DMSO.

Subsequently, to chelate Gd³⁺ ions, the 0.2 M gadolinium/0.6 M citrate/NaOH pH 8.0 (10 mL) solution was added to the solution of \oplus NP_{Ce6} according to the previously reported procedure [4]. After incubation for three days at room temperature, excess gadolinium was removed by dialysis. The Ce6 and Gd³⁺ ion-loaded cationic nanoparticle was denoted as \oplus NP_{Ce6&(DTPA-Gd)}. The concentration of Gd³⁺ was determined by inductively coupled plasma mass spectrometry (ICP-MS), indicating that the Gd³⁺ content was 3.23 wt %.

Preparation of PPC-DA/ \oplus NP_{Ce6&(DTPA-Gd)} and PPC-SA/ \oplus NP_{Ce6&(DTPA-Gd)}.

The pH_e-responsive negatively charged polymer PPC-DA or the control polymer PPC-SA was introduced onto the surface of \oplus NP_{Ce6&(DTPA-Gd)} *via* electrostatic interaction. The aqueous solution of PPC-DA (1.0 mL) at different concentrations was

added to an aqueous solution of $\oplus\text{NP}_{\text{Ce6}\&(\text{DTPA-Gd})}$ (1.0 mL, 1 mg/mL in water) at different weight ratios. The solution was allowed to stand at room temperature for 15 min before use.

Determination of ROS generation.

The S-NP, unS-NP or free Ce6 were mixed with 50 mM p-nitroso-dimethylaniline (RNO) and 100 mM imidazole in 20 mM phosphate buffer (pH 7.4), and then irradiated by a 660 nm laser (0.5 W/cm^2) for different periods of time, and reduction of RNO absorption at 440 nm was determined to reflect the production of ROS.

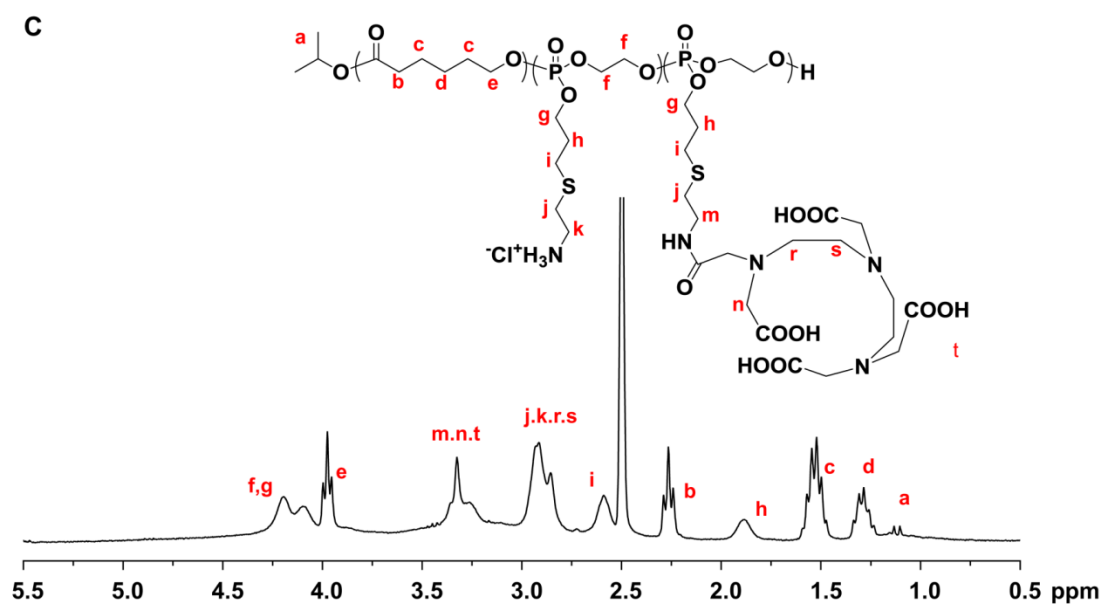
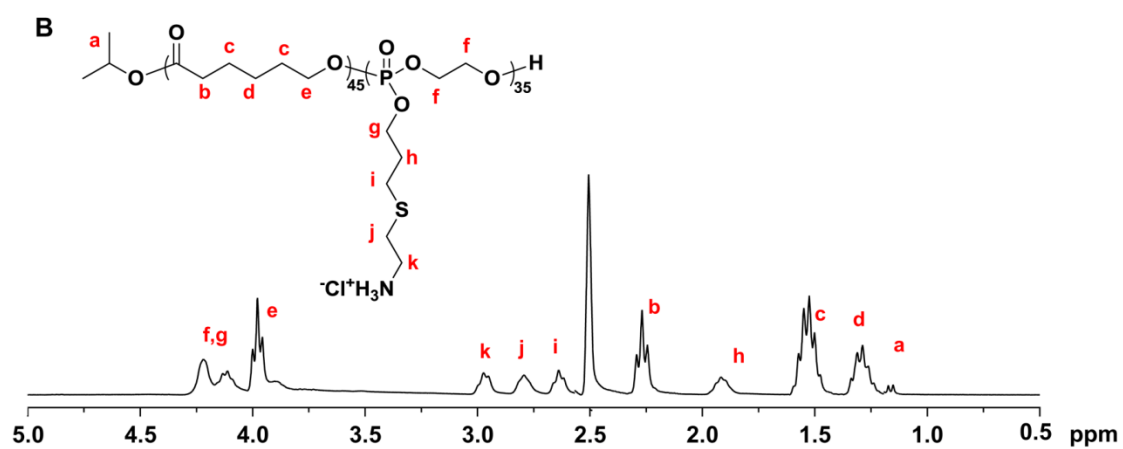
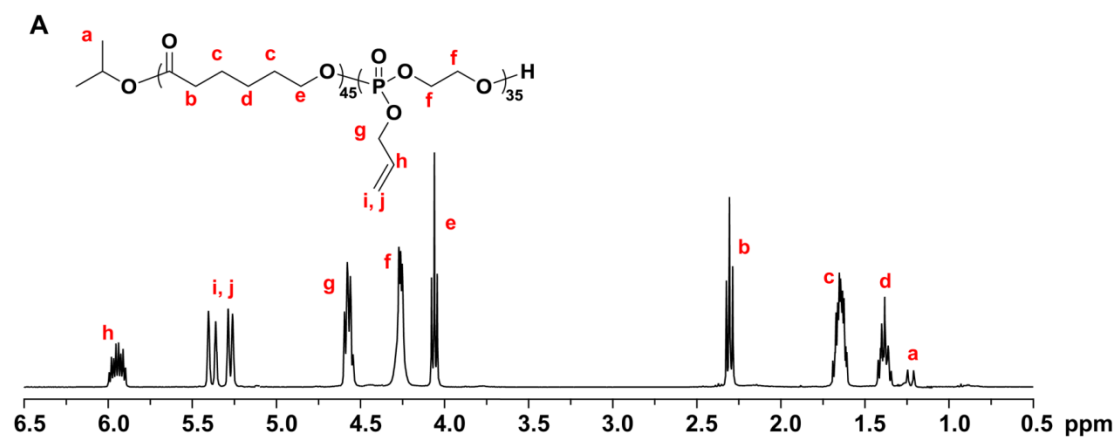


Figure S1. ^1H NMR spectra of (A) $\text{PCL}_{45}\text{-}b\text{-PAEP}_{35}$ (in CDCl_3), (B) $\text{PCL}_{45}\text{-}b\text{-PAEP}_{35}\text{-Cya}$ (in DMSO) and (C) $\text{PCL}_{45}\text{-}b\text{-PAEP}_{35}\text{-Cya/DTPA}$.

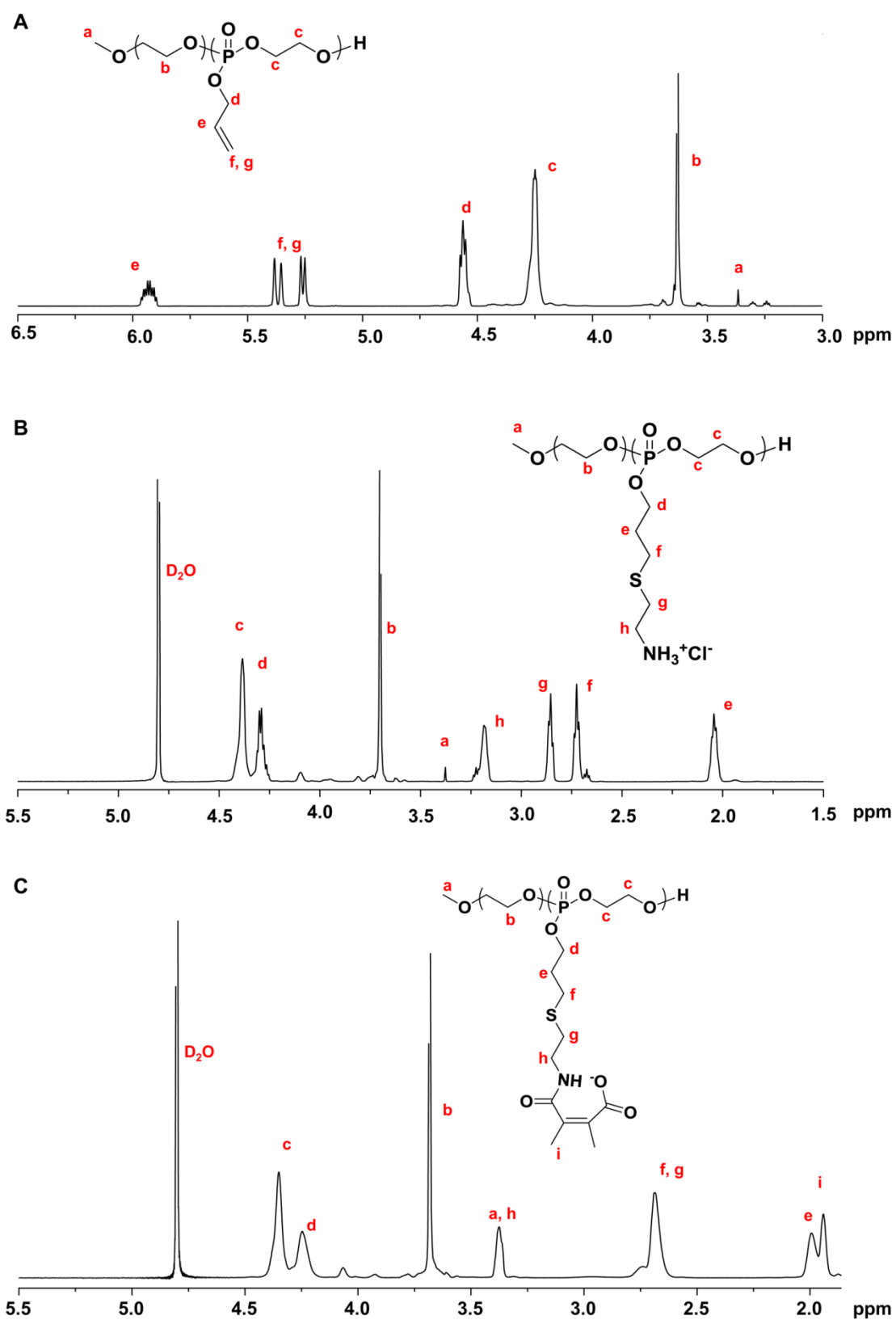


Figure S2. ^1H NMR spectra of (A) mPEG-*b*-PAEP (in CDCl_3), (B) mPEG-*b*-PAEP-Cya (in D_2O) and (C) PPC-DA (in D_2O).

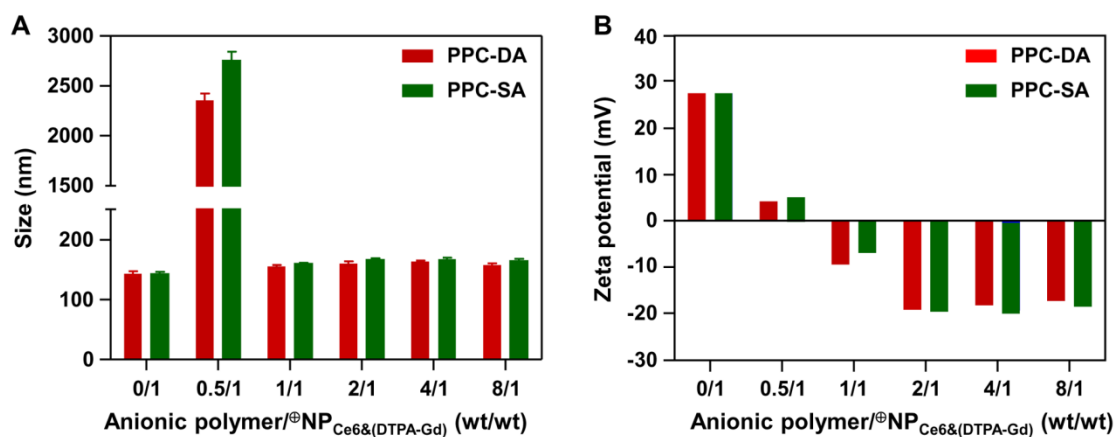


Figure S3. Effect of anionic polymer/⊕NP_{Ce6&(DTPA-Gd)} weight ratio on size (A) and zeta-potential (B) of formed nanoparticles.

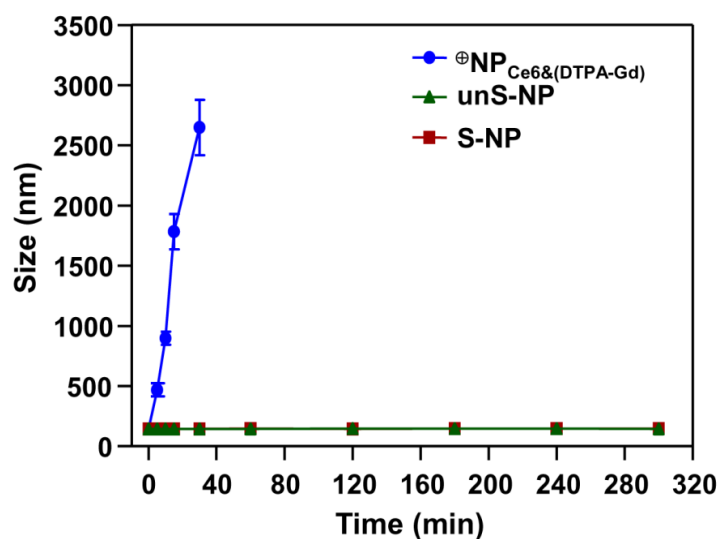


Figure S4. The size change of S-NP, unS-NP, and ⊕NP_{Ce6&(DTPA-Gd)} following incubation with PBS which contain 10% FBS.

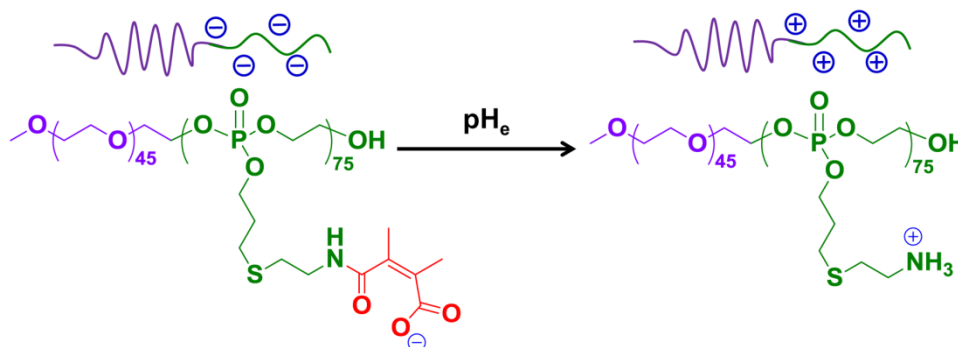


Figure S5. The pH-responsive mechanism of PPC-DA at pH_e.

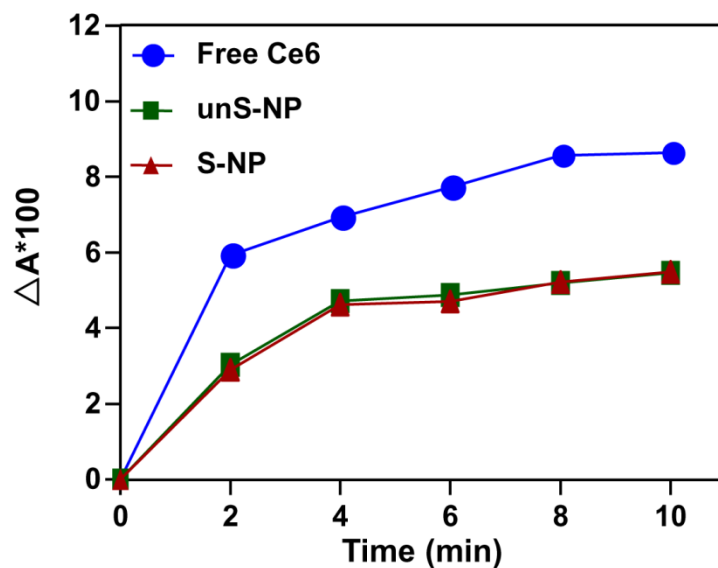


Figure S6. ROS generation by various samples indicated under the 660 nm laser irradiation. The generation of singlet oxygen was determined by the bleaching of RNO absorbance at 440 nm.

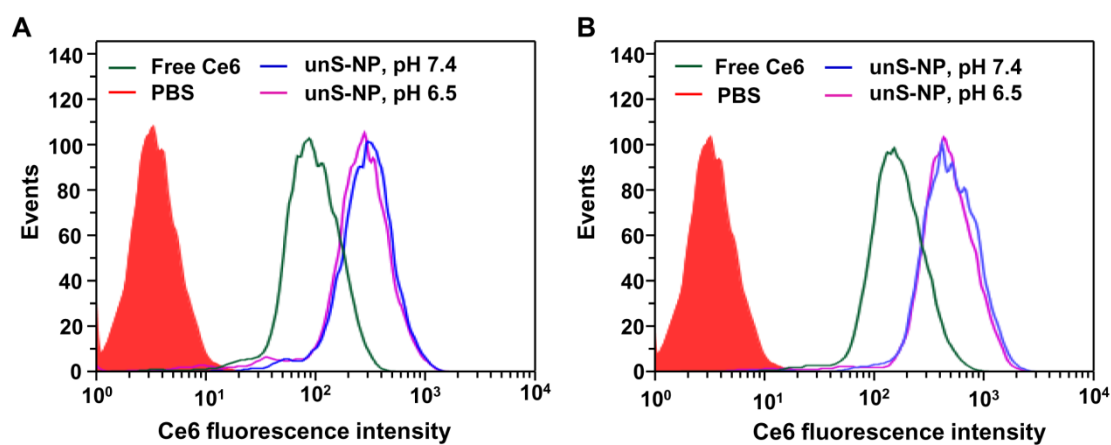


Figure S7. Flow cytometric analyses of BxPC-3 cells after incubation with unS-NP at different pH value for 2 h (A) and 4 h (B).

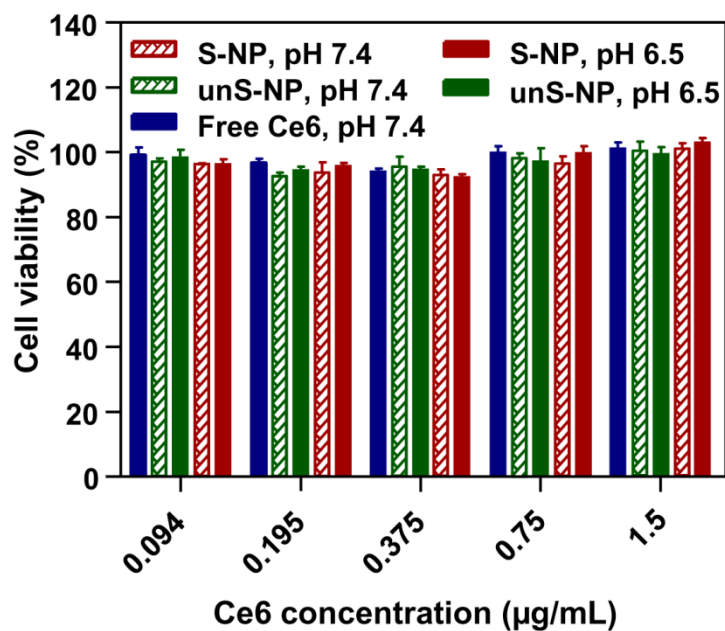


Figure S8. The cytotoxicity of unS-NP, S-NP and free Ce6 performance in BxPC-3 cells without 660-nm laser irradiation.

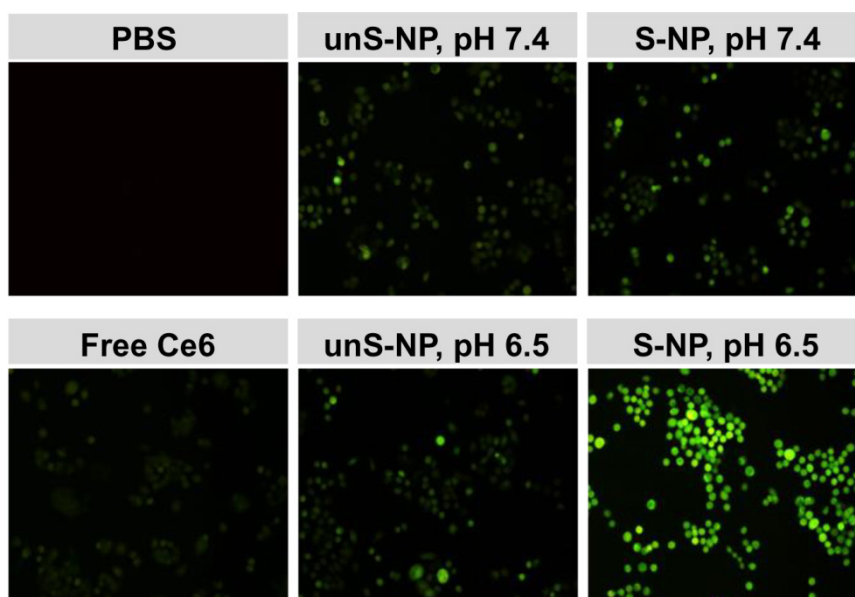


Figure S9. Fluorescence microscopy image of cells incubated with DCFH-DA and then treated with free Ce6, unS-NP and S-NP at different pH with 660-nm laser irradiation.

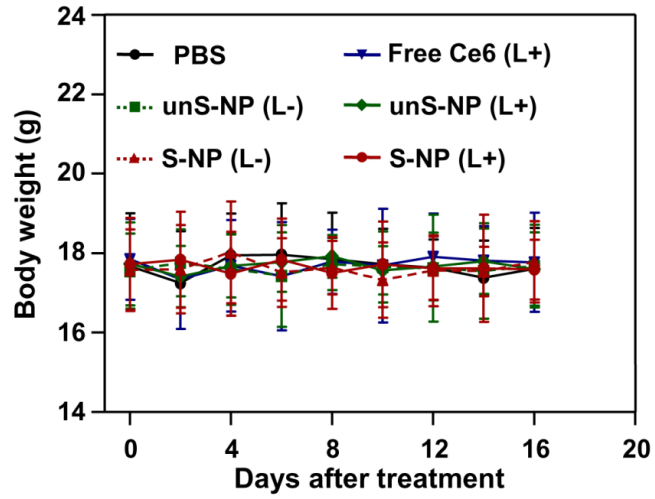


Figure S10. Body weight of mice bearing BxPC-3 tumor at different time points after treatment.

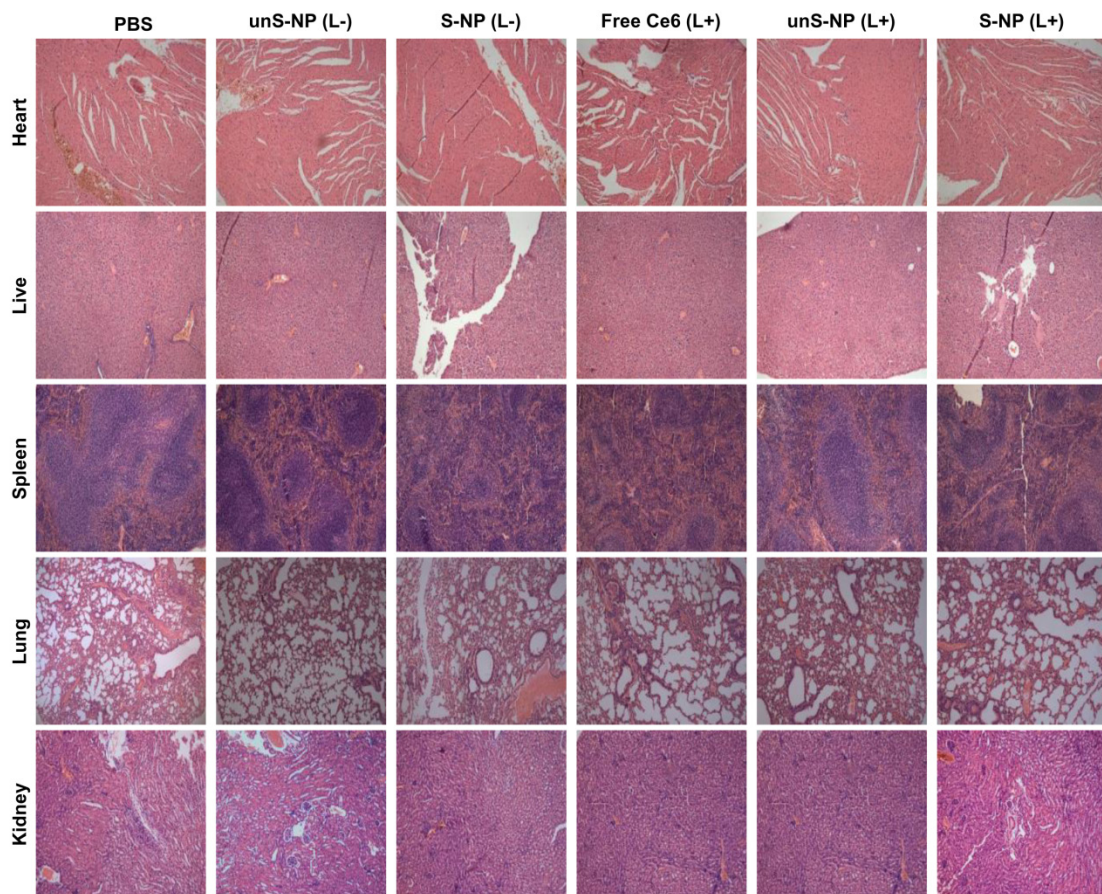


Figure S11. The H&E analysis of different organs after treatment with different formulation of Ce6.

References:

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